CHAPTER IV

4 RESULTS AND DISCUSSION

Administration of L-NAME and citicoline at different time points, i.e. before inducing ischemia (pre-ischemic), during ischemia (1 hour after the onset of ischemia) and post ischemia (3h after ischemia) revealed that pre-ischemic administration of L-NAME and citicoline ameliorated glutamate excitotoxicity better and improved neurological functioning than the during or post ischemic L-NAME and citicoline administration.

4.1 Neurological functioning and Behaviour

4.1.1 Effect of L-NAME, citicoline and combination on Neurological functioning

Rats in the IR group exhibited significant neurological deficit than sham operated (median = 4.0, p ≤0.001). IR rats exhibited neurological deficits, such as continuous spontaneous movements in all directions, decreased reaction to lateral push and circling. Pre-ischemic administration of L-NAME (median = 0.0 p ≤0.001) significantly ameliorated neurological deficits than the IR group. During (median = 0.0 p ≤0.05) and post ischemic treatment respectively (median = 2.0 p ≤0.05) has also reduced the neurological deficit significantly compared to the IR group. Among the three, pre-ischemic administration of L-NAME has significantly ameliorated neurological deficit.

Pre-ischemic (median = 0.0 p ≤0.001), during (median = 1.0 p ≤0.05) and post ischemic (median = 1.0 p ≤0.05) administration of citicoline significantly ameliorated neurological deficit than IR group. Among the three, preischemic citicoline administration exerts better neurological functioning.

Pre-ischemic administration of combination of L-NAME and citicoline has improved the neurological functioning (median = 0.0 p ≤0.001) better than pre ischemic L-NAME and citicoline group. Therefore, combination treatment has ameliorated neurological deficit better than any other treatment group. Combination of L-NAME and citicoline has
improved neurological functioning 97.2 % than the IR and individual administration of L-NAME and Citicoline has improved neurological functioning 80.9 and 90.4 % respectively. Hence, combination administration of L-NAME and Citicoline improves neurological functioning better than their individual administration (Figure 4.1). The beneficial effects of combination treatment in improving the neurological deficit is due to the reduced formation of peroxynitrite, reactive oxygen species, brain oedema and vascular damage of L-NAME. In addition to this, Citicoline is also reported to enhance the production of circulating Endothelial progenitor cells (EPCs) and thereby recovering the vasculature of the affected brain areas and promotes neurogenesis (Sobrino et al. 2011).
Figure 4.1 Effect of L-NAME, Citicoline and their Combination on Neurological Deficit
Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.05, ## - p<0.01 and ### - p<0.001), * indicates comparison between IR and treatment groups (*- p<0.05, ** - p<0.01 & *** - p<0.001).
4.1.2 Effect of L-NAME, Citicoline and combination on 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 (p ≤0.001 & p ≤0.05)]. Significant increase in the time spent (p ≤0.001) and number of visits (p ≤0.05) into open arm was observed in pre ischemic L-NAME. An insignificant increase in the time spent and number of entries into open arm was observed in the during and post ischemic administration of L-NAME.

In the citicoline treatment, pre ischemic administration has significantly increased the time spent and number of visits into the open arm (p ≤0.001 & p ≤0.05) respectively. An insignificant increase in the time spent and the number of entries into open arm was observed in both during and post ischemic citicoline rats. Pre ischemic citicoline exhibits anxiolytic behaviour in the middle cerebral artery occluded / reperfused rats.

Combination of L-NAME and citicoline administration before middle cerebral artery occlusion / reperfusion also has increased the time spent (p ≤0.001) and number of visits into the open arm (p ≤0.05). The percentage increase of the anxiolytic activity were observed to be 123.41, 133.19, 134.85 % (time spent in open arm) and 369.33, 394.33, 400.00 % (number of visits into the open arm) in the pre ischemic L-NAME, pre ischemic citicoline and combination group respectively.

Among all the treatments, pre ischemic combination administration of both L-NAME and Citicoline exerts better anxiolytic activity (Fig. 4.2 & Table 1). Anxiolytic activity of L-NAME is attributed to its ability to alleviate the release of adreno-corticotrophic hormone (ACTH) and increase the inhibitory neurotransmitter GABA (Gamma Amino Butyric Acid) (Sevgi et al. 2006 & Siripan et al. 2013). Further, Citicoline is also reported to increase the neurotransmitters dopamine and norepinephrine, which plays crucial role in anxiety (Licata et al. 2011).
Figure 4.2 Effect of L-NAME, Citicoline and their combination on Anxiety
Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p≤0.05, ## - p≤0.01 and ### - p≤0.001, • - Not significant), * indicates comparison between IR and treatment groups (* - p≤0.05, ** - p≤0.01 & *** - p≤0.001).
Table 4.1

Effect of L-NAME, Citicoline, combination on Anxiety in MCAO/R rats

<table>
<thead>
<tr>
<th>S. no</th>
<th>Group</th>
<th>Time spent in the open arm (% increase)</th>
<th>Number of visits into open arm (% increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L-NAME (Pre)</td>
<td>123.41</td>
<td>369.33</td>
</tr>
<tr>
<td>2</td>
<td>L-NAME (During)</td>
<td>34.45</td>
<td>100.00</td>
</tr>
<tr>
<td>3</td>
<td>L-NAME (Post)</td>
<td>38.49</td>
<td>86.00</td>
</tr>
<tr>
<td>4</td>
<td>Citicoline (Pre)</td>
<td>133.19</td>
<td>394.33</td>
</tr>
<tr>
<td>5</td>
<td>Citicoline (During)</td>
<td>58.02</td>
<td>114.00</td>
</tr>
<tr>
<td>6</td>
<td>Citicoline (Post)</td>
<td>53.98</td>
<td>94.33</td>
</tr>
<tr>
<td>7</td>
<td>Combination (L-NAME + Citicoline) - Pre</td>
<td>134.85</td>
<td>400.00</td>
</tr>
</tbody>
</table>

Percentage increase and decrease is in comparison with IR group
4.1.3 Effect of L-NAME, Citicoline and combination on Cognition

In the Y maze, IR group revealed significant decrease in the time spent in the novel, start and open arm (p ≤0.05, p ≤0.01 & p ≤0.001) when compared with the sham operated. Preischemic L-NAME rats had significantly spent more time in the novel arm than the start and open arm in comparison with the IR group (p ≤0.01, p ≤0.01) respectively. The time spent in the start arm was significantly decreased in the L-NAME pre ischemic group than the IR group (p ≤0.001). In the L-NAME during ischemic group, time spent in the start and open arm was decreased (p ≤0.05, p ≤0.05) with a significant increase in the time spent in the novel arm (p ≤0.01) when compared to the IR group. In L-NAME post ischemic group, significant decrease in the time spent in the start and open arm (p ≤0.05, p ≤0.05) was observed, with a significant increase in the number of entries into novel (p ≤0.01) than the IR group. The number of visits into the novel arm was also increased in the pre ischemic L-NAME group (p ≤0.05) than IR. A non significant increase in the time spent in the open arm was observed in the during and post ischemic L-NAME group.

Preischemic Citicoline rats, had significantly spent more time in novel arm than open and start arm (p ≤0.05, p ≤0.01 & p ≤0.001) respectively. A significant decrease in the time spent in the novel arm (p ≤0.01) with a non significant decrease in the time spent in the open and start arm was observed in the during ischemic L-NAME group. A nonsignificant increase in the time spent in the novel, open and start arm was observed in the post ischemic citicoline rats. However, a significant increase in the number of entries into the open arm was observed in the pre ischemic citicoline group (p ≤0.05). Whereas in the during and post ischemic citicoline group no difference was observed in the number of entries into the novel arm when compared to the IR group. Thus, administration of citicoline prior to MCAO/R improved memory.

In the combination group, a significant increase in the time spent in the novel open and start arm (p ≤0.001, p ≤0.01, p ≤0.05) was observed respectively with increased number of entries into the novel arm (p ≤0.05). The percentage increase in the novel arm entry was found to be 64.79, 65.50 and 69.23 in the pre ischemic L-NAME, citicoline and
combination group respectively. The percentage decrease in the start arm entry was found to be 34.68, 37.16 and 38.89 % in the pre ischemic L-NAME, Citicoline and combination group thereof. Percentage decrease of time spent in the open arm was found to be 35.64, 35.40 and 37.09 % respectively in the pre ischemic L-NAME, Citicoline and combination group. The percentage increase in the number of visits into the open arm was found to be 431.10, 454.12 and 469.72 % in the pre ischemic L-NAME, Citicoline and combination group respectively. Hence, among the treatment groups, combination administration of L-NAME and citicoline has improved memory in the MCAO/R rats (Fig 4.3 & Table 2).

The present finding is in corroboration with the findings that L-NAME improved cognition in the transient ischemic rats (Samson et al. 2010). 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 CA1 neurons plays crucial role in spatial working memory (Nelson & Lebessi 1997; Hartmann et al. 2005; Von Euler et al. 2006). In addition to this, Ohno also demonstrated that intrahippocampal administration of L-NAME improved memory function is ischemic rats (Ohno et al. 1994). Whereas Citicoline is known to increase the neurotransmitter dopamine and thereby improves cognition (Alvarez et al. 1999). Hurtado et al. (2007) also demonstrated that citicoline enhances the regeneration of dendrites and improves functional recovery. Evidences also show that citicoline helps in synthesizing phosphatidylcholine which maintains the integrity of cell membrane (Paul et al. 2003) and also recoups mitochondrial ATPase, membrane Na+K+ATPase and thereby impedes apoptosis and also improves cognition (Parnetti et al. 2007; Onal et al. 1997).
Figure 4.3 Effect of L-NAME, Citicoline and their combination on Cognition
Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.05, ## - p<0.001 and ### - p<0.001, ● Not significant), * indicates comparison between IR and treatment groups (* - p<0.05, ** - p<0.01 & *** - p<0.001).
Table 4.2

Effect of L-NAME, Citicoline and Combination on cognition in MCAO/R rats

<table>
<thead>
<tr>
<th>S. no</th>
<th>Group</th>
<th>Time spent in the Novel arm (% increase)</th>
<th>Time spent in the Open arm (% decrease)</th>
<th>Time spent in the Start arm (% decrease)</th>
<th>% Number of visits into open arm (% increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L-NAME (Pre)</td>
<td>64.79</td>
<td>35.64</td>
<td>34.68</td>
<td>431.10</td>
</tr>
<tr>
<td>2</td>
<td>L-NAME (During)</td>
<td>36.35</td>
<td>7.07</td>
<td>22.66</td>
<td>6.88</td>
</tr>
<tr>
<td>3</td>
<td>L-NAME (Post)</td>
<td>55.78</td>
<td>3.80</td>
<td>5.99</td>
<td>26.14</td>
</tr>
<tr>
<td>4</td>
<td>Citicoline (Pre)</td>
<td>65.50</td>
<td>35.40</td>
<td>37.16</td>
<td>454.12</td>
</tr>
<tr>
<td>5</td>
<td>Citicoline (During)</td>
<td>33.30</td>
<td>24.81</td>
<td>26.06</td>
<td>37.61</td>
</tr>
<tr>
<td>6</td>
<td>Citicoline (Post)</td>
<td>49.49</td>
<td>7.60</td>
<td>17.42</td>
<td>45.41</td>
</tr>
<tr>
<td>7</td>
<td>Combination (L-NAME + Citicoline) - Pre</td>
<td>69.23</td>
<td>37.09</td>
<td>38.89</td>
<td>469.72</td>
</tr>
</tbody>
</table>

Percentage increase and decrease is in comparison with IR group
4.1.4 Effect of L-NAME, Citicoline and combination on Motor activity

4.1.4.1 Rota Rod Test

In the rota rod test, IR rats fell faster when compared to sham operated rats (p ≤0.001). A significant increase in the fall time was observed (p ≤0.001) in the pre ischemic L-NAME group. In the during ischemic L-NAME group, a non significant increase in the fall time than the IR group was observed. Whereas, in the post ischemic L-NAME group a significant increase in the fall time was observed (p ≤0.05) when compared to the IR group.

A significant increase was observed in the fall time of pre ischemic citicoline group (p ≤0.001) than the IR. A non significant increase in the fall time was observed in the during and post ischemic citicoline groups when compared to the IR group.

Rats administered with combination of L-NAME and citicoline revealed increased fall time when compared to the pre ischemic L-NAME and citicoline group (p ≤0.001). The percentage increase in the fall time was found to be 244.23, 265.42 and 272.29 % in the pre ischemic L-NAME, citicoline and combination group respectively. Thus, among all the treatments, combination of L-NAME and citicoline has improved the motor activity better than the other treatment groups (Fig 4.4 & Table 3).

4.1.4.2 Beam Walk Test

In the beam walk assessment, the time taken to reach the goal box and the number of foot slips were found to be increased in IR rats when compared to the sham rats (p ≤0.01 & p ≤0.001). Pre ischemic administration of L-NAME has decreased the duration taken to reach the goal box and also the number of foot faults (p ≤0.01 & p ≤0.001) than IR group respectively. However, a nonsignificant decrease in the time taken to reach the goal box and number of foot faults in the during and post ischemic treatment was observed than the IR group.

Pre ischemic administration of citicoline has significantly decreased the duration taken to reach the goal box and also reduced the number of foot faults (p ≤0.01 &
p ≤0.001) than the IR group respectively. However, there was a nonsignificant decrease in the time taken to reach the goal box and significant decrease in the number of foot faults in the during citicoline group (p ≤0.05). In the post ischemic citicoline group, a non significant decrease in the number of foot faults with no effect on the time taken for reaching the goal box were observed.

In the combination treatment group, time taken and number of foot slips were significantly increased that any other treatment groups (p ≤0.001 & p ≤0.001) respectively (Figure 4.5 & Table 3). The percentage decrease in the time taken to reach the goal box in the pre ischemic L-NAME, citicoline and combination group were 52.66, 50.64 and 53.17 % respectively. A percentage decrease of 98.10, 98.22 and 98.39 % in the number of foot slips were observed in the pre ischemic L-NAME, citicoline and combination group was observed when compared to the IR group.

Alleviation of motor activity may be correlated to the antiglutamatergic and cholinergic activity of citicoline (Drago et al 1993). It was also reported that Citicoline was able to protect the motor neurons and the cerebellar granular cells against glutamate excitotoxicity mediated apoptosis (Matyja et al. 2008; Mir et al. 2003). Role of L-NAME in improved motor function may be corroborated to the decreased neuronal loss and the present finding is in corroboration with the observations of Sylwia, wherein L-NAME improved motor activity in Diazepam induced motor impairment in mice (Sylwia et al. 2008). Improvement of motor activity by L-NAME pre ischemic administration is also in corroboration with the findings of Rehni Singh and others (Rehni Singh et al. 2008; Kaur et al. 2010; Wong et al. 2005). Convalescence of the motor activity was observed in the pre ischemic citicoline group which is evident from the results of rota rod and beam walk test. Observations of the current study is in corroboration with the recent finding, that is, CDP-choline improves functional recovery and neuronal plasticity (Hurtado et al. 2007; Hurtado et al. 2008). Improvement of motor functions may also be related to the cholinergic effects of citicoline (Wurtman et al. 2000).
Figure 4.4 Effect of L-NAME, Citicoline and their combination on Motor activity (Rota Rod Test)
Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.05, ## - p<0.01 and ### - p<0.001, ● - Not significant), * indicates comparison between IR and treatment groups (* - p<0.05, ** - p<0.01 & *** - p<0.001).
Figure 4.5 Effect of L-NAME, Citicoline and their combination on Motor activity (Beam walk test)

Effect of L-NAME, Citicoline and Combination on motor activity (Beam walk test). Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.05, ## - p<0.01 and ### - p<0.001, ● - Not significant), * indicates comparison between IR and treatment groups (* - p<0.05, ** - p<0.01 & *** - p<0.001).
Table 4.3

Effect of L-NAME, Citicoline and Combination on motor activity in MCAO/R rats

<table>
<thead>
<tr>
<th>S. no</th>
<th>Group</th>
<th>Rota Rod</th>
<th>Beam Walk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fall Time (% increase)</td>
<td>Time taken (% decrease)</td>
</tr>
<tr>
<td>1</td>
<td>L-NAME (Pre)</td>
<td>244.23</td>
<td>52.66</td>
</tr>
<tr>
<td>2</td>
<td>L-NAME (During)</td>
<td>66.01</td>
<td>12.59</td>
</tr>
<tr>
<td>3</td>
<td>L-NAME (Post)</td>
<td>84.67</td>
<td>2.19</td>
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<td>4</td>
<td>Citicoline (Pre)</td>
<td>265.42</td>
<td>50.64</td>
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<tr>
<td>5</td>
<td>Citicoline (During)</td>
<td>58.27</td>
<td>14.57</td>
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<tr>
<td>6</td>
<td>Citicoline (Post)</td>
<td>83.02</td>
<td>4.80</td>
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<tr>
<td>7</td>
<td>Combination (L-NAME + Citicoline) - Pre</td>
<td>272.29</td>
<td>53.17</td>
</tr>
</tbody>
</table>

Percentage increase and decrease is in comparison with IR group
4.2 Effect of L-NAME, citicoline and combination on Neurochemical Parameters

4.2.1 Effect of L-NAME, Citicoline and combination on Glutamate & Aspartate

A significant increase in ipsilateral glutamate content was observed in the IR rats than sham operated rats (p ≤ 0.05). Glutamate content was found to be significantly reduced in pre-ischemic L-NAME rats (p ≤ 0.01). However, there was no effect on the level of glutamate in the during and post ischemic L-NAME group when compared to the IR. Hence, Pre-ischemic L-NAME treatment has significantly reduced the glutamate content.

Pre ischemic administration of citicoline has significantly alleviated glutamate content (p ≤ 0.05). Whereas, in during and post ischemic citicoline group, a slight non significant reduction in the glutamate level was observed.

In the combination administration group, a significant reduction in the glutamate level was observed (p ≤ 0.05). The percentage decrease in the glutamate level in the pre ischemic L-NAME, citicoline and combination group was found to be 56.73, 59.69 and 60.71 respectively. Hence, among all the treatment groups, combination of L-NAME and citicoline has significantly reduced the glutamate level better than pre ischemic L-NAME and citicoline group (Fig. 4.6, Table 4).

A significant increase in ipsilateral aspartate was observed in the IR rats than sham operated rats (p ≤ 0.001). Aspartate content was found to have reduced significantly in pre-ischemic L-NAME rats (p ≤ 0.05). Whereas, in the during and post ischemic group, a slight non significant reduction in the aspartate level was observed. In the citicoline group, aspartate level was significantly decreased in the preischemic treatment (p ≤ 0.01) and during ischemic treatment (p ≤ 0.05). A slight non significant reduction in the aspartate level was observed in the post citicoline group. Combination administration of L-NAME and citicoline has also significantly reduced the aspartate content (p ≤ 0.01). The percentage decrease in the aspartate level was observed to be 44.20, 43.97 and 50.11 respectively in the pre ischemic, L-NAME, citicoline and combination group.
Hence, among all the treatments, combination has greatly reduced the ipsilateral aspartate content (Fig. 4.7, Table 4). Better reduction in glutamate level by L-NAME may be correlated to early inhibition of NOS which decreases nitric oxide generation and activates cytochrome oxidase thereby restores mitochondrial function and leads to decreased glutamate level further ameliorates glutamate excitotoxicity (Cooper et al., 1994; Clementi et al., 1999, Jekabsone et al., 2007, Brown and Neher, 2010). Citicoline was also reported to exert antiglutamatergic effect by Diederich (2012) and also enhances the uptake of glutamate in cultured rat astrocytes. Besides, citicoline is also reported to play dual role in glutamate excitotoxicity, wherein it increases both neuronal ATP and astrocyte clearance of extracellular glutamate (Olivia et al. 2011).
Figure 4.6 Effect of L-NAME, Citicoline and Combination on Glutamate content
Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p≤0.05, ## - p≤0.01 and ### - p≤0.001, ● - Not significant), * indicates comparison between IR and treatment groups (*-p≤0.05, ** - p≤0.01 & *** - p≤0.001).
Figure 4.7 Effect of L-NAME, Citicoline and combination on Aspartate Level
Effect of L-NAME, Citicoline and combination treatments on Aspartate level. Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p≤0.05, ## - p≤0.01 and ### - p≤0.001, - Not significant), * indicates comparison between IR and treatment groups (* - p≤0.05, ** - p≤0.01 & *** - p≤0.001).
4.3 Effect of L-NAME, citicoline and combination on Neurochemical Parameters

4.3.1 Effect of L-NAME, Citicoline and combination on Glutamine synthetase activity

IR rats showed significant increase in GS activity than sham operated (p ≤ 0.01). GS activity of the treatment groups were decreased when compared to the IR group. Pre ischemic L-NAME administration has significantly reduced the GS activity (p ≤ 0.01) than IR group. However, there was no major effect in both during and post ischemic L-NAME group when compared to IR.

Pre ischemic citicoline administration has also greatly reduced the GS activity than pre ischemic citicoline group (p ≤ 0.001). In the during and post ischemic citicoline group a slight non significant decrease in the GS activity was observed when compared to the IR group. Combination of L-NAME and citicoline has also greatly reduced the GS activity when compared to the IR group (p ≤ 0.001) (Fig 4.8, Table 4). The percentage decrease in the GS activity was observed to be 41.98, 39.33 and 45.04 % in the pre ischemic L-NAME, citicoline and combination group respectively. Hence, combination of L-NAME and citicoline was found to exert better effect in decreasing the GS activity than individual pre ischemic administration of L-NAME and citicoline.

The decrease in GS activity may be corroborated to the decreased glutamate level. The resurgence of membrane integrity by citicoline recoups mitochondrial ATPase, membrane Na+/K+ ATPase, impedes apoptosis and thereby reduces activity of glutamine synthetase (Alvarez and Gustavo 2013). Combination administration of L-NAME and citicoline also increased the ATP and NAD level by reducing the glutamate content thereby decreasing the glutamine synthetase activity (Diederich 2012).
Fig. 4.8 Effect of L-NAME, Citicoline and combination on Glutamine Synthetase Activity

Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.05, ## - p<0.01 and ### - p<0.001, - Not significant), * indicates comparison between IR and treatment groups (* - p<0.05, ** - p<0.01 & *** - p<0.001).
4.3.2 Effect of L-NAME, Citicoline and combination on ATP & NAD

A significant decrease in the ATP content was observed in the IR group than the sham operated (p ≤0.001). ATP level has significantly increased in the preischemic L-NAME (p ≤0.001) group than the IR group (p ≤0.05). A slight non significant reduction in the ATP level was observed in the during L-NAME group. However, no obvious effect was observed in the post ischemic L-NAME group when compared to the IR.

In the citicoline group, pre, during and post ischemic citicoline has significantly increased the ATP level (p ≤0.001) compared to the IR group. Combination administration of L-NAME and citicoline has increased the ATP level significantly than the IR group and other treatment groups (p ≤0.001) (Fig 4.9, Table 4). The percentage increase in the ATP level was observed to be 288.26, 297.20 and 306.14% in the pres ischemic L-NAME, citicoline and combination group. Thus, combination of L-NAME and citicoline was found to improve the ATP content significantly better than the other treatments.

In the IR rats, NAD level was significantly decreased when compared to the sham operated (p ≤0.001). Significant increase in the NAD level was observed in the pre ischemic L-NAME (p ≤0.001) group than the IR. A slight non significant increased in the NAD content was observed in during and post ischemic L-NAME group compared to the IR. Pre ischemic citicoline administration has significantly increased the NAD level (p ≤0.001) than during (p ≤0.05) and post ischemic citicoline group (p ≤0.05) respectively compared to the IR group. Combination administration of L-NAME and citicoline has also significantly increased the NAD content (p ≤0.001). The percentage increase of NAD level in the pre ischemic L-NAME, citicoline and combination was observed to be 126.05, 143.18 and 170.70% respectively. Among all the groups, combination group was found to significantly improve the NAD content (Fig 5.0, Table 4).

L-NAME inhibits nitric oxide synthases and thereby decreases the expression of PARP which sustains ATP level (Valerie, 2009). Citicoline enhances the production of phospholipids and thereby restores the membrane integrity and thereby restores the ATP and NAD level
(Alvarez & Gustavo 2013). In addition to this, evidences show that red blood cells liberate ATP in response to flow-induced deformation and stimulates the formation of nitric oxide from endothelium, which reinstates cerebral vascular flow and thereby restores the ATP and NAD level (Sarah et al., 2014).
Figure 4.9 Effect of L-NAME, Citicoline and combination on ATP
Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.05, ## - p<0.01 and ### - p<0.001, ●- Not significant), * indicates comparison between IR and treatment groups (* - p<0.05, ** - p<0.01 & *** - p<0.001).
Figure 5.0 Effect of L-NAME, Citicoline and combination on NAD
Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p≤0.05, ## - p≤0.01 and ### - p≤0.001, ● - Not significant), * indicates comparison between IR and treatment groups (* - p≤0.05, ** - p≤0.01 & *** - p≤0.001).
4.3.3 Effect of L-NAME, Citicoline and combination on Nitrate / Nitrite content

Nitrate/nitrite was significantly increased in the IR group compared to sham operated (p ≤0.001). A significant reduction in the nitrate/nitrite content was observed in all phases of L-NAME administration (p ≤0.001) compared to the IR group. Amidst the three treatments, pre-ischemic administration of L-NAME has exerted slightly better reduction of nitrate/nitrite content than IR group.

In the citicoline group, nitrate / nitrite content was significantly reduced in the pre, during and post ischemic citicoline administration (p ≤0.001, p ≤0.01, p ≤0.01) than the IR. Combination treatment of L-NAME and citicoline has also significantly decreased the Nitrate / Nitrite content (p ≤0.001).

The percentage decrease in the nitrate / nitrite level in the pre ischemic L-NAME, citicoline and combination group was observed to be 60.09, 59.33 and 77.46% respectively. Therefore, among all the treatments, combination treatment has significantly decreased the Nitrate / nitrite content (Fig 5.1, Table 4). L-NAME inhibits NOS, and thereby decreases the formation of nitric oxide. Findings of the present study is in corroboration with the observation of a previous study where it is reported that the reduction in nitrite / nitrate levels were due to the inhibition of Nitric oxide synthase (Paramdeep et al. 2013). The extend action of L-NAME on nitrate/nitrite content may be related to its kinetic profile. Citicoline preserves the membrane structure and thereby inhibits the excess extracellular glutamate and thereby inhibits the formation of nitric oxide (Hurtado et al. 2008).
Figure 5.1 Effect of L-NAME, citicoline and combination on ipsilateral Nitrate / Nitrite content

Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.05, ## - p<0.01 and ### - p<0.001), * indicates comparison between IR and treatment groups (* - p<0.05, ** - p<0.01 & *** - p<0.001).
4.3.4 Effect of L-NAME, Citicoline and combination on Sodium Potassium ATPase (Na\( ^+ \)K\( ^+ \)ATPase)

Na\( ^+ \)K\( ^+ \)ATPase has significantly decreased (p \( \leq \) 0.001) in the IR rats when compared to the sham operated. A slight nonsignificant increase in the Na\( ^+ \)K\( ^+ \)ATPase activity was observed in the pre, during and post ischemic L-NAME group when compared to the IR group.

In the citicoline group, pre (p \( \leq \) 0.001), during (p \( \leq \) 0.01) and post (p \( \leq \) 0.05) ischemic administration of citicoline has significantly increased the Na\( ^+ \)K\( ^+ \)ATPase level than the IR group. Combination of L-NAME and citicoline has also significantly increased the Na\( ^+ \)K\( ^+ \)ATPase level (p \( \leq \) 0.001) than the IR group.

The percentage increase in the activity of Na\( ^+ \)K\( ^+ \)ATPase was observed to be 78.60, 193.70 and 205.38% respectively. Thus, combination treatment was found to increase the Na\( ^+ \)K\( ^+ \)ATPase than the other groups (Fig 5.2, Table 4). Since, L-NAME does not play any role in maintaining the integrity of the sodium potassium pump, the findings of the current investigation reveals that the activity of Na\( ^+ \)/K\( ^+ \)ATPase is not improved significantly in all the L-NAME groups. The resurgence of membrane integrity by citicoline recoups mitochondrial ATPase and membrane Na\( ^+ \)/K\( ^+ \)ATPase. Similar findings were reported in earlier research wherein Citicoline is reported to increase Na\( ^+ \)/K\( ^+ \)ATPase and further inhibited apoptosis and reduced glutamate excitotoxicity (Secades et al. 2006).
Figure 5.2 Effect of L-NAME, Citicoline and combination on Sodium Potassium ATPase (Na\textsuperscript{+}K\textsuperscript{+}ATPase) Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - $p \leq 0.05$, ## - $p \leq 0.01$ and ### - $p \leq 0.001$, ● - Not significant), * indicates comparison between IR and treatment groups (* - $p \leq 0.05$, ** - $p \leq 0.01$ & *** - $p \leq 0.001$).
4.3.5 Effect of L-NAME, citicoline and combination on super oxide dismutase

Super oxide dismutase was significantly reduced in the IR group when compared to the sham operated (p ≤0.01). Pre (p ≤0.01) and during (p ≤0.05) ischemic administration of L-NAME has significantly increased the super oxide dismutase level than the IR group. Post ischemic L-NAME administration was found to non significantly increase the super oxide dismutase level when compared to the IR group.

Pre (p ≤0.01), during (p ≤0.05) and post (p ≤0.05) ischemic administration of citicoline was found to increase the super oxide dismutase level when compared to the IR group. Combination administration of L-NAME and citicoline has significantly increased the super oxide dismutase activity (p ≤0.01) than the IR group (Fig 5.3, Table 4). Percentage increase in the super oxide dismutase level was found to be 136.78, 144.59 and 147.78\% respectively. Thus, combination of L-NAME and citicoline was found to significantly improve the superoxide dismutase level than pre ischemic L-NAME and citicoline groups. The increased activity of super oxide dismutase may be correlated to the antioxidant potential of L-NAME (Seif-el-Nasr & Fahim, 2001). Other investigations have also revealed the ability of citicoline to increase the super oxide dismutase activity (Ke et al. 2010; Menku et al. 2010; Qian et al. 2014). However, the clear mechanism of induction of super oxide dismutase activity by L-NAME and citicoline is not clearly understood.
Figure 5.3 Effect of L-NAME, Citicoline and combination on super oxide dismutase
Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p≤0.05, ## - p≤0.01 and ### - p≤0.001, ● - Not significant), * indicates comparison between IR and treatment groups (#- p≤0.05, ** - p≤0.01 & *** - p≤0.001).
4.3.6 Effect of L-NAME, Citicoline and combination on Reduced Glutathione

Reduced glutathione was significantly decreased in the IR group than the sham operated group (p ≤ 0.001). Pre ischemic administration of L-NAME had significantly increased the reduced glutathione level than the IR group (p ≤ 0.01). A non significant increase in the reduced glutathione level was observed in the during and post ischemic L-NAME group when compared to the IR.

Pre (p ≤ 0.01) and during (p ≤ 0.01) ischemic administration of citicoline has also increased reduced glutathione level than the IR group. A non significant increase in the reduced glutathione level was observed in the post ischemic citicoline group. Combination treatment has increased the reduced glutathione level significantly (p ≤ 0.001) than any other group Fig 5.4, Table 4). Percentage increase in the reduced glutathione level was observed to be 249.31, 319.17 and 330.13% in the pre ischemic L-NAME, citicoline and combination group. Hence, combination of L-NAME and citicoline was found increase the reduced glutathione level than preischemic L-NAME and citicoline.

Findings of the present study is in line with previous investigation findings. Several investigators reported that citicoline inhibits fatty acid release and augments phosphatidyl choline synthesis and thereby increases the glutathione reductase activity. The antioxidant property of L-NAME has also been reported in previous studies (Seif-el-Nasr & Fahim, 2001 & Oluwatosin et al. 2010).
Figure 5.4 Effect of L-NAME, Citicoline and combination on Reduced Glutathione Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.05, ## - p<0.01 and ### - p<0.001, ●- Not significant), * indicates comparison between IR and treatment groups (*- p<0.05, ** - p<0.01 & *** - p<0.001).
TABLE 4.4

Effect of L-NAME, citicoline and combination on Biochemical Parameters in MCAO/R rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Glutamate (% decrease)</th>
<th>Aspartate (% decrease)</th>
<th>Glutamine Synthetase (% decrease)</th>
<th>ATP (% increase)</th>
<th>NAD (% increase)</th>
<th>Nitrate / Nitrite (% decrease)</th>
<th>Na+K+ ATPase (% increase)</th>
<th>SOD (% increase)</th>
<th>GSH (% increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-NAME (Pre)</td>
<td>56.73</td>
<td>44.20</td>
<td>41.98</td>
<td>288.26</td>
<td>126.05</td>
<td>60.08</td>
<td>78.60</td>
<td>136.78</td>
<td>249.31</td>
</tr>
<tr>
<td>L-NAME (During)</td>
<td>7.44</td>
<td>30.82</td>
<td>15.26</td>
<td>89.94</td>
<td>53.67</td>
<td>54.96</td>
<td>76.69</td>
<td>78.73</td>
<td>139.72</td>
</tr>
<tr>
<td>L-NAME (Post)</td>
<td>6.67</td>
<td>22.21</td>
<td>16.33</td>
<td>25.69</td>
<td>51.26</td>
<td>51.28</td>
<td>74.41</td>
<td>70.03</td>
<td>99.59</td>
</tr>
<tr>
<td>Citicoline (Pre)</td>
<td>59.69</td>
<td>43.97</td>
<td>39.33</td>
<td>297.20</td>
<td>143.18</td>
<td>59.33</td>
<td>193.70</td>
<td>144.59</td>
<td>319.17</td>
</tr>
<tr>
<td>Citicoline (During)</td>
<td>23.87</td>
<td>33.23</td>
<td>25.94</td>
<td>137.43</td>
<td>80.01</td>
<td>35.39</td>
<td>129.82</td>
<td>128.78</td>
<td>309.58</td>
</tr>
<tr>
<td>Citicoline (Post)</td>
<td>19.25</td>
<td>29.10</td>
<td>19.36</td>
<td>110.55</td>
<td>79.15</td>
<td>36.71</td>
<td>99.69</td>
<td>119.56</td>
<td>161.64</td>
</tr>
<tr>
<td>Combination</td>
<td>60.71</td>
<td>50.11</td>
<td>45.04</td>
<td>306.14</td>
<td>170.70</td>
<td>77.46</td>
<td>205.38</td>
<td>147.78</td>
<td>330.13</td>
</tr>
</tbody>
</table>

Percentage increase and decrease is in comparison with IR
4.4. Western Blotting

4.4.1. Effect of L-NAME, Citicoline and combination on TNF-α and IL-10

TNF-α was upregulated in the IR group than the sham operated (p ≤0.01). Pre-ischemic administration of L-NAME has down regulated the expression of TNF-α (p ≤0.01) and upregulated the IL-10 expression (p ≤0.01) than the IR group. A non significant decrease in the expression of TNF-α and increase in IL-10 expression was observed in during and post ischemic L-NAME groups than the IR group respectively.

In the citicoline group, pre ischemic administration of citicoline has significantly reduced the expression of TNF-α (p ≤0.05) and increased the expression of IL-10 (p ≤0.05) than IR group. A slight non significant decrease in the expression of TNF-α and increase in expression of IL-10 was observed in both during and post ischemic Citicoline group. Combination of L-NAME and citicoline has significantly down regulated the expression of TNF-α (p ≤0.05) and up regulated the expression of IL-10 (p ≤0.05) than any other group (Fig. 5.5, Table 5). Combination of administration of L-NAME and citicoline has considerably decreased the protein expression of TNF-α and increased IL-10 expressions.

This may be corroborated to the fact that TNF-α exerts neurotoxicity only in the presence of elevated inducible nitric oxide synthase (iNOS) and L-NAME is known to inhibit NOS (Zhou et al., 2013). L-NAME also decreases the production of nitrate/nitrite content by inhibiting iNOS which further down regulates the expression of TNF-α. This finding also coincides with the report of Chen et al. (2014). The potential of citicoline to increase and decrease the expression of TNF-α and IL-10 may be related to its ability to decline the accumulation of free fatty acids which in turn down regulates the expression of proinflammatory markers. Citicoline also exhibits anti inflammatory activity by attenuating the phospholipase A2 activation (Adibhatla et al. 2002).
Figure 5.5 Effect of L-NAME, Citicoline and combination on TNF-α
Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p<0.05, ## - p<0.01 and ### - p<0.001, ● - Not significant), * indicates comparison between IR and treatment groups (* - p<0.05, ** - p<0.01 & *** - p<0.001).
Figure 5.5 Effect of L-NAME, Citicoline and combination on IL-10
Data expressed as Mean±SEM, # indicates comparison between sham and IR (# - p≤0.05, ## - p≤0.01 and ### - p≤0.001, ● - Not significant), * indicates comparison between IR and treatment groups (* - p≤0.05, ** - p≤0.01 & *** - p≤0.001).
4.5. Effect of L-NAME, citicoline and combination on brain infarction and neuronal damage

4.5.1 Effect of L-NAME, citicoline and combination on TTC staining

Severe infarction of about 45.80 % was observed in the IR groups when compared to the sham operated. Infarction of about 10.99, 29.47 and 29.84 % was observed in pre, during and post ischemic administration of L-NAME. In the citicoline group, 10.31, 17.35 and 25.08 % infarction was observed in the pre, during and post ischemic group respectively. Whereas in the combination administration of L-NAME and citicoline infarction of about 8.62 % was observed. Hence, it is evident from the present findings that combination treatment poses synergistic neuroprotective activity (Fig 5.6).

The decreased infarct volume i.e. increased uptake of TTC stain in brain sections of the combination group reveals that early inhibition of nitric oxide load and protection of membrane stabilization could restore mitochondrial function. Singh et al. (2010) ascertained that L-NAME treatment significantly reversed the mitochondrial complex-I activity and caspase-3 activity. This is in corroboration with the finding that pre ischemic administration of L-NAME significantly reduces the infarct volume in TTC staining (Nagafuji et al., 1993). Inhibition of NOS by L-NAME also decreases extracellular glutamate and increases neuronal viability, and thereby confers neuroprotection and neuronal plasticity (Stoll et al., 2000). On the other hand, L-NAME decreases NO generation and inhibits the formation of peroxynitrite and thereby inhibits DNA disruption, which inturn down regulates PARP1 and increases NAD content and protects the neurons from damage (Tsai et al., 2007). Reduction in cerebral infarct volume by L-NAME at doses 1, 3 and 10 mg/kg b.w. is also demonstrated by Kamii et al (1996). Citicoline increases the synthesis of phosphotidyl choline and sphingomyelin, decreases the release of free fatty acids and inactivates phospholipase A2 thereby restores the membrane integrity, this is evident from the reduced brain infarction in the citicoline and combination group.
Figure 5.6 Effect of L-NAME, citicoline and combination on Brain Infarction
White area denotes infarction
Figure 5.6 Effect of L-NAME, Citicoline and Combination on Brain Infarction
White area denotes infarction

L-NAME - Pre

L-NAME - During

L-NAME - Post
Figure 5.6 Effect of L-NAME, Citicoline and Combination on Brain Infarction
White area denotes infarction
Combination - Pre

Figure 5.6 Effect of L-NAME, Citicoline and Combination on Brain Infarction
White area denotes infarction

8.62%
4.5.2 Effect of L-NAME, citicoline and combination on Neuronal damage (Histopathology)

Occlusion of middle cerebral artery in rats produced severe neuronal damage in the ischemic reperfusion rats (IR) when compared to the 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 ipsilateral striatum of the IR group also appeared severely vacuolated with dissolution of the neurophil.

In the L-NAME group evident reduction of neuronal damage was revealed in the pre ischemic group. The striatum of the pre ischemic group appeared moderately vacuolated with few dark stained cells. In L-NAME during ischemic group, infarction at the pre optic area was observed to be larger with discernible vacuolation and nuclear shrinkage in the penumbra. Infarction with severe necrosis was observed in the striatum of during ischemic L-NAME group. Histology examination of the L-NAME 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 27.1 %, 55.0 % and 65.2 % was observed in the L-NAME pre, during and post ischemic groups respectively.

Evident reduction in neuronal damage was revealed in the pre ischemic citicoline group, where in, the striatum appeared mildly vacuolated with few dark stained cells. In the, citicoline 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 were reduced better when compared to the IR group. Amongst all the three treatments, substantial reduction in the neuronal damage was observed in the Citicoline pre ischemic treatment.
Apparent reduction in neuronal damage was revealed in the combination group. The striatum of the combination group revealed only very few dark stained neurons and mild vacuolation. Combination treatment also exhibited mild cortical necrosis. Preoptic area and the hypothalamus appeared normal. Neuronal damage of was reduced to about 10.8 % only in the group treated with Citicoline and L-NAME combination. Neuronal damage in all the treatments (L-NAME and Citicoline) were reduced better when compared to the IR group. Amongst all the treatments, substantial reduction in the neuronal damage was observed in the combination treatment (Fig. 5.7 & 5.8).

With regard to histopathology, better reduction of neuronal damage in combination group may be related to simultaneous membrane stabilizing property by citicoline and the reduction of nitric oxide generation by L-NAME. Inhibition of NOS by L-NAME reduces the generation of nitric oxide, prevents nitrosative stress and sustains mitochondrial integrity and thereby greatly decreases the neuronal damage. 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 earlier studies (Ashwal et al. 1994 & Mishiya et al. 1999).
Figure 5.7 Effect of L-NAME, Citicoline and Combination on Neuronal Damage - Cortex, Hippocampus & Hypothalamus
Magnification: a = 15 x, b = 400 x (Arrow indicates Infarction)
Figure 5.7  Effect of L-NAME, Citicoline and Combination on Neuronal Damage - Cortex, Hippocampus & Hypothalamus
Magnification: a = 15 x, b = 400 x (Arrow indicates Infarction)
Citicoline - Pre ischemic

Citicoline - During ischemic

Citicoline - Post ischemic

Figure 5.7 Effect of L-NAME, Citicoline and Combination on Neuronal Damage - Cortex, Hippocampus & Hypothalamus
Magnification: a = 15 x, b = 400 x (Arrow indicates Infarction)
Figure 5.7 Effect of L-NAME, Citicoline and Combination on Neuronal Damage - Cortex, Hippocampus & Hypothalamus
Magnification: a = 15 x, b = 400 x
Figure 5.8  Effect of L-NAME, Citicoline and Combination on Neuronal Damage - Striatum
Magnification:  a = 15 x, b = 400 x
Figure 5.8  Effect of L-NAME, Citicoline and Combination on Neuronal Damage
- Striatum
Magnification:  a = 15 x, b = 400 x (Arrow indicates Infarction)
Figure 5.8 Effect of L-NAME, Citicoline and Combination on Neuronal Damage - Striatum
Magnification: a = 15 x, b = 400 x (Arrow indicates Infarction)
Combination - Pre ischemic

Figure 5.8 Effect of L-NAME, Citicoline and Combination on Neuronal Damage - Striatum
Magnification: a = 15 x, b = 400 x
4.5.3 Effect of L-NAME, Citicoline and combination on the Expression of GFAP in different brain regions

A significant increase in the Glial Fibrillary Acidic Protein (GFAP) positive cells was observed in the IR group when compared with sham operated. GFAP positive cells were increased in the cortex, hippocampus, dentate gyrus, hypothalamus and the striatum of IR group when compared to sham operated. GFAP positive cells in all the regions of brain were decreased in the L-NAME (Pre, during and post), citicoline (pre, during and post) and in the combination group than the IR group. Among the different regions of the brain, GFAP positive cells were intensely increased in the dentate gyrus region compared to other brain regions (Fig. 5.9, 6.0, 6.1, 6.2, 6.3). The reduction in the GFAP positive cells indicates decreased astrogliosis which may be corroborated to the neurological outcome. Current findings were in corroboration with the results of investigation performed by Kalayci et al. (2005), in which it is reported that the GFAP positive cells were decreased in L-NAME treated rats. 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 (Diederich et al., 2012) and also decreases GFAP expression in the peri infarct area after stroke (Alvarez and Gustavo, 2013).
Figure 5.9 Effect of L-NAME, Citicoline and Combination on GFAP Expression in Ipsilateral Hypothalamus
Magnification: A - 4x, B - 40X, Bar: A - 500 μm, B - 50 μm
Figure 5.9 Effect of L-NAME, Citicoline and Combination on GFAP Expression in Ipsilateral Hypothalamus
Magnification: A- 4x, B-40X, Bar: A- 500 μm, B-50 μm
Figure 5.9 Effect of L-NAME, Citicoline and Combination on GFAP Expression in Ipsilateral Hypothalamus
Magnification : A- 4x, B-40X, Bar : A- 500 μm, B-50 μm
Figure 5.9 Effect of L-NAME, Citicoline and Combination on GFAP Expression in Ipsilateral Hypothalamus
Magnification: A- 4x, B-40X, Bar: A- 500 μm, B-50 μm
Figure 6.0 Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Cortex
Magnification - 20x, Bar = 50 μm
Figure 6.0 Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Cortex
Magnification - 20x, Bar = 50 μm
Figure 6.0 Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Cortex
Magnification - 20x, Bar = 50 μm
Figure 6.0  Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Cortex
Magnification - 20x, Bar = 50  μm
Figure 6.1 Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Hippocampus
Magnification - 20x, Bar = 50 μm
Figure 6.1 Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Hippocampus
Magnification - 20x, Bar = 50 μm
Figure 6.1 Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Hippocampus
Magnification - 20x, Bar = 50 μm
Figure 6.1 Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Hippocampus
Magnification - 20x, Bar = 50 μm
Figure 6.2 Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Dentate Gyrus
Magnification - 20x, Bar = 50 μm
Figure 6.2 Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Dentate Gyrus
Magnification - 20x, Bar = 50 μm
Figure 6.2 Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Dentate Gyrus
Magnification - 20x, Bar = 50 μm
Figure 6.2 Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Dentate Gyrus
Magnification - 20x, Bar = 50 μm
Sham

Ischemic Reperfusion

Figure 6.3 Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Striatum
Magnification - 20x, Bar = 50 μm
Figure 6.3 Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Striatum
Magnification - 20x, Bar = 50 μm
Figure 6.3 Effect of L-NAME, Citicoline and Combination on GFAP Expression in the Striatum
Magnification - 20x, Bar = 50 μm
4.6 CONCLUSION

In the current study the best suitable therapeutic time window of L-NAME (NOS inhibitor) and citicoline (Membrane stabilizer) was investigated by administering them at different time of ischemia (before, during and after ischemia) in middle cerebral artery occluded / reperfused Sprague Dawley rats. Since, L-NAME and Citicoline was found to be effective in the pre ischemic phase, combination of these two drugs were administered at pre ischemic phase in the MCAO/R rats. The neuroprotective effect of the candidates were measured in terms of level of glutamate, aspartate, glutamine synthetase, Nitrate/Nitrite, Sodium potassium ATPase, ATP, NAD, reduced glutathione, superoxide dismutase, neurological functioning, anxiety, cognition and motor activity. In addition to this, the expression of TNF- α, IL-10 and GFAP was studied, brain infarction volume was also determined and histopathology of brain was performed using H&E staining.

Present investigation revealed the following findings,

- Combination treatment improves neurological functioning better than individual administration. Among citicoline and L-NAME, pre ischemic citicoline improves neurological deficits than the other groups of citicoline and L-NAME
- Combination treatment improves cognition and motor activity, and reduces anxiety better than individual treatment. Among citicoline and L-NAME, pre ischemic citicoline exerts better effect than the other groups.
- Combination treatment ameliorates glutamate excitotoxicity, decreases aspartate, glutamine synthetase and nitrate / nitrite than the citicoline and L-NAME groups. Citicoline also exhibits better neuroprotection in pre ischemic phase than citicoline and L-NAME group
- Combination administration of L-NAME and citicoline increases ATP, NAD, superoxide dismutase and reduced glutathione than the other groups of citicoline and L-NAME. Citicoline works better at the pre ischemic phase than the other citicoline and L-NAME groups.
- Combination treatment upregulates the expression of IL-10 and down regulates the expression of TNF-α than the other citicoline and L-NAME groups. Pre ischemic citicoline functions better than the other citicoline and L-NAME groups.
• Combination treatment decreased expression of GFAP, reduced brain infarction and neuronal death than the other citicoline and L-NAME group. Pre ischemic citicoline exhibits better function than L-NAME.

Hence, it is concluded that combination of L-NAME and citicoline exhibits synergistic neuroprotective effect. However, among L-NAME and citicoline, citicoline exerts better neuroprotective effect, in particular, pre ischemic administration of citicoline exhibits better effect than the other groups of citicoline and L-NAME. Therefore, it is evident from the present results that L-NAME and citicoline exhibits synergistic effect and it is also apparent that multiple targeting aids better in the treatment of cerebral ischemia. Thus, it is concluded that combination of L-NAME and citicoline may be used as a prophylactic agent in the patients with known risk of stroke such as hypertension, cardiac abnormalities such as patent foramen ovale (PFO) and atrial septal aneurysm (ASA), carotid artery stenosis and transient ischemic attacks.