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Original Article

Palliative Action of *Woodfordia fruticosa* Leaves Containing Woodfruticosin on Biological Redox Imbalance and Anemia in CFA–Induced Arthritic Rats

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ABSTRACT

Background: Rheumatoid Arthritis (RA) is a chronic disorder affecting musculoskeletal and autoimmune system with inflammatory characteristics. Anemia is most prevailing, among other extra-articular complications in RA. These complications are analogous to exorbitant inflammation and biological redox imbalance which ultimately affects the quality life and life expectancy of RA patients. **Objectives:** The study was planned to investigate the palliative action of *Woodfordia fruticosa* (WF) leaf extracts and isolation from active fraction, on articular/ extra-articular complications in rats with Complete Freund's Adjuvant (CFA) induced arthritis. **Materials and Methods:** The action of Petroleum ether (WFP) and Ethanolic (WFE) extracts of WF was assessed in CFA induced adult Lewis rats (180-200gm) arthritic rats. In prophylactic treatment they received WF extracts 100mg/kg p.o. for 28 successive days and therapeutic treatment from the 14th day of CFA injection. Active extract was then fractionated by solvent-solvent extraction and chromatographic techniques. The isolated compound was characterized by TLC and various spectroscopic studies. **Results:** WFP significantly alleviated the severity of arthritis in animals than WFE in 28 days. Remarkable reduction ($p< 0.001$) in various complications was observed including paw volume (85.42%), loss in body weight, erythrocyte sedimentation rate, anemia, locomotor activity, and lipid peroxidation of hepatic/ synovial tissues and increment in SOD (U/mg) activities. A compound was isolated and characterized by UV, TLC, FTIR, LCMS and NMR spectroscopic techniques. **Conclusion:** WFP at 100mg/kg was advantageous in the superintendence of RA intricacies which was also confirmed by the histological and radiological studies. And a tannin compound Woodfruticosin was isolated.

Keywords: Rheumatoid arthritis, Chromatography, Isolation, Spectroscopy, Woodfruticosin.

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INTRODUCTION

Globally a part of the world's population suffers from Rheumatoid Arthritis (RA), a chronic inflammatory condition marked by immune-mediated inflammatory sinusitis that causes vasculogenesis, malformation of joints, the destruction of cartilage and bone, hyperplasia, and functional impairment. All of these signs significantly lower quality of life and increase the risk of depression. Joint swelling, synovial hyperplasia, pain, altered morphology, and the development of pannus are all symptoms of arthritis, a bilateral condition that primarily affects the ankles.[1,2] This autoimmune disorder is chronic and progressing with unidentified sources, and manifests as stiff synovial joints, swelling, and pain.[3] There is no known cause of the illness that results in its development. About 0.75% of adults in India and 1% of adults worldwide suffer from this illness.[4] An inflammatory reaction, inflammation of joints, joint damage and hypercellular synovial tissues are the pathological ensigns of RA.[5,6] In RA conditions, an imbalance between proinflammatory and anti-inflammatory states leads to synovial membrane inflammation and joint damage.[7] The prevalent extra-articular intricacy in RA is anemia which is usually associated to anemia of inflammation.[8] The pervasiveness of those intricacies is contemplated as a mortality predictor in RA patients,[9] they are relevant to excessive inflammation and biological redox imbalance spurred on, respectively by inflammatory mediators and free radicals, which are produced throughout the course of illness.[10] Free radicals e.g., Reactive Oxygen Species (ROS) and Nitrogen Species (RNS) have detrimental effects on vital organs and synovial joints. So, the intricacies of arthritis may

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be minimized by the free radical scavenging approach using antioxidants.[11]

The use of NSAIDs like etoricoxib, DMARDs like methotrexate, hydroxychloroquine, sulphasalazine, and leflunomide, corticosteroids like methylprednisolone and prednisolone, immuno-suppressants, and biological agents have all been linked to side effects, regardless of improvements in the effectiveness of conventional treatment options for this condition.[12] However, despite a growth in usage, there is no proof of the efficacy and safety of these supplementary therapies.[13]

Woodfordia fruticosa Kurz. (dhataki) is well known in various Asian parts for its medicinal and therapeutic values belonging to the family *Lythraceae*.[14] Its flowers and leaves are used in traditional medicines for hemothermia, fever, seminal weakness, persistent dysentery, and menorrhagia.[15,16] The dried and powdered flowers promote granulation in ulcers.[17] While chemical analyses of several WF flower extracts have revealed the existence of many phytoconstituents such as saponins, flavonoids,[18] tannins, polyphenols,[19] and alkaloids.

Therefore, the purpose of the current investigation was to determine if WF extracts had any ameliorative effects on articular or extra-articular problems in rats with CFA-induced arthritis (particularly in cellular oxidative stress and anemia).

MATERIALS AND METHODS

Instruments and chemicals

Diclofenac sodium (≥99%, Bio-Techne India Private Limited, 4454/50), EDTA (99.0%, Merck Laboratories Pvt. Ltd., 6381-92-6), Complete Freund's adjuvant was obtained from Sigma Aldrich Chemical Co., Bengaluru (F5881), DTNB (>98%, HPLC, Tokyo Chemical Industry Co. Ltd., 69-78-3, D0944), Trichloro acetic Acid (99%, 76-03-9), Thio-Barbituric Acid (504-17-6, Loba Chemie Pvt. Ltd., Mumbai). All additional substances utilized in the investigation were of analytical grade. Instruments employed included a rotatory flash evaporator (IKA RV10), trinocular microscope, UV-spectrophotometer (Pharmaspecs shimadzu), hot plate (Lyzer India), actophotometer (Teknik), and cooling centrifuge (Remi-electrotechnic limited).

Plant material and extraction procedure

The plant was gathered in Bhopal at Vindhya Herbals, barkheda pathani and identified by morphological and microscopic evaluation. The plant was authenticated by Dr. Zia Ul Hasan (botanist), Safia Science College, Bhopal (M.P.) with a voucher Specimen no. 316/Bot/Safia/12. The microscopic feature of the Transverse Section (T.S.) of the leaf was also studied.

In order to prevent the chemical components of the leaves from being destroyed by high temperatures, they were washed and dried in the shade. Using petroleum ether, chloroform, ethanol, and water as consecutive solvents, the WF leaves relatively coarse powder was extracted (Figure 1). By using a rota-vacuum evaporator under vacuum, the final traces of solvent are eliminated. The resultant dried extracts were weighed, the percentage (w/w) was computed, and they were then kept for later use in a container that was firmly closed.[20]

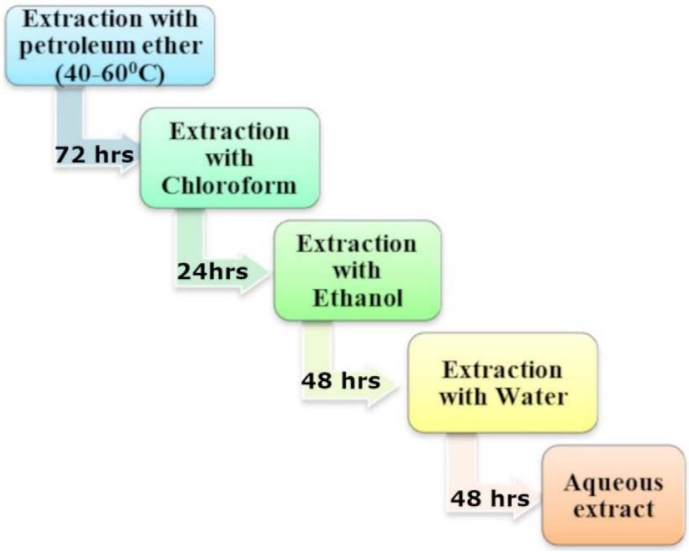

Figure 1: Successive solvent extraction procedure for *Woodfordia fruticosa* leaves.

Phytochemical analysis

The extracts were examined for phytochemical determinations of proteins, carbohydrates, oils, amino acids, steroids, triterpenoids, fats, glycosides, saponins, flavonoids, alkaloid, phenolic compounds, tannins and volatile oils, according to the method described by Harborne.[21] The WFP and WFE extracts were chosen for further investigations after preliminary analysis.

Animals

Healthy albino Lewis rats of either sex, 12 weeks old, and weight between 180 and 200 g were utilized in the experiment for the assessment of the acute oral toxicity and anti-arthritic potential. The rats were utilized after an adaptation period of ten days to the lab environment and were fed with standard feed and water at predetermined intervals. According to the guidelines, set forth by the committee for the purposes of control and supervision of experiments on animals, the Institutional Animal Ethical Committee authorized the study (CPCSEA1413/PO/E/S/11, SBRL/IAEC/June/2014/16), Every animal was housed in a standard cage, held at 25°C, and given a 12 hr cycle of light and darkness. Prior to the trial, the animals were kept fasting for a period of 12 hr.

Experimental design and treatment schedule

Acute oral toxicity test and dose determination

Acute oral toxicity test for an oral dose of WF was determined at different doses e.g. 5, 50, 300, and 1000 mg/kg body weight. As per the OECD guidelines 420, the maximum recommended dose was used to test. The control group received only the vehicle 0.5% CMC. Using an intubation cannula, the extract suspensions in 0.5% CMC were given by gavage as a single dosage. Three animals in total were used for each dosage level that was investigated. For every 48 to 72 hr, the animals were checked for mortality and overall behavioral changes. Every day in the afternoon for 1 hr, the behavioral shifts were monitored (10 to 11 a.m.). Following administration of various doses of extracts, the animals were continuously observed for the first 4 hr, then regularly for the following 6 hr, and once more after 24, 48, and 72 hr. In the treatment group, there was no mortality rate at the single dosage of 1000 mg/kg administered via gavage, but above that there is, so 1/10th of this dose (100 mg/ kg) was taken for the study.[22]

Induction of arthritis

Each set of seven Lewis rats weighed 180 ± 20 g and consisted of six rats of either sex, were formed. First group treated as control (received saline water only), second group treated as (negative control CFA-induced) arthritic control, third group was treated with the standard Diclofenac sodium drug (a positive control/ anti-arthritic control group) (suspended in 0.5% CMC), fourth group and sixth group received ethereal extract (WFP) and alcoholic extract (WFE) of *Woodfordia fruticosa* extract for twenty eight days from the day of induction (0-28 days treatment), respectively, fifth and seventh group received ethereal (WFP) and alcoholic extract (WFE) of *Woodfordia fruticosa* for fourteen days from the fourteenth day of induction (14th to 28th day treatment), respectively (Table 1). Weekly observations of variations in body weight were noted.

Induction of arthritis in rats was achieved by injecting intradermally 0.1 mL of CFA into hind paw's sub plantar area of the animals.[23] On the 29th day of the experiment, blood was collected through the retro-orbital puncture method for analysis of biochemical parameters, and then all animals were euthanized through cervical decapitation.

Assessment Parameters for Arthritis

Development of the arthritic disease condition e.g., hind paw swelling was monitored using vernier calipers, and body weight variation was determined by weighing. Measurement of the rear paw volume were made on days 0, 7, 14, 21, and 28 (after injection of CFA) to determine the severity of swelling and improvements in RA conditions. The movement of animals was determined under locomotor activity.[24] It was determined before the induction (0 days) and continuously for 7 days, intervals up to 28 days of the protocol, by using actophotometer. The animals were weighed and marked. The equipment was turned on and checked for the proper working of photocells for accurate counting. Animals from each group were placed on the instrument for 10 min and basal activity score was recorded for all animals.

Table 1: Grouping of animals.

Sl. No.	Group	Details
1	Group I (GI)	Sod. CMC 0.5% w/v in distilled water 5 mL/kg, p.o.
2	Group II (GII)	*Mycobacterium butyricum* in paraffin oil 10 mg/mL.
3	Group III (GIII)	Suspension of Diclofenac sodium in 0.5% w/v sodium CMC, 5mg/kg p.o.
4	Group IV (GIV)	Ethereal extract of *Woodfordia fruticosa* leaves (WFP) for 28 days, 100 mg/kg p.o. body weight (Treatment-1).
5	Group V (GV)	Ethereal extract of WFP for 14 days, 100 mg/kg p.o. body weight (Treatment-2).
6	Group VI (GVI)	Ethanolic extract of WFE for 28 days, 100 mg/kg p.o. body weight (Treatment-3).
7	Group VII (GVII)	Ethanolic extract of WFE for 14 days, 100 mg/kg p.o. body weight (Treatment-4).

Hematological Assessment

The experimental and rat blood was subjected to determine the hemoglobin content by Erba autoanalyzer, pre and post stages of arthritis induction. The hemoglobin content of different groups was collated with the control group.

Blood from rats was taken into a blood collection tube by an orbital puncture on the seventh, fourteenth, twenty-first, and twenty-eighth days to determine the Erythrocyte Sedimentation Rate (ESR).[25] By using enzymatic testing kits, the antioxidant indices Lipid Peroxidation (LPO) and Superoxide Dismutase (SOD) were measured in plasma.[26]

Radiographic and Histopathological Analysis

The adjoining joints of interphalangeal region were collected at the end of the study from two animals per group to study histopathological changes. Washed and stored samples (10% formalin) were treated with eosin-hemotoxylin stain, then examined microscopically under 100 X magnifications. Swelling in soft tissues, tightened joint spaces, and bony erosions were

examined in the hind legs of the experimental animals (arthritis induced) by X-ray radiographic studies.

Statistical Analysis

The values were expressed as the mean ± standard error mean (n=6). GraphPad Software Inc, San Diego, CA, USA was used for the statistical study of data which included one-way Analysis of Variance (ANOVA) and Dunnett's test. $p<0.05$ (or less) was used to determine whether the results were statistically significant.

Isolation and Characterization of Phytoconstituent

The powdered WF leaves were successively extracted with solvent in increasing polarity e.g., petroleum ether, chloroform, ethanol, and water. The ethanolic extract (10g) was then fractionated by solvent-solvent extraction technique with increasing polarity of solvents (ether, benzene, chloroform and acetone 40 mL each). The residue obtained from acetone fraction (9.46g) was then taken for column chromatographic separations on silica gel by gradient elution of solvent system (~400 mL each) chloroform and ethanol (A3:1, B1:1 and C4:6). The eluents obtained from system C were pooled and the obtained residue was fractionated using solvent-solvent extraction process with solvents of increasing polarity (ether, benzene, chloroform and acetone 30 mL each). The residue obtained from the acetone fraction was then purified with solid-phase extraction technique using a silica column and water. TLC and spectrometric analysis (UV, FTIR, LCMS and NMR) of the obtained residue (380mg) were performed for characterization.

RESULTS

Morphological and Microscopic analysis

Morphological and microscopic characterization helped in the identification and confirmation of crude woodfordia leaves. The size, shape, color, odor, microscopic arrangement of tissues in leaves was studied and compared with the standards confirming the identity of the drug (Figures 2-4).

Preliminary phytochemical screening

After successive solvent extraction following percentage yield was obtained: petroleum ether extract (greenish black 10.78%w/w), Chloroform extract (dark green 9.96%w/w), Ethanolic extract (brownish black 21.43%w/w) and aqueous extract (brownish black 17.72%w/w). According to preliminary phytochemical testing, petroleum ether extract includes steroids and lipids, whereas chloroform extract contains saponins, proteins, and steroids. Tannins, phenolic substances, and flavanoids were found in ethanolic extract. Tannins, saponins, polysaccharides, flavanoids, and phenolic substances were found in aqueous extracts. The presence of phytoconstituents observed was known for their therapeutic potentials and good

Figure 2: *Woodfordia fruticosa* leaves (WFL).

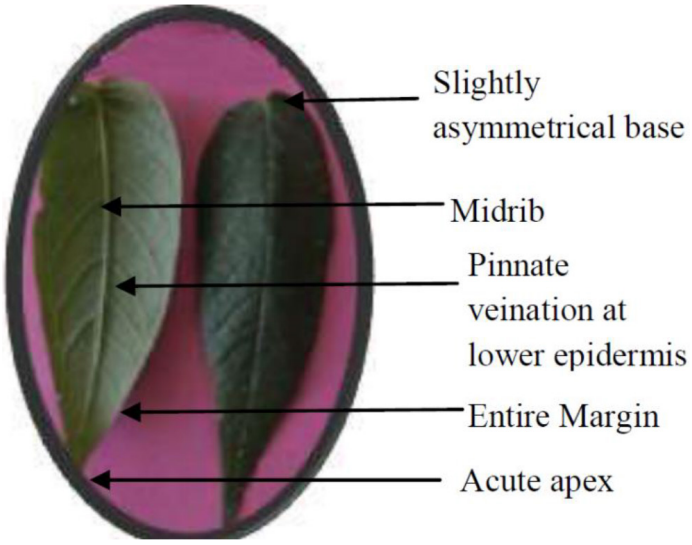

Figure 3: Leaves of *Woodfordia fruticosa*.

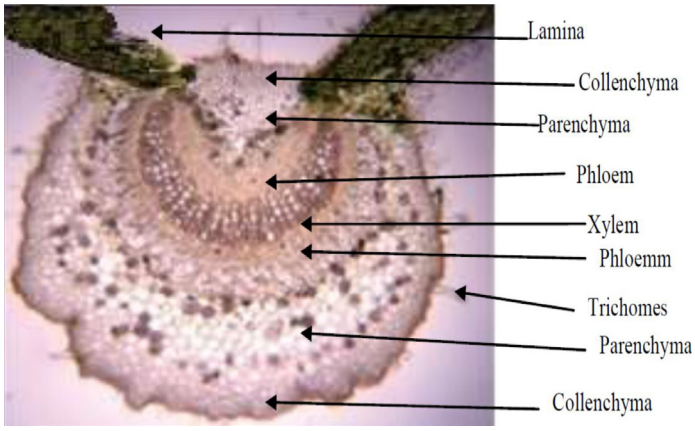

Figure 4: Microscopy of WFL.

Table 2: Qualitative analysis of different extracts of *Woodfordia fruticosa* leaves.

Sl. No.	Chemical Test	Pet Ether	$CHCl_3$	Ethanol	Aqueous
1.	CARBOHYDRATE				
	1Molish, 2Fehling, 3Benedict test	-	-	-	3
2.	PROTEIN				
	1Biuret, 2Millon's test	-	1	-	-
3.	AMINO ACID				
	1Nihydrin test	-	-	-	-
4.	FATS AND OILS				
	1Filter paper test	1	-	-	-
5.	STEROID AND TRITERPENOIDS				
	1Salkowski reaction, 2Libermann-Burchard reaction	1,2	2	-	-
6.	GLYCOSIDES AND SAPONINS				
	1Raymonds test, 2Keller-Killani test, 3Foam test	-	3	-	3
7	FLAVONOIDS AND PHENOLIC COMPOUNDS				
	1Shinoda test, 2Lead acetate test	-	-	1,2	2
8.	ALKALOIDS				
	1Dragendorff's, 2Mayer's, 3Wagner's test	-	-	-	-
9.	VOLATILE OIL				
	1Sudan red	-	-	-	-
10.	TANNINS				
	1Vanillin HCl, 2Gelatin, 3Ferric chloride test	-	-	1,3	1,3

'-' means negative result, '1,2,3' means positive results of the specific mentioned test.

yield of extracts suggested for the selection of petroleum ether and ethanolic extract of WF for evaluation of pharmacological potential (Table 2).

Regulatory effect of WFP and WFE on hind paw volume

Vernier calipers were used to measure the animals' hind paw volume on days 0, 7, 14, 21, and 28 of the experiment. In the current investigation, oedema and erythema were the inflammatory symptoms that emerged during 24 hr duration and increased subsequently in hind paws injected with CFA. Treatment groups receiving WF extracts demonstrated significantly reduced arthritic inflammation in rats when compared with vehicle and negative control groups Figures 5 and 6. In terms of mean variations in paw volume over the course of 28 days, the WFP extract (0.12±0.05) performed better than WFE (0.43±0.09). The percentage inhibition in paw volume of animals treated with WF extracts was observed in the following order: GIV (85.42%) >GV (81.31%) >GVI (50.13%) >GVII (48.42%).

Regulatory effects of WFP and WFE on body weight loss

The body weight of arthritic animals was considerably modulated when correlated with the control group of animals showing a

Figure 5: CFA induced inflammation in rats.

relationship between the degree of weight loss and the joint inflammation levels. The arthritic rats experienced substantial weight loss in the 1st week following adjuvant injection, followed by weeks of normal weight gain, but the Treatment 1st group and group treated with standard drug showed less loss in body weight (Figure 7). During the course of treatment, WFP (GIV and GV) substantially ($p<0.001$) reduced the body weight loss in arthritic rats. WFP extracts enhanced the gains in body weight of arthritic rats starting from day 0 and showed a correlation with meal

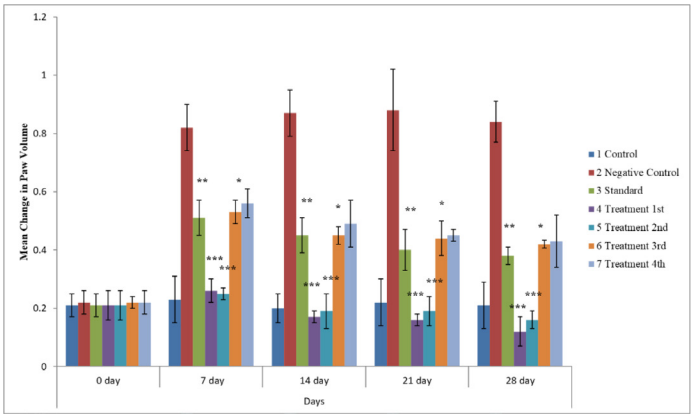

Figure 6: Effect of WF extracts on mean change in paw volume. *n* = 6 animals in each cohort; values are presented as Mean ± S.E.M. ANOVA, Dunnet's test ***p < 0.001, **p <0.01, *p <0.05 (treated groups Vs arthritic control group II).

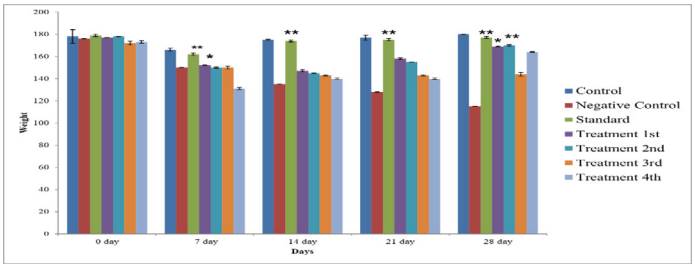

Figure 7: Effect of WF extracts on animal's body weight. ***n*** = 6 animals in each cohort; values are presented as Mean ± S.E.M. ANOVA, Dunnet's test **p < 0.001, *p <0.05 (treated groups Vs arthritic control group II).

consumption. With food consumption, WFP extracts improved the body weight gain of arthritic rats starting on day 0.

Regulatory effect of WFP and WFE on Movement

Movement of the rats was studied by the locomotor activity using actophotometer on the day of 0,7th, 14th, 21st, and 28th day. The WF extracts showed ameliorated motility improvement (p<0.001) in the rats which suggests the recuperations in arthritic intricacies (Figure 8). WFP extract comparatively showed more impressive results than WFE and exhibited palliative results in 28 days.

Modulatory action of WFP and WFE on anemic rats

On days 0, 7, 14, 21, and 28 of the experiment, haemoglobin content was measured, and arthritic rats had a significant drop in Hb content when compared to other group rats (Figure 9). In arthritic rats the Hb content was significantly reduced (p<0.001) by 14.34±0.58 to 12.84±0.07. Administration of WFP extract for 0-28 days significantly reduced the anemic state of arthritic rats. This palliative effect of his WFP was greater than WFE compared to the standard drug. Hb content was observed in different groups over 28 days, it was higher in GI (14.47±0.16) and lower in GII (12.84±0.07). The other groups have following order: GVII< GVI< GV< GIV< GIII.

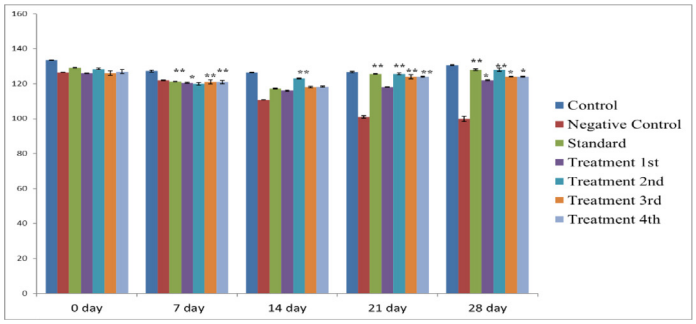

Figure 8: Effect of WF extracts on Movement. *n* = 6 animals in each cohort; values are presented as Mean ± S.E.M. ANOVA, Dunnet's test **p < 0.001, *p <0.05 (treated groups Vs arthritic control group II).

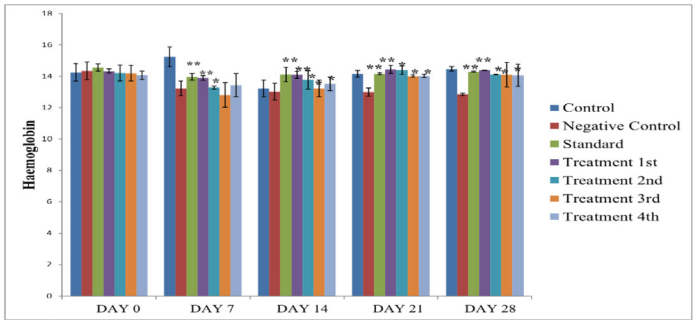

Figure 9: Effect of WF extracts on hemoglobin content. *n* = 6 animals in each cohort; values are presented as Mean ± S.E.M. ANOVA, Dunnet's test **p < 0.001, *p <0.05 (treated groups Vs arthritic control group II).

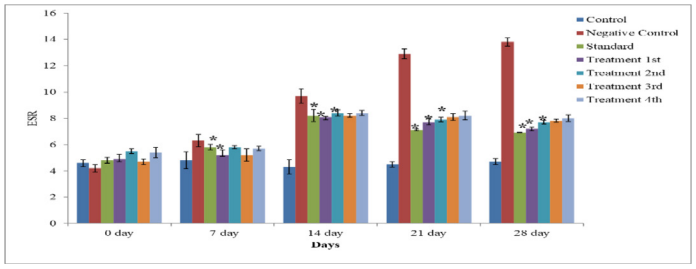

Figure 10: Effect of *WF* extracts on ESR. *n* = 6 animals in each cohort; values are presented as Mean ± S.E.M. ANOVA, Dunnet's test **p < 0.001, *p <0.05 (treated groups Vs arthritic control group II).

Modulatory action of WFP and WFE on ESR

ESR was substantially reduced in WF-treated groups in comparison to the group treated with vehicle. On day 28, ESR values dropped from 8.10 millimeters per hour (mm/h) to 7.2 mm/h in the group IV and 8.4 to 7.7 (mm/h) in group V in the WFP treatment group. The levels of ESR increased significantly from 4.2 to 13.8 in 28 days in the negative control group Figure 10.

Modulatory action of WFP and WFE on oxidative stress

However, lipid peroxidation was significantly reduced, as measured by MDA in the liver. Statistically, this decrease in MDA

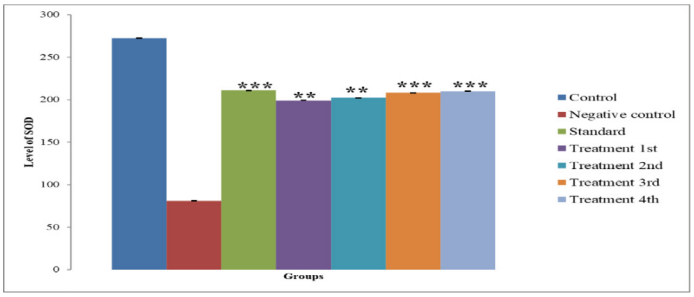

Figure 11: Effects of *WF* extracts on SOD activity. $n = 6$ animals in each cohort; values are presented as Mean ± S.E.M. ANOVA, Dunnet's test ***$p < 0.001$, **$p < 0.05$ (treated groups Vs arthritic control group II).

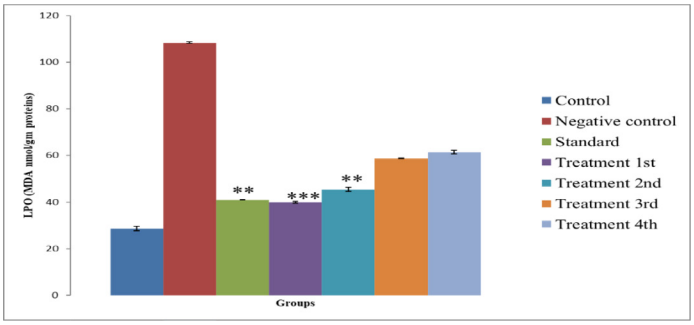

Figure 12: Effects of *WF* extracts on LPO activity. $n = 6$ animals in each cohort; values are presented as Mean ± S.E.M. ANOVA, Dunnet's test ***$p < 0.001$, **$p < 0.05$ (treated groups Vs arthritic control group II).

was significant ($p < 0.001$) when compared to the control group with arthritis Figures 11 and 12. WFE (210 ± 0.12) was found more effective than WFP (199 ± 0.14) when compared with the healthy control group at twenty eighth day. SOD levels were significantly ($p < 0.001$) reduced in arthritic rat group (81 ± 0.05) than the control group (272.60 ± 0.04) of animals. In contrast, treatment with WF in rats with arthritis revealed a remarkable increase in SOD levels during the developing phase ($p < 0.05$). In comparison to healthy control animals (28.7 ± 0.11), lipid peroxidation (MDA) levels were considerably higher ($p < 0.001$) in arthritic animals (108.4 ± 0.35) and lower in GIV and GV (WFP treated groups). In contrast, treatment with WF in arthritic animals showed remarkable reduction in LPO levels during the developing phase (39.9 ± 0.98) (Figure 12).

Radiographic analysis after 28 days of CFA induction

Radiological imaging tool is helpful in understanding the severity and extent of the disease. In the present study, observations showed that ankle joints have no deformities whereas in the negative control group bony destruction, narrowing of the joint spaces, and swelling in soft tissues were observed (Figures 13A to G). The group receiving standard medication prevented this bone destruction and had less joint swelling. There was no swelling of the joint and no bony destruction observed in the treatment 1st to 4th groups (e.g., GIV and GVII). In comparison to treatment II, IV, and the negative control groups, treatment I and treatment III

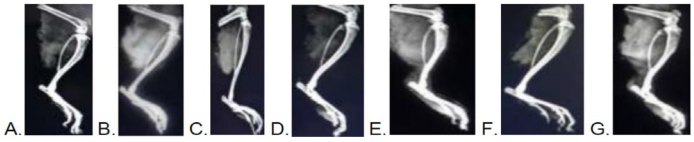

Figure 13: Radiology of Hind Paw. Where A. Normal Control (GI), B. Negative Control (GII), C. Standard group (GIII), D. Treatment-I (GIV), E. Treatment-II (GV), F. (GVI) Treatment-III, G. (GVII) Treatment-IV.

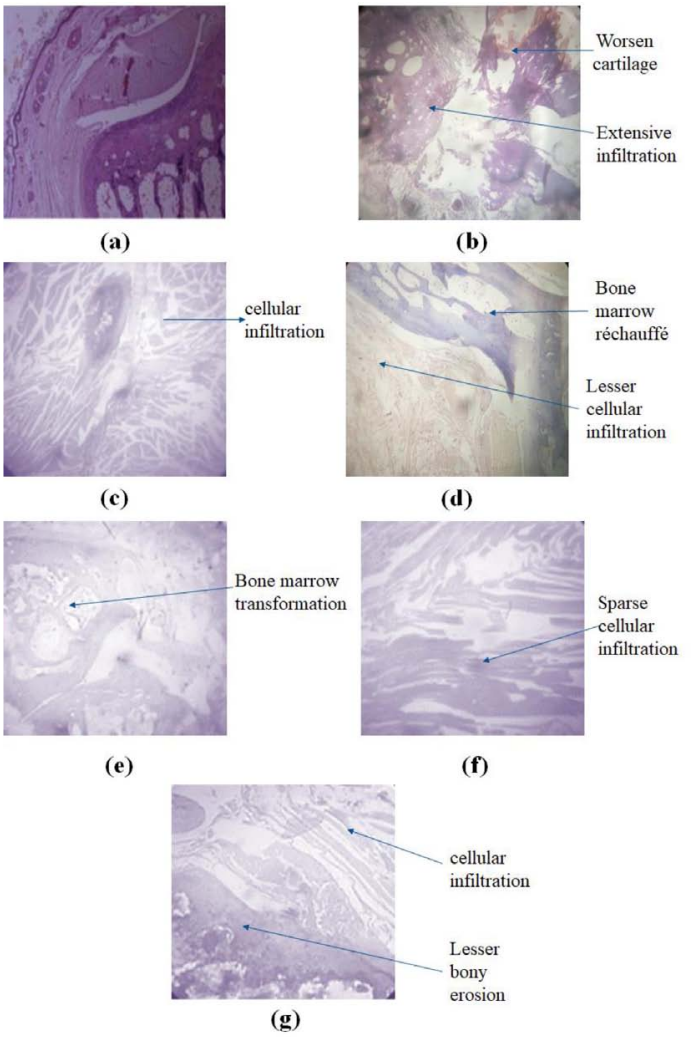

Figure 14: Histopathological presentation of joints. a= (Normal) Control Group, b= Arthritic (Negative control) Group, c= Standard drug Group, d=WFP 100mg treated 1st (test group-I, GIV), e= WFP 100mg treated 2nd Group (test group-II, GV), f= WFE 100mg treated (test group-III, GVI) and g= WFE 100mg treated (test group-IV, GVII).

groups demonstrated a substantial reduction in the destruction of bone, lessening of soft tissue swelling, and narrowing of the joint space.

Histopathology of joints

Figures 14a to g represent the changes observed in histopathology of adjoining joints of interphalangeal region of different groups, Group I (a) the control group showed the normal histological

pattern of the ankle joint. The animals of negative control group II showed prominent abnormalities in joints including extensive congregation of infiltrated inflammatory exudations on the articular surface, development of edema, worsening of cartilage with erosion and destruction of bone marrow (Figure 14). Regular bone marrow with minimal cellular infiltrations was observed in the standard drug-treated group III (c). The test group IV and VI (d and f) Twenty-eight days of treatment resulted in fewer signs of inflammation, including sparse cellular infiltration, lack of edema formation, and normal bone marrow. In broad perspective, the inflammation in ankle joints of rats were prevented significantly in twenty-eight days and there was no degeneration of ankle joints noticed in drug treated groups when was compared with GII, the negative control group.

Characterization of compound

The λ_{max} of the isolated molecule in UV-spectroscopic analysis was observed. It was 219nm and 269nm, demonstrating the existence of conjugated unsaturation in it (Figure 15). The off-white amorphous powdered compound showed single spot in TLC studies in solvent system nButyl alcohol:Acetic acid:Water (4:2:4) with visualizing agent. The FTIR spectra of compound showed characteristic bands at 3431.22, 3292.70, 2974.25 (free OH and OH stretch), 1718.46 (C=O stretch, unsaturated conjugated acid), 1631.34 (C=C-C phenyl aromatic ring stretch), 1454.85 (aromatic ring stretch), 1394.52 (OH bending, phenol), 1265.37 (primary alcohol OH in-plane bend), 1228.29, 1176.30 (aromatic C-H in-plane bend), 1090.39 (C-O cyclic ether large rings) (Figure 16). The mass spectra showed the molecular ion peak at m/z 1721 $(M+H)^+$. ^{1}H-NMR (500 MHz, CD_3COCD_3) δ ppm: 3.792 (1H, dd, 6-H), 3.961 (1H, d, 6'H), 4.095 (1H, ddd, 5'H), 4.363 (1H, d,1'H), 4.752 (1H, ddd, 5'H), 4.913 (1H, dd, 6'H), 5.028 (1H, t, 4'H), 5.265 (1H, dd, 6-H), 5.407 (1H, t, 3'H), 5.519 (1H, dd, 2'-H), 5.882 (1H, dd, 2-H), 6.056 (1H, t, 4-H), 6.173 (1H, t, 3-H), 6.364 (1H, s, 2-6H), 6.448 (1H, s, 6-6H), 6.673 (1H, s, 6'-6-H), 6.856 (1H, 4-6-H), 7.062 (1H, s, 2'-6-H), 7.109 (1H, d, 1-H), 7.291 (1H,

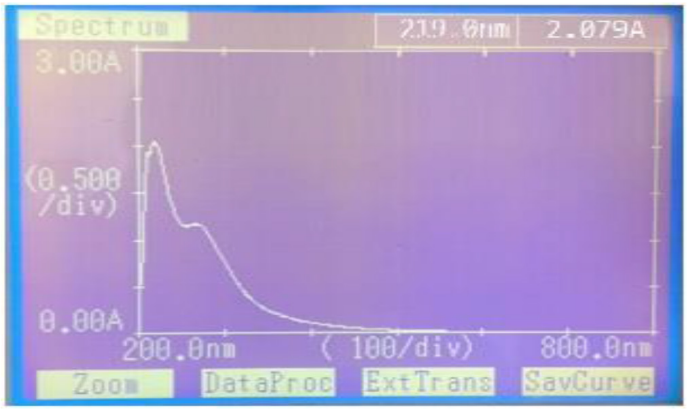

Figure 15: Ultraviolet Spectra of the Isolate from WF.

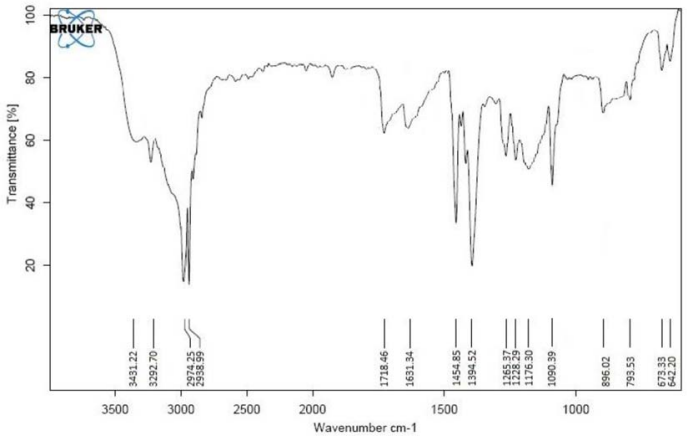

Figure 16: FTIR Spectra of the Isolate from WF.

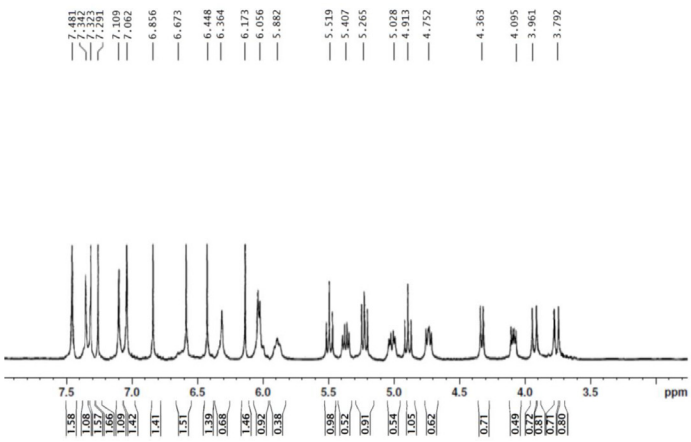

Figure 17: ^{1}H NMR Spectra of the Isolate from WF.

s, 4'-6-H), 7.323 (2H, s, 1-2-H, 1-6-H), 7.342 (2H, s, 3-2-H, 3-6-H), 7.481 (2H, s, 3'-2-H, 3'-6-H) (Figure 17).

^{13}C-NMR (500 MHz, CD_3COCD_3) δ ppm: 91.88 (C-1), 73.52 (C-2), 71.93 (C-3), 70.25 (C-4), 72.92 (C-5), 63.34 (C-6), 96.25 (C-1'), 75.29 (C-2'), 73.71 (C-3'), 74.44 (C-4'), 72.45 (C-5'), 65.97 (C-6') (sugars); 122.08 (C-1-1), 110.9 (C-1-2), 146.58 (C-1-3), 139.98 (C-1-4), 146.58 (C-1-5), 110.9 (C-1-6) (galloyl carbons); 165.49 (C-1-7) carbonyl carbon group; 121.25 (C-2-1), 143.47 (C-2-2), 140.51(C-2-3), 138.52 (C-2-4), 138.61(C-2-5), 109.39 (C-2-6) (valoneoyl carbons); 168.23 (C-2-7) carbonyl carbon group; 122.02 (C-3-1), 111.02 (C-3-2), 145.94(C-3-3), 139.64 (C-3-4), 145.94(C-3-5), 111.02 (C-3-6) (galloyl carbons); 168.23(C-3-7) carbonyl carbon group; 126.8 (C-4-1), 116.09 (C-4-2), 145.15 (C-4-3), 137.05 (C-4-4), 145.87 (C-4-5), 108.6 (C-4-6) valoneoyl carbons; 168.68 (C-4-7) carbonyl carbon group; 127.53 (C-5-1), 117.65 (C-5-2), 145.18 (C-5-3), 135.94 (C-5-4), 148.33 (C-5-5), 106.51 (C-5-6) valoneoyl carbons; 168.42 (C-5-7) carbonyl carbon group; 119.00 (C-2'-1), 144.21(C-2'-2), 145.37 (C-2'-3), 134.3 (C-2'-4), 139.4 (C-2'-5), 109.65 (C-2'-6) valoneoyl carbons; 167.27 (C-2'-7) carbonyl carbon group; 122.02 (C-3'-1), 111.5 (C-3'-2), 146.08 (C-3'-3), 139.4 (C-3'-4), 146.08 (C-3'-5),

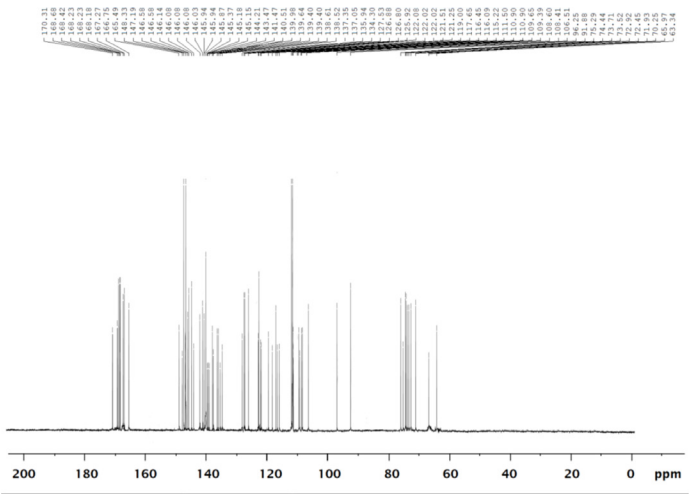

Figure 18: ^{13}C NMR Spectra of the Isolate from WF.

111.5 (C-3'-6) galloyl carbons; 166.75 (C-3'-7) carbonyl carbon group; 126.88 (C-4'-1), 121.51 (C-4'-2), 146.08 (C-4'-3), 141.47 (C-4'-4), 147.19 (C-4'-5), 115.22 (C-4'-6) valoneoyl carbons; 168.18 (C-4'-7) carbonyl carbon group; 125.92 (C-5'-1), 116.45 (C-5'-2), 146.14 (C-5'-3), 137.35 (C-5'-4), 146.03 (C-5'-5), 108.41 (C-5'-6) valoneoyl carbons; 170.31 (C-5'-7) carbonyl carbon group (Figure 18).

DISCUSSION

CFA-induced arthritis has been shown to be valuable in assessing novelty of human RA therapeutics as worldwide population is suffering.[27,28] The CFA-induced model is commonly applied to evaluate the anti-arthritic potential and anti-inflammatory prospects of drugs used to treat RA.[29] These models have many similitudes to human RA, making them more suitable for research.[30]

Paw swelling, an index was used to measure the intensity of inflammation in rats with CFA induction, and reduced swelling in paw is a hallmark of the anti-arthritic action of various drugs. This was used to measure the potential of WF extract at a dose of 100 mg/kg p.o. The WF-administered group revealed remarkable reduction in paw volume, especially WFP, when compared to the negative arthritic control group and WFE Figures 5-6. The ameliorating potential of WF extract (from day 0) on the paw edema seen in arthritic animals was due to interleukin (IL-1β) and pro-inflammatory cytokines (e.g., Tumor Necrosis Factor (TNF-α). They alter the production and generation of other inflammatory mediators such as NO and Prostaglandins (PGE2) and free radicals by increasing the expression levels of the triggering enzymes Cyclooxygenase (COX-2) and NO synthase, respectively.[31,32]

There is an association among the degree of inflammatory signs and changes in weight of body (Figure 7).[33] Decreased weight gain might be because of anorexia, decreased intestinal absorptive capacity (because of oxidative harm to the gut), as well as metabolic aggravations resulting from excessive production of free radicals and systemic pro-inflammatory mediators.[34,35] Weight gain increment is a simple and suitable measure for recuperation of arthritis in CFA animal model.

The significant decrease in musculoskeletal number in rats of CFA model could be caused by the immune system stimulation against the invading antigens, and the corresponding increase in the WF-treated group indicated its immunomodulatory action.[36] The movement of activity observed under actophotometer, notably in arthritic animals was reduced because of the pain in joints and stiffness (Figure 8).

Blood examinations like hemoglobin, packed cell volume, and white blood cell count reflect the function of bone marrow.[37] From the observations, it is evident that the decrement in hemoglobin level suggests the condition of anemia in rats with arthritis. This was suggested by the reports on analogous arthritis models. Abnormal iron accumulation in synovial tissues and the reticuloendothelial system, and unresponsiveness of the bone marrow to anemic conditions were the two most important causes (Figure 9).[38] The changes in blood pathological parameters of the CFA animal models reflected normocytic normochromic anemia without having unequal-sized RBCs. This may be because of the oxidative harm, gastrointestinal hemorrhage, or suppression of erythropoiesis in bone marrow by the pro-inflammatory cytokines, e.g., particularly TNF-α, IL-β, IL-6, or both. WF extracts, especially WFP, significantly reduced anemia in arthritic rats.[39]

Increased ESR is a hallmark of chronic arthritis. As the drug lowers the ESR the conditions are getting improved (Figure 10).

Peroxidation of lipids is key etiological cause for damage of cell membrane, and elevated MPO and MDA levels cause oxidative damage to the intestinal mucosa via lipid peroxidation.[40] The polyunsaturated fatty acids found in the membrane lipids are the primary targets of ROS, that are damaged and cause lipid peroxidation. In our study, the elevated MDA levels in arthritic animals was observed, which is the final product of decomposed lipid peroxidation. These elevated levels of MDA were an indicator of ROS production, and the outcomes agreed with the earlier statements. In our research, administering WFP to arthritic rats significantly reduced plasma MDA levels. There was significant reduction in SOD activity in arthritic animals than the animals without arthritis. The enormous generation of Reactive Oxygen Species (ROS) may have depleted SOD and sensitized synovial fluid and joint collagen to oxygen radical impairment in arthritic animals. However, administration of WFP to arthritic animals significantly increased SOD activity and decreased LPO levels, approaching normal controls, indicating ROS scavenging or antioxidant properties 11 and 12.[41]

Distinctive abnormalities like edematous tissue, bone marrow destruction, degeneration of the cartilage, and extensive accumulation of infiltrated inflammatory exudations in the articular surface were shown in the joint of negative control arthritic rat group II. The rat joints in group treated with standard drug had shown regular bone marrow conditions despite lower cellular infiltration. The WFP treatment for 28 days showed less signs of inflammation, such as sparse cellular infiltration, no edema formation, and normal bone marrow, while WFP treatment for 14 days showed less cartilage destruction and cellular infiltration of the joint surface.[42] Inflammatory features of rat ankle joints were significantly prevented in the 28-day drug-treated group compared with the 14-day drug-treated group. None of the drug-treated groups showed signs of ankle deterioration (Figure 13).

The severity of the disease can be determined by radiographic changes in RA status. Early radiographic signs of arthritis include soft tissue swelling, whereas major radiographic changes like bone erosion and narrowing of the joint space are only visible in later stages of the disease (Figure 14).[43-46] WFP treatment for 28 days provided significant protection against bone destruction due to mild soft tissue swelling and joint space narrowing compared to the group treated with WFP for 14 days. The experimental findings of the research demonstrated that WFP extract has significant anti-arthritic activity against rheumatoid arthritis caused by CFA.

The compound isolated from WF active fraction was characterized and identified by thin layer chromatography, UV, FTIR, LCMS, ^{1}H NMR and ^{13}C NMR spectroscopic studies (Figures 15-19). The data reflects the structure of compound has glucose, galloyl and valoneoyl groups will be $C_{75}H_{52}O_{48}$, D-Glucose, cyclic 4-2:6-2'-(4-(6-carboxy-2,3,4-trihydroxyphenoxy)-4',5,5',6,6'-pentahydroxy(1,1'-biphenyl)-2,2'-dicarboxylate) 3-(3,4,5-trihydroxybenzoate), cyclic diester with alpha-D-glucopyranose cyclic 4-2':6-2-(4-(6-carboxy-2,3,4-trihydroxyphenoxy)-4',5,5',6,6'-pentahydroxy(1,1'-biphenyl)-2,2'-dicarboxylate) 1,3-bis(3,4,5-trihydroxybenzoate) which is recognized as woodfruticosin.[47] The structure of the isolated compound was interpreted as woodfruticosin with comparing the spectra in available spectral libraries.[48]

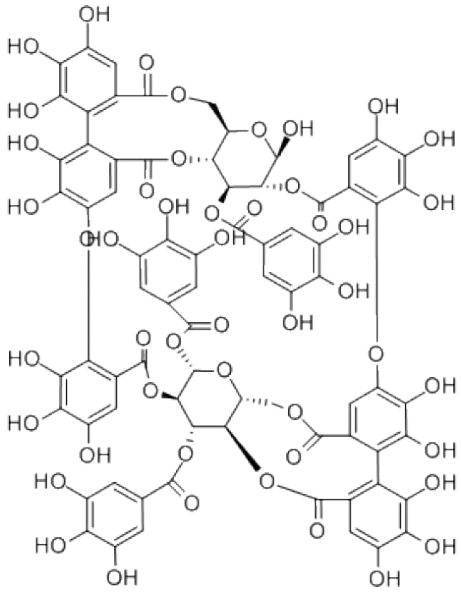

Figure 19: Woodfruticosin.

CONCLUSION

In conclusion, WFP (100 mg/kg) was more effective than WFE in alleviating extra-articular/ joint oxidative damage and anemia in the CFA animal model, exclusively on the day of arthritis induction with the treatment initiation. WF extract has shown the presence of tannins and a compound woodfruticosin was isolated.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

WF: *Woodfordia fruticosa*; **WFP:** Ethereal extract of *Woodfordia fruticosa* leaves; **WFE:** Ethanolic extract of *Woodfordia fruticosa* leaves; **CFA:** Complete Freund's adjuvant; **Hb:** Hemoglobin; **LPO:** Lipid peroxidase; **SOD:** Superoxide dismutase; **MDA:** Malondialdehyde; **ANOVA:** Analysis of Variance; **TLC:** Thin layer chromatography; **UV:** Ultraviolet Spectroscopy; **FTIR:** Fourier Transform Infrared Spectroscopy; **LCMS:** Liquid Chromatography Mass Spectrophotometer; **^{1}H NMR:** Proton Nuclear Magnetic Resonance; **^{13}CNMR:** Carbon Nuclear Magnetic Resonance.

SUMMARY

In the CFA-induced arthritic rats, the extracts of *Woodfordia fruticosa* leaves obtained after sequential solvent extraction demonstrated substantial antiarthritic potential. The assertion was confirmed by the histological and radiological examinations of the joint. The biological redox imbalance and the anemic state predisposed with the arthritic complications was greatly impacted by the extracts of woodfordia leaves. A tannin molecule woodfruticosin was isolated from woodfordia leaves extract with solvent-solvent partitioning, column and thin layer chromatographic techniques. The purified phyto-molecule was

characterized by the spectroscopic methods like UV, FTIR, LCMS, ^{13}C NMR and ^{1}H NMR techniques.

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