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Biological activity of *Bauhinia racemosa* against Diabetes and Interlinked Disorders like Obesity and Hyperlipidemia

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Abstract

Background: *Bauhinia racemosa* Lam. (BR) has been used widely for the treatment of diabetes since ancient times. Along with its medicinal importance; the tree having great traditional value in India. The protocol was performed using Wistar rats to determine the effect of petroleum ether extract of BR leaves on blood glucose level, adipose tissue and lipid profile. The study was further evaluted to determine insulin level, glucose tolerance, food and water intake of the animals.

Methods: Wistar rats of both sexes were selected for the study. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ) 45 mg/kg. Animals were divided into five groups namely control group, diabetic group, reference group (glibenclamide) and two test groups BR-1 (250 mg/kg extract) and BR-2 (500 mg/kg) each group containing 6 animals. Daily dosing was performed for 28 days and feed and water consumption was observed regularly at the end of the study.

Results: The main finding of the study for the test extract was that the petroleum ether extract of BR leaves prevented a rise in blood glucose level in STZ induced diabetic animal. Furthermore, extract showed significant antiadipogenic and antihyperlipidemic effect. It improved lipid profile by decreasing the levels of serum triglycerides, total cholesterol, low-density lipoprotein (LDL) and increasing high-density lipoprotein (HDL) cholesterol. Extract exhibited the concentration-dependent inhibitory effect with an IC_{50} value of 660.26 μg/ml.

Conclusions: It can be concluded from the study that BR extract posses signigicant antidiabetic activity by reducing blood glucose level of diabetic rats. It also normalized adipose tissues and lipid level. Due to its potential, it may be an effective drug for treatment of diabetes and related complications like obesity and dyslipidemia.

Keywords: Adipocyte differentiation assay, Oral glucose tolerance test, Diabetes, Hyperlipidemia, Obesity

Background

Diabetes mellitus is a metabolic disorder characterized by abnormal carbohydrate, protein and fat metabolism due to deficient insulin action on target tissues which results in hyperglycemia [1]. Diabetes mainly is of two types Type-1 and Type-2 diabetes i.e. insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) respectively [2, 3]. According to International Federation of Diabetes report it is estimated that 387 million people have been suffering from diabetes till 2014, in which 90% cases of type-2 diabetes. The numbers of diabetic people are expected to rise to 592 million by 2035 [4]. Increase incidence of visceral obesity is closely associated with the risk of diabetes mainly Type-2 diabetes [5]. There is a pronounced correlation between an increased quantity of visceral fat, metabolic disorder and heart related diseases [6].

Bauhinia racemosa Lam. (BR) belongs to the family *Caesalpiniaceae*, popularly known as 'Sonpatti' in India. Its leaves are simple, bilobed, and alternate; stipules small, caducous, petiole 10–33 mm long, slender, pubescent, swollen at the base and at the tip. Poor and harsh climate is favorable for its growth of rare medicinal tree. The tree is widely distributed throughout India, Srilanka, Ceylon, China and Timor [7]. The deciduous tree is propagated easily from seed. Almost each & every part

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of this tree has some medicinal values. The tree has small, creamy white or yellow colored flowers in axillary or terminal racemes. The flowers of BR are laxative & seeds are anti-bacterial [8] and [9]. BR commonly used in the treatment of diabetes, however, its other species like *Bauhinia vahlii, Bauhinia variegata, Bauhinia purpurea, Bauhinia tomentosa* has been used as antidiarrhoeal, antispasmodic, anti-inflammatory, carminative, vermicidal and hepatoprotective activity [10, 11]. The tree yields a useful gum & fibers, bark is used for dyeing. Juice of BR which is extracted from the tender shoots is mixed with mother's milk and used to clean and cool eyes. The bark fiber is used to make ropes to make ladders and to tie cows [12].

The phytochemistry of *Bahuinia* revealed the presence of a new tetracyclic lupeol, betulin, β-sitosterol, and tetracyclic 2, 2-dimethyl chroman isolated from the roots [13]. Stem bark revealed the presence of alkaloids, glycosides, carbohydrates, saponins, flavonoids, triterpenoids (ß-amyrin), anthroqunonine, steroid (ß-sitosterol) stilbene (resveratrol) [14] and [15]. It has been reported that different plant isolates possess insulin releasing, pancreatic beta cells re-generating and fighting the insulin resistance problems.

Methods

Collection, authentication and extraction

Leaves of BR were collected from forest of Ambikapur (23.1200° N, 83.2000° E) district of Chhattisgarh in the month of December 2014 authenticated by Dr. N. K. Satti, Department of Natural Products, Institute of Integrative Medicine, Jammu, and leaves were deposited in the herbarium of the Institute. 1 kg leaves of BR were extracted with petroleum ether by cold percolation at b.p. 40–60 °C and percentage yield was found to be 6.42%. The extract was dried with the help of evaporator (Buchi, USA) under reduced pressure, temperature (37 – 40 °C). Dried extract was placed in an airtight container and further used for studies as antidiabetic, antiadipogenic, and hypolipidemic studies [16].

Phytochemical screening

The petroleum ether extract of BR leaves was subjected to different chemical tests separately for identification of various active constituents employing standard protocols recommended by WHO [17].

Experimental animals

Wistar rats were selected for the study weighing 170–190 g. Experimental protocol was prepared to take the minimum number of animals for in-vivo and acute toxicity study and selected animals were approved by the Institutional Animal Ethical Committee (IAEC reg no-68/99/CPCSEA/reg) of Indian Institute of Integrative Medicine (IIIM), CSIR, Jammu. The animals kept in the arrangement of 12 h light and 12 h dark conditions with temperature maintained at 22 ± 2 °C and humidity 47 ± 65%. Pelleted diet was used for animals feeding throughout the study and water *ad libitum*. Animals were divided into five different groups as normal control (NC), diabetic control (DC), reference group (RG), test group-1 (BR-1) (250 mg/kg), test group-2 (BR-2) (500 mg/kg) each group containing six animals.

Cell line

3T3-L1 cell line was used for evaluating *in vitro* antiadipogenic activity which was procured from National Centre for Cell Science (NCCS), Pune, India.

Chemicals used

Dexamethasone, Dimethyl sulfoxide (DMSO), Dulbecco's modified Eagle's medium (DMEM), Fetal bovine serum (FBS), Gentamycin, Insulin, Penicillin, Phosphate Buffer Saline (PBS), Roswell Park Memorial Institute medium (RPMI), Streptomycin, Trypsin, Sodium Pyruvate were purchased from Sigma Chem. Co., USA. Other chemicals are Cholesterol (Qualigens fine chem., glaxo India Ltd.), Ethylenediamine tetraacetic acid, disodium salt (EDTA) (HiMedia Laboratories Pvt. Ltd., Mumbai), Formaldehyde (Qualigens fine chem., glaxo India Ltd.), 3-isobutyl-1-methylxanthine (IBMX), Isopropyl alcohol (Sisco Research Laboratories. Pvt. Ltd., Mumbai), Sodium bicarbonate ($NaHCO_3$) (HiMedia Laboratories Pvt. Ltd., Mumbai), Oil Red O Dye, Blood glucose, Triglyceride, Total Cholesterol, FFA estimation kits (Siemens Medical Solutions Diagnostics Ltd., Baroda, Gujarat, India), Rat Insulin ELISA kit (Mercodia, Sweden), Simavastatin, Glibenclamide (Nicholas Piramal Research Limited Mumbai).

Pharmacological screening methods

In-vitro methods

In-vitro cytotoxicity assay 3T3-L1 cell line was assayed by Neutral Red Assay in order to determine *in vitro* toxicity of petroleum ether extract of BR-1 and BR-2. The cell line was cultured in DMEM complete media using 150 cm^2 culture flasks with temperature maintained at 37 °C allowing 5% CO_2 and relative humidity more than 90%. Test extract was diluted appropriately in DMEM medium, were added to the cells and again incubated for 48 h. Cells viability was determined using Neutral Red Assay procedure. Briefly, cells were seeded 2.5×10^4 cells/well at temperature maintained 37 °C and allowed to grow undistributed for 24 h then cell counts were made by using the Trypan Blue exclusion method to determine viability of cells [18]. Cell containing extract was exposed in the presence and absence of UV light. Cell observed under UV light was labelled as light extract

(LE) however dark extract (DE) was named when extract observed in dark. After incubating with samples, the cells were washed with saline and incubated for 90 min with the medium containing Neutral Red (166 ug/ml). On addition of isopranol (0.33% HCl) to lyses the cells; as a result, the incorporated dye was liberated from the viable cells. The absorbance was measured at 540 nm on a Microplate Reader (Molecular Devices, SPECTRAMAX plus 384). All the assays were performed in triplicate. The above procedure was performed as per our previous work [18]. Viability of cell was calculated by the using the formula below at 540 nm absorbance in light and dark conditions.

$$\%\ Viability\ = \frac{\text{OD sample X 100}}{\text{OD negative control}}$$

Here, OD (Optical Density/Absorbance), OD sample = absorbance of cell at different dose, OD negative control = absorbance of 100% viable cell

Dose response curve for the toxicity studies Dose response curve of BR extract for 3T3-L1 cell line is shown in Fig. 1. Extract exhibited concentration-dependent inhibitory effect with an IC_{50} value of 660.26 μg/ml.

Adipocyte Differentiation assay Differentiation was induced by subculture and seeding of preadipocyte cell line. Cells were harvested from 25 mm tissue culture flask when the cells around 70 to 80% confluent by using trypsinization. 96 well plate (2 x 10^3 cells/well cell density and volume of media per well 100 μl) tissue culture vessels was used for seeding of cells in complete media. Cells were growing for 2 days to reach 100% confluency. Cells were kept for another 48 h in this state, to arrest the cell division, then treated with adipocyte differentiation media (1 μM Dexamethasone, 0.5 mM 3-Isobutyl-1-methylxanthine, 10 μg/ml Insulin and complete media). Cells were feeded with adipocyte differentiation media after post confluency. Test extract was evaluated in different dose of 50, 100 and 200 μg/ml at day 0 for adipocyte differentiation assay and kept the cells for 96 h in this state. All the media were discarded after 96 h by gentle pipetting. Cells were easily detachable from plate at this stage. At 4^{th} day adipocyte differentiation media

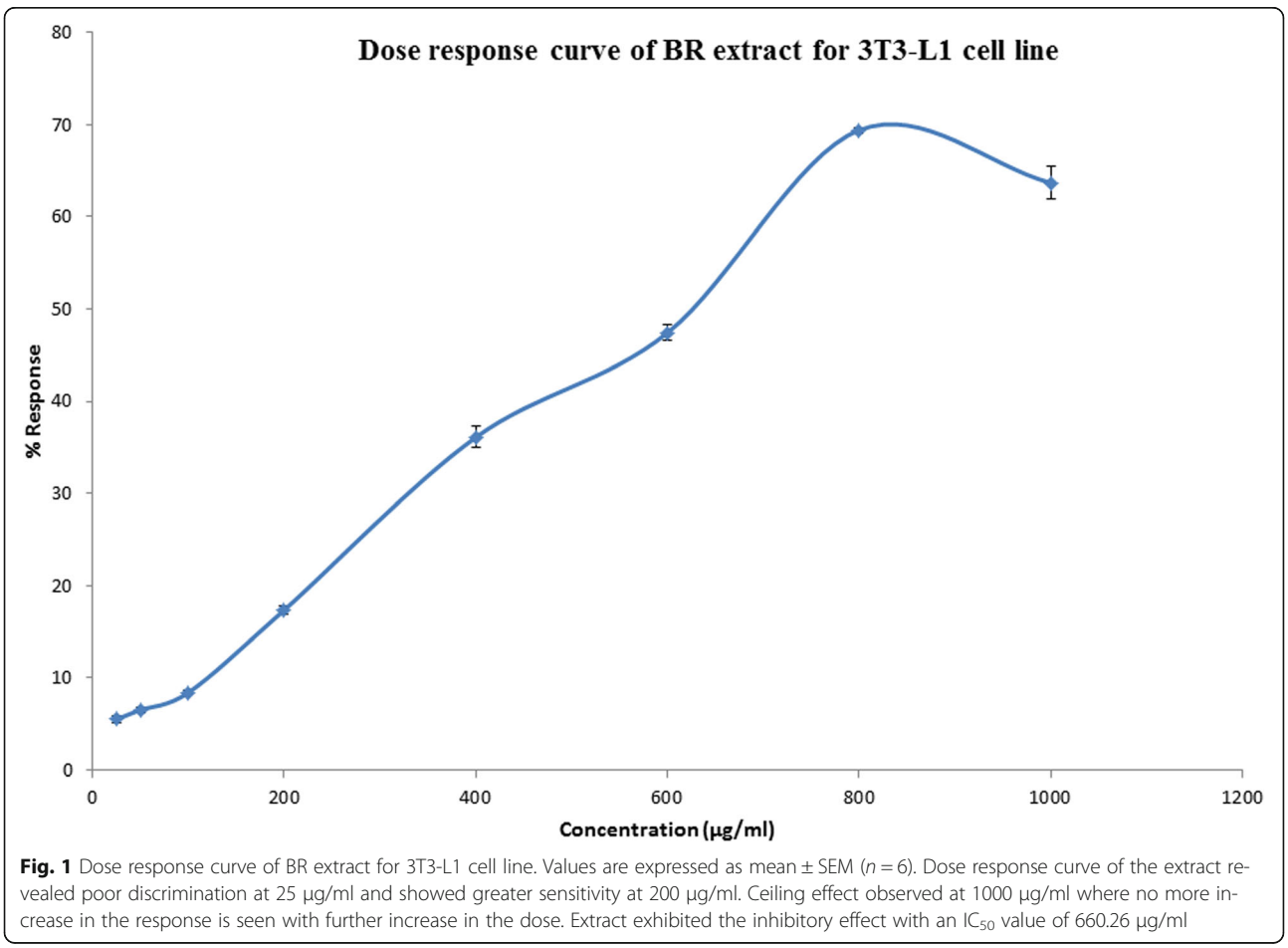

Fig. 1 Dose response curve of BR extract for 3T3-L1 cell line. Values are expressed as mean ± SEM ($n = 6$). Dose response curve of the extract revealed poor discrimination at 25 μg/ml and showed greater sensitivity at 200 μg/ml. Ceiling effect observed at 1000 μg/ml where no more increase in the response is seen with further increase in the dose. Extract exhibited the inhibitory effect with an IC_{50} value of 660.26 μg/ml

was added by subsequent changes in every 2 days. Lipid droplets were visible inside the cells after 8^{th} day and droplets enlarges at around the 14^{th} day. After day 8, water was removed and kept it in dry. Elute oil Red O by adding 100% isopropanol incubated for 10 min OD measurement at 540 nm in spectrophotometer [19]. Extract was evaluated at both light and dark conditions at 540 nm absorbance and determine percentage cell inhibition using the formula below:

$$\%\,\text{Cell inhibition} = \frac{\text{Control absorbance- Test absorbance}}{\text{Control absorbance}} \times 100$$

In-vivo experimental methods

Acute toxicity study The acute toxicity study was carried out on male Wistar rats weighing 150–200 g by administering extract orally at one of the levels (150, 500, 1000, 2000 mg/kg) once only. The dose that shows toxicity signs/morality is the toxic dose and $1/10^{th}$ of this toxic dose is considered for therapeutic exploration [20]. Toxicity study of extracts was performed as per OECD guideline No.423.

Eighteen hours fasted Rat model Male Wistar rats, six animals in each group were fasted overnight. Blood glucose determination was done initially at 0 h prior to drug treatment and finally at 3 h after drug treatment has over. Animals were divided into four groups, namely normal control 1% v/v Tween 80 (10 ml/kg), reference group (glibenclamide; 5.0 mg/kg), test group; BR-1 (250 mg/kg extract) and BR-2 (500 mg/kg extract).

Oral Glucose Tolerance Test (OGTT) Oral glucose tolerance test was performed on overnight fasted male Wistar rats. Animals were divided into different groups as normal control (untreated), glucose primed control, reference group (glucose primed + glibenclamide), test groups (glucose primed + test extract). Glucose was given in the dose of 1.5 g/kg of 10% solution to all groups except normal control. Estimation of blood was done at 0 min (prior to any treatment), 30 min and 90 min [21].

Induction of diabetes in rats Diabetes was induced by single intraperitoneal injection of freshly prepared STZ (65 mg/kg body weight) in 0.1 M citrate buffer (pH 4.5) in a volume of 1 ml/kg body weight. Nicotinamide was given in the dose of 110 mg/kg prior to induction of diabetes to reduce the beta cytotoxic effect of streptozotocin. Diabetes was established in these STZ treated rats over a period of 4 days. The control animals were treated with citrate buffer (pH 4.5). After 4 days blood was collected from the retro-orbital route and the plasma glucose level of each rat was determined. Animals were evaluate by using glucometer and considered diabetic when fasting blood glucose (FSG) level higher than 250 mg/dL [22].

Treatment Protocol for STZ induced diabetic rats model The diabetic rats were divided randomly into five different groups, each containing six animals. Normal and Diabetic control animal groups received 1% v/v Tween 80 (1 ml/kg p.o.), Reference group received glibenclamide 5.0 mg/kg p.o. and extract of BR was given in the doses of 250 and 500 mg/kg in 1% v/v of Tween 80 (1 ml/kg p.o.) for a period of 28 days to different treatment groups. The blood sample was collected on 0, 7, 14, 21 and 28 days.

Blood glucose Blood glucose was estimated by glucose oxidase/peroxidase (GOD/POD) method using a commercially available enzymatic kit from Siemens Medical Solutions Diagnostics Ltd., Baroda and Gujarat, India. The absorbance of the test samples and reference was measured against blank at 505 nm spectrophotometrically. The concentration of the glucose was calculated using the following formula:

$$\text{Concentration of glucose} = \frac{\text{Optical density of test}}{\text{Optical density of standard } \left(\frac{\text{mg}}{\text{dl}}\right)} \times 100$$

Insulin ELISA The principle of the Rat Insulin ELISA is based on a solid phase two-site enzyme immune assay. It is based on the direct Sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample reacted with peroxidase-conjugated anti-insulin antibodies and bound to microtitration well. A simple washing step removes the unbound enzyme labeled antibody. The bound conjugate was detected by reaction with 3, 3β, 5, 5β-tetramethylbenzidine. The reaction was stopped by adding acid to give a colorimetric endpoint that was read spectrophotometrically [23].

Lipid profile Different lipid parameters in blood like Triglycerides (TGs), Total Cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL) were estimated using suitable assay procedures. Triglycerides are the chemical form in which most fat exists in food as well as in the body. Triglycerides (TGs) were determined by enzymatic colorimetric method (Adipogenesis assay kit). Triglyceride concentration in the sample was measured at 546 nm. The determination of serum cholesterol was considered to be significant in Diabetes Mellitus and various other diseases. Total cholesterol (TC) was estimated by the enzymatic method as

described by [24]. HDL and LDL were estimated using commercial kits procured from Bayer Diagnostics & Siemens Healthcare, India with the aid of a clinical chemistry analyser Chem-7 (Erba, Manheim, Germany).

Statistical analysis All values were expressed as Mean ± SEM. The results were analyzed statistically using two way analysis of variance (ANOVA) followed by Bonferroni post-tests to calculate the level of significance. Values are expressed as mean ± SEM (Number of animals, $n = 6$); significantly different at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, when compared with diabetic control group.

Result

Phytochemical screening

The preliminary phytochemical screening of BR petroleum ether extract revealed the presence of saponins, sterols, carbohydrates, flavonoids, triterpenes and proteins.

In-vitro methods

In vitro toxicity of BR extract on 3T3-L1 cell lines

The BR extract was exposed to two different conditions of dark (DE) and light (LE) conditions. *In vitro* toxicity of BR extract was evaluated on 3T3-L1 cell lines by Neutral Red assay at different concentrations of 200, 100, 50 and 25 μg/ml; all the concentration showed more than 75% viability. However the extract exposed in dark condition exhibited more viability of cells at different concentrations as compared to light condition (Table 1).

Antiadipogenic activity of BR extract on 3T3-L1 cell line

BR extract showed significant antiadipogenic activity in both dark and light conditions. However the percentage inhibition of adipocyte cells by the extract in dark condition at the concentration 200 μg/ml was found to be 41.66%, which was lesser than the standard drug simvastatin (51.83%). The antiadipogenic effect of the BR extract on 3T3-L1 cell Line is shown in Table 2.

In-vivo experimental methods

Acute toxicity study

An acute toxicity study revealed that BR extract did not produce any toxic sign and symptoms even no any mortality when administered per orally to rats at a dose up to 2000 mg/kg. According to the dose safety level upto 2000 mg/kg two doses of BR extract, i.e. 250 and 500 mg/kg were selected for in-vivo antidiabetic study.

Table 1 *In vitro* toxicity of BR extract on 3T3-L1 cell lines

Group	% Viability			
	Concentration (μg/ml)			
	25	50	100	200
DE	93.33 ± 0.84	88.00 ± 0.73	83.33 ± 0.71	79.00 ± 0.25
LE	91.33 ± 1.11	85.00 ± 1.06	80.16 ± 0.60	76.66 ± 0.66

LE = BR extract evaluated under UV light, DE = BR extract evaluated in dark condition. Values are expressed as mean ± SEM (Number of experiments = 6) significantly different at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Table 2 Antiadipogenic activity of BR extract on 3T3-L1 cell Lines

S. N.	Group	% Inhibition Concentration (μg/ml)		
		50	100	200
1.	LE	20.66 ± 1.28	31.16 ± 1.47	39.33 ± 0.88
2.	DE	23.16 ± 0.65	33.83 ± 1.32	41.66 ± 1.05
3.	Simvastatin	32.50 ± 1.36	41.33 ± 0.49	51.83 ± 1.85

LE = BR extract evaluated under UV light (Light extract), DE = BR extract evaluated in dark condition (Dark extract)
Values are expressed as mean ± SEM (Number of experiments = 6) significantly different at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Effect of BR extract on eighteen hours fasted Rat model

The BR extract was found effective in reducing blood glucose level on eighteen hour fasted rat model. However reduction in blood glucose level by the extract BR-2 was found to be 58.8 mg/dl, which was nearly equal to reference drug glibenclamide (51.4 mg/dl) after 3 h. The results of reduction in blood glucose level by BR extracts have been summarized in Fig. 2.

Effect of BR extract on Oral Glucose Tolerance Test (OGTT)

In this model blood glucose level was evaluated on three time points, i.e. 0 min, 30 min and 90 min in glucose primed rats. Reduction in glucose level by BR-2 was significant at 90 min i.e. 115.65 mg/dl, which was closer to 90.44 mg/dl of reference group. Glucose level was increased at 30 min in all treatment groups, but it was reduced in a significant manner at 90 min (Table 3).

STZ Induced diabetes model

Effect of BR extract on fasting blood glucose level

Administration of the BR extract to STZ induced diabetic rats caused a significant reduction in fasting serum glucose (FSG) at level in the doses of 250 and 500 mg/kg. Reduction in FSG level was dose dependent, i.e. 134.70 mg/dl for BR-2 and 143.88 mg/dl for BR-1 as revealed on day 28th. Weekly change in FSG level of different dose groups and its comparison with the diabetic control group has been summarized in the Table 4.

Effect of BR extract on insulin level in diabetic rats

Insulin level was measured on day 1 was considered as initial reading and finally on day 28 as a final reading. BR extract caused a significant rise in the level of insulin in STZ induced diabetic rats. Insulin level was found to be 0.49 mg/dl for BR-2 extract on day 28 which was closer to the reference group i.e. glibenclamide (0.58 mg/dl). Effect of the BR extract on insulin level of

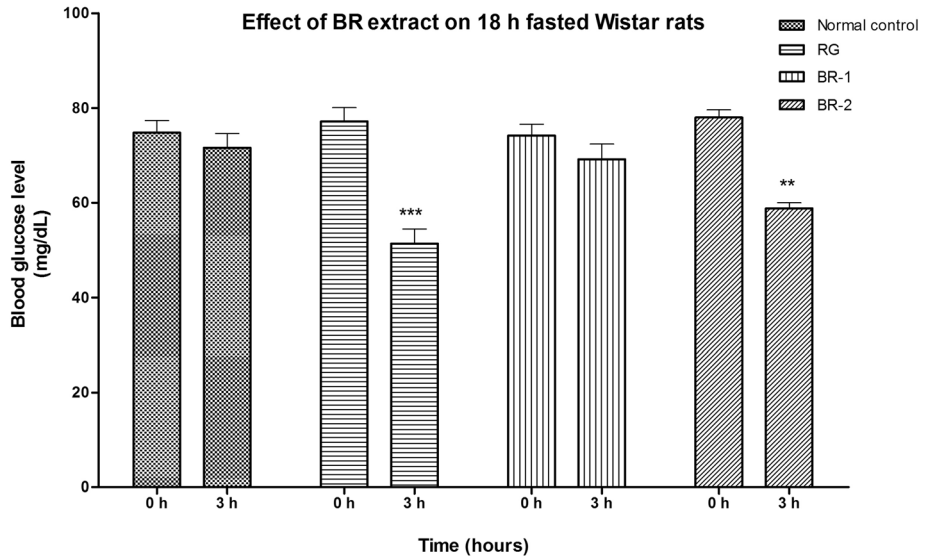

Fig. 2 Effect of BR extract on 18 h fasted Wistar rats. NC = Normal control, RG = Reference group (glibenclamide), BR-1 = BR extract in dose of 250 mg/kg, BR-2 = BR extract in dose of 500 mg/kg. Values are expressed as mean ± SEM (Number of animals, $n = 6$); significantly different at, $^{*}p < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to control group at 0 h blood glucose level

STZ induced diabetic rats is graphically summarized in Fig. 3.

Effect of BR extract on lipid profile in diabetic rats

STZ-induced diabetic rats having an increased amount of cholesterol, LDL and triglyceride as compared to their respective values in control animals. Administration of glibenclamide and BR-2 extract caused a significant reduction in the lipid profile of diabetic rats, i.e. 53.75 mg/dl and 57.66 mg/dl respectively. BR-2 extract exhibited an increase in the level of HDL (52.74 mg/dl) with a decrease in TG (87.34 mg/dl) and TC (117.38 mg/dl) levels. Lipid profile of STZ-induced diabetic rats has been summarized in Table 5.

Effect of BR extract on body weight of diabetic rats

STZ caused an abrupt decrease in body weight of animals. However treatment of diabetic animals with BR extract revealed a significant rise in body weight in a weekly pattern and the results were closer to the reference group animals (Fig. 4). At the end of the study on day 28 the body weight of BR-2 treated animals was found to be 183.48 gm which was equal to reference group animals i.e. 184.31 gm.

Discussion

This study suggested that BR possess definite antidiabetic, hypolipidemic and antiadipogenic activity in STZ-induced diabetic rats. BR extract showed a dose dependent fall in FSG with 250 and 500 mg/kg dose in experimental diabetic animals. Moreover, the daily administration of the BR extract to STZ-diabetic rats for four weeks caused a significant reduction in food and water intakes, and an increase in the body weight. STZ is a cytotoxic compound which induces

Table 3 Effect of BR extract on blood glucose level of glucose primed rats

S. N.	Group	Dose (mg/kg)	Blood Glucose Level (mg/dl)		
			0 min	30 min[#]	90 min[#]
1.	NC	Untreated	77.71 ± 0.87	78.02 ± 0.79	78.68 ± 1.13
2.	GPC	Vehicle	81.32 ± 1.08	172.91 ± 1.85***	178.65 ± 1.13***
3.	RG + glucose	5.0	79.34 ± 0.80	152.87 ± 2.12***	90.44 ± 2.42***
4.	BR-1+ glucose	250	82.30 ± 1.15	157.78 ± 2.45***	123.57 ± 3.49***
5.	BR-2+ glucose	500	81.97 ± 1.04	152.92 ± 1.02***	115.65 ± 1.45***

[#]Time post glucose administration. NC = Normal control, GPC = glucose primed control, RG = Reference group (glibenclamide), BR-1 = BR extract in dose of 250 mg/kg, BR-2 = BR extract in dose of 500 mg/kg

Values are expressed as mean ± SEM (Number of animals = 6) significantly different at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Table 4 Effect of BR extract on STZ induced diabetes in rats

S. N.	Group	Dose (mg/kg)	Blood Glucose Level (mg/dL)				
			Day 0	Day 7	Day 14	Day 21	Day 28
	NC	untreated	79.12 ± 0.78	89.83 ± 1.10	86.36 ± 1.03	87.60 ± 1.43	87.46 ± 2.37
	DC	vehicle	80.20 ± 0.56	370.04 ± 6.28	338.30 ± 13.18	297.97 ± 2.66	297.76 ± 2.97
	RG	5.0	81.35 ± 0.49	353.33 ± 3.49**	261.48 ± 2.69***	182.82 ± 3.18***	122.70 ± 3.26***
	BR-1	250	81.37 ± 0.47	361.00 ± 2.80	281.10 ± 2.55***	204.49 ± 3.79***	143.88 ± 2.06***
	BR-2	500	79.72 ± 0.74	355.41 ± 3.17*	264.23 ± 2.56***	191.24 ± 2.69***	134.70 ± 1.58***

NC = Normal control, DC = Diabetic control, RG = Reference group (glibenclamide), BR-1 = BR extract in dose of 250 mg/kg, BR-2 = BR extract in dose of 500 mg/kg
Values are expressed as mean ± SEM (Number of animals = 6) significantly different at *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

diabetes by damaging β pancreatic cells that causes a reduction in insulin release. [25], reported that extracts of medicinal plants causes activation of β cells as an antidiabetic effect and hence showing insulinogenic action. STZ causes persistent hyperglycemia through destruction of pancreatic β-cells (type-I diabetes mellitus). The possible mechanism through which BR extracts showed an antidiabetic effect might have been due to increased utilization of glucose by peripheral tissues, improved sensitivity of target tissues for insulin or it may be due to β-cell stimulation. Extract of BR exhibited hypocholesterolemic and hypotriglyceridemic effects, while increasing the levels of HDL in diabetic rats. These findings have been well justified by various reports in the literature stating that some medicinal plants show antidiabetic as well as hypolipidemic effects [26].

The elevation of serum insulin in BR extract treated STZ-induced diabetic rats could either be due to the insulinotropic substances present in the extract, which induce the intact functional β-cells to produce insulin, or the protection of the functional β-cells from further deterioration so that they remain active and produce insulin. Similarly the extracts of *Medicago sativa* [27], *Eucalyptus globulus* [25, 28] and *Sambucus nigra* [29] have been reported to possess antidiabetic property by insulin-releasing action. Since insulin inhibits the activity of Glc-6-Pase in the liver of diabetic rats and controls hepatic glucose production (HGP), the insulinotropic effect of BR might play an immense role in the control of diabetes in STZ-induced diabetic rats. The suppression of Glc-6-P hydrolysis could also be one of the reasons for the hypoglycemic effect of the BR extract in diabetic rats. The improvement in lipid

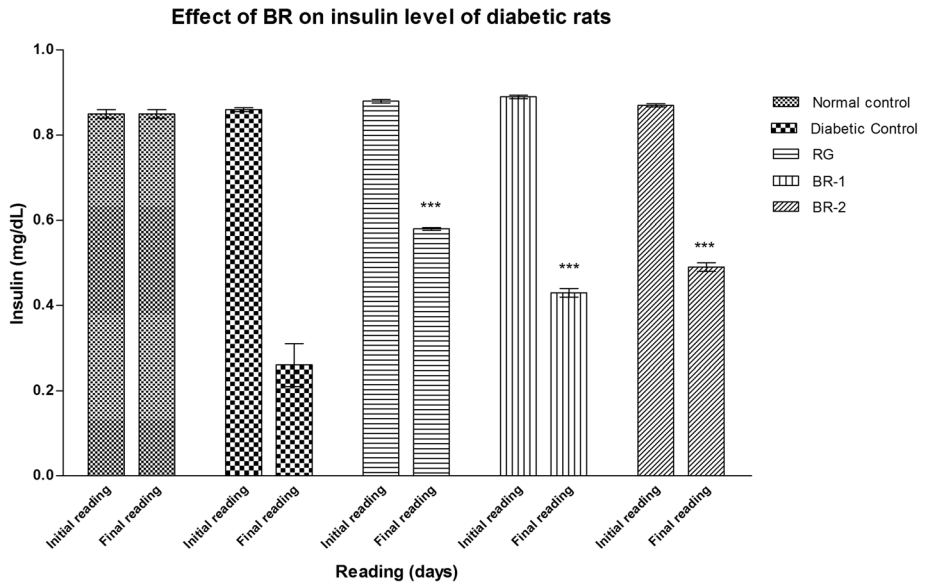

Fig. 3 Effect of BR extract on Insulin Level of STZ induce diabetes rats. NC = Normal control, DC = Diabetic control, RG = Reference group (glibenclamide), BR-1 = BR extract in dose of 250 mg/kg, BR-2 = BR extract in dose of 500 mg/kg. Values are expressed as mean ± SEM (Number of animals, $n = 6$); significantly different at, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ when compared with diabetic control group

Table 5 Effect of BR extract on Lipid profile of STZ induced diabetes in rats

S. N.	Group	Dose (mg/kg)	Lipid profile (mg/dL)			
			TG	TC	HDL	LDL
1	NC	Untreated	88.90 ± 0.92	97.08 ± 1.003	57.37 ± 0.57	47.52 ± 0.57
2	DC	Vehicle	106.47 ± 2.12	148.91 ± 0.80	33.39 ± 1.27	105.16 ± 3.61
3	RG	5.0	85.88 ± 0.34***	106.00 ± 0.40***	57.30 ± 0.65***	53.75 ± 0.78***
4	BR-1	250	89.84 ± 0.78***	126.29 ± 1.37***	45.14 ± 1.32***	60.14 ± 1.31***
5	BR-2	500	87.34 ± 0.77***	117.38 ± 0.75***	52.74 ± 0.44***	57.66 ± 1.21***

NC = Normal control, DC = Diabetic control, RG = Reference group (glibenclamide), BR-1 = BR extract in dose of 250 mg/kg, BR-2 = BR extract in dose of 500 mg/kg
Values are expressed as mean ± SEM (Number of animals = 6) significantly different at *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

profile by these extracts also supports their antidiabetic activity along with antihyperlipidemic activity [30].

BR extract showed more than 75% of viability of cells during in-vitro study. The extract of BR revealed significant inhibition in differentiated cell as compared to non-differentiated cell in 3T3-L1 cell line [31]. Improvement in lipid profile reduces the possibilities of diabetes induced obesity and cardiovascular diseases [32]. BR extract normalized the amount of triglycerides (TG) and total cholesterol (TC) in STZ-induced diabetic rats and may be effective for the treatment of cardiovascular disease like coronary artery disease, atherosclerosis etc. Triglycerides (TG) are prepared from carbohydrates as a major energy source or derived from fats, by taking food [33]. Calories present in food is not absorbed immediately by tissues; it is converted into TG and transported to fat cells for storage. Release of triglycerides from fat tissues is regulated by hormones to meet the body's needs for energy between meals. Excess triglycerides in plasma are called hypertriglyceridemia. Elevation in triglyceride level may be a consequence of another disease like untreated diabetes mellitus. STZ-induced diabetes and adipocyte differentiation assay have been used as a model to evaluate the effect of test products on lipid/fat metabolism [34, 35]. To develop the antidiabetic drugs and antiadipogenic drugs the lipid centric approach has been widely used.

Conclusion

It is revealed from the present study that BR extract possess antidiabetic activity due to decreasing blood glucose level of STZ-induced diabetic rats. Furthemore, BR extract also exhibits lowering effect on lipid level and adipose tissue. Due to this property the extract showed significant antiadipogenic and hypolipidemic effect. Pharmacologically, the extract improved the lipid profile by decreasing the levels of serum triglycerides, total cholesterol, LDL and increasing HDL cholesterol.

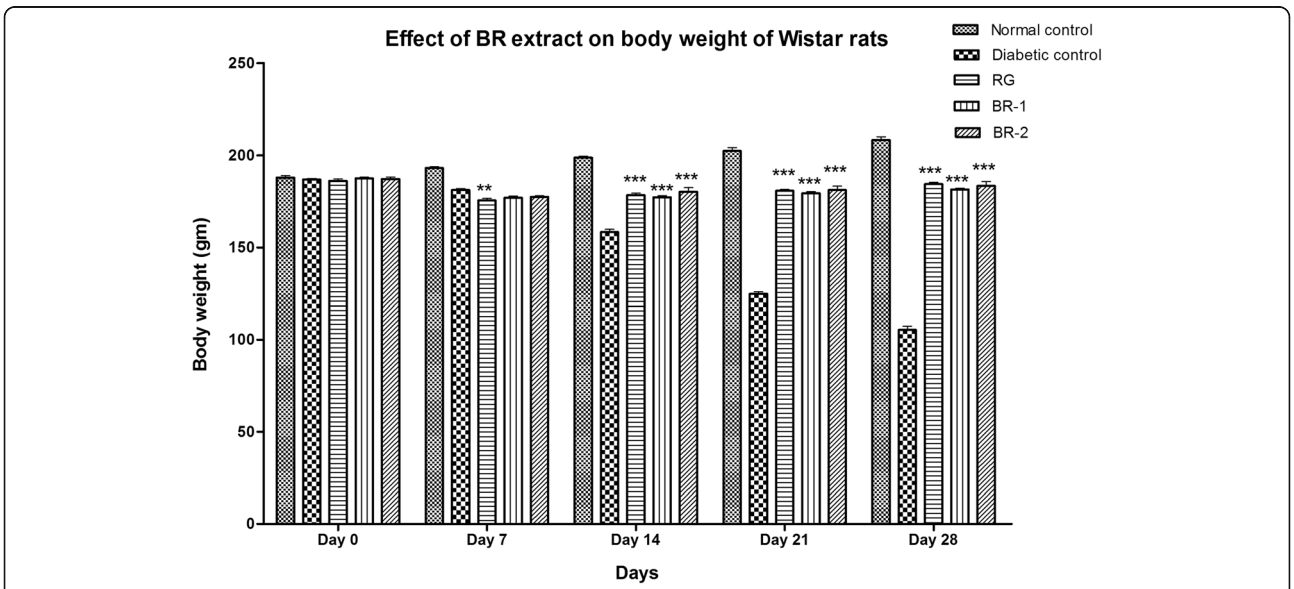

Fig. 4 Effect of BR extract on body weight of diabetic rats. NC = Normal control, DC = Diabetic control, RG = Reference group (glibenclamide), BR-1 = BR extract in dose of 250 mg/kg, BR-2 = BR extract in dose of 500 mg/kg. Values are expressed in mean ± S.E.M. Where $n = 6$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; compared with diabetic control group

Conclusively, these effects of BR may be useful for the development of potent herbal medicine for diabetes. The relevance of anti-obesity mechanism of BR extractmay be more appropriate. Further studies to isolate, identify and characterize the active principle(s) are in progress.

Abbreviations

BR: *Bauhinia racemosa*; BR-1: test group-1; BR-2: test group-2; DC: diabetic control; DE: dark extract; DMEM: Dulbecco's modified Eagle's medium; DMSO: Dexamethasone, Dimethyl sulfoxide; EDTA: Ethylenediamine tetraacetic acid; FBS: Fetal bovine serum; HDL: High density lipoprotein; IDDM: Insulin dependent diabetes mellitus; LDL: Low density lipoprotein; LE: Light extract; NC: Normal control; NIDDM: Non-insulin dependent diabetes mellitus; RG: Reference group; RPMI: Roswell Park Memorial Institute medium; TC: Total cholesterol; TG: Triglycerides

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Authors' contributions

VK carried out the pharmacology evaluation, participated in the dosing, animal care, feeding etc. KR carried out the in-vitro antiadipogenic studies. PJ participated in the design of the study, performed the statistical analysis and drafted manuscript. ZA conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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