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Effect of Consumption of *Corchorus olitorius* L., in Carbon Tetrachloride-Induced Liver Damage in Male Wistar Rats

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ABSTRACT

Carbon tetrachloride (CCl_4) has been extensively used in experimental models to demonstrate its hepatotoxic potential. Humans are often exposed to it where it is used in petrol additives, refrigerants, catalyst in polymer formation and in pesticides. In this study, the effect of leaves of *Corchorus olitorius* L. in CCl_4-induced liver damage in male wistar rats was assessed using alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), plasma total protein, superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST), reduced glutathione (GSH), Packed Cell Volume (PCV), hemoglobin (Hb) and White Blood Cell (WBC) as well as histological assay. Thirty five male wistar rats distributed into seven groups of five rats each were used in this study. The 1 mL kg^{-1} body weight of CCl_4 was administered orally thrice in a week to hepatotoxic groups. Animals in all the groups were either fed control diet or *C. olitorius*-supplemented diets (COSD). It was observed from the result of this study that exposure to CCl_4 and *Corchorus olitorius* L., produced a significant increase ($p<0.05$) in ALP activity and plasma total protein in some groups, no significant change ($p>0.05$) in AST, ALP and SOD activities and a significant decrease ($p<0.05$) in GST and catalase activities in the non-hepatotoxic groups while GSH increased significantly in all the groups. PCV, Hb and WBC count were not significantly different ($p>0.05$) and microscopic examination showed severe histological damage in hepatotoxic groups fed with *C. olitorius*-supplemented diet. These observations indicate that regular consumption of unprocessed *C. olitorius* L., may further enhance the hepatotoxic potential of CCl_4 in humans.

Key words: *Corchorus olitorius* L., carbon tetrachloride, biochemical parameters, wistar rats

INTRODUCTION

The liver is the largest glandular organ of the body with a weight of about 1.5 kg and reddish brown in color, having a wide range of functions such as detoxification of harmful substances, synthesis of protein, urea formation, glycogen storage and production of enzymes involved in digestive processes (O'Grady, 2000). The liver is prone to damage because of its high functional and biochemical activities. Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. Xenobiotic metabolizing enzymes such as cytochrome P450-dependent monooxygenases localized in hepatocytes carry out oxidative reactions which produce reactive metabolites that can induce liver injury and present with asymptomatic elevation of hepatic enzymes (Hodgson and Levi, 2004; Jaeschke *et al*., 2002). Excessive inflammatory

response of neutrophils to microbial infection or tissue trauma can also aggravate existing liver injury (Chen *et al*., 2007; Malhi *et al*., 2006). Injury to the hepatocytes and bile ducts consequently results in the accumulation of bile acid which further promotes liver damage (Patel *et al*., 1998). Liver damage is a global health challenge having high morbidity and mortality and presents both economic and psychological challenges. The incidence of liver damage is on the increase particularly in developing countries. As such its management and prevention remain a top priority for the scientific community all around the world.

Epidemiological studies have shown that lifestyle such as dietary pattern is implicated in the etiology of several forms of liver injury (Dossus and Kaaks, 2008; Kruk, 2007; Friedenreich, 2001). *Corchorus olitorius* is a common vegetable widely consumed in South west, Middle belt and Northern part of Nigeria. It is used in the preparation of soups and sauce and has been employed in the treatment of diseases such as fever, diahorrea, diabetes as well as portal hypertension (Shittu *et al*., 2006; Oboh *et al*., 2012). Its therapeutic properties have been attributed to the presence of non-nutrients or bioactive phytochemicals (Oboh *et al*., 2012). In addition, some phytotoxins have been identified in the plant such as calcium oxalate which may present with conditions such as kidney stone as well as electrolyte imbalance (Musa and Ogbadoyi, 2012). However, the widespread consumption of this vegetable may show its possible hepatoprotective property or otherwise mask its apparent hepatotoxicty. This formed the basis of this study aimed at investigating the effects of *C. olitorius* on the biochemical and histological parameters in carbon tetrachloride-induced liver damage in male wistar rats.

MATERIALS AND METHODS

Collection and processing of plant materials: The leaves of *C. olitorius* L., were purchased from local vendors in Ilepo market Lagos State, Nigeria. They were authenticated by Dr. Conrad Omonhinmin of Applied Biology and Biotechnology Unit of the Department of Biological Sciences, Covenant University. The leaves were carefully picked and air-dried for about four weeks and ground into coarse powder using a mixer grinder.

Animals: A total of thirty five apparently healthy albino wistar male rats of an average weight of 120 g were purchased from the Animal Breeding Center of the Federal University of Agriculture, Abeokuta, Nigeria. The animals were housed under tropical temperature and humidity conditions with an alternating 12 h light and dark cycle. They were allowed access to food and water *ad libitum* prior and during the experimental period. All experimental procedures were carried out in compliance with Guidelines for the Care and Use of Laboratory Animals prescribed and approved by Covenant University Ethics Committee.

Assay kits and other reagents: The assay kits for alanine transaminase, aspartate transaminase and alkaline phosphatase were obtained from Randox Laboratories Ltd., UK. Carbon tetrachloride was purchased from British Drug Houses (Poole, Dorset, UK). Silymarin was from Micro labs Limited, India. All other chemicals were of analytical grade.

Formulation of experimental diets: A control and two experimental diets namely diet A, B and C, respectively were compounded using a slight modification of the method adopted by Emeka and Obidoa (2009) as shown in Table 1. Diet A (control diet) did not contain leaves of *C. olitorius*. Diet B (5% COSD) and C (10% COSD) were prepared by supplementing with 5% and 10% leaves of *C. olitorius*, respectively.

Table 1: Composition of experimental diets (g/100 g)

Feedstuff	Diet A (Control diet)	Diet B (5% COSD)	Diet C (10% COSD)
Maize (flour)	50	50	500
Groundnut cake	9.6	9.6	9.6
Fish meal	6	6	6
Wheat offal	26	21	16
5% *C. olitorius* leaves	-	5	-
10% *C. olitorius* leaves	-	-	10
Bone meal	2.0	2.0	2.0
Oil	4.0	4.0	4.0
Oyster shell	2.0	2.0	2.0
Salt	0.2	0.2	0.2
Broiler premix	0.2	0.2	0.2
Total (approx)	100.00	100.00	100.00

Table 2: Animal grouping and treatment

Experimental groups	Group name	Treatment	Feeding
I	Normal control (NC-group)	Olive oil	Diet A
II	CCl_4-group (Negative control)	CCl_4 in olive oil (1 mg kg^{-1} body weight)	Diet A
III	Silymarin-group (Positive control)	CCl_4 in olive oil (1 mg kg^{-1} body weight) +Silymarin (50 mg kg^{-1})	Diet A
IV	5% COSD-group	CCl_4 in olive oil (1 mg kg^{-1} body weight)	Diet B
V	10% COSD-group	CCl_4 in olive oil (1 mg kg^{-1} body weight)	Diet C
VI	5% COSD-normal	Olive oil	Diet B
VII	10% COSD-normal	Olive oil	Diet C

Experimental design: The rats were acclimatized for two weeks and thereafter weighed and randomly distributed into seven groups of five animals each (Table 2). Liver damage was induced in animals in group II, III, IV and V by oral administration of CCl_4 1 mL kg^{-1} body weight dissolved in olive oil (1:1) three times per week for 16 weeks (Fujii *et al.*, 2010) Silymarin was used the standard drug for liver damage and was administered daily. The normal control group (I) was administered olive oil (vehicle) only and fed diet A (control diet). The CCl_4-group (negative control) was administered CCl_4 in olive oil (1 mg kg^{-1} body weight) and fed diet A. The Silymarin-group (positive control) was administered CCl_4 in olive oil (1 mg kg^{-1} body weight), silymarin (50 mg kg^{-1}) and fed diet A. The 5% COSD-group and 10% COSD-group were administered CCl_4 in olive oil (1 mg kg^{-1} body weight) and fed diet B and C, respectively. The 5% COSD-normal and 10% COSD-normal were administered olive oil (1 mg kg^{-1} body weight) and fed diet B and C, respectively. The animals were allowed free access to feed and water *ad libitum* throughout the period of experiment that lasted for sixteen weeks. The animals were weighed three times weekly throughout the period of the experiment.

Collection of blood and liver: At the end of the experimental period, the rats were fasted overnight and sacrificed under mild euthanasia with pentobarbital. Blood samples for haematological and biochemical analyses were withdrawn from the rats through the retro-orbital venous plexus into EDTA and lithium-heparinized tubes, respectively. The blood in the heparinized tubes was centrifuged at 3000 rpm for about 15 min and the plasma separated and stored at -20°C until analysis. The liver was subsequently excised, rinsed in 0.9% normal saline, dried and

weighed. Approximated 1.5 g portion of each liver samples were preserved in 10% buffered formal saline in sterile tubes for each animal and correctly labeled for histopathological examination. For biochemical assay, the liver was cut very thinly with sterile scalpel blade and homogenized in phosphate buffered saline (1:10 w/v) pH 7.0. The homogenates were centrifuged at 5000 rpm for 10 min and the supernatants stored at -18°C to be used for analysis.

Determination of liver enzymes: The activities of alanine transaminase and aspartate transaminase were assayed by methods described by Reitman and Frankel (1957). Alkaline phosphatase was assayed by the method of Wright *et al.* (1972).

Determination of antioxidant capacity: Superoxide dismutase, catalase, glutathione-s-transferase and reduced glutathione levels were determined by the methods Zou *et al.* (1986), Beers and Sizer (1952), Habig *et al.* (1974), Ellman (1959) and Beutler *et al.* (1963), respectively. One unit of SOD activity was defined as the quantity of SOD required to inhibit 50% of reaction and expressed as U mg^{-1} protein. The activity of CAT was expressed as mol of H_2O_2 consumed/min/mg protein. GST activity was expressed as nmol CDNB-GSH conjugate/min/mg protein. The GSH was expressed as mol NADPH consumed/min/mg protein. Hemoglobin and WBC were determined according to standard methods described by Lewis *et al.* (2001). Plasma total protein concentration was determined by the Biuret method of Gornall *et al.* (1949).

Determination of haematological parameters: Haematological parameters including percentage packed cell volume (PCV%) and hemoglobin concentration (Hb) were determined with a hematocrit machine while the White Blood Cell (WBC) count was done with a hemocytometer according to the method described by Tietz *et al.* (1994).

Histopathological examination: Liver sections fixed in 10% buffered formalin were dehydrated in gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections of 4-5 μm thick were prepared using a rotary microtome and then stained with haematoxylin and eosin dye. The tissues were observed under the microscope at magnification of 400 for cell necrosis, fatty change, hyaline degeneration, ballooning degeneration, infiltration of kupffer cells and lymphocytes. The extent of CCl_4 -induced liver damage was evaluated as a function of pathologic lesions in the stained liver sections. The tissue slices were interpreted by a consultant pathologist at the Lagos University Teaching Hospital, Nigeria.

Statistic analysis: The data analysis was performed using the statistical package for social science (SPSS software version 13.0 for Windows). Data was expressed as Means±S.D. The effects of the treatments were evaluated statistically using the one-way analysis of variance (one-way ANOVA) test and this was followed by *post hoc* LSD to correct for multiple comparison of the groups.

RESULTS

Effects of *C. olitorius* on activity of liver enzymes and plasma protein: The ALT activity was significantly decreased in 5% COSD-normal while it was increased in 10% COSD-normal (Table 3). There were no significant changes ($p<0.05$) in AST activities in all the groups. However, ALP activity was increased in 10% COSD-group and 5% COSD-normal while it significantly decreased in 10% COSD-normal (Table 3).

The total plasma protein concentration of the negative control and majority of the hepatotoxic and non-hepatotoxic groups was significantly ($p<0.05$) increased compared to the positive control group (Table 3).

The elevated total plasma protein concentration may be suggestive that the vegetable may provide potential hepatoprotective effect and maintain protein synthesis capacity of the hepatocytes.

Effects of *C. olitorius* on antioxidant capacity: Superoxide dismutase (SOD) activity level was not significantly ($p<0.05$) different among the groups. There was a significant decrease ($p<0.05$) in GST activity in the 5% COSD-normal and 10% COSD-normal compared with the normal and negative control groups (Table 4).

Catalase activity was significantly ($p<0.05$) decreased in the normal control compared with the negative control. It was significantly decreased in all the hepatotoxic groups. Reduced glutathione (GSH) level was significantly ($p<0.05$) increased in the hepatotoxic groups compared with the normal and negative control. The GSH level was significantly ($p<0.05$) increased in 10% COSD-normal group (Table 4).

Effect of *C. olitorius* on weight and hematological parameters: There was a significant ($p<0.05$) decrease in weight of the 5% COSD-normal and 10% COSD-normal groups fed diet B and C compared to the negative control group but no significant differences in body weight changes among the other groups (Table 5).

Table 3: Effects of *Corchorus olitorius* on activity of liver enzymes and plasma protein

Experimental groups	ALT (U mg^{-1} protein)	AST (U mg^{-1} protein)	ALP (IU)	Protein (g dL^{-1})
Normal control (NC-group)	61.44±17.57	58.95±6.67	264.00±2.30	$35.07±1.49^{b}$
CCl_4-group (Negative control)	39.26±23.65	69.93±4.48	219.88±199.26	$40.48±7.12^{b}$
Silymarin-group (Positive control)	50.82±39.62	59.28±7.28	226.09±73.83	$27.27±3.39^{ca}$
5% COSD-group	$73.13±3.67^{a}$	67.60±6.64	197.34±13.34	$36.14±5.34^{b}$
10% COSD-group	39.37±7.19	49.62±14.33	$353.28±37.72^{ab}$	$37.45±5.00^{b}$
5% COSD-normal	$34.93±4.51^{c}$	48.14±8.30	$366.16±81.88^{ab}$	34.60±1.88
10% COSD-normal	$77.86±10.08^{ab}$	57.89±8.56	$136.16±15.32^{c}$	34.21±2.37

Data was presented as Means±SD of five rats. a: significant ($p<0.05$) compared to CCl_4-group, b: significant ($p>0.05$) compared to Silymarin-group, c: significant ($p>0.05$) compared to normal control-group, ALT: Alanine transaminase, AST: Aspartate trasaminase, ALP: Alkaline phosphatase glutathione, COSD: *Corchorus olitorius*-supplemented diet

Table 4: Effects of *Corchorus olitorius* on antioxidant markers

Experimental groups	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	GSH (μM)	GST (U mg^{-1} protein)
Normal control (NC-group)	1.26±0.46	$0.50±0.03^{a}$	$1.81±0.03^{b}$	0.14±0.02
CCl_4-group (Negative control)	1.75±0.67	$1.06±1.46^{c}$	2.34±0.26	$0.20±0.08^{b}$
Silymarin-group (Positive control)	3.04±4.40	0.49±0.38	$2.61±0.46^{c}$	$0.10±0.07^{a}$
5% COSD-group	1.27±0.77	$0.09±0.06^{a}$	$3.17±0.39^{ca}$	0.15±0.02
10% COSD-group	1.55±1.51	$0.14±0.07^{a}$	$2.54±0.41^{c}$	0.15±0.05
5% COSD-normal	2.05±0.92	0.48±0.17	2.16±0.40	$0.04±0.04^{ca}$
10% COSD-normal	0.75±0.14	$0.05±0.01^{a}$	$2.89±0.46^{c}$	$0.10±0.01^{a}$

Data was presented as Means±SD of five rats. a: significant ($p>0.05$) compared to CCl_4-group, b: significant ($p>0.05$) compared to Silymarin-group, c: significant ($p>0.05$) compared to normal control-group, SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione, GST: Glutathione-S-transferase, COSD: *Corchorus olitorius*-supplemented diet

Table 5: Effects of *Corchorus olitorius* L., on weight and hematological parameters

Experimental groups	Weight gain (%)	PCV (%)	Hb (g dL^{-1})	WBC (10^3 mm^{-3})
Normal control (NC-group)	35.38±4.16	73.33±5.51[a]	23.66±1.78[a]	9.30±9.96
CCL_4-group (Negative control)	47.57±7.89[b]	56.00±16.00*	18.06±5.17*	7.80±2.11
Silymarin-group (Positive control)	36.10±6.69[a]	57.00±13.00	18.39±4.20	8.77±3.17
5% COSD-group	37.46±25.73[a]	60.00±7.81	19.36±2.52	7.77±4.73
10% COSD-group	43.85±10.77	60.67±12.50	19.57±4.04	7.00±5.50
5% COSD-normal	26.62±13.88[a]	47.67±11.24*	15.38±3.62*	15.33±3.23[a]
10% COSD-normal	40.16±9.40[a]	59.00±21.00	19.03±6.78	4.50±1.40

Data was presented as Means±SD of five rats. a: significant (p>0.05) compared to CCl_4-group, b: significant (p>0.05) compared to Silymarin-group, c: significant (p>0.05) compared to Normal control-group, PCV: Packed cell volume, Hb: Hemoglobin, WBC: White blood cell, COSD, *Corchorus olitorius*-supplemented diet

Table 6: Histological features of the liver of experimental male rats

Groups	Parenchyma	Necrosis	Periportal inflammation	Periportal fibrosis
G1	Diffuse congestion	-	-	-
G2-3	Diffuse congestion	-	Mild	-
G4-5	Congestion, fatty change	-	Moderate	Present
G10	Congestion	-	Moderate	-
G11-12	Congestion	-	Moderate	Present

G1: Histological feature of the normal control male rat, G2: Histological feature of CCl_4 negative control, G3: Histological feature of CCl_4 positive control group (with silymarin), G4: Histological feature of 5% COSD-group, G5: Histological feature of 10% COSD-group, G10: Histological feature of 5% COSD-normal, G11: Histological feature of 10% COSD-normal

PCV and Hb concentration in negative control decreased significantly ($p<0.05$) compared with the normal control but not significant ($p<0.05$) change in the 5% COSD-group and 10% COSD-groups (Table 5). They were significantly decreased ($p<0.05$) in 5% COSD-normal group compared with the normal control. The WBC count was non-significantly different in all the groups except in 5% COSD-normal which was significantly ($p<0.05$) increased compared with the negative control (Table 5).

Effects of *C. olitorius* L., on histological features of livers of experimental rats: The histological features of the experimental animals are described (Table 6) and shown in Fig. 1a-e. Histological features of the normal control group showed normal hepatic architecture (Fig. 1a) while the negative control showed marked but diffused parenchymal congestion with mild lymphocytic infiltration (Fig. 1b). The 5% COSD-group exhibited periportal fibrosis with moderate lymphocytic infiltration and hepatic fatty changes (Fig. 1c). The 5% COSD-normal and 10% COSD-normal exhibited periportal congestion, dilated hepatic arteries filled with blood (Fig. 1d and e). However, necrosis was absent in all the groups.

In Fig. 1a, Histological section showed normal hepatic architecture with mild, Diffused Parenchymal Congestion (DPC). In Fig. 1b, histological section showed normal hepatic architecture with Marked, Diffused Parenchymal Congestion (MDPC) and mild Lymphocytic Infiltration (LI) around the portal tracts. In Fig. 1c, histological section showed moderate Lymphocytic Infiltration (LI) and microvesicular Fatty Changes (FC). In Fig. 1d, histological section showed Periportal Fibrosis (PF) with moderate Lymphocytic Infiltration (LI). In Fig. 1e, histological section showed Periportal Fibrosis (PF) with moderate Lymphocytic Infiltration (LI).

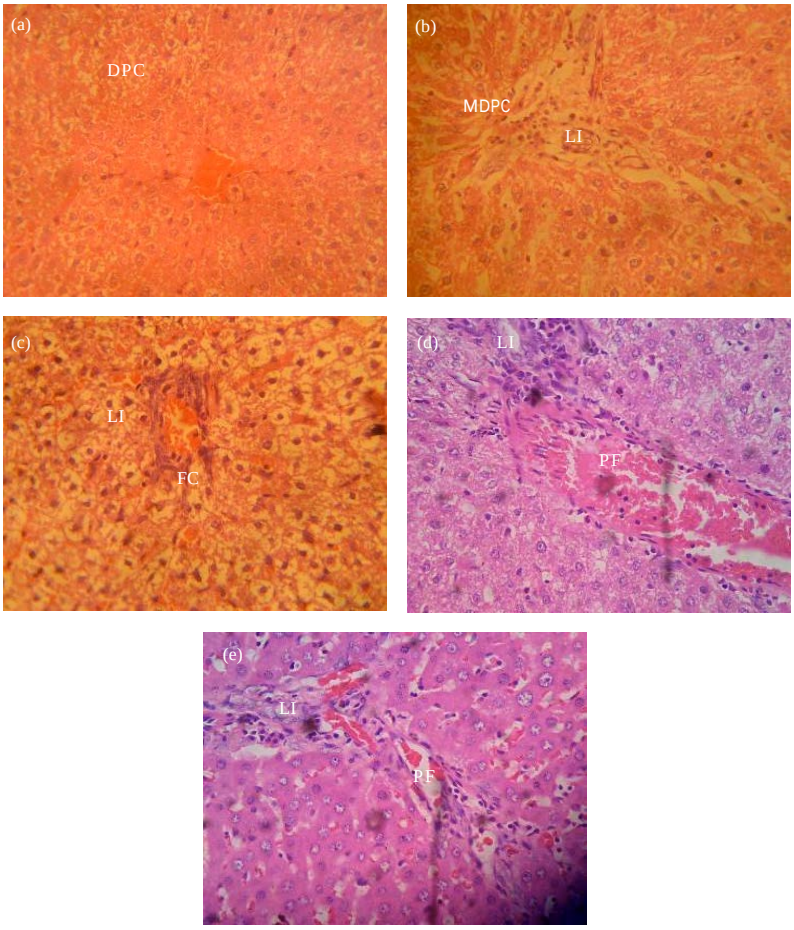

Fig. 1(a-e): Histological observation of the liver section of (a) Non-hepatotoxic control (x400), (b) Hepatotoxic control and positive (silymarin group) control (x400), (c) Hepatotoxic group fed *Corchorus olitorius*-supplemented diet (COSD) (x400), (d) Non-hepatotoxic group fed 5% *Corchorus olitorius*-supplemented diet (COSD) (x400) and (e) Non-hepatotoxic group fed 10%*Corchorus olitorius*-supplemented diet (COSD) (x400)

DISCUSSION

Carbon tetrachloride (CCl_4) has been well established as a potent inducer of hepatotoxicity via its metabolic activation in the liver to a reactive free radical trichloromethyl peroxyl ($\bullet CCl_3$). On the other hand, consumption of several vegetables have been reported to be beneficial in the perturbation of the deleterious effect of toxins such as CCl_4 through induction of detoxification systems such GSH/GST system (Wark *et al*., 2004). In this study, the weight gain in all the treatment groups especially those given only COSD were non-significant. This could be as a result of the high fibre content of the plants which help to lengthen digestion time and thus increase satiety (Noonan and Savage, 1999). The fibre content would also help to increase fecal weight and excretion. Generally, dietary fibres have been found to be associated with weight loss. Nutritionally, it is known that vegetables are low in calories and this can explain the non-significance weight gain observed in this study (Oboh *et al*., 2012). Hence, supplementation of staple foods with these plants

may be beneficial to weight loss. The non-significant weight gain in the hepatotoxic groups may be attributed to the toxic effect of CCl_4 which reduced the functionality of hepatic cells and the liver with respect to carbohydrate, protein and lipid metabolism. The result from this study showed an increase in the activity of the liver enzymes which includes ALT, AST and ALP in the hepatotoxic groups including those fed with COSD. The rise in the activity of these enzymes is suggestive of toxicity to the liver which may have been caused by anti-nutrients present in the unprocessed vegetables such as calcium oxalate, hydrogen cyanide, phytate and nitrate leading to membrane leakage and consequently increased serum level of the enzymes (Musa and Ogbadoyi, 2012). These antinutrients have been implicated in several health problems such as kidney stones as well as electrolyte imbalance (Okon and Akpanyung, 2005). *Corchorus olitorius* L., has been classified as a high nitrate vegetable and hence its regular consumption may present with metheamoglobinemia and cancers (Galler, 1997). A decrease in total protein is an important indicator of the liver damage. The decrease in total protein in some of the hepatotoxic groups may be attributed to toxic effect of CCl_4 on Endoplasmic Reticulum (ER) which in turn affects the protein synthesis capacity of the cells (Ron and Walter, 2007). Stressed ER is unable to efficiently maintain a balance between synthesized and unfolded protein. This results in inflammation and consequently dysfunctional cells.

The histopathological results which showed congestion, hepatic fatty change and fibrosis in the groups fed with diets supplemented with the vegetable further confirms the apparent toxicity of the vegetable to the liver as seen in the elevated liver enzymes.

The significant decrease in PCV and Hb concentration in 5% COSD-group may be linked to the development of metheamoglobinemia as a result of nitrate toxicity from regular consumption of the raw vegetable (Musa and Ogbadoyi, 2012). High nitrate in the blood has been found to cause oxidation of the hemoglobin thus reducing oxygen transport to body tissues. This condition may lead to unconsciousness and consequently death (Chan, 2011). This is also suggestive that consumption of *C. olitorius* L., especially in their unprocessed form may not contribute to red blood cell synthesis (Shaarawy *et al*., 2009). White Blood Cell (WBC) count was also significantly increased in the negative control compared to the normal control. This could be attributed to increased neutrophils in the blood which is also an index of liver toxicity (Saba *et al*., 2010). The increase in WBC in groups fed COSD could be suggestive that long term consumption of this vegetable may compromise the immune system (Oyedeji and Bolarinwa, 2013).

Superoxide dismutase (SOD) activity level in most of the treatment groups did not significantly change and this may imply that the consumption of the unprocessed vegetables may not enhance the induction of this antioxidant enzyme. The SOD as an antioxidant enzyme helps in mopping up of ROS. The SOD induction has been linked with the consumption of vegetables such as broccoli, spinach and carrot which possess high antioxidant potential. Their phenolic content scavenges superoxides and peroxyl radicals there by sparing SOD (Rice-Evans *et al*., 1997). Hence, *C. olitorius* L., may not contain phytochemicals capable of inducing SOD. On the other hand hepatoprotective potential of the vegetable may be dose-dependent.

A decreased GST activity level serves as platform for increased susceptibility to hepatic injury and cancer. This suggests that consumption of the unprocessed *C. olitorius* L., may not be protective against liver damage perhaps due to the high antinutrients content of the plant. The presence of phytate, oxalate and cyanides may be responsible for the low activity of GST. The GST which is largely localized in the centrilobular regions of the hepatocytes is important in detoxification process by conjugating activated xenobiotics and harmful oxidants with glutathione

to improve their detoxification and elimination (Ziglari and Allameh, 2013). Although, phenolic content of vegetables such as garlic, broccoli and carrot have been reported to induce the enzyme (Lampe *et al*., 2000), *C. olitorius* L., may not contain sufficient quantity to induce GST activity or their antioxidant properties may have been masked by the hepatic damage that may have been induced by the presence of the CCl_4 and the antinutrients (Hodgson and Levi, 2004).

Catalase activity in blood was not significantly different among the groups. It was however significantly ($p<0.05$) reduced in the liver of almost all the groups compared with the hepatotoxic negative control. Catalase together with glutathione peroxidase (GPx) helps in mopping up ROS thus protecting the hemoglobin from oxidative stress. The reduction in catalase activity may play a role in host defense response to oxidative stress. An intervention study reported that catalase activity may not be significantly affected by dietary intervention (Dragsted *et al*., 2004). Another report has shown that catalase activity may be decreased with increase in intake of some fruits and vegetables (Ahn *et al*., 2006). Thus, it may be inferred that the consumption of *C. olitorius* L., did not significantly affect catalase activity. Reduced glutathione (GSH) is a tripeptide that is vital in the scavenging of free radicals and detoxification of harmful substances. During oxidative stress, glutathione level in the blood decreases leading to reduction of life-sustaining antioxidants. A damage to the liver results in a substantially low level of GSH. The GSH level of group fed COSD was increased significantly suggesting that consumption of *C. olitorius* L., could greatly improve free radical scavenging antioxidant molecules in the system and hence disease prevention (Demirkol *et al*., 2004).

CONCLUSION

From the observations of this study, it can subtly conclude that the regular consumption of *C. olitorius* L., may induce or aggravate hepatotoxicity and may play a role in liver damage in humans. This may be attributed to the presence of some antinutrients at elevated concentration in the vegetable. However, the processing of these plant foods through cooking and other methods prior their consumption may possibly lower their hepatotoxic potential in humans. The increase in GSH content by these plants may be of physiological importance especially in increasing the antioxidant capacity of their consumers: The role of GSH as an endogenous antioxidant in preventing free radical mediated disease formation cannot be overemphasized.

This study has revealed the importance of proper processing (blanching and cooking prior consumption) of most plant foods (including *C. olitorius* L.) especially those that contain potential toxic compounds. These may mask its potential to cause liver damage. Based on the result of this study it is recommended that care should be taken in the consumption of this vegetables as well as its usage as therapeutic plant. The vegetable should also be properly processed to eliminate or reduce the antinutrient contents such as oxalate, nitrate and cyanide which readily accumulate in the plant parts and lead to health problems including degenerative diseases in humans.

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