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Original Article

Evaluation of Antihyperglycemic and Antihyperlipidemic Activity of Leaf Extracts of *Breynia vitis-idaea* in Alloxan Induced Diabetic Rats

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ABSTRACT

Aim: The present study was carried out to evaluate the hypoglycemic and hypolipidemic activity of alcohol and aqueous leaf extracts of *Breynia vitis-idaea* (Burm. F.) Fischer in alloxan induced diabetic rats. **Methods:** Diabetes was induced into albino Wistar rats by intraperitonial administration of alloxan (120 mg/kg). Normal and diabetic rats divided into different groups of six each. Alcohol and aqueous leaf extracts (300 mg/kg and 600 mg/kg) and standard drug (Glibenclamide 500 µg/kg) was administered orally for 21 days. Blood samples were collected from overnight fasted normal and diabetic rats on 0th, 7th, 14th and 21st days of treatment. Hypoglycemic activity was evaluated by measuring serum glucose level and glycosylated haemoglobin level after dosing with aqueous and alcohol extracts. Hypolipidemic activity was evaluated by measuring various biochemical parameters like total cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein, high density lipoprotein and phospholipids. **Results:** Both the extracts significantly ($P<0.001$, $p<0.01$) reduced fasting blood glucose of alloxan diabetic rats in a dose-related manner, when compared to control and standard. They also have a significant recovery in the levels of parameters measured in lipid profile, when compared to control and standard group. **Conclusion:** The present investigation established pharmacological evidence to support the folklore claim that it is used as hypoglycemic and hypolipidemic agent.

Key words: Alloxan, *Breynia vitis-idaea,* Glibenclamide, Hypoglycemic, Hypolipidemic.

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INTRODUCTION

Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia, lipoprotein abnormalities, raised basal metabolic rate, defect in reactive oxygen species scavenging enzymes and altered intermediary metabolism of major food substances. Diabetes is a major degenerative disease having complications which include hypertension, atherosclerosis and microcirculatory disorders.[1] The increasing incidence of the disease worldwide may be due to sedentary life style, unhealthy diet, obesity and other predisposing risk factors.[2] It is projected to become of the world's main disablers and killers, as the number of people with diabetes multiplies worldwide. The disease has taken an ever increasing share of national and international healthcare budgets.[3]

The World Health Organization has also recommended the evaluation of the effective use of plants, because of the modern drugs are not safe. The synthetic hypoglycemic agents used in clinical practices have serious side effects like haematological effects, coma, disturbances of liver and kidney.[4] It is widely accepted that the most challenging goal in the management of diabetes is to achieve blood glucose level as close to normal as possible. However, in the indigenous system of medicine good numbers of plants are mentioned for the cure of diabetes and some of them have been experimentally evaluated and active principles were isolated. In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant material for their potential medicinal value.[5]

Many medicinal plants used in ethnomedical practices in India are known or little known to scientific world. The main objective of the study was to assess the hypoglycemic and hypolipidemic potential of leaves of Coral berry tree *Breynia vitis-idaea* belonging to the family Euphorbiaceae. It is known as "Surasaruni" in Hindi.[6]

Breynia vitis-idaea (Burm.f.) is an evergreen 1.5-5 m tall glabrous tree or large erect shrub with horizontal branches found in the Gangetic plain, Western Peninsula, China, Malay Peninsula and Sri Lanka. These plants are planted as ornamental hedge in garden. Leaves are 1-3 cm long, elliptic to elliptic-ovate, alternate dark brown or black when dry. Bark is yellowish grey, flowers are small, greenish yellow or pink. The fruits are fleshy, pink to red which turns black when ripe and measures 2-3 mm in diameter. The seeds are black and have a very hard seed coat. Root contains β-sitosterol. Leaves contain triacontane, ceryl alcohol, lanosterol, pentatriacontanoic acid. A new sulphur containing spiroketal glycoside, breynin I and a new terpenic glycoside, breyniaionoside E together with 10 known compounds were isolated from the aerial parts of *Breynia vitis-idaea.* A decoction of the roots is employed as mouthwash for toothache. Leaves applied as poultice to hasten suppuration. Leaf juice given after parturition to prevent haemorrhage. Dried leaves smoked like tobacco to relief in tonsilitis. Astringent bark used to guard against haemorrhage.[7-9] Aqueous, ethanol extracts of plant proves the anti-cancer action in HEPG2 cell lines against carbon tetra chloride induced toxicity in cell line.[10] Leaves warmed along with leaves of *Dodonaea viscosa*, *Dalbergia paniculata* and applied for 2-3 days regularly to cure swelling of legs and testis.[11] In the current literature, there is not much data concerning the effect of *Breynia vitis-idaea* on the blood glucose level and parameters used in lipid profile. Therefore, the present study has been planned to investigate the effect of extracts in alloxan induced diabetic rats and to compare it with diabetic untreated and glibenclamide as a reference standard.

MATERIALS AND METHODS

Drugs and chemicals

The following drugs and chemicals were used in the experiment: Alloxan monohydrate was purchased from Central Drug house, New Delhi, India and Glibenclamide was purchased from Bal Pharma, Bangalore, India. Diagnostic kits of Glycosylated haemoglobin was purchased from Coral Clinical System, Goa, India and diagnostic kits of cholesterol, phospholipids, triglycerides, HDL, VLDL and LDL were purchased from Span diagnostics Ltd., Surat, India and rest all other reagents and chemicals were of analytical grade.

Plant material

The leaves of *Breynia vitis-idaea* were collected from the vicinity of Tirunnelveli (Tamil Nadu, India). Taxonomic identification was carried out by V. Chelladurai, Research Officer-Botany (Retired scientist-CCRAS). A voucher specimen (JC Nagar) was deposited in the herbarium of the department of Pharmacognosy in the college for future reference.

The collected leaves were washed thoroughly in tap water to remove any unwanted matter and then dried under shade for one week. After complete drying, leaves were pulverized into coarse powder. The powder stored in airtight container in cool and dark place to prevent deterioration by elevated temperature, light and moisture.

Preparation of crude extracts

Coarsely powdered, shade dried leaves of *Breynia vitis-idaea* was charged into a soxhlet apparatus and successive hot extraction was carried out using ethanol (70% v/v) for 24 h. The

liquid extract was concentrated in rotary flash evaporator at a temperature not exceeding 50°C (yield 12.65% w/w). The alcohol extract was formulated as a suspension in distilled water using 2% v/v Tween-80 as suspending agent for animal studies.

The aqueous extract was prepared by maceration method. The coarsely powder of leaves kept with chloroform water for 24 h. The macerate was filtered and filtrate concentrated in rotary flash evaporator (yield 15.3% w/w). Aqueous extract was formulated by dissolving in distilled water for animal studies. The extracts were preserved in desiccators for further experiments.

Animals used

Swiss albino mice weighing 20-30 g and albino rats (Wistar strain) weighing 170 ± 10 g of either sex were used for the study. The animals were procured and housed in the animal house at least 2 weeks prior to the study, for acclimatization. Animal house was well maintained under standard hygienic conditions, at a temperature (15-20 ± 5°C), room humidity (60% ± 10%) with 12 h day and night cycle with food and water *ad libitum*. All the pharmacological experiments were as per CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) norms after obtaining approval of the Institutional Animal Ethics Committee (Reg. No. 870/ac/08/CPCSEA).

Acute toxicity studies

These studies were carried out to study the acute toxic effects and determine minimum lethal dose of the drug extracts. Swiss albino mice of either sex weighing between 20-30 g fasted overnight, were used for the study. Each extract was orally administered at doses of 30, 100, 300, 1000 and 3000 mg/kg body weight to separate groups of mice. Subsequent to administration of drug extracts, the animals were observed closely for the first three hours, for any toxic manifestations, like increased motor activity, salivation, clonic convulsions, coma and death. Subsequently observations were made at regular intervals for 24 h. The animals were under further investigation up to a period of 1 week.[12]

Induction of diabetes mellitus

In the present work alloxan monohydrate was used to induce hyperglycemia in animals at the dose of 120 mg/kg body weight by intraperitonial injection.[13] The fasting blood glucose levels were determined after 72 h of alloxan administration. Rats having blood glucose level above 200 mg/dl were selected for the study. Diabetic rats were divided in six groups; each group comprised of six rats.[14]

Group 1-Normal control.

Group 2–Positive control-Untreated alloxan diabetic rats.

Group 3–Standard-Alloxan diabetic rats treated with glibenclamide (500 µg/kg, p.o.)

Group 4-Alloxan diabetic rats treated with aqueous extract (300 mg/kg, p.o)

Group 5-Alloxan diabetic rats treated with aqueous extract (600 mg/kg, p.o)

Group 6-Alloxan diabetic rats treated with alcohol extract (300 mg/kg, p.o)

Group 7-Alloxan diabetic rats treated with alcohol extract (600 mg/kg, p.o)

Doses of aqueous extract, alcohol extract, standard drug and normal saline were calculated according to the body weight of each animal. Suspension of extracts, standard drug and normal saline were administered orally to each animal using stainless steel feeding needle fitted on a plastic syringe. The treatment schedule was once daily for 21 days and animals were fed on laboratory diet of pellet chow and water *ad libitum*. They were fasted for 18 h prior to blood withdrawal.

Determination of hypoglycemic activity

Blood samples were collected by orbital sinus puncture under mild ether anaesthesia. Serum was separated by centrifuging blood at 6000 rpm for 15 min. Serum glucose estimation was performed on 0^{th}, 7^{th}, 14^{th} and 21^{st} day by end point method using Autochem Nexgen semi autoanalyzer (Span diagnostics, Surat, India) with the help of glucometer (Glucochek, Surat, India). On 21^{st} day estimation of glycosylated hemoglobin was also performed using UV- visible spectrophotometer (Systronic 2203) with the help of Glycohemoglobin reagent kit.

Determination of hypolipidemic activity

Blood samples were collected by orbital sinus puncture under mild ether anaesthesia on 21^{st} day from the start of treatment. Serum was separated and analyzed for various biochemical parameters–Cholesterol, Triglycerides, HDL, LDL, VLDL and Phospholipids by using various kits.

Statistical analysis

The data obtained were statistically analyzed by one way analysis of variance (ANOVA) and expressed as mean ± S.E.M. followed by Tukey Kramer Multiple Comparison Test using instat software.

RESULTS

Acute toxicity studies

Acute toxicity study revealed the nontoxic nature for both the extracts. There was no mortality and no toxic reactions found at any of the doses tested until the end of the study period. As per OECD guidelines, therapeutic range was considered between 1/10 to 1/5 times of LD_{50}. Accordingly, 300 mg/kg and 600 mg/kg BW doses for both the extracts were selected for determination of pharmacological studies.

Hypoglycemic activity

Hypoglycemic activity of aqueous and alcohol extracts of *Breynia vitis-idaea* were evaluated in alloxan induced diabetic rats. Administration

of alloxan increases the serum glucose level in normal rats. The effects of extracts and glibenclamide on serum glucose level in diabetic rats are depicted in Table 1. The fall in serum glucose levels of the extracts and glibenclamide treated groups were compared with that of positive control (diabetic untreated) group. Both aqueous and alcohol extracts showed significant hypoglycemic effect in comparison with positive control group on 7th day itself. The continuous treatment for three weeks leads to a dose dependent fall in serum glucose level. The dose of 600 mg of both the extracts decreases the serum glucose level towards normal level. The concentrations of serum glycosylated haemoglobin level in diabetic rats are depicted in Table 2. The concentration of serum glycosylated haemoglobin level also found significant when compared to positive control group.

Administration of 300 mg/kg of aqueous extract showed 13.61%, 41.94%, 60.89% decline in glucose levels of experimental animals on day 7th, 14th, 21st respectively. The glycosylated haemoglobin level was found 52.25% less, when compared to diabetic control group. Administration of 600 mg/kg of aqueous extract showed 20.04%, 47.83%, 66.62% decline in glucose levels of experimental animals on day 7th, 14th, 21st respectively. The glycosylated haemoglobin level was found 59.25% less, when compared to diabetic control group. Administration of 300 mg/kg of alcohol extract showed 17.47%, 44.37%, 63.37% decline in glucose levels of experimental animals on day 7th, 14th, 21st respectively. The glycosylated haemoglobin level was found 52.75% less, when compared to diabetic control group. Administration of 600 mg/kg of alcohol extract showed 19.34%, 45.44%, 63.67% decline in glucose levels of experimental animals on day 7th, 14th, 21st respectively. The glycosylated haemoglobin level was found 59% less, when compared to diabetic control group.

Hypolipidemic activity

The lipid profile of normal control, positive control, glibenclamide and extracts treated diabetic rats are depicted in Table 3 and 4. In alloxan induced diabetic rats there was a significant increase in total cholesterol, triglycerides, phospholipids, LDL-cholesterol, VLDL-cholesterol and significant decrease in HDL-cholesterol in serum, when compared to normal control. The extracts and glibenclamide treated rats were significantly decrease the total cholesterol, triglycerides, phospholipids, LDL-cholesterol, VLDL-cholesterol and increase the HDL-cholesterol on day 21. Both the extracts showed almost same effect on serum glucose level. In the diabetic untreated rats the lipid profile levels remained higher without much change during the study period of 21 days.

DISCUSSION

Type I diabetes mellitus is a chronic disease characters by high blood glucose level due to an absolute or relative deficiency or circulating insulin levels. Various types of oral hypoglycemic agents are available along with insulin for treating diabetes mellitus. It is generally accepted that sufonylureas including glibenclamide, produce hypoglycaemia by stimulating the pancreatic β-cells to release more insulin. Reducing hepatic insulin clearance, stimulate the release of somatostatin and suppressing the secretion of glucagon. Sulfonylureas have also been shown to suppress hepatic gluconeogenisis.[15-16]

Table 1: Effect of aqueous and alcohol extracts of leaves of *Breynia vitis-idaea* on serum glucose level in alloxan induced diabetic rats

Groups	Serum glucose level on			
	0th day	7th day	14th day	21st day
Normal control	118.98±3.04	122.28±2.92	117.74±3.05	118.35±3.59
Positive control	389.80±5.72	409.91±13.89	412.31±13.17	406.21±9.58
Std. (Glibenclamide)	379.89±25.47	240.66±15.98***	178.35±14.21***	113.45±2.93***
Aq. Ex. (300 mg/kg)	384.79±9.01	332.41±8.9***	223.40±9.30***	150.46±6.15***
Aq. Ex. (600 mg/kg)	400.13±9.01	319.91±9.41**	208.72±7.59***	133.56±3.78***
Alc. Ex. (300 mg/kg)	393.18±10.49	324.48±10.18***	218.69±10.73***	144.01±5.46***
Alc. Ex. (600 mg/kg)	369.01±12.83	297.64±12.55***	201.31±3.86***	134.04±4.24***

Std.-Standard, Aq. Ex.-Aqueous Extract, Alc. Ex.-Alcohol Extract.
Values expressed as Mean ± SEM. One way ANOVA (***p<0.001, **p<0.01)
Tukey Kramer Multiple Comparison Test in comparison with diabetic control group.

Table 2: Effect of aqueous and alcohol extracts of leaves of *Breynia vitis-idaea* on glycosylated hemoglobin conc. on 21st day

Groups	Glycosylated hemoglobin conc.
Normal control	3.92±0.04
Positive control	12.0±0.15
Standard (Glibenclamide)	4.13±0.18***
Aqueous extract (300 mg/kg)	5.73±0.17***
Aqueous extract (600 mg/kg)	4.89±0.07***
Alcohol extract (300 mg/kg)	5.67±0.16***
Alcohol extract (600 mg/kg)	4.92±0.05***

Values expressed as Mean ± SEM. One way ANOVA (*** p<0.001).
Tukey Kramer Multiple Comparison Test in comparison with diabetic control group.

Table 3: Effect of aqueous and alcohol extracts of leaves of *Breynia vitis-idaea* on different parameter in alloxan induced diabetic rats on 21st day

Groups	Serum HDL	Serum LDL	Serum VLDL
Normal control	22.26±1.21	35.67±1.53	14.11±0.85
Positive control	14.29±0.46	71.01±2.41	33.06±2.0
Std. (Glibenclamide)	20.07±0.87***	39.41±2.18***	16.73±0.68***
Aq. Ex. (300 mg/kg)	17.23±0.44**	50.22±1.06***	25.56±1.44**
Aq. Ex. (600 mg/kg)	19.19±0.31***	43.78±2.04***	19.60±0.75***
Alc. Ex. (300 mg/kg)	17.27±0.53**	48.60±1.38***	25.97±1.02**
Alc. Ex. (600 mg/kg)	19.03±0.39***	42.46±0.91***	17.79±0.55***

Values expressed as Mean ± SEM. One way ANOVA (*** p<0.001, ** p<0.01)
Tukey Kramer Multiple Comparison Test in comparison with diabetic control group.

Table 4: Effect of aqueous and alcohol extracts of leaves of *Breynia vitis-idaea* on different parameter in alloxan induced diabetic rats on 21st day.

Groups	Cholesterol	Triglycerides	Phospholipids
Normal control	91.65±2.92	93.75±2.16	153.24±3.36
Positive control	197.66±3.63	172.35±2.42	252.20±4.29
Std. (Glibenclamide)	122.82±2.69***	116.36±3.02***	169.82±2.19***
Aq. Ex. (300 mg/kg)	145.97±3.57***	141.76±2.88***	190.09±2.99***
Aq. Ex. (600 mg/kg)	135.99±1.82***	122.54±1.55***	177.20±2.53***
Alc. Ex. (300 mg/kg)	144.16±1.89***	138.57±3.27***	188.93±2.87***
Alc. Ex. (600 mg/kg)	128.16±1.83***	122.09±1.66***	174.02±3.01***

Values expressed as Mean ± SEM. One way ANOVA (*** p<0.001)
Tukey Kramer Multiple Comparison Test in comparison with diabetic control group.

The present study focused the scientific explanation about the hypoglycemic and hypolipidemic activity for both the extracts of leaves of *Breynia vitis-idaea* for the management of alloxan induced diabetes. Experimental animals were made diabetic using alloxan. Alloxan is a toxic glucose analogue, which selectively destroys insulin producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin dependent diabetes mellitus in these animals, with characteristics similar to type-1 diabetes in humans. In diabetic rats, alloxan led the elevation of fasting blood glucose level, which was maintained over a period of 2-3 weeks. Decrease in blood glucose level may be due to the regeneration of β-cells of the pancreas which was destroyed by alloxan.[17-18]

Lipid play an important role in the pathogenesis of complications involved with diabetes mellitus. The elevated level of serum cholesterol, LDL, VLDL, triglycerides and reduced level of HDL possess to be a rises of factor for developing microvascular complication leading atherosclerosis and cardiovascular diseases like coronary heart disease. The abnormal high concentration of serum lipid in diabetic mainly due to increased mobilization of free fatty acids from peripheral fat depots, since insulin inhibits the hormone sensitive lipase, insulin deficiency or insulin resistance may be responsible for dislipidimia.

The present studies provide the introductory approach for the evaluation of its traditional preparations in order to scientifically validate the therapeutic use of *Breynia vitis-idaea* in the control of diabetes as well as maintenance of various biochemical parameters.

CONCLUSION

Screening of Ayurvedic drugs/plants for biological activity assumes prime importance to establish physiological action of the drug. To obtain required evidence that will demonstrate drug's safety and effectiveness for its proposed use, a carefully designed and progressive sequence of preclinical (animal) and clinical (human) studies are undertaken.

This study indicated that both of the extracts of *Breynia vitis-idaea* have potential to decrease blood glucose level as well as improving hyperlipidaemia and to reduce the complications associated with experimental diabetes. This study also supports the folklore usefulness of this plant in the treatment of diabetes. It can be concluded that the roots of this plant could be further investigated for antidiabetic bioactive principles.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

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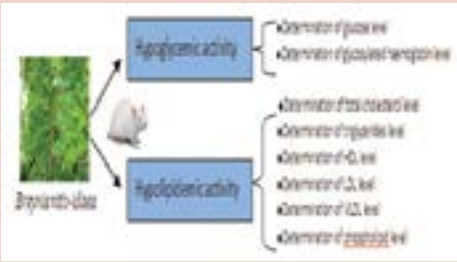
PICTORIAL ABSTRACT



SUMMARY

- Administration of alcohol and aqueous extracts of *Breynia vitis-idaea* to the alloxan induced diabetic rats decrease the level of blood glucose, glycosylated haemoglobin.
- It also brings the cholesterol, phospholipids, triglycerides, HDL, VLDL and LDL level towards normal value.
- Administration of alcohol and aqueous extracts of *Breynia vitis-idaea* to the alloxan induced diabetic rats decrease the level of blood glucose, glycosylated haemoglobin. It also brings the cholesterol, phospholipids, triglycerides, HDL, VLDL and LDL level towards normal value.

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