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Full Length Research Paper

Evaluation of anti arthritic potential of *Hybanthus enneaspermus*

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The effect of alcoholic and aqueous extracts of the whole plant of *Hybanthus enneaspermus* Muell (Violaceae) on freund's adjuvant induced arthritis was evaluated. The percentage of yield was found to be 12.8 and 10.6% for alcoholic and aqueous extracts respectively. Both the extracts significantly ($p < 0.001$) decrease the paw thickness at the end of 30 days treatment. Though in acute phase inflammation both of them show the same potency in chronic phase alcoholic extract exhibit more potency than the aqueous extracts. At the end of the studies the alcoholic extract shows more pronounce effect (59.4%) as comparable to aqueous extract (57.4%). The phytochemical analysis revels the presence of alkaloids, flavonoids, glycosides, phenols, carbohydrates and tannins in the extracts. The increase in body weight was observed in tested animals as comparable to the control. This result support the folkore use of this plant against the inflammmatory conditions like arthritis.

Key words: *Hybanthus enneaspermus,* adjuvant induced arthritis, whole plant, acute and chronic phase inflammation, body weight.

INTRODUCTION

Rheumatoid arthritis is a systemic autoimmune disorder characterized by polyarticular symmetrical arthritis. Various inflammatory mediators produce joint inflammation with pain function loss, joint destruction and permanent deformity after certain time if remained untreated. This disease has a world wide distribution but its pathogenesis is not clearly understood (Harris et al., 1990) although there are few anti-rheumatic drugs showing effectiveness on the treatment of rheumatoid arthritis, the side effect and toxicity call for new and more effective natural drugs (Scott et al., 1998). Rat adjuvant arthritis is an experimental model of polyarthritis which has been widely used for preclinical tests for numerous anti-arthritic agents which are in preclinical and clinical investigations. The pattern of inflammation by freund's adjuvant is similar pattern as arthritis in mammals. (Pearson, 1956; Carlson et al., 1985; Benslay et al., 1991). *Hybanthus enneaspermus* Muell belongs to family Violaceae is an herb or a shrub distributed in the tropical and subtropical regions of world. It is an herb often with woody troches found in the warmer parts of India. The plant is popularly known as *Ratanpurus* (Hindi). Traditionally the plant is used as an aphrodisiac, demulcent, tonic, diuretic, in urinary infections, diarrhoea, leucorrhoea, dysuria, in inflammation and sterility (Yoganarasimhan, 2000) an infusion of the plant extract is given in case of cholera. The plant has been reported to have anti-inflammatory, antitussive, antiplasmodial, antimicrobial (Rajakaruna et al., 2002; Weniger et al., 2004) anti convulsant and free radical scavenging activity (Hemlatha et al., 2003). The plant is reported to contain aurantiamide acetate, isoaborinol, -sitosterol and triterpene (Prakash, 1999; Retnam, 2003). In folklore the plant is used in case of gonorrhoea, urinary infections and in inflammatory conditions. Whether these claims are valid or not should be probed scientifically to establish the rational use. With this aim we carried out investigation of this plants potential against arthritis induced by freund's adjuvant.

MATERIALS AND METHODS

Plant material

The plant was collected from rural belt of Bhubaneswar, Orissa and was authenticated in the department of Botany, Utkal University, Bhubaneswar. The plant was collected in bulk and washed with tap

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water to remove the soil and dirt particles and then shade dried. The dried plant materials were milled into coarse powder by a mechanical grinder and sieved in sieve 20. The coarse powder was taken for extraction in soxhlet apparatus and fine powder for maceration.

Preparation of extracts

Alcoholic extract

The powdered plant (1 kg) was exhaustively extracted by Soxhlet apparatus with 95% ethanol at a temp 70°C up to 72 h. The total ethanolic extract was filtered and concentrated by distillation process. The concentrated mass was dried vacuum till constant weight.

Aqueous extract

The powdered plant material (1 kg) was macerated with chloroform water (1:9) for seven days. Chloroform-water was used to prevent the growth of microorganism in the extract. The extractive was filtered and concentrated over a water bath and further dried in vacuum oven till constant weight.

Experimental animals

Adult male albino mice 20 – 25 gm and rats 150 – 200 gms were used for the study. Animals were kept in the animal house of University Department of Pharmaceutical Sciences, Bhubaneswar, maintained under standard husbandry condition with free access to food and water *ad libitum.* All the experiments in this study were approved by institutional animal ethical committee with CPCSEA registration number IAEC/999/UDPS, Utkal University.

Acute toxicity studies

Oral acute toxicity studies were carried out with Albino mice weighing 20 – 25 gm, with 2 mice per dose group. The extracts were administered as per the staircase method (Ghosh, 1984; Reed, 1938). The mice were fed with alcoholic and aqueous extract of *H. enneaspermus* separately suspended in 2% of gum acacia at dose 1000, 2000, 3000, 4000, 5000 mg/kg bodyweight. The animals were observed continuously for 2 h for the gross behavioral changes and then intermittently once in every 2 h and finally at the end of 24 and 72 h to note for any signs of toxicity including death.

Freund's adjuvant induced arthritis

Freund's adjuvant induced Arthritis model was used to access 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 2% of gum acacia, Group II received Diclofenac sodium (50 mg/kg p.o) served as reference standard and Group III and IV received the ethanol and water extracts at a conc. of 500 mg/kg. Arthritis was induced by injecting 0.05 ml (0.5% w/v) of freund's adjuvant into the left hind paw .0.5% of w/v of freund's adjuvant was prepared by triturating 1 mg dead spores of *Mycobacterium tuberculai* in 2 ml of liquid paraffin. Drug treatment was started from the initial day, that is, from the day of adjuvant injection (0 day), 30 min before adjuvant injection and continued till 21st day. Paw thickness was measured on 1st, 2nd, 4th, 7th, 10th, 15th, 21st, 30th days by using verneir calliper (Tijani et al., 2008). The mean changes in injected paw edema with respect to initial paw volume, were calculated on respective days and the percentage of inhibition of paw edema with respect to untreated group was calculated on respective days following formula:

Percentage inhibition = 100[(T- C)/ C]
C = Difference in paw thickness in control group (C-Co).
T = Difference in paw thickness in test group (T-To).
Co = Initial thickness of control.
C = Thickness in time t in control.
To = Initial thickness of test.
T = Thickness in time t.

The body weight of the animals were measured by digital balance (CE,th-750) to access the course of the disease at the initial day before induction and at the end of 30th day.

Statistical analysis

Values were presented as mean ± S.E.M. Statistical analyses were performed by T-test for two independent variables or by ANOVA followed by the significance of differences between groups was determined by Student's t-test or ANOVA followed by Dunnett's multiple comparison.

RESULTS

From the extraction the yield was found to be 12.8% for alcoholic and 10.6% for aqueous extracts. From the acute toxicity study it was found that both the extracts are safe up to 5000 mg/kg so one tenth of this dose was consider as the evaluation dose. Preliminary phytochemi-cal screening revel the presence of alkaloids, flavonoids, glycosides, phenols, carbohydrates and tannins.

Table 1 shows the effect of extract on freund's adjuvant model induced arthritis. In the 30 days study it was found that both alcoholic and aqueous extract significantly $P < 0.001$ decrease the chronic inflammation induced by adjuvant shown as decrease in paw thickness. Standard diclofenac sodium significantly decrease the paw thickness from the 1st day after induction of freund's adjuvant, where as the extracts significantly decrease the thickness after 4th day. From Table 2, it was found that the alcoholic extract has got highest percentage of inhibition 59.4% of paw edema as compared to aqueous extract which is 57.4% at the end of 30 days. Standard Diclofenac sodium decreases the paw edema by 72.4%. A loss of body weight observes during the arthritis condition. Standard drug, alcoholic and aqueous extract significantly increases the body weight of the animal as compare to control which is depicted in Table 3.

DISCUSSION

The present study was carried out to see the efficiency of Indian herbal source against a chronic inflammatory disease, that is, arthritis. In the present study, rats were selected to induce arthritis because they develop a chronic swelling in multiple joints due to accumulation of inflammatory cells, erosion of joint cartilage and bone

Table 1. Effect of *H. enneaspermus* extracts on freund's adjuvant complete induced arthritis paw thickness (in cm).

Treatment	0 day	1st day	2nd day	4th day	7th day	10th day	15th day	21st day	30th day
Control	0.49±0.01	0.88±0.01	1.04±0.01	1.04±0.01	1.05±0.05	1.08±0.05	1.11±0.04	1.15±0.02	1.18±0.02
Standard	0.46±0.01	0.80±0.03*	0.72±0.03**	0.73±0.04**	0.71±0.04**	0.69±0.03**	0.69±0.03**	0.66±0.03**	0.65±0.04**
Aqueous extracts	0.45±0.01	0.88±0.03	0.84±0.01	0.85±0.02*	0.83±0.02**	0.80±0.01**	0.74±0.01**	0.73±0.02**	0.73±0.02**
Alcoholic extract	0.46±0.01	0.89±0.01	0.84±0.01	0.83±0.01*	0.83±0.01**	0.83±0.01***	0.79±0.01**	0.77±0.01**	0.75±0.01**

Values expressed in avg ± S.E.M of 6 rats.
*p < 0.05 for control untreated Vs treated.
**p < 0.01 for control untreated Vs treated.
***p < 0.001 for control untreated Vs treat.

Table 2. Percentage inhibition on Freund's adjuvant complete induced arthritis.

Treatment	1st day	2nd day	4th day	7th day	10th day	15th day	21st day	30th day
Standard (s)	12.8%	52.7%	50.0%	55.0%	61.0%	62.9%	66.6%	72.4%
Aqueous extracts	-	30.9%	32.7%	33.9%	37.2%	46.7%	53.0%	57.4%
Alcoholic extract	-	29.0%	27.2%	32.1%	40.6%	53.2%	57.5%	59.4%

Table 3. Changes in body weight in adjuvant induced arthritis in rats.

Group	Mean body weight (gm)		Mean changes in body weight (±SEM)
	Before induction	On 30th day	
Control	154±2.36	167±4.4	13.3±1.667
Standard	155.8±1.4	198.5±3.8	42.7±2.582**
Alcohol extract	150.8±3.6	173.2±4.2	22.4±4.595**
Aqueous extract	151.6±3.2	176.5±3.6	24.9±1.537*

All Values expressed in Avg ± S.E.M of 6 rats.
*p < 0.05 for control untreated Vs treatment.
**p < 0.01 for control untreated Vs treatment.
***p < 0.001 for control untreated Vs Treatment.

destruction. It has close similarities to human rheumatoid diseases (Singh et al., 1996). The determination of paw swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation and the therapeutic effects of drugs. The freund's adjuvant model is chosen as it develop chronic swelling in multiple joints with influence of inflammatory cells with erosion of joint cartilage and bone destruction. Chronic inflammation involves the release of number of mediators like cytokines (IL-1B and TNF-), GM-CSF, interferon's and PGDF. These mediators are responsible for the pain, destruction of bone and cartilage that can lead to severe disability

(Eric et al., 1996).

However standard drug, aqueous and alcoholic extract significantly suppressed the swelling of the paws in both acute and chronic phase which may be due to the suppression of inflammatory mediator released due to induction of freund's adjuvant. Though the actual mechanism of suppressing inflammation is not known but it can be correlated with the presence of alkaloids and flavonoids in suppressing the inflammation and antioxidant activity.

Changes in the body weight have also been used to access the course of the disease and the response to therapy of anti-inflammatory drugs (Winder et al., 1969). 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. Earlier findings suggest that absorption of 14C- glucose and 14C-leucine in rat's intestine was reduced in the case of inflamed rats (Somasundaran et al., 1983) but on the treatment with anti-inflammatory drugs, the decrease in absorption was nullified and it shows that the anti-inflammatory drugs correct the decreased/deranged absorption capacity of intestine during inflammation.

The increased body weight during treatment of standard drug, aqueous and alcoholic extracts may be due to the restoration of absorption capacity of intestine. From the results observed from the current investigation, it is concluded that the aqueous and alcoholic extract of *H. enneaspermus* possesses potentially useful anti-arthritic activity since it give a positive result in controlling inflammation in adjuvant induced arthritic model in rats.

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