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Effects of Liquidambar Orientalis Oil on Liver and Kidneys in Heathy Wistar Rats

R. Kıyıcı[1], B.K. Karaayvaz[2], H.A. Akkan[3], M. Karaca[3], Z. Özyıldız[4], A. Kart[5], S. Garlı[6] 10.18805/IJAR.DRF-364

ABSTRACT

Background: Nowadays, the tendency towards alternative herbal therapies has increased due to the side effects of drugs. However, since the contents, method of use, dosage and toxic effects of herbal products are not fully known, undesirable situations are occasionally encountered. However, there are many experimental studies on the cytotoxicity of *L. orientalis* oil, most of these studies are *in vivo* modelling. In this study, the cytotoxic effects of *L. orientalis* oil on the liver and kidneys were investigated in healthy rats by oral administered.

Methods: The study material consisted of 18 female Wistar albino rats (190-280 g) aged 8-10 weeks. All animals were housed under optimal temperature, light and humidity conditions and fed with normal rat pellet food ad libitum. Housing conditions were checked daily. Three groups were organized as *L. orientalis* oil, olive oil and control group, respectively. Each group consisted of 6 rats.

L. orientalis oil group: *L. orientalis* oil (150 mg/kg) dissolved olive oil (0.6 mL).

Olive oil group: Olive oil (0.6 mL).

Control group: Empty gavage.

The entire trial was performed by intragastric gavage once a day for 14 days.

Result: The obtained data showed that serum biochemical parameters remained within normal ranges in oral use of Liquidambar orientalis oil (150 mg/kg) and did not show any difference with the control and olive oil groups. No pathological changes related to cytotoxicity were found in the samples taken from liver and kidney tissues.

Key words: Biochemistry, Histopathology, Kidney, *Liquidambar orientalis,* Liver.

INTRODUCTION

There are four phylogenetic sweetgum species known in the world "*L. orientalis* Mill., *L. formosana* Hance, *L. styraciflua* and *L. acalycina*". *Liquidambar spp.* trees are important natural resources containing beneficial compounds with medicinal and many other economic values. The resinous essence obtained by scraping the bark of the *Liquidambar spp.* tree and essential oils obtained from its leaves have been used in medical treatment for many years (Gürbüz *et al*., 2013).

Liquidambar orientalis (*L. orientalis*, Sweetgum, Akgunluk) is a relict endemic plant that grows in the southwest of Turkey, especially in the Marmaris and Koycegiz regions. Both the resin obtained by scraping the bark and the essential oils obtained from the leaves have been used for centuries by people in this region for cough, skin and stomach complaints. *L. orientalis* oil is actually a plant sap obtained from bark of this tree by scraping and is also called styrax, storax, styrax liquids, levant styrax, orientalis frankincense gum. Although commercial preparations are available, it is also sold as no name in herbal shops in the region (Moerman, 1998). Anti-inflammatory, antiulserigenic and antioxidant properties of *L. orientalis* have been previously demonstrated by experimental studies. Although the use of herbal medicine has become very popular in recent years, toxic effects caused by incorrect usage of dosage, method and exposure are frequently encountered. (Arslan and ªahin, 2016; Gürbüz *et al*., 2013).

[1]Department of Plant and Animal Production, Food, Agriculture and Livestock Vocational School, Mehmet Akif Ersoy University, Burdur, Türkiye.

[2]Laboratory and Veterinary Health Associate Degree Program, Food, Agriculture and Livestock Vocational School, Mehmet Akif Ersoy University, Burdur, Türkiye.

[3]Department of Veterinary Internal Medicine, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Türkiye.

[4]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Türkiye.

[5]Department of Veterinary Pharmacology, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Türkiye.

[6]Experimental Animals Application and Research Center, Mehmet Akif Ersoy University, Burdur, Türkiye.

Corresponding Author: R. Kıyıcı, Department of Plant and Animal Production, Mehmet Akif Ersoy University, Food, Agriculture and Livestock Vocational School, Burdur, Türkiye.
Email: rkiyici@mehmetakif.edu.tr

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Liquidambar spp. trees are important natural resources with medicinal and economic value because they contain compounds beneficial for human health (Moerman, 1998). The sap, also called Storax, has proven

to be a potent alternative antimicrobial agent against multidrug resistant bacteria. It has also been reported that Asian storax contains cinnamic acid, cinnamyl cinnamate, 3-phenylpropyl cinnamate, ethyl cinnamate, benzyl cinnamate, styrene and vanillin (Moerman, 1998; Guenther, 1952). Cinnamic acid has been reported previously to have antimicrobial and antioxidant properties (Sova, 2012). In addition, the leaves, bark and seeds of *Liquidambar spp.* tree also have beneficial compounds such as shikimic acid, which is the precursor of oseltamivir phosphate to the production of its antiviral active ingredient. Other extracts from *Liquidambar spp.* trees have been reported to have antioxidant, anti-inflammatory and chemopreventive potential (Sharun *et al*., 2021). Some compounds from *Liquidambar spp.* extract have been reported to suppress hypertension in mice. Studies have shown that extracts obtained from *Liquidambar spp.* seeds have anticonvulsant effects, which indicates that these extracts may be a potential drug that can be used in the treatment of epilepsy. In addition *Liquidambar spp.* juices have antifungal activity against various phytopathogenic fungi and to be an effective treatment to evade nematodes and yellow mosquitoes from the plant. Therefore, it is reported that these extracts can be used as an alternative option to some harmful drugs such as pesticides and antifungal agents. The list of products obtained from *Liquidambar spp.* trees can be increased by conducting more intensive and extensive research on this tree, which is abundant in our country (Lingbeck *et al*., 2015).

Liver and kidney are important organs in which toxins are detoxified and evaded from the body. Abnormalities in AST, ALP, ALT, GGT for the liver and urea and creatinine parameters for the kidney are used as toxicity indicators. (Okoro and Farate, 2019; Nwose *et al*., 2019; Damiati, 2019; Rodríguez *et al*., 2019; Ribeiro *et al*., 2019; Wilkerson and Ogunbodede, 2019).

The aim of this study is to examine the effects of orally administered *L. orientalis* oil on the liver and kidneys of healthy rats using biochemical and histopathological methods.

MATERIALS AND METHODS

Plant material

The *L. orientalis* oil purchased from a commercial company. (Koycegiz Yoresel Sigla Yagi Gunluk, Ozcakirlar Gida Sanayi ve Ticaret, Koycegiz, Türkiye). *L. orientalis* oil was dissolved with olive oil.

Experimental animals and study groups

In the study, 18 female Wistar breed albino rats, 8-10 weeks old, 190-280 gr, obtained from the Experimental Animals Unit of Burdur Mehmet Akif Ersoy University, were used. An ethics committee report was obtained from Animal Experiments Local Ethics Committee (dated 15.09.2021 and numbered 812).

All animals were housed under optimal temperature, light and humidity conditions and fed with normal rat pellet food ad libitum. Housing conditions were checked daily. Three groups were organized as *L. orientalis* oil, olive oil and control group, respectively. Each group consisted of 6 rats.

L. orientalis oil group: *L. orientalis* oil 150 mg/kg (Gürbüz *et al*, 2013) dissolved olive oil (0.6 mL).
Olive oil group: Olive oil (0.6 mL).
Control group: Empty gavage.

The entire trial was performed by intragastric gavage once a day for 14 days.

Blood analysis

On the 14th day of the study, blood samples from the heart were taken into serum tubes under anesthesia and the serums were separated. AST, GGT, creatinine, urea, BUN, bilirubin levels were measured with Erba xl 1000 device as per the manufacturer's instructions.

Statistical analysis

Statistical analysis of blood serum results obtained in this study was performed with One-way ANOVA test in SPSS 22.00 package program. Differences between groups were compared with Duncan test. Values with $P<0.05$ were considered statistically significant.

Histopathological examination

The rats were sacrificed by cervical dislocation method under xylazine-ketamine anesthesia. After macroscopic examination of the liver and kidneys, the tissues were fixed in 10% buffered formaldehyde. After fixation, they were trimmed and placed in routine tissue follow-up and embedded in paraffin blocks. Sections of 5 µm thickness were taken from paraffin blocks and stained with routine Hematoxylin and Eosin staining. Microphotographs were taken under a light microscope.

RESULTS AND DISCUSSION

Clinical Findings: Some of the rats administered *L. orientalis* oil and olive oil showed softening in feces consistency. Apart from this, no adverse effects were found.

In this study, no statistical difference was found between the groups in the serum biochemical values used for the evaluation of liver and kidney functions.

Serum biochemical analyzes

AST, GGT, creatinine, urea, BUN, bilirubin levels of serum samples obtained on the 14th day of the study are given in Table 1.

Histopathological findings

Macroscopic findings

All groups were initially examined macroscopically and no difference was observed among the groups.

Microscopic findings

Liver

The animals in the experimental and control groups were examined in terms of vena centralis, vena interlobularis

and the state of the sinusoids, the arrangement of the remark cords, hyperplasia of Kupffer cells, degenerative and necrotic changes in hepatocytes and hyperplasia of the bile ducts. At the end of the examination, no difference was observed in the livers between the control and study groups (Fig 1).

Kidney

In the histopathological appearance of the kidneys of the animals in the groups, the status of the glomerulus and Bowman's spaces, degenerative and necrotic changes in the tubular epithelium, shed epithelial cells and proteinous masses in the tubular lumens were evaluated. At the end of the examination, no difference was observed between the control and study groups (Fig 2).

The antimicrobial (Sova, 2012), antifungal (Lee *et al*., 2009), anticoagulant (Yang *et al*., 2011), antihepatotoxic (Konno *et al*., 1998) and anticarcinogenic (Jeong HG, 1999; Tan *et al*., 2003) properties of *L. orientalis* have been previously reported. It has also been shown to accelerate wound healing in mice (Öçsel *et al*., 2012) and reduce hypertension (Ohno *et al*., 2008).

Although there are many studies investigating the effects of plant extracts on healthy rats (Kandemir *et al*., 2017; Mercan *et al*., 2017; El Hendy and Al Gemeai, 2014; Oduola *et al*., 2010), no study has been found evaluating the effects of *L. orientalis* on the kidney and liver when oral administered. The most relevant study on this topic investigated the protective effects on different doses of *L. orientalis* oil on carbon tetrachloride (CCl4)-induced liver damage in rats, but were not evaluated effects on the livers of healthy rats (Aydýngöz *et al*., 2013).

This study revealed that *L. orientalis* oil did not have a cytotoxic effect on the liver and kidney when administered orally to healthy rats, as seen in Table 1. However, since there is no study about effects of oral administration of *L. orientalis* oil on various organs and systems in healthy rats, it could not be discussed.

Table 1: AST, GGT, creatinine, urea, BUN, total bilirubin values in serum samples obtained from rats on the 14th day of the study.

Parameter	Control	Olive oil	Sweetgum oil (Sweetgum oil dissolved in olive oil)	P values
Urea	9.20±2.40	9.51±1.27	10.71±1.89	>0.05
BUN	25.16±6.40	26.00±3.34	29.50±5.16	>0.05
Creatinine	0.65±0.09	0.66±0.01	0.66±0.03	>0.05
AST	145.81±10.90	145.35±12.78	153.68±17.88	>0.05
GGT	1.46±0.16	1.65±0.63	1.10±0.46	>0.05
Total bilirubin	0.07±0.02	0.09±0.01	0.08±0.31	>0.05

Values with P<0.05 are statistically significant.

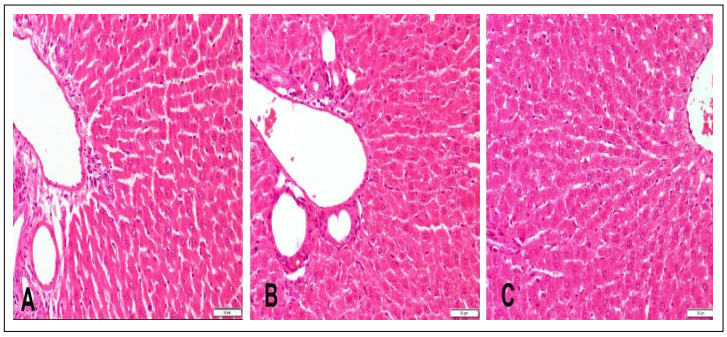

Fig 1: Liver, H and EX 200, bar: 50 µ. A. Control group, B. Sweetgum oil group, C. Olive oil group.

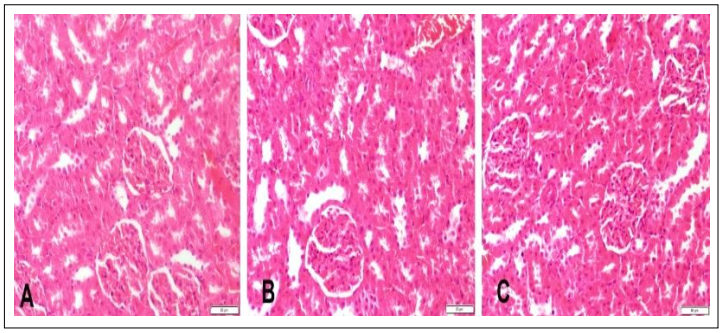

Fig 2: Kidney, H and EX 200, bar: 50 µ. A. Control group, B. Sweetgum oil group, C. Olive oil group.

In this study, the data obtained from serum biochemical and histopathological methods revealed that *L. orientalis* oil did not cause any toxic effects on the liver and kidney when administered orally to healthy rats.

CONCLUSION

In the present study, as a result of biochemical and histopathological examinations performed after 14 days of oral use of *L. orientalis* oil in Wistar albino rats, it was revealed that *L. orientalis* oil did not show a toxic effect on kidney and liver.

At the end of the study, no difference was observed histopathologically between the control and study groups in the liver and kidney tissues.

The results of this study showed that *L. orientalis* oil obtained from *L. orientalis* Mill. does not cause any side effects on liver and kidneys in certain dose by oral route.

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Ethical statement

This study was approved by the Local Ethics Committee of Burdur Mehmet Akif University (Approval Number: 15.09.2021/812).

Conflict of interest

The authors are declared that there is no conflict of interest.

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