Possible Neuroprotective Effect of Red Beetroot Extract on Visual Cortex Injury Induced by Cerebral Ischemic/Reperfusion Injury in Albino Rats

Original Article

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ABSTRACT

Introduction: Worldwide, stroke is among the leading causes of death and disability. The most common type of stroke is cerebral ischemia. Strokes have a high impact on vision. Loss of vision can be the worst residual effect following cerebral infarction. Health promoting properties of red beetroot have become widely known in recent years. In addition to its potent antioxidant properties, it also has anti-inflammatory and vascular protective effects.

Aim of the Work: The purpose of this study was to explore the prophylactic mechanisms of beetroot extract against cerebral ischemia/reperfusion (I/R) injury in the visual cortex of albino rats.

Materials and Methods: In our study, a total of 40 albino rats were used. They were allotted into four groups: Group I (control group), Group II (Red beetroot extract; RBE group), Group III (I/R group) and Group IV (Beetroot extract + I/R group). By the end of the experiment, the visual cortex was extracted and subjected to histological, immunohistochemical, and morphometric analyses.

Results: Red beetroot extract alleviated the ischemic induced visual cortex neurodegenerative changes. Neuronal cells showed a significant increase in toluidine blue color intensity. It protected the visual cortex against cerebral ischemia/reperfusion injury via down-regulation of caspase-3, TNF-α, iNOS and GFAP and up-regulation of HSP70 and PCNA immunoreaction.

Conclusion: According to the results of the current study, beetroot extract may be a potential neuroprotective agent in I/R damage of visual cortex. It protects the visual cortex from oxidative stress, inflammation, and apoptosis resulting from I/R damage. It provides an experimental basis for the application of beetroot extract in the treatment of cerebral ischemic diseases.

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Key Words: Beetroot , ischemic/reperfusion , rat, visual cortex.

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INTRODUCTION

Strokes, both ischemic and hemorrhagic, are among the leading causes of disability and death worldwide. Cerebral ischemia is the most common type of stroke, explicating nearly 80% of stroke cases[1]. The majority of stroke cases, regardless of whether they are ischemic or hemorrhagic, occur in low- and middle-income countries, where the average age of incidence and fatality being 6 years lower than in high-income countries, resulting in greater disability[2].

Chronic cerebral hypoperfusion plays a role in many types of cognitive impairments including Alzheimer's disease[3]. Reductions in cerebral blood flow and the resulting pathological processes significantly impair white matter, resulting in gray matter atrophy as revealed by neuroimaging[4,5].

Vision occupies a significant portion of the central nervous system. Consequently, strokes have a significant impact on vision. After a cerebral infarction, visual loss is often the most serious long-term consequence[6]. After a stroke, visual impairment leads to depression and loss of independence and reduces quality of life[7].

The aim of acute stroke treatment is to restore blood flow, which is referred to as "reperfusion". Spontaneous reperfusion, on the other hand, occurs in about 50-70% of ischemic stroke patients[8]. Ischemia-reperfusion injury (IRI) is a complicated and varied condition. Many factors influence the injury, including collateral circulations, duration of ischemia, gender, age, comorbidities, changes in systemic blood pressure and genetic predispositio [9,10].

Reperfusion injury occurs through a variety of mechanisms. These include oxidative stress, leukocyte infiltration, mitochondrial mechanisms, platelet activation, complement activation, and disruption of the blood-brain barrier (BBB), resulting in neurological dysfunctions and severe neuron death[11]. Furthermore, the production

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of reactive oxygen and nitrogen species (ROS and RNS) increases with reperfusion, for which the brain exhibits high sensitivity as it causes oxidative stress, resulting in memory deficits after a stroke[12].

Clinically, tissue plasminogen activator has been used to treat acute cerebral ischemia[13]. Unfortunately, the severe hemorrhagic transformation injury and recurrent blood flow have limited its use in clinical practice[14]. As a result of the complicated pathological processes of cerebral ischemia/reperfusion, existing therapeutic strategies have not been effective. Therefore, finding alternative therapies to treat stroke patients remains a significant challenge worldwide[15].

Red beetroot, also called Beta vulgaris rubra, is gaining attention in recent years as a health promoting food. Even though beetroot has recently gained scientific attention, it has been used as a natural medicine since Roman times[16]. The potent antioxidant, anti-inflammatory, and vascular-protective properties of beetroot have been documented in a number of in *vitro* and in *vivo* studies in humans and animals. To date, human studies have reported that beetroot supplementation reduces blood pressure, oxidative stress, and inflammation, maintain endothelial function, and restores cerebrovascular function[17].

To the best of our knowledge, there have been little published researches about the influence of beetroot extract against cerebral ischemia/reperfusion (I/R) injury. So, this study aimed at exploring the prophylactic effects of beetroot extract against cerebral I/R injury in rat visual cortex based on histological and immunohistochemical studies.

MATERIALS AND METHODS

Chemicals

Fresh red beetroots (Beta vulgaris) were obtained from a local market in Menoufia, Egypt. One kilogram of Beta vulgaris roots were chopped into small pieces of about 1 cm each and then deeply macerated by immersing them in 70% ethanol (1.5 L) for three successive days. A rotatory evaporator was used to concentrate the alcoholic extract under reduced pressure until complete drying (Biochemistry Department, Science University, Menoufia, Egypt). Finally, the resulted extract (150 gram) was suspended in distilled water.

Animals

In this study, a total of forty Sprague-Dawley albino male rats weighing between 200 and 250 grams were utilized (Ain shams Animal House, Cairo, Egypt). Two weeks before the experiment the rats were left for acclimation, kept under controlled conditions of temperature and humidity with free access to food and clean water, as well as a 12-hour cycle of light and darkness. All experimental steps and animal maintenance were conducted consistent with the roles and the guidelines of the Research Ethics Committee, Menoufia University, Faculty of Medicine with the ethical approval number (6/2021ANAT2).

Experimental design

The animals were allocated randomly into 4 groups (each included 10 rats):

Group I: consisted of 10 rats and were subdivided into 2 subgroups:

- Ia: (control) (n=5): animals were fed a regular diet.
- Ib: (Sham operated group) (n = 5): the rats were subjected to a sham surgical procedure on day 15 and left without treatment.

Group II: Beetroot extract group: The rats received red beetroot extract (RBE) (500 mg/kg/day/orally) for 22 days[18].

Group III: Ischemic/Reperfusion (I/R) group: This group subjected to occlusion of right common carotid artery (CCAO) for 90 minutes followed by reperfusion for one week[19].

Group IV: Beetroot extract + I/R group: The rats received RBE (500 mg/kg/day/orally) for 22 days then were underwent the same surgical procedure as group III followed by reperfusion for one week.

Surgical procedure

The standard method for inducing cerebral ischemia/reperfusion was used[19]. Anesthesia was administered to rats that were fasted overnight using thiopental sodium (30 mg/kg). An incision was made in the midline ventral region of the throat. The right common carotid artery was identified and released from both the surrounding tissue and from the vagus nerve. Global cerebral ischemia was produced by blocking the right common carotid artery with a clamp. The clamp was removed after 90 minutes to allow blood to flow through the carotid artery again. The rats were kept at 37 °C on a heated surgical platform. Sterile conditions were maintained throughout the surgical procedure at Ain Shams University, Faculty of medicine, Egypt. Only operation without clamp insertion was performed in Sham operated group.

After the experiment ended, the animals had been anesthetized by the use of ketamine (90 mg/kg) and xylazine (15 mg/kg) intraperitoneal then decapitated. The occipital (visual) cortex was extracted and subjected to histological, immunohistochemical, and morphometric analyses.

Histopathological assessment

Histological study

Visual cortex was fixed in 10% buffered formaldehyde solution. After being hydrated and cleared, the specimens were embedded in paraffin. Serial coronal sections were cut 5 µm thick for normal histological examination by staining them with hematoxylin and eosin (H & E) and with toluidine blue (TB) to detect Nissl's granules[20].

Immunohistochemical study

Paraffin sections on poly-L-lysin coated slides were deparaffinized and rehydrated. Sections were inserted into 3% hydrogen peroxide (H_2O_2) in order to block endogenous peroxidase. The microwave antigen retrieval procedure was done. The sections were incubated with primary anti Caspase-3 antibody; an apoptotic marker cytoplasmic expression, (rabbit polyclonal antibody, Dako, Carpinteria California, USA); anti-Tumor necrosis factor-alpha (TNF-α) antibody, inflammatory cytokine, expressed in the cytoplasm of granular cells, (mouse monoclonal antibody, Gene tex company, Cairo, Egypt); anti-inducible nitric oxide synthase (iNOS) antibody, a marker for oxidative stress, (rabbit polyclonal, Lab vision); anti Heat shock protein antibody (HSP70 antibody; a marker for oxidative stress-cytoplasmic and nuclear expression, (rabbit polyclonal antibody, Midco Trade Company, Giza, Egypt); anti Glial fibrillary acidic protein (GFAP) antibody; a marker for astrocyte activation, (rabbit polyclonal antibody, Midco Trade Company, Giza, Egypt), and anti-Proliferating cell nuclear antigen (PCNA) antibody, a marker of cellular regeneration, (Sigma-Aldrich, St Louis, Missouri, USA). The biotinylated polyvalent secondary antibody was then applied. Incubation of the sections with streptavidin-peroxidase was subsequently performed. Afterward, DAB substrate chromogen (3,3′-diaminobenzidine tetrahydrochloride) was then applied. Hematoxylin was used to counterstain the slides to be examined under a light microscope[21].

Morphometric assessment

With the use of Image J software, version K 1.45, the color intensity of toluidine blue, number of Caspase-3, TNF-α, iNOS, HSP70, GFAP and PCNA immune positive cells were measured. For every parameter, ten non-overlapping fields (x400) were examined for five different rats/experimental groups.

Statistical analysis

Mean ± SD was used to present the collected data. SPSS (Inc., Chicago, IL, USA) version 23 on an IBM compatible computer was used to analyze the data. The data was analyzed using one-way ANOVA then post hoc Bonferroni test. Statistics were deemed significant at the level of $p \leq 0.05$.

RESULTS

Examination of subgroups in group I and Beetroot extract group showed the same histological findings and revealed no statistically significant variance in all the studied parameters between them. So, we considered all as a control group.

H&E stain

Sections of the control group stained by H&E showed the six layers forming the visual cortical area (I, II, III, IV, V and VI; the outer molecular, the external granular, the external pyramidal, the inner granular, the inner pyramidal and the polymorphic layers respectively). The neuropil revealed pyramidal cells, granular cells, neuroglia, and blood capillaries. Pyramidal cells showed vesicular nuclei, basophilic cytoplasm, and long apical dendrites. Rounded in shape granular cells were noticed with large vesicular open face rounded nuclei. Different types of neuroglia with dense nuclei can also be detected (Figures 1A,B).

The visual cortex of rats exposed to I/R injury showed multiple shrunken neurons with dark nuclei surrounded by vacuolated pale areas, others showed ghost like appearance. The neuropil appeared vacuolated and showed vascular dilatation. Small multinucleated acidophilic masses surrounded by a clear space were noticed. Numerous neuroglia: most of them surrounded by a halo could be seen (Figure 1C).

Rats treated with Beetroot extract before exposure to I/R, visual cortex obtained from them displayed improvement in nerve cells. Most of the pyramidal, granular and neuroglia cells were nearly similar to that of the control group. Occasional pyramidal cells were still affected which appeared shrunken and nuclei was darkly stained and surrounded by pericellular halos. Also, blood capillaries were still dilated (Figure 1D).

Toluidine blue stain

The control group visual cortex exhibited dark blue staining for the dense Nissl's granules in the neuronal cell cytoplasm (Figure 2A). Toluidine blue staining color intensity in the I/R group was significantly reduced when compared to the control ($P < 0.001$) (Figure 2B, 2D). Compared to I/R group, beetroot extract protection increased the color intensity of toluidine blue significantly ($P<0.001$) (Figure 2C, 2D).

Immunohistochemical findings

In visual cortex of the control group, low levels of Caspase-3 and TNF-α immunoreactivity was detected. In I/R group, number of Caspase-3 and TNF-α immunostained cells increased significantly. This increase was significantly decreased in the beetroot extract + I/R group (group IV) (Figure 3).

This was associated with significant increase in iNOS in the visual cortex of the I/R group compared to the control group. A significant decrease in number of cells was observed in beetroot extract + I/R group compared with the I/R group (Figure 4).

HSP70 expression analysis was implemented to assert the effects of hypoxia. Compared to control group, HSP70 immunoreactivity was elevated in the I/R group. Moreover, beetroot extract treatment before exposure to I/R (group IV) further enhanced Hsp70 expression (Figure 4).

GFAP positive immunoreactivity was significantly increased compared with the control group. On contrast, this immunoreactivity significantly downregulated in beetroot extract + I/R group compared to the I/R group (Figure 5).

In control rats, cell proliferation in the visual cortex was identified based on PCNA immunoreactivity. In I/R group, PCNA immunoreactivity level was significantly reduced than in control rats. However, the level observed in beetroot extract + I/R group was significantly elevated than I/R group (Figure 5).

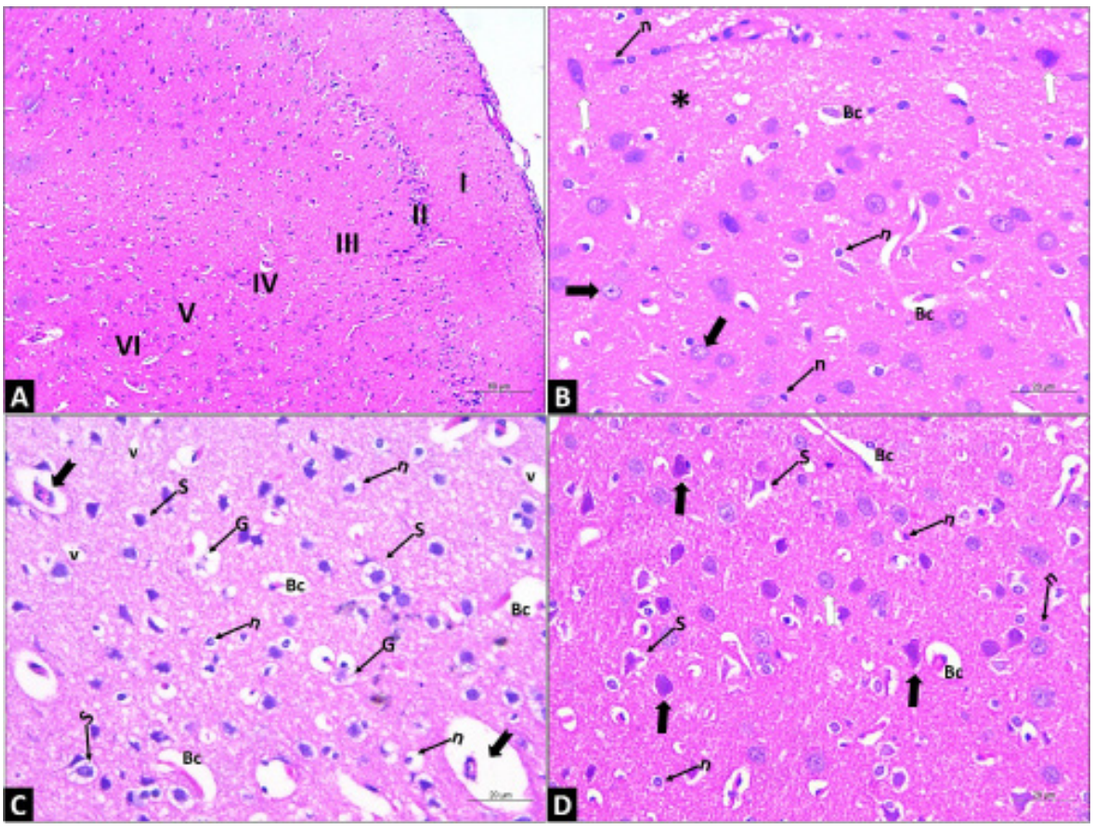

Fig. 1: Representative hematoxylin and eosin staining of the rat visual cerebral cortex of different groups: Control rats (A) showing the general histological structure of the visual cortex (six layers); outer molecular (I), external granular (II), external pyramidal (III), internal granular (IV), internal pyramidal (V), and polymorphic (VI) (H&E, Scale bar: 80 µm, 200x). (B) The neuropil (asterisk) of the both internal granular and pyramidal layers of visual cortex of the control group showing open face rounded nuclei of pyramidal cells, their cytoplasm was basophilic, and shows long apical dendrites (white arrows). Rounded granular cells (black arrows) with open face nucleus and little cytoplasm could be seen. Different types of neuroglia cells (n) with dark stained nucleus and blood capillaries (Bc) could be seen. (C) The internal granular and internal pyramidal layers of visual cortex of I/R group: shows shrunken neurons (S) with pyknotic nuclei surrounded by clear halos are clearly seen. Some cells appear as ghosts (G) with loss of nuclear details. Notice the dark marginal nuclei (notched arrows) in two acidophilic mass surrounded by clear space. The neuropil shows vacuolations (v) and dilated blood capillaries (Bc). Numerous neuroglia (n): most of them surrounded by a halo could be seen. (D) Beetroot extract + I/R group: both internal granular and pyramidal layers of visual cortex shows pyramidal cells (black arrows), granular cells (white arrows) and neuroglia (n) with almost normal morphology; however, few shrunken pyramidal cells (S) and dilated blood capillaries (Bc) could be detected. (B, C, & D; H&E, Scale bar: 20 µm, 400x).

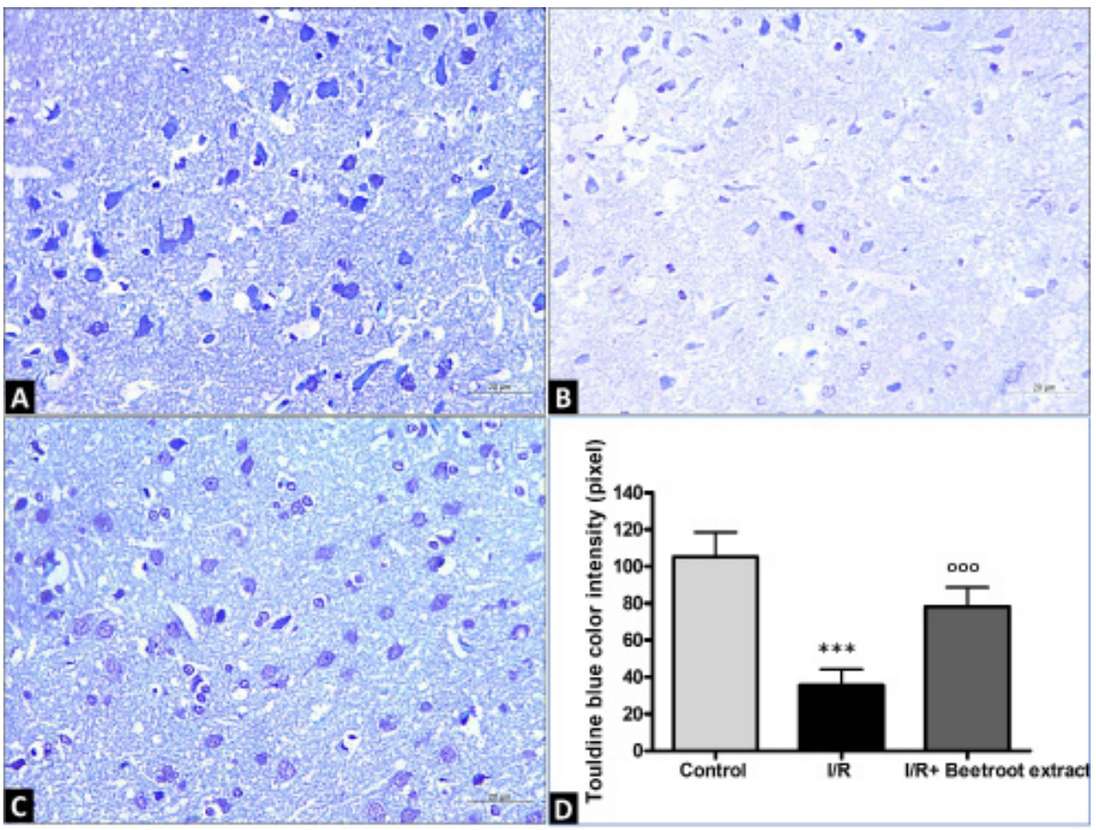

Fig. 2: Representative staining of the rat visual cortex of different experimental groups with toluidine blue: A. Control group: the neuronal cells exit dark blue staining. B. I/R group: comparatively to the control group, neuronal cells had a significantly reduced color intensity. C. Beetroot extract treatment attenuated I/R induced reduction in the toluidine blue color intensity (A, B & C; Toluidine blue, Scale bar: 20 µm, 400x). D. *** $P<0.001$, compared with control group, ᵒᵒᵒ $P<0.001$, compared with I/R group. Statistical analysis.

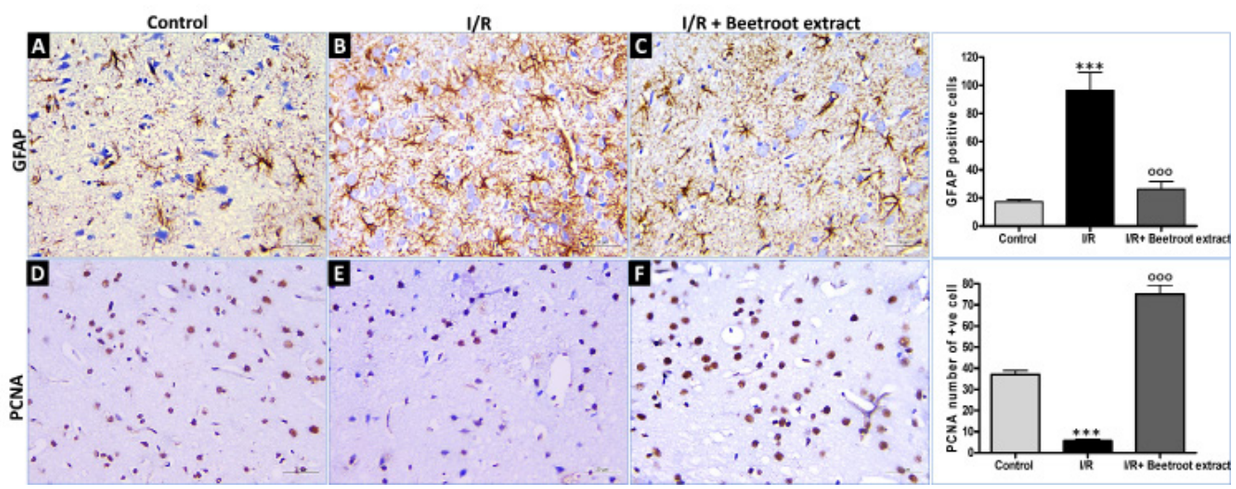

Fig. 3: Immunostained image of the rat visual cortex representative of different experimental groups showing that the Caspase-3 (A-C) and TNF-α (D-F) immunoreactions were downregulated in Beetroot extract +I/R group comparing to the I/R group (Scale bar: 20 µm, 400x). *** $P<0.001$, in comparison with the control group; ᵒᵒᵒ $P<0.001$, compared to the I/R group.

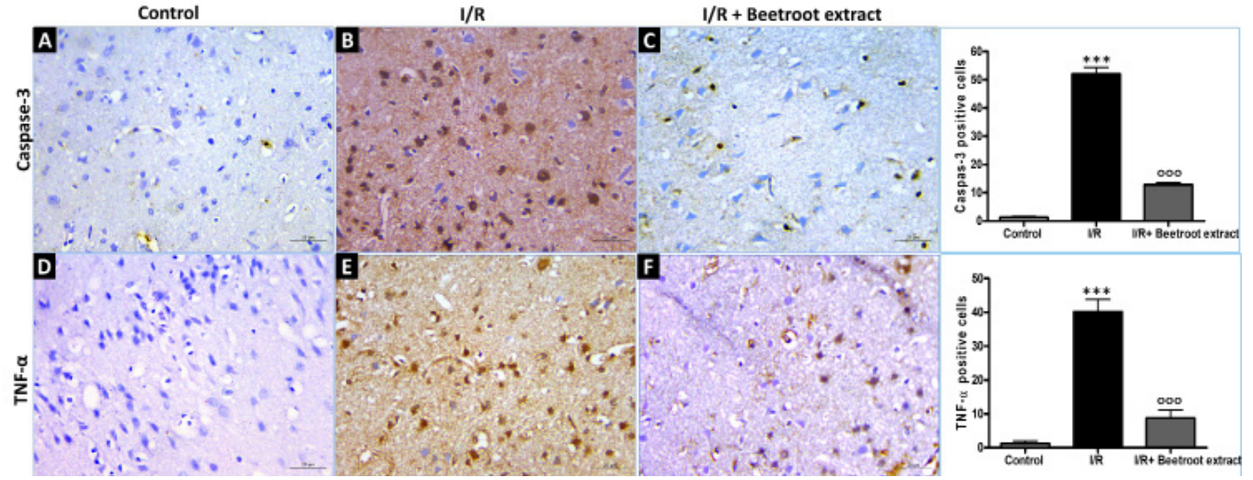

Fig. 4: Immunostained image of the rat visual cortex representative of different experimental groups showing that iNOS (A-C) immunoreaction was downregulated in Beetroot extract +I/R group. However, HSP70 was upregulated (D-F) compared to the I/R group (Scale bar: 20 µm, 400x). ** P<0.01 and *** P<0.001, In comparison with the control group; ° P<0.5 and °°° P<0.001, In comparison with the I/R group.

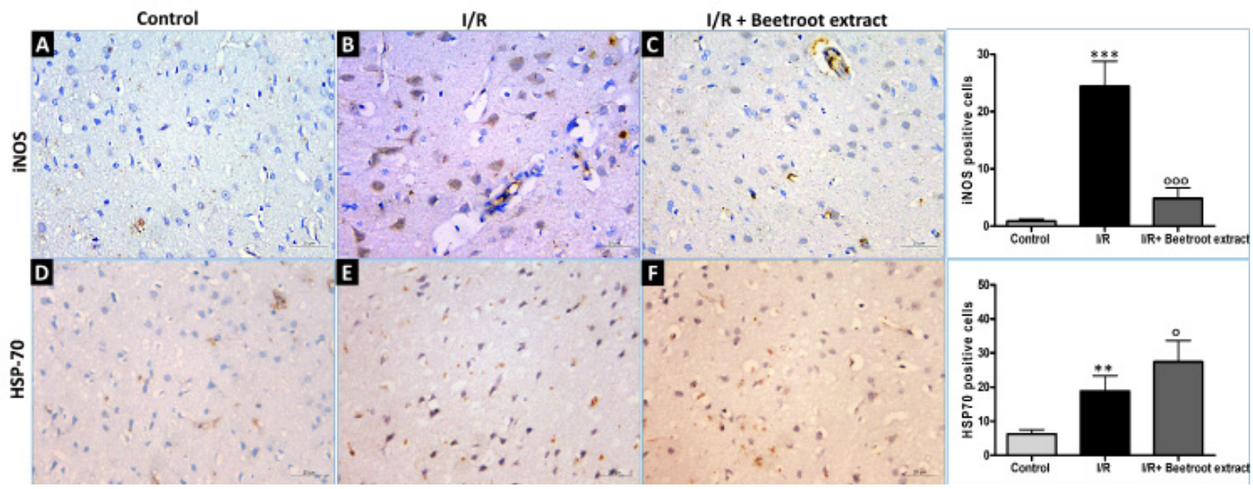

Fig. 5: Immunostained image of the rat visual cortex representative of different experimental groups showing that GFAP (A-C) immunoreactions was downregulated in Beetroot extract + I/R group and PCNA was upregulated (D-F) compared to the I/R group (Scale bar: 20 µm, 400x). *** P<0.001, in comparison with the control group; °°° P<0.001, in comparison with the I/R group.

DISCUSSION

The ischemic cerebral vascular disease (ICVD), which results from insufficient blood flow in the vessels supplying the brain, is a deadly and disabling disease, with males suffering a higher mortality rate than females. Therapies for ICVD, like thrombolytic tissue plasminogen activator, have a narrow therapeutic window. Consequently, development of new therapies is essential to improve ICVD outcome[22].

Due to the poor prognosis associated with ischemic stroke, research is being conducted to investigate protective therapies for its patients to prevent neurological complications[23]. Consequently, the present study was planned to investigate the probable protective effect of beetroot extract on the post-ischemic visual cortex neurodegeneration.

In the existing study, H&E-stained sections of the visual cortex of the I/R group (group III) showed multiple shrunken neurons with dark nuclei surrounded by vacuolated pale areas, others showed ghost like appearance. The neuropil appeared vacuolated and showed vascular dilatation. Small multinucleated acidophilic masses surrounded by a clear space were also noticed. This was consistent with Zhang *et al*. (2020)[24] who demonstrated that I/R injury caused cytotoxic cerebral edema, this was due to damage to blood-brain barrier and inflammatory response. The tissue injury can take the pattern of selective neuronal necrosis or infarction, the later resulting in damage to neurons, glia and blood vessels[25]. In addition, Bassiony *et al*. (2015)[26] explained that dark nuclei can be apoptotic nuclei resulting from numerous degenerated neurons that have fused together.

In the current study, visual cortex sections were exposed to the toluidine blue stain for the assessment of neuronal cell loss. It showed a decrease in the intensity of Nissl's granules in the cytoplasm of the neurons of I/R group compared to the con¬trol group. The same results were reported by Elsayed *et al*. (2021)[23] and Yu *et al*. (2016)[27].

In the present study, apoptotic changes were confirmed by the significant up-regulation of caspase-3 immune reaction in neurons of I/R group. This was in accordance with Ravindran and Kurian (2019)[28] who noticed that after ischemia-reperfusion injury in rat, caspase-3 activity in the brain was significantly elevated by 24 hours. Also, Zhang *et al*. (2020)[24] reported that in the I/R-injured rat brain, caspase-3 expression was significantly increased. This was explained by Liu *et al*. (2013)[29] who stated that injury caused by ischemia/reperfusion increased caspase-3 activity or increased its expression, which resulted in neuronal apoptosis.

In the present work, HSP-70 and TNF-α immunohistochemistry showed a statistically significant increased expression in the visual cortex of the I/R group. This was in agreement with Tirapelli *et al*. (2010)[30] who reported increased expression in the HSP70 after experimental ischemia suggesting its involvement with cerebral ischemia. The heat shock protein family is induced in the brain by various insults such as excitotoxin exposure, elevated body temperature, trauma, and cerebral ischemia[31]. Experimental studies have shown that HSP70 protects the brain and brain cells from traumatic brain injury, cerebral ischemia and other insults to the brain[32]. Furthermore, HSP70 has been shown to play a crucial role in cytoprotection, by inhibiting apoptosis and regulating immune responses[33].

Tumor necrosis factor alpha is a cytokine that is produced by the brain and systemically[34]. TNF-α is essential for acute inflammatory responses, and it is thought to exacerbate strokes[35]. Blood and cerebrospinal fluid levels rise immediately after a stroke[36]. The same finding was reported by Zhao and Xu (2020)[37] who observed increased levels of TNF-α in the serum of cerebral I/R injury rat model.

Oxidative stress is an imbalance between ROS production and antioxidant defenses leading to oxidative damage[38]. In view of cerebral oxidative stress, in the present study, a striking increase in the oxidant molecule nitric oxide (NO) was noticed in the I/R group. This was in accordance with Gürsoy-Özdemir *et al*. (2000)[39] who supported the hypothesis that along with the neuronal NO deleterious effect during ischemia, NO generation during reperfusion has a significant role in the development of reperfusion injury, possibly by producing peroxynitrite.

Quantitative analysis of GFAP immunoreactive neurons exhibited a significant increase in the number of GFAP immunoreactive neu¬rons in I/R group. Previous studies showed that damaged neurons induce astrogliosis[40]. This agrees with Elsayed *et al*. (2021)[23] who reported a significant increase in active GFAP-positive cells in the dentate gyrus in CCAO rats. In addition Zhao *et al*. (2018)[41] demonstrated that 28 days after I/R injury, astrocytes in the boundary zone had increased and hypertrophied and there was a glial scar surrounding the ischemic core area. Choudhury and Ding (2016)[42] stated that one important event that occurs during the post-ischemic stage is reactive astrogliosis and glial scar formation.

The proliferating cell nuclear antigen [PCNA] is a histochemical marker of cellular regeneration[43]. Originally, PCNA was detected in nuclei of cells throughout DNA synthesis[44] and during the process of repairing DNA damage[45]. In the present work, the level of PCNA immunoreactivity was significantly low in I/R group compared to control rats. This was in contrary with Dief *et al*. (2018)[46] who reported the proliferation of brain tissue in peri-infarct zones of middle cerebral artery occlusion (MCAO) rats, which was correlated with blood vessels sprouting. Furthermore, PNCA and Ki-67 immunostaining was detected in several cells in the lesion, indicating new proliferation of several cell types. However, Dief *et al*. (2018)[46] assumed that functional recovery of rats following MCAO was not attributable to neuronal regeneration, but rather to the fact that brains of rats, particularly that of young rats used in the study, is able to life-long plasticity. They also recommended additional studies be conducted in order to determine whether certain neurotrophic factors may increase the morphological or the functional recovery process.

In our study the beneficial effect of beetroot extract against cerebral ischemia/reperfusion injury was examined based on histological and immunohistochemical assessment. Sections of group IV visual cortex stained with H&E revealed improvement in nerve cells. Utmost of the pyramidal, granular and neuroglia cells were as that of the control group. This agrees with Albasher *et al*. (2020)[47] who found that pretreatment with red beetroot extract improved the changes in the brain's histopathology induced by organophosphorus insecticides. In addition, Hajihosseini *et al*. (2017)[48] showed that by enhancing cholinergic activity in the brain, scopolamin-induced neuronal damage and memory dysfunction were improved by beetroot leaf extract. In addition, beta vulgaris leaf extract was able to enhance cholinergic neurotransmission in rats that have diminished memory[49]. Our study proved that beetroot extract protected the visual cortex against cerebral ischemia/reperfusion injury via down-regulation of caspase-3, TNF-α, iNOS and GFAP and up-regulation of HSP70 and PCNA immunoreaction within the brain tissue. Therefore, via the previous mechanisms, beetroot extract protected the visual cortex through its antioxidant, anti-apoptotic and anti-inflammatory effects.

In the existing study, beetroot extract decreased caspase-3 expression in the cortical tissue. This agrees with Albasher *et al*. (2020)[47] who reported that beetroot extract downregulated the levels of caspase-3, Bax and

cytochrome c in chlorpyrifos intoxicated rats' brain tissue. In addition, El Gamal *et al*. (2014)[18] revealed that beetroot extract inhibited NF-κB activation, NF-κB DNA binding and kidney tubular apoptosis/necrosis conferred by renal damage caused by gentamycin. Moreover, beetroot extract administration in group IV resulted in up-regulation of HSP70 compared with group III. Ravagnan *et al*. (2001) [50] demonstrated that when HSP70 is overexpressed, events occurring downstream of caspase activation can be hindered. In our study, beetroot extracts appeared to facilitate the inhibition of apoptosis through the upregulation of HSP70.

In the current study, an immune response was induced in the brain following cerebral ischemia/reperfusion as evidenced by increases in TNF-α levels in the visual cortex. Beetroot extracts and their active compounds, betalains, perform anti-inflammatory functions facilitated by the signaling of proinflammatory cytokines[17]. In addition, beetroot treatment inhibited TNF-α and IL-6 induced by gentamicin in the renal tissue by inactivating NF-κB[18].

Among all body organs, the brain's low antioxidant molecule level, high lipid content, and high oxygen consumption closely correlate with the progression of oxidative stress conditions[51]. Due to its antioxidant content, especially betalain[17], beetroot extract decreased iNOS levels in the visual cortex of rats in group IV. A variety of free radicals can be eliminated by beetroot's antioxidant properties[52]. The ability of beetroot to activate nuclear factor (erythroid-derived 2)-like 2, which in turn activates antioxidant enzyme genes, has been attributed to the increase in antioxidant enzyme levels[53].

This was in correlation with Matias *et al*. (2016)[54] who demonstrated that flavonoids target astrocytes and encourage the release of brain-derived neurotrophic factor (BDNF) and inhibit the release of glial fibrillary acidic protein (GFAP) in the central nervous system. This may elucidate the capability of beetroot extract to reduce GFAP levels following cerebral I/R injury in the current study.

The present study showed a significant up-regulation of PCNA expression in group IV compared to group III. This was in accordance with Cho *et al*. (2017)[55] who observed significant increase in Ki-67 expression following beetroot treatment, hematopoietic cell proliferation index, positive cells in irradiated mice. They concluded that administration of beetroot extract with γ-ray radiation can lower DNA damage as well as lead to increased proliferation and stimulation of hematopoietic progenitor cells, which suggests that beetroot has protective effects against radiation damage.

CONCLUSION

Beetroot extract protects the visual cortex from oxidative stress, inflammation, and apoptosis resulting from I/R damage. It provides an experimental base for the application of beetroot extract in the treatment of cerebral ischemic diseases and would serve as a theoretical basis for further development. However, a future study has to investigate the precise molecular mechanisms of beetroot's neuroprotective effects.

CONFLICT OF INTERESTS

There are no conflicts of interest

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الملخص العربى

التأثير الوقائي العصبي المحتمل لمستخلص الشمندر الأحمر على إصابة القشرة البصرية التي يسببها نقص / إعادة التروية الدماغية في الجرذان البيضاء

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مقدمة: تعتبر السكتة الدماغية في جميع أنحاء العالم من بين الأسباب الرئيسية للوفاة والعجز. أكثر أنواع السكتات الدماغية شيوعًا هو الإقفار الدماغي. السكتات الدماغية لها تأثير كبير على الرؤية. يمكن أن يكون فقدان البصر أسوأ تأثير متبقي بعد احتشاء دماغي. أصبحت الخصائص المعززة للصحة في جذر الشمندر الأحمر معروفة على نطاق واسع في السنوات الأخيرة. بالإضافة إلى خصائصه المضادة للأكسدة القوية ، فهو أيضًا مضاد للالتهابات ووقائي للأوعية الدموية.

الهدف من الدراسه: الغرض من هذه الدراسة هو استكشاف الآليات الوقائية لمستخلص الشمندر الاحمر ضد نقص/ إعادة التروية في القشرة البصرية للجرذان البيضاء.

المواد والطرق: في دراستنا، تم استخدام أربعين جرذًا أبيض. تم تقسيمها إلى أربع مجموعات المجموعة الأولى (المجموعة الضابطة)، المجموعة الثانية (مستخلص الشمندر الأحمر)، المجموعة الثالثة (مجموعة نقص/إعادة التروية) والمجموعة الرابعة (مستخلص الشمندرالأحمر + نقص/إعادة التروية) بنهاية التجربة، تم استخلاص القشرة البصرية واخضاعها للتحليلات النسيجية والهيستوكيميائية مناعية والمورفومترية.

النتائج: خفف مستخلص الشمندر الأحمر من التغيرات التنكسية العصبية الناتجة عن نقص تروية القشرة البصرية. أظهرت الخلايا العصبية زيادة في عدد حبيبات نيسل. انه يحمي القشرة البصرية ضد نقص /إعادة التروية الدماغية عن طريق نقص caspase-3 و TNF-α و iNOS و GFAP وزيادة HSP70 و PCNA .

الخلاصة: وفقًا لنتائج الدراسة الحالية، قد يكون مستخلص جذر الشمندر عاملاً محتملاً في الحماية العصبية لنقص/إعادة التروية للقشرة البصرية. يحمي القشرة البصرية من الإجهاد التأكسدي والالتهاب وموت الخلايا المبرمج الناتج عن نقص/إعادة التروية يوفر أساسًا تجريبيًا لتطبيق مستخلص الشمندر في علاج أمراض نقص تروية الدماغ.