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Hepatoprotective Activity of Aqueous *Curcuma Longa* Rhizome Extract Against Carbon Tetrachloride-Induced Liver Toxicity in Adult Wistar Rats

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Abstract

The aim of the study was to assess the protective potentials of aqueous extract of Curcuma longa rhizome on carbon tetrachloride-induced liver toxicity in adult Wistar rats. 30 male adult Wistar rats weighing between 180 and 250 g were used in the study and were randomly assigned into 6 groups containing 5 rats each. The study lasted for eight weeks; Group 1 served as control and received no administration. Group 2 received 1 ml/kg body weight of carbon tetrachloride; group 3 received 200 mg/kg body weight of Curcuma longa extract; group 4 received 400 mg/kg body weight of Curcuma longa extract; group 5 received 1 ml/kg body weight of carbon tetrachloride and 200 mg/kg body weight of Curcuma longa; group 6 received 1 ml/kg body weight of carbon tetrachloride and 400 mg/kg body weight of Curcuma longa. Carbon tetrachloride group showed statistical significant increase in liver enzyme activities compared to with control. All treated groups showed statistical significant low liver enzyme activities compared with Carbon tetrachloride. Carbon tetrachloride group showed statistical significant alteration in the antioxidant activities of the liver compared with control group. In all the treated groups, antioxidant activities were normal compared with Carbon tetrachloride. H&E stain revealed carbon tetrachloride caused grade 2 steatosis. Groups 1, 3, 4, and 5 showed grade 0 steatosis. Groups 6 and 7 showed grade 1 steatosis. Masson's trichrome stain revealed carbon tetrachloride caused grade 3 fibrosis. Groups 1, 3, 4, and 5 showed Grade 0 fibrosis. Groups 6 and 7 showed grade 2 fibrosis. Conclusively, aqueous extract of Curcuma longa rhizome showed protective potentials against carbon tetrachloride – induced liver toxicity in adult Wistar rats.

Keywords: Hepato-toxicity; Turmeric; hepatoprotective; antioxidants; histomorphology.

INTRODUCTION

The liver is a major organ that is solely present in vertebrate animals and performs various important biological functions such as detoxification, and the synthesis of proteins and biochemicals essential for digestion and growth of the organism (Elias and Bengelsdorf, 1952; Abdel-Misih and Bloomston, 2010). It is the largest and heaviest gland in the body and, after the skin, the largest and heaviest single organ (Moore, 2013).

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Hepatotoxicity occurs when the activities of drugs, chemicals and other toxins cause significant damage/insults to the liver which can lead to cirrhosis, fibrosis and even liver cancers when left unattended (Gulati *et al*., 2018). Carbon tetrachloride ($CCl_{4)}$ induces hepatotoxicity by free radical formation of depending on the partial pressure of oxygen. CCl_3^* and CCl_2^* radicals form in low partial pressure that leads to covalent metabolite binding, which mostly affects the metabolism of lipids (decreased transport out of the hepatocyte, increased synthesis) and ultimately causes steatosis or fatty liver (Scholten *et al*., 2015). In contrast, CCl_3-OO^* radical forms in high oxygen partial pressure with consequent lipid peroxidation, that push the cell from steatosis to apoptosis and in hepatocytes, protein synthesis is also suppressed by CCl_4, which leads to loss of structural and functional integrity of cells (Unsal *et al*., 2021).

Curcuma longa, which belongs to Zingiberaceae family, grown in tropical and subtropical regions throughout the world, is a perennial, erect and leafy plant with very large, lily like leave that grows up to 1.2 m long (Ansari *et al*., 2020). It has been called one of the most useful herbal medicinal plants. Extensive researches have proven that most activities of the *Curcuma longa* are due to its principal constituent, a curcuminoid called curcumin (Fuloria *et al*., 2022). It possesses various important properties with antioxidant activities and is useful in conditions such as inflammation, ulcer and cancer. It has been confirmed to possess antifungal, antimicrobial, nephroprotective and hepatoprotective activities (Ansari *et al*., 2020). Also, it exerts protective and in some cases curative potential against conditions such as diabetes, allergies, arthritis, Alzheimer's disease, various cancer, and other chronic and hard curable diseases (Nasri *et al*., 2014). Salama *et al.* (2013) had demonstrated hepatoprotective effect of ethanolic extract of *Curcuma longa* on thioacetamide induced liver cirrhosis in rats. The antioxidant potential of *Curcuma longa* had been demonstrated by Reddy and Lokesh (1994) where they showed that dietary turmeric lowers iron-induced lipid peroxidation in the rat liver by enhancing the activities of antioxidant enzymes. Studies have demonstrated that curcumin modulates NF-kβ activity (Singh and Aggarwal, 1995). Furthermore, the scavenging curcumin to ability on a variety of ROX (Maheswari *et al*., 2006) makes this compound a suitable tool to be studied in CCl4-induced liver damage. Despite several studies in the hepatoprotective effects of *Curcuma longa,* there is still paucity of data on the protective potential of its aqueous rhizome extract against CCl_4-induced liver fibrosis by histologically demonstrating the amount of collagen in liver tissues. This study therefore investigated the protective potentials of aqueous extract of *Curcuma longa* rhizome on Carbon tetrachloride – induced liver toxicity in adult Wistar rats.

MATERIALS AND METHODS

Collection and preparation of plant extract

Fresh rhizomes of *Curcuma longa* were purchased from local traders from Edaiken market in Egor Local Government Area of Benin City, Edo State, Nigeria. They were identified at the Department of Plant Biology and Biotechnology, Faculty of Life Science University of Benin, Benin City, Edo State, Nigeria and Herbarium number (UBH-C397) was assigned. The rhizomes were then washed, dried, pulverized and stored in cool dry place. The pulverized extract was then macerated in water for about 48 hours at room temperature and filtered with Watman filter paper. The filtrate was lyophilized at Natural Product Research Lab, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria, following the method described by Kumar (2019).

Experimental animals
Thirty-five (35) adult male Wistar rats weighing between 180 and 250 g were purchased kept, and maintained in standard laboratory condition at room temperature (35^{o} C), humidity and under twelve-hour dark-light cycle at the Department of Anatomy Animal Holding Facility, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. The animals were fed on standard feed Grower mash diet (Vital feeds Nigeria Ltd) and clean tap water *ad libitum*.

Experimental design
Thirty (30) adult male Wistar rats weighing between 180 and 250 g were randomly assigned into six (6) groups comprising five (5) Wistar rats each. Group 1 served as control in the experiment receiving no administration. Group 2 received 1 ml/kg body weight of CCl_4 mixed in olive oil (50/50 %) three times weekly for eight (8) weeks. Group 3 received 200 mg/kg body weight of *Curcuma longa* once daily for eight (8) weeks. Group 4 received 400 mg/kg body weight of *Curcuma longa* once daily for eight (8) weeks. Group 5 received 1 ml/kg body weight of CCl_4 three times weekly and 200 mg/kg body weight of *Curcuma longa* once daily for eight (8) weeks. Group 6 received 1 ml/kg body weight of CCl_4 three times weekly and 400 mg/kg body weight of *Curcuma longa* once daily for eight weeks.

Animal sacrifice and collection of samples
After eight (8) weeks of administration, the animals were sacrificed under chloroform anesthesia and a midline incision was made on the anterior abdominal wall. Blood samples were collected through the abdominal aorta into plain sample tubes for liver biochemical and antioxidant investigations. The blood samples for serum biochemical assays were centrifuged at 3000 g for 10 min to obtain serum, which was later used for the estimation of biochemical parameters. The liver tissue was also collected for histopathological examinations.

Biochemical parameters
Liver function parameters Alanine transaminase (ALT) and aspartate aminotransferase (AST) activities were measured by Reitman and Frankel (1957) method while serum alkaline phosphatase (ALP) was assayed by the method of Englehardt (1970). Serum total and direct bilirubin (T. Bil. and D. Bil.) levels were assayed by Jendrassik and Grof (1938) method. All parameters were assayed using commercially available kits.

Antioxidant enzymes
Superoxide dismutase activity was determined using the method of Misra and Fridovich (1972). Catalase activity was determined in the serum by the method of Cohen (1970). Reduced glutathione (GSH) activity was determined using the method described by Ellman (1959). Malondialdehyde (MDA) level in the serum was determined according to the method of Buege and Aust (1978).

Histological analysis
The Liver was excised, fixed in 10% formal saline solution and processed via routine histological procedure. The tissues were dehydrated by passing them through ascending grade of alcohol (70%, 90% and 100%) and processed following standard procedure as recommended by Drury and Wallington 1980. Hematoxylin and Eosin staining (Drury and Wallington, 1980) was used to evaluate hepatic steatosis in the liver. Masson's trichrome staining described by Sheehan and Hrapchak (1980) was used to evaluate fibrosis in the liver. A pathologist blindly classified the grade of steatosis according to Kleiner and Brunt (2012) and grade of fibrosis according to Batts and Ludwig (1995).

Statistical analysis

The data were analyzed using IBM statistical Package for Social Sciences, Version 23 (manufactured by International Business Corporations {IBM}; released in 2015). Results were presented as (mean ± SEM). The parameters for all groups were compared using analyses of variance (ANOVA). *Post hoc* analysis was done using Least Square Difference (LSD). Differences in means were considered significant at 95% confidence level (that is when probability was less than 0.05 {P < 005}).

RESULTS

Table 1 shows the result of the liver function test of the treated groups. From the table, there was a statistically significant elevation ($p < 0.05$) of aspartate aminotransferase, alanine transaminase, alkaline phosphate, direct bilirubin and total bilirubin in the CCl_4 treated group compared with the control group. Furthermore, while there were no statistical significant difference ($p > 0.05$) in the liver function parameters between other experimental groups and the control group, the liver function parameters were significantly reduced ($p < 0.05$) in *Curcuma longa* treated groups compared with the CCl_4 treated group.

Table 1: Effect of CCl_4.and *Curcuma longa* on Serum liver function biomarkers

GROUP	AST (U/L)	ALT (U/L)	ALP (U/L)	D. BIL. (µmol/L)	T. BIL. (µmol/L)
1	11.52+0.20^a	33.23+3.59^a	50.56+1.92^a	124.75+0.58^a	33.15+0.83^a
2	17.33+1.13^b	69.65+8.19^b	71.67+0.01^b	152.25+1.73^b	55.18+0.10^b
3	10.58+0.46^a	34.13+7.39^a	52.49+5.83^a	128.58+2.38^a	31.26+7.21^a
4	13.28+0.02^a	34.93+2.11^a	55.53+0.32^a	129.03+0.20^a	31.72+1.74^a
5	12.51+2.79^a	39.51+4.82^a	53.00+4.04^a	125.00+3.70^a	41.22+0.76^a
6	12.02+0.42^a	32.07+0.61^a	48.61+2.15^a	127.13+1.45^a	34.80+5.00^a

* Groups with the same superscript are significant while groups with unlike superscript are not significant

Table 2 shows the result of the liver oxidative stress parameters. From the table, there was a significant elevation ($p < 0.05$) in malondialdehyde and significant reduction in superoxide dismutase, catalase, and reduced glutathione activities in the CCl_4 treated group compared with the control group. Furthermore, while there were no statistically significant difference ($p > 0.05$) in malondialdehyde, superoxide dismutase, catalase and reduced glutathione activities between the experimental groups and the control group, the malondialdehyde activity was significantly reduced ($p < 0.05$) while superoxide dismutase, catalase and reduced glutathione activities were significantly elevated ($p < 0.05$) in all the *Curcuma longa* treated groups compared with the CCl_4 treated group.

Table 2: Effect of CCl_4.and *Curcuma longa* on liver oxidative stress parameters

GROUP	MDA (µmol/L)	SOD (U/ml)	CAT (MU/1)	GSH (mM)
1	354.22+22.17^a	3.07+0.49^a	0.37+0.01^a	0.68+0.05^a
2	531.01+5.96^b	1.18+0.29^b	0.01+0.01^b	0.37+0.04^b
3	333.57+3.88^a	3.58+0.46^a	0.40+0.01^a	0.77+0.06^a
4	320.86+2.48^a	3.28+0.20^a	0.38+0.01^a	0.81+0.11^a
5	332.52+7.65^a	2.81+0.05^a	0.34+0.01^a	0.59+0.07^a
6	324.82+4.88^a	2.93+0.28^a	0.33+0.03^a	0.61+0.04^a

* Groups with the same superscript are significant while groups with unlike superscript are not significant.

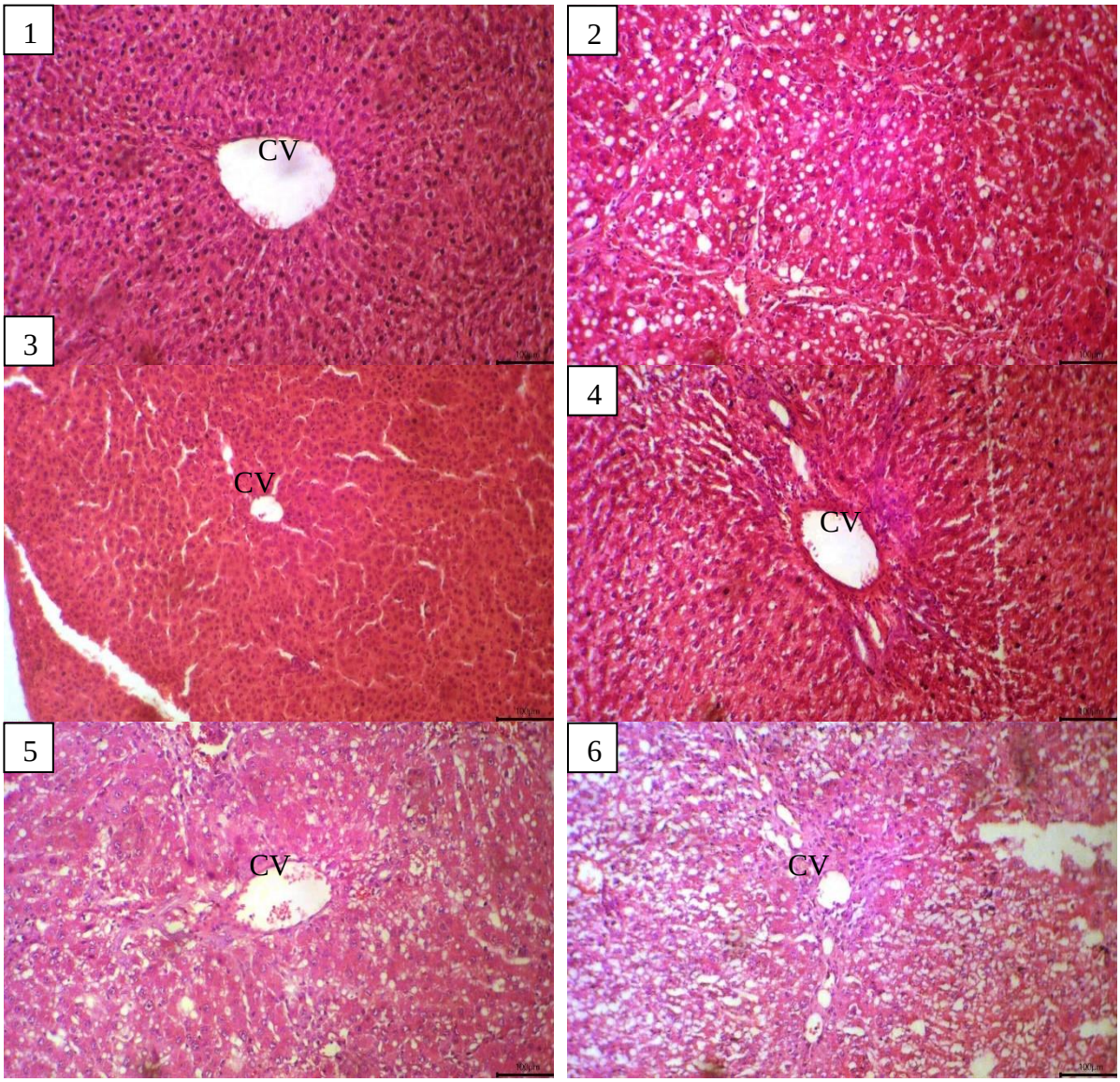

Plate A: representative photomicrographs of the experimental animals: 1 (control), 2 (CCl_4 only), 3 (200 mg/kg body weight of *Curcuma longa* only), 4 (400 mg/kg body weight of *Curcuma longa* only), 5 (CCl_4 + 200 mg/kg body weight of *Curcuma longa*), 6 (CCl_4 + 400 mg/kg body weight of *Curcuma longa*)). Groups 1, 3 and 4 no evidence of steatosis. Group 2 had grade 2 (grade 2/3: 34-66% of hepatocytes) microvesicular and macrovesicular steatosis in zone 3 in all rats. Groups 5 and 6 had grade 1 steatosis (grade 1/3: 5-33% of hepatocytes) in all animals; CV = central vein (H&E; Objective Lens: 10X; Scale bar = 100 µm)

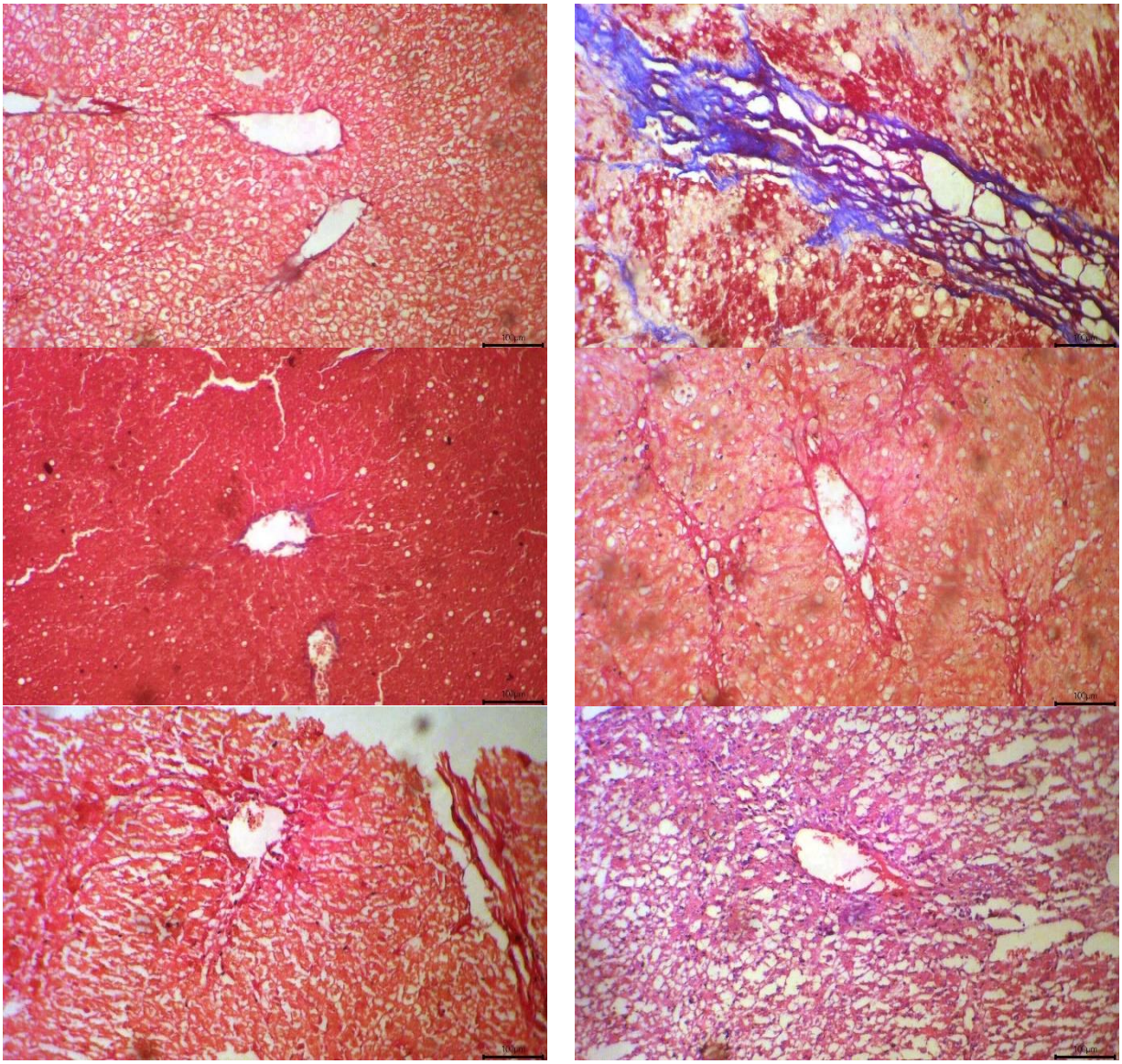

Plate B: representative photomicrographs of the experimental animals: 1 (control), 2 (CCl_4 only), 3 (200 mg/kg body weight of *Curcuma longa* only), 4 (400 mg/kg body weight of *Curcuma longa* only), 5 (CCl_4 + 200 mg/kg body weight of *Curcuma longa*), 6 (CCl_4 + 400 mg/kg body weight of *Curcuma longa*). Control group shows no evidence of collagen deposition around the central vein. CCl_4-treated group had grade III fibrosis (Batts and Ludwig, 1995). Other groups (3-6) show normal histological features similar to the control group with no evidence of collagen deposition around the central vein (Masson trichrome; Objective Lens: 10X; Scale bar = 100 µm).

DISCUSSION

The liver is a vital organ in the body that is responsible for detoxifying harmful substances (Mescher, 2018). Liver injury occurs due to activation of these harmful substances (xenobiotics and drugs) to chemically active metabolites (Unsal *et al.,* 2021).

CCl_4 is a chemical toxicant known to cause liver toxicity by causing intracellular and intramembranous lipid destruction as a result of enhanced lipid peroxidation, and increased membrane permeability due to its break-down products such as reactive aldehyde (Srinivasan *et al*., 2005; Unsal *et al.,* 2021).

Natural antioxidants that possess the abilities to eliminate free radicals and protect the liver from oxidative stress have been demonstrated to be in many food and plants including vegetables and medicinal plants (Li *et al*., 2015). Unsal *et al*., (2020) reported that natural antioxidants reduce the risk of CCl_4-induced liver injury, and chemical hepatitis. Unsal *et al*.,

(2021). Furthermore, Unsal *et al*., (2021) reported that antioxidants neutralize the harmful effects of free radicals induced by CCl_4 on liver cells and modulate biochemical changes in liver tissue.
Curcuma longa, which belongs to Zingiberaceae family, is one of such plants that possess important antioxidants useful as a medicinal plant (Fuloria *et al*., 2022). It has been demonstrated to possess anti-diabetic (Hajavi *et al*., 2017), cardiovasculo-protective (Wang *et al*., 2018; Gao *et al*., 2019; Li *et al*., 2020), antioxidant (Fuloria *et al*., 2022), and other therapeutic properties.

In this study, the histology of the control group showed normal liver architecture and no collagen deposition. The histological findings showed that there were macrovesicular and microvesicular steatosis on H & E evaluation and grade III fibrosis (Batts and Ludwig, 1995) with Masson's trichrome evaluation in Group 2 treated with 1 ml/kg body weight of CCl_4 in olive oil (50/50 %). These findings are in accordance with the reports of Scholten *et al*., (2015) that CCl_4 causes steatosis and Boll *et al*., (2001) that CCl_4 also causes fibrosis. On treatment with CCl_4 + *Curcuma longa* 200 mg/kg body weight and CCl_4 + *Curcuma longa* 400 mg/kg body weight, sections began to show better architecture and reduced collagen deposition when compared with CCl_4 treated group. These findings are in line with the report of *Curcuma longa* hepatoprotection by Fuloria *et al*., (2022)

The increase in the activities of liver enzymes seen in this study may be due to the increased hepatocyte membrane permeability cause by CCl_4 intoxication (Lawal *et al*., 2015; Unsal *et al*., 2021). This agrees with various studies that have shown that CCl_4 intoxication causes liver enzymes to leak through hepatocyte membrane into extracellular spaces (Lawal *et al*., 2015; Ibrahim *et al*., 2020; Unsal *et al*., 2021). The decrease in the activities of liver enzymes in CCl_4 + *Curcuma longa* 200 mg/kg body weight and CCl_4 + *Curcuma longa* 400 mg/kg body weight treated groups agrees with the findings of Ibrahim *et al*., (2020) that *Curcuma longa* exerts protection on the structural integrity of hepatocellular membranes.

The accumulation of bilirubin in the circulation can be attributed to the liver's inability to conjugate bilirubin with glucuronide resulting in unconjugated bilirubin accumulation in the blood which reflects liver damage (Usunobun *et al*., 2020; Mostafa *et al*., 2022). In this study, the hepatoprotective abilities of *Curcuma longa* is seen in the significant decrease of the activities of direct and total bilirubin in CCl_4 + *Curcuma longa* 200 mg/kg body weight and CCl_4 + *Curcuma longa* 400 mg/kg body weight treated groups when compared with CCl_4 group as is in line with Nasri *et al*., (2014) and Fuloria *et al*., (2022).

In accordance with the findings of Nasri *et al*., (2014) and Alkinani *et al*., (2021), in this study, *Curcuma longa* exerted hepatoprotection on the antioxidant system of the liver seen in CCl_4 + *Curcuma longa* 200 mg/kg body weight and CCl_4 + *Curcuma longa* 400 mg/kg body weight treated groups when compared with CCl_4 group can be attributed to the significant abundance of flavanoid present in the extract (Umar *et al*., 2019; Aye *et al*., 2019).

CONCLUSION

The aqueous extract of *Curcuma longa* rhizome exerted protective potentials against CCl_4 – induced liver toxicity in adult Wistar rats through antioxidant and hepatoprotective activities. More studies can be done with histochemical staining to confirm the intensity of carbon tetrachloride – induced liver toxicity and the protective potentials of aqueous extract of *Curcuma longa* rhizome in adult Wistar rats.

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