Original Research Article

Biochemical effects of *Telfairia occidentalis* leaf extracts against copper-induced oxidative stress and histopathological abnormalities

Abstract

This study was carried out to investigate the protective effect of oral administration of *T. occidentalis* against copper induced oxidative stress. Forty two adult male Wistar rats were divided equally into six groups. Group 1 were orally gavaged with standard animal feed only, group 2-6 in addition to normal feed received oral treatment of 0.3mg/kg b.w copper daily, group 3 and 4 received 250mg/kg b.w and 500mg/kg b.w aqueous leaf extract of *T. occidentalis* respectively, while group 5 and 6 were treated with 250mg/kg and 500mg/kg b.w methanol extract of *T. occidentalis*. The oral administration of 500mg/kg b.w proved most effective in the restoration of the antioxidant enzyme status, while the hepatoprotective activity of the extracts was completely effective on the AST levels, total bilirubin, and serum albumin levels. The results for renal function markers show that only 500mg/kg b.w methanol extract completely restored the urea and potassium levels. This indicates that *T. occidentalis* could ameliorate the antioxidant status in copper-induced oxidative stress.

Keywords: Methanol extract; oxidative stress; hepatotoprotective activity; antioxidant enzyme; renal function markers

1.0 Introduction

In humans, the involvement of oxidative stress in the development of certain diseases and/or exacerbation of their symptoms has been well documented. They are initiated by free radicals like nitric oxide, hydrogen peroxide, superoxide anions, and hydroxyl radicals. Free radicals and reactive oxygen species have been reported to cause the disruption of the integrity of biological systems, thereby contributing to the pathogenesis of some diseases like cancer, diabetes, arthritis, and atherosclerosis (~~Ames, 1983; Feher *et al*., 1987;~~ Aruoma, 1998). The damaging effects of these free radicals are however cushioned by some endogenous antioxidants like glutathione reductase, catalase, tocopherol, superoxide dismutase, ascorbic acid etc. That notwithstanding, these endogenous antioxidants have been found to be insufficient in the complete prevention of the effects of free radicals. In such events where the activities of these antioxidants are inadequate, the administration of exogenous antioxidants becomes imperative. Unfortunately, many reports have argued that the administration of synthetic exogenous antioxidants evoke numerous side effects, whereas natural plant sources of antioxidants exhibit very strong antioxidant effects due to the presence of components such as phenols, flavonoids, flavonols, proanthocyanins, lycopene etc (Eseyin *et al*., 2014)**.** In most African countries like Nigeria, plants play several roles in traditional medicine due to abundance of different natural products. The ingestion of these plant sources of natural products have been identified as the hallmark of disease prevention (Johnson, 2001; Virgilli *et al*., 2001; Adedapo *et al*., 2008). Nowadays, researchers tailor their studies towards the potential health effects of the phytochemical constituent of these foods and vegetables (Wei and Shiow, 2001)**.** Plants are good sources of antioxidants, and *Telfairia occidentalis* have been one of the most investigated due to its antioxidant properties and free radical scavenging potentials (Oboh and Akindahunsi, 2004; Iweala and Obidoa, 2009; Kayode *et al*., 2009). *Telfairia occidentalis* is a member of the cucurbitaceae family and is indigenous to South East Nigeria. Common names for the plant include Fluted gourd, Fluted pumpkin, and Ugu. The fluted gourd grows in many nations of West Africa but is mainly cultivated in Nigeria, used primarily in soups and herbal medicines. The leaves of *T. occidentalis* are attributed to be nutritious and medicinal, and as a result, are consumed in different parts of the country. Several extracts of the leaf have been reported to elevate the antioxidant enzyme levels both in vivo and in vitro, and scavenge, prevent or suppress the production of free radicals (Oboh and Akindahunsi, 2004; Oboh *et al*., 2006; Kayode *et al*., 2009; Kayode, *et al*., 2010). Studies have specifically attributed the antioxidant effects of *T. occidentalis* leaf to its high ascorbic acid and phenol content (Oboh, 2005). Hamza *et al*., (2013) have argued that the extracts of *T. occidentalis* gotten from the usage of different enzyme solvents offered protective and chemopreventive effects on oxidative stress induced organs like brain, liver, and kidney. *T. occidentalis* is known to contain high superoxide and hydroxyl radical scavenging potentials, high total antioxidant and phenol content, and lipid peroxidation inhibition potentials (Hamza *et al*., 2013)**.** In addition, Nwanna and Oboh, (2007) have reported the hepatoprotective characteristics of polyphenol contents of *T. occidentalis* leaves on acetaminophen induced liver damage. Therefore, it is possible that by consuming the

leaves of *T. occidentalis*, the provision of sufficient antioxidants that can prevent or cushion the effects of oxidants and promote the general well being of animals, is assured. It is to this end that this study was carried out to evaluate the modulatory effect of aqueous and methanol leaf extracts of *T. occidentalis* against copper-induced oxidative stress and histopathological abnormalities.

1.1 Materials and methods

1.1.1 Collection of plant samples

Fresh pumpkin leaves were bought from Choba Market in Rivers State Nigeria, authentication of the leaves was carried out in the Department of Plant Science and Biotechnology, University of Port Harcourt Choba, Rivers State, Nigeria, and was sun dried for 4 days before being ground into powdery form using an electronic grinding machine (Dade:DFT-50).

1.1.2 Preparation of Aqueous Extract

A 300g of the powdered *Telfairia occidentalis* was measured and soaked in 600ml of distilled water for 48 hours and afterwards sieved using a muslin cloth before being filtered with Whatmann paper size 1. The filtrate was thus concentrated using Rotory Evaporator at 40°C.

1.1.3 Preparation of Methanolic Extract

The powdered *Telfairia occidentalis* (300g) was measured and soaked in 600ml of 75% methanol for 48 hours and afterwards sieved using a muslin cloth before filtering with Whatmann paper size 1.The filtrate was thus concentrated using Rotory Evaporator at 40°C.

1.1.4 Chemicals and Reagents

They include the following: Copper sulphate, Chloroform (BDH chemicals Ltd), methanol 95% (SIGMA), Formalin 10%, Biochemical reagent kits (MINDRAY) and Finisher feed (Top Feed Ltd).

1.1.5 Experimental Animal

Forty two (42) male Wister rats weighing 170-200g were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt. The animals were acclimatized to house conditions for seven days (7), and provided with normal feed (Top feed grower's mash) and clean water *ad libitum*. Animal experiments were conducted in accordance with the internationally accepted principle for laboratory animal use and care (NIEHS, 1985)**.**

1.1.6 Experimental Design

Animals were weighed and randomly assigned into six equal groups, namely

Group 1: Received only normal feed and water

Group 2: Received intraperitoneal injection of 0.3mg/kg body weight of copper sulphate once daily to induce oxidative stress, with no further treatment administered.

Group 3: Received intraperitoneal injection of 0.3mg/kg body weight of copper sulphate once daily, and was treated with 250mg/kg body weight of the aqueous extract of *Telfairia Occidentalis* leaves

Group 4: Received intraperitoneal injection of 0.3mg/kg body weight of copper sulphate once daily, and was treated with 500mg/kg body weight of the aqueous extract of *Telfairia Occidentalis* leaves

Group 5: Received intraperitoneal injection of 0.3mg/kg body weight of copper sulphate once daily, and was treated with 250mg/kg body weight of the methanolic extract of *Telfairia Occidentalis* leaves.

Group 6: Received intraperitoneal injection of 0.3mg/kg body weight of copper sulphate once daily, and was treated with 500mg/kg body weight of the methanolic extract of *Telfairia Occidentalis* leaves.

All groups were fed with the same animal feed and water *ad libitum*.

After 28 days, the animals to be sacrificed were first subjected to inhalational anesthesia with chloroform followed by cervical dislocation. Each of the experimental animals were placed on a dissecting slab, and cut along the thorax down the abdominal region. Blood was collected via cardiac puncture and dispensed into the Heparin bottle for biochemical assays.

1.1.7 Biochemical Parameters

1.1.7.1 Determination of oxidative stress parameters

1.1.7.2 Determination of Malondialdehyde (Ohkawa *et al.,* 1979)

Normal saline (0.5ml) was pipetted into a test tube containing 0.5ml of the serum sample. About 2ml of thiobarituric acid (TBA)/trichloroacetic acid (TCA) mixture was added, allowed to boil for 1 hour, cooled to room temperature, and centrifuged at 4000rpm for 5min. The clear supernatant was read at 535nm.

1.1.7.3 Determination of Catalase activity (Aebi, 1984)

Distilled water (2.5ml) was pipetted into test tube containing 0.5ml H_2O_2, and about 40µl sample was added and mixed thoroughly**.** Rate of decomposition of hydrogen peroxide was read at 240nm at 30sec interval for 5 mins.

1.1.7.4 Estimation of Superoxide Dismutase (SOD) activity (McCord and Fridovich (1969)

Sample extract (20ml) and 2.5 ml of 0.05 M carbonate buffer (pH 10.2) were mixed together and equilibrated in the spectrophotometer. In addition, 0.3 ml of 0.3 mM freshly prepared adrenaline was added and mixed by

inversion. The increase in absorbance at 480 nm was monitored spectrophotometrically at 30 seconds intervals for 3mins.

1.1.7.5 Estimation of Glutathione (GSH) (Sedlak and Lindsay, 1968)

To a test tube containing 0.5ml of the sample was added 0.5ml (50%) of TCA and the solution was mixed and centrifuged at 2.0 x 10^3rpm. Then, 1ml of the supernatant was mixed with 2ml of 0.01m DTBN reagent (Ellman's reagent) and kept away from direct light for 15 to 20 minutes. The absorbance at 412nm was recorded. Then, standard glutathione was added to a mixture of 1.5ml phosphate buffer and 2ml of DTBN, and absorbance was read at 412nm after 15 minutes. The concentrations of glutathione (µg/ml) were traced from the standard curve for glutathione.

1.1.7.6 Determination of Glutathione Peroxidase (GPx) (Paglia and Valentine, 1967)

Glutathione peroxidase (GSH-px) activity in the sample was measured using Randox GSH-px kit according to the method of Paglia and Valentine, (1967).

1.1.8 Liver Function Test

Liver Enzymes

Concentrations of AST, ALT, and ALP were obtained by kinetic methods with kits from Mindray test kits (Mindray Medical International Limited, China) using a double-beam spectrophotometer. Other reagents used were of analytical grade.

1.1.9 Renal Function Test

1.1.9.1 Determination of Creatinine

A Modified method according to Bartels and Bolmer (1971) was used to determine the level of Creatinine in the samples. Mindray test kits (Mindray Medical International Limited, China) was used for the analysis.

1.1.9.2 Determination of Urea

Urease-glutamate Dehydrogenase -UV method according to Berthelot's method (Weatherburn, 1967) was used to determine the level of Urea in the samples. Mindray test kits (Mindray Medical International Limited, China was used for the analysis.

1.1.9.3 Determination of Serum Abumin (ALB) level

Bromcresol green (BCG) method was used to determine the level of Plasma Albumin in the samples according to the method of Doumas *et al.,* (1971).

1.1.9.4 Determination of Serum Total Protein (TP)

Biuret method was used to determine the level of total protein in the samples according to the method of Flack and Woollen, (19845) and Tietz, (2005)

1.1.9.5 Determination of Total Bilirubin (T-BIL) concentration

Jendrassik-Grof method (Jendrassik and Grof, 1938) of Mindray test kit was used to determine the level of Total Bilirubin in the samples.

1.1.9.6 Determination of serum electrolyte

Na^+ and K^+ were determined by flame photometery using Jenway P7 Flame photometer. Chloride ion activities of serum enzymes were determined using diagnostic kits (Quinica Clinica Applicada S.A Spain) following Reitman and Frankel (1957) method.

1.1.9.7 Histological Examination

The liver and kidneys of a rat from each group after being removed from surrounding tissues were fixed in 10% formal saline and after 72 h the organs were dehydrated in graded alcohol (20%, 30%, 50%, 70%, 95%), 5 min each, cleared in xylene, and embedded in paraffin. The resulting blocks were completely sectioned and randomized. The selected sections were stained in haemotoxylin and eosin and the slides were then examined at magnification of ×400 under optical microscope.

Statistical analysis

All data were subjected to statistical analysis. Values are reported as Mean ± Standard error of mean (SEM) while one way ANOVA was used to test for differences between treatment groups using Statistical Package for Social Sciences (SPSS) version 20. The results were considered significant at p-values of less than 0.05 ($p<0.05$).

Results and Discussion

The results of administration of *T. occidentalis* extracts on MDA levels of Wistar rats with copper-induced oxidative stress (Fig.1) shown below, reveals a significant derangement of antioxidant system on exposure to copper. Response related increase in the levels of MDA on exposure to inducers of oxidative stress is well documented**.**

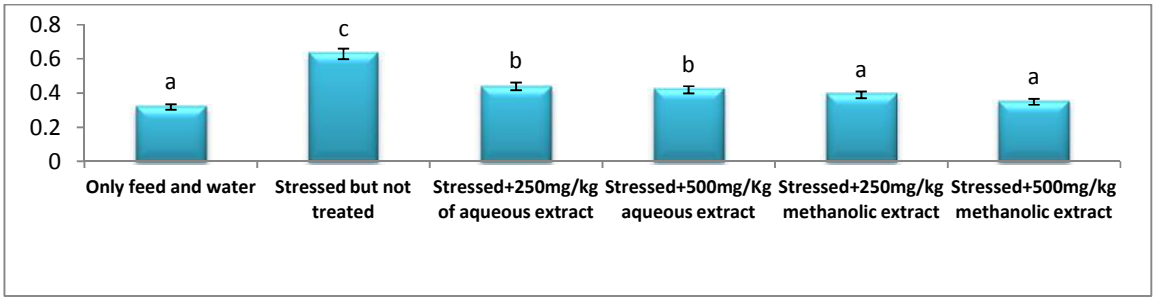


Fig.1: Effect of *T. occidentalis* extracts on malondialdehyde levels (umol/L) of Wistar rats with copper-induced oxidative stress.
***Columns bearing similar letters (a-c) are not significantly (p<0.05) different**
***Stressed implies received intraperitoneally, 0.3mg/kg body weight of copper sulphate once daily**

The results of administration of *T. occidentalis* extracts on MDA levels of Wistar rats with copper-induced oxidative stress (Fig.1) shows a significant derangement of antioxidant system on exposure to copper. Response related increase in the levels of MDA on exposure to inducers of oxidative stress is well documented**.** Ali and Atalla, (2014) reported a significant increase in MDA levels after administration of Dimpylate. Monosodium glutamate, paracetamol, and carbon tetrachloride when used as oxidative stress inducers, all significantly increased the MDA levels which were in agreement with this present study (Moselhy and Ali, 2009; Amel, 2011; Omotayo *et al*., 2015). Dervis *et al*., (2003) similarly reported an increase in MDA levels after exposure to copper. MDA is also an indicator for lipid peroxidation (Amel, 2011). The results of this present study showed that the significant decrease in the levels of MDA on treatment with aqueous extract of *T. occidentalis* was not concentration dependent. Further, methanol extracts at both concentration used completely restored the activity of MDA when compared to the control group. This complete restorative activity of *T. occidentalis* on MDA was on par with *Matricaria chamomilla* extracts (Ali and Atalla, 2014), *Coeletura aegyptiaca* (Amel, 2011), and cinnamon extracts (Moselhy and Ali, 2009). The increase in MDA levels was for the augmentation of oxidative stress in tissues but the significant ameliorative potentials of both aqueous and methanol extracts of *T. occidentalis* alleviated this increase.

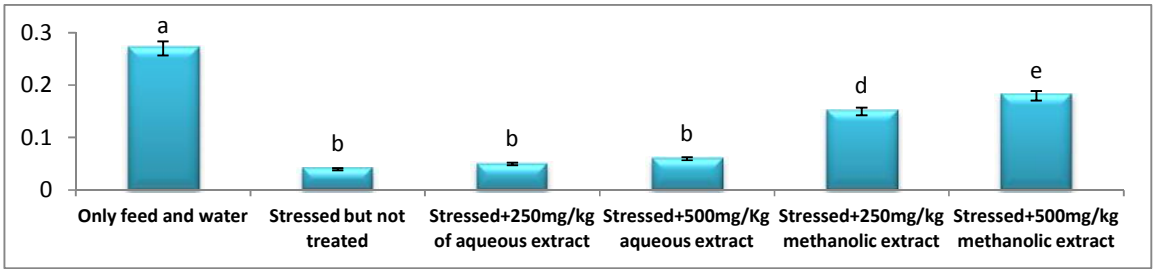


Fig. 2: Effect of *T. occidentalis* extracts on superoxide dismutase levels (umol/L) of Wistar rats with copper-induced oxidative stress.
***Columns bearing similar letters (a-e) are not significantly (p<0.05) different**
***Stressed implies received intraperitoneally, 0.3mg/kg body weight of copper sulphate once daily**

A significant decrease in the activity of SOD was observed on treatment with copper (Fig. 2). The administration of both 250mg/kg b.w and 500mg/kg b.w aqueous extracts of *T. occidentalis* was found ineffective in restoring the activity of SOD. Treatment with 250mg/kg b.w methanol leaf extract of *T. occidentalis* significantly increased the concentration of SOD which increased significantly on further increment

to 500mg/kg b.w administered methanol leaf extract. However, the restoration of the activity of SOD on administration of 500mg/kg b.w methanol leaf extract was not absolute, as shown in Fig. 2. Ethanol extracts of *Mangifera indica* stem barks offered more SOD restorative potentials (Omotayo *et al*., 2013) than methanol extracts of *T. Occidentalis* in this study. Although in line with this present study, *Tinospora cordifolia*, and *Emblica officinalis* were also unable to completely restore the activity of SOD against Cd-induced oxidative stress and alcohol-induced oxidative stress respectively (Damodara *et al*., 2007; Padma *et al*., 2016). Superoxide dismutase catalyzes the dismutation of superoxide anion to hydrogen peroxide, for further detoxification to water by catalase (Mukherjee *et al.,* 2003). The decrease in activity of SOD after treatment with copper in this present study implies that the synthesized antioxidant enzyme has been suppressed. This is in line with the findings of Omotayo *et al*., (2015).

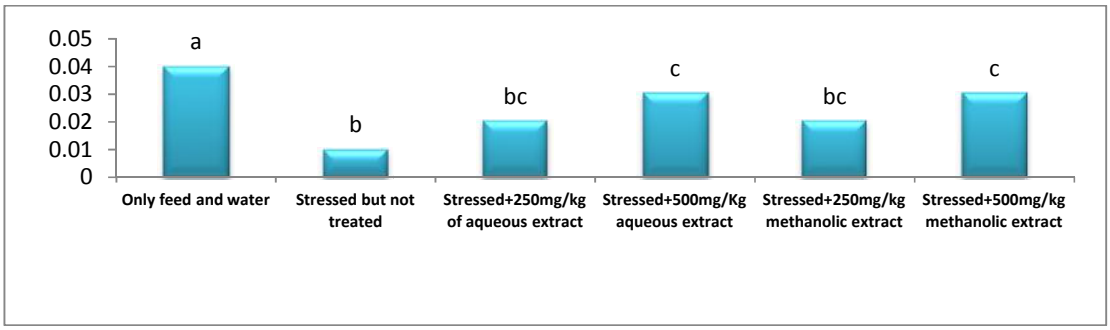


Fig. 3: Effect of *T. occidentalis* extracts on glutathione levels (ug/L) of Wistar rats with copper-induced oxidative stress.
***Columns bearing similar letters (a-c) are not significantly (p<0.05) different**
***Stressed implies received intraperitoneally, 0.3mg/kg body weight of copper sulphate once daily**

The effect of *T. occidentalis* extracts on glutathione levels (ug/L) of Wistar rats with copper-induced oxidative stress was shown in Fig. 3. Copper-induced oxidative stress significantly reduced the glutathione levels which significantly increased after the administration of 500mg/kg b.w aqueous and methanol leaf extract. Dose level of 200mg/kg b.w extract of *Baphia nitida* was able to significantly restore the activity of diazepam induced oxidative stress in rats (Akande *et al*., 2011). Khan *et al*., (2012) reported that 200mg/kg b.w various fractions of *L. procumbens* suppressed the toxic effects of $KBrO_3$-induced oxidative stress, while reversing the activity of the enzymes near to the control group. In line with the findings of this present study, Khan *et al*., (2011) and Sahreen *et al*., (2011) have also reported similar findings with other plant extracts. Fuentealba *et al*., (1993) posited that glutathione is activated in advanced exposure to copper. This implies that the toxicity of copper could strongly depend on the significant reduction in the intracellular levels of glutathione.

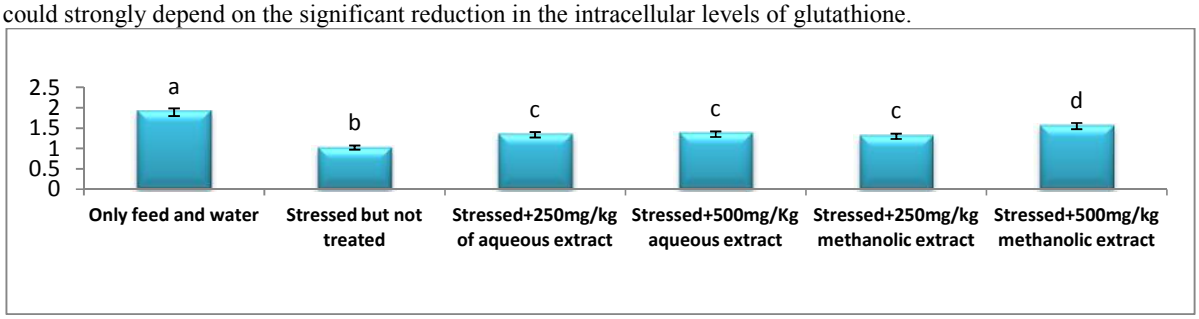


Fig. 4: Effect of *T. occidentalis* extracts on glutathione peroxidase (GPx) levels (ug/L) of Wistar rats with copper-induced oxidative stress.
***Columns bearing similar letters (a-d) are not significantly (p<0.05) different**
***Stressed implies received intraperitoneally, 0.3mg/kg body weight of copper sulphate once daily**

The GPx levels of Wistar rats with copper-induced oxidative stress after treatment with aqueous and methanol extracts of *T. occidentalis* was shown in Fig. 4. The significant decrease in GPx levels after treatment with copper was slightly ameliorated by the administration of 250 and 500mg/kg b.w aqueous leaf extract of *T. occidentalis*, and 250mg/kg b.w methanol leaf extract of *T. occidental*. Treatment with 500mg/kg b.w methanol leaf extract of *T. occidentalis*, proved most effective (Fig. 4) but was still significantly lower than the GPx levels

of the control group. GPx is regarded as one of the key enzymes for the detoxification of reactive oxygen species. *Matricaria chamomilla* did not completely restore the activity of GPx (Ali and Atalla, 2014), but grape seed proanthocyanidine extracts did completely restore the Gpx activities against oxidative stress induced by cisplatin in rats. The restorative potentials of *Tinospora cordifolia* extract on Cd-induced oxidative stress in rats, compared significantly to the control group (Padma *et al*., 2016)**.**

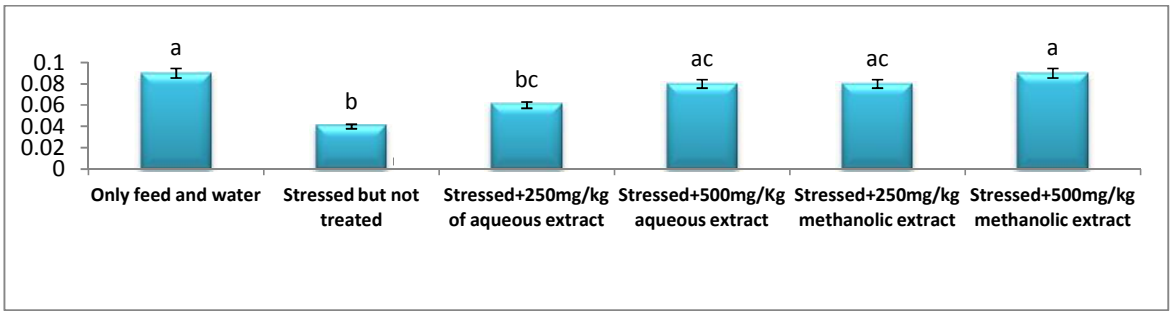


Fig. 5: Effect of *T. occidentalis* extracts on catalase levels (u/ml)of Wistar rats with copper-induced oxidative stress.
***Columns bearing similar letters (a-c) are not significantly (p<0.05) different**
***Stressed implies received intraperitoneally, 0.3mg/kg body weight of copper sulphate once daily**

Catalase plays a central role in the decomposition of hydrogen peroxide to water and oxygen (Gaetani *et al*., 1996). From the statistical point of view, treatment with 250mg/kg b.w aqueous leaf extract of *T. occidentalis* was ineffective, while no significant difference was recorded for the catalase activity after treatment with 500mg/kg b.w aqueous leaf extract and 250mg/kg b.w methanol extract of *T. occidentalis*. Total restoration of the activity of catalase was achieved on administration of 500mg/kg b.w methanol extract as shown in Fig. 5. Omoregie and Osagie, (2012) have reported that the supplementation of protein deficient diet with methanol extracts of *A. hybridus, J. tajorensis, G. Africana, T. triangulare,* all enhanced the activity of catalase. On a similar note, 200mg/kg b.w of *B. nitida* ethanol extracts similarly restored the activities of catalase.

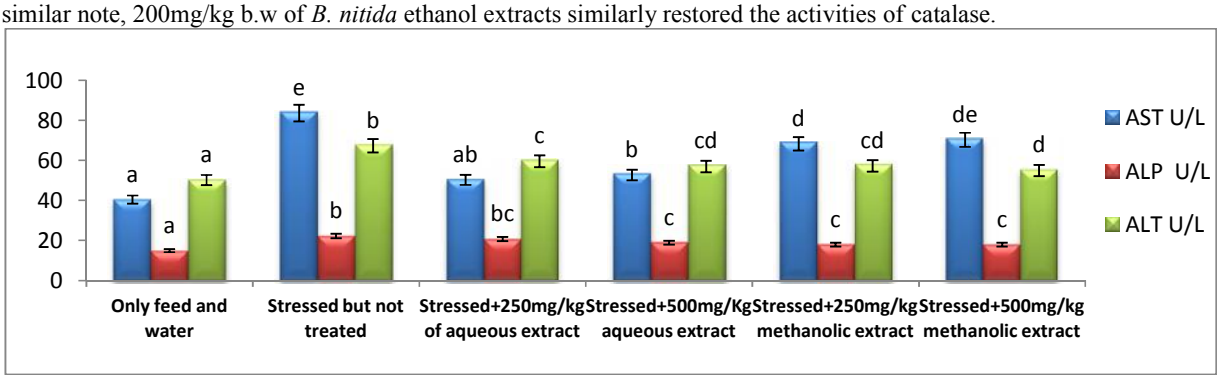


Fig. 6: Effect of *T. occidentalis* aqueous and methanolic extracts on liver markers (U/L) of Wistar rats with copper-induced oxidative stress
***Columns bearing similar letters (a-e) separately for each enzyme, are not significantly (p<0.05) different**
***Stressed implies received intraperitoneally, 0.3mg/kg body weight of copper sulphate once daily**

The effect of *T. occidentalis* aqueous and methanolic extracts on liver markers (U/L) of Wistar rats with copper-induced oxidative stress are shown in Fig. 6. It is well documented that oxidants generally cause harm to the liver tissues. AST and ALT are cytosolic enzymes and are important biomarkers of liver damage. In this present study, the administration of copper significantly elevated all liver enzyme markers. The elevation in the plasma levels of transaminases on treatment with copper indicates impairment of liver functions. Transaminases are regarded as the most sensitive biomarkers with direct implication in the extent of cellular distortion and toxicity because their cytoplasmic location enables their release into the circulation after cellular damage (Stockham and Scott, 2002). Alterations in AST and ALT are reported in hepatic disease and in myocardial infarction (Yagmurca *et al.,* 2003). In this present study, alterations in the AST levels were shown to be completely restored only by 250mg/kg b.w of the aqueous leaf extract of *T. occidentalis*. However, significant differences were recorded for other treatment groups, but none were comparable to the control group. Treatment using 250mg/kg b.w of aqueous leaf extract did not significantly attenuate the toxicity posed by copper on ALT (Fig. 6). However, treatment using 500mg/kg b.w aqueous extract, 250mg/kg b.w of methanol leaf extract, and

500mg/kg b.w of methanol leaf extract showed significant hepatoprotective potentials. The results have thus shown that hepatoprotective activities of these extracts is considered to be dose dependent especially for AST and ALT while ALP were unaffected by dose. The findings Omotayo *et al*., (2015) and Malami *et al.,* (2014) is in line with the findings of this present study showing a decrease in activity of ALT, AST, ALP in rats with CCl_4 induced hepatotoxicity and treated with aqueous stem bark extract of *M. indica*.

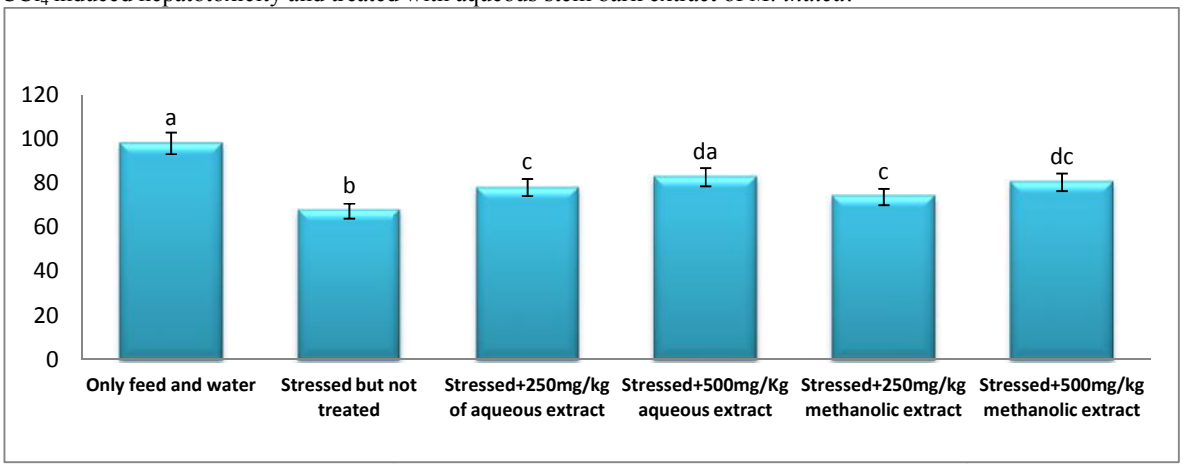


Fig. 7: Total protein levels (g/L) of *T. occidentalis* extract- treated-Wistar-rats with copper-induced oxidative stress.
***Columns bearing similar letters (a-d) are not significantly (p<0.05) different**
***Stressed implies received intraperitoneally, 0.3mg/kg body weight of copper sulphate once daily**

The restoration of total protein levels, to normal state after treatment with copper and extracts, proved to be concentration dependent. The total protein levels after treatment with 500mg/kg b.w aqueous extract were comparable to that of the control group (Fig. 7). The functional status of the liver is reflected by total protein (Pachathundikandi and Varghese, 2006) because the liver is enriched with machineries for the synthesis of serum proteins excluding γ-globulins, hence, liver damage is characterized by hypoproteinemia which can affect the whole physiological status of animals (Iweala and Osundiya, 2010; Kanchana and Mohammed, 2011). In this present study, a significant reduction in total protein observed in exclusively copper treated groups in relation to control group indicates hypoproteinemia, while the significant increase in the aqueous and methanol treated groups suggests increased protein synthesis. Serum total proteins have also being reported to have significantly decreased in Dimpylate intoxicated rats as compared to the group not treated with the oxidant (Alli and Atalla, 2014). On another note, Yousef *et al*., (2009) found that the plasma total protein significantly decreased after administration of Cisplatin but significantly increased in comparison with the control group after treatment with grape seed proanthocyanidin extract.

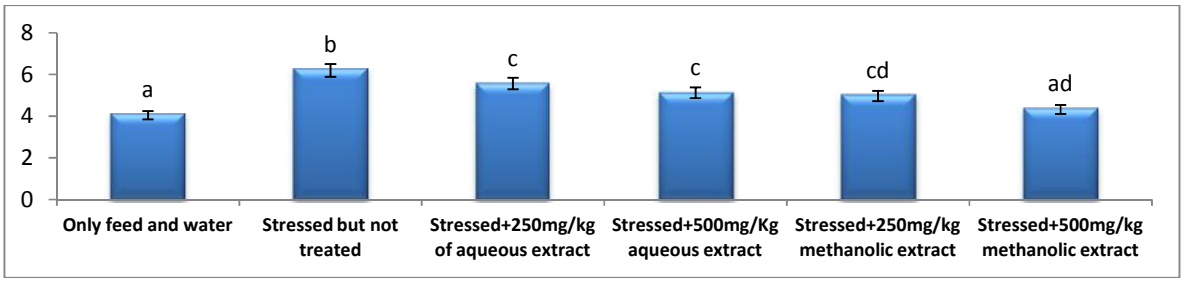


Fig. 8: Bilirubin levels (µmol/L) of *T. occidentalis* extract- treated Wistar rats with copper-induced oxidative stress.
***Columns bearing similar letters (a-d) are not significantly (p<0.05) different**
***Stressed implies received intraperitoneally, 0.3mg/kg body weight of copper sulphate once daily**

Figure 8 shows the bilirubin levels (Umol/L) of *T. occidentalis* extract- treated Wistar rats with copper-induced oxidative stress. Oxidative stress have been shown to increase the bilirubin levels, as indicated in Fig. 8 from the treatment group subjected only to intraperitoneal administration of copper. According to Sanjiv, (2002) the level of bilirubin, which is a major breakdown product of haemoglobin, increases on account of liver damage which manifests as skin discolouration. The reports of Anosike *et al*., (2002), in agreement with the findings of

this study, showed that oxidative stress induced by CCl_4 elevated the total bilirubin levels. A similar report has been found for ethanol induced oxidative stress on bilirubin levels (Sudha *et al*., 2012). The results of this present study show that the restoration of total bilirubin by methanol extracts of *T. occidentalis* occurred in a dose dependent manner up to 500mg/kg b.w. Anosike *et al*., (2012) using ethanol extract of *Pyrenacantha staudti* reported complete restoration of total bilirubin levels at 400mg/kg b.w. Amelioration of copper induced oxidative stress depletion of total bilirubin by 500mg/kg b.w methanol leaf extract of *T. occidentalis* further show its protective effect against copper induced liver toxicity. The extract may have exerted its hepatoprotective effects by enhancing bilirubin uptake by the liver and subsequent secretion into the bile ducts.

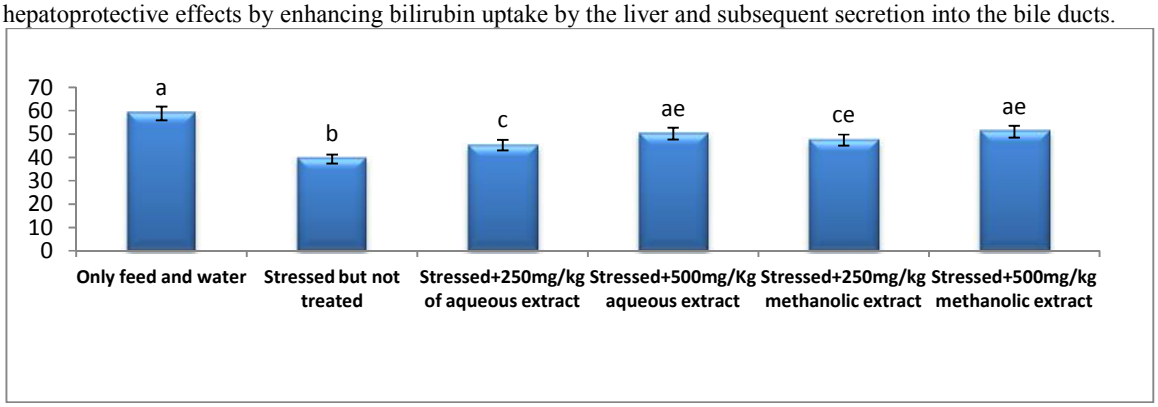


Fig. 9: Albumin levels (g/L) of *T. occidentalis* extract- treated Wistar rats with copper-induced oxidative stress.
***Columns bearing similar letters (a-e) are not significantly (p<0.05) different**
***Stressed implies received intraperitoneally, 0.3mg/kg body weight of copper sulphate once daily**

The results for the albumin levels (g/L) of *T. occidentalis* extract- treated Wistar rats with copper-induced oxidative stress are shown in Fig. 8. The significant reduction in albumin levels on treatment with copper is in line with other similar studies. Ojelabi *et al*., (2012) reported a derangement in albumin activity during cadmium mediated oxidative stress. Yakubu *et al.,* (2012) also reported a significant reduction in albumin levels on intubation of acetaminophen as an oxidant. In this present result, the LSD test carried out showed that only 500mg/kg b.w aqueous leaf extract and 500mg/kg b.w methanol extract completely restored the albumin activities. Similarly, Ebenyi *et al*., (2012) revealed that the extract of *A. sativum* produced a significant increase ($p < 0.05$) in albumin level in rats with paracetamol-induced oxidative stress, in a dose dependent manner. This was in consonance with the findings of this present study. Albumin is important in reducing the bioavailability and toxicity of many substances, by binding them. This binding potential of albumin to xenobiotics is reduced in occurrence of hypoalbuminaemia which causes the blockage of the binding sites by various metabolites. Although this hepatoprotective activity of albumin cannot be related to any specific phytochemical constituent of the extracts, some of the constituent phytochemicals like flavonoids and phenols are known to poses antioxidant properties.

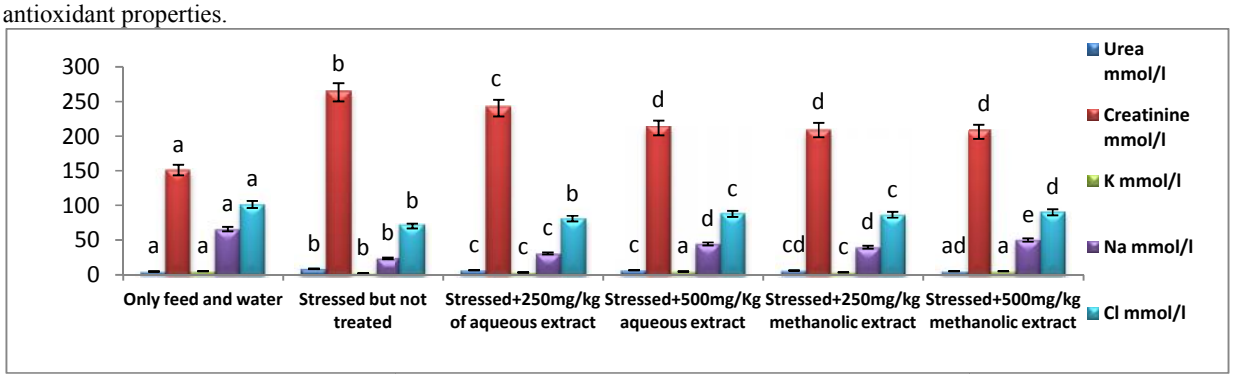


Fig. 10: Effect of *T. occidentalis* extracts on electrolyte markers (mmol/l) of Wistar rats with copper-induced oxidative stress.
***Columns bearing similar letters (a-e) separately for each marker, are not significantly (p<0.05) different**
***Stressed implies received intraperitoneally, 0.3mg/kg body weight of copper sulphate once daily**

The pre-treatment of *T. occidentalis* aqueous and methanol leaf extract on copper induced oxidative stress on electrolyte markers were shown in Fig. 10. The results showed a significant increase in urea levels from

4.73mmol/l to 8.77mmol/l on induction of oxidative stress. A similar oxidative stress induced increase was obtained for the creatinine levels on treatment with copper. Administration of both 250mg/kg b.w and 500mg/kg b.w aqueous leaf extract were unable to restore the activities of urea and creatinine, though caused a significant reduction in their levels when compared to the exclusively copper treated group, with only creatinine showing a dose dependent amelioration on aqueous leaf extract treatment. In contrast, according to Nwangwa, (2012) *Carica papaya* seed extracts were able to completely reverse the CCl_4 induced renal toxicity in rats monitored through their urea and creatinine levels. The results of treatment with methanol leaf extract on creatinine as shown in figure 10 of this present study indicated the inability of the concentrations used to completely restore the creatinine levels, but significantly reduced the serum urea levels comparable to the control group. Oxidative stress conversely reduced the serum potassium, sodium, and chloride levels as shown in Fig. 9. However, on administration of 500mg/kg b.w of both extracts, the potassium levels were completely restored, while none of the concentrations used in this present study were sufficient to completely restore the serum concentration of Na^+ and Cl^- after copper induced oxidative stress. Ali *et al*., (2015) also noted a significant alteration in selected serum electrolytes in the rats exposed to ethanol induced oxidative stress. The findings of this present study were in agreement with the report of Grover *et al.,* (2001) on the effect of *Piper guineense*, and *G. latifolium* during oxidative stress.

HISTOPATHOLOGY EXAMINATION OF KIDNEY

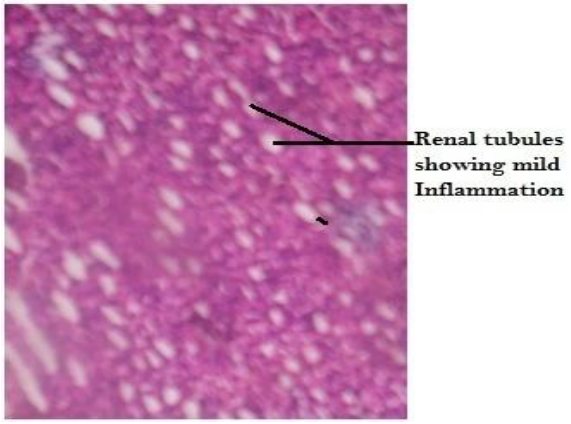


Plate A

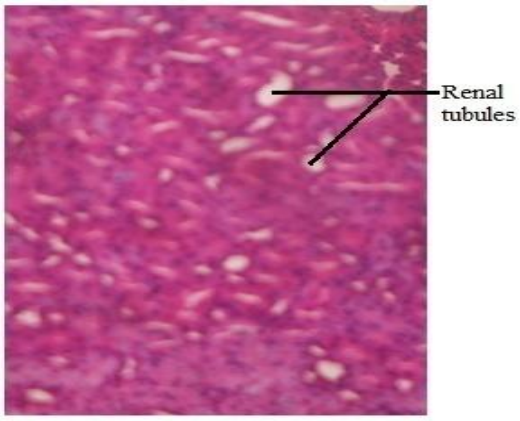


Plate B

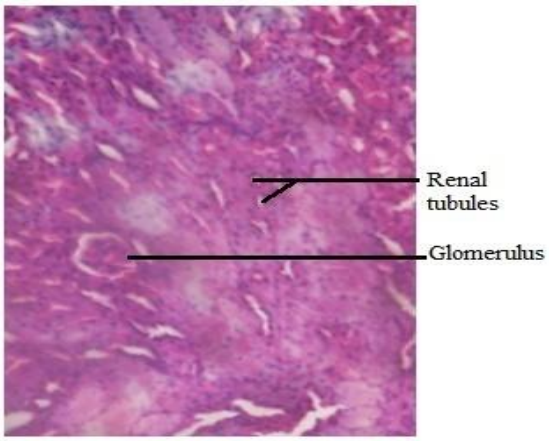


Plate C

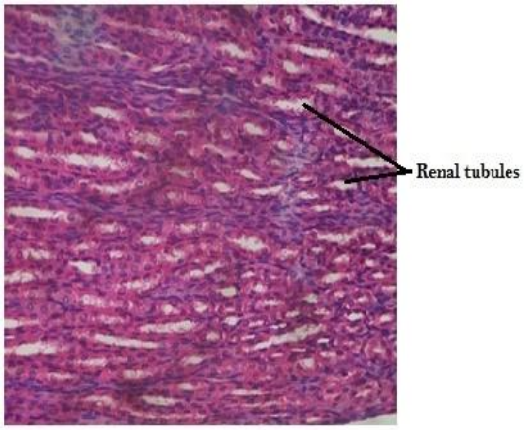
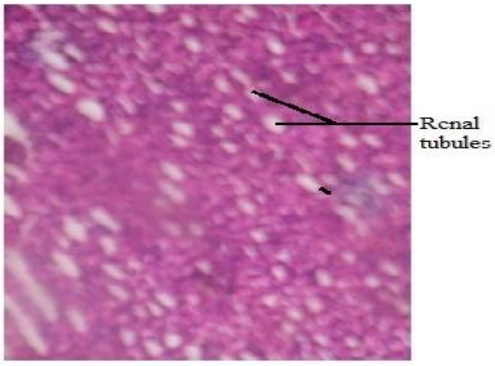


Plate D



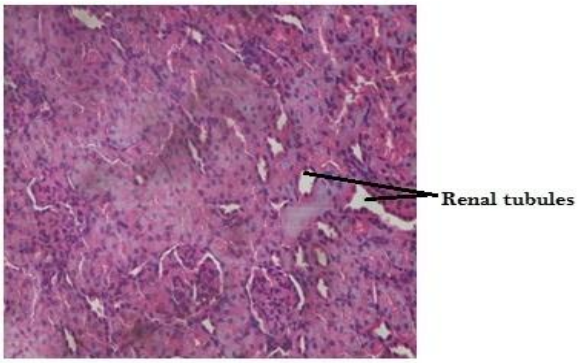
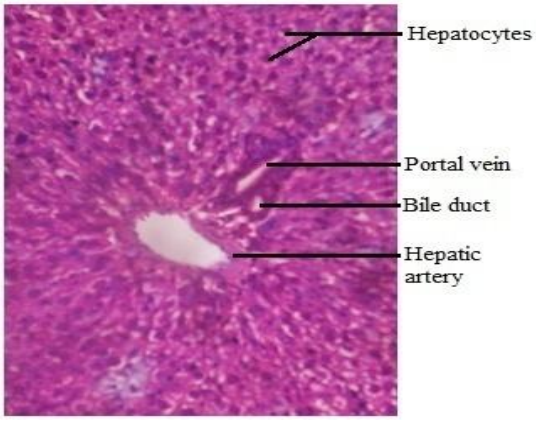


Plate E **Plate F**

The histological section of the kidney tissue of the rat in Plate A, shows normal renal tubules while the histological section of the kidney of the rat orally treated with 0.3mg/kg body weight of copper sulphate but not treated with the extracts, shows inflammation in renal tubules (Plate B). Histological section of the kidney of the rats induced with 0.3mg/kg body weight of Cu and treated with aqueous extract of 250mg/kg body weight showed ameliorations (Plate C) and increment to 500mg/kg body weight of *Telfairia occidentalis* showed normal renal tubules (Plate D). Treatment with methanolic extract of 250mg/kg body weight of *Telfairia occidentalis* coupled with copper (0.3mg/kg) showed normal renal tubules (Plate E) and an increase to 500mg/kg body weight of *Telfairia occidentalis* showed normal renal tubules (Plate F).

HISTOPATHOLOGY EXAMINATION OF LIVER



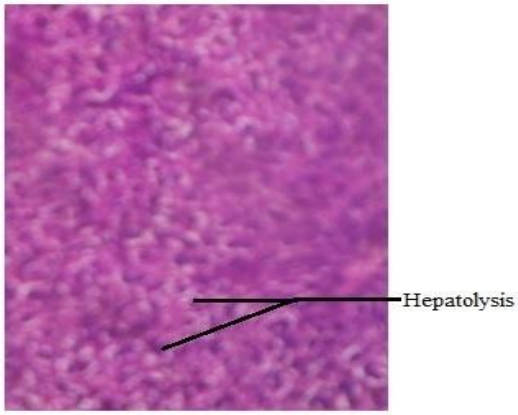


Plate A **Plate B**

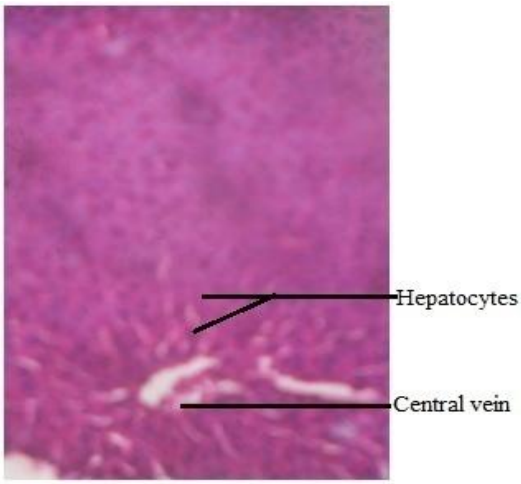


Plate C

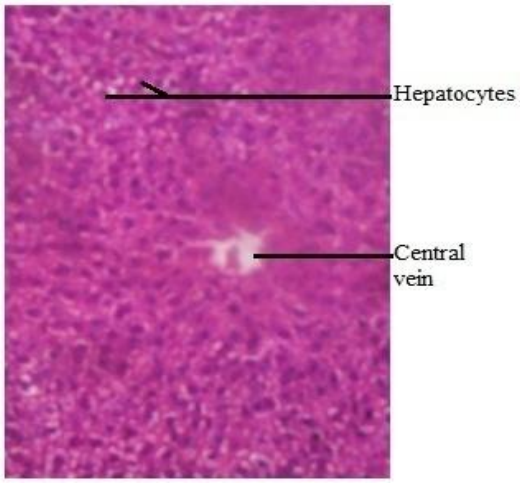


Plate D

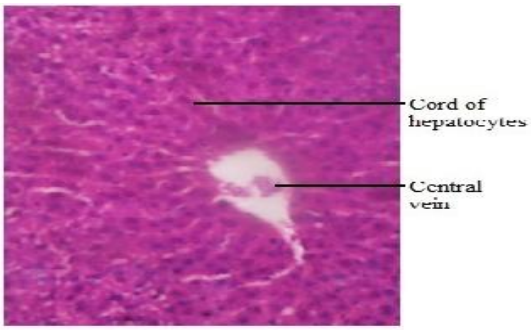


Plate E

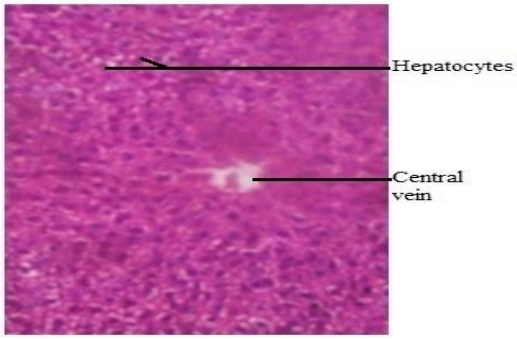


Plate F

Histological study of the liver tissue of the rat in the group 1 (Plate A) showed normal hepatocytes and portal vein and on administration of 0.3mg/kg body weight of copper sulphate without any extract treatments, showed destruction of liver cells as seen in Plate B. The administration of Cu (0.3mg/kg) body weight with 250mg aqueous *T. occidentalis* extract in Plate C on histological section of liver of the rats shows recovering liver tissues central vein and cords of hepatocytes. Histological section of the liver of the rats induced with Cu (0.3mg/kg) body weight and treated with aqueous extract of 500mg/kg body weight of *Telfairia occidentalis* shows normal liver tissues central vein and cords of hepatocytes (Plate D) while histological section of the liver of the rats induced with Cu(0.3mg/kg) body weight and treated with methanolic extract of 250mg/kg body weight of *Telfairia occidentalis* (Plate D) showed restoration of liver tissues central vein and cords of hepatocytes while histological section of the liver of the rats induced with Cu (0.3mg/kg) body weight and treated with methanolic extract of 500mg/kg body weight of *Telfairia occidentalis* (Plate E) showed restoration of liver tissues central vein and cords of hepatocytes.

Conclusion

The results obtained in this study indicated that the administration of copper, induced hepatotoxicity, renal toxicity, and histopathological abnormalities as shown in the levels of SOD, GSH, GPx, catalase and elevated levels of MDA. Elevated levels of AST, ALP, ALT and bilirubin, and reduced levels of albumin, and total proteins affirmed induced toxicity. Elevated levels of urea and creatinine and decrease in concentration of serum electrolytes indicated disruption in renal functionality on treatment with copper. Oral administration of 500mg/kg b.w methanol extract of *T. occidentalis* had the most restorative effect among other treatments on these biochemical parameters. This suggests that *T. occidentalis* could ameliorate the antioxidant status in copper-induced oxidative stress.

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