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ANTIOXIDANT EFFECT OF POMEGRANATE TOTAL PEEL EXTRACT ON OXIDATIVE DAMAGE CAUSED BY CISPLATIN IN RAT LIVER

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ABSTRACT

Cisplatin as an antineoplastic drug shows the considerable hepatotoxicity on the liver in high doses. The involvement of oxidative stresses has been identified in Cisplatin cytotoxicity. Due to the antioxidant properties of pomegranate peel, this study was designed to evaluate antioxidant effects of Pomegranate total peel extract (PPE) against oxidative stress induced by Cisplatin in rat.

In this laboratory experimental study, 60 male Wistar rats were randomly divided into four groups. Group 1 was selected as a control. Groups 2 and 4 were gavaged by PPE (500 mg/kg) for 15 consecutive days. Groups 3 and 4 received single-dose Cisplatin (7.5 mg/kg) on the tenth day of experiment intraperitoneally and the administration of pomegranate peel continued during other 5 days in groups 2 and 4. Finally, the histological scores of Malondialdehyde (MDA), reduced Glutathione as well as activity of Superoxidase Dismutase, Catalase and Glutathione Peroxidase and Reductase enzymes were measured in homogenate of the hepatic tissue. Finally, these findings were compared with histopathological results.

Pomegranate peel extract could increase the scores of hepatic antioxidants and decrease significantly amount of MDA in group 4. The histological changes were also consistent with enzymatic findings histopathologically.

Antioxidant function of pomegranate caused to protect rats liver against oxidative stress induced by Cisplatin.

Keywords: Pomegranate peel, Cisplatin, oxidative stress, hepatic antioxidants, rat

INTRODUCTION

The human body daily is exposed to a lot of free radicals. Free radicals by the induction of undesirable oxidation damage to the cellular membrane, proteins, destroy DNA and lead to cell death [1]. Environmental pollution, stress, smoking, inadequate nutrition radiations are of external factors that lead to the production of free radicals [2] . However, free radicals are an integral part of the normal function in the body's cells. Cells need oxygen to produce energy in a process so called to the mitochondrial respiration that cells take oxygen, burn it and released energy and free radicals are produced during this process [3,4] .The human body is equipped with antioxidants immune system to counter free radicals. Glutathione Peroxidase, Catalase and Superoxidase Dismutase are antioxidant enzymes that neutralize the toxic intermediates prevent damage of free radicals [5]. Therefore, to cope with oxidative stress, there is need to consume adequate amounts of antioxidants which by neutralizing free radicals, maintain and rehabilitate function of cell within the normal range. Nowadays, researchers' attention has been shifted from traditional antioxidants such as vitamin C, vitamin E and beta-carotene towards other phytochemicals [6]. Including these phytochemicals can be noted polyphenol compounds that possess anti-cancer effects [7], anti-ischemic, anti-allergic, anti-hepatotoxicity and anti-inflammatory [8].

Cisplatin is one of the most important anti-cancer drugs that frequently are used to treat the different types of cancer. This drug has the potent anticancer property against a wide range of malignancies including ovarian, testicular, cervical, bladder, pulmonary cancers and other tumors resistant to anti-cancer therapeutic regimens. Despite of the clinical beneficial effects of Cisplatin in the treatment of carcinoma, this drug has numerous toxic side effects including nephrotoxicity, nervous system toxicity and ototoxicity [9]. The renal toxic effects of Cisplatin are very serious and using it is restricted to the dose so that in using from its high doses, hydrotherapy and co-administration of the diuretic medications in order to reduce its renal toxicity are recommended [10]. In the invasive therapeutic protocols which high doses of Cisplatin to inhibit tumor are used, also hepatotoxicity of drug appears.

It should be noted when low doses of drug are used repeatedly, hepatic toxic effects continue to occur [11]. However, hepatotoxicity induced by Cisplatin less has been considered and little information about the mechanisms predisposing this damage is available. It has been reported that oxidative stresses through production of reactive oxygen species (ROS) [12], the reduction of the antioxidant enzymes immune system including antioxidant enzymes and non-enzymatic molecule reduced Glutathione are fundamental changes that occur following treatment with Cisplatin [13]. Furthermore, dysfunction in the mitochondrial structure and function, the incidence of apoptosis [14] and involvement of prior-inflammatory genes such as COX-2 and iNOS (inducible Nitric Oxide Synthetase) may have an important role in the mechanism of Cisplatin-induced hepatotoxicity [15]. Several studies to evaluate the protective effects of the different chemical compounds in order to reduce the toxic effects of Cisplatin have been conducted, but unfortunately some of the compounds which as Chemoprotector to reduce the adverse and toxic effects of Cisplatin in the therapeutic protocols are used, cause to reduce its anti-cancer effects and some other do not completely eliminate toxic effects of this drug [16].

In contrast to all toxic and harmful effects of drugs such as Cisplatin, compounds are found that can partly inhibit toxicity caused by misuse of such compounds. Including these compounds are the active substances of plant extracts. The determinant point is that in the hepatic damage, increasing score of free radicals is seen and the damage of the hepatic cells is due to increasing production of free radicals and reducing the functional potential of these cells. There are the different antioxidant agents of organs that protect them against oxidative agents [17].

Pomegranate with the scientific name of L. Punica granatum from the family of Punicaceae is a plant that has been considered in traditional medicine of many nations, especially in the Middle East for many years [18]. Previous studies have shown different effects of this plant. Including this effects are effects of the anti-cancer [19,20,21,22], inhibiting apoptosis processes and cellular anti-proliferation [23,24,25], anti-bacterial, anti-fungal and anti-virus [26,27,28], cardiovascular protection [29], decreasing blood pressure [30], anti-hyperlipidemia [31,32,33], anti-inflammation [34,35], anti-diabetes [33,36], protection against radiation UV [37,38,39], males infertility treatment and recovery of spermatogenesis [40], weight loss and obesity treatment

[41], neuroprotective, particularly in infants exposed to hypoxic conditions at delivery time [42,43,44] and Alzheimer treatment [45] which have been proven about pomegranate total extract and or compounds purified from it.

This plant due to having high scores of polyphenolic unique combinations, the attention of many researchers has attracted in recent years [46]. Antioxidant high capacity of pomegranate in vitro is more related to high scores of polyphenolic compounds such as Ellagic acid, Gallic acid, Anthocyanins, Pany Kalagin and Pany Calin as well as flavonoid and anti-cyanide compounds [47,48,49]. Studies have shown that these compounds function through chelating ions involved in the oxidation process and or eliminating free radicals [50]. Studies have also shown that pomegranate juice is rich in vitamin C that is effective in antioxidant activity of plant [51].

Considering the extent of use from Cisplatin and due to being toxicity, misuse of this prevalent drug as a result of hepatic and renal impairment that can lead to death, achievement to appropriate solutions to prevent and decrease the possible toxicity poisonings caused by use of Cisplatin seems completely necessary. Unfortunately, despite the fact that until now preventive effects and detoxifying of some medicinal plants and their various ingredients affecting druge-induced hepatic damages has been investigated, but only a few extracts or active ingredient in the elimination of hepatotoxicity have been identified [52,53].Therefore, given the cheapness of medicinal plants, presence of their active compounds and ease of access to them [54,55], medicinal plants still can be a viable option in relation to researches to study hepatic detoxifying induced by effects of these drugs and or other toxic compounds [52,53].

Considering the therapeutic beneficial and numerous effects of pomegranate, it is believed that this plant can protect liver against oxidative and toxic effects of Cisplatin. In any case, according to the literature review, there isn't a study about the protective effects of pomegranate total extract against Cisplatin-induced hepatotoxicity.

Therefore, present study first has been designed to evaluate the protective effects of pomegranate total extract against Cisplatin-induced hepatotoxicity. It should be explained that cause of selecting this plant, on one hand is because of its high antioxidant function that according to studies conducted is equal to 3 times the antioxidant effect of green tea extract [56] that so far, according to general belief in

scientific circles, green tea was considered almost the most powerful natural antioxidant.

MATERIALS AND METHODS

This experimental study was conducted on 60 Wistar rats with weight of 210 ± 30 g and age of 7 weeks. Rats from the breeding and keeping center of experimental animals 's Razi Vaccine & Serum Institution of Country North West were prepared and in the same condition and maintained in special cages of keeping rats for 12 hours of light and 12 hours of darkness in 23 ± 2 ° C temperature. Animals' feeding was conducted by special pellets for experimental animals and food and water were freely available. 1 week before the start of the study, animals were treated for compatibility with the environment. In this study, to investigate the renal protective effects of pomegranate, pomegranate peel extract as coated tablets with 225 mg weight that are available by product' Amin - Iran Pharmaceutical Company were used.

The physiological serum was used to dissolve dried pomegranate extract. In this experimental study, animals were randomly divided into six groups of ten in individual cages. After 7 days of adaptation to environmental conditions, the rats were randomly divided into 4 groups of 15 as follows:

1) Healthy control group receiving 10 ml / kg sodium chloride 0.9% (Normal Control; NC) for 15 days;

2) Group treated with pomegranate peel extract (Pomegranate Peel Extract (PPE)) for 15 days at a dose of 500 mg / kg /day food;

3) Control group with the toxic drug of Cisplatin (Toxicant Control; Cis) for 15 day, 0.9 % sodium chloride orally / 10 days of Cisplatin intraperitoneal (Mylan - France) to the extent of 7.5 mg / kg in 10 ml / kg normal saline [58].

4) Group treated with extract and toxicant (PPE + Cis): 15 days, pomegranate peel extract orally / 10 days of Cisplatin intraperitoneal.

Histopathological studies:

At the end of the fifteenth day, all rats were killed by method of cervical decapitation. Rats liver samples were immediately removed and part of the left lobe diaphragmatic side of hepatic samples for stabilization were placed in 10% buffered formalin and then by using the common methods of histopathological passage and providing pathological sections, serial sections with 5μ thickness were prepared and from each cut, a section and a total of 10 sections from each sample stained with hematoxylin-eosin 3 of each sample were provided. Amount of inflammation in the portal area, hepatic

cells necrosis and inflammatory cells infiltration in semi-quantitative scale according to the method proposed by Frei et al in 1984 were evaluated. Damage severity from 0 to 4 (0: no damage, 1: least damage, 2: mild damage, 3: moderate damage and 4: severe damage) was scored (Frei et al. 1984). All ratings with a magnification × 100 and in 5 microscopic fields from each section randomly were conducted by optical microscope of Nikon model (ECLIPSE E 200, made in Japan).

Measurement of antioxidant activity*:*

Concurrent with sampling liver for the histopathological studies, another part of rats liver in cold saline was washed and homogenate 10% in 1.15% 10% of the 15/1 (w / v) KCl was prepared. Homogenate at a speed of 700 rpm and for 10 min at 4 ° C was centrifuged and floating solution to measure score of lipid peroxidation by measuring the amount of Malondialdehyde (MDA) as well as to measure the activity of antioxidant enzymes Superoxide Dismutase (SOD), Catalase, Glutathione Peroxidase (GPx) and Glutathione Reductase (GR) was used.

-MDA as a measure of lipid peroxidation in the form of TBARS (Thiobarbituric acid reacting substances) and by using from Esterbauer and Cheesman was measured and TBARS value was expressed as nanomol /mg protein [60].

-SOD activity [61] was measured by Nishikimi and was modified by the method of Kakkar [62]. Each unit of SOD activity as enzyme concentration required for inhibiting the production of color by 50%/ min under study condition was determined.

-CAT activity by Claiborne method [63] and based on the decomposition of hydrogen peroxide was examined.

-The activity of Glutathione Peroxidase by using from method's Rotruck et al [64] and based on the following reaction was measured and as µmol oxide Glutathione / min / mg protein was expressed.

H_2O_2 + 2GSH (Reduced Glutathione) $\rightarrow 2H_2O$ + GSSG (Oxide Glutathione)

Tested and oxidized Glutathione mol / min / mg protein was expressed.

-The activity of Glutathione Reductase also by using from method's Mohandas et al according to the following reaction was measured:

$NADPH + H^+ + GSSG \rightarrow NADP^+ + 2GSH$ [65].

Quantitative data obtained as the mean ± standard deviation (Mean ± SD) were presented and significant difference between groups by statistical test's one-

way Analysis Of Variance (ANOVA) and post hoc Tukey test at score α=0/05 were examined. Differences in score p <0.05 was considered significant.

RESULTS

Histopathology results:

According to Tables 1, 2 and 3 that show the microscopic observations of rats liver in the studied groups quantitatively, the cellular and lobular structure of the rats liver studied in groups 1 (NC) and 2 (PPE) seemed normal. But in rats liver of group 3 (CIS), volume of the inflammatory cells associated with inflammatory cells average infiltration around the central vein, portal veins congestion, cytoplasm vacuolization of the hepatic cells as well as hemorrhage in the interstitial space and the scattered foci of hepatocytes necrosis in different sections of the hepatic lobes was observed. In group 4 (PPE + CLP), treatment by pomegranate peel extract clearly prevented from incidence of the histopathological changes in the hepatic tissue of rats studied. However, in some rats, the poor degenerative changes especially in around the central vein is seen and outline was similar to the control group.

Table 1: Effect of pomegranate peel extract on the interstitial infiltration score of inflammatory cells in the hepatic tissue of rats in Cisplatin-induced hepatotoxicity

Interstitial infiltration score of inflammatory cells	Therapy Groups			
	PPE + CIS	CIS	PPE	NC
Score 0	6	0	13	14
Score 1	3	0	2	1
Score 2	2	2	0	0
Score 3	3	5	0	0
Score 4	1	8	0	0

0: No injuries, 1: Minimum damage, 2: Mild impairment, 3: Moderate damage and 4: Severe injury
NC: Normal Control, PPE: Pomegranate Peel Extract, CIS: Cisplatin

Table 2: Effect of pomegranate peel extract on score of hepatocytes necrosis in the hepatic tissue of rats in Cisplatin-induced hepatotoxicity

Score of hepatocytes necrosis	Therapy Groups			
	PPE + CIS	CIS	PPE	NC
Score 0	3	0	14	15
Score 1	4	0	1	0
Score 2	2	1	0	0
Score 3	3	6	0	0
Score 4	3	8	0	0

0: No injuries, 1: Minimum damage, 2: Mild impairment, 3: Moderate damage and 4: Severe injury
NC: Normal Control, PPE: Pomegranate Peel Extract, CIS: Cisplatin

Table 3: Effect of pomegranate peel extract on score of congestion and inflammation in the portal area's the hepatic tissue of rats in Cisplatin-induced hepatotoxicity

Score of congestion and inflammation in the portal area's	Therapy Groups			
	PPE + CIS	CIS	PPE	NC

Score 0	**4**	**0**	**15**	**13**
Score 1	**5**	**0**	**0**	**2**
Score 2	**2**	**3**	**0**	**0**
Score 3	**2**	**5**	**0**	**0**
Score 4	**2**	**7**	**0**	**0**

0: No injuries, 1: Minimum damage, 2: Mild impairment, 3: Moderate damage and 4: Severe injury
NC: Normal Control, PPE: Pomegranate Peel Extract, CIS: Cisplatin

RESULTS ANTIOXIDANT FACTORS:

According to Figures 1, 2, 3, 4, 5 and 6 as well as Table 4, the values of Malondialdehyde, Glutathione and antioxidant enzymes in group NC (recipient of normal saline) and PPE (recipient of pomegranate peel extract) didn't show a significant difference with each other but in the CIS group (recipient of Cisplatin), MDA score has been increased significantly ($p<0.01$) in comparison with the control group. On the other hand, the amounts of Catalase, Glutathione Peroxidase, Glutathione Reductase, Superoxide Dismutase and reduced Glutathione have been decreased significantly ($p<0.001$) in comparison with the control group.

But in PPE + CIS (group 4), treatment with pomegranate peel extract prevented from the reducing amounts of Catalase, Glutathione Peroxidase, Glutathione Reductase, Superoxide Dismutase and reduced Glutathione caused by Cisplatin. Pomegranate peel also prevented from the increase of Malondialdehyde so that the significant difference between this group and the control group (NC) was not observed.

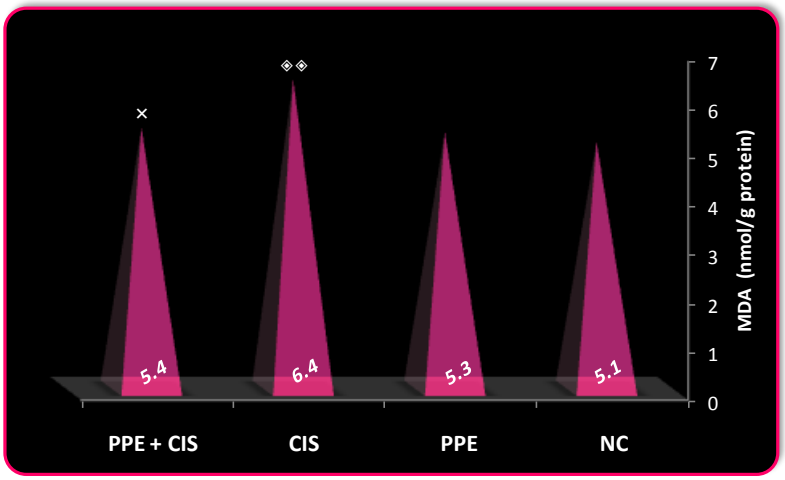

Figure 1: Comparison of rat liver MDA scores in the therapeutic groups
NC: Normal Control, PPE: Pomegranate Peel Extract, CIS: Cisplatin
Compared with Cisplatin Group ×: $p < 0.001$ / Compared with the control group ◈◈: $p < 0.001$

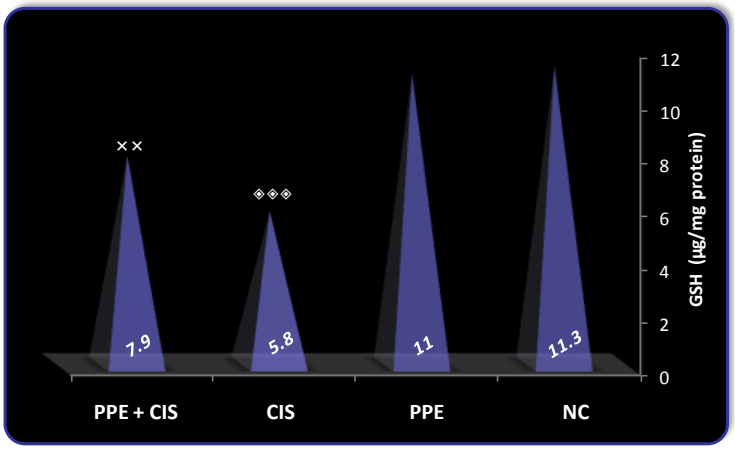

Figure 2: Comparison of rat liver GSH scores in the therapeutic groups
NC: Normal Control, PPE: Pomegranate Peel Extract, CIS: Cisplatin
Compared with Cisplatin Group ××: p < 0.001 / Compared with the control group ◈◈◈: p < 0.001

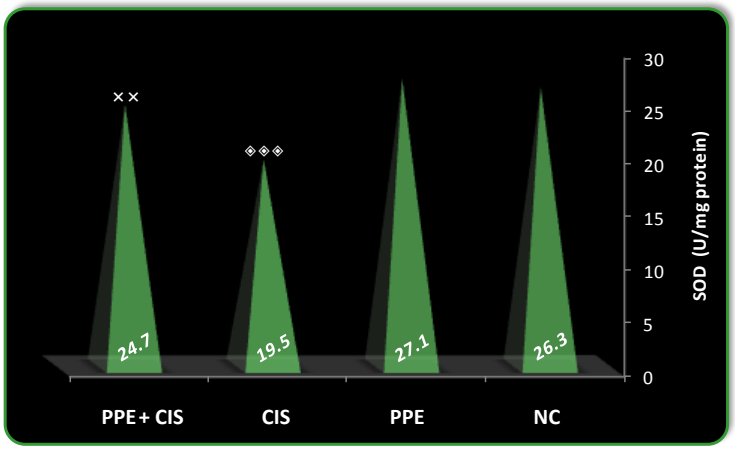

Figure 3: Comparison of rat liver SOD scores in the therapeutic groups
NC: Normal Control, PPE: Pomegranate Peel Extract, CIS: Cisplatin
Compared with Cisplatin Group ××: p < 0.001 / Compared with the control group ◈◈◈: p < 0.001

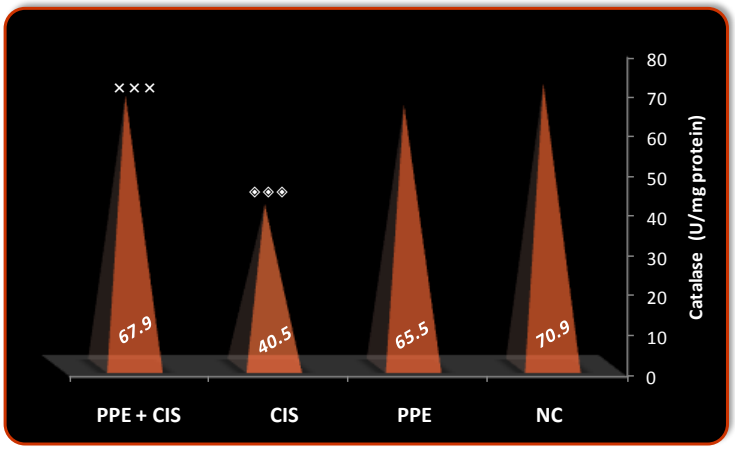

Figure 4: Comparison of rat liver Catalase scores in the therapeutic groups
NC: Normal Control, PPE: Pomegranate Peel Extract, CIS: Cisplatin
Compared with Cisplatin Group ×××: p < 0.001 / Compared with the control group ◈◈◈: p < 0.001

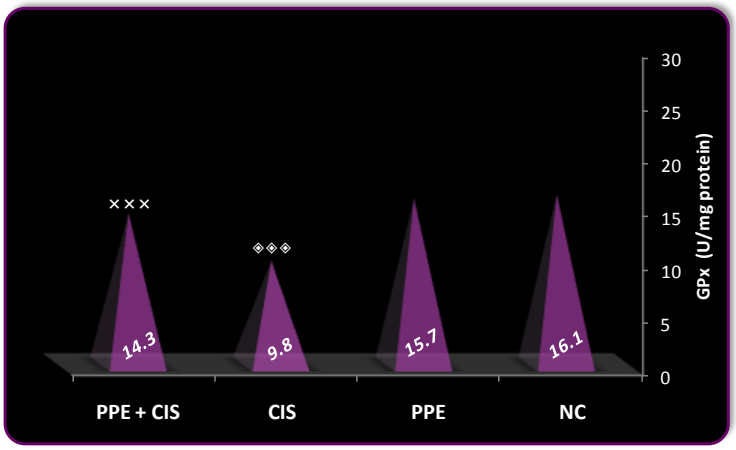

Figure 5: Comparison of rat liver GPx scores in the therapeutic groups
NC: Normal Control, PPE: Pomegranate Peel Extract, CIS: Cisplatin
Compared with Cisplatin Group ×××: p < 0.001 / Compared with the control group ◈◈◈: p < 0.001

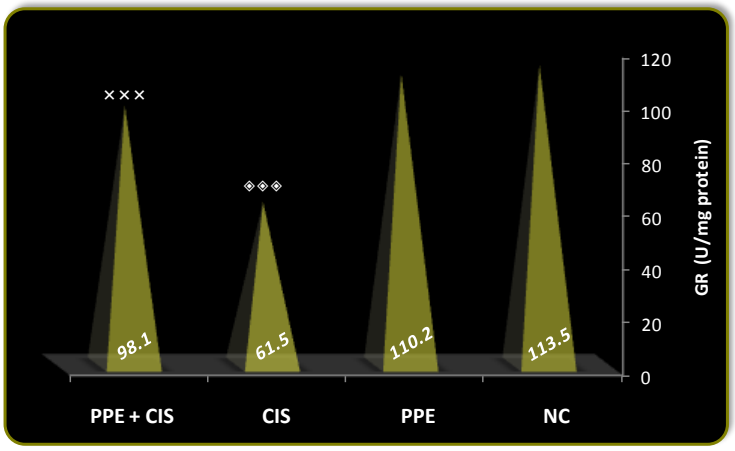

Figure 6: Comparison of rat liver GR scores in the therapeutic groups
NC: Normal Control, PPE: Pomegranate Peel Extract, CIS: Cisplatin
Compared with Cisplatin Group ×××: p < 0.001 / Compared with the control group ◈◈◈: p < 0.001

Table 4: Effect of pomegranate total peel extract on antioxidant activity of rat liver in damage induced by Cisplatin. (Values are presented as Mean ± SD.)

Groups	GR (U/mg protein)	GPx (U/mg protein)	Catalase (U/mg protein)	SOD (U/mg protein)	GSH (µg/mg protein)	MDA (nmol/g protein)
NC	113.5±2.1	16.1±2.1	70.9±1.9	26.3±0.3	11.3±0.2	5.1±0.2
PPE	110.2±2.6	15.7±1.1	65.5±2.1	27.1±0.1	11±0.6	5.3±0.4
CIS	61.5±3.5	9.8±0.6	40.5±3.6	19.5±0.6	5.8±0.4	6.4±0.1
PPE + CIS	98.1±1.8	14.3±1.3	67.9±3.5	24.7±0.2	7.9±0.1	5.4±0.6

GPx: Glutathione peroxidase, GR: Glutathione reductase, SOD: Superoxide dismutase, GSH: Reduced Glutathione, MDA: Malondialdehyde
NC: Normal Control, PPE: Pomegranate Peel Extract, CIS: Cisplatin

DISCUSSION AND CONCLUSION

The liver is one from vital organs which has the distinctive and essential role in detoxification of Xenobiotics, environmental pollutants and chemical medications. Acute hepatic failure is caused by the various factors, including viral hepatitis, toxic damages of liver

induced by toxins, medications and ischemia.

Liver is the first defense barrier's body against damage caused by corroded factors (Xenobiotics). Liver damage is caused by these agents often as hepatic necrosis (Hepaticnecrosis). Oxidative damages have fundamental role in the hepatic tissue damages that can be caused by drugs and toxins. However, the hepatic damages are caused by viral factors as well as are immune-mediated mainly through the activation of apoptotic cell death pathway [66]. Antioxidants are substances that providing presence in foods and the body, even in small amounts, can protect body against various types of oxidative damages caused by reactive oxygen species [67].

Medicinal plants due to their availability, minimal side effects and economies as the worthy alternatives of synthetic drugs have always been considered and since the last few decades, in particular have been regarded by researchers. Biological substances with plant origin constitute a branch from modern pharmacotherapy of diseases.

Although, there are the various pharmacologic agents to treat a variety of diseases, but most patients are not able to tolerate the side effects of chemical drugs and on the other hand, most plants leave very few side effects on patients. Oxidative stress is achieved from an imbalance between the production of free radicals in vivo and antioxidant defense mechanisms.

In organisms, peroxidation of lipids contained in walls of living cells is including the most important goals of free radicals. Under this condition, not only the structure of wall and its function are affected, but also some of oxidation-induced products for example, Malondialdehyde (MDA) can react with biomolecules and Cytotoxic and genotoxic effects show.

Therefore, the high presence of free radicals particularly, peroxides has a key role in the pathogenesis of a number of diseases such as diabetes, cardiovascular disease, cancer, aging and other diseases [68]. Although, nowadays, the synthetic antioxidants especially in the food industry widely are used, many of these compounds have adverse effects for human health [69,70].

For example, there are very much evidences that confirms the toxicity and dietary adverse effects of synthetic antioxidants added to foodstuff such as butyl hydroxy Anisole, butyl hydroxy toluene and third beta hydroxy Kinon. In addition, the risk of hepatic damage and incidence of cancer in laboratory animals

are from the disadvantages of using synthetic antioxidants [69,71].

Due to this fact, natural antioxidants that there are mainly medicinal herbs, fruits and vegetables, has found many advocates among consumers and it seems that are very important in prevention of a number from diseases. Considering that plants are one from the important sources of antioxidants, researches in this area are increasing.

Plants that are rich in antioxidant compounds can protect cells from oxidative damages [72]. Natural antioxidants increases power of the plasma antioxidants and reduce the risk of some diseases such as cancer, cardiac diseases and stroke [73]. Secondary metabolites derived from plants such as total phenol and flavonoid have a strong potential to remove free radicals which in all various parts of the plant such as leave, fruit, seed, root and peel are present [74]. Therefore, given the high prevalence of chronic and degenerative diseases, it is reasonable to use plants and especially, the plants that have high total phenol and flavonoid to meet antioxidants required of body.

In the present study, the increase in score of Malondialdehyde (MDA) and reduce of Glutathione reserve score (GSH) in hepatic liver following encounter with Cisplatin indicate that oxidative stress caused by free radicals is one of the possible mechanisms involved in the pathophysiology of Cisplatin-induced hepatotoxicity.

Researches' Partibha et al (2006) have shown that lipid peroxidation and depletion of reduced Glutathione (GSH) following treatment with Cisplatin in hepatic liver of rat happens that this finding is similar to our study results [75]. Reduced Glutathione is a important component of non-enzymatic antioxidant system and plays an important role in controlling the toxic effects of Cisplatin [76].

Therefore, reduce of Glutathione score can discussed as a direct factor in Cisplatin-induced lipid peroxidation. The role of oxidative stress and involvement of reactive oxygen species (ROS) in the pathogenesis of Cisplatin-induced hepatotoxicity have been demonstrated [77]. Koc et al studies have shown that Cisplatin inhibits the activity of Sup eroxide Dismutase, Catalase and Glutathione Peroxidase enzymes and increases Malondialdehyde score in rat liver [78].

In our study, changes in antioxidant immune system and score of Malondialdehyde in hepatic liver of rats were observed following treatment with Cisplatin which these results are consistent

with the results of their study. Decrease in SOD activity is a sensitive index of hepatic cells damage.

This enzyme is one of the most important factors in the enzymatic antioxidant defensive system. Superoxide Dismutase removes Superoxide anion by converting it to hydrogen peroxide and thus, reduces its toxic effects [79]. In the present study, the score of Superoxide Dismutase in rats treated with Cisplatin due to abundant formation of Superoxide anions was decreased significantly and then, enzymes activity of hydrogen peroxide scraper i.e. Catalase and Glutathione Peroxidase also in these rats was significantly decreased. It seems that the inactivation of Superoxide Dismutase (SOD) Superoxide increased anions leads to deactivation of Catalase and Glutathione Peroxidase enzymes.

Catalase by hydrogen peroxide decomposition protects tissues against very active radicals of hydroxyl. Therefore, the decrease in CAT activity may lead to some devastating effects caused by Superoxide and hydrogen peroxide radicals [80]. Glutathione Reductase is a hepatic cytosolic enzyme which is involved in reducing of Glutathione oxide (GSSG) as a final product of Glutathione Peroxidase activity on reduced Glutathione (GSH) [81].

In our study, consumption of pomegranate total peel extract prevented the reduction of Superoxide Dismutase, Catalase, Glutathione Peroxidase caused by Cisplatin. This event may happen as a result of the removal of radicals by the extract which has caused the preservation and conservation of these enzymes.

Furthermore, following treatment with Cisplatin, a significant decrease was achieved in score of Glutathione Peroxidase which led to the availability of Glutathione Reductase to substrate, and thus, decreased activity of Glutathione Reductase. It seems that pomegranate total peel extract with Cisplatin again establishes activity of Glutathione Reductase and causes consumption of Glutathione oxidize to form reduced Glutathione and increasing the detoxification of active metabolites by conjugation with reduced Glutathione.

The results of present study confirm report of other researchers about antioxidant effects and free radical elimination of antioxidant plant compounds [82]. In researches that by Ajith et al have been conducted about the protective effects of polyphenolic compounds contained in plants against Cisplatin nephrotoxicity, have been shown that this extract through reduce of oxidative stresses applies its own protective role against Cisplatin so that

reduces the amount of MDA and increases score of reduced Glutathione and antioxidant enzymes activity of the immune system such as Superoxide Dismutase, Catalase and Glutathione Peroxidase [83].

The action mechanism of these compounds in their study is consistent with the results of present study. Chaturvedula et al (2011) have emphasized presence of high scores of polyphenolic compounds in this plant [46]. Furthermore, according to studies' Wang et al (2004), Seeram et al (2005) and Lansky et al (2006), high antioxidant capacity of pomegranate in vitro is more related to high score of polyphenolic compounds such Ellagic acid, Gallic acid, Anthocyanins, Pany Kalagin and Pany Calin and flavonoid and antioxidant compounds [47,48,49].

In a study by Nugteren et al (1987) has been shown that these compounds through chelating ions involved in the oxidation process and or by eliminating free radicals function [50]. Ellagic acid and Gallic acid are from compounds contained in the peel pomegranate that Ellagic acid is a dimer derivative of Gallic acid and mainly is found in high plants such as fruits and dried fruits [84,85]. Elliott et al (1999) showed that Ellagic has of anti-mutation, antiviral skin bleaching and antioxidant property and currently in Country of Japan as an antioxidant is added to food [86].

The study's El-Nemr et al (1990) has shown that pomegranate juice is rich in vitamin C which is effective in antioxidant activity of plant [51]. Mavlyanov et al (1997) also have known the presence of vitamin C high scores along with phenolic compounds such as Poonykalazyn, Poony Calin, Gallic acid, Lagic acid Anthocyanin involved in antioxidant powerful function of pomegranate [87].

These compounds mainly are observed in fresh fruit formed [88,89] and with enlargement of the fruit, its antioxidant activity is also reduced that is due to the reduction in the amounts of ascorbic acid and its phenolic compounds [90]. It has been also shown that the amounts of phenolic compounds and ascorbic acid vary among various pomegranate cultivars [91].

In relation to the pharmacological effects of these compounds, Ignarro et al (2006) determined that pomegranate juice can prevent oxidative damage of nitric oxide and cause to enhance nitric oxide biological function [92]. In addition, in the study's Schubert et al (2002), inhibiting the activity of nuclear factor Kappa - Beta (NF-κB) as a natural antioxidant in vascular endothelial cells by pomegranate has been proven [93]. Zafar Rasceel

(2009) noted in a study that the pomegranate fruit extract can prevent activity of MAP Kinase (MAPK) and factor Kappa - Beta (NF-κB) in human Ku812cellular class [94]. It was found in a study Lunn et al (2006) that Ellagi Tannins contained in pomegranate juice can cause to inhibit inflammatory signals in cancer cells of colon increased [95].

It was determined in study's Aviram et al (2002) that flavonoids contained in pomegranate juice extract can prevent LDL oxidation and cardiovascular diseases in mouse and human [96]. Hayek et al (2001) claimed that the prescription of pomegranate juice supplement in atherosclerotic mice causes to reduce macrophage lipid peroxidation and inhibit the accumulation of cholesterol and atherosclerosis in them [97].

Also, it was showed in a study conducted by Aviram et al (2000) in human and atherosclerotic mice that pomegranate juice consumption causes to reduce oxidative stress, platelet aggregation and the low-density lipoproteins [98]. According to what was said, probably pomegranate peel extract through its own antioxidant properties protects liver against the toxic oxidative effects of Cisplatin.

Therefore, after conducting clinical trials and obtaining positive results, pomegranate peel total extract can as a herbal medicine to prevent hepatic oxidative damages caused by Cisplatin in patients with cancer be recommended and used as an available source with antioxidant property as supplement and food additive and or through pharmaceutical industries simultaneously with Cisplatin. Whether or not this plant causes to reduce the therapeutic effect of Cisplatin remains unknown in this study and possibility to compare from view of effects in subjects that are with neoplasia has not been provided.

Furthermore, how to affect the different doses of extract and identify accurately the main active substance or substances, location as well as molecular and cellular mechanism or mechanisms involved in its pharmacological action remains unknown and requires future and wider studies.

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