Methylsulfonylmethane (MSM) as a New Target for the Treatment of Glycerol-Induced Nephropathy

Shaza Anwar Al Laham*

Department of Pharmacology & Toxicology, Faculty of Pharmacy, Damascus University, Syria

Abstract: *Objective*: Methylsulfonylmethane (MSM) is a naturally occurring nutrient composed of sulfur, oxygen and methyl groups. MSM may have anti-inflammatory and free radical scavenging activity. The present work was done to investigate the possible cure effect of methylsulfonylmethane on glycerol-induced acute renal failure (ARF) in rats.

Methodes: After water deprivation, a kidney injury was induced in rats by intramuscular administration of glycerol 10 mL/kg (50% vol./vol. in saline). Several parameters including macroscopic score, histopathological and biochemical were determined to assess the degree of treatment.

Results: Results showed that MSM decreased macroscopic and microscopic kidney's injury scores caused by glycerol. MSM also significantly reduced urea and creatinine levels compared to glycerol-induced ARF group.

Conclusion: MSM as a natural product has a curing effect against glycerol-induced myoglobinuria.

Keywords: Methylsulfonylmethane (MSM), Acute renal failure, Myoglobinuria.

1. INTRODUCTION

Acute renal failure (ARF) is a syndrome characterized by an acute loss of renal function. Despite the reversibility of this loss in most patients who survive, mortality from ARF remains high (over 50%). Therefore, the search for effective therapy to accelerate recovery and attempts to prevent ARF have attracted much attention [1].

Acute renal failure (ARF) is characterized by a rapid, potentially reversible, decline in renal function, including a rapid fall in glomerular filtration rate (GFR) and retention of nitrogenous waste products over a period of hours or days.

Rhabdomyolysis is the syndrome characterized by the breakdown of striated muscle with the massive release of myoglobulin into the extracellular fluid and circulation leading to filtration of myoglobulin to renal tubules [2], which forms obstructing tubular casts, thus Rhabdomyolysis provokes acute tubular necrosis (ATN). Myoglobin also leads to intra-renal vasoconstriction due to nitric oxide scavenging and through hypovolemia [3]. The large numbers of disorders known to cause rhabdomyolysis include intrinsic muscle dysfunction (including trauma, burns, intrinsic muscle disease, and excessive physical exertion), metabolic disorders, hypoxia, drugs, toxins, infections, temperature extremes and idiopathic disorders [2].

*Address correspondence to this author at the Damascus University-Damascus, Syria; Tel: 009630933375655; E-mail: lahamshaza@gmail.com

Methylsulfonylmethane (MSM) also known as dimethyl sulfone and methyl sulfone, is an organic sulfur-containing the compound that occurs naturally. MSM is found in small amounts in many foods, including unpasteurized milk, grains, meat, eggs, and fish [4], in bovine milk; and in human urine (4-11 mg/day of MSM are normally excreted in the urine)[5]. Also, found in human cerebrospinal fluid and plasma.

MSM may have anti-inflammatory activities, chemopreventive properties, prostacyclin (PGI2) synthesis inhibition, anti-atherosclerotic action, salutary effect on eicosanoid metabolism, and free radical scavenging activity [6]. Also, is known as a potent antioxidant/anti-inflammatory compound [7]. Methylsulfonylmethane is one of the least toxic substances in biology, similar in toxicity to water [8].

The purpose of this present study was to evaluate the curative effects of MSM supplementation on the glycerol-induced ARF in rats, for the first time.

2. MATERIAL & METHOD

Wister rats weighing (250-300g) were adapted for one week before any experimental procedures and were fed with standard commercial rat pellets and allowed water ad libitum. They were kept at controlled environmental conditions (temperature 23 ± 2°C, humidity 55 ± 15%, lighting regimen of 12-h light: 12-h dark). All methods performed in this study were in accordance with regulatory guidance on the care and use of experimental animals.

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2.1. Experimental Design and Treatment Protocol

Three groups of rats (n = 6 for each group), were employed in this study: group 1(N), served as a control; group 2 (G), was only given Glycerol (surechem products LTD) (50%, 10 mL/kg, i.m.); group 3 (M), was given Glycerol plus MSM (Panvo Organics Pvt Ltd) (400 mg/kg), starting after 60 mints of the glycerol injections.

After 24 h (hours) of water deprivation, on the first day, in groups (G) and (M), a kidney injury was induced in rats by intramuscular administration of glycerol 10 mL/kg (50% vol./vol. in saline) [9], at a single dose injected in the hind limb muscles of the rats, half of the dose was administered in each hind limb muscle. While group (N) had an equal volume of normal saline (10 ml/kg, i.m.). Drinking water and food then resumed ad libitum. After 60 minutes of glycerol injection, for consecutive 6 days, orally administered using an intubation needle was done, by saline for the group (N), and (G); and MSM (400 mg/kg) for the group (M) suspended with normal saline [10]. An hour after the last dose, the animals were sacrificed under deep ethyl ether anesthesia (surechem products LTD). The blood samples and kidney tissues were harvested for future biochemical and pathology analyses.

2.2. Renal Function Tests

Blood samples were collected by heart puncture. Serum was separated for renal function tests (serum urea and creatinine concentrations).

Serum Creatinine Concentration

Creatinine concentrations in plasma samples were measured with a rate-blanked and compensated picric acid colorimetric assay (CREA, Roche/Hitachi Modular p analyzer). In this enzymatic method, creatinine is converted to creatine under the activity of creatininase. In alkaline solution, creatinine forms a yellow-orange complex with picrate. The absorbance of samples and standard were measured twice (after 30 sec (seconds) and 90 sec) spectrophotometrically (Hitachi U-1800) at 505 nm. The concentrations calculated as per the manufacturer's protocol.

Serum Urea Concentration

The principle of the urea measuring, using the Roche/Hitachi Modular p analyzer kit, based on the change in the intensity of staining ammonia compounds with sodium salicylate and sodium hypochlorite, which is directly proportional to the concentration of urea in the sample. The colored complex measured by spectrophotometrically at 340 nm, as per the manufacturer's protocol.

2.3. Macroscopic and Histological Evaluation of Kidneys

The abdominal cavities of the rats were opened; Kidneys were immediately collected and washed in cold normal saline, and longitudinally sectioned. Kidney sections appearance were examined macroscopically.

For microscopic evaluation, tissues were fixed in 15% paraformaldehyde for 24 hours. After fixation, tissues were dehydrated in a graded ethanol, cleared in xylene, and then embedded in paraffin using an automated histology processor following standard histological protocols. Replicate sections were cut at 5 µm thickness on a rotary microtome and stained with hematoxylin and eosin on an automated stainer.

Samples were blindly analyzed to determine the extent of kidney injury based on the technique outlined by Erdogan *et al.* [11]. The examinations focused on the kidney proximal tubules were graded for the degree of renal damage, based on the following parameters: tubular cell necrosis and apoptosis, cytoplasmic vacuole formation, and tubular dilatation. Interstitial edema and medullary congestion were also assessed. The severity of these lesions was determined based on the percentage of involvement of the kidney. Higher scores represent more severe damage, with the maximum score being 4: [0, histopathological changes <10%; 1, (10%–25%); 2, (25%–50%); 3, (50%–75%); and 4, (75%–100%)]. It was also examined the renal glomerular injury, hemorrhage, inflammation, fibrinoid and hyaline dystrophies, where the presence of these injuries was grade 1, and their absence was grade 0. The mean score for each parameter was determined and subjected to statistical analysis.

3. STATISTICAL ANALYSIS

Results were expressed as (mean ± standard deviation SD). Statistical analysis was performed using the GraphPad Prism (Version 6) statistical package. Comparisons between the groups for parametric were performed using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests, for serum creatinine, and urea concentration; Statistical significance was set at $p < 0.0001$. Lesion score and histological score (non-parametric values) were analyzed using the Mann Whitney U test, and the frequency of categorical binary data was evaluated

using Fisher's exact test. P values <0.05 were considered as statistically significant.

4. RESULTS

4.1. Effects of MSM on Kidney Function

As shown in Table **1**, the serum levels of urea and creatinine in (G) rats were significantly higher than those in (N) rats did. While administration of MSM significantly reduced the levels of urea and creatinine compared with that of (G) rats (P< 0.0001).

Table 1: Renal Function: Serum Level of Urea and Creatinine Concentrations

Groups	Urea mmol/L	Creatinine µmol/L
Normal (N)	33.85 ± 9.237	0.398 ± 0.088
Glycerol (G)	111.7 ± 21.09 χ	1.87 ± 0.265 χ
MSM (M)	51 ± 6.261 η	0.628 ± 0.065 η

χ **P < 0.0001 vs. N; η **P < 0.0001 vs. G.

4.2. Macroscopic and Histological Results

Macroscopic Evaluation

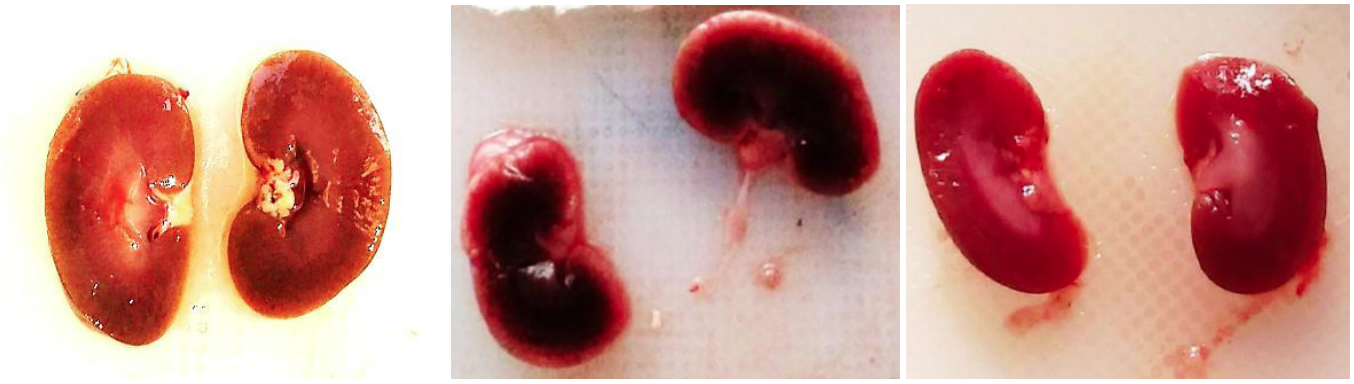
Kidneys have normal macroscopically appearance and color, in control group (N). Kidneys in injured group (G) were bigger than in control group, with different macroscopic morphology. They showed yellow cortex with dark brown medulla. Noticed congestion and edema. While the normal macroscopically appearance and color, was in the group (M) (Figure **1**).

Histological Evaluation

Under the light microscope, the kidney sections from the control group of rats did not show any damage, with the glomeruli and tubuli having a normal appearance (Figure **2**. N). In group (G) rats injected with glycerol, severe lesions were seen in renal tubules, which show dilatation, vacuolation and typical apoptotic morphology including swelling, deformation of their lined epithelial cells (Figure **2**, G1, G2). Renal tubular scores were significantly higher compared to control group (N), (P<0.05). There is also mild to moderate medullary congestion and interstitial edema. The histological scores of these lesions were significantly higher than the control group (P<0.05) (Figure **3**). The glomerular injuries were observed in this group represented as mesangial extracellular matrix deformation, necrosis, and glomerular capillary congestion, these lesions' scores were significantly higher than of the control group (N), (P<0.05). Fibrinoid and hyaline dystrophies also observed in this group specimen (Table **2**) and were significantly different from the control group (N) (P<0.05). The signs of inflammatory lesions also observed in this group represented as mononuclear and polymorphonuclear leukocytes infiltration in the tubules and interstitium, these lesions were no significantly compared control group. Hemorrhage and coagulation in renal vessels were also observed in some samples, (Table **3**). However, in the MSM group, the glomeruli and proximal tubuli appearance were normal in some rats, and renal tubules slightly enlarged, with infiltration of inflammatory cells, and glomerular capillaries congestion in other rats. As compared to (G) group, the histological scores of this futures were significantly lower than of the injured group (G), (P<0.05). Except for fibrinoid dystrophy, and inflammatory lesions, hemorrhage, and coagulation in renal vessels were no significantly compared (G) group. (Figure **2** M1,M2; Table **2**).

5. DISCUSSION

The most commonly used model for studying ARF is a rat receiving a single intramuscular injection of glycerol, which induces rhabdomyolysis. A number of



A-N B-G C-M

Figure 1: Representative macroscopic kidney changes: (N) normal group; (G) glycerine group; (M) MSM group.

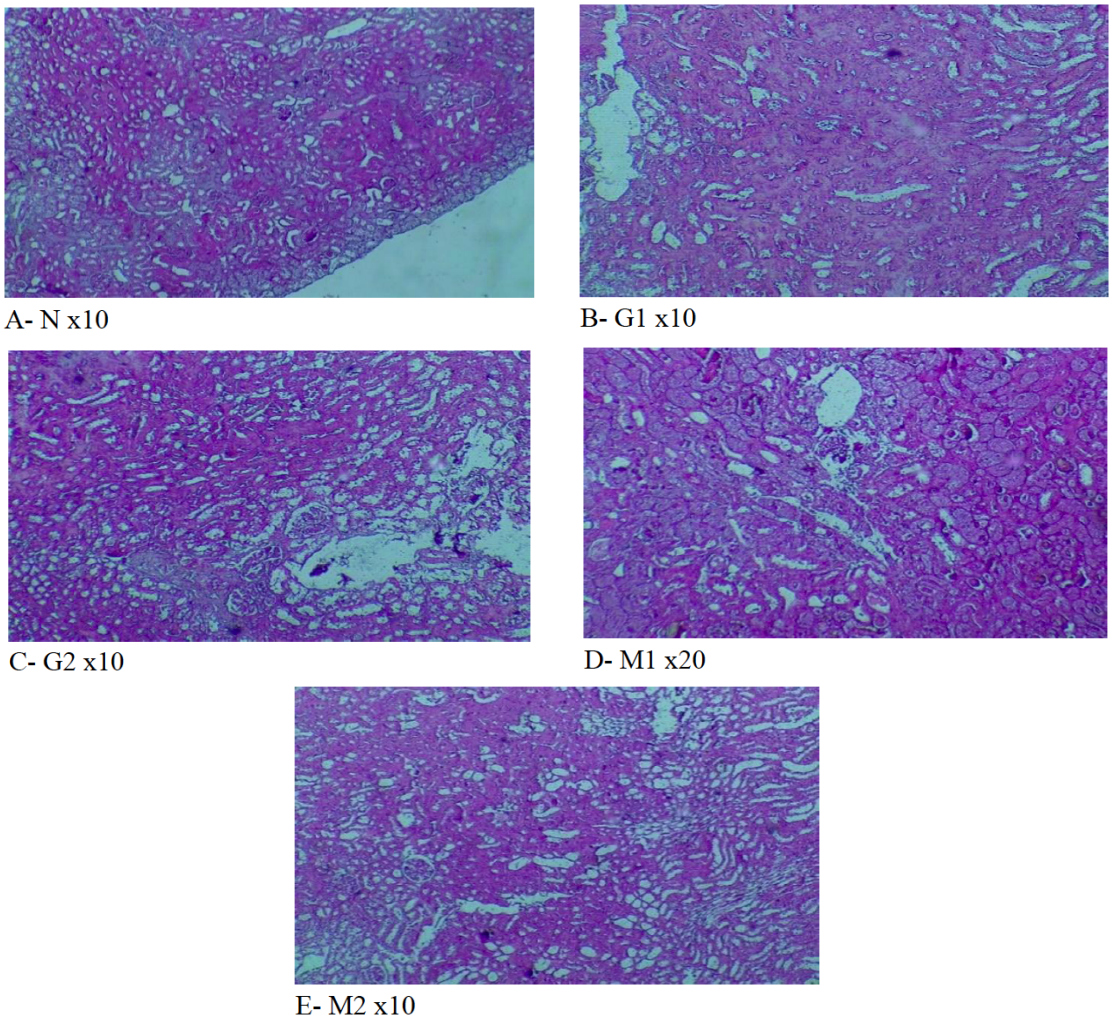


Figure 2: Representative histomorphological kidney changes: (N) normal group; (G) glycerine group; (M) MSM group. All photomicrographs were taken at a magnification of 10x, except M1 was 20x. (N) represent Kidney section of a control rat showing normal architecture. (G1), and (G2) represent Kidney section of a glycerol treated rats showing glomerular deformation, tubular dilatation, vacuolation, swelling and degeneration of their lined epithelial cells, vascular congestion, fibrinoid distrophy and hyaline dystrophy. (M1), and (M2) represent Kidney section of MSM treated rats showing the enhancement in tubular and glomerular injuries and other pathologic alterations.

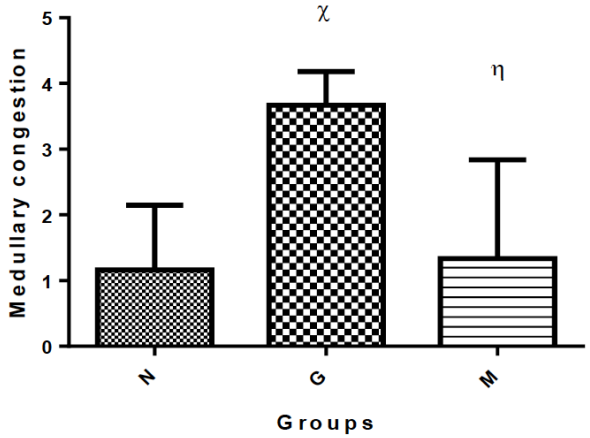


Figure 3: Effect of MSM on the histological score of damage on medullary congestion.

Each column represented the mean±SD.

χ significant vs. N group *P=0.0011< 0.05; η significant vs. G group *P=0.0108<0.05.

N: control group; G: glycerol, M: MSM.

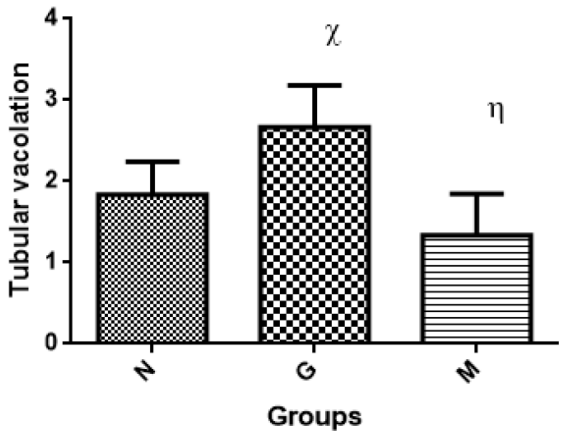


Figure 4: Effect of MSM on the histological score of damage on tubular vacuolation.

Each column represented the mean±SD.

χ significant vs. N group *P=0.0227<0.05; η significant vs. G group *P=0.0065<0.05.

N: control group; G: glycerol, M: MSM.

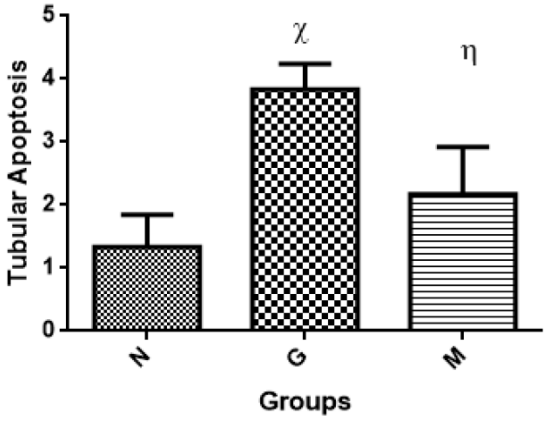


Figure 5: Effect of MSM on the histological score of damage on tubular apoptosis.

Each column represented the mean±SD.

χ significant vs. N group *P=0.0011<0.05; η significant vs. G group *P=0.0032<0.05.

N: control group; G: glycerol, M: MSM.

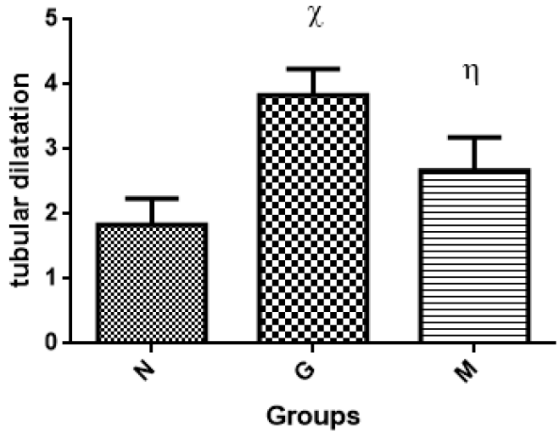


Figure 6: Effect of MSM on the histological score of damage on tubular dilatation.

Each column represented the mean±SD.

χ significant vs. N group *P=0.0011<0.05; η significant vs. G group *P=0.0011<0.05.

N: control group; G: glycerol, M: MSM.

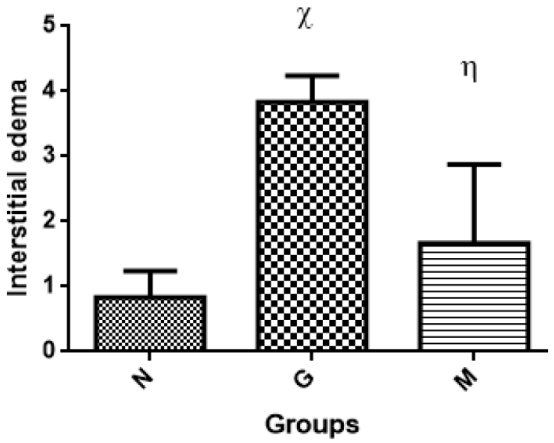


Figure 7: Effect of MSM on the histological score of damage interstitial edema.

Each column represented the mean±SD.

χ significant vs. N group *P=0.0076<0.05; η significant vs. G group *P=0.0076<0.05.

N: control group; G: glycerol, M: MSM.

studies have shown that rhabdomyolysis-induced myoglobinuric ARF [1]. The pathogenesis of glycerol-induced myoglobinuric acute renal failure involves ischemia, vascular congestion and reactive oxygen metabolites [12], and tubular necrosis [3]. Renal injury associated with ischemia/reperfusion results from a dynamic process involving the vasculature and tubules. There is a complex activation of signaling cascades resulting in hemodynamic alterations, leukocyte accumulation, and direct injury to the tubule epithelial cells followed by a repair process that can restore normal morphology and function [13].

The results of this study showed that glycerol administration produced a typical pattern of nephrotoxicity, which was manifested by the increased level of serum levels of urea and creatinine, the results were in agreement with many studies such as Stefanovic [14], and Manikandan [12]. However, MSM administration showed a significant decrease in their levels.

There was a good correlation between macroscopic and histological scores in each study group. The

Table 2: Effects of MSM on the Glomerular Injury, Fibrinoid Dystrophy, and Hyaline Dystrophy, Expressed as the Frequency of Injured Animals in each Group

feature	Glomerular injury			Fibrinoid dystrophy			Hyaline dystrophy		
Groups	injury	No injury	Significance	injury	No injury	Significance	injury	No injury	Significance
N	0	6		0	6		0	6	
G	5	1	θ	0	6		6	0	χ
M	5	1	γ	0	6		0	6	η

χ *P=0.0011<0.05 as compared to N group; η *P=0.0011<0.05 as compared to G group.

θ *P=0.0076<0.05 as compared to N group; γ *P=0.0076<0.05 as compared to G group.

N: control group;G: glycerol, M: MSM.

Table 3: Effects of MSM on Acute Inflammation, Hemorrhage, and Coagulation, Expressed as the Frequency of Injured Animals in each Group

Feature	Acute inflammation			Hemorrhage			Coagulation		
Groups	injury	No injury	Significance	Injury	No injury	Significance	injury	No injury	Significance
N	0	6		0	6		0	6	
G	0	6		0	6		4	2	θ
M	0	6		2	4		2	4	

θ *P=0.0076<0.05 as compared to N group. N: control group, G: glycerol, M: MSM.

histological studies of the kidney from glycerol-treated rats showed damaged glomerular structure, hemorrhagic and hyaline cast deposits, tubular necrosis, vacuolar degeneration changes in the epithelial cells, cellular proliferation with fibrosis, thickening of capillary walls and glomerular tuft atrophy, as showed in Manikandan's study [12].

Animals treated with MSM for 400mg/kg body weight showed a normal architecture similar to the control animals. As some studies displayed the MSM properties, like Methylsulfonylmethane (MSM), an organic sulfur-containing compound, inhibits LPS-induced release of pro-inflammatory mediators in murine macrophages through downregulation of nuclear factor kappa-light-chain-enhancer of activated B cells ($NF\text{-}_KB$) signaling [4]. MSM may have anti-inflammatory activities, chemopreventive properties, prostacyclin (PGI2) synthesis inhibition, anti-atherosclerotic action, salutary effect on eicosanoid metabolism, and free radical scavenging activity [6]. MSM suppression the expression of inflammatory mediators such as NO, PGE2, IL-6, and TNF-α, as well as iNOS and COX-2 [4]. Unlikeness renal TNF-α mRNA is increased after only 30 min of ischemia, and the TNF-α transcription factor, nuclear factor kappa B (NFκB) is activated after 15 min of ischemia [13].

The present study demonstrated that MSM efficiently suppressed renal dysfunction. Both renal function tests were relatively better in MSM group, in addition, the macroscopic and histological scores. Taken together, these results provide strong evidence for the curative effect of MSM in glycerol-induced ARF. The effect is probably due to the anti-inflammatory effect of MSM. This may provide therapeutic opportunities for treating myoglobinuric ARF in humans. To our knowledge, this is the first study to show that MSM healing ARF.

CONCLUSION

These findings show novel beneficial effects of MSM on ARF injury. Demonstrated that 400 mg/kg of MSM efficiently suppressed renal dysfunction and tissue injury. Renal function tests and histopathologic scores were relatively better in MSM group.

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