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Research article

Curcumin alleviates liver injury and altered monoamines level in cerebral cortex of sepsis-induced rats

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Key words: Sepsis; curcumin; oxidative stress; inflammation, monoamines.

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Abstract

Sepsis imposes a great economic burden on healthcare systems worldwide. It may occur after abdominal surgery, burns, hemorrhage or trauma and remained a major challenge for researchers and clinicians. Natural plant products were used as remedies in alternative medicine throughout the world. About 75% of individuals in developing countries depend on natural products for healthcare. Curcumin (Cur), is a polyphenol isolated from *Curcuma longa* having wide spectrum of biological functions. Cur has been used as a potential therapeutic agent in some pathological conditions and against many diseases. The present study was designed to explore whether Cur protects the antioxidant system and the immune response of liver, and the brain neurotransmitters against inflammation produced by sepsis induction. Sepsis-induced toxicity in adult albino rats was detected by the Complete Blood Count, physiological parameters as liver enzymes in serum; aspartate and alanine aminotransferases, oxidative stress (oxidant and antioxidant) in liver tissue, Nitrites, Nitrates, Malondialdehyde, Glutathione disulfide, Glutathione, Catalase and Super Oxide Dismutase levels as well as immunological studies; Tumor Necrosis Factor-alpha and Prostaglandin E2 levels in both serum and liver tissue, molecular studies (reverse transcriptase polymerase chain reaction RT-PCR) for Interleukin 8, Prostaglandin-E 2, Cyclooxygenase-2 and inducible Nitric Oxide Synthase genes, and supported by histopathological examination of liver tissue. Neurochemical studies estimated levels of Norepinephrine, Dopamine and Serotonin in the cerebral cortex of rats' for all groups. The present results indicated that Cur was effective in alleviating sepsis-induced severe oxidative stress and also, improved the immune response of liver and alleviate septic neurotoxic problems.

Introduction

Liver has a central and pivotal role in metabolism and in the immunological homeostasis. It is responsible for more than 200 important physiological functions, making liver critical for host survival after severe injury. Sepsis was reported as a combination of clinical symptoms and systemic inflammation related to an infectious insult [1]. Pathogens, toxins, or inflammatory mediators, all of these, may lead to liver injury in sepsis. The injury Progression of injury begins from active hepatocellular dysfunction, damage and then to liver failure [2]. Pathogenesis of acute liver injury involves interplay of oxidative stress, apoptosis, inflammation, and necrosis which leads to molecular processes [3]. Sepsis causes various complications, as cardiac dysfunction, liver disorder, kidney injury and brain injury, as well. Septic patients with those complications suffer from the brain dysfunction, known as septic encephalopathy, are reported earlier and more frequent than those in other systems [4]. Therefore, it is considered as a severe disease, as it is associated with high mortality [5]. Sepsis is reported to trigger oxidative stress, inflammation, and cell death in the brain with clinical manifestation ranges from mild delirium to coma [4].

Natural products, nowadays, provide a supply for new leads in treating different types of diseases such as cancer, inflammation and liver diseases. More than half of all pharmaceutical products were discovered from natural compounds or their derivatives [6]. *Curcuma longa* (turmeric) is a traditional herbal medicine with long history, connected with the *Zingiberaceae's* family, was

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used in cooking in India and other parts of the world [7]. It was, also, used for treatment of inflammatory conditions in China and Southeast Asia [8]. Curcumin (Cur) a hydrophobic polyphenol, one of the curcuminoids, an active constituent and famous flavonoids found in turmeric. Research on Cur for its chemoprophylaxis and anti-inflammatory properties has been on the rise rapidly in the last decade [9].

Findings from cultured cells, animal models, and human clinical trials treated with turmeric and Cur showed their effectiveness in treating immune system disorders [10], neurodegenerative disorder [11], gastrointestinal diseases and bacterial diseases [12]. As Cur controls inflammation, cell growth and apoptosis, most of the anti-oxidant and anti-inflammatory activities are attributed to it [13].

Till now, the therapy for liver and brain injuries due to sepsis is lacking as the mechanism of sepsis is complex and the late therapies targeting a single molecular fail to cure the disease. The role of Cur in sepsis-induced brain injury has not been extensively investigated. Therefore, the present study explores the protective effect of Cur on sepsis-induced liver and brain injuries and whether Cur regulates COX-2/PGE2 and IL-8 mRNA expression in liver of sepsis-induced rats *via* the COX pathway. Also, if the inflammatory changes in liver after induction of sepsis that may lead to the progression of the disease as well as the accompanying changes in brain neurotransmitters leading to brain injury were ameliorated by Cur treatment.

Materials and methods

Experimental animals

Forty healthy adult male albino rats, each weighing 150-200 g were used in the present study. Animals were brought from the laboratory animal breeding of national organization of drug control and research (NODCAR), Giza, Egypt. Rats were kept under strictly hygienic conditions for acclimatization under properly controlled environmental conditions in the animal houseat ambient temperature (22-25ºC), controlled humidity and adjusted light/dark rhythm. They were kept in plastic cages with stainless steel wire lids of adequate size each comprising 10 animals allowing free spontaneous motility and were kept through the period of the experiment. Rats were fed with a standard diet with composition authorized by Association of Official Analytical Chemist (AOAC), which consists of about 78.5% carbohydrates (including about 50% crude cellulose fibers), 15.2% protein, 3.2% lipids, 2.1% salt mixture and 1% multi vitamins. Rats were allowed to free access of food and water *adlibitum*. The National Institute of Health guidelines for animal health and accommodation [14] were supervened.

Induction of sepsis

Sepsis was induced in rats by the cecal slurry method. The cecal slurry was prepared according to [15] by mixing cecal contents obtained from donor rats with 5% dextrose in water (D5W) to yield 200 mg cecal material/5 ml. The slurry was prepared fresh and used within 2 hours in which 200 mg cecal material/kg was intraperitoneally (i.p.) injected.

Curcumin

Curcumin powder (curcuminoid; 95.02%) was obtained from Sigma chemical company (Sigma, Aldrich, USA), product number C7727, CAS nomber458-3703. Cur powder was suspended in 0.5% carboxyl methyl cellulose (CMC) just before administration as 250 mg/kg/day [16].

Experimental design and animal grouping

The experimental animals used in this study (40 adult male albino rats) were randomly divided into four equal groups; each group consists of 10 animals and was being administrated the treatment as follow:

I- Control Group (CON, n= 10): Animals of this group were received an oral administration of 0.5% CMC every day for 14days followed by 5% dextrose (D5W) on the 15thday.

II- Sepsis Group (SEP, n= 10): Animals of this group were received an oral administration of 0.5% CMC every day for 14 days. Then sepsis was induced experimentally on the 15th day by using the cecal slurry method.

III-Curcumin Group (CUR, n= 10): Animals of this group were orally administered Cur in a dose of 250 mg⁄kg⁄day for 14 days. Rats were received 5% dextrose (D5W) on the 15thday.

IV- Curcumin & Sepsis Group (CUR & SEP, n=10): Animals of this group were orally administrated the Cur (250 mg⁄kg⁄day) for 14 days and then sepsis was induced experimentally on the 15th day by using the cecal slurry method.

Animals of all groups were sacrificed on the 16th day post treatment, and blood was collected in tubes containing (EDTA), as an anticoagulant, for further biochemical investigations. Liver and cerebral cortex of each animal were quickly excised using a frozen ice plate and were kept at -80ºC for further investigations. After being washed in saline, liver tissue samples were preserved directly in formalin for histological investigations.

Tissues sampling preparation

Cerebral cortex (CC) was excised from brain of each animal from each group after decapitation, taken and homogenized in iced 10% KOH with glass homogenizer then centrifuged at 3500 rpm for 5 min. Tissue supernatants were obtained and processed for the estimation of neurotransmitters (NE, DA and 5-HT) using HPLC. Liver tissue samples were also taken from all rats, dissected, washed in ice-cold saline. Liver tissue sample

were taken and homogenized in phosphate buffer (pH: 7.4) then centrifuged at 3500 rpm for 5 min. Tissue supernatants were obtained and processed for the estimation of oxidative stress markers, TNF-α and PGE2 levels. Another liver tissue sample was taken from each rat and frozen at -80ºC for determination of mRNA expression of IL-8, COX-2 and iNOS, using real-time PCR.

Estimation of complete blood count (CBC)

The CBC was performed on Sysmex KX-21N automated hematology analyzer with a white blood cell (WBC) differential count, a peripheral smear was, also, prepared to be examined microscopically.

Biochemical analysis

Determination of liver function tests in sera

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated by a kinetic method according to the international Federation of Clinical Chemistry (IFCC) [17], using kits from Spectrum, Catalog No.261005.

Estimation of oxidant/anti-oxidant stress markers

Determination of malonaldehyde (MDA), glutathione (GSH) and glutathione disulfide (GSSG) contents and the nitrite (NO^{2-}) and nitrate (NO^{3-}) ratio were performed using HPLC. Liver samples were analyzed on an Agilent HP 1100 series HPLC apparatus (USA). The analytical column was anion exchange PRP-X100 Hamilton, 150 x 4.1 mm, 10 µm. The mobile phase was a mixture of 0.1 M NaCl-methanol, at a volume ration 45:55 respectively; the flow rate was 2 ml/min, wavelength adjusted to 230nm.

Determination of super oxide dismutase and catalase activities in liver homogenate

The hepatic Super Oxide Dismutase (SOD) activity was determined using spectrophotometer, according to the procedure of [18], depending on the ability of the enzyme to inhibit phenazinemethosulphate mediated reaction of nitrobluetetrazolium dye. Meanwhile, hepatic Catalase (CAT) activity was determined using the method of [19] by following the decrease in the absorbance at 240 nm due to the decomposition of H_2O_2. The difference in the absorbance per unit time is a measure of the CAT activity.

Estimation of TNF-α in serum and in liver homogenate

TNF-α level was assayed using ELISA kit purchased from Koma Biotech Company; Catalog No. K0331196. The ELISA kit of TNF-α contained all the necessary reagents required for performing the quantitative measurement.

Estimation of prostaglandin E-2 in serum and in liver homogenate

Prostaglandin E2 (PGE2) kit was purchased from SunLong Biotech Co., LTD. Catalog No. SL0601Ra. The ELISA kit used is Sandwich-ELISA as the method.

Quantitative real-time PCR

RNA extraction

Total RNA of hepatic tissue was isolated using Qiagen tissue extraction kit (Qiagen, USA) according to instructions of manufacture.

cDNA synthesis

Moloney murine leukemia virus (MMLV) reverse transcriptase was used for synthesis of cDNA from RNA. The total RNA (0.5–2 µg) was used for cDNA conversion using high capacity cDNA reverse transcription kit from Fermentas (USA).

Real-time qPCR

Real-time qPCR amplification and analysis were performed using an Applied Biosystem with software version 3.1 (StepOne™, USA), using SYBR Green I. Relative values of gene expression were normalized to β-actin. Primer sequences and accession numbers of the genes were provided in table 1.

Table 1. Forward and reverse primer sequences for iNOS, COX2, IL-8 and *β*-actin.

	Primer sequence	Gene bank accession number
iNOS	Forward primer: 5′-GACCAGAAACTGTCTCACCTG-′3 Reverse primer: 5′-CGAACATCGAACGTCTCACA-′3	NM_012611.1
COX2	Forward primer: 5′- CCATGTCAAAACCGTGGTGAATG-′3 Reverse primer: 5′-ATGGGAGTTGGGCAGTCATCAG-′3	NM_021838.2
IL-8	Forward primer: 5′-ATGCCTCGTGCTGTCTGACC-′3 Reverse primer: 5′-CCATCTTTAGGAAGACACGGGTT-′3	NM_001277073.1
***β*-Actin**	Forward primer: 5′-TATCCTGGCCTCACTGTCCA-′3 Reverse primer: 5′-AACGCAGCTCAGTAACAGTC-′3	NM_031144.3

Neurochemical studies

Determination of monoamines concentrations using HPLC

Estimation of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in cerebral cortex of the brain of all treated rats were carried out using HPLC; all samples were analyzed on an Agilent HP1100 series HPLC apparatus (USA). HPLC system consisted of quaternary pump, a column oven, Rheodine injector 20µ loop, UV variable wavelength detector, using program purchased from chemstation software.

Using solid phase extraction Chromabond column NH_2 phase Catalog No. 730031, the sample was immediately extracted from the trace lipids. The sample was injected directly into an aqua column 150 mm X 4.6 mm X 5µ C18, purchased from Phenomenex (USA) under the following condition: mobile phase: 20 mM potassium phosphate, pH 2.5, flow rate: 1.5 ml/min, UV: 280 nm. NE, DA and 5-HT were separated after few minutes. Each monoamine position and concentration from the sample was determined as compared to that of the standard, the content of each monoamine was expressed as µg per gram brain tissue [20].

Histopathological examination

Liver Specimens were fixed in 10% neutral-buffered formal saline for 72 hours. Specimens of 6 µm thickness were serially sectioned and stained with Haematoxylin and eosin according to the method described [21].

Statistical analysis

Results were evaluated statistically according to statistical analysis; data were presented as mean ± SEM (standard error mean) using SPSS (Statistical Package for Social Science) version 16. Variables were statistically analyzed by one-way analysis of variance (ANOVA) test. When differences were significant, Post hoc test (LSD, Least Significant Difference) was performed to find the individual differences between groups. Statistical difference with values of $p<0.05$ considered statistically significant.

Results

Effect of sepsis and/or Cur on CBC

The sepsis-induced toxicity in all animals groups were detected by measuring CBC. As illustrated in table 2, Sepsis induction showed significant changes at $p<0.05$ as RBC's count, Hb% content, platelets and neutrophils were decreased with a percentage difference of -14.6%, -15.3%, -48.1% and -24.0%, respectively, while increased WBC's whole count with a percentage difference of 128.6% represented with increased lymphocytes with a significant change of 48.6% and in eosinophils with a significant change of 44.6%. If compared to Sepsis group, Cur-Sepsis treated rats exhibited a decrease in WBC's whole count, precisely in neutrophils, together with an increase in lymphocytes, those changes were of a significant change at $p<0.05$, which indicated the ameliorative effect of Cur.

Table 2. The Protective Effect of Curcumin (Cur) on Complete Blood Picture (CBC) Against Sepsis-Induced Experimentally in Male Albino Rats.

Parameters	Experimental groups			
	Control	SEP	CURC	CURC & SEP
RBCs (10^6/cmm)	4.03 ± 0.007	3.44 ± 0.006 (-14.6%)[a]	3.87 ± 0.004 (-3.9%)	3.62 ± 0.0041 (-10.1%)[a]
WBCs (10^3/cmm)	7.90 ± 0.042	18.04 ± 0.073 (128.6%)[a]	7.94 ± 0.013 (0.7%)	13.88 ± 0.057 (75.9%)[a,b]
Platelets (10^3/cmm)	441.80 ± 0.264	229.60 ± 0.256 (-48.1%)[a]	441.40 ± 0.231 (-0.1%)	210.60 ± 0.276 (-52.4%)[a]
Haemoglubin (g/dL)	12.18 ± 0.0212	10.32 ± 0.0176 (-15.3%)[a]	11.60 ± 0.0110 (-4.7%)	10.86 ± 0.0122 (-10.8%)[a]
Neutrophils (%)	65 ± 0.028	49 ± 0.107 (-24.0%)[a]	63 ± 0.047 (-3.1%)	33 ±0.114 (-48.5%)[a,b]
lymphocytes (%)	30 ± 0.036	44 ± 0.063 (48.6%)[a]	35 ± 0.114 (17.3%)[a]	61 ±0.081 (104.7%)[a,b]
Monocytes (%)	4 ± 0.0313	4 ± 0.0407 (4.8%)	3 ± 0.0407 (-30.5%)	4 ± 0.0356 (-10.2%)
Eosinophils (%)	2 ± 0.0261	3 ± 0.0356 (44.6%)	2 ± 0.0261 (0.0%)	2 ± 0.0280 (33.7%)

Data expressed as mean ±SEM

( ): % Difference with respect to control value

a: significant changes at $p<0.05$ as compared to control group. b: significant changes at $p<0.05$ as compared to sepsis group.

Effect of sepsis and/or Cur on ALT and AST

As depicted in figure 1, sepsis induction in rats showed an increased level of ALT and AST, with a significant change at $p<0.05$, if compared with the control values. Cur-treated rats increased significantly ALT level and decreased AST level non-significantly. Cur administration to sepsis-induced rats decreased both enzyme levels, with a significant change in both ALT and AST levels.

Effect of sepsis and/or Cur on oxidant\antioxidant status in liver of rats

In the present study, sepsis induction to rats elicited sharp increases of significant change at $p<0.05$ in MDA, GSSG, $NO2^-$ and $NO3^-$ levels in livers of septic rats when compared to the control values (Figure 2). Meanwhile, the antioxidant molecule GSH showed a marked decrease in its content accompanied with sharp decreases with significant change at $p<0.05$ in CAT and SOD activities. Data concerning Cur+sepsis-treated rats indicated that, though Cur treatment did not completely ameliorate the changes observed in sepsis-induced rats, as MDA, GSSG and $NO2^-$ levels remained significantly elevated with respect to the control values. In addition, Cur treatment to septic rats increased GSH level with non- significant change, moreover, CAT and SOD activities also, increased being of a significant change at $p<0.05$ with respect to Sepsis group.

Effect of sepsis and/or Cur on inflammatory markers of rats

The present results in figure 3 showed that sepsis induction in rats induced dramatic increase, in TNF-α and PGE2 levels in both serum and liver being of a significant change at $p<0.05$. Meanwhile, Cur treatment to septic rats exerted a strong lowering effect on TNF-α and PGE2 levels, though still elevated significantly than the control values.

Constant with the biochemical findings, sepsis induction increased the expression of IL-8, COX-2 (mediates inflammation through production of prostaglandins) and iNOS in liver tissue of septic rats. Meanwhile, Cur showed a sharp inhibitory effect on IL-8, COX-2 and iNOS mRNA expression of liver septic rats, being of a significant change at $p<0.05$, with respect to Sep-induced rats.

Effect of sepsis and/or Cur on monoamines levels in cerebral cortex of rats

The present results in table 3 indicate that sepsis decreased NE, DA and 5-HT levels in the CC, with a significant change at $p<0.05$ when compared to the control values. Meanwhile, though Cur-sepsis treated rats showed significant increase in their CC levels of NE and 5- HT, when compared to control rats, Cur treatment to septic rats revealed improved NE, DA and 5-HT levels in brain of septic rats, with a significant change at $p<0.05$, indicating the ameliorative and the protective role of Cur on brain neurotransmitter during sepsis.

Histopathological findings

Histopathological examination of liver tissue were undertaken to monitor the changes in liver architecture in septic rats, Cur group and Cur-sepsis treated rats (Figure 4). Liver injury in sepsis was confirmed by histological changes in the liver. Sepsis induction to rats caused hydropic changes in cytoplasm of most hepatocytes. Many nuclei showed karyolysis and karyorrhexis. Fibrosis with cellular infiltration was also observed around blood vessels (Figures 4-B), focal aggregation of inflammatory cells and dilatation with congestion of blood vessels. Cur treatment alone slightly affected liver tissue by increasing the number of pyknotic nuclei and mild dilatation of blood sinusoids (figure 4-C). Cur treatment to septic rats was found to reduce the cellular changes and to a lesser extent the cellular infiltration and fibrosis (Figure 4-D). A low degree of amelioration represented by hydropic changes, dilatation and congestion of blood vessels and sinusoids were still observed.

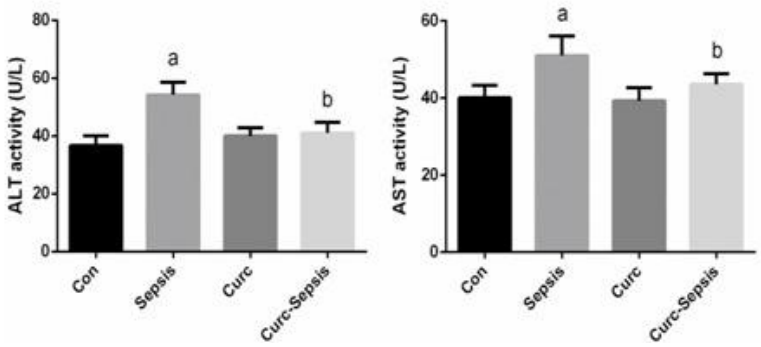

Figure 1. The Protective Effect of Curcumin (Cur) on Aspartate Aminotransferase (AST) and Alanine Amino Transferase (ALT) Against Sepsis-Induced Experimentally in Male Albino Rats. Values Represent the Mean ± SEM (*n* = 10). a: significant changes at p<0.05 as compared to control group. b: significant changes at p<0.05 as compared to sepsis group.

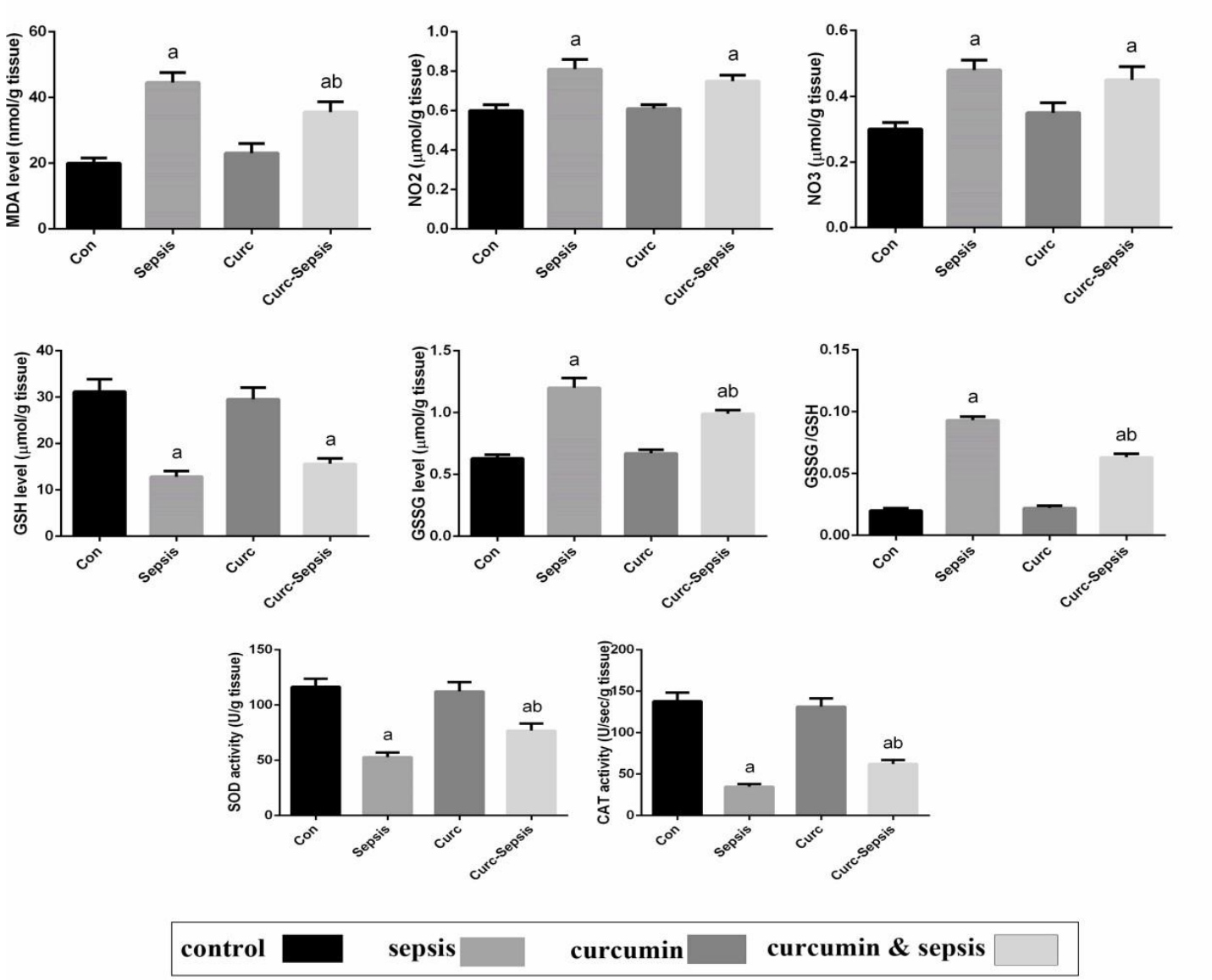

Figure 2. The Protective Effect of Curcumin (Cur) on Oxidative/Antioxidant Stress Markers in Liver Tissue of Septic Rats. Values Represent the Mean ± SEM (n = 10). a: significant changes at p<0.05 as compared to control group. b: significant changes at p<0.05 as compared to sepsis group.

Table 3. The Protective Effect of Curcumin (Cur) on Monoamines (Norepinephrine NE, Dopamine DA, Serotonin 5-HT) in Brain Cortex Tissue Against Sepsis-Induced Experimentally in Male Albino Rats.

Parameters	Experimental groups			
	Control	SEP	CURC	CURC&SEP
NE μg/g tissue	0.86±0.0013	0.53±0.0016[a]	0.83±0.0030	0.64±0.0020[a,b]
DA μg/g tissue	1.84±0.0023	1.41±0.0047[a]	1.81±0.0033	1.66±0.0082[b]
5-HT μg/g tissue	0.67±0.0008	0.41±0.0014[a]	0.60±0.0009[a]	0.47±0.0006[a,b]

Data expressed as mean ±SEM
( ): % Difference with respect to control value
a: significant changes at p<0.05 as compared to control group. b: significant changes at p<0.05 as compared to sepsis group.

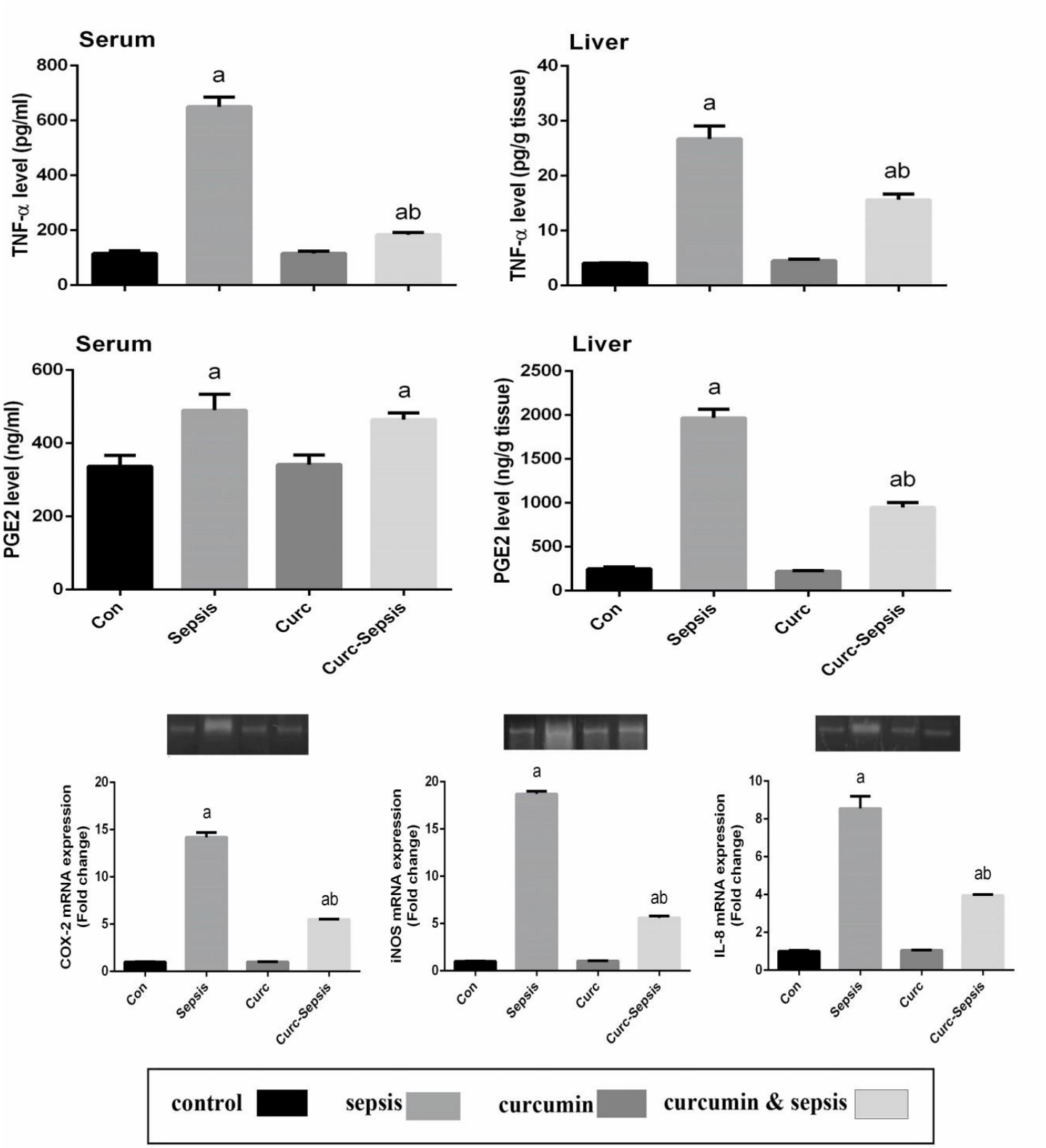

Figure 3. The Protective Effect of Curcumin (Cur) on Inflammatory Markers in Liver Tissue of Septic Rats. Values Represent the Mean ± SEM (n = 10). a: significant changes at $p<0.05$ as compared to control group. b: significant changes at $p<0.05$ as compared to sepsis group.

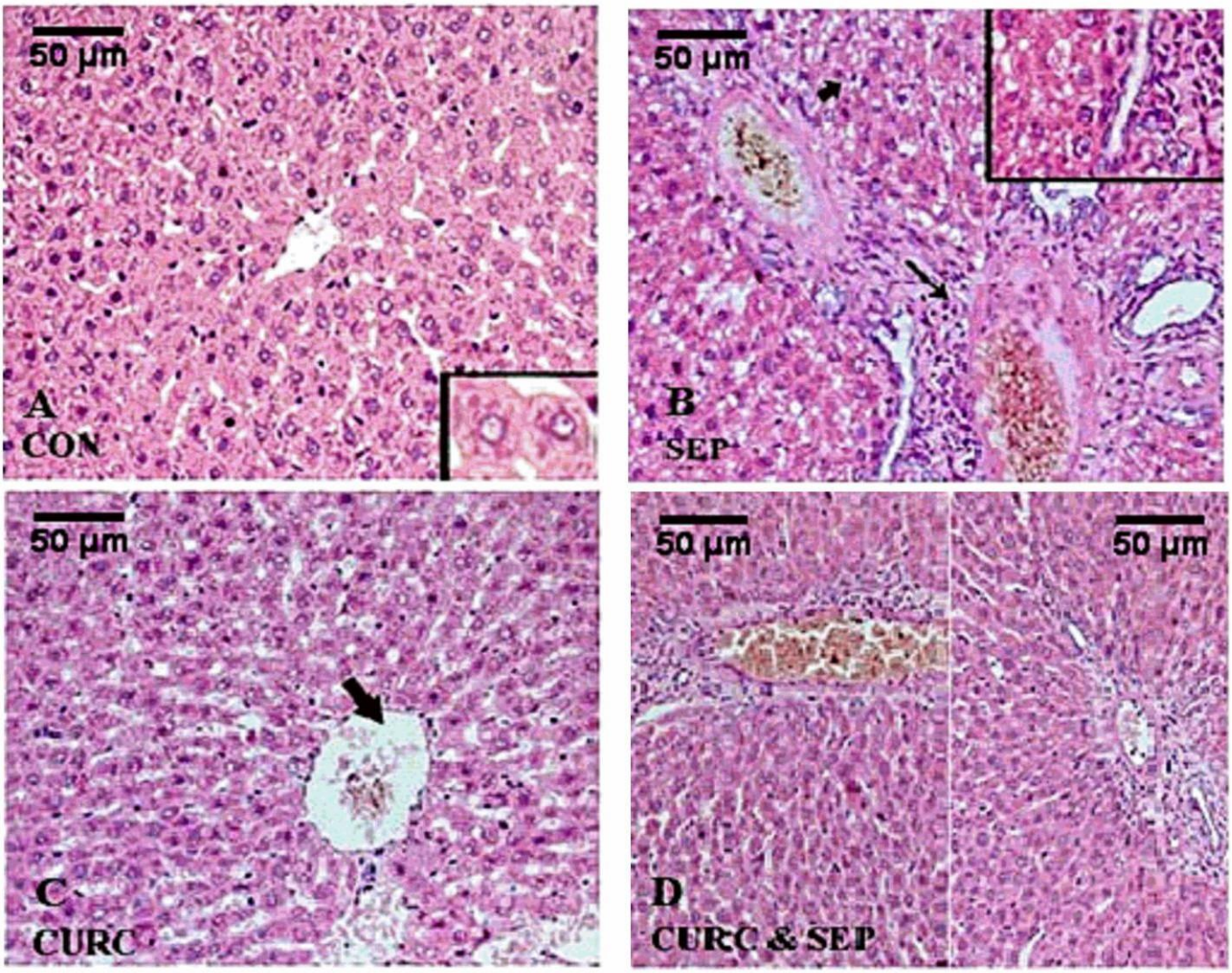

Figure 4. The Protective Effect of Curcumin (Cur) on Hepatic Tissue of Sepsis-Induced Rats. (CON) Control group (Figure 4-A), SEP Sepsis Group (Figure 4-B) Indicated hydropic changes in hepatocytes, fibrosis with cellular infiltration, karyolysis and karyorrhexis nuclei and focal aggregation, dilatation with congestion of blood vessels. (Cur) Curcumin Group showed increased number of pyknotic nuclei and mild dilatation of blood sinusoids (Figure 4-C). (Cur+SEP) Curcumin and Sepsis Group, Cur treatment to sepsis-induced rats reduced cellular changes; cellular infiltration and fibrosis (Figure 4-D), but a lower degree of hydropic changes, dilatation and congestion were still observe.

Discussion

Severe sepsis is accompanied with a systemic inflammatory response syndrome (SIRS) which was characterized by ROS overproduction and increased levels of proinflammatory cytokines, which contribute individually, or in combination to the recruitment of leukocyte and subsequent organ damage in sepsis [22]. Oxidative stress was responsible of liver damage induced by many agents, including viral infections, drugs, alcohol, environmental pollutants and dietary components, resulting in liver injury progression leading to fibrosis of liver and finally, cirrhosis [6]. Imbalanced generation and degradation of ROS cause oxidative stress and consequently generation of free radicals and cellular damage [6]. The present results indicated rats liver injury after sepsis induction, which was obvious by significant increases of ALT and AST levels, and by increasing markers of oxidative stress in liver as; MDA, GSSG, NO_2^- and NO_3^- ratio, while decreasing the antioxidant GSH content, along with the antioxidant enzymes CAT and SOD levels.

Several studies have documented the potency of Cur as antioxidant [23], hepato- and nephroprotectant [24], antimicrobial [25], anti-inflammatory [26] and its potential anti- depressant properties [27]. The hepatoprotective effects of Cur were assessed in the present study, on ALT and AST levels and liver histology were, also, considered. Results of the present study indicated that Cur treatment to sepsis-induced liver

injured rats decreased liver injury evidenced by decreasing the elevated ALT and AST levels. Several studies have documented the protective ability of Cur against oxidative stress and on liver injury induced by different agents *in vitro* and *in vivo* [28-29]. Cur was found to lower ALT, AST and alkaline phosphatase levels and prevent liver toxicity [30]. Recently, Cur was found to improve CCl_4-induced lipid peroxidation, decreased serum ALT and AST levels and attenuated the increase in liver MDA level, thus protecting it against injury [31]. Cur was, also, documented as a scavenger of different types of ROS free radicals in liver *via* its phenolic, diketone and methoxy group [6]. Cur, in the present study, markedly attenuated both the decrease of cellular antioxidants and the increase of oxidant parameters in liver of sepsis-induced rats.

The antioxidant defence systems, including the non-enzymatic antioxidant molecule (GSH) and enzymatic activities such as SOD and CAT play an important role in preventing liver damage [32]. Cur treatment controlled the balance between oxidant and antioxidant [33]. Also, Cur was found to up-regulate the activities of SOD, CAT and the level of GSH, either in kidneys of glycerol-induced nephrotoxicity or liver of diethylnitrosamine induced hepatocarcinogenesis [34]. Earlier study showed that, Cur Pre- and post-treatment were found to prevent oxidative stress in methotrexate-induced oxidative stress in rats by inhibiting ROS production and ameliorating SOD and CAT as antioxidant enzymes. Moreover, oxidative stress resulting from cisplatin-induced experimental inflammation was found to be reduced by Cur [35]. GSH level and SOD and CAT activities were also increased by Cur treatment and decreased MDA level in an immobilization-induced stress rat model [36]. The protective effect of Cur could be attributed directly to scavenging O_2 and H_2O_2. The bio-membrane protective effect due to peroxidative damage was, also, linked to Cur- ROS scavenging ability [37].

Liver was found to have a central role in the systemic response to critical illness, through both; elimination of pathogenic microorganisms and toxins from circulation, and the acute phase reaction also, the release of liver-derived cytokines, inflammatory mediators, and coagulation cascade components [2]. Bacterial phagocytosis and clearance from liver were carried out by multiple types of liver cells [38]. Kupffer cells, liver sinusoidal endothelial cells, and stellate cells, are the defence line against blood-borne bacteria in the liver, therefore, protecting the liver and the whole body. The hepatic reticuloendothelialsystem, efficiently traps and eliminates it. Kupffer cells carryout the clearance of bacteria and soluble bacterial products through exhibiting a high endocytic and phagocytic capacity [39]. Clearance of bacteria from the bloodstream is carried out through the cooperation of Kupffer cells with platelets and neutrophils [40].

Following harmful bacterial attack, the releasing rate of pro-inflammatory mediators, secondary mediators after tissue injury, NO and ROS from Kupffer cells increase [41]. As Kupffer cells were responsible for generating inflammatory cytokines in early sepsis, Liver is considered a source of inflammatory mediators, thus mediating sepsis-induced liver injury [39]. Results of the present study demonstrated that sepsis induction decreased RBC's count, Hb% content, platelets and neutrophils with a marked increase in WBC's count specially; lymphocytes and eosinophils, compared to control group. As documented earlier, severe sepsis was characterized by white blood cell count abnormality, fever, and presumed infection and high heart rate. Sepsis was , also, found to trigger production of various array of cytokines for controlling infection while their excessive production lead to tissue and organ injury, and the anti-inflammatory being critical in regulating the overall immune response and in establishing homeostasis, therefore, dysregulation of both trigger pathogenesis[42].

The oxidant-antioxidant state in the present work was accompanied with increased TNF-α, PGE2 levels in serum and liver tissue homogenate and increased expression of IL-8, COX-2 and iNOS mRNA in liver of sepsis-induced rats. Results of the present study, are in agreement with earlier studies, indicating increased COX-2 , IL-8 and PGE2 levels after sepsis induction, which contribute to acute and chronic inflammation, oxidative stress, bacterial or viral infection, and cancer [43].

Inflammation was documented as an adaptive physiological response induced by deleterious conditions including infection and tissue injuries. It is considered to be the product of complex series of responses triggered by the immune system [2]. Hyper inflammation has an early phase, followed or overlapped by a prolonged state of immune-suppression [44], referred to as sepsis-induced immunoparalysis [45], which is characterized by impaired innate and adaptive immune responses, thus playing a pivotal role in the pathogenesis of tissue damage, multiple organ failure, and finally death [45]. So, the liver-mediated immune response to sepsis acts as a double-edged sword: it cleared bacteria and toxins but caused inflammation, immunosuppression and organ damage [2]. During sepsis, various pathogens and damage associated molecular patterns cause immoderate activation, of platelets (produced from bone marrow mega karyocytes as a nucleate cells), also it play a role in regulation of inflammatory response [1]. The release of the pro-inflammatory factors from platelets granules into the surrounding or their transfer to plasma membrane, such as interleukins, monocyte chemo-attractant protein, platelet factor, to activate more distant platelets and immune cells thus playing a deleterious role in the dissemination of coagulopathy and inflammatory responses in sepsis [46].

Neutrophils, the chemotactic factors in the site of infection, are considered the first line cell of defence

against the bacterial and fungal pathogens, and recruit to the site of infection. Chemokine IL-8 plays a major role in neutrophils activation, influence the chemotaxis of immune cells, an inflammatory mediator in response to viral or bacterial pathogen, as potential biological marker in fibrosis and ALT levels [47], and also, in tissue repair mechanisms such as angiogenesis and cell proliferation. Up-regulation of IL-8 at the transcriptional level, on receiving inflammatory stimuli, in many different cell types; fibroblasts, monocytes, and hepatocytes, could lead to protection of cells from inflammatory stimuli effects [48]. Moreover, a significant increase in serum IL-8 levels in patients with sepsis was documented [49].

TNF-α, a cytokine with many biological effects, released by macrophages, monocytes, T lymphocytes, and other cells, also, synthesized in many tissues playing critical roles in several biological processes, as host resistance to infection and inflammatory responses. Both pro-inflammatory cytokines TNF-α and IL-8, showed increased levels, in response to pathogen infection, in sera and livers of septic rats. With abnormal aggregation of neutrophils in capillaries, especially those in liver sinusoids, leading to microvascular occlusion, impaired neutrophils recruitment to the infectious part, and damaged neutrophil result in tissue ischemia and consequently multiple organ failure [2].

Altered signaling pathways, increase the levels of inflammatory markers. COX-2, PGE2 and IL-8 mRNA were, also, up-regulated by sepsis as depicted from the present results. TNF-α stimulate acute phase reaction, inducing apoptotic cell death, and inhibiting viral replication and tumorigenesis. Many transcription factors, such as; TNF-α, nuclear-factor kappa B (NF-κB), and IL-6 regulate IL-8 expression [50]. Moreover, IL-8 itself was found to up-regulate some tumor genes, such as COX-2 (rate-limiting enzyme, involved in inflammation, cellular proliferation, anti-apoptosis activity, and tumorigenesis), lipooxygenase-5, and phospholipase A2, thus promoting development of cancer [51]. Cytokine production in sepsis is affected by TLRs signaling, as those receptors activate platelets to release TNF-α as immune-modulatory agent, promoting neutrophils and endothelial cells activation. Moreover, liopopolysaccharides binding to TLRs lead to their activation, which in turn activates c-Jun N-terminal kinase, NF-κB and AP-1, causing binding to promoters of inflammatory cytokines, leading to huge cytokines production in sepsis [52]. So, the inappropriate activated platelets are major contributors in initiation of disseminated intravascular coagulation leading to the platelet adhesion thus reducing oxygen supply and enhancing inflammatory cytokine networks [53].

Cur was known to be a highly pleiotropic molecule interacting with unlimited inflammatory molecular targets, especially in clinical trials as well as *in vitro* and *in vivo* studies indicating its potential therapeutic effect. Cur has been reported to have anti- inflammatory, anti-apoptotic and anti-bacterial functions [24]. Cur is a strong inhibitor of reactive oxygen-generating enzymes; as lipoxygenase, COX, and iNOS. Cur markedly reduced the hemolysis and lipid peroxidation of erythrocytes and acts as a scavenger of NO by blocking the enzyme that produces it, thus exerting a promoter activity [54]. NO and its producer iNOS, down-regulate neutrophil migration; through down-regulation of vascular cell adhesion molecule [55]; also, interaction of NO with ROS molecules, leading to peroxynitrite formation which decrease neutrophil chemotactic activity and leukocyte endothelium interaction, and finally, induction of hemeoxygenase (HO)-1 expression by NO, impair neutrophil rolling and adhesion [56].

The present study indicated that, Cur significantly decreased and attenuated the elevations of TNF-α and PGE2 levels in serum and liver of sepsis-induced rats and also, COX-2, iNOS and IL-8 mRNA expression in liver of sepsis-induced rats. Several studies showed that Cur, *in vitro*, inhibited the expression of COX-2 mRNA and production of prostanoids in cancerous cells. Cur also inhibited COX-2 expression in mouse macrophage cell line exposed to LPS [57].

Inactivation of two transcription factor genes, activator protein-1 (AP-1; mediating cell proliferation) and nuclear factor-κB (NF-κB; mediating immune activity, inflammation, collagenase and cell proliferation) may explain the inhibitory effects of Cur on proinflammatory gene expression and inhibition of inflammation [58]. Moreover, Cur inhibited PGE2 production, completely, by suppressing the proteinase-activated receptor-2 which triggers PGE2 production by attenuating both COX-2 upregulation and NF-κB signals [59].

Sepsis caused high levels of oxygen free radicals and inflammatory mediators, which are occurred secondary to increased membrane permeability caused by damage to the liver cell structures and mitochondrial membranes. Sodium pump dysfunction leads to sodium retention, hepatocyte swelling, and finally hepatocyte apoptosis [2], as indicated in histopathological analysis of the present work. Liver injury in mice using galactosamine /lipopolysaccharide (LPS) was mitigated by Cur treatment by inhibition of oxidative stress and mitochondrial dysfunction-mediated caspase-3 [60]. In addition, Cur also, attenuated liver injury induced by chronic ethanol through inhibition of oxidative stress *via* mitogen-activated protein kinase (MAPK)/nuclear factor E2-related factor-2 pathway in mice [61]. Cur was found to have a dual role in apoptosis, showing different reactions in different cells. The underlying mechanism involves increased SOD vitality, regulation of inflammatory and anti-inflammatory cytokine expression. Moreover, Cur pretreatment of human umbilical vein endothelial cell line suppressed oxidative stress, (Akt) phosphorylation and cell death thus protecting cells (during early epileptogenesis) against ROS-induced

damage inducing autophagy and microtubule-associated protein expression, inhibiting apoptosis and inducing autophagy *via* Akt/mTOR signalling pathway activation [62].

Brain removes or inactivates potentially damaging agents or tissues by a purpose of inflammation, so, it responds to injury, infection, or disease through neuroinflammation. Glias of the CNS, and lymphocytes, monocytes, and macrophages of the hematopoietic system, mediate this inflammatory response. Brain over-activation has severe consequences including depressive-like behaviour. Major depressive disorder showed alterations in immunological markers, with increased pro-inflammatory cytokines levels. Moreover, chronic inflammation changes brain cells and synaptic plasticity causing neurodegeneration, coupled with a reduction in neuroprotection, leading to dementia, especially in older people [63].

As a result of high levels of pro-inflammatory cytokines, the "sickness behaviour" symptoms were recorded including; depression, reduction in locomotor activity, anhedonia, anorexia and cognitive disturbances. A delayed and progressive loss in dopaminergic neurons of substantia nigra were observed in earlier studies, due to increased pro-inflammatory response [64]. Changes in behaviour similar to depression, major depression, and neurodegeneration could arise from unregulated inflammation and increased levels of pro- inflammatory cytokines, while attenuation of the inflammatory response was found to reduce the depressive symptoms [65]. Moreover, inflammation may be a common mediator of observed death conditions due to depression and chronic pain [64]. Furthermore, it was suggested that neuroinflammation was suppressed by NE and those NE uptake inhibitors' therapeutically efficient in treating depression may be partially related to this mechanism [66].

The present results indicated that sepsis-induction decreased DA, NE and 5-HT levels in CC of rats. Moreover, studies indicated that enhanced inflammatory markers level from peripheral immune cells and serum proteins may enter the central nervous system through blood brain barrier (BBB) leakage, as endothelial cells injury as well as astrocytes lead to BBB disruption, therefore, triggering the leakage of immune cells and inflammatory mediators which are in turn enhance the inflammatory responses leading to aggravated brain injury [67]. Patients with sepsis-induced brain injury have a higher mortality rate as sepsis triggers cell death, due to overproduction of pro-inflammatory cytokines, ROS production, and mitochondrial dysfunction, leading to organ damage [68].

Neurological diseases were reported to be ameliorated by Cur treatment. Cur protected rat neurons in the cortex against oxyhemoglobin-induced injury *via* attenuating oxidative stress. By decreasing ROS production and preserving mitochondrial membrane potential, Cur remarkably attenuated mitochondrial oxidative damage after sepsis induction, as mitochondrial oxidative damage participates in the increased loss of mitochondrial function efficiency, which, in turn, further aggravates the mitochondrial oxidative damage and ultimately aggravates sepsis-induced brain damage [69]. Elevation of both; mitochondrial membrane potential and mitochondrial complex I activity, improved survival rate and attenuated brain edema after Cur treatment to septic mice. Yun *et al.*, [70] indicated that Cur control the reverse destructive processes as an antioxidant, anti-apoptotic, anti-inflammatory and immunomodulatory compound.

Conclusion

The present findings provide evidence for the antioxidant and anti-inflammatory activities of Cur in septic rats. The present results indicated that this protection could be attributed to: (i) Cur attenuated inflammatory responses, thus preventing liver and brain injuries; (ii) Cur- mediated quenching of free radicals and maintenance or improvement of endogenous antioxidant defence systems.

Conflict of Interest

There is no conflict between this work and any other works.

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