

Anti-inflammatory, analgesic and anti-lipid peroxidative properties of *Wattakaka volubilis* (Linn.f.) Stapf.

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Abstract

The ethanolic extract of *Wattakaka volubilis* (Linn.f.) Stapf. was screened for its anti-inflammatory and analgesic effects in animals. The extract showed a significant inhibition of carrageenan-induced rat paw edema and acetic acid-induced writhing in mice compared to the standard anti-inflammatory and analgesic drug, indomethacin. The extract also showed potent *in vitro* inhibition of FeCl₂-ascorbic acid-stimulated mice liver lipid peroxidation.

Keywords: *Wattakaka volubilis*, Anti-inflammatory, Analgesic, Lipid peroxidation, Indomethacin

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Wattakaka volubilis

Introduction

The importance of medicinal plants in traditional health care practices, providing clues to new areas of drug research and biodiversity conservation is now well recognized. Inflammation is a complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells and irritants. It is the protective attempt by the organism to remove the injurious stimuli as well as initiate healing process for the tissue and considered to be the major cause of rheumatoid arthritis. Drugs currently used for management of pain and inflammatory conditions present toxic side effects on chronic administration. Therefore, attempts are being taken to study promising plants which may lead to develop newer or safer drugs¹.

Wattakaka volubilis (Linn.f.) Stapf. (Family—Asclepiadaceae) is a large climber with green flowers in

drooping umbels, with smooth bark and ash-coloured, leaves rounded at the base. It is found in India and South East Asia. The root is applied to snake bites and given to women to cure headache after child birth and the leaves are applied to boils and abscesses to promote suppuration. It is emetic, diaphoretic and diuretic². Traditional healers of Kerala use its leaves to treat inflammatory and painful conditions³. However, till date no scientific validation of these properties has been reported. Hence, the present study was taken up to investigate the anti-inflammatory, analgesic and lipid peroxidation properties of leaves of this plant.

Materials and Methods

Plant material

The plant of *W. volubilis* were collected from Kollam, Kerala and identified with the help of taxonomist.

Some authors have treated name of this plant as a synonym for *Dregea volubilis* (Linn.f.) Benth. ex Hook.f., but we have followed some floras available in this area. Voucher specimens of the plant were deposited at the herbarium of the Institute (TBGT 57027 dated 15 May 2007).

Preparation of plant extract

The leaves were separated from the plant and washed, shade-dried and powdered. The powder (100g) was extracted with 1000 ml of 95% ethanol overnight in a conical flask, at room temperature on a shaker. The ethanolic extract was finely filtered and the solvent was evaporated completely on a rotary evaporator. The yield of the crude extract

obtained was 5.62g. It was referred to as WV. For administration orally, the crude extract WV was suspended in 0.5% Tween-80 to concentrations of 250 and 500 mg/kg.

Experimental animals

Wistar rats (110-340g, fasted) and Swiss albino mice (20-35g), of either sex were used and grouped and housed in poly-acrylic cages (six animals per cage) and maintained under standard laboratory conditions (temperature 24-28°C, relative humidity 60-70% and 12 h dark-light cycles). They were fed commercial rat feed (Lipton India Ltd, Mumbai, India) and boiled water was given *ad libitum*. All animal experiments were carried out according to NIH guidelines, after getting the approval of the Institute's Animal Ethics Committee (Registration No 25-1/99/AWD 176/ CPCSEA dtd 29/09/1999).

Carrageenan-induced rat paw edema

Edema was induced in rats according to the method of Winter *et al* (1962)⁴. Briefly, 0.1ml of 1% carrageenan (Sigma Chemical Company, USA) was injected into the right hind paw, under the plantar aponeurosis (Group1-carrageenan controls). In a separate group of animals (Group-2), indomethacin (5mg/kg) was administered orally. The plant extract in two doses (250 and 500mg/kg) was administered orally to the animals of Group-3 and 4, 30 min before carrageenan injection. The hind paw volume was measured plethysmographically just before and three hours after carrageenan administration. The difference in left and right paw volumes indicated the degree of inflammation. The anti-inflammatory activity of the plant

extract was estimated as the degree of edema inhibition.

Acetic acid - induced writhing assay

Analgesic responses were assessed by the method of Koster *et al* (1959)⁵. Swiss albino mice were divided into 4 groups, each group containing 6 animals. Control group (Group1) received a single dose of 0.5% Tween-80 (0.5ml) orally. The toxin control group (Group 2) was administered with a single dose of 0.5 ml indomethacin orally. Groups 3 and 4 received a single dose of WV at two concentrations (250 and 500mg/kg). After 20 min, 0.5% acetic acid (0.25ml) was administered intraperitoneally to all the groups. The number of writhes per animal was counted for 30 min, 5 minutes after treatment with acetic acid.

Anti-lipid peroxidation studies

Anti-lipid peroxidant effect of WV was studied *in vitro* following the modified method of Yoshiuki *et al* (1981)⁶ and Masao *et al* (1993)⁷. Briefly, 0.5g of the rat liver tissue was sliced and homogenized with 10ml of 150 mM KCl-Tris-HCl buffer (pH 7.2). Then, 0.25 ml of liver homogenate was taken and added in control, induced and sample tubes. This was followed by addition of 0.15ml Tris HCl buffer (pH 7.2), 0.05ml of 1 mM ascorbic acid (AA), and 0.05 ml of 4mM FeCl₂ to the induced tubes and also 0.025, 0.05 and 0.1ml of WV extract was taken in the sample tube. The mixture was incubated at 37°C for 1h in capped tubes. Afterwards, 0.5ml of 0.1N HCl, 0.2 ml of 9.8% sodium dodecyl sulphate (SDS), 0.9 ml of distilled water and 2ml of 0.6% thiobarbituric acid (TBA) were added to

each tube and the tubes were vigorously shaken. Following this, all the tubes were placed in a boiling water bath at 100°C for 30 min. After cooling, the flocculent precipitate was removed by adding 5ml of n-butanol and was mixed well. They were centrifuged at 1500rpm for 20 minutes. The supernatant was collected and the absorbance was measured at 532 nm.

Behavioural and toxic effects

Three groups of 10 mice were administered orally, 500, 1000 and 1500 mg/kg of WV. They were observed continuously for 1h for any gross behavioural changes, symptoms of toxicity and mortality, if any and intermittently for the next 6 h and then again, 24h after dosing with extract.

Statistical analysis

The analysis was carried out using the Students 't'-test (Snedecor and Cochran., 1980)⁸. Results were reported as mean \pm S.D. and the 't' test was used to evaluate difference between groups with $P \leq 0.01$, considered as significant.

Results

Anti-inflammatory activity

WV at both the doses used in the study (250 and 500mg/kg) significantly inhibited the carrageenan-induced paw edema in rats. At 250mg/kg dose, there was 75% inhibition and at 500mg/kg dose, 83.33% inhibition was obtained after 3h of carrageenan injection. Indomethacin (5mg/kg) produced 83.33% inhibition of edema formation (Table 1)

Analgesic activity

WV at both the doses used in the

Table 1: Effect of ethanolic extract of *Wattakaka volubilis* (WV) leaves and indomethacin on carrageenan-induced paw edema in rats

Treatment	Oral dose (mg/kg)	Differences in paw volume at 3 h. (ml)	Percentage inhibition of edema (%)
Carrageenan control	—	0.60 ± 0.02	—
WV	250	0.15 ± 0.01**	75.00
WV	500	0.10 ± 0.02**	83.33
Indomethacin	5	0.10 ± 0.01**	83.33

Values are the mean ± S.D, n = 6. **P≤0.01, compared to carrageenan control

Table 2 : Effect of ethanolic extract of *Wattakaka volubilis* (WV) leaves extract and indomethacin on acetic acid-induced writhing response in mice

Treatment	Oral dose (mg/kg)	Mean number of writhes in 30 min	Percentage inhibition of writhing (%)
Acetic acid control	—	48.00 ± 2.00	—
WV	250	9.00 ± 5.00**	81.25
WV	500	7.00 ± 2.00**	85.42
Indomethacin	5	6.00 ± 1.00**	87.5

Values are the mean ± S.D, n = 6, ** P≤0.01, compared to acetic acid control

Table 3: Inhibitory effect of *Wattakaka volubilis* (WV) on FeCl₂-ascorbic acid (AA) -induced lipid peroxidation in rat liver homogenate *in vitro*

Group	Concentration µg/ml)	MDA	MDA inhibition (%)
Normal control	—	0.782 ± 0.01	—
FeCl ₂ -AA control	—	2.460 ± 0.09	—
FeCl ₂ -AA +WV	50	0.635 ± 0.03**	74.19
FeCl ₂ -AA + WV	100	0.654 ± 0.05**	73.41
FeCl ₂ -AA +WV	150	0.667 ± 0.07**	72.89

Values are the mean ± S.D, n = 3. **P≤0.01 compared FeCl₂ control

study significantly inhibited acetic acid-induced writhing response in mice, dose dependently. Writhing response inhibition at the dose of 250 mg/kg was

81.25%. The extent of writhing response inhibition at 500mg/kg dose was 85.42%. Indomethacin, the positive control used in the study, which is a known analgesic

agent produced an inhibition of 87.5% (Table 2).

In vitro anti-lipid peroxidation effects

WV at 50µg/ml showed the highest percentage of inhibition of FeCl₂-AA stimulated rat liver lipid peroxidation *in vitro*. There was a significant increase in malondialdehyde (MDA) in FeCl₂-AA treated rat liver homogenate, compared to the normal one without FeCl₂-AA (Table 3). It was found to be effective in decreasing MDA production *in vitro* in rat liver homogenate treated with FeCl₂-AA treated mixture showing its antilipid peroxidant effect. There was decreased inhibition of MDA with increased concentration of WV beyond 100µg/ml.

Toxicity study

In the toxicity study, no mortality occurred within 24h with the 3 doses of WV tested. The LD₅₀ was therefore, greater than 1500mg/kg p.o in mice (data not shown).

Discussion

Although several agents are known to treat chronic inflammatory diseases, prolonged use of these agents should be avoided due to serious or adverse side effects. Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects. Plants are used for treating various diseases like rheumatism, fever, infection, edema, etc. Analgesic drugs available in the market today also exert a wide range of side effects. The study of plant species traditionally used as pain killers should still be seen as a logical and fruitful research strategy, in search of analgesic

drugs⁹⁻¹². Inflammation is a complex process and various mediators e.g. prostaglandin, leucotrienes, platelet activating factor, etc. have been reported to be involved in the development of inflammatory diseases.

The results of present study revealed that the time course of edema development in carrageenan induced paw edema models in rats is generally represented by a biphasic curve⁴. The first phase occurs within an hour of injection and is partly due to the trauma of injection and also due to the serotonin component¹³. Prostaglandins (PGs) play a major role in development of the second phase of reaction which is measured around 3h time¹⁴. The presence of PG in the inflammatory exudates from the injected foot can be demonstrated¹⁵. Carrageenan-induced paw edema model is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit the enzyme, cyclooxygenase involved in prostaglandin synthesis¹⁶. Based on these reports, it can be inferred that the inhibitory effect of WV on carrageenan-induced inflammation in rats could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis.

Acetic acid-induced writhing response in mice is a simple, rapid and reliable model to evaluate peripheral type of analgesic action of herbal and other drugs¹⁷. The present study showed that the plant possessed potent analgesic property. The abdominal constriction is related to the sensitization of nociceptive receptors by prostaglandins. It is, therefore, possible that the plant exerts analgesic effect

probably by inhibiting synthesis or action of prostaglandins.

WV showed significant *in vitro* lipid peroxidation in rat liver homogenate. It is evident that non-enzymatic lipid peroxidation occurs during the experimental inflammation in rats. Lipid peroxides may be pro-inflammatory and can damage the tissues directly¹⁸. Protection against free radical lipid peroxidation by plant extracts is of great significance for their traditional use against inflammatory disorders, many of which are associated with membrane damage and tissue recovery¹⁹. Lipid peroxidation results in mitochondrial swelling and disintegration²⁰. Disintegration of lysosomes has been correlated with the peroxidative decomposition of lysosomal lipids²¹. It can, therefore, be concluded from the present study that the beneficial effects of WV may be from its role in stabilization of lysosomes and its antioxidant activities. The combination of anti-inflammatory and analgesic effects of WV indicates the likelihood of intervention of prostaglandin synthesis as prostaglandins have been established as a common mediator in all these responses. However, this possibility needs to be investigated in detail. WV is non-toxic which is supported by its extensive use in traditional medicine of Kerala to treat inflammatory and painful conditions.

Phytochemical reports on WV have shown the presence of glucosides and alkaloids in roots which are considered to produce significant antioxidant, anti-inflammatory and analgesic effects^{22, 23}. Further studies are warranted on these lines to pinpoint the chemicals and their exact mechanism of action.

Conclusion

It can be concluded from present study that *W. volubilis* leaf extract can be used for the development of a herbal drug for anti-inflammatory and analgesic conditions and warrants further studies to decipher its exact mechanism of action.

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