Magnesium sulfate ameliorates cerebral ischemia reperfusion injury via interfering with inflammatory and oxidative pathways

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Abstract

Magnesium sulfate has neuroprotective effects and decrease overall neuronal firing. It is also decrease firing of excitable tissues outside the brain. Moreover, it has anti-inflammatory effect. The purpose of this study was to investigate the anti-inflammatory and antioxidant effects of magnesium sulfate in rat brain following ischemia reperfusion stress.Twenty four rats were grouped into 4 groups: The first (sham group), the second (control) and the third group(control-vehicle) and the forth (treated with Magnesium sulfate). Animals in the second group underwent bilateral common carotid artery ligation without treatment, whereas the forth group were injected with magnesium sulfate 250mg/kg intraperitoneally before procedure. Brain homogenate were prepared after the procedure for measurement of cerebral level of IL-6, IL-9, MCP-1 and ICAM.Our study demonstrated that cerebral level of IL-9 in control group was 163.3 ± 30.4 pg/mg and it significantly decreased in magnesium sulfate treated group (21.8 ± 1.72 pg/mg). Cerebral level of MCP-1 in the control group was 109.05 ± 18.2 pg/mg, while it significantly reduced in magnesium sulfate treated group (38.16 ± 3.54 pg/mg). Mean cerebral levels of ICAM of control was 362.8 ± 26.81 pg/mg while mean cerebral level of ICAM in treated group was 35.5 ± 4.71 pg/mg.Magnesium sulfate significantly decreased cerebral inflammatory markers IL-6,IL-9, MCP-1 and ICAM in global ischemia model in rats and regressed I/R injury.

Keywords: Magnesium sulfate,Global cerebral ischemia, IL-9, ICAM, MCP-1

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Introduction

Stroke is major cause of death and disability over the world. Its incidence is increasing in Middle East to a serious problem [1, 2]. Ischemic stroke is the main etiological form. It is due to thrombus originated from atheromatous plaque [3]. The initial event in atherosclerosis is oxidative stress and endothelial dysfunction. This will lead to decrease in nitric oxide and prostacyclin. These substances maintain normal function of endothelium but after the loss of them platelet aggregation and release of inflammatory mediators happens [4].

Brain ischemia occurs when cerebral blood flow is reduced to a low level by certain pathological conditions, such as stroke or cardiac arrest [5, 6, 7]. The brain critically depends on a continuous supply of oxygen and glucose, more so than any other organ. While the brain represents only 2% of total body weight, it receives 15% of the total cardiac output. This high oxygen and energy demand is largely due to the necessity for active maintenance of ion gradients (i.e., Na^+/K^+ATPase) in excitable neurons [8]. Neuronal discharge and release of neurotransmitters and neuropeptides all require exceptionally large amounts of energy [9]. Thus, due to its high-energy demand, coupled with its limited capacity to store energy, the brain is uniquely sensitive to reductions in blood flow [8].

Magnesium sulfate has been used in a variety of neurological diseases like status epilepticus and eclampsia of pregnancy [10]. It has neuroprotective effect and decreases evoked potential of neurons [11]. It also blocks action potential in cardiomyocytes thus it used in certain types of arrhythmia as antiarrhythmic drug [12]. This drug has anti-inflammatory effect [13]. It also decreases cerebral edema and maintains blood brain barrier [14]. It dilates cerebral blood vessels [15]. Magnesium sulfate has beneficial effect in acute stroke patient [16]. Antenatal magnesium sulfate therapy given to women at risk of preterm birth is neuroprotective against motor disorders in childhood for the preterm fetus [17]. Costantine et al,2009 found that fetal exposure to magnesium sulfate in women at risk of preterm delivery significantly reduces the risk of cerebral palsy without increasing the risk of death [18].

Furthermore, magnesium sulfate decreases neuron apoptosis after cerebral ischemia-reperfusion injury [19]. Aim of the study: This study was designed to reveal the neuroprotective effect of magnesium sulfate against ischemia reperfusion injury.

Method

Animals and Study Design

A total of 24 Adult Sprague-Dawley rats [20] weighing (150-220 g) were purchased from Animal Resource Center, College of Veterinary Medicine-University of Kufa. They were housed in the animal house of Kufa College of Medicine in a temperature-controlled (25°±1C) room (humidity was kept at 60–65%) with alternating 12-h light/12-h dark cycles and were allowed free access to water and chow diet until the start of experiments. After the 1st week of localization the rats were distributed randomly into 3 groups as follow:

- Sham group: Rats underwent the same anesthetic and surgical procedures for an identical period of time, but without bilateral common carotid artery occlusion (BCCAO) .
- Control group (induced-untreated): Rats underwent anesthesia and surgery with bilateral common carotid artery occlusion (BCCAO) for 30 min. and then reperfusion for 1 hour but without drugs.
- Control - Vehicle group: For 10 days before surgery rats received daily intraperitoneally (IP) with normal saline (0.9% Nacl) (0.5 ml) [13, 21]. Then, anesthesia and surgery with bilateral common carotid artery occlusion (BCCAO) for 30 min. and later reperfusion for 1 hour.
- Magnesium sulfate (treated): Rats received 270mg/kg of magnesium sulfate before the surgery(13) , then anesthesia and surgery with bilateral common carotid artery occlusion (BCCAO) for 30 min. and later reperfusion for 1 hour

Induction of global brain ischemia

Induction of global ischemia by bilateral common carotid artery occlusion [22, 23]. Rats were maintained at approx. 37°C under a light bulb and under general anesthesia ketamine &xylazine (80mg/kg & 5mg/kg intraperitoneally) [24]. Animals were placed on the back in the supine position. A small median incision was made in the neck and both carotid arteries were separated from vagal nerves, then exposed bilaterally and occluded by using vascular clamp and clamped for 30 min. In the reperfusion, the

clamp were removed after ischemia and reperfusion was allowed to take place for 1 hour.

Preparation of samples and measurement of cerebral level of cytokines and chemokine

Following decapitation, the brain was removed and washed in cold 0.9% saline, kept on ice and subsequently blotted on filter paper, then weighed and homogenised using a high intensity ultrasonic liquid processor and brain tissues were homogenized in ice-cold 1:10 (w/v) 0.1 M phosphate-buffered saline (PBS) (pH 7.4), containing protease inhibitor cocktail and 0.2% Triton X-100 for 30 seconds (Famakin et al., 2012) [25]. Homogenisation procedure was performed as quickly as possible under completely standardized conditions 10% (w/v) of the homogenates were centrifuged at 14,000×g for 20 min at 4°C and supernatant was kept on ice until assayed by using ELISA technique after obtaining the standard curve for above parameters.

Tissue Sampling for Histopathology

Coronal brain sections were fixed with 10% formalin and embedded in paraffin wax and cut into longitudinal section of 5µm thickness The sections were stained with haemotoxylin and eosin dye for histopathological observation (Chandrashekhar et al., 2010) [26].

Histological analysis and damage scoring of brain

The histological observations (evaluated by a pathologist using a double-blind method) were scored using a pathological scoring scale (Pokela, 2003) [27] as follows: 0 (normal): no morphological signs of damage; 1(slight): edema or eosinophilic or dark neurons(pyknotic) or dark/shrunk cerebellar Purkinje cells; 2(moderate): at least two small hemorrhages and 3(severe): clearly infarctive foci (local necrosis).

Measurement of infarction area

Rats were sacrificed after 30min of BCCAO and 1 hour of reperfusion tissue damage or the infarction area was measured by 2,3,5-triphenyltetrazolium chloride(TTC) staining method according to Bederson et al. (1986) [28]. Animals were decapitated and the brain was quickly removed for 2,3,5-triphenyltetrazolium chloride (TTC) staining.

Preparation of brain slices

The brains were quickly isolated, the edematous or soft brains were placed in a freezer at -20°C for up to 20 minutes to facilitate sectioning, and the brain was sliced into uniform coronal section.

TTC staining (For staining by the immersion method)

TTC was dissolved in PBS at 2% (w/ v) concentration and used immediately for staining brain slices. For staining by the immersion method the TTC solution was prepared by dissolved TTC 2% (W/V) in PBS (phosphate buffer saline) (0.2 M Na2HPO4 and 0.2 M NaH2PO4, pH 7.4-7.6), with 37°C (Isayama et al., 1991) [29]. TTC solution was prepared immediately before use. The sections were put in a glass petri dish containing a shallow layer of 2% TTC, and glass cover slips wetted with the TTC solution were placed on top of each slice. To ensure even staining, the top and bottom surfaces of the section were in contact with the glass. The dishes were covered with aluminum foil to prevent exposure to light because TTC is light sensitive and incubated at 37°C for 30 minutes. The TTC solution was then replaced with 10% buffered formalin (phosphate-buffered formalin, PBF). To prevent distortion and fixed, brain slices were kept flat in the Petri dish or immersion in 10% phosphate-buffered formalin (PBF) overnight as reported by Bederson et al. (1986)(28) . The fixed brain sections were photographed and analysis by image analysis software (Digimizer), the unstained areas of the fixed brain sections were defined as infracted. Then the cerebral infarction area was observed and compared between various treatment groups and control group

Statistical analyses

Statistical analyses were performed using SPSS 20.0 for windows.lnc. Analysis of variant (ANOVA) was used for analysis of data to compare between mean of the four groups. The results are expressed as mean ± SEM. In all tests;$P<0.05$ was considered to be statistically significant.

Results

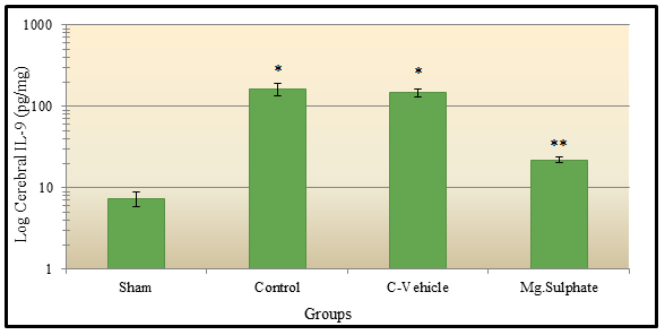

Figure 1.

The Error bar chart shows the difference in mean± SEM values of cerebral IL-9 level (pg/mg) in the four experimental groups at the end of the experiment (No. of animals = 6 in each group). $^{*}P<0.05$ *vs*. sham group; $^{**}P<0.05$ *vs*. Ctrl and vehicle group.

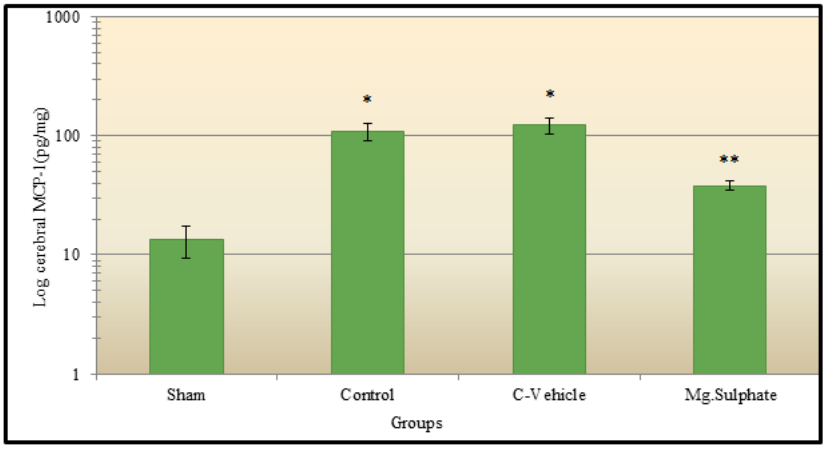

Figure 2.

The Error bar chart shows the difference in mean± SEM values of cerebral MCP-1 level (pg/mg) in the four experimental groups at the end of the experiment (No. of animals = 6 in each group). $P<0.05$ vs. sham group, $^{**}P<0.05$ vs. Ctrl and vehicle group.

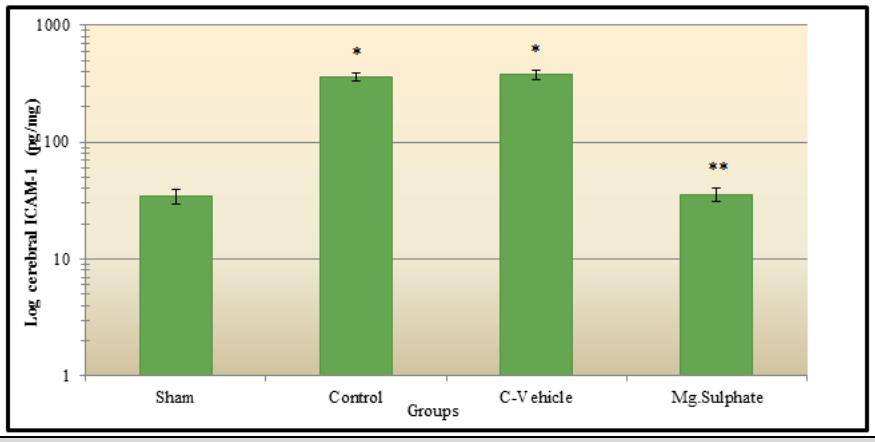

Figure 3.

The Error bar chart shows the difference in mean± SEM values of cerebral ICAM-1 level (pg/mg) in the four experimental groups at the end of the experiment (No. of animals = 6 in each group). $^{*}P<0.05$ *vs*. sham group, $^{**}P<0.05$ *vs*. Ctrl and vehicle group.

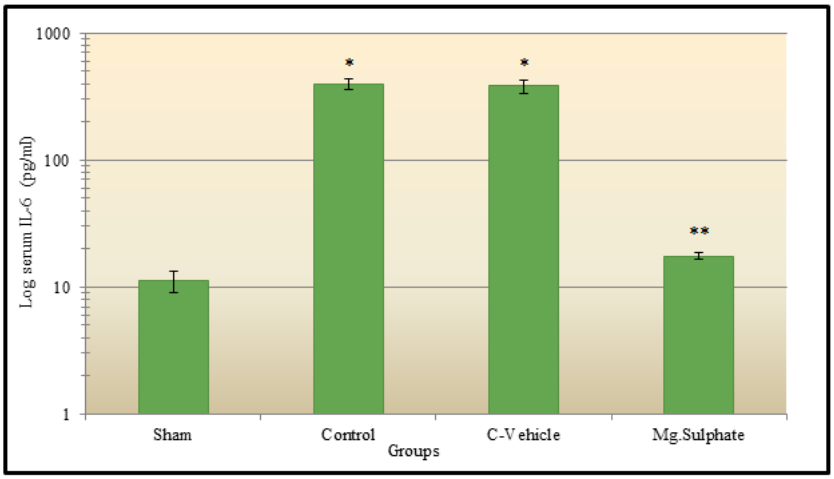

Figure 4.

The Error bar chart shows the difference in mean± SEM values of cerebral IL-6 level (pg/ml) in the four experimental groups at the end of the experiment (No. of animals = 6 in each group).$^{*}P<0.05$ *vs*. sham group, $^{**}P<0.05$ *vs*. Ctrl and vehicle group

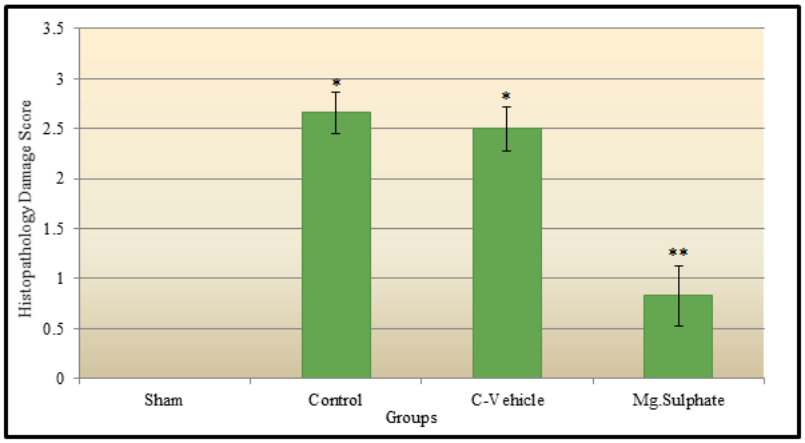

Figure 5.

The Error bar chart shows the difference in mean± SEM values of Cerebral Histopathology Damage Score in the four experimental groups at the end of the experiment (No. of animals = 6 in each group). $^{*}P<0.05$ *vs*. sham group, $^{**}P<0.05$ *vs*. Ctrl and vehicle group.

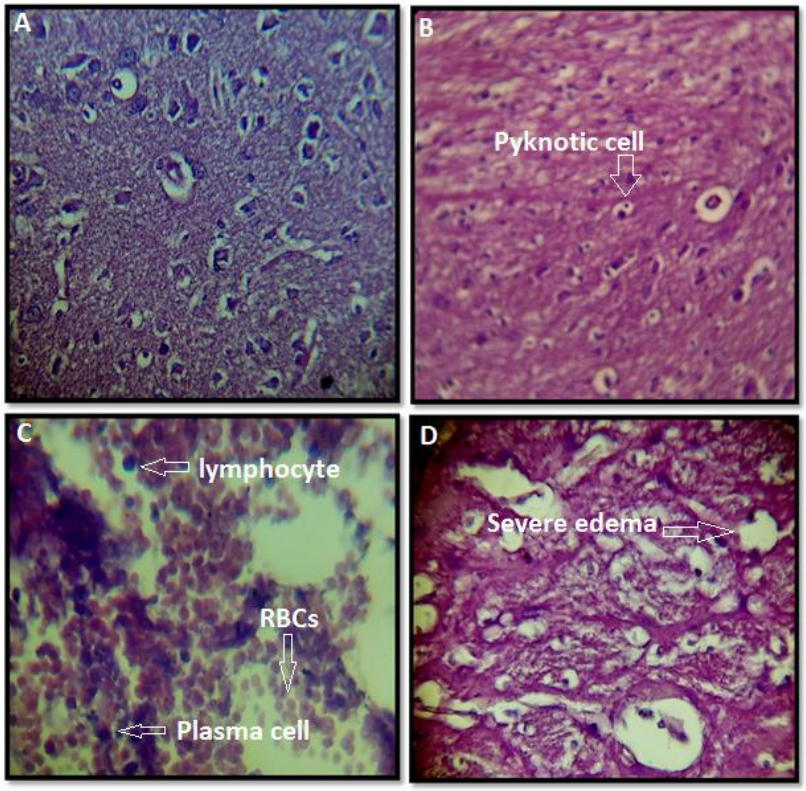

Figure 6.

Histopathological photography of rat brain showing different pathological grades of cerebral I/R injury. (A): Normal, (B): slight, (C): moderate and (D): severe

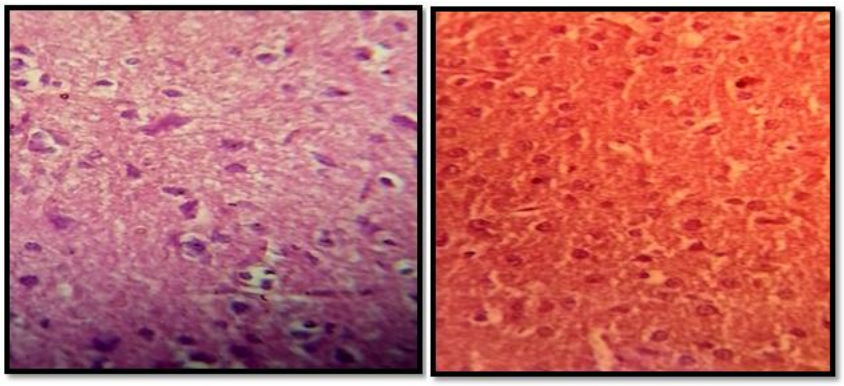

Figure 7.

Histopathological photography of rat brain treated with magnesium sulfate shows histopathological score of normal to slight I/R injury.

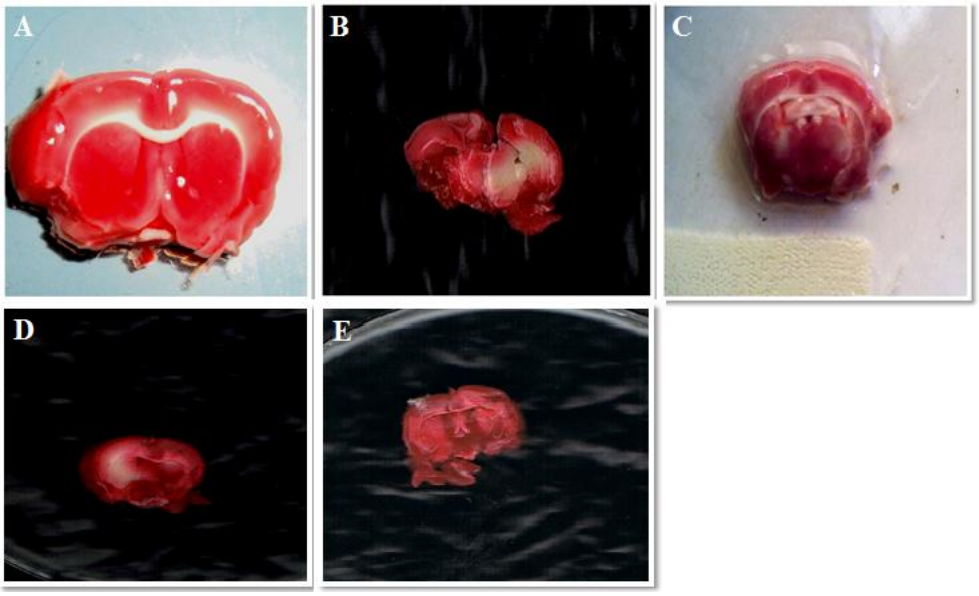

Figure 8.

Slices of rat brain from different groups stained with TTC stain to determine the infarction size. (A): Normal Sham group. (B): Control group, infarction size 30%. (C): Control-Vehicle, infarction size 25%. (D) and (E): Magnesium sulfate treated group, infarction size 15% and normal (respectively).

In control group, serum level of IL-9 was 163.3 ± 30.4 pg/ml in comparison to sham group which was 7.35 ± 1.45pg/mg (P<0.05) as shown in figure (1). Magnesium sulfate significantly decreases cerebral IL-9 to 21.8±1.72 ng/mg (P<0.05).

Multiple comparisons among the groups are shown in figure (1).Cerebral level of MCP-1 was significantly higher in control group (109.05 ± 18.2 pg/mg) in comparison to sham group (13.31±3.88pg/mg) (P<0.05). Magnesium sulfate significantly decreases cerebral level of MCP-1 to 38.16 ± 3.54pg/mg(P<0.05) as shown in figure (2).

The increase in cerebral level of ICAM-1 was significantly higher in control group (362.8 ± 26.81pg/mg) in comparison to sham group (34.3 ± 4.99 pg/mg)(P<0.05) as shown in figure (3). In control group, cerebral level of IL-6 was 396.7 ± 38.2pg/mg in comparison to sham group which was 7.35 ± 1.45pg/mg (P<0.05) as shown in figure (4). Magnesium sulfate significantly decreases cerebral IL-6 to 17.5 ± 1.11 ng/mg (P<0.05).

Control group showed cerebral histopathological damage score of 2.66 ± 0.21. In comparison to that, magnesium sulfate treated group showed histopathological score of 0.83 ± 0.30.

Discussion

In this study, a significant increase in inflammatory mediator (IL-9) level in cerebral tissue. During search in internet, there was no previous study regarding role of IL-9 in cerebral ischemia reperfusion injury. Ischemia reperfusion injury of the brain brings a systemic inflammatory response causes further damage by the inflammatory mediators like interleukins, chemotactic factors and adhesion molecules like IL-9, MCP-1 and ICAM-1 [30].

The inhibition of this inflammatory response may limit the neuronal damage and subsequently decrease the extent of ischemia reperfusion injury [31]. The extent of cerebral damage following cerebral infarction belongs to some extent to the degree of damage by ischemia reperfusion injury following restoration of blood flow following spontaneous regression of the arterial thrombus [32].

The reperfusion injury is too far extent is due to inflammatory process. Generally, cytokines and their receptors are nearly undetectable under normal conditions.

However following cerebral ischemia, proinflammatory cytokines are quickly and highly up-regulated in the brain [33, 34]. In addition, it has been shown that peripherally derived cytokines are involved in brain inflammation. Thus, peripherally derived mononuclear phagocytes, T-lymphocytes, NK cells and polymorphonuclear leukocytes produce and secrete cytokines and might contribute to inflammation of the CNS [35].

Inflammation plays an important role in acute ischemic stroke (AIS), indicating important interactions between the nervous and immune systems [36]. Interleukin-9 (IL-9) is a multifunctional cytokine produced by activated TH2 clones in vitro and during TH2-like T cell responses in vivo [37]. Elevated mean IL-9 serum levels have been observed in human neonates who will later develop cerebral palsy [38]. Ormstad et al. (2011) showed that a significant elevation in IL-9 in the acute ischemic stroke [39].The findings of elevated levels of IL-9 in acute ischemic stroke AIS patients are novel. Chemokine that has been associated with ischemia/reperfusion injury is chemoattractant protein-1 (MCP-1).

The MCP-1 levels are increased in the cerebrospinal fluid of stroke patients [40]. Expression of chemokines following focal ischemia is thought to have a deleterious role by increasing leukocyte infiltration [41]. MCP-1 is a major factor driving leukocyte infiltration in the brain parenchyma [42]. There is increasing evidence that cellular adhesion molecules (CAMs) play an important role in the pathophysiology of acute ischemic stroke [43]. There is increasing evidence that cellular adhesion molecules (CAMs) play an important role in the pathophysiology of acute ischemic stroke [43]. Patients with acute ischemic stroke had higher soluble intercellular adhesion molecule-1 (sICAM-1) levels compared to patients without cardiovascular disease . Moreover, sICAM-1 levels were significantly higher in patients who died compared to those who survived [44].

Magnesium sulfate decreases inflammation through its blocking effect on l-type calcium channels [45]. It also block NMDA receptor and acts as NMDA receptor antagonist and limit NMDA mediated brain injury during stroke [46]. Magnesium sulfate administration ameliorates inflammatory response and decrease cytokines in both maternal and fetal compartments associated with preterm labor [18].

In conclusion, we find that magnesium sulfate reduces inflammatory response following ischemia reperfusion injury of the brain. We recommend further study for other inflammatory markers like IL-10 and further study for effect of magnesium sulfate on NMDA receptor. In addition to that further study for evaluation of effect of magnesium sulfate on TNF-α and complement system.

Competing interests

The authors declare that there is no conflict of interest.

Author Contributions

All authors wrote, read and approved the final manuscript.

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