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AMELIORATION OF CCL_4 –INDUCED NEPHROTOXICITY IN RAT BY FLAVONOID, ALKALOIDS, SAPONIN, AND TANNINS EXTRACTED FROM *COMBRETUM DOLICHOPENTALUM*

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ABSTRACT

Combretum dolichopentalum is a shrub commonly used as herbal therapy for women after parturition. It has been used to manage mild perturbation of the kidney induced by CCl_4. The aim of this study was to compare the effects of flavonoids, alkaloids, saponins, and tannin (FAST) extracted from *C. dolichopentalum* on CCl_4-induced renal damage. Sixty- five (65) rats were sorted into nine groups, and allowed food and water *ad libitum*. Group I control (water), group II CCl_4, group III was pre-treated with 250 mg/kg crude ethanol extract of *C. dolichopentalum* (EECD), group IV was pre-treated with 500 mg/kg crude EECD, group V was pre-treated with 50 mg/kg b.w silymarin, group VI to IX were pre-treated with 100 mg/kg b.w each of flavonoid, alkaloid, saponin, and tannin extracted from *C. dolichopentalum* respectively for seven days. On the 8th day, all animals (except the normal) were intoxicated with 0.4 ml/kg body weight CCl_4 in liquid paraffin (2:1). All the rats were killed 48 h after CCl_4 administration, and kidneys were excised and used for determination of histopathological and biochemical parameters. Intoxicating rats with CCl_4 induced histological changes causing glomerular and tubular degeneration. CCl_4 also caused marked elevation in serum MDA, K^+ and urea. A significant decrease in Na^+ and no significant changes in Cl^- were observed. Treating animals with EECD and phytochemicals such as flavonoid, alkaloid, saponin and tannin extracted from *C. dolichopentalum* led to an improvement, in both biochemical indices and histopathological injuries, however, flavonoid and alkaloid components demonstrated a more significant reduction in MDA and urea, and tannin in K^+. All phytochemical components including the crude extract normalised Na^+. Moreover, it is interesting to note that though there were no significant differences between the normal, intoxicated group and crude extract in Cl^- concentration, all phytochemical components (FAST) increased Cl^- concentration significantly. Data from the current study indicates that *C. dolichopentalum* influence electrolyte balance. Furthermore, EECD especially flavonoid and alkaloid extract of *C. dolichopentalum* clearly protects the kidney from CCl_4-induced nephrotoxicity.

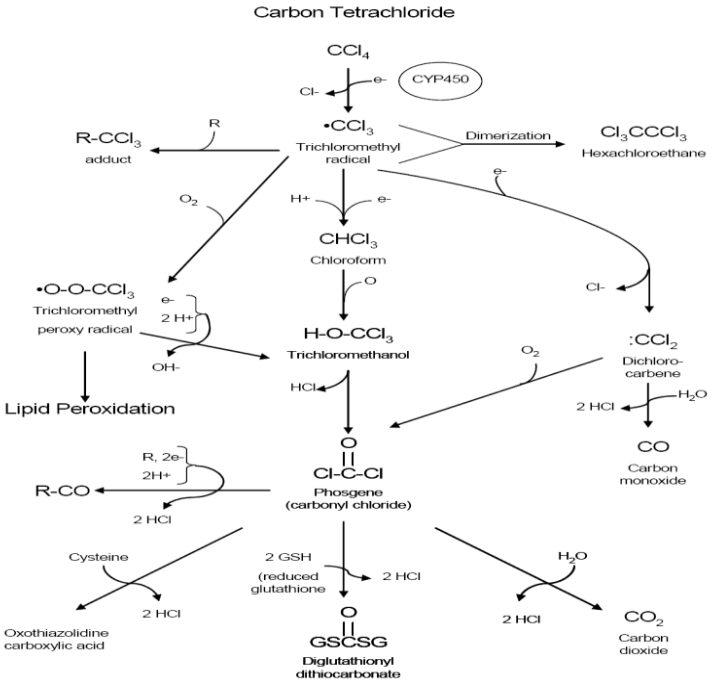
Subject: Biochemistry, Toxicology, Histology

KEYWORDS: Carbon tetrachloride, Nephrotoxicity, Phytochemicals, Electrolytes.

1.0 INTRODUCTION

As the main excretory organ the kidney is a target for intoxication. The renal damage caused by CCl_4 is necrosis of the proximal tubular epithelium, which may be accompanied by swelling of tubular epithelium, fatty infiltration and, casts in the tubular lumen.[1] The mechanism of renal toxicity (Figure 1) is thought to be a result of a toxic free radical intermediate (trichloromethyl radical) of cytochrome P-450 metabolism of CCl_4.[2,3] This radical can bind to cellular molecules (nucleic acid, protein, lipid) and form DNA adducts. This binding alters the permeability of the plasma membrane, mitochondria, and endoplasmic reticulum, resulting in cell damage[4], elevates free fatty acids, triglycerides, and cholesterol in the liver and kidney of rats[5,6] and cause male genotoxic effects in mouse bone marrow and germ cells.[7] Also, CCl_4-induced damage includes adverse alterations of the endogenous antioxidants.[8] These antioxidants are vital substances possessing the ability to protect the body from damage caused by free radical-induced oxidative

stress.[9] There is need to employ the use of natural antioxidants against chemically induced toxicities. Among these natural sources include *C. dolichopentalum* which contains a wide range of phytochemicals that exhibit a wide range of beneficial effects in a multitude of diseases.[10] *C. dolichopentalum* is composed of flavonoids such as kaempferol, apigenin, isorhamnetin beside others[11] not excluding rutin.[12] There are suggestions that rutin exhibits antioxidant, anti-inflammatory, anti-apoptotic, antihyperuricemic, and antihyperlipidemic potentials.[13,14,15,16,17] It has been reported that rutin prevented methotrexate-induced hepatic injury in rats[18], used for the treatment of hyperglycemic complications[19] and displayed a promising effect against oxidative assault associated with male infertility.[20] Furthermore, apigenin has been found to possess affinity for the opioid receptors, acting in the nanomolar range, as a non-selective antagonist of all three opioid receptors.[21] Therefore, the present work investigates the phytochemical components with the best nephrotoxic potentials against CCl_4-induced renal toxicity.



CYP450, usually CYP2E1, but also CYP3A; R = acceptor molecule, such as protein or lipid.

Figure 1: Metabolism of CCl_4 [ACGIH 2001].[55]

2.0 MATERIALS AND METHODS

2.1 Chemicals and reagents

Carbon tetrachloride CCl_4 was purchased from Sigma Chemicals (St. Louis, MO, USA).

2.2 Collection and processing of Plant Sample

Fresh leaves *of C. dolichopentalum* were harvested from a farm in Obinze in Owerri West Local Government Area of Imo State, Nigeria. The plant was authenticated by Mr. A. Ozioko, of the Bioresource Development and Conservation Program (BDCP), Research Centre at the University of Nigeria, Nsukka, Enugu State, Nigeria. The fresh leaves were plucked from their stems, washed with distilled water and allowed to dry at room temperature. The dried plant samples were pulverized (using electric blender) and stored in an airtight container kept in a desiccator for 3 days.

2.3 Ethanol extraction of plant

Three hundred grams of the pulverized dried plant sample was extracted with 1.75 L of 80% ethanol by maceration for 2 days, this was carried out in three separate cans of 100 g each and then pulled together. Following filtration using a sieve, the filtrate was further filtered using a Whatman No. 1 filter paper. The concentration of the extract was achieved using a rotary evaporator under mild temperature and reduced pressure and labelled ethanol extract of *C. dolichopentalum* (EECD).

2.4 Extraction of Flavonoids, Alkaloid, Saponin, and Tannin (FAST) for *In Vivo* Studies

2.4.1 Alkaloid extraction

Fifty gram (50 g) quantity of the sample was weighed into a 1000 ml beaker and 500 ml of 29% acetic acid in ethanol was added and allowed to stand for 6 hr. This was filtered and the filtrate was concentrated over a water bath to one quarter of the original volume. The alkaloid was precipitated out using concentrated ammonium hydroxide which was added drop by drop until precipitation was complete. The solution was allowed to settle and the precipitate was collected by filtration using Whatman No. 1 filter paper.[22]

2.4.2 Saponin extraction

Fifty grams (50 g) of the plant sample was weighed into a 1000 ml beaker and 500 ml of 20% ethanol was added and stirred using a glass rod. The mixture was heated over water bath for 4 hr with continuous stirring while the temperature was maintained at 55°C. The mixture was filtered and the residue was re-extracted with 500 ml of 20% ethanol. The combined extract was reduced to 40 ml over water bath at 90°C. The concentrated extract was transferred into a 250 ml separation funnel and 50 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This process was repeated thrice and 60 ml of n-butanol was added. The mixture was washed twice with a 10 ml of 5% sodium chloride. The remaining solution was heated over water bath and the residue dried to constant weight.[22]

2.4.3 Flavonoid extraction

Fifty grams (50 g) of the plant sample was extracted repeatedly with 500 ml of 80% aqueous methanol at room temperature. The solution obtained was filtered with Whatman no 45 filter paper. The combined filtrates were later transferred into a crucible and evaporated to dryness over a water bath.[23]

2.4.4 Tannin extraction

Fifty grams (50 g) of the plant sample was extracted with 500 ml of water by maceration. The aqueous extract was extracted thrice with ethyl acetate to eliminate neutral substances. The extract was brought to pH 2 by the addition of concentrated HCl and re- extracted with ethyl acetate. This was later evaporated to dryness.

2.5 Animals

Wistar albino rats were purchased from the Animal House of the Department of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria. The animals were acclimatized for 7 days at room temperature in metal cages under 12/12-hour light and dark and were fed and maintained *ad libitum* on water and rat pellets (Pfizer Feeds, Aba, Nigeria). This study was conducted in accordance with laws and regulations for handling experimental animals as was approved by the Department of Biochemistry, FUTO.

Sixty-five (65) Wistar albino rats were weighed and sorted into nine groups (Table 2.5.1) of seven animals each, so that their average weights were approximately equal. The animals were housed in metal cages. After 7 days' acclimatization on rat pellets, they were weighed, and the weights used to calculate amount of extracts to be administered and other treatments to be used.

2.5.1 Experimental design.

Groups	Group Identity	Treatment
I	Normal control (NC)	feed and water
II	Positive control (PC)	feed, water and CCl_4
III	Treated group (T_{250})	250 mg/kg body weight of EECD and CCl_4
IV	Treated group (T_{500})	500 mg/kg body weight of EECD and CCl_4
V	Sylimarin group	50 mg/kg body weight of sylimarin and CCl_4
VI	Flavonoid group	100 mg/kg body weight of flavonoid and CCl_4
VII	Saponin group	100 mg/kg body weight of saponin and CCl_4
VIII	Alkaloid group	100 mg/kg body weight of alkaloid and CCl_4
IX	Tannin group	100 mg/kg body weight of Tannin and CCl_4

EECD and FAST were administered daily by oral gavage, for 7 days. The dosage of administration of the extract was adopted from the acute toxicity studies carried out earlier. The animals were allowed food and water *ad libitum*. At the end of 7 days pre-treatment with EECD, flavonoids, alkaloids, saponins, and tannins extracted from *C. dolichopentalum*, the animals in all the groups (except normal control) were intoxicated with 0.4 ml/kg body weight $CC1_4$ in liquid paraffin (2:1). The CCl_4 was allowed to act on the animals for 48 hrs. After overnight fast the rats were subjected to a light anaesthesia with dimethyltetrachloride and blood collected by cardiac puncture. The kidneys of the animals were obtained, washed in 1.15% KCl buffered solution and dabbed with paper, weighed and prepared for biochemical and histopathology studies.

2.6 Blood collection

Blood samples of each animal were taken by cardiac puncture, allowed 45 minutes to clot at room temperature. After centrifugation at 600 x g for 15 minutes, the serum collected was used to assay for electrolytes and some biochemical parameters.

2.7 Methods

Malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) to form a red or pink coloured complex which in acid solution, absorbs maximally at 532 nm.[24] Urea is hydrolyzed by Urease to produce ammonia and CO_2 in a modified Berthelot reaction in the presence of nitroprusside.[25] Sodium is precipitated as triple salt, sodium magnesium uranyl acetate, the excess uranium reacts with ferricyanide producing a chromophore whose intensity varies inversely as the concentration of sodium

in the test specimen.[26] The concentration of chloride was determined according to the method of[26], chloride ion displace thiocyanate from non-ionized mercuric thiocyanate, thiocyanate released reacts with ferric ions to form a chromophore whose intensity is directly proportional to the chloride concentration. Potassium was estimated by preparing a colloidal suspension whose turbidity is proportional to potassium concentration using sodium tetraphenylboron.[27] The method described by Okoro[28] with minor modifications was used for the histology of the kidney, following tissue collection, fixation, processing, sectioning, staining and mounting.

3.0 RESULT

3.1 Effect of EECD and FAST on MDA and Urea

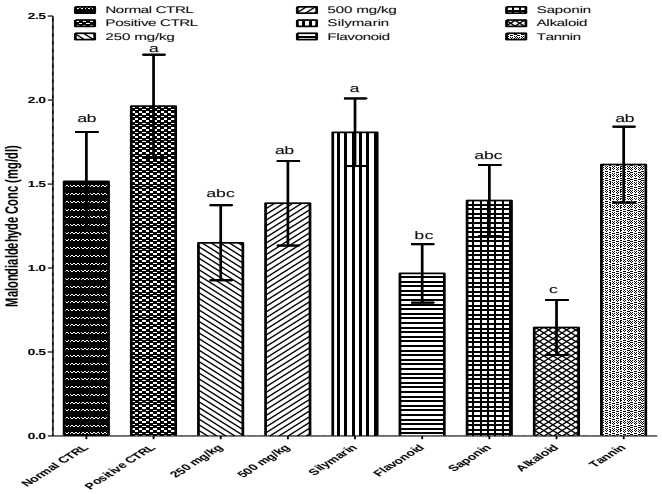


Figure 22: Effect of EECD in Malondialdehyde (MDA) Concentration in CCl_4 -Induced Oxidative Stress. Bars bearing different letters are statistically significant (p<0.05)

Figure 1: Effect of EECD and FAST on malondialdehyde (MDA) concentration in CCl_4 -induced oxidative stress. Bars bearing different letters are statistically significant (p<0.05).

The evidences in the result as shown in figure 1, there was a significant (p<0.05) increase in the positive control group compared to the normal. A significant (P<0.05) decrease in the flavonoid and alkaloid control were also observed. Nevertheless, there was no significant difference (p>0.05) among the normal, 500 mg/kg b.w EECD and tannin groups. There was also no significant difference (p>0.05) between the 250 mg/kg b.w EECD and the saponin treated groups.

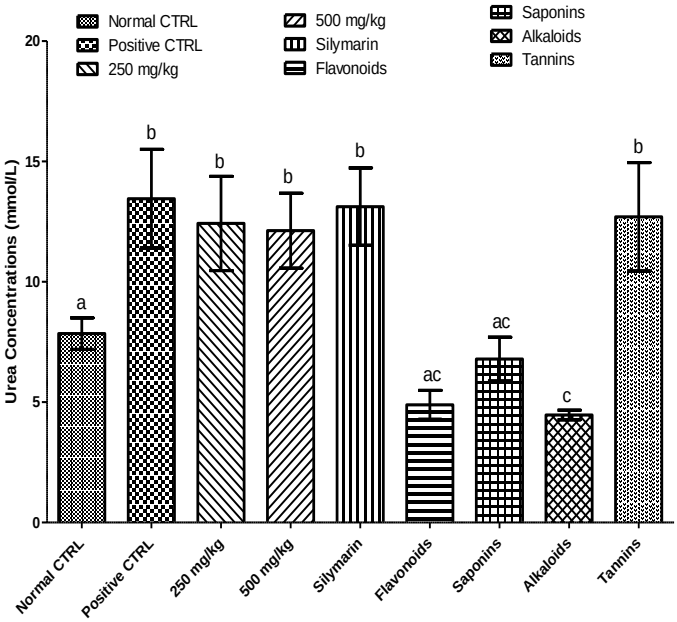


Figure 2: Effect of EECD and FAST on serum urea concentration in CCl_4 induced nephrotoxicity. Bars bearing different letters are statistically significant (p<0.05).

Figure 2 shows a significant ($p<0.05$) increase in the positive control group compared to the normal, the flavonoid, the saponin, and the alkaloid groups. However, there was no significant ($p>0.05$) difference among the positive control, the EECD treated groups, the silymarin as well as the tannin groups.

3.2 Effect of EECD and FAST on some kidney and electrolytes parameters

Figure 3 shows the effect of EECD and FAST on sodium concentration in CCl_4 -induced nephrotoxicity.

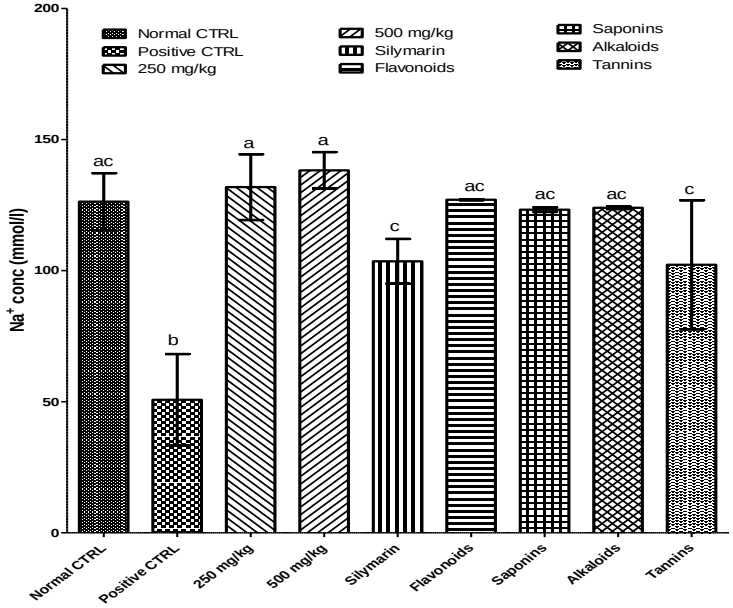


Figure 3: Effect of EECD and FAST on sodium concentration in CCl_4 -induced nephrotoxicity. Bars bearing different letters are statistically significant ($p<0.05$).

Figure 3 shows a significant ($P<0.05$) decrease in sodium concentration of the positive control group compared to the normal and all the treated groups including the silymarin group. Also observed was a non significant ($p>0.05$) difference among the normal, the flavonoid, saponin, and tannin control. There was also a non significant ($p>0.05$) difference between the silymarin and the tannin groups.

3.3 Effect of EECD and FAST on some kidney and electrolytes parameters

Figure 4 shows the effect of EECD and FAST on potassium concentration in CCl_4 -induced nephrotoxicity.

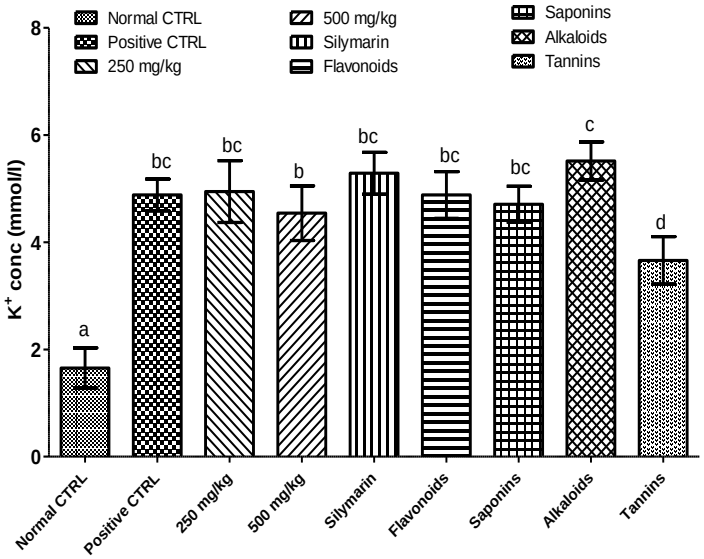


Figure 4: Effect of EECD and FAST on potassium concentration in CCl_4- induced nephrotoxicity. Bars bearing different letters are statistically significant ($p<0.05$).

Figure 4 shows a significant (P<0.05) increase in potassium concentration of the positive control group compared to the normal group. A significant difference was also observed between the positive control group, the alkaloid and the tannin groups. However a non significant (p>0.05) difference was observed between the positive control and the EECD at 250 mg/kg, the silymarin, the flavonoid as well as the alkaloid control.

Figure 5 shows effect of EECD and FAST on chloride concentration in CCl_4 -induced oxidative stress.

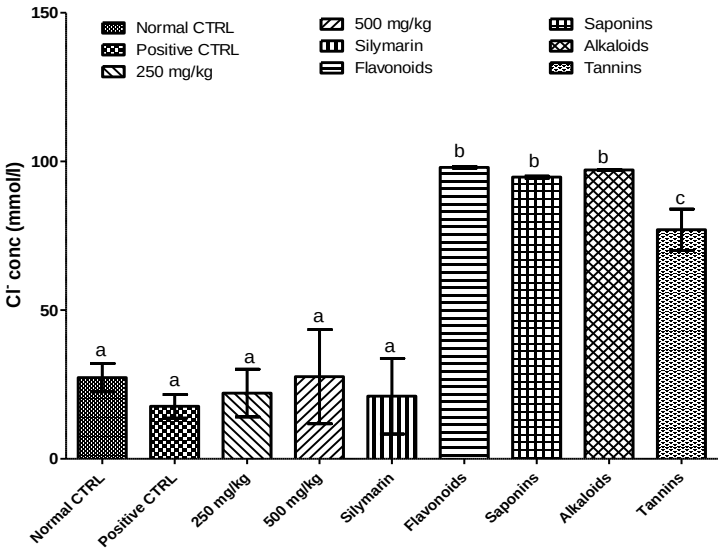
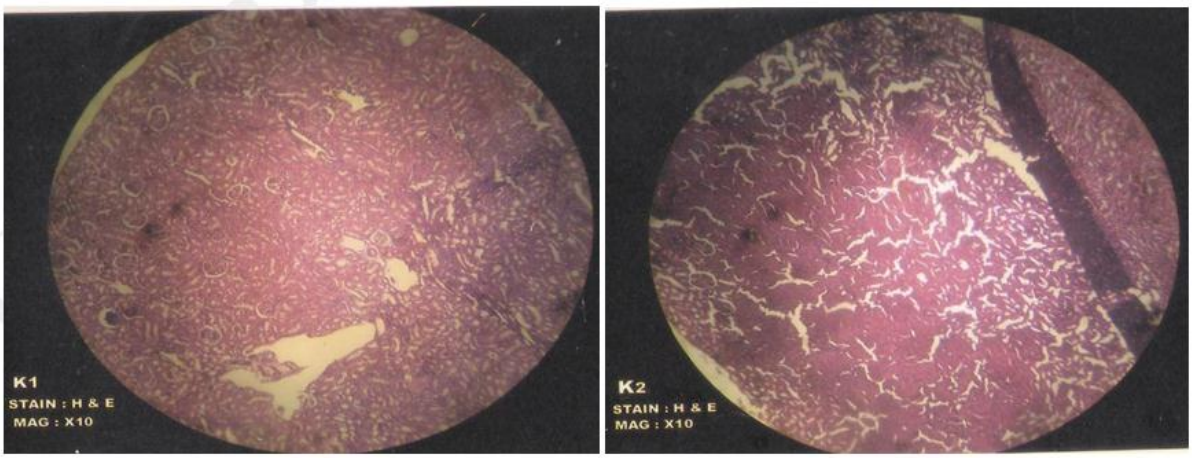


Figure 5: Effect of EECD and FAST on chloride concentration in CCl_4 -induced oxidative stress. Bars bearing different letters are statistically significant (p<0.05).

The result as shown in Figure 5 reveals a significant (P<0.05) increase in chloride concentration of the flavonoid, saponin, alkaloid and tannin groups compared to the positive control group. There was no significant (p>0.05) difference among the normal, the positive control, the EECD treated groups as well as the silymarin groups.

3.4 Histological Characteristics of kidney samples



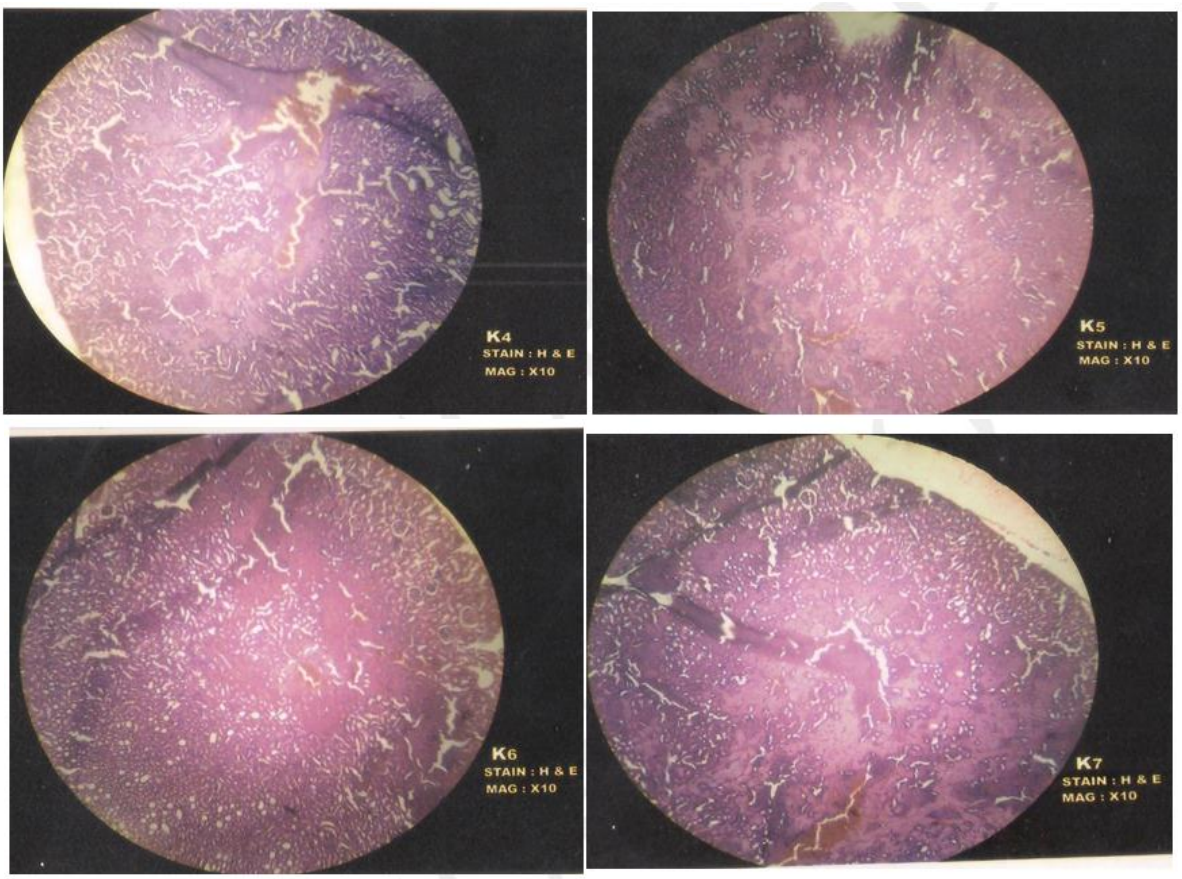


Figure 6: Light microphotographs of HE-stained sections of rat kidneys. K1=Normal, K2= positive control, K4= saponin, K5= 250 mg/kg, K6= 500 mg/kg, K7= Silymarin

The kidney section of the normal group shows normal appearance of tubules, glomeruli, tubular and interstitial cells. However, the kidney section of rat in positive control indicates glomerular and tubular degenerations, interstitial inflammation and oedema, congestion of the tubular cell and marked collagen deposition. Also shown was acute tubular necrosis. The saponin group indicated a more severe damage to the glomerular and tubular cells compared to the intoxicated group. However, normal glomeruli and tubular cells were observed in the kidneys of EECD treated rats with 500 mg/kg b.w EECD showing better protection.

4.0 DISCUSSION

Some of the functions of the kidney include getting rid of waste products of metabolism and maintaining salt-water balance. These functions cannot be fulfilled if the kidney suffers a chemical assault from potent toxicant such as CCl_4.[29] Humans are exposed to CCl_4 via oral, inhalation and dermal routes in the form of very stable chlorinated hydrocarbon used as solvent for oils and fats, as a refrigerant and as a dry-cleaning agent.[30]

The observed significant increase ($P<0.05$) in the concentration of MDA in the group of rats intoxicated with CCl_4 (positive control) shows that CCl_4 was able to penetrate hepatocytes, cytosol, mitochondrial and the endoplasmic reticulum (ER) of hepatocytes which contain xenobiotic reducing enzymes (electron transfer chain and NADPH dependent reductase catalysed reaction) a site for CCl_4 metabolism prior to its toxicity.[31,32,4] The group of rats pre-treated with EECD and *C. dolichopentalum* derived phytochemicals (flavonoids, alkaloids, saponins, tannins (FAST) then intoxicated with CCl_4 showed decreased MDA concentrations, this is in line with the work done by.[5,33] The reduction of CCl_4 induced oxidative stress by the anti-peroxidative activity of the EECD, might be due to the plant extract ability to prevent the penetration of CCl_4 into the liver cells, as it contains appreciable concentration of tannins.[12] Tannins are known to 'tar' the outermost layer of the mucosa, rendering it less permeable and resistant to chemical or mechanical injury or irritation. The effect of pre-treatment of the animals with FAST indicates that alkaloids and flavonoids extracts showed better inhibitory effect against the activation of CCl_4. This may be due to the presence of the flavonoid called rutin.[12] This is in line with Elsawy et al.[1], who used rutin to ameliorates carbon tetrachloride-induced hepatorenal toxicity and hypogonadism in male rats. The presence of Lunamarine, a quinoline alkaloid in *C. dolichopentalum* may be responsible for its anti- modulatory actions.[12]

The ability of the kidneys to excrete urea and maintain salt and water balance was assessed by estimating the serum concentrations of urea, K^+, Na^+, Cl^-. The

measurement of urea concentration is an important investigation in diagnosing kidney damage and its cause. A marked increase in urea as shown in the intoxicated group is indicative of damaged renal function.[1] Slight increases in urea may occur when there is any condition associated with increased protein breakdown among other reasons or inability of the kidneys to effectively excrete urea. EECD offered protection to the kidney as is evident in the dose dependent reduction of serum urea concentration. Furthermore, pretreatment with alkaloids and flavonoids appeared to stimulate the kidney to effectively excrete urea, resulting in lower concentration of urea in the alkaloid and flavonoid control groups. Rutin a plant derived-polyphenolic bioflavonoid in *C. dolichopentalum* has been reported to modulate nephrotoxicity through its regulatory effect on apoptotic pathways including inhibition of several activation of caspases.[34,17] Radwan et al.,[16] also indicated that the nephroprotective effect of rutin might be valuable in improving the therapeutic index of cisplatin.

Sodium, potassium and chloride are the main electrolytes responsible for fluid osmolarity (osmotic pressure); they also influence ionic strength and thus, the solubility of proteins and other substituents. Na^+ is the main regulator of the osmotic pressure in body fluids. Therefore, a severe loss of H_2O and salt from the body can lead to a state of shock and low blood pressure, muscular weakness, wrinkled skin and decreased extracellular fluid (ECF) volume. Conditions that can cause hyponatremia owing to loss of NaCl as shown in the intoxicated group include diarrhoea and vomiting; abnormal increase in urine excretion due to reduced aldosterone production or excess water retention will also dilute the sodium in the ECF. Loss of water and electrolyte is characterized by reduced Na^+, increased urea, raised PCV and haemoglobin and decreased plasma proteins.[35] In kidney dysfunction, Na^+ is not reabsorbed and is thus excreted in the urine. EECD and FAS group had sodium ion concentrations very close to that of the normal control, with the tannin and silymarin group tagging along. *C. dolichopentalum* leaf contains the alkaloid sparteine[12], which has been shown to be a sodium channel blocker[35], and thus possesses a potential to regulate Na^+, this can be beneficial in controlling blood pressure. Notwithstanding, mineral analysis on *C. dolichopentalum* leaves shows the presence of a very high concentration of sodium.[36] This may explain the balance in sodium ion concentration achieved in the EECD and FAST treated groups. Sodium is concerned with fluid balance (retains water in ECF), neuromuscular excitability, acid-base balance, maintenance of viscosity of blood. Quercetin, a flavonoid in *C. dolichopentalum*[11] down regulates the renal expression of epithelial Na^+ channel (ENaC) in hypertensive Dahl salt-sensitive rats, and this effect is associated with a reduction in systolic blood pressure.[37] The ENaC plays a special role in the kidney, regulating Na^+ reabsorption in renal tubules.[38]

Sodium in the body is mainly associated with the anions chloride (Cl^-). A significant increase in chloride ion concentration was observed in the FAST treated groups despite the non-significant change amongst the normal, positive, and EECD treated group. Quercetin stimulates the Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1), a key ion transporter regulating cytosolic Cl^- concentration.[39] NKCC1 activation affects several body and cellular functions, such as renal Na^+ reabsorption, thereby regulating the concentration and volume of extracellular fluid.[38] Chloride is important in the production of HCl in the gastric juice. This indicates that *C. dolichopentalum* leaf extract might be an effective chloride regulator.[40] also reported an increase in serum chloride ion using *Datura metel* leaf when administered to test animals.

The concentration of electrolytes depends on the degree of tubular reabsorption and secretion required to maintain electrostatic equilibrium. The positive control group showed significant ($P<0.05$) increase in serum concentration of K^+. This could be as a result of the inhibition of the activity of the Na^+/K^+ pump. Any substantial increase in the extracellular (EC) concentrations of K^+ will lower the chemical gradients for K^+ and, thus the membrane potential, causing a depolarization of the membrane. If EC [K^+] rises too far, this will interfere with normal heart and nervous function. EECD pre-treated rats showed maintenance of electrolyte balance in the face of CCl_4 intoxication. Flavonoid, alkaloid and saponin showed a non-significant change in K^+ when administered. This is in line with the work of Imo et al.[40] who reported an increase in serum K^+ ion when *Datura metel* leafs were administered. The tannin extract group showed a better effect as K^+ regulator compared to flavonoid, saponin and alkaloid groups in an attempt to normalize K^+ concentration. Sparteine, an alkaloid has also been shown to be an antiarrythymic agent.[41] Thus the higher concentration of K^+ observed in the alkaloid control group shows that the presence of alkaloids other than sparteine could be contributing synergistically to its rise. Ujowundu et al.,[36] also reported the presence of high concentration of potassium in *C. dolichopentalum* thus, hyperkalemic patients should cut back on *C. dolichopentalum* leaves.

Histopathological studies of the kidney showing glomerular and tubular degenerations, interstitial inflammation and oedema congestion of the tubular cell and marked collagen deposition in the intoxicated group. Accidental exposure to CCl_4 may result in swelling of tubular epithelium, fatty infiltration and, casts in the tubular lumen.[42,43] Failure of the kidney to perform its essential functions: may be due to trauma, any condition that impairs the flow of blood to the kidneys; certain toxic substances such as compounds of mercury, CCI_4, ethylene glycol; bacterial toxins; glomerulonephritis, and acute obstruction of the urinary tract.

Following assault by CCl_4 and any other toxicant (44) on the kidney, an inflammatory process in response to the injury on the kidney is initiated by local vasodilation that increases blood flow, followed by increased vascular permeability that enables plasma to move out to the capillaries and into the tissue, producing local oedema. Neutrophils and later monocytes infiltrate the injured tissue. Mediators of inflammation acting as signals and involved in intercellular cross talk can directly participate in tissue destruction if released in excess quantities.[45] Flavonoids exerts renoprotective actions that may be of interest in diseases such as glomerulonephritis and chemically-induced kidney insufficiency[38] as shown in the EECD, flavonoid, alkaloid and tannin treated group. Flavonoids prevent or attenuate the renal injury associated with arterial hypertension, both by decreasing blood pressure and by acting directly on the renal parenchyma.[38] Several other flavonoids have shown renal protective effects against numerous nephrotoxic agents that frequently cause acute or chronic kidney injury. Dillard and German,[46] also reports that luteolin, a component of *C. dolichopentalum*[11] possess anti-inflammatory activities. The general and common mechanism of action of flavonoids is their attenuation of renal oxidative stress and inflammation. Flavonoids have been reported to protect rat or mouse kidney against nephrotoxic agents.[47,48,49,50] However, kidneys of the group of rats treated with the saponin extract of *C. dolichopentalum* indicated a more severe acute tubular necrosis. This is in line with the findings of Brandao-Costa et al.[51], who revealed the nephrotoxicity of a crude extract of saponin isolated from *Filicium decipiens* seeds on male Wistar rats. Many saponin glycosides exhibit toxic effect at higher doses over an extended period causing excessive salivation, loss of appetite, and removing surface membranes.[52,53] Oral toxicity of saponins in warm blooded animals is reportedly low due to its low absorbtion from the intestinal tract. The finding in this study,[53] however, shows that saponins can be absorbed from the gastrointestinal tract. Saponins are usually not associated with renal damage, but when absorbed, their membrane-permeabilizing effect may possibly be detrimental to the renal epithelial cells.

CONCLUSION

The presence of rutin and lunamarin may be responsible for the anti-proliferative and neuroprotective properties of *C. dolichopentalum*. Sparteine and quercetin *in C. dolichopentalum* leaf possesses a potential to regulate Na^+, this can be beneficial in controlling blood pressure. Notwithstanding, the high concentration of sodium and potassium in *C. dolichopentalum* and its ability to induce re-absorption of chloride could recommend it for electrolyte therapy. However, that cannot be said of the crude saponin portion of *C. dolichopentalum*.

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