ANTI-INFLAMMATORY ACTIVITY OF VARIOUS EXTRACTS OF *LEUCAS ASPERA* BY PAW OEDEMA METHOD, COTTON PELLET GRANULOMA METHOD AND ULCER INDEX STUDY OF ALBINO RAT.

[1]Sudhakara reddy M, [2]Udaykiran V, [3]Sivasankar R, [4]Jayasankar A, [5]Subahan M
[1]Department of Zoology,Loyola degree college, Pulivendula. A.P.
[2,3,5]Department of Zoology, S.V.Univesity., Tirupati.A.P
[4]P.V.K.N.Govt.College,Chittoor,A.P

ABSRTACT

Leucas aspera, an annual herb found throughout India as a weed in cultivated fields used in the treatment of anti- inflammatory and antimicrobial studies, respectively Pathogen free, Wistar strain albino rats were used in the present study, The four extracts of *Leucas aspera* tested for their anti-inflammatory activity by paw oedema method, the ethanol extract at a dose of 400 mg/kg body weight showed significant activity five hours of the treatment (0.833 ± 0.042) when compared to reference standard (0.87 ± 0.021). The activity of petroleum ether, chloroform and water extracts are comparable with that of standard diclofenac sodium. Similarly the extracts of *Leucas aspera* tested for their anti-inflammatory activity by Cotton pellet granuloma method, the activity of ethanol extract is comparable with that of standard diclofenac sodium. Petroleum ether, chloroform and distilled water extracts showed moderate activity when compared to standard drug diclofenac sodium. Similarly the standard anti-inflammatory drug at a dose of 150 mg/kg body weight potentially increased the ulceration. The ulcer index of petroleum ether extract at a dose of 400 mg/kg body weight is comparable to that of control. The ulcer index of chloroform, ethanol and distilled water extracts of *Leucas aspera* at a dose of 400 mg/kg body weight is significantly reduced. The various extracts and semipurified fraction F_5 of ethanol extract showed significant inhibition activity compared to control. The inhibition activity of other extracts is moderate and near to that of control group.

Key words: *Leucas aspera*, anti-inflammatory activities. Cotton pellet granuloma, Ulcer index, Wistar strain albino rats.

[1]**Corresponding Author:**
Dr. M. Sudhakara reddy
Reader in Dept.of Zoology,
Loyola Degree College
Pulivendula.A.P.
[3]**Co-Author:**
Dr.R.Sivasankar (Fellow of UGC-MRP)
Dept.of zoology
S.V.University, Tirupati.

INTRODUCTION

Leucas aspera is a species within the *Leucas* genus and the *Lamiaceae* family. Although the species has many different common names depending on the region in which it is located, it is most commonly known as Thumbai. Found throughout India, it is known for its various uses in the fields of medicine and agriculture. *Leucas aspera* is reported to have antifungal, prostaglandin inhibitory, antioxidant, antimicrobial, antinociceptive and cytotoxic activities (Prajapati MS,2012). *Leucas aspera* is a very common weed commonly found throughout India and the Philippines is used as an insecticide that can reach heights of 15–60 cm (R. Srinivasan 2011). Definitely, the plant kingdom still holds many species of plants containing constituents of medicinal value which have yet to be discovered. Large numbers of plants are constantly being screened for their possible pharmacological value. *Leucas aspera* Spreng (Fig.1) is an annual herb found throughout India as a weed in cultivated fields, waste lands and roadsides. Its leaves, roots, stalks, young shoots and flowers have been used for wide range of medicinal properties (Kirtikar and Basu, 1991).

The present study Literature survey revealed that ethanol extract of leaves of *Leucas aspera* has been found to possess antibacterial activity (Shirazi, 1947), whereas chloroform and petroleum ether extracts possess fungistatic as well as fungicidal activities (Thakur *et al.*, 1987). This plant has long been used as an antipyretic remedies in south India, the leaves are said to be useful in chronic rheumatism. The juice is used as an external application for psoriasis, chronic skin eruptions and painful swellings, flowers are given with honey for cough and cold in children in NorthBengal (Kirtikarand Basu, 1991) This context triggered us to review and organize the literature and, in this review we explained anti-inflammatory activities along with pharmacological investigations and also emphasized the current applications and future scope of *L. aspera* as a medicinal plant.

Fig.1. *Leucas aspera Linn*

Medicinal properties of *Leucas aspera*:

Even today the different parts of the plant *Leucas aspera* is being used in folk medicine for the following health care purposes. (Sudhakara reddy M (2007)

a) Asthma: Grind the leaves of *Leucas aspera* with rock salt into a paste. Prepare tablets with this paste. Take one tablet in each time twice daily with a spoonful ginger juice or honey.

b) Conjunctivities: Squeeze the leaves of *Leucas aspera* and extract the juice put one to two drops of juice in eyes twice daily for two days only.

c) Fever (malaria): Grind together a entire plant of *Leucas aspera* and leaves of Nyctancthes arbortristis and extract at the juice. Take two teaspoonfuls in each time once daily for two days only.

d) Headache: Grind the leaves of *Leucas aspera* and extract the juice. Inhale the juice through nostrils to instant relief from headache.

e) Jaundice: Grind the leaves of *Leucas aspera* and extract the juice after filtration. Take one teaspoonful twice daily for one week with half teaspoon honey.

f) Diabetes: Grind together the flowers of a single plant of *Leucas aspera* and 21 No. cloves with a paste. Take the paste once daily till cure.

g) Mouse bite: Grind the plant of *Leucas aspera* with seeds of *Piper nigrum* into a paste. Mix the paste in water and prepare a drink. Take the drink thrice daily for three days; note (a) Take two number of *Leucas aspera* and 21 No. *Piper nigrum* seeds for adult (b) Take one plant of *Leucas aspera* and 2 No of *Piper nigrum* seeds for children.

h) Otalgia: Squeeze the leaves of *Leucas aspera* and extract the juice and warm the juice gently. Put this inside the ear drop by drop twice daily for three days.

i) Skin diseases (Itches): Apply the leaf juice of *Leucas aspera* on itches area once daily till cured.

j) Ozena: Grind the leaves of *Leucas aspera* and extract the juice. Put 2-3 drops of the juice inside the nostril as nasal drop twice daily for 2-3 days.

k) Snake bite: Grind the leaves of *Leucas aspera* and extract the juice. Put 3-4 drops of the juice in each nostril once for one day only.

l) Toothache: Chew the leaves of *Leucas aspera* with common salt to instant relief from toothache.

m) Wound: Grind the dried leaves of *Leucas aspera* into powder. Apply the powder once daily on wound for 2-3 days (or) grind together the leaves and inflorescence and the rhizome of *curcuma longa* into paste. Apply the paste locally on wounds in poultry.

Biological and chemical properties of *Leucas aspera*:

The whole plant powder of *Leucas aspera* has been reported to have insecticidal properties and Methyl-*O*-acetyloleolanolate, Methyl-*O*-acetyl ursolate and *β*-sitosterol acetate compounds were isolated (Chaudhry and Ghosh, 1969). The glycosides containing galactose and D-fructose have been isolated from aqueous extract of the plant (Chatterjee and Patil, 1994). Mixture of the sterols having β-sitosterol and other uncharacterised compounds have been isolated from the benzene extract of the whole plant of *Leucas aspera*. This mixture has shown antibacterial activity against *micrococcus pyogenes* and *Escherichia coli* (Chatterjee and Patil, 1994; Khaleque, 1970). The mixture of essential oils which has antifungal activity against various pathogenic fungi was isolated (Narasimha Rao *et al.*, 1972). A triterpenoid leucolactone and two long chain compounds, I-hydroxy tetratriacontan-4-one, and 32-methyl-tetratriacontan-8-ol, were isolated from root and shoot part of *Leucas aspera*, respectively. Two new aliphatic enols were isolated from the shoots of the same plant 28-Hydroxy pentatriacontan-7-one and 7-hydroxy dotriacontan-2-one has been also isolated together with 5-acetoxytriacontan and β-sitosterol (Mishra *et al.*, 1992).

The plant *Leucas aspera* has been screened for its antifertility activity. The various extracts of the same plant have been studied for their fungistatic and fungicidal activities. The smoke of the leaves prevents filarial vector mosquito (Pandian, 1994).

Even though, there are few reports on the isolation of chemical constituents from the various extracts of *Leucas aspera*, so far very little is known about its biological activities. Therefore, in the present study, we report the anti-inflammatory and analgesic activities of various extracts of *Leucas aspera* by different models like, acute, subacute and chronic by using rat as an animal model. Efforts were also made to isolate the active fraction from ethanol extract of *L. aspera* by applying column chromatography and HPLC. The purified compound so obtained was also tested for its anti-inflammatory activity.

MATERIAL AND METHOD:

Collection of the Plant Material:

The fresh whole plant of *Leucas aspera* was collected in the month of July and August in and around Sri Venkateswara University campus and authenticated at the Herbarium (Voucher No. 210), Department of Botany, S.V.University, Tirupati. Collected plants were immediately sprayed with alcohol to cease the enzymatic degradation of secondary metabolites. They were chopped into small fragments, thick stem, roots, twigs and other

woody parts were chopped into 1" to 2" pieces and split longitudinally into several sections to enable early drying under shade. Chopping fresh plant materials at once after collection hastens drying and advantages on reduction in bulk and drying speed. The plant was kept under shade for drying for about 7 days.

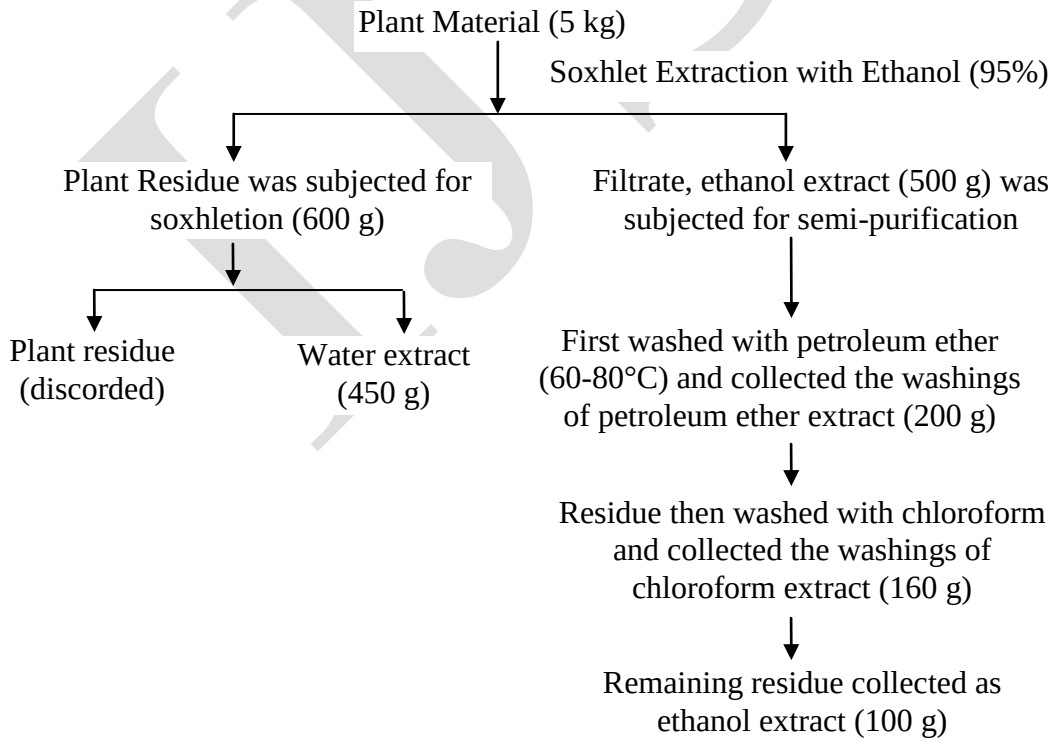
Extraction of Whole Plant:

The shade dried whole plant material (5 kg) was powdered and extracted with ethanol (95%). The ethanol extract (500 g) was concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature (40-50°C). The ethanol extract was purified as detailed below:

Animals:

Pathogen free, Healthy wistar strain albino rats weighing between 150-200 g were selected in the present study, the usage of animals was approved by the Institutional Animal Ethics Committee. The rats were housed in clean polypropylene cages under hygienic conditions with photoperiod of 12 hours light and 12 hours dark. The rats were fed with standard laboratory chow (Hindustan lever Ltd, Mumbai) and water *ad libitum*.

EXPERIMENTAL DESIGN:



STATISTICAL ANALYSIS:

Statistical analysis has been carried out using student's't' test and one way ANOVA followed by Dunnet's test .The data was analyzed for the significance and the results were presented with the P-value.

RESULTS AND DISCUSSION:

1. ACUTE INFLAMMATION METHOD:(paw oedema method)

Anti-inflammatory activity was evaluated by carrageenan - induced (C.A.Winter *et al.,* 1957) rat hind paw oedema method (Katzung, 1999). Albino rats of either sex weighing between 150-200 g were divided into six groups of six animals each. The first group served as the control and received vehicle only (Tween-80, 1%), second group of animals were administered with standard drug diclofenac sodium 150 mg/kg body weight, orally. The animal of the third, fourth, fifth and sixth groups were treated with petroleum ether, chloroform, ethanol (95%) and distilled water extracts of *Leucas aspera* at a dose of 400 mg/kg body weight, orally. A mark was made on both the hind paws just below the tibio-tarsal junction so that every time the paw could be dipped in the mercury column of plethysmograph upto the mark to ensure constant paw volume. After 30 min of above treatment an inflammatory oedema was induced in the left hind paw by injection of 0.5 ml of carrageenan (1%, w/v) in the planter tissue of the paw of all animals. The right paw served as a reference to non-inflammed paw for comparison. The initial paw volume was measured plethysmographically with in 30 sec. of the injection. The relative increase in the paw served as a reference to non-inflammed paw for comparison. The initial paw volume was measured plethysmographically with 30 sec. of the injection. The relative increase in the paw volume was measured in control, standard and treated group, 4 hr after carrageen injection. The percent increase in paw volume over the initial reading was also calculated. This increase is paw volume in animals treated with standard drug and the four extracts of *Leucas aspera* were compared with the increase in paw volume of untreated control animals after 4 hr. Thus, percent inhibition of paw volume in treated animals were used for calculating the percent inhibition of oedema of the control group using the formula % inhibition = (1-Vt / Vc) 100, where Vt and Vc are mean relative changes in the paw volume of the test and control, respectively. The results are summarized in the Table 1.

Among the four extracts of *Leucas aspera* tested for their anti-inflammatory activity by paw oedema method, the ethanol extract at a dose of 400 mg/kg body weight showed significant activity five hours of the treatment (0.833 ± 0.042) when compared to reference standard (0.87 ± 0.021). The activity of petroleum ether, chloroform and water extracts are comparable with that of standard diclofenacsodium.(Table.1).

2. SUBACUTE INFLAMMATION (COTTON PELLET GRANULOMA):

Subacute inflammation was produced by cotton pellet induced granuloma in rats. (Winter CA, Porter CC, 1957; Turner RA 1965). Foreign body induced Granuloma method was developed by D'Arey *et al.*, 1960 (Turner, 1996) with some modifications. Healthy rats of either sex weighing between 150-200 g were selected and hair from the axillae and grain region were removed with the help of clips. Under lighter ether anaesthesia a small incision was made in axillae groin region respectively and two sterile cotton pellet weighing 10 mg and two sterile grass piths (25 x 2 mm) each were implanted subcutaneously. The incisions were sutured and animals were caged individually after recovery from anaesthesia. Aseptic precautions were taken throughout the experiment. These rats were divided into six groups of six animals each. Group first received Tween-80 solution (1%) and served as control. Group second received diclofenac sodium solution at a dose of 150 mg/kg body weight and served as reference standard. Groups three, four, five and six received petroleum ether, chloroform, ethanol and distilled water extracts, respectively at a dose of 400 mg/kg body weight. The treatment was started on the day of implantation of cotton pellet and grass piths and continued for successive ten days.

On the 11^{th} day, the rats were sacrified with an over dose of anaesthesia to remove the cotton pellets and grass piths. The cotton pellets free from extraneous tissue were dried overnight at 60°C to note their dry weight. Net granuloma formation was calculated by substracting initial weight of cotton pellets (10 mg) from the weights noted. (Table.2.1 and Table2.2) As suggested by Despas quate and Meli (1965), mean granuloma dry weight for each animal was calculated and expressed as mg/100 g body weight (BW). Mean granuloma dry weight was calculated for various groups for the sake of comparison. The results obtained are detailed in (Table-2.2).

The results obtained from the present study reveals that among ten fractions of ethanol extract of *Leucas aspera* (F_1-F_{10}) screened for the anti-inflammatory activity by paw oedema method. Only, fraction F_5 showed significant activity compared to reference standard

diclofenac sodium and fractions F_1, F_2, F_3, F_6, F_8 showed moderate activity, while fractions F_4, F_7, F_9 and F_{10} showed comparable activity at 5^{th} hour of the treatment ,The results obtained are detailed on (Table-2.1).

Similarly among the four extracts of *Leucas aspera* tested for their anti-inflammatory activity by cotton pellet granuloma method, the activity of ethanol extract is comparable with that of standard diclofenac sodium. Petroleum ether, chloroform and distilled water extracts showed moderate activity when compared to standard drug diclofenac sodium.

The above results are supported by the adrenal granuloma dry weight of experimental rats and the percentage inhibition of dry granuloma weight.

3. SUBACUTE INFLAMMATION (ULCEROGENIC EFFECT IN RATS)

Study of ulcerogenic potential by scoring the gastric lesions (Vogel H. Gerhard (2002).Healthy Rats were divided into six groups weighing between 150-200 g were selected. These rats were divided into six groups of six animals each. Group first received Tween-80 solution (1%) and served as control. Group second received diclofenac sodium solution at a dose of 150 mg/kg body weight and served as reference standard. Groups three, four, five and six received petroleum ether, chloroform, ethanol and distilled water extracts of *Leucas aspera* respectively at a dos of 400 mg/kg body weight. The treatment was started on the day of implantation of cotton pellet and grass piths and continued for successive ten days. On the 11^{th} day, the rats were sacrificed with an over dose of anesthesia and stomach was opened along the greater curvature and the mucosa was gently washed with normal saline avoiding injuries to the mucosa. The number of ulcers in all the groups was counted with the help of magnifying glass and severity was calculated by using orbitary system (Gupta *et al.,* 1999)

i) Denuded epithelium = 10
ii) Petechial and frank haemorrhages = 20
iii) One or two ulcers = 30
iv) Multiple ulcers = 40
v) Perforated ulcers = 50

Petroleum ether, chloroform, ethanol, distilled water extracts and standard anti-inflammatory drug diclofenac sodium were subjected for their ulcer index potency. The standard anti-inflammatory drug at a dose of 150 mg/kg body weight potentially increased the ulceration. The ulcer index of petroleum ether extract at a dose of 400 mg/kg body weight is comparable to that of control. The ulcer index of chloroform, ethanol and distilled water extracts of *Leucas aspera* at a dose of 400 mg/kg body weight is significantly reduced. (Table.3).

The anti-inflammatory activity of various extracts of *Leucas aspera* assessed by different available methods indicates that, the ethanol extracts and fraction F_5 of ethanol extract of *Leucas aspera* found to show significant anti-inflammatory activity at a dose of 400 mg/kg body weight. This significant anti-inflammatory activity of ethanol extract and fraction F_5 of ethanol extract in induced acute and subacute inflammation is due to the presence of lead molecules in them. These lead molecules inhibit the process of inflammation by inhibiting vascular and cellular events by inhibiting any one are all chemical mediators which cause inflammation. The moderate or less anti-inflammatory activity of petroleum ether, chloroform, water and other fractions of ethanol extract indicates that they contain the lead molecules in lesser concentrations. (Table.3).

All clinically practiced and being practiced non-steroidal anti-inflammatory drugs cause gastric ulceration hence, it is essential to study the ulcer index produced by the extracts of *Leucas aspera* at that particular dose. The reference standard diclofenac sodium caused 100% ulceration in experimental rats whereas all the extracts of *L. aspera* caused ulceration even at lesser extent than the control group. This study clearly indicates that different extracts of *L. aspera* especially ethanol extract showed good anti-inflammatory activity and safe in causing ulceration. (Table.3)

CONCLUSION

Leucas aspera, an annual herb found throughout India as a weed in cultivated fields used in folk medicine for the treatment of asthma, conjuctivities, headache, jaundice, skin diseases etc.,The anti-inflammatory action of triterpenoids has been reported by many researchers (B.Vazquez,1996; N.Suh,1998). Therefore, it seems that anti-inflammatory profile of *Leucas aspera* might be related to the triterpenoids and phenols. In conclusion, the whole plant extract of *Leucas aspera*, which contains triterpenoids, phenols, possesses anti-inflammatory effects. However, the plant *L. aspera* may hence be used as lead compounds for designing potent anti-inflammatory drug which can be used for treatment of various diseases.

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Table-1: Data showing the anti-inflammatory activity of various extracts of *Leucas aspera* by paw oedema method

Group	No. of Animals	Average b.w.	Dose (mg/kg)	Mean paw volume in ml ± S.E.				
				0 hr	½ hr	1 hr	3 hr	5 hr
Control (Tween-80)	06	185	1 ml (1%) 10 mg	1.48±0.016	1.48±0.016	1.56±0.021	1.76±0.021	1.66±0.021
Standard (Diclofenac sodium)	06	190	150	1.46±0.021	1.36±0.021**	1.16±0.021**	0.98±0.016**	0.87±0.021**
Pet. Ether extract	06	180	400	1.45±0.022	1.33±0.03**	1.08±0.03**	1.03±0.021**	0.92±0.017**
Chloroform extract	06	185	400	1.45±0.022	1.30±0.025**	1.18±0.03**	1.066±0.042**	0.90±0.036**
Ethanol extract	06	190	400	1.45±0.022	1.28±0.016**	1.15±0.022**	1.03±0.042**	0.833±0.042**
Water extract	06	195	400	1.45±0.022	1.25±0.022**	1.08±0.03**	1.016±0.017**	0.90±0.025**

(n=6) in each group
All drugs were given orally 30 minutes prior to carrageenan. Student's 't' test: P<0.05*, P<0.01** and P<0.001***
Dunnet's test: All the treated groups had significant (P<0.01) anti-inflammatory activity.
** Indicates significant difference at P<0.01 when compared to control.

Table (2.1) Data showing the anti-inflammatory activity of various fractions of ethanol extract of *Leucas aspera* by paw oedema method

Group	No. of Animals	Average b.w.	Dose (mg/kg)	Mean paw volume in ml ± S.E.				
				0 hr	½ hr	1 hr	3 hr	5 hr
Control (Tween-80)	06	185	1 ml (1%) 10 mg	1.48±0.017	1.48±0.017	1.57±0.021	1.76±0.021	1.66±0.021
Standard (Diclofenac sodium)	06	190	150	1.46±0.021	1.36±0.021**	1.16±0.021**	0.98±0.017**	0.87±0.021**
F_1	06	185	400	1.55±0.022	1.45±0.022	1.25±0.22**	1.05±0.022**	0.98±0.017**
F_2	06	175	400	1.53±0.021	1.38±0.017*	1.23±0.021**	1.11±0.030**	0.95±0.034**
F_3	06	170	400	1.41±0.030	1.31±0.030	1.18±0.017**	1.06±0.021**	0.93±0.033**
F_4	06	185	400	1.45±0.022	1.33±0.021**	1.23±0.021**	1.03±0.021**	0.90±0.025**
F_5	06	190	400	1.48±0.030	1.33±0.033**	1.18±0.016**	1.05±0.022**	0.81±0.047**
F_6	06	180	400	1.46±0.021	1.35±0.022**	1.23±0.021**	1.05±0.022**	0.95±0.022**
F_7	06	175	400	1.51±0.030	1.36±0.021**	1.23±0.021**	1.10±0.025**	0.91±0.047**

F_8	06	170	400	0.55±0.022	1.45±0.034	1.23±0.033**	1.13±0.033**	0.96±0.021**
F_9	06	180	400	1.46±0.021	1.28±0.016**	1.16±0.025**	1.05±0.022**	0.91±0.030**
F_{10}	06	190	400	1.46±0.021	1.31±0.030**	1.15±0.022**	1.05±0.032**	0.90±0.036**

(F_1-F_{10} are different column fractions of alcohol extract of *L. aspera*)
* Indicates significant difference at P<0.05, compared to control.
** Indicates significant difference at P<0.01 when compared to control.

Table-2.2: Effect of various treatments on cotton pellet granuloma

Groups	Drugs & Dose mg/kg B.W.	Granuloma dry weight (mg/100 g B.W.) mean ±S.E.	% inhibition	P-value
1	Control	19.50±0.42	-	-
2	Diclofenac sodium	9.83±0.47	49.58	<0.01**
3	Petroleum ether	12.00±0.57	38.46	<0.01**
4	Chloroform	11.50±0.42	41.02	<0.01**
5	Alcohol	10.33±0.49	47.02	<0.01**
6	Distilled water	12.33±0.49	36.76	<0.01**

(n=6) in each group
All the drugs were administered orally, once daily, for ten days.
Dunnet's test: All the treated groups significantly (p<0.01) reduced granuloma dry weight.

Table-3: Effect of various treatments on ulcer index

Groups	Drugs & Dose mg/kg B.W.	No. of Ulcers / No. of Rats used	Main ulcer index S.E.M.	% of animals with ulcer	P-value
1	Control (Tween 80)	3/6	10.33±0.98	50%	-
2	Diclofenac sodium	6/6	35.00±3.16	100%	<0.01**
3	Petroleum ether	2/6	9.50±0.42	33.33%	NS
4	Chloroform	1/6	8.50±0.42	16.66%	NS
5	Alcohol	1/6	6.50±0.56	16.66%	NS
6	Distilled water	1/6	9.00±0.57	16.66%	NS

(n=6) in each group P<0.01**
All the drugs were administered orally, once daily, for ten days.
Dunnet's test: None of the treated groups except diclofenac sodium showed significant ulceration.