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Research Article

Protective Effects of *Curcuma longa* (Linn) Rhizoids Ethyl Acetate Extract against Alcohol Induced Oxidative Stress and Nephrotoxicity in Female Wistar Rats

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Abstract

Aim: The aim of this study was to assess the protective effect of *Curcuma longa* rhizoids ethyl acetate extract (CLREAE) against alcohol-induced oxidative stress and nephrotoxicity.

Methods: Thirty female (30) Wistar rats were divided randomly into six groups. Groups 1, 2, 3, 4, 5 and 6were administered with normal saline; 20% ethanol; 100 mg of CLREAE + 20% ethanol; 200 mg of CLREAE + 20%; 350 mg of CLREAE + 20% ethanol and 350 mg of CLREAE respectively for 14 days.

Results: There was a significant ($p < 0.05$) decrease in the SOD, CAT and GPx activities and GSH concentration of rat treated with only 20% ethanol when compared to the normal control group, but there was a significant ($P < 0.05$) increase in the groups pretreated with different doses of the CLREAE when compared to groups administered with only 20% ethanol. Also, treatment with the CLREAE resulted in a significant ($p < 0.05$) decrease in the renal biomarkers (creatinine and urea) when compared to the normal control group. But there was a significant ($p < 0.05$) increase in the groups pretreated with different doses of the CLREAE when compared to groups administered with 20% ethanol only. Kidney MDA level significantly ($p < 0.05$) decreased in rats pretreated with different doses of CLREAE compared with the normal control. The results of the histology show that they were a physiologic recovery in the kidney tissues as groups treated with different doses of the CLREAE showed signs of protection against toxicity evident from reduced necrosis of tubular and glomerular epithelial.

Conclusion: The study suggested the CLREAE has protective effects against alcohol-induced oxidative stress and nephrotoxicity in female Wistar rats via *in vivo* free radical scavenging ability.

Keywords: *Nephrotoxicity; CL; Curcuma longa; Oxidative Stress; Alcohol; Ethyl Acetate*

Introduction

Nephrotoxicity is the toxicity in the kidneys. It is a poisonous effect of some substances, both toxic chemicals and medications, on renal function. Risk factors for nephrotoxicity that have been identified in several studies include alcoholism, high blood pressure, diabetes mellitus, exposure to occupational nephrotoxins and chronic use of analgesics [1]. Alcohol and its metabolites go through kidneys and are excreted into urine, and its content in the urine is higher than that of the blood and the liver. The kidney is often involved in the development, maintenance and counter regulation of complex electrolyte disturbances [2]. Regular alcohol consumption raises blood pressure

which is a risk factor for renal damage. Structural and functional abnormalities of the kidney are reported with increasing frequency in the fetal alcohol syndrome seen in children who have been prenatally exposed to ethanol. The kidneys are efficiently designed organs that perform two primary tasks in the body: excretion of metabolic end products and precise regulation of body fluid constituents [3]. As they accomplish these tasks, the kidneys form and collect urine, which exits through the ureters to the bladder [4]. Nephrotoxicity defines the damaging effects of xenobiotics on the kidney. Alcohol consumption has been linked to high incidences of coronary heart disease (CHD) and chronic kidney disease (CKD) [5]. The toxicity of alcohol is associated with its metabolism through alcohol dehydrogenase (ADH) which converts ethanol to the toxic acetaldehyde which is finally oxidized to acetate through aldehyde dehydrogenase (ALDH). Acetaldehyde is a toxic by-product metabolite of ethanol that leads to liver damage [6]. Acute alcohol intoxication or chronic alcoholism leads to renal damage [7]. Other studies report similar findings, showing that the incidence of kidney disease is comparable or even lower in heavier drinkers (more than 210 g/week alcohol consumption) than in those who drink moderately (70 - 210 g/week alcohol consumption) [8]. In contrast, some studies find that heavy alcohol consumption may predict poorer outcome in patients with chronic kidney diseases [9]. For example, White and colleagues [9] reported that heavier drinkers (those consuming more than 30g of alcohol/week) were at higher risk of incident albuminuria, which is typically a symptom of kidney disease. The growing body of evidence suggests that chronic ethanol exposure induces functional alterations and structural damage in the kidneys [10]. From a functional perspective, after ethanol exposure, disorganization of proximal tubules, disorientation of microvilli and luminal casts, expansion of the mesangial matrix of glomeruli, occlusion of proximal convoluted tubules, as well as partial degeneration of proximal tubule cells along with reduced height of the cells have been observed in previous studies [10]. While the mechanism of alcohol-induced cell injury and diseases remain to be investigated, recent studies indicate that reactive oxygen species (ROS) may play an important role. Reactive oxygen species are able to cause various cellular injuries, such as DNA damage, lipid peroxidation and protein modification. Cellular systems are protected from ROS-induced cell injuries by an array of defense composed of various anti-oxidants with different functions. When the ROS present in the cellular system overpower the defense systems, they will cause oxidative stress or cell injury, leading to development of diseases [11]. The production of ROS is usually in balance with the availability and cellular localization of anti-oxidant enzymes and thiols, such as superoxide dismutase (SOD), CAT, glutathione peroxidase (Gpx) and glutathione (GSH). GSH synthesis is dependent on ATP but the maintenance of its reducing power is dependent on NADPH and the pentose phosphate pathway [12]. *In vivo* studies have found accumulated oxidative damage occurs from decreased levels of these endogenous anti-oxidants rather increase ROS production [13]. To optimize the kidney health status and to reduce the risk of oxidative stress-based diseases, a plant extract reach in natural antioxidants is recommended. Therefore, research work has been undertaken to uncover potential new sources of natural plant materials. For years, there have been studies based on the use of natural compounds plant-derived as potential therapeutic agents for various diseases in humans. Curcumin is a phenolic compound extracted from *Curcuma longa* rhizome commonly used in Asia as a spice, pigment and additive. Numerous studies have shown that curcumin has broad biological functions particularly antioxidant and anti inflammatory. In fact, it has been established that curcumin is a bifunctional antioxidant; it exerts antioxidant activity in a direct and an indirect way by scavenging reactive oxygen species and inducing an antioxidant response, respectively. The renoprotective effect of curcumin has been evaluated in several experimental models including diabetic nephropathy, chronic renal failure, ischemia and reperfusion and nephrotoxicity induced by compounds such as gentamicin, adriamycin, chloroquine, iron nitrilotriacetate, sodium fluoride, hexavalent chromium and cisplatin. It has been shown recently in a model of chronic renal failure that curcumin exerts a therapeutic effect; in fact, it reverts not only systemic alterations but also glomerular hemodynamic changes. Another recent finding shows that the renoprotective effect of curcumin is associated to preservation of function and redox balance of mitochondria. Taking together, these studies attribute the protective effect of curcumin in the kidney to the induction of the master regulator of antioxidant response nuclear factor erythroid-derived 2 (Nrf2), inhibition of mitochondrial dysfunction, attenuation of inflammatory response, preservation of antioxidant enzymes and prevention of oxidative stress.

Materials and Methods

Apparatus and biochemical instruments

The apparatus and instruments used for the experiment include micropipette, cotton wool, tissue paper, heparinized tubes, beakers, Eppendorf tubes, measuring cylinder, conical flask, disposable gloves, dissecting kit, needles and syringes (5 ml and 10 ml), weighing balance, spectrophotometer (Spectrum 23A), centrifuge, homogenizer, Chloroform-methanol (2:1v/v), diethyl ether, normal saline, distilled water, 0.05M potassium chloride (KCl), creatine reagent kits, urea reagent kits were all purchased at Libertas laboratory services, Camp, Abeokuta.

Animals

Thirty (30) female Wister rats weighing between 150 - 220g were obtained from Tayo farm, Ajibode, University of Ibadan, Ibadan. The animals were housed in well- ventilated wooden cages at room temperature (28 - 30°C), light and humidity, where they were allowed free access to maximum feeding of animal feed and water *ad libitum*. They were acclimatized for two weeks before the commencement of the experiment. The animals were randomly distributed into six groups of five animals each as shown in the table below. All animals experiment were approved by the Nigeria animal and use committee (NACUC) of the federal University of Agriculture Abeokuta, and the methods were carried out in accordance with the approved guidelines.

Plant collection and preparation

Curcuma longa rhizomes were harvested from Ajasa farms, Idi-Ori Village, Ile-Ise Awo, Abeokuta, the plant specimen was authenticated by the Department of Botany, Federal University of Agriculture, Abeokuta, as *Curcuma longa* (Family: Zingiberaceae). The plant specimen matches with the Herbarium specimen no: FUNAAB H-0065.

Figure : *Turmeric rhizoids in it (a) natural form (b) grounded form.*

Extraction of the plant materials

The rhizomes were rinsed properly, cut into small sizes and air-dried to remove moisture present in them. After they were dried, the rhizomes were ground into powdery form using a mechanical blender. One hundred milligram (1000 mg) of the powdery turmeric

was weighed using an analytical weighing balance and treated with a suitable solvent, ethyl acetate which was measured to be 2000 ml. The mixture was left to stay for 3 days in a shaker at room temperature. The solution was filtered using a Whatman No.1 filter paper. Subsequently, the solvent was evaporated using a rotary evaporator under reduced pressure at a controlled temperature 50°C. The semi extracts were stored at 4°C until when needed for use.

Experimental design

The animals were divided into six groups containing five (5) animals each.

Groups	Treatment
1	Control (Normal saline)
2	20% Ethanol only
3	100 mg/kg body weight of extract + 5.22 mg/kg body weight 20% ethanol
4	200 mg/kg body weight of extract + 5.22 mg/kg body weight 20% ethanol
5	350 mg/kg body weight of extract + 5.22 mg/kg body weight 20 % ethanol
6	350 mg/kg body weight of extract

Table 1: *Animal grouping.*

Sacrifice

Food and water were withdrawn from the animals 24 hours before the sacrifice. The rats were anaesthetized with diethyl ether in a desiccator and then sacrificed.

Collection of blood

Blood was collected from the inferior vena cava of the heart of the animals into plain centrifuge tubes and was allowed to stand for 1 hour. Serum was prepared by centrifugation at 4000 rpm for 10 minutes in a centrifuge. The clear supernatant was stored at -4°C for other biochemical assays.

Harvesting of organs

The rats were then dissected from the abdominal to the thoracic region using dissecting scissors and forceps. The essential organ (kidney) was then harvested from the rats, rinsed in normal saline solution and kept in ice-cold cooler box.

Homogenization of organs

The harvested kidney was cut into a weight of 0.2g and were homogenized in 1.8 ml of Sucrose-Tris-EDTA buffer. This is then centrifuged at 4000 rpm for 10 minutes, after which the supernatants were collected into Eppendorf tubes and stored in a cooler box at 4°C

Statistical analysis

Quantitative data were analyzed using one-way analysis of variance (ANOVA), followed with a post hoc (Duncan) test for significant values. P-value < 0.005 was considered statistically significant. Statistical package for the social sciences application software, SPSS version 20 was used for statistical analysis and the charts were plotted using Microsoft-Excel application software 08. Data are expressed as mean **± standard error of mean (SEM).**

Results

Groups (n = 5)	GSH (µmol/mg protein)	GPx (U/mg protein)	SOD (U/mg protein)	Catalase (U/mg protein)
1	1.96 ± 0.06	0.93 ± 0.04	25.74 ± 1.17	0.47 ± 0.05[b]
2	1.29 ±0.06[a]	0.71 ± 0.03	15.59 ± 0.40[a]	0.36 ± 0.02[a]
3	1.77 ± 0.08	0.84 ± 0.06[b]	15.80 ± 0.95[a]	0.36 ± 0.03[a]
4	1.65 ± 0.07	0.81 ± 0.02[b]	19.06 ± 0.63[b]	0.45 ± 0.06[b]
5	1.50 ± 0.07[b]	0.77 ± 0.08[a]	21.31 ± 0.74	0.44 ± 0.06[b]
6	2.29 ± 0.04	0.79 ± 0.06[a]	23.96 ± 1.28	0.99 ± 0.14

Table 2: *Effect of ethyl acetate extract of Curcuma longa on antioxidant parameters; GSH, GPx, Catalase, SOD in the kidney of alcohol induced female Wistar rats.*
Results are mean ± SEM
a: Controls are compared with ethanol treated groups. p < 0.05.
b: The ethanol treated group is compared with the Curcuma longa extract + ethanol treated groups. p < 0.05.

There was a significant (P < 0.05) decrease in the SOD, CAT and GPx activities and GSH concentration when the groups administered with 20% ethanol were compared to the normal control group, but there was a significant (P < 0.05) increase in the groups pretreated with different doses of the extract when compared to groups administered with 20% ethanol only.

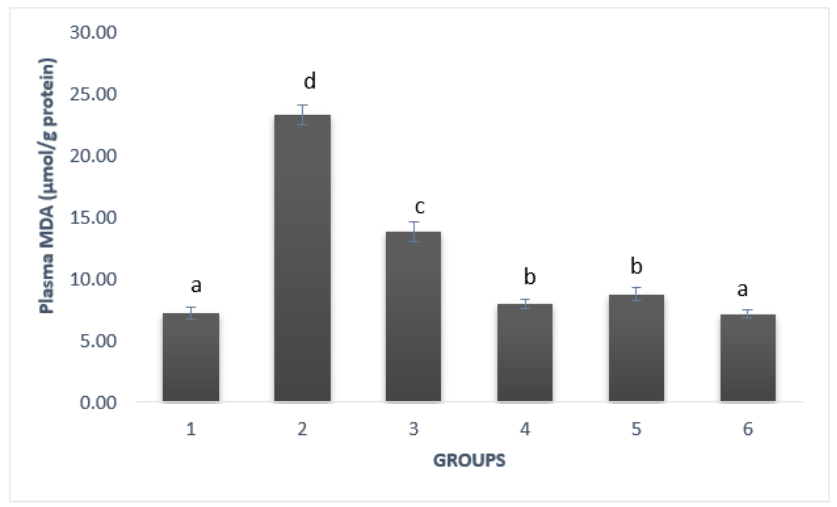

Figure 1: *Effect of ethyl acetate extract of Curcuma longa on malondialdehyde in the plasma of alcohol-induced female Wistar rats. Results are Mean ± SEM.*
a: Controls are compared with ethanol treated groups. p < 0.05.
b: The ethanol treated group is compared with the Curcuma longa extract + ethanol treated groups. p < 0.05.

The MDA level in the plasma significantly ($P < 0.05$) decreased in groups pretreated with different doses of ethyl acetate *Curcuma longa* extract before administration of 20% ethanol as compared to animals administered with 20% alcohol alone, but there was a significant ($P < 0.05$) increase when compared to the normal control group.

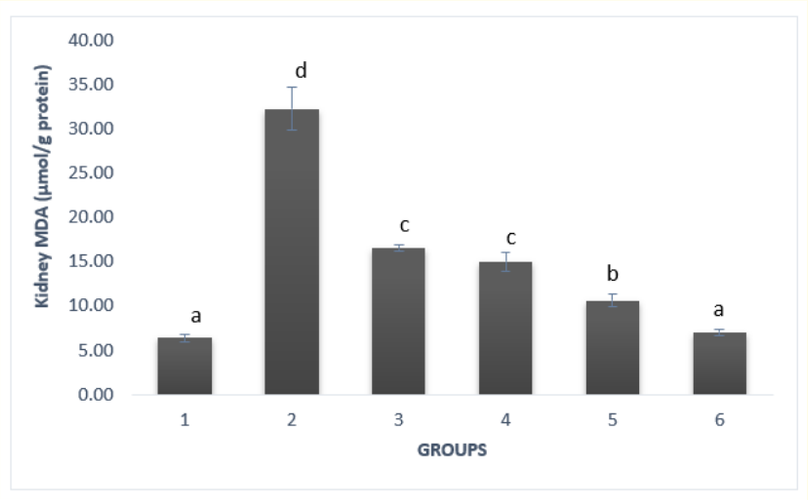

Figure 2: *Effect of ethyl acetate extract of Curcuma longa on malondialdehyde in the kidney of alcohol-induced female Wistar rats. Results are Mean ± SEM.*

a: Controls are compared with ethanol treated groups. $p < 0.05$

b: The ethanol treated group is compared with the Curcuma longa extract + ethanol treated groups. $p < 0.05$.

The MDA level in the kidney significantly ($P < 0.05$) decreased in groups pretreated with different doses of ethyl acetate *Curcuma longa* extract before administration of 20% ethanol as compared to animals administered with 20% alcohol alone, but there was a significant ($P < 0.05$) increase when compared to the normal control group.

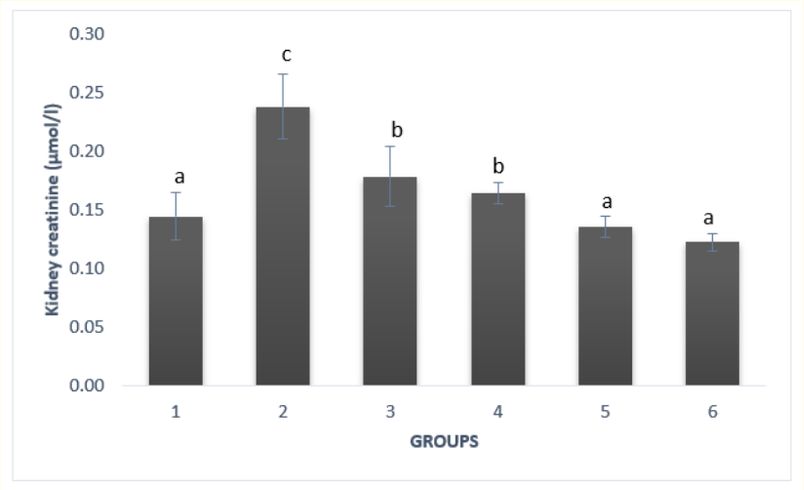

Figure 3: *Effect of ethyl acetate extract of Curcuma longa on creatinine level in the kidney of alcohol-induced female Wistar rats. Results are Mean ± SEM.*

a: Controls are compared with ethanol treated groups. $p < 0.05$

b: The ethanol treated group is compared with the Curcuma longa extract + ethanol treated groups. $p < 0.05$.

Treatment with extract resulted in a significant ($P < 0.05$) increase in the renal level of the creatinine when the group administered with 20% ethanol only was compared to the normal control group, but there was a significant ($P < 0.05$) decrease in the groups pretreated with different doses of the extract when compared to groups administered with 20% ethanol only.

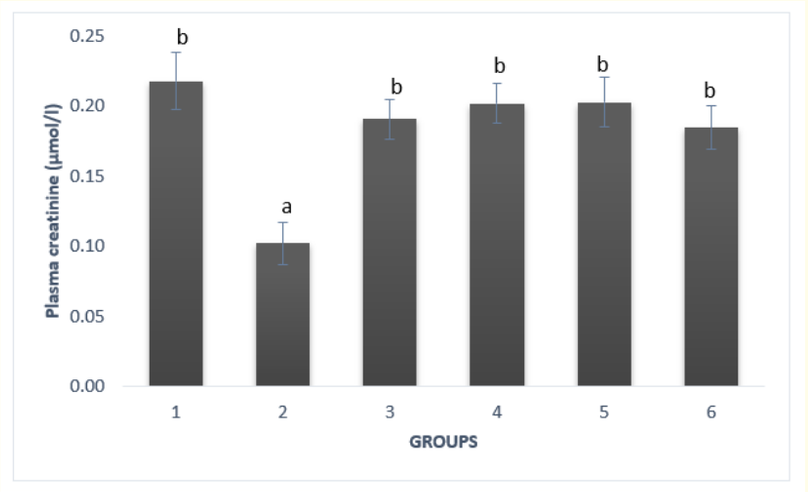

Figure 4: *Effect of ethyl acetate extract of Curcuma longa on creatinine in the plasma of alcohol-induced female Wistar rats. Results are Mean ± SEM.*

a: Controls are compared with ethanol treated groups. p < 0.05.

b: The ethanol treated group is compared with the Curcuma longa extract + ethanol treated groups. p < 0.05.

Treatment with extract resulted in a significant ($P < 0.05$) decrease in the plasma level of creatinine when the group administered with 20% ethanol only was compared to the normal control group, but there was a significant ($P < 0.05$) increase in the groups pretreated with different doses of the extract when compared to groups administered with 20% ethanol only.

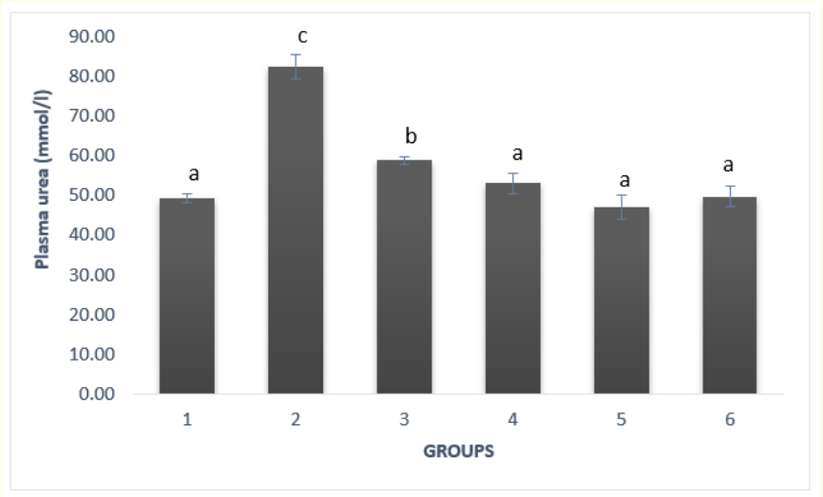

Figure 5: *Effect of ethyl acetate extract of Curcuma longa on urea in the plasma of alcohol-induced female Wistar rats. Results are Mean ± SEM.*

a: Controls are compared with ethanol treated groups. p < 0.05.

b: The ethanol treated group is compared with the Curcuma longa extract + ethanol treated groups. p < 0.05.

Treatment with extract resulted in a significant ($P < 0.05$) increase in the plasma level of urea when the group administered with 20% ethanol only was compared to the normal control group, but there was a significant ($P < 0.05$) decrease in the groups pretreated with different doses of the extract when compared to groups administered with 20% ethanol only.

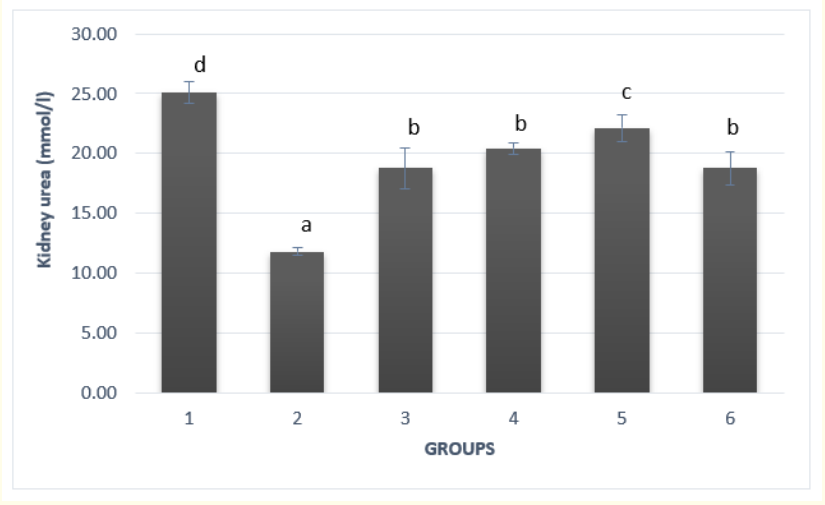

Figure 6: *Effect of ethyl acetate extract of Curcuma longa on urea in the plasma of alcohol-induced female Wistar rats. Results are Mean ± SEM.*

a: Controls are compared with ethanol treated groups. p < 0.05.

b: The ethanol treated group is compared with the Curcuma longa extract + ethanol treated groups. p < 0.05.

Treatment with extract resulted in a significant ($P < 0.05$) decrease in the kidney level of urea when the group administered with 20% ethanol only was compared to the normal control group, but there was a significant ($P < 0.05$) increase in the groups pretreated with different doses of the extract when compared to groups administered with 20% ethanol only.

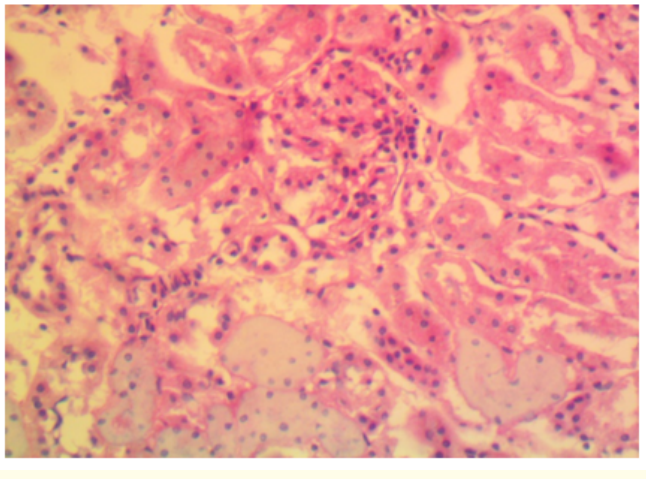

Figure 7: *Section of the kidney showing moderate necrosis of tubular (arrow) and glomerular (arrow head) epithelial cells and proteinaceous materials in the tubules (x400; H & E).*

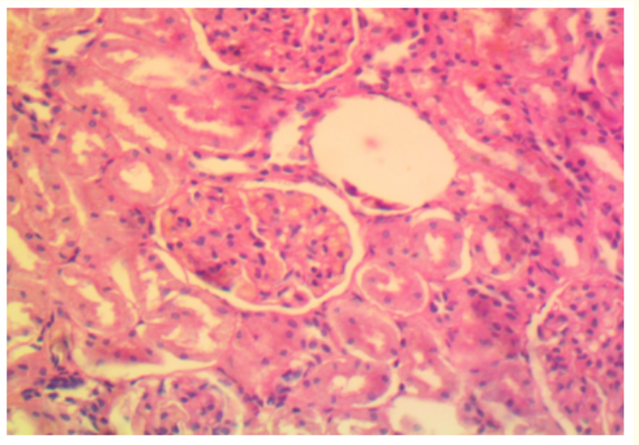

Figure 8: *Section of the kidney showing severe necrosis of tubular (arrow) and glomerular epithelia cells (arrow head) (x400; H & E).*

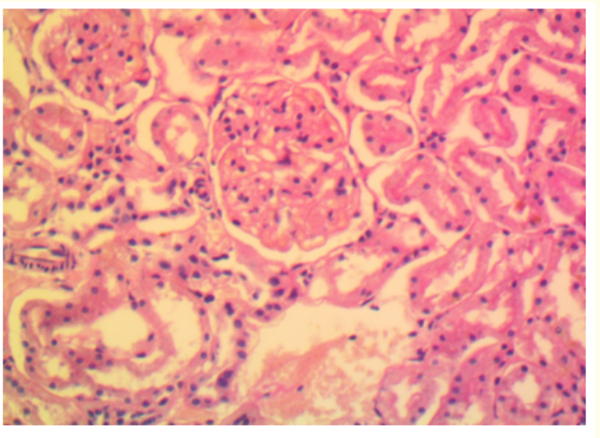

Figure 9: *Section of the kidney showing mild necrosis of tubular (arrow) and glomerular (arrow head) epithelial cells (x400; H & E).*

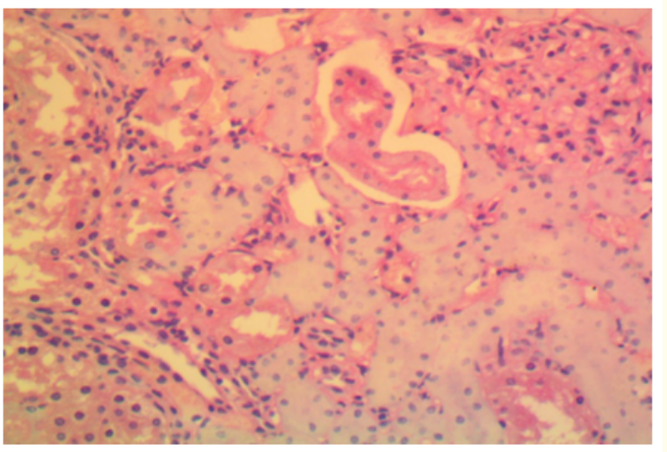

Figure 10: *Section of the kidney showing moderate necrosis of tubular (arrow) and glomerular (arrow head) epithelial cells (x400; H & E).*

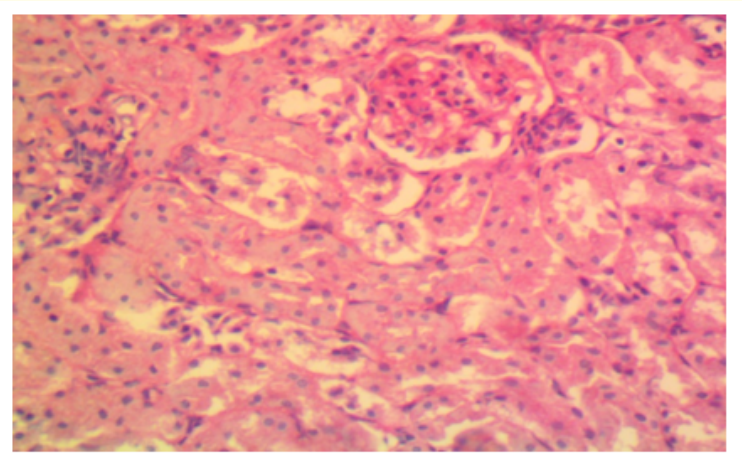

Figure 11: *Section of the kidney showing moderate necrosis of tubular (arrow) and glomerular (arrow head) epithelial cells (x400; H & E).*

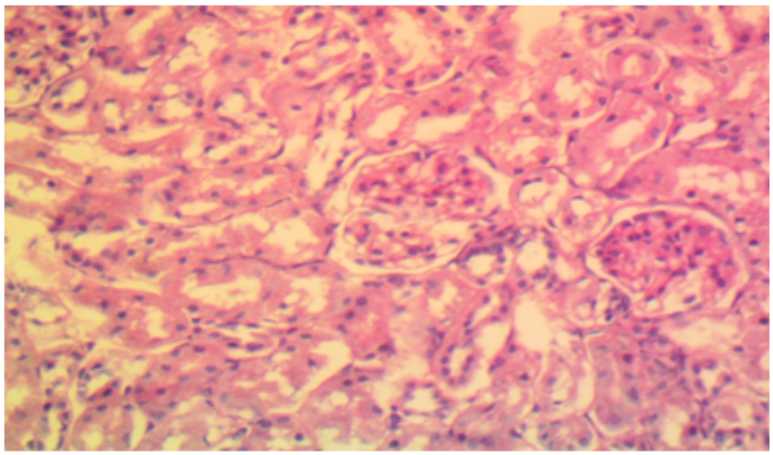

Figure 12: *Section of the kidney showing moderate necrosis of tubular (arrow) and glomerular (arrow head) epithelial cells (x400; H & E).*

Discussion

Medicinal plants are of therapeutic values and pharmacological interest that remain the main source of active drugs from natural sources. Studies indicate that functional groups associated to *Curcuma longa* chemical structure including bis-α, βunsaturated β-diketone, two methoxy groups, two phenolic hydroxy groups and two double-conjugated bonds might play an essential role in antiproliferative and

anti-inflammatory activities. *Curcuma longa* has keto-enol tautomer's, of which keto form predominates in acid and neutral solutions and enol form in alkaline solutions. In the present study, ethanol treatment induced a significant ($P < 0.05$) decrease in Reduced GSH content and SOD, GPx and CAT activities in the kidney of rats when compared to the control group. These changes were markedly reversed by treatment with *Curcuma longa* extract. The reduction in the activities of these antioxidant enzymes may be due to the inhibition of their synthesis by some reactive molecules generated during ethanol metabolism. It could also be as a result of oxidation of the enzymatic proteins by the generated reactive oxygen species. GSH plays a significant role in both scavenging reactive oxygen species (ROS) and in the detoxification of xenobiotics [14]. The decrease of this endogenous antioxidant is obviously connected with ethanol-induced oxidative stress, which is characterized by the generation of toxic acetaldehyde and other reactive molecules in the cell. The obtained result agrees with the findings of Hussain K [15] and Molina-Jijón E., *et al*. [16] who reported that chronic ethanol treatment caused a significant reduction in Renal GSH level. The observed increase in the reduced GSH level in rats co-treated with *Curcuma longa* extract and ethanol is likely due to the combined protective effects of the extract and the endogenous GSH. It may have also resulted from the induction of glutathione reductase which plays a critical role in the reduction of oxidized glutathione to reduced glutathione at the expense of NADPH and GSH- GSSG cycle in the cell.

Treatment with extract also resulted in a significant ($P < 0.05$) decrease in the renal activities of the kidney biomarkers; creatinine and urea when the groups administered with 20% ethanol were compared to the normal control group, but there was a significant ($P < 0.05$) increase in the groups pretreated with different doses of the extract when compared to groups administered with 20% ethanol only. *Curcuma longa* have been proved to show strong anti-oxidant [17]. This possibly accounts for its anti-oxidant property which enabled it to protect the kidney against the disastrous effects of free radicals and reactive oxygen species (ROS). A number of systems that generate reactive aldehyde species and reactive oxygen species are activated by chronic consumption of alcohol [18]. These results suggest that *Curcuma longa* extract significantly inhibits kidney derangement induced by ethanol. The investigated plant is rich in polyphenolic compounds such as flavonoids as part of its secondary metabolites [19]. This possibly accounts for its anti-oxidant property which enabled it to protect the kidney against the disastrous effects of free radicals and reactive oxygen species (ROS). Oxidative stress results from a disturbance in the balance between generated oxidants and anti-oxidants in favor of the oxidants. This is often caused by an increase in the generation of reactive oxygen species (ROS) and a decrease in the activity of anti-oxidant system [20]. According to Albano E [21] and Cederbaum AI [22] chronic alcohol consumption does not only activate free radical generation, but also alters the levels of both enzymatic and non-enzymatic endogenous antioxidant systems. This results in oxidative stress with cascade of effects, thus, affecting both functional and structural integrity of cell and organelle membranes [23]. The ethyl acetate *Curcuma longa* extract tested was able to suppress alcohol-induced oxidative stress in the rats.

The MDA level in the kidney significantly ($P < 0.05$) decreased in groups pretreated with different doses of ethyl acetate *Curcuma longa* extract before administration of 20% ethanol as compared to animals administered with 20% alcohol alone, but there was a significant ($P < 0.05$) increase when compared to the normal control group. A number of systems that generate reactive aldehyde species and reactive oxygen species are activated by chronic consumption of alcohol [24]. This is consistent with the findings in this study which showed a significant increase ($P < 0.05$) in renal malondialdehyde concentration in rats treated with ethanol relative to control. Alcohol metabolism which occurs primarily in the liver and kidney is characterized with the formation of free radicals and reactive oxygen species. As such, the observed high level of MDA in the kidney could be adduced to the generation of free radicals resulting in the peroxidation of membrane lipids. Moreover, the main pathway for alcohol metabolism involves the enzyme alcohol dehydrogenase (ADH) [25]. ADH metabolizes alcohol into toxic acetaldehyde, whose interaction with cell proteins and lipids can result in free radical generation and renocellular damage. Conversely, co-treatment of 100 mg/kg bwt/day to 350mg/kg bwt/day of *Curcuma longa* extract with ethanol caused a marked ($P < 0.05$) reduction in renal MDA level, when compared with rats treated with ethanol alone (Figure). According to the reports of Kumar Hari KB and Kuttan R [26] and Kassuya CA., *et al*. [27] arrays of antioxidant phytochemicals present in a plant extract are responsible for

the decrease in the level of lipid peroxidation (LPO). The antioxidant molecules in *Curcuma longa* are likely responsible for the free radical scavenging activity exhibited by the extract in this study [28]. Histopathological examination of the kidney section of the rats in the ethanol-treated group revealed an intense distortion of the renal architecture. The renal cells were found to be damaged in the ethanol treated rats whereas negligible damage was seen in rats co-treated with the extract. From the histological results, it is obvious that the was a physiologic recovery in the kidney tissues as groups treated with different doses of the extract showed signs of protection against toxicity evident from reduced necrosis of tubular and glomerular epithelial.

Conclusion

From this study, it can be suggested that *Curcuma longa* extract elicit protection against alcohol-induced nephrotoxicity and oxidative damage in rats possibly by acting as an *in vivo* free radical scavenger or through induction of antioxidant enzymes, drug detoxifying enzymes, and prevention of excessive stimulation of lipid peroxidation.

Conflict of Interest

The authors declare no conflict of interest about this work.

Acknowledgement

This work was carried in the department of biochemistry at college of bioscience, Federal University of Agriculture Abeokuta. In addition, the authors would like to thank all the colleagues and staff for their contributions and valuable discussions that enriched this study.

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Volume 9 Issue 2 February 2021