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Hypoglycemic Potential of *Bridelia retusa* Bark in Albino Rats

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ABSTRACT

In the present study, *Bridelia retusa* Bark was screened for their hypoglycemic potential on normal, oral glucose tolerance study and alloxan induced rats. Albino rats were divided into groups (n = 6) received different treatments consisting of control, methanolic, pet-ether and n-butanol extracts (at a dose of 200 and 400 mg kg^{-1}) and standard drug Glibenclamide (5 mg kg^{-1} p.o.). Estimation of blood glucose level was done by GOD/POD method. Results indicated that *B. retusa* extracts does not affect blood glucose level on normal rats but in Alloxan induced diabetic rats n-butanol extract showed significant ($p<0.001$) fall of blood sugar level. However, the effect of drug was less than standard. Phytochemical analysis has revealed the presence of steroids, triterpenoids, tannins and flavonoids as major constituents.

Key words: Antidiabetic, *Bridelia retusa*, alloxan-induced diabetes, oral glucose tolerance test, glibenclamide

INTRODUCTION

Diabetes mellitus is one of the common metabolic disorders and 1.3% of the population suffers from this disease throughout the world (Raghunathan and Raghunathan, 1992). Insulin and oral hypoglycemic agents like sulphonylureas and biguanides are still the major players in the management of the disease. Due to lack of insulin, hyperglycemia and glycosuria almost invariably occur. The search for a curative agent against this disease resulted in the introduction of several hypoglycemic agents. Some of which are used therapeutically. However, various harmful side effects and weak effectiveness of them made their use limited and the search to find more effective agents continues. Investigation in the plant kingdom culminated in the discovery of many herbal hypoglycemic agents. One of them is taken for investigation in this study.

Bark of *Bridelia retusa* (airyshawii) belonging to family Euphorbiaceae commonly known as Asana. Khaja is a shrub or climbers is found to be distributed through out the hotter parts of India. Bark is a valuable astringent and used in the form of a liniment in rheumatism (Kirtikar and Basu, 1935). It exhibited antiviral, hypoglycemic, hypotensive, antifertility activity and removal of urinary concretions properties in pharmacological trials (Anonymous, 1958). It is reported as anti inflammatory activity in animal model (Mehare and Hatapakki, 2003). Earlier work was reported for wound healing activity on leaves of *Bridelia retusa* (Bagad, 2007). Plant has also been reported to have an action as a contraceptive and antibacterial property against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Shigella dysenterae* and *Pseudomonas*

aeruginosa (Adhav *et al*., 2002). Aqueous and alcoholic extracts of its bark were investigated for acute antiinflammatory activity in carrageenan-induced rat paw oedema. The bark extract is used by the tribal to develop sterility and it is used as a contraceptive. One or two drops of fruit extract are poured in ear to cure earache (Jain *et al*., 2005). Objective of the present study was to evaluate the effect of methanolic, pet ether and n-butanol extracts of *Bridelia retusa* (airyshawii) bark on different parameters related to hypoglycemic action in albino rats.

MATERIALS AND METHODS

Collection and authentication of plant material: The fresh bark of *Bridelia retusa* Spreng was collected in the month of August (2007) from Ranipur (Toranmal) of Nandurbar District (MS), India. It was identified, confirmed and authenticated by Dr. D.A. Patil, H.O.D. of Botany, Dr. P. R. Ghogrey College, Dhule. (MS). A voucher specimen of the bark was deposited in department for future reference.

Preparation of test extract solution: Dried powdered bark of *Bridelia retusa* were extracted with methanol and then methanolic extract dissolve in sufficient amount of water and then fractioned with pet ether 60-80°, n-butanol and finally all obtained extracts was concentrated to dryness in a vacuum dryer and subjected to preliminary Phytochemical screening. For pharmacological studies, dried extract were mixed with 0.3% CMC solution, triturated and adjusted to final volume to get required concentration.

Test animals: Three months old Wistar albino rats of either sex having weight of 200-250 g were procured from R.C.Patel Institute of Pharmaceutical Education and research, Shirpur. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±27°C and 35-60% humidity). Standard palletized feed and tap water were provided *ad libitum*. The study was conducted in accordance with Institutional Animal Ethical Committee, Registration no (RCPCOP/IAEC2007-8/9) under CPCSEA.

Normal glycemic test (Jafri *et al*., 1999; Khosia *et al*., 1995): Fasted rats divided into seven groups, each group contain six animals. Groups divided as follows:

Group I vehicle control, groups II and III received methanolic extract of bark of *Bridelia retusa* at a dose of 200 and 400 mg kg^{-1}, respectively, Groups IV and V received pet-ether extract at 200 and 400 mg kg^{-1}, respectively while Group VI and VII received n-butanol extract at a dose of 200 and 400 mg kg^{-1}, respectively.

After 1 h of extract administration, blood sample were collected from the retro-orbital plexus at 30, 90, 120 min. Serum was separated and blood glucose levels were measured immediately by the glucose oxidase method.

Oral Glucose Tolerance Test (OGTT) (Stumvoll *et al*., 2000): Animals were divided into eight groups of six rats each. Group I served as control received distilled water. Group II received Glibenclamide at dose of 5 mg kg^{-1} b.wt. as reference drug. Groups III-VIII were treated with methanol extract, petroleum ether extract and n-butanol extract of bark of *Bridelia retusa* at dose of 200 and 400 mg kg^{-1} b.wt. respectively as a fine suspension orally. Thirty minutes after administration of the drug, all animal groups received glucose (2 g kg^{-1} b.wt., p.o.) Blood samples were collected for blood glucose levels determination.

Alloxan-induced hyperglycemia (Szkudelski, 2001): Rats were made diabetic by single i.p. injection of 100 mg kg^{-1} body weight of alloxan monohydrate (Sigma chemicals, USA; 5% w/v in normal saline). Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were kept for next 24 h on 5% w/v glucose solution to prevent hypoglycemia. Five days later blood samples were drawn and glucose levels were determined to confirm development of diabetes (>250 mg dL^{-1}). The diabetic rats were divided into eight groups, each containing six animals. Group I served as control received distilled water. Group II received Glibenclamide (Alembic Ltd. Baroda, India) at dose of 5 mg kg^{-1} b.wt. as reference drug. Groups III-VIII were treated with methanol extract, petroleum ether extract and n-butanol extract of bark of *Bridelia retusa* at dose of 200 and 400 mg kg^{-1} b.wt., respectively as a fine suspension orally. Treatment with drugs started on 6th day of diabetics induced (i.e., day 1) and was continued for 12 days. No sign of toxicity was noticed on behavior and general health of the animals when exposed to extracts. Blood samples were drawn at three day intervals till end of study. Blood glucose estimation and body weight measurements were done on day 1, 3, 6, 9 and 12 days of the study.

Collection of blood: The animals were anesthetized with anesthetic ether and with the help of small capillary the retro-orbital vein was punctured and 1 mL blood was collected into the Eppendrof tube.

Separation of serum: The blood collected in Eppendrof tube was allowed to clot for 30 min. The tubes were kept for the centrifugation (Remi Centrifuge R 24) at 2000 rpm for 10 min. The serum was separated and stored at 2-8°C, until it was used for the estimation of biochemical parameter by glucose oxidase determination methods.

RESULTS

Normal glycemic test: All the extracts of bark of *B.retusa* do not show any significant reduction in the blood glucose level on the normal rats at 90 and 120 min (Table 1).

Oral glucose tolerance test in normal rats: Glucose loaded normal rats increased serum glucose levels from 81.65±0.61 to 102.72±1.15 at 0 and 90 min. The methanol, petroleum ether and butanol extract ($p<0.001$) have shown significant increase in glucose tolerance. The blood glucose levels were reduced considerably within 90 min of the drug administration. The methanol and butanol extracts reduced the glucose levels to normal. Maximum effect was observed in butanol extract (Table 1).

Alloxan induced diabetes in rats: Administration of alloxan (100 mg kg^{-1}, i.p.) led to elevation of fasting blood glucose levels, along with significant decrease in body weight, which was maintained over a period of 12 days. Similar to glibenclamide, administration of methanol, petroleum ether and butanol extract significantly ($p<0.001$) decreased the elevated blood glucose level in comparison with untreated diabetic rats (Table 2). The 12th days of daily treatment of various extracts of *B. retusa* led to dose dependent fall in blood glucose level by 20-36% effect seems to reach maximum at 12 days of treatment. Alloxan induced diabetic rats showed reduction in body weight during 12 days which was restored by glibenclamide and all test samples on 9th and 12th days (Fig. 1).

Table 1: Effect of different extracts of bark of *B. retusa* on blood glucose level in normal rats

		Time (min)		
Groups	Dose mg kg^{-1} b.wt.	0	90	120
Control	-	61.23±0.71	61.56±0.91	60.98±0.31
Methanolic extract	200	62.21±0.66	62.12±0.80	62.03±0.43
	400	59.62±0.63	59.23±0.60	59.01±0.66
Pet-ether extract	200	61.58±0.62	61.12±0.61	61.10±0.75
	400	61.13±0.60	60.78±0.22	60.23±0.53
n-butanol extract	200	61.48±0.84	60.13±0.59	59.96±0.89
	400	59.73±0.64	58.13±0.70	58.06±0.54

Values are Mean±SE (n = 6)

Table 2: Effect of different extracts of bark of *B. retusa* on blood glucose level (mg dL^{-1}) in Alloxan (100 mg kg^{-1}) induced diabetes rats

	Blood glucose level (mg dL^{-1} at days)				
Groups and dose (mg kg^{-1}) b.wt.	1	3	6	9	12
Control	265.87±1.05	268.41±0.61	272.22±1.94	274.35±1.32	277.78±1.06
Glu.+Gli.	267.07±1.33	240.23±2.80***	215.07±2.05***	190.58±1.94***	142.46±3.52***
Methanolic extract 200	266.67±1.07	265.45±1.27^{NS}	255.57±2.41***	233.18±2.85***	210.71±2.17***
Methanolic extract 400	263.85±1.70	260.56±1.46*	245.23±2.13***	228.62±2.12***	202.39±2.65***
Pet ether extract 200	268.27±2.23	266.53±1.21^{NS}	262.30±2.63^{NS}	255.29±1.49***	220.64±1.85***
Pet ether extract 400	264.66±1.88	262.55±1.75^{NS}	256.75±3.93**	245.09±1.91***	208.33±1.93***
n-butanol extract 200	265.47±1.90	260.15±1.76*	249.60±2.45***	224.71±1.54***	198.81±2.21***
n-butanol extract 400	267.47±0.93	259.37±1.26**	233.34±1.95***	203.13±1.90***	172.61±1.33***

Values are Mean±SE (n = 6) ANOVA: p<0.0001. Tukey-Kramur Multiple comparison post hoc-test: *p<0.05, **p<0.01, ***p<0.001, NS: Non significant as compare with control

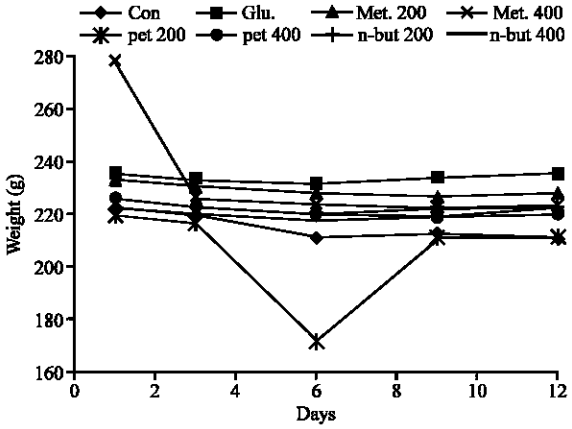

Fig. 1: Effect on body weight on alloxan induced rats at days

DISCUSSION

In adult animals, alloxan selectively destroys the pancreatic insulin-secreting β-cells. The main mechanism of action of glibenclamide is the stimulation of insulin release. It has been described that glibenclamide is effective in moderate diabetic state and ineffective in severe diabetic animals where pancreatic β-cells are almost totally destroyed (Suba *et al*., 2004). In the diabetic model rats,

the extracts of *B. retusa* showed an anytihyperglycemic effect comparable to that of glibenclamide. Thus, the extract may act on β-cells like sulfonylurea drugs to stimulate insulin secretion. Similar results have been reported with ethanolic extract of *Bridelia ndellensis* stem bark (Sokeng *et al.*, 2005). As the extracts of *B. retusa* did not show any hypoglycemic effect in normal glycemic rats on fasting condition. On the other hand, the hypoglycemic effect of the extracts in the glucose-fed rats may be accounted in part, by an inhibition of intestinal glucose absorption and the stimulation of the glucagonlike peptide (GLP-1) which is also a glucose-dependent insulin secretagogue (Goke *et al.*, 1995).

All extracts of *B. retusa* exhibited significant anti-hyperglycemic activity (especially n-butanol extracts) in alloxan-induced hyperglycemic rats with changes in body weight. They also improved condition of DM as indicated by parameter like body weight.

Phytochemical investigation of *B. retusa* extracts shows the presence of steroids, triterpenoids, Flavonoids, saponins, tannins and phenolics. Different mechanisms of action to reduce blood glucose levels with the help of plant extract already exist. Some plants exhibit properties similar to the well known sulfonylurea drugs like Glibenclamide. In this study, *B. retusa* extracts produced hypoglycemic effects may be due to the presence of phytosterols and triterpenoids. Also some flavonoids and saponins present in the medicinal plants may significantly reduce the blood glucose levels (Diatewa *et al.*, 2004; Abdel-Hassan *et al.*, 2000). A study on the hypoglycemic properties of *Bridelia ferruginea* extract revealed blood glucose level were lowered due to flavonoids, β-sitosterol, quercetin, quercetin-3-glycoside and epigallocatechin isolated from it (Sokeng *et al.*, 2005). Therefore, it was demonstrated hypoglycemic activity of *B. retusa*, which belongs to the same genus, is likely to contain such compounds responsible for the observed antihyperglycemic and hypoglycemic effects. Further chemical and pharmacological investigations are in progress to elucidate in detail the active principles and the mechanism of action of this plant extract.

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