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Research Article

Assessing the Effect of *Portulaca oleracea* Seed Extract for Alleviating Collagen Type-II Induced Arthritis in Female Wistar Rat

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ABSTRACT

Rheumatoid arthritis is a sign of progressive degradation of cartilage, subchondral bone, and small joints, as well as the persistence of synovitis and the formation of pannus. This research intends to assess the purported antiarthritic effects of an extract from the seeds of *Portulaca oleracea.* Female wistar albino rats (140–200 g) were used and assigned to five groups: Group I administrated NS (10 mL/kg), group II received 0.2 mL of CoII-IFA, group III received 300 mg/kg of fish oil, and groups IV & V administrated 100 and 200 mg/kg of methanolic extract of *P. oleracea* (MePO). During the experiment, the rats' weight, arthritic score, and footpad edema were evaluated to determine the severity of their arthritis. Later, blood samples were collected from the animals, which were then analyzed for hematological, pro-inflammatory, antioxidant, and histological parameters. Key point: A dose-dependent reduction was seen in rats treated with a methanolic extract of *Portulaca* seeds. Levels of hematological and pro-inflammatory cytokines were considerably reduced by treatment. Although the standard drug and 200 mg/kg of MePO had anti-inflammatory effects, the latter were more pronounced at this dose. The two side by side showed that the treatment groups of RBC, WBC, NL-ratio, and ML-ratio levels were normalized. Further histology confirmed the reduction of joint deformity, edema, formation of pannus, and infiltration of neutrophils in the MePO groups in contrast to arthritic rats. It is hypothesized that *P. oleracea* may reduce arthritis and can be used as an adjuvant therapy or incorporated it into your diet with the main course of treatment.

Introduction

Rheumatoid arthritis (RA) is an autoimmune condition that may lead to joint destruction, inflammatory polyarthritis, etc. As a result of inflammation and toxin production in the synovium, cartilage deteriorates.[1] Inflammation may affect several organs; however, it most often affects the synovium, which is the inner surface of the joint. Damage to bones, cartilage, and joints may result from synovial fluid inflammation. Notable symptoms include redness, swelling, and discomfort. The frequency of arthritis is three times greater in men than in women, and it affects one percent globally.[2] Inflammation of the synovium may be a complex interplay of many factors, including genetics, environmental factors, and the immune system. Immunological dysregulation and impaired self-tolerance are the end outcomes. Macrophages secrete eicosanoids, which include prostaglandins, leukotriene, and cytokines and reactive oxygen species.[3,4] Inflammatory diseases control antioxidant metabolism and alter mediators in immune cells and macrophages by suppressing cyclooxygenase and lactate dehydrogenase. Biological response modifiers, corticosteroids, disease modifying anti-rheumatic drugs (DMARDS), non-steroidal anti-inflammatory drugs (NSAIDs), and rheumatoid arthritis are the most common prescribed medicines for this condition. Unwanted effects may be experienced with any of these medications. In their pursuit of effective, long-

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lasting anti-inflammatory medications, researchers are turning to time-honored methods.[5]

Portulaca oleracea has a long history of culinary and medicinal uses, dating back at least 2,000 years. The Portulaceae family counts the annual plant *P. oleracea* among its members. Although their provenance is unclear, archaeobotanical artifacts have been discovered at several ancient sites. There are around 60 medical uses for the plant and thirty biological ones. As an alternative medicine, purslane is said to contain medicinal properties of the plant, including the roots, stems, leaves, and seeds. This particular plant is cultivated for its edible leaves. Purslane, pigweed, Kurfa, Andrachni, Baralunia, and many other names are associated with it. Its spongy, spherical stems are around six inches in height and adorned with clusters of tiny, dark-green, oval- or wedge-shaped leaves. In the summer months of June and July, it produces yellow blossoms. Many diverse parts of the world are native to the purslane plant, including Europe, Asia, and Oceania. Purslane plants are said to have a huge therapeutic value like; anti-inflammatory, antibacterial, antioxidant, anti-diabetic, and hepatoprotective properties. In traditional Unani medicine, the purslane plant has a long history of usage for a variety of conditions, including skin disorders, fever, diarrhea, hemorrhoids, spleen diseases, and more. Modern times have also seen Purslane's incorporation into cosmetics.[6]

From a clinical, pathological, immunological, and histological standpoint, the CIA model is the most faithful representation of actual RA compared to other arthritis diagnostic models.[7] New medicines for RA are developed in part using CIA, which also helps us understand the etiology of RA in people.

Arthritis conditions tremendously impact people's health in India and worldwide. Several herbal remedies like turmeric, ginger, bark extract and many more are promoted today for treating disease, relieving inflammatory consequences and overall health wellbeings by modification or booster of immune clock. Therefore, the current study aims to establish the link between the proposed outcome and the administration of *P. oleracea* in arthritis rats.

Materials And Methods

Procurement and Authentication of *P. oleracea*

The plant seed was procured from Indian Jadi Booti, Noida, India. It was authenticated from the seeds of the *P. oleracea* shrub by Dr. Noorunnisa Begum, Head of the Centre for Herbal Gardens at FRLHT in Yelahanka, Bangalore, with the reference number FRLHT Acc. No. 5886.

Extraction and Sample Preparation of *P. oleracea*

The seeds of *P. oleracea* were air-dried, made powder and passed through a coarse 10/40 mesh screen. The seed powder was then extracted using the soxhlet method. A reflux condenser was used to extract the 250 g of powder using three seven-hour cycles and 1000 mL of methanol. The 50% reduction in volume was then filtered and evaporated completely by using a rotational vacuum, and the semi-residue yield was found to be 7.4%, which was then used to continue the experiment.

Experimental Animals

The antiarthritic activity was carried out using female Wistar albino rats (140–200 g). With a temperature of 25 ± 2°C, normal conditions were maintained for the animals, including a humidity level of 40 to 45% and a 12-hour light/dark cycle. We gave them conventional feed pellets and water when they needed it. Protocol number was assigned by the IAEC, KCP Bengaluru, and the experiment was authorized by the CPCSEA in New Delhi, India: (KCP/IAEC/11/22-23/03/22/12/22).

Experimental Design

There were a grand total of 66 female wistar albino rats distributed among the five groups.

Group I: The normal group received a saline solution at a dosage of 1-mL per 100 g of body weight.

Group II: As a disease control, rats received collagen type II-IFA to induce arthritis.[8,9]

Group III: As a standard therapy, arthritic rats were given orally 300 mg/kg body weight of fish oil.[10]

Group IV: As a test drug, arthritic rats were given *P. oleracea* extract (100 mg/kg b.w., orally).[11]

Group V: As a test drug, arthritic rats were given *P. oleracea* extract (200 mg/kg b.w., orally).[11]

Dose Selection

Based on the results of an earlier study,[11] 100 & 200 mg/kg of body weight were chosen for the current study.

Induction of Arthritis by using Collagen Type II (CoII) in Rat's

To induce arthritis, we used CoII and an incomplete Freund's adjuvant (IFA). Collagen was diluted using a 2 mg/mL chilled 0.1 M acetic acid solution. The night before, we cooled the combination to a temperature of 4°C. Collagen is emulsified in acetic acid with same amount of IFA, and the combination is called an inciting agent. From the mixture, 0.1 mL was injected into regions above each limb. On the seventh day after the first immunization, each animal was given a booster injection of the same mixture on dose of 0.1 mL.

All animals were received the injection in the same method. The saline, Fish oil and plant extract were administered orally *via* an intragastric tube, beginning on the 20th day after the booster immunization and continuing until the 44th day. In order to conduct a histological investigation biochemical observation, blood and ankle joint samples were collected from rats that were euthanized on the 45th day.[8,9]

Arthritic score

The severity of the arthritis were evaluated as follows: Ankle or wrist swelling in grade 3, mild reddening or swelling in one paw finger in grade 2, moderate swelling in one or more paw fingers in grade 3, and severe arthritic swelling in the fingers and wrist in degree 4 are the possible outcomes on a scale from 0 to 4. Swelling in the paws and wrists manifests itself in these ways. Arthritis scores in rats induced by CIA may go as high as 8.[12]

Body weight

The body weight change was measured with a digital scale from day 1 to day 44.[12]

Hematological Parameters

The standard laboratory methods were followed to collect the blood samples from animals to assess the Hb, RBC, and WBC counts, NL-ratio, ML-ratio, and rheumatoid factor (RF).[12]

Detection of pro-inflammatory cytokines

Briefly, a sandwich ELISA test was performed using the BD kit, Bioscience as instructed by the manufacturer to assay the amounts of IL-6 and TNF-α cytokines. [13]

Measurement of serum antioxidants enzyme study

Both lipid peroxidase (LPO) and superoxide dismutase (SOD) have their antioxidant activities assessed using standard methods. [14]

Lipid peroxidation assay

Ohkawa et al. was used for the purpose of quantifying lipid peroxides. Finally, 100 mL of the 10% tissue homogenate, which had been previously made according to the above-mentioned protocol, was mixed with 0.8% TBA, 0.1% SDS and 20% acetic acid. The OD of MDA was measured at 532 nm after 30 minutes of heating and cooling. The extraction process made use of N-butanol-pyridine. Protein concentration is measured by MDA in nmol/mg.

Superoxide dismutase

In order to combine 2.78 mL of pH 10.2 sodium carbonate buffers with 100 µL of 1 mM EDTA and Twenty microliter of tissue supernatant, incubate at 30°C for 45 minutes. The response was started by adding 100 mL of adrenaline. After three minutes, the absorbance was measured at 480 nm. As a control, sucrose was used. The superoxide dismutase (SOD) activity was measured in units per mg of protein to ensure accuracy.

Histopathological Analysis

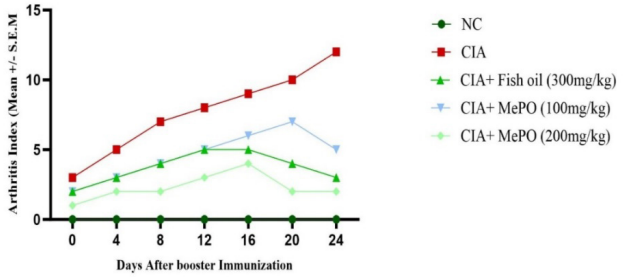
Knee joints were removed from the animals, and then they were let to soak in 10% formalin for 12 to 24 hours. The microtome was used to slice the blocks into 5 µm-thick slices. The next steps would include cleaning it with xylene and drying it with ethanol. After that, it would be set in paraffin wax and used to make blocks. They were processed in an alcohol-xylene series and stained by H&E.[15]

Statistical Analysis

The findings from each group are shown as the Mean ± SEM (n = 6) rats. The statistical analysis was carried out using Graph Pad Prism, version 10. Data analysis used a one-way ANOVA followed by Tukey's test to check for differences between the groups and the significance threshold was $p < 0.05$.

Results And Discussion

Reducing inflammation and discomfort of illness from progressing is the aim of therapy. Glucocorticoids, NSAIDs, systemic DMARDs, etc., may be used to manage acute therapy.[16] Unfortunately, there may be unintended side effects from taking some medications for an extended period of time; therefore, it may be necessary to seek out adjuvant therapy or alternative treatments in order to control the course of the illness. Plants and natural products provide priceless ideas and models for the development of novel pharmaceuticals.[17] Chemicals with a wide variety of molecular structures and biological roles may be abundant in these substances. Isolated from nature are both the pure native compounds and the semisynthetic equivalents of several medical medications. The majority of the rats started showing indications of arthritis twelve days following the initial vaccine. On the twelfth day, the CIA animals' total weight decreased but their paw volume increased. On the 19th day, the sickness rate was 100%. The rats were given 300 mg/kg of oral fish oil (Group III or standard) on day 20 as part of their treatment. Groups III & IV rats were given 100 and 200 mg/kg of *P. oleracea* methanolic extract on days 33 to 44, respectively. Day 4 saw an increase in scores across the board for the arthritis-induced rats, despite the lack of inflammation seen in the control group. Group II animals, who did not experience any symptoms of arthritis, had the highest arthritic score (13.74 ± 0.060) until the 36th day. Following that, the index began to fall before eventually leveling out at an average of 11.41 ± 0.348 until day 44. Up to day 20, the arthritis index in group III (CIA animals given fish oil). The treated animals were shown a significant decrease in their arthritic index. Beginning on day 24, their arthritic index was 3.52 ± 0.035. Group V (CIA rats given 200 mg/kg of MePO) had a higher arthritic index before treatment. Starting on day 21, the animals' arthritic index gradually decreased until day 40. The animals' arthritic index was 2.001 ± 0.065 at 44 days of age, which was the lowest recorded. The pictograph showing arthritis and swelling of the footpad in the CIA rats was confirmed, and it was found that Groups III and V of rats given fish oil and a methanolic extract of *P. oleracea* had significantly lower arthritis scores ($p < 0.0001$) compared to group II of rats given the

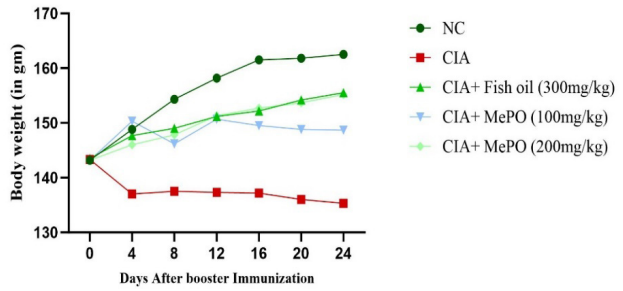


The data are presented as the mean plus standard error of the mean (SEM), with six rats per group.

NC-Normal control; CIA-Collagen induced arthritis; MePO- Methanolic extract of *P. oleracea*

Fig. 1: Rats treated with a methanolic extract of *P. oleracea* for chronic inflammatory arthritis were scored macroscopically

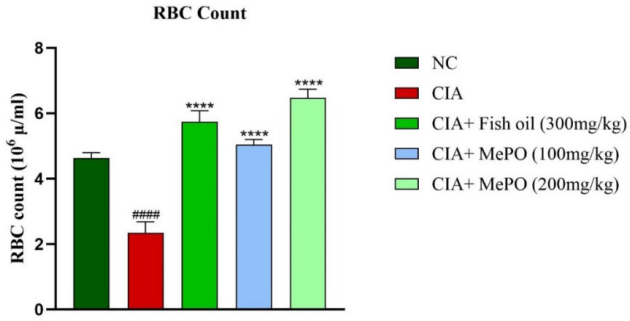
Fig. 2: Diagram depicting collagenase type II induced arthritis and footpad swelling in rats



The Mean and SEM are provided for each group, which consists of six rats.

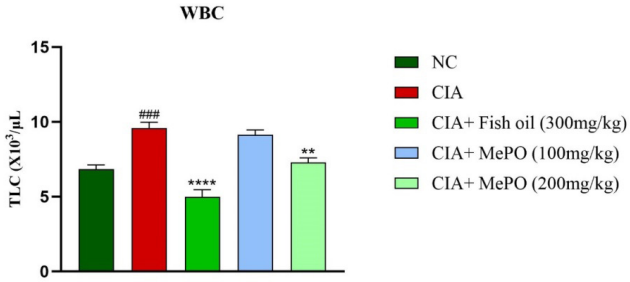
Fig. 3: Weight loss or gain in CIA-treated rats fed a methanolic extract of *P. oleracea*

same treatment (Figs 1 and 2). Paw edema and arthritis score computation are easy approaches to quantifying



The results, with six rats in each group, are shown as the Mean ± Standard Error of Mean (SEM). The results demonstrate statistical significance when compared to normal saline (***$p < 0.0001$) and Disease Control (****$p < 0.0001$) (CIA).

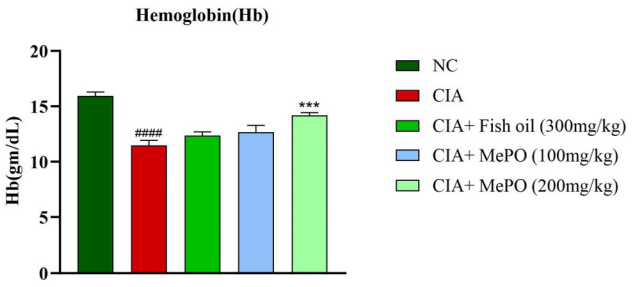
Fig. 4: RBC Level in CIA treated rat with *P. oleracea* extract



Given that there are six rats per group, all results are presented as the mean with or without the standard error of the mean (SEM). For statistical purposes, a p-value below 0.001 is deemed significant. Comparing with the Normal saline control and the Disease control, the CIA, respectively, yielded statistical significance levels of **$p < 0.01$ and ****$p < 0.0001$, respectively.

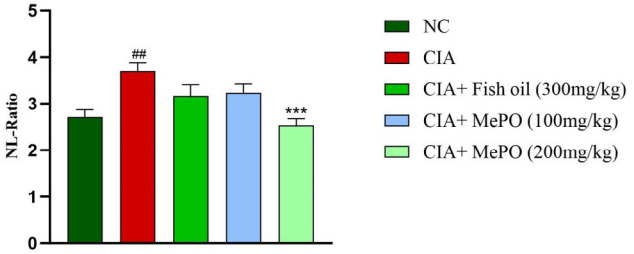
Fig. 5: WBC Level in CIA treated rat with *P. oleracea* extract

inflammation levels. When the number of granulocytes and monocytes increases, the paws enlarge.[18] Rats weighing 140 to 200 g were used in this study. The CIA rats' weight increased consistently up to day 4. Every rat that had arthritis lost weight. Due to severe arthritis, the group II CIA rats' body weight decreased by 135 ± 2.90 g until day 44. Rats in group III were administered fish oil (CIA rats), their body weight gradually decreased until day 20, after which it increased to 155.5 ± 2.99 on day 44. From day 24 to day 44, rats in group IV and V (CIA rats) that were given a methanolic extract of *P. oleracea* at doses of 100 & 200 mg/kg, respectively, saw a steady increase in body weight (148.7 ± 2.10 and 155.2 ± 3.02) (Fig 3). The RBC level decreases noticeably in the CIA arthritis control group, while it increases noticeably in all treatment groups $p < 0.0001$ (Fig. 4). Within the arthritic control group (CIA), there is a notable decrease in white blood cell count in comparison to all treatment groups, $p < 0.0001$ in the standard group, $p < 0.01$ in MePO 200 mg/kg (Fig. 5). The results showed that the groups who got the treatment had considerably greater hemoglobin levels ($p < 0.001$ in MePO 200 mg/kg), in contrast to the control group with



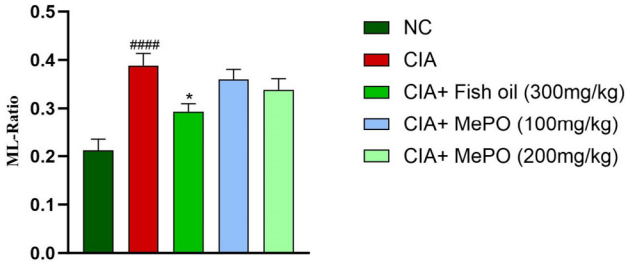
Notable differences were seen when comparing the results to the normal saline and disease control groups, and these differences are shown as the average plus or minus the standard error of the mean (SEM) (CIA).

Fig. 6: Hb Level in CIA treated rat with *P. oleracea* extract



The data are presented as Mean ± Standard Error of Mean (SEM), with six rats per group. *$p < 0.01$ vs. Normal saline control, and ***$p < 0.001$ vs. Disease control, CIA.

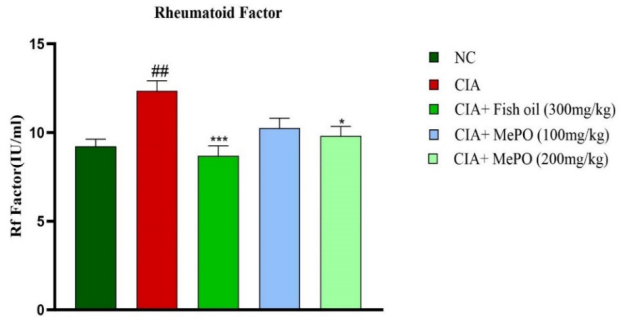
Fig. 7: NL- Ratio in CIA treated rat with *P. oleracea* extract



The Mean ± Standard Error of Mean (SEM) is shown to demonstrate the findings, with six rats in each group. *When compared with Normal saline, disease control, and the CIA ($p < 0.05$), the results are favourable. The threshold of significance is $p < 0.0001$ when contrasted with the normal control group.

Fig. 8: ML- ratio in CIA treated rat with *P. oleracea* extract

arthritis (CIA), which had significantly lower levels (Fig 6). All other groups had a decrease in NL-Ratio, except for the arthritic control group (CIA), which had an elevated one ($p < 0.001$ in MePO 200 mg/kg) (Fig. 7). The ML-Ratio shows a considerable rise in the arthritic control group (CIA), but a slight reduction in all therapeutic groups ($p<0.05$) (Fig. 8). A greater NL-ratio, lower hemoglobin levels, and a lower red blood cell count were seen in the control group of arthritic rats. Anemia is the underlying condition to which each of these signs and symptoms points. [19] There



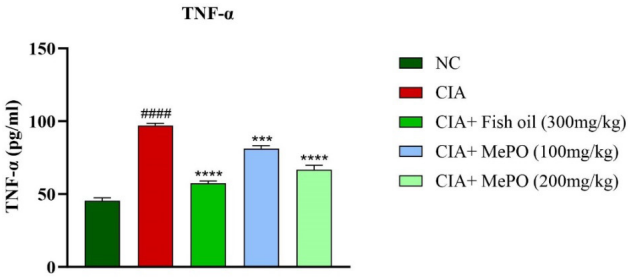
The data are shown as Mean ± Standard Error of Mean (SEM) with six rats per group, in comparison to the Normal control group. The significance levels for the comparisons are ##$p < 0.01$, *vs. Disease Control, CIA, and Normal Saline, with $p < 0.05$ and ***$p < 0.001$, respectively.

Fig. 9: RF factor in CIA treated rat with *P. oleracea* extract

was a significant improvement in the anaemia-stricken groups that received MePO treatment. As evidence of MePO's immunomodulatory activity, the leukocyte count rose dramatically in rats with CIA and fell sharply in those treated with the compound. The immune system going into overdrive in reaction to foreign antigens could explain the sharp increase in white blood cell count. These studies show the antiarthritic capabilities of *P. oleracea* conclusively. The blood plasma may include the antibody rheumatoid factor. The amount of radiofrequency (RF) was very high in cases with arthritis. It will start to go down when you get the right treatment. The results showed that the arthritis control group's RF value was higher than the other groups (CIA). In comparison to the group that received arthritis therapy, the groups that were given 200 mg/kg and fish oil had a considerably reduced RF value ($p < 0.001$ & $p < 0.05$, respectively) (Fig. 9). When B-cells and plasma cells invade the synovium of rheumatoid arthritis (RA) patients, they create RF, a crucial marker of the amount of IgM in the serum of ill hosts. Serum RF levels were found to be significantly lower in animals treated with MePO compared to ill-control animals. Research using *P. oleracea* extract has shown anti-inflammatory properties, which may be due to the plant's antioxidants and flavonoids. Invasion of macrophages, leukocytes, and fibroblasts caused synovitis in the model being discussed. Synovitis is characterized by an inflammatory response that is mediated by the immune system and arthritis. All of these cells possess the capability to generate inflammatory mediators upon activation, including PGE2 cytokines such as; TNF-α, IL-6, and IL-1. Also, these mediators set in motion the production of many proteolytic enzymes, which initiate the deterioration of cartilage and bone, respectively. Reducing TNF-α production would suggest that MePO slows down the development of arthritis. Turmoil fibroblast proliferation and cartilage degradation, two pathogenic processes in rheumatoid arthritis, have been associated with TNF-α. Additionally, studies have shown that it possesses pro-inflammatory and immuno-

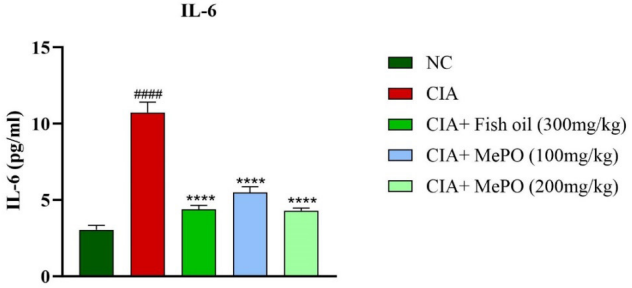
potentiating properties. Beyond its activity as an inflammatory cytokine, TNF-α is very effective in bone loss and plays a significant role in the development of RA. Recent research on animals with rheumatoid arthritis has shown that TNF-α is present in the synovial fluid, plasma, and tissues. The immune system's macro- and microenvironments are both important in the beginning and progression of arthritis. Cytokines are not only crucial in arthritic conditions, but they also play a key function in recruiting and activating certain types of leukocytes. Inflammatory mediators, prognostic markers, and cytokines are vital players in the arthritic milieu. To simulate arthritis in this experiment, rats were inoculated with type II collagen. The remarkable parallels to RA in humans triggered this move. There seem to be two separate phases of CFA-induced arthritis. When leukocytes move to the wounded area, they produce mediators such as IL-1, TNF-α, and prostaglandins, which promote edema in the acute phase. When cell-mediated immunity is present, the immune response enters its secondary phase. [20]

After previously being shown to be elevated, the CIA rats showed a substantial reduction in TNF-α and IL-6 levels when administered 100 and 200 mg/kg of *P. oleracea*, respectively. *P. oleracea* 100 & 200 mg/kg both suppressed these variables to a similar extent when given alone (Figs. 10 and 11). TNF-α has the ability to significantly increase IL-1 levels in both the synovial fluid and membrane cells. The breakdown of bone and cartilage is caused by the activation of monocytes and synoviocytes by these inflammatory mediators. The breakdown is aided by their ability to trigger a cascade of inflammatory processes. Consequently, IL-1 has a role in the progression of RA-related synovitis. As stated before, novel approaches have been developed to combat autoimmune arthritis by reducing the effects of TNF-α and IL-6. [21] The oxidative stress state of animals that have generated collagen type II may be illuminated by measuring their antioxidant levels. According to research in the scientific community, the antioxidant defense is formed by SOD and LPO. Low levels of antioxidants are an indicator of systemic and cellular oxidative stress. [22] Comparing the CIA group to the groups treated with MePO and fish oil revealed notably lower SOD levels, while the CIA group did have much greater levels overall. *P. oleracea* reduced LPO levels in the serum of several experimental animals, as seen in Fig. 12. The LPO levels were higher in the CIA rats compared to other experimental groups. Compared to rats given CIA



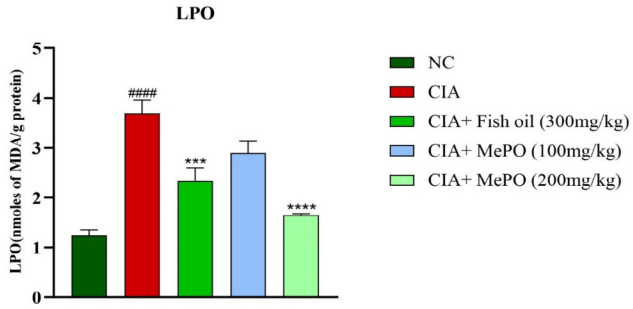
The results, with six rats in each group, are shown as the Mean ± Standard Error of Mean (SEM). The means are considerably different from the Normal Saline control and the CIA disease control (p<0.0001).

Fig. 10: Serum cytokines assessment; TNF- α Level in CIA treated rat with *P. oleracea* extract



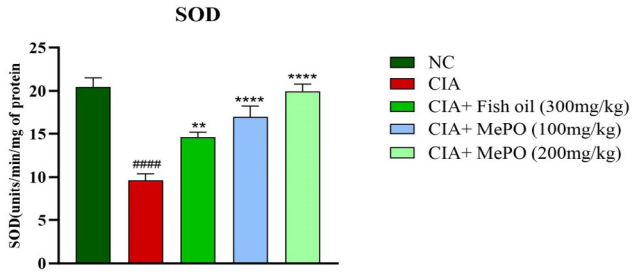
Each group consists of six rats, and the data are shown as the Mean ± Standard Error of Mean (SEM). According to the data, there is a statistically significant difference when compared to Normal Saline (####$p < 0.0001$) and Disease control (****$p < 0.0001$) (CIA).

Fig. 11: Serum cytokines assessment; IL-6 level in CIA treated rat with *P. oleracea* extract



The results, which are shown as the mean plus or minus the standard error of the mean (n=6 rats per group), include a $p < 0.0001$ compared to the normal saline control, a ***$p < 0.001$ compared to the disease control, and a ****$p < 0.0001$ compared to the CIA control.

Fig. 12: Evaluation of serum antioxidants and lipoxygenase (LPO) levels in rats treated with CIA and extract from *P. oleracea*



Each group consists of six rats, and the data are shown as the Mean ± Standard Error of Mean (SEM). The results of ####$p < 0.0001$ indicate a substantial difference when compared to Normal Saline, with **$p < 0.01$ and ****$p < 0.0001$. The CIA is in charge of illnesses prevention.

Fig. 13: Blood antioxidant evaluation; superoxide dismutase (SOD) level in CIA-treated rats given extract from *P. oleracea*

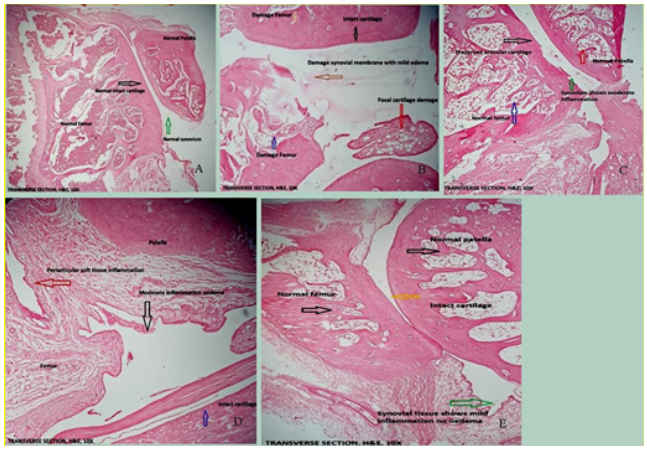


Fig. 14: Tumor analysis of the tibio-tarsal joint in rats given CIA and those given a treatment; A) Normal control, normal saline, B) Disease Control, CIA, C) Std. drug treatment, Fish oil (300 mg/kg), D) Plant extract, *P. oleracea* extract (100 mg/kg), and E) *P. oleracea extract* (200 mg/kg).

alone, animals in groups V and III supplemented with *P. oleracea* and fish oil had significantly lower levels of LPO. Several experimental animals had their blood levels of superoxide dismutase (SOD) changed by *P. oleracea,* as seen in Fig. 13. Results showed that the CIA group had the lowest levels of SOD when compared to the other groups in the experiment. Rats in Groups V and III had significantly elevated levels of SOD after supplementation with fish oil and *P. oleracea*. On the other hand, rats that were given just CIA were also examined. Fish oil is a well-liked medication for the prevention of inflammatory bowel disease in dogs (CIA). Paw edema, lesions, and histological abnormalities are halted in their tracks by this medicine. The FOP's inhibitory and modulatory effects on nitric oxide and cytokines involved in complicated, inflammatory responses may account for these results. Specifically, IL-10 is overexpressed, whereas TNF, IL-1, IL-2, GM-SCF, and IL-6 are downregulated. Based on these considerations, we draw the conclusion that fish oil may be a viable alternative therapy option for arthritis, which might alleviate the symptoms of the illness and its progression.[23] Supporting the protective influence of MePO in arthritis, light microscopy showed much reduced damage to the synovial lining cell layer, and histological tests showed an improvement in the synovial membrane. Synovial hyperplasia, pannus development, cartilage degradation, bone erosion, localized cartilage injury, and narrowing of the joint space were among the abnormalities discovered on histological analysis of the tibio-tarsal joint of the CIA rats (Fig. 14). The synovium and soft tissue in both joints were normal in the normal group of rats, and the articular surfaces and joint spaces were also normal. Cartilage degeneration, mild hyperplasia symptoms, and pannus were the 300 mg/kg fish oil treatment outcomes. Administration of MEPO at a dosage of 200 mg/kg, rats' tibia tarsal joints exhibited minor indications of cellular infiltration. There was also no evidence of cartilage or joint injury that we could detect. Acute and chronic inflammatory cells in the dermis, muscle, and the region around the joint, which extended into the thigh, were discovered throughout the course of the disease control assessment, which also revealed periarticular inflammation, which showed up as edema. The synovium shows signs of mild inflammation and edema. Damage to the cartilage seems to be localized. The inflammation resulted in an abundance of lymphocytes. Some articular cartilage remained in the knee joint, and significant edema and inflammation migrated from the synovium to the periarticular soft tissues. In addition, there were no lymphocytes in the synovium. When given 100 mg/kg of *P. oleracea*, the cartilage in the knee joint remains intact. The periarticular soft tissues enlarge and thicken due to moderate to severe inflammation in the synovial tissue. The 200 mg/kg dose of *P. oleracea* improved the cartilage in the knee. There was modest inflammation in the synovial tissue, but no edema or thickening of the periarticular soft tissue was seen. An evaluation of the patella and femur revealed no abnormalities, and the absence of inflammatory cells was confirmed.

Conclusion

To conclude, *P. oleracea* has many significant impacts on arthritis rats. These effects include a reduction in joint inflammation, a slowdown of the disease's progression, and protection of cartilage and bone from deterioration. The findings demonstrated that average body weight increased significantly, RF factor, footpad edema, and LPO levels all decreased. Also, there was a reduction in both arthritis scores and edema in the footpads. Enzymatic antioxidant SOD was found to be elevated, whereas IL-6 and TNF-α were found to be reduced. Histological analysis of bone tissue showed decreased inflammation, lymphocyte buildup, cartilage degradation, and disruption of the cell layer lining in treated animals in contrast to control arthritic rats. Although *P. oleracea* has more of a history as an anti-rheumatic drug, its phytochemical profile gives hope that it may also have anti-arthritis properties. So, it's possible that *P. oleracea* can be taken as an adjuvant therapy to manage overall health and well-being.

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