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Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 4, Issue 3, 2012

Research Article

HYPOGLYCEMIC, HYPOLIPPIDEMIC AND ANTIOXIDANT EFFECTS OF LEAVES METHANOLIC EXTRACT OF *BACCAUREA RAMIFLORA*

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Received: 18 May 2012, Revised and Accepted: 15 Jun 2012

ABSTRACT

The present study was designed for investigating the hypoglycemic, hypolipidemic and antioxidant activity of the leaves of *B. ramiflora*. Antioxidant potential was assayed by measuring the free radical scavenging activity using 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Diabetes was induced in adult albino rats of both sexes by intra peritoneal (i.p) injection of alloxan (120 mg/kg). Methanolic extract of *B. ramiflora* leaves (200 mg/kg) was administered as a single dose per day to the diabetic rats for 14 days. The control group received distilled water for the same duration. Blood glucose levels and serum lipid profiles were measured in the diabetic and non-diabetic rats. The methanolic extract showed potent free radical scavenging activity with IC_{50} value of 23.83 (μg/ml). It produced substantial hypoglycemia and reduced the elevated blood glucose level in the diabetic rats towards normal and it was statistically highly significant ($p<0.005$). Except HDL, the methanolic extract decreased the level of cholesterol, triglycerides and LDL and this reduction was statistically highly significant ($p<0.005$). The present study on this plant established that the leaves of *B. ramiflora* possess hypoglycemic, hypolipidemic and antioxidant activity.

Keywords: *B. ramiflora,* Hypoglycemic, Hypolipidemic, Antioxidant, DPPH

INTRODUCTION

Diabetes mellitus is a syndrome characterized by a chronic increase in blood glucose and is usually associated with a loss of weight, energy and biochemical alterations of glucose and lipid metabolism [1, 2]. To reduce the risk of late complications and negative outcomes of diabetes mellitus, such as blindness, renal failure and limb amputation, the control not only in blood glucose levels, but also lipid levels is necessary [3].

Despite the considerable treads that have been made in understanding and management of diabetes, the disease and disease related complications are increasing unabated [4]. Currently the global prevalence of diabetes mellitus is estimated to be 150 million and this figure is expected to increase to over 300 million by 2025 [5].

On the contrary in spite of the availability of known antidiabetic medicines, remedies from medicinal plants are used with success to treat this disease [6]. It has also been reported that before the advent of insulin injections and other pharmaceutical preparation, healers relied heavily upon medicinal plants and herbs to treat diabetes 7.

Traditionally many plants are used in the treatment of diabetes throughout the world, especially in Africa. This is because the plant drugs are frequently considered to be less toxic and have fewer side-effects than the synthetic drugs [8]. Based on the World Health Organization (WHO) recommendations, hypoglycaemic agents of plant origin used in traditional medicine have received renewed attention [9].

From the beginning of the last century, evidence of the lipid lowering properties of medicinal plants has been accumulated [10]. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes [11], but only a few have received scientific scrutiny. From the estimated 350,000 plant species worldwide only a small percentage has been investigated phytochemically and an even smaller percentage has been properly studied in terms of their pharmacological properties[12].

Baccaurea ramiflora belonging to the family Euphorbiaceae is a tall evergreen tree growing widely in the highland of India, Burma, Thailand, Vietnam, Laos, Cambodia, Malaysia and China [13]. It is utilised in Chinese Dai medicine as an antiphlogistic and anodyne against rheumatoid arthritis, cellulites, and abscesses [14]. An ethanolic extract of the leaves evidently showed antioxidant activities. For the genus Baccaurea LOUR., belonging to the tribe Scepeae, so far only one study exists, in which ten compounds, comprising among others a prenylated flavonol, a flavonoid and a lignan, isolated from the leaves of *Baccauera ramiflora* were screened for their antioxidant activities [15].

The fruit was reported to possess antiviral & antioxidant and the stem bark of the plant was reported to have diuretic activity [16, 17]. The stems and the leaves evidently showed antioxidant activities. Two new phenols, 6'- O-vanilloylisotachioside & 6'- O-vanilloyltachioside were isolated from the leaves and three new compounds, 4'-O-(6- O-vanilloyl)-beta-D-glucopyranosyl tachioside D, 6'-O-vanilloylpicraquassioside D & 6'-O-vanilloylicariside B were identified from the stems of the plant [15, 18].

To the best of our knowledge, previously no study has been reported on the hypoglycemic and hypolipidemic activity of the leaves of *B. ramiflora*. The present study was designed to examine the hypoglycemic, hypolipidemic and antioxidant activity of the metabolic extract from *Baccaurea ramiflora* leaves in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Collection of the plant

The fresh leaves of the plant *Baccaurea ramiflora* were collected during the month of July 2009 from the area of Narsingdi, Dhaka, Bangladesh and were identified by a botanist.

Drying and Pulverization

The fresh leaves of the plant were washed with water to remove adhering dirt and then cut into small pieces, sun dried for 4 days. After complete drying, the entire portion was pulverized into a coarse powder with the help of a grinding machine and was stored in an airtight container for further use.

Extraction of Plant Material

The powdered 100 g of *Baccaurea ramiflora* were extracted with 3 times with methanol in a flat bottom glass container, through occasional shaking and stirring for 7 days. The final extracts were

passed through No. 1 Whatman filter paper (Whatman Ltd., UK). The filtrates obtained were concentrated under vacuum in a rotary evaporator at 40 °C and stored at 4°C for further use.

Drugs, Chemicals and Reagents

Metformin was purchased from Square Pharmaceuticals Ltd. Dhaka, Bangladesh. All other reagents, assay kits and chemicals used in this work were purchased from Sigma Chemical Co. St Louis, MO, USA.

Experimental animals and their Management

Forty four-week old albino rats (*Rattus novergicus*: Sprague-Dawley strain,) of both sexes were used for the experiments. These animals were apparently healthy and weighed 80-100 g. The rats were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental condition for one week for acclimatization after their purchase and fed ICDDR; B formulated rodent food and water ad labium. They were housed individually in cages and were kept at constant room temperature (25.0 ± 3.0ºC), humidity 35-60% and 12 hours light and 12 hours dark cycle. Excreta were removed from the cages on every day. The animals were divided into four groups having 6 rats in each group.

Induction of diabetes mellitus and measurement of plasma glucose

Preparation of Alloxan solution

At first body weight of the rats were measured. Then required amount of Alloxan was measured according to the dose of 120 mg of Alloxan per kg body weight. Then calculated amount of Alloxan was dissolved in 0.1 ml of sterile normal saline water.

Induction of diabetes by injecting alloxan

The rats were injected Alloxan monohydrate, dissolved in sterile normal saline water at a dose of 120 mg/kg body weight intraperitoneally once a day. Alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release; therefore the rats were treated with glucose solution orally. After few days rats with moderate diabetes having glycosuria and hyperglycemia that is blood glucose level exceed normal level.

Preparation of dosage of active drug

Metformin hydrochloride was in microcrystalline form and freely soluble in water. The dosage was prepared in solution form using sterilized water in such a concentration that each 0.1ml contained metformin hydrochloride that is equal to 100 mg/kg/day, since metformin is effective in such dose in case of humans.

Measurement of blood glucose level

Glucose concentration was measured in a blood sample obtained from tail puncture, with a glucometer (One touch Ultra, Life Scan, Inc, USA). Only animals that had a blood glucose concentration higher than 10 mM after 72 hours of treatment with alloxan were used for the study. Control rats were injected with normal water only.

Blood Samples Collection and Preparation of Plasma for lipid profile evaluation

At the end of 14 days treatment, after 24 h fasting, blood samples were collected from post vena cava of the rats anaesthetizing with Ketamine (500 mg/kg body, intra peritoneal) and transferred into heparinised tubes immediately. Blood was then centrifuged at 4,000 *g* for 10 min using bench top centrifuge (MSE Minor, England). The supernatant serum samples were collected using dry Pasteur pipette and stored in the refrigerator for further analyses. All analyses were completed within 24 h of sample collection.

Determination of lipid profile

Triglycerides, Total Cholesterol and HDL concentration were evaluated according to the instruction of manufacturer of assay kits (purchased from Sigma Chemical Co, St Louis, MO, USA). According to Friedewald's formula, VLDL and LDL were calculated as: VLDL cholesterol = TG/5 and LDL cholesterol = TC – (VLDL+HDL cholesterol) [19].

Screening of free radical scavenging activity

Free radical scavenging activity of the leaves of *B. ramiflora* was determined based on their scavenging potential of the stable DPPH free radical in both qualitative and quantitative assay.

i) Qualitative assay: A suitably diluted stock solutions of extracts were spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve both polar and non-polar components of the extracts. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved band was observed for 10 minutes and the color changes (yellow on purple background) were noted [20].

ii) Quantitative assay: The antioxidant activity of the leaves extract of *B. ramiflora* was determined using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay [21, 23, 24]. DPPH offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic anti-oxidants [22]. DPPH solution was prepared in 95% methanol. The crude extracts of B. *ramiflora* were mixed with 95% methanol to prepare the stock solution (5 mg/50mL).

The concentration of the sample solutions was 100μg/ml. The test samples were prepared from stock solution by dilution with methanol to attain a concentration of 20 μg/ml, 40 μg/ml, 60 μg/ml, 80 μg/ml and 100 μg/ml. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing *B. ramiflora* extract and after 20 min, the absorbance was taken at 517 nm. Ascorbic acid was used as a positive control. The DPPH solution without sample solution was used as control. 95% methanol was used as blank. Percent scavenging of the DPPH free radical was measured using the following equation-

$$\% \text{ DPPH radical scavenging } (\%) = [1-(As/Ac)] \times 100.$$

Where, Ac=absorbance of control, As =absorbance of sample solution.

Then percentage of inhibition was plotted against respective concentrations used and IC_{50} value was calculated from the graph using Microsoft Excel 2007.

Statistical analysis

The value of glucose (mmol/l) and lipid profile parameters (mg/dl) were expressed as Mean ± SEM (standard error of mean) and analyzed for ANOVA and post hoc Dunnet's t-test. SPSS (Statistical Package for Social Science) for WINDOWS (Ver. 18) was applied for the analysis of data. Differences between groups were considered significant at $P < 0.05$, 0.001 levels.

RESULTS

The hypoglycemic effect of *Baccaurea ramiflora* leaves is shown in Table 1. It was found that it decreased blood glucose level in alloxan induced diabetic rats and produced substantial hypoglycemic effects. Incase of lipid profile, the plant extract decreased the level of cholesterol, triglycerides, LDL (Table 2). The decrease in cholesterol, triglycerides, LDL level was statistically highly significant ($p<0.005$) in comparison with control. There was slight increase in HDL level in comparison with control. But it was statistically non significant. In case of radical scavenging activity, it showed potent antioxidant activity with IC_{50} value of 23.83 μg/ml. The IC_{50} value of standard (Ascorbic Acid) was 15.93 μg/ml (Graph 1).

Table 1: The effect of *B. ramiflora* leaves methanolic extract on Fasting Blood glucose of both diabetic and non diabetic rats

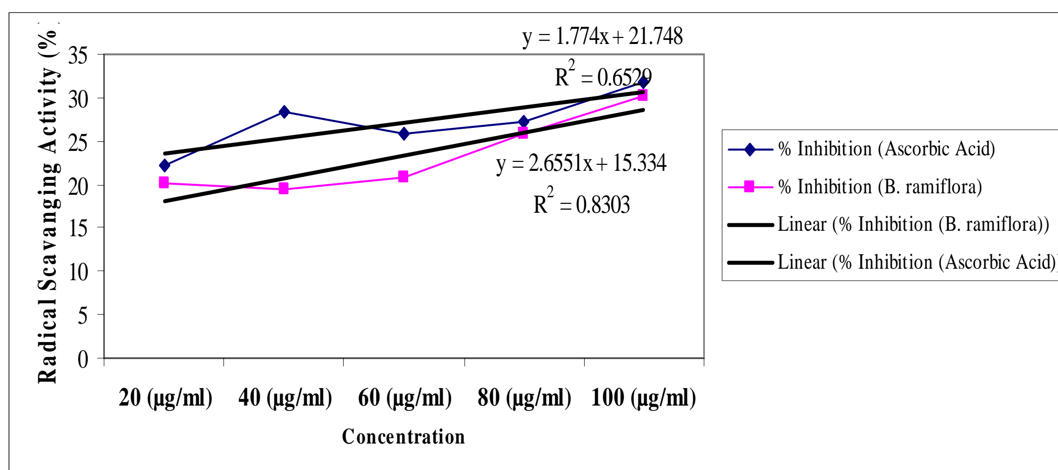
Groups	1st week	3rd week	Decrease / increase (%)
	Glucose (mmol/ l)	Glucose (mmol/ l)	
Group 1 (200 mg/kg)	14.52 ± 0.62	7.25 ± 0.37	50.06, decrease
Group 2 Metformin (100 mg/ kg/day)	15 ± 0.16	6.275 ± 0.18	58.17, decrease
Group 3 (Diabetic Control)	11.5 ± 0.67	15.4 ± 0.69	33.91, increase
Group 4 (Control)	6 ± 0.1	6.25 ± 0.12	4.00, increase

All the values (mmol/l) are expressed as Mean ± SEM (standard error of mean). Differences between groups were considered significant at $P < 0.05$, 0.001 levels.

Table 2: The effect of *B. ramiflora* leaves methanolic extract on lipid profile parameters of both diabetic and non diabetic rats

Parameters	Control (n=6)	Diabetic control (n=6)	Standard control (metformin 100 mg/ kg/day)	Extract, 200 mg /kg (n=6)
Triglycerides	60 ± 6.6	102 ± 6.6	66.13 ± 6.7	70.415 ± 1.88***
Total cholesterol	180.7 ± 19.4	259.7 ± 19.4	218.01 ± 16.67	219.41 ± 7.22***
LDL	190.3 ± 22.8	300.3 ± 22.8	255.328 ± 17.77	230.59 ± 3.19***
HDL	9.6 ± 1.7	14.6 ± 1.7	12.13 ± 0.64	10.45 ± 1.06 NS

All the values (mg/dl) are expressed as Mean ± SEM (standard error of mean). Differences between groups were considered significant at $P < 0.05$, 0.001 levels.



Graph 1: The Free radical scavenging activity of *B. ramiflora* methanolic extract and ascorbic acid.

DISCUSSION

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted [25]. Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas [26, 27]. It causes a massive reduction in insulin release by the destruction of b-cells of the islets of langerhans, thereby inducing hyperglycaemia [28].

Except hyperglycemia diabetes mellitus usually produces many complications, such as hyperlipidemia, hyperinsulinemia, hypertension, obesity, atherosclerosis, and even cardiovascular disease [29, 30]. Diabetes has been found to be associated with indices of oxidative damage. Hyperglycemia can lead to the glycation of tissue proteins. Glycation and glucose auto-oxidation generate hydrogen peroxides, hydroxyl radicals and protein-reactive ketoaldehydes. Hyperglycemia can also lead to increased lipid peroxidation, superoxide production, glycation of the lipoproteins, oxidative DNA damage, and so on. Antioxidants can provide defense against free radical damage.

It is assumed that the antioxidants may have a role in the prevention of diabetes [31]. Diabetic complications can be prevented or retarded by administration of appropriate antioxidants, in addition to traditional therapeutic principles [32]. High levels of TC (total cholesterol) and TG (triglycerides) are major risk factors for atherosclerosis and coronary heart disease. An increase in HDL-c is associated with a decrease in atherosclerotic and coronary risk [33].

The mechanism(s) of hypolipidemic and hypoglycemic actions of the *B. ramiflora* extract are not known, but may involve insulin, since in addition to causing hypoglycemia, insulin lowers lipid levels and normalizes plasma lipids in alloxan induced diabetic rats [34, 35]. The effect of *B. ramiflora* leaves on hyperglycemia and lipid profile may be due its potent free radical scavenging activity. And the free radical scavenging activity may be due to the presence of flavonoids, tannins, terpenes and steroids that has been reported earlier by other authors.

CONCLUSION

From the present study it can be concluded that, the leaves of *B. ramiflora* possess hypoglycemic, hypolipidemic and antioxidant activity. The next step would be to isolate the particular compounds responsible for the observed activities and identification of probable mechanism for this.

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