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ORIGINAL ARTICLE

The possible protective role of platelet-rich plasma against cisplatin-induced hepatotoxicity in rat model

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ABSTRACT

Keywords: Hepatotoxicity, Platelet-Rich Plasma, Cisplatin

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Background: Cisplatin is a potent chemotherapeutic agent used to treat a variety of cancers .The major side effect of cisplatin is its hepatotoxicity. its mechanism was due to the affection of oxidant-antioxidant system. **Aim of study**: To investigate the therapeutic role of PRP against cisplatin-induced hepatotoxicity in rat model. **Materials and Methods**: 30 adult male rats , divided into 3 groups; control group; cisplatin-treated (5mg/kg, single i.p. dose); and PRP (0.5ml/kg s.c/ every other day/ for 1 week) and cisplatin-treated. **Results**: The cisplatin group showed marked pathological changes with loss of architecture, apoptotic hepatocytes, pyknotic nuclei. Dilated congested portal vein with polymorphic cellular infiltration, increased collagen fibers deposition, and a weak positive PAS reaction were observed. Immunohistochemical analyses showed strong positive caspase- 3 reactions and weak positive VEGF immunoreaction in cisplatin treated group. Group III revealed improvement of the parenchymal architecture with strong PAS reaction and minimal collagen fibers deposition. Weak positive caspase-3 reaction and strong positive VEGF reaction were noticed in PRP treated group. **Conclusion**: PRP treatment could ameliorate the destructive effects of cisplatin on the liver tissue, improve the histopathological changes and can normalize the uniform hepatic parenchymal architecture.

INTRODUCTION:

Cancer is one of the most dangerous health problems which face the world **(Sumit, 2019)**. Chemotherapy is an effective and wide spread way of cancer treatment in which one or more chemotherapeutic agents are used **(Donna and Robert, 2009).** Cisplatin (CP) is one of the platinum-based alkylating drugs, which has been cited as being among the most used cytotoxic anticancer medication **(Zhu et al., 2016).** It is commonly used clinically against testicular, ovarian, cervical, as well as bladder malignancies **(Hassan et al., 2020).** It is thought to kill cancer- cells, primarily by forming DNA adducts, causing G2 cell cycle arrest, and finally triggering apoptosis **(Ezz-Din et al., 2011)**. In spite of its significant anticancer activity, the clinical use of cisplatin is often limited by its undesirable side effects such as nephrotoxicity, neuropathy, cardiotoxicity and ototoxicity at high doses **(Man et al., 2020).** Hepatotoxicity had been certained as another important dose-limiting side effect during CP-based treatment protocols **(Dkhil et al., 2013)**Steatosis,necrosis

(steatohepatitis) and hepatocellular liver injury were described in patients who developed liver enzyme

elevations, 4 weeks after starting a regimen of cisplatin **(Dkhil et al., 2013).** Oxidative stress plays a serious role in cisplatin induced hepatotoxicity. The increase in generation of reactive oxygen species (ROS) or decrease in antioxidant activity leads to cell death and toxicity **(Yilmaz et al., 2005).**

Platelet rich plasma (PRP) is defined as an autologous product made of whole blood through centrifugation process producing high platelet concentrate in a small volume of plasma **(Andi et al., 2020).** This high platelet concentrate can lead to high growth factor levels that play important roles in the nature of thrombosis, hemostasis and wound healing **(Dhurat &Sukesh, 2014).** Growth factors on the platelets have effect on chemotaxis, differentiation, proliferation, proliferation, and synthetic activity of cells, which regulate physiological remodeling and healing **(Andi et al., 2020).**

In addition to proteins needed for hemostasis, natural cocktail of growth factors and bioactive substances are present within α-granules in the PRP which orchestrating tissue regeneration. These growth factors include platelet derived growth factor (PDGF), transforming growth factor-β1 (TGF-β1), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), (b-FGF), insulin-like growth factor (IGF) and connective tissue growth factor (CTGF) **(Dessoukey et al., 2021).**

The platelet-rich plasma (PRP) used in tissue regeneration serves as a developing area for clinicians and researchers. Due to the application of this autologous technology, PRP has potential therapeutic value in different medical fields **(Anitua et al., 2012)**. PRP has been used predominantly in the musculoskeletal field in sports injuries, cardiac surgery, pediatric surgery, gynecology, urology, plastic surgery, ophthalmology **(Andia et al., 2015),** and in dermatology; as in tissue regeneration, wound healing, scar revision **(Rubina &Ramon, 2018).** Further, some studies have reported that platelets accumulate in the liver under some kinds of pathologic conditions, like ischemia/reperfusion injury **(Pak et al., 2010)** and liver cirrhosis **(Zaldivar et al., 2010).**

MATERIALS AND METHODS

Animals:

The present study was carried out on 30 adult male albino rats weighing (150-250gm). The experiment was performed at the Animal and Experimental House, Faculty of Medicine, Assiut University. The rats were housed in separate cages (10 rats each), after an acclimatization period for two weeks. The animals were allowed to standardized laboratory diet and water ad libitum throughout the experiment.

Chemicals:

1. **Cisplatin** (CDDP): supplied as vials; each contains 50mg/50ml, purchased from Merck company, Germany). It was given as a single intra peritoneal (I.P) dose (5 mg/kg).
2. **Platelet Rich Plasma** (PRP): was supplied from the blood of donor rats in the animal house of Assiut University. It was given as a S.C dose (0.5ml/ kg every other day/ for 1 week).

Preparing Platelet-Rich Plasma

Ten rats were used as blood donors and 2 mL blood was collected using aseptic techniques from the retro-orbital plexus into tubes containing 0.3 ml (EDTA) anticoagulant. Two centrifugations were performed to the blood; the first at 14000 rpm for 15 min at 73C, to separate erythrocytes; and the second at 2000 rpm for 10 min to concentrate the platelets **(Kazemnejad et al., 2008).** This procedure was done in the laboratory of the animal house of Assiut University. The PRP were freshly prepared each time, and used within one hour after preparation **(Kwon et al., 2012).**

Experimental design:

The rats were randomly divided into 3 groups (10 rats, each), as follows:

1-Group I (control group)

2-Group II (cisplatin-treated): each rat was injected with a single dose of cisplatin (5 mg/kg/ i.p)

3-Group III (cisplatin and PRP-treated): each rat was injected I.P with a single dose of cisplatin (5 mg/kg). Then on the 7th day each rat was injected subcutaneously with (0.5ml/kg) of PRP, every other day for one week. At the end of the experiment, the rats of each group were sacrificed and the liver was excised and fixed in 10% formalin solution for preparation of paraffin blocks and sections and then processed for **histopathological examination (Bancroft and Gamble, 2008) and immunohistochemical analysis for** Caspase- 3 and Vascular endothelial growth factor (VEGF) staining **(Alturkistani et al., 2016).**

RESULTS

A-Histological results

H&E-stained liver sections of **control** group showed uniform hepatic parenchymal architecture consists of classic hepatic lobules. Each lobule containing cords of regularly arranged hepatocyte plates radiated from a central vein and separated by narrow finely arranged blood sinusoids with portal area at the corners of this hepatic lobule**.** The hepatocytes appeared polyhedral in shape, with central rounded vesicular basophilic, nuclei, prominent nucleoli and acidophilic cytoplasm. The portal area contained branches from portal vein, bile duct and hepatic artery (Figs. 1a, b& c).

The Cisplatin treated sections characterized by diffuse disturbed hepatic architecture with disorganized and dissolutions of hepatic cords or with thin cords, widely separated by irregular dilated sinusoids. Most hepatocytes around the central vein and portal triad within the lobule appeared degenerated or apoptotic with more eosinophilic cytoplasm and small deeply stained basophilic nuclei. Other hepatocytes showed hydropic degeneration and appeared swollen with small darkly stained or hyperchromatic degenerated nuclei and vacuolated cytoplasm (Figs.1d, e& f).

Liver sections of PRP+ Cisplatin -treated group demonstrated preservation of uniform hepatic parenchymal architecture. Apparently, normal hepatocytes were arranged in regularly radiating cords from the mildly dilated central vein in the hepatic lobule. Most hepatocytes showed well-preserved cytoplasm and central vesicular nuclei, although few ones appeared apoptotic or exhibited vacuolated cytoplasm with pyknotic or lysed nuclei. Characterized numerous binucleated hepatocytes were evident in this group around the central vein and portal area. The cords were separated by regularly directed normal hepatic sinusoids, but some appeared mildly dilated and were lined with prominent Von-Kupffer cells. The portal area showed apparently normal portal vein and bile ductulus (Figs. 1g, h, i).

Masson's trichrome-stained liver sections in control group demonstrated a thin layer of collagen fibers around the central vein and adjoining hepatic sinusoids. A small amount of Masson's trichrome blue staining of collagen fibers was also seen surrounding the elements of the portal triad (Fig 2 a).

Cisplatin treated group revealed an increase in the amount collagen fibers (fibrosis) throughout the hepatic lobule. Expanded periportal areas were observed in the liver and there was an increase in collagen fiber depositions and blue staining of Masson's trichrome around the portal tracts (periportal fibrosis), as well as around the central veins, as compared to control.(Fig 2 b).

PRP-Cisplatin treated group revealed mild collagen fibers deposition within the hepatic lobule and the amount of blue Masson's trichrome staining was comparable to the control, around the central vein as well as the portal tract and the sinusoids (Fig 2 c).

PAS-stained liver sections: Strong positive PAS reaction of the glycogen content was seen throughout the different zones of the hepatic lobules in control rats. This positive reaction appeared in the form of red glycogen containing granules in the cytoplasm of the hepatocytes around central vein and the portal area as well as in the wall of the vessels of the hepatic lobules (Fig. 3 a, b).

Cisplatin treated group showed marked diminished PAS reaction in most of hepatic lobules. Most of hepatocytes showed non obvious or weak positive PAS reaction in their cytoplasm (Fig.3c, d).

The PRP treated group showed moderate to strong positive PAS reaction in the hepatocytes around the central vein and or portal area and their endothelial walls, comparable to control (Fig.3e,f).

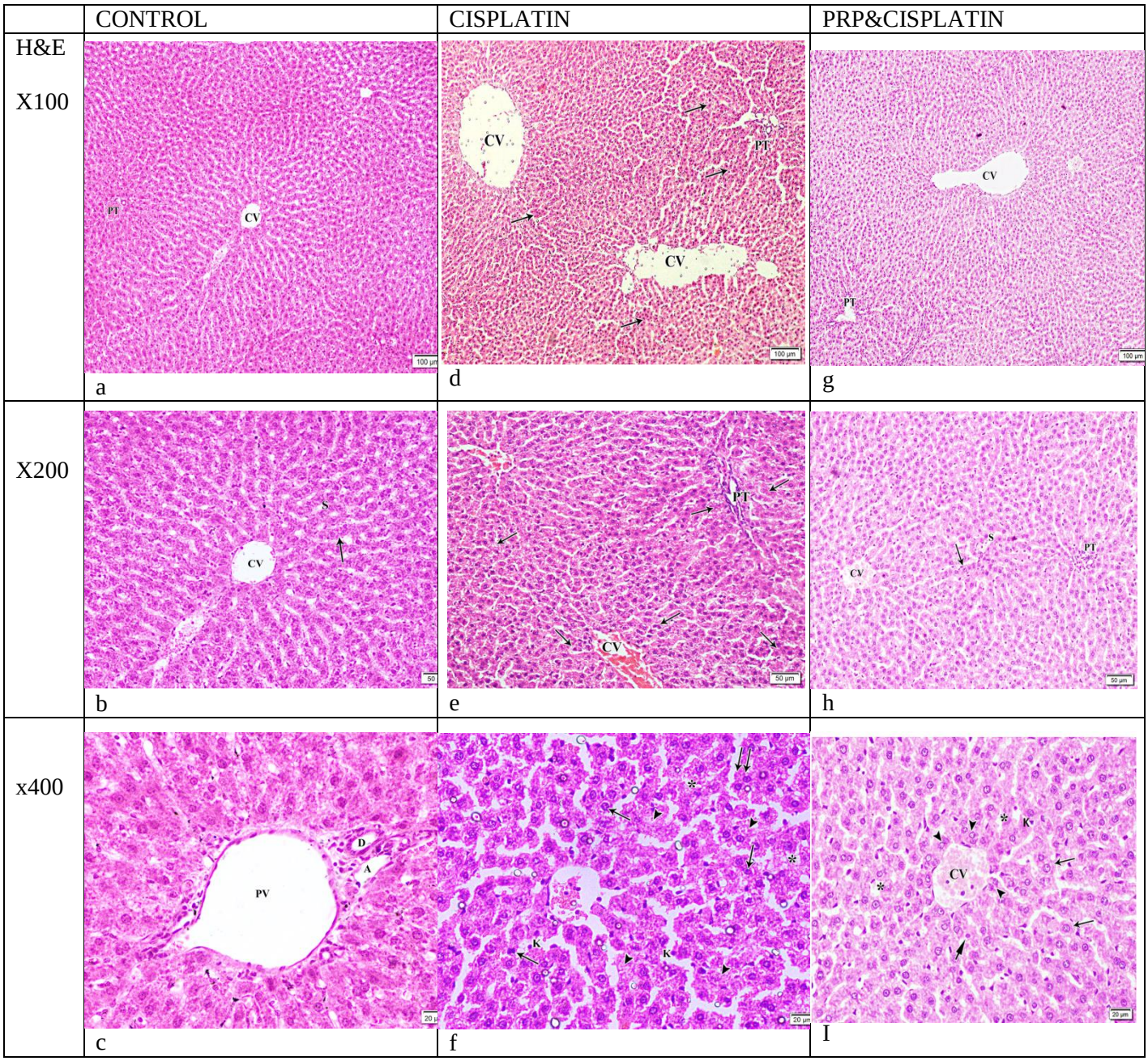


Fig.1. H&E stained liver sections of the experimental groups showing normal classic liver architecture of the control group (a,b,c); marked dissolutions of the architecture, dilatation and congestion of the central vein and hepatic sinusoids with variable degenerative changes in the hepatocytes of Cisplatin-treated group (d,e,f); obvious improvement of the liver structure and normalization of the hepatocytes, sinusoids and portal area (g,h,i) in PRP-treated group. CV: central vein S: hepatic sinusoid, PT: portal tract, PV: portal vein, A: hepatic artery, D: bile duct, K: proliferating Kupffer cells, (*) focal area of necrosis, arrow: degenerative cells, arrow head: binucleated hepatocytes.

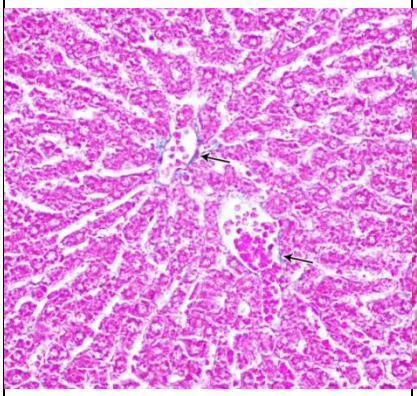
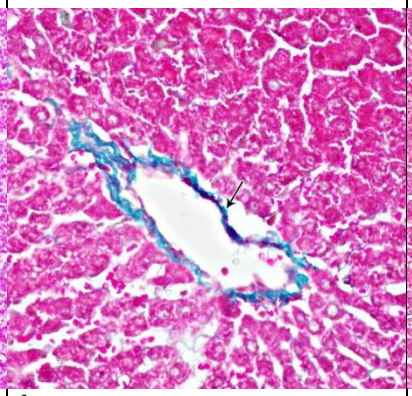
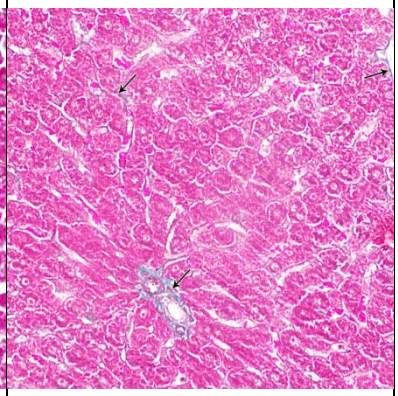
	CONTROL	CISPLATIN	PRP&CISPLATIN
MASSON TRICHROME STAINING X400			
	a	b	c

Fig 2. Masson' trichrome stained liver sections showing minimal collagen fibers in the control group (A), marked increase of collagen fibers in cisplatin-treated group (B) and mild collagen deposition in PRP-treated group (C).

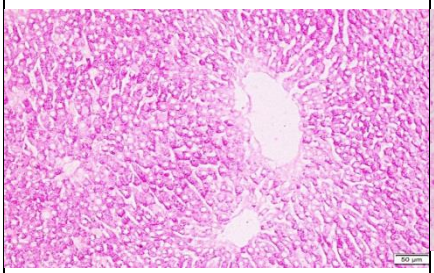
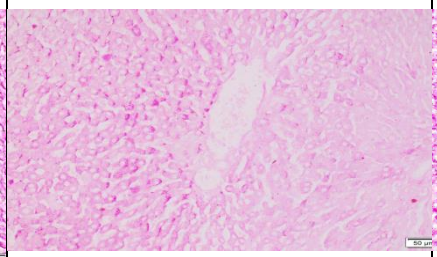
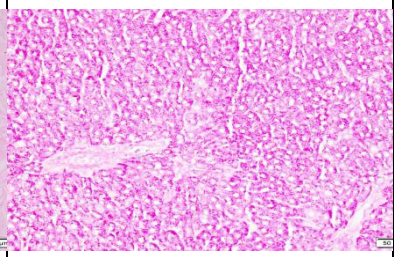
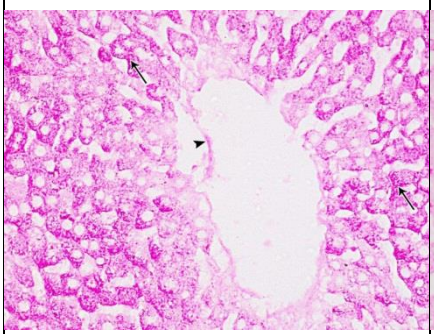
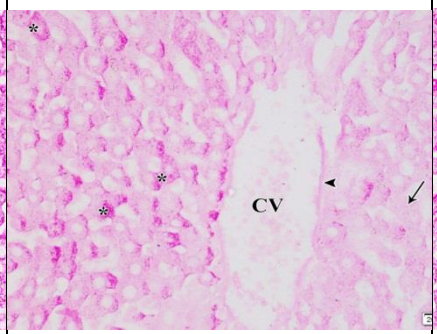
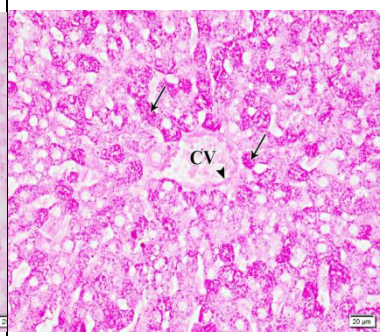
	CONTROL	CISPLATIN	PRP&CISPLATIN
PSA STAINING X200			
	a	c	e
X400			
	b	d	f

Fig. 3: PAS-stained sections showing positive PAS reaction in the hepatocytes of the control group (a,b), weak reaction(*) or depletion (arrow) in cisplatin treated group(c,d) and strong reaction in PRP-treated group (e,f).

B- Immunohistochemical results

Caspase-3 immunoexpression

Liver-stained sections of the control group revealed occasional weak positive immunoreaction in few sporadic cells and some sinusoidal Kupffer cells as well as in the endothelial lining of portal tract and some sinusoids of the hepatic lobule (Fig. 4a,b). The cisplatin group showed marked increase in the positivity of caspase-3 immunoreaction within the hepatic lobule as compared to control group (Fig 4. c). The vacuolated and apoptotic hepatocytes, surrounding the Portal tract and the central vein showed the strong brown immunoreactions of caspase-3 immunoreactivity predominantly in their cytoplasmic

remnants and in few nuclei (Fig.4c, d). A strong positive caspase-3 immunoreaction was also revealed in the endothelial lining of the vessels at the central vein, portal tract and hepatic sinusoids (Fig.4c,d). With PRP treatment, a marked diminished in caspase-3 immunoexpression was observed except for a positive reaction in few apoptotic hepatocytes within the hepatic lobule. Caspase-3 expression was extremely reduced in hepatocytes either in intensity of brown positive hepatocytes or distribution among hepatocytes. This faint positive immunoreaction was mainly observed in cells around the central vein and to some extent around the portal area and in endothelial lining (Fig.4e, f).

VEGF immunoreaction

Positive VEGF immunoreaction was noted in the endothelial lining of the central vein, portal vein and hepatic sinusoids as well as in the cytoplasm of the adjoining nearby hepatocytes and in few sporadic cells within the hepatic lobule of the control group (Fig.5 a, b). Diminished VEGF immunoreaction was observed throughout the hepatic lobule of cisplatin treated sections, except for in some sporadic apoptotic cells and the endothelial lining (Fig.5 c, d). Meanwhile, positive VEGF immunoexpression was demonstrated with variant intensity, throughout the hepatic lobule of PRP-Cisplatin treated sections. The positive reaction was more obvious expressed in the endothelial lining of the vessels and in the nearby hepatocytes (Fig.5 e). Moreover, the positive VEGF immunoreaction was strongly expressed in the hepatocytes, surrounding the central vein (Fig.5 e, f).

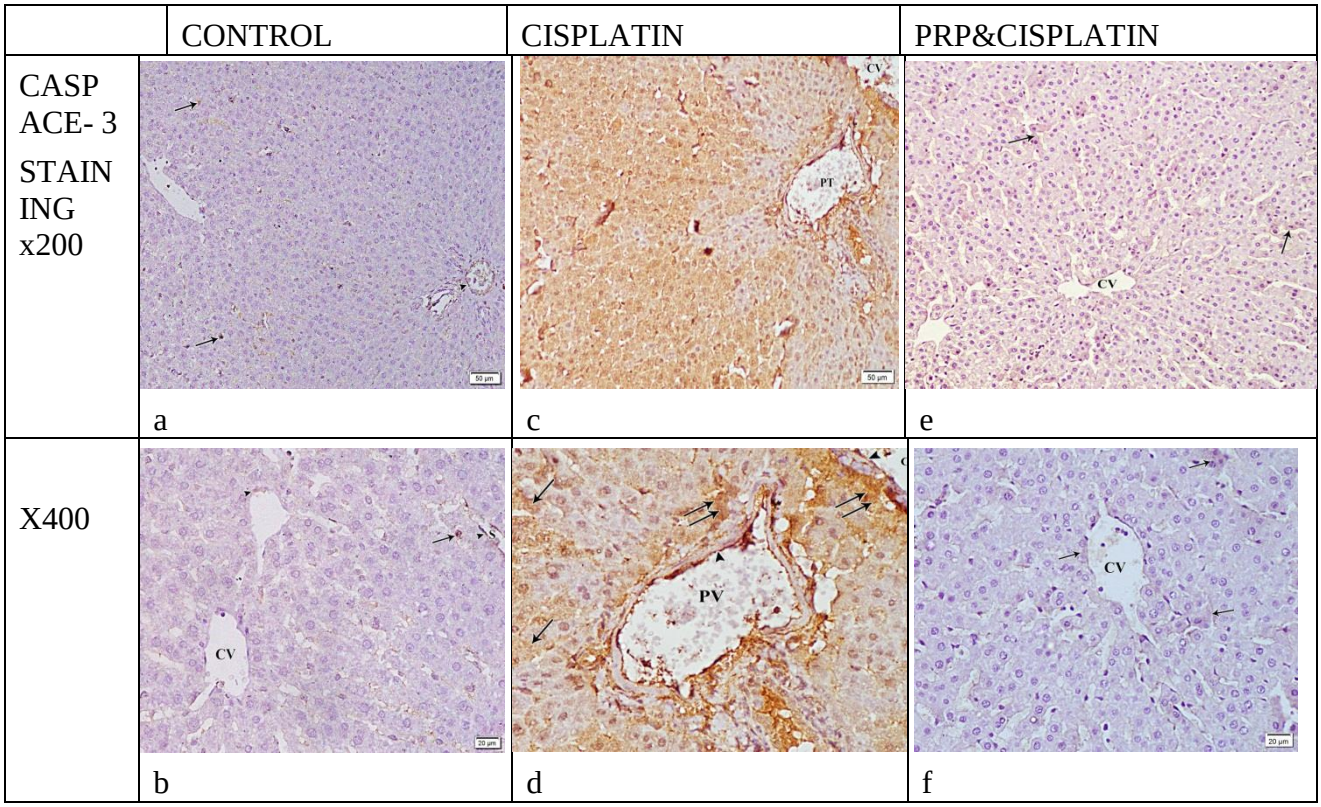


Fig4. Caspase- 3 immunoexpression: Negative reaction in the hepatocytes of the control group (a,b), strong reaction in cisplatin-treated group (c,d) and mild reaction in sporadic cells of PRP-treated group (e,f).

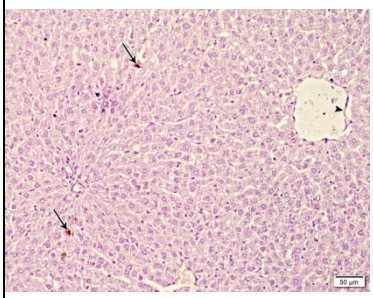
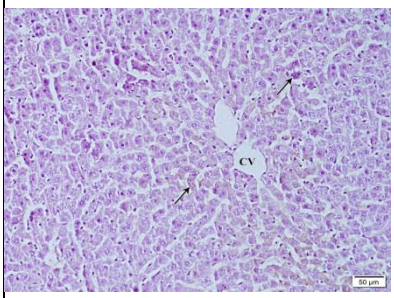
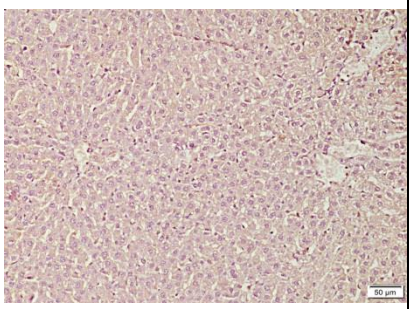
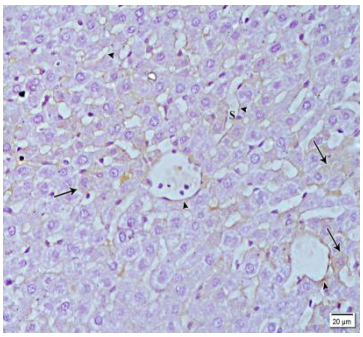
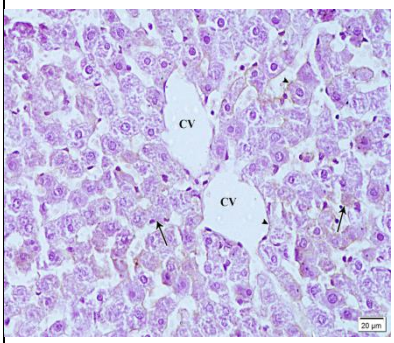
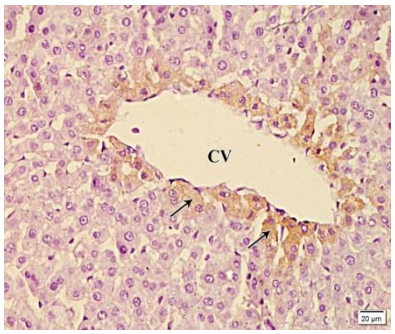
	CONTROL	CISPLATIN	PRP&CISPLATIN
VEGF STAINING X200	 a	 c	 e
X400	 b	 d	 f

Fig5.VEGF immunoexpression: positive VEGF reaction in the endothelial lining of the hepatic lobule and nearby hepatocytes, in the control group (a,b), diminished VEGF immunoreaction in cisplatin-treated group (c,d) and strong positive VEGF reaction in PRP-treated group (e,f).

DISCUSSIONS

Cisplatin has been cited as being among the most used cytotoxic anticancer medication due to its broader efficacy in the treatment of various types of cancers **(Zhu et al., 2016)**. The clinical use of cisplatin is often limited by its undesirable side effects such as nephrotoxicity and ototoxicity **(Man et al., 2020).**

Generation of highly reactive oxygen species (ROS) may be the underlying mechanism of hepatotoxicity induced by cisplatin. ROS could damage biological molecules such as lipid, protein, DNA, and eventually impair cell integrity. Thus cisplatin can cause oxidative damage to the liver **(Palipoch et al., 2014).** In addition to functional and structural mitochondrial injury, and apoptosis, involvement of proinflammatory genes such as COX-2, and inducible nitric oxide synthase (iNOS), may play some important role in the mechanism of cisplatin hepatotoxicity **(Ezz-Din et al., 2011).**

Hepatotoxicity can also occur when cisplatin is administered at high doses and or at low doses of cisplatin, probably due to cumulative effect in the liver which cause massive hepatic toxicity, including dissolution of hepatic cords, focal inflammatory lesions and necrosis **(Singh et al., 2015).**

Accordingly, our current results showed many histopathological abnormalities in cisplatin (5 mg/Kg) treated liver in the form of marked disruption of hepatic cords and dilated blood sinusoids with prominent Kupffer cells. Hepatocellular apoptosis and necrosis with cytoplasmic vacuolations and nuclear pyknosis were prominent features in our study. In consistent, **Aboraya et al. (2022)** indicated that cisplatin treatment resulted in a severe array of events of hepatotoxicity. **AbdRashid et al. (2021**) reported hepatocellular vacuolation, sinusoidal dilatations and cytoplasmic changes around the central vein in cisplatin-induced liver toxicity. Moreover, **Alrashed and El-Kordy (2019) & Fathy et al. (2022)** in their histological study of cisplatin treated rats with a single dose of 10mg/Kg showed sinusoidal congestion, vacuolar degeneration disorganization of hepatocytes around central vein, along with multiple necrotic foci. **SAH et al. (2022)** reported that the most intense necrotic area, congestion and mononuclear cell infiltration was in the cisplatin group when compared to the other groups. They mentioned with **Aydin et al. (2003)** that this inflammatory reaction could be related to the oxidative stress and destruction of the endothelial cells by the free radicals.

Moreover, our result is confirmed by **Mir et al. (2015),** who showed that the significant increase in the average activity of serum enzymes (ALT, AST, ALP) in cisplatin treated groups, was related to hepatocytes cell membrane damage and enzymes leak from the hepatocytes.

Apoptosis is a common feature of hepatotoxicity following the administration of hepatotoxins which were proved to induce a significant deterioration in the liver **(Al-Hamdany and Al-Hubaity, 2014)**. Apoptosis was an important finding in the present study which is evidenced by diminished cell size, pyknotic nuclei and more eosinophilic cytoplasm with reduced volume. **Alrashed and El-Kordy (2019)** indicated that the observed cytoplasmic and nuclear changes in hepatocytes of cisplatin-treated animals could be the result of induction of oxidative stress and damage of DNA that causes degeneration and death of hepatocytes. The larger number of apoptotic cells in the hepatotoxicity induced by cisplatin, may switch to secondary necrosis **Alrashed and El-Kordy, (2019)**.

The prominent Kupffer cells, in the present study and that of **Omar et al. (2016)** and **Taghizadeh et al. (2021)** could be correlated with the amount of injury to the hepatic tissue induced by cisplatin intoxication and represents a defensive mechanism of detoxification. Kupffer cell hyperplasia was established as a contributor to hepatic oxidative stress **(EL Kalawy et al., 2017)**.

The cisplatin induced hepatocellular degenerations, in this study was distributed throughout the hepatic lobule, but mainly occupied the pericentral area around the central venules. This is supported by **Alrashed and El-Kordy (2019) & Fathy et al. (2022),** who demonstrated that the variable degenerative changes as well as the extensive disorganization in hepatocytes and the sinusoidal dilatations were common around the central vein in cisplatin-induced liver toxicity.

The present study showed that cisplatin induces reduction in hepatocytes glycogen content throughout the hepatic lobule in a heterogeneous pattern. **Kara & Kilitci (2022)** supported our results and showed significant decrease in the area percentage of PAS reaction in the hepatocytes of cisplatin group as

compared to the control group. The synthesis and storage of glycogen granules are the functions of normal hepatocytes. Glycogen depletion noticed in the liver may be attributed to the oxidative stress and mitochondrial dysfunction caused by anticancer drug. The oxidative stress leads to depression in the cytochrome oxidase and dehydrogenase enzymes activities and this was reflected on the liver glycogen through enhancing glycogenolysis **(El Kalawy et al., 2017)**.

Masson's trichrome stained liver sections in this study demonstrated an increase in collagen depositions around the blood vessels of cisplatin group. This is in agreement with previous report of **Alrashed and El-Kordy (2019) & SAH et al. (2022).** Perivascular round cell infiltration, associated with membrane changes of endothelial lining cells manifesting periportal fibrosis **(El-Sayyad et al., 2009).** Marked accumulation of collagen fibers and proliferating bile ducts are the resultant of ROS generation (**Alrashed and El-Kordy, (2019)**. The inflammatory cytokines, released by the inflammatory cells led to deposition of connective tissue (mainly collagen I), causing fibrosis in the inflamed area, this explained the significant increase of percentage of Masson's trichrome positively stained area **(Abd-Elhafiz & Issa, 2021)**.

Apoptosis is a gene-regulated event related to special morphological changes. Two main families of proteins, including cysteine protease called caspase enzyme (especially caspase 3, 8, 9) and Bcl-2 family are believed to participate in apoptotic mechanism. Caspase 3 is the most important member of caspase family, which is responsible for many biochemical manifestations of apoptosis that lead to cleavage of nuclear and cytosolic substrates, chromatin condensation, frangmentation of DNA, and apoptotic body **(Karadeniz et al., 2011).**

Anti-caspase-3 antibody was used in this study for immunohistochemical detection of apoptotic hepatocytes and revealed a prominent increase in Caspase-3 immunoexpression in cisplatin group compared to the control one. The positive reaction was demonstrated predominantly in the cytoplasm and/or little nuclear immunoreactivity of the hepatocytes as well as in the endothelial lining within the lobule. Administration Cisplatin (7.5 mg/kg) in rats for 7 days resulted in a marked increase in the liver expression of caspase-3 protein relative to controls **(Eid and El-Shitany, 2021)** and the increase in the number of caspase-3 positive cells was found to be statistically significant in cisplatin group **(SAH et al., 2022). Taghizadeh et al. (2021)** mentioned that **t**he intensity of the brown color indicates the activity of caspase-3 in hepatocytes.

Cisplatin is thought to kill cells primarily by forming DNA adducts, causing G2 arrest in the cell cycle, triggering apoptosis **(Abd Rashid et al., 2021). Neamatallah et al. (2018); Hassan et al. (2020) & Abd-Elhafiz & Issa (2021)** confirmed that cisplatin administration is accompanied by apoptosis and caspase activation is the essential step for the initiation of apoptosis induced by various stimuli. The hepatic oxidative stress induced by the cisplatin would result in apoptosis, that confirmed by increased hepatic caspase-3 expression **(Aboraya et al., 2022)** and enhanced caspase-3 gene expression in rat liver tissue **(Fathy et al., 2022)**.

Weak VEGF immunoreaction was expressed in cisplatin treated group in this study. This faint VEGF reaction was observed in cytoplasm of the hepatocytes, around the central vein and portal triad as well as in the endothelial lining, within the hepatic lobule**.** However, **El- Sharouny et al., (2019)** demonstrated a strong VEGF immunoreaction in cisplatin treated liver. They noticed a marked increase in the mean value of area % of VEGF immunoexpression in cisplatin group, which was statistically significant, compared to the control. VEGF is a mitogen for vascular endothelial cells and regulates vascular pathophysiology, including vasodilatation, vascular permeability, migration, and survival of endothelial cells. The expression of VEGF and its receptors is not restricted to vascular endothelial cells; VEGF is secreted by several epithelia, where it modulates cell growth by autocrine and paracrine mechanisms **(Franchitto et al., 2013)**. Moreover, VEGFs are potent inductors of vascularization, development and growth of several cancers, and are upregulated in many human cancers. **Song Zhong et al. (2007)** demonstrated that cisplatin inhibited VEGF expression in human ovarian cancer cells and found that cisplatin inhibited the VEGF reporter activity in a dose-dependent manner, indicating that cisplatin inhibited transcriptional activation of VEGF. Cisplatin may inhibit proliferation of endothelial cells in vitro and is thought also to trigger a degenerative process of medium thickness vessel walls, thus causing occlusive vascular disease in

the long term **(Morlese et al., 2007)**. This may explain the weak VEGF immunoreaction in cisplatin treated rat in this study.

Platelet-rich plasma (PRP) has grown as an attractive biologic instrument in regenerative medicine for its powerful healing properties **(Andi et al., 2020)**. PRP is a powerful therapeutic option for its ability to deliver a great variety of biologically active growth factors (GFs) to the site of injury and is characterized by its simplicity, effectiveness, safety, and constant availability **(Dhurat & Sukesh., 2014)**.

This product has proven its efficacy in multiple studies, but its effect on cisplatin-induced hepatotoxicity has not yet been elucidated **(Knezevic et al., 2016)**. Hepatic GF mediates cellular proliferation, migration, survival, and tissue regeneration. So, it could be anticipated that PRP administration as a natural cocktail of GFs with cisplatin would improve liver recovery **(Knezevic et al., 2016)**.

Our present result showed that PRP treatment in a dose of (0.5ml/kg), every other day for 1 week improves and recovers the histopathological abnormalities induced by cisplatin in liver tissue. The HE stained sections showed preservation of the hepatic parenchymal architecture and most hepatocytes appeared with large vesicular nuclei and apparently arranged in regularly radiating cords from the mildly dilated central vein and separated by narrow regular hepatic sinusoids, with apparent normal Kupffer cells. The portal area and its components appeared normal in the hepatic lobule. Confirmedly, **Hesami et al. (2014); Abdel Fattah et al. (2018) & El-Sharouny (2019)** in their histological study of platelet treated rats with a single dose of 0.5mg/Kg showed obvious improvement of the liver structure and normalization of the hepatocytes, sinusoids and portal area. Meanwhile, in accordance to **Arafa et al. (2014); El-Sharouny et al. (2019)** studies, the present study revealed few apoptotic hepatocytes or with vacuolated cytoplasm and or pyknotic nuclei, as well as few necrotic areas in PRP treated hepatic lobule. Characterized numerous binucleated hepatocytes or hepatocytes with proliferating nuclei were evident in this group around the central vein and portal area. These results with the previous ones may indicate the curative effect of PRP on the cisplatin induced toxicity (**Salem et al., 2018; El-Sharouny et al., 2019 & Dessoukey, et al., 2021).**

The PAS-staining in the present study as well as in the study of **Ton& Robert (2016)** showed that administration of PRP to cisplatin treated animals lead to restoration of the glycogen contents in the cytoplasm of hepatocytes, throughout the hepatic lobules of PRP treated sections, comparable to the control ones. **El-Sharouny et al.,(2019)** reported that the synthesis and storage of glycogen granules are the functions of normal hepatocytes and PRP appeared to be histologically effective in the regeneration of liver hepatocytes.

The administration of PRP to cisplatin induced hepatic fibrosis resulted into marked improvement in liver structure and mild collagen fibers deposition was revealed in **Masson's trichrome** stained sections in the present work. The amount of blue Masson's trichrome staining was comparable to the control, around the central vein and portal area. This result is confirmed by **El-Sharouny et al., (2019)** and **Abd Elzaher et al. (2021)**. This could be due to the fact that PRP increase the intracellular expression of the anti-inflammatory mediators (IL-4, IL-10, and IL-13) which is known to play a major role in inhibiting inflammation and decreasing IL-1β-mediated catabolic effect **(Moghadam et al., 2017).** Moreover, IGF1 is one of the growth factors in PRP which stimulates the release of growth hormones, which help in tissue repair, as well as the progenitor cells came with the vessels **(Textor, 2014; Kholodkova & Romak, 2016).** They also declared that the progenitor cells in the PRP differentiate in the hepatic tissue into hepatocytes and stellate cells, thus preventing formation of connective tissue components. This capacity of PRP to ameliorate cisplatin induced hepatotoxicity is represented by the improved histological results in PRP group. It may be mediated through natural cocktail of growth factors and bioactive substances within α-granules in the PRP which orchestrating tissue regeneration. These growth factors include platelet derived growth factor (PDGF), transforming growth factor-β1 (TGF-β1), epidermal growth factor (EGF), VEGF, (b-FGF) and connective tissue growth factor (CTGF) **(Halpern et al., 2012)**. Moreover, others reported that PRP decreased the expression of mRNA of fibrosis associated genes including TGF-β, in addition to, inflammatory related gene NF-kβ and liver IL-8. They also, reported a significant increase in the anti-apoptotic marker Bcl-2 after PRP treatment, confirming PRP role in liver regeneration **(Salem et al., 2018).**

Anti-caspase-3antibody immunohistochemical stained sections, in this study showed a marked diminished in caspase-3 immunoexpression in hepatic tissue, after administration of PRP to cisplatin treated liver, except for a weak reaction in few hepatocytes and in the hepatic sinusoidal lining. Caspase-3 expression was extremely reduced in hepatocytes either in intensity of brown positive hepatocytes or distribution among hepatocytes. Our findings match the criteria reported by **Salem et al. (2018)** & **El-Sharouny et al.,(2019)** who demonstrated a suppression of apoptosis in cisplatin treated hepatic lobule after PRP treatment. The significant decrease in the area fraction of apoptosis in PRP group compared to cisplatin group. This could be due to the fact that PRP has anti-apoptotic activities via down regulating the expression of apoptotic genes as DAPK1 and BIM mRNA and inhibiting p53, BAX, and caspase-3 levels. In addition, PRP may suppress caspase-3 by enhancing the PI3K/Akt pathway which curbs reactive oxygen species generation, thereby downregulating nuclear factor Kappa B (NF-κB) activation and increasing resistance to oxidation **(Dessoukey et al., 2021)**.

Clear positive **VEGF** immunoexpression was demonstrated in this study, with variant intensity, throughout the hepatic lobule, of **PRP**- treated sections, compared to the cisplatin group. The positive reaction was more obvious expressed in the endothelial lining of the vessels and in the nearby hepatocytes, mainly surrounding the central vein. While, **El-Sharouny et al., (2019)** demonstrated a less VEGF immunoreaction in PRP treated group liver sections, as compared to the cisplatin treated one. They showed that, the mean value of area % of VEGF immunoexpression in PRP group (10.4±3.8) was less than of cisplatin group (14.3±2.3). **Ohkohchi., et al. (2012)** proved that immediate contact between platelets and hepatocytes could evoke the release of soluble factors from platelets, for example IGF-1, VEGF and HGF, which are considered crucial mediators for liver recovery. The powerful influence of high concentrations of biologically active substances involved in the neoangiogenesis led to growth of new blood vessels that created a unique framework for the diseased tissue (**Textor, 2014; Kholodkova & Romak, 2016)**.

This result could be related to the fact that PRP extract promotes angiogenesis through the angiopoietin1-Tie2 pathway that stimulates growth, migration, and differentiation of endothelial cells **(Dessoukey et al., 2021).** Natural cocktail of growth factors and the progenitor cells in the PRP promote the differentiation of hepatocytes and liver regeneration (**Salem et al., 2018)**.

CONCLUSIONS

The results of this study concluded that the PRP treatment could ameliorate the destructive effects of cisplatin on the liver tissue and improve the induced histopathological changes. PRP can normalize the uniform hepatic parenchymal architecture (reduces the apoptosis, necrosis and degeneration and fibrosis). It can improve the function of the liver (restores the glycogen content of the hepatocytes).

Platelet therapy can open a new horizon to develop novel strategies for the treatments of liver diseases. Overall, platelet rich plasma can be used as a complementary procedure to decrease the destructive effects of hepatotoxicants.

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