CHAPTER-4

EFFECT OF ARFHI ON PANCREAS, LIVER AND KIDNEY OF NORMAL AND DIABETIC RATS: HISTOLOGICAL EXAMINATION AND TOXICITY EVALUATION.

INTRODUCTION

Herbal medicines have good curative effect on certain diseases especially for diabetes mellitus which needs continuous medication throughout the life. Present day allopathic medicines are costlier and having more side effects which could cause severe damages to the vital organs. Hence, finding a suitable herbal medicine for diabetes mellitus is very important in the current situation (Boopathy raja et al., 2010). Herbal medicines, containing active ingredients extracted from aerial or underground parts of plants are widely used in health-care or as dietary supplements. One of the major drawbacks of these medicines is limited bioavailability, being poorly absorbed if taken orally (Chawla et al., 2013).

The lack of access to effective, cheap, safe and user friendly medicines has resulted in an ever increasing number of people using herbal medicines. There is a belief that these medicines are safe because they are natural. Herbal remedies have become popular over the past decade and they are widely used for the treatment and prevention of various diseases. Many people mistakenly think that all medicinal herbs, being natural, are generally safe and free from undesirable side effects while acting as an effective agent. The use of herbal medicine is still poorly understood by the public. Now a days, toxicity and safety of medicinal herbs is one of the most discussed topics as herbal products have become popular worldwide. Before a Phytomedicine is brought into a clinical trial program, its safety profile must be assured by conducting a series of nonclinical, pharmacological and toxicological investigations (Merlin et al., 2004). Naturally the expected benefits of herbal remedy should outweigh its potential risks. Insufficient evidence of safety of an herbal product is enough to stop further development, as it may carry serious health risks (Woolf., 2003).

Most reports of toxic effects due to the use of herbal medicines and dietary supplements are associated with hepatotoxicity, although reports of other toxic effects including kidney, nervous system, blood, cardiovascular, dermatologic effects, mutagenicity and carcinogenicity
have also been published in medical literature (Pak et al., 2004; Niggemann and Gruber, 2003). The liver in vertebrate body performs many vital functions, including metabolic and detoxification activities. A number of chemical agents and routine drugs produce cellular as well as metabolic liver injury. Therefore, many herbal and other indigenous sources have been adequately explored for the safe and effective hepatoprotective action. Relatively recently, the liver has been recognized as a major target of injury in patients with insulin resistance or the metabolic syndrome.

Pancreas is a compound tubular alveolar, partly exocrine and partly endocrine gland. The endocrine part of the pancreas is in the form of serous acini, secreting the secretions into intralobular duct. The endocrine part of the pancreas is in the form of numerous rounded collections of cells known as islets of langerhans, embedded within the exocrine part. Each islet is separated by the surrounding alveoli by a thin layer of reticular tissue. The average islet in rats is 150 µm in diameter and contains about 45 ng of insulin. There are four major endocrine cell types in mammalian islets, the insulin producing \( \beta \) cells, the glucagon producing \( \alpha \)-cells, the somatostatin producing \( \delta \) cells and pancreatic polypeptide producing pp-cells. The \( \beta \) cells are polyhedral, being truncated pyramids and are usually well granulated with secretory granules 250-300nm in diameter.

It has been estimated that each rat \( \beta \) cell contains about 10,000 granules. There are two forms of insulin granules: electron dense mature granules and moderately dense immature granules (Bonner-Weir and Smith, 1994). Microscopic examination shows abundant patches of \( \beta \) cells in the pancreas of normal rats which are absent in diabetic pancreas (Anil et al., 1996). Selective destruction of \( \beta \) cells is observed in alloxan or Streptozotocin induced diabetic rats. Lytic and vascular changes of cellular components are also observed in diabetes. Small and shrunken islets and destruction of \( \beta \) cells are observed in the diabetic condition (Mitra et al., 1996). Insulitis, with heavy lymphocytic infiltration in and around the islets may be present and is more commonly seen in islets containing residual \( \beta \) cells in T1DM.

Liver is considered to be consisting of large number of hexagonal lobules. Each lobule consists of a central vein, from which cords or rows of liver cells radiate like spokes of a wheel. Each lobule is delineated by a connective tissue (Chaudhari et al., 1998). Microscopic examination of normal liver shows the glycogen granules as reddish purplish material in
hepatocytes with Periodic acid-Schiff (PAS) staining (Mitra et al., 1996). But diabetic liver shows decreased deposits of glycogen granules. Histology of liver during diabetes shows structural alterations in the liver due to lack of insulin. In liver cells the sinusoidal spaces and the vein lumen are enlarged. The major alterations are thickening of the wall of the blood vessels and capillaries in diabetic state. The distortion in the usual arrangement of the hepatic cells may be brought about by the increase in the lumen of the veins which might have pushed the surrounding cells (Anil and Paulose, 1995; Anil et al., 1996). The fibrosis observed in diabetic liver shows the extensive damage of liver cells which is replaced by the fibrous tissue (Balazs and Halmos, 1985).

Kidney is covered by a capsule, below which there is a cortex, occupying upper 4/5th of the kidney. Inner to cortex there is medulla. In the cortex circular structures called renal capsules are present, surrounding which are tubules in various shapes. The dark rounded thick walled tubules are parts of the proximal convoluted tubule. The lumen of the proximal convoluted tubule is small and indistinct. The light thin wall tubule with distinct lumen is called distal convoluted tubule (Singh et al., 1992). The kidney in a newly diagnosed diabetic subject is enlarged (Mogensen and Andersen, 1973). This was also confirmed in alloxan or Streptozotocin induced diabetes in rats (Seyer-Hansen et al., 1983). Kidney sections of diabetic animals showed thickening on the walls of the nephron filling their lumen along with glomerulopathy. Diuresis is a common feature associated with diabetes which may be the reason for structural changes observed with glomerulus (Anil and Paulose, 1995; Anil et al., 1996). Renal failure is an important complication of diabetes. It causes death in more than 10% of all diabetics and in over 50% of those who develop diabetes in childhood (Anderson, 1985). The most important contribution to this high mortality is diabetic glomerulosclerosis, which can also give rise to the nephritic syndrome. Hyalin thickening of afferent glomerular arterioles is also very common in diabetes. Deposition of hyaline in the mesangium of all the glomerular lobules, with the associated thickening of the glomerular capillary basement membrane is present in the diabetic kidney.

Glomerulosclerosis is especially common in early onset diabetes (Anderson et al., 1985). The hallmark of diabetes is thickening of basement membrane of glomerular capillaries. Also thickening of tubular BM has been demonstrated (David Woodrow et al., 1991) in diabetes.
Four distinct lesions can be recognized by light microscopy in glomeruli of diabetic patients in addition to alterations in the thickening and configuration of BMs. Among that nodular lesion, which was initially described by Kimmelstiel - Wilson syndrome in 1936, is considered to be pathognomonic of diabetes mellitus. The nodules generally associated with some degree of diffuse glomerulosclerosis which is characterized by diffuse deposition of BM like material in mesangium of the entire glomerulus. Mesangial cell proliferation can frequently be seen, but with the progression of the lesion there is compression and disappearance of cells owing to the replacement by increasing amount of mesangial matrix. Mesangial expansion rather than nodules, caused by excess deposition of BM- like material may have a potential effect on vascular potency by reducing the lumina of capillaries and thus impeding blood flow (Reddi et al., 1990, Camerini et al., 1990).

Some investigators reported that thickening of GBM is a late phenomenon, while others believe that it occurs early in the diabetes. Osterby et al., (1991) reported that thickening of GBM in T1DM remains normal at the clinical onset of diabetes. With increasing duration of disease, BM becomes thickened, leading eventually to the total occlusion of many diseased glomeruli. Thickening of Bowman’s capsule and tubular BM also occurs early in the disease. It is generally believed that the thickening of the GBM is a slow and gradual process, initially demonstrable by electron microscopy and later on light microscopy. Its incidence and severity increase with duration of diabetes. Atheroma is very common after severe diabetes. Other renal changes in diabetes include papillary necrosis greatly aggregating renal failure. Secondary atrophy of the tubules and intestinal fibrosis result from the glomurular lesion.

Streptozotocin is well known for its selective pancreatic islet β cell cytotoxicity and in many animal species; STZ induces diabetes that resembles human hyperglycemic non-ketotic diabetes mellitus (Weir et al., 1981). Further rats treated with STZ display many of the features in human subjects with uncontrollable DM and are invaluable when studying the mechanisms by which hyperglycemia may contribute to microvascular complications such as neuropathy, nephropathy and retinopathy (Obrosova et al., 2005). The functioning of pancreas, liver and kidney may be affected due to decreased levels of insulin, hyperglycemia and its consequences. In the present investigation the histological changes in these tissues of diabetic rats and the effect of treatment with ARFHI on these were studied.
Numerous biochemical analysis has been used as standard safety tests. These include assays of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) and Alkaline phosphatase (ALP), which have proven to be very good indicators of liver toxicity (Friedman et al., 1996). Measurement of serum creatinine and urea reflects the functioning of kidneys (Smith et al., 2006). Hence measurement of these parameters enables study of the toxic effects of the drug on the liver and kidney.

Alanine transaminase (ALT) (EC 2.6.1.2) is also called as serum glutamate pyruvate transaminase (SGPT). ALT is found in serum and in various bodily tissues, but is most commonly associated with the liver. It catalyzes the transfer of an amino group from alanine to α-ketoglutarate. It is commonly measured clinically as a part of diagnostic liver function tests, to determine liver health. Significantly elevated levels of ALT often suggest the existence of other medical problems such as viral hepatitis, congestive heart failure, liver damage, biliary duct problems, infectious mononucleosis, or myopathy. For this reason, ALT is commonly used as a way of screening for liver problems. ALT levels can also increase in response to strenuous physical exercise. When elevated ALT levels are found in the blood, the possible underlying causes can be further narrowed down by measuring other enzymes. For example, elevated ALT levels due to liver cell damage can be distinguished from biliary duct problems by measuring alkaline phosphatase.

Aspartate transaminase (AST) also called serum glutamate oxaloacetate transaminase (SGOT) (EC 2.6.1.1) is similar to alanine transaminase (ALT) in that it is another enzyme associated with liver parenchyma cells. It facilitates the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate, and vice-versa. Two isoenzymes are present in humans. They have high similarity.

- SGOT1, the cytosolic isoenzyme derived mainly from red blood cells and heart.
- SGOT2, the mitochondrial isoenzyme is predominantly present in liver.

AST is raised in acute liver damage. It is also present in red blood cells and cardiac muscle, skeletal muscle, kidney and brain tissue, and may be elevated due to damage to those sources as well. AST (SGOT) is commonly measured clinically as a part of diagnostic liver function tests, to determine liver health.
Alkaline Phosphatase (ALP) (EC 3.1.3.1) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules including nucleotides, proteins and amino acids. ALP is zinc containing metallo enzyme; it is activated by Mg$^{2+}$ and other divalent ions. Active centre of alkaline phosphatase include serine residue. As the name suggests, alkaline phosphatases are most effective in alkaline environment. Its alternative name is orthophosphoric monoester phosphohydrolase. It is widely distributed in the body but is particularly associated with bone (osteoblasts), small intestine (mucosal cells), liver (cells of biliary system), placenta and Kidney (proximal convoluted tubules). The bone, liver and kidney isoforms have the same amino acid sequence (coded on chromosome 1) but differ in their carbohydrate content; the intestinal and placental forms also have their primary structures in common (coded on chromosome 2). ALP present in normal plasma is approximately equally of hepatobiliary and bone origin. ALP activity is increased in the plasma of diabetic case (Cantor et al., 1947; Hough et al., 1981). Poor diabetic control may relate to bone/liver ALP, whereas hyperphagia may mainly increase intestinal ALP.

The present study was taken up to evaluate the effect of treatment with ARFHI on liver and kidney functions and histological changes in the diabetic as well as normal rats.

**MATERIALS AND METHODS**

**Evaluation of the acute toxicity of the ARFHI in normal rats**

Acute toxicity of ARFHI was evaluated in healthy wistar male albino rats, according to the guidelines set by Organization for Economic Cooperation and Development (OECD) (Bala et al., 2010) mentioned in chapter 1.

Induction of diabetes in male wistar rats was made as described in the chapter “Materials and Methods”. Rats with the fasting blood glucose levels of $\geq$ 250mg /dL were taken for the experiment. The rats were divided into 5 groups with 6 rats in each group.

**Group 1:** Normal untreated rats  
**Group 2:** Normal rats treated with 750 mg ARFHI /kg bw /day /for 28days  
**Group 3:** Diabetic untreated rats  
**Group 4:** Diabetic rats treated with 750 mg ARFHI /kg bw /day /for 28 days  
**Group 5:** Diabetic rats treated with 20 mg of glibenclamide /kg bw /day/ for 28 days.
The animals in group 2 and 4 were given daily oral dose of 750 mg ARFHI/kg bw/day for 28 days, while group 1 and 3 rats were given water alone and group 5 rats were treated with glibenclamide at a dose of 20 mg/kg bw at morning time for a period of 28 days. All the 5 groups were sacrificed on the last day of treatment by cervical dislocation and then blood, pancreas, liver and kidney were collected and the tissues were stored in 10% formalin after washing 3 times with normal saline. Serum was separated immediately and then stored for further biochemical investigations. The activities of SGOT, SGPT, ALP and the levels of urea, creatinine were estimated in serum by using the methods given in the “Materials and Methods” chapter.

**Histological studies**

Histological parameters were studied with the help of pathologist at the Department of Pathology, S.V. Veterinary University, Tirupati, A.P, India. The tissues were washed, dehydrated with alcohol, cleared with xylene and paraffin blocks were made. Serial sections of 5μm thickness were cut using a rotary microtone. The sections were then deparaffinised with xylene and hydrated in descending grades of alcohol. The slides were then transferred to haematoxylin for 10 min, followed by rinsing with water, differentiated in 1% acid alcohol, rinsed in water, bleuing in running tap water or 1% lithium carbonate. Later they were counter stained with eosin, rinsed with water, dehydrated with ascending grades of alcohol, cleared with xylene and mounted on glass slides. These slides were examined under 10X and 40X using microscope.

**RESULTS**

**Effect of long term treatment with ARFHI on hepatic and renal function markers**

Fig 4.0 and Table 4.0 show the levels of hepatic and renal functional markers respectively in all the experimental groups of rats. Diabetic rats showed elevated activities of SGOT, SGPT and ALP. Renal function markers such as urea and creatinine in plasma were also increased in the diabetic rats when compared to normals. Treatment of diabetic rats with the ARFHI at a dose of 750 mg/kg bw for 28 days resulted in a significant reduction in their blood urea and creatinine in diabetic treated group. In addition the treatment also significantly reduced the activities of SGOT, SGPT and ALP. Similar effects were observed with glibenclamide, but they were less in magnitude when compared to those with ARFHI. There were no significant changes in the levels of hepatic and renal function markers in the normal treated rats.
Fig. 4.0 Effect of the ARFHI on SGOT, SGPT and ALP in normal and experimental diabetic rats. Values are given as mean ± S.D.

Table 4.0 Effect of ARFHI on the plasma urea and creatinine levels of the normal and diabetic rats after 28 days treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
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<tbody>
<tr>
<td>1</td>
<td>33.83±5.63</td>
<td>0.400±0.141</td>
</tr>
<tr>
<td>2</td>
<td>29.00±3.16</td>
<td>0.466±0.121</td>
</tr>
<tr>
<td>3</td>
<td>85.66±3.55</td>
<td>1.200±0.141</td>
</tr>
<tr>
<td>4</td>
<td>48.00±3.16</td>
<td>0.900±0.141</td>
</tr>
<tr>
<td>5</td>
<td>54.08±1.36</td>
<td>1.000±0.357</td>
</tr>
<tr>
<td>F value</td>
<td>225.67</td>
<td>17.79</td>
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<tr>
<td>P value</td>
<td>0.000</td>
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Values are given as mean ± S.D from six rats in each group. Values not sharing a common superscript letter differ significantly at p < 0.01 (DMRT).
Histopathology

Histological findings in pancreas, liver and kidney in normal rats, diabetic rats and diabetic rats treated with the ARFHI and glibenclamide are given below.

PANCREAS

**Fig 4.1 & 4.2** are the photomicrographs (10x and 40x respectively) of the pancreas of a normal rat showing the normal architecture and normal islets of Langerhans. **Fig 4.3 & 4.4** are the photomicrographs (10x and 40x respectively) of the pancreas of diabetic untreated rats. **Fig 4.5 & 4.6** are the photomicrographs (10x and 40x respectively) of the pancreas of a normal rat treated with ARFHI, which shows the normal architecture as in the normal rats. **Fig 4.7 & Fig 4.8** are the photomicrographs (10x and 40x respectively) of the pancreas of diabetic rats treated with ARFHI Insulitis with lymphocytic infiltrations, atrophy and destruction of β-cells were markedly seen in the pancreas of diabetic rats. Regenerative changes in tissue architecture of pancreas were observed in the pancreas of diabetic rats treated with ARFHI by recovery of the damaged islets and an improvement in number of beta cells. **Fig 4.9 & Fig 4.10** are the photomicrographs (10x and 40x respectively) of pancreas diabetic rat treated with glibenclamide showing regenerative changes in tissue architecture.

LIVER

**Fig 4.11 & 4.12** are the photomicrographs (10x and 40x respectively) of the liver of a normal rat showing the normal hepatic architecture with normal central vein, prominent nucleus and normal hepatocytes. **Fig 4.13 & 4.14** are the photomicrographs (10x and 40x respectively) of the liver of diabetic untreated rats, which show degenerative liver with severe congestion of central vein, hemorrhages in the sinusoidal spaces and granular appearance of the hepatocytes (degenerative change) with cloudy swelling (hazzy nucleus). **Fig 4.15 & 4.16** are the photomicrographs (10x and 40x respectively) of the liver of a normal rat treated with ARFHI, which show the normal architecture similar to normal rats. **Fig 4.17 & 4.18** are the photomicrographs (10x and 40x respectively) of the liver of diabetic rats treated with ARFHI, showing normal liver architecture with slight congestions in central vein, normal sinusoidal spaces and normal hepatocytes. **Fig 4.19 & Fig 4.20** are the photomicrographs (10x and 40x respectively) of the liver of diabetic rats treated with glibenclamide showing regenerative changes in tissue architecture.
respectively) of the diabetic rats treated with glibenclamide showing normal liver architecture with slight congestions in central vein, normal sinusoids and normal hepatocytes.

**KIDNEY**

**Fig 4.21 & 4.22** are the photomicrographs (10x and 40x respectively) of the kidney of a normal rat showing normal architecture of kidney with normal glomeruli and normal tubular epithelial cells. **Fig 4.23 & 4.24** are the photomicrographs (10x and 40x respectively) of the kidney of diabetic untreated rats showing atrophy of the glomeruli, necrotic tubular epithelial cells and dark pyknotic nuclei. Hemorrhage was seen with in the Bowman’s space due to glomerular damage. There was degeneration of glomeruli with wider Bowmen’s spaces and diffuse vacuolation of the tissues. **Fig 4.25 & 4.26** are the photomicrographs (10x and 40x respectively) of the kidney of a normal rat treated with ARFHI, which show the normal architecture similar to normal rats. **Fig 4.27 & Fig 4.28** are the photomicrographs (10x and 40x respectively) of the kidney of diabetic rats treated with ARFHI showing normal glomeruli, normal intertubular vessels and tubular epithelial cells indicating regenerative changes in rats treated with ARFHI. **Fig 4.29 & Fig 4.30** are the photomicrographs (10x and 40x respectively) of the diabetic rats treated with glibenclamide showing similar changes as in those treated with ARFHI.

**Evaluation of the acute toxicity of ARFHI in normal rats**

The various observations showed the normal behavior of the treated rats. No toxic effects were observed even at the dose of 3000 mg ARFHI/kg bw. Hence there were no lethal effects in any of the groups, treated with the highest dose of ARFHI.
EC = ENDOCRINE
EX = EXOCRINE
SC = SEROUS CELLS
CC = CENTROACINAR CELLS
Fig. 4.3: Diabetic rat pancreas 10X

Fig. 4.4: Diabetic rat pancreas 40X

NC = NECROTIC CHANGE
V = VACUOLIZATION
C = CONGESTION
V = VACUOLIZATION
PANCREAS

Fig. 4.5: ARFHI treated normal rat pancreas 10X

Fig. 4.6: ARFHI treated normal rat pancreas 40X

EC = ENDOCRINE
EX = EXOCRINE
SC = SEROUS CELLS
CC = CENTROACINAR CELLS
PANCREAS

Fig. 4.7: ARFHI treated diabetic rat pancreas 10X

Fig. 4.8: ARFHI treated diabetic rat pancreas 40X

EC = ENDOCRINE
EX = EXOCRINE

EC = ENDOCRINE
SC = SEROUS CELLS
Fig. 4.9: Glibenclamide treated diabetic rat pancreas 10X

Fig. 4.10: Glibenclamide treated diabetic rat pancreas 40X

EC = ENDOCRINE

SC = SEROUS CELLS
<table>
<thead>
<tr>
<th>LIVER</th>
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<tr>
<td><img src="image1" alt="Fig. 4.11: Normal rat liver 10X" /></td>
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</table>

H = HEPATOCYTE  
CV = CENTRAL VEIN
Fig. 4.13: Diabetic rat liver 10X

Fig. 4.14: Diabetic rat liver 40X

C = CONGESTION
NC = NECROTIC CHANGE
LIVER

Fig. 4.15: ARFHI treated normal rat liver 10X

Fig. 4.16: ARFHI treated normal rat liver 40X

CV = CENTRAL VEIN
S = SINUSOIDS

BD = BILLARY DUCT
H = HEPATOCYTE
LIVER

Fig. 4.17: ARFHI treated diabetic rat liver 10X

Fig. 4.18: ARFHI treated diabetic rat liver 40X

S = SINUSOIDS
CV = CENTRAL VEIN
BD = BILLYARY DUCT
**LIVER**

<table>
<thead>
<tr>
<th>Fig.4.19: Glibenclamide treated diabetic rat liver 10X</th>
<th>Fig.4.20: Glibenclamide treated diabetic rat liver 40X</th>
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<tr>
<td><img src="image1.png" alt="Image 1" /></td>
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CV = CENTRAL VEIN  
H = HEPATOCYTE  
S = SINUSOIDS  
CV = CENTRAL VEIN
# KIDNEY

<table>
<thead>
<tr>
<th>Fig. 4.21: Normal rat kidney 10X</th>
<th>Fig. 4.22: Normal rat kidney 40X</th>
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<tbody>
<tr>
<td>BC = BOWMAN'S CAPSULE</td>
<td>BC = BOWMAN'S CAPSULE</td>
</tr>
<tr>
<td>RT = RENAL TUBULE</td>
<td>PN = PICNOTIC NUCLEI</td>
</tr>
</tbody>
</table>

"BC" = Bowman's Capsule
"RT" = Renal Tubule
"PN" = Picnotic Nuclei
DBC = DISTRACTIVE BOWMAN’S CAPSULE
C = CONGESTION
KIDNEY

Fig. 4.25: ARFHI treated normal rat kidney 10X

Fig. 4.26: ARFHI treated normal rat kidney 40X

BC = BOWMAN’S CAPSULE

D = DISTAL

191
<table>
<thead>
<tr>
<th>Fig.4.27: ARFHI treated diabetic rat kidney 10X</th>
<th>Fig.4.28: ARFHI treated diabetic rat kidney 40X</th>
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<tbody>
<tr>
<td>BC = BOWMAN’S CAPSULE</td>
<td>PN = PICNOTIC NUCLEI</td>
</tr>
<tr>
<td>RT = RENAL TUBULE</td>
<td>BC = BOWMAN’S CAPSULE</td>
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**KIDNEY**
KIDNEY

Fig. 4.29: Glibenclamide treated diabetic rat kidney 10X

RT = RENAL TUBULE
BC = BOWMAN’S CAPSULE

Fig. 4.30: Glibenclamide treated diabetic rat kidney 40X

G = GLOMERULI
BC = BOWMAN’S CAPSULE
DISCUSSION

Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include measurement of activity of serum aminotransferases, alkaline phosphatase (ALP), bilirubin and albumin levels and prothrombin time. Aminotransferases, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. Alkaline phosphatase (ALP), acts as a marker of biliary function and cholestasis. The elevated levels of aminotransferases are mainly due to acute viral hepatitis, ischemic hepatitis, or drug or toxin-induced liver injury.

Measurement of activities of aminotransferases (AST and ALT) and ALP is of clinical and toxicological importance, as changes in their activities are indicative of tissue damage by toxicants or in disease conditions (Singh et al., 2001). Bopanna et al., (1997) and Eskander et al., (1995) demonstrated that the administration of several herbal extracts could restore the changes in the activities of serum enzymes like alkaline phosphatase (ALP), acid phosphatase and transaminases (AST and ALT).

Serum ALT, AST and ALP levels were determined to evaluate the hepatic functions (Degirmenchi et al., 2002). The increase in aminotransferases levels may be due to the cellular damage in the liver caused by STZ-induced diabetes. Although ALT is also present in mitochondria and cytosol, the mitochondrial form is low in activity and is very unstable. The detailed mechanism by which enzymes are released from the cytosol and mitochondria of hepatocytes is not completely known. Experimental studies have shown that subtle membrane changes are sufficient to allow passage of intracellular enzymes to the extracellular space (Garella et al., 1997). Very large concentration gradient between the hepatocytes and the sinusoidal space usually exists for enzymes. Cell damage increases permeability causing cytosolic isoenzymes to spill into the sinusoids and from there into the peripheral blood (Garella et al., 1997).

In the present study a significant increase in the activities of serum AST, ALT and ALP were observed in diabetic untreated rats compared to normal control rats. Yanardag et al., (2005) and Rajasekaran et al., (2006) reported that in the STZ diabetic rats, the levels of SGOT and
SGPT activities were significantly increased. Shinde and Goyal (2003), also reported that STZ induced an elevation of serum level of hepatic enzymes in diabetic rats, indicating the hepatotoxic effect of Streptozotocin. ALT and AST are directly associated with the conversion of amino acids to ketoacids and the increased protein catabolism accompanying gluconeogenesis and urea formation that are seen in diabetic state might be responsible for the elevation of these aminotrasferases.

In our study, treatment of diabetic rats for 28 days with ARFHI lowered the serum AST, ALT and ALP activities in diabetic rats. Our findings are in agreement with those of Prakasam et al., (2004). ARFHI has protective effect against liver toxicity caused by STZ. Treatment with ARFHI in normal rats for 28 days did not produce any changes in the activities of these enzymes indicating no hepatotoxicity of ARFHI. When compared with glibenclamide, ARFHI could produce higher reducing effect on the SGOT, SGPT and ALP activities in the diabetic rats.

In this study elevated levels of blood urea and creatinine were observed in diabetic untreated rats, which are considered as significant markers of renal dysfunction (Bethesda et al., 2001). In diabetic subjects negative nitrogen balance with enhanced tissue proteolysis and decreased protein synthesis can contribute to increased serum urea and creatinine levels, indicating impaired renal functions in diabetic animals (Jensen et al., 1981). After the treatment with ARFHI a significant reduction in the levels of urea and creatinine were observed in the diabetic treated rats. It indicates that ARFHI is preventing the renal damage in diabetic rats and the protective effect of ARFHI on the kidneys is higher than glibenclamide.

Kaleem et al., (2008) reported that oral administration of Annona squamosa extract for 30 days significantly lowered blood urea, uric acid, creatinine levels and the activities of AST, ALT and ALP in STZ-diabetic rats. Can et al., (2004) proposed the use of Aloe gel extract in preference to Aloe pulp extract because aloe pulp extract have the tendency to decrease ALT but increases ALP. Both ALP and ALT activities were decreased upon treatment with aloe gel extract. Ramesh and Pugalendi (2006) reported that treatment with umbelliferone (7-hydroxycoumarin) decreased the activities of AST, ALT and ALP in STZ induced diabetic rats, by its insulin secretory and antioxidant properties. Atef et al., (2007) reported that administration of ginger and clove oils together or individually significantly decrease the liver function markers (AST, ALT and ALP activities) and renal function markers (Creatinine, urea and uric acid levels)
in Streptozotocin induced diabetic rats. Das and Sarma (2009) reported the hepatoprotective activity of the ethanolic extract of the pulp of 
*Eugenia Jambolana* (Jamun) in albino rats. The improvement in the renal and hepatic functions after treatment with ARFHI in diabetic rats could be due to its beneficial effects on insulin secretion, glycemic control and oxidative stress in diabetes.

**Histopathological studies**

The histological sections of the pancreas, liver and kidney tissues were observed to know the effect of ARFHI fed in non-diabetic and diabetic rats. This was done to observe any protective or harmful effect of ARFHI on non-diabetic and diabetic rats.

The decrease in cellularity within islets of Langerhans observed in diabetic rats in the present study reflects the cytotoxicity of Streptozotocin (Szkudelski et al., 2001). Streptozotocin destroys β-cells selectively and a single adequate dose produces long lasting hyperglycemia and insulin deficiency (Szaleczky et al., 1999). Previous studies have reported that Streptozotocin enters the β-cells via a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of Streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of NAD⁺ and ATP. Enhanced ATP dephosphorylation after Streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are generated. Furthermore, Streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the Streptozotocin action, β-cells undergo destruction by necrosis (Szkudelski 2001). Other studies indicated that cytotoxic effects of Streptozotocin are dependent upon DNA alkylation by site-specific action with DNA bases (Benneth and Pegg, 1981) and by free-radical generation during Streptozotocin metabolism (Bolzan and Bianchi, 2002).

In the present study β-cells in some islets were found to be fusiform. The change in the shape of cells can be attributed to the partial damage caused by Streptozotocin. Aybar et al., (2001) have reported that use of lower dose of Streptozotocin produced an incomplete destruction of pancreatic β-cells even though rats became permanently diabetic. The regeneration
of the β-cells of the STZ-destructed islets upon treatment with AR FHI, is probably due to the fact that pancreas contains stable (Quiescent) cells which have the capacity of regeneration. Therefore, the surviving cells can proliferate to replace the lost cells (Kumar et al., 1992).

The changes in the liver in diabetic rabbits induced by Streptozotocin have been reported earlier (Mitra et al., 1996). The diabetic liver showed degeneration and congestion. In diabetes, degradation of liver glycogen and gluconeogenesis are increased while glucose utilization is inhibited. Increased levels of glucose 6-phosphatase in the liver, facilitating glucose release into the blood. As a result, the liver continues to produce glucose even with severe hyperglycemia. Under these circumstances the normal liver would shut off to deposit glycogen (Sherlock and Dooley, 1993).

Hamilton, (1987) indicated that diabetes mellitus is one of the most common causes of fatty liver where fats are accumulated in the hepatocytes; he indicates that fat comprises as much as 40% of liver weight in patients with diabetes mellitus (It is 5% in normal liver). Herman et al., (1999) related the hepatomegaly observed in STZ-induced diabetes in rats to the hyperplasia in early phase and to the decreased apoptosis in the later stage. Itho et al., (1979) have considered that fatty infiltration of liver as a precursor of cirrhosis in diabetic patients. Falchuk et al., (1980) showed hepatic fatty steatosis and pericentral fibrosis in diabetic patients. They recognized that hepatocytes were markedly swollen and suggested that these abnormalities may represent an intermediate lesion between fatty steatosis and cirrhosis. Nanji et al., (1986) mentioned that the damage was mainly to the plasma membrane of the hepatocytes and could be attributed to the elevation in aspartate aminotransferases in patients with fatty infiltrations.

In the present study Sinusoidal haemorrhages, Vasculations in the hepatocytes (fatty changes), Granular appearance of the hepatocytes (degenerative change) and cloudy swelling (hazy nucleus) and inflammation were noticed in the liver of diabetic rats. Our histological findings are in agreement with the degenerative structural changes reported in liver tissues as a result of insulin depletion (Can et al., 2004) in neonatal STZ (100 mg/kg) - induced Type-2 diabetic rat models. Can et al., (2004), observed an increase in degeneration in central veins to portal veins, excess vacuolization, granular appearance in the cytoplasm, dilations in the
sinusoids and moderate hyperemia. These changes were reduced in ARFHI-fed rats. This may be due to beneficial and protective effect of ARFHI on liver tissues of diabetic rats.

The main function of the kidneys is to excrete the waste products of metabolism and to regulate the body concentration of water and salt. Histologically STZ-diabetic control rats showed marked multifocal clarifications and vacuolations in their kidneys. Moreover, it has been reported that Streptozotocin does not possess any significant nephrotoxic potential (Floretto et al., 1998). All structural changes in kidneys resulting from STZ administration in rats can thus be attributed to altered metabolism in diabetes (Rasch et al., 1980). In a study reported by Bolkent et al., (2004) in the neonatal STZ (100 mg/kg STZ)-induced Type-2 diabetes, alteration in the structural integrity of the apical membrane of proximal tubules of the kidney tissue in the diabetic rats was observed. Normoglycaemia in diabetic rats with ARFHI treatment in this study could ameliorate the glomerular and tubular lesions that characterize diabetic nephropathy. The improvement of renal morphology and function in STZ diabetic rats after treatment with ARFHI in the present investigation could be attributed to its antidiabetic action resulting in alleviation of altered metabolic status in animals. However, the excellent recovery of renal function expected with treatment of ARFHI can be explained by the regenerative capability of the renal tubules (Kissane et al., 1985).

Tissue sections of healthy rats treated with ARFHI showed no pathological changes in pancreas, liver and kidney and were comparable to those of normal control rats. The standard, glibenclamide treated group also showed recovery and tended to approach the histology of the normal rats but less regenerative efficacy when compared to treatment with ARFHI was observed.

The biochemical and histological observations in the normal treated and diabetic treated rats coincide with the results of the acute toxicity study with ARFHI confirming its non toxic nature.
CONCLUSIONS

From the above results it is very clear that treatment of diabetic rats with ARFHI has significant beneficial effects on histological changes caused by diabetes mellitus. The regenerative changes in tissue architecture of pancreas, liver and kidney could be due to the effect of ARFHI on insulin secretion, glycemic control, carbohydrate and lipid metabolisms, oxidative stress and antioxidant enzyme activities in the diabetic rats treated with ARFHI for 28 days. Oral administration of ARFHI in normal rats did not show any signs of hepatic and renal toxicity. In diabetic rats the raised levels of serum urea, creatinine and the increased activities of AST, ALT and ALP were significantly reduced after treatment with ARFHI. This suggests the protective role of ARFHI against the damage of pancreas, liver and kidney during diabetes.
SUMMARY AND CONCLUSION

The results obtained from the various studies conducted on the alkaloid rich fraction of *Heliotropium indicum* (ARFHI) are summarized below.

- Checking of crude aqueous suspension of *Heliotropium indicum* whole plant at a dose of 500 mg/kg/bw has produced significant (46%) antihyperglycemic activity at 6th hour of the treatment in STZ induced diabetic rats.

- In the increasing order of polarity different solvent (Hexane, Ethyl acetate, Methanol and aqueous) extracts of *Heliotropium indicum* were prepared and screened for antihyperglycemic activity. Among these methanol extract and aqueous extract each at a dose of 500 mg/kg bw showed significant antihyperglycemic activity (31.5% and 46.8% fall in blood glucose respectively) at 6th hour of the treatment in STZ induced diabetic rats.

- Alkaloid rich fraction of *Heliotropium indicum* (ARFHI) was prepared and screened the different doses of ARFHI for its antidiabetic activity in STZ induced diabetic rats. After 6th hour of the oral administration of ARFHI at a dose of 750 mg/kg bw, 60% fall in blood glucose was observed which is much higher that of glibenclamide (a standard drug) which has caused only 39% fall in the blood glucose. None of the doses could produce hypoglycaemic activity in normal rats.

- Acute toxicity of ARFHI was evaluated in healthy male wistar albino rats, according to the OECD guidelines. The various observations showed normal behaviour of the treated rats. No toxic effects were observed even at the dose of 3000 mg ARFHI/kg bw. There were no lethal effects in any of the groups, treated with the highest dose of ARFHI.

- Phytochemical analysis revealed the presence of steroids, alkaloids, triterpenes, saponins and tannins in *Heliotropium indicum* whole plant.

- Long term treatment of diabetic rats with the ARFHI at a dose of 750 mg/kg bw for 28 days resulted in 61% reduction in FBG levels, with significant improvement in glycemic control (HbA1c).
Treatment with ARFHI prevented the loss of body weights and enhanced the hepatic glycogen levels in diabetic rats reflecting the efficacy of ARFHI in the maintenance of normal carbohydrate metabolism.

Treatment with ARFHI produced a significant rise in plasma insulin levels in diabetic rats, indicating the insulin secretagogue property of the ARFHI.

The activities of carbohydrate metabolising enzymes hexokinase, Glucose 6-phosphate dehydrogenase were significantly increased where as those of gluconeogenic enzymes glucose 6-phosphatase and fructose 1,6-bisphosphatase were significantly decreased in liver and kidney of diabetic rats after treatment with the ARFHI.

Treatment with ARFHI in diabetic rats resulted in a reversal of the increased quantities of the carbohydrate moieties of glycoproteins and glycosaminoglycans to near normal levels in plasma, liver and kidney.

The treatment with ARFHI produced significant antihyperlipidemic activity in diabetic rats by decreasing the elevated levels of serum TG, total, LDL and VLDL cholesterol and by increasing HDL cholesterol. It also exhibited inhibitory effect on HMG-CoA reductase, the key regulatory enzyme in cholesterol biosynthesis.

The long term treatment of diabetic rats with ARFHI reduced the oxidative stress as indicated by decreased levels of TBARS and CAT activity. It also increased the activities of enzymatic (SOD, GPx and GST) antioxidants and concentration of non enzymatic (GSH, Vitamin C and Vitamin E) antioxidants in plasma and tissues reflecting the antioxidant efficacy of the ARFHI.

Administration of ARFHI to normal rats did not show any signs of hepatic and renal toxicity, and in diabetic rats it decreased the raised levels of serum urea, creatinine and the activities of AST, ALT and ALP. This suggests the protective role of ARFHI against liver and kidney damage during diabetes.

Histological studies have shown the following
a. In diabetic untreated rat pancreas there was insulitis with lymphocytic infiltrations. Atrophy and destruction of β- cells were marked. The regenerative changes in tissue architecture of pancreas were observed in diabetic rat pancreas treated with ARFHI.

b. Diabetic untreated rats showed degenerative liver with severe congestion of central vein, haemorrhages in the sinusoidal spaces and granular appearance of the hepatocytes (degenerative change) with cloudy swelling (hazzy nucleus). Treatment with ARFHI in diabetic rats showed normal liver architecture with slight congestion in central vein, normal sinusoidal spaces and normal hepatocytes.

c. The kidney of diabetic untreated rats showed atrophy of glomeruli, necrotic tubular epithelial cells and dark picnotic nuclei. The kidney of diabetic rats treated with ARFHI showed normal glomeruli, normal inter tubular vessels and tubular epithelial cells indicating regenerative changes.

From all these observations it is concluded that the alkaloid rich fraction of *Heliotropium indicum* possesses significant antidiabetic activity. The antidiabetic activity of the ARFHI could be due to its stimulatory effect on insulin secretion resulting in improvement in the altered activities of carbohydrate metabolising enzymes, lipid metabolism and antioxidant defence system in the diabetic treated rats.