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Effect of *Plumbago zeylanica* L. on blood glucose and plasma antioxidant status in STZ diabetic rats.

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Abstract

The study was undertaken to evaluate the antioxidant potential of *Plumbago zeylanica* L. in streptozotocin (STZ) diabetic rats. The ethanolic extract of root of *P. zeylanica* L. at doses of 100 mg, 200 mg/kg p.o. for 21 days resulted in significant reduction in plasma TBARS and a significant elevation in plasma reduced glutathione (GSH), ascorbic acid. It is concluded that these results suggest that the ethanolic extract of root of *P. zeylanica* L. possess a strong antioxidant effect in diabetic rats.

Key words: *Plumbago zeylanica*, Streptozotocin, diabetes mellitus, TBARS, antioxidants.

1. Introduction

Diabetes mellitus is a disorder treated in Indian traditional medicine using medicinal plants [1-4]. In practice, it is being increasingly recognized to be an alternative approach to modern medicine. The World Health Organization (WHO) has also recommended that this practice should be encouraged, especially in countries where access to conventional treatment of diabetes mellitus is not adequate [5].

Currently, oxidative stress is suggested as one of the mechanisms underlying diabetes and diabetic complications [6], which results from an imbalance between radical generating and radical scavenging systems [7, 8]. The level of lipid peroxidation in cell is controlled by various cellular defense mechanisms consisted of enzymatic and non-enzymatic scavenging systems [9]. The efficacy of this defense

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mechanism is altered in diabetes [10] and hence the ineffective scavenging of free radicals may play a crucial role in determining tissue injury.

Plumbago zeylanica L. (Plumbaginaceae) is used as an irritant of the skin in the treatment of dyspepsia, piles, anasarca, diarrhoea and skin diseases[11]. The root paste is applied externally in leprosy and other skin diseases of an obstinate character and to open abscesses. It has been specially recommended in the treatment of rheumatism[12] and one of its chief ingredients has been advocated to produce hypolipidaemic effect [13].

Our preliminary experimental results were highly encouraging as they revealed that blood glucose level was significantly lowered after oral administration of ethanolic extract of root of *P. zeylanica* in STZ induced diabetes. To our knowledge, no detailed investigations have been carried out to shed light on the antioxidant property of *P. zeylanica* root extract. Thus the present investigation envisages confirming the antioxidant effect of *P. zeylanica* root extract and examining its effect on plasma antioxidant status.

2. Materials and methods

2.1. Animals

Male Wistar albino rats (Weighing 150-200g) were procured from the Animal house, Bishop Heber College, Tiruchirapalli. Animals were maintained at central animal house and were fed on standard diet with water *ad libitum*. All studies were conducted in accordance with the National Institute of Health guide lines [14].

2.2. Plant material

Root of *P. zeylanica* was collected from Kollimalai of Tamilnadu and the plant was botanically authenticated by Fr. K. M. Matthew, Director, Rapinat Herbarium, St. Joseph's College, Tiruchirapalli. Voucher Herbarium specimens were deposited in the (collection number 743, 4109, 25403) Rapinat Herbarium for future references.

2.3. Chemicals

STZ and thiobarbituric acid were obtained from Sigma Chemical Co, (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.4. Preparation of plant extract

Air-dried powder (500g) of *P. zeylanica* roots were extracted by percolation at room temperature with 70% ethanol. The extract was concentrated under reduced pressure at room temperature and dried in a vacuum desicator. The residue was extracted with distilled water and filtered. The filtrate was kept in the oven and evaporated to dryness. The dried mass (yield = 48.5g) was diluted with 50% aqueous sucrose and used in experiments.

2.5. Induction of diabetes

Rats were made diabetic by single administration of STZ (60mg/kg/i.p) [15, 16] dissolved in 0.1M-citrate buffer, pH 4.5. Forty-eight hours later, blood samples were collected and glucose levels were determined to confirm the development of diabetes. Only those animals which showed hyperglycemia (blood glucose levels > 250mg/dl) were used in the experiment.

2.6. Experiment design

2.6.1. Assessment of plant extract on glucose tolerance

The normal rats were divided in to 4 groups of 6 animals each; Group I served as control, receiving vehicle alone (2% Gum acacia, distilled water), Group II & III received the root extract of *P. zeylanica* (100 mg, 200 mg/kg/p.o) suspended in vehicle respectively. Group IV received Tolbutamide (250 mg/kg/p.o). All the animals were given glucose (2 g/kg) 30 min after dosing, blood samples were collected from the tail vein just prior to 30, 60 and 90 min after the

glucose loading and blood glucose levels were measured.

2.6.2. Assessment of plant extract on STZ diabetic rats

The diabetic rats were divided in to 5 groups of 6 animals each. Group I received vehicle alone, served as control. Group II received STZ (60mg/kg/i.p) dissolved in 0.1M-citrate buffer. Group III & Group IV received the root extract of *P. zeylanica* (100 mg, 200 mg/kg/p.o) suspended in vehicle followed by single intra-peritonial administration of STZ. Group V received Tolbutamide (250mg/kg/p.o) followed by single intra-peritonial administration of STZ.

Blood samples were collected at different time intervals (30 min, 60 min, 90 min) for the estimation of blood glucose and the experiment was continued further to study the effect of *P. zeylanica* root extract on plasma antioxidant status in STZ diabetic rats.

2.7. Biochemical analysis

After 21 days treatment, blood was collected into heparinized tubes. The plasma was separated and used to determine the biochemical parameters. Blood glucose level was measured by glucose oxidase method [17], plasma TBARS content was estimated by the method of Nichans and Samuelson [18] and oral glucose tolerance test [19], reduced GSH [20], ascorbic acid [21] were determined.

2.8. Statistical analysis

Two-way analysis of variance (ANOVA) with interaction effects was employed for analyzing the initial dose response data (Table 1,2), and one-way analysis of variance was employed for analyzing the antioxidant status and general parameters (Table-3). Inter-group comparisons were done using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals.

3. Results

The effect of ethanolic extract of root of *P. zeylanica* on glucose tolerance is shown in Table-1. In glucose fed rats (2g/kg) administration of 100, 200mg/kg p.o of *P. zeylanica* significantly increased the tolerance for glucose.

The effect of ethanolic extract of root of *P. zeylanica* in STZ diabetic rats is given in Table-2. The fasting blood glucose level in STZ

Table 1. Effect of ethanolic extract of root of *P. zeylanica* on oral glucose (2g/kg) tolerance test.

		Blood glucose (mg/dl)*			
Group	**Treatment**	**Fasting**	**30m**	**60m**	**90m**
I	Control	68.2 ± 0.8	140.2 ± 6.2	133.1 ± 5.2	120 ± 7.9
II	*P. zeylanica* (100mg/kg/p.o)	68.8 ± 1.2	128.2 ± 5.4[a]	118.4 ± 5.6[a]	98.5 ± 6.1
III	*P. zeylanica* (200mg/kg/p.o)	67.1 ± 0.9	127 ± 3.8[a]	114.5 ± 6.1[a]	88.3 ± 5.8[a]
IV	Tolbutamide (250mg/kg/p.o)	66.4 ± 0.6	129.6 ± 2.4[a]	110.2 ± 4.3[a]	77.4 ± 4.2[a]

Values are mean ± SD of six animals in each group. Values not sharing a common superscript differ significantly at $P<0.05$, Duncan's Multiple Range Test (DMRT)

Table 2. Effect of ethanolic extract of root of *P. zeylanica* on blood glucose in normal and STZ diabetic rats.

Group	Treatment	Blood glucose (mg/dl)*			
		Fasting	1h	2h	3h
I	Control	68.1 ± 2.4	64.3 ± 3.4^{a}	63.2 ± 2.4^{a}	62.8 ± 3.0^{a}
II	Diabetic control	255.6 ± 3.8^{a}	252.5 ± 3.8^{bc}	248.4 ± 3.9^{bc}	240.5 ± 2.8^{b}
III	*P. zeylanica* (100mg/kg/p.o)	243.3 ± 4.2^{a}	237.4 ± 4.1^{b}	232.5 ± 4.45^{b}	228.7 ± 3.4^{c}
IV	*P. zeylanica* (200mg/kg/p.o)	249.4 ± 5.1^{a}	244.5 ± 2.9^{bc}	237.8 ± 7.2^{bc}	218.5 ± 5.8^{cd}
V	Tolbutamide (250mg/kg/p.o)	244.5 ± 4.4^{a}	240.6 ± 2.5^{c}	228.7 ± 8.4^{c}	217.4 ± 6.2^{d}

Values are mean ± SD of six animals in each group. Values not sharing a common superscript differ significantly at P<0.05, Duncan's Multiple Range Test (DMRT)

Table 3. Effect of ethanolic extract of root of *P. zeylanica* on blood glucose, plasma TBARS, plasma GSH and ascorbic acid in normal and STZ diabetic rats.

Group	Treatment	Blood glucose (mg/dl)*		TBARS	GSH	Ascorbic acid
		Initial	Final	(nmol/ml)	(mg/dl)	(mg/dl)
I	Control	68.1 ± 2.4	74.5 ± 3.8^{a}	2.4 ± 0.8^{a}	25.4 ± 2.6^{a}	1.85 ± 0.2^{a}
II	Diabetic control	245.6 ± 3.8	288.5 ± 8.9^{b}	3.2 ± 0.4^{b}	16.4 ± 2.4^{b}	1.0 ± 0.4^{b}
III	*P. zeylanica* (100mg/kg/p.o)	243.3 ± 4.2	154.6 ± 9.1^{c}	2.8 ± 0.5^{bc}	19.5 ± 2.1^{c}	1.45 ± 0.3^{b}
IV	*P. zeylanica* (200mg/kg/p.o)	249.4 ± 5.1	132.4 ± 8.8^{d}	2.4 ± 0.6^{ac}	23.8 ± 2.3^{a}	1.65 ± 0.2^{a}
V	Tolbutamide (250mg/kg/p.o)	244.5 ± 4.4	115.5 ± 9.7^{e}	2.2 ± 0.3^{a}	24.2 ± 2.2^{a}	1.7 ± 0.1^{a}

Values are mean ± SD of six animals in each group. Values not sharing a common superscript differ significantly at P<0.05, Duncan's Multiple Range Test (DMRT)

diabetic rats was 240-260mg/dl. The initial reduction in blood glucose was observed 2h after administration of *P. zeylanica* extract. Table-3 depicts the levels of blood glucose, plasma TBARS, plasma GSH and ascorbic acid in control and experimental animals. The plasma TBARS are significantly elevated in diabetic rats as compared to normal rats. Administration of *P. zeylanica* extract (100mg, 200mg/kg) and tolbutamide 250mg/kg induced significant reduction in the plasma TBARS as compared to diabetic rats. The plasma GSH and ascorbic acid are significantly lowered in diabetic rats as compared to control animals. Administration of *P. zeylanica* extract (100mg, 200mg/kg) and tolbutamide 250mg/kg increased significantly the GSH, and ascorbic acid as compared with diabetic rats.

Discussion

The results of the present study indicate that the ethanolic extract of root of *P. zeylanica* was found to reduce the glucose level in normal glucose loaded animals and in animals made diabetic with STZ. The STZ induction in adult animals produces a type II diabetes model. STZ selectively destroys the pancreatic insulin secreting β-cells that leaves less active pancreatic cells and results in diabetes mellitus [22].

In our study the administration of ethanolic extract of root of *P. zeylanica* significantly reduces blood glucose level in normal and STZ rats and remarkably improves oral glucose tolerance in normal rats. The possible mechanism by which the root of *P. zeylanica* brings about its hypoglycemic action may be by potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the β-cells of islet of Langerhans or its release from bound insulin. In this context a number of other plants have also been observed to have hypoglycemic effects [23-25].

Oral administration of ethanolic extract of root of P. zeylanica showed antioxidant effect in STZ diabetic rats. In the present study we observed an increase in the levels of plasma TBARS which is an index of lipid peroxidation in the STZ diabetic rats. The increased levels of plasma TBARS confirm the possibility that the major function of the extract is the protection of the vital tissues including liver, kidney, brain and pancreas thereby reducing the causation of diabetes. Griesmacher *et al* [26] reported that an increase in the levels of lipid peroxides and TBARS in plasma is generally thought to be the consequence of their increased production and liberation in to the circulation.

Vitamin-C is an excellent water-soluble antioxidant that primarily scavenges oxygen radicals [27]. Vitamin-C has been reported to contribute to up to 24% of the total peroxy radical trapping antioxidant activity [28]. We have observed a decreased level of Vitamin- C in the diabetic rats. This decreased level could be due to increased utilization of Vitamin- C in deactivation of the increased levels of reactive oxygen species or to the decrease in GSH level, since the GSH is required for the recycling of Vitamin- C [29].

GSH is a metabolic regulator and putative indicator of health. We observed lower level of plasma GSH in STZ diabetic rats. It appears that generation of oxygen radicals by increased levels of glucose causes increased utilization of GSH. Other workers have also reported decreased level of plasma GSH in STZ diabetic rats [30, 31]. In conclusion, the ethanolic extract of root of *P. zeylanica* offers a strong hypoglycemic effect and antioxidant protection in STZ induced diabetic rats.

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