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Original Article

HEPATOPROTECTIVE AND HYPOGLYCEMIC ACTIVITIES OF *VIBURNUM OPULUS* L.

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Abstract

In the present study, we investigated the hepatoprotective effect of the water extract of Viburnum opulus L. (VO) on carbon tetrachloride (CCL_4)- induced hepatotoxicity in rats, hypoglycemic activity and acute lethality of the same extract in mice.

Biochemical parameters of hepatic damage such as serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin concentrations were determined. CCl_4 (0.8 mL/kg i.p for 7 days) treatment increased the serum AST, ALT, ALP and bilirubin levels significantly as compared to controls. Treatment of animals with silibinin (50 mg/kg) +CCl_4 (0.8 mL/kg i.p.) and VO (100 mg/kg, i.p.) +CCl_4 (0.8 mL/kg i.p.) for 7 days lowered the levels of ALT and AST but was not significant. It was observed that ALP levels higher than normal values. The results of biochemical tests were also confirmed by histopathological examination. VO showed a few balooning degeneration, apoptosis, centrilobular necrosis in the liver tissue and bridging necrosis similar to silibinin-treated group. To compare hypoglycemic activity of VO, glibenclamide was used as the reference agent. However, VO has hypoglycemic activity neither in diabetic mice nor in their healty controls.

The present study revealed that the water extract of Viburnum opulus had slight hepatoprotective effect on carbon tetrachloride-induced acute liver toxicity in rats and no hypoglycemic activity in mice. The LD_{50} of VO was determined as 5.447 g/kg.

Key words: *Viburnum opulus, Caprifoliaceae, Hepatoprotective activity, Hypoglycaemic activity, Median lethal dose.*

Viburnum opulus L. Yapraklarının Hepatoprotektif ve Hipoglisemik Aktivitesi

Bu çalışmada Viburnum opulus L. (VO) bitkisinin sulu ekstresinin sıçanlardaki karbon tetraklorür (CCl_4) nedenli hepatotoksisite üzerine hepatoprotektif etkisi, hipoglisemik aktivitesi ve akut letalitesi araştırılmıştır.

Serum aspartat amino transferaz (AST), alanin amino transferaz (ALT), alkalin fosfataz (ALP) ve bilirubin konsantrasyonu gibi karaciğer hasarının biyokimyasal parametrelerine bakılmıştır. CCl_4 (0.8 mL/kg i.p., 7 gün) grubunda serum AST, ALT, ALP ve bilirubin seviyeleri kontrol grubuyla kıyaslandığında anlamlı seviyede arttığı görülmüştür. Sıçanlara silibinin (50 mg/kg)+CCl_4 (0.8 mL/kg i.p.) ve VO (100 mg/kg, i.p.) + CCl_4 (0.8 mL/kg i.p.) 7 gün boyunca uygulandığında, tek başına CCl_4 uygulandığındaki yükselen serum ALT ve AST seviyeleri düşmüştür, ancak bu düşüş anlamlı değildir. ALP değerleri normal değerlerden daha yüksek olduğu görülmüştür. Biyokimyasal testlerin sonuçları histopatolojik incelemelerle de doğrulanmıştır. VO karaciğerde daha az balon dejenerasyonu, apoptozis ve sentrilobular nekroz ve silibinin uygulanmış gruba benzer köprüleşme nekrozu göstermiştir.

VO'nun hipoglisemik aktivitesini değerlendirmek için glibenklamid referans madde olarak kullanılmıştır. Fakat VO, sağlıklı ve diyabetik farelerde hipoglisemik aktivite göstermemiştir.

Bu çalışmada Viburnum opulus'un sulu ekstresinin karbontetraklorürle indüklenerek karaciğerde toksisite oluşturulan sıçanlarda zayıf hepatoprotektif etki gösterdiği ve farelerde herhangi bir hipoglisemik aktivite göstermediği sonucuna varılmıştır.VO'nun LD50 değeri 5.447 g/kg olarak saptanmıştır.

Anahtar kelimeler: *Viburnum opulus, Caprifoliaceae, Hepatoprotektif aktivite, Hipoglisemik aktivite, Ortalama letal doz*

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INTRODUCTION

The genus *Viburnum* (Caprifoliaceae) is composed of more than 230 species distributed from South America to South-East Asia, the majority of them being endemic (1). The plant genus is represented by four species in the flora of Turkey; *Viburnum opulus* L., *V. orientale* Pallas, and *V. lantana* L.and *V. tinus* L. (2,3).

In Central Anatolia, a traditional beverage named "***gilaburu"*** has been prepared from the fruits of *V. opulus* whose fruits have a dark-red color and edible. The fruits of *V. opulus* have been used as an antidiabetic, and the bark of *V. lantana* has been used as a rubefiant and analgesic in Turkish folk medicine (4,5). Oxidative stress which is defined as an in balance between the generation oxidants and antioxidant defence capacity of the body (6). The preventive effect of *V. dilatatum* Thunb. on oxidative damage has been found in rats plasma, liver and stomach subjected to stress (5) and streptozotocin-induced diabetic rats (7). In addition, the effects of *V. dilatatum* on antioxidant enzymes in plasma, liver, stomach have been examined in a previous study whose results have suggested that ingestion of this plant might contribute to reduce the consumption of antioxidant enzymes, such as superoxide dismutase, catalase, glutation peroxidase and glutation (8). The alcoholic extracts of *V. erubescens* Wall. have been studied for antiviral activity, as well (9). Some iridoid aldehydes isolated from *V. luzonicum* Rolfe have exhibited moderate inhibitory activity against HeLa S3 cancer cells (10).

The genus *Viburnum* is known to contain triterpenoids (11-13), diterpenoids (14,15), sesquiterpenes (16) , iridoids (17-20) and polyphenols (21,22).

In our previous studies, we have tested the antioxidant, antinociceptive and anti-inflammatory effects of *V. lantana* and *V. opulus* (23-25). The hepatoprotective and hypoglycemic activities of *V. lantana* have also been studied by us (26). We also determined amentoflavone, salicin and chlorogenic acid contents in different organs of *V. opulus* and *V. lantana* by using HPLC (27,28).

Thus, as a continuation of our research on this genus (23-28) we now report the hepatoprotective and hypoglycemic effects of *V. opulus*. These activities have not been investigated before on this species.

EXPERIMENTAL

Plant material

V. opulus L. was collected in 2005 from flowering plants near Kayseri (Turkey). Taxonomic identity of the plant was confirmed by Prof. Dr. H. Duman, a plant taxonomist in the Department of Biological Sciences, Faculty of Art and Science, Gazi University, Ankara, Turkey. Voucher specimens were kept in the Herbarium of Ankara University, Faculty of Pharmacy (AEF No 23696).

Preparation of extract

Air-dried and powdered leaves of the plant were extracted with water. The aqueous extract was prepared by macerating 100 g of plant powder in 1000 mL cold distilled water for 1 day. The macerate was evaporated and lyophilized. The extract yield was 22.4 g/ 100g (w/w).

Animals

Male and female Sprague-Dawley rats (200-250 g) and Swiss albino mice (20-24 g) were maintained in the Animal House of YüzüncüYıl University, Faculty of Medicine.The animals were breed in our institutional animal house but the lineage originally obtained from Ankara Health Protection Institute (a governmental organisation). The animals were housed in standard cages (48 cm x 35 cm x 22 cm), at room temparature (22±2 ^{0}C) with artificial light from 7.00 am to 7.00 pm, and provided with pelleted food (Van Animal Feed Factory, Van-TURKEY) and water *ad libitum*. The protocol for the study was approved by the Ethical Committee of Yüzüncü Yıl University Faculty of Medicine Animal Breeding and Research (2005/06-02).

Drugs and Chemicals

Carbon tetrachloride (Merck, Darmstadt, Germany), olive oil (Fluka, Steinheim, Germany), alloxan, silibinin (Sigma, Steinheim, Germany) and glibenclamide (Nobel, İstanbul, Turkey). CCl_4 was dissolved in olive oil and silibinin was solved with ethyl alcohol.

Acute toxicity test

Male and female mice were randomly assigned to nine groups with six animals in each group. First group was treated with isotonic saline solution (ISS; 0.9% NaCl) and considered as control and the other eight groups were treated with *Viburnum opulus* given intraperitoneally (*i.p).* in increasing dosages of 0.20, 0.32, 0.40, 0.80, 1.60, 3.20, 4.80 and 6.40 mg/kg body weight. The mortality in each cage was assessed 72 h after administration of *V. opulus*. The percentage mortalities were converted to probits. Regression lines were fitted by the method of least squares, and confidence limits for the LD_1, LD_{10}, LD_{50}, LD_{90}, and LD_{99} values were calculated by the method of Litchfield and Wilcoxon (29) and Kouadio et al. (30).

Carbon tetrachloride model for evaluation of hepatoprotective activity

The carbon tetrachloride (CCl_4) model described by *Handa and Sharma* (31) was used for scheduling the dose regimen. 0.8 mL/kg, *i.p.* of carbon tetrachloride diluted in olive oil (1:1 dilution) was employed for inducing liver toxicity.

Thirty-six rats of either both sexes were distributed into six groups of six animals each. Group I, which served as control, received isotonic saline solution (ISS) by intraperitoneal administration (*i.p.*). Group II (olive oil control) received olive oil (0.8 mL/kg) *i.p.* once daily for 7 days. Group III received ethanol 0.2 mL/kg, group IV received silibinin 50 mg/kg (32). Group V received CCl_4:olive oil (1:1) 0.8 mL/kg and group VI received aqueous extract of VO 100 mg/kg + CCl_4:olive oil (1:1) 0.8 mL/kg intraperitoneally (*i.p.*) at the same time, once daily and simultaneously for seven days. All the animals were observed daily and any dead animals were subjected to post-mortem examination to find the cause of death. The rats were killed after 24 hours from the last examination done on the seventh day. At the end of the treatment, blood samples were collected by direct cardiac puncture and the serum was used for the assay of the marker enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin.

Body weights of the rats were measured daily for eight days. Daily changes in body weights as percentages were recorded.

Assessment of liver function

The serum AST, ALT, ALP and bilirubin concentrations were determined with a commercial slides using a Vitros D60 II autoanalyzer.

Histopathological examination of the liver

The livers of the experimental animals were fixed in 10 % neutral buffered-formalin prior to routine processing in paraffin-embedded blocks. Sections (4 μm thick) were cut and stained using Hematoxylin-eosin (HE) and Masson's Trichrome stain. Histological damage was expressed using the following score system; 0: absent; +:mild; ++:moderate; +++:severe (26).

Preparation of alloxan diabetic mice

Mice were fasted for 18 h. diabetes was induced by an *i.p.* injection of 150 mg/kg of alloxan monohydrate in ISS. This procedure were repeated three times (33). After 7 days of the last treatment, mice with blood glucose levels of 200 mL/dL and over were taken into the study (34). Animals were randomly divided into three groups of six animals each. Group I mice received 0.1 mL ISS *i.p.* The animals of group II were treated orally with glibenclamide, used as a standard at a dose of 3.0 mg/kg. Group III received *i.p.* with 100 mg/kg body weight of VO. Blood glucose levels were determined before treatment, 1, 2, 4 and 24 h after treatment by applying glucose oxidase peroxidase method (Abbott, United Kingdom). The blood was taken from tail ven by scalpel blade. The same protocol described above for normal mice was followed also in this case, three groups of six animals each were used.

Statistical analyses

Results are reported as mean±SEM (standard error of mean). Body weight changes and histopathological findings were evaluated by Chi-square test. The total variation was analysed by performing one-way analysis of variance (ANOVA). Tukey's HSD (honestly significant difference), LSD (least significant diffeence), Dunnett and Tamhane's T2 tests were used for determining significance. Probability levels of less than 0.05 were considered significant.

RESULTS

Acute toxicity test

Mice were used to determine the *i.p.* LD_{50} value of *V. opulus*. The LD_{50} value of the extract was found to be 5.447 g/kg in mice. These data enabled us to select the dose to be administrated to rats for assessing its hepatoprotective activity. The results of lethal doses are shown in Table 1.

Table 1. Lethal doses of *Viburnum opulus.*

Lethal doses	Dose (g/kg)
LD_1	2.345
LD_{10}	3.424
LD_{50}	5.447
LD_{90}	8.665
LD_{99}	11.463

Effects of VO on AST, ALT, ALP and bilirubin levels

The results of hepatoprotective effect of VO on intoxicated rats are shown in Table 2. In the CCl_4 treated group serum AST, ALT, ALP and bilirubin levels were quite high. The VO treated group had significantly lower levels of ALT, AST, ALP and bilirubin when compared with the CCl_4 group similar to silibinin treated group.

Effects of VO on the rat body weight

The effect of VO on the body weight of CCl_4-induced rats is shown in Table 3. The percentual daily body weight changes indicated that the CCl_4-treated group and VO-group had the same weight loss.

Table 2. The effect of *Viburnum opulus* on serum levels of AST, ALT, ALP and total bilirubin.

Treatment	ALT	AST	ALP	T. Bilirubin
	Serum (U/L)	Serum (U/L)	Serum (U/L)	mg/dL
Control	43.5±2.1	177.0±15.6	408.2±36.9	0.06±0.01
Olive oil	46.8±3.4	127.8±16.9	539.7±45.6	0.04±0.01
Ethanol	52.3±9.6	169.0±1.7	295.8±21.8	0.07±0.01
Silibinin	205.8±66.4	708.8±183.4	316.3±35.7	0.26±0.51
CCl_4	[abcd] 959.4±152.1	[abc] 1931.9±303.6	[a] 154.9±14.1	0.33±0.17
Viburnum opulus	[abc] 500.8±82.7	1158.2±217.3	[a] 174.8±11.3	0.05±0.01
F-value	17.278	13.649	26.582	1.187
p-value	0.000	0.000	0.000	0.337

The results of post-hoc Tukey's HSD test:
a: $p<0.05$ with respect to control.
b: $p<0.05$ with respect to olive oil.
c: $p<0.05$ with respect to ethanol.
d: $p<0.05$ with respect to silibinin.

Table 3. Standardized daily changes in body weight of the rats.

Groups	Body weight changes (%)
Control	8.87
Olive oil	-1.72
Ethanol	0.51
Silibinin	[a] -3.49
CCl_4	[a] -11.82
Viburnum opulus	[a] -10.24

a: $p<0.05$ with respect to control.

Histopathological examination

Histopathological examination demonstrated that in ISS and olive oil treated liver, no alterations were observed. But, CCl_4 group (compared to ISS control and olive oil control group) induces ballooning degeneration, centrilobular necrosis, bridging necrosis and apoptosis in hepatocytes (Figure 1). Silibinin treated liver (compared to CCl_4 control group) did show remarkable recovery on ballooning degeneration and apoptosis (Figure 2). Histopathological examination of VO-treated group showed less ballooning degeneration and apoptosis. Centrilobular necrosis had not been found in VO group (Figure 3). The results of the histopathological studies showed in Table 4.

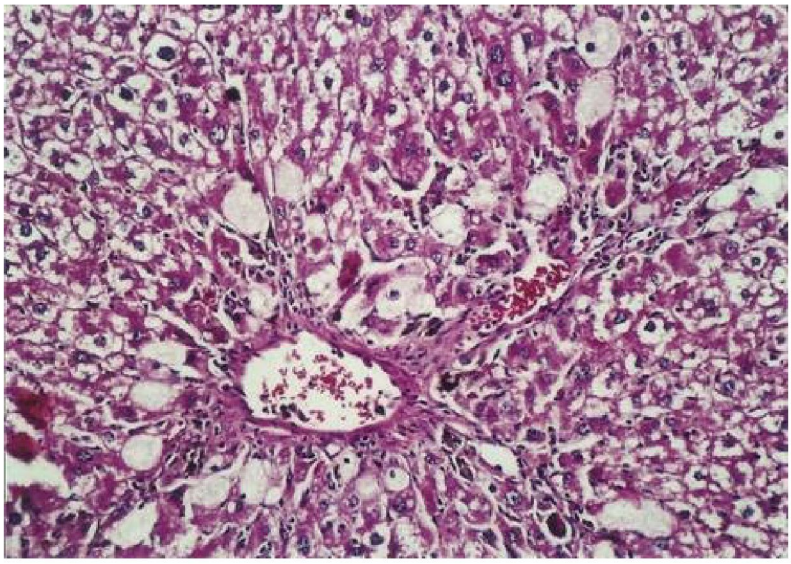

Figure 1. Numerous ballooned hepatocytes are seen in the liver (Hematoxylin-eosin stain, original magnification, HE, x50)

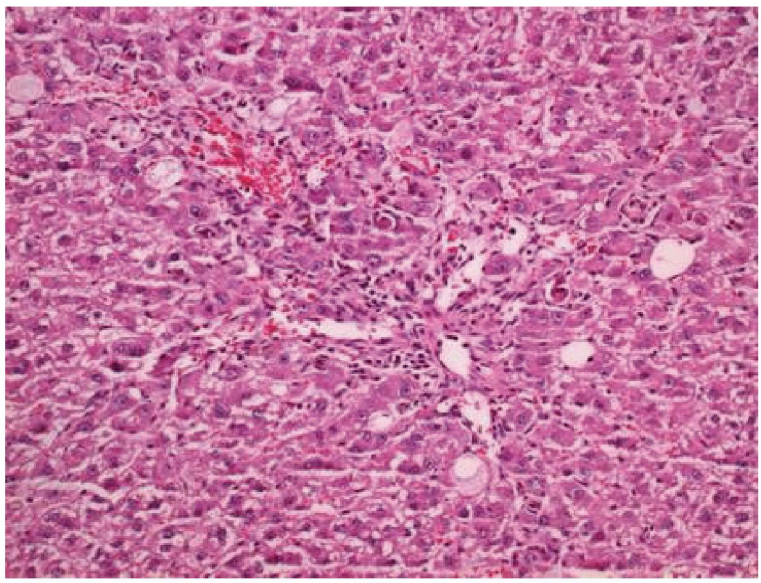

Figure 2. The liver tissue of silibinin groups (Hematoxylin-eosin stain, original magnification, HE, X20)

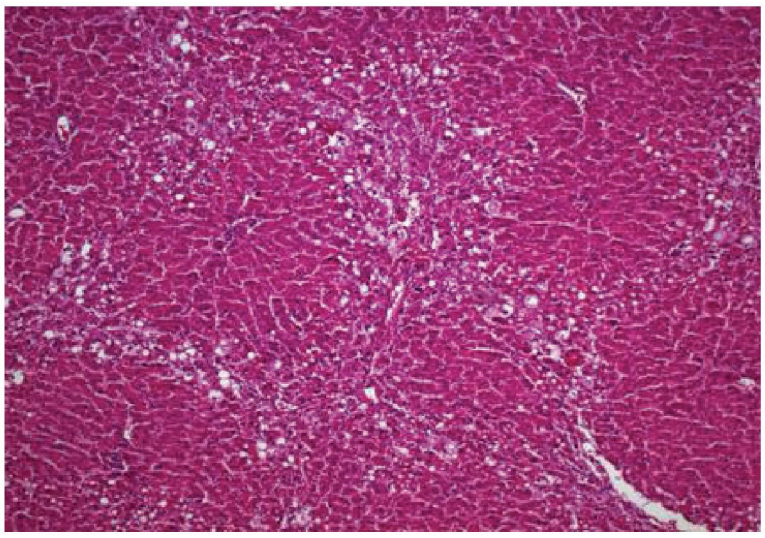

Figure 3. The liver tissue of *Viburnum opulus* groups (Hematoxylin-eosin stain, original magnification, HE, X10).

Table 4. Standartized results histopathological evaluation of study groups in rats.

Groups	Ballooning degeneration	Apoptosis and/or necrosis of hepatocytes	Bridging necrosis	Total score
Control	0	0	0	0
Olive oil	0	0	0	0
Ethanol	0	0	0	0
Silibinin	14	7	2	[a] 23
CCl_4	15	15	15	[ab] 45
V. opulus	17	11	2	[ac] 30

a: $p<0.05$ with respect to control.
b: $p<0.05$ with respect to silibinin.
c: $p<0.05$ with respect to CCl_4.

Hypoglycemic Activity

In table 5 glibenclamide treatment significantly decreased the blood glucose levels in diabetic mice, starting from the second hour. On the other hand, VO had no effect in terms of lowering blood glucose. Table 6 shows that blood glucose levels of normal mice were in normal values during the study. Only the blood sample taken in the second hour from VO-treated group was found to have a significant higher value of blood glucose when compared with control and glibenclamide-treated groups.

Table 5. Blood glucose levels in glibenclamide, *V. opulus* and control groups of mice with alloxane-induced diabetes.

Groups	Fasting blood glucose (mg/dL) (change %)				
	Before treatment	1st hour	2nd hour	4th hour	24th hour
Control	337.20±23.5	318.40±25.3	308.00±34.2	225.00±34.5	205.40±19.4
Glibenclamide	267.33±37.7	197.83±47.4	[a]150.50±39.8	[a]101.83±10.7	90.17±15.4
V. opulus	295.40±13.8	260.40±13.3	224.80±18.2	163.00±30.7	192.60±67.2
F values	1.510	3.095	5.678	5.856	2.780
P values	0.257	0.080	0.017	0.015	0.099

Post-hoc LSD test: a: $p<0.05$ compared to ISS group.

Table 6. Blood glucose levels in glibenclamide, *V. opulus* and control groups of healthy mice.

Groups	Fasting blood glucose (mg/dL) (change %)				
	Before treatment	1st hour	2nd hour	4th hour	24th hour
Control	91.5±12.8	72.8±7.2	60.5±4.2	61.3±4.2	54.5±3.1
Glibenclamide	68.8±1.4	59.3±4.9	59.0±3.7	53.3±3.0	49.8±2.0
V. opulus	91.0±6.5	79.6±3.8	77.8±10.0	63.0±3.4	[ab]72.4±5.0
F values	2.463	3.894	2.110	2.121	9.910
P values	0.135	0.056	0.172	0.171	0.004

Post-hoc LSD test: a: $p<0.05$ compared to ISS group.
b: $p<0.05$ compared to glibenclamide group.

DISCUSSION

This is the first study demostrating hepatoprotective and hypoglicemic activities of VO.

The partial hepatoprotective effect of VO was evidenced by the amelioration of biochemical indicators of liver damage and the pathological disturbances caused by CCl_4 alone. This feature of VO, however, was not as strong as that of silibinin.

Horváth et al. (32) have reported that silibinin and/or vitamin E modulates the cellular immunoresponse and restores impaired liver function following partial hepatectomy, presumably through their antioxidant capacity although it is not clear which mechanism terminates this process.

Mohamed et al. (35) have also reported the hepatoprotective activity of *Viburnum tinus* L. using the same method. They have found that the levels of ALT and AST enzymes are significantly reduced by treatment with the extract in a dose-dependent manner. Treatment with 25 mg/kg (*i.p.*) of *V. tinus* extract showed no significant change in serum ALT and AST levels, while its high dose, i.e., 50 mg/kg caused a significant hepatoprotection, evidenced by improvement in ALT and AST values.

In our previous study (26) hepatoprotective and hypoglycemic activities of *Viburnum lantana* (VL) leaf extract had a similar effect at the 100 mg/kg dose. VL has been ameliorated the levels of AST, ALT and ALP elevated by the CCl_4 treatment alone and the results of biochemical tests have also been confirmed by histopathological examination. However, VL had no hypoglycemic activity in alloxan diabetic mice. The results of this study confirm the previous results in this regard.

Iwai et al. reported that, the preventive effect of *V. dilatatum* Thunb. on oxidative damage was found in rats subjected to stres (5) and in streptozotocin diabetic rats (7).

The hepatic damage induced by CCl_4 is well known to be mediated by its free radical metabolites such as CCl_3 and Cl_3COO, which could be readily interact with unsaturated membrane lipid to produce lipid peroxidation and/or with other critical cellular macromolecules leading to a cell damage (36,37). Thus, it has been suggested that the potential hepatoprotective activity of VO against CCl_4 –induced hepatic damage may be due to its antioxidant capacity. Being releated to this feature, in a previous study, we have also investigated free radical scavenging effect of *V. opulus* by the 1,1'-diphenyl-2-picryhydrazyl (DPPH) scavenging and superoxide anion scavenging methods (25). The branch extracts of *V. opulus* inhibit superoxide anion in a concentration dependent manner. The fruit extracts of *V.opulus* are free of any scavenging effect on superoxide anion formation. *V. opulus* leaf extract showed moderate scavenger effect on superoxide anion formation. All tested extracts exhibit scavenging effect on DPPH radical. When compared with butylated hydroxytoluene (BHT), *V. opulus* branch, fruit and leaf, extracts indicate the highest DPPH radical scavenging activities with IC_{50} values of 14, 57, 250 µg/ml, respectively.

In our another studies, *V. opulus* and *V. lantana* extracts were also analysed for their salicin, chlorogenic acid, and amentoflavone contents using HPLC with a water, tetrahydrofurane, and orthophosphoric acid (97.7:1.8:0.5, v/v/v) solvent system (27-28) . Our results show that VO extract was enriched in salicin and chlorogenic acid. However, the VL extract contained more amentoflavone than the VO extract.

As we indicated before, *Viburnum* species contain triterpenoids, diterpenoids, sesquiterpenoids, iridoids, and polyphenols. A synergism could therefore be possible among these substances (23,24,26-28).

The results obtained herein have also revealed that VO had no hypoglycemic activity in alloxan-diabetic mice (Table 5). However the aqueous extract of the fruits of *V. opulus* have been used as hypoglycemic agent in Turkish traditional medicine as a drink (gilaburu). Further studies are needed to clarify this inconsistency.

The intraperitoneal LD_{50} value of *Viburnum opulus* extract was determined to be 5.447 g/kg (Table 1). The VO extract seems to be quite safe due to its high LD_{50} value.

In conclusion, the present study reveals that the water extract of *Viburnum opulus* possesses promising hepatoprotective activity. At this stage we do not know the reason(s) behind this observations. Neverthless, it could be due to glycosides, terpenes, and polyphenols within the extract (1,10,12,13,16).

In order to elucidate the mechanism(s) by which VO extact components exhibit hepatoprotective effect, which we demostrated in this study, furher studies with the isolated components will follow.

For the reason that we focused on just the investigated dose which is lack of hypoglycemic effect; in the further studies we are going to check the higher doses for evaluating this effect.

REFERENCES

1. **Lobstein, A., Haan-Archipoff, G., Englert, J., Kuhry, J.G., Anton, R.,** "Chemotaxonomical investigation in the genus *Viburnum*", *Phytochemistry,* 50, 1170-1175, **1999.**
2. **Davis, P.H.,** Flora of Turkey and the East Aegean Islands, Vol. 4, pp. 543-544, Edinburgh University Press, Edinburgh, **1972.**
3. **Davis, P.H., Mill, R.R., Tan, K.,** Flora of Turkey and the East Aegean Islands, Vol. 10 (Supp), pp. 154, Edinburgh University Press, Edinburgh, **1988.**
4. **Baytop, T.,** Therapy with Medicinal Plants in Turkey, (2nd ed.), pp. 210, Nobel Tıp Kitabevleri, İstanbul, **1999.**
5. **Iwai, K., Onodera, A., Matsue, H.,** "Antioxidant acitivity and inhibitory effect of Gamazumi (*Viburnum dilatatum* Thunb.) on oxidative damage induced by water immersion restraint stress in rats", *Int. J. Food Sci. Nutr.*, 52, 443-451, **2001.**
6. **Şendoğdu, N., Aslan, M., Deliorman Orhan, D., Ergun, F., Yeşilada, E., "**Antidiabetic and antioxidant effects of Vitis vinifera L. leaves in streptozotocin-diabetic rats", *Turkish J. Pharm. Sci.,* 3, 7-18, **2006.**
7. **Iwai, K., Onodera, A., Matsue, H.,** "Inhibitory effects of *Viburnum dilatatum* Thunb. (gamazumi) on oxidation and hyperglycemia in rats with streptozocin-induced diabetes", *J Agric Food Chem*, 52, 1002-1007, **2004.**
8. **Kim, M., Iwai, K., Matsue, H.,** "Phenolic compositions of *Viburnum dilatatum* Thunb. fruits and their antiradical properties", *J. Food Comp. Anal.*, 18, 789-802. **2005.**
9. **Dhar, M.L., Dhar, M.M., Dhawan, B.N., Mehrotra, B.N., Ray, C.,** "Screening of Indian plants for biological activity", *Indian J. Exp. Biol.,* 6, 232-247, **1968.**
10. **Fukuyama, Y., Minoshima, Y., Kishimoto, Y., Chen, I.S., Takahashi, H., Esumi, T.,** "Cytotoxic iridoid aldehydes from Taiwanese *Viburnum luzonicum*", *Chem. Pharm. Bull.*, 53, 125-127, **2005.**
11. **Kagawa, M., Minami, H., Nakahara, M., Takahashi, H., Takaoka, S., Fukuyama, Y.,** "Oleanane-type triterpenes from *Viburnum awabuki", Phytochemistry,* 47, 1337-1341, **1998.**
12. **Machida, K., Kikuchi, M.,** "Viburnols: Six novel triterpenoids from *Viburnum dilatatum*", *Tetrahedron Lett.*, 38, 571-574, **1997.**
13. **Fukuyama, Y., Minami, H., Fujii, H., Tajima, M.,** "Triterpenoids from *Viburnum suspensum*", *Phytochemistry,* 60, 765-768, **2002.**
14. **Kubo, M., Chen, I.S., Fukuyama, Y.,** "Vibsane-type diterpenes from Taiwanese *Viburnum odoratissimum*", *Chem. Pharm. Bull.,* 49, 242-245, **2001.**

15. **Fukuyama, Y., Kubo, M., Minami, H., Yuasa, H., Matsuo, A., Fujii T, Morisaki M, Harada, K.,** "Rearranged vibsane-type diterpenes from *Viburnum awabuki* and phytochemical reaction of vibsanin B", *Chem. Pharm. Bull.*, 53, 72-80, **2005.**
16. **Fukuyama, Y., Minami, H., Ichikawa, R., Takeuchi, K., Kodama, M.,** "Hydroperoxylated guaiane-type sesquiterpenes from *Viburnum awabuki*", *Phytochemistry*, 42, 741-746, **1996.**
17. **Iwagawa, T., Hase, T.,** "An iridoid acetylalloside from *Viburnum japonicum*", *Phytochemistry*, 25, 1227-1229, **1986.**
18. **Iwagawa, T., Yaguchi, S., Hase, T.,** "Iridoid glucosides from *Viburnum suspensum*", *Phytochemistry*, 29, 310-312, **1990.**
19. **Çalış, İ., Yürüker, A., Rüegger, H., Wright, A.D., Sticher, O.,** "Lantanoside, a monocyclic C_{10} iridoid glucoside from *Viburnum lantana*", *Phytochemistry*, 38, 163-165, **1995.**
20. **Tomassini, L., Foddai, S., Nicoletti, M., Cometa, M.F., Palazzino, G., Galeffi, C.,** "Iridoid glucosides from *Viburnum ayavacense*", *Phytochemistry*, 46, 901 905, **1997.**
21. **Parveen, M., Khan, M.S., Ilyas, S., Ilyas, M.,** "Luteolin 3′-xylosyl(1→2) glucoside from *Viburnum grandifolium*", *Phytochemistry* ,49, 2535-2538, **1998.**
22. **Lobstein, A., Weniger, B., Malécot, V., Um, B.H., Alzate, F., Anton, R.,** "Polyphenolic content of two Colombian *Viburnum* species (Caprifoliaceae)", *Biochem. Syst. Ecol.*, 31, 95-97, **2003.**
23. **Sever Yılmaz, B., Saltan Çitoğlu, G., Altun, M.L., Özbek, H.,** "Antinociceptive and anti-inflammatory activities of *Viburnum lantana* L.", *Pharm. Biol.*, 45, 241-245, **2007**.
24. **Altun, M. L., Saltan Çitoğlu, G., Sever Yılmaz, B., Özbek, Ö.,** "Antinociceptive and anti-inflammatory activities of *Viburnum opulus* L.", *Pharm. Biol.*, 47 (7), 653-658., **2009.**
25. **Altun, M.L., Saltan Çitoğlu, G., Sever Yılmaz, B., Çoban, T.,** "Antioxidant Properties of *Viburnum opulus* and *Viburnum lantana* Growing in Turkey", *Int. J. Food Sci. Nutr.*, 59, 175-180, **2008.**
26. **Sever Yılmaz, B., Saltan Çitoğlu, G., Altun, M.L., Özbek, H., Bayram, İ., Cengiz, N., Altınyay, Ç.,** "Hepatoprotective and hypoglycemic activity of *Viburnum lantana* L.", *Turkish J. Pharm. Sci.*, 3, 151-165, **2006.**
27. **Altun, M.L., Sever Yılmaz, B., Saltan Çitoğlu, G.,** "*Viburnum opulus* L. ve *Viburnum lantana* L.'da amentoflavon YBSK analizi", Ankara Ecz. Fak. Derg. (Journal of Faculty of Pharmacy of Ankara University), 36, 161-169, **2007.**
28. **Altun, M.L., Sever Yılmaz, B.,** "HPLC Method for the Analysis of Salicin and Chlorogenic Acid of *Viburnum opulus* L. and *Viburnum lantana*", Chem. Nat. Comp., 43, 205-207, **2007.**
29. **Litchfield, J.T., Wilcoxon, F.W.J.,** "A simplified method of evaluating dose-effect experiments" *J. Pharmacol. Exp. Ther.*, 96, 99-113, **1949.**
30. **Kouadio, F., Kanko, C., Juge, M., Grimaud, N., Jean, A., N'Guessan, Y.T., Petit, J.Y.,** "Analgesic and anti-inflammatory activities of an extract from *Parkia biglobosa* used in traditional medicine in the Ivory Coast", *Phytother. Res.*, 14, 635-637, **2000.**
31. **Handa, S.S., Sharma, A.,** "Hepatoprotective activity of andrographolide from *Andrographis paniculata* against carbon tetrachloride", *Indian J. Med. Res.* [B], 92, 276-283, **1990.**
32. **Horváth, M.É., González-Cabello, R., Blázovics, A., Looij, M., Barta, I., Müzes, G., Gergely, P., Fehér, J.,** "Effect of silibinin and vitamin E on restoration of cellular immune response after partial hepatectomy", *J. Ethnopharmacol.* ,77, 227-232, **2001.**
33. **Rodriguez, H., Perez, R.M., Muñoz, H., Perez, C., Miranda, R.,** "Inducción de diabetes en raton por medio de aloxana" *Acta. Med.*, 11, 33-36, **1975.**
34. **Singh, S.N., Vats, P., Suri S. et al.,** "Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats", *J. Ethnopharmacol.*,*76*, 269–277, **2001.**

35. **Mohamed, M.A., Mohamed, S.A., Moharram, F.A., El-Sayed, M.M., Baiuomy,A.R.,** "Phytochemical constituents and hepatoprotective avtivity of *Viburnum tinus*", *Phytochemistry*, 66, 2780-2786, **2005.**
36. **Snyder, R., Andrews, L.S.,** Toxic effects of solvents and vapors. In: Klassen, C.D. (Ed.), Toxicology: The Basic Science of Poissons, pp. 737-772, Mc Graw-Hill, New York, **1996.**
37. **Özbek, H., Çitoğlu, G.S., Dülger, H., Uğraş, S., Sever, B.,** "Hepatoprotective and anti-inflammatory activities of *Ballota glandulossima*", *J. Ethnopharmacol.*, 95,143-149, **2004.**

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