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Anti-Inflammatory Activity of Mono Ammonium Glycyrrhizinate Liquorice in Rheumatoid Arthritis against Complete Freund's Adjuvant Induced Arthritis in Female Albino Wistar Rats

Eswar Kumar K.¹ and Nagabharathi M.^{2*}

¹Andhra University College of Pharmacy, Vishakhapatnam, Andhra Pradesh, India

²Vignan Institute of Pharmaceutical Technology, Duvvada, Vishakhapatnam, India

*Corresponding Author

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Abstract: The present study was conducted to evaluate the potential of mono ammonium glycyrrhizinate liquorice root extract in rheumatoid arthritis and explore its possible mechanism of action. Paw and sciatic nerve samples were used to estimate total protein, catalase enzyme and GSH (Glutathione S-Transferase). Significant ($p < 0.01$) reduction in protein content, catalase enzyme and Reduced Glutathione (GSH) clearly indicate that mono ammonium glycyrrhizinate has anti-inflammatory property. The present study suggests that mono ammonium glycyrrhizinate shows protective activity in CFA-induced arthritis, which may be due to its improved haematological properties as well as its antioxidant and anti-inflammatory properties.

Keywords: Anti-inflammatory activity, Mono Ammonium Glycyrrhizinate, Rheumatoid Arthritis, Complete Freund's adjuvant, Protein, Catalase, Glutathione S-Transferase

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that primarily targets the joints, causing inflammation, pain, and potentially joint damage (Lee *et al.*, 2017). It is characterized by the fact that the immune system mistakenly attacks the synovial membrane, the membrane that surrounds the joints. RA often affects multiple joints and its symptoms include joint pain,

stiffness, swelling, and fatigue. (Silman and Pearson, 2002). Early diagnosis and treatment are important to manage RA and prevent joint damage. Although the exact cause is not fully understood, a combination of genetic, environmental and hormonal factors is believed to contribute to its development (Van der Linden *et al.*, 2010). RA can have a significant impact on a

Table 1: Experimental design

Groups	Treatment	Dose mg
Group I	Normal control (NC)	none
Group II	Disease Control (DC)	CFA 100 μ l (5 mg/ml)
Group III	CFA + Low dose of Test compound	CFA+50 mg/kg
Group IV	CFA + High dose of Test compound	CFA+100 mg/kg

person's daily life and may require lifestyle modifications and on-going medical care for effective management. Mono-ammonium glycyrrhizinate, commonly abbreviated as MAG, is a chemical compound derived from the root of the licorice plant (*Glycyrrhiza glabra*), a plant native to parts of Europe and Asia. This compound stands out for its unique properties and various applications in the pharmaceutical and food industries. Mono ammonium glycyrrhizinate is a natural compound extracted from licorice root (Moura *et al.*, 2015). Licorice has been used for centuries in traditional medicine for its potential health benefits. MAG usually appears as a white or slightly yellow powder. It has a sweet taste similar to liquorice, making it suitable for use as a flavouring agent. (Wang *et al.*, 2023). The present study was conducted to evaluate the potential of mono ammonium glycyrrhizinate liquorice root extract in rheumatoid arthritis and explore its possible mechanism of action. Paw and sciatic nerve samples were used to estimate total protein, catalase enzyme and GSH (Glutathione S-Transferase).

Materials and Methods

Plant Material:

Mono Ammonium Glycyrrhizinate powdered roots (mesh size 250) have been supplied by Pharmaceutical shop, Jadavji Lallubhai and Co., Hyderabad, India.

Experimental Animals:

Animals were obtained from the Mahaveer Enterprises, Hyderabad. Adult female Wistar rats, (200-210 g) were used in the present study.

Animals were maintained under standard laboratory conditions (12:12 h light/darkness; at 23 \pm 1°C) with standard animal diet and water available *ad libitum*. Our Institute was approved by CPCSEA for conducting animal experiments with the registration number: 516/01/A/CPCSEA.

Experimental Design:

Before the experiment, the rats were acclimatized to the laboratory conditions for a period of two weeks, and then rats were kept fasted for 18 h prior to the experiment with water *ad libitum*. Rats were randomly divided into four groups of six animals. Group I rats were normal control group, Group II were disease control, Group III were treatment group 1 (low dose of test compound), and Group IV were treatment group 2 (High dose of test compound) (Table 1).

Dose selection:

The acute oral toxicity study was done according to OECD426 Guidelines. The studies were conducted on female albino Wistar rats weighing between 25-35g and were divided into 4 groups containing 6 rats each. They were fasted overnight and maintained with water *ad libitum*. The selected animals were administered at a dose level of 2000 mg/kg body weight.

Evaluation of Rheumatoid Arthritis:

Paw and sciatic nerve samples were used to estimate all parameters. The tissues were weighed and homogenate prepared by using the TWEEN buffer pH 8. Prepared 10% homogenate.

(i) Protein was estimated by the method of Lowry *et al.* (1955).

Table 2: Calibration Curve of Bovine Serum Albumin (BSA)

BSA Concentration	Protein Estimation (μg)			
	Set 1	Set 2	Mean	Mean after blanking
blank	0.060	0.056	0.058	-
40 μl	0.125	0.128	0.068	0.068
80 μl	0.186	0.187	0.128	0.064
120 μl	0.285	0.234	0.181	0.060
160 μl	0.299	0.284	0.233	0.058
200 μl	0.342	0.349	0.287	0.057

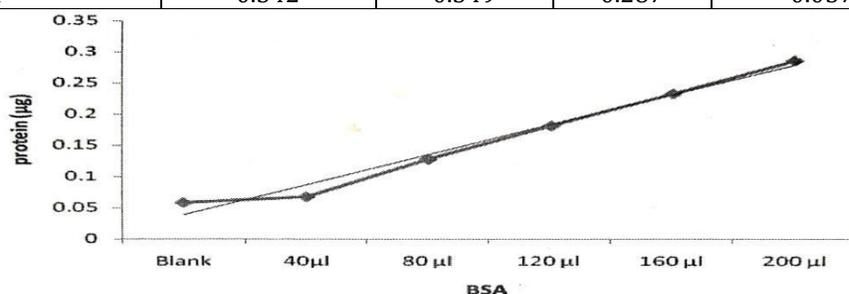


Fig. 1: standard curve of maltose for protein estimation.

Table 3: Effect of MAG on total soluble protein in sciatic nerve of CFA induced rheumatoid arthritis in albino Wistar rats

Groups	Protein (mg/dl) (sciatic nerve)						Mean \pm SEM
	R1	R2	R3	R4	R5	R6	
Control	135.4	143.05	141.17	120.5	140.9	145.3	137.72 \pm 3.69
Disease control (CFA)	70.5	82.3	58.8	65.7	58.8	73.9	68.33 \pm 3.73###
MAG (50 mg/kg)	95.2	115.8	110.7	98.5	115.9	118.5	109.1 \pm 4.03*
MAG (100 mg/kg)	115.5	135.7	130.8	115.7	120.7	131.3	124.95 \pm 3.57***

All values represented MEAN \pm SEM, n=6, ***P<0.001, **P<0.01, *P<0.05 and ^{ns}P<0.05 when compared to Disease Control and ###p<0.001 when disease control compared with Control Group, using one-way ANOVA-Dunnett's multiple comparison test.

(ii) Catalase activity was measured using the method of Aebi et al. (1974) Activity of catalase was expressed as moles of H₂O₂ metabolized/mg protein/min.

(iii) Estimation of Reduced Glutathione (GSH) was done by Glutathione colorimetric assay method.

Statistical analysis:

All data are represented as mean \pm SEM. Statistical analysis was performed using Graph Pad Prism software, 6.0 utilizing One-way ANOVA followed by Dunnett's multiple comparison tests. P value <0.001 was considered significant.

Results

For protein estimation, maltose standard curve was plotted using two sets (Fig. 1).

Effect of MAG on total soluble protein in sciatic nerve:

The animals in control group showed normal values of total soluble protein. Sciatic nerve samples of disease control show little decrease in protein content, whereas MGA (50 mg/kg and 100 mg/kg) showed significantly (p<0.001) raised level of protein than disease control (Table 3).

The animals in control group showed normal

Table 4: Effect of MAG on total soluble protein in paw of CFA induced rheumatoid arthritis in albino Wistar rats

Groups	Protein (mg/dl) (paw)						Mean ± SEM
	R1	R2	R3	R4	R5	R6	
Control	350	308.8	367.6	426.4	485.2	320.3	376.38±27.58
Disease control (CFA)	120	164.7	162.3	110.8	172.3	157.5	147.93±10.53###
MAG (50 mg/kg)	226.4	294.1	217.6	210.9	182.7	220.9	225.43±15.09**
MAG (100 mg/kg)	282.3	256.4	230.6	250.3	298.2	250.7	261.41± 10.00***

All values represented Mean±SEM, n=6, ***P<0.001, **P<0.01, *P<0.05 and ^{ns}P<0.05 when compared to Disease Control and ###p<0.001 when disease control compared with Control Group, using one-way ANOVA-Dunnett's multiple comparison test.

Table 5: effect of MAG on total soluble protein in sciatic nerve & paw of CFA induced rheumatoid arthritis in albino Wistar rats

Groups	Soluble protein (mg/dl)	
	Mean ±SEM	
	Sciatic nerve	paw
Control	137.72±3.69	376.38±27.58
Disease control (CFA)	68.33±3.73###	147.93±10.53###
MAG(50 mg/kg)	109.1±4.03*	225.43±15.09**
MAG(100 mg/kg)	124.95±3.57***	261.41±10.00***

All values represented Mean±SEM, n=6, ***P<0.001, **P<0.01, *P<0.05 and ^{ns}P<0.05 when compared to Disease Control and ###p<0.001 when disease control compared with Control Group, using one-way ANOVA-Dunnett's multiple comparison test.

Table 6: effect of MAG on catalase in sciatic nerve of CFA induced rheumatoid arthritis in albino Wistar rats

Groups	Protein (mg/dl) (sciatic nerve)						Mean ± SEM
	R1	R2	R3	R4	R5	R6	
Control	6.1	6	5.8	5.8	5.6	6.3	5.93±0.10
Disease control (CFA)	2.9	2.1	3.5	2	3.3	2.5	2.71±0.25###
MAG (50 mg/kg)	3.8	3.5	4.3	3.8	4.5	3.9	3.96±0.14**
MAG (100 mg/kg)	5.2	3.5	4.7	4.5	5.2	4.1	4.53± 0.26***

All values represented MEAN±SEM, n=6, ***P<0.001, **P<0.01, *P<0.05 and ^{ns}P<0.05 when compared to Disease Control and ###p<0.001 when disease control compared with Control Group, using one-way ANOVA-Dunnett's multiple comparison test.

values of total soluble protein. Sciatic nerve and paw samples of disease control show minor decrease in protein content, whereas MGA (50

mg/kg) and MGA (100 mg/kg) showed significantly (p<0.001) raised level of protein than disease control (Table 4).

Table 7: Effect of MAG on catalase in paw of CFA induced rheumatoid arthritis in albino Wistar rats

Groups	Protein (mg/dl) (sciatic nerve)						Mean ± SEM
	R1	R2	R3	R4	R5	R6	
Control	5.9	5.9	6.5	5.5	6.8	7.5	6.35±0.29
Disease control (CFA)	2.9	2.5	3.5	2.2	3.9	2	2.83±0.30###
MAG (50 mg/kg)	4.9	3.9	4.5	3	4.4	3.8	4.08±0.27**
MAG (100 mg/kg)	5.5	4.5	6	4.3	5.7	5.3	5.21± 0.27***

All values represented MEAN±SEM, n=6, ***P<0.001, **P<0.01, *P<0.05 and ^{ns}P<0.05 when compared to Disease Control and ###p<0.001 when disease control compared with Control Group, using one-way ANOVA-Dunnett's multiple comparison test.

Table 8: Effect of MAG on Catalase in paw and sciatica of CFA induced rheumatoid arthritis in albino Wistar rats

Groups	Catalase (µmol/mg of protein)	
	Sciatic nerve	paw
Control	6.35±0.29	0.52±0.03
Disease control (CFA)	2.83±0.30###	1.27±0.19###
MAG(50mg/kg)	4.08±0.27**	0.69±0.01**
MAG(100mg/kg)	5.21±0.27***	0.43±0.02***

All values represented MEAN±SEM, n=6, ***P<0.001, **P<0.01, *P<0.05 and ^{ns}P<0.05 when compared to Disease Control and ###p<0.001 when disease control compared with Control Group, using one-way ANOVA-Dunnett's multiple comparison test.

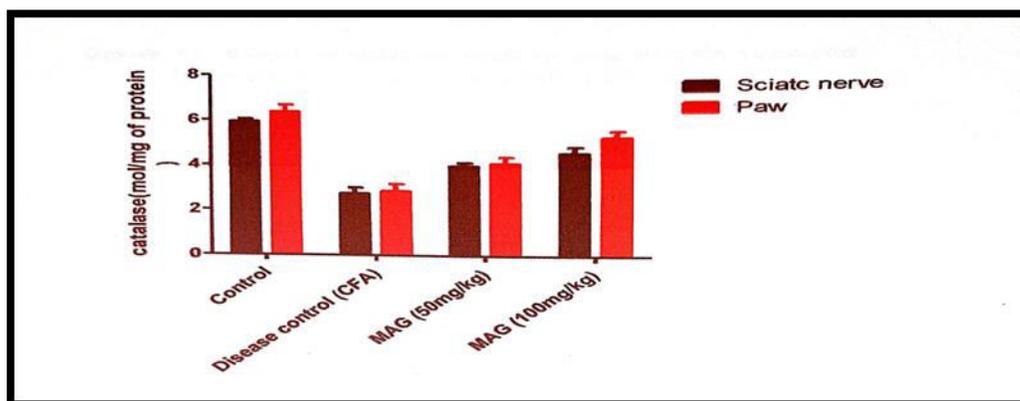


Fig. 2: Effect of MAG on catalase in paw & sciatica of CFA induced rheumatoid arthritis in albino Wistar rats.

The animals in control group showed normal values of catalase enzyme. Sciatic nerve and paw samples of disease control show minor decrease in catalase content, whereas MGA (50 mg/kg) and MGA (100 mg/kg) showed significantly (p<0.001) raised level of catalase enzyme than disease control (Tables 6, 7; Fig. 2).

The animals in control group showed normal values of GST level. Sciatic nerve and paw samples of disease control show decrease in GSH content, whereas MGA (50 mg/kg) and MGA (100 mg/kg) showed significantly (p<0.001) raised level of GSH than disease control (Tables 8, 9; Fig. 3).

Table 9: effect of MAG on GSH in paw of CFA induced rheumatoid arthritis in albino Wistar rats

Groups	GSH ($\mu\text{mol}/\text{min}/\mu\text{g}$) (sciatic nerve)						Mean \pm SEM
	R1	R2	R3	R4	R5	R6	
Control	2.53	2.6	2.4	2.32	2.62	2.6	2.51 \pm 0.05
Disease control (CFA)	0.9	0.7	1.1	0.9	0.11	1	0.785 \pm 0.14###
MAG (50 mg/kg)	1.4	1	1.9	1.2	1.2	1.7	1.4 \pm 0.13**
MAG (100 mg/kg)	1.9	2.5	2.1	1.9	2.2	2.4	2.16 \pm 0.102***

All values represented MEAN \pm SEM, n=6, ***P<0.001, **P<0.01, *P<0.05 and ^{ns}P<0.05 when compared to Disease Control and ###p<0.001 when disease control compared with Control Group, using one-way ANOVA-Dunnett's multiple comparison test.

Table 10: Effect of MAG on GSH in sciatica of CFA induced rheumatoid arthritis in albino Wistar rats

Groups	GSH ($\mu\text{mol}/\text{min}/\mu\text{g}$) (sciatic nerve)						Mean \pm SEM
	R1	R2	R3	R4	R5	R6	
Control	2.53	2.6	2.4	2.32	2.62	2.6	2.51 \pm 0.05
Disease control (CFA)	0.9	0.7	1.1	0.9	0.11	1	0.785 \pm 0.14###
MAG (50 mg/kg)	1.4	1	1.9	1.2	1.2	1.7	1.4 \pm 0.13***
MAG (100 mg/kg)	1.9	2.5	2.1	1.9	2.2	2.4	2.16 \pm 0.102***

All values represented MEAN \pm SEM, n=6, ***P<0.001, **P<0.01, *P<0.05 and ^{ns}P<0.05 when compared to Disease Control and ###p<0.001 when disease control compared with Control Group, using one-way ANOVA-Dunnett's multiple comparison test.

Table 11: Effect of MAG on GSH in sciatica& paw of CFA induced rheumatoid arthritis in albino Wistar rats

Groups	GSH ($\mu\text{mol}/\text{mg}$ of protein)	
	Sciatic nerve	paw
Control	2.51 \pm 0.05	2.81 \pm 0.30
Disease control (CFA)	0.785 \pm 0.14###	0.62 \pm 0.08###
MAG(50mg/kg)	1.4 \pm 0.139**	1.28 \pm 0.08***
MAG(100mg/kg)	2.16 \pm 0.102***	0.43 \pm 0.08***

All values represented MEAN \pm SEM, n=6, ***P<0.001, **P<0.01, *P<0.05 and ^{ns}P<0.05 when compared to Disease Control and ###p<0.001 when disease control compared with Control Group, using one-way ANOVA-Dunnett's multiple comparison test.

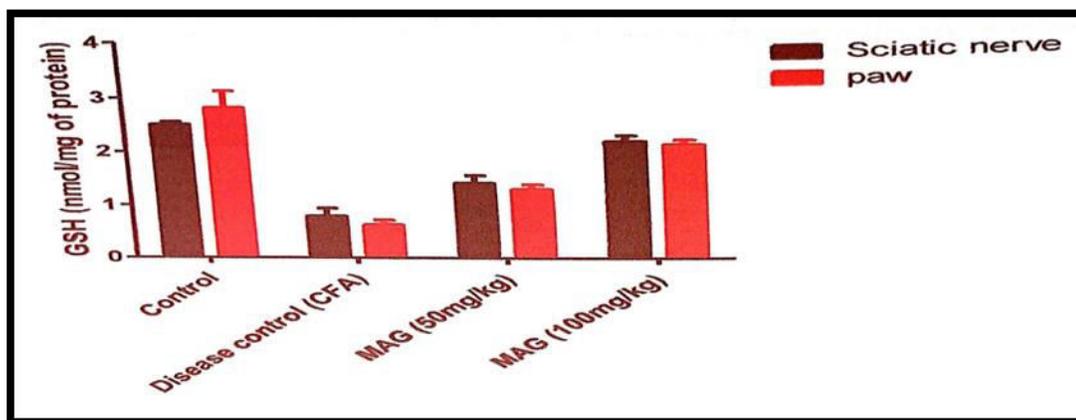


Fig. 3: Effect of MAG on GSH in sciatica & paw of CFA induced rheumatoid arthritis in albino Wistar rats.

Discussion

Rheumatoid arthritis (RA) is an abnormal autoimmune disease that causes deformity, and is characterized by chronic inflammation and joint structure damage. CFA has recently become a popular tool to observe the efficacy of herbal medicines for treating arthritis. Free radicals are indirectly implicated in joint damage because they also play an important role as secondary messengers in inflammatory and immunological cellular responses in RA. The total ROS, H_2O_2 , O_2^- , and OH were higher in RA patients regardless of the sample used-cell exposure to increased oxidative stress becomes refractory to several stimuli including those for growth and death and may perpetuate the abnormal immune response (Hassan *et al.*, 2011). SOD, CAT, GSH, is considered as an important antioxidant defense system in the body. It functions as an intracellular reductant in redox reactions taking place in the human body. It protects cellular components from damage caused by ROS. Low concentration of GSH found in the plasma of RA patients is in agreement with some other studies as well. (Dalle-Donne *et al.*, 2003) GSH content has also been reported to be reduced in the liver of arthritic rats. On the other hand, free radicals can degrade directly the joint cartilage, attacking its proteoglycan and inhibiting its synthesis, oxidative damage of hyaluronic acid and lipid peroxidation products and oxidation of low-density lipoproteins and carbonyl

increment resulting from protein oxidation have also been demonstrated in RA as well as DNA damage. (Hadjigogos *et al.*, 2003)

Conclusion

Significant elevations of CAT and GSH were observed with MAG 100 mg/kg treatment in CFA induced arthritis in rats (paw and sciatic nerve). The mono ammonium glycyrrhizinate showed protective activity in CFA induced arthritis which might be due to their enhanced haematological profile, antioxidant and anti-inflammatory activity.

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