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ANTI-DIABETIC IMPACT OF 'AJU MBAISE' HERBAL COCKTAIL IN STREPTOZOTOCIN-INDUCED DIABETIC FEMALE RATS

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ABSTRACT

This study evaluated the hypoglycaemic potential of 'Aju Mbaise' herbal mixture. Female Wistar rats of 40 – 50 g and fifty-four (54) in number were obtained and housed at the animal house of the Department of Pharmacology, University of Port Harcourt. They were assembled into 6 groups of 9 rats each. Group I served as the normal control (NC) while the remaining five groups were induced with diabetes type 2 using high-fat diet (HFD) for about 8 weeks and streptozotocin at 35 mg/kg body weight. Group II was the diabetic control (DC) while the other groups (III, IV, V & VI) were administered 7.2 mg/kg metformin and the cocktail extract at three different concentrations of 500 mg/kg, 250 mg/kg, and 100 mg/kg respectively. Biochemical tests were conducted after twelve weeks of treatment. The blood glucose concentration was estimated using the Accu-check glucometer and glucose strips while the rest biomarkers were analyzed using their specific test kits. Diabetes mellitus was confirmed after HFD and STZ administration. Result obtained showed that the normal and metformin control groups recorded significant ($p<0.05$) lower blood glucose, liver enzymes (AST, ALT, & ALP), urea, and creatinine, as well as higher total protein concentrations when compared to the diabetic control and treated groups. Also, significant ($p<0.05$) reduction in blood glucose, liver enzymes, urea, and creatinine, as well as increase in total protein concentrations was recorded by the treated groups when compared to the diabetic control group. This study revealed the potential hypoglycaemic effect of 'Aju Mbaise' herbal cocktail.

Keywords: Aju Mbaise, Hypoglycaemic, Metformin, Glucometer, and Streptozotocin.

INTRODUCTION

Persistent upsurge in diabetes mellitus (DM) and its complications still remain a global health challenge that requires utmost attention. According to Banday *et al.* (2020), DM is a metabolic disorder characterized by hyperglycemia that manifests as a consequence of defects in insulin secretion, insulin action, or both. According to Jwad and AL-Fatlawi (2022), this ailment results from rise in blood glucose, which thus destroys many body systems, prevalently the veins and nerves. Insulin inefficiency as found in type-2 DM (T2D) stimulates constant hyperglycaemia with disorder of carbohydrate, fat, and protein metabolism (Guan *et al*., 2018). The pathologic hallmark

of DM leads to both microvascular and macrovascular complications (Sun *et al.*, 2022). Some of these complications include neuropathy, nephropathy, retinopathy, foot ulcer disease (FUD), hearing impairment, and Alzheimer's disease (Onyeji, 2022). Globally, about 193 million diabetics remain undiagnosed, predisposing them to the development of several long-term complications of untreated chronic hyperglycemia (Unnikrishnan and Misra, 2021). According to a critical evaluation of mortality patterns by race described by Sareen *et al.* (2018), blacks had a higher mortality rate than whites, and it also affects individuals of all ages. This serious ailment (DM) has been regulated with the use of insulin and other hypoglycemic agents (antidiabetic drugs) such as the biguanides, meglitinides, sulfonylureas, alpha-glucosidase inhibitors, and thiazolidinediones. According to Nnadiukwu *et al.* (2016), these antidiabetic drugs effectively control blood glucose level when administered appropriately, but also are wearisome, expensive, and have shown numerous adverse effects. Therefore, there is need for adoption of plant and its product for the treatment and/or control of DM. According to Verma *et al.* (2018), high blood glucose can be dealt with the utilization of herbs, and the anti-diabetic actions can be accomplished through; stimulation of insulin synthesis and acting like insulin, regeneration of damaged pancreatic beta cells, improvement of insulin sensitivity, altering the activities of enzymes that catalyze glucose metabolism, and by inhibition of glucose absorption. 'Aju Mbaise' is a herbal cocktail composed of combination of leaves, roots and trunk of different medicinal plants wrapped together. According to Ogueke *et al.* (2016), the cocktail effectively detoxifies, and sanitizes the womb, as well as reduces stomach to its original size and shape in good time when administered to women after childbirth. It also increases the mineral and vitamin concentrations in lactating mothers (Ezejindu and Iro, 2017). According to Nnadiukwu *et al.* (2019), the cocktail is made up of *Sphenocentrum jollynum, Cnestis ferruginea, Xylopia aethiopica, Uvaria chamae, Palisota hirsuta, Scleria sp., Napoleona sp., Dialium guineense, Combretum racemosun, and Heterotis rotundifolia*. These plants have shown their individual therapeutic effects against so many diseases. Amidu *et al.* (2008), reported the presence of alkaloids, terpenoids, and flavonoids in the ethanol root concentrate of *Sphenocentrum jollynum. S. jollyanum* possesses anti-diabetic (Alese *et al.*, 2014), antioxidant (Olorunnisola and Afolayan, 2013), anti-inflammatory (Olorunnisola *et al.*, 2017), anti-allergic (Olorunnisola *et al.*, 2017), anti-bacterial (Koleosho *et al.*, 2013), anti-viral (Moody *et al.*, 2002) and anti-malaria (Olorunnisola and Afolayan, 2013). *Cnestis ferruginea* has been found to contain isoflavone, coumarin, and anthocyanins which have been ascribed to be responsible for antidiabetic activities in some plants (Bhatt *et al.*, 2016). Adisa *et al.* (2014), reported the in vivo hypoglycaemic activity of methanol leaf concentrates of *C. ferruginea* in STZ-induced diabetic rodents. Hypoglycaemic and hypocholesterolemic activities of *C. ferruginea* leaves was also reported by Ndip *et al.* (2013). Onocha *et al.* (2005), revealed alkaloids, steroids, saponins and tannins in *Combretum racemosun* extracts. According to Nsuadi *et al.* (2012), *C. racemosun* possesses anti-inflammatory, vasorelaxant, and trypanocidal properties. *Dialium guineense* possesses antimalaria, anti-inflammatory, and antioxidant properties (Nijveldt *et al.*, 2001). According to Etekpo *et al.* (2018), the phytochemical screening of *Heterotis rotundifolia* revealed the presence of phenolic and flavonoic compounds, which is attributed to its antioxidant activity. According to Chah *et al.* (2006), the leaves and seeds extracts of *Napoleona imperialis* contains tannins, glycosides, saponins and proteins and have showed bactericidal action. Esimone *et al.* (2005), reported that methanolic extract of *N. imperialis* showed a fast wound healing property. The anti-inflammatory, antipyretic, and anti-oxidant

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effects of *Palisota hirsuta* was reported by Boakye-Gyasi *et al.* (2011). Okwuosa *et al.* (2012), reported the hypoglycaemic, antifungal, bacteriostatic and antimalaria properties of *Uvaria chamae*. Achigan-Dako *et al.* (2010), likewise prescribed the root as a potential remedy for amenorrhoea, avert miscarriage; and quell labour torments. The medicinal abilities of these individual plants are attributed to the bioactive compounds present in them. Thus, the collective bioactive compounds of these plants will have a tremendous wide therapeutic effect. Previous study by Nnadiukwu *et al.* (2019), revealed that the herbal cocktail of 'Aju Mbaise' is rich in various phytochemicals (alkaloids, flavonoids, glycosides, hydrogen cyanide, phenols, saponins, steroids, tannins, and terpenoids), vitamins, mineral, and other dietary nutrients essential for preservation of good health and better life. Ogueke *et al.* (2016), also reported the presence of bioactive compounds such as alkaloids, tannins, flavonoids, and saponins as well as minerals (potassium, calcium, magnesium, sodium, iron, zinc, phosphorus, copper, and manganese) in Aju Mbaise herbal mixture. The present study was designed to investigate the anti-diabetic capacity of 'Aju Mbaise' herbal cocktail considering the therapeutic effects of the individual plants that constitute the cocktail.

MATERIALS AND METHODS

Reagents

All the biochemical reagents, chemicals and materials used in this research work were of standard analytical grade. Streptozotocin (STZ) was purchased from Sigma Chemicals Co. St. Louis, MO, USA. The biochemical reagents kits were manufactured by Randox Laboratories Ltd., County Antrim, United Kingdom. Metformin was a product of Merck Serono, Milano, Italy.

Collection and Identification of Plant Samples

Fresh samples of the plants that made up 'Aju Mbaise' were gathered at Obodo Ujichi, Ahiazu and Amuzi, Ahiara Towns, in Ahiazu Mbaise L.G.A, of Imo State, Nigeria. The plant samples were identified as *Cnestis ferruginea*, *Xylopia aethiopica*, *Uvaria chamae*, *Palisota hirsuta*, *Scleria sp*., *Napoleona imperialis*, *Dialium guineense*, *Combretum racemosun*, and *Heterotis rotundifolia* by Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria. The fresh plants after collection were air dried, sliced into pieces and pulverised before the extraction with ethanol. The resultant extract was weighed, kept in clean bottles and stored until usage.

Preparation of high fat diet (HFD)

The HFD was prepared according to the method of Liu (2018), using standard laboratory chow (Top feed) growers mash, lard and sucrose in the ratio of 3:1:1 respectively.

Experimental Animals

A total of fifty-four (54) female Wistar rats were used. This gender was preferred as they mostly consume this herbal cocktail. The rats were acquired from the Department of Veterinary Medicine, University of Nigeria, Nsukka (UNN), Enugu State, Nigeria. They were aged 3 months and weighs between 40 – 50 g at the time of procurement. The experimental rats were housed in the animal house of the Department of Pharmacology, University of Port Harcourt, Rivers State, Nigeria, and were left for seven (7) days to conform to the experimental conditions during which they were given normal feed (Top feeds-grower's mash) and clean water. The animals were handled in line with the global guiding principles for the care and use of animals for scientific purposes. The Research work was approved by the Research Ethics Committee of the University of Port Harcourt with authorization number: UPH/CEREMAD/ REC/MM64/003.

Induction of Type 2 DM

A single dose intraperitoneal injection of 35 $mgkg^{-1}$ body weight (b.w) of STZ prepared with normal saline was used to induce type-2 DM to the experimental rats in Groups II to VI. Hyperglycaemia manifestation in the rats was allowed for 7 days after the STZ injection. Fasting blood glucose was checked to ascertain DM before the inception of treatment (oral administration of metformin and the extract) which was done daily for a period of twelve (12) weeks. At every 4 weeks interval, 3 animals from each group were fasted overnight, anaesthetized, sacrificed, and blood samples collected for biochemistry analyses. The blood samples were centrifuged at 3000 revolutions per minute (rpm) for 10 minutes at room temperature, and the respective serum collected and used for the outlined biochemical assays. The organs (liver, and kidney) were harvested for histopathological analysis and preserved in a sample bottle containing 10% formalin.

Experimental Design

The experimental animals were assembled into six groups of nine animals each. The diabetic animals in groups III to VI were treated with metformin 7.2 $mgkg^{-1}$ b.w, and three different doses of the cocktail extract as shown in the table below;

Table 1: Groupings of the Experimental animals

Groups	Code	Treatment
I.	**Negative Control**	The animals in this group were not induced with diabetes but were given distilled water and normal feed throughout the experiment.
II.	**Positive Control**	The animals in this group were made diabetic, received water and normal feed but remained untreated throughout the experiment.
III.	**Metformin Treated**	Diabetic animals treated with 7.2 $mgkg^{-1}$ b.w metformin
iv.	**500 mg/kg Extract**	Diabetic animals treated with 500 $mgkg^{-1}$ b.w of the cocktail extract
v.	**250 mg/kg Extract**	Diabetic animals treated with 250 $mgkg^{-1}$ b.w of the cocktail extract
vi.	**100 mg/kg Extract**	Diabetic animals treated with 100 $mgkg^{-1}$ b.w of the cocktail extract

Procedure for Biochemical Assays

The blood glucose concentration was determined with Accu-check glucometer and glucose strips. Other biomarkers such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total protein, total bilirubin, conjugated bilirubin, albumin, urea and creatinine were determined using their appropriate Randox reagent Test kits, and the absorbance of the samples read with Advanced Microprocessor UV-VIS Spectrophotometer (Single Beam -295). The concentrations of the electrolytes; sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO_3^-) were estimated according to the procedure presented in their respective Teco Diagnostic test kits manuals.

Histological investigation

Histological examination studies were done on the liver and kidney tissues of the experimental rats to evaluate any possible alteration that might have occurred in these tissues within the experimental period. These organs were fixed in 10% formal saline, to exterminate any bacteria present and ensure that the tissue does not rot/damage. Samples were dehydrated in graded alcohol series (from a lower to higher concentration), and thin uniform sections prepared with a rotary microtome. The slides were then viewed

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under a microscope and photomicrograph captured with a high-imaged quality camera at x400 magnification.

Statistical analysis

Values were reported as Mean ± standard error of mean (SEM), while Duncan Test of one way ANOVA was used to test for significant differences between treatment groups using the Statistical Package for the Social Sciences (SPSS) (version 25.0). The results were considered significant at ($p<0.05$).

RESULTS

The results of the biochemical analysis which includes blood glucose level, AST, ALT, ALP, total protein, albumin, total bilirubin, conjugated bilirubin, urea, creatinine, and electrolytes (sodium, potassium, chloride and bicarbonate) were presented in Tables 2 – 15 respectively.

Effect of 'Aju Mbaise' herbal extract on blood glucose level of Streptozotocine (STZ) induced diabetic Wistar rats

From Table 2, a significant reduction in blood glucose level was seen in the groups that were given the standard anti-diabetic medication (metformin) and the herbal extracts as treatment progressed. The diabetic control group (DC) has blood glucose that is significantly higher ($p=0.01$) when compared to the normal control (NC) group and the treated groups throughout the experimental period. The NC group maintained normal blood glucose.

Table 2: Effect of 'Aju Mbaise' herbal extract on Blood Glucose level of STZ induced diabetic Wistar rats

	Glucose (mg/dl)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	63.67 ± 1.45^{a}	70.67 ± 5.70^{a}	78.67 ± 1.76^{a}
DC	314.67 ± 36.34^{c}	305.33 ± 34.23^{c}	271.00 ± 54.37^{b}
Metformin	70.00 ± 15.87^{a}	83.33 ± 10.48^{a}	77.00 ± 14.18^{a}
500 mg Extract	124.67 ± 31.67^{ab}	115.33 ± 29.54^{ab}	107.00 ± 23.12^{a}
250 mg Extract	168.67 ± 45.32^{ab}	152.33 ± 37.26^{ab}	147.00 ± 31.53^{a}
100 mg Extract	201.67 ± 36.16^{b}	173.00 ± 13.05^{b}	150.00 ± 46.29^{a}

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ concentrate of the cocktail herbal mixture.

Effect of 'Aju Mbaise' herbal extract on the hepatic markers of STZ induced diabetic Wistar rats

From the result displayed in Table 3-9, AST activity of the NC group was seen to be significantly lower ($p=0.01$) than the rest of the experimental groups in the 4th and 8th weeks. The AST activity of the DC group was higher than that of the other groups in weeks 8 and 12. There was an increase in AST activity in the DC group as the experiment advances. Also, the treated groups recorded a progressive decline in AST activity as treatment progresses (see Table 3). The ALT activity of the DC was higher than the rest groups at weeks 8 and 12 respectively. There was a progressive rise in ALT activity of the DC group, as well as a progressive decline in ALT activity of the treated groups as experiment progresses. The ALT activity of the groups administered the herbal mixture were lower than that of the metformin treated group (see Table 4). In Table 5, the ALP of the DC group was seen to be higher than the rest groups. Additionally, a perceptible rise in

ALP activity was seen in the DC group as experiment advances, in contrast to a declined ALP activity recorded in the herbal cocktail treated groups. The NC group maintained an ALP activity lower than the other experimental groups. From the result shown in Table 6, the total protein (TP) concentration of the DC group was lower than the other groups throughout the experiment. The TP of the metformin treated group was higher than the groups administered the herbal cocktail extract in weeks 4 and 8. From the result shown in Table 7, the albumin concentration of the DC group was lower than the other groups throughout the experiment. The albumin concentration of the group that received 250 $mgkg^{-1}$ b. w. of the herbal cocktail extract was higher than the other groups after the twelfth week. The total bilirubin (T.Bil) concentration of the NC group was lower than the other experimental groups. Likewise, the T.Bil concentration of the DC group was higher than the other experimental groups. The T.Bil of the herbal cocktail treated groups declined as treatment advances (see Table 8). From the result displayed on Table 9, conjugated bilirubin concentration of the NC group was lower than the other experimental groups throughout the duration of the experiment. Likewise, conjugated bilirubin concentration of the DC group was seen to be higher than the other experimental groups during the experimental period apart from the group that was administered 250 $mgkg^{-1}$ b. w. of the herbal cocktail extract in week 8.

Table 3: Effect of 'Aju Mbaise' herbal extract on Aspartate Transaminase (AST) activity of STZ induced diabetic Wistar rats

	AST (U/L)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	74.67 ± 4.06^{a}	76.00 ± 3.06^{a}	68.00 ± 7.21^{a}
DC	106.33 ± 3.84^{b}	111.67 ± 7.22^{b}	128.00 ± 15.72^{c}
Metformin	109.33 ± 9.21^{b}	99.67 ± 2.73^{b}	102.33 ± 12.55^{bc}
500 mg Extract	98.00 ± 6.11^{ab}	105.33 ± 5.70^{b}	78.00 ± 7.57^{ab}
250 mg Extract	107.00 ± 13.43^{b}	99.67 ± 7.13^{b}	87.67 ± 7.75^{ab}
100 mg Extract	114.33 ± 9.13^{b}	107.00 ± 6.08^{b}	93.00 ± 2.65^{ab}

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ concentrate of the cocktail herbal mixture.

Table 4: Effect of 'Aju Mbaise' herbal mixture on Alanine Transaminase (ALT) activity of STZ induced diabetic Wistar rats

	ALT (U/L)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	35.33 ± 5.46^{a}	41.33 ± 4.67^{a}	32.67 ± 0.67^{a}
DC	56.33 ± 14.43^{a}	60.67 ± 10.41^{b}	66.33 ± 19.10^{b}
Metformin	39.67 ± 1.20^{a}	45.67 ± 5.24^{ab}	37.67 ± 2.96^{a}
500 mg Extract	34.00 ± 5.29^{a}	38.67 ± 1.76^{a}	34.67 ± 5.46^{a}
250 mg Extract	34.00 ± 3.79^{a}	38.67 ± 2.85^{a}	37.33 ± 2.60^{a}
100 mg Extract	38.00 ± 5.29^{a}	42.00 ± 3.46^{a}	36.67 ± 3.53^{a}

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

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Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ concentrate of the cocktail herbal mixture.

Table 5: Effect of 'Aju Mbaise' herbal extract on Alkaline Phosphatase (ALP) activity of STZ induced diabetic Wistar rats

	ALP (U/L)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	61.67 ± 8.09^{a}	67.00 ± 4.58^{a}	59.33 ± 0.88^{a}
DC	123.00 ± 12.66^{b}	130.33 ± 6.17^{c}	140.00 ± 20.55^{c}
Metformin	87.33 ± 16.95^{ab}	92.33 ± 13.45^{ab}	88.00 ± 15.63^{ab}
500 mg Extract	75.33 ± 4.81^{a}	70.00 ± 6.11^{a}	74.00 ± 4.16^{ab}
250 mg Extract	105.33 ± 22.04^{ab}	112.00 ± 15.53^{bc}	95.33 ± 18.52^{ab}
100 mg Extract	124.00 ± 8.08^{b}	110.67 ± 7.51^{bc}	108.67 ± 8.11^{bc}

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ concentrate of the cocktail herbal mixture.

Table 6: Effect of 'Aju Mbaise' herbal extract on Total Protein concentration of STZ induced diabetic Wistar rats

	Total Protein (g/dl)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	6.73 ± 0.09^{b}	6.93 ± 0.15^{c}	7.47 ± 0.43^{b}
DC	5.27 ± 0.64^{a}	4.40 ± 0.42^{a}	4.77 ± 0.83^{a}
Metformin	6.57 ± 0.41^{b}	7.30 ± 0.38^{c}	5.77 ± 0.47^{ab}
500 mg Extract	6.20 ± 0.21^{ab}	6.67 ± 0.27^{bc}	5.50 ± 0.64^{ab}
250 mg Extract	6.10 ± 0.21^{ab}	6.40 ± 0.12^{bc}	6.43 ± 0.89^{ab}
100 mg Extract	5.70 ± 0.47^{ab}	5.90 ± 0.29^{b}	6.13 ± 0.65^{ab}

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ concentrate of the cocktail herbal mixture.

Table 7: Effect of 'Aju Mbaise' herbal extract on Albumin concentration of STZ induced diabetic Wistar rats

	Albumin (g/dl)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	4.33 ± 0.19^{b}	4.20 ± 0.26^{a}	4.40 ± 0.40^{a}
DC	2.93 ± 0.35^{a}	3.07 ± 0.48^{a}	3.07 ± 0.27^{a}
Metformin	3.97 ± 0.33^{ab}	4.23 ± 0.37^{a}	3.77 ± 0.18^{a}
500 mg Extract	4.30 ± 0.52^{b}	4.23 ± 0.58^{a}	4.93 ± 0.82^{a}
250 mg Extract	3.70 ± 0.12^{ab}	3.77 ± 0.23^{a}	5.17 ± 0.72^{a}
100 mg Extract	3.77 ± 0.30^{ab}	3.97 ± 0.12^{a}	4.50 ± 0.97^{a}

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ concentrate of the cocktail herbal mixture.

Table 8: Effect of 'Aju Mbaise' herbal extract on Total Bilirubin concentration of STZ induced diabetic Wistar rats

	Total Bilirubin (µmol/l)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	11.67 ± 3.71^{a}	11.33 ± 1.45^{a}	10.33 ± 4.48^{a}
DC	25.33 ± 1.86^{b}	26.67 ± 1.45^{c}	18.33 ± 2.96^{a}
Metformin	16.67 ± 5.24^{ab}	18.33 ± 4.37^{ab}	14.67 ± 6.23^{a}
500 mg Extract	20.33 ± 2.03^{ab}	18.33 ± 0.88^{ab}	11.67 ± 4.26^{a}
250 mg Extract	25.00 ± 1.53^{b}	22.00 ± 2.31^{bc}	13.00 ± 6.56^{a}
100 mg Extract	23.67 ± 1.45^{b}	23.67 ± 1.45^{bc}	12.00 ± 6.03^{a}

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ concentrate of the cocktail herbal mixture.

Table 9: Effect of 'Aju Mbaise' herbal extract on Conjugated Bilirubin concentration of STZ induced diabetic Wistar rats

	Conjugated Bilirubin (µmol/l)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	5.27 ± 0.33^{a}	5.57 ± 0.24^{a}	4.47 ± 1.24^{a}
DC	12.27 ± 3.26^{a}	11.63 ± 2.69^{ab}	12.03 ± 4.30^{a}
Metformin	9.60 ± 0.75^{a}	10.80 ± 0.96^{ab}	8.60 ± 1.81^{a}
500 mg Extract	10.97 ± 1.88^{a}	9.97 ± 0.95^{ab}	10.10 ± 3.01^{a}
250 mg Extract	9.20 ± 4.24^{a}	14.47 ± 0.79^{b}	7.90 ± 0.95^{a}
100 mg Extract	10.50 ± 3.12^{a}	10.50 ± 3.12^{ab}	9.50 ± 3.58^{a}

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ concentrate of the cocktail herbal mixture.

Effect of 'Aju Mbaise' herbal extract on renal function of STZ induced diabetic Wistar rats

From the result displayed in Table 10, urea concentration of the DC group was seen to be significantly higher ($p<0.05$) than the rest of the experimental groups. A progressive decrease in urea level among the treated groups was seen as treatment advances. The group administered 250 $mgkg^{-1}$ b. w. of the herbal extract has a lower urea concentration than the other groups throughout the experimental period. From the result presented in Table 11, creatinine concentration of the DC group was seen to be higher than the other experimental groups, and increases as the experiment advances. Also, there was a decline in creatinine concentration among the treated groups at week 12. The group administered 500 $mgkg^{-1}$ b. w. of the herbal extract has a lower creatinine concentration than the other groups after the 12 weeks experimental period. From the result displayed in Table 12, sodium ion concentration of the NC group was significantly higher ($p=0.02$) than the herbal extract treated groups at weeks 4 and 8. The metformin treated group recorded higher sodium ion concentration than the groups administered the herbal extract at weeks 4 and

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8. An increase in sodium ion concentration among the herbal extract treated groups was recorded as treatment advances. The potassium ion concentration of the NC group was lower than the other experimental groups in week 8. The potassium ion concentration of the DC group was lower than the other experimental groups in weeks 4 and 12. The potassium ion concentration across the groups was at the lowest in week 12 when compared to weeks 4 and 8 (see Table 13). From the result displayed in Table 14, chloride ion concentration of NC group was higher than the other experimental groups throughout the experimental period, while the DC group recorded the lowest chloride ion concentration after the twelfth week of analysis. The metformin treated group recorded a higher Cl^- concentration than the groups that received the herbal extract, aside in week 12 when the Cl^- concentration of the group treated with 500 $mgkg^{-1}$ b. w. of the herbal extract was higher. From the result presented in Table 15, a significant decrease ($p<0.05$) was recorded in week 8, when the bicarbonate ion concentration of the DC group was compared with the other experimental groups with the exception of the NC and the group that received 100 $mgkg^{-1}$ b. w. of the herbal extract.

Table 10: Effect of 'Aju Mbaise' herbal extract on Urea concentration of STZ induced diabetic Wistar rats

	Urea (mmol/l)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	6.07 ± 0.50^{a}	5.57 ± 0.37^{ab}	5.87 ± 0.34^{a}
DC	8.33 ± 0.30^{a}	8.10 ± 0.10^{c}	8.10 ± 0.29^{a}
Metformin	6.57 ± 0.76^{a}	6.70 ± 0.67^{abc}	5.83 ± 1.11^{a}
500 mg Extract	6.70 ± 0.75^{a}	5.97 ± 0.09^{ab}	5.63 ± 1.29^{a}
250 mg Extract	5.70 ± 0.62^{a}	5.33 ± 0.29^{a}	5.37 ± 0.38^{a}
100 mg Extract	6.97 ± 1.49^{a}	7.37 ± 1.13^{bc}	5.70 ± 1.81^{a}

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.
Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ concentrate of the cocktail herbal mixture.

Table 11: Effect of 'Aju Mbaise' herbal extract on Creatinine concentration of STZ induced diabetic Wistar rats

	Creatinine (µmol/l)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	115.00 ± 10.41^{a}	110.00 ± 5.77^{a}	116.33 ± 15.30^{ab}
DC	163.33 ± 11.67^{b}	166.00 ± 9.71^{c}	172.33 ± 5.49^{b}
Metformin	121.00 ± 17.35^{a}	123.67 ± 15.71^{ab}	120.00 ± 18.03^{ab}
500 mg Extract	138.33 ± 11.67^{ab}	129.00 ± 4.58^{ab}	106.67 ± 26.69^{a}
250 mg Extract	131.67 ± 10.37^{ab}	134.33 ± 8.84^{ab}	121.33 ± 2.73^{ab}
100 mg Extract	150.67 ± 12.33^{ab}	141.67 ± 3.76^{bc}	118.33 ± 28.43^{ab}

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.
Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ concentrate of the cocktail herbal mixture.

Table 12: Effect of 'Aju Mbaise' herbal extract on Sodium ion (Na^+) concentration of STZ induced diabetic Wistar rats

	Sodium (mmol/l)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	146.00 ± 9.07^{c}	154.33 ± 1.33^{b}	148.33 ± 10.49^{a}
DC	139.67 ± 5.61^{bc}	132.00 ± 3.00^{a}	123.33 ± 9.40^{a}
Metformin	142.67 ± 4.98^{c}	149.00 ± 4.04^{b}	135.00 ± 4.04^{a}
500 mg Extract	120.67 ± 2.33^{a}	125.00 ± 2.89^{a}	131.67 ± 7.26^{a}
250 mg Extract	123.33 ± 4.48^{ab}	129.00 ± 4.04^{a}	139.00 ± 3.51^{a}
100 mg Extract	119.67 ± 5.24^{a}	126.33 ± 2.73^{a}	129.67 ± 9.87^{a}

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ concentrate of the cocktail herbal mixture.

Table 13: Effect of 'Aju Mbaise' herbal extract on Potassium ion (K^+) concentration of STZ induced diabetic Wistar rats

	Potassium (mmol/l)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	5.13 ± 0.34^{a}	4.93 ± 0.15^{a}	4.60 ± 0.40^{a}
DC	4.93 ± 0.43^{a}	5.13 ± 0.23^{a}	3.60 ± 0.98^{a}
Metformin	5.20 ± 0.21^{a}	5.40 ± 0.06^{a}	4.47 ± 0.43^{a}
500 mg Extract	5.20 ± 0.64^{a}	5.60 ± 0.35^{a}	3.97 ± 0.19^{a}
250 mg Extract	5.33 ± 0.34^{a}	5.77 ± 0.34^{a}	4.33 ± 0.58^{a}
100 mg Extract	4.97 ± 0.15^{a}	5.50 ± 0.40^{a}	4.67 ± 0.24^{a}

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ concentrate of the cocktail herbal mixture.

Table 14: Effect of 'Aju Mbaise' herbal extract on Chloride ion (Cl^-) concentration of STZ induced diabetic Wistar rats

	Chloride (mmol/l)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	105.33 ± 25.73^{a}	116.67 ± 14.44^{a}	103.00 ± 24.79^{a}
DC	80.00 ± 17.93^{a}	84.67 ± 13.92^{a}	92.67 ± 27.24^{a}
Metformin	97.67 ± 24.97^{a}	107.67 ± 15.06^{a}	95.33 ± 24.83^{a}
500 mg Extract	72.33 ± 16.02^{a}	82.67 ± 10.09^{a}	101.00 ± 27.06^{a}
250 mg Extract	73.67 ± 13.64^{a}	85.33 ± 19.13^{a}	93.67 ± 25.78^{a}
100 mg Extract	68.00 ± 12.06^{a}	94.33 ± 26.42^{a}	94.00 ± 28.31^{a}

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ concentrate of the cocktail herbal mixture.

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Table 15: Effect of 'Aju Mbaise' herbal extract on Bicarbonate ion (HCO_3^-) concentration of STZ induced diabetic Wistar rats

	Bicarbonate (mmol/l)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	26.00 ± 1.15^{a}	28.00 ± 1.15^{ab}	28.00 ± 1.15^{a}
DC	26.33 ± 1.45^{a}	24.33 ± 0.88^{a}	26.33 ± 1.76^{a}
Metformin	28.00 ± 0.58^{a}	29.33 ± 1.45^{b}	28.33 ± 0.33^{a}
500 mg Extract	27.33 ± 1.86^{a}	29.00 ± 2.00^{b}	28.00 ± 1.15^{a}
250 mg Extract	28.33 ± 1.67^{a}	31.33 ± 1.33^{b}	26.67 ± 1.76^{a}
100 mg Extract	26.67 ± 0.88^{a}	28.00 ± 0.58^{ab}	26.67 ± 0.88^{a}

Group values (Mean ± SEM) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500 mg Extract** = Treated with 500 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **250 mg Extract** = Treated with 250 $mgkg^{-1}$ concentrate of the cocktail herbal mixture; **100 mg Extract** = Treated with 100 $mgkg^{-1}$ concentrate of the cocktail herbal mixture.

Histopathology Results

Histological examination of the liver and kidney of the experimental animals are shown in Plates 1- 24. The results showed the observations recorded in the different tissues. From the results below, Plates 1- 6 show the liver histology of the various groups after 4 weeks of treatment. A normal liver photomicrograph of normal control (NC) animal showing hepatic sinusoids and cords of hepatocytes radiating away from the Central Vein (CV) was seen in Plate 1. Plate 2 shows a mildly distorted liver tissue of diabetic control (DC) animal with congested portal vein (PV) and inflamed cells. Plate 3 shows histological normal hepatic tissue of metformin treated animal with a partially congested central vein. Plate 4 shows normal hepatic tissue of animal treated with 500 $mgkg^{-1}$ b. w. extract, with a partially congested portal vein. Plates 5 and 6 shows mildly distorted liver tissues of animals treated with 250 $mgkg^{-1}$ b. w. and 100 $mgkg^{-1}$ b. w. extracts respectively, with congested central vein (CV) and inflamed cells. Plates 7- 12 show liver histology of the various groups after the 12th week of treatment. Plate 7 represents a normal liver histology photomicrograph of NC animal with hepatic sinusoids & cords of hepatocytes radiating away from a partially congested central vein (CV). Plate 8 shows a distorted liver tissue of DC animal with congested portal vein (PV) with inflamed hepatic cells. Plate 9 shows normal liver tissue photomicrograph of metformin treated animal with congested central vein. Plate 10 shows a normal liver tissue photomicrograph of animal treated with 500 $mgkg^{-1}$ b. w. extract, with congested central vein. Plate 11 represents a normal liver tissue photomicrograph of animal treated with 250 $mgkg^{-1}$ b. w. extract, with cords of hepatocytes radiating away from the central vein. Plate 12 shows normal liver tissue photomicrograph of animal treated with 100 $mgkg^{-1}$ b. w. extract, with hepatocytes, sinusoid and a portal vein.

Plates 13 - 18 show the renal histology of the various groups after 4 weeks of treatment. Plate 13 shows normal renal tissue photomicrograph of NC animal with renal tubules (RT) lined by simple epithelial cells and glomeruli (G) containing mesangial cells and capillaries. Plate 14 reveals tubulo-glomerular mutilated renal tissue photomicrograph of DC animal with distorted renal tubules and obliterated Bowman's capsular spaces. Plates 15 shows mildly distorted kidney photomicrograph of metformin treated animal with partitioned glomerular tufts. Plate 16 reveals a mildly distorted glomerular renal tissue photomicrograph of animal treated with 500 $mgkg^{-1}$ b. w. extract, with partitioned glomerular tufts. Plate 17 shows an abnormal renal tissue photomicrograph of animal

treated with 250 $mgkg^{-1}$ b. w. extract, with occluded Bowman's capsular spaces. Plate 18 shows histological distorted renal tissue photomicrograph of animal treated with 100 $mgkg^{-1}$ b. w. extracts, with distorted glomeruli and blocked Bowman's capsular spaces. Plates 19 - 24 show renal histology of the various groups after 12 weeks of treatment. Plate 19 represents normal renal tissue photomicrograph of NC animal with histological normal glomeruli and renal tubules (RT). Plate 20 shows an abnormal renal tissue photomicrograph of DC animal with obliterated Bowman's capsular spaces and distorted renal tubules. Plates 21 - 24 revealed a normal histological renal tissue photomicrograph of animals treated with metformin, 500 $mgkg^{-1}$ b. w. extract, 250 $mgkg^{-1}$ b. w. extract, and 100 $mgkg^{-1}$ b. w. extract respectively with normal renal tubules and glomeruli.

Architecture of hepatic tissues of Streptozotocine (STZ) induced diabetic rats after 4 weeks of treatment with metformin and Aju Mbaise herbal cocktail extract

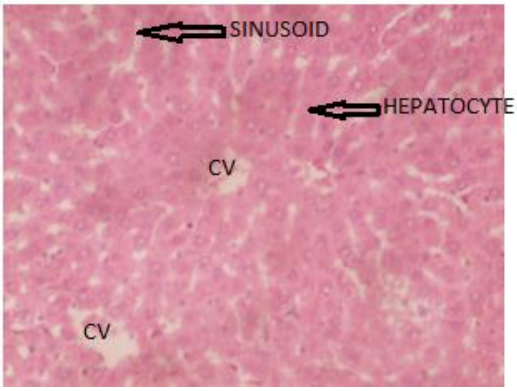
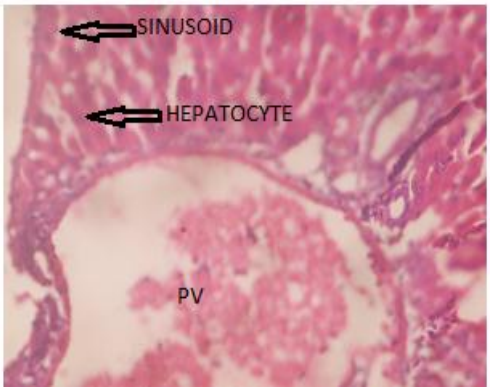


Plate 1



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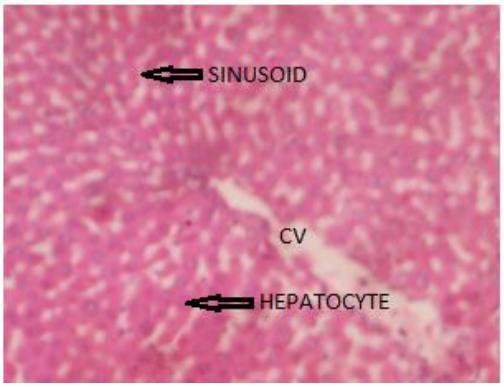

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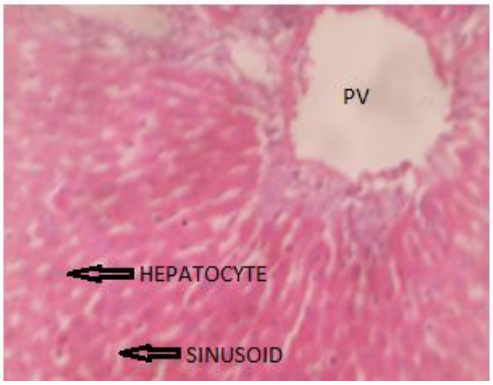

Plate 4

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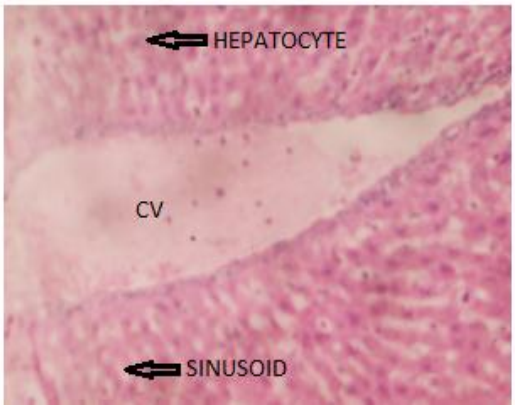

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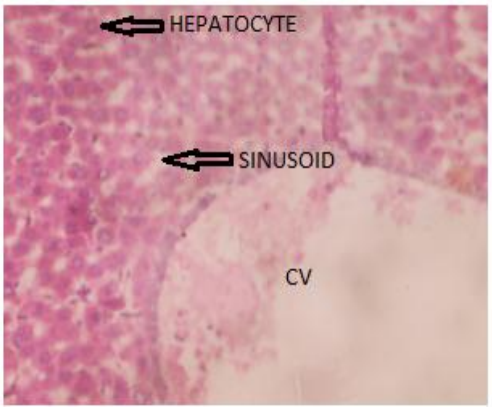
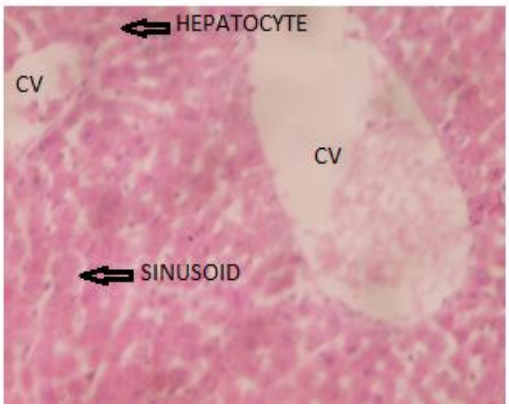


Plate 6

Architecture of hepatic tissues of STZ induced diabetic rats after 12 weeks of treatment with metformin and Aju Mbaise herbal cocktail extract



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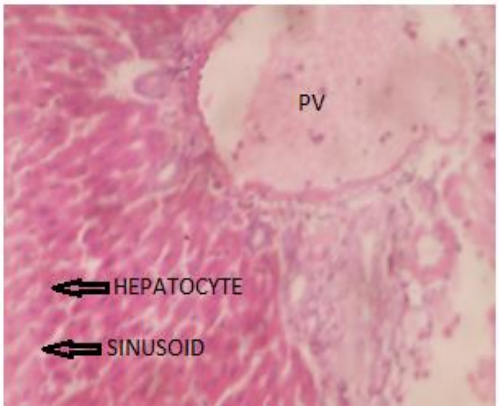

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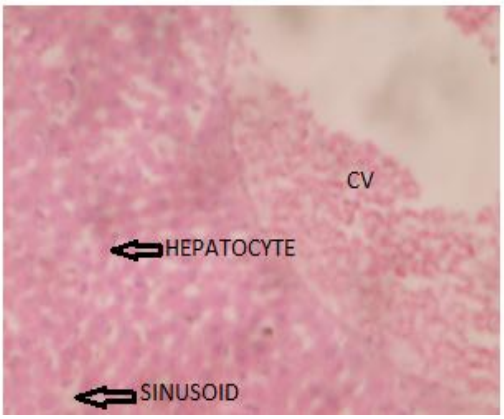

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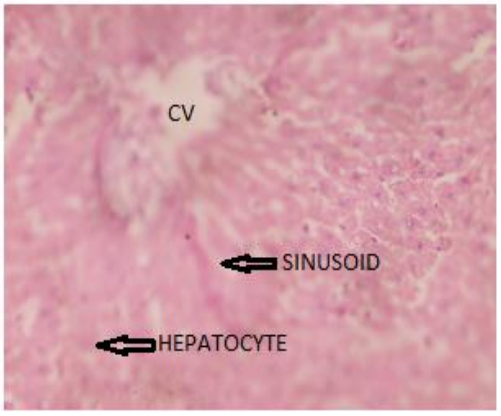
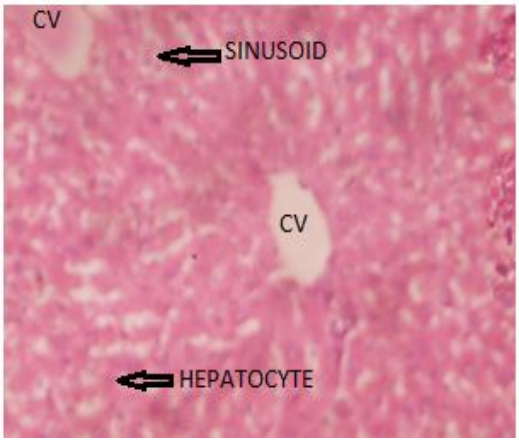


Plate 10



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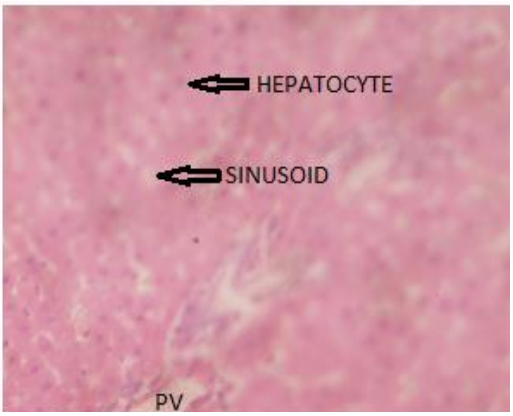
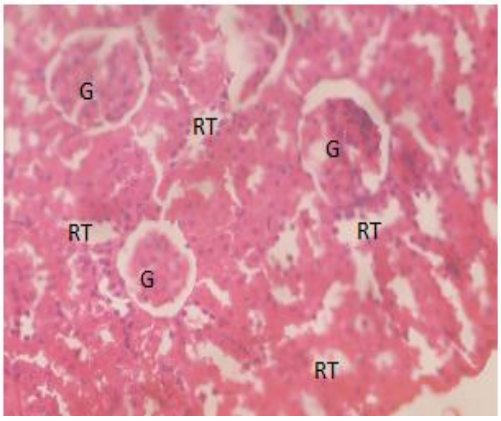


Plate 12

Architecture of renal tissues of STZ induced diabetic rats after 4 weeks of treatment with metformin and Aju Mbaise herbal cocktail extract



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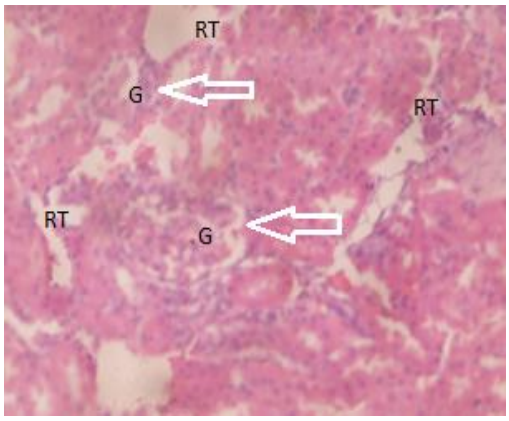

Plate 14

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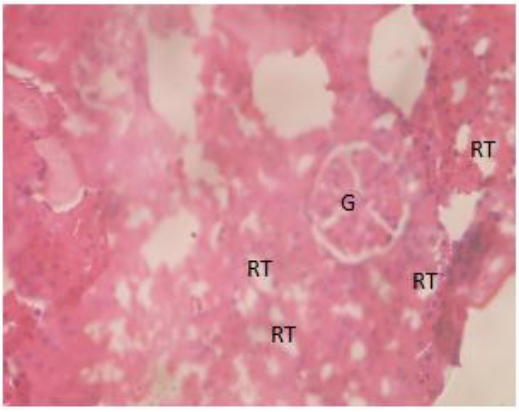

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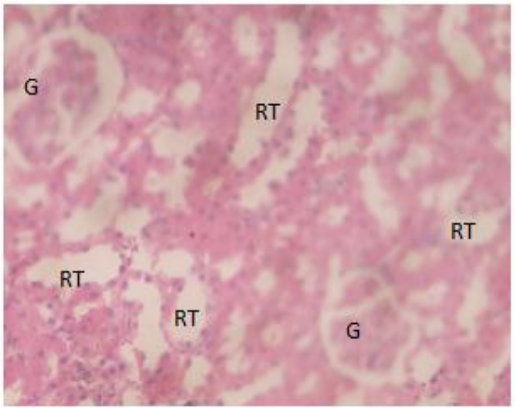

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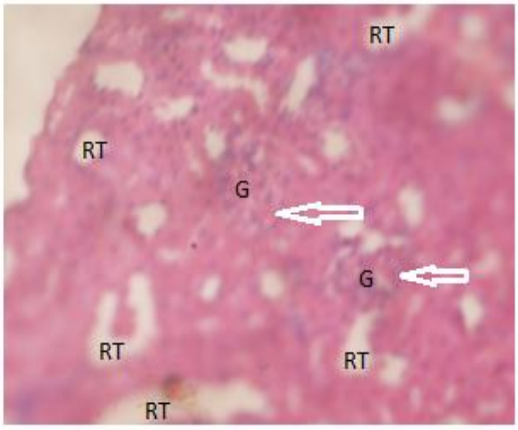

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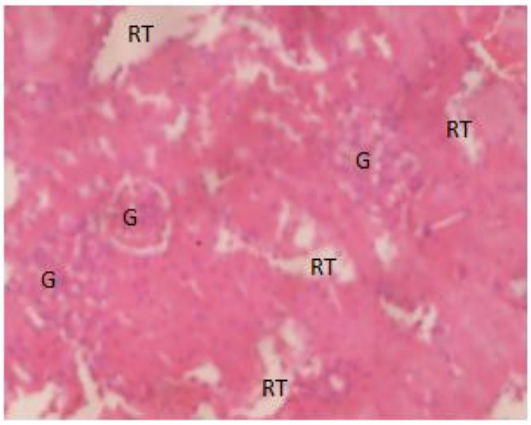
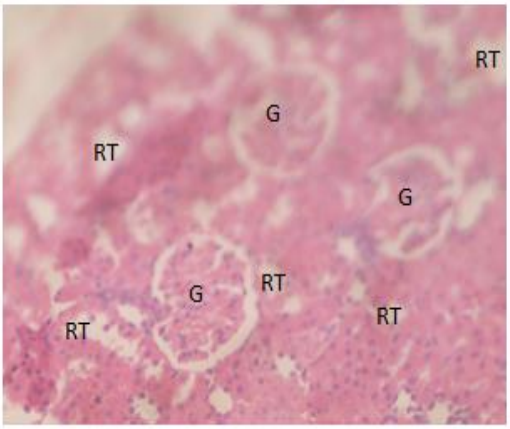


Plate 18

Architecture of renal tissues of STZ induced diabetic rats after 12 weeks of treatment with metformin and Aju Mbaise herbal cocktail extract



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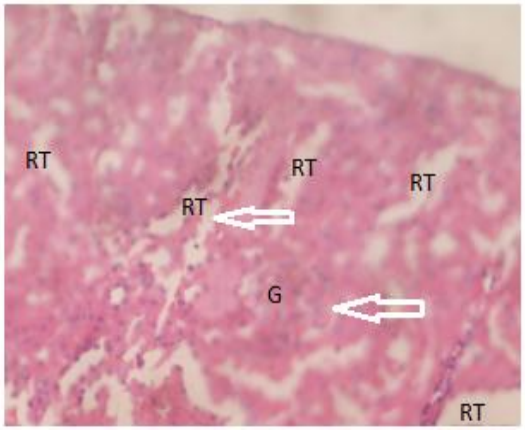

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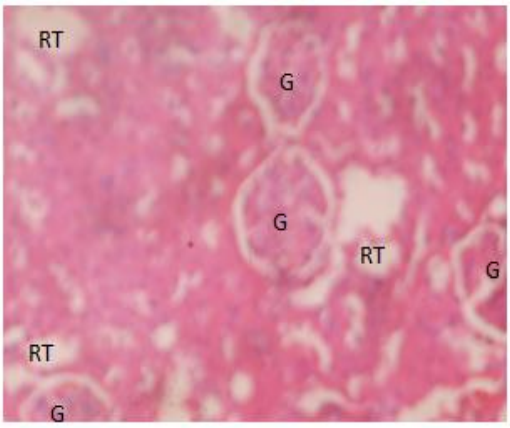

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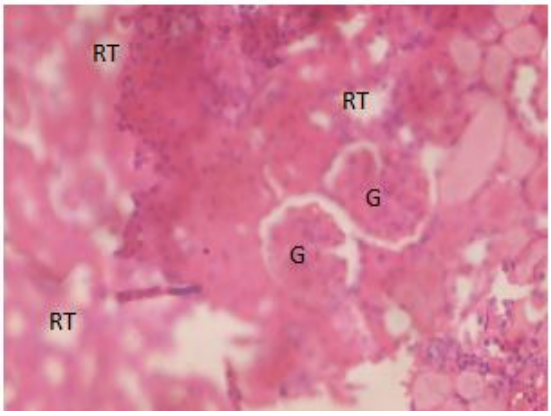

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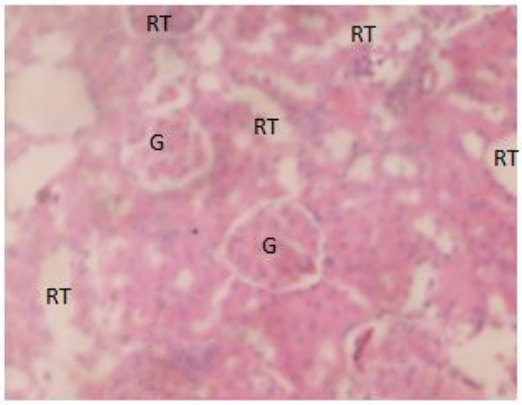

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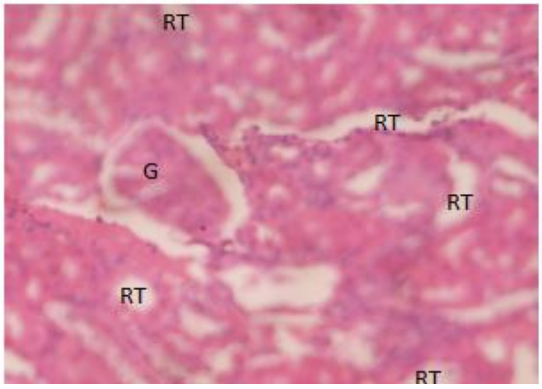

Plate 24

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DISCUSSION

According to Nnadiukwu *et al.* (2019), DM is a known chronic human autoimmune disease that is commonly associated with an abnormal high blood sugar (glucose) level. Thus, blood glucose assay is the most prominent and fundamental biomarker utilized in diagnosing and proving DM. According to Wang *et al.* (2013), DM induced with high fat diet (HFD) and low dose STZ is marked by critical rise in serum glucose, cholesterol, triglyceride, LDL, urea and creatinine and declined level of insulin and HDL. Administration of HFD with a low dose (35 $mgkg^{-1}$) STZ can cause type 2 diabetes (T2D) in Wistar albino rats. From the study, the diabetic animals were treated with a standard antidiabetic drug (metformin) and three (3) different concentrations of the extracts of 'Aju Mbaise' herbal mixture. The result revealed that the herbal extract lowered blood glucose level, though not as proficient and viable as the standard antidiabetic drug metformin. This significant decrease in glucose level might be attributed to the presence of flavonoids (Sok Yen *et al.,* 2021), phenols (de Paulo Farias *et al.,* 2021) and terpenoids (Ghani, 2019) in the extract of the herbal mixture. This outcome is in agreement with the report of Eze *et al.* (2019) that antidiabetic properties of medicinal plants are subject to the actions of the constituent secondary metabolites. According to Li *et al.* (2020), reduction in blood glucose level may occur by enhanced glucose uptake, stimulation of pancreatic β-cells to release insulin or by inhibiting the activities of pancreatic amylase and gluconeogenesis. A further hypoglycaemic action of the herbal extract may be by lipolytic activities in the liver and muscle tissues. This response is common with some antidiabetic drugs that act as transcription components that improve recycling of glycerol in the fat tissue rather than the hepatic and muscle tissues. In diabetes condition, amylase significantly metabolizes long chain carbohydrate compounds into simpler glucose, maltose and other absorbable monosaccharides. Thus, amylase inhibition would reduce the rate of glucose release and absorption in the small intestine and consequently suppress postprandial hyperglycemia (Hanhineva *et al.,* 2010). Liver function tests (LFTs) are group of tests usually used to inspect for liver disorder, and its progression, as well as estimate the possible effects of potential anti-hepatotoxic agents (Shakeel *et al.,* 2022). Elevated activities of AST, ALT, and ALP were noted in this investigation. Thus, substantiates the report of Nnadiukwu *et al.* (2016), that liver enzyme activities increase in diabetic condition because of their release during plasma membrane and cellular damage. The herbal extract was able to reduce the concentration of these enzymes in the animals as treatment advanced. The declined liver enzyme function observed in the treated groups signifies a protective potential of the herbal extract against liver toxicity. This demonstrates that the herbal cocktail extract has a hepatoprotective property. Total protein concentration was higher in the NC and all the treated animals than the DC animals as treatment advanced. As indicated by Yassin *et al.* (2015), decrease in serum total protein might be due to decreased protein synthesis and assimilation. Albumin is a plasma protein synthesized in the liver and flows in the circulation system. It is an essential determinant of gravity of certain infections (Farag and Ebrahim, 2020). According to Sheinenzon *et al.* (2021), reduced albumin concentration might be linked to decrease in its synthesis in the liver. From this study, a decrease in albumin concentration was seen when the DC group was compared with other experimental groups that had higher albumin levels. The findings as made in this study proved that the herbal mixture elevated the serum albumin levels of the diabetic treated animals. Bilirubin is an end-product of heme degradation (Graw, 2023). Total serum bilirubin is found to be high in animals with haemolytic anaemia, and this increase is majorly caused by prominent indirect-reacting bilirubin. The rate at which bilirubin

is elevated in haemolytic anaemia is dependent on the rate of red cell destruction and the capacity of the liver to excrete the newly synthesized bilirubin (Hansen *et al.*, 2020). From this study, serum bilirubin level was increased in the DC group when compared to the NC and the other treated animals throughout the experiment. Moreover, reduced concentrations of total and direct bilirubin were observed in the treated groups. Urea is a nitrogenous compound that accumulates in the plasma when normal renal excretion is obstructed. The urea and creatinine level of the DC animals were higher than the NC and the treated animals. A significant increase in serum urea and creatinine concentration indicates an impaired renal function of diabetic animals (Nnadiukwu *et al.*, 2016). The elevated levels of urea and creatinine in DC animals when conmpared with the NC animals is consonant with the reports of Olorunnisola *et al.* (2017), which posited that DM may lead to renal malfunctioning which might be due to oxidative stress and the stimulation of gluconeogenesis as alternative glucose supply route resulting from insulin deficiency. According to Rawitch and Baynes (2022), gluconeogenesis is persistent by improved proteolysis which releases the amino group (NH_2) of glucogenic amino acids that are deaminated in the liver resulting in elevated urea concentration. The significant decrease in urea witnessed after treatment can be attributed to the ability of the herbal mixture extract to reduce glucose concentration and thus increase insulin effect causing a decrease in proteolysis (Gabriel and Idu, 2021). According to Bennet *et al.* (2017), the kidney also maintains constant electrolyte balance in the blood irrespective of some alterations in the human body. A lower plasma electrolyte level in the DC than the NC and the treated animals were recorded in this work, and this is in consonant with the reports of Govindappa (2015). The herbal extract increased the electrolyte level of the treated animals. Subsequently, this herbal mixture could be employed to improve depleted electrolyte levels, and reducing elevated urea and creatinine levels common in diabetic conditions. This study has also shown that the administration of HFD and low dose of injection of STZ can actually damage and/or cause significant alterations in the liver, and kidney. This is in consonant with the reports of Nnadiukwu *et al.* (2016), that diabetogenic agents such as alloxan, has the tendency to cause liver, renal and pancreatic damage. The anomalies in the diseased tissues include mild distortion of the liver tissue due to congested portal and central veins as well as inflamed hepatocytes, distorted renal tubules and obliterated Bowman's capsular spaces. All these were remedied after the administration of the therapies; thus, there was a positive effect of the herbal mixture of 'Aju Mbaise' on the organs assessed. The ability of the Aju Mbaise herbal mixture extract to manifest these actions in diabetic animals could be attributed to its high flavonoids, terpenoids and phenolic constituents as well as minerals and vitamins (D and E). Derakhshanian *et al.* (2017), posited that trace elements like cobalt, boron, chromium, copper, sulfur, iodine, zinc and molybdenum enhance insulin action by activating insulin receptor sites. According to Berridge (2017), vitamin D plays a significant role in maintaining normal release of insulin by the pancreatic beta cells (β-cells), and its deficiency contributes to both the initial insulin resistance and the subsequent onset of diabetes caused by β-cell death. Also according to Pazdro and Burgess (2010), vitamin E has shown protection against lipid peroxidation in oxidative stress-induced diabetes complication.

CONCLUSION

This study has shown that prolonged (8 weeks) administration of high fat diet (HFD), and thereafter 35 $mgkg^{-1}$ b. w. intraperitoneal injection of STZ can cause type-2 diabetes mellitus. This was also confirmed with the histological assessment of the liver and kidney which revealed that HFD-STZ model of diabetes caused an obliterated hepatocytes,

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distorted renal tubules and obliterated Bowman's capsular spaces of the kidney. The study revealed the herbal cocktail extract activity in restoring the damaged organs as treatment progressed. The study showed the herbal cocktail extract potential in regulating blood glucose level, renal and liver function markers especially the liver enzymes activities. This study also recorded that the ethanol extract of 'Aju Mbaise' herbal cocktail at 500 mg/kg b. w. is safe in the management of type 2 diabetes mellitus, but not to be compared with the activity and efficacy of metformin.

Competing Interests

Authors have declared that no competing interests exist.

Authors' Contributions

Nnadiukwu, T. A., and Monago-Ighorodje, C. C, designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript.

Nnadiukwu, T. A., managed the analyses of the study.

Nnadiukwu, T. A., and Chuku, L. C, managed the literature searches.

All authors read and approved the final manuscript.

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