EFFECT OF *TERMINALIA ARJUNA* STEM BARK EXTRACT ON THE ACTIVITIES OF MARKER ENZYMES IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Insight of evidence that some complications of diabetes mellitus due to hyperglycemia, we investigated the effect of *T.arjuna* bark extract on serum, liver and kidney marker enzymes in alloxan - induced diabetic rats. *T. arjuna* was administered orally at a doses of 250 and 500 mg/kg body weight for 30 days, after which serum liver and kidney tissues were assayed for the degree of pathological changes by means of markers such as alkaline phosphatase (ALP), acid phosphatase (ACP), alanine amino transferase (ALT), aspartate amino transferase (AST) and lactate dehydrogenase (LDH) resulted in a significant reduction in serum and tissue of liver and kidney marker enzymes when compared with control rats *T. arjuna* at a dose of 500 mg/kg body weight exhibited higher efficacy.

Keywords: *Terminalia arjuna*, hyperglycemia, alloxan diabetes, marker enzymes.

INTRODUCTION

Diabetes mellitus is a major disease affecting nearly 10% of the population. In spite of the introduction of hypoglycemic agents, diabetes and related complications continue to be a major medical problem. Furthermore, insulin resistance or decreased level of insulin is a characteristic feature of cirrhosis[1]. The acute hyperglycemic causes life threatening ketoacidosis and also chronic hyperglycemia leads to conditions like retinopathy, nephropathy and peripheral insufficiencies. Liver disease is one of the leading causes of death in persons with diabetes[2].

Alloxan produces oxygen radicals in the body, which cause pancreatic injury and could be responsible for increased blood sugar seen in the animals[3]. However, it is found that action is not specific to pancrease as other organs such as liver, kidney and haemopoietic system also affected by alloxan, administration as seen from the elevation of marker enzymes[4].

Many indigenous Indian medicinal plants have been found to be successfully used to manage diabetes. The incidence of which is very high all over the world especially in India. The reason is that plant drugs could be effective and at the same time have less or no side effects. The medicinal plant *T.arjuna* is widely used in the indigenous system of medicine practiced in India for several ailments[5, 6.] Thus there is no reports on the ability of *T.arjuna* bark extract in serum liver and kidney marker enzymes in diabetes. Hence, the present study has been under taken to evaluate the protective role of ethanolic extract of *T.arjuna* on serum, liver and kidney marker enzymes such as ALP, ACP, ALT, AST and LDH in alloxan-induced diabetic rats.

METHODS

Plant material and preparation of 50% ethanolic extract

The wet *Terminalia arjuna* bark were collected from Siruvani coastal of Agali in Kerala. The specimen was identified and certified by Botanical Survey of India (BSI) Coimbatore.

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 serum and tissue marker enzymes were evaluated by oral administration of the extract on 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 h but had free access to water. Clearance for the handling of experimental animals was obtained from the Ethical committee constituted for the purpose (CPCSEANO: 659/02/a).

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[7]. After 72 hours of alloxan injection, the diabetic rats (glucose level > 250 mg/dl) were separated and used for the study [8].

Experimental procedure

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 drug was administered for the period of 30 days.

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.

Biochemical estimations

Serum glucose was estimated by GOD / POD method [9]. Activities of ALP[10] (alkaline phosphatase), ACP[10] (acid phosphatase) ALT[11] (alanine amino transaminase, AST (aspartate amino transaminase and LDH[12] (lactate dehydrogenase) were assayed in serum, liver and kidney of experimental animals.

RESULT

As shown in Table 1, the levels of glucose in serum of alloxan induced diabetes rats were found to be significantly elevated as compared with control rats. Oral administration of *T.arjuna* (250 and 500 mg/kg body weight) restored the glucose level to the near normal in diabetic rats.

Table 2 depicts the activities of serum marker enzymes in diabetes when

compared to control rats. The increased activity of ALP, ACP, ALT, AST and LDH after *T. arjuna* treatment at a dose of 250 and 500 mg/kg body weight become increased to near normal. There was no significant change in *T. arjuna* (250 and 500 mg/kg body weight) treated control rats in the above mentioned parameters.

Table 3 shows the activities of ALP, ACP, ALT, AST and LDH in liver and kidney tissues of different groups of experimental animals. The activities were increased significantly in diabetic animals when compared with control rats. Oral administration of *T.arjuna* bark extract at a dose of 250 and 500 mg/kg body weight were lowered the activities. There was no significant change in the activities of these enzymes in *T.arjuna* treated control rats.

DISCUSSION

From the observations made in Table 1 it is clear that the administration of *T.arjuna* brings down the serum glucose levels considerably in alloxan induced diabetic animal. The glucose lowering activity observed in the diabetic animals may be due to the stimulation of β - cells of pancreatic islets[13]

Estimating the activities of serum and tissue marker enzymes like ALP, ACP, AST, ALT and LDH can make assessment of liver function, when liver and kidney cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released in to the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage[14]

In the present study we have observed an increased in the level of serum liver and kidney marker enzymes in alloxan induced diabetes indicating that alloxan administration produced hepatic damage and an increase in membrane transport is evidenced by enhanced activities of membrane bound enzymes, ALP and ACP in the kidney[15]. ALP and ACP are ubiquitous in nature, their primary role of extra cellular phosphatases is to provide inorganic phosphate for cell growth by hydrolysis of external phosphate esters which do not penetrate the cytoplasmic membrane[16] and also ALP is the prototype of these enzymes that reflect the pathological alteration in biliary flow[17]. Oral administration of *T.arjuna* bark extract to lower the elevated serum enzyme level.

The serum, liver and kidney levels of AST and ALT have also found to become elevated in diabetic induced animals, whenever disease process affects liver cell integrity[18]. Transaminases (AST &ALT) which are active in absence of insulin because of availability of amino acids in to blood of diabetes are responsible for the increased gluconeogenesis and ketogenesis observed in diabetics[19]. Oral administration of *T.arjuna* bark extract (250 & 500 mg/kg body weight) to lower the elevated serum enzyme levels. In view of this the extract mediated reduction in the levels of ALT and AST towards the respective normal values is an indication of stabilization of plasma membrane as well as repair of hepatic, tissue damage caused by alloxan. This effect is in agreement with the commonly possesses isoenzymes recognized as markers for liver and muscle legions accepted view that serum level of transaminases return to normal with restore the normal function of liver and kidney[20].

LDH is a cytosolic enzyme is involved in biochemical regulation (i.e. interconversion of lactate to pyruvate) reactions of the body tissues and fluids, LDH products the cofactor (NAD^+ / NADH) for glycolytic enzymes[21]. Serum, liver & kidney LDH is almost doubled in alloxan induced diabetic animals. This is line with finding of others[22]. Administration of *T.arjuna bark* extract the LDH level was found to decreased and this was similar to that of normal rats. The decreases of LDH activity in serum, liver and kidney there by decreasing the endogenous glucose production.

In conclusion it can be suggested that the oral administration of *T.arjuna* bark extract can revert the increased activity of marker enzymes it is found in diabetic rats which clearly indicates the protective role of *T.arjuna* bark extract in diabetes mellitus. More over the dose at 500 mg/kg body weight have more efficacy. However *T.arjuna* does not alter the assayed parameter in control rats, there by indicating its non-toxic nature in the applied dosage.

Table 1. Effect of *Terminalia arjuna* stem bark on serum glucose, levels in alloxan induced diabetic 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[b]	302.67 ± 22.35[f]	125.60 ± 24.73[e]	82.50 ± 04.72[a]	106.67 ± 0.625[b]	113.17 ± 14.25[b]

Values are expressed as Mean ± SD (n=6) Means followed by a common letter are not significantly differ at p<0.05 (DMRT).

Table 2. Effect of *Terminalia arjuna* stem bark on serum marker enzymes in alloxan – induced diabetic 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						
ALP[1]	406 ± 1.63[d]	546.33 ± 8.00[f]	374.38 ± 3.88[a]	426.38 ± 8.41[e]	402 ± 2.56[d]	401.12 ± 2.52[d]
ACP[2]	125.24 ± 2.31[e]	225.25 ± 2.83[f]	113.69 ± 2.40[a]	124.24 ± 4.48[c]	123.20 ± 2.74[e]	124.24 ± 5.55[e]
AST[3]	54.82 ± 1.88[c]	183.63 ± 7.93[f]	49.31 ± 5.12[a]	57.64 ± 4.27[e]	53.93 ± 3.13[c]	56.37 ± 2.88[c]
ALT[4]	69.38 ± 4.02[c]	212.17 ± 3.39[f]	76.28 ± 2.81[d]	111.85 ± 8.66[e]	65.34 ± 2.97[c]	67.51 ± 2.81[c]
LDH[5]	357.28 ± 23.74[d]	705.38 ± 58.26[f]	237.29 ± 41.68[e]	340.69 ± 25.98[a]	335.89 ± 11.69[d]	335.33 ± 4.74[d]

Values are expressed as Mean ± SD (n=6) Means followed by a common letter are not significantly differ at p<0.05 (DMRT).

Units:

1. µ moles of phenol liberated / L
2. µ moles of pyruvate liberated / L
3. µ moles of phosphorous liberated / L
4. µ moles of pyruvate liberated / L
5. µ moles of pyruvate liberated / L

Table 3. Effect of *Terminalia arjuna* stem bark on liver and kidney marker enzymes in alloxan – induced diabetic 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
Liver						
ALP[1]	11.88 ± 0.57[d]	48.57 ± 6.14[f]	5.38 ± 0.89[a]	10.05 ± 0.84[b]	12.21 ± 0.41[d]	10.36 ± 0.57[d]
ACP[2]	13.09 ± 0.38[c]	31.11 ± 3.24[f]	13.74 ± 0.48[d]	16.41 ± 0.47[e]	12.97 ± 0.80[c]	13.04 ± 1.68[c]
AST[3]	15.23 ± 1.33[b]	44.54 ± 1.52[f]	23.52 ± 2.54[e]	16.08 ± 1.21[d]	14.82 ± 0.72[b]	15.73 ± 0.95[b]
ALT[4]	12.43 ± 0.26[c]	24.06 ± 0.50[f]	11.05 ± 1.34[a]	12.65 ± 0.31[e]	12.16 ± 0.40[c]	12.59 ± 0.41[c]
LDH[5]	84.20 ± 2.83[e]	179.08 ± 4.62[f]	69.75 ± 3.90[a]	78.75 ± 11.95[b]	80.62 ± 4.48[e]	80.66 ± 0.55[e]
Kidney						
ALP[1]	18.88 ± 0.90[b]	54.11 ± 0.79[f]	20.84 ± 1.38[d]	32.78 ± 2.05[e]	18.38 ± 1.02[b]	19.02 ± 0.59[b]
ACP[2]	20.78 ± 0.13[d]	42.56 ± 0.79[f]	17.25 ± 1.71[a]	23.35 ± 1.12[e]	19.64 ± 1.24[d]	20.68 ± 0.60[d]
AST[3]	20.85 ± 1.20[c]	32.61 ± 3.06[f]	22.64 ± 0.27[e]	18.87 ± 1.55[a]	22.42 ± 1.14[c]	19.81 ± 1.66[c]
ALT[4]	7.45 ± 0.71[d]	17.35 ± 2.47[f]	5.20 ± 0.50[a]	7.05 ± 0.67[c]	6.59 ± 0.19[d]	7.94 ± 0.37[d]
LDH[5]	111.13 ± 10.91[d]	203.44 ± 34.78[f]	94.51 ± 5.01[a]	112.60 ± 6.90[e]	107.13 ± 14.62[d]	103.62 ± 3.24[d]

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

Means followed by a common letter are not significantly differ at p<0.05 (DMRT).

Units:
1. n moles of phenol liberated / min / mg protein
2. n moles of phenol liberated / min / mg protein
3. µ moles of pyruvate liberated / min / mg protein
4. µ moles of pyruvate liberated / min / mg protein
5. µ moles of pyruvate liberated / min / mg protein

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