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ANTIDIABETIC EFFECT OF *T. ARJUNA* BARK EXTRACT IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The present study was carried out to evaluate the antidiabetic effect of T.arjuna stembark extract and to study the activities of hexokinase, aldolase and phosphoglucoisomerase, and gluconeogenic enzymes such as glucose-6-phosphatase and fructose -1,6-diphosphatase in liver and kidney of normal and alloxan induced diabetic rats. Oral administration of ethanolic extract of bark (250 and 500mg/kg body weight) for 30 days, resulted in significant decrease of blood glucose from 302.67 ± 22.35 to 82.50 ± 04.72 and in a decrease in the activities of glucose-6-phosphatase, fructose-1,6-disphosphatase, aldolase and an increase in the activity of phosphoglucoisomerase and hexokinase in tissues. However, in the case of 250 mg / kg body weight of extract, less activity was observed. The study clearly shows that the bark extract of T.arjuna *possesses potent antidiabetic activity.*

KEY WORDS

Terminalia arjuna, gluconeogenic, alloxan, aldolase, antidiabetic

INTRODUCTION

Diabetes mellitus is a major public health problem in the developed as well as developing countries. It is ranked seventh among the leading causes of death, and third when its fatal complications are taken in to account (1). Traditional antidiabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities or as simple dietary adjuncts to existing therapies (2).

Herbal treatments are becoming increasing by popular as the herbal preparations have no or least side effects(3). *Terminala arjuna* is an important medicinal plant widely used in the preparation of Ayurvedic formulations used against several ailments. The use of *Terminalia arjuna* bark in the management of hypercholesterolaemia has been widely reported (4-9). The pharmacological studies have shown antiviral (10) anti mutagenic (11) antiplague formation (I2), anticancer (13) and hypotensive properties (14) and abnormal platelet activity (15) diabetes in human trial (16), (17), (18).

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There were no reports on the ability of *T.arjuna* bark on gluconeogenic and glycolytic enzymes in diabetes. Present investigation aims to study the regulation of carbohydrate metabolic and catabolic enzymes in liver and kidneys of normal and alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant material and preparation of 50% ethanolic extract

The wet *Terminalia arjuna* bark was collected from Siruvani coastel of Agali in Kerala. The specimen was certified by Botanical Survey of India (BSI) Coimbatore, and by the Department of Pharmacognosy and Phytochemistry, J.S.S College of Pharmacy Ooty, Tamil Nadu, India.

Terminalia arjuna was used in the form of crude 50% ethanol extract and this extract was prepared according to the traditional system of medicine. The shade dried and coarsely powdered stem bark (1kg) was extracted with 50% alcohol in the cold for 72 hours. The extract was filtered and distilled on water bath, a reddish brown syrupy mass was obtained and it was finally dried at low temperature under reduced pressure in a rotary evaporator. A crude residue (75g) was obtained giving a yield of 7.5%. The antidiabetic effects were evaluated by oral administration of the extract to the alloxan induced diabetic rats.

Animals

Male albino rats of Wistar strain weighing about 150 – 200 g obtained from the Medical College of Trichur (Kerala) were used for the study. They were fed a standard rat pellet diet (Sai Durga feeds, Bangalore) and water was provided *adlibitum* and maintained under standard laboratory conditions. (Temperature 24-28°C, relative humidity 60 - 70%) Animals described as fasted were deprived of food for 16 hours but had free access to water.

Alloxan Induced diabetes

Diabetes was induced by a single ip injection of 120 mg/kg of alloxan monohydrate (S.D Fine – Chem. Ltd., Mumbai, India), in sterile saline (19). After 72 hours of alloxan injection, the diabetic rats (glucose level > 250 mg/dl) were separated and used for the study(20).

Experimental set up

The animals were divided in to 6 groups of 6 each. Group I served as normal healthy control. Group II (untreated diabetic control). Group III diabetic rats given *T.arjuna* bark extract (250 mg/kg body weight). Group IV diabetic rats given *T.arjuna* bark extract (500 mg/kg body weight). Group V control rats given *T.arjuna* bark extract (250 mg/kg body weight) Group VI control rats given *T.arjuna* bark extract (500 mg/kg body weight). The crude extract was administered for a period of 30 days.

Collection of blood, kidney and liver from the rat

After the experimental regimen, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected on decapitation and serum was separated by centrifugation (for 20 min at 2000 rpm). The liver and kidney were excised immediately and thoroughly washed in ice - cold saline. The serum and tissues were collected and used for biochemical experiments.

Estimation of biochemical parameters

Serum glucose was estimated by GOD/POD method (21), hexokinase (22), phosphoglucoisomerase(23), aldolase (24), glucose-6-phosphatase (25) and fructose-1,6-disphosphatase (26) were assayed in liver and kidney.

RESULTS AND DISCUSSION

As shown in (Table 1) the levels of glucose in serum of alloxan induced diabetic rats were found to be significantly elevated as compared with control rats. Oral administration of *T.arjuna* (250 and 500 mg/kg body weight) for 30 days showed significant ($p<0.05$) reduction in glucose.

The activities of glycolytic enzymes (hexokinase, aldolase, phosphoglucoisomerase) in the liver and kidney of control and experimental rats are shown in (Tables 2 and 3).

The activity of hexokinase and phosphoglucoisomerase were seen significantly decreased, where as the activity of aldolase was seen significantly increased in diabetic rats, when compared with control rats ($p<0.05$). Oral administration of *T.arjuna* (250 and 500 mg/kg body weight) for 30 days significantly reversed these values to normal.

The activities of gluconeogenic enzymes (glucose 6-phosphatase and fructose-1, 6-diphosphatase) in the liver and kidney of control and experimental animals are shown in (Tables 4 and 5).

The acitivity of gluconeogenic enzymes, glucose-6-phosphatase and fructose-1, 6-diphosphatase (liver and kidney) were found significantly elevated in diabetic rats when compared with control rats ($p<0.05$). Oral administration of *T.arjuna* (250 mg and 500 mg/kg body weight) for 30 days brought back the activity of the above enzymes to the near normal level. However, drug alone treated rats (250 and 500 mg/kg body weight) did not show any significant ($p>0.05$)

Table 1
Effect of *T. arjuna* stem bark on serum glucose, of control and experimental rats.

Parameters	Group 1 Control	Group II Diabetic	Group III Diabetic+ TA 250mg/kg	Group IV Diabetic+ TA 500mg/kg	Group V Control+ TA 250mg/kg	Group VI Control+ TA 500mg/kg
Serum Glucose (mg/dl)	98.33 ± 02.66	302.67±22.35a*	125.60 ± 24.73b*	82.50 ± 04.72cf*	106.67 ± 0.625d^{ns}	113.17 ± 14.25e^{ns}

Values are expressed as Mean ± SD (n=6)
Statistical comparison : a : Group I and Group II b : Group II and Group III c : Group II and Group IV
d : Group I and Group V e : Group I and Group VI f :Group III and Group IV
*P<0.05 ns- non significant

Table 2
Effect of *T.arjuna* on the activities of glycolytic enzymes in liver of control and experimental rats.

Parameters	Group 1 Control	Group II Diabetic	Group III Diabetic+ TA 250mg/kg	Group IV Diabetic+ TA 500mg/kg	Group V Control+ TA 250mg/kg	Group VI Control+ TA 500mg/kg
Hexokinase (nmoles of glucose -6-phosphate formed/min/mg/protein)	402.67 ± 46.65	82.80 ± 1.14a*	409.33 ± 12.75b*	194.49 ± 7.58cf*	377.03 ± 5.82d^{ns}	399.96 ±20.12e^{ns}
Aldolase (nmoles of glyceraldehyde formed/ min / mg protein)	155.13 ± 5.48	242.49 ± 10.87a*	160.61 ± 5.23b*	151.60 ±10.15cf*	152.66 ± 4.78d^{ns}	158.25 ± 7.85e^{ns}
Phosphoglucoisomerase (nmoles of fructose formed /min/mg protein)	47.78 ± 0.91	24.42 ± 1.25a*	28.75 ± 0.55b*	34.95 ± 0.59cf*	47.06 ± 0.81d^{ns}	47.24 ± 0.37e^{ns}

Values are expressed as Mean ± SD (n=6)
Statistical comparison : a : Group I and Group II b : Group II and Group III c : Group II and Group IV
d : Group I and Group V e : Group I and Group VI f :Group III and Group IV
*P<0.05 ns- non significant

changes when compared with control animals. In our study, administration of *T.arjuna* bark extract resulted in a significant reduction in blood glucose level, when compared with diabetic control animals. The extract containing 500 mg/kg body weight showed a better glucose level reduction than 250 mg/kg body weight. The mechanism may be through the stimulation of b-cell for elevated secretion of insulin, there by increasing the utilization of glucose in various tissues (27).

Liver functions as a “glucostat” and plays a vital role in the maintenance of blood glucose level and hence it is of interest to examine the possible role of *T.arjuna* on key enzymes of carbohydrate metabolism in liver. Liver is the candidate organ involved in glucose homeostasis. It is the main site for glycolysis, a process where glucose is degraded and gluconeogenesis, where glucose is synthesized from lactate, amino acids and glycerol. These are the two important complementary events that balance the glucose load in our body (28).

Hexokinase is the prime enzyme catalysing glucose phosphorylation. The first step in glycolysis (29) is severely impaired during diabetes (30). Impairment of hexokinase activity suggest the impaired oxidation of glucose via glycolysis

Table 3
Effect of *T.arjuna* on the activities of glycolytic enzymes in kidney of control and experimental rats.

Parameters	Group 1 Control	Group II Diabetic	Group III Diabetic+ TA 250mg/kg	Group IV Diabetic+ TA 500mg/kg	Group V Control+ TA 250mg/kg	Group VI Control+ TA 500mg/kg
Hexokinase (nmoles of glucose -6-phosphate formed/min/mg/protein)	304.05 ± 16.87	67.84 ± 1.31a*	287.48 ± 36.67b*	218.63 ± 35.53cf*	300.96 ± 8.80d^{ns}	302.13 ± 14.86e^{ns}
Aldolase (nmoles of glyceraldehyde formed/ min / mg protein)	221.94 ± 8.89	272.23 ± 8.89*	205.13 ± 3.40b*	225.62 ± 2.20cf*	226.67 ± 27.48d^{ns}	219.29 ± 13.8e^{ns}
Phosphoglucoisomerase (nmoles of fructose formed /min/mg protein)	37.07 ± 1.28	15.94 ± 0.84a*	38.08 ± 0.55b*	26.11 ± 0.27cf*	37.36 ± 0.32d^{ns}	37.34 ± 0.27e^{ns}

Values are expressed as Mean ± SD (n=6)
Statistical comparison : a : Group I and Group II b : Group II and Group III c : Group II and Group IV
d : Group I and Group V e : Group I and Group VI f :Group III and Group IV
*P<0.05 ns- non significant

Table 4
Effect of *T. arjuna* on the activity of gluconeogenic enzymes in liver of control and experimental rats.

Parameters	Group 1 Control	Group II Diabetic	Group III Diabetic+ TA 250mg/kg	Group IV Diabetic+ TA 500mg/kg	Group V Control+ TA 250mg/kg	Group VI Control+ TA 500mg/kg
Glucose-6-phosphatase (nmoles of Pi liberated / min / mg protein)	39.46 ± 0.85	109.89 ± 4.71a*	46.71 ± 2.44b*	40.94 ± 3.05cf*	38.59 ± 0.62d^{ns}	38.30 ± 1.61e^{ns}
Fructose-1, 6-diphosphatase (nmoles of Pi iberated / min / mg protein)	26.44 ± 4.18	346.79 ± 17.02a*	41.37 ± 8.57b*	24.85 ± 2.75cf*	18.38 ± 1.38d^{ns}	24.56 ± 5.94e^{ns}

Values are expressed as Mean ± SD (n=6)
Statistical comparison : a : Group I and Group II b : Group II and Group III c : Group II and Group IV
d : Group I and Group V e : Group I and Group VI f :Group III and Group IV
*P<0.05 ns- non significant

leading to its accumulation resulting in hyperglycemia.

Insulin influences the intracellular utilization of glucose in a number of ways. Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase and phosphofructokinase. Hexokinase is universally present in cells of all types. Hexokinase catalyses the conversion of glucose to glucose 6-phosphate and plays a central role in the maintenance of glucose homeostasis. In the liver, this above enzyme is an important regulator of glucose storage and disposal (31).

The hexokinase activity was found to be decreased in diabetic rats which may be due to insulin deficiency (insulin stimulates and activates hexokinase). Treatment with *T.arjuna* elevated the activity of hexokinase in the liver. *T.arjuna* may stimulate insulin secretion, which may activate hexokinase, there by increasing utilization of glucose leading to decreased blood sugar levels.

Activity of phosphoglucoisomerase and ATP dependent phosphofructokinase enzyme are reported to be under regulation by citrate (32), which is a TCA cycle intermediate. Decrease in activity of phosphoglucoisomerase might be expected to inhibit the proportion of glucose 6-phosphate metabolized via the glycolytic pathway. (33).

Aldolase, another key enzyme in the glycolytic pathway, increases in diabetes and this may be due to cell impairment and necrosis (34). In experimental diabetes the cells are subjected to alloxan induced-damage and very often exhibit glycolysis after a period of increased oxygen uptake.

Table 5
Effect of *T. arjuna* on the activity of gluconeogenic enzymes in kidney of control and experimental rats.

Parameters	Group 1 Control	Group II Diabetic	Group III Diabetic+ TA 250mg/kg	Group IV Diabetic+ TA 500mg/kg	Group V Control+ TA 250mg/kg	Group VI Control+ TA 500mg/kg
Glucose-6-phosphatase (nmoles of Pi liberated / min / mg protein)	49.06 ± 7.17	133.88 ± 4.52a*	38.84 ± 1.73b*	40.77 ± 02cf*	38.07 ± 1.07d^{ns}	53.05 ± 8.61e^{ns}
Fructose-1, 6-diphosphatase (nmoles of Pi iberated / min / mg protein)	20.81 ± 1.78	218.11 ± 16.14a*	55.17 ± 10.92b*	31.10 ± 2.45cf*	30.81 ± 3.61d^{ns}	24.66 ± 3.38e^{ns}

Values are expressed as Mean ± SD (n=6)
Statistical comparison : a : Group I and Group II b : Group II and Group III c : Group II and Group IV
d : Group I and Group V e : Group I and Group VI f :Group III and Group IV
*P<0.05; ns- non significant

Fructose-1,6-diphosphatase and glucose-6-phosphatase are important regulatory enzymes in gluconeogenesis. In diabetic animals the enzyme levels were observed to increase (34). The increased activities of glucose 6-phosphatase and fructose-1,6-diphosphatase in liver and kidney of the alloxan induced diabetic rats may be due to insulin insufficiency.

Insulin decreases gluconeogenesis by decreasing the activities of key enzymes, such as glucose-6-phosphatase, fructose-1, 6-diphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxylase (35). In *Terminalia* treated rats, these two enzymes (glucose-6-phosphatase, fructose-1,6-diphosphatase) were seen significantly reduced in liver and kidney. This may be due to increased insulin secretion, which is responsible for the repression of the gluconeogenic key enzymes.

From the present study, it is concluded that *T.arjuna* bark extract exhibited antidiabetic activity by enhancing the peripheral utilization of glucose by correcting the impaired liver and kidney glycolysis and by limiting its gluconeogenic formation similar to insulin. This effect may be due to the presence of tannin, saponin, flavonoids and other constituents presence in the bark, which could act synergistically or independently in enhancing the activity of glycolytic and gluconeogenic enzymes.

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