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Antioxidant and hepatoprotective potentials of curcuminoid isolates from turmeric (*Curcuma longa*) rhizome on CCl_4-induced hepatic damage in Wistar rats

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ABSTRACT

The present study examined the protective effects of curcuminoid isolates from *Curcuma longa* against carbon tetrachloride (CCL_4)-induced hepatic injury in rats. The hepatoprotective effect of the crude extract (150, 300 and 600 mg/kg bw) and curcuminoids (75, 150 and 300 mg bw) was evident by significant increases in the serum antioxidative defence capacities (super oxide dismutase, reduced glutathione, catalase) and reduction in biomarker enzymes of liver integrity (aspartate transaminase, alanine transaminase and alkaline phosphatase) in comparison to the results obtained in the CCL_4-untreated animals. Some of these parameters were completely restored by pre-treatments with curcuminoids. Similarly, the curcuminoids increases the concentrations of total proteins, albumins and ameliorated histological changes observed in CCL_4 injured rats. Therefore, curcuminoid could be considered a novel candidate for the development of new drug against liver diseases.

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KEYWORDS
Antioxidants; hepatoprotective; *Curcuma longa*; curcuminoids; carbon tetrachloride

1. Introduction

Liver diseases are significant worldwide issues, accounting for the deaths of hundreds of thousands of patients each year [1]. More than 10% of the global population suffers from liver diseases and its mortal end-stage generally follows cirrhosis and liver cancer [2]. The continual environmental exposure to toxicants, dietary xenobiotics and the body's complex biochemical reactions result in the production of free radicals, such as reactive nitrogen species (RNS) and reactive oxygen species, (ROS) under different pathophysiological conditions [3]. The over-generation of these oxygen or/and nitrogen-derived reactive species coupled with antioxidant deficiency leads to oxidative stress which has been implicated in a multitude of organ dysfunctions [4]. However, the major cause of hepatic dysfunction is drug-induced oxidative stress and liver toxicity [5].

Carbon tetrachloride (CCl_4) is a well-known hepatotoxic drug commonly used for acute or chronic liver damage in a wide variety of laboratory animals [5–6–7]. The best-characterized form of xenobiotics-induced free radical-mediated liver disease is the liver damage caused in rats by CCl_4 [8]. CCl_4-induced liver damage through the generation of ROS causes oxidative stress and consequently cellular damage [9]. The potency or viability of any hepatoprotective drug is, therefore, related to its ability to either reduce the generation of free radicals, harmful effects or maintain the normal hepatic physiological mechanism [10].

Although there is a notable development in modern medicine, hepatic disease remains a global health problem, thus the search for new drugs is still ongoing [11]. So far, no effective treatments in conventional or synthetic medicine give protection to the liver against damage or help to regenerate hepatic cells [12]. Some synthetic antioxidants, such as butylated hydroxy-toluene (BHT), butylated hydroxy-anisole (BHA) and tertiary butyl hydroquinone (TBHQ), have been documented to produce toxins or act as carcinogens. Because of this fact efforts are being made to find suitable curative agents in natural products for the treatment of liver diseases.

Plant-derived medicines have played a key role in both ancient and contemporary healthcare of many cultures. As a natural defence mechanism against disease and infection, thousands of secondary metabolites are produced by higher plants [13]. These products have pharmacological or biological activity which can be utilized in the discovery and design of pharmaceutical drugs. Of at least tens of thousands of globally introduced small-molecule drugs, the origins of most can be traced to natural goods [14]. Antioxidants

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extracted from medicinal plants are a logical therapeutic approach for treating liver diseases that are safer, cheaper and more efficient for the population impacted [15].

Turmeric (*Curcuma longa*) is a rhizomatous perennial herb (family; Zingiberaceae) commonly used as food spices, body cleanser and as medicine for treatments of several disorders including anorexia, cough, sinusitis, asthma, anthelmintic, gonorrhoea, renal and hepatic diseases [16]. Curcuminoid is a major bioactive phenolic compound derived from *C. longa*. It is composed of curcumin (1,7-bis(4-hydroxy-3-methoxypheny1)-l,6-heptadiene-3,5-dione) and its derivatives bis-demethoxy-curcumin (BDMC) and dimethoxy-curcumin (DMC), which have been widely reported for anticancer, anti-inflammatory, antioxidant, antimutagenic, wound healing, gastroprotective and antimicrobial activities [17–24]. The curcumin is classified as generally recognized as safe (GRAS) by the US Food and Drug Administration [16]. The present study, therefore, aims to evaluate its antioxidant and hepatoprotective potentials in CCl_4-induced hepatic damage.

2. Materials and methods

2.1. Plant materials

C. longa rhizomes were obtained from the Kure market in Minna, Nigeria. The rhizomes were washed, dried at room temperature and were blended with an electric blender. The powder was sealed and stored in polyethene bag until required for use.

2.2. Experimental animals

Adult rats (48–168 g) were obtained from ABU, Zaria, Nigeria. The animals were comfortably housed under normal environmental conditions and had free access to commercial pellets of feed and water.

2.3. Crude extract and curcuminoid isolation

Crude extract and curcuminoids were extracted according to the method of Nabati et al. [25]. A 100 g pulverized turmeric rhizome was extracted with 500 mL of methanol using a Soxhlet extractor. Curcuminoids from the pulverized turmeric rhizomes were isolated first, extracting oleoresin using acetone. Curcuminoids were then precipitated using petroleum aether and filtered with Whiteman filter paper.

2.4. Phytochemical and acute toxicity screening

Turmeric rhizome crude extract was screened for quantitative phytochemicals using standard procedures [26,27], while acute toxicity was conducted in accordance with Lorke's method [28].

2.5. Experimental design for hepatoprotective study

A total of 45 rats were allotted to nine (I–IX) groups ($n = 5$). Groups I–III were administered crude extract at doses of 150, 300 and 600 mg/kg bw, groups IV–VI were administered curcuminoids at 75, 150 and 300 mg/kg bw, respectively. Groups VII and VII received 100 mg/kg bw silymarin and normal saline, respectively, while group IX serves as the control group. Treatments were given for 168 h at an interval of 24 h (10:00 am). CCl_4 (2 ml/kg bw) was given once 6 h after the 7th extract/curcuminoid treatment regime and the animals were sacrificed after 24 h [29]. Collection and processing of samples (blood and liver) for histological and biochemical studies was carried out, as described by Shittu et al. [30].

2.6. Determination of biochemical parameters

The method of Misra and Fridovich [31] was used to determine SOD activity. The method of Sinha [32] was used to determine the catalase (CAT) activity. The activity of Glutathione peroxidase (GPx) in serum was determined by using the method of Beutler et al. [33]. The activities or concentrations of aspartate transaminase (AST), alanine transaminase [34], alkaline phosphatase [35], total proteins [36] and albumin [37] were determined by standard methods.

2.7. Data analysis

Data were analysed using SPSS. Means were compared and differences were separated using ANOVA ($P < 0.05$) and Duncan's Multiple Range Test.

3. Results

3.1. Phytochemical and acute toxicity of crude extract of C. longa

Saponin (1742.63 ± 1.94 mg/100 g) was the most abundant phytochemical composition of *C. longa*, while tannin (7.01 ± 0.23 mg/100 g) was the least abundant one (Table 1). The turmeric extract had a safe dose and LD_{50} of 1000 mg/kg bw and > 5000 mg/kg bw, respectively.

3.2. Biomarker enzymes

The activities of AST (Figure 1), ALT (Figure 2) and ALP (Figure 3) were higher ($p < 0.05$) in the serum of

Table 1. Phytochemical compositions of *C. longa*.

Parameters	Compositions (mg/100 g)
Phenols	213.41 ± 1.36^{c}
Flavonoids	97.24 ± 0.64^{b}
Tannins	7.01 ± 0.23^{a}
Alkaloids	97.24 ± 0.64^{b}
Saponins	1742.63 ± 1.94^{d}

Note: Values are mean ± SEM ($n = 3$).

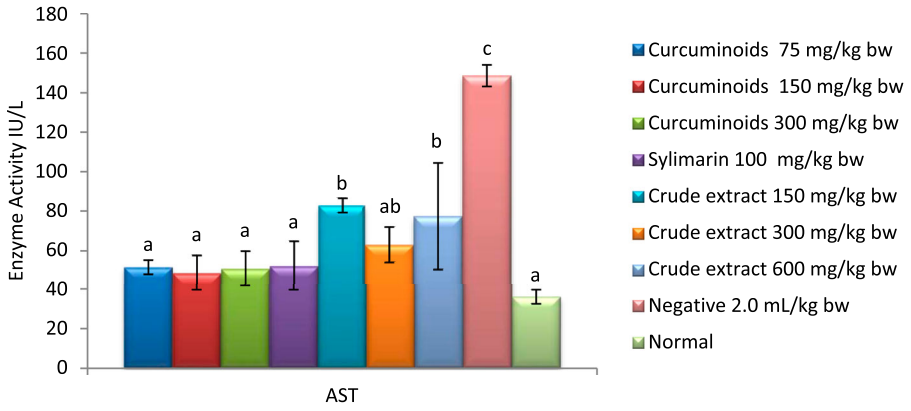

Figure 1. Effect of *C. longa* and Curcuminoids on serum AST in CCl_4-induced hepatotoxic rats. Bar denotes Mean ± SEM ($n = 5$). Statistical differences ($p < 0.05$) are denoted by the different alphabetical superscripts.

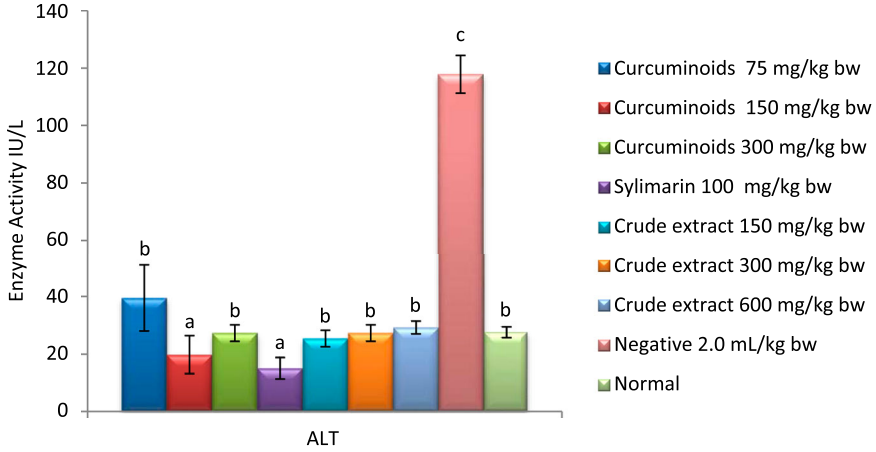

Figure 2. Effect of *C. longa* and Curcuminoids on serum ALT in CCl_4-induced hepatotoxic rats. Bar denotes Mean ± SEM ($n = 5$). Statistical differences ($p < 0.05$) are denoted by the different alphabetical superscripts.

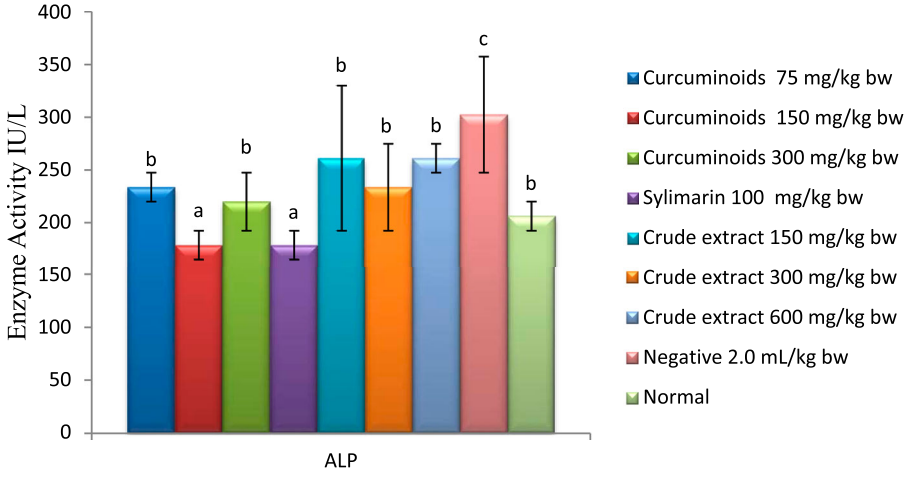

Figure 3. Effect of *C. longa* and Curcuminoids on serum ALP in CCl_4-induced hepatotoxic rats. Bar denotes Mean ± SEM ($n = 5$). Statistical differences ($p < 0.05$) are denoted by the different alphabetical superscripts.

CCl_4 hepatotoxic rats relative to the control group. Pretreatment with the crude extract and curcuminoids significantly reduces the serum enzyme activities relative to the untreated group. Similarly, the curcuminoids and silymarin treatments significantly restored the activities of AST (Figure 1) to the normal levels. Crude extract (300 mg/kg bw) and curcuminoids increase the serum total proteins (Figure 4) and albumin (Figure 5) concentrations when compared with the control and untreated group ($p < 0.05$).

3.3. Antioxidant enzymes

The activities of SOD (Figure 6) and GPx (Figure 7) were significantly ($p < 0.05$) lower in the serum of CCl_4-mediated hepatotoxic rats relative to the control

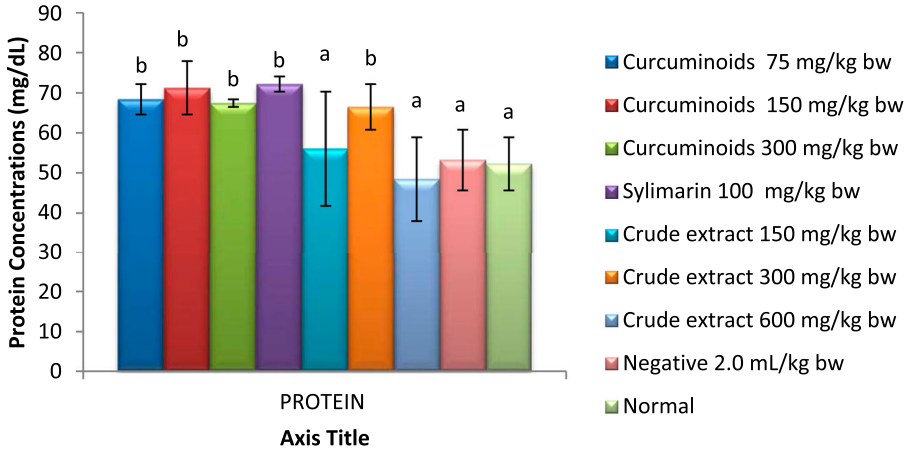

Figure 4. Effect of *C. longa* and Curcuminoids on serum proteins in CCl_4-induced hepatotoxic rats. Bar denotes Mean $\pm$ SEM ($n = 5$). Statistical differences ($p < 0.05$) are denoted by the different alphabetical superscripts.

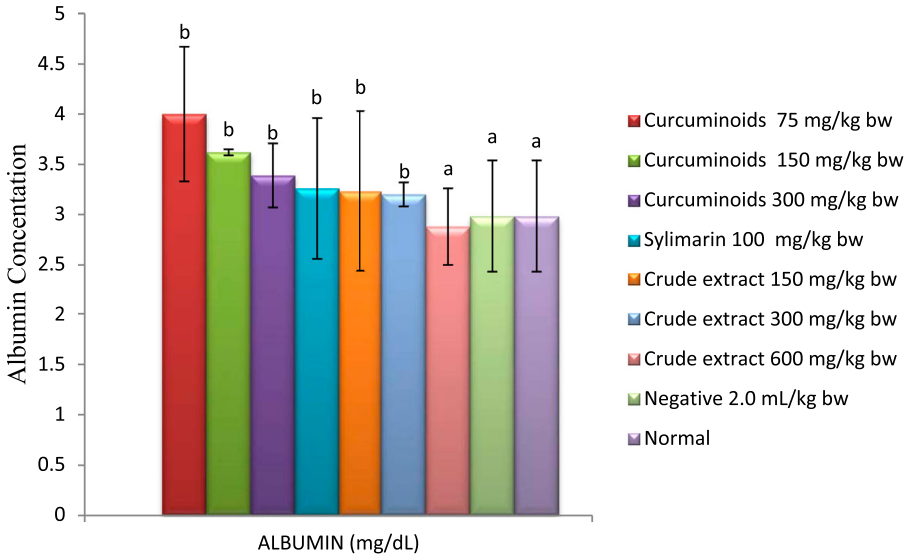

Figure 5. Effect of *C. longa* and Curcuminoids on serum albumin in CCl_4-induced hepatotoxic rats. Bar denotes Mean $\pm$ SEM ($n = 5$). Statistical differences ($p < 0.05$) are denoted by the different alphabetical superscripts.

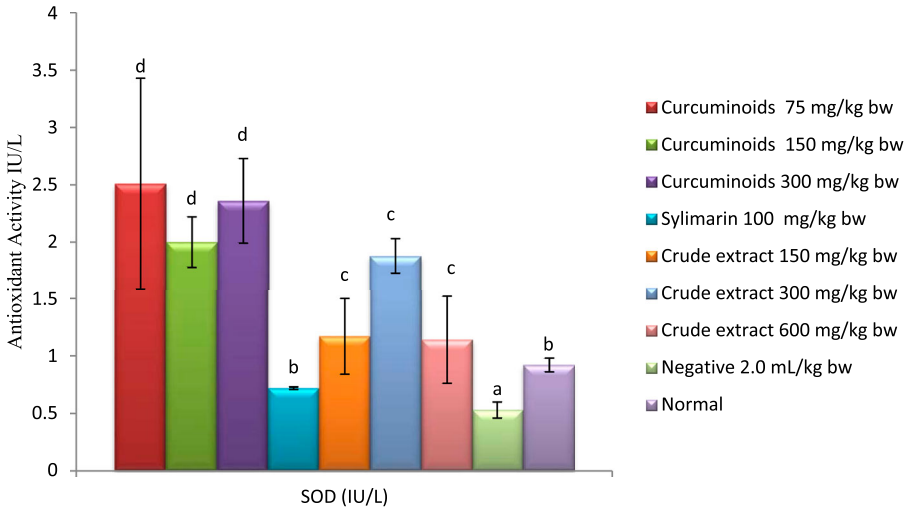

Figure 6. Effect of *C. longa* and Curcuminoids on serum SOD in CCl_4-induced hepatotoxic rats. Bar denotes Mean $\pm$ SEM ($n = 5$). Statistical differences ($p < 0.05$) are denoted by the different alphabetical superscripts.

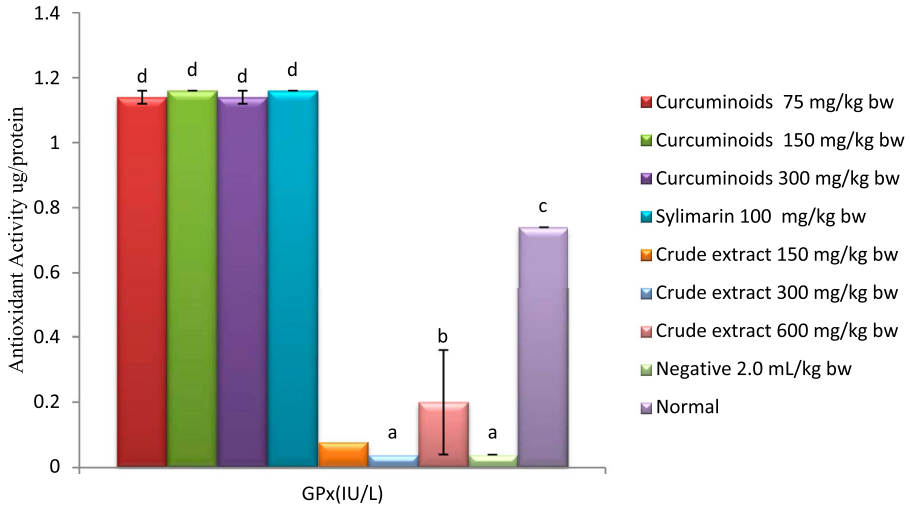

Figure 7. Effect of *C. longa* and Curcuminoids on serum GPx in CCl_4-induced hepatotoxic rats. Bar denotes Mean ± SEM ($n = 5$). Statistical differences ($p < 0.05$) are denoted by the different alphabetical superscripts.

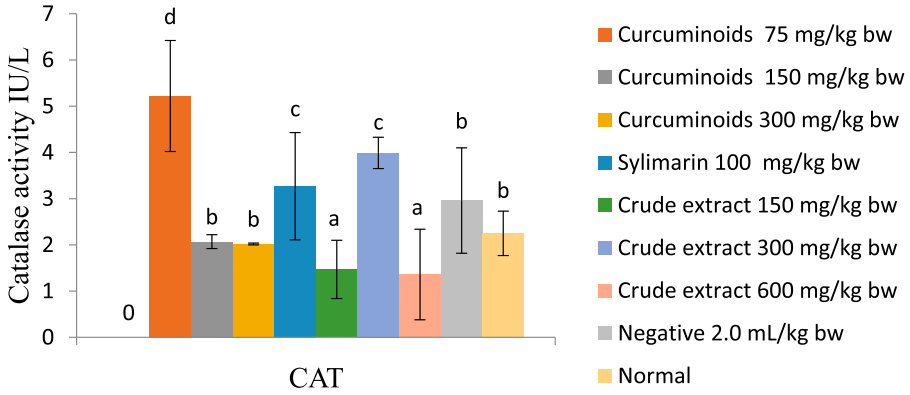

Figure 8. Effect of *C. longa* and Curcuminoids on serum CAT in CCl_4-induced hepatotoxic rats. Bar denotes Mean ± SEM ($n = 5$). Statistical differences ($p < 0.05$) are denoted by the different alphabetical superscripts.

group. Pre-treatment of rats with the crude extract and curcuminoids increases the serum SOD and GPx activities relative to the untreated group. Furthermore, pre-treatment of rats with the crude extract and curcuminoids increases ($p < 0.05$) the SOD activities when compared with the normal control group (Figure 6), while curcuminoid increases ($p < 0.05$) the GPx activities (Figure 7). Catalase activities in untreated control and normal control were comparable ($p > 0.05$). Treatments with 75 mg/kg bw curcuminoids, silymarin (100 mg/kg bw) and 300 mg/kg bw crude extract significantly ($p < 0.05$) increase the catalase activities when compared with normal and untreated control groups (Figure 8).

4. Discussion

Phytochemicals usually have a medicinal potential and serve as a blueprint for drug discovery and developments [38]. High levels of phytochemicals, including alkaloids, flavonoids, phenols and saponins, in *C. longa* rhizome established in this study, are an indication of its potential for medicine and therapy. This is because the pharmacological impacts of plants are based on these compounds [14]. Tannins, phenols and flavonoids have recorded antimicrobial, anti-viral, anticancer, antioxidant, immunomodulatory and anti-inflammatory activities [39,40,41].

Scientific reports have indicated that $CC1_4$ intoxication compromised the integrity of hepatocyte and consequently led to the release of enzymes into the blood/serum [29,42]. Biomarker enzymes, including AST, ALT and ALP, are, therefore, used to indicate the liver's physiological state during CCL_4 assaults. The significant increases in the activities of AST (Figure 1), ALT (Figure 2) and ALP (Figure 3) in the serum of CCl_4 group rats are an indication of leakages of these enzymes from the liver due to compromised integrity [43]. However, crude extract of *C. longa* and curcuminoids decreases ($p < 0.05$) the serum enzyme activities when compared with the untreated group. This finding suggests that the extract was able to reverse the injurious effects of $CC1_4$, or did not enable CCl_4 to cause pronounced injury to the cells [44]. Results of the present study also indicated that curcuminoids and silymarin restored the activities of AST (Figure 1) to the normal levels. This effect suggests that liver integrity has been preserved by the curcuminoids despite the CCL_4 intoxication.

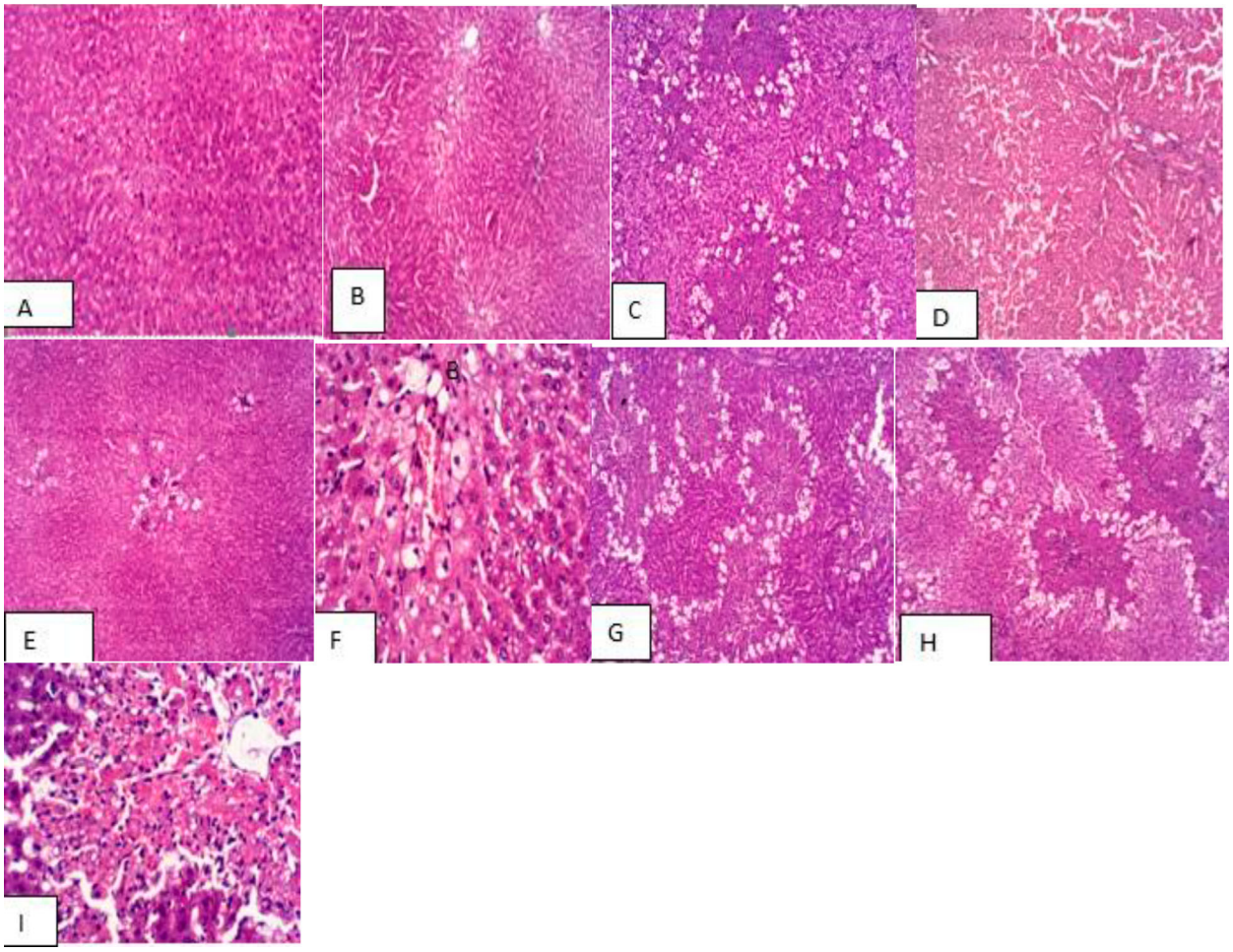

Figure 9. Histology of liver sections of *C. longa* and curcuminoids-treated CCL_4-intoxicated rats. A = control, B = silymarin (100 mg/kgbw). C–E = curcuminoids (75–300 mg/kgbw), F–H = crude extract (150–600 mg/kgbw), I: Untreated control. Magnification 400×.

The elevated serum levels of ALP in CCL_4-intoxicated rats could be attributed to activation of the enzyme molecule *in situ*, this will pose a consequential effect on cells whose activities depend on phosphate esters [43,45]. However, the fact the activity of ALP in silymarin (100 mg/kg) and curcuminoids (600 mg/kg) treated as compared well with the control value suggest that the curcuminoids have some functions in preserving the structural integrity of hepatocellular membrane.

The levels of serum albumins and proteins are vital indicators of impaired or normal functions of the hepatocyte [46]. Although no significant alterations were found ($p > 0.05$) in the serum levels of albumins and proteins in CCL4-intoxicated and untreated rats when compared with the normal control, the increase in total proteins following pretreatment with the curcuminoids could be attributed to enhance the functionality of the liver [47].

Studies have implicated free radicals and oxidative stress in toxicant-induced liver damage [13]. Therefore, effective antioxidant treatment is important in improving or salvaging a liver from drug-induced toxicity. In the present study, the levels of oxidative stress induced by CCL4 were indicated by significant decreases in SOD (Figure 6) and GPx (Figure 7) in serum of CCl_4-mediated hepatotoxic rats when compared with normal control rats. GPx and SOD are major ROS scavenging enzymes in the hepatic system. Interestingly, the curcuminoids causes an increase in ($p < 0.05$) SOD, catalase and GPx activities than the levels found in normal control rats. This is an indication that in addition to protecting the liver against toxicants, curcuminoids also enhance the antioxidant system of the animals, this will positively enhance the animal capability to fight future infection or contact with toxicant [48]. The biochemical findings in this study were also confirmed by histopathological observation (Figure 9) which demonstrated that the curcuminoids protected the liver against CCl_4-induced histological distortion of the liver. This further confirms our initial claim that curcuminoids could serve as a promising agent in salvaging the liver against chemical toxicants and might further expand its therapeutic value in other diseases.

Conclusion

The present study demonstrated that curcuminoids could be considered a novel candidate for the

development of a new drug against liver diseases. However, mechanistic experiments and clinical studies are necessary to confirm our findings.

Ethical statements

Animals were handled in accordance with the ethical principles governing the use of laboratory animals as contained in the Canadian Council on Animal Care Guidelines and Protocol Review.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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