CHAPTER 6

DISCUSSION

Oxidative stress is the imbalance between intracellular production of free radicals and the cellular defense mechanism. Increase in lipid peroxides or ROS (Reactive oxygen species) occurs in diabetes. Free radicals like superoxide anion & hydroxyl radical exert their toxic effects by acting on DNA, membrane proteins, and lipids. Liver enzymes SGOT, SGPT, ALP are present in high concentration in the normal hepatocytes of the liver, and these enzymes are leaked into the circulation as a result of damage to cell membrane of hepatocytes [133].

ACD improves the liver function by decreasing the levels of SGOT, SGPT in diabetic treated rats, indicating its hepatoprotective effect. ALP acts as a marker of Biliary function [98]. Reduction in ALP levels in ACD treated diabetic rats further validates its hepatoprotective effect. Treatment of normal rats with ACD maintained the levels of hepatic enzymes thereby showing its non-toxic nature. Histopathological examination of liver sections of STZ diabetic rats showed marked hepatocytes necrosis, fatty degeneration, and extensive vacuolization and distorted liver structure. Treatment with ACD restored the normal architecture of liver tissue in STZ diabetic rats, thereby proving its hepatoprotective role. No changes were found in the liver histopathology of normal rats treated with ACD, indicating its non-toxic nature. Treatment with glibenclamide also restored the near normal architecture of liver tissue in STZ diabetic rats, but showed the presence of vascular congestion of the central vein and few hepatocytes nuclei vacuolization.

In this study, a marked decrease in the activities of Catalase (CAT), Superoxide dismutase (SOD) and glutathione Peroxidase (GPX) in the liver of untreated diabetic rats were observed. The functions of these antioxidant enzymes are interconnected and a decrease of their activities results in the accumulation of Lipid peroxides and increases oxidative stress in diabetic rats. Treatment of rats with ACD ameliorated the STZ induced
decrease in the activities of GPx, SOD and CAT, which lead to the support of antioxidant properties of C. dactylon as demonstrated in both Invitro and in vivo models.

In this study, levels of hepatic enzymes were elevated in the diabetic group showing damage of hepatocytes, by means of elevation of endotoxins which pass through the intestinal wall into the portal blood, thereby enter into the liver, these endotoxins stimulate Kupffer cells to produce reactive oxygen species (ROS) and pro-inflammatory cytokines such as TNF-α and IL-1β, both cytokines are important mediators in inflammation, leading to cell death [134]. The significant decrease in the levels of hepatic enzymes observed in the present study, when treated with aqueous extract of Cynodon dactylon in diabetic group, exhibited its protective effect in the liver tissue, which indicates the membrane stabilizing action of the extract, which further prevented leakage of intracellular hepatic enzymes.

Histopathological observation has shown the restoration of normal liver morphology in the Cynodon dactylon treated diabetic rats, regeneration of reticular fibres as well as reduction in the collagen fibres in the liver section of the extract treated diabetic rats. In the diabetic condition, hepatic stellate cells are activated, resulting in increased accumulation of collagen fibres in the hepatic stellate cells [135]. After activation of hepatic stellate cells, matrix metalloprotease 2 (MMP-2) and tissue inhibitor metalloprotease 1, 2 (TMMP-1, 2) increased with simultaneous decrease in matrix metalloprotease 1 (MMP-1). The elevated MMP-2 activates the breakdown of collagen fibres type III & IV in the normal liver, elevated TMMP-1, 2 and decreased MMP-1, inhibit the degradation of collagen type I fiber in the scar tissue, consequently the accumulation of numerous collagen fibres may lead to liver fibrosis and cirrhosis [136].

The Phytochemicals like flavonoids, polyphenols present in the Cynodon dactylon extract might have inhibited the free radical generation process and mopped up circulating free radicals causing histological lesions and complications of Diabetes, thereby resulting in the regeneration of hepatic cells. The Phytochemical present in the Cynodon dactylon may be responsible for the protective effect of liver tissue of streptozotocin diabetic induced liver damage in wistar rats, possibly through their anti-oxidant activity.
Diabetes mellitus is associated with progressive metabolic derangement, worsening glycaemic control and morphological changes in the pancreas, liver and other organs. Oxidative stress is known to play a significant role in the induction of these processes. Pancreatic insulin reserve is an important parameter of islet function with tight coupling between insulin secretion and production being necessary for adequate functioning of pancreatic $\beta$-cells. Insulin deficiency in diabetes mellitus leads to accumulation of lipids especially TG and TC in diabetic patients [96]. High concentration of cholesterol in human serum is one of the primary factors in the development of atherosclerosis. Marked hyperlipidaemia in diabetic state may be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depot. Further in diabetic state, there is inactivation of lipoprotein lipase by which free fatty acids are converted into phospholipids and cholesterol, which are finally discharged into blood, causing an elevation of serum phospholipids levels.

Induction of diabetes with STZ was related with the reduced rate of body weight gain and the increased food and water intakes. Treatment with ACD prevented the reduction in body weight in diabetic rats, which also lowered food and water intakes. Body weight is one of the general indicators of metabolic regulation for diabetes; Gluconeogenesis in cells is stimulated to compensate for the reduced level of glucose, which results in a decrease in the body weight.

Induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to increased muscle wasting [137] and due to loss of tissue proteins [138]. Diabetic rats treated with ACD showed improvement in body weight as compared to the diabetic control, which may be due to its protective effect of controlling muscle wasting, i.e., reversal of gluconeogenesis and may also be due to the improvement in insulin secretion, thereby possible glycaemic control.

DM, Characterized by persistent hyperglycemia caused by any of the several possible causes, is the most prominent disorder related to a failed blood sugar regulation. There was a significant elevation in the serum glucose in the diabetic control rats compared to the control group. An increase in the water intake was necessary to depress the elevation of the osmotic pressure due to the elevated blood glucose level. The Antihyperglycaemic effect of the ACD may be due to the enhancement of the Insulin effect
of Plasma caused by increased pancreatic secretion of insulin from the insulin-secreting \( \beta \) cells or release of bound insulin.

Mammalian cells are constantly exposed to ROS as a result of normal metabolic process occurring during aerobic respiration. STZ is taken up by the pancreatic \( \beta \) cells via glucose transporter GLUT-2. The main cause of STZ induced \( \beta \) cell death is via formation of extensive poly ADP ribose, depletion of cellular nicotine adenine dinucleotide and ATP and generation of ROS [139]. Diabetes arises from irreversible destruction of the \( \beta \) islet cells of the islets of langerhans in the pancreas, causing deregulation or insulin reduction [140].

The pancreas of ACD treated diabetic rats showed improved islet morphology; and the pancreatic islets of the ACD treated diabetic rats showed significant increase in the insulin secreting \( \beta \) – cells. Therefore, the antihyperglycaemic activity of ACD may be due to the substances present in the ACD which stimulate insulin secretion and / or protect the intact functional \( \beta \) – cells from STZ– induced destruction.

Glycosylated haemoglobin levels were found to be increased in the untreated diabetic control group. Increased non-enzymatic and auto-oxidative glycosylation is one of the possible mechanisms linking hyperglycemia and the vascular complications of diabetes [141]. Diabetic rats showed higher levels of Glycated haemoglobin indicating their poor glycaemic control. Treatment with ACD showed significant decrease in Glycosylated haemoglobin levels, which could be due to an improvement in insulin secretion.

In this study, 45 days of treatment with aqueous extract of \( C.dactylon \) at a dose of (500 mg/kg, body weight) had significantly reduced the fasting blood glucose levels and increased plasma insulin in STZ –induced diabetic rats. Blood glucose lowering potential of aqueous extract of \( C.dactylon \) might be due to activation of \( \beta \)- cells giving insulinogenic effect through the stimulation of regeneration process and reactivation of \( \beta \)- cells, also the presence of flavonoids in the extract may be responsible for the stimulation of glucose uptake in peripheral tissues and regulation of activity and/or expression of the rate limiting enzymes involved in carbohydrate metabolism [142]. In the present study, treatment with aqueous extract of \( C.dactylon \) at a dose of 500 mg/kg, bodyweight, markedly decreased serum TC, TG and LDL levels by 57%, 61%, and 75%, as high levels of LDL cholesterol
predispose to atherosclerotic state. HDL levels were also significantly increased by more than 80% in *C. dactylon* aqueous extract treated rats. These results were similar to those reported by many authors on medicinal plants, which exhibited antihyperlipidemic activity.

The biochemical findings of this study were correlated with the histopathological changes in the pancreatic islets of normal and experimental animals, which reveal that treatment of diabetic rats with 500 mg/kg, bodyweight of aqueous extract of *C. dactylon* showed substantial recovery of pancreatic islet architecture, which also showed improvement in mass and density of the islets. The observed histopathological findings were similar to the findings, reported by very few authors [78, 142]. Histological examination of Masson’s trichrome stained sections of STZ- induced diabetic rats showed increased collagen deposition mainly around pancreatic ductules; whereas collagen bundles were fairly similar to control rats in pancreatic sections of the *C. dactylon* treated STZ- induced diabetic rats. This increased collagen could be attributed to the chronic accompanying auto-immune reactions, as insulin dependent diabetes mellitus is characterized by the activation of autoimmunity towards β-cells.

In diabetic rats the aqueous extract of *C. dactylon* at a dose of 500 mg/kg, bodyweight exhibited significant hypoglycaemic and antihyperlipidemic effect, shown biochemically and histologically. The aqueous extract of *C. dactylon* exhibits ameliorative effects almost similar to the standard group (glibenclamide 5 mg/kg, bodyweight). Histopathological observations made in this study also warrant that aqueous extract of *C. dactylon* is effective in reducing the islet cellular toxicity induced by Streptozotocin (STZ).