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Evaluation of anti-inflammatory effect of methanolic & chloroform Extracts and pure molecules (Quercetin) of roots of *Vitex trifoliata* in experimental animals

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Abstract

The objective of present study was evaluating the methanolic & chloroform extract anti-inflammatory activity of vitex trifoliate. Acute oral toxicity studies in mice of either sex (20-30 g) revealed that the extracts up to 2000 mg/kg have not produced any mortality in experimental animals. Since methanolic extracts have produced lower activity than chloroform extracts which almost equal active with standard drug, the chloroform extract was further subjected to phytochemical evaluation to isolate the pure molecules which are responsible for anti-inflammatory activity. The results were expressed as maximal paw oedema (maximal peak during the 6 h) and as total paw oedema (area under the time-course curve) and presented as mean ± s.e.m., n=5.

Keywords: Vitex trifoliate roots, Carrageenan, inflammation, ibuprofen, methanolic & chloroform extract.

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1. Introduction

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation [1]. Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow [2]. Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflamemation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation[3] .whereas prostaglandins are detectable in the late phase of inflammation[4]. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g.

hydrocortisone. All of these drugs possess well known side and toxic effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. Exploring the healing power of plants is an ancient concept. For centuries people have been trying to alleviate and treat disease with different plant extracts and formulations [5]. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs [6]. Screening of the plants for their biological activity is done on the basis of either their chemotaxonomic investigation or ethnobotanical knowledge for a particular disease. Identification of a particular compound against a specific disease is a challenging long process. Importance of the plant lies in their biologically active principles. Vitex trifoliate (Verbenaceae) is an aromatic shubby tree which can grow to 4m. in the Seychelles, bearing trifoliate leaves, or more rarely 1-2 leaves.

It is found from chintapalli, anantagiri forest, vizag to nallamadala forests in Andhra Pradesh. The practice of herbal medicine dates back to the very earliest periods of known human history. There is evidence that herbs have been used in the treatment of diseases and for revitalizing the body system in almost all ancient civilizations. There is a rapid progress in various fields of human activity, the field of medicine and its allied sciences. All these have made rapid strides. The present study on Indian medicinal plant has been aimed to focus for their anti-inflammatory potentials. The idea stemmed from the following fact according to folkloric use, the plants were not screened for the treatment of inflammatory conditions. On the basis of the survey of ethnomedical, folkloric information and literature, the following plants were selected Vitex trifoliate[7, 8, 9].Exhaustive and up to date review of literature for anti-inflammatory their methods of screening and pharmacological review of the selected plant were conducted. Dried powdered roots of Vitex trifoliate were separately extracted in a Soxhlet apparatus for 6 h successively with chloroform and methanol. The concentrate is dried under vaccum in a rotary evaporator.[10].All the extracts and the individual compounds have to be screened for anti-inflammatory activity using carrageenan-induced rat paw oedema method. To assess the folk claims and to find out the active constituents responsible for anti-inflammatory activity[11, 12,13,14,15].

2. Materials and Method

Animals:

Wistar albino rats of either sex weighing between 150-200 g were obtained from National Institute of Nutrition, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of 25 $\pm$ 2^0C with an alternating 12h light-dark cycle and relative humidity of 50`±15%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee and by the Regulatory body of the government (Regd no. 516/01/A/CPCSEA). They were fed with standard laboratory diet (supplied by Ratan Brothers, India) and water ad libitum during the experiment.

Chemicals:

Methanol, chloroform, Sodium Carboxy Methyl Cellulose was pharmaceutical grade, purchased from S.D fine chem., Mumbai. carrageenan, ibuprofen purchased from sigma chemicals, st.louis, MO, USA.

Collection & Preparation of extract:

The dried and powdered roots of V.trifoliata were extracted by Soxhlet apparatus succesively with Chloroform and methanol. Methanolic and Chloroform extracts were tested for carrageenan induced paw oedema. The extracts which produced significant anti-inflammatory effect were subjected to column chromatography. The pure molecule VT (Quercetin) obtained from Chloroform extract has been studied for anti-inflammatory effects on carrageenan-induced paw oedema.

Methodology:

Wistar rats (150-200 g, purchased from National Institute of Nutrition, Hyderabad, Andhra Pradesh, India) were used. Oedema was induced by injecting, subcutaneously (s.c) into the sub plantar tissue of the left hind paw of each rat, 0.1 mL of 1% carrageenan suspension in saline. The right hind paws of the same rats received 0.1 mL of saline alone in the same manner as control. Before the induction of oedema, the thickness of the both paws of each rat between lower and upper surface was measured using an instrument consisting of a graduated micrometer combined with a constant loaded lever system to magnify the small changes in paw thickness during the course of the experiment. The measurements were then taken at 1h intervals after the induction of the oedema for up to 6hs.Oedema was monitored as the percentage increase in paw thickness in the carragenin injected paw. To assess the effect of saline on the oedema produced, the percentage increase in paw thickness produced in the saline injected paw was subtracted from that of carrageenan injected left paw [16]. The percentage increase in paw thickness was plotted against the time (h) and the maximal oedema response induced during the 6hs was determined. The paw oedema was constantly increased during 4 hours and reached peak of oedema at 4^{th} hour. At the 5^{th} and 6^{th} hour, the oedema was gradually reduced (fig 1).

The total oedema response as the area under the time course curves (AUC) was also determined. The % Increase in paw thickness can be measured by using the formula $[(Y_t-Y_o)/Y_o] \times 100$
Where, Y_t = Paw thickness at time t h (after injection) and Yo = Paw thickness at time 0 h (before injection).

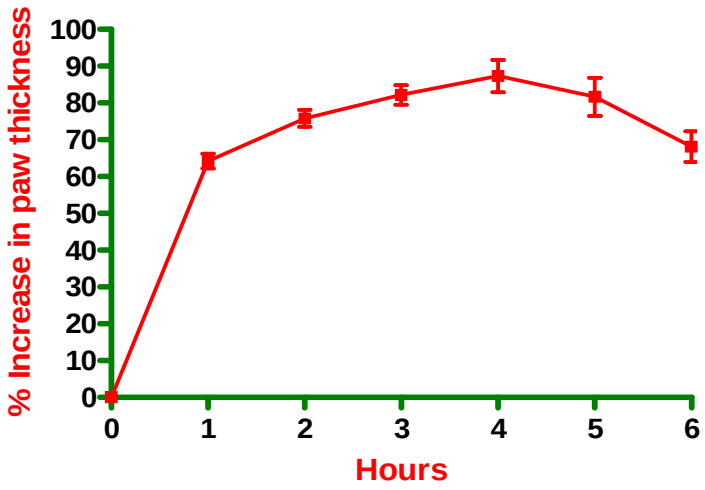
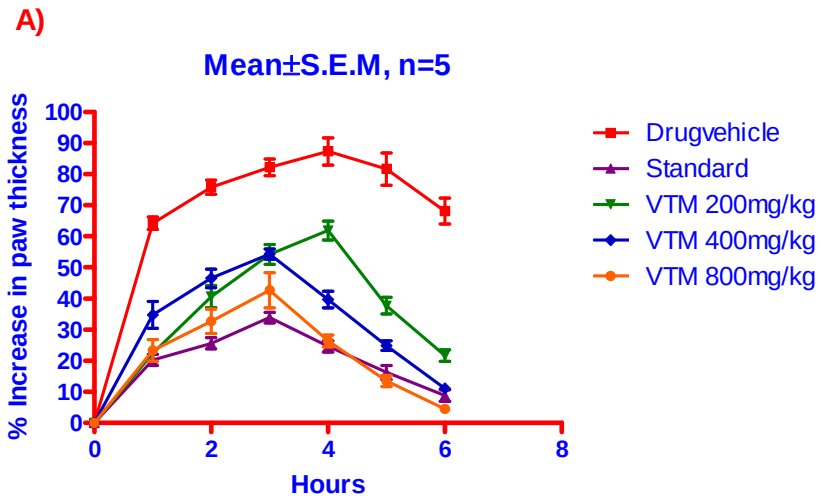


Fig1. Progression of the carrageenan-induced rat paw oedema over 6 h as monitored with Zeitlin's apparatus

Assessment of drug effects
For screening purposes, drugs (extract or compounds) in sodium carboxy methyl cellulose were always predosed to rats prior to the induction of carrageenan paw oedema. The drug actions were evaluated by comparing the maximal paw oedema response during 6h (monitored as % increase in paw thickness) in the drug treated groups with that produced in the drug vehicle (control) treated group. The total (area under the time course curve, AUC, calculated using Trapezoid Rule) oedema response as the area under the time course curve, produced in the drug treated groups was also compared with that from the control group. [17,18,19,20].

3. Results and Discussion

The extract of Vitex trifoliate was tested for in vivo studies for their ability to reduce the anti-inflammatory response in carrageenan induced rat paw oedema in rats. The results of reduction of rat paw odema were shown in table 1. Maximal paw oedema during 6h & total AUC paws oedema during 6h for group I (control) was measured as 0.0 ± 4.39 & 0.0 ± 5.49. Fig-2 shows that the ibuprofen and methanolic crude extract significantly inhibited the maximal oedema response by 61.32 ±1.72, 29.45 ± 3.0, 38.07 ± 1.61 and 51.34 ± 5.64 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 70.50 ± 1.83 and 44.84 ± 1.76 , 48.80 ± 2.43 and 66.20 ± 2.67 respectively over 6 h when compared to the control group treated with drug vehicle.



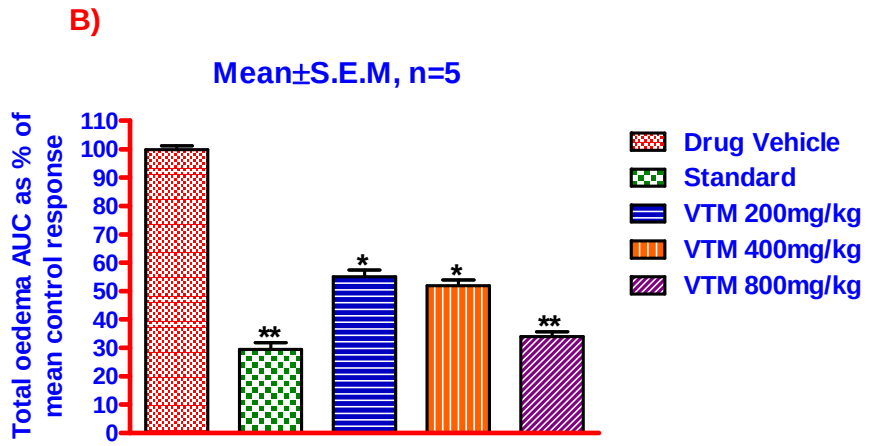
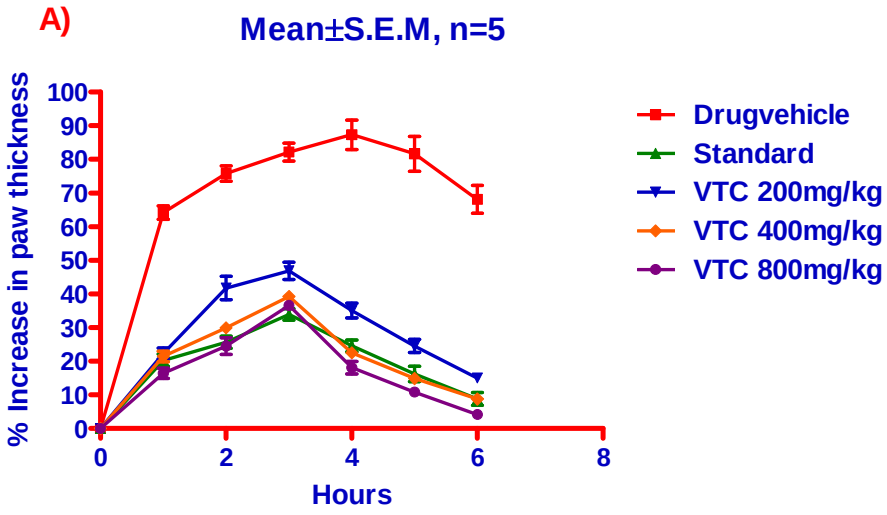


Fig2. Effect of the methanolic crude extract of VTM 200,400,800mg/kg along with Ibuprofen (2.5 x 10^{-5}moles/kg body wt.) on A) the maximal and B) the total paw oedema in carrageenan induced rats.



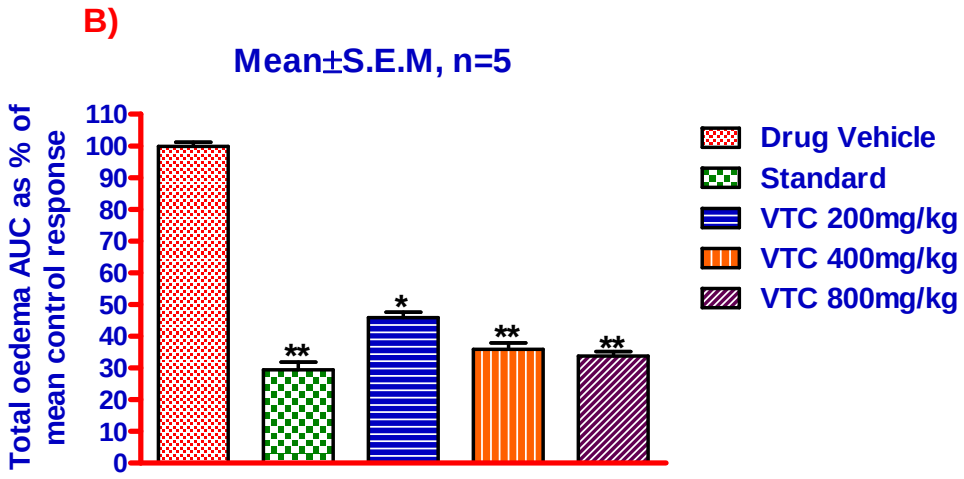


Fig 3. Effect of the chloroform crude extract of VTC 200,400,800mg/kg along with Ibuprofen (2.5 x 10^{-5}moles/kg body wt.) on A) the maximal and B) the total paw oedema in carrageenan induced rats.

Fig-3 shows that the ibuprofen and chloroform crude extract significantly inhibited the maximal oedema response by 61.32 ±1.72, 46.55 ± 2.59, 55.25 ± 0.83 and 58.29 ± 0.87 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 70.50 ± 1.83 and 53.85 ± 3.25 , 68.05 ± 1.54 and 68.97 ± 4.91 respectively over 6 h when compared to the control group treated with drug vehicle at first and second phases of inflammation.

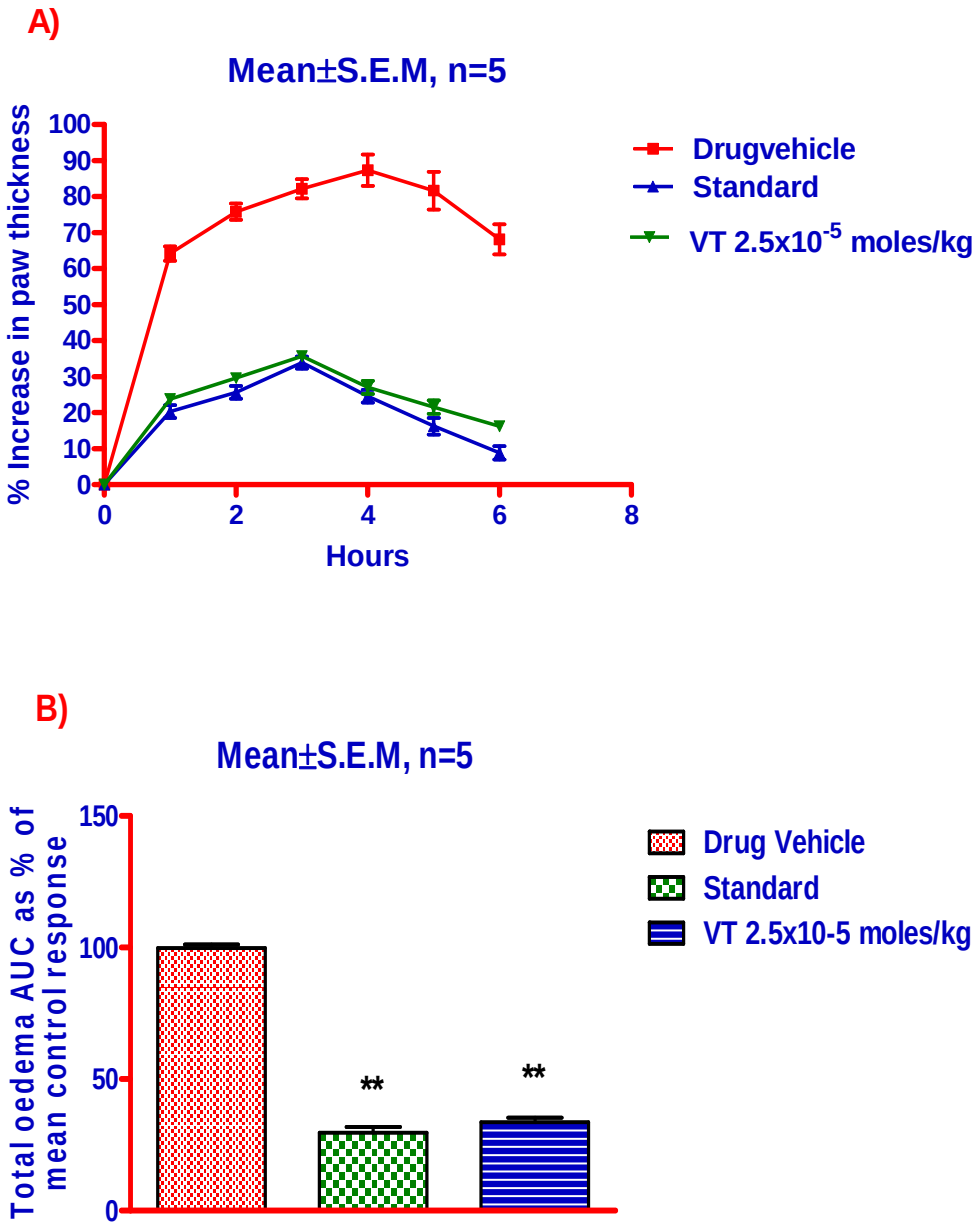


Fig 4. Effect of the pure molecule VT-3(Quercetin) isolated from *V.trifoliata* along with ibuprofen (2.5 x 10^{-5}moles/kg body wt.) on A) the maximal and B) the total paw oedema in carrageenan induced rats.

Fig.4 shows that the ibuprofen and pure compound VT-3(Quercetin) significantly inhibited the maximal oedema response by 61.32 ±1.72 and 59.26 ± 1.40 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 70.50 ± 1.83 and 66.18 ± 1.93 respectively over 6 h. The pure molecule exhibited significant reduction in reducing paw oedema when compared to the control group at all evaluated intervals of time.

Table 1. Percentage inhibition of carrageenan induced paw oedema in rats by prophylactic treatment with the methanolic & chloroforms Extracts and pure molecules (Quercetin) of roots of *V. trifoliata* and Ibuprofen

Treatment	Dose	Percentage inhibition of the maximal paw oedema during 6h.	Percentage inhibition of total AUC paws oedema during 6h.
Group I	Drug vehicle(1% Sodium CMC)	0.0 ± 4.39	0.0 ± 5.49
Group II	Ibuprofen $2.5x10^{-5}$ moles/kg	61.32 ± 1.72**	70.5 ± 1.83**
Group III	Received methanolic extract of *V.trifoliata* 200 mg/kg	29.45 ± 3.0*	44.84 ± 1.76*
Group IV	Received methanolic extract of *V.trifoliata* 400 mg/kg	38.07 ± 1.61*	48.8 ± 2.43*
Group V	Received methanolic extract of *V.trifoliata* 800 mg/kg	51.34 ± 5.64**	66.2 ± 2.67**
Group VI	Received chloroform extract of *V.trifoliata* 200 mg/kg	46.55 ± 2.59*	53.85 ± 3.25*
Group VII	Received chloroform extract of *V.trifoliata* 400 mg/kg	55.25 ± 0.83**	68.05 ± 1.54**
Group VIII	Received chloroform extract of *V.trifoliata* 800 mg/kg	58.29 ± 0.87**	68.97 ± 4.91**
Group IX	Received VT-3(Quercetin) from *.trifoliata* at 2.5 X 10^{-5} moles/kg	59.26 ± 1.40**	66.18 ± 1.93**

Significance:*P<0.05, **P<0.01

The results suggested that the standard drug Ibuprofen significantly inhibited paw oedema compared to drug vehicle, where as the methanolic extract of V.trifoliata produced significant and dose dependant effect in reducing paw oedema at the two phases of inflammation. Whereas the Chloroform extract of V.trifoliata produced significant and dose dependant effect in reducing paw oedema at the two phases of inflammation when compared to the drug vehicle treated group. The results suggested that both standard Ibuprofen and pure molecule VT-3(Quercetin) significantly inhibited paw oedema compared to drug vehicle treated group. The pure molecule exhibited almost 97% equipotency compared to standard drug treated group.

4. Conclusion

Thus, it can be concluded that doses 400 and 800 mg/kg were exhibited highly significant (p<0.01) activity, where as the dose 200 mg/kg exhibited a significant (p<0.05) effect when compared to drug vehicle treated control group. Methanolic extracts of vitex trifoliate can shows significance lesser effect as compared to the chloroform extract of vitex trifoliate. The pure molecules VT (Quercetin) from V.trifoliata at the dose of $2.5x10^{-5}$ moles/kg had shown significant (P<0.01) reduction in paw oedema, when compared to control group treated with drug vehicle.

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