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Allium Cepa Attenuates Ischemia Reperfusion Induced Brain Injury

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Abstract

Objectives: Cerebral stroke continues to be a leading cause of death and disability without efficacious therapy. *Allium cepa* have potential antioxidant effects and its neuroprotectiveness can be effective against ischemia-reperfusion induced brain injury.

Materials and Methods: This study carried out the neuroprotective effect of aqueous extract of *Allium cepa* leaves (200 mg/ kg, 400 mg/ kg body weight, p.o.) and vitamin E as reference standard drug on 30 min induced ischemia, followed by reperfusion by performing the neurobehavioral tests such as, neurodeficit score, hanging wire test, beam walk test, rota rod test and elevated plus maze test. The biochemical parameters were also measured in animals brain are glutathione, superoxide dismutase, catalase, nitric oxide and lipid peroxidation products in control and treated rats.

Results: The aqueous extract of *Allium cepa* leaves (200mg/kg, 400mg/kg body weight, p.o.) treated groups showed a statistical significant reduction in neurological deficit score, increase in the grasping ability and forelimb strength, improved motor performance, increased grip strength but no significant improvement in memory functions. The biochemical parameters in the rats brain showed a significant increase in glutathione level ($P < 0.05$, $P < 0.01$) an increased levels of SOD ($P < 0.01$), catalase ($P < 0.0.1$) and significant decrease in the total nitrite ($P < 0.05$, $P < 0.01$) and lipid peroxidation ($P < 0.01$).

Conclusion: Results from the study shows that *Allium cepa* extract has produced neuroprotection due to its antioxidant potential on ischemia reperfusion induced brain injury.

Key Words: *Brain ischemia-reperfusion, Allium cepa, neuroprotection, oxidative stress*

Introduction

Cerebral stroke continues to be a leading cause of death and disability worldwide due to the limited efficacy of current therapy. [1] There is a great need for curative treatments and good prevention protocols for populations at high stroke risk. [2] Brain stroke is a sudden loss of brain function usually caused by a blocked or leakage of a blood vessel. It develops from a complex cascade of cellular events that ultimately leads to cerebral infarction [3] and causes sudden loss of vision, balance, coordination, speech and memory.[4] Severe strokes may lead to sudden death. Cerebral ischemia (stroke) triggers a complex series of biochemical and molecular mechanisms that impairs the neurologic functions through breakdown of cellular integrity mediated by excitotoxic glutamatergic signalling, ionic imbalance, free-radical reactions, etc.[5] After cerebral ischemia/reperfusion, the reactive oxygen species (ROS) production is dramatically increased and overwhelms endogenous antioxidant systems, leading to oxidative stress. The brain is particularly vulnerable to oxidative stress injury due to its high consumption of oxygen, abundant polyunsaturated fatty acids and low levels of endogenous antioxidants. [6] It has been suggested that free radicals are produced during or after cerebral ischemia. [7] These radicals have the capacity to initiate destructive reactions on biological membranes as well as on other cellular structures. Therefore, they may play an important role in causing extensive secondary damage in brain tissue and possibly death. ROS have been reported to activate lysosomal enzymes, which may contribute to neuronal injury. [8] *Allium cepa L.* (onion) belongs to the family Alliaceae. The onion leaves are rich in flavonoids quercetin and isorhamnetin, [9] it also contains significant quantities of polyphenols, anthocyanins, kaempferol and luteolin. Onion is more used as food and medicine. Traditionally it is used in common cold, in expelling phlegm, promoting sweating, in hypertension, diarrhea, fungal infection, colon cancer and as an antimicrobial agent.

[10] Earlier studies reported the beneficial effects of onion bulbs extract on brain ischemia induced edema and blood brain barrier dysfunction, an another study investigated and proved the protective effect of methanolic extract of outer scales and edible portion of *Allium cepa* on ischemia reperfusion induced cerebral injury. The flavonoids are the known antioxidants and as *A. cepa L.* contains high levels of flavonoids. Therefore, in this study the antioxidant effects of aqueous leaves extract of *A. cepa L.* on ischemia reperfusion induced brain injury with regards to neurobehavioral and biochemical parameters were studied.

Materials and Methods

Materials and subjects

The *Allium cepa L.* (onion) leaves coarse powder was defatted with petroleum ether at (60-80^{o}C) by Soxhelation. The marc was boiled with water (55-60^{o} C) at low flame for 3-4 hours, and then it is allowed to cool and filtered. The marc was concentrated under reduce pressure, appropriate concentrations of the extract were made in distilled water. The dry extract was stored in an air tight container for pharmacological evaluation and phytochemical investigation. Ten weeks old Albino Wistar rats of either sex weighing 225-275 g were used. The animals were housed under controlled housing conditions and were exposed to 12-h light-dark cycles. Before the experiment the rats were allowed to acclimatize for 10 days. The animals had free access to pellet basal diet and water *ad libitum*. The rats were habituated to the laboratory conditions for 36 hours prior to the experimental study in order to reduce any nonspecific stress.

Acute Toxicity

Toxicity study up and down procedure was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) guidelines 423, 425-430. Two groups of Wistar rats (n=3) were fasted overnight with water *ad libitum* and food was withheld for 3-4 hrs after oral administration of the extract. One group of animals was treated with starting dose of 2000 mg/kg body weight orally and the maximum dose of 5000 mg/kg body weight was administered to second group. A normal saline was administered to the third group and all the animals were observed individually. Clinical signs including changes in eyes, skin fur and mucous membranes were observed. The gross behaviors like body positions, rearing, tremors, locomotion and gait were observed and recorded. The effect of extract on pain response, stereotypy, passivity, grip strength, righting reflex, vocalization, body weight and water intake was observed and recorded. No mortality was seen with this dose. As per OECD guidelines the substance might be considered to have an LD_{50} value above 2000mg/kg and 5000mg/kg body weight.

Preparation of doses

From Acute oral toxicity study, it was found that aqueous leaves extract of *Allium cepa L.* was safe at limit dose 5000 mg/kg and 2000 mg/kg body weight, therefore a dose less than 1/10th of this dose i.e. 400 mg/kg & below were used in subsequent study for the aqueous extract of *Allium cepa* leaves.

Experimental Method

The experiment was carried out in accordance and adherence with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Institutional Animal Ethics Committee (IAEC) the approval no. was CPCSEA/IAEC/P'col-26/2012-13/62, dated November 3, 2012. Utmost steps were exercised to reduce pain and discomfort to the animals during experimental procedures. Rats were anesthetized with ketamine (50 mg/ml/kg) injected intra-peritoneally. A small cut was made on the neck region and the neck muscles were retracted for isolation of the common carotid artery. The internal carotid artery was subsequently isolated and 30 min of ischemia was given to the rat by blocking the internal carotid artery with micro-vascular clip. After the ischemic period, the neck muscles were stitched and an antibiotic was applied. [11] The test extract suspension was administered to the respective group of rats described below for a period of eight days. The animals were randomly divided into six groups, each group comprising of 06 animals. Group 1 (sham) animals served as normal control and received vehicle (saline 10ml/kg orally) for 8 days. The ischemia reperfusion was produced in Groups 2-6. After the induction of 30 min ischemia and reperfusion, Group 2 rats were administered with vehicle (saline 10ml/kg orally for 8 days). Group 3 animals were administered with vitamin E (50 mg/kg orally). Group 4 and 5 animals were administered with aqueous extract of *Allium cepa* leaves (200 mg/kg and 400 mg/kg orally) respectively for 8 days. The saline, aqueous extract of *Allium cepa* leaves and vitamin E were administered with the help of oral gavage needle. Seven additional days of post ischemic survival time were provided. On 16th and 17th day of the total study period the behavioral studies were carried out. After the completion of behavioral tests, the rats were sacrificed by an overdose of ketamine (75 mg/ml/kg)

injected intra-peritoneally as an anesthetic. The brains were isolated. The isolated brains were frozen for biochemical tests.

Neurobehavioral Test

Neurodeficit score

The neurological status of the animals was assessed using the methods described by Bederson *et al*. [12] Accordingly, four categories of neurological findings were observed and noted: 0 = no observed neurological deficit; 1 = contralateral forelimb flexion with wrist flexion and shoulder adduction; 2 = reduced resistance to lateral push; and 3 = circling movements towards the ipsilateral side.

Hanging wire

The experimental animals were hanged freely by their forelimbs on a metallic wire stretched between 2 poles, 45 cm above a foam sheet. The time (in seconds), until the animal fell down, was recorded. 2 min of cut off time was selected. This task was used as a measure of grasping ability and forelimb strength.

Beam walk test

The fore and hind limbs motor co-ordination was appraised by using beam walk test. The rat was individually placed on a beam walk apparatus (Inco) made up of a wooden bar 60 cm long and 1.5 cm wide, height 50 cm. The motor performance of rat scored on scale ranging from 0 to 4. This is a special test for animals subjected to cerebral ischemia and reperfusion. For motor incordination, Number of foot slip; Number of falls; distance travelled along beam was studied.

Rota rod test

By performing rota rod test the sensorimotor performance was evaluated. All animals were tested for their ability to remain on the rotating bar at a speed of 20 revolutions/min (rpm) on a Rotarod apparatus (Inco). Each animal was trained for a minimum of three trials. After 8 post ischemic days the animals were tested for motor impairment. Latency to fall off from the rotating rod was noted for each trial with a 5 min maximum to termination of the trials.

Memory Test

Elevated plus maze

The elevated plus-maze apparatus (Inco) for rat consists of a central platform connected to two open arms and two enclosed arms. The maze is elevated to a height of 50 cm from the floor. During training trials the animal was placed at the end of an open arm, facing away from the central platform of the maze. The time taken by the animal to move from open arm and cross the line marked in enclosed arm with all four paws was recorded as transfer latency time (TL). In case the rats did not enter the enclosed arm within 90 s, it was gently pushed into the enclosed arm and a TL of 90 s was assigned to it. The animal was allowed to remain in the maze for the duration of 10 s. The TL measured on the plus maze on the first day serves as an index of acquisition, whereas the TL measured after 24 h of acquisition trial was taken as an index of retrieval.

Biochemical Estimation

In this study 6 rats per group were used. After completion of the behavioral parameters, the brains were isolated. Five brains were used for biochemical tests. The pooling of brain was not done for biochemical tests. The small quantity of homogenate of individual brain was made in saline and further divided in tubes and used for various biochemical tests.

Preparation of Post Mitochondrial Supernatant (PMS)

The brain tissues were homogenized in chilled potassium phosphate buffer (50mM, pH 7.4) using a Remi homogenizer. The homogenate was centrifuged in a refrigerated centrifuge at (10,500 rpm) for 20 minutes at 4°C to obtain the PMS, the PMS was used for the estimation of enzymes such as superoxide dismutase, catalase and nitric oxide, a nonenzyme parameter.Glutathione levels were measured by the method of Elman (1959). [13] SOD activity was measured by the method of Marklund (1985). [14] Catalase activity was assayed by the method of Clairborne (1985). [15] Nitric oxide was estimated by an indirect measurement of nitrite, nitrate and total nitrite in rat brain PMS. The total nitrite contents of the sample were measured from the calibration curve for nitrite. The total nitrite was estimated by the method of Green *et al* (1982). [16] The lipid peroxidation (malondialdehyde) in the homogenate was determined by the method of Ohkawa *et al* (1979). [17]

The results were expressed as nmol of MDA/mg protein. The total protein was estimated by Lowry's *et al* (1959) method. [18]

Histopathological studies

One brain from each group was washed with ice cold buffered saline solution (pH 7.4) and preserved in 4% formaldehyde in 0.1 moles/liter phosphate buffer solution (PBS, pH 7.4). The brains were embedded in

paraffin and cut into sections (7 µm thickness). Brain sections obtained 1.5mm behind the bregma in the coronal plain were stained with haematoxylin-eosin (H-E) using standard methods. The normal morphology and presence and nature of ischemic damage were verified by a pathologist.

Statistical analyses

Behavioral data were analyzed by analysis of variance (ANOVA) followed by post Dunnett's test. It was carried out by using Graph Pad Instat followed by Dunnett's test at level of significance $P < 0.05$ value. All data were shown as the mean ± standard error of the mean. Statistical analysis was performed using Graph Pad statistical software (Graph Pad Software, Inc. California, USA).

Results

Behavioral result

After the ischemia reperfusion injury the animals showed loss of balance, motor incordination and memory loss. The neurobehavioral condition of the animals was evaluated by the behavioral tests.

Allium cepa Reduces Neurological Score and Improves Motor Performance in Ischemic Rats

In the ischemic animals a significant increase in neurological deficit score as compared to sham group was observed, whereas treatment with *Allium cepa* (400 mg/kg body weight) showed a statistical significant reduction in the neurological score as compared to the ischemic group animals. A significant decrease on the grasping ability and forelimb strength, grip strength, a significant impairment in motor coordination was observed in the ischemic group of animals as compared to the sham group. Treatment with aqueous extract of *Allium cepa* showed a statistical significant increase on the grasping ability and forelimb strength ($P < 0.01$) in the hanging wire test with (400 mg/kg body weight), in grip strength with 200 mg/kg ($P < 0.05$) and 400 mg/kg ($P < 0.01$) in the rota rod test and a significant improvement in the motor coordination with a decrease in the no. of foot slips with 200 mg/kg and 400 mg/kg ($P < 0.01$) respectively, a decrease in the No. of falls with 200 mg/kg ($P < 0.05$) and 400 mg/kg ($P < 0.05$) and an increase in the distance travelled with 200 mg/kg and 400 mg/kg ($P < 0.01$) in the beam walk test respectively [Table 1, 2].

Allium cepa has not Restored Ischemia Reperfusion Induced Memory Deficits

In the ischemic animals a statistical significant increase in initial latency ($P < 0.05$), first retention latency ($P < 0.01$) and second retention latency ($P < 0.01$) was observed in the plus maze test as compared to the sham group animals. Treatment with aqueous extract of *Allium cepa* showed no significant reduction in the transfer latency with both the doses in the plus maze task. *Allium cepa* extract has not shown protective effects on ischemia reperfusion induced impairment in the retrieval of memory [Table 3].

Allium Cepa Protects Brain of Ischemic Animals by Antioxidant Mechanism

In the ischemic animals a statistical significant decrease in brain glutathione, superoxide dismutase and catalase also a statistical significant increase in the levels of nitric oxide and lipid peroxidation product malondialdehyde was observed in the Group 2 animals. Treatment with the two doses of *Allium cepa* extract caused a significant increase in the glutathione levels with 200 mg/kg ($P < 0.05$) and 400 mg/kg ($P < 0.01$), SOD ($P < 0.01$) with both the doses, catalase ($P < 0.01$) with both the doses. A significant decrease in nitric oxide with 200 mg/kg ($P < 0.05$) and 400 mg/kg ($P < 0.01$) and MDA ($P < 0.01$) with both the doses used [Table 4].

Histopathological findings

The vehicle treated rat showed normal architecture of neurons [Figure - A]. Ischemia-reperfusion brain injury has caused complete loss of neurons in the rat brain of Group 2 [Figure - B, C]. Vitamin E treated rat brain also shows reduced histopathological abnormalities and shows partial loss of neurons [Figure - D]. *Allium cepa* extract (200 mg/kg) treated rat brain showed partial loss of neurons [Figure - E]. *Allium cepa* extract (400 mg/kg) treated rat brain showed reduction in the ischemia reperfusion induced histopathological abnormalities and partial loss of neurons [Figure - F].

Discussion

Cerebral ischemia is caused by a deficiency in blood supply to a part of the brain, which in turn triggers various pathophysiological changes. The ischemic brain injury causes temporary loss of oxygen and energy supply and also the generation of ROS. [19] The major pathobiological mechanisms of ischemia/reperfusion (IR) injury include excitotoxicity, oxidative stress, inflammation and apoptosis. [20] Recently the existence of a novel apoptotic pathway in the endoplasmic reticulum (ER) has been demonstrated and the activation of caspase-12 can lead to apoptosis. The overall process of ischemic brain injury is extremely complex. [21] The

brain is very susceptible to the damage caused by oxidative stress, due to its rapid oxidative metabolic activity, high polyunsaturated fatty acid content, relatively low antioxidant capacity, and inadequate neuronal cell repair activity. [20] The inflammatory response to brain injury plays a vital role in the pathogenesis of stroke, characterized by peripheral leukocytes infiltration and neurotoxicity to the cerebral parenchyma. [22] Infiltrated leukocytes exacerbate brain injury. [23]

Allium cepa L. has been reported to have antimicrobial, antispasmodic, anticholesterolaemic, hypotensive, hypoglycemic, antiasthmatic, anticancer and antioxidant properties. From the literature it was found that onion contains polyphenols, anthocyanins, flavonoids, quercetin, kaempferol and their glycosides. [10] These chemical constituents are having potent antioxidant property. Thus, the present study was undertaken to find an effective agent for the treatment of ischemic brain injury where *Allium cepa L.* may prove as a neuroprotective agent against ischemia reperfusion induced brain injury. The antioxidant phytoconstituents present in *Allium cepa L.* may prove to be beneficial treatment for ischemia reperfusion induced brain injury.

In the present study from the behavioral result it was found that there were changes in the neurological status, significant decrease in grasping ability, forelimb strength, loss of balance and co-ordination i.e. motor performance in the ischemic group when compared with sham control animals. The protective effect of two oral doses of aqueous extract of *Allium cepa* i.e. 200mg/kg and 400mg/kg was evaluated in I/R rats with vitamin E as a reference standard. In case of behavioral parameter it was found that there is a significant improvement in neurological status, significant increase in grasping ability and forelimb strength, improvement in balance and in co-ordination i.e. motor performance but no significant changes in memory.

The damage of the central nervous system was evaluated by the neurological deficit scores. Our study demonstrated that aqueous extract of *Allium cepa* significantly decreased the neurodeficit scores. The results with biochemical parameters show that ischemia reperfusion injury has caused oxidative stress. In the ischemic brain, it was found that certain levels of free radicals were already produced during ischemia (without reperfusion). Then, upon reperfusion, a burst of free radical production was observed, this indicates that free radicals produced upon reperfusion may be the direct cause of the subsequent lipid peroxidation. [24] As ROS attacks on biological membrane, leading to oxidative destruction of polyunsaturated fatty acids (PUFAs). These PUFAs are rich in brain, leading to oxygen free radical induced lipid peroxidation (LPO). [19]

Malondialdehyde (MDA) is one of the most sensitive indicators of lipid peroxidation. [25] There was significant increase in the level of MDA in global cerebral ischemia in gerbils indicating oxidative stress. Reperfusion injury in rat brain led to significant increase in MDA levels. [19] The results of the present study, indicates that *Allium cepa* ameliorated LPO, as it significantly decreased the brain tissue MDA level, an effect that could be attributed to its capacity to scavenge reactive oxygen species (ROS). One of the ROS that elevates in cerebral ischemia is nitric oxide radical (NO^-). NO is beneficial as a messenger or modulator, but in conditions such as oxidative stress, it is potentially toxic. Nitric oxide is a molecular mediator of inflammation. The toxic effects of NO may be attributed to $ONOO^-$, which is a reaction product of NO with superoxide (O_2^-). Nitric oxide synthase (NOS) activity and NO release are greatly increased in the acutely ischemic brain. NOS activity increases approximately ten-fold from baseline 10 min after middle cerebral artery occlusion of rat. [19] NO level was significantly increased in response to ischemia/reperfusion in the ischemic group but was found to be reduced in the *Allium cepa* extract treated groups. Thus diminishing of NO by *Allium cepa* might reduce the potential for damage to neurons and supporting elements in the brain observed with ischemia and reperfusion.

Normally there is a balance between the reactive oxygen species generation and the endogenous antioxidant systems superoxide dismutase (SOD), Catalase and glutathione (GSH). Superoxide dismutase is the primary line of defense against tissue damage caused by reactive oxygen species, it catalyzes the dismutation of superoxide anion to hydrogen peroxide and prevents the formation of the hydroxyl radical. [26] However, SOD activity was found to be decreased in the ischemic group, may be due to increased ROS production. Treatment with aqueous extract of *Allium cepa* with a dose 200mg/kg and 400mg/kg significantly increased the level of SOD. Catalase and glutathione peroxidase are generally regarded as the second line of defense by dismutating peroxide into water and molecular oxygen. [26] The reduction in the catalase activity in the ischemic group may result in the accumulation of free radicals leading to oxidative stress. Groups treated with aqueous extract of *Allium cepa* (200mg/kg and 400 mg/kg) was found with increased brain tissue catalase enzyme activity as compared to the ischemic group. GSH, an endogenous antioxidant found in all animals, GSH inhibition in cerebral

ischemia increases the susceptibility of plasma membranes towards peroxide attacks. The loss of GSH and formation of protein–glutathione mixed disulfide (PrSSG) and (GSSG) in the brain results to various membrane dysfunctions, such as inhibition of Na^+/K^+-ATPase activity. [26] Administration of aqueous extract of *Allium cepa* significantly increased the tissue glutathione level. The histopathological changes of brain were assessed by hematoxylin-eosin (H-E) staining in the brain slices. Histopathological studies revealed that ischemia reperfusion caused neuronal loss in the ischemic group. However treatment with vitamin E and aqueous extract of *Allium cepa* leaves showed partial loss of neuron as compared to the ischemic group. Thus administration of aqueous extract of *Allium cepa* leaves after the ischemia reperfusion injury ameliorated histopathological consequences of IR.

Allium cepa has a neuroprotective effect on ischemia reperfusion induced brain injury. The neuroprotective effect is attributed to its ability to scavenge free radical, inhibit nitric oxide production which is an inflammatory mediator, and lipid peroxidation by depleting malondialdehyde (MDA). *Allium cepa* also potentiated the antioxidant defense system by increasing the SOD, catalase and tissue glutathione level. Thus the exact mechanism of action of *Allium cepa* is hypothesized to be its antioxidant activity.

The results and observations indicate that internal carotid artery occlusion causes behavioral deficit, and free radical generation and accumulation leading to oxidative stress. These effects are produced due to weakening of the antioxidant defense system. Therefore, the present study provides the evidence that *Allium cepa* may be useful in the treatment of cerebral stroke. From the observation we conclude that *Allium cepa* attenuates the ischemia-reperfusion induced neurological deficit, decrease in motor performance on beam walk test, hanging wire and rota red test and produced neuroprotective effect through the antioxidant mechanism. Further phytochemical evaluation with isolated purified phytoconstituents of *Allium cepa* may be needed for validating its neuroprotective potential

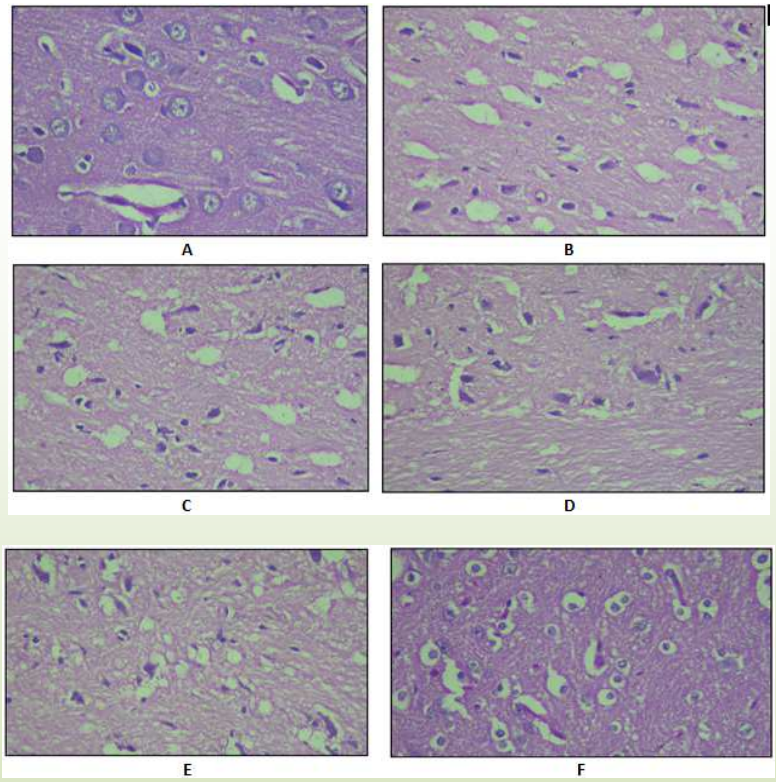

Fig. 1: Photomicrographs of rat brain (H & E stain) Respective treatment showing following changes in brain section (A) sham control rat brain showing normal architecture of neurons (B&C) vehicle treated rat brain showing ischemia reperfusion induced injury showing neuronal loss. (D) vitamin E treated rat brain showing partial loss of neurons (E) aqueous extract of *Allium cepa* leaves (200mg/kg) treated rat brain showing partial loss of neurons. (F) aqueous extract of *Allium cepa* leaves (400mg/kg) treated rat brain showing partial neuronal loss. Magnification 400X

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Table 1: Effect of aqueous leaves extract of *Allium cepa* (ALAC) and vitamin E on neurobehavioral tests in ischemia-reperfusion injury in rats

GROUPS	Group name	Neurodeficit mean score	Hanging wire (mean fall time in sec)	Rotarod (mean fall time in sec)	Beam Walk Test		
					No. of Foot Slip	No. of Falls	Distance Travelled (cm)
1	Sham (control)	0	27.5 ± 3.22	160 ± 19.403	1.6 ± 0.6708	0.3 ± 0.2108	34.6 ± 8.032
2	Ischemia	6.0	6.5 ± 0.6387**	28.66 ± 4.910**	10.2 ± 2.182**	4.8 ± 1.249**	5.8 ± 2.056**
3	Vitamin E + ischemia	3.6	22.16 ± 5.394$	73.5 ± 7.615$	5.9 ± 1.014$	2.0 ± 0.9309$	41.5 ± 7.766†
4	ALAC 200 + ischemia	1.4	19.16 ± 3.833#	69.33 ± 4.937$	4.1 ± 0.3073†	1.3 ± 0.4261$	45.0 ± 5.354†
5	ALAC 400 + ischemia	0.3	26.3 ± 5.542†	82.66 ± 10.607†	3.0 ± 0.3651†	1.8 ± 0.4014$	48.0 ± 4.885†

ALAC and Vitamin E improves the neurobehavioral outcomes in ischemic rats. Neurobehavioral tests such as neurodeficit scores, hanging wire and rota rod test and beam walk test results showed impairment of neurobehavioral deficit in ischemic rat. The neurobehavioral outcomes such as neurodeficit score, grasping ability & forelimb strength, muscle grip strength by rota rod and motor coordination and function by beam walk test of the treated rats showed a significant improvement with high dose of ALAC and vitamin E. The data mentioned is of n=5 in each group and the values are mean ± S.E.M. Data were analyzed by ANOVA followed by Dunnett's test. (*) and (**) indicates a significant difference for ischemia group vs sham control group at $P<0.05$ and $P<0.01$. $^{\$, \dagger}$ indicates a significant difference for treated group vs ischemic group at $P<0.05$ and $P<0.01$. #Nonsignificance. ALAC= Aqueous leaves extract of *Allium cepa*, SEM=Standard error of mean.

Table 2: Effect of aqueous leaves extract of *Allium cepa* (ALAC) and vitamin E on neurobehavioral tests (beam walk test) in ischemia-reperfusion injury in rats

GROUPS	Group name	No. of Foot Slip	No. of Falls	Distance travelled (cm)
1	Sham (control)	1.6 ± 0.6708	0.3 ± 0.2108	34.6 ± 8.032
2	Ischemia	10.2 ± 2.182**	4.8 ± 1.249**	5.8 ± 2.056**
3	Vitamin E + ischemia	5.9 ± 1.014$	2.0 ± 0.9309$	41.5 ± 7.766†
4	ALAC 200 + ischemia	4.1 ± 0.3073†	1.3 ± 0.4261$	45.0 ± 5.354†
5	ALAC 400 + ischemia	3.0 ± 0.3651†	1.8 ± 0.4014$	48.0 ± 4.885†

ALAC and Vitamin E improves the neurobehavioral outcomes with regards to beam walk test in ischemic rats. In beam walk test the results showed impairment of motor performance in ischemic rat. Improvement in motor coordination and function was observed in the treated rats with both the doses of ALAC and vitamin E. The data mentioned is of n=5 in each group and the values are mean ± S.E.M. Data were analyzed by ANOVA followed by Dunnett's test. (*) and (**) indicates a significant difference for ischemia group vs sham control group at $P<0.05$ and $P<0.01$. $^{\$, \dagger}$ indicates a significant difference for treated group vs ischemic group at $P<0.05$ and $P<0.01$. ALAC= Aqueous leaves extract of *Allium cepa*, SEM=Standard error of mean.

Table 3: Effect of aqueous leaves extract of *Allium cepa* (ALAC) and vitamin E on Elevated plus maze in ischemia-reperfusion injury in rats

GROUPS	Group name	Initial Latency	First Retention Latency (after 2 hr)	Second Retention Latency (after 24 hr)
1	Sham (control)	33.33 ± 8.3	73.5 ± 7.384	63.0 ± 3.077
2	Ischemia	75.5 ± 9.179*	23.33 ± 14.364**	10.0 ± 2.989**
3	Vitamin E + ischemia	61.0 ± 11.402 $^{\#}$	45.16 ± 14.45$^{\#}$	8.33 ± 1.801$^{\#}$
4	ALAC 200 + ischemia	66.166 ± 11.044$^{\#}$	30.5 ± 8.192$^{\#}$	7.66 ± 1.585$^{\#}$
5	ALAC 400 + ischemia	61.166 ± 10.628 $^{\#}$	14.83 ± 3.280$^{\#}$	5.33 ± 0.8028$^{\#}$

The effect of two doses of ALAC 200 mg/kg and 400 mg/kg orally and vitamin E 50 mg/kg orally for 7 days in ischemic rats. The transfer latency was significantly increased in ischemic group as compared to sham control group. But no significant reduction in the transfer latency was observed by treatment with ALAC and vitamin E as compared to ischemic animals. In the plus maze task, ALAC and vitamin E has not shown protective effects on ischemia induced impairment in the retrieval of memory. The data mentioned is of n=5 in each group and the values are mean ± S.E.M. Data was analyzed by ANOVA followed by Dunnett's test. (*) and (**) indicates a significant difference for ischemia group vs sham control group at $P<0.05$ and $P<0.01$. $^{\#}$Nonsignificance. ALAC= Aqueous leaves extract of *Allium cepa*, SEM=Standard error of mean.

Table 4: Effects of aqueous leaves extract of *Allium cepa* (ALAC) and vitamin E on glutathione, SOD, catalase Nitric oxide and MDA in brain tissue of ischemia-reperfusion injury in rats

GROUPS	Group name	Glutathione µmole/g brain	SOD U/mg protein	Catalase nmol of H_2O_2 consumed/min/mg protein	Nitric oxide (µmols)	MDA nmol of MDA g^{-1} Protein
1	Sham (control)	5.228 ± 0.374	110.982 ± 7.97	600.6344 ± 33.71	32.1 ± 3.90	4.426 ± 0.518
2	Ischemia	3.032 ± 0.827*	53.012 ± 4.10**	295.106 ± 26.17**	50.44 ± 6.33*	6.536 ± 0.753*
3	Vitamin E + ischemia	5.448 ± 0.387$^{\$}$	98.294 ± 3.66$^{\$}$	481.304 ± 26.59†	27.76 ± 7.14$^{\$}$	4.378 ± 0.450$^{\$}$
4	ALAC 200 + ischemia	5.258 ± 0.289$^{\$}$	113.232 ± 20.32†	480.876 ± 51.62†	29.24 ± 2.62$^{\$}$	3.328 ± 0.136†
5	ALAC 400 + ischemia	6.096 ± 0.737†	124.338 ± 14.19†	536.954 ± 32.19†	24.31 ± 2.06†	3.028 ± 0.253†

The levels of glutathione and nitric oxide were significantly decreased and the enzyme activities of SOD and catalase were significantly decreased and an increase in MDA levels was observed in ischemic group as compared to sham control. The glutathione and nitric oxide levels were significantly increased, activities of superoxide dismutase and catalase enzymes were significantly increased and a reduction in the levels of MDA was seen by ALAC and vitamin E treatment as compared to group 2. The data mentioned is of n=5 in each group and the values are mean ± S.E.M. Data were analyzed by ANOVA followed by Dunnett's test. (*) and (**) indicates a significant difference for ischemia group vs sham control group at $P<0.05$ and $P<0.01$. $^{\$, \dagger}$ indicates a significant difference for treated group vs ischemic group at $P<0.05$ and $P<0.01$. $^{\#}$Nonsignificance. ALAC= Aqueous leaves extract of *Allium cepa*, SEM=Standard error of mean

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