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THE HISTO-PROTECTIVE EFFECTS OF THE ROOT EXTRACTS OF *SALACIA NITIDA* BENTH ON TISSUES OF ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Aim: This study was carried out to determine the histo-protective and regenerative effects seen on the liver, tissue and pancreas in alloxan induced diabetic rats after administration of *Salacia nitida* Benth. **Study design**: Experimental Animal Study. **Place and Duration of Study:** Department of Pharmacology, University of Port Harcourt, Rivers State from July 2016 to February 2017. **Methodology**: This study was carried out to investigate the potential tissue protective effects of the root extracts of *Salacia nitida* Benth in alloxan induced diabetic rats. Fifty five adult Wistar rats randomly divided into eleven groups of five rats each. The method of successive extraction was used. The anti-diabetic study was evaluated in twenty-one days, comprising of two phases: induction phase and treatment phase. On day 21 of treatment with *S. nitida* extracts, the animals were sacrificed and histological analysis carried out. **Results**: the histolgical examination of the kidney, liver and pancreas of alloxan induced diabetic rats indicated a protective effect after administration of the dicholoromethane extract with less diabetes induced degenerative damage seen. **Conclusion**: the dicholoromethane extract showed histoprotective effects as seen with the regeneration of damaged cells on exposure of the rats to alloxan.

KEYWORDS: Alloxan, *Salacia nitida*, histology, blood glucose, type I, type II.

1.0 INTRODUCTION

Diabetes mellitus (DM) also referred to as either type I or type II diabetes is described as a metabolic disease with a genetic component with associated endocrine disorders, persistent and or recurrent hyperglycaemia as a major characteristic.[1] Whether it is T1 or T2 diabetes, those affected are at greater risk of developing complications, thus the need to shield patients from the principal underlying feature, which is persistent hyperglycaemia. Prolonged hyperglycaemia predisposes the individual to significant morbidities associated with microvascular complications such as retinopathy, nephropathy and neuropathy which affect the eyes, kidneys and nerves respectively and macrovascular complications such as ischemic heart and peripheral vascular disease and stroke. These conditions are known to reduce the life expectancy and the quality of life of the affected individual. The American Diabetes Association (ADA) assessed the national expenditure on diabetes in the USA for 2002 to be $US 132 billion, increasing to $US 192 billion in 2020.[2]

Apart from fasting hyper-glycaemia, a few organs develop complications in diabetes. The conditions and main organs affected by diabetes are; glycogenosis and dextrinosis and steatohepatitis (liver), atherosclerosis and microangiopathy (blood vessels), retinopathy (eyes), nephropathy (kidneys), neuropathy (cerebrum and peripheral nerves).[3-5] These harmful effects are isolated into microvascular complications like in the kidneys, nerves and eye[6] and macrovascular in the blood vessels. Microvascular complications can be defined as retinopathy, which requires photocoagulation, vitreous haemorrhage, lethal or non-lethal renal failure.[7] Wallace (1999) states because macrovascular complications in diabetes affects the major arteries, its dreariness and mortality far exceed the dangers of microvascular complications in more advanced individuals with diabetes.[8] According to Turner et al (1996), the UK Prospective Diabetes study (UKPDS) observed that 9% T2DM patients developed microvascular disease after 9 years of follow-up, when compared with 20% for macrovascular complications.[9] In particular, the survey demonstrated the need for optimal control to prevent the development of microvascular and macrovascular complications. In all, patients with prolonged diabetes mellitus will present with microvascular or macrovascular degeneration.[10] The development and subsequent advancement of diabetic nephropathy occurs because of poor diabetic control, which over the years leads to the damage of the kidney because the filtration capacity of the kidney is lost.[11] Diabetic nephropathy can otherwise be called Kimmelstiel-Wilson syndrome, which is a disorder in which the albumin excretion is greater than 300mg in 24 hours (>300 mg/day) and is affirmed on no less than two events 3-6 months apart.

There is arterial hypertension and intercapillary glomerulonephritis which is perpetual and progressive impairment of the kidney function.[12] The liver is a vital organ in the regulation of blood glucose level. In cases of prolonged diabetes, the liver morphology and function are altered.[13] The presence of fat in the liver known as Non- Alcoholic Fatty liver increases the risks of both cardiovascular disease and type 2 diabetes. It was discovered that impaired insulin action for the most part result in non-alcoholic fatty liver disease, including steatosis and steatohepatitis.[14] In this century, diabetes has been regarded as one of the world's principal health emergencies with a higher prevalence among the world populace within ages 20 to 79 years having either type 1 or type 2 diabetes.[15] The primary undesirable impact of insulin is hypo-glycaemia.[16]

The main point of treatment in type II diabetes has dependably been to lower blood glucose levels utilizing several approaches, such as the use of Sulphonylureas which stimulate pancreatic islets cells to increase insulin, Metformin which modulate glucose production by slowing the process in the liver, Thiazolidines which enhances insulin activities, α-glucosidase inhibitors which interferes with gut glucose absorption and Insulin which lowers the production of glucose and enhances glucose uptake . Most of these therapeutic agents have been reported to induce tissue toxicity and thus hamper efficacy and tolerability; with the propensity to bring about increase in weight. A few of these drugs cause hypo-glycaemia and not very many address underlying defects, for example, weight gain and insulin. In this manner, there is requirement for more up to date therapeutic approaches.[17] Aside, from having a number of side effects, the orthodox oral hypoglycemic drugs have not successfully being able to manage nor control the long term complications (microvascular and macrovascular).[18-19] In this manner, there is requirement for more up to date therapeutic approaches.[17] Undesirable effects are also noticed among the classes of anti-diabetic drugs thus it is imperative that safer, more specific and more sustainably effective hypoglycaemic agents for diabetes treatment be developed.[20] Therefore, research on the prevention and treatment of diabetes and its complications has become a major public health issue. Furthermore, World Health Organization[21] has suggested that traditional plants be assessed for diabetes. Herbal medicines have been used by patients in diabetes and other disorders because they have fewer side effects and have the potential to impart therapeutic effects.[22] One of such plants is *Salacia nitida*. It is a genus of plants in the Celastraceae family. They are woody climbers actually found in tropical districts. A few species in this genus has been utilized in conventional medicine, for example, in the Ayurvedic system from India. Some of the chemical constituents from the root bark of this plant include but not restricted to polyphenols like salacinol, kotalanol and mangiferin.[23] A study indicated that the root extracts of *Salacia nitida* showed anti-diabetic properties in alloxan induced diabetic rats.[24] This study was aimed at investigating the histo-protective and regenerative effects seen on the liver, tissue and pancreas in alloxan induced diabetic rats after administration of extracts of *Salacia nitida* Benth.

2.0 MATERIALS AND METHOD

2.1 Plant material

The roots of *Salacia nitida* plant used in this study was collected fresh from a local area in Omuokiri of Aluu, Rivers, Nigeria. Its herbarium number is UPHCO288. It was then processed that is washed and reduced to smaller sizes and air –dried. It was then ground into powder.

2.2 Preparation of extracts

About 1850g of the powdered root was sequentially extracted by method as in.[24]

2.3 Animals

Fifty five (55) adult Wistar rats males and females which were obtained from the animal house of the University of Port Harcourt, were weighed (150-250g). The animals were kept for one week to become acclimatized to laboratory conditions before inception of the study. They were fed with standard pelletized feed (Premier Feed Mill Co. Ltd, Ibadan) and clean water was given to them under clean conditions. The animals were chosen randomly. The approval of the Animal Ethical Committee of University of Port Harcourt was sought before the start of the study. Every one of the protocols and investigations were consistent with the ethical standards and rules.

2.4 Hypoglycaemic Activity

2.4.1 Experimental induction of diabetes; Diabetes was induced in the animals after they were deprived of food for 12 hours (overnight fast) by an intraperitoneal injection of alloxan at 150 mg kg^{-1} b.wt which was solubilized using 100ml of saline solution before injection.[25] The animals were then fed after an hour. 72 hours after administration of the alloxan, blood samples were collected by the clipping of the tail and the blood glucose levels estimated. The control group received saline alone. For further experimental study, groups of 5 rats each were separated based on their blood glucose levels >150mg/dl.[26-27]

2.4.2 Experimental design

Fifty five (55) rats were divided into Eleven (11) groups of animals containing five rats each;

Group I: Saline (untreated/ normal control).

Group II: Hyperglycaemic rats- Diabetic control.

Group III: Hyperglycaemic rats + Glibenclamide (10mg/kg/day p.o. once daily).

Group IV: Hyperglycaemic rats + dicholoromethane extract of *Salacia nitida* root; low dose (250 mg/kg/b.wt p.o once daily).

Group V: Hyperglycaemic rats + dicholoromethane extract of *Salacia nitida* root; high dose (500 mg/kg/b.wt p.o once daily).

Group VI: Hyperglycemic rats + ethyl acetate extract of *Salacia nitida* root; low dose (250 mg/kg/b.wt p.o once daily).
Group VII: Hyperglycemic rats + ethyl acetate extract of *Salacia nitida* root; high dose (500mg/kg/b.wt p.o once daily).
Group VIII: Hyperglycemic rats + methanol extract of *Salacia nitida* root; low dose (250mg/kg/b.wt p.o once daily).
Group IX: Hyperglycemic rats + methanol extract of *Salacia nitida* root; high dose (500mg/kg/b.wt p.o once daily.
Group X: Hyperglycemic rats + aqueous extract of *Salacia nitida* root; low dose (250mg/kg/b.wt p.o once daily).
Group XI: Hyperglycemic rats + aqueous extract of *Salacia nitida* root; high dose (500mg/kg/b.wt p.o once daily).

Blood glucose levels were monitored by collecting blood into the test kit (Accu-Chek Active Test Meter, Roche Diagnostics). Blood was collected by making small incisions on the tails of the rats. The drug and extracts were administered for a period of 21 days using orogastric tube.

2.5 Histopathological Analysis
On day 21 of treatment with *S. nitida* extracts, the animals were sacrificed under ether anaesthesia, Histopathological analyses were then carried out to observe any liver, kidney and pancreas toxicity. The organs of choice were excised and preserved in 10% formalin solution at that point; the organs were dehydrated in graded ascending alcohol. Xylene was used to remove the alcohol for proper infiltration and embedding in paraffin block cassettes. Thin sections of the tissues in transverse axis were cut using microtome. Sections of the tissues were then deparaffinised and stained using Haematoxylin and Eosin (H&E) stain. They slides were then viewed light microscope to observe any structural changes in the right and left lobes of the liver, kidney and pancreas. Images of the slides were taken and recorded for comparison. This process was carried out by a histochemist and histopathologist.

2.6 Statistical Analysis
Statistical analysis of results was carried out making use of SPSS (Statistical Product and Service Solution), version 20.0. Thereafter, results were presented as Mean±Standard Error of Mean. ANOVA followed by the LSD (Least Significant Difference), was used to determine significant differences across the groups. The difference in the mean values of the groups was the criteria for the pharmacological activities and was considered significant at $p \leq 0.05$.

3.0 RESULTS

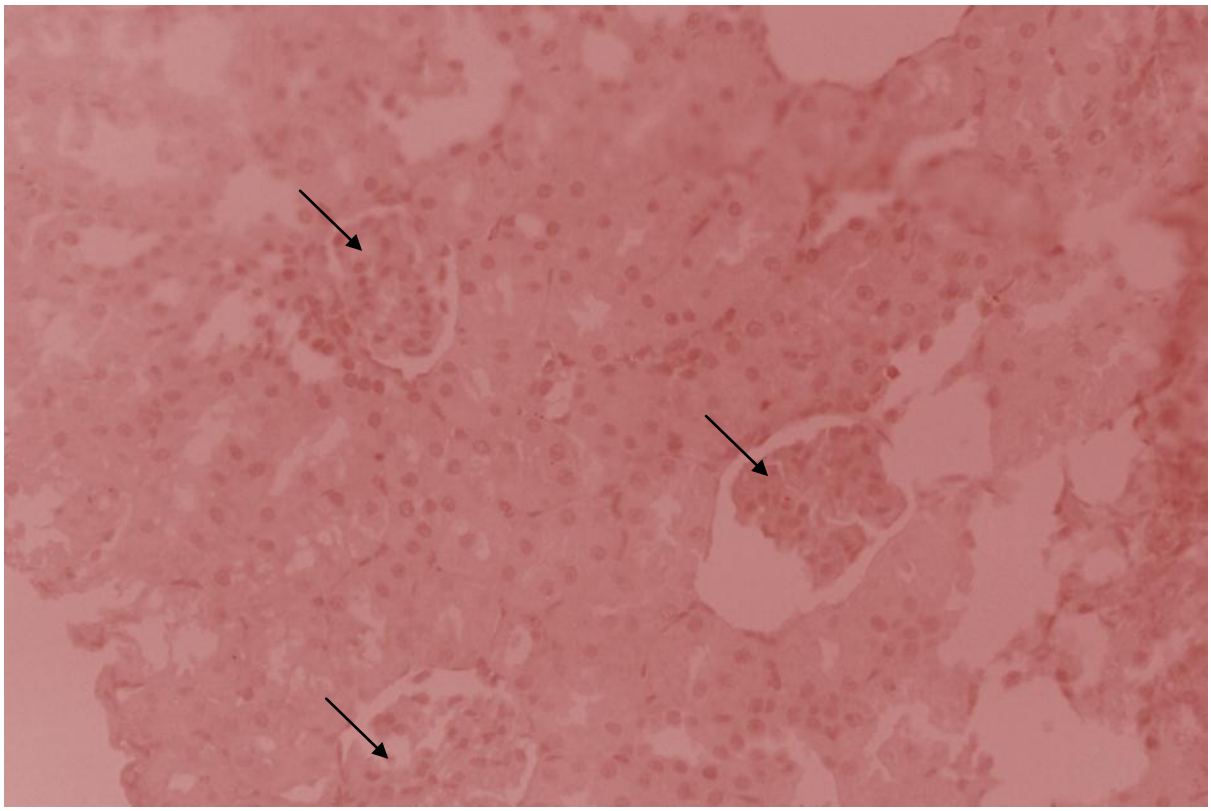

Fig. 1a: H&E section (x40) of untreated kidney showing diffuse mesangial sclerosis in the glomeruli (arrows) and foci of tubular necrosis consistent with diabetes mellitus.

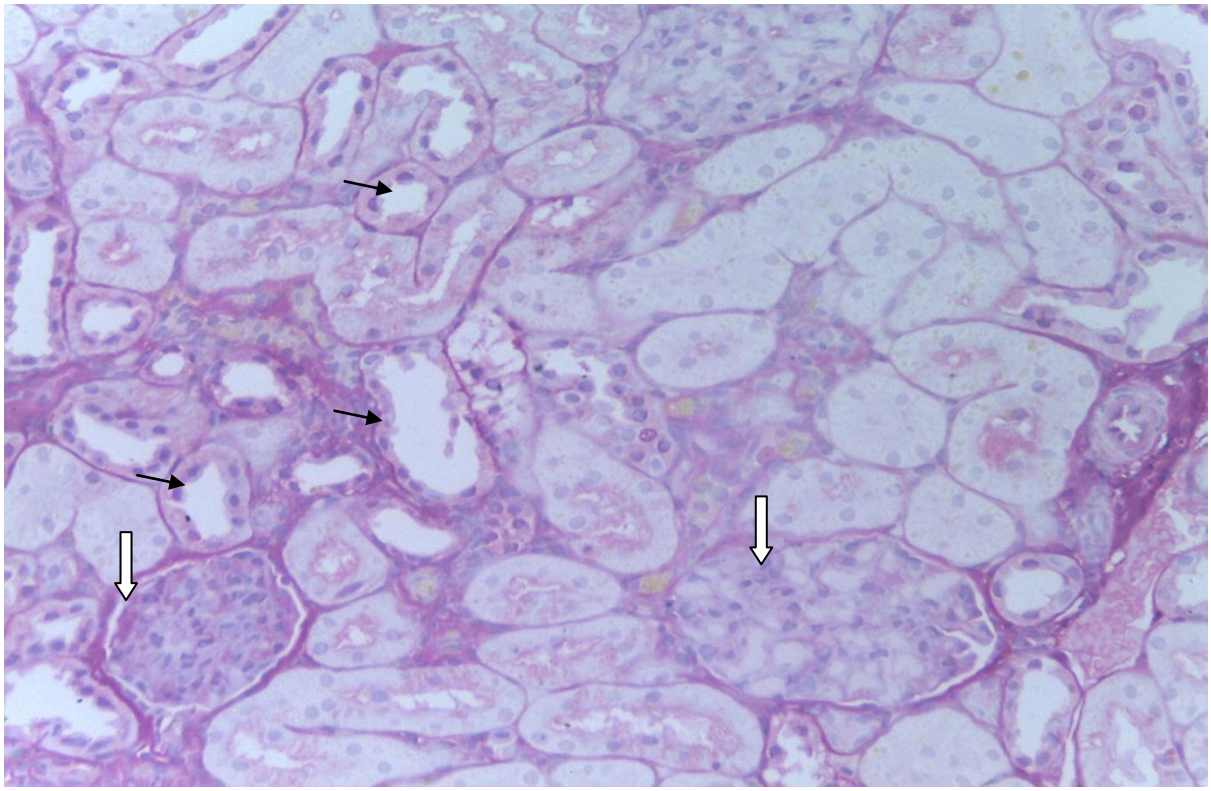

Fig 1b: H&E section(x40) of kidney treated with DICHLOROMETHANE extract show diffuse increase in mesangial matrix (leading to swelling of the glomeruli- white arrows) and sclerosis. There is tubular basement thickening (black arrows) also. The lining epithelium and vascular channels appear normal.

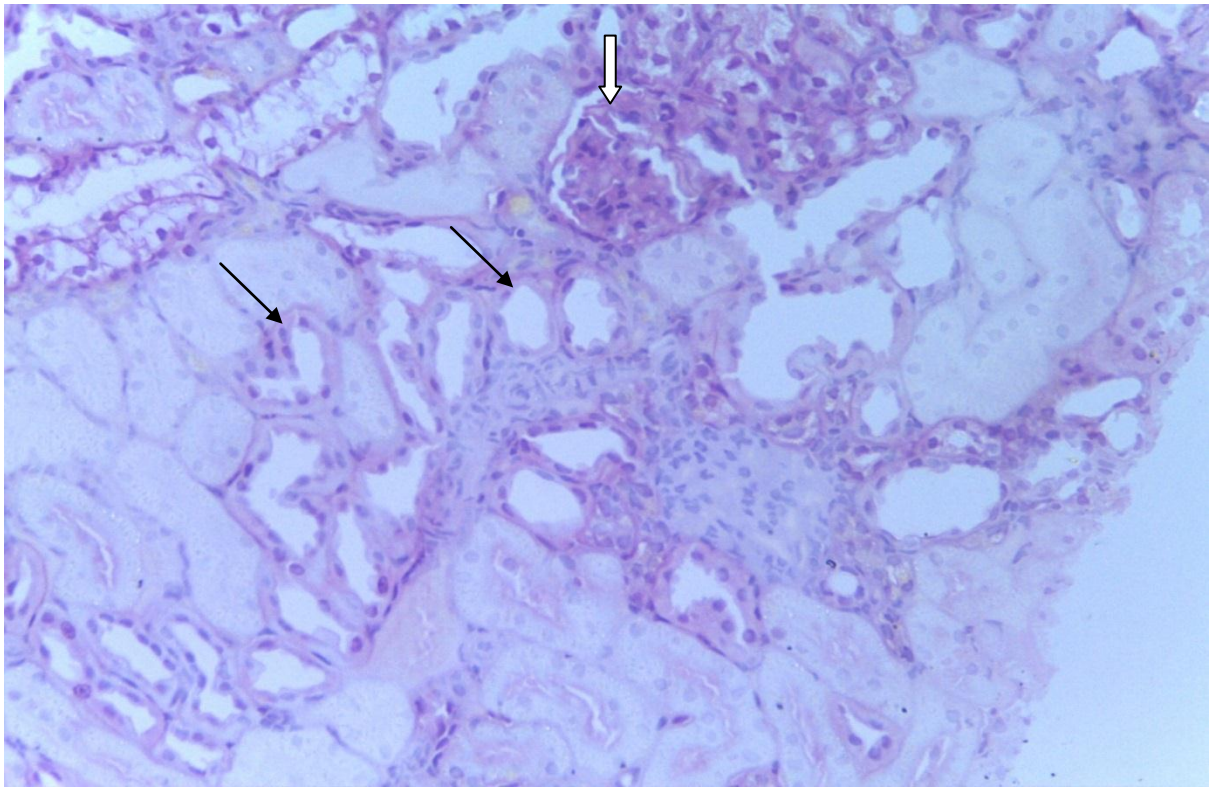

Fig 1c: H&E section(x40) of kidney treated with GLIBENCLAMIDE show mild glomerular sclerosis (white arrow) and thickened tubular basement membrane (black arrows). The vascular channels are unremarkable.

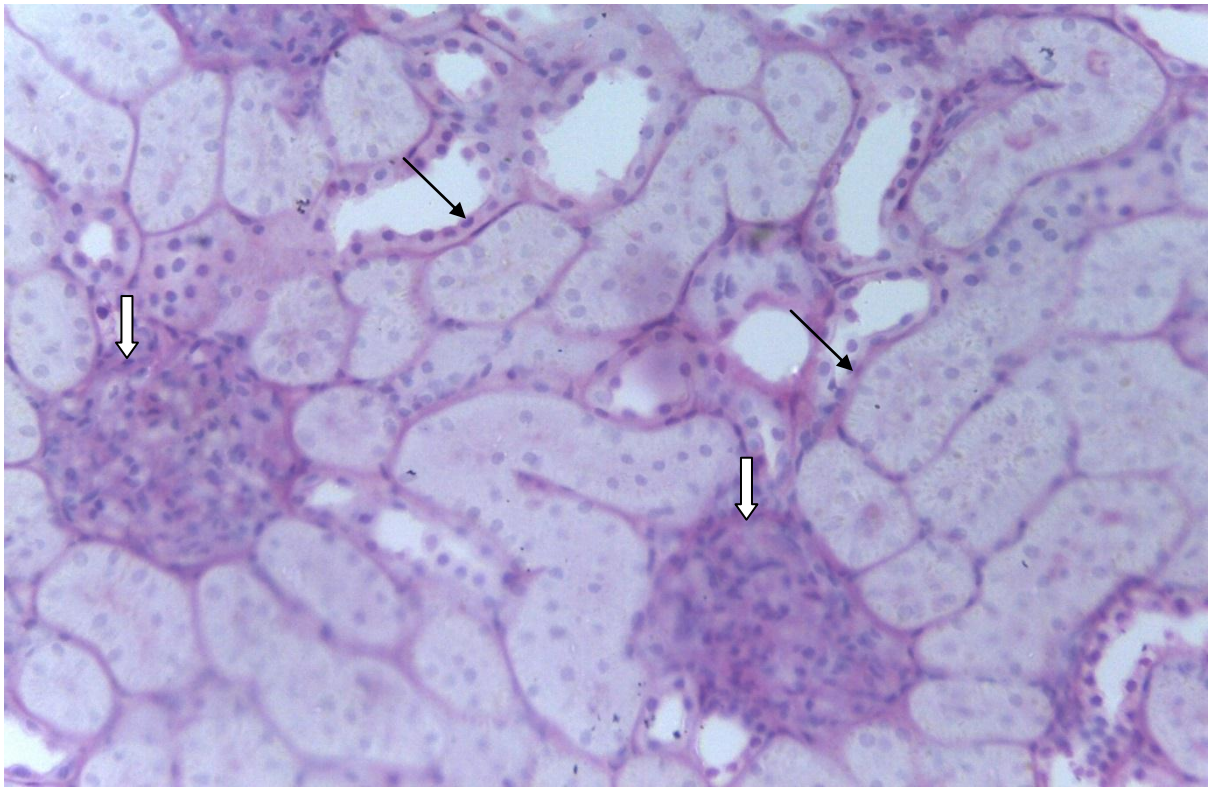

Fig. 1d: H&E section (x40) of kidney treated with ETHYL ACETATE extract show increased mesangial matrix (white arrows) obliterating the Bowman's capsule. There is glomerusclerosis also. The tubules show thickened basement membrane (black arrows) but healthy lining epithelial cells. The vascular channels are unremarkable.

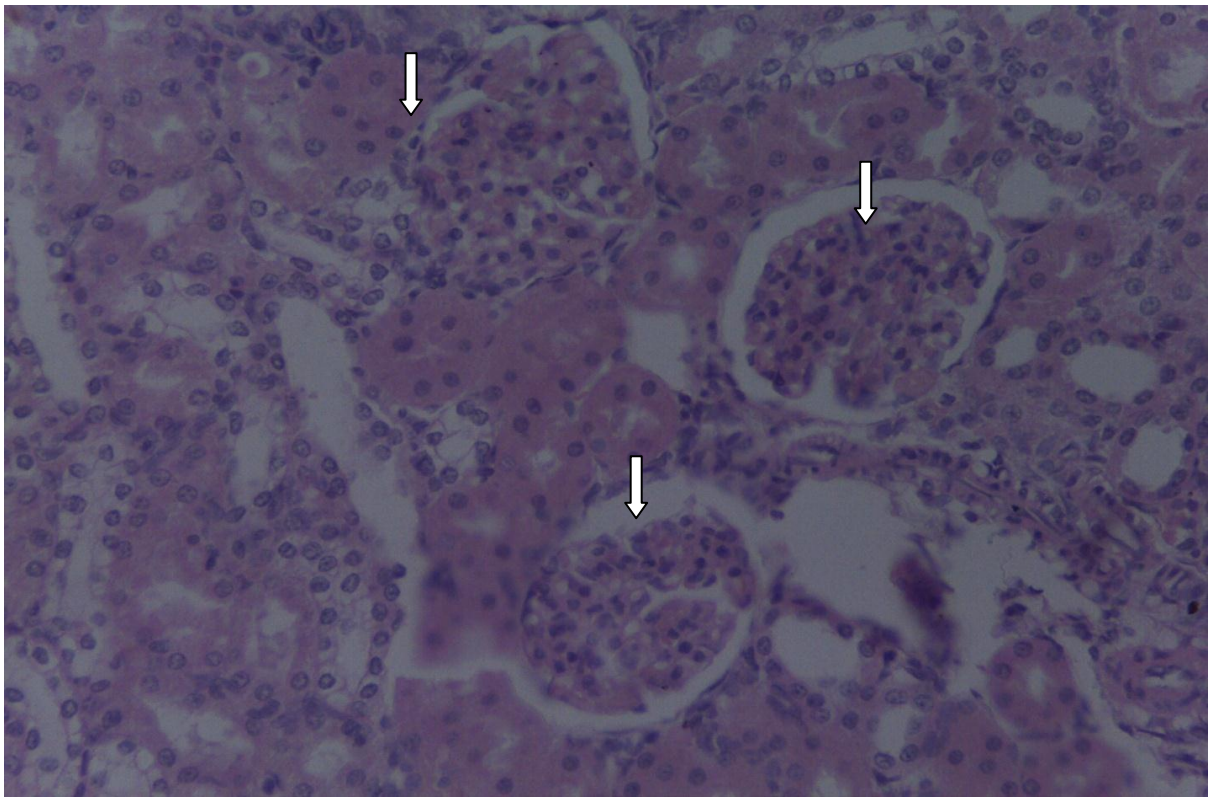

Fig. 1e: H&E section (x40) of normal kidney with normal appearing glomeruli (arrows), tubules and vascular channels.

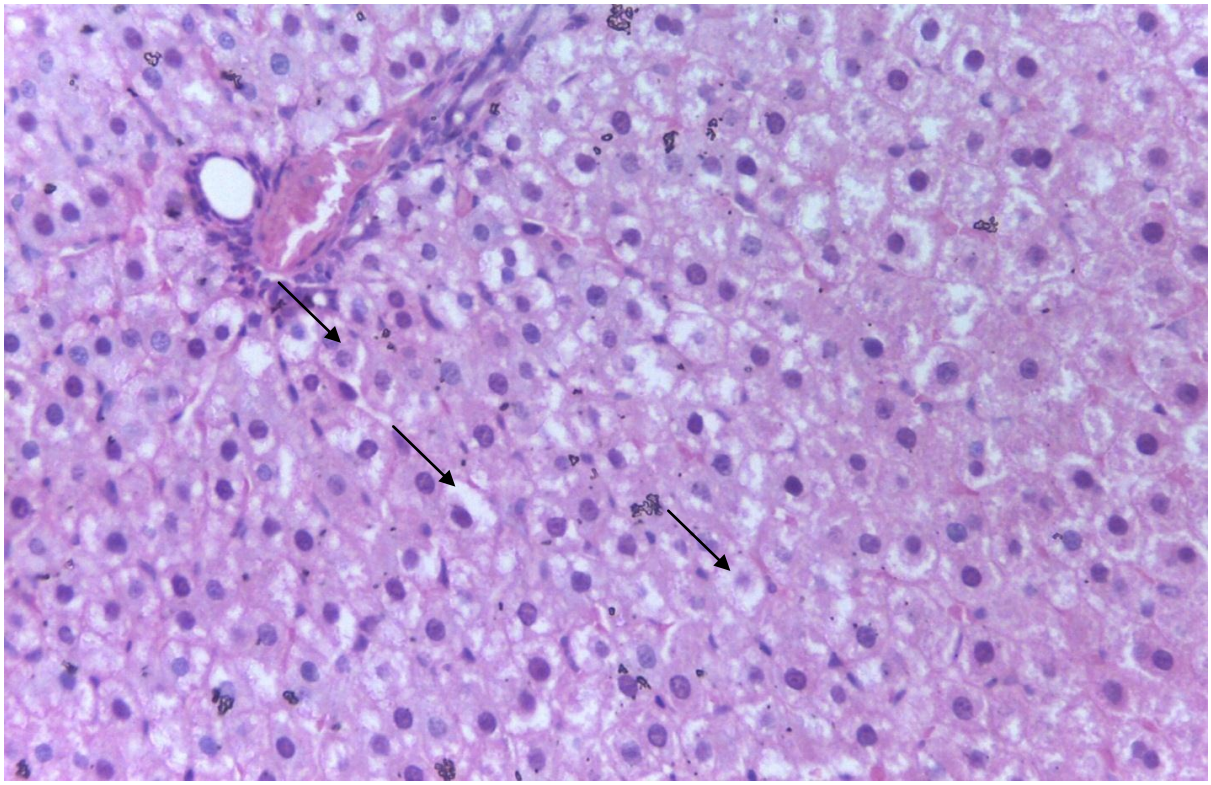

Fig. 2a: H&E section (x40) of untreated liver showing moderate to severe fatty change (arrows) consistent with diabetes mellitus.

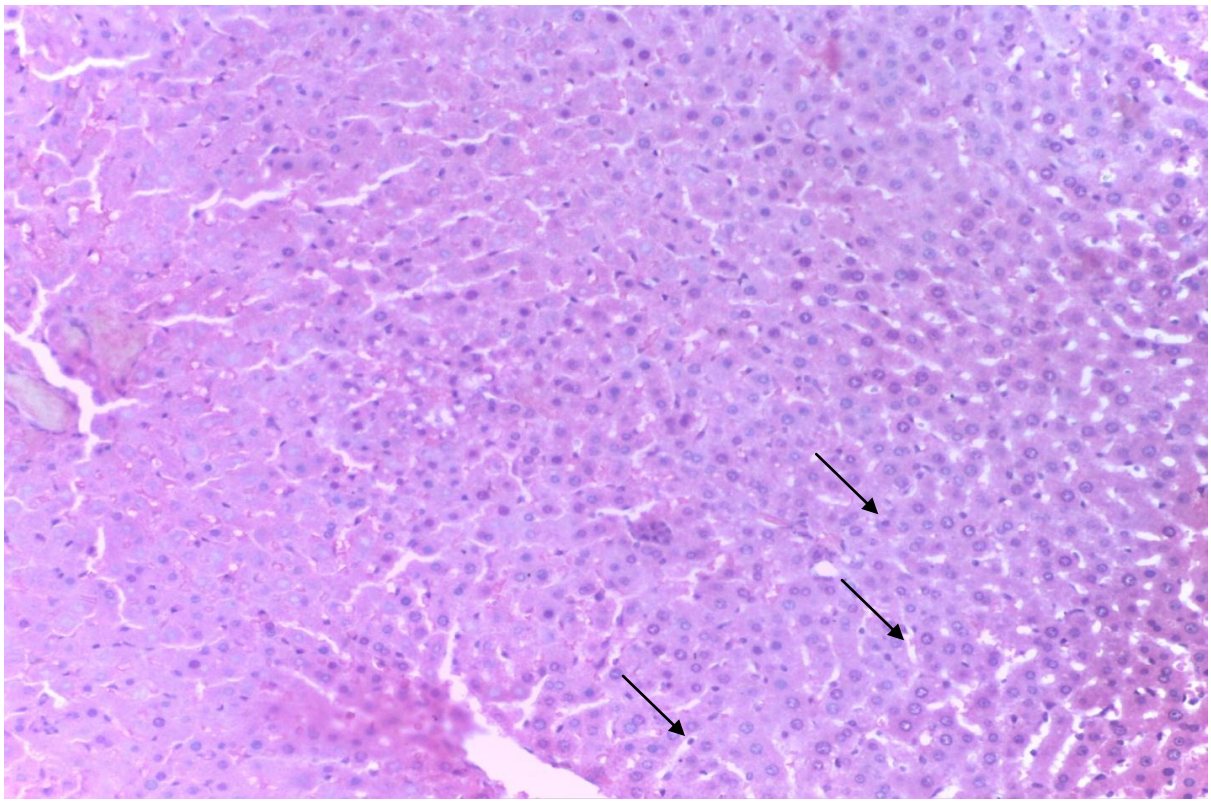

Fig. 2b: H&E section (x40) of liver treated with dichloromethane extract show normal lobular architecture and normal appearing plates of hepatocytes and diffuse scanty mononuclear inflammatory cells infiltration (arrows).

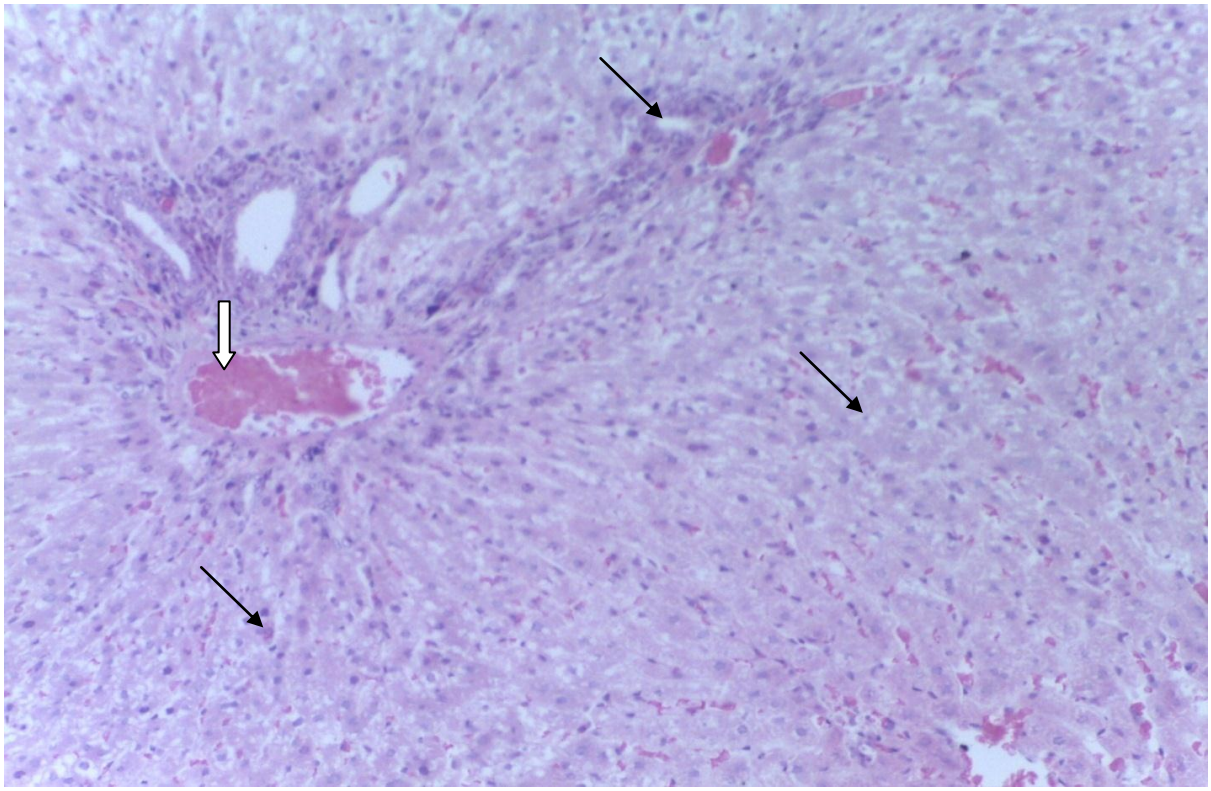

Fig. 2c: H&E section(x40) of liver treated with glibenclamide show normal lobular architecture, mild hepatocyte feathery degeneration (black arrow) and diffuse mononuclear inflammatory cells infiltrate. There is vascular congestion (white arrow) also.

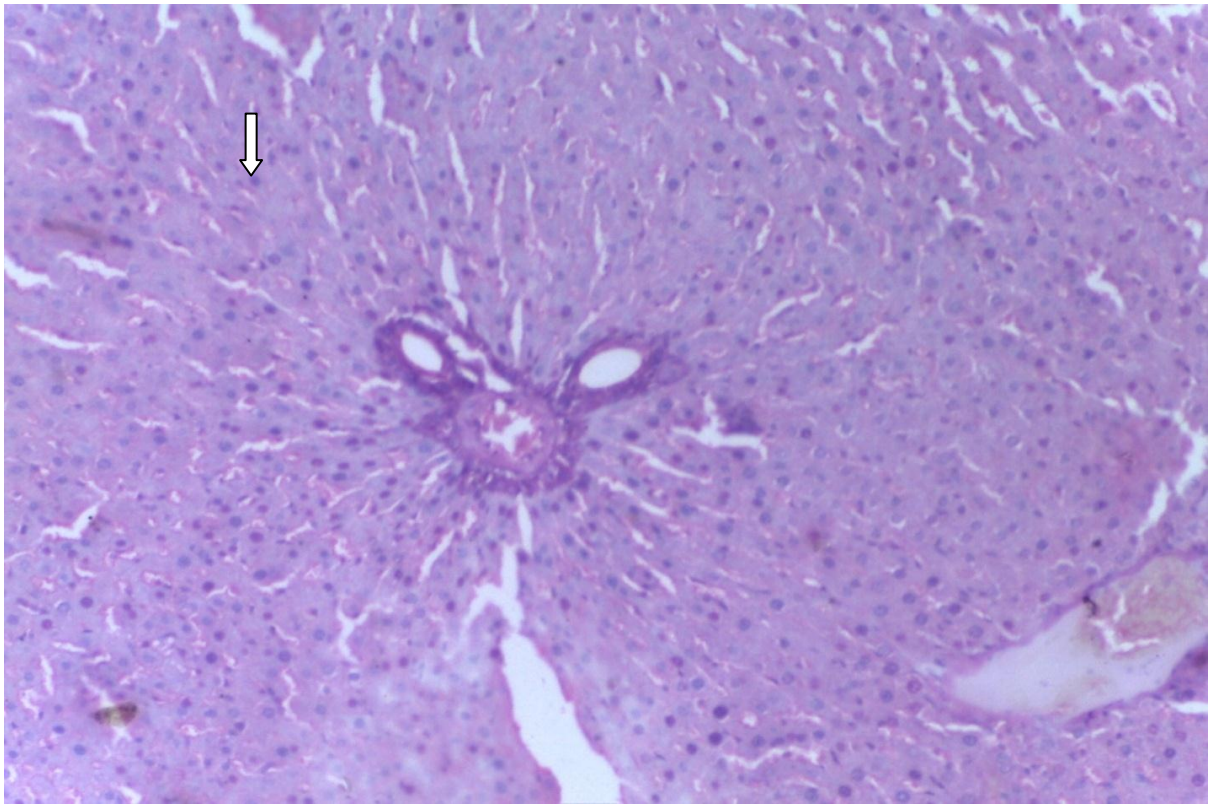

Fig. 2d: H&E section (x40) of normal liver showing lobular architecture and normal plates of hepatocytes (arrow).

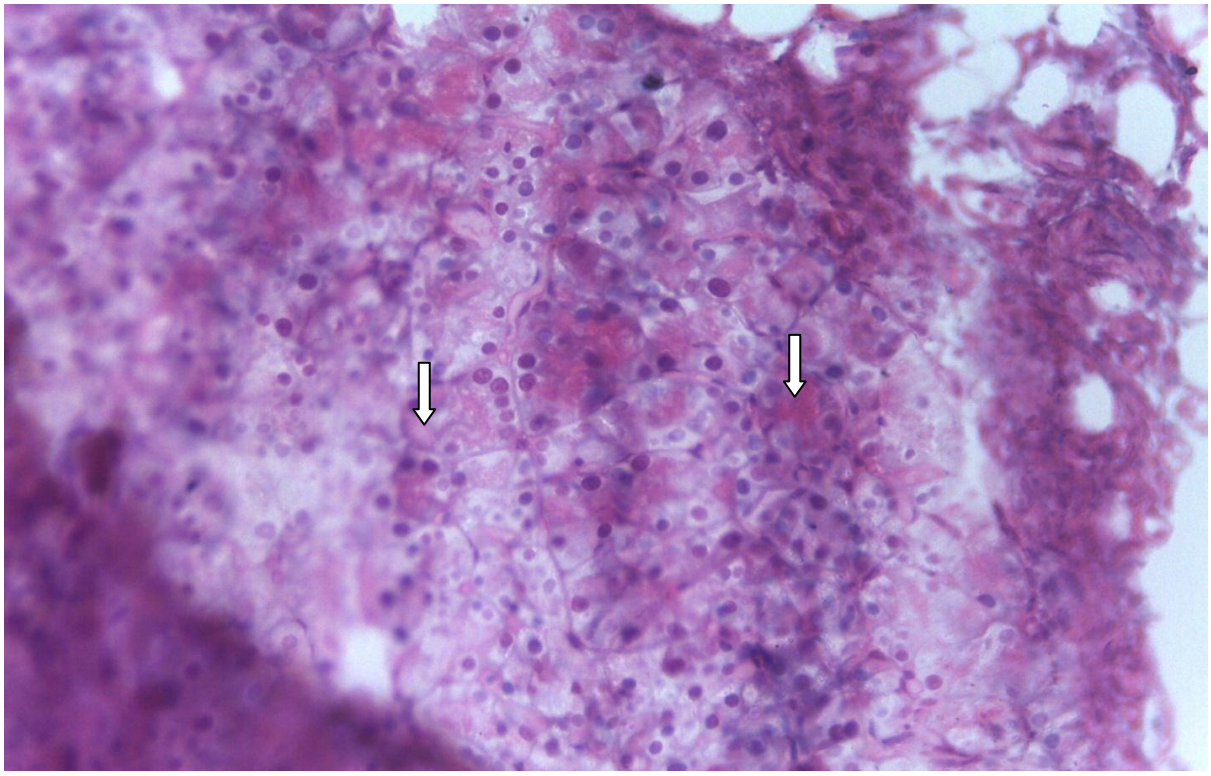

Fig. 3a: H&E section (x40) of untreated pancreas show depleted islet cell mass (white arrow) and exocrine gland hyperplasia.

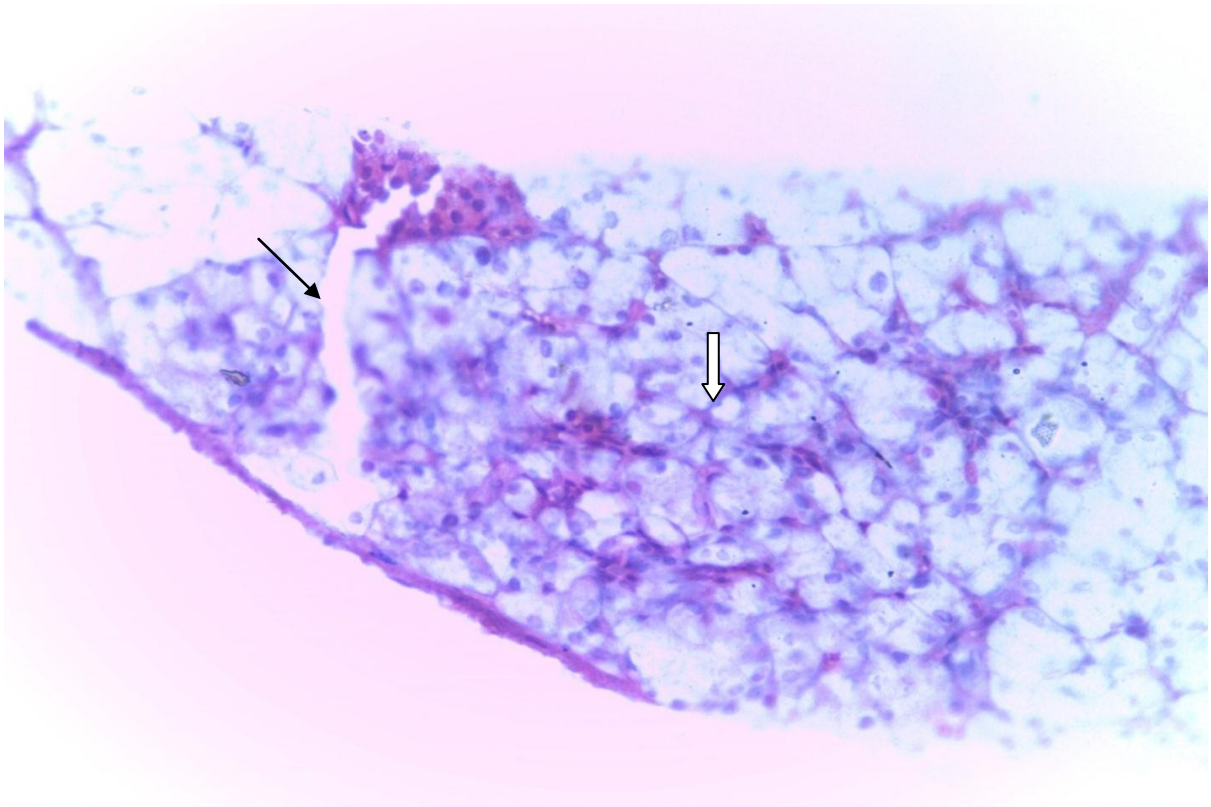

Fig. 3b: H&E section (x40) of pancreas treated with dichloromethane extract decreased islet cell mass. The exocrine glands (white arrows) are normal.

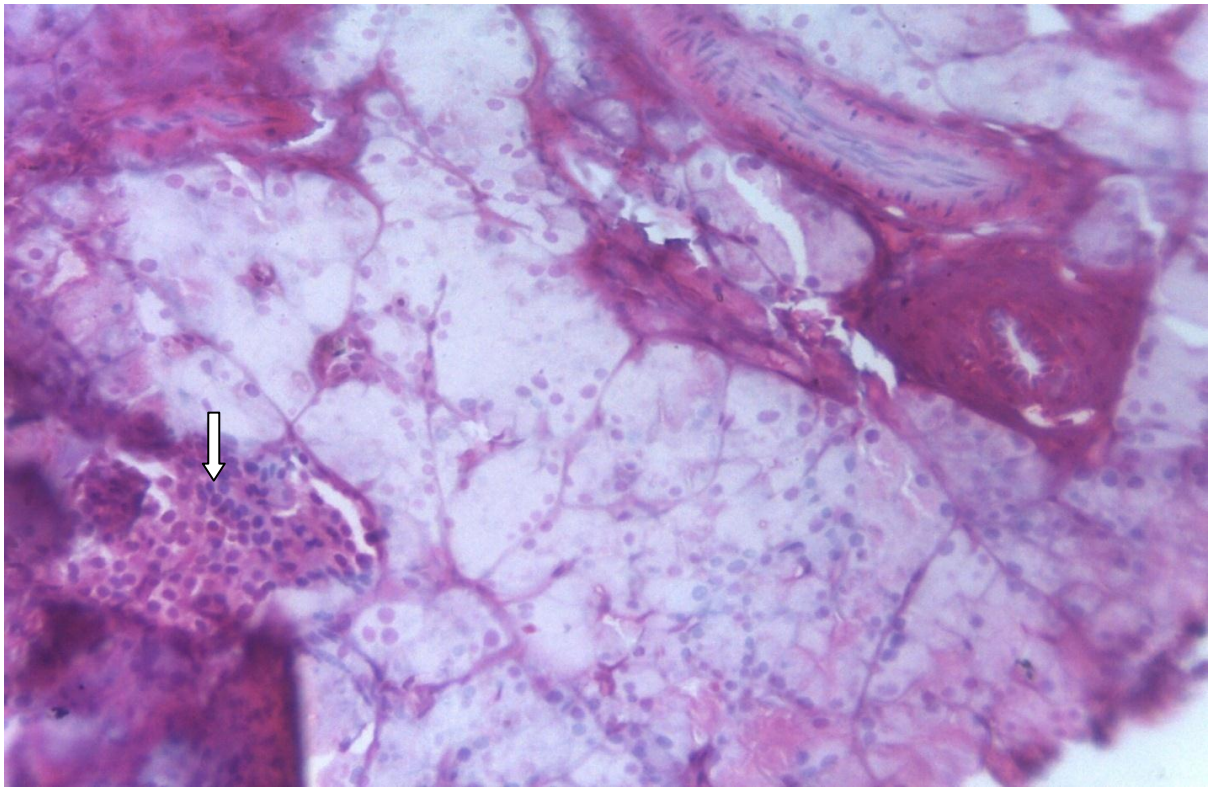

Fig. 3c: H&E section(x40) of pancreas treated with glibenclamide show healthy and normal appearing islet cell mass (white arrow) and exocrine glands.

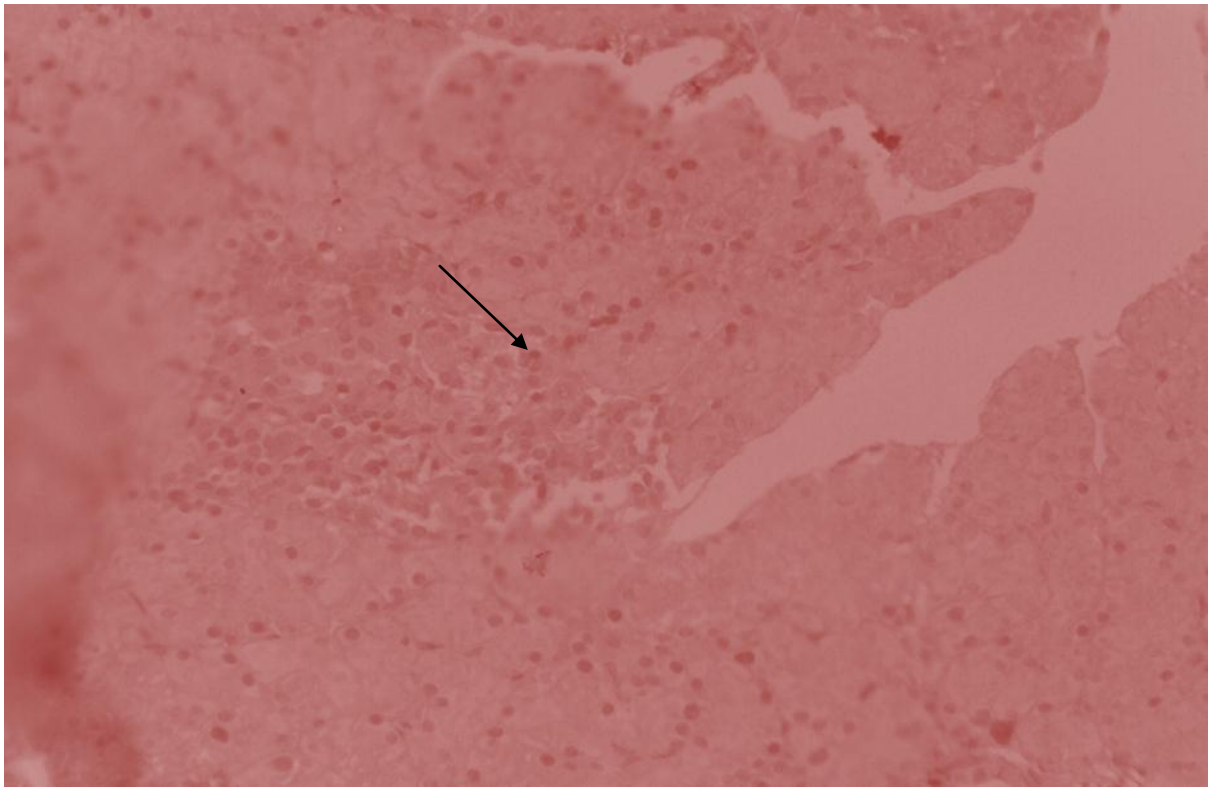

Fig. 3d: H&E section (x40) of pancreas treated with aqueous extract showing normal appearing islet cell mass (arrow) and exocrine glands.

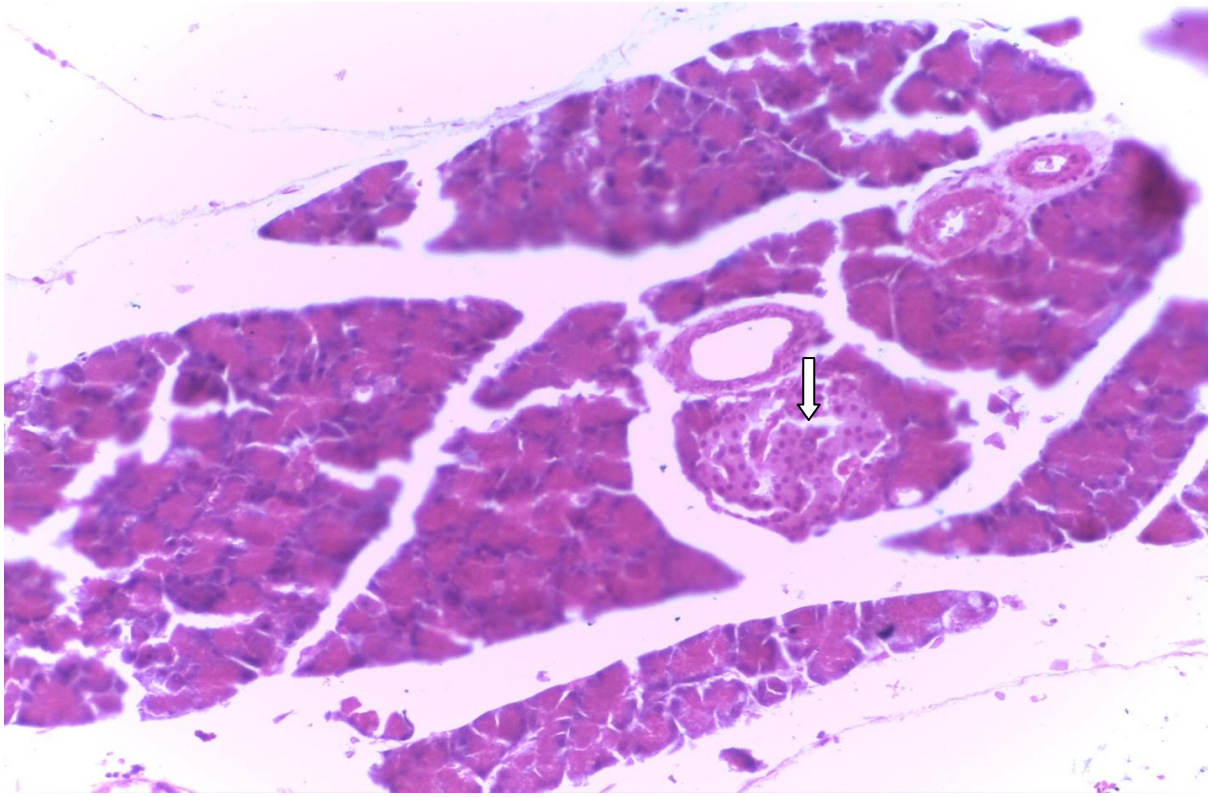

Fig. 3e: H&E section (x40) of normal pancreas showing normal islet cell mass (arrow) and exocrine glands.

4.0 DISCUSSION

Diabetes mellitus has several causes amongst which is unhealthy diet, sedentary lifestyle, urbanization, aging and obesity,[28] complications may result if this condition stays untreated. Such complications include but not limited to cardiovascular risks, Diabetic Retinopathy, Diabetic Ketosis, Renal failure and cataract formation.[29-32]

Fig 1a-3e shows the histopathological examination of Hematoxylin and Eosin stained sections of the pancreas, Kidney and liver of all the rats in this study.

The histological examination of the kidneys of the untreated diabetic rats showed the presence of thickening of the glomerular basement membrane, diffuse mesangial expansion and diabetic glomerulosclerosis. On administration of the dicholoromethane extract it showed that the destructive morphological changes was upturned to normal while with the ethyl acetate extract was shown to have returned to near normal with thickened basement but healthy lining epithelial cells. This positive change may be deduced as a result of the protective effects of the Salacia nitida extract and it decreased the alloxan-induced oxidative stress that causes the fatty degeneration and the aggregation of the inflammatory cells in the kidney. Similar findings in studies by Tohid Hassanalilou et al(2007) showed that on the administration of mulberry extract it was capable of ameliorating the complications of diabetes seen in the kidneys.[33] In another study by Rahimi-Madiseh et al found less severe glomerular damage in the group that was treated with the M.nigra extract and it was implied that this administration was likely to prevent kidney tissue damage in diabetic rats and this fruit seems to be beneficial to patients with diabetes.[34]

The liver has complex roles in the function of the body. One of such is the metabolism of excess glucose into glycogen for storage (glycogen can later be converted back to glucose for energy) and to balance and make glucose as needed).[35] It is exposed to reactive oxygen species due to oxidative damage resulting from diabetes.[36] Histological examination shows normal liver architecture with normal hepatocytes of diabetic rats when they were supplemented with dicholoromethane extract at different doses unlike the untreated alloxan-diabetic liver, which showed damaged central vein and moderate to severe fatty change consistent with diabetes mellitus. These results indicated that Salacia nitida extract may greatly improve hepatocyte degeneration which is associated with development of diabetes as seen in a similar study carried out making use of *Bryonia multiflora*.[37]

Pancreas is one of the vital organs meant for polysaccharide metabolism; it helps to maintain the homeostasis of blood glucose through insulin synthesis. The effect of administration of Alloxan on experimental models causes pancreatic damage which is demonstrated with structural and functional alterations such as disorganization of pancreatic architecture, and depletion of insulin producing cells.[38-40]

The histology of the panceas showed changes after administration of alloxan. Such changes included depleted islet cell mass and exocrine gland hyperplasia which was a representation of destructed β-cells and vacuolated pancreatic acini. Similar results were observed in.[41] In this study, the histological images of the pancreatic tissue of the rats after treatment with aqueous extracts and dicholoromethane revealed a significant increase in the number of pancreatic β-cells with no adipocyte deposit. There were also normal sized cells of islets of Langerhans

5.0 CONCLUSION

The Dicholoromethyl Extract was found efficient in controlling diabetic micro and macro vascular complications. The histological examination of the kidney, liver and pancreas indicated the protective effect of Salacia nitida Benth; unveiling reduced diabetes induced degenerative damage

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