Assessment of antidiabetic potential of leaf extract of *Bauhinia variegata* Linn. in Type-I and Type-II diabetes

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Abstract

Aim: *Bauhinia variegata* Linn., syn: *Kovidara* and *Kachnar,* is a medium-sized deciduous tree generally found in sub-Himalayan region of India. Just about all parts of this plant are used in traditional medicine for the treatment of various ailments. A metabolic disorder, diabetes mellitus is one of the most common disorders. The current study was intended to appraise the efficacy of the ethanolic extract (EE) of *B. variegata* leaves in animal models of Type I and Type II diabetes. **Materials and Methods:** Type I diabetes was induced by alloxan at the dose of 150 mg/kg (i.p.) in male albino Wistar rats while Type II diabetes was induced by high-fat diet and alloxan at the dose of 130 mg/kg (i.p.). Diabetic animals were treated with EE at the dose of 250, 500, and 1000 mg/kg. Glipizide (5 mg/kg) was used as standard treatment drug. **Results:** Treatment was given for 28 days. Parameters evaluated were body weight, plasma glucose, cholesterol, triglyceride, aspartate aminotransferase, alanine transaminase (ALT), alkaline phosphatase (ALP), total proteins, albumin, creatinine, and blood urea nitrogen (BUN). In Type II diabetes, high-density lipoprotein levels in plasma and plasma insulin level were also evaluated. EE was also found to decrease cholesterol, triglyceride, creatinine, and BUN level in both types of diabetes. EE did not show any significant effect on plasma levels of aspirate aminotransferase, ALT, and ALP. EE was found to increase the albumin and total protein levels. **Conclusion:** Results obtained indicate that the EE of the leaves of *B. variegata* Linn. was able to lower the increased blood glucose levels in the selected animal models.

Key words: Alloxan, *Bauhinia variegata* Linn., blood urea nitrogen, ethanolic extract, glipizide

INTRODUCTION

About 250 species of *Bauhinia variegate* Linn. grow in the tropical regions of the world. It includes shrubs, trees, and vines that are frequently planted for their showy flowers and ornamental foliage.[1] *B. variegate* Linn. is native to southeastern Asia and grows throughout India and China. It is most commonly cultivated in India.[2] *B. variegate* Linn. (Caesalpiniaceae) is a medium-sized deciduous tree, known as (Sanskrit) *Kanchanara*, (Hindi) *Kovidara,* and (Marathi) *Raktakanchan*. Almost all parts are used in traditional medicine for the treatment of various ailments such as asthma, ulcer, diabetes mellitus, a chronic metabolic disorder of insulin deficiency, or ineffectiveness, constitute a global public health burden and predictions estimate that India, China, and the United States will have the largest number of diabetic people by 2030.[1] The search for plant-based products for control of diabetes mellitus continues, and the World Health Organization has also long back recommended herbal treatment of diabetes mellitus.[2] *B. variegate* Linn. (Family: Fabaceae), vernacularly called Kachnara, is an herbaceous medicinal plant, found throughout India. The leaves of the many *B. variegata s*pecies [Figure 1] are used in antidiabetic treatments by many populations of the world.[3]

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Figure 1: Leaves of *Bauhinia variegata* Linn

In India, stem bark is used as an antidiabetic in the Ayurvedic system of medicine.[4]

B. variegata, belonging to the family Caesalpiniaceae, has been extensively utilized for its therapeutic assets. Numerous phytochemicals are recognized from various parts of *B. variegata* which belongs to different classes of phytochemicals, i.e., flavonoids, glycosides, terpenes, and steroids. Leaves of *B. variegata* contain naringenin (a flavonoid aglycone),[5] quercetin 3-methyl ether, luteolin, rutin, isoquercitrin, and daucosterol.[6] Quite a lot of pharmacological activities of various parts of *B. variegata* are studied by researchers who described that the root extract possesses anti-inflammatory activity.[7] Stem has been reported to possess antitumor and antiulcer activities. Leaves have been found to possess antihyperlipidemic activity[8] along with *in vitro* antioxidant,[9] antibacterial, and antifungal actions.[10] It is also reported that the ethanolic extract (EE) of leaves of the plant induces insulin secretion in insulin-secreting cell line.[11] Insulin-like proteins have been isolated from leaves of *B. variegata* possessing blood glucose depressing action.[12,13]

Findings showed that the EE of leaves of *B. variegata* has significant activity in Type I diabetes.[14] Thus, the present study was intended to assess the efficacy of EE of *B. variegata* leaves in Type I and Type II diabetes which can be well utilized to affirm the use of leaves of *B. variegata* in the treatment of Type I and Type II diabetes.

MATERIALS AND METHODS

Chemicals

Alloxan was procured from Sigma-Aldrich, USA, and Glipizide was obtained as gift sample from Torrent Pharmaceutical Ltd., Mumbai. Diagnostic kits were acquired from Genome Diagnostics Pvt. Ltd., India. All other chemicals used were of analytical grade.

Plant Material

B. variegata fresh leaves were collected from Bundelkhand University Campus Dist., Jhansi (U.P.). The plant material was taxonomically identified and authenticated by Dr. R.V. Singh, Scientist and Head, Herbarium and Museum, Central Council Research Ayurveda and Siddha (CCRAS), Gwalior, with Ref. no. CCRAS/16/036).

Preparation of Extract

The dried leaves of *B. variegata* were powdered and an EE was prepared by means of a double maceration method. Grounded leaves (500 g) were macerated with 2000 ml of ethanol for 7 days after which the extract was filtered through muslin cloth, and the filtrate was stored in refrigerator till next processing. The marc was again kept for maceration with fresh distilled water for 7 days which was again filtered through muslin cloth after 7 days. The filtrate obtained during this step was mixed with the filtrate obtained in the first step. The extract was concentrated on a water bath and was stored in a refrigerator until further use.[15]

Determination of Total Phenolic Content

The concentration of phenolics in plant extracts was determined using spectrophotometric method ethanolic solution of the extract in the concentration of 1 mg/ml and was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of ethanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water, and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml ethanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water, and 2.5 ml of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at λ_{max} = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid, and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then, the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).[16]

Determination of Total Flavonoid Content

The content of flavonoids in the examined plant extracts was determined using spectrophotometric method. The sample contained 1 ml of ethanol solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution dissolved in ethanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λ_{max} = 415 nm. The samples were prepared in triplicate for each analysis, and the mean

value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin, and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of rutin equivalent (mg of RU/g of extract).[17]

Determination of Quercetin in EE by High-performance Thin-layer Chromatography (HPTLC) Method

Quercetin was analyzed from EE using HPTLC method on CAMAG (Switzerland) HPTLC system comprising a sample applicator, extract solution (50 mg/ml) and quercetin solutions of concentration 10, 20, 40, 60, and 80 mg/ml prepared in water. Volume of 10 ml of these solutions was applied as 8 mm bands at a spraying rate of 50 s/µl. TLC plate (10 cm × 15 cm) coated with 0.2 mm layer of silica gel 60 F254 (Merck, Germany) was used for the development of HPTLC.

The mobile phase for ascending development of TLC plates was comprised n-hexane:ethyl acetate:formic acid:acetic acid in the ratio of 3:7:0.2:0.2. Densitogram was recorded by scanning the plate at 254 nm.[18]

Experimental Animals

The adult male albino rats of weight 180–240 g were selected for the study. All animals were procured from disease-free animal house, Institute of Pharmacy, Bundelkhand University, Jhansi. All research experiments were approved by CPCSEA and IAEC with approval no. BU/Pharm/IAEC/16/a/01. The animals were housed in polypropylene cages, 5 per cage with free access to standard laboratory diet and water *ad libitum*. The rats were maintained under standard laboratory conditions at 25 ± 2°C relative humidity 50 ± 15% and normal photoperiod (12 h dark/12 h light) were used for experiment.

Effect of EE in Alloxan-induced Type I Diabetic Rats

Type I diabetes was persuaded in experimental animals by an intraperitoneal (i.p.) injection of alloxan at a dose of 150 mg/kg in normal saline solution (1 ml/kg) to overnight fasted rat. Fasting blood glucose levels were measured after 5 days, animals with blood concentration >250 mg/dl were considered to be diabetic.[19]

Treatment for Type I Diabetes Study

Six normal non-diabetic animals were kept to normal control group which was named as "Group I." This group was given 1% solution of sodium carboxymethyl cellulose (CMC)

as vehicle. On the basis of plasma glucose level and body weight, the diabetic animals were randomly assigned into five groups (*n* = 6).

Diabetic control animals were assigned to Group II which was administered with 1% CMC solution. Diabetic rats in Groups III, IV, and V were treated with EE at dose of 250, 500, and 1000 mg/kg (p.o.) of body weight, respectively. Diabetic animals in Group VI received treatment with standard drug glipizide at dose of 5 mg/kg (p.o.).[20] Extract and standard drugs were prepared freshly in 1% CMC solution before administration. All animals received the respective treatment for 28 days.

Parameters Assessed

Body weight

Any variation in the body weight of the animal model was carefully monitored, and reports were generated weekly.

Plasma glucose

Samples of blood were withdrawn from retro-orbital plexus in microcentrifuge tubes using pre-heparin zed rat bleeding capillaries on the 0, 7, 14, 21, and 28th day, and plasma glucose level was assessed using market available diagnostic kit (Robustserv Pharmaceutical Devices Pvt. Ltd., Bengaluru, India).

Biochemical parameters

Blood samples were collected on the 28th day and were analyzed to obtain plasma supernatant and many biochemical parameters such as cholesterol, triglycerides, aspirate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphates (ALPs), total proteins, albumin, creatinine, and blood urea nitrogen (BUN) levels using commercially available diagnostic kits.

Oral glucose tolerance test (OGTT)

OGTT was performed on the 28th day.[14] After 60 min of treatment, 20% glucose solution (2 g/kg) was orally administrated to the experimental animals. Plasma glucose was assessed at 0, 30, 60, 90, and 120 min after glucose administration.

Effect of EE in High-fat Diet (HFD) - Alloxan-induced Type II Diabetic Rats

Type II diabetes induction

Male albino rats were used to induce Type II diabetes described by Srinivasan using combination of HFD and intraperitoneal injection of low-dose (100 mg/kg) alloxan.[21] Animals received standard pellet diets considered as normal control and diabetic animals received HFD throughout the

study. After 14 days of HFD, rats were administered with 100 mg/kg of alloxan (alloxan was dissolved in normal saline solution).

Plasma glucose levels were measured after 7 days of standard drug administration. Animals with a plasma glucose level >250 mg/dl were considered as diabetic and were selected for the study.

Treatment

Group I was assigned as normal control animals ($n = 6$) and received 1% solution of sodium CMC. Type II diabetic animals were randomly assigned into five groups containing six animals in each. Diabetic control animals were assigned to Group II which was administered with 1% CMC solution. Diabetic rats in Groups III, IV, and V were treated with EE at dose of 250, 500, and 1000 mg/kg (p.o.) of body weight, respectively. Diabetic animals in Group VI received treatment with standard drug glipizide at dose of 5 mg/kg (p.o.).[21] Extract and standard drugs were prepared freshly in 1% CMC solution before administration. All animals received the respective treatment for 28 days.

Parameters Assessment

Body weight

On 0, 7, 14, 21, and 28th day of treatment, the experimental animals were weighed.

Plasma glucose level

Plasma glucose was assessed on day 0, 7, 14, 21, and 28 of treatment.

Biochemical parameters

After 28 days of treatment, blood samples were analyzed for various biochemical parameters which included cholesterol, triglycerides, high-density lipoproteins (HDL), AST, ALT, ALP, total proteins, albumin, creatinine, and BUN.

At the end of the study, plasma insulin level was estimated using rat insulin ELISA kit as per the instructions provided by manufacturer (Mercodia, Sweden). ELISA washer (Erba Smart Wash-III, Germany) and ELISA reader (Erba Microscan, Germany) were used for the estimation.

Statistical Analysis

All values are expressed as mean ± standard error of the mean. Statistical analysis was performed by one-way analysis of variance. The results were considered statistically significant if $P < 0.05$.

RESULTS

Total Phenolic Content

The total phenolic content of EE was found to be 2.33 ± 0.085 mg of gallic acid equivalent/g of extract.

Total Flavonoid Content

The total flavonoid content of EE was found to be 184.2 ± 4.88 mg of quercetin equivalent/g of extract.

Quercetin Content

From HPTLC analysis, it was found that the EE content was 0.32 ± 0.04% w/w of quercetin.

Effect of EE in Alloxan-induced Type I Diabetic Rats

Body weight

Body weight of normal control animals was 265.2 ± 2.41 g which come down to 146.2 ± 5.9 g in diabetic control animals. Body weight was found to increase after treatment with EE at dose of 500 and 1000 mg/g to 172.4 ± 4.99 g ($P < 0.05$) and 180.4 ± 6.56 g ($P < 0.01$), respectively, when compared with diabetic control. Figure 2 depicts the effect of EE on body weight of animals in Type I diabetes study on the 28th day.

Plasma glucose level

Plasma glucose level was elevated in diabetic control animals (421.49 ± 12.29 mg/dl) significantly ($P < 0.0001$) when compared to normal animals (84.21 ± 1.3 mg/dl).

EE at the dose of 500 and 1000 mg/kg decreased the plasma glucose level to 325 ± 7.3 mg/dl and 301 ± 6.1 mg/dl, respectively. At the end of the study, plasma glucose level in glipizide-treated rats was 240 ± 5.3 mg/dl ($P < 0.001$). Effect of EE on plasma glucose level is shown in Figure 3 which shows plasma glucose levels on the 28th day.

Biochemical parameters

The effect of EE of various biochemical parameters is presented in Table 1. Total cholesterol level was significantly increased ($P < 0.001$) in diabetic control animals when compared with normal animals. EE decreased the total cholesterol to 92.3 ± 2.71 mg/dl ($P < 0.01$) at the dose of 1000 mg/kg when compared to diabetic control. Value of triglyceride level in diabetic control group was increased to 105.2 ± 2.6 mg/dl.

EE at the dose of 500 mg/kg and 1000 mg/kg decreased triglyceride level to 93.433 ± 2.246 mg/dl ($P < 0.05$) and 93.422 ± 2.26 mg/dl ($P < 0.001$), respectively. Creatinine

Parameters	Normal control	Diabetic control	Diabetic+EE (250 mg/kg)	Diabetic EE (500 mg/kg)	Diabetic+EE (1000 mg/kg)	Diabetic+glipizide (5 mg/kg)
TC (mg/dl)	75.10±2.65	106.5±3.05###	102.1±2.51	94.6±2.82	92.3±2.71**	91.18±3.54*
TG (mg/dl)	75.41±3.15	105.2±2.6###	101.6±2.25	93.422±2.22*	88.5±3.23***	89.53±1.56***
AST (IU/L)	171.1±4.59	249.9±7.08###	252.3±6.41	249.1±8.09	248.9±8.41	213.8±7.4*
ALT (IU/L)	57.56±2.85	73.66±2.95#	72.51±2.19	71.95±3.32	70.02±3.90	68.28±2.46
ALP (IU/L)	83.73±2.60	167.8±2.18###	165.9±4.62	161.5±3.03	154.5±4.86	157.0±4.05
TP (g/dl)	9.4±0.26	6.57±0.35###	6.78±0.32	6.82±0.21	7.79±0.11*	7.73±0.20*
ALB (g/dl)	4.54±0.17	2.55±0.32###	2.65±0.16	2.63±0.23	3.52±0.21	3.75±0.220
CK (mg/dl)	0.79±0.026	0.958±0.033###	0.94±0.023	0.90±0.023	0.84±0.025*	0.88±0.021
BUN (mg/dl)	25.22±1.37	51.52±1.98###	50.54±1.31	50.56±1.47	44.56±1.41*	35.73±1.39***

Table 1: Effect of EE of *B. variegata* on biochemical parameters in Type 1 diabetes

Values are expressed as Mean±SEM (TC: Total cholesterol, TG: Triglyceride, AST: Aspartate aminotransferase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, TP: Total protein, ALB: Albumin, CK: Creatinine, BUN: Blood urea nitrogen). *$P<0.05$, **$P<0.01$, ***$P<0.001$ when compared with diabetic control. #$P<0.05$. ###$P<0.001$ when compared to normal control. SEM: Standard error of the mean, EE: Ethanolic extract, *B. variegata: Bauhinia variegata*
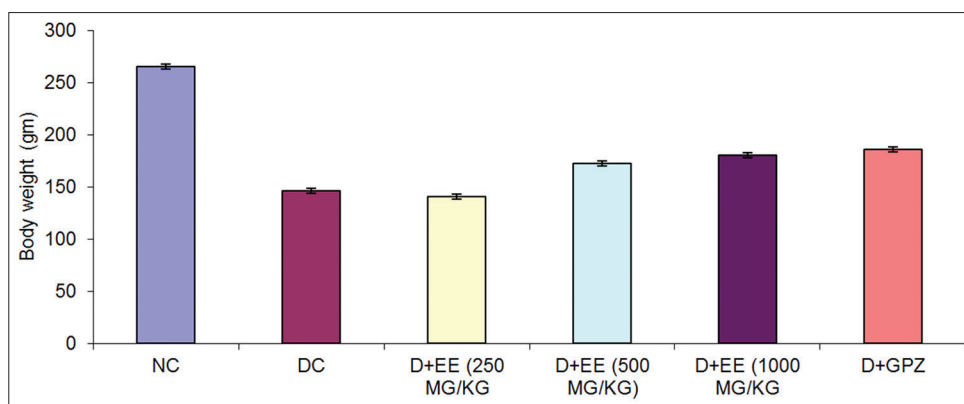

Figure 2: Effect of ethanolic extract (EE) of *Bauhinia variegata* on body weight in Type 1 diabetes on 28 days. NC: Normal control, DC: Diabetic control, diabetes+EE, (250 mg/kg), diabetes+EE, (500 mg/kg), diabetes+EE, (1000 mg/kg), diabetes+glipizide. Values are expressed as Mean±standard error of the mean. *$P<0.0$, **$P<0.01$, ***$P<0.001$ when compared with diabetic control. ### $P<0.001$ when compared to normal control
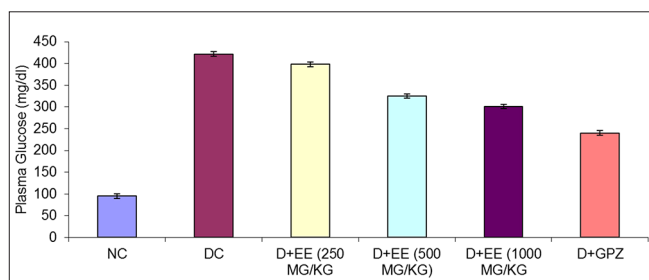

Figure 3: Effect of ethanolic extract (EE) of *Bauhinia variegata* on plasma glucose in Type 1 diabetes on the 28th day.NC: Normal control, DC: Diabetic control, diabetes+EE, (250 mg/kg), diabetes+EE, (500 mg/kg), diabetes+EE, (1000 mg/kg), diabetes+glipizide. Values are expressed as Mean±standard error of the mean. *$P<0.0$, **$P<0.01$, ***$P<0.001$ when compared with diabetic control. ###$P<0.001$ when compared to normal control

level was increased $0.958 ± 0.033$ mg/dl ($P < 0.001$) in diabetic animals when compared to normal control. EE at the dose of 1000 mg/kg decreased the creatinine level to

$0.84 ± 0.025$ mg/dl ($P < 0.05$). BUN was decreased to $44.56 ± 1.41$ mg/dl ($P < 0.5$) at dose of 1000 mg/kg which was increased to $51.52 ± 1.98$ mg/dl in diabetic animals.

EE did not show any significant effect on AST, ALT, and ALP at selected dose levels. Total protein and albumin level was declined to $6.67 ± 0.35$ g/dl and to $2.77 ± 0.12$ g/dl, respectively, in diabetic control animals. EE at the dose of 1000 mg/kg increased the total protein to $7.79 ± 0.11$ g/dl ($P < 0.01$). No significant effect was observed on plasma albumin level after treatment with EE at all doses.

Effect of EE in HFD - Alloxan-induced Type II Diabetic Rats

Body weight

From Figure 4, we can state that no significant change was observed in normal, diabetic, and treatment groups of the animals. Figure 3 displays the body weight of animals.
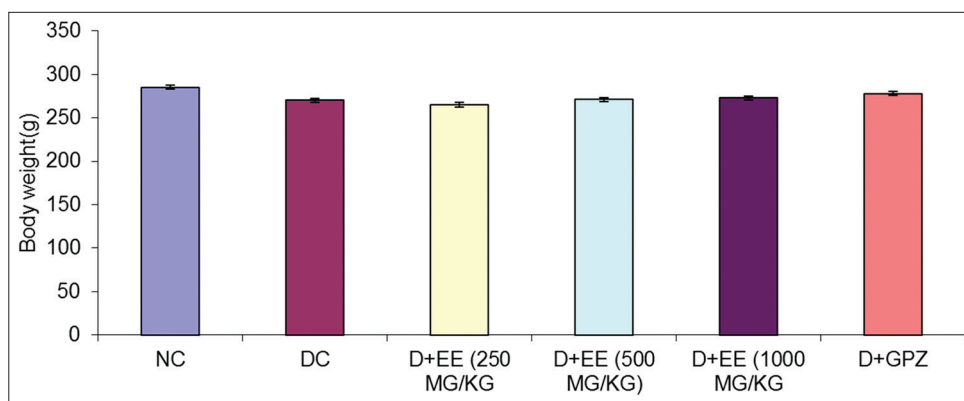
Figure 4: Effect of ethanolic extract (EE) of *Bauhinia variegata* on body weight in Type II diabetes. NC: Normal control, DC: Diabetic control, diabetes+EE, (250 mg/kg), diabetes+EE, (500 mg/kg), diabetes+EE, (1000 mg/kg), diabetes+glipizide. Values are expressed as Mean±standard error of the mean. *P<0.0, **P<0.01, ***P<0.001 when compared with diabetic control. ###P<0.001 when compared to normal control
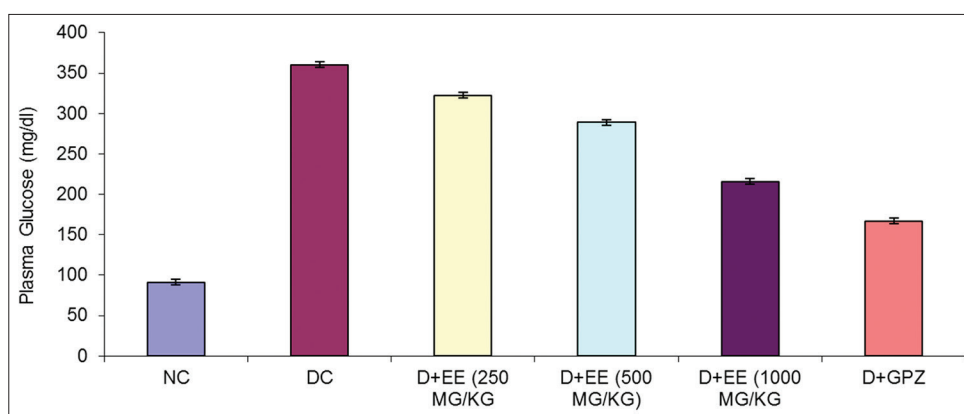

Figure 5: Effect of ethanolic extract (EE) of *Bauhinia variegata* on plasma glucose in Type II diabetes. NC: Normal control, DC: Diabetic control, diabetes+EE, (250 mg/kg), diabetes+EE, (500 mg/kg), diabetes+EE, (1000 mg/kg), diabetes+glipizide. Values are expressed as Mean±standard error of the mean. **P<0.01, ***P<0.001 when compared with diabetic control. ###P<0.001 when compared to normal control

Plasma glucose level

Plasma glucose level of normal control animals was 91.2 ± 3.4mg/dl which was markedly increased in diabetic control group to 360.3 ± 3.11 mg/dl ($P < 0.001$). Treatment with EE 500 and 1000 mg/kg for 28 days lowered the plasma glucose level significantly to 289 ± 13.33 mg/dl ($P < 0.01$) and 215.4 ± 3.50 mg/dl ($P < 0.001$), respectively, when compared to diabetic rats.

At the end of the study, plasma glucose level in glipizide-treated rats was 167.2 ± 4.68 mg/dl ($P < 0.001$). The result of EE treatment on plasma glucose level of Type II diabetic rats is shown in Figure 5.

Biochemical parameters

Total cholesterol level was found to be 139.60 ± 1.76 mg/dl ($P < 0.001$) in diabetic control group when compared to normal animals (71.50±2.37 mg/dl). Cholesterol level was significantly decreased after treatment with EE at the dose of 1000 mg/kg to 115.8 ± 3.33 mg/dl ($P < 0.001$) when compared to diabetic control. Glipizide had little effect on total cholesterol level

compared to EE. The effect of EE on biochemical parameters of type II diabetic rats is shown in Table 2.

The diabetic control animals exhibited high triglyceride level, which was found to be 135.80 ± 2.07 mg/dl ($P < 0.001$) when compared to normal animals. EE at the dose of 500 and 1000 mg/kg decreased the triglyceride level to 122.7 ± 2.29 mg/dl ($P < 0.05$) and 104.2 ± 3.41 mg/dl ($P < 0.001$), respectively, compared to diabetic control. Glipizide also significantly decreased triglyceride level to 111.0 ± 2.07 mg/dl ($P < 0.001$).

Sinking of HDL level to 26.16 ± 0.88 mg/dl ($P < 0.001$) was efficient in diabetic control animals in comparison to normal animals (37.83 ± 0.89 mg/dl). HDL level was increased to 30.09 ± 1.03 mg/dl ($P < 0.01$) and 32.36 ± 0.72 mg/dl ($P < 0.001$) after treatment with EE at the dose of 500 and 1000 mg/kg, respectively. Glipizide had no significant effect on HDL level.

A significant upsurge in AST and ALT levels was observed to 245.7 ± 5.33 IU/L and 79.82 ± 1.79IU/L, respectively, in diabetic animals. An increase in ALP level was also detected

Parameters	Normal control	Diabetic control	Diabetic+EE (250 mg/kg)	Diabetic+EE (500 mg/kg)	Diabetic+EE (1000 mg/kg)	Diabetic+glipizide (5 mg/kg)
TC (mg/dl)	72.50±2.48	126.60±1.76###	133.6±2.86	131.1±2.85	115.8±3.33***	128.70±2.41*
TG (mg/dl)	49.59±2.77	135.80±2.07###	127.1±2.95	119.7±2.23*	105.2±3.21***	111.0±2.01***
HDL (mg/dl)	37.83±0.89	26.16±0.88###	26.26±0.48	30.09±1.43**	32.36±0.72***	25.80±0.52
AST (IU/L)	164.5±4.87	245.7±5.33###	245.1±5.2	238.8±6.5	239.2±6.8	210.6±6.14**
ALT (IU/L)	61.51±2.40	79.82±1.79###	74.61±2.481	72.03±3.072	67.01±3.596	68.61±3.28*
ALP (IU/L)	82.2±2.50	163.20±2.52###	159.5±3.70	166.6±2.86	154.4±3.49	159.80±3.77
TP (g/dl)	8.69±0.23	5.94±0.13###	6.25±0.30	6.41±0.17	6.73±0.23*	6.98±0.30*
ALB (g/dl)	5.81±0.34	2.91±0.20###	3.07±0.19	3.86±0.15	3.93±0.14*	3.97±0.12*
CK (mg/dl)	0.69±0.02	1.11±0.019###	1.134±0.037	0.975±0.425	0.938±0.041*	0.94±0.037*
BUN (mg/dl)	21.37±1.03	36.35±1.40###	39±1.34	35.55±1.23	30.45±1.19**	26.86±1.24***
Plasma insulin (pmol/l)	241.4±10.95	213.1±9.34###	206.4±8.84	210.4±11.11	225.8±7.01	244.8±9.38*

Table 2: Effect of EE of *Bauhinia variegata* on biochemical parameters in Type 2 diabetes

Values are expressed as Mean±SEM (TC: Total cholesterol, TG: Triglyceride, HDL: High-density lipoproteins, AST: Aspartate aminotransferase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, TP: Total protein, ALB: Albumin, CK: Creatinine, BUN: Blood urea nitrogen). *$P<0.05$, **$P<0.01$, ***$P<0.001$ when compared with diabetic control. ###$P<0.05$, $P<0.001$ when compared to normal control. SEM: Standard error of the mean, EE: Ethanolic extract, *B. variegata: Bauhinia variegata*

to 163.20 ± 2.52 IU/L in diabetic control animals when equated to that of normal animals. There was no significant effect on AST, ALT, and ALP levels of the EE.

Total protein and albumin levels were found to decreased in diabetic animals 5.94 ± 0.13 g/dl ($P < 0.001$) and 2.91 ± 0.20 g/dl ($P < 0.001$), respectively, when compared to normal animals. EE at the dose of 1000 mg/kg increases total protein level to 6.93 ± 0.23 g/dl ($P < 0.05$) and albumin level to 3.93 ± 0.14 g/dl ($P < 0.05$).

Creatinine level was significantly elevated in diabetic control group 1.11 ± 0.019 mg/dl ($P < 0.001$) when compared to normal control group. Creatinine level decreased to 0.938 ± 0.041 mg/dl ($P < 0.05$) after treatment with 1000 mg/kg EE.

BUN level was increased to 36.35 ± 1.40 mg/dl ($P < 0.001$) in diabetic animals which was decreased to 30.45 ± 1.19 mg/dl ($P < 0.01$) in EE 1000 mg/kg treated group.

Reduction in plasma insulin level was pragmatic in diabetic control animals. Plasma insulin level in diabetic animals was found to be 213.4 ± 9.34 pmol/L ($P < 0.05$) when compared to normal animals (241.4 ± 10.95 pmol/L). EE did not show a significant effect on plasma insulin level when compared to diabetic animals. Glipizide had increased the plasma insulin level significantly to 244.8 ± 9.38 pmol/L ($P < 0.05$) when compared to diabetic animals.

DISCUSSION

Alloxan is a very unstable chemical compound[21] with a molecular shape resembling glucose.[22,23] Both alloxan and glucose are hydrophilic and do not penetrate the lipid bilayer of the plasma membrane. The alloxan molecule is structurally

so like glucose that the GLUT2 glucose transporter in the beta-cell plasma membrane accepts this glycomimetic and transports it into the cytosol.

Alloxan does not inhibit the function of the transporter[24] and can therefore selectively enter beta cells in an unrestricted manner.[25,26,27] It is therefore not toxic to insulin-producing cells that do not express this transporter. The half-life of alloxan is short;[28,29] in aqueous solution it spontaneously decomposes into non-diabetogenic alloxanic acid within minutes.[21] Because of this, it must be taken up and accumulated quickly in the beta cell,[25] and is therefore ineffective when blood flow to the pancreas is interrupted for the first few minutes after alloxan injection.[30-32] N-substituted alloxan derivatives with a long carbon side chain, such as butyl alloxan, differ chemically from alloxan in that they are lipophilic.[33]

Chemically, alloxan is oxygenated pyrimidine derivative beta-cell toxic glucose analog used for induction of diabetes mellitus in experimental animals. Treatment of the rats with alloxan induces the diabetes along with other unwanted biochemical and pathological changes in both Types I and II diabetes. Significant change was observed in body weight after treatment with EE.

Plasma glucose levels were found to decrease significantly in both types of diabetes after treatment with EE of *B. variegata* leaves for 28 days. Results of OGTT also support these results. EE of *B. variegata* leaves at dose of 1000 mg/kg showed maximum improvement in glucose tolerance when compared to diabetic animals. Effect of EE 1000 mg/kg was as good as that of glipizide. Plasma glucose lowering activity of EE was observed without altering plasma insulin level significantly.

Lipid profile of the diabetic animals was found to improve significantly after treatment with EE of *B. variegata* leaves.

EE at the dose of 1000 mg/kg decreased the cholesterol and triglyceride levels in Type I as well as Type II diabetic animals while increasing HDL level in Type II diabetic animals at the dose of 500 and 1000 mg/kg in both the types of diabetes.

EE of *B. variegata* leaves did not show any significant effect on AST, ALT, and ALP levels. EE showed significant increase in total protein and albumin in diabetic rats at the dose of 1000 mg/kg. EE had also decreased the concentration of creatinine and BUN which may be due to improvement in kidney function.

Various *in vitro* and *in vivo* studies have proved the use of flavonoids containing medicinal plants in the treatment of diabetes mellitus.[34–36] This includes flavonoids such as quercetin, rutin, and kaempferol.[37,38] *B. variegata* leaves are a good source of flavonoids.[39] This bioactivity of extract may be attributed to the presence of flavonoids in the extract.

CONCLUSION

From the various studies conducted and results obtained, it can be concluded that the EE of *B. variegata* leaves possesses antidiabetic activity in Type I as well as Type II diabetes.

Findings in the current study say that the EE of *B. variegata* leaves exhibited considerable effect on the various biochemical parameters such as AST, ALT, and ALP levels, concentration of creatinine and BUN, as well as leading to reduction in the blood glucose levels in experimental animal models. From the future standpoints of the contemporary study, histopathological appraisal of the pancreas to monitor any degenerative and necrotic vicissitudes can be steered.

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REFERENCES

1. Larson SS. Pollen morphology of Thai species of *Bauhinia Caesalpiniaceae.* Grana 1974;14:114-31.
2. The Controller of Publication. The Ayurvedic Pharmacopoeia of India. Vol. 1. New Delhi: The Controller of Publication; 2001. p. 321-2.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol. 2. Dehradun: International Book Distributors; 1999. p. 891-902.
4. Rajkapoor E, Jayakar B, Murugesh N. Antitumor activity of *Bauhinia variegata* on Dalton's ascetic lymphoma. J Ethanopharmacol 2003;89:91.
5. Arafa R. Naringenin as an antidermatophyte flavonoid aglycone from Bauhinia variegata. Al-Azhar J Microbiol 2001;51:17-23.
6. Dondaity S, Mahdy M, Hamouly M, Ammar H. Chemical and or biological studies of *Bauhinia variegata* L. and *Cleome droserifolia* Forssk. Del. growing in Egypt. Egypt J Biomed Sci 2005;19:181-203.
7. Yadava RN, Reddy VM. Anti-Inflammatory activity of a novel flavonol glycoside from the *Bauhinia variegata* Linn. Nat Prod Res 2003;17:165-9.
8. Kumar D, Parcha V, Dhulia I, Maithani A. Effect and valuation of anti-hyperlipidemic activity of methanol extract of *Bauhinia variegata* Linn. leaves in triton WR-1339 Tyloxapol induced hyperlipidemic rats. J Pharm Res 2011;4:58-60.
9. Mishra A, Kumar S, Bhargava A, Sharma B, Pandey AK. Studies on *in vitro* antioxidant and antistaphylococcal activities of some important medicinal plants Cell. Mol Biol 2011;57:16-25.
10. Ali MS, Azhar I, Amtul Z. Antimicrobial screening of some *Caesalpiniaceae*. Fitoterapia 1999;70:299-302.
11. Frankish N, DeSousa MF, Mills C, Sheridan H. Enhancement of insulin release from the beta-cell line INS-1 by an ethanolic extract of *Bauhinia variegata* and its major constituent roseoside. Planta Med 2010;76:995-7.
12. Rashid KI. Detection of insulin-like protein and some active compounds in *Bauhinia variegata* Linn. leaf ethanolic extracts and the effect in reducing blood glucose levels in Mice. IOSR J Pharm 2014;4:1-5.
13. Khot PR, Agrawal SL, Patole AM, Tundulwar MR, Tenpe CR, Rathod SS. Antidiabetic and antioxidant effect of ethanolic extract of *Bauhinia varigata* leaves in streptozotocin induced diabetic rats, Inventi Rapid. Ethnopharmacology 2012;14:12-3.
14. Kulkarni YA, Garud MS. Effect of *Bauhinia variegata* Linn. *Caesalpiniaceae* extract in streptozotocin induced type I diabetic rats. Orient Pharm Exp Med 2015;15:191-8.
15. Kulkarni YA, Panjabi R, Patel V, Tawade A, Gokhale A. Effect of *Gmelinaarborea* Roxb. in experimentally induced inflammation and nociception. J Ayurveda Integr Med 2013;4:152.
16. Singleton VL, Orthofer R, LamuelaRaventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 1999;299:152-78.
17. Quettier DC, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx MC, *et al*. Phenolic compounds and antioxidant activities of buckwheat *Fagopyrum esculentum* Moench Hulls and Flour; J. Ethnopharmacol. 2000;72:35-42.
18. Chakraborthy SG, Ghorpade PM. Determination of quercetin by HPTLC in *Calendula officinalis* extract. Int

J Pharma Biosci 2010;1:1-4.

19. Trivedi NA. Effect of *Bauhinia variegata* Linn. on blood glucose and lipid profile in alloxan induced diabetes rats. Indian J Pharmacol 2004;36:373-6.

20. Lenzen S. The mechanisms of alloxan and streptozotocin induced diabetes. Diabetologia 2008;51:216-26.

21. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of highfatdiet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol Res 2005;52:313-20.

22. Weaver DC, Barry CD, Mcdaniel ML, Marshall GR, Lacy PE. Molecular requirements for recognition at gluco-receptor for insulin release. Mol Pharmacol 1979;16:361-8.

23. Gorus FK, Malaisse WJ, Pipeleers DG. Selective uptake of alloxan by pancreatic B-cells. Biochem J 1982;208:513-5.

24. Elsner M, Tiedge M, Guldbakke B, Munday R, Lenzen S. Importance of the GLUT2 glucose transporter for pancreatic beta cell toxicity of alloxan. Diabetologia 2002;45:1542-9.

25. Hammarström L, Hellman B, Ullberg S. On the accumulation of alloxan in the pancreatic beta-cells. Diabetologia 1967;3:340-4.

26. Boquist L, Nelson L, Lorentzon R. Uptake of labeled alloxan in mouse organs and mitochondria *in vivo* and *in vitro*. Endocrinology 1983;113:943-8.

27. Malaisse WJ, Doherty M, Ladriere L, Lagae FM. Pancreatic uptake of [2-^{14}C] alloxan. Int J Mol Med 2001;7:311-5.

28. Bloch KO, Zemel R, Bloch OV, Grief H, Vardi P. Streptozotocin and alloxan-based selection improves toxin resistance of insulin-producing RINm cells. Int J Exp Diabetes Res 2000;1:211-9.

29. Patterson JW, Lazarow A, Levey S. Alloxan and dialuric acid their stabilities and ultraviolet absorption spectra. J Biol Chem 1949;177:187-96.

30. Gomori G, Goldner MG. Acute nature of alloxan damage. Proc Soc Exp Biol NY 1945;58:232-3.

31. Bailey CC, Collins-Williams J, Le Compte PM. Effect of alloxan in rabbits with temporary occlusion of the arteries to the pancreas. Proc Soc Exp Biol 1950;71:580-3.

32. Hellman B, Diderholm H. The diabetogenic effect of alloxan after elimination of extra-pancreatic factors. Acta Endocrinol Copenh 1955;20:81-7.

33. Munday R, Ludwig K, Lenzen S. The relationship between the physicochemical properties and the biological effects of alloxan and several N-alkyl substituted alloxan derivatives. J Endocrinol 1993;139:153-63.

34. Nicolle E, Souard F, Faure P, Boumendjel B. Flavonoids as promising lead compounds in type 2 diabetes mellitus: Molecules of interest and structure activity relationship. Curr Med Chem 2011;18:2661-72.

35. Patel MB, Mishra SH. Hypoglycemic activity of C-glycosyl flavonoid from *Enicostemma hyssopifolium*. Pharm Biol 2011;49:383-91.

36. Mohan S, Nandhakumar L. Role of various flavonoids: Hypotheses on novel approach to treat diabetes. J Med Hypotheses Ideas 2014;8:1-6.

37. Kobori M, Masumoto S, Akimoto Y, Takahashi Y. Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. Mol Nutr Food Res 2009;53:859-68.

38. Prasath GS, Subramanian SP. Modulatory effects of fisetin a bioflavonoid, on hyperglycemia by attenuating the key enzymes of carbohydrate metabolism in hepatic and renal tissues in streptozotocin-induced diabetic rats. Eur J Pharmacol 2011;668:492-6.

39. Babua PV, Liub D, Gilbertc ER. Recent advances in understanding the anti-diabetic actions of dietary flavonoids. J Nutr Biochem 2013;24:1777-89.

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