

Evaluation of anti arthritic activity of aqueous extract of *Hibiscus Platinifolius* in albino rats

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ABSTRACT

Rheumatoid arthritis is a chronic, inflammatory disorder that may affect many tissues and organs, but principally attacks flexible (synovial) joints. The process produces an inflammatory responses of the capsule around the joints, secondary swelling of the synovial cells. The aim of the present study was to investigate anti-arthritic activity of leaves of *Hibiscus platinifolius* linn on male Wistar rats and estimation of paw edema, body weight measurement and measurement of activity of marker enzymes like alanine trans aminases(SGPT)and serum glutamate oxalo acetate transferases (SGOT) in serum by using Aqueous extract of hibiscus platinifolius line. The study of anti-arthritic activity involves induction of arthritis to rats of all groups using FCA and turpentine oil induced in i.p route, followed by subsequent treatment with aqueous extraction at two different doses. i.e AEHP 200mg/kg and AEHP400mg/kg respectively. Diclofenac sodium is used as a reference standard. paw edemas, paw height, paw volume were estimated from the serum by using Freund's complete adjuvant(FCA) and turpentine oil induced arthritis .The biochemical parameters were increased in all arthritic rats, there parameters were decreased by the administration of aqueous extraction of *Hibiscus platinifolius* at dose of 200mg and 400 mg respectively. From this study it has been concluded that the aqueous extract of leaves of *Hibiscus platinifolius* having good anti-arthritic activity, which is comparable to Diclofenac sodium.

Key words: *Hibiscus platinifolius* linn, Anti-Arthritic, Rats, Paw volume, Paw width, and Paw height.

INTRODUCTION

Hibiscus platanifolius Linn (Malvaceae) known as Mapleleaved mallow is an important medicinal plant. It is an evergreen tree, growing up to 10 m tall. Leaves are alternate, simple, stipulate, petiolate and ovate to lanceolate, often with a toothed or lobed margin. Leaves are usually 3-5 lobed, 6-12 × 5-12 cm. Leaf are palmately veined, entire or various lobed. Flowers are pale pink, with each petal having a deep pink base. Flowers are about 3-5 inches across. Sepals are leathery, hairy. Stamen-column is 2-2.5cm long, pale pink. Maple leaved mallow is native to India.

MATERIALS AND METHODS

Preparation of extraction: The *Hibiscus platinifolius* plant was collected during the march 2013 from Sri Venkateshwara University, Tirupati, India. The plant was authenticated by Dr. Madhava Chetty, Department of Botany and voucher specimen of the plant were preserved at institute herbarium library. Plant was separately washed, wiped-dry, and subsequently reduced to a coarse powder. About 100 g of the plant material were separately extracted for 24 h aqueous with intermittent vigorous shaking. The extracts were filtered, concentrated with a rotary evaporator and dried over a water bath at 45°C shaking. The extracts were filtered, concentrated with a rotary evaporator and dried over a water bath at 45°C. The residue from the plant parts were used for experimental analysis.

Acute toxicity: The acute toxicity of the Aqueous extract of *Hibiscus platinifolius* was determined as per the OECD guideline no. 423 (Organization for Economic Cooperation and Development). It was observed that the test extract was not mortal even at a dose of 2000 mg/kg body weight. Hence, 200 mg/kg and 400 mg/kg doses were selected for further study.

1st Model:

Experimental protocol design:

Assessment of the parameters:

A) Effect of Aqueous extract *Hibiscus platinifolius* paw oedema against FCA induced chronic arthritis in rats:

The FCA induced chronic anti-arthritic activity of Aqueous extract of *Hibiscus platinifolius* 200mg/kg and 400mg/kg was carried out and Wistar albino rats (150-200g) were divided into 4 groups, contains 6 animals in each group.

Group I. Control group received vehicle (10 % v/v tween 80; p.o.) + FCA induced changes in rat paw oedema

Group II. Effect of Diclofenac (10 mg/kg; p.o.) on FCA induced changes in rat paw oedema

Group III. AEHP (200 mg/kg; p.o.) on FCA induced changes in rat paw oedema

Group IV. AEHP (400 mg/kg; p.o.) on FCA induced changes in rat paw oedema.

Adjuvant arthritis was induced by the sub-plantar injection of 0.1ml of Freund's complete adjuvant (FCA) on day zero (kalia et al). Everyday animals were carefully and thoroughly inspected, by

examining the affected paw and the animals general status. In FCA induced animals, the sub-plantar injection of FCA produces local oedema after few hours with a progressive increase reaching its maximum upto 21st thday. On 0th, 7th, 14th, and 21st day the paw-oedema volume of each rat was measured thoroughly in all groups. Inflammation in the paw oedema was measured by using Digital Plethysmometer (7140 UGO Basile). The change in the paw-oedema volume for the Aqueous extract of *Hibiscus Platinifolius* was summarized in the Table1.

The percentage inhibition of paw oedema volume of each treated groups is calculated by using the following equation, Percentage inhibition,

$$\% = (V_c - V_t / V_c) 100$$

Where, V_t = Mean paw volume of each treated group

V_c = Mean paw volume of control group

The width and height of the right paw of each rat was measured with a digital Vernier caliper ruler before and on subsequent testing days, after the induction of arthritis according to Andersen et al.

Table 1: Effect of Aqueous extract of *Hibiscus platinifolius* on Body weight against FCA induced chronic arthritis in rats

Groups	Body weight			
	0 day	7 th day	14 th day	21 st day
Control (10% tween80)	163.2±1.138	154.5± 2.405	141.7± 0.918	137.7± 0.988
Diclofenac (10 mg/kg)	154.7±1.647	168.2±1.493**	180.5±1.544***	179.3±1.764***
AEHP 200mg/kg	163.3±1.054	177.7±1.054*	189.3±2.333**	183.5±1.765***
AEHP 400mg/kg	159.5±1.708	181.5±1.708**	188.3±1.764**	183.5±2.604***

All values are expressed as mean ± SEM, n=6, One way Analysis Variance (ANOVA) followed by Dunnett's multiple comparison test; *** $p < 0.001$ as compared to control group; AEHP200mg/kg, AEHP 400mg/kg.

Table 2: Effect of Aqueous extract of *Hibiscus platinifolius* on paw oedema volume against FCA induced chronic arthritis in rats

Groups	Paw oedema volume (ml) (%EI)				%inhibition of Oedema
	1 st day	7 th day	14 st day	21 st day	
Control 10% tween80)	0.74±0.01	0.87±0.05	0.92±0.01	1.02±0.03	-----
Diclofenac(10 mg/kg)	0.64±0.01ns	0.48±0.02**	0.37±0.01***	0.31±0.01***	69.55
AEHP 200 mg/kg	0.75±0.01ns	0.78±0.03*	0.71±0.02**	0.69±0.01***	45.58
AEHP 400 mg/kg	0.71±0.00ns	0.74±0.01*	0.68±0.01***	0.64±0.03***	32.58

2nd model: (turpentine oil): turpentine oil induced granuloma pouch in rat: Subcutaneous dorsal granuloma pouch was made in ether anaesthetized rats by injecting 2 ml of air, followed by injection of 0.5 ml of turpentine oil into it. All drugs were administered orally one hour prior to turpentine oil injection and continued for seven consecutive days. On day 7, the pouch was opened under anesthesia, the amount of exudate was taken out with a syringe, and later on the volume was measured and compared with those of the control and standard group.

The Turpentine induced Acute anti-arthritis activity of Aqueous extract *Hibiscus platanifolius* 200mg/kg and

400mg/kg was carried out on Wistar albino rats (150-200g) were divided into 4 groups, contains 6 animals in each group.

Group I. Control group received vehicle (10 % v/v tween 80; p.o.) + Turpentine induced changes in rat paw oedema

Group II. Effect of Diclofenac (10 mg/kg; p.o.) on Turpentine induced changes in rat paw oedema

Group III. AEHP (200 mg/kg; p.o.) on Turpentine induced changes in rat paw oedema

Group IV. AEHP (400 mg/kg; p.o.) on Turpentine induced changes in rat paw oedema

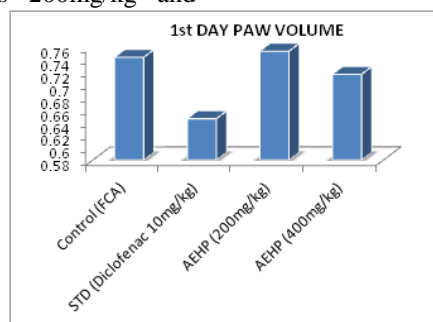


Figure.1.Effect of Aqueous extract of *Hibiscus platinifolius* on paw oedema volume against FCA induced chronic arthritis in rats

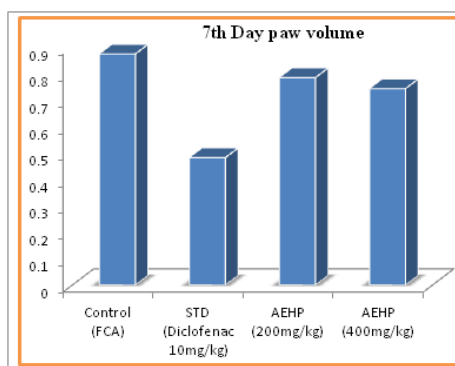


Figure.2. Effect of Aqueous extract of Hibiscus platinifolius on paw oedema volume against FCA induced chronic arthritis in rats

All values are expressed as mean \pm SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison, * $p < 0.05$, ** $p < 0.01$ as compared to control group; AEHP 200mg/kg, AEHP 400mg/kg.

Statistical analysis: The data are expressed as mean \pm SEM. Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Dunnett's Multiple comparison test (DMCT). The results were considered statistically significant if the p values

Turpentine oil model:

Effect of Aqueous extract of Hibiscus platinifolius on paw oedema volume against Turpentine induced granuloma pouch in rats: The treatment of AEHP (200 and 400 mg/kg) significantly reduced the volume of exudates ($P < 0.001$) in turpentine oil-induced granuloma pouch dose dependently, which was comparable with the effect of diclofenac ($P < 0.001$).

RESULTS AND DISCUSSION

FCA Model:

A. Effect of Aqueous extract of Hibiscus platinifolius on Body weight against FCA induced chronic arthritis in rats: Body weight is one of the parameter in arthritis there is significant difference between body weights of each groups immediately after induction of arthritis in animals. Thereafter control group showed significant decrease in body weight after 3rd week ($p < 0.001$) as compared to standard and extract treated groups. However there is significant increase body weight in treated groups after 3rd week. standard group ($p < 0.001$), AEHP [200mg/kg ($p < 0.001$)] and AEHP [400mg/kg ($p < 0.001$)] as compared to control group.

B. Effect of Aqueous extract of Hibiscus platinifolius on paw oedema volume against FCA induced chronic arthritis in rats: In FCA induced chronic arthritis model, control group animals showed increased paw oedema gradually upto 21st day. The test extract AEHP 200mg/kg and AEHP 400mg/kg and Diclofenac showed significant reduced right paw oedema ($p < 0.001$), as compared to control group.

C. Effect of Aqueous extract of Hibiscus platinifolius on FCA induced rat chronic arthritis serum biochemical parameters: The biochemical marker ALT, AST and ALP was increased significantly in Control (FCA) group. Diclofenac and test extract AEHP 200mg/kg and AEHP 400mg/kg was showed significantly ($p < 0.001$) decreased in ALT, AST and ALP level as compared to control group.

NSAID's are widely used clinically for RA. However, despite their great number, their therapeutic efficacy seems to be hampered by the presence of a number of undesired and often serious side effects. Selective COX-2 inhibitors make alternative approach to arthritic treatment with reduced GI side effects, but on long term treatment leads to serious cardiovascular and thrombotic side effects. However, a series of new biological monoclonal antibodies (anti-TNF, anti-IL-1Ra anti-CD 20, anti-IL-2, IL-4) were preferred for RA but these are highly expensive.

The author has immensely fascinated by these aspects of drug research and looked for some new safer anti-inflammatory and anti-rheumatic drugs represents a new challenging goal for acute and chronic inflammatory conditions. This is the dissertation work consists of four chapters with title of "Evaluation of AEHP as potential anti-inflammatory and antiarthritic agents".

The chronic inflammation involves the release of various inflammatory mediators like cytokines (IL-1 α and TNF- α), granulocyte monocytes colony stimulating factor (GM-CSF), platelet derived growth factor (PDGF) and others. These mediators are responsible for the pain, destruction of cartilage and leads to severe disability. Paw swelling is one of the major factors in assessing the degree of inflammation and efficacy of the drugs.

Adjuvant induced arthritis is non-specific immune response within the joint can also result in inflammatory and erosive disease. Paw swelling is an index of

measuring the anti-arthritic activity of various drugs and it is employed here to determine the activity of AEHP 200mg/kg and AEHP 400mg/kg. Reference standard Diclofenac sodium, AEHP 200mg/kg and AEHP 400mg/kg administered groups showed marked reduction in paw volume when compared with the arthritic control group by inhibiting the release of inflammatory mediators.

The cytoplasmic enzymes like AST and ALT serves as indicators and suggestive for disturbances of the cellular integrity induced by pathological conditions. These enzymes are used as sensitive markers for evaluation of protective activity, these markers attribute towards persistent inflammation. The increased enzyme activity may result from one of the several mechanisms which include the release of various enzymes from leukocytes, from necrotic or inflamed synovial tissue and production and release of an increased amount of enzymes due to altered synovial tissue. A positive correlation observed between the leukocytes in the field and the enzyme levels is considered as evidence for the release of enzymes from the leukocytes. A loss of semi-permeability of the synovial membrane has also been correlated with the significant elevation of enzyme levels. In present study, the decreased level of cytoplasmic enzymes ALT supports the protective role of the AEHP 200mg/kg, 400mg/kg and Diclofenac sodium. Granuloma pouch technique was modified using turpentine oil as irritant. An aseptic inflammation resulting in large volume of haemorrhage exudate is elicited which resembles the sub-acute type of inflammation. Turpentine oil-induced granuloma pouch offer a model for exudative type of inflammation. Though, the chemical mediators of this type of response are unknown, protein synthesis is necessary for the formation of granuloma. AEHP has show potential inhibitory action on exudates formation. Kinin is said to be the main mediator of granuloma, as it not only vasodilate but also increase the vascular permeability in the early stages of inflammation. Thus, AEHP may possess anti-kinin like activity.

CONCLUSION

Therefore drugs appear to be effective against FCA induced arthritis and Turpentine induced Inflammation. This finding justifies the usefulness of AEHP in the treatment of inflammatory diseases associated like arthritis. It is concluded that Aqueous extract of *Hibiscus platinifolius* possess significant anti-arthritic activity, which is comparable to diclofenac sodium.

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