ABANT IZZET BAYSAL UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

EFFECT OF ANTIOXIDANT MORIN ON XENOBIOTIC METABOLIZING ENZYMES IN THE LIVER OF 7,12-DIMETHYLBENZ[A]ANTHRACENE AND ENDOSULFAN TREATED HEALTHY AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

DOCTOR OF PHILOSOPHY

CANAN SAPMAZ

BOLU, SEPTEMBER 2015

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APPROVAL OF THE THESIS

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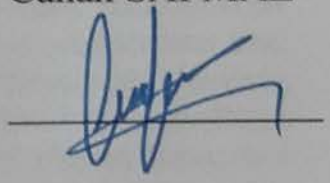
To my family

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Canan SAPMAZ



ABSTRACT

EFFECT OF ANTIOXIDANT MORIN ON XENOBIOTIC METABOLIZING ENZYMES IN THE LIVER OF 7,12-DIMETHYLBENZ[A]ANTHRACENE AND ENDOSULFAN TREATED HEALTHY AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

PHD THESIS
CANAN SAPMAZ
ABANT IZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF CHEMISTRY
(SUPERVISOR: PROF. DR. AZRA BOZCAARMUTLU)

BOLU, SEPTEMBER 2015

Living organisms are exposed to different chemicals at the same time in their daily lives. Some of these chemicals are beneficial but some of them are harmful for us. 7,12-Dimethylbenz[a]anthracene (DMBA) is a lipid soluble molecule, producing toxic and carcinogenic effects within the body. Endosulfan is an organochlorine pesticide, used extensively in the agricultural areas against insects. It may exist in air, freshwater, sea water, soil samples and foods. It is possible to be exposed both DMBA and endosulfan with smoking cigarette and breathing polluted air in our daily lives. Morin is a dietary flavonoid having chemoprotective and antioxidant properties in living organisms. Diabetes mellitus is a metabolic disorder causing damage in organs. The effects of morin have not been determined in the presence of toxic chemicals and diabetes. Therefore, this study is aimed to determine the effect of morin on xenobiotic metabolizing enzymes in the presence of DMBA and endosulfan in normal and diabetic rats. For this purpose 120 male Wistar rats (weighing 170-245 g) were randomly selected and divided into 16 groups (8 groups were composed of normal rats and the other 8 groups were diabetic rats). The rats in control group were treated with corn oil three times in a week. Morin administration was started at 1st day of the administration period and continued through the experimental period with 25 mg/kg body weight morin with the frequency of three times in a week. DMBA-treated groups were gavaged with 30 mg/kg body weight DMBA at 12th, 19th and 26th days of the administration period. Endosulfan treatment was started at the 12th day of the administration period at a dose of 5 mg/kg body weight endosulfan. This treatment was continued through the experimental period with the frequency of three times in a week. Rats in normal rat groups were killed by cervical dislocation on the 54th day of the administration period and rats in diabetic rat groups were killed by cervical dislocation on the 43rd day of the administration period. Microsomes and cytosols were prepared for each liver tissue by differential centrifugation. Cytochrome P4501A1 (CYP1A1) associated 7-

ethoxyresorufin O-deethylase (EROD) activities increased in all treatment groups. Rats in morin, DMBA and endosulfan group had 1.6, 1.8 and 1.6 fold higher EROD activities than those of the control group. The highest EROD activities measured in liver microsomes of normal rats were obtained from DMBA+endosulfan+morin group which was significantly higher (2.6-fold) than the activity obtained from the control group ($p<0.05$). Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase (MROD) activities of endosulfan, morin and endosulfan+morin groups were not different from the MROD activities of the control. DMBA treatment significantly increased MROD activity in normal rats (1.5-fold). Co-administration of DMBA and endosulfan also increased MROD activities in normal rat group (1.8-fold). Morin treatment decreased the effect of endosulfan and DMBA on MROD activity. Morin, endosulfan and DMBA treatments did not produce significant effect on cytochrome P4502B associated 7-pentoxyresorufin O-depentylase (PROD) activities in normal rat groups. Cytochrome P4502E associated aniline 4-hydroxylase activities of rats significantly increased with endosulfan and DMBA administration. Their effects on CYP2E decreased with administration of these chemicals with morin. Cytochrome P4503A associated erythromycin N-demethylase activities increased with endosulfan, DMBA and morin treatment. The highest activities were measured in DMBA+endosulfan+morin group in normal rats. Glutathione S-transferase activities increased in DMBA treated groups. Catalase and glutathione reductase activities were not affected from morin and endosulfan treatments. DMBA treatment significantly increased catalase and glutathione reductase activities. The induction of the diabetes mellitus by intraperitoneal injection of streptozotocin significantly increased EROD, MROD, PROD, erythromycin N-demethylase, glutathione S-transferase, catalase and glutathione reductase activities of rat liver microsomes and cytosols compared to the control group ($p<0.05$). Endosulfan and DMBA treatments increased EROD and MROD activities in diabetic rats. Morin treatment decreased EROD activities in endosulfan and DMBA treated diabetic rats. MROD activities increased with endosulfan and DMBA treatments in diabetic rats. Morin treatment decreased MROD activities in diabetic rats treated with endosulfan and DMBA. Aniline 4-hydroxylase, erythromycin N-demethylase, glutathione S-transferase and catalase activities decreased with endosulfan and DMBA treatments in diabetic rats. Besides the protein analysis, liver tissues were also evaluated by histopathological analysis. The histopathological studies indicated that morin treatment was not modified the appearance of the liver cell. Co-administration of endosulfan and DMBA increased tissue damage in the liver. Morin treatment reduced the endosulfan and DMBA induced liver tissue damages both in normal and diabetic rats. In conclusion, co-administration of morin with endosulfan and DMBA decreased the toxic effects of endosulfan and DMBA in normal and diabetic rats.

KEYWORDS: Cytochrome P450 (CYP), 7,12-Dimethylbenz[a]anthracene, Endosulfan, Morin, Diabetes, Liver, Rat.

ÖZET

ANTİOKSİDAN MORİNİN, 7,12-DİMETİLBENZ[A]ANTRASEN VE ENDOSÜLFANA MARUZ BIRAKILMIŞ SAĞLIKLI VE STREPTOZOTOSİN İNDÜKLENMİŞ DİYABETİK SIÇANLARIN KARACİĞERİNDE KSENOBİYOTİK METABOLİZE EDEN ENZİMLER ÜZERİNE ETKİLERİ

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(TEZ DANIŞMANI: PROF. DR. AZRA BOZCAARMUTLU)

BOLU, EYLÜL - 2015

Canlı organizmalar günlük hayatlarında aynı anda çeşitli kimyasallara maruz kalmaktadır. Bu kimyasalların bazıları bizim için faydalıyken bazıları zararlıdır. 7,12-Dimetilbenz[a]antrasen (DMBA) vücutta toksik ve kanserojen etki yapan yağda çözünür bir moleküldür. Endosülfan tarım alanlarında böceklere karşı yaygın olarak kullanılan organoklorlu pestisittir. Endosülfan havada, tatlı su kaynaklarında, deniz suyunda, toprak örneklerinde ve gıdalarda bulunabilmektedir. Günlük hayatımızda her iki kimyasala da sigara tüketimi ve kirli havanın solunması ile maruz kalmak mümkündür. Morin canlı organizmalarda kemoprotektif ve antioksidan özelliklere sahip bitkisel kökenli bir flavonoiddir. Diyabet organlarda hasara neden olan bir metabolik hastalıktır. Toksik kimyasalların ve diyabet hastalığının varlığında morinin etkileri henüz belirlenmemiştir. Bu çalışmada, DMBA ve endosülfana maruz kalmış normal ve diyabetik sıçanlarda ksenobiyotik metabolize eden enzimler üzerine morinin etkisini belirlemek amaçlanmıştır. Bu amaçla, 120 erkek Wistar sıçanı (170-245 g ağırlığında) rastgele dağıtılarak 16 grup (8 grup normal sıçanlar ve diğer 8 grup diyabetik sıçanlar içermek üzere) oluşturulmuştur. Kontrol grubundaki sıçanlara haftada üç kez mısır yağı verilmiştir. Morin deneysel sürecin ilk gününde sıçanlara verilmeye başlanmış ve deney süreci boyunca haftada üç defa 25 mg/kg vücut ağırlığı morin olacak şekilde verilmiştir. DMBA uygulanan gruplara 30 mg/kg vücut ağırlığında DMBA deney sürecinin 12, 19 ve 26. günlerinde ağız yoluyla verilmiştir. Endosülfan uygulamasına (5 mg/kg vücut ağırlığında endosülfan) deney sürecinin 12. gününde başlanmış olup bu uygulama deney sürecinde haftada üç kez olacak şekilde sürdürülmüştür. Normal sıçanlar deney sürecinin 54. gününde, diyabetik sıçanlar deney sürecinin 43. gününde servikal dislokasyon ile öldürülmüştür. Mikrozom ve sitozoller her bir doku örneğinden

ayrı ayrı hazırlanmıştır. Sitokrom P4501A1 (CYP1A1) ilişkili 7-etoksiresorufin O-deetilaz (EROD) aktivitesi tüm deney gruplarında artmıştır. Morin, DMBA ve endosülfan gruplarındaki sıçanlarda kontrol grubuna kıyasla 1.6, 1.8 ve 1.6 kat daha yüksek EROD aktivitesi belirlenmiştir. En yüksek EROD aktivitesi kontrol grubuna kıyasla DMBA+endosülfan+morin grubundan elde edilen mikrozomlarda (2.6 kat) belirlenmiştir. Sitokrom P4501A2 aktivitesi ile ilişkili 7-metoksiresorufın O-demetilaz (MROD) aktivitesi endosülfan, morin ve endosülfan + morin gruplarında kontrol grubuna kıyasla farklılık göstermemiştir. Normal sıçanlarda DMBA uygulaması MROD aktivitesini anlamlı şekilde (1.5-kat) arttırmıştır. Normal sıçanlada DMBA ve endosülfanın birlikte uygulanması MROD aktivitesini 1.8 kat arttırmıştır. Morin uygulaması MROD aktivitesini üzerine endosülfan ve DMBA etkisini azaltmıştır. Morin, endosülfan ve DMBA sitokrom P4502B ile ilişkili 7-pentoksiresorufin O-dependilaz (PROD) aktivitesi üzerinde değişikliğe sebep olmamıştır. Sitokrom P4502E ilişkili anilin 4-hidroksilaz aktivisi endosülfan ve DMBA verilen sıçanlarda önemli miktarda artmıştır. Bu kimyasalların CYP2E üzerindeki etkileri morin uygulaması ile azalmıştır. Sitokrom P4503A ilişkili eritromisin N-demetilaz aktiviteleri endosülfan, DMBA ve morin uygulamarı ile artmıştır. Normal sıçanlarda en yüksek eritromisin N-demetilaz aktiviteleri DMBA + endosülfan + morin grubunda ölçülmüştür. Glutatyon S-transferaz aktivitesi DMBA verilen gruplarda artmıştır. Katalaz ve glutatyon redüktaz aktiviteleri morin ve endosülfan uygulamalarından etkilenmemiştir. Katalaz ve glutatyon redüktaz aktiviteleri DMBA uygulaması ile önemli ölçüde artmıştır. İntraperitoneal streptozotosin enjeksiyonu ile oluşturulmuş diabetes mellitus sıçan karaciğer mikrozomlarında ve sitozollerde kontrol grubuna gore EROD, MROD, PROD, eritromisin, N-demetilaz, glutatyon S-transferaz, katalaz ve glutatyon redüktaz aktivitelerinde anlamlı artışa sebep olmuştur ($p < 0.05$). Endosülfan ve DMBA uygulamaları diyabetik sıçanlarda EROD ve MROD aktivitelerini artırmıştır. Morin uygulaması endosülfan ve DMBA verilen diyabetik sıçanlarda EROD aktivitesini azaltmıştır. Diyabetik sıçanlarda MROD aktivitesi endosülfan ve DMBA uygulamalarıyla artmıştır. Morin uygulaması endosülfan ve DMBA verilen diyabetik sıçanlarda MROD aktivitesini azaltmıştır. Anilin 4-hidroksilaz, eritromisin N-demetilaz, glutatyon S-transferaz ve katalaz aktiviteleri endosülfan ve DMBA verilen diyabetli sıçan gruplarında azalmıştır. Protein analizleri yanında karaciğer dokuları histopatolojik olarak da incelenmiştir. Histopatolojik çalışmalar morin uygulamasının karaciğer hücre görünümünü modifiye etmediğini göstermiştir. Endosülfan ve DMBA'nın birlikte verilmesi karaciğerde doku hasarını arttırmıştır. Morin verilmesi hem normal hem de diyabetli sıçanlarda endosülfan ve DMBA ile oluşan doku hasarını azaltmıştır. Sonuç olarak, morinin endosülfan ve DMBA ile birlikte verilmesi endosülfan ve DMBA'nın toksik etkilerini normal ve diyabetli sıçanda düşürmüştür.

ANAHTAR KELİMELER: Sitokrom P450 (CYP), 7,12-Dimetilbenz[a]antrasen, Endosülfan, Morin, Diyabet, Karaciğer, Sıçan.

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LIST OF ABBREVIATIONS AND SYMBOLS

ε-ACA : ε-Amino caproic acid
A4H : Aniline 4-hydroxylase activity
Ah : Aromatic hydrocarbon
AhR : Aryl hydrocarbon receptor
ANOVA : One-way analysis of variance
ARE : Antioxidant (or electrophile) response element
ARNT : Aryl hydrocarbon receptor nuclear translocator
BCIP : 5-bromo-4-chloro-3-indolylphosphate
BIS : N,N'-methylene bisacrylamide
BSA : Bovine serum albumin
CAR : Constitutive/active androstane receptor
CAT : Catalase
CDNB : 1-chloro-2,4-dinitrobenzene
CO : Carbon monoxide
CYP : Cytochrome P450
DDT : Dichlorodiphenyltrichloroethane
DEA : Diethanolamine
DMBA : 7,12-Dimethylbenz[a]anthracene
DMSO : Dimethyl sulfoxide
DNB_SG : 1-glutathione-2,4-dinitrobenzene
EDTA : Ethylenediaminetetraacetic acid disodium salt dihydrate
ERND : Erythromycin N-demethylase
EROD : 7-ethoxyresorufin O-deethylase activities
GPx : Glutathione peroxidase
GR : Glutathione reductase
GRred : Reduced glutathione reductase
GRE : Glucocorticoid response element
GSH : Reduced glutathione
GSSG : Oxidized glutathione
GSTs : Glutathione S-transferases

HEPES : N-2-hydroxyethylpiperazine-N'-2-ethansulphonic acid

HSP90 : A heat shock protein of 90 kDa

MDMA : Methylenedioxymethamphetamine

mRNA : Messenger RNA

MROD : 7-methoxyresorufin O-demethylase activities

$NADP^+$: β-nicotinamide adenine dinucleotide phosphate disodium salt

NADPH : Nicotinamide adenine dinucleotide phosphate reduced form

NBT : Nitro blue tetrazolium chloride

NBT/BCIP : Nitro blue tetrazolium/5-bromo-4-chloro-3-indolylphosphate

NDMA : N-nitrosodimethylamine

OCPs : Organochlorine pesticides

p23 : A co-chaperone protein of 23 kDa

PAHs : Polycyclic aromatic hydrocarbons

PCBs : Polychlorinated biphenyls

PCDDs : Polychlorinated dibenzo-p-dioxins

PCDFs : Polychlorinated dibenzofurans

PHAH : Polyhalogenated aromatic hydrocarbons

PMS : Phenazine methosulfate

PMSF : Phenylmethanesulfonyl fluoride

POPs : Persistent organic pollutants

Por : Porphyrin ring

ROS : Reactive oxygen species

PROD : 7-Pentoxyresorufin O-depentylase activity

PVC : Polyvinyl chloride

PXR : Pregnane X receptor

SDS : Sodium dodecyl sulfate

SDS-PAGE : Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SEM : Standard error of mean

SOD : Superoxide dismutase

TBST : Tris Buffered Saline plus Tween 20 solution

TCDD : Tetrachlorodibenzo-para-dioxin

TEMED : N, N, N', N' tetramethylethylene diamine

Tris : 2-amino-2-(hydroxymethyl)-1,3-propanediol

Tween 20 : Polyxyethylene sorbitan monolaurate

UDP : Uridine diphosphate

VDR : Vitamin D receptor

XAP2 : X-associated protein 2

XRE : Xenobiotic response element

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1. INTRODUCTION

The human body is continuously exposed to xenobiotics (chemicals that are foreign to living organisms). Xenobiotics may accumulate in tissues or may be converted to more toxic chemicals or water-soluble metabolites by biotransformation enzymes. Xenobiotics may be harmful not only by their direct effects on the organisms but also indirectly producing some diseases and developmental abnormalities in organisms. Therefore, it is very important to determine the effects of xenobiotics in living organisms.

1.1 Persistent Organic Compounds

Persistent organic pollutants (POPs) are chemicals that have properties including high toxicity, bioaccumulation through food web and persistence in the environment. They resist environmental breakdown via biological and chemical processes and undergo a slow decomposition by photolysis. POPs are lipophilic chemicals and accumulate in the body fat of living organisms. They become more concentrated at the highest trophic levels of food chain. They cause adverse effects to human health and the environment.

POPs can be divided into two categories as intentionally and unintentionally produced chemicals. Intentionally produced chemicals are organochlorine compounds produced as wanted products by different reactions and used in industrial processes and agricultural areas. They can be divided into two types as industrial chemicals and organochlorine pesticides (OCPs). Polychlorinated biphenyls (PCBs) are chemicals used in industrial applications. They are used as plasticizers, hydraulic and heat exchanger fluids and additives to paints and lubricants and are also present in electrical transformers and large capacitors. Organochlorine pesticides include a variety of multipurpose chlorinated chemicals such as insecticides, herbicides and

fungicides. These compounds can be separated into four groups. The first group includes chloroethylene derivatives and dichlorodiphenyltrichloroethane (DDT) and its analogues (eg, dicofol, methoxychlor) are examples for this group. The second group includes hexachlorocyclohexane (ie, benzene hexachloride) and its isomers (eg, lindane, gamma-hexachlorocyclohexane). The third group includes chlorinated cyclodiene compounds such as endosulfan, chlordane, heptachlor, aldrin, dieldrin, endrin, isobenzan. The fourth group includes toxaphene (ie, chlorinated camphene, camphechlor) (El-Shahawi et al., 2010; Manahan, 2002). Unintentionally produced chemicals are produced as unwanted by-products of combustion or chemical process. They are divided into three types as polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Polycyclic aromatic hydrocarbons consist of two or more aromatic rings and do not contain any heteroatoms or carry any substituents. PAHs are formed by pyrolysis and the incomplete combustion of other hydrocarbons and released from industries, domestic oil furnaces, gasoline and diesel engines. The living organisms are exposed to polyaromatic hydrocarbons by breathing polluted air, consuming polluted water and food and smoking cigarette (Bozcaarmutlu et al., 2009; Lakshmi and Subramanian, 2014; Manahan, 2002). The polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are two series of structurally similar persistent organic compounds having similar chemical properties. PCDDs and PCDFs comprise of two benzene rings and include two or one oxygen atom, respectively. The toxicity of PCDD and PCDF congeners depends on the number and positions of the chlorine atoms and congeners have chlorine atoms substituted at 2, 3, 7 and 8 position cause high toxicity within the body. They are by-products of industrial and thermal processes. PCDDs occur as by-products in the manufacture of some organochlorides, in the incineration of chlorine-containing substances such as polyvinyl chloride (PVC), in the chlorine bleaching of paper, and from natural sources such as volcanoes and forest fires. PCDFs can be formed by pyrolysis or incineration of chlorine containing products, such as PVC, PCBs, and other organochlorides, or of non-chlorine containing products in the presence of chlorine donors. The most toxic of the PCDD/PCDF congeners is 2, 3, 7, 8-tetrachlorodibenzo-para-dioxin (2,3,7,8-Cl4DD or 2,3,7,8-TCDD). Humans are easily exposed these chemicals by consuming contaminated food (El-Shahawi et al., 2010).

1.1.1 7,12-Dimethylbenz[a]anthracene

7,12-Dimethylbenz[a]anthracene (DMBA) is one of the most potent mutagenic and carcinogenic methylated polycyclic aromatic hydrocarbons (PAHs) (Figure 1.1). It is formed by methyl substitution of benz[a]anthracene and is extensively used to form DNA-DMBA adducts and induce cancer in animal models. It induces tumors in breast, skin, lung and liver and induces nephroblastomas in ovariectomized rats and erythroleukemia in rats. DMBA is produced during the incomplete combustion of carbon-containing compounds and it is possible to expose to DMBA with smoking cigarette, breathing car exhaust and furnace gases in daily life. (Clarke, 1997; Lambard et al., 1991; Lee et al., 2002; Malejka-Giganti et al., 2005; Marvanová et al., 2008; Reddy et al., 2012; Shou et al., 1996b; Sugiyama, 2002; Yang and Dower, 1975). DMBA damages many organs by affecting the activities of phase I, II, antioxidant and serum enzymes, inducing the production of reactive oxygen species and leading to DNA-adduct formation (Arora et al., 2014)

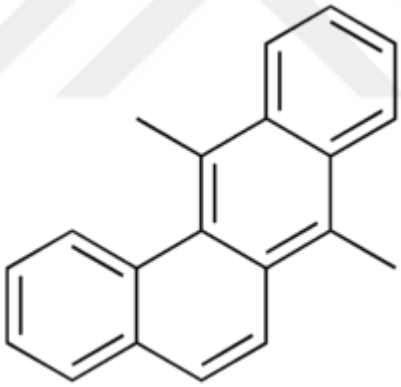


Figure 1.1. Structure of 7,12-dimethylbenz[a]anthracene

7,12-Dimethylbenz[a]anthracene is a procarcinogen and inactive by itself. The biological activity such as carcinogenic and mutagenic effects and DNA adduct formation of DMBA requires its metabolic conversion to electrophilic metabolites by mixed function oxidases (Shou et al, 1996b).

The conversion of DMBA to its ultimate carcinogenic metabolites catalyzed by cytochrome P450s and epoxide hydrolase. CYP1A1, CYP1B1, CYP2B1,

CYP2E1 and CYP2C6 are the members of cytochrome P450 system that involved in metabolism of DMBA (Lambard et al., 1991; Szaefer et al., 2011; Lakshmi and Subramanian; 2014). DMBA metabolism occurs predominantly in liver by participation of CYP1A1. 7,12-DMBA-3,4-diol-1,2-epoxide is the ultimate carcinogenic metabolite of DMBA produced by the reaction catayzed by either CYP1A1 or CYP1B1 (Christou et al., 1995; Kleiner et al., 2004; Lambard et al., 1991; Wislocki et al., 1980). Figure 1.2. shows the metabolic activation of 7,12-DMBA to the carcinogenic metabolites 7,12-DMBA-3,4-diol, 7,12-DMBA-3,4-diol-1,2-epoxide in the presence of CYP1A1 and epoxide hydrolase.

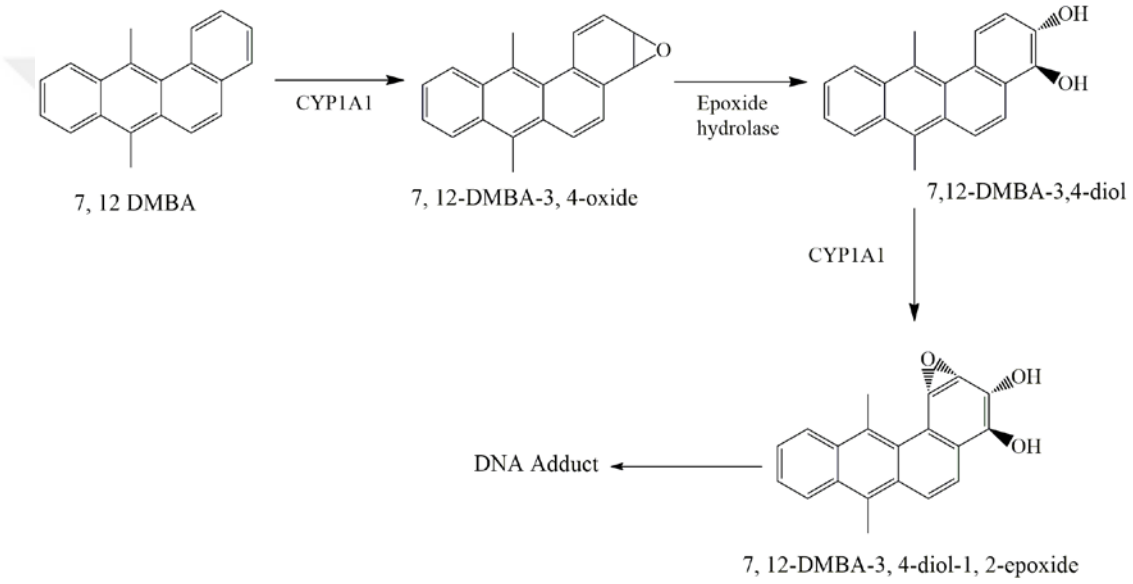


Figure 1.2. Metabolic activation of 7,12-DMBA to the carcinogenic metabolites (taken from Shimada and Fujii-Kuriyama, 2004 and modified).

1.1.2 Endosulfan

Endosulfan (6, 7, 8, 9,10 ,10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzadioxathiepin-3-oxide) is a manufactured organochlorine pesticide which is used to control a number of insects on food crops such as coffee, grains, tea, fruits, and vegetables and on non-food crops such as tobacco and cotton. Because of its widespread usage and its potential for environmental transport,

endosulfan may exist in air, freshwater, sea water, soil samples and foods. Non-target organisms are also affected by endosulfan. Humans are exposed to this pesticide by consuming contaminated food, water and cigarettes, dermal contact and inhalation. (Ozkoc et al., 2007; Lonsway et al., 1997; Nizamlioglu et al., 2005; Sofuoğlu et al., 2004; Uboh et al., 2011; Umar et al., 2012; Yenisoy-Karakaş, 2006)

The technical grade endosulfan is a mixture of two stereoisomers, α and β endosulfan with a mixed proportion of 70% and 30%, respectively. Endosulfan is an endocrine disruptor and affects the immune system, binds to estrogen receptor and affects the reproduction system by decreasing the testosterone level (Lemaire et al., 2006; Saiyed et al., 2003; Singh and Pandey, 1990). It has been observed in laboratory animals that endosulfan is toxic to the liver, kidney, nervous system, and reproductive organs (Casabar et al., 2010; Gupta and Chandra, 1977; Hack et al., 1995; Paul et al., 1994; Sinha et al., 1997).

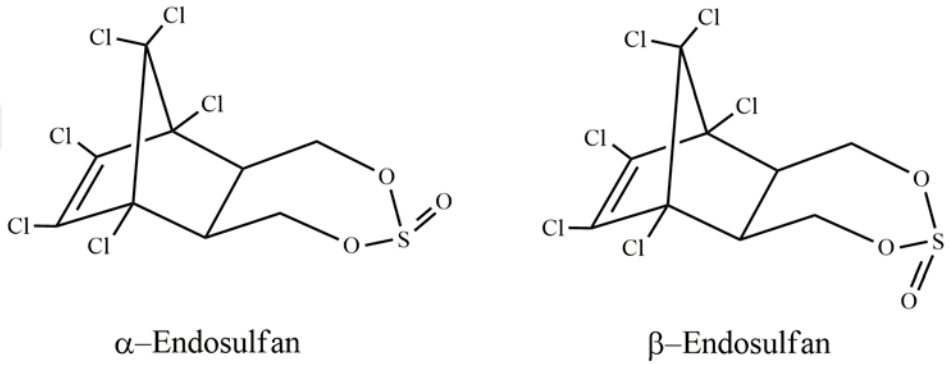


Figure 1.3. Structure of endosulfan

It is metabolized to endosulfan sulfate, endosulfan diol, endosulfan ether, endosulfan lactone, and endosulfan hydroxyether by oxidation or hydrolysis reactions (Martinez Vidal et al., 1998). Endosulfan is converted to endosulfan sulfate as a result of oxidation, biotransformation, or photolysis. Endosulfan sulfate is nearly as toxic as endosulfan and does not undergo any further degradation. Endosulfan diol is non-toxic metabolite of endosulfan produced by hydrolysis. This metabolite can be further degraded to non-toxic endosulfan ether, endosulfan hydroxyether and endosulfan lactone. Therefore, production of endosulfan diol is an important detoxification pathway of endosulfan (Kennedy et al., 2001; Khan, 2012; Li et al.,

2009). Endosulfan sulfate is the single metabolite of α-endosulfan metabolism. The metabolism of α-endosulfan is mediated by CYP2B6, CYP3A4, and CYP3A5 (Casabar et al., 2006; Lee et al., 2006) and that of β-isomer is mediated by CYP3A4 and CYP3A5 (Lee et al., 2006). The proposed metabolic pathway for endosulfan based on animal studies is shown in Figure 1.4.

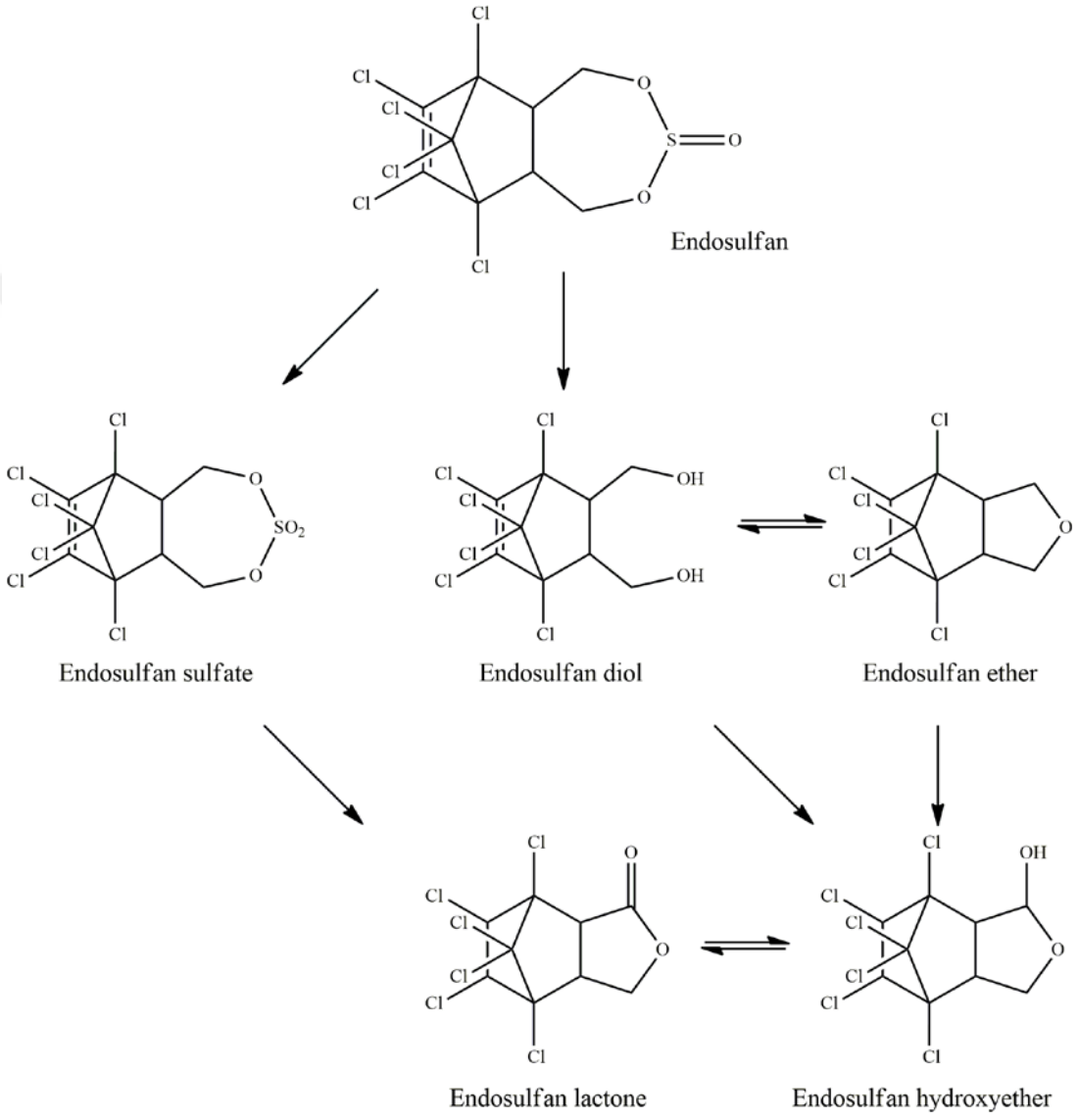


Figure 1.4. Products of endosulfan metabolism (taken from ATSDR, 2000)

1.2 Biotransformation

Biotransformation is the enzyme-catalyzed metabolic conversion of endogenous and xenobiotic compounds to increase their water solubility in order to facilitate the excretion of these compounds from the body (Lech and Vodicnik, 1985). The biotransformation reactions of xenobiotics occur in two stages, called phase I and phase II. (Williams, 1971)

Phase I biotransformation reactions involve oxidation, reduction and hydrolysis reactions. In these reactions, xenobiotics are converted to metabolites that are sufficiently ionized, or hydrophilic, to be either eliminated from the body without further biotransformation or by the addition of a functional group (–OH, –NH2, – SH or –COOH) to the parent compound xenobiotics are converted to an intermediate metabolite that is ready for Phase II biotransformation. The most important enzyme system involved in Phase I reactions is cytochrome P450 (CYP)-dependent mixed function oxidase system. They catalyze most of the oxidative biotransformation of xenobiotics (Coulthard and Boddy, 2004; Parkinson et al., 2010).

In Phase II biotransformation reactions, which involve glucuronidation, sulfonation, acetylation, methylation, conjugation with glutathione, and conjugation with amino acids (such as glycine, taurine, and glutamic acid), hydrophilicity of xenobiotics are increased and xenobiotics are converted to more water-soluble form which can be excreted from the body more easily than the parent compound or Phase I metabolite. Glutathione S-transferases, sulfotransferases, UDP-glucuronyl transferases and N-acetyl transferases are the most important Phase II enzymes and they take part in conjugation reactions. Xenobiotics having functional group such as –COOH, –OH or $–NH_2$ are not metabolized by Phase I biotransformation reactions and undergo direct metabolism by conjugative Phase II enzymes. Biotransformation of xenobiotic plays an important role for maintaining homeostasis during exposure of organisms to small foreign molecules, such as drugs (Coulthard and Boddy, 2004; Parkinson et al., 2010).

1.2.1 Cytochrome P450s (Monooxygenases)

Cytochrome P450-dependent mixed function oxidase system is a membrane-bound, multi-component, electron transport system. It is a member of phase I enzyme system and involved in the biotransformation of endogenous compounds such as steroids, fatty acids, vitamins and bile acids as well as exogenous compounds such as drugs, pesticides and environmental pollutants (Arınç and Philpot, 1976; Arınç et al., 2005; Arınç et al., 2007; Bozcaarmutlu et al., 2009; Bozcaarmutlu et al., 2014a; Bozcaarmutlu et al., 2014b; Bozcaarmutlu et al.,2015; Gonzalez, 1988; Guengerich, 1990; Lu and Levin, 1974; Sole´ and Livingstone, 2005).

Cytochrome P450s have the characteristic Soret peak formed by absorbance of light at wavelengths near 450 nm when the heme iron is reduced with sodium dithionate and complexed to carbon monoxide. The name cytochrome P450 is derived from the phenomena, pigment with an absorbance at 450nm (Omura and Sato, 1964 a, b).

Cytochrome P450s are present in liver, intestine, kidney, lungs, brain, skin, prostate gland, placenta, brain etc. and play important roles either in the detoxification of xenobiotics by converting them to their more water soluble metabolites or in biological activation of xenobiotics by converting them to more toxic products (Martignoni et al., 2006). They serve as a powerful oxidizing catalyst and participate in different oxidation reactions including hydroxylation, dealkylation, oxidation, dehalogenation, epoxidation, deamination etc. (Guengerich, 1993). The common reaction catalyzed by these heme proteins is a monooxygenase reaction. In the reaction catalyzed by cytochrome P450 system, one atmospheric oxygen atom is incorporated into an organic substrate (RH) while the other oxygen atom is reduced to water in the presence of reduced cofactor ($NADPH,H^+$) (Figure 1.4).

$$R\text{-}H + O_2 + NADPH + H^+ \rightleftharpoons R\text{-}OH + H_2O + NADP^+$$

Figure 1.5. General reaction catalyzed by the cytochrome P450s.

The cytochrome P450 catalytic cycle occurs in the presence of NADPH reductase, cytochrome *b5* and membrane phospholipids. NADPH reductase and cytochrome *b5* serve as electron source in whole reaction (Siroka and Drastichova, 2004). Microsomal cytochrome P450 catalytic cycle is shown in Figure 1.5.

The enzyme contains heme in the ferric (Fe^{+3}) form at ground state (Dawson, 1988). Xenobiotic binds to the cytochrome at ground state and this binding alters the conformation for the electron transfer from NADPH via a nearby flavoprotein, NADPH cytochrome P450 reductase to the heme. It is followed by entrance of one electron from NADPH to the system catalyzed by NADPH-cytochrome P450 reductase to produce a ferrous (Fe^{2+}) form and generate a reduced cytochrome-substrate complex. Molecular oxygen then binds to the reduced cytochrome-substrate complex and a ternary complex of xenobiotic, cytochrome, and oxygen is formed. The ternary complex receives another electron, either through the same flavoprotein as mentioned before or through a different flavoprotein in which the electron is first passed through cytochrome *b5*, another cytochrome present in the endoplasmic reticulum and peroxide intermediate is formed. The pathway containing cytochrome *b5* for the second electron can also use NADH as the pyridine nucleotide electron donor. The addition of the second electron to the ternary complex results production of water and a perferryl-oxygen (FeO^{3+}) (which is equivalent to Fe^{V+}). The enzyme then remove two electrons from the recipient substrate and this results insertion of one atom of oxygen into the substrate to yield the product and conversion of cytochrome P450 to its ground state ferric (Fe^{3+}) form (Penning, 2011).

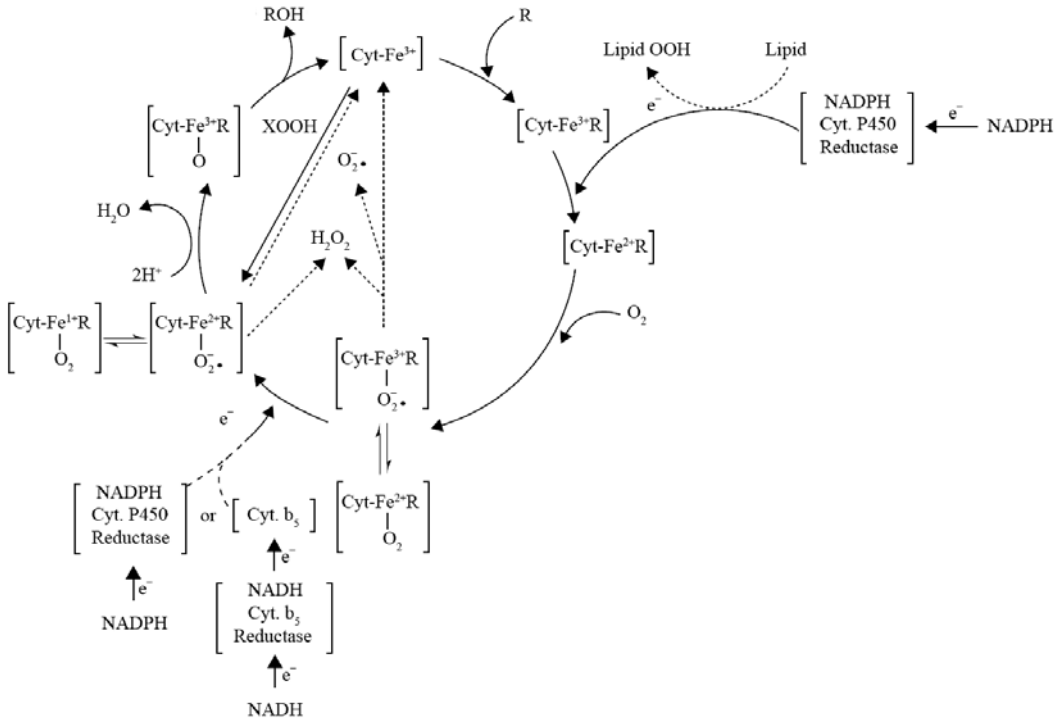


Figure 1.6. Microsomal cytochrome P450 catalytic cycle (taken from Rose and Hodgson, 2004).

Cytochrome P450 monooxygenases are a superfamily of heme-thiolate proteins and more than 20000 P450 sequences have been identified (as of July 2013, Dr. Nelson's Cytochrome P450 homepage; http://drnelson.uthsc.edu/CytochromeP450.html). There are 18 mammalian cytochrome P450 (CYP) families and human genome contains encoded 57 CYP genes (Nelson and Nebert, 2011; Nebert et al., 2013).

The nomenclature of cytochrome P450s are based on percentage of amino acid sequence identity. Cytochrome P450s are sorted into families and subfamilies. According to nomenclature guidelines, cytochrome P450 genes are indicated as CYP (or cyp in the case of mouse genes). Enzymes sharing more than or equal to 40 percent amino acid sequence similarity are assigned to a particular family designated by an Arabic numeral. Enzymes sharing more than or equal to 55 percent amino acid sequence identity in the case of mammalian proteins and 46 percent amino acid sequence identity in the case of non-mammalian proteins are assigned to a particular

subfamily designated by a letter. The second Arabic numeral followed the subfamily designation indicates individual isoforms. The name of the gene is written in italics, whereas the enzyme is not (Nebert et al., 1987; Rose and Hodgson, 2004).

Cytochrome P450 catalyzed reaction may modify parent compound to its equally active metabolite, its inactive metabolite (detoxification) or toxic metabolite (bioactivation). Both endogenous and exogenous molecules are metabolized by cytochrome P450 enzymes (Guengerich, 1990; Nebert and Gonzalez, 1987; Nelson, 2003; Arinc and Bozcaarmutlu, 2003; Hodgson and Rose, 2007; Zanger and Schwab, 2013). Among the substrates metabolized by cytochrome P450s, xenobiotics are mainly metabolized by CYP1A, CYP2B, CYP3A, CYP2C and CYP2E subfamilies (Nelson and Nebert, 2011; Nebert et al., 2013).

1.2.1.1 CYP1A Subfamily

In human and rodents, the CYP1 enzyme family comprises three genes in two subfamily called CYP1A and CYP1B. CYP1B is mainly expressed in extrahepatic tissues (Guengerich, 2015). The CYP1A subfamily consists of two members, CYP1A1 and CYP1A2, sharing more than 70% identity in their amino acid sequence in humans (Badal and Delgoda, 2014; http://drnelson.uthsc.edu/CytochromeP450.html). The human CYP1A enzymes shows more than 80% identity with rat (83 and 80%, respectively, for CYP1A1 and CYP1A2), mouse (83 and 80%, respectively for CYP1A1 and CYP1A2), dog (84% for CYP1A2) and monkey (95% for both CYP1A1 and CYP1A2) in their amino acid sequences (Martignoni et al., 2006). CYP1A1 is expressed in mainly extrahepatic tissues of mouse, rat and human. It is predominantly found in small intestine, lung, placenta and kidney and weakly in liver (Bullock et al.1995; Choudhary et al. 2003; Ding and Kaminsky, 2003; Guengerich et al., 1997; Hakkola et al., 1996; Shimada et al., 1996; Parkinson, 1996). The main organ in which CYP1A2 is expressed is liver. CYP1A2 is also expressed at low levels in extrahepatic tissues in human, rat and mouse (Choudhary et al. 2003; Ding and Kaminsky, 2003; Shimada et al., 1989).

The enzymes in CYP1A family play important role in the metabolism of both endogenous and exogenous compounds. CYP1A1 and CYP1A2 are involved in the conversion of chemicals to highly reactive metabolites which cause carcinogenicity in experimental animals and humans while their corresponding parent compounds are chemically inactive (Androutsopoulos et al., 2009; Conney, 1982; Guengerich and Shimada, 1991). Enzymes in CYP1A subfamily catalyses the metabolic activations several compounds such as DMBA, aflatoxin B1, PAHs, polyhalogenated aromatic hydrocarbons (PHAH), heterocyclic amines found in charred meat and tobacco-related N-nitrosamines (Androutsopoulos et al., 2009; Nebert et al., 2004; Nebert and Dalton,2006). CYP1A1 is generally involved in the biotransformation of environmental chemicals such as benzo[a]pyrene, 7,12-dimethylbenz[a]anthracene and PCBs (Wahlang et al., 2015). The hormones including 17B estradiol and melatonin and inflammatory mediators such as arachidonic acid and eicosapentoic acid are the endogenous substrates that CYP1A1 plays critical role in their metabolism (Arnold et al., 2010; Badal and Delgoda, 2014; Lee et al., 2003; Ma et al., 2005; Rendic and Guengerich, 2012; Schwarz et al., 2004; Shimada and Fujii-Kuriyama, 2004). CYP1A2 is found in the metabolism of aromatic amines, polycyclic aromatic hydrocarbons and some drugs such as acetaminophen, caffeine, clozapine, phenacetin, tacrine, and theophylline (Anzenbacher and Anzenbacherová, 2001; Martignoni et al., 2006; Rendic and Guengerich, 2012). Polycyclic aromatic hydrocarbons (charbroiled meat, cigarette smoke) polychlorinated biphenyls, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), caffein in coffee, phenobarbital and other barbiturates, omeprazole, rifampicine and ritonavir are commonly known inducers and cimetidine, α-naphthoflavone, fluvoxamine, erythromycin, disulfiram, enoxacin, furafylline and ciprofloxacin are commonly known inhibitors of enzymes in CYP1A subfamily (Tanaka, 1998; Guengerich, 2015; Zanger and Schwab, 2013)

PAHs and polyhalogenated hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) also induce CYP1A through the aryl hydrocarbon receptor (AhR) and aryl hydrocarbon receptor nuclear translocator (ARNT) (Nebert et al., 2000). The activation mechanism of CYP1A gene expression regulated by the heterodimer composed of the AhR and ARNT was proposed by Poland and Knutson (1982). The aryl hydrocarbon receptor exists in the cell cytoplasm as a multiprotein complex containing two molecules of heat-shock protein (HSP90, a heat shock protein of 90

kDa), XAP2 (X-associated protein 2 (Meyer et al., 1998)) (also referred to as AIP or ara9 (Carver and Bradfield, 1997; Ma and Whitlock, 1997)) and p23 (a co-chaperone protein of 23 kDa) (Kazlauskas et al., 1999). AhR is a ligand-activated transcription factor and HSP90 binding maintains unliganded AhR in the cytoplasm in a configuration that facilitates ligand binding. The hydrophobic inducer (ligand) passively diffuses into the cell and enzyme induction is initiated by the binding of this specific xenobiotic, such as PCDDs, PCBs, PCDFs and PAHs, to the multiprotein complex (Poland and Knutson, 1982; Whitlock et al., 1996; Denison and Heath-Pagliuso, 1998; Whitlock, 1999; Denison et al., 2002; Gotoh et al., 2015). Upon this binding, HSP90 molecule, XAP2, and p23 release from the complex and ligand-AhR complex translocates into the nucleus of the cell. The liganded AhR dimerizes with a nuclear protein, ARNT within the nucleus, thereby AhR:ARNT heterodimer is generated (Henry and Gasciewicz, 1993; Hord and Perdew, 1994; Pollenz et al., 1994; Hankinson, 1995; Whitlock et al., 1996; Denison et al., 2002). Formation of the AhR:ARNT heterodimer converts the complex into its high affinity DNA binding form (Probst et al., 1993; Hankinson, 1995; Denison et al., 2002). This complex binds to its specific DNA recognition site, known as the xenobiotic response element (XRE) and this binding initiates synthesis of the messenger RNA (mRNA) which is responsible for transcribing the appropriate sequence for CYP1A synthesis. The cytochrome P450 molecule is then incorporated into the membrane of the endoplasmic reticulum (Denison et al., 1988; Stegeman and Hahn, 1994; Whitlock et al., 1996; Whitlock, 1999; Denison et al., 2002). The regulation of CYP1A by the cytosolic aryl hydrocarbon receptor (AhR) is shown in Figure 1.7.

Aryl hydrocarbons such as DMBA are not only substrate of the enzyme but also ligand of the AhR. The presence of such aryl hydrocarbons increases mRNA expression of the CYP1A1 enzyme via AhR receptor which mediates increase in the expression of the CYP1A1 enzyme (Willey et al., 1997; Badal and Delgoda, 2014).

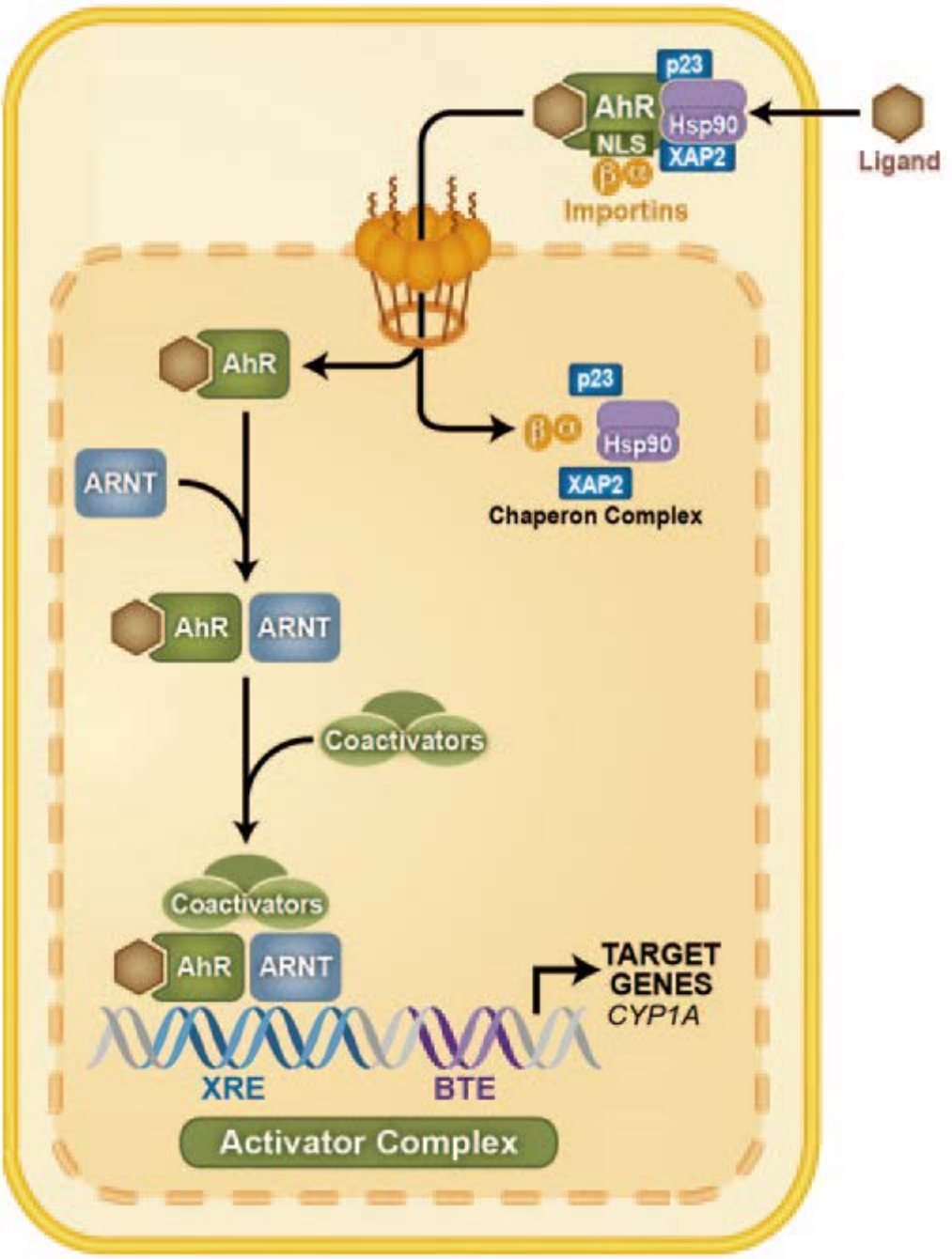


Figure 1.7. The regulation of CYP1A by the cytosolic aromatic hydrocarbon (Ah) receptor (taken from Gotoh et al., 2015).

1.2.1.2 CYP2B Subfamily

CYP2B subfamily includes two genes called CYP2B6 and CYP2B7 in humans. CYP2B6 is primarily expressed in liver and detected in some extrahepatic tissues such as lung, intestine, kidney and hearth (Anzenbacher and Anzenbacherová, 2001; Hodgson and Rose, 2007; Martignoni et al., 2006; Guengerich, 2015; Zanger and Schwab, 2013). CYP2B7 is mainly expressed in extrahepatic tissues. Two CYP2B isoenzymes CYP2B1 and CYP2B2 are found in rats. They are structurally related isoenzymes and show 97% identity with very similar substrate specificities. Both CYP2B1 and CYP2B2 are expressed constitutively in the liver and extrahepatic tissues such as small intestine and lungs (Martignoni et al., 2006). Although the genomic structures of the human and rat CYP2B genes are different, CYP2B is strongly induced by phenobarbital in both rats and humans. This strong inducibility by phenobarbital is a distinctive property of CYP2B genes and similar mechanisms are observed in humans and rats (Martignoni et al., 2006; Zanger and Schwab, 2013). CYP2B1 rat is ortholog to human CYP2B6 and shows 76% identity with human CYP2B6 (Yamano et al., 1989).

Many diverse chemicals are metabolized by CYP2B6. It participates the metabolism of several important drugs including the prodrug cyclophosphamide, the antiretroviral drugs efavirenz and nevirapine, the antidepressant and smoking cessation agent bupropion, the benzodiazepine diazepam, the antimalarial artemisinin, anesthetics like propofol and ketamine, the synthetic opioid methadone, as well as including ecstasy (MDMA, 3,4 - methylenedioxymethamphetamine) and nicotine (Zanger et al., 2007; Zanger and Schwab, 2013). CYP2B6 plays important role in the metabolism of not only the clinically used drugs but also environmental chemicals such as PAHs such as benzo[a]pyrene (Shou et al., 1994), dibenzo[a,l]pyrene (Shou et al., 1996a), substituted PAHs such as 6-aminochrysene (Yamazaki et al., 1994), 7,12- dimethylbenz[a]anthracene (Shou et al., 1996b), PCBs such as 2,2',4,4',5,5'-hexachlorobiphenyl (Ariyoshi et al., 1995) , insecticides such as α-endosulfan (Casabar et al., 2006; Lee et al., 2006), malathion (Buratti et al., 2005), methoxychlor (Dehal and Kupfer, 1994) and parathion (Butler and Murray, 1997), herbicides such as alachlor, metolachlor, acetochlor, and butachlor (Coleman et al., 1999; Coleman et al., 2000) , and some industrial chemicals such as n-hexane

(Crosbie et al., 1997) and toluene (Kim et al., 1997; Nakajima et al., 1997). Besides these chemicals, CYP2B6 is also induced by phenobarbital, phenytoin, ritonavir, carbamazepine and rifampin (Guengerich, 2015, Tanaka, 1998). Proadifen (SKF-525A), thio-TEPA, clopidogrel, 3-Isopropenyl-3-methyl diamantane, ticlopidine, phenylethylpiperidine are some inhibitors of CYP2B (Ekins and Wrighton, 1999; Parkinson et al., 2010; Zanger and Schwab, 2013)

CYP2B6 gene expression is regulated by constitutive/active androstane receptor (CAR, originally named MB67). Phenobarbital antagonizes epidermal growth factor receptor signaling to indirectly activate the constitutive/active androstane receptor (CAR, originally named MB67). In addition to CAR, pregnane X receptor (PXR, originally named NR1I2) also activates CYP2B6 gene expression (Casabar et al., 2010; Gotoh et al., 2015).

1.2.1.3 CYP2E Subfamily

CYP2E1 is the only gene of the CYP2E subfamily showing strong conservation among species. Human CYP2E1 shares an amino acid sequence identity of 80% with rat, mouse and dog CYP2E1 and of 96% with monkey CYP2E1 (Martignoni et al., 2006). It is constitutively expressed in liver and also detected in many extrahepatic tissues including lung, small intestine, brain, nasal mucosa, pancreas, kidney cortex, testis, ovaries, the gastrointestinal tract and cardiac tissue (Ding and Kaminsky, 2003; Ferguson and Tyndale, 2011; Hukkanen et al., 2002; Joshi and Tyndale, 2006; Kazakoff et al., 1994; Lieber, 1997; Michaud et al., 2010; Norton et al., 1998; Thelen and Dressman, 2009; Upadhya et al., 2000; Warner and Gustafsson, 1994).

CYP2E1 metabolizes a large number of low-molecular-weight endogenous such as long chain fatty acids and exogenous compounds (molecular weight <100). CYP2E1 is also known as ethanol-inducible cytochrome P450 isoenzyme. Ethanol, acetone, benzene, isoniazid, pyrazole and pyridine are commonly known inducers and disulfiram, 4-methylpyrazole, isothiocyanates, chlormethiazole and diethyldithiocarbamate are commonly known inhibitors of CYP2E1. The metabolism

of several industrial chemicals (e.g. acetone, benzene, carbon tetrachloride, chloroform, pyridine and vinylidine chloride), halogenated anaesthetics (e.g. halothane, sevoflurane, enflurane and isoflurane), drugs (e.g. acetylsalicylic acid (aspirin), acetaminophen (paracetamol), chlorzoxazone and trimethadione) are mainly catalyzed by CYP2E1 (Arinç et al., 2000; Arslan et al., 2011; Guengerich et al., 1991; Guengerich and Shimada, 1991; Guengerich, 2015; Klotz and Ammon, 1998; Lieber, 1999; Tanaka et al., 2000; Yamazaki et al., 1995).

CYP2E1 is involved in bioactivation of many carcinogens, procarcinogens, and protoxins including *N*-alkylnitrosamines, benzene, carbon tetrachloride and *N*-alkylformamides. Together with CYP3A4, it converts acetaminophen (paracetamol) to reactive quinoneimine, which can bind to hepatic and renal proteins. A procarcinogen and food contaminant, N-nitrosodimethylamine (NDMA), is converted to its carcinogenic form by N-demethylase activity associated with CYP2E1 (Anzenbacher and Anzenbacherová, 2001; Arinç et al., 2007; Guengerich 2015). Besides that, CYP2E1 plays a detoxification role in ethanol metabolism by preventing ethanol to reach excessive levels (Kataoka et al., 2005)

In addition to the chemical induction of CYP2E1, it is also induced under diverse pathophysiological conditions including diabetes, obesity, starvation, alcoholic and non-alcoholic liver disease (Aubert et al., 2011; Caro and Cederbaum, 2004; Ioannides et al., 1988; Kataoka et al., 2005; Lucas et al., 1998; Mandl et al., 1995; Raucy et al., 1990; Raza et al., 2004; Schenkman et al., 1989; Tanaka et al., 2000; Zanger and Schwab, 2013). Oxygen exposure also causes induction in the expression of this enzyme (Anzenbacher and Anzenbacherová, 2001).

1.2.1.4 CYP3A Subfamily

The CYP3A subfamily is the most important of all human drug-metabolizing enzymes. CYP3A comprises four genes called CYP3A4, CYP3A5, CYP3A7 and CYP3A43. CYP3A7 is a foetal enzyme and the expression of this isoenzyme is silenced after birth. It is also found in adult endometrium and placenta (Greenblatt et al., 2008; Westlind et al., 2001; Zanger and Schwab, 2013). CYP3A43 is mainly

expressed in prostate and detected in liver at very low levels. The contribution of this isoforms to drug metabolism is not at any higher extent due to the low expression level in the liver (Gellner et al., 2001; Greenblatt et al., 2008). CYP3A5 is the main CYP3A form in human kidney and also expressed in liver, gastrointestinal enteric mucosa, prostate and kidney. It shows 83% amino acid sequence identity with CYP3A4. The biotransformation of approximately 50% of therapeutic drugs currently on the market or development is catalyzed by CYP3A4 which is the most abundant cytochrome P450 in human liver. It is also expressed in some extrahepatic tissues, including lung, stomach, colon, brain, and adrenal (at very low levels). In contrast to other CYP3A isoenzymes, CYP3A4 has not been reported to be expressed in kidney, prostate, testis, or thymus (Greenblatt et al., 2008; Guengerich, 2015; Martignoni et al., 2006).

In rats, CYP3A subfamily consists of five isoenzymes as CYP3A1/3A23, CYP3A2, CYP3A9, CYP3A18, and CYP3A62. CYP3A2, CYP3A18 and CYP3A9 are expressed in a sex-specific manner in rats. CYP3A1 and CYP3A2 isoenzymes are expressed only in the liver. CYP3A2 and CYP3A18 are male specific and CYP3A9 female specific forms of this subfamily. CYP3A9 and CYP3A18 are mainly expressed in the liver and also detected in the intestinal tract at low levels (Martignoni et al., 2006). CYP3A62 is predominant CYP3A form in the intestinal tract and the expression profile of CYP3A62 is similar to that of human CYP3A4 and rat CYP3A9 (Martignoni et al., 2006; Matsubara et al., 2004). The catalytic competence of rat CYP3A2 is generally similar with human CYP3A4. Rat CYP3A2 exhibits a 73% homology of the amino acid sequence, some substrate preference and functional analogies to human CYP3A4. Both rat CYP3A2 and human CYP3A4 are involved in the metabolism of erythromycin, nifedipine, lidocaine, testosterone, aflatoxin B_1 and benzo[a]pyrene. The human and rat CYP3A subfamily isoforms are inhibited by ketoconazole, troleandomycin and erythromycin (Wójcikowski et al., 2012).

CYP3A4, which has large and flexible active site, participates the metabolism of extremely broad range of chemicals. It is found in the metabolism of either large substrates such as immunosuppressants (e.g. cyclosporin A and tacrolimus), antibiotics (e.g. erythromycin), and anticancer drugs (taxol) or smaller molecules

such as ifosfamide, tamoxifen, benzodiazepines, several statins, antidepressants, opioids and etc. (Guengerich, 2015; Hendrychová et al., 2011; Scott and Halpert, 2005; Zanger and Schwab, 2013). Because CYP3A4 is found in the metabolism of large spectrum of drugs, undesirable drug interactions may be observed. Interactions are not only observed between drug-drug metabolisms but also observed between the constituents of the diet and drug metabolisms. The inhibitory effect of grapefruit juice to the drugs metabolized by CYP3A4 is the well-known example for this situation (Anzenbacher and Anzenbacherová, 2001; He et al., 1998; Guengerich, 2015).

The expression of CYP3A genes is regulated by glucocorticoid receptor (GR), pregnane X receptor (PXR), constitutive androstane receptor (CAR) and vitamin D receptor (VDR) (Guengerich, 2015; Zanger and Schwab, 2013). Table 1.1 illustrates substrates, inhibitors and inducers of CYP3A4.

Table 1.1. Substrates, inhibitors and inducers of CYP3A4 (Taken from Parkinson, 1996).

Substrates			Inhibitors	Inducers
Acetaminophen	Erythromycin	Retinoic acid	Amprenavir	Carbamazepine
Aldrin	Ethinylestradiol	Saquinavir	Clotrimazole	Dexamethasone
Alfentanil	Etoposide	Steroids (e.g., cortisol)	Delavirdine	Glutethimide
Amiodarone	Flutamide	Tacrolimus (FK 506)	Ethinylestradiol	Nevirapine
Aminopyrine	Hydroxyarginine	Tamoxifen	Fluoxetine	Phenobarbital
Amprenavir	Ifosphamide	Taxol	Gestodene	Phenytoin
Antipyrine	Imipramine	Teniposide	Indinavir	Rifabutin
Astemizole	Indinavir	Terfenadine	Itraconazole	Rifampin
Benzphetamine	Lansoprazole	Tetrahydrocannabinol	Ketoconazole	Ritonavir
Budesonide	Lidocaine	Theophylline	Miconazole	St. John’s Wort
Carbamazepine	Loratadine	Toremifene	Nelfinavir	Sulfadimidine
Celecoxib	Losartan	Triazolam	Nicardipine	Sulfinpyrazone
Cisapride	Lovastatin	Trimethadone	Ritonavir	Troglitazone
Cyclophosphamide	Midazolam	Troleandomycin	Saquinavir	Troleandomycin
Cyclosporin	Nelfinavir	Verapamil	Troleandomycin	
Dapsone	Nicardipine	Warfarin	Verapamil	
Delavirdine	Nifedipine	Zatosetron		
Digitoxin	Omeprazole	Zonisamide		
Diltiazem	Quinidine			
Diazepam	Rapamycin			

1.2.2 Glutathione S-transferases

The glutathione S-transferases (GSTs) are a family of enzymes found in organisms as diverse as mammals, insects, plants, fish, birds. This family is the member of phase II enzyme system and plays a key role in the detoxification of endogenous and exogenous electrophilic compounds and provides protection against electrophiles and products of oxidative stress. Human GSTs are expressed and characterized as three major families called cytosolic, microsomal, or mitochondrial (Dostalek and Stark, 2012; Hayes and Pulford 1995; Mannervik et al., 2005; Sheehan et al., 2001; Sherratt and Hayes, 2001).

Cytosolic GST family is the soluble form constituting approximately 4% of total soluble protein in the liver (Eaton and Bammler, 1999; Mannervik et al., 1985). Cytosolic GSTs are dimeric proteins containing two subunits having molecular weight of 23-30 kDa. The soluble human GSTs are categorized into eight main classes as alpha, mu,pi, sigma, theta, kappa, omega and zeta based on their substrate specificity, chemical affinity, amino acid sequence kinetic behavior, and structural properties (Hayes and Strange, 2000; Coulthard and Boddy, 2004; Dostalek and Stark, 2012; Sherratt and Hayes, 2001).

Many endogenous (e.g., prostaglandins, cholesterol - 5,6 – oxide, dopaminochrome) and exogenous compounds (PAH epoxides, 7-hydroxymethylbenz[a]anthracene sulfate, acrolein) act as GSTs substrates. The GSTs catalyze the conjugation of tripeptide glutathione (γ-Glu-Cys-Gly) with the sulfur atom of Cys into a wide variety of chemicals with electrophilic functional groups. The conjugation reactions occur as either addition reactions, in which glutathione is added to an activated double bond or strained ring system or displacement reactions, in which glutathione displaces an electron-withdrawing group. The substrates undergo displacement reactions contains halide, sulfate, sulfonate, phosphate, or a nitro group (i.e., good leaving groups) attached to an allylic or benzylic carbon atom. The binding of reduced glutathione (GSH) to electrophilic chemicals results water soluble GSH S-conjugates and this product is eliminated from the body (Coulthard

and Boddy, 2004; Dostalek and Stark, 2012; Mannervik et al., 1985; Mannervik and Danielson, 1988; Mannervik et al. 2005; Parkinson, 1996)

The activation mechanism of glutathione S-transferases (GSTs) expressions is controlled by regulatory elements such as the glucocorticoid response element (GRE), antioxidant (or electrophile) response element (ARE), and the xenobiotic response element (XRE) (Coulthard and Boddy, 2004; Hayes and Pulford 1995).

1.3 The Oxidative Stress and Antioxidant Enzymes

Oxidative stress occurs as a result of the imbalance between antioxidant capacity of a cell and the production of reactive oxygen species (ROS). This imbalance leads to oxidative damage to proteins, molecules, and DNA and cell injury or death within the body (Mates, 2000; Rahman et al., 2012; Sies, 1997). Reactive oxygen species are highly toxic oxygen derived reactive molecules and free radicals. Many ROS are originating from exogenous sources such as environmental pollutants, consumption of alcohol and cigarette and exposure to ionizing radiation. Besides exogenous sources, they are produced during normal cellular functions by cellular respiration in which oxygen molecules are reduced to superoxide due to the release of electrons from the electron transport chain and as metabolic by-products of metabolic processes involving enzymatic reactions catalyzed by microsomal cytochrome P450s, xanthine oxidase and nitric oxide synthetase. Superoxide ($O_2^{\bullet -}$), hydroxyl ($^{\bullet}OH$) and hydroperoxyl ($HO_2^{\bullet}$) are examples for radicalic ROS and hydrogen peroxide (H_2O_2), hypochlorus acid (HOCl), hypobromus acid (HOBr), ozone (O_3) and singlet oxygen ($^1O_2$) are examples for non-radicalic ROS (Brieger et al., 2012; Castro and Freeman, 2001; Davies, 1995; Sies, 1997).

Antioxidant defense system regulates ROS levels within the body and protects cells against adverse effects of these molecules. Antioxidant defense system consists of enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) and low molecular-weight antioxidants such as ascorbate, α-tocopherol and glutathione (GSH), cysteine, thioredoxin and vitamins (Halliwell, 1995; Rahman et al., 2012)

1.3.1 Catalase

Catalase (CAT) is a heme-containing antioxidant enzyme comprising four identical subunits of 59.7 kDa. Each subunit contains 500 amino acid residues, one heme group, namely ferriprotoporphyrin IX at redox center and a tightly bound NADPH molecule. The presence of NADPH prevents formation of inactive form of catalase (Kirkman and Gaetani, 1984; Kirkman and Gaetani, 2007; Kodydková et al., 2014).

Catalase is present in several mammalian and non-mammalian cells. It is found at the highest concentrations in mammalian erythrocytes and liver and occasionally in the kidney. In tissues such as liver, catalase is found predominantly in peroxisomes (Bebe and Panemangalore, 2003; Deisseroth and Dounce, 1970; Quan et al., 1986).

Catalase belongs to the class of oxidoreductases which converts toxic hydrogen peroxide (H_2O_2) to water and regulates intracellular hydrogen peroxide levels. Degradation of hydrogen peroxide in the presence of catalase takes place in two steps. In the first step, one H_2O_2 molecule oxidizes heme to an oxyferryl species in which one oxidation equivalent is removed from the iron and one from the porphyrin ring (Por) to generate a porphyrin cation radical. The second hydrogen peroxide molecule is used as a reductant of compound I to regenerate the resting state enzyme, water and oxygen. In the second step, compound I is oxidized by a second peroxide molecule I to regenerate the resting state enzyme, water and oxygen. In this way two molecules of hydrogen peroxide are converted into two molecules of water and one molecule of oxygen (Chelikani et al., 2004; Goyal et al., 2012). The main reactions catalyzed by catalase enzyme are given in Figure 1.8.

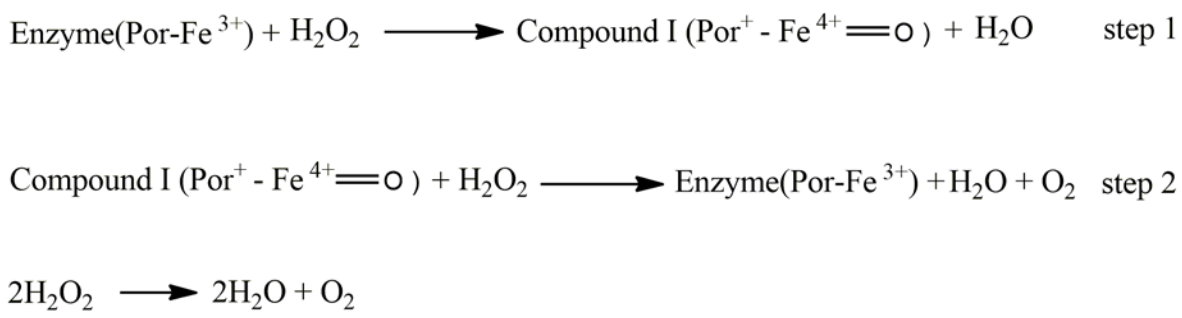


Figure 1.8. The main reactions catalyzed by catalase enzyme

1.3.2 Glutathione Reductase

Glutathione reductase (GR) is an antioxidant enzyme which is found in all cell types. It has been detected chloroplasts, mitochondria, cytosol and peroxisomes. It is a dimeric flavoprotein consisting of two identical subunits with 52 kDa molecular weight each. It is crucial for cellular survival since it is directly involved in responses against oxidative stress, heavy metals and herbicides.

Glutathione reductase catalyzes the reduction of glutathione disulfide (GSSG) to sulfhydryl form glutathione (GSH). Reduced glutathione (GSH) contributes to the removal of reactive electrophiles through conjugation and scavenging ROS directly or in a reaction catalyzed by glutathione peroxidase, thereby GSH represents an important role in protecting cells against oxygen free radicals. Glutathione reductase maintains the intracellular glutathione level and this feature is critical to the cell against oxidative stress (Carlberg and Mannervick, 1985; Deponte, 2013).

Glutathione reductase catalyzed reactions require NADPH. First of all, glutathione reductase and NADPH interact with each other to produce reduced glutathione reductase (GRred). Then, GRred interacts with oxidized glutathione (GSSG), resulting in a disulfide interchange. This interaction produces a molecule of GSH and the GRred-SG complex. Finally, GRred-SG complex rearrange to produce second molecule of GSH and the oxidized form of glutathione reductase (Carlberg et

al., 1981; Carlberg and Mannervick, 1985; Deponte, 2013). General reaction mechanism was given in Figure 1.9.

Figure 1.9. The main reaction catalyzed by glutathione reductase

1.4 Flavonoids

Flavonoids are polyphenolic molecules abundant in plants. Most of the flavonoids have specific color. By the help of this color, plant attracts pollinating insects. Besides this role, some of the flavonoids have functions in UV filtration, nitrogen fixation, cell cycle inhibition, and as chemical messenger. The flavonoids can be classified into eight major subtypes, which include flavans, flavanones, isoflavanones, flavones, isoflavones, anthocyanidines, chalcones and flavonolignans (Hodek et al., 2002). Figure 1.10 illustrates the structures of basic flavonoid skeleton. According to the report of Hodek et al. (2002), more than 8000 compounds of flavonoids have been identified. Derivatives of flavonoids have hydroxyl or methyl groups at different positions in the basic structures.

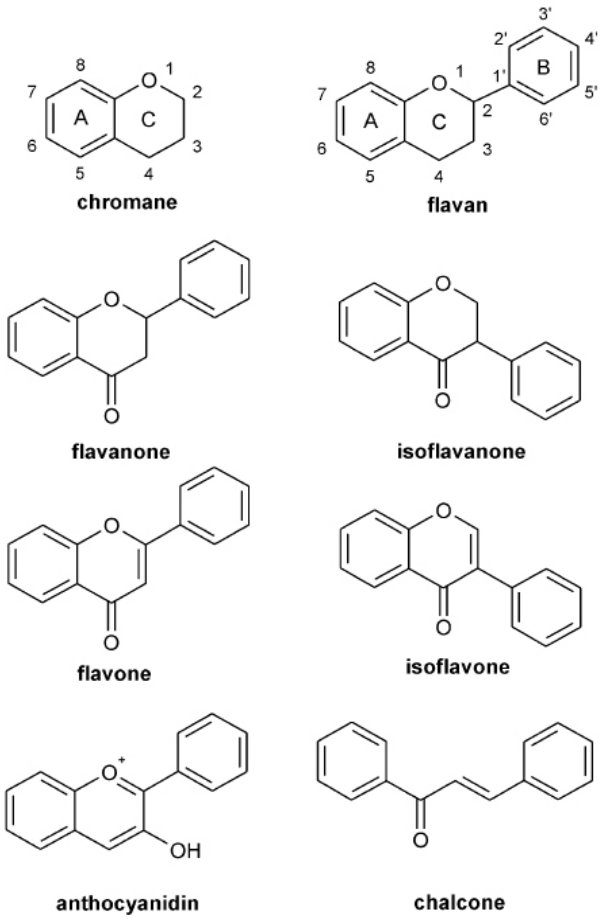


Figure 1.10. Structures of basic flavonoid skeleton (The figure was taken from Hodek et al., 2002)

Flavonoids produce a wide variety of physiological and biological effects in living organisms. They have antioxidant, antibacterial, antiinflammatory, antiallergic, antimutagenic, anticarcinogenic, antiviral properties and enzyme-modifying activity (Birt et al., 2001; Cotelle et al., 1996; Digiovanni 1990; Havsteen, 1983; Hodek et al., 2002; Jovanovic et al., 1994; Middleton et al., 2000; Middleton and Kandaswami, 1992; Moon et al., 2006; Ratty and Das 1988; Vernet and Siess 1986). In addition, flavonoids have antioxidant and free-radical scavenging activities (Chatuphonprasert et al., 2010). Flavonoids show modulatory effects on monooxygenase activities in liver microsomes of rat, rabbit and human (Huang et al., 1981; Raucy and Johnson, 1985; Siess et al., 1989; Tsyrlov et al., 1994). Flavonoids may stimulate or inhibit the monooxygenase activities. In general, flavonoids possessing hydroxyl groups often inhibit P450-dependent monooxygenase activity *in*

vitro, whereas those lacking hydroxyl groups can stimulate this activity (Buening et al., 1981; Lasker et al., 1984; Tsyrlov et al., 1994). However, the studies also indicated that the effects of flavonoids may change *in vitro* and *in vivo* studies. For example, quercetin inhibits P450-dependent B[a]P hydroxylase activity in liver microsomes from rat and human (Buening et al., 1981; Sousa and Marletta, 1985; Wiebel et al. 1974). However, quercetin increases mRNA expression of CYP1A1 in human breast cancer cells (Ciolino et al., 1999). Kaempferol and hesperitin have inhibitory effects on CYP1A1, while diosmin and diosmetin have stimulatory effects on CYP1A1 (Ciolino et al., 1998; Kang et al., 1999; Zhai et al., 1998). Apigenin, chrysin, galangin, luteolin, and morin synergistically enhanced β-NF-induced CYP1A1 expression at 24 h, but considerably suppressed it at 9 h (Chatuphonprasert et al., 2010). The determination of effects of flavonoids on cytochrome P450 system is important since cytochrome P450s have roles in the metabolism of pollutants, drugs, food additives and endogenous molecules such as hormones.

1.4.1 Morin

Morin (3,5,7,2′,4′-pentahydroxyflavone) is a flavonoid especially abundant in onion, seed weeds and is found in almond (*Prunus dulcis*), fustic (*Chlorophora tinctoria*), guava (*P. guajava L.*), red wine, and Osage orange (Nandhakumar et al., 2012). Morin is structurally related with quercetin, both having OH in position 3, a resorcinol moiety, and a carbonyl group in position 4; the only difference between them is the hydroxylation pattern on B-ring, which is meta in morin but ortho in quercetin. Figure 1.11 illustrates the structure of morin.

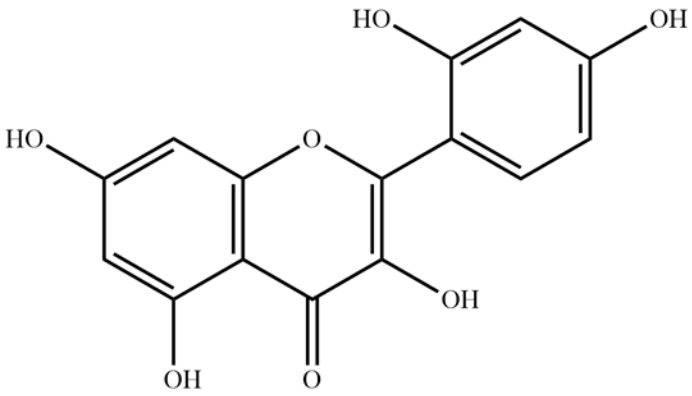


Figure 1.11. The structure of morin

Morin has different functions in living organisms including antioxidant, antiinflammatory, chemopreventive agent, cell proliferation inhibitor, neuroprotective reagent and anti-promotion properties (Mendoza-Wilson et al., 2011; Nandhakumar et al., 2012; Subash and Subramanian, 2009) Morin has five hydroxyl groups in its structure (at the position 3, 5, 7, 2′,4′). These hydroxyl groups of morin have a role in scavenging the reactive oxygen species which initiate lipid peroxidation (Cholbi et al., 1991; Sreedharan et al., 2009). The potential role of morin in the chemoprevention of cancer has been shown in DMBA treated rats (Nandhakumar et al., 2012). In this study, elevated serum markers are reduced by morin in DMBA induced cancerous group of rats (Nandhakumar et al., 2012). The beneficial role of morin has been shown in diabetes mellitus. It has been shown that morin (30 mg/kg/day) decreased glucose level and increased insulin level in diabetic rats. In addition, morin is considered as non toxic chemical since morin treatment does not alter the normal architecture of liver and brain (Subash and Subramanian, 2009)

1.5 Diabetes Mellitus

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of insulin (Maritim et al., 2003). According to International Diabetes Federation, 8.3% of the world's population suffers from this disease and this ratio is

increasing due to population growth, aging, urbanization, low physical activity and the high prevalence of obesity (Magliano et al., 2015; Veghari et al., 2010; Wild et al., 2004).

Diabetic complications are many including nephropathy, neuropathy, retinopathy, cardiovascular diseases, alteration of bone and mineral metabolism (Abuohashish et al., 2013). High concentration of glucose level in blood affects firstly kidneys, circulatory system and nervous system and causes damage in all other organs. In addition, it causes early ageing in tissues and organs. Experimental animal studies have been shown that persistent hyperglycemia causes an increase in the production of reactive oxygen species and may disturb oxidant/antioxidant ratio and increase in free radical formation within the body (Baynes and Thorpe, 1999; Saxena et al., 1993).

Two major classes of diabetes mellitus are proposed; Type 1 and Type 2 diabetes. In type I, β-cell destruction leads to diabetes mellitus and insulin is required for survival. Type 1 comprises immune-mediated and idiopathic diabetes mellitus. Immune-mediated diabetes results from cell-mediated autoimmune destruction of the insulin-producing β-cells. Idiopathic diabetes mellitus patients have permanent insulinopenia. Type 2 is the most common type of diabetes. It is characterized by disorder of insulin resistance and insulin secretion. Most patients do not require insulin injection and oral anti-diabetic agents in the treatment of Type 2 diabetes (Magliano et al., 2015).

Diabetic animal model may be developed by either surgical method by removing pancreas out of the body or non-surgical methods by administration of chemicals such as alloxan and streptozotocin (Rees and Alcolado, 2005). Streptozotocin (2-deoxi-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose)) is a naturally occurring N-nitroso derivative of D-glucoseamine (Figure 1.4). The administration of streptozotocin at a dose of 60mg/kg initiates an autoimmune process that results in the destruction of the Langerhans islets beta cells and clinical diabetes produced within 2-4 days (Akbarzadeh et al., 2007).

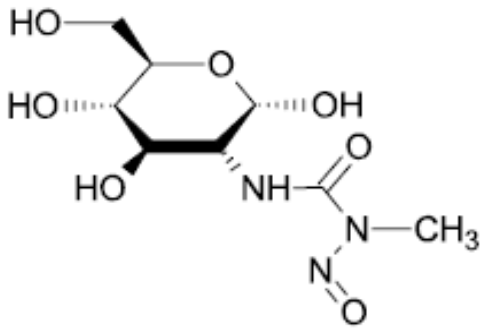


Figure 1.4. Chemical structure of Streptozotocin.

In the treatments of diabetes generally chemical drugs or insulin are used. However, the results of epidemiological studies indicates that insulin and insulin analogues, especially insulin glargine, increases the risk of cancer (Bronsveld et al., 2015). Recently, the studies in the treatment of diabetes mellitus is focused on plant derived molecules especially on phenolic compounds to protect the body against the effects of diabetes (Crozier et al., 2009; Dembinska-Kiec et al., 2008). It has been shown that one of the flavonoid quercetin has protective effects in diabetes by decreasing oxidative stress, preserving pancreatic β-cell integrity, possibly through the alteration of lipid peroxidation, nitric oxide production antioxidant enzyme activities (Coskun et al., 2005). Beneficial role of morin in the regulation of glucose level disrupted in diabetes has been shown in rats (Abuohashish et al., 2013). However, the effects of morin on cytochrome P450 system have not been studied in diabetic rats.

2. AIM AND SCOPE OF THE STUDY

Endosulfan and 7,12-dimethylbenz(a)anthracene (DMBA) are lipid soluble toxic molecules causing metabolic disorders. 7,12-Dimethylbenz(a)anthracene is a chemical present in tobacco smoke. Endosulfan is an organochlorine pesticide. Organochlorine pesticides (OCPs) are chlorinated hydrocarbons that are used extensively in the agricultural areas against insects. Since these lipid soluble chemicals can not be metabolized easily, they accumulate in the environment and cause persistent organic pollution in ecosystem. It is possible to be exposed to both these chemicals by smoking cigarette. Diabetes mellitus is a metabolic disorder characterized by high blood glucose level. High glucose level in diabetes leads to the generation of free radicals and disturbs oxidant /antioxidant ratio within the body. Flavonoids are polyphenolic compounds that are found in plants. They have antioxidant activities. Flavonoids possess their antioxidant activities by scavenging of free radicals and inhibiting enzymatic reactions responsible from the production of free radicals. Morin is one of the flavonoids obtained from plants. Recently, the studies have been focused on the treatment of metabolic disorders by plant derived molecules. The effects of morin on the antioxidant enzyme systems and free radical generating systems have not been studied in the liver of 7,12-dimethylbenz[a]anthracene and endosulfan treated healthy and streptozotocin induced diabetic rats.

In this study our aim was

1- to measure the effects of endosulfan on cytochrome P450 system and antioxidant enzymes in rat livers,
2- to measure the effects of 7,12-dimethylbenz(a)anthracene on cytochrome P450 system and antioxidant enzymes in rat livers,
3- to measure the effect of morin on cytochrome P450 system and antioxidant enzymes in rat livers,
4- to show chemoprotective effect of morin in endosulfan and/or 7,12-dimethylbenz(a)anthracene treated rats,

5- to determine the effect of morin on cytochrome P450 system and antioxidant enzymes in streptozotocin (STZ) induced diabetic rats,
6- to determine the effects of endosulfan and 7,12-dimethylbenz(a) anthracene treatment on cytochrome P450 system and antioxidant enzymes in streptozotocin induced diabetic rats, and
7- to determine the effects of morin on cytochrome P450 system and antioxidant enzymes in endosulfan and 7,12-dimethylbenz(a)anthracene treated streptozotocin induced diabetic rats.

The effects of these chemicals were determined by measuring phase I enzyme activities (7-ethoxyresorufin O-deethylase (CYP1A1), 7-methoxyresorufin O-demethylase (CYP1A2), 7-pentoxyresorufin O-depentylase (CYP2B), aniline 4-hydroxylase (CYP2E) and erythromycin N-demethylase (CYP3A)), phase II enzyme activity (glutathione S-transferase) and the oxidative stress biomarker enzyme activities (catalase and glutathione reductase). The effects of these chemicals on the cytochrome P450 system were also measured at the protein level by using polyclonal antibodies produced against CYP1A, CYP2B, CYP2E and CYP3A. In addition to biochemical parameters, histopathological studies were also carried out to show pathological changes.

3. MATERIALS AND METHODS

3.1 Materials

Aniline ($C_6H_5NH_2$; 101261), bromophenol blue ($C_{19}H_{10}BrO_5S$; 108122), diethanolamine (DEA; $C_4H_{11}NO_2$; 803116), N,N-dimethylformamide ($HCON(CH_3)_2$; 102937), dimethyl sulfoxide (DMSO; $(CH_3)_2SO$; 116743), ethanol (C_2H_5OH; 100983), formaldehyde (CH_2O; 344198), glacial acetic acid (CH_3COOH; 100056) glycerol ($C_3H_8O_3$; 104092), hydrochloric acid (HCl; 100314), hydrogen peroxide (H_2O_2; 108600) magnesium chloride hexahydrate ($MgCl_2.6H_2O$; 105833), methanol (CH_3OH; 106007), nitro blue tetrazolium chloride (NBT; $C_{40}H_{30}Cl_2N_{10}O_6$; 124823), potassium chloride (KCl; 104936), potassium dihydrogen phosphate (KH_2PO_4; 104871), dipotassium hydrogen phosphate (K_2HPO_4:105101), potassium sodium tartrate tetrahydrate ($C_4H_4KNaO_6.4H_2O$; 108087), sodium carbonate (Na_2CO_3; 106392), sodium chloride (NaCl; 106404), sodium hydroxide (NaOH; 106462), N, N, N', N' tetrametylethylene diamine (TEMED; $C_6H_{16}N_2$; 110732), tricloroacetic acid (CCl_3COOH; 100807), triton X-100 ($C_{14}H_{21}(C_2H_4O)_nOH$; 112298) and zinc chloride ($ZnCl_2$; 108816) were purchased from Merck KGaA, Darmstadt, Germany.

Acetylacetone ($CH_3COCH_2COCH_3$; 00900), acrylamide (C_3H_5NO; A8887), ε-amino caproic acid (ε-ACA; $C_6H_{13}NO_2$ A2504), p-aminophenol ($H_2NC_6H_4OH$; 35837), 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris; T1378), ammonium acetate ($CH_3CO_2NH_4$; A1542) anti-rabbit IgG alkaline phosphatase antibody (A3687), bovine serum albumin (BSA; A7511 or A7888), 1-chloro-2,4-dinitrobenzene ($ClC_6H_3(NO_2)_2$; 24438), copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$; C7631), 7,12-dimethylbenz[a]anthracene (DMBA; $C_{20}H_{16}$; D3254), erythromycin ($C_{37}H_{67}NO_{13}$; E0774), ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA; $C_{10}H_{14}N_2Na_2O_8.2H_2O$; E5134), Folin-Ciocalteu's phenol reagent (F9252), N-2-hydroxyethylpiperazine-N'-2-ethansulphonic acid (HEPES; $C_8H_{18}N_2O_4S$; H3375), glycine ($C_2H_5NO_2$; G7126), β-mercaptoethanol (M6250),

N,N'-methylene bisacrylamide (BIS; $C_7H_{10}N_2O_2$; M7256), morin hydrate ($C_{15}H_{10}O_7.xH_2O$; M4008), perchloric acid ($HClO_4$; 30755), phenylmethanesulfonyl fluoride (PMSF; $C_7H_7FO_2S$; P7626), L-glutathione oxidized disodium salt (GSSG; G4626), L-glutathione reduced (GSH; G6013), resorufin ethyl ether ($C_{14}H_{11}NO_3$; E3763), resorufin methyl ether ($C_{13}H_9NO_3$; M1544), resorufin pentyl ether ($C_{17}H_{17}NO_3$; P0928), resorufin sodium salt (23,015-4) and streptozotocin ($C_8H_{15}N_3O_7$; S0130) were purchased from Sigma-Aldrich, Saint Louis, Missouri, USA.

5-bromo-4-chloro-3-indolylphosphate disodium salt (BCIP; 203788), glucose-6-phosphate dehydrogenase (346774), α-D-glucose-6-phosphate monosodium salt (346764), polyxyethylene sorbitan monolaurate (Tween 20; 655204), sodium dodecyl sulfate (SDS; 428023) were purchased from Calbiochem, San Diego, California, USA.

Ammonium persulfate ($(NH_4)_2S_2O_8$; AC327081000) was purchased from Acros Organics, Geel, Belgium.

β-nicotinamide adenine dinucleotide phosphate disodium salt ($NADP^+$; $C_{21}H_{27}N_7NaO_{17}P_3$; A1394), β-Nicotinamide adenine dinucleotide phosphate reduced tetrasodium salt (NADPH; $C_{21}H_{26}N_7Na_4O_{17}P_3$; A1395) phenazine methosulfate (PMS;$C_{14}H_{14}N_2O_4S$; A2212) were purchased from Applichem GmbH, Darmstadt, Germany.

Trans blot tranfer medium (pure nitrocellulose membrane; 162-0115) were purchased from Bio-Rad Laboratories, Richmond, California, USA.

Monoclonal anti-rat CYP1A1 (MABS19), monoclonal anti-rat CYP2B1/2BC.B/C4 (MAB10037), polyclonal anti-rat CYP2E1 (AB1252) and monoclonal anti-rat CYP3A4/3A1/3A11 (MAB10041) were purchased from Millipore, Billera, MA, USA.

Endosulfan (İmpasülfan 35 WP; IMPA Agriculture and Veterinary Medicine Company, Istanbul, Turkey) was obtained as from Bolu Directorate of Provincial Food Agriculture and Livestock**.**

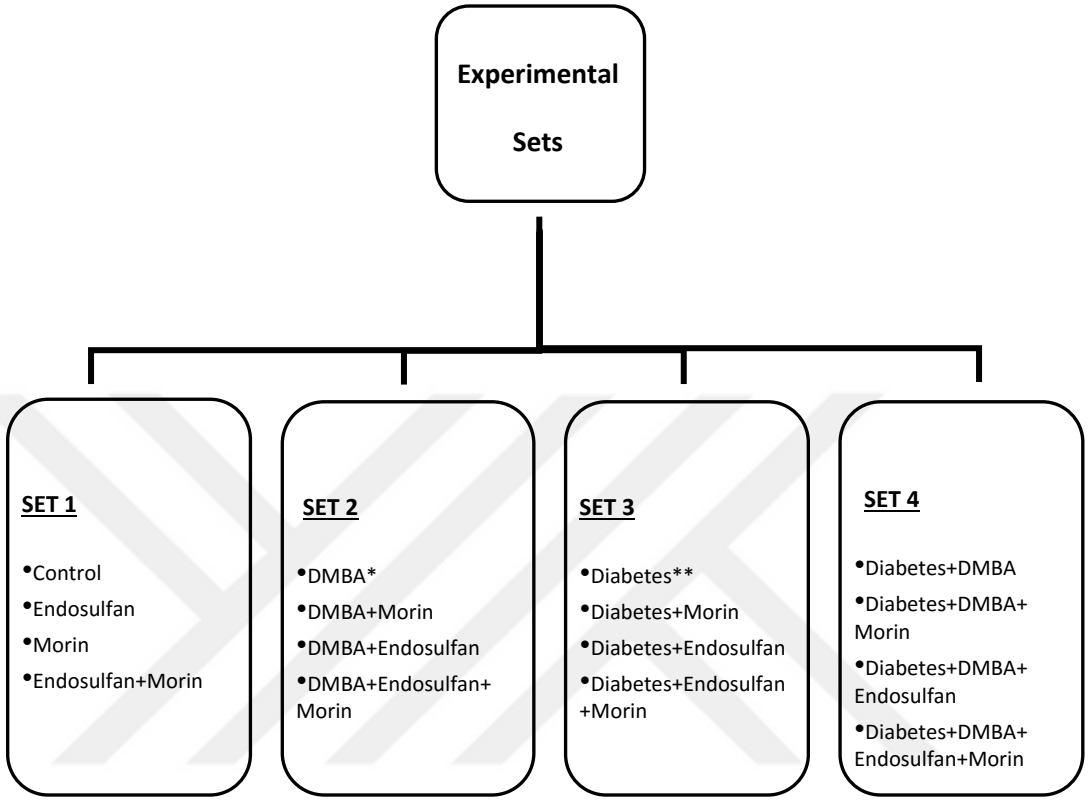
All the other chemicals were of analytical grade and were obtained from commercial sources at the highest grade of purity available.

3.2 Animals and Treatments

Two months old adult female (n=12) and male (n=5) Wistar rats were purchased from Kobay Experimental Animals Laboratory to breed new generations. They were housed in plastic cages depending on their sex in Experimental Animal Unit of Abant Izzet Baysal University. After an acclimatization period of 10 days, rats were mated in a combination of two or three female with a male (2:1 or 3:1) to obtain adequate number of male rats. All rats were maintained at 22-24 °C with 55-60 % moisture ratio on a 12 hour light and 12 hour dark cycle, with access to water and standard food ad *libitum*. The animal-based studies involving their treatments and care were carried out with ethical approval obtained from the Animal Care Ethics Committee of Abant Izzet Baysal University Medical School (Process number: 2011/10) and conducted in accordance with the Declaration of Helsinki.

The 2.5-month-old adult Wistar male rats, weighing 170-255 g, were used in this study. In order to minimize litter effects, rats were randomly selected and separated into groups. Animal studies comprised four different sets each including four different groups (Figure 3.1). The first experimental set consisted of control (group 1), endosulfan (group 2), morin (group 3) and endosulfan+morin (group 4) groups. The second experimental set consisted of 7,12-dimethylbenz[a]anthracene (DMBA; group 5), DMBA+morin (group 6), DMBA+endosulfan (group 7), and DMBA+endosulfan+morin (group 8) groups. The third experimental set consisted of diabetes mellitus (group 9), diabetes mellitus+endosulfan (group 10), diabetes mellitus+morin (group 11) and diabetes mellitus+endosulfan+morin (group 12) groups. The fourth experimental set consisted of diabetes mellitus+DMBA (group 13), diabetes mellitus+DMBA+morin (group 14), diabetes mellitus+DMBA+

endosulfan (group 15), and diabetes mellitus+DMBA+endosulfan+morin (group 16) groups. Each set was carried out at different periods of this study and experimental animals of each set were mated at proper time before used.



*7,12-Dimethylbenz[a]anthracene
**Diabetes mellitus

Figure 3.1. Schematic representation of the sets in animal studies.

Each experimental group of set 1 and set 2 included seven animals at the beginning of the chemical treatments. The chemical treatments including DMBA, endosulfan, and morin were carried out on healthy rats in set 1 and set 2. Each experimental group of set 3 and set 4 included eight animals at the beginning of the chemical treatments. The chemical treatments including DMBA, endosulfan, and morin were carried out in streptozotocin induced diabetic rats found in set 3 and set 4.

7,12-Dimethylbenz[a]anthracene, endosulfan, and morin treatments were carried out by oral gavage and streptozotocin treatments were carried out by single intraperitoneal injection. Before treatments, the rats were deprived of food and water

overnight (at least 12 h). The blood glucose levels of rats were measured on the 1st day of study using Accu-Chek Active test strip in Accu-Chek Active test meter. In diabetic groups (group 9-16), diabetes was induced on the 7th day of the experimental period by a single intraperitoneal injection of buffered solution (0.1 mol/L citrate, pH 4.5) of streptozotocin at doses of 60.0 mg/kg body weight (b.wt.). Four days after streptozotocin injection (11th day of this study), the blood glucose levels of rats in diabetic groups (group 1-9) were measured and animals having blood glucose levels more than 250 mg/dL were considered as diabetic and used for this study. The blood glucose levels of rat after streptozotocin injection were between 272 mg/dL and 600 mg/dL.

Experimental design of set 1 and set 2 (groups 1-8) was given in Figure 3.2 and experimental design of set 3 and set 4 (groups 9-16) was given in Figure 3.3. The rats in control (group 1) and diabetes mellitus (group 9) groups were gavaged with 0.5 mL of corn oil three times in a week during the experimental period. The rats in DMBA-treated groups (groups 5, 6, 7, 8, 13, 14, 15, and 16) were gavaged with 30.0 mg/kg b.wt. DMBA in totally 0.5 mL of corn oil at 12th, 19th and 26th days of the study. Except for 12th, 19 th and 26 th days, 0.5 mL of corn oil was given to these rats during the administration period with the frequency of three times in a week. Endosulfan-treated groups (groups 2, 4, 7, 8, 10, 12, 15, and 16) were gavaged with 0.5 mL of corn oil three times in a week up to twelfth day of the administration period. The administration of endosulfan was started at the twelfth day at a dose of 5.0 mg/kg b.wt. endosulfan in totally 0.5 mL of corn oil and this treatment was continued through the experimental period with the frequency of three times in a week. Morin-treated groups (groups 3, 4, 6, 8, 11, 12, 14, and 16) were gavaged with 0.5 mL of corn oil and 25.0 mg/kg b.wt. morin in 0.2 mL drinking water three times in a week. Rats in group 1-8 were sacrificed on the 54th day of the experimental period of this study by cervical dislocation without using any anesthetics. Figure 3.2 illustrates the schematic representation of animal studies carried out on rats in groups 1-8 (set 1 and set 2).

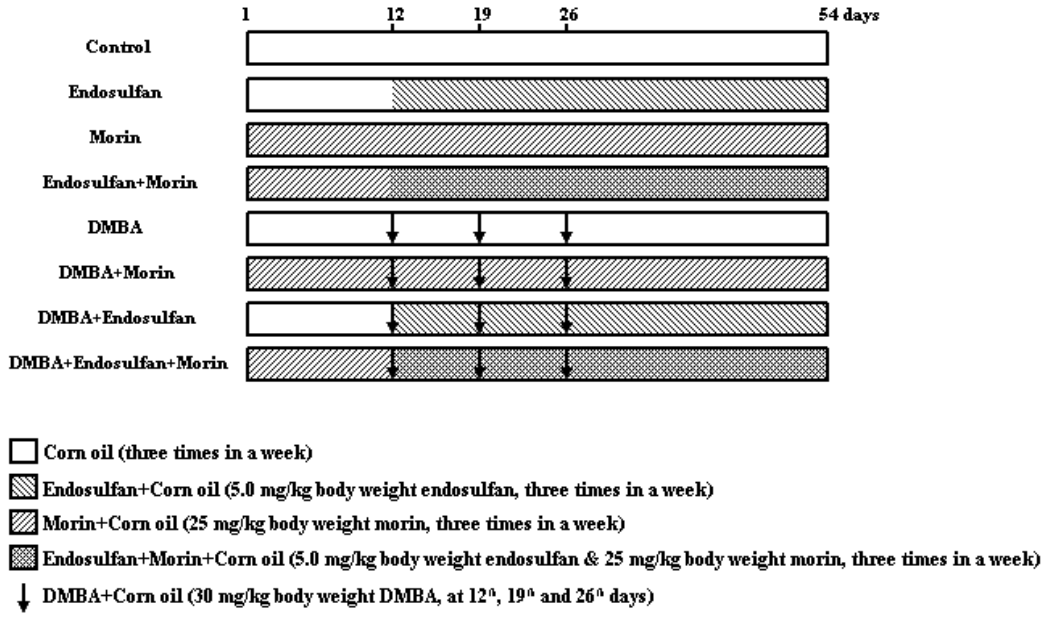


Figure 3.2. Schematic representation of animal studies carried out on rats in groups 1-8.

The chemical treatments of rats in groups 9-16 were performed at the same conditions with that in groups 1-8. However animal deaths were observed in group 9-16 during the animal studies. Therefore, rats in these groups were sacrificed on the 43rd day of the experimental period of this study by cervical dislocation without using any anesthetics. Figure 3.3 illustrates the schematic representation of animal studies carried out on rats in groups 9-16 (set 3 and set 4).

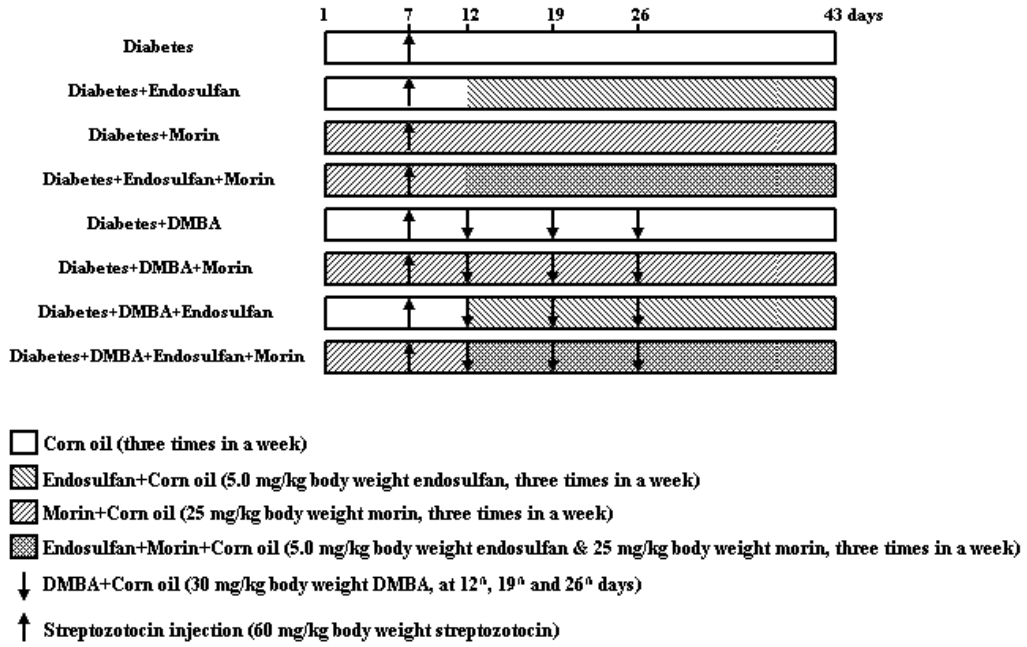


Figure 3.3. Schematic representation of animal studies carried out on rats in groups 9-16.

After sacrification, the liver and lung tissues of rats were removed immediately. A small section was removed from the same region of each liver and lung tissue. These tissue sections were stored in 10% formaldehyde solution for histopathological studies. The remaining parts of the livers and lungs were first wrapped by freezing bags and covered by aluminum foil and frozen by putting into liquid nitrogen and then stored in a freezer at - 80 $^{\circ}$C until used.

3.3 Methods

3.3.1 Preparation of Rat Liver and Lung Microsomes and Cytosols

The microsomes and cytosols of rat livers and lungs were prepared by the method described by Arınç et al. (1991) with some modifications. The livers and lungs were taken from freezer (- 80 $^{\circ}$C) and thawed on ice and all subsequent steps were carried out in 0-4°C ice bath. In order to remove as much blood as possible, tissues were washed first with cold distilled water, and then with cold 1.15% KCl

solution. Then they were drained on a filter paper, weighed and cut into small pieces with scissors. The resulting liver and lung tissue minces were homogenized in 1.15% KCl solution containing 2.0 mM EDTA pH 7.4, 0.25 mM ε-ACA, 0.1 mM PMSF at a volume equal to 3.0 times the weight of tissues. This homogenization step was performed by using glass homogenizer packed in crushed ice, coupled motor (Black & Decker, V850 multispeed drill)-driven Teflon pestle at 2400 rpm, with fifteen passes.

The homogenate was centrifuged at 10000×g by using Sigma 3-30K Refrigerated Centrifuge, Saint Louis, Missouri, USA with 12156 rotor for 20 minutes. The supernatant fraction was filtered through two layers of cheesecloth. The pellet containing cell debris, nuclei and mitochondria was discarded. The obtained supernatant solution containing endoplasmic reticulum and other fractions of the cell was centrifuged at 45000 rpm (105000×g) for 60 minutes using Beckman Optima L-90K ultracentrifuge (Beckman Coulter Inc., Fullerton, California, USA) with 70.1 Ti rotor. At the end of the centrifugation, the obtained pellet fractions were microsomes and supernatant fractions were cytosols. Cytosols were taken into Eppendorf tubes and stored in freezer at - 80 $^{\circ}$C until used. The packed microsomal pellet was suspended in 1.15% KCl solution containing 2.0 mM EDTA, pH 7.4 and resedimented by ultracentrifugation at 45000 rpm (105000×g) for 60 minutes. The supernatant fraction was discarded. Then the washed microsomal pellet was resuspended in 25% glycerol containing 1.0 mM EDTA pH 7.4 at a volume of 1.0 mL for each gram of rat tissues. Resuspended microsome was homogenized manually using the teflon-glass homogenizer in order to obtain homogenous suspension. Microsome was separated into small aliquots and put into Eppendorf tubes. Aliquots of microsomes were stored in freezer at -80 $^{\circ}$C after gassing with nitrogen.

3.3.2 Protein Determination

Concentrations of microsomal and cytosolic proteins were determined by the method of Lowry et al. (1951). Before cytosolic protein determination, liver cytosols were recentrifuged at 20000 rpm (Sigma 3-30K Refrigerated Centrifuge, Saint Louis,

Missouri, USA) by using 12154 rotor for 30 minutes. The supernatant fractions were taken and the protein concentrations of cytosols were determined using this phase. The centrifuged cytosols were stored at -80 °C until used for glutathione S-transferase and antioxidant enzyme activity measurements. In the determination of protein concentration, the rat liver microsomes and cytosols were diluted to 1:250 and the rat lung microsomes were diluted to 1:30. An aliquot of 0.10, 0.25 and 0.50 mL of samples (microsomes or cytosols) were mixed with distilled water in order to complete the volume to 0.50 mL in test tubes. Bovine serum albumin was used as a standard. Bovine serum albumin solutions were prepared at five different concentrations consisting of 0.020, 0.050, 0.100, 0.150 and 0.200 mg/mL BSA. 0.50 mL of each standard solution was taken into standard tubes separately. After that, all tubes were mixed with 2.5 mL of alkaline copper reagent which was prepared freshly by mixing 2.0% copper sulfate, 2.0% sodium potassium tartrate and 20% Na_2CO_3 in 0.1 N NaOH with the proportion of 1:1:100 in the written order and incubated at room temperature for 10 minutes. Then, 0.25 mL of 1.0 N Folin-Ciocalteu's phenol reagent was added to the tubes and incubated at room temperature for 30 minutes. The resulting color intensity was measured by using spectrophotometer (Jasco V-530 UV/VIS Spectrophotometer, Jasco International Corporation, Tokyo, Japan) at 660 nm. The standard calibration curve of BSA from 20 to 200 μg/mL was plotted and used for the determination of protein concentration of samples.

3.3.3 Determination of 7-Ethoxyresorufin O-Deethylase Enzyme Activity of Rat Liver and Lung Microsomes

Cytochrome P4501A (CYP1A) was determined at the level of enzyme activity by measuring 7-ethoxyresorufin O-deethylase activities (EROD) of rat liver and lung microsomes. CYP1A has a role in biotransformation of chemicals to their highly reactive forms. EROD activity is induced in the presece of many chemicals such as polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and dioxins. CYP1A associated 7-ethoxyresorufin O-deethylase activities of rat liver and lung microsomes were determined by the method described by Burke and Mayer (1974) with some modifications. The principle of the method is based on the

conversion of 7-ethoxyresorufin to 7-hydroxyresorufin catalyzed by monooxygenases in the presence of molecular oxygen and NADPH (Figure 3.4).

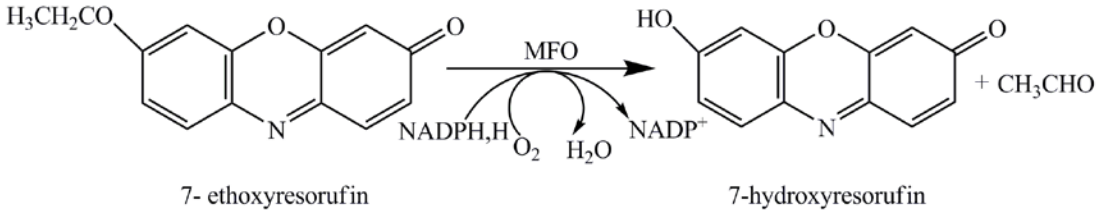


Figure 3.4. 7-Ethoxyresorufin O-deethylase reaction.

Assay conditions were optimized for rat liver and lung microsomes by testing different substrate concentrations, enzyme concentrations, cofactor concentrations, pH, and temperature. EROD activities of each microsome were measured in duplicates and sometimes in triplicates at optimum conditions. In this method, resorufin solution was used as a standard and 7-ethoxyresorufin solution was used as a substrate. Firstly, 0.5 mM stock 7-ethoxyresorufin solution and 1.0 mM stock resorufin standard solution were prepared in dimethyl sulfoxide (DMSO). Then 50 μM daily 7-ethoxyresorufin solution and 5.0 μM daily resorufin solution were prepared by diluting stock solutions with 0.2 M potassium phosphate buffer pH 7.4 containing 0.2 M NaCl. A typical reaction mixture contained 0.1 M potassium phosphate buffer pH 7.4, 0.1 M NaCl, 2.4 mg BSA, 5.0 μM 7-ethoxyresorufin, 100 μg microsomal protein, 0.5 mM NADPH generating system (prepared by incubation of 2.5 mM $MgCl_2$, 2.5 mM glucose-6-phosphate, 14.6 mM HEPES, pH 7.8, 0.5 U glucose-6-phosphate dehydrogenase and 0.5 mM $NADP^+$ for 5 minutes at 37 $^{\circ}$C) in a final volume of 2.0 mL. The reaction components except NADPH generating system were added into a test tube and incubated for 2 minutes at 37 $^{\circ}$C in a water bath. The reaction was initiated by the addition of NADPH generating system and followed for fifteen minutes at 37 $^{\circ}$C in spectrofluorometer (Hitachi F-4500, Hitachi High-Technologies Corporation, Tokyo, Japan) at 535 nm (excitation) and 585 nm (emission) wavelengths. Finally 50 pmol resorufin was added to the reaction mixture and the increase in fluorescence was recorded. The specific EROD activity was calculated using the fluorescence increase caused by the addition of resorufin.

3.3.4 Determination of 7-Methoxyresorufin O-Demethylase Enzyme Activity of Rat Liver Microsomes

Cytochrome P4501A2 (CYP1A2) was also determined at the level of enzyme activity by measuring 7-methoxyresorufin O-demethylase activities (MROD) of rat liver. MROD activity is induced in the presece of many chemicals such as polyaromatic hydrocarbons and aromatic amines. Cytochrome P4501A2 (CYP1A2) associated 7-methoxyresorufin O-demethylase activities (MROD) of rat liver microsomes were determined by the method described by Burke and Mayer (1974) with some modifications. The principle of the method is based on the conversion of 7-methoxyresorufin to 7-hydroxyresorufin catalyzed by monooxygenases in the presence of molecular oxygen and NADPH (Figure 3.5).

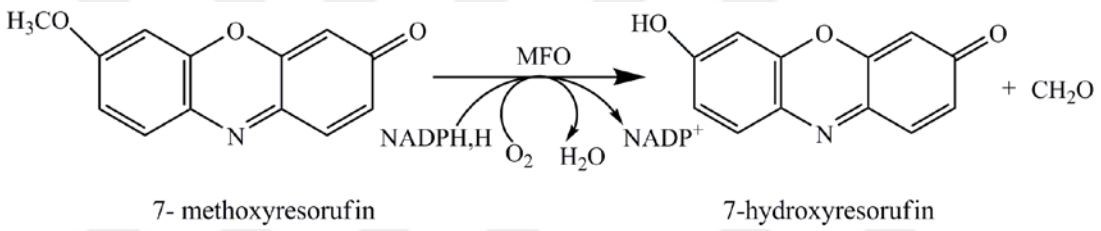


Figure 3.5. 7-Methoxyresorufin O-demethylase reaction.

Assay conditions were optimized for rat liver microsomes by testing different substrate concentrations, enzyme concentrations, cofactor concentrations, pH, and temperature. MROD activities of each microsome were measured in duplicates and sometimes in triplicates at optimum conditions. In this method, resorufin solution was used as a standard and 7-methoxyresorufin solution was used as a substrate. Firstly, 0.5 mM stock 7-methoxyresorufin solution and 1.0 mM stock resorufin standard solution were prepared in dimethyl sulfoxide (DMSO). Then 50 μM daily 7-methoxyresorufin solution and 5.0 μM daily resorufin solution were prepared by diluting stock solutions with 0.2 M potassium phosphate buffer pH 7.4 containing 0.2 M NaCl. A typical reaction mixture contained 0.1 M potassium phosphate buffer pH 7.4, 0.1 M NaCl, 1.2 mg BSA, 5.0 μM 7-methoxyresorufin, 50 μg microsomal protein, 0.5 mM NADPH generating system (prepared by incubation of 2.5 mM $MgCl_2$, 2.5 mM glucose-6-phosphate, 14.6 mM HEPES, pH 7.8, 0.5 U glucose-6-

phosphate dehydrogenase and 0.5 mM $NADP^+$ for 5 minutes at 37 °C) in a final volume of 2.0 mL. The reaction components except NADPH generating system were added into a test tube and incubated for 2 minutes at 37 °C in a water bath. The reaction was initiated by the addition of NADPH generating system and followed for fifteen minutes at 37 °C in spectrofluorometer (Hitachi F-4500, Hitachi High-Technologies Corporation, Tokyo, Japan) at 535 nm (excitation) and 585 nm (emission) wavelengths. Finally a known amount of resorufin (50 pmol) was added to the reaction mixture and the increase in fluorescence was recorded. The specific MROD activity was calculated using the fluorescence increase caused by the addition of resorufin.

3.3.5 Determination of 7-Pentoxyresorufin O-Depentylase Enzyme Activity of Rat Liver Microsomes

7-Pentoxyresorufin O-depentylase activity (PROD) is a cytochrome P4502B (CYP2B) associated enzyme activity. CYP2B has a role in the metabolism of endogenous molecules such as testosterone and androstedione. CYP2B associated 7-pentoxyresorufin O-depentylase activities of rat liver microsomes were determined by the method described by Burke and Mayer (1974) with some modifications. The principle of the method is based on the dealkylation of 7-pentoxyresorufin catalyzed by monooxygenases in the presence of molecular oxygen and NADPH (Figure 3.6).

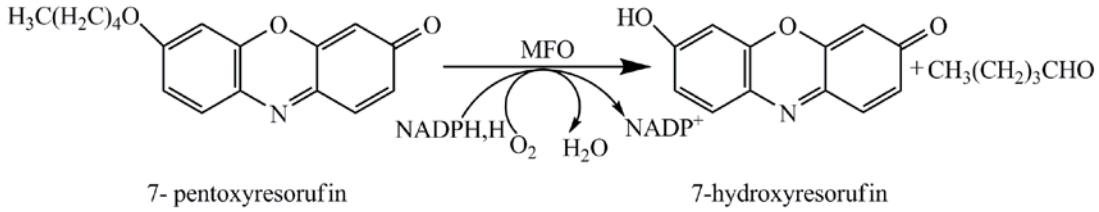


Figure 3.6. 7-Pentoxyresorufin O-depentylase reaction.

Assay conditions were optimized for rat liver microsomes by testing different substrate concentrations, enzyme concentrations, cofactor concentrations, pH, and temperature. PROD activities of each microsome were measured in duplicates and sometimes in triplicates at optimum conditions. In this method, resorufin solution was used as a standard and 7-pentoxyresorufin solution was used as a substrate.

Firstly, 0.5 mM stock 7-pentoxyresorufin solution and 1.0 mM stock resorufin standard solution were prepared in dimethyl sulfoxide (DMSO). Then 100 μM daily 7- pentoxyresorufin solution and 5.0 μM daily resorufin solution were prepared by diluting stock solutions with 0.2 M potassium phosphate buffer pH 7.5 containing 0.2 M NaCl. A typical reaction mixture contained 0.1 M potassium phosphate buffer pH 7.5, 0.1 M NaCl, 1.2 mg BSA, 10 μM 7-pentoxyresorufin, 150 μg microsomal protein, 0.5 mM NADPH generating system (prepared by incubation of 2.5 mM $MgCl_2$, 2.5 mM glucose-6-phosphate, 14.6 mM HEPES, pH 7.8, 0.5 U glucose-6-phosphate dehydrogenase and 0.5 mM $NADP^+$ for 5 minutes at 37 $^{\circ}C$) in a final volume of 2.0 mL. The reaction components except NADPH generating system were added into a test tube and incubated for 2 minutes at 37 $^{\circ}C$ in a water bath. The reaction was initiated by the addition of NADPH generating system and followed for five minutes at 37 $^{\circ}C$ in spectrofluorometer (Hitachi F-4500, Hitachi High-Technologies Corporation, Tokyo, Japan) at 535 nm (excitation) and 585 nm (emission) wavelengths. Finally a known amount of resorufin (50 pmol) was added into the reaction mixture and the increase in fluorescence was recorded. The specific PROD activity was calculated using the fluorescence increase caused by the addition of resorufin.

3.3.6 Determination of Aniline 4-Hydroxylase Enzyme Activity of Rat Liver Microsomes

Cytochrome P4502E (CYP2E) was determined at the level of enzyme activity by measuring aniline 4-hydroxylase activity (A4H) of rat liver microsomes. CYP2E has a role in the metabolism of low-molecular-weight-chemicals such as ethanol. It generally takes part in bioactivation reactions. CYP2E associated aniline 4-hydroxylase activities of rat liver microsomes were determined by the method described by Imai et al. (1966). The principle of the method is based on the hydroxylation of aniline catalyzed by monooxygenases in the presence of molecular oxygen and NADPH (Figure 3.7).

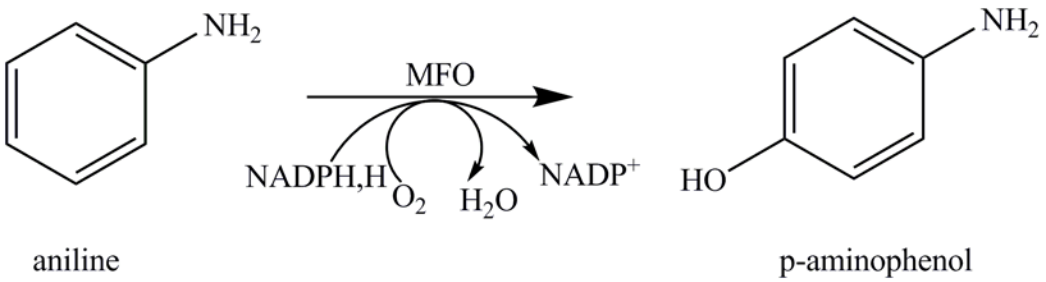


Figure 3.7. Aniline 4-hydroxylase reaction.

Assay conditions for aniline 4-hydroxylase were optimized for rat liver microsomes by testing different substrate concentrations, enzyme concentrations, cofactor concentrations, pH, and temperature. Aniline 4-hydroxylase activities of each microsome were measured in duplicates at optimum conditions. A typical assay mixture contained 100 mM HEPES buffer pH 7.5, 8.0 mM aniline, 2.5 mM $MgCl_2$, 1.5 mg microsomal protein and 0.5 mM NADPH generating system (prepared by incubation of 2.5 mM $MgCl_2$, 2.5 mM glucose-6-phosphate, 14.6 mM HEPES, pH 7.8, 0.5 U glucose-6-phosphate dehydrogenase and 0.5 mM $NADP^+$ for 5 minutes at 37 $^{\circ}$C) in a final volume of 0.5 mL. Zero time blanks were prepared for each reaction mixture by adding 0.25 mL of 20% trichloroacetic acid solution before the addition of cofactor. The reaction was initiated by the addition of 0.075 mL of NADPH generating system to incubation mixture and to zero time blank tubes. The reaction tubes and zero time blank tubes were incubated at 37°C for 25 minutes with moderate shaking in water bath. After 25 minutes, the enzymatic reaction was stopped by addition of 0.25 mL of 20% trichloroacetic acid solution to the reaction tubes. The contents of the tubes were transferred into Eppendorf tubes and were centrifuged at 12000xg (Sigma 3-30K Refrigerated Centrifuge, Saint Louis, Missouri, USA) for 30 minutes at 4°C for the removal of denatured microsomal proteins.

p-Aminophenol solution was used as standard. 0.125 mM p-aminophenol solution was prepared daily and standards were prepared by using this solution at four different concentrations (5.0, 12.5, 25.0, and 37.5 nmoles). The standard tubes contained p-aminophenol and other incubation constituents were run under the same conditions as for the reaction tubes.

After centrifugation and preparation of standards, 0.5 mL aliquots of each tube were transferred to test tubes and mixed with 0.25 mL 20 % Na_2CO_3 and with 0.25 mL 4% phenol in 0.4 N NaOH, respectively. The mixture was incubated at 37°C for 30 minutes in a water bath. The intensity of blue color developed was measured at 630 nm using spectrophotometer (Jasco V-530 UV/VIS Spectrophotometer, Jasco International Corporation, Tokyo, Japan). The specific aniline 4-hydroxylase activity was calculated using the standard calibration curve of p-aminophenol.

3.3.7 Determination of Erythromycin N-Demethylase Enzyme Activity of Rat Liver Microsomes

Cytochrome P4503A (CYP3A) was determined at the level of enzyme activity by measuring erythromycin N-demethylase (ERND) activities of rat liver microsomes. Cytochrome P4503A is responsible from the metabolism of most of the drugs and generally takes part in detoxification reactions. CYP3A associated erythromycin N-demethylase activities of rat liver microsomes were determined by measuring the quantity of formaldehyde formed colorimetrically according to the method of Nash (1953) as modified by Cochin and Axelrod (1959). The principle of the method is based on the N-dealkylation reaction of erythromycin catalyzed by monooxygenases in the presence of molecular oxygen and NADPH (Figure 3.8).

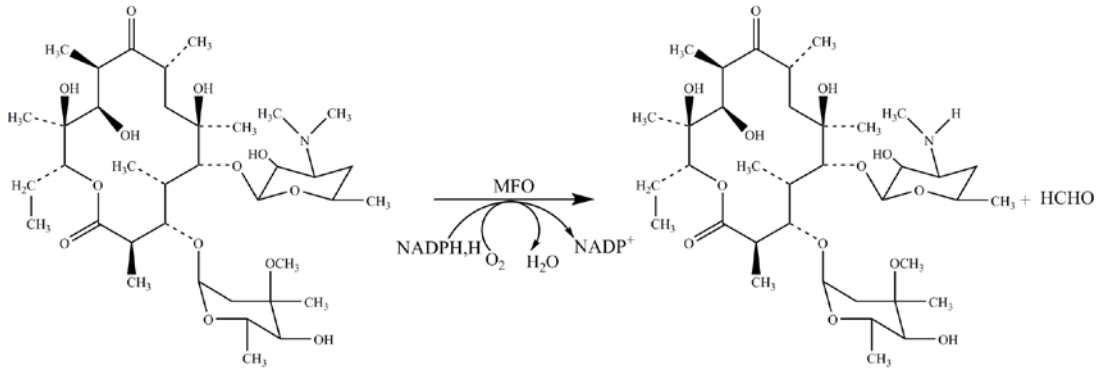


Figure 3.8. Erythromycin N-demethylase reaction.

Assay conditions were optimized for rat liver microsomes by testing different substrate concentrations, enzyme concentrations, cofactor concentrations, pH, and temperature. ERND activities of microsomes were measured in triplicates at optimum conditions. A typical assay mixture contained 100 mM HEPES buffer pH 7.8, 1.0 mM erythromycin (prepared by dissolving 73.4 mg of erythromycin in 50 % ethanol), 3.0 mg microsomal protein and 0.5 mM NADPH generating system (prepared by incubation of 2.5 mM $MgCl_2$, 2.5 mM glucose-6-phosphate, 14.6 mM HEPES, pH 7.8, 0.5 U glucose-6-phosphate dehdyrogenase and 0.5 mM $NADP^+$ for 5 minutes at 37 $^{\circ}$C) in a final volume of 0.5 mL. Zero time blanks were prepared for each reaction mixture by adding 0.5 mL of 0.75 N perchloric acid solution before the addition of cofactor. The reaction was initiated by the addition of 0.150 mL of NADPH generating system to the incubation mixture and to zero time blank tubes. The reaction was carried out at 37°C for 15 minutes with moderate shaking in water bath. After 15 minutes, the enzymatic reaction was stopped by the addition of 0.5 mL 0.75 N perchloric acid solution. The contents of the tubes were transferred into Eppendorf tubes and were centrifuged at 15000xg (Sigma 3-30K Refrigerated Centrifuge, Saint Louis, Missouri, USA) for 20 minutes at 4°C for the removal of denatured microsomal proteins.

Formaldehyde solution was used as a standard in this method. 0.5 mM formaldehyde solution was prepared daily and standards were prepared by using this solution at four different concentrations (0.012, 0.025, 0.050, and 0.100 mM). The standard tubes contained formaldehyde and other incubation constituents were run under the same conditions as for the reaction tubes.

Nash reagent was prepared just before use by the addition of 0.4 mL of acetylacetone to 100 mL solution containing 30.8 g ammonium acetate and 0.6 mL of glacial acetic acid. After centrifugation and preparation of standards, 0.5 mL aliquots of standard tubes and reaction tubes were transferred to test tubes and mixed with 0.375 mL Nash reagent. All tubes were incubated at 50°C for 10 minutes in a water bath. The intensity of yellow color developed was measured at 412 nm using spectrophotometer (Jasco V-530 UV/VIS Spectrophotometer, Jasco International

Corporation, Tokyo, Japan). The specific ERND activity was calculated using the standard calibration curve of formaldehyde.

3.3.8 Determination of Total Glutathione S-transferase Enzyme Activity of Rat Liver Cytosols

Glutathione S-transferases (GSTs) are members of phase II enzymes and takes part in detoxification reactions of some endogenous and exogenous compounds. GSTs catalyze the conjugation of the sulphydryl group of GSH and an electrophilic atom of a xenobiotic compound. These conjugation reactions results in the formation of less toxic products and water-soluble compouds which are readily excreted. Total glutathione S-transferase (GST) activities of rat liver cytosols were determined according to the method of Habig *et al.* (1974). The principle of the method is based on the formation of 1-glutathione-2,4-dinitrobenzene (DNB_SG) complex from the conjugation reaction between 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) catalyzed by GSTs (Figure 3.9).

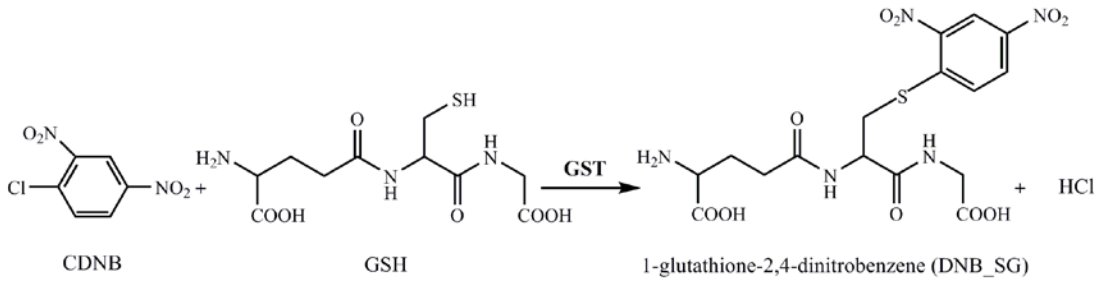


Figure 3.9. Total glutathione S-transferase reaction.

Assay conditions were optimized for rat liver microsomes by testing different substrate concentrations, enzyme concentrations, cofactor concentrations, pH, and temperature. GST activities of each cytosol were measured in duplicates and sometimes in triplicates at optimum conditions. A typical reaction mixture contained 2.50 mL of 100 mM potassium phosphate buffer, pH 7.5, 0.20 mL of 30 mM GSH, 0.15 mL enzyme source (100-200 fold diluted cytosols with 10 mM pH 7.5 phosphate buffer). The reaction was initiated by the addition of 0.15 mL of 20 mM CDNB into reaction medium and the rate of reaction was followed against a blank

cuvette containing potassium phosphate buffer, pH 7.5 instead of enzyme source by measuring the formation of 1-glutathione-2,4-dinitrobenzene (DNB_SG) complex spectrophotometrically at 340 nm for 2 minutes (Jasco V-530 UV/VIS Spectrophotometer, Jasco International Corporation, Tokyo, Japan). The specific total GST activity was calculated as nmole 1-glutathione-2,4-dinitrobenzene conjugate formed per milligram protein in one minute at 25 $^{\circ}$C, using 9.6 mM $^{-1}$ cm $^{-1}$ as an extinction coefficient (ε_{340}).

3.3.9 Determination of Catalase Enzyme Activity of Rat Liver Cytosols

Catalase (CAT) is an oxidative stress biomarker enzyme and has a role in the detoxification of hydrogen peroxide to water. Catalase activities of rat liver cytosols were determined according to the method of Aebi (1984). Catalase activity measurements provide information about the existence of the oxidative stress. The principle of the method is based on the decomposition of H_2O_2 to water and oxygen in the presence of catalase enzyme (Figure 3.10).

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

Figure 3.10. Catalase reaction.

Assay conditions were optimized for rat liver microsomes by testing different substrate concentrations, enzyme concentrations, cofactor concentrations, pH, and temperature. Catalase activities of each cytosol were measured in duplicates and sometimes in triplicates at optimum conditions. In a typical enzyme activity measurement, rat liver cytosol was pretreated with 1% Triton X-100 for 10 minutes (1:10 dilution). Then the mixture was diluted further to make a total dilution of 5000 or 6000 with 50 mM phosphate buffer, pH 7.4. A typical reaction mixture contained 2.0 mL of 5000 or 6000 times diluted rat liver cytosol in 50 mM phosphate buffer, pH 7.4 and 40 mM H_2O_2 H_2O_2 (prepared in 50 mM phosphate buffer, pH 7.4) in a final volume of 3.0 mL. The reaction was initiated by the addition of H_2O_2 and the rate of reaction was followed against a blank cuvette containing potassium phosphate buffer, pH 7.4 instead of substrate at 240 nm for 1 minute (Jasco V-530 UV/VIS

Spectrophotometer, Jasco International Corporation, Tokyo, Japan). The specific catalase activity was calculated as µmole H_2O_2 consumed per milligram protein in one minute at 25 °C using 0.0364 mM^{-1} cm^{-1} as an extinction coefficient (ε_{240}).

3.3.10 Determination of Glutathione Reductase Enzyme Activity of Rat Liver Cytosols

Glutathione reductase (GR) is another oxidative stress biomarker enzyme which reduces glutathione disulfide (GSSG) to the sulfhydryl form of glutathione (GSH) by NADPH. The determination of glutathione reductase activity also provides information about the existence of oxidative stress. Glutathione reductase activity of rat liver cytosols was determined according to the method of Carlberg and Mannervick (1985). The principle of the method is based on the NADPH-dependent reduction of oxidized glutathione (GSSG) to glutathione (GSH) in the presence of glutathione reductase (Figure 3.11).

$$ROH + GSSG + NADPH + H^+ \xrightarrow{GR} ROOH + 2GSH + NADP^+$$

Figure 3.11. Glutathione reductase reaction.

Assay conditions were optimized for rat liver microsomes by testing different substrate concentrations, enzyme concentrations, cofactor concentrations, pH, and temperature. Glutathione reductase activities of each cytosol were measured in duplicates at optimum conditions. A typical reaction mixture contained 0.1 M potassium phosphate buffer, pH 7.5, 0.5 mM EDTA, pH 7.5, 0.1 mM NADPH, 0.020 mL enzyme (2 fold diluted cytosol), 1.0 mM oxidized glutathione (GSSG) and distilled water in a final volume of 2.0 mL. The reaction was initiated by the addition of oxidized glutathione (GSSG) and the reduced glutathione (GSH) formation was followed against a blank cuvette containing distilled water in place of substrate GSSG. The consumption of NADPH was followed spectrophotometrically at 340 nm for 5 minutes (Jasco V-530 UV/VIS Spectrophotometer, Jasco International Corporation, Tokyo, Japan). The specific enzyme activity was calculated as nmole

NADPH oxidized per milligram protein in one minute at 25 $^{\circ}$C, using 6.22 $\mu M^{-1} cm^{-1}$ as an extinction coefficient (ε_{340}).

3.3.11 Western Blot Analysis

Cytochrome P450s of rat liver microsomes were determined at the protein level by western blot analysis. Monoclonal anti-rat CYP1A1, monoclonal anti-rat CYP2B1/2BC.B/C4, polyclonal anti-rat CYP2E1, and monoclonal anti-rat CYP3A4/3A1/3A11 (Millipore, Billera, MA, USA) were used as primary antibodies.

The first step of this method is the separation of microsomal proteins by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS), in a discontinuous buffer system as described by Laemmli (1970). The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by using 4% stacking gel and 8.5% separating gel. These gel solutions were prepared just before use. The stacking gel consisted of 1.3 mL of gel solution (29.2% acrylamide containing 0.8% N,N'-methylene bisacrylamide solution), 6.1 mL of distilled water, 2.5 mL of stacking gel buffer (0.5 M Tris-HCl, pH 6.8), 0.1 mL of 10% SDS solution, 0.05 mL of 10% ammonium persulfate solution and 0.01 mL of N, N, N', N' tetrametylethylene diamine (TEMED). The separating gel consisted of 8.5 mL of gel solution (29.2% acrylamide containing 0.8% N,N'-methylene bisacrylamide solution), 13.55 mL of distilled water, 7.5 mL of separating gel buffer (1.5 M Tris-HCl, pH 8.8), 0.3 mL of 10% SDS solution, 0.15 mL of 10% ammonium persulfate solution and 0.015 mL of N, N, N', N' tetramethylethylene diamine (TEMED). Gels were polymerized between two glass plates in central running module (SDS-PAGE setup). First separating gel solution was prepared and immediately transferred into the space between glass plates up to the desired height. Before the polymerization has started, the surface of this solution was covered by the addition of 2-methylpropanol in order to produce smooth surface on top of the separating gel, so that bands are straight and uniform. After polymerization, the aqueous layer above the separating gel was poured off completely. Then stacking gel solution was prepared and immediately transferred over the separating gel and comb was placed between glass plates. After polymerization of stacking gel, comb was

removed and wells were rinsed with ten times diluted electrode running buffer (0.25 M Tris, 1.92 M Glycine) containing SDS (1.0 gram per 1.0 liter solution). The prepared gel set up was placed in outer buffer chamber (Scie-Plas Mini Electro-Blotting Unit) including appropriate amount of ten times diluted electrode running buffer (0.25 M Tris, 1.92 M Glycine) containing SDS (1.0 gram per 1.0 liter solution).

After preparing gel setup, liver microsomes were diluted 1:3 (3 part sample and 1 part buffer) with 4× sample dilution buffer containing 0.25 M Tris-HCl, pH 6.8, 8% SDS, 40% glycerol, 20% β-mercaptoethanol, and 0.01% bromophenol blue. The sample containing tubes were boiled in a boiling water bath for 2 minutes exactly. Then each sample was loaded into the corresponding well with the help of a Hamilton syringe. In western blot analysis of CYP1A, CYP2B, CYP2E, and CYP3A, all sample wells were loaded with 120 µg, 120 µg, 80 µg, and 160 µg microsomal protein, respectively.

After application of the samples, outer buffer chamber was connected to the power supply (Elite 200, Wealtec Corp., Sparks, NV, USA) for separation of proteins in an electrical field and electrophoresis was run at 10 mA constant current and corresponding voltage. When electrophoresis was completed, gel was removed from the outer buffer chamber for western blot analysis which was carried out as described by Towbin et al. (1979) with some modifications. In order to adjust the final size of gel and remove the buffer salts and SDS used in the SDS-PAGE, gel was equilibrated in transfer buffer (25 mM Tris, 192 mM glycine and 20% methanol) for 15 minutes with constant shaking. Nitrocellulose membrane was cut 1 cm larger then the dimension of the gel and two pieces of filter paper (Whatman #1) were cut 1 cm larger then the dimension of the membrane. Before the preparation of transfer sandwich, nitrocellulose membrane, two filter papers and fiber pads of the transfer sandwich were placed in transfer buffer and saturated with transfer buffer solution. Western blot sandwich was prepared by placing the transfer sandwich materials in the order of compression cassette, two fibre pads, filter paper, gel, nitrocellulose membrane, filter paper, two fibre pads, and compression cassette. In this step, all the transfer sandwich materials should be overlapped each other and air bubbles between gel and membrane should be removed in order to transfer all proteins from gel

through the membrane. Then, the sandwich was put into the outer buffer chamber (Scie-Plas Mini Electro-Blotting Unit) in such a way that the gel was toward the negative electrode and membrane toward the positive electrode. The outer buffer chamber was filled with cold transfer buffer and connected to the power supply. Voltage was set to constant 90V and transfer process was carried out in the cold room at 4°C for 90 minutes.

At the end of the transfer process, the membrane having the transferred protein on it, i.e. “blot” was obtained. The blot was taken from the outer buffer chamber, placed into a plastic dish in such a way that protein side facing up and washed with Tris Buffered Saline plus Tween 20 solution (TBST; 20 mM Tris-HCl, pH 7.4, 0.5 M NaCl, and 0.05 % Tween 20) for 10 minutes with constant shaking in order to remove the salts and buffers of transfer medium. Then the blot was incubated with blocking solution (5% Non-Fat Dry-Milk in TBST) for 40 minutes with constant shaking in order to fill the empty spaces between bound proteins. This step prevents the non-specific binding of antibodies on the membrane. In the western blot analysis of CYP1A, CYP2B and CYP3A, the blot was incubated with primary antibody for 2 hours. In the western blot analysis of CYP2E, the blot was incubated with primary antibody for 1 hour with constant shaking. As primary antibody, 500 times diluted monoclonal anti-rat CYP1A1, monoclonal anti-rat CYP2B1/2BC.B/C4, polyclonal anti-rat CYP2E1, and monoclonal anti-rat CYP3A4/3A1/3A11 were used. After incubation of blot in primary antibody, the blot was washed 3 times with 100 mL TBST for 5 minutes each to remove excess primary antibody from the membrane. The blot was then incubated with secondary antibody conjugated with marker enzyme-alkaline phosphatase (anti-rabbit IgG-ALP conjugate, 1:10000 ratio) for 1 hour with constant shaking. The blot was washed three times with TBST for 5 minutes each to remove excess secondary antibody from the membrane. The removal of the excess antibody prevents the non-specific reaction between excess antibody and substrate solution. Finally, blot was incubated with nitro blue tetrazolium/5-bromo-4-chloro-3-indolylphosphate (NBT/BCIP) substrate solution as described by Ey and Ashman (1986) to visualize the specifically bound antibodies. NBT/BCIP substrate solution was freshly prepared from three solutions-solution A, solution B, and solution C. Solution A contained 2.67 mL of 1.5 M Tris-HCl, pH 8.8, 4.0 mL of 1 M NaCl, 0.82 mL of 100 mM $MgCl_2$, 0.04 mL of 100 mM $ZnCl_2$, 0.096 mL of

diethanolamine, 12.2 mg NBT, and distilled water in a final volume of 40 mL. The pH of the solution was adjusted to 9.55 with saturated Tris before completing to final volume. Solution B was 2.0 mg/mL phenazine methosulfate solution. Solution C was prepared by dissolving 5.44 mg BCIP in 0.136 mL N, N-dimethyl formamide. Finally NBT/BCIP substrate solution was prepared by mixing first solution A with solution C and then 0.268 mL of solution B. The blot was incubated with this solution until the bands appeared. Then the solution was discarded and blot was washed with distilled water. Finally, blot was dried under air, covered by filter paper and stored at dark. The final images of the blots were photographed using computer based gel imaging instrument (MiniLumi, DNR Bio-Imaging Systems Ltd, Jerusalem, Israel), and densities of bands was analyzed using The Scion Image Software for Windows (Version 4.0.2, Scion Corporation, Maryland, USA) as a quantitative tool to determine cytochrome P450 protein levels.

3.3.12 Histopathological studies

After dissecting, a small piece of the tissue was cut from each liver and lung tissues and stored in the small bottles including 10% formaldehyde solution for histopathological studies. The tissue sections were taken from the same region of liver and lung. In order to obtain microscopic sections, the tissues were embedded in paraffin. Firstly, paraffin blocks were prepared. Tissues fixed in 10% formaldehyde solution were rinsed in running water for 3-4 hours. In order to remove water, tissues were dehydrated by passing them through a series of increasing alcohol concentrations. Dehydration process were carried out by incubating the tissues in 70% ethanol solution for 2 hours, in 80% ethanol solution for 2 hours, in 96% ethanol solution for 1 hour, in 96% ethanol solution for 1 hour, in 100% ethanol solution for 1 hour, and in 100% ethanol solution for 1 hour, respectively. Ethanol and paraffin are immiscible and the presence of ethanol inhibits the infiltration of paraffin into the tissues. Therefore a subsequent step was required in order to remove alcohol, which was called clearing. For this purpose, dehydrated tissues were cleared by placing them in xylene for 1 hour and this step was repeated one more time. The clearing step was followed by infiltration of paraffin. Firstly, tissues were incubated with 1:1 xylene-paraffin mixture for 30 minutes at 64 $^{\circ}$C in oven. Then they were

incubated with paraffin for 1 hour at 64 °C in oven and this step was repeated one more time. After that rubber moulds were filled with molten paraffin and tissue samples thoroughly infiltrated with paraffin were embedded into the molten paraffin. They were cooled in refrigerator and blocks were prepared. The prepared blocks were sectioned at 4-5 μm thickness by using a Leica RM 2125 RT (Leica, Nussloch, Germany) rotary microtome. Sections were stained with hematoxylin and eosin in order to assess general histopathological changes. First of all, these sections were deparaffinized for staining. Before deparaffinization, sections were incubated at 64 °C in oven for 1 hour. After that they were incubated in xylene for 2 minutes and this step was repeated one more time. Then they were incubated in 100% ethanol for 1 minute and this step was repeated one more time. The sections were incubated in 96% ethanol for 1 minute and this step was repeated one more time. After these steps, the sections were rinsed under running water, incubated in hematoxylin for 3 minutes, and rinsed under running water, respectively. The washed tissue sections were inserted in acid-ethanol mixture for two times. Then they were rinsed under running water, incubated in diluted ammonia for 10-15 seconds, rinsed under running water, and incubated in 80% ethanol solution for 1 minute, respectively. These sections were incubated in eosin for 1 minute. After that they were incubated in 96% ethanol for 2 minutes and this step was repeated one more time. The sections were incubated in 100% ethanol for 2 minutes and this step was repeated one more time. Finally, the sections were incubated in xylene for 10 minutes. They were examined and photographed by using BH2 Olympus photomicroscope (Olympus, Tokyo, Japan).

2.3.12. Statistical Analysis

All data were expressed as mean ± standard error of mean in the tables. Data were tested for normal distribution using the Shapiro-Wilk test. The parametric data were analyzed by one-way analysis of variance (ANOVA) statistically. When significant differences were observed, Tukey HSD post-hoc test was applied. The non-parametric data were analyzed by Kruskal-Wallis H test. The analyses were carried out using the SPSS statistical package (SPSS Statistics 17.0).

4. RESULTS

In this study, 120 adult male Wistar rats were used to determine the effects of chronic exposure of 7,12-dimethylbenz[a]anthracene (DMBA), endosulfan, and morin on healthy (group 1-8) and diabetic rats (group 9-16). DMBA, endosulfan and morin were given separately or in double or triple combinations to rats by oral gavage and diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin as described in "Materials and Methods" section. Each experimental group of healthy rats (group 1-8) included seven animals and each experimental group of diabetic rats (group 9-16) included eight animals at the beginning of the chemical treatments. There were no animal deaths during the chemical treatment studies of healthy rats. The total number of healthy rats on which experiments were performed was 56. The total number of diabetic rats (group 9-16) at the beginning of the animal studies was 62. Animal deaths were observed in streptozotocin induced diabetic rats (group 9-16). These deaths started after streptozotocin induction and continued though the study. The total number of diabetic rats on which experiments were performed was 26.

The effects of DMBA, endosulfan, and morin on healthy and diabetic rats were determined by phase I (7-ethoxyresorufin O-deethylase (CYP1A1), 7-methoxyresorufin O-demethylase (CYP1A2), 7-pentoxyresorufin O-dependentylase (CYP2B), aniline 4-hydroxylase (CYP2E) and erythromycin N-demethylase (CYP3A)), a phase II (glutathione S-transferase), and oxidative stress biomarker (catalase and glutathione reductase) enzyme activities.

4.1 Effects of 7,12-Dimethylbenz[a]anthracene, Endosulfan, and Morin Treatments on Liver Enzyme Activities of Healthy and Diabetic Rats

4.1.1 7-Ethoxyresorufin O-Deethylase Activity of Rat Liver Microsomes

Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase (EROD) activities were measured in each rat liver microsomes and the specific EROD activities were calculated as pmole resorufin formed per milligram protein in one minute at 37 $^{\circ}$C, using the fluorescence increase caused by the addition of resorufin. EROD activities of groups were expressed as average EROD activity±standard error of mean (SEM).

The average specific 7-ethoxyresorufin O-deethylase activity of rats in control group (group 1) was 71±7 pmol/min/mg protein (N=7) (Table 4.1). Rats in morin group (group 3) had 1.6-fold higher EROD activities than those in control group and the average of specific EROD activity measured in this group was found to be 112±6 pmol/min/mg protein (Table 4.3). The average EROD activity of rats in endosulfan+morin (group 4) group was 121±9 pmol/min/mg protein (N=7) (Table 4.4). This activity was 1.7-fold higher than the average EROD activity of rats in control group and this increase was statistically significant ($p<0.05$). DMBA treatment caused a significant 1.8-fold increase in EROD activity in rat liver microsomes ($p<0.05$). The average EROD activity of DMBA-treated group (group 5) was 126±6 pmol/min/mg protein (N=7) (Table 4.5). The significantly different results were also observed in DMBA+morin (group 6) and DMBA+endosulfan (group 7) groups ($p<0.05$). The average EROD activity obtained from rats in DMBA+morin group was 156±12 pmol/min/mg protein (N=7) (Table 4.6) and that in DMBA+endosulfan group was 151±15 pmol/min/mg protein (N=7) (Table 4.7). These activities were 2.2- and 2.1-fold greater than the average EROD activity obtained from control group, respectively. The highest EROD activities measured in liver microsomes of healthy rats were obtained from DMBA+endosulfan+morin group (group 8), which was 185±13 pmol/min/mg protein (N=7) (Table 4.8). This

activity was significantly higher (2.6-fold) than the activity obtained from the control group ($p<0.05$). Figure 4.1 illustrates EROD activity results in healthy rats.

Table 4.1. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from control group (group 1).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	27	96
2	25	62
3	29	62
4	22	41
5	32	62
6	21	83
7	22	88
Average Activity±SEM N=7		71±7

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.2. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from endosulfan group (group 2).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	26	126
2	18	139
3	28	103
4	31	120
5	29	71
6	34	111
7	23	129
Average Activity±SEM N=7		114±8

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.3. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from morin group (group 3).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	27	97
2	26	97
3	24	120
4	20	118
5	24	105
6	26	103
7	24	142
Average Activity±SEM N=7		112±6

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.4. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from endosulfan+morin group (group 4).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	29	93
2	27	115
3	28	98
4	28	162
5	29	133
6	30	116
7	27	131
Average Activity±SEM N=7		121±9

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.5. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene group (group 5).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	18	111
2	20	154
3	18	125
4	20	113
5	16	135
6	20	127
7	19	116
Average Activity±SEM N=7		126±6

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.6. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+morin group (group 6).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	18	166
2	15	139
3	20	111
4	14	209
5	18	179
6	15	138
7	18	153
Average Activity±SEM N=7		156±12

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.7. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+endosulfan group (group 7).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	17	137
2	16	110
3	21	174
4	17	186
5	20	215
6	23	116
7	23	122
Average Activity±SEM N=7		151±15

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.8. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 8).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	21	195
2	25	192
3	22	217
4	28	174
5	20	224
6	24	177
7	24	118
Average Activity±SEM N= 7		185±13

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

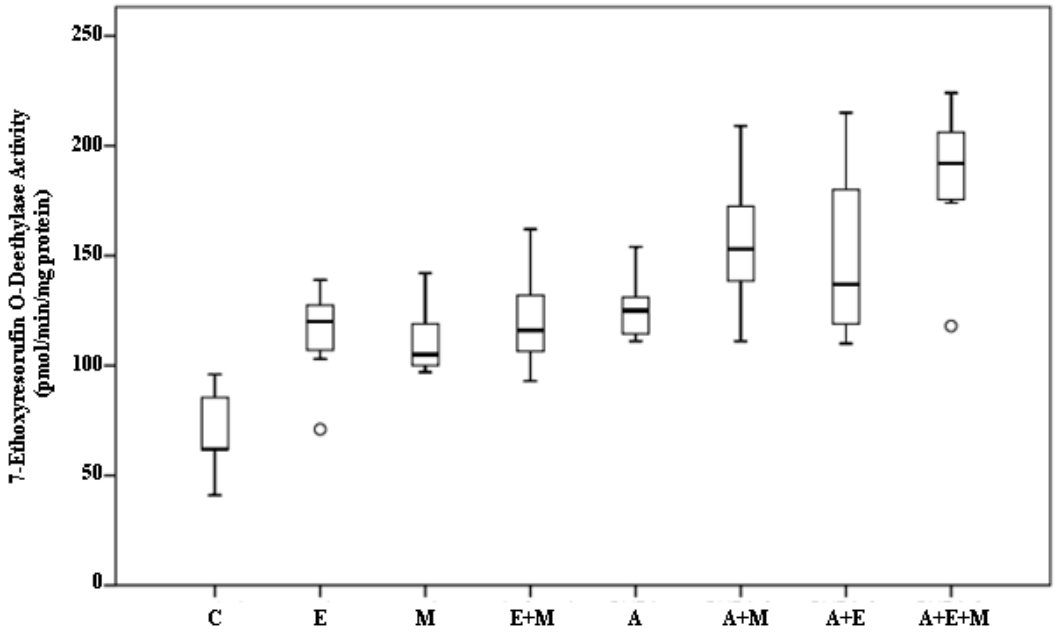


Figure 4.1. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities of rat liver microsomes obtained from group 1-8. C (Control), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

The induction of the diabetes mellitus by intraperitoneal injection of streptozotocin significantly increased EROD activity of rat liver microsomes by 3.4-fold compared to the control group ($p<0.05$). The average EROD activities measured in diabetes mellitus group (group 9) was 245±22 pmol/min/mg protein (N=5) (Table 4.9). The average of EROD activities obtained from rats in diabetes mellitus+endosulfan group (group 10) was 292±22 pmol/min/mg protein (N=7) (Table 4.10) and that in diabetes mellitus+morin group (group 11) was 272±12 pmol/min/mg protein (N=3) (Table 4.11). These activities were 1.2- and 1.1-fold greater than the average EROD activity obtained from the diabetes mellitus group, respectively. Rats in diabetes mellitus+endosulfan+morin group (group 12) had 243 pmol/min/mg protein (N=2) average enzyme activity (Table 4.12). The average specific EROD activity of rats in diabetes mellitus+DMBA group (group 13) was 346±20 pmol/min/mg protein (N=3) (Table 4.13). Rats in diabetes mellitus+DMBA group had 1.4-fold higher EROD activities than those in diabetes mellitus group, however, this difference was not statistically significant ($p<0.05$). The average enzyme activities of rat liver microsomes obtained from diabetes mellitus+DMBA+morin (group 14), diabetes mellitus+DMBA+endosulfan (group

15), and diabetes mellitus+DMBA+endosulfan+morin (group 16) groups were 202±26 pmol/min/mg protein (N=3), 198 pmol/min/mg protein (N=2), and 168 pmol/min/mg protein (N=1), respectively (Table 4.14, 3.15, and 3.16). These results showed that co-administration of DMBA+morin, DMBA+endosulfan, and DMBA+endosulfan+morin to diabetic rats caused a decrease in EROD activities of rat liver microsomes compared to the diabetes mellitus group. Figure 4.2 illustrates EROD activity results in diabetic rats.

Table 4.9. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from diabetes mellitus group (group 9).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	14	308
2	15	270
3	23	206
4	17	255
5	21	187
Average Activity±SEM N=5		245±22

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.10. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from diabetes mellitus+endosulfan group (group 10).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	20	279
2	21	289
3	18	246
4	17	329
5	19	199
6	19	331
7	21	368
Average Activity±SEM N=7		292±22

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.11. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from diabetes mellitus+morin group (group 11).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	18	297
2	20	257
3	18	263
Average Activity±SEM N=3		272±12

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.12. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from diabetes mellitus+endosulfan+morin group (group 12).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	14	252
2	22	233
Average Activity N=2		243

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.13. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene group (group 13).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	22	355
2	25	307
3	20	375
Average Activity±SEM N=3		346±20

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.14. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from diabetes mellitus, 7,12-dimethylbenz[a]anthracene+morin group (group 14).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	15	190
2	17	163
3	15	252
Average Activity±SEM N=3		202±26

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.15. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan group (group 15).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	15	158
2	19	238
Average Activity N=2		198

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.16. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 16).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	18	168

Each microsome fraction was prepared from single rat liver.

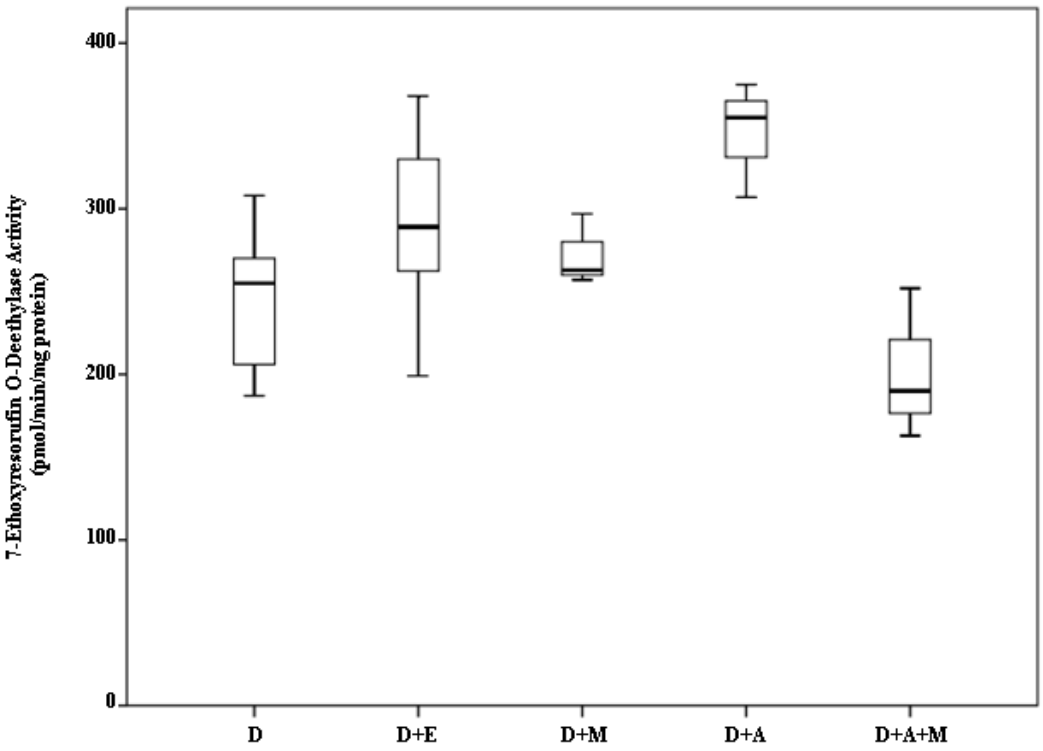


Figure 4.2. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities of rat liver microsomes obtained from group 9-16). D (Diabetes mellitus), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

4.1.2 7-Methoxyresorufin O-Demethylase Activity of Rat Liver Microsomes

Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase (MROD) activities were measured in each rat liver microsomes and the specific MROD activities were calculated as pmole resorufin formed per milligram protein in one minute at 37 ºC.

The average specific 7-methoxyresorufin O-demethylase activity of rats in control group (group 1) was 34.0±0.7 pmol/min/mg protein (N=7) (Table 4.17). The average MROD activities of rats in endosulfan (group 2) and endosulfan+morin (group 4) groups were similar to control group and found to be 36.0±4.3 pmol/min/mg protein (N=7) and 33.8±3.9 pmol/min/mg protein (N=7), respectively (Table 4.18 and 3.20). The average MROD activity obtained from rats in morin group (group 3) was 41.4±1.2 pmol/min/mg protein (N=7) (Table 4.19). Therefore, there were no significant differences in the enzyme activities measured in liver microsomes of endosulfan, morin, and endosulfan+morin groups compared with that of the control group. Rats in DMBA group (group 5) had 1.5-fold higher MROD activities than those of the control group. The average of specific MROD activity obtained from this group was 52.1±1.9 pmol/min/mg protein (Table 4.21), which was significantly different from the average enzyme activity of the control group ($p<0.05$). Moreover, significant differences were observed in the average MROD activities of rats treated with DMBA+morin and DMBA+endosulfan+morin compared to rats in the control group ($p<0.05$). The enzyme activities of rats in DMBA+morin (group 6) and DMBA+endosulfan+morin (group 8) groups were similar to each other and were found to be 58.8±4.1 pmol/min/mg protein (N=7) and 58.1±2.4 pmol/min/mg protein (N=7), respectively (Table 4.22 and 3.24). The average MROD activities of rats in these groups were 1.7-fold higher than that in the control group. The highest MROD activities measured in liver microsomes of healthy rats were obtained from DMBA+endosulfan group (group 7), which was 68.6±6.3 pmol/min/mg protein (N=7) (Table 4.23). Co-administration of DMBA and endosulfan caused significant 1.8-fold increase in the average MROD activity of rat liver microsomes ($p<0.05$). Figure 4.3 illustrates MROD activity results in healthy rats.

Table 4.17. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from control group (group 1).

Sample Number	Average Activity (pmol/min/mg protein)
1	33.4
2	36.8
3	32.3
4	31.8
5	33.8
6	35.0
7	35.1
Average Activity±SEM N= 7	34.0±0.7

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.18. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from endosulfan group (group 2).

Sample Number	Average Activity (pmol/min/mg protein)
1	34.1
2	51.9
3	33.4
4	37.2
5	25.2
6	20.8
7	49.4
Average Activity±SEM N= 7	36.0±4.3

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.192. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from morin group (group 3).

Sample Number	Average Activity (pmol/min/mg protein)
1	37.2
2	40.1
3	42.3
4	40.4
5	42.3
6	40.4
7	47.3
Average Activity±SEM N= 7	41.4±1.2

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.20. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from endosulfan+morin group (group 4).

Sample Number	Average Activity (pmol/min/mg protein)
1	27.6
2	25.5
3	27.9
4	30.0
5	30.0
6	53.4
7	42.5
Average Activity±SEM N= 7	33.8±3.9

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.21. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene group (group 5).

Sample Number	Average Activity (pmol/min/mg protein)
1	52.6
2	53.7
3	43.0
4	48.3
5	53.2
6	56.7
7	57.1
Average Activity±SEM N= 7	52.1±1.9

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.22. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+morin group (group 6).

Sample Number	Average Activity (pmol/min/mg protein)
1	70.9
2	59.0
3	57.1
4	53.4
5	68.8
6	63.9
7	38.7
Average Activity±SEM N= 7	58.8±4.1

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.23. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+endosulfan group (group 7).

Sample Number	Average Activity (pmol/min/mg protein)
1	73.5
2	51.5
3	72.5
4	79.1
5	77.4
6	86.6
7	39.8
Average Activity±SEM N= 7	68.6±6.3

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.24. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 8).

Sample Number	Average Activity (pmol/min/mg protein)
1	66.7
2	64.8
3	60.0
4	55.6
5	58.8
6	49.8
7	51.1
Average Activity±SEM N= 7	58.1±2.4

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

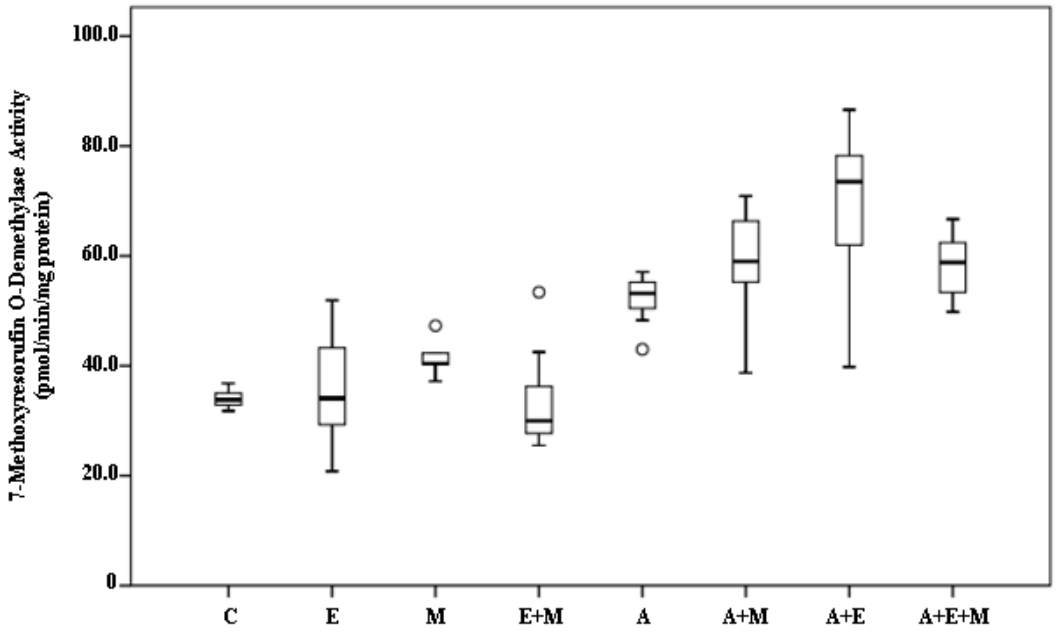


Figure 4.3. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from group 1-8. C (Control), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

The average specific 7-methoxyresorufin O-demethylase activity of rats in diabetes mellitus group (group 9) was 59.4±4.5 pmol/min/mg protein (N=5) (Table 4.25). The intraperitoneal injection of streptozotocin significantly increased MROD activity of rat liver microsomes by 1.7-fold compared to the control group ($p<0.05$). The average MROD activities obtained from rats in diabetes mellitus+endosulfan (group 10), diabetes mellitus+morin (group 11), diabetes mellitus+endosulfan+morin (group 12), and diabetes mellitus+DMBA (group 13) groups were 84.9±11.3 pmol/min/mg protein (N=7), 77.5±10.6 pmol/min/mg protein (N=3), 69.9 pmol/min/mg protein (N=2), and 85.6±3.7 pmol/min/mg protein (N=3), respectively (Table 4.26, 3.27, 3.28, and 3.29). The chemical treatments carried out to these groups increased the enzyme activities of rat liver microsomes by 1.4-fold in diabetes mellitus+endosulfan and diabetes mellitus+DMBA groups, 1.3-fold in diabetes mellitus+morin group, and 1.2-fold in diabetes mellitus+endosulfan+morin group. According to results obtained from these groups, these increases were not statistically significant ($p<0.05$). The administrations of DMBA+morin, DMBA+endosulfan, and DMBA+endosulfan+morin decreased MROD activity in diabetic rats. The average enzyme activities of rat liver microsomes obtained from

diabetes mellitus+DMBA+morin (group 14), diabetes mellitus+DMBA+endosulfan (group 15), and diabetes mellitus+DMBA+endosulfan+morin (group 16) groups were 42.8±4.1 pmol/min/mg protein (N=3), 46.0 pmol/min/mg protein (N=2), and 29.7 pmol/min/mg protein (N=1) (Table 4.30, 3.31, and 3.32), respectively. Figure 4.4 illustrates MROD activity results in diabetic rats.

Table 4.25. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from diabetes mellitus group (group 9).

Sample Number	Average Activity (pmol/min/mg protein)
1	75.9
2	61.8
3	52.1
4	56.2
5	50.9
Average Activity±SEM N= 5	59.4±4.5

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.26. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from diabetes mellitus+endosulfan group (group 10).

Sample Number	Average Activity (pmol/min/mg protein)
1	83.0
2	85.4
3	50.7
4	106.8
5	41.9
6	102.2
7	124.1
Average Activity±SEM N= 7	84.9±11.3

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.27. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from diabetes mellitus+morin group (group 11).

Sample Number	Average Activity (pmol/min/mg protein)
1	97.9
2	72.4
3	62.2
Average Activity±SEM N=3	77.5±10.6

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.28. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from diabetes mellitus+endosulfan+morin group (group 12).

Sample Number	Average Activity (pmol/min/mg protein)
1	70.8
2	69.0
Average Activity N=2	69.9

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.29. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene group (group 13).

Sample Number	Average Activity (pmol/min/mg protein)
1	91.7
2	78.9
3	86.1
Average Activity±SEM N=3	85.6±3.7

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.30. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from diabetes mellitus, 7,12-dimethylbenz[a]anthracene+morin group (group 14).

Sample Number	Average Activity (pmol/min/mg protein)
1	50.4
2	36.2
3	41.7
Average Activity±SEM N=3	42.8±4.1

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.31. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan group (group 15).

Sample Number	Average Activity (pmol/min/mg protein)
1	43.0
2	48.9
Average Activity N=2	46.0

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.32. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 16).

Sample Number	Average Activity (pmol/min/mg protein)
1	29.7

Each microsome fraction was prepared from single rat liver.

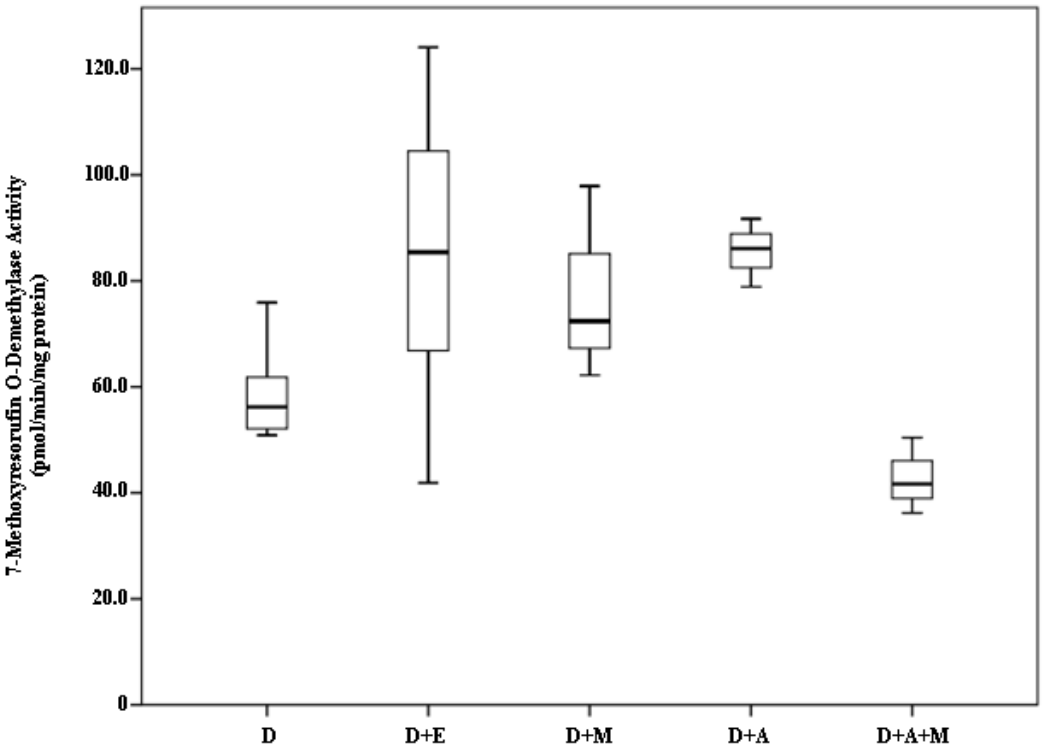


Figure 4.4. Cytochrome P4501A2 associated 7-methoxyresorufin O-demethylase activities of rat liver microsomes obtained from group 9-16. D (Diabetes mellitus), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

4.1.3 7-Pentoxyresorufin O-Depentylase Activity of Rat Liver Microsomes

Cytochrome P4502B associated 7-pentoxyresorufin O-depentylase (PROD) activities were measured in each rat liver microsomes and the specific PROD activities were expressed as pmole resorufin formed per milligram protein in one minute at 37 ºC.

The average specific 7-pentoxyresorufin O-depentylase activity of rats in the control group (group 1) was 18.0±1.0 pmol/min/mg protein (N=7) (Table 4.33). The administrations of endosulfan, DMBA, and DMBA+endosulfan slightly decreased PROD activity in healthy rats. The average PROD activities obtained from endosulfan (group 2), DMBA (group 5), and DMBA+ endosulfan (group 7) groups were 15.3±0.9 pmol/min/mg protein (N=7), 16.9±0.7 pmol/min/mg protein (N=7), and 16.4±1.4 pmol/min/mg protein (N=7), respectively (Table 4.34, 3.37, and 3.39). The average PROD activities of rats in morin (group 3) and DMBA+morin (group 6) groups were similar to rats in the control group and found to be 17.6±0.8 pmol/min/mg protein (N=7) and 18.5±1.0 pmol/min/mg protein (N=7), respectively (Table 4.35 and 3.38). The administrations of endosulfan+morin and DMBA+endosulfan+morin slightly increased PROD activities in healthy rats. The average PROD activities obtained from rats in endosulfan+morin (group 4) and DMBA+endosulfan+morin (group 8) were 19.1±1.2 pmol/min/mg protein (N=7) and 19.8±0.8 pmol/min/mg protein (N=7) (Table 4.36 and 3.40). The PROD activities of rats in DMBA+ endosulfan+morin group was significantly different from endosulfan group. Figure 4.5 illustrates PROD activity results in healthy rats.

Table 4.33. Cytochrome P4502B associated 7-pentoxyresorufin O-dependentylase activities of rat liver microsomes obtained from control group (group 1).

Sample Number	Average Activity (pmol/min/mg protein)
1	18.1
2	17.6
3	16.3
4	21.4
5	17.7
6	13.9
7	21.3
Average Activity±SEM N=7	18.0±1.0

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.34. Cytochrome P4502B associated 7-pentoxyresorufin O-dependentylase activities of rat liver microsomes obtained from endosulfan group (group 2).

Sample Number	Average Activity (pmol/min/mg protein)
1	13.9
2	15.8
3	15.6
4	13.3
5	13.3
6	15.5
7	19.8
Average Activity±SEM N=7	15.3±0.9

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.35. Cytochrome P4502B associated 7-pentoxyresorufin O-dependentylase activities of rat liver microsomes obtained from morin group (group 3).

Sample Number	Average Activity (pmol/min/mg protein)
1	15.0
2	17.0
3	17.6
4	18.7
5	19.1
6	15.1
7	20.5
Average Activity±SEM N=7	17.6±0.8

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.36. Cytochrome P4502B associated 7-pentoxyresorufin O-depentylase activities of rat liver microsomes obtained from endosulfan+morin group (group 4).

Sample Number	Average Activity (pmol/min/mg protein)
1	16.9
2	16.9
3	16.5
4	19.5
5	17.3
6	23.3
7	23.6
Average Activity±SEM N=7	19.1±1.2

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.37. Cytochrome P4502B associated 7-pentoxyresorufin O-depentylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene group (group 5).

Sample Number	Average Activity (pmol/min/mg protein)
1	17.4
2	20.2
3	18.0
4	15.7
5	15.7
6	15.7
7	15.7
Average Activity±SEM N=7	16.9±0.7

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.38. Cytochrome P4502B associated 7-pentoxyresorufin O-dependentylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+morin group (group 6).

Sample Number	Average Activity (pmol/min/mg protein)
1	19.0
2	19.2
3	14.2
4	20.6
5	22.2
6	16.9
7	17.6
Average Activity±SEM N=7	18.5±1.0

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.39. Cytochrome P4502B associated 7-pentoxyresorufin O-dependentylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+endosulfan group (group 7).

Sample Number	Average Activity (pmol/min/mg protein)
1	21.4
2	13.7
3	16.6
4	12.7
5	20.5
6	17.3
7	12.6
Average Activity±SEM N=7	16.4±1.4

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.40. Cytochrome P4502B associated 7-pentoxyresorufin O-dependentylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 8).

Sample Number	Average Activity (pmol/min/mg protein)
1	20.8
2	18.8
3	23.3
4	18.3
5	17.4
6	21.2
7	19.1
Average Activity±SEM N=7	19.8±0.8

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

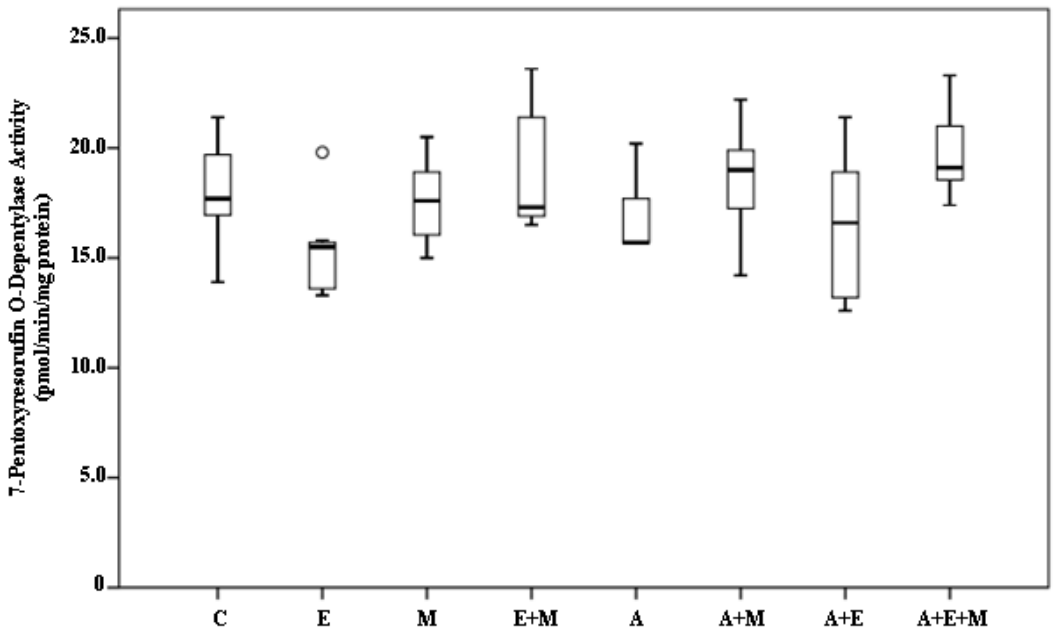


Figure 4.5. Cytochrome P4502B associated 7-pentoxyresorufin O-dependentylase activities of rat liver microsomes obtained from group 1-8. C (Control), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

The average specific 7-pentoxyresorufin O-depentylase activity of rats in diabetes mellitus group (group 9) was 23.5±3.3 pmol/min/mg protein (N=5) (Table 4.41). The intraperitoneal injection of streptozotocin caused 1.3-fold increase in PROD activities of rat liver microsomes, however this increase was not significant with respect to control group ($p<0.05$). In diabetic rats, the administrations of endosulfan and morin increased PROD activities of rat liver microsomes by 1.2-fold in diabetes mellitus+endosulfan group (group 10) and 1.5-fold diabetes mellitus+morin group (group 11). The average enzyme activities of diabetes mellitus+endosulfan and diabetes mellitus+morin were 36.9±8.6 pmol/min/mg protein (N=7) and 35.3±5.4 pmol/min/mg protein (N=3), respectively (Table 4.42 and 3.43). Endosulfan+ morin and DMBA treatments of diabetic rats did not altered the average PROD activities of rat liver microsomes and these activities were 24.8 pmol/min/mg protein (N=2) in mellitus+endosulfan+morin group (group 12) (Table 4.44) and 22.6±0.8 pmol/min/mg protein (N=3) in diabetes mellitus+DMBA group (group 13) (Table 4.45). The average enzyme activities of rat liver microsomes obtained from diabetes mellitus + DMBA + morin (group 14), diabetes mellitus + DMBA + endosulfan (group 15), and diabetes mellitus+DMBA+endosulfan+morin (group 16) groups were 13.6±1.4 pmol/min/mg protein (N=3), 18.5 pmol/min/mg protein (N=2), and 15.7 pmol/min/mg protein (N=1) (Table 4.46, 3.47, and 3.48), respectively. The chemical treatments carried out to these groups decreased the enzyme activities of rat liver microsomes, however, these decreases were not statistically significant ($p<0.05$). Figure 4.6 illustrates PROD activity results in diabetic rats.

Table 4.41. Cytochrome P4502B associated 7-pentoxyresorufin O-depentylase activities of rat liver microsomes obtained from diabetes mellitus group (group 9).

Sample Number	Average Activity (pmol/min/mg protein)
1	33.9
2	28.5
3	16.8
4	20.2
5	18.2
Average Activity±SEM N=5	23.5±3.3

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.42. Cytochrome P4502B associated 7-pentoxyresorufin O-depentylase activities of rat liver microsomes obtained from diabetes mellitus+endosulfan group (group 10).

Sample Number	Average Activity (pmol/min/mg protein)
1	83.1
2	36.7
3	23.9
4	46.9
5	17.3
6	20.8
7	29.7
Average Activity±SEM N=7	36.9±8.6

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.43. Cytochrome P4502B associated 7-pentoxyresorufin O-depentylase activities of rat liver microsomes obtained from diabetes mellitus+morin group (group 11).

Sample Number	Average Activity (pmol/min/mg protein)
1	34.1
2	45.1
3	26.6
Average Activity±SEM N=3	35.3±5.4

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.44. Cytochrome P4502B associated 7-pentoxyresorufin O-depentylase activities of rat liver microsomes obtained from diabetes mellitus+endosulfan+morin group (group 12).

Sample Number	Average Activity (pmol/min/mg protein)
1	15.0
2	34.6
Average Activity N=2	24.8

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.45. Cytochrome P4502B associated 7-pentoxyresorufin O-depentylase activities of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene group (group 13).

Sample Number	Average Activity (pmol/min/mg protein)
1	22.7
2	21.2
3	23.8
Average Activity±SEM N=3	22.6±0.8

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.46. Cytochrome P4502B associated 7-pentoxyresorufin O-depentylase activities of rat liver microsomes obtained from diabetes mellitus, 7,12-dimethylbenz[a]anthracene+morin group (group 14).

Sample Number	Average Activity (pmol/min/mg protein)
1	15.6
2	10.9
3	14.2
Average Activity±SEM N=3	13.6±1.4

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.47. Cytochrome P4502B associated 7-pentoxyresorufin O-depentylase activities of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan group (group 15).

Sample Number	Average Activity (pmol/min/mg protein)
1	15.6
2	21.4
Average Activity N=2	18.5

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.48. Cytochrome P4502B associated 7-pentoxyresorufin O-depentylase activities of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 16).

Sample Number	Average Activity (pmol/min/mg protein)
1	15.7

Each microsome fraction was prepared from single rat liver.

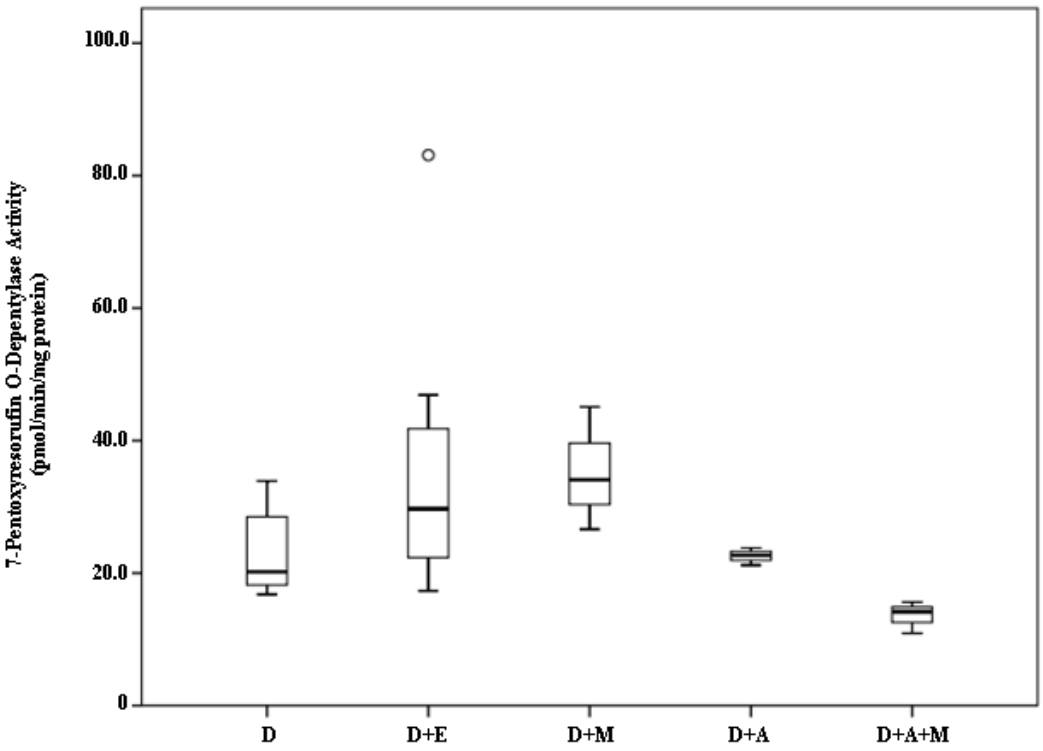


Figure 4.6. Cytochrome P4502B associated 7-pentoxyresorufin O-dependentylase activities of rat liver microsomes obtained from group 9-16. D (Diabetes mellitus), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

4.1.4 Aniline 4-Hydroxylase Activity of Rat Liver Microsomes

Cytochrome P4502E associated aniline 4-hydroxylase (A4H) activities were measured in each rat liver microsomes and the specific aniline 4-hydroxylase activities were expressed as nmole p-aminophenol formed per milligram protein in one minute at 37 ºC.

The average specific aniline 4-hydroxylase activity of rats in the control group (group 1) was 0.70±0.03 mol/min/mg protein (N=7) (Table 4.49). The average aniline 4-hydroxylase activities of rats in endosulfan (group 2) and DMBA+morin (group 6) groups were similar to each other. The average aniline 4-hydroxylase activity was found to be 0.79±0.02 nmol/min/mg protein (N=7) in endosulfan group (group 2) (Table 4.50). The average aniline 4-hydroxylase activity of rats in DMBA+morin group (group 6) was 0.77±0.03 nmol/min/mg protein (N=7) (Table

4.54). Aniline 4-hydroxylase activities of rats in these groups were 1.1-fold higher than that in the control group. The average aniline 4-hydroxylase activity obtained from rats in morin (group 3) and endosulfan+morin (group 4) groups were 0.64±0.03 nmol/min/mg protein (N=7), and 0.74±0.02 nmol/min/mg protein (N=7), respectively (Table 4.51 and 3.52). Statistically significant differences (1.2-fold) were observed in the average aniline 4-hydroxylase activities of rats treated with DMBA, DMBA+endosulfan, and DMBA+endosulfan+morin compared to rats in the control group ($p<0.05$). The average aniline 4-hydroxylase activities of rat liver microsomes in DMBA (group 5), DMBA+endosulfan (group 7), and DMBA+endosulfan+morin (group 8) were 0.84±0.02 nmol/min/mg protein (N=7), 0.87±0.04 nmol/min/mg protein (N=7), and 0.86±0.02 nmol/min/mg protein (N=7) (Table 4.53, 3.55, and 3.56), respectively. Figure 4.7 illustrates aniline 4-hydroxylase activity results in healthy rats.

Table 4.49. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from control group (group 1).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.75
2	0.79
3	0.73
4	0.65
5	0.71
6	0.59
7	0.65
Average Activity±SEM N=7	0.70±0.03

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.50. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from endosulfan group (group 2).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.83
2	0.80
3	0.77
4	0.80
5	0.86
6	0.70
7	0.79
Average Activity±SEM N=7	0.79±0.02

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.51. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from morin group (group 3).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.7
2	0.73
3	0.69
4	0.55
5	0.63
6	0.57
7	0.64
Average Activity±SEM N=7	0.64±0.03

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.52. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from endosulfan+morin group (group 4).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.8
2	0.79
3	0.73
4	0.71
5	0.73
6	0.71
7	0.7
Average Activity±SEM N=7	0.74±0.02

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.53. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene group (group 5).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.76
2	0.84
3	0.78
4	0.81
5	0.87
6	0.89
7	0.92
Average Activity±SEM N=7	0.84±0.02

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.54. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+morin group (group 6).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.86
2	0.78
3	0.67
4	0.79
5	0.89
6	0.66
7	0.72
Average Activity±SEM N=7	0.77±0.03

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.55. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+endosulfan group (group 7).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.96
2	0.83
3	1.0
4	0.71
5	0.94
6	0.86
7	0.76
Average Activity±SEM N=7	0.87±0.04

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.56. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 8).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.95
2	0.89
3	0.9
4	0.82
5	0.77
6	0.83
7	0.88
Average Activity±SEM N=7	0.86±0.02

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

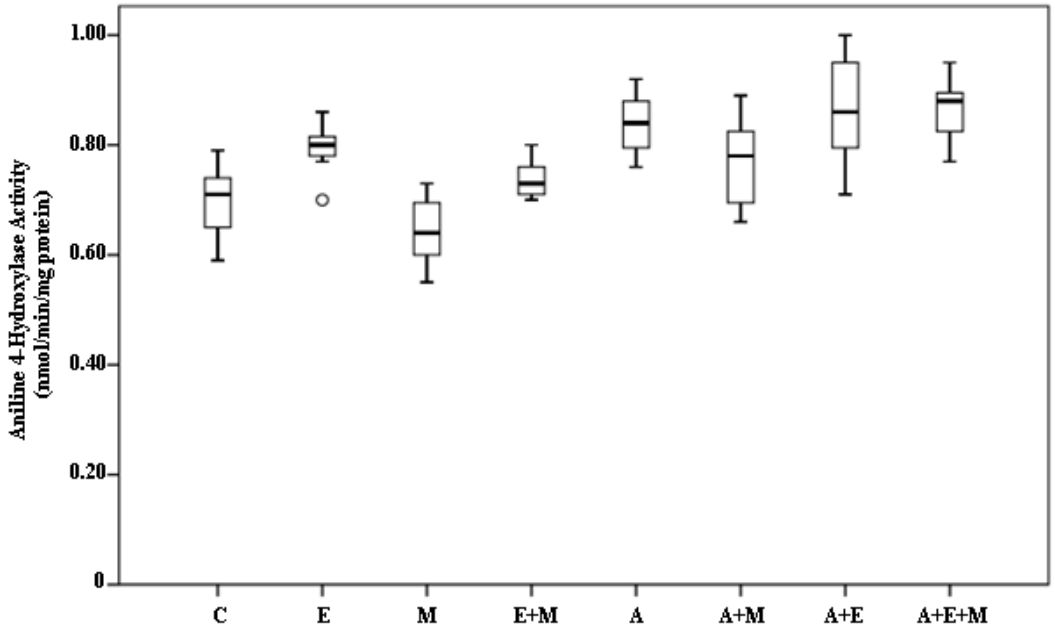


Figure 4.7. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from group 1-8. C (Control), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

The average specific aniline 4-hydroxylase activity of rats in diabetes mellitus group (group 9) was 0.70±0.06 nmol/min/mg protein (N=5) (Table 4.57). The induction of the diabetes mellitus by intraperitoneal injection of streptozotocin did not alter the average aniline 4-hydroxylase activity of rat liver microsomes. The average aniline 4-hydroxylase activity of rats in diabetes mellitus+endosulfan (group 10) group was similar to diabetes mellitus group. The activity was 0.71±0.03 nmol/min/mg protein (N=7) in this group (Table 4.58). The average aniline 4-hydroxylase activities obtained from rats in diabetes mellitus+morin (group 11), diabetes mellitus+endosulfan+morin (group 12), diabetes mellitus+DMBA (group 13), and diabetes mellitus+DMBA+endosulfan (group 15) groups were 0.64±0.06 nmol/min/mg protein (N=3), 0.59 nmol/min/mg protein (N=2), 0.63±0.05 nmol/min/mg protein (N=3), and 0.58 nmol/min/mg protein (N=2), respectively (Table 4.59, 3.60, 3.61,and 3.63). The average aniline 4-hydroxylase activities of these groups were lower than the average enzyme activity of diabetes mellitus group; however, these decreases were not statistically significant. A significant decrease was observed in the average aniline 4-hydroxylase activity of rat liver microsomes obtained from diabetes mellitus+DMBA+morin group (group 14) ($p<0.05$). The average aniline 4-hydroxylase activity of this group was 0.46±0.02 nmol/min/mg protein (N=3) (Table 4.62). The enzyme activity of diabetes mellitus+DMBA+endosulfan+morin group (group 16) was measured as 0.84 nmol/min/mg protein (N=1) (Table 4.64). Figure 4.8 illustrates aniline 4-hydroxylase activity results in diabetic rats.

Table 4.57. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from diabetes mellitus group (group 9).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.79
2	0.84
3	0.57
4	0.73
5	0.57
Average Activity±SEM N=5	0.70±0.06

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.58. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from diabetes mellitus+endosulfan group (group 10).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.66
2	0.75
3	0.75
4	0.75
5	0.58
6	0.69
7	0.76
Average Activity±SEM N=7	0.71±0.03

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.59. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from diabetes mellitus+morin group (group 11).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.69
2	0.53
3	0.70
Average Activity±SEM N=3	0.64±0.06

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.60. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from diabetes mellitus+endosulfan+morin group (group 12).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.51
2	0.66
Average Activity N=2	0.59

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.61. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene group (group 13).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.63
2	0.54
3	0.72
Average Activity±SEM N=3	0.63±0.05

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.62. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from diabetes mellitus, 7,12-dimethylbenz[a]anthracene+morin group (group 14).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.51
2	0.43
3	0.45
Average Activity±SEM N=3	0.46±0.02

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.63. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan group (group 15).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.43
2	0.73
Average Activity N=2	0.58

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.64. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 16).

Sample Number	Average Activity (nmol/min/mg protein)
3	0.84

Each microsome fraction was prepared from single rat liver.

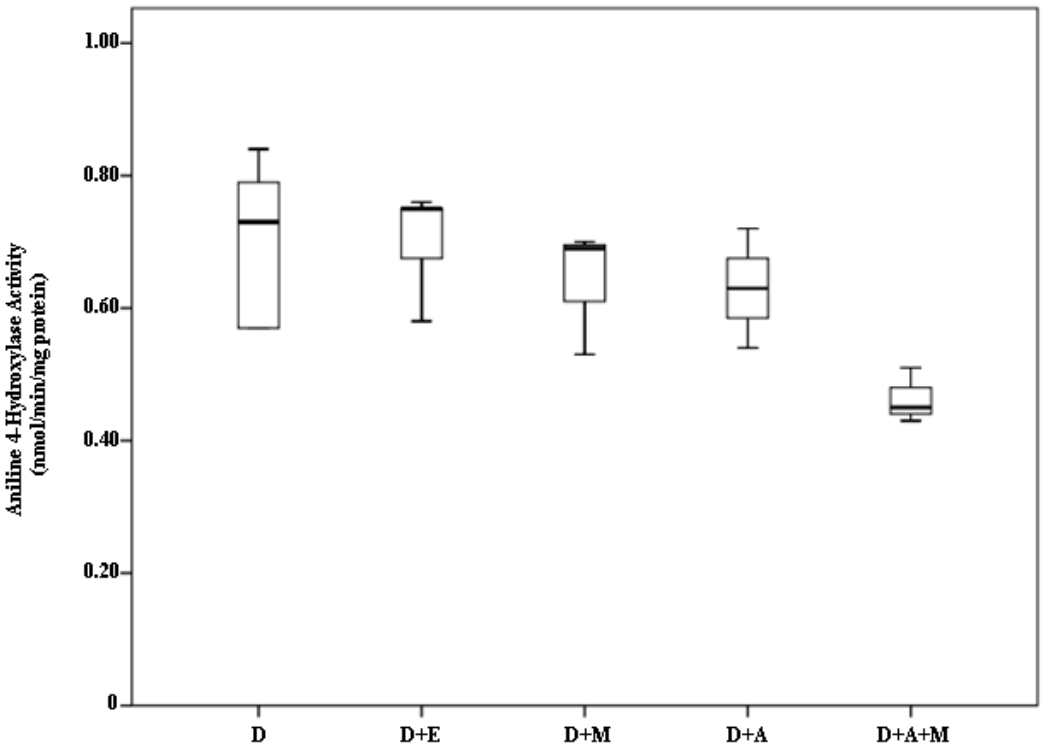


Figure 4.8. Cytochrome P4502E1 associated aniline 4-hydroxylase activities of rat liver microsomes obtained from group 9-16. D (Diabetes mellitus), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

4.1.5 Erythromycin N-Demethylase Activity of Rat Liver Microsomes

Cytochrome P4503A associated erythromycin N-demethylase (ERND) activities were measured in each rat liver microsomes and the specific erythromycin N-demethylase activities were expressed as nmole formaldehyde formed per milligram protein in one minute at 37 ºC.

The average specific erythromycin N-demethylase activity of rats in the control group (group 1) was 0.38±0.01 nmol/min/mg protein (N=7) (Table 4.65). Rats in endosulfan (group 2), endosulfan+morin (group 4), and DMBA (group 5) groups had 1.3-fold higher erythromycin N-demethylase activities than the rats in the control group and the average of specific erythromycin N-demethylase activities obtained from these groups were 0.50±0.02 nmol/min/mg protein (N=7), 0.48±0.04 nmol/min/mg protein (N=7), and 0.48±0.03 nmol/min/mg protein (N=7),

respectively (Table 4.66, 3.68, and 3.69). The administrations of morin, DMBA+morin, and DMBA+endosulfan increased average erythromycin N-demethylase activities of rat liver microsomes by 1.2-fold compared to the control group. The average enzyme activities of rat liver microsomes were 0.44±0.03 nmol/min/mg protein (N=7) (Table 4.67) in morin group (group 3), 0.44±0.04 nmol/min/mg protein (N=7) (Table 4.70) in DMBA+morin group (group 6), and 0.46±0.03 nmol/min/mg protein (N=7) (Table 4.71) in DMBA+endosulfan (group 7). Although these chemical treatments increased the enzyme activities of rat liver microsomes, these differences were not found to be statistically significant. The highest average erythromycin N-demethylase activity measured in liver microsomes of healthy rats was obtained from DMBA+endosulfan+morin group (group 8), which was 0.54±0.03 nmol/min/mg protein (N=7) (Table 4.72). The administration of DMBA, endosulfan, and morin together caused statistically significant (1.4-fold) increase in the average erythromycin N-demethylase activity of rat liver microsomes ($p<0.05$). Figure 4.9 illustrates erythromycin N-demethylase activity results in healthy rats.

Table 4.65. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from control group (group 1).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.37
2	0.37
3	0.39
4	0.40
5	0.35
6	0.39
7	0.41
Average Activity±SEM N=7	0.38±0.01

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.66. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from endosulfan group (group 2).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.55
2	0.42
3	0.48
4	0.59
5	0.53
6	0.52
7	0.43
Average Activity±SEM N=7	0.50±0.02

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.67. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from morin group (group 3).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.43
2	0.38
3	0.34
4	0.63
5	0.46
6	0.44
7	0.42
Average Activity±SEM N=7	0.44±0.03

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.68. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from endosulfan+morin group (group 4).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.44
2	0.48
3	0.30
4	0.53
5	0.46
6	0.56
7	0.62
Average Activity±SEM N=7	0.48±0.04

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.69. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene group (group 5).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.63
2	0.48
3	0.44
4	0.40
5	0.54
6	0.46
7	0.43
Average Activity±SEM N=7	0.48±0.03

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.70. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+morin group (group 6).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.51
2	0.40
3	0.29
4	0.51
5	0.46
6	0.36
7	0.55
Average Activity±SEM N=7	0.44±0.04

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.71. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+endosulfan group (group 7).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.47
2	0.33
3	0.59
4	0.46
5	0.51
6	0.41
7	0.44
Average Activity±SEM N=7	0.46±0.03

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.72. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from 7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 8).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.44
2	0.66
3	0.49
4	0.57
5	0.59
6	0.53
7	0.53
Average Activity±SEM N=7	0.54±0.03

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

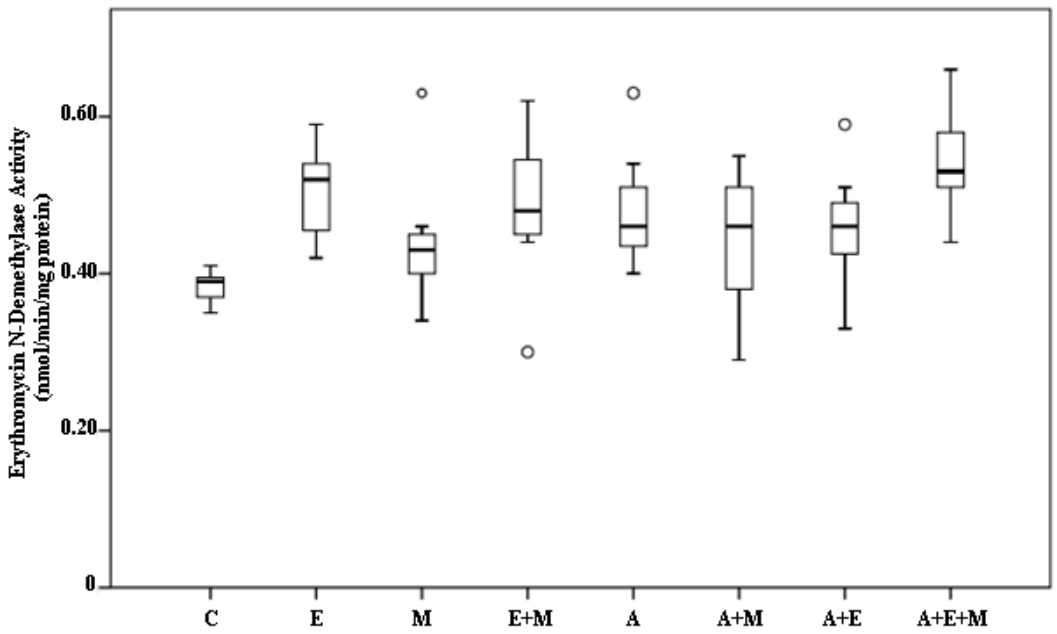


Figure 4.9. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from group 1-8. C (Control), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

The induction of the diabetes mellitus by intraperitoneal injection of streptozotocin significantly increased erythromycin N-demethylase activity of rat liver microsomes by 1.9-fold compared to the control group ($p<0.05$). This activity measured in diabetes mellitus group (group 9) was 0.68±0.02 nmol/min/mg protein (N=5) (Table 4.73). The chemical treatments carried out to diabetic rats decreased the enzyme activities of rat liver microsomes and the obtained average erythromycin N-demethylase activities of these diabetic groups were lower than the average enzyme activity of diabetes mellitus group. The average enzyme activities of rat liver microsomes were 0.44±0.03 nmol/min/mg protein (N=7) in diabetes mellitus+endosulfan group (group 10) (Table 4.74), 0.52±0.15 nmol/min/mg protein (N=3) in diabetes mellitus+morin group (group 11) (Table 4.75), 0.48 nmol/min/mg protein (N=2) in diabetes mellitus+endosulfan+morin group (group 12) (Table 4.76), 0.37±0.02 nmol/min/mg protein (N=3) in diabetes mellitus+DMBA group (group 13) (Table 4.77), 0.31 nmol/min/mg protein (N=2) in diabetes mellitus+DMBA+morin group (group 14) (Table 4.78), 0.55 nmol/min/mg protein (N=2) in diabetes mellitus+DMBA+endosulfan (group 15) (Table 4.79), and 0.58 nmol/min/mg protein (N=1) in diabetes mellitus+DMBA+endosulfan+morin (group 16) (Table 4.80). Figure 4.10 illustrates erythromycin N-demethylase activity results in diabetic rats.

Table 4.73. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from diabetes mellitus group (group 9).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.70
2	0.66
3	0.72
4	0.72
5	0.59
Average Activity±SEM N=5	0.68±0.02

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.74. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from diabetes mellitus+endosulfan group (group 10).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.49
2	0.55
3	0.34
4	0.45
5	0.54
6	0.35
7	0.36
Average Activity±SEM N=7	0.44±0.03

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.75. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from diabetes mellitus+morin group (group 11).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.41
2	0.34
3	0.81
Average Activity±SEM N=3	0.52±0.15

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.76. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from diabetes mellitus+endosulfan+morin group (group 12).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.29
2	0.66
Average Activity N=2	0.48

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.77. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene group (group 13).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.39
2	0.38
3	0.33
Average Activity±SEM N=3	0.37±0.02

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.78. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from diabetes mellitus, 7,12-dimethylbenz[a]anthracene+morin group (group 14).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.26
2	0.35
3	N.D.
Average Activity N=2	0.31

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.79. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan group (group 15).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.33
2	0.76
Average Activity N=2	0.55

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.80. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 16).

Sample Number	Average Activity (nmol/min/mg protein)
1	0.58

Each microsome fraction was prepared from single rat liver.

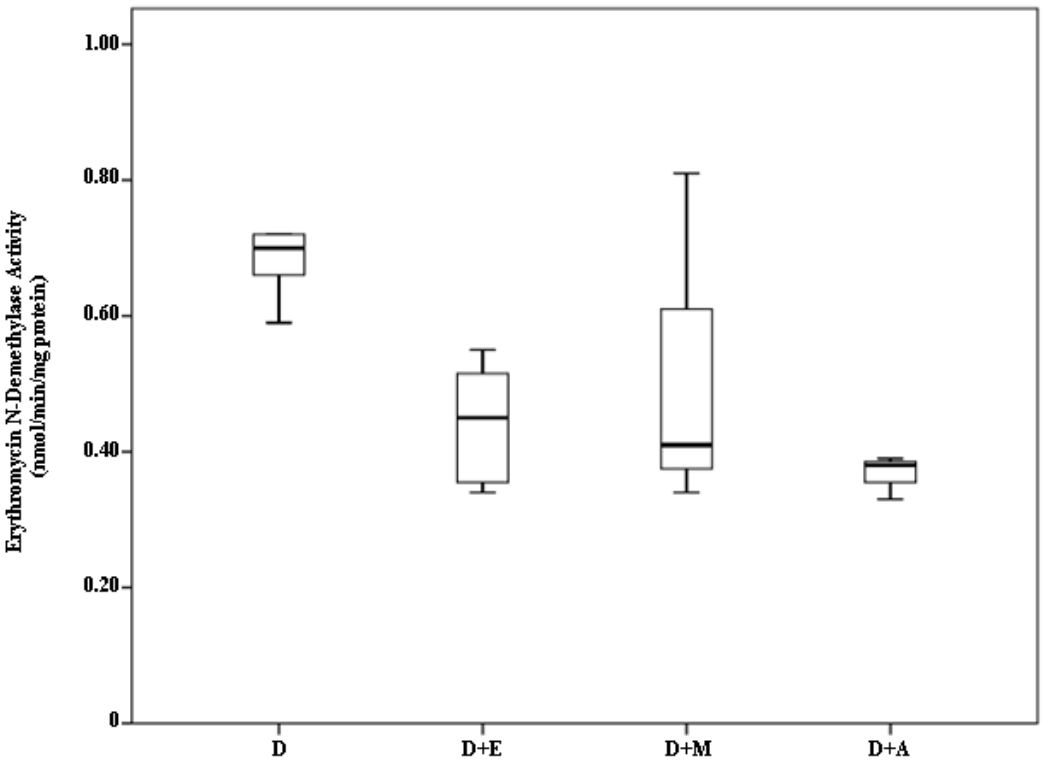


Figure 4.10. Cytochrome P4503A associated erythromycin N-demethylase activities of rat liver microsomes obtained from group 9-16). D (Diabetes mellitus), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

4.1.6 Total Glutathione S-Transferase Activity of Rat Liver Cytosols

Total glutathione S-transferase (GST) activities were measured in each rat liver cytosols and the specific total glutathione S-transferase activities were calculated as nmole 1-glutathione-2,4-dinitrobenzene conjugate formed per milligram protein in one minute at 25 $^{\circ}$C, using 9.6 mM $^{-1}$ cm $^{-1}$ as an extinction coefficient (ε_{340}).

The average total glutathione S-transferase activity of rats in the control group (group 1) was 365±14 nmol/min/mg protein (N=7) (Table 4.81). Similar average glutathione S-transferase activity was obtained from rats in endosulfan group (group 2). The average enzyme activity of this group was 365±24 nmol/min/mg protein (N=7) (Table 4.82). The average glutathione S-transferase activities obtained from rats in endosulfan+morin group (group 4) was 460±10 nmol/min/mg protein (N=7) (Table 4.84). This activity was significantly different from the control group

and 1.3-fold greater than the average glutathione S-transferase activity obtained from the control group ($p<0.05$). The average glutathione S-transferase activities of rats in DMBA+morin (group 6) and DMBA+endosulfan (group 7) groups were similar to each other and were 577±25 nmol/min/mg protein (N=7) and 585±23 nmol/min/mg protein (N=7), respectively (Table 4.86 and 3.87). These activities were significantly different (1.6-fold higher) from the activity obtained from the control group ($p<0.05$). The highest total glutathione S-transferase activities measured in the liver cytosols of healthy rats were obtained from DMBA (group 5) and DMBA+endosulfan+morin (group 8) groups, which were 651±57 nmol/min/mg protein (N=7) and 649±33 nmol/min/mg protein (N=7), respectively (Table 4.85 and 3.88). These treatments caused significant increase (1.8-fold) in the average glutathione S-transferase activities of rat liver cytosols ($p<0.05$). Figure 4.11 illustrates total glutathione S-transferase activity results in healthy rats.

Table 4.81. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from control group (group 1).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	33	418
2	32	337
3	34	338
4	29	336
5	34	413
6	29	380
7	34	332
Average Activity±SEM N= 7		365±14

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.82. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from endosulfan group (group 2).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	31	377
2	28	232
3	37	442
4	39	372
5	33	392
6	36	380
7	31	359
Average Activity±SEM N= 7		365±24

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.83. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from morin group (group 3).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	27	453
2	31	321
3	33	483
4	27	432
5	31	514
6	33	348
7	30	457
Average Activity±SEM N= 7		430±27

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.84. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from endosulfan+morin group (group 4).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	32	468
2	30	469
3	28	459
4	31	412
5	33	492
6	31	482
7	31	436
Average Activity±SEM N= 7		460±10

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.85. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from 7,12-dimethylbenz[a]anthracene group (group 5).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	29	670
2	28	787
3	25	452
4	31	544
5	22	818
6	22	786
7	29	501
Average Activity±SEM N= 7		651±57

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.86. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from 7,12-dimethylbenz[a]anthracene+morin group (group 6).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	28	642
2	24	548
3	28	576
4	26	499
5	22	671
6	26	500
7	27	600
Average Activity±SEM N= 7		577±25

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.87. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from 7,12-dimethylbenz[a]anthracene+endosulfan group (group 7).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	29	610
2	27	509
3	30	586
4	28	695
5	34	593
6	32	566
7	29	535
Average Activity±SEM N= 7		585±23

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.88. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from 7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 8).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	28	634
2	32	627
3	30	568
4	31	711
5	24	585
6	21	603
7	32	816
Average Activity±SEM N= 7		649±33

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

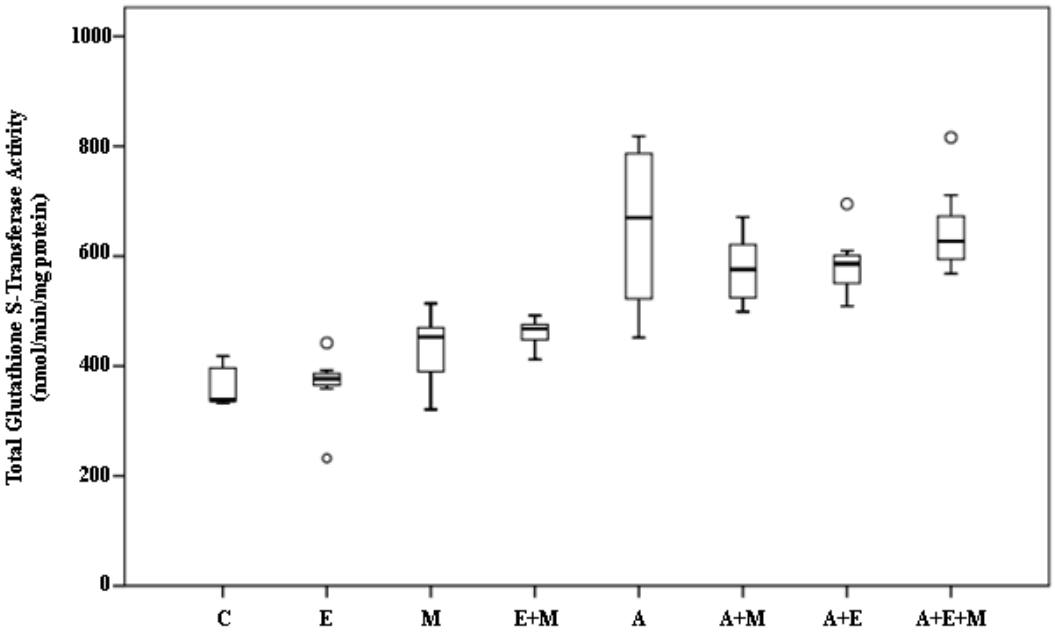


Figure 4.11. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from group 1-8. C (Control), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

The average total glutathione S-transferase activity of rats in diabetes mellitus group (group 9) was 820±43 nmol/min/mg protein (N=5) (Table 4.89). The intraperitoneal injection of streptozotocin significantly increased (2.2-fold) glutathione S-transferase activities of rat liver cytosols compared to the control group ($p<0.05$). Significant decreases were observed in the average glutathione S-transferase activities of rat liver cytosols obtained from diabetes mellitus+morin (group 11), diabetes mellitus+DMBA (group 13), and diabetes mellitus+DMBA+morin groups (group 14) ($p<0.05$). The average total glutathione S-transferase activities of rat liver cytosols were 499±31 nmol/min/mg protein (N=3) in diabetes mellitus+morin group (group 11) (Table 4.91), 591±41 nmol/min/mg protein (N=3) in diabetes mellitus+DMBA group (group 13) (Table 4.93), and 529±30 nmol/min/mg protein (N=3) in diabetes mellitus+DMBA+morin group (group 14) (Table 4.94). Tables 3.89-3.96 show cytosolic total glutathione S-transferase activities obtained from the chemical treatment studies of diabetic rats. Figure 4.12 illustrates total glutathione S-transferase activity results in diabetic rats.

Table 4.89. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from diabetes mellitus group (group 9).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	22	795
2	20	888
3	24	751
4	27	720
5	24	947
Average Activity±SEM N= 5		820±43

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.90. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from diabetes mellitus+endosulfan group (group 10).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	24	816
2	23	868
3	23	605
4	24	720
5	25	626
6	24	532
7	29	584
Average Activity±SEM N= 7		679±48

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.91. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from diabetes mellitus+morin group (group 11).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	27	513
2	27	440
3	28	543
Average Activity±SEM N= 3		499±31

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.92. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from diabetes mellitus+endosulfan+morin group (group 12).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	26	523
2	29	579
Average Activity N= 2		551

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.93. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene group (group 13).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	27	542
2	28	559
3	26	673
Average Activity±SEM N= 3		591±41

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.94. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from diabetes mellitus, 7,12-dimethylbenz[a]anthracene+morin group (group 14).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	23	488
2	24	588
3	17	511
Average Activity±SEM N= 3		529±30

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.95. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan group (group 15).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	25	568
2	22	655
Average Activity N= 2		612

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.96. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 16).

Sample Number	Protein Concentration (mg/mL)	Average Activity (nmol/min/mg protein)
1	19	879

Each cytosol fraction was prepared from single rat liver.

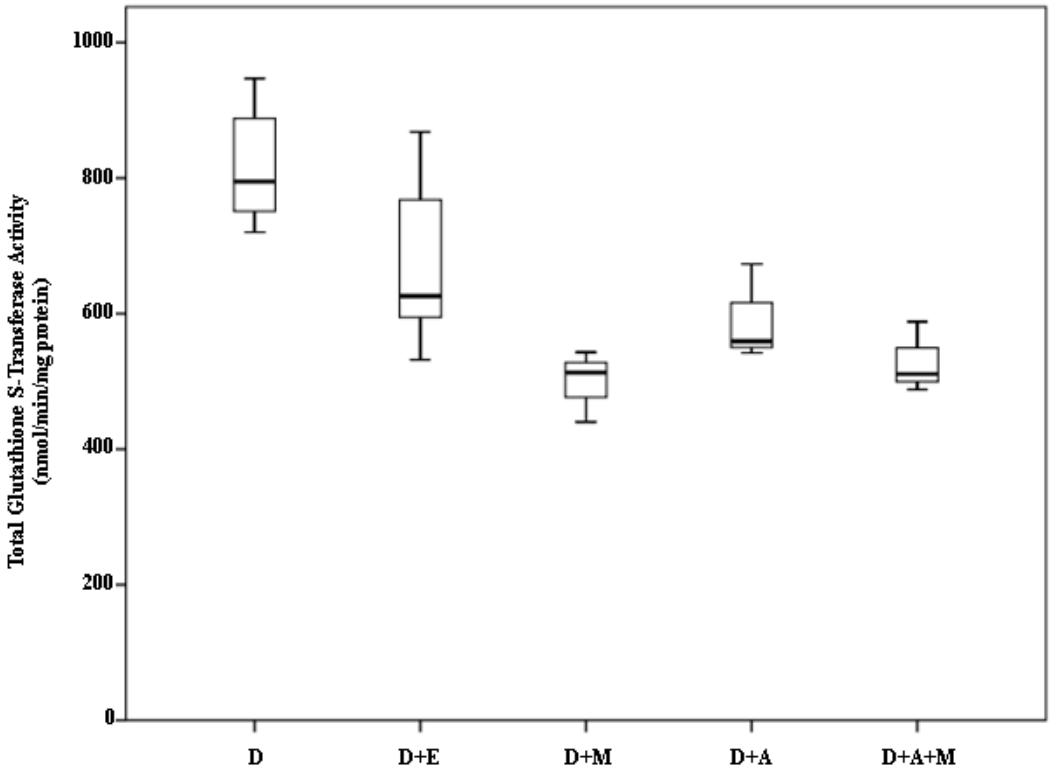


Figure 4.12. Total glutathione S-transferase activities and protein concentrations of rat liver cytosols obtained from group 9-16. D (Diabetes mellitus), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

4.1.7 Catalase Activity of Rat Liver Cytosols

Catalase (CAT) activities were measured in each rat liver cytosols and the specific catalase activities were calculated as nmole H_2O_2 consumed per milligram protein in one minute at 25 $^{\circ}$C using 0.0364 mM $^{-1}$ cm $^{-1}$ as an extinction coefficient (ε_{240}).

The average specific catalase activity of rats in the control group (group 1) was 618±29 nmol/min/mg protein (N=7) (Table 4.97). The administration of endosulfan, morin, and endosulfan+morin did not significantly alter the average catalase activities in healthy rats ($p<0.05$). The average catalase activities of rats in endosulfan (group 2), morin (group 3) and endosulfan+morin (group 4) groups were 540±38 µmol/min/mg protein (N=7), 569±34 µmol/min/mg protein (N=7) and 612±48 µmol/min/mg protein (N=7), respectively (Table 4.98, 3.99, and 3.100).

Significant increase (1.4-fold) were observed in the average catalase activities of rats in DMBA and DMBA+morin groups compared to rats in the control group ($p<0.05$). The highest catalase activities measured in liver cytosols of healthy rats were obtained from these groups. The average of specific catalase activities obtained from DMBA (group 5) and DMBA+morin (group 6) groups were 896±55 µmol/min/mg protein (N=7) and 843±37 µmol/min/mg protein (N=7), respectively (Table 4.101 and 3.102). Administration of endosulfan and co-administration of endosulfan and morin decreased the catalase activities of DMBA-treated rats. The average catalase activities obtained from rats in DMBA+endosulfan group (group 7) was 749±37 µmol/min/mg protein (N=7) (Table 4.103) and that in DMBA+endosulfan+morin group (group 8) was 677±25 µmol/min/mg protein (N=7) (Table 4.104). Figure 4.13 illustrates catalase activity results in healthy rats.

Table 4.97. Catalase activities of rat liver cytosols obtained from control group (group 1).

Sample Number	Average Activity (µmol/min/mg protein)
1	673
2	589
3	647
4	465
5	593
6	676
7	681
Average Activity±SEM N=7	618±29

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.98. Catalase activities of rat liver cytosols obtained from endosulfan group (group 2).

Sample Number	Average Activity (µmol/min/mg protein)
1	510
2	382
3	618
4	666
5	475
6	507
7	619
Average Activity±SEM N=7	540±38

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.99. Catalase activities of rat liver cytosols obtained from morin group (group 3).

Sample Number	Average Activity (µmol/min/mg protein)
1	486
2	428
3	572
4	683
5	546
6	620
7	645
Average Activity±SEM N=7	569±34

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.100. Catalase activities of rat liver cytosols obtained from endosulfan+morin group (group 4).

Sample Number	Average Activity (µmol/min/mg protein)
1	763
2	579
3	556
4	482
5	566
6	815
7	520
Average Activity±SEM N=7	612±48

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.101. Catalase activities of rat liver cytosols obtained from 7,12-dimethylbenz[a]anthracene group (group 5).

Sample Number	Average Activity (µmol/min/mg protein)
1	1065
2	789
3	926
4	785
5	967
6	1056
7	683
Average Activity±SEM N=7	896±55

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.102. Catalase activities of rat liver cytosols obtained from 7,12-dimethylbenz[a]anthracene+morin group (group 6).

Sample Number	Average Activity (µmol/min/mg protein)
1	858
2	970
3	837
4	675
5	894
6	899
7	765
Average Activity±SEM N=7	843±37

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.103. Catalase activities of rat liver cytosols obtained from 7,12-dimethylbenz[a]anthracene+endosulfan group (group 7).

Sample Number	Average Activity (µmol/min/mg protein)
1	830
2	808
3	628
4	708
5	632
6	882
7	752
Average Activity±SEM N=7	749±37

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.104. Catalase activities of rat liver cytosols obtained from 7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 8).

Sample Number	Average Activity (µmol/min/mg protein)
1	657
2	786
3	564
4	668
5	708
6	693
7	661
Average Activity±SEM N=7	677±25

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

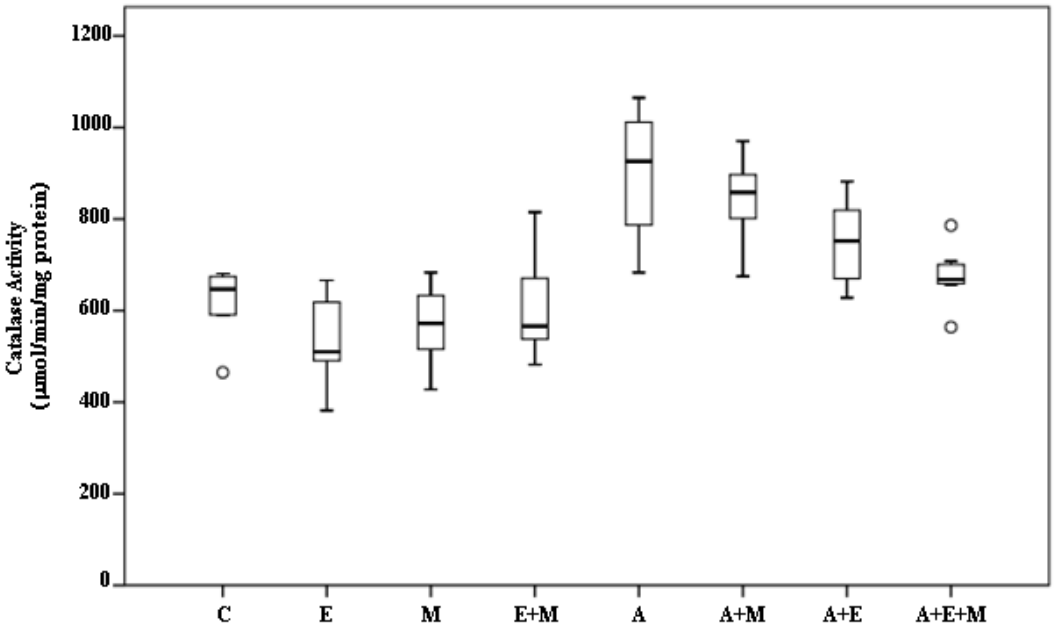


Figure 4.13. Catalase activities of rat liver cytosols obtained from group 1-8. C (Control), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

The average specific catalase activities of rats in diabetes mellitus group (group 9) was 776±25 µmol/min/mg protein (N=5) (Table 4.105). This activity was 1.2-fold greater than the average catalase activity obtained from the control group. Tables 3.105-3.112 show cytosolic catalase activities obtained from the chemical treatment studies of the diabetic rat groups. Morin and DMBA+morin treatments caused significant decreases in the average catalase activities of diabetic rat liver cytosols ($p<0.05$). The average catalase activities of rat liver cytosols obtained from diabetes mellitus+morin (group 11) and diabetes mellitus+DMBA+morin group (group 14) were 565±34 µmol/min/mg protein (N=3), and 360±34 µmol/min/mg protein (N=3), respectively (Table 4.107 and 3.110). Figure 4.14 illustrates catalase activity results in diabetic rats.

Table 4.105. Catalase activities of rat liver cytosols obtained from diabetes mellitus group (group 9).

Sample Number	Average Activity (µmol/min/mg protein)
1	744
2	748
3	853
4	721
5	814
Average Activity±SEM N=5	776±25

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.106. Catalase activities of rat liver cytosols obtained from diabetes mellitus+endosulfan group (group 10).

Sample Number	Average Activity (µmol/min/mg protein)
1	655
2	829
3	813
4	746
5	685
6	746
7	641
Average Activity±SEM N=7	731±28

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.107. Catalase activities of rat liver cytosols obtained from diabetes mellitus+morin group (group 11).

Sample Number	Average Activity (µmol/min/mg protein)
1	621
2	503
3	571
Average Activity±SEM N=3	565±34

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.108. Catalase activities of rat liver cytosols obtained from diabetes mellitus+endosulfan+morin group (group 12).

Sample Number	Average Activity (µmol/min/mg protein)
1	555
2	570
Average Activity N=2	563

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.109. Catalase activities of rat liver cytosols obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene group (group 13).

Sample Number	Average Activity (µmol/min/mg protein)
1	468
2	583
3	582
Average Activity±SEM N=3	544±38

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.110. Catalase activities of rat liver cytosols obtained from diabetes mellitus, 7,12-dimethylbenz[a]anthracene+morin group (group 14).

Sample Number	Average Activity (µmol/min/mg protein)
1	293
2	384
3	403
Average Activity±SEM N=3	360±34

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.111. Catalase activities of rat liver cytosols obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan group (group 15).

Sample Number	Average Activity (µmol/min/mg protein)
1	359
2	760
Average Activity N=2	560

Each microsome fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.112. Catalase activities of rat liver cytosols obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 16).

Sample Number	Average Activity (µmol/min/mg protein)
1	1123

Each microsome fraction was prepared from single rat liver.

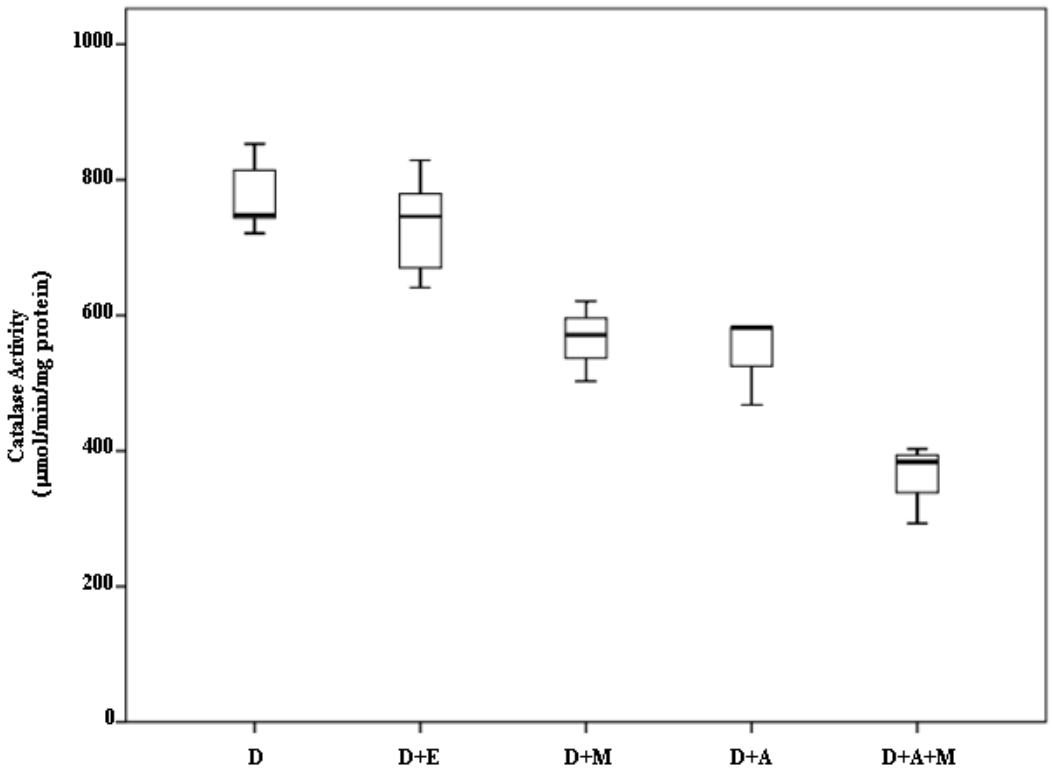


Figure 4.14. Catalase activities of rat liver cytosols obtained from group 9-16. D (Diabetes mellitus), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

4.1.8 Glutathione Reductase Activity of Rat Liver Cytosols

Glutathione reductase activities were measured in each rat liver cytosols and the specific glutathione reductase activities were calculated as nmole NADPH oxidized per milligram protein in one minute at 25 $^{\circ}$C, using 6.22 µM $^{-1}$ cm $^{-1}$ as an extinction coefficient (ε_{340}).

The average specific glutathione reductase activities of rats in the control group (group 1) was 46.7±2.2 nmol/min/mg protein (N=7) (Table 4.113). The average glutathione reductase activities of rats in endosulfan (group 2) and endosulfan+morin (group 4) groups were similar to the control group. The enzyme activities of rat liver cytosols obtained from endosulfan (group 2) and endosulfan+morin (group 4) groups were 46.3±1.7 nmol/min/mg protein (N=7) and 46.3±1.2 nmol/min/mg protein (N=7), respectively (Table 4.114 and 3.116). The

average glutathione reductase activities of rats in morin (group 3), DMBA+endosulfan (group 7), and DMBA+endosulfan+morin (group 8) groups were 49.7±1.6 nmol/min/mg protein (N=7), 51.5±1.5 nmol/min/mg protein (N=7), and 54.1±1.7 nmol/min/mg protein (N=7), respectively (Table 4.115, 3.119, and 3.120). The administrations of morin, DMBA+endosulfan, and DMBA+endosulfan+morin slightly increased the average glutathione reductase activities in healthy rats. The average glutathione reductase activities obtained from rats in DMBA (group 5) was 59.2±2.6 nmol/min/mg protein (N=7) (Table 4.117) and that in DMBA+morin group (group 6) was 55.8±1.6 nmol/min/mg protein (N=7) (Table 4.118). The enzyme activities observed in these groups were significantly different from that in the control group ($p<0.05$) and they were 1.3- and 1.2-fold greater than the average glutathione reductase activities obtained from the control group, respectively. Figure 4.15 illustrates glutathione reductase activity results in healthy rats.

Table 4.113. Glutathione reductase activities of rat liver cytosols obtained from control group (group 1).

Sample Number	Average Activity (nmol/min/mg protein)
1	50.8
2	37.6
3	50.2
4	41.1
5	52.1
6	51.6
7	43.2
Average Activity±SEM N= 7	46.7±2.2

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.114. Glutathione reductase activities of rat liver cytosols obtained from endosulfan group (group 2).

Sample Number	Average Activity (nmol/min/mg protein)
1	47.7
2	36.4
3	50.4
4	46.2
5	46.2
6	48.9
7	48.2
Average Activity±SEM N= 7	46.3±1.7

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.115. Glutathione reductase activities of rat liver cytosols obtained from morin group (group 3).

Sample Number	Average Activity (nmol/min/mg protein)
1	52.1
2	44
3	48
4	52.8
5	56.2
6	45.3
7	49.3
Average Activity±SEM N= 7	49.7±1.6

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.116. Glutathione reductase activities of rat liver cytosols obtained from endosulfan+morin group (group 4).

Sample Number	Average Activity (nmol/min/mg protein)
1	47.6
2	51.9
3	44.1
4	42.7
5	47.4
6	46.4
7	43.9
Average Activity±SEM N= 7	46.3±1.2

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.117. Glutathione reductase activities of rat liver cytosols obtained from 7,12-dimethylbenz[a]anthracene group (group 5).

Sample Number	Average Activity (nmol/min/mg protein)
1	56.9
2	53.2
3	66.2
4	51.3
5	63.1
6	68.8
7	55.1
Average Activity±SEM N= 7	59.2±2.6

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.118. Glutathione reductase activities of rat liver cytosols obtained from 7,12-dimethylbenz[a]anthracene+morin group (group 6).

Sample Number	Average Activity (nmol/min/mg protein)
1	59.3
2	55.7
3	58.8
4	49.3
5	60.3
6	50.6
7	56.7
Average Activity±SEM N= 7	55.8±1.6

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.119. Glutathione reductase activities of rat liver cytosols obtained from 7,12-dimethylbenz[a]anthracene+endosulfan group (group 7).

Sample Number	Average Activity (nmol/min/mg protein)
1	59.6
2	49.9
3	52.4
4	51.1
5	48.7
6	51.3
7	47.6
Average Activity±SEM N= 7	51.5±1.5

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.120. Glutathione reductase activities of rat liver cytosols obtained from 7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 8).

Sample Number	Average Activity (nmol/min/mg protein)
1	56.6
2	49
3	47.8
4	56.2
5	55.6
6	52.9
7	60.7
Average Activity±SEM N= 7	54.1±1.7

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

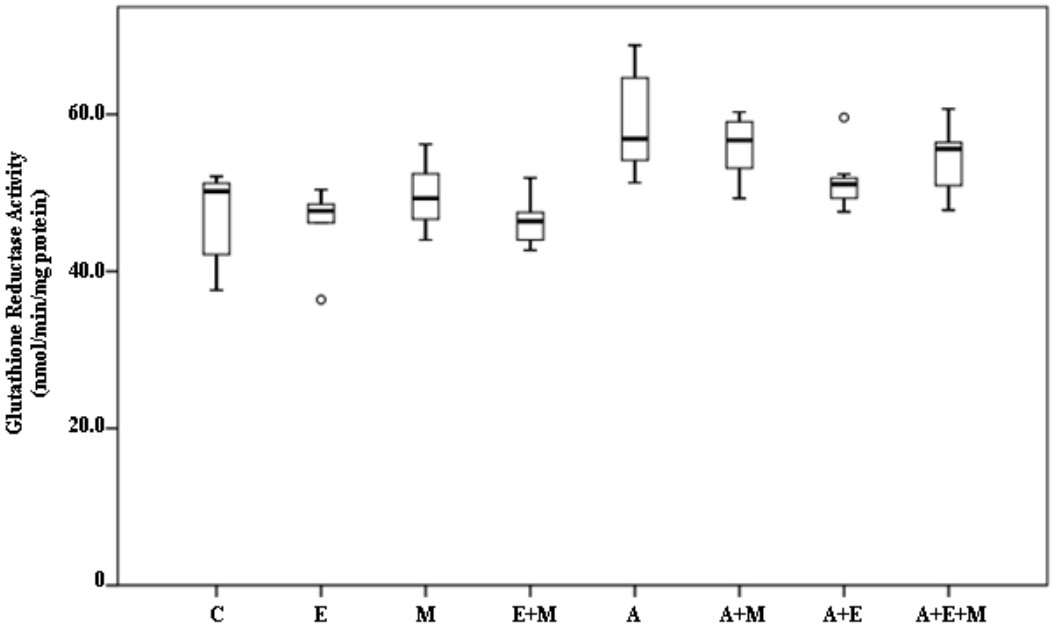


Figure 4.15. Glutathione reductase activities of rat liver cytosols obtained from group 1-8. C (Control), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

The induction of diabetes mellitus by intraperitoneal injection of streptozotocin increased glutathione reductase activities of rat liver cytosols by 1.2-fold compared to the control group. Tables 3.121-3.128 show cytosolic glutathione reductase activities obtained from the chemical treatment studies of diabetic rats. This activity measured in diabetes mellitus group (group 9) was 56.0±1.6 nmol/min/mg protein (N=5) (Table 4.121). The administration of 7,12-dimethylbenz[a]anthracene, endosulfan, and morin did not significantly altered the glutathione reductase activities in diabetic rats ($p<0.05$). Figure 4.16 illustrates glutathione reductase activity results in diabetic rats.

Table 4.121. Glutathione reductase activities of rat liver cytosols obtained from diabetes mellitus group (group 9).

Sample Number	Average Activity (nmol/min/mg protein)
1	55.6
2	60
3	58
4	50.2
5	56.4
Average Activity±SEM N= 5	56.0±1.6

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.122. Glutathione reductase activities of rat liver cytosols obtained from diabetes mellitus+endosulfan group (group 10).

Sample Number	Average Activity (nmol/min/mg protein)
1	59.2
2	62.5
3	56.7
4	60.8
5	56.9
6	64.1
7	62.8
Average Activity±SEM N= 7	60.4±1.1

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.123. Glutathione reductase activities of rat liver cytosols obtained from diabetes mellitus+morin group (group 11).

Sample Number	Average Activity (nmol/min/mg protein)
1	62.9
2	49.6
3	52.3
Average Activity±SEM N= 3	54.9±4.1

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.124. Glutathione reductase activities of rat liver cytosols obtained from diabetes mellitus+endosulfan+morin group (group 12).

Sample Number	Average Activity (nmol/min/mg protein)
1	54.4
2	60.6
Average Activity N= 2	57.5

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.125. Glutathione reductase activities of rat liver cytosols obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene group (group 13).

Sample Number	Average Activity (nmol/min/mg protein)
1	56.9
2	59.7
3	59.8
Average Activity±SEM N= 3	58.8±1.0

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.126. Glutathione reductase activities of rat liver cytosols obtained from diabetes mellitus, 7,12-dimethylbenz[a]anthracene+morin group (group 14).

Sample Number	Average Activity (nmol/min/mg protein)
1	45.5
2	59.6
3	53.7
Average Activity±SEM N= 3	52.9±4.1

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.127. Glutathione reductase activities of rat liver cytosols obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan group (group 15).

Sample Number	Average Activity (nmol/min/mg protein)
1	56.1
2	67.2
Average Activity N= 2	61.7

Each cytosol fraction was prepared from single rat liver. N indicates the number of rat liver in this group.

Table 4.128. Glutathione reductase activities of rat liver cytosols obtained from diabetes mellitus+7,12-dimethylbenz[a]anthracene+endosulfan+morin group (group 16).

Sample Number	Average Activity (nmol/min/mg protein)
1	81.6

Each cytosol fraction was prepared from single rat liver.

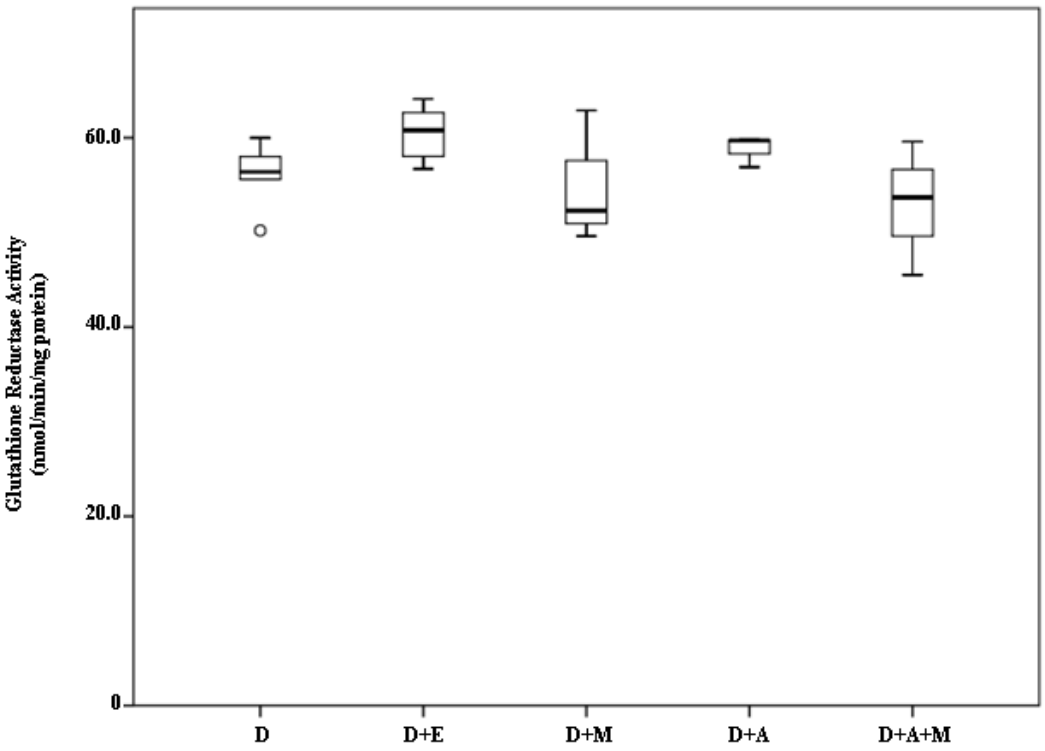


Figure 4.16. Glutathione reductase activities of rat liver cytosols obtained from group 9-16. D (Diabetes mellitus), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

4.2 7-Ethoxyresorufin O-Deethylase Activities of the Healthy Rats Lung Microsomes Obtained from DMBA and Morin Treated Groups

The administration of morin and DMBA caused significantly changes in cytochrome P4501A1 enzyme activities of rat liver microsomes, as mentioned before. Lung is another important organ in the mammals. In order to obtain information about the effects of morin and 7,12-dimethylbenz[a]anthracene treatments on cytochrome P4501A1 enzyme activity of rat lung, cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase (EROD) activities were measured in each rat lung microsomes obtained from control (group 1), morin (group 3), DMBA (group 5) and DMBA+morin (group 6) groups.

The average specific 7-ethoxyresorufin O-deethylase activity of rat lung microsomes obtained from the control group (group 1) was 4.0±0.2 pmol/min/mg protein (N=7) (Table 4.129). The administration of 7,12-dimethylbenz[a]anthracene

and morin did not significantly altered the EROD activities in treatment groups ($p<0.05$). The average EROD activities obtained from rats in morin (group 3), DMBA (group 5) and DMBA+morin (group 6) groups were 3.7±0.3 pmol/min/mg protein (N=7), 4.0±0.3 pmol/min/mg protein (N=7), and 4.1±0.3 pmol/min/mg protein (N=7), respectively (Table 4.130, 3.131, and 3.132). Morin, DMBA and DMBA+morin treatments did not alter the EROD activities in the rat lung tissues. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities of rat lung microsomes were given in Figure 4.17.

Table 4.129. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat lung microsomes obtained from control group (group 1).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	7	4.1
2	6	4.3
3	4	3.8
4	6	3.1
5	4	3.6
6	5	4.2
7	5	4.8
Average Activity±SEM N=7		4.0±0.2

Each microsome fraction was prepared from single rat lung. N indicates the number of rat lung in this group.

Table 4.130. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat lung microsomes obtained from morin group (group 3).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	5	3.5
2	4	3.1
3	5	3.7
4	4	3.3
5	3	3.4
6	7	5.2
7	5	3.4
Average Activity±SEM N=7		3.7±0.3

Each microsome fraction was prepared from single rat lung. N indicates the number of rat lung in this group.

Table 4.131. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat lung microsomes obtained from 7,12-dimethylbenz[a]anthracene group (group 5).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	5	4.1
2	4	3.4
3	5	4.1
4	4	3.0
5	5	4.3
6	5	5.3
7	5	3.9
Average Activity±SEM N=7		4.0±0.3

Each microsome fraction was prepared from single rat lung. N indicates the number of rat lung in this group.

Table 4.132. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities and protein concentrations of rat lung microsomes obtained from 7,12-dimethylbenz[a]anthracene+morin group (group 6).

Sample Number	Protein Concentration (mg/mL)	Average Activity (pmol/min/mg protein)
1	4	5.1
2	4	4.0
3	4	4.8
4	7	4.1
5	4	3.9
6	5	3.3
7	6	3.4
Average Activity±SEM N=7		4.1±0.3

Each microsome fraction was prepared from single rat lung. N indicates the number of rat lung in this group.

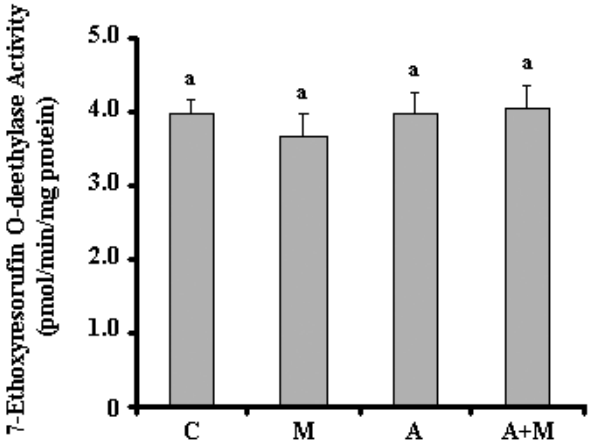


Figure 4.17. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities of rat lung microsomes. Homogenous subsets (groups did not differ significantly) were indicated by the same letters ($p<0.05$). C (Control), M (Morin), A (7,12-dimethylbenz[a]anthracene).

4.3 Western Blot Analysis of Cytochrome P450 Proteins of Healthy and Streptozotocin induced Diabetic Rats

The western blot analyses were performed by using the suitable cytochrome P450 antibodies having cross-reactivity with rat to determine the effects of DMBA, endosulfan, and morin on the specific cytochrome P450s at the protein level. Monoclonal anti-rat CYP1A1, polyclonal anti-rat CYP2E and monoclonal anti-rat CYP3A (Millipore, Billera, MA, USA) were used as primary antibodies and anti-rabbit IgG-ALP conjugated alkaline phosphatase was used as secondary antibody. Microsomal cytochrome P450 protein levels were quantified by densitometric analysis and the intensities of each band were quantified as relative peak area (R.P.A) by Scion Image software program.

In determination of cytochrome P4501A1 protein level by western blot analysis, 1:500 diluted monoclonal antibody (anti-rat CYP1A1 IgG) was used. All sample wells were loaded with 120 µg microsomal protein. Cytochrome P4501A1 associated 7-ethoxyresorufin O-deethylase activities of samples loaded were between 62 pmol/min/mg protein and 224 pmol/min/mg protein. The highest EROD activity of the samples loaded was 224 pmol/min/mg protein, however there were no cross reactivity in wells between cytochrome P4501A1 in rat liver microsomes and monoclonal anti-rat CYP1A1. This result showed that, microsomal CYP1A1 protein levels were not enough to be detected by western blot analysis.

In determination of cytochrome P4502B protein level by western blot analysis, 1:500 diluted monoclonal antibody (anti-rat CYP2B1/2BC.B/C4) was used. All sample wells were loaded with 120 µg microsomal protein. Cytochrome P4502B associated 7-pentoxyresorufin O-dependentylase activities of samples loaded were between 15.5 pmol/min/mg protein and 83.1 pmol/min/mg protein. Cross-reactivities between cytochrome P4502B in rat liver microsomes and monoclonal anti-rat CYP2B were observed in wells. However microsomal CYP2B protein levels of rat liver microsomes and their band intensities were not enough to be quantified by densitometric analysis.

In the determination of cytochrome P4503A4 protein level by western blot analysis, 1:500 diluted monoclonal antibody (anti-rat CYP3A4/3A1/3A11 IgG) was used. All sample wells were loaded with 160 µg microsomal protein. Cytochrome P4503A associated erythromycin N-demethylase activities of samples loaded were between 0.35 nmol/min/mg protein and 0.66 nmol/min/mg protein. Although the highest ERND activity of the samples loaded was 0.66 nmol/min/mg protein, cross reactivity between cytochrome P4503A in rat liver microsomes and monoclonal anti-rat CYP3A were not observed in wells. This result showed that, microsomal CYP3A protein levels were also not enough to be detected by western blot analysis.

In the determination of cytochrome P4502E1 protein level by western blot analysis, 1:500 diluted polyclonal antibody (anti-rat CYP2E1 IgG) was used. All sample wells were loaded with 80 µg microsomal protein. Cross-reactivities between cytochrome P4502E1 in rat liver microsomes and polyclonal anti-rat CYP2E1 were observed in wells and related image was given in Figure 4.18. CYP2E1 protein levels of rat liver microsomes were quantified by densitometric analysis. Cytochrome P4502E1 protein levels and respective CYP2E1 associated aniline 4-hydroxylase activities of samples loaded were given in Table 4.133. According to the densitometric analysis, band intensities of each group were similar to control group. The results of western blot analysis also confirmed the enzyme activity results and indicated that chemical treatments of rats did not significantly altered the CYP2E1 at protein level.

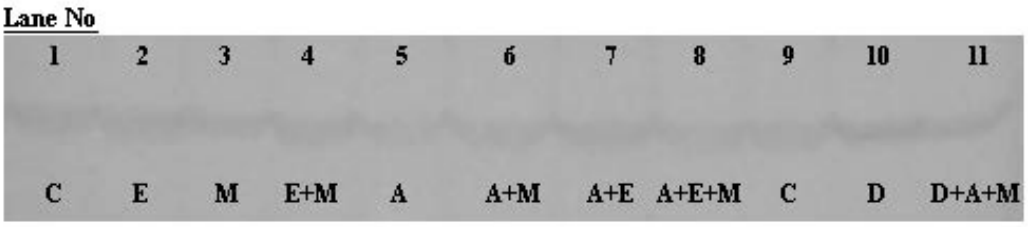


Figure 4.18. Immunochemical detection of microsomal cytochrome P4502E1 of rat livers. D (Diabetes mellitus), E (Endosulfan), M (Morin), A (7,12-dimethylbenz[a]anthracene).

Table 4.133. Cytochrome P4502E1 protein levels and respective CYP2E1 associated aniline 4-hydroxylase activities of rat liver microsomes loaded.

Lane No	Aniline 4-Hydroxylase Activity (nmol/min/mg protein)	Relative Peak Area (RPA)
1	0.71	778
2	0.79	884
3	0.64	738
4	0.71	883
5	0.89	688
6	0.86	739
7	0.86	843
8	0.88	778
9	0.73	852
10	0.73	947
11	0.43	726

4.4 Determination of Pathological Effects of Chemical Treatments on Healthy and Diabetic Rats

Histopathological studies were also carried out to observe the pathological effects of 7,12-dimethylbenz[a]anthracene (DMBA), endosulfan, and morin on healthy and diabetic rats. After dissecting, a small part of each liver and lung tissues was removed and fixed in 10% formaldehyde solution. Sections were prepared from each tissue as described in "Materials and Methods" and stained with hematoxylin and eosin in order to assess general histopathological changes.

4.4.1 Histopathological Studies of Liver Tissues

Histopathological studies were carried out for liver tissues obtained from each group. The pathological changes were interpreted by comparing the observations of

treatment groups with that of the control group. Figure 4.19 illustrates the appearance of liver cells in control group. In this section, portal space and the hepatocytes of rat liver tissues were seen normal in the control group. Figure 4.20 illustrates the appearance of liver cells in endosulfan group. In the livers of rats treated with endosulfan, congestion of sinusoids, vacuolated hepatocytes, and increase in the number of Kupffer cells were observed. In addition, the cells undergoing mitosis were found in endosulfan treatment group.

The histopathological studies indicated that morin treatment was not modified the appearance of the liver cell. The appearance of liver cells observed in morin group was similar to that in the control group (Figure 4.21). The number of Kupffer cells and congestion of sinusoids observed in endosulfan+morin group were lesser than that in endosulfan group. Moreover, vacuolated hepatocytes were not observed in endosulfan+morin group (Figure 4.22).

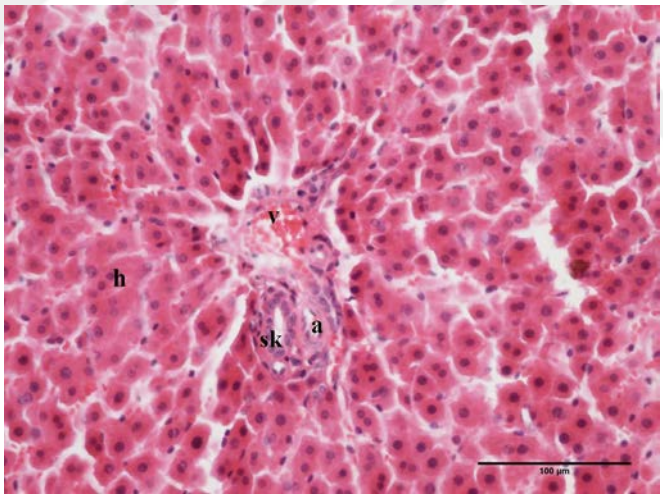
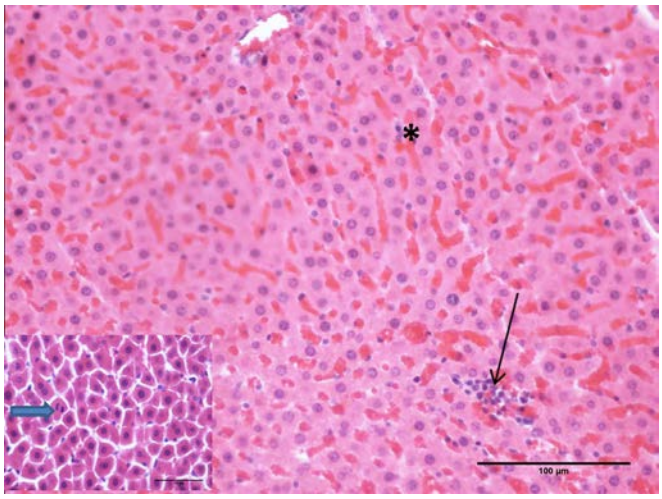
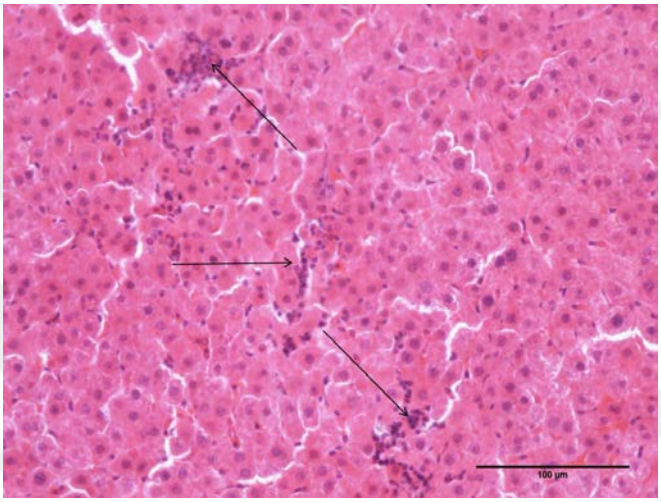


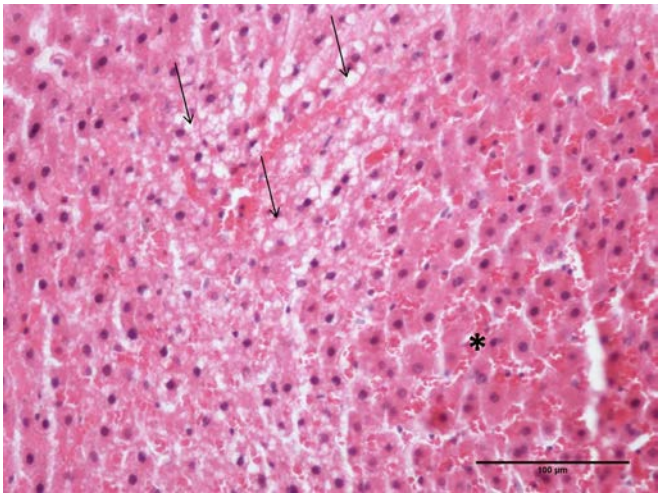
Figure 4.19. Representative liver section obtained from control group (group 1). Hepatocyte (h), bile duct (sk), vena porta branches (v), arterial branches (a).



A



B



C

Figure 4.20. Representative liver sections obtained from endosulfan group (group 2). A) Congestion of sinusoids (star marked), Kupffer cells (arrow marked). Hepatocytes undergoing mitosis (thick arrow marked). B) Kupffer cells (arrow marked) C) Congestion of sinusoids (star marked), vacuolated hepatocytes (arrow marked).

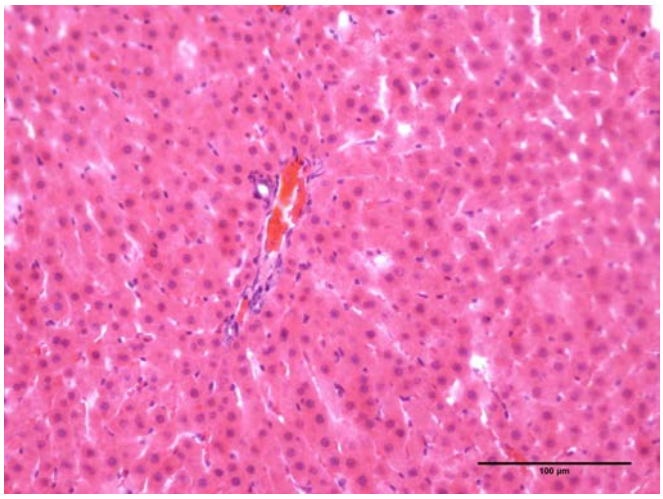


Figure 4.21. Representative liver section obtained from morin group (group 3).

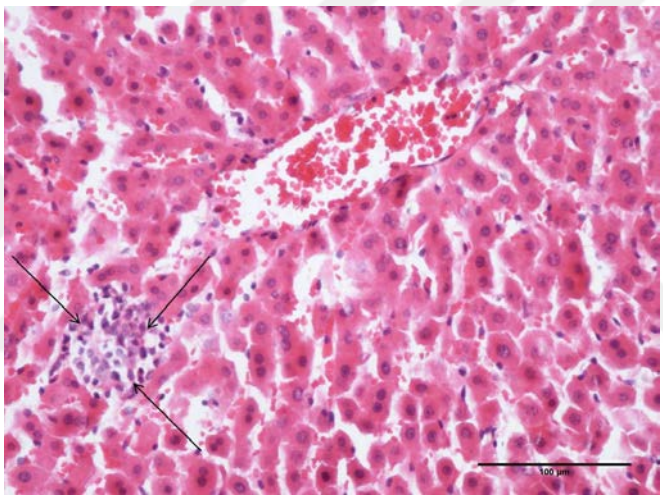


Figure 4.22. Representative liver section obtained from endosulfan+morin group (group 4). Kupffer cells (arrow marked)

A large number of inflammatory cells were observed in the livers of rats treated with DMBA in comparison with the control group (Figure 4.23A). When the image was enlarged, it was observed that the numbers of mononuclear cells were higher in the inflammation areas and some of the hepatocytes had hyperchromatic nucleus. In addition, the numbers of hepatocytes having double nuclei in the tissue sections of this group were higher than that of the control group (Figure 4.23B). Apoptotic bodies were also observed in liver tissues obtained from DMBA group (Figure 4.23C). In the liver tissues obtained from the rats treated with both DMBA and endosulfan, necrotic areas and disruption in hepatocytes were observed. In addition, the number of hyperchromatic hepatocytes and inflammatory cells increased (Figure 4.25). These results indicated that the level of the liver tissue

disruption obtained from this group was higher than the liver tissue obtained from rat treated with DMBA. In the liver tissues obtained from rats treated with both DMBA and morin, necrotic areas were not observed. Moreover, the number of inflammatory cells and hepatocytes having hyperchromatic nuclei in liver tissues of this group were less than that in the liver obtained from rats treated with DMBA (Figure 4.24). In the tissues obtained from the group treated with DMBA+endosulfan+morin, enlargement in sinusoids was observed. In this group, hyperchromatic nuclei of hepatocytes were distinctive however the numbers of inflammatory cells were less than DMBA group (Figure 4.26A). In addition, congestion of the liver sinusoids draws attention in the liver tissues obtained from this group (Figure 4.26B).

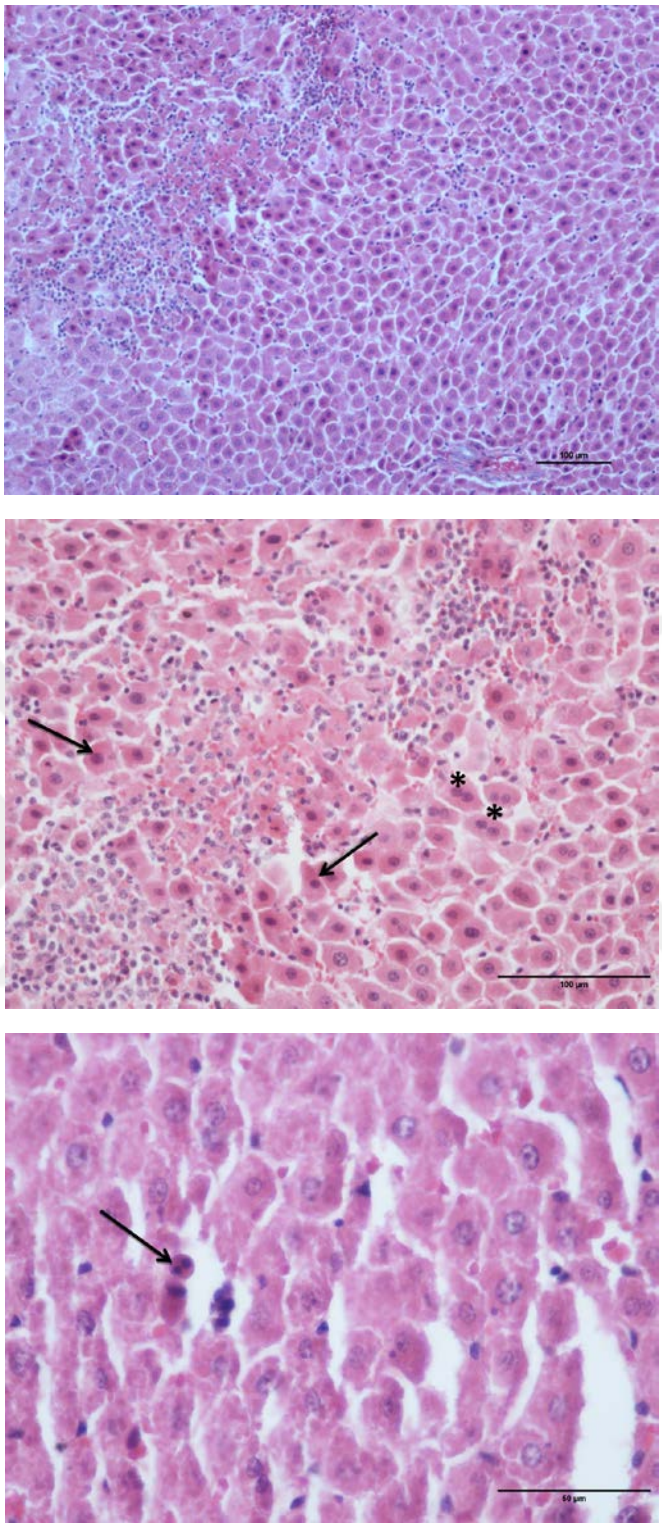


Figure 4.23. Representative liver sections obtained from DMBA group (group 5). B) Hyperchromatic nucleus (arrow marked), hepatocytes having double nuclei (star marked) C) Apoptotic bodies (arrow marked).

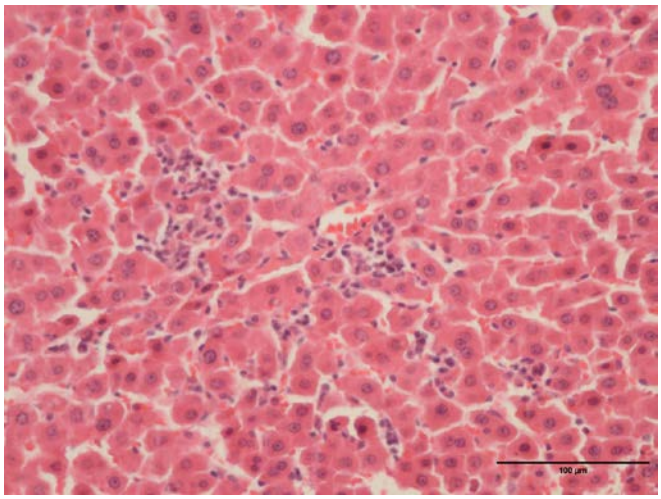


Figure 4.24. Representative liver section obtained from DMBA+morin group (group 6).

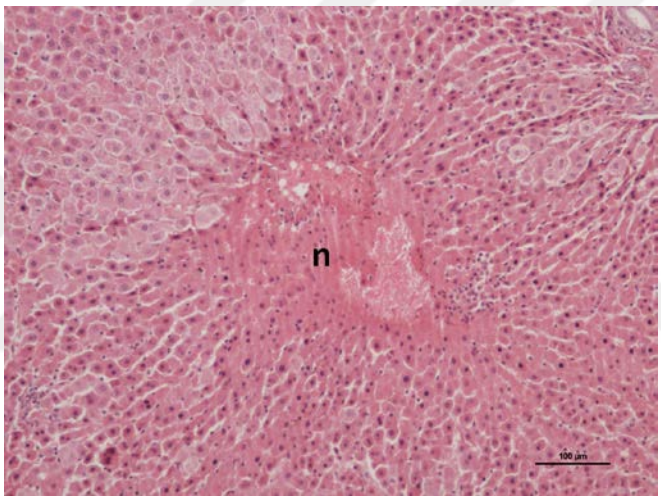
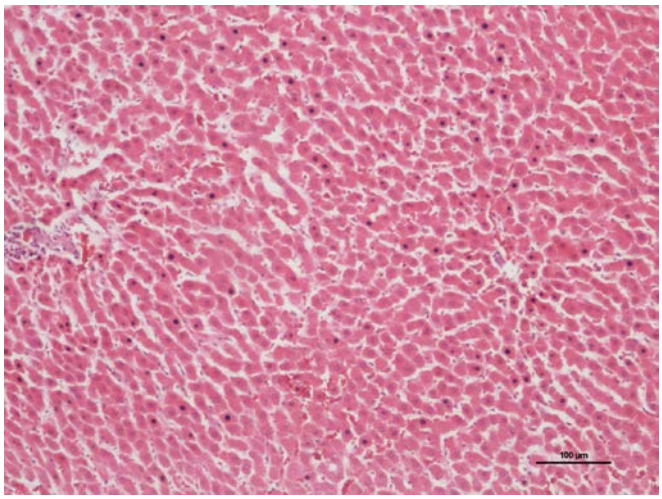


Figure 4.25. Representative liver section obtained from DMBA+endosulfan group (group 7). Necrotic areas (n)


A

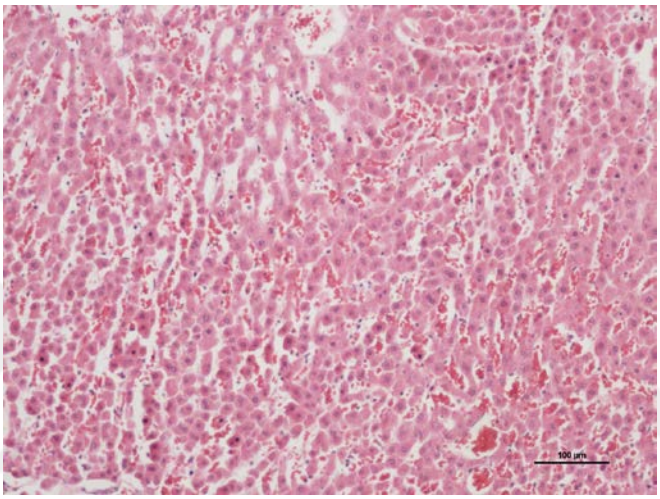
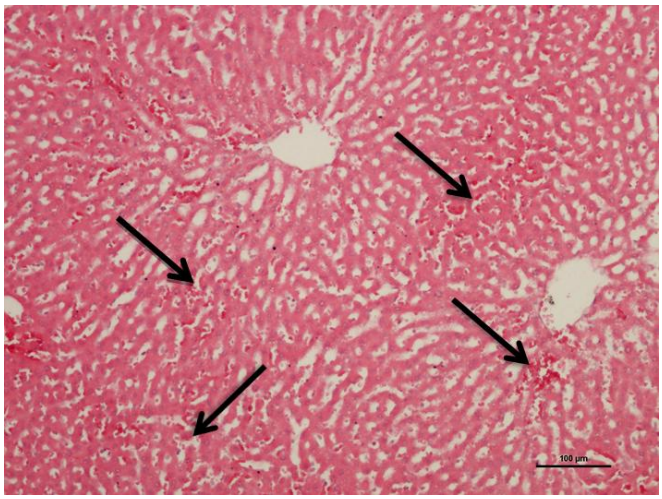

B

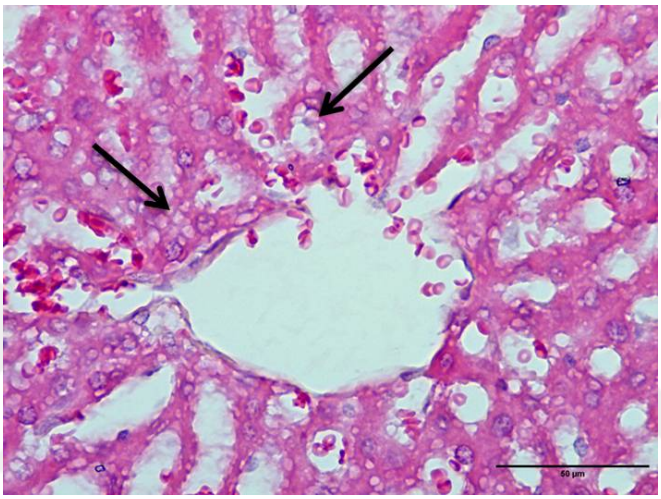
Figure 4.26. Representative liver sections obtained from DMBA+endosulfan+morin group (group 8).

In the liver tissues obtained from diabetes mellitus group, enlargement in sinusoids, congestion, hemorrhage (Figure 4.27A), and vacuolization in hepatocytes (Figure 4.27B) were observed. In addition, the number of Kupffer cells in the tissue sections of diabetes mellitus was higher than the control group (Figure 4.27C). In the tissues obtained from the diabetic group treated with endosulfan, bile duct poliferation in portal areas, enlargement in sinusoids, and hemorrhage were observed (Figure 4.28A). Furthermore, necrotic areas were seen in portal areas of hepatocytes (Figure 4.28B). Congestion of the liver sinusoids and hemorrhage in the liver tissue obtained from diabetes mellitus+morin group were less than that in the liver tissue obtained from diabetes mellitus (Figure 4.29). When the liver tissues obtained from diabetes mellitus+endosulfan+morin group were compared with diabetes mellitus+endosulfan group, it was observed that the level of the enlargement in sinusoids and hemorrhage decreased with morin treatment (Figure 4.30A). However,

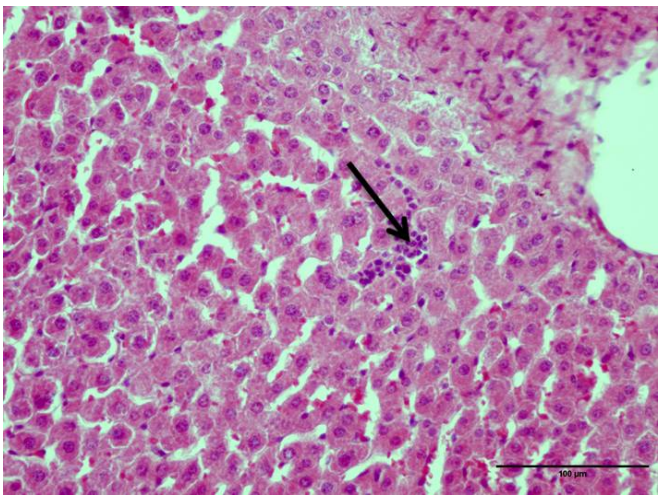
the vacuolization in hepatocytes and increase in the number of the Kupffer cells were observed in some areas (Figure 4.30B). Similar to diabetes mellitus+endosulfan group, congestion of the liver sinusoids, hemorrhage and vacuolization in hepatocytes were also observed in the liver tissues obtained from diabetes mellitus+DMBA group (Figure 4.31). In the liver tissues obtained from diabetes mellitus+DMBA+morin group, the level of hemorrhage decreased with morin treatment. However, the number of inflammatory cells increased (Figure 4.32A). Another decrease was observed in the vacuolization in hepatocytes. In the same group, large numbers of bile ducts were seen in portal areas (Figure 4.32B). In the liver tissue obtained from diabetes mellitus+DMBA+endosulfan group, a remarkable increase in the number of hepatocytes was observed. Another remarkable increase was observed in the connective tissues in portal area. Furthermore, hemorrhage and occurrence of bridging in portal area were seen in liver tissues obtained from this group (Figure 4.33). In the liver tissues obtained from diabetes mellitus+DMBA+endosulfan+morin group, congestions of the liver sinusoids and vena centralis were observed. In some areas, unusually increased number of Kupffer cells was observed.



A

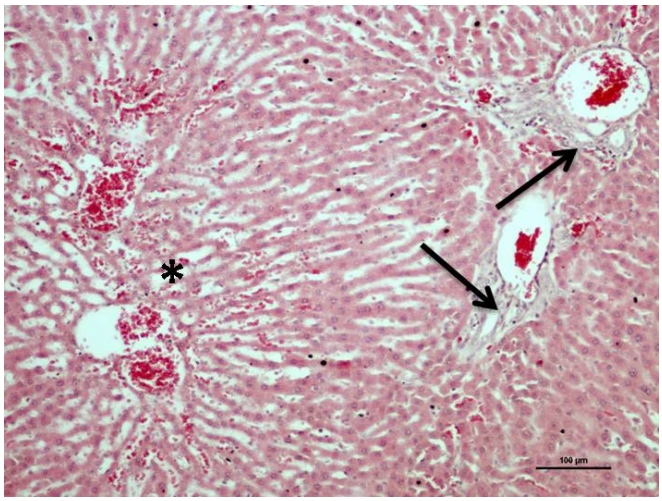


B

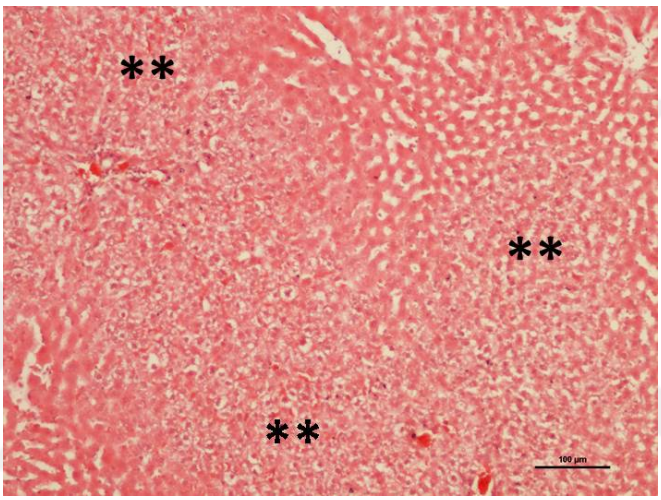


C

Figure 4.27. Representative liver sections obtained from diabetes mellitus group (group 9). A) Enlargement in sinusoids, congestion, hemorrhage (arrow marked) B) Vacuolization in hepatocytes (arrow marked) C) Kupffer cells (arrow marked).



A



B

Figure 4.28. Representative liver sections obtained from diabetes mellitus+endosulfan group (group 10). A) Bile duct poliferation in portal areas (arrow marked), enlargement in sinusoids (star marked) B) Occurrence of necrotic areas in portal areas (double star marked)

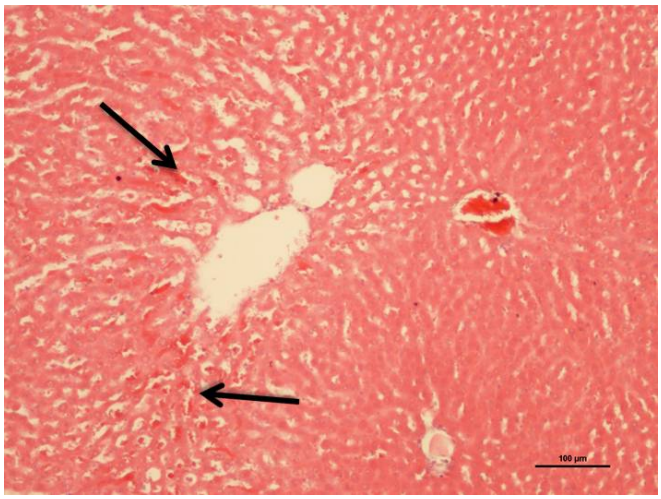
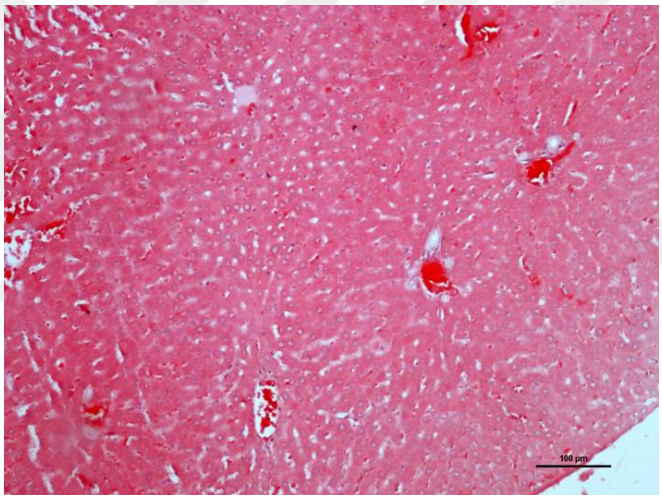
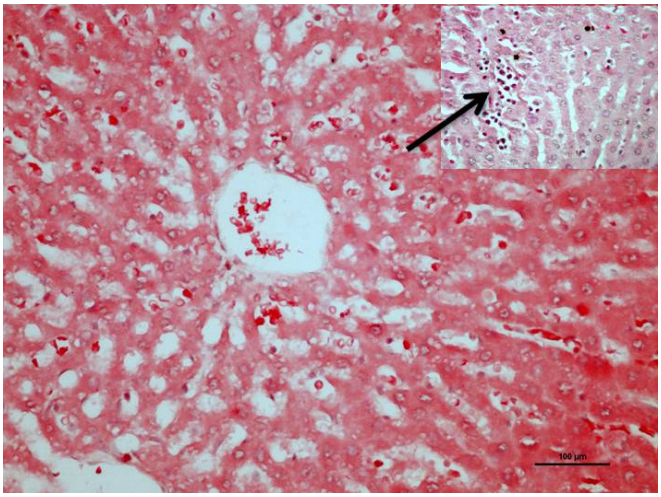


Figure 4.29. Representative liver section obtained from diabetes mellitus+morin group (group 11). Congestion of the liver sinusoids and hemorrhage (arrow marked)



A



B

Figure 4.30. Representative liver sections obtained from diabetes mellitus+endosulfan+morin group (group 12). B) Kupffer cells (arrows).

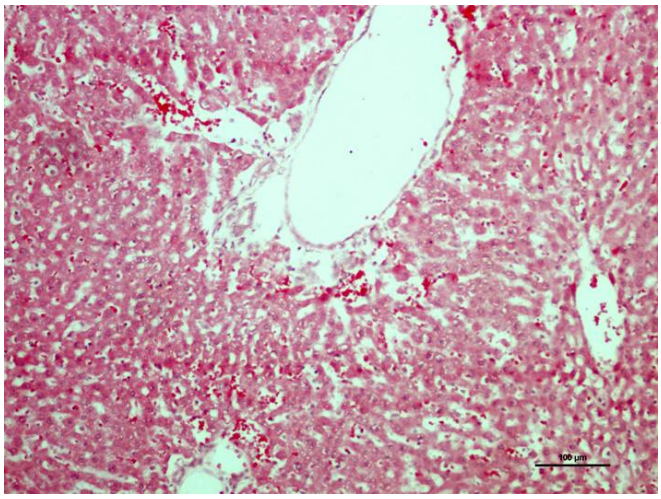
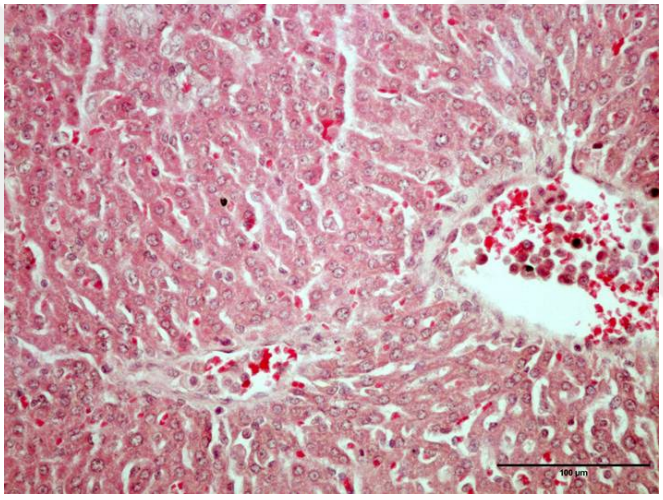
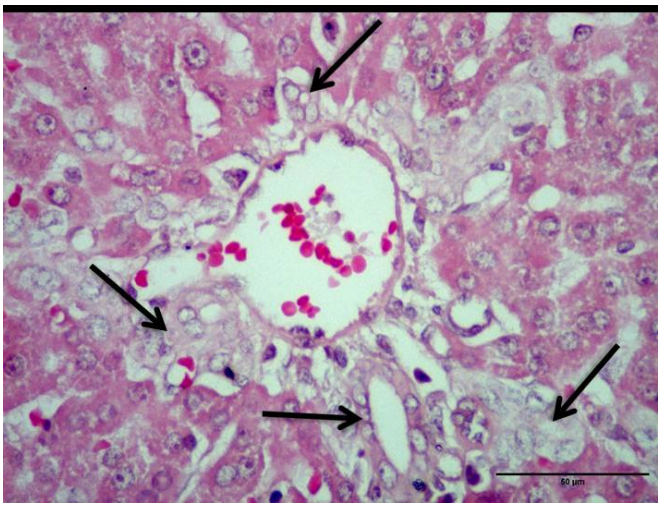


Figure 4.31. Representative liver section obtained from diabetes mellitus+DMBA group (group 13).



A



B

Figure 4.32. Representative liver sections obtained from diabetes mellitus+DMBA+morin group (group 14). B) Bile ducts in portal areas (arrow marked)

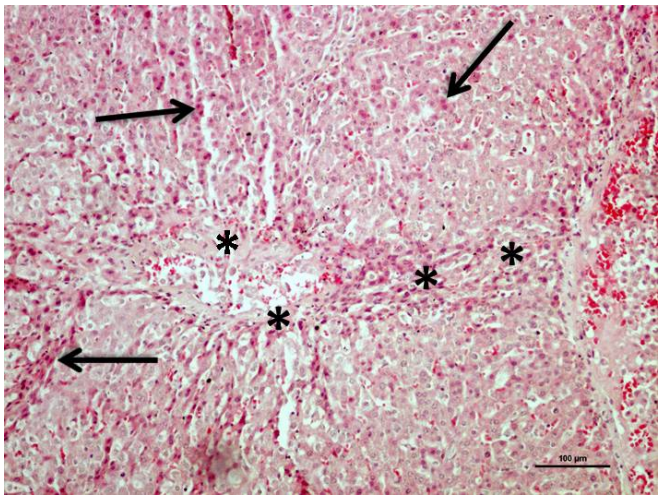
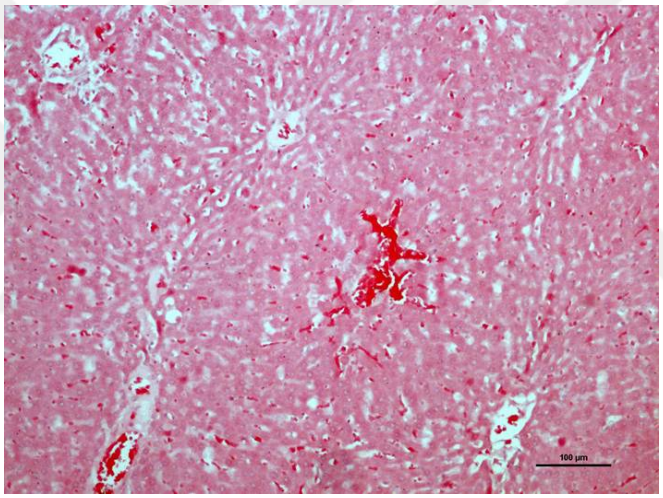
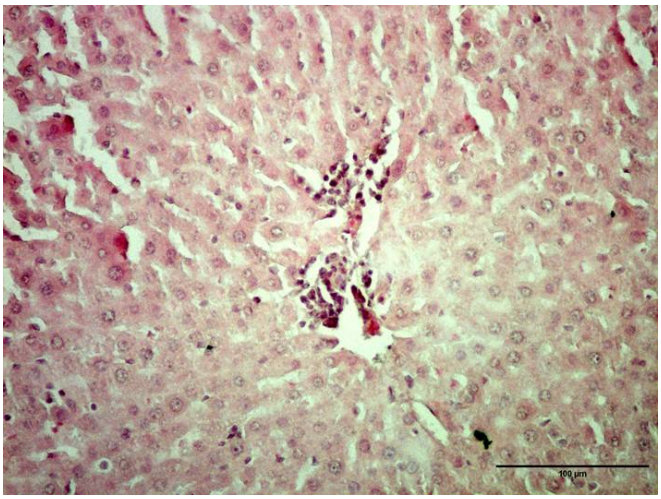


Figure 4.33. Representative liver section obtained from diabetes mellitus+DMBA+endosulfan group (group 15). Hepatocytes (arrow marked), bridging in portal area (star marked).



A



B

Figure 4.34. Representative liver sections obtained from diabetes mellitus+DMBA+endosulfan+morin group (group 16). Hepatocytes (arrow marked), bridging in portal area (star marked).

4.4.2 Histopathological Studies of Lung Tissues

Histopathological studies carried out for liver tissues indicated that the administration of DMBA caused histological damage in rat liver tissues. In addition, morin reduced the DMBA induced liver damage in DMBA+morin group. Therefore histopathological studies were also carried out for lung tissues obtained from DMBA, morin, and DMBA+morin treated groups to determine the pathological effects of these chemicals on another important organ, lung, in mammals. The pathological changes were interpreted by comparing the observations of treated groups with that of the control group. Figure 4.35 illustrates the normal appearance of lung cells in control group. The histopathological studies indicated that the appearances of the lung cells obtained from morin-treated group gave similar results as observed in the control groups (Figure 4.36). Figures 3.37 and 3.38 illustrate the appearance of lung cells obtained from DMBA-treated group. In the sections obtained from this group, increase in inflammatory cells, proliferation in hemorrhage, damage in parenchymal tissue, thickening of the walls between the alveoli were seen. Alveolar sacs were not distinguishable in the lung sections obtained from DMBA-treated group (Figure 4.37A and 4.37B). In addition, large number of macrophages were observed in bronchioles (Figure 4.38A) and alveoli (Figure 4.38B). Figure 4.39 illustrates the appearance of lung cells in DMBA+morin group. Hemorrhage was seen in some places of lung sections obtained from this group. In the sections obtained from DMBA+morin group, thickness of the walls between the alveoli was lower than that obtained from DMBA group. In addition, alveolar structures were more distinguishable in the lung sections obtained from DMBA+morin group compared to DMBA group and macrophages were not observed in bronchioles and alveoli in this group. Consequently, DMBA induced damage in the rat lung tissue and morin-treatment carried out to rats in DMBA+morin groups reduced the inflammation and hemorrhage. However morin treatment was not completely reduced DMBA caused tissue damage in the lungs.

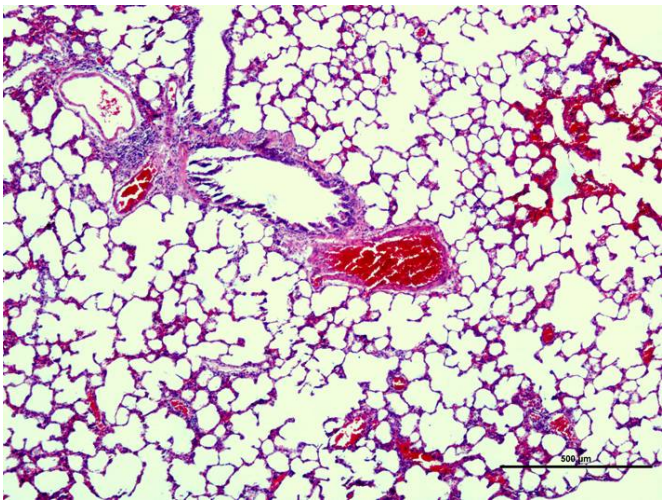


Figure 4.35. Representative lung section obtained from control group (group 1).

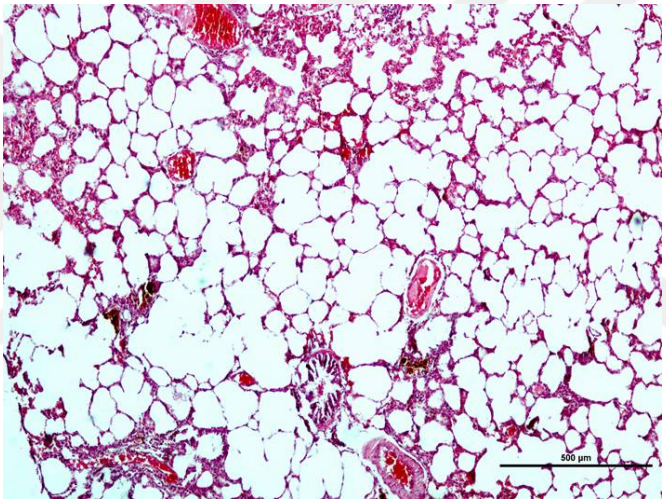


Figure 4.36. Representative lung section obtained from morin group (group 3).

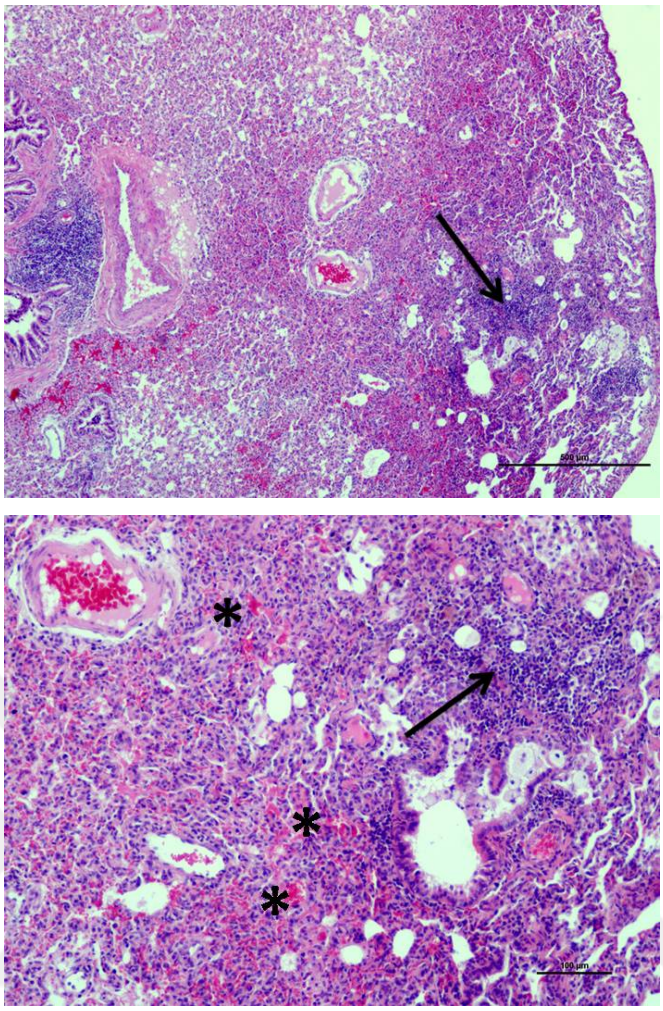


Figure 4.37. Representative lung sections obtained from DMBA group (group 5). Inflammatory cells (arrow marked), hemorrhage (star marked).

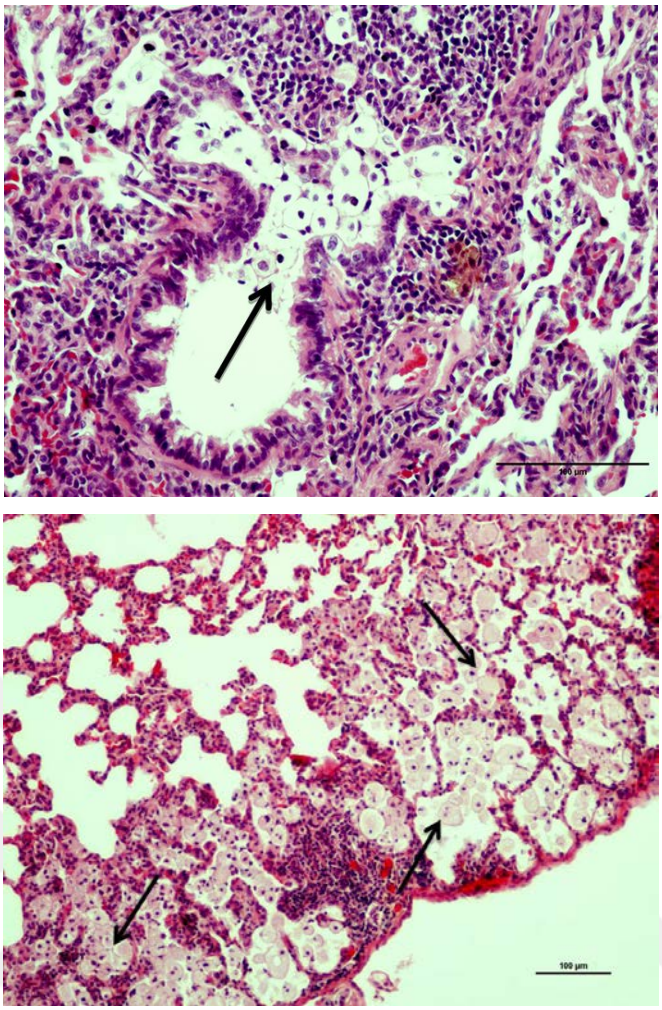


Figure 4.38. Representative lung sections obtained from DMBA group (group 5). Macrophages (arrow marked).

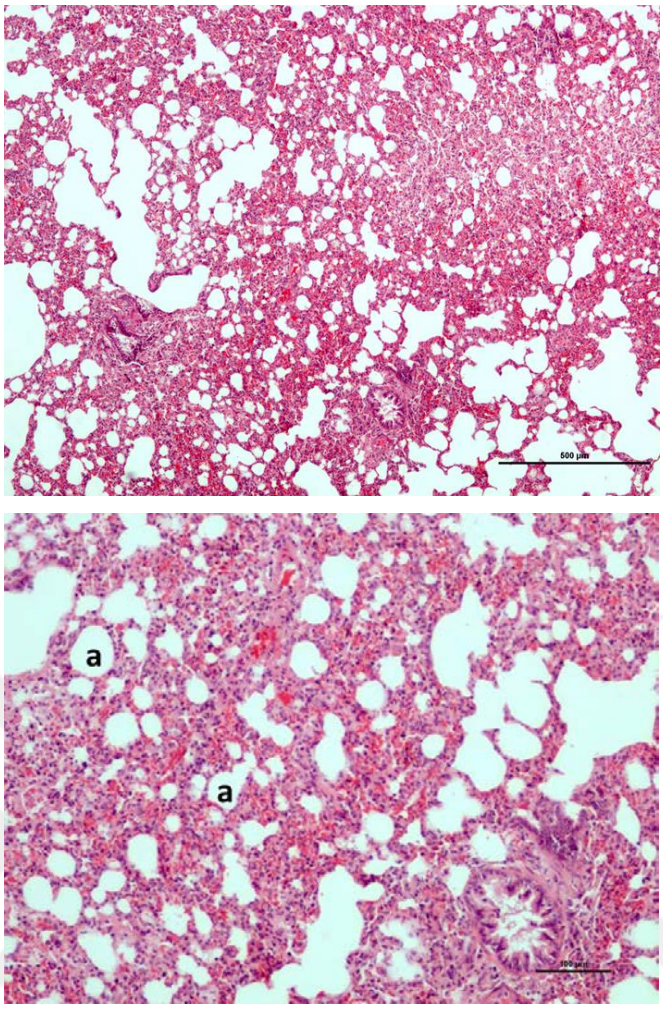


Figure 4.39. Representative lung sections obtained from DMBA+morin group (group 6). Alveolar structures (a).

5. DISCUSSIONS

All living organisms are exposed to many different xenobiotics in our daily lives. We may be exposed to these chemicals trough breathing air, drinking water, eating food and smoking cigarette. Some of these chemicals are beneficial, but some of them are toxic for us. We may be exposed to beneficial chemicals and toxic chemicals together in our daily lives. Today, it has been well known that beneficial chemicals may also cause toxic effects by inhibiting important enzyme catalyzed reactions as in the case of naringenin. Naringenin is a flavanone generally found in grapefruit. It has many beneficial roles including antioxidant, anti-inflammatory, anti-carcinogenic effects in living organisms. However, it is an inhibitor of cytochrome P4503A (CYP3A) (Lu et al., 2011). CYP3A is the major enzyme in the liver responsible from the metabolism of drugs. Inhibition of CYP3A with naringenin decreases metabolism of drugs and causes accumulation of CYP3A substrates in the body. These accumulated drugs may lead to toxic effects in overdose. Biochemical mechanism of this toxicity involves the inhibition of xenobiotic metabolizing enzyme activities. Some of the xenobiotics have roles in the stimulation of bioactivation reactions. It is therefore important to study effects of different chemicals on xenobiotic metabolizing enzymes.

Xenobiotics are metabolized by phase I and phase II reactions within the body. Oxidative reactions of phase I reactions are mainly catalyzed by cytochrome P450 depended monooxygenases. Cytochrome P450 (CYP) is a superfamily of proteins involved in oxidative metabolism of both endogenous (steroids, fatty acids, prostaglandins, biogenic amines, and retinoids) and exogenous compounds (drugs, alcohols, organic solvents, anesthetic agents, dyes, environmental pollutants, and chemicals) (Arinc and Bozcaarmutlu, 2003; Guengerich, 1990; Hodgson and Rose, 2007; Nebert and Gonzalez, 1987; Nelson, 2003; Zanger and Schwab, 2013). Cytochrome P450s are present in animals, plants, fungi, lower eukaryotes and bacteria. More than 20000 distinct sequences were named in 2013 according to Dr. Nelson's Home Page records (http://drnelson.uthsc.edu/CytochromeP450.html).

Among the cytochrome P450s, CYP1A is important since it is induced with environmental pollutants including polycyclic aromatic hydrocarbons, polychlorinated biphenyls (PCBs) and dioxins. CYP2B and CYP3A are important cytochrome P450 enzymes involved in xenobiotic metabolism and endogenous molecules such as hormones. CYP2E is another cytochrome P450 involved in xenobiotic metabolism including small molecules such as alcohols.

Phase II biotransformation reactions are conjugation reactions in the xenobiotic metabolism. Glutathione-S-transferases (GSTs) are a group of enzymes involved in the conjugation reactions of xenobiotics. They catalyze the conjugation of phase I metabolites, environmental carcinogens and epoxide intermediates with reduced glutathione (GSH) (Gallagher et al., 1996; Martínez- Gómez et al., 2006; Wu and Dong, 2012). During metabolic reactions, reactive oxygen species (ROS) are produced and these highly reactive molecules may interact with DNA or the other macromolecules present in the cell. Antioxidant enzyme activities including catalase, superoxide dismutase, glutathione reductase and glutathione peroxidase neutralize toxic effects of ROS (van der Oost et al., 2003).

Endosulfan and 7,12-dimethylbenz[a]anthracene (DMBA) are the toxic chemicals used in this study. These chemicals are chosen especially in this study because it is possible to be exposed both of these chemicals in our daily lives. We may be exposed to endosulfan by eating food contaminated with endosulfan, skin contact with contaminated soil, smoking cigarettes made from contaminated tobacco or by inhalation during its application (Lonsway et al., 1997; Oboh et al., 2011). DMBA is a methylated polycyclic aromatic hydrocarbon (PAH) present in the tobacco smoke and produced during incomplete combustion of carbon containing compounds (Girolami et al., 2008). It is also used as a model chemical to create cancer in the experimental animals.

In the near future it is possible to use plant derived antioxidant molecules in our daily lives in the prevention of diseases. However, the role of plant derived materials on the toxic effects of chemicals has not been well defined. Morin (3,5,7,2′,4′-pentahydroxyflavone) is an antioxidant molecule present in onion, apple, almond (*Prunus dulcis*), fustic (*Chlorophora tinctoria*), guava (*P. guajava L.*), red

wine, and Osage orange (Aggarwal and Shishodia, 2006; Nandhakumar et al., 2012; Romero et al., 2002; Subhashinee et al., 2006). Mechanism of chemopreventive effect of morin on toxic effects of endosulfan and DMBA was studied in this study in normal healthy rats and streptozotocin induced diabetic rats.

In this study, 120 rats were used to determine the effect of endosulfan, DMBA, morin and their double and triple combinations on xenobiotic metabolizing enzyme activities in normal and diabetic rats. The effects of these chemicals on the cytochrome P450 system were also measured at the protein level by using monoclonal and polyclonal antibodies produced against CYP1A, CYP2B, CYP2E and CYP3A. In addition to biochemical parameters, histopathological studies were carried out to show pathological changes.

In this study, test chemicals were given by gavage. The same route of exposure was specifically chosen for endosulfan, DMBA and morin. The effects of these chemicals were determined in the liver. Liver is the major site for the metabolism of xenobiotics. It has been reported that endosulfan causes liver tissue damage independent of the route of exposure (Uboh et al., 2011). Gastrointestinal route can be considered as the major absorption route for endosulfan since we are mainly exposed to endosulfan by eating contaminated foods. From the gastrointestinal tract, endosulfan is directly transferred to liver. It is metabolized into its toxic metabolite endosulfan sulphate in the liver (Silva and Gammon, 2009). Liver is considered as a main target organ for the endosulfan toxicity (Dubois et al., 1996). DMBA is a procarcinogen molecule. It requires metabolic activation to its ultimate carcinogenic metabolite, DMBA-3,4-dihydrodiol-1,2-epoxide by liver enzymes (Kleiner et al., 2004). Most of the activated metabolites are formed in the liver. Therefore liver plays a major role in the tumour initiating process of DMBA (Girolami et al., 2008; Szaefer et al., 2014).

In our study, endosulfan increased CYP1A associated ethoxyresorufin O-deethylase (EROD), CYP3A associated erythromycin N-demethylase and CYP2E associated aniline 4-hydroxylase activities in the liver of normal healthy rats. Endosulfan is metabolized in the liver to reactive intermediates by cytochrome P450 system (Silva and Gammon, 2009). It has been shown that CYP3A and CYP2B are

the primary enzymes catalyzing the metabolism of endosulfan in human liver (Lee et al., 2006). In an acute study, endosulfan increases CYP2B and CYP3A protein levels in human hepatocyte cell cuture system (HepG2 cells) in a dose dependent manner after 72 hr exposure period (Casabar et al., 2006; Casabar et al., 2010). This induction in CYP3A and CYP2B is mediated by pregnane X receptor (PXR) (Casabar et al., 2010). In our study, CYP3A activities significantly increased in rat liver treated with endosulfan compare to control activity. This result is in agreement with those of Casabar et al., (2006), Dubois et al., (1996), Lee et al., (2006), Lemaire et al., (2004). It is clear that endosulfan regulates its own metabolism by increasing CYP3A protein level. CYP3A is the most abundant cytochrome P450 in the liver and it plays important role in the metabolism of xenobiotics including drugs and endogenous molecules such as hormones (Casabar et al., 2010; Usmani et al., 2003). It has been reported that testosterone metabolism and clearance rate increase with endosulfan treatment in rodents (Casabar et al., 2010; Singh and Pandey, 1989; Wilson and Leblanc, 1998). It is clear that increase in CYP3A level with endosulfan treatment increases its own metabolism and may modify the metabolism of CYP3A related substrates including drugs and hormones.

In this study, CYP2B associated pentoxyresorufin O-depenthylase (PROD) activity was not affected from endosulfan administration. There are contradictory results in the literature about the role of CYP2B in the endosulfan metabolism. The results obtained in this study are in agreement with the study of Kocarek et al., 1991. In their study, endosulfan induced neither CYP2B1 nor CYP2B2. Endosulfan at the doses of 5 mM increased PROD activity in HepG2 cells (Dehn et al., 2005). In another acute study carried out in human hepatocyte, CYP2B6 is reported as one of the cytochrome P450s responsible from the metabolism of endosulfan (Casabar et al. 2006). CYP2B6 is expressed in the human liver. There is no CYP2B6 expression in rat liver. However, CYP2B1 and CYP2B2 are expressed cytochrome P450s in rat liver (Martignoni et al., 2006). The difference between human and rat results can be explained with interspecies differences or route of drug administration.

CYP1A is another cytochrome P450 having a role in the pesticide metabolism. Chlordane, DDT, aldrin, kepone and toxaphene increase CYP1A associated EROD activities in rats (Bozcaarmutlu et al., 2014b; Nims et al., 1998;

Oropeza-Hernandez et al., 2003). CYP1A1 and CYP1A2 are the members of CYP1A present in rat, mouse, dog and human (Martignoni et al., 2006). CYP1A amino acid sequence is highly conserved between species and more than 80% amino acid sequence identity have been reported between man and rats (Martignoni et al., 2006). In this study, the highest increase in enzyme activities was determined in CYP1A1 related ethoxyresorufin O-deethylase (EROD) activity in endosulfan treated rats. The study related with the role of CYP1A in endosulfan metabolism is restricted with the study of Dehn et al (2005). Elevated EROD activities have been reported in HepG2 cells treated with 5 mM endosulfan after 24 hour of exposure (Dehn et al., 2005). The results of our study suggest that CYP1A also participates endosulfan metabolism. CYP1A is responsible from the metabolism of toxic chemicals and it converts toxic chemical to more toxic metabolites. CYP1A induction may have role in the toxic metabolite formation and endosulfan toxicity.

CYP2E associated aniline 4-hydroxylase activity increased in the endosulfan treated group with respect to control group, though this increase was not statistically significant. Elevated aniline 4-hydroxylase activities have been shown in rodents treated with endosulfan in the other studies (Narayan et al., 1990; Robacker et al., 1981; Silva and Gammon, 2009). CYP2E have a role in metabolism of small xenobiotics including ethanol and drugs. It has important roles in both detoxification and bioactivation of xenobiotics. Although its role is less than the other cytochrome P450s, the role of CYP2E was determined in the endosulfan metabolism in this study.

Endosulfan metabolism produces highly reactive free radicals that cause oxidative stress (Sohn et al., 2004). It has been reported that endosulfan administration modifies antioxidant enzyme activities such as superoxide dismutase, catalase, glutathione peroxidise and glutathione S-transferase (Bebe and Panemangalore, 2003; El-Shenawy and Nahla, 2010; Kalender et al., 2005; Pal et al., 2009). However, glutathione S-transferase, catalase and glutathione reductase activities were not affected from endosulfan treatment in this study. It is clear that the amount of free radical generated from endosulfan dosage is tolerated with available antioxidant enzyme activities.

Besides the biochemical parameters, histopathological analyses were also performed in the liver tissues of endosulfan treated rats. Endosulfan treatment caused congestion of sinusoids. In addition, vacuolated hepatocytes and the cells undergoing mitosis were observed. In addition, the number of Kupffer cells increased in liver tissue sections of endosulfan treated rats. It is clear that endosulfan treatment modified the appearance of normal liver cells.

In this study, second test chemical was 7,12-dimethylbenz[a]anthracene (DMBA). DMBA is a methyl substituted polycyclic aromatic hydrocarbon derived from benz[a]anthracene (Shou et al., 1996b). It is widely used as a model chemical carcinogen in animal studies. Carcinogenic activity of DMBA is related with the metabolic activation of DMBA by cytochrome P450 enzymes to reactive intermediates such as DMBA-3,4-dihydrodiol-1,2-epoxide (Kleiner et al., 2004; Lakshmi and Subramanian, 2014; Miyata et al., 2001). This epoxide binds to DNA and initiates DNA adduct formation and carcinogenesis (Miyata et al., 2001). In our study, statistically significant induction in cytochrome P450 activities were measured in CYP1A associated EROD and MROD and CYP3A associated erythromycin N-demethylase activities in rats treated with DMBA. It has been reported that DMBA is mainly metabolized in the liver by CYP1A (Girolami et al., 2008). Besides these cytochrome P450 enzyme, CYP2B6, CYP2C9, CYP2C8, CYP2E1, CYP3A4, and CYP3A5 are also involved in DMBA metabolism at different degrees in human liver (Lakshmi and Subramanian, 2014; Lambard et al., 1991; Szaefer et al., 2011). In our study, CYP2B related PROD activity was not affected from DMBA treatment. There are contradictory results about the induction of PROD activity in the literature. In rat liver, PROD activity increases with 25 mg/kg DMBA administration. But the induction is much less than induction of EROD (Szaefer et al., 2011). Significant induction is reported for PROD activity in mammary tissue of rats (Lakshmi and Subramanian, 2014). At high doses such as 30 mg/kg as we used in our study, PROD activity is not affected from DMBA administration in mice (Liu et al., 2011).

In our study, DMBA treatment also induced glutathione S-transferase in normal rats. Glutathione S-transferases catalyze the conjugation reactions of many xenobiotics including epoxides. The role of GST in the detoxification of DMBA has been shown in different studies (Coles and Ketterer, 1999; Szaefer et al., 2011). In

these studies, GST activities increase in rats treated with DMBA. As a result of phase I reactions DMBA is metabolized into epoxides and hydroxydiols. It is clear that some of the metabolites of DMBA are metabolized by GSTs. In our study, antioxidant enzyme activities are also measured in rat liver. Catalase and glutathione reductase enzyme activities also increased with DMBA treatment. Catalase plays a role in the removal of hydrogen peroxide to oxygen and water. Glutathione reductase has a role in the regeneration of reduced glutathione and regulation of GSH/GSSH ratio. The increase in catalase and glutathione reductase activities indicates that DMBA administration stimulates antioxidant enzyme activities. Antioxidant enzyme activities are induced in the presence of free radicals. It is clear that free radical generation increases with DMBA treatment. Similar results have been also reported for catalase and glutathione reductase activities in DMBA treated rats (Kaur and Arora, 2013; Nanta and Kale, 2011).

In histopathological analysis, a large number of inflammatory cells were observed in the livers of rats treated with DMBA in comparison with the control group. In addition, the numbers of hepatocytes having hyperchromatic nucleus and double nuclei increased in the tissue sections of DMBA treated rats. Besides these, apoptotic bodies were also observed in liver tissues obtained from DMBA group. It is clear that DMBA treatment causes tissue damage in the liver of rats.

Combine effects of endosulfan and DMBA were tested on xenobiotic metabolizing enzyme activities for the first time in this study. EROD activities of endosulfan+DMBA group were higher than those of endosulfan alone. MROD activities of endosulfan+DMBA group were higher than those of endosulfan and DMBA alone. Aniline 4-hydroxylase activities of endosulfan+DMBA group were slightly higher than those of endosulfan and DMBA alone. Aniline 4- hydroxylase activities of endosulfan+DMBA group were significantly different from those of the control. GST activities of endosulfan+DMBA group were significantly different from those of endosulfan alone, but GST activities of endosulfan+DMBA group were not different from those of DMBA alone. Similar to GST activity, catalase and glutathione reductase activities of endosulfan+DMBA group were significantly different from those of endosulfan. However these activities were less than those of DMBA alone. Higher activities in endosulfan+DMBA group than endosulfan and

DMBA alone were found in EROD, MROD and aniline 4-hydroylase activities. It is clear that stimulation of CYP1A and CYP2E enzyme activities increases in the presence of both of these chemicals. However, the co-administration of endosulfan and DMBA produced activities less than the additive effect. Glutathione S-transferase, catalase and glutathione reductase activities were less than those of DMBA alone. GST and antioxidant enzyme activities were required for detoxification of toxic metabolites. The increase in the toxic effect with co-administration of endosulfan and DMBA was also observed in histopathological analysis. Necrotic areas and disruption in hepatocytes were observed. In addition, the number of hyperchromatic hepatocytes and inflammatory cells was higher in the tissue sections obtained from co-administration of endosulfan and DMBA group than endosulfan and DMBA alone.

Recently, studies have focused on cancer chemoprevention with plant derived natural compounds. The strategy bases on the modulation of xenobiotic metabolizing enzyme activities by plant derived molecules (Girolami et al., 2008). Morin is a flavanoid present in many plants including onion. In this study, morin was specifically chosen since it has minimal toxicity even at higher dose (Abuohashish et al., 2013). In the literature, inhibitory effect of morin has been shown on ECOD activity *in vitro* study (Moon et al., 1998). Recently, the studies are mainly focused on the metabolism of flavonoids to understand the mechanism of chemoprotective role of flavonoids in the prevention of carcinogenesis. It has been reported in several studies that flavonoids can either inhibit or induce metabolic enzymes depending on their structure, dosage and the experimental conditions (Choi et al., 2006; Hodek et al., 2002; Hong-Mook and Jun-Shik, 2009; Shin et al., 2006). Several flavonoids by themselves are inducers of CYP1A1 mRNA (Androutsopoulos et al., 2010; Chatuphonprasert et al., 2010). They are metabolized by CYP1A enzyme and act as agonist for aryl hydrocarbon receptor (AhR) (Chatuphonprasert et al., 2010). Avalibility of hydroxyl groups in the structure of flavonoids increase their chance to be metabolized by cytochrome P450 system (Androutsopoulos et al., 2010; Chatuphonprasert et al., 2010). In our study, morin increased CYP1A related EROD and MROD activities in morin treated rats. The result of this study suggests that CYP1A may involve in the metabolism of morin. The other xenobiotic metabolizing enzyme activities were not affected from the morin treatment. Similarly, morin

administration at the doses of 30 mg/kg is not modified antioxidant enzyme activities including catalase (Subash and Subramanian, 2009). In addition, the histopathological studies indicated that morin treatment was not modified the appearance of the liver cell. Normal appearance of the liver after morin administration has also been reported by Subash and Subramanian (2009).

Co-administration of morin and endosulfan increased CYP1A associated EROD activity compare to control activity. But, elevated activity was not different from those of endosulfan and morin administration alone. EROD activity of DMBA+morin group was significantly different from the control activity. In addition, morin administration together with DMBA increased EROD activity. However, additive effect was not observed in EROD activity in this group. The highest EROD activity was observed in the case of triple combination of these chemicals. Elevated EROD activities with the combinations of morin indicate that morin, endosulfan and DMBA stimulate the synthesis of CYP1A in rat liver. We observed stimulatory effects of these chemicals when we gave them alone. We expected to see additive effect in the combinations of these chemicals. However, EROD activities measured in triple administration of these chemicals were less than the additive effect. Similar increase in MROD activity was observed in DMBA + morin and DMBA + endosulfan + morin groups. The additive effect was not observed, though MROD activities increased with double (endosulfan + morin) and triple administration of chemicals (DMBA + endosulfan + morin). The reason might be related with AhR receptor. CYP1A induction is under the control of AhR receptor. Molecules may compete with each other to bind the same receptor side and the presence of the other molecules may prevent interaction of the other with AhR receptor.

Aniline 4-hydroxylase activities were decreased in endosulfan + morin and DMBA + morin groups than those of endosulfan and DMBA alone. Aniline 4-hydroxylase activities in triple administration of these chemicals were not statistically different from endosulfan and DMBA administration alone. Similarly, morin administrations together with endosulfan and DMBA were not produced significant changes in erythromycin N-demethylase activity. Morin treatment decreased GST activities DMBA + morin and DMBA + endosulfan + morin groups

compare to DMBA alone. Similar decrease was also observed in catalase and glutathione reductase activities. This decrease in antioxidant enzyme activities might be related with radical scavenger role of morin. The studies related with the co-administration of DMBA and morin are limited and restricted with few studies. In the studies of Nandhakumar et al., (2012), antioxidant enzyme activities decrease with DMBA treatment. Morin treatment restores these activities. In the studies of Yang et al., (2013), inhibition of CYP3A activity with morin has been shown in DMBA treated rats. In the liver tissues obtained from rats treated with both DMBA and morin, necrotic areas were not observed. Moreover, the number of inflammatory cells and hepatocytes having hyperchromatic nuclei in liver tissues of this group was less than that in the liver obtained from rats treated with DMBA. In the tissues obtained from the group treated with DMBA+endosulfan+morin, enlargement in sinusoids was observed. In this group, hyperchromatic nuclei were distinctive however the number of inflammatory cells was less than DMBA group. It is clear that endosulfan and DMBA stimulates inflammation in the liver tissues and morin reduce their effects. The results of this study indicate that morin administration increased metabolic elimination of endosulfan and DMBA by inducing CYP1A activity and reduced elevated antioxidant enzyme activities by its own radical scavenger role. Histopathology results supported biochemical analysis that morin reduced toxic effects of endosulfan and DMBA in the liver cells.

In this study, the effects of endosulfan, DMBA and morin were determined in the presence of metabolic disease, diabetes mellitus. Experimental diabetes was induced in diabetic rats groups by single interiperitonal administration of streptozotocin (60 mg/kg). Streptozotocin is a well-known drug used to induce experimental diabetes in rodents to investigate metabolic and pharmacological changes associated with diabetes (AlSharari et al., 2014; Kamboj et al., 2010; Lenzen; 2008). Streptozotocin (60 mg/kg) leads to destruction of the Langerhans islet beta cells and induces experimental diabetes mellitus in 2-4 days (Akbarzadeh et al., 2007). In our study, animal mortality was not observed in normal rat groups. However, % 58 of rats present in diabetic rats groups was died throughout the chemical administration period. In our study, the xenobiotic metabolizing enzyme activities were measured in the liver of 26 diabetic rats. High mortality rate has been generally observed in experimentally induced diabetes in rodents. In our study, high

mortality rates were generally observed in the double and triple chemical combination groups in diabetic rat groups. In these groups, mortality may result from the toxic effect of chemicals, gastrointestinal administration of chemicals by gavage or diabetes itself.

Diabetes mellitus is characterized with several clinical metabolic problems including hyperglycemia. Xenobiotic metabolizing enzyme activities are also affected from diabetes. In our study, the induction of diabetes mellitus by intraperitoneal injection of streptozotocin significantly increased EROD, MROD, PROD and erythromycin N-demethylase activities with respect to those of control group of normal rats. The increase in CYP1A, CYP2B, CYP3A and CYP2E proteins have been shown in different studies in streptozocin induced diabetic rats (Barnett et al., 1993; Kim et al., 2005; Raza et al., 1996; Raza et al., 2000; Shimojo et al., 1993; Sindhu et al., 2006; Thummel et al., 1990). Besides the cytochrome P450 system, glutathione S-transferase and antioxidant enzyme activities, catalase and glutathione reductase, increased in streptozotocin induced diabetic rats in our study. The increase in free radical production and impaired antioxidant system are widely observed in diabetes mellitus (Baynes et al., 1999; Chang et al., 1993; Maritim et al., 2003; McLennan et al., 1991; Saxena et al., 1993). The increase in the liver glutathione S-transferase and antioxidant enzyme activities have been reported in different studies in streptozotocin induced diabetic rats (Aragno et al., 1999; Caballero et al., 2000; Raza et al., 1996). In addition, severe tissue damage including enlargement in sinusoids, hemorrhage, and vacuolization in hepatocytes was observed in histopathological analysis of liver tissues of diabetic rats. In addition, the number of kupffer cells in the tissue sections of diabetic rats was higher than those of the control group. It is clear that diabetes itself causes tissue damage.

Diabetic individuals are also exposed to pollutants in their daily lives. The effects of endosulfan and DMBA alone on xenobiotic metabolizing enzyme activities were studied in diabetic rats for the first time in this study. In streptozotocin induced diabetic rats, administration of endosulfan and DMBA increased EROD and MROD activities as observed in normal rat groups. However, erythromycin N-demethylase and glutathione S-transferase activities decreased in diabetic rat groups with the administration of endosulfan and DMBA. Aniline 4-hydroxylase, catalase and

glutathione reductase activities were not affected from endosulfan and DMBA treatments. In histopathological analysis, severe tissue damages including hemorrhage, vacuolization in hepatocytes and necrotic areas were detected in the endosulfan and DMBA treated rats. Tissue damage increased in the diabetic rats treated with co-administration of endosuldan and DMBA. Furthermore, hemorrhage and occurrence of bridging in portal area were seen in liver tissues obtained from this group.

In this study, the effects of morin on the xenobiotic metabolizing enzyme activities were also studied in streptozotocin induced diabetic rats. The effects of morin on the complications of diabetes have been studied in different studies (Fang et al., 2003; Ola et al., 2014). Treatment of diabetes is generally achieved by insulin or drugs such as glibenclamide. These drugs have many side effects. The protective effects of morin against diabetes have been shown in rats (AlSharari; 2014; Fang et al., 2003; Ola et al., 2014). The effect of morin was determined on cytochrome P450 system for the first time in diabetic rats in this study. Morin administration increased CYP1A associated EROD and MROD activities and CYP2B related PROD activities though these increase in the activities were not statistically different from those of the diabetic rats. Oxidative stress related enzyme activities, glutathione S-transferase, catalase and glutathione reductase decreased in morin treated diabetic rat group. This decrease in the antioxidant activities might be related with the radical scavenger role of morin. The effect of morin on catalase has been determined in streptozotocin induced diabetic rats (Ola et al., 2014; Zhang et al., 2009). In these studies, catalase activity increases with the treatment of morin in diabetic rats. If morin is an antioxidant molecule and effective free radical scavenger, the cell does not need to produce new antioxidant enzymes. The results of histopathological analysis also supported the results of biochemical analysis. The tissue damage observed in endosulfan and DMBA treatments was reduced by co-administration of morin. It was observed that the level of hemorrhage decreased with morin treatment. Another decrease was observed in the vacuolization in hepatocytes.

6. CONCLUSIONS

The combined effects of endosulfan, DMBA and morin were determined on xenobiotic metabolizing enzyme activities for the first time in normal and diabetic rats in this study. The combined effects of chemicals are the important topics of pharmacology, toxicology and physiology. The usage of endosulfan has been banned in most of the developed countries. However, its usage continues in many others. Recent studies clearly indicate that endosulfan is present in fish, sea water, fruits, vegetables, milk and human (Bedi et al., 2015; Bozcaarmutlu et al., 2014; Miglioranza et al., 2013; Shen et al., 2007; Yin et al., 2015). DMBA is one of the carcinogen chemical present in tobacco smoke. It is possible to be exposed both of these molecules at the same time in our daily lives. The combined effects of these chemicals have not been tested in test organisms, human being or cell culture systems. In this study, the results clearly indicated that co-administration of endosulfan and DMBA increased toxic effects of one another by inducing specific cytochrome P450 enzymes activities. Morin administration increased CYP1A level in the liver. It is clear that metabolism of CYP1A related molecules increases with morin. The other enzyme activities were not affected from morin administration. In addition, morin itself did not produce toxic effect in liver tissue. All enzyme activities were induced in diabetic rats. It is clear that diabetes itself affects xenobiotic metabolizing enzyme activities. Morin administration reduced some of the stimulated enzyme activities. Co-administration of endosulfan and DMBA with morin decreased the toxic effects of endosulfan and DMBA both in normal and diabetic rats. In this study, chemopreventive effect of morin was shown in the presence of endosulfan and DMBA on xenobiotic metabolizing enzyme activities for the first time in normal and streptozotocin induced diabetic rats.

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7. CURRICULUM VITAE

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Bozcaarmutlu A, Sapmaz C, Aygun Z, Arınç E (2009) "Assessment of pollution in the West Black Sea Coast of Turkey using biomarker responses in fish", Marine Environmental Research, 67:167-176.

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Bozcaarmutlu A, Turna S, Sapmaz-Erden C, Yenisoy-Karakas S, Arinç E "Characterization of Aldrin Epoxidation in Flathead Mullet (Mugil Cephalus) Liver Microsomes; Involvement of CYP3A", 47th Congress of the European Societies of Toxicology, 28-31 August, 2011, Paris, France.

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