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Research Article

# EFFECT OF HIGH DOSES OF ROSUVASTATIN IN MIA-INDUCED OSTEOARTHRITIS

**Jyoti Bajaad, Rajvinder Kaur, Krishna Reddy V. Bijjem***

Department of pharmacology, ISF college of pharmacy, Moga-142001,

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## ABSTRACT

*Statins exert favorable effects on hyperlipidemia but may also possess anti-inflammatory effects. Here, we explored the effects of rosuvastatin in a model of Monosodium iodoacetate-induced arthritis in rat. Palmer et al. were unable to show any preventive effect using oral atorvastatin or subcutaneous rosuvastatin in murine collagen model. So the present study was designed to check effect of rosuvastatin at various doses in MIA –induced osteoarthritis.*

***Key Words:*** *Hyperlipidemia, Rosuvastatin, MIA –induced osteoarthritis, Anti-inflammatory effects*

## INTRODUCTION

Osteoarthritis (OA) is a "condition characterized by focal areas of loss of articular cartilage within the synovial joints, associated with hypertrophy of the bone (osteophytes and subchondral bone sclerosis) and thickening of the capsule"[1]. The American College of Rheumatology (ACR) defined osteoarthritis as a heterogeneous group of conditions that leads to joint signs and symptoms which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone at the joint margins [2]. About 80% to 90% of individuals of both sexes have evidence of OA by the age of 65 yrs [1]. A pan-European survey showed that one in five adults is suffering from chronic pain and among those; osteoarthritis was the primary cause of pain in more than 30% of respondents [3].

*Corresponding author:
**Dr B.V.Krishan.Reddy**
M. Pharm., PhD.Associate professor
Department of pharmacology,
**ISF college of pharmacy, Moga-142001,**
Phone: +91-9501930177
Fax: +91-1636236564
jyotibajaad@gmail.com

### *Pathophysiology of Osteoarthritis*

Cartilage is constantly remodelling itself in a process of autolysis and repair that is mediated by chondrocytes. In OA, the balance of remodelling may first get misbalanced because of a biomechanical factor, a genetic factor, or another unidentified factor (idiopathic cause). As the process advances, the newly formed cartilage is thinner and weaker than normal cartilage. This weaker cartilage allows greater mechanical stress to be transmitted to subchondral bone, causing microfractures and other bony trauma. It also transmits increased pressure to the chondrocytes, which release additional degrading mediators, such as proinflammatory cytokines ( interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α))[4], that impact the cartilage matrix by altering chondrocyte metabolism and ultimately affecting all the cmponents of the joint, including the subchondral bone, synovium, meniscus, ligaments, and supporting neuromuscular apparatus. It has been deduced from the recent studies that the protease(s) are responsible for initiating matrix

degradation. The involvement of MMP such as collagenase-1 (MMP- 1), stromelysin-1 (MMP-3), gelatinase 92 kd (MMP-9), collagenase-3 (MMP-13) andaggrecanase, might be responsible for the proteolysis of cartilage proteoglycan (aggrecan) [5-6] . MMP-13 is the most proficient at hydrolysing bonds in type II collagen, the most abundant collagen in articular cartilage [7-8].

Several post-receptor signaling pathways have also been implicated in the modulation of the proinflammatory cytokines and MMPs. Mitogen- activated protein kinases (MAPK) p38 and ERK 1/2, and nuclear factor-κB (NF-κB) appear to be the major pathways involved. The cartilage eventually becomes hypocellular. Remodelling and hypertrophy of bone also occur, leading to the bony sclerosis and development of osteophytes, which may alter the joint and restrict movement.

***Anti-inflammatory effect of statins***

Statins are a group of drugs that inhibit 3-hydroxy-methyl-glutaryl coenzyme A (HMG-CoA) reductase, the enzyme responsible for the conversion of HMG-CoA to mevalonate, the rate-limiting step in de novo cholesterol synthesis [9]. Therefore statins are most commonly used drugs in the treatment of dyslipidemia and prevention of coronary artery disease [10,11-12].

Statins inhibit not only the biosynthesis of cholesterol but also of isoprenoid intermediates such as geranyl-geranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP). GGPP and FPP attachments for the post-translational modification (isoprenylation) of several proteins, including the small GTP-binding proteins Ras, Rac, and Rho. Isoprenylation is essential for activation and intracellular transport of proteins crucial for various cellular functions, such as maintenance of cell shape, motility, factor secretion, differentiation, and proliferation [13]. Most of the effects of statins, other than their lipid lowering activity have been correlated with their anti-inflammatory activity [14]. The anti-inflammatory effects of statins can result from their capacity to interfere with the mevalonate pathway and inhibit prenylation of Rho family GTPases[15,16-20]. Statins have been recognized as anti-inflammatory drugs since the first clinical observation of pravastatin decreasing the incidence of severe acute rejections, therefore, significantly improving the 1-year survival in heart transplant recipients [21]. Various findings suggest that statins also exert anti-inflammatory by regulating the immune system. The immunomodulatory properties of statins could be exerted through interference in the expression and function of a variety of immune relevant molecules. Fluvastatin and simvastatin have recently been shown to inhibit MMP-9 (gelatinase B) activity and secretion by macrophages [22].This effect is reversed by the addition of mevalonate, suggesting that it is mediated by HMG CoA reductase inhibition. Fluvastatin appears to decrease MMP-1 expression in human vascular endothelial cells. This effect is also seen with lovastatin and again is completely blocked by co-incubation with mevalonate [23]. In a collagen-induced arthritis model in mice, Palmer et al. were unable to show any preventive effect using oral atorvastatin or subcutaneous rosuvastatin.

## MATERIAL AND METHODS

***Drugs and Chemicals***

Monosodium iodoacetate (MIA) a product of Sigma Aldrich Ltd, St. Louis, USA. Rosuvastatin a product of Ranbaxy Ltd., Gurgaon, Haryana, India; was prepared by dissolving it in normal saline. Rosuvastatin was administered once daily at doses (3,10 and 30 mg/kg, i.p.) starting from 24 hours before MIA and was continued for next 21 days.

***Animals and groups***

Age matched young male/female Wistar rats, weighing 180-250g, were used. Rats were fed on standard chow diet and water *ad libitum.* They were acclimatized in animal house and were exposed to 12 hour day/night cycle at the temperature of 25±2ºC. Animals were divided in 4 groups (n=6).

Group I: Vehicle treated monosodiumiodoacetate (MIA) control.

Group II: Effect of rosuvastatin (3 mg/kg) in MIA induced osteoarthritic rats.

Group III: Effect of rosuvastatin (10 mg/kg) in MIA induced osteoarthritic rats.

Group IV: Effect of rosuvastatin (30 mg/kg) in MIA induced osteoarthritic rats.

***Induction of Experimental Osteoarthritis***

On day 0, after clipping the hairs covering the patellar area of the right knee (ipsilateral, IL), animals were anaesthetised and placed in the supine position. After disinfecting the skin with a povidone–iodine solution, a single injection of 25 µl sterile 0.9% sodium chloride (NaCl, saline) containing 2 mg of monosodium iodoacetate (MIA, Sigma, UK) was administered through the patellar tendon (also known as the infra-patellar ligament) using a 27G needle.

***Assessment of mechanical allodynia (Von Frey test)***

The threshold for touch sensitivity was measured in both hind paws, using an automated apparatus for applying reproducible light touch (Dynamic plantar Aesthesiometer 37400-002; UgoBasile, Comerio, Italy). Animals were placed in their compartments on the metal mesh surface. After a short period, in which they showed exploratory behavior, they remained still in a resting position and at this time the test began. With the help of an adjustable angled mirror, the touch stimulator unit was placed beneath the selected hind paw to position the filament below the plantar surface of the animal. When the unit is started, the electrodynamic actuator lifts the stainless steel filament, which touches the plantar surface and begins to exert an upward force below the threshold of feeling. The force increases, until the animal moves its paw or until the point at which greatest present force is met. The maximum value of force in grams (45 g) was previously fixed.

***Assessment of Thermal Hyperalgesia***

Hyperalgesia to thermal stimulation was determined using a Plantar Test Apparatus (37370-002 UgoBasile, Comerio, Italy) modeled as described by Hargreaves et al. (1988). Rats were placed individually in Plexiglas cubicles mounted on a glass surface maintained at 25±2C. During this time, the rats initially demonstrated exploratory behavior, but subsequently stopped exploring and stood quietly with occasional bouts of grooming. A thermal stimulus, in the form of radiant heat emitted from a focused projection bulb, which was located under the glass floor, was focused onto the plantar surface of the right hind paw (ipsilateral paw), and paw withdrawal latencies (PWLs) were recorded. A cut-off latency of 20s was imposed to avoid tissue damage. PWLs were measured in duplicate for the right hind paw (contralateral paw) of each animal, and the mean of the two values was used for analysis. Those animals showing latencies equal to 20s were discarded. Finally, mean basal latencies of 13–15 s were also established [23-24]. The elapsed time until the brisk withdrawal of the hind paw from the thermal stimulus was recorded automatically using photodiode motor sensors.

***Assessment of the hind paw volume and paw thickness***

The paw volume, up to the ankle joint, was recorded before (day 0), and at pre-decided days for the MIA-treated group for the 21 day observation period. The paw volume in the treatment groups was recorded before (day 0), and at 3, 5, 7, 14 and 21 days after the administration of MIA and the drugs were administered from day 1 to day 21. Measurement of both the hind paws was taken so as to obtain the absolute increase in paw volume by using mercury plethysmogram (INCO, Ambala, India) . Paw thickness was determined using the calibrated digital calipers on day 0, 3, 5, 7, 14 and 21.

***Measurement of Mobility***

The scoring of mobility was performed by modifying the evaluation scale reported by Butler et al. (1992): score 6, walks normally; score 5, walks being protective toward the

ipsilateral hind paw (touches the ipsilateral hind paw fully on the floor); score 4, walks being protective toward the ipsilateral hind paw (touches only the toe of the ipsilateral hind paw on the floor); score 3, walks being protective toward both hind paws (touches the contralateral hind paw fully on the floor); score 2, walks being protective toward both hind paws (touches only the toe of the contralateral hind paw on the floor); score 1, crawls only using the fore paws; and score 0, does not move.

***Assesment of joint swelling***

The knee and ankle joint diameter was measured using calibrated digital calipers.

***Collection of blood samples in rats***

In this study, blood was collected on day 0 and and at the end of treatment schedule on day 21st for estimation of levels of total cholesterol, High density lipoprotein (HDL), Low density lipoprotein (LDL), Very low density lipoprotein (VLDL) and triglycerides in blood serum.

The total cholesterol and HDL was estimated by cholesterol oxidase peroxidase CHOD-POD method using commercially available kit (Coral Clinical System, Goa, India). The serum triglyceride was estimated by glycerophosphate oxidase peroxidase GPO-PAP method using commercially available kit (Coral Clinical System, Goa, India).

***Histological examination***

On 21st day after MIA injection, rats were over-anaesthetized with phenobarbitone and sacrificed. Knee joint was removed and fixed in 10% neutral buffered formalin, decalcified by 10% formic acid and embedded in paraffin. 5 µm sections of femorotibial joint was stained by hematoxylin and eosin [25].

## RESULTS

**Effect of rosuvastatin (3, 10 and 30 mg/kg) on mechanical hyperalgesia and thermal hyperalgesia in MIA-induced osteoarthritis in Wistar rats**

An intra-articular injection of MIA (2mg) significantly decreased the mechanical threshold (MT) and paw withdrawal latency (PWL) by the 3rd days, which then remained constant during the 21 days observation period. However, treatment with rosuvastatin (3 and 10mg/kg, i.p) significantly increased the mechanical threshold and PWL in a dose-dependent manner as compared to MIA control group. In contrast, treatment with rosuvastatin 30mg/kg, i.p, did not significantly increase MT and PWL as compared to MIA control group (Fig. 1 and 2)

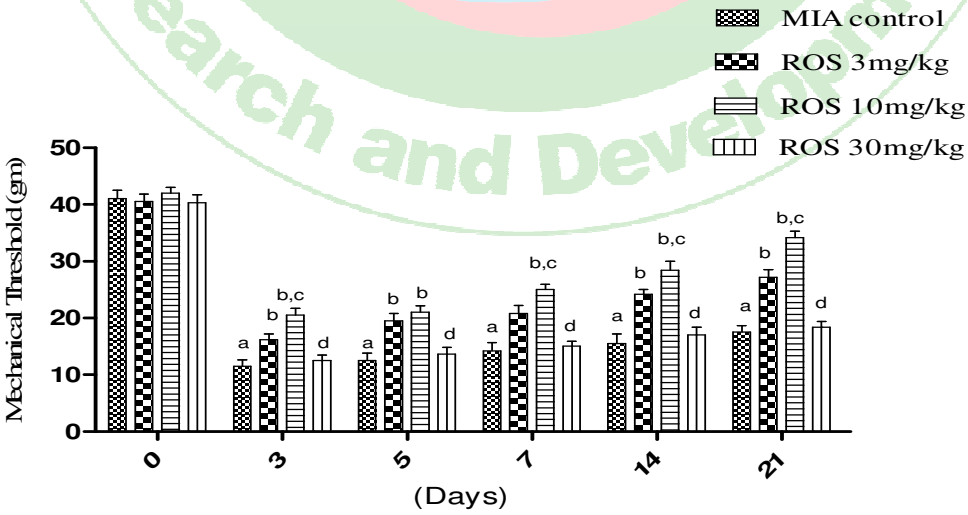

**Fig.1 Effect of various doses of rosuvastatin on MIA induced mechanical hyperalgesia in Wistar rats.** All values are expressed as mean± SEM. a=p<0.05 vs day 0, b=p<0.05 vs MIA Control, c=p<0.05 vs ROS 3mg/kg, d=p>0.05 vs MIA Control.

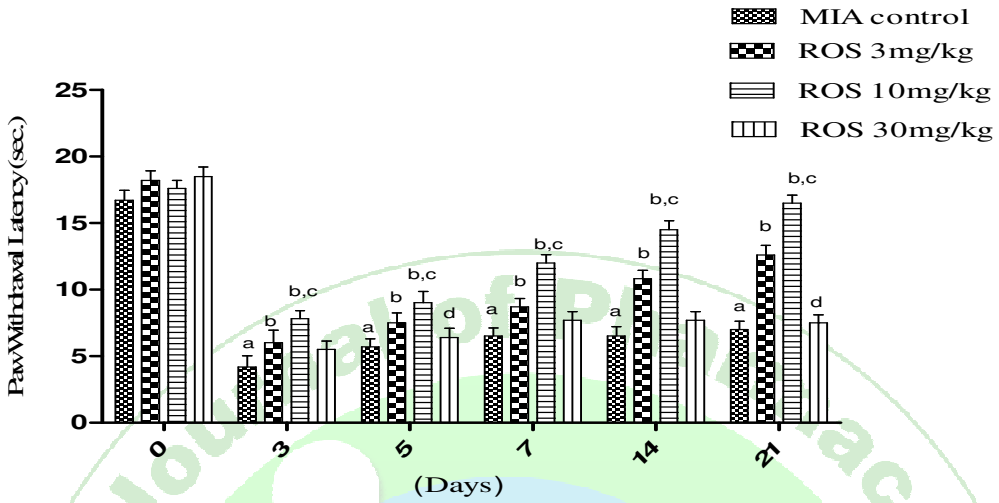


**Fig.2 Effect of various doses of rosuvastatin on MIA induced thermal hyperalgesia in Wistar rats.** All values are expressed as mean± SEM. a=p<0.05 vs day 0, b=p<0.05 vs MIA Control, c=p<0.05 vs ROS 3mg/kg, d=p>0.05 vs MIA Control.

**Effect of rosuvastatin (3, 10, 30 mg/kg) on paw volume, paw thickness and joint diameter in MIA-induced osteoarthritis in Wistar rats**

An intra-articular injection of MIA (2mg) significantly increased the paw volume, paw thickness and joint diameter during the first 3 days, which then regressed during the 21 days observation period. However treatment with, rosuvastatin (3and 10mg/kg, i.p) significantly reduced the paw volume, paw thickness and joint diameter as compared to MIA control group. In contrast rosuvastatin 30mg/kg, i.p, did not significantly decrease paw volume, paw thicknessas and knee joint diameter as compared to MIA control group (Table 1 and 2, Fig.3).

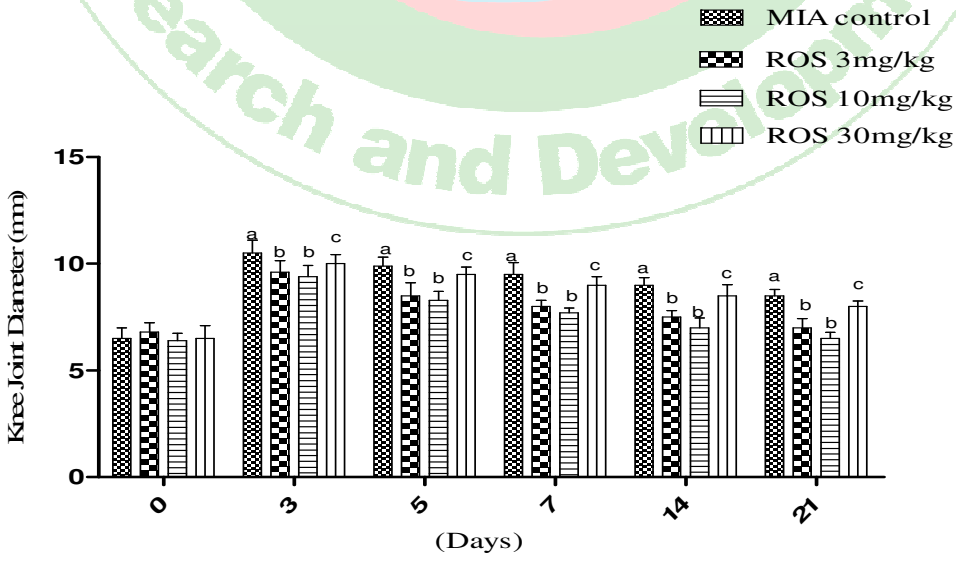

**Fig.3 Effect of various doses of rosuvastatin on MIA-induced joint edema in Wistar rats.** All values are expressed as mean± SEM. a= p<0.05 vs day 0, b=p<0.05 vs MIA Control, c=p> 0.05 MIA control

**Table 1. Effect of various doses of rosuvastatin on MIA induced paw edema in Wistar rats**

| *Days* | *0* | *3* | *5* | *7* | *14* | *21* |
|---|---|---|---|---|---|---|
| MIA Control | 1.2± 0.11 | 2.1± 0.10[a] | 1.95± 0.12[a] | 1.82± 0.11[a] | 1.65± 0.10[a] | 1.53± 0.09[a] |
| ROS 3mg/kg | 1.28± 0.11 | 1.86± 0.07[b] | 1.63± 0.08[b] | 1.55± 0.06[b] | 1.45± 0.11[b] | 1.35± 0.10[b] |
| ROS 10mg/kg | 1.3± 0.10 | 1.7± 0.12[b,c] | 1.48± 0.10[b] | 1.42± 0.09[b] | 1.35± 0.06[b] | 1.28± 0.07[b] |
| ROS 30 mg/kg | 1.24± 0.10 | 1.9± 0.12 | 1.87± 0.10[d] | 1.75± 0.09[d] | 1.56± 0.11[d] | 1.45± 0.12[d] |

All values are expressed as mean± SEM. a=p<0.05 vs day 0, b=p<0.05 vs MIA Control, c=p<0.05 vs ROS 3mg/kg d=p>0.05 vs MIA Control.

**Table 2. Effect of various doses of rosuvastatin on MIA induced paw thickness in Wistar rats**

| Days | 0 | 3 | 5 | 7 | 14 | 21 |
|---|---|---|---|---|---|---|
| MIA Control | 4.8± 0.53 | 6.6± 0.32[a] | 6.1± 0.25[a] | 5.8±0.29[a] | 5.5±0.48[a] | 5.2± 0.31[a] |
| ROS 3mg/kg | 4.5± 0.57 | 5.5± 0.34[b] | 5.1± 0.24[b] | 4.9± 0.39b | 4.58± 0.46[b] | 4.43± 0.32[b] |
| ROS 10mg/kg | 4.3± 0.41 | 5.1± 0.32[b] | 4.75± 0.32[b] | 4.5± 0.25[b] | 4.35± 0.36[b] | 4.30± 0.31[b] |
| ROS 30 mg/kg | 4.45± 0.42 | 6.0± 0.21 | 5.75± 0.31[c] | 5.10± 0.28 | 4.80± 0.40 | 4.70± 0.42[c] |

All values are expressed as mean± SEM. ) a=p<0.05 vs day 0, b=p<0.05 vs MIA Control, c=p>0.05 vs MIA Control.

**Effect of rosuvastatin (3, 10 and 30 mg/kg) on mobility behavior in MIA-induced osteoarthritis in Wistar rats**

An intra-articular injection of MIA (2mg) significantly decreased the mobility behavior (Table 3). However, treatment with rosuvastatin (3 and 10 mg/kg, i.p) produced significant increase in mobility score of the animal, in comparison to MIA control group. Whereas, treatment with rosuvastatin 30 mg/kg, i.p did not increase mobility score as compared to rosuvastatin 3 and 10mg/kg group (Table 3).

**Table 3. Effect of MIA and various doses of rosuvastatin on mobility score in wistar rats**

| Days | 0 | 3 | 5 | 7 | 14 | 21 |
|---|---|---|---|---|---|---|
| CFA Control | 6± 0 | 3.4± 0.18[a] | 3.1± 0.19[a] | 2.9±0.20[a] | 2.45±0.21[a] | 2.20± 0.18[a] |
| ROS 3mg/kg | 6± 0 | 3.6± 0.25 | 3.9± 0.21[b] | 4.2± 0.22[b] | 4.25± 0.18[b] | 5.0± 0.21[b] |
| ROS 10mg/kg | 6± 0 | 3.8± 0.15[b] | 4.3± 0.23[b,c] | 4.5± 0.19[b,c] | 4.6± 0.22[b,c] | 5.5± 0.19[b,c] |

| | | | | | | |
|---|---|---|---|---|---|---|
| ROS 30 mg/kg | 6± 0 | 3.6± 0.13[d] | 3.4± 0.15 | 3.2± 0.25 | 2.8± 0.15 | 2.6± 0.14 |

All values are expressed as mean± SEM. ) a=p<0.05 vs day 0, b=p<0.05 vs MIA Control, c=p>0.05 vs MIA Control.

**Effect of arthritis and pharmacological intervention on serum total cholesterol, triglycerides, LDL and HDL level**

No significant changes in total cholesterol, triglycerides, LDL and HDL were observed in MIA-administered rats as compared to basal values (Table 4).

**Effect of rosuvastatin (3, 10 and 30 mg/kg) on histological changes in MIA-induced osteoarthritis in Wistar rats.**

The histological examination of femorotibial joints revealed the presence of multifocal collapse and fragmentation of bony trabeculae. In addition, focal extensive areas of subchondral bone marrow were replaced by loosely arranged spindle cells and increased numbers of osteoclasts were observed in vehicle treated MIA control.

Whereas with the treatment of rosuvastatin (3, 10 and 30 mg/kg) there was significant reduction in the collapse and fragmentation of the bony trabeculae and also in the number of osteoclasts as compared to MIA treated control group (Fig.4).

| S.No. | Total Cholesterol | | Triglycerides | | HDL | | LDL | (mg/dl) |
|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 21 | Day 0 | Day 21 | Day 0 | Day 21 | Day 0 | Day 21 |
| MIA Control | 44± 4.56 | 45.66±4.32 | 29± 3.57 | 30.5± 3.39 | 22.6± 1.42 | 21.6±2.42 | 12.6±1.54 | 13±1.6 |
| MIA+ROS 3mg/kg | 43.5±4.50 | 43.8±4.49 | 28.5±2.25 | 29.6± 4.56 | 23.2± 2.48 | 21.9± 2.53 | 12.2±1.25 | 13.4±1.42 |
| MIA+ROS10mg/kg | 40.5± 5.45 | 39.5±3.38 | 28±2.34 | 28± 5.29 | 22.3± 2.03 | 23±2.2[a] | 12±1.55 | 13±1.54 |
| MIA+ROS30mg/kg | 42.1±3.50 | 38± 3.38 | 28.4±2.03 | 29±3.36 | 22±2.64 | 23.6±2.56 | 13±1.50 | 12.5±1.45 |

**Table.4 Effect of MIA and pharmacological interventions on serum Total cholesterol, triglycerides, LDL and HDL level**

All values are expressed as mean± SEM.

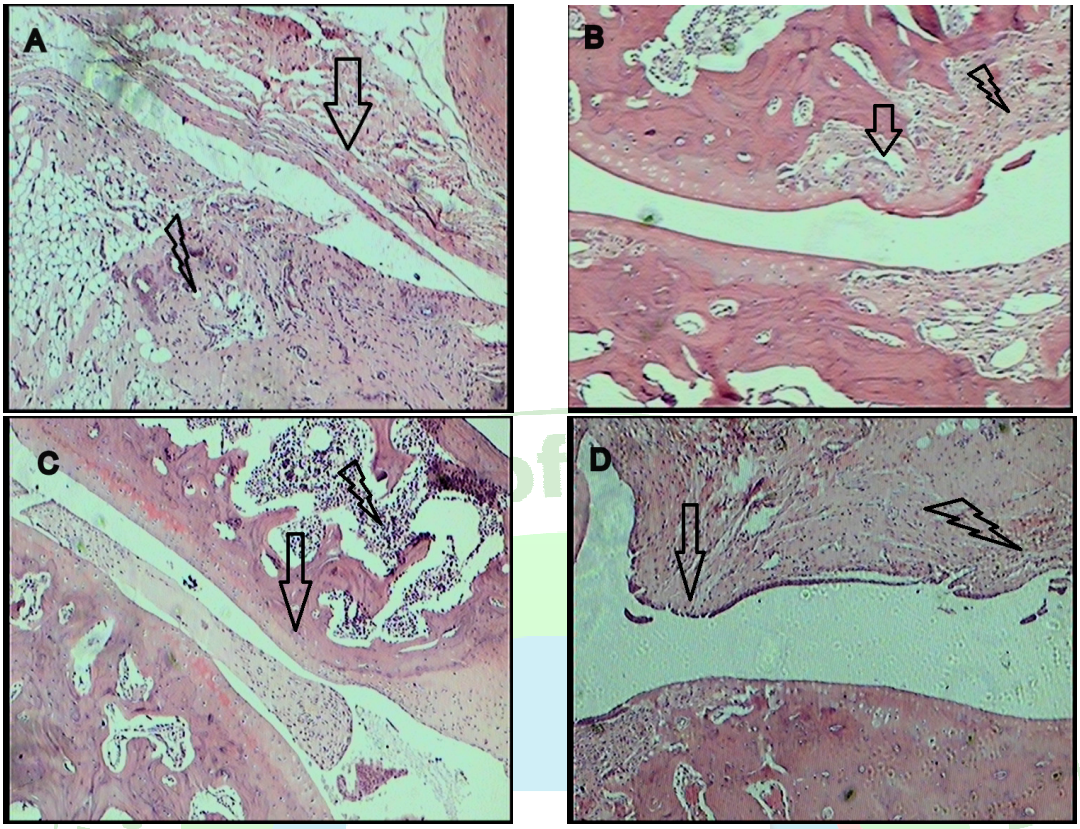


**Fig. 4 Effect of various doses of rosuvastatin on histological changes in MIA-induced-osteoarthritis in Wistar rats. A, MIA Control; B, rosuvastatin 3mg/kg: C rosuvastatin 10mg/kg; D, rosuvastatin 30mg/kg.**

## DISCUSSION

The single intra-articular injection of monosodium iodoacetate (MIA) in wistar rats was commonly used experimental model of osteoarthritis (OA) as it consistently reproduces the joint pathology and pain symptoms similar to OA patients [21, 27, 28]. MIA is an inhibitor of glyceraldehyde-3-phosphate dehydrogenase activity, and also the glycolysis inhibition had shown to induce chondrocyte death [29]. Intra-articular injection of varying doses of MIA (0.5 mg to 3.0 mg) has shown to induces chondrocyte death in the articular cartilage of rodent and nonrodent species[21, 27, 28]. The advantage of MIA model is that rapidly produces the clinical and pathological features of osteoarthritis. When used in rats, the model reproduces cartilage lesions with loss of proteoglycan matrix and functional joint impairment similar to human OA. In cartilage, lesions are characterized by chondrocyte necrosis, cell cloning (chondrones), fibrillation, loss of stainable proteoglycan matrix, and erosion with exposure of subchondral bone [27].

Estimation of paw volume, paw thickness and joint diameter has been used as index of inflammation . Mechanical allodynia, thermal hyperalgesia, pain on joint movement (joint hyperalgesia) and decreased locomotion which are prominent features in arthritic pain [30] were also measured. The decreased paw withdrawal latency to the thermal stimuli [24] and decrease mechanical threshold to the von frey filament, have been documented to be index of arthritic pain.

The patients with rheumatoid arthritis had evidence of a mild dyslipidaemia with higher levels of total cholesterol, LDL and triglycerides, and lower levels of HDL compared to healthy controls [31,32-33]. This atherogenic lipid profile may be due to higher levels of cholesteryl ester transfer protein (CETP) activity that decreases HDL levels by transferring cholesteryl ester from anti-

atherogenic HDL to atherogenic LDL . However , it has not been observed in case of Osteoarthritis in this study.

Statins have anti-inflammatory properties, regardless of their ability to lower cholesterol [34]. Further, evidence of the anti-inflammatory effects of statins relates to the inhibition of COX-2 expression [35]. Statins also up-regulate the expression and the activity of nitric oxide synthase, explaining the antinociceptive effect of rosuvastatin. Furthermore, the antinociceptive effect of statins may be due to the inhibition of the cytokines and prostaglandin release . Statins are also known to attenuate the secretion of pro-inflammatory cytokine interleukins (IL-1, 2, 4, 5, 10, 12), interferon-γ, and tumor necrosis factor-α (TNF-α), decrease the activity of cyclooxygenase-2 (COX-2), thromboxanes A2, and throm-boxanes B2, and enhance the synthesis of prostacyclin which may contribute to decrease platelet activation [10]. Statins including rosuvastatin (40 mg/kg, i.p) have no preventive or curative effects in the attenuation of autoimmunity in murine experimental arthritis . Simvastatin, have been reported to increase proinflammatory cytokine production by monocytes and dendritic cells [36-37]

In the present study, intra-articular administration of MIA (2 mg) produced mechanical hyperalgesia, thermal hyperalgesia, and increase in joint diameter, paw volume, paw thickness, and decrease in pain scores. Rosuvastatin (3,10 mg/kg) significantly increased the paw withdrawal latency towards mechanical and thermal stimuli but rosuvastatin (10 mg/kg) did not produce any significant effect on mechanical and thermal hyperalgesia. Rosuvastatin (3,10 mg/kg) decreased joint diameter, paw volume and paw thickness. MIA administration did not produce any significant changes in total cholesterol, triglycerides, LDL and HDL level when compared to normal values on day 0.

Hence it may be concluded, though high doses of statins are beneficial in hyperlipidemia to decrease the incidence of cardiovascular risks but rosuvastatin at high dose (30 mg/kg) is not beneficial in producing anti-inflammatory and analgesic effect in both rheumatoid arthritis and osteoarthritis.

## REFERENCE


1. *WHO Scientific Group. The burden of musculoskeletal conditions at the start of the new millenium.World Health Organisation; 2003.*
2. *Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K. Development of criteria for the classification and reporting of OA. Classification of Osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986; 29: 1039-49.*
3. *Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. Eur J Pain 2006; 10:287–333.*
4. *Scher JU, PillingerMH, Abramson SB. Nitric oxide synthases and osteoarthritis. Curr Rheumatol Rep 2007;9: 9–15.*
5. *Wu YS,Hu YY, Yang RF. Thematrixmetalloproteinases as pharmacological target in osteoarthritis: statinsmay be of therapeutic benefit.Med Hypotheses 2007;69:557–9.*
6. *Nagase H, Woessner Jr JF. Matrix metalloproteinases. J Biol Chem 1999;274:21491–4.*
7. *Ulrich-Vinther M, Maloney MD, Schwarz EM, et al. Articular cartilage biology. J Am Acad Orthop Surg 2003;11:421–30.*
8. *Mitchell PG, Magna HA, Reeves LM. Cloning, expression, and type II col-lagenolytic activity ofmatrixmetalloproteinase-13 fromhuman osteoarthritic cartilage. J Clin Invest 1996;97:761–8.*
9. *Schachter, M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. Fundam Clin Pharmacol. 2005;19:117–25.*
10. *Schönbeck, U., Libby, P. Inflammation, immunity, and HMG-CoA reductase inhibitors: statins as antiinflammatory agents? Circulation. 2004;109:18–26.*
11. *Liao, J.K., Laufs, U. Pleiotropic effects of statins. Annu Rev Pharmacol Toxicol. 2005;45:89–118.*
12. *Greenwood, J., Walters, C.E., Pryce, G., Kanuga, N., Beraud, E., Baker, D. Lovastatin inhibits brain endothelial cell Rho-mediated lymphocyte migration and attenuates experimental autoimmune encephalomyelitis. FASEB J. 2003;17:905–7.*
13. *Mackay, D.J., Hall, A. Rho GTPases. J Biol Chem. 1998;273:20685.*
14. *van der Most, P.J., Dolga, A.M., Nijholt, I.M., Luiten, P.G., Eisel, U.L. Statins: mechanisms of neuroprotection. Prog Neurobiol. 2009;88:64–75.*
15. *Essig, M., Nguyen, G., Prie, D., Escoubet, B., Sraer, J.D., Friedlander, G. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors increase fibrinolytic activity in rat aortic endothelial cells. Role of geranylgeranylation and Rho proteins. Circ Res. 1998;83:683–90.*
16. *Ortego, M., Bustos, C., Hernandez-Presa, M.A., Tunon, J., Diaz, C., Hernandez, G. Atorvastatin reduces NF-kappaB activation and chemokine expression in vascular smooth muscle cells and*

*mononuclear cells. Atherosclerosis. 1999;147:253–61.*

17. *Hausding, M., Witteck, A., Rodriguez-Pascual, F., von Eichel- Streiber, C., Forstermann, U., Kleinert, H. Inhibition of small G proteins of the rho family by statins or clostridiumdifficile toxin B enhances cytokine-mediated induction of NO synthase II. Br J Pharmacol. 2000;131:553–61.*

18. *Danesh, F R., Sadeghi, M.M., Amro, N., Philips, C., Zeng, L., Lin, S. 3-Hydroxy-3-methylglutaryl CoA reductase inhibitors prevent high glucose-induced proliferation of mesangial cells via modulation of Rho GTPase/p21 signaling pathway: implications for diabetic nephropathy. Proc Natl Acad Sci USA. 2002;99:8301–5.*

19. *Eto, M., Kozai, T., Cosentino, F., Joch, H., Luscher, T.F. Statin prevents tissue factor expression in human endothelial cells: role of Rho/Rho-kinase and Akt pathways. Circulation. 2002;105:1756–9.*

20. *Masamura, K., Oida, K., Kanehara, H., Suzuki, J., Horie, S., Ishii, H. Pitavastatin-induced thrombomodulin expression by endothelial cells acts via inhibition of small G proteins of the Rho family. Arterioscler Thromb Vasc Biol. 2003;23:512–7.*
21. *Kobashigawa, J.A., Katznelson, S., Laks, H., Johnson, J.A., Yeatman, L., Wang, X.M., Chia, D., Terasaki, P.I., Sabad, A., Cogert, G.A., Trosian, K., Hamilton, M.A., Moriguchi, J.D., Kawata, N., Hage, A., Drinkwater, D.C., Stevenson, L.W.:Effect of pravastatin on outcomes after cardiac transplantation. N Engl J Med. 1995; 333:621–627.*
22. *Bellosta, S., Via, D, Canavesi, M., Pfister, P., Fumagalli, R., Paoletti, R., Bernini, F. HMG-CoA reductase inhibitors reduce MMP-9 secretion by macrophages. Arterioscler Thromb Vasc. Biol. 1998;18:1671– 1678.*
23. *Ikeda, U., Shimpo, M., Ohki, R., Inaba, H., Takahashi, M., Yamamoto, K., Shimada, K. Fluvastatin inhibits matrix metalloproteinase-1 expres-sion in human vascular endothelial cells. Hypertension. 2000;36: 325–329.*
24. *Hargreaves, K., Dubner, R., Brown, F., Flores, C. and Joris, J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain., 1988; 32: 77–88.*
25. *Mene´ndez, L., Andre´s-Trelles, F., Hidalgo, A., Baamonde, A. Involvement of spinal k-opioid receptors in a type of footshock-induced analgesia in mice. Brain Res., 1993; 611: 264–271.*
26. *Kobayashi Kiyoshi, Imaizumi Rei, Sumichika Hiroshi, Tanaka Hideki, Goda Maki. Sodium iodoacetate induced experimental osteoarthritis and associated painmodel in rats. J. Vet. Med. Sci., 2003; 65(11): 1195-1199.*
27. *Guzman E. Roberto, Evans G. Mark, Bove Susan, Morenko Brandy and Kilgore Kenneth. Mono-Iodoacetate-Induced Histologic Changes in Subchondral Bone and Articular Cartilage of Rat Femorotibial Joints: An Animal Model of Osteoarthritis, Toxicol Pathol, 2003; 31: 619.*
28. *Combe, B., Cosso, B., Clot, J., Bonneau, M. and Sany, J. Human placenta-eluted gammaglobulins in immunomodulating treatment of rheumatoid arthritis. Am J Med., 1985, 78, 920-8.*
29. *Cournil C, Liagre B, Grosin L, Vol C, Abid A. Overexpression and induction of heat shock protein (Hsp) 70 protects in vitro and in vivo from mono-iodoacetate (MIA)-induced chondrocytes death. Arthritis 2001; 3(1): 41.*
30. *Tatsuo, M.A.K.F., Carvalho, W.M., Silva, C.V., Miranda, A.E.G., Ferreira, S.H., Francischi, J.N., Analgesic and antiinflammatory effects of dipyrone in rat adjuvant arthritis model. Inflammation, 1994; 18: 399–405.*
31. *Georgiadis, A.N., Papavasiliou, E.C., Lourida, E.S., Alamanos, Y., Kostara, C., Tselepis, A.D., Drosos, A.A., Atherogenic lipid profile is a feature characteristic of patients with early rheumatoid arthritis: effect of early treatment — a prospective, controlled study, Arthritis Res. Ther. 2006; 8: 82.*
32. *Jonsson, S.W., Backman, C., Johnson, O., Karp, K., Lundström, E., Sundqvist, K.G., Dahlqvist, S.R., Increased prevalence of atherosclerosis in patients with medium term rheumatoid arthritis. J. Rheumatol, 2001; 28: 2597–2602.*
33. *Park, Y.B., Lee, S.K., Lee, W.K., Suh, C.H., Lee, C.W., Lee, C.H., Lipid profiles in untreated patients with rheumatoid arthritis. J. Rheumatol, 1999; 26: 1701–1704.*
34. *Dinarello, C.A. Anti-inflammatory agents: present and future. Cell. 2010;140:935–50.*
35. *Hernández-Presa, M.A., Martín-Ventura, J.L., Ortego, M., Gómez-Hernández, A., Tuñón, J., Hernández-Vargas, P. Atorvastatin reduces the expression of cyclooxygenase-2 in a rabbit model of atherosclerosis and in cultured vascular smooth muscle cells. Atherosclerosis. 2002;160:49–58.*
36. *Kiener PA, Davis PM, Murray JL, Youssef S, Rankin BM, Kowala M. Stimulation of inflammatory responses in vitro and in vivo by lipophillic HMG/CoA reductase inhibitors. Int Immunopharmacol 2001; 1:105-18.*
37. *Sun D, Fernandes G. Lovastatin inhibits bonemarrow derived dendritic crell maturation and upregulates proinflammatory cytokines production. Cell Immunol 2003;223:52-62*