Chapter 4

Histopathological Studies of Plant Extract Treated and Control Diabetic Rats.
**Introduction**

According to the studies carried out by many researchers for the management of diabetes which is the prominent disorder which involves the mal function of pancreas, kidney and liver. During the study, the investigation was carried out to analysis the architecture and the morphology of the normal, standard and control experimental rats with plant extracts taken up in the study.

Hematoxylin and eosin stain section of pancreas, kidney and liver were checked for the normal morphology and architecture after the treatment of plant extracts. The distortion and chronic inflammation of islets cells of pancreas, hepatic lobules and central vein damage in liver and tubular congestion, structure damage and hemorrhage to the glomeruli in kidney were taken into concern after the treatment of plant extracts.

*In vivo* studies are of important fact to assays the activity of the compounds present in the plant extracts taken up in the present study. The histopathology section revealed the details of morphology, normal function and rejuvenation of the cells that leads to the normal functioning can be very well understood.

**Histopathological studies** (Talukder *et al.*, 2007)

The liver, pancreas and kidney were removed from the animals and the tissues were stored in 10% formalin. Histopathological studies were carried out in J.S.S Medical College, Mysore.

**Procedure:**

Tissue preparation

a. Dehydration: Tissues are dehydrated by using different strength of alcohol such as

   - 70% alcohol for 1 h
   - 90% alcohol for 1 h
   - 100% alcohol for 1 h

b. Clearing: The clearing of tissue was done by using xylene and tissues were minced into pieces which was kept in xylene solution for 2 h.
c. Impregnation with wax: The tissue was impregnated along with paraffin wax and was kept at 54-60°C (Melting point temperature of paraffin wax) in heating cups for 1 h. The volume of wax depends on volume of tissues used mostly it will be 25-30 times volume of tissues.

d. Blocking or embedding in paraffin wax: Impregnated tissues were placed in a mould with their labels and then melted wax was poured in it and allowed to settle and solidify.

e. Section in Microtome: The tissue block was cut to the thickness of 3-5 µm using rotator microtome. They were flattened on warm water and mounted onto albumerised slides and allowed to fix.

**Staining procedure:**

**Hematoxylin and eosin staining**

1. The sections fixed on slides were put in xylene for 3 min.
2. Then transferred to absolute alcohol for 3 min.
3. Transferred to 80% alcohol for 2 min.
4. Placed in 50% alcohol for 2 min.
5. The slides were washed in running tap water for 1 min and put in Harris’s Hematoxylin for 5-7 min.
6. Wash in running tap water for 30 min.
7. Wash excess dye in 1% acid alcohol by continuous agitation for 15 sec.
8. Wash in running tap water for 30 min.
9. Give 2-3 dips in ammonia water solution until tissues attain a blue colour.
10. Wash in running tap water for 30 min.
11. Counter stain with eosin for 3-5 min.
12. Wash in running tap water for 30 min.
13. Dehydrate by keeping in increasing concentration of alcohol (2-3 min in 50%, 70%, 95% and absolute alcohol).
14. Clear it in xylene and cover slip is placed over.
15. The section was studied for the cell characteristic using a Binocular Microscope.
Results:

Pancreas

Normal: Figure 4 are the photomicrographs of pancreas of normal rats showed normal pancreatic architecture.

Control: Islets cells at the amidst of exocrine pancreas tissue figure 4.1 lymphocytic inflation where observed. β-cells were destructed with atrophy. Shrieked islets cells were also found prominent.

Standard: Figure 4.2 Pancreases of diabetic rats treated with glabni-clamide was taken as standard showed thickened vasculative with normal pancreatic tissue.

Eosin and hematoxylin stained section of pancreas of diabetic rats that are treated with ethanol extracts of *S. xantocarpum* root, *S. xantocarpum* fruit and *P. kurroa* rhizome at 250 and 500 mg were studied for 28 days. The results revealed that at 250 mg/kg b.t. *S. xantocarpum* root extract showed the mild distortion of islets cell with normal exocrine pancreas in the stained section figure 4.3. *Solanum xantocarpum* fruit extract treated pancreas of diabetic rats showed normal pancreatic morphology (Fig. 4.4). Islets cells of pancreas showed increased density (Hyperplasia) in the diabetic treated with extracts of *P. kurroa* rhizome (Fig. 4.5).
Pancreas

Figure 4: Normal rat Pancreas (40X)

Figure 4.1: Diabetic control rat Pancreas (40X)

Figure 4.2: Standard (Glibenclamide) treated rat pancreas (40X)

Figure 4.3: *S.xanthocarpum* root treated 250mg/kg b.w diabetic rat pancreas (40X)

Figure 4.4: *S.xanthocarpum* fruit treated 250mg/kg b.w diabetic rat pancreas (40X)

Figure 4.5: *P.kurroa* 250mg/kg b.w diabetic rat pancreas (40X)
The combination of the extract was found to be promising in regenerating the tissue architecture of islets cells of pancreas. The experimental rats that were treated with root and fruit combination showed normal pancreatic morphology.

*Solanum xantocarpum* root + *S. xantocarpum* fruit showed normal pancreatic morphology (Fig. 4.6). *Solanum xantocarpum* root + *P. kurroa* showed similar results like *S. xantocarpum* root + *S. xantocarpum* fruit (Fig. 4.7). *Picrorhiza kurroa* + *S. xantocarpum* fruit exhibited the increase in number of islets cells that leading to a condition hyperplasia (Fig. 4.8).

Ethanolic plants extracts at 500 mg/kg. b.w revealed that *S. xantocarpum* root treated pancreas showed mild islet cells density (Fig. 4.9). *Solanum xantocarpum* fruit treated pancreatic section showed relative islet cells density (Fig. 4.10). Similar results were observed in *P. kurroa* rhizome (Fig. 4.11).
Figure 4.6: *S. xantocarpum* root + *S. xantocarpum* fruit 250mg/kg b.w diabetic rat pancreas (40X)

Figure 4.7: *P. kurroa* + *S. xantocarpum* root 250mg/kg b.w diabetic rat pancreas (40X)

Figure 4.8: *S. xantocarpum* fruit + *P. kurroa* 250mg/kg b.w diabetic rat pancreas (40X)

Figure 4.9: *S. xantocarpum* root 500mg/kg b.w diabetic rat pancreas (40X)

Figure 4.10: *S. xantocarpum* fruit 500mg/kg b.w diabetic rat pancreas (40X)

Figure 4.11: *P. kurroa* 500mg/kg b.w diabetic rat pancreas (40X)
Solanum xantocarpum root + S. xantocarpum fruit, P. kurroa + S. xantocarpum root and P. kurroa + S. xantocarpum fruit showed increased islets cells density with normal architecture of pancreas which was found to be more prominent among the combination at higher concentration (Figs. 4.12-4.14).

Figure 4.12: S. xantocarpum root + S. fruit 500mg/kg b.w diabetic rat pancreas (40X)

Figure 4.13: P. kurroa + S. xanthocarpum root 500mg/kg b.w diabetic rat pancreas (40X)

Figure 4.14: S. xanthocarpum fruit + P. kurroa 500mg/kg b.w diabetic rat pancreas (40X)
Kidney

Normal: Histopathology of kidney in the normal rats showed normal architecture of tubules and glomerulei (Fig. 4.15).

Control: In the control revealed destruction of tubules, chronic inflammation along with structural damage in glomeruli (Fig. 4.16).

Standard: In standard section of kidney was similar to the normal kidney of the experimental rats with normal morphology of tubules and glomerulei (Fig. 4.17).

The plant extracts at 250 mg/kg bw treated experimental rats showed destruction of tubules, chronic inflammation along with structural damage in glomeruli in the kidney.

The plant extracts of S. xantocarpum root, S. xantocarpum fruit and P. kurroa rhizome at 250 mg concentration were treated to be the experimental rats. The kidney were subjected to histopathological studies and it revealed that, S. xantocarpum root treated rats exhibited the tubular congestion was found to be mild and normal morphology of the kidney (Fig. 4.18). Solanum xantocarpum fruit treated studies revealed thickened tubular lining with hemorrhagic foci (Fig. 4.19). Picrorhiza kurroa rhizome treated rats showed mild distortion in tubules and congestion in glomeruli (Fig. 4.20).
Kidney

Figure 4.15: Normal rat kidney (40X)

Figure 4.16: Diabetic control rat kidney (40X)

Figure 4.17: Standard (Glibenclamide) treated rat kidney (40X)

Figure 4.18: *S.xanthocarpum* root 250mg/kg b.w diabetic rat kidney (40X)

Figure 4.19: *S.xanthocarpum* fruit 250mg/kg b.w diabetic rat kidney (40X)

Figure 4.20: *P.kurroa* 250mg/kg b.w diabetic rat kidney (40X)
In the *S. xantocarpum* root + *S. xantocarpum* fruit combination mild distorted tubules with chronic inflammation was observed (Fig. 4.21). *Picrorhiza kurroa* + *S. xantocarpum* root showed tubules of the kidney showed dilated cystically (Fig. 4.22). A similar result was also observed by *P. kurroa* + *S. xantocarpum* fruit (Fig. 4.23).

The plant extract treated at 500 mg/kg bw, *S. xantocarpum* root showed similar results as in 250 mg/kg bw with tubular lining thickened, chronic inflammation but normal glomeruli (Fig. 4.24). *Solanum xantocarpum* fruit the condition found to be improved with chronic inflammation, glomeruli having congestion with mild distorted tubules (Fig. 4.25). *Picrorhiza kurroa* rhizome at this concentration showed normal morphology in the kidney architecture (Fig. 4.26).
Figure 4.21: *S.xanthocarpum* fruit + *S.xanthocarpum* root
250mg/kg b.w diabetic rat kidney (40X)

Figure 4.22: *P.kurroa* + *S.xanthocarpum* root
250mg/kg b.w diabetic rat kidney (40X)

Figure 4.23: *S.xanthocarpum* fruit + pk
250mg/kg b.w diabetic rat kidney (40X)

Figure 4.24: *S.xanthocarpum* root
500mg/kg b.w diabetic rat kidney (40X)

Figure 4.25: *S.xanthocarpum* fruit
500mg/kg b.w diabetic rat kidney (40X)

Figure 4.26: *P.kurroa*
500mg/kg b.w diabetic rat kidney (40X)
In the *S. xantocarpum* root+ *S. xantocarpum* fruit combination at 500 mg/kg bw showed only congestion with tubular hemorrhage (Fig. 4.27). *Picrorhiza kurroa* + *S. xantocarpum* root showed congestion with tubular hemorrhage like *S. xantocarpum* fruit + *S. xantocarpum* root treated kidney architecture (Fig. 4.28). *Picrorhiza kurroa* + *S. xantocarpum* fruit exhibited normal architecture which was predominating with mild chronic inflammation (Fig. 4.29).
Liver

Liver that was subjected for histopathological studies revealed that in normal rats the morphology of hepatic lobules and central vein were appeared to be normal (Fig. 4.30). In the control rats the distortion was observed in hepatic parachymatious cells along with chronic inflammation (Fig. 4.31). In the standard rats, histopathological section shows the normal morphology (Fig. 4.32).

In the plants extracts at 250 mg/kg bw *S. xantocarpum* root treated rats revealed the mild distortion of hepatocytes with dilated central vein (Fig. 4.33). *Solanum xantocarpum* fruit treated sections showed only central vein dilation however the hepatocytes were normal (Fig. 4.34). *Picrorhiza kurrooa* treated liver also showed the similar characteristic that of *S. xantocarpum* fruit treated rats (Fig. 4.35).
Figure 4.30: Normal rat liver (40X)

Figure 4.31: Diabetic control rat liver (40X)

Figure 4.32: Standard (Glibenclamide) treated rat kidney (40X)

Figure 4.33: *S. xanthocarpum* 250mg/kg b.w diabetic rat liver (40X)

Figure 4.34: *S. xanthocarpum* fruit 250mg/kg b.w diabetic rat liver (40X)

Figure 4.35: *P. kurroa* 250mg/kg b.w diabetic rat liver (40X)
In the plant extract combination of *S. xantocarpum* root+ *S. xantocarpum* fruit distorted lobules and fatty changes were prominent (Fig. 4.36). *Picrorhiza kurroa* + *S. xantocarpum* root showed only mild distortion of hepatocytes was seen in liver section treated with this concentration (Fig. 4.37). *Picrorhiza kurroa* + *S. xantocarpum* fruit exhibited normal morphology was predominant with mild distortion of lobules was observed (Fig. 4.38).

In the plant extract at 500 mg/kg bw *S. xantocarpum* root treated liver revealed slight improvement at higher dosage however congestive centrilobular zone was noticed (Fig. 4.39). In the *S. xantocarpum* fruit treated rats liver sections revealed similar kind of dilated central vein with normal hepatocytes like that of 250 mg/kg bw treated liver cells (Fig. 4.40). *Picrorhiza kurroa* exhibited normal morphology of hepatic cells with mild inflammation, a slight improvement in the regeneration of cells at the higher dosage treated hepatic cells (Fig. 4.41).
Figure 4.36: *S.xanthocarpum* root + *S.xanthocarpum* fruit
250mg/kg b.w diabetic rat liver (40X)

Figure 4.37: *S.xanthocarpum* root + *P.kurroa*
250mg/kg b.w diabetic rat liver (40X)

Figure 4.38: *S.xanthocarpum* fruit + *P.kurroa*
250mg/kg b.w diabetic rat liver (40X)

Figure 4.39: *S.xanthocarpum* root
500mg/kg b.w diabetic rat liver (40X)

Figure 4.40: *S.xanthocarpum* fruit
500mg/kg b.w diabetic rat liver (40X)

Figure 4.41: *P.kurroa*
500mg/kg b.w diabetic rat liver (40X)
Solanum xantocarpum root + S. xantocarpum fruit combination showed central vein was found dilated with mild distortion of hepatocytes was observed in the extract treated liver cells (Fig. 4.42). In P. kurroa + S. xantocarpum root, the section revealed the normal morphology with mild inflammation (Fig. 4.43). Picrorhiza kurroa + S. xantocarpum fruit the combination and concentration revealed normal hepatic morphology the results was found to be evident compared to all the other extracts treated to the experimental models (Fig. 4.44).

Figure 4.42: S.xanthocarpum root + S.xanthocarpum fruit 500mg/kg b.w diabetic rat liver (40X)

Figure 4.43: S.xanthocarpum root+ P.kurroa 500mg/kg b.w diabetic rat liver (40X)

Figure 4.44: S.xanthocarpum fruit+ P.kurroa 500mg/kg b.w diabetic rat liver (40X)
Discussion of pancreas:

The normal control rats showed normal tissue architecture. The islets were rounded and elongated with evenly distributed cytoplasm with lightly stained nucleus which surrounded by acinar cells.

In the diabetes induced rats the pancreas architecture was found degenerative in general and islets of langerhans in the pathological feature.

The diabetic rats without any treatment showed the degenerative and necrotic change in the tissue architecture including the shrunken islets of langerhans. The nucleus of necrotic cells showed pyknosis or marginal hyperchromatic. This may be due to hydropic degeneration and degranulation in the cytoplasmic in necrotic cells, some of the tissue exhibited dark eosinophilic cytoplasm. The least insulin immune reactivity was observed in the some of the β-cells in the islets of langerhans. The results were concurred with Coskun et al. (2005) and Karaca et al. (2010).

Abdel-moneim et al., (2012) has reported that increase in the islets number which was found to be attributed due to the hypoglycemic effect on Nigell sativa which increase the glucose reobservation from the intestine during the time course. However the treated rats in the present study showed remarkable increase in β-cells number and decrease in their diameter including the nuclear diameter. In the present study S. xantocarpum fruit, S. xantocarpum root and rhizome of P. kurroa and the S. xantocarpum fruit + S. xantocarpum root, S. xantocarpum fruit + rhizome of P. kurroa and S. xantocarpum root + rhizome of P. kurroa were experimented in the laboratory rats. Among the plants extracts was found to be effective in bringing about the stimulatory effect on the division of β-cells, restore insulin production and also block the diabetogenic action of STZ. The results were on par with Augusti and Sheela (1996) and Milionis et al. (2005), where they have found the similar kind of results with the treatment of Nigella sativa.

According to Augusti and sheela (1996) some plants in singles and in combination may bring about synergetic activity in stimulating β-cells and also accelerate the glucose reobservation as the combination of extract may exhibit the antioxidant effect.
In the STZ induced rats there was an increasing in serum glucose that was in concomitant decrease in serum insulin level which resulted in pancreatic β-cells necrosis. This enhances ATP dephosphorylation and results in superoxide, hydrogen sulphide, hydroxyl radical generation (Gupta et al., 2005).

The plants in singles and in combination showed the protection to the majority of cells in the langerhans islets cells. These were moderate insulin antigen +ve veithy towards many β-cells. This antigen +ve veithy was found to exhibit more by the synergetic activity of combination in general *S. xantocarpum* fruit + *P. kurroa*, *S. xantocarpum* root + *P. kurroa* and in single by *P. kurroa* in particular.

**Kidney**

The kidney of the normal experimental rats exhibited the normal glomureli with thin glomerular basement membrane. Normal cell architecture with capsular were also seen. Major detectable abnormality in histopathology of the cell architecture in the kidney was not observed. However in the diabetic induced models the histopathology reveals diffused degenerative tubules with multifocal of hemorrhages.

In glabinclamide animal model systems all the above said are seen to the minimum changes in kidney cell architecture.

Morphological and functional changes are exhibited by the kidney during the onset of the diseases. The preliminary effect during the diabetic factor that is associated with hyperglycemia. These are responsible for dilation of proximal and distil tubules in the cortex. The individual response factor which is associated with the inflammatory process being the secondary effect. Diuresis is the feature which is associated with diabetic directly proportion to the structural changes seen in glomeruleus. Sever hyperglycemia causes renal damage. Several researchers have mentioned the lesion of kidney with the probable mechanism. The kidney sections of the experimental rats have shown lesions, glomerulosclerosis, glomerular membrane thickening, arteriolar hyalinization and widespread tubular necrosis. This glomerulosclerosis results in decreased kidney function, finally renal failure and diabetic nephropathy (O’Donnella et al., 1998). The tissue architecture including the cortex, medulla and tubules were found degenerated and necrotic in the nephron of
the diabetic animal models. The glomerulosis was emptied and distal tubular also damaged in diabetic nephron in the present study (Selvan et al., 2008).

The better recovery of the renal recovery as expected with the treatment of S. xantocarpum fruit, S. xantocarpum root and rhizome of P. kurroa singly and the in combination S. xantocarpum fruit + S. xantocarpum root, S. xantocarpum fruit + rhizome of P. kurroa and S. xantocarpum root + rhizome of P. kurroa can be explained by the regenerative capability of the renal tubules. Similar observation has been made by Thakran et al. (2004), with another herb Trigonella foenum graecum seed power. In the present study the herbal combination S. xantocarpum fruit + P. kurroa, S. xantocarpum root + P. kurroa followed by single P. kurroa reversed diabetic state at the tissue level with simultaneously metabolic normalization proved to be potential herbal combination as an antidiabetic assort.

Liver

Histopathological studies of the hepatic cells in the liver of normal experimental rats exhibited normal tissue architecture without degenerative and necrotic changes. In the diabetic induced rats there was the degeneration of hepatocytes which was found to pronounce with the loss of the normal architecture in diabetic induced rats.

In the present study, the histopathological architecture has shown that sinusoidal spaces are dilated in diabetic rats with multifocal fatty degeneration. The research finding is in par with Nakanckar et al. (2013). Necrosis of the cell was observed with the complete degeneration of liver cells including irregular appearance due to oozing of cellular content resulting in cell death.

The portal tracts showed portal triad with portal vein, hepatic artery and bile duct the tissue architecture was found distorted in the arrangement in the arrangement of the cell in central vein, per portal fatty infiltration with focal necrosis in hepatocytes.

The tissue architecture was found degenerative in very few of the standard of the standard drug treated experimental rats.
In the group of experimental rats treated with *S. xantocarpum* fruit, *S. xantocarpum* root and rhizome of *P. kurroa* singly and the in combination *S. xantocarpum* fruit + *S. xantocarpum* root, *S. xantocarpum* fruit + rhizome of *P. kurroa* and *S. xantocarpum* root + rhizome of *P. kurroa*, normal and effect areas of liver in patches adjacent to each other was observed. Among the extracts, rhizome of *P. kurroa* in particular and *S. xantocarpum* fruit + *P. kurroa*, *S. xantocarpum* root + *P. kurroa* in general showed less degenerative changes, necrosis and hemorrhage with consequence appearance of leucocytes *i.e.*, is essential for the hepatoprotective effect brought about by the extracts in singles and in combination. Similar observations are made by Malar and Bai (2009) with *Phyllanthus emblica*. 