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Antioxidant and Antidiabetic Properties of *Vitex nigundo* L. Leaves

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Abstract: *Vitex Nigundo* Linn. is credited with important medicinal activities such as antioxidant, anti-inflammatory, analgesic, anticonvulsant etc. The purpose of the presents study was to assess the therapy effect of *V. Nigundo* leaves extract on glucose level, total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, SGPT and SGOT in blood serum of diabetic animals. Ethyl alcohol extract of *V. Nigundo* leaves in doses of 200 and 300 $mgkg^{-1}$ body weights was daily administered orally by gavages to mice for 21 days to evaluate their antihyperglycemic and antihyperlipidemic effects on normal and streptozotocin-induced diabetic mice. Ethyl alcohol extract of *V. Nigundo* leaves in doses of 300 $mgkg^{-1}$ body weights showed ($P<0.01$) more significant activity than the ethyl alcohol extract of *V. Nigundo* leaves in doses of 200 $mgkg^{-1}$ body weights. The study validates scientifically the widely claimed use of *V. Nigundo* leaves as an ethnomedicine to treat diabetes mellitus.

Keywords: *Vitex nigundo*, Antidiabetic, Diabetes Mellitus, Hyperglycemic, Hyperlipidemic

1. Introduction

Diabetes Mellitus (DM) is a chronic disease resulted either from the production of insufficient insulin by pancreas or the body's ineffective use of insulin. There are more than 220 million diabetic people worldwide, and it was estimated in 2005 that 1.1 million people died from diabetes with almost 80% of these deaths occurred in low and middle-income countries (WHO, 2011) [1]. World Health Organization (WHO) projected that death caused by diabetes will double between 2005 and 2030.

Realizing the seriousness of this scenario, researchers worldwide are putting endless efforts in searching for complementary and alternative medicine therapies (CAM) for diabetes. Herbs, dietary supplements, and mind-body medicine are the most commonly used and studied CAM modalities to treat diabetes [2]. *Allium sativum* (garlic), *Coccinia cordifolia* (ivy gourd), *Momordica charantia* (bitter melon), *Opuntia streptacantha* (prickly pear cactus), *Gymnema sylvestre* (gymnema), *Panex ginseng* (ginseng), aloe vera, and *Trigonella foenum graecum* (fenugreek) are some examples of herbs and biologically based practices used for diabetes [2]. *Tamarindus indica* [3], *Artemisia herba alba* [4] and *Moring aoleifera* [5] were proven scientifically in diminution of fasting blood sugar level of diabetic animals.

Vitex negundo Linn. belonging to family *Verbenaceae* is a large aromatic shrub. Countries it is indigenous to include Afghanistan, Bangladesh, Bhutan, Cambodia, China, India, Indonesia, Japan, Korea, Kenya, Madagascar, Malaysia, Mozambique, Myanmar, Nepal, Pakistan, the Philippines, Sri Lanka, Taiwan, Tanzania, Thailand, and Vietnam [6]. *Vitex negundo* leaves have been used as anti-inflammatory, analgesic and antihistamine [7]. The leaves possess antimicrobial activities [8], hepatoprotective potentials [9], antioxidant activity [10]. This plant also has anticancer and antitumor [11-13] properties. A limited research work of *Vitex negundo* plant extract on the complications of diabetes

were studied [14]. The antiglycemic activity of petroleum ether, methanol and aqueous extract of *Vitex nigundo* stem have been studied [15]. However, after oral administration of *Vitex nigundo* L. leaves ethanol extract, the glycemic and atherogenic complications of diabetes have not been studied. Our aim was to investigate the effects of *Vitex nigundo* leave extract onglycogenic status including, plasma glucose, plasma lipid profile including total cholesterol, triglycerides, HDL, LDL and SGPT, SGOT in diabetic mice.

2. Materials and Methods

2.1. Plant Material

The *Vitex negundo* Linn. leaves were collected from the University of Rajshahi campus. Plant specimens were identified by Dr. A. H. M. Mahbubur Rahman, Associate professor and taxonomist department of Botany, university of Rajshahi. A voucher specimen with reference number #33 was deposited in the Department of Botany, University of Rajshahi. The leaves were cut into small pieces, sun dried and ground into a powder with an electric grinder. The powder was packed and stored in a refrigerator at 4 °C until used.

2.2. Extraction of Vitex negundo Linn. Leaves Extract

Fresh leaves of *Vitex negundo* Linn. (local name 'Nishinda') were dried completely under mild sun, and grinded with an electric grinder into coarse powder and used for extraction.

In cold extraction, the coarse power was submerged in ethyl alcohol (95%) since ethyl alcohol is the most common solvent for extracting most of the constituents present in herbal material. Flat bottom flasks were used for this purpose which were kept at room temperature and allowed to stand for several days (5-7) with occasional shaking and stirring. When the solvent became concentrated, the contents were filtered through cotton and then through filter paper. Then the solvent i.e., ethanol was allowed to evaporate using shaking machine for several days (7-10). The crude extracts of the plants were obtained, which were preserved for further use.

2.3. Determination of Antioxidant Compounds

The content of total phenolics of methanolic extract of *Vitex negundo* was determined using Folin-ciocalteu reagent (FCR) as oxidizing agent and ascorbic acid (AA) as standard [16].

The content of total flavonoids of methanolic extract was determined by aluminum chloride colorimetric method. Quercetin was used as standard [17].

2.4. Test Animal

Albino mice were selected as experimental animal to carry out this study. Mice weighting about 25-30gm were collected from the Animal Resource Division of ICDDR'B Mohakhali, Dhaka.

2.5. Maintenance of Animal

Thirty (30) mice were randomly divided into five groups. Each mouse was numbered with a permanent marker for experimental purpose, weighed and recorded. Five cages (containing 6 mice) were kept in the animal house of Biochemistry Department, Rajshahi University. The animals were fed on standard laboratory diet with water for 21 days. The diet consisted of wheat 40%, wheat brane 19%, rice polish 4%, fish meal 10%, til oil cake 10%, gram 6%, mashcolai 6%, skim milk powder 4%, soyabean oil 1%, salt 0.5%, molases 0.5% (percent of ingredients used in the ration). Vitamins and minerals will be added 2.5 kg/ton of pellet.

2.6. Experimental Animals

Thirty male Albino mice of the same age and body weights of 25-30gm were purchased from the International Centre for Diarrheal Disease Research, Bangladesh and used in the experiments. The mice were housed in cages (six mice per cage) at an ambient temperature of 25-30 °C and 45-55% relative humidity with a 12-h dark and light cycle for one week prior to the experimental work. The mice had free access to a standard pellet diet and water *ad libitum*. The institutional Animal Ethics Committee approved this study.

2.7. Induction of Diabetes Mellitus

Diabetes mellitus was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) in 0.1 M citrate buffer (pH 4.5) at a dose of 60 $mgkg^{-1}$ body weight (b.w.) per mice to a group of overnight-fasted mice. Diabetics developed and stabilized in STZ-treated mice over a period of 7 days. The mice were allowed to drink a 5% glucose solution overnight to prevent the hypoglycemia. Healthy mice received an equivalent amount of citrate buffer intraperitoneally and were regarded as control mice. Seven days after STZ administration, the fasting blood glucose (FBG) levels of each mouse were determined and severely diabetic mice with FBG levels of>200 mg/dl (11.5 mmol/dl) were employed in the study.

2.8. Experimental Design and Blood Collection

A long-term study of 21 days was conducted on the severely diabetic mice. Thirty mice were divided into five groups of six mice each. Group I and Group II were non-diabetic and diabetic control mice that were fed a standard mice diet. Group III and Group IV consisted of diabetic mice that were fed a standard diet and were treated with *V. Nigundo* L. leaves extract at doses of 200 $mgkg^{-1}$ b.w. and 300 $mgkg^{-1}$ b.w., respectively. Group V was composed of diabetic mice that were treated with a 0.1-ml solution of the oral hypoglycemic drug, glibenclamide (0.5 $mgkg^{-1}$ b.w.), and a normal diet.

Before the administration of the *V. Nigundo*supplement,

the basal blood glucose levels were measured in each of the groups. After 21 days of the experiment, all mice were anesthetized with chloroform vapor, and blood samples were withdrawn by cardiac puncture. Aliquots of blood were transferred to screw-capped bottles that contained a fluoride/oxalate anticoagulant for the determination of blood serum glucose, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, SGPT, SGOT and total protein concentrations. All analyses were performed within 24 hours of blood collection.

The blood samples were collected from ventricle of heart. Blood was kept about 20 minutes at room temperature, for coagulation. After centrifugation at 3000 rpm for 10 minutes at 4°C, serum was drawn off and stored at -80°C until the experiments were performed.

2.9. Analytical Procedure

Blood glucose concentrations were estimated according to the glucose oxidase method using a reagent kit (Randox Laboratory Ltd., UK). Serum total cholesterol and HDL-cholesterol concentrations were measured according to the CHOD-PAP method, using a commercial kit. Serum LDL-cholesterol concentrations were also estimated using the CHOD-PAP method after precipitation with magnesium sulfate and phosphotungstic acid. Levels of SGPT and SGOT activity were estimated using an SGPT and SGOT assay kit, respectively. Triglyceride concentrations were measured using the GPO-PAP method with a commercial kit.

2.10. Statistical Investigation

The results are expressed as the mean ± standard deviation (S.D.) of triplicate analyses. All statistical comparisons were performed using a one-way analysis of variance (ANOVA) followed by a multiple two-tailed t-test. Differences were considered significant at a *P* level of 0.05 or lower.

3. Results

The amount of total phenolic content and flavonoid content was expressed as mg/100g of fresh weight. Total phenolic and flavonoid content of *Vitex negundo* L. leaves were found 95.188±5.338 mg of AA/100g and 122.86±4.04 mg q/100g dry extract respectively (Figure 1). Total phenolic content was determined by plotting ascorbic acid as standard curve (Figure 2) and the flavanoid content was determined by plotting quercetin as standard curve (Figure 3).

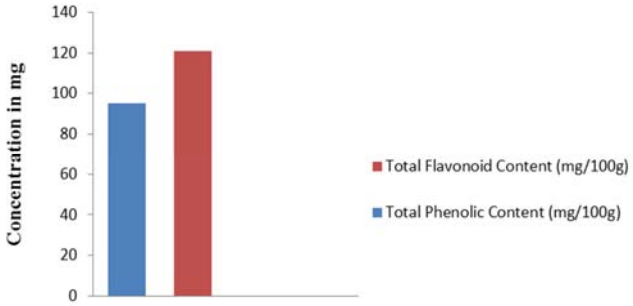

Figure 1. *Total phenolic and flavonoid content of V. negundo* L. *leaves.*

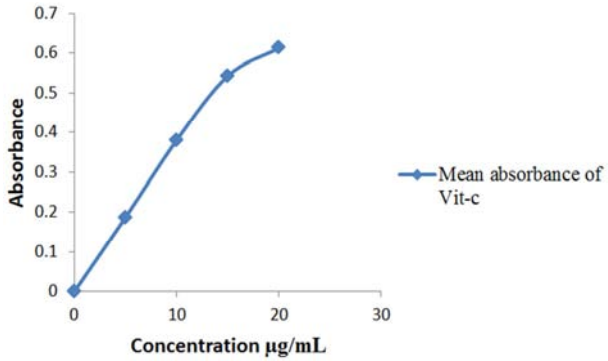

Figure 2. *Standard curve of Ascorbic acid for the determination of total phenolic content.*

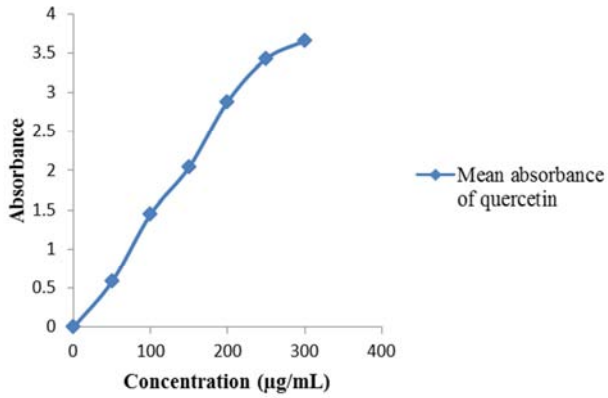

Figure 3. *Standard curve of quercetin for the determination of total flavonoids compounds.*

Hypoglycemic Effect of Vitex negundo L. Leaves

Table 1 shows that the animal blood sugar level was increased in diabetic untreated mice as compared to non-diabetic normal mice. After the oral administration of ethanol extract of Vitex negundo L. leaves, the blood sugar level return to normal as compared to diabetic mice.

Table 1. *Effects of the leaves extract of V. nigundo* L. *leaves on serum glucose in experimental mice.*

Groups (treatmentanddoses)	Serumglucoselevel (mmolL^{-1})			
	Initialday	7day	14day	21day
I (non-diabetic)	5.44±0.568	5.51±0.172	5.63±0.27	5.59±0.419
II (diabetic)	19.23±0.635*	20.61±0.475*	22.38±0.724*	24.87±1.005*
III (extract, 200mgkg^{-1}b.w.)	20.32±0.502^{a**}	18.55±1.103**	16.19±1.13**	14.92±0.738*
IV (extract, 300mgkg^{-1}b.w.)	20.28±0.741**	17.740.249^{a**}	15.81±0.31**	13.45±0.856**
V (glibenclamide, 0.5mgkg^{-1}b.w.)	21.85±0.879**	17.15±0.776**	12.33±0.785**	9.65±0.665**

Blood glucose level in the treated mice were significantly different from nondiabetic and diabetic groups at P<0.001; a: P<0.05, *indicated the difference from nondiabetic group; whereas **indicated the difference from diabetic group.

Comparing the blood sugar level in streptozotocin induced diabetic mice, ethanol extract administered subject showed significant reduction of blood glucose level at $P<0.001$, (Table 1). In 7th to 21st days, plant extract at the dose of 200 mg/kg b.w. administration group's glucose levels maintained 9.99% - 40.01% lower than the diabetic control group. In the 7th, 14th and 21st days of experiment with 300 mg/kg b.w. glucose level was decreased by 13.93%, 29.36%, and 45.91% respectively whereas by supplementing glibenclamide it was 16.77%, 44.91%, 61.19% in those respective days compared to diabetic group.

Changes in Body Weight of Mice

Table 2 shows that the body weight was decreased in diabetic untreated mice as compared to normal mice. After the oral administration of ethanol extract of *Vitex negundo*L. leaves the body weight was elevated. In case of 300 mg/kg b.w., the increase in body weight was 10.51%, which was significant ($P<0.05$) than the 200 mg/kg b.w. treatment in which case body weight increased 7.55% ($P<0.005$). Increase of body weight by 300 mg/kg b.w. was as near as the glibenclamide treatment (12.01%, $P<0.001$) (Table 2).

Table 2. *Body weight Effects of the ethyl alcohol leaves extract of V. nigundo* L. *on mice body weight.*

Groups (treatment and doses)	Body weight (g)			
	Initial day	7day	14day	21day
I (non-diabetic)	26.66±0.586	27.82±0.302	28.95±0.188	29.61±0.607
II (diabetic)	26.13±0.435	25.42±0.589^{a*}	24.57±0.282^{a*}	23.65±0.749
III (extract, 200mgkg^{-1}b.w.)	25.89±0.756	25.90±0.316^{a**}	26.33±0.643^{a**}	26.98±0.673
IV (extract, 300mgkg^{-1}b.w.)	26.08±0.608	26.51±0.701^{b**}	27.05±0.235^{a**}	27.65±1.222
V (glibenclamide, 0.5mgkg^{-1}b.w.)	26.34±0.514	26.68±0.513^{a**}	27.53±0.573^{a**}	28.15±0.648

Body weight in the treated mice were significantly different from nondiabetic and diabetic groups at a: P<0.001; b: P<0.05* indicated the difference from nondiabetic group; whereas** indicated the difference from diabetic group

Lipid profile

Table 3 shows that serum total cholesterol, triglyceride, and LDL-cholesterol levels were significantly increased and the HDL -cholesterol level decreased in diabetic untreated mice as compared to normal mice. After the oral administration of ethanol extract of Vitex negundo leaves the elevated serum total cholesterol, triglyceride, and LDL-cholesterol levels were decreased and the HDL -cholesterol level was increased. Table 3 represented the changes of lipid profile by the administration of Vitex negundo leaves extract at the dose of 200 mg/kg b.w., 300 mg/kg b.w. and glibenclamide in streptozotocin induced diabetic mice. Cholesterol (P<0.001) level was observed to reduce 7.81% and 9.52% for the treatment of leaves extract at the dose of 200 mg/kg b.w. and 300 mg/kg b.w. respectively in diabetic mice whereas in case of glibenclamide, it was 12.69% (P<0.001). LDL level was reduced for 200mg/kg b.w., 300 mg/kg b.w. and glibenclamide treatment at 7.04% (P<0.01), 8.84% (P<0.001) and 11.22% (P<0.001) whereas TG level was increased significantly (P<0.05) at 10.23% (P<0.05), 15.37% (P<0.001) and 22.39% (P<0.001) respectively. A little bit different scenario was observed for HDL level. Glibenclamide reduced the HDL level more significantly (24.23%, P<0.01) than 200 mg/kg b.w. (6.61%, P<0.05) and 300 mg/kg b.w. (13.66%, P<0.05).

Table 3. *Effects of the ethyl alcohol extract of V. nigundo* L. *leaves on serum total cholesterol, triglyceride, and HDL - and LDL – cholesterol levels in experimental mice.*

Groups (treatment and doses)	Total cholesterol (mmolL^{-1})	Triglyceride (mmolL^{-1})	HDL-cholesterol (mmolL^{-1})	LDL-cholesterol (mmolL^{-1})
I (non-diabetic)	5.545±0.114	1.406±0.093	0.303±0.0175	4.613±0.143
II (diabetic)	6.557±0.154^{a*}	1.965±0.123^{a*}	0.227±0.027^{a*}	5.427±0.125^{a*}
III (extract, 200mgkg^{-1}b.w.)	6.045±0.167	1.768±0.108^{b**}	0.242±0.023	5.045±0.156^{c**}
IV (extract, 300mgkg^{-1}b.w.)	5.933±0.156^{a**}	1.663±0.019^{a**}	0.258±0.017	4.947±0.151^{a**}
V (glibenclamide, 0.5mgkg^{-1}b.w.)	5.725±0.149^{a**}	1.525±0.103^{a**}	0.282±0.018^{c**}	4.818±0.169^{a**}

Serum Cholesterol, Triglycerides, HDL and LDL in the treated mice were significantly different from nondiabetic and diabetic groups at a: P<0.001; b: P<0.05; c: P<0.01 *indicated the difference from nondiabetic group; whereas **indicated the difference from diabetic group.

SGPT and SGOT Level

Table 4 shows that the enzyme SGPT and SGOT was increased in diabetic untreated mice as compared to normal mice. After the oral administration of ethanol extract of *Vitex negundo* L. leaves the body weight was decreased.

There was a significant increase of SGPT and SGOT level after diabetes induction which was compensated by leaves extract of 200 mg/kg b.w. and 300 mg/kg b.w. The reduction of SGPT by leaves extract of 300 mg/kg b.w. and 200 mg/kg b.w. was 14.65% (P<0.01) and 17.14% (P<0.001) respectively whereas 24.28% (P<0.001) for glibenclamide. The reduction of SGOT level was highly significant for 300 mg/kg b.w. (33.19%, P<0.001) than 200mg/kg b.w. treatment in which case SGOT increased 29.79% (P<0.001). Increase

of SGOT by 300 mg/kg b.w. was as near as the glibenclamide treatment (42.39%, P<0.001) (Table 4).

Table 4. *Effects of the extract of V. nigundo L. leaves on enzyme level in experimental mice.*

Groups (treatment and doses)	SGPT (U/L)	SGOT (U/L)
I (non-diabetic)	52.83±2.48	41.33±2.8
II (diabetic)	86.5±6.09*	83.33±2.58*
III (extract, 200mgkg^{-1})	73.833±4.622^{a**}	58.5±5.468**
IV (extract, 300mgkg^{-1})	71.667±4.926**	55.667±5.68**
V (glibenclamide, 0.5mgkg^{-1})	65.5±4.59^{b**}	48.0±5.44**

Serum SGPT and SGOT in the treated mice were significantly different from nondiabetic and diabetic groups at P<0.001; a: P<0.01 *indicated the difference from non diabetic group; whereas ** indicated the difference from diabetic group.

4. Discussion

In the present study, diabetes was induced in mice by injecting streptozotocin (65 mg/kg) intraperitoneally. Streptozotocin causes diabetes mellitus in a wide variety of animal species by damaging insulin secreting β-cell, resulting in a decrease in endogenous insulin release, which paves the way for the decreased utilization of glucose by the tissues [18]. Hypercholesterolemia and hypertriglyceridemia are common complications of diabetes mellitus in addition to hyperglycemia [19]. This work has done to evaluate the effects and comparison of ethanolic extract of *Vitex negundo* Linn. leaves extract at the dose of 200 mg/kg b.w. and 300 mg/kg b.w. on body weight, blood glucose, serum enzymes (SGPT, SGOT), serum total cholesterol (TC), serum triglyceride (TG), serum HDL and LDL in STZ-induced diabetic mice.

To determine whether there was a statistically significant difference in hyperglycemia achieved by the extracts on the 21st day, student's t-test was applied and compared with the control group. A significant reduction in blood glucose of 14.92 mmol/L, 13.45 mmol/L (P<0.001) and 10.65 mmol/L (P<0.01) was observed after 21 days of treatment with the ethanol extracts of *Vitex negundo* Linn. leaves (200 and 300 mg/kg b.w.) and glibenclamide (0.05 mg/kg b.w.) respectively. Body weight is also increase in 26.99 gm (P>0.05), 27.66 gm (P<0.05) and 29.153 gm (P<0.05) was observed after 21 days of treatment with the ethanol extracts of *Vitex negundo* Linn. Leaves (200 and 300 mg/kg b.w.) and glibenclamide (0.05 mg/kg b.w.) respectively.

These results have shown that the *Vitex negundo* Linn. leaves extract possess blood glucose lowering effect in STZ-induced hyperglycemic mice. Thus, the folk use of *Vitex negundo* Linn. leaves extract for the control of diabetes may be validated by this study. A previous study reported that the efficacy of *Vitex negundo* Linn. leaves in the treatment of diabetes [14]. The leaves extract of *Vitex negundo* Linn. was also useful in reducing the serum lipid profile (Total cholesterol, TG, HDL). Our findings were consistent with the result of Islam et al and Kanti et al. [20, 21] their results confirmed hypoglycemic and hypolipidemic activity of plant extract in diabetic mice. The level of HDL-C increased and LDL-C level decreased significantly after administration of leave extract is an indication of the reduction of the risk of coronary heart disease and this might be due to the presence of polyphenol in *Vitex negundo* Linn. leaves extract [21, 22] Serum enzymes, SGPT and SGOT levels were increased after the administration of leave extract. This finding is similar to the finding reported by Islam et al [20]. It is also observed from the study that *Vitex negundo* Linn. leaves extract (200 mg/kg b.w.) is more effective than *Vitex negundo* Linn. leaves extract (300 mg/kg b.w.) for the control of diabetes in comparison with standard.

5. Conclusion

From the studies it was found that crude ethanol extracts of *Vitex negundo* leaves showed significant hypoglycemic and hypolipidemic activity against streptozotocin induced diabetic mice. This study revealed that *Vitex negundo* leaves contain secondary metabolites like phenols and flavonoids and those have antioxidant actions. In the control of diabetes and its complications they have beneficial effects. The plant extract also demonstrated significant lowering effects on SGPT and SGOT enzymes in liver function in diabetic rats. In conclusion, the *Vitex negundo* leaves extract as substitute for synthetic drugs in the treatment of diabetes.

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