Protective Effect of *Punica granatum* Peel Extract Against Pentachlorophenol-Induced Oxidative Stress, Cytogenetic Toxicity and Hepatic Damage in Rats

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Abstract: Pomegranate is an edible fruit rich source of polyphenolic compounds, which have the antioxidant activity. This study aimed to evaluate the protective effect of *Punica granatum* extract against pentachlorophenol-induced oxidative stress, cytogenetic and hepatic injury in rats. Male rats were divided into six groups, group I control, group II and III received low and high doses of *P. granatum* (200 & 400 mg/kg/day). Group IV received PCP; group V and VI received low and high doses of *P. granatum* then PCP for 30 days. The level of MDA was significantly elevated in PCP group, pre-treatment with extract caused reduction in the malondialdehyde (MDA) and brought them near to normal. Cytogenetic analyses results of pentachlorophenol (PCP) treated group recorded significant increase in the frequencies of total structural chromosomal aberrations, chromosomal deletions and centric fusions in somatic and germinal animal cells. Pretreated with the extract and PCP in a dose-dependent manner was showed significant decrease in different types of chromosomal aberrations. The histological examination of liver sections treated with PCP showed vacuolization, degeneration of the cytoplasm, necrosis, fatty degeneration and interstitial fibrosis which were ameliorative by extract. Also PCP treatment showed decreased in the DNA content, with increase in activation of caspase-3 and pretreatment with *P. granatum* extract showed increased in the DNA content and caspase-3. It could be concluded that *P. granatum* peels extract protect against PCP-induced oxidative stress, cytogenetic toxicity and hepatic injury.

Key words: *Punica granatum*; Pentachlorophenol; Liver; Cytogenetic; Histopathology; DNA content.

INTRODUCTION

Pentachlorophenol (PCP) is a well-known organochlorine compound mainly used as a general herbicide, pesticide and wood preservative, as well as an insecticide and broad-spectrum biocide (ATSDR, 2001). PCP as a priority pollutant due to its slow and incomplete biodegradation (Gupta et al., 2002; Chen et al., 2004). The general population may be exposed to PCP primarily through the ingestion of water and food (Jorens and Schepens, 1993) but rather high doses are attained in occupational settings (Seiler, 1991). Thus, health effects of PCP among workers, as well as among the general population, are of great concern. Severe exposures to PCP may result in an acute and often fatal intoxication, both in humans and experimental animals, uncoupling of oxidative phosphorylation in mitochondria and the generation of reactive oxygen species (ROS) would be the principal mechanism of action (Proudfoot, 2003). PCP is readily absorbed across the skin, lungs and gastrointestinal lining (Reigart and Roberts, 1999). Studies showed that PCP possesses endocrine-disrupting functions (Louise and Gerald, 1996; Benjamin et al., 2002; Chen et al., 2004) and could exert its immunotoxical function, as well as humoral immune parameters such as serum IgM production.

Metabolic studies carried out in rodents or human liver homogenates have indicated that PCP undergoes oxidative dechlorination to form tetrachlorohydroquinone (TCHQ) (Renner and Hopfer, 1990; Wang et al., 2000). TCHQ seems to be a more toxic form of the xenobiotic that is able to induce DNA strand breakage, protein adducts and depletion of glutathione content in liver tissue (Wang and Lin, 1995; Wang et al., 1997). PCP is able to induce oxidative stress and apoptosis, cell cycle arrest as consequence of DNA damage (p53), mitogenic response (cyclin D1) and apoptosis (caspase 3) (Dorsey et al., 2006). Caspase-3, the most prevalent caspase in the cell and an important member of a family of cysteine proteases (Yuan et al, 1993), has been widely reported to be the key executioner caspase in apoptosis (Cohen, 1997). Active caspase-3 cleaves many
substrates such as poly (ADPribose) polymerase-1 (PARP) and ICAD (inhibitor of caspase-activated DNase) that result in key morphological alterations involved in apoptosis (Fischer et al., 2003).

Oxidative stress plays a critical role in cancer, inflammatory, cardiovascular and neurodegenerative diseases as well as in aging, endogenous protection against oxidative stress is achieved by enzymes that catalytically remove free radicals and other reactive species (Storz, 2005). There is an intimate relationship between nutrition and the antioxidant defense system, as some exogenous low molecular weight antioxidants may be supplied by the diet. These two main systems of the antioxidant defense act in coordination, their levels being regulated by each other, to avoid oxidative stress events (Masella et al., 2005).

Punica granatum, (Punicaceae) or Pomegranate, is an edible fruit cultivated in Mediterranean countries, Asian countries and some parts of the United States. Pomegranate has been extensively used as a folk medicine by many cultures (Langley, 2000). Pomegranate fruit is a rich source of two types of polyphenolic compounds: anthocyanins (such as delphinidin, cyanidin pelargonidin) and hydrolyzable tannins (such as punicalin, pedunculagin, punicalagin, gallagic and ellagic acid esters of glucose), which account for 92% of the antioxidant activity of the whole fruit (Gil et al., 2000). The plant possesses an immense therapeutic value. A number of biological activities such as antitumour (Afaq et al., 2005), antibacterial (Prashanth et al., 2001), anti diarrhoeal (Das et al., 1999), antifungal (Dutta et al., 1998), antiulcer (Gharzouli et al., 1999) have been reported with various extracts/components of different parts of this plant. Pomegranate is now gaining importance because of its potent antioxidant activity. Some potent antioxidants have been isolated from the fruit juice and have been found to be bioavailable, effective and safe (Cerda et al., 2003). Pomegranate extract inhibits lipid peroxidation at lower concentrations than vitamin E (Rosenblat et al., 2003). Pomegranate wine and fruit constituents inhibit nuclear factor k B (NFkB), a transcription factor activated by reactive oxygen species (ROS) and hence implicated in pathophysiology of numerous diseases (Afaq et al., 2005). Pomegranate extract has no side effects and no known drug interactions and prevents liver fibrosis (Thresiamma and Kuttan, 1996).

The purpose of the present study was to study the ability of pomegranate peels extract to scavenge various reactive oxygen species and inhibit lipid per oxidation. Also, the possible preventive effects of pomegranate extract was determined against pentachlorophenol induced oxidative stress, cytogenetic effect, histopathological changes, DNA content and caspase 3 in liver of rats.

MATERIAL AND METHODS

Plant Material:
The fruits of Punica granatum were collected from the local market in Cairo, Egypt in April, 2008 and identified by experts in National Herbarium reference.

Extraction:
The rind of Punica granatum was manually separated from the whole fruits after cleaning, then dried in hot-air oven, powdered and extracted with a mixture of methanol: water (7:3, v/v) by a Soxhlet apparatus at 65 °C. The solvent was completely removed and the dried crude extract thus obtained was used for investigation. Crude extract was then dissolved in saline each 1ml contains 200mg of methanloic extract of P. granatum.

Phytochemical Analysis:
The aqueous methanol extract of the Punica granatum peels was subjected to qualitative chemical screening for the identification of the tannins, and flavonoids using standard procedures (Trease & Evans, 2001).

Test for Tannins:
The aqueous methanol extract (1 mL) was mixed with 10mL of distilled water and filtered. Ferric chloride reagent (3 drops) was added to the filtrate. A blue-black or green precipitate confirmed the presence of gallic tannins or catechol tannins, respectively.

Test for Flavonoids:
A portion of the aqueous methanol extract (2 mL) was heated, and metallic magnesium and concentrated hydrochloric acid (5 drops) were added. A red or orange coloration indicated the presence of flavonoids.

Chemicals:
Pentachlorophenol (C6Cl5OH, CAS No. 87-86-5, Lot No. 01530TS), with purity 98.0% was purchased from Sigma-Aldrich Chemical CO., (St. Louis, Missouri). All other chemicals used in the study from the highest purity commercially available from Merck and BDH chemical (Germany).
Animals:
Healthy adult male Sprague-Dawley rats with an average body weight of 170 ± 20 g were obtained from the animal house of the National Research Centre. The animals were housed under standard laboratory conditions (12 h light and 12 h dark) in a room with controlled temperature (24±3°C) during the experimental period. The rats were provided free standard pellet diet and water ad libitum. Animal care and handling was in-accordance with the guidelines set by the World Health Organization, Geneva, Switzerland. With the approval of the committee for animal care at the National Research Centre, Egypt.

Experimental Design:
After one week of acclimation, the animals were divided into six groups, 6 rats each and were treated as follows:
- Group I: Controls received the vehicle of normal saline (2 ml/kg).
- Group II: Received methanolic extract of *P. granatum* at a dose of 200 mg/kg/day orally for 30 days.
- Group III: Received methanolic extract of *P. granatum* at a dose of 400 mg/kg/day orally for 30 days.
- Group IV: Received PCP 7.3 mg/kg/day orally for 30 days.
- Group V: Received methanolic extract of *P. granatum* at a dose of 200 mg/kg/day then PCP for 30 days.
- Group VI: Received methanolic extract of *P. granatum* at a dose of 400 mg/kg/day then PCP for 30 days.

At the end of experimental period, blood samples were collected from the retro-orbital vein plexus and direct cardiac puncture, under ether anesthesia in sterile tubes and centrifuged at 3500 rpm for 15 min and Serum was separated for measurement of malondialdehyde (MDA). After the collection of blood samples all animals were sacrificed by cervical dislocation; femur and testis of each animal were dissected for cytogenetic, and liver for studies histopathological changes and DNA content.

Serum Lipid Peroxidation Level:
It was estimated by the measurement of malondialdehyde (MDA) by spectrophotometric method (Satoh, 1978) using commercial kits (Biodiagnostic reagent kits, Egypt). The level of lipid peroxidation was expressed as μmol/ml.

Cytogenetic Analyses:
Two hours before sacrifice, the animals were injected with 0.5 mg of colchicine.

Chromosome Analysis in Somatic Cells:
Femora were removed and bone marrow cells were aspirated using saline solution. Metaphase spreads were prepared by using Preston *et al.* (1987) method. Fifty metaphase spreads per animal were analyzed for studying the chromosome aberrations

Chromosome Analysis in Germ Cells:
Spermatocyte cells were prepared according to Brewen and Preston (1978) for meiotic chromosomal analysis.

Histopathological and Histochemical Studies:
After draining the blood, liver samples were excised, washed with normal saline and fixed in 10% buffered neutral formalin and paraffin embedded. Paraffin sections were taken at 5 µm thickness processed in alcohol-xylene series and was stained with alum hematoxylin and eosin for the histological examination. For histochemical studies, other sections from liver were stained with Feulgen stain for DNA content (Drury *et al.*, 1980).

Immunohistochemistry for Caspase-3:
Immunohistochemical staining of anti-caspase-3 antibody was performed by streptavidin-biotin. Sections of four µm thick were deparaffinized and incubated with fresh 0.3% hydrogen peroxide in methanol for 30 min at room temperature. The specimens were then incubated with anti-caspase-3 antibody as the primer antibody at a 1:100 dilution. The specimens were counterstained with H and E. Negative controls were prepared by substituting normal mouse serum for each primary antibody.

Statistical Analysis:
The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as significant.
Results:

Effects of Pomegranate Extract Pretreatment on MDA:

There was a significant increase in serum MDA level of PCP intoxicated rats as compared to control group. However, pre-treatment with *P. granatum* at a dose of (200 and 400 mg/kg) caused marked significantly (p<0.05) protection, evidence by reduction in the MDA levels and brought them near to normal level Fig (1).

![Fig. 1: Effect of low and high dose of *Punica granatum* extract pretreatment on serum MDA levels in pentachlorophenol toxicity.](image)

Chromosome Examination in Bone Marrow Cells:

The results of the frequencies of total structural chromosomal aberrations in the two groups of animals treated with low and high dose of *P. granatum* extract showed no significance compared with control group. The animals treated with PCP observed statistical significant (P<0.001) increase in the frequencies of all types of chromosomal aberrations compared to control groups. The total aberrations in animals treated with PCP were 50.8±0.94 compared to 1.50±0.84, 2.78±2.37, 2.20±1.61 in the groups I, II and III, respectively (Table, 1).

Groups V and VI of animals treated with low and high doses of *P. granatum* extract (200 and 400mg/kg, respectively) and PCP showed statistical significant (P<0.001) decrease in all types of chromosomal aberrations compared to group treated with PCP. The total structural chromosomal aberrations decreased from 50.8±0.94 in animals treated with PCP to 5.71±0.41 and 2.80±0.88 in animals treated with low and high doses of Pomegranate extract and PCP, respectively. Comparative study between the groups of animals treated with low and high doses of *P. granatum* extract and PCP revealed that the different types of chromosomal aberrations decreases in animals treated with high dose of Pomegranate extract (400mg/kg) compared with low dose (200mg/kg). Centric fusion was the most type of aberrations decrease by high dose of Pomegranate extract (Table, 1).

Table 1: The mean value of different chromosomal aberration was induced by PCP in bone marrow of rats and protective effect of *Punica granatum* extract in different doses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>No. of examined cells</th>
<th>Structural chromosomal aberration</th>
<th>Total aberration</th>
<th>Numerical aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gap</td>
<td></td>
<td>2n-1</td>
</tr>
<tr>
<td>Control Group</td>
<td>5</td>
<td>250</td>
<td>0.20±0.44*</td>
<td>0.31±0.1a</td>
<td>1.50±0.84</td>
</tr>
<tr>
<td>Low dose of pomegranate extract treated group</td>
<td>5</td>
<td>250</td>
<td>0.61±0.81*</td>
<td>0.60±0.51</td>
<td>2.78±2.3</td>
</tr>
<tr>
<td>High dose of pomegranate extract treated group</td>
<td>5</td>
<td>250</td>
<td>0.42±0.67*</td>
<td>0.48±0.22</td>
<td>2.20±1.8</td>
</tr>
<tr>
<td>PCP treated group</td>
<td>5</td>
<td>250</td>
<td>15.01±1.57***</td>
<td>16.6±0.94**</td>
<td>50.8±0.94 ***</td>
</tr>
<tr>
<td>Low dose of pomegranate extract+ PCP treated group</td>
<td>5</td>
<td>250</td>
<td>6.37±0.33 **</td>
<td>5.71±0.41 **</td>
<td>5.71±0.41 **</td>
</tr>
<tr>
<td>High dose of pomegranate extract+ PCP treated group</td>
<td>5</td>
<td>250</td>
<td>4.80±0.85 ***</td>
<td>3.11±1.14 ***</td>
<td>2.80±0.88 **</td>
</tr>
</tbody>
</table>

Within each column, means superscript with the same letter are not significantly different. (b)Significantly different from control group (c) Significantly different from PCP - treated group

***P<0.001
Chromosome Examination in Spermatocyte Cells:

The results of chromosomal aberrations induced in rat’s spermatocytes by low dose of *P. granatum* extract, high dose of extract, PCP, low dose of extract and high dose of extract followed by PCP were tabulated in Table 2. Chromosome aberrations of spermatocyte cells showed as chain, autosomal univalent and x-y univalent. Statistical analysis between control group and the groups received Pomegranate extract illustrated that there was no significance differences in the frequencies of different types of chromosomal aberrations. While animals treated with PCP showed statistical significant (P<0.001) increase in the frequencies of autosomal univalent, x-y univalent, and total structural chromosome aberrations compared to control group.

Chromosomal aberrations of rat’s spermatocytes treated with low and high doses of *P. granatum* extract and PCP were decreased compared to the rats treated with PCP. This decrease was 11.96±2.25, 4.40±1.81 compared to 25.81±2.11, respectively. The difference in chromosomal aberrations of rat’s spermatocytes and PCP were decreased compared to the rats treated with PCP. This decrease was 11.96±2.25, 4.40±1.81 was evident in the higher dose of P.

Within each column, means superscript with the same letter are not significantly different. (b) Significantly different from control group (c) Significantly different from PCP - treated group ** P<0.01 and ***P<0.001

### Table 2: The mean value of different chromosomal aberration was induced by PCP in spermatocytes of rats and protective effect of Punica granatum extract in different doses.

<table>
<thead>
<tr>
<th>Treatment Doses</th>
<th>No. of Animals</th>
<th>No. of examined Cells</th>
<th>Structural chromosomal aberrations</th>
<th>Total aberration</th>
<th>Numerical Aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total aberration</td>
<td>Chromosome</td>
<td>Univalent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Autosomal</td>
<td>X-Y Univalent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Autosomal</td>
<td>X-Y Univalent</td>
</tr>
<tr>
<td>Control Group</td>
<td>5</td>
<td>250</td>
<td>0.60±0.90</td>
<td>0.40±0.54</td>
<td>1.00±0.87</td>
</tr>
<tr>
<td>Low dose of pomegranate extract treated group</td>
<td>5</td>
<td>250</td>
<td>0.51±0.11</td>
<td>0.3±0.22</td>
<td>2.98±0.54</td>
</tr>
<tr>
<td>High dose of pomegranate extract treated group</td>
<td>5</td>
<td>250</td>
<td>0.32±0.42</td>
<td>0.8±0.55</td>
<td>1.63±1.36</td>
</tr>
<tr>
<td>PCP treated group</td>
<td>5</td>
<td>250</td>
<td>0.41±0.45</td>
<td>1.10±1.30</td>
<td>4.40±1.44</td>
</tr>
<tr>
<td>Low dose of pomegranate extract+ PCP treated group</td>
<td>5</td>
<td>250</td>
<td>3.8±0.41</td>
<td>4.60±1.14</td>
<td>11.96±2.23</td>
</tr>
<tr>
<td>High dose of pomegranate extract+ PCP treated group</td>
<td>5</td>
<td>250</td>
<td>1.40±0.45</td>
<td>2.20±0.85</td>
<td>4.40±1.14</td>
</tr>
</tbody>
</table>

Histopathological Studies:

The histological examination of liver sections in the control rat showed a normal hepatic architecture with distinct hepatic cells, sinusoidal spaces and a central vein, portal tract with prominent nucleus (Fig.2 A). There were no abnormalities or histological changes in the liver of rats treated with two doses of *P. granatum* (200 & 400 mg/kg/day). Histopathological examination of the liver section of the rats treated with PCP showed hepatic architecture was disturbed due to distortion of the hepatic cords and an intense centrilobular necrosis and vacuolization of the cytoplasm (Fig. 2 B). The central vein, portal vein and hepatic artery were congested with mild degree fibrous tissue proliferation and hepatocellular degeneration (Fig. 2 C). The nuclei of most of the hepatocytes appeared with numerous dense clumps of chromatin and some nuclei appeared pyknotic (Fig. 2 D).

Liver sections of rat administrated of *P. granatum* extract at dose (200 & 400 mg/kg) and PCP clearly showed a remarkable improvement it was evident from the formation of normal hepatic cells and central veins with the absence of necrosis and vacuoles., which are comparable with PCP toxicity groups. The nuclei were normal indicating the recovery of the liver (Fig. 2 E & F). All these results indicate a hepatoprotective potential of *P. granatum* extract showed a dose dependent activity which was confirmed by histopathological examination.

Histochemical Studies for DNA Content:

The histochemical investigation of liver control rats showed that normal distribution of DNA content and chromatin substances of the cells were stained by Feulgen stain (Fig.3 A). Examination of liver sections of rat treated with PCP exhibited a decrease in DNA content in liver cells as compared to control (Fig. 3 B & C). The nuclei appeared larger and more irregular in shape than control group with very little peripheral condensed chromatin. Meanwhile, clumped chromatin was also observed in some hepatocytes. In the liver section of rat treated with *P. granatum* at dose (200 & 400 mg/kg) and PCP showed marked improvement in DNA content was evident in the higher dose of *P. granatum* when compared to PCP group (Fig. 3 D & E).
Fig. 2: Representative photomicrographs of liver sections stained by H & E. A: Section taken from liver of control rats shows a preserved architecture with central veins (CV), polygonal hepatocytes (H) with their rounded nuclei (N) and blood sinusoids (S) (X400). B, C and D: Sections taken from liver of PCP treated rats. B: Shows loss of the normal architecture with dilated, congested central vein and portal tract ( astric), fibrosis ( long arrow), leucocytic infiltration and hydrobic degeneration of hepatocytes (arrow head) (X400). C: Shows fatty change, hydrobic degeneration of hepatocytes (arrow head) as well as focal hepatic necrosis replaced by mononuclear cells infiltration (long arrow) and congested blood sinusoids (astric) (X400). D: Shows portal triaditis with thin fibrous bridges radiating from the portal tract (F). Liver cells show congested blood sinusoids (arrow head). Note the increase in mitotic figures (M) (X400). E: Sections taken from liver of rat treated with *P. granatum* (200mg/kg/day) followed by PCP shows almost normal liver histology with slight dilated in sinusoids (S), few pyknotic cells (arrow head) and lesser degrees of inflammation (X400). F: Sections taken from liver of rat treated with *P. granatum* (400mg/kg/day) followed by PCP shows near to normal arrangement of the hepatic cords, regeneration in the hepatocytes and many binucleated liver cells (BN) (X400).
Fig. 3: Representative light photomicrographs of liver sections stained by Feulgen technique for DNA content. A: Section taken from liver of control rats shows red purple colored particles in the nucleoplasm of hepatocytes and Kupffer cells (x 400). B and C: Sections taken from liver of PCP treated rats. B: Shows a marked depletion of DNA in the nuclei as indicated by the weak magenta (x 400). C: Shows a marked depletion of DNA in the hepatocytes as indicated by the weak magenta and pleomorphism of nuclei (x 400). D: Sections taken from liver of rat treated with *P. granatum* (200mg/kg/day) followed by PCP shows an increase in DNA content of nuclei (x 400). E: Sections taken from liver of rat treated with *P. granatum* (400mg/kg/day) followed by PCP shows DNA content more or less like control (x 400).

**Immunohistochemistry for Caspase-3:**

Subsequently, we examined whether *P. granatum* possessed the anti-apoptotic effects against PCP caused liver injury by analysis of capase-3 in the liver. We observed caspase-3 expression in the cytoplasm of liver cells. Expression of caspase-3 was not observed in control liver (Fig. 4 A). By comparison, strong expression of caspase-3 was observed in PCP group as shown in (Fig. 4 B) and gradually decreased in the treated groups with *P. granatum* (200 & 400 mg/kg) (Fig. 4 C & D). The expressions of caspase-3 on rat treated with PCP and *P. granatum* (400 mg/kg) were highly decreased than the expressions observed in the group treated with *P. granatum* (200 mg/kg).
Fig. 4: Expression of caspase-3 immunohistochemical staining (X 400). A: A section obtained from liver of control rat, shows Caspase-3-immunolabeled hepatocytes were rarely present. B: A section obtained from liver of rat treated with PCP shows an increased number of caspase-3 immunolabeled hepatocytes were observed around central veins, identified by brown staining. C: Sections taken from liver of rat treated with \textit{P. granatum} (200mg/kg/day) followed by PCP D: Sections taken from liver of rat treated with \textit{P. granatum} (400mg/kg/day) followed by PCP shows Caspase-3-immunolabeled cells were slight decrease compared to rats treated by PCP.

**Discussion:**

In the present study there was a statistical significant increase in MDA content in PCP intoxicated animals. Pre-treatment with \textit{P. granatum} extract (200 & 400 mg/kg/day) significantly prevented the increase in MDA levels and brought them near to normal level. These results were in-agreement with Dong \textit{et al.}, (2009) and Han \textit{et al.}, (2009) they observed there was a significant increase in cellular malondialdehyde (MDA) concentration after PCP treatments. Luo \textit{et al.}, (2009) revealed that, a strong positive correlation between PCP liver concentrations and MDA level and its metabolites tetrachlorohydroquinone (TCHQ) induce hydroxyl radical formation which increase oxidative damage.

The elevation of MDA content might have resulted from an increase of reactive oxygen species (ROS) as a result of stress condition in the rats with PCP intoxication. It is known that the elevation of lipid peroxidation after some xenobiotic is consumed, and followed often by the superoxide overproduction, which after dismutation produce singlet oxygen and hydrogen peroxide, and it can be easily converted later into the reactive OH. Both single oxygen and OH radical have a high potential to initiate free radicals chain reactions of lipid peroxidation (Halliwell, 1996).

These results clearly suggested that inhibition of PCP-induced lipid peroxidation in rat liver microsomes were related to their abilities of phenolic and flavonoid compounds present in extracts of \textit{P. granatum} to inhibit hepatic oxidative enzymes (cytochrome P450 system) (Chidambara-Murthy \textit{et al.},2002; Ajaikumar \textit{et al.}, 2005). MDA levels were lowered significantly by the treatment of the rats with the ethanolic extracts of pomegranate peel, when compared with CCl4-treated group (Osman \textit{et al.}, 2011). Also, increases in tissue MDA levels due to bile duct ligation (BDL) were reduced back to control levels by pomegranate peel extract (PPE) treatment (Toklu \textit{et al.}, 2007).

In the present study, cytogenetic results in bone marrow cells showed that highly significant of the frequencies of total structural chromosome aberrations, frequencies of deletions and centric fusions and in spermatocyte cells there were significant differences in the frequencies of autosomal univalent and x-y univalent in rats treated with PCP compared to control group. These results in-agreement with Rui-xia \textit{et al.}, (2007) who reported sodium pentachlorophenol could induce DNA damage, chromosome aberration of cells of hamster.
ovary and including single strand or double-strand breaks on the cultured epithelial cells of human nasal mucosa (Milowska et al., 2003; Tisch et al., 2005). Farah et al., (2006) stated that, PCP was able to produce aberration of chromatid and chromosome types in a significant manner. In rats were pretreated with low and high doses of P. granatum extract showed that attenuation of changes in the PCP group. Valadare et al., (2010) demonstrated that P. granatum has a preventive effect against chromosome fragmentation and/or damage to the mitotic apparatus, probably due to its free radical scavenging capability.

In the present study, histopathological changes including vacuolization of the hepatocytes, focal hepatocellular degeneration, necrosis, fatty degeneration and interstitial fibrosis with inflammatory infiltration were observed in the liver of rats exposed to PCP. These results were confirmed by the data of Umemura et al., (2002), who reported the PCP caused hepatocellular centrilobular hypertrophy, vacuolation, were evident in the 10-, 30-, and 60-mg/kg/day dose group animals (Bernard et al., 2002), cellular swelling, vacuolar degeneration (Villena et al., 1992) and periportal fibrosis (NTP, 1989).

Examination of liver sections of rat treated with PCP exhibited a decrease in DNA content and increase in activation of caspase-3 in liver cells as compared to control. The major metabolite of PCP, tetrachlorohydroquinone (TCHQ), was reported to induce 8-oxodG formation in liver DNA of mice during DNA replication, leading to point mutations (Cheng et al., 1992, Dahlhaus, et al 1994) and subsequent oncogene activation (Le Page et al., 1995, Kamiya et al., 1992).

PCP increases the consumption of oxygen and It is caused by the loss of mitochondrial breathing as a result of the impairment of oxidative phosphorylation. High consumption of oxygen in those conditions can contribute to the process of excessive production of the reactive oxygen species (ROS), which could explain the mechanism of PCP toxicity in the case of DNA damage (Pavlica et al., 2001). ROS generate different types of DNA damage, such as the damage as well as single and double strand breaks both in in vivo and in vitro systems (Dahlhaus and Appel, 1993). However, PCP can induce apoptosis with its typical characteristics of nuclear shrinkage, condensation, caspase-3 activation and breakage as well as formation of apoptotic bodies, and further experiment demonstrated that PCP-induced apoptosis occurred in a dose-dependent manner (Wispyriyono et al., 2002; Fernández et al., 2005) The mechanisms underlying the role of oxidative stress in apoptosis may include high levels of ROS directly increasing caspase activity, disrupting intracellular Ca2+ homeostasis and resulting in the ATP depletion due to the close relationship between ROS and mitochondria (McConkey, 1998; Thayyullathil et al., 2008).

Guo et al., (2007) reported that, Pomegranate extracts has antioxidant activity and prevent DNA damage. Lamar et al., (2005) revealed that, the P. granatum whole fruit extract could sequestrate reactive oxygen species caused by hydrogen peroxide, a mechanism that allows it to protect the DNA against the lesions provoked by this agent. The antimutagenic effect of the bioactive pomegranate compounds has been demonstrated by a decrease in the frequency of genotoxicant-induced chromosomal aberrations in bone marrow cells of mice and rats (Alekperov, 2002).

In the present study P. granatum extract pretreated groups showed normal hepatic cells and central veins and decrease the liver fibrosis, Also, pre-treatment with P. granatum extract ameliorated the DNA content and decease of activation caspase-3 comparable to the PCP values. In agreement with present study, (Toklu et al., 2007) who reported pomegranate peel extract administration reduced the hepatic collagen content in the bile duct ligation (BDL) rats. Thus, pomegranate peel extract administration alleviated the BDL-induced oxidative injury of the liver and improved the hepatic structure and function. It therefore seems likely that pomegranate peel extract, with its antioxidant and antifibrotic properties may be of potential therapeutic value in protecting the liver from fibrosis and oxidative injury due to biliary obstruction (Toklu et al., 2007). Pomegranate flower and fruits extract also exhibited scavenging ability for ROS. It potently scavenged (O2.), (H2O2), (OH) and (NO), with its potent free radical quenching capacity, was expected to inhibit oxidative damage to biomolecules (Noda et al., 2002).

The pomegranate extract significantly inhibited ferric nitrilriacetate (Fe-NTA) induced oxidative stress and also protected from necrosis and other pathological changes and maintain hepatic architecture (Kaur et al., 2006). Similar hepatoprotective effects have been reported with pomegranate peel extract, which inhibited CCl4, induced oxidative stress and hepatic injury (Chidambara -Murthy et al., 2002). Droge, (2002) reported that certain flavonoids and phenols have protective effect on liver damage due to its antioxidant properties. Antioxidant potential of pomegranate juice and peel, seeds extracts is attributed to their high polyphenolics content (Seeram et al., 2005) and possess a potent antioxidant activity (Kaur et al., 2006).

**Conclusion:**

There is clear evidence from this study that oxidative damage plays a major role in PCP-induced chromosomal aberration, histopathological changes and DNA damage in liver, as evidenced by significant an elevation lipid peroxidation end products. The pretreatment with extract of pomegranate peels showed a preventive effect against chromosome fragmentation and/or damage to the mitotic apparatus and oxidative DNA damage, probably due to its free radical scavenging capability. Also, this study clear that pomegranate peels
extract has hepatoprotective effect by restoring the histopathological changes in liver to normal hepatic architecture. The extract has a huge amount of polyphenolics, to which its antioxidant activity may be ascribed.

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