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RESEARCH ARTICLE

# THE EFFECT OF *BAUHINIA VARIEGATA* ETHANOLIC EXTRACT ON MONOSODIUM IODOACETATE-INDUCED OSTEOARTHRITIS IN MALE ALBINO RATS

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## ABSTRACT

Osteoarthritis (OA) is characterized by the breakdown of cartilage in joints. Most currently-used drugs to treat OA induce gastrointestinal troubles and renal impairment. The current study investigated the alleviative effects of the ethanolic crude extract of *Bauhinia variegata* (BV) leaves on monosodium iodoacetate (MIA)-induced OA. Ten groups of male Wistar albino rats (*Rattus norvegicus*; 5 rats/group) were randomly allotted into the control group that (G1) received distilled water; groups treated orally/daily with 200, 400, or 600 mg of BV extract/kg body weight, or the reference drug (250 mg glucosamine sulfate/kg body weight) for 4 weeks (G2-G5, respectively); group treated with 40 mg MIA/mL once by intra-articular injection (G6); and groups treated with MIA + the different doses of BV extract or the reference drug (orally/daily for 4 weeks) after 4 weeks of MIA injection (G7-G10, respectively). The results showed significant increases in serum calcium and phosphorus levels, and significant decreases in the activity of serum alkaline phosphatase, the levels of serum interleukin-6, prostaglandin E2, leptin, and type II collagen, as well as the levels of cyclooxygenase 2 and inducible nitric oxide synthase in knee joints**,** in the MIA groups treated with BV extract (in a dose-dependent manner) compared with the MIA group. The X-ray imaging showed a significant increase in joint space in the MIA groups treated with the high doses of BV extract compared with the MIA group. The present study suggested that BV extract may be promising in the treatment of OA.

## INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease that mostly affects older people's weight-bearing joints, especially the knees and hips[1]. The slow breakdown of articular cartilage and the joint space narrowing that characterize OA are responsible for the excruciating pain that patients experience during weight-bearing activities, which in turn limits their range of motion and has a negative impact on their quality of life[2]. Inflammation is responsible for the structural changes that take place during OA progression, as mechanical stress results from stimulation of pro-inflammatory cytokines[3]. These cytokines are produced in the syno-

vium by chondrocytes inducing extracellular matrix degeneration, as well as chondrocytes apoptosis[4].

In fact, there is no fundamental treatment for OA, most therapy theories depend on decreasing inflammation by non-steroidal anti-inflammatory drugs (NSAIDS), pain management and replacing joints in severe cases[1,5]. Unfortunately, frequency of drugs intake caused undesirable effects including gastric, renal, and hepatic problems[6]. Therefore, new drugs are required that not only decline the pain or stop/limit degeneration, but also treat structural joint degeneration with less/no side effects. Thus, traditional herbal medicinal sources have been widely studied as alternative therapeutic agents to improve joint structure and function and kill pain as well[7].

Among the Fabaceae family of plants, *Bauhinia variegata* Linn. (BV) is highly regarded for its traditional therapeutic uses. The BV plant is native to many Asian nations, including India, Pakistan, South China, Thailand, Vietnam, Cambodia, Laos, and Burma[8], and its tree reaches a height of 20 to 30 feet. Mountain ebony, orchid tree, Kachnar (Hindi), Raktakanchan (Marathi) (English) is other names for BV[8]. Traditionally, it is used for ulcers and skin diseases treatment[9], while its roots are used as snake anti-venom[10]. This plant is also used for treating several diseases, as inflammation[11]. BV has been reported to have many biological activities including antioxidant, anti-cancer, antibacterial, and antiarthritic, besides, its hepatoprotective effect[12,13]. However, the BV's anti-inflammatory effect against OA has not been investigated so far. Thus, this study intended to estimate the possible anti-inflammatory effect of the ethanolic crude extract of *Bauhinia variegata* against monosodium iodoacetate (MIA)-induced OA in male albino rats.

## MATERIALS AND METHODS

### Animals

Fifty mature male Wistar albino rats (*Rattus norvegicus*) weighing around 150-170 g were purchased from the Lab Animal House of the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. Rats were housed in a standard plastic cage (three rats/cage) under standard condition and maintained at room temperature (25.0±1.0ºC) with natural day light rhythm. Animals were kept two weeks for acclimatization before starting the experiment, fed on standard rodent pellets, and given water supply *ad libitum* during the experimental period.

### BV collection and extract preparation

In November and December of 2017, BV fresh leaves were collected from the garden of the Botany Department at the Faculty of Science at Suez Canal University. These leaves were then validated by a botanist from the Faculty of Science at Suez Canal University. Fresh leaves of BV were thoroughly washed in running water to clean it from dirt and dust, dried in shades for one week and then dried leaves were grinded using a mechanical grinder to get a fine powder. The obtained 3.5 kg of the dried material was extracted with solvent (ethanol 75%). The ethanolic extract was subjected to a vacuum and concentrated with a rotary flash evaporator. The extract was then labeled and weighed. The extract yield was calculated using the following formula[14]: Yield (% fresh weight) = [Weight dried sample (g) × 100] / [Weight fresh sample (g)]

### Phytochemical screening of BV crude extract

Based on the methods reported by Chien *et al*.[15], the extract were analyzed by gas chromatography-mass spectrometry (GC-MS) at Al-Azhar University's Regional Center for Mycology and Biotechnology, Cairo, Egypt. The GC was interfaced to a Polaris Q mass spectrometer (Thermo Finnigan, Hertfordshire, UK). The electron ionization device with ionization energy of 70eV was used for GC-MS detection. The carrier gas was helium gas with a constant flow rate of 1.21 mL/min and a purity level of 99.99 percent.

The injector temperature was set to 200ºC and the mass transfer line temperature was set to 240ºC. The oven was heated from 70 to 220ºC at a rate of 10ºC/minute, and then kept at a constant temperature for 1.0 minute, before being allowed to reach 300ºC after 10 minutes. Using a split ratio of 1:40 and a mass range of 50-650 atomic mass units, 2 µL of each diluted sample was manually injected in the split less mode (amu). The entire GC-MS process takes 50 minutes.

Peak area normalization was utilized to determine the relative abundance of the extract's constituents. Components were identified by first comparing their mass spectra to a spectrometer database (Wiley and NIST libraries were used as library sources to match the components found in the plant material) and then by determining their Kovats indices, which were determined by calculating the retention times of the components.

**Toxicity of BV crude extract**

The Lorke[16] technique was used to conduct an acute toxicity analysis of BV crude extract. Nine rats were randomly split into three groups of three animals, and each group received either 10, 100, or 1000 mg extract/kg body weight (orally through gavage) in the first phase of the study. After 24 hours, the rats were examined for any symptoms of illness or death. In the second stage, the process was carried out once more, this time with three rats. The rats were randomly split into three groups of one animal, and each group received either 1600, 2900, or 5000 mg extract/kg body weight[17]. For a whole day, the rats were tracked for any indications of toxicity or death.

**Induction of OA**

In accordance with Marker and Pomonis[18], OA was produced in male rats by intra-articular injections of MIA (Sigma-Aldrich, St. Louis, MO, USA) diluted in an isotonic saline to a final dosage of 40 mg/mL, using smaller syringes with 27 gauge needles. The powder or solution containing MIA must be handled with the proper safety equipment, as it is very poisonous. The redness, edema, and hypersensitivity to unpleasant stimuli were localized to the injected joint. Inflammation developed shortly after the injection and reached its peak between 12 and 24 hours later. MIA-inflamed rats had normal grooming and activity levels, and hyperalgesia appeared to have negligible impact on their regular behavior.

**Experimental design**

The experimental animals (n=50) were randomly allotted into 10 groups (5 rats/ group) as follow: The control group (G1) received distilled water; groups treated orally/daily with the low-dose (200 mg), moderate-dose (400 mg), and high-dose (600 mg) of BV extract/kg body weight (G2-G4, respectively) for 4 weeks; group treated orally/daily with the reference drug, 250 mg/kg glucosamine sulfate/kg body weight (G5) for 4 weeks; group treated with 40 mg MIA/mL once by intra-articular injection (G6) for OA induction; groups treated with MIA + the different doses of BV extracts (orally/daily for 4 weeks) after 4 weeks of MIA injection (G6-G9, respectively); group treated with MIA + the reference drug (orally/daily for 4 weeks) after 4 weeks of MIA injection (G10).

**X-ray imaging of the knee joint**

The degradation of cartilage and sub-chondral bones was evaluated by X-ray radiography at the end of the experimental period. All animals were generally anesthetized by intramuscular injection of a mixture of 5% ketamine and 0.05% xylazine. The animals were examined radiologically mediolateral position for the right stifle joint, the rats were laid in the supine position with 45° of hip flexion, using Listem X-ray generating tube (Listem machine with Fuji cr unit; Wonju-si, Gangwon-do, Republic of Korea) at Eliman scan laboratory, Ismailia, Egypt. Joint space width (JSW) measurements of radiographic images were achieved using a Java plug-in (Image J software, v. 1.47t, NIH Clinical Center, Bethesda, MD, USA).

**Blood and tissue sampling**

Blood samples were collected from retro-orbital plexus *via* capillary tube from animals for determination of the biochemical parameters[19]. The blood sampling was performed under effect of light diethyl ether anesthesia (Aledani et al., 2022)[20]. The samples were centrifuged at 1000 ×*g* for 10 minutes and the clear serum was separated carefully, collected, and stored in eppendorf tubes at −20ºC until estimation of serum chemistry. After that, rats were euthanized by an overdose of diethyl ether, then scarified and the whole knee joints were removed, some was fixed immediately with 10% formalin for histological examination and others were preserved in freezer for analysis of bio-markers in cartilaginous tissue. Photographs of all experimental groups were taken and the observations were blindly recorded. Small pieces of tissue were homogenized in phosphate-buffered saline (10 mg tissue/ 100 µL PBS). The homogenates were then centrifuged for 15 minutes at 2800 ×*g*. The supernatants were carefully collected and either analyzed right away or froze the samples in the freezer.

**Biochemical analysis**

Serum calcium level was assayed using a colorimetric calcium assay kit (Abnova, Taipei City; Taiwan; catalog number: KA0812), while serum phosphate level was measured by Atlas Medical assay kit (Cambridge, UK). Serum alkaline phosphatase (ALP) activity was assayed using QuantiChrom ALP assay kit (DALP-250; BioAssay Systems, Hayward, CA, USA). The serum interleukin-6 (IL-6), prostaglandin E2 (PGE2), leptin, collagen type 2 (CoL-2) levels were measured using commercially available ELISA kits specific for rats provided by MyBioSource, Inc., San Diego, CA, USA (catalog numbers: MBS355410, MBS705028, MBS701500, MBS728101, respectively). In addition, cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS) in knee joints were measured using kits provided by MyBioSource, Inc. (catalog numbers: MBS020734, and MBS723326, respectively).

**Histological examination**

The knee joints were dissected free of skin and muscle, placed in 10% formalin, then transferred to a decalcification solution (20% EDTA, for a week) on a plate shaker at room temperature. The samples were thoroughly rinsed and processed with graded alcohol dehydration then embedded frontally in paraffin and serially sectioned at 7 $\mu$m[21]. Tissue sections were stained with hemato-xylin and eosin (H&E), examined by light microscopy, and photographed.

**Statistical analysis**

One way ANOVA test was applied for the statistical analysis for the present data of all experimental groups according to the mathematical principles described by Campbell[22] followed by post-hoc comparisons (Tukey HSD test) using SPSS statistics version 20. The result was considered to be significant when $P<0.05$.

## RESULTS

**Phytochemical characterization using GC-MS**

The GC-MS chromatogram was able to identify thirty phytochemicals as constituents of the BV extract (Figure 1). Of these compounds, 9,12,15-octadeca-trienoic acid, ethyl ester, (Z,Z,Z) was the major compound (43.64%). This was followed by phytol (17.29%), nonadeca-diene (6.06%), heptacosane (CAS) (5.6%), squalene (3.95%), and stearic acid (3.86%) as shown in Table "1". The other chemical substances of BV extract comprised a pro-portion that was less than 2% (Table 1). The quantity of plant materials after they had been dried (291.55g) and pulverized was used to calculate the percentage of crude ethanol extract yield (3500g). The amount of crude ethanol extract obtained from BV leaves was 8.33% of the total yield.

**Biosafety of BV crude extract**

There was no mortality recorded in the 24 hours of observation period in all groups,

the crude extract at doses 10, 100, 1000, 1600, 2900, and 5000 mg extract/kg body weight had no significant adverse effect on male rats; they showed no change in the general appearance during the observation period. All of the doses were well-tolerated and exhibited normal behavior.

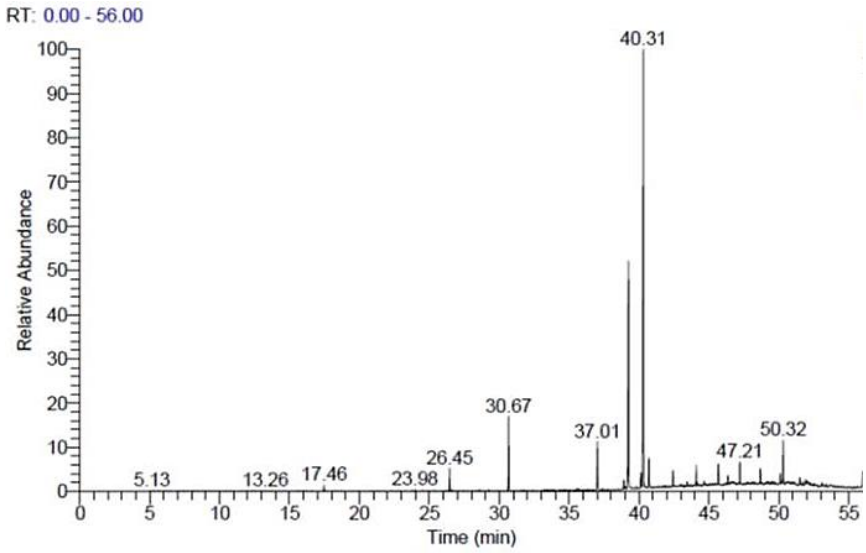


**Figure 1:** GC-MS chromatogram showing the phytochemical components of the ethanolic extract of *Bauhinia variegata* leaves.

**Table (1):** Phytocomponents identified in the ethanolic crude extract of *Bauhinia variegata* leaves by GC-MS.

| RT [a] Time (minute) | Compound Name | Molecular Formula | Molecular Weight | Peak Activity Area (%) |
|---|---|---|---|---|
| 17.46 | 2-Cyclohexylcyclohexanone | $C_{12}H_{20}O$ | 180 | 0.52 |
| 26.45 | Butylated hydroxytoluene | $C_{15}H_{24}O$ | 220 | 2.02 |
| 30.67 | Nonadecadiene | $C_{19}H_{36}$ | 264 | 6.06 |
| 37.01 | Stearic acid | $C_{18}H_{36}O_2$ | 284 | 3.86 |
| 38.91 | Methyl palmitoleate | $C_{17}H_{32}O_2$ | 268 | 1.00 |
| 39.23 | Phytol | $C_{20}H_{40}O$ | 296 | 17.29 |
| 40.14 | Linoleic acid ethyl ester | $C_{20}H_{36}O_2$ | 308 | 1.37 |
| 40.31 | 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z) | $C_{20}H_{34}O_2$ | 306 | 43.64 |
| 40.71 | Octadecanoic acid ethyl ester | $C_{20}H_{40}O_2$ | 312 | 2.56 |
| 42.43 | Tricosane (CAS) | $C_{23}H_{48}$ | 324 | 1.23 |
| 44.65 | Simvastatin acid | $C_{25}H_{40}O_6$ | 436 | 0.55 |
| 45.68 | Docosane (CAS) | $C_{22}H_{46}$ | 310 | 1.63 |
| 46.35 | Diisooctyl phthalate | $C_{24}H_{38}O_4$ | 390 | 0.80 |
| 47.21 | Heptacosane (CAS) | $C_{27}H_{56}$ | 380 | 5.6 |
| 50.32 | Squalene | $C_{30}H_{50}$ | 410 | 3.95 |
| 51.89 | 1-Heptatriacotanol | $C_{37}H_{76}O$ | 536 | 0.61 |
| 52.03 | 2-Octylcyclopropaneoctanal | $C_{19}H_{36}O$ | 280 | 0.80 |
| 53.10 | Dotriacontane (CAS) | $C_{32}H_{66}$ | 450 | 1.35 |

[a]RT: retention time of the chemicals determined based on peaks in the GC-MS spectrometer; substances are presented in the order of their elution from a TG5MS column.

**Effect of BV extract on X-ray radiography of knee joint of OA rats**

The control group showed normal appearance of intact articulation and normal joint space (Figure 2A). The MIA group had gradually progressing alterations to the knee joint, as damage in articular cartilage and bone surface with obvious degeneration of the tibial lateral plateau and narrow in joint space (NJS) compared with the control group (Figure 2B). Oral treatments of BV extract at 200, 400, and 600 mg/kg body weight were able to reduce alterations in rat knees of MIA-induced OA and showed significance improvement especially at the high dose of BV extract (Figures 2C-E). However, treatment of MIA rats with standard drug displayed mild improvement in knees compared with the MIA rats (Figure 2F).

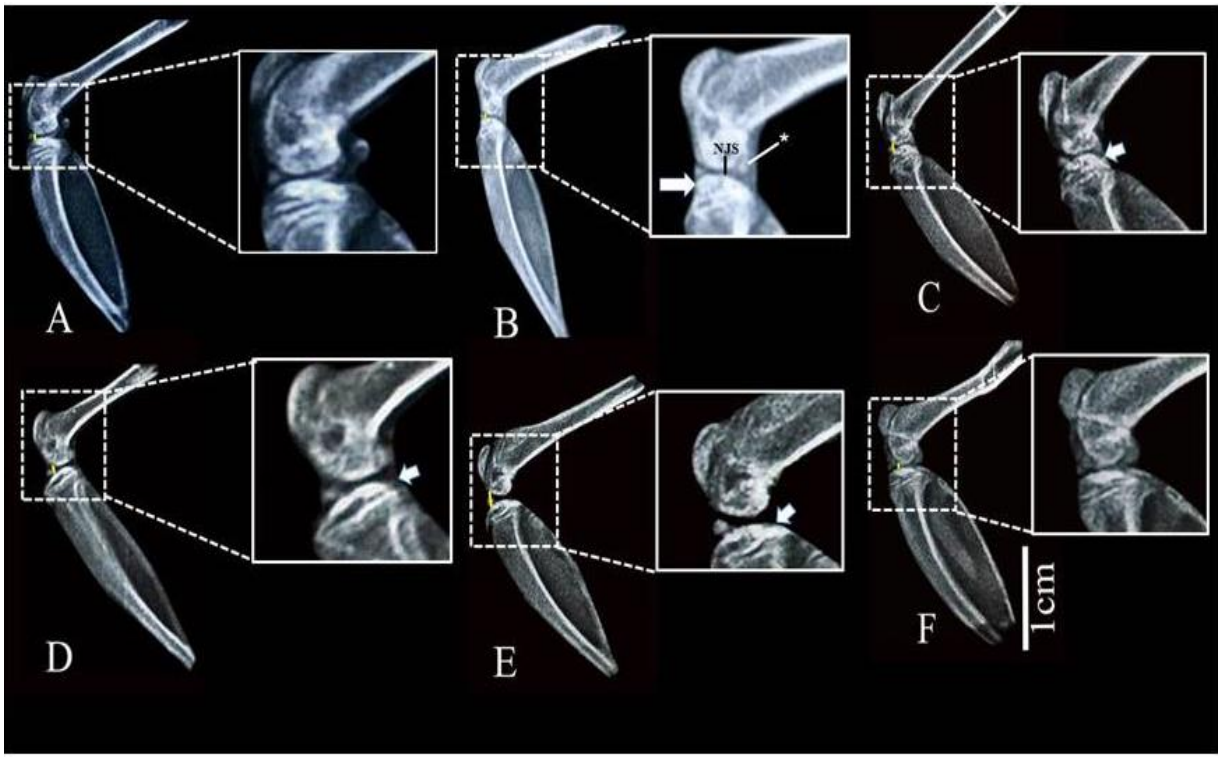


**Figure 2:** X-ray radiographic images from medial-lateral view of the right knee showing the efficacy of the ethanolic *Bauhinia variegata* (BV) extract in reducing the development of rat osteoarthritis. **(A)** Representative image for the control rat or rat treated with tested extract displayed intact articulation and normal joint space. **(B**) Obvious degeneration of the tibial lateral plateau (arrow) and narrow in joint space (NJS) besides development of bone spur in femoral condyles (*). The articular degeneration was reduced after 4 weeks of treatment with BV **(C)** 200 mg/kg body weight; **(D)** 400 mg/kg body weight, and **(E)** 600 mg/kg body weight as indicated by the arrow. **(F)** Monosodium iodoacetate-treated rats received the standard drug displayed mild improvement.

**Effect of BV extract on biochemical parameters of OA rats**

The results showed significant increases ($P$<0.05) in serum calcium and phosphorus levels in the MIA groups treated with BV extract (especially at the moderate and high doses) compared with the MIA group (Table 2). In addition, the results showed significant decreases ($P$<0.05) in the activity of serum ALP the levels of serum IL-6, PGE2, leptin, and type II collagen, as well as the COX2 and iNOS levels in knee joints, in the MIA groups treated with BV extract (especially at the moderate and high doses) compared with the MIA group (Tables 2 and 3).

**Table (2):** Effect of the ethanolic *Bauhinia variegata* (BV) extract on serum Ca, phosphate ($PO_4^{3-}$), leptin, interleukin-6 (IL-6) levels and the activity of alkaline phosphatase (ALP) in osteoarthritis (OA) male rats.

| Groups | | Ca (mg/dL) | $PO_4^{3-}$ (mg/dL) | ALP activity (U/L) | Leptin (ng/mL) | IL-6 (pg/mL) |
|---|---|---|---|---|---|---|
| Control | Mean±SE | 9.57±0.51 | 4.29±0.29 | 51.0±1.73 | 5.53±0.29 | 27.90±1.90 |
| OA | Mean±SE | 4.30±0.17[a] | 1.35±0.14[a] | 294.0±17.32[a] | 12.25±0.56[a] | 117.2±3.46[a] |
| | % of change[1] | −55.06% | −68.53% | +476.47% | +121.51% | +320.07 |
| BV (low-dose) | Mean±SE | 8.78±0.04 | 4.02±0.27 | 49.0±1.73 | 5.40±0.36 | 30.20±0.57 |
| | % of change[1] | −8.25% | −6.29% | −3.92% | −2.35% | +8.24% |
| BV (moderate-dose) | Mean±SE | 9.18±0.85 | 4.21±0.51 | 47.50±2.59 | 6.10±0.23 | 29.0±1.73 |
| | % of change[1] | −4.07% | −1.86% | −6.86% | +10.30% | +3.94% |
| BV (high-dose) | Mean±SE | 8.75±0.13 | 4.85±0.14 | 47.50±4.33 | 6.33±0.57 | 29.33±2.96 |
| | % of change[1] | −8.56% | +13.05% | −6.86% | +14.46% | +5.12% |
| Glucosamine sulfate | Mean±SE | 8.13±0.40 | 4.40±0.40 | 72.63±6.26 | 5.40±0.36 | 30.66±4.05 |
| | % of change[1] | −15.04% | +2.56% | +42.41% | −2.35% | +9.89% |
| OA+BV (low-dose) | Mean±SE | 5.08±0.34 | 2.63±0.17[b] | 220.0±2.30[b] | 10.23±0.55 | 84.15±4.58[b] |
| | % of change[2] | +18.13% | +94.81% | −25.17% | −16.48% | −28.19% |
| OA+BV (moderate-dose) | Mean±SE | 6.52±0.70[b] | 3.26±0.42[b] | 93.33±6.69[b] | 8.85±0.95[b] | 81.50±3.52[b] |
| | % of change[2] | +51.62% | +141.48% | −68.25% | −27.75% | −30.46% |
| OA+BV (high-dose) | Mean±SE | 7.84±0.60[b] | 3.69±0.20[b] | 76.33±6.83[b] | 7.60±0.17[b] | 59.25±1.64[b] |
| | % of change[2] | +82.32% | +173.33% | −74.03% | −37.95% | −49.44% |
| OA+Glucosamine sulfate | Mean±SE | 4.80±0.23 | 2.91±0.44[b] | 71.0±1.73[b] | 11.26±0.90 | 68.25±1.06[b] |
| | % of change[2] | +11.62% | +115.55% | −75.85% | −8.08% | −41.76% |

Values were expressed as mean ± standard error (SE) for five rats in each group. **[1]**The percentage of changes of the BV extract- and glucosamine sulfate-treated groups were relative to the control group. **[2]**The percentage of changes of the OA+BV groups are relative to the OA group. [a]$P<0.05$: a significant different from the control group. [b]$P<0.05$ a significant different from the OA group. Other values proved no significance.

**Histopathological examination**

Histopathological examination of the knees of the control group revealed the presence of a healthy chondrocyte population, as well as an intact cartilage surface (Figure 3A). In the joint tissues of MIA group, abnormalities including synovial hyperplasia (pannus), inflammatory cell infiltration, cartilage deterioration, and bone disintegration were seen (Figure 3B). When rats with MIA were given the low-dose of BV extract as treatment, the results showed that the cartilage on the joint surface was mostly intact, while the synovial membrane in the joint tissues had experienced only slight damage (Figure 3D). In MIA rats received the moderate-dose of BV extract, less severe histopathological changes were recorded including preservation of the joint's surface layer and synovial space (Figure 3E). The MIA group received the high-dose of BV extract showed the most significant

**Table (3):** Effect of the ethanolic *Bauhinia variegata* (BV) extract on serum prostaglandin E2 (PGE2) and type II collagen (CoL-2) levels, as well as cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS) in knee joint of osteoarthritis (OA) male rats.

| Groups | | PGE2 (pg/mL) | CoL-2 (ng/mL) | COX2 (ng/g tissue) | iNOS (ng/g tissue) |
|---|---|---|---|---|---|
| Control | Mean±SE | 11.50±0.51 | 13.35±0.43 | 1.67±0.10 | 1.72±0.15 |
| OA | Mean±SE | 75.70±2.54[a] | 31.75±3.26[a] | 12.45±0.08[a] | 13.70±0.17[a] |
| | % of change[1] | +558.26% | +137.82% | +645.50% | +696.51% |
| BV (low-dose) | Mean±SE | 11.95±0.02 | 13.20±0.63 | 1.60±0.11 | 1.88±0.24 |
| | % of change[1] | +3.91% | −1.12% | −4.19% | +9.30% |
| BV (moderate-dose) | Mean±SE | 11.56±1.03 | 10.55±0.66 | 1.98±0.24 | 2.20±0.28 |
| | % of change[1] | +0.52% | −20.97% | +18.56% | +27.90% |
| BV (high-dose) | Mean±SE | 11.12±1.07 | 12.45±1.06 | 2.51±0.51 | 1.65±0.20 |
| | % of change[1] | −3.30% | −6.74% | +50.29% | −4.06% |
| Glucosamine sulfate | Mean±SE | 10.80±0.17 | 14.50±0.80 | 3.31±0.62 | 1.68±0.26 |
| | % of change[1] | −6.08% | +8.61% | +98.20% | −2.32% |
| OA+BV (low-dose) | Mean±SE | 61.15±2.28[b] | 24.75±0.77[b] | 10.60±0.23 | 8.70±0.17[b] |
| | % of change[2] | −19.22% | −22.04% | −14.85% | −36.49% |
| OA+BV (moderate-dose) | Mean±SE | 44.65±1.47[b] | 18.80±0.75[b] | 6.58±0.48[b] | 5.40±0.05[b] |
| | % of change[2] | −41.01% | −40.78% | −44.97% | −60.58% |
| OA+BV (high-dose) | Mean±SE | 44.66±5.17[b] | 12.00±0.80[b] | 3.70±0.17[b] | 3.15±0.14[b] |
| | % of change[2] | −41.00% | −62.20% | −70.28% | −77.00% |
| OA+Glucosamine sulfate | Mean±SE | 49.15±1.70[b] | 20.20±0.63[b] | 5.66±1.03[b] | 8.15±0.31[b] |
| | % of change[2] | −35.07% | −36.37% | −54.53% | −40.51% |

Values were expressed as mean ± standard error (SE) for five rats in each group. **[1]**The percentage of changes of the BV extract- and glucosamine sulfate-treated groups were relative to the control group. **[2]**The percentage of changes of the OA+BV groups are relative to the OA group. [a]$P<0.05$: a significant different from the control group. [b]$P<0.05$ a significant different from the OA group. Other values proved no significance.

improvement in terms of the reduction of pathological symptoms (Figure 3F). The histological score based on the intact cartilage surface, synovial lymphocytic infiltration, and subchondral bone erosion shown in Figure ‘‘4’’ indicated that the MIA-treated animals BV extract (especially at the moderate and high doses) retained cartilage surface intact, while synovial lymphocytic infiltration and subchondral bone erosion were significantly decreased ($P<0.05$) as compared with the MIA group in a dose-dependent manner.

## DISCUSSION

The phytochemical examination of the BV extract showed the presence of components with well-documented therapeutic and physiological effects. The ethanolic extract of BV was found to include two significant chemical components, according to GC-MS results: 9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z,Z) and phytol. Nonadecyl styrene, heptacosane, squalene, stearic acid, octadecanoic acid ethyl ester, and butylated hydroxytoluene are the next most abundant chemicals. It has been suggested that the polyenoic fatty acid compound 9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z,Z) has many biological activities[23]. Kumar *et al.*[24] found that the methyl ester of 9,12,15-octadecatrienoic acid (Z,Z,Z) had hepatoprotective, anti-inflammatory, hypocholesterolemic, and cancer-preventive properties. Diterpene phytol was promise as an antibacterial, anti-inflammatory, anti-cancer, and diuretic treatment. Results from treating arthritis with phytol suggested that reactive oxygen species (ROS) are a potential new class of medicines for treating chronic

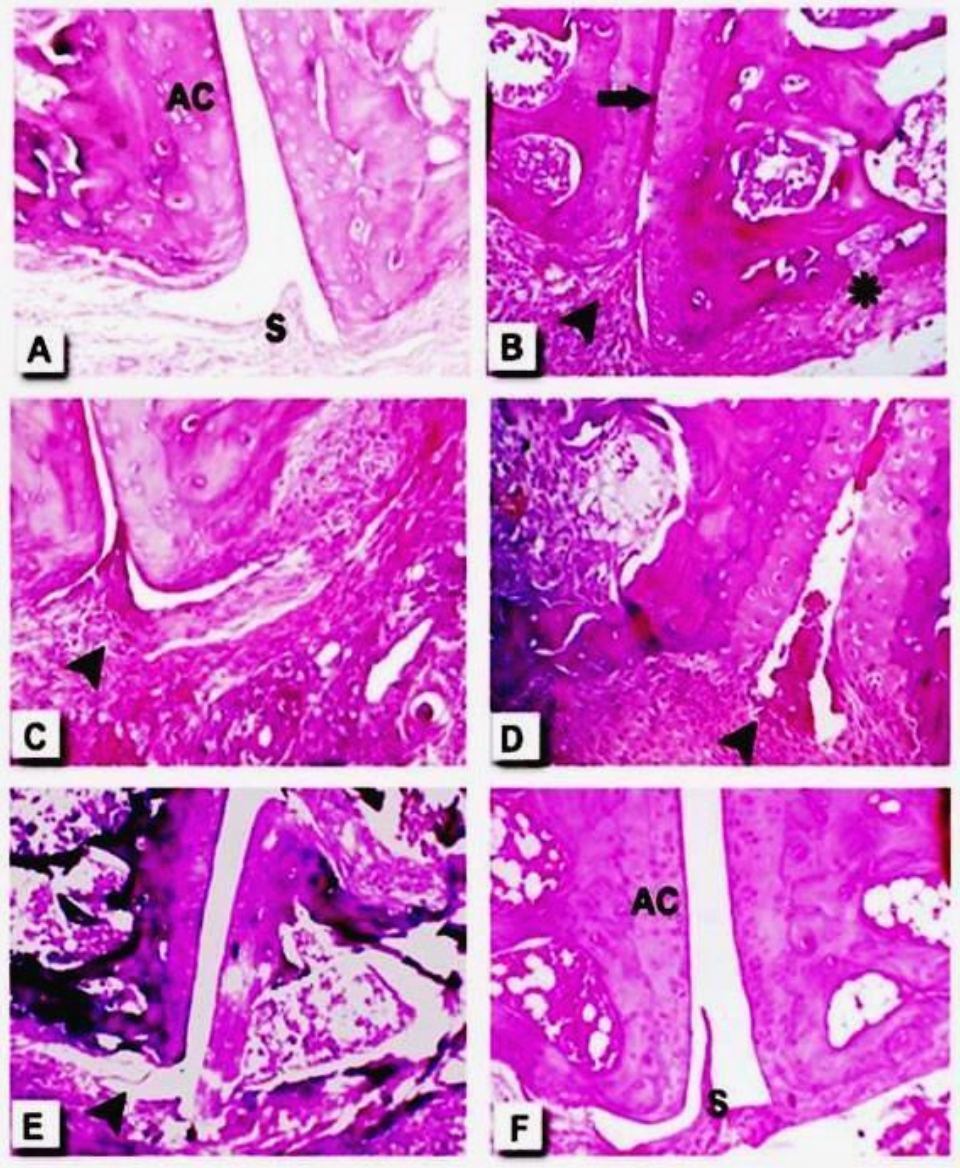


**Figure 3:** Histological slides of the knee joint (stained with hematoxylin and eosin) of the control and monosodium iodoacetate (MIA)-treated rats rat received different doses of the ethanolic *Bauhinia variegata* (BV) extract. **(A)** Control rat exhibited an undamaged cartilage surface and a normal population of chondrocytes in the articular cartilage (AC), as well as a normal synovial space around the joint (S). **(B)** The joints of MIA rat exhibited more pronounced synovial hypertrophy and hyperplasia (the arrow head), as well as more pronounced infiltration of the synovial tissues (*). This is accompanied by the breakdown of bone and cartilage (shown by the arrow), as well as the development of pannus, which invades the subchondral bone. **(C)** Part of a joint in MIA group received glucosamine sulfate. A slight reduction in the amount of synovial inflammatory cell infiltration (arrow head) was found. **(D)** A knee joint segment taken from MIA group received 200 mg BV extract/kg body weight exhibited a reduction in the amount of synovial inflammatory cell infiltration and synovial lining hyperplasia (arrow head). **(E)** A knee joint segment taken from MIA group received 400 mg BV extract/kg body weight showed the articular cartilage surface layer was intact and the synovial space around the joint is normal (arrow head). **(F**) A knee joint segment taken from MIA group received 600 mg BV extract/kg body weight showed the articular cartilage (AC) surface was intact and the synovial space around the joint was found to be normal (S), magnification = 200×.

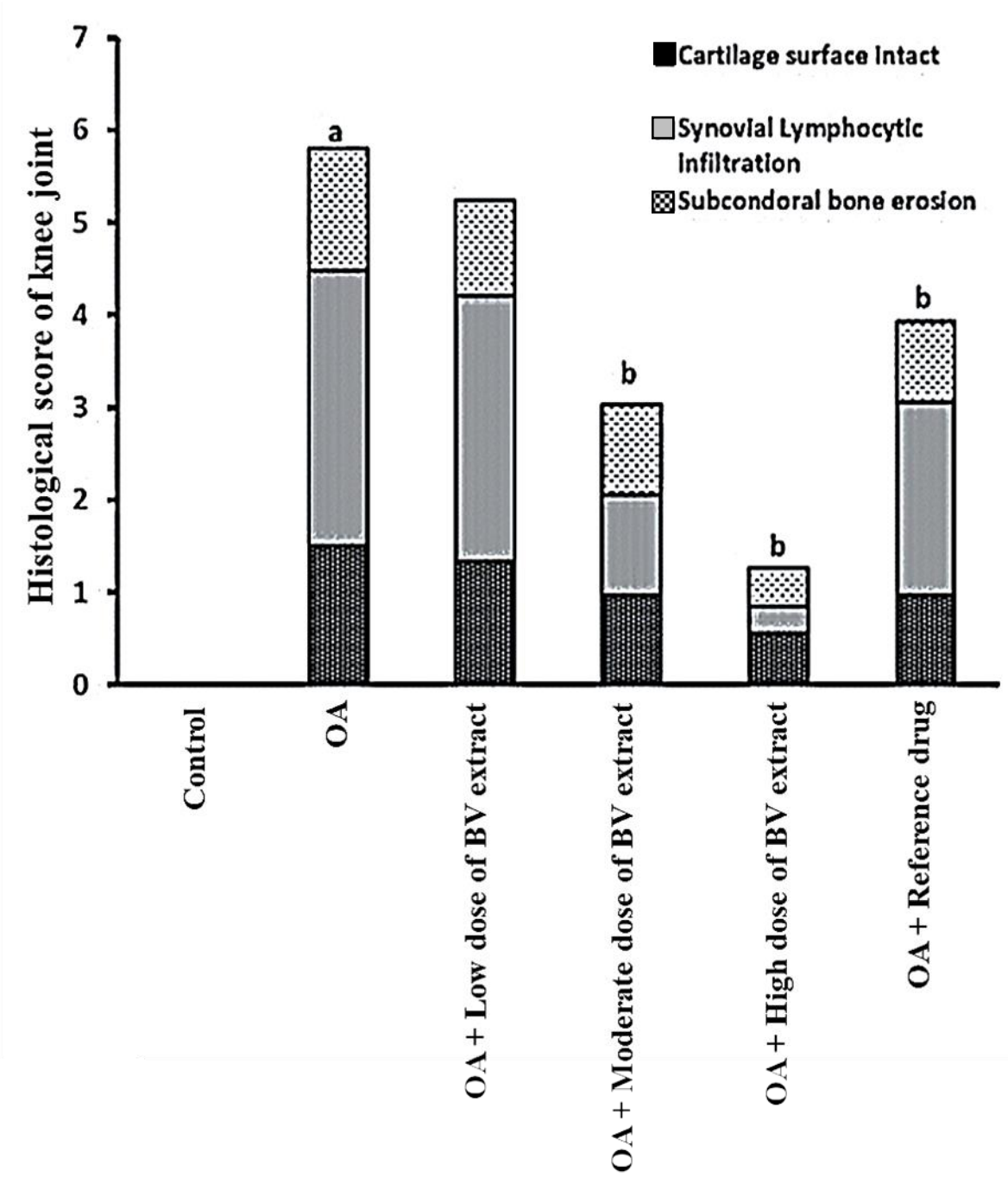


**Figure 4:** Histological score of knee joint in the control rats, *Bauhinia variegata* (BV) extract-treated osteoarthritic rats, and reference drug-treated osteoarthritic rats. The letters a and b denote statistically significant differences of the total histological score of knee joints when compared with the control and OA model groups, respectively ($P<0.05$).

inflammatory illnesses[25]. The leaves of *Flueggea leucopyrus*[26] contain phytol that has been shown to be beneficial in varying degrees of arthritic inflammation. Butylated hydroxytoluene (BHT) is a popular anti-oxidant utilized in many different applications, including a preservative in food and an anti-inflammatory agent[27]. Squalene has been proposed as a tri-terpene molecule with potential antimicrobial, anticancer, anti-HIV, and immunostimulant effects. It has been shown that squalene possesses antioxidant capabilities because it is resistant to peroxidation and works as a singlet

oxygen scavenger[28]. The present result demonstrated that all used BV extract doses (10, 100, 1000, 1600, 2900, and 5000 mg extract/kg body weight) didn't induce any mortality cases in male rats. Moreover, no changes, in general appearance, occurred during the observation period indicating that BV was well-tolerated (orally) in rats up to 5000 mg/kg body weight, this came in agreement with Makadiya *et al.*[29].

An injection of MIA into a rodent's knee joint is a widely used experimental model to study OA[30]. The BV crude extract showed anti-inflammatory effect in the MIA-induced OA rat model in the current study. The high doses of BV extract induced a significant increase in serum calcium and phosphorus level in MIA animals. Even though there is no direct evidence that calcium or phosphorus plays a role in the pathogenesis of OA, the present results may support that calcium and phosphorus are involved in physiological and pathological processes of chondrocyte.

Animals with MIA had elevated serum ALP activity, but MIA-induced OA rats treated with BV extract showed a drop dramatically in serum ALP activity. Leakage of ALP from damaged cells into the circulation has been hypothesized to account for its enhanced activity. A study by Kendall *et al.*[31] compared 15 individuals with rheumatoid arthritis with elevated serum ALP activity to the same number of patients with serum normal ALP activity and found that the former group had more active illness.

The IL-6 levels were found to be significantly lower in BV-treated MIA rats compared with untreated MIA animals. Synovial fluid from people with OA has been shown to have much higher levels of pro-inflammatory cytokines than that of people without OA, and these cytokines have been shown to play crucial roles in promoting the catabolic processes in OA that lead to cartilage deterioration[32]. One of the primary proinflammatory cytokines found to have a role in the pathogenesis of OA is IL-6, which causes joint and cartilage degradation by encouraging the activation of osteoclasts and the differentiation of mesenchymal cells into chondroblasts[33]. Additionally, earlier research indicated that TNF-α has comparable to or synergistic with IL-6 in producing matrix-degrading enzymes and inhibiting proteoglycan synthesis, leading to loss of cartilage[33,34]. Many studies have shown that the anti-inflammatory medications that can block the production of these cytokines may help manage OA[32]. Thus, we measured the blood levels of pro-inflammatory cytokines like IL-6 in MIA-induced OA rats to examine the anti-inflammatory effects of BV and discovered that BV treatment lowered this cytokine, while MIA raised this parameter. The data of the current study suggested that BV may be able to inhibit inflammatory reactions and, in turn, minimize articular cartilage degradation probably by decreasing IL-6.

In the pathophysiology of OA, PGE2 plays a crucial role as an inflammatory disease mediator by inhibiting the formation of extracellular matrix and reducing the proliferation of chondrocytes[35]. The results of the present study demonstrated that the serum level of PGE2 was significantly higher in the MIA group. However, BV effectively reduced MIA-induced elevation of PGE2 production by decreasing iNOS and COX2 level. Yan *et al.*[36] found that leptin and its receptor were significantly elevated in serum and synovial fluid of patients with severe OA and positively correlated with the severity of the disease suggesting that leptin plays an important role in OA development. The present study showed also that treatment of OA by BV extract may control leptin activity by decreasing significantly its levels in serum of MIA-induced OA rats.

A significant decrease in serum CoL-2 level in MIA animals treated with different doses of BV extract has been recorded in the current study. These results suggested the anti-OA activity of BV extract. Increased serum CoL-2 levels in MIA-induced OA mice compared with the control mice

suggested a faster rate of CoL-2 breakdown in OA. Increased levels of CoL-2 breakdown products in the blood and urine of OA patients are consistent with this observation[37]. Treatment with BV extract slowed the progression of the cartilage damage *via* decreasing significantly the levels of serum CoL-2 in MIA-induced OA animals treated with extracts. This maybe confirmed by the result of GC-MS indicating the presence of components like butylated hydroxytoluene and octadecanoic acid ethyl ester that have free radical scavengers activity leading to a reduction in CoL-2 breakdown.

The results revealed a significant decrease in iNOS level in MIA animals treated with different doses of BV extract. These results suggested the chondroprotective and OA suppressive effects of BV extract *via* inhibition of iNOS due to presence of bioactive secondary metabolites derived from BV. Epigallocatechin-3-gallate (EGCG) and Wogonin are two examples of plant-derived polyphenolic substances that have been hypothesized to reduce NO generation in IL-1-stimulated human OA chondrocytes by downregulating iNOS expression[38]. Inhibition of iNOS and anti-inflammatory actions in chondrocytes has also been attributed to several dietary polyphenols, including gallic acid, silibinin, carvacrol, and sinapic acid[39-42].

Consistent with the X-ray findings, the histological analysis of the knee joint of rats revealed synovial hyperplasia (pannus), inflammatory cell infiltration, cartilage degradation, and bone loss in the MIA group. Degeneration of the articular cartilage and subchondral bone, as well as necrosis of the chondrocyte, were seen, which was consistent with earlier studies describing the histological alterations of the articular cartilage in the Tibiofibular joint following MIA injection[43-45]. The histopathological data suggested that BV extract protected cartilage and stops chondrocyte cell degeneration by blocking the production of inflammatory mediators such PGE2, COX2, and iNOS. Knee degeneration was analyzed radiographically, and it was shown that OA rats' knee joints exhibited bone damage, cartilage degeneration, decreased joint space, and instability. This result was in commitment with those found by de Morais *et al*.[46]. In a 30-day study, BV extract (600 mg/kg body weight) reduced MIA's negative effects on OA animals and improved their X-ray images of their articular surfaces. The X-ray analysis confirmed that after being absorbed in the small intestine, BV extract had a direct regenerative impact on the cartilage in the knee joint, mending damage caused by wear and tear.

In conclusion, BV extract at 400 and 600 mg/kg body weight demonstrated promising anti-OA action by reducing inflammation in a rat model of MIA-induced OA, as shown by its effect on inflammatory markers over a range of dosages. In light of BV and its components' exceptional biological activity, further studies for discovering other mechanisms of anti-OA action of BV extract and its bioactive components such as those related to oxidative stress is recommended.

## COMPLIANCE WITH ETHICAL STANDARDS

The study was conducted according to approval REC107/2022 from the Scientific Research Ethics Committee of the Faculty of Science, Suez Canal University, and all efforts were made to minimize animal suffering during the handling.

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## CONFLICT OF INTEREST

The authors declare no competing interests.

## AUTHORS' CONTRIBUTIONS

All authors contributed to the study's design. Material preparation, data collection, and analysis were performed by MAH, HMT, RAE, and HT. The first draft of the manuscript was written by RAE, MAH, and

HMT. Prior versions of the manuscript were discussed by all authors. Each author has reviewed and approved the final version of the manuscript.

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# تأثير المستخلص الإيثانولي لنبات خف الجمل "*Bauhinia vriegata*" على التهاب المفاصل الناجم عن يودواسيتات أحادي الصوديوم في ذكور الجِرذان المهقاء

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يتميز اِلتهاب المفاصل بتآكل الغضاريف، معظم الأدوية المستخدمة حاليًا في العلاج تسبب اضطراب الجهاز الهضمي وقصور كُلوي. بحثت هذه الدراسة في التأثيرات المُخففة للمستخلص الإيثانولي لأوراق نبات خف الجمل ضد اِلتهاب المفاصل المُستحث بيودواسيتات أحادي الصوديوم في ذكور الجِرذان المهقاء (*Rattus norvegicus*). تم تقسيم "بشكل عشوائي" ذكور جِرذان "Wistar" المهقاء إلى عشر مجموعات (5 جِرذ/مجموعة) إلى المجموعة الضابطة (G1)، التي تلقت الماء المقطر؛ المجموعات المعاملة عن طريق الفم/يوميًا بجرعات 200 أو 400 أو 600 مجم من مستخلص خف الجمل/كجم من وزن الجسم، أو الدواء المرجعي (250 مجم كبريتات الجلوكوزامين/كجم من وزن الجسم) لمدة 4 أسابيع (G5-G2، على التوالي)؛ المجموعة المعاملة بجرعة "40 مجم" يودواسيتات أحادي الصوديوم/مل مرة واحدة عن طريق الحقن داخل المفصل (G6)؛ والمجموعات المعاملة بـكل من يودواسيتات أحادي الصوديوم + الجرعات المختلفة من مستخلص خف الجمل أو الدواء المرجعي (عن طريق الفم/يوميًا لمدة 4 أسابيع) بعد 4 أسابيع من حقن يودواسيتات أحادي الصوديوم (G10-G7، على التوالي). أظهرت النتائج زيادات ذات دلالة إحصائية في مستويات الكالسيوم والفوسفور، وانخفاض ذو دلالة إحصائية في نشاط الفوسفاتاز القلوي، ومستويات إنترلوكين 6، والبروستاجلاندين-E2، واللبتين، والكولاجين من الطراز الثاني في مصل الدم، وكذلك مستويات إنزيمات الأكسدة الحلقية 2 ومخلّق أكسيد النتريك (iNOS) في مفاصل الركبة في المجموعات المعاملة بـكل من يودواسيتات أحادي الصوديوم ومستخلص خف الجمل (بطريقة تعتمد على الجرعة). كما أظهر التصوير بالأشعة السينية زيادة كبيرة في مساحة المفصل في مجموعة يودواسيتات أحادي الصوديوم المعاملة بالجرعات العالية من مستخلص خف الجمل مقارنة بمجموعة يودواسيتات أحادي الصوديوم. واقترحت الدراسة الحالية أن مستخلص خف الجمل قد يكون واعدًا في علاج المراحل المبكرة من التهاب المفاصل.