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Research Article

HISTO-REPARATION OF HEPATIC PARENCHYMA OF EXPERIMENTAL WISTAR RATS EXPOSED TO CCL_4-INDUCED HEPATOPATHY AND TREATED WITH VITAMİN B_2 AND GREEN TEA EXTRACT

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ABSTRACT. Natural antioxidants often exhibit protective or ameliorative effect against tissue toxins. The present study was carried out to comparatively assess ameliorative activity of Vitamin B_2 (VB_2) and green tea extract (GTE) against CCl_4-induced hepatic tissue damage through evaluation of serum ALT and AST levels and histomorphological changes in hepatic tissue of study animals. CCl_4 exposure resulted in significant weight loss, increased serum levels of ALT and AST and prominent hepatic histopathological changes in form of necrosis, inflammation, steatosis, and ballooning degeneration. Treatment with VB_2 and GTE extract comparatively showed significant amelioration of hepatopathic effects of CCl_4 exposure indicated by marginal weight loss, marginal increase in serum ALT and AST levels as well as prominent reparation of hepatic histomorphology.

Keywords: *Vitamin B2, green tea, carbon Tetrachloride, hepatopathy, wistar rats*

INTRODUCTION

In the day to day activities of humans, there is a persistent exposure to different chemical agents that are applied for various domestic or commercial purposes. One of such chemical agents is the Carbon Tetrachloride (CCl_4) with wide range of domestic applications including its usage as solvent, refrigerant, in dry-cleaning and many more. However, pathologies of tissues, like nervous, renal and hepatic tissues, have been associated with CCl_4-exposure. The CCl_4 is usually metabolized in the hepatic tissue by cytochrome P450 (from endoplasmic reticulum) leading to the formation of reactive oxygen species such as trichloromethyl (CCl_3) and peroxytrichloromethyl (CCl_3O_2) radicals [1]. These highly reactive radicals bind to membrane lipids or proteins of hepatic cells or organelles to initiate the oxidative hepatopathic effects of the CCl_4 [2]. Some hepatic tissue pathologies due to CCl_4-exposure include necrosis, steatosis, inflammation and fibrosis [3, 4]. Essentially, the CCl_4 has remained one of the most commonly used chemical agents for experimentally inducing hepatic tissue pathologies in order to probe the efficacy of hepato-protective or reparative effects of therapeutic agents or mixtures. These potential hepato-protective or reparative agents include natural antioxidants like vitamin B_2 (VB_2) or riboflavin and therapeutic mixtures like green tea extract (GTE). As a natural antioxidant, the VB_2 is synthesized by plants and some micro-organisms but usually taken as an essential dietary supplement in humans where it function as a vital part of the antioxidant machinery [5]. Furthermore, the green tea has been described as

the second most common drink especially in some parts of the world while the green tea extract (GTE) derived from the plant (*Camellia sinensis*) leaves has been reported to exhibit prominent antioxidant properties [6, 7]. The study by Zhen et al, opined that GTE may provide a potent curation or prevention against oxidative stress-induced hepatic damage and fibrosis [8]. In this study, the objective was to comparatively assess and correlate hepatic histomorphology of experimental Wistar rats *in vivo* during ameliorative activity of VB_2 and GTE against CCl_4-induced hepatic tissue damage.

MATERIALS AND METHODS

Chemical reagents used

The chemical reagents used in this study were purchased from Bristol Scientific Co. Ltd. (Lagos, Nigeria) and Sigma Chemical Co. (St Louis, MO, USA). Green tea was manufactured in China and purchased locally in the form of 50 g packet from Steven Chuks Global Associate Ltd. (Lagos, Nigeria).

Preparation of chemical reagents

The CCl_4 was dissolved in olive oil (ratio 1:1) to obtain the experimental hepatotoxic-inducing agents. Twenty (50 g) packets of green tea were emptied into a clean container and macerated in distilled water with constant agitation every 12 hours. After 72 hours, the mixture was filtered, evaporated to dryness to obtain the GTE. The aqueous preparation of VB_2 was prepared by dissolution of known concentration of VB_2 in a given volume of distilled water.

Study animals

Thirty male albino Wistar rats, weighing between 155-180 g, were used for this study. The study animals were kept in animal cages under hygienic conditions, room temperature of 25±2ºC and relative humidity of 45 - 50% within the Central Animal House Facility, Igbinedion University, Okada, Edo State, Nigeria. The study animals were fed on standard animal feed and granted free access to drinking water *ad libitum* throughout the study period. The approval for this study was granted by Research and Ethics Committee of Igbinedion University, Okada, Edo State, Nigeria (Reference Number: 12/013952/HSC). All experimental procedures were in compliance with International guidelines for experimental animal handling and care.

Study design

The study animals were grouped into five (I-V), each group composed of six animals (n=6). Group I animals served as normal control and was administered with distilled water (2 ml/kg body weight) orally for 15 days. Group II animals served as standard control and was administered with olive oil (1 ml/kg body weight) intraperitoneally (IP) once every 3 days (5 doses). Group III served as positive test control and administered with CCl_4 in olive oil (1.5 ml/kg body weight) IP once every 3 days (5 doses). Group IV was administered with CCl_4 in olive oil (as in group III above) + VB_2 (100 mg/kg) orally everyday for 15 days. Group V was administered with CCl_4 in olive oil (as in group III

above) + GTE (10 mg/kg) orally everyday for 15 days. The total body weight of study animals were recorded during the 0, 5, 10 and 15 days of study.

Serum biochemical assay

After the study period, blood sample of study animals was collected through the retro-orbital sinus, centrifuged at 4000 rpm for 15 minutes to derive serum sample which was used to determine the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) following the method by Reitman and Frankel [9].

Hepatic tissue collection and processing

After the 15-day study period, the animals were sacrificed and their hepatic tissues were collected, weighed and processed for sectioning. The relative organ weight was calculated as the ratio of total organ weight to total body weight expressed as percentage. The processing of hepatic tissue of study animals involved fixation in 10% Neutral Buffered Formalin, dehydration in alcohol (two changes each of 70%, 90% and absolute alcohol), clearing in xylene for 30minutes and embedding in molten paraffin wax to form hepatic tissue blocks.

Hepatic tissue sectioning and staining

5 μm thick sections of hepatic tissue were made from the tissue blocks by using rotary microtome and the sections were mounted on microscope slides. Histological staining of tissue sections was done by using the Haematoxylin and Eosin (H & E) technique. The H & E staining of hepatic tissue sections involved dewaxing in xylene, hydration with alcohol and distilled water, staining with Haematoxylin, rinsing in water, differentiation in 1% acid alcohol (1% Hydrochloric acid in 70% alcohol), blueing in Scoh's tap water, rinsing in water, counterstaining in Eosin, rinsing in water, dehydration in alcohol, clearing in xylene and mounting with Distrene polystyrene xylene (DPX) [10].

Hepatic tissue histomorphological study

Stained hepatic tissue sections were examined under microscope to assess observable histomorphological features in the hepatic tissues of study animals. Photomicrographs of hepatic tissue sections were generated using 10 Megapixel digital camera for microscope. The image-J software by National Institute of Health (NIH, Bethesda, MA, USA) was used to evaluate observable histopathological features including necrosis, inflammation, steatosis and ballooning degeneration within the hepatic tissue of study animals.

Statistical analysis

All experimental data recorded during this study were analysed using Statistical Package for Social Science (IBM-SPSS, version 20) (IBM Corp, NY, USA) and statistical results were presented as mean ± standard error of mean (SEM). Comparison of statistical results was done using *t*-test while multiple comparisons were done using one way analysis of variance (ANOVA) with $p < 0.05$ regarded as statistically significant probability level.

RESULTS AND DISCUSSION

Evaluation of total body weight of study animals

The mean values of the total body weight of study animals in normal control Group I, standard control Group II, test control Group III, and treatment Groups IV and V measured at days 0, 5, 10 and 15 of study period were given in Figure 1. Comparatively, the mean total body weight of study animals showed steady increase in Groups I and II, significant reduction in Group III but non-significant decrease in Group IV and V during the study period.

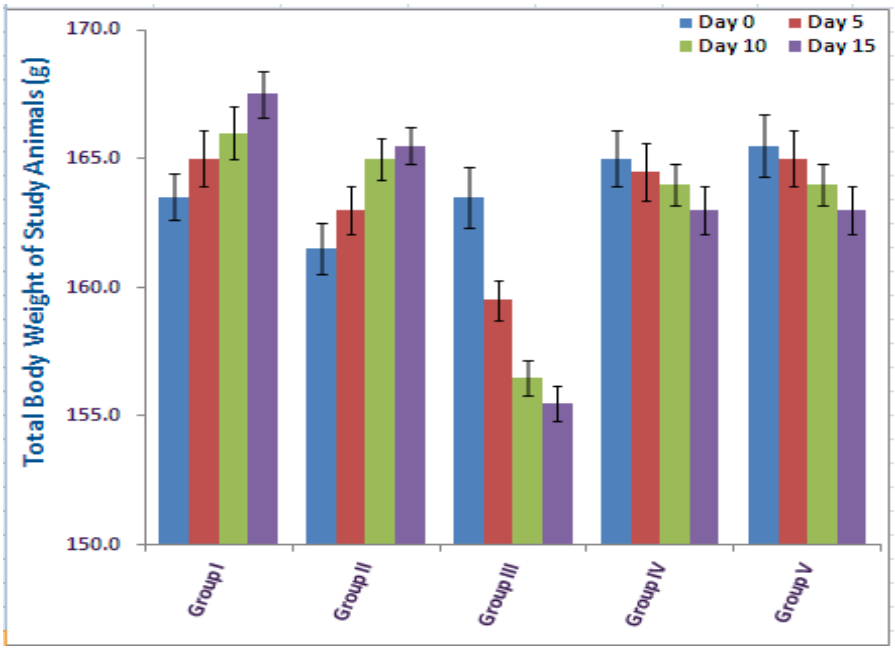


Fig. 1. *Mean body weight of study animals in normal control Group I, standard control Group II, test control Group III and treatment Groups IV and V.*

Evaluation of organ weight and relative organ weight of study animals

The mean values of hepatic tissue weight and relative organ weight of study animals in normal control Group I, standard control Group II, test control Group III, and treatment Groups IV and V were given in Figure 2. Comparatively, the mean organ weight and relative organ weight of test control Group III showed significant reduction relative to control Groups I and II while the treatment Groups IV and V showed non- significant reduction.

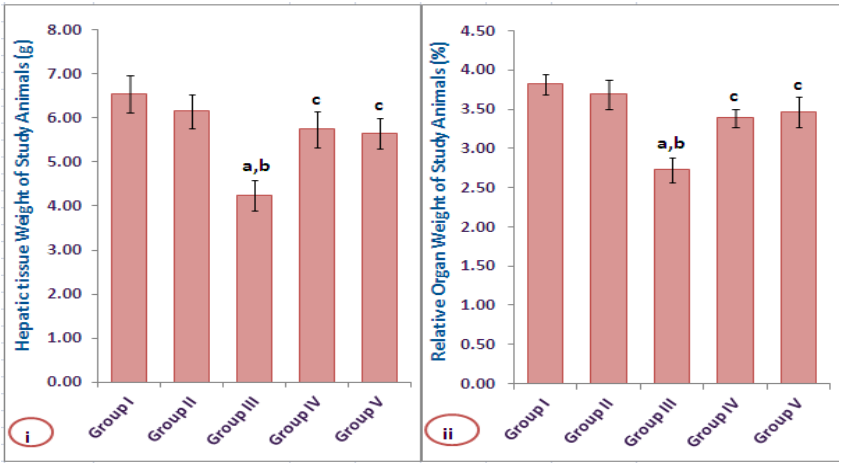


Fig. 2. *Mean values of hepatic tissue weight (i) and relative organ weight (ii) of study animals in normal control Group I, standard control Group II, test control Group III and treatment Groups IV and V. (a, b and c indicated significant difference at $p<0.05$ when compared with Group I, II and III respectively).*

Biochemical assay results

The mean values of ALT and AST concentrations in the serum of study animals in normal control Group I, standard control Group II, test control Group III, and treatment Groups IV and V were given in Figure 3. The serum concentrations of ALT and AST showed significant increase in test control group III in comparison to values in control Groups I and II while the increased values in treatment Groups IV and V were comparatively non-significant.

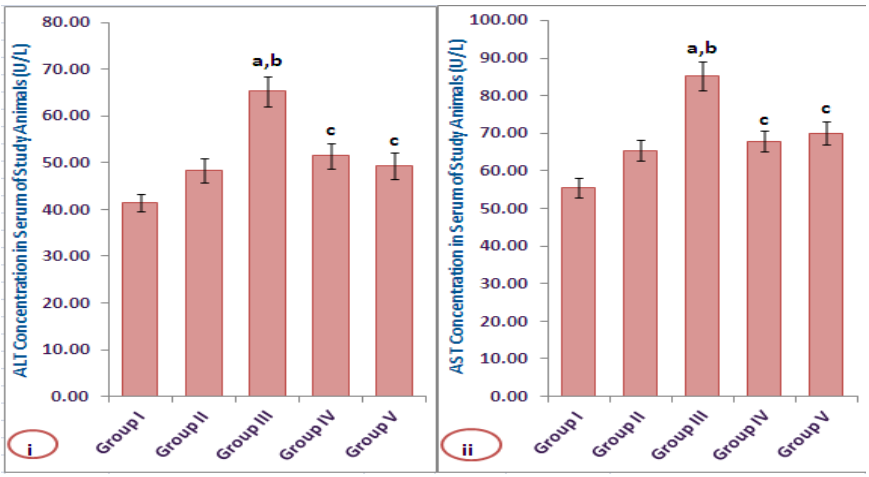


Fig. 3. *Mean values of serum ALT (i) and AST (ii) concentrations in normal control Group I, standard control Group II, test control Group III and treatment Groups IV and V. (a, b and c indicated significant difference at $p<0.05$ when compared with Group I, II and III respectively).*

Hepatic histopathological results

The microscopic examination revealed different histomorphological features of hepatic tissue in normal control Group I, standard control Group II, test control Group III and treatment Groups IV and V animals (Figures 4). The evaluated histopathological features included necrosis, inflammation, steatosis and ballooning degeneration in the hepatic parenchyma of study animals (Figure 5 and 6).

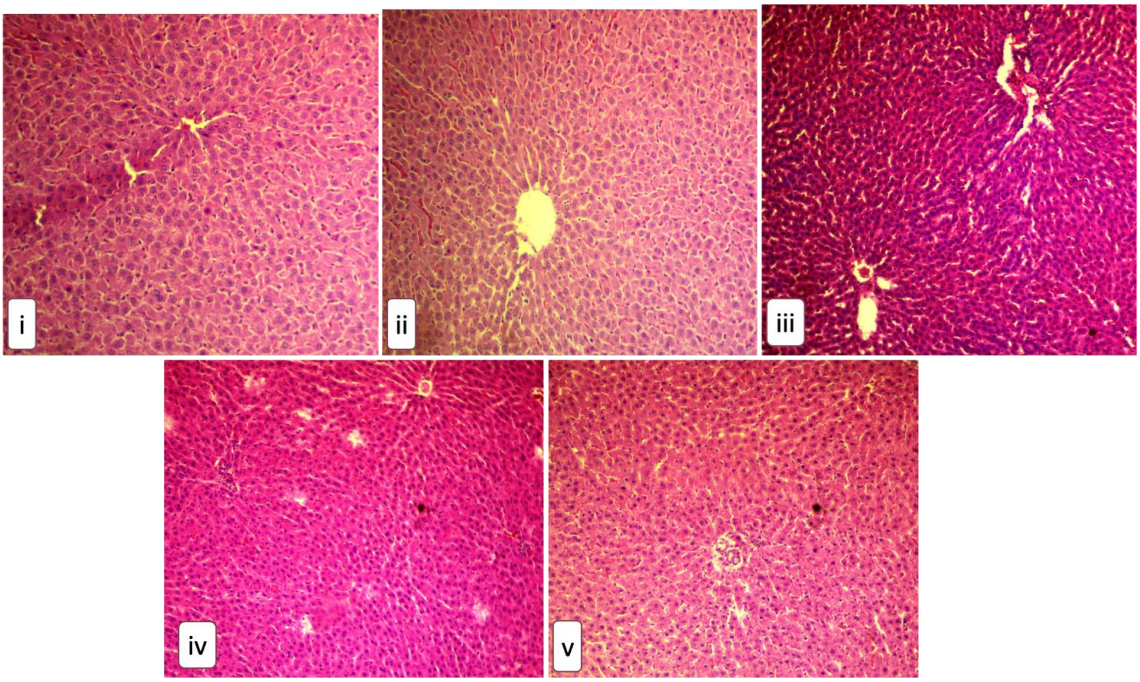


Fig. 4. *Representative photomicrograph of hepatic tissue of study animals (H&E X100). (i = normal control Group I, ii = standard control Group II, iii = test control Group III, iv = treatment Group IV and v = treatment Group V).*

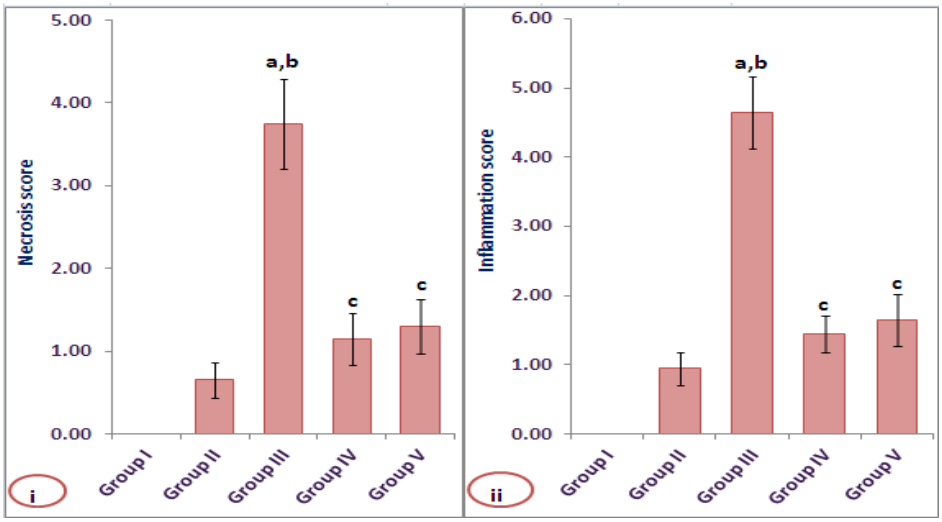


Fig. 5*. Evaluation of necrosis (i) and inflammation (ii) in the hepatic parenchyma of study animals in normal control Group I, standard control Group II, test control Group III and treatment Groups IV and V. (a, b and c indicated significant difference at $p<0.05$ when compared with Group I, II and III respectively).*

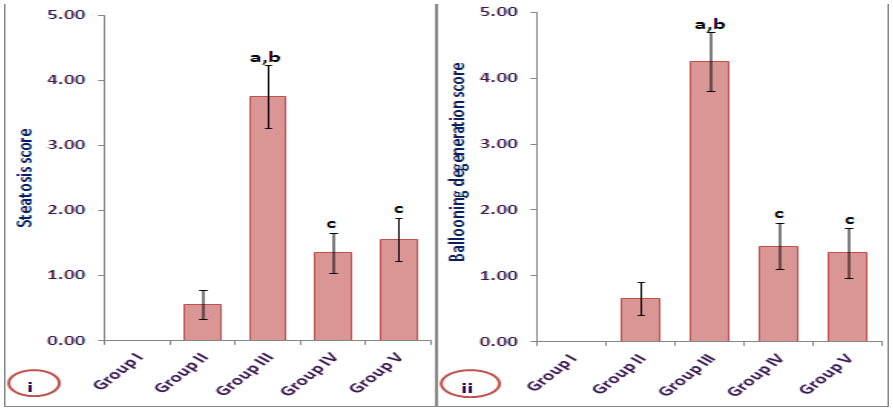


***Fig. 6**. Evaluation of steatosis (i) and ballooning degeneration (ii) in the hepatic parenchyma of study animals in normal control Group I, standard control Group II, test control Group III and treatment Groups IV and V. (a, b and c indicated significant difference at $p<0.05$ when compared with Group I, II and III respectively).*

Essentially, the CCl_4 exposure results in significant elevation of lipid peroxidation which in turn leads to membrane degradation of cells (including hepatic cells) that contributes to its cytotoxic effects including hepatopathy [11, 12]. From the findings of this study (Figures 1-3), the CCl_4 exposure exerted inverse influence on body and organ weight gain and caused significant ($p<0.05$) elevation of serum biomarkers of hepatic tissue damage (ALT and AST) in test control Group III animals. However, the treatment Groups IV and V showed comparable outcomes with those of normal and standard control Groups I and II.

Generally, therapeutic agents exert their hepato-protective or reparative effects against CCl_4-induced hepatic tissue damage through significant reduction in reactive radicals produced leading to disruption of lipid peroxidation that follows CCl_4 exposure [13]. The VB_2 plays a key role in the body antioxidant machinery by functioning as coenzyme for activation of glutathione reductase (GR) enzyme and the activated GR in turn converts oxidized glutathione into its reduced form [14, 15]. The reduced glutathione function as potent endogenous antioxidants that deactivates reactive oxygen species produced in different cells [16]. These include the toxic reactive oxygen species produced in hepatic tissues following CCl_4 exposure.

Furthermore, the potent antioxidant and anti-lipid peroxidation activities of GTE constitute vital cellular and biochemical bases of its therapeutic applications [17-21]. Its antioxidant activity, due to its potent free radical scavenging ability and supported by chelation of metal ions by constituent catechins, thereby enhanced its cytoprotective effects against damaging effects of cytotoxins [22, 23]. These radicals also include the highly reactive oxidative radicals produced in hepatic tissues due to CCl_4 exposure. Consequently, the hepatic tissue damage following CCl_4 exposure presented varying degrees of hepatic histomorphological alterations. According to results of this study (Figures 4-6), the CCl_4 exposure caused deleterious alterations in hepatic histomorphology as observed in significant ($p<0.05$) elevations in hepatic necrosis, inflammation, steatosis and ballooning degeneration in test control Group III study animals relative to the normal and standard control Groups I and II. However, the

treatment Groups IV and V animals showed comparable hepatic histomorphology with those of normal and standard control Groups I and II.

Hepatotoxins usually cause disruption of hepatic tissue histomorphology following acute or chronic exposure [24]. Essentially, anti-hepatotoxic properties exhibited by anti-hepatotoxins include preservation of hepatic tissue histomorphology against deleterious effect of hepatotoxins or reparation of hepatic tissue histomorphology following exposure to hepatotoxins. The study by Wang et al. described anti-hepatotoxic activities of Cannabidiol against hepatotoxic agent (chronic alcohol exposure) to include attenuation of steatosis, inflammation and neutrophil-mediated injury in hepatic tissues of experimental animals [25]. Further study by Uzzi and Grillo [26] reported anti-hepatotoxic activities of *Cassia occidentalis* extract against hepatotoxic agent (paracetamol) to include amelioration of hepatic vascular congestion, inflammation and peri-portal oedema. The findings from another study showed that factors that result in deficiency of Apoptosis-stimulating protein 2 of p53 will in turn prevent hepatic tissue injury via inhibition of inflammation and cell death after exposure to CCl_4 hepatotoxicity [27]. In another study, propolis extract was reported to exhibit protective effects on the hepatic tissue morphology against alcohol-induced steatosis in experimental animals [28]. Among the hepatic histomorphological changes described in a study by Alrashed and El-kordy on hepatoprotective activity of panax ginseng against cisplatin-induced hepatotoxicity were significant reductions in hepatocyte vacuolization, sinusoids dilation and collagen deposition [29]. According to the study by Layman et al., [30], other hepatic histomorphological activities that characterize the hepatoprotective effect of green rooibos extract include anti-steatic and anti-inflammatory activities. The inhibition of apoptosis and reduction of inflammation in hepatic tissue was also reported during hepatoprotective activity of 1-O-(4-hydroxymethylphenyl)-α-L-rhamnopyranoside and essential oil of *Cinnamomum verum* bark against CCl_4 hepatotoxicity [31, 32].

Therefore, an improved hepatic histomorphology following exposure to hepatotoxic agent is a key marker of anti-hepatotoxic effect of the therapeutic agent. According to the findings of this study, treatment with VB_2 and GTE comparatively resulted in significant improvement of hepatic histomorphological characteristics of experimental animals following exposure to CCl_4-induced hepatopathy.

CONCLUSION

Based on the findings of this study, the VB2 and GTE comparatively exhibited potent ameliorative effects against hepatotoxic effects of Carbon Tetrachloride exposure essentially due to their active antioxidant activities leading to histomorphological reparation of hepatic tissue of exposed and treated experimental animals.

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