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Full Length Research Article

Effect of *T. Arjuna* Stem Bark Extract on Histopathology of Liver, Kidney and Pancreas of Alloxan-Induced Diabetic Rats

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ABSTRACT

The present study examined the effect of ethanolic extract (250 and 500 mg/kg body weight) of Terminalia arjuna stem bark in alloxan - induced diabetic rats for 30 days, and its histopathological study was investigated in the liver, kidney and pancreatic tissues sections. Pathological lesions were evoked in cells of diabetic rats. The extract improve the liver, kidney and pancreas function and reduce lesions associated with diabetic state in alloxan induced rats. The effect of oral administration of T. arjuna at a dose of 500 mg / kg body weight was more efficacy than the 250 mg/kg body weight. The results indicate the extract exhibit the protective effect on tissues, and proves its potentials as an antidiabetic agent. **(Afr. J. Biomed. Res. 9: 189 – 197)**

Keywords: *Teminalia arjuna,* histopathology, β- cell, lesions, alloxan

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INTRODUCTION

In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus, which is a syndrome resulting from a variable interaction of hereditory and environmental factors and characterized by abnormal insulin secretion or insulin receptor or post - receptor events affecting metabolism involving carbohydrates, proteins and fats in addition to damaging β-cells of pancreas, liver and kidney in some cases (Ghosh, 2001). Patients depend on insulin for management of IDDM. Without insulin, they develop degenerative complications such as microangiopathy, nephropathy and retinopathy. Diabetic nephropathy is the most important cause of death in type 1 diabetic patients, of whom, 30 – 40% eventually develop end stage renal failure (Giorgino *et al*., 2004). Liver disease is one of the leading cause of death in persons with type 2 diabetes. The standardized mortality rate for death from liver disease is greater than that of cardiovascular disease. The spectrum of liver disease in type 2 diabetes ranges from nonalcoholic fatty liver disease to cirrhosis and hepatocellular carcinoma (Keith *et al*., 2004).

Experimental type 1 diabetes induced with streptozotcin or alloxan in rats display many features seen in human subjects with uncontrolled diabetes mellitus(Chattopadhyay *et al* ., 1997).

Terminalia arjuna belongs to the family *Combretacea*, (Roxb Wight Arn) is a large evergreen tree. The tree is found throughout Indian subcontinent, Myanmar, and Sri Lanka particularly in topical moist deciduous and dry deciduous forest. Naturally *arjun* favours river banks and the sides of water bodies (Purohi *et al*.,2004).The bark has been used in Indian native Ayurvedic medicine for over three centuries, primarily as a cardiotonic. Clinical evolution of this botanical medicine indicates it can be of benefit in the treatment of coronary disease, heart failure, and possibly hypercholestrolemia has been widely reported (Tiwari *et al*., 1989, Pathak *et al*., 1990, Khanna *et al*., 1996, Shaila *et al*., 1997a and Ram *et al*., 1997).

The importance of using herbal medicine in treating various diseases like diabetes mellitus looks lucurative. Meagre work has been done in *T. arjuna* on diabetes mellitus and histopathological studies. On the same line I have chosen such a wide medicinal characteristics plant *T. arjuna* stem bark extract in alloxan-induced diabetes. Focusing their abilities on the tissues protective effects.

MATERIALS AND METHODS

Plant material and preparation of 50% ethanolic extract

The wet *Terminalia arjuna* bark were collected from Siruvani coastel of Agali in Kerala, during september 2003 and were carefully identified and certified by Botanical survey of India (BSI) Coimbatore.

Terminalia arjuna was used in the form of crude 50% ethanolic 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% ethanol (1.5L) 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%. When needed, the crude extract was suspended in distilled water and used in the study.

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%) Animal described as fasted were deprived of food for 16 h but had free access to water. Ethical clearance for the handling of experimental animals was obtained from the 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 FineChe. Ltd., Mumbai, India), in sterile saline (Ravivijayavargia *et al*.,2003). After 72 hours of

alloxan injection, the diabetic rats (glucose level> 250 mg/dl) were separated and used for the study (Perfumi *et al*.,1996).

Administration of plant extract

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 rat 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.

Collection of blood liver kidney and pancreas

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. The kidney and liver 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 measured by GOD / POD method (Trinder 1969).

Histopathology

The liver, kidney and pancreas were preserved in 20% formalin immediately after removal from the animal.

Tissue processing

Liver, kidney and pancreatic tissues were placed in 10% formalin (diluted to 10% with normal saline) for 1 hr to rectify shrinkage due to high concentration of formalin. The tissues were dehydrated by ascending grades of isopropyl alcohol by immersing in 80% isopropanol overnight and 100% isopropyl alcohol for 1 hour. The dehydrated tissues were cleared in two changes of xylene, 1 hour each. The wax impregnated tissues were embedded in paraffin blocks using the same grade wax. The paraffin blocks were morented and cut with rotary microtome at 3 micron thickness. The sections were floated on a tissue floatation bath at 40°C and taken on glass slides and smeared with equal parts of egg albumin and glycerol. The sections were then melted in an incubator at 60°C and after 5 min the sections were allowed to cool.

Tissue staining

The sections were deparaffinised by immersing in xylene for 10 min in horizontal staining jar. The deparaffinised sectons were washed in 100% isopropyl alcohol and stained in Ehrlich's hematoxylin for 8 min in horizontal staining jar. After staining in hematoxylin, the sections were washed in tap water and dipped in acid alcohol to remove excess stain (8.3% HCl in 70% alcohol). The sections were then placed in running tap water for 10 min for blueing (slow alkalization). The sections were counter stained in 1% aqueous eosin (1 gm in 100 ml tapwater) for 1 min and the excess stain was washed in tap water and the sections were allowed to dry. Complete dehydration of stained sections was ensured by placing the sections in the incubator at 60°C for 5 min. When the sections were cooled, they were mounted in DPX mount having the optical index of glass (the sections were wetted in xylene and inverted on to the mount and placed on the cover slip).

The architecture was observed low power objective under microscope. The cell injury and over aspects were observed under high power dry objective (Dunn 1974).

Statistical evaluation

Statistical evaluation was done using one- way analysis of variance (ANOVA) followed, by Duncan's Multiple Range Test (DMRT). Statistical significance was set at ($P<0.05$).

RESULTS

Effect of *Terminalia arjuna* (ethanalic extract) on serum glucose.

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

Effect of *Terminalia arjuna* (ethanolic extract) on liver kidney and pancreas histopathology.

In the normal liver tissue section shows sinusoidal cards of hepatocytes with central vein and portal tracts. The portal tracts show portal triad with portal vein, hepatic artery and bile duct, where as the diabetic rat liver tissue section shows distortion in the arrangement of cells around the central vein, periportal fatty infiltration with focal necrosis of hepatocytes (Figure 1 a and b). The bark extract (250 and 500 mg / kg body weight) treated brought back the cellular arrangement around the central vein and reduced necrosis. Also it helped to bring the blood vessels to normal condition (Figure 1 c and d). The group V and VI did not show any significant change of liver, when compared with group I (figure 1 e and f).

Kidney sections of diabetic rat showed tubular damage, proteinuria and haemorrhage. Haemorrhage is seen with in the Bowman's space due to glomerular damage (Figure 2 a and b). In *Terminalia* bark extract (250 and 500 mg / kg body weight) treated diabetic kidney, the damaged capillary loops with increase in the thickness of the wall, glomeruli and tubules without proteinuria and haemorrhage (Figure 2 c and d) Group V and VI did not alter the structure of kidney, when compared with group I (Figure 2 e and f).

The light microscropic examination by specific staining of pancreas in normal tissues section shows lobules of exocrine acini, interlobular ducts and occasional islets of langerhan which is not observed in alloxan induced diabetic pancreas (Figure 3 a & b). In *T.arjuna* bark extact (250 and 500mg body weight) treated pancreas the cells seem to have gathered together and small preserved islets similar to the normal (Figure 3 c and d). The group V & VI did not shows any significant change of pancreas, when compared with normal pancreas (Figure 3 e and f).

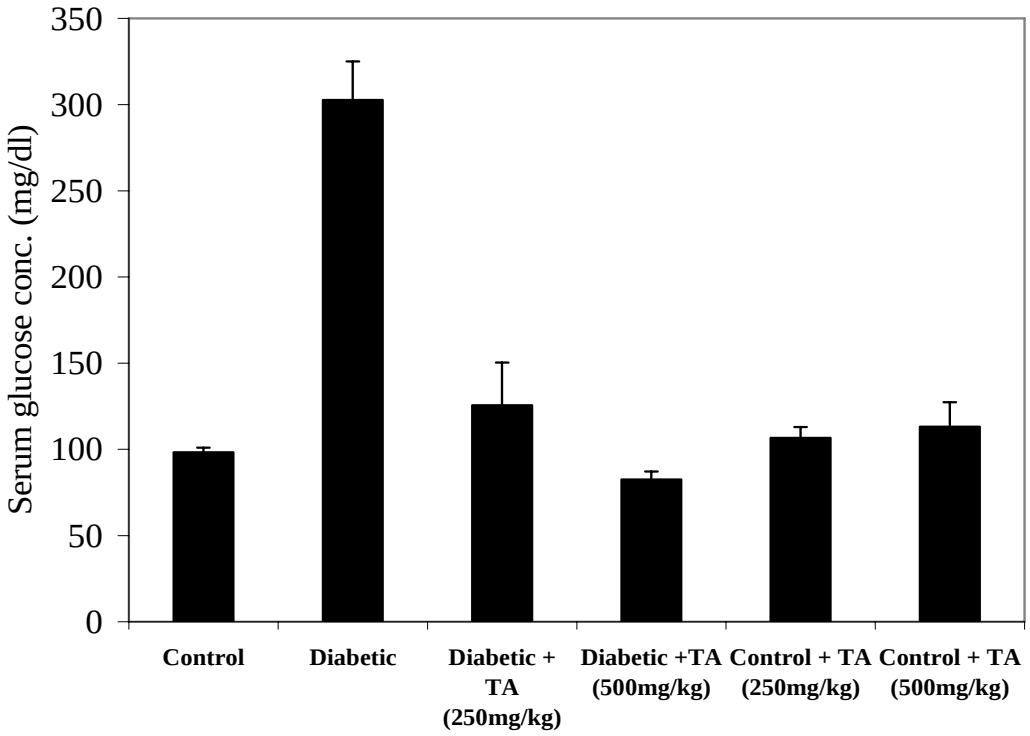

Fig. 1.
Effect of *Terminalia arjuna* stem bark on serum glucose in alloxan-induced diabetic rats

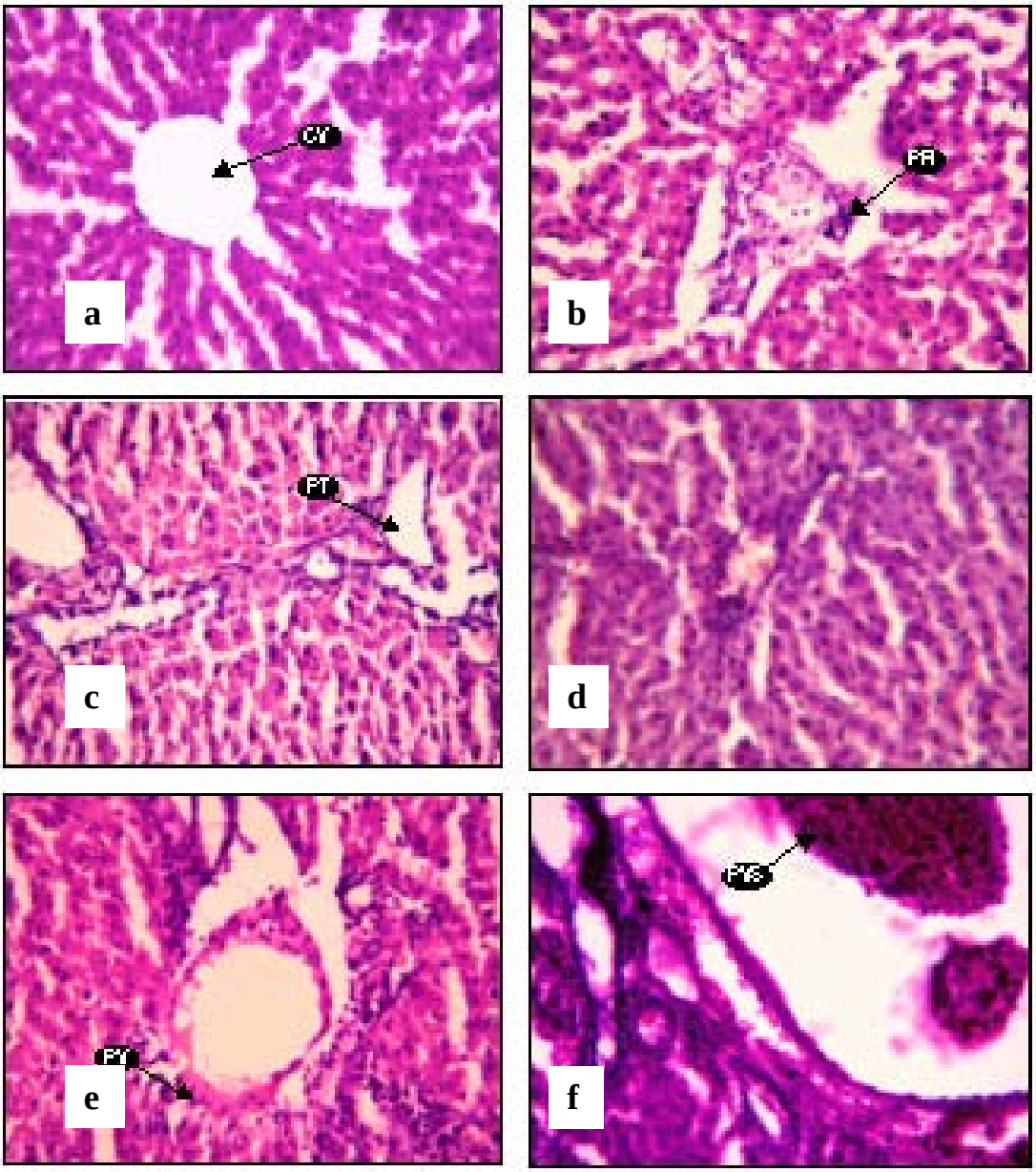

Plate 1

Histopathological Changes in Liver of Control and Experimental rats. **a. Group I -** Normal liver showing the central vein (CV)with radiating cords of hepatocytes; **b.Group II -** Diabetic liver shows periportal fatty infiltration (PFI) with focal fat necrosis; **c. Group III -** Shows normal portal tract (PT) ; **d. Group IV** Portal track showing normal features; **e. Group V -** Congested and edematous portal vein (PV) with mild haemorrhage; **f. Group VI -**Portal vein shows haemorrhage in the lumen in the perivenular space (PVS)

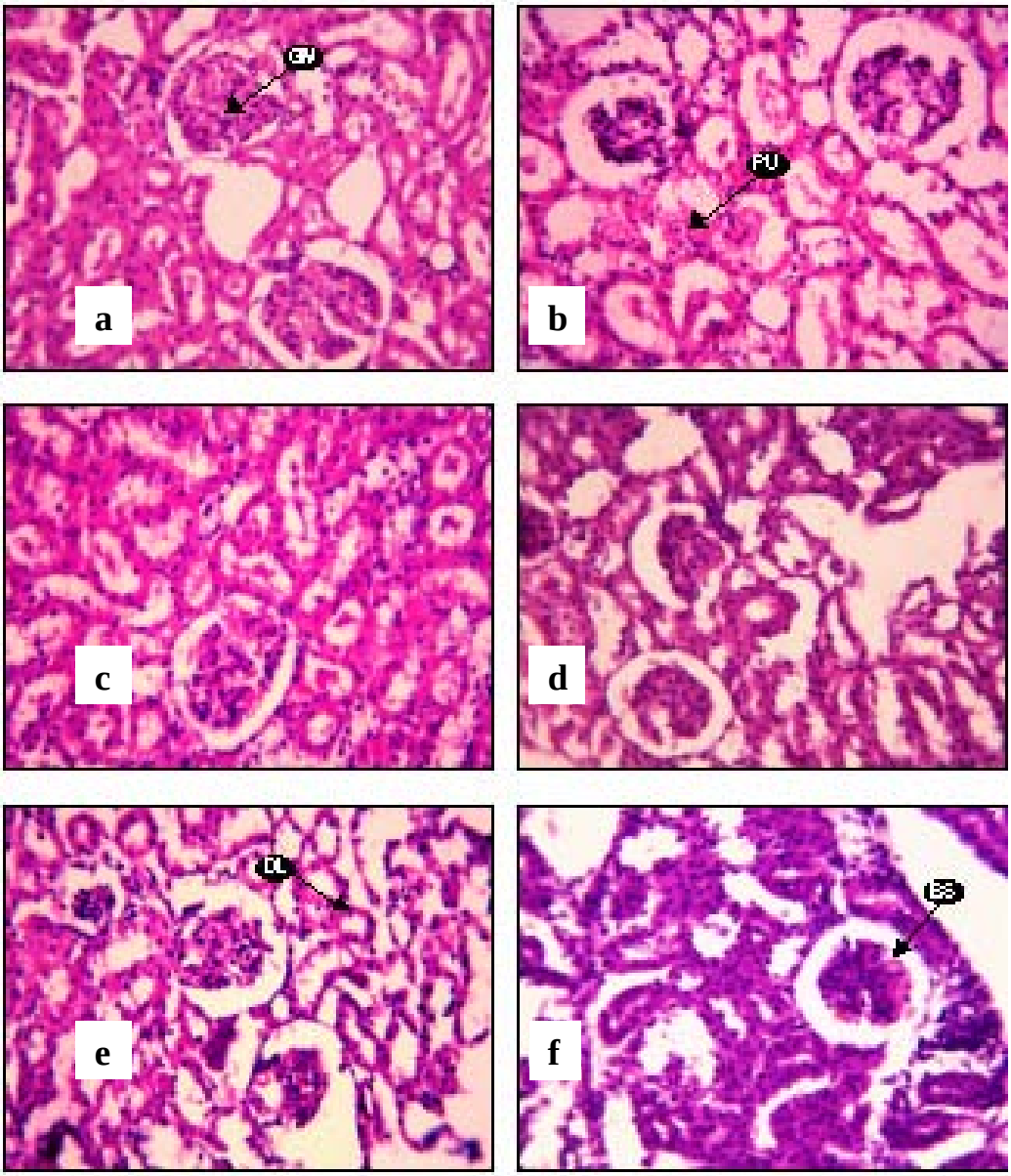

Plate 2

Histopathological Changes in Kidney of Control and experimental rats. Normal rat kidney show glomeruli (GM) and proximal convoluted tubules. **b. Group II** shows tubular damage proteinuria (PU) and hemorrhage; **c. Group III** tubules show proteinuria and glomerular damage; **d. Group IV** - Shows glomeruli and tubules without proteinuria and haemorrhage; **e. Group V**-Glomerulus shows some dilated loops (DL) and suffused RBCs; **f. Group VI -** Glomerulus shows suffused capillary loops with RBCs and expansion by red cells spillage in to Bowman's space (BS)

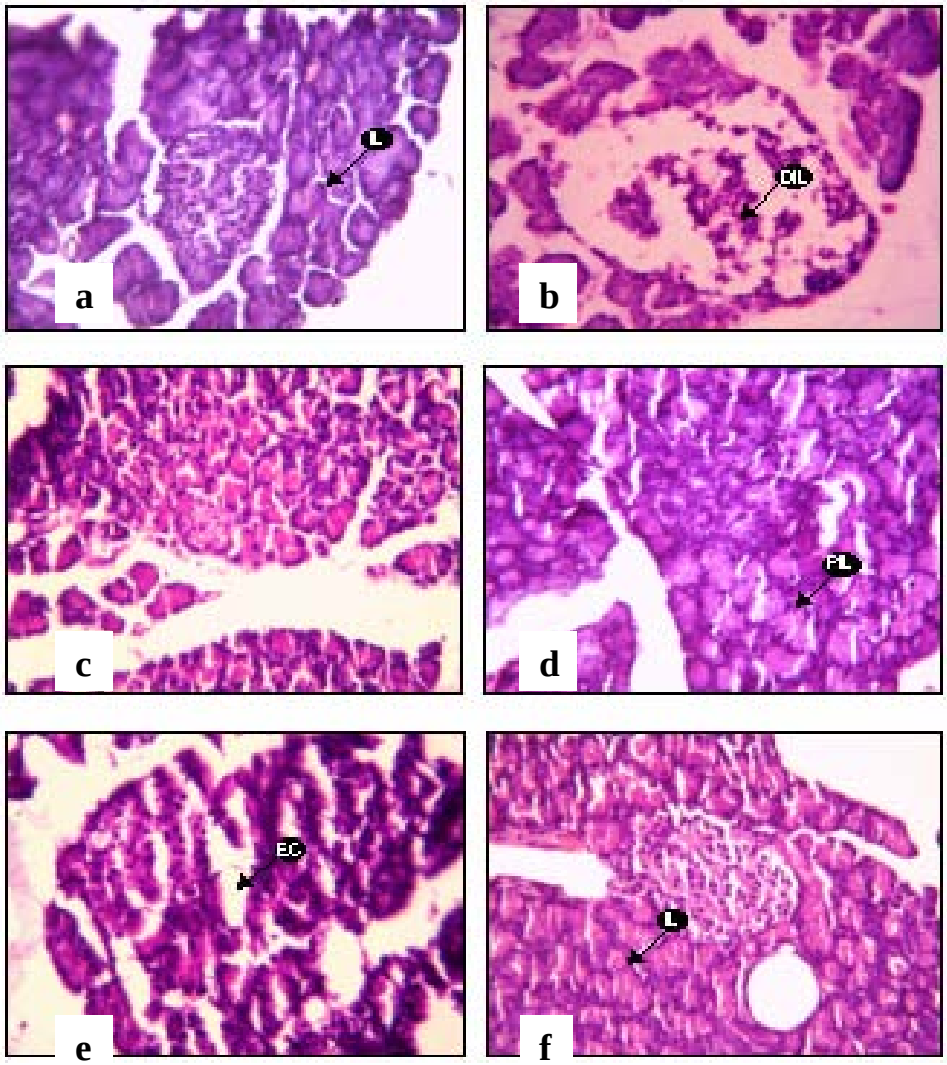

Figure 3
Histopathological Changes Of Pancreas Of Control and experimental Rats. **a. Group I -** Pancreas showing exocrine acini and endocrine islets (IL); **b. Group II -** Shows depleted islets (DIL); **c. Group III -** Shows exocrine acini and small preserved islets; **d. Group IV** - Shows preserved islets (PIL); **e. Group V-** Preserved islets and exocrine (EC) are seen; **f. Group VI** - Pancreas showing islets (IL)

DISCUSSION

Our findings reveal that the significant decrease of serum glucose level in extract treated diabetic rats. Alloxan not only destroys the pancreatic β-cells but causes kidney damage, which is however reversible, while streptozotocin selectively destroys pancreatic insulin secreting β-cells (Gilman *et al.*,1990). The degenerative changes in the histology of liver and kidney brought about by alloxan administration are similar to earlier observations (Shanmugasundarm *et al.*, 1983, Leegwates *et al.*, 1984, Ghosh *et al.*, 2001 and Thakran *et al.*, 2004). Histologically, liver section of alloxan induced diabetic rats showed marked structural alterations in the liver as a result of absence of insulin. The major alteration was periportal fatty infliteration, necrosis of hepatocytes. This damage is partially reversed by the *T.arjuna* bark extract treatment and is similar to that observed by *Gymnema sylvestre* therapy in alloxan diabetic

rabbits by Shanmugasundaram *et al(1983)* and *Vinca rosea* extract in alloxan- induced diabetic rats by Ghosh *et al*.(2001). The kidney histopathology data of alloxan induced diabetic rats showed marked tubular damage, haemorrhage in the Bowman's space due to glomerular damage. The results indicate a primary and a secondary effect of the diabetic state on the kidney of the rat. The primary effect, the diabetes factor was associated with hyperglycaemia and was responsible for dilatation of proximal and distal tubules in the cortex. The secondary effect, named the individual response factor, was associated with inflammatory processes (Leegwates *et al*., 1984). Diuresis is a common feature associated with diabetes which may be the reason for structural changes observed with glomerulus (Das *et al*., 1996). The excellent recovery of renal function expected with treatment of *T. arjuna* can be explained by the regenerative capability of the renal tubules. Similar results have been observed with the treatment of alloxan induced diabetic rats with *Trigonella foenum graecum* seed powder by Thakran *et al*. (2004). The role of *T.arjuna* in reversing the diabetic state at the cellular level besides the metabolic normalization further proves its potential as an antidiabetic assert.

The present study revealed that the immediate action of alloxan induced diabetes by destroying β-cells even at a single dose of 120 mg/kg of body weight. The ultra structure of alloxan diabetic pancreas showed considerable reduction in the islet langerhans and depleted islets. These are in agreement with earlier reports (Gholamali *et al*., 2005 and Ghosh *et al*., 2001). The diabetic rats showed pancreatic islet regeneration. The regenerative effect of the pancreatic cells by *Terminalia arjuna* via exocrine cells of pancreas may enlighten the positive effects of these agent on the production of insulin.

Conclusion

The goal of these studies was to evaluate the effect of *Terminalia arjuna* (50% ethanolic extract) on development of liver, kidney and pancreatic tissue damage or complications in alloxan – induced diabetic rats. Our data show that the *Terminalia arjuna* extract was found to effectively improve the liver, kidney and pancreas function and reduced the lesions associated with diabetic state in alloxan – diabetic rats. Further more, the effect of oral *T. arjuna* at the dose 500mg/kg body weight was more efficacy than 250 mg/kg body weight.

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