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ANTIDIABETIC ACTIVITY OF METHANOLIC EXTRACT OF BARK OF *BAUHINIA PURPUREA*

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ABSTRACT

The present investigation was carried out to study the antidiabetic effect of the methanolic extract of bark of *Bauhinia purpurea* in Streptozotocin induced diabetic model. The antidiabetic activity was evaluated in normal & Streptozotocin induced diabetic rats. Decreased blood glucose level of the test animals showed that the extract exhibited significant antidiabetic activity when compared to diabetic control group. The results also indicated dose dependent effect. The antidiabetic activity produced by the extract might be due to the increased uptake of glucose at the tissue level or due to an increase in pancreatic beta cell function or due to inhibition of intestinal absorption of glucose.

INTRODUCTION: Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of insulin produced.

Such deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves. As the number of people with diabetes multiply worldwide, the disease takes an ever-increasing proportion of national and international health care budgets. It is projected to become one of the world's main disablers and killers within the next 25 years.

Regions with greatest potential are Asia and Africa, where DM rates could rise to two to three-folds than the present rates. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations [1].

Bauhinia purpurea (family: Caesalpiniaceae) is a medium sized, evergreen, ornamental tree found throughout India. The leaves are rigidly sub-coriaceous, glabrous and shallowly cordate. The purple colored flowers of the species distinguishes it specifically from other species of *Bauhinia*. It is presently being used for ailments such as sores, wounds, diarrhea, dropsy, pain, rheumatism, convulsions, delirium, septicemia and so on [2]. Its decoctions are recommended for ulcers as a useful wash [3]. The aerial parts of the plant are reported to contain flavanone glycosides, foliar flavonoids, 6-butyl-3-hydroxy flavanone, amino acids, phenyl fatty ester, lutine and β- sitosterol [4-9].

Flavonoids are polyphenolic compounds are widely distributed in the plant kingdom. They are reported to exhibit various pharmacological activities such as CNS activity, cardiotonic activity, lipid-lowering activity, antioxidant activity, hepatoprotective activity, and hypoglycemic activity [10]. These active constituents and the above mentioned activities in turn appear to correlate with some other biological activities.

In view of alleged antidiabetic potential of *Bauhinia purpurea*, we have investigated effect of methanolic extract of its bark on fasting blood sugar levels in Streptozotocin (STZ) induced diabetic rats. STZ [2-deoxy- 2- {3- (methyl- 3- nitrosoureido)- D-gluco-pyranose}] is synthesized by *Streptomycetes achromogenes* and is used to induce both insulin-dependent and non-insulin dependent DM. STZ is also efficacious after intraperitoneal administration of a dose between 40-60 mg/kg body weight or even a higher dose, but single dose below 40 mg/kg body weight may be ineffective [11, 12].

MATERIALS AND METHODS:

Plant Material: The bark of *B. purpurea* was collected from the surrounding areas of Greater Noida in June, 2010. The plant was identified and authenticated (Voucher No NHCP/ NBPGR/2010-20 dated 10th May 2010) by Dr. Anjula Pandey, Principal Scientist, National Bureau of Plant and Genomic Resources (NBPGR), New Delhi, India. A copy of the herbarium has been preserved in the Department of Pharmaceutical Technology, NIET, Greater Noida, India, for future reference. The part was sun dried after washing and then ground to a coarse powder in a mechanical grinder.

Extraction Procedure: The powdered bark was macerated with different polar and non polar solvents for seven days. After which each extract was subjected to phytochemical screening using standard methods [13].

Since the methanolic extract had the maximum constituents henceforth, methanol was chosen as the solvent for extraction.

The coarse powder of the bark (32.81 g) was extracted in a soxhlet apparatus with methanol and the solvent was removed by controlled evaporation under reduced pressure on a heating mantle at temperature below 60^{o}C. The crude extract thus obtained was tested for its antidiabetic potentiality.

The extract was used to prepare suspensions of 50 mg/kg and 100 mg/kg concentrations using water and Tween 80 : Tween 20 (1:1) for treatment.

Drugs: STZ was obtained from SRL Lab, Delhi, India. All other chemicals used for the study were of analytical grade.

Animals: Male Wistar rats weighing between 150 and 200 g were used for antidiabetic activity. Toxicity study was carried out on Albino mice (25-30 g) as per the OECD guidelines and the study was approved by the Institutional Animals Ethics Committee (CPCSEA) (Approval code no. 1121/ac/CPCSEA/07/NIET/IAEC /2010/32P/16). Animals were fed a standard pellet (Lipton India Ltd., Mumbai, India) and water *ad-libitum*. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under 24-28^{o}C temperature, 60-70% relative humidity and 12 h day and night cycle. Animals described as fasted were deprived of food for 16 h but had free access to water.

Sample collection: Blood samples were collected by retro-orbital plexus puncture method and blood glucose levels were estimated using an electronic glucometer (Morepen, Baddi, HP, India).

Effect of *Bauhinia purpurea bark* Extract on STZ Induced DM [14]: Diabetes was induced in rats by tail vein injection of STZ (50 mg/kg), dissolved in citrate buffer. One group of 6 identical rats was kept without STZ administration as normal control, group I. Forty eight hours after STZ administration, blood samples were drawn by retro-orbital puncture method and glucose levels were determined to confirm diabetes. The diabetic rats exhibiting blood glucose levels in the range of 140 mg/dl or more were selected for the studies. These diabetic rats were sub-divided into 3 groups as follows:

Group II rats, served as diabetic control (STZ induced) were given 0.5 ml of 5% Tween 80 in place of the extract;

Group III diabetic rats were given 50 mg/kg *B. purpurea* bark extract in 0.5 ml 5% Tween 80;

Group IV diabetic rats were given 100 mg/kg *B. purpurea* bark extract in 0.5 ml 5% Tween 80;

Group V diabetic rats were given 0.5 ml of 5% Tween 80 containing Glibenclamide (500 µg/kg).

The dose (500 µg/kg) of Glibenclamide was selected based on previous reports.[15] The normal control group of rats (group I) were given 0.5 ml of 5% Tween 80 only.

Each of the control and the test groups consisted of 6 animals. The treatments were continued daily for 15 days. Blood was collected by retro-orbital puncture method for glucose estimation just before drug administration on the 1st day and 1 h after drug administration on days 4, 7 and 10.

Statistical Analysis: The results were expressed as Mean ± SEM. Comparison between the groups was made by analysis of variance (ANOVA), followed by Dunnett's test. A value of $P < 0.001$ was considered significant [16].

RESULTS AND DISCUSSION:

Preliminary Phytochemical Screening: The results of the preliminary phytochemical screening of the various extracts of *B. purpurea* bark were depicted in **Table 1**, which showed the presence of alkaloids, glycosides, saponins, phytosterols, phenolic compounds and tannins in the methanolic extract of the part used.

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF VARIOUS BARK EXTRACTS OF *BAUHINIA PURPUREA*

Plant constituents	Petroleum ether extract	Chloroform extract	Acetic acid extract	Acetone extract	Methanol extract	Aqueous extract	Benzene extract
Alkaloids	-	+	+	+	+	+	-
Carbohydrates	-	-	-	-	-	-	-
Glycosides	-	-	+	+	+	-	-
Saponins	+	+	+	+	+	+	+
Phenolic compounds & Tannins	-	-	-	+	+	+	-
Flavonoids	-	-	-	-	+	-	-
Phytosterols	+	+	+	+	+	+	+

Yield: After soxhleting the bark powder (32.81 g) with methanol, a semisolid, dark, viscous crude extract with 16.39% w/w yield was obtained, which was subjected to further antidiabetic studies.

The Effect of Methanolic Extract of *Bauhinia purpurea* Bark on STZ induced Diabetic Rats: Administration of STZ (50 mg/kg, i.p.) led to many folds elevation of fasting blood glucose levels, which was maintained over a period of 2 weeks. Two weeks of daily treatment of methanolic extract of *B. purpurea* led to a dose-dependent fall in blood sugar levels. Vehicle control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 15 days (**Table 2**). The effect of methanolic bark extract of *Bauhinia purpurea* on glucose level in STZ induced diabetic rats are summarized in **Table 3** , where a significant reduction ($P<0.001$) in blood glucose level was observed in the drug treated animals, when compared to the diabetic control group.

TABLE 2: EFFECT OF METHANOLIC EXTRACT OF BARK OF *BAUHINIA PURPUREA* ON BODY WEIGHT (G) ON STZ (50 MG/KG, I.P.) INDUCED DIABETES IN RATS

Groups	Average body weight (g) of rats				
	1st day	4th day	7th day	10th day	15th day
Normal control (0.5 ml of 5% Tween 80)	180.50 ± 0.61	182.00 ± 0.44	182.67 ± 0.21	182.50 ±0.34	184.67 ± 0.21
STZ induced diabetic control	201.00 ± 0.68*	174.33 ± 0.33*	160.67 ± 0.49*	153.50 ± 0.34*	145.33 ± 0.33*
STZ + MEBP (50 mg/kg)	196.33 ± 0.61*	181.67 ± 0.42*	177.00 ± 1.03*	174.50 ± 0.34*	174.33 ± 0.21*
STZ + MEBP (100 mg/kg)	180.67 ± 0.66*	174.33 ± 0.33*	171.67 ± 0.21*	169.33 ± 0.33*	169.00 ± 0.34*
STZ + Glibenclamide (0.5 mg/kg)	189.33 ± 0.84*	185.33 ± 0.33*	182.00 ± 0.25*	179.50 ± 0.22*	177.67 ± 0.21*

Values are mean ± SEM for groups of six animals each; * $P < 0.01$ as compared to normal control on corresponding day

TABLE 3: EFFECT OF METHANOLIC EXTRACT OF BARK OF *BAUHINIA PURPUREA* ON GLUCOSE LEVEL IN STZ INDUCED DIABETIC RATS

Groups	Glucose levels (mg/dl)				
	1st day	4th day	7th day	10th day	15th day
Normal control (0.5 ml of 5% Tween 80)	134.50 ± 0.4282	134.33 ± 0.4944	134.00 ±0.5774	134.17 ± 0.5426	134.83 ±0.5426
STZ induced diabetic control	146.00 ± 0.4472$^{a\#}$	151.50 ± 2.460^{a*}	158.00 ±2.309 ^{a*}	161.67 ± 1.116^{a*}	164.00 ±0.7746^{a*}
STZ + MEBP (50 mg/kg)	154.50 ± 0.4282^{b*}	140.83 ± 2.272^{b**}	134.83 ±1.621 ^{b**}	128.83 ±0.7923 ^{b**}	128.50 ±0.7638 ^{b**}
STZ + MEBP (100 mg/kg)	155.67 ± 0.8433^{b*}	143.83 ± 1.838^{b**}	130.17 ±0.6540 ^{b**}	129.17 ±0.4773 ^{b**}	129.17 ±0.4773 ^{b**}
STZ + Glibenclamide (0.5 mg/kg)	155.33 ± 0.4944*	139.33 ± 1.256**	131.00 ±1.155 **	126.33 ±1.054 **	125.33 ±1.520 **

Values are mean ± SEM, 6 rats in each group; STZ (50 mg/kg) was injected to control and all other drug treated groups; [a] STZ induced diabetic group vs normal group, #p>0.05; [b] extract treated group vs STZ induced diabetic group, *p<0.01; **p<0.001

CONCLUSION: The extract has exhibited antidiabetic property in streptozotocin induced diabetic rats, as evident from the glucose levels. The hypoglycemic activity may be ascribed to the presence of flavonoids, which have been shown to inhibit cyclooxygenases and promote β-cell regeneration besides having insulin secretary property [17-19]. The results of the present study suggests that the methanolic extract of the bark of *B. purpurea* illustrates significant hypoglycemic activity, which may be due to the presence of flavonoids in it, as claimed by earlier reports.

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