EFFECT OF FICUS GLOMERATA ROXB LATEX ON BEHAVIOURAL OF ADJUVANT-INDUCED ARTHRITIC RATS.

*Madan Kaushik, I.S.Muchchandi, H.R. Chitme, V. M. Chandrasheker
*Department of Pharmacology, S.D.M. College of Pharmacy, Rajound (HR) -136044
Department of Pharmacology, H. S .K. College of Pharmacy, Bagalkot (Karnataka) -587101

Abstract

Arthritic is one of the major disease affecting most population. Ficus Glomerata Roxb latex was evaluated for its antiarthritic activity on FCA induced arthritic rats. These are broadly categorized as psychosis, neurosis & mood disorder. All the animals were subjected to open field test before the induction of arthritic & there after every week up to 6 weeks (42 days) rats were placed in an open field in the sound attenuated room. The floor was white poly vinyl with a black gride dividing open field 84 squares (10x10cm) illumination was provided by a bulb (60w) placed above center of the field, while the rest of room was in darkness. The rat was initially placed in the center of the field and observed for 5 min in all test. Latency time to start explores the open field (second) horizontal locomotor activity (grids lines crossed), vertical locomotors activity (rearing), grooming (rubbing the nose its fore paws & preening), incidence of defecation (number of boluses), & number of urinations was recorded. Control group was compared with normal, indomethacin and latex treated groups compared with control. The results showed significant ($p<0.01$) increase in ambulation behavior on 6th week. Latency time has shown gradual and significant ($p<0.05$) delay in exploration ability. Grooming behavior was completely variable. A significant ($p<0.05$) decrease in frequency of urination and defecation is observed. The open field test for Ficus Glomerata Roxb Latex has shown appreciable result by impairing intension for improvement, weight bearing capacity and increased in threshold of pain.

Key words : Latency time, horizontal locomotor, rearing, grooming, Ficus Glomerata Roxb, antiarthritic and Indomethacin

1. Introduction

Rheumatoid arthritis (RA) is most common form of chronic autoimmune disease characterized by a relapsing and remitting course of joint inflammation. It has worldwide prevalence of about 1% of the adult's population. It is more being in women than the men, with an annual incidence of 3 per 10,000 adults. Recently, it has been reported that microorganism including bacteria, viruses, fungi, parasites, bacterial DNA and bacterial toxin may exacerbate the inflammatory response at the joint and bone. *M. tuberculosis* and *M. leprae* are the most severe and more common mycobacterium causing joint and bone diseases.

Ficus glomerata roxb (*Moraceae*) is commonly known as Gular, traditionally its latex is useful in different disorders and has been recommended to be useful in pain, inflammation, wound healing, piles, diarrhoea, dysentery, astringent, stomachic, carminative. Recently, the plant has been studied scientifically for its analgesic, antipyretic, antidiabetic, hepatoprotective, antifungal and antibacterial activities. In the literature no scientific work has so far been reported on the antiarthritic activity of latex of this plant. The present paper reports the antiarithritic activity of latex of this plant.

2. Materials and method

Plant material

Ficus glomerata roxb latex was collected during October to November 2006 from Karnal (Haryana, India) and was authenticated by plant Taxonomist Prof. V. V. Siddhalingappanavar. The latex was collected from tree by a diagonal cut angled downward made through the bark; these cuts extend the trunk. The latex exuding from the cut was collected in a small cup. The amount of latex obtained on each tapping was about 0.5 ml.

Drugs and chemicals

Freund's Complete Adjuvant (FCA) composed of 1mg/ml heat killed *Mycobacterium tuberculosis* were purchased from Sigma Aldrich,

*** Corresponding Author:**
Madan Kaushik,
Department of Pharmacology, S.D.M. College of Pharmacy, Rajound (HR) -136044

Bangalore (India). Indomethacin was obtained from U-Medico Laboratories Pvt. Ltd., Vapi, Gujarat (India). All other chemicals were of analytical grade and were obtained from Qualigen Fine Chemicals, Mumbai (India).

Experimental animals:

Female albino rats (Wistar strain) weighing 150-170 g were used for the present study. Food and water were supplied (*ad libitum*) and the animals were kept in a 12 h: 12h light and dark cycle. All the animals were maintained under controlled temperature (27±2^{0}C). All the experiments were conducted in accordance with the direction of Institutional Animal Ethics committee, CPCSEA, Government of India (Approval No.: HSKCP/IAEC/ 2005-6/1-8). Due to painful condition imposed on animals, the numbers of subjects used were restricted to the minimum six per group that allowed reliable statistical analysis of the results.

Acute Oral Toxicity

Healthy nulliporus female Swiss albino mice weighing 15-30 g, starved over night were divided into 3 groups (n =3) and were fed with increasing dose (30, 300, and 3000 mg/kg) of the latex. The toxicity was evaluated as per the Guidelines for non clinical-toxicity, Investigation of Herbal medicine (Annexure -1) given by the Ministry of Health and family Welfare, Govt. of India and OECD guideline 425. The total latex administered orally in doses up to 3 g/kg did not show any signs of toxicity and mortality in mice, when observed for 14 days after administration. The doses selected were 50, 100, 200, 400 and 500mg/kg.

Preparation of test solution and administration drugs

Test solution of latex prepared in normal saline was administered orally using oral catheter from day 1 to day 42. Dose calculation was based on w/w of the latex. The animals were divided into 8 groups of 6 animals each .The first group served as Normal group and recieved normal diet and water *ad libitum*. The second and third group recieved normal saline (10ml/kg) and indomethacin (10mg/kg) respectively and served as control and standard group respectively. The fourth, fifth, sixth, seventh and eighth group recieved latex solution orally at 50, 100, 200, 400 and 500mg/kg dose respectively and served as test groups.

Open Field Test behavior

For behavioural observations all the animals were subjected to open field test before the induction of arthritis and there after every week up to (42-days). Briefly, rat was placed in an open field in the sound-attenuated room. The floor was white polyvinyl with a black grid dividing open field into 84 squares (10×10). Illumination was provided by a bulb (60 W) placed above the center of the field, while the rest of the room was darkened. The rat was initially placed in the corner or in center of field and observed for 5 minutes in all tests, latency time to start explore the open field (sec), horizontal locomotor activity (grids lines crossed), vertical locomotor activity (rearing), grooming (rubbing the nose with its forepaws and preening), incidence of defecation (number of boluses), and number of urinations and defecations were recorded. Between trials the box was cleaned with wet sponge and tissue paper. All the observations were made between 18.00 and 20.00 h.

Statistical Analysis:

All the values are expressed as mean ± SEM, Statistical analysis by one way ANOVA followed by Dunnets test, $P< 0.05$ was considered as significant.

3. Results and discussion

All test parameters shown in table-1, the rat of the control group treated with FCA alone shown a gradual decrease in ambulatory behaviour and it was significant ($P<0.01$) on 6th week of the study, when compared to ambulatory behaviour prior to induction of arthritis. Indomethacin treatment has no significant effect on improvement in mobility condition, however, the animals of this group have significantly ($p<0.05$) declined ambulatory behaviour up to 5th week of study, but it caused complete reversal of arthritis anti-ambulatory behaviour on 6th week. Treatment of arthritic rat with 100 mg/kg dose of latex has shown significant ($p<0.05$) improvement in ambulatory behaviours from 1st week to last day of the study. The toxic dose 500 mg/kg has shown significant increase in number of horizontal movements in the 1st week but it failed to maintain its effect from 2nd week of the study. The result of the statistical analysis has shown no significant difference in ambulatory behaviour of arthritic rats when treated with 50, 200, and 400 mg/kg dose of latex.

Effect of *Ficus Glomerata Roxb* showed gradual decrease in spontaneous activity in control group of animals as the rearing behavior of arthritic rats decreased significantly ($P< 0.05$) from 3rd week to last week of the study. Treatment with indomethacin and latex has shown no significant change in rearing behaviour of arthritic rats throughout the study. And the grooming activity of arthritic rats, the result obtained by this study is completely variable in term of control and treatment group of animals. The latency time to explore in FCA-induced arthritic rats has shown gradual and significant ($p<0.05$) delay in exploration ability. Treatment of arthritic rats with indomethacin for 42 days has shown an appreciable and significant decrease in latency time to explore the open field. Treatment of FCA-induced arthritic rats with all the doses of latex significantly improved the condition by decreasing latency time to explore the open field from 1st week to throughout the study. The angiogenic and anxiolytic effect of treatment on FCA-induced arthritic rats was studied by considering the frequency of urination and defecation in five minute of exploratory FCA, induced anxiogenic behaviour significantly ($p<0.05$) in 3rd week of the study till the completion, when compared to normal group of animals. Indomethacin treatment has no significant effect till the first week of the study. However, it has shown significant anxiolytic effect by decreasing the frequency of urination and defecation from 2nd week to 6th week of the study. Significant ($P< 0.05$) decrease in frequency of urination and defecation was observed by the treatment of all doses of the latex from 1st week of the study. Based on these results and previous studies we validate the traditional and folk claims on the use of *Ficus glomerata* latex showed the effect on behavioural parameters in the treatment of arthritis.

4.Conclusion

It has also been suggested that FCA-induced rheumatoid arthritis has a wide spread effect on physiological homeostasis due to the severe discomfort in animals. In our experiments, the control arthritic rats treated with saline shown significant and gradual decrease in ambulatory and rearing behaviour. Whereas, significant gradual increase in number of grooming and increased in frequency of urination and defecation along with increased latency time to explore was observed when they have been exposed to the open field test. Treatment of arthritic animals with latex and indomethacin significantly improved ambulatory behaviour without affecting on rearing and also shown significant reduction in grooming and frequency of urination and defecation. The latency time to explore the open field was significantly ($p<0.05$) reduced in all the arthritic animals of treatment groups. These observations supports the efficacy of latex treatment in behavior modulation induced by arthritis by decreasing irritation, anxiety, ability to bear the pressure on inflamed paw, increased intention to walk. This shows the possible applicability of the latex in symptomatic treatment of arthritis .

Table: 1 : Effect of *Ficus glomerata* latex on behavioural of adjuvant-induced arthritic rats

Parameters	Treatment	0 week	1st week	2nd week	3rd week	4th week	5th week	6th week
Ambulatory	Normal	↑↓	↑	↓	↓	↓	↓	↓
	Control	↑↓	↓	↓	↑	↓	↓	↓**
	Indomethacin 10 mg	↑↓	↓	↓	↓	↓	↓*	↑
	Latex 50 mg	↑↓	↓	↓	↓	↓	↓	↓
	Latex 100 mg	↑↓	↑*	↑**	↑**	↑**	↑	↑**
	Latex 200 mg	↑↓	↑	↑	↓	↑	↑	↑
	Latex 400 mg	↑↓	↑	↑	↑	↓	↓	↓
	Latex 500 mg	↑↓	↑	0	↑	↑*	↓*	↓*
Rearing	Normal	↑↓	↓	↑	↓	↓	↓**	↓**
	Control	↑↓	0	↓	↓*	↓*	↓**	↓**
	Indomethacin 10 mg	↑↓	↓	↓	↓	↓	↓	↑
	Latex 50 mg	↑↓	↓	0	↓	↑	↓	↓*
	Latex 100 mg	↑↓	↓**	↓	0	0	0	↑
	Latex 200 mg	↑↓	↑	↑	↑	↑	↑	↑
	Latex 400 mg	↑↓	↓	↓	↓	↓	↓	↓
	Latex 500 mg	↑↓	↓	0	↓	0	↓*	↓**
Grooming	Normal	↑↓	↑	↑**	↓**	↓	↓	↓**
	Control	↑↓	↓**	↑*	↓	↓	↓**	↓**
	Indomethacin 10 mg	↑↓	↑	↑	0	0	↑	↑
	Latex 50 mg	↑↓	↑	↓**	↑**	↓**	↓**	↓**
	Latex 100 mg	↑↓	↑	↑	↑**	0	↑	↓
	Latex 200 mg	↑↓	↑	↑	↑	↑	↑**	0
	Latex 400 mg	↑↓	↑**	↑**	↑	↑	↓**	↓**
	Latex 500 mg	↑↓	↑	↑	0	↓*	↓**	↓**
Latency time to explore	Normal	↑↓	↑	↑	↑	↓**	↓**	↓**
	Control	↑↓	↑	↑	↑*	↑*	↑*	↑*
	Indomethacin 10 mg	↑↓	↓*	↓*	↓**	↓**	↓**	↓**
	Latex 50 mg	↑↓	↓**	↓**	↓**	↓**	↓**	↓**
	Latex 100 mg	↑↓	↓	↓*	↓**	↓**	↓**	↓**
	Latex 200 mg	↑↓	↑	0	↑*	↑*	↓**	↓
	Latex 400 mg	↑↓	↑	↓**	↓	↓**	↓**	↓**
	Latex 500 mg	↑↓	↓**	↓**	↓**	↓**	↓**	↓**
Urination,	Normal	↑↓	↓	↑	0	↓	↓	↓*
	Control	↑↓	↓	↓	↓*	↓*	↓*	↓*
	Indomethacin 10 mg	↑↓	↑	↓*	↓*	↓	↓*	↓*
	Latex 50 mg	↑↓	↓*	↓*	↓	↓	↓*	↓*
	Latex 100 mg	↑↓	↑	↓*	↓*	↓	↓*	↓*
	Latex 200 mg	↑↓	↓*	↓*	↓*	↓*	↓	↓*
	Latex 400 mg	↑↓	↓*	↓*	↓*	↓*	↓	↓*
	Latex 500 mg	↑↓	↓*	↑*	0	↓*	↓*	↓*
Defecation	Normal	↑↓	0	↓	↓	↓	↓	↓
	Control	↑↓	↓	↓	↓*	↑*	↓*	↓*
	Indomethacin 10 mg	↑↓	↓	↓*	↓*	↓*	↓*	↓*
	Latex 50 mg	↑↓	↓	↓*	↓*	↓*	↓*	↓*
	Latex 100 mg	↑↓	↓*	↓*	↓*	↓*	↓*	↓*
	Latex 200 mg	↑↓	↓*	↓*	↓*	↓	↓	↓*
	Latex 400 mg	↑↓	↓*	↓	↓*	↓	↓**	↓*
	Latex 500 mg	↑↓	↑	↓*	↓*	↓	↓*	↓*

Al the values are expressed as mean ± SEM, Statistical analysis by one way ANOVA followed by Dunnets test.*$P<0.05$,**<0.01 and ***$P<0.001$ as comparison to control

REFERENCES

Aktar MS, Qureshi. Phytopharmacological evaluation of Ficus glomerata Roxb fruit for hypoglycemic activity in normal and diabetic rabbits. Pak J Pharm Sci 1988 Jul; 1(2):87-96.

Axford SJ. Joint and bone infections. Rehum Inter J 2006 Oct; 34(10):405-412.

Calvino B, Bernard MO, Bars DL. Parallel clinical and behavioural studies of adjuvant-induced arthritis in the rats: possible relationship with chronic pain. Beh Bra Res 1987; 24:11-29.

Costa MD, Sutter PD, Gybel J, Vanhess J. Adjuvant induced arthritis in rats: A possible animal model of chronic pain. Pain 1981; 10:173-185.

Dimitrijevic M, Laban O, Djuric VJ, Stanijevic S, Miletic T, Jovanovic VK, Todorovic C, et al. Behaviour and severity of adjuvant–arthritis in four strains. Bra Beh Immuni 2001; 5:255-65.

Evans WC. Evans D. Trease and Evans pharmacogonacy. 5th ed. Philadelphia: WB saunder company limited; 2002. p. 240-241.

Ferber PC, Fischer RW, Immunization of laboratory animals, 3R-Info-Bulletin 11; Research Foundation Switzerland [Online]. 1999 Mar [cited 2004]; Available from:URL:http://www.forschung3r.ch/en/information/jb04.

Guidelines for Toxicity Investigation of Herbal Medicine (Annexure-1).Department of Ayurveda, Yoga, & Naturopathy, Unani, Siddha and Homeopathy, Ministry of health and Family Welfare, Government of India 2005. [Online]. 2005 Aug 19 [cited 2005]; Available from:URL:http://indianmedecine.nic.in.

Khan N, Sultana S. Modulatory Effect of Ficus racemosa: Diminution of Potassium Bromate-Induced Renal Oxidative Injury and Cell Proliferation Response. Basic and Clin Pharmacol Toxicol 2005 Nov; 97(5):282-8.

Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 10th ed. Pune: Nirali Parkashan; 1998. p. 152-153.

Kehlen A, Pachnio A, Thiele K, Langer J. Gene expression induced by interleukin-17 in fibroblast-like synoviocytes of patient with rheumatoid arthritis: upregulation proteins TSG-6. Arthritic Res Ther 2000 Apr 28; (5):184-185.

Mandal SC, Saha BP, Pal M. Antiinflammatory, analgesic and antipyretic activity in rodents of plant extracts used in African medicine. Phytother Res 2000 Jun; 14(4):278-80.

Mandal SC, Maity TK. Das J, Pal M, Saha BP. Hepatoprotective activity of Ficus racemosa leaf extract on liver damage caused by carbon tetrachloride in rats. Phytother Res 1999 Oct; 13(5):430-2.

Mandal SC, Saha BP, Pal M. Studies on antibacterial activity of Ficus racemosa Linn leaf extract. Phytother Res 2002 Jun 8; 14(5):278-280.

Qudhia P. Ficus glomerata as medicinal herb in Chhattisgarh, India (Online).
2003 Research note. Available form:
URL:http://www.botanical.com/site/column_poudhia/127_doomar.html.

Spector TD. Rheumatoid arthritis. Rheum Dis Clin North Am 1990; 16:513-537.

Sliman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. Arthritis Res Ther 2002; 4(3):265-272.

Sharma PV. Dravyaguna-Vijana. Varansi: Chaukhambha Bharati Academy; 2001.p. 666-667.

The wealth of India, Raw-Material,volume-v:SP-W, CSIR, New Delhi, India, 1999, 35-35

Vonshak A, Barazani O, Sathiyammoorthy P, Shalev R, Varday D, et al. Screening South Indian medicinal plants for antifungal activity against cutaneous pathogens. Phytother Res 2003 Nov; 17(9):3-5.

Vogel HG, Vogel WH, Sholkens BA, Sandow J, Muller G, Vogel WF. Drug discovery and Evaluation. 2nd ed. New York: springer-verlag Berlin Heidelberg; 2002.