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# *Punica granatum* L *var nana*: A Hepatoprotective and Curative Agent Against $CCl_4$ Induced Hepatotoxicity in Rats

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**Abstract**

Since prehistoric times, medicinal plants and their extracts had been used as prolific sources of medications. Pomegranate is a symbol of life, longevity and in Aurvedic medicine; pomegranate is considered a pharmacy up to itself. Despite several previous studies centered to the phytochemistry and pharmacological actions of all *Punica granatum* L. components, however, to date, few reports about the variety *nana*, which implies that this species is yet under-investigated. *Punica granatum L. var. nana* is a dwarf variety of *Punica granatum* popularly planted as an ornamental plant in gardens. This study aims to determining the biomedical potentiality of the methanolic extract of *Punica granatum L. var. nana* leaves as source of botanical hepatoprotective and hepatocurative agents. The methanolic extract exhibited hepatoprotective and curative effects against $CCl_4$ induced hepatotoxicity in rats and both effects were preventive and curative. The histopathological studies support the hepatoprotective and curative effects by restoring the normal tissue architecture. Furthermore, this extract was found to be safe by using experimental albino rats suggesting that it may be utilized as a potential source of some beneficial bioactive compounds.

**Key words:** *Punica granatum L. var. nana*, *Lythraceae*, $CCl_4$, hepatoprotective activity.

## Highlights

- Pomegranate is a rich producer of divers pharmacologically active compounds.
- Methanolic extract of *P. granatum* L. *var nana* is a promising hepatoprotective and curative agent against $CCl_4$ induced hepatic failure.
- Methanolic extract of *P. granatum* L. *var nana* is safe and showed no signs of toxicity which confirmed via biochemical and histopathological investigations.
- Pomegranate's hepatoprotective activity is linked to highly content of phenolics compounds.

## 1. Introduction

For thousands of years, medicinal plants have been used widely for the treatment of a variety of human health problems based on their highly effective bioactive constituents [1, 2]. Liver is an amazing and critical organ, highly effective for several pathogenic factors and metabolic pathways [3, 4, 5]. Hepatic injury and liver disorders continue to be a widely major health problem although the enormous develop in modern medicine [6, 7]. Carbon tetrachloride ($CCl_4$) is a classical and powerful hepatotoxin, widely used in animal experimental model to persuade liver damage comparable with hepatic injury in humans [8, 9, 10, 11]. The liver damage induced by $CCl_4$ is based on the fact of producing reactive oxygen species (ROS) causing oxidative stress and hepatic failure or fibrosis [12, 13, 14]. $CCl_4$ converted to free radicals i.e. $CCl_3^{\bullet}$ and $CCl_3OO^{\bullet}$ by the hepatic cytochrome $P_{450}$ during the hepatotoxicity which react with proteins, lipids and DNA [15, 16],

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destroying the cell membranes structure [17], cells inflammation and activation of neutrophils which cause the death of hepatic cells [18, 19].

The pomegranate is one of most important dietary sources. It disclosed a myriad of pharmacological potentialities e.g. helminthiasis, redox regulation and anticancer [20]. Historically, several parts of the pomegranate have been used in the tradition medicine e.g. fruit rind has been used to treat dysentery and hemorrhage [21], menstrual irregularities [22] and anti-diabetic [23]; Leaves and flowers have been used to treat respiratory diseases, hepatic problems, cough, fever, throat diseases, increase sperms, slightly astringent, increase strength and memory [24]; Peels has been used to treat diarrhea and intestinal parasites [25] eye and ear inflammation [24], general tonic [26] and antipyretic and analgesic [27].

*Punica granatum* L *var. nana* is a cultivated dwarfed species usually planted in gardens as an ornamental plant and used as a bonsai due to its small leaves, flowers, and fruits. Morphologically, it is a deciduous shrub and it is found to be different from the edible pomegranate with its orange flowers and having a plenty of petals, its fruits are teeny and not consumable and it is shorter (3-4 m) [28]. Phytochemical analysis of its methanolic extract showed its rich content of phenolic constituents [29]. Although plant extracts rich in phenolic compounds have been showed hepatoprotective effect and prevent liver damage effectively [4], but to the best of our knowledge, the hepatoprotective and curative effects of *Punica granatum* L *var. nana* against $CCl_4$-liver injury have not been determined.

The present work aims to estimate the hepatoprotective and curative effects of the methanolic extract of *Punica granatum* L *var. nana* leaves against $CCl_4$-induced liver injury in rats.

## 2. Material and methods

2.1. Plant material

In May 2009, at the flowering stage, leaves of *Punica granatum* L. *var nana* (Family; *Lythraceae*) have been collected from El-Tahreir garden, Bani-Suef governorate, Egypt. The collected plant has been identified by Dr. T. Labeb, Herbarium of Orman garden, Horticulture Research Institute, Giza, Egypt for the botanical identification. A voucher specimen (leaves) was deposited at the Herbarium of the Biochemistry Department, Faculty of Agriculture, Fayoum University, Egypt.

350g of dried leaves were ground and exhaustively extracted with MeOH/$H_2O$ (4:1) at room temperature. The extract filtrated and evaporated under reduced pressure to yield 75g crud extract. Subsequentially, it was defatted using chloroform to afford 71.3g cure defatted material which stored for further biological evaluation.

2.2. In vivo toxicity, hepatoprotective and curative activities

2.2.1. Animals

Female albino rats weighing 180-220g were obtained from the Faculty of Agriculture, Minia University, Egypt. They were housed under stander condition with food and water adlibitumun and animal were handled in accordance with (NIH guide for the care and use of laboratory animals, NIH Publication No. 85-23, 1985, revised 1996) and guiding principles for research involving animals [30] till the end of the experiment (6 weeks).

2.2.2. Study protocol

Animals were divided randomly into eleven equal groups of eight rats each: negative control (-ve) orally received daily 1 mL $dH_2O$.; Toxicity of the extract groups, $E_{60}$, $E_{120}$ and $E_{240}$, orally received daily 60, 120 and 240 mg/kg of the extract dissolved in 1 mL $dH_2O$, respectively; positive control group (+ve) rats were intraperitoneally injected twice weekly with $CCl_4$ at a dose of 2 mL/kg as 50% paraffin's oil solution and orally received daily 1 mL $dH_2O$; Hepato-curative effects of the extract against $CCl_4$, $CCl_4$+$E_{60}$, $CCl_4$+$E_{120}$, $CCl_4$+$E_{240}$, intraperitoneally injected for ten days daily with $CCl_4$ at a dose of 2 ml/kg as 50% paraffin's oil solution, so as the groups to have hepatic failure, and then intraperitoneally injected twice weekly with $CCl_4$ at a dose of 2 ml/kg as 50% paraffin's oil solution and orally received daily 60, 120 and 240 mg/Kg of the extract dissolved in 1 mL $dH_2O$, respectively. Hepato-protective effects of the extract against $CCl_4$, $E_{60}$+$CCl_4$, $E_{120}$+$CCl_4$, $E_{240}$+$CCl_4$, orally received daily 60, 120 and 240 mg/kg of the extract dissolved in 1 mL $dH_2O$ for ten days and then intraperitoneally injected twice weekly with $CCl_4$ at a dose of 2 mL/kg as 50% paraffin's oil solution and orally received daily 60, 120 and 240 mg/kg of the extract dissolved in 1 mL $dH_2O$, respectively. At the end of experiment, the rats were anesthetized by ether inhalation, blood samples were collected from the retro-orbital venous plexus and serum samples were collected from the coagulated blood after centrifugation (3000 rpm, 4 °C, 15 min), the obtained serum was used for the biochemical analysis. Immediately after blood collection, animals were scarified by cervical decapitation under ether anesthesia, livers and kidneys were excised and washed with cold saline to remove the blood and were stored in 10% formalin for histopathological examination.

2.2.3. Biochemical Analysis

2.2.3.1. Liver function tests determination

Alanine amino transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)

and albumin were determined according to Murray, [31], Young, [32], Beifield and Goldberg, [33] and Doumas *et al*, [34] respectively using diagnostic kits obtained from Roche Diagnostics Ltd (Germany).

2.2.3.2. Lipid profile determination

Cholesterol, HDL-C and triacylglycerol were estimated by the colorimetric enzymatic method according to Allain *et al.* [35], Lopes-Virella *et al.* [36] and Glick *et al.* [37] respectively. The kits were supplied from Biocon Diagnostic (Germany).LDL-cholesterol was determined utilizing this equation: LDL-C (mg/dl) = Total cholesterol - (HDL-C+ TG/5) according to Friedewald *et al.* [38].

2.2.3.3. Oxidative stress determination

The lipid peroxidation is a good way for evaluation the tissue damage caused by oxidant stress. Hence levels of malondialdehyde (MDA) measured using the method of Ohkawa *et al.* [39] using enzymatic colorimetric procedure Kit from BioDiagnostic Co., Egypt.

2.2.3.4. Kidney Function tests determination

Urea and creatinine were determined in serum sample calorimetrically according to Fawcett and Soctt [40] and Bartles *et al.* [41] respectively using enzymatic colorimetric procedures Kits from BioDiagnostic Co., Egypt.

2.2.3.5. Histopathological Examination

Liver and kidney samples were fixed in 10% neutral formalin followed by dehydration in ascending grades of alcohol, clearing in xylene and embedding in Paraffin wax. Liver and kidney sections (5 µm thickness) were prepared with lecia RM rotary microtome (Leica instrument, China). The sections have been stained with hematoxylin and eosin (H&E), mounted on glass slides with canda balsam (Sigma, USA) and examined under a light microscope (Olympus CX31, Japan) at 4 × 100 magnification for histopathological changes determination lymphocytes, myeloid cells, hyperplasia and necrosis.

2.3. Statistical Analysis

Data were analyzed using ANOVA procedures in Genstat statistical package (Discovery 4) (VSN International Ltd, Oxford, UK). Difference between means was compared using least significant difference test (LSD) at 1% level.

## 3. Results and Discussion

Liver failure is one of the most frequent diseases worldwide, causing a high rate of mortality. It is distinguished by liver function loss quickly, necrosis and a massive number of hepatocytes. Several studies have been performed to disclose effective medications and treatment for liver diseases but still few useful liver drugs are available practically right now. Several herbal-based natural medicines are available now in the market for liver disorders treatment such as silymarin from *Silybum marianum* and other preparations from multi herbal ingredient [2].

Carbon tetrachloride was selected to induce liver fibrosis in rats due to its ability to cause hepatocytes damage including cell membranes dissolution and free radicals production including trichloromethyl radicals ($CCl_3^{\bullet}$) and chlorine radicals ($Cl^{\bullet}$), binding covalently to proteins macromolecular in the cell and in cell membrane [42]. Furthermore, destroying of lipidperoxidation of cell membranes due to the reaction of $CCl_4$ with $O_2$ forming $CCl_3O_2^{\bullet}$, which resulted in the elevation of liver marker enzymes activities i.e. ALT, AST and ALP in blood serum [42], MDA level elevation [43] and the significant increase of total cholesterol, triglycerides, LDL and significant decrease of HDL level [44].

### 3.1. Effect of experimental treatments on Liver marker enzymes activities

To assess the edibility and safety, curative and hepatoprotective effect of the methanolic extract, the necrosis activities markers i.e. ALT, AST and ALP have been measured in blood serum, the results are shown in Table (1). In the toxicity of the extract groups, $E_{60}$, $E_{120}$ and $E_{240}$, the liver marker enzymes were either lower or slightly higher than those recorded for animals of negative control group.

Rats treated with $CCl_4$, the levels of ALT, AST and ALP were markedly raised by 235.9 %, 144.6 % and 364.9 %, respectively when compared with -ve control group indicating the severity of hepatic injury and congestion caused by $CCl_4$.

In the hepato-curative groups, methanolic extract was given after ten days of $CCl_4$ treatment, the administration of the extract at dose 60, 120 or 240 mg/kg, caused dose dependent reduction of raised AST levels by 32.2 %, 39.8 % and 46 %, respectively when compared to +ve control group.

The same doses decreased ALT and ALP by 31.3 %, 39.7 % and 45.9 %, respectively for ALT and 10.1 %, 29.2 % and 43.01 %, respectively for ALP. Also, hepato-protective treatment groups i.e. ($E_{60}$+CCl4, $E_{120}$+$CCl_4$, $E_{240}$+$CCl_4$) pre-treated with 60, 120 and 240 respectively for ten days before $CCl_4$ injection, suppressed the AST, ALT and ALP activities up to or lower than the level the rats of -ve control group.

Indeed, the reduction in the activities of serum AST, ALT and ALP by oral administration methanolic extract of *P. granatum L. var. nana* leaves after or before $CCl_4$ administration is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by $CCl_4$. These results are in agreement with the commonly accepted view indicating that the serum levels of liver marker enzymes activities return to

normal with healing of the hepatic parenchyma and the generation of the hepatocytes [45].

### 3.2. Effect of experimental treatments on blood serum biochemical, Albumin (liver function) and lipid profile

The hepatic synthetic function was assessed by determine albumin concentration in serum, albumin levels in sera obtained are shown in Table (2). Slight differences in albumin concentration level were observed between the negative control group and groups treated with toxicity groups. These results indicated that the oral administration of the methanolic extract of *P. granatum L. var. nana* showed no harmful or toxic effects at dose of 60, 120 and 240 mg/kg, as evident from normal serum albumin level compared with the -ve control group. The serum albumin concentration in rats treated with $CCl_4$ was decreased to the half when compared to -ve control group. In $CCl_4$, treatment, lesioned hepatocytes, protein synthesis is deficient; this is reflected in a decreased synthesis of hepatic lipoproteins and hence in an accumulation of neutral lipids in the liver with the consequent fatty infiltration [46]. In agreement to our data [47], found significant decrease in serum albumin level for $CCl_4$ administration as in + ve control group.

Regarding, the hepato-curative groups, the administration of the extract at dose 60, 120 or 240 mg/kg, cause an increase in the level of albumin concentration by 49.8, 67.8 and 72.2 % respectively when compared with + ve control group, whereas hepato-protective treatment groups, pre-treated with the methanolic extract for ten days before $CCl_4$ injection revealed albumin concentration level very close to those observed for -ve control group. Effects of the defatted methanolic extract on serum lipid profile level are shown in Table (2). The level of lipid profile in rats of groups treated with methanolic extract only at the dose 60, 120 and 240 mg/kg were either lower or slightly higher with dose dependent than those recorded for rats of –ve control group, which indicated that no harmful or toxic effect of this extract on lipid profile.

In + ve control group the administration of $CCl_4$ showed significantly increased the concentration of TC, LDL and TG by 201.2%, 203.9% and 289.6%, respectively when compared with -ve control group. On the other hand, HDL concentration significantly decreased by 47.97%. High levels of serum TC, LDL and TG, and lower concentration of HDL were induced with $CCl_4$ in rats, consistent with results recorded from previous studies [44, 48].

**Table 1.** Effect of different treatments on blood serum hepatic enzymes activities (ALT, AST and ALP)

| Treatments | AST (U/ml) | ALT (U/ml) | ALP (U/ml) |
|---|---|---|---|
| -ve | $51.0\pm0.29^{g}$ | $54.5\pm0.29^{f}$ | $22.8\pm0.43^{h}$ |
| $E_{60}$ | $47.5\pm0.23^{h}$ | $51.5\pm0.15^{h}$ | $20.1\pm0.05^{i}$ |
| $E_{120}$ | $51.5\pm0.18^{g}$ | $53.3\pm0.09^{g}$ | $22.5\pm0.27^{h}$ |
| $E_{240}$ | $54.4\pm0.1^{ef}$ | $57.0\pm0.04^{e}$ | $28.5\pm0.27^{f}$ |
| +ve | $171.3\pm0.16^{a}$ | $133.3\pm0.14^{a}$ | $106.0\pm2.31^{a}$ |
| $CCl_4+E_{60}$ | $116.1\pm0.09^{b}$ | $91.6\pm0.12^{b}$ | $95.3\pm0.25^{b}$ |
| $CCl_4+E_{120}$ | $103\pm0.12^{c}$ | $80.4\pm0.26^{c}$ | $75.1\pm1.01^{c}$ |
| $CCl_4+E_{240}$ | $92.5\pm0.2^{d}$ | $72.1\pm0.13^{d}$ | $60.4\pm0.23^{d}$ |
| $E_{60}+CCl_4$ | $53.3\pm0.07^{f}$ | $53.2\pm0.06^{g}$ | $15.9\pm0.03^{j}$ |
| $E_{120}+CCl_4$ | $54.3\pm0.1^{ef}$ | $54.0\pm0.05^{fg}$ | $25.5\pm1.44^{g}$ |
| $E_{240}+CCl_4$ | $55.6\pm0.11^{e}$ | $56.4\pm0.1^{e}$ | $30.2\pm0.01^{e}$ |

Data are means (n=5) ± SE. Means followed by the same letter in each column are not significantly different according to the LSD test ($P\leq0.01$).

**Table 2.** Effect of experimental treatments on blood serum biochemical, Albumin (liver function) and lipid profile

| Experimental Treatments | Albumin (g/dl) | Total cholesterol (mmol/l) | Triglycerides (mg/dl) | LDL (mg/dl) | HDL (mg/dl) |
|---|---|---|---|---|---|
| -ve | $4.81\pm0.1^{b}$ | $84.75\pm0.14^{h}$ | $76.35\pm0.09^{e}$ | $54.74\pm0.14^{i}$ | $55.26\pm0.0^{d}$ |
| $E_{60}$ | $4.02\pm0.04^{c}$ | $85.01\pm0.06^{h}$ | $75.75\pm0.09^{e}$ | $50.16\pm0.03^{k}$ | $52.02\pm0.0^{e}$ |
| $E_{120}$ | $4.75\pm0.1^{b}$ | $87.59\pm0.22^{f}$ | $78.54\pm0.03^{d}$ | $53.29\pm0.11^{j}$ | $57.59\pm0.0^{c}$ |
| $E_{240}$ | $5.25\pm0.09^{a}$ | $88.54\pm0.14^{e}$ | $80.31\pm0.12^{c}$ | $56.01\pm0.06^{h}$ | $60.43\pm0.1^{b}$ |
| +ve | $2.27\pm0.16^{e}$ | $255.25\pm0.14^{a}$ | $297.44\pm0.14^{a}$ | $166.34\pm0.0^{a}$ | $28.75\pm0.14^{i}$ |
| $CCl_4+E_{60}$ | $3.40\pm0.1^{d}$ | $142.50\pm0.11^{b}$ | $87.00\pm0.06^{b}$ | $89.00\pm0.27^{c}$ | $65.91\pm0.1^{a}$ |
| $CCl_4+E_{120}$ | $3.81\pm0.04^{c}$ | $119.00\pm2.31^{c}$ | $46.96\pm0.03^{g}$ | $86.00\pm0.58^{d}$ | $43.28\pm0.1^{g}$ |
| $CCl_4+E_{240}$ | $3.91\pm0.05^{c}$ | $86.29\pm1.44^{g}$ | $45.37\pm0.08^{h}$ | $84.54\pm0.58^{e}$ | $40.45\pm0.2^{h}$ |
| $E_{60}+CCl_4$ | $4.55\pm0.1^{b}$ | $92.75\pm1.3^{d}$ | $52.49\pm0.06^{f}$ | $101.33\pm1.8^{b}$ | $40.63\pm0.1^{h}$ |
| $E_{120}+CCl_4$ | $4.60\pm0.0^{b}$ | $89.25\pm0.14^{e}$ | $46.33\pm1.2^{g}$ | $83.17\pm1.59^{f}$ | $44.00\pm0.58^{f}$ |
| $E_{240}+CCl_4$ | $4.70\pm0.3^{b}$ | $86.75\pm0.43^{g}$ | $36.65\pm0.14^{i}$ | $61.83\pm1.01^{g}$ | $53.38\pm1.8^{d}$ |

Data are means (n=5) ± SE. Means followed by the same letter in each column are not significantly different according to the LSD test ($P\leq0.01$).

The obtained results may be attributed to the increase of the non-essential fatty acids resulted from lipid peroxidation induced by $CCl_3^{\bullet}$ and $CCl_3O_2^{\bullet}$ free radicals.

The oral administration of the methanolic extract at the dose of 60, 120 and 240 mg/kg after ten days of $CCl_4$ treatment (hepato-curative groups) significantly decreased the concentration of TC, LDL and TG

accompanied by a significant increase in HDL level when compared to + ve control group. This effect was increased with increasing the oral administration dose of defatted methanolic extract.

A pre-treatment with the defatted methanolic extract before $CCl_4$ administration (hepato-protective treatment groups) resulted in further decrease of TC, LDL and TG and increases of HDL level in comparison with hepato-curative groups. Similar finding was reported by Khan *et al.*, which revealed that the administration of *Sonchus asper* methanolic extract significantly lowered the $CCl_4$ induced the serum levels of TC, LDL and TG, while elevating HDL levels [49]. Inhibition of cholesterol absorption by plant extracts could be a mechanism contributing to the positive changes in plasma cholesterol lipoprotein profile and in lipid content in liver [50]. These results highlight that the methanolic extract of *P.granatum L.var.nana* had both protective and therapeutic effect on lipid metabolism disorders resulting from liver damage by $CCl_4$ treatment in rats.

3.3. Effect experimental treatments on oxidative stress parameter

The amount of aldehydic products (Malondialdehyde, MDA) generated by lipid peroxidation was quantified by the thiobarbituric reaction, which is widely used as marker of lipid peroxidation [39]. The effects of defatted methanolic extracts on serum MDA level are shown in Table (3). Notably oral administration of methanolic extract only, restored serum MDA to normal level as evident from MDA level of – ve control group, indicating no harmful or toxic effect of this extract on hepato cell membrane.

**Table 3**. Effect of experimental treatments on blood serum oxidative biomarkers (MDA)

| Experimental Treatments | MDA (nmol/ml) |
|---|---|
| -ve | $3.79\pm0.02^{b}$ |
| $E_{60}$ | $3.50\pm0.03^{bcd}$ |
| $E_{120}$ | $3.77\pm0.13^{b}$ |
| $E_{240}$ | $3.85\pm0.05^{b}$ |
| +ve | $15.29\pm0.11^{a}$ |
| $CCl_4+E_{60}$ | $3.07\pm0.08^{e}$ |
| $CCl_4+E_{120}$ | $3.38\pm0.07^{cde}$ |
| $CCl_4+E_{240}$ | $3.65\pm0.14^{bc}$ |
| $E_{60}+CCl_4$ | $3.12\pm0.08^{de}$ |
| $E_{120}+CCl_4$ | $3.22\pm0.07^{de}$ |
| $E_{240}+CCl_4$ | $3.86\pm0.03^{b}$ |

Data are means (n=5) ± SE. Means followed by the same letter in each column are not significantly different according to the LSD test ($P\leq0.01$).

In rats treated with $CCl_4$, the level of MDA was markedly increased by 303 % when compared with negative control group indicate extensive oxidation damage to liver tissues and support previous findings of other investigation [48]. Oral administration of methanolic extract after or before $CCl_4$ administration attenuated this elevation of MDA. The results indicated that the defatted methanolic extract is effectively able of protecting liver against $CCl_4$-induced oxidative damage in rats. Other studies on the protective effect of *Sonchus asper* extract and rutin against $CCl_4$-induced hepatotoxicity in rats have revealed similar finding that found to be in agreement with the previous findings [48, 49].

The free radicals produced in the cytochrome due $CCl_4$ treatment attacked and destroyed polyunsaturated fatty acids in microsomal lipids, leading to its peroxidation and covalently bind to microsomal lipids and proteins. This results in the generation of ROS (reactive oxygen species) that cause oxidation of critical thiol groups and injure mitochondria [48]. These effects dramatically change the properties of biological membranes, resulting in severe cell damage and play a significant role in the pathogenesis of diseases. Membrane lipid peroxidation induces cross linking in proteins this can be provoked by the MDA through covalently binding to the free amine function of proteins which lead to an irreversible polymerization. Thereby, it has become the key to prevent and cure hepatic damage by eliminating free radicals and preventing lipid peroxidation. The defatted methanolic extract of *Punica granatum* L *var*. *nana* exhibited free radical scavenging activity against DPPH radical ($IC_{50}$ = 25.97 $\mu g.ml^{-1}$), Furthermore, the phytochemical screening of the defatted methanolic extract revealed the presence of saponins, anthocyanins, alkaloids, tannin and flavonoids [29]. These compounds have been reported to show anti-oxidative activity against $CCl_4$-induced lipid peroxidation and have the ability to scavenge radicals such as hydroxyl, superoxide and peroxyl which are known to be important in cellular prooxidant states [48, 51].

3.4. Effect of experimental treatments on Kidney function

Changes in serum urea and creatinine have been used as important indices for evaluating the impact of chemicals on kidney function. Increasing of urea and

creatinine concentration in blood suggests the inability of kidney to excrete these waste products and consequently further suggest a decrease in glomerular filtration rate (GFR). The effect of defatted methanolic extract on the levels of serum urea and creatinine is shown in Table (4).

**Table 4**. Effect of experimental treatments on blood serum kidney function

| Experimental Treatments | Urea (mg/dl) | Creatinine (mg/dl) |
|---|---|---|
| -ve | $54\pm0.58^{b}$ | $0.53\pm0.01^{bc}$ |
| $E_{60}$ | $54.67\pm0.16^{b}$ | $0.55\pm0.01^{b}$ |
| $E_{120}$ | $54.33\pm0.08^{b}$ | $0.54\pm0.02^{bc}$ |
| $E_{240}$ | $54.12\pm0.08^{b}$ | $0.52\pm0.05^{bc}$ |
| +ve | $79.63\pm0.19^{a}$ | $1.39\pm0.11^{a}$ |
| $CCl_4+E_{60}$ | $30.85\pm0.38^{h}$ | $0.24\pm0.03^{e}$ |
| $CCl_4+E_{120}$ | $35.88\pm0.51^{g}$ | $0.34\pm0.01^{de}$ |
| $CCl_4+E_{240}$ | $37.99\pm0.28^{f}$ | $0.39\pm0.01^{cde}$ |
| $E_{60}+CCl_4$ | $43.55\pm0.26^{e}$ | $0.46\pm0.01^{bcd}$ |
| $E_{120}+CCl_4$ | $46.98\pm0.27^{d}$ | $0.5\pm0.06^{bc}$ |
| $E_{240}+CCl_4$ | $52.62\pm1.52^{c}$ | $0.52\pm0.03^{bc}$ |

Data are means (n=5) ± SE. Means followed by the same letter in each column are not significantly different according to the LSD test ($P\leq0.01$).

Orally administration of methanolic extract only showed no harmful or toxic effect on kidney function as evident from normal serum parameters i.e serum urea and creatinine contents compared with – ve control group. Carbon tetrachloride administration found to cause nephrotoxicity indicated by the increasing of urea and creatinine levels in the blood serum [48], furthermore a common manifestation on nephritic damage in acute renal failure is characterized by decline in GFR, which may have been induced by toxic chemicals including $CCl_4$ as showed in + ve control group[52].

In + ve control group rats treated with $CCl_4$, the levels of serum urea and creatinine were significantly higher than those recorded for - ve control group indicating the severity of kidney injury caused by $CCl_4$. Increasing levels of urea and creatinine in serum might occur due to $CCl_4$ induced physiological imbalance in liver and kidney [53, 54] attributed the increase in serum urea and creatinine levels to increase in renal disorders. Since the kidney has an affinity for $CCl_4$ and contains cytochrome $P_{450}$ predominantly in the cortex [55], so the mechanism of $CCl_4$ nephrotoxicity is very similar to that of the hepatotoxicity. The oral administration of the methanolic extract after ten days of $CCl_4$ treatment (hepato-curative groups) significantly decreased content of serum urea and creatinine as compared with the + ve control group. The content of serum urea and creatinine were lower in pretreatment groups with the defatted methanolic extract before $CCl_4$ administration (hepato-protective treatment groups) in comparison with those reported in the + ve control group. Whereas the groups treated with methanolic extract before $CCl_4$ administration (hepato-protective treatment groups) at the same doses increased contents of serum urea and creatinine when compared to hepato-curative treatment groups, but these levels were lower than those observed for – ve control group. It could be concluded that methanolic extract effectively relived kidney damage induced by $CCl_4$ whether they were orally administration before or after $CCl_4$ treatment.

3.5. Effect of experimental treatments on histological markers of hepatotoxicity

The effects of the defatted methanolic extract of *P.granatum L.var.nana* leaves on toxicity of the extract groups and intoxicated rats with $CCl_4$ for 6 weeks was evaluated by histopathological examination of the liver and kidney tissues.

3.5.1. Effect of experimental treatments on liver histological profile

Histopathological investigations result of liver tissues (Figure 1) showed significant correlation with the biochemical study results. The microscopic examination of the liver tissue in the -ve control group showed normal structure of the liver cells. The unit of liver tissue is the classic hepatic lobule which is hexagonal in outline; each hepatic lobule is formed of liver cells (hepatocytes). These hepatocytes are arranged in the form of liver cords or plates. These hepatic plates or cords radiate from the central vein and are formed of one or more rows of liver cells. The liver sections of rats received only the defatted methanolic extract with different doses showed that most hepatocytes had a normal structure in different zones and variable activation of Kuppfer cells.

The liver of rats in +ve control group which treated with $CCl_4$ only showed nuclear pyknosis in some hepatocytes and necrosis in other cells, hyperplasia of Kupffer cells and bile duct in association with connective tissue proliferation especially at portal areas, these were in agreement with previously reported data [56, 57].

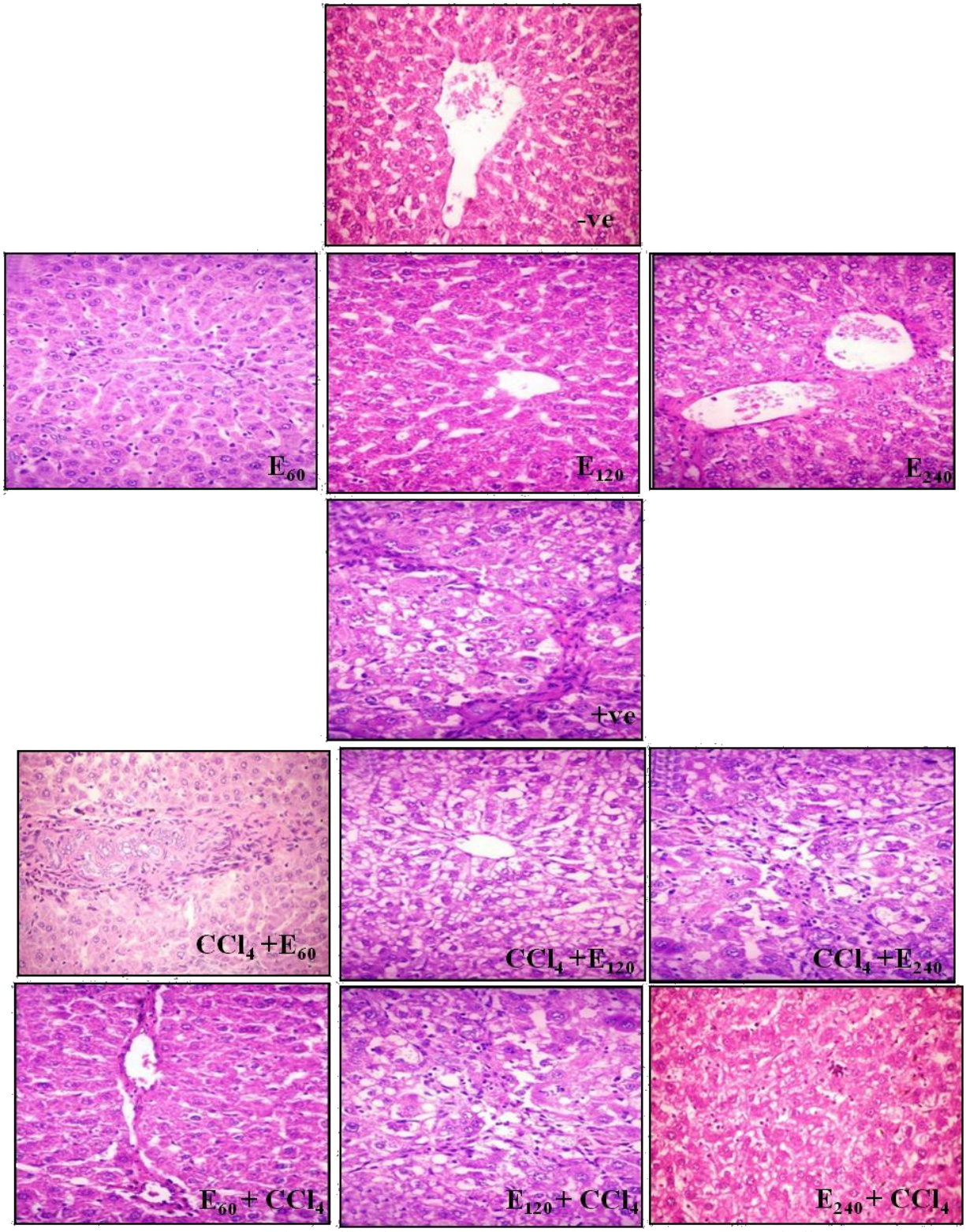


**Figure 1.** Photomicrograph for the effect of *Punica granatum* L *var. nana extract* on the liver tissue of different groups.

Hepato-curative groups, treated with the methanolic extract after $CCl_4$ treatment showed variable degree of steatosis, fibrosis, stellate cell proliferation and Kupffer cell activation in liver sections. These changes were decreased with increasing the dose.

Histopathological examination of liver sections obtained from hepato-protective treatment groups treated with the methanolic extract before $CCl_4$ treatment revealed maximum protective effect in the liver tissues against $CCl_4$ induced liver. These treatments showed a pronounced improvement in

hepatic architecture when compared with $CCl_4$ control group. The protective effect was increase with increasing the dose.

***3.5.2. Effect of experimental treatments on kidney histological profile***

Effect of experimental treatments on renal histological profile is shown in Figure (2). The cross section of the kidney from - ve control group showed normal architecture of glomeruli and renal tubules with intact nucleus.

The cross section of the kidney from the toxicity of the extract groups didn't show any histological variation from negative control once. The rats in +ve control group which received $CCl_4$ only, the microscopic screening of the samples revealed thickening of B owman"s capsule atrophy in most glomerular tufft, degenerated glomerulus and intertubelar connective tissue proliferation vacuolation and swelling of several renal epithelium which leads to narrowing of tubular lumen, examination of kidney sections from hepato-curative and hepato-protective rats revealed improvement dose-dependent in renal architecture when compared with the + ve control group.

## 4. Conclusion

In conclusion, the present study showed that, the administration of *Punica granatum L. var. nana*, methanolic extract has no signs of toxicity either for the liver or the kidney, which is confirmed through the biochemical analysis of blood serum and histopathological studies. Furthermore, the treatment of rats with carbon tetrachloride causes both hepatotoxicity and nephrotoxicity, due to the oxidative stress as resulted from the production of the free radicals. The treatment with the methanolic extract of *Punica granatum* L. *var nana* leaves attenuated the effect of carbon tetrachloride as hepatotoxin agent significantly, either through its protective or curative treatment. So, such natural extract can be used as a promising hepatoprotective and hepatocurative agent against carbon tetrachloride induced hepatic injury.

## 5. Conflicts of interest

There are no conflicts to declare.

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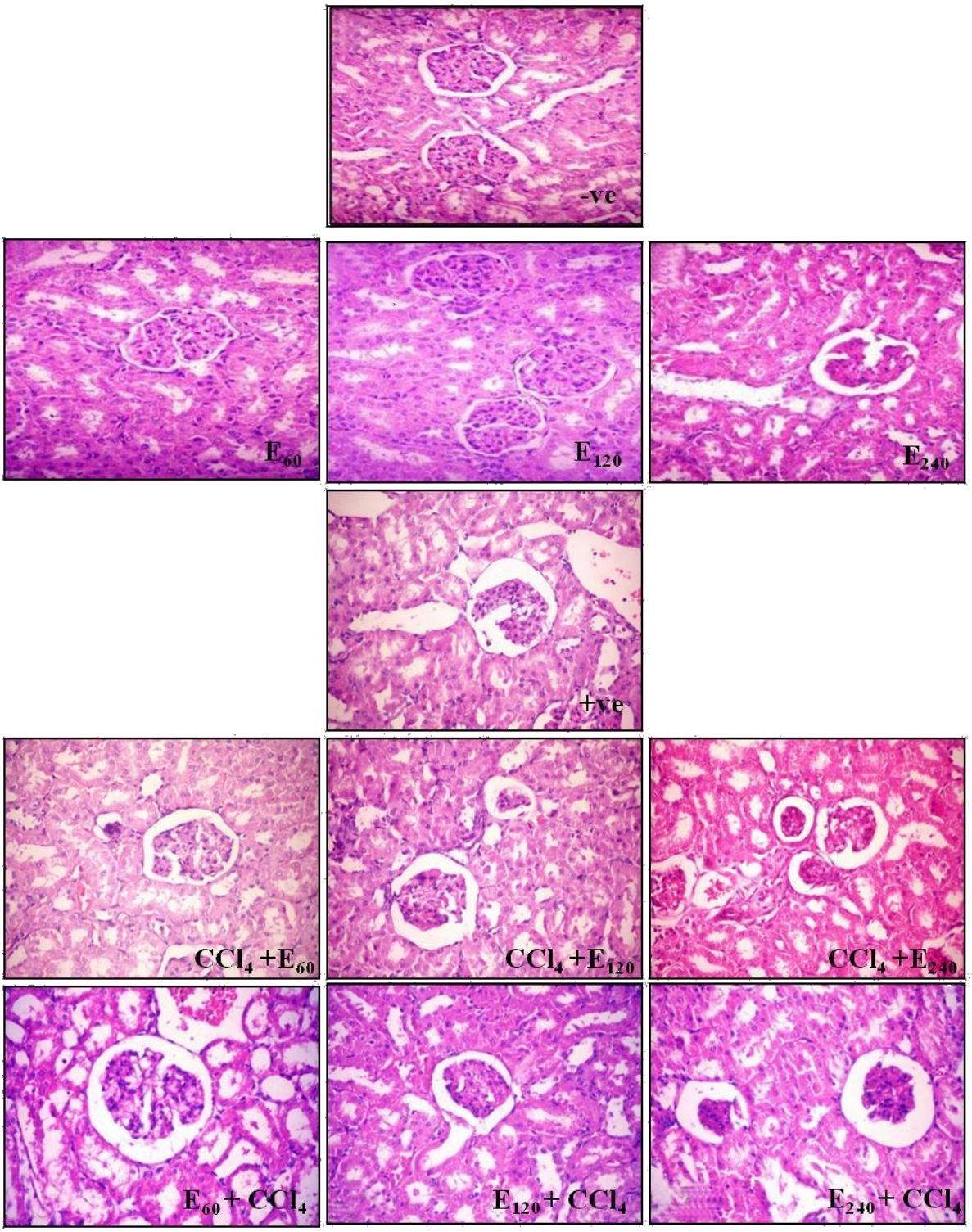


**Figure 2.** Photomicrograph for the effect of *Punica granatum* L *var. nana extract* on the kidney tissue of different groups.

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