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Anti-arthritic activity of leaves of *Calotropis gigantea* Linn.

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Abstract

Objective: To study the anti-arthritic activity of leaves of *Calotropis gigantea*. Materials and methods: Petroleum ether (40-60°), ethyl acetate, ethanol and aqueous extract of *Calotropis gigantea* leaves were tested for various preliminary phytoconstituents and were screened for anti-arthritic activities using Freund's adjuvant arthritis in albino rats. The extracts were administered orally for 21 days and the mean changes in diameter of paw were noted at regular intervals. The changes in body weight were recorded daily. On 22nd day at the end of study blood was collected and haemoglobin content, total WBC count, differential WBC count, ESR and RBC were also estimated. Results: The *Calotropis gigantea* leaves extracts reduced the hind paw oedema. In biochemical study *Calotropis gigantea* extracts showed increase in haemoglobin content as compared to adjuvant positive control group. The increased WBC count was significantly suppressed by extracts. The significant increase of ESR in adjuvant control was also restored back to normal by the extracts. Conclusions: From the results obtained in the current investigation, it may be concluded that the extract of *Calotropis gigantea* Linn. possesses potentially useful anti-arthritic activity.

Key words: *Calotropis gigantea*, anti-arthritic, adjuvant arthritis.

1. Introduction

Calotropis gigantea Linn (Asclepiadaceae) commonly known as "Arka" in Sanskrit and "Mudar" in English has been claimed in traditional literature to be valuable against a wide variety of diseases. The shrub is distributed through out India in dry waste places. Indian medicinal plants describe the use of this plant in the treatment of number of ailments including anorexia, asthma, cold and cough. Roasted leaves application is useful in painful joints or swellings [1, 2, 3].

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In the present study, the anti-arthritic activities of leaves extract of *Calotropis gigantea* have been studied using Freund's adjuvant – induced arthritis in rats.

2. Material and methods

2.1 Plant material

The leaves of *Calotropis gigantea* were collected around Belgaum city in November 2004 and were identified by Prof. R.S. Goudar, Department of Botany, KLES's R.L. Science Institute, Belgaum, Karnataka, where a voucher specimen is deposited.

2.2 Preparation of Extracts

The leaves were dried under shade reduced to a fine powder, which were subjected to hot continuous extraction in a Soxhlet extractor, successively with petroleum ether (40-60°), ethyl acetate and ethanol. Finally, the powdered material was macerated with chloroform water for 24 hr to obtain aqueous extract. Each time before extracting with next solvent, the powdered material was dried in hot air oven below 50°. Each extract was then concentrated by distilling off the solvent followed by evaporation to dryness on a water bath. All extracts were stored in refrigerator for qualitative analysis and pharmacological studies.

2.3 Animals

Albino Wistar rats of either sex weighing 150-200 gm were used. Animals were housed at a temp of 25 ± 1°C and relative humidity of 45-55 %. A 12:12 dark light cycle was followed during the experiments. All the animal experiments were approved by Animal Ethics Committee of the Institution (CPCSEA Registration No. 221)

2.4 Drugs

Freund's complete adjuvant (Sigma, Germany) Diclofenac sodium were used in the study.

2.5 Anti-arthritic activity

2.5.1 Freund's Adjuvant induced arthritis [4]

Animal were randomly divided in to six groups of six animals each (n=6), Group One served as control received 1 % Tween 80, Group Two received Diclofenac sodium (10 mg/kg po.) served as reference standard [5] and Group Three, Four, Five and Six received the crude extract of leaves of pet. ether, ethyl acetate, ethanol and aqueous po. respectively.

Arthritis was induced by injecting a 0.05 ml (0.5% w/v) suspension of killed *Mycobacterium tuberculosis* homogenized in liquid paraffin into the left hind paw. Drug treatment was started from the initial day that is from the day of adjuvant injection (0 day), 30 minutes before adjuvant injection and continued till 21st day.

Paw volume was measured on 4th, 8th, 14th and 21st day by using plethismometer. The changes in body weight were recorded daily. At 22nd day blood was withdrawn through retro orbital vein puncture of all groups by anaesthetizing the animals with di-ethyl ether and the biochemical parameters like haemoglobin content, total WBC count, differential WBC count, ESR and RBC were analyzed.

2.6 Statistical Analysis

The experimental results are represented as mean ± SEM (Standard Error Mean). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test P values <0.05 were considered significant.

3. Results and discussion

Steroids, triterpenoids, glycosides, flavonoids, carbohydrates, proteins were found to be present in *Calotropis gigantea* leaves extracts as observed by the qualitative tests. In adjuvant

induced arthritis model, rats develop a chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction and remodeling. These inflammatory changes ultimately result in the complete destruction of joint integrity and function in the affected animals [6].

The aqueous extract inhibited the rat paw oedema by 67.04 % after 21 days where as diclofenac sodium produced 68.29 % inhibition of rat paw oedema after 21 days (Table 1). The aqueous extract, petroleum ether extract and standard drug shown a significant inhibition of the paw oedema (Table 2).

The standard drug, petroleum ether, ethyl acetate, ethanol and aqueous extracts have shown the increase in haemoglobin content 14.48 ± 0 .054, 14.43 ± 0.049, 14.8 ± 0.10, 13.96 ± 0.08 and 14.22 ± 0.094 respectively when compared to control 13.47 ± 0.14 (Table 3). The increased WBC count was significantly suppressed by extracts and standard diclofenac sodium. Arthritis condition generally results in accumulation of leucocytes and release of lysosomal enzymes, the main mediators in arthritis [7]. In present study the migration of leucocytes into the inflammed area is significantly suppressed by the extracts as seen from the significant decrease in total WBC count.

Table 1. Mean changes in paw volume in adjuvant induced arthritis in rats

Group	Changes in Paw Volume			
	4th day	**8th day**	**14th day**	**21st day**
Control Tween 80 (2%)	4.802 ± 0.1331	4.770 ± 0.1984	4.763 ± 0.2546	4.168 ± 0.1015
Diclofenac sodium (10 mg/kg)	3.952* ± 0.1300	3.003** ± 0.1573	2.298** ± 0.1468	1.290** ± 0.1041
Petroleum ether extract (300 mg/kg)	4.253 ± 0.1743	3.140** ± 0.1477	2.425** ± 0.2142	1.367** ± 0.1131
Ethyl acetate extract (200 mg/kg)	4.263 ± 0.3222	3.142** ± 0.1783	3.072** ± 0.2882	2.087** ± 0.2485
Ethanol extract (300 mg/kg)	4.763 ± 0.2177	3.027** ± 0.1483	2.388** ± 0.2790	1.982** ± 0.2654
Aqueous extract (300 mg/kg)	0.443 ± 0.1696	2.347** ± 0.2194	1.763** ± 0.1993	1.328** ± 0.1674

All values are in Mean ± SEM
* $p<0.05$ = Significant, ** $p<0.01$ = More significant vs. Control; n = 6

Table 2. Percentage inhibition of Paw volume in adjuvant-induced arthritis in rats

Group	% Inhibition of Paw Volume			
	4th day	**8th day**	**14th day**	**21st day**
Diclofenac sodium (10 mg/kg)	17.58	36.72	50.85	68.29
Petroleum ether extract (300 mg/kg)	10.98	33.68	45.06	66.22
Ethyl acetate extract (200 mg/kg)	10.69	33.79	34.58	48.25
Ethanol extract (300 mg/kg)	0.63	36.24	48.90	51.13
Aqueous extract (300 mg/kg)	7.135	50.36	62.54	67.04

Table 3. Effect of Biochemical Parameters in Adjuvant induced Arthritis in Rats

Group	Hb gms%	Changes in Biochemical Parameters					ESR (mm/hr)	RBC count (million/ cu.mm)
		Total WBC count (cells/cu.mm)	Differential count %					
			Neutrophils	Lymphocytes	*Eosinophils*	*Monocytes*		
Control	13.47 ± 0.14	8517 ± 70.32	10.17 ± 1.87	89.50 ± 1.89	0.33 ± 0.21	0.0 ± 0.0	10 ± 0.68	7.17 ± 0.27
Standard	14.48 ± 0.054**	6350 ± 178.4**	42.17 ± 3.07**	54.33 ± 2.93**	2.17 ± 0.31**	2 ± 0.37**	5 ± 0.45**	7.45 ± 0.31
Petroleum ether extract	14.43 ± 0.05**	5783 ± 272.5**	35.67 ± 2.12**	60.83 ± 2.04**	1.83 ± 0.31**	1.67 ± 0.33**	4.83 ± 0.31**	7.87 ± 0.21
Ethyl acetate extract	14.8 ± 0.10**	5650 ± 403.1**	35.17 ± 3.94**	64.83 ± 3.93**	0.0 ± 0.0	0.0 ± 0.0	7.50 ± 0.99	7.31 ± 0.45
Ethanol extract	13.96 ± 0.08**	6883 ± 98.04**	46.83 ± 6.31**	50.83 ± 6.15**	1.0 ± 0.26**	1.33 ± 0.44*	6.67 ± 0.92**	6.83 ± 0.36
Aqueous extract	14.22 ± 0.10**	6553 ± 158.50**	36.17 ± 2.74**	59.67 ± 2.50**	2.00 ± 0.37**	2.00 ± 0.37**	3.83 ± 0.70**	7.38 ± 0.45

All values are in Mean ± SEM

* $p<0.05$ = Significant, ** $p<0.01$ = More significant vs. Control; n = 6

Table 4. Changes in body weight in adjuvant induced arthritis in rats

Group	Mean body weight		Mean changes in body weight (± SEM)
	0th day	21st day	
Control	158.3	166.6	08.33 ± 1.67
Standard	155.8	195.8	40.00 ± 2.58**
Petroleum ether extract	150.8	174.2	23.33 ± 4.60**
Ethyl acetate extract	151.6	162.5	10.83 ± 1.54
Ethanol extract	144.2	160.8	16.67 ± 4.01
Aqueous extract	150.8	185.8	35.00 ± 2.89**

All values are in Mean ± SEM
* $p<0.05$ = Significant, ** $p<0.01$ = More significant vs. Control; n = 6

The increased lymphocytes count in adjuvant control group was significantly restored back to normal by extracts and standard drug. The ESR count which drastically increased in arthritic control group has been remarkably counteracted by the standard and extracts, restoring it back to normal thus justifying it significant roles in arthritic conditions. Changes in the body weight have also been used to assess the course of disease and the response to the therapy of anti-inflammatory drugs [8]. During the course of experimental period, as the incidence and severity of the arthritis is increased, the changes in body weight of the rats also occur. The loss of body weight during arthritis condition was also supported by earlier observation on alterations in the metabolic activities of diseased rats [9]. An earlier finding suggests the absorption of ^{14}C – glucose and ^{14}C – leucine in rats was reduced in the case of inflamed rats [10]. But on treatment with anti-inflammatory the decrease in the absorption was nullified [11]. And this shows that the anti-inflammatory drugs correct the decrease/ deranged absorption capacity of intestine during inflammation. The significant increase in body weight during treatment of diclofenac sodium, petroleum ether extract and aqueous extract when compare to control was may be due to the restoration of absorption capacity of intestine.

From the result observed in the current investigation, it may be concluded that the extract of *Calotropis gigantea* Linn. possesses potentially useful anti-arthritic activity.

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