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Assessment of single and combined administration of herbal extracts on the hepatorenal system of diabetic rats

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ABSTRACT

This work aimed at evaluating the hepatorenal changes that occur in diabetic rats after single and combined administration of ethanol leaf extracts of *Ocimum gratissimum* and *Kalanchoe pinnata*. Thirty six (36) male adult rats was divided into six (6) groups of six (6) rats each. The group 1 was normal rats that were given only water and normal rat chow; Group 2 was untreated diabetic (DM) rats; Group 3 was DM rats that were treated with 500 mg/kg b.w. of the antidiabetic drug, metformin; Groups 4, 5 and 6 were DM rats given 200 mg/kg^{-1} b.w. of *O. gratissimum* leaves, *K. pinnata* leaves, and combined dose (ratio: 1:1 w/w) of the leaves of *O. gratissimum* + *K. pinnata* respectively. The rats were induced with diabetes mellitus (DM) through single intra-peritoneal (i.p.) injection of 150 mg/kg b.w. of alloxan monohydrate. Standard spectrophotometric methods were used to measure hepatorenal biomarkers and for histopathological studies. Serum ALT, AST and total bilirubin concentrations were significantly elevated ($p < 0.05$) while serum urea, creatinine, Na^+ and K^+ concentrations were significantly lowered ($p < 0.05$) in the rat groups treated with single and combinatorial herbal formulations (SCHF) in comparism with the untreated diabetic rat group. Histologic markers of hepatic injury and good therapeutic renal state were recorded in this study in rats treated with both SCHF. In conclusion, SCHF reversed the damages elicited on the kidney by diabetes but was not able to reverse the damage that was incurred on the liver.

***Keywords*:** liver, kidney, herbal formulation, histology, diabetes

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1. INTRODUCTION

Metabolic processes in the liver and kidney are generally necessary to maintain consistency in the internal environment of vertebrates (Singh *et al*., 2011; Gu and Manautou, 2012). Hepatocytes' metabolic processes are controlled at the molecular, organelle, cellular, and organ levels (Mello *et al*., 2016). Hepatocytes' endogenous metabolic control mechanisms include interactions with sinusoidal and Kupffer cells, regulatory enzyme activity, and organelles in charge of protein and lipid biosynthesis. In the meantime, interactions between the renal, enteric, and endocrine systems, as well as biochemical interactions between the liver and the musculature, enable exogenous control mechanisms (Chikezie *et al*., 2020). Other sources provide a summary of the metabolic heterogeneity of hepatocytes in both health and disease (Kudryavtseva *et al*., 2001; Chikezie *et al*., 2020). Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and the inducible hepatocyte smooth endoplasmic reticulum (SER) specific enzyme g-glutamyl transferase (g-GT) are non-functional plasma enzyme indicators that are used to establish the routine clinical evaluation of the functional status of hepatocytes. This test is known as the liver function test (LFT)/biliary integrity test (BIT). Additionally, blood sample concentrations of albumin, total bilirubin, and total protein are measured (Coates, 2011; Hall and Cash, 2012). The kidneys' functional unit is the nephron. The elimination of low plasma threshold substances like urea, creatinine, and uric acid is the main function of the renal tissues. Along with controlling blood electrolyte levels, the renal tissues also control extracellular fluid volume, osmolality, and the vascular system's acid-base balance. Additionally, steroid and polypeptide hormones like erythropoietin, renin, and 1, 25 dihydroxyvitamin D are biosynthesised in the kidneys (Mona *et al*., 2013; Gounden and Jialal, 2019). Reduced renal function is indicated by elevated blood levels of plasma low threshold substances. Among other blood indicators, the kidney function test (KFT) measures blood urea nitrogen (BUN) and plasma creatinine levels; elevated BUN levels in the blood are indicative of the onset and progression of renal disease (Al-Hindi *et al*., 2019). Indicators from renal and kidney function tests are used to track how well therapeutic interventions are working to restore impaired renal function (Gounden and Jialal, 2019).

Ocimum gratissimum L. belongs to the Lamiaceae family (Ogundipe *et al*., 2017). The plant is a perennial herb that grows extensively throughout the world's warm and temperate climates (Okon *et al*., 2012). The phytochemical compositions of *O. gratissimum*'s diethylether, ethyl acetate, ethanol, and aqueous leaf extracts have been thoroughly documented elsewhere (Oladosu-Ajayi *et al*., 2017; Adeshina *et al*., 2018; Sahalie *et al*., 2018). It was observed that *O. gratissimum* contained comparatively high amounts of alkaloids, flavonoids, saponins, methyl cinnamate, camphor, thymol, eugenol, linalool, xanthones, citral, terpenes, and lactones (Ofem *et al*., 2012; Adeshina *et al*., 2018; Sahalie *et al*., 2018). Extracts from *O. gratissimum* are used by practitioners of traditional medicine to treat and manage a variety of conditions, including fever, rheumatism, paralysis, epilepsy, high fever, diarrhea, sunstroke, influenza, gonorrhea, and mental illness (Chetia *et al*., 2014). Empirical studies have confirmed that practitioners of folklore medicine use *O. gratissimum* extracts to treat microbial infections (Adeshina *et al*., 2018; Sahalie *et al*., 2018; Chikezie *et al*., 2020; Ohiagu *et al*., 2021a).

Kalanchoe pinnata, also known as the "divine plant," is a succulent plant. Its leaf, stem, and root parts are rich in bioactive compounds with high therapeutic value indices across different nations (Nayak *et al*., 2010). Kalanchoe pinnata, a member of the Crassulaceae family, is also commonly referred to as Master herb, Ranakalli, Miracle leaf, Mexican Love plant, Katakataka, Mother of Thousand, Cathedral Bells, Air plant, etc. (Kaur *et al*., 2014). According to Afzal *et al*. (2013), it has a number of pharmacological properties, including antimicrobial, antioxidant, urolithiatic, and antiproliferative activity. The plant *K. pinnata* also has thrombolytic, hemoprotective, nephroprotective, gastroprotective, antihistamine, antihypertensive, and immunosuppressive properties (Bopda *et al*., 2014; Ghasi *et al*., 2011). Kidney stones are commonly treated with the juice of the leaves of *K. pinnata* in conventional medicine (Cruz *et al*., 2008). According to empirical data, harmful phytochemicals found in edible vegetables and medicinal plants are typically removed by conventional and traditional processing techniques before the plant materials are consumed (Chikezie and Ojiako, 2013; Ndidi *et al*., 2014). Some of these medicinal and edible plants have been shown to cause systemic toxicity and organ dysfunction, particularly when consumed in large amounts and in their raw form (Chikezie *et al*., 2015; Ibegbulem *et al*., 2018). Not withstanding the vast medicinal properties of *O. gratissimum* and *K. pinnata*, the present study ascertained the hepatorenal changes that occur in diabetic rats following the administration of single and combined ethanol leaf extracts of *O. gratissimum* and *K. pinnata.*

2. METHODS

2. 1. Collection and preparation of samples

Collection and preparation of the leaves for extraction was according to the methods previously described (Ojiako *et al*., 2016). All the leaves were collected between the months of October and November, 2024.

2. 2. Preparation of leaf extracts

Preparation of ethanolic leaf extracts of the two ground samples was according to the methods previously described (Ojiako *et al*., 2016). Appropriate doses of single and combinatorial leaf extracts were prepared and administered to the rats.

2. 3. Experimental animals/ethics

A commercial animal house in Owerri-North LGA, Imo State, Nigeria, sold male albino rats weighing an average of 145 g. In metal cages with adequate ventilation, the rats were kept at 28 ±2 °C and 30 to 55% relative humidity on a 12-hour light/12-hour dark cycle. They were also given unlimited access to water and pelletized standard Guinea Feed® (PSGF) (United Africa Company Nigeria Plc., Jos, Nigeria). To get used to the surroundings, they were housed for two weeks. The ethical committee on the use of animals for research at Imo State University in Owerri, Nigeria's Department of Biochemistry, gave its approval to the current study. The rats were handled in compliance with the National Institutes of Health's (NIH, 1978) standard guidelines for laboratory animal care.

2. 4. Induction of Diabetes Mellitus

To induce diabetes mellitus, rats were given a single intraperitoneal (i.p.) injection of 150 mg/kg b.w. of alloxan monohydrate (Sigma, St. Louis, USA). Rats were chosen for the experiment if their fasting plasma glucose concentration (FPGC) was greater than 110 mg/dL for five consecutive days. This was considered diabetic (Ojiako *et al*., 2016).

2. 5. Experimental Design

Six (6) groups of six (6) male albino rats each were created from the 36 total. Sixteen (16) hours before the start of treatment, the rats were not fed or given water (Ottaviano *et al.,* 2008).

The animal groups were treated by oral gavage on daily basis for 21 days as described by Pepato *et al*. (2004) and were grouped on the basis of treatments given as follows:

- Group 1: Normal rats that were given only water and normal rat chow
- Group 2: Untreated DM rats
- Group 3: DM rats that were treated with 500 mg/kg b.w. of the antidiabetic drug, metformin
- Group 4: DM rats were given 200 $mgkg^{-1}$ b.w. of *O. gratissimum* leaves
- Group 5: DM rats that were given 200 $mgkg^{-1}$ b.w. of *K. pinnata* leaves
- Group 6: DM rat that were given 200 $mgkg^{-1}$ b.w. combined dose (ratio: 1:1 w/w) of the leaves of *O. gratissimum* + *K. pinnata*

2. 6. Hepatorenal Function biomarkers

Serum concentrations of hepatorenal tissues biomarkers were measured; serum AST and ALT activities were assessed according to the methods of Henry *et al*. (1960) and Onunogbo *et al*. (2013) respectively, and serum ALP activity (Njoku *et al*., 2011), serum total bilirubin (Pearlman and Lee, 1974), serum urea (Fawcett and Scott, 1960), serum creatinine (Bonsnes and Taussky, 1945), serum sodium ion (Maruna, 1958), serum chloride ion (Skeggs and Hochstrasser, 1964) serum potassium ion (Maruna, 1958) and serum bicarbonate ion (Skeggs and Hochstrasser, 1964) concentrations were measured.

2. 7. Histopathological studies

The liver and kidney tissues were fixed in 10% formosaline to prevent putrefaction and autolysis, embedded in paraffin wax, sectioned and stained with hematoxylin and eosin (H&E) for histological analysis. The slides were examined under a light microscope (Olympus CH; Olympus, Tokyo, Japan) and photomicrographs were taken with a Leica DM 750 Camera at ×400 magnification.

2. 8. Statistical analysis

Analysis of variance (ANOVA; SPSS Version 15, IBM Corporation, New York, USA) was used to analyze the data, and the results were displayed as mean ± standard deviation of the mean (SD). At $p < 0.05$, differences between the means of the various groups were deemed significant.

3. RESULTS

3. 1. Liver Function Biomarkers

Figure 1 shows the serum ALT and AST concentrations of the experimental rat groups, and Figure 2 shows the serum ALP and total bilirubin concentrations of the experimental rat groups. The groups treated with 500 mg/kg metformin, *O. gratissiumum* leaf extract and *K. pinnata* leaf extract (Group 3, Group 4 and Group 5 respectively) had significantly higher serum ALT concentrations than that of the normal control group (Group 1) and untreated diabetic group (Group 2) ($p < 0.05$). However, there was no significant difference in serum ALT concentration of Group 1, Group 2 and the combinatorial herbal dose group (Group 6) rats ($p > 0.05$). The serum AST concentration of *O. gratissimum* leaf extract (Group 4) and *K. pinnata* leaf extract (Group 5) groups were significantly higher than the normal control (Group 1), untreated diabetic (Group 2), antidiabetic drug (Group 3) and *O. gratissimum* leaf extract (Group 4) groups ($p < 0.05$). Nevertheless, Group 6 was significantly comparable in serum AST concentration to Group 1, Group 2 and Group 3 ($p > 0.05$). The serum ALP concentration of the untreated diabetic group (Group 2) was significantly higher than that treated with *O. gratissimum* leaf extract (Group 4) and the combinatorial herbal dose (Group 6) ($p < 0.05$). All the herbal formulation treated rat groups had significantly higher serum ALP concentrations than the normal control group (Group 1) ($p < 0.05$). The serum total bilirubin concentration of all the herbal formulation treated rat groups and antidiabetic drug group (Group 3) were significantly comparable ($p > 0.05$), but were significant higher than the normal control (Group 1) and untreated diabetic (Group 2) groups ($p < 0.05$).

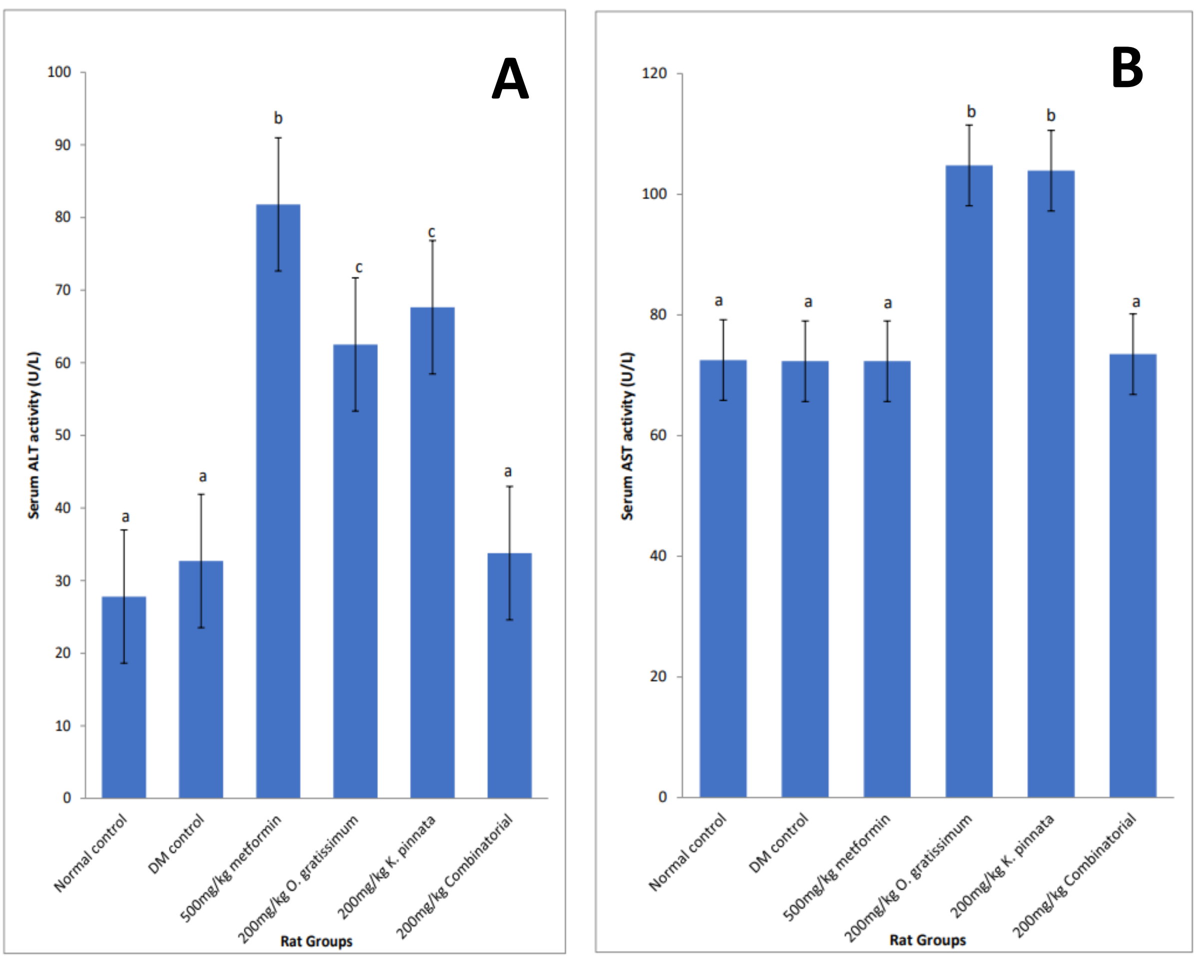


Figure 1. Effects of single and combinatorial administration of *O. gratissimum* and *K. pinnata* leaves on serum ALT (**A**); AST (**B**) concentrations in diabetic Wistar rat model. Bars with the same letter of alphabet are not significantly different (p > 0.05).

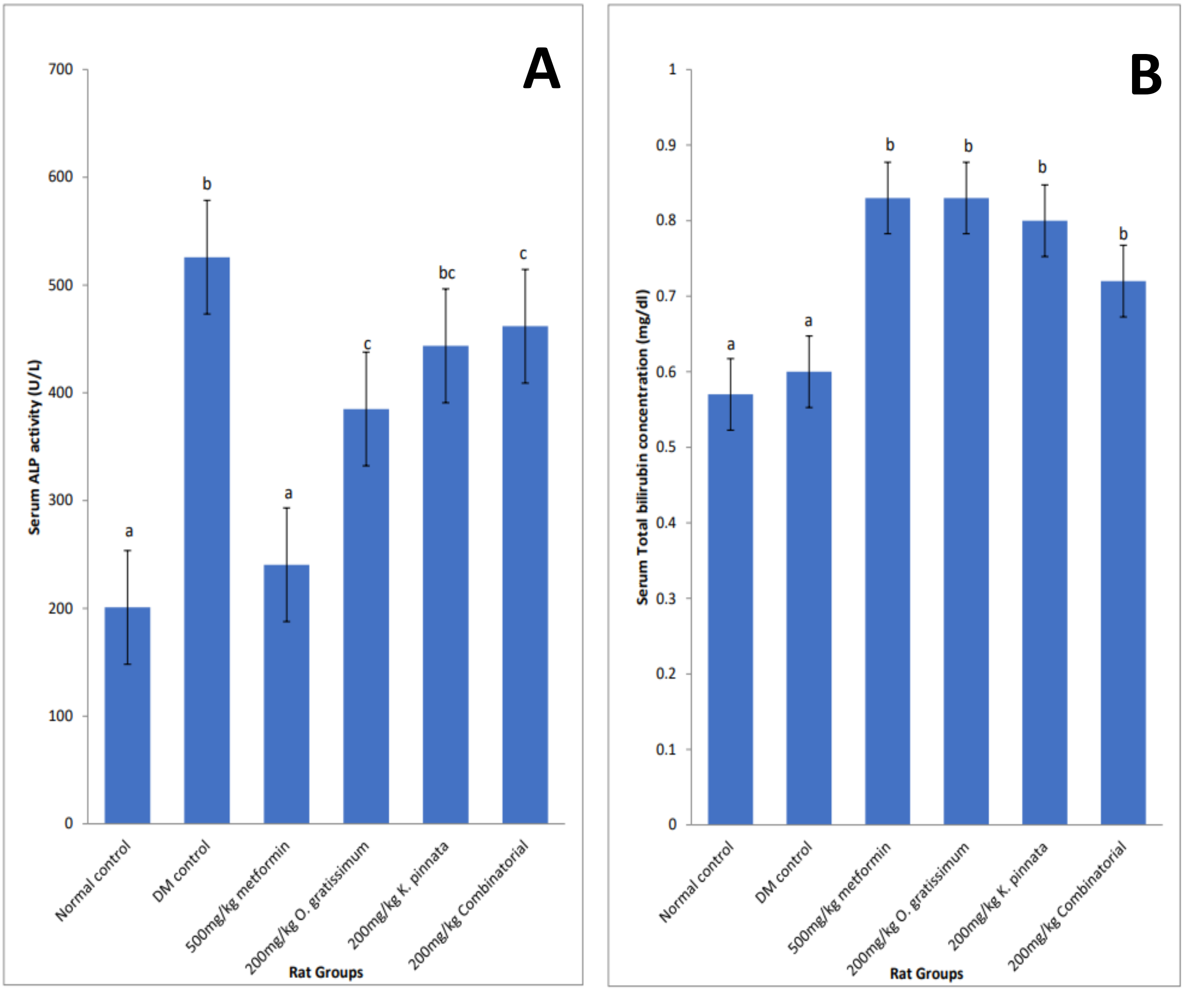
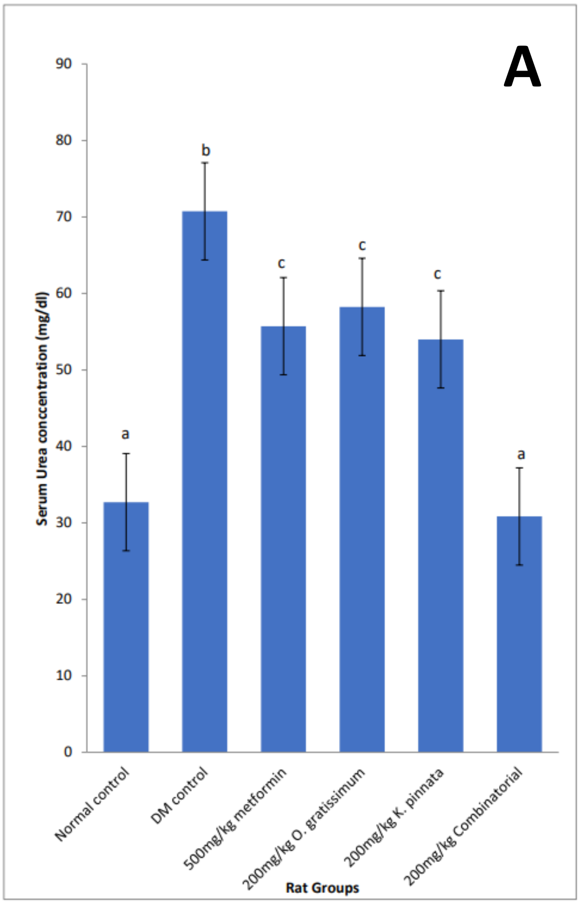


Figure 2. Effects of single and combinatorial administration of *O. gratissimum* and *K. pinnata* leaves on serum ALP (**A**); total bilirubin (**B**) concentrations in diabetic Wistar rat model. Bars with the same letter of alphabet are not significantly different ($p > 0.05$).

3. 2. Kidney Function Biomarkers

The serum urea and creatinine concentrations of the experimental rat groups is shown in Figure 3. The serum urea concentration of all the herbal formulation treated rat groups and the antidiabetic drug group (Group 3) were significantly lower than the untreated diabetic group (Group 2) ($p < 0.05$). The antidiabetic drug (Group 3), *O. gratissimum* leaf extract (Group 4) and *K. pinnata* leaf extract (Group 5) groups had significantly higher serum urea concentration than the normal control group (Group 1) ($p < 0.05$).

There was no significant difference in the serum urea concentration of Group 1 and the combinatorial herbal dose group (Group 6) ($p > 0.05$). The normal control (Group 1), antidiabetic drug (Group 3) and all the herbal formulation treated rat groups were all significant comparable in serum creatinine concentration ($p > 0.05$). Furthermore, the untreated diabetic group (Group 2) was significantly higher in serum creatinine concentration than all the herbal formulation treated rat groups ($p < 0.05$).



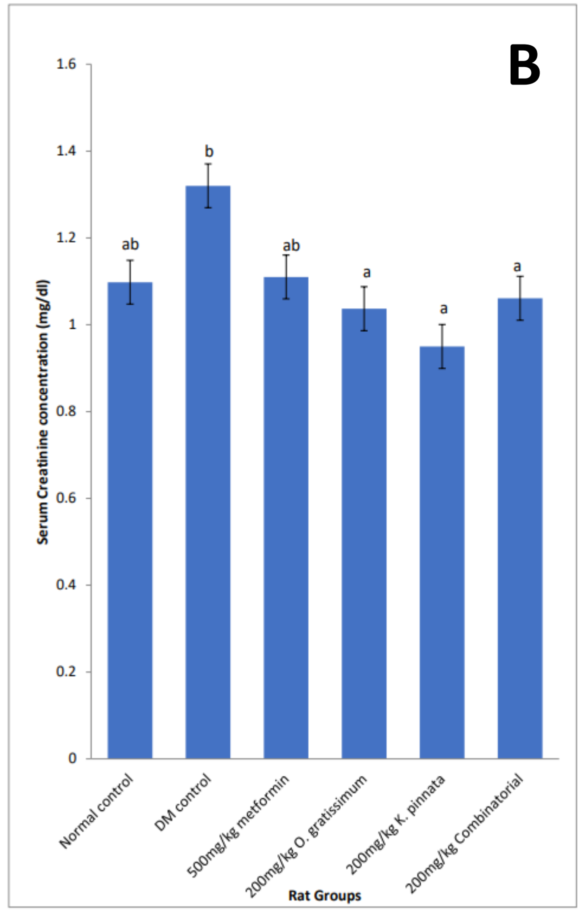


Figure 3. Effects of single and combinatorial administration of *O. gratissimum* and *K. pinnata* leaves on serum Urea (**A**); Creatinine (**B**) concentrations in diabetic Wistar rat model. Bars with the same letter of alphabet are not significantly different (p > 0.05).

Figure 4 shows the serum chloride and sodium ion concentrations of the experimental rat groups; and Figure 5 shows the serum potassium and bicarbonate ion concentrations of the experimental rat groups. There was no significant difference in serum chloride ion concentration between the normal control (Group 1), the untreated diabetic (Group 2) and all the herbal formulation treated rat groups ($p > 0.05$). Furthermore, the antidiabetic drug group (Group 3) was significantly higher in serum chloride ion concentration than the *O. gratissimum* leaf extract (Group 4) and *K. pinnata* leaf extract (Group 5) groups ($p < 0.05$). The serum sodium ion concentration of the combinatorial herbal dose group (Group 6) was significantly higher than that of the other experimental rat groups ($p < 0.05$).

The groups treated with *O. gratissimum* leaf extract (Group 4) and *K. pinnata* leaf extract (Group 5) were significantly comparable to the normal control (Group 1) and untreated antidiabetic (Group 2) groups ($p > 0.05$). The serum potassium ion concentrations of all the herbal formulation treated rat groups were significantly lower than the normal control (Group 1), untreated antidiabetic (Group 2) and antidiabetic drug (Group 3) groups ($p < 0.05$). The serum potassium ion concentration of Group 3 was significantly comparable ($p > 0.05$) to Group

2 but significantly lower than Group 1 ($p < 0.05$). There was no significant difference in the serum bicarbonate ion concentration of all the experimental rat groups ($p > 0.05$).

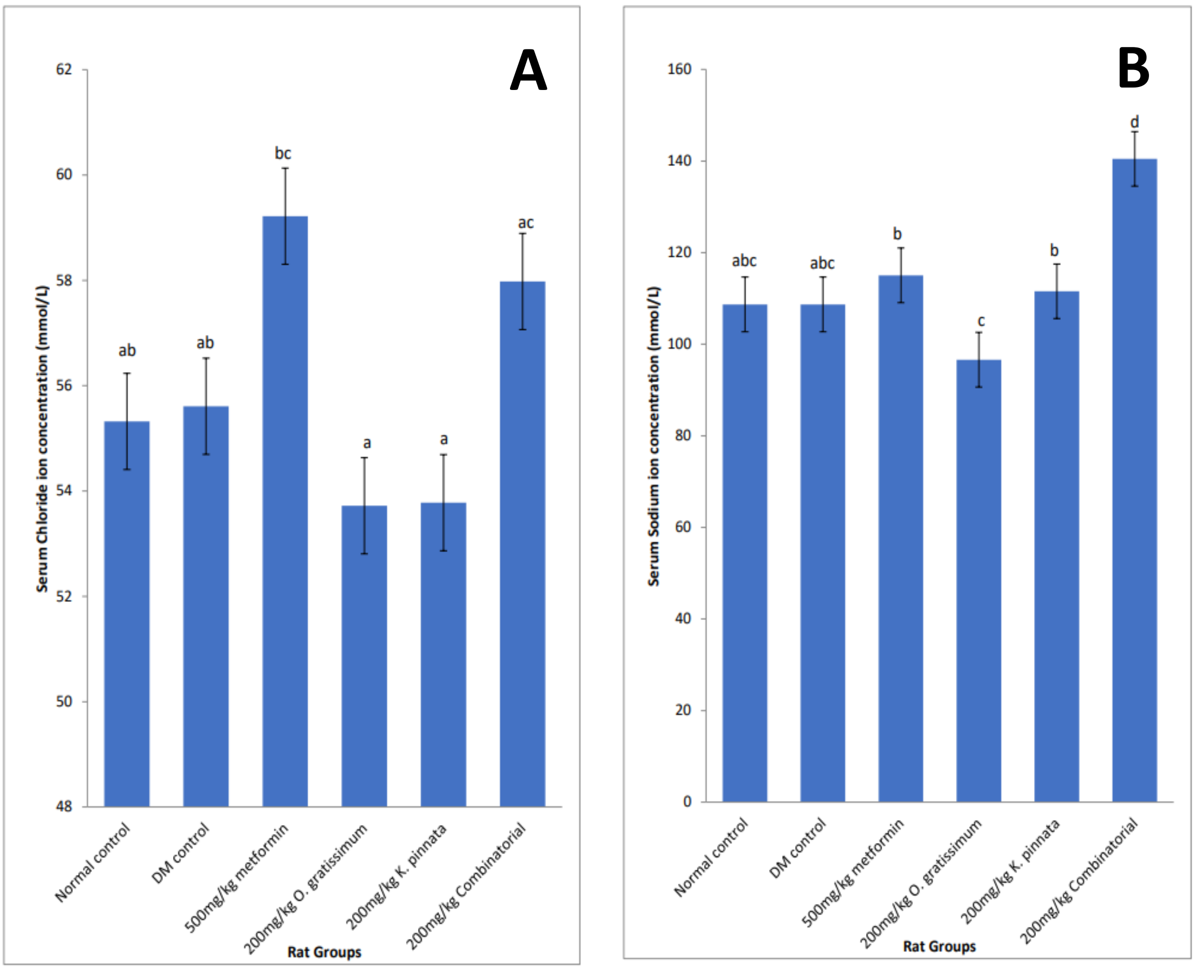


Figure 4. Effects of single and combinatorial administration of *O. gratissimum* and *K. pinnata* leaves on serum chloride (**A**); sodium (**B**) ion concentrations in diabetic Wistar rat model. Bars with the same letter of alphabet are not significantly different (p > 0.05).

3. 3. Histological analysis of the Liver Tissue Sample

Figure 6 shows the histopathology of the liver tissue sample of the experimental rat groups. The histology of the liver sample of the normal control group revealed a normal liver structure, the central vein (CV) was clear, the sinusoids (S) and hepatocytes (H) radiated hexagonally in their normal liver architecture with open faced nuclei (N) (healthy). For the untreated diabetic group, there were gross pathological changes as a gross distortion was seen (***) vividly; there was a degree of deformation seen as the hexagonal pattern was distorted, the nuclei (N) darkened and partly shrinking in size when compared with that of the normal control group. There was a regenerating liver structure as the central vein (CV) was clear in the liver tissue sample of the antidiabetic drug (metformin) treated group, the sinusoid and hepatocytes were seen radiating hexagonally, the nuclei was still seen darkened as it was still combacting with the effect. The liver tissue sample of the *O. gratissimum* leaf extract treated group was more restored than the antidiabetic drug (metformin) treated group although some slight fat cells were seen on the central vein (CV); the sinusoids (S) and the hepatocytes (H) were seen radiating hexagonally; the nuclei (N) were also seen regenerating.

There were signs of hyperactivities as some proliferation of cells (PC) were seen in the liver tissue sample of the *K. pinnata* leaf extract treated group; the two central veins were clear and the nuclei (N) showed a high degree of regeneration when compared with the untreated diabetic group.

For the liver tissue sample of the *O. gratissimum* + *K. pinnata* leaf extract treated group, there was better regeneration although it slightly came out of the distortion seen in the untreated diabetic group; the sinusoids (SS) and hepatocytes (H) gradually showed the hexagonal radiation and the nuclei (N) was gradually coming to normalcy while the central vein (CV) had some fat cells which might have been due to the hyperactivities or healing process.

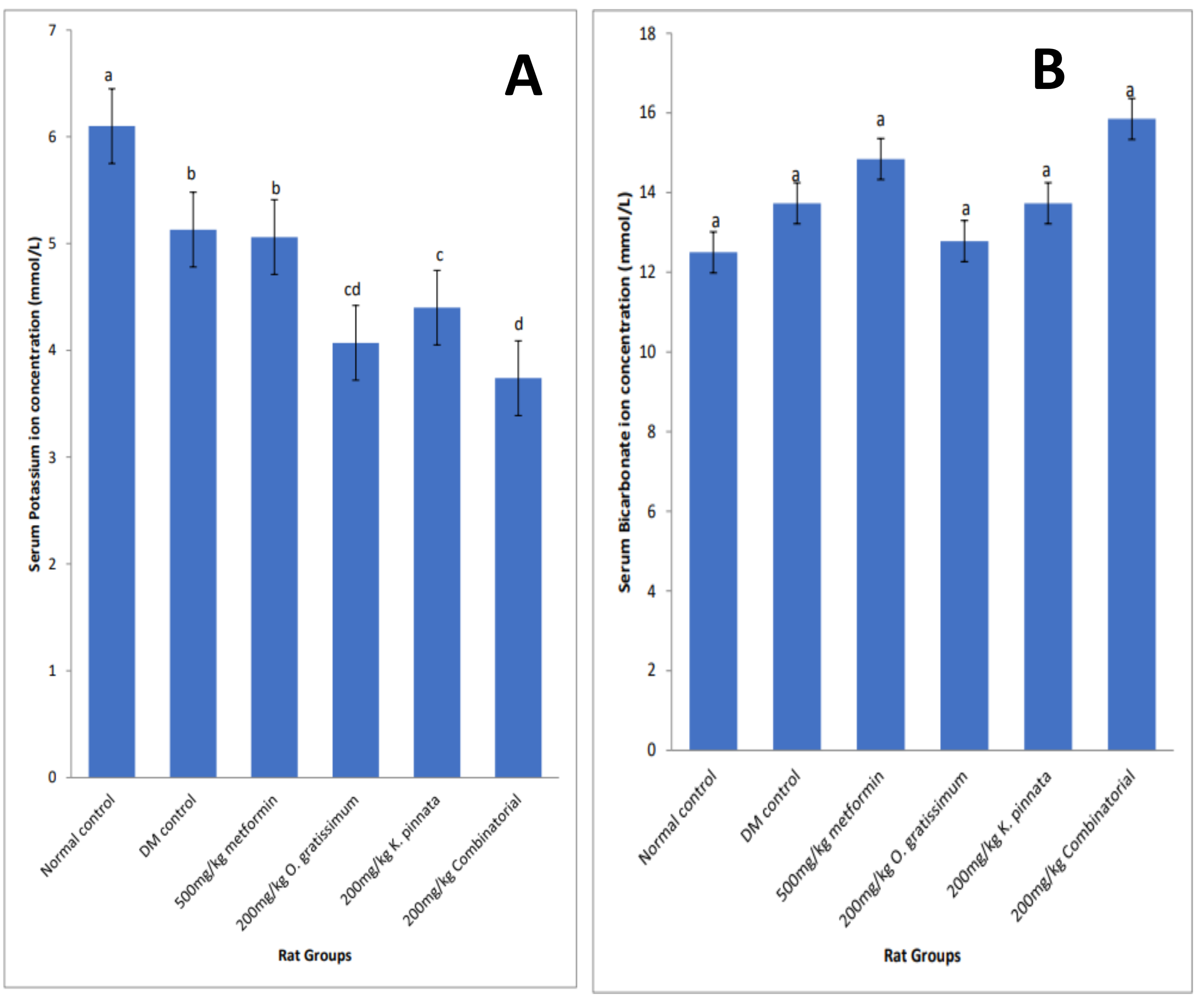


Figure 5. Effects of single and combinatorial administration of *O. gratissimum* and *K. pinnata* leaves on serum potassium (**A**); bicarbonate (**B**) ion concentrations in diabetic Wistar rat model. Bars with the same letter of alphabet are not significantly different ($p > 0.05$).

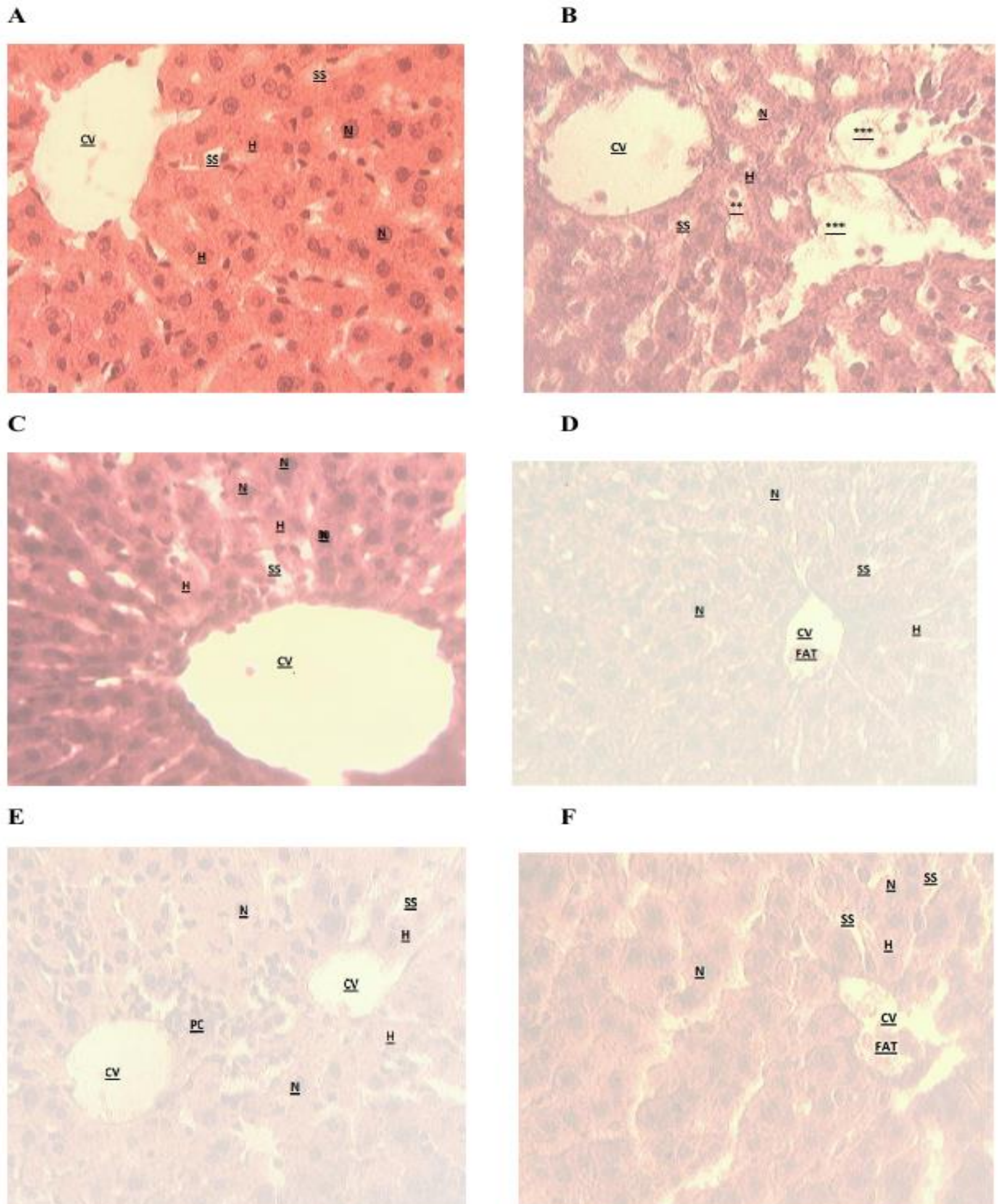


Figure 6. Histopathology of the liver (H & E stained) (**A**) normal control group (×400); (**B**) untreated diabetic group (×400); (**C**) antidiabetic drug (metformin) group (×400); (**D**) *O. gratissimum* ethanolic leaf extract treated group at 200 mg/kg (×400); (**E**) *K. pinnata* ethanolic leaf extract treated group at 200 mg/kg (×400); (**F**) *O. gratissimum* + *K. pinnata* ethanolic leaf extract treated group at 200 mg/kg (×400). CV: central vein, S: sinusoids, H: hepatocytes, N: nuclei**,** PC: proliferation of cells.

3. 4. Histological analysis of the Kidney Sample

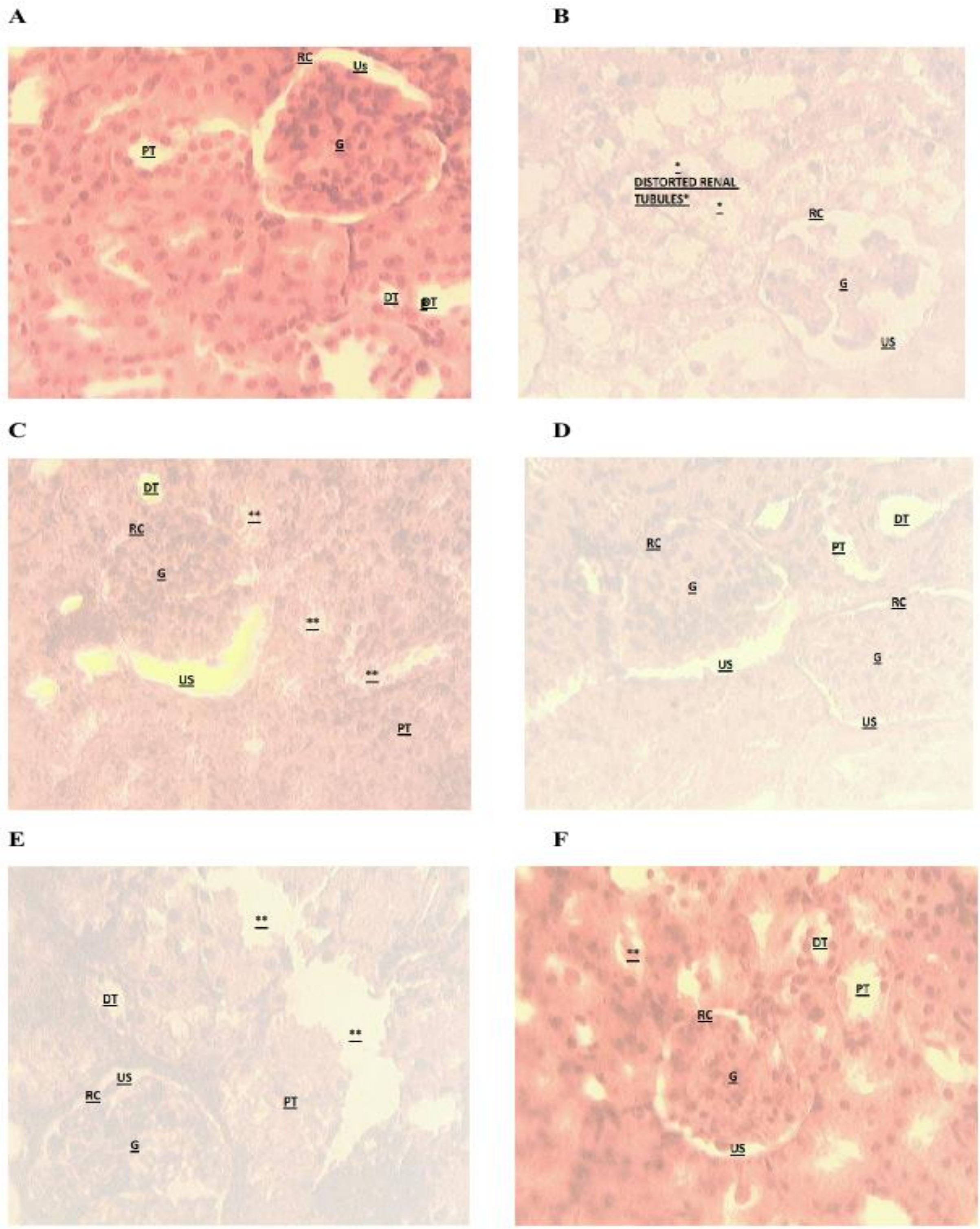


Figure 7. Histopathology of the Kidney (H & E stained) (**A**) normal control group (×400); (**B**) untreated diabetic group (×400); (**C**) antidiabetic drug (metformin) group (×400);

(**D**) *O. gratissimum* ethanolic leaf extract treated group at 200 mg/kg (×400); (**E**) *K. pinnata* ethanolic leaf extract treated group at 200 mg/kg (×400); **(F)** *O. gratissimum* + *K. pinnata* ethanolic leaf extract treated group at 200 mg/kg (×400). RC: renal corpuscle, US: urinary space. G: glomerulus, PT: proximal tubule, DT: distal tubule.

Figure 7 shows the histopathology of the kidney tissue sample of the experimental rat groups. The histology of the kidney sample of the normal control group revealed a normal cortex area the kidney, the renal corpuscle (RC), the urinary space (US) and glomerulus (G) were in good shape. Furthermore, the proximal tubule (PT) and distal tubule (DT) were all clear and healthy. There was visible pathological changes in the kidney tissue sample of the untreated diabetic group; the morphology and the structural architecture were seen with much distortion; the renal corpuscle (RC) was seen with a shrinking and splitting glomerulus and urinary space (US) while the renal tubule both proximal tubule (PT) and distal tubule (DT) were all distorted.

This antidiabetic drug (metformin) treated group showed a healing process when compared with the untreated diabetic group; the glomerulus in the renal corpuscle (RC) was regenerating as the urinary space was gradually taking its normal shape; slightly fat cells were seen in some of the tubules due to the healing process. For the kidney tissue sample of the *O. gratissimum* leaf extract treated group, a more restored kidney structure was seen as the whole features were regaining total normalcy at the upper sides while the lower side was gradually coming to normalcy; this group has a better effect in comparison to the antidiabetic drug treated group.

There were signs of hyperactivities in the kidney tissue sample of the *K. pinnata* leaf extract treated group as the healing process seems to be slow due to the partial distortions (**) seen and the entire structure was seen struggling to come alive. In the kidney tissue sample of the *O. gratissimum* + *K. pinnata* leaf extract treated group, a more excellent healing was seen as the whole features looked totally restored as in a normal kidney.

4. DISCUSSION

Due to the lack of accurate descriptions of their biochemical constituents, actual dosage, and toxicological profiles (all necessary to ensure their safe use) plants of medicinal significance are not widely accepted or incorporated into orthodox medical practices (Deng *et al*., 2013; Chikezie & Ojiako, 2015).

The toxicity profiles of the chemical components of widely used medicinal plants are unfortunately poorly understood (Barbosa *et al*., 2016). Therefore, before using medicinal plants for drug development or to increase the therapeutic efficacy of already-approved treatments, their toxicological significance must be assessed (Ohiagu *et al*., 2021b). The therapeutic efficacy of medicinal plants has been linked to their bioactive compound compositions (Chikezie *et al*., 2018).

The yellow breakdown product of normal haeme catabolism, bilirubin (previously known as haematoidin) is excreted in bile and urine, and elevated levels may indicate certain diseases like jaundice, the neurotoxicity of neonatal hyper bilirubinemia, etc. (Odiegwu *et al*., 2021).

The main reason to measure alkaline phosphatase is to check for the possibility of liver or bone disease.

In humans, alkaline phosphatase is present in all tissues throughout the body, but is especially concentrated in the liver, bile duct, kidney, bone, and placenta. An enzyme known as an amino transferase catalyzes a type of reaction between an amino acid and α-keto acid (Sharma *et al*., 2014; Odiegwu *et al*., 2021).

The present study demonstrated that both single and combinatorial herbal formulations of ethanolic leaf extract of *O. gratissimum* and *K. pinnata* elicited detrimental effects on serum levels of ALT, AST, and total bilirubin through the elevation of these liver function biomarkers in comparison with the untreated diabetic rat group. Additionally, histologic markers of hepatic injury were recorded in rats treated with both single and combinatorial herbal formulations of ethanolic leaf extract of *O. gratissimum* and *K. pinnata*. The transaminase enzymes, namely alanine transaminase (ALT) and aspartate transaminase (AST), are important in the production of various amino acids, and measuring the concentrations of various transaminases is important in the diagnosis and tracking of many diseases (Odiegwu *et al*., 2021). Diabetes has been linked to liver damage through a combination of increased oxidative stress and an aberrant inflammatory response that damages hepatocytes and activates the transcription of pro-apoptotic genes; pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α significantly contribute to the accumulation of oxidative damage products in the liver, including conjugated dienes, fluorescent pigments, and malondialdehyde (Mohamed *et al*., 2016; Ohiagu *et al*., 2024).

These results are consistent with a study by Ojo *et al*. (2013), which found that administering aqueous leaf extracts of Ocimum gratissimum at 4.5µg/kg caused hepatic damage in albino rats. The study was attributed to the combined toxicity of the phytochemical constituents, including tannin, saponins, glycosides, and alkaloids. The results of this study are supported by the study by Onaolapo and Onaolapo (2011), which found that treatment with *O. gratissimum* ethanolic leaf extract decreased blood sugar and increased biochemical and histologic markers of hepatic injury. The results of Yadav and Dixit (2003) are in conflict with the hepatic harm caused by *K. pinnata* leaf extract as reported in this study.

Since urea and creatinine have a low blood threshold, the kidneys quickly remove them from the bloodstream. According to metabolic studies, creatinine is primarily derived from muscle protein turnover, while urea is a nitrogenous waste product of dietary origin (Samra and Abcar, 2012; Rodwell, 2015). One diagnostic metric for determining the functionality of renal tissue is the speed at which urea and creatinine are removed from the bloodstream and eliminated in the urine. According to Obeten *et al*. (2013), the kidney is the primary regulator of all bodily fluids and is in charge of preserving the body's fluid and electrolyte homeostasis or equilibrium.

The results of this study were consistent with earlier findings that showed that alloxan-induced diabetic rats showed a slight normalization of serum creatinine, urea, and electrolyte concentrations by lowering malondialdehyde (MDA) levels in their kidneys (Dey *et al*., 2019; Singh *et al*., 2020). In comparison to the untreated diabetic rat group, the diabetic rat groups treated with a single and combination herbal formulation of ethanolic leaf extract of *O. gratissimum* and *K. pinnata* had lower serum urea, creatinine, chloride ion, and potassium ion concentrations. In this study, rats treated with a single and combination herbal formulation of ethanolic leaf extract of *O. gratissimum* and *K. pinnata* showed histologic markers of good therapeutic renal state. Previous research supported the current findings, showing that *O. gratissimum* extracts improved renal function and prevented kidney damage by reducing inflammation and oxidant-induced cyclophosphamide toxicity (Alabi *et al*., 2020).

5. CONCLUSION

Single and combinatorial herbal formulations of the ethanolic leaf extracts of *O. gratissimum* and *K. pinnata* reversed the damages elicited on the kidney by diabetes but was not able to reverse the damage that was incurred on the liver.

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