Modulation of IL-6 In Ameliorating Arthritic Conditions In Rats by Rosa Alba L Flower Extract and Fraction

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Abstract

Objectives: Rheumatoid arthritis characterized by joint inflammation and pain,affecting millions of peoples around the world. Traditional system of medicine had proven to be preventive and treating physical and mental illness. The aim of the study is to assess anti-arthritic potential of the plant *Rosa alba* L by considering complete freund's adjuvant, formaldehyde and turpentine oil induced arthritic model.

Methods: Ethanolic extract and its ethyl acetate fraction were considered for the study and quercetin was quantitatively estimated by high performance thin layer chromatographic (HPTLC) method. Moreover, hematological and biochemical studies were performed in blood and serum of wistar albino rat. Then histopathological studies had also been performed on rat hind paw joint.

Results: Phytochemical screening estimated the presence of carbohydrates, phenolic compounds, flavonoids, phytosterols, amino acids and fixed oil in ethanolic extract and ethyl acetate fraction of the plant. Amount of quercetin in the flowers of the plant was found to be 0.26%w/w by quantitative HPTLC method. Paw edema significantly decreased ($P<0.001$) by extract and fraction of the plant in dose dependant manner by considering formaldehyde and turpentine induced acute arthritic model. The level of proinflammatory cytokines, interleukin (IL-6) was considerably decreased ($P<0.001$) in the fraction treated group of rats at 400 mg/kg b.w by considering complete freund's adjuvant (CFA) induced arthritic model.

Conclusions: Ameliorated hematological, biochemical and histopathological parameters confirmed the antiarthritic potential of the plant.

Keywords: Complete freund's adjuvant, Interleukin, HPTLC, Quercetin, Histopathology

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INTRODUCTION

Rheumatoid arthritis (RA) is indicated as an autoimmune ailment concerned with the soreness in the joints, cartilage devastation and synovial intensification. Nowadays, broader therapeutic choices including Janus kinase inhibitors along with disease modifying antirheumatic drugs (DMARD's) and biologic agents were likely to be used for the treatment. However early diagnosis, intensive therapy considered to be useful for the treatment[1]. Globally, 20 million cases consider prevalent cases, 1.2 million cases consider incident cases and 3.4 million cases consider disability adjusted life years (DALYs)[2].

Traditional medicines and therapy being a holistic health care system treats certain class of problems resulting in overall wellbeing apart from complementary and alternative medicines (CAM) systems which aims on cause and prevention[3]. Hence, medicinal plants and derived molecules can decrease the manifestation related to RA. The plant *Rosa alba* L is considered to be an aromatic shrub and used medicinally and commercially. The aromatic water made from the plant is utilized as flavoring agent in the cake, jamand drinks[4]. Several scientific approaches determined that *R. alba* exhibits various pharmacological activities including antioxidant[5], antimicrobial[6], antifertility and teratogenic activity[7], cytotoxic and genotoxic activity[8], memory enhancing

activity[9] stress induced skin barrier disruption activity[10], bradykinin antagonist activity[11]. Chemically the plant contains geraniol, heneicosane, nonadecane, citronellol, linalool, β phenylethyl alcohol, nerol, neral, geranial, eugenol, methyleugenol, nonadecene, eicosane, and tricosane12, tannins, ellagitannins, and flavonoids13, aliphatic hydrocarbons, minerals, alcohols, aldehydes, monoterpenoids, sesqiterpene, benzyl benzoate, allo-aromadendrene, β selinene[14]. Traditionally the flowers of the plant claimed for lessening inflammation, treating cold and catarrh of nose, treating diseases of the lungs, opthalmia and rheumatism[15].

Despite the above-mentioned pharmacological action, no antiarthritic activity has been explored for the plant, thus the current work aims to explore the antiarthritic potential of *Rosa alba* L flower extracts and fractions in acute and chronic arthritic models.

MATERIALS AND METHODS

Plant collection

The flowers of the *Rosa alba* L were collected from herbal garden of United Institute of Pharmacy, Naini, Prayagraj in the month of March 2019 and air-dried at 40⁰ C. The plant was authenticated by taxonomist Dr. Arti Garg from Botanical Survey of India, Prayagraj and voucher specimen has been submitted in departmental herbarium of BSI with Accession No. 104698. Grind the air-dried material into coarse powder, again air dried under shade and kept in closed container.

Extraction and fractionation

The powdered material of dried flowers of *Rosa alba*(500g) were macerated along with petroleum ether toeliminate fatty substance; marc was additionally extracted by 50% ethanol by continuous extraction process in Soxhlet assembly at 50⁰C-60⁰C. The obtained semi solid plant extract have been subjected for drying on water bath under controlled conditions and then store in air tight container in a desiccator. Thus 80.8 g of solid residue (yield 39.8 % w/w) was obtained. Fractionation was made by amalgamating 100 g of relic with water and chloroform, ethyl acetate, methanol and aqueous (1:1) by considering successive liquid–liquid partitioning method in a separating funnel. The chloroform (RACF), ethyl acetate (RAEA), methanol (RAM) and aqueous fractions (RAAF) fractions were obtained and intensified considering rotavapor (Buchi, USA) at <400⁰C. The yield of ethyl acetate fraction was 40.6% w/w.

Phytochemical screening and TLC

RAEE and RAEA were tested for phytochemical screening and thin layer chromatography (TLC) considering Silica Gel 60 to be stationary phase[16]. Under UV chamber at 254 nm, 365 nm and visible light the plates were experimented for spots considering Toluene: ethyl acetate: formic acid (8:1:1v/v/v) in visible light. RAEE showed (04) spots and subjected for further fractionation. Then ethyl acetate (RAEA), chloroform (RACF), methanol (RAMF) and aqueous (RAAF) fractions were also tested. In RAEA, four spots were observed at visible hence considered for anti-arthritic activity.

Depiction of ethanolic extract and its fraction by HPTLC

RAEE AND RAEA were analyzed qualitatively by HPTLC method. 10 µl of 50% ethanolic extract (RAEE) and its ethyl acetate fraction (RAEA) were marked on plates. The plates were developed at 580nm using mobile phase Ethyl acetate: toluene: formic acid (8:1:1v/v/v).

Experimental animals

Young healthy male albino wistar rats were kept in the animal care of United Institute of Pharmacy, Allahabad. The experimental protocol was approved by the Institutional Animal Ethical Committee of United Institute of Pharmacy with approval number UIP/IAEC/Nov.-2020/08.

Toxicity study of the plant

The lethal median dose (LD50) assessment was performed in rats by OECD guidelines[17].

Induction of arthritis

Arthritis was induced intraplantarally in left hind paw after 30 min of drug/vehicle administration to all the rats[18].

Acute arthritic model

Seven groups (n=6) were selected for study. Group I, given vehicle, Group II given 0.1 ml of 2% formaldehyde solution/0.1ml 10%v/v turpentine oil emulsion, Group III rats treated with drug aspirin(100 mg/kg body weight), Group IV (RAEE) 200 mg/kg body weight, Group

V (RAEE) 400 mg/kg body weight, Group VI (RAEA) 200 mg/kg body weight, Group VII (RAEA) 400 mg/kg body weight.
RAEE and RAEA evaluated for anti-arthritic activity by measuring paw volume and joint diameter on day 0, 2, 4, 6, 8, 10 and every hour for up to 6 hours on formaldehyde and turpentine induced arthritic model respectively.

Chronic arthritic model

Complete Freund's adjuvant arthritic model

Parameters suggesting paw volume, body weight, joint diameter on day 0, 1, 4, 8, 12, 16, 20, 24, and day 28 were considered in this study. Inference of various parameters were considered by withdrawing the blood by retro-orbital puncture on 28th day.

Hematological and serum parameters

The parameters includinginterleukin (IL-6) were measured on 28thday[18].

Histopathological analysis

The rats were sacrificed and the joint sections were histopathologicallyanalysed[18]. The snaps of histopathological slides were captured with a Nikon E400 microscope (Chiyoda, Tokyo, Japan).

Statistical analysis

Data was expressed as mean±SD and statistical analysis was carried out by using GraphPad Prism 9.1.2 software by applying two-way ANOVA with Newman-Keuls method. P<0.001 was considered to be significant.

RESULTS

Phytochemical analysis

Carbohydrates, phenolic compounds, flavonoids, phytosterols, amino acids and fixed oil were present in 50% *Rosa alba* ethanolic extract and *Rosa alba* ethyl acetate fraction.

Toxicity of the plant extract

Rosa alba ethanolic extract (RAEE) and *Rosa alba* ethyl acetate (RAEA) fraction did not exhibit any toxicity and mortality when oral dosing was done up to 5000 mg/kg. Hence, two treatment doses were selected i.e., 200mg/kg and 400mg/kg b.w.

High-performance thin layer chromatography fingerprinting analysis

The Quantitative estimation of quercetin was performed considering ethyl acetate fraction of *Rosa alba* L at 580nm using Ethyl acetate: toluene: formic acid (8:1:1v/v/v) as the mobile phase. The HPTLC plate and densitograms were shown in Figure 1, Figure 2 and Figure 3 respectively. The retardation factor (Rf) value of standard quercetin was found to be at 0.58. The amount of quercetin by quantitative HPTLC method was found to be 0.26 %w/w.

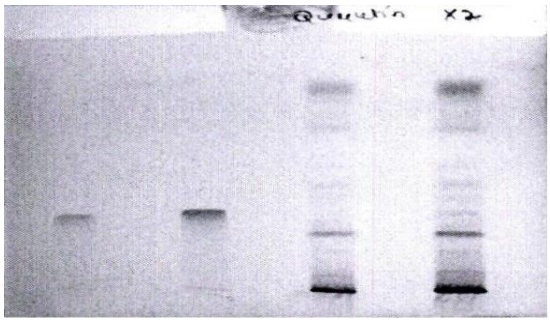

Figure 1: HPTLC fingerprinting plate of quercetin marker compound and ethyl acetate fraction (RAEA) of *Rosa alba* L (X2)

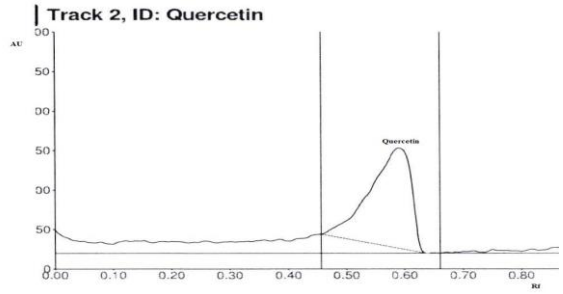

Figure 2: HPTLC densitogram of quercetin marker compound

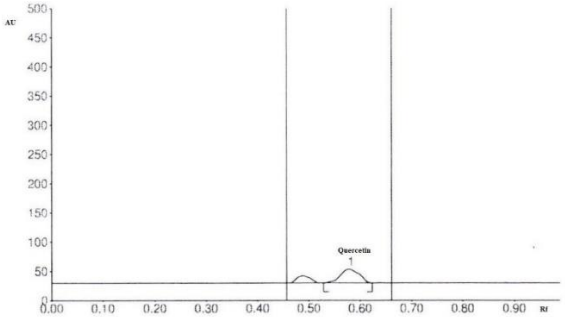

Figure 3: HPTLC fingerprinting of ethyl acetate fraction (RAEA) of *Rosa alba* L.

Effect of RAEE and RAEA on acute arthritic models

On injecting formaldehyde an acute reaction was observed followed by the hyperemic and edematous response in rats, signifying the establishment of acute arthritis. The outcome of joint odema from 0th to 10th day in rats was presented in Figure 4 after comparison with arthritic control.

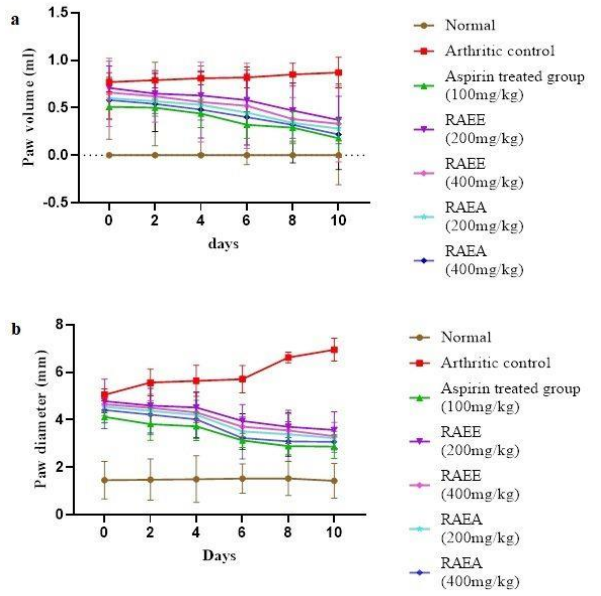

Figure 4: a) Effect of ethanolic extract and ethyl acetate fraction of *R alba* on paw volume in formaldehyde induced arthritic model b) Effect of ethanolic extract and ethyl acetate fraction of *R alba* on paw diameter in formaldehyde induced arthritic model

Diseased control rats treated turpentine had a significant ($p<0.001$) rise in paw volume and diameter as matched to healthy rats. RAEE, RAEA and aspirin lessen the distension and diameter from 3 hour onwards as compared to diseased rats as shown in Figure 5.

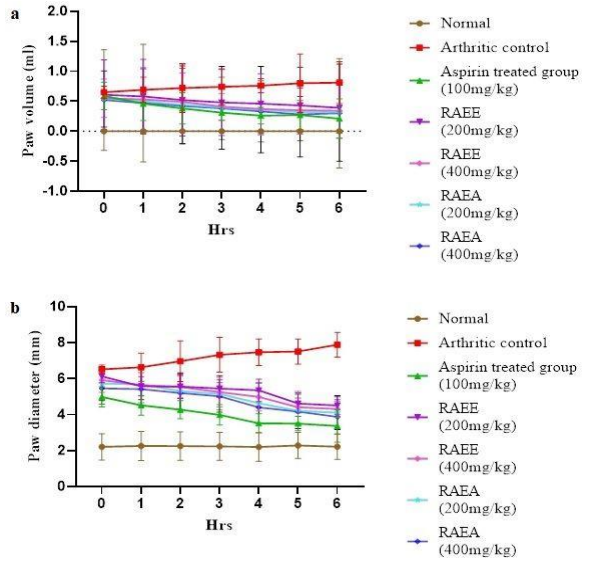

Figure 5: a) Effect of ethanolic extract and ethyl acetate fraction of *R alba* on paw volume in turpentine induced arthritic model b) Effect of ethanolic extract and ethyl acetate fraction of *R alba* on paw diameter in turpentine induced arthritic model

Effect of RAEE and RAEA in CFA induced arthritic model

Paw swelling

As demonstrated in Figure 6, rats developed persistent arthritis in their left hind paw. In paw volume, when matched to the positive control, all CFA treated rats had a significant ($p<0.001$) rise in paw volume and diameter.

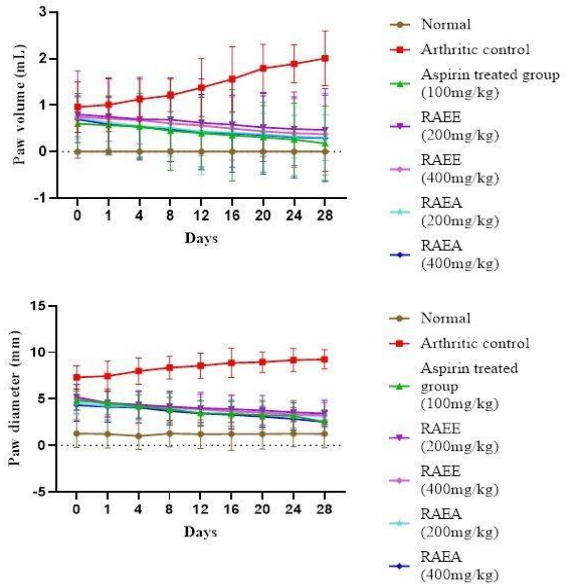

Figure 6: a) Effect of ethanolic extract and ethyl acetate fraction of *R alba* on paw volume in CFA induced arthritic model b) Effect of ethanolic extract and ethyl acetate fraction of *R alba* on paw diameter in CFA induced arthritic model

Body weight

This experiment showed that in comparison to the RAEE, RAEA and aspirin-treated groups, the rats in the arthritic control groups lost weight. On day 28, figure 7 showed increased body weight for RAEE and RAEA as compared to the arthritic control group.

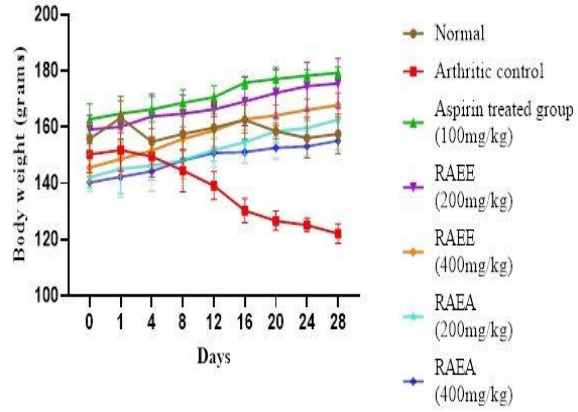

Figure 7: Effect of ethanolic extract and ethyl acetate fraction of *R alba* on body weight of rats

Hematological parameters

The blood samples of all groups were tested for blood factors, 28 days after CFA treatment to rats. The results were depicted in Figure 8.

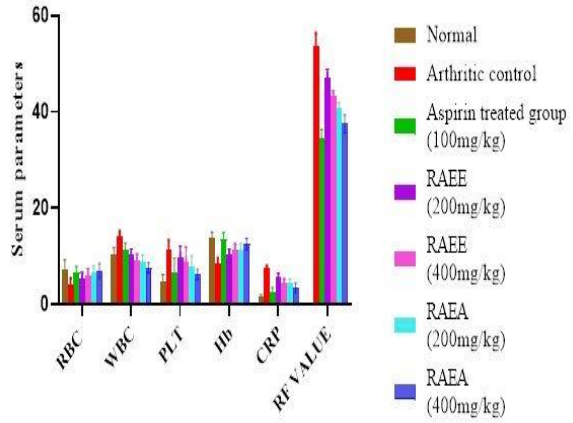

Figure 8: Effect of ethanolic extract and ethyl acetate fraction of *R alba* on hematological parameters in CFA induced arthritic model

Biochemical parameters

Aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotranferase (ALT) were measured in 28 days following CFA injection in all groups. The results were shown in Figure 9.

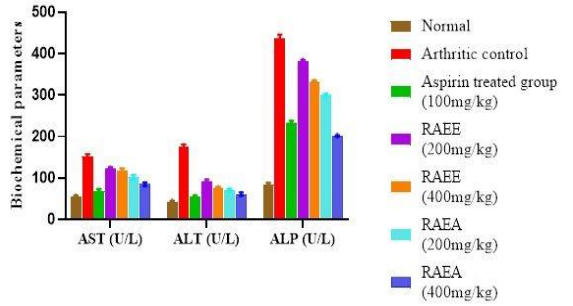

Figure 9: Effect of ethanolic extract and ethyl acetate fraction of *R alba* on biochemical parameters (AST, ALT and ALP) in CFA induced arthritic model

The effect of RAEE and RAEA on total protein and albumin/globulin (A/G) ratio also significantly ameliorated the value as shown in Figure 10.

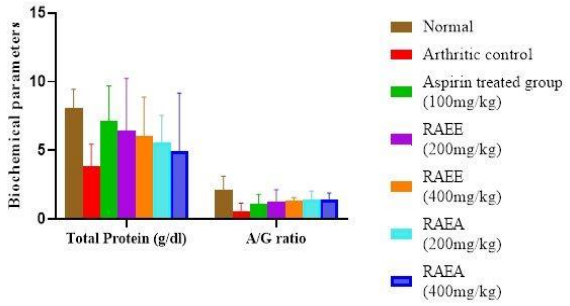

Figure 10: Effect of ethanolic extract and ethyl acetate fraction of *R alba* on biochemical parameters (total protein and A/G ratio) in CFA induced arthritic model

Interleukin-6(IL-6) was also estimated in picogram/ml (pg/ml). The result was shown in Figure 11.

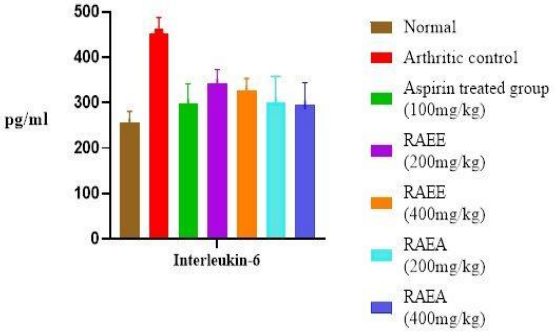

Figure 11: Effect of ethanolic extract and ethyl acetate fraction of *R alba* on biochemical parameters on biochemical parameters (interleukin) in CFA induced arthritic model

Histopathological analysis

In histopathological study, Group I epitomize integral synovial layer,absence of inflammation and bone necrosis, regular synovial cavity (Figure 12a). In the arthritic control group, deep invasion of inflamed cells, bone necrosis, amplified synovial vascularity, inflated synovial space with filtrates of plasma protein was seen (Figure 12b). Group III treated rats point up fortification together with bonemortificacion, lessened spreading of inflammatory cells, diminiaturesynovial vascularity, nonappearance of pannus along with fibrin as in (Figure 12c). Group IV exemplify bone mortificacion amid inflammatory cells, synovial gap, synovial hyperplasia and declined fibrin as in (Figure 12d). Group V showed shows necrosis of bone along with inflammatory cells, enlarged synovial gap, synovial hyperplasia and less fibrin deposit as in (Figure 12e). Group VI showed exemplified realistic consequences with diminished inflammatory cells as in (Figure 12f). Group VII showed typify worth protection along synovial

gap which congealed with reduced fibrin deposit as in (Figure 12g).

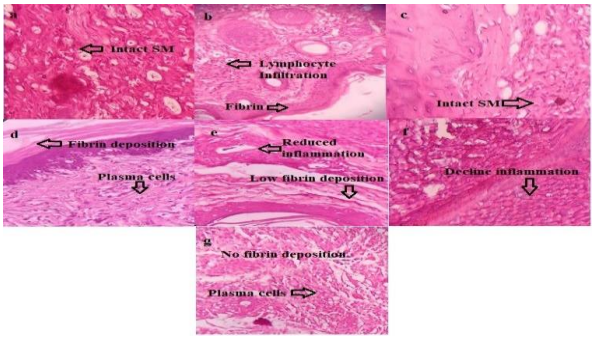

Figure12: Histopathological analysis of ank1e joint stained with haematoxylin-eosin after 28 days. Positive control (a) (b) Arthritic control (c) Aspirin control 100mg/kg b.w. (d) RAEE 200mg/kg b.w. (e) RAEE 400mg/kg b.w. (f) RAEA 200mg/kg b.w. (g) RAEA 400mg/kg b.w.(SM) synovial membrane

DISCUSSION

The plant *Rosa alba* mostly used in traditional medicine for ameliorating the symptoms associated with rheumatoid arthritis, but there are no scientific studies supporting the claimed use. Hence, the current experiment was accomplished to examine the antiarthritic effect of ethanolic extract and ethyl acetate fraction of the plant in arthritic model. Phytochemical studies and quantitative HPTLC analysis established the existence of diversified phytoconstituents including quercetin in ethyl acetate fraction of the plant (RAEA). The results on acute toxicity studies consider that *Rosa alba* is safe upto the maximum dose including 5000mg/kg hence two doses were selected viz 200mg/kg b.w and 400mg/kg b.w for the study. In acute arthritic models the animals were treated orally with the extract and its fractions. Paw swelling were estimated by using formaldehyde and turpentine induced arthritic models. Joint distension occurred as 5 histamin (5HT), kinins and prostaglandins induces arthritis by turpentine oil as inducermodel[19]. In this study inflammatory reactions retains including hyperamic and edematous reactionsuggesting formaldehyde as inducer[20]. Fallout was justified as RAEA at 400 mg/kg b.w. significantly dropped in joint enlargement.

CFA model extensively exemplifies[21] temperate cartilage destruction, bone resorption as studied in this experiment. Cytokines of both the type 1 T helper cells (Th-1) and T helper 17 cell(Th-17) phenotype suggested as indicator in the joint pathology. Taking into account, cytokines like tumor necrosis factor-alpha (TNF-α), IL-6, Interleukin-17A (IL-17A), Interleukin-21 (IL-21) and Interleukin-1β (IL-1β), IL-6 explored and signifies the chronic circumstances of the illness. Augmented release of TNFα and IL-1 by spleen cells implies declined body weight as previously explored[22] which justify diminished weight of rats in arthritic group. Limiting prostaglandin and impedition of cyclooxygenase enzyme by aspirin diminishespain[23]. Excess amount serum enzyme as ALP, ALT and AST in the serum, amplified by phagocytization of leukocytes discharges phosphatases[24]. Moving back, as level of albumin decreases hence more amount total protein in serum on account of augmented permeability of vascular tissues[25,26], RAEE and RAEA signified improved levels of globulin and low down levels of albumin designates recuperation of inflammatory situation in dose dependent manner. As per previous reports[27], anemic condition arouses since excess flow of interleukin 6 (IL6) releases hepcidin and iron cannot be librated by macrophages in the blood. The severity of rheumatoid arthritis governs the presence of Rheumatoid factor RF and augmented platelet count along with IL-6, IL-1β, Interleukin-4(IL-4) in serum that leads to pathologic thrombocytosis. A confirmed ameliorating inflammatory circumstances were odserved inn this study by increase in Hb, RBC count, decrease in RF and restoration of CRP, IL-6 and platelets levels. Elevated WBC level in arthritic rats were diminished in treated rats which designates recuperation of inflammatory situation.

Quercetin exemplifies as an anti-inflammatory and antiarthritic moiety from past many years[28]. In this study the extract and fraction of *R. alba* diminishes the elevated interleukin (IL-6) level suggesting decreased inflammatory reactions at molecular level.

Histopathological justification clarifies decreased tissue infiltration, improved texture of affected organs and tissues. This study limits in ascertaining the effect of quercetin in decreasing the manifestations associated with arthritis.

CONCLUSION

Rosa alba L. has potential antiarthritic effect in the presented experimental work. The possible effects on arthritis could probably be interrelated with the existence of flavonoid present and decrease in the interleukin (IL-6)

level in the ethyl acetate fraction treated animals. Hence this study supports the pharmacological facts to reported traditional state of the plant in the management of manifestations related to arthritis.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Animal experiments performed followed the ethical standards for the care and use of laboratory animals. The experimental protocol was approved by the Institutional Animal Ethical Committee in accordance with the "Guide for the Care and Use of Laboratory Animals" and ARRIVE guidelines. No human experiments were involved.

AVAILABILITY OF DATA AND MATERIAL

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

COMPETING INTERESTS

The authors report no financial or any other conflicts of interest in this work.

FUNDING STATEMENT

There is no funding to report

AUTHOR CONTRIBUTION

All authors made substantial contributions to the conception and design, acquisition of the data or analysis and interpretation of data ; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal ; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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