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IN VIVO ANTI INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF *OLDENLANDIA HERBACEA*

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ABSTRACT

The anti-inflammatory activity of ethanolic extracts of *Oldenlandia herbacea* were investigated, the crude ethanolic extract of *O. herbacea* compared with standard ibuprofen on carrageenan induced paw edema at different time intervals are depicted. The oral administration of ethanolic extract at 100 and 200 mg/kg bw produced significant and dose dependent inhibition of paw edema after 1 to 3 hrs of treatment. When compared with standard ibuprofen on carrageenan induced paw edema method, the percentage inhibitions of crude ethanolic extract of *O. herbacea* at 100 and 200 mg/kg after 1-3 hr shows the value of 25.56 ± 0.72 and 44.36 ± 1.32 to 57.83 ± 0.84, inhibit significantly.

KEYWORDS

Oldenlandia herbacea, Anti-inflammatory, Formalin and Carrageenan.

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INTRODUCTION

The *O. herbacea* or *Hedyotis herbacea* elongs to the family- Rubiaceae is an erect, glabrous annual shrub found in tropical regions of Africa and Asia[1]. Annual and spreading branched herb was about 1.5-60 cm. tall, with glabrous 4-ribbed stems. Leaf blades of *O. Herbacea is* 0.6-5.5 cm. x 1-3.5(5) mm and it is linear to linear-lanceolate, acute at the apex, cuneate at the base with a few setae at the margins but the petiole are not developed, stipule sheath short, rarely exceeding 0.5 mm, long but not

fimbriate. Flowers usually isostylous but in one variety markedly heterostylous and it paired at the nodes with pedicels graceful spreading at the length of 0.8-3.5 cm. *O. herbacea* extract or decoction was reported to be useful in the treatment of malaria. A decoction of the herb is used for bathing rheumatic patients and the powdered herb is administered for rheumatic fever and swellings. The herb is boiled in oil and the oil used in elephantiasis and pains in the body. The leaves are employed as expectorant in asthma and consumption and the recent review proofs that it is having antibacterial activity [2, 3].

MATERIALS AND METHOD

Preparation of Extract

Extraction was done according to standard procedures using analytical grade solvents. For ethanolic extract 250 gm, powdered herbs was taken in a pouch of filter paper and kept inside the soxhlet thistle then it was extracted with petroleum ether for 48-72 hours for defeating after that it was extracted with ethanol for 48-72 hours. Then Preliminary Phytochemical screening was performed. The extract was concentrated to dryness under vacuum to yield a dark greenish semisolid residue (yields: 3.7%) which was preserved in refrigerated condition. Furthermore, the dried ethanolic extract was used for evaluation of anti inflammatory activities.

Preliminary Phytochemical Screening[4]

The extracts were subjected to qualitative tests for identification of phytoconstituents present in it. The different tests performed are as fallows

Test of Alkaloids

A small portion of the ethanolic extract were stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate may be tested carefully with various alkaloidal reagents such as,

a. Mayer's reagent - Cream precipitate
b. Dragendroff's reagent - Orange precipitate
c. Hager's reagent - Yellow precipitate
d. Wagner's reagent - Reddish precipitate

Test for Carbohydrates and Glycosides

The minimum amount of extract were dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates and glycosides.

Molisch's Test

The filtrate was treated with 2-3 drops of 1% alcoholic alpha napthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube.

Fehling's Test

The filtrate was treated with 1ml of Fehling's solution and heated. Orange precipitate was obtained shows the presence of carbohydrates.

Another portion of the extract was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Legals, Borntrager's test to detect the presence of different glycosides.

Legal's Test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Purple colour in ammoniacal layer was observed.

Test for Phytosterol (Libermann Burchard Test)

One gram of the extract was dissolved in few drops of dry acetic acid, 3 ml of acetic anhydrade was added followed by few drops of concentrated sulphuric acid. Appearance of bluish green colour showed the presence of phytosterol.

Test for Fixed oils and Fats[5]

A small quantity of the extract was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hrs. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

Test for Tannins and Phenolic Compounds

Small quantities of extract were dissolved separately in water and tested for the presence of phenolic compounds and tannins with

1. Dilute Ferric chloride solution 5% - Violet colour
2. 1% solution of gelatin containing 10% NaCl- White precipitate
3. 10% Lead acetate solution - White precipitate.

Test for Proteins and Free Amino Acids

Small quantities of extracts were dissolved separately in a few ml of water and treated with:

1. Million's reagent - Appearance of red colour shows the presence of proteins and free amino acids.
2. Ninhydrin reagent - Appearance of purple colour shows the presence of proteins and free amino acids.
3. Biuret test - Equal volume of 5% solution of sodium hydroxide and 1% solution of copper sulphate were added. Appearance of pink colour shows the presence of proteins and free amino acids.

Test for Gums and Mucilage's

About 10ml of extract were added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

Test for Flavonoids[6]

a. With aqueous sodium hydroxide solution, blue to violet colour (Antho cyanins), yellow colour (Flavones), yellow to orange (Flavonones).
b. With concentrated sulphuric acid, yellowish orange colour (anthocyanins), yellow to orange colour (Flavones), orange to crimson (Flavonones).
c. Shinoda's Test
   The extract were dissolved separately in alcohol, to this a piece of magnesium followed by conc. hydrochoric acid drop wise were added and heated. Appearance of magenta colour shows the presence of flavonoids.

Test for lignin

With alcoholic solution, phloroglucinol and conc. hydrochloric acid, appearance of red colour shows the presence of lignin. The results of chemical tests of whole plant powder and extract were shown in Table No.1.

Experimental Animals

Evaluation of anti inflammatory activity was done by using carrageenan induced paw edema method. Albino rat weighing 180-200g either sex was used in this experiments and the animals were maintained under standard laboratory conditions with access to standard diet and water ad libitum. They were housed in polypropylene cages under standard laboratory conditions (12-h light/ 12-h dark cycle, 21 ± 2 ºC, and relative humidity 55 %). The rats were acclimatized to laboratory condition for 7 days before commencement of experiment.

***In vivo* Anti inflammatory activity (Carrageenan induced paw edema method)** [7, 8]

The four groups of six animals in each group were selected for the study

Group I: Served as control.

Group II: Treated with 100 mg/kg ethanolic extract of the *O. Herbacea*.

Group III: Treated with 200 mg/kg ethanolic extract of the *O. Herbacea*.

Group II: Served as standard and treated ibuprofen 100mg/kg.

Inflammation was induced by carrageenan in Wistar albino rats and paw volume was measured after standard and extract treatment.

Acute toxicity study[9]

Acute oral toxicity studies were performed according to OECD no. 423 guidelines. Three rat and mice of either sex were selected for the study. The animals were fasted over night for food with free access for water prior to test extract. Ethanolic extract of *O. Herbacea* were administration orally up to dose 2000 mg/kg. Individual animal was observed after dosing at least once during first 30 min., periodically during 24 hrs, with a special attention given during the first 4 hrs and daily thereafter, for a 7 days.

RESULTS AND DISCUSSION

The phytochemical screening of ethanolic extract of *O. Herbacea* revealed the presence of alkaloids, carbohydrate, glycosides, tannins, and flavonoids was shown in Table No.1. Thus, the activity of *O. Herbacea* could be due to flavonoid components. The Ethanolic extract of *O. Herbacea* did not show any toxicity and behavioral changes in rats up to 2000 mg/kg hence doses of (100 and 200 mg/kg) were selected for the present study. Carrageenan-induced rat paw edema has been a popular inflammatory model to investigate anti-inflammatory

effect of compounds has a biphasic effect[10]. The first phase is due to release of histamine and serotonin (5-HT) (0-2hr), plateau phase is maintained by a kinin like substance (3hr) and second accelerating phase of swelling is attributed to PG release. In our study, *O. Herbacea* (100, and 200 mg/kg) significantly reduced edema induced by carrageenan in all three phases. The anti-inflammatory effect of *O. Herbacea* with standard ibuprofen 100mg/kg on carrageenan induced paw edema at different time intervals are depicted in Table No.2. Oral administration of ethanolic extract produced significant and dose dependent inhibition of paw edema after 1 to 3 h of treatment when compared to control ibuprofen 100mg/kg in carrageenan induced paw edema method was shown in Figure No.1.

Table No.1: The Preliminary Phytochemical Screening of Ethanolic Extract of *O. herbacea*

S.No	Phytoconstituents	Ethanolic extract
1	Alkaloids	(+)
2	Carbohydrates	(+)
3	Glycosides	(+)
4	Flavonoids	(+)
5	Phytosterols	(+)
6	Fixed oils and Fats	(-)
7	Saponins	(-)
8	Phenolic and Tannins	(+)
9	Lignins	(+)
10	Proteins, Amino Acids	(-)
11	Gums and Mucilage	(-)

(+) Presence **(-) Absence**

Table No.2: Anti inflammatory activity of crude ethanolic extracts of *O. herbacea* (Carrageenan induced paw edema)

S.No	Treatments	Dose (mg/kg)	1h		2h		3h	
			Paw volume (ml)	% Inhibition	Paw volume (ml)	% Inhibition	Paw volume(ml)	% Inhibition
1	Control	-	0.38 ± 0.00	-	0.57 ± 0.00	-	0.71 ± 0.00	-
2	Ethanolic extract of *O. Herbacea*	100	0.27 ± 0.01[a]	25.56 ± 1.50	0.38 ± 0.00[b]	39.18 ± 0.62	0.45 ± 0.00[b]	43.65 ± 0.72
		200	0.19 ± 0.00[a]	44.36 ± 1.32	0.28 ± 0.00[b]	57.34 ± 0.84	0.32 ± 0.01[b]	57.83 ± 0.84
3	Ibuprofen	100	0.18 ± 0.01[a]	44.75 ± 1.61	0.24 ± 0.01[b]	63.52 ± 0.78	0.23 ± 0.01[b]	73.59 ± 0.74

Values are given as mean ± S.E.M. for groups of six animals each; values are statistically significant at [a]p<0.01 and [b]p< 0.001.

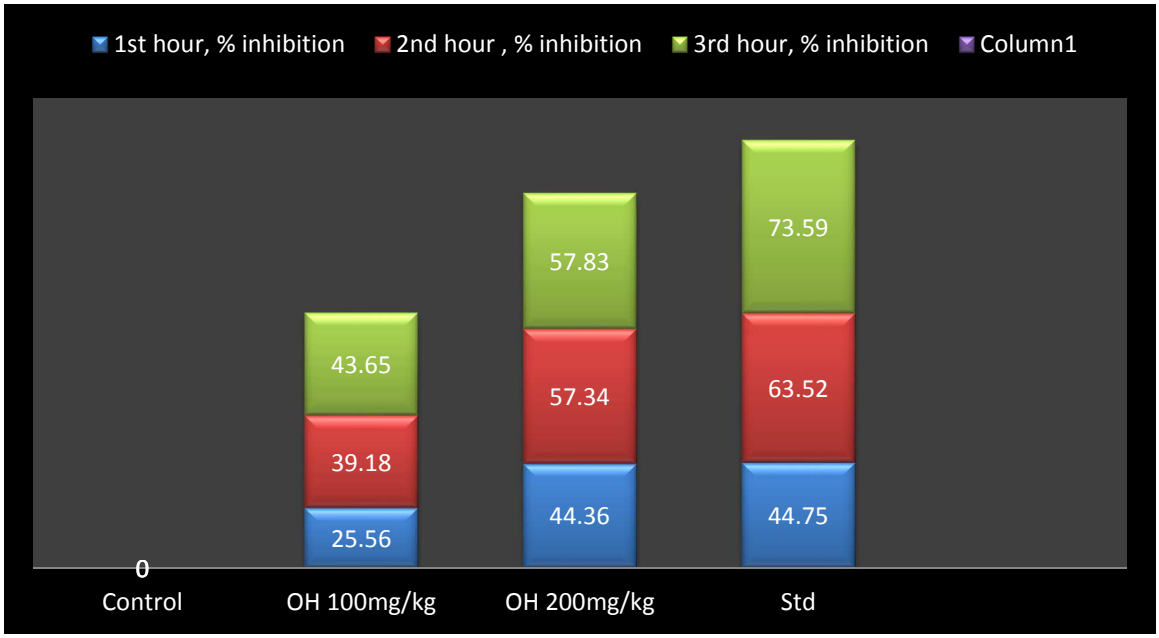

Figure No.1: Effect of ethanolic extract of *O. herbacea* 100, 200mg/kg in Carrageenan induced paw edema

CONCLUSION

On the basis of the results obtained it can be concluded that the ethanolic extract of *O. herbacea* seems to possess anti inflammatory activity in wister albino rats. Further studies are needed to be evaluated to find the potential usefulness of this extract in clinical conditions.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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