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HEPATOPROTECTIVE AND HYPOGLYCEMIC ACTIVITY OF *VIBURNUM LANTANA* L.

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Abstract

In the present study we investigated the hepatoprotective effect of the water extract of Viburnum lantana L. (VL) leaves on carbon tetrachloride (CCl4)-induced hepatotoxicity in rats, hypoglycemic activity and lethal doses 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. CCl4 (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) + CCl4 (0.8 mL/kg i.p.) or VL (100 mg/kg, i.p.) + CCl4 (0.8 mL/kg i.p.) for 7 days significantly ameliorated the levels of AST, ALT and ALP elevated by the CCl4 treatment alone. The results of biochemical tests were also confirmed by histopathological examination. The livers of the group treated with VL showed less balooning degeneration and apoptosis. Necrosis had not been observed in VL group. These results suggest that, liver damage occured in VL-treated group is less than the damage occured in silibinin-treated group.

To evaluate of hypoglicemic activity of VL, glibenclamide was used as the reference agent. But, VL has not hypoglycemic activity in healthy and diabetic mice.

Keyword: *Viburnum lantana, hepatoprotective activity, hypoglycemic activity, median lethal dose.*

Vibrinum Lantana Yapraklarının Hepatoprotektif ve Hipoglisemik Etkisi

Bu çalışmada Viburnum lantana L. (VL) yapraklarının sulu ekstresinin, sıçanlarda karbon tetraklorür (CCl4) nedenli hepatotoksisite üzerine hepatoprotektif etkisi, farelerde letal dozu ve hipoglisemik aktivitesi araştırılmıştır.

Serum aspartat amino transferaz (AST), alanin amino transferaz (ALT), alkalin fosfataz (ALP) ve bilirubin konsantrasyonu gibi karaciğer hasarının biyokimyasal parametrelerine bakılmıştır. CCl4 (0.8 mL/kg i.p., 7 gün) grubunda serum AST, ALT, ALP ve bilirubin seviyelerinin kontrol grubuyla

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kıyaslandığında anlamlı seviyede arttığı görülmüştür. Sıçanlara silibinin (50 mg/kg)+CCl4 (0.8 mL/kg i.p.) ve VL (100 mg/kg, i.p.) + CCl4 (0.8 mL/kg i.p.) 7 gün boyunca uygulandığında, tek başına CCl4 uygulandığındaki yükselen serum AST, ALT ve ALP seviyeleri anlamlı bir şekilde düşmüştür. Biyokimyasal testlerin sonuçları histopatolojik incelemelerle de doğrulanmıştır. VL uygulanan grubun karaciğerlerinde daha az balon dejenerasyonu ve apoptozis görülmüştür. Bu grupta da nekroz gözlenmemiştir. Bu sonuçlar VL uygulanan gruptaki karaciğer hasarının silibinin uygulanan grupta gözlenen hasardan daha az olduğunu göstermektedir.

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

Anahtar kelimeler: *Viburnum lantana, hepatoprotektif aktivite, hipoglisemik aktivite, ortalama letal doz.*

INTRODUCTION

The genus *Viburnum* (*Caprifoliaceae*) comprises more than 230 species distributed from South America to South-East Asia, the majority of them being endemic (1).

The plant is represented by four species in the flora of Turkey; *Viburnum opulus* L., *V. orientalis* Pallas, and *V. lantana* L. and *V. tinus* L. (2,3).

In Middle Anatolia, a traditional drink named ***gilaburu*** has been prepared from the fruits of *V. opulus*. The fruit has a dark-red color and is edible. The barks of *V. lantana* have been used in folk medicine as rubefiant and analgesic (4). The preventive effect of *V. dilatatum* on oxidative damages was found in rats plasma, liver and stomach subjected to stress (5) and streptozotocin-induced diabetic rats (6). In addition, the effect of *V. dilatatum* on antioxidant enzymes in plasma, liver, stomach was examined and the results suggested that ingestion of this plant might contribute to reduce the consumption of antioxidant enzymes, such as superoxide dismutase, catalase, glutation peroxidase and glutation (7). The alcoholic extract of *V. erubescens* Wall. has been reported to show antiviral activity (8). Some iridoid aldehytes isolated from *V. luzonicum* exhibited moderate inhibitory activity against He La S3 cancer cells (9).

The genus *Viburnum* is known to contain triterpenoids (10-12), diterpenoids (13,14), sesquiterpenes (15), iridoids (16-19) and polyphenols (20,21).

The obcective of this study was to determine the hepatoprotective and hypoglycemic effects of *V. lantana*. These activities have not been investigated before on this species.

EXPERIMENTAL

Plant material

Viburnum lantana was collected in 2005 from flowering plants near Ankara (Turkey). Voucher specimens were kept in the Herbarium of Ankara University, Faculty of Pharmacy (AEF No 23543).

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 of cold distilled water for 1 day. The macerate was evaporated and lyophilized. The extract yield was 13.45g/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 Yüzüncü Yıl 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 ^{O}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 and silibinin (Sigma, Steinheim, Germany) and glibenclamide (Nobel, İstanbul, Turkey). CCl_4 was dissolved in olive oil and silibinin was solved in ethyl alcohol.

Acute toxicity test

Male and female mice were randomly assigned to six 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 five groups were treated with *V. lantana* given intraperitoneally (i.p.) in increasing dosages of 0.20, 0.40, 0.80, 1.60 and 3.20 g/kg body weight. The mortality in each cage was assessed 72 h after administration of *V. lantana*. 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 (22), and Kouadio et al (23).

Carbontetrachloride model for evaluation of antihepatotoxic activity

The carbon tetrachloride model described by Handa and Sharma (24) 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.

Carbontetrachloride induced-hepatotoxicity

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 (25). Group V received CCl4:olive oil (1:1) 0.8 mL/kg and group VI received aqueous extract of VL 100 mg/kg + CCl4: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 during the study. 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.

Preparation of alloxan diabetic mice

Mice were fasted for 18 h. diabetes was induced by an ip injection of 150 mg/kg of alloxan monohydrate in ISS. This procedure were repeated three times (26). After 7 days of the last treatment, mice with blood glucose levels of 200 mL/dL and over were taken into the study (27).

Hypoglycemic activity in diabetic mice

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 ip with 100 mg/kg body weight of VL. 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.

Hypoglycemic activity in healthy mice

The same protocol described above for healthy mice was followed. Also in this case, three groups of six animals each were used.

Statistical analysis

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 difference) test and Tamhane's T2 tests were used for determining significance. Probability levels of less than 0.05 were considered significant.

RESULTS

Acute toxicity

Mice have been utilized to determine the i.p. LD50 value of *V. lantana*. The LD50 value of the extract was found to be 2.169 g/kg in mice. This 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 *V. lantana*.

Lethal doses	Dose (g/kg)
LD_{10}	1.448
LD_{50}	2.169
LD_{90}	3.248
LD_{99}	4.514

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

Treatment	ALT Serum (U/L)	AST Serum (U/L)	ALP Serum (U/L)	T. Bilirubin mg/dL
Control (ISS)*	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	[b]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	[abcd]1931.9±303.6	[abcd]154.9±14.1	0.33±0.17
V. lantana	[e]244.2±28.7	[e]913.3±271.9	[abd]163.5±40.0	0.18±0.12
F-value	*18.538*	*12.099*	*21.727*	*1.187*
p-value	*0.000*	*0.000*	*0.000*	*0.475*

*ISS: Isotonic saline solution.
The values are given as mean ± S.E.M. (standard error of mean).

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.
e : $p<0.05$ with respect to CCl_4.

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

Groups	Body weight changes (%)
Control (ISS)	8.87
Olive oil	-1.72
Ethanol	0.51
Silibinin	[a] -3.49
CCl_4	[a]-11.82
V. lantana	[a] -9.56

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

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

The results of hepatoprotective effect of VL on intoxicated rats are shown in Table 2. In the CCl4 treated group serum AST, ALT, ALP and bilirubin levels were quite high. The VL treated group had significantly lower levels of ALT, AST, ALP and bilirubin when compared with the CCl4 group similar to silibinin treated group.

Effects of VL on the rat body weight

The effect of VL on the body weight of CCl4-induced rats is shown in Table 3. The percentual daily body weight changes indicated that the CCl4-treated group and VL-group had the same weight loss.

Histopathological Examination

Histopathological examination demonstrated that in ISS and olive oil treated liver, no alterations were observed. But, CCl4 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 CCl4 control group) did show remarkable recovery on ballooning degeneration and apoptosis (Figure 2). Histopathological examination of VL-treated group showed less ballooning degeneration and apoptosis. Centrilobular necrosis had not been found in VL group (Figure 3). The results of the histopathological studies are given in Table 4.

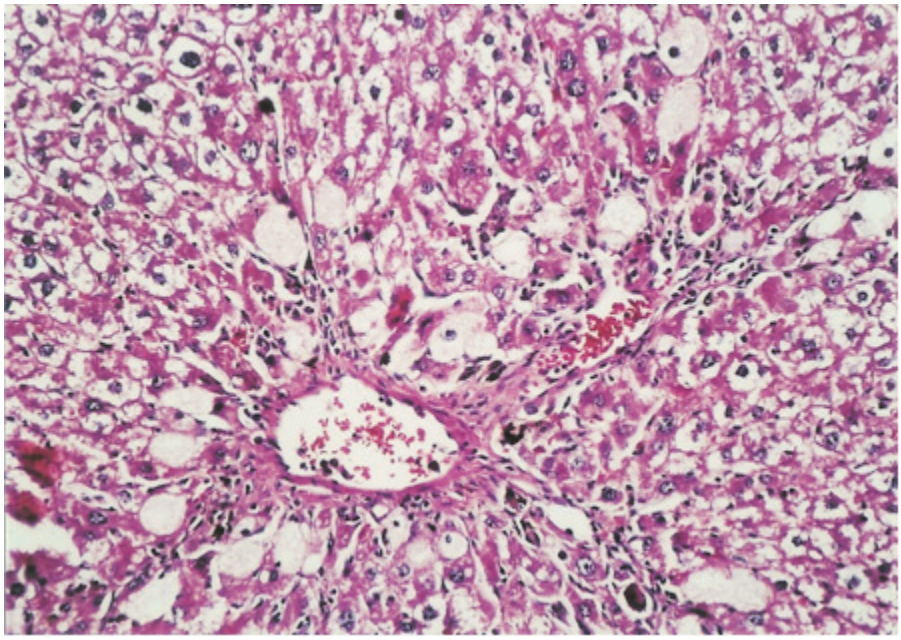

Figure 1. Numerous ballooned hepatocytes are seen in the liver (Hematoxylin-eosin stain, original magnification, HE, X20)

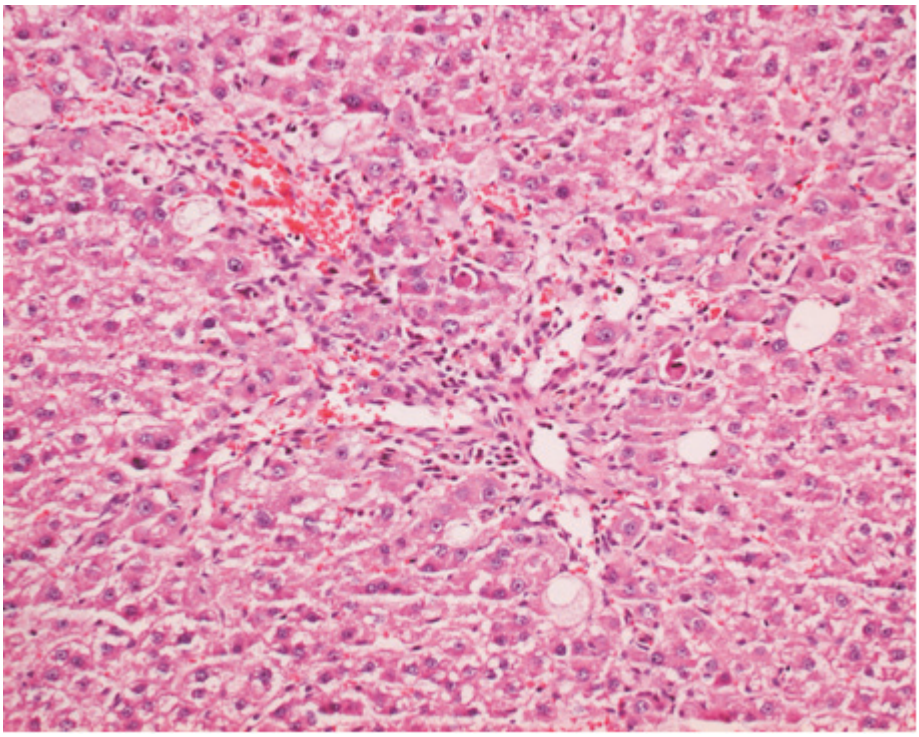

Figure 2. The liver tissue of silibinin group (Hematoxylin-eosin stain, original magnification, HE, X20)

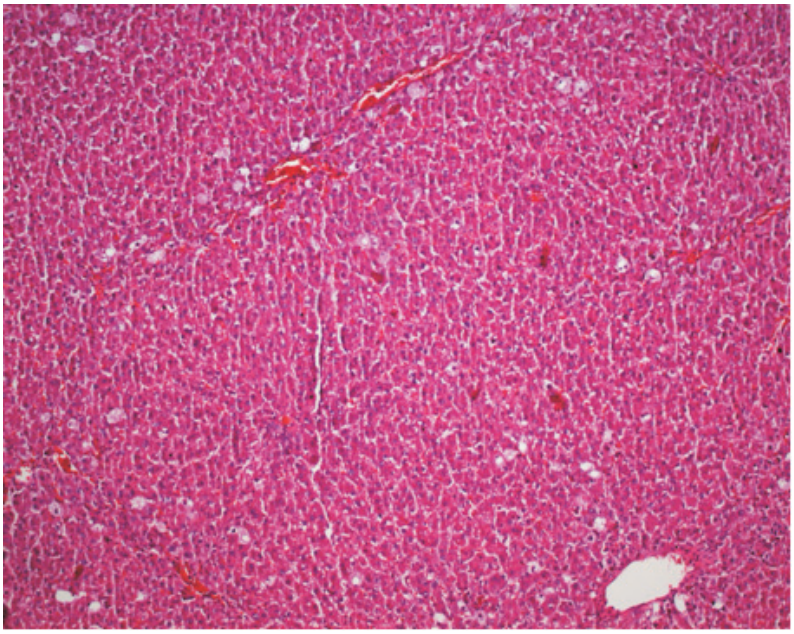

Figure 3. The liver tissue of *Viburnum lantana* group (Hematoxylin-eosin stain, original magnification, HE, X10).

Tablo 4. Standardized results of 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. lantana	8	4	0	[abc] 12

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, VL 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 VL-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. lantana* 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 (ISS)	337.20±23.5	318.40±25.3 (5.58)	308.00±34.2 (8.66)	225.00±34.5 (33.27)	205.40±19.4 (39.09)
Glibenclamide	267.33±37.7	197.83±47.4 (26.00)	[a]150.50±39.8 (43.70)	[a]101.83±10.7 (61.91)	[a]90.17±15.4 (66.27)
V. lantana	295.40±23.6	260.80±25.0 (11.71)	233.20±35.0 (21.06)	[b]197.80±28.8 (33.04)	[b]197.40±35.3 (33.18)
F values	*1.338*	*2.797*	*4.640*	*6.964*	*7.656*
P values	*0.296*	*0.098*	*0.030*	*0.009*	*0.006*

Data were represented as mean ± standart error of the mean.

Post-hoc LSD test:

a: $p<0.05$ compared to ISS group.

b: $p<0.05$ compared to glibenclamide group.

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

Groups	Fasting blood glucose (mg/dL) (change %)				
	Before treatment	1st hour	2nd hour	4th hour	24th hour
Control (ISS)	91.5±12.8	72.8±7.2 (20.44)	60.5±4.2 (33.88)	61.3±4.2 (33.01)	54.5±3.1 (40.44)
Glibenclamide	68.8±1.4	59.3±4.9 (13.81)	59.0±3.7 (14.24)	53.3±3.0 (22.53)	49.8±2.0 (27.62)
V. lantana	92.6±2.5	92.6±13.0 (0.00)	[ab] 75.6±4.6 (18.36)	62.8±4.3 (32.18)	64.8±7.2 (30.02)
F values	*3.549*	*2.965*	*4.920*	*1.633*	*2.239*
P values	*0.068*	*0.098*	*0.033*	*0.243*	*0.157*

Data were represented as mean ± standart error of the mean.

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 to show the hepatoprotective activity of VL. The intraperitoneal LD_{50} value of *V. lantana* extract was determined as 2.169 g/kg (Table 1).

When the biochemical, histopathological and body weight value results are considered, it can be suggested that *V.lantana* has partially treating effect on CCl_4-induced acute liver toxicity as strong as silibinin. The results were shown in Table 2-4 and figure 1-3. Hovath et al (25). 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 what mechanism terminates this process .

Mohamed et al (28) was also reported the hepatoprotective activity of *V. tinus* L. using the same method. In that study the levels of ALT and AST enzymes were significantly reduced by treatment with the extract in a dose-dependant manner. Treatment with 25 mg/kg (ip) of *V. tinus* extract showed no significant change in serum ALT and AST levels, while its higher dose, i.e., 50 mg/kg caused a significant hepatoprotection, evidenced by improvement of ALT and AST values.

In addition, the antioxidant properties of *V. dilatatum* THUNB. fruit squeezing solution have also been determined (5).

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 (29,30). Thus, it can be suggested that the potential hepatoprotective activity of VL against CCl_4–induced hepatic damage could be due to its antioxidant capacity. According to this feature we also investigated free radical scavenging effect of *V. lantana* by the 1,1'-diphenyl-2-picryhydrazyl (DPPH) scavenging and superoxide anion scavenging methods (31). The branch extracts of V. *lantana* inhibited superoxide anion in a concentration dependent manner. The fruit extracts of *V. lantana* did not show any scavenging effect on superoxide anion formation. *V. lantana* leaf extract showed moderate scavenger effect on superoxide anion formation. All tested extracts exhibited scavenging effect on DPPH radical. When compared with butylated hydroxytoluene (BHT), *V. lantana* leaf, branch and fruit extracts showed the highest DPPH radical scavenging activities with IC_{50} values of 14, 35, 52 and 85 μg/ml, respectively.

The results obtained herein also showed that VL had no hypoglycemic activity in alloxan-diabetic mice (Table 5). Only the blood glucose level of the sample drawn from *V. lantana* group in the second hour showed significant higher value when compared with the control and the glibenclamide-treated groups (Table 6). But since the value (75.6 mg/dL) was found to be in normal ranges, clinically it could not be significantly considered as pathologic.

In conclusion, the present study reveals that the water extract of *V. lantana* had relatively potent hepatoprotective action in rats and had no hypoglycemic activity in mice.

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