TPM Vol. 32, No. S6, 2025
ISSN: 1972-6325
https://www.tpmap.org/

Open Access

PAEONIA EMODI ROYLE ATTENUATES ADJUVANT-INDUCED ARTHRITIS THROUGH DOWNREGULATION OF INFLAMMATORY CYTOKINE SIGNALING AND BY REDUCING THE OXIDATIVE STORM

SYED IHTISHAM HAIDER[1], QAISER JABEEN[2], IRFANA LALARUKH[3*], MUHAMMAD RAMZAN KALEEM[4], MUHAMMAD MUZAMMIL RAMZAN[5], NUMERA ARSHAD[6], AISHA SHEHZAD[7], ASIM RAZA[1], NAMRA ZAREEN[1], SOHAIB IQBAL[7], MUHAMMAD AHMED KHAN[5], IJAZ ALI[5*]

[1]DEPARTMENT OF PHARMACY, MUKABBIR UNIVERSITY OF SCIENCE AND TECHNOLOGY, GUJRAT, PAKISTAN 50700
[2]DEPARTMENT OF PHARMACOLOGY, FACULTY OF PHARMACY, THE ISLAMIA UNIVERSITY OF BAHAWALPUR, BAHAWALPUR, PAKISTAN 63100.
[3]DEPARTMENT OF BOTANY, GOVERNMENT COLLEGE WOMEN UNIVERSITY FAISALABAD PAKISTAN.
[4]DEPARTMENT OF EASTERN MEDICINE, FACULTY OF MEDICAL SCIENCE GOVERNMENT COLLEGE UNIVERSITY FAISALABAD
[5]DEPARTMENT OF PHARMACOGNOSY, FACULTY OF PHARMACEUTICAL SCIENCES GOVERNMENT COLLEGE UNIVERSITY FAISALABAD.
[6]DEPARTMENT OF PHARMACY, COMSATS UNIVERSITY LAHORE CAMPUS.
[7] LECTURER PHARMACEUTICAL CHEMISTRY. LYALLPUR INSTITUTE OF ADVANCED STUDIES COLLEGE OF PHARMACY FAISALABAD.

ABSTRACT

Background: Paeonia emodi Royle is associated with the family Paeoniaceae and has traditionally been used to relieve muscular strains, headaches and rheumatic inflammation by local communities in various countries.

Study aims: The current study was set out to look at the therapeutic potential of Paeonia emodi Royle in heat induced pain and adjuvant-induced arthritis paradigm.

Methodology: Hot plate and tale immersion methods were used on rats to investigate the analgesic potential of Paeonia emodi Royle. To develop immune-mediated rheumatoid arthritis, the left hind paw of the rat was injected with complete Freund's adjuvant (CFA) and the aqueous methanolic extract of Paeonia emodi Royle (IPE) was given orally for 28 days. The extent of the paw oedema and the arthritic score in the animals were tracked over the course of the experiment. On 28th day, general hematology and blood biochemistry of animals were computed along with systemic inflammatory proteins including C-reactive proteins and rheumatoid factor. Real-time quantitative polymerase chain reaction (qPCR) was utilized to assess the expressions of interleukins (IL-1β and IL-6), tumor necrosis factor (TNF-α), nuclear factor (NF-kB) and cyclooxygenase enzymes (COX-2). PGE2 was quantified in the blood through ELISA test.

Results: At two different doses, 500 mg/Kg and 1000 mg/Kg; p.o., IPE exhibited dose-dependent pain-relieving effect in the hot plate test (with highest retention time of 12.55 ± 0.34 & 14.56 ± 0.47 seconds) and tail immersion (response time of 12.55 ± 0.38 & 13.34 ± 0.20 seconds) tests. IPE induced a significant reduction in inflamed paw and arthritic score on 14th day of CFA injection. The extract considerably curtailed the mRNA expression of TNF-α, interleukins, and COX-2 at the doses of 500 mg/Kg; p.o. ($p \leq 0.05$) and 1000 mg/Kg; p.o ($p \leq 0.001$) respectively. IPE significantly enhanced the antioxidant activities of the both superoxide dismutase (SOD) and catalase (CAT) enzyme along with significantly lowering the levels of TBARS.

Conclusion: This study concludes the Paeonia emodi Royle as a potential drug which relieves the pain and immune-mediated arthritis by downregulating pro-inflammatory cytokines as well as by modulating the oxidative storm.

Key words: Rheumatoid arthritis, TNF- α, ELISA, qPCR, Inflammation, Complete Freund's Adjuvant,

1. INTRODUCTION

Rheumatoid arthritis (RA) is a painful autoimmune disease which produces inflammation in the synovium lining of smaller joints (Hasan et al., 2019). The primary stages of the illness are characterized by the symptoms of

burning pain, flush, warmth and oedema (Lad and Bhatnagar, 2016). When the disease worsens, synovial hyperplasia and pannus development occur. Persistent bone and cartilage degeneration results in a low quality of life and reduced physical activity (Blin et al., 2021). The development of antibodies against citrullinated proteins is a key component of the pathogenesis of RA (Ahsan et al., 2021b) eliciting the relocation of neutrophils and macrophages at inflammation spot along with stimulation of monocytes. As the inflammatory process progresses, levels of TNF- α, interleukins and NF-κB get elevated respectively (Shabbir et al., 2016). Pro-inflammatory cytokines like prostaglandins, primarily prostaglandin-E2 (PGE2) and various other cytokines are released as a result of T and B cell activation. Synovium inflammation and bone deterioration are driven by polymorphic neutrophils and lymphocytes (Bala et al., 2017; Shabbir et al., 2014). Once the body's ability to compensate is impaired, reactive oxygen species (ROS) are produced simultaneously, which exacerbates synovial damage in RA leading to disastrous injury to cellular entities like proteins, lipids, cell membranes and nucleic acids (Datta et al., 2014). Modern medicine's mainstay to control inflammation and associated pain is synthetic drugs like non-steroidal anti-inflammatory drugs (NSAIDs).These drug save a negative impact on the digestive system eventually causing peptic ulcer disease (Pilotto et al., 2010). Both conventional and biological Disease-modifying antirheumatic drugs (DMARDs) and glucocorticoids are all used for the palliative therapy of rheumatoid arthritis. At the same time, these drugs also have strong injurious potential to affect the hepatic, renal and gastrointestinal cells harmfully (Oyeleke et al., 2018). Natural flora offers more effective and harmless alternates, in the form of medicinal plants, for managing inflammation and pain in RA given the fatal adverse effects of current medications (Afsar et al., 2015). Numerous studies have depicted the incredible ability of plant-based crude extracts to reduce pain and inflammation in a variety of inflammatory illnesses (Padmanabhan and Jangle, 2012; Perianayagam et al., 2004).

Paeonia emodi Royle (also known as Ood-Salib), hailing from family Paeoniaceae, is a 28 inch' tall glabrate shrub having oblong leaves. The white flowers grow on its shoots. Oval shaped fruit contains black seeds (Shazia, 2012). It is found in high-altitude areas in the Himalayas region, commonly in Northern of Pakistan, Central China, West India and Nepal (Fazal et al., 2012).

Paeonia emodi is a famous edible plant with numerous medicinal applications and is used traditionally in a variety of inflammatory disease conditions; such as rheumatic pain, headaches, muscular pains, spasms, sciatic pain and general body pains in various countries (Ahmad et al., 2018; Jamil et al., 2020). Phenolics, monoterpenes, triterpenes, steroids and a number of organic acids are the mainly reported classes of phytochemical constituents in P. emodi (Ahmad et al., 2018). Anthraquinones and oligostilbenes are also abundantly found in this plant (Tantry et al., 2012). Pharmacological activities such as neuroprotective with antiepileptic and anxiolytic activities have been studied previously in crude extracts of P. emodi (Zaidi et al., 2012). Various studies have also demonstrated the antihyperlipidemic (Vijayaraj et al., 2013), nephroprotective (Kishore et al., 2017) and hepatoprotective effects (Raish et al., 2017). Current study aims to pharmacologically assess the analgesic and antiarthritic effects of Paeonia emodi Royle crude extract (IPE) in rats, emphasizing the molecular mechanisms.

2. MATERIAL AND METHODS

2.1. Plant material

Dried rhizomes of Paeonia emodi Royle were purchased from a herbal store situated in the heart of the Gujrat city, Pakistan. Roots were identified by an expert taxonomist from the department of Botany, University of Gujrat, Pakistan. The plant specimen was submitted in the Herbarium of department of Pharmacology, faculty of Pharmacy, the Islamia University of Bahawalpur, Pakistan (voucher number PE-RT-03-21-178).

2.2. Preparation of extract

The roots were thoroughly cleaned before being prepared for coarse grinding. Coarse powder was subject to the process of maceration for 3 days in 70% methanolic solvent thrice and then filtered with muslin cloth followed by filtration using a filter paper. Under reduced pressure, the filtrate was exposed to vaporization in a rotary evaporator (Hiedolph Laborota 4000, Germany). An airtight container was used to store the viscous semisolid extract (IPE) at -20°C for further studies (Rasheed et al., 2016).

2.3. Animals

In current study, both male and female Wistar albino rats weighing from 200 and 300 grams were used. The animals were accommodated in animal house with a climate-controlled environment. A 12:12 hour light and dark cycle was followed according to protocol. Animals were given a regular feed and unlimited access to water and they were allowed to spend a week getting used to the lab environment before the start of experiment. The experimental protocols were authorized by the Islamia University of Bahawalpur's Pharmacy Animal Ethics Committee (certificate no. PAEC/21/37) (Javed et al., 2020).

2.4. Phytochemical analysis

Plants have always served as a wealthy source of diverse phytochemical components as well as their secondary metabolites. IPE was evaluated, through different tests, for screening of various phytochemicals; Identification of alkaloids was carried out using the Mayer's and Wagner's tests, glycosides by using the Keller-Kiliani, Liebermann's, and Salkowski's tests. Confirmation of flavonoids was done by performing the lead acetate and the alkaline reagent tests. Identification and quantification of tannins were carried out by using gelatin and ferric chloride tests. The Fehling's test, Molish's test, and Benedict's test were performed to determine the

carbohydrates. Xanthoproteic and Ninhydrin assays were used to examine amino acids and proteins (Akhtar et al., 2017).

2.5. In vitro antioxidant activity by DPPH assay

The antioxidant potential of IPE was determined by calculating the free radical scavenging ability of a stable 1,1 –diphenyl 2-picrylhydrazine (DPPH) free radical by using a briefly modified version of the procedure utilized by Brand-Williams et al., 1995. A milliliter of 0.1 mM DPPH methanolic extract was mixed with 1 milliliter of IPE solution with concentrations that were ranged from 200 to 1200 g/ml. L-ascorbic acid solution was used as a reference standard having the same concentrations. 1 milliliter each of DPPH and methanol was mixed and used as control. The experiment was conducted three times and each solution was left in dark for 30min throughout the reaction period (Jabeen et al., 2021). Nano Drop spectrophotometer (Denovix Inc, USA) was employed to determine the reduction in absorbance at 517nm. Percent inhibition was calculated as follow:

$$\% \text{ Inhibition} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \text{ x } 100$$

Where, 'Ac' is the absorbance of control and 'As' represents the sample absorbance.

2.6. High performance liquid chromatographic analysis

In order to qualitatively investigate the flavonoids and phenolic compounds, the HPLC technique was utilized. Throughout the experiment, fresh solutions of IPE and standard reference were prepared in methanol at 10mg/ml and 50µg/ml respectively and were maintained at 4oC throughout the experiment. A Shimadzu LC10-AT VP Liquid Chromatograph with SIL-20A auto sampler (Shimadzu Scientific Instruments, Kyoto, Japan) and SPD-10AV UV VIS Detector were used for the analysis of the samples. A Shim-Pack CLC-ODS (C-18, 25cm 4.6mm, 5m) was used for the isolation at room temperature. The solvent system used in mobile phase used was a binary system consisting of Solvent A (water: acetic acid 94:6, pH=2.2) and Solvent B (acetonitrile). The gradient elution times were as follow: 0-15min, 85% A:15% B (linear gradient, v/v,), 15-30min, 55% A45% B (linear gradient ,v/v), and 30-35min, 0% A:100% B(linear gradient , v/v equilibration). The detection wavelength was 280nm while the flow rate was 1.0ml/min. The retention periods of major peaks generated by IPE and standard solutions were compared for qualitative analysis (Jamshed and Jabeen, 2022).

2.7. Analgesic activities of crude extract of Paeonia emodi Royle (IPE)

2.7.1. Hot plate test

Hot plate method was used with minor adjustments to evaluate the analgesic potential of IPE (Kayani et al., 2016). Rats were pre-tested on a hot plate (Harvard apparatus) that was kept at a temperature of 55 ± 1°C. During the pre-testing, those animals with a latency time (the period of time a rat stays on a hot plate without licking, flicking a hind limb or jumping) more than 15 seconds were disqualified. Animals were split up into different groups at random. Tramadol (25 mg/Kg; p.o.) and diclofenac sodium (25 mg/Kg; p.o.) were administered to the standard groups, while distilled water was administered to the control group. Treatment groups received IPE at varying doses (300, 500, and 1000 mg/Kg; p.o.). Each rat was placed on a hot plate 30 minutes after the dose was administered, and the time it took the animal to respond was recorded in seconds (reaction time). The cutoff time was 30 seconds for all animals to avoid tissue injury. Each group's reaction time was noted at 0, 30, 60, 90, and 120 minutes following treatment.

Tail immersion test

In order to assess the analgesic properties of IPE, the method employed by Javed et al was utilized with minor adjustments (Javed et al., 2020). A thermostat was utilized to maintain a constant temperature of 55 ± 1°C while monitoring the tail immersion method. Each rat had its lower tail marked (3 cm) and submerged in hot water. Rats responded by withdrawing their tails from the hot water. IPE was given to the several groups orally at varied doses (300, 500, and 1000 mg/Kg; p.o.) just after basal latency testing. Following 30 minutes of treatment, the animals' responses in seconds were timed using a stopwatch at 30, 60, 90, and 120 minutes. To prevent any tissue damage, the cutoff time was set at 25 seconds.

2.8. Anti-arthritic activity of IPE

Anti-arthritic activity of IPE was estimated following our previously used method (Haider et al., 2022). There were six animals in each of the different groups that were formed from the animals. The normal and arthritic control groups received distilled water (5 ml/Kg; p.o.). The treatment groups received piroxicam (10 mg/Kg; p.o.) and IPE (300, 500, and 1000 mg/Kg; p.o.). In order to create a rheumatoid arthritis rat model, subplantar tissues of the left hind paw were administered with 200µl of Complete Freund's Adjuvant (Sigma-Aldrich, USA) in all groups except the normal control group. The first day of the trial marked the start of the treatment, which lasted for a total of 28 days.

2.8.1. Assessment of arthritis and weight variation

Digital vernier calliper was used to measure the animal's paws prior to intoxication and subsequent changes in paws sizes were noted on days 7, 14, 21 and 28. Weights of all animals were also monitored at the same intervals. Different symptoms, such as heat, redness, puffiness, tumor growth and joint flexibility, were used to gauge the severity of the inflammation. The condition was categorized as having a visual arthritic score of 0, 1, 2, 3, and 4. Using this scoring system, 0 meant there was no redness or swelling, 1 indicated minor swelling at the metatarsophalangeal joints, 2 meant that there was redness, bulge, and warmth at the interphalangeal joints, 3

meant there was swelling at the ankle joints and 4 meant that the entire paw was swollen, with stiffness also affecting the opposite paw (Ahsan et al., 2021b).

2.8.2. Screening of hematological and biochemical parameters

On the day of experiment, blood samples were obtained by euthanizing the animals followed by heart puncture. Hematological parameters such as blood cells (RBCs, WBCs and Platelets) and blood hemoglobin values (Hb) were measured using a sophisticated hematology analyzer (Sysmex, USA). To ascertain the animal's renal and hepatic health, serum creatinine (CR) and blood urea nitrogen (BUN) levels as well as the liver enzymes mainly alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) were assessed. Additionally, C-reactive proteins (CRP) and rheumatic factor (RF), two systemic inflammatory markers, were assessed using commercially available auto-analyzer kits (Selectra Pro M, France). The test procedures for each kit were followed thoroughly (Ahsan et al., 2021a).

2.8.3. Quantification of serum prostaglandin (PGE2) levels using ELISA

PGE2 levels in seum samples of the experimental animals were measured using ELISA (E-EL-0034 ELISA kit). In each of the non-specific binding (NSB) and B0 wells, 100µl of the diluent was added whereas test samples and standard solutions (100µl) each were received by their respective wells.

In addition to this, 50ul phosphate buffer was injected only in to the NSB wells. Each well (B0, standard, and test) received 50µl of the PGE2 antibody was added. With the exception of B0 wells, all other wells appeared yellow. Furthermore, after covering the plates properly, they were placed inside the shaker incubator for two hours at 500 rpm at room temperature. Additionally, 5µl of the PGE2 alkaline phosphatase was added, after washing ELISA plates with wash buffer, to all wells individually. Furthermore, p-nitrophenyl phosphate (200µl) was added as a substrate into each well followed by incubation at 25°C. Finally stop solution was added and the optical density (OD_{450}) was measured (Uttra et al., 2018).

2.8.4. Expression levels of mRNA of inflammatory markers (TNF- α, IL-1β, IL-6, NF-kB and COX2)

Extraction of the RNA was carried out from blood samples using TRIzol reagent and the subsequently quantified with the help of Nano-Drop spectrophotometer. Afterwards, by using a cDNA synthesis kit (Vivantis Technologies® Malaysia) and RNA reverse transcription methodology, the cDNA was synthesized and stored at -20 °C. In the next steps, sterile PCR tubes were taken and added 5µL qPCR master mix ROX (Simply Biologics, South Korea), cDNA (500 ng), 0.3µL each of gene specific forward and reverse primers and finally the ~10 µL nuclease-free water. The tubes were positioned in the q-PCR machine, the program was initiated and the reaction was allowed to start. There were four steps of reaction process: enzyme activation, denaturation and annealing, accomplishing in 45 cycles. In terms of fold changes, comparative gene expressions of TNF- α, IL-1 β, IL-6, NF-kB and COX-II enzyme were calculated from ΔΔCT values (Ahsan et al., 2021a; Haider et al., 2022). The GAPDH served as housekeeping gene. The primers were formed using Primer-3 input software. Primer sequences along with their amplicon size are shown in **Table 1**.

2.8.5. Determination of oxidative stress biomarkers

In order to estimate the enzymatic activity of superoxide dismutase (SOD) and catalase (CAT), serum was recovered from blood samples that were collected on the final day of the study. Malondialdehyde (MDA) levels were also measured (Jabeen et al., 2022).

i. Activity of superoxide dismutase (SOD)

To estimate the total SOD activity in serum, xanthine oxidase method was used in serum following the kit procedure (E-BC-K020-M). The results of the enzymatic activity were expressed as units/ ml.

ii. Activity of catalase enzyme (CAT)

Catalase (CAT) enzyme generally catabolizes H_2O_2 and ammonium molybdate is reported to stop this reaction which produces a yellowish complex upon reacting with residual H_2O_2. Here, the enzymatic activity of CAT was evaluated as by the formation of the yellow-colored complex at 405 nm (E-BC-K031-M). The activity of CAT was shown as units/ml (Saleem et al., 2020).

iii. Formation of malondialdehyde (MDA)

Thiobarbituric acid (TBA) underwent a chemical reaction with the MDA which is the catabolite of lipid peroxide to yield an end point reflecting a red color. The absorption peak was noted at 532 nm. Concentrations of MDA were computed as µmol/L (Akhtar et al., 2021; Ratheesh et al., 2009).

2.9. Statistical analysis

The format of each data sets was articulated as Standard error mean (±SEM). GraphPad Prism software v.8.0 was utilized for statistical analysis of differences in results of control and treatment groups. One-way and two-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed to see the comparison among groups.

3. RESULTS

3.1. Phytochemical analysis

The presence of carbohydrates, alkaloids, glycosides, flavonoids, polyphenols, terpenes and phlobatannins was indicated by the phytochemical analysis of IPE. However, neither coumarin nor any amino acids or proteins were found.

3.2. Antioxidant activity of IPE by DPPH method

Aqueous methanolic extract of Paeonia emodi Royle (IPE) revealed free radical scavenging activity in a dose-dependent manner and a maximum activity ~78.52% was seen at the concentration of 1200 µg/ml as shown in Figure 1.

3.3. Analysis of IPE by HPLC

Different compounds such as caffeic acid (Rt. 1.88), apigenin (Rt. 6.73), rutin (Rt. 17.24), epigallocatechin (Rt. 27.03), quercetin (Rt. 19.03), quercetin-rhamno-di-hexoside (Rt. 29.76), quercetin-3-O-glucopyranoside (Rt. 33.03) were found in the extract as analyzed by HPLC method (Figure 2).

3.4. Analgesic effects of IPE

3.4.1. Hot plate test

The response time of animals on hot plate who were treated with orally administered crude extract (IPE) was a dose-dependent effect. A maximal time of 8.49 ± 0.27 seconds at 300 mg/Kg; p.o., 12.57 ± 0.33 seconds at 500 mg/Kg; p.o. and 14.56 ± 0.47 seconds at 1000 mg/Kg; p.o. was recorded in comparison with 10.89 ± 0.65 and 17.59 ± 0.73 seconds by diclofenac sodium and tramadol respectively (Figure 3A).

3.4.2. Tail immersion test

Animals treated with 300, 500 and 1000 mg/Kg; p.o. of IPE took a maximum time of 9.50 ± 0.49, 12.55 ± 0.38 and 13.34 ± 0.20 seconds respectively to show their responses in tail immersion test. These findings are highly significant ($p \leq 0.001$) and are analogous to those of standard drugs diclofenac sodium (14.92 ± 0.58 sec) and tramadol (17.33 ± 1.70 seconds) as depicted in Figure 3B.

3.5. Anti-arthritic effects of IPE

3.5.1. Effect on paw oedema

Administration of Subplantar CFA exacerbated inflammation in left paw which led to the maximum swelling as seen on the 7th day. A significant increase in paw thickness and paw volume was noted in the arthritic control group at day 7–28 which was contrary to the healthy control group. Paw diameter was reduced significantly ($p \leq 0.001$) after the treatment with IPE, when compared with the arthritic control group, with maximum efficacy shown at the dose of 1000 mg/Kg; p.o. (Figure 4A).

3.5.2. Effects on arthritic score

Throughout the whole study, the normal control group did not show any inflammation in their body. The outcomes exemplified in Figure. 4B exhibited the persistent elevation in arthritic score (an indicator of severity of disease) articulated by an arthritic control group. From the day 7 onwards till the end of study, IPE effectively reduced arthritic index when compared to the arthritic control group. The highest arthritic score (3.83 ± 0.08) was observed on day 28th in the arthritic control group. This was alleviated by Piroxicam and IPE (1.61 ± 0.09). The extract reduced the arthritic score to 2.23 ± 0.09 at 1000 mg/Kg; p.o., 2.50 ± 0.10 at 500 mg/Kg; p.o. and 2.92 ± 0.11 at 300 mg/Kg; p.o. respectively.

3.5.3. Effects on weight of animals

The arthritis continued to worsen and the animal's body weight was dropping. Findings showed in Figure. 5 revealed that there was an enormous reduction in weight of the arthritic control animals as compared to normal control group ($p \leq 0.001$) from the 14th day until the end of the study. However, in rats treated with the combination of IPE and piroxicam were significantly ($p \leq 0.001$) able to maintain their body weight from day 14 to 28.

3.5.4. Effects on hematological and biochemical parameters

The study found that rat's liver enzymes (ALT, ALP and AST) as well as other blood markers including C-reactive protein (CRP) and rheumatic factor (RF) levels were elevated when they were induced arthritis using Freund's adjuvant. The arthritic control group also had a drop in the levels of Hb and RBC, while WBCs level were raised in contrast to the normal control group. It was noticed that these elevated hematological markers were dose dependent (Figure 6). The levels of these bio markers viz. ALP, ALT, AST, CRP, WBCs, and RF returned to normal significantly after treatment with piroxicam and IPE ($p \leq 0.001$). Although the blood levels of CRP and RF in arthritic control were elevated to a substantial level (38.75 ± 0.57 mg/L and 37.91 ± 0.33 IU/L respectively) as represented in Figure 7. Nonetheless, at the doses of 500 ($p \leq 0.01$) and 1000 mg/Kg; p.o. ($p \leq 0.001$) IPE was able to significantly reduce the levels of CRP and RF which was comparable to the effects of piroxicam. While piroxicam treatment had no discernible impact on kidney function tests like urea and creatinine, polyarthritis induction had a minor impact on serum creatinine and blood urea nitrogen (BUN). As shown in Figure 8B, IPE was able to significantly normalize the levels of BUN at the dose of 1000 mg/Kg; p.o. ($p \leq 0.001$).

3.5.5. Effects on oxidative stress biomarkers

Figure 9 shows the results of mitigating effects of IPE in CFA-induced oxidative stress. When compared to the normal control group (SOD: 11.66 ± 0.16 U/ml and CAT: 103.97 ± 5 U/ml), a significant reduction ($p < 0.001$) of SOD (4.15 ± 0.11 U/ml) and CAT activities (35.91 ± 2.67 U/ml) was seen in the arthritic control group. Nevertheless, as it can be noticed in Figures 9A and 9B respectively that Piroxicam at a dose of 10 mg/Kg; p.o. and IPE in a dose dependent manner significantly ($p \leq 0.001$) restored the levels of SOD and arthritic controls. When looking at the MDA levels of arthritic control group, a considerable rise was seen (32.02 ± 1.51 µM/L) when compared to the normal control group (8.31 ± 0.47 µM/L). However, treatment with piroxicam and IPE at different doses in arthritic control groups, a significant reduction in the levels of MDA was seen as represented in Figure 9C.

3.5.6. Effects on mRNA expression level of pro inflammatory cytokines

After 4 weeks of the study, the mRNA expression of several inflammatory biomarkers was identified in Wistar albino rats. The mRNA expression levels of NF-κB ($p \leq 0.001$) were found to be raised in the arthritic control group (11.23 ± 0.16 folds). Treatment with IPE at 1000 (2.91 ± 0.13 fold), 500 (4.52 ± 0.04 fold), and 300 mg/Kg; p.o. (9.29 ± 0.15 fold) and piroxicam (2.47 ± 0.18 fold) ameliorated this rise in NF-κB in treatment groups (Figure 10). The arthritic control group demonstrated an elevated levels of COX-2 expression ($p \leq 0.001$) measuring 11.50 ± 0.24-fold. When the arthritic group was treated with IPE at a dose dependent manner 300 mg/Kg; p.o. ($p \leq 0.01$), 500 ($p \leq 0.001$) and 1000 ($p \leq 0.001$), and with Piroxicam ($p \leq 0.001$), the expression of COX-2 was reduced as compared with arthritic control group (Figure 10).

A noticeable increment was seen in the expression levels of IL-6 ($p \leq 0.001$) of the arthritic control group (9.49 ± 0.18-fold) when compared to the normal control group. Nonetheless, after 4 weeks of treatment with different doses of P. emodi extract (IPE) 1000 ($p \leq 0.001$), 500 ($p \leq 0.01$) and 300 mg/Kg; p.o. ($p \leq 0.05$) a significant reduction in the IL-6 expression levels was seen. We also noticed a remarkable rise in the expression levels of TNF-α ($p \leq 0.001$) in the arthritic control group (10.98 ± 0.20-fold) which after treatment with IPE lowered to 300 mg/Kg; p.o. (6.80 ± 0.09-fold), 500 (4.75 ± 0.08-fold) and1000 (2.91 ± 0.05-fold) in arthritic rats. The arthritic control group also showed an elevated expression (11.34 ± 0.13-fold) ($p \leq 0.001$) of IL-1β (fold change) when compared to the normal control. This increased expression of IL-1β was significantly lowered by IPE at doses of 500 mg/Kg; p.o. ($p \leq 0.01$) and 1000 ($p \leq 0.001$) as shown in Figure 10.

3.5.7. Effects of IPE on joint histopathology

There was a significant demonstration of deposition of inflammatory cells and bone erosion in arthritic control group whereas the synovial tissues were intact in normal control group. IPE exhibited a highly significant reduction in accumulation of cells, bone erosion and pannus formation at the doses of 500 and 1000 mg/Kg; p.o. respectively as given in Figure 11.

4. DISCUSSION

Adjuvant-induced arthritic model in rats is a well-documented method worldwide for preclinical evaluation of drugs with proposed anti-arthritic potential because it is quite similar to human rheumatoid arthritis pathologically (Shabbir et al., 2018). Arthritis triggered by complete freund's adjuvant (CFA) comprises of two phases; the phase-1 is acute and continues for10 days while phase-2 is a chronic phase lasting for 11–28 days. First phase is initiated by the liberation of histamine, serotonin and prostanoids from immune cells (Foyet et al., 2015). In view of the detrimental effects of available therapeutic stuff to attenuate arthritis and associated pain, the researchers and practitioners are inclined towards the novel medicinal products originating from natural flora (Anilkumar, 2010).

Paeonia emodi Royle has been used traditionally for curing the numerous inflammatory disorders such as rheumatism, colic, toothache, muscular strains and headaches (Ahmad et al., 2018). In this study, an aqueous methanolic crude extract of P. emodi (IPE) was prepared, investigated for phytochemical constituents and pharmacologically evaluated for analgesic and anti-arthritic activities. The screening test discovered different alkaloids, sugars, tannins, phenols, glycosides and flavonoids. Following the HPLC analysis of the crude extract, the presence of numerous phytochemicals; such as apigenin, caffeic acid, quercetin, and epigallocatechin was revealed. Inflammation suppressing effects of apigenin (Ginwala et al., 2019), caffeic acid (Choi et al., 2018), epigallocatechin (Zhong et al., 2012) and quercetin (Rogerio et al., 2007) have been reported in previous studies. The analgesic potential of the crude extract of the IPE was analyzed using hot plate method and tail immersion method. With these techniques, hear induces local tissue damage which in result triggers the release of inflammatory mediators along with increased sensitization of nociceptors together which results in a higher magnitude of pain (Angst and Clark, 2006). IPE displayed a potent analgesic effect on the experimental animals that was on par with a centrally acting drug tramadol and even greater than non-steroidal anti-inflammatory drug diclofenac sodium. This suggests that in addition to downregulating the inflammatory mediators, the crude extract of IPE may also show its analgesic effect by regulating the pain receptors in the central nervous system.

The immune mediated arthritis also results in the upregulation of other inflammatory mediators such as TNF-α, IL-6 & IL-1β, NF-κB and cyclooxygenase enzyme (Kim et al., 2016). Tissue injury results in the production of edema followed by movement of immune cells such as macrophages, mast cells, WBCs and is produced due to tissue injury with subsequent migration of macrophages, leukocytes, mast cells and oozing of small blood vessels (Allam and Anders, 2008).

One of the major clinical manifestations of rheumatoid arthritis is anemia. An elevated key enzymes of the liver and peri-articular osteoporosis is also correlated to bone decay (Thite et al., 2014). In current study, we noted that IPE has efficiently restored the hepatic enzyme levels in treated animals in this investigation. In addition, IPE was able to preserve Hb levels in the treatment groups at various doses. It is thought to be due to the apparent healing of swollen joints. Moreover, active inflammation is indicated by the raised levels of rheumatic factor (RF) and C-reactive proteins (CRP) which are known to be the main players involved in systemic inflammation. Additionally, the high blood levels of CRP and RF indicate the progression of arthritis (Kumar et al., 2013). Studies in the past have shown that an elevated levels of IL-6 and TNF-α further enhance the production of CRP. Findings of this

study showed that IPE controlled the systemic inflammation, which is evident by lower levels of RF and CRP (Kalaiselvan and Rasool, 2016).

Paw swelling in rheumatoid arthritis has been reported previously in different studies suggesting that it is linked to inflammatory mediators specifically PGE-2 that is released during the acute phase. The macrophages and monocytes stimulate and release TNF-α, IL-6, and IL-1β in response to Complete Freund's Adjuvant (CFA). Eventually, TNF-α is also responsible for further release of inflammatory mediators such as IL-6 and IL-1β that is responsible for the recruitment of more leukocytes thus resulting infiltration and vasodilation at the site of oedema (Voon et al., 2017). Additionally, other leukocytes such as neutrophils and monocytes are attracted to these pro-inflammatory cytokines that stimulate these chemokines towards the site of inflamed joints. It is important to save bone and cartilage from destruction by blocking the release of TNF-α and other cytokines involved in gene expression of matrix metalloproteinases (Srirangan and Choy, 2010). Our current study has indicated that IPE in all three doses viz. 300mg/Kg, 500mg/Kg and 1000mg/Kg p.o. has significantly reduced the serum PGE-2 levels in addition to a marked decline in the serum expression of inflammatory mediators such as TNF-α, IL-1β, IL-6 and COX-2 enzyme when compared with the arthritic control group.

Certain enzymes activities are demonstrated to be lower in RA such as Superoxide dismutase (SOD) and catalase (CAT), while malondialdehyde (MDA) levels have been shown to be raised. This oxidative stress imparts a negative impact on gene transcription (Akhtar et al., 2021). Freund's adjuvant increases the production of reactive oxygen species (ROS) and also induces the release of pro-inflammatory cytokines thus sensitizing the immune cells (Saleem et al., 2020). As a result of this, it can be safely postulated that IPE may possibly lower the oxidative stress for its mechanism of action along with the other key mechanisms that it could lower the expression of genes responsible for production of inflammatory cytokines and cyclooxygenase enzyme (COX-2) in RA.

Phytochemical analysis of aqueous methanolic extract of Paeonia emodi Royle (IPE) has carried out and it was found to be rich in different alkaloids, phenols, glycosides and flavonoids. Presence of a vast variety of phytochemical compounds in the crude extract (IPE), having reported anti-inflammatory activities, supports the outcomes of this study. These phytochemical compounds of the IPE have attributed their ameliorating effect on joint inflammation, edema and oxidative stress and have been proven to be of medicinal value.

5. CONCLUSION

The present study concludes the Paeonia emodi Royle as a potential analgesic and anti arthritic drug to reduce inflammation as well as oxidative stress in immune mediated arthritis thus endorsing its use in rheumatism and joint disorders in native population.

Declarations and Statements

i. Conflict of Interest

None of the authors has any kind of conflict of interest.

ii. Author's Contribution

The study was designed and supervised by Qaiser Jabeen. Syed Ihtisham Haider performed all in vivo experiments and prepared initial draft. PCR analysis were performed by Awais Asif. Haseeb Ahsan performed the ELISA. Hafiz Muhammad Farhan Rasheed conducted all the hematological tests. Asim Raza performed HPLC analysis and statistical analysis was performed by Jawad Akbar khan. The article was finalized and proof read by Ammara Khan and Jawad Akbar khan.

iii. Funding

No funding or grant was acquired in this study from any local or foreign funding agency or research institute

iv. Ethical Approval

The study protocols were approved by Pharmacy Animal Ethics Committee of the Islamia University of Bahawalpur (certificate no. PAEC/21/37).

v. Availability of Original Data

All the data generated during this study is available and will be provided on reasonable request

REFERENCES

1. Afsar, T., Khan, M.R., Razak, S., Ullah, S., Mirza, B., 2015. Antipyretic, anti-inflammatory and analgesic activity of Acacia hydaspica R. Parker and its phytochemical analysis. BMC Complement Altern Med 15(1), 1-12.

2. Ahmad, M., Malik, K., Tariq, A., Zhang, G., Yaseen, G., Rashid, N., Sultana, S., Zafar, M., Ullah, K., Khan, M.P.Z., 2018. Botany, ethnomedicines, phytochemistry and pharmacology of Himalayan paeony (Paeonia emodi Royle.). Journal of ethnopharmacology 220, 197-219.

3. Ahsan, H., Haider, I., Mushtaq, M.N., Qaisar, M.N., Naqvi, F., Asif, A., 2021a. Pharmacological support to anti-arthritic prospective of physostigmine: a new approach. Inflammopharmacology 29(4), 1119-1129.

4. Ahsan, H., Irfan, H.M., Shahzad, M., Asim, M.H., Akram, M., Zafar, M.S., 2021b. Anti-rheumatic activity of pseudoephedrine (a substituted phenethylamine) in complete Freund's adjuvant-induced arthritic rats by down regulating IL-1β, IL-6 and TNF-α as well as upregulating IL-4 and IL-10. Inflammopharmacology, 1-10.

5. Akhtar, M., Sharif, A., Saleem, M., Saleem, A., Akhtar, B., Raza, M., Ijaz, H., Shabbir, M., Ali, S., Nasim, M., 2017. Genotoxic and cytotoxic potential of Alternanthera Bettzickiana, an important ethno-medicinal plant. Cellular and Molecular Biology 63(8), 109-114.
6. Akhtar, M.F., Khan, K., Saleem, A., Baig, M.M.F.A., Rasul, A., Abdel-Daim, M.M., 2021. Chemical characterization and anti-arthritic appraisal of Monotheca buxifolia methanolic extract in Complete Freund's Adjuvant-induced arthritis in Wistar rats. Inflammopharmacology 29(2), 393-408.
7. Allam, R., Anders, H.-J., 2008. The role of innate immunity in autoimmune tissue injury. Current opinion in rheumatology 20(5), 538-544.
8. Angst, M.S., Clark, J.D., 2006. Opioid-induced hyperalgesia: a qualitative systematic review. The Journal of the American Society of Anesthesiologists 104(3), 570-587.
9. Anilkumar, M., 2010. 10. Ethnomedicinal plants as anti-inflammatory and analgesic agents. Ethnomedicine: A source of complementary therapeutics, 267-293.
10. Bala, A., Mondal, C., Haldar, P.K., Khandelwal, B., 2017. Oxidative stress in inflammatory cells of patient with rheumatoid arthritis: clinical efficacy of dietary antioxidants. Inflammopharmacology 25(6), 595-607.
11. Blin, J.A., Ali, R.M., Nurdin, A., Abd Hamid, R., 2021. Quinone-rich fraction of Ardisia crispa (Thunb.) A. DC roots alters angiogenic cascade in collagen-induced arthritis. Inflammopharmacology, 1-18.
12. Brand-Williams, W., Cuvelier, M.-E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology 28(1), 25-30.
13. Choi, H.G., Tran, P.T., Lee, J.-H., Min, B.S., Kim, J.A., 2018. Anti-inflammatory activity of caffeic acid derivatives isolated from the roots of Salvia miltiorrhiza Bunge. Archives of pharmacal research 41(1), 64-70.
14. Datta, S., Kundu, S., Ghosh, P., De, S., Ghosh, A., Chatterjee, M., 2014. Correlation of oxidant status with oxidative tissue damage in patients with rheumatoid arthritis. Clinical rheumatology 33(11), 1557-1564.
15. Fazal, H., Ahmad, N., Abbasi, B.H., Abbass, N., 2012. Selected medicinal plants used in herbal industries; their toxicity against pathogenic microoraganisms. Pak. J. Bot 44(3), 1103-1109.
16. Foyet, H.S., Tsala, D.E., Bodo, J.Z.E., Carine, A.N., Heroyne, L.T., Oben, E.K., 2015. Anti-inflammatory and anti-arthritic activity of a methanol extract from Vitellaria paradoxa stem bark. Pharmacognosy Res 7(4), 367.
17. Ginwala, R., Bhavsar, R., Chigbu, D.G.I., Jain, P., Khan, Z.K., 2019. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. Antioxidants 8(2), 35.
18. Haider, S.I., Asif, A., Rasheed, H.M.F., Akram, A., Jabeen, Q., 2022. Caralluma tuberculata exhibits analgesic and anti-arthritic potential by downregulating pro-inflammatory cytokines and attenuating oxidative stress. Inflammopharmacology 30(2), 621-638.
19. Hasan, U.H., Shahzad, M., Jahan, S., Niazi, Z.R., Bukhari, I.A., Assiri, A.M., Riaz, H., 2019. Inhibitory effects of Clematis orientalis aqueous ethanol extract and fractions on inflammatory markers in complete Freund's adjuvant-induced arthritis in Sprague–Dawley rats. Inflammopharmacology 27(4), 781-797.
20. Jabeen, Q., Haider, S.I., Asif, A., Rasheed, R., Gul, S., Arshad, S., 2022. Geranium wallichianum D. Don Ex Sweet Ameliorates Rheumatoid Arthritis by Curtailing the Expression of COX-II and Inflammatory Cytokines as Well as by Alleviating the Oxidative Stress. Dose-Response 20(3), 15593258221112649.
21. Jabeen, Q., Khan, M.S., Qureshi, A.W., Rasheed, H.M.F., 2021. Effect of Abutilon indicum in thyroxine-induced hyperthyroidism in rat. ||| Bangladesh Journal of Pharmacology 16(3), 103-113.
22. Jamil, T., Bibi, Y., Zahara, K., 2020. An Insight into Endangered Himalayan Paeony (Paeonia emodi Royle): Ethnobotany, Phytochemistry and Pharmacology. Journal of Plant and Environment 2(1), 25-31.
23. Jamshed, A., Jabeen, Q., 2022. Pharmacological Evaluation of Mentha piperita Against Urolithiasis: An In Vitro and In Vivo Study. Dose-Response 20(1), 15593258211073087.
24. Javed, F., Jabeen, Q., Aslam, N., Awan, A.M., 2020. Pharmacological evaluation of analgesic, anti-inflammatory and antipyretic activities of ethanolic extract of Indigofera argentea Burm. f. Journal of Ethnopharmacology 259, 112966.
25. Kalaiselvan, S., Rasool, M.K., 2016. Triphala herbal extract suppresses inflammatory responses in LPS-stimulated RAW 264.7 macrophages and adjuvant-induced arthritic rats via inhibition of NF-κB pathway. J. Immunotoxicol. 13(4), 509-525.
26. Kayani, W.K., Dilshad, E., Ahmed, T., Ismail, H., Mirza, B., 2016. Evaluation of Ajuga bracteosa for antioxidant, anti-inflammatory, analgesic, antidepressant and anticoagulant activities. BMC complementary and alternative medicine 16(1), 1-13.
27. Kim, W., Park, S., Choi, C., Kim, Y.R., Park, I., Seo, C., Youn, D., Shin, W., Lee, Y., Choi, D., 2016. Evaluation of anti-inflammatory potential of the new ganghwaljetongyeum on adjuvant-induced inflammatory arthritis in rats. Evidence-Based Complementary and Alternative Medicine 2016.
28. Kishore, L., Kaur, N., Singh, R., 2017. Nephroprotective effect of Paeonia emodi via inhibition of advanced glycation end products and oxidative stress in streptozotocin–nicotinamide induced diabetic nephropathy. journal of food and drug analysis 25(3), 576-588.
29. Kumar, V., Verma, A., Ahmed, D., Sachan, N.K., Anwar, F., Mujeeb, M., 2013. Fostered antiarthritic upshot of moringa oleifera lam. stem bark extract in diversely induced arthritis in wistar rats with plausible mechanism. Int J Pharm Sci Res 4(10), 3894-3801.

30. Lad, H., Bhatnagar, D., 2016. Amelioration of oxidative and inflammatory changes by Swertia chirayita leaves in experimental arthritis. Inflammopharmacology 24(6), 363-375.
31. Oyeleke, S.A., Ajayi, A.M., Umukoro, S., Aderibigbe, A., Ademowo, O.G., 2018. Anti-inflammatory activity of Theobroma cacao L. stem bark ethanol extract and its fractions in experimental models. J Ethnopharmacol 222, 239-248.
32. Padmanabhan, P., Jangle, S., 2012. Evaluation of in-vitro anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. Int J Basic Appl Med 2(1), 109-116.
33. Perianayagam, J.B., Sharma, S., Joseph, A., Christina, A., 2004. Evaluation of anti-pyretic and analgesic activity of Emblica officinalis Gaertn. J Ethnopharmacol 95(1), 83-85.
34. Pilotto, A., Sancarlo, D., Addante, F., Scarcelli, C., Franceschi, M., 2010. Non-steroidal anti-inflammatory drug use in the elderly. Surg Oncol 19(3), 167-172.
35. Raish, M., Ahmad, A., Alkharfy, K.M., Jan, B.L., Mohsin, K., Ahad, A., Al-Jenoobi, F.I., Al-Mohizea, A.M., 2017. Effects of Paeonia emodi on hepatic cytochrome P450 (CYP3A2 and CYP2C11) expression and pharmacokinetics of carbamazepine in rats. Biomedicine & Pharmacotherapy 90, 694-698.
36. Rasheed, H.M.F., Rasheed, F., Qureshi, A.W., Jabeen, Q., 2016. Immunostimulant activities of the aqueous methanolic extract of Leptadenia pyrotechnica, a plant from Cholistan desert. Journal of ethnopharmacology 186, 244-250.
37. Ratheesh, M., Shyni, G.L., Helen, A., 2009. Methanolic extract of Ruta graveolens L. inhibits inflammation and oxidative stress in adjuvant induced model of arthritis in rats. Inflammopharmacology 17(2), 100-105.
38. Rogerio, A., Kanashiro, A., Fontanari, C., Da Silva, E., Lucisano-Valim, Y., Soares, E., Faccioli, L., 2007. Anti-inflammatory activity of quercetin and isoquercitrin in experimental murine allergic asthma. Inflammation research 56(10), 402-408.
39. Saleem, A., Saleem, M., Akhtar, M.F., 2020. Antioxidant, anti-inflammatory and antiarthritic potential of Moringa oleifera Lam: An ethnomedicinal plant of Moringaceae family. S. Afr. J. Bot. 128, 246-256.
40. Shabbir, A., Batool, S.A., Basheer, M.I., Shahzad, M., Sultana, K., Tareen, R.B., Iqbal, J., 2018. Ziziphora clinopodioides ameliorated rheumatoid arthritis and inflammatory paw edema in different models of acute and chronic inflammation. Biomed. Pharmacother 97, 1710-1721.
41. Shabbir, A., Shahzad, M., Ali, A., Zia-ur-Rehman, M., 2014. Anti-arthritic activity of N′-[(2, 4-dihydroxyphenyl) methylidene]-2-(3, 4-dimethyl-5, 5-dioxidopyrazolo [4, 3-c][1, 2] benzothiazin-1 (4H)-yl) acetohydrazide. European Journal of Pharmacology 738, 263-272.
42. Shabbir, A., Shahzad, M., Ali, A., Zia-ur-Rehman, M., 2016. Discovery of new benzothiazine derivative as modulator of pro-and anti-inflammatory cytokines in rheumatoid arthritis. Inflammation 39(6), 1918-1929.
43. Shazia, S., 2012. Taxonomic and Pharmacognostic authentication of problematic medicinal plants used as herbal medicine. Quaid-i-Azam University Islamabad Pakistan.
44. Srirangan, S., Choy, E.H., 2010. The role of interleukin 6 in the pathophysiology of rheumatoid arthritis. Ther. Adv. Musculoskelet. Dis 2(5), 247-256.
45. Tantry, M.A., Dar, J.A., Khuroo, M.A., Shawl, A.S., 2012. Nortriterpenoids from the roots of Paeonia emodi. Phytochemistry letters 5(2), 253-257.
46. Thite, A.T., Patil, R.R., Naik, S.R., 2014. Anti-arthritic activity profile of methanolic extract of Ficus bengalensis: Comparison with some clinically effective drugs. Biomed. Aging Pathol. 4(3), 207-217.
47. Uttra, A.M., Shahzad, M., Shabbir, A., Jahan, S., 2018. Ephedra gerardiana aqueous ethanolic extract and fractions attenuate Freund Complete Adjuvant induced arthritis in Sprague Dawley rats by downregulating PGE2, COX2, IL-1β, IL-6, TNF-α, NF-kB and upregulating IL-4 and IL-10. Journal of ethnopharmacology 224, 482-496.
48. Vijayaraj, P., Muthukumar, K., Sabarirajan, J., Nachiappan, V., 2013. Antihyperlipidemic activity of Cassia auriculata flowers in triton WR 1339 induced hyperlipidemic rats. Experimental and Toxicologic Pathology 65(1-2), 135-141.
49. Voon, F.-L., Sulaiman, M.R., Akhtar, M.N., Idris, M.F., Akira, A., Perimal, E.K., Israf, D.A., Ming-Tatt, L., 2017. Cardamonin (2′, 4′-dihydroxy-6′-methoxychalcone) isolated from Boesenbergia rotunda (L.) Mansf. inhibits CFA-induced rheumatoid arthritis in rats. Eur. J. Pharmacol. 794, 127-134.
50. Zaidi, S.A., Pathan, S.A., Singh, S., Ahmad, F., Jamil, S., Khar, R., 2012. Effect of repeated administration of Paeonia emodi wall root extract in experimental models of epilepsy and behaviour. J. Pharmacol. Toxicol 7(2), 64-77.
51. Zhong, Y., Chiou, Y.-S., Pan, M.-H., Shahidi, F., 2012. Anti-inflammatory activity of lipophilic epigallocatechin gallate (EGCG) derivatives in LPS-stimulated murine macrophages. Food chemistry 134(2), 742-748

TPM Vol. 32, No. S6, 2025
ISSN: 1972-6325
https://www.tpmap.org/

Open Access

Table 1. Sequence of Primers of Inflammatory Cytokines

S. No	Marker Name	Type	Sequence	Amplicon Size
1	COX II	Forward	TTAGGTCATCGGTGGAGAGG	217
		Reverse	GAACAGTCGCTCGTCATCCC	
2	IL-1β	Forward	AGTCTGCACAGTTCCCCAAC	230
		Reverse	AGACCTGACTTGGCAGAGGA	
3	IL-6	Forward	TACCCCAACTTCCAATGCTC	186
		Reverse	ACCACAGTGAGGAATGTCCA	
4	NF-κB	Forward	TCACCAAGCAGGAAGATGTG	161
		Reverse	GATAAGGAGTGCTGCCTTGC	
5	TNF-α	Forward	CAGGTTCCGTCCCTCTCATA	170
		Reverse	AGAAGAGGCTGAGGCACAGA	

Figures

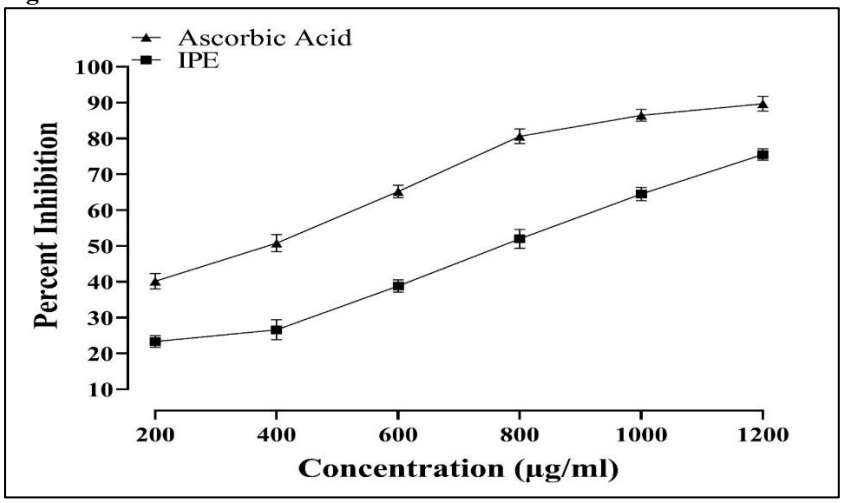
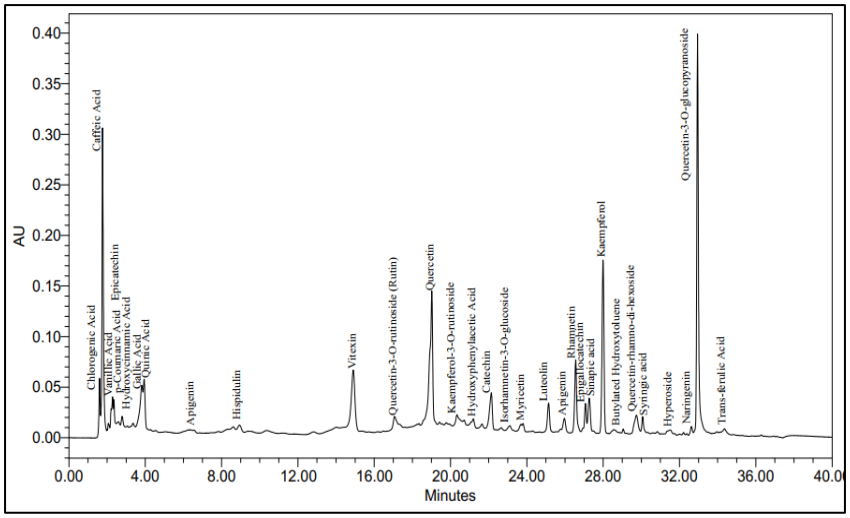


Figure 1. Dose dependent antioxidant activity of aqueous methanolic extract of Paeonia emodi Royle (IPE) compared with that of Ascorbic acid. All values (n = 3) are expressed as mean ± SEM

A



B

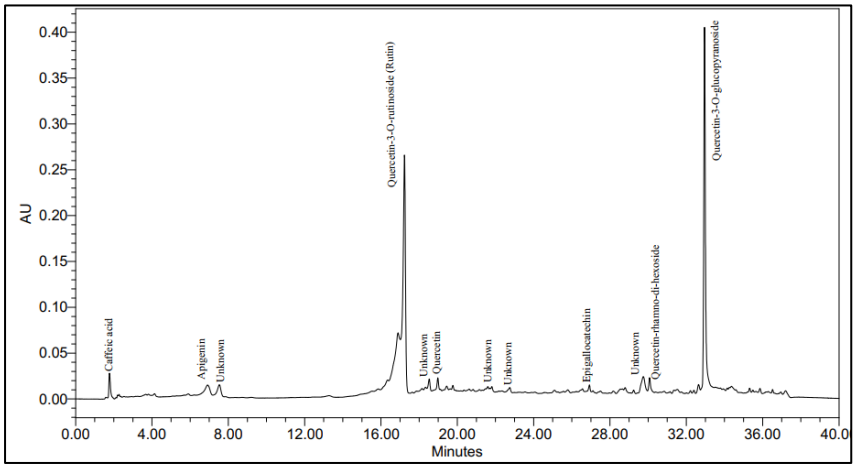


Figure 2. HPLC chromatogram of (A) standard compounds (B) aqueous methanolic extract of Paeonia emodi Royle (IPE).

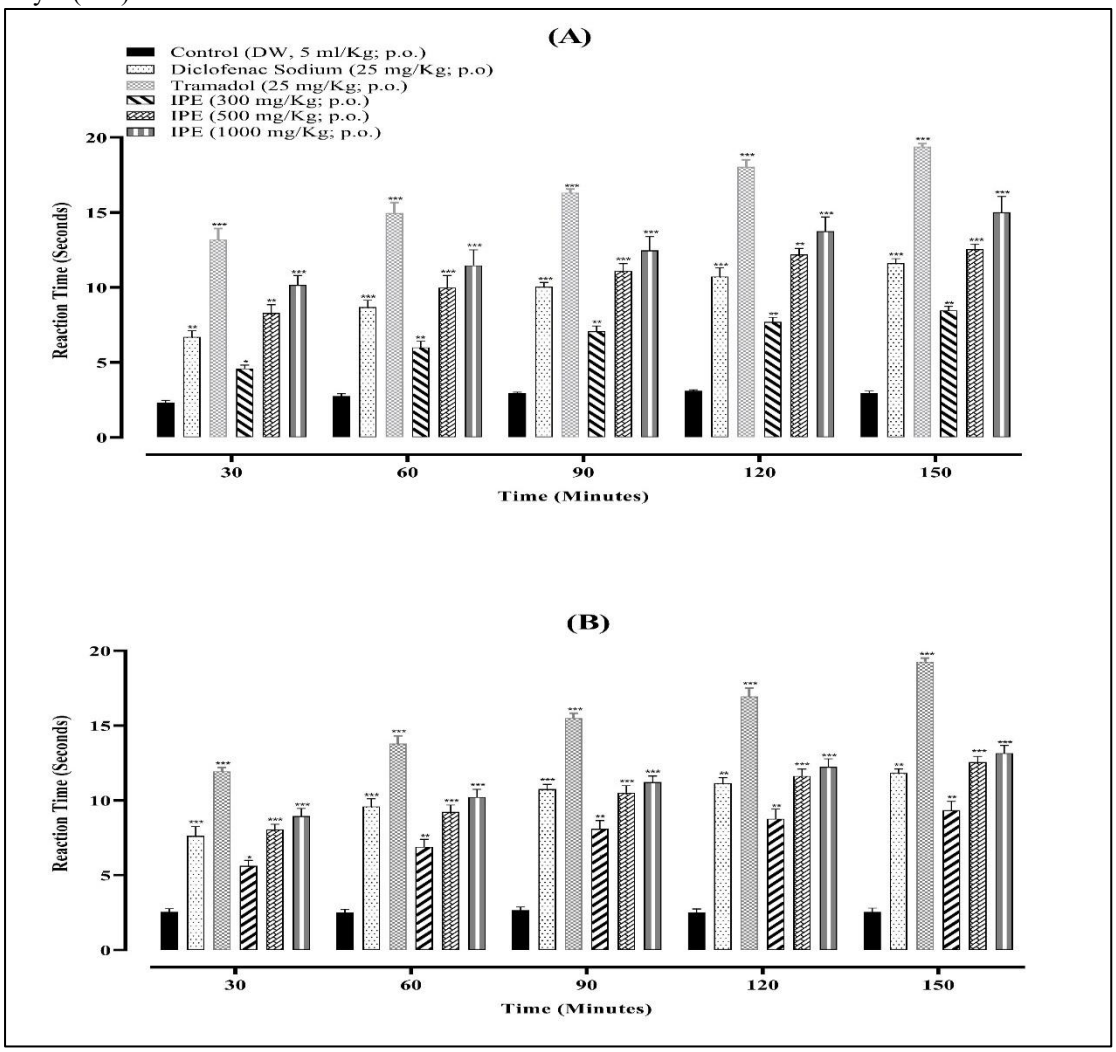


Figure 3. Effects of Paeonia emodi Royle aqueous methanolic extract IPE on time of reaction/response in seconds showed by animal in hot plate method (A) and tail Immersion test (B). All values (n = 6) are expressed as Mean ± SEM using Two-way ANOVA followed by Tukey Test. *** = $p \leq 0.001$, ** = $p \leq 0.01$, * = $p \leq 0.05$ Vs control.

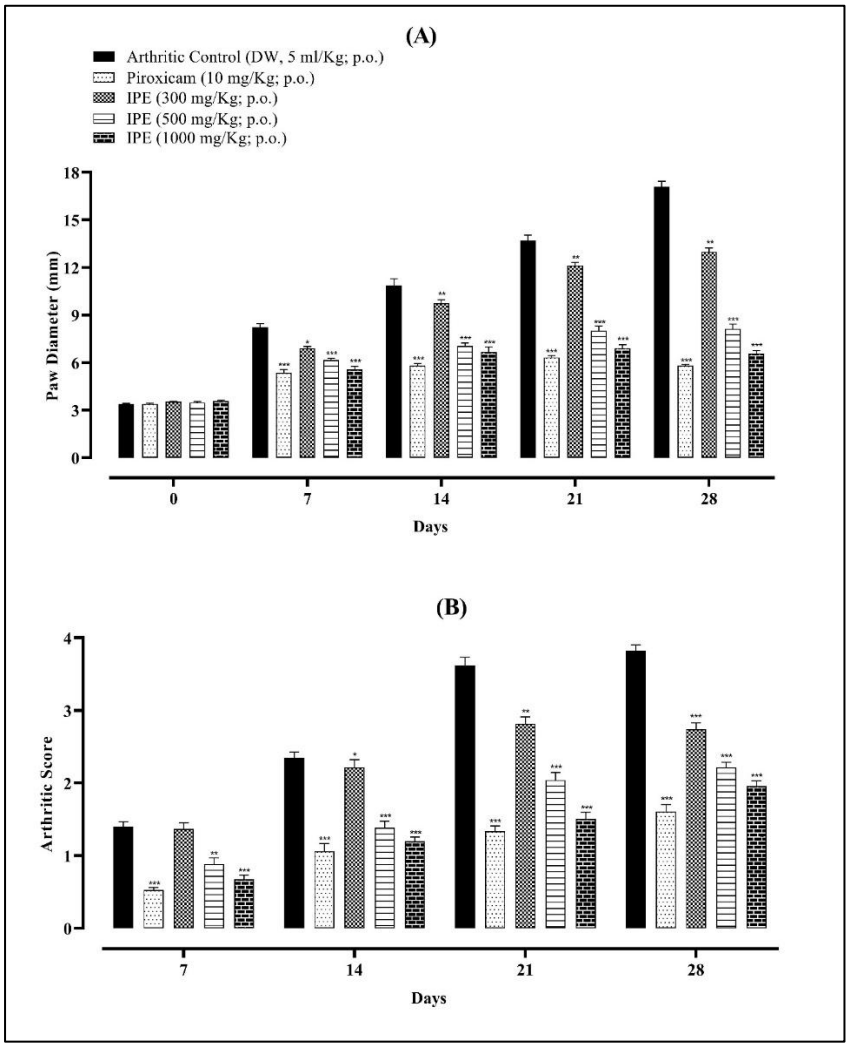


Figure 4. Effects of IPE on rat paw edema (A) measured as diameter (mm) and on arthritic index measured as score (B) in CFA-induced arthritis. All values (n = 6) are expressed as Mean ± SEM using Two-way ANOVA followed by Tukey Test. *** = $p \leq 0.001$, ** = $p \leq 0.01$, * = $p \leq 0.05$ Vs Arthritic control.

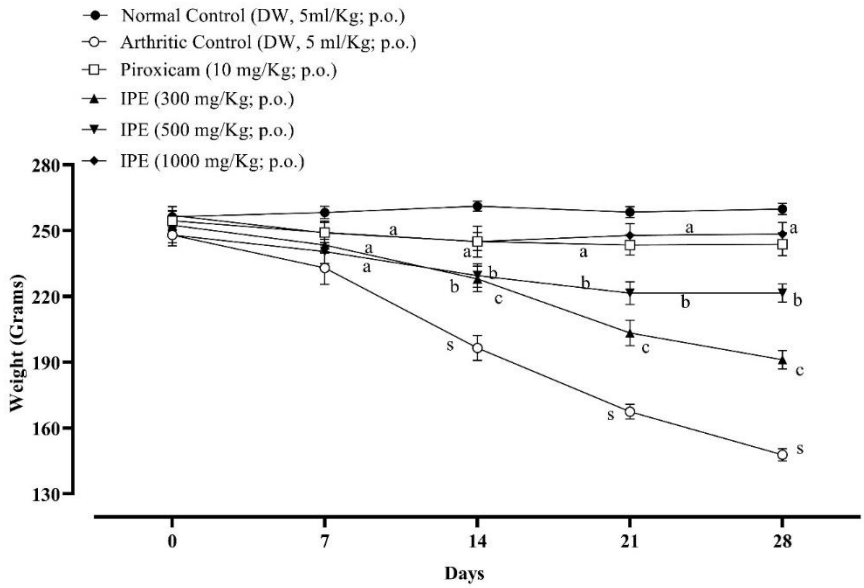


Figure 5. Effects of IPE on weight of animals in CFA-induced arthritis model. All values (n = 6) are expressed as Mean ± SEM using Two-way ANOVA followed by Tukey Test. s = $p \leq 0.001$ Vs normal control while a = $p \leq 0.001$, b = $p \leq 0.01$, c = $p \leq 0.05$ Vs arthritic control.

TPM Vol. 32, No. S6, 2025
ISSN: 1972-6325
https://www.tpmap.org/

Open Access

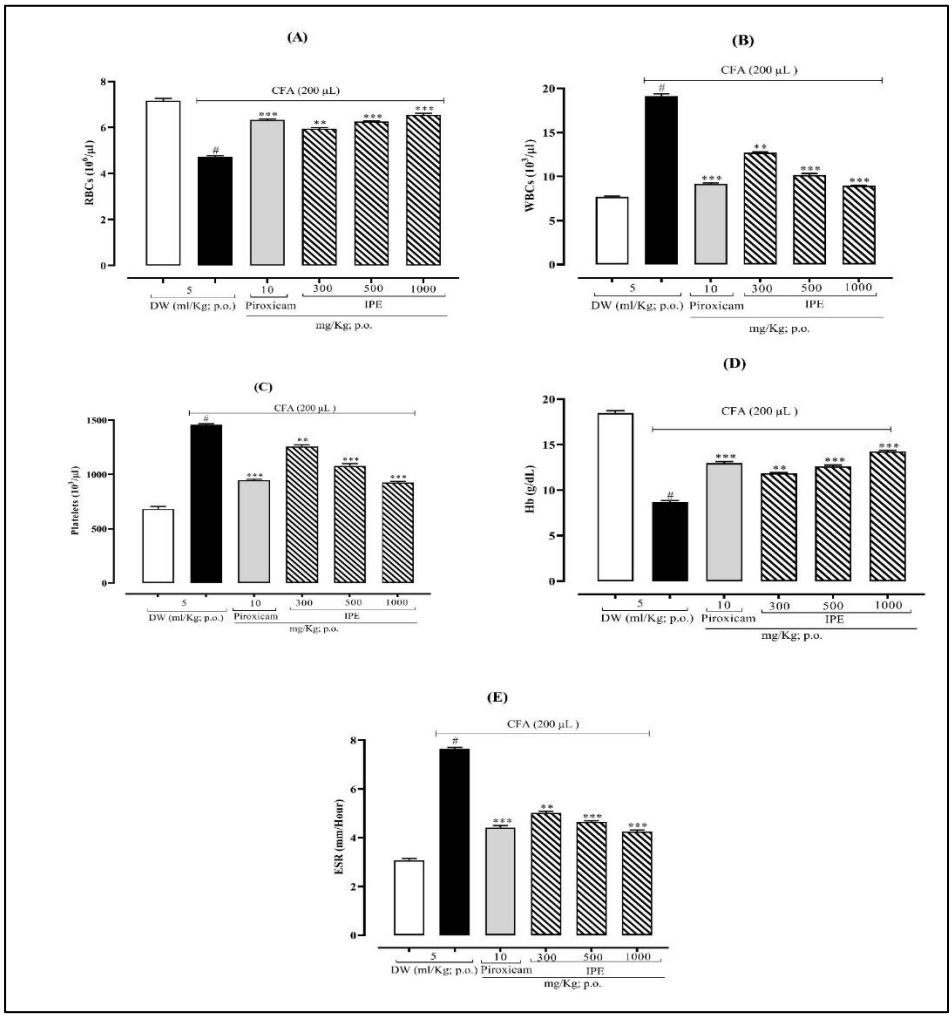


Figure 6. Effects of aqueous methanolic extract IPE on hematological parameters (A-E) in rats having CFA-induced arthritis. All values (n = 6) are expressed as Mean ± SEM using Two-way ANOVA Tukey's Post Hoc Test. # = $p \leq 0.001$ Vs normal control while *** = $p \leq 0.001$, ** = $p \leq 0.01$, * = $p \leq 0.05$ Vs Arthritic control.

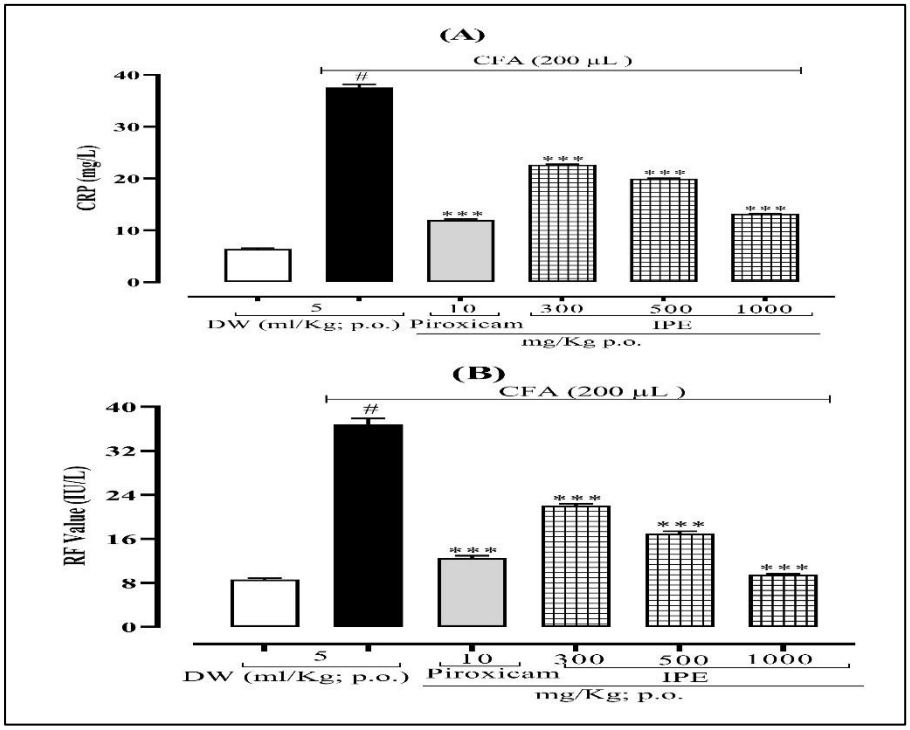


Figure 7. Effects of IPE on C-Reactive Proteins levels (A) and Rheumatic Factor values (B) in rats with CFA-induced arthritis. All values (n = 6) are expressed as Mean ± SEM using One-way ANOVA followed by Tukey's

Post Hoc Test. # = p ≤ 0.001 Vs normal control while *** = p ≤ 0.001, ** = p ≤ 0.01, * = p ≤ 0.05 Vs Arthritic control.

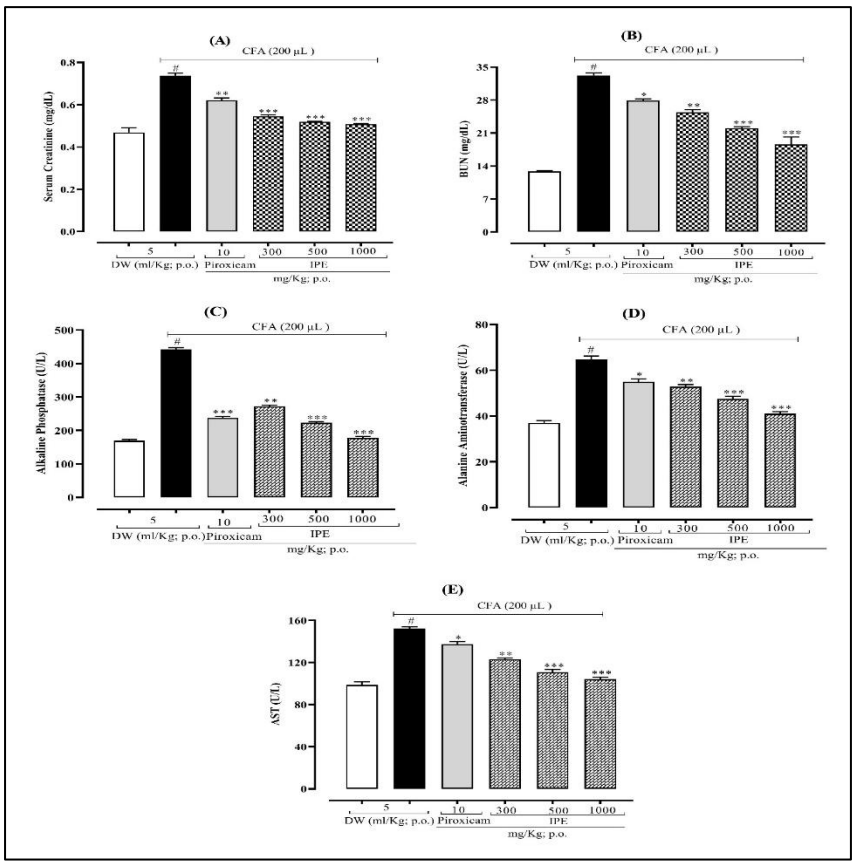


Figure 8. Effects of IPE on renal status (A-B) and hepatic enzyme levels (C-E) in rats with CFA-induced arthritis. All values (n = 6) are expressed as Mean ± SEM using One-way ANOVA followed by Tukey's Post Hoc Test. # = p ≤ 0.001 Vs normal control while *** = p ≤ 0.001, ** = p ≤ 0.01, * = p ≤ 0.05 Vs Arthritic control.

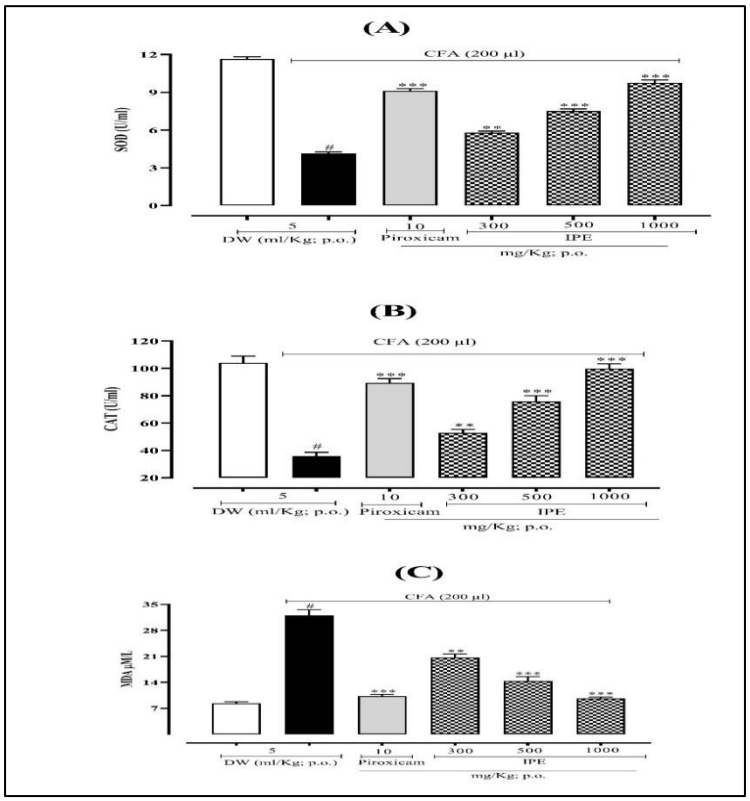


Figure 9. Effects of IPE on SOD (A), CAT (B) and MDA (C) in animals with CFA-induced arthritis. All values (n = 6) are expressed as Mean ± SEM using One-way ANOVA followed by Tukey's Post Hoc Test. # = p ≤ 0.001 Vs normal control while *** = p ≤ 0.001, ** = p ≤ 0.01, * = p ≤ 0.05 Vs Arthritic control.

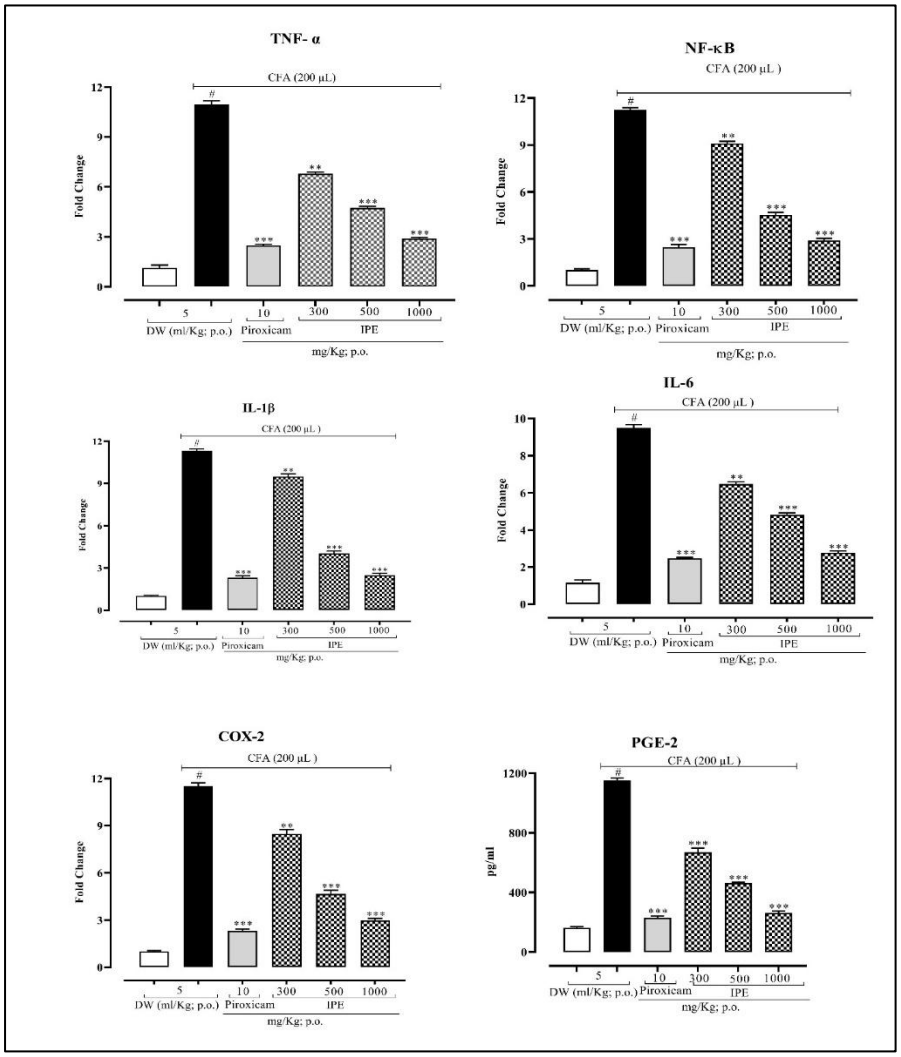


Figure 10. Effects of IPE on gene expression of COX II, inflammatory cytokines and on serum levels of PGE2 in animals with CFA-induced arthritis. All values (n = 6) are expressed as Mean ± SEM using One-way ANOVA followed by Tukey's Post Hoc Test. # = $p \leq 0.001$ Vs normal control while *** = $p \leq 0.001$, ** = $p \leq 0.01$, * = $p \leq 0.05$ Vs Arthritic control.