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ERK activation by zeranol has neuroprotective effect in cerebral ischemia reperfusion

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ABSTRACT

Aims: Incidence of stroke increases in postmenopausal women with dangerous consequences. In this study we used zeranol to protect ovariectomized (OVX) rats against cerebral I/R damage and our target is to identify the mechanism of its protection, in addition to investigating whether this mechanism inhibits inflammation (by preventing glial cell activation) and apoptosis.

Main methods: First 18 ovariectomized rats were allocated into 3 groups: I/R group, zeranol + I/R group and U0126, MEK1/2 inhibitor + zeranol + I/R group. After 24 h reperfusion, protein expression of total extracellular signal-regulated protein kinase (t-ERK1/2), phosphorylated extracellular signal-regulated protein kinase (p-ERK1/2), Bcl-2, and Bax were quantified.

Second 36 female rats were allocated into 3 groups: sham group, I/R group (after ovariectomy by 7 weeks, rats exposed to cerebral I/R) and zeranol group (after ovariectomy by 2 weeks, rats received zeranol for 5 weeks). After 24 h of reperfusion, the following parameters were measured; total nitrate/nitrite, interleukin-10, myeloperoxidase, caspase-3, and finally immunohistochemistry analysis of glial fibrillary acidic protein, cyclooxygenase-2 in cortex and hippocampus (CA1) regions were performed.

Key findings: U-0126 administration reversed the neuroprotective effect induced by zeranol through decreasing ratio of p-ERK1/2:ERK1/2 and Bcl-2/Bax in brain tissue.

Activation of ERK signaling pathway by zeranol caused reduction in brain apoptosis and inflammation.

Significance: Zeranol showed protective effect in OVX rats that were exposed to cerebral I/R by activation of ERK signaling pathway which was blocked by U0126. This protective effect in turns led to decrease inflammation and apoptosis.

1. Introduction

Cerebral ischemia leads to brain cell damage and aggravated when restoration of blood supply is done, and this is pointing to ischemia/reperfusion (I/R) damage [1]. Injury that results from cerebral I/R is due to multiple events. Mainly is owing to the lack of two main substrates (glucose and oxygen) from blood flow during ischemia that in turn results in neuronal death. Injuries following cerebral I/R are correlated with depletion of energy, rise of Ca^{2+} level, increase level of glutamate, production of free radicals, increase in inflammatory responses, and apoptosis enhancement [2].

Cerebral I/R injury causes neuroinflammation, in which microglial and astroglial are activated, in addition to liberation of cytotoxic substances such as inflammatory cytokines, matrix metalloproteinases, nitric oxide and reactive oxygen species that lead to disruption of the blood–brain barrier integrity and finally neuronal cell death [3]. Harmed cells cause secretion of more toxic agents, which trigger activation to the immune system. Therefore, sustained inflammation causes aggravation of brain damage by this endless cycle [4].

It was previously reported that pre-menopausal women are more protected against stroke than men of identical oldness [5]. Ischemia induction in ovariectomized animal, for instance the middle cerebral artery occlusion (MCAO), caused alleviation of damage that is attenuated by exogenous estrogen treatment [6]. Previous studies documented that estrogens have neuroprotective effect in various ischemia models. 17-β-estradiol (E2) has neuroprotective effect on stroke animal models and neurodegenerative diseases [7]. E2 exhibits this protective effect by several pathways such as decreases generation of reactive oxygen species either by directly acting as radical scavenging or indirectly acting through increasing expression of antioxidant factors, decreases inflammation, decreases apoptosis by regulation apoptotic factors as Bcl-2, regulating growth factor, and vascular modulation [8].

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Thus, ovariectomy will cause aggravation of dangerous consequences of cerebral I/R due to deprivation of protective effect of E2 on brain.

Phytoestrogens are akin to estrogen molecules that are abundant in many plants. They can selectively react with estrogen receptors (ERs) and cause expression of gene in estrogen response elements manner [9]. In addition to binding to ERs it has strong antioxidant activity. Dietary intakes of phytoestrogens enhance attenuation of injuries in rats after focal [10] and global ischemia [11].

A phytoestrogen, zeranol has been considered as a possible alternative for estrogen [12], which produced from reduction of zeranolenone [13]. It was found that zeranol inhibits bone loss in ovariectomized rats [14]. The cardiovascular protective effect of zeranol is confirmed, but the reports on the effect of zeranol on the nervous system are limited [12].

One of the members of the mitogen-activated protein kinase (MAPK) family is P42/44 extracellular signal-regulated protein kinase (ERK1/2) which has a significant role in cerebral ischemia. E2 supplementation decreases neuronal death caused by glutamate toxicity, while ERK signaling pathway blockage inhibits this neuroprotective effect. All studies suggested that the stimulation of ERK signaling pathway possibly will be the way of neuroprotection against ischemia [15]. Accordingly, we tried to inspect the possible role of ERK1/2 in the protective action of zeranol against cerebral I/R in OVX rat, in addition to detecting role of ERK in preventing inflammation (through inhibit glial cell activation) and apoptosis.

2. Materials and methods

2.1. Drugs and chemicals

Zeranol and U0126 (selective inhibitor of MEK1/MEK2) were obtained from Sigma Aldrich (St Louis, MO, USA). The used chemicals were of high analytical grade.

2.2. Animals

Female wistar rats (180–200 g) (8–10 weeks) were provided from the breeding unit of the Egyptian Organization of Biological products and Vaccines (Helwan, Egypt). They were caged beneath controlled conditions with free access to water and standard pellet diet (commercial available). They were habituated for a week before experiment. Animal handling and experimental protocol were accepted by the ethics committee of scientific research, Faculty of Pharmacy, Helwan University (Protocol Number: 003A-2018).

2.3. Surgical techniques

2.3.1. Ovariectomy surgery

After removing the fur from the surgical area of anesthetized rats (chloral hydrate, 360 mg/kg), it was sterilized with antiseptic. A median cut was done in abdomen, the ovaries were detected and tied with silk thread and then were dissected bilaterally [16]. Termination of oestrous cycle was assured by taking of vaginal smudge. Sham-operated group rats exposed to the same surgery technique unless ovaries dissection.

2.3.2. Exposure to cerebral I/R

OVX rats were sedated (360 mg/kg, chloral hydrate, i.p), then a ventral median cut was done in the neck to obtain the both common carotid artery after isolation from surroundings. Ischemia was initiated by ligating of the arteries for 10 min. Then, the arteries were deligated for circulation reestablishment (reperfusion for 24 h). Sham-operated group was exposed to the same surgical conditions unless artery ligation. Rectal temperature was kept at 37 ± 0.5 °C during experiment to avoid cerebral hypothermia [17].

2.4. Experimental design

2.4.1. First experiment

First experiment is interested in determining the effect of U0126 for recognizing the benefit of ERK signaling pathway in the neuroprotection caused by zeranol. The rats underwent ovariectomy and then haphazardly allocated in to 3 groups after 2 weeks of ovariectomization, 6 for each group.

Group 1: I/R group (OVX + I/R) in which rats received only vehicle for zeranol and U0126 for 5 days before I/R.
Group 2: ZER group (OVX + ZER + I/R) in which rats received zeranol (0.5 mg/kg, i.p, dissolved in olive oil) + vehicle of U0126 (DMSO) for 5 days before I/R.
Group 3: U0126 group (OVX + U0126 + ZER + I/R) in which rats recieved U0126 (0.5 mg/kg, by i.p) [18], 30 min prior to zeranol (0.5 mg/kg, i.p) [12] for 5 days before I/R.

Following 24 h reperfusion, rats' head were amputated, afterward brains were obtained, then washed with ice cold saline and were kept at − 80 °C for western analysis technique of p-ERK1/2,t-ERK1/2,Bcl-2 and Bax. Each sample (brain) was homogenized individually.

2.4.2. Second experiment

Second experiment is interested in detecting whether the neuroprotective effect of zeranol treatment through ERK activation causes prevention of inflammation by inhibiting activation of glial cell and apoptosis against cerebral I/R injury in OVX rats. Rats were haphazardly allocated into three groups, 12 for each group.

Group 1: (sham group): rats underwent the same surgical procedure without removal of ovary or occlusion of arteries, they injected vehicle of zeranol for 5 weeks (every 3 days).
Group 2: (OVX + I/R): after 2 weeks of ovariectomization, rats injected vehicle of zeranol for 5 weeks (every 3 days) then, subjected to cerebral I/R injury.
Group 3: (OVX + ZER + I/R): after 2 weeks of ovariectomization, rats injected with zeranol (0.5 mg/kg, i.p, dissolved in olive oil) [12], for 5 weeks (every 3 days), then exposed to cerebral I/R injury 24 h after last dose.

Following 24 h of reperfusion, rats' head were amputated, afterward brains were obtained, washed with ice cold saline then divided into two groups, one (n = 6 brains) was homogenized in phosphate buffer to get 10% homogenates then centrifuged, and supernatants kept at -80° C for biochemical analysis. While the other group (6 brains), was fixed in 10% neutral formalin for detecting glial fibrillary acidic protein (GFAP) & cyclooxygenase-2 (COX-2) protein expression by immunohistochemistry analysis.

2.5. Parameters

2.5.1. Determination of NOx, IL-10, MPO and CASP-3 in brain homogenates

Commercial kits were used for detecting the brain tissue levels of Total nitrate/nitrite (NO_X) (Cayman Chemical, USA, Cat. # 780001), interleukin-10 (IL-10) (Cloud-Clone Corp., USA, Cat. # SEA056Ra), myeloperoxidase (MPO) and caspase-3 (CASP-3) (CUSAIBO, USA, Cat. # CSB-E08722r; CSB-E08857r respectively). The above-mentioned parameters were measured according to the mentioned manufacturers' instructions. All kits are ELISA kit except NO_X kit is colorimetric.

2.5.2. Protein estimation

Lowry et al., 1951 [19] manner was used in detecting proteins.

2.5.3. Immunohistochemisty analysis

Immunohistochemistry analysis was used for detecting expression of COX-2 and GFAP. Section of 5 μm thickness was cut from brain tissue which was embedded in paraffin block. Sections were dewaxed and deepen in 3% H_2O_2 in methanol. Nonspecific binding sites were inhibited and then incubated at room temperature with GFAP Mouse monoclonal antibody - ready to use (Cat. # MS-280-R7), and COX-2 Rabbit polyclonal antibody (1:100 Cat. #RB-9072-R7) (Thermo Fisher Scientific, Waltham, MA, CA, USA) for 60 min, afterward sections were incubated with secondary antibody HRP Envision kit (DAKO) for 20 mins; followed by 10 min incubating with diaminobenzidine (DAB) for visualization. Finally, counter staining was done with haematoxylin, followed by dehydrating and clearing by xylene, then cover slipping for microscopic examination.

Positive cells intensity of cortical and hippocampal (CA1) GFAP& COX-2 were evaluated by Leica Qwin 500 (LEICA, Image system Ltd., Cambridge, UK). Randomly, five fields from each slide were surveyed then mean was obtained, and expressed as optical density.

2.5.4. Western blotting analysis

Western blot analysis was used for detection p-ERK1/2, t-ERK1, 2, Bcl-2 and Bax. Proteins were extracted from brain tissues by RIPA lysis buffer and Brad-fort protein assay kit (Bio Basic Inc., Canada). Proteins samples were alienated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and then transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were incubated with rabbit primary poly-clonal antibodies ERK1/2 (1:1000, Cat. # 61-7400), p-ERK1/2 (1:1000, Cat. # 44-680G), Bcl-2 (1:1000, Cat. # PAS-11379) and Bax (1:50, Cat. # PA5-38933) (Thermo Fisher Scientific, Waltham, MA, CA, USA) overnight at 4 °C. After washing, secondary polyclonal antibody attached with horseradish peroxidase (1:1000; Novus Biological, Littleton Co, USA) was incubated at room temperature with membranes for 1 h. The band compactness of the aimed proteins after normalization by beta actin was detected by using enhanced chemiluminescence detection system (Bio-Rad, Laboratories, Inc., Hercules, CA, USA) and the band intensity was detected and imaged by Chemi Doc MP Imager.

2.6. Statistical analysis

Statistically analyses were carried out using one way ANOVA followed by Tukeys' multiple comparison tests as post hoc test. GraphPad Prism software, version 5 was used for statistical analysis and graphic illustrations of data, which represented as mean ± standard deviation.

Statistically significant was considered at $p < 0.05$.

3. Results

3.1. U0126 inhibited neuroprotection induced by zeranol by decreasing relative expression ratio of p-ERK1/2 to t-ERK1/2

The relative expression ratio of p-ERK1/2 to t-ERK1/2 was significantly increased in brain tissue of zeranol group 4 fold in comparison with I/R group. In U0126 treated group, the expression ratio of p-ERK1/2 to t-ERK1/2 was significantly reduced in brain tissue by 58% in comparison with zeranol group (Fig. 1a, b).

3.2. U0126 inhibited neuroprotection induced by zeranol by decreasing relative expression ratio of Bcl2/Bax

A significant increase was observed in the relative expression ratio of Bcl2/Bax in brain tissue of zeranol group 46 fold in comparison with I/R group. In U0126 treated group the relative expression ratio of Bcl2/Bax was decreased significantly by 87% in comparison with the zeranol treated group (Fig. 2a, b).

3.3. Zeranol reduced cerebral elevation of NO_X level

NO_X level in brain tissues was remarkably elevated in I/R group by 134%. Despite, zeranol pretreatment caused a remarkable lessening in the level of NO_X in brain by 29% (Fig. 3).

3.4. Zeranol decreased cerebral elevation of MPO and IL-10 levels

As illustrated in Table 1, the level of MPO of I/R group was raised 2 fold in comparison with sham group. Moreover, the level of IL-10 in brain tissue was reduced by 32% in comparison with sham group. Even though, pretreatment with zeranol reversed these results by significantly decreasing level of MPO by 38% and significant increasing level of IL-10 by 22% in comparison with I/R group.

3.5. Zeranol decreased cortical and hippocampal expression of GFAP

I/R group caused a significant increase in the cortical and hippocampal (CA1) protein expression of GFAP by 182%, 181% respectively in comparison with sham group. On other hand, the cortical and hippocampal (CA1) protein expression of GFAP in zeranol group were reduced significantly by 40%, 35% respectively in comparison with I/R group (Fig. 4a, b, c).

3.6. Zeranol reduced cortical and hippocampal expression of COX-2

The cortical and hippocampal (CA1) protein expression of COX-2 were raised significantly in I/R group 1.2, 3.3 fold respectively in comparison with the sham group. However, the cortical and hippocampal protein expression of COX-2 in zeranol group were significantly reduced by 92%, 95% respectively in comparison with I/R group (Fig. 5a, b, c).

3.7. Zeranol decreased cerebral level of CASP-3

The brain level of CASP-3 of I/R group was remarkably increased 4.5 fold in comparison with sham group. Even though zeranol preteatment caused a noteworthy decrease in the brain level of CASP-3 by 36% in comparison with I/R group (Fig. 6).

4. Discussion

ERK1/2 is the main kinase in the signaling pathway of neuronal survival or apoptosis after cerebral ischemic injury. It also works as an early sensor of cellular stress response. It was documented that the neuroprotective effect of estrogen is principally due to activation of ERK1/2 signaling pathway [15].

In zeranol group, the phosphorylation level of ERK1/2 and Bcl-2/Bax ratio significantly increased in comparison with I/R group and these results were reversed by administration of U0126 prior to zeranol. The results agree with the study of [15] who stated that genistein as a phytoestrogen increased phosphorylation level of ERK1/2, Bcl2/Bax ratio and this was reversed by administration of U0126.

Brain ischemia inhibits phosphorylation and activation of ERK1 and its downstream cascade, sequence DNA binding factor (c-AMP response element binding protein (CREB)), which leads to decrease expression of Bcl-2 and causes stimulation death flow via caspase. While estradiol treatment in global ischemia notably upregulates phosphorylation of ERK, activation of CREB, increase expression of Bcl-2 and inhibit activation of CASP-3 [20,21] and this is similar to effect of zeranol as shown in our previous study of [22].

Cerebral I/R injury causes mitochondrial dysfunction which leads to increase production of reactive oxygen species (ROS) [23]. Oxidative stress that results from cerebral I/R leads to initiation of inflammatory pathway by activating glial cells (microglial, astrocyte). This consequently causes increase in the level of pro-inflammatory cytokines,

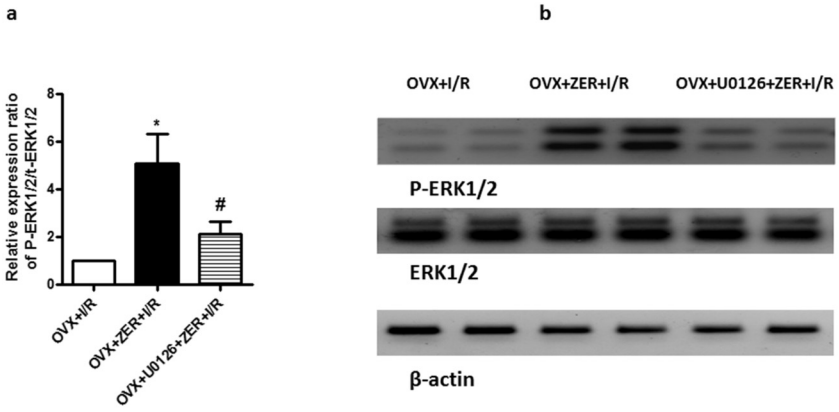


Fig. 1. (a): Effect of U0126 on neuroprotection induced by zeranol against cerebral I/R injury in OVX rats by reducing relative expression ratio of p-ERK1/2 to t-ERK1/2 after normalization to crossponding beta actin.
(b): Representative Western blots of p-ERK1/2, t-ERK1/2 and β-actin in brain following cerebral I/R injury in OVX rats showed increased phosphorylation level of ERK1/2 in zeranol group while injection U0126 before zeranol block phosphorylation of ERK1/2.
Data demonstrated by way of mean (n = 6) and S.D.
* is set against I/R group, # is set against ZER group, ($p < 0.05$).
I/R: ischemia/reperfusion, OVX: ovariectomized, ZER: zeranol.

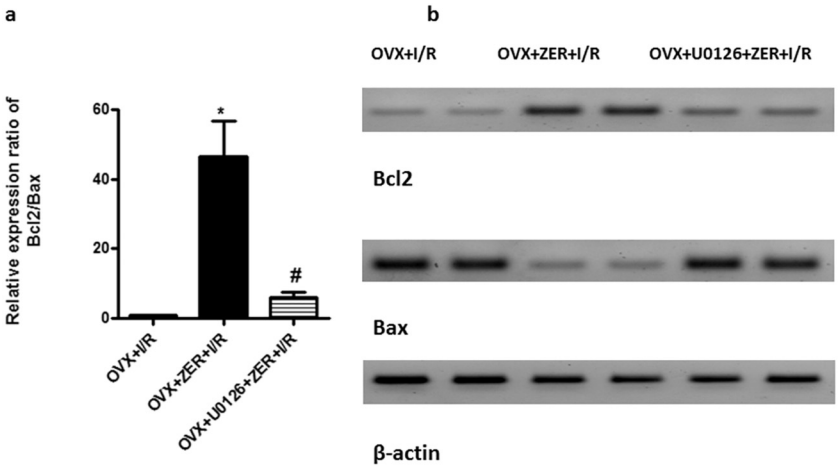


Fig. 2. (a): Effect of U0126 on neuroprotection induced by zeranol against cerebral I/R injury in OVX rats by reducing relative expression ratio of Bcl2/Bax after normalization to the crossponding beta actin.
(b): Representative western blots of Bcl-2, Bax and β-actin in brain following cerebral I/R injury in OVX rats showed increase expression of Bcl-2 and decrease expression of Bax in zeranol group while injection U0126 before zeranol reversed this action.
Data demonstrated by way of mean (n = 6) and S.D.
* is set against I/R group, # is set against ZER group, ($p < 0.05$).
I/R: ischemia/reperfusion, OVX: ovariectomized, ZER: zeranol.

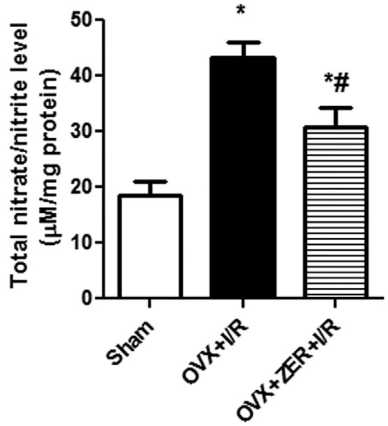


Fig. 3. The injurious effect of cerebral I/R in OVX rats on level of total nitrate/nitrite (NO_X) level was reversed by zeranol.
Data demonstrated by way of mean (n = 6) and S.D.
* is set against I/R group, # is set against ZER group, ($p < 0.05$).
I/R: ischemia/reperfusion, OVX: ovariectomized, ZER: zeranol.

augmentation of leukocyte infiltration to the injured area through up-regulating expression of adhesion molecules, and increase expression of COX-2, iNOS, GFAP that cause both necrotic and apoptotic cell death [24–26].

Biosynthesis of nitric oxide is a chief phase in pathophysiological response of the cerebral ischemia and its level augments throughout ischemia [27,28].

Our result is similar to the finding of [29] who stated that I/R in OVX rats caused a remarkable increase in the brain level of NO due to I/R injury which is aggravated by deprivation of endogenous estrogen.

Table 1
The injurious effect of cerebral I/R in OVX on levels of MPO and IL-10 was reversed by pretreatment with zeranol.

Parameters	Groups		
	Sham	OVX + I/R	OVX + ZER + I/R
MPO (ng/mg protein)	4.350 ± 0.303	14.410 ± 1.555[a]	8.900 ± 0.963[a,b]
IL-10 (Pg/mg protein)	42.490 ± 2.129	28.720 ± 1.767[a]	35.060 ± 1.098[a,b]

Data demonstrated by way of mean (n = 6) and S.D.
I/R: ischemia/reperfusion, MPO; myeloperoxidase, OVX: ovariectomized, ZER: zeranol.
[a] Is set against I/R group.
[b] Is set against ZER group, ($p < 0.05$).

We observed in the current study an increase in MPO level and this is similar to previous studies in different ischemia models as [30,31]. MPO is an enzyme found in the granules of neutrophils [32] and the increase in its activity in ischemic animals is a sign of leukocyte infiltration [33].

COX-2 is induced in case of inflammation such as ischemia. Cerebral ischemia injury causes overexpression of COX-2 in neurons, glial cells, and infiltrating leuckocytes [30]. The current study showed significant upsurge in expression of the COX-2 in cortex and hippocampus regions. Also, we observed that IL-10 level was reduced in I/R group similar to the result of [31].

Brain homeostasis is maintained by astrocytes which synthesize glial fibrillary acidic protein (GFAP). It is a protein which is involved in

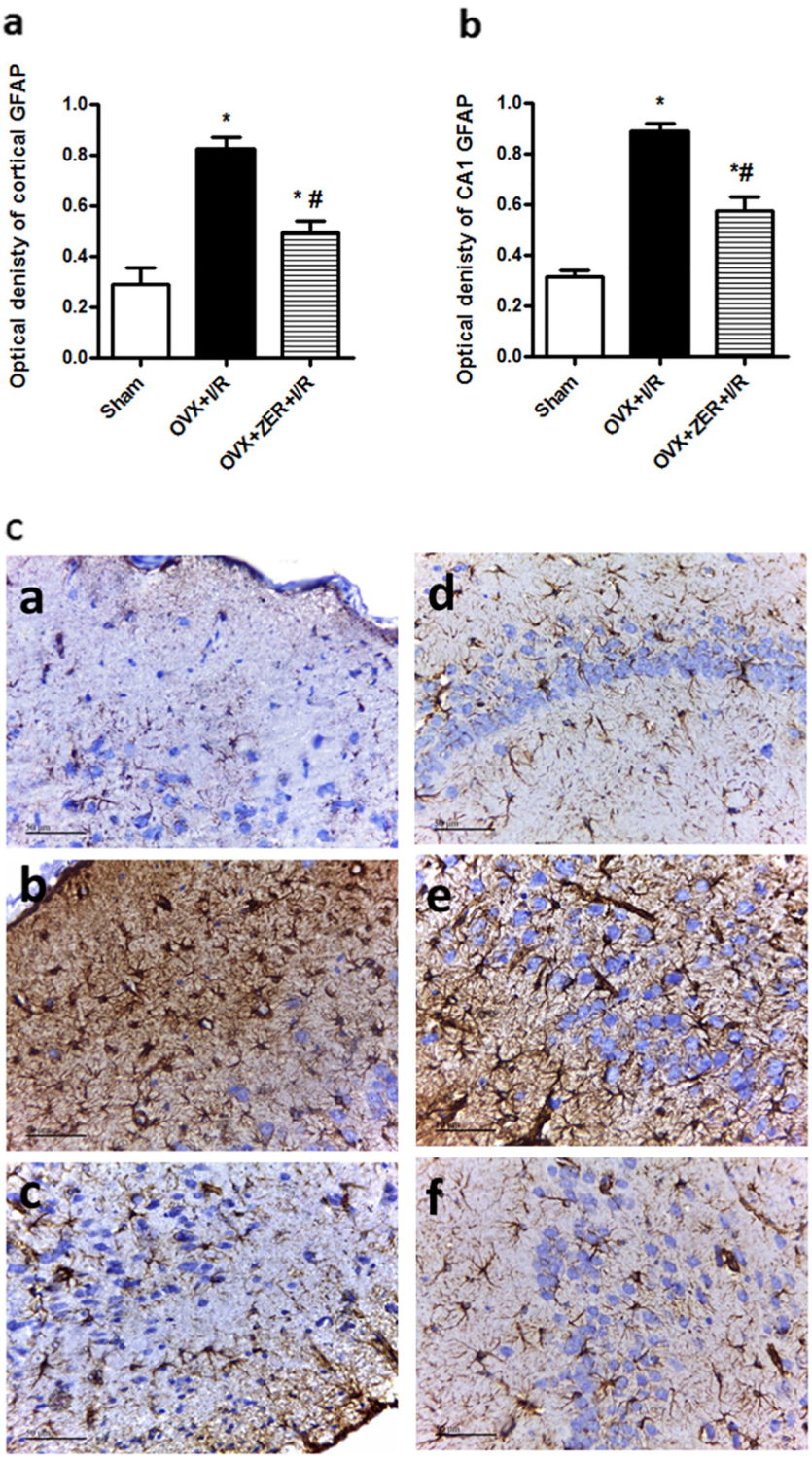


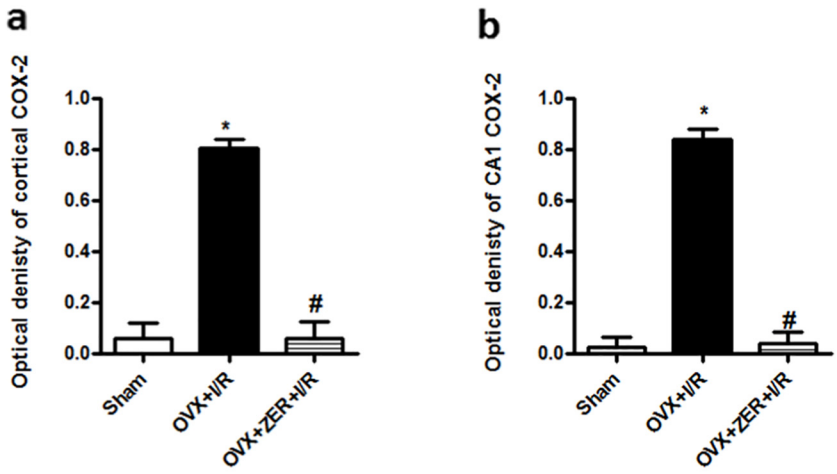
Fig. 4. (a): The injurious effect of cerebral I/R in OVX rats on cortical expression of GFAP cortical was reversed by zeranol pretreatment.
(b): The injurious effect of cerebral I/R in OVX rats on hippocampal expression of GFAP was reversed zeranol pretreatment on GFAP in hippocampus (CA1) following cerebral I/R in OVX rats.
(c):Representative photos for GFAP presenting elevation in the expression of GFAP in areas of cortex and hippocampus (CA1) of I/R group (b, e) against sham group (a, d), despite zeranol pretreatment revealed depression in the expression of GFAP in both areas (c, f) in comparison with I/R group (x40), scale bar = 50 μm.
Data were demonstrated by way of mean (n = 6) and S.D.
* is set against I/R group, # is set against ZER group, ($p < 0.05$).
GFAP: glial fibrillary acidic protein, I/R: ischemia/reperfusion, OVX: ovariectomized, ZER: zeranol.

differentiation of astrocytes from other neuronal cells [35]. In our model we found that expression of GFAP was increased which agrees with the results of [4,31,36].

Caspase-3 is an executional apoptotic effector which is activated after ischemia accompanied with continuous elevation following reperfusion [37]. Activated caspase by ROS has a role in prompting apoptosis by causing nuclear damage [38]. In cerebral ischemia or cerebral I/R, inflammatory agents up-regulated by brain tissue, also cause Caspase-3 activation and finally lead to apoptosis [39].

The current study showed noteworthy increase in the level of caspase-3 in I/R group and this agrees with the result of [34,39].

Besides antioxidant effect of zeranol [40], we found in our study that zeranol causes activation of ERK signaling pathway which up-regulates Bcl-2 expression in brain tissue after cerebral I/R. This causes increase in the stabilization of mitochondria which results in a reduction of free radical generation and consequently decreases inflammation through inhibiting glial cell activation and finally preventing apoptosis. This occurs due to Bcl-2, which inhibits free radicals generation, induces sequestration of calcium, prohibits caspase activation and prevents the proapoptotic effects as Bax and Bad as reported by



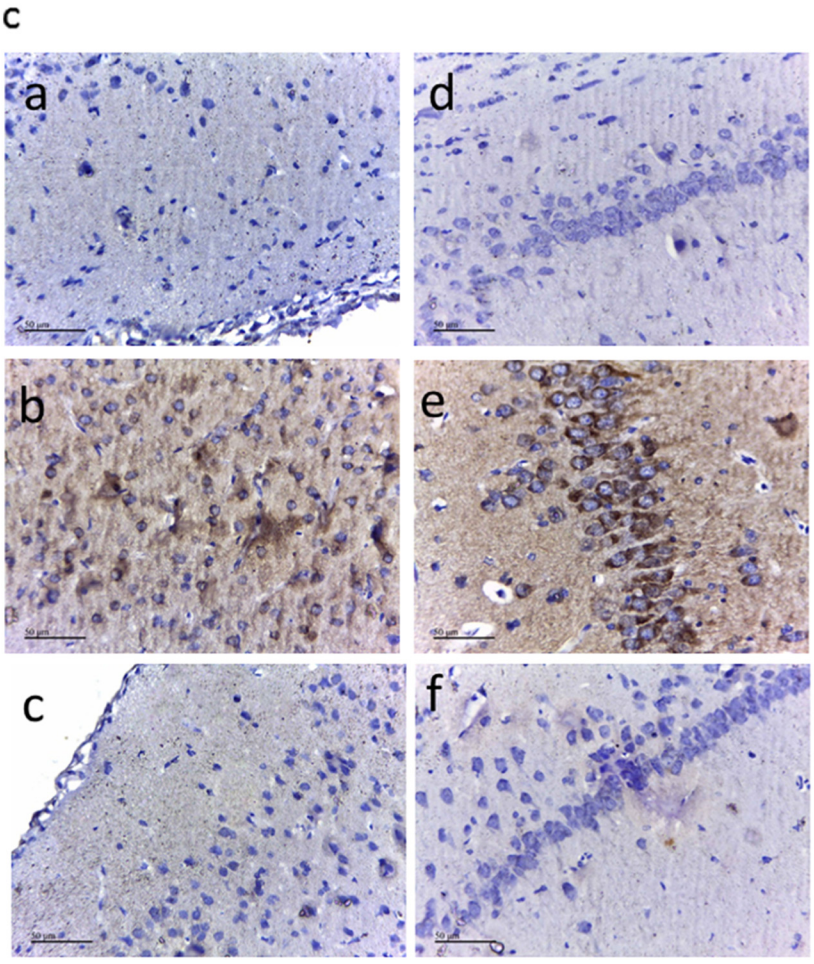


Fig. 5. (a): The injurious effect of cerebral I/R in OVX rats on cortical expression of COX-2 was reversed by pretreatment with zeranol.
(b): The injurious effect of cerebral I/R in OVX rats on hippocampal expression of COX-2 was reversed by pretreatment with zeranol.
(c): Representative photos for COX-2 presenting elevation in the expression of COX-2 in areas of cortex and hippocampus (CA1) of I/R group (b, e) against sham group (a, d), while zeranol pretreatment group revealed depression in the expression of GFAP in both areas (c, f) in comparison with I/R group (×40), scale bar = 50 μm.
Data demonstrated by way of mean (n = 6) and S.D.
* is set against I/R group, # is set against ZER group, ($p < 0.05$).
COX-2: cyclooxygenase-2, I/R: ischemia/reperfusion, OVX: ovariectomized, ZER: zeranol.

[41,42].

Alternatively, pretreatment with zeranol displayed a decrease in brain level of NO_X, MPO, IL-10. These results are similar to the studies of [24,43] respectively. It was also reported that activation of ERK pathway leaded to upregulation of IL-10 expression, which acts as triggering convertor of microglial cells by shifting from polarized M1 (inflammatory phase which release inflammatory markers) to M2 phase (anti-inflammatory phase) and this finally led to increase level of IL-10 [44]. This is what we observed in the present study where zeranol pretreatment caused activation of ERK pathway which led finally to augmentation of IL-10 level and this agreed with the finding of [45] who reported that ischemia cause decrease in level of IL-10 while treatment with exogenous estrogen reversed that through shifting microglial cell to M2 phase.

Zeranol pretreatment showed reduction in the protein expression of COX-2 and GFAP in brain tissue. These results are similar to the studies of [46,47] respectively. Finally, zeranol pretreatment caused decrease in the brain level of CASP-3 and this is similar to the study of [48].

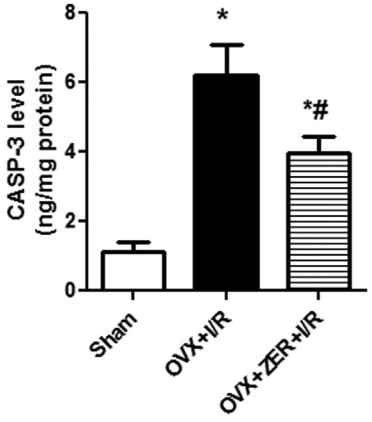


Fig. 6. The injurious effect of cerebral I/R on level of CASP-3 level was reversed by pretreatment with zeranol.
Data demonstrated by way of mean (n = 6) and S.D.
* is set against I/R group, # is set against ZER group, ($p < 0.05$).
CASP-3: caspase-3, I/R: ischemia/reperfusion, OVX: ovariectomized, ZER: zeranol.

5. Conclusion

Taken together, we reported that ERK signaling pathway activation by zeranol produce reduction in inflammation through inhibiting glial cell activation and apoptosis. This in turn, results in the neuroprotective effect of zeranol against cerebral I/R injury accompanied by ovariectomy. Additional clinical trials are needed to emphasize its effect against stroke in postmenopausal women.

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Conflict of interest

All authors announce that there are not conflicts of interest.

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Abbreviations

Bax: Bcl-2 associated x protein
Bcl-2: B-cell lymphoma 2
CA1: Cornu ammonis
COX-2: cycloxygenase-2
CREB: c-AMP response element binding protein
ERK1/2: extracellular signal regulated protein kinase 1/2
GFAP: glial fibrillary acidic protein
I/R: ischemia reperfusion
IL-10: interleukin-10
iNOS: induced nitric oxide synthetase
MEK1/2: mitogen activated protein kinase
MPO: myeloperoxidase
NO_X: total nitrate/nitrite
NO: nitric oxide
OVX: ovariectomized
p-ERK1/2: phosphorylated extracellular signal regulated protein kinase 1/2
ZER: zeranol