BOSWELLIA SERRATTA EXTRACT REDUCES ARTICULAR CARTILAGE DAMAGE AND INFLAMMATION IN OSTEOARTHRITIC RATS

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Abstract

Background: *Boswellia serrata*, have been used traditionally for the management of osteoarthritis (OA) as single *extract*s, or in other combinations. The aim of the study was to evaluate the safety and efficacy of *B. serrata* for monosodium iodoacetate (MIA)-induced osteoarthritis using rat animal model.

Methods: The *B. serrata* extract was administered orally once a day for 28 days. To confirm the OA symptoms, the Arthritis Index (AI) was assessed once each week. A histopathological evaluation of the synovial membrane, proteoglycan layer, and cartilage injury was performed. *B. serrata extract*'s anti-osteoarthritic effects were confirmed by measuring levels of pro-inflammatory mediators/cytokines and matrix metalloproteinases (MMPs) in the serum.

Results: After 28 days of treatment, two different doses of *B. serrata* extract (200mg/kg and 400mg/kg) and Ibuprofen (IB) 20mg/kg were compared with the MIA group.400mg/kg of *B. serrata* extract significantly reduced the AI score ($p<0.001$) when compared to IB 20mg/kg. 400mg/kg of *B. serrata* extract has an equivalent impact on inflammatory cytokine levels of Cyclooxygenase-2 (COX-2), Tumor Necrosis Factor (TNF-α) and MMP-2. 200mg/kg of *B. serrata* extract has a significant percentage of inhibition of COX-2, TNF-α and MMP-2 in comparison with the standard drug ($p<0.001$). There was no significant different found in the animal body weight.

Conclusion: Overall, our data suggest that *B. serrata* extract has anti-osteoarthritic effects in MIA-induced OA rats by modulating inflammatory cytokines and MMP-2.

Keywords: Osteoarthritis; *Boswellia serrata*; inflammatory cytokines; arthritis index; histopathological evaluation.

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1. Introduction

Arthritis is an inflammation of the joints that causes chronic pain and other symptoms. Among the several types of arthritis, the most frequent is osteoarthritis (OA), which has a catastrophic impact on patients' quality of life and the economy. Trauma, excess body fat, and hereditary predisposition can all exacerbate the condition. The efficacy of pharmaceutical therapies for OA is constrained. Non-steroidal anti-inflammatory medicines (NSAIDs), such as acetaminophen, ibuprofen, naproxen, and indomethacin, are frequently used to treat the condition with the goal of lowering pain and inflammation [1]. However, there are negative consequences for the digestive and cardiovascular systems from using NSAIDs regularly.

For as long as there have been humans, medicinal plants have been a goldmine of potential treatments. There are many plants that have been shown to have anti-inflammatory benefits in both animal and human experiments [2]. A variety of polyherbal formulations have also been proven to have positive effects in the treatment of osteoarthritis. There has been a lot of focus on herbs and phytochemicals as potential sources of antioxidants, hypoglycemic agents, and antihyperlipidemic agents, all of which play an important role in the development of novel therapeutic agents. Boswellic acids appear to selectively inhibit 5-LO. 5-LO causes inflammation by increasing free radical damage, calcium displacement, cell adhesion, and the migration of inflammation-producing cells to the wounded body location[3].

There has been a lot of focus on herbs and phytochemicals as potential sources of antioxidants, hypoglycemic agents, and antihyperlipidemic agents, all of which are important in the process of making new medicines[2]. *Extract* of the *B. serrata* have long been utilized in Ayurvedic medicine to treat a variety of conditions and symptoms. However, there have not been any investigations on *B. serrata* extract's toxicity, effectiveness and associated research in rat models of MIA-induced OA. Hence, this study was designed to evaluate the effects of *B. serrata* extract on articular cartilage in an MIA-induced rat model of OA and explore the potential therapeutic applications for OA.

2. Materials And Methods

2.1 Sample preparation:

The inhouse prepared *B. serrata* extract is a mixture comprising a hydro alcoholic *extract* of *Boswellia serrate* oleo gum resin.

2.2 Acute toxicity:

The OECD - 423 standards were used to conduct the acute toxicity study. We used Sprague-Dawley female rats that were between 160 and 200 g and 6 weeks old for our investigation. All of the experimental rats were kept in cages for 7 days prior to the study's start in order to get acquainted to the environment of the lab. The night before the trial started, the animals were on nil per oral. One animal at 2000 mg/kg of medicine was used for sighting investigation (p.o.), first one had lived. In the primary investigation, 2 rats received 2000 mg/kg. At 10 min, 30 min, 1, 2, 4 and 6 h after dosing, and daily for 14 days, the treated animals were observed for adverse clinical signs and mortality.

2.3 Sub-acute toxicity:

Sub-acute toxicity was characterised using OECD-407 criteria. Male SD rats weighing 150–180 g were randomly assigned to one of four groups (n = 5). The G1 (n = 5) control group, G2, G3, and G4 received 200, 500, and 1000 mg/kg of *B. serrata* extract via oral gavage daily for 28 days, respectively. Timely observation of behaviour, appearance, and other unfavourable outcomes was recorded.

2.4 Animals

Male Sprague-Dawley rats (150–190 g) aged six weeks were procured from the Biogen animal breeder (Bangalore, Karnataka). All animals had a seven-day acclimatisation period before being selected as test subjects. In terms of temperature (22 °C, 50% relative humidity, 1%), humidity, and light (12-hour cycle), the experiment was conducted in a controlled environment. The animals had unrestricted access to sterile food and water. The study complied with the IAEC's regulations (Approval number IAEC/249/2021). Alterations in body weight were monitored once every seven days.

2.5 Experimental study

After shaving and cleaning the knee with 70% alcohol, the rats' left knees were then injected with 0.9% sodium chloride in 50 L containing 3 mg MIA through a 1mL insulin syringe to cause OA (BD Medical-Diabetes Care, Franklin Lakes, NJ, USA). After three days, the rats were randomly assigned to one of five groups (n = six rats per group):

Group A: Normal control received saline
Group B: OA control
Group C: OA control treated with ibuprofen (20 mg/kg)
Group D: OA control treated with *B. serrata* extract (200 mg/kg)
Group E: OA control treated with *B. serrata* extract (400 mg/kg)

In order to administer the test substances orally, 0.5% (CMC-Na) was used as a solvent.

2.6 Histopathological investigation

Rats in all groups were anaesthetized and sacrificed when the experimental investigation was complete. For processing, a sample of the knee joint was taken from each rat and fixed in formalin (10%), embedded in paraffin, and serially sectioned at 5 mm. Hematoxylin and eosin (HE) staining was used on all tissue samples. A light microscope was used to examine the prepared slides [10].

2.7 Estimation of Pro-inflammatory cytokines

Serum was extracted from blood samples taken for the purpose of analysing pro-inflammatory cytokine concentrations. After centrifuging the blood samples at 2000 rpm for 15 minutes, the serum was removed and kept at -80 °C. Commercial ELISA kits were used to measure the levels of the inflammatory cytokines TNF-α, COX -2, and MMP-2 in the serum [10].

2.8 Statistical analysis

The mean and standard deviation of the data are displayed. One-way analysis of variance (ANOVA) was used for all data analyses, and GraphPad Prism software was used to run Dunnett's test to determine the significance.

3. Results

3.1 Acute toxicity study

Animals given 2000 mg/kg of their body weight of *B. serrata* extractshowed no signs of death, aberrant clinical symptoms, or gross pathology findings. There were also no changes in movement patterns, physical and behavioral factors, and overall appearance.

3.2 Sub-acute toxicity study

B. serrata extract therapy at 200, 500, and 1000 mg/kg doses demonstrated no toxicity, mortality, or morbidity. Throughout the course of the trial, no changes were seen in the rats' pattern of movement, physical and behavioral parameters, or overall appearance.

3.3 Effect of *B. serrata* extract on Body Weight in Monosodium Iodoacetate (MIA)-Induced Osteoarthritis in Rats

Overa period of four weeks, the animals' weights were measured on a weekly basis in order to track how *B. serrata* extract

affected their bodies. There were no noticeable differences in body weight between the disease control group and the treatment group during the treatment period. Figure 1 demonstrates that after treatment with *B. serrata* extract, there was not a noticeable shift in the amount of body weight lost or gained by the animals.

3.4 Effect of *B. serrata* extract on Arthritis Index (AI) over28 Days in MIA-Induced Osteoarthritis in Rats

We inspected each animal once a week to look for signs of OA and swelling (depicted in Table 1). The arthritis index (AI), which takes into account swelling, was highest in the MIA group compared to the other groups. After 28 days of treatment, two different doses of *B. serrata* extract (200mg/kg and 400mg/kg) and Ibuprofen (IB) 20mg/kg were compared with the MIA group. 400mg/kg of *B. serrata* extract significantly reduced the AI score ($p<0.001$) when compared to IB 20mg/kg.

3.5 Effect of *B. serrata* extract on histopathology

The presence of histopathological alterations was evaluated in all rat groups. Bone damage, synovial hyperplasia, and inflammation levels were analyzed by histopathology in the rat models. Joint modifications are depicted in Figure 2. Joint edema, synovial gap enlargement, cartilage erosion, and synovial hyperplasia were some of the histological abnormalities observed in the MIA group of rats. In contrast, synovial hyperplasia and joint edema were reduced by therapy with *B. serrata* extract 400 mg/kg compared with Ibuprofen 20 mg/kg. These results from histopathology demonstrate that *B. serrata* extract 400 mg/kg reduces the severity of MIA-induced OA in rats.

3.6 Effect of *B. serrata* extract on inflammatory cytokine levels

A major role for pro-inflammatory cytokines in the development of OA is the maintenance of tissue damage and persistent inflammation. We thus examined the impact of *B. serrata* extracton COX-2 and TNF-α in MIA-induced OA rats. When compared to the MIA-induced control group, COX-2 and TNF- α levels showed significant difference at IB 20 mg/kg, *B. serrata* extract 200 mg/kg, and *B. serrata* extract 400 mg/kg ($p<0.001$). When compared to IB 20 mg/kg, *B. serrata* extract200 mg/kg demonstrated an impact that was similar in terms of the percentage of inhibition of COX-2 and TNF-α. When compared to IB 20 mg/kg, *B. serrata* extract 400 mg/kg exhibited an equivalent impact. In comparison to the dose of the standard drug, a test drug dose of 200 mg/kg is considered highly significant ($p<0.001$) (Figure 3).

3.7 Effect of *B. serrata* extract on MMP

In order to explore the effect of *B. serrata* extract on the serum of MIA-induced rats, the protein levels of MMP-2 were measured and analyzed. MMP-2 levels indicated a significant difference at IB 20 mg/kg, *B. serrata* extract 200 mg/kg, and *B. serrata* extract 400 mg/kg as compared to the MIA-induced control group ($p<0.001$). *B. serrata* extract 400 mg/kg had an effect that was comparable to that of IB 20 mg/kg in terms of the percentage of MMP-2 inhibition it provided. *B. serrata* extract was found to be effective in reducing the expression of these markers in a dose-dependent way.

4. Discussion

Loss of articular cartilage and moderate inflammation of the tissue around the joint define osteoarthritis (OA), a painful and disabling condition[10]. As a result of the joint's heightened sensitivity to pressure and the accompanying chronic arthritic pain, OA makes movement challenging for those who suffer from it. Pain relief, joint mobility, joint strength, and reducing the

debilitating symptoms of the illness are the primary goals of OA treatment.

Synergistic actions are believed to be possible in herbal compounds that contain more than one medicinal plant. This would result in an increase in the effects that are desired[11]. On the basis of this concept, a variety of different polyherbal substances have been examined in clinical and experimental research as potential therapeutic agents in the management of osteoarthritis.

The purpose of this study was to assess the safety and efficacy of *B. serrata* extract, for the treatment of osteoarthritis. *B. serrata* extract have been shown to have anti-inflammatory benefits on their own, but their beneficial effects on osteoarthritis had yet to be researched. Degeneration of cartilage and heightened inflammation can be caused by monosodium iodoacetate (MIA), an inhibitor of glyceraldehyde-3-phosphate dehydrogenase that also disrupts cellular glycolysis[12][13][14]. Pain, edema, and stiffness are justsome of the OA symptoms triggered by inflammation's production of cytokines and their subsequent biochemical and mechanical interactions with other mediators[15]. Using MIA-induced OA as a model for assessing the effectiveness of possible modulators of OA has been standard practice for some time. The model has been implemented in several OA-related studiesdue to its ability to simulate the symptoms experienced by those living with OA[16].

In-vivo acute toxicity and sub-acute toxicity studies were used to assess the toxicity of *B. serrata* extract. The acute and sub-acute toxicity studies conducted on SD rats were unaffected at the dose range up to 2 g, implying that they were not toxic.When proteoglycan, the primary element of the ECM, is broken down by MMPs, articular cartilage degradation begins[17][18]. Using H&E staining of the articular cartilage, we could see the histological changes in the current investigation. We verified that rats with MIA-induced OA had cartilage and synovial membrane injury, as well as proteoglycan degradation. The destruction of cartilage and synovial membrane was successfully prevented by 400 mg/kg *B. serrata* extract administration. These findings support the hypothesis that *B. serrata* extract treatment inhibits cartilage degradation and proteoglycan layer breakdown, thereby mediating anti-osteoarthritic effects.It has been shown that the severity of disease progression is directly connected tothe extent to which pro-inflammatory cytokines are upregulated in the knee joints of peoplewith OA[19]. TNF- α is a pro-inflammatory cytokine that affects bone and cartilage remodeling and can cause synovial hyperplasia[20].

MIA-induced inflammation in rats increased serum TNF- α, IL-1, IL-10, COMP, and CRP and knee joint with high NF-B p65 expression. JHF, a novel polyherbal formulation, controlled these inflammatory markers, promoting chondrocyte differentiation and osteophyte development, and slowing OA progression[21]. *B. serrata extract*'s anti-inflammatory effects wereanalyzed by measuringthe pro-inflammatory mediator COX-2, pro-inflammatory cytokines TNF-α and proteases MMP, particularly MMP-2, in the serum of MIA-induced OA rats. We found that *B. serrata extract* administration, like ibuprofen, might attenuate the MIA-induced rise in cytokines. These findings suggest that *B. serrata extract* can lessen inflammatory mediators and responses, which can reducecartilage degradation.

The effects of *B. serrata* extract levels of pro-inflammatory mediators and cytokines on the development of osteoarthritis were validated in the current investigation. Additionally, *B. serrata extract*slowed the development of OA by lowering the levels of MMP expression, and preventing the breakdown of articular cartilage and the

synovial membrane. These findings showed that *B. serrata* extracthas potential therapeutic applicationsin osteoarthritis.

5. Conclusion

B. serrata extract, has been shown to have anti-osteoarthritic effects in MIA-induced OA rats by modulating inflammatory cytokines and MMP-2. Our study demonstrated the safety and efficacy of *B. serrata* extract in reducing the arthritis index, inhibiting COX-2, TNF-α and MMP-2 levels, and protecting the articular cartilage against the progression of osteoarthritis. *B. serrata extract* could potentially work as a therapeutic agent for osteoarthritis, but this first needs to be proven by clinical studieson humans.

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Conflicts of interest: The authors declared no conflict of interest.

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Table 1. Effect of *B. serrata* extract on the arthritis index for 28 days in MIA-induced osteoarthritis in rats. The data are presented as mean ± SEM (n=6/group).

	Day - 0	Day – 7	Day – 14	Day – 21	Day - 28
Normal control	0	0	0	0	0
MIA	12±0.16	11.33±0.10	10.98±0.03	10.77±0.04	10.43±0.07***
MIA + Ibuprofen 20mg/kg	11.32±0.06	11.23±0.04	11.05±0.05	10.63±0.07	10.1±0.07*
MIA + *B. serrata* extract200mg/kg	11.15±0.05	11.07±0.04	10.67±0.06	10.17±0.04	9.95±0.05***
MIA + *B. serrata* extract400mg/kg	11.45±0.06	11.3±0.04	10.83±0.06	10.4±0.10	9.9±0.06***

*Level of significance P < 0.05 *, P <0.01 **, P <0.001 ****

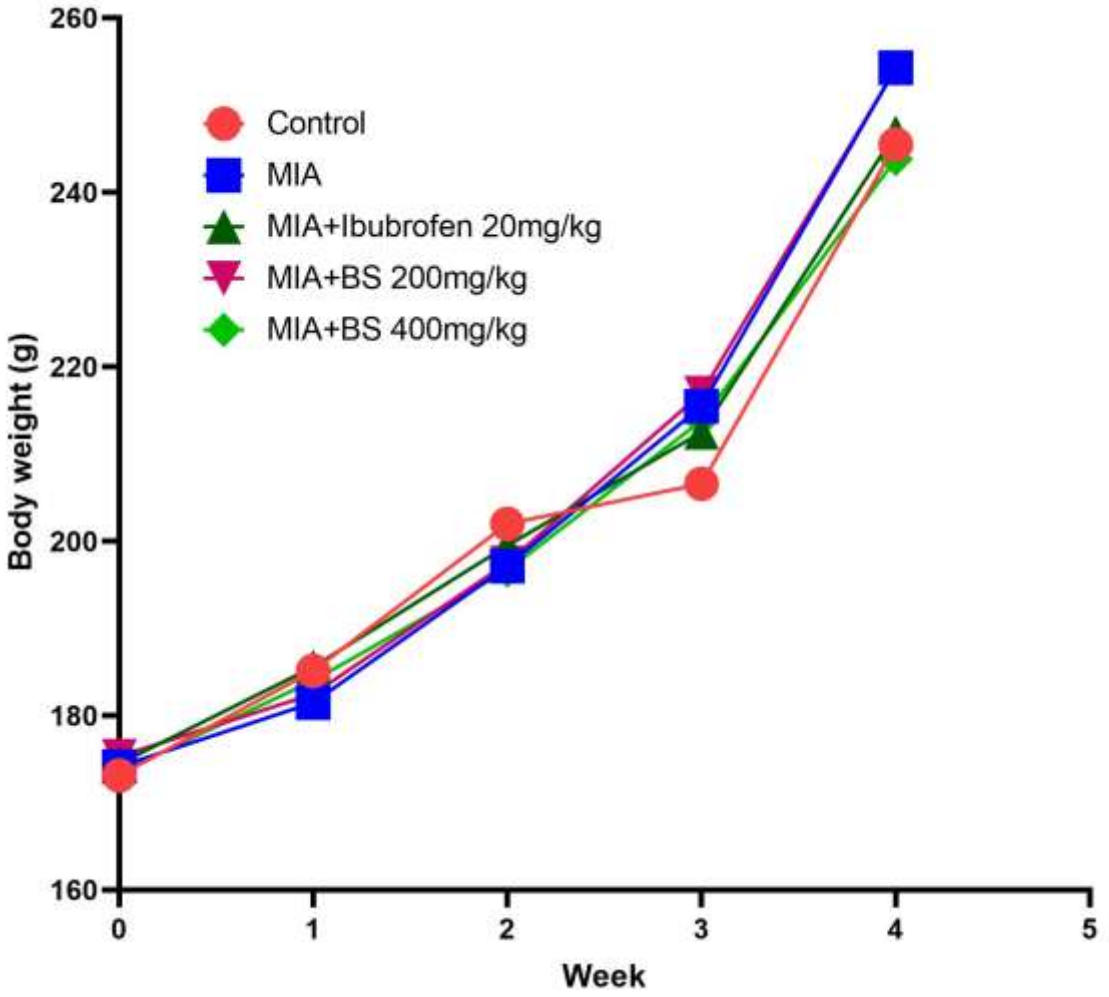

Figure1. The influence of *B. serrata* (BS) *extract* on weight gain or loss in rats with osteoarthritis caused by monosodium iodoacetate (MIA). Data are presented as the mean + SEM (n = 6/group), based on weekly measurements of body weight over the course of 4 weeks. There was no statistically significant difference between any of the groups.

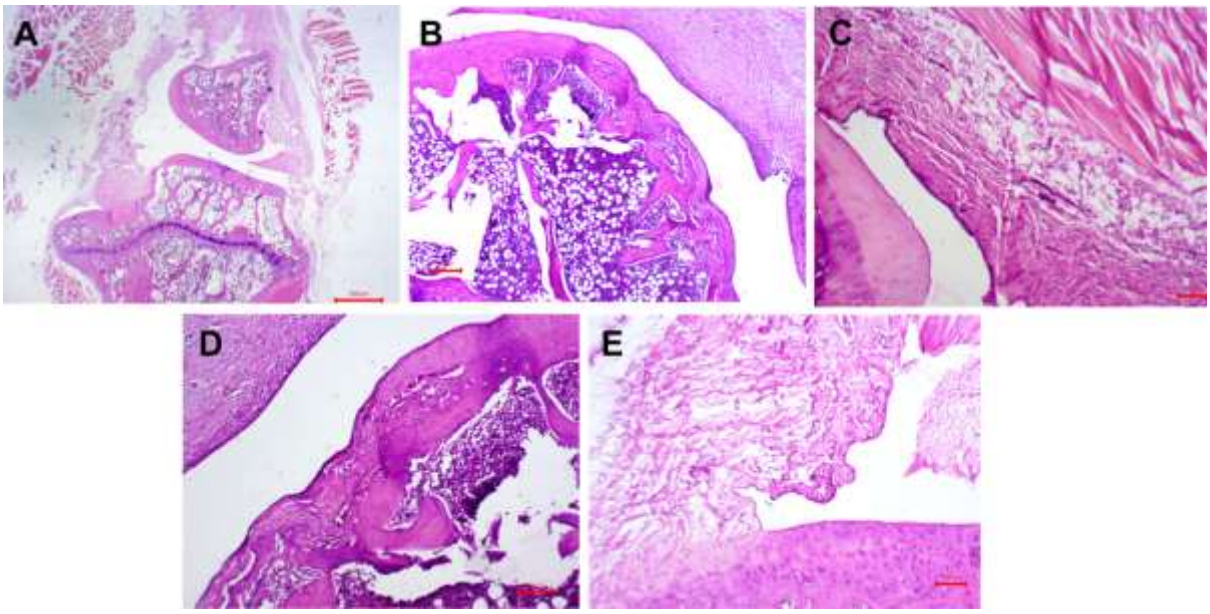

Figure 2. In a rat model of osteoarthritis, the effects of *B. serrata* extract on the histological assessment of joint activity after exposure to MIA were examined. Hematoxylin and eosin (H&E) were used to stain synovial membranes and cartilage in knee joints, (A) Control, (B) MIA, (C) MIA + Ibuprofen 20 mg/kg, (D) MIA + *B. serrata* extract 200 mg/kg, and (E) MIA + *B. serrata* extract 400 mg/kg.

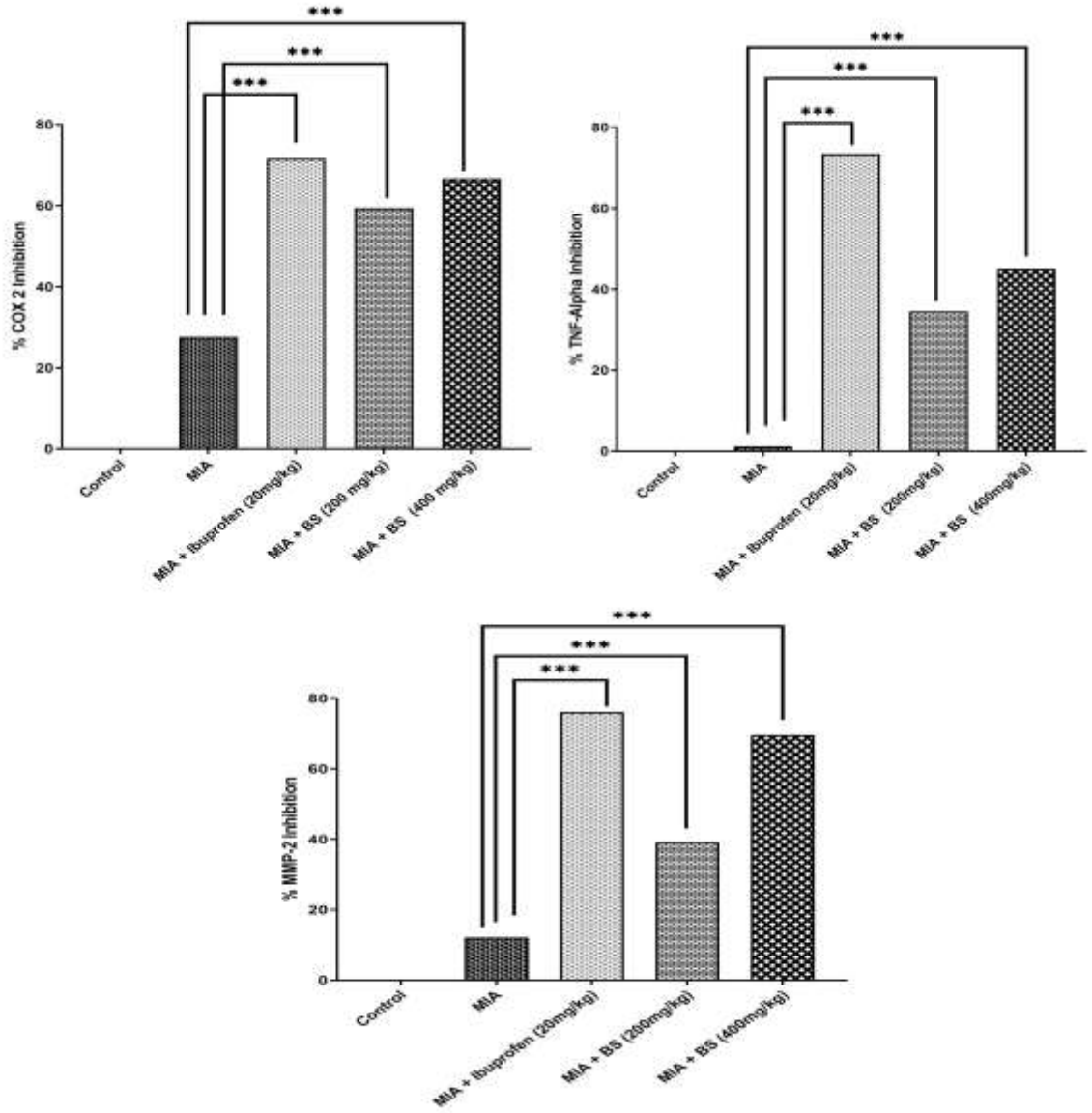

Figure 3. Inflammation-related protein expression levels in serum and the role of *B. serrata* extract (BS). The expression levels of (A) Inflammatory mediators Cyclooxegenase-2 (COX-2), (B) Pro – inflammatory cytokines tumor necrosis factor – alpha (TNF-α), (C) protein levels of matrix metalloproteinases (MMP – 2).