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RESEARCH ARTICLE

Anti-inflammatory Effect of *Woodfordia fructicosa* Leaves Ethanolic Extract on Adjuvant and Carragenan Treated Rats

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Abstract: ***Background*:** *Woodfordia fructicosa* is used traditionally for the treatment of inflammation associated with arthritis.

***Methods*:** In the present study, the anti-inflammatory activity of *W. fructicosa* (WFE) leaves ethanolic extract was assessed in Sprague Dawley rats by giving 200 mg/kg dose orally. Inflammation was studied by using carrageenan induced paw edema, Freund's adjuvant (FA) and monosodium iodo acetate (MIA) induced arthritis as animal models. Serum tumor necrosis factor-alpha (TNF-α) was estimated in blood sample of animals treated with FA. The one way ANOVA followed by Bonferroni's test was used for statistical analysis.

***Results*:** WFE significantly decreased ($P<0.05$, $P<0.001$) paw thickness in carrageenan induced paw edema and FA induced arthritis. The significant decrease in knee diameter ($P<0.001$) in MIA induced arthritis as well as inhibitory effect ($P<0.001$) on elevated TNF-α was observed.

***Conclusion*:** These results showed that the WFEexerted an inhibitory effect on TNF-α and carrageenan paw edema which may justify its traditional use in inflammatory conditions. Thus, the study shows that leaves of *W. fruticose* afford anti-inflammatory activity by preventing the inflammation in different animal models.

Keywords: Arthritis, carrageenan, ethanolic extract, freund's adjuvant induced, inflammation, monosodium iodo acetate, paw edema, tumor necrosis factor, *Woodfordia fructicosa.*

1. INTRODUCTION

W. fruticosa (*L.*) Kurz belongs to Lythraceae family occurring abundantly in India, and also in a majority of the countries of South East and Far East Asia [1]. Ayurvedic and Unani System of medicine reports its use in traditional medicine in the treatment of various ailments such as diarrhea [2], allergy [2], dysentery, intestinal parasites, skin diseases, ulcer, epistaxis, worms, *etc.* [3, 4]. Many marketed drugs comprise flowers, fruits, leaves and buds mixed with pedicels and thinner twigs of the plant and its leaves are used as a folk medicine in India and Nepal. Total 0.33% of volatile oil has been reported for *W. fruticosa* in 3 hours [5]. Many chemical compounds including tannins, flavonoids, anthraquinone glycosides, and polyphenols are reported to be present in *Woodfordia fruticosa* [6]. The presence of three dimeric hydrolysable tannins *viz*. Woodfordin A, B and C along with seven known hydrolysable tannins including oenothein B has been reported in the plant [6]. An amount of isoschimawalin 'A' is present in the tannin fraction of the plant.

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Inflammation is a complex phenomenon ever associated with cellular damage. Arthritis is one of the most common rheumatic disorders. Involvement of inflammation of synovial tissue in arthritis patients is well established with the involvement of immune cells and their cytokines in its pathogenesis [7]. Tumor necrosis factor *(TNF-α)* [7, 8] and interleukin 8 are considered as important inflammatory mediators associated with tissue damage. The anti-inflammatory activity of this plant has been reported by different researchers by using different models and extracts. Hydroethanolic extract of flowers of *W. fruticose* was evaluated for anti-inflammatory [9, 10] and antinociceptive activity [9]. Anti-inflammatory activity of flower extract has been reported against experimental asthma, induced by the combination of histamine and acetylcholine aerosol in guinea pigs [1]. Similarly,the methanolic extract of the flowers was evaluated for its analgesic and anti-inflammatory activity [11, 12]. It is reported that flavonoids, anthraquinones and macrocyclic ellagitannins from *W. fruticosa* inhibited LPS-induced *iNOS* and *COX-2* gene expression [10-13], which can be considered as one of the probable reasons for its anti-inflammatory potential. Thus on the basis of its traditional uses, reported chemical constituents and effect on *iNOS* and *COX-2* gene expression, the present investigation was designed to ascertain the effect of ethanolic extract of *W. fruticosa* on chemically induced inflammation.

2. EXPERIMENTAL

2.1. Materials and Methods

2.1.1. Plant Material and Chemicals

Leaves of *W. fruticosa* were collected in the month of October from 'Gharsi' village hills, Solan Himachal Pradesh, India. Plant was authenticated at the department of forestry, Dr. Y.S. Parmar University Solan, India and linked to UHF-Herbarium with field book number 12545. Carrageenan, Freund's adjuvant and monosodium iodo acetate were procured from Sigma Aldrich, Bangalore Genei. TNF-α was estimated using commercial ELISA kit procured from Genxbio, Delhi. All other chemicals used in present investigation were of analytical grade.

2.2. Animals

All animal experiments were performed with prior permission from Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Protocol Approval Reference No. PBRI/IAEC/12/PN-226). Sprague-Dawley rats of either sex weighing 200 ± 20 g were used in present investigation. Animals were randomly selected from animal house of PBRI and housed in a group of five in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (Golden feed, New Delhi) and water regularly. Animals were further divided in different groups with six animals in each group.

2.3. Extraction

Plant material was dried in shade and ground to coarse powder using acommercial grinder. Dried powder was defatted with petroleum ether and further extracted with ethanol at 40°C in Soxhlet's apparatus till complete exhaustion. The extract was coded as WFE and was dried in rotary vacuum evaporator. Dried extract was packed in an air tight container and preserved at 4°C till further use.

2.4. Acute Oral Toxicity (OECD 423)

WFE was dissolved in distilled water and administered orally at 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg to different animals as per the guidelines. Mortality was observed as requested in the guidelines. None of the animals showed any sign of toxicity at 2000 mg/kg, therefore a dose of 200 mg/kg (1/10th,) was selected as the dose for present *in- vivo* investigation.

2.5. Carrageenan Induced Paw Edema

Animals were divided into three groups. Animals of groups 1, 2 and 3 were given distilled water (10 ml/kg), extract (200 mg/kg) and Indomethacin (IND) (10 mg/kg), respectively, orally through oral gavage for five days. On fifth day paw edema was induced in experimental animals as per the previous reporting with slight modification [10]. Intraplantar carrageenan (0.3%) was injected into the hind left paw using a 26 gauge needle [13-15]. Paw thickness was measured with

electronic digital calipers at 1, 3 and 5 h following carrageenan administration. Percentage inhibition of edema was calculated using the formula:

% Inhibition = [(A control - A test)/ A control] x 100

2.6. Freund's Adjuvant (FA) Induced Arthritis

Newbould in 1963 induced arthritis in rats by mycobacterial adjuvant for the study of compounds of known value in the treatment of rheumatoid arthritis in man. In his work, the male pathogen free albino rats (200g) were used. The arthritis syndrome was induced by intradermal injection of dead tubercle bacilli in liquid paraffin B.P in the concentration of 5mg/ml through a no. 20 needle into the plantar surface of the right hind foot [16, 17].

The present study was completed with few modifications. Excluding group 1, rats of groups 2, 3, and 4 were administered 0.01 ml Freund's adjuvant on 0 day. Animals of groups 3 and 4 were administered IND (10 mg/kg) and WFE (200 mg/kg) respectively orally through oral gavage. Paw thickness was considered an indicator of arthritic condition. Vehicle, extract or Indomethacin was given to the animal 30 minutes before the administration of Freund's complete adjuvant and continued till the 28th day. Paw volume was measured on the 0^{th}, 7^{th}, 14^{th}, 21^{th} and 28^{th} day by using electronic digital calipers. On completion of the 28^{th} day, blood samples were collected by retro-orbital puncture and analyzed for total leukocyte counts (TLC), differential leukocyte counts (DLC), and TNF-α.

2.7. TNF-α Estimation

Serum was collected from rats treated as described above (FA-induced arthritis) and TNF-α was estimated in serum using standard protocol given by the manufacturer of a commercial kit (Genxbio) used in the present investigation.

2.8. Osteoarthritis Induced by MIA

Monosodium iodoacetate was injected intra-articularly in a total volume of 50 µl (2 mg) through the patellar ligament of the right knee for induction of experimental osteoarthritis, using a 26-gauge needle, in anaesthetized rats. The left knee joint (control) was injected with saline. Oral treatment with extract (200 mg/kg) was started on day 7 after MIA injection and was continued until the termination of the study. Another group of rats was administered IND (10 mg/kg) orally. The control group was treated with vehicle alone. Each experimental group included six animals. The diameter of the knees was measured every other day using electronic digital calipers [18].

3. STATISTICAL CALCULATION

All data were analyzed by One Way ANOVA followed by Bonferroni's test. $P<0.05$ was considered significant.

4. RESULTS AND DISCUSSION

4.1. Results

In acute oral toxicity, no mortality was observed in animals administered with WFE up to 2000 mg/kg thus $1/10^{th}$ of 2000 mg/kg *i.e.* 200 mg/kg was selected as the dose of WFE for assessment of its effect on carrageenan-induced paw edema and, FA and MIA-induced arthritis (Table **1**).

In carrageenan-induced paw edema, the effect of WFE started from the first hour of observation. The maximum edema occurred at 5 h and paw volume returned to almost baseline level by the end of 24 h, (only the 5 h data are reported in Table **2**, Fig. (**1**)). WFE significantly lowered paw edema as compared to vehicle-treated animals. The effect was more significant at the 5^{th} hour ($P<0.001$) as compared to the 1^{st} ($P<0.05$) and the 3^{rd} hour ($P<0.05$).

Table 1. Acute oral toxicity study of *W. fructicosa* ethanolic extract by using OECD-423 guidelines.

Dose	Lethality
	W. fructicosa
5 mg/Kg	0/3
50 mg/Kg	0/3
300 mg/Kg	0/3
2000 mg/Kg	0/3

Table 2. Effect of indomethacin and of *W. fruticosa* ethanolic extract on carrageenan induced paw edema.

Treatment	Dose	1 Hr	3 Hr	5 Hr
Control (Vehicle)	1 ml	$1.6 \pm 0.122^{*}$	$1.9 \pm 0.045^{*}$	$2.4 \pm 0.097^{\&}$
IND	5 mg/kg	$0.7 \pm 0.091^{\#}$	$0.6 \pm 0.094^{\#}$	$0.4 \pm 0.078^{\#}$
WFE	200 mg/kg	$1.3 \pm 0.099^{*,\#}$	$1.6 \pm 0.128^{\#}$	$1.8 \pm 0.082^{\&,\#}$

Data presented in Mean ± SEM (N=6).
* P<0.05 compared to vehicle treated group after 1 and 3rd hour.
& P<0.001 compare to vehicle treated group after 5th hour.

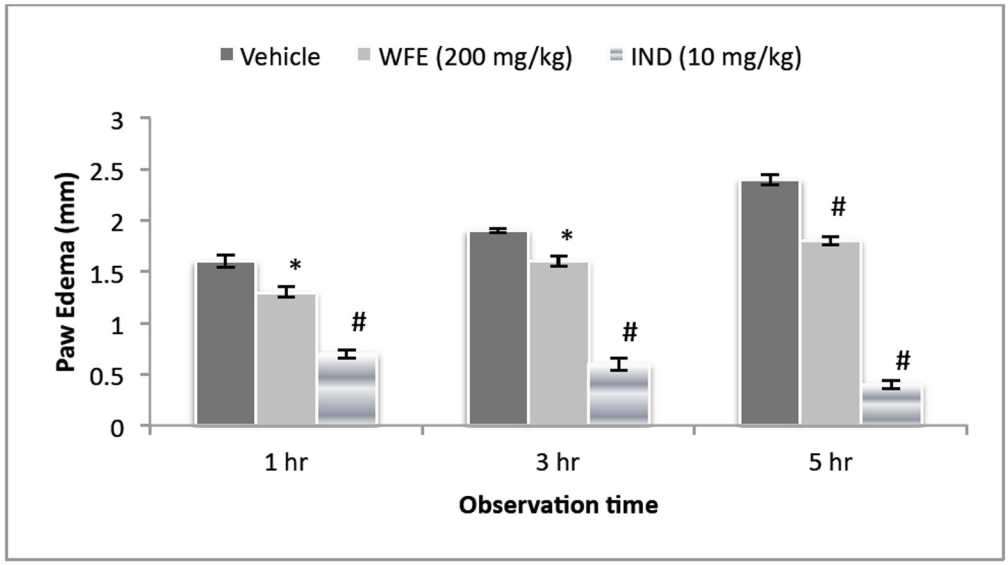

Fig. (1). Effect of WFE on carrageenan induced paw edema. Data presented in Mean ± SEM (N=6).
* P<0.05 as compared to vehicle treated group at 1st and 3rd hour.
# P<0.001 as compared to vehicle treated group at 5th hour.

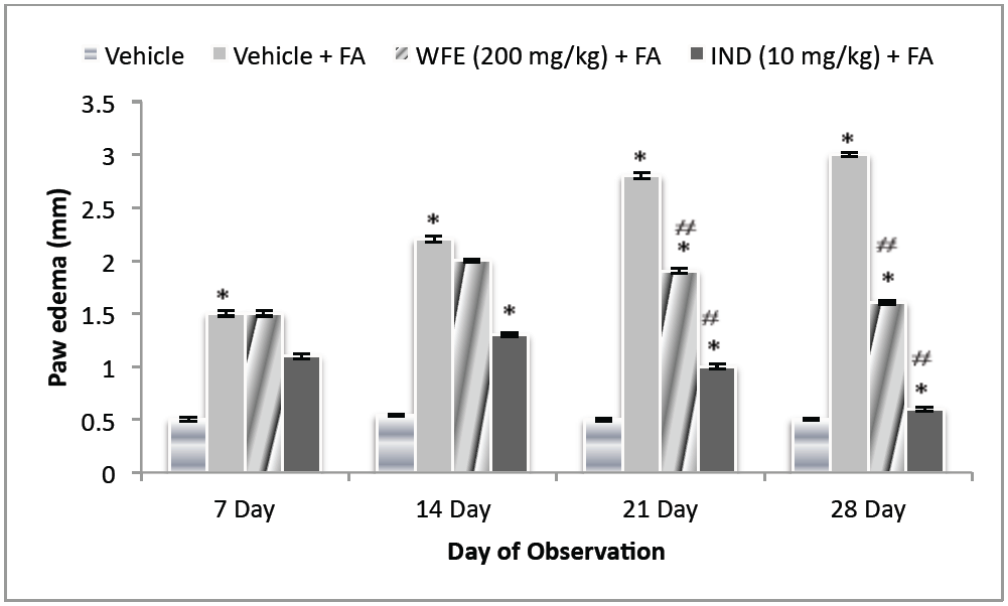

Fig. (2). Effect of WFE on Freund's adjuvant induced. Data presented in Mean ± SEM (N=6).
* P<0.001 as compared to vehicle + FA treated group.
# Showing significant values as compared to vehicle+ FA group.

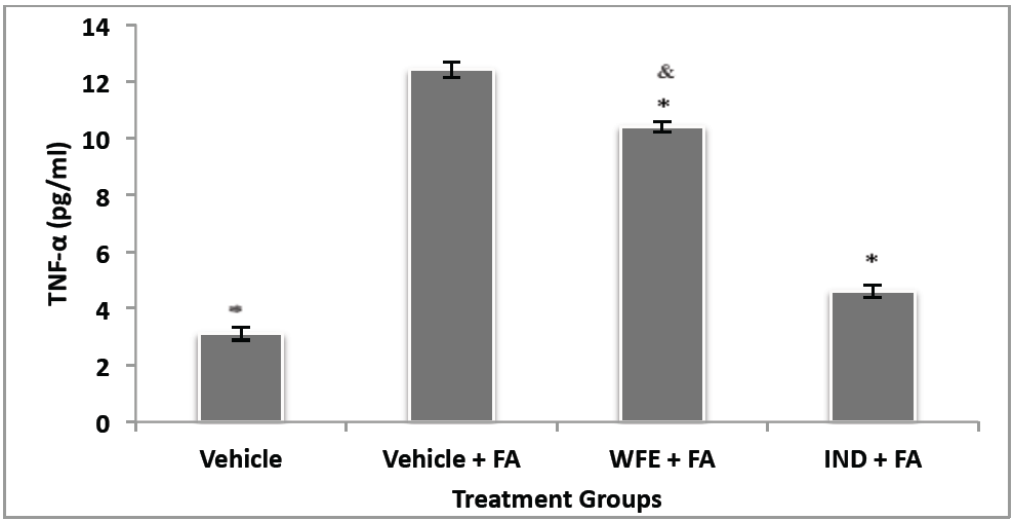

Fig. (3). Effect of WFE on FA induced elevation of TNF-α. Data presented in Mean ± SEM (N=6). [&]significant as compared to Vehicle+FA. * P<0.001 as compared to vehicle + FA treated group.

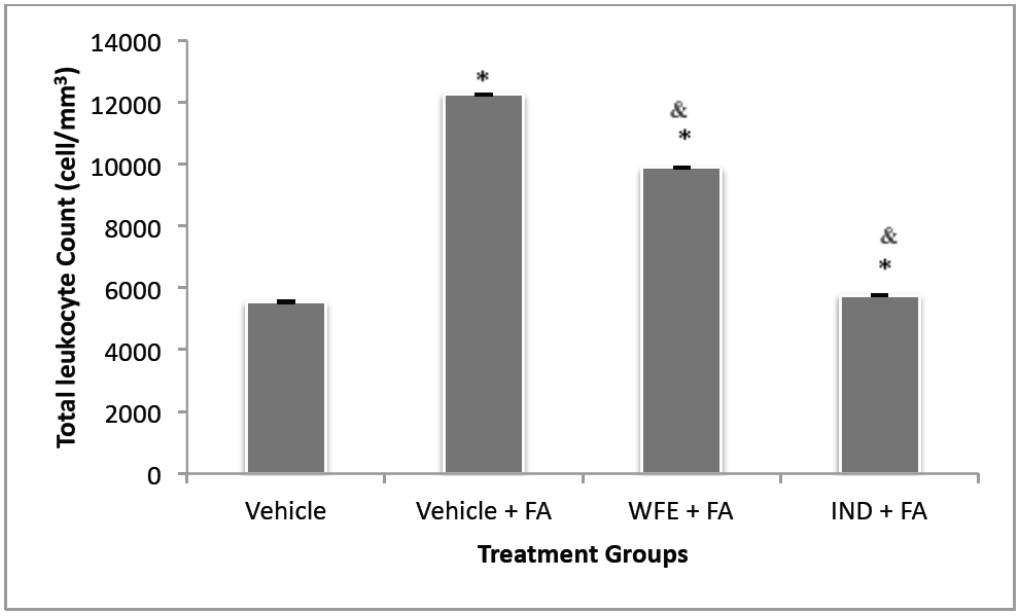

Fig. (4a). Effect of WFE on FA on TLC. Data presented in Mean ± SEM (N=6).
* P<0.001 as compared to vehicle + FA treated group. [&]Significant decrease in TLC level as compared to vehicle +FA treated group.

Investigation on FA-induced arthritic animal (Table **3**, Fig. **2**) revealed that WFE was significantly effective in reducing edema from the 14th day to the 28th day of observation. There was a progressive reduction in paw edema with an increase in time after FA administration. The TNF-α significantly increased (P<0.001) in rats treated with FA as compared to vehicle-treated rats (Table **4**, Fig. **3**). Both WFE and IND significantly reduced (P<0.001) the TNF-α levels. In FA treated animals total leukocytes count (TLC) (Table **5a**, Fig. **4a**), and differential leukocytes count (DLC) (Table **5b**, Fig. **4b**) were found to be significantly elevated (P<0.001) compared to vehicle only treated animals. In animals treated with WFE and IND, the level of TLC and DLC was significantly inferior (P<0.001) as compared to animals treated with vehicle and FA.

Osteoarthritis induced by monosodium iodoacetate in test animal revealed a gradual increase

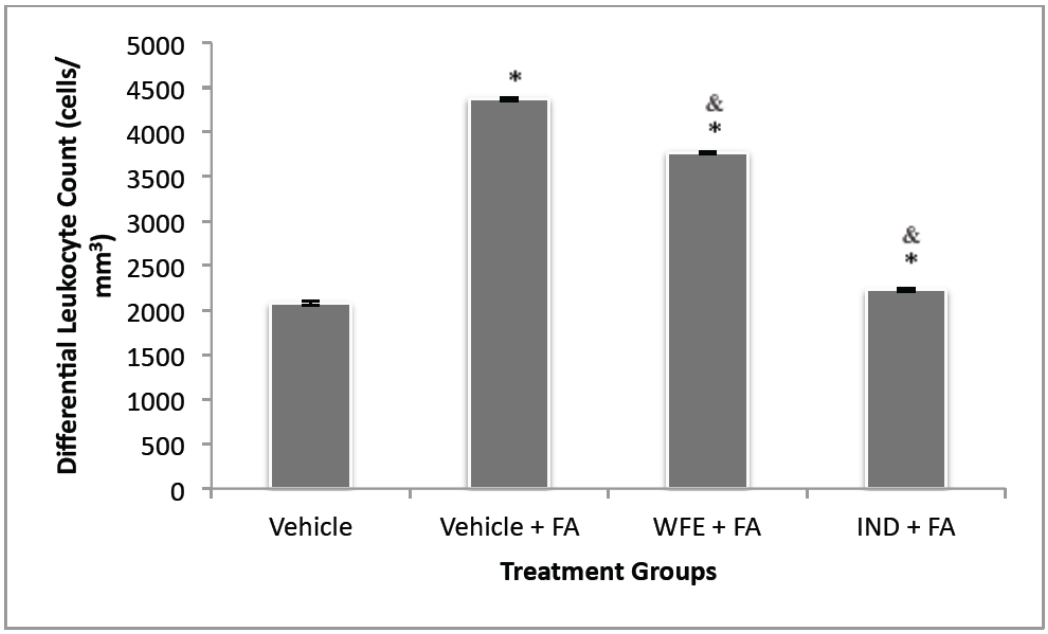

Fig. (4b). Effect of WFE on FA induced effect on DLC. Data presented in Mean ± SEM (N=6).
* P<0.001 as compared to vehicle + FA treated group.
[&]Significant decrease in DLC level as compared to vehicle +FA treated group.

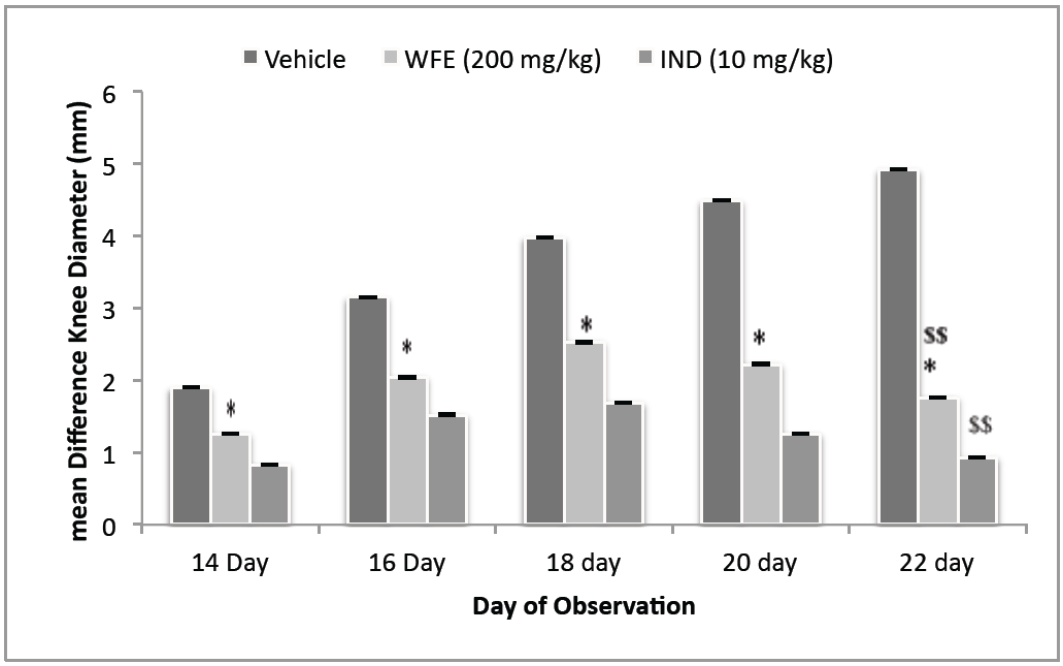

Fig. (5). Effect of WFE on MIA induced. Data presented in Mean ± SEM (N=6).
* P<0.001 as compared to vehicle treated group.
[$$]Significant decrease in knee diameter up to end of 22 day as compared to vehicle group.

in knee diameter from day 14 to day 22 (Table **6**, Fig. **5**). Administration of WEF and IND on the start from day 14 provided significant protection in osteoarthritis as indicated by decreasing knee diameter. The knee diameter increased on the 16th day as compared to the 18th day of observation in all treatment groups but in WFE and IND after the 18th day, knee diameter decreased significantly. Knee diameter of animals treated with WFE and IND was significantly inferior (P<0.001) as compared to vehicle-treated animals on each day of observation.

Table 3. Effect of WFE and IND on freund's adjuvant induced arthritis in rats.

Treatment	Dose	7 Day	14 Day	21 Day	28 Day
Vehicle	1 ml	0.5 ± 0.038	0.54 ± 0.020	0.5 ± 0.031	0.5 ± 0.026
FA	0.01 ml	1.5 ± 0.047	2.2 ± 0.051*	2.8 ± 0.062*	3.0 ± 0.037*
IND	10 mg/kg	1.1 ± 0.050*	1.3 ± 0.035*	1.0 ± 0.042*,#	0.6 ± 0.044*,#
WFE	200 mg/kg	1.5 ± 0.048	2.0 ± 0.035*	1.9 ± 0.040*,#	1.6 ± 0.033*,#

Data presented in Mean ± SEM (N=6).
*P<0.001 in comparision to FA and vehicle treated group.
Showing significant values as compared to vehicle+ FA group.

Table 4. Concentration of TNF -α after administration of IND and WFE in blood sample of rats.

Treatment	Dose	Concentration (pg/ml)
Vehicle	1 ml	3.1 ± 0.379*
FA	0.01 ml	12.4 ± 0.503*,&
IND	10 mg/kg	4.6 ± 0.379*,#
WFE	200 mg/kg	10.4 ± 0.300*,#,&

Data presented in Mean ± SEM (N=6).
* P<0.001 as compare to vehicle + FA treated group.
&significant as compared to Vehicle+FA.
less significant value as compared to FA+IND group.

Table 5a. Total leukocyte count (TLC) in blood samples from Vehicle, IND and WFE treated rats.

Treatment	Dose	TLC (Cell/mm^3)
Vehicle	1 ml	5542.5 ± 43.054
FA	0.01 ml	12268.5 ± 40.203*
IND	10 mg/kg	5777.5 ± 37.572*, &
WFE	200 mg/kg	989.7 ± 41.532*,&

Data presented in Mean ± SEM (N=6).
* P<0.001 as compare to vehicle group.
&Significant decrease in TLC level as compared to vehicle +FA treated group.

Table 5b. Differential leukocyte count (DLC) in blood samples from Vehicle, IND and WFE treated rats.

Treatment	Dose	DLC (Cell/mm^3)
Vehicle	1 ml	2077.7 ± 41.371
FA	0.01 ml	4369.7 ± 38.003*
IND	10 mg/kg	2228.2 ± 41.242*,&
WFE	200 mg/kg	3770.2 ± 29.307*,&

Data presented in Mean ± SEM (N=6).
*P<0.001 as compare vehicle and FA treated group.
&Significant decrease DLC level as compared to vehicle +FA treated group.

Table 6. Effect of WFE and IND on monosodium iodo acetate induced arthritis in rats.

Treatment	Dose	14 Day	16 Day	18 Day	20 Day	22 Day
Control (Vehicle)	1 ml	1.90 ± 0.0150	3.15 ± 0.0250	3.98 ± 0.0141	4.49 ± 0.0095	4.92 ± 0.0221
IND	10 mg/kg	0.83 ± 0.0095*	1.52 ± 0.0263*	1.69 ± 0.0095*	1.26 ± 0.0125*	0.93 ± 0.0208*,$$
WFE	200 mg/kg	1.26 ± 0.01470*	2.04 ± 0.0310*	2.53 ± 0.0275*	2.22 ± 0.0275*	1.76 ± 0.0150*,$$

Data presented in Mean ± SEM (N=6).
*P<0.001 as compare to vehicle treated group.
$$Significant decrease in knee diameter up to end of 22 day as compared to vehicle group.

4.2. Discussion

Herbs are considered to be an important source of bioactive components useful in the treatment of various ailments. Present investigation revealed that WFE possesses significant anti-inflammatory activity. *W. fructicosa* consists of flavonoids, anthraquinones and macrocyclic ellagitannins [13]. Anti-inflammatory activity of WFE observed in the present study can be attributed to the presence of these bioactive components [19-21].

The anti-inflammatory activity of this plant has been reported by taking ethanolic extract of *W. fruticosa* flowers [10]. Here the researcher used different models to induce inflammation such as carrageenan, autacoid induced hind paw edema, formaldehyde and cotton pellet granuloma in mice. Similarly, anti-inflammatory activity on ethanolic extract of the plant was evaluated on asthma induced by the combination of Histamine and acetylcholine aerosol in guinea pigs [10].

In a different study, the methanolic extract of flowers of the plant has been evaluated against carrageenan histamine, dextran, serotonin and formaldehyde induced hind paw edema [11]. Here the flower extract was tested by means of Brine shrimp bioassay. Two different doses of 400 and 600 mg/kg were evaluated for the anti-inflammatory activity against carrageenan, histamine, dextran, serotonin and formaldehyde induced rat paw edema [11].

Carrageenan-induced paw edema is one of the widely accepted models for screening of acute phase of inflammation. Mechanism of inflammation in this model can be divided into two phases, in which the first phase is associated with the release of histamine, serotonin [22], and kinins like mediators. On the other hand, the second phase is associated with the release of prostaglandins like mediators [23]. Most of the steroidal and nonsteroidal anti-inflammatory agents were found to be effective in the second phase [24-26] and they are related to COX-2 inhibition. Induction of paw edema by Carrageenan is associated with modulation of some inflammatory and proinflammatory mediators like prostaglandins, leukotrienes, histamine, bradykinin, and TNF-α [27]. In the present investigation, WFE was found to be effective in both stages of inflammation.

Onset and progression of polyarticular inflammation, bone resorption and bone proliferation are some of the reasons for wide acceptance of adjuvant-induced arthritis for investigation of the inhibitory potential of drugs acting on arthritis [26]. When FA is injected into the footpad, it allows studying the acute inflammatory reaction in that local area as well as the immunological reaction [27]. Among the various pro-inflammatory mediators TNF-α plays an important role in inflammation associated with arthritis [28]. The rationale of using anti-TNF-α therapy in patients with long-standing rheumatoid arthritis is based on the involvement of TNF-α in its pathophysiology. It is also suggested that other pro-inflammatory cytokines also get inhibited if TNF-α is decreased [29]. In the present study, it was observed that WFE not only decreased edema associated with the administration of adjuvant but it also decreased elevated TNF-α in serum. Further anti-inflammatory potential of WFE was also ascertained by using one another model of inflammation associated with arthritis, MIA-induced arthritis. After MIA injection, pain occurs during the initial stage of synovial inflammation [30]. In peripheral and cen-

tral sensory neurons cellular sensitization is having an important role in the initiation and maintenance of nociceptive transmission pain associated. In the present set of experiments, the most important investigation was that WFE was effective in the initial stage of induction as well as in the later stage [31, 32]. Thus the involvement of analgesic and anti-inflammatory activity can be attributed to the effect of WFE in MIA-induced arthritis.

The anti-inflammatory activity of *W. fructicosa* flavonoids and ellagitannins could be explained through different mechanisms, *e.g*. regulation of human gut microbiota metabolism or induction of antioxidant enzymes [33, 34].

Thus, the present study proves that the leaves of *Woodfordia fruticosa* provide anti-inflammatory and anti arthritic activity by preventing the inflammation in different animal models.

CONCLUSION

From the present investigation, it can be concluded that WFE possesses significant anti-inflammatory activity. The anti-inflammatory activity may be due to the presence of flavonoids. This study has established that ethanolic extract had a maximum anti-inflammatory activity which can be important in drug development for the formulation of single or multi-herbal drugs especially in the area of inflammation.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All animal experiments were performed with prior permission from Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal, India (approval no. PBRI/IAEC/12/PN-226)

HUMAN AND ANIMAL RIGHTS

No human were used in this study. Animal care and experiments were performed in accordance with OECD guidelines no. 423.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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