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Research Article

ANTIARTHRITIC ACTIVITY OF ROOT EXTRACTS OF *COCCULUS HIRSUTUS*

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ABSTRACT

The anti-arthritic effect of oral administration of methanolic and aqueous extracts of root (100 and 200 mg/kg, p.o., n=6) of *Cocculus hirsutus* was evaluated using Freund's adjuvant arthritis model in Wistar albino rats. The acute toxicity studies were carried out according to the CPCSEA guidelines. Arthritis was induced by injecting 0.1ml of complete Freund's adjuvant below the plantar aponeurosis of the right hind paw. Treatment with the extracts and standard started on the day of induction of inflamogens and continue up to 21 days.The body weight loss that was found during the arthritic condition was corrected on treatment with methanolic extracts of root of *Cocculus hirsutus linn*. The swelling of the paw during the secondary lesions was also markedly reduced. Various hematological parameters like total WBC count, ESR and RBC were also estimated. The results of the present study support the traditional use of this plant and it can be used as anti-arthritic drug.

Keywords: Anti-arthritic, *Cocculus hirsutus linn*, Erythro sedimentation rate, Freund's complete adjuvant.

INTRODUCTION

Herbal and natural products of folk medicine have been used for centuries in every culture throughout the world. Scientists and medical professionals have shown increased interest in this field as they recognize the true health benefits of these remedies[1]. Natural products have played an important role throughout the world in treating and preventing human diseases[2]. Rheumatoid arthritis (RA) is an autoimmune disorder characterized by synovial proliferation, inflammation, subsequent destruction like deformity of joints or destruction of cartilage and bone[3]. Rheumatoid arthritis can also cause inflammation of the tissue around the joints, as well as in other organs in the body[4]. Various phytochemical constituents from herbal plant showed beneficial effect in rheumatoid arthritis.

Cocculus hirsutus (L.) (Menispermaceae) is growing abundantly in different parts of India. Sepals are hairy therefore it is called *Cocculus hirsutus.* Commonly it is known as jaljamni[5]. Earlier investigation on the plant resulted in the isolation of several bioactive alkaloids and triterpenoids[,6,7,8,9]. *C. hirsutus* used medicinally by the Indian tribes for a wide range of ailments, including constipation and kidney problems[10,11]. The extracts of flowers, seeds, leaves and barks of *C. hirsutus* have been extensively studied for many potential uses including the anti-inflammatory and analgesic activities[12]. The present study envisaged evaluating the roots of *C. hirsutus* for its antiarthritic activity.

MATERIALS AND METHODS

Plant material

The roots of *C.hirsutus* were collected from the forests of Pavagadh, Gujarat, India and authenticated at the department of bioscience, Vallabh vidyanagar, Gujarat. The roots were air-dried separately for 1 month and the respective material was powdered.

Preparation of Extract

The petroleum ether extract of root powder was prepared using petroleum ether (40-60^{0}C) by soxhlet method at a temperature of 40-60^{0}C[13]. The methanolic extract was prepared using methanol by soxhlet method at a temperature of 40-60^{0}C. Aqueous extract was also prepared .The extracts were concentrated under vacuum and dried over anhydrous sodium sulphate. The methanolic extract yielded semisolid, viscous, dark coloured mass while aqueous extract yielded dark brown coloured mass. A suspension of methanolic extract in 1% (w/v) gum acacia was prepared for oral administration by gastric intubation method[14].

Pharmacological screening for anti-arthritic activity

Animals

For acute toxicity studies and anti-arthritic activities, Male Wister albino rats weighing between 150 g to 200 g were selected. The animals were acclimatized to standard laboratory conditions (temperature 25 ± 2^{0}C) and maintained on 12 h light, 12 h dark cycle. They were provided with regular rat chow (Lipton India Ltd., Mumbai, India) and drinking water ad libitum. The animal care and experimental protocol were in accordance with the Institutional Animal Ethical Committee (IAEC).

Determination of Acute Drug Toxicity

The acute oral toxicity study was carried out as per the guideline set by the Organization for Economic Co-operation and Development (OECD) received from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One tenth of the medium lethal dose (LD50) was taken as an effective dose. The acute toxicity of the various extracts was determined[15].

Freund's adjuvant Induced Arthritis in Rats

Freund's adjuvant induced Arthritis model[16] was used to assess the anti-arthritic activity in albino rats. Animals were randomly divided into four groups of six animals each (n=6). Group I served as control received 1% tween 80, Group II received dexamathasone (1 mg/kg p.o.) served as reference standard , Group III and IV received the crude extracts of roots of methanolic extracts (100mg/kg,p.o,200mg/kg,p.o,), Group V and VI received the crude extracts of roots of aqueous extracts (100mg/kg,p.o,200mg/kg, p.o.),respectively. Arthritis was induced by injecting a 0.05 ml (0.5% w/v) suspension of killed Mycobacterium tuberculosis bacteria (Difco) homogenized in liquid paraffin into the left hind paw. Drug treatment was started from the initial day i.e. from the day of adjuvant injection (0 day), 30 minutes before adjuvant injection and continued till 21st day. Paw volume was measured on 5th, 13th and 21st day by using plethismometer. The mean changes in injected paw edema with respect to initial paw volume, were calculated on respective days and % inhibition of paw edema with respect to untreated group was calculated. Percentage inhibition of paw volume was calculated by the formula,

$$i = (1-\Delta V_{Treated} / -\Delta V_{Control})*100$$

Where, ΔV represents the mean change in paw volume

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 diethyl ether and haematological estimation RBC and WBC count were estimated in an improved

neubauer chamber[17]. ESR was estimated by the method of westergren[18].

Statistical analysis

The statistical significance was assessed by using one-way analysis of variance (ANOVA) and followed by dunnet's comparisons test. All the data are presented as mean ± SEM and p<0.05 was considered as significant.

RESULTS

From the acute toxicity study, the LD_{50} cut-off dose for methanolic extract and aqueous extract was found to be 3000 mg/kg body weight. Hence, the therapeutic doses were taken as 100 mg/kg and 200 mg/kg body weight for methanolic extracts and aqueous extracts. Skeletal complications start with focal erosion of cartilage followed by marginal and subchondral bone loss in adjuvant-induced arthritis model. Extended joint destruction with ankylosis and generalized bone loss are characteristic for late complications[19].

The methanolic extract inhibited the rat paw edema by 68.40 whereas dexamethasone produced 71.90% inhibition of rat paw edema after 21 days (Table 1). Aqueous extract showed inhibition of the rat paw edema less than methanolic extract. As shown in (Table 2) standard drug and methanolic extracts have shown the increase in Hemoglobin content compare to control. The total WBC counts were remarkably increased in adjuvant-induced rats (Table 2 Control group). However, *C. hirsutus* root extracts and standard drug treated group significantly decreased (P<0.05) the total WBC count. 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 its significant roles in arthritic conditions. The loss of body weight observed during the arthritis condition (Table 3). The standard drug, methanolic extract and aqueous treatment significantly increased the body weight.

Table 1: Mean changes in paw volume and percentage inhibition of paw volume in Adjuvant-induced arthritis in rats

Groups	Change in paw volume				% inhibition of paw volume			
	3rd Day	5th Day	13th Day	21stDay	3rd Day	5th Day	13th Day	21st Day
Model Control	8.98±0.10	11.72±0.56	11.07±0.31	10.9±0.63	0	0	0	0
standard	6.15±0.51*	5.75±0.36*	4.33±0.25**	3.09±0.85*	31.52	50.93	60.89	71.90
100mg(Methanol)	7.06±0.33*	8.16±0.75*	6.29±0.468*	4.48±0.11*	21.39	30.38	43.17	58.94
200mg(Methanol)	6.91±0.81*	5.90±0.29**	4.55±0.91**	3.16±0.11*	23.10	49.70	58.90	68.40
100mg(Aqueous)	7.35±0.26*	9.09±0.29*	7.65±0.20*	5.23±0.28*	18.45	22.45	30.89	51.83
200mg Aqueous)	8.11±0.21*	10.13±0.29*	8.17±0.71*	4.92±0.19*	9.69	13.57	26.20	54.87

All values are expressed as *Mean ±S.E.M. *p<0.05 = Significant, n = 6;* **p< 0.01=more significant vs. control

Table 2: Changes in hematological parameters in adjuvant arthritis rats (mean ± SEM)

Parameters	Total WBC count (cells/cu.mm)	RBC count (million/cu.mm)	Hb (gm%)	ESR (mm/hr)
Model control	6.79 ±0.92	4.67 ±.05	12.98 ± 0.25	3.56 ± 0.16
Standard	6.45 ± 0.23*	4.21 ± 0.13*	13.86 ± 0.89**	4.02 ± 0.34*
100mg(methanol)	6.32 ± 0.35*	3.98 ± 0.37**	14.67 ±0.10*	4.18 ± 0.31*
200mg(methanol)	6.42 ± 0.21 **	4.04 ± 0.29*	14.12 ± 0.04**	4.23 ± 0.89*
100mg (Aqueous)	6.58 ±0.47*	3.89 ± 0.31*	13.30 ± 0.17*	4.11 ± 0.71*
200mg Aqueous)	6.66 ± 0.13	4.15 ± 0.84*	14.08 ± 0.65*	4.08 ± 0.28

All values are expressed as *Mean ±S.E.M. *p<0.05 = Significant vs. Control; n = 6;* **p< 0.01=more significant vs. control vs. control

Table 3: Changes in body weight in adjuvant arthritis rats (mean ± SEM)

Groups	Before induction	On 21st day	Mean changes on body weight((± SEM)
Model control	167.9	186.6	18.731
Standard	161	172.5	11.519**
100mg (methanolic)	155.4	172.9	17.511*
200mg(methanolic)	153.2	162.5	9.356**
100mg (Aqueous)	158.7	166.4	7.734*
200 mg (Aqueous)	153.4	165.7	10.327*

All values are i expressed as Mean ±S.E.M ; *p<0.05 = Significant, n = 6; **p< 0.01=more significant

DISCUSSION

In the present study, rats were selected to induce arthritis because animal models have played a key role in defining mechanisms and it has close similarities to human rheumatoid disease[20]. The determination of paw swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation and the therapeutic effects of drugs. Acute or chronic inflammatory processes cause an accumulation of zinc and copper in many organs, particularly in the inflamed areas. However, standard drug, and methanolic extract significantly suppressed the swelling of the paws. In the present study, the migration of leucocytes into the inflamed area was significantly suppressed by the standard drug and methanolic extract as seen from the significant decrease in total WBC count. Erythrocyte sedimentation rate (ESR) is an estimate of the suspension stability of RBC's in plasma. It is related to the number and size of the red cells and to the relative concentration of plasma proteins, especially fibrinogen, α and β globulins. Increase in the rate is an indication of active but obscure disease processes. In the studies there is an increased ESR level which is a common diagnostic feature in patient in chronic arthritis[21].

Changes in body weight have also been used to assess the course of the disease and the response to therapy of anti-inflammatory drugs. As the incidence and severity of arthritis increased, the changes in the body weights of the rats also occurred during the course of the experimental period. The increased body weight during treatment of standard drug and methanolic extracts may be due to the restoration of absorption capacity of intestine[22].

From the results observed in the current investigation, it may be concluded that the methanolic extracts of roots of *C. hirsutus* has a promising anti-arthritic activity since it was active in both the inflammation models and adjuvant. It was dose dependant and the dose of 200mg/kg was more effective than 100mg/kg bodyweight whereas methanolic extracts were more effective than aqueous extracts.

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