Research Article

Effect of Acute Immobilization Stress on Blood Brain Barrier Integrity in Adult Male Albino Rats

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Abstract
Aim of work: This research aimed to study the effect of immobilization stress; IS (one of the forms of physical and psychological stressors that individuals are frequently exposed to) on blood brain barrier (BBB) integrity, and its possible predisposition to neurodegenerative diseases and the possible mechanism. **Materials and Methods:** thirty six adult male albino rats were randomly divided into six equal groups: control, anti-oxidant (Antox), three hours IS, three hours IS + Antox, six hours IS and six hours IS + Antox groups. Anti-oxidants were administrated intra-peritoneally once daily for ٥ days in the form of both L-ascorbate, ٢٠٠ mg/kg and alpha tocopherol, ٦٠ mg/Kg before exposure to IS. **Results:** the present study showed that IS time dependently (six hours' parameters were significantly higher than three hours' parameters) produced break down of the BBB evidenced by the significantly higher levels of biomarkers of brain injury; protein S١٠٠B, and Neuron specific enolase (NSE) in both serum and brain, and brain Evans blue dye (EB) as compared with the control non-stressed group. Oxidative stress is incriminated, as the levels of malondialdehyde (MDA) and Nitric oxide (NO) were significantly higher in the brain of stressed rats and the changes were significantly lowered by anti-oxidant pretreatment which in addition, attenuated the elevated levels of catecholamines, corticosterone, and glucose produced by IS. **In conclusion:** The results support the possibility that stress is a trigger for neurodegenerative brain diseases through disruption of BBB that can be early assessed by biomarkers of brain injury. Oxidative stress is the contributing mechanism and anti-oxidant pretreatment can offer protection.
Key words: S١٠٠B protein, Neuron Specific Enolase, immobilization restraint stress, nitric oxide and malondialdehyde

Introduction
The blood brain barrier (BBB) separates blood in the brain capillaries from the interstitial fluid of the brain. It is formed of the capillary endothelium strongly bounded by tight junctions and expressing very minimal transcytosis, a thick basement membrane investing pericytes, and the feet processes of astrocytes and perivascular macrophages[١].

The BBB restricts movement of molecules between systemic circulation and interstitial brain fluid. This buffers the brain from rapid ionic and metabolic changes, protects the brain neurons from toxic substances in blood, keeps the central and peripheral neurotransmitter pools separate without cross talks in between, and maintains homeostasis of the brain microenvironment. Physiologically, passage through this brain gate occurs only by selective carrier mediated transport or by transcytosis across endothelial cells; which involves end-ocytosis at the capillary membrane and exocytosis at the abluminal border. In addition, the cerebral endothelial cells possess several enzymes to degrade neurotransmitters while their passage from blood to brain which makes the BBB also a chemical barrier[٢,٣].

Clinically, disruption of BBB is the pathological basis for many neurological diseases including: trauma, ischemia, hypertensive strokes, dementia, epilepsy and disseminated sclerosis. Furthermore, emotional and environmental stressors reportedly influence brain function and are known to be key factors in the genesis of neuropsychiatric diseases[٤,٥,٦,٧].

One of the experimental models of stress in albino rats is immobilization stress. It is not only anxiogenic, but also excitotoxic increasing the release of excito-transmitters; aspartate and glutamate and associated with oxidative stress to which the brain tissue is highly vulnerable[٨]. Therefore, we used this model in the present work to study: **١)** the effect of immobilization stress on BBB integrity and neuronal damage, **٢)** the effect of duration of stress exposure, **and ٣)** the possible contribution of free radicals.

Material and methods

I- Animals

Thirty six adult male albino rats (Sprague-Dawley strain), of average weight ٢٢٠-٢٥٠ g, about ٤ months old were used in the present study. They were purchased from the National Research Center, Cairo; Egypt. They were housed in groups of six in stainless steel cages that offered adequate space for free movement and wandering (٤٠ cm x ٤٠ cm x ٢٥ cm) at room temperature with natural dark/light cycles, and allowed free access to water and commercial rat's diet (Nile Company, Egypt) for two weeks for acclimatization. All experimental protocols were approved by the animal care committee of Minia University which coincides with international guidelines. Rats were classified randomly into the following groups (six rats each):

١- Control group (C group); in which rats were left freely wandering in their cages at room temperature.

٢- Anti-oxidant group (Antox group); in which each rat received anti-oxidants in the form of both L-ascorbate, ٢٠٠ mg/kg and alpha tocopherol, ٦٠ mg/Kg (Nile pharmaceutical co., Egypt) intra-peritoneally once daily for ٥ days[٩].

٣- Three hours immobilization stress group (٣h-IS group); in which each rat was immobilized on a wooden board by taping the four limbs to a specially prepared metal mounts for three hours[١٠,١١].

٤- Three hours immobilization stress + Anti-oxidant group (٣h-IS + Antox group); where rats were pretreated with anti-oxidants as in group ٢, before being subjected to immobilization stress for three hours as in group ٣.

٥- Six hours immobilization stress group (٦h-IS group); in which each rat was immobilized as in group ٣, but for six hours[١٢].

٦- Six hours immobilization stress + Anti-oxidant group (٦h-IS+Antox group); where rats were pretreated with antioxidants as in group ٢, before being subjected to immobilization stress for six hours as in group ٥.

II- Experimental protocol

All experiments were conducted in the morning starting at ٨ a.m., after an overnight fast. On the day of the experiment, all rats received an intra-peritoneal injection of Evans Blue (EB) dye (٢ % in normal saline solution at a dose of (٤ml/kg) half an hour before IS in stressed groups and three hours before decapitation in non-stressed groups. EB rapidly binds to plasma albumin and homogenize in blood in half an hour and blood levels are maintained for about ٢٤ hours. Since albumin does not pass the BBB under normal physiological conditions, spectrophotometric determination of EB accumulation in brain tissue is an easy and reliable way to determine BBB permeability [١٣].

At the end of all experiments according to the group, all rats were decapitated, blood was collected from the jugular vein, allowed to clot, and centrifuged and supernatant serum was collected in eppendorf tubes and stored at -٢٠ºC till the time for biochemical assay.

- ***Brain preparation***

The skulls were opened carefully to take the brain. The limbic system (hippocampus, and hypothalamus) were dissected, excised, weighed and homogenized in phosphate buffered saline by ultrasonic homogenizer (٤٧١٠ series, Chicago). After centrifugation in a cooling centrifuge (٤ºC), at ١٥٠٠٠ rpm for ٣٠ minutes, the supernatant was taken for biochemical analysis.

III- Biochemical analysis

Serum and brain homogenates were subjected to the following tests:

A- Tests to evaluate the integrity of BBB:

(١) Brain EB concentration

Brain homogenates were incubated overnight with ٥٠ % trichloroacetic acid, then centrifuged and the supernatant was

read at ٦١٠ nm wavelength using spectrophotometer (Spectronic ٢٠٠٠, Baush & Lumb) against a known standard according to *Manaenko et al.* [١٤] .

(٢) Protein S١٠٠B;
It was measured using enzyme-linked immunosorbent assay kit (ELISA; Wuhan El Arab Science Company, China). Protein S١٠٠B in the sample is sandwitched between the specific antibody coating the microtiter plate wells and the added specific antibody linked to horseradish peroxidase; an enzyme that develops coloured complex when tetramethoxypropane (TMP) is added. The intensity of colour is proportionate to protein S١٠٠B in the sample and its absorbance is read at ٤٥٠ nm using ELISA apparatus (SLT-SPECTRA, Salzpurg).

(٣) Neuron specific enolase (NSE);
It was measured using enzyme-linked immunosorbent assay kit (ELISA; Wuhan El Arab Science Company, China), with the same principle and technique as protein S١٠٠B.

B- Tests to evaluate the neuroendocrine physiological response:

(٤) Serum and brain catecholamines [١٥]**:** were determined using spectro-fluorophotometric method. It depends on oxidation of catecholamines by addition of ٠.١ normal iodine followed by addition of alkaline sulfite to stop oxidation and produce certain fluorescence that could be read at different excitation/emission wavelengths according to the type of catecholamine (norepinephrine, epinephrine and dopamine) which is proportionate to their concentration using spectroflurophotometer (Shimadzu-RF ٥٠٠٠; Japan).

(٥) Serum corticosterone[١٦]**:** The method depends on the extraction of free ١١-hydroxycorticosteroids; mainly corticosterone and cortisol by methylene chloride followed by their condensation with an acidic fluorescence reagent and measuring the induced fluorescence which is proportionate to the steroid concentration at ٥١٠ nm excitation and ٤٥٠ nm emission using spectroflurophotometer (Shimadzu-RF ٥٠٠٠; Japan) against a known standard.

(٦) Serum glucose[١٧]**;** was determined using enzymatic colorimetric method depending on enzymatic oxidation of glucose in the presence of glucose oxidase to yield hydrogen peroxide that reacts with phenol and ٤-aminiantipyrine to form a red violet colour; the intensity of which is proportionate to glucose concentration and its absorbance is measured spectrophotometrically at ٥٤٦ nm using spectrophotometer (Spectronic ٢٠٠٠, Baush and Lumb).

C- Tests to evaluate the oxidative status:

(٧) Serum and brain malondialdhyde (MDA); was determined as indicator of lipid peroxides using thiobarbituric acid colorimetric method described by *Okawa et al.* [١٨]. The absorbance was read at ٥٣٢ nm using spectrophotometer (Spectronic ٢٠٠٠, Baush and Lumb). ١,١,٣,٣-tetramethoxypropane was used to prepare a standard curve for MDA.

(٨) Brain Nitric Oxide (NO); was determined using a colorimetric nitrite assay kit (Bio-diagnostic, Egypt). It is based on the conversion of nitrate to nitrite; the stable metabolite of NO by nitrate reductase followed by the addition of Griess reagent to convert nitrite to a deep purple azo compound proportionate to NO concentration. The absorbance was read at ٥٢٠ nm using spectrophotometer (Spectronic ٢٠٠٠), and was compared with a standard curve that was simultaneously prepared using sodium nitrite of different concentrations.

Statistical analysis

Statistical analysis was performed using Graph and Prism software and significant difference between groups was done by one way ANOVA followed by Tokey-Kramer post hoc test for multiple comparisons with a value of $p \leq ٠.٠٥$ considered statistically significant.

Results

A- Evaluation of the integrity of BBB:

Tables (١), (٢), and (٣) show that stress time dependently increased significantly the serum and brain (hypothalamic and hippocampal) levels of biomarkers of BBB injury; S١٠٠B, NSE, and brain Evans Blue when compared with the control group. The levels were significantly higher after ٦h-IS as compared with ٣h-IS. Pretreatment with

antox drugs had no significant effect on these biomarkers as compared with the control group; however, they significantly reduced these levels in the stressed groups. Serum Evans Blue levels did not significantly change indicating homogenous distribution of the dye in blood that was not affected by any treatment to the rats.

B- Evaluation of the oxidative status:

Figures (١), and (٢) show that stress time dependently increased serum and brain lipid peroxides and brain NO levels, when compared with the control group. The levels were significantly higher after ٦h-IS as compared to ٣h-IS. Pretreatment with antox drugs had no significant effect on these parameters during rest as compared with the control group; however, they significantly reduced these levels in the stressed groups.

***C- Evaluation of the physiological parameters of the stress response*:**

Table (٦) and (٧) show that antox treatment had no significant effect of their own neither on the catecholamines; epinephrine, norepinephrine and dopamine of serum or brain nor on serum corticosterone and glucose levels during rest. However, they significantly ameliorated the response of increased levels of these parameters during stress (٣h- and ٦h-IS), but never inhibited it completely. The levels remained significantly higher than control group. Furthermore, prolongation of the stress period (٦h-*vs.* ٣h-) produced a significantly higher response.

Table (١): The levels of S١٠٠B protein in the brain (pg/mg brain tissue) and serum (pg/ml) of rats of the different groups.

Groups→ Sample↓	Control	Antox	٣h-IS groups		٦h-IS groups	
			٣h-IS	٣h-IS+Antox	٦h-IS	٦h-IS + Antox
Serum	٥٨±١.٢	٥٧±١.٥	٣٠٠±١١.٨^{a}	٦٤±٣.٦^{c}	٣٣٢±١٢.١^{ab}	٧٨±٣.٩^{ac}
Brain	٨٠.٤±٣.٤	٧٨.٨±٤.٧	٣٢٩± ٩.٦^{a}	٩٢.٥±٦.٣^{c}	٣٥٧±١٠.١^{ab}	٩٨.٢±٧.٣^{ac}

Data represent the mean ± S.E. of six rats/ group. Superscripts: a=significant (p≤٠.٠٥) with the control, b= significant with ٣h-stressed group, c= significant with corresponding non treated stressed group. ٣h-IS= ٣h-Immobilization stress; ٦h-IS= ٦h- Immobilization stress group; Antox= Anti-oxidant.

Table (٢): The levels of NSE protein in the brain (pg/mg brain tissue) and serum (pg/ml) of rats of the different groups.

Groups→ Sample↓	Control	Antox	٣h-IS groups		٦h-IS groups	
			٣h-IS	٣h-IS+Antox	٦h-IS	٦h-IS+Antox
Serum	٢٠± ٣.١	١٨±٢.١	١٦١±٧.٤^{a}	٢٣± ٣.٧^{c}	١٨٢±٨.٢^{ab}	٢٥± ٣.١^{c}
Brain	٥٥± ٢.٥	٥٤± ٢.٧	٥٠٠±١٠.٤^{a}	٨٣±٦.٤^{ac}	٥٣٦±١٢.٢^{ab}	٩٢.٣±٨.١^{ac}

Data represent the mean ± S.E. of six rats/ group. Superscripts: a=significant (p≤٠.٠٥) with the control, b= significant with ٣h-stressed group, c= significant with corresponding non

treated stressed group. ٣h-IS= ٣h-Immobilization stress; ٦h-IS= ٦h- Immobilization stress group; Antox= Anti-oxidant.

Table (٣): The levels of Evans Blue in serum (µg/ml) and brain (µg/g brain tissue) of rats of the different groups.

Groups→ Sample↓	Control	Antox	٣h-IS groups		٦h-IS groups	
			٣h-IS	٣h-IS + Antox	٦h-IS	٦h-IS+Antox
Serum	١٤٠٠±٥٦	١٤١٠±٤٧	١٤٢٢±٦٩	١٤١٩±٥٢	١٤٣٨±٦٨	١٤٣١±٧٦
Brain	١.٣±٠.١٧	١.٤±٠.١٥	١٨±٢[a]	٣.٣±٠.٣٥[ac]	٢٩±٣.٦[ab]	٤.٧±٠.٥١[ac]

Data represent the mean ± S.E. of six rats/ group. Superscripts: a=significant (p≤٠.٠٥) with the control, b= significant with ٣h-stressed group, c= significant with corresponding non treated stressed group. ٣h-IS= ٣h-Immobilization stress; ٦h-IS= ٦h- Immobilization stress group; Antox= Anti-oxidant.

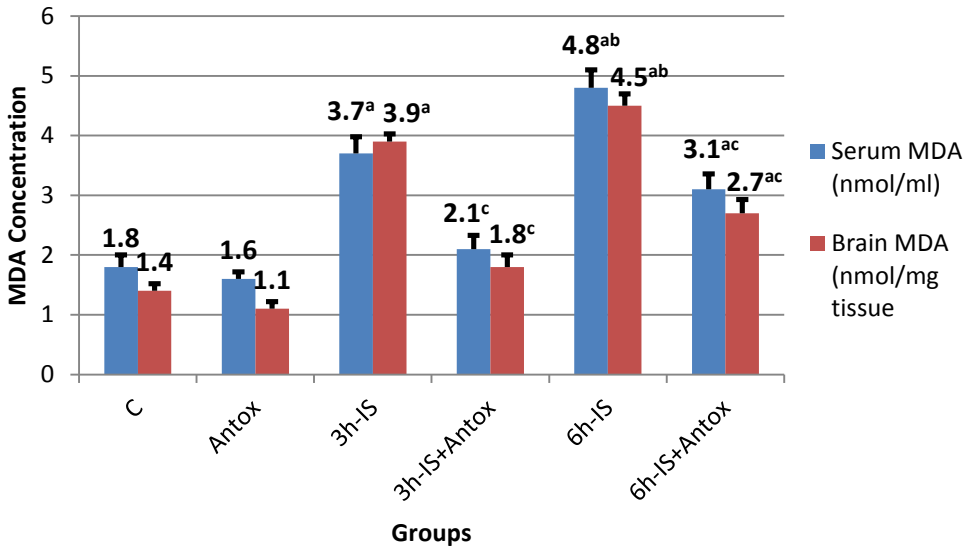

Figure ١: The levels of lipid peroxides (MDA) in serum (nmol/ml) and brain (nmol/mg brain tissue) of rats of the different groups. a=significant with the control, b= significant with ٣h-stressed group, c= significant with corresponding non treated stressed group, P≤٠.٠٥. ٣h-IS= ٣h-Immobilization stress; ٦h-IS= ٦h- Immobilization stress group; Antox= Anti-oxidant. Data are expressed as mean ± S.E.M. of ٦ rats in each group.

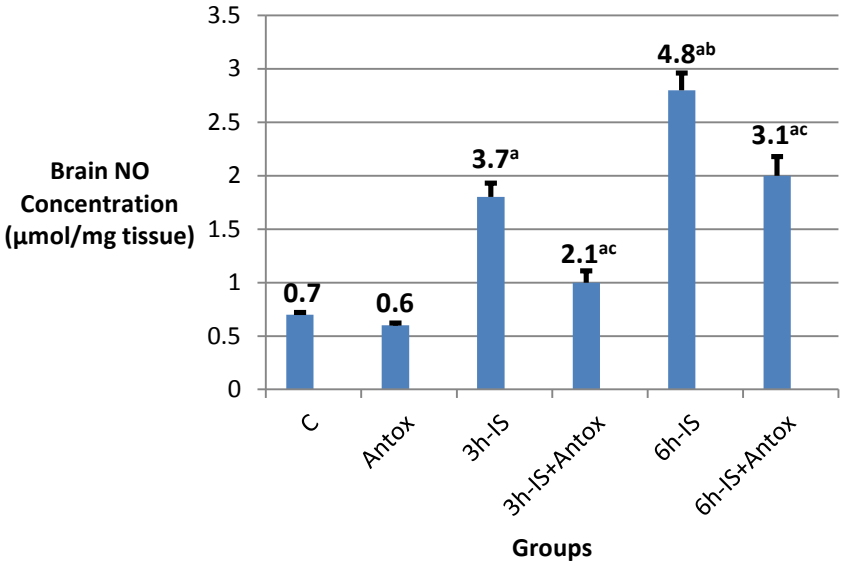

Figure ٢: The levels of Nitric oxide (NO) in brain (µmol/mg brain tissue) of rats of the different groups. a=significant with the control, b= significant with ٣h-stressed group, c= significant with corresponding non treated stressed group, P≤٠.٠٥. ٣h-IS= ٣h-Immobilization stress; ٦h-IS= ٦h- Immobilization stress group; Antox= Anti-oxidant. Data are expressed as mean ± S.E.M. of ٦ rats in each group.

Table (٦): The levels of catecholamines in serum (ng/ml) and brain (µmol/mg brain tissue) of rats of the different groups.

Groups→ / Parameter↓		Control	Antox	٣h-IS groups		٦h-IS groups	
				٣h-IS	٣h-IS + Antox	٦h-IS	٦h-IS+Antox
E	Serum	٧٩.٢±٣.١	٧٧.٢±٣.٤	١٨٩±٥.١^{a}	١٦٨±٦.١^{ac}	٢١٢± ٦.٣^{ab}	١٨٦± ٧.١^{ac}
	Brain	٣٩.٨±١.٦	٣٧.٩±١.٨	١١٧±٣.٩^{a}	٩٩±٤.٦^{ac}	١٣٢±٣.٩^{ab}	١١٢±٤.١^{ac}
NE	Serum	٦٨.٧±٢.٢	٦٥.٣±٢.٨	١٧٧±٥.٢^{a}	١٦١±٣.٩^{ac}	١٩٩±٥.٧^{ab}	١٨٢±٣.٤^{ac}
	Brain	٢٣.٧±١.٣	٢٢.٦±١.١	٨٣.٤±٢.١^{a}	٧٦.٤±٢.٣^{ac}	٨٩.٨±٢.٢^{ab}	٧٩.١±٢.٤^{ac}
D	Serum	٥٩.٢±١.٤	٥٧±٢.٤	١١١±٣.٩^{a}	١٠٠±٢.١^{ac}	١٢٧±٣.٨^{ab}	١١٤±٣.٥^{ac}
	Brain	١١.٩±١.٤	١١±٠.٩٤	٦٨.٧±٣.٣^{a}	٥٩.٢±٣^{ac}	٧٩.٥±٣.٩^{ab}	٦١.٥±٣.١^{ac}

Data represent the mean ± S.E. of six rats/ group. Superscripts: a=significant (p≤٠.٠٥) with the control, b= significant with ٣h-stressed group, c= significant with corresponding non treated stressed group. ٣h-IS= ٣h-Immobilization stress; ٦h-IS= ٦h- Immobilization stress group; Antox= Anti-oxidant; E=epinephrine; NE=norepinephrine; and D=dopamine.

Table (٧): Serum corticosterone (µg/ml) and glucose (mg/dl) levels of rats of the different groups.

Groups→ Parameter↓	Control	Antox	٣h-IS groups		٦h-IS groups	
			٣h-IS	٣h-IS + Antox	٦h-IS	٦h-IS+Antox
Corticosterone	٤٠.٨±٣.٨	٣٧±٣.١	١٣٩±٦.١^{a}	١١٨±٧.١^{ac}	١٥٦±٦.٢^{ab}	١٣٠±٦.٩^{ac}
Glucose	٩٦±٦.٥	٩٥±٧.٢	٢١٣±١٥^{a}	١٧٦±١٣.١^{ac}	٢٥٨±١٤.٢^{ab}	٢١٦±١٢.٢^{ac}

Data represent the mean ± S.E. of six rats/ group. Superscripts: a=significant ($p \leq ٠.٠٥$) with the control, b= significant with ٣h-stressed group, c= significant with corresponding non treated stressed group. ٣h-IS= ٣h-Immobilization stress; ٦h-IS= ٦h- Immobilization stress group; Antox= Anti-oxidant.

Discussion

Stressors influence brain function and are known to be key factors in the genesis of neuropsychiatric and neurodegenerative diseases. On the other hand, BBB integrity keeps the neurons of the brain away from the hazardous circulatory changes in neurotransmitters, cytotoxic mediators and ionic changes. In the present work, we aimed to study the effect of acute immobilization stress in male albino rats for ٣ and ٦ hours on BBB integrity taking in consideration that this form of stress simulates post-traumatic and/or paralytic hypokinesia in human subjects that is always associated with depression. The hippocampus and hypothalamic brain areas were selected because they represent the center for memory, learning and processing of the stress response and the neuron-endocrinal control [١٩].

It was found that protein S١٠٠B and NSE were significantly higher in the serum of stressed rats, while EB was significantly higher in the brain homogenates indicating BBB disruption due to stress. The levels were significantly higher after ٦ h as compared with the ٣ h stress period indicating progressive damage with prolongation of the stress exposure period.

Protein S١٠٠B belongs to calcium-binding protein family that is glial specific and expressed primarily by astrocytes. It is called S١٠٠ protein because it is soluble in saturated ١٠٠٪ ammonium sulphate solution and it is a dimer of two b chains that is why it is called B. It acts as a neurotrophic factor and neuronal survival protein that could function in neurite extension, and axonal proliferation. It could also be secreted peripherally by schwann cells and melanocytes[٢٠], and this may explain the basal serum control levels.

Activation of astrocytes occurs in different brain insults; infection, inflammation, ischemia and hyperglycemia with changing morphology as enlarged cell body and increased number and length of dendrites to strengthen the BBB [٢١]. This may contribute to the increased brain levels of protein S١٠٠B with stress observed in this study. However, when released into extracellular brain fluid, it does not cross the BBB to blood unless there is increased permeability or disruption as found in the present work and the work of others; *Zongo et al.* [٢٢], following head injury, and *Bargerstock et al.* [٢٣], following osmotic opening of BBB by mannitol infusion, running and jogging exercises and boxing. However, focal brain lesions may increase protein S١٠٠B in the extracellular brain fluid only without a concomitant increase in plasma if the BBB is intact[٢٤].

NSE is localized within the cytoplasm of neurons as part of the glycolytic system and is concerned with the energy production and slow axoplasmic transport. It is never

secreted in extracellular brain fluid, so its presence indicates neuronal damage. Basal physiological blood levels as found in the control group of the present work is due to its presence in peripheral cells; RBCs, lymphocytes, plasma cells, capillary endothelium, and neuroectodermal cells. Only if disruption of BBB occurs, it can cross in either direction. This finding agrees with *Cheng et al.*[٢٥]. In the stressed groups of the present work, its increase in CSF indicates neuronal damage and its increase in plasma reflects break through the BBB and is compatible with *Mrozek et al.* [٢٦] and it was progressively correlated with the duration of stress.

EB when i.p. injected in rats is rapidly reabsorbed, conjugated with plasma albumin and homogenize in blood, but never cross the BBB unless disrupted. This agrees with *Manaenko et al.* [١٤], *Yen et al.* [٢٧], *Do et al.* [١٢], and *Wang and Lai* [٢٨]. So, the higher level of EB in brain homogenates of stressed rats of the present study is a sure marker that signifies BBB disruption with immobilization stress and it was time dependent.

Pretreatment with anti-oxidants in the present study had no significant effect of their own on BBB integrity in non-stressed rats, nor on the neuro-endocrine physiological parameters tested, however, did they significantly reduce the markers of BBB disruption in stressed rats. This incriminates oxidative stress in the pathophysiologic mechanism of BBB disruption during stress. The progressive higher levels of peroxides in the stressed groups and their corresponding reduction following free radical scavenger pretreatment strongly supports this conclusion.

The brain is more susceptible to oxidative stress due to its high content of unsaturated fatty acids and the low content of anti-oxidant defenses; anti-oxidant enzymes and free radical scavengers [٢٩]. The sources of reactive oxygen species (ROS) in the brain of stressed rats include increased release of glutamate that represents about ٦٠ % of the brain excitatory transmitters. Glutamate acts on N-Methyl, D-Aspartae Receptor, (NMDA) receptor to increase **Ca^{2+}** influx into neurones with consequent activation of several protein enzymes responsible for degradation of proteins, phospholipids and DNA as well as mitochondrial dysfunction; a mechanism called excitotoxicity generating free ROS [٢٩]. Furthermore, Ca^{2+} influx can activate Ca^{2+}- dependent arachidonic acid cascade and nitric oxide synthase regenerating superoxide and peroxinitrite free radicals respectively [٣٠]. The levels of brain nitric oxide in this study were significantly higher during stress, and the role of NO in BBB disruption has been proved by *Kovacic et al.* [٣١].

The significantly higher serum and brain catecholamine levels in the stressed rats of this study, is compatible with the study of *Ahmad et al.* [١٩] who found it to be area specific in the brain; more in the frontal cortex and hippocampus and peripherally reflect the sympatho-adrenal response, however, catecholamines can never cross an intact BBB due to high monoamino oxidase (MAO) content of brain capillary endothelium, but do that when BBB is disrupted[٣]. In this study, the high levels of catecholamines in the brain of stressed rats could partially be ascribed to induction of enzymes of catecholamine biosynthesis by stress namely; tyrosine hydroxylase and Dopa decarboxylase and/or to diffusion from the blood after BBB disruption. They are hazardous due to formation of ROS whether metabolized by MAO or by auto-oxidation and support the hypothesis of Siraki and O'Brien that biogenic amines form pro-oxidant radicals [١٩].

In the present work, increased serum corticosterone during stress reflects the stimulated hypothalamo-pituitary-adrenal axis response and its metabolic importance for maintaining a higher serum glucose level particularly with prolonged stress durations (٦h) to maintain energy balance. However, glucocorticoids can act as a double faced coin and they can impair several neuronal processes necessary to survive an injury as reuptake of excito-toxic glutamate, sequestration and extrusin of cytosolic Ca^{2+}, and free radical quenching [٣٢]. The disrupted BBB as found in this work could facilitate such effects. Furthermore, *Nowacka and Obuchowicz* [٣٣]

have found an inverse relation between plasma glucocorticoids and brain trophic factors as brain derived nerve growth factor (BDNF) and vascular endothelial growth factor (VEGF) which can contribute to neuronal degeneration during stress. The hyperglycemia found in this study as a consequent metabolic response could increase the expression and activation of pro-oxidant enzymes and alters mitochondrial oxidation in favor of free radical generation. This is compatible with *kmada et al.* [٣٤], and *Jing et al.* [٣١].

Oxidative stress induced injury of the brain including the BBB may occur through breakdown of tight junction proteins by activation of matrix metalloproteinases (MMP-٢, gelatinase A and MMP-٩; gelatinase B) which catalyse proteolytic breakdown of intercellular proteins and basement membranes or through oxidative damage of cell membrane phospholipids [٣٤]. This has been morphologically proved recently by ultrasatructural study in adult rats by *Santha et al.* [٣٥] and supports our findings.

In conclusion, the present study showed that exposure of male albino rats to IS produced a time dependent break down of the BBB evidenced by the significantly and progressively higher biomarker levels (protein S١٠٠B, NSE, and EB) in both serum and brain when compared with control rats. Oxidative stress is incriminated in BBB disruption, as the levels of lipid peroxides (MDA) and NO were significantly higher in the brain of stressed rats and the changes were ameliorated by anti-oxidant pretreatment. The significantly high catecholamines, glucocorticoids and blood glucose levels during stress exposure can contribute to brain injury by generating ROS and suppressing neuronal growth factors. The results support the possibility that stress is a trigger for neurodegenerative brain diseases through disruption of BBB. Based on this conclusion, further research will be directed towards studying the effects of chronic maintained or repeated stress application and the possibility of autoimmune antibody formation against protein S١٠٠B by the peripheral immune system with consequent attacking astroglial cells when BBB is persistently damaged.

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