

# Cerebroprotective assessment of ethanolic extract of *Moringa oleifera* flowers against global cerebral ischemia reperfusion in wistar rats

Janey Alam<sup>1</sup>, Esha Yadav<sup>2</sup>, Shahanuwaz Ahammad<sup>3</sup>, Sadaf Arfi<sup>4</sup>, Ankit Shukla<sup>5</sup>, Abdul Basit<sup>6</sup>, Rahul Dev<sup>7</sup>, Ravi Kant Patel<sup>8</sup>

<sup>1,2,3,4,5,6,7</sup>Axis Institute of Pharmacy, Rooma Kanpur.

<sup>8</sup>Maya Institute of Pharmacy, Mendu Rd, Hathras.

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## Abstract

Numerous herbal medications have historically been used for their neuroprotective properties. The goal of that study's research was to find out whether female rats suffering from global cerebral ischemia and reperfusion could benefit from an ethanolic extract of the *Moringa oleifera* flower. All animals underwent a 10-day pretreatment period.

Normal control (group I) rats received 10 days of pre-treatment with 1% carboxymethyl cellulose (CMC). Ischemic control (group II), MOE low dosage (group III), MOE high dose (group IV), and standard medicine Quercetin (group V) were pre-treated with 1% CMC 1ml/kg, MOE 200mg/kg, MOE 400mg/kg, and Quercetin (group V) at 25mg/kg for 10 days respectively. On the ninth day, all behavioural parameters were evaluated. In order to produce reperfusion injury for 24 hours, the clips were detached on the tenth day after the BCCAO-induced ischemia had been inflicted for 30 minutes under anaesthesia with ketamine (50 mg/kg, i.p). The incision was then stitched up using silk sutures.

All rats were examined for neuroprotective activity and behaviour measures once the reperfusion period had ended. These parameters included neurological score, EPM for spatial memory, rota-rod, locomotor activity, and hanging wire test. As compared to the ischemic group II, group IV (high dosage) significantly ( $p < 0.01$ ) affected neurological score, Rota-rod, locomotor activity, hanging wire test, and transfer delay time (EPM test). *Moringa oleifera* flower extract (MOE) 400mg/kg exhibited more similar neuroprotective efficacy to that of the conventional medication Quercetin 25mg/kg in some ways. The transfer latency depicted in the EPM was substantially more influenced by group IV (400mg/kg) than by group V of quercetin ( $p < 0.01$ ).

Numerous oxidative stress and biochemical parameters, such as thiobarbituric acid reactive substances (TBARS), tissue glutathione (GSH), catalase activity, nitric oxide activity, and triphenyltetrazolium chloride (TTC) staining of the brain infarct area, were used to evaluate the neuro-protective potential of various compounds. Catalase activity, superoxide dismutase activity, GSH, TBARS, and NO levels were considerably higher in pre-treatment groups with MOE (200mg per kg and 400mg per kg and quercetin (25mg per kg). The study's findings were based on the histopathological outcome. A study that demonstrated comparable neuro protective efficacy against BCCAO-induced cerebral ischemia in female rats was proof that MOE had neuroprotective activity in comparison to the usual medication Quercetin.

**Keywords:** *Moringa oleifera*, Cerebroprotective assessment, Global cerebral ischemia, Neurobehavioral scores.

## 1. INTRODUCTION

A fast-growing, evergreen or deciduous plant known as *Moringa oleifera* (Moringaceae) is a wonder plant. Drumstick in English, Saguna, Sainjna in Hindi, and Subhanjana in Sanskrit.<sup>1</sup> The plant is widely distributed in the Sub-Himalayan range. It may grow between sea level and 1400 m above sea level. It is widely grown in front yards, Assam, Bengal, and foreland. Whole Asia, Africa, Caribbean region, South and central America<sup>2</sup> from Mexican region to Peru<sup>3</sup>, Brazil<sup>4</sup>, Paraguay<sup>5</sup>, Sri Lanka are all regions where drumsticks are frequently cultivated. For enhancing breastmilk, it is known as a mother's greatest friend in the Philippines, and it is rarely prescribed for anaemia. The *Moringa oleifera* tree's entire body, inclusively, flower, fruits, leaves, roots, gum, seed, bark and oil of seeds has medicinal properties for various illnesses.<sup>6</sup> This herb has historically been used as an antispasmodic, expectorant, diuretic, and antilithic. Ascorbic acid, beta-carotene, protein, and naturally occurring antioxidants such flavonoids, phenolics, and carotenoids are said to be its major nutrients isolated from root, aurantiamide acetate<sup>7</sup> and 1,3 dibenzyl urea extracted from stem,<sup>8</sup> vanillin, -sitosterol, -sitostenone, 4-hydroxy mellein, and octa-cosanoic acid.<sup>9,10,11</sup>  $\beta$ -Sitosterol, Alkaloids- moringine, moringinine obtained from both stem and bark.<sup>12</sup> Octacosanoic acid, 4-

hydroxymellein, vanillin,  $\beta$ -sitosterone.<sup>13</sup> Sucrose, amino acids, D-glucose, rhamnetin,<sup>15</sup> flavonoids and traces of alkaloids (kaempferitrin, isoquercitrin, quercetin, rhamnetin and kaempferol) and also some minerals (calcium and potassium) were extracted from *Moringa oleifera* flower.<sup>16,17</sup> Niazimicin, 4-hydroxyphenyl acetamide, [4-hydroxyphenyl acetic acid], [4-( $\alpha$ -L-rhamnosyloxy)benzyl nitrile]<sup>18</sup> [4-( $\alpha$ -L-rhamnosyloxy)benzylglucosinolate]<sup>19</sup> and moringyne were also isolated [3-O-(6-O, oleoyl-D-glucopyranosyl)]<sup>20</sup>  $\beta$ -sitosterol.<sup>21,22</sup>

The plant *Moringa oleifera* has a widely pharmacological activities<sup>23</sup>, including those regulate thyroid hormone levels and treat hyperthyroidism, as well as antioxidant, antidiabetic, diuretic, cardiovascular disease, hyperlipidaemia, antispasmodic, ulcer-protective, hepatoprotective, anti-bacterial, antitumor, anti-fungal, and other responses.<sup>24</sup>

Therefore, the present study was helm to investigating cerebroprotective assessment of ethanolic flower extract of *Moringa oleifera* flowers against global cerebral ischemia reperfusion in wistar rats. This herb was also found to have evidence of anticancer<sup>2</sup>, hepatoprotective activity<sup>26</sup>, antiulcer activity<sup>27</sup>, CNS activity<sup>28</sup> and antioxidant effects.

## 2. Methodology

### 2.1 Chemical and equipment

All chemicals and equipment were procured from Qualigens Fine Chemicals (Maharashtra) Sigma Aldrich Chemical Co. (now subsidiary of Merk), and were of analytical grade. Glutathione (Lobachemie), Rota rod (Ajanta AEI-RR-SH-04), Elevated plus maze (EPM, Orchid Scientific & Innovative India pvt ltd), Actophometer (Adarsh Int. ambala), Neurological score, Hanging wire test, centrifuge (spinwell), Ultra Violet (UV) spectrophotometer, and Thiobarbituric acid (TBA), Himedia (PharmaSpec UV-1700, shimadzu Japan).

### 2.2 Authentication and collection

Flowers of *Moringa oleifera* gathered in May 2022 from Dasauli and Basha Kursi Road Lucknow, India, and verified by a botanist at Integral University (Faculty of Pharmacy) Lucknow (1422/IU/PHAR//HRB/22/7). *Moringa oleifera* flower specimen was successfully submitted to authentication department for reference.

### 2.3 General preparation of extract from *Moringa oleifera* flowers.

Washed the flowers thoroughly and dried inside room, dried 100g flowers were extracted by cold maceration with 50% ethanol and withstand at room temperature shaking occasionally. To calculate percentage yield after filtrate concentrated under the reduced pressure by drying extract with the help of rota vapour (decibel instrument). It kept in air tight container and used for Pharmacological investigation.

### 2.4 Experimental Animal

The Central Drug Research Institute Lucknow's animal house provided female (Wistar) animals weighed up between (190-250g). Experimental animals were housed under the conventional laboratory setting for acclimatization for 12-hour cycle light/dark, feeding food pellets, and water ad-libitum. Animals used in experiments are cared for in accordance with (CPCSEA). Integral University (Faculty of Pharmacy) Lucknow and Institutional Animal Ethical Committee (IAEC), have given their approval for this project's ethical conduct.

### 2.5 Experimental Model and Protocol

All animals were separated into five groups and every group contained five animals randomly (n=5). Animals in group I that had received 1% carboxy methyl cellulose (pre-treated) served as the normal control. Animals in group II that received a vehicle pre-treatment for ten days. On the tenth day, BCCAO was used to induce ischemia for 30 minutes and after that allowing blood to flow again through the carotid arteries to cause reperfusion for 24 hours. Animals were pre-treated for 10 days with MOE low dose 200mg per kg (group III), MOE high dose 400mg per kg (group IV), and conventional medication Quercetin 25mg per kg<sup>25</sup> (group V).

All animals were examined for neurological behavioural results after the reperfusion period was over. Ketamin (50 mg/kg i.p.) was administered to all animals prior to being sacrificed under anaesthesia through decapitation. Each animal's brain was then removed, cleaned in chilled 0.9% saline, and each group brain was then weighed. The supernatant from the homogenate was recovered by centrifuging it at 3000 g for 3 minutes at 4 oC. The entire brain was immersed in a formalin solution (10%, v/v) for histological analysis.

### 2.5 Assessment of Neurological score

The elevated plus maze (EPM) test for spatial memory and neurological score, Rota-rod for rodent motor coordination, Actophotometer for measuring locomotor activity (ambulation), and Hanging wire test for measuring overall muscle function

and muscle relaxant in rodents were used to measure the behavioural parameters. Catalase activity, NO, tissue glutathione, thiobarbituric acid reactive substances (TBARS), and brain infarct area by TTC staining.

## 2.6 Histopathological Evaluation

Under anaesthesia, all animals were sacrificed and whole-brain removed and brain was immersed in formalin solution (10%, v/v). For histological examination of cerebral ischemia, neuron atrophy, neuron scarring, neuron shrinkage, and disrupted brain architecture.

## 2.7 Analysis of data

The experimental data analysed with mathematically as (SEM±Mean). For individual results were utilized Student's 't' test and compared with Pad Prism tool. Newman-Keuls test for comparison between them. Statistical significance determined to exist when probability values was lower than ( $p < 0.05$ ).

## 3. Results

The table below shown the potential of MOE (*Moringa oleifera* extract) on cerebral ischemia reperfusion. Ischemic control (group II) had a neuro-deficit score ( $3.20 \pm 0.45$ ) that was substantially higher than normal control (group I), which had a score of ( $0.0 \pm 0.0$ ). In comparison to group II, pre-treatment groups with MOE 200, 400mg/kg and conventional medication Quercetin 25mg/kg shown considerably (MOE 200mg per kg ( $p < 0.05$ ) and 400 mg per kg ( $p < 0.01$ ) significantly reduced neuro-deficit score as ( $1.10 \pm 0.23$ ), ( $0.56 \pm 0.21$ ) and ( $0.4 \pm 0.20$ ). Group IV (MOE 400mg/kg) have similar potential to group V received conventional Quercetin (25mg/kg) treatment. When compared to group II, the rats pre-treated with 400mg per kg of MOE shown a substantial change ( $p < 0.01$ ).

Group II significant reduction (using rota rod) in hanging time ( $2.987 \pm 3.12$ ) as compared to Group I ( $6.67 \pm 1.27$ ). Pre-treated with (MOE 200mg per kg ( $p < 0.05$ ) and 400 mg per kg ( $p < 0.01$ ) increased hanging latency time ( $3.29 \pm 0.24$ ) and ( $5.438 \pm 0.35$ ) as compare to group II ( $2.987 \pm 3.12$ ). Group II significantly declined hanging wire and locomotor activity ( $45.25 \pm 2.05$ ) and ( $138.2 \pm 1.03$ ) as compare to group I ( $82.36 \pm 4.7$ ) and ( $289.24 \pm 1.43$ ). Pre-treated with MOE 200, 400mg/kg significantly increased hanging wire activity and Locomotor activity ( $67.58 \pm 4.70$ ,  $78.52 \pm 5.1$  and  $198.69 \pm 1.2$ ,  $274.35 \pm 1.2$ ) as compare to group II ( $45.25 \pm 2.05$ ,  $138.2 \pm 1.03$ ). Pre-treated group IV MOE (400mg per kg) and group V standard drug Quercetin (25mg per kg) have resemblance in action.

**Table 1.A.** Effects of ethanolic extract of *M. oleifera* flower (MOE) on behaviour parameter neuro-deficit scores, Rota rod activity, hanging time and locomotor activity in ischemia reperfusion rats and pre-treated groups.

Groups	Group name	Neuro-deficit scores	Mean fall time in Sec. (From Rota rod Apparatus)	Hanging Wire (mean fall time in sec)	Locomotor activity Counts/5min
I	Sham (Control)	$0.0 \pm 0.0$	$6.67 \pm 1.21$	$82.36 \pm 4.7$	$289.24 \pm 1.43$
II	Ischemic Control	$3.2 \pm 0.45^{**}$	$2.987 \pm 3.12^{**}$	$45.25 \pm 2.05^{**}$	$138.2 \pm 1.03^{**}$
III	MOFE 200mg/kg	$1.1 \pm 0.23\$$	$3.29 \pm 0.24\$$	$67.58 \pm 4.7\$$	$198.69 \pm 1.2\$$
IV	MOF Extract 400mg/kg	$0.56 \pm 0.21\#$	$5.438 \pm 0.35\#$	$78.52 \pm 5.1\#$	$274.35 \pm 1.2\#$
V	Quercetin 25 mg/kg	$0.4 \pm 0.20\#$	$6.28 \pm 1.24\#$	$80 \pm 1.08\#$	$278.28 \pm 1.24\#$

The values were represented as Mean±SEM. Where (\*\*) denotes(p<0.01) as compared to normal control groups I; indicated (p<0.05),(\$p<0.05) and (#p<0.01) as compared to ischemic control group II.

**Table 1.B** Effect of MOE and quercetin on rats injured by cerebral ischemia-reperfusion in an elevated plus maze.

Groups	Groups	Onset or initial latency	I <sup>st</sup> retention latency	II <sup>nd</sup> retention latency (After 24 hr)
I	Sham	23±2.92	29.6±2.29	33.8±2.41
II	Ischemic Control	58.8±3.21**	61.238±3.81**	69.13±3.41**
III	MOF Extract 200mg/kg	63.8±3.01\$	68.8±3.47\$	73.5±3.03\$
IV	MOF Extract 400mg/kg	60.8±1.41#	56.8±1.51#	58.8±1.55#
V	Quercetin 25mg/kg	65.8±1.21#	60.8±1.34#	63.8±1.31#

Ischemia control (group II) significantly increased initial or onset latency (58±3.12), first retention latency (61.23±3.81) and Second retention latency (69.13±3.41) as compare to group I, (23±2.92, 29.6±2.29 and 33.8±2.41) respectively. Pre-treated with MOE 200mg/kg attenuated increased the transfer latency time (63.8±3.01, 68.8±3.47 and 73.5±3.03) as compare to ischemic control (group II). MOE 400mg/kg (group IV) and standard Quercetin drug 25mg/kg (group V) had exhibited slightly improvement in initial latency, impairment in first and second retention latency (58±3.12, 61.23±3.81 and 69.13±3.41), (60.8±1.41, 65.8±1.21), (56.8±1.51, 58.8±1.55) and (60.8±1.34, 63.8±1.31) respectively.

The results were represented as Mean±SEM(n=5). Where (\*\*) denotes (p<0.01) as compared to normal control groups I; indicated (p<0.05), (\$p<0.05) and (#p<0.01) as compared to ischemic control group II. #Non significance for pre-treated group over ischemic group.

**Table 1.C** Effects of MOF on TBARS, Glutathione, CAT and NO by cerebral ischemia reperfusion induced rats.

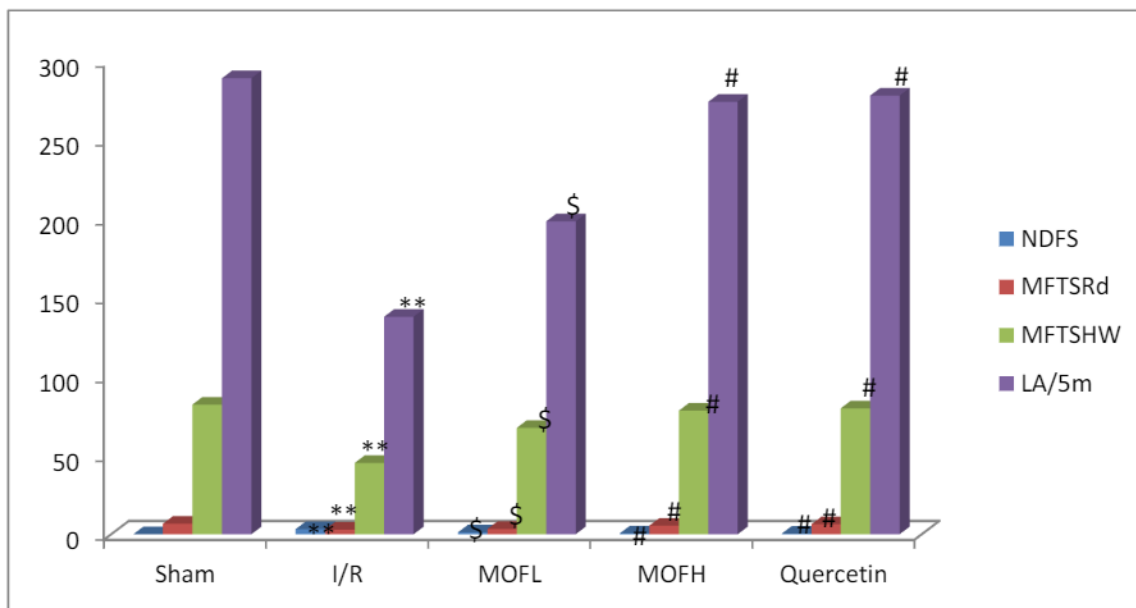
Groups	Groups	TBARS (nM of MDA)	GSH (µg/mg protein)	Catalase activity (Consumed H <sub>2</sub> O <sub>2</sub> nmol, µg/mg protein)	NO, (µg/mg protein)
I	Sham	5.93±0.13	3.29±0.08	106.35±1.03	25.94±0.55
II	Ischemic Control	15.89±0.75**	1.27±0.09**	90.79±5.01**	65.21±2.12**
III	MOF Extract 200mg/kg	9.21±0.15\$	1.98±0.91\$	138.01±1.09\$	28.33±0.33\$
IV	MOF Extract 400mg/kg	7.25±0.22#	2.93±0.78#	178.10±1.40#	23.49±0.215#
V	Quercetin 25 mg/kg	6.91±0.32#	3.01±0.29#	201.33±2.50#	24.68±0.284#

BCCAO followed by 24 hours of reperfusion dramatically elevated TBARS and nitric oxide levels, indicating oxidative stress in group II (15.89±0.75 and 65.21±2.12) as compare to group I, (5.93±0.13 and 25.94±0.55). Pre-treated group III 200mg/kg (9.21±0.15 and 28.33±0.33), group IV 400mg/kg (7.25±0.22 and 23.49±0.215) and group V standard Quercetin 25mg/kg (6.91±0.32 and 24.68±0.284) were significantly declined the level of TBARS and Nitric oxide as compared to group II. The GSH and catalase activity narrow down in brain and shown in group II (1.27±0.09 and 90.79±5.01) as compare to group I (3.29±0.08 and 106.35±1.03). Pre-treated with MOE (178.10±1.40) and Quercetin (201.33±2.50) significantly increase catalase activity as compared to group II (90.79±5.01).

The results were represented as Mean±SEM(n=5), Where (\*\*) denotes (p<0.01) as compared to normal control groups I; indicated (p<0.05), (\$p<0.05) and (#p<0.01) as compared to ischemic group. The data represent significant variation between pre-treated and the ischemia groups at (p<0.05) and (p<0.01) respectively.

**Graph 1.A.**

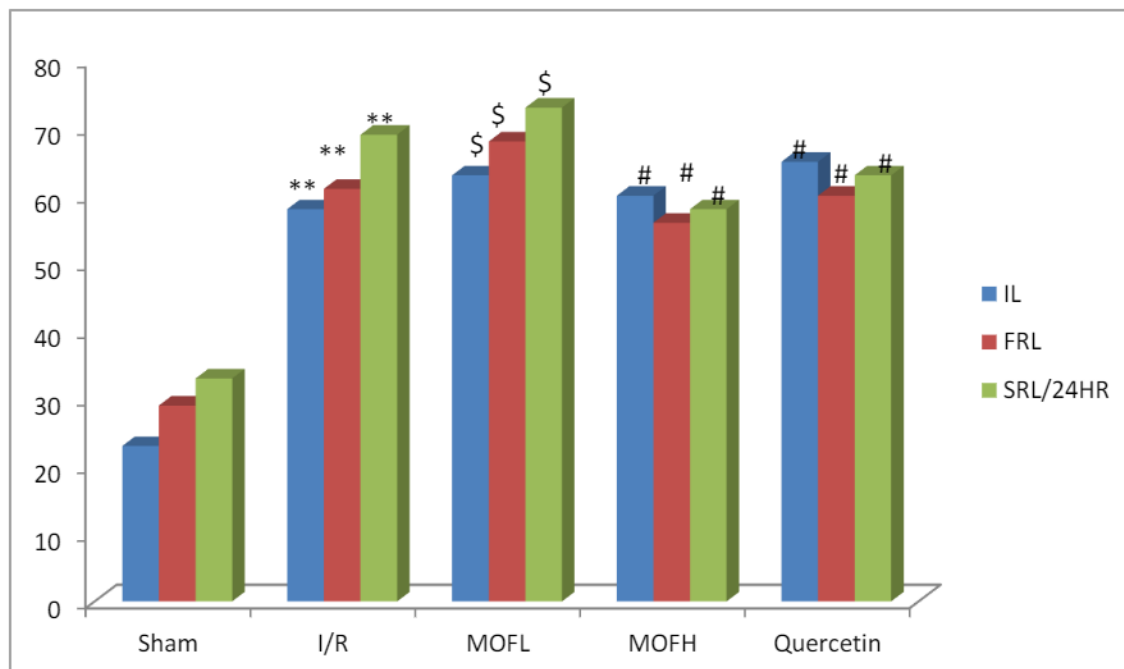
Effects of ethanolic extract of *M. oleifera* flower (MOE) on behaviour parameter neuro-deficit scores, Rota rod activity, hanging time and locomotor activity in ischemia reperfusion rats and pre-treated groups.



NDFS- Neuro-deficit scores, MFTSRD- Mean fall time in Sec from rota rod, MFTSHW- Mean fall time in sec hanging wire, LA- Locomotor activity counts/5min.

**Graph 1.B.**

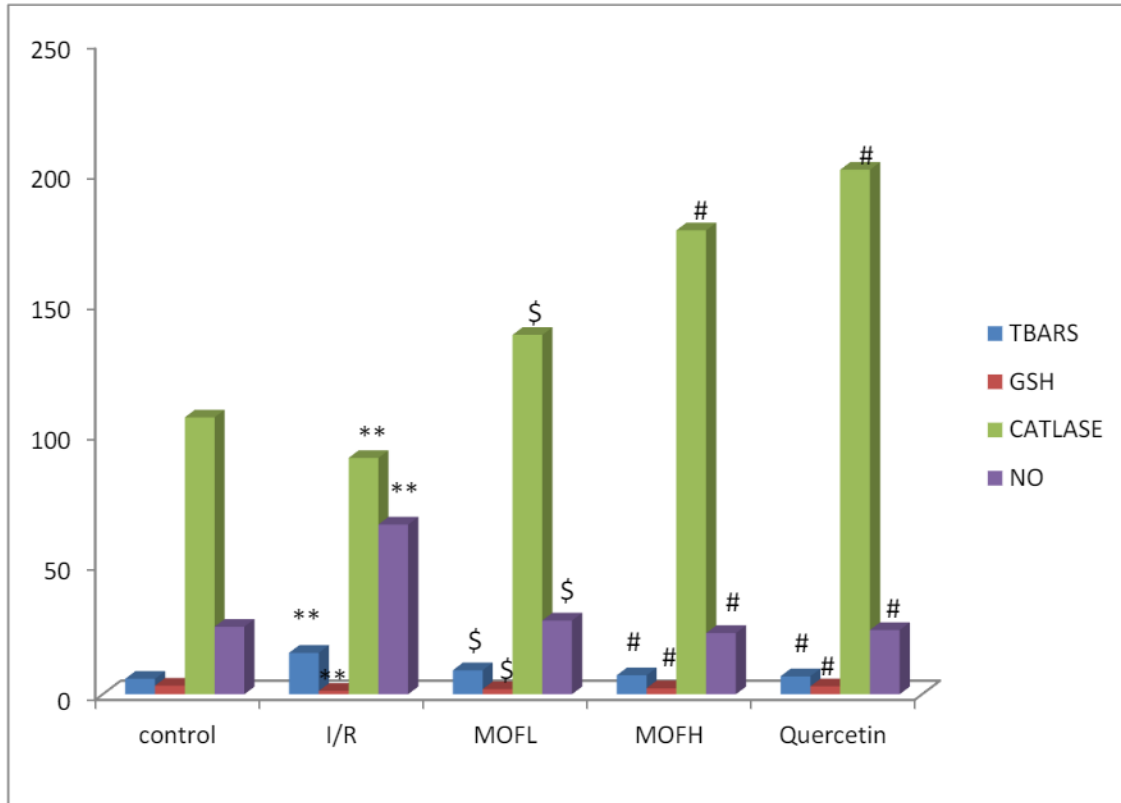
Effect of MOE and quercetin on rats injured by cerebral ischemia-reperfusion in an elevated plus maze.



IL. Initial latency, FRL- First Retention Latency. SRL- Second retention latency.

**Graph1. C**

Effects of MOF on TBARS, Glutathione, CAT and NO by cerebral ischemia reperfusion induced rats.



TBARS Thiobarbaturic Acid, GSH-Glutathione, Catalase, NO, Nitric oxide

#### 4. Discussion

Inadequate blood supply to an encephalon to fulfil metabolic demands known as brain ischemia, also known as cerebral ischemia<sup>29</sup>. This condition is caused by the blockage of a major cerebral artery by an occlusion<sup>30</sup> or an embolism, which causes tissue destruction in the affected area and also deficiency of cerebral blood flow and an OGD condition<sup>31</sup>.

The animals' locomotor activity, hanging latency time, and rota rod test for muscular relaxant action (hanging time) were considerably decreased in untreated groups as compare to pre-treated groups. BACCAO followed in each group by 24 hours reperfusion. Pre-treated animals have shown significant improvement in all behaviour and biochemical parameters as nontreated animals. The study results were revealed dose-dependent actions in pretreatment animal's groups. The doses of *Moringa oleifera* flower extract were designed as 200mg/kg/orally (low dose) and 400 mg/kg/orally (high dose) and shown their significant potential dose-dependently and also improved the locomotor activity, hanging latency time, and muscle relaxant action.

Retention transfer latency (RTL) in the normal control group I dramatically decreased after 24 hours of reperfusion, showing memory retention. The transfer delay time in the elevated plus maze was substantially longer for animals that underwent 30 minutes of BCCAO followed by 24 hours of reperfusion than it was for rats in group I who did undergo surgery. In comparative analysis of the ischemia control group II with respect to pretreatment low dose group of *Moringa oleifera* flower 200 mg/kg/orally of significantly reduced the already augmented in the transfer latency time. However, 400mg/kg of has a similar effect to conventional drug quercetin 25mg/kg/orally (Table 2).

After 24 hours, reperfusion dramatically raised TBARS levels and decreased catalase activity in the brain relative to untreated group I rats, indicating oxidative damage. Pre-treatment with *Moringa oleifera* flower at doses 200mg per kg and 400mg per kg of dramatically increased catalase activity and replenished GSH glutathione levels. When compared to group 2, the treatment with MOF and quercetin dramatically enhanced the catalase activity. In comparison to the sham control, the superoxide dismutase activity was lower in group 2 that had received a vehicle treatment. While oral administration of MOF and quercetin dramatically boosted the activity of superoxide dismutase. In group 2 ischemic rats, there was an increase in total nitrite. (Table

3)

The neuroprotective potential by MOE due to its antioxidant properties and presence of phytochemicals in MOE lessen the oxidative harm caused by challenging with 30 minutes of BCCAO and then 24 hours of reperfusion. However, it might be recommended to use MOE excessively to prevent related cognitive loss and neurodegenerative diseases.

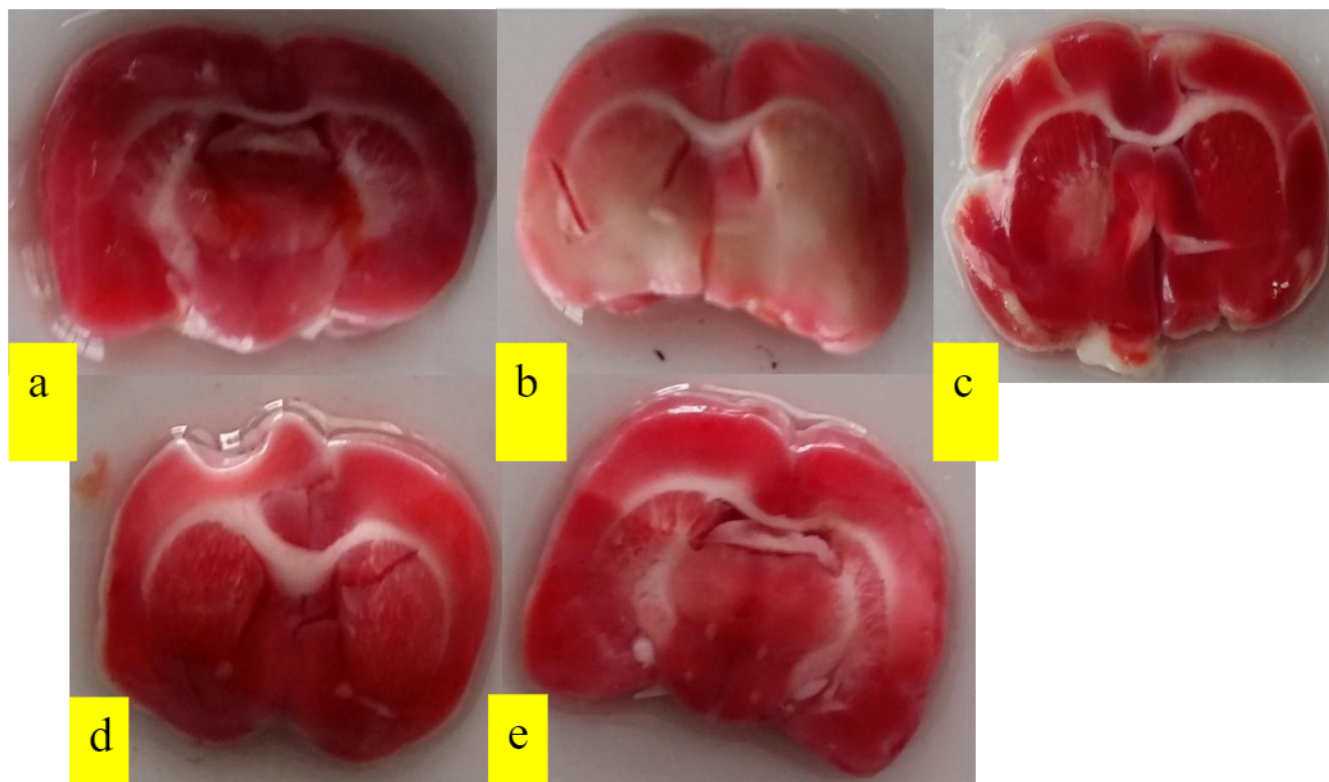
## 5. Conclusion

The *Moringa oleifera* flower's ethanolic extract (MOE) has been shown to have neuroprotective properties against global cerebral ischemia reperfusion in rats, as evidenced by the significantly improved forelimb strength, balance, and coordination (i.e., motor performance), neuro-deficit score, rotarod test, hanging wire test, and beam walk test results.

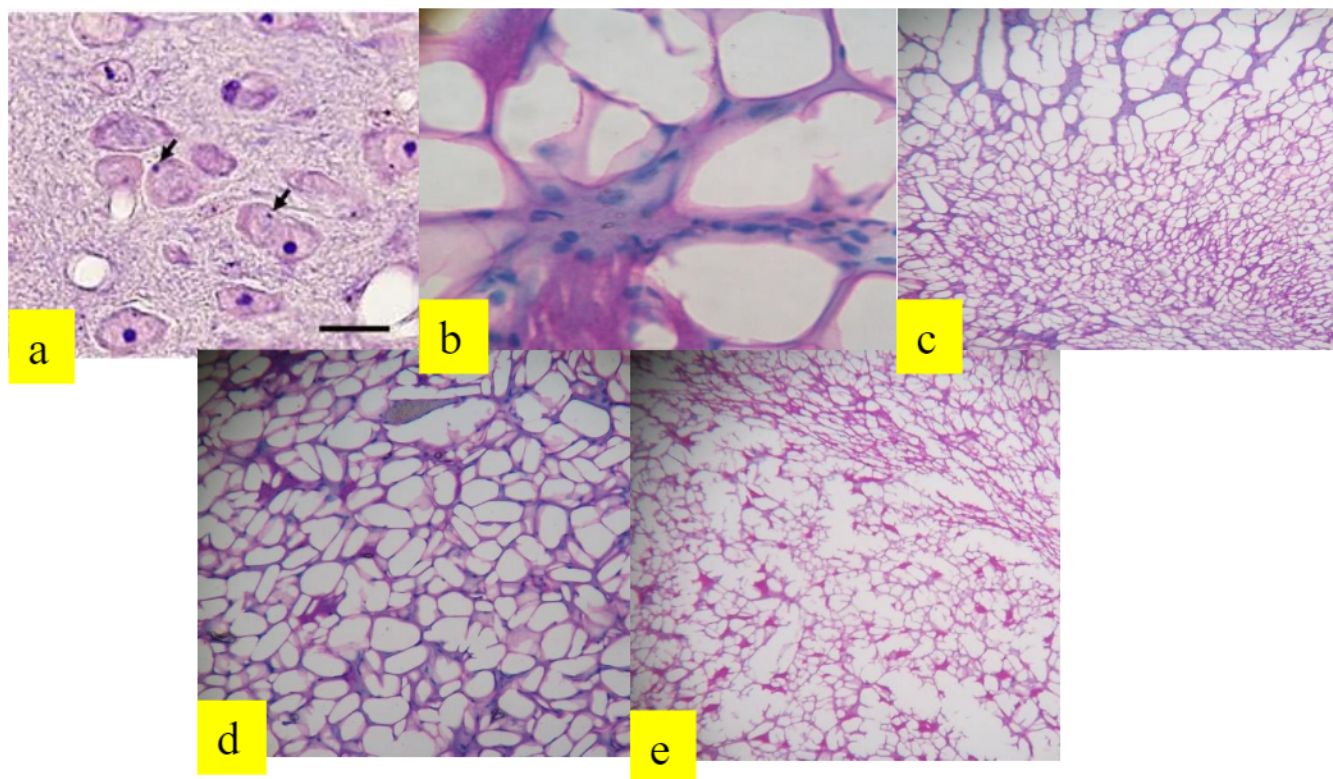
Locomotor activity, hanging latency time, elevated plus maze test, initial transfer memory, first and second retention latency, and muscle relaxant action are some of the memory tests that showed a substantial improvement in inadequate GSH glutathione and catalase activity. In contrast, therapy with MOE and quercetin resulted in a modest decrease in nitric oxide and thiobarbituric acid reactive compounds (TBARS).

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**Fig. 1.** Rats from the control and experiment groups were shown the brain's gross anatomy: Transverse section of Rat brain. a. normal control b. Ischemic control. c. 200mg per kg (low dose) d. 400mg per kg (high dose) e. Quercetin (25mg per kg)



**Fig2.** Rats from the control and experimental groups underwent brain histopathology. a. Normal control (group I), b. Ischemic control (group II), c. Low dose 200mg per kg (group III) d. High dose 400mg per kg (group IV) e. Quercetin dose 25mg per kg, (group V)

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