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Effect of *Punica granatum* seeds on Doxorubicin and Gamma Radiation -Induced Hepatotoxicity in Wistar Rats

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Abstract
Punica granatum Linn popularly known as 'Pomegranate' is a rich source of flavonoids. Flavonoids have been implicated in Hepatoprotective activity.
Objectives: To evaluate effect of *Punica granatum* seeds and its flavonoids on doxorubicin-induced hepatotoxicity in Wistar rats.
Materials and Methods: The Anthocyanidins of *Punica granatum* (100 mg/kg) extracts as potent antioxidants in improving the toxicity of doxorubicin (DOX) and/or gamma radiation in albino Wistar rats. The rats were injected with DOX (2.5 mg/kg, i.p.) and/or exposed the rats to irradiation (2 Gy, whole body) weekly, for four consecutive weeks. The antioxidant treatments were used daily *via* oral gavages for two weeks protection period and during the experiment (4 weeks). The levels of aminotranferases (ASAT, ALAT), alkaline phosphatase (ALP) and Superoxide dismutase (SOD), Catalase (CAT), Glutathione reductase (GSH) and Lipid peroxidation (TBARS) levels in liver were measured.
Result: In the present study, DOX and/or gamma radiation revealed increase in the hepatic biomarkers (ASAT, ALAT and ALP), also decreased in total protein and albumin, which were significantly decreased after pretreatment with Pomegranate (POM) (100 mg/kg), while decreased in total protein and albumin. DOX and/or gamma radiation significantly decreased levels of SOD, CAT, and GSH which were restored with POM.
Conclusion: The study concludes that POM possess potential hepatoprotective activity which may be attributated to its flavonoids (anthocyanidins), having therapeutic potential in treatment of liver disorders.

Keywords: *Punica granatum*, hepatotoxicity, doxorubicin and gamma radiation

Introduction
Doxorubicin (DOX) used for the treatment of cancer in 1969, this chemical has demonstrated high antitumor efficacy. However, its use in chemotherapy has been limited largely due to its diverse toxicities, including cardiac, renal, hematological and testicular toxicity (Gillick *et al*., 2002; [27] Yilmaz *et al*., 2006) [75] alterations of DNA and the production of free radicals (Quiles *et al*., 2002) [57], mitochondrial damage and iron-dependent oxidative damage to biological macromolecules (Thomas *et al*., 1987). DOX is an anthracyclic antibiotic with broad spectrum of anti-neoplastic activity. Oxidative stress has been demonstrated in DOX mediated myocardial, renal (Fadillioglu *et al*., 2004) [23] and liver (Kwiecien *et al*., 2006) damages. DOX injection substantially increased the peroxidative damage in the myocardium, hepatic and renal tissues and markedly increased the serum Creatinine, BUN, ALAT and ASAT (Saad *et al*., 2001) [60]. DOX treatment also induced peroxidative alterations in various tissues (Chen *et al*., 1998; [16] Todorova *et al*., 2009) [69], which was evident by significant elevation in SOD and CAT. Scientific and technological advancements have increased radiation burden in humans, since exposure to low level of radiation frequently has become common during medical diagnostic procedures, space or air travel cosmic radiation and use of certain electronic gadgets. Exposure to ionizing radiation can induce functional and structural changes in the liver (Kim & Jung, 2017) [39], mainly caused by the excess of free radicals and oxidative stress. Oxidative stress is a crucial factor in liver damage (Cichoż-Lach & Michalak, 2014) [17] Hepatocyte's lipids, proteins, and nucleic acids are among the cellular structures to be affected primarily by reactive oxygen species (ROS). It is well documented that lipid peroxidation disrupts the normal membrane structure (Niki, 2009), proteins oxidation affect signal transduction and DNA repair enzymes (Dalle-Donne *et al*., 2003) [18], and DNA oxidation leads to the formation of 8-hydroxy-deoxyguanosine (8-OHdG) and mutation in the DNA strands (Voulgaridou *et al*., 2011) [71]. These processes result in structural and functional abnormalities in the liver (Cichoż-Lach & Michalak, 2014) [17]. The effects of ROS are counteracted by antioxidants including glutathione (GSH), the most important antioxidant molecule (Valko *et al*., 2007) [70], superoxide dismutase, GSH peroxidase, and catalase (CAT). In addition to oxidative stress, alteration in the detoxification pathways can affect the integrity of the liver (Cederbaum, 2017) [15]. Detoxification inside the liver cells depends on phase 1 catalyzed by the cytochrome P450 (CYP450) enzyme group and the formation of free radicals, which are further metabolized by phase 2 involving conjugation reactions and the formation of a water-soluble component, which can be excreted through urine or bile. The phase 2 enzyme systems include both Uridine 5′-diphospho-glucuronosyltransferase and GSH transferase (GSH-T). Several studies have shown evidence of associations between induced phase 1 and/or decreased

phase 2 activities and an increased risk of disease (Hodges & Minich, 2015) [35]. Flavonoids possess a variety of biochemical and pharmacological activities such as antioxidant, antiviral, anti-carcinogenic and anti-inflammatory effects believed to be beneficial for human health (Danks *et al*., 1987) [19]. Polyphenols are the major class of pomegranate fruit phytochemicals, including flavonoids (anthocyanins), condensed tannins (proanthocyanidins) and hydrolysable tannins (ellagitannins and gallotannins) (Hernandez *et al*., 1999; [34] Gil *et al*., 2000) [26]. Pomegranate phytochemicals also show potential in chemoprevention of various types of cancers, by exerting antiproliferative effects on tumor cells (Kim *et al*., 2002) [40]. The presence of anthocyanins is responsible for the appealing bright red colour of juice and other products of pomegranate fruit (Jaiswal *et al*., 2010) [37]. Pomegranate extracts have been shown to scavenge free radicals and decreased macrophage oxidative stress and lipid peroxidation in animals (Rosenblat *et al*., 2006) [58] and increase plasma antioxidant capacity in old humans (Guo *et al*., 2008) [29]. The pomegranate seed oil makes up to 12 to 20% of the total seed weight, the oil contains maximum amount of polyunsaturated (n-3) fatty acids such as linolenic acid, The oil contains further lipids such as punicic acid, oleic acid, stearic acid and palmitic acid **(**Ozgul-Yucel, 2005; Fadavi *et al*., 2006) [22]. Several studies have showed that pomegranate as a fruit native to Middle East, has therapeutic effects as being an antioxidant (Mahattanatawee *et al.,* 2006) [46], anti-proliferation (Seeram *et al.,* 2005) [64], anti-inflammatory (Adams *et al.,* 2006) [1], anti-microbial and anti-carcinogenic effects (Lansky & Newman, 2007) [44]. The protective effects of pomegranate seed against nephrotoxicity induced by gentamicin, cisplatin and Hexachlorobutadiene had also been shown (Boroushaki *et al.,* 2014; [12] Boroushaki *et al.,* 2015) [13]. Pomegranate derived products have shown beneficial effects on the treatment and prevention of various diseases, such as cancer, cardiovascular disease, neurological disorders diabetes, Alzheimer's disease and other diseases (Hartman *et al.,* 2006; [33] Mena *et al*., 2012) [48]. Nirwane *et al.* (2014) [52] study Therapeutic effect of POM possess potential nephroprotective activity which may be attributive to its flavonoids (anthocyanidins), having preventive effective of DOX Induced Nephrotoxicity in Wistar Rats. Pomegranate fruit is highly appreciated for beneficial health effects in the form of decreasing cardiovascular and other chronic diseases due to its high contents of organic acids, vitamins, polysaccharides, essential minerals, and most importantly antioxidants (Al-Maiman & Ahmad, 2002 [6]; Longtin, 2003) [45]. The high antioxidant nature of pomegranate fruit has played a major role in its increased consumption across developed countries, especially in the form of juice and other processed products. In light of the previous hypothesis, the present study aims to investigate the potential hepatoprotective effects of *Punica granatum* L Fruit extract, as potent antioxidants, in their recommended antioxidant doses, against doxorubicin and gamma radiation induced oxidative stress in Wistar rats.

Materials and Methods

Chemicals and dosage

Doxorubicin was purchased from EIMC united pharmaceuticals, Co. Egypt as Adricin® (doxorubicin hydrochloride). The doxorubicin injection dose in the present study was 2.5 mg/kg, i.p., weekly for four consecutive weeks (10 mg/kg body weight cumulative doses).

Irradiation (R)

The Canadian Gamma cell–40 (137Cs) (National Center for Radiation Research and Technology (NCRRT), Nasr City, Egypt) was used to irradiated rats with a whole body fractioned dose (2 Gy every week for four weeks up to 8 Gy cumulative doses). The dose rate at the time of the experiment was 0.45 Gy/min (4.44 min exposure times).

Plant extract

Preparation of *Punica granatum* (POM) seeds extract

Punica granatum (POM) seeds extract were obtained from fruit purchased from a local market. Then, the seeds were dried and milled and 72% alcohol was added to the milled seeds. The obtained mixture was stored in a beaker for 3 days and mixed in the mornings and evenings. They were spread on a flat surface after 3 days to dry. Finally, the obtained extract was collected (purity percentage of the extract was 17%), dissolved in distilled water **(**Sarkaki & Rezaiei, 2013; [62] Sarkaki *et al*., 2015) [63].

Experimental Animals

Sixty-four male Wistar albino rats (120–130 g) at the beginning of this experiment were used. The rats were divided randomly and assigned into eight equal experimental groups (contains8 rats in each group) as the following: Group I: (Control) rats of this group were neither treated nor irradiated and were provided with standard diet-pellets and drinking tap water *ad libitum* during the experiment for six weeks. Group II: (POM) comprised of normal rats were daily subjected to oral administration of 100 mg/kg body weight of POM seeds alcoholic extract dissolved in saline via oral gavage tube for six weeks (Wei *et al*., 2015). Group III: (DOX) comprised of normal rats injected intra-peritoneally with four equal doses at (2.5 mg/kg body weight) of DOX for 4 times alternatively over a four weeks to make a total cumulative dosage of (10 mg/kg body weight), The first dosage was given on the seventh day from the beginning of the experiment (Fathy *et al*., 2017). Group IV: (POM+DOX) comprised of normal rats injected with DOX as in group III and administrated 100 mg/kg b.w/day of POM extract orally for two week prior exposure to DOX. The administration of the POM was extended for six weeks. Group V: (R) comprised of normal rats were exposed to whole-body with four equal doses at (2 Gy body weight) of irradiation for 4 times alternatively over a four weeks to make a total cumulative dosage of (8 Gy body weight), The first dosage was given on the seventh day from the beginning of the experiment (Fathy *et al*., 2017) [25]. Group VI: (POM+R) comprised of normal rats were exposed to whole-body with irradiation as in group V and administrated 100 mg/kg b.w/day of POM extract orally for two week prior exposure to Radiation. The administration of the POM was extended for six weeks. Group VII: (DOX-R) comprised of normal rats injected intraperitoneally with four equal doses of DOX and exposed to whole-body irradiation (DOX-R) for 4 times alternatively over a four weeks, rats of this group were irradiated following 20h of DOX injection. Group VIII: (POM+(DOX-R)) comprised of normal rats injected intraperitoneally with four equal doses of DOX and exposed to whole-body irradiation (DOX-R) as in group VII

and administrated 100 mg/kg b.w/day of POM extract orally for two week prior exposure to (DOX-R). The administration of the POM was extended for six weeks.

Collection and Preparation of Samples

Blood samples were collected at the end of the experiment from each animal under anesthesia from the retro-orbital plexus using capillary tubes. Blood samples were collected and put into non-heparinized plain tubes, which were centrifuged at 4000 rpm for 10 min. The serum samples were frozen at -80 °C for the following measurements. After sampling, animals were sacrifced and livers were isolated, dissected out and washed with isotonic saline. Each tissue was homogenized in ice-cold phosphate (0.05 M - KCl, pH 7.4) buffer solution for 30 seconds twice to yield a 10% (w/v) by using (Heidolph, Diax 9000 apparatus) homogenizer. The homogenates were centrifuged under cooling at 4000 rpm for 20 min. The supernatants were subsequently aliquoted and stored at -80 °C until used.

Biochemical Study

The liver tissue homogenates were used for the determination of hepatic thiobarbituric acid reactive substances (TBARS) (Yoshioka *et al*., 1979) [76] and hepatic reduced glutathione (GSH) (Beutler *et al*., 1963) [11]. In addition, the activities of superoxide dismutase (SOD) (Kakkar *et al*., 1984) [38] and catalase (CAT) enzymes (Aebi, 1984) were estimated in the homogenates using kits from bio-diagnostic Co., Egypt. The serum levels of transaminases (ASAT & ALAT) (Bergmeyer *et al*., 1986) [10], alkaline phosphatase (ALP) (Moss, 1982) [49], total protein (TP) (Gornal *et al*., 1949) [28] and albumin (Doumas *et al*., 1971) were estimated using kits from Egyptian Company for biotechnology spectrum, Egypt.

Statistical Analysis

Statistical package for social sciences SPSS/PC computer program (version 20, USA) was used for the statistical analysis of the present results. The results were analyzed using analysis of variance (ANOVA) one-way test followed by least significant difference test for multiple comparisons. Differences were considered statistically significant at $P<0.05$. Data are summarized as mean ± standard error.

Results

Pomegranate has been found to be effective in protecting liver toxicity induced by DOX treatment. The represented data of DOX and radiation intoxicated rats showed a significant raises ($P<0.05$) in serum ALAT, ASAT and ALP enzymes activity as compared with the control group. While a significantly decreased ($P<0.05$) might be restored to normal levels in rats pre-co-treated with POM extract in combination with DOX and radiation as compared with the corresponding value of DOX and radiation groups. The percentage of change from serum total protein and albumin in the groups treated with DOX and radiation showed a significant decrease when compared with corresponding value of control group. On the other hand, the percentage change of serum total protein and albumin in treated rats with POM extract in combination with DOX and radiation showed significant increase as compared with the corresponding value of DOX and radiation groups.

Table 1

Parameter	Liver function test									
	ALAT (U / L)		ASAT (U / L)		ALP (U / L)		Total protein (g/dl)		Albumin (g/dl)	
Groups	Mean ± SE	%change	Mean ± SE	%change	Mean ±SE	%change	Mean ±SE	%change	Mean ±SE	% change
Group I (Control)	65.52±1.12[a]		70.4±1.9[a]		115±1.3[a]		6.5±0.19[a]		4.22±0.13[a]	
Group II POM	64±0.7[a]	-2.46	70.26±1.8[a]	-0.252%	113.9±.1.8[a]	-1.62	6.7±0.14[a]	1.79	4.29±0.1[a]	1.21%
Group IIIDOX	118.1±3.2[b]	80.27	98.53±4[b]	40.29%	172±4.3[b]	49.78%	5.2±0.04[b]	--20.47%	2.87±0.08[b]	-31.99%
Group IV POM+DOX	80±0.9[c,e]	20.67	88.4±0.95[c,d]	25.84%	141.8±3[c]	22.34%	5.9±0.12[c,d]	-9.94%	3.34±0.15[c]	-18.99%
Group VR	105.5±3.6[d]	61.02	94.6±3.17[b,c]	34.36%	162.6±4.3[d]	41.34%	5.3±0.12[b]	-17.95%	3.15±0.04[b,c]	-25.39%
Group VI(POM+R)	75.78±1.2[c]	14	77±1.8[e]	9.36%	127.2±1.6[e]	9.04%	6±0.18[c]	-5.22%	3.55±0.1[c]	-15.76%
Group VII DOX+R	134.3±3.2[f]	105	112.4±1.3[f]	59.66%	177.7±2.4[b]	54.46%	4.6±0.21[e]	-29.26%	3.87±0.16[b]	-31.93%
Group VIII POM+(DOX+R)	84.8±3.2[e]	26.8	87.7±1[d]	17.92%	149.2±3.2[c]	30.43%	5.6±0.19[b,d]	-12%	3.44±0.17[c]	-17.36%

Pom = *Punica granatum*; R = *Radiation* and DOX = Doxorubicin

Data are expressed as mean ± standard error Significant start from ($P<0.05$).

Percentage changes (%) are calculated by comparing treated groups with normal control group.

The percentage of change from antioxidant and oxidative biomarker of liver homogenate in the groups treated with DOX and radiation alone or with combination showed a significant decrease in parameters of SOD, CAT and GSH when compared with corresponding value of control group. While an increased in MDA in rats treated with DOX and radiation alone or with combination when compared with corresponding value of control group. On the other hand, the percentage change of CAT, SOD and GSH in rats treated with POM extract in combination with DOX and radiation showed significant increase as compared with the corresponding value of DOX and radiation groups and also a significant decreased in MDA in rats treated with POM extract in combination with DOX and radiation when compared with the corresponding value of DOX and radiation group.

Table 2

Parameter/ Groups	Liver antioxidant bio-marker							
	TBARS(nmole/ g wet tissue)		GSH (mg /g wet Tissue)		CAT (u/mg protein)		SOD (u/mg protein)	
	Mean ±SE	%change	Mean ±SE	%change	Mean ±SE	%change	Mean ±SE	%change
Group I (Control)	196±1.58[a]		2.94±.02[a]		1.56±.02[a]		74.77±.73[a]	
Group II POM	193±1.44[a]	-1.53%	2.98±.03[a]	1.36	1.54±.02[a]	-1.28%	75.37±1.04[a]	0.8%
Group III DOX	224±1.94[b]	14.28%	2.39±.03[b]	-18.7%	1.25±.02[b]	--19.87%	65.79±.49[b,d]	-12%
Group IV DOX+POM	213±0.93[c]	8.67%	2.65±.03[c]	-9.86%	1.45±.03[a,c]	-7.05%	70.54±.48[c]	-5.66%
Group VR	221±1.52[b]	12.75%	2.47±.03[d]	-15.99%	1.34±.03[b,c]	-14.1%	66.45±.47[b]	-11.13%
Group IV(POM+R)	213±1.12[c]	8.67%	2.74±.03[e]	-6.8%	1.5±.01[a,c]	-3.85%	69.82±.45[c]	-6.62%
Group VIIDOX+R	238±1.6[d]	21.43%	2.23±.03[f]	-24.15%	1.06±.18[d]	-32.05%	60.53±.39[e]	-19.05%
Group VII (DOX+ R)+ POM	231±2.64[e]	17.86%	2.31±.03[b]	-21.43%	1.15±.02[b,d]	-26.28%	64.46±.53[d]	-13.79%

Pom = *Punica granatum*; R = *Radiation* and DOX = DoxorubicinData are expressed as mean ± standard error Significant start from (P<0.05). Percentage changes (%) are calculated by comparing treated groups with normal control group.

Discussion

Many drugs used for cancer chemotherapy are known to produce toxic side effects in multiple organ systems including the liver. DOX has long been one of the most extensively used chemotherapeutic agents for treatment of various cancers. The clinical application of this drug is, however, complicated by its potential toxicity to the liver (Zhao *et al*., 2012; [78] Afsar *et al*., 2019) [4]. Exposure to ionizing radiation is characterized by production of ROS associated with increase in lipid peroxidation and decrease in activity of body antioxidant enzymes with possible damage of cellular membrane (Azab and Nada, 2004; [7] Shedid *et al*., 2018) [65]. The DOX treatment and/or radiation exposure produces DNA damage, cytotoxicity and morphological changes causing oxidative stress and generation of ROS (Fang *et al*., 2002) [24]. The increased formation of TBARS in response to DOX and/or radiation exposure in the present study has been associated with an early marker of oxidative stress because they are correlated well with increased production of ROS (Baskol *et al*., 2006). The endogenous antioxidant system contains several natural enzymes (SOD & CAT) and non-enzyme (GSH) defenses (Halliwell *et al*., 1992) [32]. The DOX injection and/or exposure to ionizing radiation lead to depletion of these endogenous antioxidants (Adem *et al*., 2014) [2]. The SOD is the only enzyme that utilizes the free radicals as a substrate and catalyzes the reduction of superoxide radical to hydrogen peroxide (Balin and Allen, 1986) [8]. The significant decrease in SOD activity in the liver tissue of DOX and/or irradiated rats might be due to the overproduction of free radical. The enzyme is one of the self-defense mechanisms against oxidative stress where it constitutes the first line of defense against the toxic effects of ROS (Guo *et al*., 2003). The catalase enzyme converts hydrogen peroxide to water and oxygen (Balin and Allen, 1986) [8]. The present study showed a significant decrease in the activity of CAT recorded in the liver tissue of DOX and/or irradiated rats as compared with the control rats. This decrease might be due to the oxidative modification of various protein types, leading to functional alterations and physiological impact. The present results showed a significant decrease in GSH concentration in the liver tissue of DOX and/or irradiated rats as compared with the control rats. The GSH deficiency contributes to Oxidative stress (Wu *et al*., 2004) [74]. In addition, the depletion in GSH level after DOX and/or radiation exposure may be due to its diffusion through impaired cellular membranes or inhibition of GSH synthetase and glutathione reductase enzymes (Zahran *et al*., 2006) [77]. In the same concern, Srinivasan *et al*. (2007) showed that the decreased levels of GSH might be due to its utilization by the enhanced production of ROS. The oxidative stress markers in the liver of DOX and/ or irradiated rats in the present study showed increased TBARS and decreased SOD, CAT and GSH activities. The alterations in oxidative stress markers may be due to the radio-sensitizing effect that might be probably related to the inability of cells to cope with the overproduction of different free radicals (Weiss and Landauer, 2003) [73]. The clinical and diagnostic values associated with increased blood enzyme concentrations of ASAT, ALAT and ALP as well as TBIL have long been recognized (Martin and Freidman, 1998). The elevated liver marker enzymes in the blood serum reflected the radical-mediated lipid peroxidation of the liver cell membranes. These free radicals combined with the cellular proteins, which in turn, initiate lipid peroxidation (TBARS), resulting in structural changes of bio-membranes and loss of liver integrity and decreased metabolic activity (Güven and Gülmez, 2003) [30]. The increased levels of these diagnostic markers of hepatic function in DOX and/or irradiated rats are implicative of the degree of hepatocellular dysfunction. The increase in transaminases was the clearest indication of cellular leakage and loss of functional integrity of the membrane of liver cells (Elsadek *et al*., 2017) [21]. In addition, the elevated liver markers (ALAT, ASAT, ALP and TBIL) could be attributed to bile duct obstruction or infiltrative diseases of the liver (Fathy *et al*., 2017 [25]; Afsar *et al*., 2019) [4]. The results in the present work showed a significant decrease in serum total proteins and albumin in DOX and/or irradiated groups. The decrease in serum protein might be the result of damaged biological processes or due to the changes in the membrane permeability of the liver and kidney tissues, resulting in leakage of protein, especially albumin through the kidney (Ali *et al*., 2007; Haggag *et al*., 2008) [31]. Various parts of pomegranate, *Punica granatum* L, have been used for various medicinal purposes. Many studies have shown that pomegranate extract possesses antioxidant (Thitipramote *et al*., 2019) [67], immunomodulatory (Labsi *et al*., 2016) [43], antibacterial activities (Salgado *et al*., 2006) [61], etc. DOX, an anthracycline antibiotic, has a broad antitumor spectrum, and has been used against a wide variety of hematopoietic malignancies and solid tumors (Ogura, 2001) [54]. A strategy to diminish the side effects of anticancer drugs with preservation of their

chemotherapeutic efficacy is necessary. A great number of plants worldwide showed a strong antioxidant activity and a powerful scavenger activity against free radicals (Nirwane & Patil, 2012) [53]. The primary phytochemical screening of Pomegranate extract showed the presence of flavonoids, alkaloids, tannins and anthocyanidins. In present study the phytochemical screening of Anthocyanidins extract of *Punica granatum* seeds gave positive tests for flavonoids, saponins, tannins and Phenolic compound (Kokate *et al*., 1994) [41]. The results of a present study indicate that the DOX (10 mg/kg, i.p.) induce pathological changes in serum and biochemical markers, indicative of toxicity and increase in free radical production. In our study, we observed that significant rise in liver marker enzymes is an indicator of abnormal functioning of the liver. This increase suggest that an increased leakage of these enzymes from mitochondria as result of toxicity induced by treatment with DOX. Administration of DOX (10 mg/kg, i.p.) to rats significantly increased serum ASAT, ALAT and ALP. Our results are in good agreement with those previously reported (Injac *et al*., 2008; Fathy *et al*., 2017**)** [25]. The efficacy of any hepatoprotective herb is essentially dependent on its capacity of either reducing the harmful effects or maintaining the normal physiologic function which has been disturbed by hepatotoxic agents. Exogenous and endogenous protective agents with antioxidant properties were reported to show some protective effects in DOX-induced hepatotoxicity. POM is one promising agent against various toxicities associated with oxidative stress and peroxidative damage (Russo *et al*., 2002) [59]. In our current study, POM treatment against DOX toxicity significantly reduced the elevated MDA level and also normalized tissue GSH, SOD and CAT activity. Measurement of the activities of serum marker enzymes, like ASAT, ALAT and ALP, can make assessment of liver function (Bosek & Nakano, 2003) [14]. In this study the hepatoprotective effect of POM was evaluated by measuring levels of serum marker enzymes such as (alkaline phosphatise) ALP, (alanine aminotransferase) ALAT, (aspartate aminotransferase) ASAT levels in serum. Deteriorations of liver function tests (serum ALP, ALAT, ASAT) also decreased in total protein and albumin revealed hepatic dysfunction in DOX group. Similar results have been reported by Nirwane & Patil (2012) [53], for liver tissue in which DOX caused high ALAT, ASAT and ALP levels, also decreased serum ASAT, ALAT, ALP and levels of SOD, CAT, and GSH which were restored with POM treatment indicate reduced hepatocellular damage.

Conclusion

In conclusion, this study provides firm evidence that doxorubicin and radiation can adversely damage the Liver by inducing significant biochemical change. Our results raise the hope that co-administration of *Punica granatum* with doxorubicin and radiation may be a promising solution to complication of doxorubicin induced hepatotoxicity.

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