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Research Article
Effect of Berberine on Animal Arthritis-One Effective Pharmacological Agent Against the Mia Induced Osteoarthritis

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Abstract

Background and Objective: Berberine is a common plant isolated compound and used in China in the management of illness. The present study was performed to scrutinize the anti-arthritic and inflammatory activity of berberine on Osteoarthritis induced by Monosodium Iodoacetate in the experimental model of rodents. **Materials and Methods:** For the present study, Wistar rats were procured and randomly categorized into 4 groups I: Animals treated with saline water, II: Animals treated with mono Iodoacetate to induced OA: III: Animals received MIA and berberine and lastly, IV: Rats received MIA and standard drug. The therapeutic property of berberine was assessed by its impact on the LPS induced raw cell *in vitro* and *in vivo* i.e., determining the biochemical index, weight-bearing distribution and proinflammatory cytokines level. **Results:** Lipopolysaccharide-induced RAW 264.6 cells were used to investigate *in vitro* anti-inflammatory activity of berberine. The present finding indicates that berberine reduced the production of proinflammatory cytokines i.e., Nitric Oxide (NO), interleukin-6 (IL-6), interleukin-10 (IL-10), the prostaglandin E_2 (PGE_2) in RAW cells and shown anti-inflammatory activity. Furthermore, berberine reversed the proinflammatory cytokines and inflammatory mediatory production, reduced the weight-bearing distribution in the hind paw and prevents destruction of the joint tissue in the present experimental model of OA in rodents. **Conclusion:** From the whole study, it is concluded that berberine was found to be an effective pharmacological agent against MIA induced osteoarthritis.

Key words: Berberine, osteoarthritis, therapeutic agent, inflammatory, pharmacological agent

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The most common disorder that is associated with musculoskeletal tissue is Osteoarthritis (OA) that is characterized via cartilage degeneration, sclerosis in subchondral bone, osteophyte formation and inflammation in synovial fluid. Osteoarthritis also harms joints, damage the function of cartilage and irregularities in proinflammatory pathways[1]. It also damages the synovial joints cartilage that is associated with articular cartilage and subchondral bone that results in causing pain in the joints while walking and standing and letdown in movement in joints. Various approaches are available for the treatment of OA and medications show various side effects[2]. Therefore main reason behind the OA is cartilage destruction and degradation of collagen is also a parallel cause that involves the advancement of OA along with inflammation[3].

Literature cited the inhibition of inflammatory reaction that helps in the management of OA[4]. It was also documented that the traditional drugs have the potency to limits the expansion of the OA via reducing destruction to cartilage and relieves inflammation reaction and attenuates the pain in joint[5].

Scientist has proved that the isolated compounds from the plant tend to combat against various diseases. Berberine (alkaloid) is a bioactive molecule obtained from the plant *Coptidis rhizoma* and exerts several biological activities involving anticancer, antimicrobial, anti-inflammatory, antimalarial and hypoglycemic[6]. It also reduces the level of blood lipids and is helpful during hyperlipidaemia.

It also decreases the level of glucose in the blood and controls insulin sensitivity and weight loss in animals and humans during experiments. Berberine also controls diabetes mellitus in patients and also shows inflammatory effects[7]. It also exhibits anti-apoptotic properties via reducing the Caspase-3 and Caspase-9 and raise the ratio of Bcl-2/Bax in cerebral ischemia[8]. It was observed from the literature that berberine is found to be a strong anti-inflammatory and antioxidant property both *in vitro* and *in vivo*[9]. Another study already established the relationship between the inflammatory factors and expansion of chronic and chronic arthritis[10,11].

Owing to these properties, this study scrutinizes the anti-inflammatory and anti-osteoarthritis effects of berberine against monosodium Iodoacetate (MIA) induced osteoarthritis (OA) in an experimental model of rats.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Joint Surgery, The Third Hospital of Jinan Lab, China from May, 2020 to June, 2021.

Estimation of anti-inflammatory activity (*in vitro*): RAW 264.7 cells were used to determine the *in vitro* anti-inflammatory activity of berberine. Dulbecco's modified Eagle's medium (DMEM) has 5.5% of FBS and 1% antibiotics were used to culture the RAW 264.7 cells. It was incubated in 5% carbon dioxide at room temperature to induce these cells and fresh medium and lipopolysaccharide were used in both cases absence/presence of sodium dodecyl sulphate[12].

Estimation of prostaglandin (PGE_2), nitric oxide (NO) and proinflammatory cytokines: LPS was used to stimulate and SDE to culture the RAW 264.7 cells for 24 hrs. To evaluate the production of NO, Griess reagent was employed. ELISA kits were used to assess the pro-inflammatory cytokines (IL-10 and IL-6) and inflammatory mediators (PGE_2) as per instructions by the manufacturers[13].

Evaluation of anti-osteoarthritis activity *in vivo*

Animals: Wistar albino rats were used for the current study. The animals were procured and caged in the animal house under standard environmental conditions along with the proper cycle of dark and light 12:12. All the animals were fed with a standard row chow and water *ad libitum*. The protocol used for the current study is as per compliance and approved by The Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University guidelines (animal ethical number: 2020030SFX).

Experimental study: All the animals were randomly categorized into the 5 groups: Group I rats served as normal and have saline, Group II Model control, Group III Osteoarthritis rats received berberine (20 mg kg^{-1}).

Group IV Osteoarthritis rats received standard drug Indomethacin (IND) at a dose level of 2 mg kg^{-1}. Injection of monosodium Iodoacetate at a dose level of 3-50 mg μL^{-1} was given to animals for the induction of osteoarthritis. Before the supplementation of the monosodium Iodoacetate for 1 week, an animal's received an oral dose of standard drug and berberine for 4 weeks[14].

Estimation of the distribution of weight-bearing on hind-paw: To evaluate the distribution of weight-bearing on hind-paw, a capacitance tester was utilized. Rats were kept in a

measuring chamber to measure the weight-bearing force for 3 sec. OA induced group reveals a reduction in hind paws in weight-bearing tolerance. The weight distribution ratio was measured[15]:

$$\text{Weight of the right hind limb} = \frac{\text{Weight on right hind limb}}{\text{Weight on right hind limb}} \times 100$$

Proinflammatory mediators and inflammatory cytokines: Blood samples were utilized to collect serum for the determination of proinflammatory mediators and cytokines. The sample was subjected to centrifugation for twenty minutes at the speed of twenty thousand to isolate the serum and store it in a deep freezer. The level of PGE_2 and cytokines IL-6, IL-1b, TNF-α were estimated using commercially available ELISA kits[16].

Histopathological investigation: For the histopathological study, a joint of the knee was taken away from all group rats and the sample was placed in 10% formalin solution and embedded in liquid paraffin. Hematoxylin and eosin (HE) were used to stain the tissue and slides were observed under a microscope[17].

Statistical analysis: All the data were represented as Mean±SD and performed by the one way ANOVA and Dunnett method used software Graph Pad prism.

RESULTS

Effect of berberine on inflammatory and proinflammatory mediators: RAW 264.7 cells were induced by LPS and berberine impact was observed for inflammatory properties (*in vitro*) like NO, the production reduced from 78.06±0.70-22.4±0.30 in Fig. 1a and PGE_2 production reduced from 62000±500-22000±100 in Fig. 1b, Cox-2 production reduced from 1.27±0.12-0.42±0.06 in Fig. 1c. Besides, IL-10

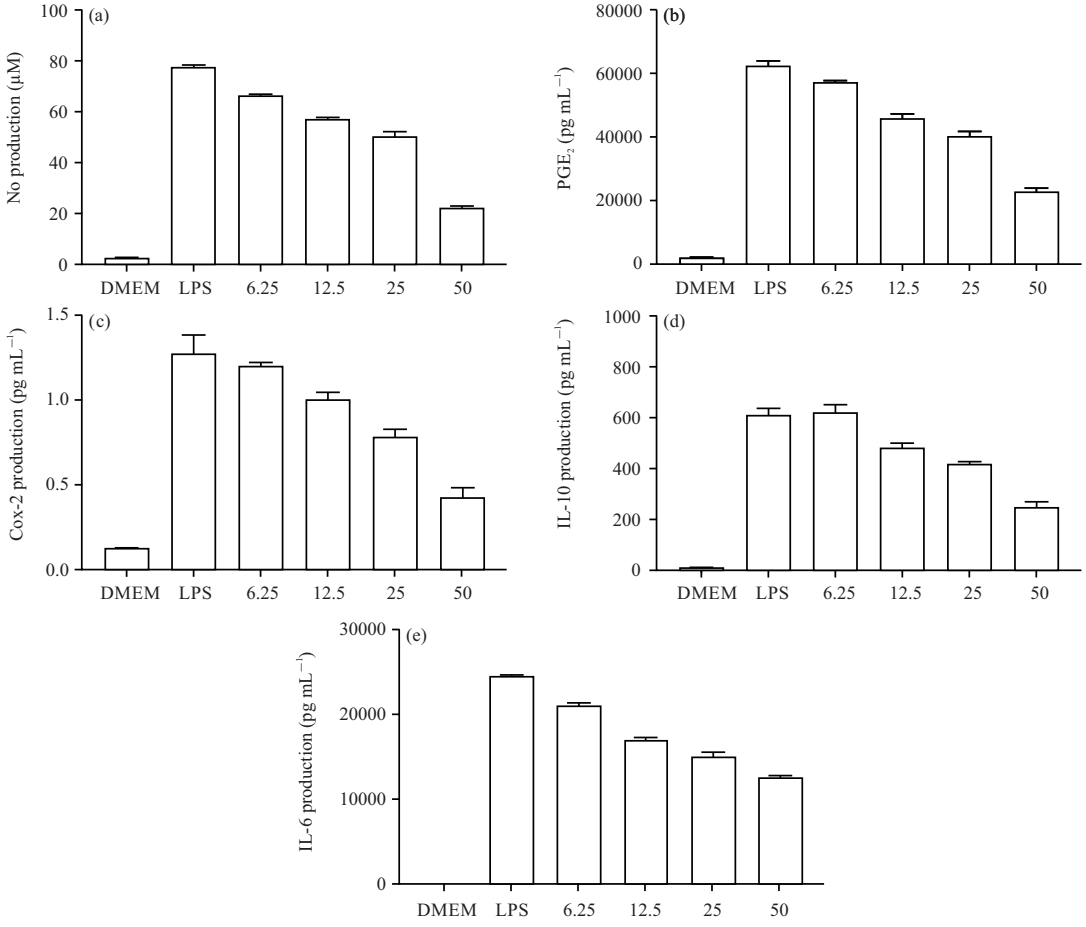

Fig. 1(a-e): Effects of berberine on the production of proinflammatory cytokines in LPS-stimulated RAW 264.7 cells, (a) NO, (b) PGE_2, (c) Cox-2, (d) IL-10 and (e) IL-6 production

production reduced from 613.33±27.3-250.00±20.0 in Fig. 1d and IL-6 production reduced from 24500±100.0-12533±200.0 in Fig. 1e, So according to the series test, we find after berberine+LPS were used to treat the cells for continuously 24 hrs, it significantly reduced the inflammatory and proinflammatory mediators in the cells. It was also observed that cell viability and was not affected by berberine and does not cause any toxic effects on the RAW 264.7 cells.

Berberine impact on rat's limb weight distribution: Figure 2 reveals the impact of berberine in hind paw weight-bearing distribution. To evaluate the proper weight distribution among the contralateral and sensitized hind limbs is the main index for joint pain in the arthritic knee. In capacitance, the tester was used for 14 days to assess the hind paw weight-bearing. In a MIA induced rats, the hind paw weight-bearing distribution was significantly reduced in comparison with the normal control group after the administration of MIA injection after 1 day and it was maintained for 11 days. In comparison to MIA, the group rats treated with berberine and standard drug (Indomethacin) displayed decreased values at 7 days. Furthermore, rats treated with berberine and standard drug (Indomethacin) show a balance among both paw hind legs and reversed the value. This figure reveals that the rear leg weight bear in the berberine treated group has been substantially restored.

Effect of berberine on inflammatory cytokine levels: This study assesses the expression of osteoarthritis as proinflammatory cytokines help in the management of cardiac inflammation and injured tissue. The current study has evaluated the impact of berberine on the level of proinflammatory cytokines for example IL-1b, TNF-α and IL-6 and inflammatory cytokines involving PGE_2 in all group rats. Model groups rats showed the raised level of inflammatory cytokine but the level of PGE_2 were considerably down-regulated by berberine from 890±32.0-640±20.0 pg mL^{-1} in Fig. 3a, then the NO production reduced from 66.06±7.0-

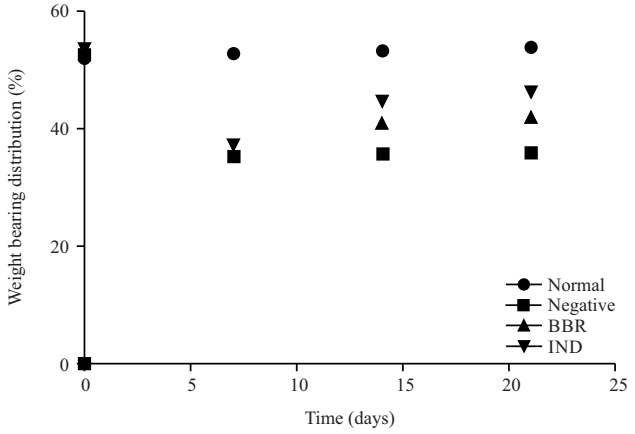

Fig. 2: Impact of berberine on alteration of the weight-bearing distribution in rats with OA

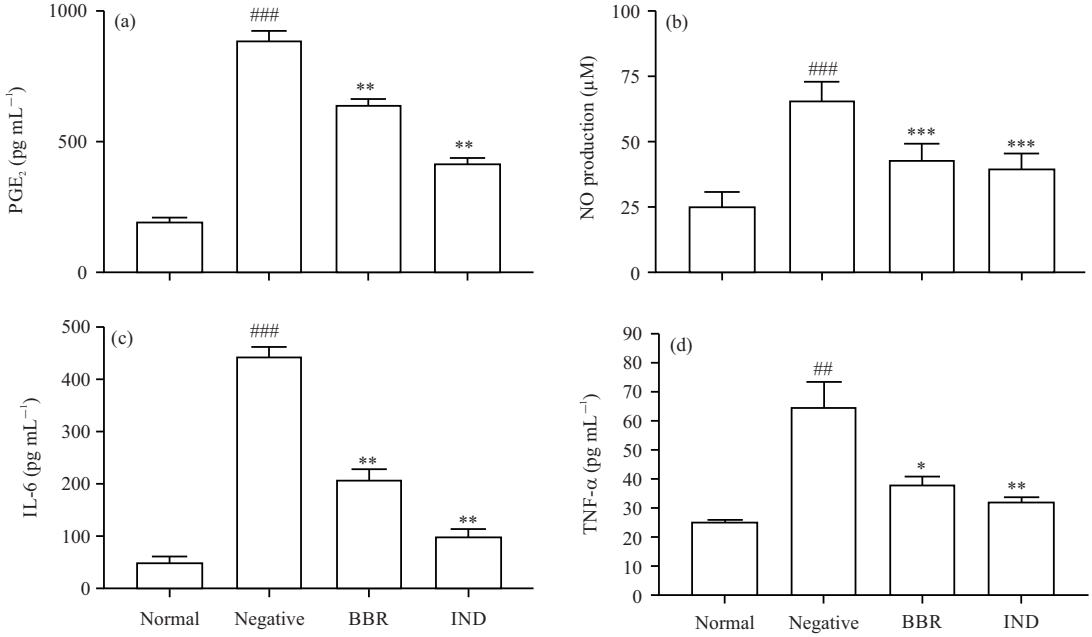

Fig. 3(a-d): Impact of berberine on the level of the proinflammatory mediators and cytokines in MIA induced osteoarthritis in rodents, (a) PGE_2, (b) NO production, (c) IL-6 and (d) TNF-α

###p<0.001 versus normal, p<0.05 versus model

43.16±5.0 µM in Fig. 3b and IL-6 changed from 446.20± 26.4-210±19.4 pg mL^{-1} in Fig. 3c and TNF-α found it reduced from 65.20±4.2-38.4±2.1 pg mL^{-1} in Fig. 3d were considerably down-regulated by berberine and standard drug after the study. The current study indicates that berberine prevents the pain induced by MIA in joints in animals were alleviating the inflammatory cytokines.

DISCUSSION

In clinical procedures, the treatment available for osteoarthritis primarily aims to minimize symptoms, protect articular mobility and reduce functionality loss. Many studies have proposed that the treatment of inflammatory arthritis has improved the symptoms of osteoarthritis by many traditional plants and its derived constituents[18-21]. Berberine exhibits different therapeutic properties, although the protective role of berberine in treatment with OA has not been demonstrated. This research has therefore been performed to examine the anti-osteoarthritis and anti-inflammatory effects of berberine in OA induced by MIA in rats.

Several cases of osteoarthritis have reported that the inflammatory mediators play a major role in cartilage degradation production and development[19]. Proinflammatory cytokines and inflammatory mediators have demonstrated a potential influence on the catabolic characteristics leading to OA pathophysiology[20-22]. Hence, this study find that the result of proinflammatory cytokines and inflammatory mediators (IL-6, NO, IL-10 and PGE_2) in RAW 264.7 cells induced lipopolysaccharide was significantly suppressed by current findings.

Several parameters such as weight-bearing parameters, proinflammatory cytokines and histology study of tissue are used as a means of evaluating the anti-osteoarthritis property on the MIA induced model of BBR. An anti-osteoarthritis activity in the MIA-induced OA model of BBR has also been assessed in the present analysis[23,24]. Pain associated with OA may be caused by due to movement in joints, generally leading to a reduction in use and decreased joint mobility[25-27]. Results of the present study have verified that berberine considerably recovers the weight-bearing capacity and inflammation in OA induced MIA-in animals. Many studies have shown that inflammation exhibits a major role during the expansion of arthritis[28-30]. The present study shows that berberine improves the OA induced by MIA in rats by inhibiting the proinflammatory cytokines and inflammatory mediator in serum that confirms the chondroprotective property of berberine in OA.

The spread of the OA and destruction in the structure of joint cartilage play a role in inflammation. The inflamed synovium generates pro-inflammatory mediators and cytokines for example NO and PGE_2, which change the balance between reparations and degradations of the cartilage matrix[31,32]. Such symptoms exacerbate the weakening of the joints and clinical signs during OA. Thus a successful approach to treating the progression and extend the production of OA will focus on the inflammatory reaction[33]. Berberine decreased the inflammatory reactions dramatically in the current study and stopped the progression of OA.

CONCLUSION

In brief, the effect of berberine on LPS-induced RAW cells was demonstrated by suppressing the development of PGE_2, NO, IL-6 and TNF-α. Berberine alleviates joint pain and rigidity, decreased inflammatory mediation and proinflammatory cytokines and restricted cartilage and subchondral bone tissue in an experimental model of MIA-induced osteoarthritis. The present finding indicates that the treatment of OA and OA related symptoms can be effectively treated with berberine.

SIGNIFICANCE STATEMENT

This study discovered the effect of berberine on LPS-induced RAW cells that can be beneficial for osteoarthritis and this study will help the researchers to uncover the critical areas of inflammatory and osteoarthritis that many researchers were not able to explore. Thus a new theory on the treatment of OA and OA related symptoms may be arrived at.

REFERENCES

1. Singh, J.A., S. Noorbaloochi, R. MacDonald and L.J. Maxwell, 2015. Chondroitin for osteoarthritis. Cochrane Database Syst. Rev., Vol. 2015. 10.1002/14651858.cd005614.pub2.
2. Kavas, A., M. Özdemir, S. Gürses, D. Keskin and A. Tezcaner, 2010. *In vitro* investigation and biomechanical modeling of the effects of plf-68 on osteoarthritis in a three-dimensional model. Biomech. Model. Mechanobiol., 10: 641-650.
3. Callahan, L.F., R.J. Cleveland, K.D. Allen and Y. Golightly, 2021. Racial/ethnic, socioeconomic and geographic disparities in the epidemiology of knee and hip osteoarthritis. Rheumatic Dis. Clin. North Am., 47: 1-20.
4. Berenbaum, F., 2013. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis). Osteoarthritis. Cartilage., 21: 16-21.

5. Vina, E.R. and C.K. Kwoh, 2018. Epidemiology of osteoarthritis: Literature update. Curr. Opin. Rheumatol., 30: 160-167.
6. Winkler, A., A. Łyskowski, S. Riedl, M. Puhl, T.M. Kutchan, P. Macheroux and K. Gruber, 2008. A concerted mechanism for berberine bridge enzyme. Nat. Chem. Biol., 4: 739-741.
7. Stähli, B.E., G.G. Camici, J. Steffel, A. Akhmedov and K. Shojaati *et al*., 2006. Paclitaxel enhances thrombin-induced endothelial tissue factor expression via c-Jun terminal NH_2 kinase activation. Circ. Res., 99: 149-155.
8. Imanshahidi, M. and H. Hosseinzadeh, 2008. Pharmacological and therapeutic effects of *Berberis vulgaris* and its active constituent, berberine. Phytother. Res., 22: 999-1012.
9. Neag, M.A., A. Mocan, J. Echeverría, R.M. Pop, C.I. Bocsan, G. Crişan and A.D. Buzoianu, 2018. Berberine: Botanical occurrence, traditional uses, extraction methods and relevance in cardiovascular, metabolic, hepatic and renal disorders. Front. Pharmacol., Vol. 9. 10.3389/fphar. 2018.00557.
10. Guerrero-Solano, J.A., O.A. Jaramillo-Morales, C. Velázquez-González, M.D. La O-Arciniega, A. Castañeda-Ovando, G. Betanzos-Cabrera and M. Bautista, 2020. Pomegranate as a potential alternative of pain management: A review. Plants, Vol. 9. 10.3390/plants9040419.
11. Powanda, M.C., M.W. Whitehouse and K.D. Rainsford, 2015. Celery seed and related extracts with antiarthritic, antiulcer and antimicrobial activities. Prog. Drug Res., 70: 133-153.
12. Alhakmani, F., S. Kumar and S.A. Khan, 2013. Estimation of total phenolic content, *in-vitro* antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. Asian Pac. J. Trop. Biomed., 3: 623-627.
13. Belwal, T., A. Bisht, H.P. Devkota, H. Ullah and H. Khan *et al*., 2020. Phytopharmacology and clinical updates of berberis species against diabetes and other metabolic diseases. Front. Pharmacol., Vol. 11. 10.3389/fphar.2020.00041.
14. Chun, J.M., H.S. Kim, A.Y. Lee, S.H. Kim and H.K. Kim, 2016. Anti-inflammatory and antiosteoarthritis effects of *Saposhnikovia divaricata* ethanolextract: *In vitro* and *in vivo* studies. Evidence-Based Compl. Alt. Med., Vol. 2016. 10.1155/2016/1984238.
15. Griffioen, M.A., V.H. Dernetz, G.S. Yang, K.A. Griffith, S.G. Dorsey and C.L. Renn, 2015. Evaluation of dynamic weight bearing for measuring nonevoked inflammatory hyperalgesia in mice. Nurs. Res., 64: 81-87.
16. Verma, A., P.C. Bhatt, G. kaithwas, N. Sethi and M. Rashid *et al*., 2016. Chemomodulatory effect melastoma malabathricum linn against chemically induced renal carcinogenesis rats via attenuation of inflammation, oxidative stress and early markers of tumor expansion. Inflammopharmacology, 24: 233-251.
17. Kumar, S., V. Kumar and O. Prakash, 2011. Antidiabetic, hypolipidemic and histopathological analysis of *Dillenia indica* (L.) leaves extract on alloxan induced diabetic rats. Asian Pac. J. Trop. Med., 4: 347-352.
18. Rahman, M., S. Beg, G. Sharma, S. Saini and R.A. Rub *et al*., 2016. Lipid-based vesicular nanocargoes as nanotherapeutic targets for the effective management of rheumatoid arthritis. Recent Pat. Anti-Infective Drug Discovery, 11: 3-15.
19. Dumond, H., N. Presle, B. Terlain, D. Mainard, D. Loeuille, P. Netter and P. Pottie, 2003. Evidence for a key role of leptin in osteoarthritis. Arthritis Rheumatism, 48: 3118-3129.
20. Kalff, K.M., M. El Mohammed, J. van Egmond, J. Veening and L. Joosten *et al*., 2010. Pre-treatment with capsaicin in a rat osteoarthritis model reduces the symptoms of pain and bone damage induced by monosodium iodoacetate. European J. Pharmacol., 1: 108-113.
21. Janusz, M.J., E.B. Hookfin, S.A. Heitmeyer, J.F. Woessner and A.J. Freemon *et al*., 2001. Moderation of iodioacetate-induced experimental osteoarthritis in rats by matrix metalloproteinase inhibitors. Osteoarthritis Cartilage, 9: 751-760.
22. Lee, D.G., S.Y. Park, W.S. Chung, J.H. Park and E. Hwang *et al*., 2015. Fucoidan prevents the progression of osteoarthritis in rats. J. Med. Food, 18: 1032-1041.
23. Combe, R., S. Bramwell and M.J. Field, 2004. The monosodium iodoacetate model of osteoarthritis: A model of chronic nociceptive pain in rats? Neurosci. Lett., 370: 236-240.
24. Si, H,B., Y. Zeng, S.Y. Liu, Z.K. Zhou and Y.N. Chen *et al*., 2017. Intra-articular injection of microrna-140 (miRNA-140) alleviates osteoarthritis (OA) progression by modulating extracellular matrix (ECM) homeostasis in rats. Osteoarthritis Cartilage, 25: 1698-1707.
25. Tang, T., T. Muneta, Y.J. Ju, A. Nimura and K. Miyazaki *et al*., 2008. Serum keratan sulfate transiently increases in the early stage of osteoarthritis during strenuous running of rats: Protective effect of intraarticular hyaluronan injection. Arthritis Res. Ther., Vol. 9. 10.1186/ar2363.
26. Pomonis, J.D., J.M. Boulet, S.L. Gottshall, S. Phillips, R. Sellers, T. Bunton and K. Walker, 2005. Development and pharmacological characterization of a rat model of osteoarthritis pain. Pain, 114: 339-346.
27. Ren, F.L., X. Guo, R.J. Zhang, S.J. Wang and H. Zuo *et al*., 2007. Effects of selenium and iodine deficiency on bone, cartilage growth plate and chondrocyte differentiation in two generations of rats. Osteoarthritis Cartilage, 15: 1171-1177.

28. Ashraf, S., P.I. Mapp and D.A. Walsh, 2011. Contributions of angiogenesis to inflammation, joint damage and pain in a rat model of osteoarthritis. Arthritis Rheumatism, 63: 2700-2710.
29. Lei, M., C. Guo, L. Hua, S. Xue, D. Yu, C. Zhang and D. Wang, 2017. Crocin attenuates joint pain and muscle dysfunction in osteoarthritis rat. Inflammation, 40: 2086-2093.
30. Park, S., L.R. Lee, J.H. Seo and S. Kang, 2016. Curcumin and tetrahydrocurcumin both prevent osteoarthritis symptoms and decrease the expressions of pro-inflammatory cytokines in estrogen-deficient rats. Genes Nutr., Vol. 11. 10.1186/s12263-016-0520-4.
31. Iovu, M., G. Dumais and P. du Souich, 2008. Anti-inflammatory activity of chondroitin sulfate. Osteoarthritis Cartilage, 16: S14-S18.
32. Calado, G.P., A.J.O. Lopes, L.M.C. Junior, F.D.A. Lima and L.A. Silva *et al*., 2015. *Chenopodium ambrosioides* L. reduces synovial inflammation and pain in experimental osteoarthritis. PLoS ONE, Vol. 10. 10.1371/journal.pone. 0141886.
33. Corciulo, C., M. Lendhey, T. Wilder, H. Schoen and A.S. Cornelissen *et al*., 2017. Endogenous adenosine maintains cartilage homeostasis and exogenous adenosine inhibits osteoarthritis progression. Nat. Commun., Vol. 8. 10.1038/ ncomms15019.