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"Transient activation of nuclear factor erythroid 2-related factor (NRF-2) induced pharmacological conditioning of brain by a NADPH: Quinone oxidoreductase 1 (NQO1) pathway"

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ABSTRACT
Background: Nuclear erythroid-related factor 2 (NRF2) antioxidant response elements (NEF) signaling pathway regulates the expression of genes whose protein products are involved in the elimination of reactive oxidants and electrophilic agents through conjugative reactions by enhancing cellular antioxidant ability in cerebral ischemia. Therefore, the present study was designed to investigate the potential pharmacological preconditioning of brain induced by Oltipraz, an NRF2 activator, in a mouse model of cerebral ischemia-reperfusion injury.
Materials and methods: Bilateral carotid artery occlusion for 17 min followed by reperfusion for 24 h was employed to produce ischemia-reperfusion-induced cerebral injury in male Swiss albino mice. Cerebral infarct size was measured using triphenyltetrazolium chloride (TTC) staining. Memory was assessed using Morris water maze test and elevated plus maze. Degree of motor in coordination was evaluated using inclined beam walk test, rota-rod test and lateral push test. The levels of TBARS, GSH and Nitrite were measured in the brain as an index of oxidative stress.
Results: Bilateral carotid artery occlusion, followed by reperfusion, produced a significant rise in cerebral infarct size, brain nitrite/nitrate levels, acetylcholinesterase activity, and thiobarbituric acid reactive species level along with a fall in glutathione. A significant impairment of memory and motor coordination was also noted. Treatment of oltipraz (1, 3 & 10 mg/kg, i.p), 24 hour prior to ischemia, significantly and dose dependently attenuated the above effects of ischemia-reperfusion injury in mice. Oltipraz-induced neuroprotective effects were significantly attenuated by administration of 5-methoxy-1,2-dimethyl-3-[(4-nitrophenoxy)methyl]indole-4,7-dione (ES936) (0.3, & 1 mg/kg, i.p), a NAD(P)H: Quinone Oxidoreductase 1 inhibitor.
Conclusions: Results indicate that oltipraz an nuclear factor erythroid 2-related factor 2 (NRF 2) activator induced preconditioning of brain, probably through NAD(P)H: Quinone Oxidoreductase 1 (NQO1) dependent pathways. Therefore, nuclear factor erythroid 2-related factor 2 can be explored as an important target to contain ischemia-reperfusion injury.

Keyword: Oltipraz, NRF2 activator, ischemia-reperfusion injury, Morris water maze, Rota-rod test, 5-methoxy-1,2-dimethyl-3-[(4-nitrophenoxy)methyl]indole-4,7-dione (ES936), NAD(P)H: Quinone Oxidoreductase 1.

I. INTRODUCTION

Ischemic brain disease or stroke is still the third-leading cause of disability and the second leading cause of death worldwide. In terms of the total number of cases, the burden increased significantly between 1990 and 2019 (70% more incident strokes, 43% more stroke deaths, 102% more common strokes), with lower and lower middle income nations bearing the majority of the burden (86% of deaths) (Feigin, Brainin et al. 2022, Jingli, Jing et al. 2022). In cerebral ischemic stroke, occlusion of a major cerebral artery by an embolus or local thrombosis can result in transient or permanent reduction of cerebral blood flow to a portion of the brain. This results in deprivation of glucose and oxygen, since the brain relies on a continuous supply of nutrients and ions via mostly carrier mediated processes across the blood brain barrier (BBB). Any irregularity in these transport mechanisms dramatically affects neuronal function and outcome after acute and chronic stroke (Shah and Abbruscato 2014). Acute ischemic stroke can be treated by clot busting and clot removal, but main failure of this treatment is the short time interval from stroke onset within which it has to be used (Dobkin and Dorsch 2013, Jivan, Ranchod et al. 2013).

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1344-1358 www.ijprajournal.com ISSN: 2249-7781

Researchers have also directed their focus from time to time so as to develop processes in order to salvage ischemic injury. Ischemic preconditioning (IPC) is a potent protective strategy introduced by Murry et al for the ischemic myocardium, which was later applied by Kitagawa et al to the ischemic neuronal injury (Murry, Jennings et al. 1986, Kitagawa, Matsumoto et al. 1990). IPC has been demonstrated in other organ systems as well including skeletal muscle, spinal cord, kidney, intestine, and liver (Goadsby and Edvinsson 1993, Pang, Yang et al. 1995, Hotter, Closa et al. 1996, Matsuyama, Chiba et al. 1997, Turman and Bates 1997). Subsequently, many other forms of preconditioning, such as ischemic, pharmacological, thermal, or gas inhalation (particularly in lung injury) have been investigated (Lloris-Carsi 1993).

Pharmacological agents, following the discovery of related molecular mechanisms of PC, are gradually replacing ischemia or hyperthermia, as the specific "targeted" pretreatment methods to protect organs from various types of injuries. Moreover, detailed mechanistic studies of these phenomenon have indicated the possibility of pharmacologically activating certain biochemical transduction systems leading to the appearance of a preconditioning like protective effect that lasts beyond the agent's elimination, a phenomenon commonly referred to as pharmacological preconditioning (Riess, Eells et al. 2004). IPC can be replicated by pharmacological agents by activating cell membrane receptors such as the adenosine, beta-adrenergic, bradykinin, calcitonin gene related peptide (CGRP), and opioid receptors, and by administering endogenous triggers such as free radicals, nitric oxide, and calcium, activating the intracellular signaling pathway (Murry, Richard et al. 1990, Liu, Thornton et al. 1991, Garlid, Paucek et al. 1997, Schulz, Post et al. 1998, Cain, Meldrum et al. 1999, Post, Schulz et al. 2000). The main advantage of pharmacological PC is its clinical feasibility over ischemic PC (Yellon and Dana 2000).

Nuclear erythroid-related factor 2 (NRF2) activation has been shown to produce a preconditioning like ameliorative effect on ischemic myocardium as well as the ischemic brain (W Thompson, V Narayanan et al. 2012, Zhang, Xiao et al. 2013). The nuclear factor erythroid 2-related factor 2 and antioxidant-response element (Nrf2-ARE) pathway is a main modulator related to inflammation and oxidative damage, which are involved in the neurodegenration after neuronal injury after neuronal injury. Previous studies have demonstrated that NRF2-ARE pathway play neural protective roles in traumatic brain injury, cerebral ischemia, and intracerebral hemorrhage models (Chen, Fang et al. 2011). The transcription factor NRF2 has been shown to be protective in stroke as a key regulator of antioxidant-responsive genes (Yu, Zhao et al. 2011). NQO1 expression and the binding activity of NRF2 to antioxidant response element (ARE) were increased during oxidative stress related to neuronal injury (Wu, Li et al. 2013). Moreover, activated NRF2 regulated the expression of NADPH-dependent reductase plays a critical role in neuroprotective mechanism (Crean, Felice et al. 2012).

The present study was conducted to determine the molecular mechanisms involved in the presumed anti-oxidative effects of oltipraz against experimental stroke. Therefore, the study has been undertaken to investigate the effect of oltipraz induced pharmacological preconditioning on the ischemic brain injury and to find out any possible link between the transduction systems of pharmacological preconditioning induced neuroprotection elicited by NADPH-dependent reductase enzymes NQO1.

II. MATERIALS AND METHODS

2.1. Animals

Swiss albino mice of either sex weighing 25±2 g, maintained on standard laboratory diet (Kisan Feeds Ltd., Mumbai, India) and having free access to tap water were employed in the present study. They were housed in the departmental animal house and were exposed to 12 hr cycle of light and dark. The experiments were conducted in a semi-sound proof laboratory. The experimental protocol was approved by institutional animal ethics committee and care of the animals was carried out as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No. 1181/ab/08/CPCSEA).

2.2. Drugs and chemicals

Oltipraz, ES936 (Sigma Aldrich Chemical Pvt. Ltd., St Louis, USA) and Chloral hydrate (Riedel-deHaen, Germany) were dissolved in. All chemicals were dissolved / diluted in sterile saline prepared in triple distilled water/ 10% dimethylsulphoxide solution in triple distilled water as appropriate. The chemicals used were of analar

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1344-1358 www.ijprajournal.com ISSN: 2249-7781

quality and all drug solutions were freshly prepared before use.

2.3. Ischemia reperfusion induced cerebral injury

Mice were anaesthetized using chloral hydrate (400 mg/kg, i.p.). A midline ventral incision was made in the neck to expose right and left common carotid arteries, which were isolated from surrounding tissue and vagus nerve. A cotton thread was passed below each of the carotid artery. Global cerebral ischemia was induced by occluding the carotid arteries. After 17 min of global cerebral ischemia, reperfusion was allowed for 24 h the incision was sutured back in layers (Himori et al., 1990). The sutured area was cleaned with 70% ethanol and was sprayed with antiseptic dusting powder. The animals were shifted individually to their home cage and were allowed to recover. A single dose of the oltipraz, an activator of NRF2, was given to induce pharmacological (drug induced) preconditioning. The pharmacological preconditioning was induced 24 h prior to the Global cerebral ischemia.

2.4. Assessment of cerebral infarct size

At the end of 24h of reperfusion after global cerebral ischemia, animals were sacrificed by spinal dislocation and the brains were removed and placed immediately in ice cold saline for 10 min. Brain samples were then sliced into uniform coronal sections of about 1 mm thickness. The slices were incubated in 1% triphenyltetrazolium chloride (TTC) at 37°C in 0.2 M tris buffer (pH 7.4) for 20 min (Bochelen, Rudin et al. 1999). TTC is converted to red formazone pigment by nicotinamine adenine dinucleotide (NAD) and lactate dehydrogenase and thus stained the viable cells deep red. The infarcted cells have lost the enzyme and cofactor and thus remained unstained dull yellow. The brain slices were placed over glass plate. A transparent plastic grid with 100 squares in 1 cm^2 was placed over it. Average area of each brain slice was calculated by counting the number of squares on either side. Similarly, number of squares falling over non-stained dull yellow area was also counted. Infarcted area was expressed as a percentage of total brain volume. Whole brain slices were weighed. Infarcted dull yellow part was dissected out and weighed. Infarct size was expressed as percentage of total wet weight of brain.

2.5. Evaluation of memory using Morris water maze

Morris water-maze (MWM) test was employed to assess memory of the animals (Morris 1984, Parle 2004). MWM consisted of large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28 ± 1° C). The water was made opaque with non toxic white colored dye. The pool was divided into four equal hypothetical quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A submerged platform (10 cm^2), painted white was placed in target quadrant 1 cm below surface of water so as to provide escape area. The position of platform was kept unaltered throughout the training session.

2.6. Acquisition trial

Each mouse was subjected to four trials on each day. A rest period of 5 min was allowed in between each trial. Four trials per day were repeated for four consecutive days. Starting position on each day to conduct four training trials was changed as described below and quadrant Q4 was maintained as target quadrant in all acquisition trials.

Table 1

Four day acquisition trial in different quadrants

Days	**Quadrant**			
Day1	Q1	Q2	Q3	Q4
Day 2	Q2	Q3	Q4	Q1
Day 3	Q3	Q4	Q1	Q2
Day 4	Q4	Q1	Q2	Q3

Escape latency time (ELT) to locate the hidden platform in water maze was noted and day 4 ELT served as an index of acquisition or learning. After recording day 4 ELT, the animal was subjected to the surgical procedure and then put to day 5 retrieval test on the Morris water-maze.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1344-1358 www.ijprajournal.com ISSN: 2249-7781

2.7. Retrieval trial

On fifth day the platform was removed. Each mouse was placed in water maze and allowed to explore the maze for 120 sec. Each animal was subjected to four such trials and each trial was started from different quadrant. Mean time spent in all three quadrants i.e. Q1, Q2 and Q3 were recorded and the time spent in the target quadrant i.e. Q4 in search of missing platform was also noted, which served as an index of retrieval. The experimenter always stood at the same position. Care was taken that relative location of water maze with respect to other objects in the laboratory serving as prominent visual clues were not disturbed during the total duration of study. All the trials were completed between 10.00 to 16.00 hrs.

2.8. Evaluation of motor coordination using Rota-rod test

Rota rod has been used to evaluate motor coordination by testing the ability of mice to remain on revolving rod (Dunham 1957). The apparatus consisted of horizontal rough metal rod of 3 cm diameter attached to a motor with variable speed. This 70 cm long rod was divided into four sections by wooden partitions. The rod was placed at a height of 50 cm to discourage the animals to jump from the rotating rod. The rate of rotation was adjusted to allow the normal mice to stay on it for five minutes. Each mouse was given five trials before the actual reading was taken. The animals staying on revolving rod for period of five minutes before the surgical procedure were selected and the test was again performed after 17 min of global cerebral ischemia followed by 24 h of reperfusion.

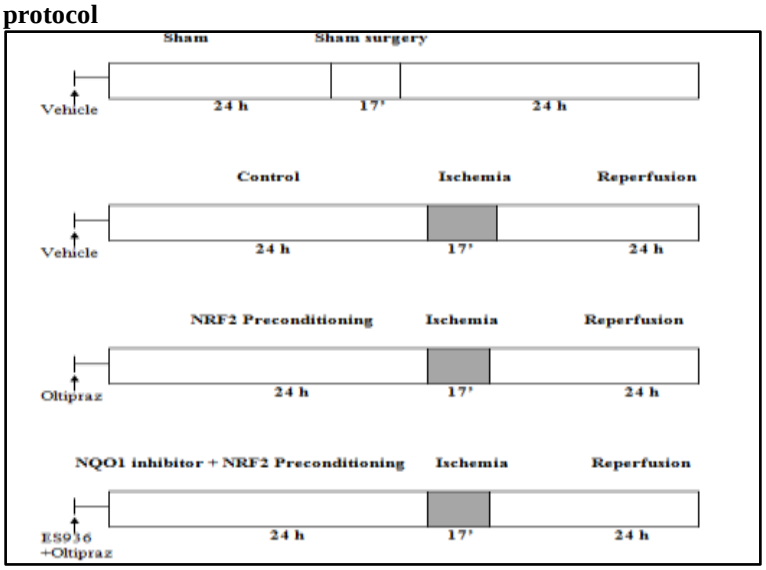
2.9. Inclined beam-walking test

Inclined beam-walking test was employed to evaluate fore and hind limb motor coordination (Feeney, Boyeson et al. 1981). Each animal was individually placed on a metallic bar 55 cm long and 1.5 cm wide, inclined at an angle of 60° from ground. The motor performance of mouse was on a scale ranging from 0 to 4. A grade of 0 was assigned to animal that could readily traverse the beam, grade 1 was given to animal demonstrating mild impairment, grade 2 was assigned to animal demonstrating moderate impairment, grade 3 was given to animal demonstrating severe impairment and grade 4 was assigned to animal completely unable to walk on the beam. Inclined beam-walking test was performed before global cerebral ischemia and 12 h, 24 h after global cerebral ischemia and reperfusion.

2.10. Lateral push test

Motor coordination was also evaluated by observing the percentage of mice showing resistance to lateral push (Bederson, Pitts et al. 1986). Mouse was placed on a rough surface for firm grip and evaluated for resistance to lateral push from either side of shoulder. The test was performed before global cerebral ischemia and 12h, 24h after global cerebral ischemia and reperfusion. Mice with increased or decreased resistance to lateral push after global ischemia were assigned + or - score respectively.

2.11. Experimental protocol



International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1344-1358 www.ijprajournal.com ISSN: 2249-7781

In total seven groups were employed and each group comprised of 8 animals.

Group I (Sham group): Mouse was subjected to surgical procedure carotid arteries were isolated and a thread was passed below them but the arteries were not occluded. After 17 min, threads were removed and the animal was sutured back and allowed to recover for 24 h.

Group II (Control group): Each mouse was subjected to 17 min global cerebral ischemia followed by reperfusion for 24 h.

Group III-V (Oltipraz preconditioning groups): Oltipraz, an NRF2 activator (1, 3 & 10 mg/kg i.p.) was administered 24 h prior to global cerebral ischemia and followed by 17 min of global cerebral ischemia and 24 h reperfusion in mouse.

Group VI-VII (ES936+ Oltipraz preconditioning groups): ES936 (0.3 & 1 mg/kg i.p.) a NQO1 inhibitor was administrated 1 h before and 6 h and 12 h following oltipraz (10 mg/kg, i.p) administration. Rest of procedure was same as described for group-III.

Table 2

Escape latency time (ELT) of animals on Morris water-maze. Values are mean ± standard error of means (S.E.M.) a= $p < 0.05$ Vs day 1 ELT

Sr. no.	Group	Day 1 ELT (in seconds)	Day 4 ELT (in seconds)
	Sham	109± 3.4	39 ± 2.9[a]
	Control	108 ± 3.3	41 ± 2.5 [a]
	Oltipraz PC (1 mg/kg, i.p)	107 ± 2.9	40 ± 2.7 [a]
	Oltipraz PC (3 mg/kg, i.p)	111 ± 2.8	40 ± 2.4 [a]
	Oltipraz PC (10 mg/kg, i.p)	107 ± 3.8	38 ± 3.2 [a]
	ES936 (0.3 mg/kg, i.p) + Oltipraz PC (10 mg/kg, i.p)	106 ± 3.9	41 ±2.7 [a]
	ES936 (1 mg/kg, i.p) + Oltipraz PC (10 mg/kg, i.p)	105 ± 3.5	38 ± 2.6 [a]

"Abbreviations: PC: preconditioning; i.p: intraperitoneal; ELT: escape latency time.

2.12. Statistical analysis

The results were expressed as mean ± standard error of means (S.E.M.). Statistical analysis for all the results was done using one-way ANOVA followed by Tukey's multiple range tests as post-hoc analysis. A value of $P<0.05$ was considered to be statistically significant.

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III. RESULTS

3.1. Effect of pharmacological preconditioning on cerebral infarct size

Global cerebral ischemia of 17 min followed by reperfusion for 24 h (I/R) produced significant increase in cerebral infarct size measured by both volume and weight method when compared to sham group. Pharmacological preconditioning with Oltipraz (1, 3 & 10 mg kg^{-1} i.p.) administered 24 h prior to ischemia-reperfusion significantly and dose dependently attenuated I/R induced rise in cerebral infarct size measured by volume and weight method (Fig. 1, 2).

3.2. Effect of ES936 on pharmacological preconditioning induced reduction in I/R cerebral infarct size

Pretreatment of ES936 (0.3 & 1 mg/kg, i.p) (An NQO1 inhibitor), significantly and dose dependently attenuated oltipraz preconditioning induced decrease in infarct size, (Fig. 1, 2).

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1344-1358 www.ijprajournal.com ISSN: 2249-7781

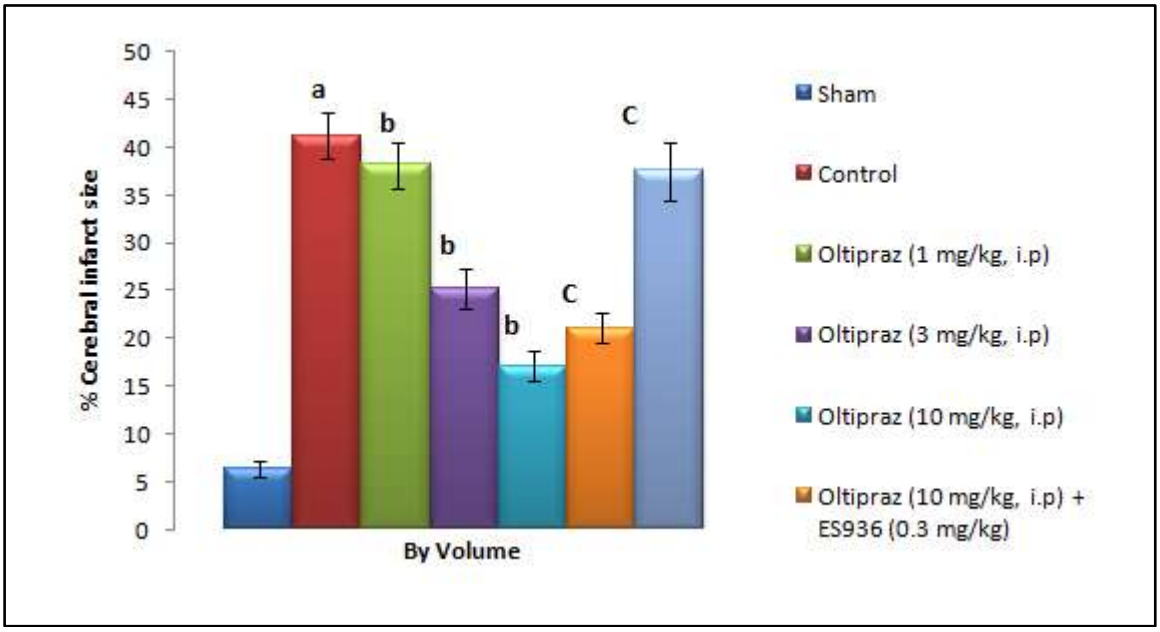

Figure 1: Effect of Pharmacological preconditioning and interventions on ischemia reperfusion induced cerebral infarct size by Volume. Values are mean ± standard error of means (S.E.M.) a = P<0.05 vs. Sham group; b = P<0.05 vs. Control group; c = P<0.05 vs. Oltipraz preconditioning (OPC) group.

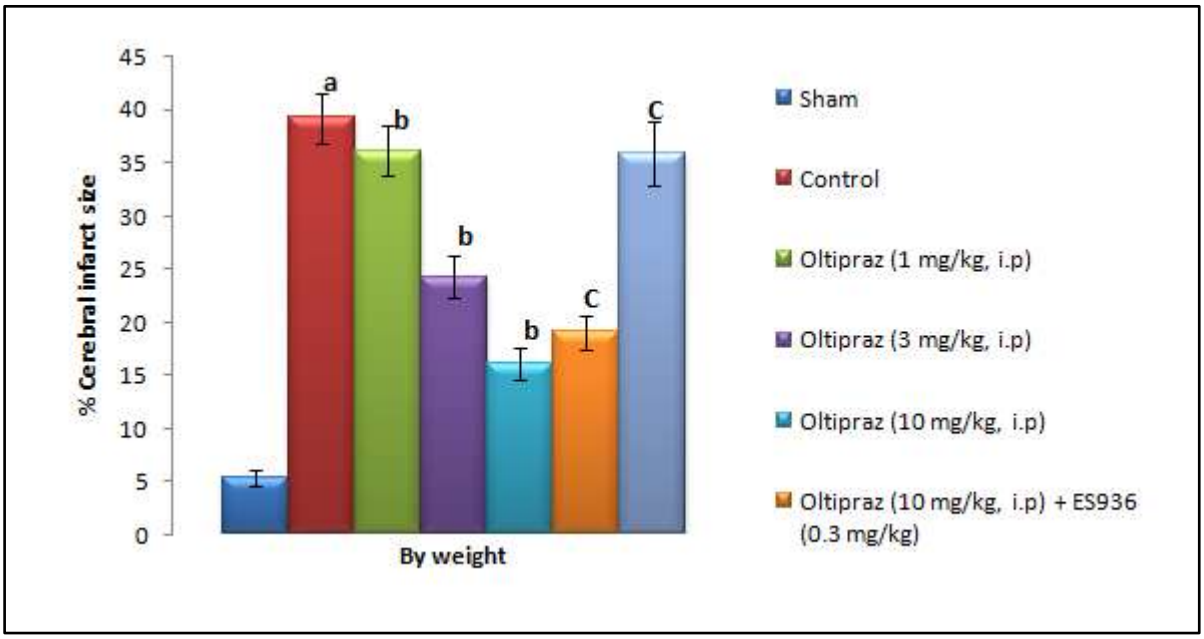

Figure 2: Effect of Pharmacological preconditioning and interventions on ischemia reperfusion induced cerebral infarct size by Weight. Values are mean ± standard error of means (S.E.M.). a = P<0.05 vs. Sham group; b = P<0.05 vs. Control group; c = P<0.05 vs. Oltipraz preconditioning (OPC) group.

3.3. Effect of pharmacological preconditioning on global cerebral ischemia and reperfusion induced impairment of memory as assessed using Morris water maze test

There was a downward trend in escape latency time (ELT) of the animals on subsequent exposure to Morris water maze indicating normal learning abilities (Table 1). The sham control mice, when subjected to retrieval test on day 5 spent

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1344-1358 www.ijprajournal.com ISSN: 2249-7781

significantly ($p<0.05$) more time in the target quadrant (Q4) in search of the missing platform as compared to the time spent in other quadrants (Q1, Q2, Q3), reflecting normal memory capacity (Fig. 3). Global cerebral ischemia and reperfusion significantly reduced day 5 time spent in target quadrant (TSTQ), when compared to sham control animals reflecting impairment of memory. Pharmacological preconditioning induced with Oltipraz (1, 3 & 10 mg kg^{-1} i.p.) produced significant and dose dependent increase ($p<0.05$) in day 5 time spent in target quadrant thus attenuating I/R induced memory impairment (Fig. 3).

3.4. Effect of ES936 on pharmacological preconditioning induced reversal of I/R memory impairment, using MWM test

Pretreatment of ES936 (0.3 & 1 mg kg^{-1}, i.p.) significantly and dose dependently attenuated oltipraz preconditioning induced rise in day 5 TSTQ of mice on MWM (Fig. 3).

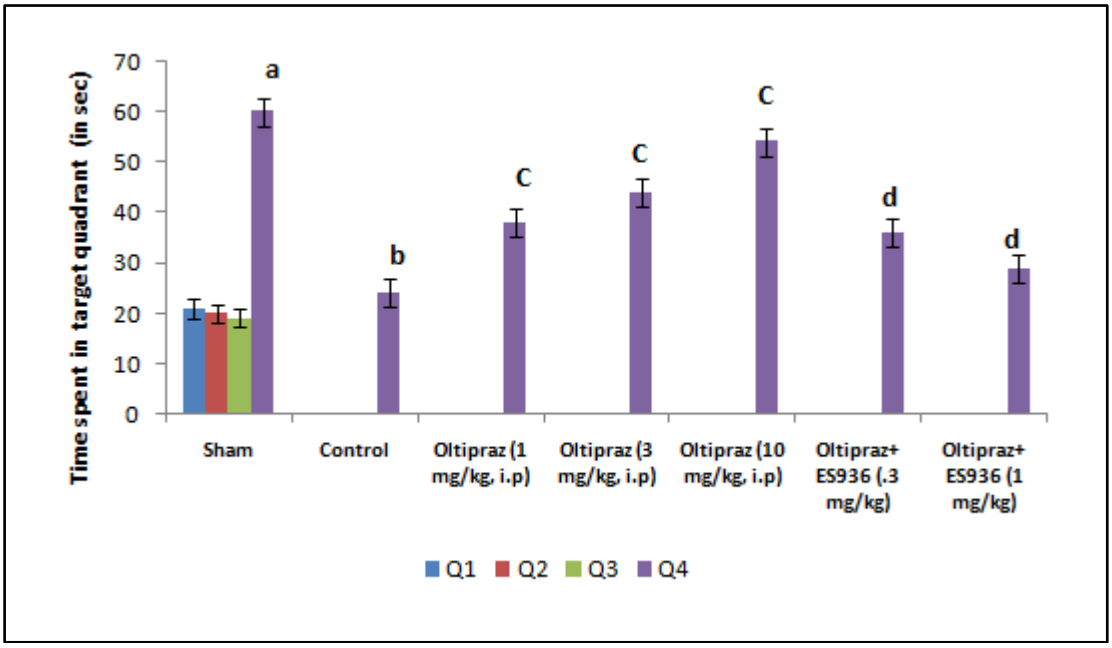

Figure 3: Effect of Pharmacological preconditioning and interventions on ischemia reperfusion induced decrease in time spent in target quadrant (TSTQ) as assessed using Morris water maze. Values are mean ± standard error of means (S.E.M.) a = $P < 0.05$ vs. time spent in other quadrant i.e. Q1, Q2, Q3 in Sham group; b = $P < 0.05$ vs. time spent in target quadrant i.e. Q-4 in Sham group; c = $P < 0.05$ vs. time spent in target quadrant in Control group; d = $P < 0.05$ vs. time spent in target quadrant in Oltipraz preconditioning (OPC) group.

3.5. Effect of pharmacological preconditioning on global cerebral ischemia and reperfusion-induced impairment of motor performance

Global cerebral I/R induced impairment of motor performance was assessed by Rota-rod test, inclined beam walking test, and lateral push response.

3.5.1. Effect on fall down time using Rota-rod test

Global cerebral ischemia of 17 min followed by reperfusion for 24 h produced significant reduction in fall down time, measured by Rota-rod test, after 24 h of reperfusion, when compared to sham group. Pharmacological preconditioning with Oltipraz (1, 3 & 10 mg kg^{-1} i.p.) administered 24 h prior to ischemic insult dose dependently attenuated I/R induced reduction in fall down time (Fig. 4).

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1344-1358 www.ijprajournal.com ISSN: 2249-7781

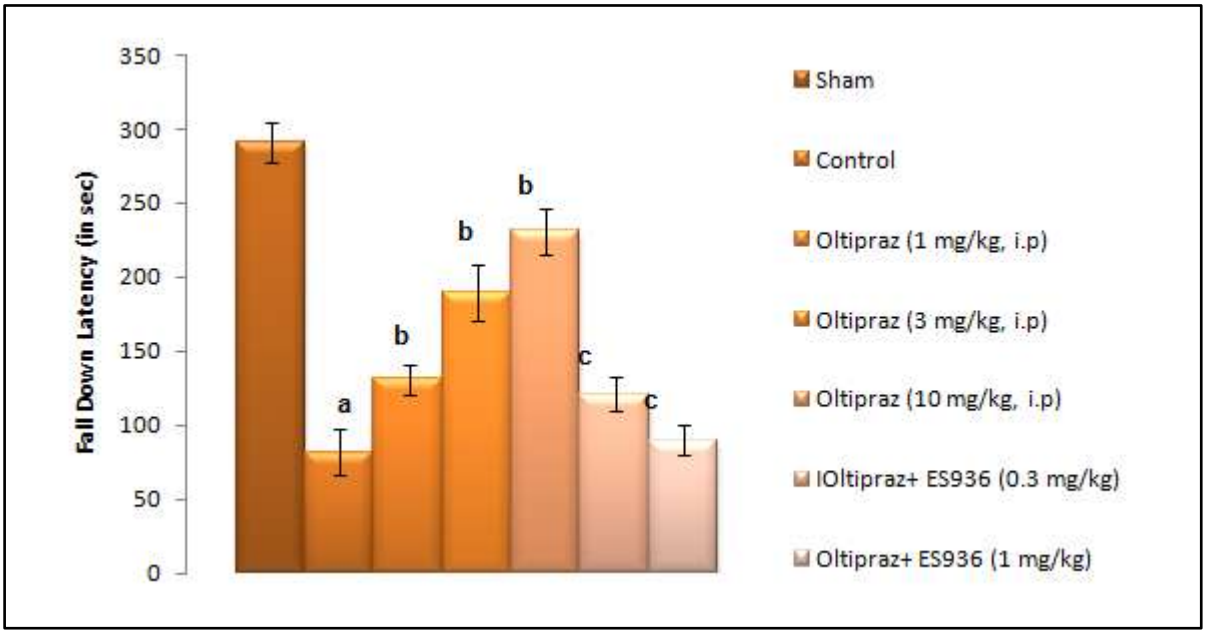

Figure 4: Effect of pharmacological preconditioning and interventions on ischemia reperfusion induced changes in motor performance (fall down time) in mice using Rota rod test. Values are mean ± standard error of means (S.E.M.). a = P<0.05 vs. Sham group; b = P<0.05 vs. Control group; c = P<0.05 vs. Oltipraz preconditioning (OPC) group.

3.5.2. Effect on motor incoordination score using inclined beam walking test

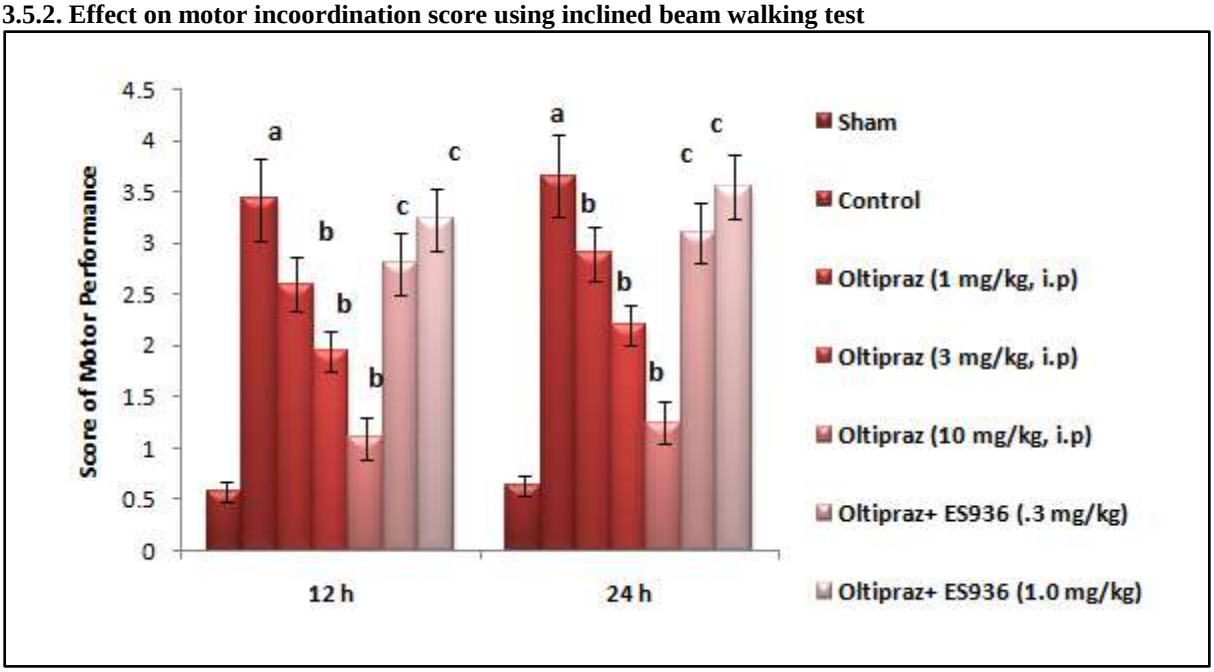

Figure 5: Effect of Pharmacological preconditioning and interventions on ischemia reperfusion induced changes in motor performance (Score of motor performance) in mice using inclined beam walk test. Values are mean ± standard error of means (S.E.M.) a = P<0.05 vs. Sham group; b = P<0.05 vs. Control group; c = P<0.05 vs. Oltipraz preconditioning (OPC) group.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1344-1358 www.ijprajournal.com ISSN: 2249-7781

Global cerebral ischemia of 17 min followed by reperfusion for 24 h produced significant rise in motor incoordination score, when compared to sham group as assessed by inclined beam walking test, after 12 h and 24 h of reperfusion. Pharmacological preconditioning with Oltipraz (1, 3 & 10 mg kg^{-1} i.p.) administered 24 h prior to ischemic insult dose dependently attenuated I/R induced increase in motor incoordination score in a significant manner (Fig. 5).

3.5.3. Effect on resistance to lateral push response

Global cerebral ischemia of 17 min followed by reperfusion for 24 h produced a significant decrease in percentage of mice exhibiting resistance to lateral push in mice noted after 12 h and 24 h of reperfusion, when compared to sham group. Pharmacological preconditioning with Oltipraz (1, 3 & 10 mg kg^{-1} i.p.) administered 24 h prior to ischemic insult dose dependently attenuated I/R induced decrease in percentage of mice demonstrating resistance to lateral push when compared to the control group (Fig. 6).

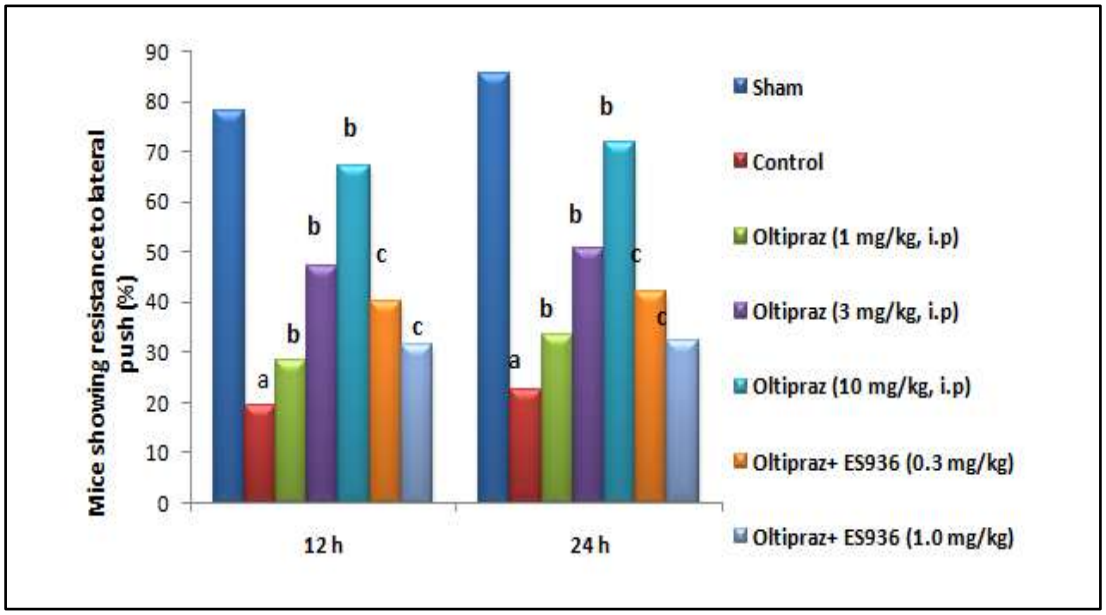

Figure 6: Effect of Pharmacological preconditioning and interventions on ischemia reperfusion induced changes in motor performance in mice using lateral push test. Values are percentage mice demonstrating resistance to lateral push test. a = P<0.05 vs. Sham group; b = P<0.05 vs. Control group; c = P<0.05 vs. Oltipraz preconditioning (OPC) group.

3.5.4. Effect of ES936 on pharmacological preconditioning induced reversal of I/R impairment of motor performance

Pretreatment of ES936 (0.3 & 1 mg/kg, i.p) significantly and dose dependently abolished oltipraz preconditioning induced decrease in motor incoordination score (Fig. 5), rise in fall down time (Fig. 4) and increase in the percentage of mice exhibiting resistance to lateral push (Fig. 6).

IV. DISCUSSION

Global cerebral ischemia and reperfusion model employed in the present study is reported to simulate the clinical situation of cerebral ischemia (de Leciñana, Díez-Tejedor et al. 2001). Cerebral ischemia has been reported to impair memory because hippocampal neurons are susceptible to the deleterious effects of ischemia and reperfusion and hippocampus is involved in regulation of memory (Jenkins, Povlishock et al. 1981). Cerebral ischemia is further documented to impair motor ability as well (Rogers, Campbell et al. 1997).

Therefore, in the present investigation we employed Morris water-maze test to assess memory and Rota-rod test, inclined beam walk test and lateral push test for evaluation of motor coordination. In our study, global cerebral ischemia/ reperfusion (I/R) produced a significant rise in infarct size and induced impairment of memory as well as of motor coordination. These

International Journal of Pharmaceutical Research and Applications
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findings are in line with earlier reports (Norio, Hiroshi et al. 1990, Gupta, Singh et al. 2003, Rehni and Singh 2007, Rehni, Singh et al. 2007). In the present study, a single injection of oltipraz, 24 h prior to the severe ischemic insult, has resulted in a considerably reduced ischemia-reperfusion induced cerebral injury as measured in the terms of cerebral infarct size and impairment of memory and motor-coordination, which is in consonance with earlier findings (Heurteaux, Lauritzen et al. 1995, Matsuyama, Chiba et al. 1997). The pathogenesis of many neurodegenerative diseases is thought to be associated with oxidative stress due to the accumulation of Reactive oxygen species. The Nrf2-ARE transcriptional pathway plays an important role in the regulation of genes that control the expression of proteins critical in the detoxication and elimination of ROS and electrophiles (Nguyen, Nioi et al. 2009). NRF2 is a member of the cap'n'collar (CNC) family of transcription factors, which also include NRF1, NRF3 and p45 NF-E2. NRF2 is a basic leucine zipper (bZIP) protein that in the nucleus hetero dimerizes with small MAF or JUN proteins followed by binding to specific DNA sites termed anti-oxidant response elements (ARE) or electrophile response elements (EpRE) (Itoh, Chiba et al. 1997, Venugopal and Jaiswal 1998). This binding can initiate transcription of various cytoprotective genes including enzymes in the glutathione defense system and proteasome subunits to prevent oxidative stress and enhance the anti oxidative capacity of brain derived neurotrophic factors (Kwak, Wakabayashi et al. 2003, Sakata, Niizuma et al. 2012), the anti-apoptotic B-cell lymphoma 2 (Niture and Jaiswal 2012), the anti-inflammatory interleukin (IL)-10, the mitochondrial transcription (co)-factors NRF-1 and peroxisome proliferator-activated receptor γ co-activator 1-α (PGC-1α) (Piantadosi, Withers et al. 2011), the iron exporter ferroportin 1 (Harada, Kanayama et al. 2011), and the autophagic protein p62 (Komatsu, Kurokawa et al. 2010). This activation of Nrf2 is an important pathway that can upregulate endogenous antioxidant production, and prevent the pathogenesis of ischemic injury. ROS has been suggested to regulate activation of Nrf2 following ischemia through kinase activation. Subsequent phosphorylation of Nrf2 enhances Nrf2 dissociation from Keap1 and allows Nrf2 to express antioxidant enzymes and other proteins to better adapt the cell to oxidative stress (Papaiahgari, Zhang et al. 2006). Nrf2 has a ubiquitous expression, as Nrf2 has been shown to induce antioxidant gene transcription in rat liver, lung, brain and heart tissue (Habeos, Ziros et al. 2008, Dreger, Westphal et al. 2009, Kikuchi, Ishii et al. 2010). Nrf2 was shown to be up-regulated following a 50% reduction in cerebral blood flow in mice; the resulting cerebral oligemia in mice led to increased oxidative stress and subsequent activation of Nrf2 in neurons predominantly in cerebellar Purkinje cells and cingulate cortex (Liverman, Cui et al. 2004). There has been extensive debate as to whether transient hypoxic stress activates Nrf2 protective pathways. A previous study demonstrated upregulation of Nrf2-targeted gene transcription following IPC in human and rat astrocytes; more importantly, the observed decrease in cell death due to induction of IPC was abrogated in homozygous Nrf2 knockout rats, suggesting that Nrf2 could mediate an important role in IPC mediated neuroprotection (Bell, Al-Mubarak et al. 2011). It is conceivable that the absence of NQO1 may result in diminished protection against O_{2}- free radicals over the lifetime of an individual, leading to increased cell damage and incidence of disease. This is especially relevant to NQO1 because of a common single nucleotide polymorphism (NQO1*2). Expression of the NQO1*2 allele results in a NQO1 protein that has minimal catalytic activity and is rapidly degraded by the ubiquitin/proteasomal system (Traver, Siegel et al. 1997, Siegel, Anwar et al. 2001) that play a prominent role attenuation of ischemic preconditioning of brain (Rehni, Singh et al. 2010). Recent studies have found that curcumin induces the expression of heme oxygenase-1 and aldose reductase (Kang, Kim et al. 2007, Pugazhenthi, Akhov et al. 2007) in vitro through a PI3K/Akt-mediated signaling pathway involving the transcription factor Nrf2 induced expression of NQO1 after focal cerebral ischemia/ reperfusion injury in rats inhibits oxidative stress. In case of MCAO there is remarkable up-regulation of phospho-Akt and NQO1 in ischemic rat brain following treatment with curcumin (5 mM), and this up-regulation was accompanied by increases in the nuclear translocation of Nrf2 and in the DNA-binding of Nrf2 to the ARE sequence (Wu, Li et al. 2013). Based on the above discussion, it may be deduced that while oltipraz an NRF2 activator preconditions the brain via NAD(P)H:quinone oxidoreductase 1 (NQO1) a chemoprotection enzyme activation linked mechanism.. This observation further suggest that these NQO1 based transduction systems involved in the respective pharmacological preconditioning evoked by

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oltipraz might actually be linked, i.e. there is an relationship between nrf2 activation and NQO1 in mediating neuroprotective effect of pharmacological preconditioning.

V. CONCLUSION

We demonstrated that oltipraz reduced oxidative stress generated by ischemia/ reperfusion (I/R) and promoted cell survival involving Nrf2/NQO1 signal pathway. Therefore, it is concluded that the neuroprotective effect of pharmacological preconditioning induced by oltipraz involves activation of NAD(P)H:quinone oxidoreductase 1. The present study has been designed to investigate the NRF2 receptors transduction systems in neuroprotective mechanism of pharmacological preconditioning Bilateral carotid artery occlusion of 17 minutes followed by reperfusion for 24 hr was employed to produced cerebral injury. Ischemic neuronal injury was assessed in the brain tissue using triphenyltetrazolium chloride (TTC) staining. Memory was assessed using Morris water maze (MWM) test. Degree of motor incoordination was evaluated using inclined beam-walk test, rota rod test and lateral push test. Oltipraz (1, 3 & 10 mg kg^{-1} i.p.) an NRF2 activator, were administered 24h before surgery in a separate group of animals to induce pharmacological preconditioning. ES936 (0.3 & 1 mg kg^{-1} i.p.) was employed as a selective inhibitor of NQO1 respectively. Based on results obtained in the present study, the following salient findings may be summarized. Global cerebral ischemia followed by reperfusion produced significant increase in neuronal injury measured in terms of infarct size by volume and weight method. A significant impairment of memory and motor coordination was also noted in these animals. Pharmacological preconditioning by oltipraz treatment produced a significant decrease in cerebral infarct size along with reversal of I/R induced impairment of memory and motor-coordination. Oltipraz induced neuroprotective effects were abolished significantly by ES936 administered 1 h & 6 h, 12 h before & following oltipraz administration. It may be concluded that neuroprotective effect of pharmacological preconditioning induced by oltipraz an activator of Nrf2 (nuclear factor E2-related factor 2), involves activation of detoxication enzyme NQO1 (NADPH: quinone oxidoreductase 1).

In summary, the Nrf2 mediated transcriptional pathway plays an important role in the regulation of genes that control the expression of proteins and enzymes critical in the detoxication and elimination of ROS and electrophiles during ischemic injury.

Conflict of interest

The authors declare that there are no conflicts of interest.

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