Evaluation of Gamma Irradiation Effect on the Oxidative Stress Factors in Septic Rats Treated With Iranian Plant Essential Oils

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ABSTRACT

Cuminum cyminum L. (CM), Zataria multiflora Boiss. (ZM) and Mentha piperita L. (MP) are traditional medicinal plants with various pharmacological properties. This study was designed to assess the role of gamma irradiation—a modern decontamination method—in hepatoprotective effects of their essential oil (E.O.) in septic rats induced by experimental cecal ligation and puncture (CLP) model. The rats were divided into 20 groups; sham-operated (SOP); CLP; CLP + CM, ZM and MP (E.O.) (100 & 200 mg/kg b.w) and CLP + gamma irradiated (10 and 25 kGy) E.O, (100 & 200 mg/kg b.w) as treatment groups. All E.O., were injected i.p immediately after sepsis induction. 24 hour after CLP, the rats were sacrificed and the liver tissue was examined considering lipid peroxidation (LP), glutathione (GSH) and myeloperoxidase (MPO) activity. The results indicated that CLP operation caused significant (P<0.05) increase in the LP and MPO levels concomitant with decreased GSH level. Administration of the E.O, (100 and 200 mg/kg b.w) extracted from non irradiated plants as well as the irradiated (10 and 25 kGy) plant E.O, could significantly (P<0.05) modulate the levels of LP, MPO and GSH. It can be concluded that all E.O, even after irradiation exposure could modulate the oxidative injury parameters related to liver damages in CLP rat model. In conclusion, the plant irradiation didn’t have any adverse effects on the hepatoprotective activities of the extracted oils.

Key words: Essential oils, Oxidative stress, Antioxidant, CLP, Gamma irradiation

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INTRODUCTION

Sepsis is a potentially life-threatening complication of a systemic infection due to the microorganism or its toxin on the blood. Sepsis is one of the most important public health concerns and is the leading cause of morbidity and mortality in intensive care unit (ICU)\(^1\). The oxygen-free radicals produced during sepsis can cause oxidative stress leading to multi-organ failure via several mechanisms including the peroxidation of membrane lipids, increasing the myeloperoxidase (MPO) activity and decreasing the glutathione (GSH) levels\(^2,6\).

In regards to the increasing occurrence of resistance and side effects of antibiotics and other synthetic drugs in sepsis treatment, medicinal plants with determined antibacterial and antioxidant activities could be appropriate alternative treatments. *Zataria multiflora* Boiss. (ZM) (Family Lamiaceae) known as Avishan-e-Shirazi geographically grow in Iran, Pakistan and Afghanistan\(^7\). It is used in Iranian traditional medicinal such as antiseptic, antispasmodic and anesthetic agent\(^8\). Its essential oils (E.O.) and extracts exhibited the antibacterial activity against the different positive and negative gram bacteria as well as strong antioxidant and protective activities\(^9-12\). *Cuminum cyminum* L. (CM) as an aromatic plant in Apiaceae family cultivated in Asia, Africa and Europe\(^13\). In folk medicine, it is widely used as carminative, antispasmodic and appetite stimulant agents\(^14\). The antioxidants, chemopreventive and antibacterial activities of MP oils have been previously confirmed\(^15-19\). *Mentha piperita* L. (MP) with common name peppermint belonging to Lamiaceae family originated from Mediterranean region and also cultivate all over the world. The plant are commonly used in folk medicine for carminative, antispasmodic, antiemetic, diaphoretic, analgesic, anti-inflammatory, stimulant, emmenagogue and anticatharrhal applications. Its essential oils are generally used externally for antipruritic, astringent, rubefacient, antiseptic and antimicrobial purposes, and for treating neuralgia, myalgia, headaches and migraines\(^19-28\).

In addition, gamma-irradiation is the process of exposing products to radiation to delay ripening, inhibit sprouting and extend shelf-life by reducing spoilage organisms, helping to meet quarantine standards for export to foreign markets\(^29\). The gamma irradiation method is allowed for the decontamination of dried herbs, spices and vegetable seasonings with a maximum overall average absorbed dose of 10 kGy but this limitation has been raised by the Food and Drug Administration (FDA) to doses of 30 kGy for these products\(^25,26\).

One of the most problems using gamma irradiation is the adverse effects on the biological activities of the medicinal plants\(^30,31\). So, the investigation of the irradiation effects on the biological and therapeutical properties of the medicinal plants cannot be ignored. Most recent studies were focused on the possible changes in the biochemical properties of medicinal plant during irradiation. Our recent studies also confirmed that the antioxidant, antibacterial and antiseptic activities of the essential oils and extracts obtained from the different medicinal plants such as *Carum carvi*, ZM, MP and CM didn’t change due to gamma irradiation up to 25 kGy\(^32-38\). In followings, the present study aimed to evaluate the effect of gamma irradiation at two doses (10 and 25 kGy) on the hepatoprotective abilities of E.O, derived from three Iranian endemic medicinal plants *e.g.* ZM aerial parts, MP leaves and CM seeds in cecal ligation and puncture CLP rat model.
MATERIALS AND METHODS

Plant irradiation and oil extraction
Zataria multiflora Boiss. aerial parts, Mentha piperita L. leaves and Cuminum cyminum L. seeds collected from Shiraz, Isfahan and Kerman cities of Iran, respectively. They were characterized by an expert botanist Mr. Bagherzadeh (Research in Forests and Rangelands institute, Isfahan, Iran). The plants were packed in 3 batches (50g) in heat-sealed polyethylene pouches and passed by a Co^{60} source for irradiation at two doses (10–maximum approved dose for decontamination of food supply- and 25 kGy- sterile dose-) using a high dose rate research irradiator (Co^{60}, Gamma cell 220 (A.E.C.L^); Canada) calibrated with Frick standard dosimeter which is installed in Radiation Application Research School of Atomic Energy Organization of Iran. The dose was controlled by the exposure time of each container to the source. The temperature and dose rate for all the samples were 22–23 °C and 0.37Gy/s, respectively. The dose range within the samples was ± 20% of the actual dose. The control and irradiated samples were stored in plastic containers at room temperature (28–30 °C) under the same conditions

In following, the CM, ZM and MP samples before and after gamma irradiation were subjected to oil extraction using a Clevenger-type apparatus. The extraction was carried out for 2 h and the oils were stored in dark glass bottles in a freezer until further use.

Animal treatments and experimental design
A total of 100 male Wistar rats (260-280 g) were used in this study. All rats were obtained from Qom Azad University’s experimental and research animal laboratory. Rats were maintained at 23°C with access to standards rat food and tap water ad libitum.

The animal studies had been approved by the Medical Ethics Committee of Tarbiat Modares University based on the World Medical Association Declaration of Helsinki. The animals were acclimated to the laboratory environment for at least 1 week prior to surgical manipulation. The rats were randomly divided in 20 groups (n=5); In Sham-operated group (Group 1) (SOP), rats undergone laparotomy and received DMSO as vehicle. In CLP group (Group 2), animals received vehicle alone after CLP operation. In CLP treated groups (Groups 3-8), E.Os of all three non irradiated plants (100 and 200 mg/kg b.w) diluted in DMSO, were injected intraperitoneally (i.p) immediately after CLP operation. In CLP treated groups (Groups, 9-20), irradiated (10 and 25 kGy) E.Os of all three plants (100 and 200 mg/kg b.w). Then, 24 h after CLP surgery, the liver tissues were removed, washed and processed for biochemical analysis. In addition, blood samples were collected for CFU count.

CLP model
Polymicrobial sepsis in rats was induced by CLP according to the method of Hubbard. Briefly, the rats were anesthetized by injection (i.p) of ketamine (90 mg/kg b.w) and xylazine (10 mg/kg b.w) mixture. A small mid abdominal incision (2–3 cm) was made and the cecum was exposed. A distended portion of the cecum just distal to the ileocecal valve was isolated, filled with fecal content, and tied with a 3-O silk suture in a manner not to disrupt bowel continuity. The ligated portion of the cecum was punctured twice with a 20-gauge needle. The cecum was then replaced in its original position within the abdomen and the abdomen was then closed with a 3-O suture in two layers. Then, the animals were allowed to recover. In the sham-operated rat, the cecum was exposed, manipulated and returned to the peritoneal cavity without being
punctured. After surgery, normal saline (3 ml/100 g b.w) was given subcutaneously to all rats to prevent dehydration.

**Colony forming units (CFUs) determination**

Blood was obtained under ether anesthesia from the heart puncture and immediately serially diluted 10-fold in sterilized salt solution. The diluted blood was transferred rapidly to BHI plates and placed into incubator for 48 h at 37°C. The number of colonies was counted using a colony counter (Sana, Iran).

**Biochemical analysis**

*Measurement of myeloperoxidase (MPO) activity in liver*

Tissue MPO activity was measured, with minor modification, by according to the procedure of Hillegas. Weighed tissue samples were homogenized in 50mM-potassium phosphate buffer (pH 6.0) and centrifuged at 41400 g for 10 min. After discarding the supernatant, the pellets were suspended in a solution containing 0.5% hexadecyl-trimethyl-ammonium bromide dissolved in 1 ml potassium phosphate buffer (pH 6.0). In following to three freeze-thaw cycles, the samples were centrifuged at 41400 g for 10 min. MPO activity was determined by adding 150 μL of the supernatant to 1150 mL of 10 mmol/L phosphate buffer (pH 6.0) and 1 mL of 1.5 mmol/L α-dianisidine hydrochloride containing hydrogen peroxide. The absorbance at 460 nm was measured for 1 min and the rate of change in the absorbance was used to calculate the activities of MPO. MPO activity was expressed as the amount of enzyme that reduces 1µmol peroxide/min.

*Measurement of TBARSs as lipid peroxidation products in liver*

The concentration of TBARS was measured spectrophotometrically using TBA reagent based on the procedure described by Buege and Aust. A weighed portion of the liver was homogenized in phosphate buffer (100 mM, pH 7.0) and used to measure the level of thiobarbituric acid reactive substances (TBARS) as indices for lipid peroxidation. The homogenate (1 mL) was added to 2 ml TBA reagent and was shaken for 15 min. The mixture was incubated for 15 min. After cooling, the mixture was centrifuged at 3000 g. The absorbance at 535 nm was measured. The results were expressed as nanomole of TBARS per gram of tissue (nmol TBARS/g liver).

*Measurement of GSH*

GSH level was estimated in liver homogenate according to the procedure of Sedlak and Lindsay. A weighed portion of the liver was homogenized in EDTA 0.02 M. The 5 ml of homogenate was immediately precipitated with 1 ml of 50% trichloroacetic acid and 4 ml distilled water; the precipitate was removed after centrifugation at 3000 g for 15 min. To determine the GSH level, the 2 ml of supernatant was mixed with 4ml Tris-buffer (0.4M), containing EDTA (0.2M) and 0.1ml 5,5-dithiobis (2-nitrobenzoic acid) (0.01 M). Absorbance was measured at 412 nm using a spectrophotometer. The results of the GSH levels in the liver were expressed as micromoles per milligram of tissue (µmol/g liver).

**Statistical analysis**

Data are presented as means ± Standard Error (SE). The results were subjected to one-way ANOVA followed by Tukey’s HSD (Honestly Significant Differences) using SPSS 22.0 software. The significance was considered as \( P<0.05 \).
RESULTS

CFU determinations in septic rats treated with CM, ZM and MP E.O, before and after gamma irradiation:
The blood bacterial load was determined by CFU count at 24 h after CLP, were shown in Table 1. CFU was significantly higher (in the blood of the CLP group in comparison to laparotomy group ($P<0.05$). Treatments of rats with non irradiated CM E.O, could significantly ($P<0.05$) decrease the bacterial load at 100 mg/kg b.w and 200 mg/kg b.w. Also, the administration of non -irradiated ZM E.O, could surprisingly diminish the CFU at 100 mg/kg b.w and 200 mg/kg b.w compared to the CLP group ($P<0.05$). Indeed, the bacterial load considerably ($P<0.05$) declined in the groups treated with 100 and 200 mg/kg b.w of MP E.O, in compared to the CLP groups. Based on our statistical analysis, there is no significant difference between 100 and 200 mg/kg b.w doses on the reduction of CFU ($P>0.05$).

Table No.1: Blood bacterial number (CFU/ml) in sepsis rats treated with Z. multiflora Boiss. (ZM), Cuminum cyminum L. (CM) and Mentha piperita L. (MP) E.O, before and after gamma irradiation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bacterial counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laparotomy (DMSO)</td>
<td>0</td>
</tr>
<tr>
<td>CLP (DMSO)</td>
<td>22172 ± 850</td>
</tr>
<tr>
<td>CLP + non-irradiated E.Os (100 mg/kg b.w)</td>
<td>4820 ± 37</td>
</tr>
<tr>
<td>CLP + Irradiated E.Os (10 kGy) (100 mg/kg b.w)</td>
<td>6240 ± 30</td>
</tr>
<tr>
<td>CLP + Irradiated E.Os (25 kGy) (100 mg/kg b.w)</td>
<td>4985 ± 20</td>
</tr>
<tr>
<td>CLP + non-irradiated E.Os (200 mg/kg b.w)</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>CLP + Irradiated E.Os (10 kGy) (200 mg/kg b.w)</td>
<td>28 ± 2.2</td>
</tr>
<tr>
<td>CLP + Irradiated E.Os (25 kGy) (200 mg/kg b.w)</td>
<td>40 ± 3</td>
</tr>
</tbody>
</table>

In Sham- operated group (SOP), rats were undergone laparotomy and received DMSO as vehicle; In CLP group, the CLP operation was done and animals received vehicle alone; CLP+ essential oils (E.Os) groups, E.Os (100 & 200 mg/kg b.w) were injected (i.p) immediately after CLP operation. Values of mean ± SEM obtained from five rats.

Oxidative injury parameters in septic rats treated with CM, ZM and MP E.O, before and after gamma irradiation:
As shown in Tables 2-4, CLP operation caused a significant increase ($P<0.05$) in TBARS as compared to the control group. Treatments of rats with essential oils extracted from the CM, ZM and MP at the doses of 100 and 200 mg/kg b.w significantly decreased the LP levels ($P<0.05$). Also, the MPO activity was
significantly elevated 24 h post CLP in the liver as compared with control group \((P<0.05)\). Administration of the rats with the E.O, obtained from plants (100 and 200 mg/kg b.w) depleted the liver MPO activity to the normal value \((P<0.05)\). In addition, the GSH concentrations diminished in CLP group as compared to the control group \((P<0.05)\). However, the GSH level increased significantly \((P<0.05)\) in the treated groups with CM, ZM and MP E.O, (100 and 200 mg/kg b.w) \((P<0.05)\).

There were no differences on suppressing the hepatic TBARS level, MPO activity as well as increasing the GSH level, between E.O, extracted from the non-irradiated plants in comparison to E.O, pulled out from irradiated ones \((P>0.05)\). In other words, the E.O, derived from irradiated CM, ZM and MP as well as non-irradiated ones could modulate the oxidative injury parameters in septic rats \((P<0.05)\). Moreover, there were no differences (for all biochemical parameters) on the efficacy of the oil between 100 mg/kg b.w and 200 mg/kg b.w \((P>0.05)\). According to the results, the differences among the irradiated groups (10 and 25 kGy) of all E.O, in all biochemical parameters (LP, GSH and MPO) weren’t considerable \((P>0.05)\).

### Table No.2: Hepatic oxidative parameters in sepsis rats treated with *Cuminum cyminum* L. (CM) E.O, before and after gamma irradiation

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nmol/gr liver)</th>
<th>MPO (U/mg liver)</th>
<th>GSH (µmol/ gr liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laparotomy (DMSO)</td>
<td>16.3 ± 0.5</td>
<td>4.2 ± 0.3</td>
<td>47.8± 3.3</td>
</tr>
<tr>
<td>CLP (DMSO)</td>
<td>28.6 ± 1.6*</td>
<td>9.6 ± 0.6*</td>
<td>11.8 ± 2.5*</td>
</tr>
<tr>
<td>CLP + non-irradiated CME.Os</td>
<td>13± 1.8**</td>
<td>5.8 ± 0.4**</td>
<td>34.5 ± 3.1**</td>
</tr>
<tr>
<td>(100 mg/kg b.w)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLP + Irradiated CM E.Os</td>
<td>15.1 ± 2.1**</td>
<td>5.8 ± 0.1**</td>
<td>34.7 ± 1.9**</td>
</tr>
<tr>
<td>(10 kGy) (100 mg/kg b.w)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLP + Irradiated CM E.Os</td>
<td>15.1 ± 2.7**</td>
<td>5.7 ± 0.4**</td>
<td>35.4 ± 2**</td>
</tr>
<tr>
<td>(25 kGy) (100 mg/kg b.w)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLP + non-irradiated CME.Os</td>
<td>13 ± 1.2**</td>
<td>6 ± 0.3**</td>
<td>35.5 ± 1.6**</td>
</tr>
<tr>
<td>(200 mg/kg b.w)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLP + Irradiated CM E.Os</td>
<td>14.4 ± 0.2**</td>
<td>5.9 ± 0.4**</td>
<td>34.8 ± 1.2**</td>
</tr>
<tr>
<td>(10 kGy) (200 mg/kg b.w)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLP + Irradiated CM E.Os</td>
<td>15.2 ± 1.4**</td>
<td>6± 0.5**</td>
<td>35± 2.7**</td>
</tr>
<tr>
<td>(25 kGy) (200 mg/kg b.w)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In Sham- operated group (SOP), rats were undergone laparotomy and received DMSO as vehicle; In CLP group, the CLP operation was done and animals received vehicle alone; CLP+ essential oils (E.O.) groups, E.Os (100 & 200 mg/kg b.w) were injected (i.p) immediately after CLP operation. Values of mean ± SEM obtained from five rats. *\(P<0.05\) is considered significantly different from laparotomy group. ** \(P<0.05\) is considered significantly different from CLP group within each group.
Table No.3: Hepatic oxidative parameters in sepsis rats treated with *Zataria multiflora* Boiss. (ZM) E.Os before and after gamma irradiation

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nmol/gr liver)</th>
<th>MPO (U/mg liver)</th>
<th>GSH (µmol/ gr liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laparotomy (DMSO)</td>
<td>16.3 ± 0.5</td>
<td>4.2 ± 0.3</td>
<td>47.8 ± 3.3</td>
</tr>
<tr>
<td>CLP (DMSO)</td>
<td>28.6 ± 1.6*</td>
<td>9.6 ± 0.6*</td>
<td>11.8 ± 2.5*</td>
</tr>
<tr>
<td>CLP + non-irradiated ZM E.Os (100 mg/kg b.w.)</td>
<td>21.9 ± 2.6**</td>
<td>5.4 ± 0.3**</td>
<td>39.3 ± 1.9**</td>
</tr>
<tr>
<td>CLP + Irradiated ZM E.Os (10 kGy) (100 mg/kg b.w.)</td>
<td>16.7 ± 3**</td>
<td>4.7 ± 0.7**</td>
<td>45.8 ± 7.6**</td>
</tr>
<tr>
<td>CLP + Irradiated ZM E.Os (25 kGy) (100 mg/kg b.w.)</td>
<td>16.9 ± 0.5**</td>
<td>5 ± 0.4**</td>
<td>44.9 ± 4.6**</td>
</tr>
<tr>
<td>CLP + non-irradiated ZM E.Os (200 mg/kg b.w.)</td>
<td>18.8± 0.5**</td>
<td>5.4 ± 0.6**</td>
<td>48.5 ± 2.8**</td>
</tr>
<tr>
<td>CLP + Irradiated ZM E.Os (10 kGy) (200 mg/kg b.w.)</td>
<td>15.7± 1.1**</td>
<td>5.3 ± 0.4**</td>
<td>39 ± 2.5**</td>
</tr>
<tr>
<td>CLP + Irradiated ZM E.Os (25 kGy) (200 mg/kg b.w.)</td>
<td>15.2 ± 0.84**</td>
<td>5.4 ± 0.5**</td>
<td>38.5 ± 3.1**</td>
</tr>
</tbody>
</table>

In Sham-operated group (SOP), rats were undergone laparotomy and received DMSO as vehicle; In CLP group, the CLP operation was done and animals received vehicle alone; CLP+ essential oils (E.O)s groups, E.Os (100 & 200 mg/kg b.w.) were injected (i.p) immediately after CLP operation. Values of mean ± SEM obtained from five rats. *P<0.05 is considered significantly different from laparatomy group. ** P<0.05 is considered significantly different from CLP group within each group.

Table No.4: Hepatic oxidative parameters in sepsis rats treated with *Mentha piperita* L. (MP) E.Os before and after gamma irradiation

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nmol/gr liver)</th>
<th>MPO (U/mg liver)</th>
<th>GSH (µmol/ gr liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laparotomy (DMSO)</td>
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<td>4.2 ± 0.3</td>
<td>47.8 ± 3.3</td>
</tr>
<tr>
<td>CLP (DMSO)</td>
<td>28.6 ± 1.6*</td>
<td>9.6 ± 0.6*</td>
<td>11.8 ± 2.5*</td>
</tr>
<tr>
<td>CLP + non-irradiated MP E.Os (100 mg/kg b.w.)</td>
<td>11.1 ± 1.6**</td>
<td>6.3 ± 0.2**</td>
<td>35.8 ± 7.2**</td>
</tr>
<tr>
<td>CLP + Irradiated MP E.Os (10 kGy) (100 mg/kg b.w.)</td>
<td>20.5 ± 3.2**</td>
<td>5.7 ± 0.4**</td>
<td>43.1 ± 3.9**</td>
</tr>
<tr>
<td>CLP + Irradiated MP E.Os (25 kGy) (100 mg/kg b.w.)</td>
<td>16± 3.6**</td>
<td>5.7 ± 0.4**</td>
<td>32.5 ± 3.3**</td>
</tr>
<tr>
<td>CLP + non-irradiated MP E.Os (200 mg/kg b.w.)</td>
<td>18.4 ± 2.1**</td>
<td>5.8 ± 0.2**</td>
<td>34.7 ± 1.7**</td>
</tr>
<tr>
<td>CLP + Irradiated MP E.Os (10 kGy) (200 mg/kg b.w.)</td>
<td>15.2 ± 1.1**</td>
<td>5.7 ± 0.3**</td>
<td>39.5 ± 7.3**</td>
</tr>
<tr>
<td>CLP + Irradiated MP E.Os (25 kGy) (200 mg/kg b.w.)</td>
<td>14 ± 0.4**</td>
<td>5.9 ± 0.2**</td>
<td>35± 5**</td>
</tr>
</tbody>
</table>

In Sham-operated group (SOP), rats were undergone laparotomy and received DMSO as vehicle; In CLP group, the CLP operation was done and animals received vehicle alone; CLP+ essential oils (E.O)s groups, E.Os (100 & 200 mg/kg b.w.) were injected (i.p) immediately after CLP operation. Values of mean ± SEM obtained from five rats. *P<0.05 is considered significantly different from laparatomy group. ** P<0.05 is considered significantly different from CLP group within each group.
DISCUSSION

Based on our previous data, the in vivo antibacterial activities of three oils extracted from CM, ZM and MP were informed by reducing the number of CFUs from the bloodstream increased significantly after CLP surgery. Also, the in vitro strong antibacterial activities of three oils against gram-positive and gram-negative bacteria by the determination the minimal inhibitory (MIC) and minimal bacterial concentrations (MBC) overlapped with the in vivo results. The evidences presented in our recent articles showed that the antioxidant capacities of the three oils remarkable in vitro system could be attributed to their main phytochemical components. In followings, in the present study, the role of gamma irradiation as a modern decontamination method in the protective effects of three oils on oxidative injury parameters in septic rats induced by CLP rat model were investigated. Our aim is to access whether the in vitro and in vivo antibacterial and antioxidant activities of three oils can protect the liver from damages even after irradiation exposure thorough the CLP method which was the most practical method of sepsis.

Our data indicated that the essential oils extracted from CM, ZM and MP could adjust antioxidant defense system by changing the oxidative stress parameters namely: LP, MPO and GSH disturbed in septic rats (Tables 1-4). It seems that the oil therapeutic effects can be mainly due to its intervention with the mediators of oxidative stress/antioxidant parameters disturbed by sepsis.

In sepsis, myeloperoxidase (MPO)—a hemoprotein secreted by activated leukocytes—amplifies the oxidizing potential of H2O2 by using it as co-substrate to generate reactive intermediates that promote LP. Increased concentrations of LP and MPO are found in rats with sepsis, and tissue MPO is a marker of LP levels that increase when septic shock is induced by CLP in rats. Our study confirmed the elevation of LP level and MPO activity in septic rats (Tables 1-4). In addition, this study indicated the oil hepatic protective activity in rats treated with three E.Os through significant decrease in the liver MPO activity caused to the suppressed hepatic LP (P<0.05) (Tables 2-4). In addition, GSH is an important constituent of intracellular protective mechanisms against oxidative stress. So, the GSH recuperation in rats treated with all the E.Os showed their hepatoprotective effects. These data are in agreement with the report of Villa who reported the protective role of GSH against sepsis. Other studies have also indicated that antioxidants such as silymarin and N-acetylcysteine (NAC), which maintain the cellular defense mechanisms, could protect oxidative tissue damage caused by sepsis. Besides, The reduction of hepatic LP concomitant with the replenishment of GSH content by all E.Os represented that the oils have great potential in maintaining the oxidative/antioxidant balance. Other studies confirmed these results, reported that *Nigella sativa* ethanolic extract prevented lung injury induced by CLP through modulating the LP, GSH levels and MPO activity. Sildenafil is a highly protective agent in preventing lung and kidney damages caused by CLP-induced sepsis via maintenance of the oxidative/antioxidant status such as LP, GSH and MPO activity. Also, curcumin protects against sepsis-induced acute lung injury in rats with counteract the inflammatory cells infiltration and, hence, ROS generation and regulate cytokine effects.

On the other hand, our data indicated that the irradiated groups (10 and 25 kGy) as same as non -irradiated ones have significant effects on the potential of hepatoprotective activities of the three E.O, (100 and 200 mg/kg b.w). It means that the oils at both doses (100 and 200 mg/kg b.w) extracted from irradiated ZM aerial parts, MP leaves and CM at 10 and 25 kGy, as well as non -irradiated ones, could modulate the hepatic antioxidant/oxidative stress statue by decreasing the MPO and...
Anti-septic activity of irradiated plant oils

LP levels concomitant with increasing GSH level (Tables 2–4). These results correlate with our recent findings that gamma irradiation to caraway seeds did not have any significant effects on the oil hepatoprotective activity in septic rats. One study reported the application of γ-radiation at doses from 5 to 30 kGy did not cause any significant changes in the total phenolic content and antioxidant activities of caraway and bay leaves extracts.

It is well known that hepatoprotective effects are associated with plants including efficient antioxidant compounds. Our GC/GC mass analysis showed that the main chemical compositions of the essential oils isolated from CM were β-pinene (24.07%), γ-terpinene-7-al (21.48%), γ-terpinene (20.09%), cumin aldehyde (19.03%) and p-cymene (10.2%)33. Also, the major compounds of ZM essential oils were thymol (61.8%), carvacrol (10.5%), p-cymene (7.5%), and γ-terpinene (4.4%)32. We also considered Persian MP with the main chemical components as menthol (50.9%), menthone (14.9%), α-gurjunene (8.7%), neo-menthol (6.5%) and 1, 8-cineole (3.7%)34. In other words, the antioxidant activities of these herbs could be attributed to their main active antioxidant components such as phenols and flavonoids; for example the existence of phenolic monoterpenes including carvacrol and thymol in ZM oils and menthone and menthol in MP oils and β-pinene and γ-terpinene in the CM oils32–34. Previous studies also confirmed the main role of antioxidants in chemopreventive effects of the natural products56–57. Antioxidants can reduce the damages of oxidative stresses by scavenging free radicals58. Other data was demonstrated that the essential oil of rosemary containing antioxidative compounds, namely 1, 8-cineole, camphor and α-pinene, possess a strong antioxidant and hepatoprotective activities59. Also, Nithianantham reported the hepatoprotective activity of Clitoria ternatea leaf may be due to its free radical scavenging and antioxidant activity, resulting from the presence of some antioxidant compounds in the extracts60. In addition, the lack of change in the composition, antioxidant and radical scavenging properties of the oils after plant gamma irradiation32–34, confirmed the association between antioxidant compounds and oil hepatoprotective activity.

In the other hand, the in vitro and in vivo antibacterial activities explained in our recent studies35–37 indicated that i.p administrations of the E.Os may have direct contact with the bacteria in the peritoneum which can validate the microbicidal effect leading to an increased bacterial clearance in 24 h post CLP. The CLP model seems to resemble qualitatively as well as quantitatively the clinical observations of vascular reactivity and inflammation during polymicrobial peritonitis, bacteremia and systemic sepsis. The cecum contains a huge concentration of microbes, which are a combination of gram-negative and gram-positive flora. The ligation of the cecum produces a source of ischemic tissue and polymicrobial infection indeed. This combination of ischemic/necrotic tissue and microbial infection distinguishes this multifactorial model from a number of other bacterial sepsis models. After replacing the punctured cecum into peritoneum, a severe peritonitis happened due to start decanting the fecal contents into the abdomen. Our results (Table 1) indicated that the bacteria will be detectable on the blood and peritoneum within 24 hours in CLP groups. While, the administration of the both non-irradiated and irradiated treatment groups including the three E.Os (100 and 20 mg/kg b.w) derived from the different medicinal plants could surprisingly decrease the CFU counts when compared with the CLP ones (P<0.05). One study reported that the administration of Myrrh (an Indian plant) led to a remarkable increase in survival and an increased bacterial clearance in mice with lethal sepsis induced by CLP.
CONCLUSION

This study indicated the effectiveness of the E.Os extracted from CM, ZM and MP at both doses (100 and 200 mg/kg b.w) by reducing the LP level and MPO activity as well as raising the GSH concentration in septic rats after CLP operation. In addition, gamma irradiations (10 and 25 kGy) were suggested as a safe method for decontamination of the three medicinal plants in order not to considerably change in the efficiency of the E.Os, indicating the potent modulatory anti-septic activity.

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