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ANTIDIABETIC, ANTIOXIDANT AND HYPOLIPIDEMIC POTENTIALS OF *M. charentia* METHANOL LEAF EXTRACT ON ALBINO RATS

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ABSTRACT

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin (type 1 diabetes) or when the body cannot effectively use the insulin it produces(type 2 diabetes). The aim of this research was to determine antidiabetic, antioxidant and hypolipidemic potentials of *M. charentia* methanol leaf extract on rats. The phytochemical screening was carried out using standard laboratoryprotocols. Alloxan was used to induced diabetes and extract was administered orally (100, 200 and 400 mg/kg body weight, for 21 days. Antidiabetic, lipid profile and antioxidant activities we determine adopting

Introduction:

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin (type 1 diabetes) or when the body cannot effectively use the insulin it produces(type 2 diabetes) (Jones*et al.*, 2009). Insulin is a hormone that regulates blood sugar. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels (Gardner and Shoback, 2011).In 2014,

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standard methods. Histological examination of liver and pancreas during 21 days of treatment was also carried out. Phytochemical screening revealed the presence of alkaloids, flavonoids, phlobatanins, saponin, glycoside, phenols and terpenoids. The extract revealed a significant ($P<0.05$) reduction in fasting blood glucose level in alloxan-induced diabetic rats. However non-significant ($P>0.05$) differences were observed in serum lipid profile levels,antioxidant vitamins and enzymes in extract treated diabetic rats, when compared with diabetic, normal and standard drug treated rats. Histopathological studies of the pancreas showed comparable regeneration of the cells by extract which were earlier necrosed by alloxan. *M. charentia* methanol leaf extract revealed an outstanding antidiabetic potential with regeneration effect on pancreatic islets of Langerhans, and also revealed a non-toxic profile at acute dose. However, the extracts exhibit minimal hypolipidemic and antioxidant activities on diabetic rats

Keywords: Alloxan, Antidiabetic, histology, antioxidants, *M. charentia,* Lipid profile.

8.5% of adults aged 18 years and older had diabetes (Srivastava *et al*., 2019). In 2019, diabetes was the direct cause of 1.5 million deaths and 48% of all deaths due to diabetes occurred before the age of 70 years (Srivastava *et al*., 2019). Biological or physiological system is continuously exposed to severalforeign substances that lead to the production of reactive species called free radicals (ROS/RNS) which by the transfer of their free unpaired electron causes the oxidation of cellular machinery(Ifeanyi, 2018). However, In order to counterattack their effects (ROS), physiological system has got endogenous antioxidant systems or it obtains exogenous antioxidants from diet that neutralizes such species and keeps the homeostasis of body(Casas-Grajales and Muriel, 2017). Any imbalance between the RS and antioxidants leads to produce a condition

known as "oxidative stress" which leadstooccurrence of pathological condition among which diabetes is a common example (Rahal *et al*., 2014). Most of the studies also reveal the inference of oxidative stress in diabetes pathogenesis by the alteration in enzymatic systems, lipid peroxidation, impaired Glutathione metabolism and decreased Vitamin C levels. Lipids, proteins, DNA damage, Glutathione, catalane and superoxide dismutase are various biomarkers of oxidative stress in diabetes mellitus (Asmat *et al*., 2016). Oxidative stress induced complications of diabetes may include stroke, neuropathy, retinopathy and nephropathy. Consequences of diabetes results in lipid abnormalities, often termed (diabetic dyslipidemia), which are typically characterized by high levels of total cholesterol (T-Chol), high levels of triglycerides (Tg), low levels of high density lipoprotein cholesterol (HDL-C) and increased levels of small dense LDL particles (Asmat *et al*., 2016).

Medicinal plants have been used in treatment of diseases since ancient times. Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines, this is because medicinal plants cultural acceptability, low toxicity profile, less expensive and availability (Sofowora *et al*., 2013).

In Nigeria, majority of people dwellingin rural area, have limited access to conventional drugs, and also financial instabilityas well as side effects are another factors contributing to low patronage of convention therapy, leading to total dependency of these people on traditional medicine, however, majority of the plant used in folklore medicine are not scientifically validated (Elujoba *et al*., 2005).Therefore the purpose of this article is to provide documented scientific evidence on herbs used as antidiabetic agent by herbal practitioners.

MATERIALS AND METHODS

Collection and Identification of Plant Samples

The plant sample was collected in July 2021 from Birnin Kebbi town, Birnin Kebbi Local Government Area of Kebbi State. It was authenticated by a Taxonomist from Department of Plant Science and Biotechnology, Kebbi

State University of Science and Technology, Aleiro, with a voucher specimen (KSUSTA/PSB/H/VOUCHER/SN) deposited in the herbarium of the same Department.

Plant Preparation and Extraction

The leaves of *M. charentia* were washed with clean water and allowed to dry under shade for two weeks. They were then grinded to coarse powder using mortar and pestle. Five hundred grams (500g) of the powdered sample were soaked in 2500mls of methanol for 72 hrs (Dupont*et al.,* 2002). They were then filtered using muslin cloth and the filtrates were evaporated using an oven set at 45^{o}C.The dried extract was stored separately in an air tight container and kept in refrigerator at 4 ^{o}C. The percentage yield of the extract was calculated using the formula.

$$\text{Percentage yield} = \frac{\text{weight of extract}}{\text{weight of ground plant material}} \times \frac{100}{1}$$

Experimental Animals

The albino (Wistar) rats used in this study were purchased from Animal House, Usmanu Danfodiyo University, Sokoto in November, 2021. The animals were transported via plastic ventilated cages to Animal House, Faculty of Science, Kebbi State University of Science and Technology, Aleiro. The rats wereacclimatized for two (2) weeks prior the commencement of the experiment. The rats were fed with standard rodent pellets and were allowed access to water ad libitum.

Phytochemical Screening

The Phytochemical screening for the presence of saponins, tannins, alkaloids, flavonoids, tannins, steroids, saponins, glycosides, cardiac glycosides, saponin glycosides, balsams, anthraguines, and volatile oil were

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carried out according to the methods described byHarbone, (1973) Trease and Evans, (1989) and Safowara, (1993)

Acute Oral Toxicity Studies (LD_{50})

The acute oral toxicity study was conducted according to the method of Organization for Economic and Cultural Development for testing of chemical (OECD, 2001). This method has two phases which are phases 1 and 2 respectively.

Phase 1

This phase requires nine animals. The nine animals are divided into three groups of three animals each. Each group of animals are administered different doses (10, 100 and 1000 mg/kg) of test substance. The animals are placed under observation for 24 hours to monitor their behavior as well as if mortality will occur.

Phase 2

This phase involves the use of three animals, which are distributed into three groups of one animal each. The animals are administered higher doses (1600, 2900 and 5000 mg/kg) of test substance and then observed for 24 hours for behavior as well as mortality). The animals were observed for signs of drowsiness, hair loos, and loss of appetite, salivation, tremors, convulsion and bulging of the eyes. The animals were thereafter observed for a period of 14 days for any signs of delayed toxicity (Lorke, 1983).

Induction of Diabetes

Diabetes was induced in the rats by intraperitoneal injection of Alloxan in a dose of 120mg/kg body weight in Normal Saline (Chougale *et al.*, 2007).

Diabetes was confirmed in the animal after 48 hours by estimation of fasting BGLs and only rats with blood glucose level above 150mg/dl were used for the study.

Fasting Blood Glucose Monitor

Fasting blood sugar was determined using Accu-check active glucometer by Roche Diagnostic according to the method of Marks and Dawson (1965). The rats were randomly divided into 6 groups (n=4) and treated as follows:

Group 1	Normal control (untreated).
Group 2	Alloxan treated (diabetic)
Group 3	Alloxan induced-diabetic rats treated with glibenclamide (0.5mg/kg).
Group 4	Alloxan induced-diabetic rats treated with extract (100mg/kg).
Group 5	Alloxan induced-diabetic rats treated with extract (200mg/kg).
Group 6	Alloxan induced-diabetic rats treated with extract (400mg/kg).

The extract was administered to the animals orally. Body weight changes were also monitored weekly throughout the experimental period. The rats were sacrificed on the twenty-second day of the experiment. Blood samples were collected in heparinised bottles for biochemical analysis

while organs (livers and pancrease) were collected for histopathology evaluation.

Antioxidant Assay

Lipid peroxidation was determined by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin *et al*. (1993), (SOD) Activity was determined using the method of Xin *et al*., (1991). The activity of catalase was assayed according to the method of Aebi (1983), glutathione (GSH) was based on the method of Jollow *et al.,* (1974), Vitamin A and C concentrationswere determined using the method of Laurence and Sobel, (1953) and vitamin E was determined as described by Barker and Frank, (1968).

Serum Lipid Profile

The determinations of serum total cholesterol (TCL) levels were done using kit product of Randox, UK (Trinder, 1969). High density lipoprotein cholesterol (HDL-C) was determinedusing precipitation method in the presence of phosphotungistic acid and magnesium chloride (Trinder, 1969). Total triglyceride was determined by the enzymatic method described by Buccolo and David (1973) using commercially available kit. Low density lipoprotein concentration was calculated using the method of Friedwald *et al*., (1972).

Histopathological Examination

Histopathology was done using the method of Drury *et al*., (1967). Liver and pancreas of the rats were harvested and preserved in 10 % formalin. The organs were fixed in 10 % buffered formalin for 72 hours. The tissues were then dehydrated in alcohol of graded concentrations and embedded in paraffin. Embedded tissues were cut into sections of 5 µm thick and these were stained with hematoxylin and eosin for photo microscopic

assessment and placed on a clean labelled microscope glass slide. The slide was mounted on an electric light microscope for examination of any possible histopathological features. Photomicrographs of the samples were then taken.

Data Analysis

The data generated from the study are present as Mean ± Standard error of mean (SEM) and subjected to one-way analysis of variance (ANOVA) and statistical difference between means were separated using Duncan multiple comparison test using statistical package for social science (SPSS) version 20. Values are considered statistically significant at $P<0.05$. Graphs are plotted using Microsoft excel and Prism software, micrographs and diagrams were presented where necessary using digital camera.

RESULTS AND DISCUSSION

RESULTS

Percentage Yield

Extraction of 800g of Momordica charentia in 2500mls of methanol yielded 4% and the extract was soluble in water, dark green in colour and with a gummy texture.

Results of Phytochemical Screening

The qualitative phytochemical screening of *Momordica charentia* methanol leaf extract is presented in Table 1.

Table 1: Phytochemical Constituent of *Momordica charentia*

PHYTOCHEMICALS	RESULTS
Alkaloids	+
Flavonoids	+

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Tannins	-
Phlobatanins	+
Saponin	+
Glycoside	+
Phenols	+
Terpenoids	+
Anthraquinone	-

KEY: + = Present, - = Not detected

Acute Toxicity (LD_{50}) Studies

There was no any visible or detectable sign of toxicity even at the maximum dose limit (5000 mg/kg). Since no any apparent sign of toxicity or mortality recorded, the LD50 is assumed to be greater than 5000mg/kg b.wt.

Effect of M. charentia Methanol Leaf Extract on Bodyweight of Diabetic Rats

The bodyweight of diabetic rats treated with *M. charentia* methanol leaf extract for 21 days showed a weekly increase in body weight in normal control, all extract and drug treatment groups from initial week (0) to last week of the experiment (week 3) Figure 1. However, in alloxan control group a slight increase in bodyweight was observed in week one (1) but subsequently a decrease in bodyweight was recorded throughout the remaining period of the experiment (week 2 and week 3).

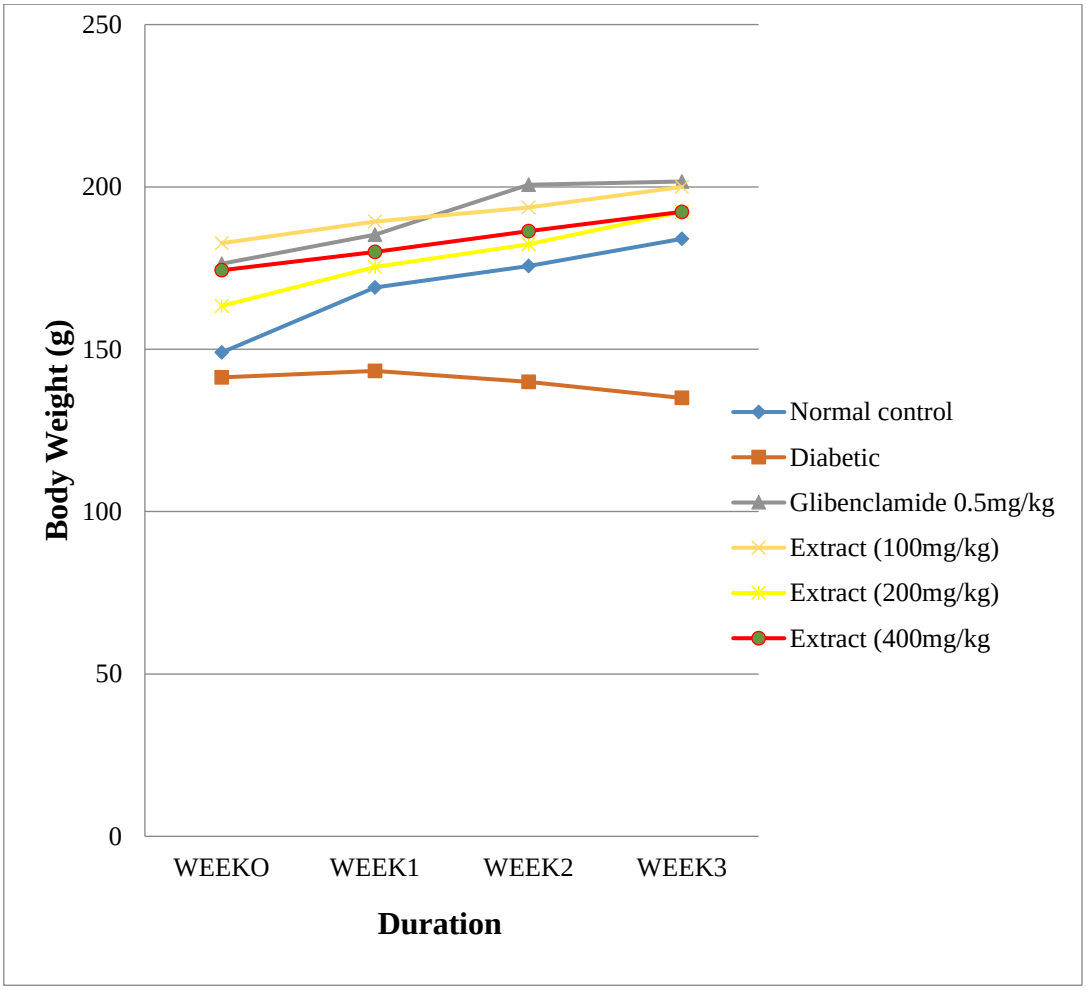

Figure 1: Bodyweight of Diabetic Rats Administered with *M. charentia* Methanol Leaf Extract for Three Weeks.

Effect of M. charentia Methanol Leaf Extract on FBS of Diabetic Rats

The effect of *M. charentia* methanol leaf extract on fasting blood sugar is presented in (Table 2). Before extract and standard drug administration (week 0), the fasting blood sugar of all the alloxan-induced groups significantly increased ($P<0.05$) compared to the normal control group. However after week 1, 2 and 3 of treatment there was significant ($P<0.05$) difference between groups treated with extract 100mg/kg, 200mg/kg and 400mg/kg when compared to diabetic control group and also, there was

significant (P<0.05) increase in diabetic control group compared to normal control. But there were no significant (P>0.05) differences in extract 100mg/kg, 200mg/kg and 400mg/kg and drug control when compared to normal control. Also at week 1-3, there was no significant (P>0.05) differences between all extract treatment groups compared to drug control.

Antioxidant Effect of *M. charentia* Methanol Leaf Extract on Diabetic Rats

Table 3 shows the antioxidant activity of *M. charentia* methanol leaf extract on diabetic induced albino rats. Malondialdehyde (MDA) showed a significant (P<0.05) increase in groups treated with 100mg/kg and 200mg/kg compared to standard drug control, diabetic control and normal control. Glutathione peroxidase (GPx) showed non-significant (P>0.05) differences between all extracttreatment groups compared to standard drug control, diabetic control and normal control. Catalase on the other hand revealed a significant (P<0.05) decreases in extracttreatment group 100mg/kg and 200mg/kg compared to normal control. The result for superoxide dismutase (SOD) revealed significant (P<0.05) increase in groups treated with extract 100mg/kg 200mg/kg, 400mg/kg, standard control and diabetic control compared to normal control. Vitamin A showed a significant decrease (P<0.05) in groups treated with 100mg/kg, 200mg/kg and 400mg/kg compared to normal control, while diabetic control group showed a non-significant decrease (P>0.05) in Vitamin A when compared to normal control group. There was also significant decrease (P<0.05) in vitamin C between all extracttreatment groups and standard control when compared to normal control but there was no significant decrease (P>0.05) in Vitamin C of diabetic control compared to normal control group. There was also significant decrease (P<0.05) in vitamin E between all extracttreatment groups and standard control when

compared to normal control but there was no significant decrease (P>0.05) in Vitamin E of diabetic control compared to normal control group

Effect of M. charentia Methanol Leaf Extract on Lipid Profile of Diabetic Rats

The results for the effect of *M. charentia* methanol leaf extraction lipid profile are presented in Table 4. The serum total cholesterol showed a significant increase (P<0.05) in extracttreatment 200 and 400mg/kg compared to standard control, diabetic control and normal control. but there no significant (P>0.05) increase in (TC) of diabetic control when compared to normal control group. There were significant increase (P<0.05) in serum high density lipoprotein, (HDL) only in group treated with extract 200mg/kg compared to standard control, diabetic control and normal control. Serum total triglyceride (TAG) revealed a significant (P<0.05) increase in extract 200 and 400mg/kg compared to standard control, diabetic control and normal control (P<0.05), but diabetic control group showed a non-significant (P>0.05) increase in TG when compared to normal control. Also serum very low density lipoprotein cholesterol (VLDL) concentration revealed a significant (P<0.05) increase only in group treated with extract 200mg/kg and 400mg/kg compared to standard control, diabetic control and normal control, , but diabetic control group showed a non-significant (P>0.05) increase in VLDL when compared to normal control. Low density lipoprotein (LDL) showed a significant (P<0.05) increase and decrease increase in groups treated with extract 100 and 400mg/kg respectively compared to standard control, diabetic control and normal control.

Table 2: Effect of *M. charentia* Methanol Leaf Extracts on Fasting Blood Sugar

Treatments	Glucose Concentration (mg/dl)			
	Week 0	Week 1	Week 2	Week 3
Normal control	73.33±2.60^{a}	86.00±3.79^{a}	95.00±4.04^{a}	91.00±2.08^{a}
Diabetic control (120mg/kg)	516.33±83.67^{b}	371.67±69.03^{b}	325.67±25.67^{b}	288.33±50.85^{b}
Standard drug (0.5mg/kg)	573.33 ±26.67^{b}	144.67±5.67^{a}	92.33±5.84^{a}	88.67±8.76^{a}
Extract (100mg/kg)	363.67±50.93^{b}	169.00±13.00^{a}	88.67±7.97^{a}	95.00±2.87^{a}
Extract (200mg/kg)	364.33±121.53^{b}	135.00±10.41^{a}	93.67±16.51^{a}	87.33±6.94^{a}
Extract (400mg/kg)	492.33±107.67^{b}	120.33±16.59^{a}	115.33±13.93^{a}	109.00±5.51^{a}

Values are presented as mean ± SD (n = 4) value having same superscript are not significantly different at (P>0.05) analysed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0.

Table 3: Antioxidant Activity of *M. charentia* Methanol Leaf Extracts

Treatments	MDA (µmole)	GSH (µmole)	CAT (µmole)	SOD (µmole)	Vitamin A (mg/dl)	Vitamin C (mg/dl)	Vitamin E (mg/dl)
Normal control distilled water 5ml/kg	59.13±1.53^{a}	54.87±0.68^{ab}	0.01±0.00^{a}	49.00±5.46^{b}	24.30±0.45^{d}	168.45±7.02^{c}	74.34±5.61^{d}
Diabetic control	69.40±0.59^{a}	61.40±3.26^{b}	0.01±0.00^{ab}	22.33±8.41^{a}	23.27±0.72^{d}	145.83±16.76^{bc}	62.61±7.95^{d}

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(120mg/kg)							
Standard drug (0.2mg/kg)	175.37±104.62^{a}	58.53±4.58^{b}	0.01 ±0.00^{abc}	12.13±0.99^{a}	19.93±0.46^{c}	120.23±4.29^{b}	47.55±3.77^{c}
Extract (100mg/kg)	367.30±2.02^{b}	48.40±0.71^{a}	0.01±0.00^{bc}	9.87±0.44^{a}	18.58±0.50^{c}	88.69±2.14^{a}	40.29±1.98^{bc}
Extract (200mg/kg)	357.83±0.56^{b}	83.03±1.02^{b}	0.01±0.00^{c}	16.13±1.04^{a}	14.47±0.58^{b}	85.71±8.81^{a}	29.91±1.67^{ab}
Extract (400mg/kg)	156.37±2.28^{a}	90.70±2.93^{b}	0.68±0.00^{ab}	24.70±6.05^{a}	11.64±1.18^{a}	74.52±8.70^{a}	25.01±1.85^{a}

Values are presented as mean ± SD (n = 4) value having same superscript are not significantly different at (P>0.05) analysed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. SOD= superoxide dismutase, CAT= catalase, GSH= glutathione reductase and MDA= malondialdehide

Table 4: Effect of *M. charentia* Methanol Leaf Extracts on Lipid Profile

Treatments	TC (mg/dl)	HDL (mg/dl)	TAG (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Normal control	346.50±0.75^{b}	101.31±0.58^{ab}	303.30±2.33^{b}	60.67±0.49^{b}	305.97±0.48^{b}

Diabetic control (120mg/kg)	374.28±16.24^{b}	55.87±21.77^{a}	336.82±15.22^{bc}	67.33±3.05^{bc}	388.13±38.22^{b}
Standard drug (0.2mg/kg)	346.09±45.00^{b}	72.53±37.23^{a}	299.30±53.05^{b}	59.80±10.60^{b}	333.30 ±92.80^{b}
Extract (100mg/kg)	256.63±0.97^{a}	141.00±1.14^{b}	191.30±5.85^{a}	38.20±1.16^{a}	152.37±2.271^{a}
Extract (200mg/kg)	462.25±2.86^{c}	230.30±1.22^{c}	400.60±3.38^{cd}	80.07±0.67^{cd}	312.01±4.33^{b}
Extract (400mg/kg)	507.53±0.38^{c}	64.20±0.56^{a}	431.03±5.55^{d}	86.17±1.10^{d}	528.50±2.29^{c}

Values are presented as mean ± SD (n = 4) value having same superscript are not significantly different at (P>0.05) analysed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0.TC= total cholesterol, TG=triacylglycerol, LDL-C=low density lipoprotein cholesterol, VLDL= very low density lipoprotein and HDL= high density lipoprotein.

Effect of *M. charentia* Methanol Leaf Extract on Histopathological Examination of Liver and Pancreas Tissues of Diabetic Rats

Histopathological examination results showed that the liver of rats in normal control group, standard drug control group, diabetic control group and groups treated at 100mg/kg, 200mg/kg, and 400 mg/kg of *M. charentia* methanol leaf extract exhibited normally distributed portal triad, central vein and hepatocytes (plate 1-6). However the pancreas of rats in normal control group showed normal exophytic glands and adequate islets

of Langerhans (Plate 7) while diabetic control groups showed atrophied islets of Langerhans (Plate 8). While all groups treated with standard drug, 100mg/kg, 200mg/kg, and 400mg/kg of *M. charentia* methanol leaf extract respectively showed islets of Langerhans with atropy and regeneration (Plate 9- 12) respectively

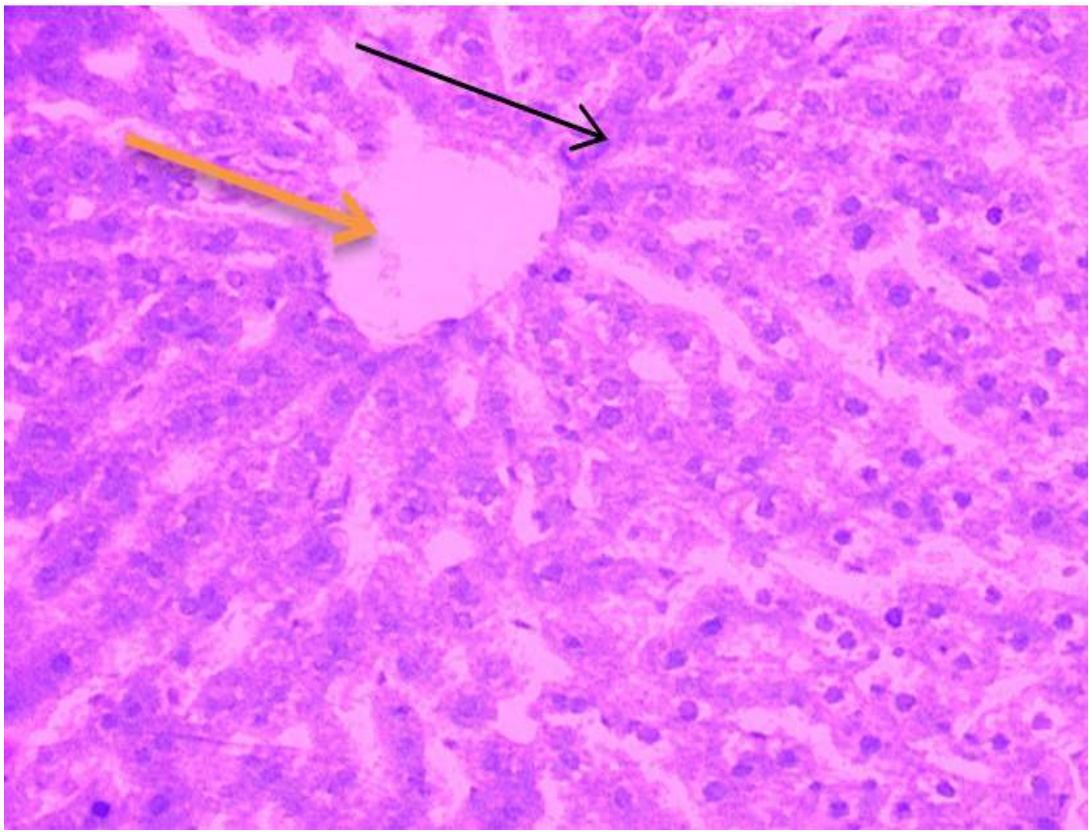

Plate 1: Photomicrograph of rat's liver obtained from control

(H and E stain, x 100 magnification). Showing normal portal triad (black arrow) and normal central vain (red arrow)

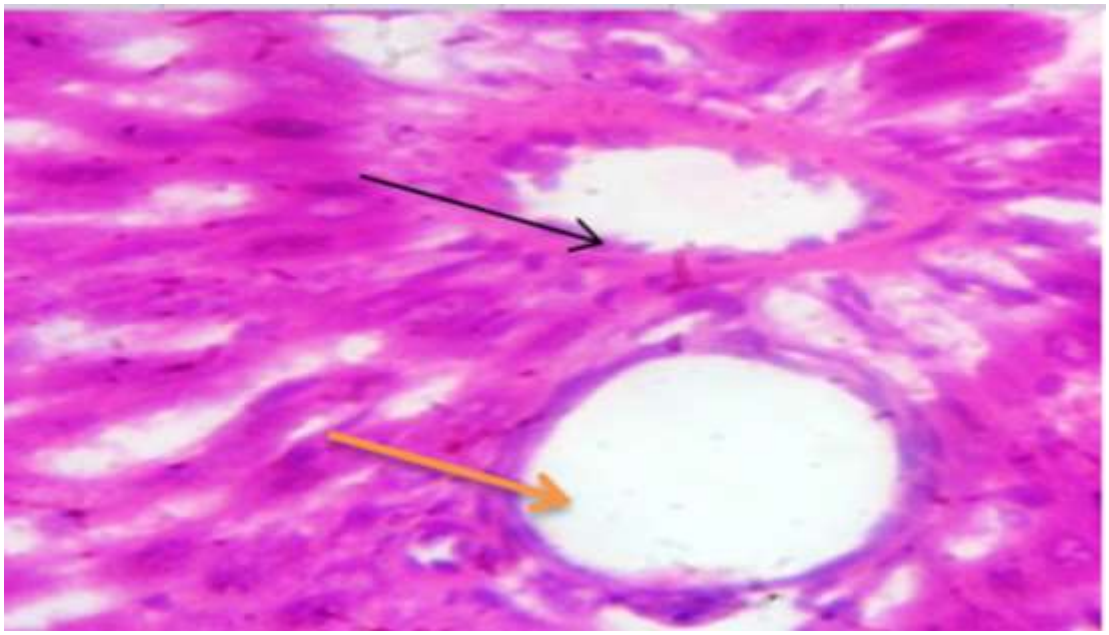

Plate 2: Photomicrograph of rat's liver obtained from alloxan control

(H and E stain, x 100 magnification). Showing normal portal triad (black arrow) and normal central vain (red arrow)

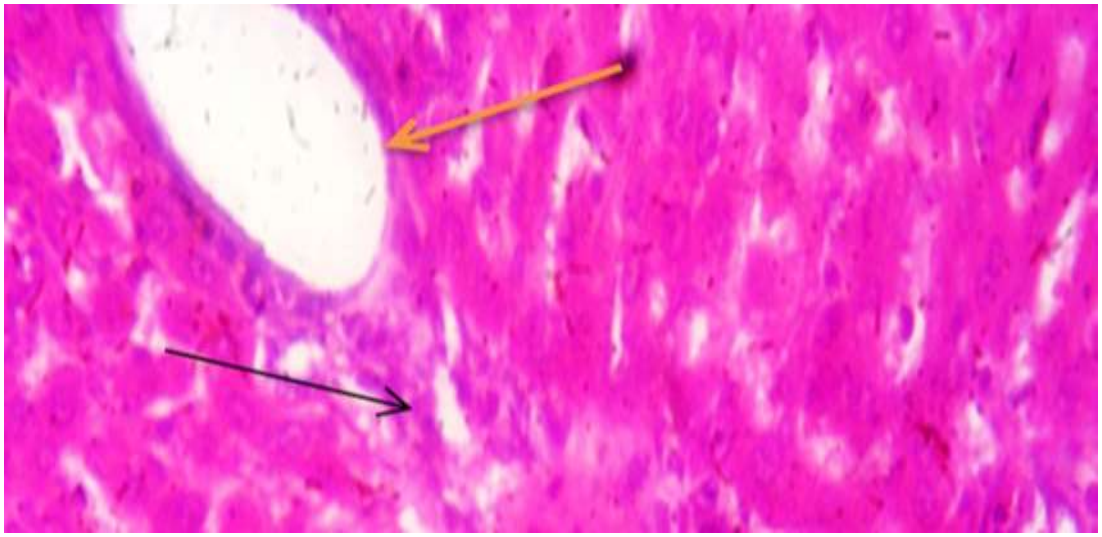

Plate 3: Photomicrograph of rat's liver obtained from group administered with glibenclamide 5 mg/kg

(H and E stain, x 100 magnification). Showing normal portal triad (black arrow) and normal central vain (red arrow)

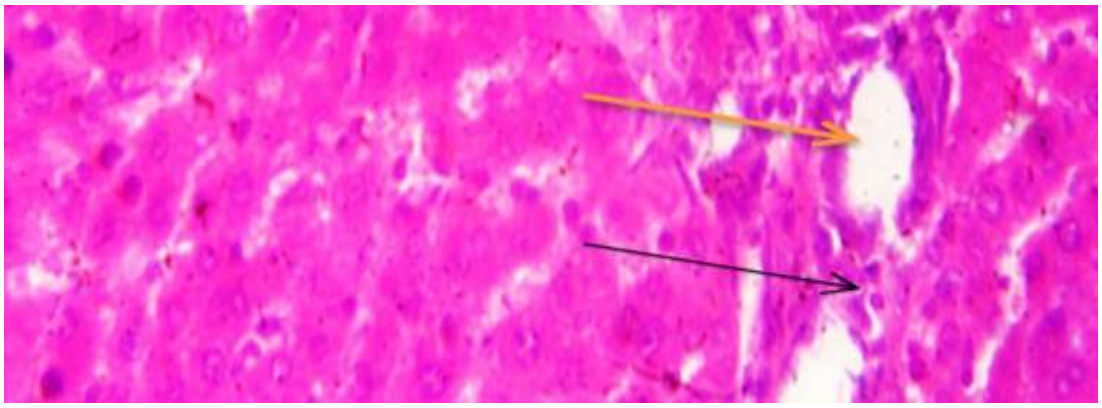

Plate 4: Photomicrograph of rat's liver obtained from group administered with 250 mg/kg *M. charentia* Methanol Leaf Extract

(H and E stain, x 100 magnification). Showing normal portal triad (black arrow) and normal central vain (red arrow)

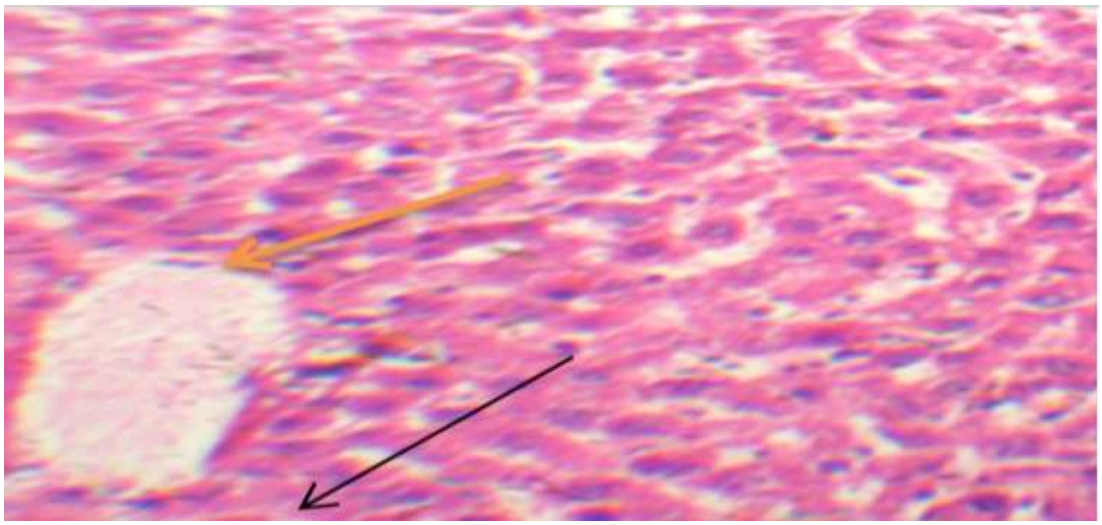

Plate 5: Photomicrograph of rat's liver obtained from group administered with 250 mg/kg *M. charentia* methanol leaf extract

(H and E stain, x 100 magnification). Showing normal portal triad (black arrow) and normal central vain (red arrow)

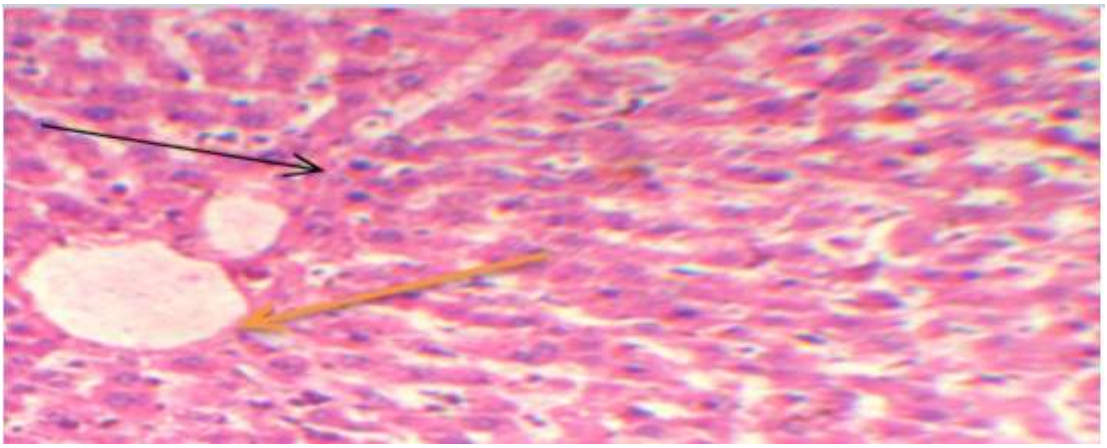

Plate 6: Photomicrograph of rat's liver obtained from group administered with 250 mg/kg *M. charentia* Methanol Leaf Extract on FBS of Diabetic Rats

(H and E stain, x 100 magnification). Showing normal portal triad (black arrow) and normal central vain (red arrow)

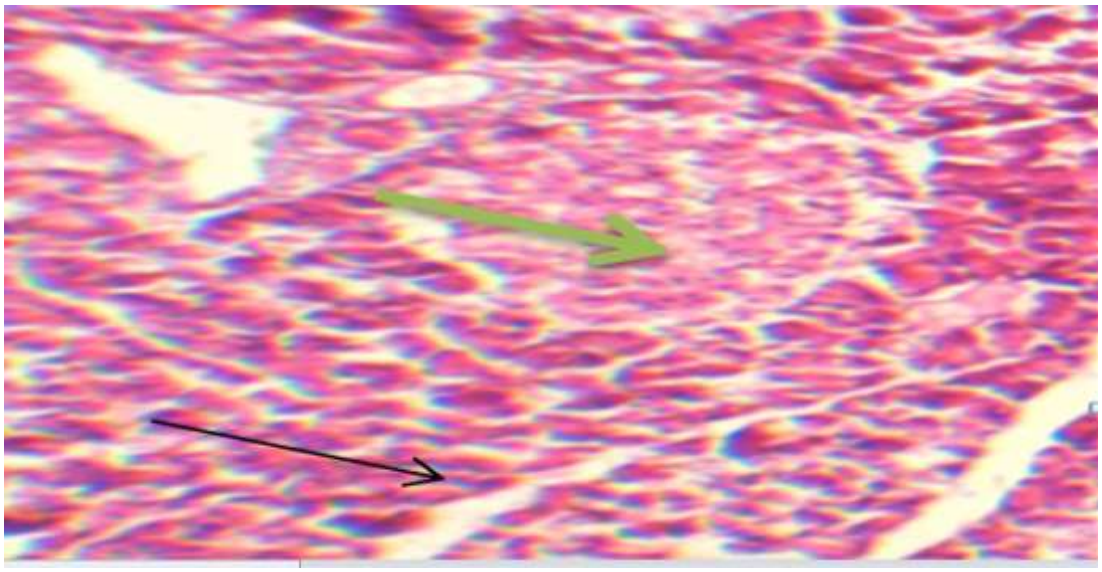

Plate 7: Photomicrograph of rat's pancreas obtained from control

Showing normal exophytic glands (black arrow) and adequate islets of (Langerhans green arrow)

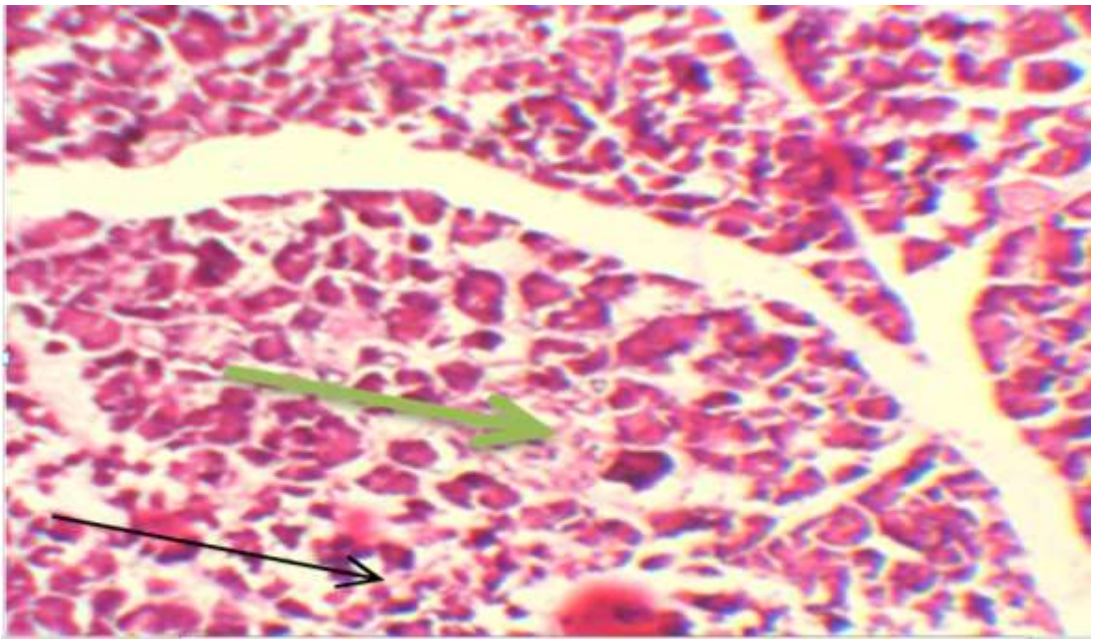

Plate 8: Photomicrograph of rat's pancreas obtained from alloxan control

Showing normal exophytic glands (black arrow) and atrophied islets of Langerhans (green arrow)

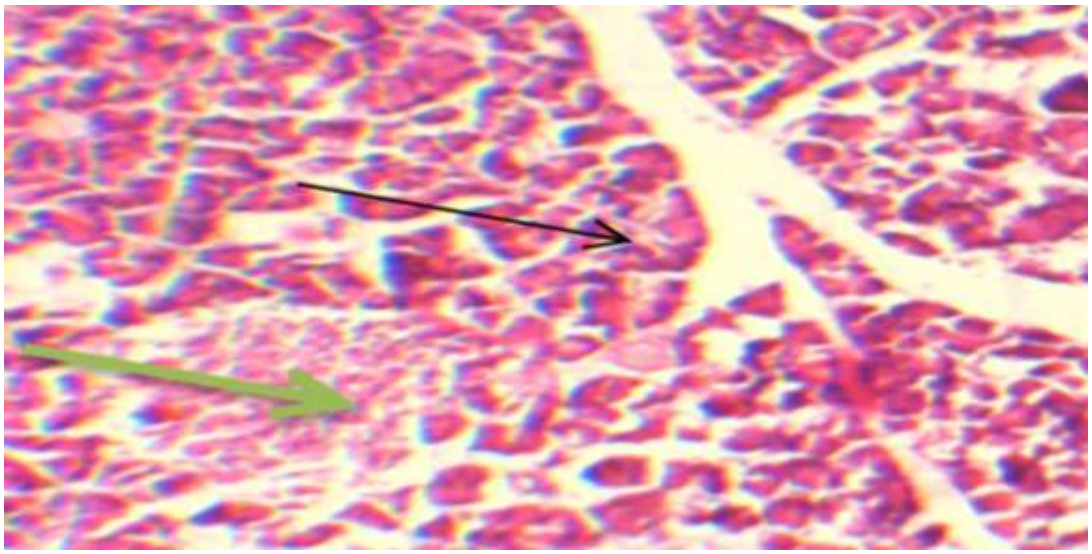

Plate 9: Photomicrograph of rat's liver obtained from group administered with glibenclamide 5mg/kg

showing normal exophytic glands (black arrow) and adequate islets of Langerhans (green arrow)

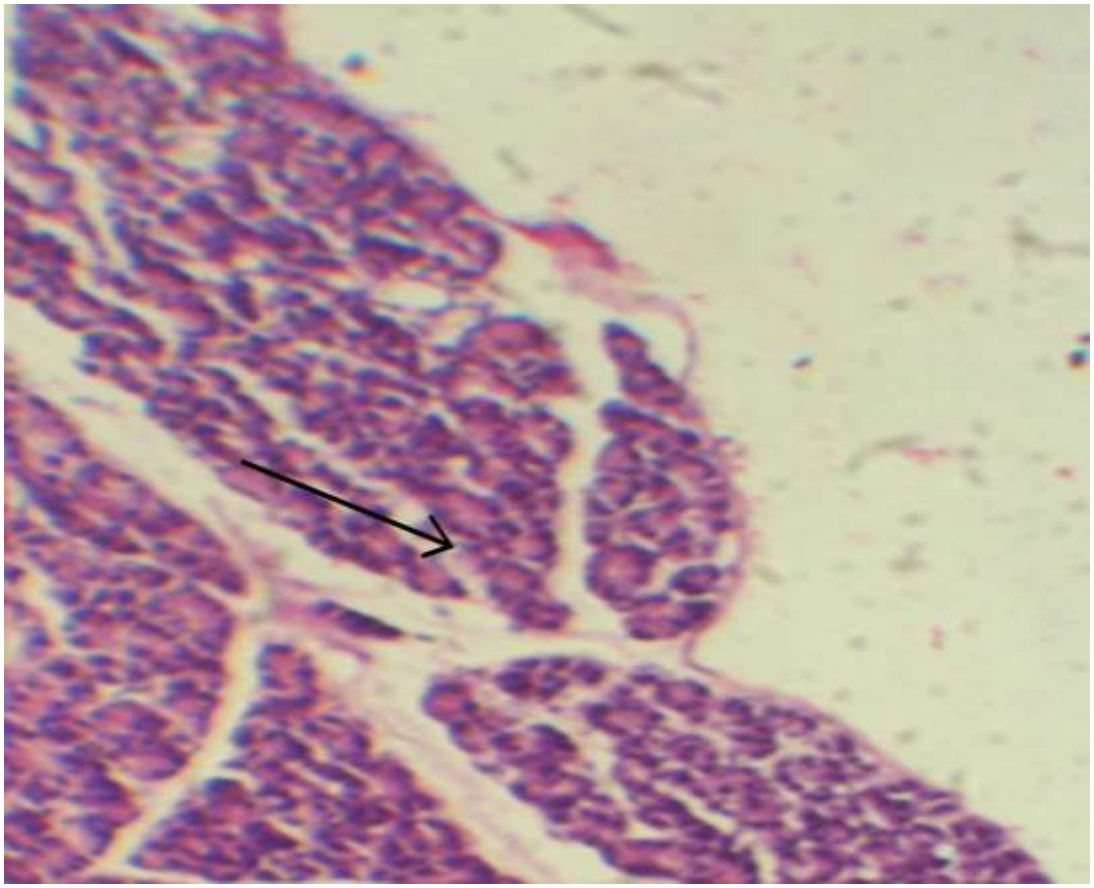

Plate 10: Photomicrograph of rat's pancreas obtained from group administered with 100 mg/kg *M. charentia* Methanol Leaf Extract

Showing normal exophytic glands (black arrow) and no visble islets of Langerhans (green arrow)

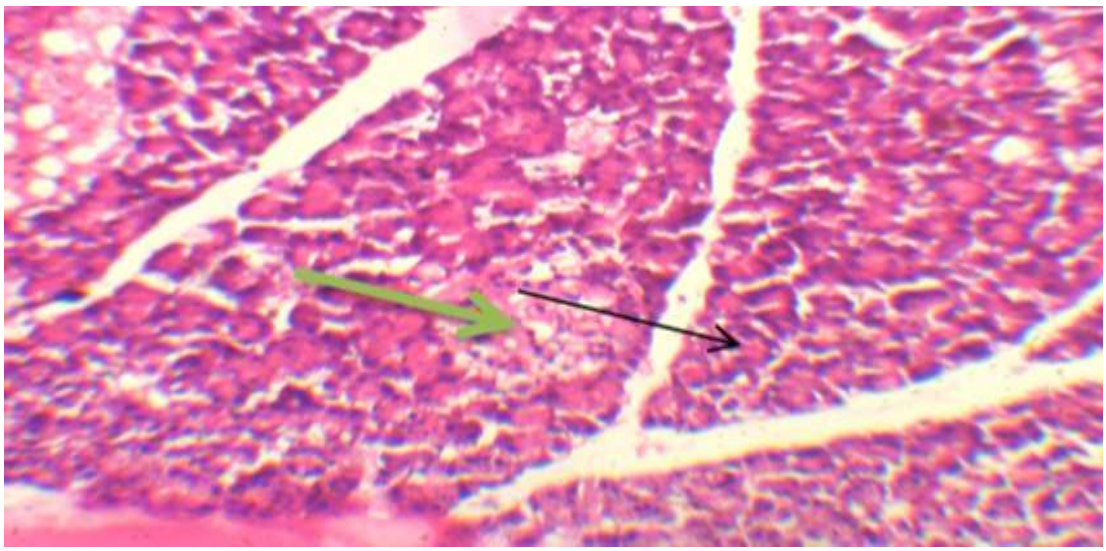

Plate 11: Photomicrograph of rat's liver obtained from group administered with 200 mg/kg *M. charentia* Methanol Leaf Extract

Showing normal exophytic glands (black arrow) and a mild atrophied islets of Langerhans(green arrow)

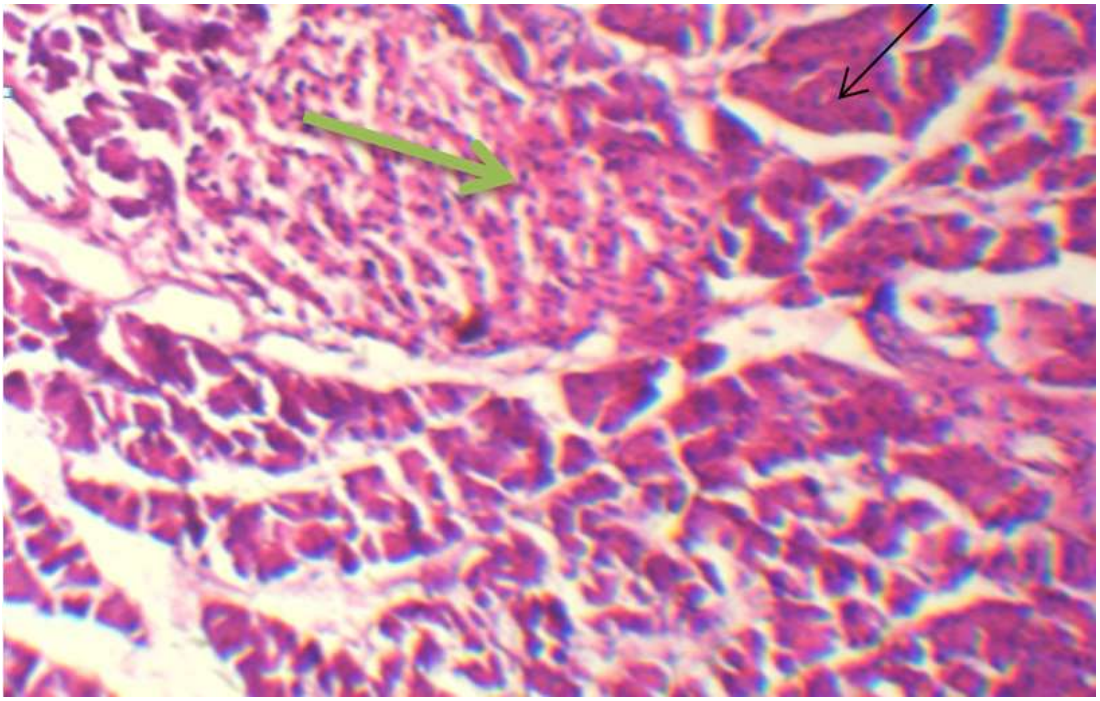

Plate 12: Photomicrograph of rat's liver obtained from group administered with 400mg/kg *M. charentia* Methanol Leaf Extract

Showing normal exophytic glands (black arrow) and normal islets of Langerhans (green arrow)

DISCUSSION

Different phytochemical have been reported to several antidiabetic activities. alkaloids have been isolated from several medicinal plants and investigated for their possible antidiabetic activity in different animal models (Mukherjee *et al.*, 2006). Alkaloids exert a wide range of antidiabetic activities through different mechanisms (Moudi*et al.*, 2013). Also, alkaloids extracted from the leaves of mulberry were found to exhibit hypoglycemic effects in streptozotocin- (STZ) induced diabetic mice (Han *et al.*, 2020). It has been reported that 1-deoxynojirimycin (DNJ), a mulberry alkaloid, reduces the activity of α-glucosidase by competitive inhibition. Upon oral administration of starch and sucrose in Kunming mice, flavonoids from mulberry leaf reduced blood glucose level and inhibited α-glucosidase activity (Kim *et al.*, 2011).According to Kumar and Patwa, (2018) phyto-compounds like flavonoids, alkaloids, tannins, steroids and glycosides is assumed to be effective to treat diabetic complications. Hence, the antidiabetic activity of *M. charentia* methanol leaf extract observed in the present study might be due to the present of these phytochemical.

In theory, the average lethal dose, or LD_{50} provides information on the amount of substance necessary to have undesirable effects on animals; and although biochemical, physiological, reproductive and behavioral effects are very useful determining toxicity, the most commonly used indicator is the death of the test organism (Saganuwan, 2017).Determination of LD_{50} (lethal dose that would kill 50% of the tested population) is usually the first step in the evaluation of the toxic characteristics of a substance (Ogbuehi*et al.*, 2015). This study showed that the LD_{50} of methanol leaf

extract of *M. charentia* is greater than 5000 mg/kg indicating that they are nontoxic at "acute dose".

Weight loss is commonly observed during diabetic condition. This is likely due to the breakdown of adipocytes and muscle tissues to replace energy lost from the body due to frequent urination and increased glycogenolysis (breakdown of glycogen to glucose) (Ene *et al.*, 2007). In this study, the untreated diabetic rats showed bodyweight lost during the entire experimentalperiod these reduction in body weights agrees with the work of Davis andHumphrey, (2012), where their n-hexane extract had similar effects on diabetic animal models.

In the present study, diabetic rats were diagnosed with extremely high fasting blood glucose levels above 150mg/dl. However, upon treatment with *M. charentia* methanol leaf extract, a weekly reduction in FBGLs was observed, just as was observed with the standard drug. This agrees with the works of Viswanathaswamy *et al.,* (2011).

Extracellular superoxide dismutase (SOD) is the major extracellular scavenger of reactive oxygen species and associated with the diabetic complication in patients with type 2 diabetes mellitus (T2DM) (Zhao *et al.*, 2018). Catalase (CAT) has been shown to be responsible for the detoxification of significant amount of hydrogen peroxides (H_2O_2) (Cheng *et al.,* 2018). Catalase is one of the regulators of free radical hydrogen peroxide metabolism. Reduced enzyme activity confirms weakened antioxidant enzyme system in diabetes (Rodriguez *et al.*, 2014). Decrease in the reduced GSH level and impairment in GSH metabolism have been reported in the erythrocyte of diabetics (Jain and McVie, 1994). Decrease in the level of GSH occurs both due to the competition between aldose reductase and glutathione reductase for NADPH, a cofactor, and increased oxidative stress (Song *et al.*, 2003). MDA is a major product of lipid peroxidation and thus an index for measuring the degree of lipid

peroxidation and alteration in the cellular redox status(Cardona, 2004). This research is in agreement with the previous study of Kakkar *et al.,*(1998), which reported the induction of diabetes in rats with alloxan results to an increase in lipid peroxidation, an indirect evidence of intensified free radical production. Controversial reports on changes in serum/plasma SOD, CAT and GSH activity of diabetic patients have been published. In some, a decrease in the activity was observed, whereas in others an increase was reported (El-barbary *et al*., 2011).In the presented study, the reduction in these enzymatic antioxidants may be due to weakened enzyme system as a result of diabetic complication.

Common antioxidants include vitamin A, C and E, non-enzymatic antioxidant they work in synergy with each other and against different types of free radicals(Habibi, 2014). The impairment of the endogenous antioxidant defense system is produced in many ways during chronic hyperglycaemia (Papachristoforou *et al*., 2020).Wu *et al*., (2022) reported that concentration of different vitamins (A, C and E) in diabetic rats were found to have decrease in there dose dependent manner. This agreed with the findings of the present study as the non-enzymatic antioxidants all decreases possibly due to the declines in the activity of enzyme system as a result of diabetic complication.

Diabetic complication is characterized by high levels of total cholesterol (T-Chol), high levels of triglycerides (Tg), low levels of high density lipoprotein cholesterol (HDL-C) and increased levels of small dense LDL particles (Bhowmik *et al*., 2018). Although the extract did not show hypolipidemic effect, but revealed an increase in levels of (T-chol), (TG), (LDL) and decreases in (HDL) which are all attributed to diabetic complications. The present study suggest that *M. charentia* methanol leaf extract have minimal hypolipidemic potential

Conclusion

In conclusion, *M. charentia* methanol leaf revealed an outstanding antidiabetic potential with regenerational effect on pancreatic islets of Langerhans, and also revealed a non-toxic profile at acute dose. However, the extracts exhibit minimal hypolipidemic and antioxidant activities on diabetic rats.

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