Neuroprotective effect of magnesium oxide/gluconate in transient global cerebral ischemia in the rat brain

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Abstract: - Ischemic stroke represents a major contributor to global morbidity and mortality rates. This study aims to explore the impact of magnesium salts on oxidative stress and the degree of cerebral damage in rats subjected to transient global cerebral ischemia. The experimental procedure involved the ligation of the common carotid arteries (CCAs) for a duration of 30 minutes, followed by a 2-hour reperfusion period. The rats received magnesium (magnesium oxide/magnesium gluconate) treatment at doses of 45 or 90 mg/kg via intraperitoneal injection, administered either prior to or subsequent to the CCAs ligation. Key biomarkers, including lipid peroxidation (malondialdehyde; MDA), nitric oxide (NO), reduced glutathione (GSH), paraoxonase-1 (PON-1) activity, glial fibrillary acidic protein (GFAP), and A-beta (Aβ) peptide levels, were assessed in the brain tissue. Additionally, a histopathological analysis was conducted. In comparison to the sham-treatment group, cerebral ischemia led to a notable increase in brain malondialdehyde levels, nitric oxide alongside significant reductions in GSH and PON-1 activity. Furthermore, there was a marked elevation in brain Aβ-peptide and GFAP levels. The findings demonstrated that magnesium administration, whether prior to CCA ligation or following reperfusion, resulted in a significant reduction in lipid peroxidation, and nitric oxide, while enhancing GSH levels and PON-1 activity. Additionally, magnesium treatment significantly decreased the levels of Aβ-peptide and GFAP. Global cerebral ischemia led to neuronal degeneration, the formation of pericellular vacuoles, the presence of apoptotic cells, pyknotic nuclei, gliosis, and congestion within cerebral blood vessels. The degree of cerebral injury was mitigated by magnesium in a dose-dependent fashion. These findings indicate that magnesium administration provides neuroprotective benefits in cases of experimental

global cerebral ischemia by diminishing the release of free radicals and the associated oxidative stress. It can be concluded that magnesium supplementation may serve as a beneficial adjunctive treatment for patients experiencing cerebral ischemia or those who have suffered a stroke, primarily due to its antioxidant properties.

Key-Words: - global cerebral ischemia; carotid ligation; magnesium; neuroprotection; oxidative stress

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1 Introduction

Global cerebral ischemia, characterized by a reduction in blood flow throughout the brain, frequently occurs in patients experiencing ventricular fibrillation or asystole lasting between 5 to 10 minutes, as well as in those undergoing complex cardiac surgeries or suffering from shock and cerebral hypoperfusion. Regardless of the underlying cause of cerebral ischemia, a series of cellular and molecular mechanisms initiate a cascade of events that lead to a "final common pathway," ultimately resulting in ischemic neuronal damage [1]. The consequences of this process include tissue hypoxia, impaired cellular function, and cell death. Prolonged episodes of ischemic insult have dire implications for neurons, extending beyond mere depletion of cellular energy reserves. The cellular alterations associated with this form of death encompass nuclear fragmentation, chromatin condensation, and shrinkage of the cell body [2].

The brain, characterized by its significant metabolic requirements, consumes approximately 20% of the body's oxygen and utilizes around 25% of its glucose. To sustain its functionality and structural integrity, the brain necessitates a continuous influx of oxygenated blood containing an adequate glucose and oxygen concentration. Consequently, brain tissue is particularly susceptible to ischemic conditions; even temporary vascular occlusion that disrupts the delivery of oxygen and glucose to cerebral tissue can trigger pathophysiological processes, ultimately leading to cell death and cerebral damage [3].

Restoration of vascular supply and reperfusion, although effective in enhancing oxygen delivery to the tissue, frequently leads to a paradoxical increase in tissue damage [2]. The molecular mechanisms underlying brain ischemia and the subsequent reperfusion that contribute to cerebral injury include oxidative stress, depletion of adenosine 5′-triphosphate (ATP), and impaired ATP resynthesis, which result in reduced energy availability. Additionally, dysfunction of the endothelial barrier alters the permeability of the blood-brain barrier, while elevated intracellular calcium levels, along with the release of inflammatory mediators and proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin-1β, and interleukin-6, are observed [4], [5].

The role of free radical-induced oxidative and nitrosative stress in cerebral ischemia/reperfusion injury is particularly significant. Neuronal membranes are rich in polyunsaturated fatty acids, which are particularly susceptible to free radical attack at carbons adjacent to double bonds. These free radicals can interact with and inflict damage on proteins, nucleic acids, lipids, and various other molecules [6]. During ischemic events, there is an increase in reactive oxygen and nitrogen species, accompanied by a depletion of cellular antioxidant defenses, such as glutathione, which normally protect cellular proteins and lipids from oxidation. Upon the restoration of oxygen supply, there is a marked increase in the production of reactive oxygen species by dysfunctional mitochondria, leading to further damage to cellular membranes and proteins that were already compromised during the initial ischemic phase [4], [7]. High concentrations of reactive species are generated from several sources, including mitochondria, the activity of cyclooxygenase enzymes, nitric oxide synthases produced by neurons, endothelial cells, and infiltrating neutrophils, as well as the hypoxia-dependent transformation of xanthine dehydrogenase into xanthine oxidase [8]. Consequently, there is an increasing interest in investigating potential neuroprotective agents that can modulate the redox state in ischemia/reperfusion injury.

Magnesium salts have demonstrated cerebroprotective effects in animal models of stroke [9]. As the fourth most prevalent mineral and the second most common intracellular divalent cation

following potassium, magnesium serves as a cofactor in more than 300 enzymatic processes related to cellular energy metabolism, protein synthesis, and the synthesis of DNA and RNA, in addition to stabilizing mitochondrial membranes [10]. The Mg^{++} ion possesses anti-inflammatory properties and functions as an antagonist to Ca^{++} [11]. In the brain, magnesium is crucial for nerve signal transmission, the maintenance of ionic homeostasis, the protection of the blood-brain barrier, and the mitigation of oxidative stress [12]. Furthermore, it plays a significant role in regulating various cell membrane receptors, such as *N*-methyl-D-aspartate (NMDA) and γ-aminobutyric acid (GABA) receptors [13]. In humans, magnesium supplementation is vital for neuronal health and a potentially valuable adjunctive treatment for Alzheimer's disease [14], with higher dietary magnesium intake linked to reduced inflammation, lower leukocyte counts, and increased volumes of grey and white matter, as well as right hippocampal volume, indicating its neuroprotective potential [15]. Additionally, hypomagnesemia has been significantly correlated with unfavorable functional outcomes in patients experiencing mild stroke severity and cardioembolic stroke [16].

In light of the above, the present study was designed to examine the effects of magnesium oxide/gluconate salt on oxidative stress and the extent of cerebral injury in rats subjected to transient global cerebral ischemia and reperfusion (I/R) injury.

2 Materials and Methods

2.1 Animals

Male Sprague-Dawley rats, with a weight range of 210-220 g, were sourced from the Animal House of the Research Centre. These rats were maintained in an environment with regulated temperature and lighting, and they had unrestricted access to standard laboratory rodent diet and water. The experimental protocols adhered to the guidelines set forth by the institutional Ethics Committee of the National Research Centre, as well as the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1996).

2.2 Drugs and Chemicals

Magnesium preparation containing magnesium oxide/magnesium gluconate (4:1) was purchased from Mepaco (Egypt). Other chemical and reagents were purchased from Sigma (USA).

2.3 Global Cerebral Ischemia

Global cerebral ischemia was induced in anesthetized rats through bilateral carotid artery occlusion, lasting for 30 minutes, followed by a reperfusion phase. Thiopental was administered at a dosage of 20 mg/kg *via* intraperitoneal (i.p.) injection. The common carotid arteries (CCAs) were surgically exposed and occluded for duration of 30 minutes using non-traumatic microvascular clips placed on each CCA just before their bifurcation. After this period, blood flow was restored and maintained for two hours. Throughout the procedure, the body temperature of the animals was regulated at 36.5 ± 0.5°C with the aid of a heating pad, and respiratory patterns were closely monitored. Following the 30-minute vascular occlusion, the clips were removed to allow for the resumption of blood flow to the affected cerebral region. Subsequently, the surgical incisions were sutured, and the animals were permitted to recover from anesthesia before being placed in a warm cage during the reperfusion phase.

2.4 Experimental Groups

Rats were randomly allocated into the following groups (6 rats each):
Group 1: Non-operated rats treated with the vehicle.
Group 2: Rats received the vehicle and underwent cerebral ischemia-reperfusion injury (I/R).
Group 3: Rats treated with magnesium at 45 mg/kg, i.p. 30 min before CCAs ligation.
Group 4: Rats treated with magnesium at 90 mg/kg, i.p. 30 min before CCAs ligation.
Group 5: Rats treated with magnesium at 45 mg/kg, i.p. 30 min after CCAs ligation.
Group 6: Rats treated with magnesium at 90 mg/kg, i.p. 30 min after CCAs ligation.

2.5 Determination of Oxidative Stress Biomarkers

Lipid peroxidation in brain homogenates was assessed by quantifying malondialdehyde (MDA) levels, following the methodology established by Ruiz-Larrea et al. [17]. This procedure involves the reaction of 2-thiobarbituric acid with MDA at a temperature of 25 °C, resulting in the formation of a red complex that exhibits a peak absorbance at 532 nm. The measurement of nitric oxide was conducted using the Griess reagent, wherein nitrate is enzymatically reduced to nitrite by nitrate reductase. The resulting nitrite subsequently interacts with the

Griess reagent to produce a purple azo compound, with absorbance readings taken at 540 nm using a spectrophotometer [18]. Additionally, reduced glutathione levels in brain homogenates were quantified according to the method described by Ellman [19]. In this assay, Ellman's reagent (DTNB; 5, 5'-dithiobis (2-nitrobenzoic acid)) reacts with the free thiol group of glutathione (GSH), yielding 2-nitro-s-mercaptobenzoic acid. The resulting chromophore displays a yellow hue, which is measured at 412 nm with a spectrophotometer.

2.6 Determination of paraoxonase-1 activity

The activity of the paraoxonase-1 enzyme as an arylesterase was assessed using phenylacetate as the substrate. In this experimental setup, the enzyme catalyzes the hydrolysis of phenyl acetate, resulting in the production of phenol. The rate of this hydrolytic reaction was quantified by measuring the increase in absorbance at 270 nm at a temperature of 25 °C, utilizing a spectrophotometer. One unit of arylesterase activity corresponds to the formation of 1 μM of phenol per minute. The enzyme activity, expressed in kU/l, is calculated using the molar extinction coefficient of 1310 M^{-1} cm^{-1} for phenol at 270 nm, pH 8.0, and 25 °C [20].

2.6 Quantification of Glial Fibrillary Acidic Protein

Brain homogenates were analyzed for GFAP using enzyme-linked immunosorbent assay (ELISA) kit from Glory Science Co., Ltd. (Del Rio, USA) according to the manufacturer's instructions.

2.7 Quantification of Amyloid Aβ Peptide

Rat amyloid beta-peptide 1-42 (Aβ 1-42) ELISA Kit (SinoGeneClon Biotech Co., Ltd) was used according to the manufacturer's instructions

2.8 Brain Histopathology

Rat brain tissue was obtained from various groups. A 10% neutral buffered formalin solution served as the fixation agent. The tissue underwent processing to produce 4 μm sections embedded in paraffin. These sections were subsequently stained with haematoxylin and eosin (H&E) to facilitate the examination of histopathological alterations under a light microscope.

2.9 Statistical Analysis

Results are presented as mean ± SEM. The analysis of the results was conducted using One Way ANOVA followed by Duncan's multiple range test. Statistical analysis was performed utilizing GraphPad Prism software, version 5 (GraphPad Software, Inc., San Diego, USA). A p-value of less than 0.05 was considered statistically significant.

3 Results

3.1 Lipid Peroxidation

In the I/R control group, brain MDA increased by 49.1% (27.5 ± 1.74 *vs.* 18.45 ± 0.82 nmol/g.tissue). Magnesium given prior to ligation of the carotid arteries significantly suppressed the rise in lipid peroxidation by 20.1% and 21.1%, respectively (21.97 ± 0.48, 21.7 ± 0.61 *vs.* 27.5 ± 1.74 nmol/g.tissue). On the other hand, post-treatment with magnesium resulted in 22.2% and 27.2% decrease in MDA levels, respectively as compared to the ischemic control group (21.40 ± 0.36, 20.01 ± 0.56 *vs.* 27.5 ± 1.74 nmol/g.tissue).

3.2 Nitric Oxide

In the I/R group, nitric oxide in the brain tissue increased by 55.5% from the saline control value of 17.43 ± 0.32 to 27.1 ± 0.56 μmol/g.tissue). Pretreatment with magnesium significantly decreased the tissue nitric oxide to 23.13 ± 0.89 and 20.27 ± 0.52 μmol/g.tissue (14.6% and 25.2% decrease), respectively. Post-treatment with magnesium, on the other hand, suppressed the increase in nitric oxide level by 19.7% and 25.1%, respectively from 27.10 ± 0.56 to 21.75 ± 0.50 and 20.29 ± 0.52 μmol/g.tissue.

3.3 Reduced Glutathione

Rats subjected to I/R showed a 38.6% decrease in the GSH brain content as compared to their saline controls (2.13 ± 0.04 *vs.* 3.47 ± 0.07 μmol/g.tissue). Pre-treatment with magnesium was found to significantly increase the GSH level by 20.2% and 23.5%, respectively (2.56 ± 0.04, 2.63 ± 0.08 *vs.* 2.13 ± 0.04 μmol/g.tissue). Similarly increments in GSH by 23.9% and 32.9% were also observed when magnesium was given after ischemia (2.64 ± 0.05, 2.83 ± 0.03 *vs.* 2.13 *vs.* 0.04 μmol/g.tissue).

3.4 Paraoxonase-1

A significant decrease in PON-1 activity by 35.4% was observed after I/R as compared to the saline control value (7.84 ± 0.23 *vs.* 12.13 ± 0.21 kU/l). PON-1 activity increased by 25.4% and 35.2% by pretreatment with magnesium (9.83 ± 0.07, 10.60 ± 0.13 *vs.* 7.84 ± 0.23 kU/l) and by 40.0% and 42.2% when magnesium was administered after CCAs occlusion (10.98 ± 0.14, 11.15 ± 0.10 *vs.* 7.84 ± 0.23 kU/l).

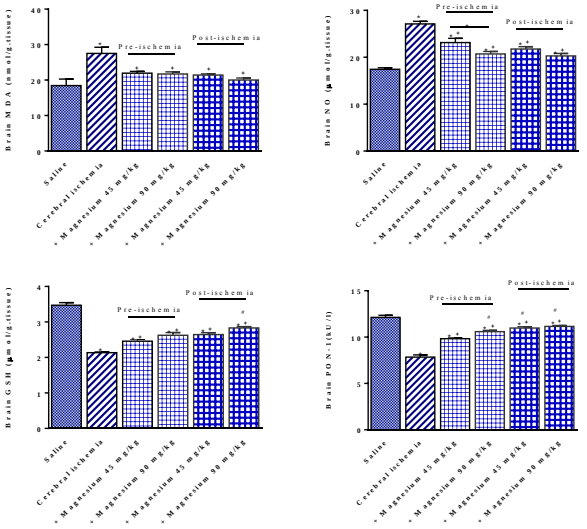

Fig. 1: Effects of pre- or post ischemic treatment with magnesium salts on oxidative stress biomarkers in rat brain. *:p<0.05 *vs.* the saline group and between different groups as shown in the figure. +: p<0.05 *vs.* the ischemia control group. #: p<0.05 *vs.* the ischemia + pretreatment with 45 mg magnesium group.

3.5 Glial Fibrillary Acidic Protein

A significant increase in GFAP brain level by 23.2% was observed after I/R from saline control value of 1.94 ± 0.07 to 2.39 ± 0.08 ng/ml. The increase in GFAP brain was significantly reduced by 16.3% and 21.8% by pre- or post-ischemic treatment with 90 mg/kg of magnesium, respectively (2.0 ± 0.03, 1.87 ± 0.006 *vs.* 2.39 ± 0.08 ng/ml).

3.6 Amyloid Aβ-Peptide

Brain Aβ-peptide level increased by 22.4% during I/R from saline control value of 2.59 ± 0.06 to 3.17 ± 0.02 pg/ml. Magnesium administration prior to CCAs occlusion significantly decreased the rise in brain Aβ-peptide by 17.4% and 20.5%, respectively (2.62 ± 0.05, 2.52 ± 0.10 *vs.* 3.17 ± 0.02 pg/ml). Similar decrements in Aβ-peptide level by 16.4% and 24.0% were observed when magnesium was given after inducing ischemia (2.65 ± 0.14, 2.41 ± 0.36 *vs.* 3.17 ± 0.02 pg/ml).

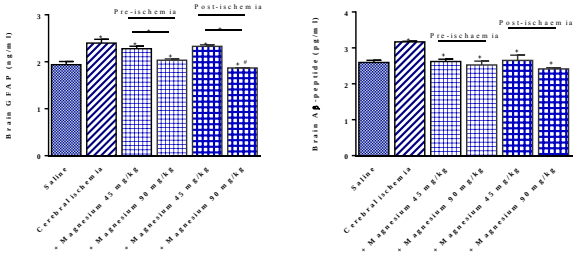

Fig. 2: Effects of pre- or post ischemic treatment with magnesium salts on GFAP or Aβ-peptide levels in rat brain. *:p<0.05 *vs.* the saline group and between different groups as shown in the figure. +: p<0.05 *vs.* the ischemia control group. #: p<0.05 *vs.* the ischemia + pretreatment with 45 mg magnesium group

3.7 Histopathological Results

Results are shown in Figs. 3 & 4, Tables 1 & 2.

3.7.1 Cerebral Cortex

The saline control group exhibited a typical histological structure of the cerebral cortex, characterized by neurons with round nuclei and intact blood vessels (Fig. 3A). In contrast, histological analysis of the cerebral cortex following I/R revealed signs of neuronal degeneration, the presence of pericellular vacuoles, apoptotic cells, pyknotic nuclei, congestion in cerebral blood vessels, and glial cells displaying either numerous lightly or darkly stained nuclei (Fig. 3B). In sections from animals that underwent I/R and received a pre-treatment of magnesium at a dosage of 45 mg/kg, there was evidence of moderate neuronal degeneration, preservation of normal blood vessels, mild pericellular vacuoles, a limited number of pyknotic nuclei, and glial cells with either many lightly or darkly stained nuclei (Fig. 3C). Conversely, sections from rats subjected to I/R and pre-treated with magnesium at 90 mg/kg demonstrated a notable enhancement in histological integrity, with minimal neuronal degeneration, normal blood vessels, a reduced number of pyknotic nuclei, and glial cells exhibiting either many lightly or darkly stained nuclei (Fig. 3D). The cerebral cortex of I/R injury rats that received post-treatment with magnesium at 45 mg/kg displayed moderate neuronal degeneration, normal blood vessels, a few pyknotic nuclei, apoptotic cells, and glial cells with either many lightly or darkly stained nuclei (Fig. 3E). Finally, sections from the cortex of I/R rats that underwent post-treatment with magnesium at 90 mg/kg revealed an improvement in histological structure, characterized by minimal neuronal degeneration, mild congestion of blood vessels, a

few pyknotic nuclei, and glial cells with either many lightly or darkly stained nuclei (Fig. 3F).

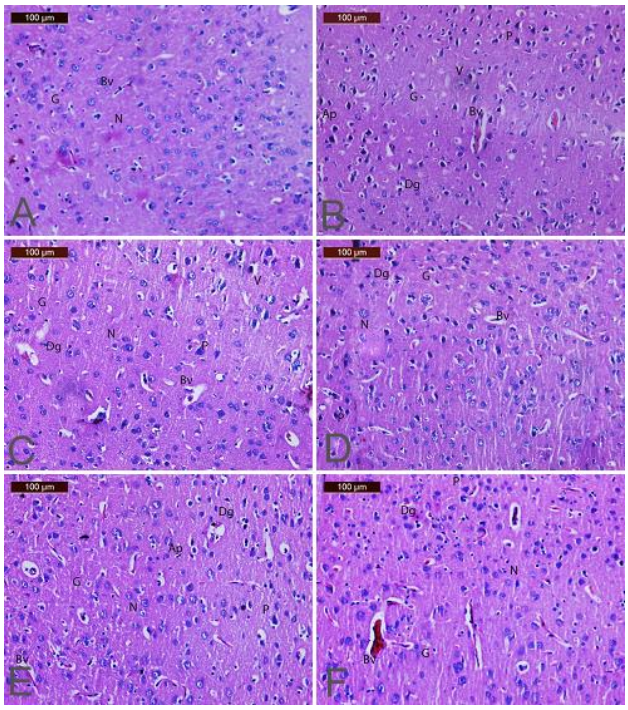

Fig. 3: Cerebral cortex sections stained with Hx & E from rats after treatment with **(A)** Saline showing normal arrangement and structure of cortical neurons, blood vessel (Bv) and glial cells (G); **(B)** Cerebral I/R showing degenerated neurons, pericellular vacuoles (V), apoptotic cells (Ap), pyknotic nuclei (P), congestion of cerebral blood vessel (Bv) and glial cells with either many lightly (G) or dark (Dg) stained nuclei; **(C)** Cerebral I/R + pre-treatment with magnesium 45 mg/kg showing moderate neuronal degeneration, normal blood vessels (Bv), mild pericellular vacuoles (V), few pyknotic nuclei (P), and glial cells with either many lightly (G) or dark (Dg) stained nuclei; **(D)** Cerebral I/R + pre-treatment with magnesium 90 mg/kg showing improvement in histological structure with minimal neuronal degeneration, normal blood vessels (Bv), few pyknotic nuclei (P), and glial cells with either many lightly (G) or dark (Dg) stained nuclei; **(E)** Cerebral I/R + post-treatment with magnesium 45 mg/kg showing moderate neuronal degeneration, normal blood vessels (Bv), few pyknotic nuclei (P), apoptotic cells (Ap) and glial cells with either many lightly (G) or dark (Dg) stained nuclei; **(F)** Cerebral I/R + post-treatment with magnesium 90 mg/kg showing improvement in histological structure with minimal neuronal degeneration, mild congestion of blood vessel (Bv), few pyknotic nuclei (P), and glial cells with either many lightly (G) or dark (Dg) stained nuclei.

3.7.1 Hippocampus

Sections from the saline-treated group showed well organized the pyramidal cells with prominent nuclei (Fig. 4A). Sections from cerebral I/R control group showed neurodegeneration and decreased thickness of pyramidal cells with some cell appeared normal nuclei and other have dark eosinophilic cytoplasm, pyknotic nuclei, apoptotic cells with mild vacuolated cells (Fig. 4B). Rats subjected to cerebral I/R and pre-treatment with magnesium 45 mg/kg showed group moderate improvement of pyramidal cells with prominent nuclei and mild pyknotic nuclei (Fig. 4C). The hippocampus from of cerebral I/R and pre-treatment with magnesium 90 mg/kg group showed improvement histological structure of pyramidal cells with prominent nuclei and very few pyknotic nuclei (Fig. 4D). Sections from cerebral I/R and post-treatment with magnesium 45 mg/kg group showed moderate improvement of histological structure of pyramidal cells with prominent nuclei and mild pyknotic nuclei (Fig. 4E). Sections from cerebral I/R and post-treatment with magnesium 90 mg/kg group showed improvement in histological structure with minimal neuronal degeneration, prominent nuclei and very few pyknotic nuclei (Fig. 4F).

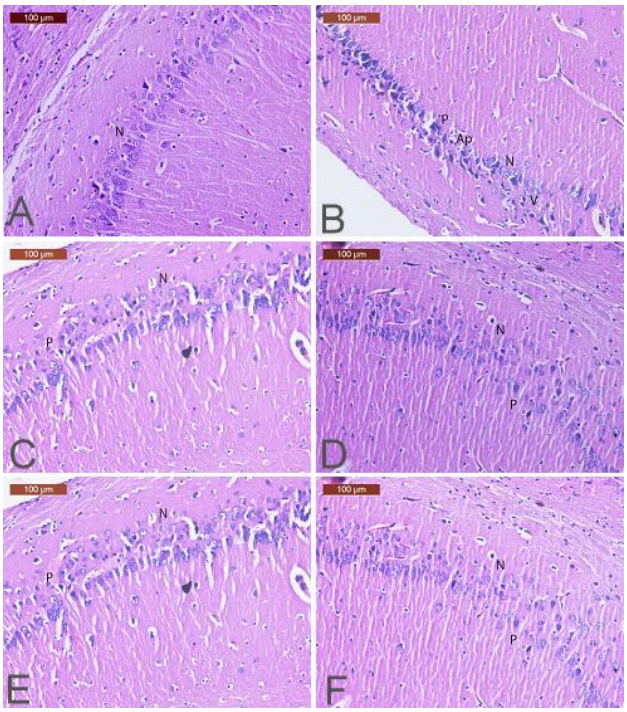

Fig. 4: Sections of the hippocampus stained with Hx & E from rats after treatment with **(A)** Saline showing well organized the pyramidal cells with prominent nuclei (N); **(B)** Cerebral I/R showing

neurodegeneration and decreased thickness of pyramidal cells with some cell appeared normal nuclei (N) and other have dark eosinophilic cytoplasm, pyknotic nuclei (P), apoptotic cells (Ap) with mild vacuolated cells (V); **(C)** Cerebral I/R + pre-treatment with magnesium 45 mg/kg showing moderate improvement of pyramidal cells with prominent nuclei (N) and mild pyknotic nuclei (P); **(D)** Cerebral I/R + pre-treatment with magnesium 90 mg/kg showing improvement histological structure of pyramidal cells with prominent nuclei (N) and very few pyknotic nuclei (P); **(E)** Cerebral I/R + post-treatment with magnesium 45 mg/kg showing moderate improvement of histological structure of pyramidal cells with prominent nuclei (N) and mild pyknotic nuclei (P); **(F)** Cerebral I/R + post-treatment with magnesium 90 mg/kg showing improvement in histological structure with minimal neuronal degeneration, prominent nuclei (N) and very few pyknotic nuclei (P).

Table 1. Semi-quantitative assessment of histological damage of the cerebral cortex in different groups.

Parameter/ Group	Neuronal degeneration	Pyknosis in neurons	Pericellular vacuoles	Apoptotic cells	Congestion of blood vessel
Control -ve	-	-	-	-	-
I/R	+++	+++	++	+++	+++
I/R + pre-treatment with magnesium 45 mg/kg	++	+	+	-	-
I/R + pre-treatment with magnesium 90 mg/kg	+	+	-	-	-
I/R + post-treatment with magnesium 45 mg/kg	++	+	-	+	-
I/R + post-treatment with magnesium 90 mg/kg	+	+	-	-	+

Histological changes scored on a 4-point scale: (-) none, (+) mild, (++) moderate, and (+++) severe damage. A minimum of 10 fields for each section of slide were examined and assigned for severity of changes using scores. Note: —, no changes; +, slight changes; ++, moderate changes; +++, severe changes.

Table 2. Semi-quantitative assessment of histological damage of the hippocampus in different groups.

Parameter/ Group	Neuronal degeneration	Pyknosis in neurons	Pericellular vacuoles	Apoptotic cells
Control -ve	-	-	-	-
I/R	+++	+++	++	++
I/R + pre-treatment with	++	+	-	-

magnesium 45 mg/kg				
I/R + pre-treatment with magnesium 90 mg/kg	+	+	-	-
I/R + post-treatment with magnesium 45 mg/kg	++	+	-	-
I/R + post-treatment with magnesium 90 mg/kg	+	+	-	-

Note: — no changes; +, slight changes; ++, moderate changes; +++, severe changes.

4 Discussion

In this investigation, the neuroprotective properties of the dietary supplement magnesium were assessed utilizing a rat model of global cerebral ischemia. The findings presented herein substantiate the magnesium's capacity to markedly reduce brain injury resulting from cerebral ischemia and subsequent reperfusion. Magnesium improved neurochemical changes and exhibited a dose-dependent reduction in histological damage within the ischemic brain tissue. Notably, the neuroprotective effects of the supplement were evident whether it was administered prior to or following the induction of ischemia. Our results thus confirm and extend previous studies describing a cerebroprotective effects for magnesium salts in experimental ischemic brain injury [21], [22].

The model of transient global cerebral ischemia induced in rats through four-vessel occlusion is widely utilized in experimental research to investigate the pathophysiological mechanisms underlying brain ischemic injury and to explore potential therapeutic strategies [23]. Unlike focal cerebral ischemia, global ischemia effectively replicates the diminished blood flow and the associated pathological processes occurring in the forebrain during instances of cardiac arrest or hypoxia. In the context of global cerebral ischemia, the absence of cerebral blood flow throughout the brain leads to neuronal damage in regions that are particularly susceptible to injury [24]. In this study, the bilateral occlusion of common carotid arteries (CCAs) for duration of 30 minutes, followed by reperfusion, resulted in oxidative stress within the brain. This was indicated by an increase in malondialdehyde, a byproduct of lipid peroxidation, and nitric oxide, alongside a reduction in reduced glutathione, which serves as the primary intracellular antioxidant and free radical scavenger in neural tissue [25]. These findings agree with previous research conducted on rodents experiencing either transient focal or global cerebral ischemia [26],[27]. Within the ischemic brain, there is a significant surge in reactive oxygen species generated by mitochondria in response to hypoxic conditions [28]. Furthermore, during the reperfusion and reoxygenation phase, there is an additional escalation in the production of oxidants due to enhanced oxygen delivery to brain tissue, leading to oxidative damage to cellular membranes, fatty acids, proteins, and DNA [2], [28].

The brain tissue is particularly susceptible to oxidative damage from free radicals due to its high levels of polyunsaturated fatty acids, the presence of redox-active metals, and oxidative byproducts of monoamine neurotransmitters, all compounded by limited antioxidant defenses. Research has highlighted the significant contribution of oxidative stress to ischemic injury in the brain [29],[30]. For instance, studies have demonstrated that mice with a 50% reduction in the activity of the antioxidant enzyme superoxide dismutase experienced a higher incidence of neuronal death and increased mortality following brain ischemia [29]. In contrast, the overexpression of this enzyme has been shown to confer protection to the brain during episodes of transient global cerebral ischemia and subsequent reperfusion [30]. Additionally, rats that received intravenous glutathione treatment post-focal brain ischemia displayed a marked decrease in infarct volume, which was linked to a reduction in the production of reactive oxygen species and an increase in the expression of the anti-apoptotic protein Bcl2 [31]. The findings of our study indicate that the administration of magnesium significantly reduced the levels of malondialdehyde, a marker of lipid peroxidation, while simultaneously enhancing

the content of reduced glutathione in the brain affected by ischemic/reperfusion injury. This suggests that magnesium may play a role in modulating the cascade of events that lead to the elevated production of reactive oxygen species.

Our findings also demonstrated a significant elevation in nitric acid levels within the brains of rats subjected to ischemia/reperfusion, which aligns with previous research indicating an increase in nitric oxide levels in the rat brain following episodes of transient focal or global cerebral ischemia [26], [32]. The rise in brain nitric oxide during ischemia is attributed to the inducible isoform of nitric oxide synthase (iNOS) present in neutrophils that infiltrate the ischemic brain, as well as from resident immune cells, specifically microglia [33]. Additionally, there is an upregulation of both neuronal and endothelial nitric oxide synthase isoforms during brain ischemia [34]. This heightened production of nitric oxide can lead to neuronal injury through the inactivation of mitochondrial electron transport chain complexes and subsequent depletion of cellular energy, a process mediated by the generation of highly toxic species such as the oxidant and nitrating peroxynitrite (ONOO-), produced by the reaction of nitric oxide and superoxide, which can oxidize or nitrosylate protein tyrosine residues [35]. The increase in nitric oxide during ischemic brain injury plays a crucial role in neuronal damage, as evidenced by the reduction in infarct volume observed in rats when iNOS was inhibited [36]. In this study, magnesium treatment led to a notable decrease in the increased levels of nitric oxide within the ischemic brain tissue. Therefore, targeting nitric oxide inhibition using magnesium may prove beneficial in mitigating neuronal loss during acute cerebral ischemia.

The present study in addition provided the first evidence of a decrease in PON-1 activity in the ischemic brain tissue. Paraoxonase-1 is an enzyme with an esterase and lactonase activities, found in various mammalian tissues, with the liver and blood exhibiting the highest levels of activity. This enzyme is produced in the liver and is transported in the bloodstream, where it is associated with high-density lipoproteins. PON1 is capable of hydrolyzing a range of organophosphorus compounds, including insecticides and nerve agents, as well as various lactones, which encompass certain drugs [37]. Additionally, it plays a role in the metabolism of toxic oxidized lipids of both low-density and high-density lipoproteins [38]. The enzyme also demonstrates peroxidase activity, which may be beneficial in addressing brain disorders characterized by elevated oxidative stress levels [39], [40]. PON1 is redox sensitive and has been shown to be inactivated by oxidative stress [41]. The reduction in activity noted in the present investigation may indicate inactivation due to heightened oxidative stress levels. Conversely, the observed restoration of PON-1 activity following magnesium treatment could be linked to diminished oxidative stress and/or may signify neuroprotective effects. Research conducted on animal models of neurodegeneration has demonstrated that the recovery of PON-1 activity correlates with a reduction in oxidative stress and the degree of neuronal injury [42], [43]. This finding implies that the enzyme could serve as a sensitive marker for the cellular redox state.

Astrocytes represent the predominant category of glial cells within the brain. These cells play a multifaceted role, not only offering structural and metabolic support to neurons but also participating in the regulation of synaptic transmission and plasticity. They are also involved in the release of neurotransmitters, the maintenance of local cerebral blood flow, and the integrity of the blood–brain barrier [44]. Astrocytes are characterized by the expression of glial fibrillary acidic protein (GFAP), which serves as a structural component of glial filaments and is crucial for maintaining their cytoskeletal architecture. GFAP is also recognized as a marker of astrocyte activation in response to central nervous system injuries, such as those caused by ischemia, trauma, or toxic substances [45]. Following brain ischemia, astrocytes undergo a series of morphological and biochemical transformations, leading to their proliferation. Reactive astrocytes exhibit a stellate shape, heightened GFAP immunoreactivity, an increased number of mitochondria, and enhanced antioxidant activities, both enzymatic and non-enzymatic [46]. Evidence of glial activation has been observed following global cerebral ischemia in rat models [47]. Notably, GFAP mRNA levels were found to be elevated in the ischemic cortex of rats subjected to ischemia followed by reperfusion, with GFAP-like immunoreactivity also identified in regions expressing GFAP mRNA. This suggests that the observed increase in GFAP may be attributed to de novo synthesis [48]. In patients experiencing an acute stroke, the protein GFAP, which is derived from astrocytes/astroglial cells, is released into the serum and has been found to correlate with both the extent of brain lesions and the neurological condition of the patient [49]. The levels of GFAP

may serve as a potential biomarker for forecasting functional outcomes one year following an acute ischemic event [50]. In the present work, GFAP measured by ELISA showed significant increase in rats subjected to the ischemic/reperfusion injury which was prevented by treatment with the higher dose of magnesium.

Substantial research indicates that the production and accumulation of toxic amyloid Aβ peptides, resulting from the sequential proteolytic cleavage of the transmembrane glycoprotein amyloid precursor protein (APP) by beta and gamma secretases, are pivotal events in the pathogenesis of Alzheimer's disease [51], [52]. These Aβ aggregates can inflict neuronal damage either directly by interacting with synapses or indirectly by activating microglia and astrocytes [51] or by inducing oxidative stress [53]. In our investigation, we noted a significant increase in Aβ-peptide levels in the brains of rats subjected to ischemia/reperfusion injury, which aligns with prior research that reported elevated beta-APP and Aβ immunoreactivity in models of experimental cerebral ischemia [54], [55]. Notably, chronic cerebral hypoperfusion has been implicated in neurodegenerative mechanisms in Alzheimer's disease via the accumulation of APP and Aβ1–42 [56], [57]. In the present study, brain Aβ levels are reduced by magnesium administration possibly due to suppressed Aβ production, indicating a possible therapeutic value of supplementation with magnesium oxide against Alzheimer's disease.

5 Conclusion

In summary, the present study indicated that the administration of magnesium salts protected cortical and hippocampal neurons against ischemia/reperfusion-induced damage, reduce oxidative stress, glial reaction, and brain Aβ levels. Collectively, these findings suggest that supplementation with magnesium salts could prove of value in patients experiencing acute cerebral ischemia as well as for controlling cerebral neurodegeneration that may lead to Alzheimer's disease in chronic cerebral hypoperfusion.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Omar Abdel-Salam and Amany Sleem designed the study. Marwan Abd El Baset conducted the experiments. Eman Youness performed the biochemical analyses, Enayat Omara performed the histopathological studies and its interpretation. Omar Abdel-Salam wrote and prepared the manuscript. Omar Abdel-Salam, Marawan Abd El Baset, Amany Sleem, Eman Youness and Enayat Omara approved the final version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest