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Neuroprotective Activity of *Valeriana officinalis* against Global Cerebral Ischemia/Reperfusion Induced Oxidative Stress in Rat

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ABSTRACT

Background: Traditionally, the plant *Valeriana officinalis* is used for various diseases via headaches, anxiety, high blood pressure, menstrual cramps, epilepsy including neuronal disorders. **Objective:** To evaluate the neuroprotective activity of *Valeriana officinalis* against global cerebral ischemia induced oxidative stress in rat. **Materials and methods:** Neuroprotective activity was carried out by oxidative stress on Sprague-Dawley rats. Evaluation of cerebroprotective activity of *Valeriana officinalis* (100, 200 and 400 mg kg^{-1} oral doses) was carried out by using the global cerebral ischemia induced by Bilateral Carotid Artery (BCA) occlusion for 30 min, followed by 24 h reperfusion. The antioxidant enzymatic and non-enzymatic levels were estimated along with cerebral infarction area, blood brain barrier disruption and histophathological studies. **Results:** The ethanol extract of *Valeriana officinals* showed neuroprotective activity by significant decrease in lipid peroxidation ($p<0.05$-$p<0.01$) and increase in superoxide dismutase ($p<0.05$), catalase, glutathione ($p<0.05$-$p<0.001$) and total thiol levels ($p<0.05$-$p<0.01$) in extract treated groups as compared to control group. Blood brain barrier disruption and cerebral infarction area was significantly reduced in extract treated groups as compared to control group and % infraction volume was reduced in ethanol extract of *Valeriana officinalis* treated groups ($p<0.001$), as compared to control group. Histopathological observation further support the potent neuroprotective activity of *Valeriana officinalis*. **Conclusion:** The ethanol extract of *Valeriana officinalis* showed neuroprotective activity against global cerebral ischemia induced oxidative stress in rat model.

Key words: *Valeriana officinalis*, flavonoids, ischemia/reperfusion, bilateral carotid artery occlusion, oxidative stress, cerebroprotective

INTRODUCTION

Neurodegenerative disease is characterized clinically by the insidious accumulation of insoluble filamentous aggregates of normally soluble proteins in the Central Nervous System (CNS) leading to neuronal death[1]. Cerebro Vascular Disease (CVD) is a neurodegenerative brain dysfunctions related to disease of the blood vessels supplying the brain that includes, some of the most common devastating disorders, such as; ischemic stroke, hemorrhagic stroke and cerebrovascular anoma[2]. They cause 2 million deaths each year and are a major cause of disability. Stroke has been ranked third most common cause of death world-wide and cerebrovascular diseases are considered to be the second most frequent cause of projected deaths in the year 2020[3].

In the global ischemia model in rats, blood supply to the brain is reduced by occluding the common carotid arteries with a reduction in blood pressure[4]. The permanent and often lethal damage of the brain is mainly the result of the death of neurons, which are particularly susceptible to any shortage of supply with metabolic energy. The immediate effect of oxygen and glucose depletion is the decrease of ATP levels, which impairs ion transport, causes the loss of membrane potential and eventually leads to the depolarization of neurons and glia. Depolarization of neurons causes the release of glutamate and other excitatory amino acids into the extracellular space, which triggers the NMDA receptors and leads to increase of Ca^{2+} in the cytosol. The Ca^{2+} is at least partly responsible for long-term pathogenic effects, which include inflammation processes and programmed cell death. After restoration of the blood flow, re oxygenation causes the production of Reactive

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Oxygen Species (ROS), which brings about additional brain damage[5]. Brain is the most susceptible organ to the damage due to oxidative stress in part, because neurons are rich in polyunsaturated fatty acids and levels of endogenous antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidases) and non-enzymes (vitamins C and E) in neuronal tissue are low[6].

Now a days an active search for new cerebro protectors is being carried out among compounds, that affect the compensatory shunt of ATP synthesis in conditions of cerebral ischemia, that modulate glutamate and GABAergic systems, regulate activity of Ca-channels and system of nitrogen oxide and also among antioxidants, neuropeptides, expression inhibitors of proinflammatory cytokines and antagonists of IL-1β-receptors[7]. Thus pharmaceutical approach for novel prevention and treatment strategies of neuro degeneration involves the use of neuroprotective agents in order to delay or stop neuronal cell death or to strengthen cellular defense system, but effective therapies still remain elusive[8]. In view of this, the polyphenolics include, flavonoids, which are found in many herbal extracts, have been shown to be strong ROS scavengers, antioxidants and protectors of neurons from lethal damage in *in vitro*[9].

By the 18th century, Valerian was widely used as, a sedative and to treat nervous disorders as well as, the "vapors" in women. Other common uses include, the treatment of headaches, anxiety, high blood pressure, menstrual cramps, epilepsy and childhood behavior problems and learning disabilities[10]. The present study has aim, to investigate the neuroprotective effect of *Valeriana officinalis* against global cerebral ischemia model in rats.

MATERIALS AND METHODS

Experimental animals: The Sprague-Dawley rats of either sex (200-260 g) and female mice (20-25 g) for acute toxicity study were obtained from the central animal house of H.S.K. College of Pharmacy and Research Centre, Bagalkot. The animals were housed at room temperature (22-28°C) with 65±10% relative humidity for 12 h dark and light cycle and given standard laboratory feed (Amruth, Sangli, Maharashtra) and water *ad libitum*. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee (HSKCP/IAEC, Clear/2013-14/1-14).

Preparation of plant extract: The dried roots and rhizomes were collected from Belgaum district of Karnatka. It was identified and authenticated in Department of Botany, Basaveshwar Science College, Bagalkot, Karnataka. Voucher specimen (B.Sc./Bot./54). The roots and rhizomes were cleaned and air dried then subjected to coarse powdering and passed through a sieve # 44 to get uniform powder size. The collected powder was successively, extracted with petroleum ether to defat and then by alcohol and water (70:30) at 60-65°C for 24 h by using soxhlet apparatus. Excess solvent was removed by solvent distillation apparatus and residue was concentrated by using Lyotrap dryer. The greenish brown solid mass of extraction was preserved in aseptic condition before performing the experiment. Based on the earlier studies, aqueous solution of *Valeriana officinalis* was prepared by using tween-80[11].

Acute toxicity study: The acute toxicity study was performed as per the method described by OECD 425 guidelines. The mice were also observed for 14 days for the other signs of toxicity, such as excitation, tremors, twitches, motor co-ordination, righting reflex and respiratory changes. No mortality was observed even at doses of 2000 mg kg^{-1} b.wt. Thus LD$_{50}$ was found as 2000 mg kg^{-1}.

Experimental protocol for global ischemia: Sprague-Dawley rats of either sex (200-260 g) were divided into five groups of 12 rats. In each groups, 6 animals used for biochemical estimation, 3 animals for infraction volume and 3 animals for blood brain barrier disruption and fed with drug or vehicle for 10 days prior to the experiment and treated as follows:

Group I : Sham (normal saline, 10 mL kg^{-1}) orally
Group II : Control (normal saline, 10 mL kg^{-1}, orally), BCA occlusion for 30 min, followed by reperfusion for 24 h
Group III : EVO (100 mg kg^{-1}), BCA: occlusion for 30 min followed by reperfusion for 24 h
Group IV : EVO (200 mg kg^{-1}), BCA: occlusion for 30 min followed by reperfusion for 24 h
Group V : EVO (400 mg kg^{-1}), BCA: occlusion for 30 min followed by reperfusion for 24 h

Induction of ischemia: Animals of group II to V were subjected Bilateral Carotid Artery (BCA) occlusion under Ketamine anesthesia (100 mg kg^{-1}, i.p). Animal were placed on the back, both carotid arteries were exposed and occluded by atraumatic clamps. The temperature was maintained around 37±0.5°C

throughout the surgical procedure and artificial ventilation (95% O_2 and 5% CO_2) provide with artificial respirator[12].

Preparation of post mitochondrial supernatant: Each animals in group were sacrificed after 10 days of treatment followed Global cerebral I/R by cervical decapitation, the brain was removed and washed in cooled 0.9% saline, kept on ice and subsequently blotted on filter paper, then weighed and homogenized in cold phosphate buffer (0.05 M, pH 7.4). The homogenates were centrifuged at 10000 rpm for 10 min at 4°C (MPW-350R, Korea) and Post-Mitochondrial Supernatant (PMS) was used for the estimation of total protein and lipid peroxidation[13]. The supernatant was again centrifuged at 15000 rpm for 1 h at 4°C. The supernatant obtained was used for further estimation of superoxide dismutase (SOD),[14] catalase (CAT),[15] glutathione (GSH)[16] and total thiols[17].

Lipid peroxidation (LPO): Thiobarbituric acid reactive substances (TBARS) in the homogenate were estimated by the method. Briefly, the 0.5 mL of 10% homogenate was incubated with 15% TCA, 0.375% TBA and 5 M HCl at 95°C for 15 min, the mixture was cooled, centrifuged and absorbance of the supernatant measured at 512 nm against appropriate blank. The amount of lipid peroxidation was determined by using $\varepsilon = 1.56 \times 105$ M^{-1} cm^{-1} and expressed as, TBARS n mol mg^{-1} of protein[18].

Superoxide dismutase (SOD): Superoxide dismutase activity was determined based on the ability of SOD to inhibit the auto-oxidation of epinephrine to adrenochrome at alkaline pH[14]. Briefly, 25 µL of the supernatant obtained from the centrifuged brain homogenate was added to a mixture of 0.1 mM epinephrine in carbonate buffer (pH 10.2) in a total volume of 1 mL and the formation of adrenochrome was measured at 295 nm. The SOD activity (U mg^{-1} of protein) was calculated by using the standard plot.

Catalase (CAT): Catalase activity was assayed by the method of Claiborne[15]. Briefly, the assay mixture consisted of 1.95 mL phosphate buffer (0.05 M, pH 7.0), 1.0 mL hydrogen peroxide (0.019 M) and 0.05 mL homogenate (10% w/v) in a total volume of 3.0 mL. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of nM H_2O_2 consumed/min/mg protein.

Glutathione (GSH): The GSH was estimated in various tissues by the method of Sedlak and Lindsay, [16]. Briefly, 5% tissue homogenate were prepared in 20 mM EDTA, pH 4.7 and 100 µL of the homogenate or pure GSH was added to 0.2 M Tris-EDTA buffer (1.0 mL, pH 8.2) and 20 mM EDTA, pH 4.7 (0.9 mL) followed by 20 µL of Ellman's reagent (10 mmol L^{-1} DTNB in ethanol). After 30 min of incubation at room temperature, samples were centrifuged and take absorbance at 412 nm GSH (nmole mg^{-1} of protein) was calculated by using, the standard plot.

Total thiols: This assay is based on the principle of formation of relatively stable yellow colour by sulfhydryl groups with DTNB. Briefly, 0.2 mL of brain homogenate was mixed with phosphate buffer (pH 8), 40 µL of 10 mM DTNB and 3.16 mL of ethanol. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blanks. The total thiol content was calculated by using $\varepsilon = 13.6 \times 1031$ cm^{-1} M^{-1} [17].

Measurement of cerebral infarct area: Three animals in each group were used for estimation of the infarct area. Evaluation of infarct area was done by 2,3,5-triphenyltetrazolium chloride (TTC) staining method. Following I/R after 24 h, animals were decapitated and the brains were removed. After the brains were placed briefly in cold saline, four coronal brain slices (2 mm thick) were made, incubated in phosphate buffered saline (pH 7.4) containing 2% TTC at 37°C for 10 min and kept in the images of the TTC stained sections were acquired by scanning by a high resolution scanner (Hewlett Packard Sacnjet 6100 C/T) and cerebral infarction volume and area was measured treatment groups and control group[13].

Blood brain barrier disruption: After 24 h of reperfusion, rats (n = 3) from each group were anesthetized with ketamine (100 mg kg^{-1} i.p) and the right jugular vein was cannulated with polyethylene tubes for the administration of Evans blue (2% in saline, 4 mL kg^{-1}). After 30 min of EB administration, animals were perfused with normal saline through same cannula to remove excess of Evans blue from blood. The brains were rapidly removed and the brains were sliced coronally into 2 mm thick sections and were scanned using, high resolution scanner. Then, the extent of BBB disruption was compared between various treatment and control groups by observing the appearance of blue colour in the brain sections[19].

Blood brain barrier disruption was carried out by the procedure described by Lam *et al.*[20] After the removal of brain, it was sliced into smaller pieces and mixing with acetone in 1% Na_2SO_4 in the ratio of 7:3. The samples were shaken gently and continuously for 24 h at room temperature. Each preparation was centrifuged for 10 min at 2000 rpm and 2 mL of the supernatant was separated for measurement of absorbance at 620 nm using UV-spectrophotometer (UV-1601, Shimadzu Corporation, Kyoto, Japan). The amount of dye recovered indicates the degree of blood brain barrier disruption, which is calculated by extrapolating with standard curve prepared with different concentrations of Evans blue solution.

Histopathological studies: Brains from control and experimental groups were fixed in 10% of formalin and embedded in paraffin wax and cut into longitudinal section of 5 μm thickness. The sections were stained with hemotoxylin and eosin dye for histopathological observation.

Statistical analysis: All the values are expressed as Mean±standard error of mean and were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test (*post hoc*) and the value p<0.05 is considered to be statistically significant.

RESULTS

Biochemical estimation: The enzymatic and non-enzymatic estimations were performed and data was summarized in Table 1. The brain homogenate showed significant reduced activity of GSH (p<0.001), Total thiols, SOD and CAT and increased in lipid peroxidation (p<0.001), in control group as compared to sham group. The ethanol extract of *Valeriana officinalis* administered groups showed significant protection in pre-treated groups by reducing the LPO (p<0.05-p<0.01) and significantly increasing the GSH

(p<0.05-p<0.001), Total thiols (p<0.01-p<0.001), SOD (p<0.01), CAT levels and showed significant protection in post-treated groups by reducing the LPO (p<0.05) and significantly increasing the GSH (p<0.01), Total thiols (p<0.05 to p<0.01), CAT (p<0.05) and SOD levels as compared to control group.

Measurement of infraction volume: A significant decrease in cerebral infarction area was observed in EVO treated groups as compared to control group, especially in caudal and rostal side. The significant increase in % infraction volume of control group (p<0.001) as compared to sham and conversely % infraction volume was reduced in EVO treated groups (p<0.001) in as compared to control group (Results were summarized in Table 2 and Fig. 1).

Blood brain barrier disruption: EVO treated groups showed potent neuroprotective effect in maintaining the blood brain barrier integrity as compared to control group. The degree of blood brain barrier disruption was significantly increase in control group (p<0.001) as compared to sham, in contrast, EVO treated groups showed significant decrease in blood brain barrier disruption (p<0.05-p<0.001) as compared with control group and the percentage inhibition of Evans blue infiltration was found to be highest at EVO 400 mg kg^{-1} (Results were summarized in Table 3 and Fig. 2).

Histopathology studies: Histopathological observation (Fig. 3) indicates the potent neuroprotection of EVO. In control group (B) showed marked increase in intracellular space, neutrophil infiltration and neuronal necrosis and disruption in architecture as compared to normal groups. In EVO treated groups (C-E) showed marked improvement by reduction of neutrophils infiltration, a compact cells with regular intracellular space and normal architecture was observed.

Table 1: Effect of ethanol extract of *Valeriana officinalis* in Global cerebral ischemia/reperfusion induced oxidative stress

Treatment groups	Lipid peroxidation (nm mg^{-1} of protein)	GSH (nm mg^{-1} of protein)	Total thiols (μm mg^{-1} of protein)	SOD (U mg^{-1} of protein)	Catalase (U mg^{-1} of protein)
Sham	124.3±32.73	32.570±18.22	75.83±12.37	400.5±138.0	0.5474±0.1224
Control	277.9±157.1[a]	7.174±2.500[a]	4.016±3.326[a]	210.2±77.84[a]	0.2181±0.1521
EVO (100 mg kg^{-1})	247.0±17.42	19.300±1.114	22.62±14.93	392.8±140.2★★	0.3661±0.1516
EVO (200 mg kg^{-1})	176.3±45.86	29.980±8.697★	48.22±18.55★★	255.3±193.4	0.4645±0.3364
EVO (400 mg kg^{-1})	138.0±43.71★	40.280±8.06★★★	80.90±22.97★★★	307.3±110.6	0.7402±0.4601

All values are expressed as Mean±SEM, n = 6, [a]p<0.001, as compared to sham group and ★p<0.05, ★★p<0.01, ★★★p<0.001, (One way Analysis of Variance (ANOVA) followed by multiple comparison Tukey's test) as compared to control group, EVO: Ethanol extract of *Valeriana officinalis*, GSH: Glutathione, SOD: Super oxide dismutase
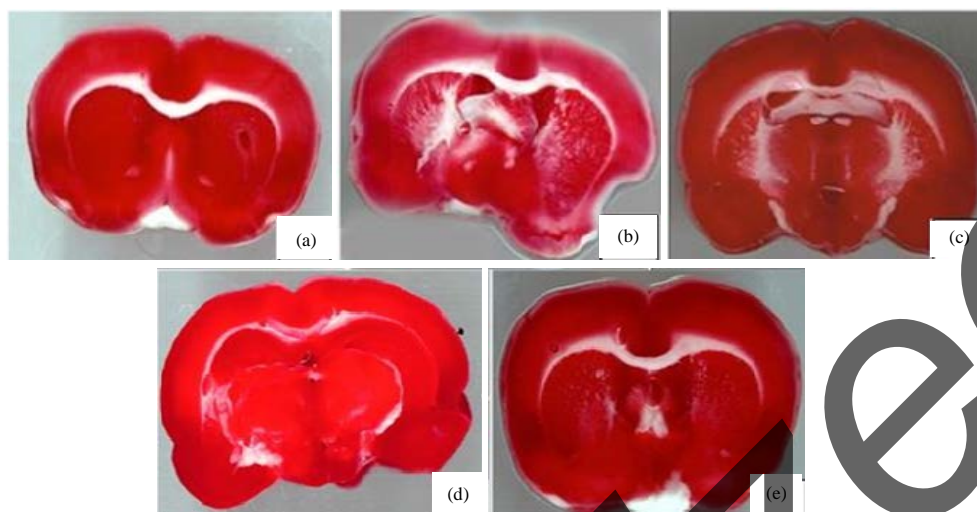
Fig. 1(a-e): Effect of ethanol extract of *Valeriana officinalis* in global cerebral ischemia/reperfusion induced oxidative stress can be evaluated by 2,3,5-Triphenyltetrazolium chloride (TTC) staining. Bran coronal sections were prepared (2 mm thickness) and then each section was stained with TTC, (a) Sham group (normal saline 10 mL kg^{-1}), (b) Control group (normal saline 10 mL kg^{-1}+ischemia 30 min followed by 24 h reperfusion), (c) EVO 100 mg kg^{-1}, (d) EVO 200 mg kg^{-1} and (e) EVO 400 mg kg^{-1}. In Fig. b a large infraction area was observed in caudate putamen. In Fig. c, d and e the infraction area of caudate putamen was markedly reduced in rats brains treated with EVO, this reduction in infraction in treated rats was mostly comparable with sham group, EVO: Ethanol extract of *Valeriana officinalis*

Table 2: Effect of ethanol extract of *Valeriana officinalis* on infarction volume

Treatment groups	Infarction volume (%)
Sham	5.20±2.062
Control	47.89±3.995
EVO (100 mg kg^{-1})	33.18±4.449
EVO (200 mg kg^{-1})	22.75±3.743★
EVO (400 mg kg^{-1})	11.46±2.085★★

All values are expressed as Mean±SEM, n = 3, [a]p<0.001, as compared to sham group, ★p<0.05, ★★p<0.01 and ★,★★p<0.001, (One way Analysis of Variance (ANOVA) followed by multiple comparison Tukey's test) as compared to control group, EVO: Ethanol extract of *Valeriana officinalis*

Table 3: Effect of ethanol extract of *Valeriana officinalis* on blood brain disruption

Treatment groups	Evan's blue concentration in brain (μg mL^{-1})	Inhibition Evan's blue brain infiltration (%)
Sham	2.371±0.3202	-
Control	4.635±0.8007[a]	-
EVO (100 mg kg^{-1})	3.638±0.5176★	21.516
EVO (200 mg kg^{-1})	3.003±0.5499★★	35.208
EVO (400 mg kg^{-1})	1.854±0.5634★★★	60.01

All values are expressed as Mean±SEM, n = 3, [a]p<0.001, as compared to sham group, ★p<0.05, ★★p<0.01 and ★,★★p<0.001, (One way Analysis of Variance (ANOVA) followed by multiple comparison Tukey's test) as compared to control group, EVO: Ethanol extract of *Valeriana officinalis*

DISCUSSION

Oxygen maintains brain function and is crucial for life. However, O_2 supplied at concentrations greater than those in normal air is highly toxic. High pressure O_2 can lead to production of ROS, which are very important mediators of cell injury and cell death. These radicals are either directly or indirectly involved in various clinical disorders, such as; neurodegenerative disorders, cancer and atherosclerosis[3].

Free radicals in the living organism are generated both enzymatically and non-enzymatically, leading to the formation of ROS. It has been reported that hydroxyl and peroxynitrite are the most potent ROS that can damage proteins, lipids and nucleic acids, resulting in the inactivation of some enzyme activities, disruption of ion homeostasis and modification of genetic apparatus and apoptotic death. Brain edema has also been studied in ischemia to assess the impact of brain damage. Cerebral edema occur, as the result of ionic imbalance across the cellular membrane due to energy failure results in neuronal depolarization, which alters ionic gradients of Na^+, K^+, Cl^-, Ca^{++}. As glutamate increases in extracellular space peri-infract depolarization occur so water shift occur resulting in cerebral edema[2].
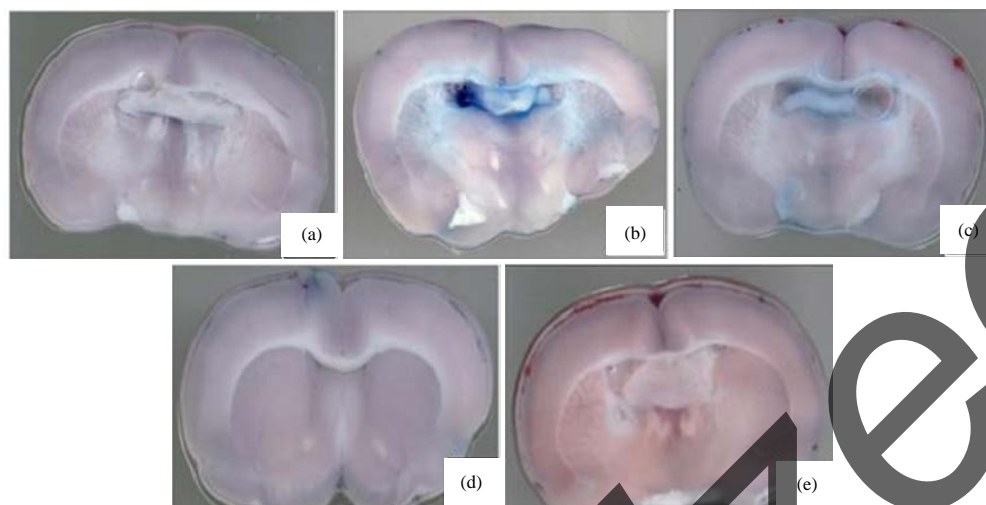
Fig. 2(a-e): Effect of ethanol extract of *Valeriana officinalis* in global cerebral ischemia/reperfusion induced blood brain disruption can be evaluated by Kumai *et al*. method. Brain coronal sections were prepared (2 mm thickness), (a) Sham group (normal saline 10 mL kg^{-1}) and jugular vein cannulated with 2% Evans blue (4 mL kg^{-1}), (b) Control (normal saline 10 mL kg^{-1}+ischemia 30 min followed by 24 h reperfusion and jugular vein cannulated with 2% Evans blue (4 mL kg^{-1}), (c) EVO 100 mg kg^{-1}, (d) EVO 200 mg kg^{-1} and (e) EVO 400 mg kg^{-1}. In ischemia, conditions due vascular endothelial growth factor blood brain barrier disruption occurs and this leads to the increase degree of blood brain barrier disruption and due to increased infiltration of dye in brain. So, in Fig. b in control right hemisphere was highly accumulated with dye this indicates high degree blood brain barrier destruction. In case Fig. c, d and e the amount of dye accumulation in right hemisphere was less which indicates blood brain barrier destructions was markedly reduced in rats brains treated with EVO, this reduction in disruption of BBB in treated rats were mostly comparable with sham group, EVO: Ethanol extract of *Valeriana officinalis*

Free radical involvement in the development of I/R-induced cerebral injury was well investigated, among which, $O_2 \bullet -$ and $OH \bullet$ are potent inducers of lipid peroxidation Excessive production of ROS can cause cellular damage and subsequent cell death, because ROS may oxidize vital cellular components such as lipids, proteins and DNA and alter several signaling pathways that ultimately promote cellular damage and death during cerebral I/R. In addition, ROS produces malondialdehyde (MDA), an end product of lipid peroxidation. The MDA reacts with TBA and was, thus, estimated as TBARS[21]. Therefore, in the present study, extent of lipid peroxidation in brain homogenate of ischemic rats (control group) was found to be elevated, but in case of EVO treated group LPO levels were significantly reduced, as compared to control group this indicates that ethanol extract of *Valeriana officinalis* has neuroprotective potential via anti oxidant defence mechanism.

Astrocytes has an important role in synthesis and maintaining GSH levels in brain. This indicates that during ischemic condition in loss of GSH levels in brain is due to impaired astrocytes function[22].

GSH levels were decreased during ischemia reperfusion condition is observed in control group of study. This decrease is may be due to impaired astrocyte function as, astrocyte have important function in maintaining and synthesis of GSH enzyme in brain. But in case of EVO treated groups GSH levels was significantly increased, as compared to control group. This suggested that ethanol extract of *Valeriana officinalis* plays a significant role in maintaining the astrocyte function, which in turn is responsible for maintaining GSH levels. The SOD and Catalase is the major enzymatic defence for aerobic cells fighting the toxic effects of super oxide radicals. In control group animals SOD, catalase and total thiols levels is significantly reduced, as compared to sham groups. The EVO
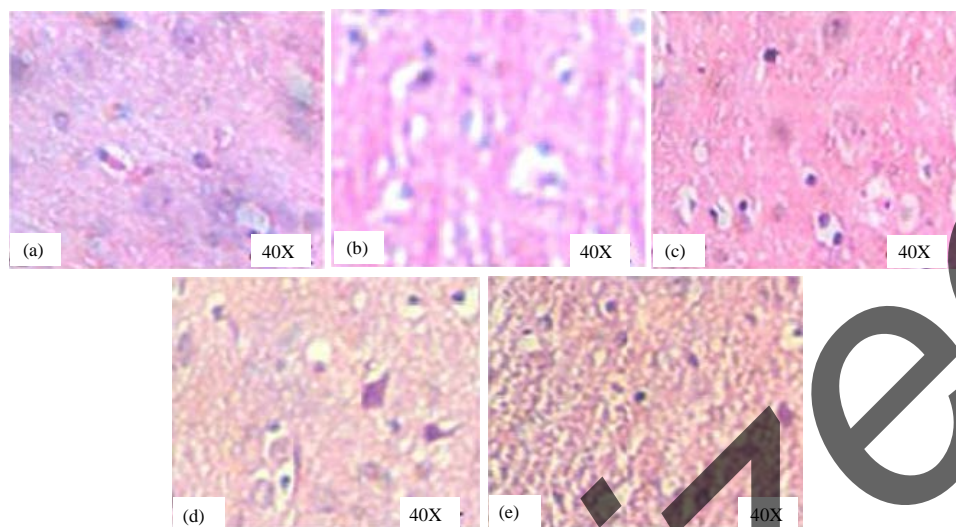
Fig. 3(a-e): Effect of ethanol extract *Valeriana officinalis* against oxidative stress induced Global cerebral ischemia/reperfusion in rat. Photographic images of brain section from different treatment groups stained with hemotoxylin and Eosin. Plates, (a) Sham group (normal saline 10 mL kg^{-1}), (b) Control group (normal saline 10 mL kg^{-1}+ischemia 30 min followed by 24 h reperfusion), (c) EVO 100 mg kg^{-1}, (d) EVO 200 mg kg^{-1} and (e) EVO 400 mg kg^{-1}. In control group, density of cells was decreased, Increased neutrophils infiltration, Cell architecture is completely altered and increased hemorrhage and neuronal cell death was observed. But in case of EVO treated groups there is significant increase cell density, decreased neutrophil infiltration, decreased neuronal death and hemorrhage, normal cellular architecture was observed,(c-e), which is comparable with sham group and EVO: Ethanol extract of *Valeriana officinalis*

treated groups showed significant increase in SOD levels but CAT levels were not showed significant increase.

Brain edema is a major and often mortal complication of brain ischemia and is led by increased vascular permeability[23]. Vascular Endothelial Growth Factor (VEGF) is a dimeric glycoprotein that is mitogenic for endothelial cells. Vascular endothelial growth factor, however, was also discovered as a vascular permeability factor,[24] are responsible to cause ischemia in brain, Cerebral ischemia leads to inflammatory cell to infiltrates from nonspecific immunologic reaction, migration of peripheral leukocytes into the brain and activation of microglia[25]. Release of inflammatory cytokines (interleukin (IL), Tumor Necrosis Factor a (TNF-a)) by ischemic neurons and glia leads to generation of adhesion molecules (selectins, integrins, intercellular adhesion molecule 1) in the cerebral vasculature, which results in breakdown of the Blood-Brain Barrier (BBB), culminating in edema formation[26,27].

In this study, the control group animals during ischemia reperfusion condition shown increased level of blood brain barrier disruption, which was evaluated by evans blue dye infiltration in brain. This increase level of blood brain barrier disruption is may be due production of high amount of vascular endothelial growth factors and generation inflammatory cytokines (interleukin (IL)-1, tumor necrosis factor a (TNF-a)) by ischemic neurons and glia leads to generation of adhesion molecules (selectins, integrins, intercellular adhesion molecule 1). In contrast, EVO treated groups showed significant protection by preventing the blood brain barrier disruption. This may be due to inhibition of production of vascular endothelial growth factors, inflammatory cytokinins and inhibiting the production of adhesion molecules.

The cerebral infarction area and histopathological studies revealed the protection against the I/R induced oxidative stress in EVO treated groups. it suggested that ethanol extract of *Valeriana officinalis* treated animals decreased the infiltration of neutrophils, reduced

intracellular space, regained normal architecture and moderate necrosis was observed. In contrast, there was a decreased cerebral infarction volume in EVO extract treated groups as compared to control group.

CONCLUSION

In conclusion, these findings suggest a potential protective role of *Valeriana officinalis* against global cerebral ischemia/reperfusion-induced brain injury. Further studies are required to pursue the interesting lead emerging from the present results to exploit the full therapeutic potential of *Valeriana officinalis*, as a neuroprotective.

ACKNOWLEDGMENTS

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