T.C.
YEDİTEPE UNIVERSITY
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DEPARTMENT OF PHYTOTHERAPY

EFFECT OF ARTICHOKE EXTRACTS ON PARACETAMOL-INDUCED HEPATOTOXICITY AND NEPHROTOXICITY: A HISTOPATHOLOGICAL EVALUATION

MASTER OF SCIENCE THESIS

ENGİN SÜMER

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ONAY

Bu tez Yeditepe Üniversitesi Lisansüstü Eğitim-Öğretim ve Sınav Yönetmeliğinin ilgili maddeleri uyarınca yukarıdaki jüri tarafından uygun görülmüş ve Enstitü Yönetim Kurulu'nun 25./08./2017 tarih ve 2017/16-119 sayılı kararı ile onaylanmıştır.

Prof. Dr. Bayram YILMAZ
Sağlık Bilimleri Enstitüsü Müdürü

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

25/08/2017

Signature

Engin Sümer

DEDICATION

To my beloved family

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Sumer, E. (2017). Effect of Artichoke Extracts on Paracetamol-induced Hepatotoxicity and Nephrotoxicity: A Histopathological Evaluation, Yeditepe University, Institute of Health Science, Department of Phytotherapy, MSc thesis, İstanbul.

The liver is the most important organ in which the basic metabolic process takes place in human body. While drugs are metabolized in the liver, they can cause liver damage. Additionally, the excretion of toxic metabolites of drugs also cause nephrotoxicity. Acute liver damage or acute hepatotoxicity can cause organ loss and/or death. Herbal extracts have been reported to show protective effects in acute hepatotoxicity models on rats. Therefore, paracetamol-induced hepatotoxicity model was used in rats, and ethanolic extracts of various parts of the artichoke plant were administered. Then, their activity on the liver and kidneys was compared with a herbal product (Legalon®) which is an available formulation on the market. Consequently, it has been shown that extracts from the artichoke receptacles and stalks give more effective results on acute hepatotoxicity and nephrotoxicity than extracts from other parts (receptacle, inner bracts, outer bracts, leaves, and stalk) of artichoke and market preparations.

Key words: acute hepatotoxicity, artichoke extracts, silymarin, liver histopathology, kidney histopathology

ÖZET

Sumer, E. (2017). Enginar Ekstrelerinin Parasetamol ile İndüklenen Hepatotoksisite ve Nefrotoksisite üzerine etkileri: Histopatolojik Değerlendirme, Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Fitoterapi Anabilim Dalı, Master Tezi, İstanbul.

Karaciğer insan vücudundaki temel metabolizma olaylarının gerçekleştiği en önemli organdır. İlaçlar karaciğerde metabolize olurken aynı zamanda karaciğer hasarına da neden olabilmektedir. Buna ek olarak, ilaçların toksik özellikteki metabolitleri de nefrotoksisiteye neden olmaktadır. Akut karaciğer hasarı ya da akut hepatotoksisite organ kayıplarına ve/veya ölüme neden olabilmektedir. Bitkisel kaynaklı ekstrelerin sıçanlar üzerinde oluşturulan akut hepatotoksisite modellerinde olumlu sonuçlar gösterdiği rapor edilmiştir. Bu nedenle sıçanlar üzerinde parasetamol ile indüklenen hepatotoksisite modeli oluşturulup, enginarın farklı kısımlarının etanolik ekstraktları hazırlanmış, karaciğer ve böbrekler üzerindeki etkinlikleri piyasa da bulunan bitkisel bir ürün (Legalon®) ile karşılaştırılmıştır. Sonuç olarak, enginarın reseptakulum ve gövdesinden elde edilen ekstraktların, piyasa preparatı ve diğer kısımların (brakte, iç yaprak, reseptakulum, kök ve kök yaprak) ekstraktlarına göre akut hepatotoksisite ve nefrotoksisite üzerine daha etkin sonuçlar verdiği gösterilmiştir.

Anahtar kelimeler: enginar ekstreleri, akut hepatotoksisite, silimarin, karaciğer histopatolojisi, böbrek histopatolojisi

SYMBOLS / ABBREVIATIONS

ALT	alanine transaminase
AST	aspartate transaminase
CMC	carboxymethyl cellulose
DPPH	1,1-diphenyl-2-picryl-hydrazyl
IFG	impaired fasting glycaemia
MDA	malondialdehyte
ROS	reactive oxygen species
TPA	12-O-tetradecanoylphorbol-13-acetate

1. INTRODUCTION

The liver as one of the crucial organs of the human body maintains and regulates its homeostasis. Hepatotoxicity refers to failure of this organ which is mostly caused by chemical agents i.e., environmental toxins, pharmacotherapy, etc. Drug-induced hepatotoxicity is a serious health problem which is the most common reason reported for withdrawal of approved medications from the market (1).

Herbal remedies offer rich sources for the management of drug induced hepatotoxicity. Several plant extracts have been shown to possess a protective effect on liver. Among these Silymarin, derived from the seeds of *Silybum marianum*, has been reported to protect hepatocytes from various toxins (acetaminophen, CCl_4 and ethanol) (2,3) and eventually several pharmaceutical formulations are available in the market; Lovenox® (2% curcumin, 80% silymarin and dandelion extract), Hepanox® capsules (silymarin, vitamins A, C, E), Legalon® (silymarin) and Lipocholine® (artichoke extract and a group of vit B).

Artichoke (*Cynara scolymus*) is one of the well-known plants as a remedy to support liver functions in traditional folk medicine. Scientific findings support its cholagogue and hepatoprotective activity (4-6).

Some previous studies demonstrated the hepatoprotective activity of receptacles of *Cynara scolymus*. For the first time, this investigation focuses on the efficacy of artichoke extracts obtained from different parts including receptacle, inner bracts, outer bracts, leaves, and stalk on paracetamol-mediated acute hepatotoxicity.

For this purpose, different parts of artichoke were extracted with solvents and acetaminophen-induced acute hepatotoxicity model was applied for the activity assessment in rats. The extracts obtained were first administered via oral route. Afterwards histopathological analysis was conducted on the liver and kidney tissue sections were investigated histopathologically.

2. GENERAL DESCRIPTION

2.1. Botanical Chapter

This section describes the botanical characteristics of *Cynara scolymus* from Asteraceae family.

2.1.1. Asteraceae

Asteraceae is the largest family of flowering plants which is represented by over 1,500 substantial genera and almost 25,000 species throughout the world. This family consists of annual to perennial herbs which are often woody at base, rarely shrubs, often with latex or oil-canals (7,8).

Members of Asteraceae exhibit different shapes of the leaves which are usually alternate or may be sometimes opposite, simple and entire to pinnately or palmately lobed or variously pinnately compound; petiolate or sessile; exstipulate. Moreover, individuals of this family are made up of dense flowering plants. Shape and state of flowers generally small, borne on the receptacle in dense terminal heads, the outer opening first, zygomorphic or actinomorphic, often both in the same capitulum, bisexual or unisexual, epigynous (8,9).

Capitula hold the flowering zone and also occur with the flowers on the receptacle which could be either tubular or ligulate. Flowers generally have 5 stamens which is located on the corolla tube and anthers are combined laterally into a cylinder round to style. Ovary inferior includes one celled basal anatropous ovule. Style is usually divided to branches which has linear and stigmatic surface. In addition to these features the characteristic fruit type of the Asteraceae family is achene (cypsela), mostly with residual pappus (8,9).

Herbs of the Asteraceae family have been classified into 6 different groups (Table 2-1) according to the dichotomous key which is represented by “Flora of Turkey and the East Aegean Islands” (9).

Table 1: Plant groups according to the dichotomous key.

All of the flowers are ligulate and ligules are 5 toothed. Latex containing herbs.	Group A
All of the flowers are not ligulate, central flowers tubular. Herbs without latex.	Group B
Spiny leaves or phyllaries	
Either leaves or phyllaries are not spiny	Group C
Herbs with radiate capitula, marginal flowers have 3 (rarely 5 or more) toothed ligules.	
Color of ligules differs from bright yellow to orange.	
Color of ligules differs from white, pink, red, purple or blue	Group D
Herbs with discoid/disciform capitula, marginal flowers are tubular sometimes elongated and radiant but usually cylindirical or ligulate.	Group E
Herbs have paleaceous or long – hairy receptacle	
Herbs have naked receptacle or margins of alveolae ciliate or toothed	Group F

2.1.1.1. *Cynara scolymus* L.

The *Cynara* genus includes perennial herbs. State of stems are erected and sparingly branched. The leaves are generally alternate, pinnately lobed, spiny or may be arachnoid hairy. Capitula is large, single and discoid in shape but originated from one to few corymbs. An involucral bract has been arranged in numerous rows. Moreover, receptacle is flat and fleshy. All of the flowers are tubular and the color of corolla blue or whitish. Anthers are spurred and filaments are hairy. Style has long branches. Achenes are oblong and angular. Pappus has mainly pulmose hairs (8,9).

Figure 1: *Cynara scolymus*

Cynara scolymus (*Cynara cardunculus* L. var. *sativa* Moris; *Cynara cardunculus* var. *scolymus* (L.)) is a perennial herb. The stem of herb grows up to 2m high with pale yellowish green color. Leaves grow up 30 – 40 x 10 – 15 cm, and extend up the stem almost to capitulum. To upper surface, leaves are green but the lower parts are greyish – green. The shape of the leaves is ovate – lanceolate in outline, pinnately lobed. Capitula is compressed – globose, 80 – 100 mm. Involucral bracts are numerous, the inner with a broad, ovate, acute, mucronate or very shortly spinose, purplish. All of the flowers are tubular and the receptacle is flat and the walls densely rigid - hairy with white bristles (8,9).

Globe artichoke (in Turkish, "enginar"). Unknown in the wild state, and generally thought to have originated from the preceding species, with which it might well be considered conspecific. Mainly cultivated in North & West Anatolia. It was first announced in Italy, Sicily and S. France. Locally, this plant species described in Istanbul and Trabzon. Its synonyms include; *Cynara cardunculus L*. var. *sativa, Cynara cardunculus var. saliva; s Cynara cardunculus var. scolymus* (9).

2.1.1.2. Ethnobotanical Data

Traditionally, *Cynara scolymus* leaf is frequently used as tea either by simmering or boiling and its dry or soft extracts has been practiced for their several health benefits. Moreover, *Cynara scolymus* other plant parts such as bracts, stem and receptacle have been eaten as a healthy vegetable for centuries.

The leaves of *Cynara scolymus* were used as infusion (1.5 g powdered dried leaf in 150 mL or 3 g in 150 ml of boiling water) 2 or 4 times daily for its cholagogue, diuretic, hepatoprotective, and cholesterol lowering effects. Moreover, various preparations (Aqueous or soft alcoholic extracts) were used its hepatoprotective, and cholesterol lowering effects (10).

Another traditional usage of leaves and flowers of *Cynara scolymus* was reported as infusion for its appetizer, diuretic, and cholagogue activities (10). Additionally, a decoction (one teacup 3 times a day for 3-4 weeks) was reported for amelioration of anorexia, appetizer, diuretic, cholagogue, nephralgia, kidney stones, and cancer (11).

2.2. Theoretical Chapter

This section presents literature reviews on *Cynara scolymus*. Moreover, this chapter gives detailed knowledge about phytochemical content and bioactivity studies.

2.2.1. Literature review on *Cynara Scolymus*

2.2.1.1. Phytochemical content

Main chemical ingredients in *Cynara scolymus* can be categorized as phenolic and terpenic compounds. Until now, phenolic acids, caffeic acids, chalcone derivatives, flavonoids, and anthocyanins are identified as phenolic constituents. When considering the terpenic ingredients, *Cynara scolymus* has sesquiterpene lactones, sesquiterpene glycosides and triterpenes.

2.2.1.1.1. Phenolic Compounds

Table 2: Phenolic acid derivatives which are presented in *Cynara scolymus*

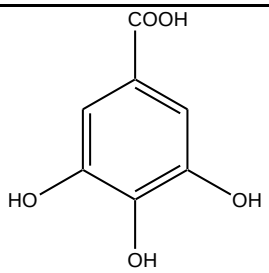
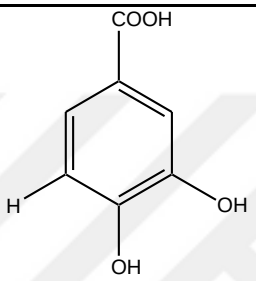
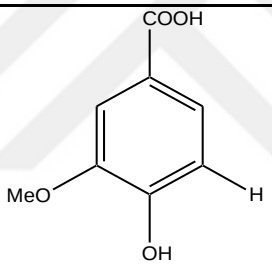
Compound	Chemical Structure	Reference
Gallic Acid		(12)
Protocathechuic acid		(12)
Vanilic acid		(13)

Table 3: Chalcone derivatives which are presented in Cynara scolymus

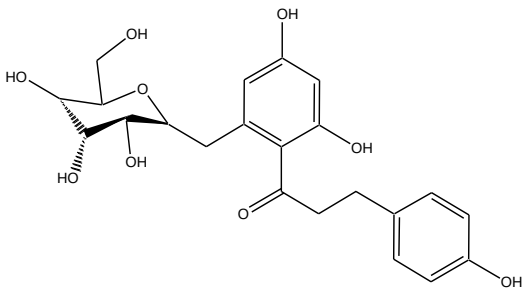
Compound	Chemical Structure	Reference
Phloretin 2-O-glucoside		(13)

Table 4: Caffeic acid derivatives which are presented in *Cynara scolymus*

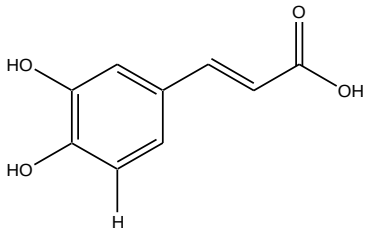
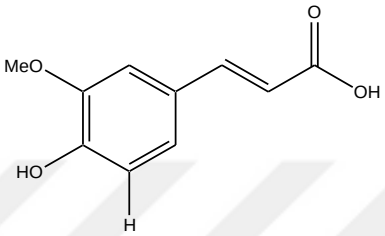
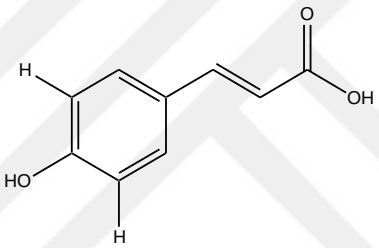
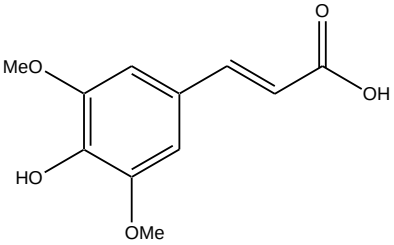
Compound	Chemical structure	Reference
Caffeic acid		(12,13)
Ferulic acid		(13)
p – coumaric acid		(12,13)
Sinapic acid		(14)

Table 5: Quinic acid derivatives which are presented in *Cynara scolymus*

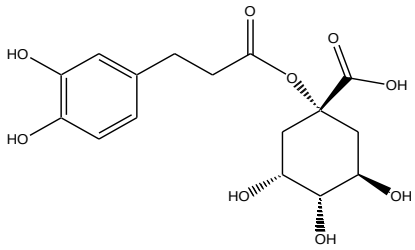
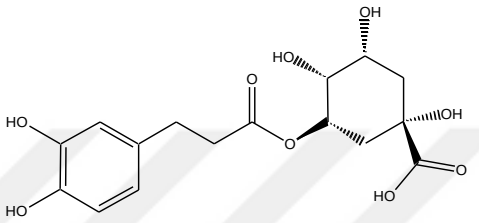
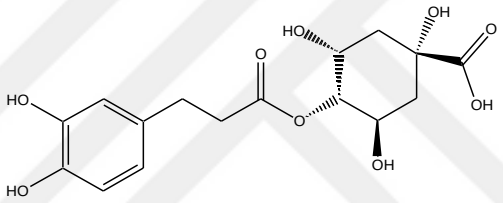
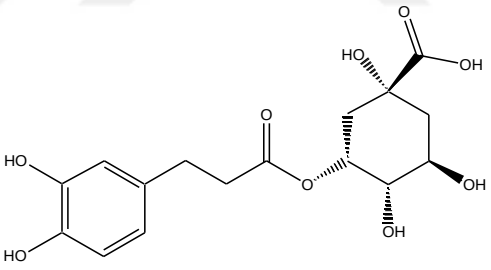
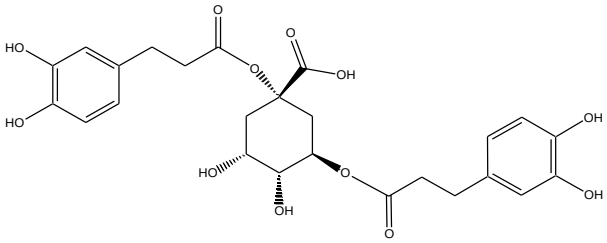
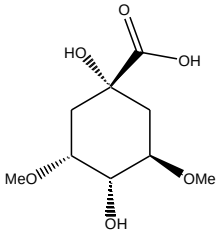
Compound	Chemical structure	Reference
1-O-caffeoylquinic acid		(15,16)
Neochlorogenic acid (3-O-caffeoylquinic acid)		(15-17)
Cryptochlorogenic acid (4-O-caffeoylquinic acid)		(15-17)
Chlorogenic acid (5-O-caffeoylquinic acid)		(15-17)
Cynarin (1,3-O-dicaffeoylquinic acid)		(15-17)
Syringic acid		(13)

Table 6: Flavonoids which are presented in *Cynara scolymus*

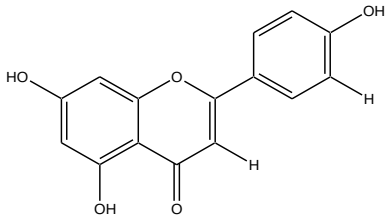
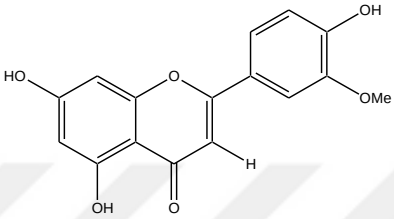
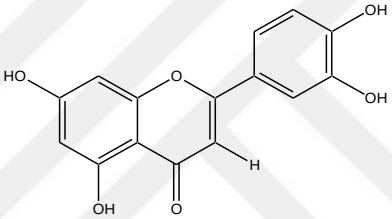
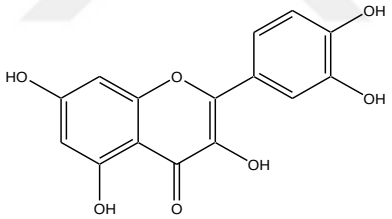
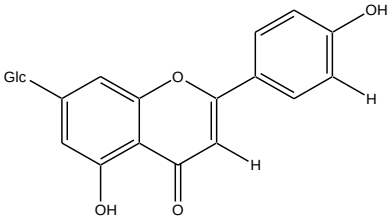
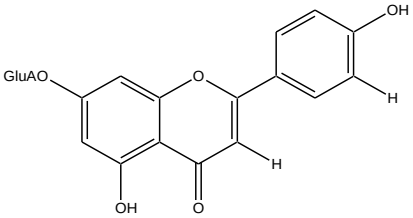
	Chemical structure	Reference
Apigenin		(12)
Chrysoeriol		(12)
Luteolin		(12,17)
Quercetin		(12)
Apigenin 7-O-glucoside		(12,15)
Apigenin 7-O-glucuronide		(12,15)

Table 6: Flavonoids which are presented in *Cynara scolymus (Cont.)*

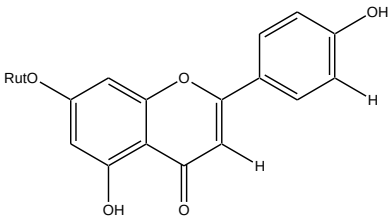
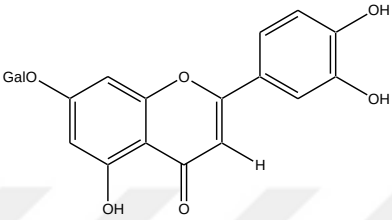
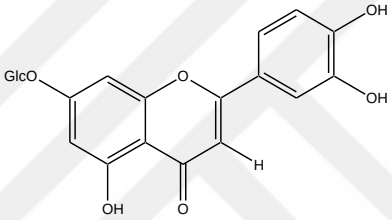
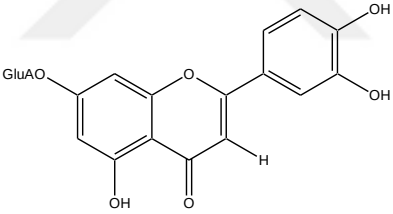
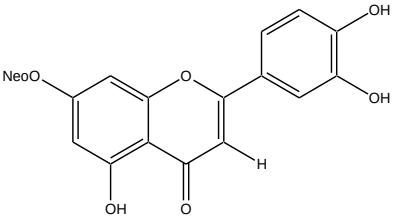
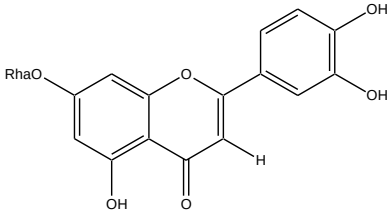
	Chemical structure	***Reference***
Apigenin 7-O-rutinoside		(12,15)
Luteolin 7-O-galactoside		(12)
Luteolin 7-O-glucoside		(12,15,17)
Luteolin 7-O-glucuronide		(12,15,17)
Luteolin 7-O-neohesperidoside		(12)
Luteolin 7-O-rhamnoside		(12)

Table 6: Flavonoids which are presented in *Cynara scolymus* (Cont.)

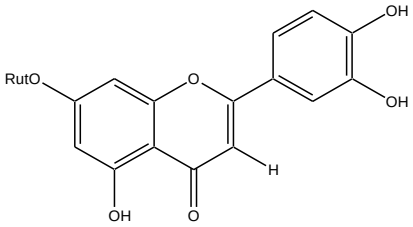
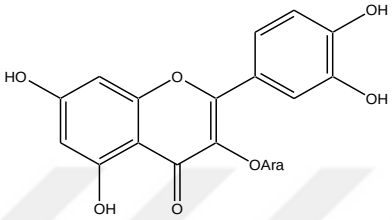
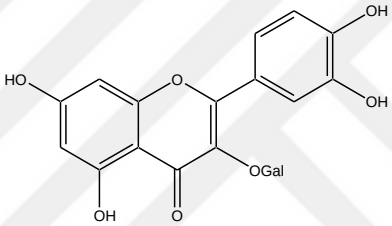
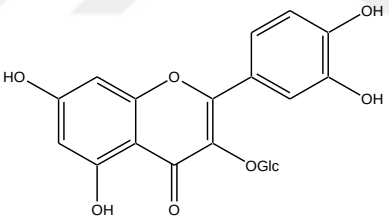
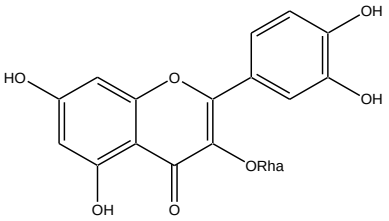
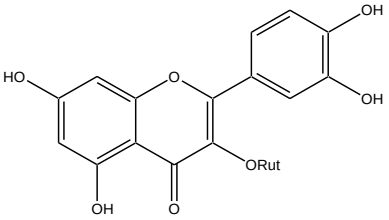
	Chemical structure	Reference
Luteolin 7-O-rutinoside		(12,17)
Avicularin (Quercetin 3-O-arabinoside)		(12)
Hyperoside (Quercetin 3-O-galactoside)		(12)
Isoquercitrin (Quercetin 3-O-glucoside)		(12)
Quercitrin (Quercetin 3-O-rhamnoside)		(12)
Rutin (Quercetin 3-O-rutinoside)		(12)

Table 6: Flavonoids which are presented in C*ynara scolymus* (Cont.)

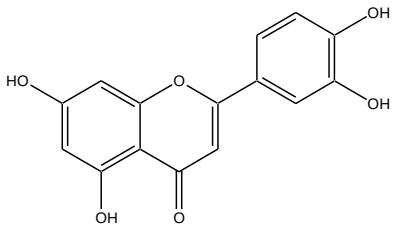
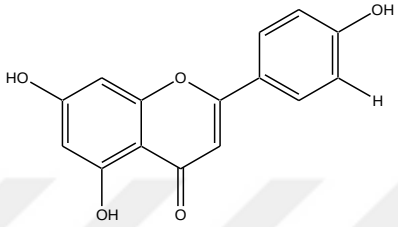
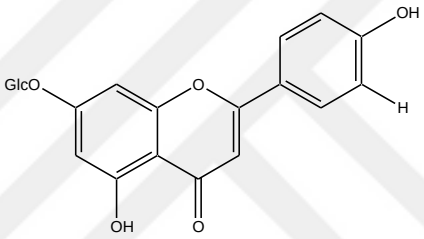
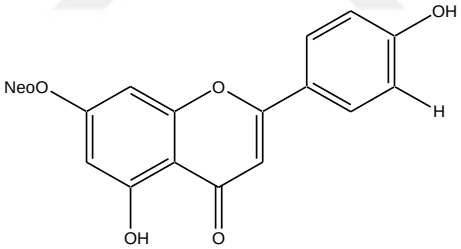
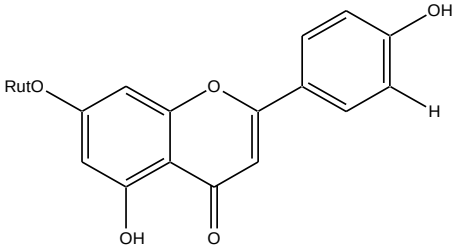
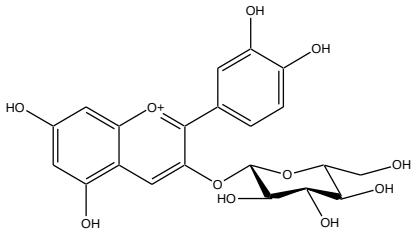
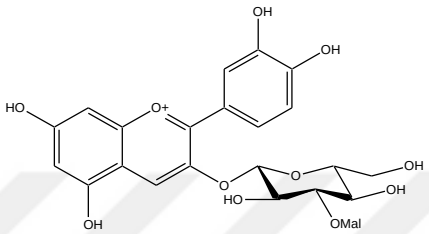
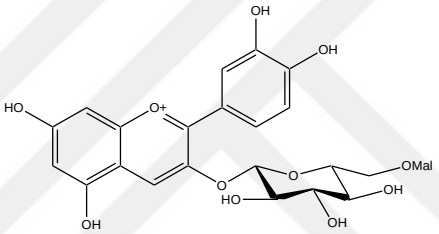
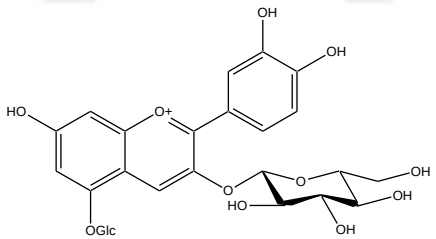
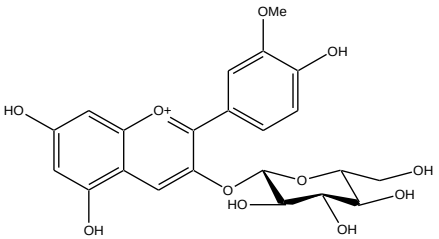
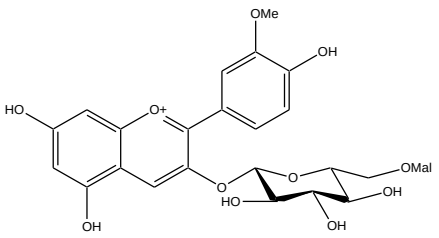
	Chemical structure	Reference
Eriodictyol		(12)
Naringenin		(12)
Prunin (Naringenin 7-O-glucoside)		(12,15)
Naringin (Naringenin 7-O-neohesperidoside)		(12)
Narirutin (Naringenin-7-O-rutinoside)		(15,16)

Table 7: Anthocyanins which are presented in C*ynara scolymus*.

	Chemical structure	Reference
Cyanidin 3-O-glucoside		(18)
Cyanidin 3-O-(3"-malonyl) glucoside		(18)
Cyanidin 3-O-(6"-malonyl) glucoside		(18)
Cyanidin 3,5-O-diglycoside		(18)
Peonidin 3-O-glucoside		(18)
Peonidin 3-O-(6"-malonyl) glucoside		(18)

2.2.1.1.2. Terpenic Compounds

Table 8: Terpenic compounds which are presented in C*ynara scolymus*

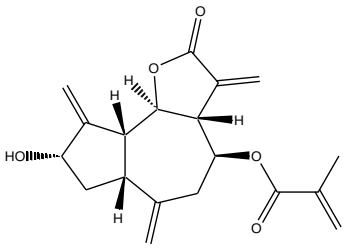
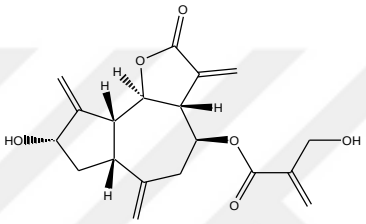
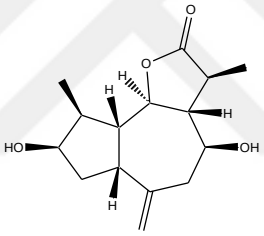
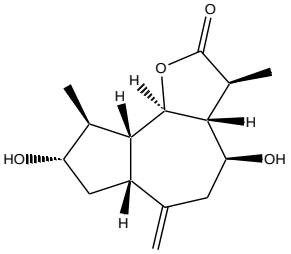
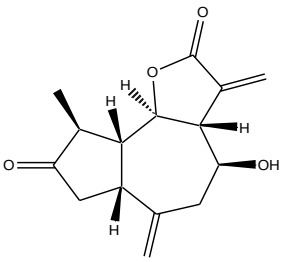
Terpenic compound	Chemical structure	Reference
Auerin (Sesquiterpene lactone)		(19)
Cynaropicrin (Sesquiterpene lactone)		(19,20)
Cynarascolide (Sesquiterpene lactone)		(19)
Isolipidol (Sesquiterpene lactone)		(19,20)
Grosheimin (Sesquiterpene lcatone)		(19)

Table 8: Terpenic compounds which are presented in C*ynara scolymus* (Cont.)

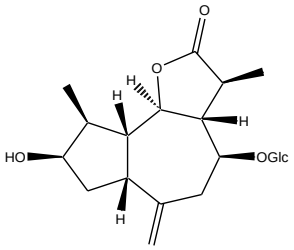
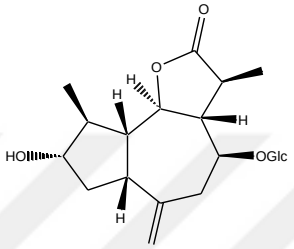
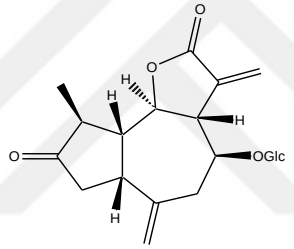
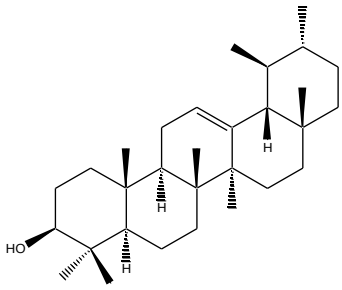
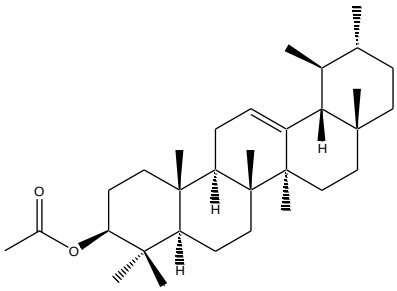
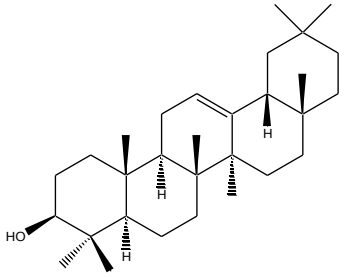
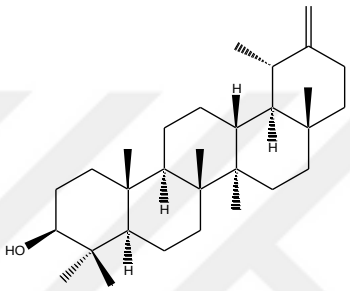
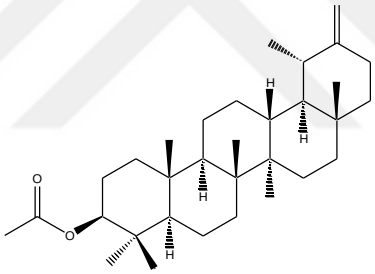
Terpenic compound	Chemical structure	Reference
Cynarascoloside A (Sesquiterpene glycoside)		(19)
Cynarascoloside B (Sesquiterpene glycoside)		(19)
Cynarascoloside C (Sesquiterpene glycoside)		(19)
α-amyrin (Triterpene)		(21)
α-amyrin acetate (Triterpene)		(21)

Table 8: Terpenic compounds which are presented in C*ynara scolymus* (Cont.)

Terpenic compound	Chemical structure	Reference
β-amyrin (Triterpene)		(21)
Taraxasterol (Triterpene)		(21)
Taraxasterol acetate (Triterpene)		(21)

2.2.1.2. Bioactivity studies

The bracts, stem and receptacle of *Cynara scolymus* have been eaten for both health and nutritional benefits. When the traditional folk medicine was investigated, *Cynara scolymus* has been used for its cholagogue and hepatoprotective effects. Moreover, the literature states that *Cynara scolymus* has antihyperlipidemic, antihyperglycemic, antidyspeptic, antimicrobial, antioxidant, anticancer, and cardioprotective activities.

2.2.1.2.1. Hepatoprotective activity

In a cell culture study *Cynara scolymus* extracts exhibit hepatoprotective activity. At the beginning of the investigation, malondialdehyte (MDA), end products of lipid peroxidation, production has been triggered in rat hepatocyte cell cultures. Then, the

aqueous extract of *Cynara scolymus* has been applied to hepatocytes. The extract applied cultures show dose dependent MDA suppression (4). Same group has also investigate the quantitative relationship between different phenolic components (caffeic acid, cynarin, cynaroside etc.) for the MDA suppression (5).

Hepatopreventive effects of *Cynara scolymus* have been investigated on oxidative stress induced hepatotoxicity animal model. At the beginning of the experiment, rats were fed on *Cynara scolymus* extract by gavage for two weeks. Then the induction step has been done. After 24 hour the animals were sacrificed. The results state that application of the *Cynara scolymus* significantly normalized the plasma transaminase activity and it was also histopathologically proved (6).

In a comparative study, hepatoprotective effects of *Cynara scolymus* extracts (bracts, receptacles and stems) have been investigated on oxidative stress induced hepatotoxicity animal model (22). Histopathological responses and MDA, aspartate transaminase (AST), alanine transaminase (ALT) levels were monitorized for the hepatoprotective activity. Receptacles and bracts extracts demonstrated highest suppressive activity on liver MDA levels.

2.2.1.2.2. Antihyperlipidemic activity

The literature states that aqueous leaf extract of *Cynara scolymus* diminishes the cholesterol biosynthesis (23). The suppression of cholesterol biosynthesis could be explained via indirect mechanisms. This statement supported with the inhibitory effect of luteolin on insulin which has impact on the cholesterol biosynthesis. Additionally, luteolin 7-O-glycoside was considered to be responsible of this indirect mechanism.

In a comparative investigation, olive oil loaded mice model was used to measure the effects of *Cynara scolymus* leaf extract, orlistat (lipase inhibitor) and clofibrate (hypolipidemic agent) on serum triglycerides. All of the agents successfully prevented the elevation of the serum triglycerides. The responsible active compounds in the C*ynara scolymus* extract were considered to be sesquiterpenes (19).

Another study states that *Cynara scolymus* leaf extract has impacts on serum and hepatic lipid levels. High cholesterol diet animal model has been applied to rats. The hypercholesterolemic rats treated with *Cynara scolymus* leaf extract. Serum cholesterol levels and triglyceride levels fell down. But liver cholesterol and triglyceride levels left unchanged. On the other hand, hepatic and cardiac MDA levels decreased (24).

In a clinical study, adult volunteers with hypercholesterolemia were used to observe the impacts of standardized *Cynara scolymus* extract on plasma lipid levels. For 3 months the candidates treated with extract or placebo. The results indicate that plasma cholesterol levels had been declined in the extract treated group while the plasma cholesterol levels rose in the placebo group (25).

2.2.1.2.3. Antihyperglycemic activity

In a study, diabetic rat model was used to investigate effects of chlorogenic acid on fasting plasma glucose, plasma and liver lipids (triglycerides and cholesterol). The findings of the research showed that chlorogenic acid diminished the postprandial glucose peak response without hypoglcemia. Also fasting plasma and liver lipid concentrations significantly decreased (26).

In another study, a placebo controlled clinical trial was performed to observe the effects of dietary supplement of *Cynarascolymus* extract. Extract treated group exhibit reduced glycemia scores. Additionally, body mass index and susceptibility to hunger score significantly reduced. The findings support that dietary supplements of *Cynara scolymus* extract has promising effects on weight control and dysglycemia problems (27).

Another double blind, placebo controlled clinical study was conducted to follow the effects of dietary *Cynara scolymus* extract on impaired fasting glycaemia (IFG) patients. Long term (8 week) treatment was applied to patients either with extract or placebo. Results revealed that glycosylated hemoglobin levels, Alc-derived average glucose, homeostatic metabolic assessment and lipidic pattern reduced in extract treated group. But, the placebo applied group did not exhibit any difference. Thus, a supplement of *Cynara scolymus* has reducing effects on glucose related metabolic parameters in IFG patients (28).

2.2.1.2.4. Antidyspeptic activity

Cynara scolymus extracts has choleretic effects which is responsible for the antidyspeptic activity. In a placebo controlled double blind study, the bile excretion volume was evaluated to explore the activity of single dose standardized *Cynara scolymus* extract. At the predetermined time intervals, the volumes of bile secretions were compared. The effective time interval for the extract was found to be 2 – 2.5 hours when it was applied postprandially. The investigators commented that the extract of *Cynara scolymus* can be recommended for the treatment of dyspepsia (29).

In an animal study the actions of *Cynara scolymus* leaf extract comparatively investigated with dehydrocholic acid (synthetic bile acid) on bile flow and bile composition. The researchers observed notable increase in bile flow which was observed to be similar with dehydrocholic acid. When the highest dose was administrated at both single and repeated applications, the bile acid increasing action of extract was found to be superior to reference group (30).

In a double blind randomized placebo controlled trial, patients with dyspepsia were treated with either *Cynara scolymus* extract or placebo. Over 1.5 month administration the life quality scores of extract treated group was found to be markedly higher than placebo treated group. The researchers noted that the extract of *Cynara scolymus* could be used for mitigating symptoms and improving the disease specific life quality in dyspeptic patients (31).

2.2.1.2.5. Antimicrobial activity

In a microbiological study, the antimicrobial action of *Cynara scolymus* leaf extracts in different solvents (chloroform, ethyl acetate and n-butanol) were investigated. Researchers noted that the n-butanol fraction exhibited the highest antimicrobial activity on seven different types of bacteria, four different types yeast and four different types of mold (32). Another investigation by same research group reported that not only leaf extracts but also different parts such as stem and head extracts demonstrate antimicrobial activity. However, the leaf extracts had greater activity than the other parts in that study (33).

The bracts of *Cynara scolymus* had also been studied for testing its antimicrobial efficacy. Bracts were extracted with ethanol and then partitioned with dichloromethane, ethyl acetate and n-butanol. Ethyl acetate extract had the most effective antimicrobial activity. Then, ethanol, chloroform, water and n-butanol extracts had the antimicrobial effects by decreasing order (34).

2.2.1.2.6. Antioxidant activity

To measure the antioxidant activity, oxidative stress model has been mostly studied. In a cell culture study, to observe the effects of aqueous and ethanolic extracts of *Cynara scolymus* intracellular oxidative stress stimulated by inflammatory mediators. The oxidation levels were followed by monitoring the levels of 2′, 7′– dichlorofluorescin parameters. Both of the extracts suppressed the reactive oxygen species (ROS) production. Thus, *Cynara scolymus* extracts have antioxidant actions on oxidative stress model (35).

In another study antioxidant activity of aqueous and organic *Cynara scolymus* extracts were investigated. Different methods were applied including DPPH radical scavenging assay, ferric reducing antioxidant power *in vivo* and human LDL oxidation *in vitro*. *Cynara scolymus* extracts inhibited LDL oxidation *in vitro*. When *in vivo* trials were conducted ferric reducing power and radical scavenging activity remained same as placebo group (36).

Another trial was conducted to investigate the antioxidant activity of *Cynara scolymus* leaf extract. To create the oxidative stress model human leukocytes were triggered with agents that generate ROS. The results illuminate that cynarin, chlorogenic acid, caffeic acid, and luteolin were responsible for the antioxidant activity of *Cynara scolymus* (37).

2.2.1.2.7. Anticancer activity

Literature indicated that methanolic extract of *Cynara scolymus* flower exhibited remarkable inhibitory effects on skin tumour formation. Moreover researchers found anti-inflammatory action against 12-O-tetradecanoylphorbol-13-acetate (TPA)-mediated inflammation on mice (21).

In a cell viability study, human hepatoma cells were used. The *Cynara scolymus* extract with polyphenolic content exhibited dose-dependent inhibitory effects on cell viability (38). Another study states that edible part of *Cynara scolymus* inhibited the cell growth of human breast cancer cell line. The researchers indicated that edible part of *Cynara scolymus* inhibited cell viability, induced apoptotic mechanisms, and showed inhibitory properties against the invasive behavior of breast cancer cell line (39).

3. MATERIAL & METHOD

3.1. Materials

3.1.1. Herbal material

The *Cynara scolymus* plants were harvested on May 12, 2016 from İzmir. The herbal material was identified by Prof. Dr. Erdem Yesilada (Faculty of Pharmacy, Yeditepe University, Istanbul, Turkey). The herbal materials were dried and separated into its parts (Table 9).

Table 9: Amount of C*ynara scolymus* parts

Parts of plant	Dried weight	Milling (powdered weight)
Outer Bracts	185,6 g	178 g
Inner Bracts	55,5 g	52
Receptacle	55,9 g	55 g
Stalk	31,8 g	28 g
Leaves	65,3 g	55 g

The herbal material was kept away from direct sunlight and dried at room temperature for two weeks. Moreover, dried herbal materials stored at -25ºC and ground to powder in a mechanic grinder and sieved before extraction. The sieve numbers according to the Ph. Eur. was 1000 (for *Cynara scolymus* leaf monograph) (40).

3.1.2. Chemical material

The chemicals are presented in Table 10.

Table 10: Chemicals and distributors

Chemical Name	Distributor
Carboxymethyl cellulose (CMC)	Sigma Aldrich
Ethanol	Sigma Aldrich
Hematoxylin-Eosin	DDK, Italy
Ketamine HCL	Richter Pharma ag
Legalon® (*Silybum marianum* extract)	Solgar
Paracetamol	Sigma Aldrich
Toluene	Sigma Aldrich

3.1.3. Equipments

The equipments are presented in Table 11.

Table 11: Equipments and manufacturers

Equipment Name	Manufacturer
Micropipette	Rainin, USA
Micropipette tips	Rainin, USA
Balance	Ohaus Explorer, USA
Centrifuge tubes	Isolab, Turkey
Grinding mill	IKA, USA
Lyophilizer	Christ Alpha 2-4 LD, Germany
Refrigerator	Arcelik, Turkey
Rotary evaporator	Heidolph, Germany

3.2. Methods

3.2.1. Extraction Method

Extraction process of plant material has been done with 8:2 Ethanol:water mixture. Plant material was extracted two times with different amounts of aqueous ethanol (8:2 Ethanol:water). Table 12 describes amounts of extraction solvent. Each extraction was carried out by continuous mixing in a reciprocating shaker for 2 hours. Then the residual parts of powdered plant material were separated via filtration and each of the filtrated extract was condensed to dryness in a rotary evaporator. The residue was reconstituted with water and was kept at – 80 ^{o}C overnight and lyophilized.

Table 12: Amounts of extraction solvents

Parts of plant	Amount of solvent (8:2 Ethanol: water) in mL	
	First extraction	Second extraction
Outer bract	1200	500
Inner bract	350	250
Receptacle	200	100
Stalk	250	100
Leaves	500	300

3.2.2. Preparation of *Cynara scolymus e*xtracts, positive and negative control dispersions

To prepare the carboxymethyl cellulose (CMC) dispersion, 5 grams of CMC was dispersed into 1000 mL water at 600 rpm for 3 hours. Then, this mixture was separated to 4 different aliquots to prepare the dispersion of extracts, to prepare paracetamol dispersion, to prepare Legalon® dispersion (positive control) and placebo (negative control) dispersion.

To treat the hepatotoxicity in rats, Legalon® suspension was used (100 mg/kg dose). To prepare Legalon® suspension, the content of Legalon® capsules was dispersed in 0.5% CMC dispersion at required dose.

To prepare the suspension of *Cynara scolymus*, lyophilized extracts were reconstituted with an aliquot of 0.5% CMC dispersion at 500 mg/kg dose.

3.2.3. Experimental design

Activity studies of *Cynara scolymus* extracts have been done with male Sprague Dawley rats. Rats were obtained from the Yeditepe University Medical School Experimental Research Center (YUDETAM) and housed at controlled room temperature (21 ± 1 °C) with a 12:12 h light-dark cycle in polypropylene cages with bedding. The experimental protocol was approved by the Ethic Committee of Yeditepe University. Rats (10 week old) were assigned to 7 groups of 7 animals each. The groups of animals and applications have been shown at Table 13. To induce hepatotoxicity in rats, paracetamol was orally administered (2000 mg/kg dose). To prepare paracetamol suspension, required amount of paracetamol was dispersed in 0.5% CMC mixture.

Table 13: The groups of animals and applications

Group	Application
Group A	Receptacle extract of *Cynara scolymus* via oral gavage at 500 mg/kg dose
Group B	Stalk extract of *Cynara scolymus* via oral gavage at 500 mg/kg dose
Group C	Inner Bract extract of *Cynara scolymus* via oral gavage at 500mg/kg dose
Group D	Outer Bract extract of *Cynara scolymus* via oral gavage at 500 mg/kg dose
Group E	Leaves extract of *Cynara scolymus* via oral gavage at 500 mg/kg dose
Group F	2 mL 0,5% CMC dispersion was applied for negative control
Group G	Legalon® dispersion was applied at 100 mg/kg dose for positive control

The following protocol was applied during the experiment:

At first day, paracetamol suspension was applied to all groups (Group A, B, C, D, E, F, and G) at 2000 mg/kg dose to induce hepatotoxicity in rats. Afterwards, 2 mL of the extracts were given to Groups A, B, C, D, and E (500 mg/kg dose). 0.5% CMC and Legalon® dispersions were applied to Group F and Group G, respectively. Animals were treated with extracts, positive and negative controls for three consecutive days. At fifth day, high dose anesthesia (with ketamine HCL; 100 mg/kg) was applied to each group for sacrification. Then, the liver and kidney tissues of the animals were taken for further analysis.

3.2.4. Histopathological Examinations:

After sacrification, liver and kidney samples were collected into 10% formaldehyde solution and stored at 4°C for histopathologic analysis. Regarding the histopathologic analysis, the tissue samples have first been washed with tap water for 2 hours and dehydrated by treating with 70%, 90%, 96% and 100% alcohol, respectively after fixation. Samples then were gotten transparent with toluene and blocked with paraffin after over the night incubation at 60°C in the oven. After that, five slices with five μm diameter were obtained from paraffin blocks and each slice was stained with hematoxylin-Eosin (H&E) dye in order to make histopathologic scoring.

3.2.4.1. Histopathologic Scoring of Kidney Samples:

Each slice was assessed in terms of bowman cavity, glomerular degeneration, proximal and distal tube degenerations, vascular congestion and interstitial edema. Each assessment was scored between 0-3. (0: no change, 1: mild, 2: moderate, 3: severe) Maximum score for each sample was 12 whereas minimum score was 0.

3.2.4.2. Histopathologic Scoring of Liver Samples:

Liver samples were prepared to facilitate comparisons of the same liver lobes of all animals. Each slice was assessed in terms of hepatic vascularization, enlargement in pyknotic nuclei and sinusoids, Kupffer cell infiltration and vascular congestion. Each assessment was scored between 0-3. (0: no change, 1: mild, 2: moderate, 3: severe) Maximum score for each sample was 12 whereas minimum score was 0. The mean value of scores obtained from all animals was statistically evaluated.

3.2.5. Statistical Analysis

The results were shown as mean values ± standard deviation (SD). One way ANOVA test was performed to find statistical significance between the groups and the groups were compared with Tukey Multiple Comparison Test. Grand Pad Prism 6 was used to perform statistical analysis. The difference was considered as statistically significant when $p<0.05$.

4. RESULTS

4.1. Extraction yield results

Extraction yields were calculated in terms of weight of powder extract. Yields of extractions were 10.40%, 5.20%, 8.96%, 12.89% and 18.05% for the receptacle, outer bract, inner bract, stalk and leaves, respectively (Table 14).

Table 14: Extraction Yields of Different Parts of *Cynara scolymus*

Part of Plant	Weight of Powdered Plant (g)	Weight of Lyophilized Extract (g)	Yield %
Receptacle	55	5.72	10.40%
Outer Bract	178	9.26	5.20%
Inner Bract	52	4.66	8.96%
Stalk	28	3.61	12.89%
Leaves	55	9.93	18.05%

4.2. Results of Histopathologic Examinations

Number of animals, number of survived animals, each score that might be indicator for pathological changes in kidney including; degeneration of bowman space and glomeruli, degeneration of proximal and distal tubules, vascular congestion, interstitial edema and for liver including; vacuolization of hepatocytes and pyknotic nuclei, enlargement of sinusoids, enlargement sinusoids, kupffer cell infiltration, vascular congestion were given in Table 15.

Table 15: Histopathological evaluations of experiment groups

Groups		A	B	C	D	E	F	G
Number of animals at the beginning (n)		7	7	7	7	7	7	7
Number of living animals (n)		5	7	6	7	4	6	7
Kidney	Degeneration of Bowman Space and Glomeruli	0/5	5/7	6/6	2/7	3/4	6/6	2/7
	Degeneration of Proximal and Distal Tubules	0/5	4/7	6/6	5/7	4/4	6/6	3/7
	Vascular Congestion	0/5	5/7	4/6	4/7	4/4	6/6	2/7
	İnterstitial Edema	0/5	5/7	3/6	4/7	4/4	6/6	3/7
Liver	Vacuolization of Hepatocytes and Pyknotic Nuclei	2/5	2/7	5/6	6/7	4/4	5/6	5/7
	Enlargement Sinusoids	0/5	0/7	5/6	6/7	3/4	5/6	5/7
	Kupffer Cell Infiltration	0/5	0/7	5/6	6/7	4/4	6/6	5/7
	Vascular Congestion	0/5	0/7	6/6	7/7	4/4	6/6	4/7

4.2.1. Results of Histopathologic Kidney Examinations

The mean values of histopathologic kidney scores were found as 1.2, 7.71, 8.0, 6.85, 10.0, 11.66 and 4.85 for group A, group B, group C, group D, group E, group F, group G, respectively. Multiple comparisons between each group were statistically done. Results showed that histopathologic kidney scores of Group A were statistically lower than other groups except Group G ($p < 0.001$). Also lower histopathologic kidney scores were detected in group B, group C, group D, group E when compared to group F as a negative control group (p values were $p<0.05$, $p<0.05$, $p<0.01$ and $p<0.01$ respectively). Furthermore, histopathologic kidney scores of group G as a positive control group (Legalon® treated group) were found to be lower than group F ($p < 0.001$) (Figure 4-2). Histopathological findings for kidney tissue were shown in Figure 2.

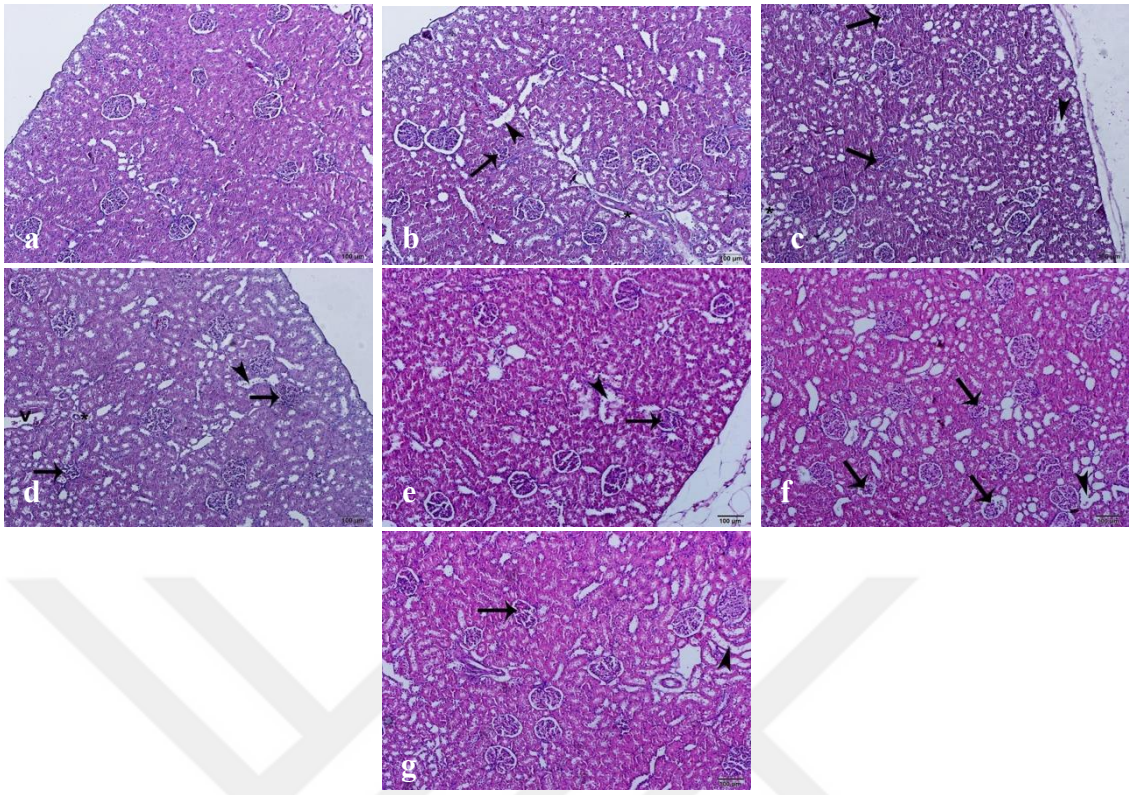


Figure 2: Histopathological findings for kidney tissue

a) Regular morphologic glomeruli and tubules are seen in group A in the kidney cortex stroma. b) Some glomeruli (arrows) and tubules (arrowheads) are damaged, edema (*) in Group B in the kidney cortex. c) A large number of glomeruli (arrows) in group C in the kidney cortex, and tubules (Arrow head) damage, edema (*) is seen. d) There are numerous glomeruli (arrows) and tubules (arrowheads) damage, edema (*), vasoconstriction (>) in the D group in the kidney cortex. e) A large number of glomeruli (arrows) and tubules (arrowheads) are seen in group E in the kidney cortex. f) There are numerous glomeruli in the F group of the kidney cortex and damage to the Bowman capsule (arrow) and tubules (arrowhead). g) There are few glomeruli (arrows) and tubules (arrowheads) in group G in the kidney cortex.

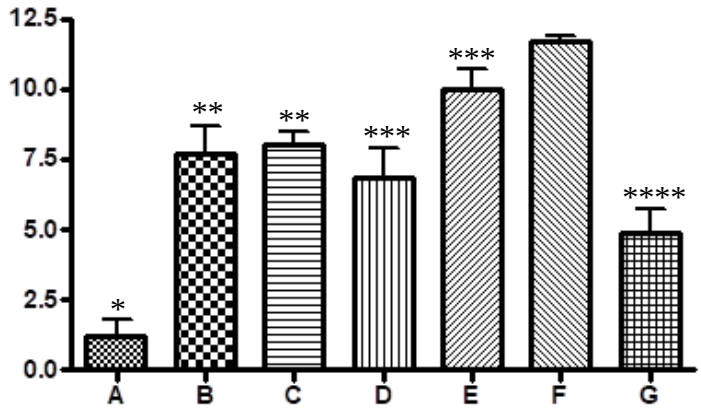


Figure 3: Histopathologic Kidney Scores for Each Groups

*Significantly different from all other groups except G ($p < 0.001$). **Significantly different from group F ($p < 0.05$). ***Significantly indifferent from group F ($p < 0.01$). ****Significantly different from group F ($p < 0.001$). Group A: 500 mg/kg receptacle, group B: 500 mg/kg stalk, group C: 500 mg/kg inner bract, group D: 500 mg/kg outer bract, group E: 500 mg/kg leaves, group F: negative controls, group G: positive controls 100 mg/kg Legalon®.

4.2.2. Results of Histopathologic Liver Examinations

The mean values of histopathologic liver scores were found as 1.20, 1.14, 9.50, 9.43, 9.25, 11.00 and 8.29 for group A, group B, group C, group D, group E, group F, group G, respectively. Multiple comparisons between each group were statistically done. According to our results, histopathologic liver scores of group A and group B were statistically lower than other groups ($p < 0.001$). No other statistically significant differences were found between groups. Figure 4-4 shows the mean histopathologic liver scores ± SD of each group. Histopathological findings for liver tissue were shown in Figure 4.

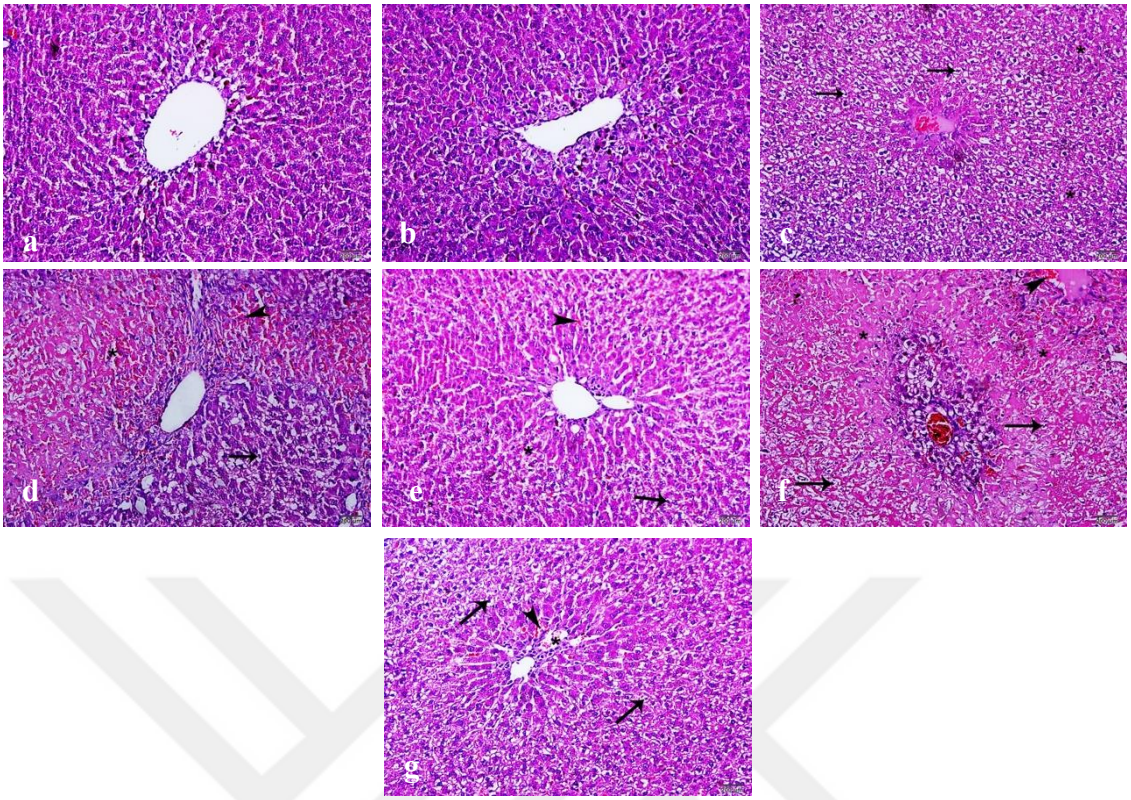


Figure 4: Histopathological findings for liver tissue

a) Hepatocytes and sinusoids are seen in radically located hepatic lobules in a uniformmorphology group A in liver tissue. b) Hepatic lobule in the normal structure in the group B of the liver and hepatocytes and sinusoids located radially in the liver are seen. c) Hepatic lobule in group C of the liver with impaired liver and picnic nucleus hepatocytes (arrow) and sinusoidal structures (*) are seen to be impaired. d) Hepatic lobules in the D group of the liver are seen as hemorrhagic (arrow head) areas in which the hepatic lobule is impaired and the picnic nucleus hepatocytes (arrow) and sinusoidal structures (*) are impaired. e) Hepatic lobule in the E liver of the liver and picnic nucleus hepatocytes (arrow), sinusoidal structures (*) are damaged, and hemorrhagic (arrow head) areas are seen. f) In the liver, there are hepatic lobules that are damaged extensively in group F and picnic nucleus hepatocytes (arrow) and impaired sinusoidal structures (*) and hemorrhagic (arrow head). g) In the liver, picnic nucleus hepatocytes (arrow) and sinusoidal structures (*) are impaired and in some places hemorrhagic (arrow head) areas are seen in group G.

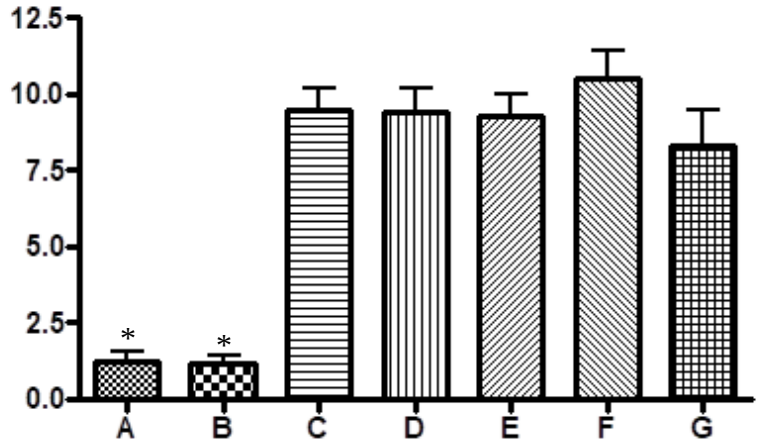


Figure 5: Histopathologic liver scores for each group.

*Significantly different from group C, D, E, F and G ($p < 0.001$). Group A: 500 mg/kg receptacle, group B: 500 mg/kg stalk, group C: 500 mg/kg inner bract, group D: 500 mg/kg outer bract, group E: 500 mg/kg leaves, group F: negative controls, group G: positive controls 100 mg/kg Legalon®.

5. DISCUSSION & CONCLUSION

Paracetamol is a well-known and frequently used compound for its analgesic and antipyretic effects (41). The hepatotoxic and nephrotoxic adverse effects of paracetamol are well-documented and generally confined to mild lesions when used acute in therapeutic doses, but it may induce severe damages in both organs when it is consumed above the therapeutic dose (42).

Metabolism and elimination of paracetamol occurs in the liver which includes phase I and phase II reactions. Principally, cytochrome P450 enzymes catalyses the metabolic reactions and converts paracetamol to N-acetyl-p-benzoquinone imine (NAPQI) (43). NAPQI is the reactive metabolite of acetaminophen and it is also mainly related with hepatotoxicity. The data has shown that CYP2E1 is the chief enzyme which is responsible for the conversion of paracetamol to NAPQI (44,45). This reactive metabolite can be detoxified via glutathione conjugation. However, excess NAPQI blocks the metabolic pathways which reduces the glutathione levels and binds the proteins. This scene results in mitochondrial dysfunction and oxidative stress. Oxidative stress makes worse the cellular injury via terminal kinase activation (46-48). This toxicological mechanism terminates with hepatocyte death.

Despite the fact that hepatic damage is the most common adverse effect of paracetamol, kidney damage has also been reported (49). In an animal model paracetamol is metabolized to p-aminophenol via deacylation reaction in the kidney. Thus, conversion of this metabolite causes paracetamol mediated nephrotoxicity (50). More recent studies have shown that kidney damage can be a result of free and non-protein derived paracetamol-cysteine complexes (51,52). These complexes are one of the breakdown products of paracetamol glutathione conjugates. This reaction is catalyzed by γ-glutamyltransferase which is highly found in the kidney. As a consequence of this conversion, free paracetamol-cysteine complexes deplete the renal glutathione, and the primary defense mechanism falls against NAPQI and ROS.

The most effective treatment option for paracetamol overdose is replenishment of liver glutathione levels. This is clinically achieved by giving N-acetyl cysteine (NAC) to the patient. The elevation of glutathione levels can scavenge excess NAPQI and interfere with protein binding. Glutathione can also decrease oxidative stress in the liver after

paracetamol overdose due to its antioxidant properties. Besides NAC treatment, several studies have shown that activated charcoal or emetic agents could be beneficial for the management of paracetamol overdose (53,54). These compounds need to be applied immediately after exposure of paracetamol overdose, because paracetamol is rapidly absorbed from the gastrointestinal tract. Dialysis and/or extracorporeal devices have been alternatively used in clinics to scavenge paracetamol from the circulation of overdosed patient and increase the clearance of toxic metabolites (55,56).

Alternatively, herbal remedies have been used for the management of health problems. The literature states that plant oriented treatment for liver disorders has promising results (57). Some of these precious medicinal plants are: *Silybum marianum* (58), *Glycyrrhiza glabra* (59), *Cynara scolymus* (4), *Berberis vulgaris* (60), *Calendula officinalis* (61), *Taraxacum officinale* (62), *Allium hirtifolium* (63), *Tragopogon porrifolius* (64), *Agrimonia eupatoria* (65), and *Prunus armeniaca* (66). The hepatoprotective agents in these plants includes silymarin, glycyrrhizin, β-sitosterol, betalain, phyllanthin, picroside, hypophyllanthin, and kutkoside. Moreover, the hepatoprotective mechanisms of these phytochemicals could be explained by antioxidant activity, free radical scavenging activity, decreasing of oxidative/nitrosative stress and inhibition of inflammatory response.

Artichoke (*Cynara scolymus*) is traditionally used for the treatment of liver and bile disorders. The extracts of artichoke contain cynarin, chlorogenic acid, and caffeic acid, other flavonoids, and polyphenols which have antioxidant activity. The literature states that plasma transaminase activities remarkably declined and histopathological changes in the liver ameliorated in rats by the help of artichoke extract (6).

In a study conducted by Shimoda and his colleagues, the anti-hyperlipidemic activity of the principal constituents from the leaves of artichoke has been examined and it has been observed that the MeOH extract from artichoke leaves shows anti-hyperlipidemic activity due to its suppressive effect on gastric emptying (19). Previous studies on artichoke leaf extract showing revealing its reductive effect on LDL oxidation due to the containment of flavonoids such as luteolin (67). Moreover, it reduces oxidative stress in endothelial cells stimulated by TNF-α (Tumor necrosis factor alpha) and oxidized LDL (35). The first study on the investigation of artichoke leaf extracts was done in 2009 and revealed that the artichoke leaf extract treatment having a positive effect on

the regulation of serum cholesterol, triglyceride levels and HC diet induced hepatic and cardiac pro-oxidant status (24).

A study conducted in hamster model to observe whether artichoke leaf extract (ALE) could lower plasma total and non-HDL cholesterol by increasing fecal excretion of neutral bile acids and sterols. The study concluded that the artichoke leaf extracts were reduced the hamster plasma cholesterol levels significantly (68). Another study published in same year demonstrated that the artichoke can be useful to decrease liver triglycerides, oxidative stress, plasma cholesterol, liver phosphatidate phosphohydrolase and triglyceride levels in hyperlipidemic rats. It has been shown that the artichoke has beneficial effects in the control of fatty liver and hyperlipidemia (69). Several studies showing that the artichoke extracts are supposed to exert a protective action to prevent carcinogenesis and atherosclerosis (70).

It has been observed that the artichoke leaf extract reduced the plasma cholesterol levels of the patients significantly (25). In another study aimed to identify the regulatory causes of artichoke extracts by using rodent models revealed that the cynaroside and particularly its aglycone luteolin were mainly responsible for inhibition of cholesterol biosynthesis by indirect inhibition of 3-HMG-CoA reductase which is the key enzyme for cholesterol biosynthesis (23).

A study investigating the dyslipidemic purpose of artichoke leaves with pear leaves, garlic extracts and their combinations reveals that only the combinations containing artichoke leaf extract inhibited HMG-CoA reductase activity significantly (71).

Moreover, another study indicates that artichoke has beneficial effects for the reduction in phosphatidate phosphohydrolase activity and liver triglyceride. Thus, it has positive effects on hyperlipidemia, oxidative stress in hyperlipidemic regimes, and abnormalities in lipid profiles (67).

Our findings indicated that receptacle and stem extracts of *Cynara scolymus* found to be effective for prevention of pathological liver changes in paracetamol-intoxicated rats. These outcomes were also in accordance with another study. Aktay G. et al. have shown the potent hepatoprotective activity of bract and receptacle extracts of *Cynara scolymus* on carbon tetrachloride treated rats (22). Moreover, receptacle and stem extracts

were found to be more effective than *Silybum marianum* extract (Legalon®) (at 100 mg/kg dose) which is known as a highly potential hepatoprotective herbal remedy.

The goal of the present study was to illuminate the possible efficacy of different parts of artichoke (*Cynara scolymus*) against paracetamol induced damage on liver and kidney. Our results showed that receptacle and stalk extracts of *Cynara scolymus* were found to be effective for prevention of pathological liver and kidney changes in paracetamol-intoxicated rats. Therefore, artichoke may be introduced as herbal alternative for liver and kidney intoxications.

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7. APPENDIX

7.1. Certificate of Animal Use in Experimental Research

DENEY HAYVANLARI KULLANIM SERTİFİKASI
CERTIFICATE of ANIMAL USE in EXPERIMENTAL RESEARCH

YEDİTEPE ÜNİVERSİTESİ

Veteriner Hekim Engin SÜMER

Yeditepe Üniversitesi Deney Hayvanları Etik Kurulunun düzenlediği 80 saatlik Laboratuvar Hayvanları kursunu tamamlamış ve kurs sınavında başarılı olmuştur

Has completed the 80 hours course and passed the examination in Laboratory Animal Science organized by the Animal Research Ethics Committee of Yeditepe University.

12-22 Kasım 2012/November 12-22,2012
İstanbul,TÜRKİYE/Istanbul,TURKEY

Prof. Dr. Ece GENÇ
Yeditepe Üniversitesi/Yeditepe University
Deney Hayvanları Etik Kurulu Başkanı /
Head of the Animal Research Ethics Committee

Prof. Dr. Nurcan BAÇ
T.C.Yeditepe Üniversitesi Rektörü
President of the Yeditepe University

7.2. Animal Research Ethics Committee Approval

YEDİTEPE ÜNİVERSİTESİ

T.C. YEDİTEPE ÜNİVERSİTESİ, DENEY HAYVANLARI ETİK KURULU
(YÜDHEK)
ETİK KURUL KARARI

Toplantı Tarihi	Karar No	İlgi	Proje Yürütücüsü
21.06.2017	615	13.06.2017	Yrd.Doç.Dr.Muhammed Hamitoğlu Prof.Dr.Erdem Yeşilada

'ENGİNAR EKSTRAKLARININ SIÇANLARDA PARACETAMOLLE İNDÜKLENMİŞ AKUT HEPATOTOKSİSİTE ÜZERİNDE HİSTOLOJİK ETKİNLİK ÇALIŞMALARI: KARACİĞER VE BÖBREK DOKULARINDA HİSTOLOJİK BİR ARAŞTIRMA' adlı bilimsel çalışma etik kurulumuzda görüşülmüş olup, çalışmanın etik kurallara uygun olduğuna oy birliğiyle karar verilmiştir.

Etik Onay Geçerlilik Süresi: 1 Yıl	**Hayvan Türü ve cinsiyeti:** **Sıçan** ♂	**Hayvan Sayısı:** 49

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