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Anti-arthritic activity of leaves of *Gymnema sylvestre* R.Br. leaves in rats

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Abstract

The present study is aimed to evaluate the leaf extracts of *Gymnema sylvestre* for acclaimed anti-arthritic activity using albino rats. The arthritic action of leaves of *Gymnema sylvestre* was studied in Freund's adjuvant induced arthritis in rats. Diclofenac sodium was used as a standard drug. The study revealed that the petroleum ether (40—60°) extract and aqueous extract of *Gymnema sylvestre* possessed significant anti-arthritic activity in all parameters of the study compared to control group. The more potent anti-arthritic activity of leaves of *Gymnema sylvestre* may be due to nature of steroids, triterpenoids and saponin glycosides. This claim demands further detailed phytochemical profile to identify the active constituent responsible for the anti-arthritic activity.

Keywords: Gymnema sylvestre, Anti-arthritic activity, Diclofenac sodium.

Introduction

Gymnema sylvestre R.Br. (Asclepiadaceae) leaves, commonly known as Gudmar is a large woody, much branched climber with pubescent young parts in dry forest up to 600 mt heights [1]. *Gymnema sylvestre* leaf has been widely used in Ayurvedic traditional medicine leaves of the plant as anti-diabetes [2], astringent, bitter, acrid, thermogenic, anti-inflammatory, anodyne, digestive and liver tonic [3]. Tannins and saponin are the chief chemical constituents present in *Gymnema sylvestre* and are known to possess anti-arthritic activity. [4]

Materials and Methods

Plant material
The leaves of *Gymnema sylvestre* was collected in August 2004 from local market of Belgaum & authenticated by Dr. G.R. Hegde, Professor and Head, P.G. Department of

Botany, Karnataka University, Dharwad. The voucher specimen (KCP/PC/205) is preserved in laboratory for reference.

Preparation of extract

The leaves were dried under shade, powdered and passed through 40 meshes and stored in closed vessel for further use. Powdered leaves were subjected to hot continuous extraction (soxhlet) with petroleum ether (40-60°). The marc was dried completely at 50°C and again loaded in the extractor and further extracted successively with ethyl acetate and alcohol. Finally, the marc was macerated with chloroform water to obtain the aqueous extract. Each extract is concentrated in vacuum under pressure using rotary flash evaporator. The different extracts were subjected to qualitative chemical investigation and were taken for pharmacological studies.

Phytochemical analysis of the extract

The extract was screened for the presence of various constituents employing standard screening test [5]. Conventional protocol for detecting the presence of glycosides, saponins, flavonoids, and tannins etc.was used. Several phytoconstituents like flavonoids, saponins and glycosides are known to promote anti-arthritic activity due to their antioxidant and antimicrobial activities [6] .

Animals

Female wistar albino rats weighing between 130-200 gm were selected between 130-200 gm were selected for the pharmacological study. The animals were kept in standard polypropylene cages. The bedding materials of cages were changed everyday. The temperature of the experimental animals room was maintained at 22±3°c and lighting was kept artificial. The study protocol was approved from Animal Ethical Committee of the Institution.

Drugs

The rats were divided into seven groups each consisting 6 rats. The first group represented normal rats. Second group that was treated as arthritic control. These two groups were received saline orally. The third group received the standard drug Diclofenac sodium at a dose of 10mg/kg [7]. 1% Tween 80 was used as a vehicle to suspend the various extracts. The fourth, fifth, sixth and seventh groups received petroleum ether, ethyl acetate, alcohol and aqueous extracts at a dose of 300 mg/kg, 200mg/kg, 30mg/kg and 300mg/kg respectively by oral route. [8].

Anti-arthritic Activity

Six groups, except normal group, were made arthritic by injecting 0.1 ml Freund's complete adjuvant (Sigma, Germany) into the subplantar region of left hind paw on day '0'. This adjuvant consists of dead mycobacterium tuberculosis bacteria suspended in heavy paraffin oil to give final concentration of 0.5 mg/ml.

Saline or extracts or Diclofenac sodium were administered orally once daily, from the initial day i.e. from the day of adjuvant injection (0 day), 30 minutes before adjuvant injection, and continued till 21st day [9].

The anti-arthritic effect of the extracts as well as Diclofenac sodium was evaluated by measuring paw volume of injected paw on 4th, 8th, 14th and 21st day of study by using Digital Plethysmometer (UGO Basile, Italy). The mean changes in injected paw volume with respect

337

to initial paw volume were calculated on respective days and % inhibition of paw volume with respect to control group was calculated [10]. The changes in body weight were recorded daily. On the day 22nd blood was withdrawn from the each animal through retroorbital vein puncture by anaesthetizing the animals with anaesthetic ether. The blood was collected into vials containing EDTA and the biochemical parameters like haemoglobin content, total WBC count, differential WBC count, ESR and RBC were analysed. [11].

Statistical Analysis
The results were analysed by using one way analysis of variance (ANOVA) followed by Dunnet's 't' test to determine the statistical significance. [12].

Results and Discussion

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 [13]. Chronic inflammation involves the release of number of mediators like cytokines (IL-Iβ and TNF-α), GM-CSF, interferons and PGDF. These mediators are responsible for the pain, destruction of bone and cartilage that can lead to severe disability [14]. It appears from our findings that the petroleum ether extract treated groups significantly reduced paw swelling may be due to inhibiting the release of above mediators or inhibiting the response of inflammatory cells or protecting the release of joint cartilage and bone destruction in chronic arthritic model.

WBC count seems to be raised in control group, but in petroleum ether extract, aqueous extract and Diclofenac sodium treated groups seems to lower (p<0.01). In arthritis condition there is a mild to moderate rise in WBC count due to release of IL-Iβ inflammatory response. IL-Iβ increases the production of both granulocyte and macrophages colony stimulating factor [15]. T-lymphocytes have been reported to play a central role in the pathogenesis of rheumatoid arthritis. These cells comprise the majority of the lymphoid cells found in the rheumatoid synovium. In arthritic condition there is a moderate elevation in lymphocyte count [7]. In all treated groups the lymphocyte count was suppressed significantly (p<0.01) as compared to control. The results indicated that the total average RBC count in the entire test materials treated group raised marginally as compared to control group. However, the changes in RBC count were found to be statistically non-significant (p>0.05), which may be due to iron deficiency or low serum iron with normal iron store. ESR is an estimate of the suspension stability of RBC's in plasma, related to the number of size of red cells and to the relative concentration of plasma proteins especially fibrinogen and the α and β globulins. Increases are an indication of active but obscure disease processes. The acute phase proteins in ESR and C-reactive protein share the property of showing elevations in the concentration in response to stress or inflammation that occurs like infection, injury, and surgery and tissue necrosis. So in arthritic condition, ESR is elevated. It appears from the study that decrease in ESR denotes the anti-arthritic activity [15].

Changes in the body weight have also been used to assess the course of the disease and the response to the therapy of anti-inflammatory drugs. During the course of the experimental period, as the incidence and severity of the arthritis is increased, the changes in body weight of the routs 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. Earlier findings suggests the absorption of ^{14}C-glucose and ^{14}C-leucine in rats intestine was reduced

338

in the case of inflamed rats. But on the treatment with anti-inflammatory drugs, the decrease in the absorption was nullified and this shows that the anti-inflammatory drugs correct the decreased/ deranged absorption capacity of intestine during inflammation. The significant increase in the body weight during treatment of Diclofenac sodium, petroleum ether extract and aqueous extract when compare to control was may be de to the restoration of absorption capacity of intestine.

Table No.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
I	Control	4.802 ±0.1331	4.770 ±0.1984	4.763 ±0.2546	4.168 ±0.1015
II	Diclofenac sodium (10mg/kg)	3.952 ±0.1300	3.003* ±0.1573	2.298* ±0.1468	1.290* ±0.1041
III	Petroleum ether extract (300mg/kg)	4.253 ±0.1743	3.140* ±0.1477	2.425* ±0.2142	1.367 ±0.1131
IV	Aqueous extract (300mg/kg)	0.443 ±0.1696	2.347* ±0.2194	1.763* ±0.1993	1.328* ±0.1674

No. of rats = 6 per group, tabular value represents mean±SE; $P<0.01$* compared I with II, III and IV

Table No.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
Standard	17.58	36.72	50.85	68.29
Petroleum ether extract	10.98	33.68	45.06	66.22
Aqueous extract	7.135	50.36	62.54	67.04

No. of rats = 6 per group, tabular value represents mean±SE; $P<0.01$ comparison with control group

Table No.3: Changes in Body Weight in Adjuvant-induced Arthritis in Rats

Group	Mean body weight		Mean changes in body weight
	0th day	21st day	
Normal	153.3	190	36.67±2.472
Control	158.3	166.6	8.33±1.667
Standard	155.8	207.5	40±2.582
Petroleum ether extract	150.8	174.2	23.33±4.595
Aqueous extract	150.83	185.8	35±2.887

No. of rats = 6 per group, tabular value represents mean±SE; $P<0.01$ comparison with control group

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Table No.4: Effect of Biochemical Parameters in Adjuvant-induced Arthritis in Rats

Sl. No.	Parameters	Control (Normal Saline)	Standard Diclofenac sodium (10mg/kg)	Test drug petroleum ether extract (300mg/kg)	Test drug aqueous extract (300mg/kg)
1.	Haemoglobin (%)	13.47 ±0.1406	14.48 ±0.05426	14.43 ±0.04944	14.22 ±0.09458
2.	Total WBC count (cu.mm)	8517 ±70.32	6350 * ±178.4	5783 ±272.5	6553* ±158.5
3.	Differential WBC count				
a)	Neutrophils (%)	10.17 ±1.1869	42.17 ±3.070	35.67 ±2.124	36.17 ±2.738
b)	Lymphocytes (%)	89.50 ±1.893	54.33* ±2.929	60.83* ±2.040	59.67* ±2.499
c)	Eosinophils (%)	0.3333 ±0.2108	2.167 ±0.3073	1.833 ±0.3073	2.0 ±0.3651
d)	Basophils	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
e)	Monocytes (%)	0.0% ±0.0	2.0% ±0.3651	1.667% ±0.3333	2.0% ±0.3651
4.	ESR (mm/hr)	10.0 ±0.6831	5.0 * ±0.4472	4.833 * ±0.3073	3.833* ±0.7032
5.	RBC (million/cu. mm)	7.168 ±0.2668	7.448 ±0.3114	7.870 ±0.2055	7.383 ±0.4515

No. of rats = 6 per group, tabular value represents mean±SE; $P<0.01$ (comparison of all parameters of control group with standard and test groups) ; RBC $p>0.05$ compared to standard

Acknowledgement

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