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The Protective Role of Neem Leaves Extract on Cisplatin-Induced Polysaccharides and Protein Depletion in Rat Liver and Kidney

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Abstract. Cisplatin, an effective antineoplastic agent, is toxic to the liver and kidney. The present study has evaluated the effect of neem (*Azadirachta indica*) leaves extract on cisplatin-induced histochemical abnormality in liver and kidney of rats. Histochemical staining of the liver and kidney sections showed weak polysaccharide and protein staining in the cisplatin-treated animals as compared to the control group. Pre, post and co-treatment of animals with methanolic extract of *A. indica* showed strong staining of polysaccharides and protein compared with the control group. These results were confirmed with image analysis, which showed that cisplatin caused significant decrease in these inclusions compared to control. Likewise, neem treated group showed significant increase in these inclusions compared with cisplatin group. These findings suggest that cisplatin induced depletion of polysaccharides and protein in the liver and kidney of rats can be reverted with neem extract.

Keywords: Cisplatin, *Azadirachta indica*, polysaccharides, protein.

INTRODUCTION

Cisplatin, cisplatinum or cis-diammine-dichloroplatinum (II) (CDDP), a platinum-based drug, is one of the most frequently used anti-neoplastic agents for various types of cancer. It has a potent anti-tumor action against wide range of malignancies, including testicular, ovarian, cervical, bladder and lung cancers as well as solid tumors resistant to other treatment regimens (Hanigan and Devarajan, 2003; Hassan *et al.*, 2012; Ghorbani *et al.*, 2012; Zhu *et al.*, 2013). Despite its clinical use fullness, cisplatin treatment has been associated with several toxic side effects, including nephrotoxicity, neurotoxicity and ototoxicity (Rabik and Dolan, 2007). In addition, alopecia, electrolyte disturbance, nausea and vomiting have been recorded in cisplatin (Lajolo *et al.*, 2009).

During the aggressive treatment protocols, higher doses of cisplatin that may be required for effective tumor suppression could also lead to hepatotoxicity, which is also encountered during low-dose repeated cisplatin therapy (Pratibha *et al.*, 2006; Lee *et al.*, 2008). Hepatotoxicity is a less-known aspect of cisplatin treatment, and there is little information about the underlying mechanism. It has been reported that oxidative stress through the generation of reactive oxygen species (ROS) (Chirino and Pedraza-Chaverri, 2009), decreased antioxidant defense system including antioxidant enzymes (Sadzuka *et al.*, 1992) and non-enzymatic molecule reduced glutathione (GSH) are major alterations in cisplatin toxicity (Zhang and Lindup, 1993). In addition, functional and structural mitochondrial damage, apoptosis, perturbation in Ca^{2+} homeostasis (Martins *et al.*, 2008) and inducible nitric oxide synthase (iNOS) may play some important role in the mechanism of cisplatin hepatotoxicity (Kart *et al.*, 2010).

Foods of plant origin with diverse medicinal properties have come under extensive study in the light of their antioxidant, antimutagenic, and anticarcinogenic effects (Khan and Mukhtar, 2008; Vinothini *et al.*, 2009). In particular, *Azadirachta indica* (neem) is a widely prevalent and highly esteemed wonder tree of the Indian subcontinent and several of its beneficial properties are reported (National Research Council, 1992; Mamoon-ur-Rashid *et al.*, 2012). Neem leaf consists of several valuable components and can be divided into two major classes: isoprenoids that include terpenoids containing limonoids, azadirone and its derivatives, c-secomeliacins, *e.g.*, azadirachtin. The

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nonisoprenoids include amino acids, polysaccharides, sulphurous compounds, polyphenolics like flavonoids and their glycosides *e.g.*, quercetin, dihydrochalcone, coumarin and tannins, aliphatic compounds (Sarkar *et al.*, 2007).

The present study was aimed at evaluating the protective activity of *Azadirachta indica* leaves extract on cisplatin-induced polysaccharides and protein depletion. This was determined by histochemical examination of liver and kidney tissues.

MATERIALS AND METHODS

*Preparation of methanolic extract of neem (*Azadirachta indica*) leaves*

Fresh matured leaves of neem tree were collected from garden in Obour City, Cairo on August 2010. The samples were identified in Botany Department, Faculty of Science, Helwan University. The leaves were cleaned, dried and powdered. The powder was used for the preparation of crude methanolic extract according to the procedure described by Manikandan *et al*. (2008) with some modifications. Air-dried powder (100 g) of *A. indica* leaves were extracted by percolation at room temperature with 70% methanol alcohol and kept in refrigerator for 24 hours. Leaves extract of *A. indica* was concentrated under reduced pressure (bath temperature 50 ºC) and dried in a vacuum evaporator. The residue was dissolved in distilled water, filtered and used in experiments.

Cisplatin

Cisplatin, [cis-$PtCl_2$ $(NH_3)_2$], was obtained from Oncotec Pharma Production GmbH as solution for infusion in vial of 25 ml (1 mg/ml).

Experimental animals

Male albino rats, weighing 120–150 g were obtained from the Animal House, The Holding Company for Biological Products and Vaccines, VACSERA, Cairo-Egypt. The animals were kept at standard housing facilities (24±1°C, 45±5% humidity and 12 h light/dark cycles). The animals were supplied with standard laboratory chow and water *ad libitum*, and left to acclimatize for 1 week before the experiments. The experimental protocol was approved by the Local Animal Care Committee and the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

The animals were divided into six groups, each of 6 as follows: Group I received an oral administration of distilled water for 5 consecutive days and served as control. Group II were orally administered with 500 mg/kg b.w. of the methanol extract of neem leaves (Dorababu *et al*., 2006) for 5 consecutive days. Group III were intraperitoneally (*i.p.*) injected with a single dose of cisplatin (5 mg/kg b.w.) according to the dose described by Choi *et al*. (2009) and left for five days. Group IV were *i.p.* injected with a single dose of cisplatin and after 24 h, these animals were received a daily oral dose of neem (500 mg/kg b.w.) for 5 consecutive days. Group V received a daily oral dose of neem (500 mg/kg b.w.) for 5 days and on the 6^{th} day these animals were injected with a single dose of cisplatin as in group IV and left for 5 days. Group VI were orally coadministered with neem and cisplatin for 5 consecutive days.

At the end of the experiment, liver and kidney were dissected out and fixed immediately for histochemical examinations.

Histochemical investigations

After fixation of tissues in 10% formal saline for 24 h, the tissues were washed in running tap water, dehydrated in ascending series of ethyl alcohol, cleared in xylene and embedded in paraffin wax (melting point of 58ºC) and stored at 4ºC until used. Histological sections (5 µm) thick were cut for carbohydrate and proteins as follows:

Periodic Acid Schiff's (PAS) technique was used for demonstration of general carbohydrates (Bancroft and Gamble, 2002). The polysaccharide materials gave magenta color. Mercuric Bromophenol Blue technique (MBB) was used for the cytochemical detection of proteins (Pearse, 1982), which acquired a deep blue color.

Quantitative measurements of PAS and protein measurements were obtained using Leica Qwin 500 Image Analyzer Computer System (England). The degree of reaction was chosen by the color detect menu and the areas of reactivity were

masked by a red binary color and area was measured at 400X magnification. Ten fields were chosen in each specimen and the mean values were obtained.

The image was transformed into a gray image, a grid of pixels each representing the intensity or brightness at that point by a range of numbers, typically from 0 (black) to 255 (white). A grayscale image is a color mode that displays image using 256 shades of gray, referred to as 8-bit grayscale image. Each color was defined as a value between 0 and 255, where 0 is the darkest (black) and 255 is the lightest (white). The strong reaction go to 0 repressent low value (high PAS or protein content), while the weak reaction go to 255 repressent high value (low PAS or protein content) in the Table I**.**

Statistical analysis

Statistical analysis involved analysis of variance (one way ANOVA) and the Student's *t*-test. The values were shown as Mean±SEM. Differences were considered significant when $P<0.05$.

RESULTS

Histochemical demonstration of polysaccharides

Liver

Examination of liver of control rat stained with PAS showed a strong PAS positive reaction, indicating the presence of large amounts of polysaccharides in the form of glycogen deposits. The reaction product was demonstrated as intense pink granular material in the cytoplasm. Most of the PAS positive products were displaced laterally towards one side of the cell (glycogen migration/flight phenomenon) caused by the effects of the fixative on the tissue. The nuclei were PAS negative reaction (Fig. 1A).

While the liver of rats treated with methanolic *A. indicia* leaves extract (500 mg/kg b.w.) for 5 days (Group I) preserved the normal contents of polysaccharides and gave a PAS reactions similar to that of control animals (Fig. 1B). Hepatic cells of liver of rats treated with a single dose of cisplatin (5 mg/kg b.w.) (Group III) demonstrated a severe depletion of carbohydrates contents in comparison to the hepatocytes of the control rats (Group I) and only a few number of cells displayed a weak to moderate reaction (Fig. 1C). Examination of liver section of the rats with pre-, post- and co-treatment of the same dose of the extract with cisplatin (Groups V, IV and VI, respectively) showed that the majority of liver cells displayed a nearly normal polysaccharides distribution (Figs. 1D, E, F).

Table I. A quantitative analysis of polysaccahrides and total protein of liver and kidney of diffirent groups of rats.

Groups	Polysaccahrides		Protein	
	Liver	Kidney	Liver	Kidney
Group I	106.43± 1.03	156.85± 1.24	118.35 ± 1.10	136.49± 0.99
Group II	117.21± 2.32	167.62± 2.98	116.16± 2.07	127.68± 2.21
Group III	169.56± 0.62*	205.33± 0.62*	186.21± 0.70*	199.16± 1.26 *
Group IV	122.10± 1.76**	172.00± 0.07**	129.21± 2.99**	142.30± 0.77**
Group V	123.54± 1.15**	163.89± 1.05**	133.66± 0.24**	133.76± 0.85**
Group VI	114.84± 2.56**	164.54± 1.38**	126.47± 1.66**	144.94± 5.86**

Data presented as mean ± S.E (n=6)

*At the P<0.05, the means are significantly different as compared with the control group

**At the P<0.05, the means are significantly different as compared with III

The mean values ± S.E of the grey level of polysaccharides content in liver of control and rats those receiving neem, cisplatin, Pre-, post- and co-treatment of the same dose of the extract with cisplatin for 5 days are 106.43±1.03, 117.21±2.32, 169.56±0.62, 122.10±1.76, 123.54±1.15, and 114.84±2.56, respectively (Table I). These data showed a significant decrease ($P<0.05$) in poly-saccharides content in rats received cisplatin as compared with control group. On the other hand, in rats treated with neem, pre-, post- and co-treatment of the same dose of the extract with cisplatin for 5 days showed significant increase ($P<0.05$) in polysaccharides content in liver as compared with cisplatin treated group.

Kidney

Examination of kidney of control rat stained with PAS technique showed the positive materials in the cortical tissues. Parietal and visceral walls of the Bowman's capsule, capillaries of the glomeruli, the basement membranes of the proximal and distal convoluted tubules and the brush borders of the proximal convoluted tubules exhibited strong positive reaction with PAS technique. The cytoplasm of the tubules was stained faintly white, while the nuclei showed PAS negative reaction (Fig. 2A).

The kidney of rats treated with methanolic *A. indicia* leaves extract (500 mg/kg) for 5 days revealed a normal PAS reaction in the glomeruli, Bowman's capsules, the basement membranes of the renal tubules and the brush borders of the proximal convoluted tubules (Fig. 2B).

Microscopic observation of kidneys of rats treated with a single dose of cisplatin (5 mg/kg b.w.) showed marked diminution in PAS positive material in the renal corpuscles and tubules (Fig. 2C).

Examination of kidney sections of the rats with Pre, post- and co- treatment of the same dose of the neem extract and cisplatin exhibited no apparent changes in the polysaccharides in both of the renal corpuscles and tubules. The stainability was more or less as in the normal one (Figs.2D, E, F).

The mean values ± S.E of the grey level of polysaccharides content in kidney of control and rats those receiving neem, cisplatin, pre-, post- and co-treatment of the same dose of the extract with cisplatin for 5 days are 156.85±1.24, 167.62±2.98, 205.33±0.62, 172.00±0.07, 163.89±1.05, and 164.54±1.38, respectively (Table I). These data showed a significant decrease (P<0.05) in polysaccharides content in kidney of rats received cisplatin as compared with control group. On the other hand, in rats treated with neem, pre-, post- and co-treatment of the same dose of the extract with cisplatin for 5 days showed significant increase (P < 0.05) in polysaccharides content in kidney as compared with cisplatin treated group.

Histochemical demonstration of total proteins

Liver

The protein contents of the liver cells of control rat (Fig. 3A) were demonstrated by the mercuric bromophenol blue method as blue granules against a light-blue ground cytoplasm, which indicate the presence of some soluble proteins. The protein granules were scattered all over the cytoplasm. The cells were limited by intensely stained plasma membranes. The nuclear envelopes and nucleoli as well as some chromatin elements were also positively stained.

The animals received neem (500 mg/kg b.w.) for 5 days did not manifest any obvious changes in the protein contents of their hepatocytes (Fig. 3B).

Hepatocytes of rats treated with a single dose of cisplatin (5 mg/kg b.w.) demonstrated a severe reduction of protein contents in comparison to the hepatocytes of the control rats and only a few number of cells displayed a weak reaction (Fig. 3C).

The protein contents and the general appearance of the hepatic cells were approximately restored after pre-, post- and co-treatment of the same dose of the extract with cisplatin, the majority of cells contained abundant well-stained proteinic granules in the cytoplasm. The staining affinity of the plasma membranes and the nuclear envelopes as well as the chromatin elements have clearly elevated than those of the previous case (Figs. 3D, E, F).

The mean values ± S.E of the grey level of protein content in liver of control and rats those receiving neem, cisplatin, pre-, post- and co-treatment of the same dose of the extract with cisplatin for 5 days are 118.35 ±1.10, 116.16±2.07, 186.21±0.70, 129.21±2.99, 133.66±0.24 and 126.47±1.66, respectively (Table I). These data showed a significant decrease (P<0.05) in protein content in rats received cisplatin as compared with control group. On the other hand, in rats treated with neem, pre-, post- and co-treatment of the same dose of the extract with cisplatin for 5 days showed significant increase (P < 0.05) in protein content in liver as compared with cisplatin treated group.

Kidney

The protein contents of the cortex of the control kidney rat were demonstrated as blue granules against a light-blue ground cytoplasm scattered in the entire cytoplasmic region. The protein contents were also showed as dark blue color in the brush borders and the plasma

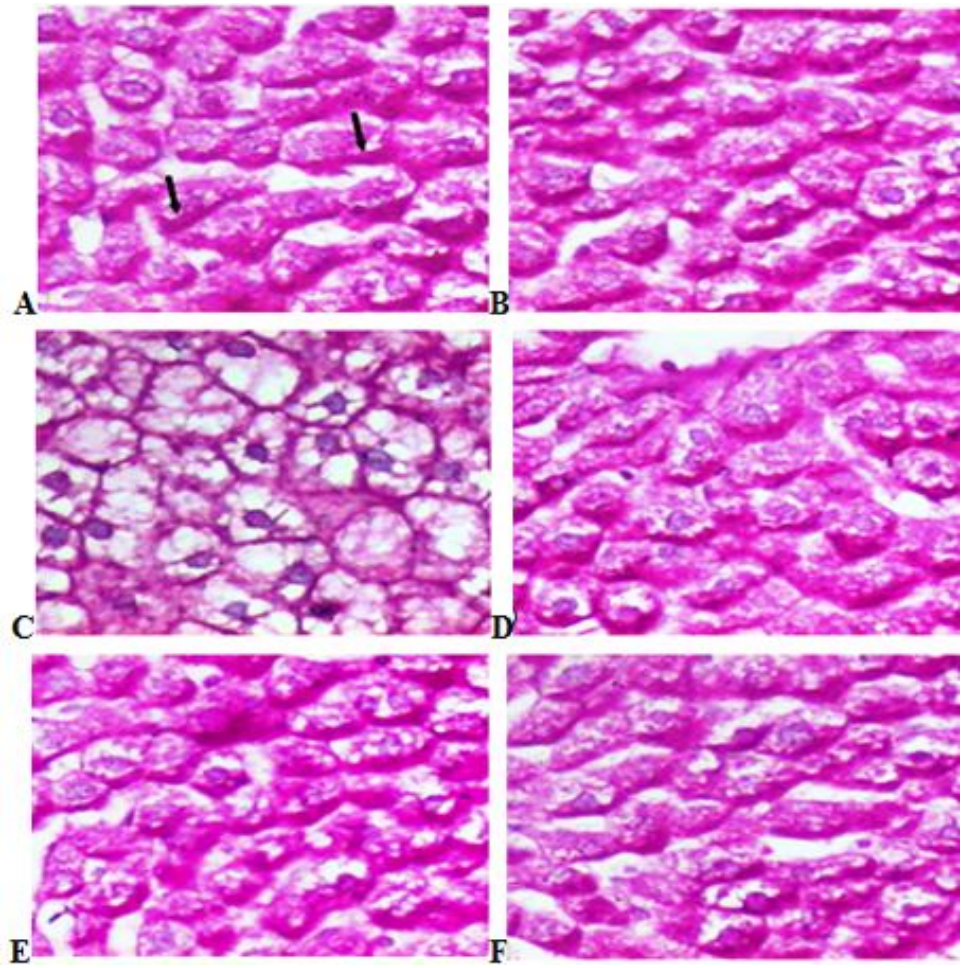

Fig. 1. Histological sections of the liver of A) control rat indicating the presence of the considerable amounts of polysaccharides (arrow), B) rats treated with daily doses of neem for 5 days shows the presence of normal amounts of carbohydrates, C) rat treated with a single dose of cisplatin shows the depletion of polysaccharides, D) rat treated with cisplatin followed and neem for 5 days shows the normal polysaccharides in some liver cells and few amounts in others, E) rat treated with successive doses of neem for 5 days before injection of a single dose of cisplatin shows more or less like the normal distribution of polysaccharides in the liver cells, F) rat treated with successive doses of neem and cisplatin for 5 days shows more carbohydrates content in hepatocytes as compared to those of the cisplatin treated rats (PAS/ H&E, x400).

membranes of the renal corpuscles and renal tubules. The glomeruli were positively stained. The nuclei contained positively stained chromatin (Fig. 4A).

The animals receiving *A. indicia* leaves extract (500 mg/kg b.w.) for 5 days did not manifest any obvious changes in the protein contents of their kidney cells (Fig. 4B).

Examination of kidney sections of the rats injected with a single dose of cisplatin (5 mg/kg b.w.) manifested obvious changes in the protein contents of their kidney cells. The glomeruli and renal tubules have lost the most protein contents and became slightly less stainable than the control animal cells (Fig. 4C).

The protein contents as well as the general appearance of the renal tissues were approximately restored after pre-, post- and co-treatment of the same dose of the extract with cisplatin; the cells of the glomeruli were again densely stained nearly like those of the controls, and the cytoplasm of the proximal and distal tubules contained abundant

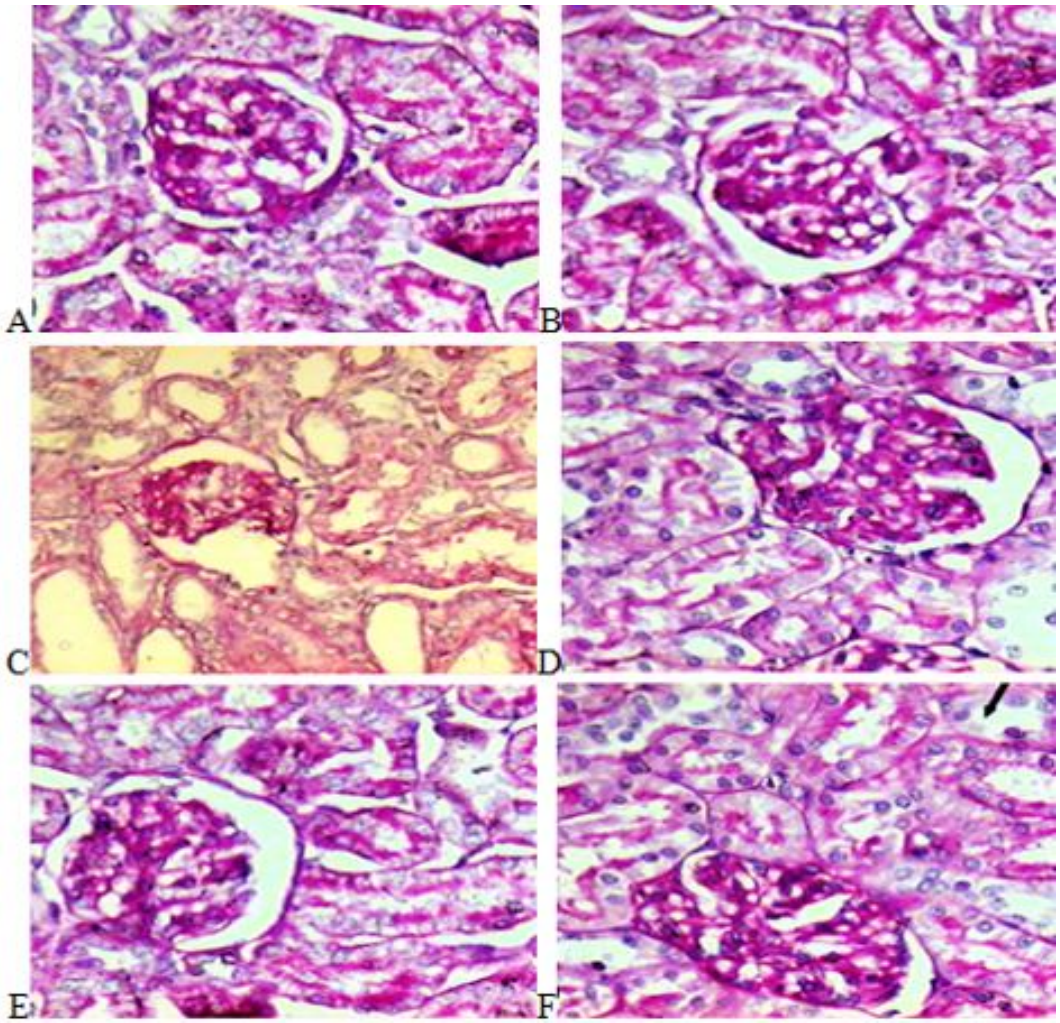

Fig. 2. Histological sections of the cortical tissue of the kidney of **A**) control rat shows the polysaccharides inclusions. **B)** rat treated with daily doses of neem for 5 days showing normal the normal distribution of the polysaccharides in the renal corpuscle and renal tubules, **C**) rat treated with a single dose of cisplatin and left for 5 days showing marked decrease in PAS reaction. The glomerulus showed faint PAS reaction. The degenerative cells and destructive brush borders showed faint stainability, **D**) rat treated with a single dose of cisplatin followed by daily doses of neem for 5 days shows the normal distribution of polysaccharides in the renal corpuscle and renal tubules, E) rat treated with successive doses of neem for 5 days before injection of a single dose of cisplatin shows the nearly normal distribution of polysaccharides in the renal corpuscle and renal tubules F) rat treated with consecutive doses of neem and cisplatin for 5 days. Notice the strong PAS reaction in the glomerulus, the basement membranes and the brush borders of the most renal tubules (PAS/ H & E, x300).

protein granules. Moreover, some cells of regenerated renal tubules displayed a slight increase in their proteins and received a darker color than the others (Figs. 4D, E, F).

The mean values ± S.E of the gray level of protein content in liver of control and rats those receiving neem, cisplatin, pre-, post- and co-treatment of the same dose of the extract with cisplatin for 5 days are 136.49±0.99, 127.68±2.21, 199.16±1.26, 142.30±0.77, 133.76±0.85 and 144.94±5.86, respectively (Table I). These data showed a significant decrease ($P < 0.05$) in protein content in rats received cisplatin as compared with control group. On the other hand, in rats treated with neem, pre-, post- and co-treatment of the same dose of the extract with cisplatin for 5 days showed

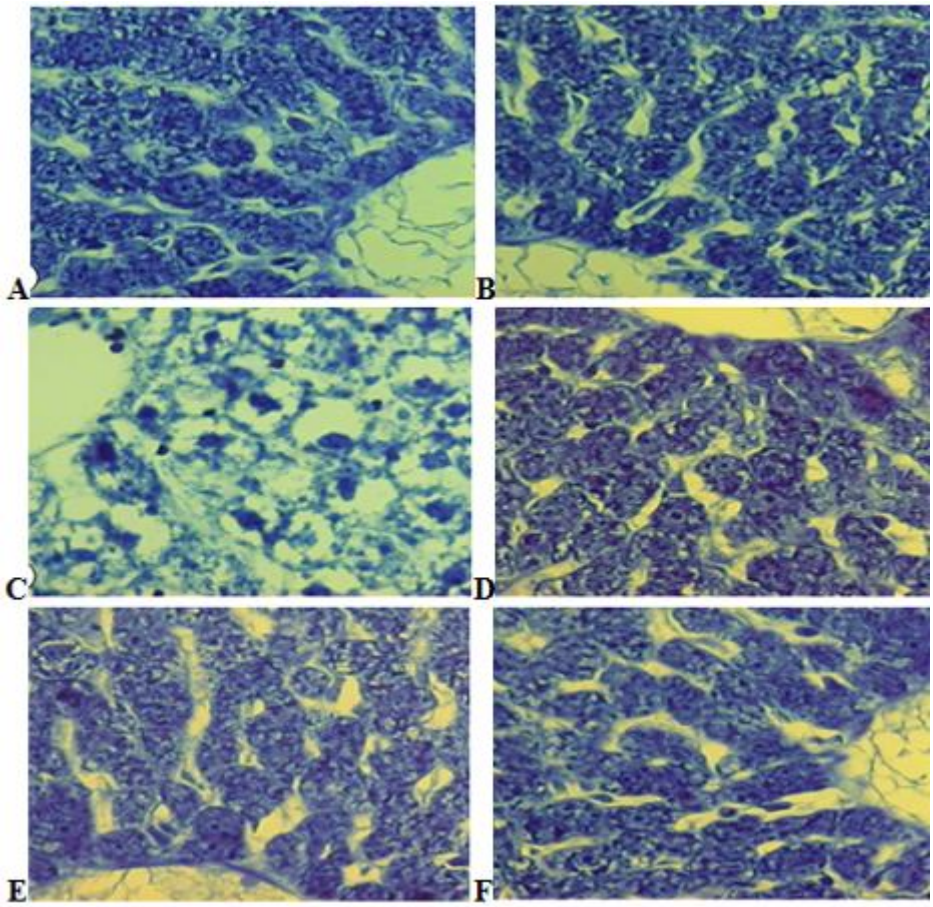

Fig. 3. Histological sections of the liver of A) control rat indicating the presence of the considerable amounts of protein in the liver cells, B) rat treated with successive doses of neem for 5 days showing the normal appearance of protein contents in the hepatocytes, C) rat treated with a single dose of cisplatin showing the dramatically affected liver cells with the depletion of protein contents in liver cells, D) rat treated with a single dose of cisplatin followed by successive doses of neem for 5 days showing the nearly normal amounts of protein in liver cells, E) rat treated with consecutive doses of neem for 5 days before injection of a single dose of cisplatin showing more or less like normal distribution of proteins in the hepatocytes, F) rat treated with daily doses of neem and cisplatin for 5 days displaying the closely normal protein contents. (MBB, x400)

significant increase ($P < 0.05$) in protein content in liver as compared with cisplatin treated group.

DISCUSSION

The liver is the centre for detoxifying any foreign compounds entering the body. So, it uniquely exposed to a wide variety of exogenous and endogenous products. These include environmental toxins and chemicals present in food or drinking (Wight, 1982). The liver is known to accumulate significant amounts of cisplatin (El-Sayyad *et al.*, 2009), thus hepatotoxicity can be associated with cisplatin treatment (Liao *et al.*, 2008).

According to the present study injection with cisplatin (5 mg/kg b.w.) induced depletion in the polysaccharides contents of the liver cells and the renal corpuscles and tubules. Also, in this study cisplatin injection of 5 mg/kg b.w. induced a severe reduction in the total proteins contents of the liver cells, the glomeruli and renal tubules in comparison to the liver and kidney of the control rats.

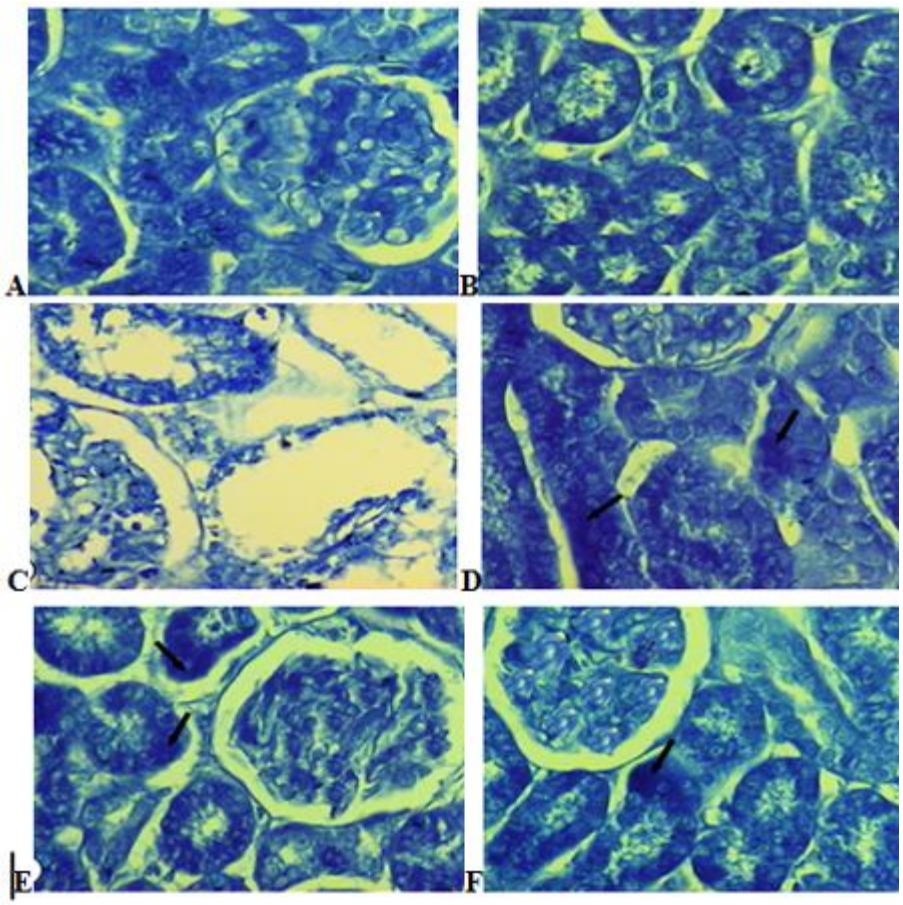

Fig. 4. Histological sections of kidneys of rats of A. control shows the normal amounts of total protein, B. treated with neem for 5 days shows the normal distribution of the proteins in the renal corpuscle and renal tubules, **C**. treated with a single dose of cisplatin and left for 5 days shows marked decrease in protein contents, **D.** treated with a single dose of cisplatin followed by successive doses of neem for 5 days shows the normal amounts of protein in the renal corpuscle and renal tubules and increment in the amount of the protein in the regenerated areas of some renal tubules (arrow), **E.** treated with consecutive doses of neem for 5 days before injection of a single dose of cisplatin shows the nearly normal protein contents in the renal corpuscle and renal tubules. Notice the darker color in the regenerative renal tubules (arrow), **F.** rat treated with consecutive doses of neem and cisplatin for 5 days. The protein contents appear more or less like the normal. Notice an increment in the amount of the protein in the regenerated areas of some renal tubules (arrow). (MBB, x400)

The present study showed decrease in the carbohydrate and protein content which can be explained by (Chen *et al.,* 1999) who stated that initiation of lipid peroxidation, necrosis and subsequent impairment in cellular metabolism collectively altered the major cellular components, including protein, and glycogen. Also, the decrease in the carbohydrate and protein content may due to the enhancement of the catabolic rate induced by cisplatin (Tikoo *et al.,* 2007; Martins *et al.,* 2008).

Cisplatin causes generation of reactive oxygen species (ROS) such as superoxide anion and hydroxyl radical (Mora *et al.,* 2003). ROS attack proteins, lipids and nucleic acids nonspecifically and induce lipid and protein oxidation (Padmavathi *et al.,* 2006; Kumaraguruparan *et al.,* 2007). Cohen and Lippard (2001) and Sadowitz *et al*. (2002) stated that cisplatin provoked inhibition of protein synthesis.

On the other hand, Reid and Li (2001) found that reactive oxygen species may activate the ubiquitin proteasome pathway. Proteasomes are

very large protein complexes located in the nucleus and the cytoplasm inside all eukaryotes (Peters *et al.,* 1994). The main function of the proteasome is to degrade unneeded or damaged proteins by proteolysis. Proteasomes are part of a major mechanism by which cells regulate the concentration of particular proteins and degrade misfolded proteins. Proteins are tagged for degradation with a small protein called ubiquitin. The tagging reaction is catalyzed by enzymes called ubiquitin ligases. Once a protein is tagged with a single ubiquitin molecule, this is a signal to other ligases to attach additional ubiquitin molecules. The result is a polyubiquitin chain that is bound by the proteasome, allowing it to degrade the tagged protein (Lodish *et al.,* 2004). Hence, the activation of the ubiquitin proteasome pathway may participate in Cisplatin - induced proteins depletion.

In general, the reduction of carbohydrates components under the effect of cisplatin could be due to the release of hydrolytic enzymes from the ruptured lysosomes under the toxic effect of the toxic agents (Shalaby, 1985). Popp and Cattley (1991) indicated that glycogen accumulation may be decreased as manifestation of toxicity, which is apparently due to impairment of enzymatic activity for glycogen catabolism or decrease in glycogen synthesis.

In the present study, the liver and kidney of animals received neem extract (500 mg/kg) for 5 days showed normal content of polysaccharides and gave a PAS reaction similar to that of control animals and did not manifest any obvious changes in the proteins content stain.

The present results are in agree with Dorababu *et al.* (2006) who documented that the aqueous extract of neem (*A. indica*) leaves showed little or no effect either on individual carbohydrates, total carbohydrates and protein. Recent study demonstrated that histopathological examination of the liver and kidney of rats showed no remarkable histopathological lesion between the controls and methanol extract of neem flowers treated rats (Kupradinun *et al.,* 2011)

In this study, histochemical results of the liver and kidney in the pre, post and co-treatments of the same dose of neem with cisplatin showed that a weak carbohydrates reaction and protein staining in the cisplatin-treated animals retained to a strong staining that appear nearly like that of the control. Moreover, some cells of regenerated renal tubules displayed a slight increase in their proteins and received a darker blue color than the others.

In the present study, the above results showed that supplementation with neem extract (500 mg/kg) prevent or reduced the histochemical alterations in the liver and kidney of the rats treated with CP to almost a normal state and these results were supported with the following earlier and recent studied.

It has been reported that oxidative stress through the generation of ROS (Chirino and Pedraza-Chaverri, 2009), decreased antioxidant defense system including antioxidant enzymes (Sadzuka *et al.,* 1992) and non-enzymatic molecule reduced glutathione (GSH) are major alterations in cisplatin toxicity (Zhang and Lindup, 1993).

The antioxidant activity of certain compounds has been suggested to protect against cisplatin-induced oxidative damage in liver (Koc *et al.,* 2005; Iraz *et al.,* 2006). Antioxidants have been shown to be protective in cisplatin nephrotoxicity (Ganczakowski *et al.,* 1995).

The leaves extract of Neem (*A. indica*) is rich in flavonoids (rutin and quercetin) (Dorababu *et al.,* 2004), flavonoglycosides, polyphenolics, tannins etc (Maity *et al*., 2009).

Flavonoid in *A. indica* is highly rich in antioxidants (Chattopadhyay *et al.,* 2004). Flavanoids have been reported to possess both antioxidant activity (Bandyopadhyay *et al.,* 2002) via scavenging free radicals (Salvayre *et al.,* 1988) and inhibition of lipid peroxidation (Dorababu *et al.,* 2004).

Neem is one of those candidate plants which has chemoprotective effect and strong anti-oxidant potential (Chattopadhyay, 2000; Sithisarn *et al.,* 2005; Ezz-Din *et al.,* 2011). In a study by Mallick *et al.* (2013), they showed that there is no alterations notoxic effect of neem leave extract on liver and kidney of rats, even with high doses exceeding the effective dose, also no apoptosis in the immune system cells and they recommend its usage in anti-cancer therapy.

Six phenolic compounds including gallic acid, benzoic acid, *p*-coumaric acid, p-

hydroxybenzoic acid, vanillic acid, and trans-cinamic acid were isolated and identified in both neem bark and leaves (Xuan *et al.*, 2004). Polyphenolics are known for their potent antioxidant and free radical scavenging properties (Krajka-Kuzniak and Baer-Dubowska, 2003). The neem leaves extract was shown to have potent antioxidant activity (Krajka-Kuzniak and Baer-Dubowska, 2003; Sithisarn *et al.* 2005).

In conclusion, the pre, post and co-treatment with the methanolic neem *(A. indica*) leaves extract were more effective in inhibiting the nephrotoxicity and hepatotoxicity as appeared in the improvements of the histochemical parameters.

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