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PREISCHEMIC ADMINISTRATION OF CITICOLINE EXERTS BETTER NEUROPROTECTION AND RESTORES NEUROCHEMICAL AND MOTOR FUNCTIONS IN MCAO/R RATS

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ABSTRACT

In the present study, the efficacy of citicoline in alleviating the ischemia/reperfusion neuronal injury was investigated in male Sprague Dawley rats. Citicoline was administered before inducing ischemia, during ischemia and after reperfusion. Animals were scored for neurological deficits, assessed for anxiety, cognition and motor activity. Sodium potassium ATPase, Glutamate, ATP, NAD was determined and western blot analysis of TNF-α and IL-10 was performed. Histopathology of the brain was performed. Citicoline has recuperated neurological deficits in the rats treated with citicoline before ischemia. Sodium potassium ATPase, ATP, NAD was increased and glutamate level was decreased in the Pre-ischemic L-NAME group than the other treatment groups. In addition to this, expression of TNF-α was down regulated, IL-10 was upregulated, and percentage neuronal damage was greatly reduced in the preischemic citicoline group. Hence, Pre-ischemic administration of Citicoline exhibits better neuroprotection in middle cerebral artery occluded / reperfused rats.

Key words: Citicoline, Glutamate, Neurological deficit, Motor activity, Cognition, Middle cerebral artery occlusion / reperfusion

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INTRODUCTION

Cerebral ischemia leads to diminished blood flow to the neurons, subsequently leading to enduring physical disabilities such as lack of balance and coordination, hemiplegia or even mortality[1]. It is reported that around 2 million neurons die every minute in the absence of an effective therapy[2]. Aforesaid complications ensue due to neuronal death owing to progression of ischemia, which is attributed to the interaction of complex pathophysiological processes such as energy failure, glutamate excitotoxicity, mitochondrial dysfunction and oxidative stress[3]. The increase in the glutamate level activates phospholipases leading to the hydrolysis of phospholipids[4] and release of arachidonic acid[5]. Subsequent oxidation of arachidonic acid contributes to the generation of reactive oxygen species[6]. Increased reactive oxygen species leads mitochondrial mutilation, which in turn results in the imbalance between the pro-oxidant and antioxidant levels augmenting oxidative stress[7]. Oxidative stress is the primary factor in neuronal death in acute brain injury, since, the disruption of the neuronal membrane is identified to be the primary and crucial cause for the debilitating ischemic events[8,9,10]. Sahota *et al.,* demonstrated citicoline as one of the promising candidates for acute neuroprotection in clinical trials[11]. In spite of the significant neuroprotection demonstrated with citicoline at preclinical levels[12,13] still citicoline is not a drug of choice in the treatment of cerebral stroke. One of the possible reasons may be the unclear information on its suitable therapeutic time window. Current investigation has been performed to determine the suitable therapeutic time window of citicoline. In the current study, citicoline was administered at three different time points, i.e. at pre (1h before the ischemia), during (1h after the onset of ischemia) and Post-ischemic (3h after reperfusion). Optimal neuroprotection afforded by citicoline is measured in terms of neurological functioning, behavior (anxiety, cognition and motor activity), biochemistry (glutamate, sodium potassium ATPase, ATP and NAD), molecular (TNF-α and IL-10) and cellular (Histopathology) parameters in middle cerebral artery occluded / reperfused (MCAO/R) rats.

MATERIALS AND METHODS

Chemicals

Citicoline (Citilin^{TM}) and 4-0 Nylon monofilament TM Ethicon was purchased from the Pharmacy of Sri Ramachandra University, India. ATP and NAD were purchased from M/s. SISCO Research Laboratories (India). Primary antibody for TNF-α (#sc-52746) and IL-10 (#sc-365858), and HRPO-conjugated goat anti-mouse secondary antibody (#sc-365858) were purchased from Santa Cruz Biotechnology (USA). All other chemicals were purchased from Himedia laboratories, India and were of analytical grade.

Animals and Ethics Approval

The study was approved by the Institutional Animal Ethics Committee (IAEC/XXXIII/SRU/250/2013). Male Sprague Dawley rats of body weight 290 to 340 g were used in the study. Rats were housed individually under controlled environment. Air exchange and air cycle were maintained at 55:45 and 12-15 cycles / h respectively. 22±3°C and 30-70% temperature and relative humidity respectively was maintained. Photoperiod of 12 h light and 12 h dark was sustained throughout the study. Animals were fed with extruded pelleted feed and purified water *ad libitum.*

Middle cerebral artery occlusion and reperfusion (MCAO / R)

Transient focal cerebral ischemia was developed in rats using middle cerebral artery occlusion / reperfusion as described by Longa *et al* with minor modifications[14]. Rats were anesthetized by injecting 350mg/kg chloral hydrate intraperitoneally. An incision was made in the upper thoracic region and the bifurcation of right common carotid artery was exposed. 4-0 nylon filament coated with 0.01% poly-l-lysine was inserted into the external carotid artery after making a nick. The filament was advanced into the internal carotid artery until a resistance is felt. The filament was held in position by tying it with the internal carotid artery and the external incision was sutured. After 2h of ischemia, the

suture is removed and the filament was slowly pulled out to allow reperfusion.

Animal grouping and experimental design

Animals were segregated into five groups, i.e., sham, IR, pre, during and Post-ischemic/ reperfusion group. Each group consisted of 12 Sprague Dawley rats. Sham operated group did not undergo the middle cerebral artery occlusion/reperfusion, the only external incision was made and the animals were sutured. The IR group underwent MCAO/R surgery. Both sham and IR group received 0.9ml of saline. Pre, during and post underwent MCAO/R surgery and received 250mg/kg b.w. of citicoline[15,16,17,18] before surgery (1h prior to surgery), during (2h after ischemia) and after surgery (3h after the onset of reperfusion). Ischemia and reperfusion time was fixed based on the earlier research[19]. 72h after reperfusion, animals were scored for neurological deficit and then sacrificed. The brains of the sacrificed animals were rapidly removed. For TTC staining brains were collected after transcardiac perfusion of saline and stored under frozen condition. For biochemical and protein expression, ipsilateral striatum of the brain was immediately dissected over an ice-cold plate following the atlas of Paxinos and Watson[20].

Assessment of neurological deficit

Neurological deficit was scored following the modified method of Bederson scoring[21]. The score was allotted as followed,

Rats showing no visible signs of neurological deficit - Score 0
Flexion of the contralateral forelimb - Score 1
Decreased defiance to lateral push - Score 2
Impulsive movement in all directions - Score 3
Circling - Score 4

Assessment of anxiety using elevated plus maze

The anxiety of the rats was assessed using elevated plus maze following modified method of Lister[22]. Elevated plus maze consists of two opposite arms in which one arm is open (50cm×10cm×0.5cm) and the other arm is closed (50cm×10cm×40cm) at both the ends respectively. An open platform of about 10x10cm is located at the center of the arms. The height of the maze is 50cm. Rats were placed on the central platform facing one of the open arms. The collective time spent and the number of visits into open arm was assessed for a period of 5 mins. An open arm entry was considered, when the rat places both the fore paws and hind paws in the open arm.

Assessment of cognition using Y- Maze

Cognition of the rats was assessed using Y-maze following the method of Akwa[23]. The three arms of the maze were randomly assigned as start arm – arm in which rat starts exploring, novel arm – which is chunked during the first exercise and opened during the second exercise and the third arm is left open during all the exercises. Ocular clues were hung in the maze during the trials and test. Rats were pre trained keeping the novel arm closed. After an h of break, test was carried out, in which the novel arm was opened and the novel vs familiar behavior of the rats were assessed by comparing the behavior in all the three arms. Rats were placed in the same arm (start arm) during pre training and test. The number of visits and the time spent in each arm was recorded for 5 mins.

Assessment of motor activity using Beam walk

Motor activity of the experimental rats was performed using beam walk test following the method of Sathiya[24]. Rats were trained before MCAO/R to pass through a narrow beam to reach a dark goal box. A bright light was placed above the narrow beam to create aversion for the rat. Animals were placed on the open end of the beam for assessment after MCAO/R. The time taken by the rat to pass through the beam, number of foot faults and the period of immobility were recorded. The maximum time of 60 seconds was provided for the rat to travel the beam.

Estimation of Sodium Potassium ATPase (Na^+K^+ ATPase)

Na^+K^+ATPase was estimated following the method of Sovoboda and Mossinger[26]. 50µl of 600mM Nacl, 50µl of 50mM KCl, 50 µl of 80 mM ATP and 50 µl of 1 mM sodium EDTA was added to 250µl of 184mM Tris HCl and pre incubated for 10 mins at 37°C and then the mixture was added to 25µl of 10% homogenate and again incubated for an hour at 37°C. The reaction was arrested by adding 10% TCA and the precipitate was centrifuged at 3500 rpm for 10 mins and released inorganic phosphorous was determined using Spectrophotometer (Perkin Elmer) at 640 nm. The protein content in the brain homogenate was estimated by the method of Lowry[25].

Estimation of Glutamate

Glutamate was estimated following the method of Babu[27]. Brain was homogenized in HCl of 0.1 N and 80% ethanol. This was then centrifuged at 4500 rpm at 25°C for 20 mins. Supernatant was collected and used for estimation of glutamate. Glutamate content was estimated using HPTLC (CAMAG – Version 1.3.4 USA). Stationary phase consisted of silica gel GF_{254} and the mobile phase was prepared using n-butanol, glacial acetic acid, water in the ratio of 65:15:25 (v/v). 0.2% ninhydrin is used for detection and wavelength of 486 nm is utilized for the determination.

ATP and NAD content

ATP and NAD were determined following the method of Zhang[28]. Brain homogenate was sonicated with ice cold per chloric acid of 0.1 N. The homogenate was then centrifuged at 14000 rpm for 5 mins under 4°C. Supernatant was collected and neutralized with 1 N NaOH and analyzed in reverse phase HPLC (Perkin Elmer). The hypersil C18 column was used as stationary phase and a mixture of KH_2PO_4 and K_2HPO_4 was used as mobile phase. 254 nm is used as the detection wavelength.

Determination of TNF-α and IL-10

Expression of TNF-α and IL-10 was determined using western blotting following the method of Lee[29] using β-actin as a marker. Brain sample was homogenized with 0.1 M ice cold Tris HCL buffer and centrifuged at 3500 rpm for 10 mins. The protein content of the supernatant was determined using Bradford reagent. Aliquots containing 50µg of protein was loaded along with β-mercaptoethanol and bromophenol blue in 12 % SDS PAGE. Seggregated proteins were transferred to PVDF membranes and blocked overnight with 3% BSA in Tris buffered saline. The membrane was then washed in Tris buffered saline and probed with primary antibodies and then again washed with Tris buffered saline thrice for 5 mins and then probed with secondary antibodies. Finally, the membrane was incubated with BCIP/NBT substrate for 10 mins. Bands were visualized under a scanner and quantified using Bio ID software (Vilber Lourmat, Marne-la-Vallee, France).

Histological examination

Animals were euthanized after reperfusion, and their brains were dissected and fixed in formalin (10%). Brain sections of about 4-5µm thickness was prepared and stained with Haematoxylin and Eosin (H&E) after processing. Changes in the cortex, hippocampus (CA1, CA2 & CA3), striatum and hypothalamus were examined and lesion scored as follows,

0-10% : No morphological changes and few dark stained cells - Score 1
11-30% : Mild oedema or dark neurons or pyknotic cells - Score 2
31-50% : Moderate number of dark neurons - Score 3
51 -70% : Moderate edema, necrosis and severe morphological changes - Score 4
71-100% : Severe oedema, necrosis and infarction - Score 5

The sum of histological scores of cortex, hippocampus (CA1, CA2 & CA3), striatum and hypothalamus was calculated and expressed as percentage damage.

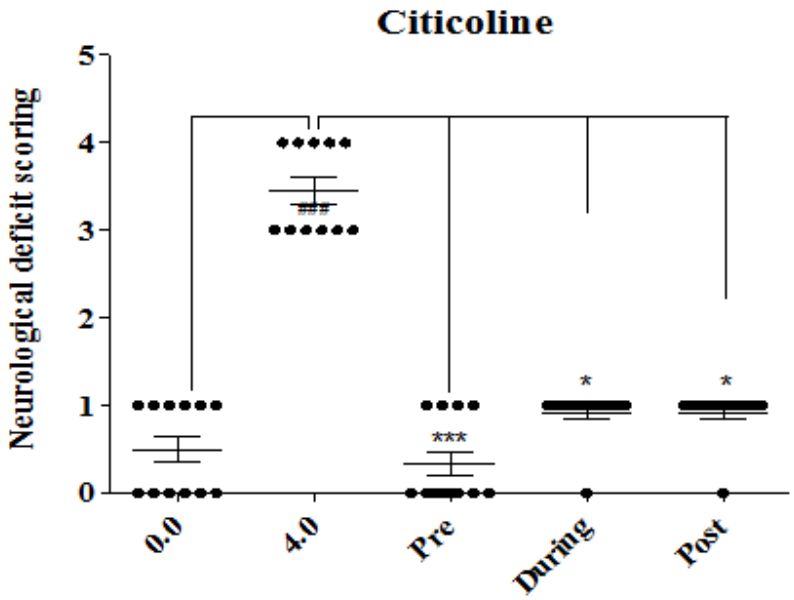
Statistical Analysis

Neurochemical, biochemical and behavioral data were expressed as mean±SEM. One way ANOVA followed by Tukey's multiple comparison tests as post hoc was performed to find the statistical differences between the groups. For neurological deficits, the values are represented as median and expressed in interquartile range. Comparisons of the neurological scores were analyzed with Mann-Whitney U test. GraphPad Prism 5 software (version 5.03) was used for all statistical calculations. Statistical significance was set at $p< 0.05$.

RESULTS

Effect of Citicoline on Neurological Deficit

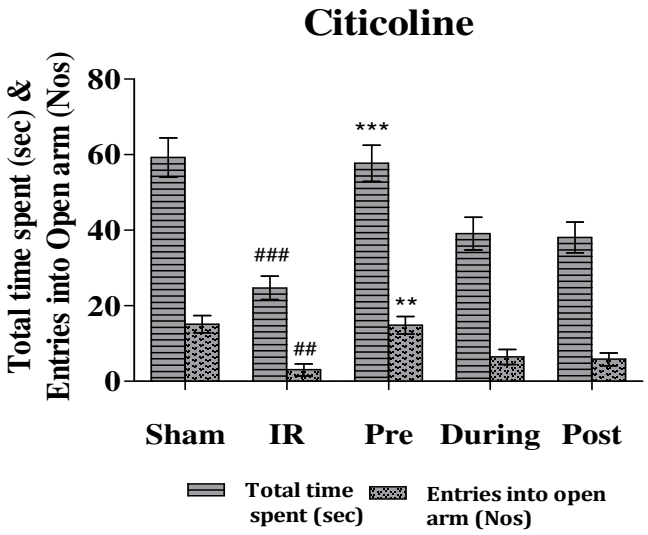
Ischemic reperfusion (IR) rats exhibited severe (median = 4.0, $p<0.001$) neurological deficits such as continuous spontaneous movements in all directions, decreased reaction to lateral push and circling when compared to the sham operated. Pre-ischemic administration of Citicoline when administered prior to ischemia significantly ameliorated neurological deficit (median = 0.0 $p<0.001$) than during and post-ischemia respectively (median = 1.0 $p<0.05$). Among the three pre-ischemic citicoline exerts better neurological functioning (Fig. 1).



*Data expressed as Mean±SEM, # indicates comparison between sham and IR (### - p<0.001), * indicates comparison between IR and treatment groups (*- p<0.5 & *** - p<0.001).*

Effect of Citicoline on Anxiety

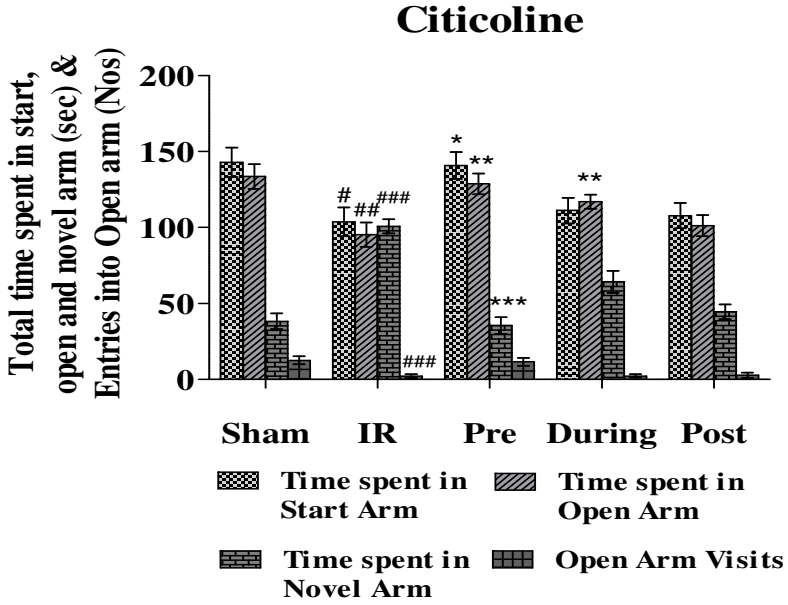
In the elevated plus maze, a significant decrease in the time spent and the number of visits into the open arm was observed in the IR group than the sham operated [(F (4, 54) = 10.76, $p<0.01$) & (F (4, 54) = 7.54, $p<0.01$)]. Pre-ischemic administration of citicoline has significantly increased the time spent in the open arm and also increased the number of visits in the open arm ($p<0.001$ & $p<0.05$). An insignificant increase in the time spent and the number of entries into open arm was observed in during and Post-ischemic rats (Fig. 2). The pre-ischemic citicoline administration shows improved behavior in elevated plus maze.



*Data expressed as Mean±SEM, # indicates comparison between sham and IR (### - p<0.001), (## - p<0.01), * indicates comparison between IR and treatment groups (**- p<0.01 & *** - p<0.001).*

Effect of Citicoline on Cognition

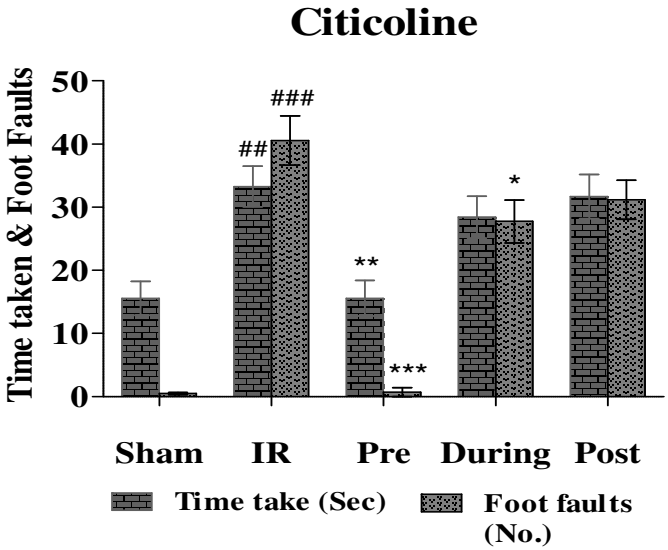
In the Y maze, time spent in the start and open remained almost the same in the IR group when compared to the sham operated [(F (4, 54) = 3.777, p<0.05), (F (4, 54) = 4.574, p<0.01). Wherein, there was a significant decrease in the entries into the novel arm in the IR group than sham operated (F (4, 54) = 37.60, p<0.001)] respectively. Pre-ischemic rats, had significantly spent more time in start and open arm, than novel arm (p<0.05, p<0.01 & p<0.001) respectively. An insignificant increase in the time spent in the start and open arm was observed in, during and post-ischemic rats. On the contrary a significant increase in the time spent in the novel arm was observed in, during ischemic group (p<0.01). However, a nonsignificant increase in the number of entries into the novel arm was observed in the post-ischemic rats respectively. Thus, administration of citicoline prior to IR improves memory (Fig. 3



*Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.5, ## - p<0.01 & ### - p<0.001), * indicates comparison between IR and treatment groups (*- p<0.5, ** - p<0.01 & *** - p<0.001).*

Effect of Citicoline on Motor activity – Beam Walk Test

In the beam walk assessment, the duration of walk on the beam and the number of foot faults was increased in the IR rats than the sham operated [F (4, 54) = 5.328, $p<0.01$ & F (4, 54) = 48.65, $p<0.001$]. Pre-ischemic administration of citicoline has decreased the duration taken to reach the goal box and also reduced the number of foot faults than during and Post-ischemic treatment ($p<0.01$ & $p<0.001$) respectively. However, there was an insignificant decrease in the time taken to reach the goal box in during and post-ischemic treatment. Whereas, a significant reduction in the number of foot faults in during ischemic group ($p<0.05$) was observed than Post-ischemic treatment. Hence, it is evident that the pre-ischemic administration of citicoline improves motor behaviour in MCAO/R rats (Fig. 4).



*Data expressed as Mean±SEM, # indicates comparison between sham and IR (## - p<0.01 & ### - p<0.001), * indicates comparison between IR and treatment groups (*- p<0.5, ** - p<0.01 & *** - p<0.001).*

Effect of Citicoline on Na^+K^+ATPase

Na^+K^+ATPase was significantly decreased [F (4, 57) = 15.10, $p<0.001$] in the ipsilateral striatum of the IR brain when compared to the sham operated. Citicoline when administered pre- ischemically has significantly increased Na^+K^+ATPase ($p<0.001$) than during and post. A slight non significant increase in Na^+K^+ATPase was observed in the groups which received citicoline during and post-ischemic. Thus, pre-ischemic administration of citicoline has improved the Na^+K^+ATPase activity (Fig. 5A).

Effect of Citicoline on Glutamate

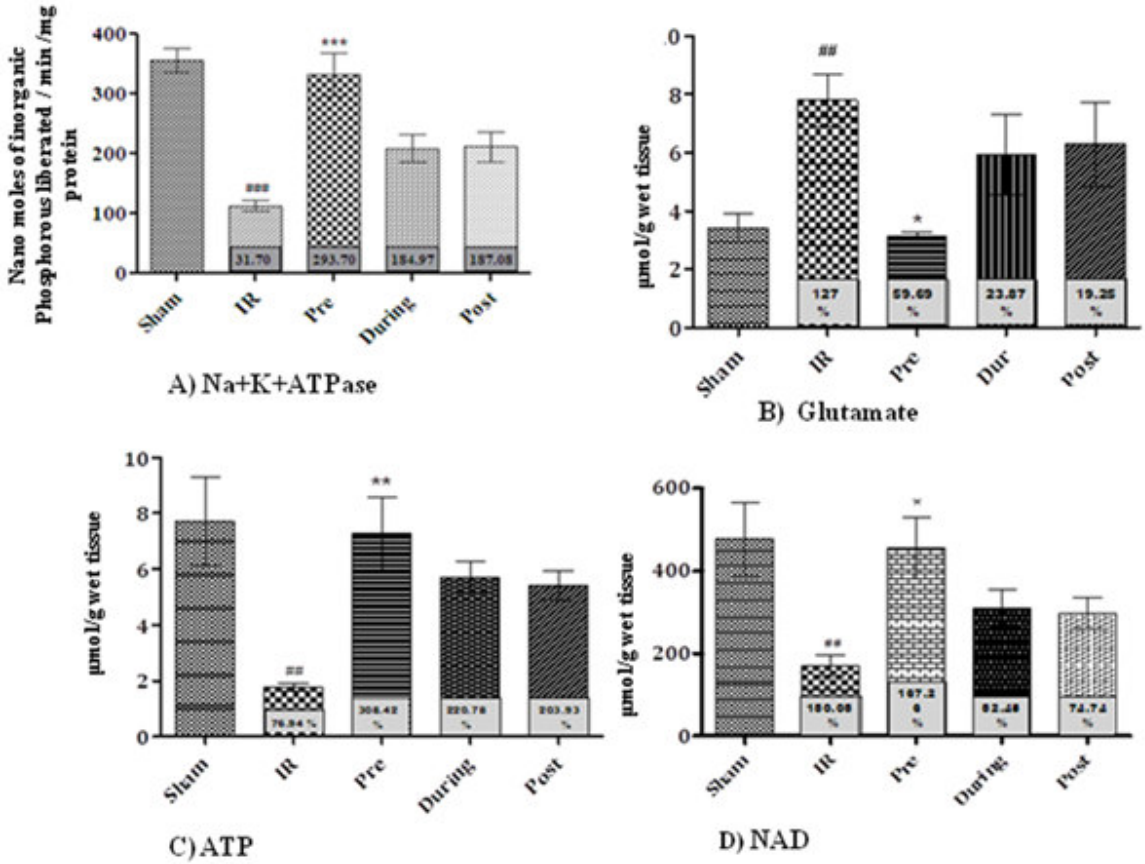
A significant increase [F (4, 54) = 3.760, $p<0.01$] in the striatal (ipsilateral) glutamate content was seen in the ischemic reperfusion (IR) group than the sham operated. Pre-ischemic administration of citicoline has significantly alleviated glutamate content ($p<0.05$). Whereas, in during and post-ischemic group, only a slight nonsignificant reduction in the glutamate level was observed. Hence, pre-ischemic administration of citicoline has significantly lowered the ipsilateral glutamate content (Fig. 5B).

Effect of Citicoline on ATP and NAD

A considerable decrease in the ATP level was observed in the brains of the IR group than the sham operated [F (4, 54) = 1.78, $p<0.01$]. Yet pre-ischemic administration of citicoline has significantly increased the ATP level than ischemic, during and Post-ischemic group respectively ($p<0.01$). A nonsignificant increase in the ATP level was observed in during and Post-ischemic group when compared to the ischemic group (Fig. 5C). Pertaining to NAD, a significant decrease in the level of NAD was

observed in the IR group than the sham operated [F (4, 54) = 4.220, p<0.01]. Whereas, a significant increase in the NAD level was observed in pre- ischemic group, than during and post-ischemic group (p<0.05). ATP and NAD levels were found to be significantly augmented in the pre-ischemic than during and Post-ischemic group. However, pre-ischemic administration of citicoline was found to augment both ATP and NAD levels when administered pre-is chemically (Fig 5D).

Fig 5. Effect of Citicoline on Na+K+ATPase, Glutamate, ATP and NAD

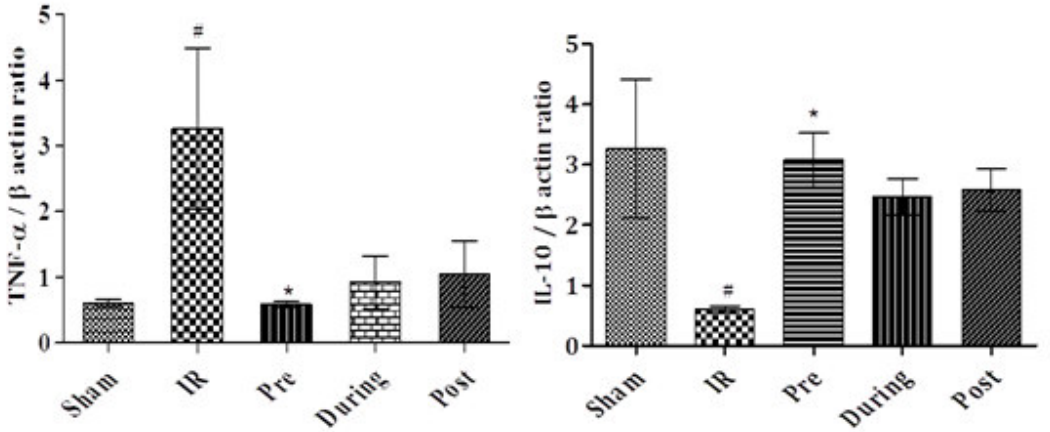


Data expressed as Mean±SEM, # indicates comparison between sham and IR (## - p<0.01 & ### - p<0.001), * indicates comparison between IR and treatment groups (*- p<0.5, ** - p<0.01 & *** - p<0.001).

Effect of Citicoline on the expression of TNF- α and IL-10

TNF-α was upregulated in the MCAO/R group than the sham operated [F (4, 54) = 3.437, p<0.05]. Pre-ischemic administration (p<0.05) of citicoline has down regulated the expression of TNF-α than during and Post-ischemic group. Slight nonsignificant decline in the expression of TNF-α was observed in during and Post-ischemic groups respectively. With regard to IL-10, it is down regulated in the MCAO/R group than the sham operated [F (4, 54) = 2.943, p<0.05]. Expression of IL-10 was also observed to be significantly up regulated in the pre-ischemic rats (p<0.05) than the ischemic group. However, during and Post-ischemic administration of citicoline had nonsignificantly up regulated the expression of IL-10. Pre-ischemic administration of citicoline has upregulated IL-10 and down regulated TNF-α than the other groups (Fig 6).

Fig 6. Effect of Citicoline on TNF-α and IL-10

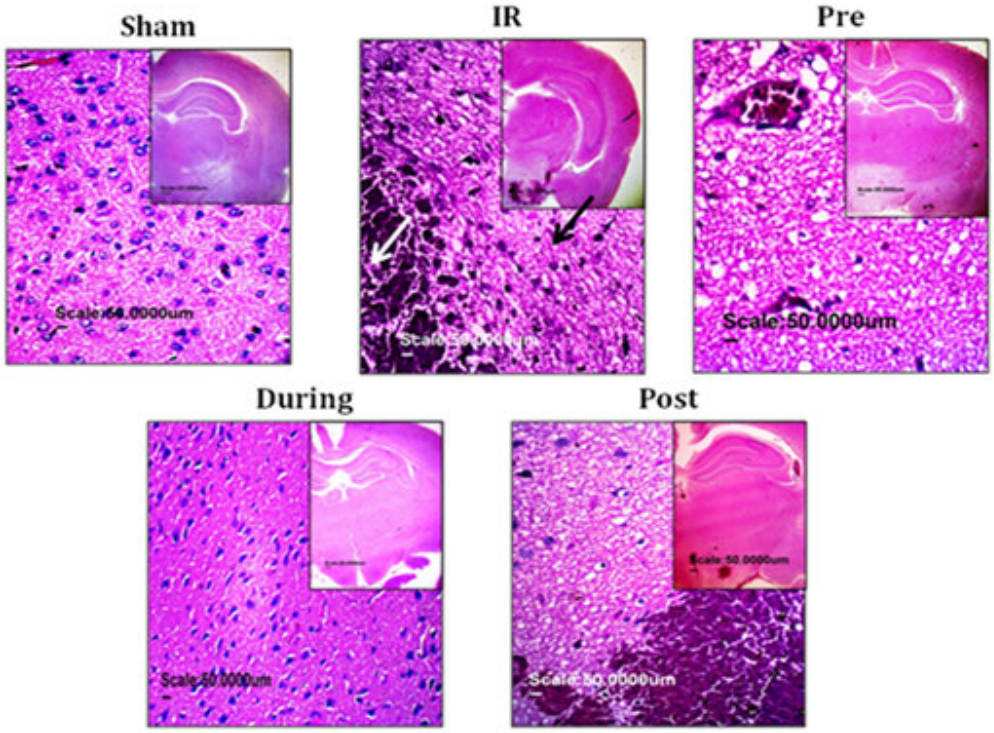


Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.5, ## - p<0.01 & ### - p<0.001), * indicates comparison between IR and treatment groups (*- p<0.5, ** - p<0.01 & *** - p<0.001).

Effect of Citicoline on Histopathology

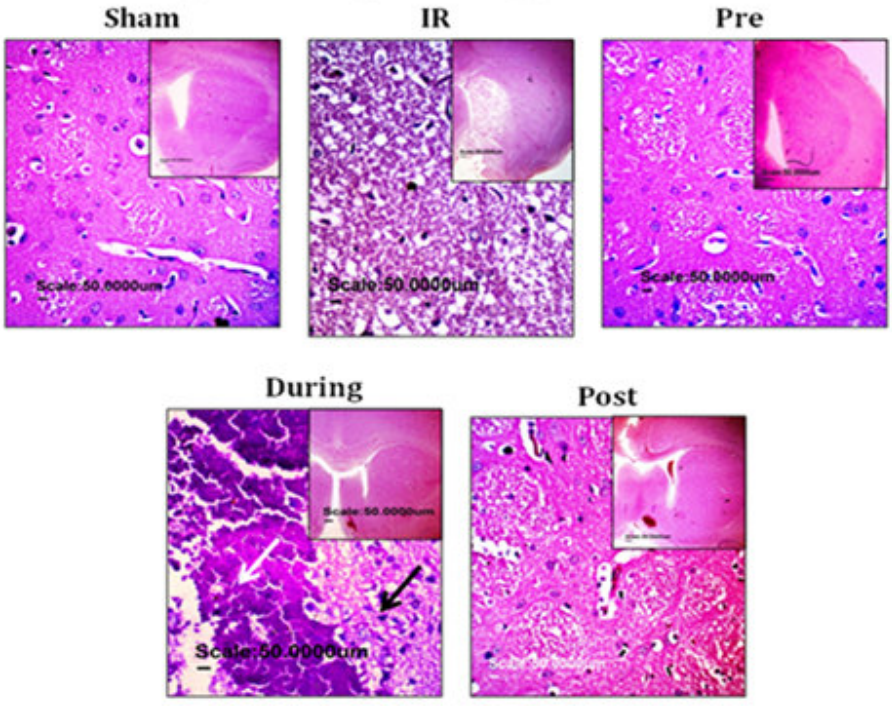
Occlusion of middle cerebral artery in rats induced severe neuronal damage in the ischemic reperfusion rats (IR) than sham operated (1.09%). The IR group exhibited about 73.5% neuronal damage characterized by necrotic focus in the hippocampus and cortex, and infarction in the hypothalamus with pyknotic cells in the penumbra. The striatum of the IR group also appeared severely vacuolated with the dissolution of the neurophil. Conspicuous reduction in neuronal damage was revealed in the pre- ischemic group, where in, the striatum appeared mildly vacuolated with few dark stained cells. In the, during ischemic group, infarct at the pre- optic area was observed, along with discernible vacuolation and nuclear shrinkage in the penumbra. In the Post-ischemic group necrotic foci in the cortex and infarction at the pre- optic area were observed. The penumbra of the infarct revealed marked vacuolation, shrunken, and degenerating cells with apparent loss of neurons. Neuronal damage of 20.5 %, 34.7 % and 44.2 % was observed in the pre-, during and post-ischemic groups respectively. Neuronal damage in all the treatments was greatly reduced when compared to the IR group. Amongst all the three treatments, substantial reduction in the neuronal damage was observed in the pre- ischemic treatment (Fig 7, 8 & 9).

Fig 7. Histopathology – Pre optic Area



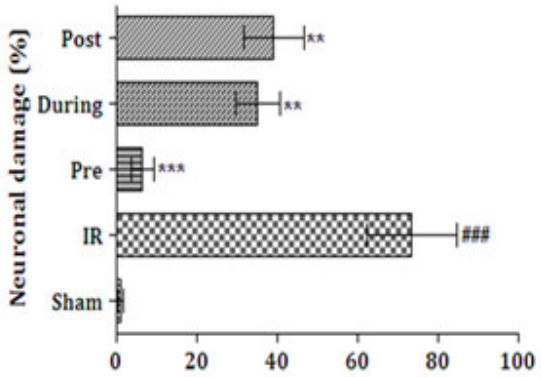
Histological changes in the pre optic area in different treatment groups of citicoline. Infarct (white arrow) and Penumbra (dark arrow) (Bar = 50 µm). Inset: Subgross view of ipsilateral brain hemisphere (Bar = 50 µm).

Fig 8. Histopathology – Striatum



Histological changes in the striatum in different treatment groups of citicoline. Infarct (white arrow) and Penumbra (dark arrow) (Bar = 50 µm). Inset: Subgross view of ipsilateral brain hemisphere (Bar = 50 µm).

Fig 9. Histopathology - Percentage damage



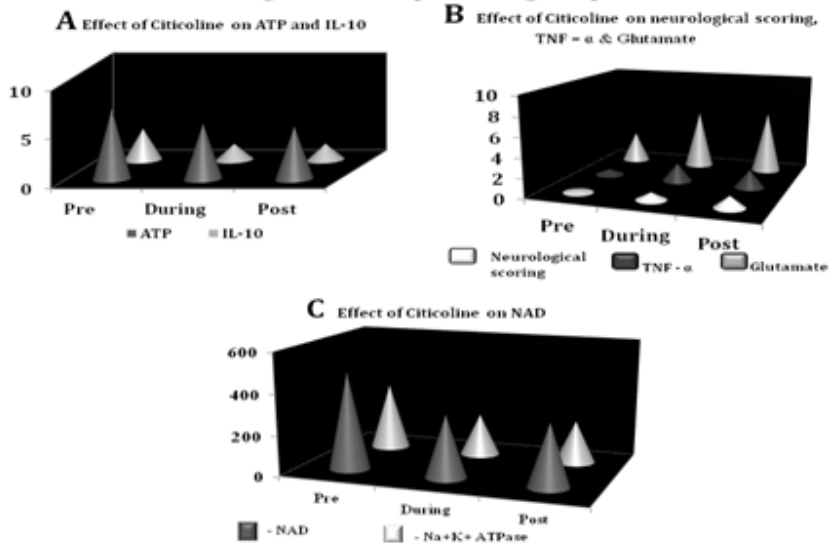
*Effect of Citicoline on percentage neuronal damage. Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.5, ## - 0.01, ### - p<0.001), * indicates comparison between IR and treatment groups (* - p<0.5, ** - 0.01, *** - p<0.001).*

DISCUSSION

Although the efficiency of citicoline has already been established preclinically, a comparative study on the effect of citicoline, a membrane stabilizer, when administered before ischemia (prophylactic) and after ischemia (treatments) has not yet been investigated. The current study has been performed as an initiative to compare the prophylactic and treatment effects of citicoline for transient focal cerebral ischemia. Various parameters such as behavior, motor function, anxiety, sodium potassium ATPase, glutamate, ATP and NAD, were studied along with histological examination. Middle cerebral artery occluded / reperfused rats underwent various behavioral tests such as an elevated plus maze, open field exploration to assess the anxiety, spontaneous and exploratory behavior respectively. Motor activity of the MCAO/R rats was assessed using rota rod and beam walk test. Besides, cognition of the experimental rats was determined using Y-maze. The current study demonstrated that pre- ischemic administration of citicoline recuperates neurological functioning and behavior in IR rats (Fig 8). Pre-ischemic administration of citicoline has significantly improvised neurological deficit and in the elevated plus maze test, it has increased the number of entries into the open arm and considerably decreased the time spent in the novel arm which exemplifies the anxiolytic behavior of the experimental rats. In addition, it was also observed that the number of ambulation was substantially increased and the immobility period was decreased in the open field experiment which demonstrates the reversal of spontaneous and exploratory behavior of the pre- ischemic animals. However, there were no changes in the rearing and grooming behavior of the treated animals. On the other hand, the time spent in the novel arm and the other two arms was significantly decreased and increased in the pre- ischemic group respectively. Convalescence of the motor activity was observed in the pre- ischemic group which is evident from the results of rota rod and beam walk test. The findings of the current study are in corroboration with the study of Hurtado (2007 & 2008) that is, persistent treatment with CDP-choline improves functional recovery and neuronal plasticity[30,31]. In addition to this, Hurtado also reported the increase in dendrites of pyramidal neurons cortex. Sobrino demonstrated that citicoline considerably increased the circulating endothelial progenitor cells (EPC), the primer of neurogenesis in a prospective study[32]. Gutierrez demonstrated that the treatment of stroke animals with CDP-choline considerably perked up the functional behavior besides the reduction in lesion volume and down regulation of low-density lipoprotein receptor-related protein (LRP)[33]. The potency of citicoline to induce neurogenesis is proposed to play vital role in restoring the neurobehavioral and motor functions of the experimental rats[34]. With regard to cognition, the prospective of

citicoline in synthesizing phosphatidylcholine, which helps in the maintenance of cell membrane integrity plays crucial role[35]. Further, the resurgence of membrane integrity also recoups mitochondrial ATPase, membrane Na+/K+ ATPase, impedes apoptosis and thereby improves cognition[36,37]. Further, the role of citicoline in improving cognition may also be related to its role in increasing phospholipids which is important in neurotransmission and also in augmenting the levels of norepinephrine and dopamine[38]. Alleviation of motor activity may be correlated with the cholinergic activity of citicoline[39]. Citicoline was able to protect the motor neurons and the cerebellar granule cells against glutamate excitotoxicity mediated apoptosis[40,41]. The neuroprotective effect of citicoline may also be attributed to its anti-glutamatergic effects. Citicoline decreased and increased the expression of TNF- α and IL-10 respectively in the current study. The reason for this may be due to the potential of citicoline to decline the accumulation of free fatty acids and in down regulating the expression of pro-inflammatory markers. Citicoline also exhibits anti inflammatory activity by attenuating the phospholipase A2 activation[42]. These findings are in corroboration with animal studies, wherein, it is demonstrated that prior intracerebral administration of citicoline reduces free fatty acids and arachidonic acid [43]. Previous studies revealed that citicoline also enhances the dilation of blood vessels leading to increased cerebral blood flow[44]. In the present study pre- ischemic administration of citicoline has decreased the neuronal damage in the histopathological studies. This finding is in corroboration with the report that immediate administration of citicoline for a period of three weeks lowered apoptosis and white matter damage[45]. In addition to this, it is also reported that citicoline increases BrdU/NeuN positive cells in the dentate gyrus, subventricular zone and in the peri-infarct area and also decreases GFAP expression in the peri infarct area after stroke[1,12]. The efficacy of citicoline in the attenuation of neurobehavioral and sensory motor deficits is attributed to its neuro rejuvenating, membrane modulating, anti inflammatory and its anti-glutamatergic potential. The ability of citicoline to exert surpassing neuroprotective and neurorepair ability may be attributed to the fact that it preserves the membrane integrity even before the onset of ischemic event and also to its impending ability to restore the neuronal affront.

Fig 10. Effect of pre, during and post citicoline



Effect of pre, during and Post-ischemic treatment of Citicoline on A) ATP AND IL-10 B)Neurological Scoring, TNF – α and Glutamate C) NAD.

CONCLUSION

It is construed from the current investigation that citicoline, a membrane stabilizer exerted better prophylactic effect in the middle cerebral artery occluded/reperfused rats by improving neurological deficit, attenuating behavior, motor activity, increasing Sodium Potassium ATPase and alleviating glutamate excitotoxicity.

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