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Antioxidant Effects of *Panax ginseng* and Zinc Against CCl_4 Induced Hepatotoxic on Rats

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Abstract: Liver fibrosis represents a health problem with significant morbidity and mortality that affects 100 million people over worldwide. It is the pathway while lead to chronic liver diseases and characterized by excess collagen and accumulation of extracellular matrix in response to chronic hepatocellular damage. Ginseng is an ancient herb which has been used as a valuable tonic and for the treatment of various diseases including hepatic disorders. In this regard, ginseng power, extracts and zinc supplementation have shown a wide array of beneficial role in the regulation of regular liver functions and the treatment of liver disorders of acute reduce the CCl_4-induced hepatotoxicity. This study was conducted on thirty six albino male rats and classified into six groups, one of them served as normal control group, while the four groups injected with CCl_4 positive control group (untreated). Group 2 received only CCl_4. Groups 3, 4, 5 and 6 were administrated 10% ginseng power, 10% ginseng power with zinc, 120 ml ginseng extract and 120 ml ginseng extract with zinc), respectively for eight weeks. The results revealed that, the positive control group showed significant decrease in final weight, weight gain, food efficiency ratio (FER), hemoglobin (HB), packed cell volume (PCV), plasma superoxide dismutase (SOD) activity and glutathione (GSH). While, significant increase was noticed in serum (AST&ALT) enzymes, alkaline phoshatase (ALP), Urea acid, creatinine levels and malondialdehyde (MDA) compared to normal control group. The treatment of 10% ginseng power, 10% ginseng power with zinc, 120 ml ginseng extract and 120 ml ginseng extract with zinc groups showed a significant increase in final weight, weight gain, food efficiency ratio (FER), hemoglobin (HB), packed cell volume (PCV), plasma superoxide dismutase (SOD) activity and glutathione (GSH). While significant decrease in serum aspartate and alanine amino transferase (AST&ALT) enzymes, alkaline phoshatase (ALP), Urea acid, creatinine levels and malondialdehyde (MDA) compared to positive control group. Histopathologically, CCl_4 caused vacuolar degeneration of hepatocytes and hepatic necrosis with inflammatory cell infiltration. The treatment of 10% GPP, 10% GPP with zinc, 120 ml ginseng extract and 120 ml ginseng extract with zinc groups different attenuated these adverse effects and markedly ameliorated histopathological and biochemical alterations caused by CCl_4 especially. This study in carried out *Panax ginseng* and zinc supplementation can reduce the CCl_4-induced hepatic toxicity, partly via anti-oxidative and anti-apoptotic process.

Key words: *Panax ginseng* · Zinc · CCl_4 · Hepatotoxicity · Liver injury

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Any body organ is a potential target for injurious effects from chemicals but some organs are more vulnerable to adverse effects than others. Liver is the first organ to metabolize all foreign compounds and hence it is susceptible to almost as many different diseases. Some are rare but there are a few, including hepatitis, cirrhosis, alcohol related disorders and liver cancer. A major cause of these disorders is due to exposure to different environmental pollutants and xenobiotics, e.g., paracetamol, carbon tetrachloride, thioacetamide, alcohol, etc [1]. Exposure may also reach the liver through its blood supply from the hepatic artery as well as the portal vein. Second, the liver has the ability to concentrates, biotransforms and excretes chemicals, irrespective of routes of exposure [2]. Carbon tetrachloride (CCl_4) intoxication in animals is an experimental model of

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oxidative stress induced hepatotoxicity and nephrotoxicity [3,4]. There is excessive generation of free radicals such as trichloromethyl and trichloromethyl peroxide radicals from the metabolic conversion of CCl_4 by cytochrome P-450 [5] (Shih *et al*., 2005). Which consequently induces oxidative changes to many cellular bio-molecules including lipid peroxidation of cell membrane in many tissues [6, 7]. Recent researches have examined the effects of plants used to support liver functions and treat diseases in the liver. Ginseng is a popular herbal medication and extract derived from the roots of a perennial plant (*Panax ginseng*) found mostly in China, Korea and Siberia.

Ginseng is used is to promote health and improve wellness, as well as to treat stress and as a mild stimulant. Ginseng has not been implicated in causing liver injury although it may have the potential of causing significant herb-drug interactions that can lead to liver injury [8]. Indeed, ginseng is sometimes used to treat acute or chronic liver injury, effective and safety. Ginseng has been affect cytochrome P450 activity and antioxidant attenuates the intensity of the oxidative stress. The health benefit of medicinal plants usually comes from the antioxidant properties of phenolic compounds in the plant. There are various phenolic compounds in plants, ranging from simple polymerized substances to highly polymerized ones. In this study, low molecular weight phenolic compounds in white ginseng were extracted using diethyl ether and ethyl acetate with medium Molecules [9] polarity. In general, phenolic compounds including vanillic acid, p-coumaric acid and ferulic acid have been reported to play an important role in the antioxidant activity of ginseng [10]. Zinc is an essential trace element in the human body, with approximately two grams in healthy adults. The daily amount of zinc required by an adult is 10–15 mg and this is absorbed primarily from the upper gastrointestinal tract, especially the small intestine [11]. Zinc is involved in the activation of approximately 300 different metallo-enzymes and metal-activated enzymes in vivo and is regarded as essential for the metabolism of nucleic acids and proteins. Therefore, it has been determined that zinc deficiency causes various pathological conditions in humans. Among these, it is known that, in patients with C-viral chronic liver disease, the blood zinc concentration decreases with progression of the disease from chronic hepatitis (CH) to compensated liver cirrhosis (LC) to decompensated LC to hepatocellular carcinoma (HCC) [12].

This study was carried out to evaluate the hepatoprotective effect of *Panax ginseng* and zinc supplementation on Ccl_4 induced liver injury in rats. For this purpose, we first outline the pharmacological effects of ginseng and zinc on the liver functions then serum levels of hepatic marker enzymes and histopathological analysis were also conducted.

MATERIALS AND METHODS

Materials

Plants: Ginseng (*Panax ginseng*) was purchased as dried material from local market in Kuwait.

Carbon Tetrachloride (CCl_4): CCl_4, Heparin, Phenobarbital and olive oil were obtained from Sigma (USA).

Octozinic: Octozinic capsules produced by October Pharma S.A.E and contain 110 zinc sulphate heptahydrate. The human therapeutic dose of zinc sulphate heptahydrate was converted to rat dose according to Paget and Barnes [13] that was 20 mg/kg body weight, dissolved in distilled water and given to rats by oral intubations. Biochemical kits were purchased from Alkan Co. for Chemicals and Biodignostics, Dokki, Giza, Egypt.

Animals: Thirty six male albino rats, Sprague-Dawley rats aged 8 weeks weighing 85-100 g were purchased from the animal house of Agricultural Research Center, Giza, Egypt. Animals were allowed to acclimate for seven days' they were fed with standard pellet diet and water ad libitum at 20-25°C under a 12 h light/dark cycle. Food was withdrawn one day before the experiment but water continued to be provided. All animal handling and experiment protocols complied with the international guidelines for the care and use of laboratory animals.

Methods

Preparation of Plant Formulations: Plant materials were milled in a mixer to give a powder and kept in dusky glass bottles in a dry location till use, according to Russo [14], who reported that plant is best kept in a dry and dark location to reduce oxidation of their contents. The fresh celery was washed with tap water, chopped into small pieces, dried with hot air oven (40–60°C) and grinded to powder [15]. Hundred grams of the powder was soaked in 600ml of 80% methanol with constant stirring by a magnetic stirrer for 48 hr. The mixture was filtered followed by removal of the solvent on the rotatory evaporator to give a dark-brown crude extract.

Experimental Design: The experiment was performed in Animal House in the Institute of Ophthalmology, Giza,

Egypt. All rats were fed for one week prior to the beginning of the experiment on basal diet. After a period of adaptation on basal diet, the rats were divided into two main groups as follows:

The First Main Group: Six rats (n = 6 rats) fed on basal diet (negative control).

The Second Main Group: Thirty rats fed on basal diet (n = 30 rats) were injected by CCl_4 intra-peritoneally (i.p.) at a dose of 0.8 mg/kg (0.5ml/kg) as a 30% olive oil solution (1ml of CCl_4 +1ml of 3% olive oil) induce acute liver damage model according to Jayasekhar *et al*. [16]. The second main group was divided into 4 sub-groups (each 6 rats) as follows:

The First Subgroup: CCl_4 group+ 10% ginseng power.

The Second Subgroup: CCl_4 group+10% ginseng power with zinc.

The Third Subgroup: CCl_4 group+120 ml ginseng extract.

The fourth Subgroup: CCl_4 group+120 ml ginseng extract with zinc

During the study, the food intake was calculated daily and the body weight gain was recorded weekly. Food and protein efficiency ratio (FER&PER) were calculated according to Chapman *et al.* [17].

Blood Sampling: At the end of the experiment period (8 weeks), rats were sacrificed after overnight fasting under ether anesthesia. Twenty four hours after the last administration, blood samples were collected by cardiac puncture from the animals, placed in heparinized tubes, allowed to clot and centrifuged at 3000×g for 10 min. Serum was carefully aspirated and transferred into clean quite fit plastic tubes and kept frozen at - 20°C until the time of analysis**.**

Biochemical Analysis

Determination of Blood Hemoglobin: Blood hemoglobin was estimated according to Drabkin [18].

Determination of Liver Functions: Estimation of ALT, AST, ALP and bilirubin levels, respectively in serum samples were measured with Boehringer Mannheim kits and a UV-rate auto-analyzer (Hitachi 736-60, Japan), were estimated according to Reitman and Frankel[19], Kind and King [20], Weichselbaum [21] and Bartholomev and Delany [22], respectively.

Determination of Kidney Functions: Blood urea and creatinine levels were measured in all samples of serum using standard kits (Randox Laboratories, UK). Urea level was estimated using the method of [23]. In alkaline medium, the ammonium ions released by urease react with salicylate and hypochloride to form green indophenols. The absorbance of samples and standards were measured by spectrophotometer at 580 nm against a reagent blank and the concentration of urea (mg/dl) was determined. Creatinine level was measured according to the procedure of Bohmer [24]. The rate of complex formation was measured photometrically at 492 nm and the concentration of serum creatinine was measured as mg/dl.

Determination of Liver Antioxidant Parameters: Livers of rats were rapidly removed and parts of them perfuse with 50 to 100 of ice cold 0.9% NaCl solution for estimation of superoxide dismutase (SOD) activity, glutathione (GSH), malondialdehyde (MDA) according to Nishikimi *et al.* [25] and Beuchamp and Fridovich [26], Habig *et al.* [27] and Ohkawa *et al.* [28], respectively.

Histopathological Analysis: Liver and kidney samples were immediately collected and fixed in 10% buffered formaldehyde solution for a period of at least 24 h before histopathological study. Samples were then embedded in paraffin wax and five-micron sections were prepared with a rotary microtome. These thin sections were stained with hematoxylin and eosin (H&E), mounted on glass slides with Canada balsam (Sigma, USA) and observed for pathological changes under a binocular microscope [29].

Statistical Analysis: The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, Statistical Soft-Ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and $p<0.05$ was used to indicate significance between different groups [30].

RESULTS AND DISCUSSION

Effect of Ginseng Treatment on Nutritional Parameters Against Ccl_4 Induced Liver Injury in Rats: The statistical data in Table 1 illustrated that, control (+ve) rat group showed a decrease in final body weight, body weight gain, food intake and food efficiency ratio. While the in

Table 1: Effect of ginseng treatment on nutritional parameters against CCL_4 -induced liver injury in rats.

		Groups				
Parameters		Initial weight (g)	Final weight (g)	Weight gain (g)	Food intake (g/d)	FER
Normal control		94.31±25. 35a	127.08±29.37a	32.76±3.5a	12.8±2.7a	0. 255±0.03a
Control positive liver injury		94.24±30.20a	111.83±35.34b	16. 6 ±5.6b	11.08±3.03b	0.145±0.15b
Subgroup	10% GP	96.32±15.99a	124.45 ±22.12a	28. 3±8.1a	12.3±2.02a	0.220±0.35a
	10% GP with zinc	93.6±6.67a	131.68±4.84a	38.07±3.07a	13.1±0.7a	0.280±0.32a
	120 ml GE	92.22±19.86a	118.08±18.08a	25. 6±3.5a	11.6±2.3a	0.220±0.06a
	120 ml GE with zinc	93.6±19.86a	124.08±34.08a	30.6±15.07a	12.5±3.5a	0.230±0.05a

Mean values in each column having different superscript (a, b) are significant.

Means with the same letter are insignificantly different. GP: ginseng power GE: ginseng extract

Table 2: Effect of ginseng treatment on blood parameters against CCL_4 induced liver injury in rats.

	-	Groups	
Parameters		HB (mg/100 ml)	PCV (mg/100 ml)
Normal control		14.60±1.40a	39.79±4.21 a
Control positive liver injury		8.12±0.21b	30.06±1.12 b
Subgroup	10% GP.	11.24±0.44a	38.02±1.35a
	10% GP with zinc	10.84±0.72a	36.02±2.18a
	120 ml GE	11.99±1.83a	37.89±2.49a
	120 ml GE with zinc	10.62±1.28a	35.21±3.83a

Mean values in each column having different superscript (a, b) are significant.

Means with the same letter are insignificantly different. GP: ginseng power GE: ginseng extract

HB: Hemoglobin. PCV: Packed cell volume.

most treatments with 10% ginseng power (GP), 10% ginseng power (GP) with zinc, 120 ml ginseng extract (GE) and 120 ml ginseng extract (GE) with zinc of CCl_4 groups showed significant increase in these parameters compared with positive control group (untreated) and non significant difference compared with normal control group. This is consistent with the results of Ramachandran *et al.* [31], who reported no significant difference in body weight gain. It has been observed in this study that retardation in body weight gain over a period is not due to low food intake but due to injury by CCl_4. While, ginseng and zinc treatment improve the body weight gain. Zinc treatments improving the body weight gain of the animals have also been reported in other studies, in which radiations or carbon tetrachloride was used to induce liver injury. The protective effects of zinc could be attributed to its ability to reduce collagen accumulation in liver and also it exerts critical physiological role in regulating the structure and function of cells [32].

Effect of Ginseng Treatment on Blood Parameters Against Ccl_4 Induced Liver Injury in Rats: As shown in Table 2, the positive control group (untreated) showed a decrease in hemoglobin and packed cell volume compared to normal control group. The 10% ginseng power (GP), 10% ginseng power (GP) with zinc, 120 ml ginseng extract (GE) group and 120 ml ginseng extract (GE) with zinc groups showed significant increase in hemoglobin and packed cell volume compared to positive control group (untreated). These results suggest that *Spirulina platensis* and *Panax ginseng* treatments may stimulate the activity of the bone marrow stem cells [33] and consequently strengthen systemic and particularly immune cellular defenses of the organism. Such nutritional supplementations with *Spirulina platensis* or *Panax ginseng* may be beneficial in humans and in animals suffering from anemia or from immune deficiency but further investigations are required for identifying active drugs supplied by these two biomedicines and for investigating their molecular actions on the regulation of the immune system and of the activity of bone marrow stem cells [1].

Effect of Ginseng Treatment on Serum Liver Parameters against CCl_4 Induced Liver Injury in Rats: As shown in Table 3, the positive control group (untreated) showed increase in serum aspartate and alanine amino transferase (AST&ALT) enzymes, alkaline phoshatase (ALP) and total bilirubin compared to normal control group. The treatment of 10% ginseng power (GP), 10% ginseng power (GP) with zinc, 120 ml ginseng extract (GE)

Table 3: Effect of ginseng treatment on serum liver parameters against CCL_4 induced liver injury in rats.

		Groups			
Parameters		ALT (µ /ml)	AST (µ /ml)	ALP (µ /ml))	Total bilirubin (mg/dl)
Normal control		13.20±1.50c	13.67±0.50c	188.10±2.00d	0.45±0.03d
Control positive liver injury		51.67±5.07a	22.80±0.89a	285.27±15.28a	1.06±0.08a
Subgroup	10% GP.	37.67±2.68b	18.43±0.51b	230.17±1.76b	0.55±0.14b
	10% GP with zinc	28.33±0.13d	17.33±0.52b	205.31±5.47c	0.57±0.09c
	120 ml GE	34.02±2.40b	1912±0.64b	191.22±7.13cd	0.46±0.03d
	120 ml GE with zinc	28.01±1.32d	18.67±0.18b	191.22±7.13cd	0.56±0.03d

Mean values in each column having different superscript (a, b, c, d, e) are significant.

Means with the same letter are insignificantly different.. GP: ginseng power GE: ginseng extract

AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. ALP: Alkaline phosphatase

and 120 ml ginseng extract (GE) with zinc groups showed a significant decrease compared to the positive control group (untreated). In liver injury, the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane, thereby causing an increased enzyme level in serum and soluble enzymes like AST will also be similarly released Administration of CCl_4 significantly raised the serum level of enzymes like AST and ALT in rats as observed in our result and this may be due to the stabilization of plasma membrane as well as repair of hepatic tissue damage [4,34]. These results may be due to the biochemical properties of some phytodrugs and extracts prepared from medicinal plants have proven hepatoprotective properties when used in the treatment of liver diseases such as hepatitis, cirrhosis, steatosis and chemical-induced liver injury. Korean ginseng (*Panax ginseng*, C.A. Meyer) is one of the most widely used medicinal plants and has a wide range of pharmacological and physiological actions. These results are in agreement with those obtained by Hye *et al*. [35] and Chidambara *et al.* [36]. As for the reason why ALT and AST levels reduced with zinc administration, the following may be suggested: It is known that zinc has an anti-oxidant effect. It has an inhibitory action on iron-dependent radical reactions and on lipid peroxidation. It has been assumed that the state of zinc deficiency in chronic liver disease first leads to an increase in hepatic phospholipids, resulting in intensification of lipid peroxidation and thereby causes hepatic cell injury Therefore, it is assumed that zinc administration inhibits lipid peroxidation and subsequently alleviates hepatic cell injury and improves the AST and ALT levels. Zinc was observed to decrease the raised activities of AST and ALT. However, there was no significant change on the serum levels of ALP. Thus, the observed effect may be as a result of antioxidant properties/activity of zinc [37, 38].

Effect of Ginseng Treatment on Kidneys Function Against CCl_4 Induced Liver Injury in Rats: Data presented in Table 4 showed that all kidneys function tests were elevated by CCl_4 administration. Urea acid and creatinine levels were found to be significantly lowered by ginseng treatment 10% ginseng power (GP), 10% ginseng power (GP) with zinc, 120 ml ginseng extract (GE) and 120 ml ginseng extract (GE) with zinc groups compared to positive control group (untreated). While the positive control group significant increase in urea acid and creatinine levels compared to normal control group. These results are in agreement with those obtained by Barakat *et al.* [39], who reported in previous studies, which this effect might be related to the antioxidative properties of ginseng, which protect the outer membrane of mammalian cells**.** The antioxidative ability of ginseng is closely related to its ginsenoside content. Ginsenosides have the ability to intercalate into the plasma membrane, change its fluidity and inhibit lipid peroxidation by chelating transition metals and scavenging ROS [40], ginsenosides thus affect membrane function, eliciting cellular responses to cytotoxic stresses [39].

Effect of Ginseng Treatment on Antioxidant Parameters Against CCl_4 Induced Liver Injury in Rats: The effects of ginseng supplementation levels on liver antioxidant enzymes activity in CCl_4 induced hepatic injury are shown in Table 5. Hepatic injury induced by CCl_4 caused significant decreased in liver antioxidant enzymes activity as superoxide dismutase SOD and glutathione GSH. The treatment of 10% ginseng power (GP), 10% ginseng power (GP) with zinc, 120 ml ginseng extract (GE) and 120 ml ginseng extract (GE) with zinc groups were a significant increasing in liver superoxide dismutase (SOD) and glutathione, while malondialdehyde (MDA) significant decreased compared to positive control group (untreated). *Panax ginseng* (GE) has been shown to

Table 4: Effect of ginseng treatment on kidneys function against CCL_4 induced liver injury in rats.

		Groups	
Parameters		Uric acid (mg/dl)	Creatinine (mg/dl)
Normal control		1.99±0.25c	1.01±0.01c
Control positive liver injury		3.76±0.48a	1.72±0.05a
Subgroup	10% GP.	3.08±0.42b	1.11±0.03a
	10% GP with zinc	2.67±0.21b	1.11±0.01b
	120 ml GE	2.88±0.56b	1.09±0.05b
	120 ml GE with zinc	2.57±0.25b	1.08±0.05b

Mean values in each column having different superscript (a, b, c, d) are significant.

Means with the same letter are insignificantly different.. GP: ginseng power GE: ginseng extract

Table 5: Effect of *Panax ginseng* treatment on antioxidant parameters against CCL_4 induced liver injury in rats.

		Groups		
Parameters		SOD (mg/dl)	GSH (mg/dl)	MDA (µmol/mg)
Normal control		16.67±1.04a	21.44±2.30a	7.12±1.98d
Control positive liver injury		8.53±1.55cd	11.04±1.56d	11.17±1.48a
Subgroup	10% GP	10.47±1.58bc	13.46±1.55b	8.06±3.21b
	10% GP with zinc	11.30±1.57b	13.90±1.55b	7.88±1.58c
	120 ml GE	10.63±1.52bc	12.42±1.53b	8.93±1.67b
	120 ml GE with zinc	11.96±3.21b	13.67±1.55b	7.93±1.48c

Mean values in each column having different superscript (a, b, c, d, e) are significant.

Means with the same letter are insignificantly different.. GP: ginseng power GE: ginseng extract.

SOD: Superoxide dismutase. GSH: Glutathione MDA: Malondialdehyde

inhibit lipid peroxidation through transition metal chelation and scavenging of hydroxyl and superoxide radicals [41]. It has been also reported that *Panax ginseng* administration increased the activity of the antioxidant enzymes SOD and GSX in rats [42]. Kumar *et al*. [43] found that administration of *Panax ginseng* root extract before irradiation significantly decreased lipid peroxidation levels and reduced the radiation damage in mice testes. Zinc is an important component of the body's antioxidant system and plays an important role in retarding the oxidative processes particularly related to diabetes mellitus. Specifically, zinc is required for the adequate formation and function of the antioxidant enzyme copper zinc superoxide dismutase (Cu, Zn, SOD) and various metallothioneins [44]. The obtained results are confirmed by the histopathological examination. Liver of normal control group showed normal histological structure of hepatic lobules, which consists of central vein and concentrically, arranged hepatocyte (Photo 1), while liver of positive control group (untreated) CCl_4 group showed vacuolar degeneration of hepatocytes and hepatic necrosis with inflammatory cell infiltration (Photo 2). Liver of group 3 showed individual necrosis of hepatocytes with dilatation of hepatic sinusoids (Photo 3)

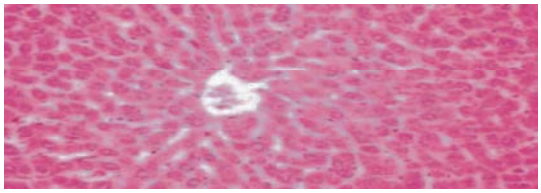

Photo 1: Liver of negative control, showing the normal histological structure (H and E X 200).

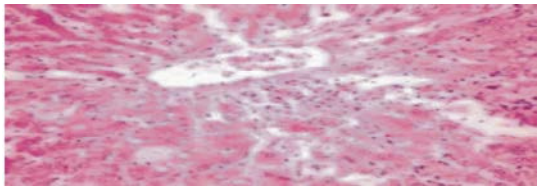

Photo 2: Liver of control (+ve) rat group showing vacuolar degeneration of hepatocytes and hepatic necrosis with inflammatory cell infiltration (H and E X 200).

and groups 4 showed slight hydropic degeneration of hepatocytes (Photo 4). While, liver of group 5 showed no histopathological changes (Photo 5). Liver of rat from group 5 showing kupffer cells activation and perivasculer mononuclear cells infiltration (Photo 6).

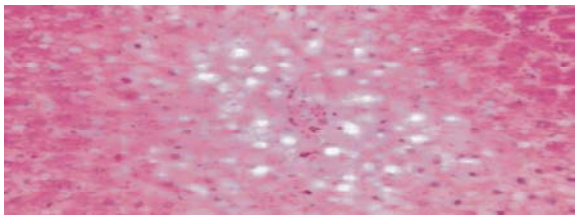

Photo 3: Liver of rat from group 3 showing individual necrosis of hepatocytes with dilatation of hepatic sinusoids (H and E X 200).

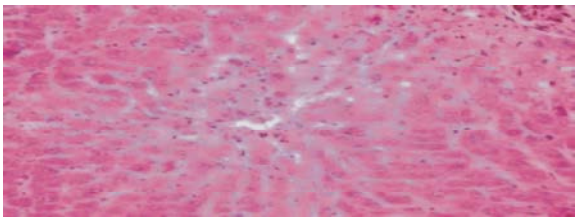

Photo 4: Liver of rat from group 4 showing slight hydropic degeneration of few hepatocytes (H and E X 200).

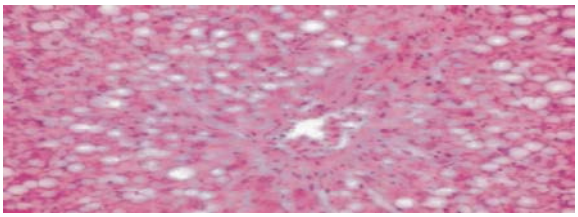

Photo 5: Liver of rat from group 5 showing no no histopathological changes (H and E X 200).

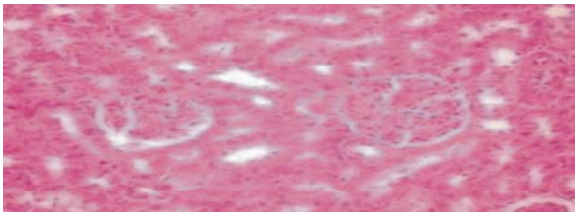

Photo 6: Liver of rat from group 5 showing kupffer cells activation and perivasculer mononuclear cells infiltration (H and E X400)

CONCLUSION

Our study has indicated that the high lights the protective role of *Panax ginseng* treatment powder, extract and zinc in maintaining the activities of enzymes involved in oxidative of stress induced in conditions of CCl_4-induced liver injury in rats. The recommended dose of *Panax ginseng* is 50 g or 600 ml per one kilogram body weight for liver patients per day (equal 10g or 120ml/kg B. wt of rats) to be used for treatment of acute liver injury.

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