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RESEARCH ARTICLE

Evaluation of Anti-Inflammatory and Anti-Arthritic Activity of *Rosa Centifolia* (Linn.) Flowers in Experimental Rats

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ABSTRACT

To evaluate anti-inflammatory and anti-arthritic activity of aqueous extract of *Rosa centifolia* (AERC) flowers in male albino rats. The anti-inflammatory activity was evaluated by administering Carrageenan (0.1 ml) into sub plantar region of rat right hind paw and the paw volume was measured at different intervals. The anti-arthritic activity was evaluated by injecting 0.1 ml Freund's Complete Adjuvant (FCA) into the right hind paw intradermally on day 1 and indomethacin(0.6 mg/kg p.o.) and AERC (400 mg/kg p.o.) treatments were continued up to 14 days. The body weight, rectal temperature, paw volume and ankle diameter were measured on alternate days, whereas RBC and WBC count, hemoglobin, serum Blood urea nitrogen(BUN), albumin, Serum glutamate pyruvate transaminase(SGPT), Serum glutamate oxaloacetate transaminase (SGOT) and Alkaline phosphatase (ALP) levels were estimated on day 14. The radiological studies of right hind paw of rats were also carried out on 14th day. The AERC showed significant reduction in paw volume which was increased by carrageenan. FCA produced loss of body weight, increase in paw volume, ankle diameter, WBC, serum BUN, SGPT, SGOT, ALP and decrease in RBC count, hemoglobin content, serum albumin levels. AERC significant reversed these changes produced by FCA. In conclusion, the aqueous extract of *Rosa centifolia* (Linn.) flowers possesses significant anti-inflammatory and anti-arthritic activity.

KEYWORDS

Rosa centifolia, anti-inflammatory, anti-arthritic, Freund's Complete Adjuvant, Carrageenan

INTRODUCTION

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli, an uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses[1].

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Rheumatoid Arthritis (RA) is a chronic, destructive inflammatory polyarticular joint and systemic autoimmune disease of unknown cause[2]. The prevalence of RA is consistent worldwide affecting, about 0.5-1.0 % of the population. It usually occurs in people between 25 and 55 year of age. Women are affected more than men at ratio of 3:1[3]. Various non-steroidal anti-inflammatory drugs (NSAID's) are widely used clinically for arthritis. However, despite their great number, their therapeutic efficacy seems to be hampered by the presence

of a number of undesired, and often serious side effects[4]. Therefore plant remedies have become increasingly popular and are often preferred over synthetically derived pharmaceuticals.

Rosa centifolia (Linn.) [Family: Rosaceae] is one such plant that is commonly found throughout India. It is extensively used as traditional medicine in Uttar Pradesh and Bihar. A decoction of flowers of rose is prescribed for inflammation of the mouth and pharynx, and ulcers of the intestine. Powder of rose buttons and seeds is used as astringent in hemorrhage and diarrheoa[5]. However, to our knowledge, there are no published scientific studies on anti-inflammatory and anti-arthritic activity of *Rosa centifolia* (Linn.) flower petals. The present study was undertaken to evaluate the anti-inflammatory and anti-arthritic activity of *Rosa centifolia* Linn. flowers in animal models of inflammation and arthritis.

MATERIALS AND METHOD

Plant material

The dried flower petals of *Rosa centifolia* (Linn.) were purchased from Amsar Private Limited, Indore, Madhya Pradesh.

Preparation of Aqueous Extract of Rosa Centifolia (Linn.)

Dried flower petals of *Rosa centifolia* (Linn.) were powdered and cold maceration was carried out using distilled water as solvent to get aqueous extract. The extract was then concentrated to dryness on water bath and stored in refrigerator until use. The extract obtained was subjected to preliminary phytochemical investigation by using standard qualitative tests[6,7].

Experimental Animals

Wistar albino rats of either sex weighing between (150-200g) were procured from central animal house of N.E.T. Pharmacy College, Raichur and kept in 12:12 hr light and dark cycle. The animals were acclimatized to laboratory conditions for 7 days. The animals were supplied with commercially available standard diet and water was allowed *ad libitum* under hygienic conditions. All animal studies were performed in accordance to guidelines of CPCSEA and Institutional Animal Ethical Committee. (576/2002/bc/IAEC/CPCSEA)

Acute Oral Toxicity Study

The acute oral toxicity of *Rosa centifolia* (Linn.) extracts was determined in female albino rats (150-200g) using revised OECD guidelines No. 425. Animals were devoid of any mortality at the highest dose of 2000 mg/kg. Hence, the 1/5th of maximum tested dose i.e. 400 mg/kg was selected as experimental dose[8].

Drugs and Chemicals Used

Freund's Complete Adjuvant (Merck specialities Pvt. Ltd, Mumbai), Chem. Kits for biochemical estimation (Erba Diagnostics, Mannheim GmbH, Germany), Anesthetic ether (Sigma solvents and Pharmaceuticals, Mumbai), Indomethacin (Newlife Healthcare, Surat), Carrageenan (Himedia Ltd., Mumbai, India).

Carrageenan Induced Paw Oedema in Rats

Male albino rats weighing between 150-200g were divided into 4 groups of 6 animals each.

Group 1: Normal control (distilled water 0.2 ml/100 g p.o.)

Group 2: Toxicant control (Carrageenan 0.1 ml of 1%)

Group 3: Carrageenan + Standard (Indomethacin 10mg/kg p.o.)

Group 4: Carrageenen + Aqueous extract of *Rosa centifolia* Linn. (400 mg/kg p.o.)

Inflammation was produced by injecting 0.1 ml of 1% carrageenan into subplantar region of rats right hind paw. The aqueous extract, standard drug and control vehicle were administered 60 min before carrageenan injection. The paw volume was measured at 0, 0.5, 1, 2, 3, 4 and 5 h interval using plethysmometer apparatus[9,10].

$$\text{Percentage Inhibition} = \frac{[Vc - Vt]}{Vc} \times 100$$

Where, Vc = paw volume in toxicant control group of rats

Vt = paw volume in treated groups of rats

Adjuvant Induced Arthritis like Inflammation in Rats

Male albino rats weighing between 150-200g were divided into 4 groups of 6 animals each.

Group 1: Normal control (distilled water 0.2 ml/100 g p.o.)

Group 2: Toxicant control (Freund's complete adjuvant 0.1 ml)

Group 3: FCA + Standard (Indomethacin 0.6 mg/kg) p.o.

Group 4: FCA + Aqueous extract of *Rosa centifolia* Linn (400 mg/kg p.o.)

Rats were injected with Freund's complete adjuvant containing 10 mg of heat killed mycobacterium tuberculosis in 1 ml paraffin oil (0.1 ml) into the left paw intradermally. Group of animals were treated with aqueous extract of *Rosa centifolia* Linn, distilled water and standard drug respectively up to 14 days from day of injection of Freund's complete adjuvant and the following parameters were recorded[11-14].

Body Weight

Body weight for each group of rats was recorded on alternative days during the period of arthritis. The mean difference in body weights in each group was calculated between the 1st day and 13th day.

% changes in body weight =

$$\frac{\text{(body weight on day 13)-(body weight on day 0)}}{\text{(body weight on day 0)}}$$

Paw Volume

The paw volume was measured 10:00 am on alternative days from day 0 to day 14 using plethysmometer apparatus for all the animals and the % inhibition of paw volume on alternate days was calculated for both the treated group of rats compared to toxicant control group of rats.

$$\% \text{ inhibition} = \frac{[Vc - Vt]}{Vc} \times 100$$

Where, Vc = paw volume in toxicant control group of rats on alternate days

Vt = paw volume in treated groups of rats on alternate days

Ankle Diameter

Changes in the ankle diameter of both injected and non injected paws from day 0 to day 14 on alternative days were assessed using a vernier scale.

Haematological Examination

On 14th day, Rats were anaesthetized with anesthetic ether and blood samples were collected by retro-orbital puncture using EDTA as anticoagulant and subjected to estimation of RBC, WBC and hemoglobin concentration.

Serum Bio-Chemical Examination

On 14th day, Rats were anaesthetized with anesthetic ether and blood samples were collected from the retro-orbital plexus without any anti-coagulant. After one hour serum was separated by centrifugation and subjected for determination of Blood Urea Nitrogen (BUN), Albumin, Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT) and Alkaline Phosphatase (ALP) by using semi auto-analyzer.

Radiological Studies

X-ray analysis of hind paw joints of the animals was carried out for evaluating the extent of bone damage.

Statistical Analysis

The values were expressed as mean ± SEM for 6 animals. The results were subjected to statistical analysis by using one-way ANOVA followed by Dunnet-'t'-test. $p<0.05$ was considered as statistically significant.

RESULTS

Phytochemical test

Phytochemical analysis of aqueous extract had shown the presence of chemical constituents like flavonoids, saponins, tannins, sterols and terpenoids, fixed oils and fats.

Table 1: Effect of aqueous extract of *Rosa centifolia* (Linn.) and indomethacin on right hind paw volume (ml) at different time intervals (h) after carrageenan administration (n=6)

Sr no	Groups	Rat hind paw volume in ml at different time intervals (h)						
		0	½	1	2	3	4	5
1	Normal control	0.85±0.02*	0.86±0.02***	0.82±0.05***	0.87±0.01***	0.89±0.07***	0.89±0.04***	0.89±0.02***
2	Toxicant control	0.95±0.02	1.13±0.04	1.38±0.02	1.46±0.03	1.61±0.04	1.54±0.14	1.43±0.05
3	Indomethacin (10 mg/kg p.o.)	0.96±0.02	1.14±0.04	1.25±0.04*	1.32±0.06	1.32±0.06***	1.28±0.06**	1.20±0.06**
4	Aqueous extract (400 mg/kg p.o.)	0.96±0.02	1.02±0.01	1.10±0.01***	1.17±0.02***	1.18±0.04***	1.14±0.04***	1.10±0.05***

Each value represents mean ± SEM of group of 6 rats. Data was analysed by using ANOVA followed by Dunnett's t test.

Where, * represents significant at $p<0.05$, **represents medium significant at $p<0.01$, *** represents highly significant at $p<0.001$ when compared with toxicant group.

Table 2: Percentage inhibition (%) of right hind paw volume (ml) produced by aqueous extract of *Rosa centifolia* Linn. and indomethacin after the Carrageenan challenge (n=6)

Sr. no.	Groups	Percentage inhibition (%) of right hind paw volume (ml) at different time interval (h)						
		0	½	1	2	3	4	5
1	Normal Control	--	--	--	--	--	--	--
2	Toxicant control	--	--	--	--	--	--	--
3	Indomethacin (10 mg/kg p.o.)	1.05	0.88	9.42	9.58	**18.01**	16.88	16.08
4	Aqueous extract (400 mg/kg p.o.)	1.05	2.65	20.28	19.86	**26.70**	25.97	23.07

Carrageenan induced Paw Oedema in Rats

In Carrageenan induced paw oedema, aqueous extract of *Rosa centifolia* (linn.) and indomethacin showed significant inhibition of paw oedema at 3 h interval and the maximum % inhibition observed for aqueous extract and indomethacin was 26.70 % and 18.01 % respectively at 3 h interval [Table 1 and 2].

Freund's Complete Adjuvant induced Arthritis in rats

Body Weight (gm)

The % changes in body weight for different groups like normal contol, toxicant control, indomethacin, aqueous extract, ethanol extract and petroleum ether extract were 14.92%, 6.23%, 17.84, 12.87%, 14.99% and 18.45% respectively [Table 3].

Paw Volume (ml)

The aqueous extract of *Rosa centifolia* (linn.) and indomethacin showed significant inhibition of paw oedema on 7th day and the maximum % inhibition observed for aqueous extract and indomethacin was 19.44 % and 14.72 % respectively on 7th day [Table 4 and 5].

Ankle Diameter (cm)

There was significant decrease in ankle diameter in aqueous extract and indomethacin treated group of rats when compared to toxicant control group of rats on day 7 and 9 and the maximum decrease was observed on 13th day [Table 6].

RBC Count (Million/Cu Mm) and Hemoglobin Content (cc/100ml)

There was a decrease in the RBC count and hemoglobin content in toxicant control group of rats when compared with the normal group of rats, which was significantly (P<0.001) reversed by aqueous extract and indomethacin treated group of rats when compared to toxicant control group of rats [Table 7].

WBC (cu/mm)

There was an increase in the WBC count in toxicant group of rats when compared with the normal group of rats. In the indomethacin and the aqueous extract fed groups, there was highly significant (p<0.001) decrease in the WBC count values when compared with the toxicant group [Table 7].

Table 3: Effect of aqueous extract of *Rosa centifolia* (Linn.) and indomethacin on the body weight (g) of rats after the Freund's Complete Adjuvant challenge (n=6).

Sr. no.	Groups → / Day ↓	Normal control	Toxicant control	Indomethacin (0.6mg/kg p.o.)	Aqueous Extract (400mg/kg p.o.)
1	1	168.2±6.95	176.5±4.17	172.0±7.46	191.8±2.54
2	3	172.0±6.89	178.0±4.23	177.0±7.29	196.2±2.71
3	5	175.3±6.68	179.8±4.09	183.0±7.28	200.2±2.56
4	7	178.3±5.89	181.3±4.23	188.8±7.04	204.5±2.52
5	9	182.2±6.22	183.2±3.97	193.8±6.69	207.7±2.89
6	11	186.5±6.82	185.2±3.90	198.2±6.82	212.0±3.02
7	13	193.3±8.04	187.5±3.99	202.7±7.11	216.5±2.84
8	% change in body weight between day 13 and day 1	14.92%	6.23%	17.84%	12.87%

Each value represents mean ± SEM of group of 6 rats. Data was analyzed by using ANOVA followed by Dunnett's t test.

Table 4: Effect of aqueous extract of *Rosa centifolia* (Linn.) and indomethacin on the right hind paw volume (ml) of rats after the Freund's Complete Adjuvant challenge (n=6)

Sr. no.	Groups → / Days ↓	Normal control	Toxicant control	Indomethacin (0.6mg/kg p.o.)	Aqueous Extract (400mg/kg p.o.)
1	**1**	0.80±0.00***	1.06±0.02	1.03±0.01	1.04±0.01
2	**3**	0.82±0.00***	1.24±0.03	1.22±0.03	1.25±0.02
3	**5**	0.83±0.00***	1.27±0.03	1.14±0.02*	1.13±0.01**
4	**7**	0.82±0.00***	1.29±0.03	1.10±0.01***	1.08±0.02***
5	**9**	0.82±0.00***	1.22±0.03	1.07±0.01**	1.03±0.02***
6	**11**	0.83±0.00***	1.16±0.02	1.05±0.01*	0.99±0.01***
7	**13**	0.84±0.00***	1.10±0.03	1.03±0.01	0.94±0.01**

Each value represents mean ± SEM of group of 6 rats. Data was analyzed by using ANOVA followed by Dunnett's t test.
Where, * represents significant at $p<0.05$, **represents medium significant at $p<0.01$, *** represents highly significant at $p<0.001$ when compared with toxicant group.

Table 5: Percentage inhibition (%) of right hind paw volume (ml) produced by aqueous extract of *Rosa centifolia* Linn. and indomethacin in rats after the Freund's Complete Adjuvant challenge (n=6)

Sr. no.	Groups ↓	Percentage inhibition (%) of paw volume (ml) on alternate days till day fourteen						
		1	**3**	**5**	**7**	**9**	**11**	**13**
1	**Normal control**	-	-	-	-	-	-	-
2	**Toxicant control**	-	-	-	-	-	-	-
3	**Indomethacin (0.6mg/kg p.o.)**	2.83	1.61	11.40	**14.72**	12.29	9.48	6.36
4	**Aqueous Extract (400mg/kg p.o.)**	1.88	-0.80	11.02	**19.44**	15.57	14.65	14.54

Table 6: Effect of aqueous extract of *Rosa centifolia* (Linn.) and indomethacin on the ankle diameter (cm) of rats after the Freund's Complete Adjuvant challenge (n=6)

Sr. no.	Groups → / Day ↓	Normal control	Toxicant control	Indomethacin (0.6mg/kg p.o.)	Aqueous Extract (400mg/kg p.o.)
1	**1**	0.63±0.00***	0.73±0.01	0.76±0.00	0.76±0.01
2	**3**	0.63±0.00***	0.86±0.01	0.90±0.00	0.89±0.02
3	**5**	0.65±0.00***	0.92±0.01	0.86±0.00**	0.88±0.01
4	**7**	0.65±0.01***	0.99±0.02	0.83±0.00***	0.86±0.01***
5	**9**	0.65±0.01***	0.91±0.01	0.82±0.00***	0.83±0.01**
6	**11**	0.64±0.01***	0.85±0.01	0.80±0.00	0.81±0.01
7	**13**	0.64±0.01***	0.88±0.01	0.79±0.00	0.79±0.01

Table 7: Effect of aqueous extract of *Rosa centifolia* (Linn.) and indomethacin on Haematological and Bio-chemical parameters measured on day 14 in group of rats (n=6)

Parameters		Groups			
		Normal control	**Toxicant control**	**Indomethacin (0.6 mg/kg p.o.)**	**Aqueous extract (400 mg/kg p.o.)**
Haematological parameters (units)	RBC count (millions/cu mm)	5.56±0.20*	4.10±0.15	5.94±0.44**	6.23±0.37***
	WBC count (Per cu mm)	7679±362.6***	12958±191.7	10092±266.3***	8117±273.2***
	Haemoglobin (gms/100 ml)	13.62±0.40**	10.72±0.35	13.33±0.51**	12.88±0.40*
Bio-chemical parameters (units)	BUN(mg/dl)	22.25±4.66	44.97±3.68	25.09±4.96*	28.02±2.35
	Albumin (g/dl)	3.83±0.06	3.21±0.12	8.30±0.96***	3.76±0.33
	SGPT (mmol/L)	52.74±19.43	103.13±70.28	66.60±12.33	57.75±5.43
	SGOT (mmol/L)	95.79±27.32	176.20±14.04	166.20±20.63	83.80±12.21*
	ALP (mmol/L)	312.10±160.6	387.11±53.67	260.50±62.78	365.50±69.83

Each value represents mean ± SEM of group of 6 rats. Data was analyzed by using ANOVA followed by Dunnett's t test.

Where, * represents significant at $p<0.05$, **represents medium significant at $p<0.01$, *** represents highly significant at $p<0.001$ when compared with toxicant group.

BUN (mg/dl)

There was increase in the BUN levels in toxicant group of rats when compared with the normal group of rats. In the indomethacin group, there was less significant (P<0.05) decrease in the BUN values whereas the aqueous extract group had shown non-significant (P>0.05) decrease in BUN levels when compared with the toxicant group [Table 7].

Albumin (g/dl)

There was decrease in the albumin levels in toxicant group of rats when compared with the normal group of rats. In the indomethacin group, there was highly significant (p<0.001) increase in the albumin levels, whereas the aqueous extract group had shown non-significant (P>0.05) increase in albumin levels when compared with the toxicant group [Table 7].

SGPT (mmol/L)

There was increase in the SGPT levels in toxicant group of rats when compared with the normal group of rats. Aqueous extract and indomethacin groups had shown non significant (P>0.05) decrease in SGPT levels when compared with the toxicant group [Table 7].

SGOT (mmol/L)

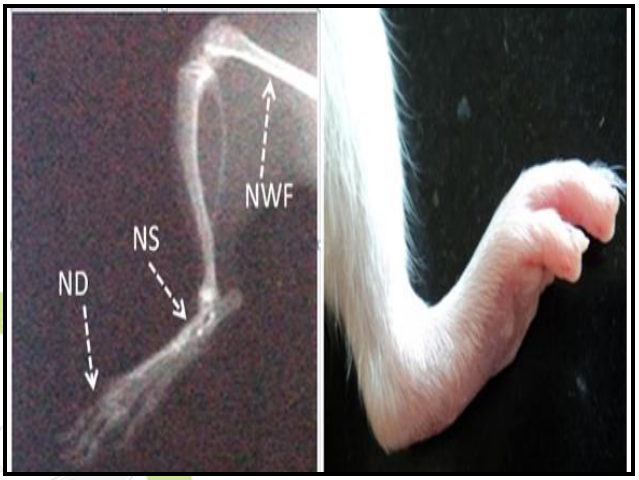
There was increase in the SGOT levels in toxicant group of rats when compared with the normal group of rats. Among aqueous extract and indomethacin group, only aqueous extract group, had shown less significant (p<0.05) decrease in the SGOT levels when compared with the toxicant group [Table 7].

ALP (mmol/L)

There was increase in the ALP levels in toxicant group of rats when compared with the normal group of rats. Aqueous extract and indomethacin groups had shown non-significant (P>0.05) decrease in ALP values when compared with the toxicant group [Table 7].

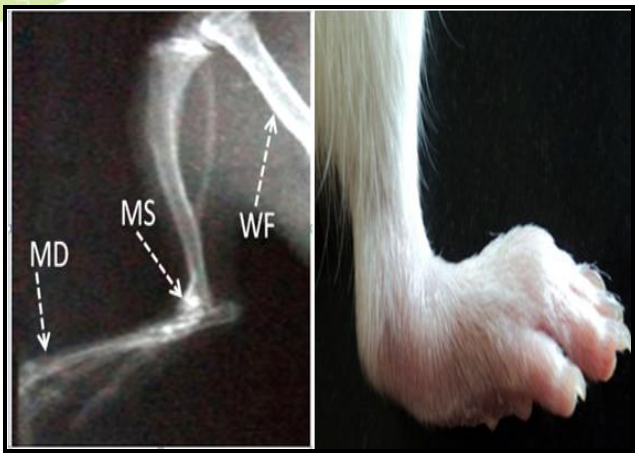
X-Ray

X-ray of toxicant group of animals showed soft tissue swelling along with narrowing of joint spaces and sign of bony destruction when compared to X-ray of normal group of animals. X-ray of indomethacin and aqueous extract treated groups had shown prevention against bony destruction by showing less soft tissue swelling and narrowing of the joint spaces (Figure 1-4).



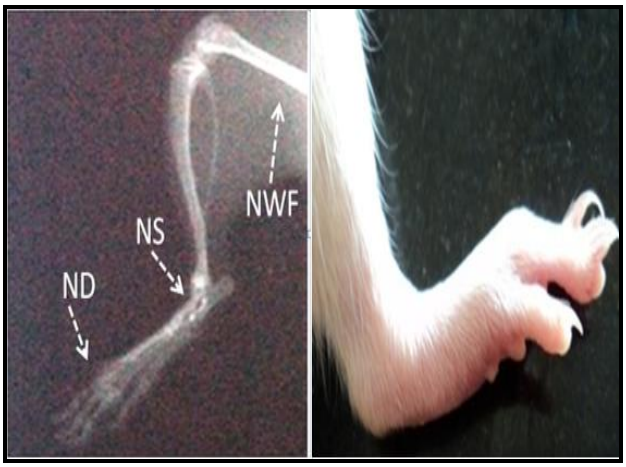
ND: No deformity, NS: No swelling, NWF: No widening of femur bone, when compared with control group rat

Figure 1: X-ray and Photograph of Normal rat on day fourteen



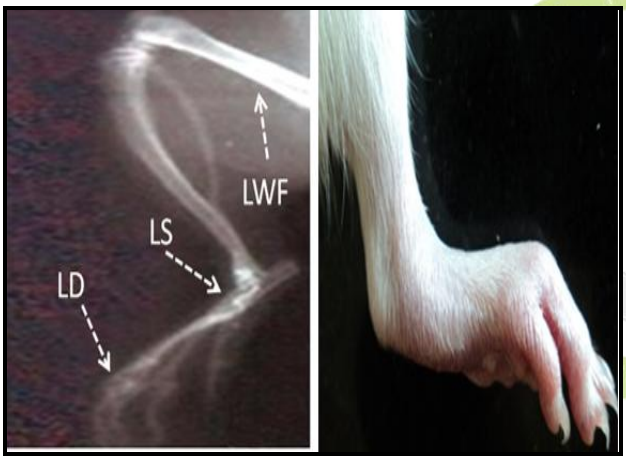
MD: More deformity, MS: More swelling, WF: Widening of femur bone

Figure 2: X-ray and Photograph of FCA induced arthritic rat on day fourteen



ND: No deformity, NS: No swelling, NWF: No widening of femur bone, when compared with control group rat

Figure 3: X-ray and Photograph of indomethacin (0.6 mg/kg p.o.) treated rat in FCA induced arthritic model on day fourteen



LD: Less deformity, LS: Less swelling, LWF: Less widening of femur bone, when compared with control group rat

Figure 4: X-ray and Photograph of aqueous extract of *Rosa centifolia* (Linn.) (400 mg/kg p.o.) Treated rat in induced arthritic model on day fourteen FCA

DISCUSSION

Carrageenan induced paw oedema is an *in vivo* model of inflammation; it was selected to assess the anti-inflammatory activity of natural products particularly in the acute phase of inflammation. oedema formation due to Carrageenan in the rat is a biphasic event. The first phase is due to the histamine and serotonin release, then peak effect is observed at 180 min due to release of kinin-like substances, and the second phase is caused by the release of protease, prostaglandin and lysosome[15]. Therefore, it can be assumed that the inhibitory effect of all the extracts and indomethacin on carrageenan-induced inflammation could be due to the inhibition of the enzyme cyclooxygenase, leading to the inhibition of prostaglandin synthesis[16]. Oral administration of the aqueous extract of *Rosa centifolia* (Linn.) flower petals suppressed the edematous response after 2 h and this effect continued upto 5 h. The observed effect was comparable with that produced by indomethacin administration.

Arthritis is a chronic inflammatory disorder that involves the release of number of mediators like cytokines (IL-IB and TNF-α), GM-CSF, interferon's and PGDF. These mediators are responsible for the pain, destruction of bone and cartilage that can lead to severe disability[17].

There are two types of models available to induce arthritis i.e. (1) type II collagen and (2) Freund's Complete Adjuvant (FCA) induced arthritis in rats. The pathological changes within the paws are very similar in both models and have similarities to the inflamed joint of rheumatoid arthritis. The low incidence and severity of polyarthritis incollagen rats limits the model to the therapeutic evaluation of anti-arthritic drugs only. In contrast, the adjuvant model with almost 100% incidence and a greater severity of arthritis is a more desirable model to test drugs in both prophylactic and therapeutic regimens. This shows that there is more incidence and severity of FCA induced arthritis, hence this model was selected[13].

In this model, rats were selected to induce arthritis because rats develop a chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid disease.[18]

Adjuvant arthritis in rat is an experimental model that shares many features with human RA, such as swelling, cartilage degradation and loss of joint function. It has been used for many

years for evaluation of anti-arthritic/anti-inflammatory agents[19].

It has been reported that changes in body weight was in response to the incidence and severity of arthritis, used to assess the onset of the disease[20]. The loss of body weight during arthritic condition was due to decrease of food intake throughout the period of study due to immobility accompanying hyperalgesia that leads to reduced absorption of ^{14}C- glucose and ^{14}C- leucine in rat's intestine during inflammation[21], but using anti-inflammatory drugs the decrease in absorption was nullified[22]. The treatment of inflamed group of rats with the aqueous extract and indomethacin corrected the decreased body weight during inflammation.

Following the injection of FCA into the right hind paw, the paw rapidly increases in size during the first three days. Thereafter the swelling diminishes slightly until about 7 or 8 days after the injection. At approximately day 10, inflamed lesions, called secondary lesions, appear in the ears, tail and in joints of hind paws. Fourteen days after the injection the lesions have usually proliferated so extensively that hind paw and ankle joint are red and swollen. Subsequently the inflammation subsides leaving pale granulomatous swellings around the joints. This condition seems nearer to human rheumatoid arthritis than any other laboratory model so far investigated[23]. After the administration of FCA, there was increase in right hind paw volume in toxicant control group of rats, which was suppressed by the aqueous extract and indomethacin treated group of rats.

It has been reported that the soft tissue swelling seen around the ankle joints was found to be due to edema of periarticular tissues, such as ligaments of joint capsules. An increase in granulocytes and monocytes was found to be associated with increase in ankle diameter of toxicant group of rats[24]. After the administration of FCA, there was increase in ankle diameter in toxicant control group of rats, which was suppressed by the aqueous extract and indomethacin treated group of rats.

In the present study, the toxicant group of rats showed a reduced RBC count and Hb level. All these conditions indicating the anaemic condition and is commonly noted in patients with chronic arthritis[25]. The two most common reasons for anaemia in arthritic patients are gastrointestinal blood loss from arthritic medication and bone-marrow changes in patients with inflammatory arthritis which prevents the release of iron for incorporation into RBCs[26,27]. The aqueous extract and indomethacin treated group showed a significant recovery from anaemic condition, by increasing the RBC count and Hb content of adjuvant rats.

In arthritis condition, there is a mild to moderate rise in WBC count due to release of IL-IB inflammatory response. IL-IB increases the production of both granulocyte and macrophages colony stimulating factor[17,28]. In the present study, the migration of leucocytes into the inflamed area was significantly suppressed by the aqueous extract and indomethacin treated group of rats as seen from significant decrease in total WBC count as compared to toxicant control group of rats.

Increased BUN levels in the toxicant group of rats indicates kidney dysfunction which may be due to the substantial fraction of blood urea in arthritic rats derived from arginine synthesized in kidneys[29]. There was decrease in BUN levels in the aqueous extract and indomethacin treated group of rats.

It has been reported that, decrease in the albumin levels in the toxicant group, might be due to the acute phases of inflammation that could merely be the reflection of the increase in capillary permeability[23]. There was increase in albumin levels in the aqueous extract and indomethacin treated group of rats.

It has been reported that, tissue damage can be measured clinically by assay of enzyme activity in sera and tissues, increase in the SGPT and SGOT levels in the toxicant group, may be due to the increase in amino transferases in serum that causes the release of enzymes from the cells of the damaged organ (liver), which is a feature of adjuvant arthritis[30]. There was non-significant

decrease in SGPT and SGOT levels in the aqueous extract and indomethacin treated group of rats, however the levels of SGOT were significantly reduced in aqueous extract treated group of rats.

It has been reported that, increase in the serum ALP levels in the toxicant group, may be due to the action of osteoblasts trying to rebuild bone mass in the affected joint, previously destroyed by the resorption activity associated to the inflammatory process[31]. There was non-significant decrease in serum ALP levels in the aqueous extract and indomethacin treated group of rats. Treatment with the aqueous extract and indomethacin, might have reduced the inflammation process and consequently the bone resorption activity.

The diagnosis of RA is usually obvious clinically and it allows therapeutic monitoring which remain the standard methods in evaluating disease progression. In arthritic rats, erosions representing bony destruction were evident on bone unprotected by cartilage, since they were directly exposed to cytokines and enzyme mediators in synovial tissue. Narrowing of spaces secondary to articular cartilage to diffuse within joints. The x-ray appearance commonly referred to as diminished joint space is the hallmark of arthritis. The diminished joint space represents a loss of articular cartilage, which may be brought by a variety of pathological mechanism. The aqueous extract and the indomethacin group had shown prevention against bony destruction by showing less soft tissue swelling and narrowing of the joint spaces[11].

The present study shows that the adverse physical, haematological, biochemical and radiological changes in arthritic group of rats were reversed to a considerable extent by oral administration of aqueous extract of *Rosa centifolia* (Linn.) flowers.

Phytochemical analysis of aqueous extract of *Rosa centifolia* (Linn.) flowers had shown the presence of chemical constituents like flavonoids, saponins, tannins, sterols and terpenoids, fixed oils and fats. Flavonoids have shown to be responsible for preventing osteoporosis, where they increase the bone mineral density[32]. Experimental studies showed that flavonoids act by inhibiting osteoclastic bone resorption both *in vitro* and *in vivo* by increasing calcium absorption from intestine and prevents bone loss by improving the balance of bone formation and resorption. The presence of flavonoids is reported to be responsible for anti-arthritic activity in nut milk extract of *Semecarpus anacardium* Linn[11] and leaf extract of plant J*usticia gendarussa*[33].

Hence, it can be assumed that anti-inflammatory and anti-arthritic activity of aqueous extract of *Rosa centifolia* (Linn.) flowers might be produced due to the presence of flavonoids.

CONCLUSION

The results showed that aqueous extract of *Rosa centifolia* (Linn.) flowers possess significant anti-inflammatory and anti-arthritic activity.

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