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ORIGINAL ARTICLES

Protective effect of a natural extract against cisplatin induced hepatotoxicity

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ABSTRACT

Cisplatin is a prominent member of the effective broad-spectrum antitumor drugs. However, its clinical usage is restricted due to some adverse side effects, such as ototoxicity, hepatotoxicity and nephrotoxicity. The objective of this study was to determine the protective effect of ginseng aqueous extract against cisplatin-induced hepatotoxicity. Adult male Wistar albino rats (100-120g) were divided into four groups (eight animals each) and treated for six weeks as follows: group (1) normal rats injected (i.p) with saline and served as control, group (2) animals orally administrated with ginseng extract (100mg/kg b.wt./day), group 3) rats injected (i.p) with cisplatin (0.4 mg/kg b.wt./day) and group 4) rats daily administrated with ginseng extract just one hour before the cisplatin injection. Results: Administration of ginseng extract in combination with cisplatin ameliorated the cisplatin-induced liver deterioration. This was monitored from the significant reduction in the level of serum ALAT, ASAT, GGT, ALP, bilirubin, cholesterol and triglycerides as well as hepatic MDA and nitric oxide coupled with an improvement in the levels of serum albumin as well as hepatic total antioxidant activity and Na/K ATPase. In conclusion, ginseng extract could play a beneficial role for prevention of cisplatin hepatotoxicity via its anti oxidative and anti-nitrosative voltage.

Key words: Tumors, cisplatin, hepatotoxicity, ginseng, albino rats.

Introduction

The majority of drugs used for the treatment of cancer today are cytotoxic (cell-killing) drugs that work by interfering in some way with the operation of the cell's DNA. Cytotoxic drugs have the potential to be very harmful to the body unless they are very specific to cancer cells - something difficult to achieve because the modifications that change a healthy cell into a cancerous one are very subtle. Cisplatin (cis-diamine-dichloroplatinum) is one of the most potent anticancer drugs used in chemotherapy. In 1978, the American Food and Drug Administration (FDA) approved cisplatin for clinical use of cancers such as bladder, cervical, esophageal, head, neck, ovarian, and testicular. However, its clinical usage is restricted due to some undesirable side effects, such as hepatotoxicity and nephrotoxicity (Nagwani, *et al*., 2010; Naqshbandi, *et al*., 2012 and Naqshbandi *et al.,* 2013). According to recent studies, nephrotoxicity and hepatotoxicity are the major dose limiting side effect in cisplatin-based chemotherapy (Fasihi, *et al*., 2012 and Mustafa, *et al*., 2012). The mechanism underlying the side effects induced by cisplatin are not understood clearly, it was considered to be attributed to the combination of multi-ways, such as the generation of reactive oxygen species (ROS), which could interfere with the antioxidant defense system and result in oxidative damage in different tissues (Florea & Büsselberg, 2011), and reaction with thiols in protein and glutathione, which could cause cell dysfunction. Oxidative stress is an important contributor to the pathophysiology of a variety of pathological conditions including inflammation and drug toxicity (Johnkennedy & Adamma, 2011). Human body has multiple mechanisms especially enzymatic or non enzymatic antioxidant systems which can protect the cellular molecules against reactive oxygen species (ROS) induced damage. However the innate defense may not be enough for severe or continued oxidative stress. Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the ROS in human body (Alipour & Wankhade, 2012).

Until now, a large number of studies have been focused on the ways for preventing the cisplatin side effects using herbal products (Hadjzadeh *et al*., 2012 and Manimozhi, *et al*., 2012). These studies suggested that the side effects of cisplatin could be protected by some plant extracts and different chemical nature. Numerous *in vitro* and animal studies have examined the interaction of *Panax ginseng* with carcinogenesis, apoptosis, angiogenesis, and metastasis (Lee *et al.,* 2005 and Yao *et al*., 2007). It was proposed an anti-inflammatory role of *Panax ginseng* in the sequence of progression to promotion in a model of carcinogenesis (Hofseth &

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Wargovich 2007). Traditionally the ginseng root, available in white or red, is used. White ginseng is prepared by air-drying after harvest, and red ginseng is prepared by a steaming or heating process (Wang *et al*., 2007 and Fishbein *et al*., 2009). The leaf, berry and other parts of ginseng are also medicinal sources (Xie *et al*., 2004 and Li *et al*., 2009).

Panax ginseng has a broad range of beneficial effects including tonic, adaptogenic, immunomodulatory, anti-inflammatory, anti-oxidant, anti-aging, anti-diabetes, anti-mutagenic, anti-cancer and neurovascular modulatory activities (Kiefer & Pantuso, 2003; Sengupta *et al.,* 2004 and Lee *et al.,* 2005). As a large number of herbs has been traditionally used to treat or reduce drug-induced complications, therefore the main objective of the present work was to explore the protective battery of *Panax ginseng* aqueous extract against cisplatin-induced hepatic damage through certain biochemical measurements in both blood and hepatic tissue.

Materials and Methods

Preparation of ginseng extract:

This study dealt with the aqueous extract of the herb rather than that of organic solvents; this due to the possible effects of the organic solvents on the conformational and configuration structure of the extract components. Specimens of Korean mountain red ginseng (*P. ginseng* C.A. Meyer) roots mainly in India and China were obtained from a local supplier, Abd El-Rahman Harraz (Bab El-Khalk zone, Cairo, Egypt).

The ginseng aqueous extract was prepared according to the extraction method of Zhang *et al.* (1995). Specimens of *Panax ginseng* roots were previously smashed, and the poweder was soaked with water (1:25 W: V) in a glass flask for three hours. The container then was placed in boiling water bath for 40 min. The solid residue was subjected to the same process once again, and then the water fractions were combined and filtered through Whatman No. 1 filter paper, and finally filtrate lypholization with freeze drier (Snijders Scientific-tilburg, Holland) under pressure, 0.1 to 0.5 mbar and temperature -35 to -41°C conditions. The dry extract was stored at -4°C until used.

Animal and Experimental Design:

Adult male Wistar albino rats (*Rattus norvegicus*) weighting 100-120g were obtained from Animal House, National Research centre, Dokki, Egypt. The animals were housed in suitable plastic cages for one week for acclimation with the new room conditions. Fresh tap water and standard rodent food pellets (Agricultural-Industrial Integration Company, Giza, Egypt) were always available. All animals were received human care in compliance with the standard insitiuation's criteria for the care and use of experimental animals. After the animals being acclimatized with the experimental room conditions, they were divided into four groups (10 animals each) as following:

Group (I): rats were daily administrated (i.p) (0.4 ml//kg b.wt) saline for six weeks and act as control.

Group (II): animals subjected to daily oral administration of ginseng aqueous extract (100 mg/kg b.w) for six weeks (Zhang *et al.,* 1995).

Group (III): animals subjected to intraperetoneally administration of cisplatin (0.4mg/kg b.wt /day) (Pratibha *et al*., 2006).

Group (IV): animals subjected to daily administration of ginseng aqueous extract on hour before intraperitonial administration of cisplatin (0.4 mg/Kg b.w) for six weeks.

Blood sampling:

At the end of the study period, animals were fasted overnight. Following diethyl ether anesthesia, and using heparinized capillary tubes, blood specimens were collected from the retro-orbital plexus into vacutainer collecting tubes and left 20 minutes to clot, then centrifuged at 3000 rpm for 10 minutes using cooling centrifuge (IEC centra-4R, International Equipment Co., USA). The sera were separated, divided into aliquots and stored at -70°C until biochemical measurements could be carried out as soon as possible.

Tissue sampling:

After blood collection, the animals were rapidly sacrificed and the liver left lobe of each animal were dissected out, washed with saline, dried, rolled in a piece of aluminum foil and stored at -70°C until homogenization and biochemical determinations.

Biochemical measurements:

In Blood:

The activity of serum aminotransferases (ALAT and ASAT) was determined according to the kinetic method described by Schumann and Klauke (2003) using instruction manual of Human reagent kits purchased from Human Gesell Schaft fur Biochemical und Diagnostic mbH, Germany. Serum GGT activity was measured according to the kinetic method described by IFCC (1983) using reagent kits purchased from Bio Systems S.A. Costa Brava 30, Barcelona, Spain. Serum alkaline phosphatase (ALP) activity was assayed according Moss & Henderson (1999) method according to the instruction manual of DiaSys reagent kits purchased from DiaSys Diagnostic systems GmbH Germany. Serum total proteins and albumin concentrations were evaluated according to the photometric systems of Johnson *et al.* (1999) using reagent kits purchased from DiaSys Diagnostic systems GmbH Germany. Serum bilirubin level was measured calorimetrically (Young, 2001) with the reagent kits purchased from Diamond Diagnostics MDSS GmbH Schiffgraben 41 30175 Hannover, Germany. Serum total cholesterol and triglycerides levels were determined according to Artiss & Zak (1997) and Cole *et al.* (1997) respectively, using DiaSys reagent kits purchased from DiaSys Diagnostic System GmbH, Germany.

In Tissue:

Malondialdehyde (MDA) is the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems and used as an indirect index for lipid peroxidation (Draper & Hadley, 1990). Lipid peroxidation level in the liver was determined chemically according to the method described by Ruiz-Larnea *et al*. (1994) on the based of MDA reaction with thiobarbituric acid (TBA) which forms a pink complex that can be measured photometrically. In this method 0.5 ml liver homogenate supernatant (1g liver tissue was homogenated in 10 ml phosphate buffer pH 7.4 and centrifuged at 5000 rpm for 10 minutes) was added to 4.5 ml TBA working reagent [0.8 g TBA was dissolved in 100 ml percloric acid 10%, and mixed with 20% trichloroacetic acid (TCA) in volume ratio 1 to 3, respectively). In a boiling and shaking water bath, the sample-reagent mixture was left for 20 minutes, then carried to cool at room temperature and centrifuged for 5 minutes at 3000 rpm. The absorbance of the clear pink supernatant was measured photometrically at 535 nm against reagent blank (0.5 ml distilled water + 4.5 ml TBA working reagent).

Nitric oxide level of the liver homogenate was determined according to the method of Montgomery et al. (1961) using the reagent kits purchased from Biodiagnostic Co., Dokki, and Giza, Egypt. Total antioxidant capacity (TAC) of the liver homogenate was estimated spectrophotometerically (Koracevic & Koracevic, 2001) with the reagent kits purchased from Biodiagnostic, Dokki, and Giza, Egypt. Finally, Na^+/K^+-adenosine triphosphatase (ATPase) activity was measured according to the chemical modified method of Tsakiris *et al* (2004).

Statistical analysis:

Comparisons between means were carried out using one way ANOVA test (Duncan) at level of $P \leq 0.01$ (Steel & Torrie, 1960) using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA.

Results:

Five animals died throughout the study period; three regarding cisplatin alone group and two from the animal group treated with cisplatin in combination with *Panax ginseng;* while no mortalities were recorded with respect to either control or ginseng animal groups.

Table (1) shows the mean values of initial and final body weights (g) as well as body weight gain of male Wistar albino rats treated with ginseng and cisplatin both alone or in combination as well as normal control. The data illustrated that administration of ginseng aqueous extract increased the body weight gain in a level close to that of control; while those treated with cisplatin alone recorded the lowest level of body weight gain. Cisplatin-ginseng combination increased the levels of body weight gain in respect to ciplatin intoxicated ones.

Table 1: Mean values of initial and final body weights (g) as well as body weight gain of treated and normal control male rats.

	Initial Body Weight (g)	Final Body Weight (g)	Body gain (g/100g b.w)
Control	168 ± 1.34	265 ± 1.42	2.22 ± 0.15
Ginseng	165 ± 1.41	263 ± 1.33	2.76 ± 0.13
Cisplatin	150 ± 1.07	163 ± 1.25	0.64 ± 0.08
CisPlt + Ginseng	158 ± 1.11	181 ± 1.41	0.93 ± 0.06

All data are presented as mean ± standard error.
CisPlt = cisplatin.

Table (2) shows that animals orally treated with the aqueous extract of *Panax ginseng* (100 mg/kg/day) for six weeks recorded non significant (p>0.05) decrease (-13.2, -11.6, -22 and -12%) in serum ALAT, ASAT, GGT and ALP activities, respectively. In contrast, animals group intoxicated intraperetoneally (i.p) with cisplatin only (0.4 mg /kg/day) for a similar period revealed a significant (p<0.01) increase (90.6, 41.7, 17.3 & 10.4%) in the same markers respectively, compared to normal animals.

With regard to rats those intoxicated with cisplatin only, the rats treated with ginseng extract combined with cisplatin showed a significant (p<0.01) decrease (-48.8, -25, -66 & -35.6 %) in serum ALAT, ASAT, GGT and ALP, respectively.

Table 2: Mean values of serum ALAT, ASAT, GGT and ALP activities of male Wistar albino rats of treated and control groups.

	ALAT	ASAT	GGT	ALP
	IU/L	IU/L	IU/L	IU/L
Control	51±1.97^{b}	195±8.03^{b}	6±0.72^{ab}	259±1 9.4^{ab}
Ginseng	44±2.8^{b}	173±4.9^{bc}	5±0.30^{b}	228±13.7^{bc}
Cisplatin	96±3.8^{a}	276±15.1^{a}	7±0.53^{a}	286±19.4^{a}
CisPlt + Ginseng	50±1.7^{b}	207±15.4^{b}	2±0.31^{c}	184±9.2^{c}

All data are presented as mean ± standard error.
The different superscript litters within the same column are significantly different.
Level of significance is p< 0.01.
CisPlt = cisplatin.

Animals orally treated with the aqueous extract of *Panax ginseng* (100 mg/kg/day) for six weeks did not affect the serum bilirubin (total or direct), total protein and albumin levels these were found close to their values of normal rats, while animals those were intoxicated with cisplatin showed a significant (p<0.01) increase in the serum level of total (116.6%) and direct (50%) bilirubin matched with non significant (p>0.01) reduction in both total proteins and albumin in comparison with normal animals. Moreover, rats those treated with ginseng extract in combination with cisplatin pointed a significant (p<0.01) decrease in serum total and direct bilirubin level (26.9 & -22.2%) respectively while serum proteins still close to normal as compared to cisplatin intoxicated rats (table 3).

Table 3: Mean values of serum total bilirubin, direct bilirubin, total protein and albumin levels of male Wistar albino rats of treated and control groups.

	Total Bili.	Direct Bili.	Total proteins	Albumin
	mg/dL	mg/dL	g/dL	g/dL
Cont	0.6 ± 0.05^{cd}	0.24 ± 0.02^{bc}	5.9 ± 0.16^{a}	3.4 ± 0.12^{a}
Gin	0.5 ± 0.04^{d}	0.18 ± 0.02^{c}	5.9 ± 0.22^{a}	3.3 ± 0.05^{a}
CisPlt.	1.3 ± 0.05^{a}	0.36 ± 0.02^{a}	5.3 ± 0.15^{a}	3.2 ± 0.08^{a}
CisPlt. + Gin	0.95 ± 0.06^{b}	0.28 ± 0.02^{b}	5.6 ± 0.2^{a}	3.2 ± 0.10^{a}

All data are presented as mean ± standard error.
The different superscript litters within the same column are significantly different.
Level of significance is p< 0.01.
CisPlt = cisplatin

Table (4) monitored that administration of 100 mg/kg/day of ginseng extract for six weeks resulted in slight (p>0.01) decrease in the level of serum total cholesterol, triglycerides and LDL-cholesterol coupled with slight rise in serum HDL-cholesterol level. Contrarily, cisplatin intoxicated-rats revealed a significant (p<0.01) elevation (18.9, 29.9 & 31.5 %) in serum total cholesterol, triglycerides and LDL-cholesterol levels respectively, matched with non significant (p>0.01) reduction (-9.3 %) in serum HDL-cholesterol level when all were compared to normal animals. With respect to rats intoxicated with cisplatin only, administration of ginseng extract in combination with cisplatin resulted in a significant (p<0.01) decrease (-13.8, -17.1 & -21.6 %) in serum total cholesterol, triglycerides and LDL-cholesterol levels respectively coupled with a slight (p>0.01) increase in HDL-cholesterol level.

Table 4: Mean values of serum total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol levels of male Wistar albino rats of treated and control groups.

	Cholesterol	Triglycerides	HDL	LDL
	mg/dl	mg/dl	mg/dl	mg/dl
Cont	79.2±2.5^{c}	70.7±2.3^{b}	23.6±0.73^{a}	41.5±1.7^{bc}
Gin	73.2±2.03^{c}	69.4±2.0^{b}	24.1±0.65^{a}	35.2±2.6^{c}
Cis	94.2±2.04^{a}	91.4±3.3^{a}	21.4±0.98^{a}	54.6±2.2^{s}
Cis+Gin	81.2±1.95^{bc}	75.7±3.8^{b}	22.0±1.2^{a}	42.8±2.1^{b}

All data are presented as mean ± standard error.
The different superscript litters within the same column are significantly different.
Level of significance is p< 0.01.
CisPlt = cisplatin.

Comparing with normal rats, administration of *Panax ginseng* extract never adverse the liver total antioxdatint capacity or the values of its oxidative stress markers (MDA and NO), while cisplatin injection significantly ($p<0.01$) lowered liver total antioxidant capacity and ATPase activity (-17.8 & -27.2 %) and increased both MDA and nitric oxide levels (96 & 70.8 %) respectively. In addition, treatment of rats with ginseng extract besides to cisplatin revealed a significant ($p<0.01$) decrease in hepatic MDA and NO (-35.3 & -16.2%) respectively; matched with a significant ($p<0.01$) increase in liver TAC and ATPaese activity (11.9 & 19.6 %) in compared to cisplatin intoxicated rats (Table 5).

Table 5: Mean values of hepatic malodialdehyde (MDA), nitric oxide (NO) and total antioxidant capacity (TAC) levels and Na^+/K^+-ATPase activity of male Wistar albino rats of treated and control groups.

	TAC	NO	MDA	Na^+/K^+-ATPase
	µmol/g	µmol/g	nmol/g	umol pi / hr / g
Control	112 ± 8.3^{abc}	137 ± 3.9^{c}	75 ± 4.2^{c}	7.7 ± 0.39^{a}
Ginseng	132 ± 9.7^{a}	135 ± 5.6^{c}	68 ± 1.9^{c}	7.9 ± 0.44^{a}
CisPlt	92 ± 6.8^{c}	234 ± 2.96^{a}	147 ± 1.8^{a}	5.6 ± 0.17^{b}
CisPlt +Ginseng	103 ± 5.7^{bc}	196 ± 9.6^{b}	95 ± 8.1^{b}	6.7 ± 0.09^{ab}

All data are presented as mean ± standard error.
The different superscript litters within the same column are significantly different.
Level of significance is $p< 0.01$.
CisPlt = cisplatin.

Discussion:

Cisplatin is the most important platinum anticancer drug, and has an activity against a variety of neoplasms, particularly head, neck, testicular, ovarian, bladder and small cell lung cancers. However, the mechanisms by which CisPt induces cytotoxicity, namely hepatotoxicity, are not completely understood. High doses of cisplatin are more effective than low doses in ovarian and colorectal cancer. Despite its significant anticancer activity, the clinical use of cisplatin is often limited by its undesirable side effects such as nephrotoxicity and hepatotoxicity (Seong *et al.,* 2005).

The present work was undertaken to study detailed mechanism of CP induced hepatotoxicity alterations and possible action mechanism of ginseng aqueous extract in preventing those deleterious changes in rat liver. CP Hepatotoxicity in this study was gauged by weight loss, The results show that single injection produced a typical pattern of hepatotoxicity as indicated by increased levels of ALAT, ASAT, ALP, GGT, bilirubin, choleaterol, triglyerides, accompanied by slight decrease albumin in the serum; and increased levels of oxidative stress markers (MDA and nitric oxide) matched ith reduction in total antioxidant activity in liver homogenate.

Oral administration of rats with ginseng extract for a period of six weeks resulted in slight increment in the body weight gain percentage which was matched with the control group, i.e. no harmful effect on the animal appetite, gastrointestinal tract physiology, food assimilation and the body weight, reflecting the biological safety of both extracts. This finding is in accordance with the previous study of Lee *et al.* (2005).

In addition, *Panax ginseng* aqueous extract did not disturb liver; this was evidenced from the values, these close to control ones, of the biochemical investigations of the serum (ALAT, ASAT, GGT, ALP, bilirubin, proteins and lipogram) as well as liver (ATPase, MDA, NO and TAC). These findings confirm the biological safety of ginseng extract and agonist the reports of many authors (Somchit *et al.,* 2005; Baum *et al.,* 2006; Karina *et al.,* 2007; Tarasub & Narula, 2008; and Naik *et al*., 2011).

Contrarily, the reduction in the body weight, as well as mortality, followed cisplatin intoxication may be due to the disturbance in the animals' appetite and gastrointestinal tract physiology, food absorption and assimilation; and . This effect may be attributed to the gastrointestinal toxicity and the reduced ingestion of food as explained by Ahmet *et al* (2005), or to the severe or prolonged emesis as suggested by Ballatori & Roila (2003) and Pratibha *et al*. (2006).

Treatment of rats with *Panax ginseng* besides to cisplatin intoxication in this study significantly restored the animals' survival rate and the lost body weight gain as a result of cisplatin intoxication. This result is confirmed with the previous reports of Raghavendran *et al.* (2011).

Nausea and vomiting are considered the foremost unpleasant side-effects of chemotherapy from the patients' viewpoint and experienced by 20–90% of cancer patients during chemotherapy (Schnell, 2003 and Kovac, 2006). In spite of rats lack the vomiting centre in brain, not capable of producing emesis (King, 1990) the rat model responds consistently to a variety of emesis-inducing stimuli, such as cisplatin, cyclophospoamide and ionizing radiation (Yamamoto *et al.,* 2002). It was reported that some antioxidants may prevent the toxic effects of drugs and other harm agents (Durak *et al.,* 2002 and Naziroglu, *et al.,* 2004). Ginseng was found to have protective effects that were attributed to their antioxidant properties by inhibiting free radical generation (Kondo *et al.,* 2006). Their conjunction with the chemotherapy may be perform an anti-emetic effect; traditional clinical experiences and in vitro and in vivo studies support anti-emetic as well as anti-anorexic effects of red

ginseng that could be improve the digestive, assimilation and metabolic functions which in turn reduces the mortality rate and weight loss (Mehendale, *et al.,* 2005).

This study pointed that cisplatin-intoxication significantly elevated serum ALAT, ASAT, GGT and ALP activities, hepatic ATPase activity and hepatic MDA and NO levels; also decreased hepatic total antioxidant capacity (TAC); consequently it reflects its hepatotoxicity potential. These findings are in constituent with that of Iraz *et al.* (2006), Iseri *et al.* (2007), Santos *et al.* (2007), Liao *et al*. (2008), Custódio *et al*. (2009), and Kart *et al.* (2010).

It was demonstrated that cisplatin interferes with mitochondrial bioenergetics, as reflected by the stimulation of mitochondrial respiration rates, depression of respiratory indexes and decreased phosphorylation rate (Custódio *et al.,* 2009) besides to its ability to increase mitochondrial inner membrane permeability to H^+ (proton leak). These effects also explain the mechanism by which the cisplatin decreases ATP content and Na^+/K^+-ATPase activity in rat liver (Santos *et al.,* 2007 and Martins *et al.,* 2008).

Moreover, the present study revealed that administration of the rats with the aqueous extract of *Panax ginseng* in line with cisplatin injection induced a significant decrease in serum ALAT, ASAT, GGT and ALP activities, hepatic ATPase activity and hepatic MDA & NO levels matched with a significant enhancement of TAC compared to the cisplatin-intoxicated group. These findings are concomitant with the reports of Liao *et al.* (2008), Custódio *et al.* (2009), Kart *et al.* (2010) who reported administration of ginseng significantly improve the liver functions disturbed by toxic compounds in rats.

Serum aminotransferases (ALAT & ASAT) are cytosolic enzymes of hepatocytes; an increase in their activities reflecting an increase in the plasma membrane permeability which in turn associated with cell death (Rosen & Keeffe, 2000). One or more mechanism could explain the cisplatin-induced hepatic disorder. Cisplatin may result in mitochondrial membrane rigidification and energetic metabolism impairment through the oxidation of a diverse set of hepatic mitochondrial components, including protein sulfhydryl groups. Additionally, cisplatin interferes with mitochondrial bioenergetics through: 1) stimulation of mitochondrial respiration rates, 2) depression of respiratory indexes and 3) decrease of phosphorylation rate. This interference is due to cisplatin ability to increase mitochondrial inner membrane permeability (Santos *et al.,* 2007 and Martins *et al.,* 2008).

Increased production of reactive oxygen species (ROS) and free radicals has been implicated in mediating CP induced toxicity (Atessahin *et al.,* 2005 and Xiao *et al.,* 2003). However, oxidative stress can occur as a result of either increased ROS generation and/or decreased antioxidant enzyme system comprising SOD, catalase and GSH-Px. These antioxidant enzymes protect the cell against cytotoxic ROS. SOD and catalase together convert superoxide radicals first to H2O2 and then to molecular oxygen and water. Other enzymes such as GSH-Px use thiol-reducing power of glutathione to reduce oxidized lipids and protein targets of ROS. Under ineffective antioxidant enzyme status, lipid peroxidation in the cellular and subcellular membranes is the inevitable outcome of ROS injury (Fadillioglu *et al.,* 2004).

Moreover, Custódio *et al.* (2009) demonstrated that cisplatin increases the sensitivity of rat liver mitochondria to Ca^{2+}-induced mitochondrial permeability transition (MPT) without interference on the Ca^{2+} uptake machinery. MPT is induced via the oxidation of protein thiol groups of the MPT complex. The most plausible hypothesis to explain cisplatin-MPT induction is concerned with the binding of cisplatin to protein thiol groups and the formation of complexes that have been described in tumor cells.

Kadikoylu *et al.* (2004) indicated the involvement of hydroxyl radicals in the mechanism of cisplatin-induced oxidative damage in liver. Hydroxyl radicals are highly reactive oxygen species, capable of reacting with proteins and abstracting a hydrogen atom from polyunsaturated fatty acids in membrane lipids to initiate lipid peroxidation (evidenced here by the elevated hepatic MDA level) which in turn impairing the hepatocyte membrane permeability, eventually leakage of the enzymes.

The elevation of hepatic MDA and NO levels suggesting oxidative stress due to ROS generation; it is one of the possible mechanisms in hepatotoxicity (Iraz *et al.,* 2006).

It was suggested that cisplatin significantly decreased the activities of SOD, catalase and GSH-Px and enhanced LPO in the hepatic tissues indicating CP-induced oxidative damage. In contrast, a marked increase in the antioxidant enzyme activities was seen when rats were fed FXO diet alone without CP administration (Naqshbandi *et al.,* 2013). In addition, the inhibition of enzymatic antioxidant (CAT, and GR) system and reduction of the non enzymatic antioxidant system such as GSH (which plays an important role in the elimination of ciplatin in liver tissue) could be included in tissue damage (Hanigan & Devarajan, 2003; Pratibha *et al.,* 2006 and Kart *et al.,* 2010).

Xanthine oxidase (XO) is found in many tissues, and the highest concentration is present in the liver and intestine. The elevated NO level as a consequence of ciplatin treatment could be attributed to elevation in the activity of xanthine dehydrogenase (XD) which converted into xanthine oxidase (XO); the later was reported to be an important source of oxygen free radicals, generating O_2^- and H_2O_2 (Harrison, 2002 and Kart *et al.,* 2010). It is also possible that XO could convert nitrite and nitrate to NO thereby nitrosative stress occurs (Chirino & Pedraza-Chaverri, 2008).

With regard to cisplatin-injected animals, treatment of rats with ginseng extract in combination with cisplatin injection significantly attenuated serum ALAT, ASAT, GGT and ALP activities, hepatic ATPase activity and hepatic MDA and NO levels and significantly up regulated hepatic total antioxidant capacity (TAC). These findings are in line with the reports of Baum *et al.* (2007); Zhang *et al.* (2008) and Naik *et al.* (2011).

It was shown that ginseng ginsebosides (Rb1, Rg1, Rg2, Rg3, Rc, Re, Rh1, and Rd have capability of scavenging reactive oxygen species and enhancing the anti-oxidative defense system to attenuate free radical-induced damage (Yokozawa *et al.,* 2004; Cheng *et al.,* 2007 and Abdel-Wahhab *et al.,* 2010).

The aqueous extract of ginseng also never adverse serum levels of bilirubin and proteins. This result point to the safe effects of this extract on the synthesis and excretion capacity of hepatic and bile canaliculi systems as well as on erythrocytes membrane i.e. RBCs fragility. This finding is in accordance with Abdel-Salam *et al* (2002); Mokhtar *et al*., (2008) and Abdel-Wahhab *et al* (2010). With respect to control rat group, significant elevation of serum bilirubin and lowered serum total protein and albumin levels were indicated as a consequence of cisplatin intoxication. This indication is consistent with the report of (Shibayama *et al.,* 2007); while treatment with ginseng extract in combination with cisplatin injection alleviated the serum level of bilirubin and proteins towards the values of normal rats. This finding agonists that of Karakus *et al* (2011).

Animals ingested the aqueous extract of *Panax ginseng* in this study for showed no disturbances in serum level of cholesterol, triglycerides, LDL and HDL. This finding is agonist that of Kim *et al* (2002b); Abdel-Salam *et al.* (2002), Baum *et al*. (2006), Mokhtar *et al*. (2008), Abdel-Wahhab *et al.* (2010) and Mahmmoud (2011). Contrarily, cisplatin intoxication significantly elevated serum level of total cholesterol, triglycerides and LDL and reduced HDL compared to normal animals. This result declares the atherogenic effect of cisplatin.

The elevated serum cholesterols and teriglycerides level herein may be attributed to one or more, of the following explanations. It was stated that, intoxication with cisplatin could cause centrilobular necrosis, which results in translocation and accumulation of fats from peripheral adipose tissue in the liver, increases hepatic synthesis of fatty acids, impaired the function of smooth endoplasmic reticulum and induce peroxisomes to catalyze β-oxidation of fatty acids converting them into Acetyl-CoA, the precursor of cholesterol biosynthesis, and decreases the release of lipoproteins (Maling *et al.*, 1962, Devarshi *et al.*, 1986 and Reddy & Rao, 2006). Also, cisplatin may activate the rate limiting enzyme, 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, which converts HMG-CoA into mevalonate which is the precursor of cholesterol biosynthesis. Cisplatin may reduce cholesterol catabolism through inactivation of 7-α hydroxylase resulted in malformation of bile acids which is the major pathway of cholesterol degradation. Therefore, malformation of bile acids, those solubilize cholesterol in an aqueous medium resulted in impaired secretion of bile (bilirubin, cholesterol bile acids). The elevation of bilirubin confirmed the disordered excretory function of hepatocytes (Tolman & Rej, 1999). It was indicated that patients with liver diseases often present with lethicin-cholesterol acyltransferase (LCAT) deficiency i.e. impaired cholesterol ester synthesis (James & Deakin, 2004).

The elevation of triglycerides (TG) level may be due to impaired removal and destruction of TG rich in lipoproteins such as VLDL, LDL, IDL and remnants (Recknagel & Lombardi, 1961), this could be confirmed by the elevated level of LDL, or to the increased hepatic synthesis of fatty acids (precursors of TG formability) (Karakus *et al*., 2011).

Chan (1992) reported that apoprotein-B 100 (Apo-B 100) [the essential structural component of very low density lipoproteins (VLDL), intermediate density lipoprotein (IDL) and low density lipoproteins (LDL)] is required for the intracellular assembly and secretion of these lipoproteins, therefore, the elevation in LDL level herein could be attributed to the increased hepatic secretion of apoprotein B-100 which could be induced by cisplatin.

Conde *et al. (*1996) and Mohammadi *et al.* (1998) stated that there is a strong correlation between apo-B and total cholesterol levels, based on this correlation elevated LDL level is a parallel result with the elevated total cholesterol level induced by cisplatin intoxication. On the other side, the reduction in HDL may be related to: the reduction in Apo-A1 which is a principle protein of HDL (i.e., impaired synthesis of HDL), its conformational changes or the elevated level of hepatic lipase (HL) which has an inverse correlation with HDL that arises from the involvement of HL in the uptake of HDL by the liver and steroid secreting tissues (Jansen *et al.,* 1980 and Colvin *et al.,* 1990).

Ginseng extracts, when ingested in combination with cisplatin, showed a significant improvement in serum lipogram. This finding is in accordance with that of many reports (Kondo *et al.,* 2000; Attele *et al.,* 2002; Wang *et al.,* 2003; Park *et al.,* 2005; Xie *et al.,* 2005 and Lee *et al.,* 2005).

Polyacetylenic compounds such as panaxynol, panaxydol, panaxydiol and panaxytriol found in the root of ginseng were found to inhibit acyl-CoA, cholesterol acyltransferase, tumor cell proliferation and platelet function as well as promote neurite outgrowth and improve scopolamine-induced memory deficit (Yamazaki *et al.,* 2001 and Kim *et al*., 2002a). Ginsenoside as one of active components of ginseng saponins may (1) accelerate serum cholesterol turnover by increased cholesterol degradation and excretion in the feces and (2) may increase LDL receptors by promoting the synthesis of LDL receptors (Yokozawa *et al.,* 2004).

Lee *et al* (2005) and Sang *et al*. (2008) demonstrated that epoxyheptadecan-4, 6-diyn-3-one (EHD), a newly isolated polyacetylene extract of ginseng, inhibits diacylglycerol acyltransferase, which is involved in the glycerol phosphate pathway, i.e. can be control the high triglyceride induced disorders, such as hypertriglycemia and obesity.

Conclusion:

From the mentioned data, it could be concluded that ginseng extract supplementation ameliorated the cisplatin-induced liver deterioration; this was monitored from the improved antioxidant capacity which consequently suppressed oxidative stress, improved liver functions and reduced cisplatin induced mortality. These results evidenced that ginseng aqueous extract has a high protective battery against the cisplatin hepatotoxicity; and can be used to enhance cisplatin efficiency as anticancer drug, i.e our findings would provide a more promising strategy for prevention of hepatotoxicity in cisplatin-based chemotherapy. However, our study is a pilot study and the results are preliminary, additional studies using animals and patients are needed.

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