Protective Effects of *Pinus halepensis* L. Essential Oil on Aspirin-induced Acute Liver and Kidney Damage in Female Wistar Albino Rats

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Abstract: Aromatic and medicinal plants are sources of natural antioxidants thanks to their secondary metabolites. Administration of *Pinus halepensis* L. (Pinaceae family) in previous studies was found to alleviate deleterious effects of aspirin-induced damage on liver and kidney. The present study, carried out on female rats, evaluates the effects of *P. halepensis* L. essential oil (EOP) on aspirin (A)-induced damage to liver and kidney. The animals used in this study were rats (n=28) divided into 4 groups of 7 each: (1) a control group (C); (2) a group given NaCl for 56 days then treated with (A) (600 mg/kg) for 4 days (A); (3) a group fed with (EOP) for 56 days then (A) for 4 days; and a group fed with only (EOP) for 56 days and given NaCl for 4 days. Estimations of biochemical parameters in blood were determined using kit methods (Spinreact). Lipid peroxidation levels (TBARS), superoxide dismutase (SOD) and catalase (CAT), glutathione peroxidase (GPx) activities were determined. Histopathological study was done by immersing pieces of both organs in a fixative solution followed by paraffin embedding and hematoxylin-eosin staining. Under our experimental conditions, Aspirin at dose 600 mg/kg body weight induced an increase of serum biochemical parameters as well as an oxidative stress in both organs. An increase occurred in TBARS by 108% and 55%, a decrease in SOD by 78% and 53%, CAT by 53% and 78%, and GPx by 78% and 51% in liver and kidney, respectively, compared to control. Administration of EOP given to rats enabled correction in these parameters. It could be concluded that the treatment with *P. halepensis* L. essential oil inhibited aspirin-induced liver and kidney damage.

Key words: biochemical estimation, oxidative stress, hepatotoxicity, histopathological study, renal toxicity

1 INTRODUCTION

Aspirin or acetylsalicylic acid, one of the widely used non-steroidal anti-inflammatory drugs, is among the most commonly consumed pharmaceutical products in the world\(^7\). It has been used as a drug for medicinal purposes such as treatment of inflammation and fever. It produces its therapeutic (anti-inflammatory and analgesic) effect and side effect (gastrointestinal ulcers) by inhibiting cyclooxygenase (COX) which is a key enzyme to catalyse the formation of prostaglandin\(^2\). However, acute overdose and long-term therapeutic administration of aspirin is very likely to lead to hepatotoxicity, renal cell cancer, gastrointestinal ulceration, nephrotoxicity\(^3,4\), and it causes death of the blood vessel tissues\(^5\).

Increasing attention has been paid to the protective effects of natural antioxidants on drug-induced toxicities especially when free radical generation is involved\(^6\). Aromatic and medicinal plants are the source of natural antioxidants by virtue of their main secondary metabolites such as polyphenol and essential oil\(^7\). Previous studies found that there are approximately 458 medicinal Taxa that have been used since ancient times in the most...
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popular treatments of diseases related to the gastrointestinal system, nervous system, cardiovascular system and dermatological conditions.

*P. halepensis* (Pinaceae family) is a tree species found all around the Mediterranean Basin. Its continental range extends from regions of North Africa (Morocco, Algeria, Tunisia, and Libya) and the Middle East (Syria, Lebanon, Jordan, Palestine, and Turkey) up to Southern Mediterranean regions in Europe (Eastern Greece, Croatia, Northern Italy, Eastern France and Eastern Spain). Previous photochemical studies concerned with this species focused on the phenolic compounds of the needles of the plant, the oil seeds, the organic compounds of stems and roots, the lipid portion of the pollen grain as well as the volatile compounds including essential oils obtained from needles.

*P. halepensis* has been used in traditional therapeutic practice such as Italian popular medicine as well as decoctions of leaves, buds, resin. Pinus spp young female cones are used to treat respiratory diseases, cough, colds and rheumatic pains. It has also been found that the resin and decoction of all pine trees have antiseptic, diuretic, rubefacient, vermifuge, antidiabetic and cicatrisant properties. The neutral lipids, glycolipids and phospholipids of seeds of *P. halepensis* have inhibitory effects on antiangiogenic diseases and are able to fight cancer. The seeds are also widely used, at least in Tunisia and similar cultural contexts, in the preparation of some antique dishes. Furthermore, they have beneficial properties such as their anti-inflammatory, antioxidiant and antineoplastic activities.

Oils of genus pine are used as fragrances in cosmetics, flavoring additives for food and beverages, scenting agents in a variety of household products, and intermediates in the synthesis of perfume chemicals. Previous studies found that the constituents of essential oil of cones of the Pinaceae family possess antioxidant analgesic and anti-HIV activities. Essential oil of *P. halepensis* has been reported to have various therapeutic properties, notably antibacterial, antifungal and herbicidal activities. It has been used in the treatment of leishmaniasis which is an infectious disease caused by different species of protozoan parasite of the genus *Leishmania* (*L.*). However, the therapeutic effect of essential oil of *P. halepensis* on aspirin-induced toxicity has not received attention in previous research. Therefore, the aim of the present study is to investigate the protective effect of essential oil of *P. halepensis* on the side effects of acute overdose of aspirin on liver and kidney of female rats by measuring some physiological parameters and determining some histopathological alterations.

2 EXPERIMENTAL PROCEDURES

2.1 Chemicals and reagents

Aspirin (A), Anhydrous sodium sulphate (Na2SO4), Butyl-hydroxytoluene (BHT); Hydrochloric acid (HCl), Hexane, Sodium chloride (NaCl), Tris-buffered saline (TBS), Thiobarbituric acid (TBA), Hydrogen peroxide (H2O2), Nitroblue tetrazolium (NBT), Phosphate buffer (PBS), Ethanol, Toluene, Paraffin, Hematoxylin and eosin were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO).

2.2 Plant material

*P. halepensis* needles were collected during October and November 2013 from the region of Sidi Aich, a suburb of Gafsa in the south west of Tunisia (Longitude: 8.8E Latitude: 34.683N Altitude: 522 m, rainfall: 150 mm/year). This plant was identified by botanists in the Faculty of Sciences of Gafsa (FSG). The collected vegetative parts were air-dried at room temperature of 25°C for 14 days in shaded and ventilated space.

2.3 Extraction and isolation of essential oil

The essential oil sample was extracted from 50 g of dry *P. halepensis* needles by hydrodistillation after grounding and immersion in 500 mL of distilled water. The extraction was carried out for 3 h. Hexane was used to recover the oil from the extraction apparatus. The organic phase was dried using anhydrous sodium sulphate until all water was evaporated and the essential oil (EOP) extract was kept at 4°C in a dark glass bottle until the accomplishment of phytochemical analyses. The yield was reported per dry material and oil results given in w/w.

2.4 Experimental protocol

2.4.1 Animals

Twenty-eight adult female Wistar rats weighing 150-200 g were bred in an animal house in the Faculty of Sciences of Sfax, Tunisia, which was approved by the Tunisian Ministry of Higher Education. The conditions of the animal house meet the required international standards. The animals were allowed to adopt to their new environment (a breeding farm) for two weeks prior to experiment and kept under photoperiods (14 h light/10 h dark) at 21-22°C. Rats were allowed free access to food pellets obtained from Société Industrielle de Conditionnement Optimisé (S.I.C.O), Sfax, Tunisia [composition: corn, Luzern, soybean, vitaminized mineral compound, dicalcium phosphate, calcium carbonate, salt-free methionine, trace elements (copper, iron, zinc, manganese, cobalt, selenium and iodine)] and water *ad libitum*. The animals were treated in accordance with the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes as well as the European Convention for the Protection of Ver-

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tebrate Animals used for Experimental and Other Scientific Purposes (Council of Europe No 123, Strasbourg, 1985).

2.4.2 Experimental design

After two weeks of acclimatization, the animals were divided into four groups of seven rats each. (C) was the control group, administered orally byavage 0.9% NaCl everyday for 60 days. (EOP) group consisted of rats orally given essential oil of *P. halepensis* (about 1.0 % w/w with dose 1 ml/kg) for 56 days, and then given NaCl for 4 days. (A) group consisted of rats treated with aspirin which was suspended in normal saline and administered orally by gastric intubation in acute dose 600 mg/kg was suspended in normal saline and administered orally by gastric intubation in acute dose 600 mg/kg (with DL50 = 5.3705 g/kg for rats) \(^{31}\); it was administered thricely a day each dose at an interval of 4 h for 4 successive days. This high dose has been used in studies investigating acute effects of aspirin in rats and mice. Such high-dose therapy is being used to treat health problems such as rheumatoid arthritis (KD, 1984) in Merchant & Modi\(^{32}\). (EOP + A) group consisted of rats administered orally by gastric intubation with essential oil of *P. halepensis* for 56 days, and then given (A) for 4 days. At the end of the experimental period, rats in each group were rapidly sacrificed by decapitation in order to minimize the handling stress. Blood was collected from all the groups by puncturing the retro-orbital plexus and allowed to clot at room temperature, and serum was separated by centrifugation at 1500 \(\times g\) for 15 min and 4°C. Liver and kidney were excised, cleaned of fat, weighed and stored at −80°C until use.

2.4.3 Determination of antioxidant enzyme activities

Rat organs (1 g), namely liver and kidney, were cut into small pieces, immersed in 2 mL ice-cold Tris-buffered saline (TBS, pH = 7.4) and centrifuged (5000 \(\times g\), 30 min, 4°C). Supernatants were collected and used in order to determine:

1. MDA, using the thiobarbituric acid assay\(^{30}\). For this assay, 125 \(\mu\)L of supernatant (S1) were mixed with 175 \(\mu\)L of 20% trichloroacetic acid containing 1% butyl-hydroxytoluene and centrifuged (1000 \(\times g\), 10 min, 4°C). Then, 200 \(\mu\)L of supernatant (S2) was mixed with 40 \(\mu\)L of HCl (0.6 M) and 160 \(\mu\)L of thiobarbituric acid (0.72 mM), and the mixture was heated at 80°C for 10 min. Absorbance was measured at 530 nm. The amount of TBARS was calculated using an extinction coefficient of 156 mM\(^{-1}\) cm\(^{-1}\) and expressed as nmol/mg protein.

2. Glutathione peroxidase (GPx) according to the method described by Flohe and Gunzler\(^{40}\). One unit of GPx was defined as oxidation by \(H_2O_2\) of 1 \(\mu\)mol of reduced glutathione peroxoide per min at a pH of 7 and a temperature of 25°C.

3. The total superoxide-dismutase (SOD) activity was determined by measuring its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT) using the method of Misra and Fridovich\(^{35}\). One unit of SOD represents the amount inhibiting the photoreduction of NBT by 50%. The activity was expressed as units/mg protein, at 25°C.

4. Catalase (CAT) using Aebi’s method\(^{39}\), the reaction mixture (1 mL) contained 100 mM phosphate buffer (pH = 7), 100 mM \(H_2O_2\), and 20 \(\mu\)L (approximately 1-1.5 mg of protein) of liver. \(H_2O_2\) decomposition was determined at 25°C by measuring the decrease in absorbance at 240 nm for 1 min. The enzyme activity was calculated using an extinction coefficient of 0.043 mM\(^{-1}\) cm\(^{-1}\) and expressed in international units (I.U.). i.e. in \(\mu\)moles \(H_2O_2\) destroyed/min/mg protein.

2.4.4 Serum marker assays

Serum glucose levels, total cholesterol, lactate dehydrogenase (LDH) activities, alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT), creatinine, urea and proteins were determined using kit methods (Spinreact).

2.4.5 Histopathological study of the liver and kidney

In order to carry out a histological study, parts of the experimental rats’ liver and kidney were immersed for 48 h in a fixative solution (solution 4% formaldehyde, in phosphate buffer, pH = 7.6), dehydrated in ascending graded series of ethanol, cleaned with toluene, immersed in paraffin, and colored with hematoxylin and eosin. The tissues were prepared then observed and examined under a light microscope.

2.5 Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). All the analyses were carried out with GraphPad Prism 6.0 for Windows (GraphPad Software, San Diego, CA). Significant differences between treatment effects were determined by one-way ANOVA, followed by Tukey’s post-hoc test for multiple comparisons with statistical significance of \(p<0.05\).

3 RESULTS

3.1 Yield of extraction

The essential oil obtained from the needles of *P. halepensis* had a strong odor and the yield of hydrodistillation was 0.18% (w/w) in relation to the dry weight of the plant.

3.2 Weight of body, liver and kidney

In this study, as shown in Table 1, the weight of body increased in all groups [C, (A), EOP, (EOP + A)] after 56 days before being treated with aspirin compared to the initial day when their weights had been 164, 13 \(\pm\) 19.34 g, 162.30 \(\pm\) 22.21 g, 150.28 \(\pm\) 19.9 g and 150.97 \(\pm\) 16.24 g, respectively. But, no statistical differences between the groups were observed. After treatment with aspirin for 4 days, in the group (A), the body weight was significantly decreased (144.03 \(\pm\) 7.56 g). Then, the liver weight de-
group decreased all these biomarkers in P. halepensis administration of essential oil of Aspirin 202.03 ± 22.96 144.03 ± 7.56 *** 194.08 ± 16.07## 178.82 ± 13.92## respectively. All values are expressed as mean ± SEM, n = 7 for each treatment group. **p < 0.01, ***p < 0.001 significantly different from C group; #p < 0.05, ##p < 0.01, ###p < 0.001 significantly different from A group.

### Liver biochemical variables

Table 3 Effect of essential oil of P. halepensis on the serum creatinine, protein, urea concentrations of kidney in rats with aspirin-induced nephrotoxicity.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>A</th>
<th>EOP</th>
<th>EOP + A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (μmol/L)</td>
<td>47.57 ± 3.06</td>
<td>70.75 ± 13.25***</td>
<td>55 ± 3.62**</td>
<td>43.71 ± 1.48##</td>
</tr>
<tr>
<td>Protein (g/L)</td>
<td>50.42 ± 7.3</td>
<td>88.57 ± 6.02***</td>
<td>55.71 ± 2.92**</td>
<td>43.85 ± 2.60##</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>8.12 ± 2.7</td>
<td>14.31 ± 2.5 ***</td>
<td>8.91 ± 2.17##</td>
<td>5.57 ± 0.13##</td>
</tr>
</tbody>
</table>

A: aspirin
EOP: essential oil of P. halepensis

All values are expressed as mean ± standard deviation (SD), n = 7 for each treatment group. ***p < 0.001 significantly different from C group; ##p < 0.01, ###p < 0.001 significantly different from A group.

3.3 Hepatic biochemical variables

Table 2 shows that serum glucose, cholesterol, ASAT, ALAT and LDH activities in (A)-treated rats were significantly higher (11.66 ± 0.57 mmol/L, 2.50 ± 0.15 mmol/L, 390.85 ± 63.02 U/L, 119.14 ± 22.31 U/L and 1581.71 ± 54.27 U/L, respectively) than those in the control group (C). Administration of essential oil of P. halepensis in (EOP + A) group decreased all these biomarkers (7.18 ± 0.37 mmol/L, 1.42 ± 0.33 mmol/L, 171.142 ± 81.92 U/L, 65.714 ± 24.70 U/L and 1107.28 ± 141.23 U/L, respectively) compared to the Aspirin group.

3.4 Renal biochemical variables

As shown in Table 3, serum creatinine, protein and urea concentration significantly increased in (A)-treated rats (70.75 ± 13.25 μmol/L, 88.57 ± 6.02 g/L and 14.31 ± 2.5 mmol/L, respectively) compared to control (C). Compared with the (A)/group, administration of P. halepensis essential oil in (EOP + A) groups resulted in a significant decrease (43.71 ± 1.48, 43.85 ± 2.60 g/L and 5.57 ± 0.13 mmol/L, respectively) in all the above mentioned biomarkers compared to (A) group.
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3.5 Lipid peroxidation Levels in liver and kidney

Figure 1 shows that TBARS level, an index of lipid peroxidation, in both liver and kidney was increased in the (A)-treated rats (by 108% and 55.7%, respectively) when compared to control. There was a significant decrease of TBARS level in the (EOP + A) group (by −67.01%, −47.26%, respectively) compared to the (A) group.

3.6 Antioxidant enzymes in liver and kidney

Activities of antioxidant enzymes SOD, CAT and GPx were found to be significantly reduced in liver and kidney of (A)-treated rats (by −78.77% and −53.84%, respectively) for SOD; (−53.58% and −78.01%, respectively) for CAT and (−78.92% and −51.51%, respectively) for GPx compared to control (C). These changes were corrected in animals treated with essential oil of P. halepensis (Fig. 2).

3.7 Histological examination

3.7.1 Histology of hepatic tissue

Based on light microscopic examinations, the architecture of liver of control animals was characterized by normal central vein and radiating cords of polyhedral-shaped hepatocytes whose cytoplasm was granulated. Histological changes appeared in the (A)-treated group, such as degenerations of hepatocytes, dilation of sinusoids, increased leukocyte infiltration and congestion of the central portal vein compared with the control group (C). The (EOP + A) group showed repairing of the liver structure (normal appearance of central vein, sinusoids and hepatocytes) compared to control group (Fig. 3).

3.7.2 Histology of kidney tissue

The histological outline of the kidney section of the rats in the (A)-treated group underwent significant degenerative changes compared with the control rats. The histological section in the control and EOP-treated rat kidneys revealed normal glomerular as well as tubule interstitial cells. However, the kidneys of A-treated animals showed histopathological damage due to a significant varying glomerular and tubular degenerations (i.e. atrophy of the glomerular and dilatation of urinary space). The (EOP + A) group showed renal repair (Fig. 4).

4 DISCUSSION

This study was undertaken to investigate the protective effects of essential oil of P. halepensis on the aspirin-induced hepatotoxicity and renal toxicity evidenced by biochemical measurement of oxidative damage and histopathological changes.

Actually, our study demonstrated that acute aspirin overdose induced increased levels of glucose, total cholesterol, LDH, AST and ALT in blood. The elevated levels of the hepatocellular enzymes were signs of aspirin-induced tissue damage. Then, the alteration of hepatic biochemical biomarkers by aspirin could be explained by an increase in plasma membrane permeability or cellular necrosis causing enzymes to leak into the blood stream. These findings are in agreement with those reported by Choi et al. who showed that aspirin induced an acute elevation of serum AST and ALT serum in rats. Moreover, aspirin treatment caused toxicity in kidney indicated by an elevation in serum creatinine, urea, and protein concentrations. These effects could be explained by the renal dysfunction associated with glomerular and tubular degenerations caused by
Fig. 2  Activities of superoxide dismutase (SOD) and catalase (CAT) and glutathione peroxidase (GPx) in liver and kidney after 2 months of treatment in controls (C), (A) aspirin treated rats, (EOP + A) aspirin-treated rats previously supplemented with essential oil of P. halepensis, (EOP) rats supplemented with essential oil of P. halepensis. Values are the mean of 7 measurements ± SD. ***p ≤ 0.001 compared to control group (C); *p ≤ 0.05 compared to treated group (A), **p ≤ 0.01 compared to treated group (A), ***p ≤ 0.001 compared to treated group (A).
aspirin poisoning or salicylism. In fact, salicylates are primarily excreted by the kidneys. Therefore, renal insufficiency or failure contributed to further accumulation of salicylates which uncoupled oxidative phosphorylation at a cellular level, producing an increased metabolic rate. In addition, aspirin induced an oxidative stress in both liver and kidney revealed by an increase in lipid peroxidation level associated with a decrease in enzymatic antioxidants as well as SOD, CAT and GPx. Furthermore, the increase of lipid peroxidation, which affected the physicochemical properties, fluidity as well as integrity of cell membrane, led to cell damage and necrosis. The histological findings reported in the present study confirmed the biochemical results. Through light microscopic examinations, it was found that aspirin-treated rats showed severe histopathological changes in both liver and kidney such as atrophy and damage of the central portal vein in liver and significant glomerular and tubular degenerations varying in kidney. The obtained results were similar to those reported by Bhattacharyya et al. indicating that lipid peroxidation increased while glutathione content as well as SOD, CAT, and GPx activities decreased in liver and kidney due to Aspirin.

The administration of the essential oil of Pinus halepensis attenuated aspirin-induced hepatotoxicity and nephrotoxicity. Indeed, after administration of EOP, the levels of glucose, cholesterol, ASAT, ALAT, LDH, creatinine, urea and protein were significantly decreased compared to aspirin-treated group. The reduction in the levels of these enzymes towards the normal value is an indication of a regeneration process.

In addition, the treatment with essential oil exerted a strong protective effect on aspirin-induced oxidative stress, as revealed by the decreased level of lipid peroxidation (TBARS), and enhanced the enzymatic antioxidant

Fig. 3 Microscopic observations of rat liver sections (H&E, C, EOP, A and EOP + A: 400 x). (C) control group showing normal parenchymal architecture, (EOP) P. halepensis treated group showing normal structure similar to control. (A) Aspirin-treated group showing diffuse central and destruction of the lobular architecture and (EOP + A) showing repairing of structure of liver. 1: degenerations of hepatocytes, 2: dilation of sinusoids, 3: increased leukocyte infiltration and congestion of the central portal vein. CV: Central vein, S: Sinusoid, H: Hepatocyte.
defense system due to increased levels of SOD, CAT and GPx activities. These parameters play an important role in maintaining the redox homeostasis under normal physiological conditions\(^{41}\). The essential oil treatment prevented the decline of these parameters previously modified by aspirin administration.

These positive changes in biochemical indices, TBARS levels, antioxidant enzyme activity observed the EOP- and aspirin-administered group are associated with the decrease in the level of free radicals due to essential oil supplementation. The tendency of these enzymes to return to nearly normal state in EOP-administered group was a clear indication of antihapatotoxicity and antinephrotoxicity. These results are in agreement with results found by Rocha \textit{et al.}\(^{42}\) indicating that terpenoids, which are major components of essential oil, lowered malondialdehyde levels and improved SOD activity in gastric mucosa. Recent studies also demonstrated that the reduction in oxidative stress and lipid peroxidation observed in the EOP-treated animals can be attributed to the vital role of essential oils as effective antioxidants\(^{41, 43-46}\). In this regard, the terpenoids are responsible for the antioxidant action of the essential oils used in this study\(^{47, 48}\). Figure 4 shows the photomicrographs of rat kidney (A) control group showing normal parenchymal architecture; (B) P. halepensis treated group showing normal structure similar to control. (A) Aspirin-treated group showing degeneration changes, (EOP + A) showing repairing of architecture of the kidney. 1: tubular degenerations, 2: Atrophy of the glomerular and dilatation of urinary space. G: Glomeruli, PT: Proximal Tubule, DT: Distal Tubule.
These molecules are the source of antioxidant capacity of the plant by scavenging free radicals. Indeed, Calleja et al.\textsuperscript{5} found that Caryophyllene, which is the common constituent of the essential oil of numerous plants and medicinal herbs, has shown high scavenging activities against hydroxyl radical and superoxide anion. Then, α-pinene is a natural compound present in the oils of many species of conifers, particularly pine trees.\textsuperscript{51} It has been reported that α-pinene could be used for the therapy of melanoma due to its great potential to induce apoptosis on cancer cells.\textsuperscript{52}

Histopathological liver and kidney sections of the rats treated with the essential oil of \textit{P. halepensis} revealed that the normal cellular architecture was retained compared with the control group, thus confirming the significant protective effect of the essential oil of \textit{P. halepensis}.

In fact, the chemical composition of essential oil of \textit{P. halepensis} varied in several findings such as those of Hamrounia et al.\textsuperscript{25} who found that the main compounds in the oil isolated by hydrodistillation from the needles grown in Tunisia were (Z)-caryophyllene (16.16-28.9\%), β-myrcene (8.5-22.9\%), α-pinene (11.7-13.14\%), β-pinene (3.13-11.8\%), bicyclogermacrene (5.2-12.37\%), α-terpinolene (8.11-11.01\%), α-humulene (2.85-5.2\%). The study of Fekih et al.\textsuperscript{24} found that the major compounds of \textit{P. halepensis} essential oil from Algeria were: myrcene (15.2\%-32.0\%), α-pinene (12.2\%-24.5\%), E-β-caryophyllene (7.0\%-17.1\%), terpinolene (1.8\%-13.3\%), 2-phenyl ethyl isovalerate (4.8\%-10.9\%), terpinene-4-ol (1.0\%-8.2\%) and sabinene (1.5\%-6.3\%). This variability is an adaptive process to the period of collection of the studied parts of the plant, particular ecologic conditions, geographical regions, climate conditions, and altitude, state of plant (fresh or dry) and method of extraction of the essential oil. Also, this variability could affect the yield of extraction of the essential oil. In our study, the results of extraction showed that the yield of hydrodistillation was 0.18\%, which is in agreement with the study of Tumen et al.\textsuperscript{55} who showed that the yield of the essential oil of \textit{P. halepensis} grown in Turkey was 0.20\%. However, this yield was different in other regions, as in three different locations at Sidi Feradj, Djelfa and Saida in Algeria, whose oils hydrodistillation yields were 0.5\%, 0.8\% and 0.9\%, respectively. Moreover, the terpenic compounds of \textit{P. halepensis} have bactericidal, fungicidal, insecticidal, antitumorogenic, pesticidal, antioxidant, analgesic and sedative effects.\textsuperscript{57} Therefore, our results showed that EOP exhibited an excellent protective effect and may be considered as a useful source of cellular defense agent in liver and kidney tissues against aspirin.

5 CONCLUSION

Based on the obtained results, this study demonstrated that aspirin induced histomorphological damage in liver and kidney by increasing serum glucose, cholesterol, ASAT, ALAT and LDH, and in kidney by increasing serum creatine, protein and urea concentration. Moreover, the essential oil of \textit{P. halepensis} offered significant protection against the toxicity of aspirin via reverting these parameters to normal values. Therefore, this finding suggests the potential of the essential oil of \textit{P. halepensis} as a novel therapeutically useful hepatoprotective and nephroprotective agent.

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