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Evaluation of anti-arthrotic activity of *Xanthoxylum oxyphyllum*, an indigenous plant

Jayant Chatterjee[1], Lipika Buragohain[1], Chandana Choudhury Barua[1], Pritam Mohan[1], Manisha Dutta[2]

[1] Department of Pharmacology and Toxicology, College of Veterinary Sciences, Assam Agricultural University, Khanapara, Assam, India

[2] Department of Food Science and Nutrition, College of Community Science, Assam Agricultural University, Jorhat, Assam, India

Abstract

Xanthoxylum oxyphyllum plant was evaluated for anti-arthrotic properties and mRNA/protein expression of few molecular markers like IL-10, NF-kB and Nrf2 in rats and mice. The Taxonomist of Assam Agricultural University identified the plant as *Zanthoxylum oxyphyllum* Edgew. which is synonym of plant *Xanthoxylum oxyphyllum* and provided collection number as 5176. The calculated LD50 was found to be below 2000 mg/kg and 1000 mg/kg was taken as the safe dose for further evaluation according to OECD-423 guidelines. Anti-arthrotic property of the plant was evaluated by the means of Adjuvant Induced Arthritis (AIA) model. Group I served as negative control and Group II served as positive control and received oral Meloxicam suspension. Group III, IV & V were administered 10, 30 & 100 mg/kg of ethanolic extract per oral, respectively. Body weight and paw volume of both paws were measured on the Ist day and Arthritis was induced by injecting 0.1 ml of FCA (Freund's Complete Adjuvant) into the left hind paw of all rats except negative control group. Paw volumes were measured to evaluate the therapeutic effect of the extract on primary lesions and inhibition of secondary lesions. Medium dose significantly increased body weight but could not inhibit primary lesions as efficiently as standard but inhibited secondary lesions by a great extent. The activation and upregulation of Nrf2 gene as confirmed by Reverse Transcriptase-PCR strengthened the antioxidant property. The ethanolic leaf extract of *Xanthoxylum oxyphyllum*, possesses Anti-arthrotic property as evidenced from the studies.

Keywords: anti-arthrotic, adjuvant induced arthritis (AIA) model, meloxicam, *Xanthoxylum oxyphyllum*, FCA (freunds complete adjuvant)

Introduction

Arthritis is one of the major causes of disability in the elderly people for getting admitted to skilled nursing facilities (Colvez & Blanchet, 1981) [2]. In general terms, it is a condition that affects the joints and surrounding tissues. It is very common in women and older adults (*Arthritis Basics* 2018) [1]. Common signs are swelling, pain, stiffness, and decreased range of motion. It can also cause permanent joint changes but damage is only seen through x-ray. Common types include Ankylosing Spondylitis, Gout, Septic arthritis, Osteoarthritis, Still's disease, Psoriatic Arthritis, Rheumatoid arthritis. Arthritis is also seen with other conditions like Lupus, Reactive arthritis, Lyme's disease etc. Drugs used for arthritis are Celecoxib, new generation NSAID used for relieving symptoms such as inflammation, swelling, stiffness, and joint pain. It's basically a COX-2 inhibitor and hence cuts out on few unwanted side effects. Drugs like DMARD's stand for disease modifying anti-rheumatic drugs are also in use nowadays. When all other medications fail Knee replacement surgery can also be considered as a last resort. Alternative therapies available for arthritis are acupuncture, massage, yoga & tai-chi (Han *et al*., 2004) [4]. A new drug called baricitinib is used for treatment and exhibits significantly less symptoms of disorder (Genovese, *et al.*, 2016) [3].

The current drugs used for treatment of arthritis have their own shortcomings. The NSAID's inhibit synthesis of protective prostaglandins (PGE2 & PGI2) through COX-1 & 2 binding thus back diffusing of H+ ions back into gastric mucosa. Deficiency of PG's reduces mucus and HCO3- secretion and may promote mucosal ischemia with increased gastric acid secretion leading to gastric mucosal damage. They also inhibit synthesis of both pro-aggregatory (TXA2) and anti-aggregatory (PGI2) prostanoids, but effect on platelet TXA2 (COX-1 generated) predominates therapeutic dose of most NSAID's inhibit platelet aggregation: bleeding time is prolonged. Risk of surgical bleeding is enhanced. Administration of NSAID's in late pregnancy has been found to promote premature closure of ductus arteriosus of infant in some cases (Tripathi, 2013) [11].

Even though steroids have been used to alleviate inflammation by a great deal, regular use of steroids has been discouraged by the practitioners due to various reasons. Although many new drugs have been introduced in the market but none show promising results in curing arthritis as many of them just alleviate symptoms. Hence keeping in mind the above shortcomings of the treatment the present study is undertaken to evaluate the anti-arthrotic properties of *Xanthoxylum oxyphyllum* plant. Local names are mezenga (assam), Timur, Bhansi (Nepal). The plants of this genus have been used by the tribals of north-east India since time immemorial for different purposes like analgesic for tooth uprooting, joint pains, and for keeping good health of the digestive, immune and joint health system of body. It is used as a local medicine and food and cultivated in India,

southern China, Bhutan Nepal, Myanmar (Medhi *et al.*, 2013) [7]. Keeping in view the above mentioned facts and theories, it was felt necessary to select the plant for this research work with the objective to evaluate the anti-arthrotic activity of *Xanthoxylum oxyphyllum* extract.

Materials and methodology

The materials and techniques used for performing the experiments are internationally acceptable. Adult healthy Wistar rats and albino mice were procured from Kolkata. All the animals were caged in polypropylene cages in small groups of 3 animals per cage. Animals had free access to standard balanced ration and clean drinking water *ad libitum*, and were kept in standard laboratory conditions. The use of experimental animals and the study protocol was duly approved by Institutional Animal Ethics Committee (IAEC) of the college.

The plant *Xanthoxylum oxyphyllum* was bought from the nearby local market and was sent to Taxonomic laboratory of Assam Agricultural University (AAU), Jorhat for authentication. Dried and crisp leaves were pulverized in the grinder, stored in air-tight containers for further use. The powdered leaves were soaked in 99% alcohol for 9 days and stirred after every 8 hours' interval for optimum extraction. Phytochemical tests were conducted on ethanolic extract of *X. oxyphyllum* as per standard procedures described by Sofowara (1993) [9], Trease and Evans (1989) [10] and Harborne (1998). Test for water solubility and acute toxicity of the extract were conducted according to standard guidelines. Evaluation of anti-arthrotic effect of ethanolic extract of *X. oxyphyllum* was done by Adjuvant Induced Arthritis method. Serum samples of the experimental animals were subjected for Western blot technique for protein expression of few molecular markers like IL-10 and NF-kB. Reverse transcriptase-PCR was done for mRNA expression of Nrf2 gene. The values were expressed as mean ± SEM and were subjected to statistical analysis by employing ANOVA using the software SAS.

Results

The ethanolic extract yield was found to be 6.38 g per 100 grams of dry powder, respectively. The extract was found to contain Alkaloids, Terpenoids, Flavonoids, Steroids, Tannins, Glycosides, phenols. It was found not to contain any Saponins. Ethanolic extract of the leaves of *X. oxyphyllum* @ 2000mg/kg body weight produced 100% mortality when administered orally mixed in the vehicle. Then 1000 mg/kg of the extract was administered per oral in 20% tween 80 solution as vehicle. All the animals survived in this category; hence it was taken as the safe dose.

Change in body weight as a measure of anti-arthrotic property of Xanthoxylum oxyphyllum

The body weight difference of different groups like Negative control, Positive control, standard, Test 1 (low dose @ 10mg/kg), Test 2 (medium dose @ 30mg/kg) and Test 3 (high dose @ 100mg/kg) are 7.60±2.29 g, -4.67±0.67g, 1.17±1.19 g, 5.83±2.91 g, 18.00 ±3.03 g and 4.17±1.76 g, respectively, where a negative sign (-) shows decrease in weight (Table 2).

Table 1: Grouping of animals to evaluate the anti-arthrotic property of ethanolic extract of *X. oxyphyllum* (Adjuvant Induced Arthritis model)

Group	No. of animals	Treatment
I	6	Negative control
II	6	Treatment control(0.1 ml of CFA in left hind paw+vehicle @20% tween80 soln)
III	6	Standard (0.1 ml of CFA in left hind paw + Meloxicam @5mg/kg)
IV	6	Test-1 (0.1 ml of CFA in left hind paw + low dose of extract @10 mg/kg)
V	6	Test-2 (0.1 ml of CFA in left hind paw + medium dose of extract @30 mg/kg)
VI	6	Test-3 (0.1 ml of CFA in left hind paw + high dose of extract @100 mg/kg)

Table 2: Effect of ethanolic extract of *x. Oxyphyllum* and meloxicam on body weight of animals in adjuvant induced arthritis:-

Treatment	Day 1 (Mean ± SE)	Day21 (Mean ± SE)	Paired Differences (Mean ± SE)	t (4)	*P-value*
N.control	75.17 ± 5.75b	82.40 ± 5.20b	7.60 ± 2.29	3.31	.030*
P.control	91.83 ± 4.45b	87.17 ± 3.97b	-4.67 ± 0.67	-7.00	.001**
Standard	98.83 ± 2.39b	100.00 ± 2.38b	1.17 ± 1.19	0.98	.374$_{NS}$
Test 1	126.50 ± 5.99a	132.33 ± 7.77a	5.83 ± 2.91	2.00	.102 $_{NS}$
Test 2	132.80 ± 9.92a	154.25 ± 12.37a	18.00 ± 3.03	5.95	.010*
Test 3	152.83 ± 8.77a	157.00 ± 8.61a	4.17 ± 1.76	2.37	.064 $_{NS}$

NS Not Significant; *Significant at P (<.05); ***Significant at P (<.01)
Numbers with different letters as superscript differ significantly

Percent (%) inhibition of swelling of injected paw volume at day 5 to evaluate primary lesions

As we can see from the values given in Table 3, standard drug Meloxicam has given maximum inhibition for left hind paw i.e. 28.3% whereas our extract at low, medium and high dose has given 5.11%, 8.11% and 3.48% respectively. Among the three groups the medium dose @ 30mg/kg has shown the maximum inhibition and is more effective than the higher dose.

Table 3: Effect of ethanolic extract of *X. oxyphyllum* and Meloxicam on injected paw edema (primary lesion) of animals in adjuvant induced arthritis

Treatment	Day 0 (Mean ± SE)	Day5 (Mean ± SE)	Paired Differences (Mean ± SE)	t (4)	*P-value*
N.control	0.53 ± 0.01a	0.55 ± 0.02a	0.01 ± 0.02	0.85	.434 NS
P.control	0.65 ± 0.02ab	1.37 ± 0.07bc	0.72 ± 0.07	9.7	<.001**
Standard (%inhibition)	0.77 ± 0.02bc	1.28 ± 0.06b	0.51 ± 0.05 (28.37%)	11.07	<.001**

Test 1 (%inhibition)	0.95 ± 0.05d	1.63 ± 0.06c	0.68 ± 0.04 (5.11)	18.48	<.001**
Test 2 (%inhibition)	0.82 ± 0.04bcd	1.48 ± 0.14bc	0.66 ± 0.13 (8.46)	5.00	.008*
Test 3 (%inhibition)	0.91 ± 0.07cd	1.6 ± 0.03c	0.69 ± 0.07 (3.48)	9.56	<.001**

NS Not Significant; Significant at P (<.05); **Significant at P (<.001)
Numbers with different letters as superscript differ significantly

Percent (%) inhibition of swelling of non-injected hind paw at day 21 to evaluate secondary lesions

Paw volume difference of right hind paw from day 0-21 of Negative control, Positive control, standard, Test 1 (low dose @ 10mg/kg), Test 2 (medium dose @ 30mg/kg) and Test 3 (high dose @ 100mg/kg) are given in Table IV. The percent inhibition of standard, low (10 mg/kg), medium (30 mg/kg) and high (100 mg/kg) dose of extract are 32.77%, 22.68%, 29.41% and 12.6% respectively. In the experiment, standard group has shown the maximum inhibition of right paw volume i.e. 32.77 % and among the three Test doses, medium dose (30 mg/kg) has shown maximum inhibition of right paw edema i.e. 29.41% which is comparable with the standard.

Table 4: Effect of ethanolic extract of *X. oxyphyllum* and Meloxicam on non-injected paw edema (secondary lesions) of animals in adjuvant induced arthritis

Treatment	Day 0 (Mean ± SE)	Day21 (Mean ± SE)	Paired Differences (Mean ± SE)	t (4)	*P-value*
N.control	0.55 ± 0.02a	0.58 ± 0.02a	0.03 ± 0.00	10.3	<.001**
P.control	0.65 ± 0.03ab	0.85 ± 0.03b	0.2 ± 0.01	22.73	<.001**
Standard (%inhibition)	0.77 ± 0.03bc	0.91 ± 0.03bc	0.13 ± 0.01 (32.77%)	9.04	<.001**
Test 1 (%inhibition)	0.93 ± 0.04c	1.08 ± 0.05c	0.15 ± 0.01 (22.68)	12.47	<.001**
Test 2 (%inhibition)	0.82 ± 0.04c	0.96 ± 0.05bc	0.14 ± 0.02 (29.41)	6.67	.003**
Test 3 (%inhibition)	0.9 ± 0.06c	1.08 ± 0.08c	0.17 ± 0.03 (12.60)	5.78	.002**

**Significant at P (<.001)
Numbers with different letters as superscript differ significantly

Evaluation of secondary lesions on 21st day with the help of arthritic score

The arthritic score is given on the basis of presence or absence of secondary lesions on different body parts of the animal and score given to Negative control, Positive control, standard, Test 1 (low dose @ 10mg/kg), Test 2 (medium dose @ 30mg/kg) and Test 3 (high dose @ 100mg/kg) (Table V). Here arthritic score of negative control is 0 as we have not induced the disease in them, but positive control has shown the highest score as there is no treatment given. As compared to the standard, low (10 mg/kg) and medium (30 mg/kg) dose of the extract have shown lower arthritic score indicating their ability to inhibit secondary lesions with not much difference with the standard. Out of the three doses of the extract, medium dose (30mg/kg) of extract has shown the best results when compared with others. Low dose (10mg/kg) of the extract has also shown better results as the score is near the standard. The high dose (100 mg/kg) of the extract has shown a much higher score than the other two groups indicating less efficiency to inhibit secondary lesions in arthritis disease.

Thus total percentage change can be calculated as:-

% inhibition of injected paw on day 5 + % inhibition of non-injected paw on day 21 + % change of arthritic index

a. Standard : 28.37 + 32.77 + 41.75 = 102.89
b. Low dose (10 mg/kg) : 5.11 + 22.68 + 33.25 = 61.04
c. Medium dose (30 mg/kg) : 8.46 + 29.41 + 41.75 = 79.62
d. High dose (100 mg/kg) : 3.48 + 12.69 + 16.6 = 32.77

Table 5: Effect of ethanolic extract of *x. Oxyphyllum* and meloxicam on arthritic score (secondary lesions) of animals in adjuvant induced arthritis

Treatment	Mean ± SE
N.control	0.00 ± 0.00 b
P.control	4.00 ± 0.47 a
Standard	2.33 ± 0.44 a
Test 1	2.67 ± 0.44 a
Test 2	2.33 ± 0.35 a
Test 3	3.33 ± 0.28 a

Means not similar by letter are significantly different.

Evaluation of anti-arthrotic property of Xanthoxylum oxyphyllum through molecular markers (IL-10, NF-kb, Nrf2)

Interleukin-10 gene expression is assessed with the help of western blotting as a measure of anti-inflammatory and anti-arthrotic activity of the plant. Upregulation of IL-10 denotes increased anti-inflammatory activity of the plant. Fig. 1, 2, 3 & 4 have been shown denoting the IL-10 activity in different group of animals in different days like 0 day, 5th day, 14th day and 21st day. As we can see in the figures, there is an upregulation of IL-10 expression in the groups receiving ethanolic extract of *X. oxyphyllum*. This shows that there is considerable anti-inflammatory activity in the test dose groups compared to the positive control.

Nuclear Factor kappa-light-chain-enhancer of activated B cells is a group of inducible transcription factors playing roles like DNA transcription, cell survival, especially playing a key role in regulating immune response to infection. Hence upregulation of this genetic factor denotes increased immunological response to inflammation which in this case is Rheumatoid arthritis in rat model. Fig. 1, 2, 3 and 4 denote the downregulation of NF-kB transcription factors indicating decrease in formation of inflammatory factors.

The Nuclear factor erythroid 2 (NFE2) - related factor 2 / (Nrf2) is a basic region leucine zipper (bZip) transcription factors which basically control regions necessary for erythropoiesis and platelet development. Nrf2 was found to be responsible for induction of a group of drug-metabolizing enzymes (DMEs) like glutathione s-transferase and NAD (P) H: Quinone oxidoreductase 1, by antioxidants and electrophiles. So basically Nrf2 functions as a xenobiotic-activated receptor (XAR) to regulate adaptive response to electrophiles and oxidants. As can be deduced from Fig. 5, 6, 7 and 8 there is an upregulation of this genetic factor named Nrf2 signalling notable anti-oxidant property. Thus the plant shows considerable antioxidant effect and thus acts as a protectant from free radical damage caused by chronic Rheumatoid arthritis, hence counteracting the inflammation factors.

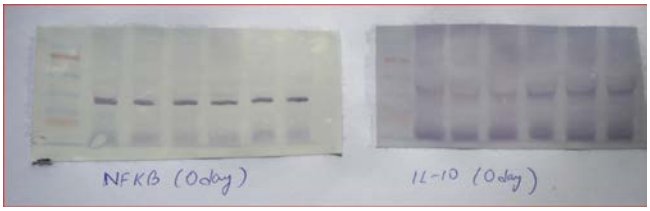


Fig 1: Bands Representing protein expression of NF-kB & IL-10 in western blotting on 0 day

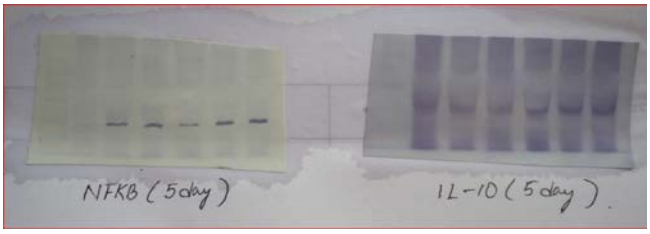


Fig 2: Bands representing protein expression of NF-kB & IL-10 in western blotting on 5TH Day

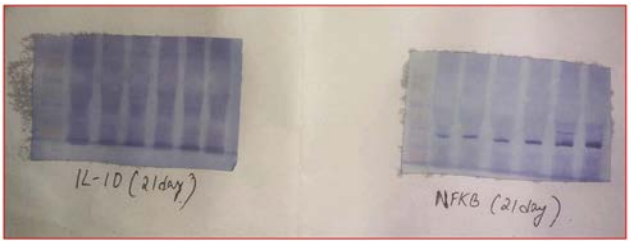


Fig 3: Bands representing protein expression of NF-kB & IL-10 in western blotting on 14TH day

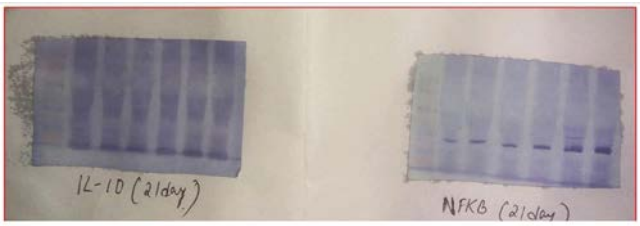
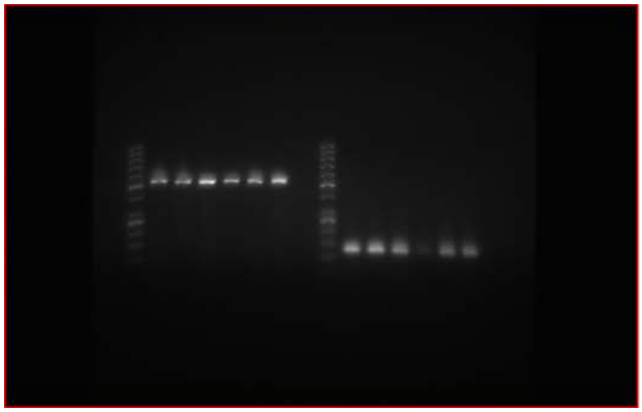
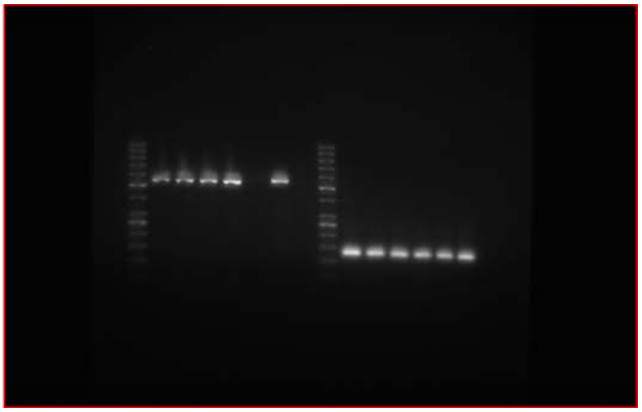


Fig 4: Bands representing protein expression of NF-kB & IL10 in western blotting on 21ST day.

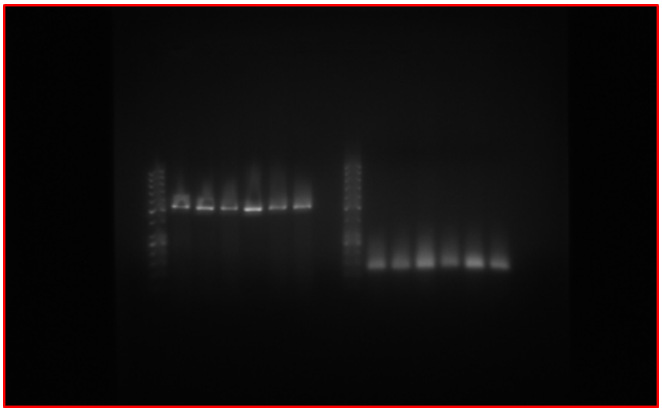


Day 0

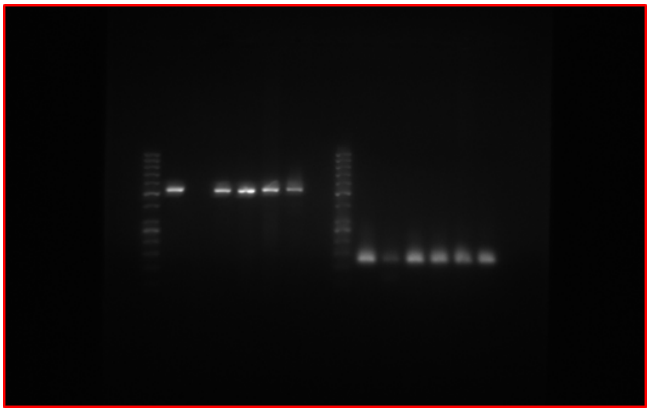


Day 5

Fig 5 & 6: Reverse transciptase - PCR Bands representing Nrf2 gene expression on day 0 and day 5 of experiment



Day 14



Day 21

Fig 7 & 8: Reverse transcriptase - PCR bands representing Nrf2 gene expression on Day 14 and Day 21 of experiment

Radiographic evaluation of the arthrotic lesions of RAT PAW

Radiographic images of the hind paws of representative animals of each group were taken with the help of X-ray machine available in clinical complex of the college. The images of different groups like negative control, positive control, standard, low dose (10mg/kg), medium dose (30mg/kg) and high dose (100mg/kg) are given in Fig. 9, 10, 11 and 12 respectively. As it can be perceived from close examination of the paws, positive control has the highest swelling compared to other groups and medium dose has given the least swelling among the test groups.

Thus, the radiographic images portray that medium dose (30 mg/kg) of ethanolic extract of *X. oxyphyllum* has shown significant anti-arthrotic properties among all the test groups.

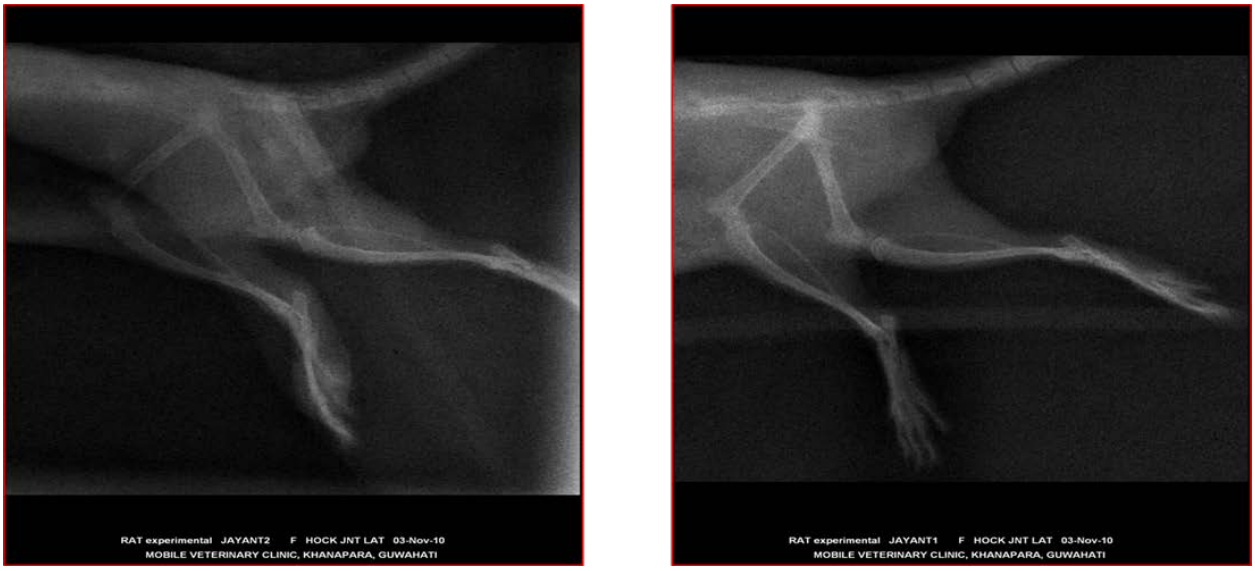


Fig 9: Digital radiograph showing PAW edema as arthrotic lesion in positive control animal

Fig 10: Digital radiograph showing paw edema as arthrotic lesion in negative control animals

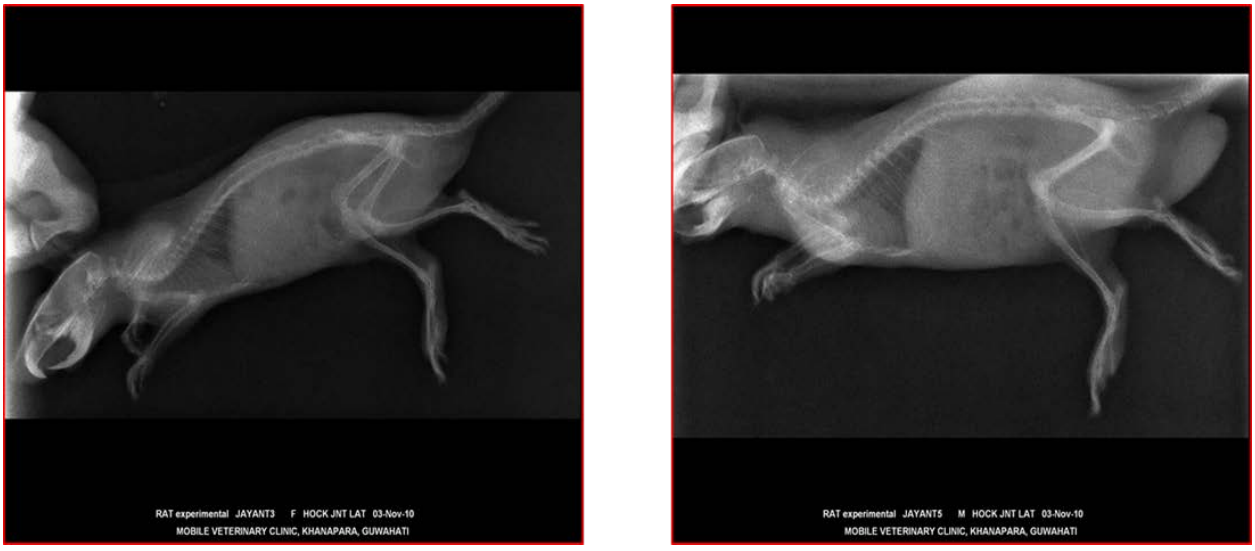


Fig 11: Digital radiograph showing paw edema as arthrotic lesion in group receiving *x. oxyphyllum* @30 mg/kg

Fig 12: Digital radiograph showing PAW EDEMA as arthrotic lesion in group receiving standard drug meloxicam

Discussion

For evaluating the anti-arthrotic properties of the ethanolic extract of *Xanthoxylum oxyphyllum*, Adjuvant-Induced Arthritis (AIA) model was followed. The difference in body weight when observed between day1 and day 21 i.e. before and after induction of disease, positive control has shown a mean decline in weight by 4.67± 0.67 grams as it was not given any treatment for RA (rheumatoid arthritis). The three doses of ethanolic extract of *Xanthoxylum oxyphyllum* i.e. 10, 30 & 100 mg/kg has shown mean increase in body weight by 5.83±2.91 g, 18.00±3.03 g and 4.17±1.76 g respectively, while the body weight gain in the standard control was 1.17±1.19 g which was found to be significantly lower than the test drug. The Freund's Complete Adjuvant (FCA) was eliciting an immunological response which affected the feed intake and body weight gain of animals. However, the standard control and the ethanolic extract of *Xanthoxylum oxyphyllum* could inhibit the negative effect of FCA on feed intake and body weight gain.

The left paw volume of animals in each group was measured on Day 0 and Day 5. On 5th day, the group receiving Meloxicam showed an inhibition of 28.37% as compared to the positive control while the test drugs @ 10, 30 & 100 mg/kg body weight showed an inhibition of 5.11%, 8.46% & 3.48%, respectively, as compared to the positive control. The inhibition of left paw volume edema signifies the ability of the drug to inhibit Primary lesions. Though the test drug @ 10, 30 & 100 mg/kg could inhibit the left paw edema, but it was not comparable to the standard drug Meloxicam. Right paw swelling of all groups were measured on Day 0 & Day 21 and compared with the positive control to determine the ability of the extract to inhibit secondary lesions. Ethanolic extract of *Xanthoxylum oxyphyllum* @ 10, 30 & 100 mg/kg could inhibit the rat paw edema @ 22.68%, 29.41% and 12.60% respectively as compared to the Positive control. In the group receiving the standard drug i.e. Meloxicam, rat paw edema inhibition was found to be 32.77%. The group receiving ethanolic extract

@ 30 mg/kg body weight showed comparable results with that of standard group.

An Arthritic score was given to all the animals as a measure of evaluating the secondary lesions on the last day i.e. 21st day of the experiment. The percent change in arthritic index was also noted as compared to positive control. The treatment control received an overall score of 4±0.47 whereas the ethanolic extract of *Xanthoxylum oxyphyllum* @ 10, 30 & 100 mg/kg received an overall score of 2.67±0.44, 2.33±0.35 and 3.33±0.28, respectively. In the group receiving standard drug i.e. Meloxicam, arthritic score was found to be 2.33±0.44. The group receiving ethanolic extract @ 30 mg/kg body weight showed similar results with that of the standard.

The total percentage change in arthritis was calculated by addition of left paw edema percent inhibition + right paw edema percent inhibition + percent change in arthritic index. The total percentage change seen in groups receiving ethanolic extract of *X. oxyphyllum* @ 10, 30 and 100 mg/kg are 61.04%, 79.62% and 32.77% respectively as compared to the Positive control. In the group receiving standard drug i.e. Meloxicam, total percentage change seen was 102.89%. The group receiving ethanolic extract @ 30 mg/kg showed comparable results with the standard.

In RA, the primary driving force behind joint inflammation is activated CD4 T helper cells and macrophages. The synovial macrophages produce pro-inflammatory cytokines like IL-1 and TNF-α which play a critical part in stimulating synovial inflammation. By upregulating the synthesis of endogenous inhibitors such as IL-1 antagonist, soluble TNF-α and IL-10. They act together to dampen the inflammatory response. As it is being observed that there is a relatively unique ability in IL-10 to downregulate the production of multiple pro-inflammatory cytokines, it leads to the idea that IL-10 would be an effective treatment for RA (Clair, 1999). In the present study, *Xanthoxylum oxyphyllum* treated group showed increased levels of IL-10 protein expression when compared with positive control group, suggesting that ethanolic extract of *Xanthoxylum oxyphyllum* might possess good anti-inflammatory activity.

Due to activation of the NF-kB pathway, there is a transactivation of a plethora of responsive genes that contribute to inflammatory responses, including TNF-α from macrophages, matrix metalloproteinases from synovial fibroblasts and chemokine that recruit immune cells to the inflamed pannus, all this occurring in the synovial cells of patients with Rheumatoid arthritis (RA). From the time of their discovery they have been linked to many different pathways like inflammation, cell survival, proliferation and differentiation. Activation of NF-kB is usually pro-inflammatory. The synovial fluid of a RA patient has been observed to have a lot of effector molecules like pro-inflammatory cytokines (such as IL-1β, IL-6, TNFα and IL-18), chemokines (such as IL-8, IP-10, MCP-1, MIP-1, and RANTES), matrix metalloproteinases (MMPs, such as MMP-1, -3, -9 and -13) and metabolic proteins (such as Cox-1, Cox-2 and iNOS). All these factors interact with each other leading to a vicious cycle of proinflammatory signals resulting in chronic and persistent inflammation (Muller-Ladner *et al.,* 2005). Factors like TNFα and many of the factors, stated above, are known to be under the control of NF-kB transcription factors. Hence, it can be deducted that NF-kB could be one of the master regulators of inflammatory cytokine production in RA. (May and Ghosh, 1998). In the current study, ethanolic extract of *Xanthoxylum oxyphyllum* treated group showed decreased expression of NF-kB, as compared with positive control group. Thus, it may be suggested that the plant extract may possess chronic anti-inflammatory properties especially anti-arthrotic in this context.

Due to induction of the vast amount of proinflammatory cytokines in the site of injury in chronic inflammation cases like in case of joint fluid in RA, there is generation of Reactive Oxygen Species (ROS) which damage macromolecules including DNA. Studies have revealed that transcription factor Nrf2 (Nuclear factor erythroid 2–related factor 2) regulates the expression of phase II detoxifying enzymes like NADPH, NAD(P)H Quinone oxidoreductase 1, glutathione peroxidase, ferritin, heme oxygenase-1, and antioxidant genes that protect cells from various injuries via their anti-inflammatory effects, thus influencing the course of disease. The ROS or free radicals are then neutralized with the help of these free radical scavenging enzymes and provide anti-inflammatory response which may lead to faster recovery from disease. In the present study, ethanolic extract of *Xanthoxylum oxyphyllum* treated group showed up-regulation of Nrf2 mRNA expression, suggesting that the plant has a good amount of anti-oxidant properties which might be due to presence of flavonoids and tannins as phytoconstituents.

X-RAY radiography of the affected limbs of a sample representative from each of the six groups was conducted to assess the bone and soft tissue conditions. It can be interpreted from the radiographs that the positive control animals had the maximum swelling and the animals receiving ethanolic extract of *X. oxyphyllum* had comparatively lesser swelling than the positive control. The group receiving 30 mg/kg of ethanolic extract of *X. oxyphyllum* had the lowest amount of swelling even compared to the standard group of animals receiving Meloxicam.

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