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ANTIDIABETIC AND HAEMATOLOGICAL EFFECT OF *MYRIANTHUS ARBOREUS* P. BEAUV. STEM BARK EXTRACT IN STREPTOZOTOCIN - INDUCED DIABETIC RATS

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Keywords:

Anti-diabetic, Hypoglycaemia, Antihyperlipidaemia, Streptozotocin, Glibenclamide, *Myrianthus arboreus*

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ABSTRACT: The increasing prevalence of diabetes mellitus worldwide is an issue of major socio-economic concern especially in Sub - Saharan Africa. Indigenous medicinal plants are used for the treatment of diabetes mellitus in most developing countries, like Ghana, but remain to be validated. In the present study, the effect of *Myrianthus arboreus* ethanol stem bark extract (MAB) (100 - 400 mg/kg) on glucose levels in streptozotocin (STZ) (45 mg/kg) induced diabetic rats was investigated using glibenclamide 5 mg/kg/day as the positive control. The effects of the extract on body weight, total protein, serum urea, serum creatinine, bilirubin, lipid profile, haematological indices and serum markers for liver function in normal, treated and untreated diabetic rats were also investigated. Induction of the diabetes in Sprague Dawley rats (150-200 g) resulted in increased levels of serum glucose, total cholesterol, triglycerides and LDL-cholesterol but decreased body weight, serum HDL-cholesterol and haemoglobin levels. Administration of the extract at the three dose levels resulted in significant (P<0.001) reduction in the levels of plasma glucose, cholesterol, LDL-cholesterol, triglycerides and serum urea and serum creatinine. Alanine transaminase (ALT) and aspartate transaminase (AST) levels were also significantly (P<0.001) decreased. The significant decrease in body weight, total protein and HDL-cholesterol which were observed in STZ-induced diabetic rats were normalized after 28 days treatment with the extract. At 200 mg/kg/day, MAB recorded significant (P<0.01) reduction in plasma glucose levels compared to glibenclamide (5 m/kg/day). Thus MAB shows significant hypoglycaemic and antihyperlipidaemic activities in STZ-induced diabetic rats justifying its use in traditional medicine.

INTRODUCTION: Diabetes is a metabolic disorder characterized by chronic hyperglycaemia and alterations in carbohydrate, protein and lipid metabolism with absolute or relative deficiencies in insulin secretion and/or insulin production [1].

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It is estimated that about 347 million are living with diabetes worldwide with the disease claiming the lives of 1.5 million people in the year 2012. More than 80% of these deaths caused by diabetes occurred in low- and medium-income countries [2]. Diabetes has been projected to become the 7th leading cause of death in the world by 2030 [3]. The effects of diabetes include long term complications and severe disabilities such as kidney disease, heart attack, stroke and neural damage leading to amputation and need for chronic care [4].

The conventional antidiabetic agents are not affordable to a larger populace in developing countries. Coupled with the side effects of these synthetic drugs, they depend on less expensive and readily available medicinal plants with less side effects for the management of the disease [5]. Most of these medicinal plants used traditionally in the management of diabetes have not been scientifically investigated to provide information on their usefulness [6].

Myrianthus arboreus P. Beauv. (Moraceae) occurs in the tropical zone of West Africa and is locally known as 'Anyankoma' by the Akans in Ghana. The leaves, fruits and stem of the plants are used in Ghana and in other parts of West Africa for the treatment of several diseases including fever anaemia, diarrhoea, dysentery, pains and cough [7]. The leaves and fruits are edible and a rich source of nutrients [8]. Decoctions prepared from the bark of the plant are used in the management of diabetes [9, 10]. *M. arboreus* has been shown to possess antimicrobial, antiamoebic, antitubercular and antioxidant activities [11, 12] which supports some of its uses in traditional medicine.

The leaves of *M. arboreus* have been shown to be potent analgesics and useful in the management of pain [13] and also have demonstrated good activity against multi-drug resistant gram-negative bacteria [14]. Not much has been done to justify the traditional use of *M. arboreus* stem bark in the treatment of diabetes although [15] showed that the stem of the plant exhibited hypoglycaemic effects in his patients. The present study was, therefore, undertaken to investigate the antidiabetic effect of the ethanol extract of the stem bark of *M. arboreus* and its effects on some biochemical and metabolic parameters in streptozotocin-induced diabetic rats.

2. MATERIALS AND METHODS:

2.1 Chemicals:

Streptozotocin (STZ) and glibenclamide were purchased from Sigma-Aldrich, (St. Louis, MO, USA). Other commercially available reagents and solvents were used as received and were of analytical grade.

2.2 Plant material and extraction: The stem bark of *M. arboreus* – voucher specimen number (KNUST/HM 1/2013/S005) was collected from Kwahu, in the Eastern Region of Ghana, in March, 2013. The plant was identified and authenticated at the Department of Herbal Medicine, College of Health Sciences, Kwame Nkrumah University of Science and Technology herbarium where voucher specimen has been deposited. The stem bark was dried at room temperature and coarsely powdered. The powder (1.5 kg) was Soxhlet extracted for 48 h using 70% ethanol and the liquid extract concentrated under reduced pressure at 40°C until the solvent for extraction was completely removed to obtain a brown residue (MAB) (yield: 5.1% $^{w}/_{w.}$).

2.3 Phytochemical screening: The powdered stem-bark of *M. arboreus* was subjected to preliminary screening for secondary metabolites present according to methods described by [16].

2.4 Experimental animals: Adult male Sprague-Dawley rats weighing between 150–200 g were obtained from Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon and allowed a period of seven days to get accustomed to the laboratory's conditions and circumstances. They were housed in stainless steel cages lined with saw dust in standard environmental conditions (temperature 25 ± 2^{o}C and 12:12 light: dark cycle) at the animal house of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology. The rats were fed on standard pellet diet and water *ad libitum*. All the rats were treated in accordance with the National Institute of Health Guidelines for the care and use of laboratory animals (NIH, Department of Health and Human Services publication No 5, revised 1985). The research protocol was done with the approval of the College of Health Sciences Ethics Committee.

2.5 Oral glucose tolerance test (OGTT): In a bid to select the optimal dose of the extract to be used in this study, a glucose tolerance test (OGTT) was first performed in normal rats using three different doses of MAB (100, 200 and 400 mg/kg body weight (bwt) for 120 min [17].

OGTT was performed in 30 overnight - fasted (18 h) normal rats divided into five groups of six rats each. Group 1 served as normal control receiving orally, distilled water. Group 2 received orally, the

reference drug glibenclamide at a dose of 5 mg/kg bwt. Groups 3, 4 and 5 received orally 100 mg/kg, 200 mg/kg and 400 mg/kg bwt of MAB dissolved in distilled water respectively. After 30 min of treatment, all the groups were orally loaded with 2 g/kg bwt of glucose. Blood samples were collected from the tail vein just prior to glucose administration and also at 30, 60 and 120 min after glucose loading. Blood glucose levels were measured using One Touch Select glucometer (LifeScan, Inc. Milpitas, CA 95035 USA).

2.6 Induction of diabetes: The animals were fasted for 16 h, and diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) (45 mg/kg) freshly dissolved in 0.1 M cold sodium citrate buffer (pH 4.5) [18, 19]. The animals were allowed to drink 5% glucose solution overnight to overcome the initial hypoglycaemia induced by the STZ. Three days after injection of STZ, fasting blood glucose was determined and the rats with blood glucose above 10.00 mmol/l [20] were used for the study. The treatment was started on the 4th day after STZ-injection and this was considered as the 1st day of treatment which continued for 28 days.

2.7 Experimental design: In the experiment, 36 rats (30 diabetic rats and 6 normal rats) divided into six groups of six rats each were used: group 1 (normal rats treated with distilled water [DW]), group 2 (diabetic rats treated with DW), group 3 (diabetic rats treated with 100 mg/kg bwt MAB, orally), group 4 (diabetic rats treated with 200 mg/kg bwt MAB, orally), group 5 (diabetic rats treated with 400 mg/kg bwt MAB, orally) and group 6 (diabetic rats treated with 5 mg/kg bwt glibenclamide, orally). The doses of *M. arboreus* stem-bark extract and standard drug were prepared fresh daily in 2% tragacanth solution. The freshly prepared suspensions were orally administered daily using gastric gavage for 28 days.

2.8 Determination of biochemical, metabolic and histopathological parameters: Blood was collected from the tail vein of the animals after application of lignocaine and the blood glucose levels measured for the first six hours using One Touch Select glucometer (Life Scan, Inc. Milpitas, CA 95035 USA). Blood glucose levels were also measured at two days intervals for 28 days. The lipid profiles of the animals, which included triglyceride (TG) levels, total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were determined using the Mindray BS-200 chemistry analyzer (www.mindray.com). Low density lipoprotein cholesterol (LDL-C) was calculated manually using the Friedewald equation [21]. Renal function tests comprising urea and creatinine tests, liver function tests involving the liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransaminase (AST), total bilirubin (T BIL), direct bilirubin and total protein (TP) were also determined using the Mindray BS-200 chemistry analyzer (www.mindray.com). Haematological indices were measured after day 28 of the experiment using the Sysmex XT 2000 Haematology auto analyzer (www.sysmex.com). The body weights of the experimental animals were also measured weekly for the 28 days.

Histological studies were conducted on the liver, lungs, pancreas and kidneys of the rats. The organs were excised after sacrificing the animals on day 29, weighed and fixed in 10% buffered formalin. They were then cut up and processed in an automated Microm Tissue Processor STP 120 (Microtom International, Walldorf, Germany). Thin sections were cut with a rotary microtome (microm) stained with Harris haematoxylin and eosin, and mounted on slides and examined using a light microscopic.

2.9 Statistical Analysis: Results were presented as mean ± S.E.M. One way ANOVA and Newman – Keuls Mutiple Comparison Test (Graphpad Prism version 5) were carried out to compare the data with the level of significance set at $p \leq 0.05$ against diabetic control.

3. RESULTS:

3.1 Effect of MAB on OGTT: Fig. 1 depicts the effect of MAB (100, 200 and 400 mg/kg bwt), p.o. on OGTT. The administration of *M. arboreus* extract at the various doses significantly ($P \leq 0.01$) prevented increase in blood glucose levels without causing a hypoglycaemic state after loading the animals with glucose solution. The extract at 400 mg/kg showed an effect comparable to that of glibenclamide (5 mg/kg).

3.2 Effect of MAB on plasma blood glucose of diabetic rats: Fig. 2 shows the effect of MAB on the blood glucose levels of STZ-induced diabetic rats 6 h after treatment. The blood glucose level of untreated diabetic rats after 6 h increased by 23.74 %. The blood glucose levels of diabetic rats treated with MAB at doses of 100, 200 and 400 mg/kg decreased by 52.28 %, 66.38 % and 69.84 % respectively. After the 6th hour, glibenclamide (5 mg/kg) lowered the blood glucose by 74.34 %. The extract at the selected doses showed significant hypoglycaemic activity ($P < 0.001$) compared to the diabetic control group 6 h after treatment.

The effect of repeated oral administration of MAB on blood glucose levels in STZ-induced diabetic rats is presented in **Fig. 3.** MAB administered at the various doses of 100, 200 and 400 mg/kg to treated diabetic rats caused a significant reduction in blood glucose levels which was related to the dose and duration of treatment. Maximum reduction occurred on day 28. The hypoglycaemic activity of MAB at 200 mg/kg was higher than that of the standard drug glibenclamide. Both the extract and glibenclamide exhibited statistically significant ($P<0.001$) hypoglycaemic activities compared to diabetic control group. There was no significant difference between the glucose levels of diabetic rats treated with MAB and the animals in the normal control group after day 12 of the experiment.

3.3 Effect of MAB on the body weight of STZ – induced diabetic rats: Streptozotocin produced significant loss in the body weights of experimental animals after administration as compared to the animals in the normal control group. The animals in the diabetic control group continued to lose weight till the end of the study. Treated animals lost weight from day 1 of the study to day 7. MAB administration at all the doses (100, 200 and 400 mg/kg) showed significant improvement in body weight after day 7 till the end of the study (**Fig. 4**).

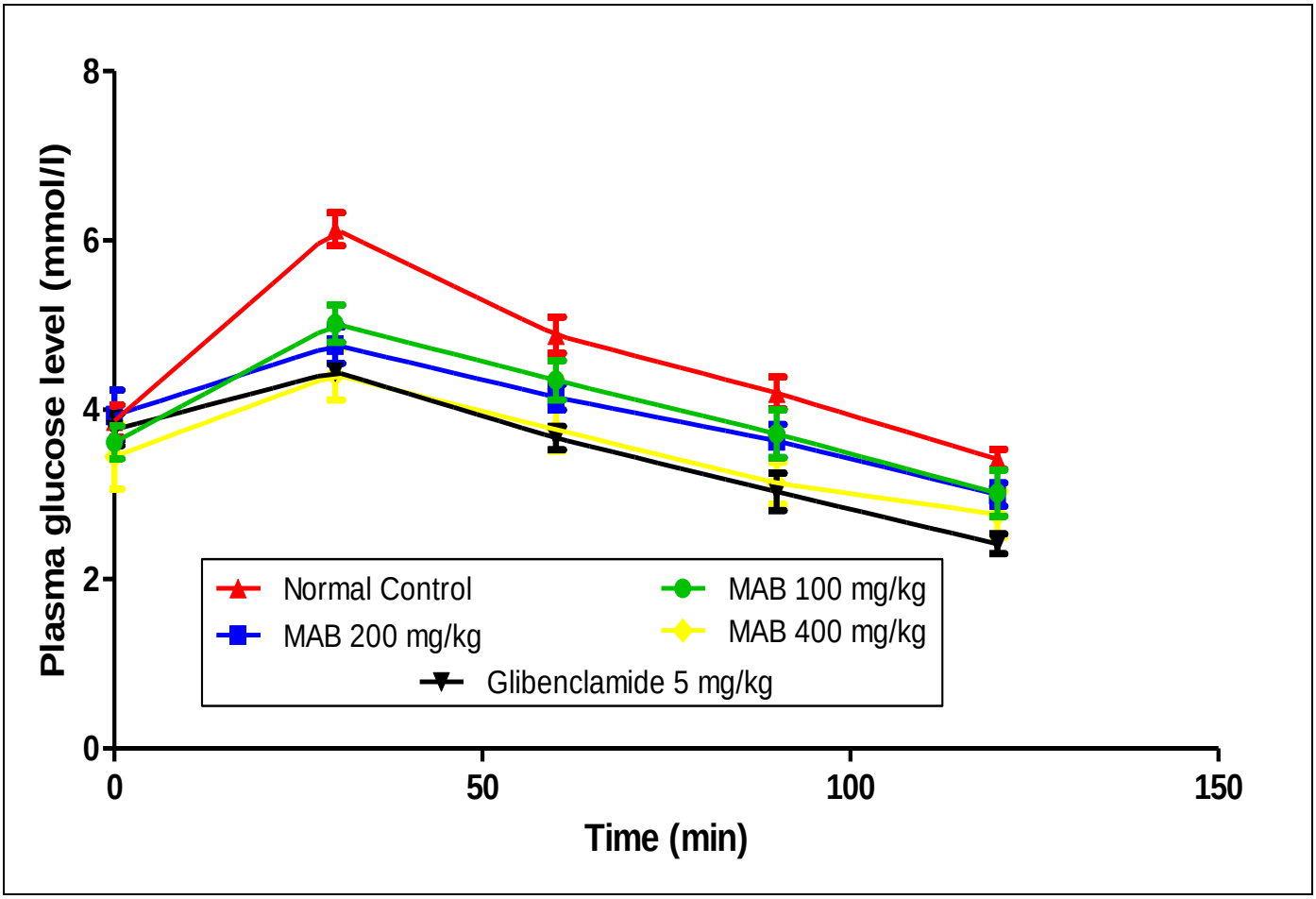


FIG. 1: EFFECT OF MAB ON ORAL GLUCOSE TOLERANCE IN EXPERIMENTAL RATS. VALUES INDICATE MEAN ± SEM (N = 6).

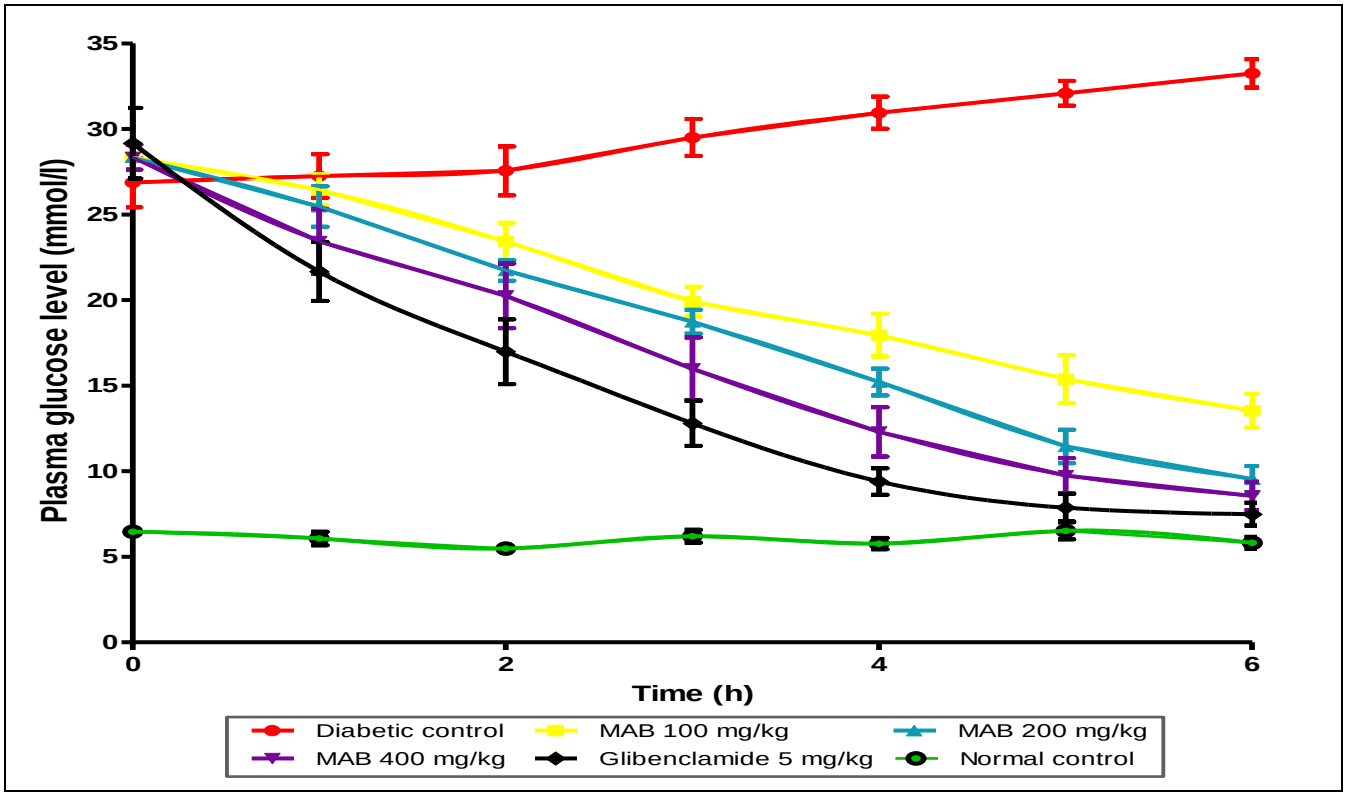


FIG. 2: EFFECTS OF MAB ON PLASMA GLUCOSE LEVELS OVER 6 H AFTER ADMINISTRATION. VALUES INDICATE MEAN ± SEM (N = 6).

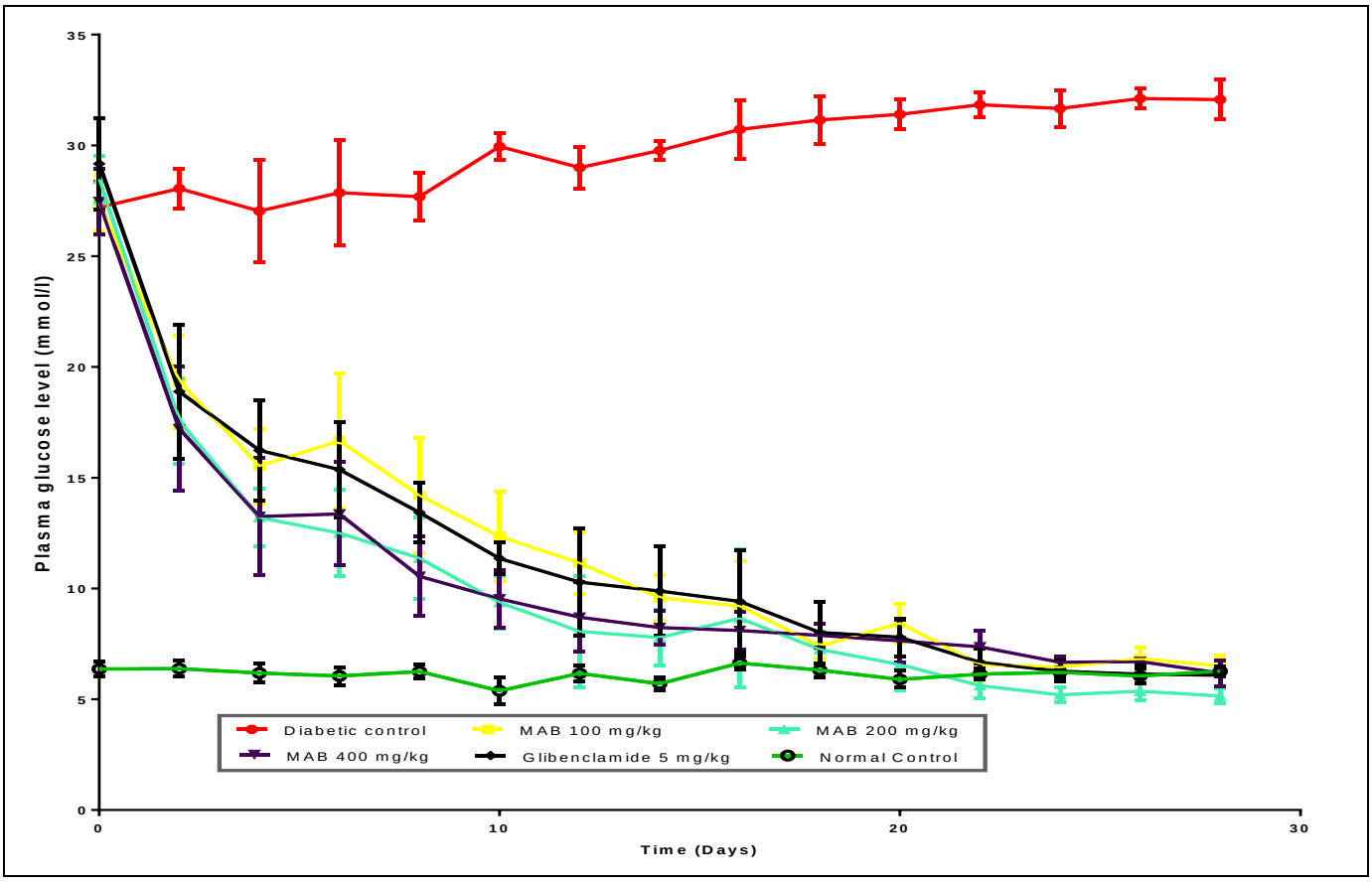


FIG. 3: EFFECT OF MAB ON PLASMA GLUCOSE LEVELS IN STZ-INDUCED DIABETIC RATS, OVER 28 DAYS PERIOD AFTER TREATMENT. VALUES INDICATE MEAN ± SEM (N = 6).

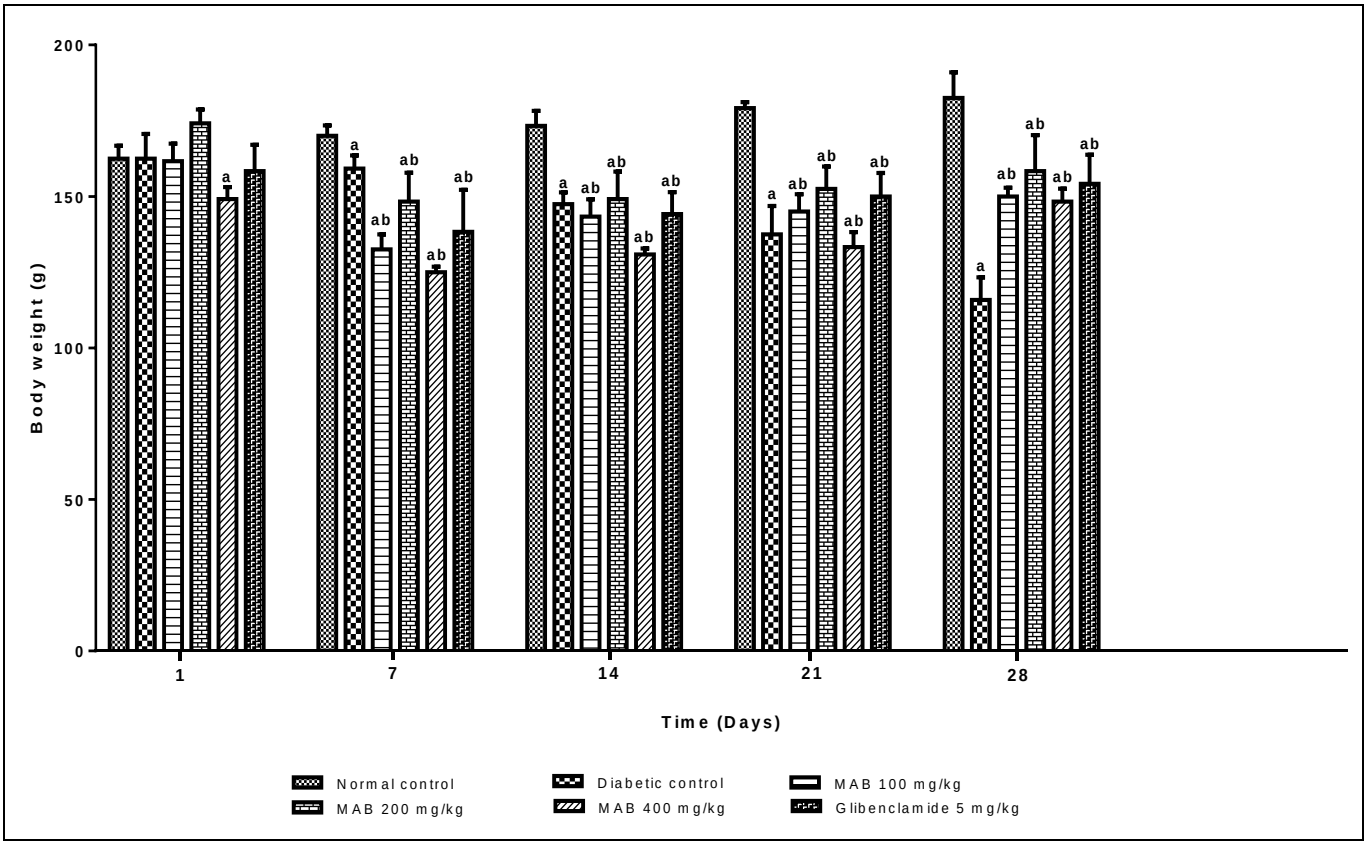


FIG. 4: EFFECT OF MAB ON BODY WEIGHT OF STZ-INDUCED DIABETIC RATS. EACH VALUE IS EXPRESSED AS MEAN ± S.E.M (n=6). ^{a}P<0.05 WHEN COMPARED TO CORRESPONDING VALUES OF THE NORMAL CONTROL. ^{b}P<0.05 WHEN COMPARED TO CORRESPONDING VALUES OF THE DIABETIC CONTROL.

3.4 Effect of MAB on water and feed intake:

Water and feed intake of the rats per body weight were measured for 28 days. After 28 days, no significant changes were observed in both feed and water intake for the animals (**Fig. 5** and **6**), although untreated diabetic rats were found to have consumed more water than the rest of the animals while rats treated with 400 mg/kg of the extract were found to have consumed more feed.

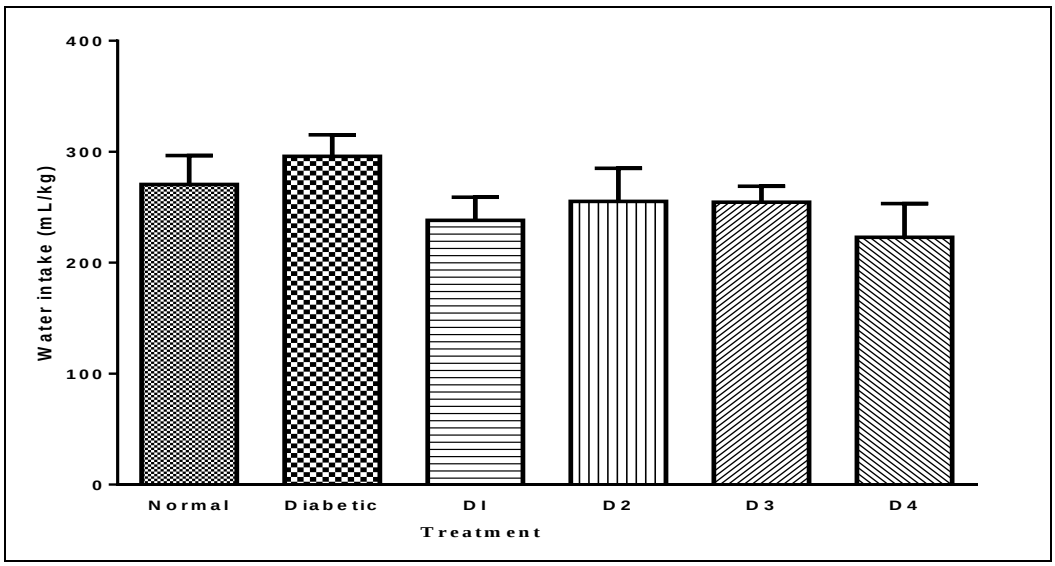


FIG. 5: EFFECT OF MAB ON WATER INTAKE OF ANIMALS

D1- diabetic rats treated with 100 mg/kg of MAB
D2- diabetic rats treated with 200 mg/kg of MAB
D3- diabetic rats treated with 400 mg/kg of MAB
D4- diabetic rats treated with Glibenclamide

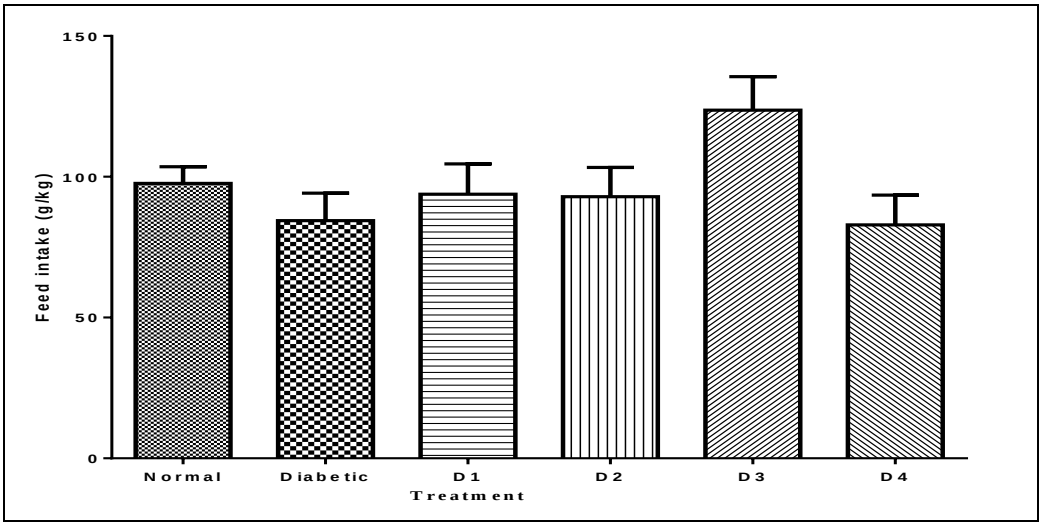


FIG. 6: EFFECT OF MAB ON FEED INTAKE OF ANIMALS
D1- diabetic rats treated with 100 mg/kg of MAB
D2- diabetic rats treated with 200 mg/kg of MAB
D3- diabetic rats treated with 400 mg/kg of MAB
D4- diabetic rats treated with Glibenclamide

3.5 Effect of MAB on the lipid profile of STZ – induced diabetic rats after 28 days: Table 1 shows the increased level of total cholesterol, triglycerides, LDL-cholesterol and decreased HDL-cholesterol in diabetic rats compared to normal control. Administration of MAB and glibenclamide for 28 days significantly reduced the total cholesterol, triglycerides and LDL-cholesterol and significantly increased the HDL-cholesterol when compared to diabetic rats.

3.6 Effect on AST, ALT, plasma protein, serum urea and serum creatinine: Table 2 shows the activities of AST, ALT and the levels of plasma protein, serum urea and serum creatinine. A significant increase in the activities of AST and ALT was observed in STZ-induced diabetic rats. After treatment with MAB at 100, 200 and 400 mg/kg, the activities of AST and ALT were significantly reduced ($P<0.001$) compared to diabetic control groups. There was a significant elevation in the levels of serum urea and serum creatinine with a significant decrease in plasma proteins when compared with corresponding values of the normal control group. However, the oral supplementation of MAB at the three doses brought the values to near normal which was similar to the effect observed with glibenclamide.

3.7 Effect on total, direct and indirect bilirubin: After 28 days of the study, there were no significant increases in bilirubin levels for both treated and untreated rats as shown in **Table 3**.

3.8 Effect of MAB on the haematological indices of diabetic rats: The number of red blood cells (RBCs), haemoglobin concentration (Hb), haematocrit (HCT), mean cell volume (MCV), mean cell haematocrit (MCH) and Mean corpuscular haemoglobin concentration (MCHC) levels in untreated diabetic rats were significantly reduced when compared to normal rats (**Table 4).** However, these were brought near normal when rats were treated with the extract. The white blood cell count in both treated and untreated rats was significantly reduced (**Table 5**) when compared to the normal. However, plasma levels of monocytes, lymphocytes, neutrophils and platelet number for all rats were all close to the normal values.

TABLE 1: LIPID PROFILE OF EXPERIMENTAL RATS AFTER 28 DAYS OF TREATMENT

Experimental Groups	Lipid profile (mg/dL)			
	Total cholesterol	Triglycerides	LDL-cholesterol	HDL-cholesterol
Normal control	79.79 ± 7.53[b]	84.73 ± 15.86[b]	26.19 ± 6.63[b]	36.66 ± 3.62[b]
Diabetic control	126.00 ± 6.41	166.30 ± 17.59	76.34 ± 6.55	16.38 ± 2.27
MAB (100 mg/kg)	69.24 ± 5.42[a]	69.24 ± 5.34[a]	45.28 ± 10.89[c]	20.12 ± 1.38
MAB (200 mg/kg)	72.54 ± 8.87[a]	56.43 ± 4.81[a]	29.27 ± 11.77[c]	31.98 ± 5.14[c]

MAB (400 mg/kg)	78.16 ± 13.03[b]	74.23 ± 18.70[a]	35.23 ± 12.43[c]	28.08 ± 5.14
Glibenclamide (5 mg/kg)	85.57 ± 4.22[b]	94.70 ± 14.25[b]	40.11 ± 3.58[c]	26.52 ± 2.27

[a]*P*<0.001 compared to corresponding values of the diabetic control.
[b]*P*<0.01 compared to corresponding values of the diabetic control.
[c]*P*<0.05 compared to corresponding values of the diabetic control.
Each value is expressed as mean ± S.E.M (n = 6)
MAB = *Myrianthus arboreus* stem bark ethanol extract.

TABLE 2: LEVELS OF AST, ALT, PLASMA PROTEIN, SERUM UREA AND SERUM CREATININE IN EXPERIMENTAL RATS AFTER 28 DAYS OF TREATMENT.

Experimental groups	AST (U/L)	ALT (U/L)	Total protein (mg/dL)	Serum urea (mg/dL)	Serum creatinine (mg/dL)
Normal control	119.60±1.50[a]	78.00±2.98[a]	8.20±0.27[a]	55.98±2.66[b]	0.73 ± 0.03 [c]
Diabetic control	268.80 ± 4.01	123.00±4.86	4.54±0.53	91.05± 8.88	0.99 ± 0.07
MAB (100 mg/kg)	151.00±9.79[a]	90.60±3.28[a]	7.38±0.31[a]	57.90±6.37[b]	0.72 ± 0.05 [c]
MAB(200mg/kg)	117.00±2.03[a]	77.20±2.70[a]	7.92±0.23[a]	56.10±5.64[b]	0.64 ± 0.02[c]
MAB (400 mg/kg)	119.60±4.26[a]	97.80±6.40[a]	7.52±0.38[a]	58.86±3.23[b]	0.79 ± 0.09 [c]
Glibenclamide (5mg/kg)	125.00±3.63[a]	94.20±5.65[a]	7.50±0.17[a]	56.46±4.08[b]	0.79 ± 0.11

[a]*P*<0.001 compared to corresponding values of the diabetic control.
[b]*P*<0.01 compared to corresponding values of the diabetic control.
[c]*P*<0.05 compared to corresponding values of the diabetic control.
Each value is expressed as mean ± S.E.M (n = 6)
MAB = *Myrianthus arboreus* stem bark ethanol extract.

TABLE 3: EFFECT OF MAB ON TOTAL, DIRECT AND INDIRECT BILIRUBIN LEVELS OF STZ-INDUCED DIABETIC RATS

Experimental groups	Total bilirubin (mg/dL)	Conjugated bilirubin (mg/dL)	Unconjugated bilirubin (mg/dL)
Normal control	0.1006 ± 0.008	0.0561 ± 0.009	0.0444 ± 0.007
Diabetic control	0.0678 ± 0.003	0.0211 ± 0.001	0.0467 ± 0.003
MAB (100 mg/kg)	0.0795 ±0.005	0.0538 ± 0.005	0.0257 ± 0.007
MAB (200 mg/kg)	0.0994 ± 0.007[b]	0.0678 ± 0.008	0.0316 ± 0.003
MAB (400 mg/kg)	0.0877 ± 0.010[b]	0.0491 ± 0.004	0.0386 ± 0.007
Glibenclamide (5mg/kg)	0.0842 ± 0.002[b]	0.0500 ± 0.002	0.0292 ± 0.000

[b]*P*<0.01 compared to corresponding values of the diabetic control.
Each value is expressed as mean ± S.E.M (n = 6)
MAB = *Myrianthus arboreus* stem bark ethanol extract

TABLE 4: EFFECT OF MAB ON RED BLOOD CELLS AND DIFFERENTIALS IN STZ - INDUCED DIABETIC RATS

Parameters	Normal control	Diabetic control	MAB (100 mg/kg)	MAB (200 mg/kg)	MAB (400 mg/kg)	Glibenclamide (5 mg/k)g
RBC($\times10^6$/µL)	6.80 ± 0.30[b]	5.16 ± 0.37	6.11 ± 0.04[c]	7.15 ± 0.19[a]	6.23 ± 0.31[c]	6.16 ± 0.31[c]
Hb (g/dL)	13.00 ± 0.37[a]	9.01 ± 0.23	10.70 ± 0.14[c]	12.85 ± 0.36[a]	10.88 ± 0.62[c]	10.56 ± 0.63[c]
HCT (%)	41.45 ± 1.24[b]	32.07± 2.04	35.33 ± 0.60	41.23± 1.16[b]	34.78 ± 1.78	36.95 ± 2.81
MCV	57.38 ± 0.50[c]	52.67 ± 0.65	60.37 ± 0.57[a]	57.08 ± 0.61[c]	53.8 ± 0.51	63.96 ± 2.68[a]
MCH	17.63±0.27	16.7 ± 0.31	18.12±0.13[c]	18.12 ±0.21[c]	17.42±0.39[c]	18.88 ± 0.45[c]
MCHC	30.93± 0.15	28.7 ± 1.26	30.10±0.24	31.60 ±0.38[c]	31.16±0.49	26.60 ± 0.85[c]

[a]*P*<0.001 compared to corresponding values of the diabetic control.
[b]*P*<0.01 compared to corresponding values of the diabetic control.
[c]*P*<0.05 compared to corresponding values of the diabetic control.
Each value is expressed as mean ± S.E.M (n = 6)
MAB = *Myrianthus arboreus* stem bark ethanol extract.

TABLE 5: EFFECT OF MAB ON WHITE BLOOD CELLS AND DIFFERENTIALS IN STZ - INDUCED DIABETIC RATS

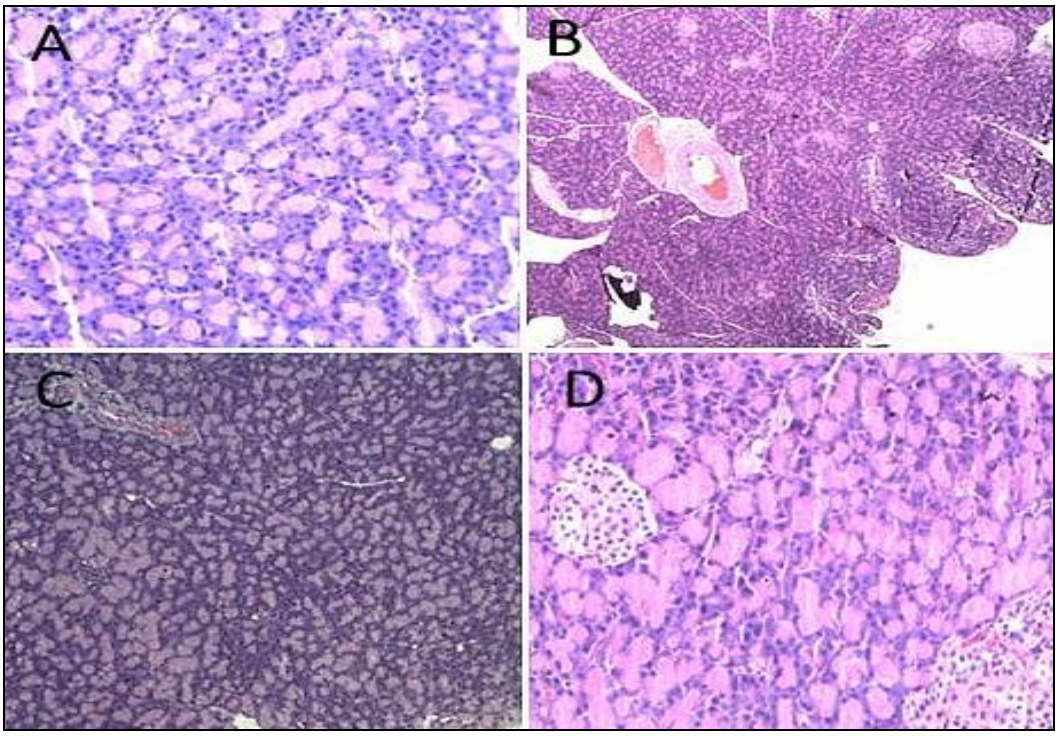
Parameters	Normal control	Diabetic control	MAB (100 mg/kg)	MAB (200 mg/kg)	MAB (400 mg/kg)	Glibenclamide (5 mg/kg)
WBC ($\times 10^3$/µL)	12.35 ± 0.75[a]	6.73 ± 0.66	6.08 ± 0.56	9.66 ± 0.93[c]	8.46 ± 1.11	7.45 ± 0.45
Lymphocytes (%)	67.77 ± 5.01	68.85 ± 2.96	66.83 ± 2.73	55.04 ± 1.82	65.08 ± 0.92	60.65 ± 2.06
Monocytes (%)	10.42 ± 2.44	11.21 ± 0.33	11.60 ± 2.18	12.64 ± 0.77	12.00 ± 0.21	10.67 ± 0.73
Neutrophils (%)	22.93 ± 3.96	22.96 ± 0.60	23.17 ± 3.10	23.91 ± 3.83	22.70 ± 0.84	24.92 ± 1.67
Platelets ($\times 10^3$/µL)	620.3 ± 35.34	597.2 ± 46.10	581.7 ± 72.23	606 ± 70.68	609 ± 40.00	661.2 ± 28.73

[a]$P<0.001$ compared to corresponding values of the diabetic control.
[b]$P<0.01$ compared to corresponding values of the diabetic control.
[c]$P<0.05$ compared to corresponding values of the diabetic control.
Each value is expressed as mean ± S.E.M (n = 6)
MAB = *Myrianthus arboreus* stem bark ethanol extract.

3.9 Pathological studies: Pathology studies on the rats is summarized in **Table 6** and **Fig. 7** and **8** below.

TABLE 6: EFFECT OF MAB ON VITAL ORGANS OF STZ - INDUCED DIABETIC RATS

Organ/dosage	Pancreas	Liver	Kidney	Lungs
Normoglycaemic	Normal islet & exocrine cells	Normal	Normal	Normal
Diabetic control	Evidence of destruction of islet cells	Increased inflammation, fatty changes and necrosis suggesting liver toxicity	Glomerular sclerosis, hyaline arteriolosclerosis and pyelonephritis	No toxic effects on the lungs
MAB 100mg/kg	Few islet cells, mass destruction of islet cells, slight attempt at regeneration	Increased inflammation, fatty changes and necrosis suggesting liver toxicity	Glomerular sclerosis, hyaline arteriolosclerosis and pyelonephritis	No toxic effects on the lungs
MAB 200mg/kg	More islet cells with some destruction, increased regeneration of cells	Inflammation, prominent sinusoids with some fatty changes (metabolic activity)	Glomerular sclerosis, hyaline arteriolosclerosis and pyelonephritis	No toxic effects on the lungs
MAB 400mg/kg	Evidence of regeneration, but toxic effect on the exocrine pancreas	No toxic effect on the liver	Glomerular sclerosis, hyaline arteriosclerosis and pyelonephritis	No toxic effects on the lungs
Glibenclamide (5mg/kg)	Evidence of destruction of islet cells and attempt at regeneration	No toxic effect on the liver	Glomerular sclerosis, hyaline arteriosclerosis and pyelonephritis	No toxic effects on the lungs



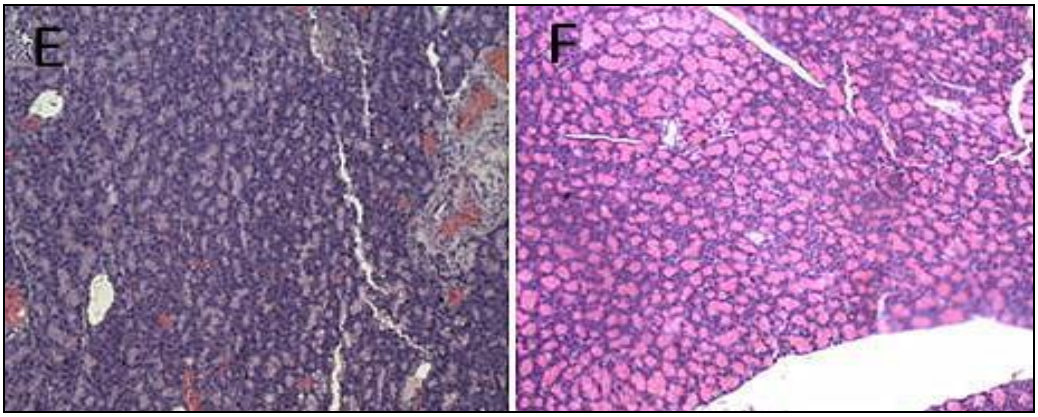


FIG. 7: HISTOLOGY OF PANCREAS IN EXPERIMENTAL RATS AFTER 28 DAYS OF TREATMENT. (A) Normal control – normal islet and exocrine cells (B) Diabetic control –evidence of destruction of islet cells. (C) Diabetic + MAB (100 mg/kg). Few islet cells, massive destruction of islet cells, slight attempt at regeneration (D) Diabetic + MAB (200 mg/kg) More islet cells with some destruction of the islet cells and increased regeneration of cells (E) Diabetic + MAB (400 mg/kg) Evidence of regeneration, but toxic effect on the exocrine pancreas.- (F) Diabetic + Glibenclamide (5 mg/kg).- Evidence of destruction of islet cells and attempt at regeneration

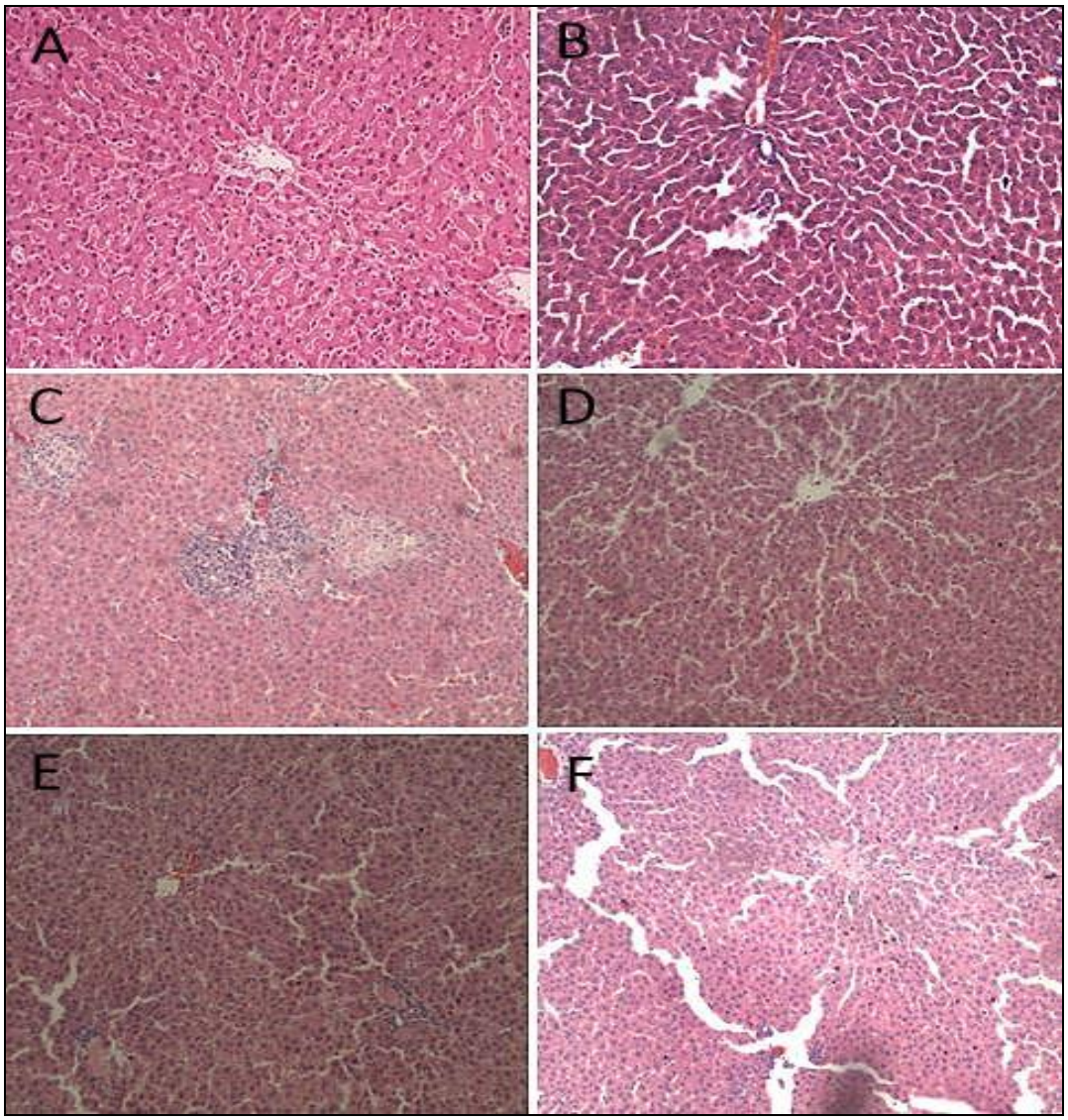


FIG. 8: HISTOLOGY OF LIVER OF EXPERIMENTAL RATS AFTER 28 DAYS OF TREATMENT. (A) Normal control - normal (B) Diabetic control - Increased inflammation, fatty changes and necrosis suggesting liver toxicity (C) Diabetic + MAB (100 mg/kg) - Increased inflammation, fatty changes and necrosis suggesting liver toxicity (D) Diabetic + MAB (200 mg/kg) - Inflammation, prominent sinusoids with some fatty changes (metabolic activity) (E) Diabetic + MAB (400 mg/kg) - No toxic effect on the liver (F) Diabetic + Glibenclamide (5 mg/kg) - No toxic effect on the liver

3.10 Phytochemical evaluation: The phytochemical screening of MAB showed the presence of flavonoids, tannins, alkaloids, phytosterols, triterpenoids, saponins and anthraquinone glycosides.

DISCUSSION: STZ-induced hyperglycaemia is a widely applied experimental diabetic model because of the ability of STZ to selectively target and destroy insulin-producing pancreatic islet β – cells. The intracellular action of STZ induces DNA strand breaks in pancreatic islet β – cells and results in islet cell death thus reducing insulin secretion [22] leading to hyperglycaemia. The study evaluated the hypoglycaemic and antihyperlipidaemic activities of MAB in STZ - induced diabetic rats and its effects on the metabolic profiles of the rats, using glibenclamide [23] as the standard hypoglycaemic agent.

The ability of MAB to lower glucose level in the oral glucose tolerance test suggests that animals treated with the extract had better glucose utilization capacity compared to those which did not receive any treatment. The increased levels of plasma glucose observed in STZ-induced diabetic rats were lowered by the administration of MAB. The reduced glucose levels suggested that MAB might exert insulin-like effect on peripheral tissues by either promoting glucose uptake mechanism or by inhibiting hepatic gluconeogenesis [24], or by absorption of glucose into the muscle and adipose tissues [25, 26] through the stimulation of a regeneration process and revitalisation of the remaining β-cells [27]. Histopathological studies of the pancreas revealed that MAB significantly improved the histological architecture of the islets of Langerhans. Groups treated with MAB showed persistence of islet cells and lesser degree of necrotic changes as compared to untreated diabetic rats.

The degeneration in the number and size of β-cells observed in diabetic rats were improved after treatment with MAB, especially at 200 mg/kg bwt. With such evidence, it is possible to assume that MAB might have stimulated insulin secretion from regenerated β-cells to improve glycaemic control. Another possibility is that MAB might have improved glycaemic control mechanisms through inhibition of glucose metabolizing enzymes such as α-amylase and α-glucosidase [28]. Our histological findings also supported these observations with maximum regeneration of islet cells occurring at 200mg/kg bwt of MAB as seen in **Fig.7D**.

STZ – induced diabetes is known to cause weight loss in animals as a result of proteinuria and insulin deficiency [29]. Insulin deficiency triggers the liver to break down protein into amino acids leading to muscle wasting and excessive weight loss [30, 31] which was observed in diabetic control rats. Treated animals started gaining weights after day 7 of the experiment. An increase in the body weight of diabetic treated rats might be due to an enhancement in glycaemic control and increased synthesis of structural proteins [32].

Alterations in lipid metabolism are some of the complications that accompany diabetes [33]. Total triglycerides (TG), total cholesterol (TC), HDL-cholesterol and LDL- cholesterol are parameters used to determine risk of cardiovascular diseases including coronary artery disease and atherosclerosis [34]. Dyslipidaemia is common among uncontrolled type 1 diabetics and has been implicated as one of the leading risk factors of cardiovascular disease [35, 36]. Defects in insulin action causes increased lipolysis in adipocytes and release of fatty acids which results in increased production of LDL and very low density lipoprotein (VLDL) particles and dyslipidaemia [37, 38]. In this study, treatment of diabetic rats with MAB improved the lipid profile by decreasing the serum levels of TC, TG and LDL-C and at the same time increased serum levels of HDL-C.

Metabolism of drugs takes place in the liver. Increased dosage of drugs generally ends up damaging the liver. Liver function tests involving marker enzymes such as AST and ALT are performed to evaluate liver function [39] or to monitor and/or detect the toxic effects of drugs and other foreign substances on the liver [40].

Increased levels of these parameters are usually due to leakages of the enzymes into blood circulation [41, 42] especially when the liver is damaged [43] or its integrity is compromised in certain conditions such as in inflammatory hepatocellular disorders [39, 44] found that the liver is necrotized in streptozotocin-induced diabetic rats.

The increase in the activities of the liver enzymes and alterations seen in the histopathological studies of the liver substantiated the hepatic damage by STZ. Treatment with MAB which exhibited hepatoprotective effect started showing evidence of hepatocyte restoration at 200mg/kg (Inflammation, prominent sinusoids with some fatty changes suggesting metabolic activity) which was better than what was observed at 100mg/kg (Increased inflammation, fatty changes and necrosis suggesting liver toxicity) with less or no toxic effect seen on the liver at 400mg/kg. This observation was substantiated by the chemical pathology findings which showed improvement with administration of the drug at 200mg/kg and 400mg/kg.

Levels of urea and creatinine in the serum are considered major indicators of renal dysfunction. The untreated diabetic rats showed significantly increased levels of urea and creatinine in the serum. However, there was significant decrease in the levels of serum urea and creatinine in the treated diabetic rats. Thus, in this study MAB prevented the progression of renal damage in diabetic rats. However, the morphological changes to support this observation is not obvious and may be because of the short time of treatment.

Ingestion of medicinal substances or plant extracts have the potential of changing normal haematological parameters [45]. Assessment of haematological parameters cannot only be used to determine the toxic effects of extracts but can also be used to explain blood relating functions of an extract or its products [46]. In this study there was significant decrease in the levels of RBC, Hb, HCT and MCV in the diabetic rats compared to the normal control rats. Thus the experimental animals became anaemic possibly due to the suppression of haemoglobin synthesis [47]. Administration of MAB improved these parameters and brought them close to normal values. The observed effects may be as a result of the extract stimulating erythropoiesis to increase the production of red blood cells [48] and the presence of antioxidant agents such as flavonoids which are able to lower lipid peroxidation and hence prevent haemolysis of red blood cells. This finding supports the traditional use of *M. arboreus* in the management of anaemia [11].

WBC count was reduced in both treated and untreated diabetic rats compared to the normal control. However, monocytes, lymphocyte, neutrophil and platelet levels of the diabetic rats were close to those of normal control rats. The observed "leukopenia" may be due to the suppressing effect of the immune system by STZ thereby reducing the number of WBCs. A reduction in the number of WBCs, could also be as a result of diabetes-induced stress [31, 47] which breaks down the rats' defensive mechanism. Diabetic rats treated with the extract at 200 mg/kg/day showed improvement in WBC count, which could probably be due to the fact that the extract contains some constituents that stimulate and/or promote the production of WBCs [30] and hence offer some form of protection to the rats' immune system.

Intraperitoneal injection of STZ leads to hyperglycaemia, which according to [47] causes a reduction in platelet number. Low platelet levels over a long period of time, can cause both internal and external haemorrhage and finally death. MAB's abilities to restore platelet levels in treated diabetic rats may probably be due to the presence of some phytochemicals that stimulate biosynthesis of clotting factors [30].

Polydipsia is one of the symptoms of diabetes. In diabetes, hyper-osmolarity and osmotic diuresis caused by hyperglycaemia triggers the thirst centres of the brain which in turn stimulates increased water intake [49]. This phenomenon was observed in the diabetic control group which showed increase in their water consumption. After 28 days of treatment, there were no significant changes in the feed and water intake of both treated and untreated diabetic rats, although it was observed that diabetic control rats consumed more water compared to normal and treated rats, while diabetic rats treated with 400 mg/kg of MAB consumed more feed. However, water consumption pattern of the treated rats was similar to that of the normal rats. Thus MAB was able to control osmolarity by normalizing plasma glucose.

Polyphagia is another symptom of diabetes that has been reported to accompany hyperglycaemia [50]. Although there were no significant changes in the feed intake of experimental animals, the feed

consumption of diabetic rats treated with 400 mg/kg of the extract increased. This could be as a result of the extract's stimulatory or modulatory effects on ghrelin, the hunger hormone that triggers the senses to consume more feed [51].

Phytochemical screening of MAB revealed the presence of tannins, flavonoids, alkaloids, triterpenoids, saponins and anthracene glycosides. Several compounds belonging to these classes of phytochemicals have demonstrated hypoglycaemic activity. *γ*-sitosterol has been shown to reduce hyperglycaemia in STZ-induced diabetic rats due to its ability to stimulate insulin secretion and inhibit gluconeogenesis [52]. Proanthocyanidin glycosides have been reported as strong antioxidants and potent hypoglycaemic agents [53-55]. The antidiabetic effect shown in this study may be attributed to synergistic or additive of these hypoglycaemic principles present in the ethanol stem bark extract of *M. arboreus*. The results of this study supports the use of the stem bark extract of *M. arboreus* as an antidiabetic agent in traditional medicine.

CONCLUSION: This study supports the use of the ethanol extract of the stem bark of *M. arboreus* as a potential adjunct dietary treatment of diabetes and as a potential source for the discovery of new orally active agent(s) for future therapy of diabetes.

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