Research Article

Investigations for antidiabetic effect of hydro alcoholic extract of *Vitex negundo* stem

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Abstract

Background: Diabetes mellitus mainly occurs due to deficiency or absence of insulin or rarely due to impairment of insulin activity causing varying degree of disruption of carbohydrate and fat metabolisum. *Vitex negundo* belonging to family Lamiaceae traditionally, used for treatment of muscle relaxant, pain reliving, anti-inflammatory, analgesic, and antiparasitic. **Objective:** This study will perform to investigate the hypoglycemic effect of stem extract of *Vitex negundo*. **Material and methods:** Hydro-alcoholic extraction of stem vitex negundo was prepared by soxhlet extraction method and futher subjected to acute oral toxicity animal received dose of 2000 mg/kg body weight, animals were observed individually for toxicity sign such as convulsion, depression mortality for 24hrs.Rat shall administer with alloxan monohydrate(120mg/kg)dissolved in sterile normal saline. When animal glucose level reach up to 200-260mg/100ml shall selected for experiment respectively. Selectively serum glucose, serum lipid and cholesterol level was determined and animal were divided into four groups. Group I (Test group) treated with stem extract (350 mg/kg)test drug, Group II(Positive control group)serve as diabetic control and were given only distilled water daily, Group III(Standard group) treated with glibenclamide (5 mg/kg) standard drug , Group IV (normal control) only 0.5% CMC solution given daily. **Results:** Extract of *Vitex negundo* (stem) also significantly reduces blood glucose level in Alloxan induced diabetic rats and compare with standard glibenclamide. Histopathology study shows that normal control have minute edema, group second have pronounced edema. **Conclusion:** Thus, it can be concluded from above that *Vitex negundo* stem extract is found to be efficacious in reducing BGL and lipid levels.

Keywords: Diabetes mellitus, *Vitex negundo*, Alloxan, Glibenclamide, karyolysis, hypoglycemic

Introduction

Diabetes mellitus is due to deficiency or absence of insulin or rarely, to impairment of insulin activity (insulin resistance) causing varying degrees of disruption of carbohydrate and fat metabolisum (Waugh and Grant, 2010). *Vitex negundo* Linn belonging to family verbenaceae. It is commonly known as Nirgundi. It is an erect shrub or small tree growing from 2 to 8cm (6.6to26.2ft) in height. Leaves palmately compound with 3-5 foliolate, leaflet lanceolate elliptic or ovate lanceolate, dark green sparsely pubucent above paler grayish pubescent beneath, lateral veins10-18 on either side of the mid rib, petiole stout, slender sometimes dischotomously or trichotomously branched about 10-30 cm long. Stem and bark occur in pieces 0.3 to 0.5 cm thick outer surface yellowish grey-rough lenticelular longitudinally channeled and transversely cracked ,inner surface is darker than outer, fracture short, taste is slightly bitter (Ladda and Magdun, 2012). Fruit is obovoid or sub-globose about 3-5 mm in diameter green glabrous black when ripe. It is commonly distributed throughout India, fairly common in wasteland on roadside, moist place near deciduous forests (Bharti et al., 2017). *Vitex negundo* has a long history of use in Indian traditional medicine particularly treatment muscle relaxant, pain reliving, antiasthma, anti-anxiety .The leaves are good for anti-inflammatory analgesic, anti-histaminic, property, snake venom whole plant is also used for antigastalgic, antiparasitic, analgesic (Ahuja et al., 2015). The plant has antifungal, anti-inflammatory, anti-bacterial and analgesic activities. According to the present investigation is an attempt to assess the bio activity of hydro-alcoholic extract of *vitex negundo* stem in experiment diabetes in rats.

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Materials and methods

Nirgundi stems (*Vitex negundo* L.) were collected from medicinal garden of MFP-PARC barked pathani Bhopal. The plant has been identified and authentication by Dr. Zia UL Hasan, Head of the botany department at Safia college of

science, Bhopal.

Standard Drug: Glibenclamide, Animals Species: Wistar albino rats (either sex) of weight 120 to 150 g.

Instruments: Soxhlet apparatus (KHERA), High precision balance (WENSAR/PG B200), Sonicator (REMI), Hot air oven (KHERA), Incubator (KHERA), Microcentrifuge (RM-12CDXMODEL), UV- Spectroscopy (Systronic double beam).

Plant stems were first cutted in small pieces initially then shade dried. Dried stem is powder by using mechanical grinder. Extraction of stem *Vitex negundo* was done by soxhlet extraction method. The 500 g of coarsely powdered stem were extracted with hydroalcoholic solvent for 72 hours at 70^{0}C and powder was dried in hot air oven at 50^{0}C. Extract was dried at room temperature and collected for the further use (Tiwari et al., 2011).

Preparation of extract formulation

A suspension formulation of hydro-alcoholic extract of *Vitex negundo* stem in 1.0% Carboxy Methyl Cellulose solution was prepared for further *in-vivo* pharmacological study

Acute oral toxicity study

Acute oral toxicity study was evaluated as per OECD guidelines (423) on Wistar albino rats. Animals were provided by Sapience Bio analytical Research Lab, Bhopal (M.P.) and experiment was done in the lab. Before experimentation rats were fasted overnight with water and libitum. Three animals were selected which receives dose of 2000mg/kg. All three animals were received dose of 2000 mg/kg body weight of hydro-alcoholic extract of *Vitex negundo* Linn stem extract by gavage using oral cannula (limit test).Animals were observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality after dosing for 24 hours, with special attention given during the first 4 hours, and thereafter, 24 hours, Administered dose was found tolerable (as no death found). Therefore, two dose levels 250 mg/kg & 350 mg/kg was selected for anti-diabetic activity (Roll et al., 1986).

Blood sample collection

At the end of study, Blood was obtained from all animal by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min. at room temperature for serum analysis. Serum was separated by centrifugation at 3000 rpm at 4˚C for 15 min. and utilized for the estimation of various bio-chemical parameters namely Serum Glucose, LDL, HDL, Cholesterol, & Triglyceride.

Alloxan induced diabetes

The rat shall administer with Alloxan monohydrate dissolved in sterile normal saline at the dose of 120mg/kg body weight i.p. (intra-peritoneally). Since Alloxan could evoke fatal hypoglycemic as a result of massive insulin release, rat shall be treated with 15 ml 20% glucose solution ip, 6h after Alloxan treatment. The rats shall then keep for next 24h with free access to 5% glucose solution to prevent hypoglycemia. After a fortnight, rat with moderate diabetes having glucosuria (indicated by benedicts test for urine) and hyperglycemia (blood glucose range of 200-260 mg/100ml) shall be selected for the experiment (Rohilla and Ali, 2012).

Determination of serum glucose level

To determine the effect of *Vitex negundo* on serum glucose level three group of animal shall be used. They shall over-night normal fasted rats (group I), normal fed rats (group II) and Alloxan induced rats (group III) .Immediately after the collection of blood samples the extract shall administer (P.O.). One hr after extract administration blood samples shall again collect, serum shall separate from sample and glucose level shall determine.

Total Serum lipid and cholesterol

For estimation of total serum lipid and cholesterol profile, serum shall isolated from blood which shall collect on the last day of treatment and serum total cholesterol (TC),Triglyceride (TG) and high density lipoprotein (HDL). LDL shall estimate by using respective diagnostic kit.

Determination of High Density Lipid (HDL) in serum

For precipitation sample and HDL reagent mix well, allow standing for 10 min at 4000 rpm after centrifugation separate the clear supernatant from the precipitate within 1hour and determining the HDL cholesterol concentration using the cholesterol reagent.mix and incubate for 5 min at 37^{0}c. Measure the absorbance of the standard and sample against the reagent blank.

$$\text{HDL Cholesterol conc. in mg/dl} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \text{X concentration of standard} \times 2$$

Determination of triglycerides

Mix the reagent and incubate for 10 min at 37^{0}c. Absorbance of standard and each test sample was determine at 505nm (500-540 nm) on bichromatic analysers against reagent blank

$$\text{Triglycerides (mg/dl)} = \frac{Absorbance of test}{Absorbance of standard} \times \text{Concentration of standard (mg/dl)}$$

Group I (Test Group): Six rats which alloxan induced diabetic rats treated orally with stem extract (350 mg/kg) test drug.

Group II (Positive Control Group): Six alloxan induced

diabetic rats and serve as diabetic control and were given only distilled water daily (120 mg/kg).

Group III (Standard Group): Six animals induced diabetic rats and shall be treated with Glibenclamide (GBC) at the dose of 5 mg/kg body weight once in a day.

Group IV (Normal Control): Six rats which serve as normal control and were given only 0.5% CMC solution daily.

After 21 days of treatment experiment shall terminated and observation made

Histopathology of Pancreas

On last day of the study the pancreas shall isolated and preserved in 10% formaline. Histopathology observation of tissues carries out at laboratory.

Results

Acute toxicity study

All extract-treated rats showed no discernible behavioral changes up to 2000 mg/kg by oral route. No mortality was observed at this dose during 72 h observation period and no significant body weight changes were observed during day's toxicity study.

Test Control Group: In 0 day blood glucose level is 100%.after administration of plant extract *Vitex negundo* (stem) on 7thday it reduce blood glucose level 13%respectively on 14 and 21 day blood glucose level reduces to 55.9% and 49.44%.

Positive Control Group: In 0 day blood glucose level is 100%.there is no administration of plant extract or standard drug. rats itself through its immunity power decreases blood glucose on 7thday it reduce blood glucose level 6.86%respectively on 14 and 21 day blood glucose level reduces to 20.17% and 32.6%.

Std. Control Group: In 0 day blood glucose level is 100%.after administration of standard drug Glibenclamide .on 7thday it reduce blood glucose level 7.8%respectively on 14 and 21 day blood glucose level reduces to 20.75% and 47.9%.

Table 1. Effect of hydro-alcoholic extract of *Vitex negundo* stems on blood glucose level

Groups	Blood Glucose conc. (mg/dl) Mean± SEM			
	0 day	7 days	14 days	21 days
Test Control	267.33±8.48***	234.5±8.88**	188.83±3.64**	135.5±10.32**
Positive Control	270.33±9.60	249±7.38	214±5.91**	182±4.73
Std. Control	248.5±11.42***	231.16±7.19***	198.83±12.19	129.16±2.79**
Normal Control	119.66±3.127**	120.5±3.528	119±3.587**	116.84±4.438**

Results are expressed as Mean±SEM (n=06)

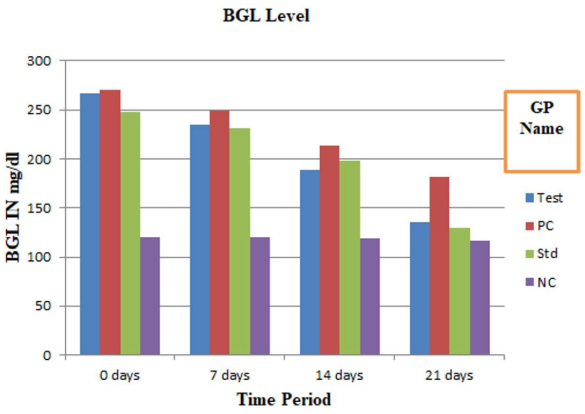

Figure 1. Effect of hydro-alcoholic extract of *Vitex negundo* stems on blood glucose level

Table 2. Effect of hydro-alcoholic extract of *Vitex negundo* stems on Lipid Parameters

Groups	Lipid Parameters Mean± SEM			
	Cholesterol (Mg/dl)	Triglyceride (Mg/dl)	HDL (Mg/dl)	LDL (Mg/dl)
Test Control	86±2.81**	125±3.58*	80±3.62**	54±4.28
Positive Control	152.16±6.76	171.5±7.37**	37.83±2.08	83.5±3.49
Std. Control	81±2.17**	115±6.67	83±3.44*	50.83±4.30*
Normal Control	86.5±3.30	101.84±5.21	57.84±3.88	69±6.37

Results are expressed as Mean±SEM (n=06)

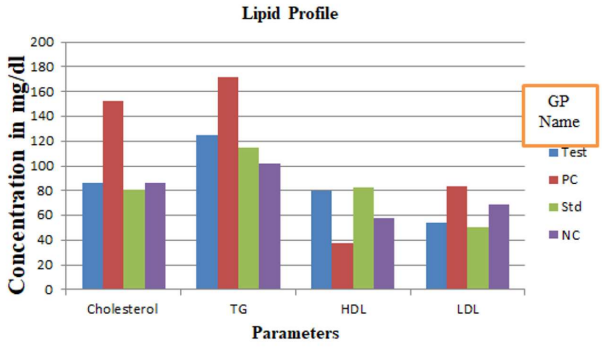

Figure 2. Effect of hydro-alcoholic extract of *Vitex negundo* stems on Lipid profile

Histopathological observations of Pancreas

Normal Control have min. Edema, Group II have pronounced edema. Alloxan caused severe necrotic changes of pancreatic islets, especially in the centre of islets. Nuclear changes, karyolysis, and severe reduction of beta cells were observed in diabetic control rats. The Pancreas of different groups was stained with hematoxylin –eosin mixture.

Pancreatic tissues from all groups were subjected to histopathological studies-The whole pancreas from each animal was removed after sacrificing the animal under

anesthesia, collected in 10% formalin solution and immediately processed by the paraffin technique. Sections of 5 µm thickness were cut and stained with hematoxylin and eosin (H and E) for histological examinations. Results are showing in figure.

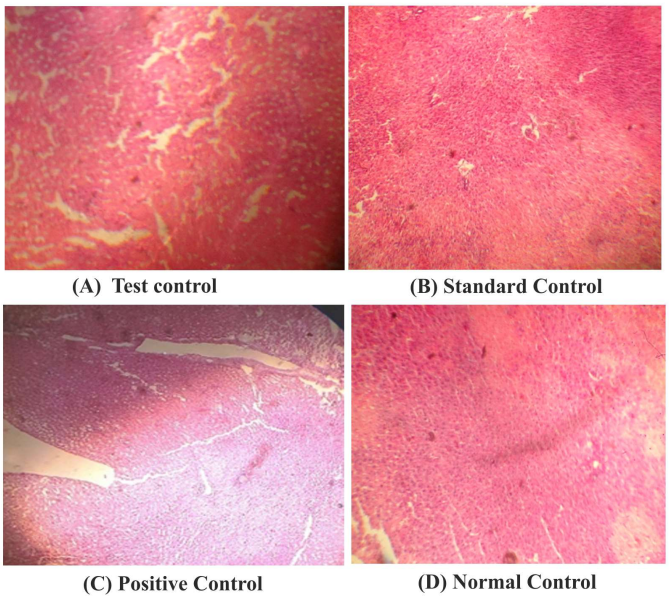

Figure. 3 Histopathological observations of pancreas after treatment with hydroalcoholic extract of *Vitex negundo* stems

Discussion

After the extraction, pharmacognostical evaluation was done. Extract was subjected to various chemical tests for preliminary identification of various phytoconstituents. The extract contains carbohydrates, alkaloids, flavonoids, glycosides, etc.

This study was undertaken; mainly to assess the protective effect of hydro alcoholic extract of stem of *Vitex negundo* against Alloxan induced Diabetes in experimental rats at dose dependent manner. Alloxan is selectively toxic to pancreatic β cells that produce insulin due to the accumulation of alloxan through the GLUT2 transporter. Though, Alloxan by itself is not toxic, but once it is infiltrated to the pancreatic b-cells through the GLUT2 transporter, Alloxan is reduced to dialuric acid in the presence of different cellular reducing agents.

The animals showing blood glucose range of greater than 200 mg dL-1 were used for the main study; the hyperglycemia was confirmed after 72 hours of Alloxan monohydrate administration (i.p.). It has been found that oral administration of test extracts at defined dose of 350 mg/kg b.w. caused a more significant potent reduction in blood glucose than other compounds in diabetic rats. However, extract at dose of 350mg/kg showed minimum glucose lowering effects as compared with 250mg/kg. This study reveals significant result of test groups, when compared with positive control (Alloxan 120 mg/kg) i.p. and standard Glibenclamide 5 mg/kg (p.o.).

Conclusion

The hydro-alcoholic extract of *vitex negundo* stem at a dose of 350 mg/kg b.w. had profound anti-diabetic effects on either sex of Alloxan induced diabetic rats. Percentage reduction in blood glucose levels of at end of final study of Groups Test control, Positive Control , and Std. Control was found to be 49.44 %, 32.6%, & 47.9 %, respectively, that is considered as remarkable and significant when compared with positive control diabetic as well as standard group.

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Conflicts of interest

The authors have no conflicts of interest.

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