Effect of Cinnamon Extract against Experimentally Induced Sepsis on The Adrenal Cortex of Adult Male Albino Rats

Original Article

Yasmin Nabil Saleh, Fatma El-Nabawia Abdel-Hady El-Safty, Noha Mohey Issa and Marwa Abdel-Samad Al-Gholam

Department of Human Anatomy and Embryology, Faculty of Medicine, Menoufia University, Menoufia, Egypt

ABSTRACT

Objectives: Sepsis is a serious worldwide condition due to its high mortality rate in hospitals. The key organ in the body's response to sepsis is the adrenal gland which responds to the adrenocorticotrophic hormone by the production of large amounts of corticosteroids, resulting in the activation of a catabolic state, immunomodulatory, anti-inflammatory effects.

Aim of the Work: To study the cinnamon extract effects on the adrenal cortex of cecal slurry-induced sepsis in rats.

Material and Methods: Sixty-six rats; six were used as donors for slurry. Five groups were formed with the rest; control (I), cinnamon extract (II), experimentally induced sepsis (III), cinnamon pretreated (IV) and cinnamon pre and post-treated septic (V) groups. sepsis induction in groups (III, IV and V) was done by cecal slurry. Scarification of animals was done at the end of the fifth week of the experiment except for group V, animals were sacrificed five days later from sepsis induction. Serum levels of aldosterone and corticosterone were measured. Also, MPO, MDA and TNF-α levels have been estimated in adrenal homogenates of all groups. H&E was performed on the adrenals in all groups, Mallory's trichrome and immunohistochemical stains for caspase3, CD44 and iNOS. Also, were examined by electron microscope. Cortical thickness, area % of collagen deposition, Caspase-3, CD44 and iNOS immunoreaction have been estimated and data were analyzed statistically.

Results: Septic group showed some deaths. But, survivors of group III revealed features of Zona glomerulosa and fasciculata inflammatory degeneration with increased levels of serum aldosterone, corticosterone, tissue MPO, MDA, and TNF-α. Also, group III revealed a marked increase in caspase3, CD44 and iNOS immunoreaction. Groups IV and V showed improvement in all previous parameters.

Conclusion: Cecal slurry-induced sepsis caused massive Zona glomerulosa and fasciculata inflammatory changes that were improved by the orally administrated cinnamon extract.

Received: 06 March 2023, **Accepted:** 27 May 2023

Key Words: Cecal slurry, cinnamon extract, cytokines, sepsis, TNF-alpha.

Corresponding Author: Yasmin Nabil Saleh , MSc, Department of Human Anatomy and Embryology, Faculty of Medicine, Menoufia University, Menoufia, Egypt, **Tel.**: +20 10 2218 5201, **E-mail:** yasmin.saleh158254@gmail.com

ISSN: 1110-0559, Vol. 47, No. 2

INTRODUCTION

Sepsis is a serious emergency, worldwide life-threatening illness, associated with multi-organ failure owing to the immune response of the body to infection[1]. Treatment of sepsis is very difficult due to misdiagnosis as a result of different infection sources, immunological responses, and pathophysiological alterations. High mortality percent is associated with sepsis, especially in intensive care unit patients. In cases of septic shock and multisystem dysfunction, the mortality rate increases by 70%[2].

Sepsis is an outcome of an exaggerated inflammatory response to infection[3]. These led to the stimulation of the immune system and the stress response that is involved in the activation of the hypothalamic-pituitary-adrenal axis (HPAA)[4,5]. In sepsis, the adrenal gland is very important in host response as the cortex produces high amounts of corticosteroids in response to adrenocorticotrophic hormone (ACTH). Corticosteroids activate catabolic pathways and produce strong anti-inflammatory, and immune-modulating action[6].

The primitive body response to sepsis stimulates cortisol release, leading to increase secretion and synthesis of pro- and anti-inflammatory agents to localize inflammation in infected tissues. But, inflammatory, apoptotic changes and ischemic damage in HPAA, may cause adrenal insufficiency[7].

Sepsis is associated with necrosis and hemorrhage within the hypothalamic pituitary adrenal axis[8,9]. This affection results in adrenal insufficiency which is characterized by decreased corticosteroid production through hypo-responsiveness of the adrenal cortex to ACTH[10].

Personal non-commercial use only. EJH copyright © 2024. All rights served *DOI: 10.21608/ejh.2023.197425.1865*

Cinnamon has proved to be a powerful antioxidant and anti-inflammatory herbal agent[11]. Cinnamon can be used to control diabetes mellitus, blood pressure, tumor growth, Alzheimer's disease, and Parkinson's disease[11]. Another interesting medical use of cinnamon might be due to its antimicrobial properties, especially its antibacterial effect[12].

So, the aim of this study was to study the histopathological changes in the adrenal cortex in response to experimentally induced sepsis and to assess the effect of cinnamon extract that was based on biochemical, histological and immunohistochemical studies.

MATERIALS AND METHODS

Animals

This study was carried out using Sixty-six Sprague-Dawley adult male albino rats weighing 200-250 grams each, obtained from Theodor Bilharz Research Institute, ElWarak, Giza, Egypt. A suitable temperature was maintained for the rats. Water and a standard diet were readily available to them. Ethics approval was obtained from the Faculty of Medicine, Menoufia University, Egypt's ethics committee on experimental animals, code no. 2/2021ANAT18.

Chemicals

Dextrose was obtained as a 5% solution from a local pharmacy (Menoufia, Egypt). Manufacturing by FIPCO, New Borg Elarab, Egypt.

The aqueous cinnamon bark extract

The extract was prepared at the faculty of science, Menoufia University, Egypt. Grinding cinnamon bark yielded a fine powder. Afterward, in 500 ml of distilled water, 50 grams of that powder were dissolved. A rotary evaporator was used to evaporate water after simple filtration[13].

Experimental design

Sixty-six rats were divided as follows: Six rats were used as donors for cecal slurry, and the others were divided into five groups

Control group (Group I): 12 adult male albino rats were included and divided into two subgroups (6 rats each)

- Subgroup IA: rats were not given any kind of treatment all over the experiment.
- Subgroup IB: rats received intraperitoneal injection by 5% dextrose for the same period of cecal slurry injection (once).

Cinnamon bark extract treated group (Group II, 12 rats): rats were given cinnamon bark aqueous extract once/day by Gastric intubation for 4 weeks and 5 days at a dose of 100 mg/kg/day dissolved in 1 ml water[10].

Experimentally induced septic group (Group III, 12 rats): 10 ml cecal slurry/kg body weight was given intraperitoneally once to rats[14,15].

Cinnamon pretreated septic group (Group IV, 12 rats): rats were given aqueous cinnamon extract at the same dose and period as group II then sepsis was initiated by cecal slurry.

Cinnamon pre and post-treated septic group (Group V, 12 rats): rats in this group received the same dose of cinnamon extract as Group II for 4 weeks and then subjected to sepsis as Group III and cinnamon extract receiving continued for another 5 days after sepsis induction.

Scarification of all animals of different groups was done at the end of the fifth week of the experiment; 48 hours after sepsis induction in groups III and IV. In group V, the animals were sacrificed 5 days later from sepsis induction. Before scarification, The animals were rendered unconscious with inhaled diethyl ether. The right, and left adrenals of each animal were dissected.

Experimental procedure

Induction of sepsis

By administering cecal slurry, sepsis was induced. A combination of xylazine (10 mg/kg) and ketamine hydrochloride (50 mg/kg) was utilized to anesthetize the rats that served as donors. The cecum was exposed after a midline abdominal incision was made. A 0.5 cm opening was made on the cecum's anti-mesenteric surface and compressed to obtain fecal material. The collected feces were weighed and immediately mixed with 5% dextrose in a 1:3 dilution[14]. The cecal slurry was used within 2 hours. It was injected intraperitoneally to rats at a dose of 10 ml/kg[15].

Biochemical study

ELISA kites (ab136933) and (ab108821) from Abcam company, USA, were used for the estimation of serum levels of aldosterone and corticosterone respectively[16,17]. Also, kits were used for estimation of myeloperoxidase (MPO) ((ab155458), Abcam company, USA), malondialdehyde (MDA) (ab238537), Abcam company, USA) and tissue tumor necrosis factor-alpha (TNF-α)(ab236712), Abcam company, USA) levels in adrenal homogenate[18,19].

Light microscopic study

1-Hematoxyline & Eosin (Hx&E) study

Fixation of samples in 10% formol saline for 2-5 days was performed, dehydration in ascending grades of ethyl alcohol, then cleared and impregnated in soft paraffin for 45 minutes followed by hard paraffin for another 45 minutes. After that, specimens were embedded in hard paraffin to obtain blocks. 5-7 micrometers in thickness paraffin sections were stained with Hx&E[19].

2- Mallory's trichrome for monitoring of collagen accumulation

Specimens were washed under water and then treated with ponceau-acid fuchsin for 3 minutes. Then counterstaining was done using aniline blue. After that, sections were washed and dehydrated[20].

3-Immunohistochemical study

Sections were prepared by boiling for 10 minutes in 10Mm, then the antigen retrieval was performed by treatment for 10 minutes in citrate buffer (Ph 6). After that sections had cooled at room temperature. Then, ultraviolet block and application of antibodies anti-caspase-3 (rabbit polyclonal antibodies (ab 4051), Abcam Company, Cairo, Egypt), anti-CD44 (rabbit polyclonal antibodies (ab 189524), Abcam Company, Cairo, Egypt) and Inducible nitric oxide synthase (iNOS) (rabbit polyclonal antibodies (ab 178945), Abcam Company, Cairo, Egypt) were performed. Immunostaining was completed by counterstaining done using Mayers hematoxylin[21]. Excluding of the primary antibodies was done to obtain negative controls. While, positive controls of Caspase-3, CD44 and iNOS were human tonsils.

4- Electron microscope study

For the transmission electron microscope study, fixation of specimens from the left adrenal was done with 2.5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4) at 4 ◦C for 2 h, and then adding 1% osmium tetroxide for post-fixation. After that, immersion of samples in serial dilution of ethanol was done. Lastly, embedding in epoxy resin (Epoxy Embedding Medium Kit; Sigma) of the fixed specimen was done. Ultramicrotome was used for cutting semi- and ultra-thin sections and stained with 1% toluidine blue. then stained with 2.5% uranyl acetate as a chief stain and counterstaining was performed by lead citrate[22].

5-Morphometric study

Six adrenal sections were selected haphazardly for morphometric analysis in the Faculty of Medicine, Menoufia University by using an Image J analyzer (version 1.43o8, National Institute of Health, USA). The obtained measurements were:

- The cortical thickness for H&E.
- The area percentage (%) of collagen in the tissue.
- The area percentage (%) of Caspase-3, CD44 and iNOS positive immunoreaction.

6- Statistical study

Tabulation and analysis of the estimated data were done by SPSS (statistical package for social science) version 23.0. Data were given as means and standard deviations. Data of studied groups were compared by ANOVA followed by post hoc test[23]. The significance of the data collected was expressed by the *P value* (probability of chance): $p<0.05$ was considered statistically significant.

RESULTS

No discernible significance was evident between the control subgroups and the group treated with cinnamon extract across all parameters

Survival rate

The survival percentage was 100% in groups I and II. But it was significantly decreased in group III. Moreover, it showed improvement in groups IV and V (Figure 1).

Biochemical findings

Serum aldosterone

Significant elevation ($P<0.001$) of serum aldosterone level was detected in an experimentally induced septic group compared to the control group. However, a significant decline ($P<0.001$) in serum aldosterone level was found in cinnamon pretreated septic and cinnamon pre and post-treated septic groups ($P< 0.001$) compared to the septic untreated group (fFigure 2A).

Serum corticosterone

In the experimentally induced septic group, significant rise ($P< 0.001$) in serum corticosterone level was found compared to the control group. Although, significant decline ($P< 0.001$) in serum corticosterone levels in both cinnamon pretreated septic and cinnamon pre and post-treated groups compared to the untreated septic group (Figure 2B).

Tissue MPO

Tissue MPO was significantly higher ($P< 0.001$) in the experimentally induced sepsis group as compared to the control group. But, significant decline ($P< 0.001$) in tissue MPO level in the cinnamon pretreated septic group compared the to untreated septic group. Also, tissue MPO level in cinnamon pre and post-treated septic groups was significantly decreased ($P< 0.001$) compared to the untreated septic group. Moreover, significant decrement ($P< 0.05$) in tissue MPO level in cinnamon pre and post-treated group compare to the cinnamon pretreated septic group (Figure 3A).

Tissue MDA

Tissue MDA level was significantly higher in the experimentally induced sepsis group ($P< 0.001$) as compared to the control group. However, MDA level in cinnamon pretreated and cinnamon pre and post-treated septic groups significantly declined ($P<0.001$) compared to the untreated septic group (Figure 3B).

Tissue TNF-alpha

There was a significant elevation ($P<0.001$) in tissue TNF alpha level in the experimentally induced septic group compared to the control group. But, significant decline ($P<0.001$) in tissue TNF-alpha level in cinnamon pretreated and cinnamon pre and post-treated septic groups compared to the untreated septic group (Figure 3C).

Light microscopic findings

HX and E findings

Adrenal sections of the control group revealed that the gland was divided into cortex and medulla, the cortex covered by a connective tissue capsule. The cortex showed three distinct zones; glomerulosa, fasiculata and reticularis.

The zona glomerulosa is arranged in groups of cells with vacuolated cytoplasm and vesicular eccentric nucleus The zona fasiculata cells are arranged in regular cords with vacuolated or pale acidophilic cytoplasm and vesicular nucleus with a prominent nucleolus. The zona reticularis formed of an irregular group of cells with deep acidophilic cytoplasm and a vesicular nucleus with prominent nucleolus (Figure 4A).

Sections of group III revealed a loss of zonation and architecture of the cortex. Vacuolation and dilated engorged sinusoids were also seen. Massive infiltration by different inflammatory cells such as; many mononuclear, macrophages and few neutrophils of ZG and ZF was seen (Figure 4B)

In group IV, sections revealed increased cortical thickness with an improvement of most of the previously mentioned histopathological findings in group III. There is a mild loss of architecture of gland cells that showed vacuolation in cytoplasm and deeply stained nuclei while other cells show normal cytoplasm and vesicular nuclei with infiltration by many inflammatory cells (Figure 4C).

Group V sections showed marked improvement of the histological findings. However, thick connective tissue capsule was found around the gland. The cells of the cortex showed normal acidophilic cytoplasm and vesicular nuclei but few cells showed vacuolation and pyknotic nuclei, dilated sinusoids and a few scattered inflammatory cells (Figure 4D).

The thickness of the adrenal cortex

The adrenal cortical thickness in the experimentally induced septic group was significantly higher (P<0.001) as compared to the control group. However, a significant decline (P<0.001) in the thickness of the adrenal cortex in cinnamon pretreated and cinnamon pre and post-treated septic groups compared to the septic untreated group was detected. The adrenal cortical thickness in the cinnamon pre and post-treated septic group showed a significant decline (P<0.001) compared to the pretreated septic group (Figure 5).

Mallory's trichrome stain

Sections of group I revealed an absence of collagen deposition around the sinusoids of the cortex and a normal amount and distribution of collagen fibers of the capsule (Figure 6A). Group III showed excessive collagen deposition in the capsule, in between the degenerated cortical cells and around the blood sinusoids (Figure 6B).

Collagen deposition in the capsule was moderately increased with mild deposition around the sinusoids of the cortex in group IV (Figure 6C). Group V revealed a mild increase in deposition of collagen fibers in the capsule with faint deposition around sinusoids in the cortex (Figure 6D).

The area percentage (%) of collagen deposition

A highly significant increment (P<0.001) in the area % of collagen deposition in the experimentally induced sepsis group as compared to control group was found. However, significant decrement (P< 0.001) in the area % of collagen deposition in cinnamon pretreated and pre and post-treated septic groups compared to the experimentally induced sepsis group. Also, the area % of collagen deposition in cinnamon pre and post-treated septic groups significantly declined (P < 0.001) compared to the cinnamon pretreated septic group (Figure 7).

Caspase-3 expression

Sections of the control group showed negative immunoreaction to caspase-3 in almost all of the adrenal cortical cells. But, the untreated septic group showed a marked increase in Caspase-3 immunoreaction. While the pretreated and pre and post-treated groups showed a reduction of Caspase-3 immunoreaction (Figures 8 a,b,c,d).

Area percentage (%) caspase-3 immunoreaction

Caspase-3 immunoreaction was significantly higher (P<0.001) in Group III compared to Group I. But, a significant decline (P< 0.001) of Caspase-3 immunoreaction in groups IV and V compared to group III was detected. Also, Caspase-3 immunoreaction in cinnamon group V showed a significant decrement (P< 0.001) compared to group IV (Figure 9 A).

CD44 immunoreaction

Sections of group I showed negative immunoreaction to CD44 in the cells of the adrenal cortex. But, the untreated septic group revealed upregulation of CD44 immunostaining. While cinnamon pretreated and pre and post-treated septic groups revealed downregulation of CD44 immunoreaction (Figures 8 e,f,g,h).

Area percentage (%) of CD44 immunoreaction

Group III expressed a significant rise (P<0.001) in the area % of CD44 immunoreaction compared to group I. But, significant decline (P<0.001) in the CD44 area % immunoreaction in groups IV and V compared to group III. The area % of CD44 immunoreaction in group V showed a significant decrement (P<0.001) compared to group IV (Figure 9B).

iNOS immunoreaction

Sections of this group I showed negative immunoreaction to iNOS the adrenal cortical cells. But, the untreated septic group showed upregulation of iNOS immunostaining. While the cinnamon pretreated and cinnamon pre and post-treated septic groups showed down-regulation of iNOS expression (Figures 8 I,j,k,l).

Area percentage (%) of iNOS immunoreaction

The area % of iNOS immunoreaction in Group III was significantly higher (P<0.001) as compared to Group I. But, a significant decline (P<0.001) in area % of iNOS immunoreaction in groups IV and V compared to group III was found. Area % of iNOS immunoreaction in group V

showed a significant decline (P<0.001) compared to group IV (Figure 9C).

Electron microscopic findings

Sections of group I showed normal distribution and echogenicity of the cytoplasm, round euchromatic nuclei, normal shape, and architecture of other cellular organelles such as mitochondria, smooth endoplasmic reticulum (SER) with intact junction complex and many lipid droplets (Figures 10,11 A). Group III showed complete rarefication of cytoplasm, shrunken irregular nuclei, partial and complete lysis of the mitochondria, dilated congested blood vessels with loss of architecture of other cellular organelles, and complete separation of junction complex (Figures 10,11B).

Sections of group IV showed moderate loss of architecture of cellular organelles, the cytoplasm showed normal areas while other areas showed rarefication and some irregular and shrunken nuclei with wide peri-nuclear space while others are normal. There was partial degeneration of mitochondria, smooth endoplasmic reticulum (SER), and other organelles. Moderate separation in intercellular junction and dilated congested sinusoids (Figures 10,11C).

Sections of this group V showed normal cytoplasm with some vacuolation, dilated sinusoids and almost normal nuclei. Other organelles appeared almost normal with intact junction complex in some cells while it was separated in other cells (Figures 10,11 D)

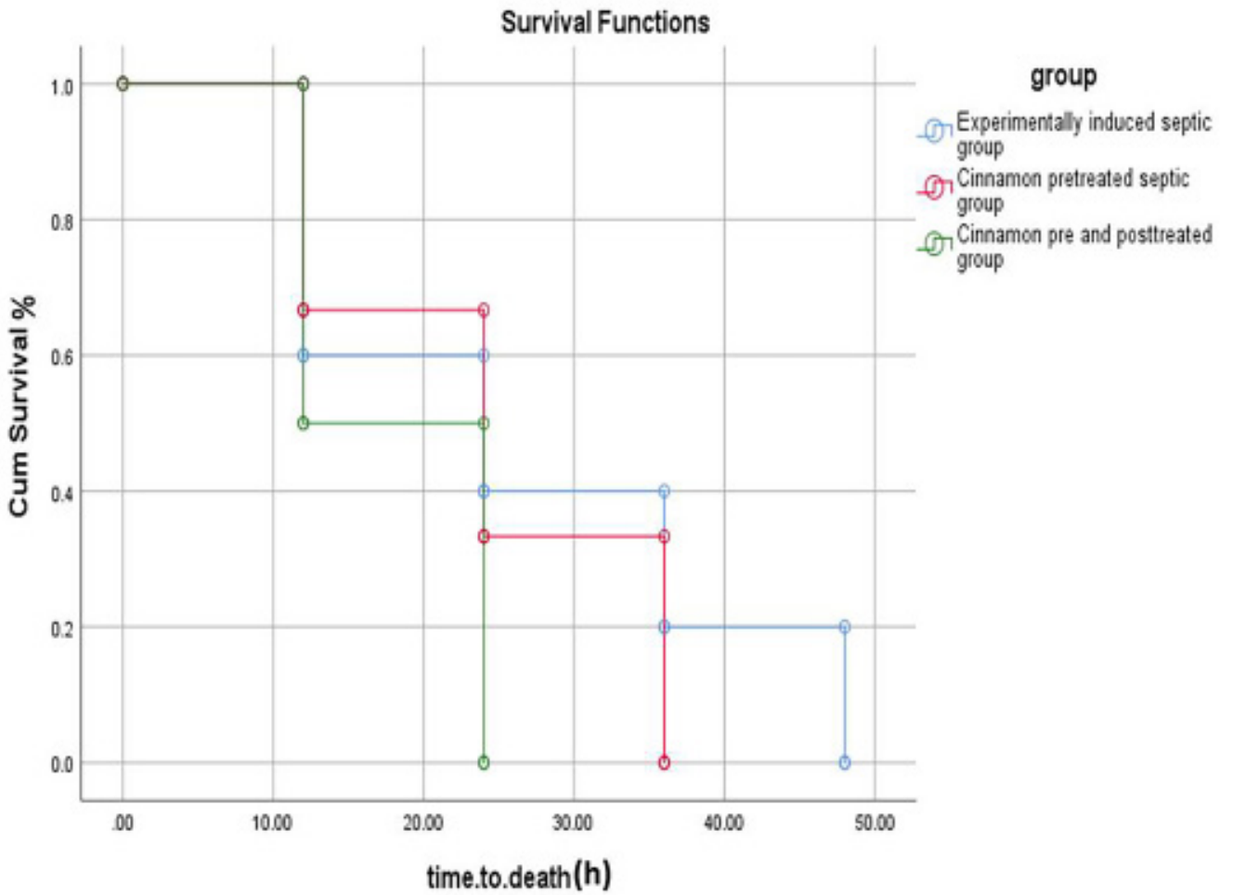

Fig. 1: Kaplan-Meier curve revealed survival% in 48 hours among the studied groups

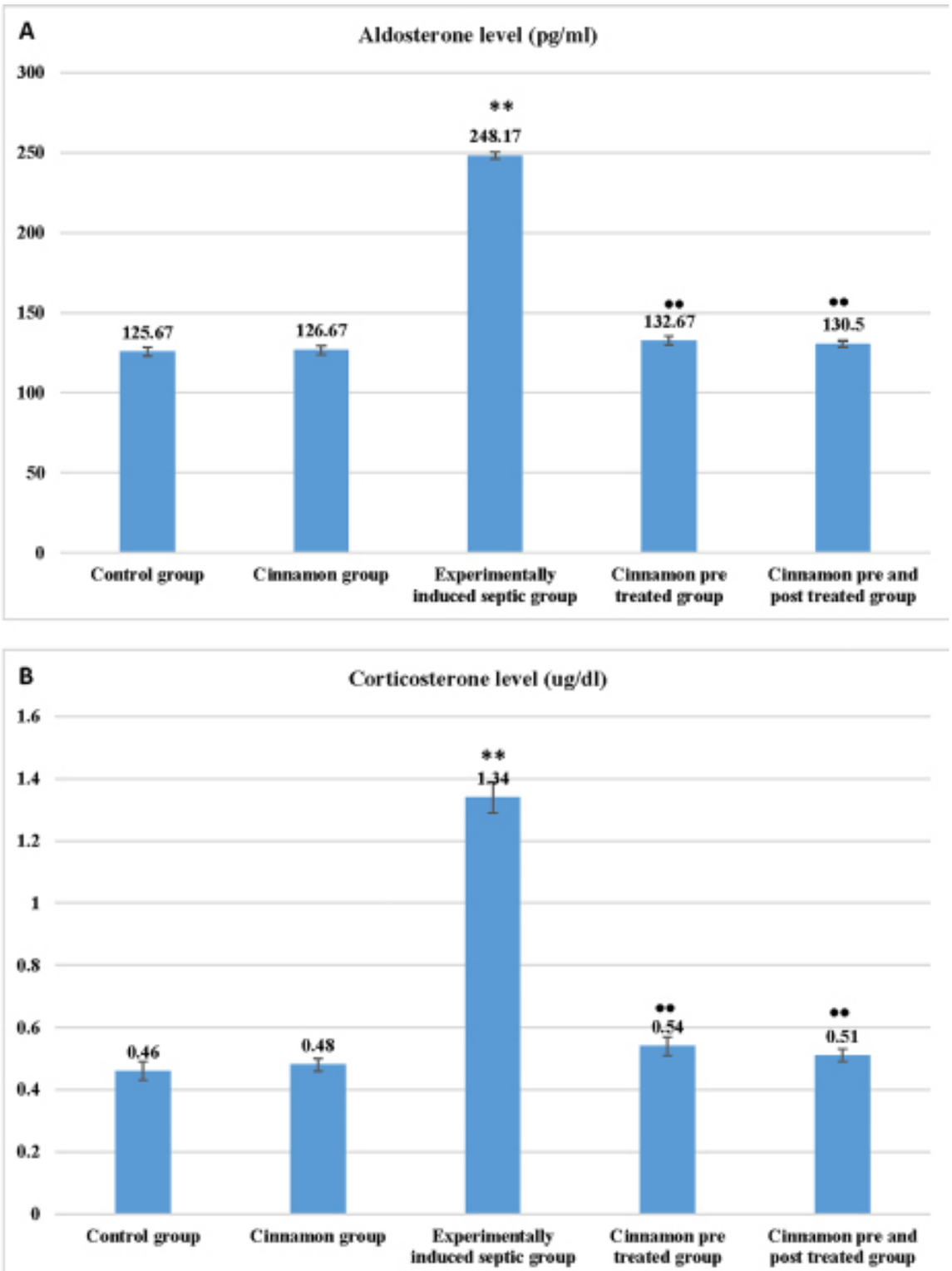

Fig. 2 (A-B): Mean serum aldosterone and mean serum corticosterone levels among the studied groups. ** Significant rise ($P < 0.001$) as compared to control group, •• Significant decline ($P < 0.001$) as compared to experimentally induced sepsis group

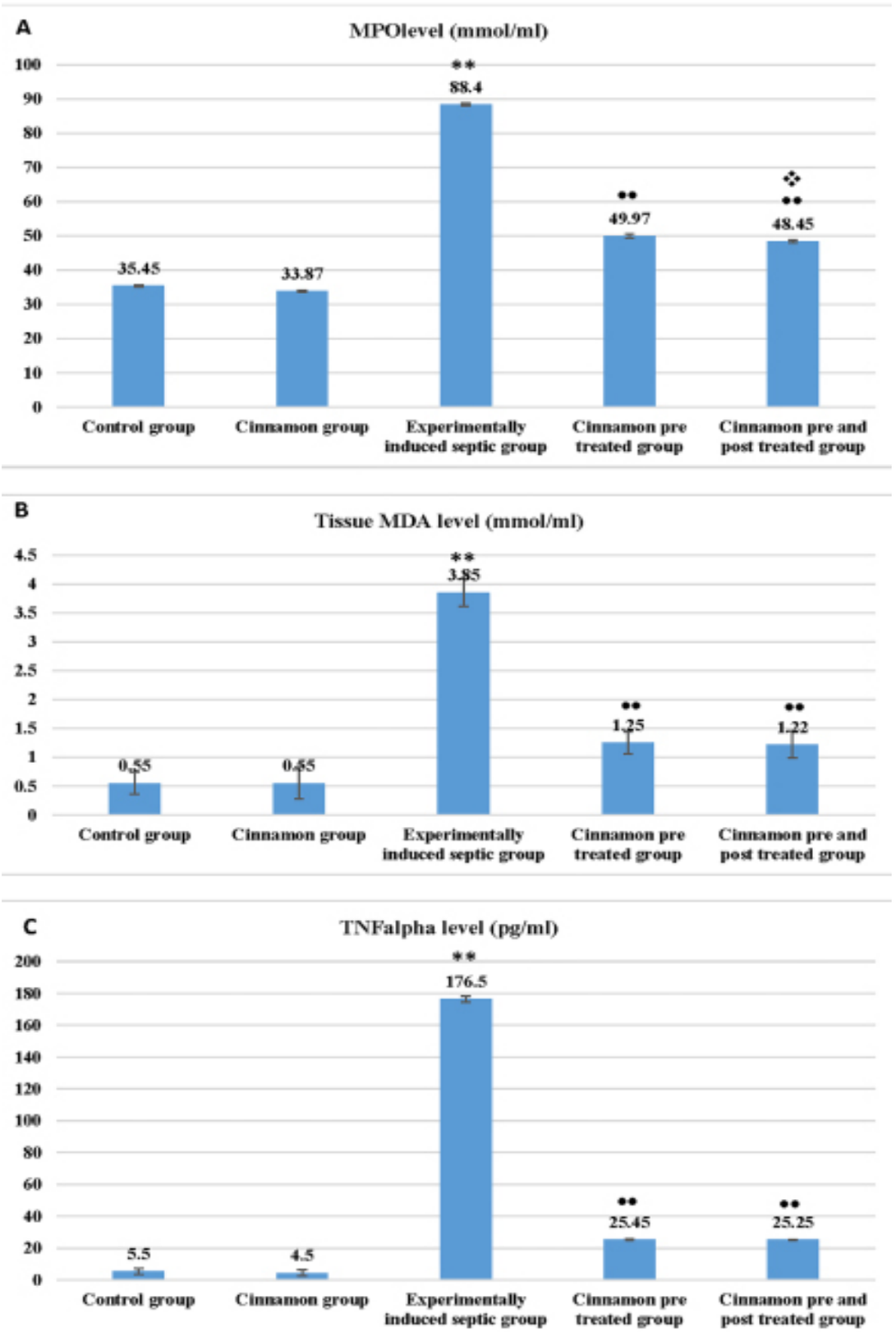

Fig. 3 (A,B,C): comparison among the studied groups regarding the tissue MPO, MDA and TNF alpha levels. ** Significant elevation ($P < 0.001$) as compared to control group, •• Significant decline ($P < 0.001$) as compared to experimentally induced sepsis group, * Significant decline ($P < 0.05$) of tissue levels of MPO in cinnamon pre and post treated septic group as compared to cinnamon pretreated septic group

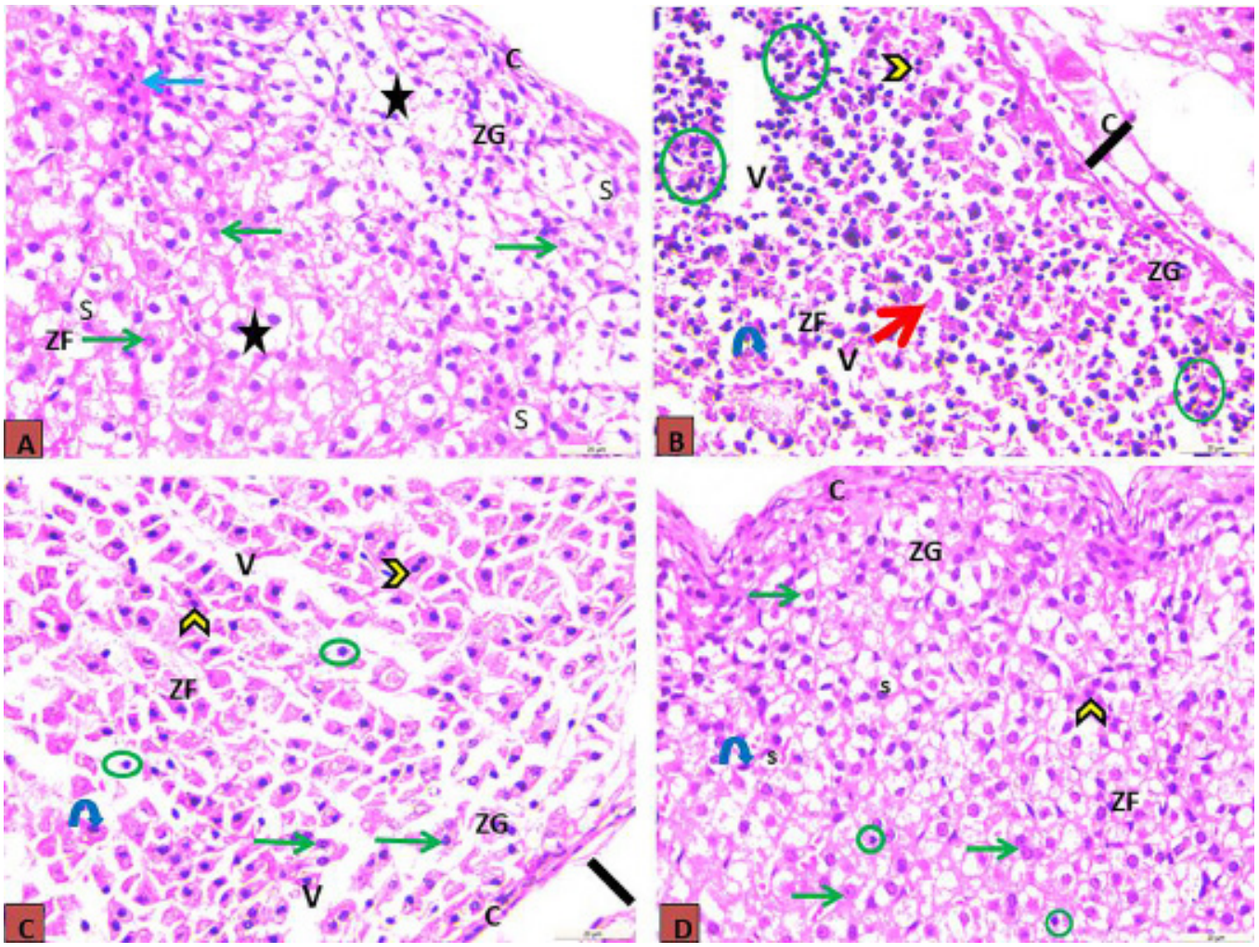

Fig. 4 (A-D): A photomicrograph of H & E stained zona glomerulosa and fasiculata of rat adrenal gland showing thin connective tissue capsule (c), cells with normal vesicular nucleus (red arrow), pale eosinophilic (blue arrow) or vacuolatd cytoplasm(star).and multiple sinusoids (S) between cells in control group (A). Thick capsule with dissociated fibers (thick black line), deeply stained nuclei in swollen (blue curved arrow) vaculated cytoplasm (V), binucleated cells (arrow head), empty degenerated cells (red arrow) and infiltration by mononuclear cells (circle) in experimentally induced sepsis group (B). Normal cells with vesicular nuclei (green arrow), binucleated cells (arrow head), large swollen cells with pyknotic nuclei (blue curved arrow), vacuolation (V) and infiltration by mononuclear cells (circle). Apparent thick capsule with wide apace between its fibers (thick line) in cinnamon pretreated septic group (C). Normal architecture of cells with vesicular nuclei (green arrow), some binucleated cells (arrow head) and some deeply stained nuclei (blue arrow), sinusoids between cells, thick capsule (C) and infiltration by mononuclear cells (circle) in cinnamon pre and post treated septic group (D). (scale bar= 20µm).

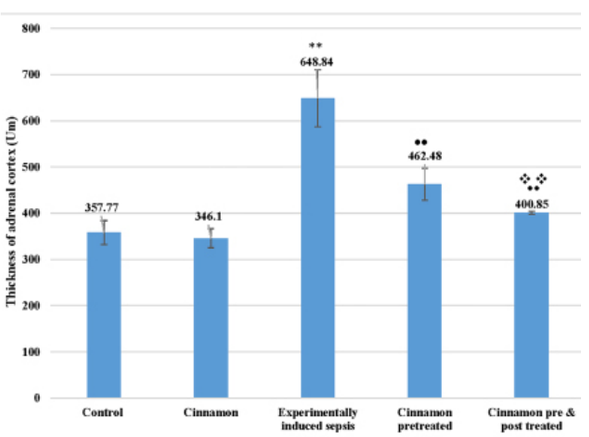

Fig. 5: Mean thickness of adrenal cortex (µm) among the studied groups. ** Significant elevation (P < 0.001) compared to control group, •• Significant decline (P < 0.001) compared to experimentally induced sepsis group, ** Significant decline (P< 0.001) in cortical thickness in cinnamon pre and post treated septic group compared to cinnamon pretreated septic group.

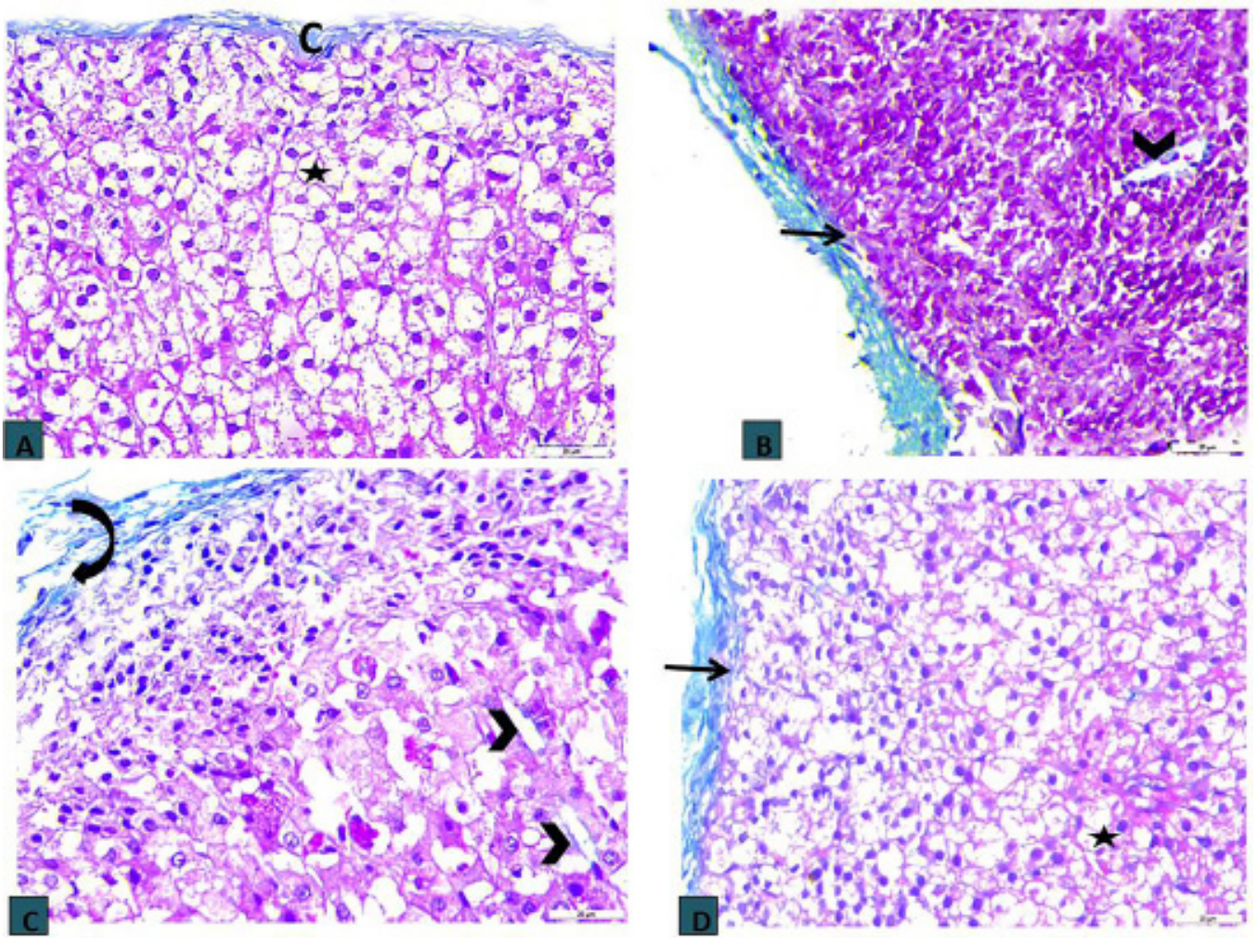

Fig. 6 (A-D): A photomicrograph of adrenal cortex stained with mallory trichrom stain revealed normal amount and distribution of collagen in capsule (c) and absence of deposition around sinusoids (star) in control group (A). Extensive increase in collagen deposition in capsule (black arrow) and around the sinusoids (arrow head) in experimentally induced sepsis group (B). Moderate increase in collagen deposition with wide space between the fibers (black arrow) and around sinusoids (arrow head) in cinnamon pretreated septic group (C). Mild increase in collagen deposition in the capsule (black arrow) with absence of deposition around the sinusoids (star) in cinnamon pre and post treated septic group (D). (Scale bar= 20µm).

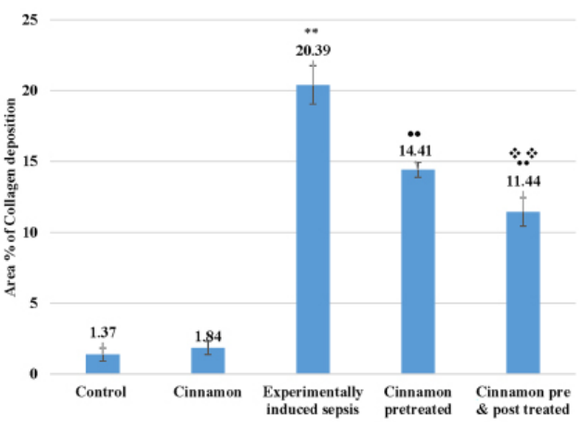

Fig. 7: The area percentage (%) of collagen deposition. ** Significant elevation (P < 0.001) compared to control group, •• Significant decline (P < 0.001) compared to experimentally induced sepsis group, ** Significant decline (P< 0.001) in area% of collagen in cinnamon pre and post treated septic group compared to cinnamon pretreated septic group.

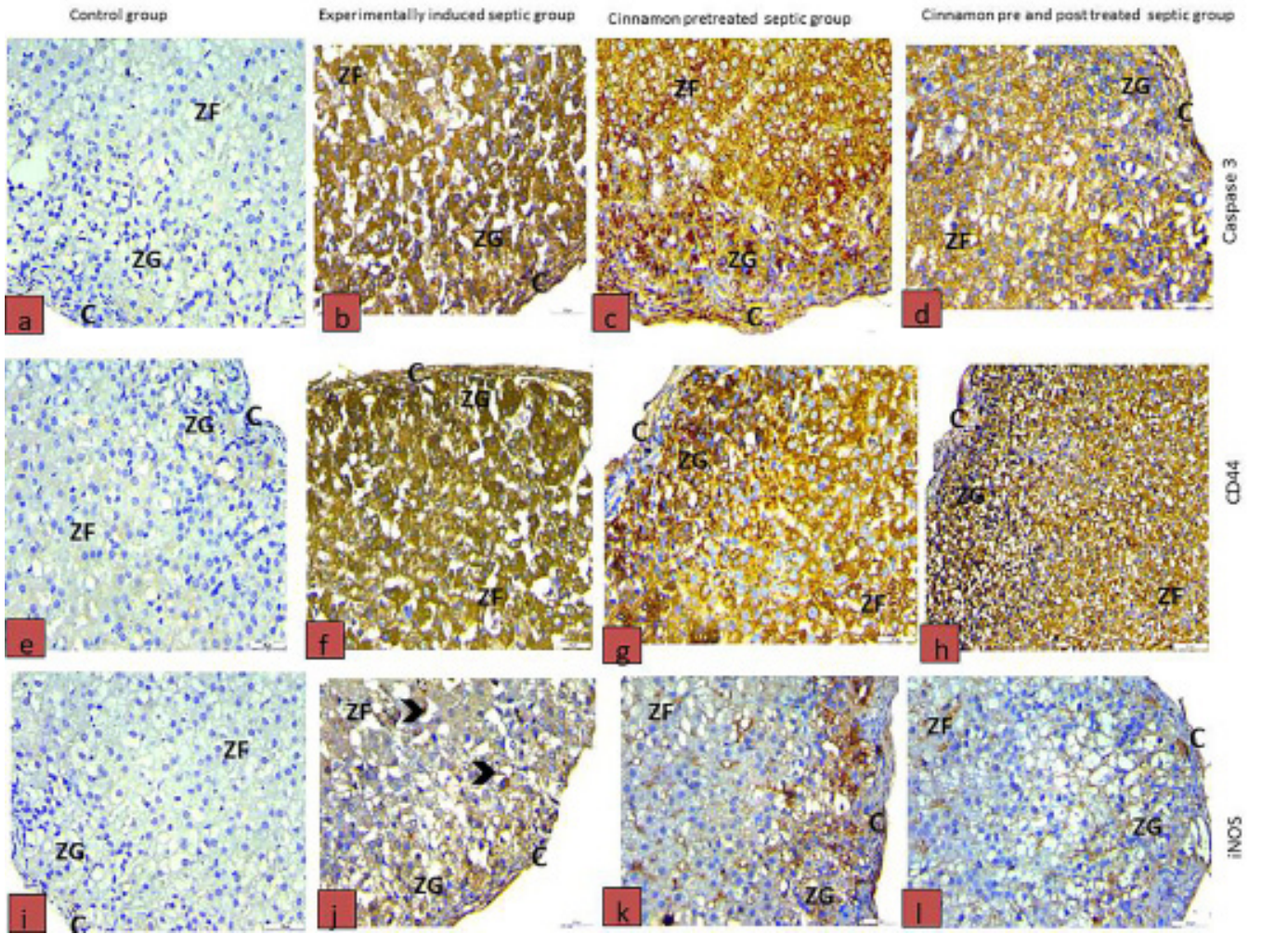

Fig. 8: photomicrograph showing negative immunoreaction of control group (a, e and i). Up regulation of immune reaction of experimentally induced septic group (b, f and j).Down regulation of immunoreaction in cinnamon pretreated and cinnamon pre and post treated groups for caspase3, CD44 and iNOS respectively. (Scale bar = 20 µm).

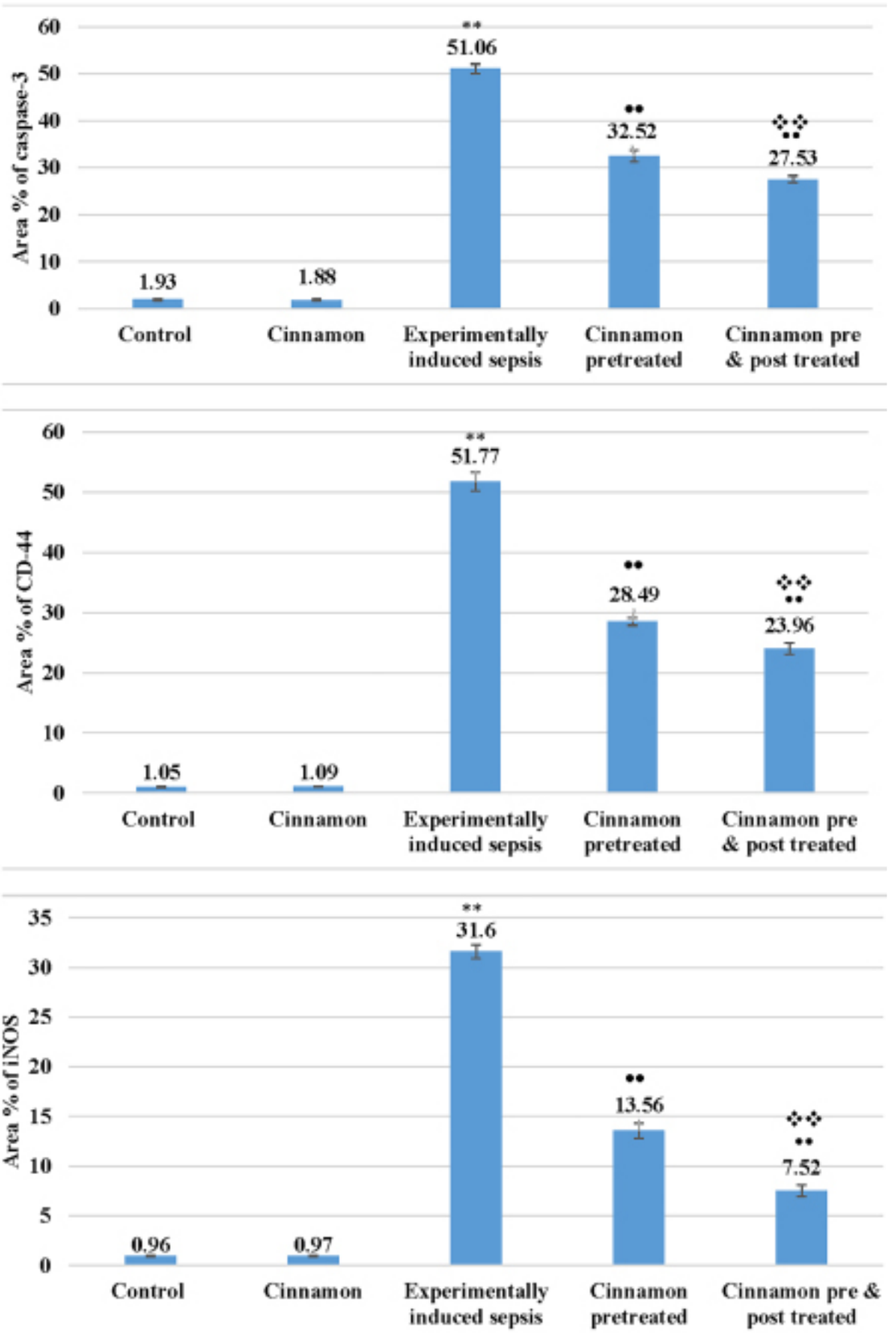

Fig. 9 (A-C): Area percentage of caspase3, CD44 and iNOS immunostaining.** Significant rise (P< 0.001) compared to control group, ** Significant decline (P< 0.00) compared to experimentally induced sepsis group, ** Significant decline (P< 0.001) compared to cinnamon pretreated septic group.

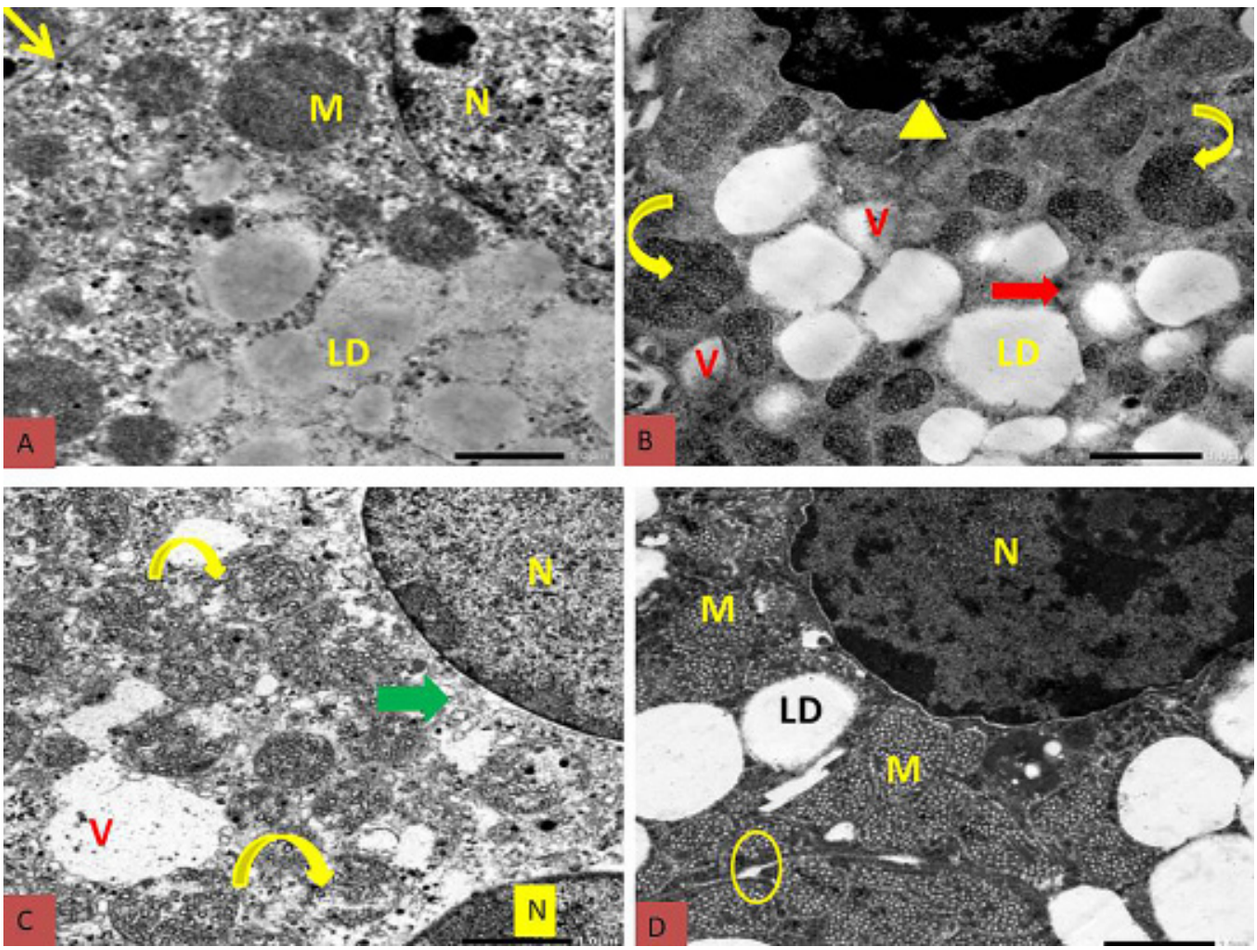

Fig. 10 (A-D): A transmission electron photomicrograph in zona glomerulosa showing regular euchromatic nucleus (N), many scattered globular mitochondria (M), many fat droplets (LD) and intact intercellular junction (yellow arrow) in control group (A).Heterochromatic nucleus with irregular nuclear envelope (*), degenerated swollen mitochondria (curved arrow). Lipofusin granules (red arrow), vacuoles and many large fat droplets (LD) in experimentally induced septic group (B).Electron lucent regular nuclei (N), partially degenerated mitochondria (twisted arrow), swollen smooth reticulum (green arrow) and large vaculated areas (V) in cinnamon pretreated septic group (C).Electron dense nucleus (N), many globular mitochondria (M) with tubule-vesicular cristae, lipid droplets (LD) and mild separated intercellular junction (circle) in cinnamon pre and post treated septic group (D). (TEM X 6000).

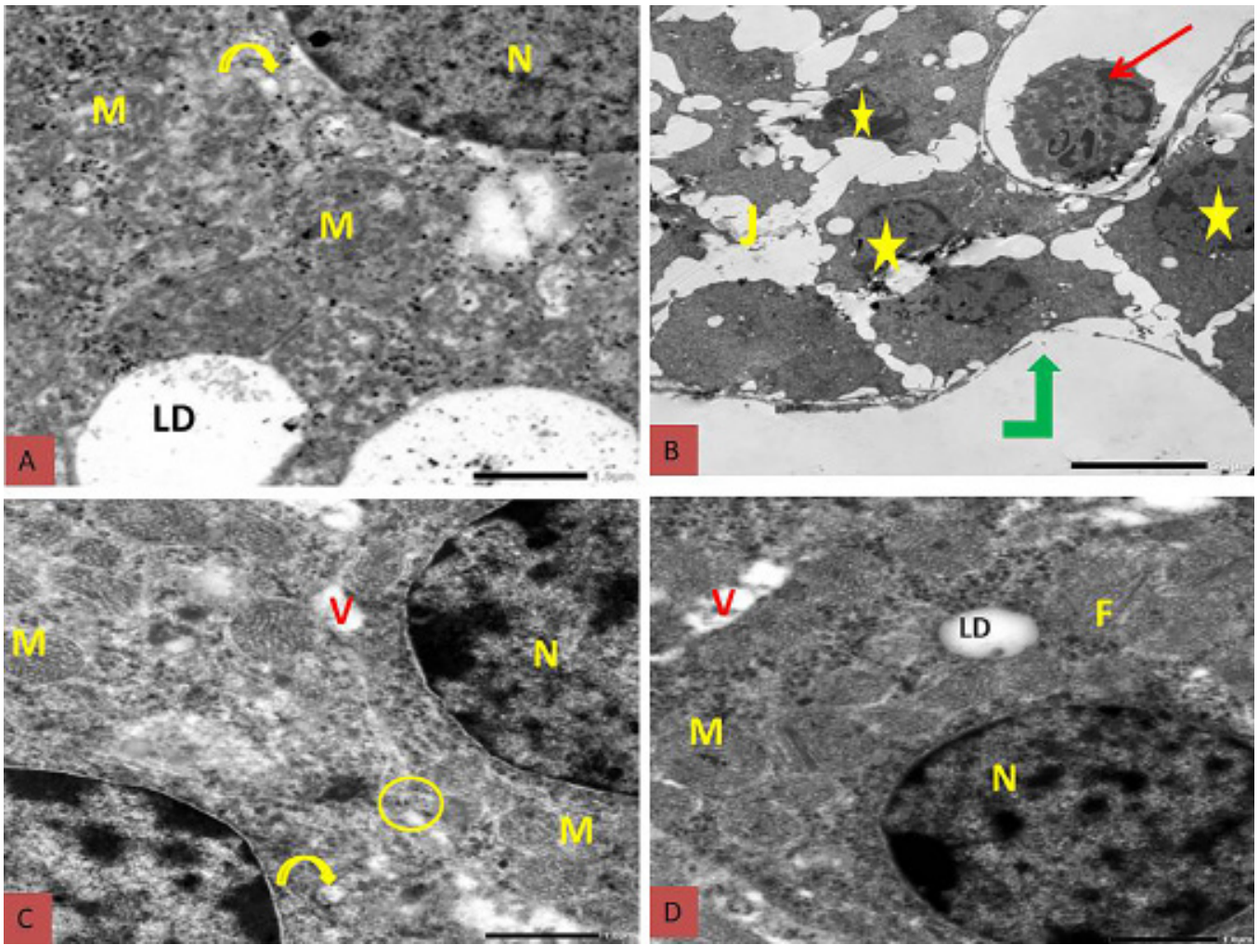

Fig. 11 (A-D): A transmission electron photomicrograph in zona fasiculata showing euchromatic nucleus (N), numerous globular mitochondria (M),well developed smooth reticulum (yellow arrow) and numerous lipid droplets (LD) in control group (A). Karryolytic nuclei (star), wide gap junction (J), dilated sinusoids (green arrow) and neutrophil (red arrow) in experimentally induced sepsis group (B). Euochromatic regular nuclei (N), partially swollen mitochondria (M), swollen smooth reticulum (yellow arrow), vaculated areas (V) and partially separated inter cellular junction (circle) in cinnamon pre and post treated septic group (C).Regular nucleus (N), many globular mitochondria (M) with tubule-vesicular cristae, lipid droplets (LD), some vacuoles (V) and collagen deposition (F) in cinnamon pre and post treated septic group (D). (TEM X6000).

DISCUSSION

Sepsis results in disturbances in the regulation of the neurogenic, endocrine, metabolic, and immune systems creating a stressful state. So, stress-accommodative systems activation is important for maintaining or restoration of body balance. The hypothalamo–pituitary–adrenal axis is an important component of this system[24,25].

Sepsis produces organ dysfunction as a result of a dysregulated host response to infection. Here, the infective agent causes an uncontrolled inflammatory reaction which results in the activation or inhibition of metabolic, hormonal, endothelial, immune, and other pathways. These produce circulatory disturbance causing multi-organ failure[26,27].

In this study, a cecal slurry model was used for the induction of sepsis to avoid the disadvantage of other methods. The dose used in this study was chosen to achieve survival 7 days after CS treatment[28].

This study has reported severe mortality in the experimentally induced septic group. Also, cinnamon pretreated and cinnamon pre and post-treated groups showed improvement in survival % which may be due to the anti-inflammatory and antimicrobial effects of cinnamon[29,30].

This study reported a significant increase in the serum aldosterone and cortisol levels in the experimentally induced septic group. These results were in harmony with Huang *et al* and Huang *et al*[31,32] which referred to the rise in hormone levels to overstimulation of the HPAA and renin-angiotensin-aldosterone system by inflammation or endotoxemia. Also, it triggered cytokines (IL-1, IL-6, and TNF-α) from stimulated peripheral immune cells, which led to the activation of different levels of the HPAA.

The current study revealed a high level of MDA in adrenal tissue homogenate. These results came in harmony with Asir *et al* and Duan *et al*[33,34] who clarified that Sepsis

is commonly associated with enhanced generation of oxygen free radicals.

MPO is a marker of neutrophils activation, in this study high MPO level was detected. Moreover, TNF-α was elevated; these results were in agreement with Sadek *et al* and Lia *et al*[35,36] who detected sudden outbursts of inflammatory cytokine TNF-α, activation and migration of neutrophils.

In the present study, both the control and the cinnamon bark extract-treated groups revealed the normal histological architecture of the adrenal gland. But, rats of the experimentally induced septic group revealed massive degenerative changes with loss of normal architecture of three cortical zones with massive infiltration by different types of inflammatory cells. These changes were in agreement with Abdel-Malak and Amin and Niemeyer *et al*[37,38] who found that all animals in septic groups showed a typical feature of inflammation and degeneration in the form of cortical cell atrophy with altered morphology. The degenerative changes observed in this study were in the form of intracellular vacuolations and pyknotic nuclei. Different inflammatory cell infiltration was detected. Pyknosis of the nuclei and vacuolation of the cytoplasm are thought to be a result of the production of reactive oxygen metabolites that cause DNA damage and lipid peroxidation[39,40].

Neutrophils and mononuclear cells were detected in the adrenal gland of the septic group. These results are due to the activation of innate immunity which is a main component of the inflammatory cascade in response to infection. In the course of infection, neutrophils are the first cells to be activated in order to decrease the infectious agent spread. The activated cells migrate to the site of infection with the secretion of big amounts of inflammatory cytokines[40,41,42].

Mallory trichrome stained sections in this study revealed a significant increase in collagen fiber deposition in the adrenal capsule with mild deposition around the sinusoids in the septic group, this may be due to high levels of ROS and inflammatory mediators[43,44]. While cinnamon-treated septic groups showed a decrease in collagen deposition compared to the septic untreated group. These findings are explained by the anti-fibrotic effects of cinnamon[45,46]. In the present study, the area % of Caspase-3 immunostaining was increased markedly in the experimentally induced sepsis group. Also, it was confirmed by ultrastructural examination the presence of apoptotic nuclei in the zona glomerulosa and fasciculata cells of this group. This observation suggested that an excessive amount of ROS led to the destruction of major cellular components; proteins, lipids, and DNA causing cell death[47]. Moreover, this study showed upregulation of the area % of CD44 immunoreaction the in experimentally induced sepsis group. The same was reported by Zhang *et al*. and Vachon *et al*[48,49]. They reported that phagocytosis mediated by CD44 plays a significant role in the clearance of apoptotic cells and hyaluronan fragments generated during inflammation.

In this study, the inflammatory status of adrenal tissue was confirmed by a significant increase in iNOS-positive cells. These findings were in line with Wang *et al*[50] who clarified that endotoxemia led to up-regulation of iNOS expression and nitric oxide production in the adrenal glands of mice.

Ultrastructurally, septic groups revealed degenerated swollen mitochondria and smooth endoplasmic reticulum. These results came in line with Bairagi *et al*[51] who observed that swelling and disruption of the cristae of the mitochondria could be explained by the accumulation of lipid granules caused by impaired steroidogenesis.

A lot of the lipofuscin pigment was detected in septic groups in the current study. These findings were observed by Elshennawy and Aboelwafa[52] who reported that the lipofuscin pigments might be explained by the inhibition of ACTH release. The appearance of abundant adhesive lipid droplets is an indicator of degenerative changes as the large amounts of lipids in the cortical cells destroy the structure and interfere with the function of the cell. Moreover, this could be caused by the disruption of cytochrome P450 enzymes due to increased ROS production, resulting in the inhibition of cholesterol biosynthesis. This, in turn, causes the accumulation of lipid droplets and cytoplasmic vacuolation of zona fasciculata and zona glomerulosa cells[53].

This study revealed a significant decrease in serum aldosterone and corticosterone levels of pretreated and pre and post-treated groups with cinnamon compared to the experimentally induced sepsis group. These findings may be a result of cinnamon supplementation which led to up-regulation of IL-1 receptor antagonist expression in both the hypothalamus and hippocampus and an antagonist of IL-1β, which have anti-inflammatory and antioxidant properties. These findings may explain the decrease in serum levels of these hormones[54].

The antioxidant activity of cinnamon has been confirmed in this study by a decrease in adrenal MDA levels in pretreated and pre and post-treated groups with cinnamon bark extract. Thota *et al*[55] reported that cinnamon exerted an antioxidant effect in patients infected with coronavirus. Moreover, the administration of cinnamon oil to rats led to an improvement in hepatic antioxidant status and MDA level with an increase in SOD and GSH[56]. Also, the anti-inflammatory effects of cinnamon have been confirmed by a decrease in TNF-α, MPO and inflammatory cells infiltration of adrenal tissue. The results are consistent with Hong *et al*[10] who clarified that aqueous cinnamon extract is rich in polyphenols as flavonoids and tannins which have a powerful anti-inflammatory effect against sepsis initiated by LPS in rats.

Abdeen *et al*[57] studied the beneficial effects of cinnamon bark extract and reported that pretreatment of cellular damage and apoptosis caused by acetaminophen in renal tissue of rats with cinnamon bark extract revealed a marked improvement in histopathological changes, which

could be due to the anti-apoptotic effect of cinnamon bark extract as a result of an increase in the antioxidant defense system and decreased oxygen-free radical activities. This anti-apoptotic effect was confirmed in our study by a significant reduction of Caspase-3 immunoreactions.

CONCLUSION

Based on our findings, it can be concluded that sepsis caused histo-pathological changes in the adrenal cortex mainly ZF through its inflammatory, oxidative stress and apoptotic effects which were mediated through modulating Caspase-3, CD44 and iNOS expression. However, cinnamon has a promising role in attenuating the obtained sepsis effects via its anti-inflammatory, anti-oxidative, and anti-apoptotic properties.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Turgman O, Schinkel M and Wiersinga W J: Host Response Biomarkers for Sepsis in the Emergency Room. Annual Update in Intensive Care and Emergency Medicine. (2023) 2023: 71-81.
2. Paoli C J, Reynolds M A, Sinha M, Gitlin M and Crouser E: Epidemiology and costs of sepsis in the United States—an analysis based on timing of diagnosis and severity level. Critical care medicine. (2018) 46 (12):1889.
3. Jennewein C, Tran N, Kanczkowski W, Heerdegen L, Kantharajah A, Dröse S and Zacharowski K: Mortality of septic mice strongly correlates with adrenal gland inflammation. Critical Care Medicine. (2016) 44(4): 190-199.
4. Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith C M, French and Levy M: Executive summary: surviving sepsis campaign: international guidelines for the management of sepsis and septic shock. Critical Care Medicine. (2021) 49 (11): 1974-1982.
5. Omar A I and Aboulkhair A G: Do microvesicles derived from adipose mesenchymal stem cells have a therapeutic potential on Escherichia coli lipopolysaccharides-induced sepsis in Zona fasciculata of adult male albino rats? A histological study. Egyptian Journal of Histology. (2018) 41(2): 204-219.
6. Kanczkowski W, Sue M, Zacharowski K, Reincke M and Bornstein S R: The role of adrenal gland microenvironment in the HPA axis function and dysfunction during sepsis. Molecular and cellular endocrinology. (2015) 408: 241-248.
7. Marino L O and Souza H P: Dysfunction of the hypothalamic-pituitary-adrenal axis in critical illness: a narrative review for emergency physicians. European Journal of Emergency Medicine. (2020) 27(6): 406.
8. Wasyluk W, Wasyluk M and Zwolak A: Sepsis as a Pan-Endocrine Illness—Endocrine Disorders in Septic Patients. Journal of Clinical Medicine. (2021) 10 (10): 2075.
9. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M and Angus DC: The third international consensus definitions for sepsis and septic shock (Sepsis-3). Jama. (2016) 315 (8): 801-810
10. Hong JW, Yang GE, Kim YB, Eom SH, Lew JH and Kang H: Anti-inflammatory activity of cinnamon water extract in *vivo* and in *vitro* LPS-induced models. BMC complementary and alternative medicine. (2021) 12 (1): 1-8.
11. Hajimonfarednejad M, Ostovar M, Raee MJ, Hashempur MH, Mayer J G and Heydari M: Cinnamon: A systematic review of adverse events. Clinical Nutrition. (2019) 38(2): 594-602.
12. Waty S and Suryanto D: Antibacterial activity of cinnamon ethanol extract (cinnamomum burmannii) and its application as a mouthwash to inhibit streptococcus growth. In IOP Conference Series: Earth and Environmental Science. (2018) 130 (1): 012049.
13. Ezzat SK, AbuElkhair MT, Mourad MI, Helal ME and Grawish ME: Effects of aqueous cinnamon extract on chemically-induced carcinoma of hamster cheek pouch mucosa. Biochemistry and biophysics reports. (2017) 12: 72-78.
14. Abd-Elrazek A, Mahmoud S and Abd ElMoneim A: The comparison between curcumin and propolis against sepsis-induced oxidative stress, inflammation, and apoptosis in kidney of adult male rat. Future Journal of Pharmaceutical Sciences. (2020) 6 (1): 1-13.
15. Lee MJ, Kim K, Jo YH, Lee JH and Hwang JE: Dose-dependent mortality and organ injury in a cecal slurry peritonitis model. Journal of Surgical Research. (2016) 206 (2): 427-434.
16. Ajdžanović VZ, Jarić IM, Živanović JB, Filipović BR, Šošić Jurjević BT, Ristić NM and Stanković SD: Histological parameters of the adrenal cortex after testosterone application in a rat model of the andropause.12:(11)31 (2016) .
17. Sadek MT, El-Abd SS and Ibrahim MA: Effect of Chronic Unpredictable Mild Stress on Adrenal Cortex of Adult Rat and The Possible Protective Role of Licorice Extract: A Histological and Immunohistochemical study. Egyptian Journal of Histology.887-901 :(4) 44 (2020) .
18. Faruk EM, Alasmari WA, Fouad H, Nafea OE and Hasan RAA: Extracellular vesicles derived from bone marrow mesenchymal stem cells repair functional and structural rat adrenal gland damage induced by fluoride. Life Sciences. (2021) 27: 119122.

19. Bancroft JD and Layton C: In Theory and Practice of histological techinques. 7th ed, Churchill Livingstone. London (2010): pp173-214.

20. Survana S, Bancroft J and Layton C: Trichrom staining in Theory and practice of histological techniques. 8th ed, Elsevier, China (2019): pp 142.

21. Bancroft JD and Gamble M: Immunohistochemical techniques in Theory and practice of histological techniques. Staining methods. 6th ed. Churchill Livingstone Edinburgh, London, Madrid, Melbourne, New York and Tokyo (2008): pp 263–325.

22. Survana S, Bancroft J and Layton C: Transmission electron microscopy in Theory and practice of histological technique, 8th edition, Elsevier, China (2019): pp 434.

23. Emsley R, Dunn G and White I R: Mediation and moderation of treatment effects in randomised controlled trials of complex interventions. Statistical methods in medical research.

24. Reyes M, Filbin MR, Bhattacharyya RP, Billman K, Eisenhaure T, Hung DT and Hacohen N: An immune-cell signature of bacterial sepsis. Nature medicine. (2020) 26 (3): 333-340.

25. Goh KH, Wang L, Yeow AYK, Poh H, Li K, Yeow JJL and Tan GYH: Artificial intelligence in sepsis early prediction and diagnosis using unstructured data in healthcare. Nature communications. (2021) 12(1): 1-10.

26. Arina P and Singer M: Pathophysiology of sepsis. Current Opinion in Anesthesiology.(2021)34(2):77-84.

27. Hollenberg SM and Singer M: Pathophysiology of sepsis-induced cardiomyopathy. Nature Reviews Cardiology. (2021) 18(6): 424-434.

28. Consoli DC, Jesse JJ, Klimo KR, Tienda AA, Putz ND, Bastarache JA and Harrison FE: A cecal slurry mouse model of sepsis leads to acute consumption of Vitamin C in the brain. Nutrients. (2020) 12(4): 911.

29. Panchal V, Gurunathan D and Muralidharan NP: Comparison of antibacterial efficacy of cinnamon extract, neem extract as irrigant and sodium hypochlorite against Enterococcus fecalis: An in *vitro* study. Indian Journal of Dental Research. (2020) 31(1): 124.

30. Gupta-Wadhwa A, Wadhwa J and Duhan J: Comparative evaluation of antimicrobial efficacy of three herbal irrigants in reducing intracanal E. faecalis populations: Anin *vitro* study. J Clin Exp Dent. (2016) 8: 230-5

31. Huang HL, Chiang MF, Lin CW and Pu HF: Lipopolysaccharide directly stimulates aldosterone production via toll-like receptor 2 and toll-like receptor 4 related PI3K/Akt pathway in rat adrenal zona glomerulosa cells. Journal of cellular biochemistry. (2010)111(4): 872-880.

32. Huang HC, Tsai MH, Lee FY, Lin TY, Chang CC, Chuang CL and Huang YH: NAFLD aggravates septic shock due to inadequate adrenal response and 11β-HSDs dysregulation in rats. Pharmaceutics. (2020) 12(5): 403.

33. Aşır F, Nergiz Y and Pala A: Vitamin E protected the mouse adrenal gland against immobilization stress. Polish Journal of Veterinary Sciences. (2022) 25(3): 447-454.

34. Duan GL, Wang CN, Liu YJ, Yu Q, Tang XL, Ni X and Zhu XY: Resveratrol alleviates endotoxemia-associated adrenal insufficiency by suppressing oxidative/nitrative stress. Endocrine Journal. (2016) 63 (6):569-580.

35. Sadek MT, El-Abd SS and Ibrahim MA (2021). Effect of Chronic Unpredictable Mild Stress on Adrenal Cortex of Adult Rat and The Possible Protective Role of Licorice Extract: A Histological and Immunohistochemical Study. Egyptian Journal of Histology, 44(4), 887-901.

36. Lai F, Zhou G, Mai S, Qin X, Liu W, Zhang Y and Han Y: Sini Decoction improves adrenal function and the short-term outcome of septic rats through downregulation of adrenal toll-like receptor 4 expression. Evidence-Based Complementary and Alternative Medicine. (2018) 2018: 9

37. Abdel Malak HW and Amin MA: Protective role of L-arginine on the suprarenal gland of adult male albino rats subjected to recurrent acute restraint stress: A histological and Immunohistochemical study. The Egyptian Journal of Anatomy. (2018) 41(1): 156-171.

38. 8-Niemeyer CS, Mescher T, Griggs R, Orlicky DJ, Wilkerson GK, Bubak AN and Nagel MA: Histopathological analysis of adrenal glands after simian varicella virus infection. Viruses. (2021) 13(7): 1245.

39. Jennewein C, Tran N, Kanczkowski W, Heerdegen L, Kantharajah A, Dröse S and Zacharowski K: Mortality of septic mice strongly correlates with adrenal gland inflammation. Critical Care Medicine. (2018) 44(4): 190-199.

40. Yu J, Xu S, Wang WX, Deng WH, Jin H, Chen XY and Sun HT: Changes of inflammation and apoptosis in adrenal gland after experimental injury in rats with acute necrotizingpancreatitis. Inflammation (2012) 35(1): 11-22.

41. Lomako VV: Blood leukocyte indices in male rats of different ages. Advances in Gerontology. (2020) 10(2): 135-141.

42. Wu B, Walker J, Spur B, Rodriguez A and Yin K: Effects of Lipoxin A4 on antimicrobial actions of neutrophils in sepsis. Prostaglandins, Leukotrienes and Essential Fatty Acids. (2015) 94: 55-64.

43. Richter K and Kietzmann T: Reactive oxygen species and fibrosis: further evidence of a significant liaison. Cell and tissue research. (2016) 365 (3): 591-605.

44. Burgoyne RA, Fisher AJ and Borthwick LA. (2021). The role of epithelial damage in the pulmonary immune response. Cells. (2020) 10(10): 27-63.

45. Eidi A, Mortazavi P, Bazargan M and Zaringhalam J: Hepatoprotective activity of cinnamon ethanolic extract against CCI4-induced liver injury in rats. Excli Journal. (2012) 11: 495.

46. Farazandeh M, Mahmoudabady M, Asghari AA and Niazmand S: Diabetic cardiomyopathy was attenuated by cinnamon treatment through the inhibition of fibro-inflammatory response and ventricular hypertrophy in diabetic rats. Journal of Food Biochemistry. (2022) e14206.

47. Soliman HM, Abd El-Haleem MR and El Tarhouny SA: Histomorphometrical and electron microscopic study of adrenocorticocytes following surgically induced extrahepatic biliary obstruction in adult female albino rats. Folia Biologica. (2015) 61(1): 14.

48. Zhang K, Hu Y and Li T: Single cell atlase of murine adrenal glands reveals immune –adrenal crosstalk during systemic candida albicans infection. Frontiers in immunology. (2022) 6466.

49. Vachon E, Martin R, Plumb J, Kwok V, Vandivier RW, Glogauer M, Kapus A, Wang X, Chow CW, Grinstein S, Downey GP. CD44 is a phagocytic receptor. Blood. 2006 May 15;107(10):4149-58.

50. Wang CN, Daun GL, Liu YJ, Yu q, Tang XL, Zhao W and Ni X: overproduction of nitric oxide by endothelial cells and macrophages contributes to mitochondrial oxidative stress in adrenocortical cells and adrenal insufficiency during endotoxemia. Free Radical Biology Medicine. (2015) 83 (31): 31-40.

51. Bairagi N, Samrat C and Chattopadhyay J: Variability in the secretion of corticotropin-releasing hormone, adrenocorticotropic hormone and cortisol and understandability of the hypothalamic-pituitary-adrenal axis dynamics – a mathematical study based on clinical evidence. Math. Med. Biol. (2008) 25: 37- 63.

52. Elshennawy WW and Aboelwafa RH: Structural and ultrastructural alterations in mammalian adrenal cortex under influence of steroidogenesis inhibitors drug. J Am Sci. (2011) 7(8): 567-76.

53. Suttie AW and Sutcliffe C.(2018): Adrenal Gland in Boormans pathology of the Rat, seconed edition, Academic press, London, P 649-667.

54. Marissal-Arvy N, Batandier C, Dallennes J, Canini F, Poulet L, Couturier K and Mormede P: Effect of a high-fat–high-fructose diet, stress and cinnamon on central expression of genes related to immune system, hypothalamic–pituitary–adrenocortical axis function and cerebral plasticity in rats. British journal of nutrition. (2014) 111(7): 1190-1201.

55. Thota SM, Balan V and Sivaramakrishnan V: Natural products as home-based prophylactic and symptom management agents in the setting of COVID-19. Phytotherapy Research. (2020) 34 (12): 3148-3167.

56. Mohammed KA, Ahmed H, Sharaf HA, El-Nekeety AA, Abdel-Aziem SH, Mehaya F M and Abdel-Wahhab MA: Encapsulation of cinnamon oil in whey protein counteracts the disturbances in biochemical parameters, gene expression, and histological picture of the liver and pancreas of diabetic rats. Environmental Science and Pollution Research. (2020) 27 (3): 2829-2843.

57. Abdeen A, Abdelkader A, Abdo M, Wareth G, Aboubakr M, Aleya L and Abdel-Daim M: Protective effect of cinnamon against acetaminophen-mediated cellular damage and apoptosis in renal tissue. Environmental Science and Pollution Research. (2019) 26(1): 240-249.

الملخص العربى

تأثير مستخلص القرفة على الإنتان المستحث تجريبياً في قشرة الغدة الكظرية لذكور الجرذان البيضاء

ياسمين نبيل صالح، فاطمة النبوية عبد الهادي الصفتي، نهي محيي عيسي، مروة عبد الصمد الغلام

قسم التشريح والاجنة-كلية الطب-جامعة المنوفية

المقدمة: يعتبر انتان الدم مشكلة عالمية خطيرة لانه السبب الرئيسي للوفيات في المستشفيات مصحوبا بفشل عضوي متعدد، وتعد الغدة الكظرية العضو المؤثر الاساسي في انتان الدم حيث تستجيب للهرمون النشط للقشرة وتفرز هرمونات تنشط عمليات التكسير، ومضادة للالتهابات، ومحسنة للمناعة.

الهدف من البحث: دراسة تاثير مستخلص القرفة علي قشرة الغدة الكظرية في انتان الدم المستحث تجريبيا في الجرذان.

المواد والطرق: ستة وستون جرذا تم استخدام ستة منهم كمتبرعين بمحتويات القولون والباقي تم تقسيمهم الي خمس مجموعات، المجموعة الاولي (الضابطة)، المجموعة الثانية (المعالجة بمستخلص القرفة)، المجموعة الثالثة (مجموعة انتان الدم المستحث تجريبيا) وشملت ١٢ جرذا وتم حقنها مرة واحدة بمحتويات القولون الصاعد المذاب في الديكستروس (١٠مجم/كجم) داخل البيرتون، المجموعة الرابعة (المعالجة بمستخلص القرفة قبل الانتان) تم اعطاءها القرفة (١٠٠مجم/كجم) مرة واحدة يوميا بالفم لمدة ٤ اسابيع و٥ ايام ثم تم استحثاث الانتان، المجموعة الخامسة (المعالجة بمستخلص القرفة قبل وبعد الانتان) تم اعطاءها القرفة (١٠٠مجم/كجم) مرة واحدة يوميا بالفم لمدة ٤ اسابيع قبل النتان المستحث وتم الاستمرار قي اعطاء القرفة ٥ ايام اخري يعد الانتان، في نهاية التجربة تم قياس مستوس الالدوستيرون والكورتيكوستيرون في الدم، وتم ذبح الجرذان واستخراج الغدة الكظرية، تم استخدامها القياس مستوي الميلوبيروكسيديز والمالوندالدهيد وعامل النخر التورمي الفا في الانسجة، وتمت دراستها هستولوجيا باستخدام صبغة الهيماتوكسلين وصبغة المالوري ترايكروم ،وتمت الدراسة الهستوكيميائية مناعية باستخدام صبغات caspase٣، CD٤٤ و iNOS، وكذلك تمت دراستها باستخدام الميكروسكوب الالكتروني.

النتائج : اظهرت نتائج المجموعة الثالثة تاثر التهابي بالغ في ZG و ZF بالاضافة الي زيادة مستوي الهرمونات والميلوبيروكسيديز والمالوندالدهيد وعامل النخر التورمي الفا وكذلك زيادة في ظهور الصبغات المناعية caspase٣، CD٤٤ و iNOS ولكن في المجموعتين الرابعة والخامسة لوحظ تحسن في كل العوامل السابقة.

الاستنتاج: يحسن مستخلص القرفة المعطي بالفم التغيرات الالتهابية الشديدة في ZG وZF الناتجة عن انتان الدم المستحث تجريبيا بمحتويات القولون.