Anti-inflammatory activity of ethanol extract of *Vitex glabrata* leaves

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Abstract: *Vitex glabrata* (*Verbenaceae*) is commonly employed for the treatment of various ailments in traditional medicine. In this study, ethanol extract of *Vitex glabrata* (EEVG) was evaluated for the anti-inflammatory activity using carrageenan-induced paw edema and cotton pellet induced granuloma formation in rat models. EEVG showed significant anti-inflammatory activity in rats in dose dependant manner. At a dose of 400 mg/kg, p.o. maximum effect was observed and was comparable ($p<0.05$) to that of diclofenac sodium (standard, 50 mg/kg, p.o.). Results of the study suggested that the anti-inflammatory activity of EEVG may be due to inhibition of prostaglandins synthesis and cessation of inflammatory events like fibroblast cell formation, neutrophils infiltration, and accumulation of fluids. Therefore, this study provides a support for the plant in the management of inflammatory related disorders.

Keywords: Anti-inflammatory activity; ethanol extract; *Vitex glabrata*.

INTRODUCTION

Vitex glabrata R.Br. (*Verbenaceae*), commonly known as smooth chaste tree, occurs in tropical and subtropical parts of India (Kirtikar and Basu, 1933). *V. glabrata* is used as food (Sinha & Lakra, 2005), for the treatment of post delivery bleeding, cramps, to control the foul odor of external genitalia (Nanda *et al*., 2002), gastrointestinal disorders, as anthelmintic, astringent, stomachic, sexual enhancer and in wound healing (Báthori and Pongrácz, 2005). In Thailand, it is used for the treatment of diarrhea, fever and as a tonic (Luecha *et al*., 2008). Aqueous extract of *V. glabrata* is reported to have Human Immunodeficiency Virus-1 (HIV-1) reverse transcriptase inhibitory activity (Woradulayapinij *et al*., 2005). Phytochemical investigations, so far on this species, has been limited to the report of ecdysteroids (20-hydroxyecdysone and 11-*α*,20-dihydroxyecdysone), luteolin-4′-*O*-*β*-D-glucopyranoside, luteolin-3′-*O*-β-D-glucopyranoside, fraxiresinol-4-*O*-*β*-D-glucopyranoside khainaoside A-C and 4-hydroxybenzoic acid (Luecha *et al*., 2008; Luecha *et al*., 2009; Werawattanametin *et al*., 1986).

Genus *Vitex* is reported to be rich in phenolic and flavonoid contents (Hernández *et al*., 1999). Plant phenolics are known to display wide range of activities including free radical scavenging and anti-inflammatory activities (Middleton *et al*., 2000). Hence, study was aimed to anti-inflammatory activity of the ethanol extract of *V. glabarata*.

MATERIALS AND METHODS

Plant material

V. glabarata leaves were collected from the Ayurvedic garden maintained by Tampcol, Chennai, Tamil Nadu, India in December 2000. It was authenticated by Prof. KN Dubey, Department of Botany, Faculty of Science, Banaras Hindu University, Varanasi and the specimen voucher (No.PCRL-38) has been preserved in the Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi for future reference.

Preparation of extract

Leaves of *V. glabrata* (2.0 kg) were air dried in shade and pulverized coarsely. Powdered plant material was defatted with petroleum ether (60-80ºC) for 24 h followed by extraction (soxhelet) with ethanol for 30h. The ethanol extract was concentrated under vacuum to give a semisolid mass (10.5% w/w). Qualitative determination for the presence of sterols, terpenes, and phenolics was carried out on extract using standard methods (Trease and Evans, 1989).

Estimation of total phenolic content

Total phenolic (flavonoid and non-flavonoid) content of EEVG was determined by using Folin-Ciocalteu (F-C) reagent and is expressed as gallic acid equivalent (GAE) in mg/g dry weight of extract (Vernon *et al*., 1999). In this assay, EEVG (1 ml, 1% w/v in methanol), F-C reagent (1 ml) and 9 ml of water were mixed in a 25 ml volumetric flask and incubated at room temperature for 5 min. Sodium carbonate solution (10 ml, 7% w/v) was added to the mixture and was diluted with double distilled water to 25 ml, mixture was then incubated for 1.5 h at room temperature. Absorbance of mixtures was measured at 765 nm against the blank (reaction mixture without EEVG).

Animals

Wistar albino rats (120-150 g) of either sex were purchased from M/S Asian Fauna store Varanasi. Animals

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were kept on a 12 h light/dark cycle at ambient temperature, were allowed *ad libtum* to standard diet and water. Rats were fasted overnight before experiments. Current guidelines for the 'care of laboratory animals and the investigation of experimental pain in conscious animals' were followed during the experiment (Zimmerman, 1983), and approval was obtained from the Institutional Ethical Committee.

Evaluation of anti-inflammatory activity by carrageenan induced rat paw edema model

The anti-inflammatory activity of EEVG was determined by carrageenan induced rat paw edema model (Winter *et al*., 1962). Rats were randomly divided into five groups (A-E) containing six rats in each group. In the sub-plantar region of right hind paw of rats, 0.1 ml carrageenan suspension (1% w/w prepared in nobrmal saline) was injected. One hour before carrageenan injection, Group A received oral dose of vehicle (Tween 20: Normal saline; 5:95, 1 ml.) and served as control group; group B received diclofenac sodium (50 mg/kg) and served as standard treatment group; and group C, D and E (served as test treatment groups) received EEVG 100, 200, and 400 mg/kg respectively. Increase in the volume of right hind paw was measured by plethysmometer after the injection of carrageenan at zero hour and after 1 h interval up to six hours. Anti-inflammatory activity of extracts was measured as their capacity to reduce paw edema volume with respect to control.

Evaluation of anti-inflammatory activity by cotton pellet induced granuloma formation model

Rats were randomly divided into five groups (A-E) containing six rats in each group. Diethyl ether was used to anaesthetize the rats after shaving off their fur. Implant of pre-weighed, sterile cotton pellets (10 mg) was made in the axilla region of rat. Group A received oral dose of vehicle (Tween 20: Normal saline; 5:95, 1 ml.), served as control group; group B received diclofenac sodium (50 mg/kg) and was served as standard treatment group; and group C, D and E (served as test treatment groups) received EEVG 100, 200, and 400 mg/kg respectively for seven days, commencing from day of cotton pellet insert. On eighth day, cotton pellets were removed and separated from surrounding tissues after diethyl ether anesthesia. The pellets were dried at 60ºC for 24 h and mean weight of granuloma tissue formed in control group (Wc) and treatment group (Wt) was obtained from the difference between the initial and final weights of each cotton pellet with its attached granulomatous tissue (Bailey *et al*., 1982). Anti-inflammatory activity of extracts was evaluated by determining the percentage inhibition of granuloma formation with respect to control group using following formula.

Percentage inhibition = $(1 - Wt/Wc) \times 100$

STATISTICAL ANALYSIS

Results were expressed as mean ± SEM; analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison test and were considered statistically significant, if $p<0.05$.

RESULTS

Phytochemical analysis of ethanol extract of *V. glabrata* revealed the occurrence of steroids, triterpenes, flavonoids and phenolics in abundance in the preliminary study.

Total phenolic content

Total phenolics content of EEVG was found to be 204.933 ± 5.79 mg (GAE/g) and was determined from regression equation of calibration curve ($y = 0.01x + 0.049$; $r^2 = 0.999$).

Anti-inflammatory activity

The anti-inflammatory effect of EEVG in carrageenan-induced paw edema model is summarized in table 1. It is clear that the injection of carrageenan suspension in vehicle treated control group causes substantial increase in the paw volume (0.54 ± 0.008 ml, 6 h), whereas time and dose dependent reduction in paw volume were observed in the EEVG treated rats. Maximum response was observed 3 h after the oral administration of EEVG at 400 mg/kg dose and was anti-inflammatory activity of EEVG was found to be comparable to that of the standard drug (diclofenac sodium). The effect of diclofenac sodium and EEVG on the cotton plate granuloma formation in rats is presented in table 2. A dose dependant, significant inhibition of granuloma formation was found in EEVG pretreatment rats (400 mg/kg p.o.) and effect of EEVG was found to be comparable with diclofenac sodium.

DISCUSSION

Plants of genus *Vitex* are found to be rich in phenolics (Hernández *et al*., 1999). In the prelimnary phytochemical analysis of ethanol extract of *V. glabrata* also showed abundance of phenolics. Therefore, it was thought worthwhile to estimate total content of phenolics in EEVG in order to investigate there effect on the activity of EEVG. Total content of phenolics was spectrophotometrically determined using method based on measurement of color intensity (at 765 nm) of blue-color complex formed due to reduction of F-C reagent by the phenolics. The total phenolics content of EEVG was found to be comparable to the ethanolic extract of fresh leaves of *V. negundo* (Lakshmanashetty *et al*., 2010) and was higher than that reported in fruits of *V doniana, V. kiniensis* and *V. fischerii* (Ochieng. & Nandwa, 2010).

The phenolic content of the plant represents the major group of plant constituents and function as a strong

Table 1: Anti-inflammatory effect of EEVG in carrageenan-induced paw edema method

Experiments	Edema volume (mm)					
	1 h	2 h	3 h	4 h	5 h	6 h
Vehicle	0.27±0.010	0.49±0.012	0.59±0.004	0.60±0.007	0.58±0.007	0.54±0.008
Diclofenac sodium	0.09±0.004*	0.16±0.003*	0.16±0.003*	0.13±0.004*	0.10±0.004*	0.05±0.005*
EEVG (100 mg/kg)	0.25±0.012	0.46±0.010	0.57±0.003‡	0.54±0.008‡	0.48±0.006*	0.45±0.005‡
EEVG (200 mg/kg)	0.23±0.005†	0.45±0.003‡	0.53±0.004*	0.49±0.007*	0.39±0.010*	0.36±0.003*
EEVG (400 mg/kg)	0.20±0.006*	0.40±0.004*	0.46±0.004*	0.26±0.021*	0.21±0.019*	0.19±0.018*

All the values were expressed as mean ± S.E.M (n=6), * = p<0.001, † = p<0.05 and ‡ = p<0.01 when compared with control group.

Table 2: Effect of ethanol extract of *V. glabrata* on cotton pellet induced granuloma

Group	Treatments	Dose (mg/kg p.o.)	Dry weight of granuloma (mg)	% Inhibition
A	Vehicle	-	60.35 ± 0.599	-
B	Diclofenac sodium	50	31.36 ± 1.162*	48.0
C	EEVG	100	51.98 ± 0.770*,†	13.8
D	-	200	47.27 ± 1.085*,†	21.6
E	-	400	37.07 ± 0.996*,‡	38.5

All the values were expressed as mean ± S.E.M (n=6), * p<0.001 when compared with control group, † p<0.001when compared with standard group, ‡ p<0.01when compared with standard group.

scavenger of free radicals. These are found to be involved in the etiology of various diseases i.e., cancer, diabetes, inflammatory disorders etc. and hence plant phenolics can play an important role in preventing/curing/mitigating diseases by regulating the production of free radicals in the cells (Croft, 1998; Conner & Grisham, 1996; Middleton *et al.,* 2000; Rahman *et al*., 2006). Moreover, the helpful property of these constituents is dependant on their nature, chemistry and amount present (Djeridane *et al*., 2006). Since, the study revealed the higher content of phenolic compounds in EEVG. Hence, it was thought meaningful to evaluate the anti-inflammatory activity of phenolics rich EEVG.

Inflammation is characterized by external symptoms like swelled and red colour patches on the skin which is triggered and progresses by complex mechanism involving several factors (Winyard & Willoughby, 2003). The anti-inflammatory activity of EEVG was determined by the carrageenan-induced paw edema and cotton pellet induced granuloma model in rats. These models are well accepted experimental models for the evaluation of acute and chronic anti-inflammatory activity respectively. Inflammatory reactions induced by carrageenan injection in early hour are mediated by secretion of histamine, 5-hydroxytryptamine, bradykinin. While in late hours, inflammation is due to synthesis of prostaglandins (PGs) etc. (Ferreira *et al*., 1974; Larsen & Henson, 1983; Vane & Booting, 1987). The maximum anti-inflammatory response, obtained 3h after EEVG treatment, suggested that the activity may be due to inhibition of PGs secretion or their synthesis. In many cells, PGs are formed due to arachidonic acid metabolism catalyzed by the cyclooxygenase (COX) enzymes. It was found that COX-2 is involved in the inflammation through various stimuli (Seibert *et al*., 1994). Hence, anti-inflammatory activity of EEVG may be due to the inhibition of COX enzymes.

The subcutaneous implantation of cotton pellet stimulated the host inflammatory responses, consequently inducing the release of inflammatory mediators and leading to tissue proliferation and granular formation. This granuloma is separated from the local tissues; cells infiltration, fibroblast cells formations and accumulation of proteins and fluid leads to an increase in weight of implanted cotton pellet. This model is often used to evaluate the effect on exudates and proliferative components of chronic inflammation (Bailey *et al*., 1982; Singh *et al*., 2009). Findings of this study revealed that EEVG inhibited the formation of granuloma. Hence, it may be insinuated that EEVG may be capable to cessation the inflammatory events like fibroblast cell formation, neutrophils infiltration and accumulation of fluids.

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