

Anti- inflammatory effect of *Vitex altissima* L.f. stem bark on Cotton pellet induced granuloma

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ABSTRACT

This paper evaluated pharmacological investigation of *Vitex altissima* L.f. [Verbenaceae] at Servaroyan hills [Yercaud] of Eastern ghats, Salem district, Tamil nadu. The information obtained can lead to directions of discovery of new drugs. For *in vivo* studies, the shade dried and powdered stem bark material was extracted in soxhlet extractor with ethanol. The present study reflected the efficacy of ethanol extract of *V. altissima* stem bark to a high extent in containing an increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharide which are natural proliferative events of granulation tissue formation. Finally reveals that the stem bark of *V.altissima* possesses potent anti- inflammatory activity.

Keywords : Anti- inflammatory, *Vitex altissima*, Cotton pellet induced granuloma, pharmacology.

INTRODUCTION

India has a rich biodiversity of medicinal plants. It also enjoys the unique position, in the usage and exporting of herbal drugs. It has 16 agroclimatic zones and 45,000 different plant species, out of which 15,000 medicinal plants are recorded so far. Medicinal plants in India have diverse consumers. They include domestic users, traditional physician, folk healers, ayurvedic drug manufacturing units, pharmacists *etc.* More than 80,000 species of Indian plants belonging to different botanical families are reported to possess medicinal properties¹(Ved *et al.*, 2000).

There is growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine² (Misar, *et al.*, 2005). A perfect example of medicinal plant credited with innumerable medicinal qualities validated by modern science and used since ancient times is *Vitex* Linn. (Verbenaceae). The genus consists of 270 species of which about 14 species are found in India and some have commercial and medicinal importance. The present study is focused on the indigenous medicinal plant *Vitex altissima*.

MATERIALS AND METHODS

Plant description

Vitex altissima is also known as Tall chaste tree. The plant is distributed throughout South India, in evergreen and deciduous forests .It is common in forest, less on slopes, down to foothills, hills above 600m. It is a densely foliaceous tree with extensive crown. A large tree grows upto 40 meters in height. Leaves compound, palmate, 3-5 foliate, winged rachis, leaflets acute, ovate, membranous, and glabrous. Flowers whitish arises from terminal panicles. Fruits ovoid drupes containing hard seed.

Collection and powder of stem bark

Fresh stem bark of *Vitex altissima* L.f. were collected from Servarayan hills of Eastern ghats, Tamil Nadu, India. The collected stem bark were cleaned to remove adhering dust and then dried under shade. The dried plant material was powdered in a Willy Mill to 60-mesh size. The stem bark powder was used for further studies.

Solvent extraction

For *in vivo* studies, the shade dried and powdered stem bark material was extracted in soxhlet extractor with ethanol after dewaxing with petroleum ether. The extract was evaporated to remove even the final traces of ethanol. The dried extract was suspended in distilled water right before use.

In vivo pharmacological studies

Experimental animals

Male wistar rats (120-150 g) and Swiss albino mice (25-30 g) used in the present study. The animals were housed in polypropylene cages (38 x 23 x 10cm) with not more than six animals per cage under standard environmental conditions of temperature ($23 \pm 1^\circ\text{C}$), relative humidity ($55 \pm 1\%$), 12 h/12 h light/dark cycle and fed with standard pellet diet and water *ad libitum*. The animals were acclimatized to the environment for two weeks prior to experimental use. Animals were fasted over night before the experimental schedule, but had free access for water *ad libitum*.

Cotton pellet-induced granuloma

For the experiment, the male wistar rats (120–150 g) were divided into four groups (n = 6). The animals were fasted overnight prior to the start of the experiment, and water *ad libitum*. Cotton pellets, weighing 30 mg each, were sterilized. Under ether anesthesia, the pellets were introduced subcutaneously through a skin incision in the back of the animals (Winter and Porter, 1957)³. The reference group (group - II) was treated with 10 mg/kg of indomethacin. At the same time, test groups of rats (group III and IV) were administered with 200 and 400 mg/ kg of the ethanolic extract of *V. altissima*. All the groups were treated orally for 7 days and the treatments started 30 min after cotton pellet implantation. On the eighth day, the animals were sacrificed with chloroform; the granulomas were removed, dried for 24 h at 60°C and the dry weights determined. The difference between the initial and final dry weights was considered to be the weight of the amount of granulomatous tissue produced (Winter *et al.*, 1962)⁴.

RESULTS AND DISCUSSION

In vivo studies

The *in vivo* studies were carried out in the ethanolic extract of *V. altissima* stem bark. The percentage yield of ethanolic extract of the stem bark sample was found to be 22%.

Effect of *V. altissima* stem bark on Cotton pellet induced granuloma

The effect of ethanolic extract of *V. altissima* stem bark against chronic inflammation was studied against cotton-pellet induced granuloma in experimental rats and the results are presented in Table 1. The test drug extract at two different concentrations (200 and 400mg/Kg b.w. p.o.) were administered orally and the activity was compared with the standard drug indomethacin (10 mg/kg). Administration of the extracts inhibited the formation of granulomatous tissue, as indicated from their decreased weight of cotton pellet in a dose dependant manner. The ethanol extract at the dose of 400 mg/kg showed the granuloma inhibition (50.50%) whereas the standard drug indomethacin showed an inhibitory percentage of 64.99%.



Table 1. Anti-inflammatory effect of *Vitex altissima* stem bark on cotton pellet induced granuloma in rats

Group	Dose (p.o.)	Initial pellet weight (mg)	Final pellet weight (mg)	Difference	Percentage protection (%)
Induced	-	30	87.51 ± 2.91	57.51 ± 2.91	---
Standard (Indomethacin)	10 mg/kg	30	50.13 ± 1.48***	20.13 ± 1.48***	64.99
<i>Vitex altissima</i>	200 mg/kg	30	73.51 ± 2.94***	43.51 ± 2.94***	24.34
<i>Vitex altissima</i>	400 mg/kg	30	58.46 ± 1.16***	28.46 ± 1.16***	50.50

Values are mean ± standard deviation (n = 6).

*Decrease in weight of cotton pellet at $P < 0.05$ when group II – IV compared to group I, ** $P < 0.01$, *** $P < 0.001$.

Cotton pellet-induced granuloma

Chronic inflammation is the reaction arising when the acute response is insufficient to eliminate the pro-inflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and infiltration of neutrophils with exudation of fluid. It occurs by means of development of proliferative cells which can either spread or form granuloma. The cotton pellet induced granuloma has been widely employed to evaluate the transudative, exudative and proliferative components of chronic inflammation because the dried weights of the pellets correlate well with the amount of granulomatous tissue (Swingle and Shideman, 1972)⁵. Efficacy of anti-inflammatory agents in chronic inflammatory states is indicated by their ability to inhibit the increase in the number of fibroblasts during granular tissue formation (Gupta *et al.*, 2003)⁶. The results of the present study reflected the efficacy of ethanol extract of *V. altissima* stem bark to a high extent in containing an increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharide which are natural proliferative events of granulation tissue formation.

Several anti-inflammatory, digestive, antinecrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have an antioxidant and/or radical scavenging mechanism as part of their activity (Repetto and Llesuy, 2002)⁷. Free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralisation by antioxidants and radical scavengers can attenuate inflammation (Geronikaki and Gavalas, 2006)⁸. Several phenolic compounds and flavonoids isolated from medicinal plants have been found to possess significant anti-nociceptive (analgesic) and/or anti-inflammatory effects (Duke, 1992)⁹. It is, therefore, possible that the anti-inflammatory effects observed with the ethanol extract of *Vitex altissima* stem bark may be attributable to its flavonoid component. The analgesic and anti-inflammatory activity is a common property of many terpenoids and sterols (Goncalves *et al.*, 2008)¹⁰. Plants of genus *Vitex* are found to be rich in phenolics (Hernández *et al.*, 1999)¹¹. Hence the observed anti-inflammatory property of ethanol extract of *Vitex altissima* might be due to the presence of phenolic compounds and their potent antioxidant activities. The anti-inflammatory efficacy of some plants of *Vitex* genus were already been reported in rat models of paw edema (Sridhar *et al.*, 2004; 2005)^{12&13}. Based on these data and the results of the present study, it is quite evident that the stem bark ethanolic extract of *Vitex altissima* possesses potent anti-inflammatory activity (Table 1).

CONCLUSION

In cotton pellet induced granuloma model, administration of the extracts inhibited the formation of granulomatous tissue, as indicated from their decreased weight of cotton pellet in a dose dependant manner. The ethanolic extract of *V. altissima* extract at a dose of 400 mg/kg depicted a significant granuloma inhibition (50.50%).

REFERENCES

1. Ved, D.K., Prathima, C.L., Morton.N. 2000. What do we know about the distribution of Indian medicinal plants. *Amruth*, 4: 2.
2. Misar, A.V., Kale, M., Joshi, M., Mujumdar, A.M. 2005. Analgesic Activity of *Dalbergia lanceolaria* barks extract in Swiss Albino Mice. *Pharmaceutical Biology*, 43(8): 723–725.
3. Winter, C.A., Porter, C.C. 1957. Effect of alterations in the side chain upon antiinflammatory and liver glycogen activities of hydrocortisone esters. *J. Am. Pharm. Assoc. Sci. Educ.*, 46: 515-519.
4. Winter, C.A., Risely, E.A., Nuss, G.W., 1962. Carrageenin-induced edema in the hind paw of rat as an assay for antiinflammatory drugs. *Proceedings of Society for Experimental Biology and Medicine*, 111: 544–547.
5. Swingle, K. F., Shideman, F. E. 1972. Phases of inflammatory response to subcutaneous implantation of cotton pellet and other modifications by certain anti-inflammatory agents. *Journal of Pharmacology and Experimental Therapeutics*, 183: 226–234.
6. Gupta, M., Mazumder, U., Ramanathan, S. K., Thangavel, S. K. 2003. Studies on antiinflammatory, analgesic and anti-pyretic properties of methanol extract of *Caesalpinia bonducella* leaves in experimental animal models. *Iranian Journal of Pharmacology and Therapeutics*, 2: 30–34.
7. Repetto, M. G., Llesuy, S. F. 2002. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Brazilian Journal of Medicine and Biological Research*, 35: 523–534.
8. Geronikaki, A. A., Gavalas, A. M. 2006. Antioxidants and anti-inflammatory diseases: synthetic and natural antioxidants with anti-inflammatory activity. *Combinatorial Chemistry and High Throughput Screening*, 9: 425–442.
9. Duke, J. A. 1992. Handbook of Biologically Active Phytochemicals and their Activities. CRC Press, Boca Raton, Florida.
10. Goncalves, J. C. R., Oliveira, F. D. S., Benedito, R. B., Sousa, D. P. D., Almeida, R. N. D. 2008. Antinociceptive activity of (-)-carvone: evidence of association with decreased peripheral nerve excitability. *Biological and Pharmacological Bulletin*, 31: 1017–1020.
11. Hernández, M.M., Heraso, C., Villarreal, M.L., Arispuro, V.I and Aranda E. 1999. Biological activities of crude plant extracts from *Vitex trifolia* L. (*Verbenaceae*). *J. Ethnopharmacol.*, 67: 37-44.
12. Sridhar C, Subbaraju G.V, Venkateswarlu, Y., Venugopal, R.T. 2004. New acylated iridoid glucosides from *Vitex altissima*. *Journal of Natural Products*, 67: 2012-2016.
13. Sridhar, C., Rao K.V., Subbaraju G.V. 2005. Flavonoids, triterpenoids and a lignan from *Vitex altissima*. *Phytochemistry*, 66(14): 1707-1712.