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# L-Carnitine ameliorates knee lesions in mono-iodoacetate induced osteoarthritis in rats



Samia Mohamed Ali<sup>a</sup>, Amr Abdel Kader Okda<sup>a</sup>, Iman Samy Dessouky<sup>b</sup>,  
Wafaa Ahmed Hewedy<sup>a</sup>, Noha Mahmoud Zahran<sup>c</sup>, Butheina Abdul-wally  
Alamrani<sup>a,\*</sup>

<sup>a</sup> *Clinical Pharmacology, Faculty of Medicine, University of Alexandria, Egypt*

<sup>b</sup> *Medical Biochemistry, Faculty of Medicine, University of Alexandria, Egypt*

<sup>c</sup> *Histology & Cell Biology, Faculty of Medicine, University of Alexandria, Egypt*

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## KEYWORDS

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IL-1 $\beta$ ;  
COMP

**Abstract Objective:** To evaluate the chondroprotective effect of L-carnitine in relation to glucosamine sulfate and in an experimental model of osteoarthritis (OA).

**Materials and methods:** Thirty-two adult male Wister albino rats weighing 150–210 g were assigned randomly into 4 groups: 8 rats in each group, group I (control group), group II (MIA induced OA group), group III (MIA induced OA + glucosamine sulfate treated group), and group IV (MIA induced OA + L-carnitine treated group). Weight, knee diameter, and knee bend score were recorded on days 0, 1, 7, 14 and 28. On day 28 all animals were sacrificed. Synovial fluid of left knee was collected, and the interleukin-1 $\beta$  (IL-1 $\beta$ ), Cartilage oligomeric matrix protein (COMP) and matrix metalloproteinase-13 (MMP-13) levels were measured by ELISA. The knee joints were removed and stained with H&E for histological evaluation.

**Results:** The pathological abnormalities attributed to MIA induced arthritis was dramatically lowered in rats treated with glucosamine or L-carnitine. Synovial fluid levels of IL-1 $\beta$ , COMP and MMP-13 were increased in OA group, and significantly reduced with glucosamine or L-carnitine treated groups.

**Conclusion:** L-Carnitine has a potential chondroprotective effect in this animal model of OA.

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## 1. Introduction

Osteoarthritis<sup>1</sup> is one of the most common forms of degenerative joint disease and a major cause of pain and disability affecting the aging population. Several factors including genetic susceptibility, obesity, injuries and inflammation of the joint have been long considered as important risk factors of the disease.<sup>2</sup>

\* Corresponding author at: Camp Ceasar, Egypt. Tel.: +20 1277640991.

E-mail address: [butheinamrani@yahoo.com](mailto:butheinamrani@yahoo.com) (B.A.-w. Alamrani).

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Under normal conditions, a dynamic equilibrium between synthesis and degradation of extracellular matrix [ECM] components is maintained.<sup>3</sup> In osteoarthritic states, however, a disruption of matrix equilibrium leads to cartilage degradation, induction of oxidative states, and eventually, apoptosis of chondrocytes.<sup>4</sup> The triggering events and exact pathologic mechanism that result in cartilage loss and degradation are not completely understood. It has been suggested that inflammatory mediators such as cytokines [IL-1 $\beta$ , TNF $\alpha$ , IL-6, IL-8, and IL-17], chemokine and reactive oxygen species<sup>5</sup> have a primarily destructive impact on articular cartilage.<sup>3</sup> These inflammatory mediators lead to increase synthesis and release of matrix metalloproteinases [MMPs] and cartilage degradation.<sup>6</sup>

Analgesics and non-steroidal anti-inflammatory drugs [NSAIDs] are the main therapeutic treatment options for OA. Unfortunately, these medications are short-term and fail to adequately address pathophysiological and biochemical mechanisms involved with cartilage degeneration and the induction of pain in arthritic joints.<sup>7</sup> The search for effective treatment/dietary supplements able to slow the progression of disease seems warranted.

L-Carnitine [LC] [3-hydroxy-4-N-trimethylaminobutyrate], the bioactive form of carnitine, is an endogenous branched nonessential amino acid derivative that plays a critical role in energy production. It transports long-chain fatty acids from the cytoplasm to mitochondria so they can be oxidized to produce energy.<sup>8</sup> L-Carnitine bioavailability is 5–18%<sup>9</sup> and eliminated from the body mainly via urinary excretion.<sup>10</sup> L-Carnitine has been shown to offer a great therapeutic potential against several chronic conditions including cardiovascular, diabetes, neurodegenerative, and inflammatory diseases.<sup>11</sup> High dose can cause nausea, vomiting, abdominal cramps, diarrhea, a “fishy” body odor and rarely muscle weakness and seizures.<sup>12</sup>

Glucosamine [GA] naturally occurring 6-carbon amino sugar, normally found in the body.<sup>13</sup> The oral bioavailability of glucosamine is about 26%. It is distributed to liver, kidney and other tissues including articular cartilage.<sup>14</sup> The mechanism of action of glucosamine is unknown. Glucosamine was demonstrated to reverse the deleterious effects of IL-1 $\beta$ , nuclear factor- $\kappa$ B and prostaglandin E2.<sup>15</sup> Glucosamine appears to be safe, with possibility of causing allergic reaction and possibly increasing the risk of developing diabetes.<sup>16</sup>

Unilateral intra-articular [i.a.] injection of mono-iodoacetate<sup>17</sup>, a chondrocyte glycolytic inhibitor, has been used to induce osteoarthritis-like changes in the articular cartilage of rodents. This minimally invasive model reproduces cartilage lesions with loss of proteoglycan matrix and functional joint impairment similar to human OA.<sup>3</sup>

In this study, knee bend test, histological examination of knee joints, in addition to synovial fluid levels of interleukin-1 $\beta$  [IL-1 $\beta$ ], and matrix metalloproteinase-13 [MMP-13] were estimated to evaluate the effect of L-carnitine, as compared to glucosamine sulfate,<sup>18</sup> in a rat model of MIA induced OA.

## 2. Materials and methods

### 2.1. Animals

Thirty-two adult male Wister rats weighing 150–210 g at the start of the experiment were purchased from Moassat Animal

House [Faculty of Medicine, Alexandria University]. Animals were fed standard rat chow with free access to water, and were acclimatized for 2 weeks before the experiment. The study protocol was approved by the Ethics Committee, Faculty of Medicine; Alexandria University, Egypt.

### 2.2. MIA-induced OA

OA was induced in rats [pre-anesthetized with ether] by a single intra-articular injection of 2 mg of mono-iodoacetate [MIA, Sigma-Aldrich, St. Louis, MO, USA] through the infrapatellar ligament into the joint space of the left knee, in a total volume of 25  $\mu$ l saline, via a 26.5-G needle.<sup>19</sup> Control rats were injected with an equivalent volume of saline.

### 2.3. Animal grouping

Rats were assigned randomly into four groups, 8 rats in each group, as follows: Group I [control group], group II [MIA induced OA group], group III [MIA induced OA/glucosamine sulfate, 250 mg/kg/day, treated group],<sup>20</sup> and group IV [MIA induced OA/L-carnitine, 100 mg/kg, treated group].<sup>21</sup>

### 2.4. Knee diameter

Knee diameter was measured using calibrated digital caliper [World Precision Instruments, Stevenage, UK] in millimeter [mm] to assess the developmental stages of OA on days 0 [pre MIA injection], 1, 7, 14, 21, and 28 [post injection].<sup>22</sup>

### 2.5. Knee bend test

The Knee-bend test was done at days 0, 1, 7, 14, 21 and 28 to evaluate the movement-induced pain caused by MIA. Briefly, we recorded the squeaks and/or struggle reactions in response to five alternate flexion and extensions of the knee joint [performed within the physiological limits of knee flexion/extension] for each rat. The score of the test was determined as follows: 0 – no responses; 0.5 – struggle to maximal flexion/extension; 1 – struggle to moderate flexion/extension or vocalizations to maximal flexion/extension; 2 – vocalizations to moderate flexion/extensions. The sum of the animal's reactions, giving maximal values of 20, represents the Knee-Bend score, an indication of the animal's movement-induced nociception. The contralateral knee was always tested first, in order to avoid an increase in the contralateral score arising from the manipulation of the injected knee. Results for both ipsilateral and contralateral knees were presented.<sup>23</sup>

### 2.6. Biochemical analysis

On day 28, rats were anaesthetized with Thiopental sodium 40 mg/kg<sup>24</sup> intraperitoneally.<sup>18</sup> The left limb of the rat was flexed over a 20 ml glass vial, then 23-gauge needle was inserted, and the limb was secured in place with tape. Sterile saline was infused intra-articularly. 2 min after infusion of 100  $\mu$ l of saline, the outflow fluid was aspirated. Synovial fluid was infused and withdrawn at a constant rate until a 400  $\mu$ l basal sample was collected in a 1.5 ml centrifuge tube. Samples were immediately centrifuged, and the supernatants were

collected and frozen at  $-20^{\circ}\text{C}$ .<sup>5</sup> The following parameters were estimated in the synovial fluid using ELISA kits: [IL-1 $\beta$ ] [BMS630, eBioscience, Inc., Vienna, Austria], [MMP-13] [SCA099Ra Uscon Life Science Inc., Houston, USA], and Cartilage oligomeric matrix protein [COMP] [SEB197Ra, Uscon Life Science Inc., Houston, USA].<sup>4,25</sup>

Briefly, micro well strips for IL-1 $\beta$ , MMP-13, and COMP were washed with wash buffer. Standard curve solution was added to standard curve wells, 50 ml of sample serum + 50 ml sample diluent and 50 ml of biotin conjugate were mixed together [mixture A] and added to the wells and incubated for 2 h at room temperature. Mixture A was removed, wells were washed and 100 ml of diluted Streptavidin-HRP [mixture B] was added and incubated for 1 h at room temperature with gentle agitation. Mixture B was removed, wells were washed and 100 ml of TMB substrate solution was added to the wells and incubated for 20 min at room temperature with gentle agitation until the highest standard curve point had developed [dark blue color]. Enzymatic reaction was stopped by the addition of 100 ml of stop solution. Plates were read on a spectrophotometer using 450 nm reference wavelengths, and cytokine concentration was determined from the standard curve. All samples and standards were run in triplicate.

### 2.7. Histopathological analysis

The knee joints of the rats were excised, and the soft tissues around it were removed. The knee joints were fixed in 10% buffered formalin, decalcified in hydrochloric acid, and embedded in paraffin. Sections [10  $\mu\text{m}$  in thickness] were stained with hematoxylin and eosin [H&E].<sup>26</sup>

### 2.8. Statistical analysis

Values are expressed as the mean  $\pm$  SD. Statistical analyses were done using the *Statistical Package of Social Sciences* [SPSS] version 20. The IL-1 $\beta$ , COMP, MMP-13 levels were tabulated and analyzed statistically by one-way ANOVA, followed by a *Post Hoc* [Scheffe] test to compare variables between different groups. While the weight, knee diameter

and knee bend score were tabulated and analyzed statistically using repeated measures ANOVA test. *p* values of less than 0.05 were considered significant.<sup>27</sup>

## 3. Results

### 3.1. Weight

Rats in group II, III and IV had a decrease in weight gain compared to control group, in first week post-MIA induction. On day 28 all studied groups had almost same weight.

### 3.2. Knee diameter, Table 1

There was a significant increase in left knee diameter in groups II, III and IV as compared to normal control group [ $p < 0.05$ ]. The maximal diameter was recorded on day 7 post-MIA injections. Treatment with GA or LC resulted in significant decrease in left knee diameter as compared to non-treated OA-induced group.

### 3.3. Knee bends score, Table 1

No sign of spontaneous nociceptive behavior or distress was found before MIA injection. Meanwhile, there was a significant increase in knee bend score in groups II, III and IV as compared to their baseline values, and to the normal control values [ $p < 0.05$ ]. Treatment with GA or LC resulted in non significant decrease, in day 14, in knee bend score when compared to OA induced group.

### 3.4. Biochemical markers

#### 3.4.1. IL-1 $\beta$ level, Table 2

IL-1 $\beta$  level was significantly increased in S.F from animals in group II as compared to group I [ $149.3 \pm 3$  pg/ml,  $56.2 \pm 19.7$  pg/ml respectively,  $p < 0.05$ ]. Meanwhile, statistically significant lower levels of IL-1 $\beta$  were obtained when MIA treated rats received either glucosamine or L-carnitine

**Table 1** Left knee diameter (mm), and knee bend score, for the studied groups.

	Groups	Days				
		1	7	14	21	28
Left knee diameter (mm)	I	0.917	0.917	0.918	0.918	0.918
	II	0.99	1.183 <sup>a</sup>	1.133 <sup>a</sup>	1.117 <sup>a</sup>	1.067 <sup>a</sup>
	III	0.914	1.014 <sup>ab</sup>	1.013 <sup>ab</sup>	1.01 <sup>ab</sup>	1 <sup>ab</sup>
	IV	0.989	1.011 <sup>ab</sup>	1.056 <sup>ab</sup>	1.033 <sup>ab</sup>	1.033
	<i>p</i> value	0.4	< 0.05*	< 0.05*	< 0.05*	< 0.05*
	F	0.99	3.48	5.412	3.772	3.387
Knee bend score	I	1.7	1.7	1.6	1.6	1.6
	II	4.2	14.2 <sup>a</sup>	5.83 <sup>a</sup>	5.8 <sup>a</sup>	4.17
	III	1.5	5.57 <sup>ab</sup>	5.43 <sup>a</sup>	5.71 <sup>a</sup>	2.14
	IV	3.89	5.56 <sup>b</sup>	5.56	4.44	3.33
	<i>p</i> value	0.3	< 0.001*	0.08	< 0.05*	0.28
	F	1.33	14.245	1.738	4.334	1.35

Note: Group I, normal control group; Group II, untreated MIA-induced OA rats; Group III, MIA-induced OA rats treated with glucosamine; Group IV, MIA-induced OA rats treated with carnitine; F, ANOVA test; \* indicates statistical significance; a, significant with normal group; b, significant with group II.



**Table 2** Levels of IL-1 $\beta$  (pg/ml), COMP (ng/ml), and MMP-13 (ng/ml) in the studied groups.

Parameter	IL-1 $\beta$ (pg/ml)	COMP (ng/ml)	MMP-13 (ng/ml)
Group I	56.2 $\pm$ 19.7	53.1 $\pm$ 14	2.4 $\pm$ 1
Group II	149.3 $\pm$ 3 <sup>a</sup>	188.2 $\pm$ 21.7 <sup>a</sup>	9 $\pm$ 3.6 <sup>a</sup>
Group III	65.2 $\pm$ 17.7 <sup>b</sup>	150.4 $\pm$ 9.6 <sup>b</sup>	3.1 $\pm$ 1.1 <sup>b</sup>
Group IV	76.2 $\pm$ 17 <sup>b</sup>	108.9 $\pm$ 15.2 <sup>b</sup>	3.4 $\pm$ 1.2 <sup>b</sup>
<i>p</i> -value	< 0.001*	< 0.001*	< 0.001*
<i>F</i>	43	71	13

Note: Group I, normal control group; Group II, untreated MIA-induced OA rats; Group III, MIA-induced OA rats treated with glucosamine; Group IV, MIA-induced OA rats treated with carnitine; *F*, ANOVA test.

\* Indicates statistical significance.

<sup>a</sup> Significant with group I.

<sup>b</sup> Significant with group II.

[65.1  $\pm$  17.7 pg/ml, 76.2  $\pm$  17 pg/ml respectively,  $p$  < 0.05 as compared to group II].

#### 3.4.2. COMP level, Table 2

COMP, a marker of joint destruction, was dramatically increased in cartilage from group II rats [171.2  $\pm$  26.6 ng/ml] as compared to group I [53.1  $\pm$  14 ng/ml]. Treatment with glucosamine sulfate or L-carnitine caused a significant decrease in COMP levels [150.4  $\pm$  9.6 ng/ml and 108.9  $\pm$  15.2 ng/ml respectively  $p$  < 0.05 when compared to group II].

#### 3.4.3. MMP-13 level, Table 2

There was a significant increase in MMP-13 level in cartilage in group II [9  $\pm$  3.6 ng/ml] as compared to group I [2.4  $\pm$  1 ng/

ml], whereas treatment with glucosamine sulfate or L-carnitine significantly abrogated this increase [3.1  $\pm$  1.1 and 3.4  $\pm$  1.2 respectively,  $p$  < 0.05 vs group II].

#### 3.5. Histological findings, Figs. 1 and 2

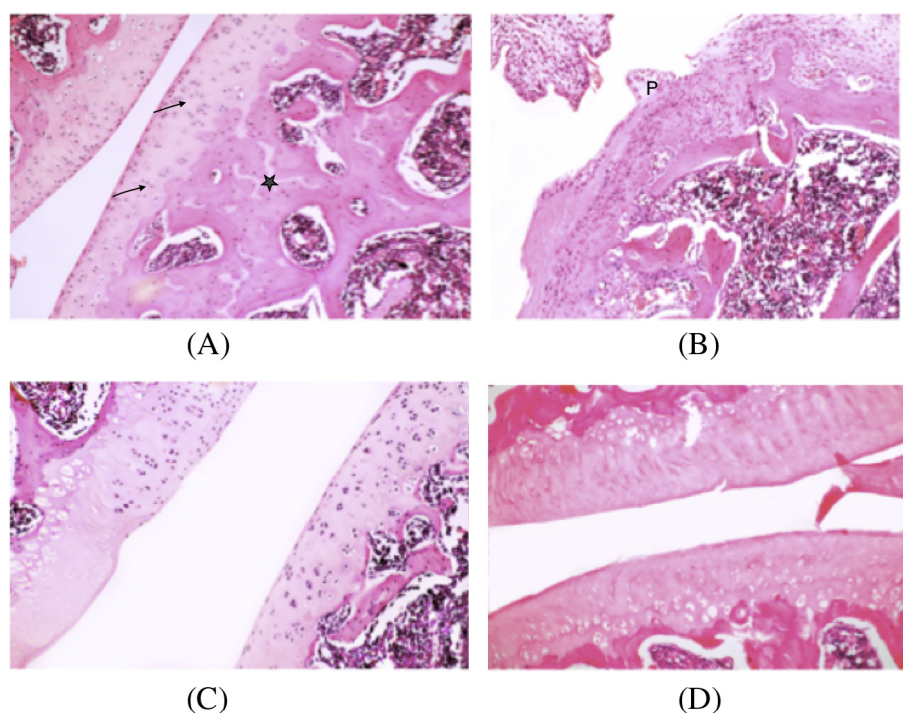
The isolated knee joints from the normal control group showed a smooth surface of the articular cartilage, with normal cellularity. In contrast, the joints from the MIA-induced OA rats showed joint space narrowing decrease in cartilage thickness, with irregular fibrillated surface, abnormal matrix intensity and chondrocytes.

Histological signs of cartilage degradation were reduced in GS, or LC treated groups. Preservation of cartilage smooth surface with almost normal thickness and matrix intensity was observed in both groups, and subchondral bone appeared with increased vascularization.

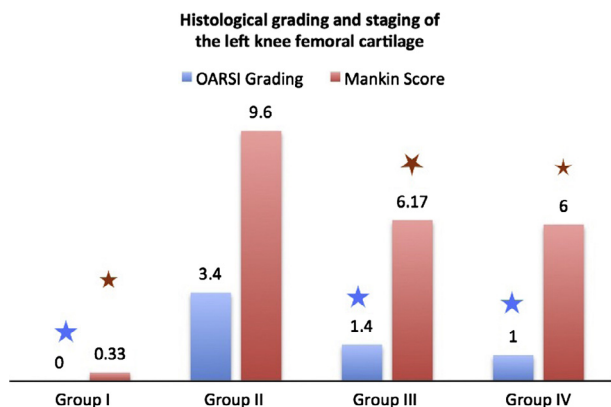
As shown in Fig. 1 [A] Group I light photomicrograph of rat knee joint of control group shows cartilage with smooth non-fibrillated superficial region, matrix and scattered chondrocytes [†]. Subchondral bone appears with more basophilic staining matrix [\*] enclosing bone trabeculae. [H&E stain. Mic. Mag.  $\times$ 100.]

[B] Group II light photomicrograph of rat knee joint of induced osteoarthritis group reveals significant difference in articular cartilage thickness with irregular fibrillated surface, abnormal matrix intensity and chondrocytes. Subchondral bone shows irregularity in matrix and shape. Notice pannus formation [P] a hyperplastic synovial villus extends over the surface of the articular cartilage as a fibrous inflammatory membrane. [H&E stain. Mic. Mag.  $\times$ 100.]

[C] Group III light photomicrograph of rat knee joint of glucosamine sulfate treated group depicts recovery of knee joint with preservation of cartilage smooth surface with normal



**Figure 1** Histological findings of all studied groups.



**Figure 2** Histopathologic evaluation of knee cartilage from control (group I), MIA-induced OA (group II), GS (group III) and LC (group IV). OARSI grading range from 1 to 6, where 1 is normal cartilage and 6 total loss of cartilage. Total mankin score is a sum of the scores for cartilage structure, cellular abnormalities, tideline and matrix Staining with toluidine blue, and total score of 15 means severe damage of cartilage while 0 means normal cartilage. According to OARSI and mankin score, both GS an LC groups show significant difference when compared to group II ( $p < 0.05$ ) \* = significant difference compared to group II.

thickness and matrix intensity. Subchondral bone appears with increased vascularization. [H&E stain. Mic. Mag.  $\times 100$ .]

[D] Group IV light photomicrograph of rat knee joint of L-carnitine treated group depicts partial recovery of knee joint with preservation of cartilage smooth surface but decreases in its thickness and matrix intensity. Subchondral bone appears with increased vascularization. [H&E stain. Mic. Mag.  $\times 100$ .]

#### 4. Discussion

Osteoarthritis is a degenerative joint disease characterized by the progressive loss of cartilage, changes in subchondral bone, and chronic pain. Analgesics and non-steroidal anti-inflammatory drugs [NSAIDs] are the main therapeutic treatment options for OA. The search for treatments able to slow the progression of disease is a great challenge and would meet an important medical need for patients with OA.

The development and progression of OA are now believed to involve inflammation, even in the early stage.<sup>1</sup> Proinflammatory cytokines and chemokine play critical roles in disturbed homeostasis in the OA cartilage matrix. Interleukin-1 $\beta$  [IL-1 $\beta$ ] among them plays a crucial role in the downregulation of the synthesis of major ECM components by inhibiting anabolic activities and increasing catabolic activities in chondrocytes.<sup>28</sup> Furthermore, IL-1 $\beta$  decreases the synthesis of a number of cytokines which contribute to inflammation, including IL-6 and IL-8.<sup>6</sup>

The MIA-induced OA animal model used in the present study is a well-characterized model and mimics pain and biochemical/structural changes associated with human OA. In our study, we demonstrated that the synovial fluid level of IL-1 $\beta$  was increased in the untreated MIA induced OA group. On the other hand, synovial fluid levels of IL-1 $\beta$  were significantly reduced after treatment with GA or LC. These results demonstrated an anti-inflammatory effect of GA, and LC.

The anti-inflammatory effects of LC are previously shown in different animal models of inflammation.<sup>29</sup>

Parallel to acute and chronic inflammatory processes, the pathophysiology of OA development includes an active biological process of matrix degradation, leading to the release of MMPs and eventually cartilage loss. MMPs can degrade native fibrillar collagens of types I, II, III, V, and XI, being involved in various pathologic conditions.<sup>30</sup> Many studies have reported increased activities of MMP-3 and MMP-13 in human OA cartilage as well as experimental animal models of OA. IL-1 $\beta$  was shown to diminish the expression of type 2 collagen and aggrecan, and increases the expression of MMP-1, -3, and -13.<sup>30</sup>

In accordance with previous studies,<sup>31</sup> we found that the synovial fluid level of MMP-13 was elevated in the untreated MIA induced OA group. Meanwhile, the levels of MMP-13 were significantly inhibited by GA, LC treatment; suggesting the potential of these agents as one of the DMOADs in OA treatment.

Cartilage oligomeric matrix protein is one of the essential components of the extracellular matrix of the cartilage. COMP regulates the cellular proliferation, apoptosis and cellular attachment in the cartilaginous tissue.<sup>32</sup> COMP has shown promise as a potential biomarker for monitoring progression of cartilage destruction, for evaluating cartilage effects of therapy, and as a prognostic tool reflecting cartilage damage.<sup>33</sup> Serum COMP levels can be detected quite early during the inflammatory process, in the synovial fluid and in the bloodstream, even before radiographic joint lesions and irreversible damage appear.<sup>32</sup>

The elevation of COMP in MIA induced OA group was concomitant with that of other animal models of arthritis.<sup>34</sup> Joosten et al., previously demonstrated that therapeutic intervention which ameliorated cartilage destruction normalized serum COMP levels in murine CIA, whereas treatment which only reduced signs of inflammation did not affect COMP levels.<sup>35</sup>

Glucosamine is one of the most frequently used alternatives worldwide due to their chondroprotective properties. Despite being considered effective by many research groups, controversy surrounds the use of glucosamine in OA. In long-term studies, no benefit of glucosamine for pain was found. The dose used in animal model was found to be much higher than that obtained through the oral ingestion of supplements.<sup>36</sup> Furthermore, in obese and diabetic patients glucosamine may cause alteration in glucose metabolism.<sup>36</sup>

In conclusion, L-carnitine is shown to be as effective as glucosamine in lowering IL-1 $\beta$  [biochemical marker of inflammation], MMP-13 and COMP [markers of cartilage destruction] in OA induced rat model. These results suggest a promising use of L-carnitine as a potential DMOAD.

#### Conflict of interest

We have no conflict of interest to declare.

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