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Research Article

Phytochemical evaluation and lipid lowering property of leaves of *Vitex negundo* linn. in hyper cholestremic rats

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ABSTRACT

The aim of the present study was to determine lipid lowering property of ethanol extract of *Vitex negundo* linn.The phytochemical studies performed as described by Wagner *et al*. Acute toxicity studies were performed initially in order to determine the safety of ethanol extract of *Vitex negundo* linn. (EEVN). In the present study the ethanol extract of *Vitex negundo* L. results a significant dose dependent reduction in Total Cholesterol and Low Density Lipoprotein level when compared with Cholesterol control group. Also all the treatment groups show a little bit increase in HDL level. Up on the triglycerides level the extracts posses a significant activity when compared to Lovastatin treated groups. The results demonstrate the effectiveness of the leaf of *Vitex negundo* L. as an anti-atherogenic agent preventing coronary artery diseases.

Keywords: Total cholesterol; triglycerides; HDL; LDL.

INTRODUCTION

Vitex negundo is a member of Verbenanceae family. It is an aromatic shrub, mainly seen in Wasteland up to 2000 meters in the Himalayas. Mostly its leaf parts were used for the study. The leaves were five follate, palmate, arranged rarely with three leaf lets. The aim of our study was to determine the phytochemical constituent present in various extract of the plant and evaluate its *invivo* hypolipidemic effect. The hypolipidemic drugs have attracted considerable attention because of their potential to prevent cardiovascular disease by retarding the accelerated atherosclerosis in hyperlipidemic individuals. (Tripathy, 2003).

MATERIALS AND METHODS

Collection and extraction

Leaves of specified plant collected locally during the month of June 2010 and were confirmed from the Dept. of Botany Govt. Victoria College, Palakkad. Leaves of the above plant was collected and dried under shade in room temperature. It was powdered mechanically and was sieved through no.20 mesh sieve. The finally powdered leaves were kept in air tight container unit the time of use. The powdered leaves (600 gm) were macerated for 24 hours on 95% v/v ethanol, and then it was subjected to percolation by using 95% v/v ethanol as menstrum. The menstrum was collected and subjected to vacuum distillation. The final yield (9.5%) was then suspended in 1% Sodium Carboxy Methyl Cellulose just before use (Shaukat Mahmud., et al 2009).

Drugs and Chemicals

The feeding cholesterol was obtained from Prowess Chemicals Pvt. Ltd., Palakkad. The standard drug Lovastatin was obtained from Dr. Reddy's Laboratories Pvt. Limited, Hyderabad. Triglyceride estimating kit was obtained from the Agape Diagnostics Pvt. Ltd., Ernakulum, Kerala. The Cholesterol estimating kit was obtained from Span Diagnostics Ltd, Surat India. All other drugs and chemicals used in the experiment were obtained commercially and were of analytical grade.

Experimental Animals

Albino rats of either sex weighing between 100 – 150 gm were used for the study. They were housed individually in polypropylene cages. The animals were fed with commercial food pellets and given drinking water ad libitum. All animal procedures have been approved by ethical committee in accordance with animal experimentation and care (817/04/AC/CSCSEA).

Phytochemical studies

The phytochemical studies were performed by Wagner et al 1984 (Harbone, 2005). The ethanol extract of *Vi-*

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tex negundo L showed a positive test for carbohydrates, glycosides, flavonoids and terpenoids.

Acute toxicity Study

Albino rats (100-150g) maintained at standard laboratory conditions was used. A total of five animals were used which received a single oral dose (4000 mg/kg of body weight) of the extract. Animals were kept overnight fasting prior to drug administration. After the administration of the extract, food was withheld for further 3-4 hrs. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 hrs (with special attention during the first 4 hrs) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal), and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrymation, perspiration, piloerection, urinary incontinence and defecation) and CNS, (drowsiness, gait, tremors and convulsions) changes.The LD_{50} of the extract as per the OECD guidelines fall under category 4 values with no signs of acute toxicity at doses of 4000 mg/kg. The biological evaluation of the extracts was carried out at a dose of the 200 mg/kg of body weight (Umamaheswari M., et.al 2007).

***IN VIVO* HYPOLIPIDEMIC ACTIVITY**

Rats were divided into 4 groups of 5 animals each. Each group except, Group-I was orally fed with cholesterol as a suspension in the coconut oil. The aqueous extract of the plant drug was orally fed as a suspension in the Carboxy Methyl Cellulose(1%).

The experiment was designed for a period of one month. Base line readings for the lipid profile was made initially and all the experimental groups were fed with cholesterol till to the end of one month except in normal control. The Serum lipid parameters has been noted on the 15th day of Cholesterol treatment, then onwards the rats in Group III, IV were treated with the respective dose of the drugs for a period of another 15 days and the serum lipid parameters are again noted to identify the effect of extract on lipid levels (Oyetayo., et. al 2006, Souza., et.al 2007).

Table 1: Dose of treated group

Group	Treatment	Dose
Group I	Normal Control	-
Group II	Hypercholestremic	70 mg/kg
Group III	Vitex negundo extract	200 mg/kg
Group IV	Lovastatin	7.2 mg/kg

Blood Collection

The animal is anaesthetized and held gently but firmly by the scruff of the neck on a solid surface so that the eye protrudes. This may help occlude the venous return from the head and neck. The orbital sinus is then penetrated with a sterile micro-capillary tube. This is pushed through the conjunctiva laterally (outer side), dorsal (above) or medially (inner side), to the back wall of the orbit where it punctures the venous sinus and so fills with blood. On withdrawal of the pipette or tube, blood exudes from the canthus where it can be collected (Dubey et.al 2005).

Serum Preparations

The serum samples used for the analysis of the lipid parameters were collected by centrifuging the blood samples. The blood sample was centrifuged for 15 min at a speed of 5000 r.p.m and the clear supernatant serum was used for the analysis.

Determination of serum Triglyceride level

The blank solution was prepared by taking 1000 µL of the working reagent. The standard solution was prepared by mixing the working reagent 1000 µL and 10 µL of the Standard triglycerides solution. Sample solution is prepared by mixing 1000 µL of the working reagent and 10 µL of the serum sample. Mixed and incubated for 5 minutes at 37^{0}C. The absorbance of sample and standard were measured against the reagent blank at 546 nm.

$$Triglycerides(mg/dl) = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times 200$$

Determination of total Cholesterol

The blank is considered as 1000 µL of cholesterol reagent. Sample solution is prepared by mixing 10 µL of the serum sample and 1000 µL of cholesterol reagent. Standard solution is prepared by mixing 10 µL of the cholesterol standard and 1000 µL of cholesterol reagent. Mixed well and Incubated at 37^{0}C for 10 minutes. The absorbance of sample and standard were measured against the reagent blank at 505 nm.

$$Total\ Cholesterol\ concentration\ (mg/dl) = \frac{Absorbance\ of\ Test}{Absorbance\ of\ standard} \times 200$$

Determination of the serum High Density Lipoprotein

Low density lipoprotein (LDL) Cholesterol, Very Low Density Lipoprotein (VLDL) cholesterol and chylomicron fractions, are precipitated by addition of Polyethylene Glycol 6000 (PEG). After centrifugation, the High Density Lipoprotein (HDL) fraction remains in the supernatant and is determined with CHOD – PAP method.

Step1: HDL – Cholesterol Separation

Test solution was prepared by taking 200 µL of the serum sample and 200 µL of the precipitating reagent. Mixed well and kept at Room Temperature (15 – 30^{0}C) for 10 minute. Centrifuged for 15 minutes at 2000 rpm and the supernatant was used for HDL – Cholesterol estimation.

Table 2: Total Cholesterol

Treatment Group	Normal Control	Cholesterol Control	Drug 200mg/kg	Lovastatin
Basic value mg/dl	144.4 ± 0.12	122.2± 0.13	138.3± 0.26	116.0± 0.40
	122.2± 0.01	184.2± 0.12	196.1± 0.45	152.3± 0.51
	144.4± 0.012	176.2± 0.24	128.0± 0.15	167.7± 0.25
	182.2± 0.11	188.8± 0.02	166.6± 0.11	200.0± 0.36
	166.6± 0.32	150.0± 0.23	*144.4± 0.12	189.3± 0.20
15th day value mg/dl	160.8± 0.02	212.2± 0.11	200.3± 0.52	199.9± 0.12
	124.2± 0.21	268.0± 0.11	205.6± 0.45	255.5± 0.11
	140.2± 0.01	250.0± 0.21	186.3± 0.14	200.3± 0.02
	176.7± 0.03	225.2± 0.36	200.2± 0.15	164.2± 0.01
	162.4± 0.13	168.4± 0.51	**199.9± 0.05	192.4± 0.12
30th day value mg/dl	155.5± 0.16	242.8± 0.16	200.3± 0.01	180.2± 0.01
	138.2± 0.45	290.2± 0.45	192.4± 0.05	200.6± 0.05
	156.2± 0.25	263.2± 0.25	***176.8± 0.12	144.4± 0.024
	192.4± 0.20	250.0± 0.20	184.3± 0.32	136.5± 0.13
	186.8± 0.25	200.0± 0.43	180.6± 0.40	127.7± 0.05

1. Values are mean ± SEM; n=3; 2. *P>0.05 when compared to normal control; 3. **P<0.05 when compared to normal control; 4. ***P<0.01 when compared to cholesterol control.

Table 3: Triglycerides

Treatment Group	Normal Control	Cholesterol Control	Drug 200mg/kg	Lovastatin
Basic value mg/dl	50.00±0.52	63.8±0.26	68.9±0.42	52.8±0.44
	66.67±0.51	52.5±0.27	*66.7±0.23	65.7±0.51
	52.87±0.22	60.2±0.38	74.8±0.21	76.8±0.12
	64.52±0.20	72.02±0.49	62.3±0.22	54.0±0.23
	67.47±0.49	56.30±0.21	50.0±0.47	58.6±0.34
15th day value mg/dl	59.1±0.16	70.2±0.49	66.4±0.44	62.7±0.24
	68.5±0.27	68.1±0.50	70.8±0.51	67.3±0.12
	74.6±0.58	62.8±0.51	68.3±0.12	69.4±0.21
	71.1±0.41	70.2±0.53	**76.4±0.34	72.9±0.53
	60.4±0.39	62.4±0.34	60.2±0.23	59.8±0.12
30th day value mg/dl	66.5±0.52	82.4±0.49	***59.3±0.42	60.1±0.56
	69.0±0.42	76.8±0.50	62.8±0.21	65.2±0.45
	70.2±0.42	90.8±0.43	60.6±0.47	58.3±0.46
	80.3±0.25	80.4±0.47	76.8±0.49	62.9±0.50
	96.4±0.37	82.4±0.43	55.4±0.52	59.6±0.54

1. Values are mean ± SEM; n=3; 2. *P>0.05 when compared to normal control; 3. **P<0.05 when compared to normal control; 4. ***P<0.01 when compared to cholesterol control.

Step 2: HDL – Cholesterol Estimation

Sample solution was prepared by mixing 100 µL of supernatant from step 1 and 1000 µL of cholesterol reagent. Standard solution was prepared by taking 100 µL of HDL – Cholestrol standard & 1000 µL of cholesterol reagent. Blank the analyzer with reagent Blank (cholesterol reagent). The absorbance of Standard was measured followed by the Sample at 505 nm (Tenpe., et.al 2007)

$$HDL\ cholesterol = \frac{Absorbance\ of\ Test}{Absorbance\ of\ standard} \times 50 \times 2^{*}$$

*(2=Dilution factor as sample is diluted 1:1 in step 1).

Determination of serum Low Density Lipoproteins

For LDL – Cholesterol using, Friedewald's equation used.

LDL Cholesterol = Total Cholesterol - Triglycerides – HDL Cholesterol

RESULTS AND DISCUSSION

Table: 1 Shows that the cholesterol fed animals shows significant increase in their blood total cholesterol level on the 15th day when compared with normal. The standard drug Lovastatin and the plant extract (200 mg/kg) were found to decrease the elevated cholesterol levels when compared to Cholesterol control group. Table: 2 Shows that the Lovastatin treated group only was found to be significantly reduce the

Table 4: High Density Lipoprotein

Treatment Group	Normal Control	Cholesterol Control	Drug 200mg/kg	Lovastatin
Basic value mg/dl	98.2±0.22	80.5±0.21	73.1±0.42	87.3±0.23
	86.4±0.13	78.4±0.13	*89.6±0.44	74.09±0.20
	83.7±0.23	62.4±0.14	94.3±0.25	83.7±0.24
	81.02±0.46	60.4±0.45	99.8±0.26	65.3±0.26
	97.9±0.40	72.3±0.27	72.6±0.37	82.4±0.47
15^{th} day value mg/dl	78.2±0.45	88.3±0.44	89.6±0.40	97.4±0.42
	84.6±0.32	86.2±0.53	87.3±0.10	86.3±0.51
	98.5±0.13	63.1±0.21	96.2±0.01	82.1±0.11
	79.6±0.32	62.5±0.22	**89.3±0.22	73.4±0.14
	94.2±0.02	79.4±0.33	86.7±0.64	92.6±0.13
30^{th} day value mg/dl	85.8±0.12	83.1±0.40	88.5±0.16	87.2±0.40
	81.0±0.52	80.4±0.10	***78.1±0.27	82.1±0.10
	93.7±0.09	62.9±0.01	90.3±0.08	70.03±0.01
	85.6±0.20	62.1±0.22	80.2±0.21	59.6±0.22
	89.2±0.12	69.8±0.64	81.09±0.61	89.2±0.33

1. Values are mean ± SEM; n=3; 2. *P>0.05 when compared to normal control; 3. **P<0.05 when compared to normal control; 4. ***P<0.01 when compared to cholesterol control.

Table 5: Low Density Lipoprotein

Treatment Group	Normal Control	Cholesterol Control	Drug 200mg/kg	Lovastatin
Basic value mg/dl	35.5±0.55	28.94±0.14	51.42±0.49	18.14±0.23
	22.46±0.01	95.3±0.21	93.16±0.50	64.26±0.20
	49.72±0.02	102.16±0.45	18.74±0.51	68.64±0.47
	88.07±0.60	113.996±0.16	*54.34±0.53	123.9±0.25
	52.20±0.45	66.4±0.27	61.8±0.54	95.18±0.25
15^{th} day value mg/dl	69.98±0.12	109.86±0.22	97.42±0.16	89.96±0.12
	25.7±0.52	168.18±0.13	104.14±0.27	155.74±0.10
	26.78±0.10	174.34±0.23	**76.44±0.08	104.32±0.86
	82.88±0.09	148.66±0.46	95.62±0.39	76.22±0.09
	56.12±0.12	76.52±0.40	101.16±0.31	87.84±0.12
30^{th} day value mg/dl	56.4±0.36	143.22±0.44	99.94±0.53	80.98±0.42
	43.4±0.37	184.04±0.27	101.8±0.17	105.46±0.08
	48.46±0.60	182.14±0.39	74.38±0.02	62.44±0.23
	90.74±0.72	171.82±0.21	***88.74±0.61	64.32±0.16
	78.32±0.81	113.72±0.31	87.62±0.63	26.58±0.42

1. Values are mean ± SEM; n=3; 2. *P>0.05 when compared to normal control; 3. **P<0.05 when compared to normal control; 4. ***P<0.01 when compared to cholesterol control.

elevated the triglyceride level when compared to cholesterol control group. But when compared to Lovastatin treated group the extract treated group was found to be having a significant activity.

Table: 3 Shows neither the extract nor the standard drug were found to decrease the elevated HDL level. And also the cholesterol & extract (200mg/kg) treated groups were found to have a significant increase in their HDL level. Table: 4 Shows that the (200mg/kg) & Lovastatin treated groups were found to be significantly reduce the elevated LDL when compared to cholesterol control on 30^{th} day.

Many plant products are increasingly recognized as having protective role in Coronary Artery Diseases (CAD), stroke through several mechanisms including antioxidant activity, hypocholesterolemic properties. Many epidemiological and clinical studies shows that lipid lowering therapy stops and prevents the events of acute Coronary Artery Diseases (CADs), reduction in LDL Triglycerides are significantly related to lipid lowering therapy. In the present study the aqueous extract of *Vitex negundo* L. results a significant dose dependent reduction in Total Cholesterol and Low Density Lipoprotein level when compared with Cholesterol control group. Also all the treatment groups show a little bit increase in HDL level. Up on the triglycerides level the extracts posses a significant activity when compared to Lovastatin treated groups. The results demonstrate the effectiveness of the leaf of *Vitex ne-*

gundo L. asan anti-atherogenic agent preventing coronary artery diseases. Further the study suggest, to identify the active chemical constituents with potential for clinical use in the prevention and treatment of atheroma and related disorders and also to evaluate the activity once again in the hypercholesteremic rats feed with pure cholesterol more than 15 days, because the triglyceride and HDL levels are not too increased within the 15 days of treatment (30 days treatment shows increasing levels of LDL as well as TG's in cholesterol control groups.)To conclude, the study suggested that the *Vitex negundo* leaf possess plasma lipid lowering activities, which might be helpful in preventing or slowing the progress of atheroma related diseases.

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