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Research Article

COMPARATIVE EVALUATION OF HYPOGLYCEMIC EFFECTS OF TWO DIFFERENT PARTS OF *BAUHUNIA PURPUREA* LINN. PLANT IN STZ-INDUCED DIABETIC ALBINO WISTAR RATS

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ABSTRACT: The present work was undertaken to study the comparative phytochemical profiles and hypoglycemic effects of *Bauhinia purpurea* Linn. Barks (BPBE) and leaves ethanolic extracts (BPLE) in albino wistar rats to validate their ethno medical use in hyperglycemia as well as to explore the better option. Phytochemicals in ethanolic extracts were analyzed by standard natural product chemistry methods. Diabetes was developed in rats by single intraperitoneal injection of Streptozotocin @ 60mg/ Kg bw. Diabetic albino wister rats (n=3) of either sex (150-200gm bw) were orally fed with the extracts once daily for 4 weeks. Glibenclamide @ 0.5mg/Kg bw was used as a positive control for comparison. Fasting blood glucose level at 0, 14th and 28th day and hemoglobin and glycosylated hemoglobin on 28th day of experiment were analyzed. Our results show that the extracts contain alkaloids, flavonoids, glycosides, terpenoids, tannins and phenolics. Rats treated with plant extracts show better glucose modulation, decreased hemoglobin glycosylation and improved hemoglobin concentration as compared to diabetic control. The hypoglycemic effect of only BPBE at 420 mgkg^{-1} on 14th and 28th day is comparable to that of standard drug glibenclamide (P>0.01). The bark extract has been observed to be more potent hypoglycemic agent than leave extract.

Key words: *Bauhinia purpurea*, Barks and leaves, Ethanol extract, Hypoglycemic, Diabetic albino wister rats.

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INTRODUCTION

Diabetes mellitus (DM), caused by inherited and/or acquired deficiency or inadequate secretion of hormone insulin (type I or insulin-dependent diabetes mellitus (IDDM)) or due to an inadequate response of target cells to insulin (type II or noninsulin-dependent diabetes mellitus (NIDDM)), or by a combination of these factors culminating in hyperglycemia is a disease as old as mankind (Wadkar *et.al* 2008). Recent reports indicate DM is today the world's leading endocrine disorder, constituting approximately six percent of the population, of which 90 percent is type II DM (Wadkar *et al*., 2008). The currently available therapeutic options, such as oral hypoglycemic and insulin, are not always satisfactory in maintaining euglycemia and in avoiding late stage DM complications. Additionally, the chronic nature of the disease and the associated complications require regular treatment and incur huge financial expenditure on the family and the healthcare system. Therefore, a need for alternative drugs that are effective, inexpensive, affordable, and safe to consume is being recognized. One of the best approaches in identifying novel antidiabetic agents is to scientifically investigate the efficacy of medicinal plants recognized to be effective in the various traditional system of medicine (Grover *et al*., 2002).

Bauhinia purpurea (BP), commonly known as purple orchid tree, belonging to the family Caesalpinaceae, sparingly grown in India. It is a species of flowering plant used in several traditional medicine systems to cure various diseases. This plant has been known to possess antibacterial, antidiabetic, analgesic, anti-inflammatory, anti-diarrheal, anticancerous, nephroprotective and thyroid hormone regulating activity. A wide range of active chemical compound including 5,6-Dihydroxy-7-methoxyflavone 6-O-β-D xylopyrano-side, bis[3,4-dihydroxy-6-methoxy-7,8-furano-5,6-monomethylalloxy]-5-C-5-biflavonyl and (4-hydroxy-7-methyl 3-C-α-L-rhamnopyranosy) - 5 - C – 5 -(4-hydroxy-7-methyl) - 3 – C - α-D-glucopyranosyl], biflavonoid, bibenzyls, dibenzoxepins, mixture of phytol fatty esters, lutein, β-sitosterol, isoquercitin and astragalin etc. (Muralikrishna *et al*., 2008, Boophong *et al*., 2007, Sabira *et al*., 2014, Chanchal *et al*., 2015) have been isolated from different parts of this plant. The present study was undertaken to testify the comparative hypoglycemic potential of this plant in type I DM rats, induced by STZ, using ethanolic extracts from two different parts of *Bauhinia purpurea* to validate its ethno medical use in hyperglycemia as well as to select more potent plant extract out of two (if any) to continue further studies with its semi purified fractions in our laboratory.

MATERIALS AND METHODS

Plant Materials

The barks and leaves of *B. purpurea* (BP) were together collected in 2013 at Shantiniketan campus of Visva-Bharati University, Birbhum district, India, in May and authenticated by Dept.of Botany, Visva-Bharati Shantiniketan, India. The herbarium for future reference has been kept in Dept. of Veterinary Pharmacology and Toxicology, WBUAFS, Belgachia, Kolkata.

Preparation of Plant Extracts

The plant materials (barks and leaves) were shade dried and pulverized in mechanical mill. The appropriate dried powdered plant materials (200 gm) were kept in 2000 ml of 90% ethanol at room temperature for 10 days.

The ethanol liquor was then filtered off using cotton plug. The filtrate obtained was subjected to repeated filtration for three times and was converted to a reduced mass on removal of ethanol under reduced pressure in a rotary vacuum evaporator. The semisolid mass is the crude ethanolic extract (CEA) of BP bark (BPBE) and leaves (BPLE). The average yield was 13.56 gm of BPBE and 12.36 gm of BPLE. Phytochemical screening of BPBE and BPLE were done according to standard natural product chemistry methods (Sarkar *et al.*, 2006, Brahmachari 2009) before storing at – 20^0C for further analysis. A known amount of each CEA was dissolved in ethanol and used for preliminary screening of hypoglycemic activity in diabetic albino wistar rats.

Chemicals and reagents

Glucose and other biochemical parameters' estimation kits were obtained from Span Diagnostics Ltd. India and Lab. care Diagnostics (India) Pvt. Ltd. Streptozotocin (STZ) was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Standard drug glibenclamide pure samples were procured from M/S Hindustan Chemicals & Pharmaceuticals, West Mumbai, India. All organic solvents were of analytical grade purchased from local companies.

Animals

The study was conducted on albino wistar rats of either sex, five to six weeks of age weighing between 150–200 g, housed in polypropylene cages at an ambient temperature of 25 ± 2°C with 12 h light and 12 h dark cycle. Rats were fed standard diets and water *ad libitum*. The animals were allowed to acclimatize to the laboratory environment for 1 week. All procedures complied with the standards for the care and use of animal subjects as stated in the guidelines laid by Institutional Animal Ethical Committee (IAEC), West Bengal University of Animal and Fishery Sciences, West Bengal, India.

Acute toxicity study

The acute oral toxicity study was carried out in accordance to the guidelines set by the Organisation for Economic Co-operation and Development (OECD 2001). Twenty seven rats were fasted overnight and divided into 9 groups (n=3 in each group). Group 1 was administered vehicle only and served as control. Group 2-5 were administered range of concentration (1500, 2000, 2500 and 3000 $mgkg^{-1}$ bw) of BPBE. Group 6-9 were administered range of concentration (1500, 2000, 2500 and 3000 $mgkg^{-1}$ bw) of BPLE. The animals were observed continuously for the first two hours, followed by once every hour up to 6 h for any changes in behavioral, neurological, and autonomic profiles and then every 24 hours up to 14 days to identify lethality. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate, convulsion, initial and final body weights, water and food intake, state of stool and body temperature. On day 15^{th} the animals were sacrificed under ether anesthesia. Hematological and serum biochemical parameters such as hemoglobin content, WBC total count, serum protein, ALT, AST, urea nitrogen, cholesterol, creatinine and alkaline phosphatase were determined. Liver, spleen, and kidneys were dissected out and observed for morphological changes. Weights of these organs were determined.

Effect of BP extract on serum glucose levels of normal fasted rats

The fasting blood sugar level of each animal was determined after overnight fasting for 18h.

Normoglycemic rats were divided into eight experimental groups (n = 3 per group). The first group of animals was given DW through canula and served as control (NC). Group II was given glibenclamide (0.5 $mgkg^{-1}$), taken as standard (PC). Group III–VIII was given an ethanolic suspension of BP extracts (bark, and leaves) orally at a dose level corresponding to their $1/5^{th}$,$1/10^{th}$ and $1/20^{th}$ of respective LD_{50} values. The fasting blood samples were collected from the tail vein just prior to and at 1, 2, 4, and 8 h after the extract administration. Serum glucose was measured by GOD/POD enzymatic method using standard biochemical kit (Span Diagnostic Ltd.).

Determination of the oral glucose tolerance test (OGTT) in normal rats

OGTT experiment was performed as explained by Gokee *et al*., (2008). The normal rats were divided into 4 groups (n = 3) and were fasted overnight (18 h). The next day the rats were administered either drinking water (NC) or glibenclamide (PC) (0.5 $mgkg^{-1}$ used as the standard drug) or $1/5^{th}$ of LD_{50} of the two extracts. Glucose (2 gkg^{-1}) was administered 30 min after feeding the extracts. Blood was withdrawn from the tail vein under ether inhalation anesthesia at 30, 60, 120 and 180 min of glucose administration and blood glucose is estimated using biochemical kits in a spectrophotometer (Systronic 125).

Induction of IDDM

The rats were fasted overnight and were administered freshly prepared STZ solution (60 mg/kg body weight dissolved in cold citrate buffer, (0.1M, pH 4.5) intraperitoneally. DM was identified by polydipsia, polyuria, and by measuring non fasting plasma glucose levels. Animals with postprandial glycemia over 225 mg/dl, 3 days after STZ administration, were considered diabetic. Whole blood was collected from the tail vein of the rats for glucose estimation in serum. Control rats received only citrate buffer. (Mondal *et al*., 2012).

Experimental design and treatment schedule

The effect of the extracts was studied in STZ-induced diabetic rats for 28 days. The rats (n = 3 per group) were divided into 9 groups: group 1: normal rats treated with vehicle alone (NC); group 2: diabetic control treated with STZ solution (60 $mgkg^{-1}$ body weight dissolved in cold citrate buffer, 0.1M, pH 4.5) intraperitoneally *(i.p.*) (DC); group 3: diabetic rats treated orally with glibenclamide @0.5mg/Kg bw (PC); group 4,5 and 6 diabetic rats were administered BPBE@$1/5^{th}$, $1/10^{th}$ and $1/20^{th}$ of its LD_{50} respectively; groups 7,8 and 9 diabetic rats were administered BPLE@$1/5^{th}$,$1/10^{th}$ and $1/20^{th}$ of its LD_{50} respectively; the vehicle, standard drug, and extracts were administered orally to respective groups once daily for 28 days.

Fasting blood samples were collected from the tail vein at 0, 14^{th}, and 28^{th} days and quantified for the blood glucose levels. On day 28, the fasted animals were euthanized under ether inhalation. The blood was collected by cardiac puncture and processed for estimation of the levels of hemoglobin(Span Diagnostic Ltd., India) and glycosylated hemoglobin (HbA_1) by using standard reagent kits (Labcare Diagnostics, India Pvt. Ltd). Briefly, 0.25 ml lysing reagent is mixed with 0.05 ml of sample, mixed well and allowed to stand at RT for 5 min. 0.1 ml hemolysate added to 3 ml resin tube and resin separator was placed in such a manner so that the rubber sleeve is approximately 3 cm above the resin level. The contents were mixed and vortexed continuously

Table 1. Phytochemical constituents of *B. purpurea* barks and leaves crude ethanolic extracts.

Plant Extracts	Phytoconstituents							
	Tannins	Alkaloids	Saponins	Glycosides	Terpenes	Flavonoids	Phenols	Anthraquinones
BPBE	+	+	-	+	+	+	+	+
BPLE	+	-	-	+	+	+	+	-

BPBE: *B. purpurea* barks extract BPLE: *B. purpurea* leaves extract. +: Present; -: Absent.

for 5 min. The resin was allowed to settle for 5 min and the resin separator was pushed down in the tube until resin is firmly packed. The supernatant was collected and absorbance was taken at 415 nm. To measure total hemoglobin, 0.02 ml of hemolysate was added to 5 ml of deionized water and mixed well. Absorbance was taken at 415nm. Glycosylated Hb% (GHb) was calculated using the formula:

(Absobance of GHb / Absobance of total hemoglobin) x 7.2

Assay temperature was 23°C.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism software package Version 6.0. The values were analyzed by analysis of variance (ANOVA) followed by Dunnett's multiple comparison test, Tukey's multiple comparison test or Sidak's multiple comparison test as applicable for the data.

RESULTS AND DISCUSSION

DM is recognized to be a common and serious metabolic disorder throughout the world. Although, oral hypoglycemic agents/ insulin are the mainstay of treatment of DM and are effective in controlling hyperglycemia, they have prominent side effects and fail to significantly alter the course of DM complications. The management efforts of hyperglycemia and the associated complications are labor intensive and challenging to both patients and the physicians. Furthermore, the economic burden associated with DM is severe to both the patient and the society (Einstein *et al*., 2012). Medicinal plants used in the various alternative and complementary systems of medicine are known to play an important role in the management of DM especially in developing countries where primary care facilities are compromised.

The detailed study of plant constituents for two crude ethanolic extracts have been done by standard natural products chemistry methods (Brahmachari 2009) and is depicted in Table 1. Bark extracts show the presence of alkaloids, phenols, tannins, terpenoids, flavonoids whereas leaves extracts show the presence of glycosides, tannins, terpenoids, flavonoids and phenolics.

The acute toxicity studies showed that the oral administration of the extracts caused death in the higher doses of 2.5 and 3 $gmkg^{-1}$ bw. So the extracts are toxic to some extent. The observed probits were plotted against log dose and LD_{50} values were obtained from the point of intersection. The LD_{50} values obtained for BPBE and BPLE were 2100 mg and 2300 mg/ kg bw. respectively.

The hypoglycemic effects of the two extracts

Table 2. Comparative effect of crude ethanolic extract of *B. purpurea* bark (BPBE) and leaves (BPLE) on fasting blood glucose level (mg%) in normal albino wistar rats at different doses.

Treatment groups	Fasting blood glucose level at different time points (hours).				
Control	0	1	2	4	8
	99.67± 1.20	98.67± 0.67	97.33± 0.66	96.67± 0.66	99.33± 1.33
Rats treated with glibenclamide @0.5 mg/Kg bw	99.67± 1.20	98.33± 1.85	85.33 ±0.66*	73.33± 1.76*	64.00± 1.15*
Rats treated with BPBE@105mg Kg^{-1} bw	84.67± 1.33*	85.33 ±1.76*	83.33 ±1.76*	85.33± 1.76*	86.00 ±2.30*
Rats treated with BPBE@210mg Kg^{-1} bw	84.67± 1.33*	79.33± 0.66*	78.00± 1.15*	76.67± 0.66*	82.00± 1.15*
Rats treated with BPBE@420mg Kg^{-1} bw	82.00± 2.00*	82.00± 1.15*	68.67± 0.66*	65.33± 0.66*	83.33± 1.33*
Rats treated with BPLE@115mg Kg^{-1} bw	95.33± 1.76	91.33± 1.33*	91.33± 1.33	94.00± 3.06	95.33 ±0.67
Rats treated with BPLE@230mg Kg^{-1} bw	84.67± 1.33*	79.33± 0.67*	77.33± 0.67*	84.67± 1.33*	84.00± 1.15*
Rats treated with BPLE@460mg Kg^{-1} bw	82.67± 1.76*	80.67± 0.67*	76.00± 2.00*	84.00±1.15*	84.67± 1.33*

All values are Mean± SEM (n=3).
* significant (P<0.01) as compared to control in a column (Two way ANOVA followed by Tukey's multiple comparison test).
BPBE: *B. purpurea* bark extract; BPLE *B. purpurea* leave extract.

were evaluated in the fasting normoglycemic animals by administering $1/5^{th}$, $1/10^{th}$ or $1/20^{th}$ of the LD_{50} dose and estimating the blood glucose levels at 1, 2, 4, and 8 h after treatment. The results showed that most of the extracts from 2 h through 8 h of the study had shown significant hypoglycemic effect (P < 0. 01) as compared with control, as shown in Table 2 and Table 2a. Tukey's multiple comparison test shows that both the extracts at most of their dose levels are more effective as compared to PC at 1 and 2 h of experiment (P<0.01). No significant difference in hypoglycemic activity was observed (P>0.01) between the higher doses ($1/5^{th}$ and $1/10^{th}$ of LD_{50}) of the two extracts when compared independently by Tukey's multiple comparison test. It is important to note that the plant extracts have shown hypoglycemic responses earlier than the standard drug glibenclamide.It may be

Table 2a. Two way ANOVA table (α =0.05) for comparative effect of crude ethanolic extract of *B. purpurea* bark (BPBE) and leaves (BPLE) on fasting blood glucose level (mg%) in normal albino wistar rats at different doses.

Source of Variation	SS	DS	MS	F	P value
Interaction	3427	28	122.4*	F (28, 64) = 24.18	P < 0.0001
Hours	843.0	4	210.7*	F (4, 64) = 41.63	P < 0.0001
Treatment	5560	7	794.2*	F (7, 16) = 97.75	P < 0.0001
Subjects (matching)	130.0	16	8.125	F (16, 64) = 1.605	P = 0.0932

*P<0.01

attributed to fast absorption of the extracts and/ or their metabolites in gut reaching the circulation and target tissues and may have potency to stimulate insulin secretion that may eventually leads to increased peripheral utilization of glucose.

Table 3 demonstrates that the groups treated with BPBE 420 $mgkg^{-1}$ showed initial rise in blood sugar up to 30 min, then the level decreased significantly (P<0.01) maintained up to 180 min. A similar pattern was also observed in case of BPLE at 460mg kg^{-1} starting from 60 min through 120min.So at these time points glucose tolerance of the animals has been significantly increased by both the extracts. As compared to control no significant difference (P>0.01) between two plant extracts was observed in this study through the entire duration of experiment. Their effects are comparable with that of standard drug, as no significant difference (P>0.01) except at 0.5 h between the standard drug and other two plant extracts was evident from two way ANOVA followed by Dunnett's multiple comparison test.

Chronic administration of two extracts of BP has revealed the fact that they at a dose dependent fashion has started exhibiting the significant (P<0.01) anti-hyperglycemic effect from 14^{th} day onwards reaching the maximum beneficial effect on 28^{th} day. (Fig.1, 2 and 3). Anti-hyperglycemic effect exhibited by all other dose levels of the both extracts except BPBE 420 $mgkg^{-1}$ on 14^{th} and 28^{th} day is not comparable to that of standard drug glibenclamide (P>0.01) as revealed by Tukey's multiple comparison test of significance of difference among treatment means following one way ANOVA. Glibenclamide is a standard antidiabetic drug that stimulates insulin secretion from beta cells of islets of Langerhans. Therefore, the present study results indicate that the bark and leaves extracts of BP may have insulin stimulating property. The earlier research works have claimed that flavonoids present in plant extracts, that has been detected in BPBE and BPLE in our experiment, inhibit cycloxygenases and promotes β-cell regeneration besides having insulin secretary property (Singh *et al*., 1976, Geeta *et al*., 1994, Gupta *et al*., 1994).The hypoglycemic effect of the extracts also satisfies the earlier reports revealed by the researchers (Pahwa *et al*., 2012;

Table 3. Comparative effect of crude ethanolic extract of *B.purpurea* bark (BPBE) and leaves (BPLE) in glucose induced hyperglycemic rats.

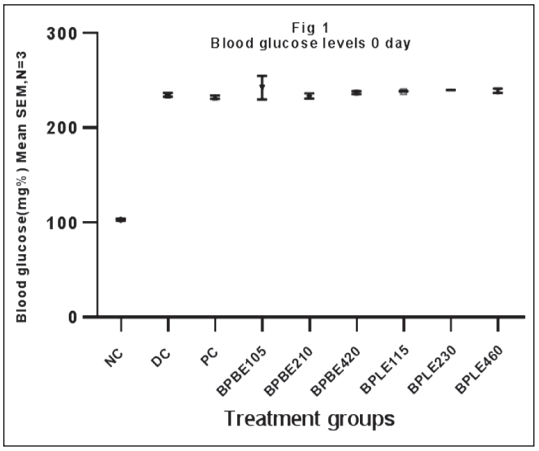
Treatment groups	Fasting blood glucose level (mg%) at different time points (minutes).				
	0	30	60	120	180
Control (NC)	101.00± 1.52	152.00± 2.30	131.66± 2.02	111.66± 0.88	108.66± 0.66
Rats treated with glibenclamide @0.5mg/Kg bw (PC)	97.33 ± 0.66	119.33±1.76*	111.33± 0.66*	104.00± 1.15	102.00±2.30
Rats treated with BPBE@420mg Kg^{-1} bw	90.00 ± 4.16*	156.00± 1.15	111.33± 4.37*	94.66 ± 2.66*	97.33 ±2.40*
Rats treated with BPLE@460mg Kg^{-1} bw	86.66 ±0.66*	146.66± 0.66	100.67± 0.66*	107.33± 0.66*	100.66 ± 0.66

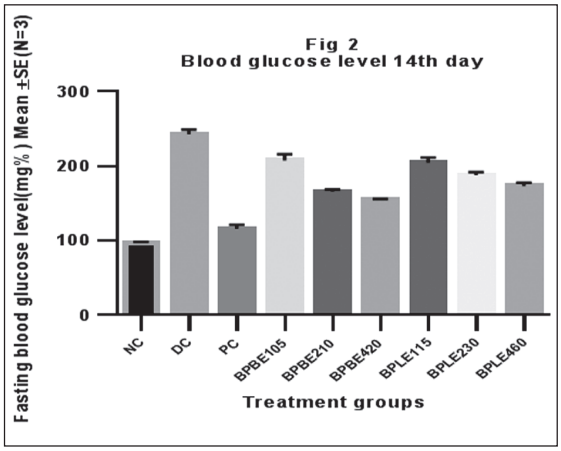
BPBE: *B.purpurea* bark extract; BPLE *B. purpurea* leave extract.
All values are Mean ± SEM (n=3).
*Significant($P<0.01$) as compared to control in a column (Two way ANOVA followed by Sidak'smultiple comparison test).

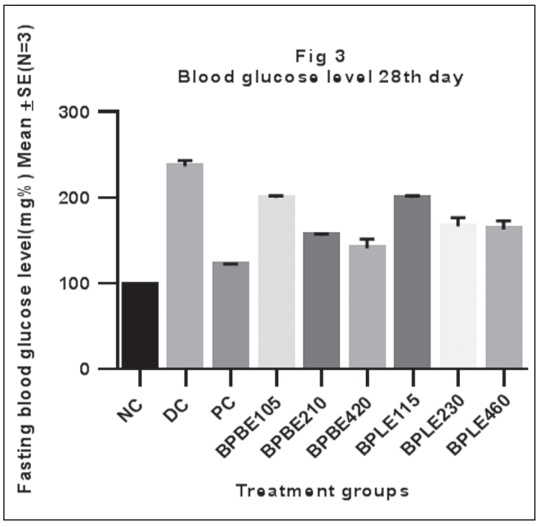
Meshram *et al*., 2013).

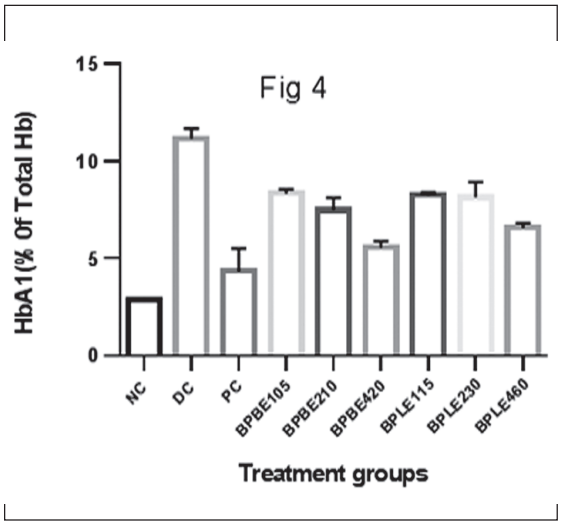
Tukey's multiple comparison test for blood glucose values also reveal that there is significant difference ($P<0.01$) in hypoglycemic effects between BPBE and BPLE at their higher dose levels($1/5^{th}$,$1/10^{th}$ of LD_{50}), the former being more potent on 14^{th} and 28^{th} day that may be due to comparatively more pancreatic beta cell stimulation. All treatment groups of the two extracts differ significantly ($P<0.01$) at all time points as compared with DC.

Similarly both BPBE and BPLE at all dose levels have significantly ($P<0.01$) reduced the glycosylated haemoglobin (HbA_1) level on 28^{th} day. However their action is not comparable with standard drug glibenclamide($P>0.01$) except BPBE at 420 $mgkg^{-1}$ as evident from Tukey's multiple comparison test of significance of difference among treatment means following one way ANOVA. It also reveals that there is no significant difference($P>0.01$) between the two same dose groups of the plant extracts in reducing glycosylation of hemoglobin that may be due to equal potency to stimulate pancreatic beta cell activity (Fig.4). Blood hemoglobin levels as compared to DC has been improved significantly ($P<0.01$) by both the plant extracts at all the dose levels with higher doses showing more beneficial effects. It is important to note that the typical characteristic of diabetes is the increase of serum glycosylated protein such as glycosylated hemoglobin (HbA_1), which is a parameter for glycemic control where glucose or other reducing sugars react with the amino residues of proteins to form Amadori products–









HbA_1 (Singh *et al*, 2001).Hence, the active constituents of these plant extracts may play a potential role in inhibiting the biosynthetic pathway of Amadori products.

The first probable hypoglycemic mechanism of action of the BP plant extracts could be linked to increase in plasma insulin. Though the exact mechanism of action of the extracts can not be concluded on the basis of present study, it could be due to increased pancreatic secretion of insulin from existing beta cells as shown in the biochemical tests, namely, glucose, and HbA_1. Second mechanism could be antioxidant property as exerted by the phytoconstituents. *Bauhinia purpurea* contain major class of secondary metabolites such as glycosides, flavonoids, saponins, triterpenoids, phenolic compounds, oxepins, fatty acids and phytosterols. From the ethanolic extract of the whole plants of *B. purpurea* two new oxepins named bauhiniastatins 1 and 2 have been isolated (Pettit *et al.*, 2006). A novel flavones glycoside,

5,6-dihydroxy-7-methoxyflavone 6-*O*-b-D-xylopyranoside was isolated from the chloroform-soluble fraction of the ethanolic extract of *B. purpurea* stems (Yadav and Tripathi 2000). The two new dimeric flavonoids namely bis [3', 4'-dihydroxy-6-methoxy - 7, 8 - furano - 5', 6'-monomethylalloxy]-5- C - 5 - biflavonyl and (4'hydroxy-7-methyl 3- C-α-rhamnopyranosyl)-5-C-5-(4'-hydroxy-7-methyl - 3 – C – α - D-glucopyranosyl) bioflavonoid with protein precipitating properties were obtained from 70% aq. acetone extract of *B. purpurea* leaves (Yadav and Bhadoria 2005). The leaves of *B. purpurea* also afforded a mixture of phytol fatty esters, leutin and β-sitosterol (Ragasa *et al*., 2004). The petroleum ether fraction of ethanolic extract (95%) of *B. purpurea* leaf gave α- amyrin caprylate on successive column chromatography with petroleum ether and chloroform which gives Liebermann-Burchard test of triterpene. The compound is characterized by spectral analysis (Verma and Chandrashekar 2009). The bark and leaves extracts prepared using different organic solvents or solvent partioning exhibited potent antioxidant activity in terms of DPPH and NO scavenging capacity *in vitro* (Urmi *et al*., 2013). Previous studies have conclusively shown that the phytochemicals such as phenolics, terpenoids, tannins, and flavanoids are potent antioxidants *in vivo* and possess hypoglycemic effects (Cazarolli *et al*., 2008, Yamasaki *et al*., 2011) The beneficial effects of diverse groups of phytochemicals in herbal remedies have been related to activities consistent with their potential use in treating diabetic disorders and complications. The *in vivo* anti-diabetic activity of plant extracts has been correlated with their flavonoid and total phenolic content (Aslan *et al*., 2006, Rauter *et al*., 2009). Glycosides, flavonoids, tannins and alkaloids have shown reliable activities that may be useful for the treatment of Type 2 diabetes (Kumar A *et al*., 2009). Also saponins, such as oleanolic acid, exhibit hypoglycemic activity and resveratrol, a phenolic compound, shows insulin-like effects in streptozotocin-induced diabetic rats (Hernández-Soto *et al*., 2005, Su *et al*., 2006). In the case of tannins, two modes of action have been proposed to explain their anti-diabetic potential. At the protein level, tannins act via insulin receptor activation leading to an increase in glucose uptake rate and lower glucose levels. At the molecular level, tannins have significant superoxide scavenging and antioxidant activity (Liu *et al*., 2005). These facts are relevant since high levels of superoxide ions in pancreatic β-cells, block insulin signaling, affecting glucose regulation (Sivitz and Yorek 2010). It is well known that certain flavonoids exhibit hypoglycemic activity and pancreas β cell regeneration ability. Thus the significant hypoglycemic effects of BPBE and BPLE may be due to the presence of more than one anti hyperglycemic principle and their synergic properties (Wolfram *et al*., 2006, Shimada *et al*., 2007, Rauter *et al*., 2010 Bansal *et al*., 2012). It was also observed in the present study that crude ethanolic extract of the BP bark was more effective than BP leaves. This difference may be attributed to a number of factors such as method of plant material extraction, use of extracting solvent and the difference in nature and amount of bioactive compounds in the extracts used. Therefore all these observations clearly validate our experimental objectives.

CONCLUSION

The results of the present study clearly establish the presence of bioactive compounds in bark and leaves of the plant *B.purpurea* having hypoglycemic efficacy in animal model. As the present study is a preliminary screening of the plant parts, a detailed study with semipurified and purified fractions of the extracts is necessary to identify the bioactive principle or a group of bioactive chemical constituents exerting such effect. The bark extracts of this plant has been found to have more potent hypoglycemic effect than leaves extract. A detailed study of biochemical parameters to establish the *in vivo* antioxidative effect of these plant extracts is necessary to correlate the antioxidative principles with hypoglycemic efficacy.

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REFERENCES

Aslan M, Deliorman Orhan D, Orhan N, Sezik E, Yesilada E (2006) *In vivo* antidiabetic and antioxidant potential of Helichrysum plicatum ssp. plicatum capitulums in streptozotocin-induced-diabetic rats. J Ethnopharmacol 109(1): 54–59.

Bansal P, Paul P, Mudgal J, Nayak PG, Pannakal ST, Priyadarsini KI, Unnikrishnan MK (2012) Antidiabetic, antihyperlipidemic and antioxidant effects of the flavonoid rich fraction of Pilea microphylla (L.) in high fat diet/streptozotocin-induced diabetes in mice. Exp Toxicol Patho 64(6): 651-658.

Brahmachari G (Editor) (2009) Natural Products: Chemistry, Biochemistry and Pharmacology, Alpha Science, Oxford, UK.

Boophong, S, Puangsombat P, Barmee A, Mahidol C, Ruchirawat S, Kittakoop P (2007) Bioactive compounds from *Bauhinia purpurea* possessing antimalarial, antimycobacterial, antifungal, anti-inflammatory and cytotoxic activities. J Nat Prod 70: 795-801.

Cazarolli LH, Zanatta L, Alberton EH, Bonorino Figueiredo MSR, Folador P, Damazio RG, Pizzolatti MG, Barreto Silva FRM (2008) Flavonoids: Cellular and Molecular Mechanism of Action in Glucose Homeostasis. Mini-Reviews in Medicinal Chemistry: 8 (10): 1032-1038.

Chanchal DK, Niranjan P, Alok S, Singh S, Saurabh (2015) An update on ayurvedic herb kachhnar (*Bauhinia purpurea* Linn.)-a review Int J Pharmacognosy 2(8): 381-390.

Einstein JW, Rais MM, Mohd MM (2012) Comparative evaluation of the antidiabetic effects of different parts of *C. fistula* Linn, a south east Asian plant. J Chem 2013: 1-10.

Geeta BS, Mathew BC, Augusti KC (1994) Hypoglycemic effects of Leuco delphinidin derivative isolated from *Licus bengalenses* (Linn.). Ind J Physiol Pharmacol 38: 220-222.

Gokee G, Haznedaroglu M Z (2008) Evaluation of anti diabetic, antioxidant and vasopressive effects of *Posidonia oceanica* extract. J Ethnopharmacol 115: 122-130.

Grover JK, Yadav S, Vats V (2002) Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol 81(1): 81-100.

Gupta SS (1994) Prospects and perspectives of natural plant products in medicine. Ind J Pharmacol 26: 1-12.

Hernández-Soto RLC, Díaz-Jiménez L, Villanueva-Ramírez S (2005) Extracción y cuantificación indirecta de las saponinas de Agave lechuguilla Torrey. In: e-Gnosis. 3: 1-9.

Kumar A, Ilavarasan R, Jayachandran T, Decaraman M, Aravindhan P, Padmanabhan N, Krishnan MRV (2009) Phytochemicals Investigation on a Tropical Plant, *Syzygium cumini* from Kattuppalayam, Erode District, Tamil Nadu, South India. Pak J Nutr 8: 83-85.

Liu X, Kim JK, Li Y, Li J, Liu F, Chen X (2005) Tannic acid stimulates glucose transport and inhibits adipocyte differentiation in 3 T3-L1 cells. J Nutr 135(2): 165-171.

Meshram SS, Hankar PR, Patil AT (2013) To study antidiabetic activity of stem bark of *Bauhinia purpurea* Linn. J Pharmacog Phytochem 2(1): 171-175.

Mondal S, Bhattacharya S, Biswas M (2012) Antidiabetic activity of Areca catechu leaf extracts against streptozotocin induced diabetic rats. J Adv Pharm Edu Res 2(1): 10-17.

Muralikrishna KS, Latha KP, Shreedhara CS, Vaidya VP, Krupanidhi AM (2008) Effect of *Bauhinia purpurea* Linn. on alloxan-induced diabetic rats and isolated frogs heart. Int J Green Pharm 2: 83-86.

Organization for Economic Co-operation and Development (OECD) (2001) 423: 1-14.

Pahwa S, Mazumder R, Bhattacharya S (2012) Antidiabetic activity of leaf of *Bauhinia purpurea*. Int J Thera Appli 2: 19-23.

Pettit GR, Numata A, Iwamoto C, Usami Y, Yamada T, Ohishi H, Cragg GM (2006) Antineoplastic agent 551 isolation and structure of Bauhiniastatins-1-4 from *Bauhinia purpurea*. J Nat Prod 69: 323-327.

Ragasa CY, Hofilena JG, Rideout J (2004) Secondary metabolite from Bauhinia purpurea. J Sci Food Agric 55: 423-431.

Rauter AP, Martins A, Lopes R, Ferreira J, Serralheiro LM, Araujo ME, Borges C, Justino J, Silva FV, Goulart M (2009) Bioactivity studies and chemical profile of the antidiabetic plant *Genista tenera*. J Ethnopharmacol 122(2): 384-393.

Rauter AP, Martins AP, Mota-Filipe H, Pinto R, Sepodes B, Justino J (2010) Antihyperglycaemic and protective effects of flavonoids on streptozotocin–induced diabetic rats. Phytoth Res 24(S2): S133-S138.

Sarkar SD, Latif Z, Gray AI (Eds) (2006) Humana Natural product Isolation, 2nd edn. Press Inc. New Jersey.

Sabira Sultana, Akram M, Asif HM, Akhtar N (2014) Complementary and Alternative approaches to treat peptic ulcer. Int Res J Pharmacy 5(5): 353-359.

Shimada M, Mochizuki K, Sakurai N, Goda T (2007) Dietary supplementation with epigallocatechin gallate elevates levels of circulating adiponectin in non-obese type 2 diabetic goto-kakizaki rats. Bioscience Biotech Biochem 71(8): 2079-2082.

Singh R, Barden A, Mori T, Beilin L (2001) Advanced glycation end-products: a review. Diabetologia 44: 129.

Singh KN, Mittal RK, Barthwall KC (1976) Hypoglycemic activity of *Acacia catechu, Acacia suma, Albizzia odoratissima* seed diets in normal albino rats. Ind J Med Res 64: 754-757.

Sivitz WI, Yorek MA (2010) Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities. Antioxidants Redox Signal 12(4): 537-577.

Su HC, Hung LM, Chen JK (2006) Resveratrol, a red wine antioxidant, possesses an insulin-like effect in streptozotocin-induced diabetic rats. Am J Physiol Endocrinol Metab 290(6): E1339-1346.

Urmi KF, Gulshanara Begum SM, Ifa T, Hamid K (2013) Comparative antioxidant activity of different parts of *Bauhinia purpurea* linn. Biol Med 5: 78-82.

Verma T, Chandrashekar KS (2009) A-amyrin caprilate - a new triterpene isolated from the leaf of *Bauhinia purpurea* linn. Asian J Res Chem 2: 569-570.

Wadkar KA, Magdum CS, Patil SS, Naikwade NS (2008) Anti-diabetic potential and indian medicinal plants. J Herbal Med Toxicol 2(1): 45-50.

Wolfram D, Raederstorff M, Preller (2006) Epigallocatechin gallate supplementation alleviates diabetes in rodents. J Nutrition 136(10): 2512-2518.

Yadav RN, Tripathi P (2000) A novel flavones glycoside from the stem of *Bauhinia purpurea*. Fitoterapia 71: 88-90.

Yadav S, Bhadoria BK (2005) Two dimeric flavonoids from *Bauhinia purpurea*. Ind J Chem 44: 2604-2607.

Yamasaki K, Hishiki R, Kato E, Kawabata J (2011) Study of Kaempferol Glycoside as an Insulin Mimic Reveals Glycon to be the key active structure. ACS Med Chem Lett 2 (1): 17-21.

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