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Pre Ischemic Administration of L-NAME Reinstates Behavior, Improves Motor Activity and Alleviates Excitotoxicity in Middle Cerebral Artery Occlusion / Reperfusion Rats

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Abstract

Objectives: To study the effect of NG nitro L Arginine Methyl Ester (L-NAME) on anxiety, cognition, motor activity and neuronal damage when administered pre, during and post ischemically in rats. **Methods:** L-NAME was administered 30 minutes before inducing ischemia (Preischemic), 1 hour after ischemia (During ischemic) and 3 hours after reperfusion (Post ischemic). After 72 hours of reperfusion, animals were assessed for anxiety using elevated plus maze test, cognition using Y-maze test and motor activity using beam walk and rota rod test. Rats were euthanized using carbon di oxide and the brains were collected. Neuronal damage was studied in the brain sections using hematoxylin and eosin stained brain sections. **Findings:** Pre ischemic administration of L-NAME has increased the time spent ($p<0.001$) and number of entries ($p<0.01$) into the open arm in the elevated plus maze than during and post ischemic administration. In the Y-maze test, pre ischemic administration of L-NAME has increased the time spent ($p<0.001$) and number of entries ($p<0.05$) in the open arm than start and novel arm. During and post ischemic administration of L-NAME has increased the time spent in open arm ($p<0.05$) however there was no change in the number of entries into the open arm. In the rota rod test, pre ischemic administration of L-NAME had significantly increased the fall time than ($p<0.001$). A non significant increase in the fall time was observed in the rota rod test in the during and post ischemic group. In the beam walk test, time taken to reach the goal box ($p<0.01$) and the number of foot faults ($p<0.001$) were decreased significantly in the pre ischemic L-NAME group than during and post ischemic administration. Pre ischemic administration of L-NAME has also significantly reduced the percentage neuronal damage. **Novelty of the study:** Though the effect of L-NAME on middle cerebral artery occluded / reperfused rats has been demonstrated in many previous studies, it fails in the clinical trials due to the lack of knowledge on appropriate therapeutic time window. In the current study, the appropriate therapeutic time window of L-NAME has been determined by administering L-NAME at three different time points i.e. pre, during and post ischemic / reperfusion. **Conclusion/Application:** L-NAME when administered pre ischemically, exerts anxiolytic effect, improves cognition and motor activity.

Keywords: Behaviour, Cerebral Ischemia, Excitotoxicity, L-NAME, Middle Cerebral Artery Occlusion / Reperfusion, Motor Activity

1. Introduction

Cerebral ischemia leads to the diminished blood flow to the brain, which subsequently leads to succinct or enduring physical disabilities such as lack of balance and coordination, and hemiplegia[1]. Aforesaid physical impair-

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ments ensue due to neuronal death owing to progression of ischemia, which is attributed to the interaction of complex pathophysiological processes such as glutamate excitotoxicity, mitochondrial dysfunction and oxidative stress[2]. Neurons of neocortex, striatum, cerebellar purkinje cells and hippocampal CA1 regions plays critical role in long term potentiation and cognition in addition to their role in sensory motor functions[3]. Though, the neurons of these regions are highly sensitive to ischemia, the primary targets are neurons of hippocampus, neocortex and striatum[4]. The increase in glutamate level due to ischemia augments the production of reactive oxygen species due to mitochondrial mutilation which consecutively results in the imbalance between the pro-oxidant and the anti-oxidant levels initiating oxidative stress[5,6]. Oxidative stress is the primary factor of neuronal death in acute brain injury[7]. On the other hand, the increasing glutamate excitotoxicity intensifies oxidative stress and also the production of nitric oxide. Nitric oxide combines with superoxide radicals and forms peroxynitrite leading to nitrosative stress. The oxidative and nitrosative stress burdens lead to the selective necrosis of hippocampal CA1 pyramidal cells[8]. The vulnerability of the neurons of hippocampal CA1 region is ascribed to the increased number of NMDA receptors[9]. The NMDA receptor of hippocampal CA1 regions gets activated by the increased glutamate level, which in turn is responsible for the motor and functional deficits[10]. Since, the motor and functional defacement ensuing cerebral ischemia may be brief or enduring, it is imperative to restore the behavioural outcome. Inhibition of Nitric oxide Synthase (NOS) restrains the generation of nitric oxide thereby alleviates nitrosative stress and curtails behavioural damage, and also allays glutamate excitotoxicity.

Hence, in the current investigation the efficiency of L-NAME (NG nitro L Arginine Methyl Ester), a NOS inhibitor in alleviating the excitotoxicity, convalescing behaviour and improving motor activity when administered pre, during and post ischemically in middle cerebral artery occluded / reperfused rats is studied.

2. Experimental Procedure

2.1 Chemicals

L-NAME was procured from Sigma, India. Nylon monofilament was purchased from the Pharmacy of Sri Ramachandra University, India. All other chemicals were purchased from Himedia laboratories, India and were of analytical grade.

2.2 Animals and Ethics Approval

The study protocol was approved by the Institutional Animal Ethics Committee of Sri Ramachandra University (IAEC/XXXIII/SRU/250/2013). Male Sprague Dawley rats of 290 – 340 g bodyweight were utilized in the investigation. Rats were housed individually in cages. An Air exchange ratio of 55:45 and 12-15 cycles of air change per hour was provided in the test rooms. Rats were maintained under 22±3°C and 30-70% temperature and relative humidity respectively. An artificial photoperiod of 12 h light and 12 h dark was maintained and the rats were supplied with pelleted feed and water ad libitum.

2.3 Middle Cerebral Artery Occlusion and Reperfusion (MCAO / R)

Middle cerebral artery occlusion and reperfusion method was used to develop transient focal cerebral ischemia in the rats following modified method of Longa et al method11. Rats were injected with 350 mg / kg chloral hydrate intraperitoneally to anesthetize. A cut was made on the upper thoracic region of the rat to expose the bifurcation of right common carotid artery. 4-0 nylon filament was coated with 0.01% poly-l-lysine and inserted into the external carotid artery after making a nick in the external carotid artery. The filament was advanced into the internal carotid artery until a slight resistance is felt. Filament was detained with the external carotid artery by tying it with non absorbable surgical suture. After 2 hours of ischemia, the suture is removed and the filament is slowly pulled out to allow reperfusion. Then the animal is sutured and maintained in a cage under a lamp to maintain the body temperature.

2.4 Animal Grouping and Experimental Design

Animals were segregated into five groups i.e., sham, ischemic / reperfusion (IR), pre, during and post ischemic / reperfusion. Each group consisted of 12 Sprague Dawley rats. Sham operated group did not undergo the middle cerebral artery occlusion / reperfusion, only external incision was made and the animals were sutured. IR group underwent MCAO/R surgery. Both

sham and IR group received 0.9 ml of saline. L-NAME was administered to the other groups at a dose of 3 mg / kg b.w. 30 minutes before ischemia (Pre), 1 hour following ischemia (during) and 3 hours after the onset of reperfusion (Post ischemic). Ischemia and reperfusion time was fixed based on the earlier research[12]. 72 hours after reperfusion behavioural assessment such as anxiety, cognition and assessment of motor activity was performed in all the groups and then sacrificed. Brains of the sacrificed animals were immediately removed and fixed in formalin (10%) for histopathology.

2.5 Assessment of Neurological and Motor Deficit, Anxiogenic Behaviour and Cognition

2.5.1 Assessment of Anxiety using Elevated Plus Maze

Anxiety in the experimental rats was assessed using elevated plus maze following the modified method of Lister[13]. Elevated plus maze is made of two arm which is made like a plus (+). One arm in the maze is open and the other arm is closed. The centre of the maze has an open platform of about 10 x 10 cm. The height of the maze from the floor is 50 cm. The rats were pretrained before MCAO / R. After surgery, the rats were placed on the central platform facing one of the open arm. The cumulative time spent and the number of visits into the open arm was assessed for 5 minutes. Open arm entry was counted when the rat places both the fore and hind limb.

2.5.2 Assessment of Cognition using Y- Maze

Experimental rats were assessed for cognition using Y-maze following the method of Akwa et al [14]. The maze consists of three arms which look like a Y. The three arms of the maze was randomly assigned as start arm (where rat are left first), novel arm (which is blocked during the first exercise and opened during test) and the other arm is open arm (which is left open during all exercises). Ocular clues were hung on the maze during the trials and test. Rats were pre trained keeping novel arm closed. After an hour of break, test was carried out, during which the novel arm is open. Novel vs familiarity of the rats was assessed by comparing the behavior in all the three arms. Rats were placed in the start arm during all assessments. Number of visits in the open arm and time spent in each arm was recorded for 5 minutes.

2.5.3 Assessment of Motor Activity using Rota Rod

The rota rod test was used to assess the motor deficit following MCAO/R in rats. Rota rod of make orchid (model: RR01) was used for the assessment. Animals were pre trained before MCAO/R. Each rat received three consecutive trials. Rota rod was started with 5 rpm and accelerated to 15 rpm within 5 minutes. The fall time of the rats were noted to assess the motor function.

2.5.4 Assessment of Motor Activity using Beam Walk

Motor activity of the experimental rats was performed using beam walk test following the method of Sathiya et al15. Beam walk apparatus consists of two beams which holds a vertical narrow beam as a platform. One end of the narrow beam consists of dark goal box. Rats were allowed to walk through the 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.

2.6 Histopathological Examination

Brain sections of about 4-5 µm thickness was prepared and stained with Haematoxylin and Eosin (H&E) after processing. Changes in the cortex, hippocampus (CA1, CA2 & CA3), striatum and hypothalamus was 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.

3. Statistical Analysis

Behavioural and percentage neuronal damage was expressed as mean±SEM. One way ANOVA followed by Tukey's multiple comparison test as post hoc was performed to find the statistical differences between the groups. GraphPad Prism 5 software (version 5.03) was used for all statistical calculations. Statistical significance was set at $p< 0.05$.

4. Results

One animal in the Ischemic / reperfusion group was found dead at 24 hours during the experiment.

4.1 Behavioural Outcomes

4.1.1 Anxiety

Significant decrease in the time spent and the number of entries into the open arm was observed in the IR group than the sham operated [(F (4, 54) = 11.08, p<0.001) & (F (4, 54) = 7.365, p<0.001)]. In the pre ischemic L-NAME group, significant increase in the time spent in the open arm and increased number of visits in the open arm was observed (p<0.001 & p<0.001) respectively. An insignificant increase in the time spent in open arm was observed in the during and post ischemic rats. Number of entries in to the open arm was also significantly increased in the pre ischemic L-NAME group (p<0.05). A non significant increase was observed in the number of visits into open arm was observed in both during and post ischemic group (Figure 1).

4.1.2 Cognition

In the Y maze, time spent in the start, open and the novel arm remained almost the same in the IR group than the sham operated [(F (4, 54) = 4.529, p<0.05), (F (4, 54) = 5.626, p<0.01) & (F (4, 54) = 22.69, p<0.001)], respectively. Preischemic rats, had significantly spent more time in novel arm than start and open arm, (p<0.05, p<0.05 & p<0.05) respectively. A nonsignificant increase in the time spent in the novel arm was observed in the during and post ischemic group than the IR group. Significant increase in the number of entries into the novel arm was observed in the pre ischemic group than ischemic (p<0.05). Whereas non significant increase in the number of entries was observed in the novel arm in both during and post ischemic group (p<0.001 & p<0.001)

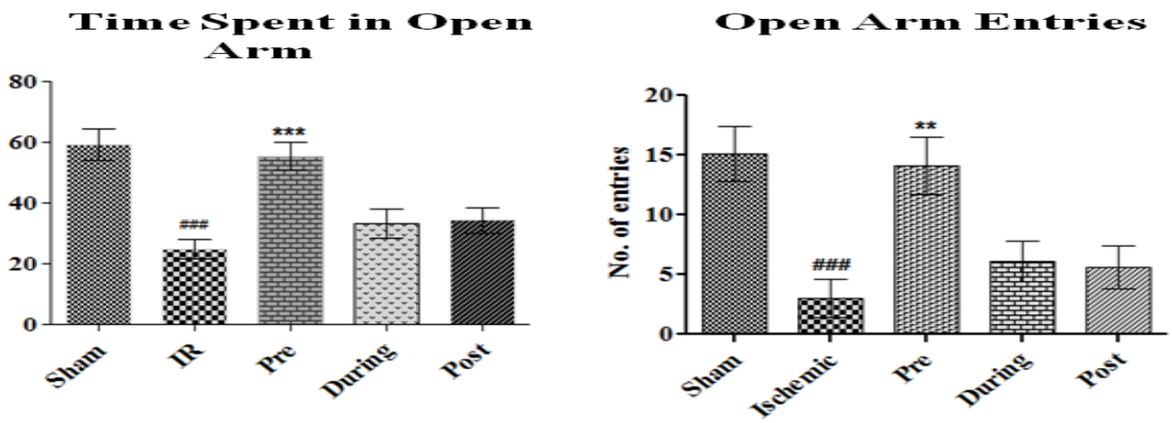

Figure 1. Effect of L-Name on Anxiety.

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

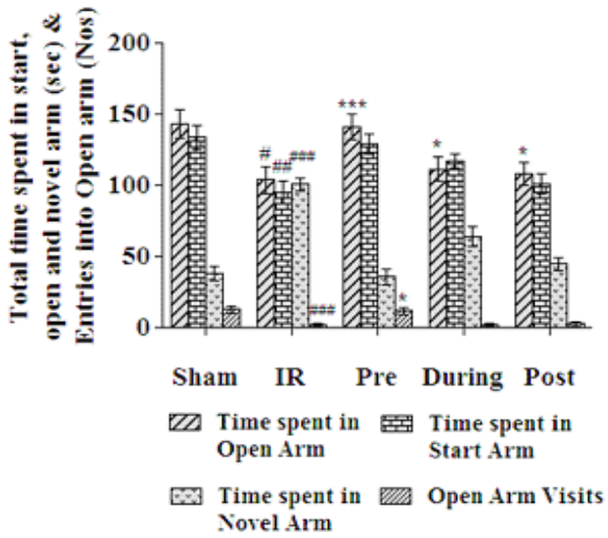

Figure 2. Effect of L-Name on Cognition.
Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.5, ## - p<0.01 and ### - p<0.01), * indicates comparison between IR and treatment groups (*- p<0.5, ** - p<0.01 & *** - p<0.001).

respectively. Thus, administration of L-NAME prior to IR improved the memory rats than during and post treatments (Figure 2).

4.1.3 Motor Activity

In the rota tod test, IR rats fell faster when compared to sham operated rats [F (4, 54) = 36.97, p<0.001]. A significant decrease in the fall time was observed (p<0.001) in the pre ischemic L-NAME group. In the during and post ischemic group, a non significant decrease was observed. . Therefore pre ischemic administration of L-NAME was found to increase the motor activity in MCAO/R rats (Figure 3a).

In the beam walk assessment, the time taken to reach the goal box and foot slips counts were found to be increased in IR rats when compared to the sham rats [F (4, 54) = 7.507, p<0.01 & F (4, 54) = 55.91, p<0.001]. Pre ischemic administration of L-NAME 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 and number of foot faults in during and post ischemic treatment. It is evident that pre ischemic

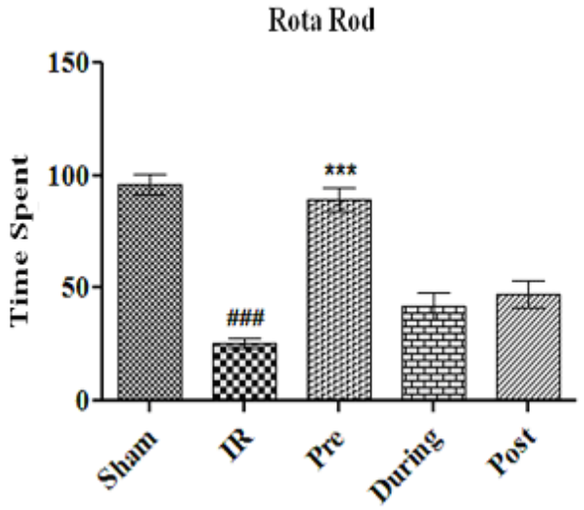

Figure 3a. Effect of L-Name on Motor Function.
Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.5, ## - p<0.01 & ### - p<0.001), * indicates comparison between IR and treatment groups (*- p<0.5, ** - p<0.01 & *** - p<0.001).

administration of L-NAME improves motor behaviour in MCAO/R rats (Figure 3b).

4.2 Histopathological Examination

Occlusion of middle cerebral artery in rats induced severe neuronal damage in the ischemic reperfusion rats (IR) than sham operated (1.09%). 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. Evident reduction in neuronal damage was revealed in the pre ischemic group, where in, the striatum appeared vacuolated with few dark stained cells. In during ischemic group, infarction at the pre optic area was observed larger with discernible vacuolation and nuclear shrinkage in the penumbra. Infarction with severe necrosis was observed in the striatum of during ischemic group. Histology examination of the post ischemic group uncovered necrotic focus in the cortex, hippocampus, and infarction in the pre optic area. Infarction was also observed in the hypothalamus. The penumbra of the infarction revealed marked vacuolation, shrunken cells and apparent loss of neurons. Neuronal damage of 50.0 %, 65.0 % and 80.0 % was observed in the pre, during and post ischemic groups respectively. Neuronal damage in all

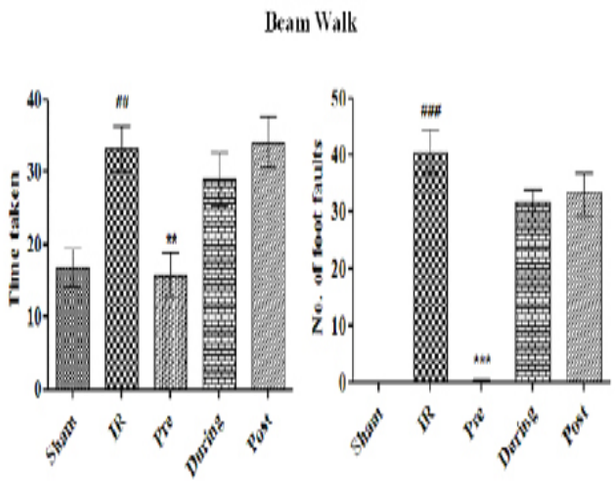

Figure 3b. Effect of L-Name on Motor Function.
Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.5, ## - p<0.01 & ### - p<0.001), * indicates comparison between IR and treatment groups (*- p<0.5, ** - p<0.01 & *** - p<0.001)"

the treatments were 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 (Figure 4 and 5).

5. Discussion

Cerebral ischemia leads to the impairment of various neuro spheres ultimately leading to wide spectrum of neurocomplications such as behavioural and motor dysfunctions[16]. Although behavioural and motor deficits are common after cerebral ischemia, it is also imperative to assess the effect of ischemia on anxiety, spontaneous behavior and cognition to understand the extent of neurobehavioral impairment. Evidences suggests that cerebral hypoperfusion leads to cognitive and memory dysfunction[17]. Yan et al., revealed that transient focal ischemia caused marked cognitive and memory dysfunctions in rats[12]. Hence, in the present study neurobehavioral and motor functioning assessments were performed to determine the ameliorating effect of L-NAME, a NOS inhibitor, when administered prior to ischemia, during ischemia and post ischemia. Middle cerebral artery occluded / reperfused rats underwent various behavioral tests such as elevated plus maze, Y-maze, beam walk and rota rod experiments. The elevated plus-maze test is widely accepted as a method for determining the anxiety of rodents[18,19]. In the elevated plus maze test, rats administered with L-NAME before inducing ischemia has shown improved anxiolytic behaviour. This is in corroboration with the finding of Mihoko demonstrated that the administration of L-NAME produced an anxiolytic effect in transient cerebral ischemia induced mice and also concluded that NOS inhibitors may be of great therapeutic value due to their effect on ischemia-induced anxiety[20]. In the Y- maze test, pre ischemic administration of L-NAME has increased the time spent by the MCAO/R rats in the open arm than the start and novel arm. The number of entries into the start arm was also increased. This result is in corroboration with the finding of Samson in which L-NAME exerted improved cognition in the transient ischemic rats[21].

The reason for improved cognition in the pre ischemic L-NAME group may be due to the decreased loss of neurons in the Hippocampal CA1 region which is evident from the histopathology results of the current study. These findings are evident from the various report of investigations, where in it is demonstrated that the CA1neurons plays crucial role in spatial working memory[22–24]. Ohno demonstrated that NOS inhibition by intrahippocampal L-NAME administration improved memory function is ischemic rats[25]. Cerebral ischemia has also been reported to cause significant decrease in the muscular strength of the limbs which was revealed by rota rod expreriments[26,27]. Pre ischemic administration of L-NAME has also improved the motor function in the middle cerebral artery occluded / reperfused rats. Improved motor activity due to L-NAME is also in corroboration with the findings of Sylvia in which it is demonstrated that L-NAME improved motor activity in Diazepam induced motor impairment in mice[26]. Improvement of motor activity by L-NAME pre ischemic administration is also in corroboration with the findings of Rehni and Singh and others[28–30].

Evident reduction in the percentage neuronal damage was observed in the pre ischemic L-NAME group. This finding is in corroboration with the results of Ashwal and Mishiya[31,32]. The reason for the better neuroprotection afforded by prior L-NAME administration may be due to the fact that it inhibits the generation of nitric oxide and there by inhibits nitrosative stress. Where as in the case of during, there is a delay in the drug to reach target site due to ischemia and in post administration, nitric oxide surge is increased by 50 % within 30 minutes of reperfusion[33]. Hence, the neuronal damage is increased in

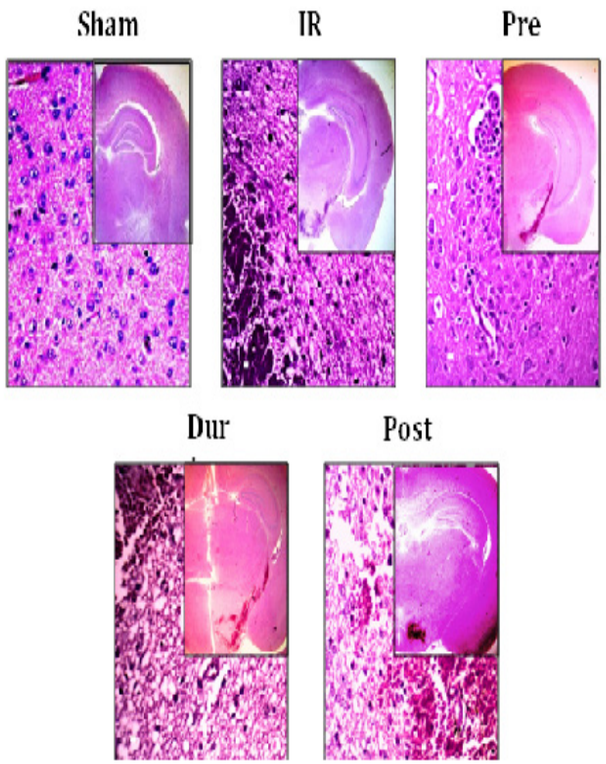

Figure 4. Histopathology – Pre optic Area.
Histological changes in the pre optic area in different treatment groups of L- NAME. Inset: Subgross view of ipsilateral brain hemisphere.

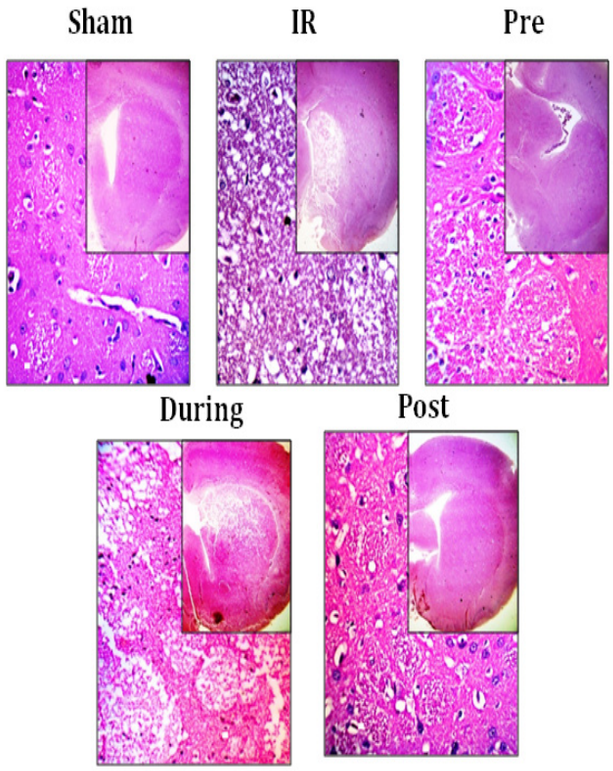

Figure 5. Histopathology – Striatum.
Histological changes in the striatum in different treatment groups of L-NAME. Inset: Subgross view of ipsilateral brain hemisphere

during and post ischemic L-NAME administration than pre ischemic. The ability of L-NAME to exert surpassing neuroprotective and neurorepair ability in prior administration may be attributed to the fact that it preserves the membrane integrity even before the onset of ischemic event and also due to its impending ability to restore the neuronal affront.

6. Conclusions

L-NAME, a NOS inhibitor when administered pre ischemically to the middle cerebral artery occluded / reperfused rats exerted anxiolytic action, improved cognition and motor function, and also reduced neuronal damage.

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8. References

1. Vespa P, Bergsneider M, Hattori N, Wu HM, Huang SC, Martin NA, Glenn TC, McArthur DL, Hovda DA. Metabolic crisis without brain ischemia is common after traumatic brain injury: a combined microdialysis and positron emission tomography study. Journal of Cerebral Blood Flow & Metabolism. 2009; 25(6):763–74.
2. Alvarez-Sabin J, Roman GC. The Role of Citicoline in Neuroprotection and Neurorepair in Ischemic Stroke. Brain Sci. 2013; 3(3):1395–414.
3. Sesso A, Belizario JE, Marques MM, Higuchi ML, Schumacher RI, Colquhoun A, Ito E, Kawakami J. Mitochondrial Swelling and Incipient Outer Membrane Rupture in Preapoptotic and Apoptotic Cells. Anat Rec. 2012; 295(10):1647–59.
4. Kirino T. Delayed neuronal death. Neuropathology. 2000; 20(s1):95–7.

5. Chan PH. Reactive oxygen radicals in signaling and damage in the ischemic brain. J Cerebral Blood Flow Metab. 2001; 21(1):2–14.
6. Floyd RA. Hensley K. Oxidative stress in brain aging: Implications for therapeutics of neurodegenerative diseases. Neurobiol-Aging. 2002; 23(5):795–807.
7. Al-Majed AA, Al-Omar FAM, Nagi MN. Neuroprotective effects of thymoquinone against transient forebrain ischemia in the rat hippocampus. Eur J Pharmacol. 2006; 543(1-3):40–7.
8. Nicole AT, Michael A, Nikolaus P. Nitric oxide: considerations for the treatment of ischemic stroke. Journal of Cerebral Blood Flow & Metabolism. 2012; 32(7):1332–46.
9. Paramdeep S, Rupinderjeet K, Amarpreet K. Clot composition and treatment approach to acute ischemic stroke: The road so far. Ann Indian Acad Neurol. 2013; 16(4):494–7.
10. Sonntag WE, Bennett C, Ingram R, Donahue A, Ingraham J, Chen H, Moore T, Brunso-Bechtold JK, Riddle D. Growth hormone and IGF-I modulate local cerebral glucose utilization and ATP levels in a model of adult-onset growth hormone deficiency. Am J Physiol Endocrinol Metab. 2006; 291(3):604–10.
11. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke. 1989; 20(1):84–91.
12. Saravana Babu C, Ramanathan M. Pre-ischemic treatment with memantine reversed the neurochemical and behavioural parameters but not energy metabolites in middle cerebral artery occluded rats. Pharmacology, Biochemistry and Behavior. 2009; 92(3):424–32.
13. Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl.). 1987; 92(2):180–5.
14. Akwa Y, Ladurelle N, Covey DF, Baulieu EE. The synthetic enantiomer of pregnenolone sulfate is very active on memory in rats and mice, even more so than its physiological neurosteroid counterpart: Distinct mechanisms? Proceedings of the National Academy of Sciences of the United States of America. 2001; 98(24):14033–7.
15. Sathiya S, Ranju V, Kalaivani P, Priya RJ, Sumathy H, Sunil AG, Babu CS. Telmisartan attenuates MPTP induced dopaminergic degeneration and motor dysfunction-through regulation of a-synuclein and neurotrophic factors (BDNF and GDNF) expression in C57BL/6J mice. Neuropharmacology. 2013; 73:98–110.
16. Cramer SC. Neurological progress repairing the human brain after stroke: I. mechanisms of spontaneous recovery. Ann Neurol. 2008; 63 (3):272–87.
17. Strong K, Mathers C, Ruth B. Preventing stroke: saving lives around the world. Lancet Neurol. 2007; 6(2):182–7
18. Yan B, Bi X, He J, Zhang Y, Thakur S, Xu H, Gendron A, Kong J, Li XM. Quetiapine attenuates spatial memory impairment and hippocampal neurodegeneration induced by bilateral common carotid artery occlusion in mice. Life Sci. 2007; 81(5):353–61.
19. Xu F, Hongbin H, Yan J, Chen H, He Q, Xu W, Zhu N, Zhang H, Zhou F, Lee K. Greatly improved neuroprotective efficiency of citicoline by stereotactic delivery in treatment of ischemic injury. Drug Deliv. 2011; 18(7):461–7.
20. Nakashima MN, Ajiki K, Nakashima K, Takahashi M. Nakashima Masakatsu Takahashi. Possible role of nitric oxide in anxiety following transient cerebral ischemia in mice. Journal of Pharmacological Sciences. 2003; 91(1):47–52.
21. Samson ML, Kajitani K, Robertson GS. Nitric-oxide synthase mediates the ability of darbepoetin alfa to attenuate pre-existing spatial working memory deficits in rats subjected to transient global ischemia. 2010; 333(2):437–44.
22. Aleman T. Role of insulin-like growth factors in neuronal plasticity and neuroprotection. Adv Exp Med Biol. 2005; 567:243–58.
23. Hartman RE, Lee JM, Zipfel GJ, Wozniak DF. Characterizing learning deficits and hippocampal neuron loss following transient global cerebral ischemia in rats. Brain Res. 2005; 1043(1-2):48–56.
24. Euler VM, Bendel O, Bueters T, Sandin J, Euler VG. Profound but transient deficits in learning and memory after global ischemia using a novel water maze test. Behav Brain Res. 2006; 166(2):204–10.
25. Drummond JC, McKay LD, Cole DJ, Patel PM. The Role of Nitric Oxide Synthase inhibition in the adverse effects of etomidate in the setting of focal cerebral ischemia in rats. Anesth Analg. 2005; 100(3):841–6
26. Mukherjee PK, Nazeer A, Kumar V, Mukherjee K, Houghton PJ. Protective effect of biflavones From Araucaria bidwillii Hook in rat cerebral ischemia/reperfusion induced oxidative stress. Behav Brain Res. 2007; 178(2):221–8.
27. Rehni AK, Singh N. Role of phosphoinositide 3-kinase in ischemic postconditioning-induced attenuation of cerebral ischemia-evoked behavioral deficits in mice. Pharmacol Rep. 2007; 59(2):192–8.
28. Sylwia T, Listos J, Fidecka S. Role of nitric oxide in the development of tolerance to diazepam-induced motor impairment in mice. Pharmacological reports. 2008; 60(4):474–80.
29. Kaur H, Jaggi AS, Singh N. Modulation of neuroprotective effect of ischemic post-conditioning by dichlorobenzamil, a Na+/Ca2+ exchanger inhibitor in mice. Biol Pharma Bull. 2010; 33(4):585–91.

30. Wong AM, Hodges H, Horsburgh K. Neural stem cell grafts reduce the extent of neuronal damage in a mouse model of global Ischemia. Brain Res. 2005; 1063(2):140–50.
31. Shen KT, Li MH, Yuan TF, Henrich C, Maro WN, Hsin YLm Freidrich BYZ, Shiang SH. Reserveratrol neuroprotective effects during focal cerebral ischemia injury via nitric oxide mechanism in rats. Journal of Vascular Surgery. 2007; 46(2):346–53.
32. Suofu Y, Clark J, Broderick J, Wagner KR, Tomsick T, Sa Y, Lu A. Peroxynitrite decomposition catalyst prevents matrix metalloproteinase activation and neurovascular injury after prolonged cerebral ischemia in rats. Journal of neurochemistry. 2010; 115(5):1266–76.
33. Uetsuka S, Fujisawa H, Yasuda H, Shima H, Suzuki M. Severe cerebral blood flow reduction inhibits nitric oxide synthesis. J Neurotrauma. 2002; 19(9):1105.