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Anti-hyperglycemic and anti-lipidperoxidative effects of *Tephrosia purpurea* leaf extract in streptozotocin induced diabetic rats

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Abstract

Objective: The main objective of the present study was to evaluate the anti-hyperglycemic and anti-lipidperoxidative effects of *Tephrosia purpurea* leaves (TpALet) in streptozotocin induced diabetic rats. **Materials and Methods :** The aqueous extract of *Tephrosia purpurea* leaves was evaluated for its anti-hyperglycemic and anti-lipidperoxidative effects in streptozotocin (50mg/kg bw) induced diabetic rats. The anti-hyperglycemic effect of the leaf extract was also compared with the standard anti-diabetic drug glibenclamide (600µg/kg bw). Glucose, glycosylated hemoglobin, total hemoglobin, lipid peroxidation by products (TBARS), enzymatic and non-enzymatic anti-oxidants in blood sample and hexokinase and glucose-6-phosphatase activities in liver were estimated calorimetrically in control and experimental animals. **Results:** "TpALet" considerably reduced the levels of blood glucose, glycosylated hemoglobin and enhanced the status of total hemoglobin, anti-oxidants and hepatic hexokinase and glucose-6-phosphatase activities. "TpALet" also showed a comparable effect to that of glibenclamide. **Conclusion:** Our results indicate that *T. purpurea* leaves have potent anti-hyperglycemic and anti-lipidperoxidative effects, which are probably due to its anti-oxidants effect.

Keywords: *Tephrosia purpurea*, streptozotocin, TBARS, anti-oxidants, TpALet.

1. Introduction

Diabetes mellitus is a clinical syndrome associated with an abnormally high blood glucose concentration due to insufficient insulin secretion or defective in insulin action. Approximately more than 150 million peoples were reported to have diabetes mellitus worldwide [1]. India is one of the leading countries for the number of people with diabetes mellitus and the current incidence of 57 million will double by the year 2025 due to its immense population [2].

Oxidative stress has been implicated in the pathogenesis of several clinical disorders including diabetes mellitus [3]. Oxygen free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins [4]. Enhanced oxidative stress has been well documented in both experimental and human diabetes mellitus [5].

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Tephrosia purpurea a sub-erect perennial herb is popularly known as “Purple tephrosia” in “English” and occurs throughout India in the bank of rivers. Experimental studies have demonstrated its anti-ulcer and hepatoprotective effects [6, 7]. Leaves of *T.purpurea* are used in Indian traditional medicine for jaundice and diabetes mellitus [8, 9]. The present study was therefore designed to focus the scientific evidence of the antidiabetic effect of *T.purpurea* leaves in streptozotocin induced diabetic rats.

2. Materials and Methods

2.1. Plant material

Leaves of *T.purpurea* were collected in and around Chidambaram, Tamil Nadu and it was botanically authenticated. A voucher specimen (AU05102) was deposited in the Department of Botany, Annamalai University, Annamalai nagar, Tamilnadu.

2.2. Preparation of plant extract

100g of fine powder of *T.purpurea* leaves was suspended in 250 ml of water for 2 hours and then heated at 60-65°C for 30 min. The extract was preserved and the process was repeated for three times with the residual powder, each time collecting the extract. The collected extract was pooled and passed through the fine cotton cloth. The filtrate upon evaporation at 40°C yielded 14% semisolid extract. This was stored at 0-4°C until used.

2.3. Preliminary phytochemical test

The preliminary investigation of *T.purpurea* leaves was found to contain flavonoids, alkaloids, glucoside rutin and lupeol.

2.4. Animals

Albino wistar male rats 7 to 8 weeks old and weighing 150-200g were used for the present study. The animals were obtained from central animal house, Rajah Muthiah Institute of Health

Sciences, Annamalai University, India and were housed in the central animal house with 12 h light and 12 h dark cycles. The animals were randomized into experimental and control groups and housed 4 or 5 in a polypropylene cages. Standard pellets obtained from Mysore Snack Feed Ltd, Mysore, India, were used as a basal diet during the experiment. The control and experimental animals were provided food and drinking water *ad libitum*.

2.5. Induction of diabetes mellitus

Diabetes mellitus was induced in Wistar rats by single intraperitoneal injection of streptozotocin (50mg/kg bw) dissolved in 0.1M-citrate buffer (pH 4.5), after overnight fasting for 12 h [10]. The diabetes was assessed by determining the blood glucose concentration within 48 hours after injection of streptozotocin. The rats with blood glucose level above 250 mg/dl were selected for experimental study.

2.6. Study design

In the experiment, a total number of 30 rats (18 diabetic rats, 6 normal rats 6 normal rats treated with “TpALet” alone) were used. The rats were divided into 5 groups of six each.

- Group I : Served as control rats and did not receive any other treatment
- Group II : Diabetic control (50mg/kg bw i.p streptozotocin)
- Group III : Diabetic rats receiving “TpALet” (600mg/kg bw) using an intra-gastric tube daily for 45 days
- Group IV : Control rats receiving “TpALet” (600mg/kg bw) using an intra-gastric tube daily for 45 days
- Group V : Diabetic rats receiving glibenclamide (600?g/kg bw) in distilled water using an intragastric tube daily for 45 days

After the experimental period, all animals were sacrificed by cervical dislocation and biochemical studies were conducted on blood, plasma, erythrocytes, erythrocyte membranes and liver of control and experimental animals in each group.

Blood glucose, glycosylated hemoglobin, total hemoglobin and plasma insulin were estimated by the methods of Sasaki *et al.*, [11], Sudhakar Nayak and Pattabhiraman [12], Drabkin and Austin [13], and ELISA using Boehringer Mannheim kit [14] respectively. TBARS in plasma, erythrocytes and erythrocyte membranes were assayed by the methods of Yagi [15] and Donnan [16]. Enzymatic antioxidants were estimated according to the methods of Kakkar *et al.*, (SOD) [17], Sinha (CAT) [18] and Rotruck *et al.*, (GPx) [19] and non enzymatic antioxidants were estimated according to the methods of Beutler and Kelley (GSH) [20], Desai (Vitamin E) [21] and Omaye *et al.*, (Vitamin C) [22]. Hexokinase and glucose-6-phosphatase activities in liver were estimated by the methods of Brandstrup *et al.*, [23] and Koida and Oda [24] respectively.

2.7. Statistical Analysis

The data are expressed as means \pm SD. Statistical comparisons were performed by one way analysis of variance [ANOVA] followed by Duncan's multiple comparisons test [DMRT]. The results were considered statistically significant if the p values were 0.05 or less.

3. Results

Table 1 shows the blood picture of control and experimental animals in each group. The levels of blood glucose and glycosylated hemoglobin were significantly increased whereas total hemoglobin and plasma insulin

levels were significantly decreased in diabetic animals (Group II) as compared to control animals (Group I). However, the above-mentioned biochemical parameters were significantly improved in diabetic rats treated with "TpALet" and glibenclamide. No statistical significance was observed between control and rats treated with "TpALet" alone. "TpALet" showed a comparable effect to that of glibenclamide.

Table 2 indicates the activities of hexokinase and glucose-6-phosphatase in liver of control and experimental animals in each group. A significant decrease in hexokinase and increase in glucose-6-phosphatase activities were noticed in liver of diabetic rats as compared to control animals. Oral administration of "TpALet" and glibenclamide decreased the glucose-6-phosphatase and increased the hexokinase activities in diabetic animals.

Table 3 presents the levels of plasma, erythrocytes and erythrocyte membranes TBARS in control and experimental animals in each group. The levels of TBARS were significantly increased in diabetic rats as compared to control rats. However, the levels of TBARS were significantly reduced in diabetic rats treated with "TpALet" and glibenclamide.

Table 4&5 show the activities of enzymatic antioxidants and levels of non-enzymatic antioxidants in plasma, erythrocytes and erythrocyte membranes respectively in control and experimental animals in each group. The levels of non-enzymatic antioxidants and activities of enzymatic antioxidants were decreased in diabetic rats as compared to control rats, which were revert back to normal range after treatment with "TpALet" and glibenclamide.

Table 1. Blood picture of control and experimental animals in each group.

Groups	Blood glucose (mg/dl)	Glycosylated hemoglobin (HbA1%)	Total hemoglobin (grams/dl)	Plasma insulin (μ U/ml)
Control	89.16 \pm 10.2 ^a	2.96 \pm 0.34 ^a	13.25 \pm 1.17 ^a	18.5 \pm 0.96 ^a
Diabetic control	266.5 \pm 15.6 ^b	6.18 \pm 0.48 ^b	8.66 \pm 1.02 ^b	10.4 \pm 0.82 ^b
Diabetes + TpALet (600 mg/kg bw)	132.5 \pm 13.1 ^{ce}	5.05 \pm 0.42 ^c	10.83 \pm 1.34 ^{ce}	13.8 \pm 0.78 ^{ce}
Control + TpALet (600 mg/kg bw)	85.06 \pm 9.07 ^a	2.84 \pm 0.31 ^a	13.86 \pm 1.24 ^a	19.6 \pm 1.1 ^a
Diabetes + glibenclamide (600 μ g/kg bw)	119.3 \pm 12.6 ^{de}	4.43 \pm 0.38 ^d	11.5 \pm 1.22 ^{de}	15.4 \pm 1.08 ^{de}

Values are expressed as mean \pm SD (n=6)

Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)

Table 2. Hexokinase and glucose-6-phosphatase activities in liver of control and experimental animals in each group

Groups	Hexokinase U*/mg protein	Glucose-6-phosphatase U**/mg protein
Control	0.25 \pm 0.02 ^a	2.45 \pm 0.09 ^a
Diabetic control	0.11 \pm 0.02 ^b	3.65 \pm 0.14 ^b
Diabetes + TpALet (600 mg/kg bw)	0.15 \pm 0.01 ^c	3.12 \pm 0.10 ^c
Control + TpALet (600 mg/kg bw)	0.27 \pm 0.03 ^a	2.24 \pm 0.11 ^a
Diabetes + glibenclamide (600 μ g/kg bw)	0.19 \pm 0.02 ^d	2.86 \pm 0.12 ^d

Values are expressed as mean \pm SD (n=6)

Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)

* μ moles of glucose phosphorylated / hour

** μ moles of inorganic phosphate liberated /min

Table 3. TBARS level in plasma, erythrocytes and erythrocyte membranes of control and experimental animals in each group

Groups	Plasma TBARS (nmol/ml plasma)	Erythrocytes TBARS (P mol/mg Hb)	Erythrocyte membranes TBARS (nmol /mg protein)
Control	1.5 \pm 0.18 ^a	0.84 \pm 0.09 ^a	0.54 \pm 0.04 ^a
Diabetic control	2.9 \pm 0.22 ^b	1.54 \pm 0.13 ^b	0.82 \pm 0.06 ^b
Diabetes + TpALet (600 mg/kg bw)	2.4 \pm 0.17 ^c	1.26 \pm 0.08 ^c	0.69 \pm 0.05 ^c
Control + TpALet (600 mg/kg bw)	1.4 \pm 0.16 ^a	0.78 \pm 0.08 ^a	0.51 \pm 0.05 ^a
Diabetes + glibenclamide (600 μ g/kg bw)	2.1 \pm 0.19 ^d	1.21 \pm 0.10 ^d	0.61 \pm 0.04 ^d

Values are expressed as mean \pm SD (n=6)

Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)

4. Discussion

Several studies have reported an increase in TBARS in experimental and human diabetes mellitus [25, 26]. Streptozotocin induced diabetes mellitus is an ideal model to study the anti-diabetic activity of medicinal plants and its constituents. Streptozotocin can induce diabetes mellitus by causing damage to pancreatic β -cells possibly by generating excess reactive oxygen species. Streptozotocin enhances lipidperoxidation process and cause DNA breaks in pancreatic β -cells of diabetic rats [27, 28]. Streptozotocin mediated membrane lipidperoxidation result in increased membrane rigidity, decreased cellular deformability, reduced erythrocyte survival, and lipid fluidity [26]. The observed increase in plasma TBARS in diabetic rats could be due to over production of lipid peroxidation by products in damaged pancreatic tissues and erythrocyte membranes with subsequent leakage into plasma.

Non-enzymatic antioxidants [vitamin E, reduced glutathione and vitamin C] and enzymatic antioxidants [superoxide dismutase (SOD), catalase(CAT), glutathione peroxidase (GPx)] form the major antioxidant defense mechanism to protect the cells against streptozotocin induced oxidative damage [26]. Several studies have shown a decline in enzymatic and non-enzymatic anti-oxidants status in plasma and erythrocytes of streptozotocin induced diabetic rats [26, 28]. Our results lend credibility to these observations. Oral administration of "TpALet" to diabetic rats significantly reduced the TBARS levels as well as improved the anti-oxidant defense mechanism in diabetic rats, which clearly indicates its potent anti-lipidperoxidative and anti-oxidants effects.

Glycosylated hemoglobin is now considered as the most reliable marker of glycemic control in diabetes mellitus [29]. Oral administration of "TpALet" to diabetic rats at a dose of 600mg/kg bw for 45 days resulted a decrease in

Table 4. Levels of enzymatic and non-enzymatic anti-oxidants in plasma of control and experimental animals in each group.

Groups	SOD (U ^A /ml)	Catalase (U ^B /ml)	GPx (U ^C /L)	GSH (mg/dl)	α - tocopherol (mg/dl)	Ascorbicacid (mg/dl)
Control	7.23±0.84 ^a	113.5±12.6 ^a	126.5±11.7 ^a	25.6±2.7 ^a	1.28±0.13 ^a	1.54±0.20 ^a
Diabetic control	4.56±0.52 ^b	74.6±9.8 ^b	84.6±9.6 ^b	15.4±1.9 ^b	0.74±0.09 ^b	1.06±0.12 ^b
Diabetes + TpALet (600 mg/kg bw)	5.27±0.62 ^{bc}	96.3±11.6 ^{cd}	108.3±11.3 ^{cd}	19.7±2.1 ^{cd}	0.93±0.11 ^c	1.23±0.14 ^c
Control + TpALet (600 mg/kg bw)	7.38±0.92 ^a	116.3±13.6 ^a	128.4±10.6 ^a	26.4±2.5 ^a	1.30±0.14 ^a	1.58±0.18 ^a
Diabetes + glibenclamide (600 μ g/kg bw)	6.24±0.58 ^{de}	106.4±13.4 ^{ad}	118.5±12.2 ^{ad}	21.3±2.3 ^{ad}	1.09±0.08 ^d	1.42±0.16 ^d

Values are expressed as mean \pm SD (n=6)

Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)

A - The amount of enzyme required to inhibit 50% NBT reduction

B - Micromoles of H₂O₂ utilized/min

C - Micromoles of glutathione utilized/min

Table 5. Levels of enzymatic and non-enzymatic anti-oxidants in erythrocytes and erythrocyte membranes of control and experimental animals in each group.

Groups	SOD (U ^A /mg Hb)	Catalase (U ^B /mg Hb)	GPx (U ^C /g Hb)	Erythrocyte membranes Vitamin E (µg/mg protein)	Erythrocyte membranes GSH (mg/dl)
Control	6.12±0.58 ^a	126.5±13.2 ^a	16.4±1.7 ^a	1.12±0.08 ^a	46.3±4.9 ^a
Diabetic control	4.26±0.41 ^b	84.6±9.2 ^b	8.6±0.96 ^b	0.74±0.06 ^b	31.4±3.8 ^b
Diabetes + TpALet (600 mg/kg bw)	4.84±0.52 ^c	95.3±11.2 ^c	9.8±1.08 ^c	0.85±0.09 ^c	39.6±4.1 ^c
Control + TpALet (600 mg/kg bw)	6.32±0.63 ^a	132.4±12.8 ^a	17.2±2.0 ^a	1.18±0.11 ^a	48.2±5.1 ^a
Diabetes + glibenclamide (600 µg/kg bw)	5.24±0.49 ^d	102.4±9.8 ^d	11.5±1.16 ^d	0.94±0.10 ^d	42.3±3.9 ^d

Values are expressed as mean ± SD (n=6)

Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

A - The amount of enzyme required to inhibit 50% NBT reduction

B - Micromoles of H₂O₂ utilized/min

C - Micromoles of glutathione utilized/min

glycosylated hemoglobin and blood sugar and an increase in the plasma insulin levels. The anti-hyperglycemic effect of “TpALet” could be due to stimulation of insulin secretion from remnant pancreatic β-cells which in turn enhance glucose utilization by hepatic and extrahepatic tissues of diabetic rats. The observed increase in total hemoglobin and decrease in blood sugar levels as revealed by decreased glycosylated hemoglobin content in “TpALet” treated diabetic

rats suggest that it improved the glycemic control mechanism. Our results thus conclude that “TpALet” has potent anti-hyperglycemic, anti-lipidperoxidative and anti-oxidant effects in experimental diabetes mellitus. The effects of *T.purpurea leaves* were also found to be much comparable to that of glibenclamide treated rats. Further studies are warranted to isolate and characterize the anti-diabetic principles from *T.purpurea leaves*.

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