

Supplementation with β -carotene aids minimize inflammation in monosodium urate crystal-induced gouty arthritis in Wistar albino rats

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Gout is a form of metabolic arthritis originated on grounds of increased accumulation of monosodium urate (MSU) crystals in joints. Current study focuses on anti-arthritis activities of β -carotene on MSU crystal-induced gouty arthritis rats in comparison with the non-steroidal anti-inflammatory drug, indomethacin. The evaluation was done by taking into account paw oedema, lysosomal enzymes, anti-oxidant enzymes, lipid peroxidation, serum biochemical parameters (uric acid, creatinine), serum cytokines (TNF- α , IL-1 β) and histopathological studies. After the induction of MSU crystals, the lysosomal enzymes were increased, antioxidant enzymes were reduced, lipid peroxidation increased and paw volume increased. β -carotene treated at a dose of 10 mg/kg of body weight stabilizes lysosomal enzymes, increases anti-oxidant enzymes, regulates lipid peroxidation and decreased paw volume. The drug β -carotene potentially influences anti-inflammatory effects in arthritic group which is evident from the reduction in the elevated levels of inflammatory cytokines, TNF- α and IL-1 β . Current study is an evidence of anti-inflammatory and anti-oxidant effects of β -carotene against MSU-crystal induced gouty arthritis rats.

Keywords: Gouty arthritis. Inflammation. β -carotene. Antioxidant. Anti-inflammatory.

INTRODUCTION

Gout is a kind of inflammatory arthritis caused due to the accumulation of high levels of uric acid in tissue and blood. This uric acid can form needle like crystals called mono sodium urate crystal (MSU). Deposition of these crystals in joints and soft tissue may result in acute arthritis and chronic arthropathy (Gonzalez, 2012). Factors that causes hyperuricemia include diet, obesity, medical conditions such as cardiovascular disorders, diabetes and kidney diseases and certain medications, surgery and also the genes that we inherit.

In India, elderly people (above 65 years) are more prone to gouty arthritis (male: female = 1:1) (Hanly *et al.*,

2009). According to current scenario, the prevalence of gouty arthritis is 3.9% in USA, 0.9% in France, 1.4-2.5% in UK, 1.4% in Germany and 3.2% (European ancestry), 6.1% (Maori ancestry) in New Zealand. Due to change in dietary and other lifestyle habits metropolitan cities and in rural areas atleast 1%-2% of adults were affected. Over past 5 decades the global burden of gouty arthritis is noticed to be increased in many parts of the world (Kuo *et al.*, 2015).

β -carotene is a red-orange pigment found in fresh fruits, vegetables which have antioxidant property to reacts with free radicals and peroxy radicals. β -carotene is partially converted into vitamin A by the enzyme dioxigenase. β -carotene inhibits the production of inflammatory cytokines like TNF- α (Tumor necrosis factor-alpha), IL-1 β (Interleukin 1 beta) and NF- κ B(nuclear factor kappa-light-chain-enhancer of activated B cells) activation reducing the inflammatory

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response (Bai *et al.*, 2005). β -carotene is partially converted into vitamin A by the enzyme dioxygenase and acts as a cardioprotective agent (Csepanyi *et al.*, 2015). The mechanism of inflammatory response is triggered by the action of binding immunoglobulins such as IgG and IgM to the monosodium urate crystal and also adhesion proteins like fibronectin and complement proteins bind to the crystal that protects it from lysis (Dalbeth *et al.*, 2005). Present study is an attempt to investigate the efficacy of β -carotene in minimizing inflammation in MSU-crystals-induced arthritis in Wistar albino rats. The need of this experiment would be essential to identify the cost efficient or effective compound with less or no side effect in treating mono sodium urate crystal-induced gouty arthritis.

MATERIAL AND METHODS

Animals

Female Wistar albino rats weighing 250-300 g, was used for this study. The female rats were easily obtained and better preferred; as the male rats were of greater demand. Animals were purchased from the Tamil Nadu Veterinary University, Chennai, India. Animals were acclimatized for a week in temperature –controlled room with a 12 hr dark-light cycle and fed commercially available pelleted feed (Hindustan Lever Ltd, Mumbai, India) and water *ad libitum*. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Culture, Government of India, and (CPCSEA) Tamil Nadu, Chennai. The Institutional animal ethical committee VIT University approved the experimental protocol followed in this study.

Drugs and chemicals

Uric acid (4 g) was dissolved in water (800 ml) by heating to 60° C and pH was adjusted to 8.9 with 0.5N NaOH. It was cooled overnight in a cold room (4° C), washed and dried. Needle-like MSU crystals obtained were suspended in a sterile saline at the concentration of 20 mg/ml (Sabina *et al.*, 2010). Monosodium urate crystals were injected intradermally with a dose of 0.2 ml (4mg) into the right footpad (Rasool *et al.*, 2006; Lemos Lima *et al.*, 2015).

β -carotene was administered orally at the dose of 10 mg/kg b.w. 1hour before the injection of MSU crystals and it was repeated for 3 days on a daily basis. Indomethacin was suspended in 2% gum acacia solution and it is also administered orally at the dose of 3 mg/kg b.w.; 1hr before the monosodium urate crystal injection and it was repeated for 3 days on a daily basis.

Experimental design

The rats were divided into four groups consists of six animals in each group

Group I	Control rat (n=6)
Group II	Gouty arthritis-induced rat (MSU-single dose (4.0 mg/0.2ml interadermal) (n=6)
Group III	Gouty arthritis -induced rat treated with β -carotene (10 mg/kg b.w per-oral route) (n=6)
Group IV	Gouty arthritis-induced rat treated with Indomethacin (3 mg/kg b.w. per-oral route) (n=6)

The dosage of the drugs was given according to the average weight of the rats.

Measurement of paw oedema

The inflammation was quantified by measuring the thickness of the rat's paw with Vernier scale at different intervals for 3 days. At the end of 72 hrs, the rats were killed by cervical dislocation. Blood from each animal was collected and serum was separated to carry out all the biochemical studies. Liver and spleen were removed and stored in saline (0.89% NaCl) at 4 °C.

Tissue homogenate

The liver and spleen isolated from each study animal was weighed 100 mg accurately and homogenized in 10 ml of 0.1M Tris-HCl buffer.

Assay of lysosomal enzymes

The enzyme acid phosphatase was assayed by using disodium phenyl phosphate as substrate (King *et al.*, 1951). β – Glucuronidase was assayed using phenolphthalein glucuronide as substrate and phenolphthalein as standard by the method of (Kawai *et al.*, 1971) β –D Galactosidase was assayed using ONGP (ortho-nitrophenyl- β -galactoside) as a substrate by the method of (Rosenblit *et al.*, 1974) N-acetyl β -D-Glucosaminidase was assayed using substrate p-nitro phenyl- β -glucuronide and standard was p-nitrophenol by the method of (Maruhn, 1976).

Assay of antioxidant parameters

Lipid peroxidation(TBARS) was determined by the method of(Högberg *et al.*, 1974) were TBA (thiobarbituric acid) was used as colouring agent. Glutathione peroxidase activity was determined by non-enzymatic method (Rotruck *et al.*, 1973) were sodiumphosphate buffer, sodium azide, reduced glutathione, hydrogen peroxide, trichloro acetic acid and DTNB (5,5'-dithio-bis-(nitrobenzoic acid). SOD was assayed by the method of (Marklund *et al.*, 1974) were pyrogallol was used as stock and analysed under 470nm of OD. Catalase was assayed by the reagent of dichromate acetic acid according to the method of (Sinha, 1972) Liver and spleen tissues total protein was estimated by the method of (Lowry *et al.*, 1951) and BSA(bovine serum albumin) was used as standard.

Measurement of serum biochemical parameters

Serum biochemical parameters such as uric acid and creatinine were estimated using commercial kits procured from AutoSpan diagnostics Ltd., India. The assays were carried out according to the manufacturer's protocol.

Measurement of serum cytokines

The levels of serum cytokines TNF- α and IL-1 β were estimated by ELISA using commercial kits procured from Sigma Aldrich Ltd., India. The assays were carried out as per the manufacturer's protocol.

Histopathology

The rat paw in each study animal was isolated for histopathological analysis and preserved in 10% formalin until further process. Sections of about 5 μ m thickness were cut using a microtome. The tissues were further processed by routine histopathological methods and stained with haematoxylin and eosin. The stained slides were examined microscopically for histopathological changes.

Statistical analysis

One way ANOVA was performed by GraphPad istat3 software where the obtained values written as mean \pm SD. Symbol (*) indicates the significance($p < 0.05$) between the groups. Student newman -kuel's test was used to analysis this method.

RESULTS

Effect of β -carotene and Indomethacin on body weight of MSU crystal-induced arthritis

Figure 1 represents the effect of β -carotene on the body weight of MSU-induced rats. It is evident that there is significant decrease in body weight of MSU-induced arthritic rats. Also, the data depicts that β -carotene treatment increases body weight significantly ($p < 0.05$; ANOVA) in MSU-induced arthritic rats. The effect of β -carotene on body weight was compared with that of the standard drug-indomethacin.

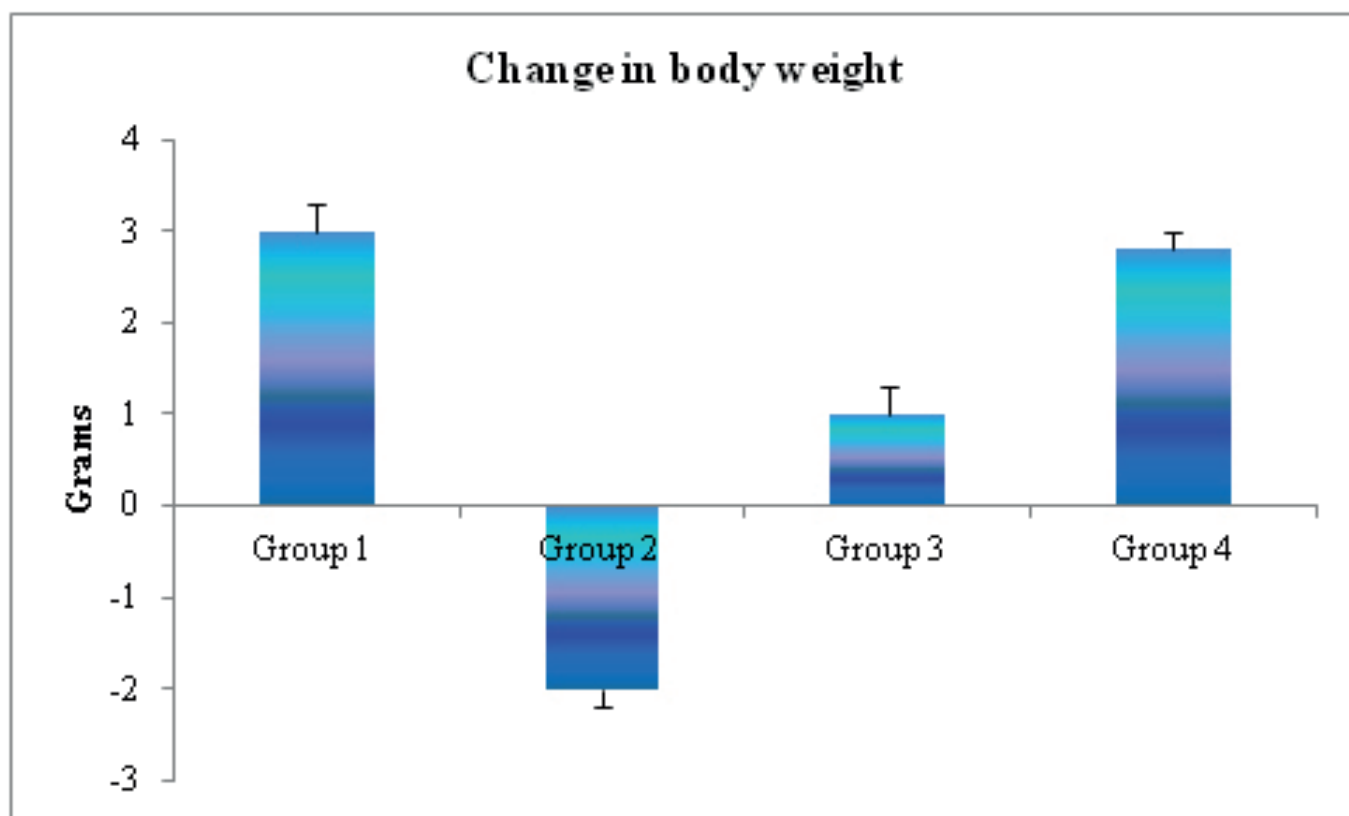


FIGURE 1 - Effect of β -carotene and Indomethacin on body weight of MSU crystal- induced arthritis N=6 animals in each group. The values are expressed as mean \pm S.D. Comparisons indicated by lower case letters were made as follows: a – group I vs. group II, III and IV; b – group II vs. group III and IV; c – group III vs. group IV. Statistical analysis was carried out by one way ANOVA followed by Student’s Newman – Kuel’s test.

Effect of β -carotene and Indomethacin on paw oedema of MSU crystal-induced arthritis

The effect of β -carotene on paw volume is shown in Figure 2. The paw volume measured from MSU-induced

rats revealed that there is a significant increase in ankle diameter. It indicates that β -carotene treatment reduces the paw diameter significantly ($p < 0.05$; ANOVA) which was comparable with that of the standard drug-indomethacin.

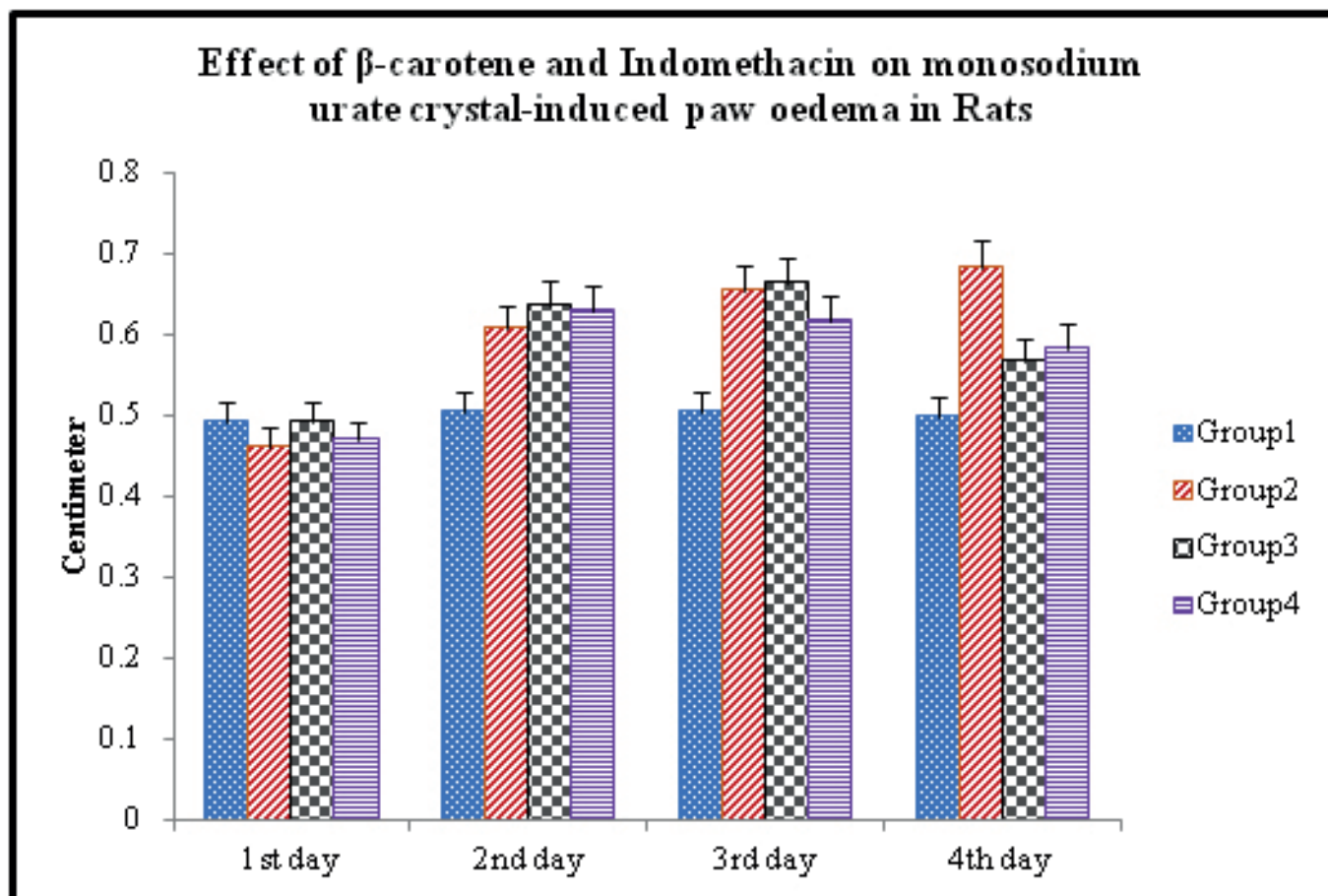


FIGURE 2 - Effect of β -carotene and Indomethacin on paw oedema of MSU crystal- induced arthritis N=6 animals in each group. The values are expressed as mean \pm S.D. Comparisons indicated by lower case letters were made as follows: a – group I vs. group II, III and IV; b – group II vs. group III and IV; c – group III vs. group IV. Statistical analysis was carried out by one way ANOVA followed by Student’s Newman – Kuel’s test.

Effect of β -carotene and Indomethacin on the activity of lysosomal enzymes of MSU crystal-induced arthritis

The effect of β -carotene on the activity of lysosomal enzymes in liver and spleen of MSU crystal-induced arthritic rats is shown in Table I. Levels of acid phosphatase, β -glucuronidase, N-acetyl

glucosaminidase and β -galactosidase were increased significantly ($p < 0.05$) in liver and spleen of MSU crystal-induced rats when compared to normal control rats. On administration of β -carotene to MSU crystal-induced rats there was significant ($p < 0.05$; ANOVA) reversal of the above changes to near normal level and the same was comparable with that of Indomethacin.

TABLE I - Effect of β -carotene and Indomethacin on the activity of lysosomal enzymes of liver and spleen of MSU crystal - induced arthritis rats

Enzymes	Group –I (Control)	Group –II (MSU crystal- induced rats)	Group –III (MSU crystal-induced rats + β -carotene 10mg/kg/b.wt)	Group –IV (MSU crystal-induced rats + Indomethacin 3 mg/kg/b.wt)
Liver				
	2.56 \pm 0.16	3.90 \pm 0.22 a*	2.90 \pm 0.20 a* b*	2.95 \pm 0.25 a* b*
β -glucuronidase (units/mg)	28.5 \pm 1.86	37.7 \pm 2.97 a*	30.5 \pm 2.45 a* b*	31.3 \pm 2.65 a* b*
N-acetyl glucosaminidase (unit/min/g)	30.4 \pm 2.36	40.5 \pm 3.11 a*	32.9 \pm 2.75 a* b*	33.5 \pm 2.90 a* b*
β -galactosidase (μ moles \times 10 ⁻²)	11.3 \pm 0.68	19.8 \pm 1.13 a*	13.7 \pm 0.75 a* b*	14.2 \pm 0.80 a* b*
Spleen				
Acid phosphatase (μ moles \times 10 ⁻²)	3.1 \pm 0.24	4.1 \pm 0.24 a*	3.45 \pm 0.30 a* b*	3.34 \pm 0.38 a* b*
β -glucuronidase (units/mg)	27.7 \pm 1.92	38.5 \pm 2.08 a*	29.7 \pm 1.60 a* b*	30.7 \pm 2.50 a* b*
N-acetyl glucosaminidase (unit/min/g)	21.5 \pm 1.50	37.4 \pm 1.96 a*	27.7 \pm 1.37 a* b*	26.7 \pm 1.87 a* b*
β -galactosidase (μ moles \times 10 ⁻²)	5.6 \pm 0.35	10.5 \pm 0.62 a*	6.5 \pm 0.22 a* b*	7.3 \pm 0.37 a* b*

N=6 animals in each group. The values are expressed as mean \pm S.D. Comparisons indicated by lower case letters were made as follows: a – group I vs. group II, III and IV; b – group II vs. group III and IV; c – group III vs. group IV. Statistical analysis was carried out by one way ANOVA followed by Student's Newman – Kuel's test. The symbols represent statistical significance at: * $p < 0.05$.

Effect of β -carotene on lipid peroxidation index and antioxidant enzymes of MSU crystal-induced arthritis

Levels of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, catalase) in liver and spleen homogenates were decreased significantly ($p < 0.05$; ANOVA) in group II rats when compared to normal control group (Table II). β -carotene administration was able to restore normal levels of the above-mentioned enzymes. The effect of β -carotene

on lipid peroxidation in liver and spleen of control and experimental rats is given in Table III. Liver and spleen homogenates of group II rats showed significant ($p < 0.05$; ANOVA) increase in lipid peroxidation levels when compared to normal control rats (group I), whereas administration of β -carotene to MSU crystal-induced rats altered the above changes by regulating the formation lipid peroxides to near normal level. The effect of β -carotene on antioxidant parameters was comparable with that of indomethacin.

TABLE II - Effect of β -carotene and Indomethacin on lipid peroxidation(mg/dl) of liver and spleen in MSU crystal -induced arthritis rats

Enzyme	Group-I (Control)	Group II (MSU crystal- induced rats)	Group III (MSU-crystal-induced rats + β -carotene 10mg/kg/b.wt)	Group IV (MSU crystal-induced rats+ Indomethacin 3mg/kg/b.wt)
Liver	2.33 \pm 0.10	3.12 \pm 0.15 a*	2.58 \pm 0.10 b*	2.55 \pm 0.19 b*
Spleen	3.15 \pm 0.17	4.50 \pm 0.25 a*	3.45 \pm 0.16 a*b*	3.65 \pm 0.30 a*b*

N=6 animals in each group. The values are expressed as mean \pm S.D. Comparisons indicated by lower case letters were made as follows: a – group I vs. group II, III and IV; b – group II vs. group III and IV; c – group III vs. group IV. Statistical analysis was carried out by one way ANOVA followed by Student's Newman – Kuel's test. The symbols represent statistical significance at: * $p < 0.05$.

TABLE III - Effect of β -carotene and Indomethacin on antioxidants enzyme of liver and spleen of MSU crystal-induced arthritis rats

Enzyme	Group-I (Control)	Group II (MSU crystal- induced rats)	Group III (MSU crystal-induced rats + β -carotene 10mg/kg/b.wt)	Group IV (MSU crystal-induced rats +Indomethacin 3mg/kg/b.wt)
Liver				
Superoxide dismutase (Units/min/mg protein)	4.1 \pm 0.2	1.9 \pm 0.11 a*	2.70 \pm 0.23 a*b*	2.87 \pm 0.25 a*b*
Glutathione peroxidase (μ g of GSH utilized/ min/mg protein)	6.88 \pm 0.62	4.68 \pm 0.27 a*	6.65 \pm 0.61 a*b*	6.80 \pm 0.45 a*b*
Catalase (Units/ min/mg protein)	13.1 \pm 0.75	7.77 \pm 0.48 a*	11.8 \pm 0.70 a*b*	10.7 \pm 0.7 a*b*
Spleen				
Superoxide dismutase (Units/min/mg protein)	2.45 \pm 0.16	1.7 \pm 0.14 a*	2.14 \pm 0.16 b*	1.89 \pm 0.14 a*b*
Glutathione peroxidase (μ g of GSH utilized/ min/mg protein)	4.7 \pm 0.38	3.4 \pm 0.15 a*	4.25 \pm 0.35 b*	4.28 \pm 0.26 b*
Catalase (Units/ min/mg protein)	10.5 \pm 0.73	5.9 \pm 0.40 a*	9.35 \pm 0.75 a*b*	8.5 \pm 0.55 a*b*

N=6 animals in each group. The values are expressed as mean \pm S.D. Comparisons indicated by lower case letters were made as follows: a – group I vs. group II, III and IV; b – group II vs. group III and IV; c – group III vs. group IV. Statistical analysis was carried out by one way ANOVA followed by Student's Newman – Kuel's test. The symbols represent statistical significance at: * $p < 0.05$.

Effect of β -carotene on serum biochemical parameters of MSU crystal-induced arthritis

The levels of serum uric acid were elevated significantly ($p < 0.05$; ANOVA) in the MSU crystals-induced arthritic rats (Figure 3). The administration of β -carotene (10 mg/kg b.w./day) caused significant ($p < 0.05$; ANOVA) reduction in the elevated levels of

uric acid in MSU crystals-induced rats. A similar effect was observed in the levels of serum creatinine where the significant rise caused by the intradermal injection of MSU crystals was brought down to near normal levels by the oral administration of β -carotene (10 mg/kg b.w./day) (Figure 4). The results of β -carotene treated group were comparable with that of the standard drug indomethacin treated group of rats.

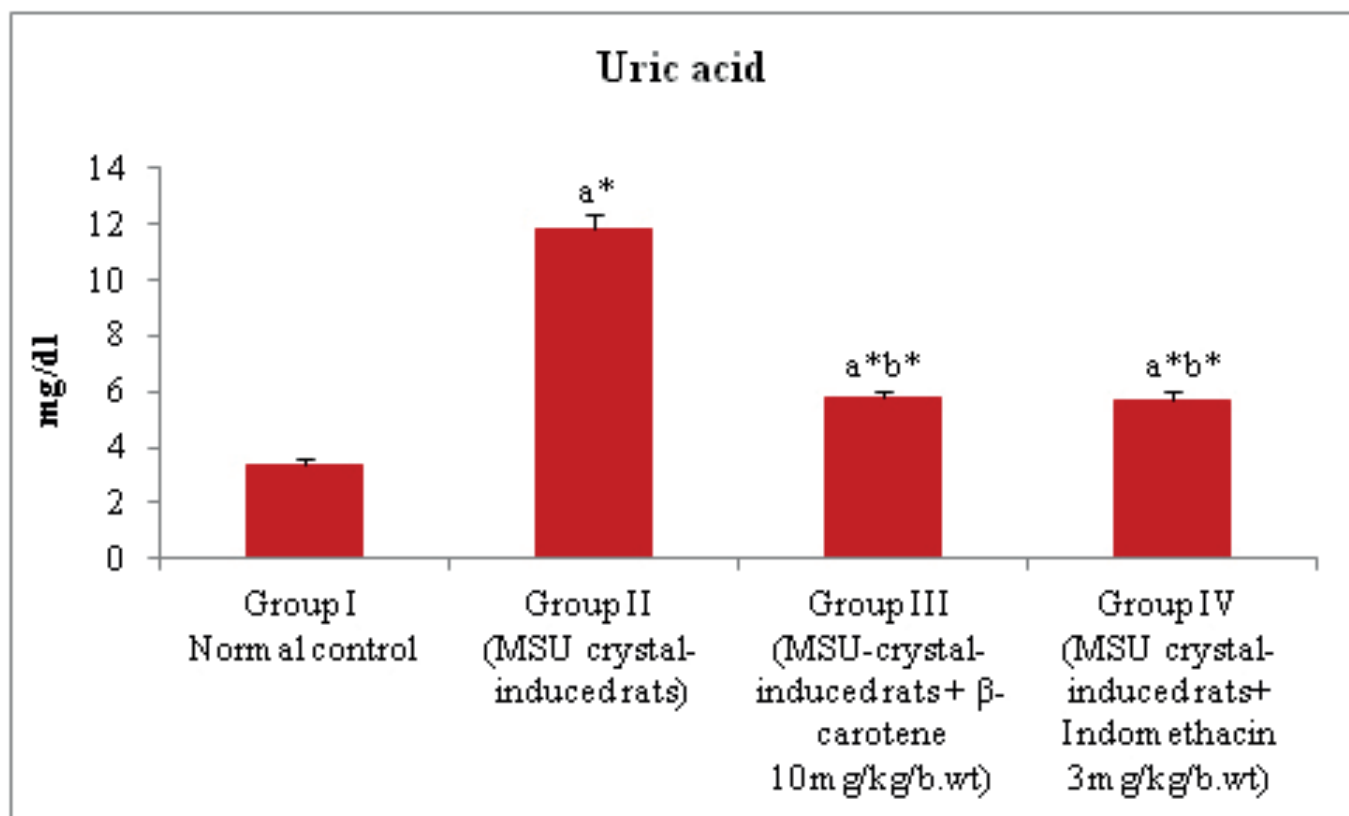


FIGURE 3 - Effect of β -carotene on serum uric acid in MSU crystal-induced arthritis

N=6 animals in each group. The values are expressed as mean \pm S.D. Comparisons indicated by lower case letters were made as follows: a – group I vs. group II, III and IV; b – group II vs. group III and IV; c – group III vs. group IV. Statistical analysis was carried out by one way ANOVA followed by Student's Newman – Kuel's test. The symbols represent statistical significance at: * $p < 0.05$.

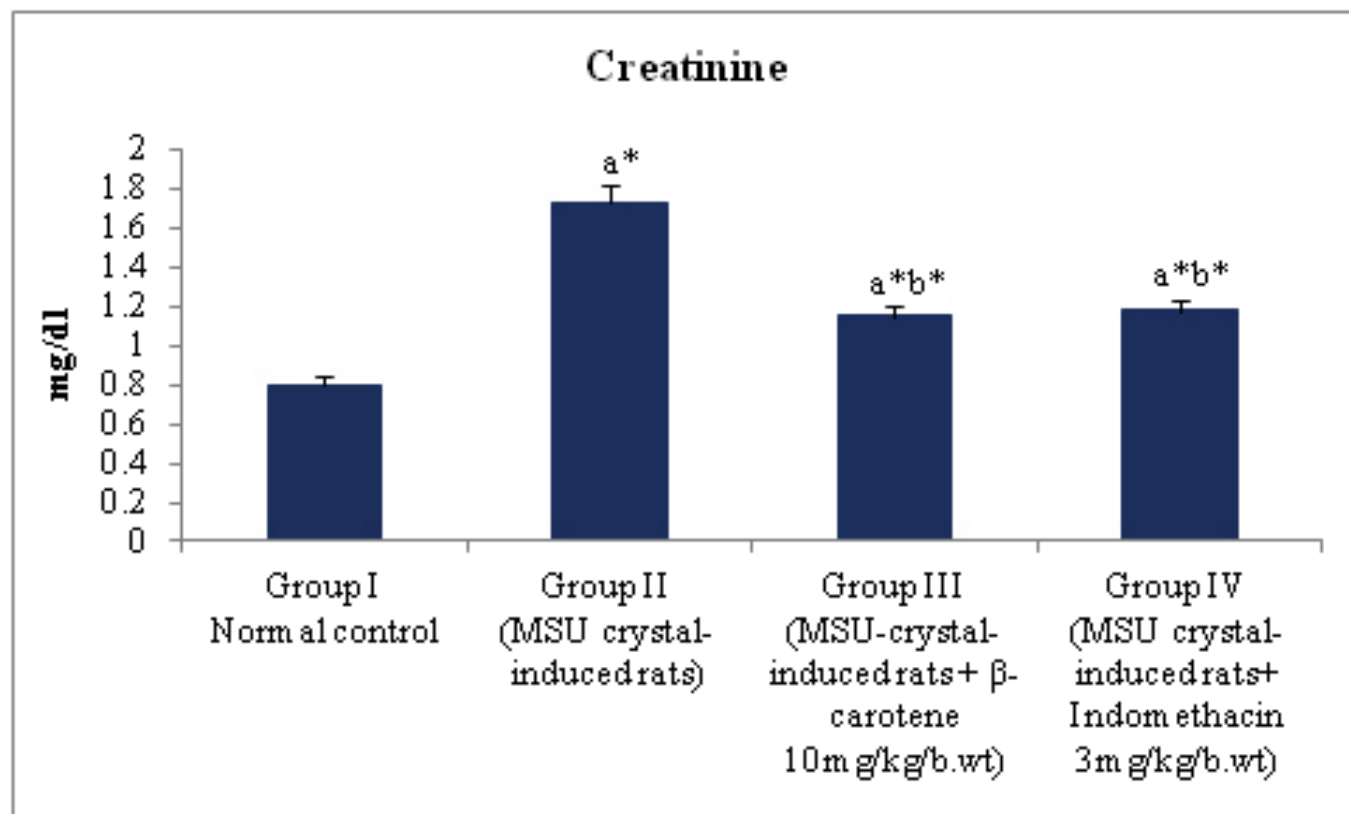


FIGURE 4 - Effect of β -carotene on serum creatinine in MSU crystal-induced arthritis

N=6 animals in each group. The values are expressed as mean \pm S.D. Comparisons indicated by lower case letters were made as follows: a – group I vs. group II, III and IV; b – group II vs. group III and IV; c – group III vs. group IV. Statistical analysis was carried out by one way ANOVA followed by Student's Newman – Kuel's test. The symbols represent statistical significance at: * $p < 0.05$.

Effect of β -carotene on serum cytokines of MSU crystal-induced arthritis

The levels of proinflammatory cytokines TNF- α and IL-1 β were found to be increased significantly in

MSU crystals-induced rats (Figure 5 and 6). The rats treated with β -carotene (10 mg/kg b.w./day) showed significant decrease in the elevated levels of the aforementioned cytokines. This effect of β -carotene was similar to that of the standard drug indomethacin.

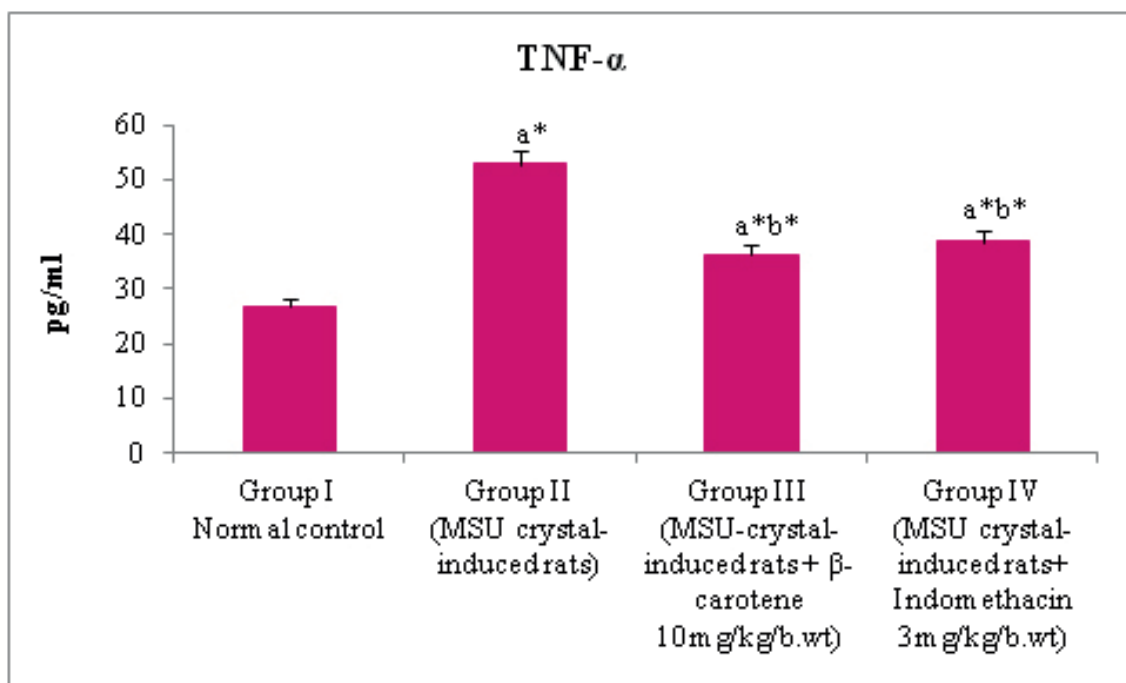


FIGURE 5 - Effect of β -carotene on serum TNF- α of MSU crystal-induced arthritis
 N=6 animals in each group. The values are expressed as mean \pm S.D. Comparisons indicated by lower case letters were made as follows: a – group I vs. group II, III and IV; b – group II vs. group III and IV; c – group III vs. group IV. Statistical analysis was carried out by one way ANOVA followed by Student’s Newman – Kuel’s test. The symbols represent statistical significance at: * $p < 0.05$.

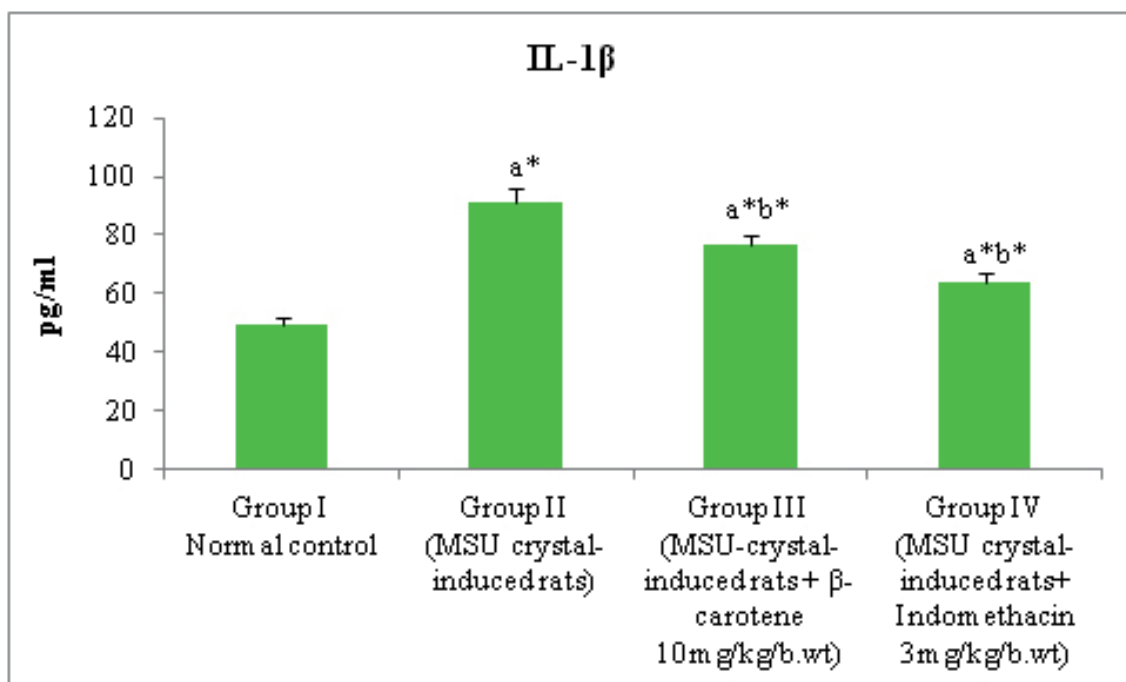


FIGURE 6 - Effect of β -carotene on serum IL-1 β of MSU crystal-induced arthritis
 N=6 animals in each group. The values are expressed as mean \pm S.D. Comparisons indicated by lower case letters were made as follows: a – group I vs. group II, III and IV; b – group II vs. group III and IV; c – group III vs. group IV. Statistical analysis was carried out by one way ANOVA followed by Student’s Newman – Kuel’s test. The symbols represent statistical significance at: * $p < 0.05$.

Effect of β -carotene on paw histopathology of MSU crystal-induced arthritic rats

Figure 7 indicates the histopathological changes in MSU crystals-induced arthritic rats. The MSU crystals-induced rat presented with synovial hyperplasia of the articular cartilage (Figure 7b). However, the

administration of β -carotene in group III rats showed to minimize inflammation and the rats also presented with diminished joint space with erosion of the articular cartilage (Figure 7c). There was no evidence of synovial hyperplasia in this group. This was comparable with the indomethacin treated rats as they showed minimal inflammation in the adjacent joint capsule (Figure 7d).

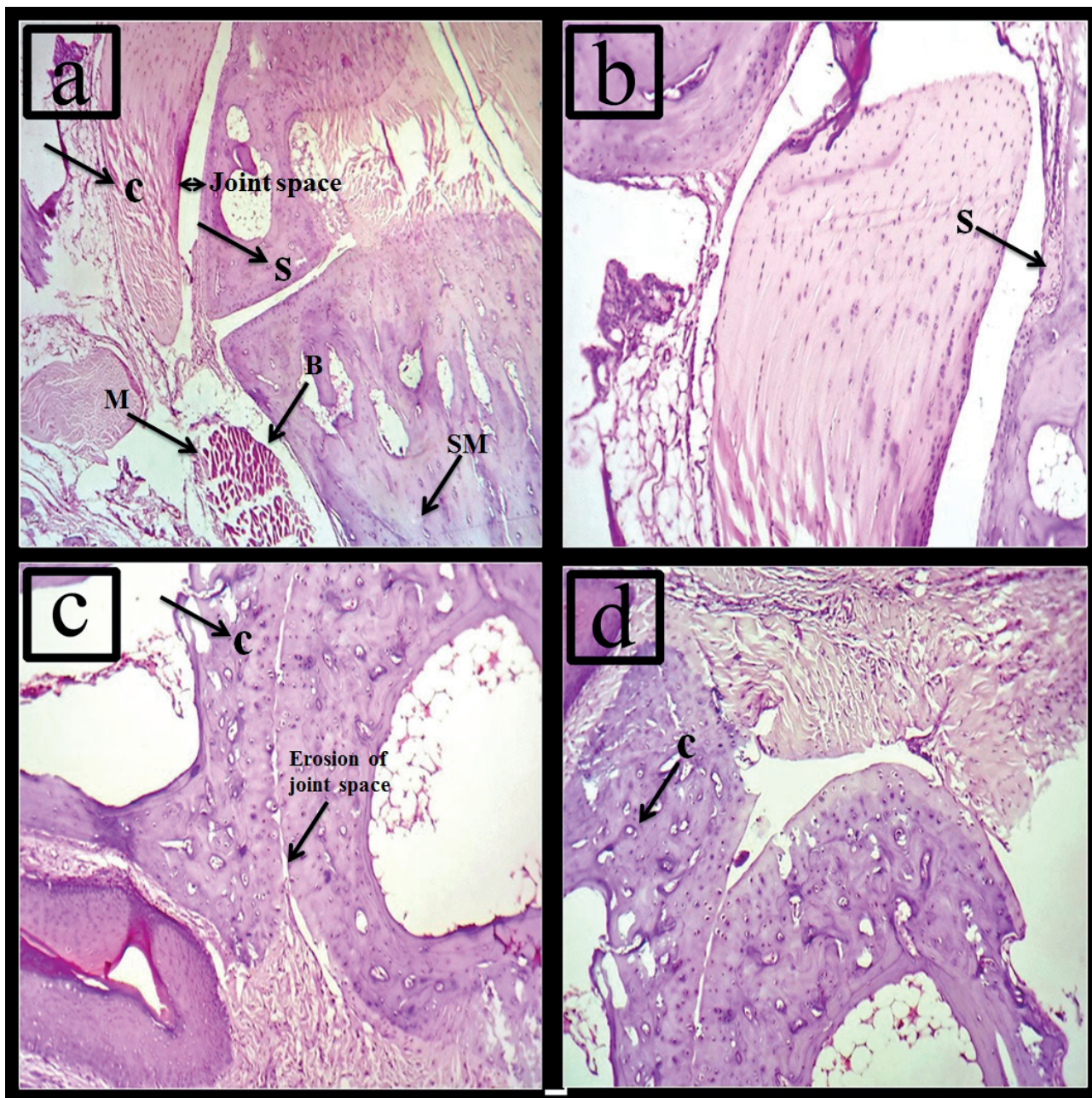


FIGURE 7 - Effect of β -carotene on paw histopathology of MSU crystal-induced arthritic rats
Haematoxylin and eosin staining: (7a) Normal appearing articular cartilage (C) and synovial tissue (S). Bone (B) and marrow (M) and adjacent skeletal muscles (SM) also appear normal (40X magnification); (7b) articular cartilage showing synovial hyperplasia (S) (100X magnification); (7c) Diminished joint space with erosion of the articular cartilage (100X magnification); (7d) Minimal inflammation in the adjacent joint capsule (C) (100X magnification).

DISCUSSION

Gout is a metabolic disorder associated with nucleic acid metabolism. It occurs due to the deposition of monosodium urate crystals in the synovial fluids of joints. This causes intense inflammation when left untreated. The high inflammatory urate crystals start culminating in the tissues resulting in hyperuricemia (Lemos Lima *et al.*, 2015; de Souza *et al.*, 2012). During the onset of this disease MSU crystal are phagocytosed by neutrophils in synovial fluid which thereby release intermediary factors involved in amplifying inflammatory reaction. In this study, when arthritis affected rats were treated with β -carotene and indomethacin it increased the body weight of the rats. Our study shown the decreased body weight in MSU-induced groups which was due to malnutrition.

MSU crystal-induced rats showed increasing levels of paw volume and lysosomal enzymes. Lysosomal enzymes play a vital role in inflammatory process. Rupturing of lysosomal enzymes is followed by discharge of hydrolytic enzymes. This process stimulates the synthesis of several inflammatory mediators like thromboxane, prostaglandins and leukotrienes (Sabina *et al.*, 2011). It is essential that the activities of lysosomal enzymes need to be stabilised to reduce the paw volume. Current study emphasises on the effect of β -carotene in the treatment of MSU crystal-induced gouty arthritis. β -carotene helps reduce the swelling of paw and stabilises lysosomal enzyme activities to near normal (Bai *et al.*, 2005). β -carotene might have reduced leukocyte migration from circulatory system into synovial cavity which is evident from histological examination (Jones *et al.*, 1991). Previous studies have shown that monocytes mature to macrophages and their response to MSU crystals to changes from pro-inflammatory to inflammatory and releases a potent anti-inflammatory cytokine for MSU induced inflammation (Orlowsky *et al.*, 2014). β -carotene will able to oxidizes the reactive oxygen species like ROO^* , $^1\text{O}_2$ and O_2^* that damage the DNA strands by rapid activation of nuclear enzyme (PARS) poly-ADP-ribosyl synthetase and also oxidized other free radical such as (NO_2^*) nitrogen dioxide, (RS^*) thiyl, (RSO_2^*) thiyl sulphonyl and (CCl_3OO^*) Halogenated peroxy radicals (Everett *et al.*, 1999). β -carotene is an antioxidant which is derived from food supplements further converted to vitamin A in our body and it is inversely proportional to the uric acid and creatinine concentration. In our study, the increased

level of uric acid and creatinine in MSU-induced group was normalized by administration of β -carotene group (Choi *et al.*, 2012). Neutrophils and macrophages are triggered to release large amounts of inflammatory cytokines after phagocytosis. Oxidative stress is high in gouty arthritis and the factors that cause might be due to hyperuricemia. β -carotene has antioxidant properties that can help neutralize the free radicals formed and reactive oxygen species potentially damaging lipids in cell membrane of MSU induced rats. It regulates the lipid peroxidation and antioxidant enzymes in liver and spleen (Sabina *et al.*, 2012).

CONCLUSION

Current study reveals the effects of β -carotene in minimizing inflammation in MSU crystal-induced gouty arthritis in Wistar albino rats. We have reported that β -carotene treatment was favourable at a dosage level of 10 mg/kg/bw had better result indicating that it (i) reduced paw oedema significantly (ii) stabilises lysosomal enzymes back to normal and increased antioxidant enzymes (iii) reduced the extend of lipid peroxidation and serum parameters (uric acid, creatinine and the cytokines $\text{TNF-}\alpha$, $\text{IL-1}\beta$). (iv) minimized histopathological changes in rat paw. Further molecular studies on analysing the other cytokines like IL-6 , CASPASE and COX are essential to understand the complete mechanism of β -carotene in attenuating MSU crystal-induced gouty arthritis.

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

ACKNOWLEDGEMENT

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