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EVALUATION OF ANTI-ARTHRITIC, ANTI-INFLAMMATORY, AND ANTIOXIDANT ACTIVITY OF POLYHERBAL FORMULATION IN SPRAGUE DAWLEY RATS

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Keywords:

Arthritic, Polyherbal formulation, Oxidative stress, Anti-inflammatory

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ABSTRACT: Objective: This work aims to evaluate in *in-vivo* the Antiarthritic and Anti-inflammatory activities and *in-vitro* antioxidant activity on Poly Herbal Formulation in rats. **Method:** Arthritis is a disorder of change in joint architecture and ligament degeneration. In this polyherbal formulation, medicinal plants were *Zingiber officinale* (33.931mg), *Piper nigrum* (33.931), *Piper longum* (33.931mg), *Spergularia daemia* (30 mg), *Delonix elates* (30 mg), *Acalypha indica* (30 mg), and *Carum copticum* (101.79 mg), which are well-known plants throughout India. They are commonly used to treat various disorders like cancer, diabetes, arthritis, rheumatoid arthritis, atherosclerosis, and chronic inflammatory disorders. The quality of finished products was evaluated as per the World Health Organization's guidelines for the quality control of herbal materials. Arthritis and inflammation were induced in both male and female Sprague-Dawley rats. Freund's complete adjuvant (FCA) is used for antiarthritic effect. Carrageenan-induced is used for the anti-inflammatory effect of polyherbal formulation (Rheumatigo) was studied at doses of 250 and 500 mg/kg and the effects were compared with prednisolone (10 mg/kg) and diclofenac (20 mg/kg). Blood samples were collected for hematological analysis at the end of the study. The histopathological analysis was carried out before terminating the study. DPPH and H2O2 methods are used in polyherbal formulations having antioxidant properties and IC50 is known. **Results:** Polyherbal formulation showed significant antiarthritic activity at 250 and 500 mg/kg, respectively, and this effect was comparable with Prednisolone's. The antiarthritic activity of polyherbal formulation is supported by hematological analysis. **Conclusion:** The polyherbal formulation showed significant antiarthritic activity in both male and female rats.

INTRODUCTION: According to WHO, 80% of the earth's population uses herbal medicines for their primary health care requirements. These herbal plants' key therapeutic components are known for their individual this herbal consist of phytochemicals which are responsible therapeutic measures today; these herbal medications have been officially recognized as alternative therapeutic systems of health and have been divided into specific departments by the government of India namely, Ayurveda, Unani, Siddha, Homeopathy, and Naturopathy [1].

More than 2500 plant species are now used to create herbal medicines in India. The use of conventional medications as folk remedies dates back more than three thousand years remedies as preparations are called pharmaceuticals [2]. Nowadays, the usage of traditional medicines has rapidly increased for the prevention and cure of many deadly diseases like Cancer, AIDS, Diabetes mellitus, Rheumatoid arthritis, *etc*, In the drug

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discovery process, medicinal plants play a vital role as 80% of the synthetic drugs are being derived from medicinal plants. Polyherbal formulations combine two or more herbal drugs to evolve different pharmacological effects. Combining herbs with different pharmacological effects leads to a synergistic effect and can treat different diseases [11].

An example of this is evaluating polyherbal formulation in Unani for its antiarthritic activity using rat arthritic models caused by formaldehyde and CFA. Inflammation is caused due to the defense activities of the immune system. It is connected to discomfort, erythema, and edema. Usually, conventional medicines are used for the treatment of inflammation. Conventional medicine like NSAIDS is the most common medicine used for treatment. As all of us know that the usage of conventional medicine leads to severe noxious effects like hepatic damage, deposition of urate crystals in the liver, kidney, heart, and spleen, *etc* and causes gastrointestinal effects like bleeding, ulceration, *etc*, conventional medicines are also very expensive, and the normal people cannot afford them.

However, the ability to use oxygen has given humans the advantage of metabolizing lipids, carbs, and proteins for energy. Costs are associated with it. Being a highly reactive atom, oxygen can form potentially harmful compounds known as free radicals or reactive oxygen species (ROS). Free radicals can destroy healthy cells in the body. Reactive oxygen species (ROS) cause cell membrane disruption by oxidizing polyunsaturated fatty acids in lipoproteins and cell membranes via metal ion-dependent hydroxyl radical production. [3]. A chronic systemic inflammatory illness known as rheumatoid arthritis (RA) causes nonspecific inflammation of the peripheral joints, articular tissue loss, and joint abnormalities. It has been found that the inflammatory cytokines, including tumour necrosis factor (TNFcanL- 1), and IL-6, are important for joint injury and inflammation throughout the progression of RA.

MATERIALS AND METHODS:

Materials:

Plant Material: The above-mentioned plants were obtained, the desired parts were dried, and the extract was prepared. Aravindh Herbals, Rajapalayam, Tamil Nadu, supplied the powdered extract. The extract was taken in two doses; the low doses were 250 mg/kg body weight and the high doses were 500 mg/kg body weight.

TABLE 1: COMPOSITION OF RHEUMATIGO OIL

S. no.	Plant name	value
1	*Zingiber officinale*	33.931mgs
2	*Piper nigrum*	33.931mgs
3	*Piper longum*	33.931mgs
4	*Carum copticum*	101.713mgs
5	*Dalmia Extensa*	101.713mgs
6	*Delonix elata*	30.000mgs

The above-mentioned plants were obtained, the desired parts were dried, and the extract was prepared. Aravindh Herbals, Rajapalayam, Tamil Nadu, supplied the powdered extract. The extract was taken in two doses; the low doses were 250 mg/kg body weight and the high doses were 500 mg/kg body weight.

Chemicals: Freund's complete adjuvant (FCA), Carrageenan, Water, DPPH, Methanol, and Hydrogen peroxide.

Drugs: Prednisolone -10mg/kg -mfg. from Pfizer ltd. Diclofenac - 20 mg/kg- mfg. from Hindustan chemicals and pharmaceuticals

Instruments: Plethysmometer, Vernier Calipers, UV Visible spectrophotometer

Animals:

Experimental Animals: Sprague Dawley rats of either sex weighing 150 – 180 grams were procured from Mahaveer Enterprises, Hyderabad. These animals were kept in proper housing with well-equipped and ventilated rooms having 12 hours dark/light cycle at Vijaya Institute of Pharmaceutical Sciences for Women (Vijayawada, India). The animals were fed a standard feed. pellet diet was also procured from Mahaveer Enterprises, Hyderabad, and had free access to distilled water except before experimentation. The Vijaya Institute OF Pharmaceutical Sciences for Women, Andhra Pradesh approved the study. CPCSEA and Institutional Animal Ethical Committee (VIPW/IAEC/1581/PO/RE/S/11/CPCSEA/Mph/0003/2021-2022) for this study. All the experimental animal procedures were carried out as per the Committee for Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Animal Models: Healthy Sprague Dawley rats of either sex weighing between 180-240 grams were selected and maintained indistinguishable conditions. They were housed in colony cages with free access to air, food, and water, except while undergoing experimentation. All the experiments were performed between the period of 10 am and 4 pm at room temperature in a noise-less well-ventilated laboratory.

METHODOLOGY:

Assessment of Anti-arthritic Activity: Grouping of animals: Each group consists of six animals of either sex and is divided into 5 groups.

Group 1: Control group treated with water.

Group 2: Diseased group induced with arthritis and treated with water. Group 3: Standard group treated with Prednisolone (10 mg/kg).

Group 4: Test-1 group treated with Rheumatigo extract (250 mg/kg).

Group 5: Test-2 group treated with Rheumatigo extract (500 mg/kg).

Complete Freund's Adjuvant-induced Arthritis: On day zero, All the groups' animals were injected into the sub-plantar region of the left hind paw with 0.1ml of complete Freund's adjuvant (CFA) except the control group. This consists of 6mg mycobacterium butyricum suspended with heavy paraffin oil by grinding with a motor and pestle to give a 6mg/ml concentration. Dosing with the test and standard compounds are administered on the same day and continued are administered on the same day and continued for 12 days. After inducing the Arthritis, the paw volume and body weight were measured using vernier calipers and a plethysmograph at 0, 4 8, 14, and 21 days. The rats were treated with the respective drugs and test samples with respective doses one hour after the experiment. The results of extracts and standards were measured and compared with the control group.

Arthritic Score [5]: The morphological characteristics of arthritis, such as redness, swelling, and erythema, were observed on alternate days from the day 13th day of the study's conclusion on day 21.

The Visual Criteria were set following is: Normal paw=0, mild swelling, and erythema = 1, swelling and erythema = 2 serve swelling and erythema =3, inability and gross deformity to use the limb = 4.

Hematological Parameter: Following the study, ether anaesthesia was used to euthanize all of the animals, who were then cervical dislocated, and blood samples were taken. Using a 23-gauge needle and a mL^{-1} syringe, blood is collected from the posterior vena cava of the heart and placed into blood tubes containing (EDTA) ethylenediaminetetraacetic acid as an anti-coagulant for haematological examination. They used to calculate the haemoglobin (Hb)(6), red blood cell (RBC) (7), and total white blood count (WBC) (8) values. The erythrocyte sedimentation rate was determined using Wintrobe method (ESR).

Histopathological Examination: The animals were slaughtered by cervical dislocation on day 21, and the ankle joints were removed and placed in 10% buffered formalin. The sections were then stained with haematoxylin and eosin after the preserve of the tissues had been declassified. The review of slides examined histological abnormalities such as soft tissue swelling, bone demineralization, cartilage erosion, and joint space narrowing.

Assessment of Anti-inflammatory Activity:

Grouping of Animals: Each group consists of six animals of either sex and is divided into 5 groups.

Group 1: Control group treated with water.

Group 2: Diseased group induced with inflammation and treated with water.

Group 3: Standard group treated with a Diclofenac (20 mg/kg).

Group 4: Test-1 group treated with Rheumatigo extract (250 mg/kg). Group 5: Test-2 group treated with Rheumatigo extract (500 mg/kg).

Carrageenan-induced Paw Edema: All the groups except the control group rats induced inflammation by using a carrageenan solution of 1% injected into the paw of the rat at the sub-plantar region. After inducing the inflammation, the

paw volume was measured by using vernier calipers and a plethysmograph at 0, 1,2,3,4,5, and 48 hours. After 48 hours reading is taken as a baseline reading. After 48 hours, the rats were treated with the respective drugs and test samples with respective doses one hour before the experiment and again inflammation was induced using carrageenan to the paw at sub plantar region and measured the paw volume was measured using a plethysmograph and vernier calipers. Dosing was continued daily for five days, and the paw volume was measured. The results of extracts and standards were measured and compared with the control group. The percentage of inhibition was calculated by using the following formula:

Percentage of inhibition = Vc – Vt / Vc. × 100

Where, Vc – Mean edema volume of control. Vt – Mean edema volume of the Test sample.

Assessment of Antioxidant Activity *In-vitro* free Radical Scavenging Activity:

DPPH (1, 1-diphenyl-2-picrylhydrazyl) Radical Scavenging Activity [9]: The ultimate concentration of DPPH was 0.1 mm after various doses of the enzyme were combined with a methanolic solution containing DPPH radicals. After giving the mixture a thorough shake and letting it stand for 30 minutes, the absorbance was calculated at 517 nm. Ascorbic acid served as the standard. The following equation was used to determine the sample's percentage of DPPH decolorization:

% decolorization = [1-(ABS sample/ABS control] x 100

Hydrogen Peroxide Scavenging (H_2O_2) Assay [10]: In phosphate buffer, H_2O_2 was made into a solution. By measuring H_2O_2 absorption at 230nm, spectrophotometry was used to calculate its concentration.

H_2O_2 was treated with different doses of enzyme for 10 minutes. The 230nm absorbance was measured compared to a blank containing phosphate buffer but on H_2O_2. The formula was used to determine the percentage scavenging of H_2O_2 and standard chemicals.

Control - Sample % scavenging of H_2O_2 = x 100 Control.

RESULTS AND DISCUSSION:

Effect of Rheumatigo on Body Weights: Body weights were recorded on days 0, 4, 8, 14, and 21. There were slightly significant differences in the body weight of all groups of animals at all points.

This is evidenced by observations, at the beginning of the treatment loss of weight in animals was found but this was recovered by administration of test samples. The data is represented in **Table 2** and the graphical representation in **Fig. 1**.

TABLE 2: EFFECT OF RHEUMATIGO ON BODY WEIGHT

Groups	Mean body weights				
	Day0	Day4	Day8	Day14	Day21
G1- Normalcontrol	170.1±4.4**	172.6±4.4**	174.33±4.4**	175.8±4.7**	175.9±4.0**
G2-DiseaseControl	135.1±4.9**	126.05±4.5**	124.51±4.8**	116.5±5.3**	112.8±5.6**
G3 -prednisolone(20mg/kg)	138.5±4.4**	146.85±2.5**	149.35±2.3**	155.5±2.2**	159.5±2.0**
G4 -Rheumatigo (250mg/kg)	122.6±6.6**	125.34±4.6**	130.35±4.2**	135.5±4.0**	140.0±3.6**
G5-Rheumatigo (500mg/kg)	151.8±2.5**	158.67±1.3**	165.17±0.9**	167.0±0.8**	169.3±0.6**

Values are Mean S.E.M., with n=6 for each group, and are significant (P< 0.05 **, P< 0.01*) when compared to the control. One-way analysis of variance (ANOVA).

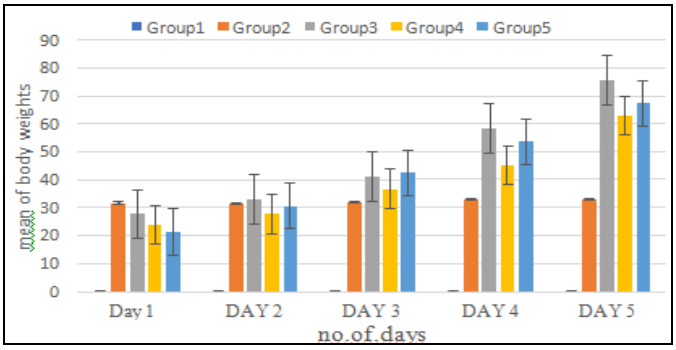


FIG. 1: MEAN VALUES OF BODY WEIGHT

Effect of Rheumatigo on Paw Volume: To examine potential basic processes and investigate the anti-arthritic effect of PHF made up of standard extracts. For purpose of this study, sub-planter injections of FCA into the left hind paw were used to cause arthritis. Both the FCA-injected and non-FCA-injected paws' elevated paw volumes suggested primary and secondary arthritic lesions, respectively. Prednisolone (DMARDS) and PHF administration at different dose (250mg/kg,

500mg/kg) effectively reduced the paw edema brought on by FCA. This is evidenced by observations of paw diameter showing a gradual decrease compared to the untreated group. The data is represented in **Table 3** and the graphical representation in **Fig. 2.**

TABLE 3: EFFECT OF RHEUMATIGO ON PAW VOLUME

Groups	Mean Paw volumes (ml)				
	Day0	Day4	Day8	Day14	Day21
G1-Normal control	0	0	0	0	0
G2-Diseased Control	1.25±0.0**	1.18±0.0**	1.18±0.5**	1.18±0.5**	1.18±0.5**
G3-prednisolone(20mg/kg)	1.08±0.4**	0.80±0.0**	0.63±0.3**	0.04±0.1**	0.29±0.2**
G4-Rheumatigo (250mg/kg)	1.16±0.0**	0.97±0.4**	0.74±0.7**	0.53±0.1**	0.48±0.2**
G5-Rheumatigo (500mg/kg)	1.13±0.1**	0.90±0.3**	0.70±0.2**	0.49±0.2**	0.38±0.1**

Values are Mean± S.E.M., with n=6 for each group, and are significant (P< 0.05 **, P< 0.01*) when compared to the control. one-way analysis of variance (ANOVA).

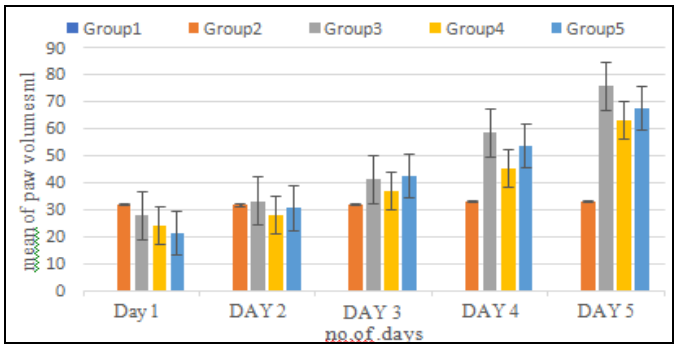


FIG. 2: EFFECT OF RHEUMATIGO ON PAW VOLUME

Effect of Rheumatigo on Paw Diameter: To examine potential basic processes and investigate the anti-arthritic effect of PHF made up of standard extracts.

For purpose of this study, sub-planter injections of FCA into the left hind paw were used to cause arthritis.

Both the FCA-injected and non-FCA-injected paws' elevated paw diameter suggested primary and secondary arthritic lesions, respectively.

Prednisolone (DMARDS) and PHF administration at different dose (250mg/kg, 500mg/kg) effectively reduced the paw edema brought on by FCA. This is evidenced by observations of paw diameter showing a gradual decrease compared to the untreated group. The data is represented in **Table 4** and the graphical representation in **Fig. 3.**

TABLE 4: EFFECT OF RHEUMATIGO ON PAW DIAMETER

Groups	Mean Paw Diameter (cm)				
	Day0	Day4	Day8	Day14	Day21
G1-Normalcontrol	0	0	0	0	0
G2-DiseaseControl	1.24±0.01**	1.23±0.29**	1.23±0.35**	1.23±0.42**	1.23±0.42**
G3-prednisolone(20mg/kg)	1.06±0.036**	0.99±0.21**	0.68±0.22**	0.38±0.07**	0.19±0.05**
G4-Rheumatigo(250mg/kg)	1.148±0.044**	1.63±0.34**	0.78±0.23**	0.54±0.15**	0.32±0.11**
G5-Rheumatigo (500mg/kg)	1.080±0.032**	1.25±0.23**	0.72±0.14**	0.43±0.32**	0.28±0.11**

Values are Mean S.E.M., with n=6 for each group, and are significant (P< 0.05 *, P< 0.01 **) when compared with control. One-way analysis of variance (ANOVA).

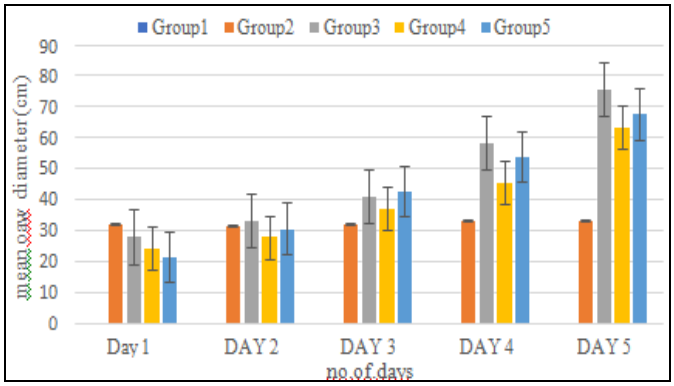


FIG. 3: EFFECT OF RHEUMATIGO ON PAW DIAMETER

Effect of Rheumatigo on Arthritic Score: In the present study, treatment with Rheumatigo 250mg/kg and 500mg/kg. 500mg/kg, was carried out which showed a gradual decrease in the score of pain observed when compared to the arthritic control in terms of arthritic score ($p \leq 0.05$ and $p \leq 0.001$ on day 13 to day 21, respectively. This is evidenced by observations of paw diameter showing a gradual decrease compared to the untreated group. The data is represented in **Table 5** and the graphical representation in **Fig. 4.**

TABLE 5: EFFECT OF RHEUMATIGO ON THE ARTHRITIC SCORE

Groups	Mean arthritic score				
	G1-Normalcontrol	G2-Diseased control	G3-Rheumatigo 250mg/kg	G4-Rheumatigo 500mg/kg	G5-Prednisolone 10mg/kg
Day 13	0.00±0.00**	3.68±0.34**	3.00±0.37**	3.29±0.39**	1.68±0.43**
Day 15	0.00±0.00**	3.51±0.33**	3.49±0.25**	3.40±0.25**	1.00±0.27**
Day 17	0.00±0.00**	3.68±0.35**	3.17±0.50**	3.00±0.36**	0.33±0.22**
Day 19	0.00±0.00**	3.83±0.18**	2.80±0.21**	2.78±0.51**	0.16±0.15**
Day 21	0.00±0.00**	3.83±0.17**	2.60±0.13**	2.45±0.40**	0.00±0.00**

Values are Mean S.E.M., with n=6 in each group, and are significant (P< 0.05**, P< 0.01 *) when compared to the control. One-way analysis of variances (ANOVA).

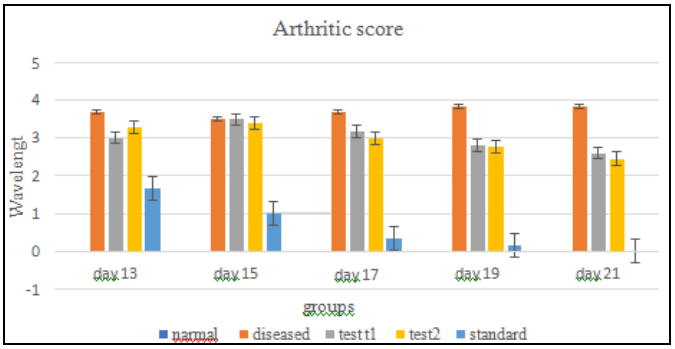


FIG. 4: EFFECT OF RHEUMATIGO ON THE ARTHRITIC SCORE

Hematological Parameters: Assessment of Hematological parameters was carried out where WBC, RBC, HB, and ESR studies were done.

It has been demonstrated that an IL-1B-mediated increase in the relevant colony-stimulating factors causes a moderate increase in WBC count in arthritic circumstances. The current investigation results show that prednisolone and rheumatoid factor treatment tend to raise RBC levels and decrease WBC levels by damaging premature RBC cells.

Other parameters like Hb % and ESR were also got modified in associated with increased endogenous protein production, such as fibrinogen and globulin by the Rheumatigo and Prednisolone treatments **Table 6** and **Fig. 5.**

TABLE 6: HAEMATOLOGICAL PARAMETERS

Groups	RBC (×106/mm3)	WBC (×103/mm3)	ESR (mm/h)	Hb (mg%)
G1-Normalcontrol	7.3 ± 0.2**	13 ± 0.4**	14 ± 0.3**	12 ± 0.4**
G2-DiseaseControl	8 ± 0.3**	7.5 ± 0.3**	12 ± 0.2**	13 ± 0.5**
G3-prednisolone(20mg/kg)	9.2 ± 0.2**	5.5 ± 0.2**	10 ± 0.2**	15 ± 0.3**
G4 -Rheumatigo(250mg/kg)	9 ± 0.1**	7.2 ± 0.3**	13 ± 0.3**	15 ± 0.4**
G5- Rheumatigo (500mg/kg)	9.8 ± 0.2**	5.6 ± 0.2**	11 ± 0.3**	16 ± 0.3**

Values are Mean S.E.M., with n=6 in each group, and are significant (P< 0.05 *, P< 0.01 **) when compared to the control. One-way analysis of variances (ANOVA).\

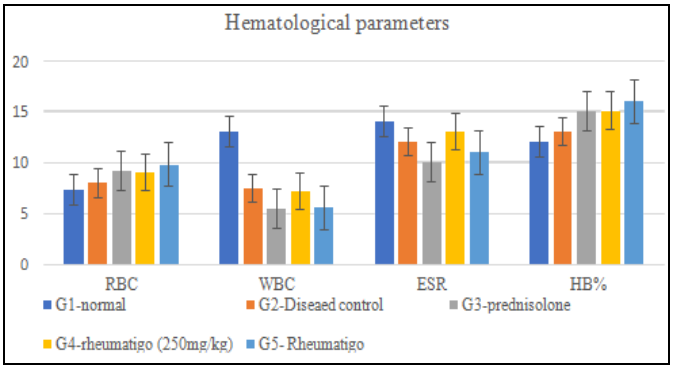
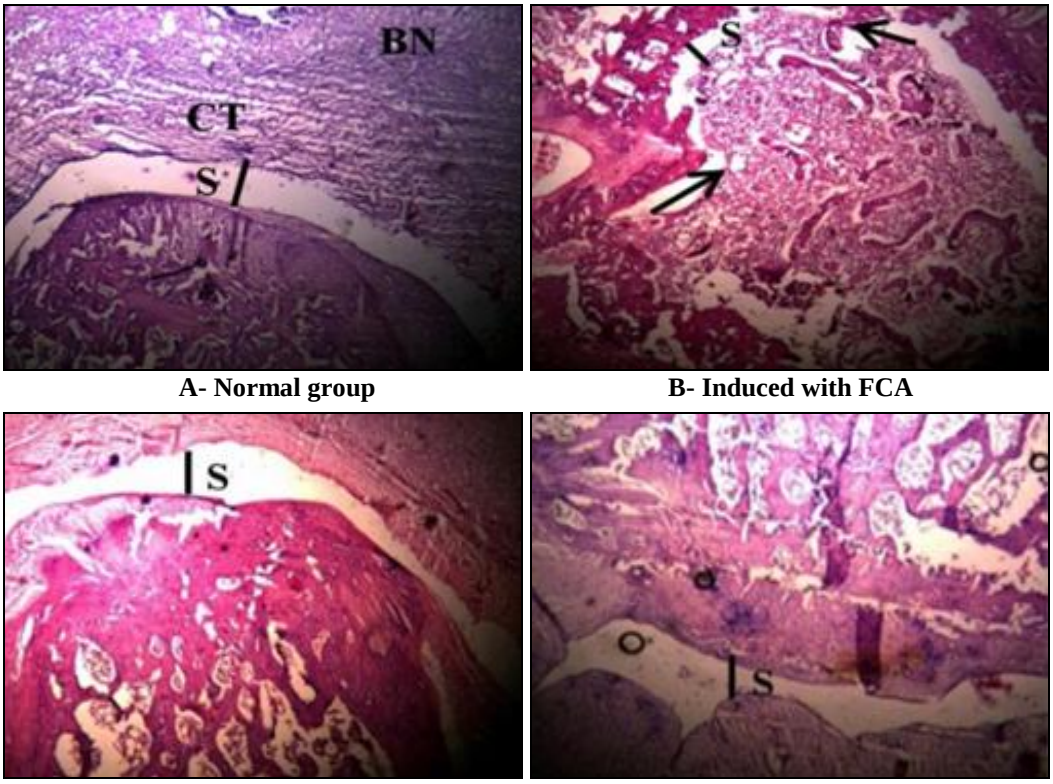


FIG. 5: HAEMATOLOGICAL PARAMETERS

Histopathology shows the histological alterations in the tarsal-tibial joints of various animal species. Synovial lining in G1 animals revealed a thin layer of flat, dormant cells and a typical synovial gap. There was no evidence of bone erosion or leukocyte infiltration. Complete Induced by Freund's Adjuvant in arthritis animals, synovial membrane cells proliferated, forming a thick, multi-layered synovial cell line with a constricted synovial space.

Additionally seen in G1, G2, and G3 were diffused synovial inflammatory cell infiltration, focal pannus formation, and another blood vessel formed in inflammatory synovial tissue.

Prednisolone-treated and Rheumatoid factor-treated groups showed low joint space narrowing, inflammatory cell infiltration and accumulation in synovial fluid, erosion of cartilage and bone, and synovial hyperplasia **Fig. 6.**



A- Normal group **B- Induced with FCA**

C-prednisolone treated group **D-Rheumatigo treated with 500mg/kg**

FIG. 6: HISTOPATHOLOGICAL STUDIES

Different animal groups can be seen in the histological sections (40) of the taros-tibial joints on the left hind paw. Group A demonstrated normal synovial space (S), articular cartilage (CT), and bone; Group B CFA-induced arthritic group showed marked infiltration of inflammatory cells, synovial hyperplasia, and marked reduction in synovial joint (shown by arrow); C prednisolone-treated group; and D -Rheumatigo 500mg/kg treated group showed less inflammatory cell infiltration, minimal synovial hyperplasia, and a modest reduction in joint space. Group A demonstrated normal synovial space (S), articular cartilage (CT), bone.

Evaluation of Anti-Inflammatory Activity:

Effect of Rheumatigo on Paw Volume: To examine potential basic processes and investigate the anti-arthritic effect of PHF made up of standard extracts.

For this study, sub-planter injections of carrageenan into the left hind paw were used to cause arthritis.

Both the carrageenan-injected and non-carrageenan-injected paws' elevated paw volumes suggested Diclofenac (NSAIDS) and PHF administration at different dose (250mg/kg, 500mg/kg) effectively reduced the paw edema brought on by carrageenan.

This is evidenced by observations of paw volume showing a percentage inhibition compared to the untreated group. The data is represented in **Table 7** and the graphical representation in **Fig. 7.**

TABLE 7: PERCENTAGE INHIBITION OF PAW EDEMA

Groups	% Inhibition of paw edema				
	Day1	Day2	Day3	Day4	Day5
G1-Normal control	0	0	0	0	0
G2-DiseaseControl	31.8±0.7**	32.3±0.6**	33.0±0.7**	32.3±0.8**	32.5±0.7**
G3-prednisolone(20mg/kg)	24.3±1.4**	35±1.06**	48.1±1.4**	54.1±1.3**	71.5±0.1**
G4-Rheumatigo (250mg/kg)	12.8±0.9**	24.8±0.6**	34.1±1.4**	46.5±1.2**	57.8±0.7**
G5-Rheumatigo (500mg/kg	23.3±0.6**	32.3±0.7**	40.3±1.4**	59.6±0.5**	73.6±1.5**

Values are % inhibition where each group has six participants and the comparison to the control is significant at P 0.05 and P 0.01*. One-way analysis of variance (ANOVA).

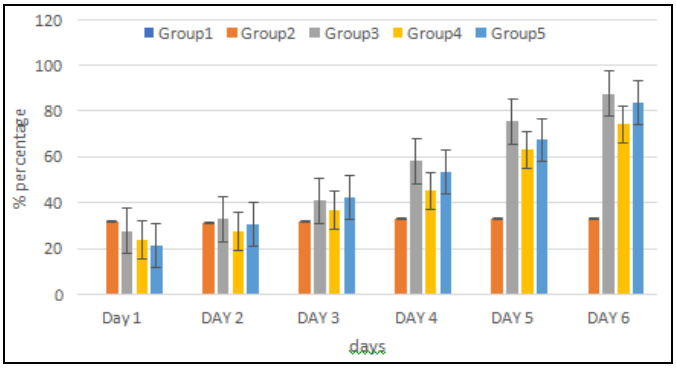


FIG. 7: PERCENTAGE INHIBITION OF PAW EDEMA

Effect of Rheumatigo on Paw Diameter by using Vernier Callipers: To examine potential basic processes and investigate the anti-arthritic effect of PHF made up of standard extracts. For this study, sub-planter injections of carrageenan into the left hind paw were used to cause arthritis. Both the carrageenan-injected and non-carrageenan injected paws' elevated paw diameter suggested Diclofenac (NSAIDS) and PHF administration at different dose (250mg/kg, 500mg/kg) effectively reduced the paw edema brought on by carrageenan.

This is evidenced by observations of paw diameter showing a percentage inhibition compared to the untreated group. The data is represented in **Table 8** and the graphical representation in **Fig. 8.**

TABLE 8: PERCENTAGE INHIBITION ON PAW DIAMETER

Groups	% Inhibition of paw edema				
	Day1	Day2	Day3	Day4	Day5
G1-Normalcontrol	0	0	0	0	0
G2-DiseaseControl	31.6±0.8**	31.5±0.8**	31.8±0.6**	33.0±0.9**	33.0±0.9**
G3-prednisolone(20mg/kg)	27.6±0.6**	33.0±0.6**	41.0±0.3**	58.3±0.1**	75.5±0.9**
G4-Rheumatigo(2500mg/kg)	23.8±0.4**	27.6±0.4**	36.6±0.6**	45.1±0.9**	63.0±0.1**
G5-Rheumatigo (500mg/kg	21.3±0.7**	30.5±0.8**	42.3±0.4**	53.5±0.2**	67.4±0.1**

Values are % inhibition where n=6 in each group, P< 0.05 **, P< 0.01 * (significant) compared with the control. Statistical analysis was done by one-way analysis of variance (ANOVA).

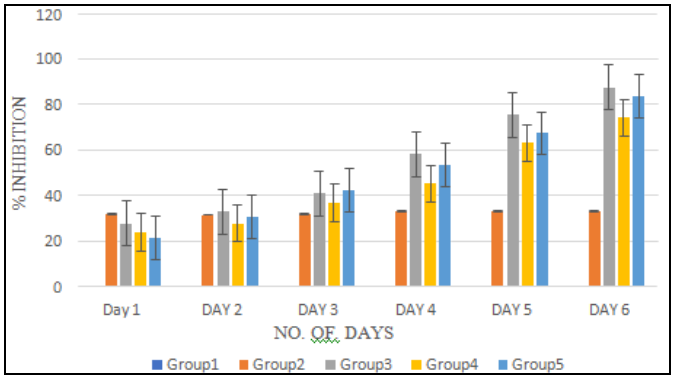


FIG. 8: PERCENTAGE INHIBITION ON PAW EDEMA

Evaluation of *In-vitro* Antioxidant Activity:

Free Radical Scavenging Activity DPPH (1, 1-diphenyl-2-picrylhydrazyl): The DPPH radical scavenging assay is an easy rapid and sensitive method for the antioxidant activity in plant extracts. The DPPH assay references compound ascorbic acid and Rheumatigo. The analysis of the radical scavenging activity of the PHF of Rheumatigo increases with increasing concentration. The IC_{50} values of the standard and test drug were found to be 397.48 and 499.01 µg/ml, respectively. In the **Table 9** and shown in the **Fig. 9.**

TABLE 9: *IN-VITRO* ANTIOXIDANT STUDY OF RHEUMATIGO BY USING DPPH ASSAY

Concentration (µg/ml)	PercentageInhibition standard	IC_{50} µg/ml of standard	Percentage Inhibition Rheumatigo	IC_{50} µg/ml of Rheumatigo
2	20.5±0.005		8.2±0.005	
4	36.7±0.005		20.6±0.5	
6	62.6±0.005	397.48	48.9±0.01	499.01
8	83.0±0.05		78.1±0.005	
10	88.9±0.00		84.8±0.005	

Data expressed as means ± SD, n=6, p≤0.001 compared with extract and standard and were found to be significant.

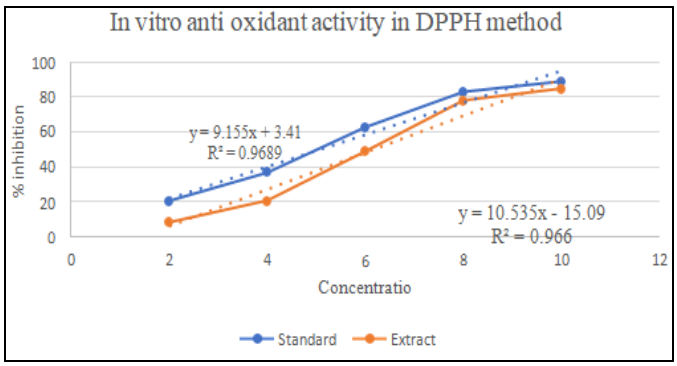


FIG. 9: THE PERCENTAGE SCAVENGING ACTIVITY OF RHEUMATIGO *IN-VITRO* ANTIOXIDANT (DPPH) ASSAY

Free Radical Scavenging Activity by Hydrogen Peroxide (H_2O_2): The H_2O_2 radical scavenging assay is an easy, rapid, and sensitive method for the antioxidant activity in plant extracts. The H_2O_2 assay references compound ascorbic acid and Rheumatigo. The analysis of the H_2O_2 radical scavenging activity of the PHF of Rheumatigo increases with increasing concentration. The IC_{50} values of the standard test drugs were found to be 49.26 and 66.82µg/ml, respectively. In **Table 10** and **Fig. 10.**

TABLE 10: *IN-VITRO* ANTIOXIDANT STUDY OF RHEUMATIGO BY USING H_2O_2 ASSAY

Concentration	Percentage Inhibition standard	IC_{50} µg/ml ofstandard	Percentage Inhibition Rheumatigo	IC_{50} µg/ml of Rheumatigo
2	21.6	49.26	9.2	66.82
4	39.7		32.9	
6	64.1		43.9	
8	96.9		56.4	
10	98.9		98.5	

Data expressed as means ± SD, n=6, p≤0.001 compared with extract and standard and were found to be significant.

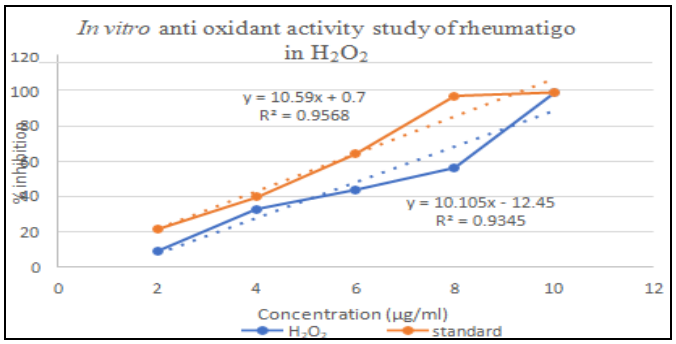


FIG. 10: THE PERCENTAGE SCAVENGING ACTIVITY OF RHEUMATIGO *IN-VITRO* ANTIOXIDANT (H_2O_2) ASSAY

CONCLUSION: The current investigation demonstrated that the extract had anti-arthritic and anti-inflammatory properties. TNF may mediate the current mode of Rheumatoid Extract- and cox-2. According to the findings, PHF efficiently reduced the volume and diameter of the hind paws in FCA-induced rats and the erosion of the bone and cartilage. The potential of PHF to reduce the levels of proinflammatory cytokines (TNF-, IL-1, and IL-6) and alter the oxidant/antioxidant balance as well as down-regulate the inflammatory marker enzymes COX2, iNOS, and transcription factor TNF-B may be responsible for its anti-arthritic properties. These findings, when considered collectively, imply that PHF has the potential to reduce clinical symptoms, enhance the quality of life of arthritic patients, and act as an anti-arthritis agent. In conclusion, 500mg/kg Rheumatigo is better than when compared to standard drugs. So, herbal medicine is beneficial the allopathy.

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REFERENCES:

1. Kiran D, Rohilla A, Rohilla S and Khan MU: *Phyllanthus amarus*: A sample therapeutic potential herb. Int J Res Ayur Pharm 2011; 2: 1096-9.15, 2020.

2. Thirumal M, Vadivelan R, Kishore G and Brahmaji VS: Aristolochiabracteolata: An overview on pharmacogenetic, phytochemical and pharmacological properties. Earth J 2012; 1: 66- 78. https://www.academia.edu/5764687/A_Review_of_Herbs_to_Treat_Skin_Disorders_in_Traditional_Siddha_Medicine
3. Gupta RK, Patel AK, Shah N, Chaudhary AK, Jha UK, Yadav UC, Gupta PK and Pakuwal U: Oxidative stress and antioxidants in disease and cancer: a review. Asian Pac J Cancer Prev 2014; 15: 4405–9. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4921101/
4. Aust SD, More house LA and Thomas CE: Role of metals in oxygen radical reactions. J Free Radic Biol Med 1985; 1: 3–25. https://doi.org/10.1016/0748-5514(85)90025-X
5. Pan T, Cheng TF, Jia YR, Li P and Li F: Anti-rheumatoid arthritis effects of traditional Chinese herb couple in adjuvant-induced arthritis in rats. J Ethnopharmacology 2017; 205: 1-7. https://doi.org/10.1016/j.jep.2017.04.020
6. Lowry Oh, Rose brough Nj, Farr Al and Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193(1): 265-75. PMID: 14907713.
7. D'Armour WL and Smith DL: A method for determining loss of pain sensation. Journal of Pharmacology and Experimental Therapeutics, 72, 74-79.
8. Docie JV. In-practical Haematology, 1958. London, J&A Churchill ltd. 1941; 38-42.
9. Blois M: Antioxidant Determinations using a Stable Free Radical. Nature 1958; 181: 1199–1200. https://doi.org/10.1038/1811199a0
10. Randall J. Ruch, Keith and Christ James E. Klaunig: Effects of culture duration on hydrogen peroxide-induced hepatocyte toxicity. Toxicology and Applied Pharmacology 1989; 100(3): 451-464. https://doi.org/10.1016/0041- 008X(89)90293-7.
11. The value and importance of plants in medicine. Evita ochel 2010; 26.

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