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AMELIORATIVE EFFECTS OF *CNESTIS FERRUGINEA* ROOT EXTRACT ON CARBON TETRACHLORIDE NEPHROTOXICITY IN ALBINO WISTAR RATS

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ABSTRACT:

The objective of this research was to assess the potential protective properties of ethanol extract derived from Cnestis ferruginea against the toxic effects by carbon tetrachloride (CCl_4) in the kidneys of rats. In this study, male albino Wistar rats were pre-treated with C. ferruginea at doses of 100 and 200 mg/kg body weight (b.w.) on a daily basis for a duration of 14 days. Subsequently, a single dosage of CCl_4 was administered intraperitoneally on the 7th day of the experiment. The rats were euthanized 24 hours after they were subjected to treatments using the extract on the 15th day. Subsequently, serum and kidneys were obtained and utilized for the analysis of biochemical parameters. Rats grouped as the control which were administered CCl_4 experienced kidney damage, as indicated by a statistically significant increase ($P < 0.05$) in the measured parameters (urea, creatinine, uric acid in serum and malondialdehyde in kidney tissue), and a significant reduction in the levels of oxidative stress marker enzymes (superoxide dismutase, catalase and glutathione peroxidase). It is worth noting that the administration of C. ferruginea to rats exposed to CCl_4 resulted in a significant reversal of the aforementioned changes, bringing them closer to a state of normalcy. The result obtained from this study suggest that C. ferruginea possess therapeutic properties which could protect the kidney against renal deterioration and its associated dysfunction.

Keywords: antioxidant, ethanol extract, reactive oxygen species, kidney, toxicity

INTRODUCTION

The kidney is an organ of utmost importance in the human body due to its essential metabolic functions, namely excretion and regulation. Kidney illness, regardless of its type, poses a significant and pressing health challenge on a global scale. The organ is vulnerable to acute injury that might compromise its physiological condition and metabolic processes due to continuous exposure to xenobiotics, especially nephrotoxins, in contemporary lifestyles (Schetz *et al*., 2005).

Carbon tetrachloride (CCl_4) is a highly potent hepatotoxin that exhibits strong lipid solubility. When CCl_4 is linked to lipids and proteins, it effectively increases the peroxidative process. According to a study conducted by Jayakumar *et al*. (2008), it has been observed that CCl_4 has the potential to induce the production of reactive oxygen species (ROS) in several tissues apart from the liver. These tissues include the kidney, heart,

lung, testis, brain, and blood. The presence of free radicals, which initiate the process of lipid peroxidation, results in cellular membrane impairment, hence contributing to many pathological alterations observed in both acute and chronic renal injuries. The primary enzyme implicated in the nephrotoxic effects generated by CCl_4 is cytochrome P450, which is specifically found in the cortical tubule cells. Additionally, there is observable evidence of heightened lipid peroxidation in the renal brush border. The impact of carbon tetrachloride (CCl_4) on renal mitochondrial activity, specifically its effect on calcium transport across mitochondrial membranes, has been investigated by Khan *et al.* (2010).

Certain substances have the ability to induce harm to renal tissue through the generation of reactive oxygen species (ROS). Carbon tetrachloride (CCl_4) has been observed to elicit the production of reactive oxygen species (ROS), diminish the levels of antioxidant defenses, and result in oxidative stress in several tissues. Carbon tetrachloride (CCl_4) has been observed to induce cellular damage in multiple organs, with a particular emphasis on the liver, kidneys, and lungs (Teschke, 2018). The generation of the CCl_3 free radical and other metabolites induces CCl_4 toxicity, resulting in cellular damage through lipid peroxidation and various other processes. According to Manno *et al*. (1996), the presence of free radicals has the potential to induce multi-level organ failure. Extensive documentation exists about the impact of reactive oxygen species (ROS) on several renal functions. One potential hypothesis for the detrimental effect of CCl_4 on renal function is attributed to the oxidative stress induced by the compound's substantial generation of reactive oxygen species (Irazabal *et al*., 2020).

Carbon tetrachloride (CCl_4) is a representative xenobiotic and a highly nephrotoxic substance frequently employed in experimental investigations to evaluate the efficacy of a test compound in mitigating or safeguarding against tissue abnormalities (Ighodaro and Akinloye, 2018). Throughout history, diverse societies across the globe have relied on the utilization of medicinal plants as a means of treating various ailments and diseases. According to Okoro *et al.* (2015), medicinal plants possess a diverse range of phytochemicals that have the potential to act as primary compounds in the exploration and development of novel pharmaceuticals.

The use of medicinal herbs have been recognised as a natural means of managing kidney diseases in humans. The presence of various secondary metabolites, also known as phytochemicals, in the body parts of these plants possess therapeutic properties against certain kidney diseases. These plants function as primary sources for promoting optimal health and are also utilized in the therapeutic and preventive measures against various disorders. The rise in the utilization of plant-based medications for illness management can potentially be attributed to various factors such as their potency, affordability and low toxicity, as suggested by Chirumbolo (2012). According to Nurul *et al*. (2015), medicinal herbs are cost-effective and powerful therapeutic interventions that can be considered relatively safe for the treatment of many diseases.

Cnestis ferruginea DC, a member of the Connaraceae family, is a woody plant that may reach a height of approximately 6 meters. It is widely distributed in the region spanning from Senegal to West Cameroon, as well as other areas within Tropical Africa. The utilization of this plant in traditional African medicine is extensive. According to Burkil (1985), the decorative value of the plant is enhanced by the presence of its red fruit. According to Ahmed (2017), the herb has historically been employed for the treatment of conjunctivitis, syphilis, gum discomfort, wounds, diarrhea, and gonorrhea.

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C. ferruginea has been recognized in the field of herbal medicine and other literary sources for its wide range of medicinal applications, including the treatment of bronchitis, tuberculosis, snakebite and syphilis (Ahmed, 2017). The plants have been documented to possess antimicrobial, antistress, laxative, antioxidant, analgesic, anti-inflammatory, aphrodisiac, and hepatoprotective properties. Research findings indicate that the remarkable effects exhibited by the plant's extract can be attributed to its composition of many bioactive metabolites, including alkaloids, flavonoids, saponins, anthraquinones, and tannins (Ajala *et al.*, 2021). Furthermore, Rahmat *et al*. (2014) have documented the potential of phenolic compounds in leaf extracts of *C. ferruginea* to mitigate liver and kidney damage induced by CCl_4. The usefulness of leaves and roots of *C. ferruginea* in traditional medicine is well recognized, but information about the roots is limited. Hence, the primary objective of this investigation was to assess the potential protective properties of an ethanolic root extract derived from *C. ferruginea* against nephrotoxicity instigated by CCl_4 in a rat model.

MATERIALS AND METHODS

Drug and Chemicals

Silymarin and carbon tetrachloride (CCl_4) were procured from Sigma chemicals, USA. The serum creatinine, urea, and uric acid kits were procured from RANDOX Laboratories Ltd., Ardmore, UK. All chemicals used in this investigation, with the exception of those specified, were of analytical grade.

Collection and Processing of Plant Materials

The roots of *Cnestis ferruginea* were obtained from a farmland located in Abraka, Delta State. They were authenticated at the Department of Plant Biology and Biotechnology, University of Benin (voucher No. UBH-C369). The samples were processed and extracted according to the methodology outlined by Okoro (2020a), and afterwards stored at a temperature of 4 °C until further utilization. Subsequently, the desiccated extract was reconstituted in distilled water in order to formulate the two dosages (200 and 100 mg/kg body weight) employed in the present investigation.

Animals

The animals utilized in this study, with a weight range of 150 - 230 g, were procured from the Anatomy Department of Delta State University, Abraka, Nigeria. They were provided with grower's mash (Top Feed, Ltd, Sapele in Delta State) and unrestricted access to water. Already established norms for ethical treatment of research animals was observed during the period of study (Olfert *et al.,* 1993).

Treatment of Animals

The study involved the use of male albino rats that were divided into five groups (Groups I-V), with each group consisting of five rats. The rats were administered oral treatment for a duration of 14 days, following the specific protocol outlined below:

Group I - Received distilled water only (negative control).

Group II- Received CCl_4 in olive oil vehicle only (positive control).

Group III- Received 100 mg/kg bw of silymarin

Group IV- Received 100 mg/ kg-day extract.

Group V- Received 200 mg/ kg-day extract.

The duration of the rat treatment spanned a period of 14 days, during which animals in groups II – V received intraperitoneal injections of CCl_4 dissolved in olive oil as a vehicle. The dosage administered was 1 ml per kilogram of body weight, and this injection occurred on the 7th day, specifically 30 minutes after the final treatment. The animals

were fasted and sacrificed through heart puncture. This procedure took place 24 hours following the administration of extract on the 15th day of the experiment. The obtained serum and kidney tissue were utilized for the assays conducted in this work.

Serum Biochemical Parameters Determination

Serum samples were utilized in the assessment of renal function through the measurement of urea, creatinine, and uric acid levels. These measurements were conducted using standard diagnostic kits (RANDOX Laboratories Ltd., Ardmore, UK).

Oxidative Stress Markers Assay

The experiments were conducted using the following procedures. The concentrations of antioxidants in liver tissue, specifically superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), were determined using the methodologies described by Marklund (1992), Sinha (1972), and Rotruck *et al*. (1973). the byproduct of Lipid peroxidation [malondialdehyde (MDA)] in liver tissue was determined using the procedure described by Ohkawa *et al*. (1979).

Statistical analysis

The data were subjected to analysis using Graph Pad Prism Version 6.0 for Windows, developed by Graph Pad Software Inc, San Diego, CA, USA. The data analysis involved the utilization of one-way ANOVA followed by the application of Turkey's post hoc test. A significance level of $P < 0.05$ was deemed to be statistically significant.

RESULT

Figure 1 shows the effect of the effect of ethanolic extracts of *C. ferruginea* and silymarin on creatinine level in the kidney of CCl_4-induced rats. The administration of the toxicant to the rats resulted to a significant increase ($p < 0.05$) in the creatinine level in the positive control rats relative to the negative control rats. However, a significant reduction ($p < 0.05$) in the level of creatinine was noticed in both the extract treated rats and the rats treated with the standard drug (groups III-V) when compared with the positive control rats. So also, significantly ($p < 0.05$) higher level of serum urea was elicited with CCl_4 intoxication as seen in the positive control compared with the negative control rats (Figure 2). But treatment of rats with the ethanolic extract of *C. ferruginea* resulted to a significant decrease ($p < 0.05$) in the urea level of rats (groups IV and V) relative to the positive control rats. In the same vein, the administration of CCl_4 to rats led to a significant increase in the serum uric acid level of positive control rats when compared with the negative control rats (Figure 3). While pre-treatment of rats with the extract prevented the CCl_4-induced elevation of uric acid level in the rats.

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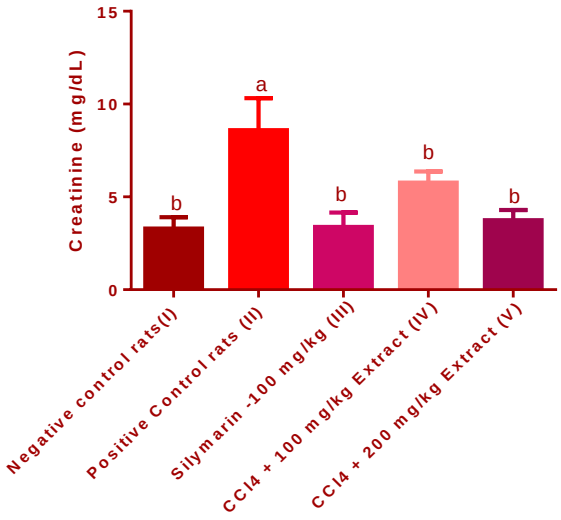

Figure 1: Effect of *C. ferruginea* root extract on creatinine level in CC_4- induced rats.

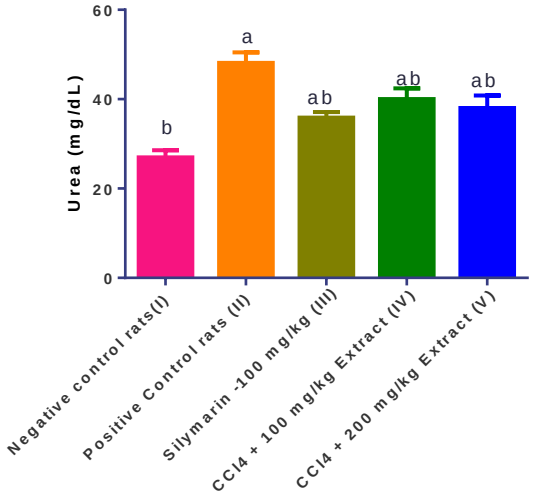

Figure 2: Effect of *C. ferruginea* root extract on urine level in CC_4- induced rats.

a p(0.05) vs negative control;

b (p<0.05) vs positive control

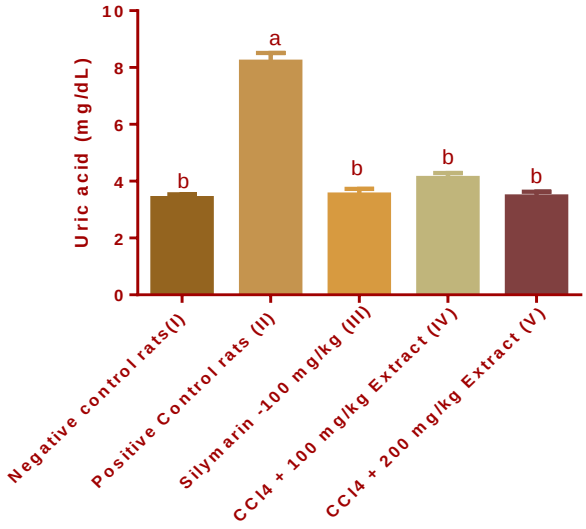

Figure 3: Effect of *C. ferruginea* root extract on uric level in CC_4- induced rats.

The effects of ethanolic extracts of *C. ferruginea* and silymarin treatment on markers of oxidative stress in the kidney of CCl_4-induced rats are shown in Figures 4-7. A significantly higher ($p < 0.05$) malondialdehyde level was observed in the positive control rats relative to the negative control group. Pre-treatment of rats with the extract resulted in significant ($p < 0.05$) reduction in the MDA level when compared with the positive control group (Figure 4). On the other hand, a significant reduction ($p < 0.05$) was observed in the activities of SOD, catalase and GPx in the kidneys of CCl_4-induced rats (positive control) relative to the rats grouped as negative control. Pre-treatment of rats with the

ethanolic extracts of *C. ferruginea* and silymarin prevented the CCl_4-induced decrease in the activities of the oxidative stress marker enzymes (Figures 5,6 and 7)..

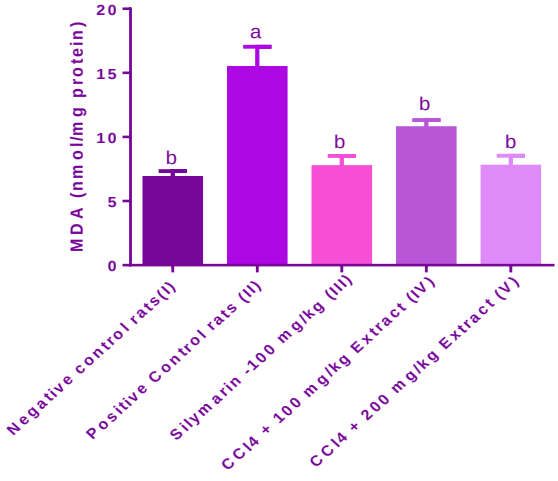

Figure 4: Effect of *C. ferruginea* root extract on malondialdehyde level in the kidney of CC_4- induced rats

a p(0.05) vs negative control;
b (p<0.05) vs positive control

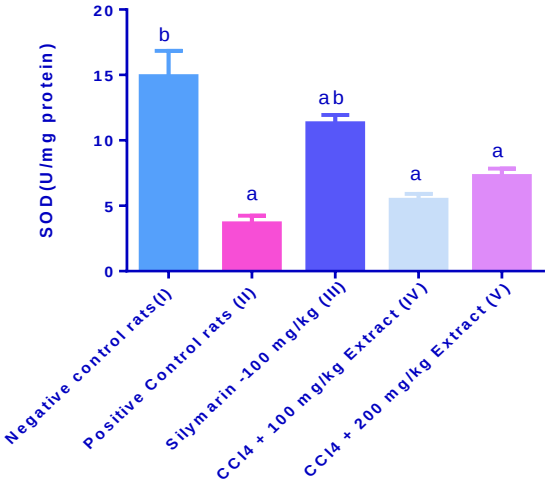

Figure 5: Effect of *C. ferruginea* root extract on superoxide dismutase level in the kidney of CC_4- induced rats

a p(0.05) vs negative control;
b (p<0.05) vs positive control

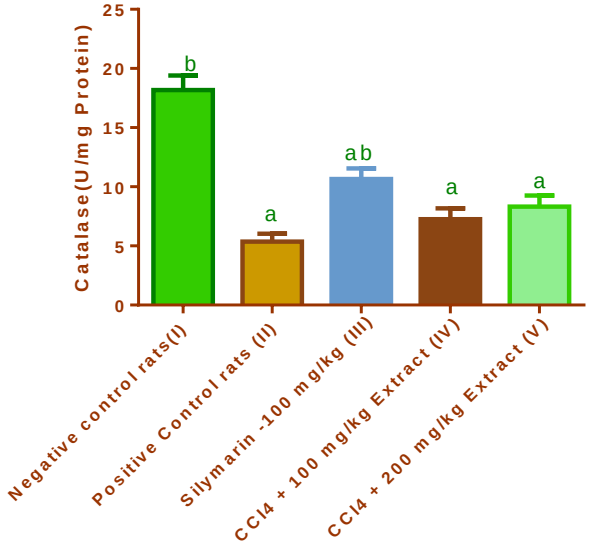

Figure 6: Effect of *C. ferruginea* root extract on catalase activity in the kidney of CC_4-induced rats
a p(0.05) vs negative control;
b (p<0.05) vs positive control

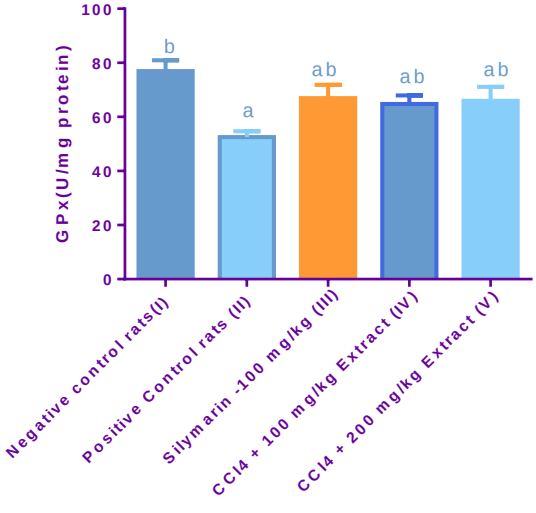

Figure 7: Effect of *C. ferruginea* root extract on glutathione peroxidase activity in the kidney of CC_4- induced rats
a p(0.05) vs negative control;
b (p<0.05) vs positive control

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DISCUSSION

Carbon tetrachloride (CCl_4) is a representative xenobiotic and a highly nephrotoxic substance frequently employed in experimental investigations to evaluate the efficacy of a test compound in mitigating or safeguarding against tissue abnormalities (Ighodaro and Akinloye, 2018). Throughout history, diverse societies across the globe have relied on the utilization of medicinal plants as a means of treating various ailments and diseases. According to Okoro *et al.* (2015), medicinal plants possess a diverse range of phytochemicals that have the potential to act as primary compounds in the exploration and development of novel pharmaceuticals. This work aimed to examine the potential protective effect of an ethanolic extract derived from the root of *C. ferruginea* against nephrotoxicity produced by CCl_4.

The result obtained from this study showed that carbon tetrachloride had nephrotoxic effect on the rats grouped as positive control. This finding aligns with the research conducted by Khan *et al*. (2009), which indicated that chronic renal injuries caused by CCl_4 intoxication were linked to elevated levels of urea and creatinine. These biomarkers were considered indicative of kidney injury, as the serum creatinine level typically remains unchanged until at least 50% of the kidney nephrons are damaged. Mishra *et al.* (2014) associated a rise in urea and creatinine in the serum with a decrease in glomerular filtration. Moreover, the elevated concentration of creatinine in the bloodstream can be ascribed to the compromised structural integrity of the nephrons (Ogeturk *et al.*, 2005). Urea and creatinine, which are nitrogenous waste products, are excreted by the kidneys. However, in cases of renal insufficiency, the process of excretion is hindered (Ozturk *et al*., 2003). Elevations in serum urea and creatinine levels are employed as a diagnostic measure for nephrotoxicity induced by carbon tetrachloride (CCl_4). The findings from this study is in agreement with other related studies which reported elevated levels of urea, uric acid, and creatinine in serum as potential markers of hepatic and/or renal damage caused by CCl_4 administration (Khan *et al*., 2009; Mahmoud, 2013).

The administration of ethanolic extract of *C. ferruginea* to rats exposed to CCl_4 resulted in the mitigation of the CCl_4-induced decrease in glomerular filtration. This was achieved by restoring the levels of urea and creatinine to normal values, comparable to those observed in the control group. A study carried out by Okokon *et al.* (2011) reported that the level of electrolytes, urea, and creatinine in serum reduced after ethanol stem extract of *Homalium lestetui* was administered on the rats in a bid to determine the nephroprotective effect of the extract on gentamicin-induced kidney injury.

The current investigation demonstrated a noteworthy decrease in superoxide dismutase levels and a considerable increase in lipid peroxides in rats treated with CCl_4, in relation to enzymatic and non-enzymatic antioxidant levels. The present study support the findings earlier reported by Khan *et al*. (2013), which documented a perturbation in antioxidant levels following the production of damage by CCl_4. The present study observed a significant decrease in antioxidant indices, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), in kidney tissues following exposure to CCl_4. Furthermore, there was a notable rise in MDA. Nevertheless, the antioxidant levels that were observed in the positive control group were hindered by pre-treatment with the ethanolic extract derived from the root of *C. ferruginea*. These findings are consistent with previous investigations reported by Khan *et al* (2010) and Khan *et al.* (2013). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are crucial antioxidant

enzymes that are involved in the elimination of free radicals within the human body (Lin *et al*., 2019). The role of oxidative stress is pivotal in the pathogenesis of renal disease. Chen *et al*. (2022) reported multiple risk factors associated with the onset of kidney disease, which encompass oxidative stress, ectopic lipid accumulation, dyslipidemia, renal cell injury, and dysfunction. The development of different clinical disorders is believed to be influenced by lipid peroxidation that is triggered by free radicals. The nephrotoxicity generated by CCl_4 is responsible for lipid peroxidation and the accumulation of malfunctioning proteins, ultimately resulting in renal impairment (Khan *et al*., 2009).

The enzyme superoxide dismutase (SOD) functions as a catalyst, facilitating the conversion of two molecules of the detrimental superoxide anion (*O2) into the benign compounds hydrogen peroxide (H_2O_2) and oxygen (O_2). Therefore, the adverse effect of superoxide anion are alleviated (Dringen *et al*., 2005). Malondialdehyde (MDA), a major secondary metabolite resulting from the process of lipid peroxidation, serves as a vital biomarker for the presence of oxidative stress. Reactive oxygen species (ROS) have been found to elevate the risk of tissue damage and induce lipid peroxidation, as evidenced by the presence of the catabolite malondialdehyde (Ng *et al*., 2007; Okoro *et al*., 2022). Earlier research has demonstrated that administration of carbon tetrachloride (CCl_4) through intraperitoneal injection leads to a notable decrease in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH), while concurrently resulting in a large elevation in the level of malondialdehyde (MDA) (Okoro *et al*., 2019).

The protective effects on the kidneys were observed with the ethanolic extract of *C. ferruginea*. The administration of CCl_4 resulted in an elevation of oxidative stress. However, it was shown that the concentration and activity of SOD, CAT, and GPx were enhanced following pre-treatment with the extract thereby leading to a reduction in oxidative stress. The findings from this study is in agreement with earlier reports by Aderogba *et al*. (2011) and Okoro (2020b). The researchers demonstrated in vitro the efficacy of antioxidant property of *C. ferruginea*. *In vivo* nephroprotective activity of ethanolic extract of *C. ferruginea* reported in this study could be attributed to the presence of phytochemicals in the plant which possess antioxidant properties (Ajala *et al*., 2021).

CONCLUSION

This study has shown that administering rat with ethanolic extract of the roots of *C. ferruginea* after the rats had been intoxicated with CCl_4 resulted in improvement of the renal function parameters. However, the extract exhibited inhibitory effects on renal function, resulting in decreased levels of MDA, while simultaneously enhancing the activity of GPx, SOD, and CAT. The results of this study indicate that the ethanolic extract of *C. ferruginea* root exhibited significant nephroprotective action, comparable to a standard drug. The observed kidney protective effect of the extract can be attributed to the presence of phytochemicals and antioxidant compounds. Findings from this study has provided additional information on the efficacy of *C. ferruginea* for therapeutic use against kidney disease.

REFERENCES

Aderogba, M.A., McGaw, L.J., Bezabih, M. and Abegaz, B.M.(2011). Isolation and characterisation of novel antioxidant constituents of *Croton zambesicus* leaf extract. *Natural Product Research*. 25(13):1224-1233.

Ahmed, H. A. (2017). Therapeutic potentials of *Cnestis ferruginea*: A review. *Journal*

Scientia Africana, Vol. 22 (No. 2), August, 2023. Pp 277-286
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

of Pharmacognosy and Phytochemistry. 6(6), 1397–1401

Ajala, L.O., Ogunlowo, O.P., Okafor, M.C., Ezem, S.N., Ogunlowo, O.M. and Adeleke, A.E. (2021). *In vitro* Insights into Prospect of *Cnestis ferruginea* Pulp Extract as an Antimicrobial Agent. *Journal of basic and applied Research in Biomedicine*, 7(1): 39-43

Burkill, H. M. (1985). The useful plants of West Tropical Africa, Vol. 1, Kew: Royal Botanic Gardens.

Chen, Z., Shrestha, R., Yang, X., Wu, X., Jia, J., Chiba, H. and Hui, S.P. (2022). Oxidative Stress and Lipid Dysregulation in Lipid Droplets: A Connection to Chronic Kidney Disease Revealed in Human Kidney Cells. *Antioxidants*, 11: 1387.

Chirumbolo, S. (2012). Role of quercetin in vascular physiology. *Canadian Journal of Physiology and Pharmacology*. 90(12):1652-7.

Dringen, R., Pawlowski, P.G. and Hirrlinger, J. (2005). Peroxide Detoxification by Brain Cells. *Journal of Neuroscience Research*, 79: 157–165.

Ighodaro, O.M. and Akinloye, O.A. (2018). *Sapium ellipticum* (Hochst) Pax leaf extract: antioxidant potential in CCl4-induced oxidative stress model. Bull Fac Pharm Cairo Univ 56(1):54e9.

Irazabal, M.V. and Torres, V.E. (2020). Reactive Oxygen Species and Redox Signaling in Chronic Kidney Disease. *Cells*, 9: 1342.

Jayakumar, T., Sakthivel, M., Thomas,P.A. and Geraldine, P. (2008).“*Pleurotus ostreatus*, an oyster mushroom, decreases the oxidative stress induced by carbon tetrachloride in rat kidneys, heart and brain,” *Chemico-Biological Interactions*, 176 (2-3): 108–120.

Khan, M.R., Rizvi, W., Khan, G.N., Khan, R.A. and Shaheen, S. (2009). Carbon Tetrachloride-Induced Nephrotoxicity in Rats: Protective Role of Digera Muricata. *Journal of Ethnopharmacology*, 122: 91–99.

Khan, R., Khan, M. and Sahreen, S. (2010) Prevention of CCl4-induced nephrotoxicity with Sonchus asper in rat. *Food and Chemical Toxicology*, 48(8-9): 2469-2476.

Khan, R.A., Khan, M.R. and Sahreen, S. (2013) Attenuation of CCl4-induced hepatic oxidative stress in rat by *Launaea procumbens*, *Experimental and Toxicologic Pathology.*, 65(3): 319-326.

Khan, R.A., Khan, M.R., Sahreen, S. and Bokhari, J. (2010). “Prevention of CCl4-induced nephrotoxicity with *Sonchus asper* in rat,” *Food and Chemical Toxicology*,48(8-9): 2469–2476.

Lin, T.A, Ke, B. J., Cheng, C.S., Wang, J. J., Wei, B. L. and Lee, C.L. (2019). Red Quinoa Bran Extracts Protects Against Carbon Tetrachloride-Induced Liver Injury and Fibrosis in Mice via Activation of Antioxidative Enzyme Systems and Blocking TGF-1 Pathway. *Nutrients*, 11: 395.

Mahmoud, M. E. E. and Samaa, S.(2013).Protective effect of ascorbic acid, biopropolis and royal jelly against aluminium toxicity in rats. *Journal of Natural Sciences Research*, 3(1):102-112.

Manno, M., Rezzadore, M., Grossi, M. and Sbrana, C. (1996) Potentiation of occupational carbon tetrachloride toxicity by ethanol abuse. *Human and Experimental Toxicology* 15(4):294–300.

Marklund, S. L. (1992). “Regulation by cytokines of extracellular superoxide dismutase and other superoxide dismutase isoenzymes in fibroblasts,” *Journal of Biological Chemistry*, 267(10): 6696–6701.

Mishra, S., Pani, S.R., Sahoo, S. (2014). Anti-nephrotoxic activity of some medicinal plants from tribal rich pockets of Odisha. *Pharmacognosy Research*. 2014; 6(3):210-217.

Ng, S. C., Anderson, A., Coker, J., Ondrus, M. (2007).Characterization of Lipid Oxidation Products in Quinoa (*Chenopodium quinoa*). *Food Chem*istry. 2007, 101, 185–192.

Nurul, H., Haruna, A., Wira, S. and Ibrahim, J. (2015). Immunomodulatory effects of selected Malaysian plants on the CD18/11a, expression and phagocytosis activities of leukocytes. *Asian Pacific Journal of Tropical Biomedicine*. 5(1):48-53.

Ogeturk, M., Kus, I., Colakoglu, N., Zararsiz, I. and Ilhan, N. (2005).Caffeic acid phenethyl ester protects kidneys against carbon tetrachloride toxicity in rats. *Journal of Ethnopharmacology*, 97(2):273-280.

Ohkawa, H., Onishi, N. and Yagi, K. (1979) Assay of lipid peroxidation in animal tissue by thiobarbituric acid reaction. *Analytical. Biochemistry*. 95: 351–358.

Okokon, J.E., Nwafor, P.A. and Noah, K.J. (2011). Nephroprotective effect of Croton zambesicus root extract against gentimicin-induced kidney injury. *Asian Pacific Journal of Tropical Medicine.*. 2011;4(12):969-972.

Okoro, I. O., Kadiri, H. E. and Inegbedion, A. (2019). *Allium cepa* extract attenuates CCl4-induced oxidative Stress in rat brain. *Thai Journal of Pharmaceutical Sciences.* 43 (1): 14-20.

Okoro, I.O. (2020a). Effects of Extraction Solvents on the Antioxidant and Phytochemical Activities of *Manihot Esculenta* Leaves. *Iranian Journal of Toxicology*. 4(1):51-58

Okoro, I.O. (2020b). Hepatoprotective effect of hydroethanol extracts of *Allium sativum* and *Carica papaya* on CCl_4-induced liver damage in rats. *Nigerian Journal of Pure and Applied Science*. 33 (1): 3639-3649.

Okoro, I.O., Okoro, E.O., Isoje, F.E. and Oyubu, G.(2022). Protective effects of *Alstonia congensis* Methanolic extract against CCl 4 induced liver damage in Wistar rats. *Scientific African*. 17 (2022) e01315.

Okoro, I., Umar, I. A., Atawodi, S. E. and Anigo, K. M. (2015). In vitro and in vivo antihyperglycemic effect of active fraction of *Cleome rutidosperma* DC. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(1):289-295

Olfert, E.D., Cross, B.M. and McWilliams, A.A. (1993). Guide to the Care and Use of Experimental Animals. 2nd ed. Ottawa, Canada: Canadian Council on Animal Care. p. 82-93

Ozturk, F., M. Ucar, I. C. Ozturk, N. Vardi and K. Batcioglu.(2003).Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats.*Urology*,62.(2):353-356.

Rahmat, A.A., Dar, F.A. and Choudhary, I.M. (2014). Protection of CCl4-Induced Liver and Kidney Damage by Phenolic Compounds in Leaf Extracts of *Cnestis ferruginea* (de Candolle). *Pharmacognosy Research*. 6(1): 19-28.

Rotruck, J.T., Pope, A.L., Ganther. H.E., Swanson, A.B., Hafeman, D.C. and Hoekstra, W.G. (1973). Selenium: biochemical roles as a component of glutathioneperoxidase, *Science*.179 (73): 588–590.

Schetz, M., Dasta, J., Goldstein, S. and Golper, T. (2005). Drug-induced acute kidney njury. *Curr Opin Crit Care* 2005; 11(6):555e65.

Sinha, A.K. (1972). Colorimetric assay of catalase. *Analytical Biochemistry*, 47: 389-394.

Teschke, R. (2018). Liver Injury by Carbon Tetrachloride Intoxication in 16 Patients Treated with Forced Ventilation to Accelerate Toxin Removal via the Lungs: A Clinical Report. *Toxics* 6(2). doi: 10.3390/toxics6020025