

Modulation of IL-6 in ameliorating arthritic conditions in rats by *Rosa alba* L flower extract and fraction

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SUMMARY

Introduction: 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. **Objective:** the objective of the study is to assess anti-arthritic potential of the plant *Rosa alba* L by considering CFA, formaldehyde and turpentine oil induced arthritic model. **Material and Method:** 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 (IL-6) were performed in blood and serum of wistar albino rat. Then histopathological studies had also been performed on rat hind paw joint. **Result:** 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. 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. **Conclusion:** Ameliorated hematological, biochemical and histopathological parameters confirmed the antiarthritic potential of the plant.

Keywords: Complete freund's adjuvant, histopathology, HPTLC, interleukin, quercetin.

RESUMEN

Modulación de IL-6 en la mejora de afecciones artríticas en ratas por extracto y fracción de flor de *Rosa alba* L

Introducción: artritis reumatoide caracterizada por inflamación y dolor articular, que afecta a millones de personas en todo el mundo. El sistema tradicional de medicina había demostrado ser preventivo y tratar enfermedades físicas y mentales. **Objetivo:** el objetivo del estudio es evaluar el potencial antiartrítico de la planta *Rosa alba* L considerando el modelo artrítico inducido por CFA, formaldehído y aceite de trementina. **Material y método:** el extracto etanólico y su fracción de acetato de etilo se consideraron para el estudio y la quercetina se estimó cuantitativamente mediante el método cromatográfico en capa fina de alto rendimiento (HPTLC). Además, se realizaron estudios hematológicos y bioquímicos (IL-6) en sangre y suero de rata albina wistar. A continuación, también se realizaron estudios histopatológicos en la articulación de la pata trasera de rata. **Resultado:** el cribado fitoquímico estimó la presencia de carbohidratos, compuestos fenólicos, flavonoides, fitoesteroles, aminoácidos y aceite fijo en el extracto etanólico y la fracción de acetato de etilo de la planta. Se encontró que la cantidad de quercetina en las flores de la planta era del 0,26% p / p por el método de HPTLC cuantitativo. El nivel de citocinas proinflamatorias, interleucina (IL-6), disminuyó considerablemente ($p < 0,001$) en el grupo de ratas tratado con fracción a 400 mg/kg de peso corporal considerando el modelo artrítico inducido por adyuvante de Freund (CFA) completo. **Conclusión:** la mejora de los parámetros hematológicos, bioquímicos e histopatológicos confirmó el potencial antiartrítico de la planta.

Palabras clave: Adyuvante completo de Freund, histopatología, HPTLC, interleucina, quercetina.

RESUMO

Modulação de IL-6 na melhora de condições artríticas em ratos por extrato e fração de flor de *Rosa alba* L

Introdução: artrite reumatóide caracterizada por inflamação e dor nas articulações, afetando milhões de pessoas em todo o mundo. O sistema tradicional de medicina provou ser preventivo e tratamento de doenças físicas e mentais. **Objetivo:** avaliar o potencial antiartrítico da planta *Rosa alba* L, considerando o modelo artrítico induzido por CFA, formaldeído e óleo de terebintina. **Material e Método:** o extrato etanólico e sua fração acetato de etila foram considerados para o estudo e a quercetina foi estimada quantitativamente pelo método de cromatografia em camada delgada de alta eficiência (HPTLC). Além disso, estudos hematológicos e bioquímicos (IL-6) foram realizados em sangue e soro de ratos wistar albinos. Em seguida, estudos histopatológicos também foram realizados na articulação da pata traseira do rato. **Resultado:** a triagem fitoquímica estimulou a presença de carboidratos, compostos fenólicos, flavonoides, fitoesteróis, aminoácidos e óleo fixo no extrato etanólico e na fração acetato de etila da planta. A quantidade de quercetina nas flores da planta foi de 0,26% p/p pelo método quantitativo de HPTLC. O nível de citocinas pró-inflamatórias, interleucina (IL-6) foi consideravelmente diminuído ($p < 0,001$) no grupo de ratos tratados com fração a 400 mg/kg b.w, considerando o modelo artrítico induzido por adjuvante completo de Freund (CFA). **Conclusão:** a melhora dos parâmetros hematológicos, bioquímicos e histopatológicos confirmou o potencial antiartrítico da planta.

Palavras-chave: Adjuvante completo de Freund, histopatologia, HPTLC, interleucina, quercetina.

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, disease modifying anti-rheumatic drugs (DMARD's), 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 which is medicinally and commercially used due to its oils and volatile products. The aromatic water made from the plant is used as a flavoring agent in the preparation of jam, cake, and 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 tricosane [12], tannins, ellagitannins, and flavonoids [13], aliphatic hydrocarbons, minerals, alcohols, aldehydes, monoterpenoids, sesquiterpene, 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 40 °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.

Drugs, chemicals and instruments

Complete Freund's adjuvant (CFA) was purchased from Sigma-Aldrich, USA. Aspirin was obtained from central drug house (CDH), New Delhi, as a gift sample. Sodium chloride, turpentine oil, formaldehyde and other chemicals and solvents taken are AR grade and were purchased from Merck specialties Pvt Ltd., Mumbai. The instru-

ments for experiment used were Plethysmometer (Orchid Scientific), Vernier caliper (Mumbai tool centre), CAMAG HPTLC system (Mutenz, Switzerland) comprising of Hamilton 100 μ l syringe, Linomat IV applicator, twin trough developing chamber (20 \times 20 cm).

Extraction and fractionation

The powdered material of dried flowers of *Rosa alba* (500 g) were macerated with petroleum ether to remove fatty substances; the marc was further exhaustively extracted with of 50% ethanol by continuous extraction process in Soxhlet assembly at 50 °C - 60 °C. 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 (RAAF) fractions were obtained and intensified considering rotavapor (Buchi, USA) at <400°C. The yield of ethyl acetate fraction was 40.6% w/w.

Preliminary phytochemical screening and TLC

RAEE and RAEA were tested for preliminary phytochemical screening and thin layer chromatography (TLC) considering Silica Gel 60 to be stationary phase by using standard procedures [16]. Under UV chamber at 254 nm, 365 nm and visible light the plates were experimented for spots considering Toluene: ethyl acetate: formic acid (8:1:1 v/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.

Characterization of ethanolic extract and its fraction

The ethanolic extract and ethyl acetate fraction were analyzed qualitatively by HPTLC fingerprinting method. 10 μ l of 50% ethanolic extract (RAEE) and its ethyl acetate fraction (RAEA) were spotted on HPTLC plates. The plates were developed at 580nm using mobile phase Toluene: ethyl acetate: formic acid (8:1:1 v/v/v).

Experimental animals

Young healthy male albino wistar rats weighing 180-210 g were kept in polypropylene cages for 12 h light and 12 h dark cycle at 25 \pm 2 °C in the animal facility of United Institute of Pharmacy, Allahabad. Prior the experiment all animals were acclimatized for five days and fed with standard laboratory pellet diet and water ad libitum. 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]. A single dose of the extract (5, 50, 300, 2000 and 5000 mg/kg) in 1% gum acacia was given orally by gavage to different group of animals (three each). The animals were regularly observed every hour during the first 12 h throughout the study period (14 days) for any abnormal changes.

Experimental induction of arthritis

Arthritis was induced intraplantarally in left hind paw after 30 min of drug/vehicle administration to all the rats. Suspensions of Standard drug aspirin, extract and its fractions were prepared in 1% w/v gum acacia and administered orally to rats. Formaldehyde solution (2% v/v) and Turpentine emulsion (10% v/v) were made in 0.9% saline.

Acute arthritic model

Seven groups of rats (n=6) were used for the study. Group I considered as positive control rats, given vehicle, Group II considered as arthritic control rats given 0.1 ml of 2% formaldehyde solution and 0.1 ml 10% v/v turpentine oil emulsion, Group III was arthritic rats treated with the standard drug aspirin at 100 mg/kg body weight, Group IV was arthritic rats treated with extract (RAEE) 200 mg/kg body weight, Group V was arthritic rats treated with extract (RAEE) 400 mg/kg body weight, Group VI was arthritic rats treated with fraction (RAEA) 200 mg/kg body weight, Group VII was arthritic rats treated with fraction (RAEA) 400 mg/kg body weight.

On day 0, 2, 4, 6, 8, and 10, the anti-arthritic activity of RAEE and RAEA was assessed on the injected paw using the parameters paw volume and joint diameter on formaldehyde induced arthritic model.

RAEE and RAEA evaluated for anti-arthritic activity by measuring paw volume and joint diameter every hour for up to 6 hours on turpentine induced arthritic model.

Chronic arthritic model

Complete Freund's adjuvant arthritic model

Anti-arthritic activity of RAEE and RAEA was evaluated on injected paw on the following parameters paw volume, joint diameter, body weight on day 0, 1, 4, 8, 12, 16, 20, 24, and day 28. Inference of various parameters were considered by withdrawing the blood by retro-orbital puncture on 28th day.

Hematological and serum parameters

Blood of the rats was withdrawn by retro-orbital puncture on 28th day. The collected blood samples were centrifuged at 2500 rpm for 15 min. The serum was collected in fresh serum tubes and stored in refrigerator (2-4 °C) after tightly capped and subjected to biochemical and hematological examination for AST, ALT, ALP levels, red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb), and platelets (PLT). Serum C-reactive protein (CRP), Rheumatoid factor (RF) and interleukin (IL-6) level. The parameters were also measured using diagnostic kits (Erba Lachema).

Histopathological analysis

The control as well as treated rats were sacrificed by using cervical dislocation and the ankle joint was removed, immediately fixed in bouin's fluid for 24 h and washed in running tap water to remove colour of bouin's fluid and dehydrated in alcohol embedded in paraffin and cut at 5 µm in a rotary microtome. The joint sections were then deparaffinized in xylene and stained with eosin-haematoxylin stain and viewed under 40X magnifications. 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 Graph-Pad Prism 9.1.2 software by applying two-way ANOVA with Newman-Keuls method. A $p < 0.001$ 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., 200 mg/kg and 400 mg/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 580 nm using Toluene: ethyl acetate: formic acid (8:1:1 v/v/v) as the

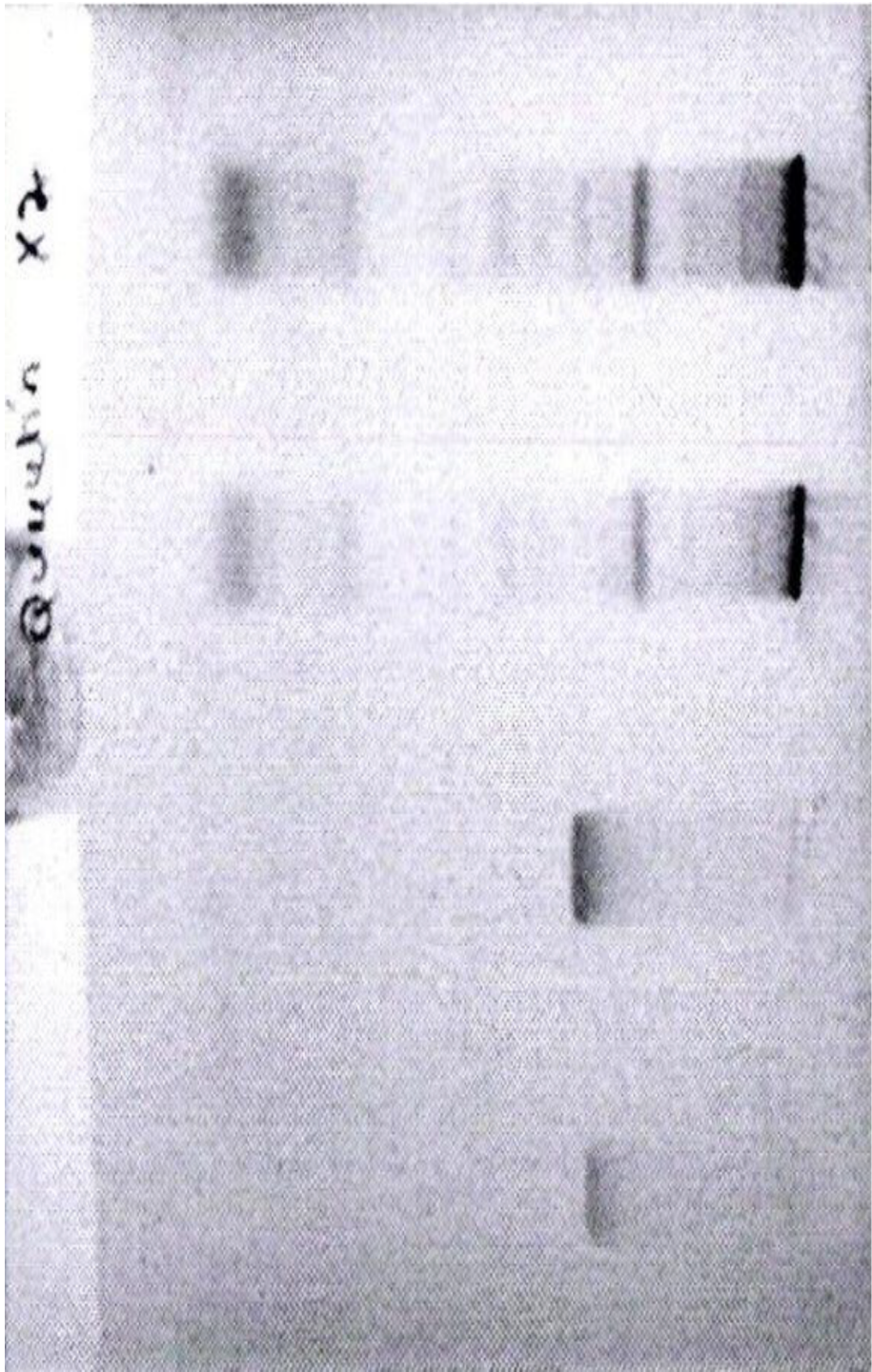


Figure 1. HPTLC fingerprinting plate of quercetin marker compound and ethyl acetate fraction (RAEA) of *Rosa alba* L.

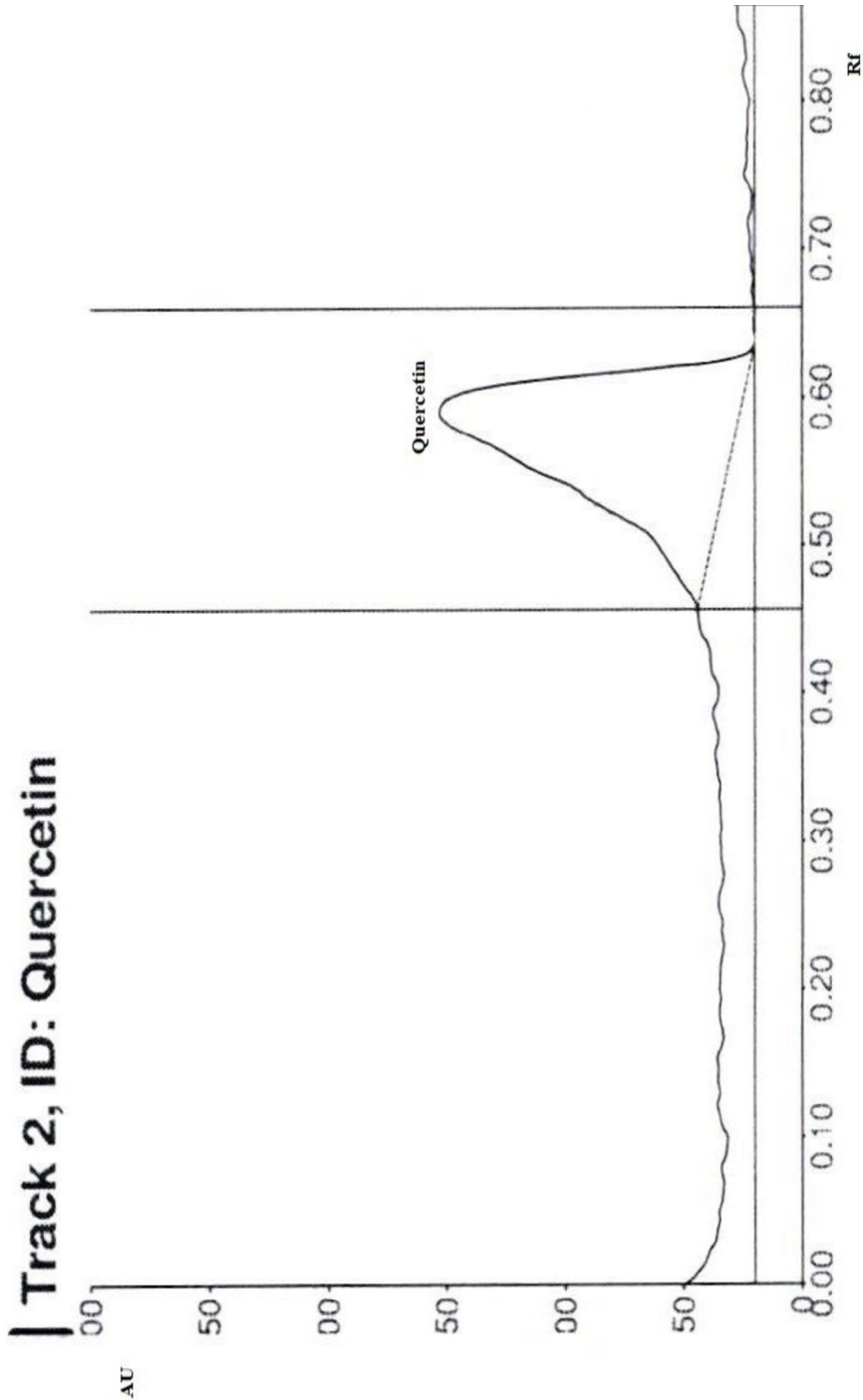


Figure 2. HPTLC densitogram of quercetin marker compound.

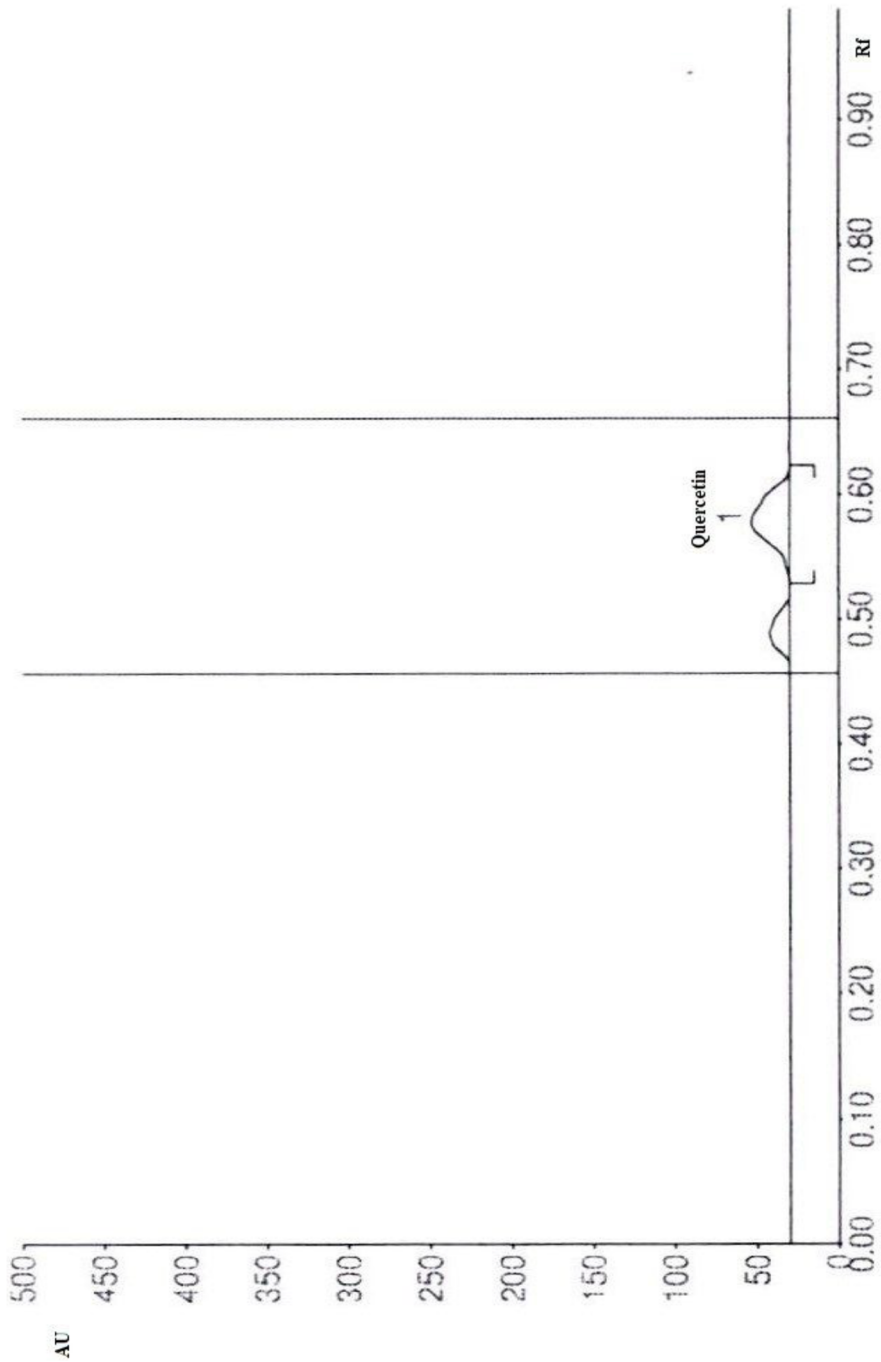


Figure 3. HPTLC densitogram of RAEA.

mobile phase. The HPTLC plate and densitograms were shown in Figures 1 to 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.

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 Fig. 4 after comparison with arthritic control.

Diseased control rats treated with formaldehyde had a significant ($p < 0.001$) rise in paw volume and diameter as matched to healthy rats. RAEA at 400mg/kg b.w and aspirin declined paw volume and diameter considerably ($p < 0.001$) from day 6 onwards matched to the disease control group. The alteration in paw volume of RAEE was (200 mg/kg; 0.37 ± 0.25 ; $p < 0.05$ and 400 mg/kg; 0.33 ± 0.40 ; $p < 0.01$), RAEA treated groups (200 mg/kg; 0.28 ± 0.43 ; $p < 0.01$ and 400 mg/kg; 0.22 ± 0.53 ; $p < 0.001$) was evident as compared to arthritic control (0.87 ± 0.16 ; $p < 0.001$) (Fig. 4a on day 10).

The variation in paw diameter of RAEE group (200 mg/kg; 3.57 ± 0.77 ; $p < 0.001$ and 400 mg/kg; 3.31 ± 0.58 ; $p < 0.001$) and for RAEA group (200 mg/kg; 3.23 ± 0.43 ; $p < 0.001$ and 400 mg/kg; 3.07 ± 0.44 ; $p < 0.001$) was obvious as compared to arthritic control (6.95 ± 0.48 ; $p < 0.001$) (Fig. 4b on day 10).

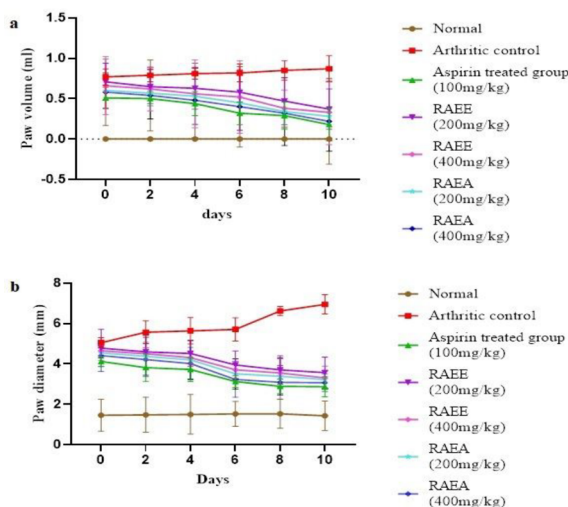


Figure 4. Effects of RAEE and RAEA paw volume and paw diameter in formaldehyde induced arthritic model.

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 Fig. 5. The alteration in paw volume of RAEE (200 mg/kg; 0.39 ± 0.42 ; $p<0.01$ and 400 mg/kg; 0.34 ± 0.45 ; $p<0.001$) and RAEA treated groups (200 mg/kg; 0.31 ± 0.81 ; $p<0.001$ and 400 mg/kg; $p<0.001$) was noticeable as compared to arthritic control (0.81 ± 0.35 ; $p<0.001$) on 6 hr in Fig. 5a. The variation in paw diameter of RAEE and RAEA treated groups (200 mg/kg; 4.51 ± 0.48 ; $p<0.001$ and 400 mg/kg; 4.31 ± 0.44 ; $p<0.001$) and (200 mg/kg; 4.19 ± 0.94 ; $p<0.001$ and 400 mg/kg; 3.88 ± 0.96 ; $p<0.001$) respectively was evident as compared to arthritic control (7.89 ± 0.69 ; $p<0.001$) on 6 hr in Fig. 5b.

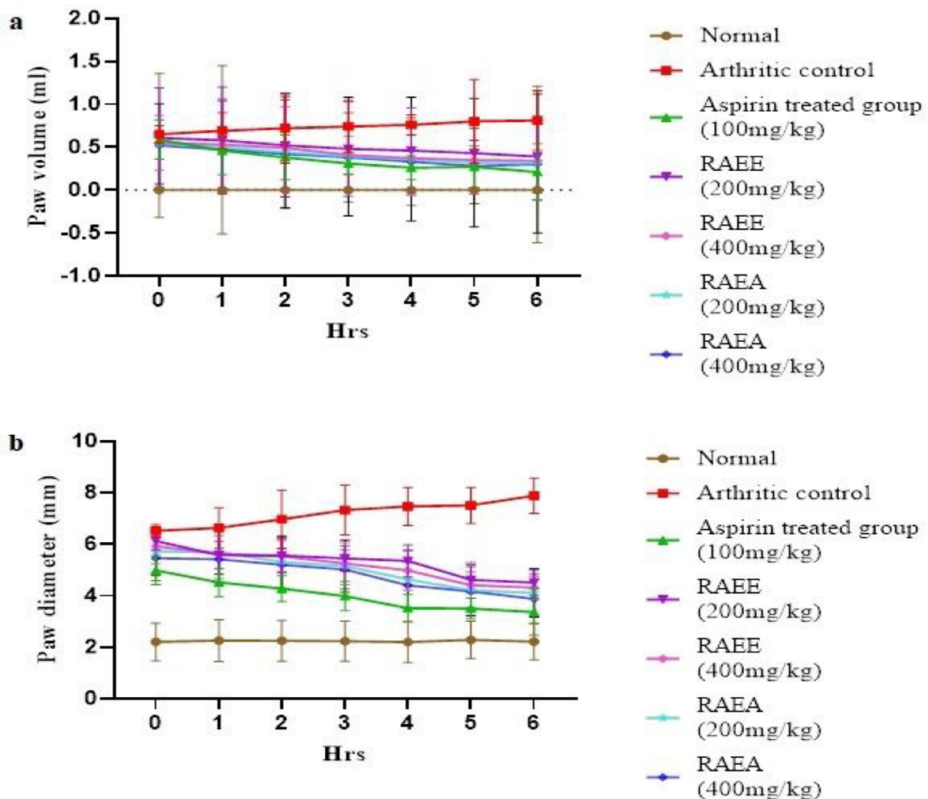


Figure 5. Effects of RAEE and RAEA paw volume and paw diameter in turpentine induced arthritic model.

Effect of RAEE and RAEA in CFA induced arthritic model

Paw swelling

As demonstrated in Fig. 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. When compared to the arthritic control group, rats given RAEE and RAEA, as well as aspirin, have significant ($p<0.001$) reduced paw volume and diameter by day 16 headlong. The alteration in paw volume of RAEE & RAEA treated groups (200 mg/kg; 0.47 ± 0.89 ; $p<0.001$ and 400 mg/kg; 0.38 ± 0.88 ; $p<0.001$) and (200 mg/kg; 0.30 ± 0.49 ; $p<0.001$ and 400 mg/kg; 0.29 ± 0.93 ; $p<0.001$) respectively was evident as compared to arthritic control (2.01 ± 0.59 ; $p<0.001$) on day 28 as shown in Fig. 6a.

The variation in paw diameter of RAEE and RAEA treated groups (200 mg/kg; 3.45 ± 1.37 ; $p<0.001$ and 400 mg/kg; 3.26 ± 1.66 ; $p<0.001$) and (200 mg/kg; 3.07 ± 1.87 ; $p<0.001$ and 400 mg/kg; 2.99 ± 1.20 ; $p<0.001$) respectively was evident as compared to arthritic control (9.25 ± 1.03 ; $p<0.001$) on day 28 as shown in Fig. 6b.

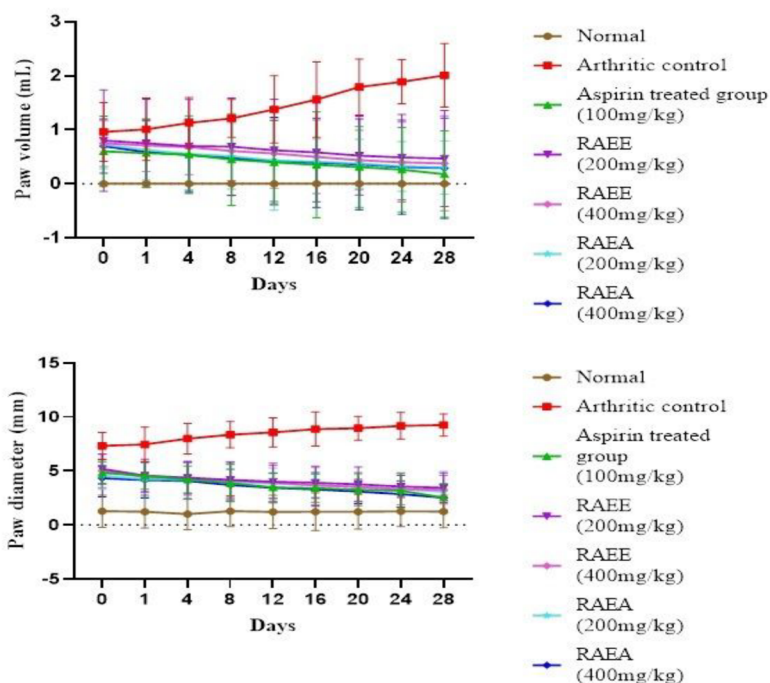


Figure 6. Effects of RAEE and RAEA paw volume and paw diameter in CFA induced arthritic model.

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. The body weight of RAEE and RAEA treated groups (200 mg/kg; 175.50 ± 5.89 ; $p < 0.001$ and 400 mg/kg; 167.83 ± 4.26 ; $p < 0.001$ and 200 mg/kg; 162.66 ± 5.71 ; $p < 0.001$ and 400 mg/kg; 155.16 ± 3.48 ; $p < 0.001$) respectively was obvious as compared to arthritic control group (122.16 ± 3.48 ; $p < 0.001$) on day 28. Figure 7 showed increased body weight for RAEE and RAEA as compared to the arthritic control group.

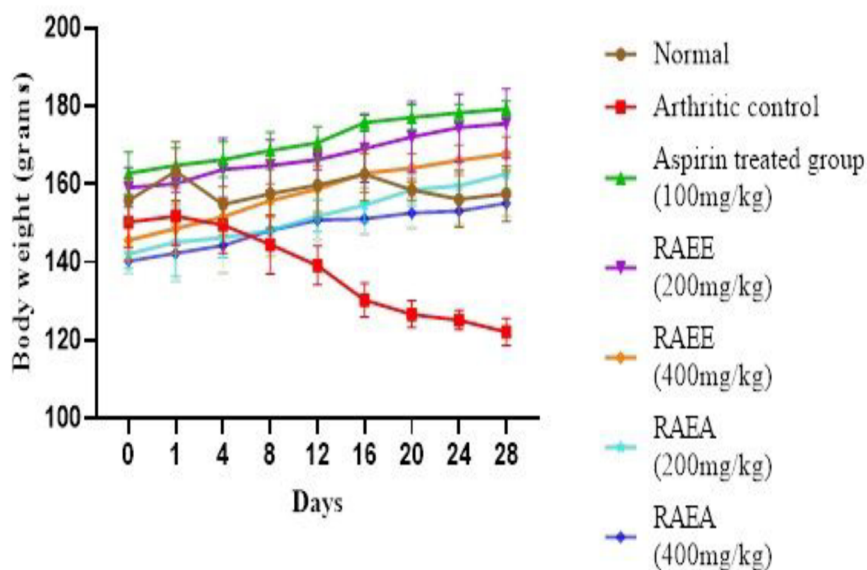


Figure 7. Effect of RAEE and RAEA on body weight in CFA induced arthritic model.

Hematological parameters

The blood samples of all groups were tested for blood factors, 28 days after CFA treatment to rats. White blood cells (WBC) (10.44 ± 1.31 - 14.22 ± 1.11), Platelet count (4.83 ± 1.35 - 11.43 ± 1.99), C-reactive proteins (CRP) levels (1.46 ± 0.51 - 7.54 ± 0.52), and (Rheumatoid factor) RF value (0.00 ± 0.00 - 53.50 ± 2.88) increased in the CFA treated group, whereas hemoglobin (13.93 ± 1.13 - 8.38 ± 1.28) and Reb blood cells (RBC) count (7.35 ± 1.86 - 3.99 ± 1.40) decreased. In the lead with arthritic control group, Group III were observed with diminution WBC as (14.22 ± 1.11 - 11.41 ± 1.33 ; $p < 0.05$), platelet count (11.43 ± 1.99 - 6.66 ± 2.94 ; $p < 0.001$), CRP levels (7.54 ± 0.52 - 2.67 ± 1.07 ; $p < 0.001$), RF value (53.50 ± 2.88 - 34.50 ± 1.87 ; $p < 0.001$) but improved Hemoglobin (Hb) value as

(8.38 ± 1.28 - 13.56 ± 1.35 ; $p < 0.001$) and RBC value as (3.99 ± 1.40 - 6.55 ± 1.32 ; $p < 0.001$). The group experimented with fraction at 400 mg/kg b.w likely to be observed with substantial rise in RBC value as (3.99 ± 1.40 - 6.95 ± 1.55 ; $p < 0.001$) and hemoglobin (8.38 ± 1.28 - 12.55 ± 1.18 ; $p < 0.001$) but dwindled WBC (14.22 ± 1.11 - 7.53 ± 1.05 ; $p < 0.001$), platelet value (11.43 ± 1.99 - 6.22 ± 1.06 ; $p < 0.001$), CRP value (7.54 ± 0.52 - 3.46 ± 0.98 ; $p < 0.001$) and Rf (53.50 ± 2.88 - 37.50 ± 1.87 ; $p < 0.001$) besides with group experimented with fraction at 200 mg/kg b.w in RBC value (3.99 ± 1.40 - 6.54 ± 1.44 ; $p < 0.001$), haemoglobin (8.38 ± 1.28 - 11.35 ± 1.24 ; $p < 0.05$), WBC (14.22 ± 1.11 - 8.88 ± 1.39 ; $p < 0.001$), platelet count (11.43 ± 1.99 - 7.82 ± 2.25 ; $p < 0.01$), CRP value (7.54 ± 0.52 - 4.26 ± 0.96 ; $p < 0.001$) and RF (53.50 ± 2.88 - 40.66 ± 1.21 ; $p < 0.001$). The group experimented with extract at 400 mg/kg b.w. anticipated with less variation in RBC value (3.99 ± 1.40 - 6.03 ± 1.40 ; $p < 0.001$), hemoglobin (8.38 ± 1.28 - 11.38 ± 1.21 ; $p < 0.05$), WBC (14.22 ± 1.11 - 9.04 ± 1.37 ; $p < 0.001$) Platelet count (11.43 ± 1.99 - 8.78 ± 3.17 ; $p < 0.05$), CRP value (7.54 ± 0.52 - 4.43 ± 0.76 ; $p < 0.001$) and RF (53.50 ± 2.88 - 43.16 ± 1.16 ; $p < 0.001$) besides with the group experimented with 200 mg/kg b.w in RBC value (3.99 ± 1.40 - 5.31 ± 1.30 ; $p < 0.05$), hemoglobin (8.38 ± 1.28 - 10.21 ± 1.23 ; $p < 0.05$), WBC (14.22 ± 1.11 - 10.46 ± 1.02 ; $p < 0.001$), platelet count (11.43 ± 1.99 - 9.88 ± 2.12 ; $p < 0.001$), CRP value (7.54 ± 0.52 - 5.53 ± 0.94 ; $p < 0.001$) and RF (53.50 ± 2.88 - 47.16 ± 1.72 ; $p < 0.001$). The results were depicted in Fig. 8.

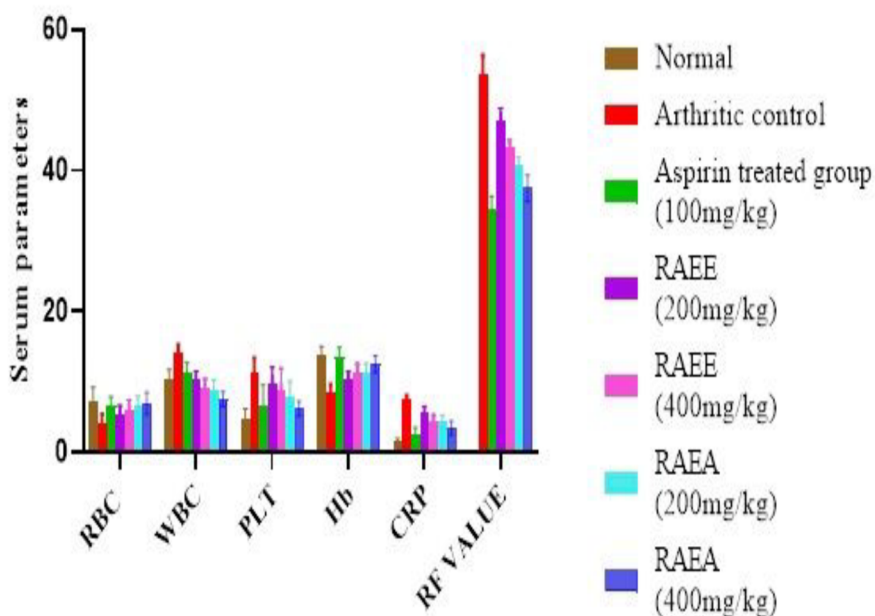


Figure 8. Effect of RAEE and RAEA on hematological parameters in CFA induced arthritic model.

Biochemical parameters

Aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) were measured in 28 days following CFA injection in all groups. The CFA-induced rat had substantially higher AST (55.00 ± 2.19 - 152.00 ± 5.54), ALT (42.97 ± 1.79 - 176.33 ± 4.50), and ALP (82.50 ± 5.75 - 437.78 ± 8.16). The group experimented with aspirin anticipated diminished levels of serum enzyme marker besides with arthritic control as values to be, AST (152.00 ± 5.54 - 68.33 ± 4.96 ; $p < 0.001$), ALT (176.33 ± 4.50 - 54.56 ± 0.53 ; $p < 0.001$), ALP (437.78 ± 8.16 - 233.16 ± 4.57 ; $p < 0.001$). Upon experimentation with extract at 200 mg/kg b.w. the levels of AST (152.00 ± 5.54 - 123.16 ± 2.92), ALT (176.33 ± 4.50 - 91.22 ± 4.78), ALP (437.78 ± 8.16 - 383 ± 2.36 ; $p < 0.05$) decreases. Experimentation with extract at 400 mg/kg b.w. AST (152.00 ± 5.54 - 118.33 ± 5.31 ; $p < 0.05$), ALT (176.33 ± 4.50 - 75.00 ± 3.52 ; $p < 0.05$), ALP (437.78 ± 8.16 - 332.33 ± 3.07 ; $p < 0.001$) were also decreases. Noteworthy variations were seen in experimented levels by Group VII, AST (152.00 ± 5.54 - 85.33 ± 4.13 ; $p < 0.001$) ALT (176.33 ± 4.50 - 61.28 ± 0.81 ; $p < 0.001$), ALP (437.78 ± 8.16 - 201.83 ± 1.83 ; $p < 0.001$) as compared with Group VI, AST (152.00 ± 5.54 - 101.66 ± 5.38 ; $p < 0.01$), ALT (176.33 ± 4.50 - 71.10 ± 2.64 ; $p < 0.01$), ALP (437.78 ± 8.16 - 299.66 ± 4.03 ; $p < 0.001$). The results were shown in Fig. 9.

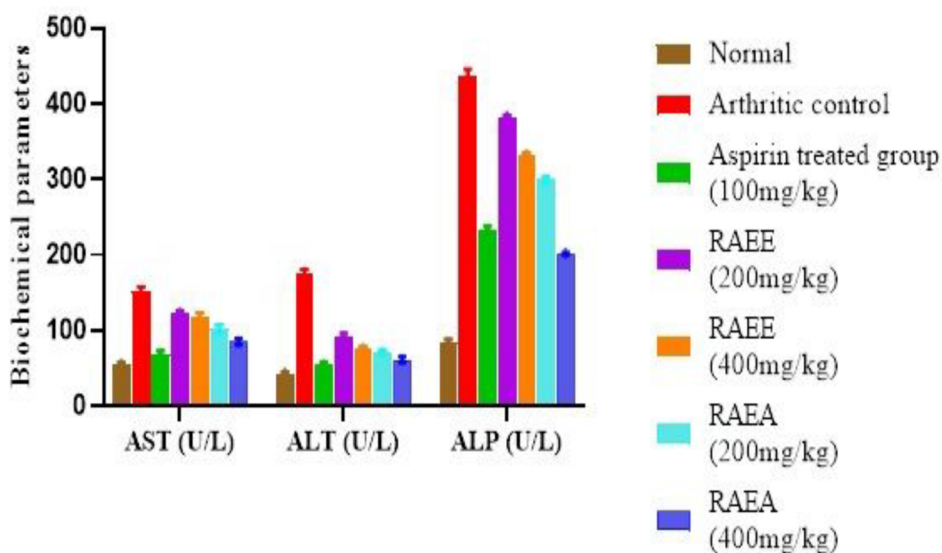


Figure 9. Effect of RAEE and RAEA on biochemical parameters in CFA induced arthritic model.

The effect of RAEE and RAEA on total protein and albumin/globulin (A/G) ratio also significantly ameliorated the value as shown in Fig. 10. For total protein the value for RAEE at 200 mg/kg was (6.45 ± 3.78) and at 400 mg/kg (6.01 ± 2.87) and for RAEA at 200 mg/kg (5.58 ± 1.96) and at 400 mg/kg (4.95 ± 4.23). For A/G ratio the value for RAEE at 200 mg/kg was (1.25 ± 0.88) and at 400 mg/kg (1.34 ± 0.22) and for RAEA at 200 mg/kg (1.39 ± 0.66) and at 400 mg/kg (1.45 ± 0.44).

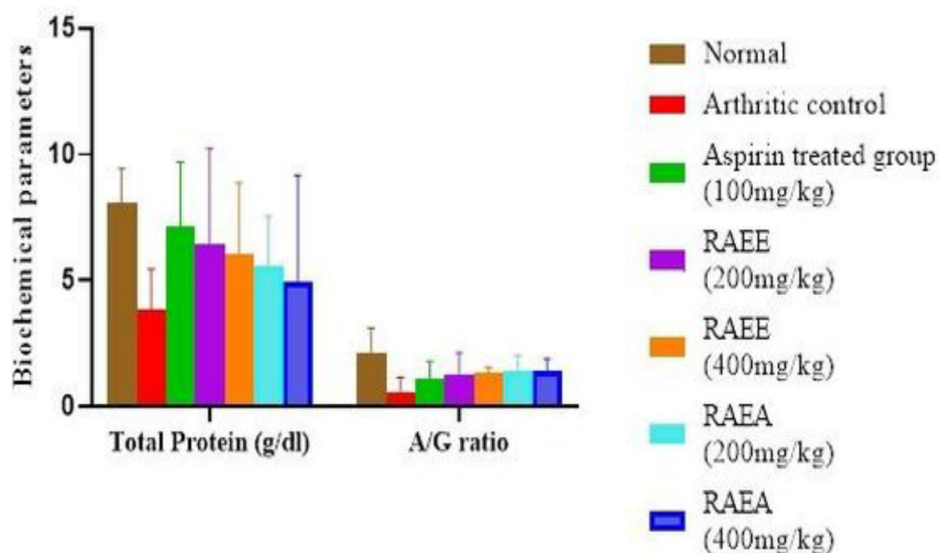


Fig. 10. Effect of RAEE and RAEA on biochemical parameters in CFA induced arthritic model.

Interleukin-6 (IL-6) was also estimated in picogram/ml (pg/ml) and the value for group II was (452.00 ± 35.54 ; $p < 0.001$) which was significantly decreased for RAEA treated group at 400 mg/kg (295.33 ± 49.13 ; $p < 0.001$) as compared to RAEE at 400 mg/kg (328.33 ± 25.31 ; $p < 0.05$). The result was shown in Fig. 11.

Histopathological analysis

In histopathological study, Group I epitomize intact synovial layer, no inflammation and no necrosis of bone, regular synovial cavity (Fig. 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 (Fig. 12b). Group III treated rats point up fortification together with necrosis of bone, lessened incursion of inflammatory cells, diminutire synovial vascularity, absence of pannus creation and

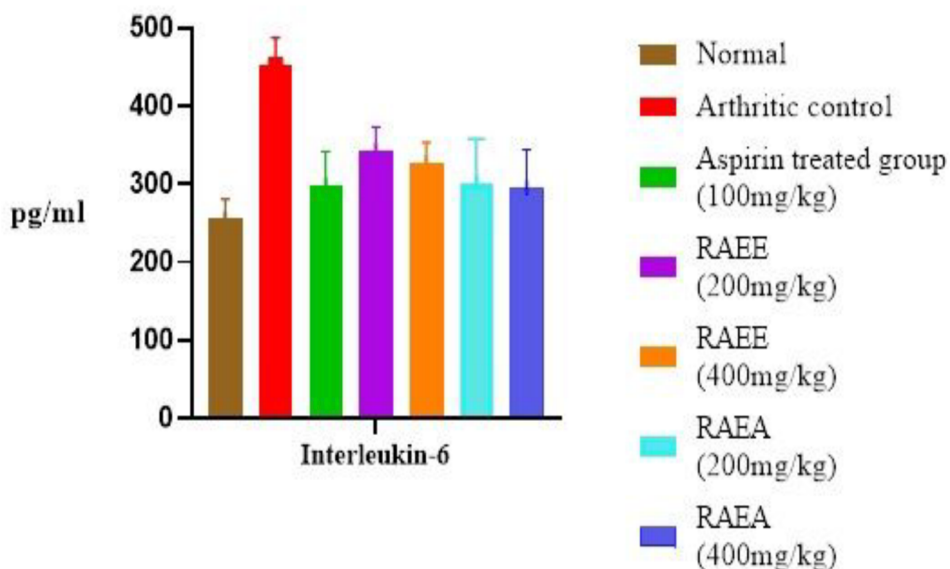


Figura 11. Effect of RAEE and RAEA on biochemical parameters interleukin in CFA induced arthritic model.

fibrin set down as in (Fig. 12c). Group IV exemplify necrosis of bone with inflammatory cells, synovial space, synovial hyperplasia and decreased fibrin deposit as in (Fig. 12d). Group V showed shows necrosis of bone along with inflammatory cells, enlarged synovial gap, synovial hyperplasia and less fibrin deposit as in (Fig. 12e). Group VI showed illustrated reasonable effect with diminished inflammatory cells as in (Fig. 12f). Group VII showed typify worth protection along synovial gap which congealed with reduced fibrin deposit as in (Fig. 12g).

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 5000 mg/kg hence two doses were selected

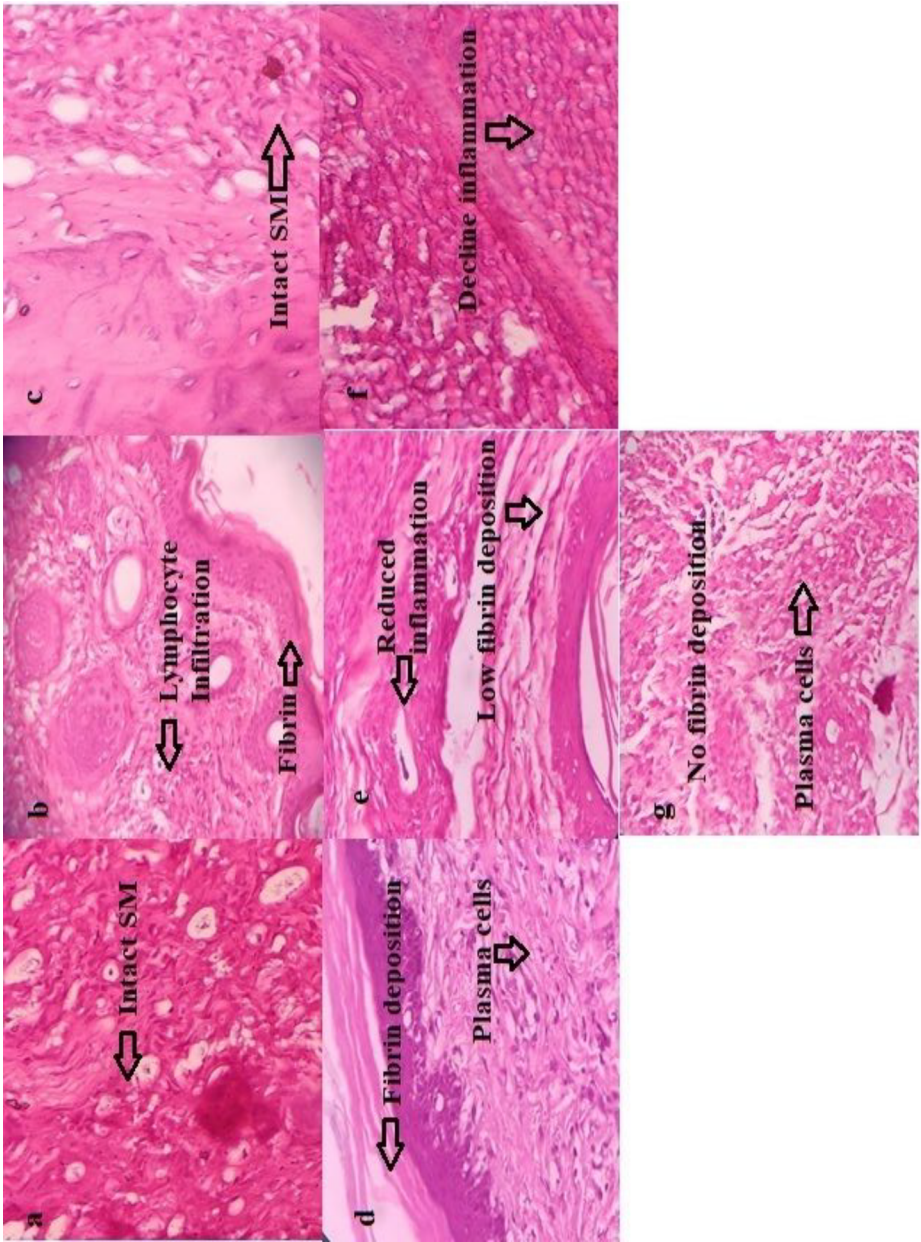


Figure 12. Histopathological analysis of ankle joint stained with haematoxylin-eosin after 28 days.

viz 200 mg/kg b.w and 400 mg/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. Turpentine induced model has been considered for antiarthritic activity since joint distension was observed due to mediators which includes 5 histamin (5HT), kinins, histamine and prostaglandins [18]. In this study these mediators might have provoked inflammatory reaction which retains for first few hours. Hyperemic and edematous response by formaldehyde is slower and chronic initially which spreads all over the joint [19]. Hence the study includes first and third day induction of arthritis by formaldehyde in the experimental animals. Results of the study justified that ethyl acetate fraction of *R. alba* at 400 mg/kg b.w. were the basis of significant drop in joint swelling in both acute models.

CFA induced arthritic model confirms RA since it resembles human pathophysiology [20] hence considered for this study as the joint pathology seen in adjuvant induced arthritis shows mild cartilage destruction, bone resorption as resemble with human RA. In this study quick, steadfast and easily reckonable polyarthritis was seen after injecting adjuvant first time. Cytokines of both the type 1 T helper cells (Th-1) and T helper 17 cell (Th-17) phenotype were believed as marker in the joint pathology. Amongst the cytokines secreted by Golgi pathway like tumor necrosis factor-alpha (TNF- α), IL-6, Interleukin-17A (IL-17A), Interleukin-21 (IL-21) and Interleukin-1 β (IL-1 β), IL-6 has been studied for the exploration of significant levels in chronic state of the disease.

Previous reports see the sights that inflammation, decreased glucose absorption, condensed intake of food and augmented release of TNF α and IL-1 by spleen cells were accountable for reduced body weight [21] hence in this study, there is a decline in the weight of rats in chronic model in arthritic control group. Ito *et al.* (2001) [22] suggests restraining of prostaglandin level and obstruct cyclooxygenase enzyme by aspirin thereby diminishing pain. Excess amount of prostaglandins have an effect on bradykinin or histamine which provokes inflammation. In this study aspirin was considered as standard drug for arthritic pain and swelling. Induction of arthritis by CFA causes mutilation in liver and kidney function and assessed by taking into consideration of larger amount serum enzyme as ALP, ALT and AST in the serum. Arthritis intended to cause discharge of chemotactic agents which pull leukocytes towards itself because of complement fixation. The ALP, ALT and AST levels in serum were augmented by phagocytization of leukocytes which releases phosphatases. Moreover bone type ALP resulting by synovial tissue may be accountable for amplified ALT and AST and ALP levels in serum [23]. In this study CFA induced arthritic model shows increased level of ALP, AST and ALT hence caused inflammation. Report claimed that arthritis is linked with decrease in plasma albumin and increase in plasma globulin on account of amplified permeability of vascular tissues to albumin as production of histamine, brad-

ypkinin and prostaglandins causes diminishing level of albumin [24]. In this study high levels of globulin and low levels of albumin in extract and fraction treated group indicates recovery of inflammatory condition. Low A/G ratio indicates overproduction of globulin and less production of albumin which indicates higher amount of total protein in the serum as discussed [25]. High A/G ratio in this study indicates recovery of inflammatory condition in extract and fraction treated groups in dose dependent manner.

In arthritic condition RBC count, WBC count, Hb, platelets, RF and CRP were important tool. As per the previous report [26], illustrates that excess flow of interleukin 6 (IL6), arouses release of hepcidin from liver cells and exerts reticence effect on liberation of iron by macrophages. These events will confiscate supply of iron to erythropoiesis grounds anemia. Rheumatoid factor RF, was taken into account to get the information about immune response and so considers as marker in RA count. Depending upon the severity of rheumatoid arthritis there is an increase in platelet count. In RA thrombopoetin level is increased in acute phase while apart from the thrombopoetin different cytokines like IL-6, IL-1 β , Interleukin-4 (IL-4) were also increased and correlated with the severity of disease which leads to pathologic thrombocytosis. In this study an increase in Hb, RBC count, decrease in RF and restoration of CRP, IL-6 and platelets showed ameliorating inflammatory condition. Augmented levels of neutrophils allied with interleukin increases WBC and acute phase proteins along with CRP in adjuvant arthritis. In this study rise in WBC count was observed in arthritic group but in extract and treated groups WBC count decreases which indicates improvement in the inflammatory condition.

Quercetin exemplifies as an anti-inflammatory and antiarthritic moiety from past many years [27]. In this study the extract and fraction of *R. alba* diminishes the elevated interleukin (IL-6) level suggesting decreased inflammatory reactions at molecular level.

Histopathology of organ signifies the texture of affected organs and tissues related to the disorder. It also justifies the toxic effect of test drugs on rodents. In this study, the tissue infiltration in extract and fraction treated groups decreased hence improvement in the arthritic conditions. 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 claim of the plant in the management of manifestations related to arthritis.

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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CONFLICTS OF INTERESTS

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

ETHICAL APPROVALS

Animal experiments performed followed the ethical standards for the care and use of laboratory animals. The experimental protocol was approved by the Institutional Ani-

mal Ethical Committee in accordance with the “Guide for the Care and Use of Laboratory Animals” and ARRIVE guidelines.

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