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STUDIES ON THE ANTI-INFLAMMATORY EFFECT OF THE AQUEOUS EXTRACT OF THE LEAVES OF *VITEX TRIFOLIA* L. IN ALBINO RATS.

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ABSTRACT

Vitex trifolia belongs to the family *lamiaceae.* The anti-inflammatory property of the aqueous extract of the leaves of *Vitex trifolia* L. was studied by carrageenan induced paw oedema, granuloma pouch and formaldehyde induced arthritis method. in albino rats. The extract *was* administered at the varying doses of 500, 1000 & 2000 mg/kg body weight per orally to three groups of six animals each. Increasing the doses of the test drug produced signicant ($p<0.001$) increased in percentage of inhibition of paw oedema and significant inhibition of exudate formation. The extract shows significant anti-inflammatory activity when compared with the control and comparable efficacy with the standard. The present study suggests that the aqueous extract of the leaves of *vitex trifolia* L. has anti-inflammatory property.

KEYWORDS: **Anti-inflammatory, carrageenan, granuloma pouch, formaldehyde arthritis**

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INTRODUCTION

The plant kingdom has many plants with properties that are conducive to health. In fact they have become a part of Indian culture and they continue to be an important source of medicine relief to the rural population .[1] *Vitex trifolia* L.(VTL; Manipuri name- Urikshibi; English name- White chaste tree/beach vitex; Hindi name- Nichinda; Sanskrit name- Jalanirgundi; Bengali name- Paniki-Shumbala) is a shrub or small tree growing from 1-4 meters in height. It is sometimes prostate or ascending in habit. The leaves are simple or 3- foliolate. In the prostate form the leaves are simple, stalkless, oblong to oblong elliptic, 4-7 cms long, 1.5-4 cms wide, pointed at both ends, smooth and shiny on the upper surface, and sparsely covered with gray hair beneath.[2] *Vitex trifolia* is traditionally used by the tribes and native medical practitioners for the treatment of various ailments including liver disorders, tumours, rheumatic pains, inflammations, sprain, fever and used in the treatment of tuberculosis.[3] Even though there are various reports of the uses of *Vitex trifolia* L. there is only few scientific data to substantiate them. Therefore, the present study has been undertaken to evaluate the anti-inflammatory properties in suitable animal experimental models.

MATERIALS AND METHODS

Plant material and extract preparation The fresh leaves of *Vitex trifolia* L. were collected from Heingang, Imphal-East during the month of july-august, 2010. The plant was identified and authenticated by Dr. Athokpam Pinokiyo, Assistant Professor, Department of Botany, DM College of Science. The plant materials were cleansed and dried under shade, powdered by a mechanical grinder. Preparation of aqueous extract was done by the method of Verma SCL and Agarwal.[4] 50gm of the powdered leaves of *Vitex trifolia* L. were extracted using soxhlet apparatus. The yield was 22%.

Acute Toxicity Texting: The animals were fed with varying doses of aqueous extract of the leaves of VTL till 3g/kgbw and they do not show any signs of toxicity or death when observed for 24hrs.

***Experimental Animals*:** Healthy albino rats of Wistar strain weighing between 100-250 gm of either sex obtained from central animal house R.I.M.S., Imphal were used in the experiment. The animals were housed in the standard rat cages and maintained on standard diet with access to water. They were acclimitized at room temperature with night and dark cycle for 1 week in the departmental animal house. The animals described as fasted, were deprived of food for 18hrs with due care to prevent coprophagy, but with water *ad libitum*. All the experimental protocols were approved by the institution animal ethics commitee.

Experimental design: The animals were divided into five groups with six animals in each group. The drugs were suspended in 2% gum acacia and administered orally.

Group	Drugs
A(Control)	2% Gum acacia (10 ml/ kg bw)
B(Test)	VTL (500mg/kg)
C(Test)	VTL (1000mg/kg)
D(Test)	VTL (2000mg/kg)
E(Standard)	Aspirin (100mg/kg)

The volumes of the medicaments are kept constant at 10ml/kg body weight of the animals. The results were compared with those of the control and standard drugs for statistical significance using one way ANOVA followed by Dunnett's 't' test.

I. *TEST OF ACUTE INFLAMMATION*

1. Carrageenan induced rat paw oedema

The method of Winter CA et al[5] was used with slight modification.The animals were fasted overnight and during the experiment but water was given *ad libitum*. Treatment groups of rats were pre-treated with test drugs and aspirin orally and control group received gum acacia suspension one hour before carrageenan injection. Freshly prepared carrageenan (1%) in 0.9% sodium chloride solution was injected in a volume of 0.1 ml into sub-plantar region of the right hind paw of the rat. The foot volume was measured in unanaesthesised rats by a plethysmometer immediately after and again at three hours after carrageenan injection and the 'volume of oedema' was recorded as the difference between two readings. The percentage of anti-inflammatory activity was then calculated by the formula of Diniz RS et al.[6]

II. SUB-ACUTE INFLAMMATION

(i). Granuloma pouch

The method described by Selye H[7] with slight modification was used. Rats were anaesthesized with ether and a subcutaneous dorsal pouch was prepared in between the shoulder blades by injecting 20 ml of air. Then 0.5 ml of turpentine oil was injected into the pouch.Treated groups of rats were treated with the test drugs, aspirin and control group received 2% gum acacia suspension orally for six days beginning from the day of pouch formation. On the seventh day the pouch was opened under ether anaesthesia and the exudates was sucked out and the amount measured. The percentage inhibition was then calculated for the different groups of drugs as compared to the control group.

III. CHRONIC INFLAMMATION

1. *Formaldehyde arthritis*

The methods described by Selye H[8] was used. A subcutaneous injection of 0.1 ml of 2% formalin was given under the plantar aponeurosis of the right hind foot of the rats. The paw volume was measured plethysmometrically for 13 days to assess the degree of inflammation. Groups of animals, as described before were treated with test drugs, gum acacia and aspirin orally daily for 13 days.

RESULT AND OBSERVATIONS

Table 1
Acute anti-inflammatory activity of aqueous extract of Vitex trifolia L. on carrageenan induced rat paw oedema.

Group	Drug dose	Mean increase in paw volume (in ml) after 3 hrs	% inhibition of paw oedema
A (Control)	**2% Gum acacia in N/S; 10 ml/kg**	**0.45 ± 0.197**	**-**
B (Test)	VTL, 500mg/kg	0.3 ± 0.136*	33.33%
C (Test)	VTL,1000mg/kg	0.25±0.113*	44.44%
D (Test)	VTL,2000mg/kg	0.18±0.0873*	60%
E(Standard)	Aspirin,100mg/kg	0.15±0.070*	66.7%

One way ANOVA F = 17.28 (p<0.01) , df = 4, 25
Value are mean±SD, n=6 in each group. *p<0.001 when compared to control.

Table 1. shows that increasing the doses of the test drug produced increase in percentage of inhibition of paw oedema. Both the test and standard drug produced highly significant inhibition of paw oedema in comparison to the control (p<0.001).

Table 2
Effect on the sub-acute inflammation of aqueous extract of Vitex trifolia L. on granuloma pouch.

Group	Dose (mg/kg),p.o	Mean volume of exudates (mean±SD) in ml	% inhibition of exudates formation
A (Control)	10ml/kg	3.5±0.43	-
B (Test)	500	2.16±0.31	38.28%
C (Test)	1000	1.83±0.48*	47.71%
D (Test)	2000	1.66±0.21*	52.57%
E (Standard)	100	1.33±0.21*	62%

One way ANOVA F =7.74(p<0.01) df = 4, 25
*Values are mean±SD, n=6 in each group; *p<0.01 when compared to control.*

Table 2. Shows that increasing concentration of the test drug produced increased inhibition of exudates formation. Both the test and the standard drug produced significant inhibition of exudate formation compared to the control group.

Table 3
Effect of the anti-inflammatory activity of the aqueous extract of Vitex trifolia L. in formaldehyde arthritis.

Group	Dose mg/kg p.o	Mean increase in paw volume(mean±SD) in ml				
		3rd day	5thday	9th day	11thday	13th day
A (Control	10ml/kg	0.56±0.27	0.6±0.03	0.64±0.02	0.67±0.03	0.61±0.03
B (Test)	500	0.36±0.04*	0.42±0.02*	0.42±0.03**	0.38±0.01**	0.37±0.02**
C (Test)	1000	0.26±0.02**	0.35±0.02**	0.38±0.01**	0.35±0.02**	0.33±0.01**
D (Test)	2000	0.24±0.01**	0.28±0.03**	0.25±0.01**	0.21±0.02**	0.2±0.02**
E(Standard)	100	0.23±0.02**	0.27±0.01**	0.22±0.01**	0.18±0.01**	0.15±0.01**

One way F(df) (p<0.01) 8.5 (4.25) 6.14258(4,25) 17(4,25) 44(4,25) 19(4,25)
*ANOVA * p<0.01, **p<0.001 when compared to control, n=6 in each group. Values = Mean±SD.*

Table 3. Shows that both test and standard drugs produced highly significant inhibition of paw oedema when compared to control. The result shows that in the control group of rats there was no significant reduction in the paw volume even on the 13th day. Whereas the aqueous extract of VTL showed significant reduction in the paw volume. The test drug at a dose of 2000mg/kg had almost similar efficacy to aspirin.

Figure 1
Showing acute-anti-inflammatory activity of aqueous extract of VTL on carrageenan induced rat paw oedema.

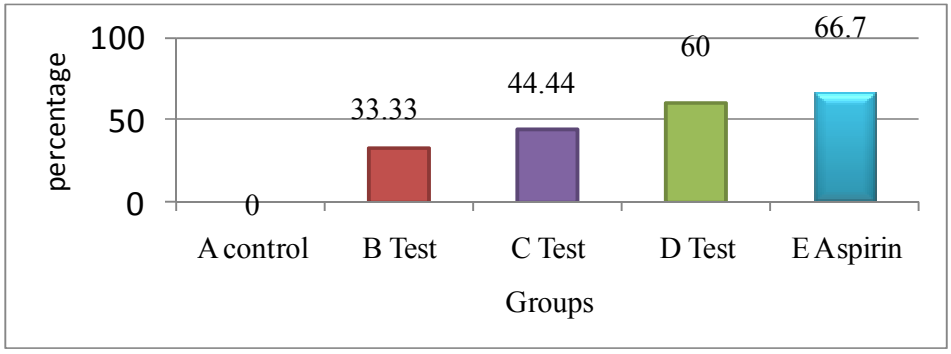


Figure 2
Showing the effect on the sub-acute inflammation of aqueous extract of VTL on granuloma pouch.

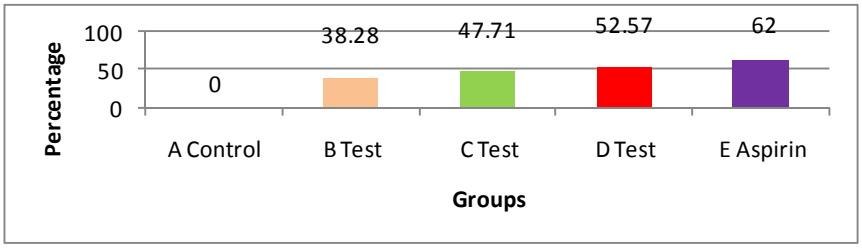
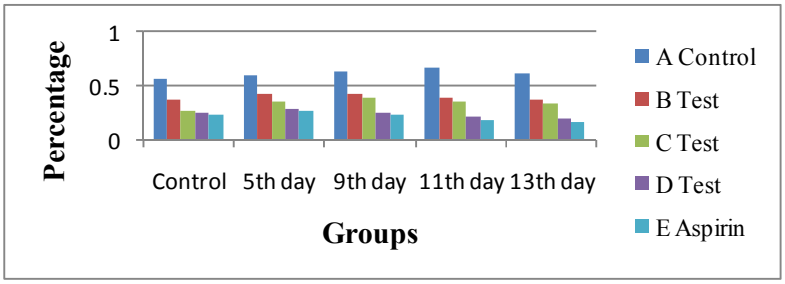


Figure 3
Showing anti-inflammatory activity of aqueous extract of VTL on chronic inflammation by formaldehyde induced arthritis



method.

DISCUSSION

The anti-inflammatory activity of VTL was tested on carrageenan induced paw oedema in albino rats which is the standard experimental model for acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effect.[9] Moreover, the model exhibits a high degree of reproducibility.[10] The carrageenan induced edema involves a biphasic response, the first phase is attributed to release of histamine, 5-HT and kinins in the 1st hr, the second phase is related to release of prostaglandin and lysosomal enzymes like substances in 2-3 hrs.[11] It appears that the aqueous extract of dried leaves of *Vitex trifolia* L. inhibit this mediators to account for its anti-inflammatory activity. Previous studies have reported the anti-inflammatory activities of flavonoids. Also, the anti-proliferative activity of flavonoids evident by decrease in granuloma weight has been reported.[12] It is therefore plausible to suggest that the significant anti-inflammatory activity of VTL in inflammation could be due to the presence of flavonoids, either alone or in combination with other constituents and it may be presumed that VTL possesses anti-kinin activity and is effective in controlling non-immunological type of inflammation. The granuloma pouch experiment was done by the method of Selye H.[7] Granuloma pouch offers a model of exudative type of inflammation. The test drug at varying doses produced significant inhibition of exudates but lesser than that of the standard drug. Formaldehyde arthritis was done by the method of Selye H .[8] From the present study it is apparent that the test drug in doses of 500mg/kg, 1000mg/kg and 2000mg/kg produced significant inhibition of paw oedema induced by formaldehyde.The test drug in dose of 2000mg/kg was found to be similar in efficacy to the standard drug aspirin(100mg/kg). It is well known that inhibition of edema induced by formalin in rats is one of the most suitable test procedure to

screen anti-arthritic and anti-inflammatory agents, as it closely resembles human arthritis.[13]Arthritis induced by formalin is a model used for the evaluation of an agent with probable anti-proliferative activity. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response.[14] The results of the formalin tests showed a possible effect of the extract on formalin induced arthritic conditions. These findings justified the usefulness of VTL in the treatment of inflammation associated diseases like arthritis. The above findings suggest that the aqueous extract of the leaves of *Vitex trifolia* L. possess significant anti-inflammatory activity against acute, sub-acute and chronic models of inflammation.

CONCLUSION

The present study reveals that the aqueous extract of the leaves of *Vitex trifolia* L. has significant anti-inflammatory activity without any significant adverse effect. Further studies involving the purification of the chemical constituents of the plant and investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with low toxicity and higher therapeutic index.

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