Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Therapeutic and Protective Efficacy of Esculetin on Inflammation and Cartilage Injury by Monosodium Iodoacetate-Induced Osteoarthritis in a Sprague-Dawley Rat Model

Jianrui Chen, Wei Liang, Hao Li, Jianzhong Huo

Orthopaedics, Taiyuan Central Hospital of Shanxi Medical University, Taiyuan, China

Submitted: 07-Jul-2022 Revised: 20-Jul-2022 Accepted: 20-Sep-2022 Published: 23-Nov-2022

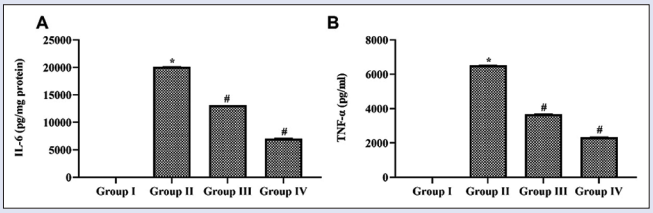
ABSTRACT

Background: Osteoarthritis (OA) is a major bone-related disease, which is characterized by joint deterioration, bone destruction, and whole joint damage, leading to permanent disability. The present study aimed to assess the anti-inflammatory and anti-osteoarthritic effects of esculetin in a monosodium iodoacetate (MI)-induced OA model in Sprague-Dawley (SD) rats. **Materials and Methods:** The anti-osteoarthritic efficacy of esculetin was determined in an MI-induced OA animal model. Rats were divided into the following groups: group I: normal control (saline), group II (MI only treated), group III (MI + esculetin), and group IV (MI + indomethacin). The potent outcome of esculetin treatment was assessed through its effects on the proinflammatory cytokines' levels, weight-bearing distribution, and histopathologic observation. Anti-inflammation efficacy of esculetin was evaluated in lipopolysaccharide (LPS)-induced Ralph and William's cell line 264.7 (RAW 264.7) cells. **Results:** *In vitro* results showed that esculetin has an anti-inflammatory efficacy through lessening the production of nitric oxide (NO), prostaglandin E2 (PGE2), tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6 in LPS-induced RAW cells. Furthermore, esculetin also reinforced the retrieval of hind limb weight-holding capacity, downregulated the generation of inflammatory mediators and proinflammatory cytokines, and shielded or protected the cartilage tissue from damage in the OA model SD rats. **Conclusion:** Esculetin seems to be a beneficial therapeutic compound for handling OA and OA-based disease conditions.

Key words: Esculetin, inflammation, monosodium iodoacetate, osteoarthritis, prostaglandin-E2

SUMMARY

- OA is one of the most common types of deteriorating diseases in joints.
- Esculetin effectively decreases the production of NO, PGE2, TNF-α, and IL-6 in LPS-administered RAW 264.7 cells
- Esculetin as well decreased hardness and joint pain, and further reduced the inflammatory and proinflammatory cytokine production and restricted the underneath cartilage bone and tissues in an MI-induced osteoarthritis SD rat model.



Abbreviations used: MI: monosodium iodoacetate; OA: osteoarthritis; NO: nitric oxide; PGE2: prostaglandin E2;TNF-α: tumor necrosis factor-alpha; IL: interleukin; LPS: lipopolysaccharide

Correspondence:

Dr. Jianzhong Huo,
Orthopaedics, Fendong District of Taiyuan Central Hospital, Taiyuan - 030000, China.
E-mail: chenjianrui970218@outlook.com
DOI: 10.4103/pm.pm_287_22

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INTRODUCTION

Osteoarthritis (OA) is one of the most common types of deteriorating diseases affecting joints. It is caused by destruction of joint cartilage and underlying bone and further disturbs the whole joint. Nearly 10 crore people suffer from OA globally.[1] The risk and occurrence of OA increase with age; further, lesions in knee joints, increase in body weight, and infectious diseases are the other risk factors contributing to OA.[2] OA is categorized by numerous risk factors with different disease conditions like inflammation, altered molecular mecha nisms, and metabolic progression, which are accountable for disease development together.[3] Previous literature reports that treating OA is challenging and joint replacement is the recommended therapeutic option in serious OA patients.[1]

Many studies revealed that proinflammatory cytokine stimulus in the cartilage tissue forms numerous structural alterations related to OA.[4-6] Moreover, the levels of proinflammatory intermediates, such as tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-1β and -6, are increased in the synovial fluid and serum of OA individuals.[7] Generally, numerous medications prescribed for OA possess side effects like nausea, vomiting, gastrointestinal bleeding and toxicity, diarrhea, and kidney toxicity.[8] Development of therapeutic drugs for OA that are effective and harmless and have lesser side effects is an urgent need. Monosodium iodoacetate (MI) is an excellent inhibitor of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which causes decreased glycolysis and altered articular cartilage connected to the histopathologic and morphologic structures of OA by delaying the incorporation of chondral assembly and induces chondrocytes' cell death. Moreover, MI causes repetitive synovial membrane thickening and subsequent infiltration of inflammatory cells, which destroys articular cartilage and persuades bone damage and chondral distortion.[9] Thus, the injection of MI into the animal knee

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Cite this article as: Chen J, Liang W, Li H, Huo J. Therapeutic and protective efficacy of esculetin on inflammation and cartilage injury by monosodium iodoacetate-Induced osteoarthritis in a sprague-dawley rat model. Phcog Mag 2022;18:926-31.

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joints is considered as an appropriate model that mimics the phenomena detected in human OA.

Lately, several studies have reported that traditional natural medicinal bases attenuate cartilage inflammation, resulting in restrained pain and enhanced functions of joints.[4,10,11] Thus, we anchored our investigation on the detection of an excellent natural compound from herbal sources with lesser side effects. Chinese natural medicines have been extensively used for centuries for the management of numerous diseases, among which esculetin is the foremost biologically dynamic component.[12] Esculetin is an excellent beneficial compound; previous studies have demonstrated its biological properties in preventing the development of tumors and its action against numerous types of tumors in both animal and human models.[13,14] Numerous studies have shown the anti-inflammatory properties of esculetin.[12,15]

Esculetin decreases the secretion of nitric oxide (NO), thus normalizing blood vessels and preventing tissue damage from inflammation; at the same time, esculetin delays soluble intercellular adhesion molecule secretion, which consequently reduces the binding of leukocytes to downregulate inflammation.[16] Moreover, esculetin effectively reduces the expression of matrix metalloproteinase in the cartilage and reduces prostaglandin E2 (PGE2) and NO levels in the synovial fluid, which subsequently delays the incidence of OA.[17] In both time-and concentration-dependent manner, esculetin effectively scavenges free radicals as well as defends against reactive oxygen species (ROS)-mediated DNA damage caused by oxidative stress.[18] Esculetin inhibits the production of proinflammatory cytokines throughout the integration among macrophages and adipocytes.[19] With the above-mentioned supportive evidence about esculetin, in the present study, we assess the anti-inflammatory and anti-osteoarthritic outcome of esculetin treatment in MI-induced OA rats.

MATERIALS AND METHODS

Chemicals and reagents

Esculetin and indomethacin were purchased from Sigma-Aldrich Co., St Louis, MO, (USA). Other chemicals like formaldehyde and other reagents used for the present study are of analytical grade (Sigma-Aldrich Co., St Louis, MO, USA).

Experimental animals

In the present study, 7-week-old male Sprague-Dawley (SD) rats were selected. The animals were obtained from the Recognized Animal House and retained in standardized environmental situations with 12-h light/dark conditions at room temperature. All rats received a regular pellet diet and clean drinking water. All investigations were performed in agreement with the institutional animal ethical committee guidelines.

OA exposure

The SD rats were anesthetized with 90 mg/kg ketamine (intraperitoneally). OA was induced by penetration of right and left knee joints with a needle (27 gauge) and syringe containing 1.2 mg of MI diluted in saline (50 mL), with reference to the standard procedure.[20]

Animal study plan

The SD rats were chosen randomly and separated into four groups with six rats in each group as follows: group I: control rats given saline; group II: OA control only; group III: OA control managed with esculetin (10 mg/kg); and group IV: OA control treated with standard anti-inflammatory drug, indomethacin (2 mg/kg). OA was induced in rats by injecting (3 mg/50 µL) MI. The MI injection was administered in the joint or articular surface of anesthetized rats. Further, the rats were orally administered with esculetin and standard drug indomethacin 1 week before the induction of MI for 4 weeks.[21]

In vitro Ralph and William's cell line 264.7 cells

The anti-inflammatory properties of esculetin were studied in Ralph and William's cell line 264.7 (RAW 264.7) cells. These cells were cultured in Dulbecco's modified Eagle's medium (DMEM) which contained 10% fetal bovine serum (FBS) and 1% antibiotics (Sigma Chemical Company, USA). The cells were incubated in a moistened atmosphere of 5% CO_2 at room temperature. Then the plates were washed or rinsed with fresh medium, and to protect the cellular environment from the entry of toxic materials, 1 µg of lipopolysaccharide (LPS) was added to the culture flask.

Assessment of NO, PGE2, and proinflammatory cytokines

The RAW 264.7 cells were cultured and stimulated with LPS for 24 h. NO production was evaluated by Griess reagent. A previously described procedure was used for the assessment of NO production.[22] The inflammatory intermediates PGE2 and TNF-α and proinflammatory intermediates like IL-6 and -1β were measured using enzyme-linked immunosorbent assay (ELISA) kits (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Hind paw weight-bearing distribution

A capacitor tester was used to observe changes in weight-holding tolerance. To measure the hind paw weight-holding capacity, the SD rats were positioned in a measurement compartment for 3–4 s for determining weight-bearing strength. The distribution ratio of animal weight was assessed by the formula: weight of right hind limb = right hind limb × 100.[23]

Investigation of serum biochemical parameters

Serum collected from SD rat blood sample was tested for important biochemical parameters. Important antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) and the levels of serum reduced glutathione (GSH) and malondialdehyde (MDA) were measured in accordance with standard diagnostic kits' protocols (Sigma Aldrich, USA).[24]

Assessment of proinflammatory cytokines

Proinflammatory cytokines were determined in the collected animal blood samples, and further, the blood samples were processed to collect serum. Serum was separated by centrifugation at 1500 rpm for 20 min; then, it was stored at −80°C for further use. Commercially available ELISA kits (Thermo Fisher Scientific, USA) were used to assess the important inflammatory mediator PGE2 and proinflammatory cytokines TNF-α, IL-1β, and IL-6 in the serum.[22]

Statistical analysis

The obtained data were presented as a mean ± standard deviation. All data investigated were compared using one-way analysis of variance. To identify the significance, Dunnett's trial was used by means of GraphPad Prism Software.

RESULTS

Effect of esculetin on inflammatory and proinflammatory mediators

Initially, we measured the effect of esculetin on prostaglandin and NO production [Figure 1a and b] in LPS-stimulated RAW 264.7 cells. In

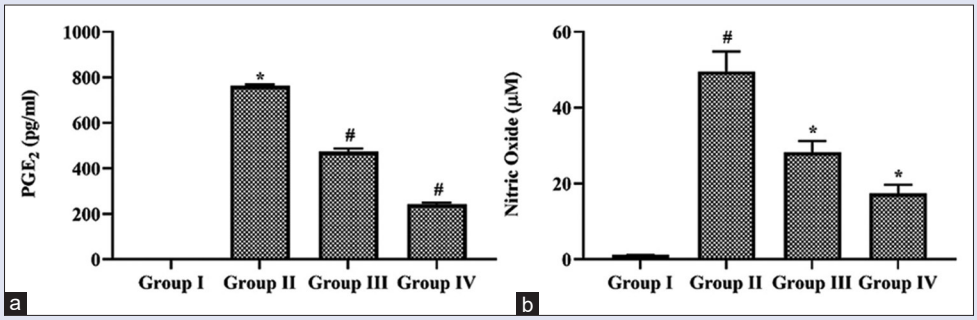

Figure 1: Effects of esculetin on the production of PGE2 and nitric oxide in LPS-induced RAW 264.7 macrophages. Effects of esculetin on the production of (a) PGE2 and (b) nitric oxide in LPS-induced RAW 264.7 macrophages. Cells were treated with esculetin (0, 5, and 10 μg/mL) along with LPS (1 μg/mL) or LPS only for 24 h. PGE2 and NO production was analyzed by ELISA. Results are expressed as the means ± SD (n = 3). *P < 0.001 versus untreated LPS and esculetin; #P < 0.05 versus LPS alone. ELISA = enzyme-linked immunosorbent assay, LPS = lipopolysaccharide, NO = nitric oxide, PGE2 = prostaglandin E2, SD = standard deviation

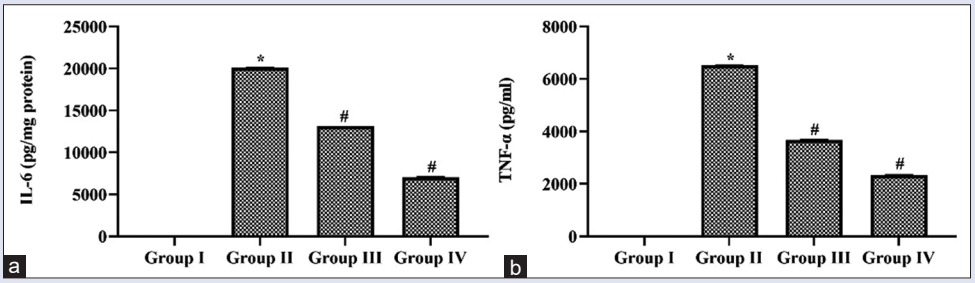

Figure 2: Effects of esculetin on the production of IL-6 and TNF-α in LPS-induced RAW 264.7 macrophages. Effects of esculetin on the production of (a) IL-6 and (b) TNF-α in LPS-induced RAW 264.7 macrophages. Cells were treated with esculetin (0, 5, and 10 μg/mL) along with LPS (1 μg/mL) or LPS only for 24 h. IL-6 and TNF-α production was measured by ELISA. Data are expressed as the means ± SD (n = 3). *P < 0.001 versus untreated LPS and esculetin; #P < 0.05 versus LPS alone. ELISA = enzyme-linked immunosorbent assay, IL = interleukin, LPS = lipopolysaccharide, SD = standard deviation, TNF-α = tumor necrosis factor-alpha

addition, its effect on IL-6 and TNF-α [Figure 2a and b] in LPS-induced RAW 264.7 cells was determined. Figures 1a-b and 2a-b, show that esculetin effectively decreased the production of PGE2, NO, IL-6, and TND-α. Also, esculetin did not disrupt cell viability and displayed no lethal properties in RAW 264.7 cells.

Results of esculetin administration on altered hind paw weight-holding distribution

Figure 3a illustrates the effect of esculetin on altered hind paw weight-bearing distribution in rats. The effect of esculetin on hind paw weight-bearing strength in animals was analyzed using a capacitor tester for 25 days. The right and left limb ratio was used to estimate the OA development. MI control group II animals showed sudden drop in weight-bearing distribution compared to group I animals which received saline. At the same time, esculetin-treated and the standard drug indomethacin–treated group III and IV rats, respectively, showed decreased values on the seventh day in comparison to MI control group II rats.

In other words, esculetin- and indomethacin-treated animals had equilibrium between the right and left hind legs and came back to normal condition. These results reveal the noteworthy recovery of hind limb weight-bearing animals in the esculetin-treated animal group. In addition, Figure 3b shows the arthritis index score also increased in MI-induced OA in group II animals. At the same time, MI induced along with esculetin-treated group III animals showed reduced arthritis index score, which indicates the efficacy of esculetin against OA.

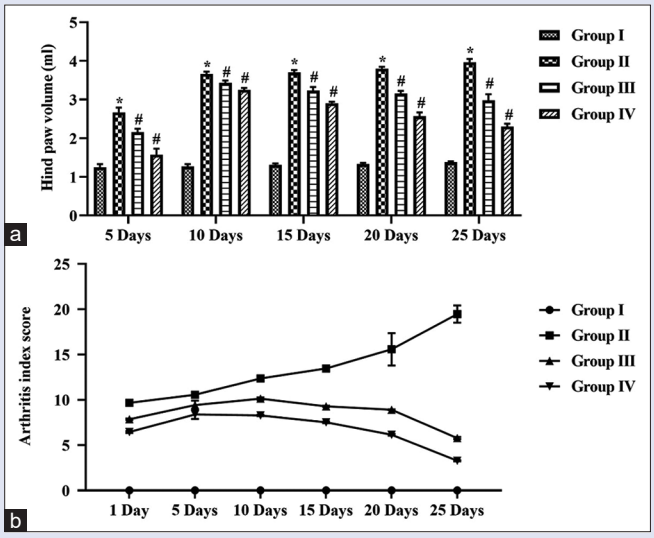

Figure 3: Effects of esculetin on changes in hind paw volume and arthritis index score in MI-induced OA in SD rats. Outcome of esculetin treatment on altered hind paw weight-holding capacity in MI-induced OA in SD rats. (a) The weight-bearing distribution ratio was estimated once a week for 25 days after the injection of MI, using a capacitance tester. Group I = normal control; group II = MI induced; group III = esculetin 10 mg/kg b.w. + MI; group IV = indomethacin 2 mg/kg b.w. (positive control). (b) Arthritic score of MI-induced SD rats. *P < 0.001 versus control; #P < 0.05 versus MI. MI = monosodium iodoacetate, OA = osteoarthritis, SD = Sprague-Dawley

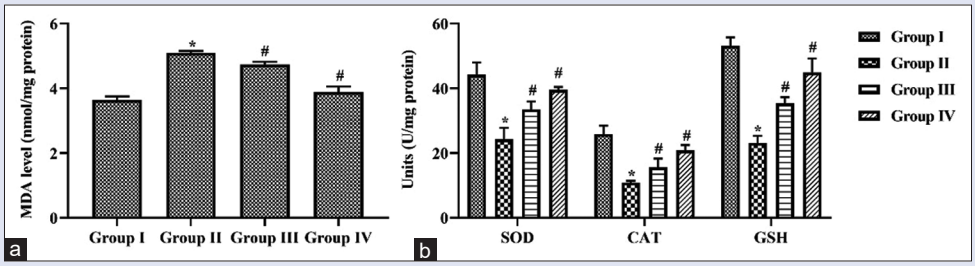

Figure 4: Efficacy of esculetin on oxidative stress markers. Results are expressed as mean with 95% CI. Substantial alteration was found in relation to control group (*) and OA group (#), $P < 0.05$. (a) MDA; (b) SOD, CAT, and GSH. *$P < 0.001$ versus control; #$P < 0.01$ versus MI. CAT = catalase, CI = confidence interval, GSH = reduced glutathione, MDA = malondialdehyde, MI = monosodium iodoacetate, OA = osteoarthritis, SOD = superoxide dismutase

Effect of esculetin on serum biochemical alterations in MI-induced arthritic rats

Figure 4a shows that the serum levels of MDA were increased significantly ($P < 0.05$) in group II arthritis rats compared to normal control group I rats. Oral administration of esculetin effectively reduced ($P < 0.05$) the development of MDA in group III rats. The same results were obtained in group IV standard drug-treated animals. Both group III and IV animals were highly comparable to OA-induced group II animals. Levels of crucial antioxidants like SOD, CAT, and GSH were decreased ($P < 0.05$) in arthritis model SD rats of group II, when compared to group I control rats, as depicted in Figure 4b, while the levels of SOD, CAT, and GSH were markedly increased ($P < 0.05$) in esculetin-treated group III rats in comparison to group II arthritis model rats. Group IV positive control rats as well showed improvement in all the above-mentioned changes in biochemical levels induced by MI.

Efficacy of esculetin on serum inflammatory cytokine levels

In maintaining tissue damage and inflammation process, proinflammatory cytokines play a significant role, especially during the expression of OA. Hence, we analyzed the efficacy of esculetin on serum inflammatory intermediates like PGE2 and crucial proinflammatory cytokines, which included IL-6, IL-1β, and TNF-α, in MI-induced OA rats. Group II rats induced with MI exhibited elevated levels of PGE2, IL-6, IL-1β, and TNF-α, and in esculetin-treated group III rats, the levels of the above inflammatory markers were reduced significantly ($P < 0.05$). Further, indomethacin-treated group IV rats as well exhibited lowered levels of inflammatory cytokines at the end of investigation. This is shown in Figures 5 and 6. Therefore, the present work proposes that esculetin shields cartilage in the MI-induced model through altering these inflammatory markers.

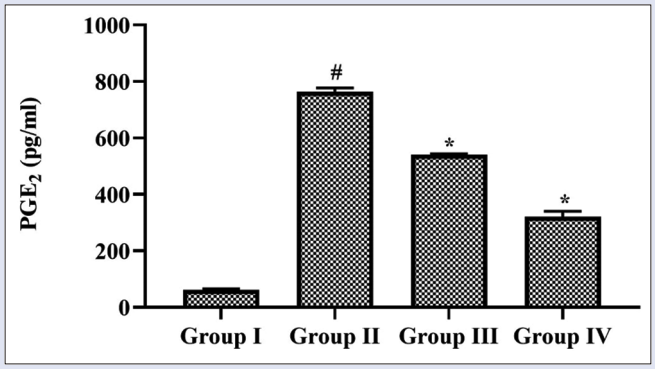

Figure 5: Effects of esculetin on the serum levels of PGE2 in MI-induced OA in SD rats. Efficacy of esculetin on the serum levels of PGE2 in MI-induced OA in SD rats. The level of PGE2 was effectively decreased by esculetin treatment in MI-induced OA in SD rats. PGE2 levels were measured by ELISA. *$P < 0.001$ versus control; #$P < 0.05$ versus MI. ELISA = enzyme-linked immunosorbent assay, MI = monosodium iodoacetate, OA = osteoarthritis, PGE2 = prostaglandin E2, SD = Sprague-Dawley

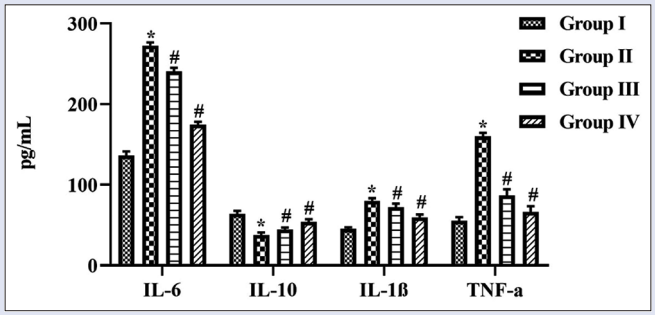

Figure 6: Effects of esculetin on the serum levels of IL-6, IL-1β, and TNF-α in MI-induced OA in SD rats, determined by ELISA. Efficacy of esculetin on the serum levels of IL-1β, IL-6, and TNF-α in MI-induced OA in SD rats. IL-1β, IL-6, and TNF-α levels were measured by ELISA. Findings revealed that esculetin effectively reduced the IL-1β, IL-6, IL-10, and TNF-α levels in MI-induced OA in SD rats. *$P < 0.001$ versus control; #$P < 0.05$ versus MI. ELISA = enzyme-linked immunosorbent assay, IL = interleukin, MI = monosodium iodoacetate, OA = osteoarthritis, SD = Sprague-Dawley, TNF-α = tumor necrosis factor-alpha

DISCUSSION

The existing treatment options for OA used in medical practice mainly aim for reduced symptoms, protection of joint flexibility, and improving functional ability. Numerous researchers have reported that many herbal-based plant constituents and compounds are used in the management of inflammation-related arthritis.[25] Esculetin displays numerous pharmacological benefits, though there is no evidence on its efficacy in affording protection against OA. Hence, the present study was performed to evaluate the anti-inflammatory and anti-osteoarthritic effects of esculetin in an MI-induced OA SD rat model. Numerous reports on OA establish that inflammatory mediators play a substantial role in the enlargement and development of cartilage damage.[26]

The inflammatory and proinflammatory cytokine molecules display a probable outcome on the catabolic effects that subsidize to the pathophysiological development of OA.[27] Our present work confirms that esculetin effectively decreases the production of the NO, PGE2, TNF-α, and IL-6 in LPS-induced RAW 264.7 cells [Figures 1 and 2]. Several parameters are used to evaluate the anti-osteoarthritic outcome

of esculetin in the MI-induced model, such as serum intermediates, inflammatory cytokines, weight-holding distribution, and histopathologic morphology. In the current study, we further evaluated the OA-mediated pain relief effect of esculetin in the MI-induced OA rat model. OA-related pain can be generated by movement in joint, which results in decreased usage and reduced joint flexibility.[28,29] Our results confirmed that esculetin markedly enhanced weight-bearing capability in MI-induced OA animals, signifying that esculetin could be valuable in the management of OA-based pain. Our present finding reveals that esculetin effectively decreases inflammation in the MI-induced OA SD rat model.

Numerous findings demonstrate that inflammation plays an important role in the development of arthritis and further confirm the chondroprotective consequence.[30,31] The present study revealed that esculetin exhibits chondroprotective properties in OA induced by MI in SD rats through inhibiting proinflammatory cytokines and inflammatory mediator molecules in serum. Further inflammation leads to the progression of OA and damage of cartilage joints.[31,32]

Proinflammatory cytokines like PGE2 and catabolic mediators such as NO are produced through the reddened synovial membrane and change the equilibrium of cartilage matrix restoration and deprivation.[33] These indications exaggerate the joint deprivation and medical symptoms caused by OA. Consequently, targeting the inflammation reaction can be an active approach to manage the development and progression of OA. In the current study, esculetin effectively decreased the inflammatory response and prevented the development of OA [Figure 6].

CONCLUSION

To conclude, esculetin has been verified as an anti-inflammatory compound through its action in repressing the levels of NO, PGE2, TNF-α, IL-6, and IL-1β in LPS-administered RAW 264.7 cells. Esculetin also decreased hardness and joint pain, further reduced the inflammatory and proinflammatory cytokine production in an MI-induced OA SD rat model. Results obtained evidently show that esculetin has anti-osteoarthritic properties. Further exploration of this context in the future could lead to the development of esculetin as an operative healing drug for the management of OA and its associated symptoms.

Acknowledgements

This work was supported by the Department of Orthopaedics, Fendong District of Taiyuan Central Hospital, Taiyuan, 030000, China.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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