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EVALUATION OF ANTI-ARTHRITIC ACTIVITY OF *EERS LINN* LEAF ON FCA INDUCED ARTHRITIC RAT MODEL

CHANDRASEKAR S*, SUMATHI L, PICHAIVEL M, PACHIAPPAN S AND SHANMUGAM G

Swamy Vivekanandha College of Pharmacy, Elayampalayam, Namakkal – 637205, India

***Corresponding Author: Dr. Sabarinath Chandrasekar: E Mail: revathi.sabari@gmail.com**

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory polyarthritis that can affect any synovial-lined diarthrodial joint, but it prefers the wrist and minor joints of the hand. The normal natural history of RA is one of increasing articular deterioration leading to joint deformities and impairment. Indeed, the understanding that tumour necrosis factor alpha plays a key role in joint inflammation and destruction has resulted in the introduction of TNF-α targeting medications, which have had a significant influence on the care of RA patients. The recognition that autoimmunity to citrullinated proteins is extremely specific for RA and may be harmful is an essential insight into the disease. According to the results of the current investigation, an ethanolic extract of *Raphanus sativus Linn* leaf may have therapeutic benefits for treating rats' arthritis. The outcomes of the present study show the benefits of *Raphanus sativus Linn* leaf in the treatment of arthritis and the reduction of clinical symptoms, such as paw volume, arthritic index, radiographic and histological investigation. The findings of the study demonstrate that the plant extract significantly reduces inflammation, protects joints, and has anti-arthritic properties.

Keywords: Rheumatoid arthritis, paw volume, arthritic index, auto-immune, anti-arthritic activity

INTRODUCTION

Rheumatoid arthritis is an autoimmune and inflammatory condition, meaning that your immune system misidentifies your body's cells as foreign substances and releases inflammatory mediators that attack the synovium. The inflamed synovium thickens and causes the joint area to become extremely painful, swollen, red, and uncomfortable to move. Inflammation of the synovium is brought on by the release of cytokines, including TNF-α, interleukin-1, and interleukin-6. The destruction of connective tissue by matrix metalloproteinase and the promotion of osteoclast generation by activated CD4+ T lymphocytes lead to joint injury **[1]**. The interaction of B- and T-cells, macrophages, chondrocytes, osteoclasts, and synovial cells, as well as the release of numerous growth factors and cytokines (such as tumour necrosis factor alpha, interleukin [IL]-1, IL-6, IL-8, and IL-17), initiates the immune inflammatory process that is characteristic of the disease. The formation of autoantibodies, most notably rheumatoid factors (RF), and the migration of T and B cells into the synovium are caused by abnormalities in the cellular and humoral immune response. Small joints are first affected, then larger joints are frequently affected as well as the bone, joints' cartilage, tendons, and ligaments. In synovial joints, a variety of immune-mediated chemicals can aggravate inflammation. Although inflammation-induced loss of articular cartilage is the main cause of RA, the precise process is yet understood **[2]**.

As complementary and alternative medicines (CAMs) gain popularity in the UK and the rest of Europe, as well as in North America and Australia, so has the usage of herbal therapies in many industrialised nations. A traditional herb known for its anti-inflammatory properties is *Raphanus sativus*. The herb's various parts have been used as diuretics, laxatives, haemorrhoids, syphilitic illness treatments, and as a supposed medication for piles. Other traditional medical uses include treating gynaecological diseases as well as anticancer, antibacterial, antidiabetic, diuretic, antifertility, hypertensive, nephroprotective, gastro protective, and hepatoprotective properties **[3]**. According to studies, *Raphanus sativus* extracts exhibit hepatoprotective, cardio-protective, anticancer, antibacterial, and anti-urolithiatic properties **[4]**.

Pathogenesis of rheumatoid arthritis

The initial symptoms of RA include immunological complexes and autoimmune disease in the joints and other organs. The synovial membrane, where the infection starts, becomes infected as a result of swelling and inflammation. The three stages of RA development are an initiation stage brought on by non-specific inflammation, an amplification stage brought on by T-cell activation,

and a chronic inflammation stage brought on by the cytokines IL-I, TNF-α, and IL-6 **[5]**. Smaller and medium-sized joints deteriorate in RA patients due to local and systemic inflammation. Several autoimmune and inflammatory mechanisms are triggered at different points in the development of RA **[6]**. The beginning of an immune response to the synovium, as well as the particular causes and mechanisms of tissue death in RA, are all influenced by both hereditary and environmental variables. Numerous ethnic groups have a higher risk of developing RA when certain genetic variants of the mononuclear complex (MHC) II are present. Human leukocyte antigens (HLAs), which are encoded by MHC II, include HLA-DR1, HLA-DR4, HLA-DR6, and HLA-DR10. These HLAs have been associated with a higher risk of RA. It is still unknown what antigen, when paired with particular MHC genetic markers, causes the immune system to overreact in RA. As putative triggers, infections and environmental elements (such cigarettes) have been put forth **[7]**. Based on the presence or lack of anti-citrullinated protein antibodies (ACPAs), there are two fundamental classifications of RA. Citrullination is a post-translational alteration that happens when the calcium-dependent enzyme peptidyl arginine-deiminase (PAD) changes a positively charged arginine into a polar but neutral citrulline. A diagnostic reference for patients with early, undifferentiated arthritis and a predictor of disease progression to RA, ACPAs are present in about 67 percent of RA patients. When compared to the ACPA-negative fraction of RA, the ACPA-positive subset exhibits a more aggressive clinical profile **[8]**.

MATERIALS AND METHODS

Collection and authentication of plant material

The leaf of *Raphanus sativus Linn* was collected in the month of November from the locality of Thalaivasal vegetable market, Salem (D.T) and were identified and authenticated by Dr. P. Radha, research officer (Botany), Siddha Medicinal Plants Garden (central council for research in siddha), Mettur, Salem- 636401.

Authentication no: R081222053S

Preparation of plant extract

The polarity of the solvents used to extract the *Raphanus sativus Linn* leaf varied. A cold maceration procedure was used for the extraction.1500 ml of ethanolic solvent extracts were combined with 100 grams of powdered *Raphanus sativus* leaves in a conical flask. Under carefully controlled climatic conditions, the conical flasks were kept with intermittent shaking for 72 hours and regular agitation. The resulting extracts were then filtered using muslin cloth. A heating mantle

was used to concentrate the filtrate while keeping the temperature below the solvents' boiling point (70^0C) **[9]**. The leftovers were then kept in a plastic screw-cap tube below room temperature until further use. It was discovered that *Raphanus sativus Linn* leaf ethanolic extract had an 84.88% yield. Raw extracts were gathered until a sticky, thick paste of extract was apparent. The extract was then kept chilled at 4°C until it was needed **[10]**.

Grouping

The animals were divided into 5 groups of 6 each.

Group I : Normal control (saline,1ml, p.o)

Group II : Arthritic control (0.1ml of *FCA* into sub plantar region)

Group III : FCA + 2mg/kg Methotrexate *p.o*

Group IV : FCA + 200 mg/kg of *EERS (Linn.)* leaf *p.o*

Group V : FCA + 400 mg/kg of *EERS (Linn.)* leaf *p.o*

Assessment of anti-arthritic activity

The following parameters were measured on certain days following the injection of Freund's Complete Adjuvant to assess the course of Freund's Complete Adjuvant-induced arthritis.

Change in Arthritis Score

A blinded observer used the visual arthritis scoring technique to record the degree of arthritis **[11]**. The arthritis score ranged from 0 to 4, with 0 denoting the least amount of swelling but still being present and 4 denoting the most edoema. This scoring method entails examining each of the four paws and assigning a unique score for each one. For assessments of the pain brought on by the arthritis, scores were given.

Change in Body weight and Paw Volume

Using a weighing balance, the body weight of each rat in each group was monitored daily until the treatment was completed. Using a plethysmograph, the swelling in the left hind paw from the ankle was monitored on the 0, 4, 8, and 21st days. By analysing the difference between day 0 paw volumes and paw volume at various time intervals, the edoema component of arthritis was determined **[12]**.

Hematological parameters

Blood was drawn through retro orbital plexus on the day of the sacrifice in order to test the following haematological parameters. Haematological auto analyser was used to analyse

haemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), lymphocyte count, mean corpuscular haemoglobin concentration (MCHC), and erythrocyte sedimentation rate (ESR).

Serological parameters

Blood was removed by retro-orbital puncture on days 9, 14, and 21. Serum was then obtained to estimate the RF and CRP factor. Spin respond (CRP-ultrasensitive) diagnostic kit was used to measure CRP. CRP is measured in mg/L. The Accucare testing kit estimates the Ra factor, however. The RF factor value is given in units of IU/ml.

Histopathology

At the conclusion of the experiment on day 21, all animals were given light chloroform anaesthesia before being cervicaly decapitated and slaughtered for histological analysis. The left hind (arthritis-induced) limb was then amputated at the knee and kept in 10% formalin after being washed with normal saline. The decalcification of the fixed tissues was followed by hematoxylin and eosin staining of slides of sagittal slices through the hind paw, which were then magnified by 4*(10). The evaluation of soft tissue swelling, bone demineralisation, pannus development, cartilage degradation, and joint space narrowing was done after reviewing the slides**.**

Statistical analysis

The information is presented as the Mean ± SEM of six replicated findings. One-way ANOVA was used to statistically assess the results, and Tukey's multiple comparison was used after that. When p 0.05, the difference was deemed significant. Prism 9.0 (Graph Pad, San Diego, California, USA) was used for all statistical analyses. data analysis software

RESULTS AND DISCUSSION

Effect of EERS on physiological and morphological parameters.

This may be explained by an increase in leptin synthesis (a cytokine-hormone) following the injection of complete Freund's adjuvant, which may also result in decreased feed intake and weight loss **[13]**. Due to the systemic or local activity of inflammatory cytokines like TNF-α and IL-1, which are largely produced by monocytes and macrophages, chronic arthritis frequently results in weight loss **[14]**. Compared to EERS 200mg/kg, the EERS 400mg/kg group saw a considerable increase in body weight. The paw volume of the ipselateral paw was used to assess the anti-inflammatory effects of EERS on rats. Following the initiation of inflammation, the peak incidence of swelling occurred between the fourth and eighth day, with an increase in paw volume that was the greatest across all groups. When compared to the FCA control, the doses of methotrexate at 2

mg/kg/week, EERS at 200 mg/kg, and EERS at 400 mg/kg, all significantly reduced the size of the animal's paws on day 21. In the eight days following the onset of arthritis, the arthritis score in the affected paw dramatically increased in all the animals. On day 21 of the study, methotrexate 2 mg/kg, EERS 200 mg/kg, and EERS 400 mg/kg of an ethanolic extract of *Raphanus sativus Linn* leaf were all compared to the normal control group and showed a statistically significant reduction in arthritis score. However, 400 mg/kg of the ethanolic extract of EERS showed inhibitory effects are shown in **(Figure 1) [15]**.

Effect of EERS on serological parameters

In this work, therapy with EERS 200mg/kg and EERS 400mg/kg considerably reduced the dramatic rise in rheumatoid factor concentration and CRP level in the arthritic rats. This demonstrated that the extracts had anti-arthritic effect, and the mechanism may be explained by the production of autoantibodies against the Fc fragment, which would prevent cartilage degradation **[16]**. When compared to the FCA control group, the serum levels of RF in the methotrexate 2 mg/kg/week, EERS 200 mg/kg, and EERS 400 mg/kg groups were all considerably lower on days 14 and 21 as shown in, **Figure 2**. CRP levels are indicators of inflammation. Monocytes are induced to secrete proinflammatory cytokines including IL-1, IL-6, and TNF-α by CRP, and phagocytic cells may also directly benefit from this proinflammatory boost. As compared to the untreated arthritic rats in this investigation, the arthritic rats treated with EERS 200mg/kg and EERS 400mg/kg had lower serum CRP levels.

Effect of EERS on haematological parameters

In this study, rats with arthritis had considerably lower levels of Hb, RBC, and concomitantly higher levels of WBC and ESR. In all therapy groups, these modifications were reversed to nearly normal levels. EERS 200mg/kg, EERS 400mg/kg, and methotrexate 2mg/kg/week all significantly increased RBC count and Hb levels in the study. In this investigation, treatment with methotrexate at 2 mg/kg/week, EERS at 200 mg/kg, and EERS at 400 mg/kg, significantly reduced the high WBC count. In this study, the methotrexate 2 mg/kg/week, EERS 200 mg/kg, and EERS 400 mg/kg, treated groups, were able to reduce the elevated ESR level shown in **Figure 3**.

Effect of EERS on biochemical parameters

When compared to the normal control, it was shown that the SGOT, SGPT, ALP, and ALT levels in the arthritic rat significantly increased. All therapy groups significantly reversed these alterations. An efficient and convenient method for evaluating the anti-arthritic action of specific

medications is the measurement of the blood levels of SGOT, SGPT, ALP, and ALT **[17]**. This study showed that decreased levels of these enzymes on methotrexate 2mg/kg/week, EERS 200mg/kg and EERS 400mg/kg treatments emphasize the decreased bone loss and organ protective role in the FCA induced arthritic rats shown in **Figure 4**.

Effect of EERS on histopathological studies

The results of a joint histopathology investigation conducted 21 days after FCA injection showed observable alterations in the joints of the hind paws. Synovial hyperplasia, leukocyte infiltration, bone and cartilage degradation, joint space narrowing, pannus development, and infiltration of inflammatory cells are some of these major changes are shown in **Figure 5**. According to histological examinations, the control group had clear synovial cavities and a smooth, monolayer synovial lining. The joint cartilage was severely destroyed and there were significant inflammatory cell infiltrations in the FCA control.

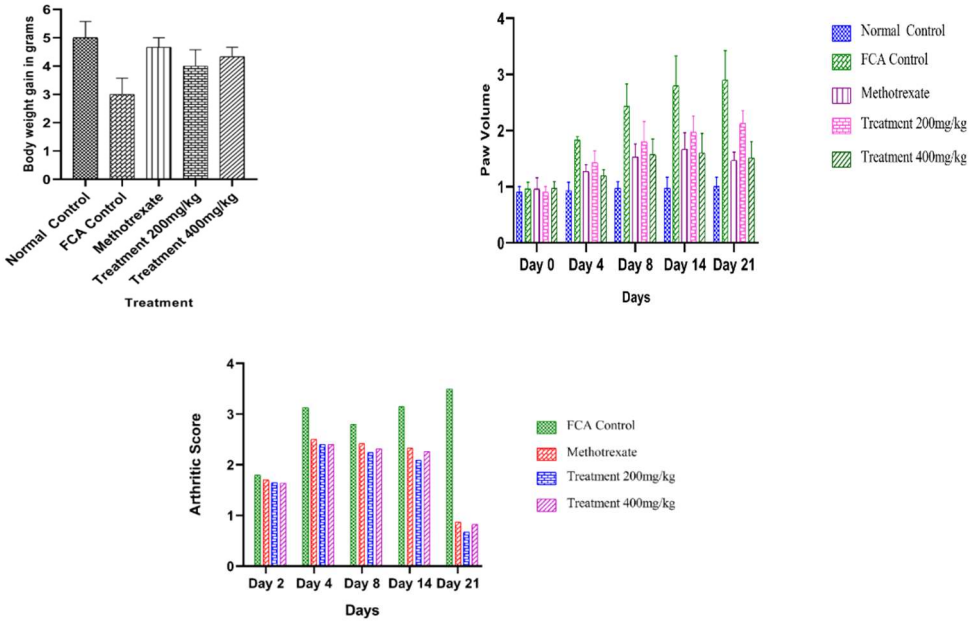


Figure 1: Effect of EERS on physiological and morphological parameters
n=6; values were expressed as Mean±SEM; *P<0.001, **P<0.01 Vs control group, #P<0.001, Vs FCA control group. Data were analysed by one-way ANOVA followed by Tukey's multiple comparison Test. Values of P<0.05 were considered significant

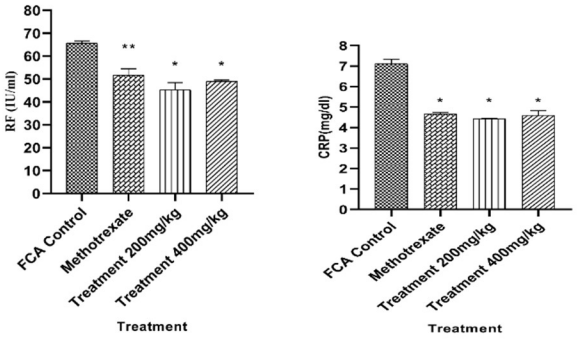


Figure 2: Effect of EERS on serological parameters
n=6; values were expressed as Mean±SEM; *P<0.001, **P<0.01 Vs control group, #P<0.001, Vs FCA control group. Data were analysed by one-way ANOVA followed by Tukey's multiple comparison Test. Values of P<0.05 were considered significant

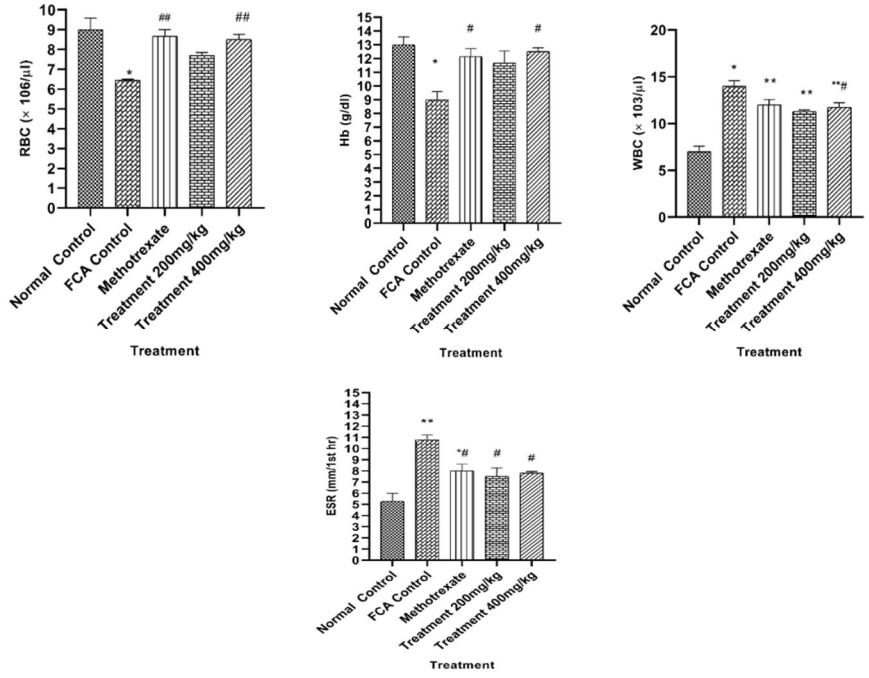


Figure 3: Effect of EERS on haematological parameters
n=6; values were expressed as Mean±SEM; *P<0.001, **P<0.01 Vs control group, #P<0.001, Vs FCA control group. Data were analysed by one-way ANOVA followed by Tukey's multiple comparison Test. Values of P<0.05 were considered significant

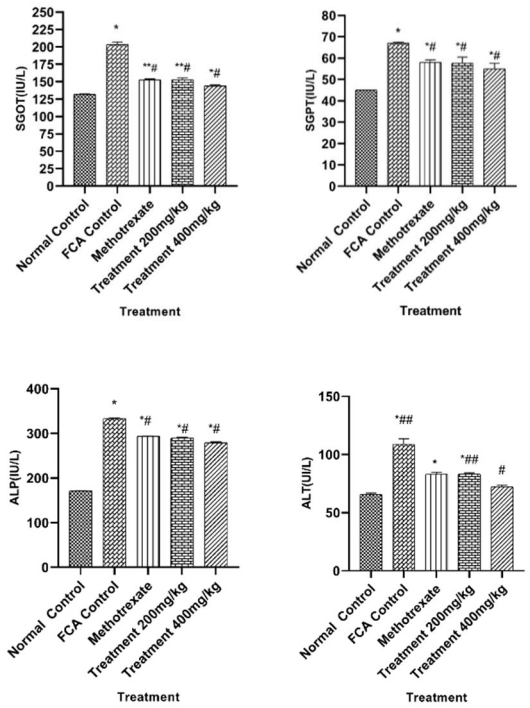
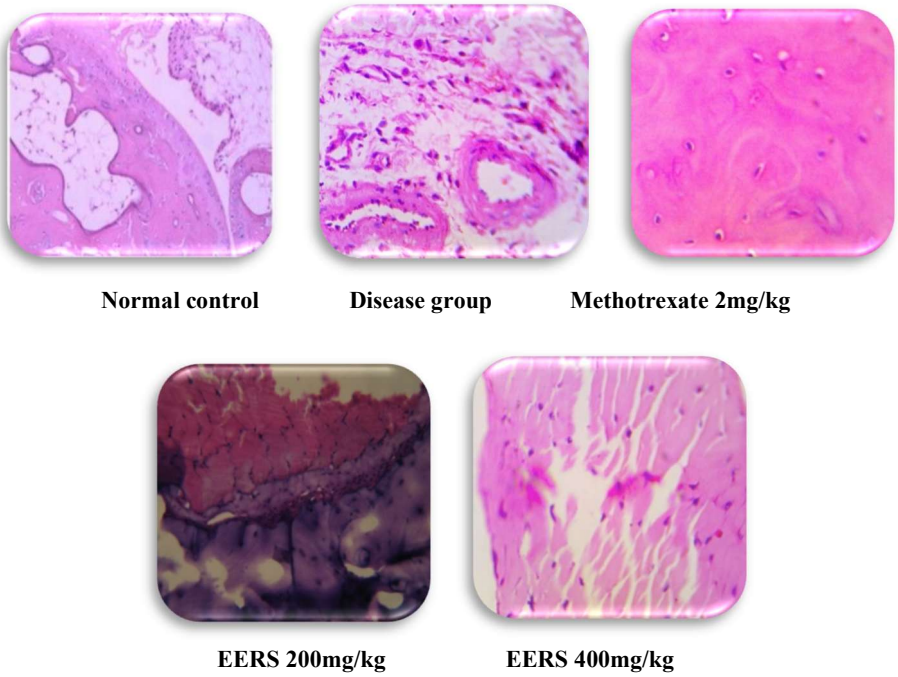


Figure 4: Effect of EERS on biochemical parameters
n=6; values were expressed as Mean±SEM; *P<0.001, **P<0.01 Vs control group, #P<0.001, Vs FCA control group. Data were analysed by one-way ANOVA followed by Tukey's multiple comparison Test. Values of P<0.05 were considered significant



Normal control **Disease group** **Methotrexate 2mg/kg**

EERS 200mg/kg **EERS 400mg/kg**

Figure 5: of EERS on tibio-tarsal joint of female Wistar Albino rats in FCA induced Rheumatoid arthritis rats

CONCLUSIONS

Based on the findings of the current investigation, we hypothesise that EERS may have anti-arthritic potential by protecting the synovial membrane, vascular permeability, preventing the degeneration of cartilage, and so enhancing health. In past investigations, it was mentioned that *R. sativus* linn leaf was traditionally used to alleviate inflammation. The findings of this study show that *R. sativus linn* leaf has positive effects on CRP, Hb, ESR, body weight, paw oedema, paw volume, arthritic index, radiographic examination, and histological examination during the recovery from arthritis. The research proved that *R. sativus linn* leaf ethanolic extract has antiarthritic action in Albino Wistar rats. To fully comprehend its anti-inflammatory mode of action, EERS should be further explored. It may also be used in the creation of pharmaceutical drugs.

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REFERENCES

[1] Choy, E. H., & Panayi, G. S. (2001). Cytokine pathways and joint inflammation in rheumatoid arthritis. *New England Journal of Medicine*, *344*(12), 907-916.

[2] Imboden, J. B. (2009). The immunopathogenesis of rheumatoid arthritis. *Annual Review of Pathology: Mechanisms of Disease*, *4*, 417-434.

[3] Aggarwal, A., Verma, S., Ghai, R., & Nagarajan, K. (2021). Potential of fruits and vegetables to treat inflammatory conditions. *Materials Today: Proceedings*, *47*, 127-134.

[4] Sugihara, T. (2022). Treatment strategies for elderly-onset rheumatoid arthritis in the new era. *Modern Rheumatology*, *32*(3), 493-499.

[5] Pichaivel, M., Sumathi, L., Kandhasamy, T., & Gnanasambandam, A. Rheumatoid Arthiritis–Recent Advances in Therapies and Treatment.

[6] Deane, K. D., Demoruelle, M. K., Kelmenson, L. B., Kuhn, K. A., Norris, J. M., & Holers, V. M. (2017). Genetic and environmental risk factors for rheumatoid arthritis. *Best practice & research Clinical rheumatology*, *31*(1), 3-18.

[7] Van de Sande, M. G. H., De Hair, M. J. H., Van Der Leij, C., Klarenbeek, P. L., Bos, W. H., Smith, M. D., ... & Tak, P. P. (2011). Different stages of rheumatoid arthritis: features of the synovium in the preclinical phase. *Annals of the rheumatic diseases*, *70*(5), 772-777.

[8] Al-Rubaye, A. F., Kadhim, M. J., & Hameed, I. H. (2017). Rheumatoid arthritis: history, stages, epidemiology, pathogenesis, diagnosis and treatment. *International Journal of Toxicological and Pharmacological Research*, *9*(2), 145-55.

[9] Rathour, S., Rawat, P., Tyagi, S., Ghosh, K., & Gupta, A. (2020). Phytochemical analysis, antioxidant and antimicrobial activity of Raphanus sativus and *Citrus limon* Peel. *International Journal of Botany Studies*, *5*(3), 452-456.

[10] Ojha, D., Maity, C., Mohapatra, P. D., & Samanta, A. (2010). In vitro antimicrobial potentialities of different solvent extracts of ethnomedicinal plants against clinically isolated. *Journal of Phytology*, *2*(4), 57-64.

[11] Ghai, C. L. (2012). *A textbook of practical physiology*. JP Medical Ltd.

[12] Bansod, M. S., Kagathara, V. G., & Somkuwar, A. D. (2010). Evaluation of analgesics and anti-inflammatory activity of a poly-herbal formulation. *Int J PharmTech Res*, *2*(2), 1520-7.

[13] Veys, E. M., Gabriel, P. A., Coigne, E., & Mielants, H. (1976). Rheumatoid factor and serum IgG, IgM and IgA levels in rheumatoid arthritis with vasculitis. *Scandinavian Journal of Rheumatology*, *5*(1), 1-6.

[14] Memon, R. A., Grunfeld, C. A. R. L., Moser, A. H., & Feingold, K. R. (1993). Tumor necrosis factor mediates the effects of endotoxin on cholesterol and triglyceride metabolism in mice. *Endocrinology*, *132*(5), 2246-2253.

[15] Kadhem, M. A. (2016). Anti-Arthritic activity of ethanolic extract of *Lawsonia inermis* in Freund'S adjuvant induced arthritic rats. *IOSR J Agric Vet Sci*, *9*, 1-6.

[16] Choy, E. H., & Panayi, G. S. (2001). Cytokine pathways and joint inflammation in rheumatoid arthritis. *New England Journal of Medicine*, *344*(12), 907-916.

[17] Obiri, D. D., Osafo, N., Ayande, P. G., & Antwi, A. O. (2014). *Xylopia aethiopica* (Annonaceae) fruit extract suppresses Freund′ s adjuvant-induced arthritis in Sprague-Dawley rats. *Journal of ethnopharmacology*, *152*(3), 522-531.