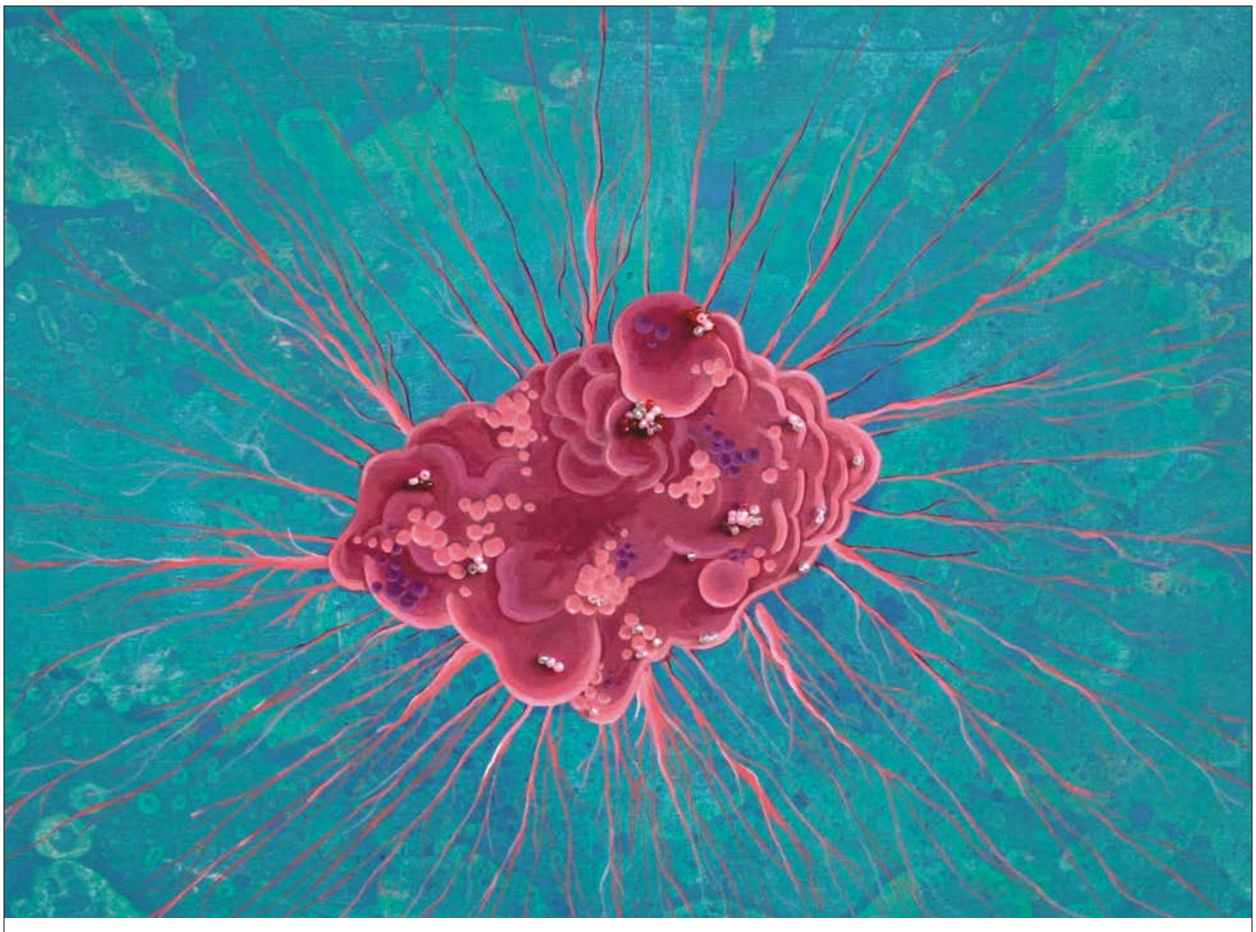

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# Cytoprotective Effect and Antioxidant Properties of Silymarin on Cisplatin Induced Hepatotoxicity in Rats: A Biochemical and Histochemical Study

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## ABSTRACT

Cisplatin (CDDP) is a potent anticancer agent associated with a variety of toxicities. The present study was carried out to investigate the cytoprotective and antioxidant activities of silymarin (SIL) on CDDP induced toxicity. Hepatotoxicity was manifested biochemically from increased activities of Aspartate Transaminase (AST), Alanine Transaminase (ALT), glucose concentration in serum, a markedly increased level of liver lipid peroxidation (malondialdehyde, MDA) associated with significantly reduced activities of the antioxidant enzymes: superoxide dismutase (SOD) and glutathione peroxidase (GPx) in CDDP group. CDDP induced histochemical alterations including: a significant depletion in glycogen stores, total protein and DNA contents in liver cells. On the other hand, post-treatment with SIL significantly decreased serum liver markers, MDA levels and increased SOD and GPx activities and mild histochemical damage than those receiving CDDP. Results suggest that pretreatment with SIL could protect liver tissues fully against CDDP toxicity, since liver markers, MDA levels; activities of antioxidant enzymes and glycogen, protein and DNA contents in liver cells were restored to normal levels. In conclusion, the cytoprotective potential of SIL in CDDP toxicity might be due to its antioxidant, metal chelating property and free radical scavenging properties, which could be useful for achieving optimum effects in CDDP induced hepatotoxicity.

**Key words:** Cisplatin, silymarin, hepatotoxicity, oxidative stress, histochemical studies

## INTRODUCTION

Cisplatin (CDDP), a platinum-containing compound, is one of the most commonly used potent anticancerous agents for the treatment of a wide range of cancers (Kintzel, 2001; Wang *et al*., 2004). In spite of, its excellent antineoplastic activity, the clinical use of CDDP is rapidly limited due to unexpected and very adverse toxic side effects that interfere with its therapeutical efficiency (Ajani, 2008; Dank *et al*., 2008). Even though the exact mode of action of CDDP induced toxicity is not completely elucidated, it is selectively taken up and accumulated in the liver and kidney cells (Stewart *et al*., 1982). Although nephrotoxicity of CDDP has been identified as the most important dose-limiting factor (Abdelmeguid *et al*., 2010a), yet little is known about CDDP induced liver injury (Zicca *et al*., 2004). The mechanism of hepatotoxicity as well as the general organ toxicities induced by cisplatin has been the focus on intense investigations (Kim *et al*., 2004; Pal *et al*., 2008). These include light microscopic examinations of the liver and other organs as well as biochemical analysis of liver enzymes (Kim *et al*., 2005; Venkatesan *et al*., 2008). The beneficial effects of a variety of antioxidants have been proved to reduce the degree of CDDP induced hepatotoxicity

(Yuce *et al*., 2007). In liver tissues, CDDP induced hepatotoxicity was found to be correlated with elevated lipid peroxidation levels (Mansour *et al*., 2006). However, oxidative stress injury is one of the most essential mechanisms that are actively involved in the pathogenesis of CDDP induced toxicity. In addition, this kind of oxidative stress may result in higher generation of reactive oxygen species, loss of mitochondrial protein-SH, decrease in the mitochondrial membrane potential (Saad *et al*., 2004) and reduction in antioxidant enzymes' activities (Mora *et al*., 2003). Therefore, CDDP induced damage was minimized by the administration of antioxidants prior to CDDP treatment (Lee *et al*., 2007).

Dietary antioxidants have found to have possible protective roles in liver cells especially against CDDP induced toxicity (Behling *et al*., 2006). Phytochemicals, including flavonoids, are naturally occurring and biologically active antioxidants that possess various protective and disease preventive actions as well as several clinical properties (Karimi *et al*., 2005). Due to their chemoprotective actions, many flavonoids and antioxidants compounds including curcumin, selenium and other dietary components have been studied for their ability to scavenge and inhibit free radical-mediated processes upon exposure to CDDP (Antunes *et al*., 2001; Silva *et al*., 2001; Singh *et al*., 2005).

Silymarin (SIL), a flavonoid complex derived from the herb milk thistle (*Silybum marianum*), has long been used as a dietary supplement for hepatoprotection due to its antioxidant activity (Laekeman *et al*., 2003; Eminzade *et al*., 2008). SIL has already effectively been applied as a protective agent in various experimental trials and both *in-vivo* and *in-vitro* experimental models of hepatotoxicty (Laekeman *et al*., 2003; Eminzade *et al*., 2008). These antioxidant properties appear to be due to their ability to scavenge free radicals and to chelate metal ions (Borsari *et al*., 2001). SIL is frequently used in the treatment of liver diseases, since SIL can protect liver cells directly by inhibiting lipid peroxidation (Mira *et al*., 1994) and preventing liver glutathione depletion; thus stabilizing the membrane permeability(Valenzuela *et al*., 1989). SIL has been shown to be safe in animal models and no significant adverse reactions were reported in human studies (Hogan *et al*., 2007).

Since, it is known that CDDP induces hepatotoxicity in the experimental animals (Mansour *et al*., 2006), previous studies have shown that SIL has the ability to ameliorate the CDDP induced liver and kidney histological and ultrastructural changes in rats (Abdelmeguid *et al*., 2010a, b). In this view, the present study was designed to evaluate the role of SIL as an antioxidant in the protection against CDDP induced hepatotoxicity at the biochemical and histochemical levels. To this end, serum liver markers' levels, as well as lipid peroxidation end products, antioxidant systems (e.g. superoxide dismutase and glutathione peroxidase), glycogen stores and total protein and DNA contents were determined in the liver tissues of rats following CDDP and/or SIL treatment.

## MATERIALS AND METHODS

**Chemicals:** Cisplatin [(CDDP) or *cis*-Dichlorodiammineplatinum (II)], silymarin (SIL), superoxide dismutase (SOD) determination kit and Glutathione Peroxidase (GPx) Cellular Activity Assay Kit were purchased from Sigma-Aldrich Chemical Company, St. Louis, MO, USA. A Commercial diagnostic kit from Bio-Diagnostic kits, Alexandia, Egypt was used for the estimation of malondialdehyde (MDA), whereas serum Aspartate Transaminase (AST), Alanine Transaminase (ALT) and glucose concentration kits were obtained from Biocon® Diagnostiks, Germany.

**Animals:** Adult male albino rats aged 10-13 weeks, weighing 140-160 g were obtained from the Faculty of Pharmacy, Beirut Arab University and were housed in stainless steel wire bottom cages at a temperature of 23±2°C and photoperiod 12 h day/12 h night. Animals were fed rat pellet diet

and tap water *ad libitum*. The laboratory animal protocol used in this study was approved by the Local Ethical Committee for animal experiments at Beirut Arab University, Lebanon. Rats were allocated randomly divided into five groups of 6 each as follows:

- **Control group:** Received no chemical treatment
- **Vehicle treated group:** Received daily intraperitoneal injection (i.p.) of propylene glycol and saline; 75/25 v/v (0.15 mL)
- **CDDP treated group:** Received single dose of CDDP 5 mg $kg^{-1}$, i.p. (Mansour *et al.*, 2006) at the beginning of the experiment
- **Post-treatment with SIL (2 h after CDDP) group:** Received single dose of CDDP (5 mg $kg^{-1}$ i.p.) followed by i.p. injection of SIL (50 mg kg $day^{-1}$) (Karimi *et al.*, 2005) for 2 weeks
- **Pretreatment with SIL (2 h before CDDP) group:** Received single dose of CDDP (5 mg $kg^{-1}$; i.p.) followed by i.p. injection of SIL (50 mg kg $day^{-1}$) (Karimi *et al.*, 2005) for 2 weeks

At the end of the experimental period (2 weeks), rats were fasted for 12 h before being anesthetized and sacrificed by cervical decapitation. Blood was collected with anticoagulant and centrifuged at 3000 r.p.m. for 15 min for serum separation. Liver tissues were isolated, cleaned from the adhering matters, washed with saline solution and cut into small pieces; i.e., some of the liver tissues were stored at -70°C for further biochemical assays; while others were immediately dropped in the suitable fixatives and were processed for histochemical studies.

**Biochemical assays:** The activities of serum aspartate transaminase (AST, GOT-AST mono, BD-GB-GOTAST mono-03) and alanine transaminase (ALT, GPT-ALT mono, BD-GB-GPTALT mono-03), were assayed kinetically and spectrophotometrically. Concentrations of glucose (Biosub® GLU, BD-461100-04) in serum were estimated calorimetrically and spectrophotometrically. The liver lipid peroxidation end product Malondialdehyde (MDA) was estimated spectrophotometrically by measuring Thiobarbituric Acid Reactive Substances (TBARS) using commercially available diagnostic kits. The absorbance of the resulted pink product can be measured at 534 nm (Satoh, 1978; Ohkawa *et al.*, 1979). In addition, the activities of superoxide dismutase (SOD, EC 1.15.1.1) and glutathione peroxidase (GPx, EC 1.11.1.9) will be determined in filtrates of liver homogenates kinetically and spectrophotometrically, respectively. The estimation and measurement of the biochemical assays were performed according to the standard procedures using their corresponding commercially diagnostic kits. The total protein concentration in liver homogenate was determined by the method of Bradford (1976) using Bovine Serum Albumin (BSA) as the standard at 595 nm.

**Histochemical studies:** Best's Carmine method (Pearse, 1968) was used to detect glycogen content in paraffin sections fixed in Carnoy fixative. Sections from materials fixed in 10% neutral formalin was stained with mercury bromophenol blue (Hg-BPB) according to Mazia *et al.* (1953) in order to detect total proteins. Feulgen nuclear reaction method (Garvin *et al.*, 1976) was used for the demonstration of DNA content in Carnoy-fixed paraffin sections.

**Statistical analysis:** Statistical analyses were performed using SPSS Version 16 statistical software package (SPSS, Cary, NC, USA). Data are presented as means with their standard error. Normality and homogeneity of the data were confirmed before ANOVA, differences among the

Table 1: Effect of silymarin (SIL) on cisplatin (CDDP) induced serum biochemical changes in control and experimental rats

| | | | | CDDP (5 mg kg$^{-1}$) + SIL (50 mg kg$^{-1}$) | |
|---|---|---|---|---|---|
| Groups | Control | Vehicle | CDDP | 2 h before CDDP | 2 h after CDDP |
| **Hepatic function indicators** | | | | | |
| AST (IU L$^{-1}$) | 32.40±4.96$^{a}$ | 27.65±4.09$^{a}$ | 304.47±38.0$^{b}$ | 195.29±12.03$^{c}$ | 181.67±11.10$^{c}$ |
| ALT (IU L$^{-1}$) | 54.13±4.53$^{a}$ | 50.53±3.36$^{a}$ | 104.5±9.55$^{b}$ | 077.69±04.25$^{c}$ | 068.25±05.93$^{a}$ |
| Glucose (mg dL$^{-1}$) | 101.05±3.20$^{a}$ | 101.25±5.90$^{a}$ | 208.57±6.70$^{b}$ | 110.88±06.20$^{a}$ | 101.44±07.39$^{a}$ |

Values are Mean±SEM for six rats in each group, Means with different superscript letters within the same parameter are significantly different

Table 2: Effect of silymarin (SIL) and cisplatin (CDDP) on the levels of lipid peroxidation and on the activities of antioxidant enzymes liver tissues of control and experimental rats

| | | | | CDDP (5 mg kg$^{-1}$) + SIL (50 mg kg$^{-1}$) | |
|---|---|---|---|---|---|
| Groups | Control | Vehicle | CDDP | 2 h before CDDP | 2 h after CDDP |
| **Lipid peroxidation** | | | | | |
| MDA (μmol g$^{-1}$ tissue) | 02.07±0.50$^{a}$ | 02.02±0.101$^{a}$ | 07.19±0.70$^{b}$ | 04.04±1.06$^{c}$ | 03.77±0.53$^{a}$ |
| **Enzymatic antioxidants** | | | | | |
| SOD (IU g$^{-1}$ tissue) | 23.82±0.80$^{a}$ | 23.46±1.00$^{a}$ | 13.61±3.27$^{b}$ | 23.40±0.48$^{a}$ | 23.25±1.13$^{a}$ |
| GPx (IU mg$^{-1}$ tissue) | 07.39±0.18$^{a}$ | 07.16±0.59$^{a}$ | 05.20±0.27$^{b}$ | 06.64±0.37$^{a}$ | 06.83±0.42$^{a}$ |

Values are Mean±SEM for six rats in each group, Means with different superscript letters within the same parameter are significantly different

experimental groups were assessed by one-way analysis of variance ANOVA and the group means were compared by LSD *post hoc* test. Values were considered statistically significant when $p<0.05$.

## RESULTS

**Biochemical investigations:** The activities of selected liver functional markers (AST and ALT) and the levels of glucose concentration in the serum of the experimental group are presented in Table 1. The results show that CDDP administration resulted in a significant increase ($p<0.05$) in the activities of serum AST and ALT as well as levels of serum glucose concentration when compared to control rats. However, administration of SIL significantly reversed these changes towards near normal. Yet, the effect of pretreatment with SIL was more effective when compared to those in post treated group, as values were significantly returned to those recorded in control group.

In CDDP treated group, the levels of MDA in the liver tissues were significantly increased ($p<0.05$) whereas the activities on enzymatic antioxidants (SOD and GPx) significantly decreased compared to control rats (Table 2). Administration of SIL 2 h after CDDP for 2 weeks significantly increased the depleted activities of SOD and GPx with significant decrease in CDDP induced lipid peroxidation (MDA) levels that did not return to normal values. On the other hand SIL pretreatment, resulted in a significant decrease in MDA levels along with significant elevation in SOD and GPX activities ($p<0.05$), when compared to CDDP alone. Thus, values approached those in control groups and did not reach statistical significance over basal values (Table 2).

**Histochemical observations:** Light microscopical examination of Best's Carmine stained slides showed remarkable alterations in most liver tissues from CDDP group. Glycogen depletion become more prominent in the cytoplasm of most liver cells of rats injected with CDDP, compared to the control liver sections (Fig. 1c). The cytoplasm exhibited a marked regression in glycogen content

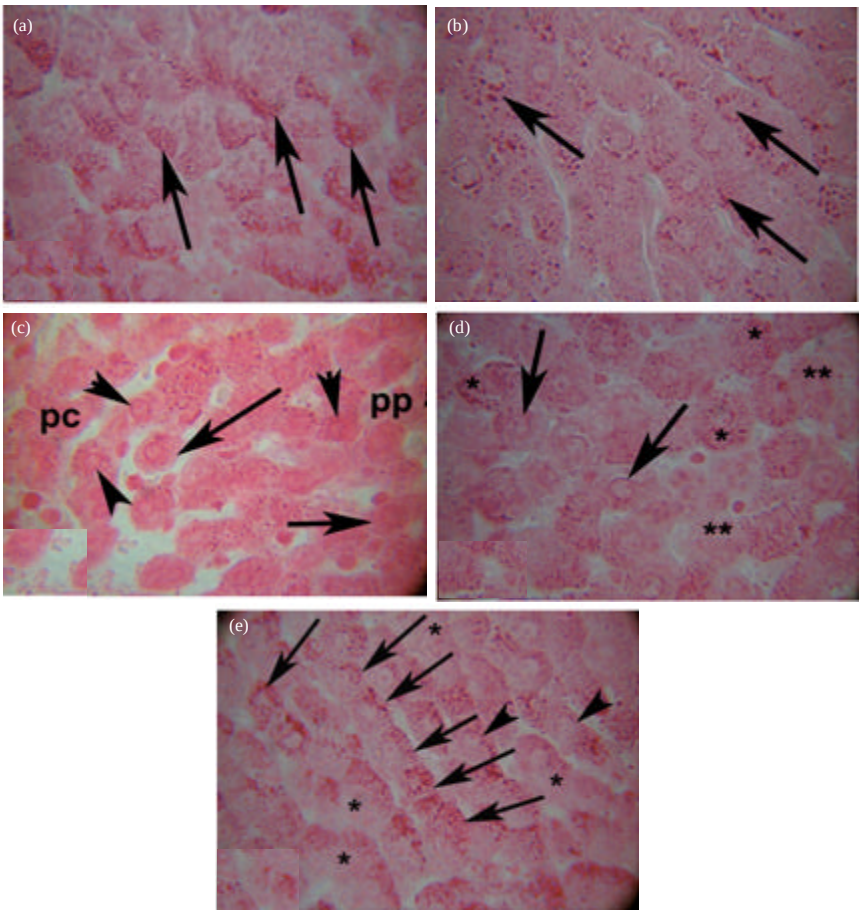


Fig. 1(a-e): Representative light micrographs of rat liver (400x) (a) Normal rat liver; showing densely red coarse glycogen granules in the cytoplasm of hepatocytes with typical glycogen flight (arrow), (b) Vehicle administrated group, notice homogenous and strong Best's-positive reaction of large sized glycogen granules within cytoplasm (arrow), (c) CDDP group, revealing depleted glycogen content within cytoplasm (arrow) of pericentral (pc) and periportal (pp) hepatocytes), nuclei with increased intensely stained glycogen deposits along nuclear envelope and nucleoli (arrow head), (d) SIL 2 h after CDDP, showing medium sized cytoplasmic glycogen granules with moderate reactivity (*), **Diffused weakly stained glycogen, nuclei with minimal glycogen deposits (arrow) and (e) SIL 2 h before CDDP, showing large cytoplasmic glycogen granules with intense reactivity and distinctive flight (arrow), negatively stained nuclei (*), minimal glycogen deposits along nuclear envelope giving weak Best's reaction (arrowhead)

with an obvious loss of reactivity whereas, most nuclei exhibited increased staining activity due to glycogen deposition along their nuclear envelope and nucleoli. Post administration of SIL increased the cytoplasmic glycogen particles, compared to CDDP group, where granules appeared moderately stained (Fig. 1d). However, the marked reduction in glycogen stores induced by CDDP was improved after pretreatment with SIL (Fig. 1e), i.e., the cytoplasm of most hepatocytes revealed numerous, coarse and intensely stained glycogen granules forming typically glycogen flight, similar to those recorded in control and vehicle groups (Fig. 1a, b). However, examination of Hg-BPB liver

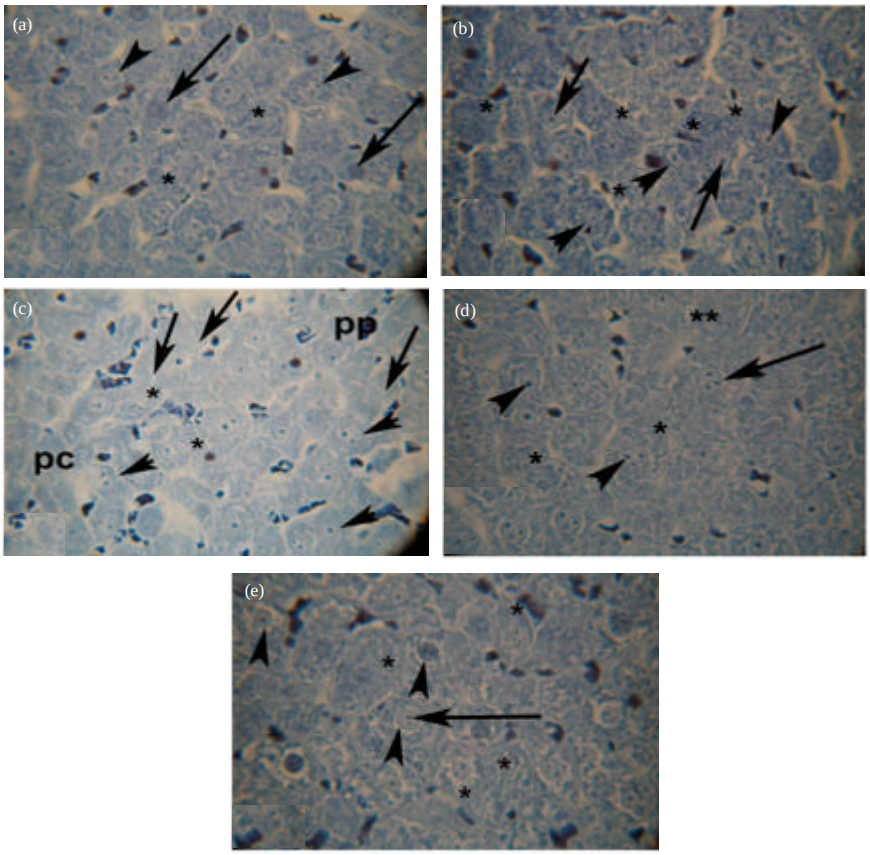


Fig. 2(a-e): Representative light micrographs of rat liver (400x) (a) Normal group; with dark blue coarse protein granules within the cytoplasm of hepatocytes (*), nucleoplasm (arrow), and along nuclear envelope (arrowhead), (b) Vehicle group, giving intense reactivity within cytoplasm (*), along nuclear envelope (arrowhead), and with nucleoli (arrow), (c) CDDP group, with reduced weakly stained (*) and negatively reactive (arrow) total protein within cytoplasm of pericentral (pc) and periportal (pp) liver cells, nuclei (arrowheads) giving weak reaction, (d) SIL 2 h after CDDP, showing moderately (asterisk) and densely (double asterisk) stained protein particles within cytoplasm, along nuclear envelope (arrow), and associated with nucleoli (arrowhead) and (e) SIL 2 h before CDDP, showing intensely reactive protein granules within cytoplasm (*), nucleolus (arrow) and along nuclear envelope (arrowhead) of liver cells

sections after 2 weeks of CDDP injection exhibited a severe depletion in the total protein content. Besides, degeneration of total protein content was manifested as minimal and weak stained protein granules within the nuclei and cytoplasm of pericentral and periportal hepatocytes (Fig. 2c) where, protein granules exhibited weak stainability. In contrast, post-treatment of SIL improved the depletion of protein content among liver cells induced by CDDP (Fig. 2d) i.e., Nuclei and cytoplasm of most cells displayed accumulation of medium-sized protein granules with moderate reactivity. However, pretreatment with SIL, recovered total protein content to normal appearance (Fig. 2e) where, protein granules were associated with the nucleoli and concentrated adjacent the nuclear envelope of most nuclei. Besides, the cytoplasm of hepatocytes possessed large-sized

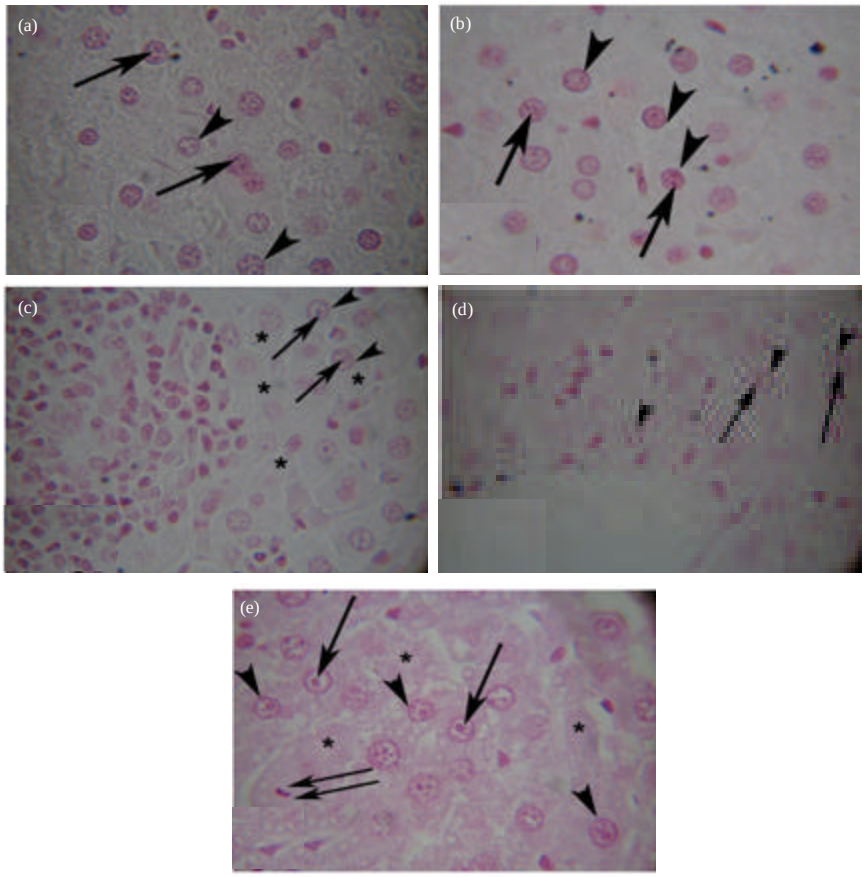


Fig. 3(a-e): Representative light micrographs of rat liver (400x) (a) Normal group; showing nuclei of hepatocytes with numerous intensely stained DNA associated with the nucleolus (arrow), and along nuclear envelope (arrowhead), (b) Vehicle group, showing coarse DNA particles with intense reactivity along nuclear envelope (arrow head), and moderately stained with the nucleoli (arrow), (c) CDDP group, nuclei with reduced weakly stained DNA particles within nucleoli (arrow) and minimally along the nuclear envelope (arrowhead), Cytoplasm (*) exhibit weak reaction to Feulgen, (d) SIL 2 h after CDDP, showing nuclei with moderately stained DNA particles along nuclear envelope (arrowhead) and associated with nucleoli (arrow) and (e) SIL 2 h before CDDP, showing nuclei with intensely reactive DNA content associated with nucleolus (arrow), and along nuclear envelope (arrowhead), Cytoplasms give weak Feulgen reaction (*), Mitotic figure: Double arrow

intensely stained protein granules similar to those recorded in control groups (Fig. 2a, b). On the other side, Feulgen nuclear reaction of liver sections of CDDP group revealed a marked depression in DNA content (Fig. 3c) where, most hepatocytes appeared diminutive and exhibited weak Feulgen reaction in their nuclei as well as in their cytoplasm. After 2 weeks, both treatments of SIL (Fig. 3d, e) increased markedly DNA contents in most hepatocytes. Thus, the nuclei maintained positive reaction to Feulgen and were characterized by a high DNA content. In addition, nuclei of most hepatocytes possessed numerous coarse and intensely stained red-purple colored particles

associated with nucleoli, scattered within the nucleoplasm and adjacent to the nuclear envelope. Whereas, the cytoplasm displayed weak reaction similar to those recorded in control groups (Fig. 3a, b). Noteworthy, pretreatment with SIL exhibited signs of nuclear division manifested by mitotic figures within the nuclei of some hepatocytes (Fig. 3e).

## DISCUSSION

CDDP, one of the most active cytotoxic agents against cancer, induces several toxicities. Hepatotoxicity is one of them and was observed during high doses of CDDP treatment (Kim *et al*., 2004; Pratibha *et al*., 2006; Abdelmeguid *et al*., 2010b). In this investigation, a single dose of CDDP (5 mg $kg^{-1}$) induced hepatotoxicity as manifested by an increase in the levels of serum ALT and AST compared to control groups. Our results confirm those previously reported by Kadikoylu *et al*. (2004), Mansour *et al*. (2006) and Lee *et al*. (2007) who stated that the administration of CDDP in albino rats induced significant impaired liver function that was estimated by an increases in the serum ALT and AST levels. Therefore, it could be suggested that liver cells that become more susceptible to CDDP toxicity. Hence, the ability of CDDP to cause a significant elevation in the activities of AST and ALT enzymes could be a secondary event following CDDP induced liver damage with the consequent leakage of ALT and AST from altered hepatocytes. Interestingly, both treatments with SIL significantly reduced the increased activities of serum ALT and AST induced by CDDP yet, it failed to return AST levels to those recorded in control groups. However, pretreatment with SIL was more efficient and protective than that of post-treatment since ALT levels were restored to baseline values after 2 weeks. Besides, improved liver function tests are likely a consequence of the lower degree of organ damage and fibrosis. This could explain the antioxidant effect of SIL and its ability to act as a free radical scavenger, thereby protecting membrane permeability (Hakova *et al*., 1996). In accordance to our findings, Ahmed *et al*. (2003) and Balahoroglu *et al*. (2008) reported that several antioxidants, such as SIL have anti-hepatotoxic activity against ALT and AST increased levels in $CCl_4$-induced hepatotoxicity in albino rats. However, Mansour *et al*. (2006) stated that the administration of SIL, 1 h before CDDP did not significantly change the indices of CDDP-induced hepatotoxicity in comparison with those animals treated with CDDP alone.

Biochemical parameters, which are sensitive indicators to alterations due to xenobiotics, can be considered as important diagnostic means in toxicological studies (Radwan *et al*., 2008). Carbohydrates are the primary and immediate source of energy (Lehninger *et al*., 2008). In this study, CDDP injection resulted in an increase in serum glucose concentration. In parallel, Best's Carmine stained liver sections in CDDP treated group revealed a remarkable reduction in the glycogen stores within the cytoplasm of most hepatocytes. This could be explained by the hepatic damage induced by CDDP in which hepatocytes are no more capable of storing glucose in the form of glycogen. This is in agreement with our previous study (Abdelmeguid *et al*., 2010b) that demonstrated a decrease in hepatic glycogen content at the ultrastrucutral level after treatment with CDDP, thus reflecting decreased gluconeogenesis by the liver (Muriel *et al*., 2001). It could be suggested that the enhanced rate of glycogenolysis resulting in increased glucose serum levels (Lehninger *et al*., 2008), was attributed to the increased stress induced by CDDP on liver tissues (Sakr and El-Abd, 2004) and reflected the important role of glucose in protecting cells from oxidative injury (Ebaid *et al*., 2007). Our results are in agreement with those reported by Miyamato *et al*. (2007) and Rosner and Okusa (2008) who demonstrated that intravenous injection of CDDP to male albino rats exerted significant elevation in serum glucose levels compared to

controls. Contradicting our findings, Bogin *et al.* (1994) postulated that two i.p. injections of CDDP resulted in a decrease in serum glucose levels in rats. On the other sides, it was clearly noticed that both treatments of SIL succeeded in returning serum glucose concentration to normal control values, thus reflecting a complete protection against CDDP induced damage. In accordance with our biochemical results, histochemical observations of Best's Carmine stained liver sections revealed that SIL pre-treatment appeared to be more effective than post-administration and it succeeded in restoring the glycogen stores within the cytoplasm of liver cells. It could be proposed that SIL ameliorates the principal functions of the liver which are related to the regulation of carbohydrate metabolism and blood glucose homeostasis. Similar results were reported by Abdel Salam *et al.* (2007) who studied the protective effect of SIL and ribavirin on $CCl_4$-induced liver toxicity in male and female rats.

Although, the mechanism of CDDP induced toxicity is not well understood, many studies documented that CDDP is taken up and accumulated in the liver cells, resulting in the enhanced production of reactive oxygen species (Mora *et al.*, 2003; Behling *et al.*, 2006). The present study demonstrated that the significant elevation in lipid peroxidation end product, MDA, was in parallel to the decrease in SOD and GPx activities in the hepatic tissue of CDDP treated rat, compared to control groups. It could be implied that CDDP is involved in altering the thiol status of the tissue with simultaneous alterations in the enzymatic antioxidants. This decrease in hepatic enzymatic activities suggests an oxidative type of injury with CDDP induced hepatotoxicity; or may be a secondary event following the CDDP induced increase in free radical production and/or decrease in lipid peroxidation protecting enzymes (Mansour *et al.*, 2006). Our speculation agreed with previous studies reported by Mora *et al.* (2003), Shimeda *et al.* (2005), Lee *et al.* (2007) who demonstrated that the activities of GPx and SOD, is potentially attributable to inactivation by the increase in Reactive Oxygen Species (ROS) or lipid peroxides when oxidative damage is extreme. In addition, Baek *et al.* (2003) and Yilmaz *et al.* (2004) demonstrated the involvement of CDDP induced mitochondrial dysfunctions in rat liver cells, particularly the inhibition of the electron transfer system. This may result in return in the enhanced generation of free radicals such as the production of superoxide anions, hydrogen peroxide and hydroxyl radicals. The hydroxyl radical is able to initiate lipid peroxidation after abstracting a hydrogen atom from polyunsaturated fatty acids in membrane lipids. These radicals can induce serious cellular injury and extensive tissue damage (Antunes *et al.*, 2000; Emerit *et al.*, 2001). Besides, hydroxyl radicals can react with macromolecules, such as membrane lipids, proteins and nucleic acids; thus changing the biological membrane integrities (Antunes *et al.*, 2000; Emerit *et al.*, 2001) and in return increasing the leakage of marker enzymes (ALT, AST) as well as glucose molecules (Grau *et al.*, 1996). These observations also support the hypothesis that part of the mechanism of toxicity in the CDDP-treated animals is related to depletion of antioxidants. In contrary to our results, Pratibha *et al.* (2006) reported that glutathione peroxidase activity showed a significant increase after CDDP therapy.

On the other hand, recent studies suggested that dietary antioxidants and some foods can attenuate CDDP induced hepatotoxicity (Kim *et al.*, 2006). In the present results, SIL pretreatment provided a full protection against CDDP induced hepatotoxicity, as liver MDA levels and SOD activities were returned to normal values. In agreement with our data, Lee *et al.* (2007) reported that the administration of SIL (100 mg $kg^{-1}$) by gastrogavage twice a day for 2 consecutive days resulted in an elevation of hepatic SOD. The free radical scavengers SOD are reported to provide partial protection against CDDP-induced structural and functional alteration in rats

(Yildirim *et al*., 2003). On the other hand, our data showed that both treatments of SIL exhibited complete protection against CDDP-induced decrease in liver GPx activities since, values were restored to those recorded in control groups. SIL is known to have hepatoprotective and anticarcinogenic effects (Kang *et al*., 2004). Our results were in close accordance to previous studies which revealed that the protective action of SIL is associated with its antioxidant properties since, SIL possibly acts as a free radical scavenger, reducing the levels of hydrogen peroxide and superoxide anion. It is suggested that SIL has a hydroxyl group at C5 in addition to the carbonyl group at C4 that may chelate ferrous iron (Aruoma 1994; Ramadan *et al*., 2002; Mansour *et al*., 2006). This chelation can play a role in increasing the activity to the level of most active scavengers, probably by site specific scavenging (Abu-Ghadeer *et al*., 2001). Besides, the free hydroxyl groups at C5 and C7 on the SIL structure can also react with peroxy radicals; thus favoring the inhibition of lipid peroxidation. In addition, SIL can act a plasma membrane stabilizer and enzyme inactivation, thus restoring enzyme activity. This may also point toward the possible de novo synthesis of these enzymes induced by the components of SIL; in addition to the glutathione system, constitute the more important defense mechanisms against damage by free radicals (Soto *et al*., 2003). This ability of SIL leads to a significant elevation in the cellular antioxidant defense machinery especially by improving the adverse effects of free radical reaction and on the increase in GSH content (Abu-Ghadeer *et al*., 2001; Ramadan *et al*., 2002). Our studies demonstrated that SIL is potent in protecting against oxidative damage induced by CDDP due to its radical scavenging capacity.

Proteins are mainly involved in the architecture of the cell (Radwan *et al*., 2008). In the present study, mercuric bromophenol blue staining revealed a sharp depression in the total protein content in both pericentral and periportal hepatocytes in rats exposed to CDDP. Thus our histochemical observation confirmed our biochemical results regarding CDDP adverse effects on protein contents. As a result, total protein content exhibited a great reduction manifested by a decrease in the enzymatic activities of both GPx and SOD. These results are in agreement and correlated with those reported by Sakr and El-Abd (2004) and Abdel Salam *et al*. (2007), who reported that $CCl_4$ reduced the liver protein content and found significant decrease of 18% in total hepatic protein in rats given $CCl_4$. Treatment with hepatotoxins like $CCl_4$caused membrane lipid peroxidation, altered lipid metabolism and decreased protein synthesis in the injured hepatocytes (Honma, 1990). Ebaid *et al*. (2007) and Abdelmeguid *et al*. (2010a, b) stated that the decrease in protein could be attributed to the disruption of lysosomal membranes under the effects of various toxicants; thus leading to the liberation of their hydrolytic enzymes in the cytoplasm. Additionally, the presence of hydrolytic enzymes could cause the lysis and dissolution of the target material within the cytoplasm. These results confirmed that of Awasthi *et al*. (1984) who found elevated lysosmal enzymatic activity accompanied by a decrease in protein and nucleic acid content in response to organophosphate insecticide. It could be proposed that due to the decreased glycogen stores observed in Best's Carmine stained liver sections in CDDP group, more energy was needed to detoxify and overcome CDDP induced stress. Thus, the next alternative source of energy to meet the increased energy demand is proteins (Lehninger *et al*., 2008). In the present investigation, Hg-BPB preparations in liver sections revealed that SIL pretreatment exhibited more protective action than SIL post-treatment. This protective action appeared in protein content within the nuclei and the cytoplasm of both pericentral and periportal hepatocytes; i.e., total protein content was completely restored and returned to normal appearance recorded in control groups. In accordance with our findings, Abdel Salam *et al*. (2007) provided evidence that the metabolic disturbance

caused by the hepatotoxin $CCl_4$ was reflected in a decrease in intracellular protein content in hepatocytes. Besides, such perturbations were improved by ribavirin monotherapy and also by combined SIL and ribavirin therapy.

In the present investigation, another histochemical change was observed as a decrease of DNA content within the nuclei of most pericentral and periportal hepatocytes in CDDP treated rats. Moreover, most of the nuclei appeared pyknotic. For that reason, the depleted and disturbed DNA in most hepatocytes indicated that these cells remained impaired by the action of CDDP. Confirming our results (Ebaid *et al.*, 2007) postulated that the anti inflammatory drug piroxicam displayed time dependant reduction in total DNA content among hepatocyte. Liver damage induced by hepatotoxins such as $CCl_4$ is associated with modifications in cell cycle-related proteins (Jeong *et al.*, 2001). In addition, it was found that the expression cell cycle-related proteins is responsible for hepatocyte regeneration in the damaged liver and may be involved in liver carcinogenesis (Jeong *et al.*, 2001). During the present study, SIL post- and pre-treatment exhibited highly protective effects in DNA content within the nuclei of most hepatic cells; i.e. nuclei appeared densely stained with high DNA particles. The highly protective effect and regenerative inducing power of SIL was greatly manifested by the increased cellular proliferation and regeneration among hepatocytes of both SIL groups. Thus, reflecting the increased evidence of mitotic figures within most nuclie. Abdel Salam *et al.* (2007) provided evidence that the elevated levels of nuclear markers appear in the nucleus during the late G1 phase with maximum expression during S-phase and drop down during G2 and M phase. Therefore, they can be an indicator of the degree of cellular proliferation and DNA synthesis (Bravo *et al.*, 1987). Besides, the level of synthesis of Proliferating Cell Nuclear Antigen (PCNA) which is a non-histone nuclear protein known as cyclin, is greatly and directly correlated with the rates of cellular proliferation and DNA synthesis (Shiina *et al.*, 1996).

In conclusion, these results suggest that CDDP causes hepatotoxicity by changing liver functional markers' activities, inducing lipid peroxidation, decreasing antioxidant system and altering glycogen and protein metabolism and DNA contents at the histochemical levels. Although the exact mechanism by which SIL prevents, to a great extent, CDDP toxicity remains to be elucidated, yet our present findings suggest that SIL pretreatment caused a generally protective and ameliorative effect against CDDP induced hepatotoxicity. SIL pretreatment reduced ALT and AST levels, glucose concentration and MDA levels, whereas antioxidant enzymes activities as well as protein, glycogen and DNA contents in liver tissues were increased and returned to control baseline values. Thus, SIL may be considered as a potentially useful candidate in the combination with chemotherapy. It is believed that SIL with its high antioxidant properties acts as a free radical scavenger, lipid peroxidation inhibitor and glutathione levels preservation.

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## REFERENCES

Abdel Salam, O.M.E., A.A. Sleem, E.A. Omara and N.S. Hassan, 2007. Effect of ribavirin alone or combined with silymarin on carbon tetrachloride induced hepatic damage in rats. Drug Target Insights, 2: 19-27.

Abdelmeguid, N.E., H.N. Chmaisse and N.S. Abou Zeinab, 2010a. Silymarin ameliorates cisplatin-induced hepatotoxicity in rats: Histopathological and ultrastructural studies. Pak. J. Biol. Sci., 13: 463-479.

Abdelmeguid, N.E., H.N. Chmaisse and N.S. Abou Zeinab, 2010b. Protective effect of silymarin on cisplatin-induced nephrotoxicity in rats. Pak. J. Nutr., 9: 624-636.

Abu-Ghadeer, A.R.M., S.E. Ali and S.A.A. Osman, 2001. Antagonistic role of silymarin against cardiotoxicity and impaired antioxidation induced by adriamycin and/or radiation exposure in albino rats. Pak. J. Biol. Sci., 4: 604-607.

Ahmed, B., S.A. Khan and T. Alam, 2003. Synthesis and antihepatotoxic activity of some heterocyclic compounds containing the 1,4-dioxane ring system. Pharmazie, 58: 173-176.

Ajani, J.A., 2008. Optimizing docetaxel chemotherapy in patients with cancer of the gastric and gastroesophageal junction: Evolution of the docetaxel, cisplatin and 5-fluorouracil regimen. Cancer, 113: 945-955.

Antunes, L.M.G., J.D.C. Darin and M.L.P. Bianchi, 2000. Protective effects of vitamin C against cisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: A dose-dependent study. Pharmacol. Res., 41: 405-411.

Antunes, L.M., J.D. Darin and N.L. Bianchi, 2001. Effects of the antioxidants curcumin or selenium on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. Pharmacol. Res., 43: 145-150.

Aruoma, O.I., 1994. Nutrition and Health aspect of free radical and antioxidants. Food Chem. Toxicol., 32: 671-683.

Awasthi, Y.C., C.A. Partridge, C. Theodore and D.D. Dao, 1984. Comparative effect of the induction of the subunits of rat liver glutathione S-transferases by butylated hydroxytoluene. Comp. Biochem. Physiol. Part C: Comp. Pharmacol., 78: 39-41.

Baek, S.M., C.H. Kwon, J.H. Kim, J.S. Woo, J.S. Jung and Y.K. Kim, 2003. Differential roles of hydrogen peroxide and hydroxyl radical in cisplatin-induced cell death in renal proximal tubular epithelial cells. J. Lab. Clin. Med., 142: 178-186.

Balahoroglu, R., H. Dulger, H. Ozbek, I. Bayram and M.R. Sekeroglu, 2008. Protective effects of antioxidants on the experimental liver and kidney toxicity in mice. Eur. J. Gen. Med., 5: 157-164.

Behling, E.B., M.C. Sendao, H.D. Francescato, L.M. Antunes, R.S. Costa and L. Bianchi-Mde, 2006. Comparative study of multiple dosage of quercetin against cisplatin-induced nephrotoxicity and oxidative stress in rat kidneys. Pharmacol. Rep., 58: 526-532.

Bogin, E., M. Marom and Y. Levi, 1994. Changes in serum, liver and kidneys of cisplatin-treated rats: Effects of antioxidants. Eur. J. Clin. Chem. Clin. Biochem., 32: 843-852.

Borsari, M., C. Gabbi, F. Ghelfi, R. Grandi, M. Saladini and F. Borella, 2001. Silybib, a new iron chelating agent. J. Inorg. Biochem., 85: 123-129.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Bravo, M.P., J. del Rey Calero and M. Conde, 1987. Silica and cancer of the bladder in males. Arch. Esp. Urol., 40: 635-637.

Dank, M., J. Zaluski, C. Barone, V. Valvere and S. Yalcin *et al.*, 2008. Randomized phase III study comparing irinotecan combined with 5-fluorouracil and folinic acid to cisplatin combined with 5-fluorouracil in chemotherapy naive patients with advanced adenocarcinoma of the stomach oresophagogastric junction. Ann. Oncol., 19: 1450-1457.

Ebaid, H., M.A. Dkhil, M.A. Danfour, A. Tohamy and M.S. Gabry, 2007. Piroxicam-induced hepatic and renal histopathological changes in mice. Libyan J. Med., 2: 82-89.

Emerit, J., C. Beaumont and F. Trivin, 2001. Iron metabolism, free radicals and oxidative injury. Biomed. Pharmacother., 55: 333-339.

Eminzade, S., F. Uras and F.V. Izzettin, 2008. Silymarin protects liver against toxic effects of anti-tuberculosis drugs in experimental animals. Nutr. Metab., 5: 18-18.

Garvin, A.J., B.J. Hall, R.M. Brissie and S.S. Spicer, 1976. Cytochemical differentiation of nucleic acids with a Schiff-methylene blue sequence. J. Histochem. Cytochem., 24: 587-590.

Grau, J.J., J. Estape, M.A. Cuchi, J.L. Firvida, J.L. Blanch and C. Ascaso, 1996. Calcium supplementation and ototoxicity in patients receiving cisplatin. Br. J. Clin. Pharmacol., 42: 233-235.

Hakova, H., E. Misurova and R. Kropacova, 1996. The effect of silymarin on concentration and total content of nucleic acids in tissues of continuously irradiated rats. Vet. Med., 41: 113-119.

Hogan, F.S., N.K. Krishnegowda, M. Mikhailova and M.S. Kahlenberg, 2007. Flavonoid, silibinin, inhibits proliferation and promotes cell-cycle arrest of human colon cancer. J. Surg. Res., 143: 58-65.

Honma, T., 1990. Effects of trichloroethylene, 1,1,1-trichloroethane and carbon tetrachloride on plasma lipoproteins of rats. Ind. Health, 28: 159-174.

Jeong, Y.Y., D.G. Mitchell, H.W. Hann, A.K. Malin, H.K. Kang, G.A. Holland and R.L. Capizzi, 2001. Hepatocellular carcinoma after systemic chemotherapy: Gadolinium-enhanced MR measurement of necrosis by volume histogram. J. Comput. Assist. Tomogr., 25: 624-628.

Kadikoylu, G., Z. Bolaman, S. Demir, M. Balkaya, N. Akalin and Y. Enli, 2004. The effects of desferrioxamine on cisplatin-induced lipid peroxidation and the activities of antioxidant enzymes in rat kidneys. Hum. Exp. Toxicol., 2: 29-34.

Kang, J.S., Y.J. Jeon, S.K. Park, K.H. Yang and H.M. Kim, 2004. Protection against lipopolysaccharide-induced sepsis and inhibition of interleukin-1beta and prostaglandin E2 synthesis by silymarin. Biochem. Pharmacol., 67: 175-181.

Karimi, G., M. Ramezani and Z. Tahoonian, 2005. Cisplatin nephrooxicity and protection by milk thistle extract in rats. Evid. Based Complement Alternat. Med., 2: 383-386.

Kim, K.S., B. Joseph, M. Inada and S. Gupta, 2005. Regulation of hepatocyte engraftment and proliferation after cytotoxic drug-induced perturbation of the rat liver. Transplantation, 80: 653-659.

Kim, S.H., K.O. Hong, W.Y. Chung, J.K. Hwang and K.K. Park, 2004. Abrogation of cisplatin-induced hepatotoxicity in mice by xanthorrhizol is related to its effect on the regulation of gene transcription. Toxicol. Applied Pharmacol., 196: 346-355.

Kim, Y.H., Y.W. Kim, Y.J. Oh, N.I. Back and S.A. Chung *et al.*, 2006. Protective effect of the ethanol extract of the roots of *Brassica rapa* oncisplatin-induced nephrotoxicity in LLC-PK 1 cells and rats. Biol. Pharm. Bull., 29: 2436-2441.

Kintzel, P.E., 2001. Anticancer drug-induced kidney disorders. Drug Saf., 24: 19-38.

Laekeman, G., S. de Coster and K. de Meyer, 2003. St. Mary's Thistle: An overview. J. Pharm. Belg., 58: 28-31.

Lee, C.K., K.K. Park, S.S. Lim, J.H. Park and W.Y. Chung, 2007. Effects of the licorice extract against tumor growth and cisplatin-induced toxicity in a mouse xenograft model of colon cancer. Biol. Pharm. Bull., 30: 2191-2195.

Lehninger, A.L., D.L. Nelson and M.M. Cox, 2008. Lehninger Principles of Biochemistry. 5th Edn., W.H. Freeman Publishers, USA., pp: 540-550.

Mansour, H.H., H.F. Hafez and N.M. Fahmy, 2006. Silymarin modulates Cisplatin-induced oxidative stress and hepatotoxicity in rats. J. Biochem. Mol. Biol., 39: 656-661.

Mazia, D., P.A. Brewer and M. Alfert, 1953. The cytochemical staining and measurement of protein with mercuric bromphenol blue. Biol. Bull., 104: 57-67.

Mira, L., M. Silva and C.F. Manso, 1994. Scavenging of reactive oxygen species by silibinin dihemisuccinate. Biochem. Pharmacol., 48: 753-759.

Miyamato, Y., K. Shimada, Y. Sakaguchi and M. Miyamato, 2007. Cisplatin (CCDP)-induced acute toxicity in an experimental model of hepatic fibrosis. J. Toxicol. Sci., 32: 311-320.

Mora, L.O., L.M. Antunes, H.D. Francescato and M. Bianchi, 2003. The effects of oral glutamine on cisplatin-induced nephrotoxicity in rats. Pharmacol. Res., 47: 517-522.

Muriel, P., N. Alba, V.M. Perez-Alvarez, M. Shibayama and V.K. Tsutsumi, 2001. Kupffer cells inhibition prevents hepatic lipid peroxidation and damage induced by carbon tetrachloride. Comp. Biochem. Physiol. C. Toxicol. Pharmacol., 130: 219-226.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

Pal, S., A.S. Sadhu, S. Patra and K.K. Mukherjea, 2008. Histological vs biochemical assessment on the toxic level and antineoplastic efficacy of a synthetic drug Pt-ATP on experimental animal models. J. Exp. Clin. Cancer Res., 27: 68-68.

Pearse, A.G.E., 1968. Histochemistry-Theoretical and Applied. 3rd Edn., Churchill Livingstone, Edinburg, London, New York.

Pratibha, R., R. Sameer, P.V. Rataboli, D.A. Bhiwgade and C.Y. Dhume, 2006. Enzymatic studies of cisplatin induced oxidative stress in hepatic tissue of rats. Eur. J. Pharmacol., 532: 290-293.

Radwan, M.A., A.E. Essawy, N.E. Abdelmeguied, S.S. Hamed and A.E. Ahmed, 2008. Biochemical and histochemical studies on the digestive gland of *Eobania vermiculata* snails treated with carbamate pesticides. Pestic. Biochem. Physiol., 90: 154-167.

Ramadan, L.A., H.M. Roushdy, G.M. Abu Senna, N.E. Amin and O.A. El-Deshw, 2002. Radioprotective effect of silymarin against radiation induced hepatotoxicty. Pharmacol. Res., 45: 447-454.

Rosner, M.H. and M. Okusa, 2008. Biomarkers in Renal Disease. 5th Edn., Nova Publishers, USA., pp: 21-45.

Saad, S.Y., T.A. Najjar and M. Alashari, 2004. Role of non-selective adenosine receptor blockade and phosphodiesterase inhibition in cisplatin-induced nephrogonadal toxicity in rats. Clin. Exp. Pharmacol. Physiol., 31: 862-867.

Sakr, S.A. and S.F. El-Abd, 2004. Effect of silymarin on histopathological and histochemical lesions induced by the antimalaril drug Chloroquine in the liver of albino mice. Int. J. Biol. Biotech., 1: 667-672.

Satoh, K., 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin. Chem. Acta, 90: 37-43.

Shiina, H., M. Igawa, H. Nagami, H. Yagi and S. Urakami *et al.*, 1996. Immunohistochemical analysis of proliferating cell nuclear antigen, p53 protein and nm23 protein and nuclear DNA content in transitional cell carcinoma of the bladder. Cancer, 78: 1762-1774.

Shimeda, Y., Y. Hirotani, Y. Akimoto, K. Shindou, Y. Ijiri, T. Nishihori and K. Tanaka, 2005. Protective effects of capsaicin against cisplatin-induced nephrotoxicity in rats. Biol. Pharm. Bull., 28: 1635-1638.

Silva, C.R., L.M. Antunes and M.D.L.P. Bianchi, 2001. Antioxidant action of bixin against cisplatin-induced chromosome aberrations and lipid peroxidation in rats. Pharmacol. Res., 43: 561-566.

Singh, D., V. Chander and K. Chopra, 2005. Protective effect of catechin on ischemiareperfusion-induced renal injury in rats. Pharmacol. Rep., 57: 70-76.

Soto, C., R. Recoba, H. Barron, C. Alvarez and L. Favari, 2003. Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. Compar. Biochem. Physiol. Part C, 136: 205-212.

Stewart, D.J., R.S. Benjamin, M. Luna, L. Feun, R. Caprioli, W. Seifert and T.L. Loo, 1982. Human tissue distribution of platinum after cis-diamminedichloroplatinum. Cancer Chemother. Pharmacol., 10: 51-54.

Valenzuela, A., M. Aspillaga, S. Vial and R. Guerra, 1989. Selectivity of Silymarin on the increase of the glutathione content in different tissues of the rat. Planta Med., 55: 420-422.

Venkatesan, P.N., P. Rajendran, G. Ekambaram and D. Sakthisekaran, 2008. Combination therapeutic effect of cisplatin along with Solanum trilobatum on benzo(a)pyrene induced experimental lung carcinogenesis. Nat. Prod. Res., 22: 1094-1106.

Wang, G., E. Reed and Q.Q. Li, 2004. Molecular basis of cellular response to cisplatin chemotherapy in non-small cell lung cancer (Review). Oncol. Rep., 12: 955-965.

Yildirim, Z., S. Sogut, E. Odaci, M. Iraz, H. Ozyurt, M. Kotuk and O. Akyol, 2003. Oral erdosteine administration attenuates cisplatin-induced renal tubular damage in rats. Pharmacol. Res., 47: 149-156.

Yilmaz, H.R., M. Iraz, S. Sogut, H. Ozyurt, Z. Yildirim, O. Akyol and S. Gergerlioglu, 2004. The effects of erdosteine on the activities of some metabolic enzymes during cisplatin-induced nephrotoxicity in rats. Pharmacol. Res., 50: 287-290.

Yuce, A., A. Atessahin, A. Ceribasi and M. Aksakal, 2007. Ellagic acid prevents cisplatin-induced oxidative stress in liver and heart tissue of rats Basic Clin. Pharmacol. Toxicol., 101: 345-349.

Zicca, Z., S. Cafaggi, M.A. Mariggi, M.O. Vannozzi and M. Ottone *et al.*, 2004. Reduction of cisplatin hepatotoxicity by procainamide hydrochloride in rats. Eur. J. Pharmacol., 442: 265-272.