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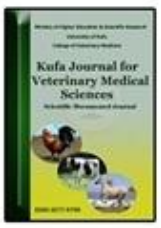

The study of neuroprotective and anti-inflammatory effects of magnesium sulfate in rats following cerebral ischemia reperfusion injury

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Abstract:

Background and objective: Magnesium sulfate has neuroprotective effects and decrease overall neuronal firing. It is also decrease firing of excitable tissues outside the brain. It is not known whether this neuroprotective effect is due to antioxidant effect, anti-inflammatory or other mechanism. In this research we study the anti-inflammatory effect of magnesium sulfate in rat brain following ischemia reperfusion stress.

Material and methods: 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. Blood samples were taken after the procedure for measurement of serum level of IL-9, MCP-1 and ICAM.

Results: Serum level of IL-9 in control group was 163.3 ± 30.4 pg/mL and it significantly decreased in magnesium sulfate treated group (21.8 ± 1.72 pg/mL). serum level of MCP-1 in the control group was 109.05 ± 18.2 pg/mL while it significantly reduced in magnesium sulfate treated group (38.16 ± 3.54 pg/mL). mean serum levels of ICAM of control was 362.8 ± 26.81 pg/mL while mean serum level of ICAM in treated group was 35.5 ± 4.71 pg/mL.

Conclusion: magnesium sulfate significantly decreases the inflammatory markers IL-9, MCP-1 and ICAM in global ischemia model in rats.

Keywords: magnesium sulfate , global cerebral ischemia, IL-9, ICAM, MCP-1.

دراسة التأثير الوقائي الدماغي و المضاد للالتهاب لدواء كبريتات المغنيسيوم في الجرذان بعد نقص التروية الدموية الدماغية و اعادة الارواء

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الخلاصة:

ان الإصابة بنقص التروية الدماغية وإعادة التروية (IRI) هي من العمليات المعقدة التي تؤدي إلى تلف الخلايا ثم موتها كما ان نقص التروية وأعادة الأرواء في الدماغ، كما هو الحال في الأجهزة الأخرى، يحفز استجابة التهابية والتي بدورها قد تؤدي إلى تفاقم مستويات الأولية لإصابات الأنسجة. أجريت هذه الدراسة لغرض التحقق من امكانية كبريتات المغنيسيوم في حماية الأعصاب و تحسين حال الإصابة الدماغية الشاملة (IRI) في نموذج الجرذان . تم اسخدام اربعة وعشرون جرذاً بالغاً استخدمت في هذه الدراسة, وقد تم توزيعهم بشكل عشوائي الى اربعة مجاميع. أظهرت نتائج المستويات المصلية لكل

من IL-9 ، وICAM-1 MCP-1 زيادة احصائية ($p<0.05$) في مجموعة السيطرة عند المقارنة بمجموعة التظاهر. كما أظهرت المعالجة بكبريتات المغنيسيوم تأثيرا احصائيا ($p<0.05$)على الالتهاب من خلال تثبيط زيادة المستويات المصلية لعوامل الألتهاب IL-9,MCP-1,ICAM-1.

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 that the neuroprotective effect of magnesium sulfate is due to its anti-inflammatory effect.

Materials and Methods:

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:

i. **Sham group** Rats underwent the same anesthetic and surgical procedures for an identical period of time ,but without bilateral common carotid artery occlusion (BCCAO).

ii. **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.

iii. **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 .

iv. **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 serum level of ICAM and IL-9 and MCP-1:

Blood samples was obtained after end of the procedure. After centrifugation, serum samples was stored in deep freeze for further analysis. Kits for measurement of ICAM, IL-9 and MCP-1 for rats was obtained. Dilution of reagents was undertaken with calculation of standard curve for each parameter. Then, enzyme linked immune-sorbent assay (ELISA) was used for measurement of serum ICAM, IL-9 and MCP-1.

Results:

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

1. **Effect of magnesium sulfate on serum level of IL-9:** 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/ml ($p<0.05$) as shown in figure (1). Magnesium sulfate significantly decreases serum IL-9 to 21.8 ± 1.72 ng/ml ($p<0.05$). Multiple comparisons among the groups are shown in table (1).

Table (1): Multiple comparisons among different group mean values of serum level of IL-9 (pg/ml) using ANOVA TEST

Group	Control	Mg Sulfate treated
Sham	-155.9*	-14.48
C-vehicle	-16.5	125*

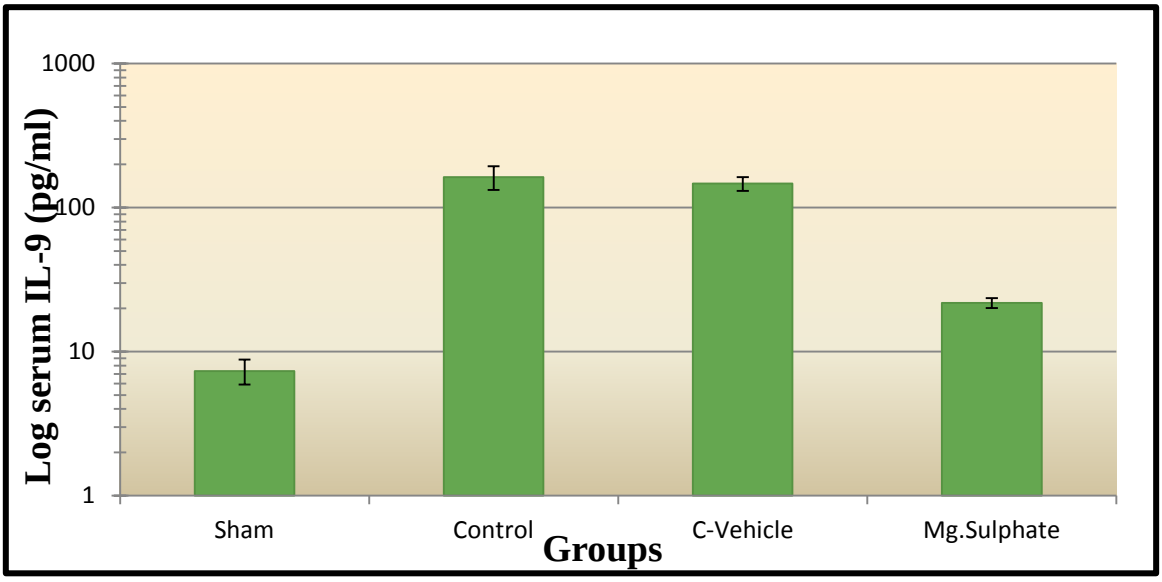

Figure (1): The Error bar chart shows the difference in mean± SEM values of serum IL-9 level (pg/ml) in the four experimental groups at the end of the experiment (No. of animals = 6 in each group).

2. Effect of magnesium sulfate on serum level of MCP-1: serum level of MCP-1 was significantly higher in control group (109.05 ± 18.2 pg/ml) in comparison to sham group (13.31 ± 3.88pg/ml) ($p<0.05$). Magnesium sulfate significantly decreases serum level of MCP-1 to 38.16 ± 3.54 pg/ml ($p<0.05$) as shown in figure (2). Multiple comparisons among the groups are shown in table 2.

Table (2): Multiple comparisons among different group mean values of serum level of MCP-1 (pg/ml) using ANOVA TEST

Group	Control	Mg Sulfate treated
Sham	-95.73*	-24.85
C-vehicle	13.9	84.78*

3. **Effect of magnesium sulfate on ICAM-1:** the increase in serum level of ICAM-1 was significantly higher in control group (362.8 ± 26.81pg/ml) in comparison to sham group (34.3 ± 4.99 pg/ml)($p<0.05$) as shown in figure (3). Multiple comparisons among the groups are shown in table (3).

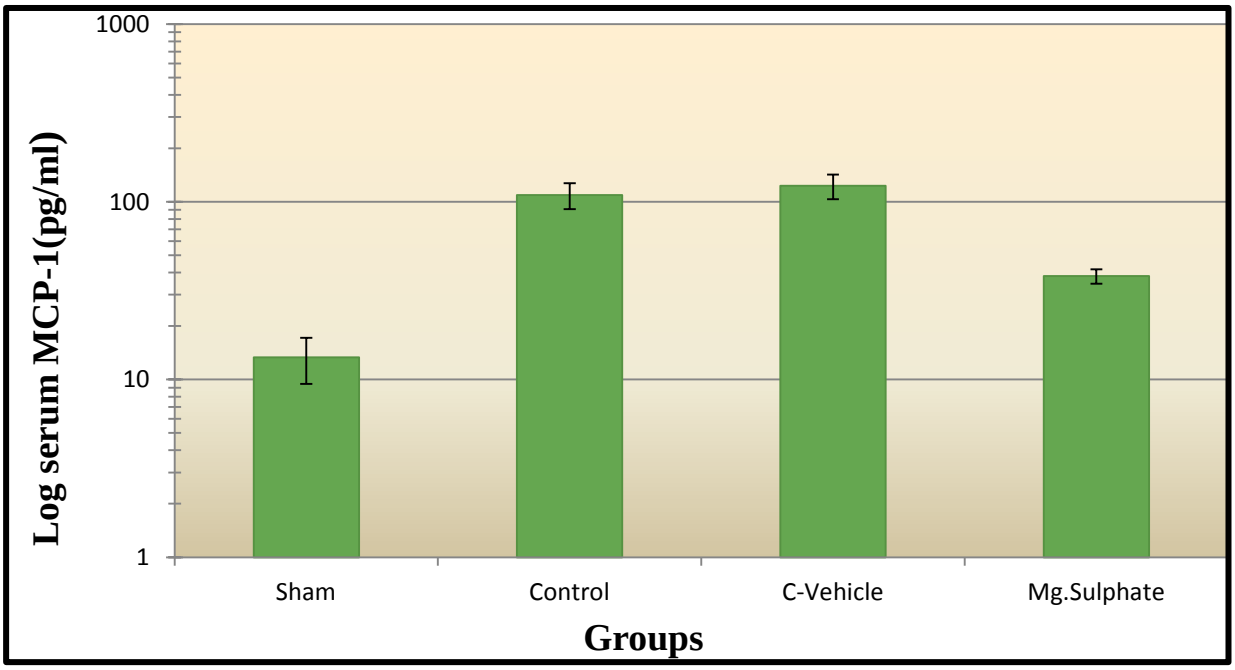

Figure (2): The Error bar chart shows the difference in mean± SEM values of serum MCP-1 level (pg/ml) in the four experimental groups at the end of the experiment (No. of animals = 6 in each group).

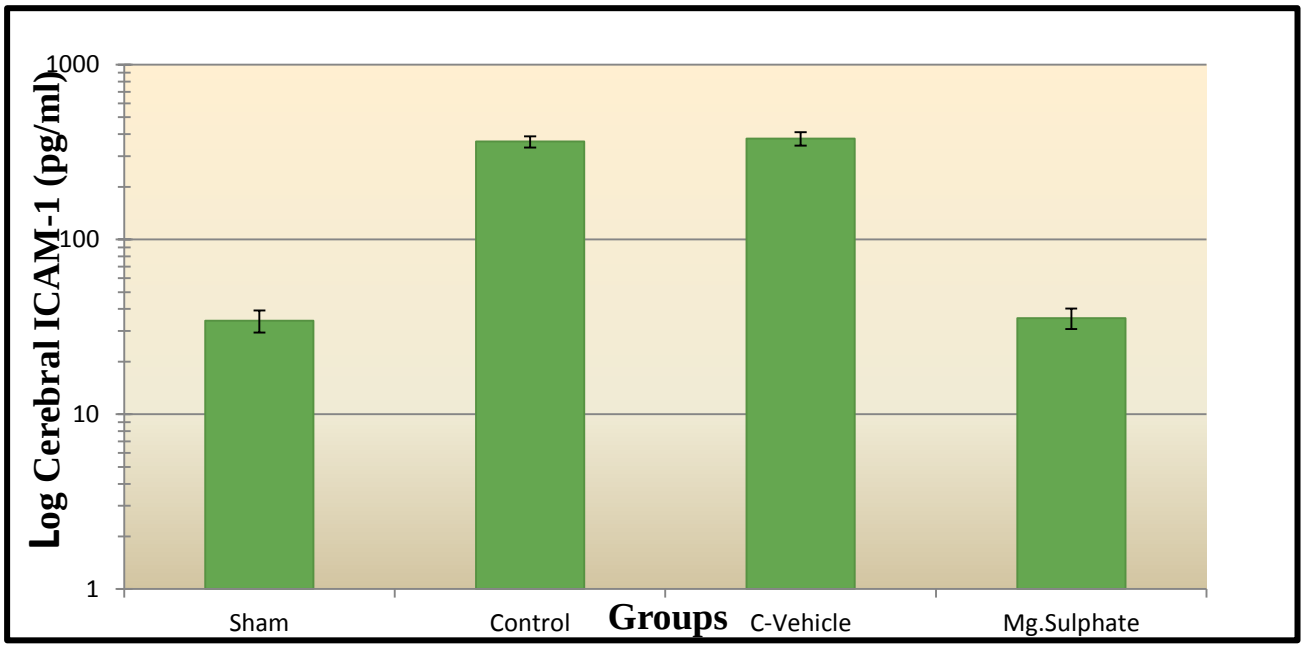

Figure (3): The Error bar chart shows the difference in mean± SEM values of serum ICAM-1 level (pg/ml) in the four experimental groups at the end of the experiment (No. of animals = 6 in each group)

Table (3): Multiple comparisons among different group mean values of serum level of ICAM-1 (pg/ml) using ANOVA TEST.

Group	Control	Mg Sulfate treated
Sham	-328.41*	-1.11
C-vehicle	14.66	341.9*

Discussion:

In this study, a significant increase in inflammatory mediator (IL-9) level in serum. 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[25]. The inhibition of this inflammatory response may limit the neuronal damage and subsequently decrease the extent of ischemia reperfusion injury[26]. 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[27]. The reperfusion injury is to 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[28,29]. 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[30] Inflammation plays an important role in acute ischemic stroke (AIS), indicating important interactions between the nervous and immune systems[31] **.** Interleukin-9 (IL-9) is a multifunctional cytokine produced by activated TH2 clones in vitro and during TH2-like T cell responses in vivo[32]**.** Elevated mean IL-9 serum levels have been observed in human neonates who will later develop cerebral palsy**[33] .** Ormstad *et al.* (2011) showed that a significant elevation in IL-9 in the acute ischemic stroke[34].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[35]. Expression of chemokines following focal ischemia is thought to have a deleterious role by increasing leukocyte infiltration[36]. MCP-1 is a major factor driving leukocyte infiltration in the brain parenchyma[37].There is increasing evidence that cellular adhesion molecules (CAMs) play an important role in the pathophysiology of acute ischemic stroke[38].There is increasing evidence that cellular adhesion molecules (CAMs) play an important role in the pathophysiology of acute ischemic stroke[38]. 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[39]**.** Magnesium sulfate decreases inflammation through its blocking effect on l-type calcium channels[40]. It also block NMDA receptor and acts as NMDA receptor antagonist and limit NMDA mediated brain injury during

stroke[41]. Magnesium sulfate administration ameliorates inflammatory response and decrease cytokines in both maternal and fetal compartments associated with preterm labor[18]. From our study we concluded 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.

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