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Original Research Article

Pharmacological evaluation of *Vernonia elaeagnifolia* (Asteraceae) leaves in hyperlipidemic albino rabbits

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Abstract

Purpose: To evaluate the antihyperlipidemic efficacy and phytochemical constituents of *Vernonia elaeagnifolia* aqueous leaf extract.

Method: Qualitative phytochemical analysis of *V. elaeagnifolia* leaves was performed. Thirty healthy albino rabbits were divided into six groups ($n = 6$). Cholesterol powder (0.5 g/kg) in 10 mL coconut oil (vehicle) was given orally to induce hyperlipidemia. The aqueous leaf extract of *Vernonia elaeagnifolia* was administered at 250 mg/kg and 500 mg/kg per oral. Lipid profile, hepatic enzymes and oxidative stress markers were evaluated.

Results: Phytochemical screening indicated the presence of tannins, proteins, flavonoids, phenols, alkaloids and saponins. Oral administration of cholesterol powder significantly ($p < 0.05$) raised the level of serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c) and triglyceride (TG) along with significant ($p < 0.05$) decrease in serum concentration of high-density lipoprotein cholesterol (HDL-c). Concentration of serum TC, LDL-c, TG and liver enzymes was significantly reduced in *V. elaeagnifolia*-treated groups. The levels of oxidative stress markers were restored to normal when the animals were treated with *V. elaeagnifolia* leaf extract; increased levels of antioxidant enzymes were observed.

Conclusion: The aqueous leaf extract of *V. elaeagnifolia* possesses antihyperlipidemic and antioxidant potentials that are dose-dependent. However, further studies are required to develop the plant for therapeutic applications.

Keywords: Hyperlipidemia, Oxidative stress markers, Cholesterol, *Vernonia elaeagnifolia*

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INTRODUCTION

Variations in normal functions of body alter the homeostasis which may lead towards serious health problems such as hypercholesterolemia [1]. Hyperlipidemia is a heterogeneous metabolic disorder. It plays an important role in the progression of life threatening cardiovascular diseases such as myocardial infarction, cardiac arrest and angina. The major accounting

problems of hyperlipidemia are increase in serum cholesterol level and oxidative stress [2]. Plaque formation takes place in the blood vessels when excess of LDL cholesterol gets accumulated in the endothelial spaces. It may worsen hypertension and normal functioning of liver and kidney [3,4].

Various types of antihyperlipidemic drugs are being used e.g. Statins (simvastatin, atorvastatin etc.), fibrates, ezetimibe, nicotinic acid and fish

oils (omega-3 fatty acids) in order to decrease the serum cholesterol level [5]. Use of these synthetic medications may lead towards severe irregularities including abnormal liver function, hyperuricemia and dry skin [6]. Statins are involved in increasing hepatic transaminase level in the serum upon prolonged use [7].

Medicinal plants have been widely used in Ayurveda system of medicine. Evidences from literature favor the use of medicinal plants because they increase the immunity of body against various diseases [8]. *V. elaeagnifolia* (Asteraceae) is a creeper. It is mostly cultivated in Asia and Europe. Plants of this family have medicinal properties against upper respiratory tract infections, stomach ulceration and skin infections [9]. Chemical constituents found in *V. elaeagnifolia* ethanolic extract are flavonoids, phenolic compounds, tannins, terpenoids, phytosterols, alkaloids, and coumarins. Conventionally *V. elaeagnifolia* leaves are being used as leech repellent [10].

Current investigation was designed to investigate the anti-hyperlipidemic activity of aqueous extract of *V. elaeagnifolia* leaves.

EXPERIMENTAL

Animals

30 healthy albino rabbits (aged, 1 year; body weight, 2.0 ± 0.2 kg) of either sex were procured from local market of Faisalabad, Pakistan. The rabbits were kept in iron cages at temperature of 25° C and 12 h dark/12 h light cycle. The rabbits were on normal feed (lucerne) and water *ad libitum*. Compliance with the rules was made by following Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) [11]. All the trials conducted on animals were approved from the Committee of Animal Experimentation by the use of First Affiliated Brooke Hospital University of Agriculture (approval ref no. XW0642/2015).

Chemicals

Cholesterol powder was purchased from Sigma Aldrich (Germany). Atorvastatin (Tab. Atorsan, 10 mg; standard drug) was procured from Novartis Pharmaceuticals Co., (Batch no, 230F11; Karachi, Pakistan).

Plant material

The leaves of *V. elaeagnifolia* were obtained from local market of Faisalabad, Pakistan. These were identified from Department of Botany,

University of Agriculture, Faisalabad. In order to remove dust from leaves, they were washed with tap water. Afterwards leaves were dried in shade. Dried leaves were ground in electric grinder to have fine powder. The grounded material was stored at 25° C in air tight container.

Animal studies

Animals were categorized into 5 groups where each group was containing 6 animals. Group 1 was normal group in which animals were fed on routine diet only; Group 2 was hyperlipidemic control and received cholesterol (0.5 g/kg) in coconut oil (10 ml); Group 3 was positive control and received cholesterol powder + atorvastatin (0.25 mg/kg) p.o; Group 4 was treated group I and received cholesterol powder + *V. elaeagnifolia* aqueous extract (250 mg/kg) p.o; group 5 was treated group II and received cholesterol powder + *V. elaeagnifolia* aqueous extract (500 mg/kg) orally.

Physicochemical analysis of plant extract

Proximate composition

Proximate composition (moisture content, dry matter, ether extract, total ash, crude protein and crude fiber) of *V. elaeagnifolia* leaves was determined according to various methods [13].

Elemental determination

One g of the powdered sample of *V. elaeagnifolia* leaves was digested with digestion mixture (nitric acid and perchloric acid) at 180 – 200 °C. Resulting transparent solvent was diluted with double distilled water up to 100 mL. This digested solution was used to determine elements through Atomic Absorption spectrophotometry (AAS; Hitachi Polarized Zeeman, Z-8200, Japan), according to the protocols described in AOAC (Association of Official Analytical Chemists; 1990).

Phytochemical screening

Phytochemical screening of *V. elaeagnifolia* leaves was done according to standard protocols for proteins, alkaloids, tannins, fixed oils, saponins and flavonoids [14].

Preparation of extract

Dried powder of leaves was added into soxhlet apparatus along with petroleum ether and extraction was carried out. The extract was subjected to hot water bath for evaporation.

Dried residue was collected and used as antihyperlipidemic drug [12].

Biochemical analysis

Blood samples were collected from jugular vein located in the neck via venous puncture. Blood was centrifuged at 1000 rpm for 10 min to isolate serum. Serum total cholesterol (TC), triglyceride (TG), HDL-c, LDL-c, ALP, ALT, AST, TAC, MDA, TOS, Catalase and SOD were measured using standard biochemical kits (DiaSys Diagnostic Systems, USA).

Histopathological analysis

Animals were sacrificed via decapitation on 45th day of experiment. Liver tissues were collected and fixed in neutral formalin solution. These tissues were processed for light microscopy according to reference methods for embedding, sectioning, mounting and staining with hematoxylin & eosin. Light microscope (Olympus optical Co., Tokyo, Japan) with 40 X objective was used to examine the tissue samples.

Statistical analysis

Statistical analysis was done by using one way ANOVA tailed by Duncan's Multiple Range test to determine statistical differences at 5% level of significance. SPSS software version 3.16 was used and results were expressed as mean \pm SE (standard error).

RESULTS

Proximate analysis

Results for the proximate composition are summarized in Table 1.

Table 1: Proximate profile of *V. elaeagnifolia* leaves

Parameter	%
Moisture content	9.5
Dry matter	90.5
Fiber content	20.8
Fat content	5.55
Crude protein	10.3
Ash content	16.2

Elemental analysis

Concentration of Sodium (Na), Cobalt (Co), Magnesium (Mg), Cadmium (Cd), Lead (Pb), Calcium (Ca), Manganese (Mn), Potassium (K), Iron (Fe) Zinc (Zn,) Nickel (Ni), and Copper (Cu) present in leaves of *V. elaeagnifolia* are expressed as mg/kg \pm SE and shown in Table 2.

Table 2: Elemental composition of *Vernonia elaeagnifolia* leaf (mg/kg, mean \pm SE)

Elements	<i>Vernonia elaeagnifolia</i>
Cobalt	4.00 \pm 0.57
Zinc	49.0 \pm 0.57
Iron	760 \pm 0.33
Manganese	45.3 \pm 0.33
Sodium	482 \pm 0.54
Magnesium	831 \pm 0.57
Nickle	3.33 \pm 0.33
Potassium	536 \pm 0.35
Copper	221 \pm 0.57
Calcium	481 \pm 0.57

Phytochemical constituents

Qualitative phytochemical analysis of *V. elaeagnifolia* evidenced the presence of saponins, proteins, phenols, flavonoids, tannins, and alkaloids as represented in Table 3.

Table 3: Phytochemical profile of *Vernonia elaeagnifolia* leaf

Test	Presence (+)
Flavonoids	+
Saponins	+
Proteins	+
Phenols	+
Tannins	+
Alkaloids	+

Biochemical profile

The amount of serum total cholesterol (TC), and triglyceride was significantly ($p < 0.05$) raised after the administration of cholesterol powder among all groups in comparison to the normal control. Serum concentration of triglyceride and total cholesterol in atorvastatin and *V. elaeagnifolia* treated groups showed significant reduction in dose dependent manner (Figure 1).

Serum concentration of LDL cholesterol was increased while significantly ($p < 0.05$) decreased in cholesterol treated groups. When treated with *V. elaeagnifolia* then it restored the levels of LDL cholesterol and HDL cholesterol in comparison to normal control (Figure 2).

Amount of hepatic enzymes; alanine transaminase, alkaline phosphatase and aspartate transaminase was elevated significantly ($p < 0.05$) in cholesterol treated groups, when treated with *V. elaeagnifolia* then concentration of hepatic enzymes was decreased near to the normal as shown in Table 4.

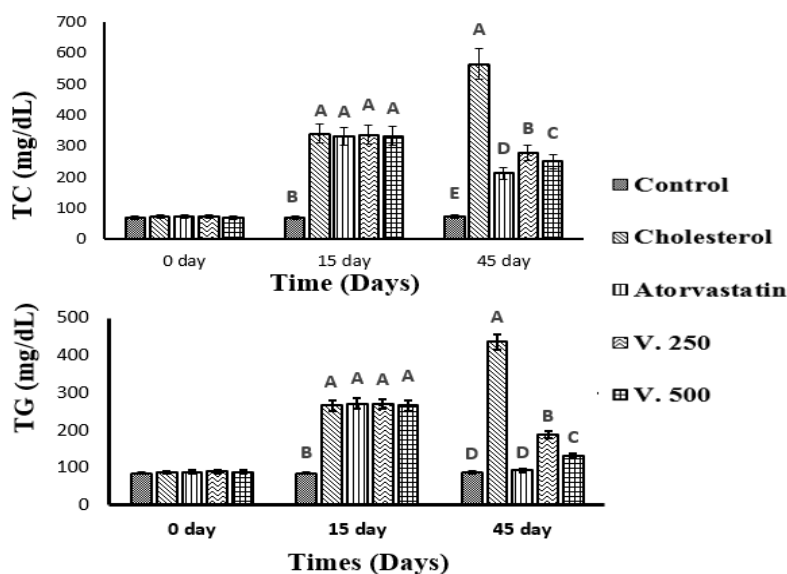


Figure 1: Effect of *V. elaeagnifolia* leaf extract on (a): serum total cholesterol (mg/dL ± SE) and (b): serum triglycerides (mg/dL ± SE). Error bars with changing letters have significant difference ($p < 0.05$) whereas; V.250 (*V. elaeagnifolia*; 250 mg/kg), V.500 (*V. elaeagnifolia*; 500 mg/kg).

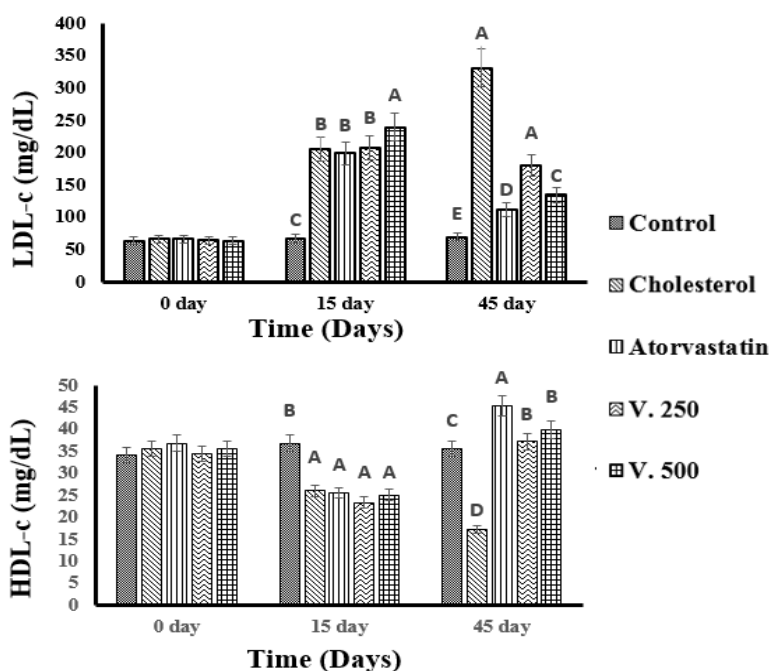


Figure 2: Effect of *V. elaeagnifolia* leaf extract on (a): LDL cholesterol (mg/dL ± SE) and (b): HDL cholesterol (mg/dL ± SE). Error bars with changing letters have significant difference ($p < 0.05$) whereas; V.250 (*V. elaeagnifolia*; 250 mg/kg), V.500 (*V. elaeagnifolia*; 500 mg/kg).

Table 4: Effect of *V. elaeagnifolia* leaf extract on hepatic enzymes

Group	Aspartate transaminase (U/L)	Alanine transaminase (U/L)	Alkaline phosphatase (U/L)
CTRL	52.00±2.67 ^D	39.83±1.47 ^D	12.66±1.89 ^A
CHOL	97.50±3.66 ^A	89.16±1.74 ^A	36.83±1.88 ^B
ATORVA	76.83±2.96 ^B	57.66±2.02 ^B	24.66±0.98 ^{CB}
V. 250	76.83±2.96 ^B	56.83±1.49 ^B	26.50±1.72 ^C
V. 500	66.83±2.84 ^C	45.33±1.05 ^C	21.33±0.88 ^D

Data is expressed as mean ± SE (n = 6); comparison is made among normal control, hyperlipidemic control and *V. elaeagnifolia* treated groups. Changing alphabets in columns exhibit data is significantly different ($p < 0.05$)

Table 5: Effect of *V. elaeagnifolia* leaf extract on antioxidant and oxidant parameters

Group	TOS	TAC	Catalase	MDA	SOD
	($\mu\text{mol/L}$)	(mmol/L)	(KU/L)	(mmol/L)	(Units/mg of protein)
CTRL	3.66 \pm 0.05 ^A	0.56 \pm 0.02 ^A	40.10 \pm 1.04 ^A	8.83 \pm 0.35 ^D	7.97 \pm 0.46 ^A
CHOL	8.08 \pm 0.24 ^B	0.25 \pm 0.01 ^D	17.93 \pm 0.64 ^D	51.42 \pm 0.85 ^A	1.08 \pm 0.05 ^D
ATORVA	4.49 \pm 0.12 ^{CB}	0.40 \pm 0.01 ^C	35.86 \pm 1.19 ^B	24.77 \pm 1.18 ^B	5.45 \pm 0.18 ^C
V. 250	4.96 \pm 0.24 ^C	0.36 \pm 0.01 ^C	30.30 \pm 1.04 ^C	23.31 \pm 0.81 ^B	5.83 \pm 0.16 ^C
V. 500	4.35 \pm 0.19 ^D	0.45 \pm 0.01 ^B	38.45 \pm 0.73 ^{AB}	13.21 \pm 0.74 ^C	6.99 \pm 0.17 ^B

Data are expressed as mean \pm SE (n = 6); comparison is made among normal control, hyperlipidemic control and *V. elaeagnifolia* treated groups. Changing alphabets in columns exhibit data is significantly different ($p < 0.05$)

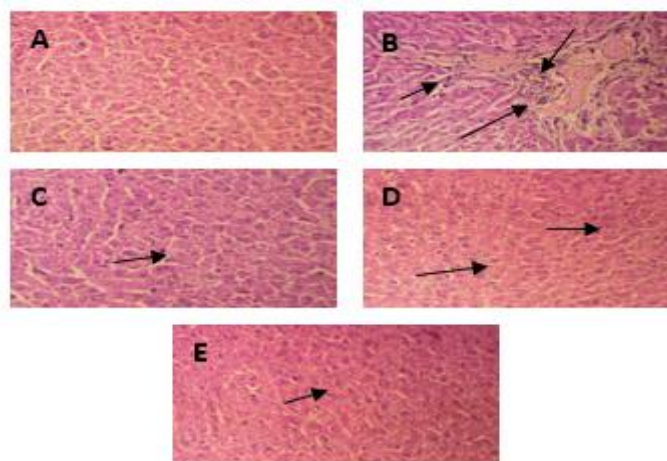


Figure 3: Histopathological features of hepatic tissue at 40 X; A: Normal control, B: Cholesterol (0.5 g/kg) treated group, C: atorvastatin (0.25 mg/kg) treated group, D: *V. elaeagnifolia* (250 mg/kg) treated group, E: *V. elaeagnifolia* (500 mg/kg) treated group

Oxidative stress markers, total oxidant status (TOS) and malondialdehyde (MDA) levels were increased when treated with cholesterol while the amount in serum was restored when *V. elaeagnifolia* was given. Administration of cholesterol increased the oxidative stress and decreased the concentration of antioxidant enzymes (Catalase; SOD) and total antioxidant capacity. Treatment with *V. elaeagnifolia* increased the antioxidant capacity and amount of antioxidant enzymes as represented in Table 5.

As shown in Figure 3, liver tissue from control rabbit showed distinctive and clear boundaries of hepatic cells. While lobular structure of hepatocytes was disrupted in hepatic tissue obtained from hyperlipidemic rabbit. In addition, necrotic and binucleate cells were present. In atorvastatin treated animal, hepatocytes were resized to normal with lesser binucleate cells. When treated with 250 mg/kg of *V. elaeagnifolia* then it reversed the state of liver injury towards normal with few hazy hepatocytes. Appearance of hepatocytes in *V. elaeagnifolia* (500 mg/kg) treated rabbit was just alike to hepatic cells of atorvastatin treated animal. Parenchyma of cells

appeared normal with mild degree of congestion (Figure 3).

DISCUSSION

Current investigation was conducted to investigate the antihyperlipidemic efficacy of *V. elaeagnifolia* aqueous leaf extract. For the induction of hyperlipidemia, cholesterol powder was administered to the animal models that increased the oxidative stress and serum cholesterol concentration. Hyperlipidemia is a major risk factor in the progression of cardiovascular diseases [15].

It was observed that micro and macro nutrients (calcium, manganese, magnesium, potassium, zinc, iron, cobalt and sodium) were present in *V. elaeagnifolia* leaves whereas heavy metals (lead, cadmium) were absent.

Previous research studies suggest that macro and micro nutrients have role in lowering the serum total cholesterol and free fatty acids. In another study it was observed that presence of calcium in sufficient amount in body enhances the process of fat digestion [16]. Calcium plays a

major role in the formation of non-esterified linkages fatty acids in the small intestine. Calcium helps in the formation of insoluble soap with esterified fatty acids. This complex becomes more water soluble and readily gets eliminated from body via feces [17]. In one investigation it was found that micronutrients (zinc, manganese and copper) potentiate the antioxidant activity of super oxide dismutase, as SOD is involved in neutralizing lipid peroxides [18].

In the present study phytochemical screening indicated the presence of major phyto-constituents (flavonoids, proteins, alkaloids, tannins, saponins and tannins). It was observed in another research that presence of these phyto-constituents exert hypolipidemic effect. Previous literature claims that intake of saponin supplementation enhance the excretion of bile salts. Cholesterol is involved in the formation of bile salts. Thus excretion of bile salts results in decreased serum cholesterol. It was reported that flavonoids are phenolic compounds that potentiate the activity of lecithin acyl transferase (LCAT) enzyme, which in turn enhances the process of fat metabolism [19].

In present investigation, serum level of TC, LDL cholesterol and TG was elevated whereas HDL concentration was reduced when treated with cholesterol (0.5 g/kg). Serum concentration of TG, LDL cholesterol, TC and HDL-c was restored to normal level when treated with *V. elaeagnifolia* aqueous leaf extract. These findings were supported by another research in which lemon juice was administered to hyperlipidemic animal model resulted in pronounced decrease in LDL cholesterol, TC and triglycerides [20].

V. elaeagnifolia significantly ($p < 0.05$) lowered the amount of TOS and MDA in the serum whereas level of antioxidant enzyme i.e. CAT and SOD was increased. Similar results were observed in another research when grape fruit juice was given to hyperlipidemic mice that resulted in decreased oxidative stress and increased serum level of antioxidant enzymes [21].

It was observed in the present study that *V. elaeagnifolia* reduced the levels of hepatic enzymes i.e. aspartate transaminase, alkaline phosphatase and alanine transaminase. These findings were supported by previous research undertaken to evaluate antihyperlipidemic effect of ginseng extract. Administration of ginseng extract restored the serum levels of hepatic enzymes, in addition concentration of serum cholesterol was also reduced [22].

CONCLUSION

The findings of this study indicate that administration of *V. elaeagnifolia* aqueous leaf extract produced anti-hyperlipidemic and antioxidant effects in rabbits. However, further investigational studies are required to elucidate the cellular mechanisms involved in hypolipidemic activity of the extract.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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