

Antioxidant Properties of Magnesium Sulfate in Rheumatoid Arthritis Patients: A Tertiary Hospital Based Experimental Study

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is an autoimmune illness that causes chronic inflammation and deformity in the joints. Oxidative stress is a crucial part of its pathophysiology. The aim of the study was to find out the antioxidant properties of Magnesium Sulfate in RA patients. **Methods:** This experimental study was carried out in the Institute of Biological Sciences (IBSc), University of Rajshahi. A total of 30 clinically diagnosed RA patients were enrolled from Department of Physical Medicine, Rajshahi Medical College and Hospital, Rajshahi during July, 2021 to June, 2022. Study subjects were divided into two groups: 15 patients were treated with conventional medicine e.g. Methotrexate, Sulfasalazine, NSAIDS and Hydroxychloroquine (control group) and 15 patients treated with conventional medicine plus Magnesium Sulfate (experimental group). Then antioxidant properties of Magnesium Sulfate was observed in experimental group. **Results:** Most the RA patients were female (53.33%) and within 40-50 years of age group (66.67%). Magnesium Sulfate had the strongest antioxidant activity at a concentration of 0.6 mmolar concentration of solution by DPPH reduction assay. Mean MDA level was decreased (2.16 ± 1.43 $\mu\text{mol/L}$) and mean percentage of reduction of MDA level was significant ($p < 0.001$) in experimental group. Neutrophil and Lymphocyte count was also decreased. **Conclusion:** Magnesium Sulfate possesses antioxidant properties. So, it might be used in rheumatoid arthritis patients to reduce the oxidative stress.

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INTRODUCTION

Rheumatoid arthritis (RA) is a long-term inflammatory illness that causes bone erosion, cartilage degradation and synovial inflammation. These symptoms can cause joint deformity and functional impairment. Even with improvements in care, the precise cause of rheumatoid arthritis is still unknown. However,

oxidative stress has come to light as a crucial component of the pathophysiology of rheumatoid arthritis, aiding in the maintenance of tissue damage and inflammatory cascades.¹ Increased oxidative stress, reduced antioxidant levels and poor antioxidant defenses are all linked to the etiology of RA.² When reactive nitrogen species (RNS) and reactive oxygen

species (ROS) exceed physiological quantities, causing damage to plasma membrane lipids, proteins and nucleic acids.³ In fact, RA patients whole blood and monocytes showed a fivefold increase in mitochondrial ROS generation, suggesting that oxidative stress is a pathogenic feature of this disease.⁴ ROS are very reactive substances that have the ability to damage proteins, lipids and nucleic acids. As a result, they play a role in the etiology of a number of chronic illnesses including rheumatoid arthritis.⁵ As usually Methotrexate was used in treatment of rheumatoid arthritis. Methotrexate can lower the number of white blood cells in blood which increases the chance of getting an infection. It can also lower the number of platelets which are necessary for proper blood clotting. Damage to cells and inflammation are the results of oxidative stress which happens due to formation of ROS and antioxidant defense mechanisms are out of balance. Due to its possible antioxidant properties of Magnesium Sulfate which is a magnesium salt containing magnesium and sulfate ion and an essential mineral, plays a vital role in various bodily function specially those related to RA.⁶ Magnesium ion can scavenges free radicles and reduce oxidative stress in cell.⁷ Magnesium has been found to possess anti-inflammatory properties. In RA, chronic inflammation in the joints leads to pain, swelling and stiffness. Magnesium may help reduce inflammation by inhibiting the production of inflammatory molecules such as cytokines and C-reactive proteins. Magnesium also helps in the absorption of calcium and vitamin-D which are essential for bone health.⁸ In rheumatoid arthritis, there is a higher risk of bone loss and osteoporosis. Magnesium is known to play a role in stress reduction and relaxation. It helps to release of stress hormones such as cortisol and promotes the production of neurotransmitters (serotonin) which is responsible for relaxation. Magnesium has been suggested to have analgesic (pain relieving) effects.⁹ It may help modulate pain signals and reduce the perception of pain. In RA, magnesium may provide additional relief from joint pain when used in combination with conventional treatment approaches. In this study,

Magnesium Sulfate used as an antioxidant which reduce oxidative stress in RA patients.^{10,11} As Magnesium Sulfate solution has been used locally, it did not produce any harmful effect comparable to medication. Therefore, Magnesium Sulfate ($MgSO_4$) may be used only for palliative management. Hence Magnesium Sulfate therapy might be another beneficial option to the physicians.

There is still controversy about the antioxidant activities of $MgSO_4$ as mentioned above. So, the aim of this study was to find out the antioxidant properties of Magnesium Sulfate in rheumatoid arthritis patients.

METHODS

This experimental study was carried out at Institute of Biological Sciences, University of Rajshahi from July, 2021 to June, 2022. A total of 30 clinically diagnosed RA patients were included from Department of Physical Medicine, Rajshahi Medical College and Hospital, Rajshahi. They were divided into two groups: Control group (n-15) treated with conventional medicine (Methotrexate, Sulfasalazine, NSAIDS and Hydroxychloroquine) and experimental group (n-15) treated with conventional medicine plus Magnesium Sulfate. Thirty (30) grams of magnesium sulfate added to one liter of lukewarm water. Hot compression was given at the site of pain by dipped washcloth in this water for 20 minutes at bedtime, twice a week for 8 weeks.¹² The American College of Rheumatology's (ACR) classification criteria for RA were used to select the study subjects. Patients with renal and hepatic failure, CVD, myocardial infarction, history of major surgery and magnesium sulphate contraindicated were excluded from this study.

Study approval was taken from ethics committee of the Institute of Biological Sciences, University of Rajshahi, Bangladesh. After taking informed consent, relevant data were collected and recorded in a preformed data sheet. Data were analyzed by using the statistical package for Social Sciences (SPSS) software, version-24. Categorical variables were summarized by using numbers and percentages while continuous

variables were summarized by means ± standard deviation (SD). Chi-square test was applied to see the relationship of age and educational status between the experimental and control groups. Unpaired t test was used to compare percentage of reduction of Malondialdehyde level and adverse events between the two groups. A *p*-value <0.05 was considered statistically significant for all tests.

Determination of antioxidant activity of Magnesium Sulfate (MgSO₄):

Antioxidant activity of MgSO₄ was determined by means of the test using DPPH (2, 2-diphenyl-1-picryl-hydrazil) radicals, which was reflected by the reduction of the absorbance of the DPPH methanol solution during the reaction with the tested solution MgSO₄. At first 0.1 mM solution of DPPH was prepared using 2mg of DPPH dissolved in 50ml of absolute methanol and thus the stock solution was prepared. DPPH (2,2-Diphenyl-1 Picrylhydrazil) is a stable free with red colour (absorbed at 517nm), if free radicals have been scavenged, DPPH was generated its colour to yellow. This assay used this character to show free radical scavenging activity.

At first 0.1 mM solution of DPPH was prepared using 3.8 mg of DPPH dissolved in 100 ml of absolute Methanol and thus prepared the stock solution 0.6 mmol/L MgSO₄ solutions.

Then various dilution of tested stock solution were prepared in the range of 0.6 mmol/L-1ml/L.

Changed in the absorbance intensity were measured using the spectrophotometer (Model 340). Changed in the absorbance intensity were measured using the spectro photometer (Model 340). In the test tubes (protected from light) 0.3 ml of tested solution and 2ml of the DPPH reagent at the concentration of 0.1 mM were added. The DPPH reagent was prepared 24 hours in advance (protected from light). After shaking the solutions in the test tubes, these were kept in the dark for 30 minutes then the absorbance was read, at the wavelength of 515 nM water (0.1ml) and methanol (0.7ml) were used as reference. Before the measurement of the absorbance of sample, the absorbance of DPPH solution was measured. The measurement was made by measuring the absorbance of 0.1ml of deionized water and 0.7 ml of DPPH radicals was water and 0.7 ml of DPPH radicals was calculated based on the formula:

$$Aa = \left(\frac{A_0 - A_i}{A_0} \right) \times 100\%$$

Where *Aa* means antioxidant activity (%), *Ai* means average absorbance of the tested solution and *A0* means average absorbance of the DPPH solution.

Determination of antioxidant activity of Magnesium Sulfate (MgSO₄) by DPPH Blank:

Number of the sample	OD on 9/3/21	OD on 10/3/21	OD on 11/3/21	OD on 18/3/21	OD on 21/3/21	OD on 23/3/21
0.6 mmolar solution	65%	65%	65%	63%	65%	56%
0.7 mmolar solution	65%	65%	65%	60%	60%	55%
0.8 mmolar solution	63%	63%	63%	56%	60%	54%
0.9 mmolar solution	63%	63%	63%	54%	50%	52%
1 molar solution	52%	61%	52%	45%	48%	50%

RESULTS

Most of the patients were primary educated, female and within 40 to 50 years of age in both groups. There were not statistically significant

(*p*>0.05) difference between the experimental and control groups in terms of age, gender, marital status and educational status (Table I).

Table I: Demographic distribution of the patients (n-30)

Variables	Experimental group (n-15)	Control group (n-15)	p-value
Age (In year)			
<40	2 (13.33%)	4 (26.67%)	>0.05
40-50	10 (66.67%)	9 (60.00%)	
>50	3 (20.00%)	2 (13.33%)	
Total	15 (100%)	15 (100%)	
Sex			
Male	05 (46.67%)	6 (40%)	>0.05
Female	8 (53.33%)	9(60%)	
Total	15(100%)	15(100%)	
Marital Status			
Married	15 (100.00%)	14 (93.30%)	>0.05
Unmarried	0 (0%)	1 (6.70%)	
Educational Status			
Primary	5 (33.30%)	7 (46.70%)	>0.05
Secondary	3 (20.00%)	2 (13.30%)	
Higher secondary	3 (20.00%)	2 (13.30%)	
Graduation	3 (20.00%)	3 (20.00%)	
Masters	1 (6.70%)	1 (6.70%)	

*Data were analyzed by Chi-square test

After two months of treatment with MgSO₄, MDA level was reduced in RA patients (Table II).

Table II: Level of MDA in the study subjects

MDA level (μmol/L)	Experimental group (n-15)	Control group (n-15)
Base line	3.89±2.07	3.36±0.93
After two months of treatment	2.16±1.43	3.19±0.77

Percentage of mean Malondialdehyde level was significantly reduced in experimental group than the control group (p <0.001) (Table III).

Table III: Percentage of mean Malondialdehyde level reduction of from baseline to two months after drug administration

Variable	Experimental group(n-15)	Control group(n-15)	p-value
Percentage of reduction of Malondialdehyde level	45.77±16.33	19.33±14.39	<0.001

*Data were expressed as mean±SD; independent t test was used.

Neutrophil and lymphocyte count were significantly decreased in experimental group compared to control group (Table IV).

Table IV: Changes of hematological and biochemical parameters of the patients

Parameters	Experimental group (n-15)	Control group (n-15)	p-value
Neutrophil (%)	66.80±3.76	71.27±5.79	0.01
Lymphocyte (%)	23.60±4.61	27.87±3.42	0.008
Eosinophil (%)	2.93±0.79	3.27±1.1	> 0.05
Monocyte (%)	1.87±0.92	2.07±0.70	> 0.05
ESR mm/hr.	44.20±3.89	46.53±5.62	> 0.05
SGPT U/L	27.13±11.82	31.60±13.18	> 0.05
Serum creatinine (mg/dl)	0.88±0.21	1.05 ± 0.24	0.03

#Data were analyzed using independent t test and were expressed as mean±SD.

Antioxidant activity of magnesium sulfate ($MgSO_4$) by DPPH reduction assay revealed that at 0.6 mmolar concentration level antioxidant activity of magnesium sulfate was highest (Table V).

Table V: In vitro determination of antioxidant activity of Magnesium Sulfate ($MgSO_4$) by DPPH reduction assay

Concentration of solution	Antioxidant activity (%)
0.6 mmolar solution	63.17±3.60
0.7 mmolar solution	61.67±4.08
0.8 mmolar solution	59.83±3.97
0.9 mmolar solution	57.50±6.16
1 molar solution	51.33±5.43

DISCUSSION

Rheumatoid arthritis (RA) is the most common form of chronic inflammatory arthritis and often results in joint damage, physical disability and oxidative stress is an important factor behind it. $MgSO_4$ is useful in reducing oxidative stress of RA patients. Most (10, 66.67%) of the patients were within 40 to 50 years in experimental group. This result is almost similar with the study conducted by Stalker et al.¹², Sankar¹³ and Deshmukh et al.¹⁴ Rheumatoid arthritis was once considered a younger person's disease but it can develop at any age. It is an autoimmune disease usually associated with smoking and obesity. Rheumatoid arthritis is common in female (53.33%) than male. Deshmukh et al.¹⁴ reported 60% patients were female. Female Immune Cells Produce More Inflammatory Cytokines like interferon-alpha and interleukin-17. These play crucial role for joint inflammation involved in rheumatoid arthritis. In contrast, the main male sex hormone testosterone increases anti-inflammatory cytokines.

In this study, mean MDA level was decreased in experimental group which was treated by magnesium sulfate plus conventional medicine. Similar finding was also found by Nour-mohammadi.¹⁵ MDA is an important biomarker of oxidative stress. This study revealed that, after two months of drug administration, mean percentage of reduction of MDA level was significantly higher in experimental group than the control group ($p < 0.001$). It indicated that Methotrexate+Magnesium Sulfate combination was very effective in comparison to only Methotrexate for rheumatoid arthritis patients. Vasanthi et al.¹⁶ reported that, MDA level was significantly reduced in RA patients treated with Magnesium Sulfate with conventional therapy. Similar results were observed by Wypartó-Wszelaki et al.¹⁷ Kalavacherla et al.,¹⁸ Gambhir et al.¹⁹ and Taraza et al.²⁰ $MgSO_4$ reduces the oxidative stress by lowering the serum MDA level in RA patients. It is apparent that patients of RA are exposed to oxidative stress and are more

prone to lipid peroxidation.²¹ Increase free radical causes overproduction of MDA which is well known marker of oxidative stress. This oxidative stress generated within an inflamed joint can produce connective tissue destruction leading to joint and periarticular deformities in rheumatoid arthritis.^{22,23} After administration of MgSO₄ containing hot water compression, neutrophil and lymphocyte count were significantly decreased in RA patients. It indicates the reduction of inflammation and oxidative stress. Antioxidant activity of Magnesium Sulfate (MgSO₄) by DPPH reduction assay revealed that, antioxidant activity was highest at 0.6 mmolar concentration. This finding is consistent with the study conducted by Jaswal et al.²⁴ There were several limitations such as sample size was only 30, purposive sampling technique was used and data were taken from only one center.

CONCLUSION

In rheumatoid arthritis patients, magnesium sulfate causes significant reduction of Malondialdehyde level. So, it might be used to treat oxidative stress in rheumatoid arthritis patients.

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