Journal Homepage: www.ijarpr.com ISSN: 3049-0103 (Online)

International Journal of Advance Research Publication and Reviews

Vol 03, Issue 9, pp 763-771, September, 2026

Evaluation of Antioxidant Activities of Fractions of Zea Mays Husk Extract Against CCL4-Induced Oxidative Stress in Wistar Rats

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Abstract

We investigated the effects of fractions of maize husk extract on CCL4 induced oxidative stress. Forty (40) Wistar rats of eight (8) groups with five (5) rats each and CCL4 induced oxidative stress was established in group 2 to 8 followed by treatment with the fractions of maize husk extract. The group 1, 2, and 3 serves as normal, oxidative stress control, silymarin control while group 4, 5, 6, 7 and 8 represents 200mg/kg of aqueous, hexane, ethylaceteta, butanol and methanol fractions of maize husk followed by the measurements of MDA, CAT and SOD, renal function test, glucose level and phytochemical screening. The fractions significantly decreased MDA and increased CAT, SOD and glucose level ($p< 0.05$) compared with CCL4-induce. The extract significantly ($p < 0.05$) reduce serum levels of creatinine, urea and chloride compared with CCL4 induced group. Histology of kidney revealed significant reductions in degenerative changes observed in the treated rats compared to untreated rats. The phytochemical screening of hexane fraction reveal presence of steroids, phenols, tetracontain and 5-Octadecene. The results showed that the fractions of Zea mays has antioxidative capacities which may be due to the antioxidant activities of their phytochemical constituents.

Key words: Histology, Silymarin, Phytochemical, phenols

1.0 Introduction

Oxidation is a crucial process of cellular metabolism that leads to the generation of free radicals and reactive oxygen species (ROS) both physiologically and pathologically (Johra et al., 2023). The reactive oxygen radicals accelerate the oxidation of biomolecules in the cells and tissues of living organisms leading to injury and death. Unrestrained generation of oxygen radicals is involved in the progression of many diseases such as liver cirrhosis, atherosclerosis, arthritis, especially rheumatoid arthritis, cancer, and many degenerative processes, including aging (Halliwell and Gutteridge, 2015). The endogenous production of oxidative enzymes like superoxide dismutase (SOD), glutathione peroxidase, and catalase (CAT) bind the free radicals and convert them into less reactive ones, thereby protecting against oxidative damage (Zulaikhah, 2017). Also, some exogenous compound such as α-tocopherol, ascorbic acid, carotenoids, and polyphenol compounds also play essential roles in sifting the free oxygen radicals, breaking down the free radicals chain reaction thereby neutralizing the reactive oxygen species (Kebede and Emire, 2019).

The liver is the main organ for metabolizing nutrients, drugs, and xenobiotics primarily by a family of cytochrome P-450 enzymes (Almazroo et al.,

2017). Therefore, the hepatic cells are vulnerable to oxidative stresses (Cichoż-Lach and Michalak, 2014) which results into the change in structure of parenchyma cells as well as extra cellular matrix of liver due to oxidative and nitrosative stress modulates immune system. Immune and inflammatory cells are recruited at the location of injury which activates non-parenchyma cells like hepatic stellate cells and kupffer cells (Dutta et el., 2018) that leads to the significant increase in levels of growth hormone, cytokines and chemokines which results to fibrosis and chronic liver diseases. However, there are several factor responsible for liver dysfunctions, the exposure to toxic reagents and drug cannot be ignored (Dutta et al., 2018). Carbon tetrachloride (CCl4) has been widely used for liver studies for long now (Dey et al., 2015). Carbon tetrachloride stimulates oxidative stress and disrupts redox homeostasis by stimulating lipid peroxidation (Adelakun et al., 2024) and this oxidative insult is followed by compromised antioxidant defenses, characterized by decreased activity of key antioxidant enzymes, including glutathione (GSH), catalase (CAT), glutathione S-transferases (GSTs), and superoxide dismutase (SOD) (Salim et al., 2025). The pathogenesis of CCl4-induced hepatorenal injury involves centrilobular necrosis, renal vacoulation, and subcellular damage, cytokine-mediated inflammatory and fibrotic responses, and hepatic metabolic dysregulation, ultimately progressing to fibrosis and, with chronic exposure, cirrhosis (Adelakun et al., 2024).

Maize is a universal prominent crop cultivated with a production of around 44 billion tonnes of grain in the years 2022–2023 (Swati *et al*., 2024). Based on the recent trend, by 2030, maize production is predictable to surpass that of wheat (Erenstein *et al*., 2021). The United States of America produces half of the world's maize, with Asia producing a third (32%) with Europe and Africa producing the majority of the rest (11 and 7.4%, respectively) (Erenstein *et al*., 2022). Maize contains series of phytochemicals in which some of them serves as antioxidants that neutralise free radicals and eliminate their potential to cause harm. The most extensively studied phytochemicals are carotenoids and flavonoids for their antioxidant potential, boost immune system, combat the risk of specific types of cancers, and promote cardiovascular, vision, bone, and neurocognitive health (Yeum and Russell, 2014). Also, all part of the maize plant from the tassels to the roots demonstration high economic and therapeutic value and the seeds, stalk, husk, and cob can be incorporated into the production of various by-products (Raghavendran *et al*., 2020). Duru (2020) reported that there are substantial amounts of minerals such as calcium, sulphur, potassium and silicon as well as some phytochemicals like phenolic compounds, steroids, anthocyanins, lunamarin, resveratrol, proanthocyanins, and flavanone found in the analyzed husk (Okokon *et al.*, 2017). They have been associated with antioxidant, anticancer and antimicrobial activities, growth hormone regulation and antihelminthic activity (Patel *et al.*, 2015).

Although several studies have explored the hepatoprotective effects of medicinal plants against carbon tetrachloride (CCl_4)-induced liver injury. Considerably, the information regarding the identification of specific plant fractions responsible for these protective effects is limited. Earlier reports have largely focused on whole-plant extracts, while relatively limited attention has been given to the hepatoprotective potential of individual fractions and the relationship between fraction composition and biological activity. In addition, although *Zea mays* husk is an abundant agricultural by-product containing active bioactive compound, its hepatoprotective effects against CCl_4-induced oxidative liver injury, particularly at the fraction level, remain insufficiently considered.

2.0 Materials and Methods

2.1 Plant Collection and Identification

Fresh corn husks were collected from the farms around Usmanu Danfodiyo University permanent site, Sokoto, Nigeria and the plant was deposited at herbarium of the Pharmacognosy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto with identification and authentication (PCG/UDUS/POAC/0010).

2.2 Plant Preparation and Extraction

Fresh corn husks collected were carefully separated from the silks, wash with clean water to remove any dust and other unwanted particles. They were allowed to shade dry on a clean surface and then pulverized to powder using a grinder. The cold maceration extraction method was adopt, where by 500 g of ground husks was macerated with 5 L of methanol for 72 hours with intermittent stirring. The mixture was sieved with a clean muslin cloth. Further filtration was carry out with Whatmann filter paper 1. The filtrate was then evaporate to dryness at low temperature (40^0C) in a hot air oven to obtain the crude methanol extract (CME) (Muntaka *et al*., 2024). The dried extract was weighed, kept in sterile container, label and process for fractionation.

2.3 Fractionation of the Crude Methanol Extract

The crude methanol extract (100 g) was suspend in distilled water and filtered; the water-soluble portion was partitioned using n-hexane (1.5 L), ethylacetate (1.5 L) and n-Butanol (1.5 L) to obtain n hexane fraction (HEF), ethyl-acetate (EAF), n-butanol (NBF) and the residual aqueous (AQF) fractions, respectively (Muntaka *et al*., 2024). The dried extract of each fraction was weighed, kept in sterile container, label and store in the refrigerator at 8^0C.

2.4 Phytochemical Screening

Qualitative Phytochemical screening and GC-MS analysis of the hexane husk fraction was carried out. The qualitative screening was performed by employing standard procedures and tests as laid down by Evans (1996) and adopted by Abdullahi *et al*., 2003 reviewed by Umar, 2017. Also GC-MS analysis was carryout as described by Shettima *et al*., (2013) using the Agilent 6890 GC-MS system procured from Agilent Technology, No. 5301 Stevens creck Blvd, Santa Clara, CA, USA.

2.5 Experimental Animals

Forty (40) Wistar Rats (150-170g) of either sex of roughly of the same age (8-10 weeks) were purchased from the Animal House, of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The animals was maintained as described by Aniagu *et al.,* (2005) in clean cage, placed in a well-ventilated room with a temperature of 26^0C to 28^0C and photoperiods of 12 hours light and 12 hours darkness. The animals was maintained on a pellet feeds obtained from Grand cereals oil mills limited, Jos, Nigeria, and were supplied with drinking water *ad libitum*. Cleaning of the animal cages was carried out weekly. All the experimental protocols were in compliance with the Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines (National

Institute of Health, 1992), and also in accordance with the recommendation of the International Association for the study of pain (IASP) (Zimmerman, 1983).

2.6 Induction of oxidative stress in Wistar rats.

Oxidative stress was induced using carbon tetrachloride (CCL4) administration of 30% CCl4 (dissolved in olive oil, v/v) at 1.0 mL/kg b.w. twice a week for 6 weeks as described by Johra et al., 2023).

2.7 Experimental design and Treatment

Forty (40) Wistar rats were randomly grouped into eight (8) groups comprising of 5 rats per group and they were treated as follows:

Group 1 (n = 5): Normal control rats (NC), untreated/ received clean water and standard rodent chow *ad-libitum*.

Group 2 (n = 5): Negative control rats (NegC), received 1mL/kg CCL4 intraperitoneal without treatment

Group 3 (n = 5): Positive control rats (PosC), received 1mL/kg CCL4 with 100 mg/kg Silymarin

Group 4 (n = 5): Aqueous test group, received 1mL/kg CCL4 and 200 mg/kg of maize husk aqueous extract.

Group 5 (n = 5): Hexane test group, received 1mL/kg CCL4 and 200 mg/kg of maize husk hexane extract

Group 6 (n = 5): Ethylacetate (EthylAce) test group, received 1mL/kg CCL4 and 200 mg/kg of maize husk ethylacetate extract

Group 7 (n = 5): Butanol test group, received 1mL/kg CCL4 and 200 mg/kg of maize husk butanol extract

Group 8 (n = 5): Methanol test group, received 1mL/kg CCL4 and 200 mg/kg of maize husk methanol extract

The appropriate doses of the treatment regiments were calculated based on a study by Okokon et al., (2017) and were administer intragastric to the animals according to their body weight by intubation using intravenous cannula tube for 14 days.

2.8 Blood Samples Collection and processing

After 2 weeks, the rats were fasted for 12 hours, and anaesthetized with 75mg/bw ketamine injection. Blood sample was collected from the animals through cardiac puncture into clean and plain bottle. The sample collected in the plain tubes was allowed to clot at room temperature and later centrifuged at 4000 revolution per minute (rpm) for 10 minutes. The sera were transferred into labelled sterile serum bottles with tighten cap and stored at -20^0C until assayed for the serum levels of oxidative stress makers and renal function tests.

2.9 Biochemical Parameters

The biochemical parameters (oxidative stress makers) were determined using ELIZA kit from Nanjing Pars Biochem Co., Ltd, China. The serum level of creatinine, urea, potassium (K^+), sodium (Na^+), chlouride (CL) and biocarbonate (HCO3) was determined spectrophotometrically (S23A spectrophotometer U.S.A) using Randox reagent kits. The glucose level (mmol/L) was determined using glucometer (Accu-chek active, Roche Diagnostic).

2.10 Identification of the compounds

The identification of components present in the hexane fraction of the plants' extract was based on direct comparison of the retention times and mass spectral data with those for standard compounds, and by computer matching with the Wiley and Nist Libraries (Adams et al., 2001 and Setzer et al., 2007).

2.11 Data analysis and data evaluation

The data obtained were compute using Microsoft Office Excel 2007 and analyse with Statistical Package for Social Sciences (SPSS) version 23.0. The results were expressed as mean ± SEM. and group comparisons were made using one-way analysis of variance (ANOVA) followed by a posthoc test. Value of equal to or less than 0.05 ($P \leq 0.05$) was considered as statistically significant.

3.0 Results and Discussion

3.1 Oxidative stress makers in CCL4 induced liver damage treated with fractions of maize husk extract.

The serum level of Malondialdehyde (MDA) in normal control significantly decreased compared to negative control ($p < 0.05$) while catalase (CAT) and super oxide dismutase (SOD) were increased in normal control compared to negative control but not significant ($p > 0.05$). The serum level of MDA decreased in all treatment groups but only hexane and methanol presented significantly decreased ($p < 0.05$) compare to negative control. Also, only the methanol indicated significant decreased in MDA against other treated groups ($p < 0.05$). The ethylacetate, hexane and methanol fractions show significantly increased CAT compare to negative control but only methanol fraction significantly increased the serum level of CAT against other groups ($p < 0.05$). However, there were no significant difference in SOD level across all the groups, although the treated groups presented increased level of SOD ($p > 0.05$).

Table 1: **Oxidative stress makers in CCL4 induced liver damage treated with fractions of maize husk extract.**

Groups	MDA (nmol/ml)	CAT (pg/ml)	SOD (ng/ml)
NC	14.00 ± 0.58	50.27 ± 3.18	50.33 ± 2.01
NegC	20.67 ± 1.45[a]	47.33 ± 2.19	49.67 ± 2.03
PosC	16.37 ± 0.88	50.00 ± 1.73	54.00 ± 5.56
Aqueous	19.00 ± 0.55[a]	51.67 ± 3.84	56.33 ± 2.19
Hexane	15.00 ± 0.58[b]	56.00 ± 3.06[b]	56.33 ± 3.71
EthylAcetate	18.00 ± 1.15	54.00 ± 1.00[b]	56.00 ± 5.03
Butanol	17.00 ± 0.43	48.00 ± 3.06	58.33 ± 6.94
Methanol	14.67 ± 1.15[bd]	67.33 ± 1.20[abcd]	55.00 ± 5.69

Values are expressed as mean±standard errors; MDA: Malondialdehyde ; CAT: Catalase:; SOD: superoxide dismutase ;; Grp 1: Normal control; Grp 2: Negative control (CCL4 only); Grp3: Positive control (Silymarin); Grp 4: 200mg/kg aqueous maize husk extract; Grp5: 200mg/kg hexane maize husk extract; Grp6: 200mg/kg ethylacetate maize husk extract; Grp7: 200mg/kg Butanol maize husk extract; Grp 8: 200mg/kg methanol maize husk extract Value differs significantly when $p < 0.05$. [a]p mean significance against NC, [b]p mean significance against NegC, [c]p significance against PosC, [a]p significance among the fractions

3.2 Renal function test parameter in CCL4 induced liver damage treated with fractions of maize husk extract.

There were decreased in serum level of urea, creatinine, Na, K, Cl-and HC03 in the normal group compared to negative control group but only creatinine was significantly ($p < 0.05$). There were significant decreased in serum level of urea, creatinine and CL- in treated group compared to negative control ($p < 0.05$). Hence there were no significant different in the serum level of Na, K, and HCO3 in the negative control groups compared to the positive control group and fractions of maize husk extract treated group ($p > 0.05$), although the treated groups decreased the level of Na and HC03.

Table 2: Renal function test parameter in CCL4 induced liver damage treated with fractions of maize husk extract.

Groups	Urea (mmol/L)	Creatinine (mmol/L)	Na (mmol/L)	K (mmol/L)	CL (mmol/L)	HCO3 (mmol/L)
NC	4.77 ± 0.15	0.63 ± 0.03	141.67 ± 0.88	4.1t ± 0.19	101.33 ± 0.88	23.33 ± 0.88
NegC	6.93 ± 0.18	1.07 ± 0.09[a]	143.33 ± 2.33	4.33 ± 0.12	106.00 ± 1.53	24.04 ± 0.35
PosC	5.70 ± 0.58	0.83 ± 0.03[b]	141.00 ± 1.15	4.50 ± 0.15	98.67 ± 0.33[b]	23.00 ± 1.53
Aqueous	4.47 ± 0.49[b]	0.72 ± 0.04[b]	143.67 ± 1.86	4.53 ± 0.18	99.00 ± 1.00[b]	24.33 ± 0.33
Hexane	5.67 ± 0.62	0.83 ± 0.03	139.00 ± 2.52	4.53 ± 0.15	100.43 ± 1.33[b]	23.33 ± 1.20
EthylAcet	3.83 ± 0.34[b]	0.85 ± 0.16[b]	141.00 ± 1.53	4.33 ± 0.19	99.33 ± 0.88[b]	22.33 ± 1.22
Butanol	4.60 ± 0.66	0.79 ± 0.09[b]	138.00 ± 1.00	4.50 ± 0.25	97.33 ± 1.20[b]	23.00 ± 1.53
Methanol	4.50 ± 0.35[b]	0.80 ± 0.10[b]	138.00 ± 1.16	4.13 ± 0.15	99.14 ± 0.41[b]	21.67 ± 1.20

Values are expressed as mean ± standard errors; Na: Sodium; K: Potassium; CL-: Chloride; HCO3: Biocrbonate; Grp 1: Normal control; Grp 2: Negative control (CCL4 only); Grp3: Positive control (Silymarin); Grp 4: 200mg/kg aqueous maize husk extract; Grp5: 200mg/kg hexane maize husk extract; Grp6: 200mg/kg ethylacetate maize husk extract; Grp7: 200mg/kg Butanol maize husk extract; Grp 8: 200mg/kg methanol maize husk extract Value differs significantly when $p < 0.05$. [a]p mean significance against NC, [b]p mean significance against NegC, [c]p significance against PosC, [a]p significance among the fractions

3.3 The glucose level in CCL4 induced liver damage treated with fractions of maize husk extract.

As presented in figure 1, the aqueous, butanol and methanol fractions significantly increased glucose level compared to negative control ($p < 0.05$) but positive control, hexane and ethylacetate fraction increased the glucose level but were not significant. ($p > 0.05$). Also, the glucose level significantly decreased in negative control, positive control, hexane fraction and ethyiacetate fraction compared to normal control ($p < 0.05$).

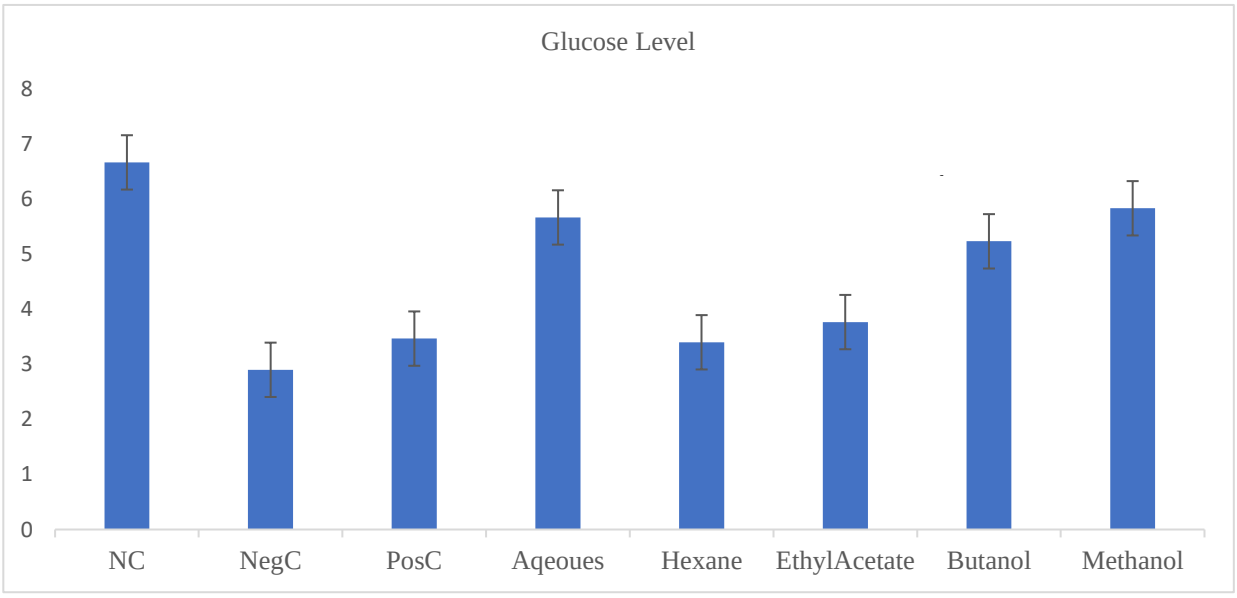


Figure 1: Serum glucose level in CCL4 induced liver damage treated with fractions of maize husk extract.

Grp 1: Normal control; Grp 2: Negative control (CCL4 only); Grp3: Positive control (Silymarin); Grp 4: 200mg/kg aqueous maize husk extract; Grp5: 200mg/kg hexane maize husk extract; Grp6: 200mg/kg ethylacetate maize husk extract; Grp7: 200mg/kg Butanol maize husk extract; Grp 8: 200mg/kg methanol maize husk extract Value differs significantly when $p < 0.05$. [a]p mean significance against NC, [b]p mean significance against NegC, [c]p significance against PosC, [a]p significance among the fractions

3.4 Qualitative analysis of the phytochemical constituents in hexane extract of Maize husk

The qualitative analysis of the phytochemical constituents in hexane fraction extract of maize husk reveal the moderate presence of alkaloids, presence of steroid, trace presence of carbohydrate and phenols while flavonoid, cardiac glycosides, tannins, saponins, protein and anthraquinine were absence.

Table 3: Qualitative analysis of the phytochemical constituents in hexane extract of Maize husk

Compounds	Test	Result
Tannins	Ferric chloride test	-
Saponins	Froth test	-
Flavonoids	Ferric chloride test	--
Cardiac glycosides	Keller-Killiani test	-
Steroids	Salkowski test	+
Alkaloids	Meyers test	++
	Wagner test	++
Carbohydrate	Molich' test	Trace
Phenols	Ferric chloride test	Trace
Protein	Xanthoproteic test	-
Anthraquinine	Borntrager's Test	-

Key: + = Present; ++ = Moderate; +++ = Significantly present;

3.5 Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of the phytochemical constituents in butanol extract of maize husk

Gas chromatography-mass spectroscopy analysis of the phytochemical analysis of one the fraction (aqueous) of Zea mays revealed the presence of 16 compounds (Table 4). The compounds present include 5-Octadecene, Tetracontane, 3-Heptadecene, Carbonic acid, Octadecyl prop-1-en, Hexadecene, 1-Heptadecene, Cycloeicosane, 3-Eicosene and 5-Eicosene

Table 4: Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of the phytochemical constituents in hexane extract of maize husk

Peak	Retention Time	Area%	Compound Name	Quality
1	4.451	9.56 5	5-Octadecene	95%
			Tetracontane	91%
2	5.086	4.30 5	3-Heptadecene	90%
3	5.481	11.02 5	Carbonic acid	91%
			octadecyl prop-1-en	91%
4	6.705	13.79 7	7-Hexadecene	90%
			1-Heptadecene	90%

Peak	Retention Time	Area%	Compound Name	Quality
5	6.946	9.71	Cycloeicosane,	95%
			5-Eicosene	95%
6	7.426	27.19	3-Eicosene	93%
7	9.555	12.28	1-Octadecene	91%

3.6 Photomicrographs of kidney Sections Stained with Haematoxylin and Eosin (H&E)

The photomicrographs demonstrate the renal parenchyma and structures across sections group 1-8. Normal control shows parenchyma containing numerous tubular profiles (T), glomerular structures (G), and an arterial structure (ART), with relatively prominent tubular with interstitial spaces. Negative control (CCL4) demonstrates predominantly tubular renal parenchyma with an arterial structure and conspicuous cytoplasmic vacuolation (VAC). Positive control shows renal corpuscular with defined glomerular structures and tubular profiles within the renal parenchyma, with a relatively preserved general tissue arrangement. Aqueous extract demonstrates glomerular, distal tubular, proximal convoluted tubular, and arterial structures, allowing clear identification of the major components of the renal parenchyma. Hexane extract indicate renal tubular structures, renal corpuscular structures (RC), and a peritubular zone (PZ), with variation in the appearance of the tubular and interstitial compartments. Ethylacetate demonstrates a prominent central vascular/structural space with surrounding renal parenchyma with arterial structure. Butanol extract predented numerous tubular profiles, an arterial structure and a glomerular structure within the renal parenchyma while methanol extract indicate parenchyma, glomerular structures and an arterial structure.

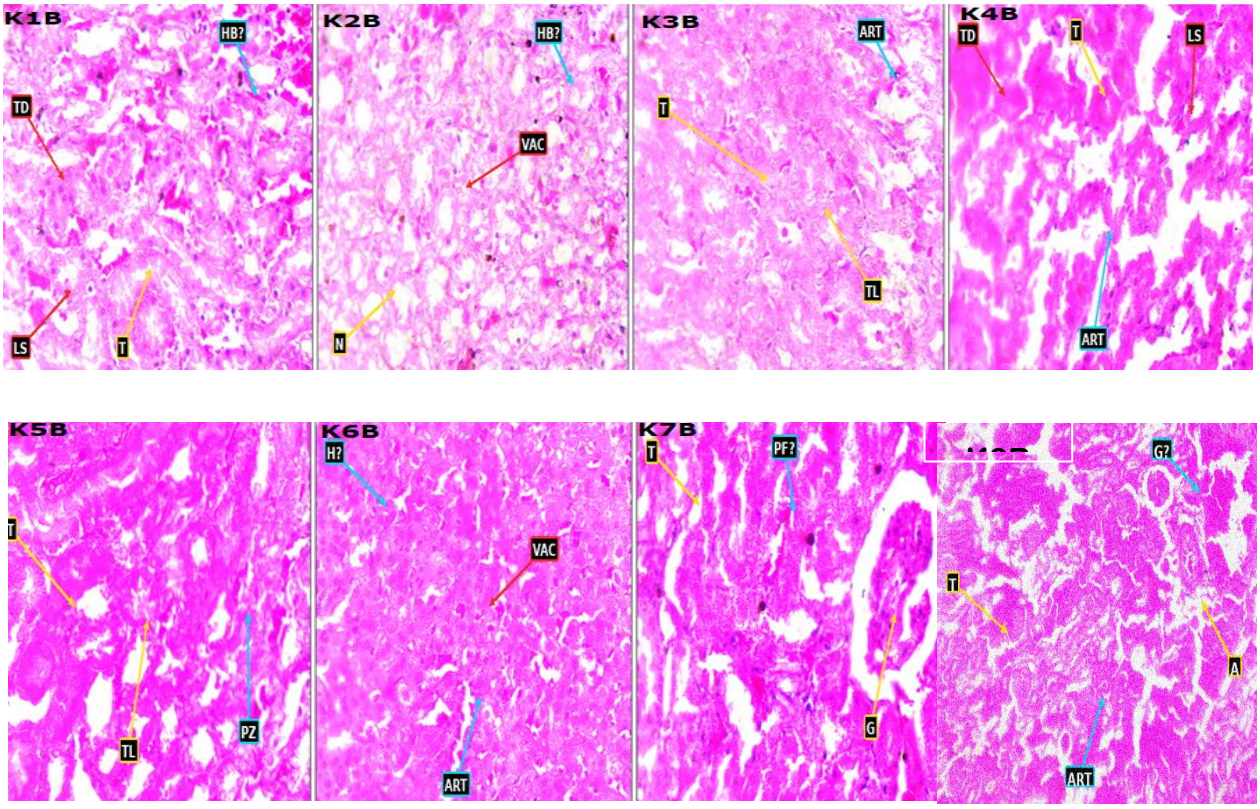


Plate 2A-G: Histomorphological features of the kidney sections (K1–K7) at ×100 magnification following H&E staining showing glomeruli (G) with renal corpuscles (RC), renal tubules (T), proximal convoluted tubules (PCT), distal tubules (DT), and arterial structures (ART), as indicated by the arrows. The sections demonstrate preserved recognition of the major renal components with variable tubular and parenchymal alterations with conspicuous cytoplasmic vacuolation in K2.

Acknowledgement

The authors appreciatively acknowledge the staff at Medical Laboratory Science Department, Ladoke Akintola University Technology Ogbomoso and Akintola University of Technology, Ogbomosho, Oyo State, Nigeria and the staff at Department of Chemical Pathology, School of Medical Laboratory

Science, Usmanu Danfodiyo University, Sokoto, Nigeria.

Declaration of Competing Interests:

"The author has no competing interest"

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