Systemic localization of vascular inflammation biomarkers in rats with depression

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SUMMARY

This study aims at investigating the influence of depression on vascular inflammation in the vital organs of the body. Thirty male Sprague-Dawley rats were used as control and experimental groups. Forced swimming protocol was used for 21 days to initiate depression in the experimental rats. Depression was evaluated through automatically measuring the rats' locomotor activity by the 6-minutes forced swimming test (FST) and by analyzing their serum corticosterone levels. Sera were collected from all rats before sacrifice and then tissue specimens of heart, lung, kidney, and liver were collected after sacrifice. The mean corticosterone level and the mean immobility duration were significantly increased in depression group. Through using the enzyme linked immunosorbent assay (ELISA), the serum levels of inflammatory biomarkers, tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF), and inducible nitric oxide synthase (iNOS) were measured, and their tissue expression were examined. The area percentage of immune expression was measured using the Image J program.

The mean serum levels of TNF-α and iNOS elevated significantly in the depression group compared to the control, whereas serum level of VEGF decreased significantly in depressed rats compared to control ones. Intense immuno-expression of inflammatory cytokines was detected in the endothelium of blood vessels of all examined tissues in depression group compared to control ones. Except for TNF-α expression in the lung tissues, the area percentage of immune expression of all the examined inflammatory cytokines were significantly increased in all the examined tissues. The study demonstrated that vasculitis can be a harmful outcome of stress and depression.

Key words: Depression – Vasculitis – Inflammatory cytokines - Vital organs

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INTRODUCTION

Depression is a common disorder affecting a wide range of the population, it is one of the commonest psychiatric diseases all over the world (Pedersen et al., 2014). According to the WHO, depression is the second largest healthcare problem in the disabled population (Moon et al., 2012). There is a great association between chronic stress, its subsequent depression, and the development of vascular diseases (Taylor et al., 2013).

The relationship between depression and inflammation is believed to be bidirectional. Vascular depression is a common term, postulating that vascular inflammation and endothelial dysfunction might induce depression symptoms and precipitate depressive syndrome (Taylor et al., 2013). Depression related modification like stress response, and neurotransmitter imbalances can enhance inflammation and increase the risk of vascular damage by increasing the levels of proinflammatory cytokines and reducing anti-inflammatory cytokine levels (Kristen, 2022).

Depression activates proinflammatory cytokines release, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which are strongly related to endothelial dysfunction and vasculitis (Black and Garbutt, 2002; Doney et al., 2022; Saadat et al., 2022). TNF-α is a crucial vascular inflammatory biomarker that engages inflammatory immune cells and advances tissue destruction. It plays critical role in enhancing chronic uncontrolled proinflammatory mediators' production (Parlindungan et al., 2023). TNF-α has multiple effects that control various intracellular functions and play an essential role in the regulation of emotions assumed to be involved in depression (Jang et al., 2021).

Vascular endothelial growth factor (VEGF) is a potent angiogenic agent. It is considered the master regulator for cytokines of angiogenesis and vasculogenesis, as it promotes endothelial cell migration and survival, as well as the formation and maintenance of capillary fenestrations (Maharaj et al., 2006; Xie et al., 2017). VEGF-A is secreted by endothelial cells, macrophages, and activated T cells (Ferrara, 2004). It is located in the central nervous system (CNS), and it is capable of crossing the blood-brain barrier, playing a vital role in the pathomechanism of depression disorder (Halmai et al., 2013; Rigal et al., 2020).

Nitric oxide (NO) is an essential signaling molecule, which mediates a variety of essential physiological processes. It is produced by inducible nitric oxide synthase (iNOS) and has been implicated in various hypoxic diseases, vascular injury, and inflammation (Cassini-Vieira et al., 2015). Its expression was detected in both serum and cortical tissues of depressed mice, and was aggravated in post-stroke depression patients (Peng et al., 2012; Wang et al., 2021).

The widespread vascular impacts of depression on the body offer a fascinating avenue to investigate cellular and molecular processes in different organs throughout the disease progression. This study was designed to investigate the influence of depression on the body's vasculature by analyzing the expression of biomarkers associated with vascular inflammation (namely TNF-α, VEGF, and iNOS) in key tissues such as the lung, kidney, liver, and heart after induction of depression in a rat model. The underlying hypothesis is that depression triggers a broad-spectrum inflammatory and damaging response in the vascular systems across various vital body organs.

MATERIALS AND METHODS

Animal model preparation

Thirty male Sprague-Dawley rats, each 150-250 g weight, were used in this controlled experiment. Animal experiments were conducted at the medical college research lab, Al-Rayan colleges and Taibah University, Saudi Arabia. All animal handling and procedures were carried out following the EC Directive 86/609/EEC for animal manipulations (https://www.researchgate.net/publication/10962580_Directive_86609EEC_on_the_Protection_of_Animals_Used_for_Experimental_and_Other_Scientific_Purposes) and the guidelines of the National Committee of Bio Ethics in Saudi Arabia (https://ncbe.kacst.edu.sa/media/xphlbp1e/كتاب-الأخلاقيات-إنجليزي.pdf). Ethical approval was taken from IRB of Al-Rayan Colleges (HA-03-M-122-038).

Thirty-five rats were purchased from Taibah University experimental animal center. They were fed with a standard pellet diet and water ad

libitum and noted for any abnormal behavior either in their movement or food intake. Thirty of the most apparently normal behavioral rats were included in the study. The selected rats were individually housed in pathogen-free conditions at 20-22°C and 45-55% humidity in a twelve hours light/dark cycle (Garrett et al., 2012).

Experimental groups:

Rats were divided randomly into two groups (15 rats each):

1. Control group: the rats were maintained on a standard chow diet for 3 weeks, including the weekends. Following this, their locomotor activity was evaluated with a 6-minute forced swimming test (FST) and then sacrificed immediately.
2. Depression group: The rats underwent the FS protocol daily for three weeks, including weekends (21 days). Following this, their locomotor activities were evaluated with a 6-minute FST and then sacrificed immediately.

In forced swimming protocol, the rats were forced to swim individually inside 60 x 22 cm glass cylinders with 45 cm water depth for 15 min daily for 21 days, temperature was maintained at a 25-28°C. The rats were let to dry in a 32°C heated enclosure before returning to their cages (Porsolt et al., 1978; Eldomiaty et al., 2017).

Assessment of the depression in the rats

The depression state of the rats was evaluated through two methods (Petit-Demouliere et al., 2005; Eldomiaty et al., 2023):

1. Detection of corticosterone level in sera collected from rats just before killing: the sera were collected from the retro-orbital vein, centrifuged at 4°C, and stored at -80°C till handled for determination of the level of corticosterone using ELISA kits (ALPCO Diag-nostics, Orangeburg, NY, USA following the manufacturer's recommendations.
2. Automatic Measurement of the locomotor activity of the rats: the locomotor activities of the rats were traced through computer software (Ethovision XT version: 8.0) during FST to detect the immobility duration in seconds.

Venous blood sample collection and determination of serum levels of inflammatory biomarkers

One hour before sacrifice, blood samples were collected from tail veins of the rats of each group. Centrifugation and serum storage took place at -20°C regarding Nyakoe et al. (2009). Serum concentrations of TNF-α, VEGF and iNOS were calculated using enzyme linked immunosorbent assay (ELISA) kits (R&D Systems, cat no; RTA00 and RRV00, Nuoyuan, Shanghai, China and MY-BIOSOURCE, cat no; MBS263618 respectively) in both control and depression-induced groups. The optical absorbance was read at 450 nm (TECAN, Switzerland). The concentrations were calculated from standard curves and analyzed with One-way ANOVA; different samples were compared.

Dissection, collection, and immunostaining of the tissues for analysis

At the assigned time, the rats were sacrificed, fresh samples from the cardiac wall, lung, kidney, and liver were collected, fixed, and embedded in paraffin blocks. Sections (5 µm) were collected, stained utilizing primary polyclonal antibodies adverse to the selected inflammatory cytokines (anti-VEGF Rabbit polyclonal antibody; Abcam; cat no: ab53465, anti TNF-α Rabbit polyclonal antibody; BIO-RAD; cat no: AAR33 and anti-iNOS Rabbit polyclonal antibody; Abcam; cat no: ab3523) 1:100 dilution. Tissue samples were fixed with 4% paraformaldehyde in PBS at pH 7.4 for 10 min at RT after scarification. They were permeabilized for 10 min with 0.15 % Triton X-100 in 0.1% bovine serum albumin (BSA) in phosphate buffer saline (PBS), blocked with 5% goat serum for 30 min and incubated with the antibodies overnight at 4°C. The tissue samples were then incubated with the secondary antibodies, washed with PBS, and counterstained with hematoxylin and viewed under a light microscope. Stained sections were examined using an Olympus BX 36 Bright field Automated microscope. The images were digitized in a 2448 × 1920-pixel matrix using a DP27 color digital video camera to detect the development of vascular injury through exploring inflammatory biomarkers expression.

Measuring the area percentage of immune stained sections

The area percentage of brown-colored VEGF, TNF-α and iNOS immunohistochemically stained sections were measured by the Image J program (National Institute of Health, Bethesda, Maryland, USA), to separate the brown stained color pixels (DAB) from the background (H&E-stained pixels) in the selected images. Non-overlapped sectors of five stained slides of all the groups and images at ×200 magnification with a clear contrast and consistent DAB and H&E stain and saved as JPEG format were used. To define color threshold range for the brown stained areas, the saturation and the brightness for the selected images were adjusted. Color deconvolution was applied to separate colors and DAB was analyzed; then threshold was used to measure the area of immunohistochemical positivity using the method mentioned in Yuan and Munson (Yuan and Munson, 2017).

Statistical analysis

The statistical analysis was conducted employing the IBM SPSS (version 21) statistical package. All data are conveyed as the means ± standard errors of the means (SEM). For Assessment of the depression (corticosterone level and the locomotor activity), comparisons between groups were conducted using the Independent Samples test with 95% confidence interval of the difference. For comparing the area percentage of immune stained sections, one-way analysis of variance (ANOVA) followed by Bonferroni pairwise was used. For all tests, the level of significance and comparisons were determined < 0.05.

RESULTS

Evaluation of the depressive state

The mean corticosterone serum level was significantly elevated in the depression group (2.89±0. 082pg/ml) compared to the control (2.53±0.027 pg/ml) ($p < 0.001$) (Fig. 1A).

The mean immobility duration was significantly augmented in the depression group (273.67±6.30 s) compared to the control (71.67±2.99 s) ($p < 0.001$) (Fig. 1B).

Serum levels of TNF-α, VEGF and iNOS (Fig. 2)

The mean of TNF-α serum levels increased significantly in the depression group (327.47 ±3.00 ng/L) compared to control group (278.20±4.39 ng/L) ($p < 0.001$). Also, the mean serum levels of iNOS increased significantly in the depression group (14.99±0.59 ng/ml) compared to control group (5.47±0.13 ng/ml) ($p < 0.001$), whereas the mean serum level of VEGF decreased significantly in serum of depressed rats (136.20 ±3.20 pg/ml) compared with control ones (171.33 ±2.87) pg/ml) ($p < 0.001$).

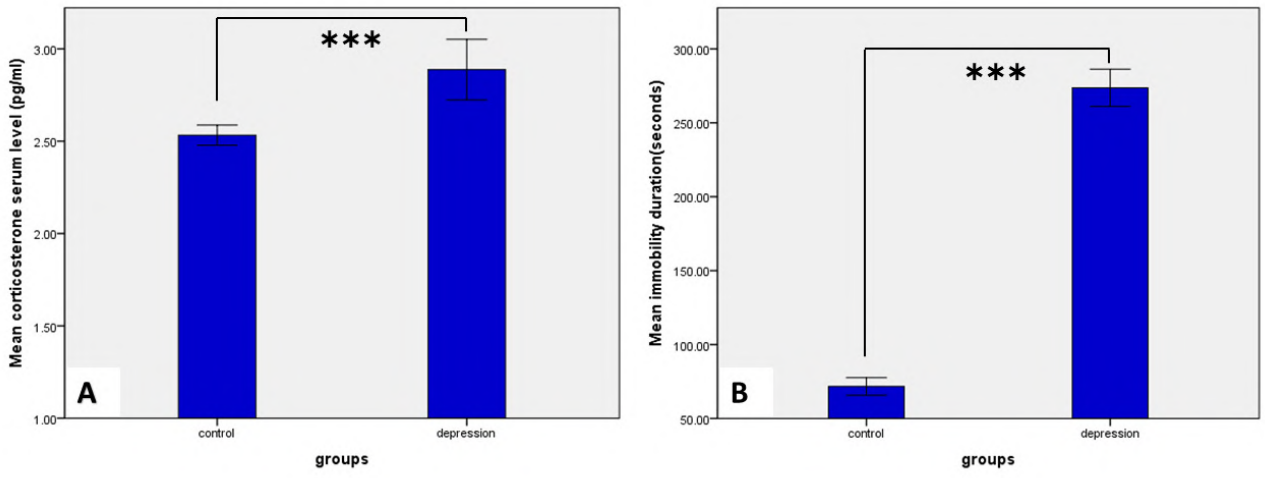

Fig. 1.- Graphs illustrating the mean corticosterone levels in control and depression groups (15 rats each) (**A**) and showing the immobility duration for control and depression groups (15 rats each) during the 6-min forced swimming test (**B**). Data are represented as mean ± SEM. *** means significance at P<0.001.

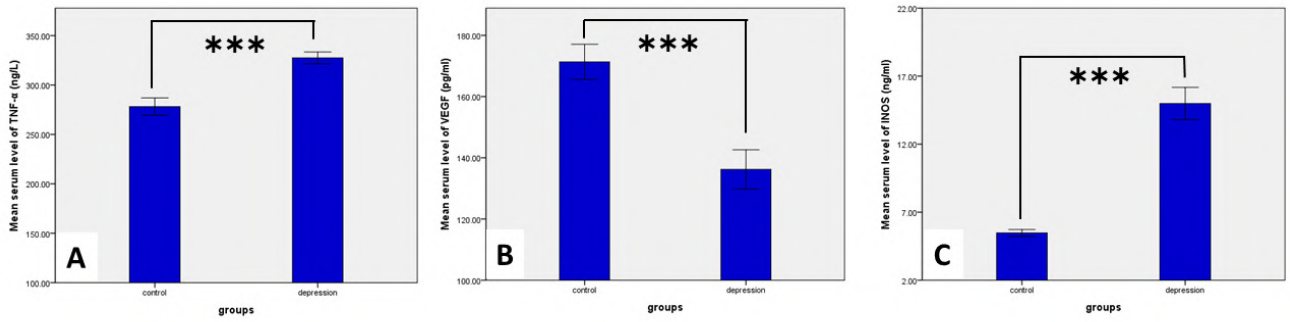

Fig. 2.- Graphs showing the mean serum levels of TNF-α (**A**), VEGF (**B**) and iNOS (**C**) in control and depression groups (15 rats each). Data are represented as mean + SEM. *** means significance at P<0.001.

Immunohistochemistry results

TNF-α, VEGF and iNOS expression in the heart tissue (Fig. 3):

The sarcoplasm of control sections showed weak TNF-α immunoreactivity in the cardiomyocytes and moderate immunostaining of the blood vessels' endothelium. In the depression group, intense immunostaining was detected in the blood vessels' endothelium and some cardiomyocytes, especially those close to the blood vessels.

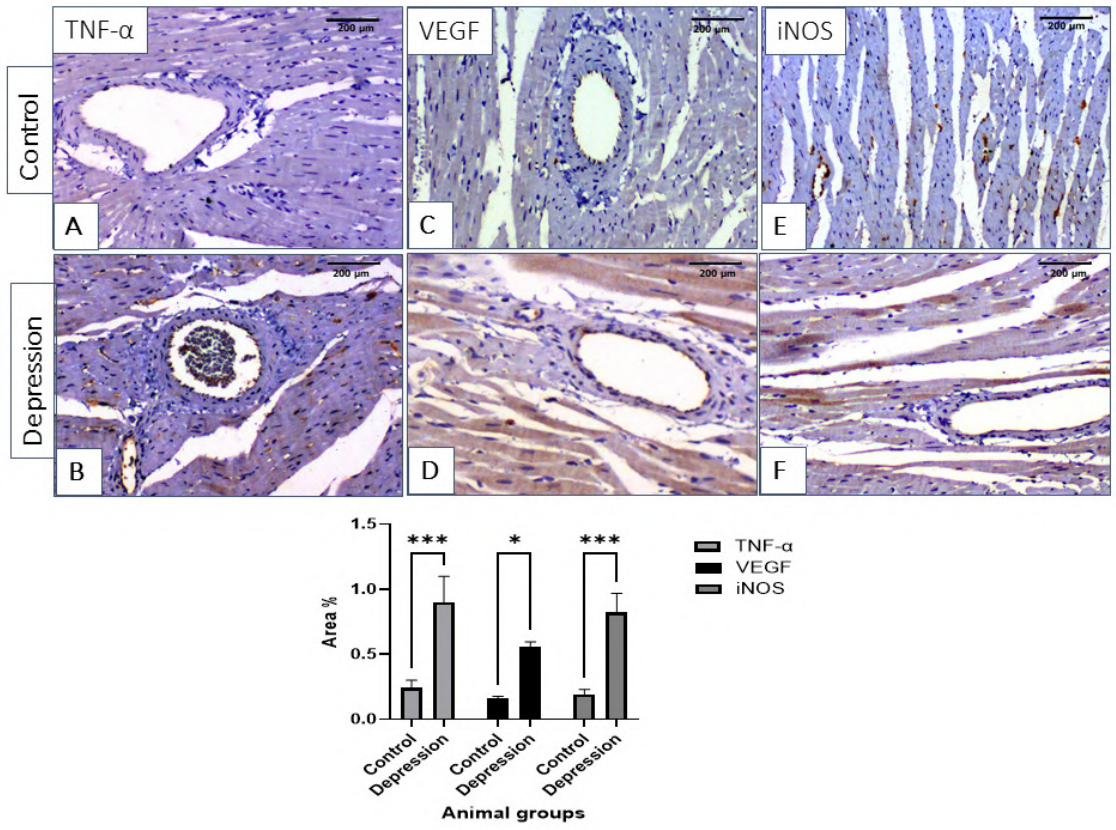

Fig. 3.- Sections of rat's heart after TNF-α, VEGF & iNOS immunohistochemical technique: (**A, B**) control and depression groups of TNF-α stained sections, in which the expression of TNF-α in the endothelium of blood vessels and some cardiomyocytes increased in depression group compared to the control group. (**C, D**) control and depression groups of VEGF-stained sections, in which the expression of VEGF in the endothelium of blood vessels and cardiomyocytes increased compared to the control group. (**E, F**) control and depression groups of iNOS stained sections, in which the expression of iNOS increased in the endothelium of blood vessels in depression group compared to control group. (**G**) Histogram illustrating the quantitative analysis of the intensity of TNF-α, VEGF & iNOS stain in different experimental groups. The depression group demonstrated a significant increase in TNF-α VEGF, & iNOS area % as compared to the control group. Data are represented as mean ± SEM. * means significance at p< 0.05 while *** means significance at p< 0.001. Scale bars = 200 µm.

In control sections, VEGF was uniformly and weakly expressed in sarcoplasm of cardiomyocytes, with moderate immunostaining of endothelium of blood vessels. In the depression group, intense immunostaining was found in the endothelium of blood vessels and cardiomyocytes.

Immune-stained section for iNOS expression showed weak immunostaining in the sarcoplasm of cardiomyocytes of control group, which became intense in cells of the depression group. The blood vessels' endothelium expressed moderate immunostaining in the control group, but intense expression appeared in the depression group.

Measuring the intensity of immuno-expression through the area percentage of immuno-expression revealed that the depression group demonstrated significant increases in the means of area percentage of TNF-α (0.9 ± 0.22) as compared to the control (0.24 ± 0.07), VEGF (0.55 ± 0.11) in comparison with the control group (0.2 ± 0.05), and iNOS (0.82 ± 0.19) compared to the control (0.19 ± 0.08), ($p < 0.001$, $p < 0.05$, $p < 0.001$ respectively).

TNF-α, VEGF and iNOS expression in the lung tissue (Fig. 4):

TNF-α immunostaining expression was weak in lining alveolar and bronchioles of control group which moderately increased in depression group.

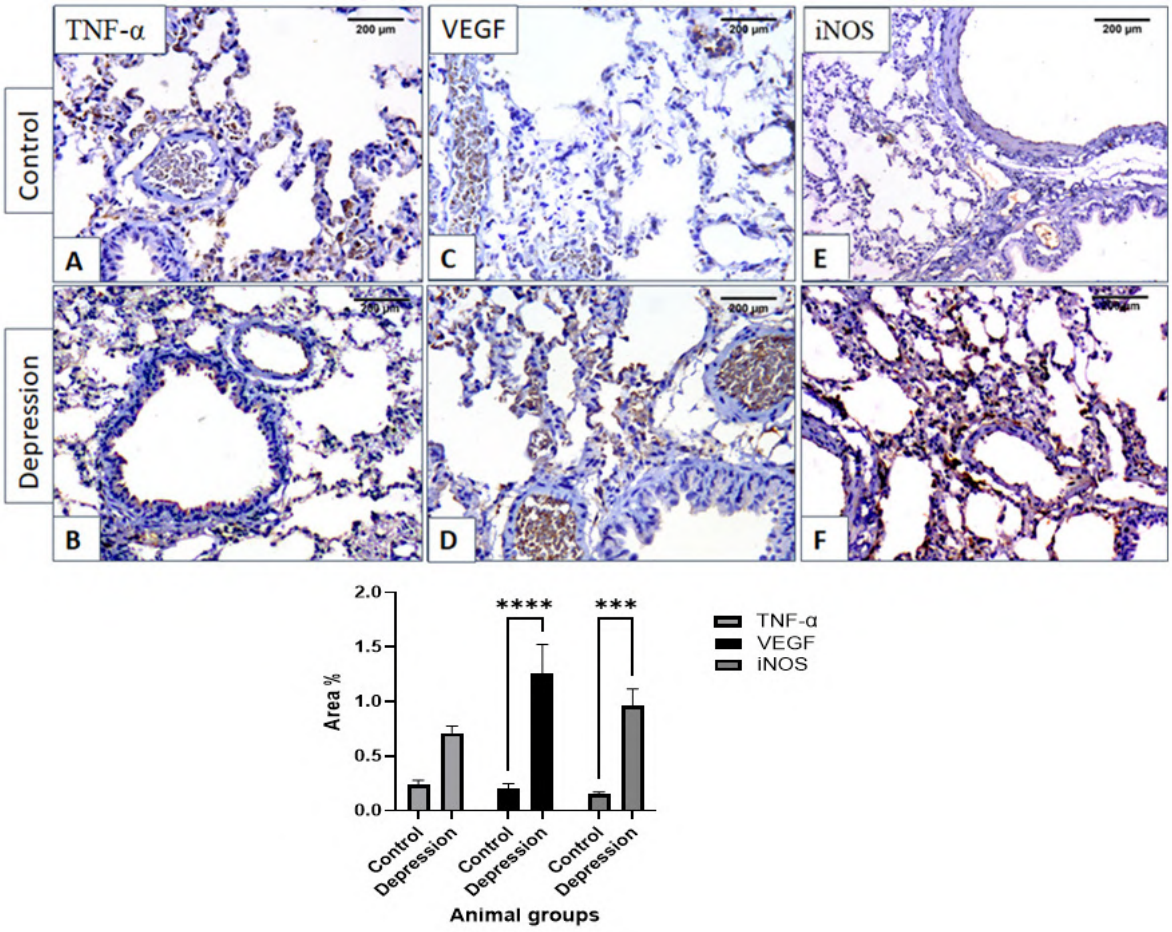

Fig. 4.- Sections of rat's lung after TNF-α, VEGF & iNOS immunohistochemical technique: (**A, B**) control and depression groups of TNF-α stained sections, in which there is a little increase of TNFα expression in alveolar epithelium, and bronchioles in depression group compared to the control group. (**C, D**) Control and depression group of VEGF-stained sections showing the increase VEGF expression in the terminal gas exchange alveoli and the epithelium of small vessels and bronchioles of the alveolus in the depression group compared to the control group. (**E, F**) Control and depression group of iNOS stained sections showing the increased expression of iNOS in the endothelial cells of pulmonary vessels, vascular smooth muscle cells and in the epithelium of bronchiolar epithelial in depression group compared to control group. (**G**) Histogram illustrating the quantitative analysis of the intensity of TNF-α, VEGF & iNOS stains in different experimental groups. The depression group demonstrated a significant increase in VEGF & iNOS area percentage as compared to the control group. However, the TNF-α area percentage showed non-significant differences. Data are represented as mean + SEM. *** means significance at $p< 0.001$ while **** means significance at $p< 0.0001$. Scale bars = 200 µm.

VEGF expression in control sections was weak and appeared in the terminal gas exchange alveoli, in the endothelium of some blood vessels, and the epithelium of bronchioles. The reaction became intense in the depression group.

Weak iNOS immunostaining was detected in all types of pulmonary vessels (arteries, veins, and capillaries) endothelial cells, smooth muscle cells of lung vessels and in the bronchiolar epithelial cells of the control group. In the depression group, the intensity of iNOS-positive expression was high as compared with the control.

When measuring the area percentage of immuno-expression, the depression group demonstrated significant increases in the means of area % for VEGF (1.26 ± 0.32) as compared to the control group (0.2 ± 0.06), of iNOS (0.96 ± 0.24) compared to the control group (0.15 ± 0.07) ($p < 0.0001$, $p < 0.001$ respectively). However, the mean area percentage for TNF-α expression showed a non-significant difference between groups.

TNF-α, VEGF and iNOS expression in the kidney tissue (Fig. 5):

Weak glomerular and tubular TNF-α immune-expression was detected in control sections. Meanwhile, in the depression group, intense TNF-α immunostaining was observed in both renal glomeruli and tubular epithelial cells.

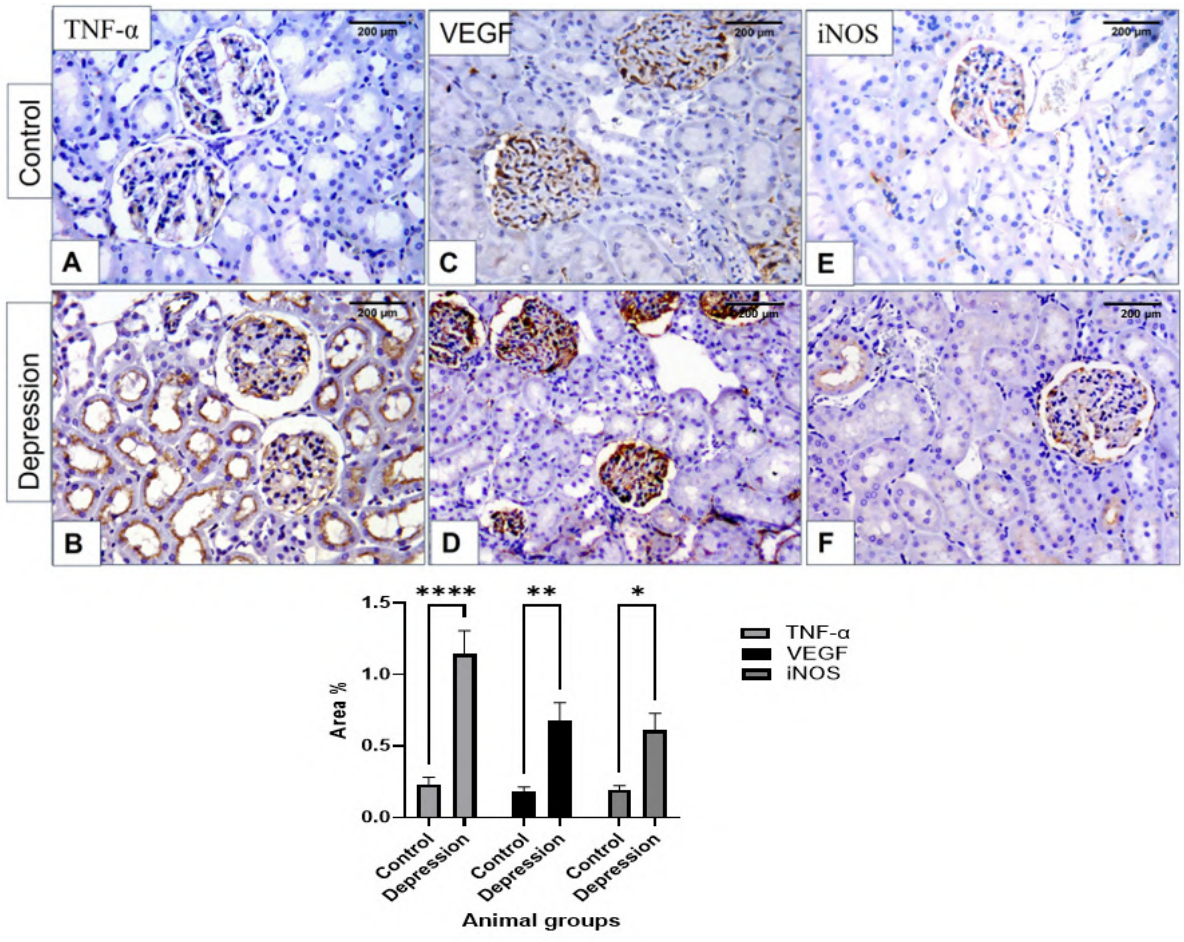

Fig. 5.- Sections of rat's Kidney after TNF-α, VEGF & iNOS immunohistochemical technique. (**A, B**) control and depression groups of TNF-α stained sections, showing the increased expression of TNF-α in renal glomeruli and tubular epithelial cells in depression group compared to the control group. (**C, D**) Control and depression group of VEGF-stained sections showing the increased VEGF expression in the epithelial glomerular cells, and blood vessels of the depression groups compared to the control group. (**E, F**) control and depression group of iNOS stained sections showing the increased iNOS expression in renal glomerular endothelial cells and capsule and in tubular cells of the depression group compared to control group. (**G**) Histogram illustrating the quantitative analysis of the intensity of TNF-α, VEGF & iNOS stain in different experimental groups. The depression group demonstrated a significant increase in TNFα, VEGF & iNOS area percentage as compared to the control group. Data are represented as mean ± SEM. * means significance at $p< 0.05$, ** means significance at $p< 0.01$ while **** means significance at $p< 0.0001$. Scale bars = 200 µm.

VEGF immune expression was weak in the glomeruli of the control sections, but it was obviously increased in the epithelial glomerular cells, blood vessels of the depression groups.

Weak iNOS expression in the glomerular endothelial cells and in the visceral and parietal glomerular capsule was found in the control group. In the depression group, the expression appeared in the renal tubule cells and increased in the glomerular endothelial cells and capsule.

Measuring the intensity of immuno-expression through the area percentage of immuno-expression, the depression group demonstrated a significant increase in the means of area percentage for TNF-α (1.2 ± 0.52) as compared to control (0.2 ± 0.16), VEGF (0.65 ± 0.39) as compared to control group (0.15 ± 0.1), and iNOS (0.55 ± 0.38) as compared to the control group (0.2 ± 0.1)($p < 0.0001$, $p < 0.01$, $p < 0.05$ respectively).

TNF-α, VEGF and iNOS expression in the liver tissue (Fig. 6):

TNF-α immune expression of the control group showed weak intracytoplasmic TNF-α positive hepatocytes, Kupfer cells, and endothelium of blood sinusoids. The depression group showed strong intracytoplasmic TNF-α expression hepatocyte, especially those surrounding the central vein, with strong expression in the endothelium of blood sinusoids and Kupfer cells.

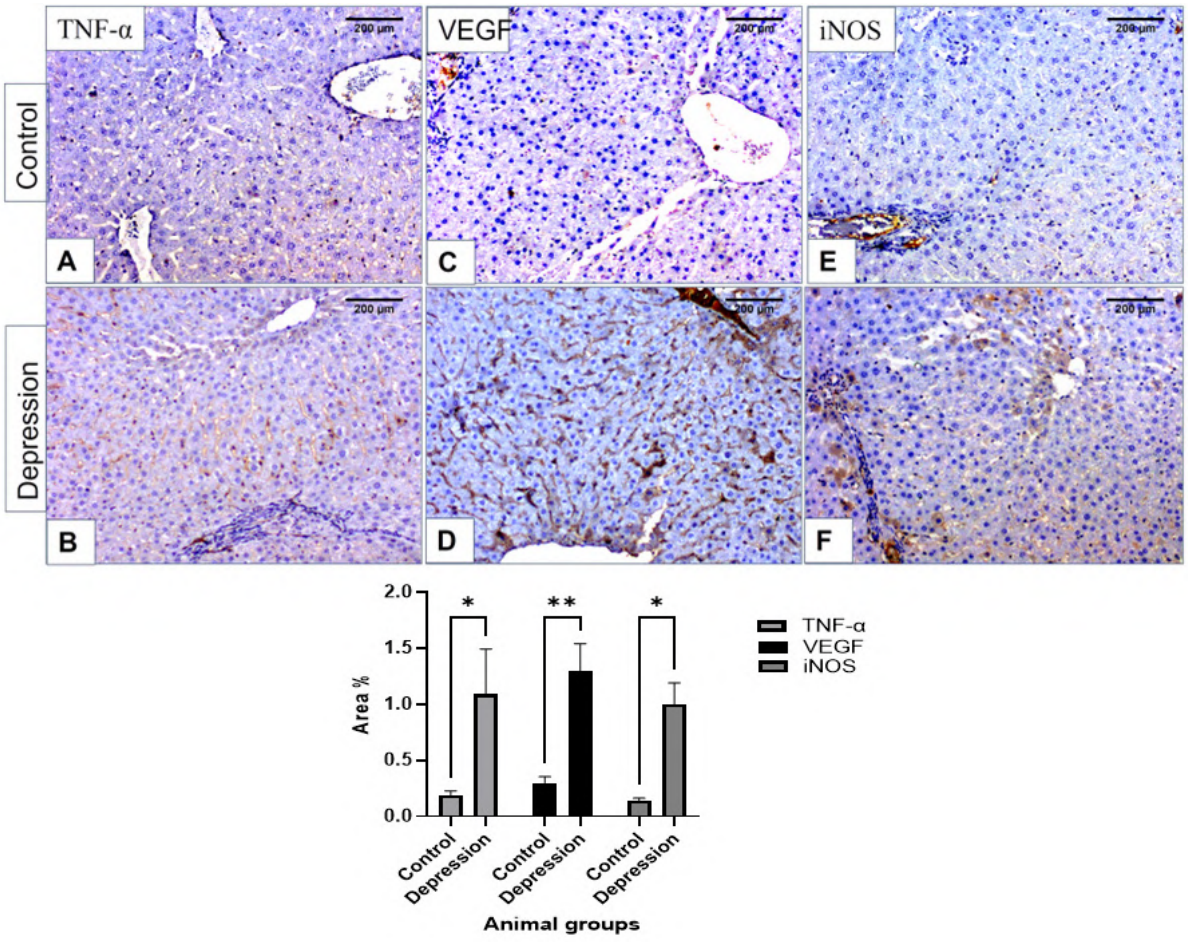

Fig. 6.- Sections of rat's liver after TNF-α, VEGF & iNOS immunohistochemical technique. (**A, B**) control and depression groups of TNFα stained sections, showing the increased intracytoplasmic expression of TNF-α in the hepatocyte, especially those surrounding the central veins and the strong expression in the blood sinusoids and Kupfer cells of the depression group compared to the control group. (**C, D**) Control and depression group of VEGF-stained sections showing the increased VEGF expression in the hepatocytes and cells of blood sinusoids in the depression group compared to the control group. (**E, F**) control and depression group of iNOS stained sections showing the increased iNOS expression in the endothelium of blood vessels and hepatocytes especially in zones surrounding the central vein and portal tract in the depression group compared to control group. (**G**) Histogram illustrating the quantitative analysis of the intensity of TNF-α, VEGF & iNOS expression in different experimental groups. The depression group demonstrated a significant increase in TNFα, VEGF & iNOS area % as compared to the control group. Data are represented as mean ± SEM. Data are represented as mean ± SEM. * means significance at $p< 0.05$ while ** means significance at $p< 0.01$. Scale bars = 200 µm.

Control sections showed moderate VEGF expression in blood sinusoids and uniformly weak expression in hepatocytes. However, in the depression group, VEGF immuno-expression was obviously increased in the endothelium of blood sinusoids and hepatocytes.

In control sections, weak iNOS immune expression was uniformly distributed in hepatocytes with moderate expression in the blood vessels' endothelium and in the epithelium of adjacent bile duct. In the depression group, intense expression was observed in the endothelium of blood vessels, and moderate intracytoplasmic hepatocytes expression appeared especially in zones surrounding the central veins and portal tract.

When measuring the intensity of immuno-expression through the area% of immuno-expression, the depression group demonstrated significant increases in the means of area % for TNFα (0.8 ± 1.27) as compared to the control group (0.2 ± 0.12), of VEGF (1.15 ± 0.77) as compared to the control group (0.25 ± 0.17), and iNOS (0.8 ± 0.6) as compared to the control group (0.1 ± 0.07) ($p < 0.05$, $p < 0.01$, $p < 0.05$ respectively).

DISCUSSION

The study hypothesized the generalized vascular inflammatory effect of depression on various body systems and tried to explore this through investigating the expression of vascular inflammatory biomarkers (TNF-α, VEGF and iNOS) (Zhang et al., 2009; Shaik-Dasthagirisaheb et al., 2013) in vital parenchymal tissues (heart, lung, kidney, and liver) after induction of depression in rats, and through studying the changed serum levels of these biomarkers in the rats with depression.

In this study, the TNF-α serum level increased significantly in the depressed rats, and its expression was significantly increased in the blood vessels' endothelium and cardiomyocytes of the heart, in renal glomeruli and tubular epithelial cells of the kidney, as well as in the blood sinusoids, Kupfer cells and hepatocyte surrounding the central vein of the liver tissue. However, the TNF-α expression did not significantly increase in the lung epithelial tissues.

The significant elevation of serum TNF-α levels in depressed rats, accompanied by heightened expression in the endothelium of blood vessels in multiple vital organs, underlines the systemic nature of inflammation associated with depression. The elevated TNF-α expression leads to the reactive oxygen species (ROS) generation, which induces endothelial dysfunction and vasculitis in various pathological conditions (Zhang et al., 2009). This systemic inflammatory response has raised the recognition that inflammation may represent a common mechanism of the disease (Miller et al., 2010). However, in this study, TNF-α was predominantly expressed in the vascular endothelium after induction of depression, and so vascular inflammation might be a detrimental consequence of depression.

In the heart tissue, the elevated TNF-α expression levels in the endothelium of blood vessels and cardiomyocytes can denote endothelial dysfunction and other cardiac ailments (Libby et al., 2002). Depression itself has long been correlated to an increased risk of cardiovascular disease, and the observed increase in TNF-α might provide a mechanistic link, indicating a direct pathophysiological pathway of depression (Mosovich et al., 2008). The increased TNF-α expression in the renal glomeruli and tubular epithelial cells of the kidney could denote vascular impairment of the kidney as was reported by Bautista et al., who suggested that TNF-α could mediate renal damage in various pathological states (Bautista et al., 2005). In the liver tissue, the TNF-α elevated expression in the blood sinusoids can denote vascular affection and hepatic inflammation (Tilg and Moschen, 2010).

On the other hand, the non-significant TNF-α expression in the lung vascular tissue reported in this study can be attributed to the molecular mechanisms included in the control of tissue damage in the lung, as was described by Xu et al., which can directly inhibit TNF-α-induced effects and attenuate the severity of lung injury (Meng et al., 2020; Xu et al., 2023).

In the present study, there were unexpected significant decrease of the serum VEGF level in the depressed rats, though its significant increased expression in the blood vessels' endothelium of

all the examined tissues. The increased tissue expression is in accordance with the reported pivotal role of VEGF as a regulator of angiogenesis, vascular permeability and tissue repair in various pathological states, including neurodegenerative diseases (Shibuya, 2011). At the same time, VEGF is proved to be a mediator of inflammation and pathological angiogenesis (Shaik-Dasthagirisaheb et al., 2013), so we can depict its increased expression in the endothelium due to its role in inflammatory processes.

On the other hand, the decreased serum level of VEGF detected in this study is in accordance with some recent studies that strongly suggested that neuronal degeneration can be due to lower circulation level of VEGF in neuronal cells and that pro-angiogenic therapy could be beneficial in treating ischemic heart and brain diseases (Oosthuyse et al., 2001; Storkebaum E et al., 2005). From other point of view, the decreased VEGF serum level does not necessarily mirror its tissue expression, as tissues can locally upregulate VEGF expression in response to local factors like hypoxia, without a corresponding increase in the systemic levels. Further, and in consistent with our results, certain stress conditions might lead to sequestration or increased uptake of VEGF in the tissues, leading to decrease of its serum levels (Shibuya, 2011).

The elevated VEGF in the heart tissue might indicate attempts at angiogenesis to tolerate the increased metabolic demand or reduced oxygen supply due to the increased proinflammatory cytokines and inflammatory processes in cardiovascular system in depression (Grippo and Johnson, 2009). Also, in the lung tissues, the reported increased VEGF expression in the endothelial cells reflects the role of this cytokine in the maintenance and repair of the alveolar structures affected by the raised inflammatory cytokines. This might suggest a reparative or defensive response against potential alveolar damage in the context of depression (Compernolle et al., 2002). In the kidney, the elevated VEGF expression in the glomerular epithelium can indicate a stress response for the potential damage produced by the increased proinflammatory cytokines in depression. Its expression can denote a protective restoration of endothelial cells and glomerular capillary circumference through the tubular repair mechanisms (Stevens et al., 2017). Elevated VEGF expression in blood sinusoids of the liver can indicate increased vascular permeability or angiogenesis, which might be a response to metabolic changes or altered detoxification demands in depression (Apte et al., 2019). Importantly, the increased VEGF expression can represent a protective mechanism for the liver sinusoidal endothelial cells, as it plays a special role in other growth factors' tissue release from hepatic endothelial cells, to protect the hepatocytes from the damage caused by the hepatotoxin (LeCouter et al., 2003).

The significant increased serum level and tissues expression of iNOS in endothelial tissues of depressed rats, offers a convincing insight for the potential connection between depression and vasculitis. The excessive production of NO, particularly from iNOS, has been associated with pro-inflammatory and oxidative processes (Förstermann and Sessa, 2012). Its association with development of depression and its severity is recently documented by Wang et al. (2021), and hense, the increased serum levels and tissue expression of iNOS can denote overproduction of NO that might exacerbate endothelial dysfunction, predisposing to inflammatory vasculitis that can impact various organ systems (Pacher et al., 2007; Wang et al., 2021).

The elevated iNOS in cardiomyocytes could indicate cardiac stress, potentially linking depression to cardiovascular diseases as was reported by Paulus and Tschope. Besides, its upregulation in pulmonary vessels and bronchiolar epithelial cells might relate depression to pulmonary complications, such as pulmonary hypertension or bronchitis seen in some patients with vasculitis (Han et al., 2004; Paulus and Tschöpe, 2013). The enhanced iNOS expression in renal tissues might predispose to renal inflammation or nephritis which is a common manifestation in certain types of vasculitis, while its increased expression in the liver's blood vessels and bile ducts might signify hepatic inflammation or cholangitis (Yaylak et al., 2008; Sedaghat et al., 2019). The systemic increase of iNOS expression in depressed rats

might suggest that depression could predispose or exacerbate vascular inflammation or vasculitis. (Pober et al., 2009).

The association of depression with systemic inflammation, particularly vascular inflammation can be considered a double-edged blade, because though depression can elevate pro-inflammatory markers like TNF-α and iNOS, these inflammatory cascades could further accentuate depressive symptoms, establishing a vicious cycle (Pober et al., 2009; Miller et al., 2010).

The study aimed to display the reverse of the conventional understanding of the association between depression and vascular inflammation. As the study demonstrated that vasculitis can be a harmful outcome resulting from stress and depression, and that vascular inflammation can be a serious reaction associated with the onset of depression.

More research is necessary to investigate the direct causative link between depression and vascular inflammation. Studies should focus on the functional implications of these vascular changes and whether they represent adaptive or maladaptive responses in the context of depression. The observed upregulation of the pro-inflammatory cytokines in endothelial lining of the vital organs can serve as a basis for future development of endothelial protective therapies that could help treatment of depression associated with chronic proinflammatory diseases.

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