



Research Paper

The effect of essential oil of *Hyptis crenata* on sepsis-induced hypothalamus injury in rats

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ABSTRACT

Hypothalamic impairments are associated with severe clinical complications caused by sepsis, including insulin resistance, heart rate variability losses and autonomic dysfunction. Studies have shown that neuroinflammation and oxidative stress are critical to the pathogenesis of neurological disorders. Essential oils have been used to treat neurological disturbances. Previous studies have shown that the essential oil of *Hyptis crenata* (EOHc), which belongs to the lamiaceae family, has significant anti-inflammatory and antioxidant properties. The aim of present study was to evaluate the effect of EOHc on hypothalamic injury induced by sepsis. Wistar rats were subjected to Sham and CLP surgical procedures. EOHc (300 mg/kg) was orally administered 12 and 24 h after sepsis induction. The animals were euthanized 48 h after surgery and their hypothalamus were removed. TNF- α , IL- β and IL-6 levels in the hypothalamus were measured through ELISA. Malondialdehyde levels and catalase activity were measured to assess oxidative stress. The morphology of the hypothalamus was analyzed by means of HE staining. EOHc inhibited TNF- α and IL- β level increase in the hypothalamus, inflammatory cell infiltration, epithelial cell loss and lipid peroxidation in CLP – operated rats as compared with the control group. Data in the current study evidenced EOHc effectiveness to help rule out hypothalamus sepsis-induced injury.

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INTRODUCTION

Sepsis is an abnormal host response to infection that results in life-threatening organ dysfunctions (Marshall, 2018; Lelubre and Vincent, 2018). Neurological dysfunctions are a common sequela in critically-ill patients with sepsis and septic shock (Hensley and Prescott, 2018; Robba et al., 2018; Schmutzhard and Pfausler, 2017). Clinical and experimental studies have shown that the hypothalamus is a brain region susceptible to sepsis-induced injuries observed in the hypothalamus of septic patients who are featured by ischemic lesions and bleeding, often followed by selective and delayed neuronal death (Hensley and Prescott, 2018; Meneses et al., 2019; Santos-Junior et al., 2018; Schmutzhard and Pfausler, 2017). In addition, changes in preoptic and

paraventricular hypothalamic areas contribute to neuronal networks that mediate sympathetic activation due to fever and disruption in the Hypothalamic-Pituitary-Adrenal (HPA) axis. This process leads to impaired vasopressin and oxytocin secretion, as well as to neurological sepsis-associated complications (Peeters et al., 2018; da Costa et al., 2017). Previous studies have described persistent neuroinflammation featured by long time-course increase in proinflammatory cytokine levels, mainly tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1) and interleukin-6 (IL-6), in the hypothalamus, during experimental sepsis (Cojocar et al., 2003; Griton and Konsman, 2018; Lelubre and Vincent, 2018; Santos-Junior et

al., 2018). Moreover, oxidative stress in earlier sepsis phases due to increasing neurotoxic agents, such as reactive oxygen and nitrogen species, free radicals and other neurotoxic substances produced in central nervous and hepatic cells (hepatic encephalopathy), appears to play critical role in the pathogenesis of sepsis-induced neurological damage (Catalao et al., 2017; Griton and Konsman, 2018; Robba et al., 2018).

Evidences of neuronal proinflammatory mediators and oxidative stress contributions to the pathogenesis and development of sepsis-induced organ failures encouraged the adoption of novel therapeutic tools. New bioactive compounds have been experimentally tested to assesses their positive influence to enhance the current treatments applied to neurological disorders caused by sepsis (Griton and Konsman, 2018; Lelubre and Vincent, 2018; Meneses et al., 2019; Sun et al., 2019). Accordingly, essential oils, which are plant-derived biological compounds often linked to defense mechanisms against biotic and abiotic stress, have shown promising results as neuroprotective agent (Szwajgier et al., 2017). Several medicinal plants and their derivatives have been used to treat neurological disorders. For centuries, Chinese and Persian traditional medicine have been using aromatic plants, as well as their essential oils in herbal formulations developed to treat different neurological disturbances, such as anxiety, depression, neurological pain and convulsion (Cavalcante et al., 2018; Parvez, 2018). The neuroprotective effects of aromatic plants and of their volatile constituents have been attributed to different action mechanisms, including the upregulation of monoamine neurotransmitters, the inhibition of monoamine oxidases, the blockage of 5-HT_{1A} receptor and the promotion of adrenocorticotropin secretion (Ayaz et al., 2017; Hassan et al., 2017).

Species *Hyptis crenata* Pohl ex Benth, which belongs to Lamiaceae family, is distributed in Northern and Northeastern Brazil, where it is popularly used to treat gastrointestinal and liver conditions. The species has presented pharmacological properties capable of help treating sepsis and its clinical complications (McNeil et al., 2011; Xu et al., 2013). Violante et al. (2012) have reported significant bactericidal activity against *Staphylococcus aureus* and *Enterococcus faecalis*, as well fungicidal activity against *Cryptococcus neoformans*, *Candida glabrata* and *Candida tropicalis* due to the use of essential oil of *H. crenata* (EOHc). Previous studies carried out by the present research group showed that EOHc - composed of camphor (32.78%), 1,8-cineole (18.02%), α -pinene (13.%) and β -caryophyllene (12.86%) - reduced ethanol and indomethacin-induced gastric mucosa lesions (Diniz et al., 2013). The hepatoprotective effect followed by EOHc-induced antioxidant activity in mice with liver dysfunction induced by *Cecum Ligation and Puncture* model of sepsis (CLP model) has been also observed (Lima et al., 2018). The aim of present study was to evaluate the therapeutic role played by the essential oil of *H. crenata* (EOHc) in

hypothalamic changes induced by CLP model of sepsis, by taking into account previous pharmacological properties attributed to EOHc and the reported role played by oxidative stress and inflammatory mediators in the pathogenesis of sepsis-induced organ dysfunctions.

MATERIALS AND METHODS

Plant material

H. crenata shoot samples were collected (March, 2016) in São Raimundo das Mangabeiras City, Maranhão State, Brazil. Plant identification was confirmed by Dr. Oriel Herrera Bonilla. The voucher specimen (N.000106) was deposited in Marlene Freitas da Silva herbarium. The essential oil of *H. crenata* Pohl ex Benth (EOCz) shoot was isolated from freshly chopped leaves through steam distillation and chemically analyzed by means of gas chromatography-mass spectrometry (GC/MS). The chromatographic analysis was performed in Hewlett-Packard 6971 equipment (Palo alto, CA, USA) under analytical conditions previously described by Diniz et al. (2013).

Animals

Female Wistar rats (200-250g) were provided by the bioscience unit of Superior Institute of Biomedical Sciences of State University of Ceará. The animals were housed under standard conditions, had free access to standard feed and water. All animals used in the current study (n=40) were kept at room temperature (22 $\pm$ 2°C) under 14/10 h light/dark cycle. All herein described procedures were previously approved by the Local Ethics Committee on Animal Research of State University of Ceará (n. 07227619-3).

Cecal ligation and puncture sepsis model (CLP)

Sepsis was induced by cecal ligation and puncture (CLP) of the polymicrobial sepsis model, as previously described by Rittirsch et al. (2009). The animals were anesthetized with intraperitoneally administered ketamine (15 mg/ kg) and xylazine (7.5 mg/kg); subsequently, a 3-cm midline incision was made in the anterior abdomen to expose the cecum. Fecal content was dragged to the cecum ligation and held below the ileocecal junction pole with 5/0 Prolene thread (Ethicon), but it did not cause bowel obstruction. Cecum was punctured 10 times with 18-gauge needle and slightly compressed until a small drop of stool came out of it. Cecum was repositioned at the abdomen and the incisions in the peritoneal wall and in the skin were closed. Sham-operated animals subjected to identical laparotomy, without cecal

puncture, composed the control group. Soon after the surgery, 2 ml of 0.9% NaCl solution was subcutaneously administered in all animals.

Experimental design

Animals were randomly divided into four groups and treated as follows:

- 1). *Group 1 (Sham group)*: Sham-operated rats (n=7) were orally pretreated through gavage - with vehicle (0.1% tween-80 solution) -, at 12 and 24 h post-surgical procedure.
- 2). *Group 2 (Sham-EOHc group)*: Sham-operated rats (n=7) were orally pretreated through gavage, with EOHc (300 mg/kg), at 12 and 24 h post-surgical procedure.
- 3). *Group 3 (CLP group)*: CLP-operated rats (n=14) were orally pretreated through gavage, with vehicle (0.1% tween-80 solution), at 12 and 24 h post-surgical procedure.
- 4). *Group 4 (CLP-EOHc group)*: CLP-operated rats (n=16) were orally pretreated through gavage, with EOHc (300 mg/kg), at 12 and 24 h post-surgical procedure.

Animals were euthanized through carbon dioxide inhalation 48 h after surgery, their hypothalamus was removed by means of careful dissection and stored at - 80°C, for further analytical analysis.

Body temperature measurement

Core body temperature of each animal was taken with the aid of rectal probe (pediatric clinical digital thermometer – Techline®, TS model 101 – China). Mean body temperatures - taken for 2 days before surgery - were used as baseline record to compare different scores calculated for these same animals 48 h after CLP or sham surgical procedure. All animals remained in the laboratory; measurements were always taken at 09:00 am.

Cytokine concentrations

TNF- α , IL 1- β and IL-6 levels in the hypothalamus were assessed by means of enzyme-linked immunosorbent assay (ELISA) techniques. The hypothalamus samples collected from four experimental groups 48 h after surgical procedures were homogenized in 1 ml of phosphate-buffered saline (PBS) solution (0.4 mmol/L NaCl and 10 mmol/L NaPO₄) added with antiproteases [0.1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 0.1 mmol/L benzethonium chloride, 10 mmol/L EDTA, 20 kallikrein inhibitor unit [aprotinin A] and 0.05% Tween 20]. Samples were then centrifuged for 10 min at 3000 *g* and the supernatant was immediately used in ELISA assays, based

on recommendations by the manufacturer (R&D Systems, Minneapolis, MN, USA). Results were expressed in pictograms per milligram of protein.

Histopathological evaluation

Hypothalamus samples were fixed in 10% (vol/vol) neutral buffered formalin, dehydrated and embedded in paraffin for 24 h for histological evaluation. Thereafter, samples were transferred to 70% alcohol until processing time. Subsequently, 4- μ m-thick sections were coated with paraffin, stained with hematoxylin and eosin (H&E) and analyzed according to criteria previously described by Ijomone and Obi (2013), with some modifications. Inflammatory cells (0-3), hemorrhagic damage (0-3) and epithelial cell loss (0-3) were the assessed parameters.

Catalase (CAT)

Catalase activity in hypothalamus supernatants followed the protocol described by Aebi (1984), which is based on determining the catalase constant hydrogen peroxide decomposition rate. Decomposition was monitored through spectrophotometry, at 240 nm, for 6 min, in reaction medium consisting of 20 mM hydrogen peroxide and 100 mM PBS buffer, at pH 7.40. Absorbance was measured in BioMate 3S UV-visible spectrophotometer (Thermo Fisher Scientific®, Waltham, MA – USA); results were expressed in millimolar of hydrogen peroxide consumed/min per milligram of protein.

Lipid peroxidation

Lipid peroxidation was determined through the method described by Kowalczyk and Stryjecka (2002). Thiobarbituric acid reactive substances (TBARS) were used as lipid peroxidation indicators based on malondialdehyde (MDA) production from lipid hydroperoxides. The calibration curve was plotted by using 1,1,3,3-tetramethoxypropane as standard. Samples were incubated for 1 h at 95°C in reaction medium consisting of 14 mM sodium dodecyl sulfate, 1.25 M acetic acid and 18 mM thiobarbituric acid and, subsequently, read at 535 nm in BioMate 3S UV-visible spectrophotometer (Thermo Fisher Scientific®, Waltham, MA – USA), after acclimating for 5 min at room temperature. Results were expressed in micromoles of MDA per milligram of protein.

Statistical analysis

Results were expressed in mean $\pm$ standard error of the mean (S.E.M.). The *P* values were calculated through one-

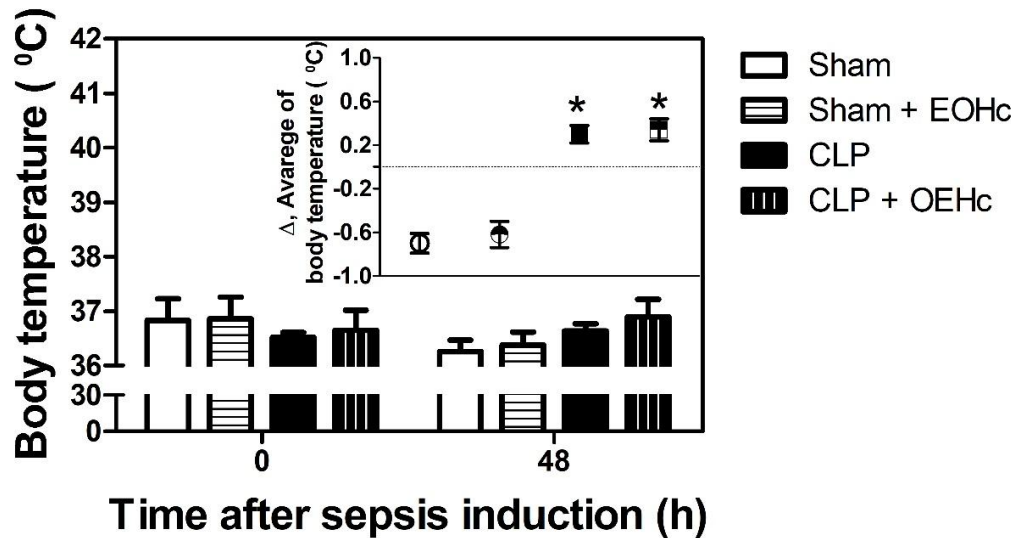


Figure 1: Effect of EOHC on sepsis-induced thermoregulation changes. At 0h, body temperatures from all animals were recorded with the aid of rectal probe to determine the baseline. Soon after, the animals were Sham- or CLP-operated. At 12 and 24h post-surgery, Sham-operated rats (n=7) and CLP-operated rats (n=7) were orally treated with vehicle or vehicle + EOHC (300 mg/kg). At 48h, diurnal body temperatures from experimental groups were similarly recorded and compared among experimental groups or with the baseline of the same animals previously recorded (*Inset*). Data represent means $\pm$ SEM. * $p < 0.05$ versus control group.

way ANOVA, with Bonferroni post-test correction. Histological assessment was analyzed by means of Kruskal-Wallis nonparametric test, followed by Dunn's test, for multiple comparisons. The analyses were performed in GraphPad Prism 6 - p values < 0.05 were significant.

RESULTS AND DISCUSSION

Although evidences that hypothalamic function impairments cause clinical complications linked to the high mortality rate recorded for septic patients, debilitating sequels in septic survivors, such as insulin resistance, heart rate variability loss and autonomic dysfunction, sepsis-induced hypothalamus injuries remain underdiagnosed and mistreated (Pinto et al., 2017; Rorato et al., 2017; Hensley and Prescott, 2018; Schmutzhard and Pfausler, 2017). The aim of the current study was to evaluate the effect of essential oil extracted from *H. crenata* (EOHC) on hypothalamus injury induced by CLP sepsis model in rats.

The effect of EOHC on sepsis-induced thermoregulation changes was initially evaluated through body temperature records taken with the aid of rectal probe, 0 and 48 h after surgical procedures. Although no significant difference of the diurnal body temperature was observed among experimental groups at 0 and 48 h (Figure 1), a decreased diurnal body temperature values recorded from baseline (0h) to 48 h was observed in the Sham group ($\Delta = -0.70 \pm 0.09^\circ\text{C}$). In contrast, CLP-operated rats showed higher body temperature values than the baseline ($\Delta = 0.30 \pm 0.08^\circ\text{C}$), as

shown Figure 1 (*in set.*). The EOHC (300 mg/kg) treatment did not have significant effect on diurnal body temperature changes observed in normal and septic rats. Neither the Sham nor the CLP groups treated with EOHC showed significant diurnal body temperature difference in comparison to their respective control groups; this outcome suggests that EOHC does not affect the thermoregulatory mechanism (Figure 1). However, it cannot be discarded that EOHC has some effect on the sepsis-induced changes in body temperature. The thermoregulatory activity of EOHC must be further evaluated if one takes into consideration the mean body temperature values, which may vary due to different factors, including severe ensuing infection and temporal sepsis progression pattern (Annane, 2018; Lee et al., 2012). Such studies are necessary because no body temperature was herein recorded at early time points and/or under other experimental protocols that could lead to intense hyper/hypothermia. On the other hand, lack of EOHC effect on fever caused by sepsis can be a pharmacological advantage, since recent studies have related that the moderated hyperthermia is associated with reduced death rates recorded for septic patients (Lee et al., 2012; Drewry et al., 2017).

Preliminary findings about the presence of an inflammatory process and of oxidative stress in the pathophysiology of sepsis-induced organ failures have provided new pathways and treatment targets to improve clinical outcomes (Catalao et al., 2017; Griton and Konsman, 2018; Lelubre and Vincent, 2018; Robba et al., 2018).

Tumor necrosis factor Alpha (TNF- α), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6) are pro-inflammatory cytokines that mediate the initial innate immune system responses to injuries or infections by increasing the production of different inflammatory mediators and polymorphonuclear defense cell infiltration in infection sites (Griton and Konsman, 2018; Cojocaru et al., 2003; Robba et al., 2018). High TNF and IL-1 β levels have been found in endotoxin-related Gram-negative sepsis; these two cytokines are effective endotoxin themselves, since they induce septic shock (Singh et al., 2018). Reis et al. (2017) have reported microglial activation due to formation of intracellular multiprotein complexes that, in their turn, activate IL-1 β and lead to significant increase in the production and expression of TNF- α , IL-6, which are reactive oxygen species (ROS) and nitric oxide (NO). Although neurological disorders have been often described at early sepsis phases, recent studies have shown that the time-course of increased cytokine protein depends on the assessed brain region (Freund et al., 1986; Correa et al., 2017). Santos-Junior *et al.* (2019) have shown that IL-1 β , IL-6 and TNF α proteins are high at 3 – 6 h after CLP induction, but they return to baseline values after 72 h; this outcome shows sustained and persistent neuroinflammation in the hypothalamus of CLP-operated rats. The herein adopted analytical parameters and the 48 h post-CLP set as time point to evaluate the potential neuroprotective effect of EOHc, were chosen based on the long-term inflammation course in rats' hypothalamus during experimental sepsis (CLP model), as mentioned above. Moreover, antibiotics were not administered in order to allow the natural pathophysiological course of sepsis to progress within a manageable 48 h study time (Dejager et al., 2011).

Several studies in literature have shown that essential oils extracted from different plant species (that is, *Nigella sativa*, *Lavandula angustifolia*, *Eucalyptus globulus*, *Mentha piperita*, *Rosmarinus officinalis* and *Piper nigrum*) have protection against neurological damage observed in different models of neurologic diseases (Parvez, 2018; Hassan et al., 2017). The neuroprotective effects of essential oils have been attributed to significant pro-inflammatory cytokine reduction, including TNF- α and IL-1 β ; moreover, they stimulate simultaneous increase in anti-inflammatory cytokines such as interleukin-8 (IL-8) (Ayaz et al., 2017). In addition, essential oils have antioxidant properties that result from the presence of an aromatic phenolic ring in their molecular structures, which promotes electron donation and hydrogen atom transfer to free radicals; Therefore, they act as free-radical scavengers reducing agents and single oxygen formation quenchers (Bialecka-Florjanczyk et al., 2018; Wu et al., 2018). Previous studies performed by the current research group have shown that the oral treatment with EOHc, at dose of 300mg/kg, can inhibit gastric lesions in ethanol- and indomethacin-induced ulcer models (Diniz et al., 2013).

EOHc (300 mg/kg) reduced the mortality rate recorded for CLP-operated rats and significantly mitigated serum level rise in liver injury biomarkers, such as ALP, ALT, and total bilirubin; it also ruled out changes in oxidative stress and hepatic histopathological changes promoted by CLP sepsis model (Lima et al., 2018). The effect of EOHc on important inflammatory mediators directly involved in sepsis-induced organ injuries were assessed based on key inflammation and oxidative stress' roles in the pathogenesis of organ impairment caused by sepsis and by preliminary pharmacological properties attributed to EOHc (Catalao et al., 2017; Griton and Konsman, 2018; Hensley and Prescott, 2018; Lelubre and Vincent, 2018).

Pro-inflammatory cytokines (TNF- α , IL-1 β and IL-6), oxidative stress biomarkers and morphological analysis were performed 48 h after CLP to assess EOHc (300 mg/kg) effect on important inflammatory mediators associated with sepsis-induced organ dysfunctions. Figure 2 shows CLP-operated rats presenting significant ($p < 0.05$) TNF- α and IL-1 β (9.36 ± 0.55 and 281.12 ± 15.13 $\mu\text{g}/\text{mg}$ of protein, respectively) increase in rats' hypothalamus in comparison to Sham-operated rats (6.48 ± 0.99 and 245.47 ± 8.93 $\mu\text{g}/\text{mg}$ of protein, respectively). Interestingly, the oral treatment with EOHc (300 mg/kg) inhibited IL-1 β and TNF- α level increase, as well as the inflammatory cell infiltration and epithelial loss in the hypothalamus of CLP-operated rats treated with EOHc in comparison to the control group, as illustrated in Figures 2 and 3. The EOHc treatment has inhibited ($p < 0.05$) tissue TNF- α and IL-1 β level increase due to sepsis, as evidenced by the TNF- α and IL-1 β (6.97 ± 0.79 and 243.12 ± 4.08 $\mu\text{g}/\text{mg}$ of protein, respectively) levels recorded for the hypothalamus of CLP-operated rats treated with OEHC, which was similar to that of Sham-operated animals (Figure 2A and B). There was no statistical difference in IL-6 levels in hypothalamus tissue, among experimental groups 48 h after surgical procedures (Figure 2C).

Inflammation and oxidative stress are involved in detrimental pathways activated during sepsis; eventually, these factors lead to organ dysfunction and death. Oxidative stress is mainly triggered by the simulation of pro-inflammatory cytokines and by multiple noxious processes taking place during sepsis, such as direct cell damage induced by reactive oxygen species or by the activation of the gene expression leading to amplified inflammatory response (Bavunoglu et al., 2016; Prauchner, 2017).

There was significant ($p < 0.05$) catalase (CAT) activity decrease (383.02 ± 19.67 mM/min/mg of protein) accompanied by increased MDA levels (18.42 ± 1.41 $\mu\text{mol}/\text{mg}$ tissue) in the hypothalamus of CLP-operated rats in comparison to Sham-operated rats (434.13 ± 7.30 mM/min/mg of protein and 14.11 ± 0.50 $\mu\text{mol}/\text{mg}$ tissue, respectively), 48 h after CLP (see Figure 3). The oral treatment with EOHc (300 mg/kg) did not have any effect on CAT activity (Figure 3A), whereas CLP-operated rats treated with EOHc showed hypothalamus MDA level (14.02

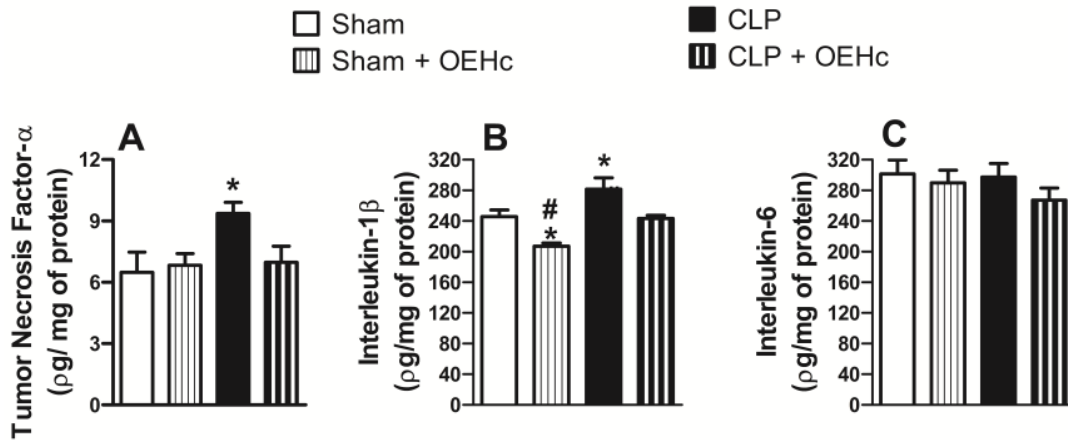


Figure 2: Effect of EOHC on sepsis-induced alterations in the hypothalamic levels of TNF- α (A), IL- β (B) and IL-6 (C), at 48 h post-CLP. At 12 and 24h post-surgery, Sham-operated rats (n=7) and CLP-operated rats (n=7) were orally treated with vehicle or vehicle + EOHC (300 mg/kg). At 48h, the animals were euthanized and the hypothalamus removed for measuring of tissue pro-inflammatory cytokines. *p<0.05 versus control group.

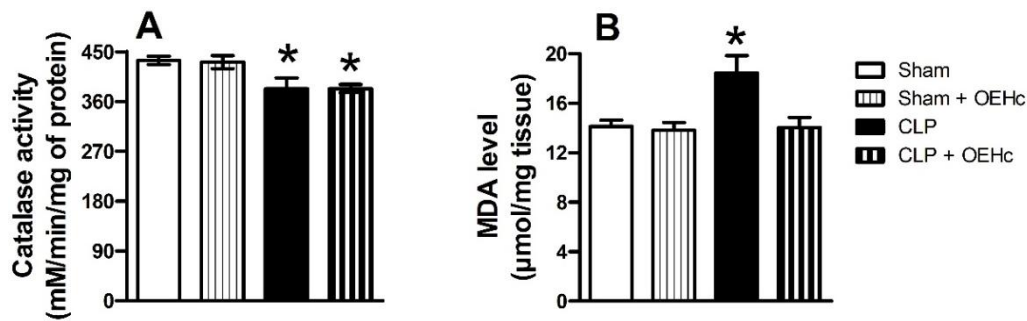


Figure 3: Effect of EOHC on sepsis-induced alterations in the hypothalamic catalase (CAT) activity (A) and malondialdehyde (MDA) levels (B), at 48h post-CLP. At 12 and 24 h post-surgery, Sham-operated rats (n=7) and CLP-operated rats (n=7) were orally treated with vehicle or vehicle + EOHC (300 mg/kg). At 48h, the animals were euthanized and the hypothalamus removed for measuring of CAT activity and MDA levels. *p<0.05 versus control group.

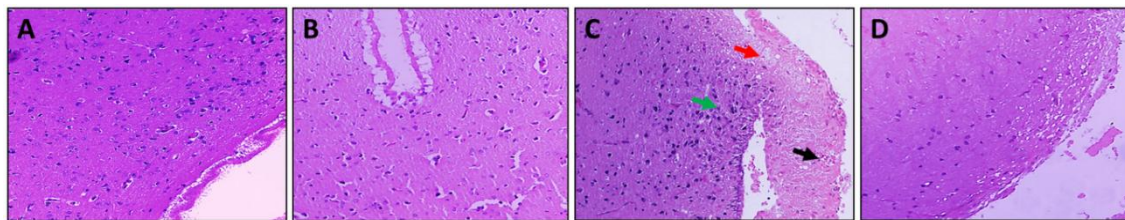


Figure 4: Effect of EOHC on sepsis-induced histomorphological changes observed in hypothalamus slices stained with hematoxylin and eosin (H&E), at 48h post-CLP. Sham (A), Sham + OEHC (B), CLP (C) and CLP + OEHC (D) groups. The oral treatment with EOHC (300 mg/kg) occurred at 12 and 24h post-surgery. At 48h, the animals were euthanized and the hypothalamus removed for histological evaluation. Inflammatory cell infiltration (green arrow), hemorrhagic damage (black arrow) and epithelial cell loss (red arrow).

$\pm 0.81 \mu\text{mol/mg tissue}$) similar to that of the Sham group (Figure 3B); this outcome indicated that EOHC significantly inhibited ($p<0.05$) hypothalamic lipid peroxidation increase due to sepsis.

The histological analysis of H&E-stained hypothalamic sections illustrated in figure 4 and the histological change scores recorded for each group in table 1 provide evidences of inflammation in the hypothalamus of CLP-operated rats,

Table 1: Histological score of sepsis-induced hypothalamus lesions assessed by HE stain.

Experimental group	Inflammatory cells (score, 0–3)	Hemorrhagic damage (score, 0–3)	Epithelial cell loss (score, 0–3)	Total (score, 0–9)
Sham	0 (0)	0 (0)	0.5 (0-1)	0.5
Sham + EOHC	0 (0)	0 (0)	0.5 (0-1)	0.5
CLP	2.5 (1-3)*	2 (1-3)*	2.5 (1-3)*	7
CLP + EOHC	0.5 (0-1)#	1 (1-1)	0.5 (0-1)#	2

Data shown are medians with minimal and maximal scores shown in parentheses. Kruskal–Wallis nonparametric test, followed by Dunn's test for multiple comparisons.

*p < 0.05 versus Sham group; #p < 0.05 versus CLP group.

as well as the neuroprotective effect of EOHC observed in previous parameters (Figures. 2 and 3). No obvious morphological changes, or significant inflammation, were observed in the hypothalamus H&E-staining sections of the Sham and Sham-EOHC groups, as illustrated in Figure 4A and B. CLP-operated animals presented inflammatory cell infiltration, hemorrhagic damage and epithelial cell loss (Figure 4C); there was significant cell infiltration and lower epithelial cell loss (*p<0.05) in the H&E staining hypothalamus sections of CLP-operated rats treated with EOHC in comparison to CLP-operated rats (see Figure 4C and D).

CONCLUSION

Based on the current results, the oral treatment with EOHC (300 mg/kg) inhibited important factors often involved in the pathogenic mechanism of sepsis-induced tissue damage. EOHC led to significant proinflammatory cytokine level decrease, inflammatory cell infiltration and lipidic peroxidation in the hypothalamus of septic rats 48 h after CLP. To the best of our knowledge, this is the first study focused on evaluating the effect of essential oils on sepsis-induced hypothalamus injury. However, further investigations are needed to clarify the pharmacological mechanisms of EOHC, which has neuroprotective effect against sepsis-induced hypothalamus injury. Data in the present study has provided evidences that the oral treatment with EOHC had anti-inflammatory and antioxidant action in the hypothalamus of CLP-operated rats, 48 h after CLP. Based on the current results, EOHC is a potential therapeutic tool to treat hypothalamic injuries caused by sepsis, as well as an alternative, or adjuvant protocol to conventional therapies, since it collaborates to improve treatment outcomes.

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