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Research Article

EFFECT OF METHANOLIC EXTRACTS OF *BLUMEA ERIANTHA* DC LEAVES ON PROTEIN METABOLISM AND MARKER ENZYMES IN STREPTOZOTOCIN- INDUCED HYPERGLYCEMIC ANIMALS

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ABSTRACT

The object of present study to investigate the possible protective effect of methanolic extracts of Blumea eriantha DC leaves on various biochemical markers in streptozotocin (STZ)-induced hyperglycemia in rats. STZ treatment (60 mg/kg/i.p) caused a hyperglycemic state that led to various physiological and biochemical alterations. Blood levels of glucose, urea, uric acid and creatinine, plasma levels of albumin and albumin/globulin ratio and the activities of diagnostic marker enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (γ -GT) in plasma, liver and kidney were markedly altered in STZ diabetic rats. Oral administration of *Blumea eriantha* DC (250, 500 mg/kg/p.o) for 21 days restored all these biochemical parameters to near normal levels. Thus, the present results have shown that methanolic extracts of *Blumea eriantha* DC leaves has the antihyperglycemic effect and consequently may alleviate liver and renal damage associated with STZ-induced diabetes in rats.

Keywords: *Blumea eriantha* DC; Asteraceae; Streptozotocin; Diabetes mellitus; Protein metabolism.

INTRODUCTION

In a recent paper, we reported the elevated levels of plasma thiobarbituric acid reactive substance (TBARS) and hydroperoxide (an index of tissue injury) in streptozotocin (STZ) diabetic rats. The higher levels of these substances suggest an increased rate of tissue injury in STZ diabetic rats. Diabetes mellitus (DM) is also grossly reflected by profound changes in protein metabolism and by a negative nitrogen (N) balance and loss of nitrogen from most organs [(1)]. Increased urea nitrogen production in diabetes may be accounted for by enhanced catabolism of both liver and plasma proteins [(2,3)]. Additionaly Bopanna et al. [(4)] and Eskander et al. [(5)] demonstrated that the administration of several herb extracts could restore the changes in the activities of serum enzymes like alkaline phosphates (ALP), acid phosphates and transaminases: aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

The plant *Blumea eriantha* DC is commonly known as 'Nimurdi' (Marathi), Kukronda in Hindi, a slender herb, dichotomously branched, covered with white and silky hair, distributed in Karnataka, Maharashtra, U.P., M.P., Bihar and Orissa. It is perennial herb upto 1m in height, leaves are 2.0-19.0 cm × 0.6- 6.0 cm, lower obovate, short petiolated, upper elliptic- ovate to oblanceolate, cordate clasping; capitula axillary or terminal, with numerous yellow florets, rarely bisexual marginal female, achenes brown, shining, sparsely pilos with white pappus.[1]Juice of herb used as 'carminative'. Leaves along *Vitex negundo* Linn and *Careya arborea* Roxb as Fomentation, sudorific, diuretic and emmenagogue. [(13)]Plant is used in Rheumatic pain[3] leaves in cough and common cold. "Erianthin" isolated from flower characterized as 5-hydroxy-3,3',4',6,7, pentamethoxyflavone, essential oil possessing a camphor like smell and consisting d-carvotanacetone and erianthin isolated from seeds also.[(14)] The essential oil extracted from leaves and stem showing potent antibacterial activity, antifungal, insecticidal.[(6)] .[(7)] .[(8)] .[(9)] .[(10)] .[(11)].LD50 of 50% ethanolic extract of plant was found to be >100mg/kg in mice. [(12)]. In the traditional medicinal system, the leaves, flowers and tender shoot are said to be cooling and demulcent; they are used in the form of decoction for leprosy. The leaves of *Blumea eriantha* DC was undertaken to verify the claim and evaluate the anti-diabetic property. [(13)] In the current literature, there is no much data concerning the effect of the *Blumea eriantha* DC on the biochemical parameters and the activities of enzymes which are abnormally altered due to DM. Therefore, the present study aims to examine the influence of oral administration of the methanolic leaves extract of *Blumea eriantha* DC on the levels of some biochemical parameters and the activities of some enzymes in serum, liver, and kidney in STZ-induced diabetic rats.

MATERIALS AND METHODS

Animals

Male Wister albino rats (160-200g) were taken from the animal house of KNIMT, SULTANPUR. And acclimatised under standard environmental conditions (12-h light/dark cycles at 25-28 C, 60-80% relative humidity), and fed with a standard diet (Hindustan Lever, India) and water ad libitum. All the studies were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals [(14)].

Preparation of plant extract

The shade dried leaves of *Blumea eriantha* DC were ground into fine powder with grinder. Then the powder passed from 10 no. sieve and suspended in equal amount of water and stirred intermittently and left overnight. The macerated pulp was then filtered through a coarse sieve and the filtrate was dried at reduced temperature. This dry mass (yield 8.5 g/kg of powdered Leaves) was served as the methanolic extract of *Blumea eriantha* DC for experiment.

Induction of hyperglycemia

Rats were made hyperglycemic by single administration of STZ (60 mg/kg/i.p) purchased from Sigma Chemical Co. (St. Louis, MO, USA) dissolved in 0.1-M citrate buffer, pH 4.5. After 48 h, blood sample were collected and glucose levels were determined to confirm the development of hyperglycemia. Only those animals which having hyperglycemia (blood glucose levels>240 mg/dl) were used in the experiment.

Experimental protocol

Hyperglycemia was induced in animals. After the induction of hyperglycemia, rats were divided into 4 groups of 6 animals each (group II-V). Group I received the vehicle alone and was served as the control. Group II received STZ (60 mg/kg/i.p) dissolved in 0.1-M citrate buffer. Group III & Group IV received the methanolic extract of *Blumea eriantha* DC (250 mg, 500 mg/kg/p.o) once daily for 21 days. Group V received tolbutamide (250 mg/kg/p.o) once daily for 21 days.After 21 days of treatment, the animals were killed by cervical dislocation. Blood was collected into heparinzed tubes, and the plasma and serum were separated by centrifugation. The liver and kidney were quickly removed, washed in ice cold, isotonic saline and blotted individually on ash-free filter paper and weighed were measured. The tissues were then homogenized in 0.1 M Tris-HCl buffer, pH 7.4. The homogenate was used for the estimation of proteins, enzymes, and other parameters.

Estimation of biochemical parameters

Plasma glucose level was measured by the method of Sasaki and Sonae [15] and serum concentration of urea, uric acid and creatinine were determined by Autoanalyser using reagent kit obtained from Boehringer (Mannheim, Germany). The protein content in plasma, liver and kidney was measured by the method of Lowry et al. [16]. The albumin and globulin content in plasma were determined by the method described by Reinhold [17]. The enzymes (AST, ALT and ALP) were assayed by the method of King and Armstrong [18] and γ-glutamyl transpeptidase (γ -GT) was assayed by the method of Rosalki and Rau [19].

Statistical Analysis

Values were presented as Means ± SD. Data were analyzed using analysis of variance (ANOVA) and group means were compared with Duncan's multiple range test (DMRT) using SPSS (Statistical Package for Social Science).

RESULTS

Table 1 showing the liver and kidney weights in control and STZ diabetic rats. Methanolic extract of *Blumea eriantha* DC restored the liver weight to near normal. The kidney weight was increased in diabetic rats and methanolic extract of *Blumea eriantha* DC normalized the kidney weight in STZ diabetes. Table 2 shows the blood levels of urea, uric acid, and creatinine in plasma of normal and STZ diabetic rats. These biochemical variables were significantly elevated in STZ diabetic rats ($p<0.05$) when compared to control animals. Oral administration of methanolic extract of *Blumea eriantha* DC (250, 500 mg/kg/ p.o) for 21 days significantly lowered urea, uric acid and creatinine levels in STZ diabetic rats.

Table 1: Effect of methanolic extract of *Blumea eriantha* D.C.leaves on liver and kidney weight in control and experimental animal

Gr	Treatment(mg/kg/po)	Liver wt (g)	liver wt/100g body wt	Kidney wt.(g)	Kidney wt /100g body wt
1	Control(2%gum acacia)	6.82±0.31 [a]	3.32±0.31 [a]	1.34±1.12 [a]	0.51±0.02 [a]
2	Diabetic control	4.30±0.13 [b]	2.65±0.18 [b]	1.50±0.44 [b]	0.54±0.33 [b]
3	Diabetic +*B. eriantha250*	5.75±0.31 [c]	3.12±0.34 [c]	1.35±0.05 [c]	0.78±0.82 [c]
4	Diabetic +*B. eriantha500*	5.92±0.15 [d]	3.42±0.14	1.15±0.05 [d]	0.65±0.05 [d]
5	Diabetic +*tolbutamide250*	6.13±0.07 [d]	3.60±0.15	1.32±0.07 [d]	0.66±0.34 [d]

Values are given as means ±SD of six animals in each group. Values not sharing a common superscript(a, b ,c and d.) differ significantly at $p<0.05$, Duncan's multiple range test (DMRT)

The levels of protein, plasma albumin and Albumin/Globulin ratio in normal and STZ diabetic rats are shown in Table 2. The level of protein in plasma was found to be reducing in diabetic animals ($p<0.05$) when compared to control animals. The lowered level of protein, after methanolic extract of *Blumea eriantha* DC treatment, increased to near normal. The levels of albumin and albumin/globulin ratio in plasma were decreased in diabetic animals. These lowered levels of plasma albumin and albumin/globulin ratio level were comes back significantly in methanolic extract of *Blumea eriantha* DC treated diabetic rats.

Table 2: Effect of methanolic extract of *Blumea eriantha* D.C. leaves on blood level of urea, uric acid,creatinine, serum protein, albumin and A/G ratio in control.

Gr	Treatment(mg/kg/po)	Urea(mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Serum protein (mg/dl)	Albumin (mg/dl)	A/G ratio
1	Control(2%gum acacia)	23.66±2.0 [a]	1.4±0.1 [a]	0.95±0.19 [a]	6.62±0.83 [a]	5.7±0.11 [a]	1.24±0.14 [a]
2	Diabetic control	37.0±0.72 [b]	1.9±0.1 [b]	2.17±0.35 [b]	5.5±0.17 [b]	1.5±1.0 [b]	0.8±0.15 [b]
3	Diabetic +*B. eriantha250*	29.1±2.8 [c]	1.6±0,4 [c]	1.78±0.15 [c]	6.9±0.23 [c]	2.1±0.15 [c]	0.96±0.15 [c]
4	Diabetic +*B. eriantha500*	23.0±0.23 [d]	1.33±0.2 [d]	1.35±0.01 [d]	6.93±0.50 [d]	5.2±0.26 [d]	1.2±0.3 [d]
5	Diabetic +*tolbutamide250*	21.7±1.9 [d]	1.19±0.8 [d]	1.26±0.2 [d]	6.23±0.5 [d]	5.3±0.25 [d]	1.12±0.32 [d]

Values are given as means ±SD of six animals in each group. Values not sharing a common superscript (a, b, c and d.) differ significantly at $p<0.05$, Duncan's multiple range test (DMRT)

Tables 3, 4 and 5 show the activities of AST, ALT, ALP and γ -GT in plasma, liver and kidney of the control and STZ diabetic rats, respectively. The activities of these enzymes were found to be significantly increased ($p<0.05$) in plasma and liver of diabetic rats. In the kidney of diabetic animals, the activities of ALP and γ-GT were increased while the activities of AST and ALT were not altered. Oral administration of methanolic extract of *Blumea eriantha* DC (250, 500 mg/kg/p.o) for 21 days resulted in the near normalization of the activities of AST, ALT, ALP and γ -GT in plasma, liver and kidney of diabetic rats.

Table 3: Effect of methanolic extract of *Blumea eriantha* D.C.leaves on serum diagnostic marker enzyme in control and experimental animal

Gr	Treatment(mg/kg/po)	AST(Ux)	ALT(Ux)	ALP(Uy)	γ -GT(Uz)
1	Control(2%gum acacia)	76.0±0.39 [a]	21.36±1.4 [a]	74.34±2.25 [a]	12.6±1.70 [a]
2	Diabetic control	110±5.0 [b]	58.66±0.04 [b]	135.13±5.0 [b]	24.64±2.2 [b]

3	Diabetic +*B. eriantha250*	94.34±3.70 [c]	41.24±2.9	115.3±1.28 [c]	18.6±1.9 [c]
4	Diabetic +*B. eriantha500*	84.24±3.70 [d]	31.67±3.12 [d]	98.34±3.72 [d]	16.8±1.63 [d]
5	Diabetic +*tolbutamide250*	84.8±3.14 [d]	26.1±2.26 [d]	87.5±5.0 [d]	16.5±1.27 [d]

Values are given as means ±SD of six animals in each group. Values not sharing a common superscript (a, b, c and d.) differ significantly at p<0.05, Duncan's multiple range test (DMRT)

Ux = mmol of pyruvate liberated/h Uy = mmol of phenol liberated/min, Uz = mmol of p-nitroanilide liberated/min

Table 4: Effect of methanolic extract of *Blumea eriantha* D.C.leaves on liver and kidney transaminases in control and experimental animal

Gr	Treatment(mg/kg/po)	AST(Ux)-Liver	AST(Ux) -Kidney	ALT(Ux)-Liver	ALT(Ux)-Kidney
1	Control(2%gum acacia)	645.0±17.4 [a]	760±14.5 [a]	934.0±19.4 [a]	850.5±22.8 [a]
2	Diabetic control	870.2±16.0 [b]	740.3±17.6 [b]	1250.2±16.33 [b]	854.4±20.2 [b]
3	Diabetic +*B. eriantha250*	748.0±20.3 [c]	749±13.4 [c]	1042.2±13.0 [c]	814.2±18.6 [c]
4	Diabetic +*B. eriantha500*	675.2±14.5 [d]	731.2±14.5	1002.3±19.3 [d]	864.4±9.24
5	Diabetic +*tolbutamide250*	644.2±12.5 [d]	761.4±20.2	945.3±11.5 [d]	870.6±16.4

Values are given as means ±SD of six animals in each group. Values not sharing a common superscript(a, b ,c and d.) differ significantly at p<0.05, Duncan's multiple range test (DMRT) Ux = mmol of pyruvate liberated/h

Table 5: Effect of methanolic extract of *Blumea eriantha* D.C.leaves on liver and kidney ALP and γGT control and experimental animal

Gr	Treatment(mg/kg/po)	ALP (Uy)-Liver	ALP(Uy)-Kidney	γ-GT (Uz)-Liver	γ-GT(Uz) -Kidney
1	Control(2%gum acacia)	0.18 ±0.02 [a]	0.25±0.03 [a]	3.24±0.24 [a]	2.58±0.18 [a]
2	Diabetic control	0.30±0.08 [b]	0.47±0.3 [b]	5.46±0.44 [b]	5.36±0.38 [b]
3	Diabetic +*B. eriantha250*	0.23±0.04 [c]	0.36±0.05 [c]	4.34±0.35 [c]	5.83±0.32 [c]
4	Diabetic +*B. eriantha500*	0.22±0.03 [d]	0.34±0.02 [d]	3.54±0.36 [d]	3.36±0.34 [d]
5	Diabetic +*tolbutamide250*	0.21±0.03 [d]	0.28±0.02 [d]	3.56±0.24 [d]	3.05±0.28 [d]

Values are given as means ±SD of six animals in each group. Values not sharing a common superscript (a, b, c and d.) differ significantly at p<0.05, Duncan's multiple range test (DMRT)

Uy = mmol of phenol liberated/min, Uz = mmol of p-nitroanilide liberated/min

DISCUSSION

The present study showing the hypoglycemic and protective effects of methanolic extract of *Blumea eriantha* DC leaves in the liver and kidney of STZ diabetic rats. We have observed a significant weight gain in methanolic extract of *Blumea eriantha* DC treated diabetic rats when compared with untreated animals. This observation shows the anabolic effect of methanolic extract of *Blumea eriantha* DC on body weight of the diabetic animals. A decrease in the liver weight observed in diabetic animals might be due to an increased breakdown of glycogen and/or pronounced gluconeogenesis. After 21 days of treatment with methanolic extract of *Blumea eriantha* DC in diabetic animals, an increase in the liver weight was observed. This result agrees well with the result of Jefferson et al. [20] who has reported that insulin therapy can increase the accumulation of glycogen in diabetic liver. Seyer-Hansen [30] and Esterby and Gundersen [21]) reported 15% rise in whole kidney weight within 72 h of induction of STZ in experimental diabetic rats. We have also observed an increased whole kidney weight in diabetic animals when compared with normal control animals. This is due to the glomerular cell proliferation accompanying glomerular enlargement in the early phase of STZ-induced diabetes in rats. In our present study, oral administration of methanolic extract of *Blumea eriantha* DC significantly decreases the kidney weight to near normal value. This might be due to the protective effect of methanolic extract of *Blumea eriantha* DC on glomerular cells in STZ-induced diabetic rats.The diabetic hyperglycemia induces the elevation of plasma levels of urea, uric acid and creatinine, which are considered as the significant markers of renal dysfunction [1]. The results in Table 2 show significant (p<0.05) increase in the level of plasma urea and creatinine in the diabetic rats when compared with respective control rats, while after the treatment of STZ diabetic rats with the methanolic extract of *Blumea eriantha* DC (250, 500 mg/kg), the levels of urea, uric acid and creatinine were significantly (p<0.05) decreased.The results of the present study demonstrated that the treatment of diabetic rats with the methanolic extract of *Blumea eriantha* DC caused a noticeable elevation in the plasma TP and A levels as compared with their normal levels. It has been established that insulin stimulates the incorporation of amino acids into proteins [5]. Treatment with methanolic extract of *Blumea eriantha* DC (250 and 500 mg/kg) or tolbutamide (250 mg/kg) normalized these enzymes activities. Similarly, increased activities of aspartate and alanine transaminase in diabetic liver were also reported by Jorda et al. [3].Elevated activity of ALP was observed in STZ diabetic rats. Prince et al. [41] have also reported increased ALP activity in experimentally diabetic rats. The increased activity of this enzyme in plasma may be a result of diabetes-induced damage to the tissues. methanolic extract of *Blumea eriantha* DC (250 and 500 mg/kg) treatment restored the activity of these enzymes to near normal by reducing their induction in diabetes. γ -GT catalyzes the transfer of the γ -glutamyl group from γ -glutamyl peptides to another peptide or L-amino acids or to water. Increased activity of γ-GT in STZ-induced diabetic rats was lowered to near normal by methanolic extract of *Blumea eriantha* DC (250 and 500 mg/kg) treatment that indicates the possible prevention of necrosis by methanolic extract of *Blumea eriantha* DC treatment.

In conclusion, the present study concluded that the methanolic extract of *Blumea eriantha* DC leaves could influence the protein metabolism and marker enzymes in STZ induced diabetic rats. Further, methanolic extract of *Blumea eriantha* DC ameliorated the impaired renal function and inhibited the liver damage associated with STZ diabetes in rats.

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