Effect of Cyclooxygenase Inhibitors on Neurological and Oxidative Damage in Experimentally Induced Acute Cerebral Ischemia/Reperfusion In Rats

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Abstract

Background: Neuroinflammation is one of the key pathological events involved in the progression of brain damage caused by cerebral ischemia. Metabolism of arachidonic acid through cyclooxygenase (COX) enzymes are involved in neuroinflammation events.

Aim of the Work: The purpose of this study was to determine whether the COX inhibitors, indomethacin (5mg/kg/IP), valeryl salicylate (VAS) (20mg/kg/IP) and nimesulide (12mg/kg/IP) could effectively ameliorate neurological and oxidative damage induced in animal model of cerebral ischemia in rat.

Material and Methods: Cerebral ischemia was induced by occlusion of left common carotid artery for 60min, stroke index was calculated. Behavioral tests were performed. Rats were sacrificed then, malondialdehyde (MDA), glutathione peroxidase (GHPx), superoxide dismutase (SOD) and areas of cerebral infarction were measured.

Results: Treatment immediately after ischemia with nimesulide and indomethacin significantly reduced the infarct size and improved neurological deficit. While VAS has no effect, nimesulide only significantly decreased lipid peroxidation measured as MDA and a significant increase GHPx and SOD. In the same time, indomethacin and VAS have no effect on these measures.

Conclusion: The interest is the findings that nimesulide (COX2 inhibitor) & indomethacin (non selective COX inhibitor) have early therapeutic effect against cerebral ischemia. The antioxidant effect may be one of the mechanisms of both drugs which appears significantly in nimesulide only, rather than indomethacin. This may be due to the action of indomethacin on COX1 beside COX2 which has confounding effects resulting from COX1 inhibition and masks its action as antioxidant or through other mediators. ROS play a pathogenic role in ischemic injury but we cannot rule out the participation of other mediators or other sources of ROS that contribute and support the hypothesis that other components beside oxygen radical are determinants of ischemic brain death. This components as vascular reactivity, non oxygen based radical, inflammatory mediators and glutamate receptor.

Key Words: Cyclooxygenase (COX) – Valeryl salicylate (VAS) – Malondialdehyde (MDA) – Glutathione peroxidase (GHPx) – Superoxide dismutase (SOD) – Reactive oxygen species (ROS).

Introduction

ISCHEMIC stroke is a leading cause of death and long-standing disability. Stroke is the second leading cause of death worldwide [1]. A variety of destructive mechanisms are involved including oxygen radical generation, calcium overload, cytotoxicity and apoptosis as well as the generation of inflammatory mediators [2]. All these neuroinflammatory mechanisms have been demonstrated to contribute ischemic brain injury [3,4]. A large number of studies indicate that blockade of the neuroinflammatory process dramatically reduces ischemic brain injury [5,9].

Large amounts of free arachidonic acid are released during ischemic brain damage through the concert action of phospholipase [10]. Metabolism of arachidonic acid through the cyclooxygenase (COX) pathway produces huge amounts of pro-inflammatory prostanoids and reactive oxygen species (ROS), which are key mediators of the inflammatory process [11]. Several studies suggest that cyclooxygenase (COX) plays a pivotal role in the progression of ischemic brain damage [8,9]. Oxidative stress is thought to be a key event in the pathogenesis of cerebral ischemia, overproduction of reactive oxygen species (ROS) during ischemia-reperfusion could cause an imbalance between oxidative and antioxidative process [12]. There is increasing evidence supporting the hypothesis that drugs have antioxidant activity can provide beneficial effect against neurodegenerative changes associated with cerebral ischemia [13].
The biological effects of non steroidal anti-inflammatory drugs are mediated by inhibition of COX enzymes. There are two different isoforms of COX enzyme. COX-1 and COX-2 have been identified [11]. The role of each COX isoform in ischemic brain injury is still controversial. This study aimed at investigating therapeutic potential of inhibition of both COX enzymes. We will provide the relative contribution of each COX isozyme utilizing selective COX1 inhibition by valeryl salicylate (VAS), selective COX2 inhibition by nimesulide and inhibition of both COX by indomethacin in treatment of cerebral ischemia.

Accordingly, stroke index, behavioral tests and cerebral infarction area measuring were used as a marker for cerebral ischemia [7].

Additionally, we investigated the possible mechanisms as antioxidant status and lipid peroxidation, we assayed serum, malondialdehyde (MDA) level as a marker of lipid peroxidation, superoxide dismutase (SOD) enzyme activities and glutathione peroxidase (GHPx) enzyme activity as antioxidants.

Material and Methods

Animals:

Adult male albino rats, with initial body weight ranging from (150-200g) were used. Rats were purchased from Experimental Animal Breeding Farm, Helwan. All animals were housed in a controlled laboratory conditions at 20-25ºC in a 12h light/dark cycle and had free access to food and water. They were allowed for one week acclimatization period before to their use in the experiment at Pharmacology Department, Faculty of Medicine, Banha University. About 2-3 rats have died after induction of cerebral ischemia.

Experimental groups:

Rats were divided into 5 groups. Each consisted of 12 rats and distributed as follows:

Group (1): A sham operated group.

Group (2): An ischemic group treated with vehicle 2% polyvinylpyrrolidone solution in saline in VAS & nimesulide groups [7,14] and physiological saline in indomethacin [15].

Group (3): An ischemic group treated with VAS 20mg/kg; ip, immediately after ischemia with additional 3 doses at 6,12,18h afterischemia/reperfusion [14].

Group (4): An ischemic group treated with nimesulide 12mg/kg; ip, immediately after ischemia with additional 3 doses at 6,12,18h after ischemia/reperfusion [7].

Group (5): An ischemic group treated with indomethacin 5mg/kg, IP, immediately after ischemia [15].

Drugs and chemicals:

VAS, nimesulide, indomethacin are products of Sigma Chemical Co. St Louis, Missouri, as powders.

Polyvinylpyrrolidone (Alfa chemical Co) as white powder.

Induction of cerebral ischemia[16]:

Animals were anaesthetized with urethane (1.25gm/kg, IP). The left common carotid artery was exposed through a ventral midline skin incision and the artery isolated from related nerves and vessels. It was occluded for 60min with a small clip. Rats were injected I.M with penicillin G (25,000IU) before surgery to prevent risk of infection.

Calculation of stroke index (S.I) [17]:

All animals were allowed to recover from anesthesia. Careful observation of all animal especially their behavior continuously before and after operation. The S.I score was determined using Table (1).

Behavioral tests:

Behavioral tests were performed in all animal groups before induction of cerebral ischemia and one day (24h) after stroke.

Forelimb placing test[18]:

The behavioral test was a vibrissae-elicited forelimb placing test. Animal were held by their torsos, which allowed the forelimb to hang free. The animal was gently moved up and down before the placing test to facilitate muscle relaxation and eliminate any struggling movement. Independent testing of each forelimb was induced by brushing
the respective vibrissae on the corner edge of a countertop. Intact animals place the forelimb ipsilateral to the stimulated vibrissae quickly onto the countertop.

Depending on the extent of injury, placing of the forelimb contralateral to the injury in response to contralateral vibrissae contact with countertop may be impaired. In the experiments each rat was tested 10 times for each forelimb, and the percentage of trials in which the rat placed the appropriate forelimb on the edge of the countertop in response to the vibrissae stimulation was determined.

The circling test [19]:

The rats were held gently by the tail suspended one meter above the floor, and observed forelimb flexion and the score for contralateral side. Normal rats extend both forelimbs towards the floor. Body posture degree according to Neurological Examination Grading system:

- Normal-grade 0-No observation.
- Moderate-grade 1-right forelimb flexion.
- Severe-grade 2-decreased resistance to lateral push (and right forelimb flexion) without circling.
- Severe-grade 3-same behavioral as grad 2 with circling to right.

Biochemical assay:

After behavioral test had done, rats (n=6) were deeply anesthetized with diethyl ether and perfused transcardially with ice cold saline to flush all blood components from the vasculature. Brains were quickly removed from animals, kept in ice cold saline, the brains homogenized in ice cold 20mm Tris-HCl buffer and centrifuged for 10min. The supernatant was collected, frozen at –70°C and employed for biochemical analysis.

- MDA as lipid peroxidation assay using method of Esterbauer and Cheese [20].
- SOD activity (colorimetric enzymatic assay kit) [21].
- GHPx activity [22].

Histological analysis:

The animals (n=6) were sacrificed under anesthesia. The brains were removed from the skull and placed in 4% formalin in 0.1m phosphate-buffered saline (pH 7.4) for 24h. The brains were embedded in paraffin wax and coronally sectioned into six slices. The area of cerebral infarction in each animal was measured in coronal sections stained with hematoxylin and eosin by computarized image analysis [23,24]. infarct volumes were calculated by multiplying the infarcted area by the slice thickness and summing the volume of the six slices. To eliminate the contribution of postsischemic edema to the volume of injury, infarct volume measurements were corrected for swelling according to the method of Lin et al. [25] & Zhang and Iadecola [26]. Infarct volumes are expressed as a percentage of the contralateral (control) hemisphere.

Statistical analysis:

All parameters were expressed as mean ±SE. Statistical analysis was performed with one-way ANOVA followed by student t-test. The result was considered significant when \( p<0.05 \) [27].

Results

Stroke index score (SI):

Fig. (1) & Table (2) show that SI in vehicle treated ischemic rats were significantly elevated 23.65±1.6 in comparison to sham operated group value 4.75±0.08. In VAS (20mg/kg/ip) treated ischemic group shows insignificant reduction in SI (22.92± 2.5) in comparison to vehicle treated ischemic group. While nimesulide treated ischemic group & indomethacin treated ischemic group showed significant reduction in SI in comparison to vehicle treated ischemic group which were 6.6±0.05 & 8.2±2.2 respectively with percentage change 72% & 65%. Nimesulide is better than indomethacin.

The circling test:

Neurological Examination grading system was in grade 3 in vehicle treated ischemic rats than sham group which in grade 0. VAS treated ischemic group showed no change in this grading system and represented by grade 3.

While, in nimesulide treated ischemic group and indomethacin treated ischemic group were grade 1 & 2 respectively. Nimesulide is better than indomethacin.

Cerebral ischemic animals showed a strong and persistent tendency to turn their upper bodies' posture to the side contralateral to the injured hemisphere and showed flexion of the forelimb contralateral to the injured hemisphere, while they were suspended by the tail. Whereas, sham operated group showed extention of both forelimbs with no bias of their upper bodies' posture.

Fore limb placing test:

Fig. (2) & Table (3) show the placing test (PT) from contralateral forelimb was 85 ±7.1 percent before cerebral ischemia and it was significantly
decrease to 22.9 ± 1.7 percent after ischemia in vehicle treated ischemic group. While, VAS treated ischemic group was 21.7 ± 1.5 percent with insignificant change.

In nimesulide treated ischemic group and indomethacin treated ischemic group, PT became 76.96±4.05 & 59.4±2.1 percent significant in comparison to vehicle treated ischemic group respectively.

Cerebral ischemic animals took longer to place a forelimb contralateral to the injury in response to contralateral vibrissae contact with countertop than ipsilateral vibrissae (p<0.05). Sham operated group showed no significant difference at any measurement.

**Biochemical parameters:**

The data presented in Tables (4,5,6) & Figs. (3,4,5) demonstrate that vehicle treated ischemic group showed significant (p<0.05) increase in MDA and also, showed significant (p<0.05) decrease in SOD and GHPx level as compared to sham operated control group. Treatment with VAS (20mg/kg/IP) and indomethacin (5mg/kg/IP) just after ischemia had no significant effect in comparison to vehicle treated ischemic group in these parameters. In the same time, nimesulide treatment (12mg/kg/IP) immediately after ischemia, significantly (p<0.05) reduced MDA in comparison to vehicle treated ischemic group with percentage change 38.5% Fig. (4) & (Table 4).

Also, Figs. (5,3). Tables (5,6) showed significantly increased GHPx and SOD antioxidant activity in comparison to vehicle treated ischemic group with percentage change 52.3% and 54.3% respectively.

**Histological analysis:**

Histological analysis in the present work confirmed the effect of nimesulide and indomethacin against cerebral ischemia and exclude VAS Fig. (6). Cerebral ischemia produced marked increased infarct area in ipsilateral cortex 71.8 ±5% of vehicle treated ischemic group. Just postischemic administration of nimesulide and indomethacin significantly (p<0.05) reduced infarct area which was 19.44±1.8% and 41.2±3.8% respectively versus vehicle treated ischemic gr oup. Reduced edema and separation of cells with microglial cell infiltration. VAS had an insignificant effect versus vehicle treated ischemic group. Sham operated group showed no changes than normal neuronal cells.

**Table (2): Effect of COX inhibitors on stroke index one day after cerebral ischemia.**

<table>
<thead>
<tr>
<th>Indomethacin treated ischemic</th>
<th>Nimesulide treated ischemic</th>
<th>VAS treated ischemic</th>
<th>Ischemic operated</th>
<th>Sham operated</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.2±2.2**</td>
<td>6.6±0.05*</td>
<td>22.9±2.5</td>
<td>23.65±1.6*</td>
<td>4.75±0.08</td>
<td>SI</td>
</tr>
<tr>
<td>65%</td>
<td>72%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

According to “modified Stroke index” by (Ohno et al., 1984). Data are represented as Mean ± SE.

* Significant at p<0.05 compared to Sham operated group.

**Table (3): Effect of COX inhibitors forelimb placing score (%) one day after ischemia.**

<table>
<thead>
<tr>
<th>Indomethacin treated ischemic</th>
<th>Nimesulide treated ischemic</th>
<th>VAS treated ischemic</th>
<th>Ischemic operated</th>
<th>Sham operated</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>59.4±2.1**</td>
<td>76.96±4.05**</td>
<td>21.7±1.5</td>
<td>22.9±1.7*</td>
<td>85±7.1</td>
<td>Forelimb placing score (%)</td>
</tr>
</tbody>
</table>

Data are represented as Mean ± SE.

* Significant at p<0.05 compared to Sham operated group.

**Table (4): Effect of selective inhibitors COX1 valeryl salicylate (VAS) (20mg/kg/IP) & COX2 nimesulide (12/mg/kg/IP) and non selective indomethacin (5mg/kg/IP) on malondialdhyde (MDA) in cerebral ischemic rats.**

<table>
<thead>
<tr>
<th>Indomethacin treated group</th>
<th>Nimesulide treated ischemic group</th>
<th>VAS treated ischemic group</th>
<th>Vehicle treated ischemic group</th>
<th>Sham operated group</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.87±0.2</td>
<td>1.82±0.21**</td>
<td>3.01±0.03</td>
<td>2.96±0.16*</td>
<td>1.32±0.24</td>
<td>MDA nMol/gm protein Percentage change</td>
</tr>
</tbody>
</table>

* Significant at (p<0.05) in comparison to sham operator control group.

** Significant at (p<0.05) in comparison to vehicle treated ischemic group.

Data are represented as mean ± SE.
Table (5): Effect of selective inhibitors COX1 valeryl salicylate (VAS) (20mg/kg/IP) & COX2 nimesulide (12mg/kg/IP) and non selective indomethacin (5mg/kg/IP) on glutathione peroxidase (GHPx) in cerebral ischemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Indomethacin treated group</th>
<th>Nimesulide treated ischemic group</th>
<th>VAS treated ischemic group</th>
<th>Vehicle treated ischemic group</th>
<th>Sham operated group</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12.24±1.52</td>
<td>20.42±0.02**</td>
<td>14.63±1.52</td>
<td>13.40±1.31*</td>
<td>21.36±2.18</td>
<td>GHPx nmol NADPH oxidized/mg protein/min</td>
</tr>
<tr>
<td>Percentage change</td>
<td>52.3%</td>
<td>37.2%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant at (p<0.05) in comparison to sham operator control group. **Significant at (p<0.05) in comparison to vehicle treated ischemic group.

Table (6): Effect of selective inhibitors COX1 valeryl salicylate (VAS) (20mg/kg/IP) & COX2 nimesulide (12mg/kg/IP) and non selective indomethacin (5mg/kg/IP) on serum superoxide dismutase (SOD) activity in cerebral ischemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Indomethacin treated group</th>
<th>Nimesulide treated ischemic group</th>
<th>Vehicle treated ischemic group</th>
<th>Sham operated group</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>101.55±6.87</td>
<td>169±5.24**</td>
<td>103.32±8.69</td>
<td>106.22±9.86*</td>
<td>SOD U/Mol</td>
</tr>
<tr>
<td>Percentage change</td>
<td>59.9%</td>
<td>54.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant at (p<0.05) in comparison to sham operator control group. **Significant at (p<0.05) in comparison to vehicle treated ischemic group.

According to “modified Stroke index” by (Ohno et al., 1984).

* Significant compared to Sham operated group.
**Significant compared to Ischemic operated group.

Fig. (1): Effect of selective COX inhibitors, COX1 valenyl salicylate (20mg/kg/IP), COX2 nimesulide (12mg/kg/IP) and non selective indomethacin (5mg/kg/IP) on stroke index in cerebral ischemic rat.

* Significant compared to sham operated group.
**Significant compared to vehicle treated ischemic group.

Fig. (2): Effect of selective COX inhibitors, COX1 valenyl salicylate (20mg/kg/IP), COX2 nimesulide (12mg/kg/IP) and non selective indomethacin (5mg/kg/IP) on forelimb placing score in cerebral ischemic rat.

* Significant at (p<0.05) in comparison to sham operator control group.
**Significant at (p<0.05) in comparison to vehicle treated ischemic group.

Data are represented as mean ± SE.

Fig. (3): Effect of selective COX inhibitors, COX1 valenyl salicylate (20mg/kg/IP), COX2 nimesulide (12mg/kg/IP) and non selective indomethacin (5mg/kg/IP) on SOD in cerebral ischemic rat.

* Significant compared to sham operated group.
**Significant compared to vehicle treated ischemic group.

Data are represented as Mean ± SE.

Fig. (4): Effect of selective COX inhibitors, COX1 valenyl salicylate (20mg/kg/IP), COX2 nimesulide (12mg/kg/IP) and non selective indomethacin (5mg/kg/IP) on MDA in cerebral ischemic rat.

* Significant compared to sham operated group.
**Significant compared to vehicle treated ischemic group.

Data are represented as Mean ± SE.
Fig. (5): Effect of selective COX inhibitors, COX1 valenyl salicylate (20mg/kg/IP), COX2 nimesulide (12mg/kg/IP) and non selective indomethacin (5mg/kg/IP) on GHPx in cerebral ischemic rat. Data are represented as Mean ± SE. * Significant compared to sham operated group. ** Significant compared to vehicle treated ischemic group.

Fig. (6): Photomicrographs of ipsilateral cortex on day 4 after cerebral ischemia in rat showing neuronal cell loss or degeneration.
A- Sham operated group showing normal architecture of cerebral cortex sections.
B- Vehicle treated ischemic group showing degeneration and apoptosis in nerve cell and inflammatory cellular infiltration.
C- VAS (20mg/kg/IP) treated ischemic group showing no improvement of ischemia.
D- Nimesulide (12mg/kg/IP) treated ischemic group showing improvement of necrosis and inflammatory cellular infiltration.
E- Indomethacin (5mg/kg/IP) treated ischemic group showing improvement of necrosis and inflammatory cellular infiltration (Hx & E X400).
**Discussion**

Non steroidal anti-inflammatory drugs (NSAIDs) are therapeutic agents of first choice for treatment of inflammation, pain and fever [28]. Recently NSAIDs have gained further attention as potential tools to enhance neurogenerative process in adult mammalian brain. Also, NSAIDs can modulate brain restoration process [29]. Additionally, NSAIDs act through inhibition of cyclooxygenase (COX) enzymes which are responsible for metabolism of arachidonic acid. Metabolism of arachidonic acid through COX enzymes is known to be actively involved in neuroinflammatory events lead to neuronal death after ischemia. COX1 & COX2 expression are dramatically induced by ischemia and appear to be an effecter of tissue damage [30]. Oxidative stress is thought to be the key event in the pathogenesis of cerebral ischemia. Previous studies have shown that one of the primary sources of ROS in the ischemic brain is through the metabolism of arachidonic acid by COX enzymes [31,32]. The COX enzyme possesses two active sites, a cyclooxygenase site which catalyzes the conversion of arachidonic acid to prostaglandin PGG2 intermediate, and a peroxidase site, which reduces the PGG2 intermediate to PGH2 and can increase Nk-B signaling independently of cyclooxygenase activity [33].

In last decade, the potential role of cyclooxygenase activity in neurodegenerative disease is still controversial. Studies aimed to improving our knowledge of mechanisms of action of COX enzymes and physiological and pathological function of COXs and particularly the therapeutic potential of its inhibition [34].

In the present study we investigated the possible neurological efficacy and antioxidant mechanism of action of cyclooxygenase inhibitors in a model of cerebral ischemia in rats.

The present work showed evidentially the infarct lesion after cerebral ischemia in regarding to sham operated group, this was in accordance with [14] who demonstrated infarct lesion after left common carotid occlusion. As regard to neurological examination, there were deterioration in stroke index and neurological tests by occlusion of left common carotid artery. It is important to emphasize the fact that these parameters were obviously after induction of ischemia. These were in consistence with Candelario-Jalil et al. [7,30] who demonstrated impairment in neurological deficits score and rotarod performance in animal models of transient and permanent focal cerebral ischemia, global brain ischemia, embolic stroke and chronic cerebral hypoperfusion.

From the results of the present work, administration of nimesulide to ischemic group, reduced elevation in stroke index and improved the neurological impairment in comparison with vehicle treated ischemic group, this was in accordance with results of Candelario-Jalil et al. [7,30] who reported that nimesulide COX2 inhibitor highly effective in animal models of cerebral ischemia. Nimesulide has been shown to reduce infarction and improve neurological function. Ahmad et al. [36] demonstrated that COX2 inhibition with valdecoxib is effective when initiated both before and after middle cerebral artery occlusion, it decreases infarction volume. In the same time, valeryl salicylate treated ischemic group failed to confer any neuroprotective effect when administrated immediately after ischemia in comparison with vehicle treated ischemic group. These results are in agreement with Candelario-Jalil et al. [14] who stated that cyclooxygenase inhibitor valeryl salicylate had no any protective effect in cortical and subcortical areas of cerebral infarction. Also, Nogawa et al. [4] and Koisinlaho et al. [37] reported that COX1 is not upregulate after ischemic brain injury so, no role of COX1 inhibitor. Also, Iadecola et al. [38] and Lin et al. [39] stated that Cox1 lacking mice are susceptible for focal cerebral ischemia. COX1 is important for normalized cells and COX1 inhibitor is detrimental. Cheung et al. [40] found that COX1 gene deletion has been shown not to affect ischemic brain injury. In contradicting to our results, Schwab et al. [41] reported that COX1 inhibitor valeryl salicylate reduced neuronal death by a blockade of transcription factor. Our findings indicated that valeryl salicylate is not effective, tempt us to suggest that COX2 activity plays important role in progression of ischemic brain injury while COX1 activity plays important role in normal cellular function. In indomethacin (non selective COX inhibitor) treated ischemic group showed significant improvement in stroke index, neurological tests and reduced infarct area in comparison with vehicle treated ischemic group. This result was in agreement with [42,43] who observed that high doses of non selective cyclooxygenase inhibitors (indomethacin, piroxicam) which block both COX1 & COX2 reduced delayed necrosis after global cerebral ischemia. Nagama et al. [44] supported this result through his observation of reduction of infarct volume after administration of non selective COX inhibitor. Miyamoto et al. [45] confirmed the result of the present study, he stated that preischemic administration of indomethacin 5mg/kg/IP significantly rescued hippocampal CA1
neuron from 9±6 cell/mm in ischemia to 87±7 cell/mm in indomethacin treated group. In addition, non selective COX inhibitor naproxen ameliorated hippocampal parenchymal cell death and edema formation mediated by excessive activation of neuronal NMDA receptors in vivo whether this protection occurred via COX2 or not [48]. In contradicting to the present study, Sutherland et al. [47] reported that indomethacin treatment in cerebral ischemic rats failed to produce a significant beneficial effect. Kourainakis et al. [53] added that the cells of diclofenac (non selective inhibitor) treated group not protected in cerebral ischemia. The failure of indomethacin to prevent ischemic neuronal injury explained by hypoperfusion. The reperfusion is associated with an imbalance between the production of thromboxane A2 and prostacyclin. Preferential synthesis of thromboxane A2 may result in vasoconstriction. This may explain, in part, the postischemic hypoperfusion syndrome. Postischemic hypoperfusion may not be a major factor in the initiation or propagation of ischemic neuronal injury [47]. Results from several studies also suggest that the marked and sustained expression of inflammation-related enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) plays an important role in the events that amplify cerebral injury after ischemia [13,57,58].

In this study, we explored the mechanisms of cyclooxygenase inhibitors by studying markers of oxidative stress. Simony et al. [12] reported that the exhaustions of intracellular antioxidants which are capable of preferentially conjugated with toxic metabolites and free radicals lead to overproduction of reactive oxygen species during ischemia so, this lead to imbalance between oxidative and antioxidative process in the body. In our study, GHPx in ischemic group was remarkably reduced reflecting that the potency of antioxidation in injured cells was altered. These results agree quite well with that of Candelario-Jalil et al. [48] who indicated that accumulation of biomarkers of oxidative damage and depletion of antioxidant reserves occur in hippocampal after initial ischemic episode. In the present study, we found that the rats subjected to left common carotid artery occlusion had significant lower level of SOD compared with sham operated group. Superoxide dismutase (SOD) acts as a cellular defense element against potentially harmful effects of superoxide ions by catalyzing the dismutation of these ions [49]. In the same time, we measured malondialdehyde (MDA) as an indicator of lipid peroxidation and hence, the oxidative stress state. We found that the ischemic group induced by left common carotid occlusion showed significant increasing in MDA versus sham operated group. Similar observation has been reported in Candelario-Jalil et al. [32] who demonstrated increasing MDA after induction of ischemia. It is well established that reactive oxygen species are produced after cerebral ischemia and play a critical role in the resulting tissue damage. Cyclooxygenase enzymes play a role in ischemic brain injury and also a potential source of oxidative stress [50,51]. In the other hand, Manabe et al. [52] and Heo et al. [53] stated that increase production of ROS is related to ischemic micro-circulatory injury and prostnoid mainly prostaglandin E2 and not free radical are the pathogenic factors mediating brain injury. Others reported that COX2 activity is a major source of ROS during neuro-inflammation both in vitro and in vivo [54,56]. This may explain by contribution of additional sources of ROS, including NADPH oxidases containing other nox homologues, mitochondrial enzymes, lipoxygenases, p450 enzymes, xanthine oxidase, as well as NOS uncoupling from substrate and cofactor depletion [13,57,58].

In the present work, treatment of rats with valeryl salicylate, nimesulide and indomethacin had variable results. Nimesulide only counteracted GHPx and SOD depletion and lipid peroxidation. It induced a significant increase in GHPx & SOD levels and decrease MDA level in nimesulide treated ischemic group when compared with vehicle treated ischemic group. These findings support the premise that nimesulide can guard against the sequences of oxidative stress. So, the antioxidant properties of nimesulide may be involved in the mechanism of action through which it exerts its effect. In agreement with our results, Candelario-Jalil et al. [14] found that rofecoxib (selective COX2 inhibitor) was effective in restoring the significant reduction in the level of GSH and the increase GSSG and lipid peroxidation that result from transient cerebral ischemia. Additionally, Candelario-Jalil et al. [32] reported that nimesulide significantly reduced hippocampal glutathione depletion and lipid peroxidation as assesses by the levels of MDA even when the treatment delayed 6 h after ischemia. Kunz et al. [38] showed that COX2 is not a major source of oxygen radicals after cerebral ischemia, raise the possibility that other COX2 reaction products including prostanoid or non oxygen based radicals, mediated the COX2 dependent component of the injury. Also, indomethacin has significant beneficial effect on reduction of infarction and improvement of neurological function but no significant amelioration in biochemical parameters of oxidative stress. In agreement to our result, Beasley et al. [59] demonstrated that indomethacin
significantly attenuates the ischemia-induced increase in eNOS suggests that cyclooxygenase-dependent mechanisms are involved in regulating this enzyme. In contradicting to the present work, Miyamoto et al. [18] revealed that indomethacin may protect neurons by attenuating oxidative stress and reperfusion injury in ischemic insult. The result of the present study may explain through indomethacin action on COX1 beside COX2 which has confounding effects resulting from COX1 inhibition and may masks its action as antioxidant through COX2. Furthermore, indomethacin, one of the agents studied most extensively, has profound effects on cerebral blood flow and vascular reactivity.

**Conclusion:** In summary, the present study has evaluated for the first time the neuroprotective and oxidative effects of the COX inhibitors in induced cerebral ischemia. nimesulide and indomethacin, showing beneficial effects on reduction of infarct volume and improvement of functional recovery. This ability to diminish ischemic damage may be due to antioxidant effect. Nimesulide is better than indomethacin may be due to its specific action on COX2. Indomethacin action on COX1 beside COX2 which has confounding effects resulting from COX1 inhibition and may masks its action as antioxidant through COX2. These results have important implications for the therapeutic potential in the treatment of cerebral ischemia. We need more investigations to know all possible mechanisms contribute in cerebral ischemia. Further studies required to demonstrate the chronic use and possible side effect on long run.

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