Full Length Articles

AMELIORATIVE EFFECT OF *MURRAYA KOENIGII* LEAF (CURRY LEAF) ON ADJUVANT INDUCED ARTHRITIS IN WISTAR RATS

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ABSTRACT

The different parts of Murraya koenigii leaf are traditionally used for treatment of wide variety of ailments. The present study was designed to evaluate the effect of methanolic extract of curry leaves on growth and haemato-biochemical parameters in adjuvant induced arthritis. Sixty Wistar rats were divided into 6 groups containing ten each (n=10), Group I- Sham control, Group II- Plant extract only, Group III- Complete Freund's Adjuvant (CFA) only, Group IV- CFA + ibuprofen, Group V- Prophylactic group, Group VI- Therapeutic group. Rheumatoid arthritis (RA) was induced by sub-plantar injection of Complete Freund's Adjuvant (CFA). The body weight gain and feed intake were measured at weekly intervals upto 28 days and blood was collected on 28th day for haematology and serum biochemistry. The analysis of parameters on body weight, feed intake and haemato-biochemistry revealed that curry leaf extract had a considerable effect in preventing and ameliorating the severity of arthritis. Methanolic extract of curry leaves appears to be a promising natural remedy of RA.

Keywords: Complete Freund's adjuvant (CFA), Rheumatoid arthritis (RA), Curry leaf extract.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease that often affects symmetrical bilateral joints (Rajaram *et al*., 2015). Uncontrolled RA is characterised

by increasing synovial, cartilage and bone deterioration that is perhaps accompanied by extra-articular symptoms (Shi *et al*., 2015; Bhalekar *et al*., 2015). RA may evolve to a severe impairment with immediate negative effects on lifestyle and an increase in death rate (Kapetanovic *et al*., 2011; Gomes *et al*., 2013). Although 0.5 to 2.0 % of the population are clinically diagnosed as RA cases (Goulielmos *et al*., 2016), all ages might suffer from RA. The incidence is mostly in those over 40 years, particularly among women, who are two to three times more likely to suffer from RA than men (Crowson *et al*., 2011).

The ultimate goal of RA treatment is to stop or at least limit joint deterioration, relieve discomfort and maintain normal joint functions (Guruprasad *et al*., 2015). RA requires lifelong treatment because it is a chronic illness. Drugs like disease-modifying anti-rheumatoid drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids help to reduce joint inflammation and pain, but regretfully, 30 % of patients do not respond to treatment (Vijayalaxmi *et al*., 2015). The high cost and numerous adverse effects of the treatment are noteworthy (Zampeli *et al*., 2015). As a result, more patients prefer using natural product remedies (Gutierrez - Rebolledo *et al*., 2015).

Medicinal plants and herbs are a rich source of phytochemicals that are found to be useful in the prevention, treatment and amelioration of a wide range of medical disorders. *Murraya koenigii*, one among them has a number of bioactive components because of which it has been demonstrated to be medicinally important plant but has scant scientific literature on its curative properties against rheumatoid arthritis. It is used as medicinal plant and its extract can be used for therapeutic or prophylactic purposes. Secondary metabolites from curry leaf such as alkaloids, flavonoids, terpenoids, vitamins, tannins and its active constituents, are crucial for the prophylactic and therapeutic characteristics. They all have physiological effects on the body at various stages of development and keep the body healthy (Balakrishnan *et al.,* 2020).

Lack of scientific data to back up the claimed uses, which are sometimes based exclusively on traditional knowledge, is a matter of concern. *In vivo* examination in laboratory animal models of the conventional claims made is essential. To assess the anti-arthritic effectiveness of novel natural and synthetic medicines, many induced animal models for RA were used. Complete Freund's adjuvant (CFA) induced arthritis is a classic experimental method for inducing chronic immune-pathological rheumatoid arthritis in laboratory animals with a pathological mechanism and cellular immunity response that are similar to those in humans (Nair *et al*., 2012). This disease model also has other benefits, including superior disease manifestation and disease progression compared to other arthritis-induced models. It is also very predictive in testing the efficacy of novel compounds as anti-RA (Hawkins *et al*., 2015; Tuncel *et al*., 2016). The present study was undertaken to find out the ameliorative

effect of *Murraya koenogii* against experimentally induced arthritis in Wistar rats.

MATERIALS AND METHODS

Animals

Male and female Wistar rats of 6-8 weeks old, weighing 180-230 grams were obtained from Laboratory Animal Medicine Unit, Centre for Animal Health Studies, TANUVAS, Chennai. The animals were housed in centralised laboratory animal house, Madras Veterinary College, Vepery, Chennai. They were placed in polypropylene cages and acclimatized for a week under standard conditions of temperature (22±3° C) and humidity (50-70 %) with a 12 hours light-dark cycle. The animals had free access to a standard pellet diet and water *ad-libitum*. All the protocols were approved by Institutional Animal Ethical Committee (Institutional Animal Ethical Committee Approval letter number Lr.No.508/DFBS/IAEC/2022. dated:05.05.2022).

Experimental design

The animals were randomly grouped into six different groups of ten animals per group (n=10) viz., Group I - sham control, Group II- Plant extract only (300 mg/kg B.W), Group III – Complete Freund's Adjuvant (CFA-0.25 ml) only, group IV– CFA (0.25 ml)+ ibuprofen at 53 mg/kg/day as reference standard, Group V (Prophylactic group) - CFA (0.25 ml) + 300 mg/kg/day of curry leaf extract from day one of induction and Group VI (Therapeutic group) - CFA (0.25 ml)+300 mg/kg/day of curry leaf extract from fourteenth day of induction.

GROUP	TREATMENT	DOSE	NO. OF ANIMALS
I	Sham Control	0.1 ml PBS was injected in the plantar region of the right hind paw of rats	10
II	*Murraya koenigii* leaf extract	300 mg /kg B.W orally for 28 days	10
III	Arthritic control	0.1ml Complete Freund's Adjuvant (CFA) was injected in the plantar region of the right hind paw of rats	10
IV	Standard drug control (Ibuprofen)	0.1ml CFA + 53 mg/kg B.W of Ibuprofen orally	10
V	Prophylactic	0.1ml CFA +300 mg/kg B.W of *Murraya koenigii* leaf extract orally from day one of induction	10
VI	Therapeutic	0.1 ml CFA +300 mg/kg B.W of *Murraya koenigii* leaf extract orally from 14th day of induction	10
	Total		60

Induction of arthritis

Arthritis was induced by injection of 0.1ml of Complete Freund's Adjuvant into the sub-plantar region of the left hind paw of each rat. Each ml of Complete Freund's Adjuvant contains 10 mg of heat killed *Mycobacterium tuberculosis* and dried in 0.85 ml paraffin oil and 0.15 ml mannide mono-oleate.

Feed intake

Feed intake (g) of all the groups was recorded daily for 30 days with the help of weighing balance and the average weekly feed intake was calculated by the formula given by Manzoor *et al*. (2020).

Feed consumption (g) = Total feed consumed during the week (g)/Number of rats fed during the week.

Body weight

The body weight for each animal was recorded at weekly interval for 4 weeks by means of weighing balance (g) and gain in body weight (BWG) was measured by using standard formula given by Manzoor *et al*. (2020).

Gain in body weight (g) = Final Weight-Initial weight (g)

Blood sample collection

For the estimation of different haematological and biochemical parameters, the rats were anaesthetized at the end of the experimental study and blood samples were collected via puncturing the retro-orbital plexus.

The blood samples were centrifuged at 10,000 rpm for 10 min to collect the serum for estimation of different biochemical parameters.

Haematology

The haematological parameters like packed cell volume (PCV), haemoglobin (Hb), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelet count were analysed by using auto-analyser BC vet 2800.

Serum biochemistry

Serum biochemical parameters such as Alanine amino-transferase (ALT), Aspartate amino-transferase (AST), Alkaline Phosphatase (ALP), Total Protein, Albumin, Creatinine, Blood Urea Nitrogen (BUN), Calcium and Phosphorous were estimated by using A15 Biosystem auto-biochemical analyser.

Statistical analysis

The data generated from the different parameters of the experimental study were subjected to one way analysis of variance (ANOVA) test using statistical package for the social sciences (SPSS) software version 20 for the window.

RESULTS AND DISCUSSION

Body weight

The data regarding the effect of various treatments on body weight (g) of rats before induction of arthritis and on 1st, 2nd, 3rd and 4th week of study in different treatment groups are mentioned in Table 1 and Fig 1. During the first 3 weeks, the changes in body weight showed no significant difference (P>0.05). At 28thday of study, the body weight in arthritic group was significantly lower when compared to control and treated groups. There was a significant difference (P<0.05) in body weight gain between the groups. The body weight gain in arthritic group was significantly lower (P<0.05) as compared to control and treated groups. Significant increase (P<0.01) in body weight gain was observed in prophylactic and therapeutic groups compared to arthritis induced group.

A preliminary marker for determining the severity of the illness and the body's reaction to anti-inflammatory drug therapy is the change in body weight (Winder *et al*., 2005). CFA induced group rats showed reduced body weight while curry leaf treated groups showed increased body weight suggesting the anti-arthritic effect. The decreased body weight gain in CFA induced arthritic group might be due to deficient absorption of nutrient via intestine whereas the increased body weight gain in treatment groups suggest normalizing of the intestine (Jalalpure *et al*., 2011).

Feed intake

The weekly changes in feed intake of different groups of rats is represented in Table 2 and Fig 2. There is a significant difference (P<0.05) between the groups during the first 2 weeks of study. There is a highly significant difference (P<0.01) in feed intake between the groups during last 2 weeks of the study. The feed intake is significantly lower (P<0.01) in arthritic group compared to control and treated groups during the last 2 weeks of the study. Highly significant increase (P<0.01) observed in feed intake of prophylactic and therapeutic group compared to arthritic and standard drug treated group during 3rd and 4th week of study. According to reports RA caused decreased appetite and severe pain which interferes with the normal feed intake of animals (Roubenoff *et al*., 1997; Yoshizaki *et al*., 1998).

Haematology

The changes in the haematological parameters like haemoglobin, RBC, PCV, WBC and platelets are presented in the Table 3. There is a significant difference between the groups in HB and WBC count. There was also significant difference (P<0.01) in MCV, MCH, MCHC levels between the groups. WBC count was significantly increased (P<0.05) in arthritic control group and significantly reduced (P<0.05) in treated groups. The significant (P<0.05) increase in leukocyte count in adjuvant-induced arthritic rats might be attributable to the immune system's stimulation against foreign antigens. Additionally, an increase in IL-1 leads to an increase in granulocyte and macrophage

Table 1. Mean (±SE) weekly body weight in grams (g) and total weight gain in 4 weeks in CFA induced arthritic and different treatment models in Wistar rats (n=10)

Groups	Initial weight (g)	1st week (g)	2nd week (g)	3rd week (g)	4thweek (g)	Body weight gain (g)
Control	194.80± 5.85	208.20±5.67	215.1±6.6	236.60±3.99	247.20^{b}±6.10	52.40^{b}±5.86
Control+ *M.koenigii* extract	190.60±8.03	203.50±8.84	211.90±8.90	237.10±3.76	246.40^{b}±4.14	55.80^{b}±6.99
CFA	186.50±5.60	194.20±6.05	198.50±6.06	213.50±7.63	219.10^{a}±4.40	32.60^{a}±4.62
CFA+IBU	193.60±7.29	197.90±9.67	205.50±9.5	219.10±7.78	244.70^{b}±4.88	51.10^{b}±7.75
CFA+ Leaf extract (Prophylactic)	187.30±11.3	192.40±10.7	198.90^{a}11.1	221.80±6.47	238.30^{b}±6.50	51.00^{b}±13.05
CFA+ Leaf extract (Therapeutic)	197.40±8.82	196.10±7.95	204.90±8.86	222.20±8.05	246.30^{b}±4.50	49.10^{b}±7.59
P value	0.918	0.762	0.707	0.069	0.002	0.002
Sig	NS	NS	NS	NS	*	*

Means bearing similar superscripts do not differ significantly within a column,
One-way ANOVA-Ducan's test;* - P<0.05, NS- Non significant.

Table 2. Mean (±SE) weekly feed intake in grams (g) in 4 weeks in CFA induced arthritic and different treatment models in Wistar rats (n=10)

Groups	1st week (g)	2nd week (g)	3rd week (g)	4th week (g)
Control	$19.75^{c}\pm0.38$	$21.25^{c}\pm0.51$	$22.90^{a}\pm0.40$	$23.55^{a}\pm0.20$
Control+Leaf Extract	$19.25^{bc}\pm0.33$	$20.75^{bc}\pm0.46$	$22.80^{b}\pm0.04$	$23.50^{b}\pm0.22$
CFA	$18.00^{a}\pm0.01$	$19.30^{a}\pm0.53$	$19.80^{c}\pm0.13$	$19.90^{b}\pm0.04$
CFA+IBU	$18.65^{ab}\pm0.20$	$19.80^{ab}\pm0.13$	$20.50^{c}\pm0.22$	$21.92^{c}\pm0.15$
CFA+Leaf extract (Prophylactic)	$18.65^{ab}\pm0.38$	$19.50^{a}\pm0.22$	$21.50^{d}\pm0.22$	$22.83^{d}\pm0.10$
CFA+Leaf extract (Therapeutic)	$18.35^{a}\pm.0145$	$19.30^{a}\pm0.13$	$21.25^{d}\pm0.11$	$21.75^{d}\pm0.11$
P value	0.02	0.03	0.00	0.00
Sig	*	*	**	**

Means bearing similar superscripts do not differ significantly within a column, One-way ANOVA-Ducan's test;* - P<0.05, **- P<0.01.

colony-stimulating factors, which is linked to an increase in TLC. The corresponding decrease in curry leaf extract-treated groups demonstrated the immune-modulating effect of curry leaf (Ekambaram *et al*., 2010). The PCV, RBC and platelet count showed no significant difference between control and treated groups.

Serum biochemistry

The levels of serum biochemical parameters are presented in Table 4. The total protein values between the groups showed a highly significant difference (P<0.01). Significant difference was observed in total protein and albumin value between the arthritic and treated groups. The total protein value of arthritic group was lower than control and treated groups. Arthritic control group had decreased level of albumin compared to that of sham control and treated groups. In the present study, there was no significant difference in the values of AST, BUN, creatinine, calcium and phosphorus among the groups. As a result of the adjuvant-induced arthritis, the level of albumin decreases and alterations in plasma protein concentrations occur (Cawthorne *et al*., 1976). Furthermore, it was proposed that the inflammatory mediators of histamine,

Table 3. Mean (±SE) haematological parameters after 4 weeks study in CFA induced arthritic and different treatment models in Wistar rats

Group	Hb (g/dl)	PCV (%)	RBC ($x10^6$ /µl)	WBC ($x10^3$ /µl)	PLT ($x10^5$µl)	MCV (fL)	MCH (pg)	MCHC (%)
Control	13.57^{a}±.09	41.17± 0.35	8.29± 0.15	12.15^{a}± 1802.69	10.40± 28421.9	49.65^{a} ±0.01	16.36^{b} ±0.01	32.96^{f} ±0.00
Control+ Leaf extract	13.50^{ab}±0.07	40.98± 0.33	7.99± 0.03	12.26^{a}± 1481.12	10.36± 21409.1	51.29^{c} ±0.04	16.88^{d} ±0.00	32.44^{d} ±0.02
CFA	13.08^{abc}±0.05	41.26± 0.57	8.13± 0.07	19.01^{b}± 897.79	10.72± 9996.88	51.77^{d} ±0.01	16.08^{a} ±0.00	31.72^{b} ±0.05
CFA+IBU	12.86^{bc}±0.19	41.41± 0.697	7.99± 0.118	13.24^{a}± 1939.75	10.46± 7922.1	51.79^{d} ±0.09	16.39^{b} ±0.10	31.05^{a} ±0.00
CFA + Leaf extract (Prophylactic)	13.28^{cd}±0.03	41.78± 0.48	8.20± 0.03	15.28^{a}± 1939.75	11.18± 35836.4	50.97^{b} ±0.02	16.19^{a} ±0.00	31.78^{c} ±0.01
CFA+ Leaf extract (Therapeutic)	13.18^{d}±0.13	40.47± 0.48	7.91± 0.13	12.66^{a}± 1461.60	10.75± 52765.6	51.18^{c} ±0.09	16.65^{c} ±0.17	32.09^{e}± 0.08
P value	0.000	0.492	0.90	0.003	0.262	0.000	0.000	0.000
Significance	**	NS	NS	*	NS	**	**	**

Means bearing similar superscripts do not differ significantly within a column (P<0.05)-One-way ANOVA-Ducan's test;* - P<0.05, **-P<0.01, NS- Non significant.

Table 4. Mean (±SE) serum biochemical parameters after 4 weeks study in CFA induced arthritic and different treatment models in Wistar rats

Group	TP (g/dl)	ALB (g/dl)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	BUN (mg/dl)	Creatinine (mg/dl)	Ca (mg/dl)	P (mg/dl)
Control	6.09^{b}± .131	4.05^{b} ± 0.17	49.30^{ab}± 2.02	139.20± 6.92	159.00^{a} ± 12.31	12.79± 0.23	0.33± 0.002	10.39± 0.34	6.42± 0.32
Control+ Leaf Extract	6.32^{b}± .197	4.29^{b} ± 0.18	53.40^{ab}± 2.17	139.20± 3.45	181.50^{a}± 10.7	12.56± 0.58	0.31± 0.005	10.58± 0.29	6.47± 0.20
CFA	4.56^{a}± .232	3.21^{a} ± 0.25	65.20^{c}± 3.120	144.10± 4.49	252.30^{b} ± 7.92	12.97± 0.37	0.34± 0.004	11.66± 0.24	6.98± 0.84
CFA+IBU	5.94^{b}± .069	4.20^{b} ± 0.17	56.30^{b}± 2.097	139.40± 3.83	177.70^{a} ± 20.35	13.06± 0.53	0.33± 0.012	10.74± 0.44	6.62± 0.03
CFA+ Leaf extract (Prophylactic)	6.03^{b}± .126	4.27^{b} ± 0.27	48.00^{a}± 1.460	142.30± 3.25	171.60^{a} ± 11.30	12.90± 0.20	0.33± 0.005	10.47± 0.39	5.90± 0.25
CFA+ Leaf extract (Therapeutic)	6.22^{b}± .114	4.21^{b} ± 0.09	50.40^{ab}± 2.35	142.10± 4.18	199.90^{a} ± 18.36	12.00± 0.19	0.35± 0.009	11.14± 0.24	6.63± 0.31
P value	0.00	0.04	0.00	0.957	0.00	0.417	0.037	0.89	0.132
Significance	**	*	**	NS	**	NS	NS	NS	NS

Means bearing similar superscripts do not differ significantly within a column, One-way ANOVA- Ducan's test;*- P<0.05, **- P<0.01, NS- Non significant.

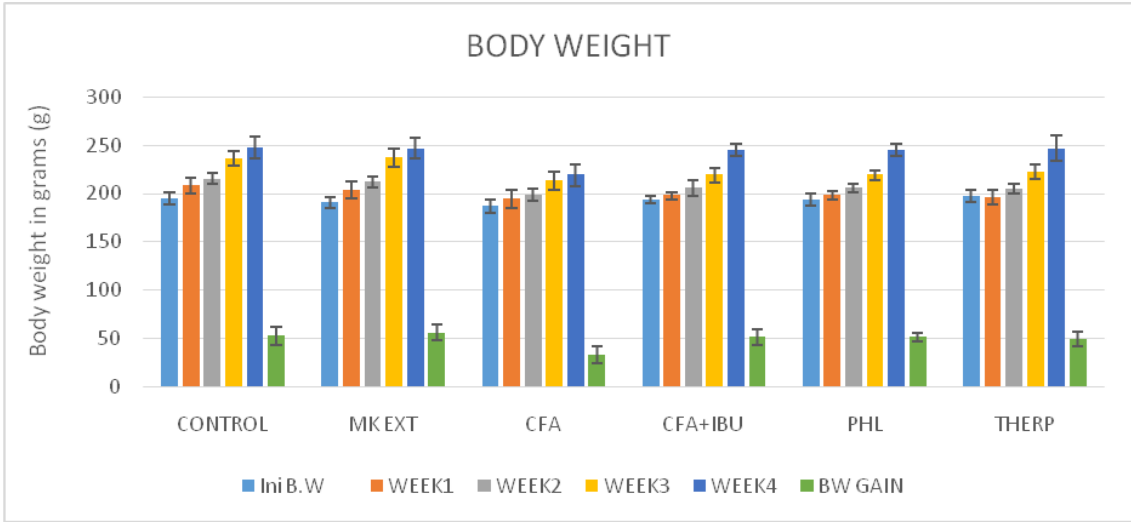

Fig 1. Mean (±SE) weekly body weight gain and total weight gain in grams (g) in 4 weeks in CFA induced arthritic and different treatment models in Wistar rats (n=10)

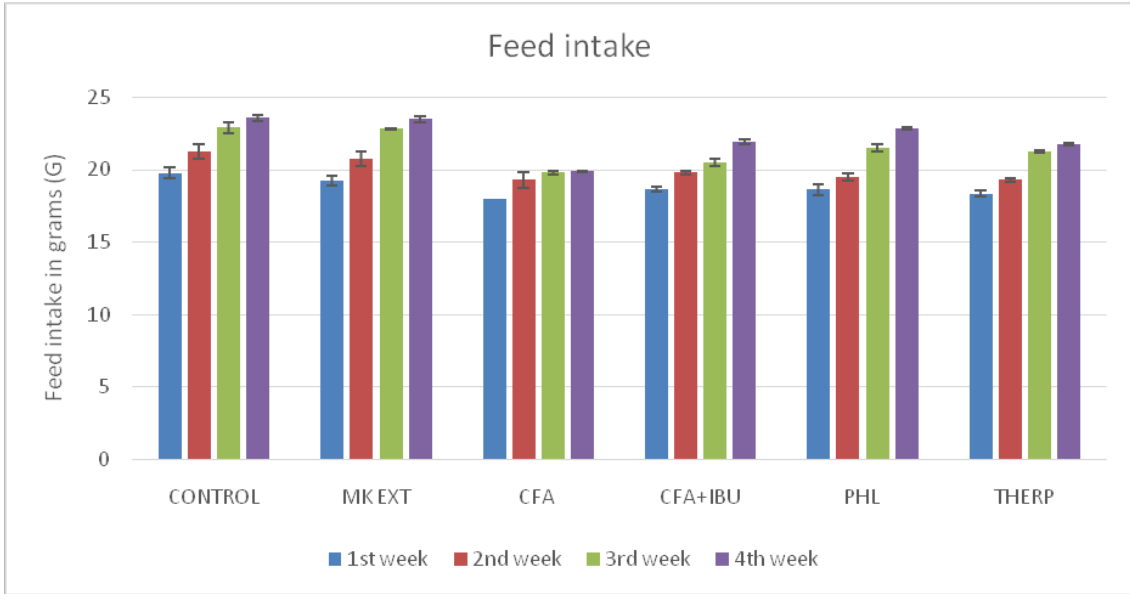

Fig 2. Mean (±SE) weekly feed intake in grams (g) in 4 weeks in CFA induced arthritic and different treatment models in Wistar rats (n=10)

bradykinin and prostaglandins generated during inflammation increased the vascular permeability to albumin, causing albumin levels in the serum to decrease (Kohn and Barchet 1976).

The liver enzyme ALT values were significantly differing among the groups. The level of ALT was significantly ($P<0.01$) increased in arthritic control group. The values of ALT in treated groups were similar to those of control group. The levels of serum enzyme ALP were significantly increased ($P<0.01$) in arthritic control group against the control and treated groups. In treated groups there was a significant decrease in the level of ALP. Since impaired liver function is known to be a feature of adjuvant arthritis, the activity of the enzymes ALT and ALP was dramatically enhanced in arthritic rats. The lipid peroxidation of the hepatocytes membrane caused by free radicals is thought to be the cause of this damage to the hepatic cells. At the site of inflammation, it was discovered that free radicals were created in considerable quantities (Gotia *et al*., 2001). Similarly, Complete Freund's Adjuvant injection into rats caused a significant release of these reactive oxygen species (Cascao *et al*., 2014). Significant decrease in the ALP levels in treated groups compared to the arthritis group indicated that curry leaf extract reversed the inflammatory changes caused by adjuvant.

CONCLUSION

The present study concluded that curry leaf has a potent effect, in preventing the haemato-biochemical alterations caused by adjuvant-induced arthritis in rats, as well as a favourable effect on feed intake and body weight gain. As a result, it may be considered that curry leaf is very much effective in attenuating the changes produced in chronic arthritis. According to the findings, curry leaf could be used to prevent severity of inflammatory and chronic arthritic conditions.

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