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Research Article

Effect of Walnut Seed Skin Against Main Organ Damage Caused by Hyperlipidemia

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Abstract

Hyperlipidemia, manifested as hypercholesterolemia and/or hypertriglyceridemia, is a condition characterized by a decrease in HDL levels with an increase in one or more of plasma lipids, including triglycerides cholesterol, cholesterol esters, phospholipids, and/or plasma lipoproteins including VLDL and LDL. It is also a major problem leading to obesity, stroke, myocardial infarction, atherosclerosis, type 2 diabetes, degenerative joint disease and circulatory disease and has become one of the most common causes of morbidity and mortality worldwide. Current anti-hyperlipidemic therapies mainly include statins and fibrates. These drugs used firstly inhibit cholesterol biosynthesis and correct the changed blood lipid profile. Secondly, it acts by increasing the clearance of triglyceride-rich lipoproteins. In addition to hepatotoxicity, myopathy, rhabdomyolysis and some undesirable side effects during pregnancy have also been reported. While these side effects limit the use of statins in hyperlipidemia, they also support the research of medicinal plant products with hypolipidemic-phyto-active compounds. The study of plants is a useful strategy for the emergence of new molecules that have an important and enhanced effect on lipid metabolism. For this reason, in the current study, considering the many beneficial effects of walnuts supported by the literature, the unused part of the walnut seed skin (WSS) is focused on. It was investigated whether ethanolic extraction of walnut seed skin (E-WSS) had a protective effect in male Wistar-Albino rats in which hyperlipidemia was induced. The histopathological evaluation of the effects of E-WSS on kidney tissue, the content of which was defined by GC-MS in our previous study, was performed in this study. In the analysis, it was concluded that E-WSS extract had a significant protective effect on the kidney tissue of rats with hyperlipidemia when compared to the healthy control group.
When the final evaluation was made, a contribution was made to the literature on the use of a natural compound that does not show toxic effects against hyperlipidemia.

Keywords: Kidney, Hyperlipidemia, Walnut seed skin, Rat

Hiperlipideminin Neden Olduğu Ana Organ Hasarına Karşı Ceviz Tohumu Kabuğunun Etkisi

Öz

Hiperkolesterolemi ve/veya hipertrigliseridemi olarak kendini gösteren hiperlipidemi, trigliseritler kolesterol, kolesterol esterleri, fosfolipidler ve/veya VLDL ve LDL dâhil plazma lipoproteinleri dâhil olmak üzere plazma lipidlerinden bir veya daha fazlasında artış ile birlikte HDL seviyelerinde azalma ile karakterize edilen bir durumdur. Aynı zamanda obezite, felç, miyokard enfarktüsü, ateroskleroz, tip 2 diyabet, dejeneratif eklem hastalığı ve dolaşım hastalığına yol açan önemli bir sorundur ve dünya çapında en yaygın morbidite ve mortalite nedenlerinden biri haline gelmiştir. Mevcut anti-hiperlipidemik tedaviler esas olarak statinleri ve fibratları içerir. Kullanılan bu ilaçlar ilk olarak kolesterol biyosentezini inhibe ederek değişen kan lipid profilini düzeltir. İkinci olarak da trigliseritten zengin lipoproteinlerin temizlenmesini artırarak etki eder. Hepatotoksisitenin yanısıra miyopati, rabdomiyoliz ve hamilelik esnasında istenmeyen birtakım yan etkilerinin olduğu da bildirilmiştir. Meydana gelen bu yan etkiler statinlerin hiperlipidemide kullanımını sınırlandırmakla beraber, hipolipidemik-fito-aktif bileşiklere sahip tıbbi bitki ürünlerinin araştırılmasını da desteklemektedir. Bitkilerin araştırılması, lipid metabolizması üzerinde önemli ve gelişmiş bir etkiye sahip yeni moleküllerin ortaya çıkması için yararlı bir strajedir. Bu nedenle mevcut çalışmada, cevizin literatürle desteklenen birçok faydalı etkisi göz önüne alınarak kullanılmayan kısım olan ceviz tohum kabuğu (CTK) üzerine yoğunlaşılmıştır. Ceviz tohum kabuğunun etanolik ekstraksiyonunun (E-CTK), hiperlipideminin indüklendiği erkek Wistar-Albino ratlarda koruyucu etkisinin var olup olmadığı araştırılmıştır. Daha önce yaptığımız çalışmada GC-MS ile içeriği tanımlanan E-CTK'nın böbrek dokusunda ki etkilerinin histopatolojik değerlendirmeleri ise bu çalışmada yapılmıştır.

Yapılan analizde, E-CTK ekstraktının sağlıklı kontrol grubu ile kıyaslandığında hiperlipidemili ratların böbrek dokusunda önemli bir koruyucu etkiye sahip olduğu sonucuna varılmıştır.
Nihai değerlendirme yapıldığında, hiperlipidemi hastalığına karşı toksik etki göstermeyen doğal bir bileşiğin kullanımına yönelik literatüre katkıda bulunulmuştur.
Anahtar Kelimeler: Böbrek, Hiperlipidemi, Ceviz tohum kabuğu, Rat

1. Introduction

Hypercholesterolemia is a life-threatening disease that develops with high lipid content in the bloodstream. Lipids play a vital role in the body's muscle growth, but abnormal blood fat levels greatly increase the risk factor for developing coronary heart diseases (Sunil et al., 2012; Ghassan, 2014; Hashem et al., 2021; Tripathi, 2013). Cardiovascular diseases are responsible for one third of the total deaths in the world, but it is believed to be the leading cause of mortality in the coming years (Jørgensen, 2013). Hyperlipidemia results from elevations of total cholesterol (TC), triglyceride, very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) in plasma. Hyperlipidemia is also caused by a decreased level of high-density lipoprotein (HDL) in the blood. Hyperlipidemia with high levels of lipoproteins is measured by the initiation and progression of plaque formation in the arteries, which can cause thrombosis and myocardial infarction (Tripathi, 2013; Thomas and Kamath, 2017). In order to keep these negative situations under control, it is necessary to reduce the lipid level. Statins and fibrates used due to their anti-hyperlipidemic effects, on the other hand, may produce an unexpected toxic effect besides their therapeutic effects (niacin, clofibrate, gemfibrozil, atorvastatin, cholestyramine, colestipol and probucol) (Hashem et al., 2021; Abu-Raghif et al., 2015). For this reason, the use of herbal products has become widespread due to the absence of undesirable side effects, their economic and easy accessibility. The development of more than half of the drugs used from natural products also plays an important role in the discovery of drugs (Xu et al., 2019).

Walnut, (*Juglans regia* L.), belongs to the Juglandaceae family. It is an important tree species with economic, ecological and oil

recovery in the world. It is widely available in many places in America, Europe and Asia. Thirteen walnut species have been described, but this number can reach up to 300 with local varieties (Cao and Li, 2017). It is considered nutraceutical because it contains proteins and essential unsaturated fatty acids, as well as tocopherols, sterols and polyphenols with well-known antioxidant, anti-inflammatory and antibacterial properties (Alasalvar and Bolling, 2015). Walnut seed skin (WSS) evaluated in this study is another important by-product that reveals the in vitro biological potential through bioactive phytochemicals that separate the two halves of the walnut kernel (Rusu et al., 2018). The aim of this study was to evaluate whether walnut seed shell extract has a protective effect on the kidney tissues of rats with hyperlipidemia induced by injection of Triton WR-1339.

2. Material and Method

2.1. Plant Material

The WSS used was obtained from walnuts belonging to Posof county in Ardahan province. In Atatürk University Genetics laboratory, after drying, it was turned into fine powder with a mixer grinder and stored at -18℃.

2.2. Preparation of WSS Extract

Ethanol extraction (E-WSS) of WSS was performed according to the method of Kotan *et al.* (Kotan et al., 2010).

2.3. Anti-Hyperlipidemic Activity

Before starting the experiment, rats were acclimated to laboratory conditions for one week. The rats were then randomly divided into five groups of six rats each (It is shown in Table 1).

B1: Healthy control group; physiological water [2.5 mL/kg, intraperitoneally (i.p)] administered group.

B2: Walnut seed husk (150 mg) (E-WSS) group; The group given walnut seed skin extract [150 mg/kg, oral dose (o.d)] 30 minutes before physiological water (2.5 mL/kg, i.p) administration.

B3: Walnut seed husk (300 mg) (E-WSS) group; The group given walnut seed skin extract [300 mg/kg, oral dose (o.d)] 30 minutes before physiological water (2.5 mL/kg, i.p) administration

B4: Hyperlipidemic group (HL). The group that received physiological water 30 minutes before Triton WR-1339 (400 mg/kg, 2.5 mL/kg, i.p) administration.

B5: Hyperlipidemic + Walnut seed skin (300 mg) (HL + E-WSS). Group that received walnut seed skin extract (300 mg/kg, o.d) 30 minutes before administration of Triton WR-1339 (400 mg/kg, 2.5 mL/kg, i.p).

Rats were fed rat chow pellets and water ad libitum (Sikarwar and Patil, 2012). After fasting for 12 hours, a single dose of Triton WR-1339 was injected intraperitoneally at a dose of 400 mg/kg and hyperlipidemic was made.

Table 1. Study groups

Groups	Healty Control	WSS 1 (150 mg)	WSS 2 (300 mg)	Positive Control (Triton WR-1339, 400 mg/kg, 2.5 mL/kg)	Triton WR-1339 + WSS 2 (300 mg)
B1	+	-	-	-	-
B2	-	+	-	-	-
B3	-	-	+	-	-
B4	-	-	-	+	-
B5	-	-	+	-	+

2.4. Histopathological Examination – Preparation of Tissues for Light Microscopy

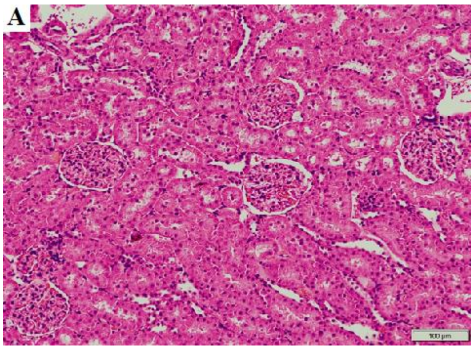
After the last application, the sacrification process was applied. After the kidney tissue was gently removed, it was washed with normal saline (0.9% NaCl). The washed tissue was fixed in 10% normal saline, buffered with formalin solution, and allowed to dry for one day. The dehydration process was achieved by passing the kidney tissue through a sequential series of alcohol and xylene. After the treatment with alcohol and xylene, the tissues were fixed in pure paraffin wax. Tissue sections of 4 µm thickness were taken with a microtome device. To observe the general histological structure, all kidney sections were stained with hematoxylin and eosin and placed on glass slides. Kidney tissue sections were examined using an Olympus B×60 microscope. Findings were photographed at e×200 magnification (Ziad et al., 2013).

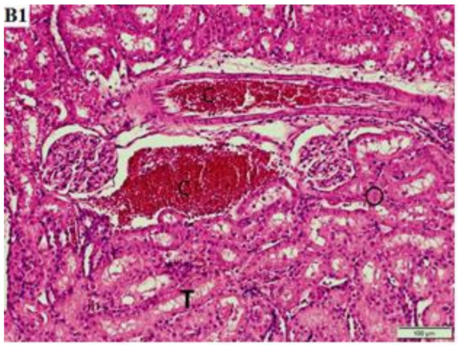
3. Results and Discussion

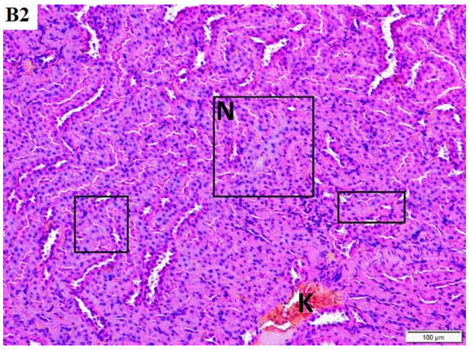
3.1. Histopathological Evaluation

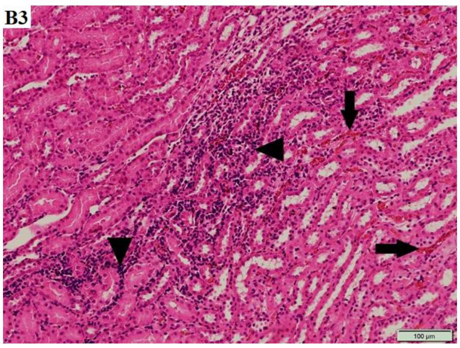
Histopathological examination was performed in the dissected section of kidney tissues and various histopathological findings were observed in kidney tissues obtained from rats injected with Triton WR-1339.

No pathological findings were observed in the group treated/protected with WSS (300 mg).









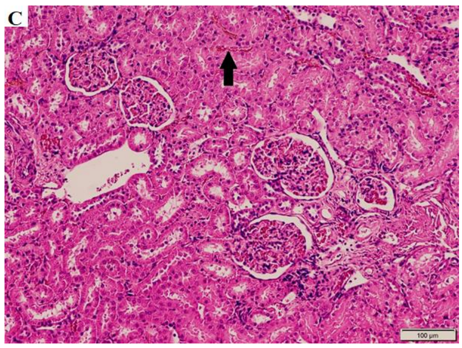


Figure 1A. Healthy control kidney tissue: normal histological image.

Figure 2B1-B3. Control group kidney tissue of the patient with hyperlipidemia with 400 mg/kg, 2.5 mL/kg Triton WR-1339 application: Increased damaged histological image (C: congestion, T: tubular dilation, Circle: apoptosis, N: necrosis, Thick arrow: hemorrhage, Arrowhead: mononuclear cell infiltration).

Figure 3C. Renal tissue in the treatment group in which 400 mg/kg, 2.5 mL/kg Triton WR-1339 and 300 mg E-WSS were administered together: Reduced damaged histological image (thick arrow: hemorrhage).,

Table 2. Histopathological findings and grades in kidney tissue

Groups	Healty Control	Positive Control	Treatment Group
	Kidney	Kidney	Kidney
Congestion	---	+++	---
Tubular Dilatation	---	++	---
Apoptosis	---	++	---
Necrosis	---	+++	---
Hemorrhage	---	+++	++
Mononuclear Cell İnfiltration	---	++	---

*Histopathological results; none (---), mild (+), moderate (++) and severe (+++

In Figures 1, 2 and 3, the histological structure of the kidney belonging to the administration groups is shown. Histological sections from healthy control rats showed normal glomerulus structures, urinary cavities, proximal, distal, and collecting tubules (Fig. 1A). After the application of Triton WR-1339, obvious damage was detected in the examination of the kidney tissue. These damages include changes such as congestion, tubular dilatation, apoptosis, necrosis, and damage to the surface epithelium. Hemorrhage and mononuclear cell infiltration were also detected. Moreover; tubules (both proximal and distal convoluted) showed many changes (Fig. 2B2). Finally; hemorrhage and mononuclear cell infiltration were seen in the interstitium (Figure 2B3). However, the histological change in kidney tissues of rats treated with Triton WR-1339 + E-WSS (300 mg) was mostly reduced compared to damage caused by Triton WR-1339, with a high-scale improvement in the group, little interstitial bleeding and appeared to have an almost normal appearance (Fig. 3C). Cholesterol, triglycerides (TG), and high-density lipoproteins (HDL-C) are essential components of the human biological system.

3.2. Discussion

Cholesterol is an unsaturated alcoholic compound belonging to the steroid family, which maintains the normal physiological functions of animal cells and is a necessary element of cell membrane activity (Tsoupras et al., 2018). It is important for the synthesis of cholesterol, adrenaline hormone and gonadal steroidal hormones. Triglycerides (TG) are fatty acid esters of glycerol that serve as fat stores in humans and animals (Ravi, 2021). Cholesterol and triglycerides are non-polar lipid substances that are insoluble in water. Therefore, lipoproteins act as a special carrier for the transport of cholesterol and triglycerides in plasma (Blanco and Blanco, 2017).

In this study, Triton WR-1339 was used as an acute model for the preliminary and rapid screening of hypolipidemic agents in inducing hyperlipidemia in rats. This nonionic detergent binds TG-rich lipoproteins, reducing the rate of lipoprotein lipase-mediated TG hydrolysis and thereby reducing the uptake of blood TGs by tissues (Millar et al., 2005). It also increases hepatic cholesterol biogenesis and 3-hydroxy, 3-methyl-glutaryl CoA (HMG CoA) reductase activity (Oh et al., 2006). However, Triton has also been reported to cause the cleavage of apolipoprotein A-I (apoA-I) and apoC-II from HDL-C (Millar, 2015). It is clear that the hyperlipidemia induced by Triton WR-1339 is the most real and greatest health hazard factor (Saravanan and Pari, 2015). After intraperitoneal injection of Triton WR-1339, histological examinations on the kidney showed some findings such as congestion, tubular dilatation, apoptosis, necrosis, hemorrhage, and mononuclear cell infiltration compared to the control group. Given these changes, it seems likely that hyperlipidemia plays a pathogenic role in the development of kidney diseases (Vázquez-Pérez et al., 2001).

In the present review, it was clearly revealed that Triton WR-1339 directly triggered kidney damage by histopathological examination. Figure 2 (B1-B3) revealed that Triton WR-1339 caused severe histopathological changes in the kidney.

Herbal medicinal products, vitamin E etc. It is well known that nutritional supplements play an important role in the etiology of hyperlipidemia. These products, like lipid-lowering drugs, are known to interfere with the lipid biosynthesis pathway, lipid excretion, and the lipid metabolism pathway of the excretory phase (Shattat, 2015). Since WSS can be used in traditional medicine for the treatment of chronic diseases such as diabetes, gout and cancer, its reliability has been tested many times in studies (Schlesinger et al., 2009). The lipid-lowering effect of the bioactive compounds of WSS used in this study may be due to their ability to interact with the substrate emulsion, inhibiting lipolysis and blocking lipase adsorption onto the substrate surface, which may result in changes in substrate surface properties. Madic *et al.* (2021), it was concluded that the histopathological changes in the kidney tissue, in which hyperlipidemia was induced, were more effective than standard drugs in their treatment using a polyherbal mixture extract (Madić et al., 2021). Azhar *et al.* (2015) also found that the aqueous extract of walnut leaves had a lipid-lowering effect in hypercholesterolemic rats, and this result is in line with our study (Azhar et al., 2015).

4. Conclusions and Recommendations

Food and agricultural industry by-products can represent valuable and inexpensive sources of bioactive compounds. Therefore, our study aimed to increase knowledge about walnut seed husk, a by-product that currently has limited use. In the current study, the histopathological effects of E-WSS, which is rich in bioactive compounds, were evaluated using a Triton WR-1339-induced hyperlipidemia model in rats. It has been determined that walnut seed skin has protective effects on kidney tissue due to its ability to correct hyperlipidemia.

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