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ANTI-DIABETIC AND HYPOLIPIDEMIC ACTIVITY OF METHANOLIC EXTRACT OF *ARISTOLOCHIA BRACTEOLATA* ON STREPTOZOTOCIN INDUCED DIABETIC RAT MODEL

M. Ganga Raju * and T. Hema Sundar Reddy

Gokaraju Rangaraju College of Pharmacy, Hyderabad, Telangana, India.

Keywords:

Aristolochia bracteolata, Glibenclamide, Anti-diabetic, Hypolipidemic, Streptozotocin

Correspondence to Author:
M. Ganga Raju

Associate professor,
Department of Pharmacology,
Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, Telangana, India.

E-mail: mgrpharma@gmail.com

ABSTRACT: The objective of the present study was to evaluate the anti-diabetic and hypolipidemic activity of methanolic extract of whole plant of *Aristolochia bracteolata* (MEAB) against streptozotocin (STZ) induced diabetic rat model. Glibenclamide was used as standard drug. The MEAB was administered orally at a dose of 200 & 400 mg/kg bd. wt and blood glucose levels were determined on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day. MEAB was found to significantly reduce blood glucose levels in streptozotocin induced diabetic rats. Reduction in blood glucose levels were observed from 7^{th} day onwards, after continuous administration of the extract. The effect of MEAB on serum lipid profile like total cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoprotein (HDL) were also measured on 28^{th} day in the diabetic rats. There was significant reduction in total cholesterol, LDL cholesterol, VLDL cholesterol and improvement in HDL cholesterol in diabetic rats. The results indicated that *Aristolochia bracteolata* possesses significant anti-diabetic and hypolipidemic activity.

INTRODUCTION: Diabetes mellitus is a complex disorder that characterized by hyperglycemia resulting from malfunction in insulin secretion and/or insulin action both causing by impaired metabolism of glucose, lipids and protein [1]. Diabetes mainly characterized by hyperglycemia, ketoacidosis, hypertriglyceridemia. The characteristic symptoms of diabetes are polyuria, polydipsia, polyphagia and unexpected weight loss [2]. Abnormalities in lipid profile are one of the most common complications in diabetes mellitus, which is found in about 40% of diabetes [3].

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Diabetes induction causes increase in cholesterol, triglycerides, LDL and VLDL [4]. The level of serum lipids is usually elevated in diabetes mellitus and such an elevation represents the risk factor for coronary heart disease [5]. Most of the hypoglycemic agents used in allopathic medicines are reported to have side effects in the long run. Therefore, there is a need to search for effective and safe drugs for diabetes [6]. The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world.

The World Health Organization also recommended and encouraged this practice especially in countries where access to the conventional treatment of diabetes is not adequate. There is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents [7].

Aristolochia bracteolate is a shrub distributed throughout India, belongs to the family Aristolochiaceae [8]. It is a creeper plant and slender perennial. Stems 30-45 cm. long, weak, prostrate, branched, striate, glabrous. Leaves 3.8-7.5 cm. long and as broad as long, broadly ovate, usually obtuse [9]. *Aristolochia bracteolate* is an herbal medicine for the treatment of various diseases such as gastric stimulant, anti-inflammatory agents, antifungal, antibacterial activity and cancer. A literature survey revealed that methanolic extract of whole plant of *Aristolochia bracteolata* is endowed with various chemical components such as phenolic compounds, flavonoids, triterpenoids, alkaloids, steroids, cardiac glycosides, saponins, aristolochic acid-A & aristolochic acid-D [10].

The present study is carried out to investigate the effect of oral administration of methanolic extract of whole plant of *Aristolochia bracteolata* on blood glucose levels and lipid profile in streptozotocin induced diabetic rats.

MATERIALS AND METHODS:

Materials: Streptozotocin (STZ) was purchased from SVR Chemicals Ltd (Hyderabad). Glucose kit & lipid profile kits were purchased from K. K. Diagnostics (Hyderabad). All other chemicals were obtained from Dwarakamai Enterprises (Hyderabad).

Animals: Inbred colony of adult wistar albino rats (150-200 g) were used for the present study. They were kept in polypropylene cages at 25 ±2° C, with relative humidity 45-55% under 12 h light and dark cycles. All the animals were acclimatized to the laboratory conditions for a week before use. They were fed with standard animal feed and water *ad libitum.* All the pharmacological experimental protocols were approved by the Institutional Animal Ethics Committee (Reg. No.1175/PO/Ere/ S/08/CPCSEA), Gokaraju Rangaraju College of Pharmacy.

Plant material and preparation of extract: The plant *Aristolochia bracteolate* was collected in January 2016 from the Nallamalla forest region, near to Velugodu, Kurnool Dist. The procured plant materials were authenticated by a botanist. The whole plant was washed, dried at room temperature in the dark and then finely grind to a powder. Then the powder was extracted with the methanol in the ratio of 1:6 by simple distillation technique. The solvent was completely removed under reducing pressure and a semisolid mass (yield: 9.4%, w/w) was obtained and stored for further study.

Preliminary phytochemical analysis: The preliminary phytochemical studies were performed for testing different chemical groups present in methanolic extract of *Aristolochia bracteolata*. Phytochemical screening gave positive results for phenolic compounds, flavonoids, triterpenoids, alkaloids, steroids, cardiac glycosides, saponins [11].

Acute toxicity study: Acute toxicity studies were carried out as per Organisation for Economic Co-operation and Development guidelines 425. Methanolic extract of *Aristolochia bracteolata* (2000 mg/kg. bd. wt) was dissolved in distilled water and given *p.o* to overnight fasted mice (n=5) and the animals were observed individually for their health status and signs of any abnormalities. The animals were observed for clinical signs (tremors, convulsions, lethargy and coma), gross behavioural changes and mortality after 30 min, 1, 2, 3 and 24 hr. daily cage side observations were continued for a period of 14 days [12].

Streptozotocin induced diabetic rat model: Diabetes was induced in healthy wistar albino rats by using streptozotocin at a dose of 50 mg/kg bd. wt. Streptozotocin was dissolved in water for injection and administered *intraperitoneally*. After 48 h of Streptozotocin injection, blood samples were withdrawn from retro orbital plexus and plasma glucose levels were determined. The animals with blood glucose levels above 250 mg/dL were selected for conducting the present study.

These rats were divided into 5 groups of 6 animals in each group. Group- I received saline (normal control), group- II received 0.5 ml of 5% Tween 80 (Diabetic control), group- III received MEAB (200 mg/kg bd. wt.), group- IV received MEAB (400 mg/kg bd. wt.) & group- V received glibenclamide (0.5 mg/kg). The treatment was continued daily for 28 days. At the predetermined time intervals i.e. 0^{th}, 7^{th}, 14^{th}, 21^{st}, 28^{th} days blood samples were collected from the animals of all the groups and

subjected for analysis of glucose on 0th, 7th, 14th, 21st, 28th days & other biochemical parameters such as TC, TG, LDL, VLDL, HDL levels were determined only on 28th day.

Statistical analysis: All results were expressed as the mean ± SEM (standard error of the mean). The results were analysed for statistical significance by one-way analysis of variance (ANOVA) followed by Dunnett's test using Graph Pad Prism version 5.0 (Graph Pad Software, USA).

RESULTS:

Phytochemical screening: The preliminary Phytochemical screening of the *Aristolochia bracteolata* showed the presence of phenolic compounds, flavonoids, triterpenoids, alkaloids, steroids, cardiac glycosides, saponins.

Acute toxicity studies: Using methanolic extract of *Aristolochia bracteolata* acute toxicity studies were performed as per OECD guidelines 425. The oral administration of methanolic extract of *Aristolochia bracteolata* did not exhibit any signs of toxicity and mortality even upto 2000 mg/kg. bd. wt. All animals were safe even after 14 days of observation.

Streptozotocin induced diabetic rat model:

Effect of extract on body weight: Table 1 shows that a significant decrease was observed in the body weight of diabetic rats compared with normal control rats. Treatment with extract of whole plant of *Aristolochia bracteolata* and glibenclamide, the body weight gain was improved.

TABLE 1: EFFECT OF METHANOLIC EXTRACT OF *ARISTOLOCHIA BRACTEOLATA* ON BODY WEIGHT BY STZ INDUCED DIABETIC RATS

Group	Treatment	Body weight (gm) Mean±SEM				
		0th Day	7th Day	14th Day	21st Day	28th Day
I	Normal control	176±0.966	183.1±1.301	191.6±2.185	198.1±2.914	202.5±2.974
II	Diabetic control	176.1±2.056	173.3±1.819	163.6±1.498	159.5±1.335	155±1.366
III	MEAB(200 mg/kg)	174.3±1.229*	173.8±1.137*	176.1±1.137*	180.5±1.979*	184.5±1.892*
IV	MEAB(400 mg/kg)	174.3±1.17*	174.1±1.351*	180.3±2.076*	183.3±2.260*	189±2.569*
V	Glibenclamide(0.5 mg/kg)	174.3±0.802*	175.5±0.806*	183.1±1.740*	187±2.160*	192.8±2.056*

Values are mean±SEM, n=6 when compared with diabetic control ***p<0.001, **p<0.01, *p<0.05.

Effect of extract on blood glucose level: Effect of methanolic extract of whole plant of *Aristolochia bracteolata* on serum glucose levels in diabetic rats was depicted in **Table 2**. In animals treated with streptozotocin (50 mg/kg *i.p*) (Group II), a significant increase in serum glucose level was observed on 0th, 7th, 14th, 21st, and 28th day when compared with normal rats (Group I). Group V received glibenclamide (0.5 mg/kg *p.o.*) showed decrease in serum glucose level when compared with diabetic control rats (Group II). After the oral administration of methanolic extract of *Aristolochia bracteolata* in diabetic rats, a significant reduction in blood glucose levels was observed on the 7th, 14th, 21st and 28th day compared with diabetic control rats (Group II).

TABLE 2: ANTI-HYPERGLYCAEMIC ACTIVITY OF METHANOLIC EXTRACT OF *ARISTOLOCHIA BRACTEOLATA* ON STZ INDUCED DIABETIC RATS

Group	Treatment	Lipid Profile (mg/dL)				
		Total Cholesterol	Triglyceride	HDL	LDL	VLDL
I	Normal control	90.50±1.648	63.83±1.222	37.16±0.307	40.56±1.659	12.76±0.244
II	Diabetic control	142.83±1.6	108.5±1.176	23.33±0.988	97.8±1.626	23.03±1.373
III	MEAB(200 mg/kg)	114.83±1.536*	90.83±0.6 *	28.66±0.421*	68±1.628*	18.16±0.120*
IV	MEAB(400 mg/kg)	104.16±1.514**	82.66±0.843**	32.66±0.614**	54.96±1.591**	16.53±0.168 **
V	Glibenclamide (0.5 mg/kg)	94.16±1.137*	75.5±1.408*	36.33±0.881*	42.73±1.430*	15.1±0.281*

Values are mean±SEM, n=6 when compared with diabetic control ***p<0.001, **p<0.01, *p<0.05.

Effect of extract on blood lipid profile: The lipid profiles in control and experimental rats are depicted in **(Table 3**) in STZ induced diabetic rats. The diabetic control rats (Group II) showed significant increase in serum triglycerides, total cholesterol, very low density lipoproteins (VLDL)

and low density lipoproteins (LDL) while decrease in High density lipoproteins (HDL) when compared with normal (Group I). Standard Glibenclamide (Group V) was reduced total cholesterol, triglycerides, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and increased high density lipoproteins (HDL) when compared with diabetic control (Group II). The methanolic extracts of *Aristolochia bracteolata* showed significant decrease in total cholesterol, triglycerides, LDL, VLDL, and significant increase in HDL when compared with diabetic control group (Group II). All these effects were observed on 28th day. The present experimental results indicated that methanolic extracts of *Aristolochia bracteolata* exhibited a potent Anti-hyperlipidaemic activity property in STZ induced diabetic rats.

TABLE 3: ANTI-HYPERLIPIDEMIC ACTIVITY OF METHANOLIC EXTRACT OF *ARISTOLOCHIA BRACTEOLATA* ON STZ INDUCED DIABETIC RATS.

Group	Treatment	Lipid Profile (mg/dL)				
		Total Cholesterol	Triglyceride	HDL	LDL	VLDL
I	Normal control	90.50±1.648	63.83±1.222	37.16±0.307	40.56±1.659	12.76±0.244
II	Diabetic control	142.83±1.6	108.5±1.176	23.33±0.988	97.8±1.626	23.03±1.373
III	MEAB(200 mg/kg)	114.83±1.536*	90.83±0.6 *	28.66±0.421*	68±1.628*	18.16±0.120*
IV	MEAB(400 mg/kg)	104.16±1.514**	82.66±0.843**	32.66±0.614**	54.96±1.591**	16.53±0.168 **
V	Glibenclamide (0.5 mg/kg)	94.16±1.137*	75.5±1.408*	36.33±0.881*	42.73±1.430*	15.1±0.281*

Values are mean±SEM, n=6 when compared with diabetic control ***p<0.001, **p<0.01, *p<0.05.

DISCUSSION: Diabetes mellitus is one of the most common chronic disease and is associated with hyperglycaemia, hyperlipidemia and co-morbidities such as obesity, hypertension. Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes. Streptozotocin (2-deoxy-2-(3-methyl-3-nitrosouredia) – D - gluco pyranose) is a potent diabetogenic agent and widely used for inducing diabetes in a variety of animals by the selective necrosis of pancreatic cells resulting in a disease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues.

Glibenclamide has been used for many years to treat diabetes by stimulate insulin secretion from pancreatic β-cells.

In the present study, diabetic rats have lower body weight, high blood glucose and lipid levels as compared to normal rats.

The present data indicated that methanolic extract of *Aristolochia bracteolata* at 200 mg/kg & 400 mg/kg significantly reduced the elevated fasting blood glucose levels & increased body weight in dose dependent manner in diabetic animals.

The possible mechanism by which methanolic extract of *Aristolochia bracteolata* reduced hypoglycemic action might be due to presence of phenolic and flavonoids. These phytochemical constituents might be increasing either the pancreatic secretion of insulin from β-cells of islets of Langerhans or increased peripheral utilization of glucose [13, 14].

Lipids play a vital role in the pathogenesis of diabetes mellitus. The levels of serum lipids are usually elevated in diabetes cases and such an elevation represents the risk factor of coronary heart disease. High levels of total cholesterol and more importantly LDL-cholesterol in blood are major coronary risk factor. The abnormal high concentration of serum lipids in the diabetic subjects is due, mainly to the increase in the mobilization of free fatty acids from the peripheral fat depots. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia.

The methanolic extract of *Aristolochia bracteolata* at 200 mg/kg & 400 mg/kg significantly reduced the elevated levels of total cholesterol, triglycerides, LDL and VLDL and also increases the HDL levels dose dependent manner in diabetic animals.

The phytochemical and pharmacological studies indicated that the methanolic extract of *Aristolochia bracteolata* contain phenolic, flavonoids. These phytochemicals might be decreased cholesterogenesis and fatty acid synthesis [15].

CONCLUSION: The result of the present investigation is quite encouraging on oral administration of methanolic extract of *Aristolochia bracteolata* at a dose of 200 mg/kg bd. wt. and 400 mg/kg bd. wt. for 28 days in STZ induced diabetic rats. There was significant decrease in elevated blood glucose levels when compared with diabetic control group. Methanolic extract of *Aristolochia bracteolata* has also lowered the total cholesterol, triglycerides, LDL and VLDL levels and increases the levels of HDL when compared to that of diabetic control group in dose dependent manner. The present study revealed that methanolic extract of *Aristolochia bracteolata* possesses significant antidiabetic and hypolipidemic activity. However further studies are required to confirm the exact mechanism of action and to isolate the phytochemical constituents responsible for these activities.

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CONFLICT OF INTEREST: None

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