Research Paper

Morin Attenuates Interleukin-1β-Induced Inflammatory Response in Primary Rat Chondrocytes by Suppressing the Overproduction of Nitric Oxide and Matrix Metalloproteinases

JING SHI*, WEI YAO[1] AND SHILEI PANG[2]

Department of Osteoarthroplasty, Cangzhou Hospital of Integrated TCM-WM, Cangzhou City, Hebei Province, 061001, [1]Intervention Department, Cangzhou Hospital of Integrated TCM-WM , Cangzhou City, Hebei Province, 061001, [2]Department of Orthopedics and Trauma, Cangzhou Hospital of Integrated TCM-WM, Cangzhou City, Hebei Province, 061001, China

The present investigation was aimed to evaluate the antiosteoarthritic effect of morin in isolated primary rat chondrocytes in the presence of interleukin-1β. Results obtained showed that morin effectively attenuated in vitro the production of inflammatory mediators, nitric oxide and prostaglandin E2. Moreover, morin also significantly decreased the levels of matrix metalloproteinases. Furthermore, in monosodium iodoacetate-induced osteoarthritis in mice, it was found that morin at 25 and 50 mg/kg, significantly reduced the heat hyperalgesia and increased the spontaneous motility in mice on days 7, 14, and 21. Besides, morin also significantly reduced the level of proinflammatory cytokines in the serum of osteoarthritic mice. Collectively, these findings demonstrated that morin could be a potential chondroprotective that could be used in the treatment of osteoarthritis.

Key words: Osteoarthritis, morin, interleukin-1β, chondrocytes, nitric oxide, matrix metalloproteinases, monosodium iodoacetate, hyperalgesia, spontaneous motility

Osteoarthritis (OA) is an incessant joint disease described by dynamic cartilage degeneration, synovial aggravation, subchondral bone sclerosis, and osteophyte development[1,2]. A complex system of cytokines and proteolytic enzymes cause degradation of the extracellular matrix proteins of cartilage, for example, type II collagen, proteoglycans and hyaluronic acid[3]. Usually, OA is an aging-related disease and not considered to be a sort of inflammatory disease. It is generally an acute phase inflammation that is superimposed with cartilage fragments. However, cartilage OA is an inflammatory condition where increased production of inflammatory mediators such as NO and PGE_2 and proinflammatory cytokines (IL-1β and TNF-α) plays a crucial role in pathogenesis[4]. Moreover, it has been demonstrated that IL-1β causes chondrocytes to release matrix metalloproteinases (MMPs) leading to chondrocyte degradation[5]. Thus, suppressing inflammatory mediators and MMPs may lessen the progression of OA.

In rodents, monosodium iodoacetate (MIA) induces OA that resembles OA in humans as far as the histology of cartilage and pain-related behavior[6]. MIA is a metabolic inhibitor that prevents cell glycolysis pathway and subsequently, instigates cell death by restraining the activity of glyceraldehyde-3-phosphate dehydrogenase in chondrocytes[7]. Intra-articular infusion of MIA prompts a decrease in the number of chondrocytes and ensuing histological and morphological articular modifications, which are resembled human OA[8-10].

Morin, 2',3',4',5,7-pentahydroxyflavon, is a dietary bioflavonoid, originally isolated from the plants of Moraceae family[11]. There has been evidence that morin has beneficial effects on several diseases. It

***Address for correspondence**
E-mail: shijingrenjie@sina.com

Accepted 14 January 2020
Revised 15 December 2019
Received 10 October 2019
Indian J Pharm Sci 2020;82(2):242-248

has demonstrated to be effective against a wide range of pathological conditions that include Alzheimer's disease[12], Parkinson's disease[13], ischemia[14], cancer[15-17] and cardiovascular abnormalities[18]. In addition, morin also shown to exert antioxidant[19], antiinflammatory[20,21], antihypertensive[22,23] and antibacterial[24] activities.

It has been demonstrated that flavonoids are abundantly present in citrus plants. Many flavonoids, such as naringin, hesperidin, nobiletin, naringenin and narirutin are isolated from citrus fruits[25]. These flavonoids were found to have amazing antiinflammatory and antioxidant activities. Because of the antiinflammatory nature of flavonoids, the present study is designed to evaluate the impact of morin on IL-1β-induced inflammatory response in primary rat chondrocytes and MIA-induced OA in mice.

MATERIAL AND METHODS

All reagents used in present research were of analytical grade. Morin, type IV collagenase, IL1-β, and kits for NO, PGE_2, TNF-α, and IL1-β all were obtained from Sigma-Aldrich, USA. Pronase was acquired from Roche, Basel, Switzerland. Dulbecco's Modified Eagle's Medium and fetal bovine serum was procured from Gibco Inc., NY, USA. 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Thermo Fisher Scientific, USA. Cytotoxicity assay and estimation of inflammatory markers were performed on a microplate reader (Thermo Fisher Scientific, USA). Eddy's hot plate and actophotometer was procured from Ugo Basile, Italy to determine heat hyperalgesia and spontaneous motility of OA mice.

Primary chondrocyte culture:

Primary rat chondrocytes (PRCs) were isolated from the knee joint cartilage of 6 w old male Wistar rats. The knee joint was sequentially digested with pronase (10 g/l) for 30 min and then type IV collagenase (1 g/l) for 6 h[26]. The obtained chondrocytes were filtered through a mesh screen to prepare a single-cell suspension and then centrifuged at 1500×g for 10 min. The cells were then transferred into a culture flask. Chondrocytes were maintained in Dulbecco's modified Eagle's medium. Monolayer cultures were established in Petri dishes at a concentration of 6×10^6 cells/ml in the medium supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml) and 10% fetal bovine serum (FBS) and incubated at 37 in a humidified incubator containing 5% CO2.

Cell viability assay:

PRCs were seeded into a 96-well microplate at a density of 5×10^3 cells per well. After overnight incubation, cells were treated with different concentrations of morin for 24 h in the presence of IL-1β at 10 mg/ml. Then, the supernatant was removed and 100 µl of MTT was added into each well. After 4 h incubation, the supernatant was again discarded and 100 µl of DMSO was added into each well to dissolve the formazan crystals. After 10 min incubation, the optical density was measured at 570 nm in a microplate reader. Percent viability was calculated using the Eqn., % viability = $((A_{treat}-A_{background})/(A_{untreat}-A_{background}))\times100$, where A_{treat} is the absorbance of the treated wells which consisted of cells with a sample in culture media and MTT, $A_{background}$ is the absorbance of the background control wells consisted of culture media and MTT and $A_{untreat}$ is the absorbance of untreated wells which consisted of cells in culture media and MTT.

NO measurement and ELISA assay:

PRCs were pretreated with different concentrations of morin for 1 h, followed by the treatment with IL-1β at 10 mg/ml for 24 h. The levels of NO in the supernatant were measured using the Griess reaction[27]. Besides, the levels of PGE_2, MMP-1 and MMP-13 released from the cultured PRCs were estimated by commercially available ELISA kits, according to the instructions of the manufacturer.

Experimental animals:

Swiss albino mice (22-25 g) were used in the experiment. Mice were housed in polypropylene cages under a 12 h day/night cycle at a temperature of 22±1°. Water and food were provided *ad libitum*. All experimental procedures were performed according to the ethics committee of Cangzhou Hospital of Integrated TCM-WM, Cangzhou City, Hebei Province, China (Ethics Number: HBZX201907173).

Induction of OA:

On day 0, mice were anesthetized with ketamine hydrochloride (50 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and the right knee was shaved and disinfected with 70 % ethanol followed by povidone-iodide. A single injection of 50 µl sterile normal saline containing 0.3 mg MIA was given into the right knee joint through infrapatellar ligament with a 300 µl syringe fitted with a 29 G needle[28]. Mice were divided into 5 experimental

groups, I, II, III, IV, and V each consisting of 6 mice. Group I and II mice were considered as normal control and MIA control, respectively. On day 0, mice of all groups except normal control were injected with a single dose of MIA to the right knee joint cavity. From day 0 to 28, mice in group III were administered with etoricoxib (standard, 10 mg/kg, p.o.) and group IV and V were administered orally 25 and 50 mg/kg of morin daily, through oral gavage. Pain assessment was determined on day 0 (before administration of MIA), 7, 14, and 21 of the experiment on the Eddy's hot plate and spontaneous motor activity was also determined using the actophotometer.

Heat hyperalgesia:

Sensitivity to heat was evaluated using Eddy's hot plate. The planter side of the right paw was placed on the hot plate (55°) and the paw withdrawal latency was noted in seconds. The maximum cut-off time was kept at 15 s to prevent paw damage due to heat[29].

Spontaneous motor activity:

Spontaneous motor activity of mice in each group was determined using an actophotometer before recording the pain parameter. The number of interruptions of infrared beams while moving or standing of animals were counted for 5 min[29].

Estimation of proinflammatory cytokines:

Mice were kept fasting for 24 h and blood was collected through retro-orbital sinus puncture, under mild ether anesthesia. Then it was centrifuged at 2500 rpm for 15 min. The serum obtained was used for the estimation of proinflammatory cytokines (TNF-α and IL-1β) using commercial ELISA kits.

Statistical analysis:

Results were expressed Mean±SEM from 5 animals in each group. A comparison between the groups was made using one-way analysis of variance (ANOVA), followed by Tukey Multiple Comparison Test using GraphPad Prism version 8.1. $P<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Table 1 showed the effect of morin on PRCs in presence of IL-1β using the MTT assay. MTT assay showed that morin at concentrations of 50 and 100 µg/ml did not affect the viability of PRCs after 24 h of incubation. Thus, 50 and 100 µg/ml concentrations were selected for further studies. The effect of morin on the production of NO and PGE_2 was detected to evaluate the antiinflammatory potential of morin. PRCs were pretreated with various concentrations of morin for 1 h before the 24 h challenge with IL-1β at 10 mg/ml. Then the level of NO in the supernatant was measured using Griess reaction and the level of PGE_2 was measured using an ELISA kit, respectively. It was found that the production of NO and PGE_2 was significantly reduced by morin (Table 2). Moreover, the levels of MMP-1 and MMP-13 were measured as MMPs play an important role in cartilage degradation. The results demonstrated that increased release of MMP-1 and MMP-13 in PRCs culture supernatants was detected after stimulation of IL-1β at 10 mg/ml. However, the enhanced production of MMPs was suppressed significantly by morin (Table 2).

TABLE 1: EFFECT OF MORIN ON VIABILITY OF PRCs IN PRESENCE OF IL-1β

morin (µg/ml)	Percent cell viability
50	99.92±0.05
100	99.83±0.12
200	98.10±0.14
300	95.29±0.25
600	80.41±0.13
1000	51.56±0.11
2000	26.95±0.26

PRCs is primary rat chondrocytes, IL-1ß is interleukin-1ß, and SD is standard deviation for n=3 observations

The effect of morin on heat hyperalgesia in MIA-induced OA in mice is summarized in Table 3. The paw withdrawal latency was measured on days 0, 7, 14, and 21. In MIA control group there was a significant decrease in paw withdrawal latency on days 7, 14, and 21. On day 0, no significant increase in the paw withdrawal latency was observed in mice treated with etoricoxib (10 mg/kg) or morin (25 and 50 mg/kg). On days 7, 14, and 21 a significant increase in paw withdrawal latency was observed in mice treated with etoricoxib (10 mg/kg) and morin (25 and 50 mg/kg). In MIA-induced OA, a significant decrease in spontaneous motility was observed on days 7, 14, and 21 (Table 4). Mice treated with etoricoxib (10 mg/kg) and morin (25 and 50 mg/kg) showed a significant improvement in spontaneous motility. The most prominent increase in spontaneous motility was observed on day 21 in the mice treated with etoricoxib (10 mg/kg) and morin (50 mg/kg). Proinflammatory cytokines assume a conspicuous role in the upkeep of tissue damage and chronic inflammation in OA. Along these lines, the impact of morin on proinflammatory

TABLE 2: EFFECT OF MORIN ON NO, PGE2, MMP-1, AND MMP-13 IN PRCs

Treatment	NO (µmol)	PGE_2 (pg/ml)	MMP-1 (ng/ml)	MMP-13 (ng/ml)
Control	84.29±1.72	1182.41±16.93	215.75±2.86	184.40±3.68
Morin (50 µg/ml)	48.78±0.95[a]	804.62±20.83[a]	119.36±1.87[a]	124.83±2.17[a]
Morin (100 µg/ml)	34.93±1.08[a]	653.37±18.27[a]	76.36±1.61[a]	94.12±0.81[a]

NO is nitric oxide, PGE_2 is prostaglandin E_2, MMP is matrix metalloproteinase and SEM is standard error of the mean. Values are expressed as mean±SEM (n=6). [a]p<0.05 considered statistically significant as compared to control

TABLE 3: EFFECT OF MORIN ON HEAT HYPERALGESIA IN MIA-INDUCED OA IN MICE

Groups	Treatment	Paw withdrawal latency (s)			
		Day 0	Day 7	Day 14	Day 21
I	Normal control	5.18±0.34	4.90±0.30	5.22±0.23	5.31±0.18
II	MIA control	5.47±0.28	3.98±0.13[a]	3.62±0.14[a]	3.21±0.12[a]
III	Etoricoxib (10 mg/kg; p.o.)	5.53±0.24	6.34±0.19[c]	6.47±0.10[c]	6.63±0.17[c]
IV	Morin (25 mg/kg; p.o.)	5.24±0.29	5.79±0.25[c]	6.04±0.50[c]	6.39±0.33[c]
V	Morin (50 mg/kg; p.o.)	5.12±0.17	6.05±0.17[c]	6.53±0.14[c]	6.61±0.17[c]

MIA is mono-iodoacetate and SEM is standard error of the mean. Values are expressed as mean±SEM (n=6). [a]p<0.05 considered statistically significant as compared to normal control group; [c]p<0.05 considered statistically significant as compared to MIA control group.

TABLE 4: EFFECT OF MORIN ON SPONTANEOUS MOTILITY IN MIA-INDUCED OA IN MICE

Groups	Treatment	Spontaneous movements (number of interruptions/5 min)			
		Day 0	Day 7	Day 14	Day 21
I	Normal control	599.60±17.07	608.40±14.21	613.00±29.97	608.00±21.53
II	MIA control	604.43±29.24	503.62±24.91[a]	446.80±25.30[a]	405.32±18.44[a]
III	Etoricoxib (10 mg/kg)	615.67±26.71	590.81±18.39[c]	608.83±21.07[c]	618.13±18.72[c]
IV	Morin (25 mg/kg)	617.80±16.75	558.59±18.60[c]	577.35±14.93[c]	589.95±12.47[c]
V	Morin (50 mg/kg)	607.12±12.80	571.44±17.69[c]	585.88±21.10[c]	598.93±19.26[c]

MIA is mono-iodoacetate and SEM is standard error of the mean. Values are expressed as mean±SEM (n=6). [a]p<0.05 considered statistically significant as compared to normal control group; [c]p<0.05 considered statistically significant as compared to MIA control group.

TABLE 5: EFFECT OF MORIN ON SERUM PROINFLAMMATORY CYTOKINES IN MIA-INDUCED OA IN MICE

Groups	Treatment	TNF-α (pg/ml)	IL-1ß (pg/ml)
I	Normal control	66.86±5.01	33.582±2.49
II	MIA control	278.64±10.76[b]	183.52±5.67[b]
III	Etoricoxib (10 mg/kg)	121.36±10.67[d]	44.07±3.13[d]
IV	Morin (25 mg/kg)	93.86±10.33[d]	97.18±3.48[d]
V	Morin (50 mg/kg)	73.58±13.73[d]	58.46±7.12[d]

MIA is mono-iodoacetate, TNF-α is tumor necrosis factor-alpha, IL-1ß is interleukin-1ß and SEM is standard error of the mean. Values are expressed as mean±SEM (n=6). [b]p<0.05 considered statistically significant as compared to normal control group; [d]p<0.05 considered statistically significant as compared to MIA control group

cytokines, including TNF-α and IL-1β (Table 5) in MIA-induced OA mice was investigated. MIA control group mice showed high levels of TNF-α and IL-1β. However, mice treated with etoricoxib (10 mg/kg) and morin (25 and 50 mg/kg) showed a significant decrease in proinflammatory cytokines level.

The accessible treatment for OA is required in clinical practice, which can decrease the symptoms, improve joint mobility and limit the loss of functional ability. Numerous scientists guaranteed that herbal medicines utilized in the treatment of joint inflammation could improve OA[30]. There is no report of morin on inflammatory response in PRCs was found. Therefore, the present study was performed to evaluate the effect of morin on IL1β-induced inflammatory response in PRCs and MIA-initiated OA in mice. The effect of morin on IL-1β-induced inflammatory response in PRCs was investigated to find out whether morin could reduce the elevated levels of inflammatory mediators (NO and PGE_2). The increase in the level of NO and PGE_2 caused by IL-1β could induce the development of OA[31] and suppressing the expression of such inflammatory mediators might inhibit the development of OA. Therefore, the effect of morin on the secretion of NO and PGE_2 from PRCs in the presence of IL-1β was investigated. IL-1β induced OA development by degrading the extracellular matrix (ECM) components, which play a pivotal role in the development of OA[32]. Elevated IL-1β increases iNOS and COX-2 expressions, leading to elevation of NO and PGE2 levels[33]. Moreover, an increase in the level of NO can also induce MMP production and activation[34]. PGE_2 also exerted OA by inhibiting chondrocyte proliferation and synthesis of ECM[35]. MMPs are proteases produced by chondrocytes in response to IL-1β and cause degradation

of ECM, leading to the progression of OA[36,37]. Further, MMP-1 and MMP-13 were shown to play an important role in the development of OA. Thus, the agent that inhibits the production of inflammatory mediators and MMPs can be used as a new therapeutic agent for OA. In the present study, inflammatory mediators such as NO and PGE_2 were significantly reduced by morin in PRCs, which probably was due to a decrease in iNOS and COX-2 expressions. Besides this, elevated levels of MMP-1 and MMP-13 were suppressed by morin treatment. MIA affects the metabolism of chondrocytes and also causes bone cartilage lesions, cartilage degradation, and chondrocyte apoptosis[38,39]. In the *in vivo* model, results demonstrated the development of heat hyperalgesia at 7, 14 and 21 d post-MIA treatment. Little is known concerning the mechanisms of MIA-induced joint pain; however, it might be due to early inflammatory responses in the joint. The acute inflammatory reaction in the MIA model lasted around 3 d during which time trophic components are probably increased in the joint which upregulates the nociceptive receptor leads to pain[40]. It has been reported that on day 7, pain is initiated predominantly by inflammation in the MIA-induced OA model and later this inflammation plays a major role in pain and it is almost certainly brought about by biochemical factors, which influencing articular cartilage and subchondral bone[41]. In the present study, on days 7, 14 and 21 were taken for pain determination to see the impact of morin and etoricoxib in MIA-induced OA rats. Our outcomes showed that both etoricoxib and morin mitigated heat hyperalgesia on days 7, 14, and 21. Morin has been reported to decrease inflammation by suppression of NF-κB-regulated gene expression[20,21]. Thus, it could be concluded that morin might have decreased the synthesis of inflammatory mediators or expression of inflammatory genes responsible for hyperalgesia in the MIA model of OA. The decrease of spontaneous motility in the MIA-induced OA model operationally characterizes analgesia as a medication actuated increment in spontaneous motility. It is significant as a target proportion of pain-relieving adequacy that isn't dependent on an evoked stimulus-response[9]. Such perceptions were the basis to examine the impact of morin on spontaneous motility in MIA-induced OA mice. Because of the decrease of hyperalgesia by etoricoxib and morin, spontaneous motility of mice was brought to normal in treatment groups in contrast to MIA-treated group proposing absence of depressant impact of morin on CNS and the antihyperalgesic effect of morin was seen without any locomotor disability.

Proinflammatory cytokines expect a prominent role in the upkeep of tissue damage and constant inflammation in OA. It has been demonstrated that the upregulation of proinflammatory genes, for example, IL-1β, iNOS, and COX2 in chondrocytes are responsible at the beginning period of MIA-induced OA[42]. In an *in vitro* antiinflammatory study, morin inhibited monosodium urate-induced inflammatory reaction in RAW 264.7 macrophages through the suppression of inflammatory mediators, intracellular ROS production and NF-κB enhancement[43]. Hence, in the present *in vivo* study decrease in TNF-α and IL-1β levels in morin-treated mice might be due to the downregulation of proinflammatory genes.

In conclusion, these results on the *in vitro* model demonstrated morin's potential to reduce the activity of inflammatory mediators like NO and MMPs in PRCs challenged with IL-1β. Moreover, in the *in vivo* study morin mitigated pain and hyperalgesia and improved spontaneous motility in MIA-induced OA mice, which indicated the antihyperalgesic effect of morin without any locomotor disability. Furthermore, the present study also showed the ability of morin to reduce TNF-α and IL-1β in MIA-induced OA mice, which could probably be due to its ability to suppress the expressions of proinflammatory genes.

ACKNOWLEDGEMENTS

The authors would like to thank Department of Osteoarthroplasty, Cangzhou Hospital of Integrated TCM-WM, Cangzhou City, China for providing necessary research facilities to carry out the work.

Conflict of interest:

The authors declare no conflict of interest.

REFERENCES

1. Kumar V, Ai-Abbasi FA, Ahmed D, Verma A, Mujeeb M, Anwar F. *Paederia foetida* Linn. inhibits adjuvant-induced arthritis by suppression of PGE_2 and COX-2 expression via nuclear factor-κB. Food Funct 2015;6:1652-66.
2. Rahman M, Beg S, Verma A, Al Abbasi FA, Anwar F, Saini S, *et al.* Phytoconstituents as pharmacotherapeutics in rheumatoid arthritis: Challenges and scope of nanomedicine in its effective delivery. J Pharm Pharmacol 2017;69(1):1-14.
3. Rengel Y, Ospelt C, Gay S. Proteinases in the joint: clinical relevance of proteinases in joint destruction. Arthritis Res Ther 2007;9:221-30.
4. Chien TY, Huang KH, Lee CJ, Tsai PW, Wang CC. Antinociceptive and anti-Inflammatory Effects of zerumbone against mono-iodoacetate-induced arthritis. Int J Mol Sci 2016;17:249.
5. Chabane N, Zayed N, Afif H, Mfuna-Endam L, Benderdour M, Boileau C, *et al.* Histone deacetylase inhibitors suppress

interleukin-1β-induced nitric oxide and prostaglandin E2 production in human chondrocytes. Osteoarthr Cartil 2008;16:1267-74.
6. Combe R, Bramwell S, Field MJ. The monosodium iodoacetate model of osteoarthritis: a model of chronic nociceptive pain in rats? Neurosci Lett 2004;370:236-40.
7. Kuyinu EL, Narayanan G, Nair LS, Laurencin CT. Animal models of osteoarthritis: classification, update, and measurement of outcomes. J Orthop Surg Res 2016;11:19.
8. Kelly S, Dunham JP, Murray F, Read S, Donaldson LF, Lawson SN. Spontaneous firing in C-fibers and increased mechanical sensitivity in A-fibers of knee joint-associated mechanoreceptive primary afferent neurones during MIA-induced osteoarthritis in the rat. Osteoarthr Cartil 2012;20:305-13.
9. Guzman RE, Evans MG, Bove S, Morenko B, Kilgore K. Mono-iodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis. ToxicolPathol. 2003 Nov-Dec;31(6):619-24.
10. Bove SE, Calcaterra SL, Brooker RM, Huber CM, Guzman RE, Juneau PL, *et al.* Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. Osteoarthr Cartil 2003;11:821-30.
11. Sreedharan V, Venkatachalam KK, Namasivayam N. Effect of morin on tissue lipid peroxidation and antioxidant status in 1, 2-dimethylhydrazine induced experimental colon carcinogenesis. Invest New Drugs 2009;27:21-30.
12. Lemkul JA, Bevan DR. Destabilizing Alzheimer's Aβ42 protofibrils with morin: Mechanistic insights from molecular dynamics simulations. Biochem 2010;49(18):3935-46.
13. Caselli A, Cirri P, Santi A, Paoli P. Morin: A promising natural drug. Curr Med Chem 2016;23(8):774-91.
14. Al-Numair KS, Chandramohan G, Alsaif MA, Veeramani C, El Newehy AS. Morin, a flavonoid, on lipid peroxidation and antioxidant status in experimental myocardial ischemic rats. Afr J Tradit Complement Altern Med 2014 Apr 3;11(3):14-20.
15. Birt DF, Hendrich S, Wang W. Dietary agents in cancer prevention: Flavonoids and isoflavonoids. Pharmacol Ther 2001;90(2-3):157-77.
16. Zhang S, Morris ME. Effects of the flavonoids biochanin A, morin, phloretin, and silymarin on P-glycoprotein-mediated transport. J Pharmacol Exp Ther 2003;304(3):1258-67.
17. Zhang, S, Yang X, Morris ME. Flavonoids are inhibitors of breast cancer resistance protein (ABCG2)-mediated transport. Mol Pharmacol 2004;65(5):1208-16.
18. García-Lafuente A, Guillamón E, Villares A, Rostagno MA, Martínez JA. Flavonoids as anti-inflammatory agents: Implications in cancer and cardiovascular disease. Inflamm Res 2009;58(9):537-52.
19. Lian TW, Wang L, Lo YH, Huang IJ, Wu MJ. Fisetin, morin and myricetin attenuate CD36 expression and oxLDL uptake in U937-derived macrophages. Biochim Biophys Acta 2008;1781(10):601-9.
20. Manna SK, Aggarwal RS, Sethi G, Aggarwal BB, Ramesh GT. Morin (3,5,7,2',4'-Pentahydroxyflavone) abolishes nuclear factor-kappaB activation induced by various carcinogens and inflammatory stimuli, leading to suppression of nuclear factor-kappaB-regulated gene expression and up-regulation of apoptosis. Clin Cancer Res 2007;13(7):2290-7.
21. Tianzhu Z, Shihai Y, Juan D. The effects of morin on lipopolysaccharide-induced acute lung injury by suppressing the lung NLRP3 inflammasome. Inflamm 2014;37(6):1976-83.
22. Prahalathan P, Kumar S, Raja B. Morin attenuates blood pressure and oxidative stress in deoxycorticosterone acetate-salt hypertensive rats: a biochemical and histopathological evaluation. Metab Clin Exp 2012;61(8):1087-99.
23. Kang DG, Moon MK, Sohn EJ, Lee DH, Lee HS. Effects of morin on blood pressure and metabolic changes in fructose-induced hypertensive rats. Biol Pharm Bull 2004;27(11):1779-83.
24. Kang SS, Kim JG, Lee TH, Oh KB. Flavonols inhibit sortases and sortase-mediated Staphylococcus aureus clumping to fibrinogen. Biol Pharm Bull 2006;29(8):1751-5.
25. Alam MA, Subhan N, Rahman MM, Uddin SJ, Reza HM, Sarker SD. Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. Adv Nutr 2014;5(4):404-17.
26. Ahmad N, Chen LC, Gordon MA, Laskin JD, Laskin DL. Regulation of cyclooxygenase-2 by nitric oxide in activated hepatic macrophages during acute endotoxemia. J Leukoc Biol 2002;71:1005-11.
27. Sanjeewa KK, Fernando IP, Kim EA, Ahn G, Jee Y, Jeon YJ. Anti-inflammatory activity of a sulfated polysaccharide isolated from an enzymatic digest of brown seaweed Sargassum horneri in RAW 264.7 cells. Nutr Res Pract 2017;11:3-10.
28. Vermeirsch H, Biermans R, Salmon PL, Meert TF. Evaluation of pain behavior and bone destruction in two arthritic models in guinea pig and rat. Pharmacol Biochem Behav 2007;87:349-59.
29. Kumari RR, More AS, Gupta G, Lingaraju MC, Balaganur V, Kumar P, *et al.* Effect of alcoholic extract of Entadapursaetha DC on monosodium iodoacetate-induced osteoarthritis pain in rats. Indian J Med Res 2015 Apr;141(4):454-62.
30. Rahman M, Beg S, Sharma G, Saini S, Rub RA, Aneja P, *et al.* Lipid-based Vesicular Nanocargoes as Nanotherapeutic Targets for the Effective Management of Rheumatoid Arthritis. Recent Pat Antiinfect Drug Discov 2016;11(1):3-15.
31. Brittberg M, Gomoll AH, Canseco JA, Far J, Lind M, Hui J. Cartilage repair in the degenerative ageing knee. Acta Orthop 2016;87:26-38.
32. Largo R, Alvarez-Soria MA, Diez-Ortego I, Calvo E, Sanchez-Pernaute O, Egido J, *et al.* Glucosamine inhibits IL-1β-induced NFkB activation in human osteoarthritic chondrocytes. Osteoarthr Cartil 2003;11:290-8.
33. Koch B, Baum W, Burmester GR, Rohwer P, Reinke M, Zacher J, *et al.* Prostaglandin E2, interleukin 1 and gamma interferon production of mononuclear cells of patients with inflammatory and degenerative joint diseases. Z Rheumatol 1988;48:194-9.
34. Sasaki K, Hattori T, Fujisawa T, Takahashi K, Inoue H, Takigawa M. Nitric oxide mediates interleukin-1-induced gene expression of matrix metalloproteinases and basic fibroblast growth factor in cultured rabbit articular chondrocytes. J Biochem 1998;123:431-9.
35. Brinckerhoff CE, Matrisian LM. Matrix metalloproteinases: A tale of a frog that became a prince. Nat Rev Mol Cell Biol 2002;3:207-14.
36. Martel-Pelletier J, Boileau C, Pelletier JP, Roughley PJ. Cartilage in normal and osteoarthritis conditions. Best Pract Res Clin Rheumatol 2008;22:351-84.
37. Blasioli DJ, Kaplan DL. The roles of catabolic factors in the development of osteoarthritis. Tissue Eng Part B Rev 2014;20:355-63.
38. Guingamp C, Gegout-Pottie P, Philippe L, Terlain B, Netter P, Gillet P. Mono-iodoacetate-induced experimental osteoarthritis:

A dose-response study of loss of mobility, morphology, and biochemistry. Arthritis Rheum 1997;40:1670-9.

39. Fitzpatrick LR, Green C, Maines LW, Smith CD. Experimental osteoarthritis in rats is attenuated by ABC294640, a selective inhibitor of sphingosine kinase-2. Pharmacol 2011;87:135-43.
40. Schuelert N, McDougall JJ. Grading of monosodium iodoacetate-induced osteoarthritis reveals a concentration-dependent sensitization of nociceptors in the knee joint of the rat. Neurosci Lett 2009;465(2):184-8.
41. Beyreuther B, Callizot N, Stöhr T. Antinociceptive efficacy of lacosamide in the monosodium iodoacetate rat model for osteoarthritis pain. Arthritis Res Ther 2007;9(1):R14.
42. Dumond H, Presle N, Pottie P, Pacquelet S, Terlain B, Netter P, *et al.* Site specific changes in gene expression and cartilage metabolism during early experimental osteoarthritis. Osteoarthr Cartil 2004;2:284-95.
43. Dhanasekar C, Kalaiselvan S, Rasool M. Morin, a Bioflavonoid Suppresses Monosodium Urate Crystal-Induced Inflammatory Immune Response in RAW 264.7 Macrophages through the Inhibition of Inflammatory Mediators, Intracellular ROS Levels and NF-κB Activation. PLoS One. 2015;10(12):e0145093.