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Phytochemical Investigation and Hypoglycemic effects of *Vitex negundo.*

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Research Article

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Keywords: Hypoglycemic, *Vitex negundo , Antidiabetic activity*.

ABSTRACT

We evaluated the hypoglycemic effects of methanol ratio extract of *Vitex negundo* stems the hypoglycemic effect was evaluated in Streptozotocin induced diabetic rats. The extract of *Vitex negundo* also significantly ($P<0.01$) reduced blood glucose level in Streptozotocin induced diabetic rats twelve hours after administration.

INTRODUCTION

It is a metabolic disorder characterized by hyperglycaemia, glycosuria, hyperlipemia, negative nitrogen balance and sometimes ketonemia. A wide-spread pathological change in thickening of capillary basement membrane, increase in vessel wall matrix and cellular proliferation resulting in vascular complications like lumen narrowing, early atherosclerosis, sclerosis of glomercular capillaries, retinopathy, neuropathy and peripheral vascular insufficiency. Diabetes mellitus is a group of endocrine syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins, and an increased risk of complications from vascular disease. Most patients can be classified clinically as having either type I diabetes mellitus (type I DM formerly known as insulin dependent diabetes of IDDM) or type II diabetes mellitus (type II DM formerly known as non-insulin dependent diabetes of NIDDM).

Vitex negundo, belonging to family Verbenaceae (which comprises 75 genera and nearly 2500 species), commonly known as five leaved chaste tree (Eng), Nirgundi (Hindi), is a deciduous shrub, occur in tropical to temperate regions (up to 2200 m from east to west) grows gregariously in wastelands and is also widely used as a hedge plant. The leaves have five leaf lets in a palmately arrangement, which are lanceolate, 4–10 cm long, hairy beneath and pointed at both ends, quadrangular whites fine tomentum, the leaves are 3-5 foliate, leaflets are lanceolate (5-10 cm), acute terminal leaflet (16-32 mm) with petiolate having 1-1.3 cm long, with a very short petiolate. The bluish purple flowers are numerous. The fruit is succulent, black when ripe, rounded and about 4 mm in diameter. A decoction of the stems of *Vitex negundo* is used in the treatment of burns and scalds [1].

MATERIALS AND METHOD

Plant material

The plant *Vitex negundo* was collected from Haridwar, Uttarakhand. The plant was identified and authenticated by Dr. H.B. Singh Scientist F and Head, Raw Materials Herbarium and Museum at National Institute of Science Communication and Information Resources, New Delhi (NISCAIR). The stem bark was air dried at School of Pharmaceutical Sciences, Shobhit University, Meerut.With ref. no. NISCAIR/RHMD/ CONSULT 2008 /9/ 1104/135.

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Preparation of Extract

The drug was dried in shade and coarsely powdered. The powdered material (100 gm) was successively extracted with petroleum ether, methanol and aqueous in a soxhlet apparatus. The extracts were subjected to removal of solvents by distillation and then dried on a water bath at temp 50±5^{0}C.

Determination of extractive

The percentages of extractives of the powdered stem bark parts of *Vitex negundo* with different solvents were determined.

Preparation of Crude Extracts

The powdered drug (100 g) was extracted with petroleum ether (60-80^{0}C), methanol and aqueous in a soxhlet extraction apparatus. The extraction was continued till a few drops of the last portion of the eluents did not leave any perceptible residue on drying on filter paper. The extract was filtered and solvent removed by distillation. The concentrated extract was dried on water bath. It was kept in vacuum desicators and weighed.

Table 1: Percent extractive of the powered stem bark of (*Vitex negundo)*

Solvent	Extraction period (Hrs)	Colour of the extract	Weight of extract	% yield
Petroleum ether (60-80^o C)	48	Light brownish	2.0 gm	2.07
Methanol	72	Dark greenish	7.62 gm	7.9
Water	72	Dark brownish	8.52 gm	9.51

Petroleum ether extract, (2.0 g, 2.07 % yield), methanol extract (7.62 g, 7.9 % yield), and aqueous extract (8.52 g, 9.51 % yield). Petroleum ether extract was light brownish in color, methanol extract was dark greenish in color and aqueous extract was dark brownish in color.

Qualitative chemical identification test

The following Qualitative chemical tests for identifying various phytoconstituents present were carried out in various extract of plant of *Vitex negundo.*

Table 2: Qualitative chemical analysis of various extract of *Vitex negundo* stem bark

Phytoconstituents	Successive extraction		
	Petroleum ether	Methanol	Aqueous
Carbohydrate	–	+	–
Alkaloid	–	+	+
Flavanoid	–	+	+
Steroid	–	+	+
Tannins	–	+	+
Phenolic compounds	–	+	+
Saponins	–	+	–
Anthraquinones	–	–	+
Detection of fixed oils and fats	+	–	–

+ Present
- Absent

The tests were done to find the presence of the active chemical constituents such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, fixed oils and fats.

Isolation of methanol extract of *Vitex negundo* by column chromatography

Silica gel (60-120 mesh) for column chromatography 100 g was packed in a column of suitable dimensions. The methanol extract 5.0 g was dissolved in methanol 10 ml and mixed with silica gel G 6.0 g and dried. The dried extract absorbed on silica gel G was transferred on top of the prepared column. The elution was carried out with solvents of increasing polarity at the rate of 60 drops per minute. The fractions of 25ml each were collect. The fraction was monitored by the TLC and those with identical TLC pattern were pooled. The details are given in table 3.

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Table 3: Column chromatography of methanol extract

Fraction no.	Eluent used	Nature of the residue	TLC Rf value
1-5	Chloroform:methanol 99:1	White	_
5-10	Chloroform:methanol 98:2	Pink	0.7
11-15	Chloroform:methanol 97:3	Light green	0.5, 0.6, 0.3
16-20	Chloroform:methanol 96:4	Green	0.3, 0.7, 0.5
21-25	Chloroform:methanol 95:5	Dark green	0.4, 0.6, 0.3, 0.2
26-30	Chloroform:methanol 94:6	Dark green	0.5, 0.3, 0.7, 0.8
31-35	Chloroform:methanol 93:7	Light yellow	0.2, 0.4
36-40	Chloroform:methanol 92:8	Yellow	0.4, 0.5, 0.7, 0.6
41-45	Chloroform:methanol 91:9	Yellow	0.6, 0.5, 0.9, 0.7

Thin layer chromatography of methanol extract

The thin layer chromatography of different fractions was performed using silica gel G plates. The following solvent systems were tried and the details are given in table 3.6

Table 4: Different solvents ratio used for thin layer chromatography

No.	Solvents	Solvent ratio
A	Chloroform:methanol	5:5
B	Ethyl acetate:formic acid:glacial acetic acid	100:11:11:27
C	Chloroform:acetone:formic acid	75:16.5:8.5
D	Benzene:pyridine:formic acid	72:18:10
E	Ethyl acetate:methanol:water	100:17:13
F	Chloroform:benzene	75:25
G	Chloroform: ethyl acetate	60:40
H	Chloroform:methanol:water	64:50:10
I	Methanol:chloroform	5:5
J	Hexane: ethyl acetate	50:50
K	Benzene:diethyl ether	50:50
L	Toluene: ethyl acetate:acetic acid	80:18:2, 60:38:2
M	Ethyl acetate: chloroform: water	30:30:40
N	Toluene: chloroform:ethanol	28.5:57:14.5

Out of the solvent tried A, B, C, I, L and N were found to most satisfactory. Detection of spot was done by visualizing plates under ultraviolet light where fluorescent spot appeared. The fractions 5-10 showing identical TLC pattern were mixed. The fractions 36-40 and 41-45 showing a no. of compounds in small concentrate were not taken for further studies. Residue from fraction 5-10 was kept in chloroform which deposited at the bottom a pinkish coloured solid showed single spot on TLC [2,3].

Characterization of isolated compound of methanol extract of *Vitex negundo*

Classically, many different colour reactions and solubility properties have been used to characterize different types of chemical constituents. These have been described in standard text reference.

Among the various spectroscopic techniques employed for the characterization and structure determination of constituents such as FTIR spectroscopy, NMR spectroscopy and Mass spectroscopy.

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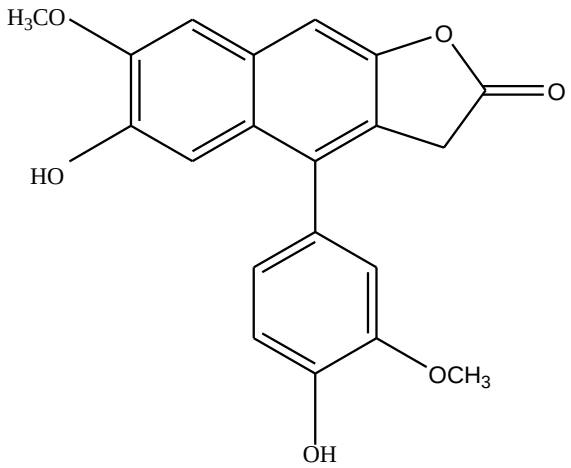
Isolated compounds of methanolic extract of *Vitex negundo* stem bark



Negundin A

Molecular formula $C_{20}H_{16}O_6$

Figure 1: Chemical structure of lignans (Negundin A)

Animals

The dried extract in dose of 100 mg/kg body weight was used for Anti diabetic activity studies in swiss albino mice. Male swiss albino mice of body weight were taken before and after experiment with the help of single pan balance 22-28 g were used for the study. The animals were housed in clean metabolic cages and maintained in controlled temperature (27± 2^0C) and light cycle (12 hrs. light and 12 hrs. dark). They were fed with standard pellet diet (Gold mohar brand, Lipton India Ltd.) and water. The protocol was approved by Institutional animal ethics committee. registration no.837/ac/04/CPCSEA.

Drugs

These drugs was used for the inducted of diabetes and was obtained from different pharmaceutical organization;

Table 5: List of Chemicals

S.No.	Chemical and solvent	Manufacture
1.	Streptozotocin	Sisco Research Laboratories Pvt. Ltd. Mumbai, India.
2.	Glibenclamide	Aventis Pharma Limited, Goa
3.	Dispovan-1ml	Hindustan syringes and medical devices Ltd. Faridabad
4.	Formaldehyde solution	Rankem, RFCL Ltd. New Delhi, India

Extraction of Plant

Methanolic extract of *Vitex negundo* stem bark and isolated compound (Negundin A) of methanolic extract.

Streptozotocin

Streptozotocin (STZ) is a naturally occurring nitrosourea product of *Streptomyces achromogenes.* Usually, the intraperitoneal injection of a single dose (25 mg/kg body weight) of it exerts direct toxicity on β cells resulting in necrosis within 48-72 h and causes a permanent hyperglycemia. STZ breaks nuclear DNA strand of the islet cells.

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Glibenclamide

Single dose of glibenclamide provokes a brisk release of insulin from pancreas. It acts on β-cell membrane leading to enhance calcium flux across it, hence degranulation. After chronic administration the insulinemic action of glibenclamide declines, but improvement in glucose tolerance is maintained. Thus it is an oral antidiabetic preparation with an efficient hypoglycemic action.

Mice were divided into the following groups.

Group I Treated of 6 mice which served as normal control and were given only distilled water daily.

Group II Treated of 6 STZ dose (25mg/kg) induced diabetic mice and served as diabetic control and were given distilled water only.

Group III Treated of 6 STZ induced diabetic mice and were treated orally with methanolic extract of *Vitex negundo* stem bark at the dose of 100 mg/kg body weight daily for 15 days, once a day.

Group IV Treated of 6 STZ induced diabetic mice and were treated orally with isolated compound (Negundin A) of methanolic extract of *Vitex negundo* stem bark at the dose of 100 mg/kg body weight daily for 15 days, once a day.

Group V Treated of 6 STZ induced diabetic mice and were given Glibenclamide (GBC) at the dose of 10 mg/kg body weight daily for 15 days, once a day. After 15 days of herbal treatment experiments were terminated and observations were made. Body weight was taken before and after experiment with the help of single pan balance. Blood glucose level was estimated on 0 day and 15th day of experiment with the help of glucometer using strip method and blood was taken from tip of the tail. Fresh urine was collected by slightly pressing the tail and back of the rat. Glucose and ketone in urine was checked using keto-diastix strips on 0 and 15th day of experiment. After termination of experiment the mice from all the groups were anesthetized and dissected out. Pancreas and liver were fixed in bouin's fluid and histological preparations were made. 5µ thick sections were cut and stained with haematoxylene and eosin. Statistical analyses were done with the help of (post hoc Dunnett's multiple comparison test). Animal housing, care and application of experimental procedures were in accordance with institutional animal ethic guidelines . [2,4,5,6,7,8]

Body weight

Diabetes is characterized by weight lose. Streptozotocin administration brings marked reduction in body weight of mice. This reduction is generally statistically significant when compared with normal control group.

Table 6:Change in body weight (gm) of mice treated with methanol extract and Negundin A on day 0.

Groups	1st Mice	2nd Mice	3rd Mice	4th Mice	5th Mice	6th Mice
Normal	26	25	27	24	26	25
Diabetic	25	23	26	27	24	25
Standard	26	23	25	28	26	24
Methanol extract	27	25	24	26	25	26
Negundin A	26	24	27	26	28	26

Table 7: Change in body weight (gm) of mice treated with methanol extract and Negundin A on day 15th.

Groups	1st Mice	2nd Mice	3rd Mice	4th Mice	5th Mice	6th Mice
Normal	24	25	26	24	25	26
Diabetic	24	_	24	26	25	23
Standard	27	26	_	27	26	27
Methanol extract	28	27	_	25	26	27
Negundin A	28	26	26	28	_	27

Table 8: Change in body weight (gm) of mice treated with methanol extract and Negundin A and dose mg/kg on 0 to 15th day.

Groups	Dose / mg/kg	Day 0	Day 15	Change
Normal	1 ml vehicle	25.5	25	-0.5
Diabetic	25 mg/kg	25	24.4	-0.6
Standard	10 mg/kg	25.33	26.6	1.27
Methanol ext.	100 mg/kg	25.5	26.6	1.1
Negundin A	100 mg/kg	26.16	27	0.84

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Blood glucose level

Diabetes is characterized by increase in blood glucose level. Streptozotocin administration brought about marked increase in blood glucose level of mice. This is generally found to be statistically significant when compared with normal control group.

Table 9: Effect of methanolic extracts of *Vitex negundo* and Negundin A fasting blood glucose level (mg/dl) in normal control, diabetic control and Streptozotocin induced diabetes mice on 0 day.

Groups	1st Mice	2nd Mice	3rd Mice	4th Mice	5th Mice	6th Mice
Normal	105	115	110	114	105	110
Diabetic	280	255	240	260	385	300
Standard	325	330	295	280	315	330
Methanol extract	340	320	300	350	290	280
Negundin A	350	320	360	330	300	290

Table 10: Effect of methanolic extracts of *Vitex negundo* and Negundin A fasting blood glucose level (mg/dl) in normal control, diabetic control and Streptozotocin induced diabetes mice on 7th day.

Groups	1st Mice	2nd Mice	3rd Mice	4th Mice	5th Mice	6th Mice
Normal	115	105	100	110	100	115
Diabetic	285	260	250	295	365	310
Standard	245	250	255	220	275	285
Methanol extract	280	270	260	295	240	245
Negundin A	250	275	285	290	250	230

Table 11: Effect of methanolic extracts of *Vitex negundo* and Negundin A fasting blood glucose level (mg/dl) in normal control, diabetic control and Streptozotocin induced diabetes mice on 15th day.

Groups	1st Mice	2nd Mice	3rd Mice	4th Mice	5th Mice	6th Mice
Normal	109	110	100	115	116	105
Diabetic	320	_	350	395	395	370
Standard	185	190	_	170	175	180
Methanol extract	200	195	_	210	170	185
Negundin A	110	115	110	120	_	105

Table 12: Effect of methanolic extracts of *Vitex negundo* and Negundin A fasting blood glucose level (mg/dl) in normal control, diabetic control and Streptozotocin induced diabetes mice on 0 day, 7th day and 15th day.

Groups	0 day	7th day	15th day
Normal	109.83±4.26	107.07±28.39	109.66±6.05
Diabetic	286.66±52.50	294.16±75.88	366±52.50
Standard	312.33±20.68	255.23±78.84	180±9.35
Methanol extract	313.33±28.05	265±28.04	192±20.68
Negundin A	325±27.39	263.33±27.38	112±27.39

Statistical analysis

Values are given as mean ± standard deviation for groups of five animals. The results were analyzed by one way analysis of variance (ANOVA) followed by post hoc Dunnett's multiple comparison test. Differences between means were considered to be significant at ($p < 0.01$).

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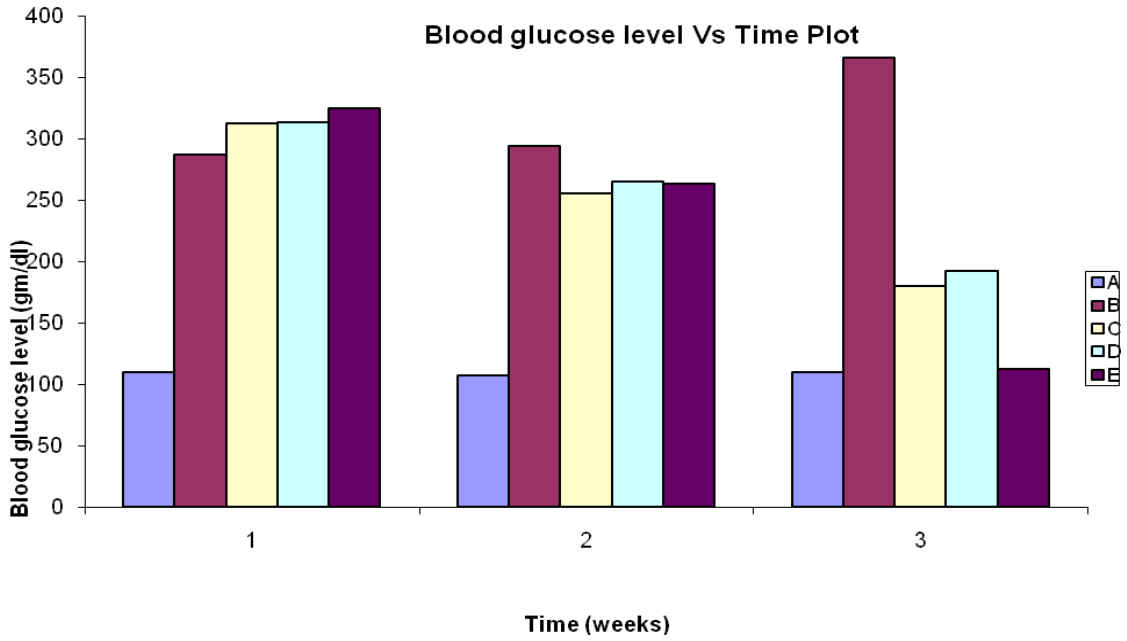

Figure 2: Histogram showing changes in blood glucose level following treatments of methanolic extracts of *Vitex negundo,* isolated compound and GBC on blood glucose level in STZ induced diabetic mice.

A (normal),
B (diabetic),
C (standard),
D (methanolic extract), and
E (isolated compound) respectively.

RESULT AND DISCUSSION

From the extract of petroleum ether, methanol and aqueous extract of dried stem bark of *Vitex negundo*. The percentage yield was found to be 2.07%, 7.9% and 9.51% respectively. The change in body weight of control and experimental groups of mice treated with methanolic extract of *vitex negundo* and *negundin A*. STZ induced (25mg/kg body weight) mice showed loss in body weight as compared to methanol extract of *vitex negundo* and *negundin A*.

The body weight of normal control treated mice, did not show any significance difference (-0.5 mg) change on the 15th day. The body weight of diabetic control mice showed decrease in their body weight (-0.6) after two weeks. Increase glucose level in diabetic control groups were found to be highly statically significance when compare to their respective normal control groups. These raised levels of blood glucose in diabetic mice were declined after oral feeding of methanolic extract of *vitex negundo*. When comparisons were made between diabetic and drug treated animal, blood glucose level were found to be declined sharply on 15th day.

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