# Considering the effect of *Rosa Damascena* essential oil on oxidative stress and COX-2 gene expression in liver of septic rats

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## ABSTRACT

**Objectives:** Sepsis is a clinical illness with the high rate of mortality in all over the world. Oxidative stress considered as the main phenomenon which occurs in sepsis. Rosa Damascena supposed as an ancient herbal planet with high pharmacological activities.

**Materials and Methods:** CLP operation as a standard model was done to induce sepsis in rats. Male adult rats were randomly divided into 5 groups. Rosa Damascena E.Os (50 and 100 mg/kg.bw), was gavaged orally for 14 days, and in the day 15$^{th}$, CLP was performed. After 24 hours, the blood samples and liver tissues were removed in order to measure oxidative stress (MPO, MDA, GSH, GST, FRAP) and biochemical parameters (ALP, AST, ALP, bilirubin) together with plasma PGE2 and COX-2 expression.

**Results:** We deduced that E.Os had capable to modulate all of the oxidative stress, antioxidant and anti-inflammatory parameters induced by CLP as characterized by the elevations in MPO and MDA levels as well as increases in AST and ALT concentrations concomitant with PGE2 and COX-2 increments. The antioxidant defense system such as GSH and FRAP was also increased in E.O treated groups.

**Conclusion:** Our results showed that E.Os has antioxidative and hepatoprotective activities through reducing the oxidative injury in sepsis caused by CLP.

**Key words:** Sepsis, Rosa Damascena, Oxidative stress, CLP, Hepatoprotective activity

## INTRODUCTION

Sepsis is one of the ancient and serious clinical diseases with high mortality in critically ill patients.[1,2] Although, all of the parasite and microorganism are potent to make sepsis, the most famous and effective cause of pathogenesis of sepsis is Gram-negative, Gram- positive bacteria and fungi.[3] Sepsis is the result of assaulting microbial pathogen or their products, like toxins in the bloodstream, expressed by the systemic inflammatory response to the infection.[4,5]

In the beginning stages of sepsis, pathogenic microorganism stimulates the body to make a large amount of chemokine and cytokines.[6] The imbalance between pro-inflammatory and anti-inflammatory parameters, are responsible of susceptibility and outcome of sepsis.[7] By increasing the oxidative stress and reactive oxygen species (ROS), body's antioxidant system is impaired, leading to affect the cells, mitochondria and finally causes organ dysfunction.[8]

To investigate the pathogenesis of sepsis and associated mechanism, these three methods are usually utilized: 1-injection and using lipopolysaccharides (LPS) and other exogenous toxin like zymogens 2- applying different fatal and vivid pathogen 3-elimination or changing endogenous protective barrier, like CLP and CASP (Colon Ascendens Stent Peritonitis) .[9] All of them try to simulate pathophysiological changes, which are closely similar to septic shock.[10] Among them, Cecal Ligation and Puncture (CLP), is a most accepted method to imitate human sepsis, which considered as a gold standard model. For the first time, Chaudhry in 1970 developed CLP among others.[11] It is a surgery done by cecum ligation and piercing, which can cause penetration microbial and pathogen factors, and finally making sepsis.[12]

Because of increased oxidant and inflammatory element, a substitute for chemical drugs, with high curative features and less side effect, is noteworthy.[13] *Damask Rose (Rosa Damascena Mill*) is an ancient herbal drug, used for treatment illnesses in the past.[14] It belongs to the Rosaceae family and considered as an ornamental plant, which is famous for the king of flowers.[15] Nowadays, this plant is cultivated all over the world for its products; essential oil and rosewater.[16]

On the other hand, the researcher confirmed some of the pharmacological properties such as antioxidant, anti-HIV and anti-bacterial activities.[17] Different Features of *Rosa Damascena* was proposed by archaic people to cure bleeding, digestive problems and treatment abdominal pain in traditional medicine.[16] Many studies have proved the antioxidant activity and other therapeutic effect of extract or essential oils

of *Rosa Damascena*.[18,19] They reported that hydroalcoholic extract of *Rosa Damascena* has analgesic and anti-inflammatory effects made by formalin-induced method.[13] The role of Rosa essential oils as a natural antioxidant with preventing effect of oxidative damage were also confirmed.[20] Meanwhile, there is no research on the therapeutic effect of *Rosa damascena* plant on hepatic injures especially induced by sepsis. So, this present study was done to evaluate the hepatoprotective and antioxidant activities of *Rosa damascena* essential oil against oxidative stress made by Cecal Ligation and Puncture (CLP) in the rat.

## MATERIALS AND METHODS

### Essential oil extraction

*Rosa Damascena* essential oils were prepared from Barij Essence pharmaceutical Co, Kashan Iran**.** A voucher specimen (Batch No: 714043, sample Serial No: AE932009) has been deposited at the Barij Essence company.

### GC and GC-mass analysis of *Rosa Damascena* essential oils

The *Rosa Damascena* essential oils was qualitatively and quantitatively determined with GC of thermo Finnegan Trace GC (thermo electron co, Waltham, MA, USA), consisting in AI/AS 3000 auto sampler, equipped with mass spectrometer of thermo Finnegan Auto Mass quadruple (thermo Electron).

GC analyzer equipped with TR5 fused silica column (30 m, 0.025 mm, 0.025 m coating thick). Analytical conditions were as follows: the column temperature was initiated at 50 ˚c for 1 min, then planned to rise to 280 ˚C at a rate of 10 ˚C/min held by using isothermal process. Helium was performed as the carrier gas at the rate of 1.0 ml/min. Identification of the constituent, was based on the comparison of GC retention, with those stored in Wiley mass spectra library.

### Free radical scavenging activity (DPPH assay)

Radical scavenging activity of *Rosa Damascena* essential oils were determined by DPPH assay. The absorbance of DPPH was read against the blank at 517 nm. The inhibitory effects of the essential oil in percent (I %) was calculated by the following equation:

I % = (Ablank – Asample/Ablank) ×100

### β-Carotene/linoleic acid bleaching assay

To determine antioxidant activity of *Rosa Damascena* essential oils, β-carotene/linoleic acid bleaching assay was performed. The oxidation of the β-carotene was monitored spectrophotometrically by measuring absorbance at 470 nm over a 60 min period. The antioxidant activity of essential oil was expressed as inhibition percentage with reference to the control after 60 min of incubation, using the following equation:

AA =100(DRC - DRS)/DRC

Where, AA = antioxidant activity; DRC = degradation rate of control = [ln(a/b)/60]; DRS = degradation rate of sample = [ln(a/b)/60]; a = absorbance at time 0; b = absorbance at 60 min.

**Treatment of animals**

Two month old male Wistar rats (200 - 250 gr), were purchased from animal breeding house of Pasteur institute, and were kept in animal facility, at the Qom Azad University animal room. Animals were acclimatized for two weeks in the standard condition, kept in photoperiod (12 h light/ 12 h dark) at 20 - 25 ºc. All the rats had free access, which were fed by laboratory pallet diet and drinking water *adlibitum*.

The animals were divided into 5 groups (n = 10): 1. Negative control (C): in the negative control group, dimethyl sulfoxide (DMSO) -as solvent of essential oils- was orally administrated to rats for 14 days following laparotomy surgery.

2. CLP group: as control group, the rats received DMSO for 14 days following CLP surgery.

3 & 4. CLP + *Rosa Damascena* E.Os (50 & 100 mg/kg.bw): the rats received orally essential oil of *Rosa Damascena* once a day for 14 days dissolved in DMSO following CLP surgery.

5. CLP + IND (2 mg/kg.bw): the rats treated with indomethacin once a day for 14 days next to CLP surgery.

**Sepsis induction**

Sepsis was induced by CLP operation. The rats were anesthetized with i.p. injection of ketamine and xylazine. Under sterile conditions, the cecum was exposed through 1 to 2 cm incision of the lower left abdomen, ligated on the ileocecal valve with a 3 - 0 silk, and then was punctured twice, with a 20 gauge needle. The cecum was replaced in the peritoneum and the abdomen was closed with surgical staples. The

rats were injected with 5 ml of saline s. c. for fluid resuscitation and were placed on a warming position until they recovered from anesthesia.

**Preparation of tissue homogenate and plasma**

After 24 h, the rats in all groups were anesthetized with diethyl ether. Blood samples were withdrawn with heparinized syringe, followed by centrifugation at 3000 *g* for 10 min. Plasma samples were disserved and maintained at -20 ºC for the biochemical purposes. In addition, the liver tissues were removed and homogenized completely with buffer phosphate in ice bath to evaluate oxidative stress biomarkers. The portion of that was also fixed in 10% formalin, for histopathological studies.

**Determination of the oxidative stress and antioxidant parameters in the liver homogenate**

**GSH estimation**

The level of reduced glutathione (GSH), as an antioxidant factor, was measured in liver homogenate as Seldak and Lindsay method.[21] The GSH level in samples was calculated by plotting a standard curve of absorbance against different concentration of GSH standard solution.

**Measurement of tissue myeloperoxidase (MPO) activity**

The extent of neutrophil accumulation in the liver was evaluated by assaying myeloperoxidase activity. MPO activity in the liver was measured by procedure of Hillegas et al.[22]

**Measurement of tissue Malondialdehyde (MDA)**

To investigate the rate of lipid peroxidation, the concentration of MDA was measured spectrophotometrically according the method of Buege and Aust.[23]

**Measurement of tissue GST activity**

Glutathione-S-transferase (GST) activity was measured according to Habig et al.[24] by investigating the conjugation of 1-chloro-2, 4-dinitrobenzene (CDNB), with reduced glutathione. The conjugation is done by an increase in the absorbance at 340 nm.

**Ferric reducing ability of plasma (FRAP)**

The total antioxidant activity of plasma was measured by using FRAP assay based on the Benzie method.[25] According to this, at low pH, ferric tripyridyltriazine complex ($Fe_3$-TPTZ), is reduced to the ferrous ($Fe_2$) form. In this reaction, intense blue color is created with the absorption at 593 nm.

**Determination of PGE2 concentration**

Prostaglandin E2 (PGE2) assessment was done by ELISA kit (BioAssay System) according to the kit instruction.

**Determination of COX-2 gene expression using quantitative real time PCR (RT–PCR)**

Total RNA extracted from the rat livers, by using RNA total kit (BioBasic Inc, Canada) surveyed quantifiably by Nano Drop 2000. Then, to synthetic cDNA, each sample was reverse transcripted into compulsory DNA (cDNA), by using PrimeScript$^{tm}$ RT reg Kit (TaKaRa and oligo dt primers). cDNA was stored at – 20 °C until use.

In order to use specific primers of COX-2, Gene Runner software version 3.05 and primer 3 servers was applied.

Expression of COX-2 genes was investigated with Real time PCR system (Rotor-Gene Q-QIAGEN). The RT-PCR analyses was done using SYBER green real time PCR Master Mix. The reaction compositions are Taq polymerase, dNTP, $MgCl_2$, SYBER green I dye, 0.2 µl primers, 0.5 µl cDNA and 10 µl H2O. The reaction condition was designed by an initial degeneration stage at 95 °C for 2 min, 40 cycles at 95 °C for 15 s, 60 °C for 20 s, and then held 72 °C for 20 s. Each sample was measured in triplicate. At the completion of each run, melting curves for the amplicons were measured by raising the temperature by 0.3 °C from 57 to 95 °C while monitoring fluorescence. The comparative cycle threshold Ct method was used for the relative quantification of gene expression. Evaluation of the relative expression of mRNA was done and normalized to GAPDH reported as a housekeeping gene.

**Assessment of liver injures parameters**

To determine the functional injures of liver caused by polymicrobial sepsis, the plasma levels of ALT, AST, ALP and total bilirubin were evaluated by using Pars Azmoon kit, IRAN.

**Histological analysis**

Tissue samples were fixed with 10 % buffered formaldehyde, dehydrated and embedded in paraffin. Liver section (5 µm) was stained with haematoxylin and eosin (H&E) examined under light microscopy (Olympus CX31RBSF) to assess the hepatic changes.

The quantitative and semi-quantitative histological analysis also used for scoring the histopathological variables by a veterinary pathologist. The mean numbers of

marginated and infiltrated neutrophils were counted in the 10 random high power fields of the microscope. Thereafter, scoring was performed between 0 - 4 as following: score 0 = 0 up to 9 neutrophils, score 1 = 10 up to 19 neutrophils, score 2 = 20 up to 29 neutrophils, score 3 = 30 up to 39 neutrophils, score 4 = more than 40 neutrophils. In addition, mononuclear cell infiltrations and kupffer cell hyperplasia scorings were as follows: score 0 = normal condition, score 1= the mild changes, score 2 = the average changes, score 3 = the severe changes, score 4 = more severe changes.

**Statistical analysis**

The data of this study was analyzed statistically with SPSS v.19. Analysis of data was expressed as mean ± SE. One way analysis of variance (ANOVA) was applied to compare the mean values. P-value of less than 0.05 was statistically considered as significant value.

## RESULTS

**Essential oil analysis & antioxidant activities**

By the result of using GC-MS analysis, 24 chemical components were identified. The major constituents of *Rosa Damascena* essential oils are characterized as Citronellol (66.11%), trans-geraniol (11.56%), Phenylethyl alcohol (5.33%), and other constituent such as linalool, pinene, citral, methyl eugenol and geranene, presented in Table 1.

The DPPH and β-carotene-linoleic acid bleaching assays were done to evaluate the antioxidant activities of *Rosa Damascena* essential oils. Essential oil was capable to reduce concentration of DPPH free radical, which was higher, as compare to Trolex (Figure 1). In addition, the β-carotene-linoleic acid bleaching test shows high antioxidant activity in comparison with the positive control (BHT) (Figure 2).

**The effect of *Rosa Damascena* essential oil on the oxidative stress and antioxidant parameters**

According to the data presented in Table 2, hepatic GSH level was decreased due to CLP operation ($p < 0.05$). Accordingly, similar to the indomethacin treated group, *Rosa Damascena* essential oils (50 and 100 mg/kg.bw) followed by CLP operation, increase the level of GSH to reach in control group($p < 0.05$).

CLP operation induced significant increases in MPO activity as compared to the control group ($p < 0.05$). On the other hand, decreases in MPO activity was seen in both groups treated with *Rosa Damasecna* essential oils (50 and 100 mg/kg.bw) ($p < 0.05$) as shown in indomethacin group (Table 2).

Hepatic malondialdehyde (MDA) level was significantly evaluated as an indicator of lipid peroxidation in CLP group, in comparison to the control group ($p < 0.05$, Table 2). Treatment of rats with essential oils significantly decreased the level of MDA in liver ($p < 0.05$, Table 2).

Concerning the effect of sepsis on FRAP level (Table 2), indicated that CLP caused significant decrease in FRAP level in comparison to the control group ($p < 0.05$). In addition, treated rats with *Rosa Damascena* essential oils in doses of 50 and 100 mg/kg.bw, increase the level of FRAP to the ideal condition ($p < 0.05$, Table 2).

CLP operation did not change the GST activity (Table 2), but treatment of rats with 50 and 100 mg/kg.bw of *Rosa Damascena* essential oils increase the activity of GST in compare to control and CLP groups ($p < 0.05$).

***Rosa Damascena* essential oils inhibited CLP-induced concentration of PGE-2**

In the present study, the evaluation of PGE2 concentration clearly indicated that CLP make significant increase in plasma level of PGE2, as compared to the control group ($p < 0.05$, Table 3). In contrast, treatment of rats with *Rosa Damascena* essential oils (50 and 100 mg/kg.bw) significantly decreased plasma PGE2 concentration in comparison to the CLP group ($p < 0.05$).

***Rosa Damascena* essential oil inhibited CLP-induced expression of COX-2**

Table 3 shows the alteration of COX-2 expression in different groups. The rats that undergone CLP, showed significant increase ($p < 0.05$) in COX2 expression as compared to control group. In contrast, the rat received *Rosa Damascena* essential oils in 50 and 100 mg/kg.bw followed by CLP operation, attenuated the level of COX-2 expression ($p < 0.05$, Table 3), as also seen in indomethacin treated group which was lower than CLP group.

**The effect of *Rosa Damascena* essential oils on plasma biomarker for liver injuries**

As shown in Figure 3, the plasma level of AST and ALT significantly increased ($p < 0.05$) in CLP group in comparison to the control group (Figures 3A&B), but, after treatment with essential oils in doses of 50 and 100 mg/kg.bw, the level of these

markers was decreased ($p < 0.05$). Meanwhile, plasma ALP and total bilirubin didn't have considerable differences throughout the experiment in all groups (Figures 3C& D).

**Histological findings**

Histopathologic assessment of the liver specimens revealed that there were some mild changes consist of congestion and granular degeneration of the hepatocytes in control group (Figure 4A). Whereas, severe congestion, interstitial edema and also margination of neutrophils in the venules and sinusoids were observed in the CLP group. Neutrophils and mononuclear cells were also infiltrated in the portal tracts and sinusoids in the septic group. Kupffer cell hyperplasia and granular degeneration were the other observed changes in the CLP group. There weren't any signs of necrosis in hepatocytes. All the changes in the CLP group revealed a kind of hepatitis called Non Specific Reactive Hepatitis (Figures 4B1&B2). However, the E.O treatments obviously reduced neutrophils infiltration in the portal tract and parenchyma of the liver tissues (Figures 4C&D). In addition, in indomethacin group, there isn't any sign of neutrophil infiltration in the portal tract or in the parenchyma (Figure 4E).

As shown in Table 4, the CLP group obviously showed the neutrophil margination and infiltration, mononuclear cell infiltration and kupffer cell hyperplasia as compared with the controle group ($p \leq 0.05$). Concerning portal inflammation, it was also meaningful in the CLP group in comparison with the control group ($p \leq 0.05$). However, there weren't obvious difference regarding granular degeneration and inflammatory foci between all study groups ($p > 0.05$). To confirm the results seen in Figure 4, all the treatment groups prominently reduced neutrophil margination and infiltration, mononuclear cells infiltration, kupffer cell hyperplasia and portal inflammation in comparing with the CLP group ($p \leq 0.05$).

## DISCUSSION

Sepsis is a generalized inflammatory response of different parasites and their toxins in the body and can be divulged as a main part of systemic inflammatory response system (SIRS). Nowadays, sepsis is the major causes of death in intensive care unit. Importance of sepsis in terms of mortality and morbidity and difficulties to treatment, make it to the public health concern.[26] Oxidative stress can define on incongruence

between oxidants and body's antioxidant system.[27] It can activate immune systems that promote destructive effect on lipid, protein and DNA. Antioxidant defense system can confront with sepsis and its consequences by combating with free radicals. The notable rate of antioxidant activity in treating sepsis was proved by different studies. Therefore, in the current study, the antioxidant and hepatoprotective activity of *Rosa Damascena* essential oils were investigated, at the first time, on the *in vivo* sepsis system induced by CLP operation through assessing the main antioxidant/oxidative stress and anti-inflammatory parameters.

In this study, increased in ALT, AST, MPO, MDA, as well as PGE2 and COX-2 expression ($p < 0.05$) concomitant with decreased in FRAP and GSH levels ($p < 0.05$) were observed in septic rats (Tables 2, 3 and Figure 3) which are modulated with oil treatments.

Polymorphonuclear leukocytes (PMN) infiltration following systemic inflammatory response could be created during sepsis, which can result in vascular as well as parenchymal cell dysfunctions. Leucocyte aggregation and activated neutrophil leading up to tissue injures, was increased by the effect of oxidant and ROS elements. Activated neutrophil can cause to MPO secretion and also free radical production,[28] meaning MPO assay as a valuable indicator to evaluate neutrophil accumulation and severity of inflammation in sepsis which was reversed in rats treated with antioxidative *Rosa Damascena* essential oils (50 and 100 mg/kg.bw) ($p < 0.05$) (Table 2). Our results are in conjunction with other studies, which showed that MPO activity as the neutrophil accumulations was elevated by sepsis, while pretreatment with selenium, *n*-acetylcysteine and simvastatin decrease MPO activity by scavenging free radical generation, which suppressed the severity of sepsis.[29,30,10]

One of the consequences of sepsis is an event called lipid peroxidation which considered as the main oxidative stress parameter which was increased by the impact of free radicals and MPO increment.[10] Lipid peroxidation can cause cell and mitochondria damages, which initiate protein degeneration, cell lysis and necrosis.[31] Treatment of rats with the essential oils decreases ($p < 0.05$) the lipid peroxidation which eventually result in diminishing MDA level (Table 2). Our results were confirmed by Ozdulger et al, reported that MPO activity and MDA concentration was increased in lung by sepsis, which is showed the intensity of neutrophils infiltration and oxidative stress condition in rat.[32] Reported that, applying estrogen could

diminish MDA concentration, and as a result decreasing in lipid peroxidation, which protected ileum and liver from sepsis-induced and oxidative stress condition.

It is a thought that there is relation between lipid peroxidation and GSH depletion in sepsis. GSH is considered as an important natural antioxidant system that can deal with ROS production which neutralized them. By establishing oxidative stress condition in the body, ROS changed the antioxidant power. In other word, antioxidant defense system in the body was capitulated leading to the increased lipid peroxidation concomitant with changing in GSH and antioxidant enzymes such as GST, CAT and SOD.[33] In our study, we observed that CLP induction caused decreasing in GSH level in comparison to the control group ($p < 0.05$, Table 2). On the contrast, treatment with *Rosa Damascena* essential oil, restore the level of GSH in ideal concentration ($p < 0.05$) indicated that inhibiting GSH depletion has curative efficiency that increases the defense ability system which keeps the body from oxidative stress by protecting from lipid peroxidation. Our results are in agreement with the Bouzenna et al.[34] who reported that administrating *citrus lemon* essential oil which has hepatoprotective effect on the oxidative stress caused by aspirin as revealed by decreasing in lipid peroxidation and amplifying the antioxidant defense system (GSH, CAT and GPx). Also, this study revealed a significant decline in ferric reducing ability of plasma (FRAP) in sepsis, while, administration of the essential oils caused a significant increase ($p < 0.05$) in the plasma FRAP (Table 2). Decrease of FRAP levels, as a factor in oxidative stress/antioxidant balancing, leads to increased resistance and/or decreased susceptibility of the liver to free radical attack.[35] In contrast, glutathione-s-transferase (GST) activities in liver were not change ($p > 0.05$) in all treated groups (Table 2) indicated not probable effective role of this enzyme in the detoxification of septic rat.

On the other hand, in sepsis, changing in cytokine levels are clearly was seen, showing the imbalances between pro-inflammatory and anti-inflammatory cytokines.[36] It seems that expression of COX-2 increased by pro-inflammatory cytokines which simulate PGE2 level (Table 3). COX-2 is an enzyme which can simulate production of prostaglandins like PGE2, which can initiate oxidative damage on tissue.[37] Increases in COX-2 expression stimulated the production of PGE2 resulting to increasing its plasma level. In other words, COX-2 expression is the key principle to immunologic disharmony in septic rats, so ceasing the COX-2 expression

diminishes the effect of oxidative stress.[38] In our study, treatment of rats with *Rosa Damascena* essential oils has an inhibitory effect on the production of cytokines and ROS leading to the decreased COX-2 expression together with plasma PGE2 level. Huang et al.[37] reported that LPS has the negative effect on COX-2 expression and PGE2 production. LPS significantly increases the level of PGE2 levels and COX-2 transcription and also production of TNF-α, IL-1β, IL-6 and IL-10. They proved that *Bupivacaine* significantly decreases COX-2 expression, PGE2 and cytokines production.

On the other side, the plasma levels of ALT and AST which considered as the most important biomarkers of liver enzymes can be considerably elevated by hepatocellular injures.[39] Hepatic injures is characterized by irregularly biomarkers of liver that can confirm by elevation of AST and ALT levels. Elevation in concentration of these enzymes indicated the loss of integrity of liver, apoptosis and necrosis. By CLP operation, concentration of these enzymes increased as compared to the control group ($p < 0.05$, Figures 3A&B). Liver injures in pathologic study, justify this increscent (Fig. 4). After hepatic injures, these enzymes released by cellular necrosis and the elevation of these enzymes showed the rate of damages on integrity of liver. Treatment of rats with *Rosa Damascena* essential oils as well as indomethacin had ability to reduce the hepatoinjures by diminishing these biomarkers (Table 4 and Figures 3&4).

On the other hand, alkaline phosphatase (ALP), is in the relation of injures in bile duct and integrity of cell membrane. Total bilirubin also considered as a parameter of liver injures.[39] On this study, serum level of ALP and total bilirubin had not any differences in CLP-induction group and other groups ($p < 0.05$, Figures 3C&D) indicating that CLP operation does not sufficient damage on liver and biliary ducts.

This study indicated the probable relationship between *in vitro* antioxidative effects of *Rosa Damascena* essential oils and *in vivo* anti-inflammatory effects. Citranellol (66.11%), trans geraniol (11.56%), Phenylethyl alcohol (5.33%) are the main constituent of the essential oils (Table 1) which possessed antioxidant activities proved by DPPH and β-carotene/linoleic acid bleaching assays. Considering recent studies, these components are completely related to its antioxidant and radical scavenging ability of the essential oil reported that fresh juice of *Rosa Damascena*

*Mill* has antioxidant potential against rats who received $CCl_4$, as a toxic material that can causes hepato injures.[18]

In summary, this study confirmed that *Rosa Damascena* essential oils has antioxidative and hepatoprotective effects, against CLP-induced sepsis, caused by reactive species. Effect of essential oils in decreasing oxidative stress parameters, improvement the antioxidant defense system and also restoring the ideal concentration of inflammatory parameters, proved this vigorous ability of *Rosa Damascena* essential oils.

## DECLARATION OF INTEREST

This research was conducted by the research deputy grant of Qom Branch, Islamic Azad University.

## ETHICAL APPROVAL

This Ethics Committee was based on the World Medical Association Declaration of Helsinki (Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964).

## REFERENCES

1. Derek C, Angus MPH, van der Poll T. Severe Sepsis and Septic Shock. N Engl J Med. 2013; 369: 840-51.

2. Peters K, Unger RE, Brunner J, Kirkpatrick J. Molecular basis of endothelial dysfunction in sepsis. Cardiovasc Res. 2003; 60: 49-57.

3. Peters J, Cohen J. Sepsis. Med J. 2013; 41(11): 667-669.

4. Cho SY, Choi JH. Biomarkers of sepsis. Infect Chemother. 2014; 46(1): 1-12.

5. Nelson GE, Mave V, Gupta A. Biomarkers for sepsis: A review with special attention to India. Biomed Res Int. 2014; 2014: 264351.

6. Li Sh, Zhu FX, Zhao XJ, An YZ. The immunoprotective activity of interleukin-33 in a mouse model of cecal ligation and puncture-induced sepsis. Immunol Lett. 2016; 169: 1-7.

7. Koh HJ, Joo J, Cho ML, Her YM, Hwang JE, Lee J. Pro-inflammatory and anti-inflammatory cytokine balance in patients with cirrhotic hepatitis during live-donor liver transplant. Exp Clin Transplant. 2013; 1: 39-43.

8. Lowes DA, Webster NR, Murphy MP, Galley HF. Antioxidants that protect mitochondria reduce interleukin-6 and oxidative stress, improve mitochondrial function, and reduce biochemical markers of organ dysfunction in a rat model of acute sepsis. Br J Anaesth. 2013; 110(3): 472-80.
9. Dejager L, Pinheiro I, Dejonckheere E, Libert C. Cecal ligation and puncture: the gold standard model for polymicrobial sepsis? Trends Microbiol. 2011; 19(4): 198-208.
10. Song T, Yin H, Chen J, Huang L, Jiang J, He T, et al. Survival advantage depends on cecal volume rather than cecal length in a mouse model of cecal ligation and puncture. J Surg Res. 2016; 203(2): 476-482.
11. Schabbauer G. Polymicrobial sepsis models: CLP versus CASP. Drug Discov Today Dis Models. 2012; 9(1): 17-21.
12. Cuenca AG, Delano MJ, Kelly-Scumpia KM, Moldawer LL, Efron PA. Cecal Ligation and Puncture. Curr Protoc Immunol. 2010; Chapter 19: Unit 19: 13.
13. Hajhashemi V, Ghannadi A, Hajiloo M. Analgesic and Anti-inflammatory Effects of Rosa damascene Hydroalcoholic Extract and its Essential Oil in Animal Models, Iranian J Pharm Res. 2010; 9 (2): 163-168.
14. Mahboubi M. Rosa damascena as holy ancient herb with novel applications. J Tradit Complement Med. 2015; 6(1): 10-16.
15. Dolati K, Rakhshandeh H, Shafei MN. Antidepressant-like effect of aqueous extract from *Rosa damascena* in mice. Avicenna J Phytomed. 2011; 1(2): 91-97.
16. Akbari M, Kazerani HR, Kamrani A, Mohri M. A preliminary study on some potential toxic effects of Rosa damascena Mill. Iran J Vet Res. 2013; 14(3): 232-236.
17. Gholamhoseinian A, Fallaha H, Sharififar F. Inhibitory effect of methanol extract of Rosa Damascena Mill. Flowers on a-glucosidase activity and postprandial hyperglycemia in normal and diabetic rats. Phytomedicine. 2009;16: 935-941.
18. Achuthan CR, Babu BH. Padikkala Antioxidant and Hepatoprotective Effects of Rosa damascena. Pharm Biol. 2003; 41(5): 357-361.
19. Baydar NG, Baydar H. Phenolic compounds, antiradical activity and antioxidant capacity of oil-bearing rose (Rosa Damascena Mill.) extracts. Ind Crops Prod. 2013; 41: 375-380.

20. Yassa N, Masoomi F, Rohani Rankouhi SE, Hadjiakhoondi A. Chemical Composition and Antioxidant Activity of the Extract and Essential oil of Rosa damascena from Iran, Population of Guilan. DARU J Pharm Sci. 2009; 17: 175-180.
21. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968; 25(1): 192-205.
22. Hillegass LM., Griswold DE, Brickson B, Albrightson-Winslow C. Assessment of myeloperoxidase activity in whole rat kidney. J Pharmacol Methods. 1990; 24(4): 285-295.
23. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978; 52: 302-10.
24. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974; 249(22): 7130-9.
25. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996; 239(1): 70-6.
26. Zolali E, Hamishehkar H, Maleki-Dizaji N, Majidi Zolbanin N, Ghavimi H, Kouhsoltani M, et al. Selenium effect on oxidative stress factors in septic rats. Adv Pharm Bull. 2014; 4(3): 289-293.
27. Abd El Tawab AM, Shahin NN, Abdel Mohsen MM. Protective effect of Saturejamontana extracts on cyclophosphamide-induced testicular injury in rats. Chem Biol Interact. 2014; 5(224): 196-205.
28. Makled MN, El-Awady MS, Abdelaziz RR, Atwan N, Guns ET, Gameil NM, et al. Pomegranate protects liver against cecal ligation and puncture-induced oxidative stress and inflammation in rats through TLR4/NF-B pathway inhibition. Environ Toxicol Pharmacol. 2016; 43: 182-192.
29. Cuzzocrea S, Mazzon E, Dugo L, Serrano I, Ciccolo A, Centorrino T, et al. Protective effects of n-acetylcysteine on lung injury and red blood cell modification induced by carrageenan in the rat. FASEB J. 2001; 15(7): 1187-200.
30. Mohamadin A.M, Elberry AA, Abdel Gawad HS, Morsy GM, Al-Abbasi FA. Protective effects of simvastatin, a lipid-lowering agent, against oxidative damage in experimental diabetic rats. J Lipids. 2011; 2011: 1-13.
31. Sener G, Arbak S, Kurtaran P, Gedik N, Yeğen BC. Estrogen protects the liver and intestines against sepsis-induced injury in rats. J Surg Res. 2005; 128: 70-78.

32. Ozdulger A, Cinel I, Koksel O, Cinel L, Avlan D, Unlu A, et al. The protective effect of N-acetylcysteine on apoptotic lung injury in cecal ligation and puncture-induced sepsis model. SHOCK. 2003; 19(4): 366-372.
33. Zhong W, Qian K, Xiong J, Ma K, Wang A, Zou Y. Curcumin alleviates lipopolysaccharide-induced sepsis and liver failure by suppression of oxidative stress-related inflammation via PI3K/AKT and NF-kB related signaling. Biomed Pharmacother. 2016; 83: 302-313.
34. Bouzenna H, Dhabi S, Sammut N, Rjeibi I, Talarmin H, Elfeki A, et al. The protective effect of Citrus limon essential oil on hepatotoxicity and nephrotoxicity induced by aspirin in rats. Biomed Pharmacother. 2016; 83: 1327-1334.
35. Dadkhah A, Fatemi F, Kazemnejad S, Rasmi Y, Ashrafi-Helan J, Allameh A. Differential effects of acetaminophen on enzymatic and non-enzymatic antioxidant factors and plasma total antioxidant capacity in developing and adult rats. Mol Cell Biochem. 2006; 281: 145-52.
36. Frencken JF, Van Vught LA, Peelen LM, Ong DS, Klouwenberg PMK, Horn J, et al. An Unbalanced Inflammatory Cytokine Response Is Not Associated With Mortality Following Sepsis: A Prospective Cohort Study. Critical care medicine. 2017; 45(5): e493-e499.
37. Huang YH, Tsai PS, Huang CJ. Bupivacaine inhibits COX-2 expression, PGE2, and cytokine production in endotoxin-activated macrophages. Acta Anaesthesiol Scand. 2008; 52: 530-535.
38. Bin Li MM, Min Li Y, Li X, Shi B, Ming Yan He MM, Xiao Liang Zhu MM, et al. COX-2 inhibition improves immune system homeostasis and decreases liver damage in septic rats. J Surg Res. 2009; 157: 43-47.
39. Hussein RRS, Soliman RH, Abdelhaleem Ali AM, Tawfeik MH, Abdelrahim MEA. Effect of antiepileptic drugs on liver enzymes. Beni-Seuf Univ J Appl Sci. 2013; 2(1): 14-19.

## FIGURE CAPTIONS

Figure 1: DPPH radical-scavenging activity of *Rosa Damascena* essential oil.
The different letters are significantly different at the $p < 0.05$ level. Samples were done in triplicate ($n = 3$).

Figure 2**:** β-Carotene/linoleic acid bleaching assay of *Rosa Damascena* essential oil The different letters are significantly different at the p **<** 0.05 level. Samples were done in triplicate (n = 3).

Figure 3: A) concentration of aspartate transaminase (AST) in different groups B) concentration of alanine transaminase (ALT) in different groups C) concentration of alkaline phosphatase (ALP) in different groups D) concentration of bilirubin in different groups. In control group rats undergo just laparotomy after 14 days administering DMSO as orally. In CLP group animals received just DMSO for 14 days. RD50 and RD100, essential oil (50 and 100 mg/kg.bw) was administered orally in 14 days and CLP operation was done. IND, treated as CLP group, with the differences that rats received indomethacin orally incubation. Values represent Mean ± SD of the each group.

RD: *Rosa Damascena*

*P < 0.05 is considered significantly different from control group within each parameter.

**P < 0.05 is considered significantly different from CLP group within each parameter

Figure 4: Histopathological studies. A) Control group, the portal tract and the hepatocytes in normal condition. B1) CLP group, neutrophil infiltration in the portal tract (arrows). B2) CLP group, neutrophil infiltration in the sinusoids which can be seen easily with their dark nucli (arrows). C) RD 50, the liver in normal condition without any neutrophil infiltration. H&E, 400*. D) RD 100 group, the portal tract and parenchyma in normal condition without any neutrophil infiltration. H&E, 400*.E) Indomethacin group, there isn't any sign of neutrophil infiltration in the portal tract or in the parenchyma. H&E, 400*.

**Table 1:** GC analysis of the *Rosa Damascena* essential oils

| | Coumpond | Percentage | RI |
|---|---|---|---|
| 1 | α-Pinene | 1.81 | 920.967742 |
| 2 | Sabinene | 0.08 | 953.225806 |
| 3 | β-Pinene | 0.3 | 956.989247 |
| 4 | Myrcene | 0.36 | 965.591398 |
| 5 | Linalool | 1.42 | 1056.45161 |
| 6 | Rose oxide (Isomer) | 0.47 | 1065.5914 |
| 7 | Phenylethyl alcohol | 5.33 | 1072.04301 |
| 8 | Rose oxide (Isomer) | 0.22 | 1079.56989 |
| 9 | Citronellol | 66.11 | 1162.84153 |
| 10 | Carvone | 0.76 | 1176.50273 |
| 11 | Trans Geraniol | 11.56 | 1181.42077 |
| 12 | Citral | 1.11 | 1194.53552 |
| 13 | Citronellol acetate | 0.69 | 1252.40964 |
| 14 | Eugenol | 0.83 | 1263.25301 |
| 15 | Nerol acetate | 0.89 | 1277.71084 |
| 16 | Methyl eugenol | 2.36 | 1297.59036 |
| 17 | Caryophyllene (isomer) | 0.69 | 1315.06024 |
| 18 | α-Guaiene | 0.54 | 1326.50602 |
| 19 | Caryophyllene (isomer) | 0.52 | 1340.96386 |
| 20 | Germacrene | 1.44 | 1362.04819 |
| 21 | Bisabolene | 0.25 | 1373.49398 |
| 22 | Bulenensene | 0.41 | 1378.31325 |
| 23 | Tetradecane | 1.41 | 1510.13514 |
| 24 | Farnesol | 0.45 | 1534.45946 |

**Figure 1**

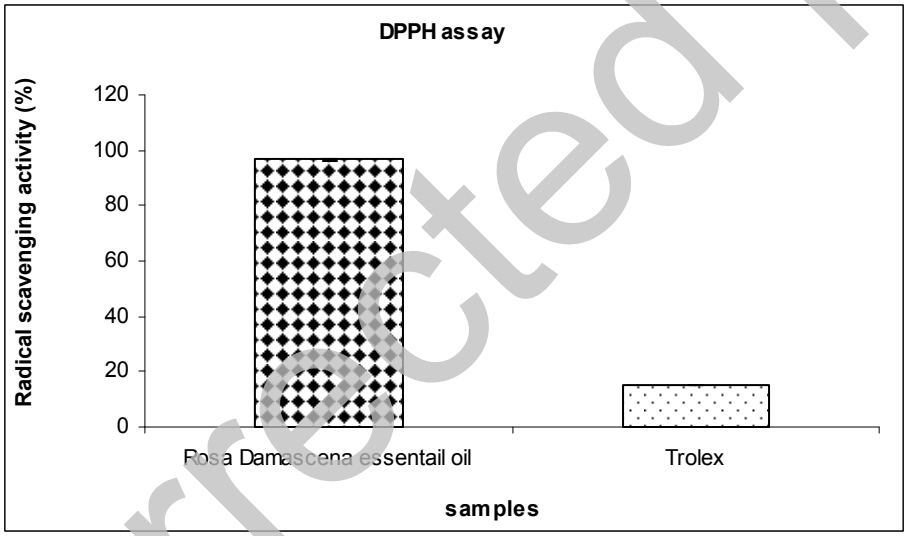


**Figure 2**

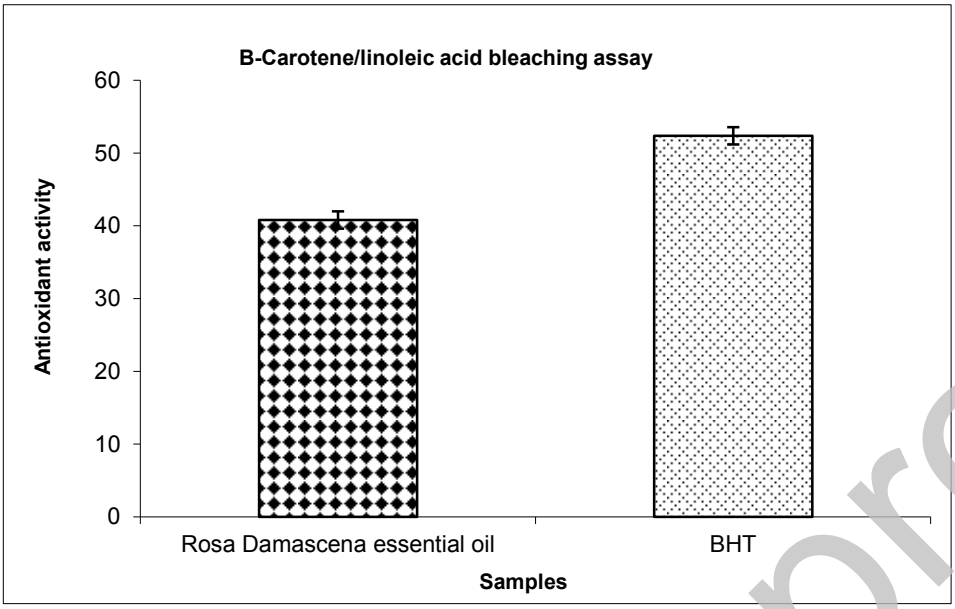


**Table 2:** Effect of *Rosa Damascena* essential oil on oxidative stress parameters

| Groups | GST (n mol/min/ mg protein) | GSH (n mol/ mg protein) | MDA (n mol/ mg protein) | MPO (U/mg protein) | FRAP (µmol/L) |
|---|---|---|---|---|---|
| Control | 1126 ± 61.61 | 11.42 ± 1.1 | 10.34 ± 1.18 | 9.46 ± 0.7 | 407 ± 21.76 |
| CLP | 1173 ± 32.11 | 7.28 ± 0.67* | 18.51 ± 1.53* | 26.13 ± 0.7* | 257 ± 10.98* |
| RD50 | 1838 ± 38.39 | 13.51 ± 0.78** | 12.66 ± 0.93** | 11.06 ± 0.92** | 377 ± 9.8** |
| RD100 | 2044 ± 85.88 | 13.58 ± 0.61** | 11.78 ± 0.84** | 10.46 ± 0.98** | 367 ± 12.18** |
| IND | 1076 ± 48.22 | 11.26 ± 0.95** | 11.8 ± 0.87** | 6.58 ± 0.2** | 280 ± 18.2 |

* P < 0.05 is considered significantly between control group and CLP group. **P < 0.05 is considered significantly between CLP group and treated groups. Data are presented as mean ± SD.

**Table 3:** Effect of *Rosa Damascena* essential oil on COX-2 expression and PGE2 level in CLP rats

| Groups | PGE2 (ng/ml) | COX-2 expression ($log_{10}$) |
|---|---|---|
| Control | 508 ± 26.7 | 0 ± 0.03 |
| CLP | 796 ± 20.7* | 0.43 ± 0.05* |
| RD50 | 632 ± 23.9** | 0.21 ± 0.03** |
| RD100 | 531 ± 8.2** | 0.25 ± 0.04** |
| IND | 536 ± 32.8** | 0.15 ± 0.01** |

*P < 0.05 is considered significantly between control group and CLP group. **P < 0.05 is considered significantly between CLP group and treated groups. Data are presented as mean ± SD

**Figure 3**

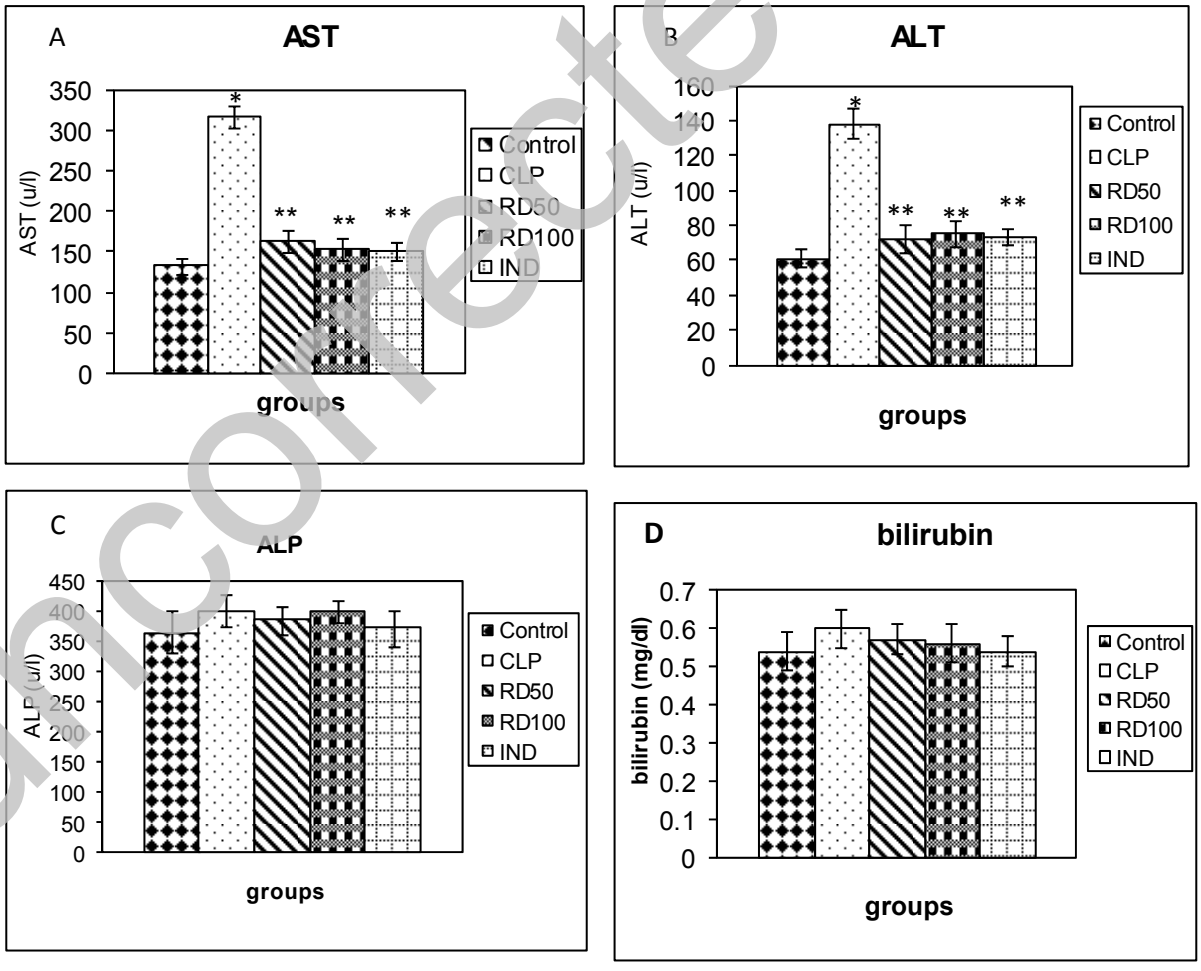

**Table 4:** Mean values and standard error of histopathologic variables of the liver specimens in the study groups

| Study groups | Neutrophil margination and infiltration | Granular degeneration | Inflammatory foci | Mononuclear cells infiltration &kupffer cell hyperplasia | Portal inflammation |
|---|---|---|---|---|---|
| Control | 0 ± 0 | 0.4 ± 0.24 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| CLP | 2.75 ± 0.25* | 0.75 ± 0.75 | 1.5 ± 0.86 | 3 ± 0.4* | 2.25 ± 0.25* |
| RD50 | 1.4 ± 0.24** | 0.2 ± 0.2 | 0.2 ± 0.2 | 1.2 ± 0.2** | 0.6 ± 0.24** |
| RD100 | 0.8 ± 0.2** | 0.2 ± 0.2 | 0 ± 0 | 1.6 ± 0.24** | 1 ± 0** |
| IND | 0.5 ± 0.28** | 0 ± 0 | 0 ± 0 | 1 ± 0** | 0.25 ± 0.25** |

*: Having significant difference in comparison with the control group
**: Having significant difference in comparison with the CLP group

**Figure 4**