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Chitosan/vitamin C combination protects against hepatocellular damage caused by carbon tetrachloride in rats

Omar M E Abdel Salam[1*], **Nermeen Shaffie**[2], **Amany A Sleem**[3]

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Author Affiliation:
[1]Department of Toxicology and Narcotics, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, Egypt
[2]Department of Pathology, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, Egypt
[3]Department of Pharmacology, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, Egypt

[*]**Corresponding author**
Department of Toxicology and Narcotics, National Research Centre, Tahrir St., Dokki Cairo,
Egypt
Email: omasalam@hotmail.com

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ABSTRACT

Chitosan is derived from chitin, a polysaccharide found in the exoskeleton of shellfish. In this study the effect of chitosan/vitamin C combination (CS/Vit C) on liver damage caused by the hepatotoxic agent carbon tetrachloride (CCl_4) in rats was examined. Rats were treated with CCl_4–olive oil (1:1, v/v) at a dose of 2.8 ml/kg by gavage. Chitosan/vitamin C at doses of 54 or 108 mg/kg (vitamin C content 9 and 18 mg/kg) was given once daily orally for one week, starting at time of administration of CCl_4. Hepatic injury was assessed by measuring the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities in serum. Oxidative stress biomarkers: lipid peroxidation (malondialdehyde; MDA), reduced glutathione (GSH), and nitric oxide levels were measured in the liver. Additionally, paraoxonase 1 (PON-1) activity was determined in the liver and serum. Haematoxylin and eosin staining of liver sections, immunohistochemical staining of anti-caspase-3 antibodies and DNA ploidy studies were also done. Results showed that CS/Vit C given to CCl_4-treated rats exerted hepatic protective effect, causing a significant decrease in the CCl_4-induced elevation in serum aminotransferases and alkaline phosphatase activities. Moreover, CS/Vit C caused a significant decrease in hepatic MDA and nitric oxide levels along with significant increase in reduced glutathione content. The histopathologic study revealed marked amelioration of vacuolar degeneration and necrosis in CS/Vit C -treated rats compared with the CCl_4 controls. Chitosan/vitamin C exerted marked antiapoptotic effect decreasing caspase-3 stained liver cells and protected against the CCl_4- induced decrease in DNA values (hypoploidy). These results suggested that CS/Vit C via its antioxidant and apoptotic actions might be a useful agent in the treatment of liver disease.

Keywords: chitosan; vitamin C; liver injury; carbon tetrachloride; anti-oxidant; anti- apoptotic

1. INTRODUCTION

The liver is the largest organ in the body that plays essential roles in carbohydrate, lipid and protein metabolism. It is also the major site for the

detoxification of xenobiotics which makes the target for toxic and metabolic injury (Dancygier and Strassburg, 2010). Liver diseases whether caused by viral agents, drugs, ethanol and other toxins, ischaemia, metabolic or cholestatic disorders share a common pathogenetic mechanism of hepatocellular damage which is oxidative stress (Lieber et al., 1997; Madan et al., 2006; Lee et al., 2010; Ahmed et al., 2013). Normally, oxygen free radicals are by products of aerobic metabolism. In the mitochondria, superoxide ($O_2^{\bullet-}$) and hydrogen peroxide are produced by leaking electrons from the electron transport chain to molecular oxygen. Superoxide then can result in the formation of hydrogen peroxide (H_2O_2) by superoxide dismutase or react with nitric oxide forming peroxynitrite ($ONOO^-$) a strong oxidant. Hydrogen peroxide in turn may be reduced to the hydroxyl radical ($HO^{\bullet}$) (Figueira, 2013). During pathological liver conditions, there is excessive formation of reactive oxygen metabolites and other reactive species from such sources as Kupffer cells, the resident macrophages, infiltrating neutrophils and detoxification of drugs and toxins (Vascotto and Tiribelli, 2015). When the production of free radicals exceed the capacity of antioxidants in the liver, oxidative stress is said to be present, which may cause oxidative damage to cell membrane lipids, enzyme proteins and nucleic acids with consequent cellular metabolic dysfunction and damage (Halliwell, 2007). In the cell several antioxidant mechanisms operate in order to overcome the consequences of oxidative stress. These include enzymes like superoxide dismutase, catalase and glutathione peroxidase and the low molecular weight antioxidants such as reduced glutathione, carotenoids, α-tocopherol (vitamin E) and ascorbic acid (vitamin C) (Sies, 1997).

Vitamin C or L- ascorbic acid is water soluble vitamin provided by fruits and vegetables in diet (Counsell, 1993). Vitamin C is absorbed from the intestinal lumen and renal tubules and transported in plasma as free ascorbate. It is transported into tissues by sodium-dependent vitamin C transporter (Wilson, 2005). In the body, vitamin C is present in aqueous compartments eg., cytosol, plasma, and other body fluids. It is an important antioxidant which protects against lipid peroxidation by scavenging free radicals such as the hydroxyl radical, superoxide anion, hypochlorous acid, and peroxynitrite (Halliwell, 1996; Carr and Frei, 1999). It also acts to regenerate α-tocopherol, beta-carotenes, glutathione, and urate from their respective radical species. The replenishment of the reduced forms of these antioxidant molecules contributes to cell defense against oxidative stress (Halliwell, 1996).

Chitosan is a deacetylated derivative of chitin a polysaccharide present in fungal cell walls and exoskeletons of crustaceans. Chitosan consists of varying amounts of β (1→4) linked residues of N-acetyl-2 amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose residues. When taken orally, chitosan is not absorbed and thus is not bioavailable. It is approved in some countries as functional dietary supplement for reducing body weight or plasma lipids due to its ability to bind fat. Other biomedical applications include wound dressings, drug delivery systems, and space filling implants (Dutta et al., 2004; Riva et al., 2011).

The aim of this study was therefore to investigate the effect of chitosan/vitamin C preparation on liver damage evoked by CCl_4 in the rat. Carbon tetrachloride is an industrial solvent capable of causing severe hepatic toxicity (Weber, 2003). Acute intoxication in experimental animals is a widely used model to produce toxic hepatitis with the aim of investigating pathogenetic mechanisms involved and potential therapeutics.

2. MATERIALS AND METHODS

Animals

The study was conducted using Sprague–Dawley rats of both sexes (150–160 g of body weight). Rats were fed with standard laboratory chow and water ad libitum. The animal experiments were done in accordance to the Ethics Committee of the National Research Centre and the Guide for Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

Drugs and Chemicals

Carbon tertrachloride (Sigma, St Louis, MO, USA) and chitosan/vitamin C (chitosan 500 mg & ascorbic acid 100 mg) (DBK Pharmaceutical, Cairo, Egypt) were used in the experiments. The rest of chemicals and reagents were of the analytical grade (Sigma, St Louis, MO, USA). The doses of chitosan for rats used in the study were based upon the human dose after conversion to that of rat according to Paget and Barnes conversion tables (1964). The dose of CCl_4 used in the study was based on previous observations (Abdel-Salam et al., 2013).

Experimental Groups

Rats were randomly assigned into 4 groups, each of 6 animals. Group 1 (normal control) received the vehicle (olive oil). Hepatic injury was induced in groups 2,3 &4 by treating rats by gavage with CCl_4–olive oil (1:1, v/v) at a dose of 2.8 ml/kg through an orogastric tube. Rats were administered half the initial dose of CCl_4 (0.14 ml/100 g body weight), 3 days after the first dose of CCl_4 in

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order to maintain hepatic tissue damage. Starting on the first day of CCl_4 administration, rats were treated with either saline (group 2: positive control), or CS/Vit C at doses of 54 or 108 mg/kg, along with CCl_4 (groups 3 & 4) orally once a day and for 1 week thereafter. The CS/Vit C doses were in the ratio of 45/9 mg/kg or 90/18 mg/kg. Rats had free access to food and drinking water during the study. Rats were killed 7 days after drug or saline administration by decapitation under ether anaesthesia.

Biochemical Studies

Serum Liver Enzymes

At the end of the study, retro-orbital vein plexus blood samples were obtained under light ether anaesthesia. The activities of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were measured according to Reitman-Frankel colorimetric transaminase procedure (Crowley, 1967) whereas colorimetric determination of alkaline phosphatase activity was done according to the method of Belfield and Goldberg (1971), using commercially available kits (BioMérieux, France).

Liver Lipid Peroxidation

The measurement of malondialdehyde (MDA) was used to determine the extent of lipid peroxidation in the liver tissue. Malondialdehyde was determined by measuring thiobarbituric reactive species. In this assay, thiobarbituric acid reactive substances (TBARS) react with thiobarbituric acid to produce TBA-MDA adduct with a red color that can be determined using spectrophotometer at 532 nm (Nair and Turner, 1984).

Liver Reduced Glutathione

In this assay, Ellman's reagent (DTNB; 5, 5′-dithiobis (2-nitrobenzoic acid)) is reduced by the sulfhydryl groups of GSH to produce 2-nitro-s-mercaptobenzoic acid. The nitromercaptobenzoic acid anion has an intense yellow color and the absorption can be measured at 412 nm using a spectrophotometer (Ellman et al., 1959).

Liver Nitric Oxide

Nitric oxide levels were measured using the Griess reaction. In this method, nitrate is converted to nitrite with the enzyme nitrate reductase. This is followed by reaction of nitrite with Griess reagent to form a purple azo compound, the absorbance of which is measured at 540 nm with spectrophotometer (Archer, 1993).

Serum and Liver Paraoxonase 1 Activity

The arylesterase activity of paraoxonase was determined in liver supernatants and serum. In this assay, phenyl acetate used as a substrate is cleaved by the arylesterase/paraoxonase to yield phenol, the rate of its formation is determined by monitoring the increase in absorbance at 270 nm at a temperature of 25°C. One unit of arylesterase activity is considered equal to 1 μM of phenol formed per minute. The activity of PON-1 is expressed in kU/L (based on the extinction coefficient of phenol of 1,310 M^{-1} cm^{-1} at 270 nm, pH 8.0, and 25°C) (Haagen and Brock, 1982).

Histopathological Studies

Representative liver samples were washed thoroughly with formal saline and then fixed in 10% neutral-buffered formal saline for 72 hours at least. The specimens were washed in tap water for half an hour, dehydrated in ascending grades of alcohol (70% - 90% - 95% - absolute), cleared in xylene and then embedded in paraffin wax. Serial sections of 6 μm thick were cut and stained with haematoxylin and eosin (Hx & E) for histopathological investigation (Drury and Walligton, 1980).

Caspase-3 Immunoreactivity

Immunohistochemical staining of anti-caspase-3 antibodies was performed with streptavidin-biotin. Sections of 4 μm thick were deparaffinized and incubated with fresh 0.3% hydrogen peroxide in methanol for 30 min at room temperature. The specimens were then incubated with anti caspase-3 antibodies as the primer antibody at a 1: 100 dilution. The specimens were counter stained with H & E. All sections were investigated by the light microscope.

DNA Ploidy Studies

Further sections were stained with Feulgen stain (Feulgen and Rosenbeck, 1942) for DNA studies and countered stained with Light green. DNA analysis was performed by Leica Quin 500 image cytometry in the Pathology Department, National Research Center. For each section (100-120) cells were randomly measured. The threshold values were defined by measuring control cells. The results

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are presented as histograms and tables which demonstrate the percentage of the diploid cells (2C), the triploid cells (3C), the tetraploid cells (4C) and the aneuploid cells (>5C). The DNA histogram is classified according to Danque et al. (1993).

Statistical Analysis

Results are expressed as mean ± SE. Data were statistically analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test for multiple group comparison. GraphPad Prism 6 for Windows (GraphPad Prism Software Inc., San Diego, CA, USA) was used. Statistical significance was considered at a probability value of less than 0.05.

3. RESULTS

Biochemistry Results

Serum Liver Enzymes

In CC_4-treated rats, the activities of AST and ALT in serum were significantly increased by 105.3% (116.2 ± 6.4 vs. 56.6 ± 1.6U/l) and 74.6% (119.8 ± 3.9 vs. 68.6 ± 1.6 U/l), respectively, compared to the corresponding vehicle control values. Meanwhile, the activity of ALP in serum was raised by 71.9% from 76.4 ± 1.6 to 131.3 ± 4.8 IU/l. The treatment of rats with Cs/Vit C at doses of 54 or 108 mg/kg significantly decreased serum enzyme activities. Serum ALT decreased by 37.2% and 57.8% from 116.2 ± 7.5 U/l in the CCl_4 control to 73.0 ± 2.7 U/l and 49.0 ± 2.6 U/l, respectively, in the CCl_4 + Cs/Vit C treatment groups. Serum AST decreased by 14.1% and 30.0%from CCl_4 control value of 119.8 ± 3.9 U/l to 102.9 ± 3.7 U/l and 84.0 ± 3.1 U/l, respectively. In addition, there was a significant decrease in serum ALP activity by 13.8% and 32.6% from 131.3 ± 4.8 in the CCl_4 control group to 114.9 ± 3.0 and 89.8 ± 4.1 in the CCl_4 + Cs/Vit C groups (Fig. 1).

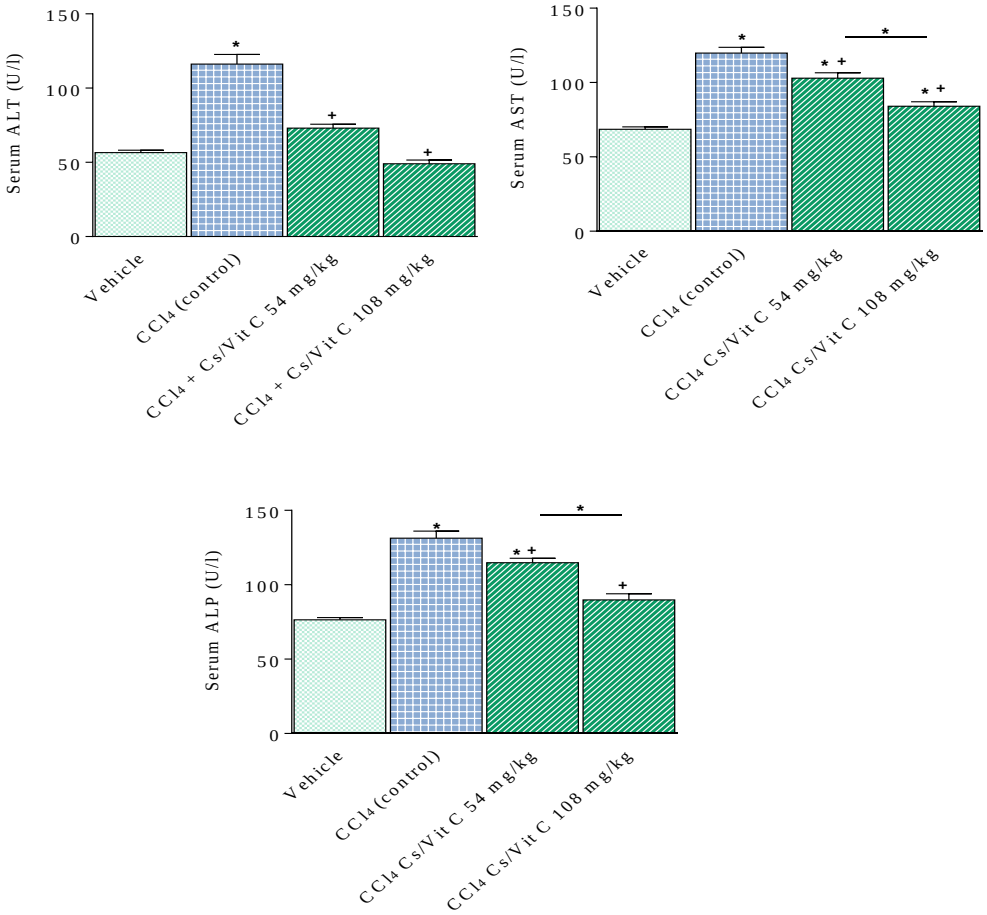

Figure 1 Effect of Cs/Vit C on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase activities in CCl_4-treated rats. *: P<0.05 vs. vehicle control. +: P<0.05 vs. CCl_4 only group and between different groups as indicated in the graph.

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Liver Lipid Peroxidation

Rats treated with CCl_4 alone showed significantly increased liver MDA level by 120.8% compared to the vehicle group (60.5 ± 3.5 vs. 27.4 ± 1.6 nmol/g. tissue). In groups treated with Cs/Vit C , MDA significantly decreased by 23.6% and 40.7%, respectively (from 60.5 ± 3.5 to 46.2 ± 1.4 and 35.9 ± 1.0 nmol/g. tissue) (Fig. 2).

Liver Nitric Oxide

In rats treated with CCl_4, the level of nitric oxide increased by 84.5% (55.9 ± 2.6 vs. 30.3 ± 1.2 µmol/g. tissue) compared with the vehicle group. The administration of Cs/Vit C resulted in significant decreases in nitric oxide level by 27.0% and 32.7% (from 55.9 ± 2.6 in the CCl_4 only group to 40.8 ± 2.8 and 37.6 ± 2.0 µmol/g. tissue in the Cs/Vit C treated groups) (Fig. 2).

Liver Reduced Glutathione

In rats treated with CCl_4, there was a significant decrease in liver GSH by 58.6% compared to the vehicle control (2.37 ± 0.15 vs. 5.72 ± 0.32 µmol/g. tissue). Cs/Vit C given at doses of 54 or 108 mg/kg caused a significant increase in GSH levels by 33.0% and 68.8%, respectively, compared to the CCl_4 control group (3.16 ± 0.18 and 4.0 ± 0.34 *vs.* CCl_4 control value of 2.37 ± 0.15 µmol/ g. tissue) (Fig. 2).

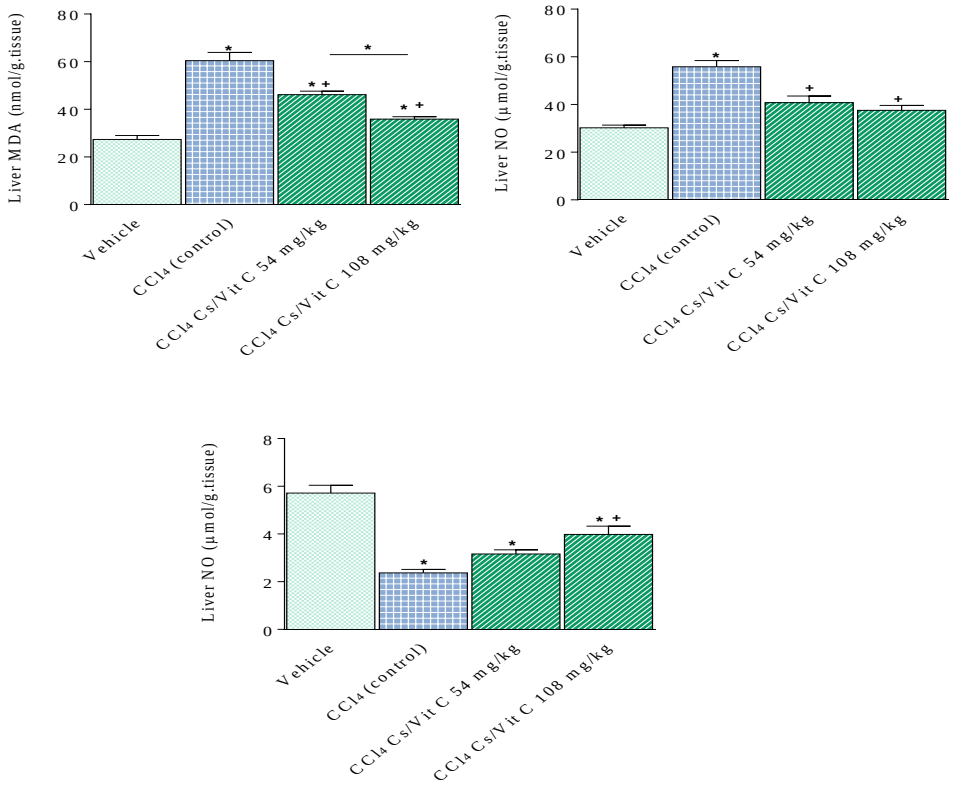

Figure 2 Effect of Cs/Vit C on oxidative stress markers in liver tissue: malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH) in CCl_4-treated rats. *: P<0.05 vs. vehicle control. +: P<0.05 vs. CCl_4 only group and between different groups as indicated in the graph.

Serum and Liver Paraoxonase-1

In CCl_4 only treated rats, both serum and liver PON-1 activity showed significant decrease by 54.5% (108.3 ± 2.7 *vs.* 237.7 ± 5.2 kU/l) and 45.8% (18.8 ± 1.2 *vs.* 34.7 ± 1.3 kU/l) compared to the corresponding vehicle control values. In rats treated with Cs/Vit C at doses of 54 or 108 mg/kg, serum PON-1 activity increased by 38.0% and 81.0% (149.4 ± 10 and 195.9 ± 9.1 *vs.* 108.3 ± 2.7 kU/l). There were also significant increments in liver PON-1 activity in rats treated with Cs/Vit C compared to the CCl_4 control group (23.4% and 47.3% increases: 23.2 ± 1.3 and 27.7 ± 1.6 *vs.* 18.8 ± 1.2 kU/l (Fig. 3).

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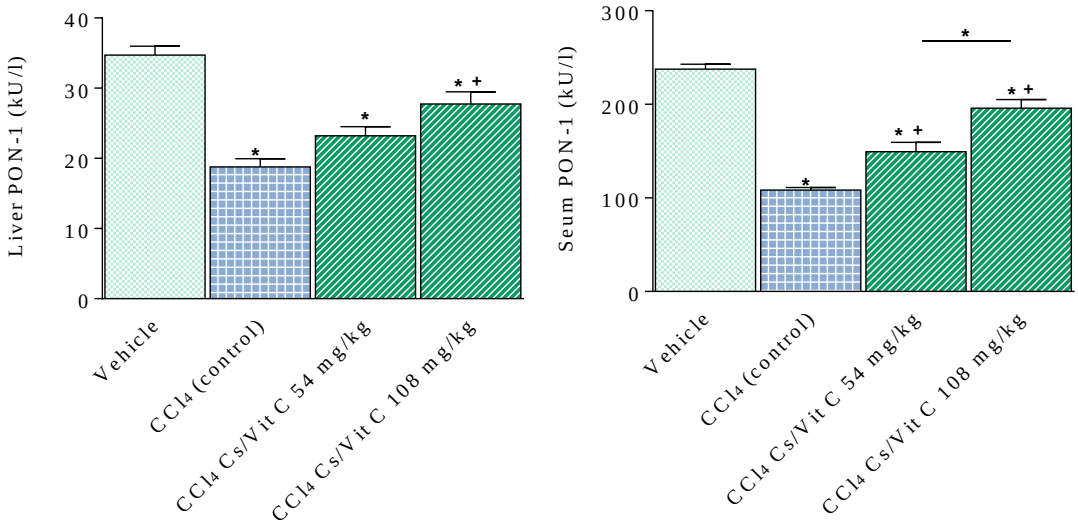

Figure 3 Effect of Cs/Vit C on paraoxonase-1 activities in serum and liver tissue in CCl_4-treated rats. *: P<0.05 vs. vehicle control. +: P<0.05 vs. CCl_4 only group and between different groups as indicated in the graph.

Histopathological Results

By examining sections in this study it was clarified that liver sections from vehicle-treated rats exhibited normal hepatic architecture (Fig. 4A). On the other hand, CCl_4 caused severe damaging effect on the hepatocytes and liver tissue in the form of severe degeneration, areas of necrosis and marked vacuolar degeneration of of hepatocytes (Fig. 4B). In CCl_4-treated rats, co-administration of Cs/Vit C resulted in a dose-dependent ameliorating effect on the damaging effect of CCl_4 that appeared as marked reduction of vacuolar degeneration and cellular infiltration (Fig. 4C & D).

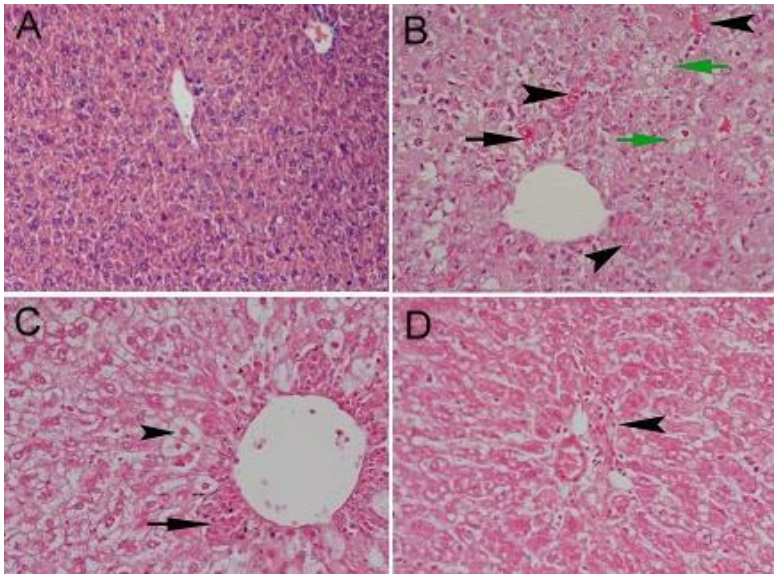

Figure 4 A photomicrograph of Hx & E stained sections of rat liver tissue after treatment with (A) Vehicle showing the normal structure of hepatic tissue. (B) CCl_4 showing severe degenerative effect of the drug on liver tissue in the form of marked vacuolar degeneration of many hepatocytes (green arrow), acidified hepatocytes (black arrow), congestion of blood sinusoids and areas of necrosis (arrowhead). (C) CCl4 and 54 mg/kg Cs/Vit C showing restriction of cellular infiltration around central vein (arrow). Only a few hepatocytes show small pyknotic nuclei with vacuolar degeneration (arrowhead), while most of them are with normal nuclei. (D) CCl4 and 108 mg/kg Cs/Vit C shows very minimal cellular infiltration in the portal area (arrowhead) with marked reduction of vacuolar degeneration of hepatocytes.

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Caspase-3 Immunostaining

The immunohistochemical investigations using anti-caspase-3 antibody revealed that compared with the vehicle-treated group showing negative result for the stain in the hepatocytes (Fig. 5A), the administration of CCl_4 caused apoptosis to many hepatocytes (Fig. 5B). Cs/Vit C exerted marked protective effect against the damaging effect of CCl_4 evidenced by marked reduction of the positively stained apoptotic cells (Fig. 5C & D).

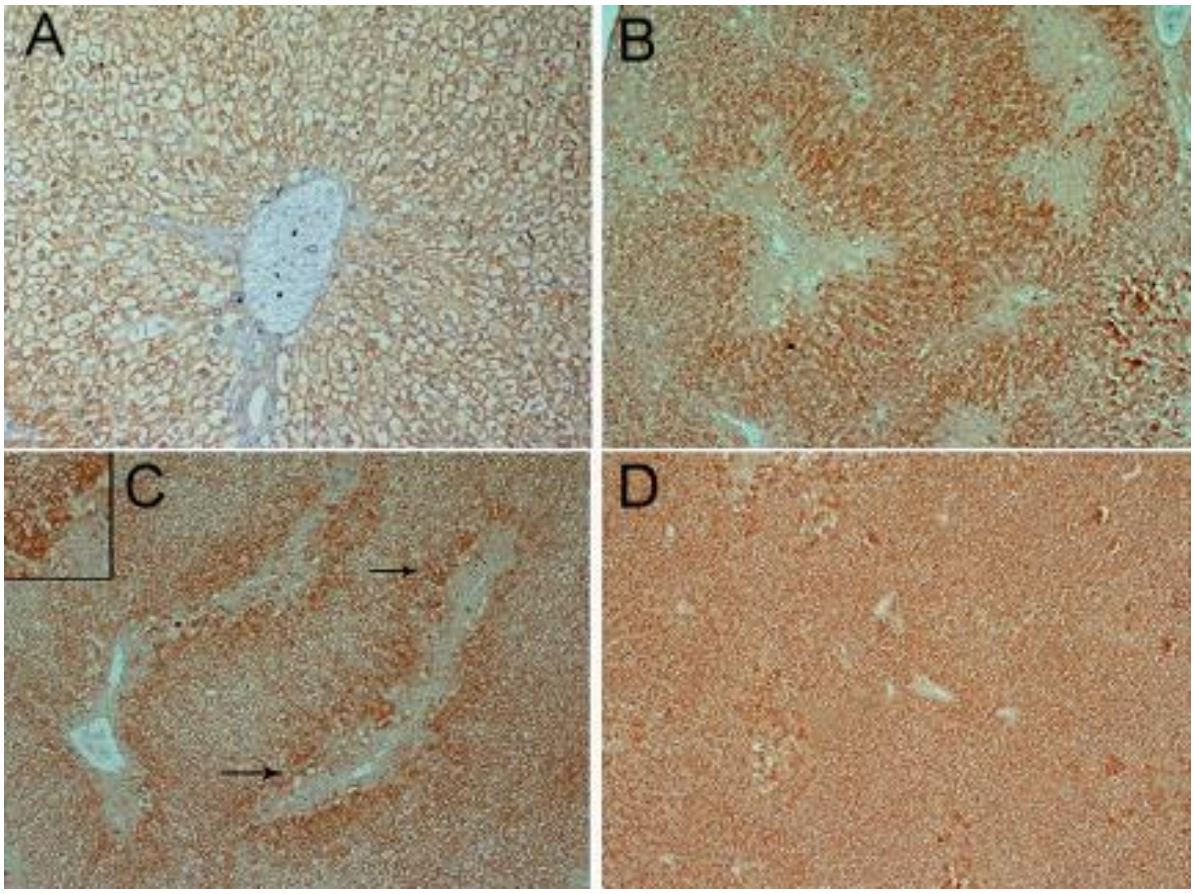

Figure 5 A photomicrograph of a section of rat liver tissue stained with anti-caspase-3 antibody after treatment with (A) Vehicle showing negative result for the stain in the hepatocytes. (B) CCl_4 showing many hepatocytes with positive immune- reaction to the stain. (C) CCl_4 and 54 mg/kg Cs/Vit C showing mild reduction of the cells that give positive reaction with the stain (arrow). (D) CCl_4 and 108 mg/kg Cs/Vit C shows marked reduction of positively stained cells.

DNA Ploidy Results

In the present study, the Qwine 500 image analyzer was used to evaluate the DNA content of examined cells. The image analysis system automatically express the DNA content of each individual cell measured then gave the percentage of each cell out of the total number of cells examined. Also, it classifies the cells into four groups; diploid (2C), proliferating cells (3C), tetraploid (4C) and aneuploid cells (>5C). The proliferating cells were further subclassified into; (<10%) low proliferation index, (10-20%) medium proliferation index and (>20%) high proliferation index. The normal distribution of DNA content in the liver cells of the vehicle control group showed that 13.1 % of the examined cells contained DNA (<1.5C), 75.52% contained diploid DNA value (2C), 11.35% contained (3C) DNA value (medium Proliferation Index) and 0.0% of the examined cells at (4C) area (table 1). Examination of cells from the CCl_4 control group showed a decrease in DNA content (hypoploidy) compared to the control. In the group treated with CCl_4 and 54 mg/kg of Cs/Vit C, 0.962% of cells contained (<1.5C) DNA value, 35.577% of cells contained (2C), 35.577% of cells contained (3C) (high proliferating index). In the group treated with CCl_4 and 108 mg/kg of Cs/Vit C, 16.34% of cells contained DNA value (< 1.5 C), 64.42% of cells contained (2C) DNA value, while 13.46% 0f cells contained (3C) DNA value (medium proliferating index) and 4.80% of cells were in (4C) area (table 1). These results indicate that chitosan given to CCl_4-treated rats resulted in DNA values comparable to the control values especially with high dose of the drug, while the CCl_4 control group showed decreased DNA values (hypoploidy).

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Table 1 DNA content of different treated groups

	DNA index (total)	< 1.5 C	DNA Index	1.5 – 2.5 C	DNA Index	2.5 – 3.5 C	DNA Index	3.5 – 4.5 C	DNA Index	>4.5 C	DNA Index
Vehicle	1.00	13.100%	0.602	75.520%	1.007	11.351%	1.402	0.0%	-	0.0%	-
CCl_4 control	0.602	90.124%	0.547	10.161%	0.832	0.0%	-	0.0%	-	0.0%	-
CCl_4 +54 mg/kg Cs/Vit C	1.448	0.962%	0.717	35.577%	1.018	35.577%	1.018	21.154%	1.959	6.731%	1.352
CCl_4 + 108 mg/kg of Cs/Vit C	1.086	16.346%	0.625	64.423%	1.038	13.462%	1.397	4.808%	1.988	0.962%	3.311

4. DISCUSSION

In this study, the potential hepatic protective effects of Cs/Vit C preparation containing small dose of Vit C was examined in the CCl_4 model of acute liver injury. The study provided evidence that Cs/Vit C exerts dose-dependent amelioration of liver damage. The increments in activities of serum transaminases, which are considered a surrogate marker of hepatic cell injury and reflects the extent of liver tissue damage is decreased by co-administering Cs/Vit C. There was also decreased extent of the histologic liver damage with amelioration of necrosis and vacuolar degeneration of hepatocytes. Caspase-3 immunohistochemistry showed strong immunoreactivity in response to CCl_4, while very few hepatocytes with caspase-3 immunolabeling were observed after treatment with Cs/Vit C, hereby, indicating decreased apoptosis. Cs/Vit C in addition protected against the decrease in DNA (hypoploidy) induced by CCl_4.

Oxidative stress is considered a major pathogenetic mechanism that underlies hepatocyte death in several diseases of the liver. In the liver, reactive oxygen and nitrogen species and other free radicals are released by Kupffer cells, mitochondria, infiltrating neutrophils, and cytochromes P450 (Vascotto and Tiribelli, 2015). The CCl_4-induced hepatic injury is mediated by its reactive intermediate metabolite, the trichloromethyl radical ($CCl^{\bullet}_3$) which binds covalently to cell membrane lipids and causes extensive lipid peroxidation, cell protein and DNA damage leading eventually to cellular impairment (Weber et al., 2003). In the present study, CCl_4 administration was associated with marked increase in the lipid peroxidation end product, MDA, indicating free radical mediated attack on membrane lipids. There was also depletion of the hepatic content of reduced glutathione (GSH), a major antioxidant in the liver which maintains the redox-balance in the cell. This decrease in hepatic GSH is likely to be caused by the loss of heptocytes with impaired synthetic ability of the liver and/or consumption of the antioxidant by the increase in the formation of reactive oxygen metabolites. Reduced glutathione is important in the protection of cells against damage from reactive oxygen metabolites, electrophiles, and free radicals formed during xenobiotic metabolism by acting as a direct antioxidant or indirectly in reactions involving such enzymes as glutathione reductase and glutathione peroxidase, maintaing the reduced state of the sulfdydryl groups of cell proteins (Wu et al., 2004; Yuan and Kaplowitz, 2009). The decrease in hepatic GSH will increase the susceptibility of liver cells to further oxidative insults and the exacerbation of the toxicant-induced liver damage result is the development of oxidative stress and oxidative damage to the cell biomolecules (Lu, 2020).

Our results also demonstrate increased hepatic nitric oxide content in CCl_4-treated rats which is in accordance with other studies (Abdel-Salam et al., 2014). The increase in nitric oxide formation in response to toxic damage and inflammation which occur secondary to CCl_4 oxidative injury is largely caused by increased expression of the inducible form of nitric oxide synthase enzyme (iNOS). This produces large amounts of nitric oxide for relatively longer time compared with that released by the endothelial isoform (eNOS) (Clemens, 1999). Whereas small amounts of nitric oxide constitutively generated by eNOS are important in maintaining the microcirculation and endothelial integrity and therefore protective, large amounts formed by iNOS could be detrimental to liver cells (Laskin et al., 2001). Nitric oxide reacts with superoxide ($O_2^{\bullet-}$) produced within the mitochondrial respiratory chain to yield peroxynitrite (ONOO–), a highly reactive species, capable of S-nitrosylation of thiol groups or nitration of tyrosine residues of proteins. (Rockey and Shah, 2004).

In the present study, we showed marked decrease in paraoxonase-1 (PON-1) activity in serum and liver tissue of CCl_4-treated rats which is in agreement with previously published studies (Abdel-Salam et al., 2014, 2021). Paraoxonase-1 is synthesized in the

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liver and found mainly in plasma bound to high density lipoproteins, having an important role in preventing the oxidation of low density lipoporoteins (Mackness et al., 1993). The enzyme which has lactonase and esterase activities is involved in detoxification of many xenobiotics and organophosphate insecticides (Furlong et al., 2016). Serum PON-1 activity which is decreased in a number of liver disorders such as acute (Bindu et al., 2011) and chronic liver diseases (Keskin et al., 2009) may be a good indicator of liver functioning (Ferre et al., 2002). In liver of mice receiving high fat/cholesterol diet, the deficiency of PON-1 was associated with increased lipid peroxidation, protein carbonyls and 8-oxo-20-deoxyguanosine, indicating a role or the enzyme in protection of the liver against oxidative stress (Gracia-Heredia et al., 2013). The decrease in PON-1 activity in CCl_4-treated animals most probably reflects impaired biosynthetic capacity of liver tissue. It could be also the result of oxidative inactivation of the enzyme. This was shown to be caused by the formation of mixed disulfide between a protein thiol and oidized glutathione (Rozenberg and Aviram, 2006).

Chitosan is being used as a food supplement with the aim to reduce fat absorption and treat obesity (Maezaki et al., 1993; Colombo and Sciutto, 1996). An important biomedical application, however, is its use in drug delivery (Riva et al., 2011). Chitosan has been reported to have protective effects in experimental models of liver toxicity eg., acetaminophen liver damage (Ozcelik et al., 2014). In this study we have shown that small doses of Vit C bound to chitosan were able to protect the liver tissue against the deleterious effect of the hepatotoxicant CCl_4. It is not clear whether chitosan could be absorbed so as to affect liver parameters. Chitosan has been shown to enhance the absorptive transport of glucosamine through cell tight junction (Qian et al., 2013). Thus, it is more likely, that chitosan led to improved solubility and bioavailability of Vit C, thereby, enhancing the hepatic protective effects of the small doses of Vit C.

5. CONCLUSIONS

The present study has demonstrated that the administration of CS/Vit C protected against liver injury induced by CCl_4 in the rat. CS/Vit C displayed antioxidant and antiapoptotic actions and prevented the CCl_4-induced decrease in DNA in liver cells. These data suggest that CS/Vit C might be a useful agent in the treatment of liver disease.

Author contribution

O.M.E.A.S. and A.A.S. conducted the research and biochemical studies, N.S. performed the histopathology and its interpretation, O.M.E.A.S. prepares the manuscript, O.M.E.A.S. and A.A.S. and N.S. approved the final version of the manuscript.

Ethical approval for animal studies

The animal experiments were done in accordance to the Ethics Committee of the National Research Centre and the Guide for Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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