Anti-Inflammatory Effects of Deuterium-Depleted Water plus *Rosa damascene* Mill. Essential Oil via Cyclooxygenase-2 Pathway in Rats

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ABSTRACT

Objectives: Natural medicine has been proposed in treating of sepsis in the worldwide. So, in this study, the effect of deuterium-depleted water (DDW) alone and adjuvant with *Rosa damascene* Mill. essential oils (RD) was considered through the evaluation of oxidative stress/antioxidant parameters and the expression of cyclooxygenase-2 (COX-2) inflammatory gene in liver damage caused by sepsis.

Materials and Methods: The rats were randomly divided into 5 groups: 1) laparotomy (LAP) group; 2) cecal ligation and puncture (CLP) group; 3) DDWs (15ppm and 30 ppm doses) groups; 4) DDWs (15ppm and 30 ppm doses) plus RD (100mg/kg.bw); 5) indomethacin (2 mg/kg.bw) as a positive control. The treatments were daily done for two weeks and creation of CLP model was occurred on the 15th day. Then, the animals were killed and their liver tissue were separated for histopathologic and biochemical assessments.

Results: Our results demonstrated that the treatment of animals with DDWs and DDWs plus RD were effective due to regulating the oxidative stress/antioxidant parameters including lipid peroxidation (LP), glutathione (GSH), glutathione s-transferases (GST), myeloperoxidase (MPO), ferric reducing ability of plasma (FRAP) and inflammatory parameters such as prostaglandin E2 (PGE2) and COX-2. Pathological studies also showed that sepsis led to the liver tissue injuries, which can be reduced by treatments.

Conclusion: Sepsis caused oxidative stress resulting in the liver tissue, but the administration of DDWs and DDWs plus RD can be useful to prevent and improve these injuries.
Key words: Deuterium-depleted water, *Rosa damascene* Mill., Essential oil, CLP, Oxidative stress/antioxidant parameters, Sepsis

INTRODUCTION

Natural products are increasingly becoming one of the most important resources for replacing the chemical compounds. They will undergo continual use toward meeting the urgent need to develop effective drugs, and they will play a leading role in the discovery of drugs for treating human diseases, especially chronic diorders.[1]

Deuterium-depleted water (DDW) known as light water has less concentration of the naturally occurring deuterium (20–25 ppm vs. 150 ppm).[2] The use of DDW for a longer period may reduce the concentration of deuterium in liquids and tissues of organisms due to isotopic exchange reactions. These reactions may impact cellular cycle and cell proliferation[3,4]. Previous studies represented that a decrease amount of deuterium in drinking water has possessed different biological activities such as anticancer, antioxidant and chemo preventive properties. [5-7]

On the other hand, our recent study reported that *Rosa (R.) damascene* Mill. essential oils with main chemical compositions; citranellol (66.11 %), transgeraniol (11.56 %), and phenylethyl alcohol (5.33 %) had antioxidant and anti-inflammatory effects in sepsis model.[8] *R. damascena* Mill. belonging to the family of Roseaceae[9], is one of the most valuable sources of flavors and fragrances worldwide which possessed some applications in medicine and food industry.[10]

A study showed that *R. damascena* Mill. has beneficial effects on the treatment of various disorders *e.g.* inflammatory reactions, premenstrual breast tenderness and spasms.[11] It is traditionally used for the treatment of abdominal and chest pain and depression.[12] Seveval biological activities of *R. damascene* Mill. essential oil have also been reported including the analgesic, anti-tussive, antidepressant, antispasmodic, antioxidant and anti-HIV activities.[13-16] A study showed that the oil extracted from *R. damascene* Mill. exhibited the antimicrobial

activity against a large number of microorganisms especially against *Proteus vulgaris* and *Klebsiella pneumonia*.[17]

Regarding the beneficial therapeutic properties of these natural products, their probable anti-inflammatory and antioxidant effects on treating severe diseases such as sepsis should be considered.

Sepsis is a systemic body reaction to invasive microorganisms like bacteria and fungi. It is one of the top ten main causes of death among all patients admitted to the hospital. It causes inflammation, microvascular damage and coagulopathy, haemodynamic instability, multiple organ dysfunction and immunosuppression. It is an important medical problem all over the world and is the most common cause of death among critically ill patients.[18, 19] The cecal ligation and puncture (CLP) model as a stable, repetitive, and applicable model leads to the pollution of the abdominal cavity by bacteria-carrying intestinal contents and induces a wide range of systemic inflammatory responses leading to sepsis.[20, 21] In the CLP model, bacteria spreading from infection sites and entering the bloodstream are rapidly trapped in the many organs such as liver, kidney, lung, and spleen and bound to the surface of specific target cells and macrophages in the target organ and subsequently killed by infiltrated neutroph.[22, 23] The organs are damaged in mice with lethal sepsis induced by CLP and also in humans with sepsis. This injury is mainly associated with ineffective bacterial clearance, leading to bacterial dissemination and high mortality rates (Moreno et al. 2006).[24] Several reports have demonstrated that inflammatory cytokines can serve as both makers and mediators of the severity of sepsis and the elevated levels of these cytokines predict mortality following CLP. [24-26]

Regarding to the increase of resistance and side effects of antibiotics and synthetic drugs in sepsis treatment, natural products with high antibacterial and antioxidant capacities could be a suitable alternative. In the current study, the inflammation was induced by CLP inflammatory model in rats in order to consider the preventive anti-inflammatory effects of DDW alone and concomitant with RD through the estimation of cyclooxygenase-2 (COX-2) gene expression and prostaglandin E2 (PGE2) as well as the assessment of oxidative stress/antioxidant parameters such as glutathione (GSH), lipid peroxidation (LP), glutathione s-transferases (GST), myeloperoxidase (MPO) and ferric reducing ability of plasma (FRAP).

MATERIALS AND METHODS

Plant materials and DDW preparations

DDW (15 and 30 ppm) prepared from Atomic Energy Organization of Iran was applied for our study. Also, the essential oils from *R. damascene* Mill. (RD) were prepared from Barij Essence Pharmaceutical Co, Kashan Iran (Batch No: 714043, sample Serial No: AE932009).

Animals

The study was carried out on 70 male Wistar rats (200-250 g). Rats were kept under standard condition (12 h light/ 12 h dark) at 20-25 ºC for two weeks. The animal studies had been approved by the Medical Ethics Committee of Tarbiat Modares University based on the World Medical Association Declaration of Helsinki. CLP model was used to cause sepsis in rats.[8]

The rats (10 rats in each group) were randomly divided into 7 groups: 1) laparotomy (LAP) group (laparotomy) as a negative control group; 2) CLP group as a control group; 3) DDWs: the rats received orally DDW (at dose of 15 ppm and 30 ppm) for two weeks; 4) DDWs + RD: the rats received RD at 100 mg/kg.bw dose plus DDW 15 ppm and 30 ppm for two weeks 5) Indomethacin: the rats received orally 2 mg/kg.bw indomethacin as positive control group. After 15 days, CLP surgery was done in all groups.

After 24 h of CLP surgery, rats were anesthetized and the heparinated blood samples were collected by heart puncture from all the animals and centrifuged at 3000 *g* for 10 min to obtain the plasma. Then, the rats were killed and liver were removed and processed for histological and biochemical assays.

Assessment of PGE2

Plasma prostaglandin E2 level was measured using the Enzyme-linked Immunosorbent assay kit (ELISA Kit; BioAssay System) according to the producer's instructions.

Assessment of COX-2 gene expression

Total RNA from liver tissues was prepared with the RNA total kit (BioBasic Inc, Canada). cDNA was synthesized with PrimeScript ™ RT reagent kit (Takara bio Inc, Japan) and oligo dt primers (Takara bio Inc, Japan), according to the manufacturer's protocol.

Then, the primers for PCR were designed with the Gene Runner software Version 3.05 and primer 3 servers (COX2 forward: 5'ACCTCTGCGATGCTCTTC3'; COX2 reverse: 5' AGGAATCTCGGCGTAGTAC3'; GAPDH forward: 5' TGCCAGCCTCGTCTCATAG 3'; GAPDH reverse: 5' ACTGTGCCGTTGAACTTGC 3'). Blast N searches were used to check

primer specificity. The cDNA samples were amplified by PCR amplification and then checked by 2.5% agarose gel electrophoresis to ensure whether PCRs contained a product with the expected size.

The relative-expression of selected gene was carried out with real-time PCR System (Rotor-Gene Q- QIAGEN). The reaction mixture contained of 5 µL SYBR Green real-time PCR Master Mix (QIAGEN) which encloses Taq DNA polymerase, dNTP, $MgCl_2$ and SYBR Green I dye, 0.2 µL of a 10 mM solution of sense/anti-sense primer, 0.5 µL of template cDNA added with H_2O to a total of 10 µL. The negative controls were also designed as above excluded cDNA. Thermal cycling conditions were carried out by an initial denaturation stage at 95°C for 2 min, followed by 40 cycles at 95°C for 15 s, 60°C for 20 s, and 72°C for 20 s. 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. All expression data were normalized using GAPDH expression as the internal standard.

Assessment of Antioxidant and liver parameters

GSH test

It was estimated in liver homogenates according to the procedure of Seldak and Lindsay (1968). [27] A weighed portion of the liver was homogenized in ethylenediaminetetraacetic acid (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 supernatantwas 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.

LPtest

The concentration of thiobarbituric acid reactive substances (TBARS) as an indicator for LP production was measured spectrophotometrically using thiobarbituric acid (TBA) reagent based on the procedure described by Buege and Aust (1978).[28]

GST test

GST was measured spectrophotometrically using 1-chloro-2, 4-dinitrobenzene (CDNB) (a general substrate) according to the procedures described by Habig (1974). [29]

FRAP test

FRAP was performed using 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) reagent as described by Benzie and Strain (1996).[30] FRAP level was calculated by plotting a standard curve of absorbance against μmol/L concentration of Fe (II) standard solution.

MPO test

MPO activity was measured, with minor modification, according to the procedure of Hillegass (1990). [31]

Assessment of liver enzymes

To confirm the liver function and injury, serum alanine transminase (ALT), asparate transminase (AST) (Pars Azmoon, Co, Iran), alkaline phasphatase (ALP) (Ziest Chem Diagnostics Co, Iran) and total bilirubin (BILI) (Darman Faraz Kave Co, Iran) were determined spectrophotometrically according to the procedure described in the kit purchased.

Histological analysis

Liver biopsies were collected from all the control and treated animals after 24 h sepsis induction. The tissue samples were fixed in 10% phosphate-buffered saline of formaldehyde solution. Dehydration was performed in graded ethanol, embedded in paraffin, section 5 μm was stained with haematoxylin and eosin (H&E). For histopathological analysis, the sections were examined by light microscopy (Olympus CX31RBSF). The histological changes were quantitatively and semi-quantitatively analyzed by a veterinary pathologist. The histologic index was scored 0 (minimal) to 4 (maximal); 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. Scoring system included: Zero (0) for normal condition, 1+ for the mild changes, 2+ for the average changes, 3+ for severe changes and 4+ score for more severe changes.

Statistical analysis

The analysis of data was performed statistically with Statistical Package for the Social Sciences (SPSS) v.19. Analysis of data was expressed as mean ± SE. One way analysis of variance (ANOVA) was applied to compare the mean values. The normal distribution of the data was examined by Kolmogorov-Smirnov normality test. P-value of less than 0.05 was statistically considered as significant value.

Results

Effect of DDWs and DDWs plus RD on PGE2 and COX-2 in sepsis

The results indicated that the level of PGE2 value increased as evident by significant rise ($P<0.05$) in the level of COX-2 in CLP group. However, the administration of DDWs reduced considerably ($P<0.05$) the level of COX-2 compared to CLP group. Indeed, the PGE2 level returned significantly ($P<0.05$) to the normal levels after using DDWs plus RD ($P<0.05$) but there was no significant changes ($P>0.05$) in COX-2 gene expression. Likewise, indomethacin as a positive control group decreased significantly ($P<0.05$) the levels of PGE2 and COX-2 gene expression when compared to CLP group (Table 1).

Effect of DDWs and DDWs plus RD on oxidative stress/antioxidant parameters in sepsis

As shown in Table 2, the levels of LP and MPO significantly ($P<0.05$) increased in the CLP group, while the level of FRAP went down remarkably ($P<0.05$). Also, sepsis led to a significant decrease in the liver GSH as compared to the LAP group ($P<0.05$). The DDWs and DDWs plus RD restored the levels of LP, MPO, and GSH in comparison to CLP group. But, the administration of DDWs plus RD could return the level of FRAP to the normal one ($P>0.05$). Meanwhile, Administration of indomethacin to rats showed the same results of treatment groups ($P<0.05$). Whereas, GST level did not show any significant changes ($P>0.05$) in all groups even after using Indomethacin as a positive control group (Table 2).

Effect of DDWs and DDWs plus RD on liver enzymes in sepsis

In this study the levels of AST and ALT significantly increased ($P<0.05$) as compared to LAP group (Table 3). In contrast, the rats pretreated with DDWs and DDWs plus RD surprisingly ($P<0.05$) restored the AST and ALT levels as compared to CLP group. Similarly, indomethacin (2 mg/kg.bw) as positive control group could return the levels of AST and ALT to the normal levels ($P<0.05$) (Table 2). Whereas, the levels of ALP and BILI had no remarkable changes in all groups even after using DDWs and DDWs plus RD (Table 3).

Histological findings

There were some mild changes of the hepatocytes in LAP group (Fig. 1A). 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 change 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 (Fig. 1B1&B2). The treated groups improved the histopathological lesions except DDW30 plus RD treated group. The portal tract and the parenchyma were nearly in normal condition in DDW15 and DDW30 treated groups (Fig. 1 C&D). Also, the presence of a few neutrophils in the sinusoids of DDW15 plus RD treated group was observed (Fig. 1. E). However, there were neutrophil infiltrations in the sinusoids in the DDW30 plus RD treated group. The kupffer cells which show hypertrophia and hyperplasia are also obvious in this group (Fig. 1 F). Also, in indomethacin group, reduced amount of neutrophils were seen (Fig. 1 G).

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 LAP group ($P \leq 0.05$). Concerning portal inflammation, it was also meaningful in the CLP group in comparison with the LAP group ($P \leq 0.05$). However, there weren't obvious difference regarding granular degeneration and inflammatory foci between all study groups ($P > 0.05$). All the treatment groups, except E.O100+DDW30 treated group 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

Our previous results demonstrated that medicinal plant with bioactive constituents such as *R. damascene* Mill. significantly affect oxidative stress/antioxidant parameters and detoxifying enzymes as well as COX-2 gene expression.[8] There is also an evidence indicating the hepatoprotective activity of deuterium depleted water against acetaminophen.[7] Following this, the present study was designed to consider, for the first time, the therapeutic efficacy of DDWs and DDWs plus RD against liver injury induced by CLP in septic rats.

Our results revealed that the sepsis induced by CLP rose significantly ($P < 0.05$) the levels of LP and MPO along with PGE2 level and COX-2 expression. Likewise, the levels of AST and ALT activities went up sharply due to CLP surgery compared with LAP group (Table 3). While, there was a considerable decrease in the amount of GSH and FRAP (as an important factor in the oxidative stress/antioxidant balancing) in comparison with the LAP group (Tables. 1, 2 & Fig. 1).

Sepsis reflects a systemic inflammatory syndrome in response to an infection and represents the leading cause of death in the intensive care unit. During the process of sepsis, the liver plays an important role in defensive responses to scavenge bacteria and produce inflammatory mediator.[32] Recent studies have also observed that the liver dysfunction as an early event in sepsis.[33] The hepatocellular liver enzymes; AST and ALT have been regarded as markers of liver injury (Alonso et al., 2014). Our results (Table 3) clearly showed that the sepsis increased the liver enzymes such as AST and ALT caused by liver damage. Biochemical results with the histological findings (Fig. 1) confirmed that the pathophysiological changes in the liver function damaged by sepsis. Other studies also proved that there is a direct link between the oxidative stress conditions and organ injuries in the CLP model.[34,35] In addition, initiation of oxidative stress was identified by the increase of MDA level.[36] GSH also plays a principal role in preventing the cells from oxidative damages.[367] Therefore, the fall of GSH level in hepatic in septic groups and the raise of LP demonstrated that sepsis promoted destruction in balancing antioxidants and oxidative stress. Whereas, MPO is a protein in neutrophils that participates in the early inflammatory process in patients with sepsis [38, 39], its elevation in septic animals concomitant with LP production leaded to hepatic dysfunction.

Furthermore, COX-2 as an early expressed gene is not only detected in most normal tissues, but also it is induced by stimuli such as pro-inflammatory cytokines [40] leading to PGE2 production which acts on neurons and contributes to the systemic responses to inflammation [41]. In our study, the increase in the level of COX-2 expression was detected in septic rats as well as PGE2 concentration in plasma level compared to the control group (Table 1).

Regarding to the importance of septic treatment, studies confirmed that the main role of antioxidants in reducing the tissue damages due to scavenge the free radicals [8, 42- 44]. To confirm, our results demonstrated that the administration of DDWs and DDWs plus RD were effective in sepsis treatment, where the levels of LP, MPO and GSH returned to the normal levels. Also, these treatments significantly ($P<0.05$) protected the the liver (based on histological analysis) and decreased the AST and ALT levels as compared to CLP group. The PGE2 level also fell considerably ($P<0.05$) after using DDWs alone and the combination of DDWs with RD. The COX-2 gene expression diminished when the rats treated with only DDWs. While, there was no considerable changes in DDWs plus RD groups which may be due to transient state of expression of some genes in sepsis. In fact, the natural agents (DDWs and DDWs+RD) protect the liver from injuries in sepsis model as potent as indomethacin -

non-steroidal anti-inflammatory drug (NSAID)- used clinically for their anti-inflammatory, anti-pyretic and analgesic properties (Table 2, 3 & Fig 1).

The reduction of deuterium content in the body's liquids due to isotope metabolism reactions is the main effect of DDW as light water. The decrease of this element's concentration in erythrocytes, in blood plasma and in homogenate of laboratory animals' hearts can be occurred with the use of water with low deuterium content. Such changes induce in their turn recover of pro-oxidant/anti-oxidant system balance and decrease of pro-oxidant load in organism which is further accompanied with higher immunity of laboratory animals. [45 456]

One study proved that DDW with its antioxidant property was effective in controlling of liver against acetaminophen toxicity.[47] We demonstrated that DDW alone and combination with *Satureja rechingeri* essential oil had synergistic effects in prevention of acetaminophen induced hepatotoxicity in rat due to the reduction of oxidative stresses.[7] Other research was reported that the DDW pre-treatment protecteed the liver from the chromium toxicity by restoring the levels of AST and ALT activities.[48] Furthermore, DDW have anticancer action due to the influence on gene expression regulation and consequently on protein biosynthesis.[49]

Moreover, the protective effects of the oils may be due to its antioxidant activity and free radical scavenging effects of phenolic compounds and flavonoids presented in the oils. Our current study indicated the *in vivo* anti-inflammatory activities of *Rosa damascena* essential oils may be associated with their antioxidant compounds namely citranellol, trans geraniol and phenylethyl alcohol as the main constituents of the essential oils which proved antioxidant activities by DPPH and β-carotene/linoleic acid bleaching assays.[8] Previous study also revealed that the essential oil of rosemary containing antioxidant compounds has a strong antioxidant and hepatoprotective activities by modulating the malondialdehyde (MDA) and GSH levels and also catalase (CAT), peroxidase (Px), glutathione peroxidase (GPx), and glutathione reductase (GR) activities. The study showed that hepatoprotective activity can be attributed to 1, 8-cineole, as its major compound as well.[50] Nithianantham et al., also (2011) reported that the hepatoprtective activity of *Clitoria ternatea* leaf may be due to its free radical-scavenging and antioxidant activity.[51] One study reported that the treatment rats with ethyl acetate extracted from *A. cochinchinesis* root suppressed inflammatory responses through inhibition of NO, COX-2 and reactive oxygen species (ROS) productions.[52]

CONCLUSION

The current findings indicated that the pretreatment of rats with DDWs and DDWs plus RD exerted beneficial effects on the prevention of liver damages, induced by CLP inflammatory model, through not only reducing the levels of liver enzymes and oxidative stress/antioxidant parameters, but also through the balance of COX-2 and PGE2 levels. The histopathological studies proved that the hepatic injuries were improved via the administration of DDWs and DDWs plus RD as well.

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FIGURE CAPTION

Fig 1: Histopathological studies. A) LAP 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) DDW15 group, the portal tract and the parenchyma in normal condition. H&E, 400*. D) DDW30 group, the portal tract and the parenchyma in normal condition. H&E, 400*. E) DDW15+RD, presence of a few neutrophils in the sinusoids (thin arrows). Kupffer cells also could be seen in the picture (thick arrows). H&E,400*. F) DDW30+RD group,neutrophils infiltration in the sinusoids (thin arrows). The Kupffer cells which show hypertrophia and hyperplasia also are obvious (thick arrows). H&E, 400*. G) Indomethacin group, a few infiltrated neutrophils (arrows) could be seen in the picture. H&E, 400*.

Table 1: The effect of DDWs and DDWs+RD on PGE2 and COX-2 gene expression in septic rats

Groups	PGE2 (ng/ml)	COX-2 gene expression
LAP	508 ± 26.7	0± 0.03
CLP	796 ± 20.7[a]	0.43 ± 0.05[a]
DDW15	584 ± 18.4[b]	0.32 ± 0.03[b]
DDW30	709 ± 18[b]	0.23 ± 0.02[b]
RD100+DDW15	486 ± 24.6[b]	0.48± 0.05
RD100+DDW30	530 ± 17.4[b]	0.45 ± 0.05
Indomethacin	536± 32.8[b]	0.15± 0.11[b]

[a] P<0.05 is considered significantly between LAP and CLP groups. [b] P<0.05 is considered significantly between CLP and treatment groups.

Table 2: The effect of DDWs and DDWs+RD on onoxidative stress/antioxidant parameters in septic rats

Groups	LP (pmol/mg protein)	GSH (nmol/mg protein)	MPO (U/mg protein)	GST (nmol/min/mg protein)	FRAP (µmol/L)
LAP	10.34 ± 1.18	11.42 ± 1.1	9.46 ± 0.7	1126 ± 61.61	407 ± 21.76
CLP	18.51 ± 1.53[a]	7.28 ± 0.67[a]	26.13 ± 0.7[a]	1173 ± 32.11	257 ± 10.98[a]
DDW15	11.95 ± 1[b]	9.86 ± 0.75[b]	18.9± 0.98[b]	1355 ± 46.02	265 ± 15.54
DDW30	12.39 ± 1.04[b]	9.65 ± 0.75[b]	18.34± 1.89[b]	1246 ± 52.97	246 ± 9.7
RD+DDW15	10.23 ± 0.908[b]	13.9 ± 1.03[b]	10.34± 0.75[b]	1976 ± 66.67	374 ± 7.33[b]
RD+DDW30	11.15± 0.86[b]	14.25 ± 1[b]	11.24± 0.8[b]	1878 ± 45.09	383 ± 6.2[b]
Indomethacin	11.8± 0.87[b]	11.26± 0.95[b]	6.58 ± 0.2[b]	1076± 48.22	280± 18.2[b]

[a]P<0.05 is considered significantly between LAP and CLP groups. [b]P<0.05 is considered significantly between CLP and treatment groups.

Table 3: The effect of DDWs and DDWs+RD the on liver enzymes in septic rats

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	BILI (mg/dl)
LAP	132 ± 9.58	61 ± 5.35	364 ± 33.8	0.54± 0.05
CLP	317 ± 13.58[a]	136 ± 8.76[a]	400 ± 25.8	0.6 ± 0.05
DDW15	168 ± 11.76[b]	74 ± 7.63[b]	394 ± 33	0.59± 0.04
DDW30	171 ± 9.91[b]	78 ± 8.01[b]	377 ± 30.8	0.58 ± 0.04
RD+DDW15	135 ± 7.92[b]	64 ± 5.93[b]	357 ± 27.4	0.55± 0.04
RD+DDW30	143 ± 10.06[b]	67 ± 6.65[b]	368 ± 20.5	0.55 ± 0.04
Indomethacin	150± 11.72[b]	73± 4.48[b]	371± 30	0.54± 0.04

[a] $P<0.05$ is considered significantly between LAP and CLP groups.[b] $P<0.05$ is considered significantly between CLP and treatment groups.

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

Groups	Neutrophil margination and infiltration	Granular degeneration	Inflammatory foci	Mononuclear cells infiltration &kupffer cell hyperplasia	Portal inflammation
LAP	0 ± 0	0.4 ± 0.24	0 ± 0	0 ± 0	0 ± 0
CLP	2.75 ± 0.25[a]	0.75 ± 0.75	1.5 ± 0.86	3 ± 0.4[a]	2.25 ± 0.25[a]
DDW15	0.4 ± 0.24[b]	0.4 ± 0.24	0 ± 0	1.4 ± 0.4[b]	0.4 ± 0.24[b]
DDW30	1 ± 0[b]	0 ± 0	0.8 ± 0.8	0.8 ± 0.2[b]	0.2 ± 0.2[b]
RD+DDW15	1.4 ± 0.24[b]	0 ± 0	0.2 ± 0.2	1.4 ± 0.24[b]	1 ± 0[b]
RD+DDW30	3 ± 0	0.8 ± 0.37	0.4 ± 0.4	3 ± 0	3 ± 0
Indomethacin	1. 6 ± 0.16[b]	0.29 ± 0.19	0.45 ± 0.21	1.3 ± 0.21[b]	0.5 ± 0.21[b]

[a]P<0.05 is considered significantly between LAP and CLP groups. [b] P<0.05 is considered significantly between CLP and treatmentgroups.

Fig. 1: