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Preliminary screening of *Waltheria indica* (L.) plant for its anti-inflammatory activity

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Abstract

The investigation on anti-inflammatory activity of the various extract of *Waltheria indica* L. was reported to find out the pharmacological basis for its ethnomedical use. The anti-inflammatory activity of the pet ether (PEW) and methanol (MEW) extracts of the leaves of *Waltheria indica* L. (Malvaceae)were evaluated by using in vivo (Carrageenan & histamine induced rat paw edema, cotton pellet granuloma test) models. It was observed that, all the extracts showed significant activity in the in-vivo model at the dose of 500 mg/kg b.w. orally, when compared with control and standard drugs. Of the two extracts tested, methanol extract MEW showed most significant activity well in comparison to the standard drug. Therefore, present study suggests, potential of leaves of *Waltheria indica* L. in both models of acute and chronic inflammation.

Key words: Anti-inflammatory, *Waltheria indica* L., Delayed type hypersensitivity

Introduction

Inflammation is part of the complex biological response of body tissues. It is an attempt of self-protection; the aim being to remove harmful stimuli, including damaged cells, irritants, or pathogens - and begin the healing process. Inflammation does not mean infection, even when an infection causes inflammation. Infection is caused by a bacterium, virus or fungus, while inflammation is the body's response to it. Inflammation involves liberation of chemical mediators that include histamine, 5-HT, bradykinin and eicosanoids [1]. The Greek term for inflammation was *phlegmone*, "the fiery thing" (phlox = flame) [2]. This process increases the blood flow to the areas of injury or infection and thereby stimulates nerves and cause pain [3].

Waltheria indica is a woody plant with a hard or annual stump(4). The plant is wide spread in subtropical and tropical regions [4].*Waltheria indica* L. commonly known as “Sleepy morning” belong to the family Sterculiaceae [5]. Although plantgrowingin various regions of the world, shrub native to India, Hawaii, North America and Africa. It has erect, branching herbs or under shrubs; densely stellate-tomentose all over. Leaves 2-5 x 1-2.5 cm, elliptic-ovate to oblong, base cordate, margin serrate, apex rounded, venation impressed above and prominent beneath; petiole to 2.3 cm long. Flowers in axillary and terminal subsessile clusters. Calyx tube 2-4 mm long, campanulate; lobes 5. Petals 5, yellow, 4-5 mm long, obovate-obtuse. Stamens 5, connate below. Ovary villous, 1-celled; ovules 2; style c.1.5 mm long; stigma penicillate. Capsule 3-4 x 2 mm, obovoid, 2-valved, enclosed in calyx. Seed 1, c. 2 mm long, obovoid. *Waltheria indica* possess therapeutic potential in the treatment of malaria [6], infectious diseases(5), (e.g., lungs infection due to *Klebsiella pneumonia*, diarrhea due to *Candida albicans* or *Escherichia coli* [7], prevention of oxidative stress [8] and as analgesic (5).Further studies are necessary to explore phytochemicals responsible for the pharmacological effects and the mechanisms of action. The present study was undertaken to investigate the possible anti-inflammatory activity of all successive extracts of *Waltheria indica* L.

Experimental

Plant material

The plant material was collected from the villagepachora of Dist. Jalgaon, Maharashtra, in the month of September and was authenticated by Dr. D.A.Patil, Taxonomist, Department of Botany, S.S.V.P.S College of Science, Dhule, Maharashtra India.

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Preparation of extract

The aerial parts of *W. indica* were dried in air, powdered using pulverizer and passed through sieve no.40. The successive extraction of power was done by soxhlet apparatus with pet ether (60-80%) followed by methanol (95%), filtered and concentrated in vacuum evaporator. The dried extracts were then collected and preserved in desiccator before use. Pet ether extract (PEW) was emulsified using tween 80 and other extract were prepared in aqueous base for in vivo studies.

Animals

Male wistar rats weighing 180-220 g and swissalbino mice of both sexes weighing 15-22g were used throughout these experiments. They were housed in standard cages (six animal per cage) at temperature of 22±2^{o}C and 12:12 h light dark cycle. The animals were provided with pellet diet and water *ad libitum*. The study was approved by Institutional Animal Ethical Committee (IAEC) of Sri Satya Sai University of Technology & Medical Science, Sehore, MP, India, registered under CPCSEA.

Acute Toxicity study

Seventy rats and mice were divided into seven groups of ten animals each. One group served as control and received 0.9% NaCl alone (10ml/kg) given intraperitoneally (i.p.), while the remaining six groups were treated with increasing doses of the methanolic extract dissolved in water; 100, 500, 1000, 1500, and 2000 and 5000 mg/kg (i.p.), respectively. The mortality rate within a 24 h period was determined and the $LD_{50}$was estimated according to the method described by Miller and Tainter [9,10]. with slight modifications. According to the result of acute toxicity test, the doses of 250 and500 mg/kg were chosen for experiments.

Carragenan induced rat paw edema

The anti-inflammatory activity of various extracts of *W. Indica* on Carragenaninduced rat paw edema was determined according to Winter et al. and various researchers with slight modifications [11-14].Wistar albino rats with a body weight between 180-200 gm were divided into six groups (n=6), the animals were starved overnight before the experiment; the rats received water *ad libitum*. Control group received saline, the standard group received Diclofenac sodium(5 mg/kg) and the test groups received various extracts of *W. Indica* (MEW and PEW) at the dose of 250 and 500 mg/kgp.o. Thirty minutes after the administration of various substances, edema was induced by injecting 0.1 ml of 1% carrageen an (Sigma Chemical Co.) in distilled water into the sub-planetor tissue of the left hind paw of each rat.. The paw volume was measured at 0,60,120 and 180 min using the plethysmometer (UgoBasile, Italy). The percentage inhibition of paw volume in extract-treated groups was compared with the control group (treated with vehicle).

Cotton pellet granuloma test

Extracts of *W. Indica* (PEW,MEW) in chronic inflammation was studied using cotton pellet-induced granuloma test [12-16]. Wister albino rats with a body weight between 180-200 gm were divided into six groups (n=6), Cotton pellets weighing 15±1 mg were autoclaved and implanted subcutaneously into both sides of the groin region of each rat. Control group received the normal saline, 5 ml/kg p.o. The extract, MEW and PEW at concentration of 250 and 500 mg/kg was administered orally for 7 days. Standard treated group received diclofenac (10 mg/kg)p.o. for the same period. On the 8th day, the animals were sacrificed and the pellets together with the granuloma tissue were carefully removed and dried in an oven at 60^{0}C. The dry pellets were weighed and the mean weight of the granuloma tissue formed around each pellet was determined. The level of inhibition of granuloma tissue development was calculated using the relation:

$$\text{Percent inhibition} = \frac{(Tc - Tt)}{Tc} \times 100$$

Where Tc = weight of granuloma tissue of control group
Tt = weight of granuloma tissue of treated group

Statistical analysis

All data were represented as mean± SEM and as percentage. Results were statistically evaluated using ANNOVA followed by multiple comparison Dunnette's test. $P < 0.05$ was considered significant.

Results

Effect of Carragenan induced rat paw edema

Various extracts of *W. Indica* (PEW and MEW)aerial parts significantly reduced the rat paw edema induced by carrageen an by 54% and 59% at a higher dose of 500mg/kg,3 hours after the injection of noxious agent. It was observed that themethanolic extract (MEW) at the higher dose of 500 mg/kgexhibited comparable results with that of standard diclofenac sodium which inhibited the rat paw edema by 62% (Table1).

Table-1a: Mean increase in paw volume in Carrageenan induced acute inflammation

Group	Dose (mg/kg)	Mean increase in paw volume (ml)		
		1hr	2hr	3hr
Negative Control	----	0.48 ± 0.04	0.76 ± 0.10	1.12 ± 0.03
Diclofenac sodium	10	0.20 ± 0.01	0.24 ± 0.02	0.38 ± 0.05
PEW250	250	0.30 ± 0.06	0.45 ± 0.03	0.49 ± 0.09
PEW500	500	0.29 ± 0.03	0.41 ± 0.08	0.42 ± 0.01
MEW250	250	0.25 ± 0.04	0.39 ± 0.01	0.84 ± 0.03
MEW500	500	0.22 ± 0.07	0.30 ± 0.02	0.69 ± 0.04

Values are expressed as Mean ± SEM, n=6
Data analyzed by One-way ANOVA followed by Dunnette's test
Figures in parentheses indicate the % anti-inflammatory activity, ** P < 0.01, * P < 0.05
MEW (*W. indica* methanol extract), PEW (*W. indica*pet.ether extract)

Effect of Cotton pellet granuloma test

Various extracts of *W. Indica* (PEW and MEW)aerial parts showed significant (P<0.01) anti-inflammatory activity in cotton-pellet induced granuloma by inhibiting the formation of fibroblast by 22% and 38% at a higher dose of 500 mg/kg by PEW and MEW extracts respectively. It was observed that methanolic extract (MEW) exhibited comparable results with that of standard diclofenac sodium which showed an inhibition of 44% (Table 2).

Table-2: Percent inhibition in cotton pellet-induced granuloma in rats

Group	Dose (mg/kg)	Weight of dried cotton pellet (mg)	% Inhibition
Control	Normal Saline (10ml/kg)	64.58± 2.898	--
Diclofenac sodium	5	35.96±2.19**	44.32
PEW	250	40.70±1.66**	35.38
	500	50.53±1.32**	22.84
MEW	250	44.29±1.92**	36.53
	500	38.23±2.16**	38.17

Values are expressed as Mean ± SEM, n=6
Data analyzed by One-way ANOVA followed by Dunnette's test
Figures in parentheses indicate the % anti-inflammatory activity, ** P < 0.01, * P < 0.05
MEW (*W. indica* methanol extract), PEW (*W. indica*pet.ether extract)

Discussion

The overall results showed that the methanolic extract (MEW) from *W. indica,* aerial parts exhibited a significant anti-inflammatory activity against animal models in mice and rats induced by various factors. Since it is evident that carrageenan induced edema is commonly used as an experimental model for acute inflammation and is believed to be biphasic, of which first phaseis mediated by the release of histamine and 5-HT in the early stage followed by kinin release and then prostaglandin in the latter phase(1). The significant ameliorative activity of the extracts observed in the present study may be due to inhibition of the mediators of inflammation such as histamine, serotonin and prostaglandin.

W. indica significantly (P< 0.01) inhibited carrageenan induced rat paw edema. These results suggested *W. indica* play an important role in acute phase of inflammation and its action was partly related to histamine. So it may be suggested that its anti-inflammatory activity is possibly backed by its anti- 5-HT activity which is responsible for the same.

Proliferation of fibroblasts as well as multiplication of small blood vessels is an indication of repair phase of inflammatory process. Such proliferating cells produce a highly vascularised and red mass known as granulation tissue [16]. Granuloma of chronic inflammation comprises an accumulation of modified macrophages arranged in small clusters or nodular collections or surrounded by a cuff of lymphocyte [14-17]. Investigation of various extract revealed that the methanolic extract exerted significant (P<0.01) effect on the granulomatous inflammation. Other extract also showed significant anti-inflammatory activity in the chronic model of cotton pellet induced granuloma model which reflected its efficacy to inhibit the increase in the number of fibroblast and synthesis of collagen and muco-polysaccharides during granuloma model tissue formation(1).

Phytochemical investigation of methanolic extract (MEW) extract revealed the presence of saponins (both steroidal and triterpenoidal) and sapogenin (aglycone) like oleonolic acid [18]. While the pet ether extract (PEW) was found to possess β-

sitosterol and lupeol. Saponins are known to possess anti-inflammatory activity. Triterpenic acid like oleanolic acid is knownfor its anti-inflammatory activity [19,20]. β-sitosterol and lupeol are also known for their action on inflammation [12-14]. Thus the significant anti-inflammatory activity may be due to saponins and sapogenins present in the extract.

Based on the results it can be concluded that the anti-inflammatory property of the aerial parts of *W. indica* is mainly due to the presence of saponins and steroids like β-sit sterol, which in part seems to be connected to its protective action on inflammatory mediators. The results helped in giving pharmacological evidence to the ethno medical claims of *W. indica*. It can also be concluded that *W. Indica* could be a good candidate for the development of new anti-inflammatory drug.

References

[1]. Mazumder U, Gupta M, Manikandan L, Bhattacharya S, Haldar P, Roy S. Evaluation of anti-inflammatory activity of Vernonia cinerea Less. extract in rats. Phytomedicine. 2003;10(2):185-8.

[2]. Ryan GB, Majno G. Acute inflammation. A review. The American journal of pathology. 1977;86(1):183.

[3]. Vogel HG, Vogel WH. Drug Discovery and Evaluation: Pharmacological Assays: Springer Berlin Heidelberg; 2013.

[4]. Zongo F, Ribuot C, Boumendjel A, Guissou I. Botany, traditional uses, phytochemistry and pharmacology of *Waltheria indica* L. (syn. Waltheria americana): A review. Journal of Ethnopharmacology. 2013;148(1):14-26.

[5]. Olajuyigbe O, Babalola A, Afolayan A. Antibacterial and phytochemical screening of crude ethanolic extracts of *Waltheria indica* Linn. African Journal of Microbiology Research. 2011;5(22):3760-4.

[6]. Rao YK, Fang S-H, Tzeng Y-M. Inhibitory Effects of the Flavonoids Isolated from *Waltheria indica* on the Production of NO, TNF-. ALPHA. and IL-12 in Activated Macrophages. Biological and Pharmaceutical Bulletin. 2005;28(5):912-5.

[7]. Mathabe M, Nikolova R, Lall N, Nyazema N. Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. Journal of ethnopharmacology. 2006;105(1):286-93.

[8]. Rao YK, Geethangili M, Fang S-H, Tzeng Y-M. Antioxidant and cytotoxic activities of naturally occurring phenolic and related compounds: a comparative study. Food and Chemical Toxicology. 2007;45(9):1770-6.

[9]. Miller L, Tainter M. Estimation of EC50 and its error by means of log. Probit Graph. Paper Proc Soc Exp Biol Med. 1944;26:1-9.

[10]. Franzotti E, Santos C, Rodrigues H, Mourao R, Andrade M, Antoniolli A. Anti-inflammatory, analgesic activity and acute toxicity of Sida cordifolia L.(Malva-branca). Journal of Ethnopharmacology. 2000;72(1):273-7.

[11]. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Experimental Biology and Medicine. 1962;111(3):544-7.

[12]. Agarwal R, Rangari V. Antiinflammatory and antiarthritic activities of lupeol and 19 alpha-H lupeol isolated from Strobilanthus callosus and Strobilanthus ixiocephala roots. Indian Journal of Pharmacology. 2003;35(6):384-7.

[13]. Ahn E-K, Jeon H-J, Lim E-J, Jung H-J, Park E-H. Anti-inflammatory and anti-angiogenic activities of Gastrodia elata Blume. Journal of ethnopharmacology. 2007;110(3):476-82.

[14]. Okoli CO, Akah PA. Mechanisms of the anti-inflammatory activity of the leaf extracts of Culcasia scandens P. Beauv (Araceae). Pharmacology Biochemistry and Behavior. 2004;79(3):473-81.

[15]. Srimal R, Dhawan B. Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti-inflammatory agent. Journal of pharmacy and pharmacology. 1973;25(6):447-52.

[16]. Fang QM, Zhang H, Cao Y, Wang C. Anti-inflammatory and free radical scavenging activities of ethanol extracts of three seeds used as “Bolengguazi”. Journal of Ethnopharmacology. 2007;114(1):61-5.

[17]. Okoli C, Akah P, Nwafor S, Anisiobi A, Ibegbunam I, Erojikwe O. Anti-inflammatory activity of hexane leaf extract of Aspilia africana CD Adams. Journal of ethnopharmacology. 2007;109(2):219-25.

[18]. Farooq M, Varshney I, Khan MS. Saponins and Sapogenins IV: Isolation of Oleanolic Acid from Sesbania Aegyptica Pers. Journal of the American Pharmaceutical Association (Scientific ed). 1959;48(8):466-8.

[19]. Giner-Larza EM, Máñez S, Recio MC, Giner RM, Prieto JM, Cerdá-Nicolás M, et al. Oleanonic acid, a 3-oxotriterpene from Pistacia, inhibits leukotriene synthesis and has anti-inflammatory activity. European journal of pharmacology. 2001;428(1):137-43.

[20]. Kim K-A, Lee J-S, Park H-J, Kim J-W, Kim C-J, Shim I-S, et al. Inhibition of cytochrome P450 activities by oleanolic acid and ursolic acid in human liver microsomes. Life sciences. 2004;74(22):2769-79.