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The Protective Role Of *Panax ginseng* On Hepatotoxicity Induced By Carbon Tetrachloride In Male Rats

Abdallah M. Sliai

Department of Biology, Turabah University College, Taif University, Saudi Arabia

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Abstract

The present study aimed to investigate the possible protective effects of *Panax ginseng* C. A. MEYER (Araliaceae) on liver damage caused by carbon tetrachloride in rats. The histopathological changes and certain liver enzymes were investigated. The results showed that carbon tetrachloride causes elevated serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels production of the liver and decreased nuclear area and nuclear volume in the liver of CCl_4-treated rats. Histopathological observations indicated severe damage in the liver. *Panax ginseng* co-treatment to the CCl_4-administered rats attenuated the increase of the liver enzymes and restored the mean values of the nuclear area and nuclear volume and the morphological damage in the liver was reduced. It is therefore suggested that the *Panax ginseng* can provide a protective effect against acute hepatic injury caused by CCl_4 in rats.

* **Corresponding Author:** Abdallah M. Sliai ✉ a.sliai@tu.edu.sa

Introduction

Carbon tetrachloride (CCl_4) is a colorless, volatile, non-inflammable liquid that is produced by the mixture of chlorine with chloroform in the presence of light. CCl_4 is a common environmental pollutant. Workers are at high risk of exposure to high levels through inhalation and dermal contact. Carbon tetrachloride (CCl_4) is widely used in the dry-cleaning industry and is a highly toxic chemical agent. CCl4 causes cellular damage in multiple organs, mostly in the liver, kidneys, and lungs (Teschke *et al.*, 2018). The toxic effects of CCl_4 on the liver have been extensively studied. The overproduction of free radicals is toxic to hepatocytes and initiates reactive oxygen species causing hepatocyte death and hepatic damage. It is the common model for highly reactive free radicals that can induce liver injury as liver necrosis, hepatocyte apoptosis and fibrosis (Fan *et al.*, 2018; Simeonova *et al.*, 2001; Zhu *et al.*, 2021). Weber *et al.*, 2003 mentioned that CCL_4 causes acute liver damage like necrosis and steatosis. This action is due to free radicals release, which are trichloromethyl and peroxy trichloromethyl radicals. Anti-oxidative Treatment was proposed to be a potential means of preventing or attenuating toxic liver injury (Higuchi and Gores, 2003; Singh, 2005; We *et al.*, 2022). Anti-oxidative Treatment was proposed to be a potential means of preventing or attenuating toxic liver injury. In recent years, there has been a worldwide trend toward the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables (Wang and Jiao, 2000). Ginseng is one of the most widely used medicinal plants, particularly in traditional oriental medicine, and has a wide range of pharmacological and physiological actions and antioxidant activity that protects from toxic substances. Ginseng is a traditional herbal remedy used in Chinese medicine for thousands of years (Loo *et al.*, 2004). One of the most commonly used and researched ginsengs is *Panax ginseng*. The main active components of *Panax ginseng* are ginsenosides, which have been shown to have a variety of beneficial effects, including anti-inflammatory, antioxidant, and anticancer effects (Kampen *et al.*, 2003). Moreover, it was found that ginseng protects from toxic substances (Lee *et al.*, 2005) and human diseases (Yang *et al.*, 1993) by several different mechanisms. Recent studies have demonstrated that ginseng intake is associated with reduced risk factors for cancers (Xiaoguang *et al.*, 1998). Many authors demonstrated that ginseng could recover the liver damage produced by toxins (Lin *et al.*, 2003; Gum *et al.*, 2007).

The aim of the current research was to assess the toxic effect of CCL_4 on the liver of adult male albino rats and the possible protective role of Ginseng**.**

Materials and methods

Experimental animals

Twenty-four adult male Sprague–Dawley rats weighing about 120 - 150 g were obtained from the animal house. The animals were housed in individual suspended stainless steel cages at 22 ± 2 ˚C with a 12 hours light/dark cycle and allowed to acclimatize for a period of 15 days prior to experimental use. Throughout the experiment, the rats were allowed free access to feed.

The rats were divided into four different groups comprising six animals each:

Group I: normal control has received an injection of vehicle (olive oil) alone once daily.

Group II: received orally *Panax ginseng* extract (20 mg/kg b.w) suspended in corn oil.

Group III: received subcutaneous injections of CCl_4 in olive oil at a dose of 3 ml/kg twice a week to induce toxicity (Basu *et al.*, 2003).

Group IV: received subcutaneous injections of CCl_4 in olive oil at a dose of 3 ml/kg twice a week to induce toxicity. Rats were pretreated orally with *Panax ginseng* extract (20 mg/kg b.w) once daily and six hours before the dose of CCl_4.

Sample collection

Animals were sacrificed 24 h after the last injection. Blood was collected, allowed to clot and centrifuged at 3000×*g* for 10 min to obtain serum. All serum samples were kept at −70 °C before the determination of the biochemical parameters. Serum levels of (ALT)

and (AST) as markers of hepatic function were determined according to the method of Reitman and Frakel (1957) and the activity of serum alkaline phosphatase (ALP) was determined according to the method of Kind and King (1954).

Histological examination

Animals were sacrificed, liver tissues were removed immediately and fixed in 10% neutral formalin solution for at least 24 h, then processed, embedded in paraffin wax and sectioned (5µm) thickness for histopathological examination. Sections were stained with haematoxylin and eosin (H&E) using the standard techniques and then analyzed under a light microscope.

Histomorphometrical measurements

For the morphometrical study, three slides from the liver of each group (6 sections per slide) were measured. Liver measurements included nuclear area and nuclear volume. Histological sections were studied by using a research microscope equipped with a video camera and connected to a PC-based image analysis system. Sigma Scan Pro (version 5.0, Jendel Scientific, SPSS Inc., Chicago, USA) was used for image analysis and morphometrical data acquisition.

Statistical analysis

For statistical analysis, univariate analysis of variance was used to test differences between values. All values are given as means ± standard error. The values were considered significant when $p<0.05$. All statistical analyses were performed using SPSS.

Results

Liver function (Table 1)

Serum ALT and AST and ALP levels were elevated at 24 h following CCl4 administration. Elevation of ALT, AST, and ALP was significant ($p<0.05$) in comparison with the normal control group. Pretreatment with *Panax ginseng* extract remarkably decreased the levels of serum ALT, AST and ALP in the liver of CCl4-treated rats, although the enzyme levels were still higher than in the control. Differences were significant at ($p<0.05$) compared with the CCl_4 group.

Table 1. Liver functions of rats of the different groups.

parameters / Groups	ALT µ/ml	AST µ/ml	ALP µ/l
Control group	82.27 ± 2.33	198.73 ±5.98	75.46 ±2.02
Extract group	83.7 ± 2.21	201.54 ± 5.38	78.12
CCl_4 group	165.37 ± 1.78 *	297.12 ± 5.95 *	124.7 ± 1.65 *
Extract & CCl_4 group	112.62 ± 1.81**	241.63 ± 4.21 **	111.71 ± 2.62 **

Data presented as means ± Standard Error

* Significant difference at P<0.05 compared with control group.

** Significant difference at P<0.05 compared with CCl4 group.

Histopathological findings

The microscopic examinations of sections of the liver of control and extract-treated rats showed the normal structure of the hepatic lobule. The central vein is surrounded by hepatocytes with eosinophilic cytoplasm and distinct nuclei.

The hepatic sinusoids are shown between the hepatocytes (Fig. 1A). The histopathological examination of the liver of CCl_4-treated rats revealed obvious changes versus control animals. The arrangement of the anastomosing plates of hepatocytes was disrupted and there was disorganization of the hepatic cords and severe hepatocyte necrosis. In the central vein region, many liver cells were ballooned with multiple vacuolations in their cytoplasm and invasion of infiltrative inflammatory cells (Fig. 1B).

Treatment with extract was able to ameliorate the CCl_4-induced liver injuries and improved typical histological appearance (Fig. 1C).

Histomorphometrical findings

Data obtained from histomorphometrical measurements of the liver (Table 2) revealed that CCL4 administration reduced the mean value of nuclear area (27%) compared to those of controls. Statistically, the inhibition was significant ($p<0.05$). Extract Treatment to the CCl_4-administered rats restored the nuclear area (14%); the stimulation was statistically significant ($p<0.05$). The nuclear volume was reduced by (30%), in the CCl_4-treated rats versus those of controls. Statistically, the inhibition was significant with a level of $p<0.05$. Co-treatment with extract restored the nuclear volume (16%). The stimulation was statistically significant ($p<0.05$).

Table 2. Histomorphometry of the liver.

parameters / Groups	Nuclear area (sq pixel)	Nuclear volume (cubic pixel)
Control group	365 ± 18	3544 ± 320
Extract group	358 ± 17	3497 ± 332
CCl_4 group	266 ± 20*	2475 ± 287*
% Reduction vs. control	27%	30%
Extract & CCl4 group	308 ± 21**	2935 ± 255**
% Stimulation vs. CCl4 -treated	14%	16%

Data presented as means ± Standard Error

* Significant difference at P<0.05 compared with control group.

** Significant difference at P<0.05 compared with CCl4 group.

Discussion

Theoxidant–antioxidant system is in equilibrium in normal healthy conditions. Disturbance of this balance may cause tissue injury and oxidative damage to membrane lipids and other cellular components (Wolf, 1999). Liver injury was associated with oxidative stress, a cellular imbalance between the production and elimination of free radicals (Amin and Hamza, 2005). Cleavage of carbon–chloride bond of carbon tetrachloride leads to the formation of trichloromethyl peroxy radical, which can cause pathogenesis of liver injury (Cheeseman *et al.*, 1985). The CCl_3 radical alkylates cellular proteins and polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides, leading to liver damage (Bishayee *et al.*, 1995). The overproduction of free radicals resulting from oxidative stress could directly injure the hepatocellular membrane by lipid peroxidation, followed by the massive release of inflammatory mediators or cytokines, which eventually lead to liver injuries. Our results showed that CCl_4 administration caused severe acute liver damage in rats, demonstrated by significant elevation of serum AST, ALT levels and histopathological changes, which was consistent with the findings of other investigators (Basu, 2003). Histological observations basically support the results obtained from serum enzyme assays. The liver of CCl_4-intoxicated rats showed massive fatty changes, necrosis, infiltration of lymphocytes around the central vein and loss of cellular boundaries. The abnormally higher level of serum ALT, AST and ALP observed in our study is the consequence of carbon tetrachloride-induced liver dysfunction and denotes the damage to the hepatic cells (Singh *et al.*, 1999). In accordance with (Wang *et al.*, 1997), results stated that the serum ALT and AST levels were significantly increased at 12, 24 and 48 h in the CCl_4-treated mice. It has been reported that fatty accumulation in the liver following CCl_4 poisoning is the result of an imbalance between lipid synthesis and degradations and failure of triglycerides to move as very low-density lipoproteins from the liver to the circulation (Boll *et al.*, 2001a, b).

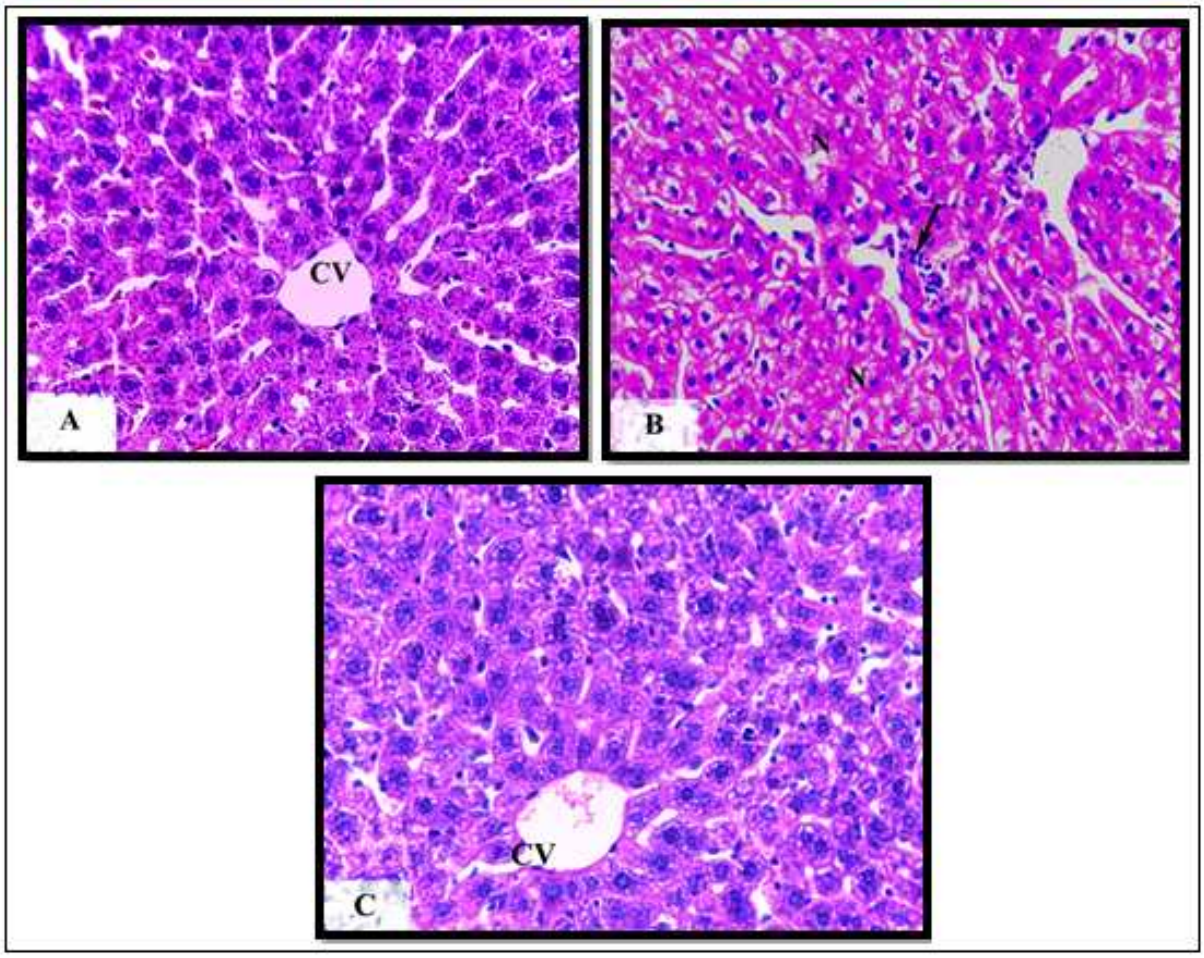

Fig. 1. A. A photomicrograph of a T.S. of control liver of rats showing the normal structure of the hepatic lobules. The central vein (CV) is surrounded by the hepatocyte. (H & E, X 400)

Fig. 1B. A photomicrograph of a T.S. of liver of CCl_4-administration rats showing dilated vein , disrupted of hepatocytes, distinct area of necrosis (N). and the lymphocytic infiltration (arrow). (H & E, X 400)

Fig. 1C. A photomicrograph of a T.S. of liver of CCl_4-administratiom rats treated with *Panax ginseng* extract showing that the hepatocytes are more or less as control. (H & E, X 400).

In the present study, animals treated with CCl_4 plus ginseng showed a marked improvement in all the tested biochemical parameters as well as the histological picture of the liver. This is in accordance with Kim *et al.*, 1997 reported that ginseng has a potent protective action against CCl4-induced toxicity. Also, Mannaa *et al.*, 2006 reported that ginseng has protective effects against toxic substances such as PCBs-induced liver. Ginseng extracts may increase the biosynthesis of protein and nucleic acid and enhance the reduction and elimination of toxic effects as well as stimulates the regeneration of cells and improves inflammation (Kim *et al.*, 1997; Mannaa *et al.*, 2006). Zhang *et al.*, 1996 showed that hydroxyl radicals formed by the Fenton reaction were inhibited by ginseng extract.

Conclusion

In conclusion, the current study revealed that CCl_4 induced severe toxic effects on the liver, as indicated by the elevation of serum biochemical parameters as well as pathological and morphometric alterations of hepatocytes. *Panax ginseng* extract exhibits a potential protective effect against CCl_4 -induced stress.

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