

Bronchoprotective, Bronchodilatory and Anti-Inflammatory Activity of Ethanolic Extract from *Woodfordia fruticosa* (Kurz.) Flowers

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ABSTRACT

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The present investigation was aimed to investigate the bronchoprotective, bronchodilatory and anti-inflammatory effect of ethanol extract of *Woodfordia fruticosa* dried flower (WF-EE). WF-EE exhibited significant and dose dependent bronchoprotective, bronchodilatory and anti-inflammatory effect. WF-EE at 200 mg/kg prolonged the latent period and exhibited protection against experimental asthma induced by the combination of histamine and acetylcholine aerosol in guinea pigs. WF-EE (1 mg/ml) exhibited 100 % bronchodilation (complete bronchorelaxation) at concentrations 1.6 and 3.2 mg against acetylcholine and histamine induced contraction respectively. Administration of extract (100 or 200 mg/kg, p.o.) to rats showed significant inhibition of carrageenan as well as egg albumin induced paw edema. The anti-inflammatory effect of extract at 200 mg/kg was comparable to standard drug indomethacin (10 mg/kg, p.o.). Moreover, WF-EE has not shown any toxicity symptoms and mortality up to 2000 mg/kg, p.o. in rats, when evaluated for the acute toxicity study of WF-EE as per OECD guidelines. These results suggest the anti-asthmatic (bronchoprotective as well as bronchodilatory) and anti-inflammatory potential of WF-EE along with safety margin for oral administration.

Keywords: *Woodfordia fruticosa*, asthma, bronchodilation, guinea pig trachea, anti-inflammatory, carrageenan, egg-albumin.

INTRODUCTION

Woodfordia fruticosa (Kurz), family Lythraceae is a straggling leafy shrub, distributed abundantly throughout the India, as well as in a majority of the East Asian countries¹. In India, different parts of *Woodfordia fruticosa* (WF) are commonly used in traditional systems of medicines like Ayurveda and Unani². The traditional and pharmacological claims of WF dried flowers can be ascribed to its important bioactive phytoconstituents such as different types of tannins³⁻⁶, sterols, anthroquinones, saponins and flavonoids^{7,8}.

Traditional reports indicate use of dried WF flowers as an astringent tonic in the disorders of mucus membrane, paste of WF flowers is also claimed to be used in the central Indian province for the treatment of cough, which point towards its possible bronchoprotective effect. In addition, WF flowers are extensively used by tribal people for its wound, ulcer healing, analgesic and anti-rheumatic properties⁹⁻¹¹. Preclinical data from various studies indicates that, dried WF flower extract possess antipyretic, anti-inflammatory effect in

cotton pellet induced granuloma model¹², antitumor^{13, 14}, antiviral¹⁵, immunomodulatory¹⁶, antifertility¹⁷ and antibacterial¹⁸ activity. All these reports suggest the potential of WF flowers as an important pharmacological target for further investigation.

However, there was no experimental reports apropos to anti-asthmatic (bronchoprotective and bronchodilatory) effect of WF flower extract. Therefore, the present investigation was undertaken to evaluate bronchoprotective effect against acetylcholine and histamine aerosol induced bronchospasm, bronchodilatory effect using histamine or acetylcholine induced contraction on isolated guinea pig tracheal chain¹⁹. Moreover, the inflammatory conditions are important factors in aggravation of asthma; hence anti-inflammatory potential of WF-EE was examined in the acute models of inflammation induced by carrageenan²⁰ or egg albumin²¹ in rats. The present investigation demonstrated significant bronchoprotective, bronchodilatory and anti-inflammatory effect of WF-EE.

MATERIALS AND METHODS

Collection and extraction of WF flowers

WF flowers were collected from the outskirts of Nagpur Dist., Maharashtra, India and authenticated from Botanical Survey of India (BSI), Pune, Maharashtra, India. Reference No.

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BSI/WC/Tech.12008/ 272, authentication No., MHG-1. Shade dried flowers were coarsely powdered (500 g), defatted using petroleum ether (60-80 °C) and extracted exhaustively with ethanol (95%) using Soxhlet apparatus. Obtained extract was collected, filtered through Whatman filter paper (No. 42) and concentrated over water bath to obtain sticky extract (31.28 g) referred as WF-EE.

Chemicals

Histamine, acetylcholine and carrageenan were purchased from Hi-Media, Mumbai, India. Carboxy methyl cellulose (CMC), solvents for extraction; petroleum ether (60-80 °C) and ethanol were procured from Loba Chemicals, Mumbai, India. Indomethacin and aminophylline were generously gifted by Wokhardt Pharmaceuticals, Aurangabad, India. All other chemicals used in the present investigation were of AR grade and purchased from Loba Chemicals Mumbai, India.

Animals

Albino rats (200-250 g) and guinea pigs (300-400 g) were kept under standard 12:12 h light/dark cycle in a temperature-controlled (24 ± 1 °C) environment with *ad libitum* access to rodent chow (Lipton, India) and water. Experimental protocols were approved by Institutional Animal Ethical Committee (IAEC) Constituted for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) by Ministry of Environment and Forests, Government of India, New Delhi (IAEC approval No. 536/02/C/CPCSEA).

EXPERIMENTAL

Acute toxicity study:

The limit dose test of up and down system according to OECD/OCDE test guidelines on acute oral toxicity (No. 423) was studied at a limit dose of 2 g/kg body weight (p.o.) for WF-EE²². Three rats were selected (one male and two female) such that the weight differences do not exceed ± 10% of the mean initial weight of the sample population. The rats were fasted for food, over-night with free access to water prior to administration of WF-EE (2 g/kg), suspended in 1.0 %, w/v CMC and the access to food was reinstated after 3-4 h. Thereafter, individual rat was observed after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h and daily for a total of 14 days. The rats were observed for systemic and behavioral toxicity patterns as described in OECD/OCDE test guidelines. At the end of toxicity study, all surviving animals were sacrificed.

Effect of WF-EE on Histamine and acetylcholine induced bronchospasm in guinea pigs (Evaluation of bronchoprotective effect)¹⁹

Guinea pigs of either sex were kept fasting for 12-24 h before

the commencement of experiment and only water was provided *ad libitum*. To screen the sensitivity and suitability, animals were placed in a plexiglass chamber (histamine chamber) and challenged with mixture of equal volume of 0.1% histamine hydrochloride and 2% acetylcholine chloride, under the average pressure of 450±50 mmHg for 15 sec. The time to onset of respiratory distress (preconvulsive time) during the challenge was measured, the guinea pigs were considered to be insensitive and discarded with preconvulsive time of more than 120 sec. The adequate and sensitive guinea pigs were randomly allotted in control, standard and treatment groups (n=4). Animals were grouped as Group I (vehicle, 10 ml/kg) Group II (Aminophylline; AMN 10 mg/kg), Group III (WF-EE 100 mg/kg) and Group IV (WF-EE 200 mg/kg). The animals of negative control group administered distilled water orally and the positive control group was administered with aminophylline (10 mg/kg) by gastric perfusion, the other groups were treated with 100 and 200 mg/kg doses of WF-EE respectively. All animals in treatment and standard groups were treated with a single dose daily for 3 days prior to the challenge; the last dose was administered 1 h before the bronchial challenge. The delitescence of convulsion (latency period) and tumble numbers for each animal during challenge (within a 6 min exposure period of aerosol) were recorded. Aerosol provoked a bronchospastic reaction in all animals within three minutes. The delay in the appearance of the bronchospastic reaction was considered as bronchoprotective effect. Protection from convulsion was expressed relative to control.

$$\text{Percentage protection} = [1 - (T_1/T_2)] \times 100$$

Where,

T₁- preconvulsive breathing time (sec) in control group

T₂ - preconvulsive breathing time (sec) after the administration of test compound/ standard drug

Effect of WF-EE on the spasmogen induced contraction on guinea-pig isolated trachea:

Bronchodilatory effect of WF-EE was evaluated as described by Vogel¹⁹; guinea pigs were sacrificed by a blow on the head and exsanguinated. The section of trachea was separated from adjacent tissue to obtain tracheal rings which were tied to get chain of 3-4 individual tracheas. The chain was mounted in a 20 ml organ bath containing Krebs-Henseleit (K-H) solution of the composition (mM): NaCl 118.4, KCl 4.7, KH₂PO₄ 1.2, NaHCO₃ 25.0, CaCl₂ 2.5, MgSO₄ 1.2, glucose 11.1, pH 7.4 ± 0.05 and temperature of bath was maintained at 37 ± 1 °C. Tissue was suspended under isotonic tension of 0.5 g and allowed to equilibrate for at least 1 h before commencing the experiment, during which the K-H solution was replaced after every 10 min. After the equilibrium period contraction was

induced by adding the acetylcholine or histamine (1 µg/ml). Thereafter, the WF-EE (1 mg/ml) was added serially in increasing doses to the tissue bath until complete bronchodilation was observed. At the end of experimentation, the effect of WF-EE on pre-contracted tracheal chain was expressed as percent bronchodilation.

Evaluation of Anti-inflammatory effect using carrageenan and egg albumin-induced inflammation:

The anti-inflammatory activity of WF-EE was determined with the help of two different models *viz.* carrageenan and egg-albumin induced inflammation test²⁰⁻²¹. Male Albino rats were fasted for 24 h before the commencement of experiment but water was provided *ad libitum*. Freshly prepared carrageenan 0.1 ml (1 % suspension in normal saline) or 0.1 ml/kg of fresh egg-albumin was injected into the plantar region of hind paw of the rats to induce inflammation. For each model, animals were grouped as; group I (vehicle, 10 ml/kg), group II (indomethacin 10 mg/kg), group III (WF-EE 100 mg/kg) and group IV (WF-EE 200 mg/kg) for each group n=6. The WF-EE and indomethacin were suspended with 1 % CMC suspension (w/v) and administered orally 1 h before the carrageenan or egg-albumin injection. Change in paw volume was measured after administration of phlogistic agent at 1, 3 and 5 h in carrageenan and at 20, 40, 60, 80, 100 and 120 min after egg albumin injection.

Statistical Analysis:

Results were expressed as mean ± S. E. M. of four observation for bronchoprotective & bronchodilatory studies. While for anti-inflammatory studies mean S.E.M. of 6 observations. The data of bronchodilation study was analyzed by one-way ANOVA followed by post hoc Dunnett's multiple comparison test. The results of anti-inflammatory activity were analyzed by two-way ANOVA followed by post hoc Bonferroni post-tests (Factor I: treatment; Factor II: time). A value of P<0.05 was considered to be statistically significant in all the cases.

RESULTS AND DISCUSSION

According to Bousquet J *et al.*²², two important targets that should be controlled in the treatment as well as management of asthma are bronchoconstriction and exacerbated inflammatory conditions. Therefore, the present study evaluates bronchoprotective, bronchodilatory and anti-inflammatory potential of WF-EE.

Acute toxicity studies of WF-EE according to OECD/OCDE²³ guideline, exhibited significant safety margin of WF-EE as indicated by lack of systemic and behavioral toxicity up to 2000 mg/kg (p.o.). At this dose no adverse effect was observed during first 30 min, 24 h and even up to 14 days after administration of extract. Hence, randomly two doses were selected i.e., 100 and 200 mg/kg for the bronchoprotective and anti-inflammatory studies.

In bronchoprotective study WF-EE at 200 mg/kg produced significant prolongation of preconvulsive time (latency period) and decreased the tumble numbers with percentage protection of 52.24 in acetylcholine and histamine aerosol challenged guinea pigs as compared to vehicle. The result was better than that of the standard drug aminophylline with percentage protection of 45.44. However, at the low dose (100 mg/kg) this extract did not exhibit significant bronchoprotective effect with percentage protection of 15.95. This suggests dose dependent bronchoprotective effects of WF-EE against bronchoconstriction induced by histamine and acetylcholine aerosol. The effects of the WF-EE and aminophylline on sensitive guinea pigs exposed to mixture spray of 0.1% histamine and 2% acetylcholine chloride are shown in Table 1.

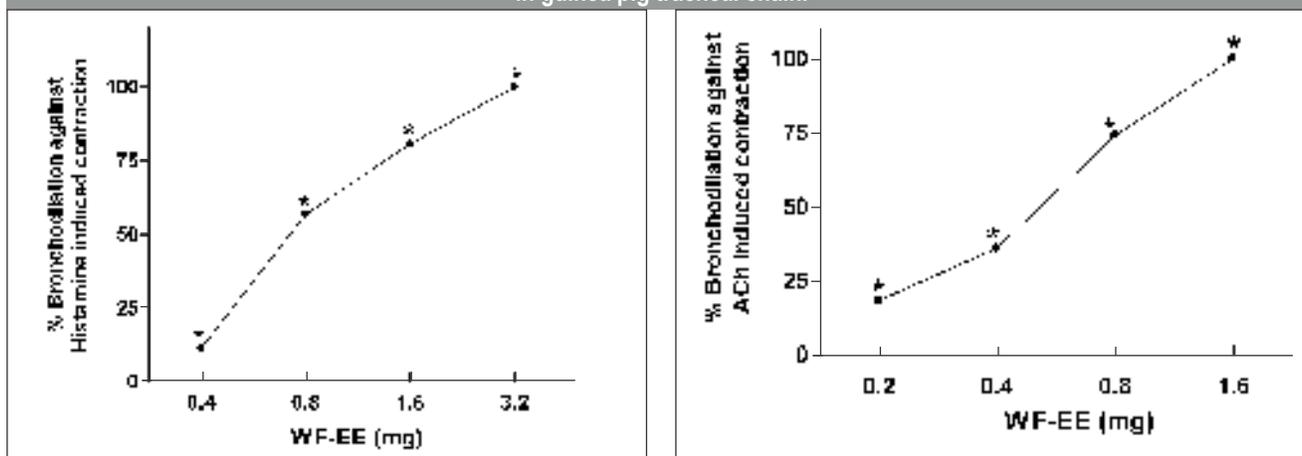
In the pursuit to assess the bronchodilatory potential of WF-EE, guinea pig tracheal chain was contracted by histamine or acetylcholine (1µg/ml). WF-EE showed significant (P<0.001) bronchodilatory activity in the precontracted tracheal chain preparation, as indicated by attenuation of the histamine as well as acetylcholine-induced contractions in concentration dependent manner. WF-EE produced 100 %

Table 1: Bronchoprotective effect of WF-EE on acetylcholine and histamine aerosol induced bronchospasm in guinea pigs.

Treatment group	Dose (mg/kg)	Tumble No.	Latency (sec)		% Protection
			Before	After	
Control		14	88 ± 13	89 ± 14	
WF-EE	100	11	79 ± 13	94 ± 09 ns	15.95
	200	05	85 ± 16	178 ± 22**	52.24
AMN	10	08	90 ± 18	162 ± 27*	45.44

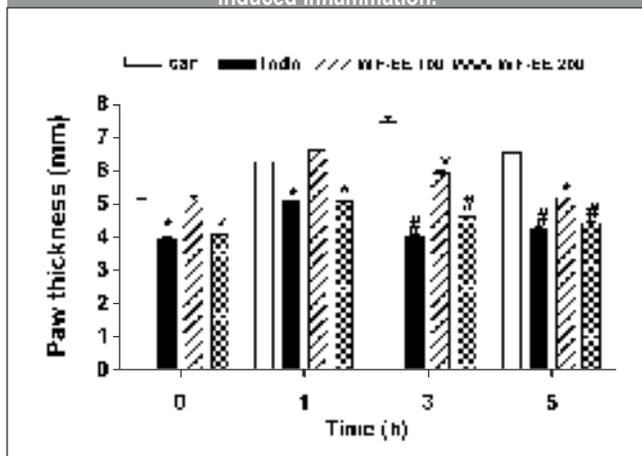
The WF-EE extract (100 or 200 mg/kg; p. o.) was administered daily for 3 days and 1 h before the aerosol challenge. Prolongation of latency period and tumble numbers were recorded in test animals. Values represent the mean ± S. E. M. of four independent replicates. ns- not significant, *p < 0.05 and **p < 0.001; indicates the different levels of significance when compared against control group.

Fig.1 a and b :Bronchodilatory effect of WF-EE on [a] histamine (1 µg/ml) or [b] acetylcholine (1 µg/ml) induced contraction in guinea pig tracheal chain.



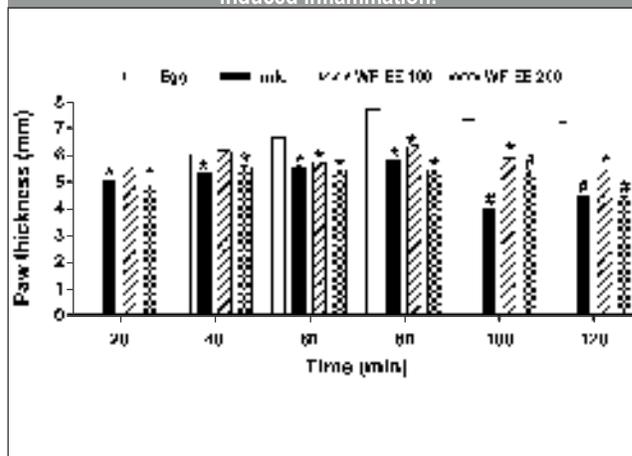
WF-EE (1 mg/kg) was added in increasing volumes i.e. 0.2, 0.4, 0.8, 1.6 and 3.2 ml in the tissue bath after maximum contraction was induced in tracheal chain by the above spasmogens. The values are expressed as mean ± S. E. M. of data from guinea pig tracheal chain (n= 6). *P<0.01vs histamine (55 mm was considered 100 %) or acetylcholine (45 mm was considered 100 %) induced contractions (one-way ANOVA followed by post hoc Dunnett's multiple comparison test).

Fig.2: Anti-inflammatory effect of WF-EE on carrageenan induced inflammation.



The WF-EE extract (100 or 200 mg/kg) was administered orally 1 h before carrageenan injection. Change in paw volume was measured at 0, 1, 3 and 5 h after carrageenan injection. Each bar represents the mean of paw volume (in mm) ± S. E. M. of data from 6 animals. Level of significance was indicated as *P<0.01 or #P<0.001 vs. control group (Two-way ANOVA followed by Bonferroni post-tests).

Fig. 3: Anti-inflammatory effect of WF-EE on egg-albumin induced inflammation.



The WF-EE extract (100 or 200 mg/kg) was administered orally 1 h before egg albumin injection. Change in paw volume was measured at 20, 40, 60, 80, 100 and 120 min after egg-albumin injection. Each bar represents the mean of paw volume (in mm) ± S. E. M. of data from 6 animals. Level of significance was indicated as *P<0.01 or #P<0.001 vs. control group (Two-way ANOVA followed by Bonferroni post-tests).

bronchodilation (complete bronchorelaxation) against histamine and acetylcholine induced contraction at 3.2 mg (Fig 1a), at 1.6 mg (Fig 1b) respectively. Thus, WF-EE exhibits significant bronchodilatory activity in guinea pig pre-contracted tracheal chain preparation by counteracting acetylcholine and histamine induced contraction. WF flowers have been reported to contain saponins, tannins and flavonoids^{7, 8} and these constituent might participate in the observed anti-asthmatic effect of WF-EE^{24, 25}. Therefore, it

can be contemplated that WF-EE possesses both anticholinergic and anti-histaminergic activity. It may be emphasized that WF-EE as a promising target for the control as well as management of the complications arising due to bronchoconstriction, as evident in asthma.

As asthma is characterized with increasing airway obstruction caused by bronchospasm, bronchoconstriction, inflammation and edema of the bronchia, the present

investigation further attempts to explore the anti-inflammatory potential of WF-EE using *in-vivo* carrageenan or egg albumin models. The carrageenan-induced rat paw edema is categorized into two phase phenomenon based on the time, release and the type of mediators involved. The first hour after carrageenan injection is considered as an initial phase which is attributed to the release of histamine and 5-HT. While 3-5 h after carrageenan injection is the second phase and contributed by induction of prostaglandins, bradykinins, protease and lysosome, which acts as mediators of edema formation^{26,27}. The present research report demonstrates inhibition of carrageenan induced inflammation in both phases by WF-EE at 200 mg/kg dose. While, at 100 mg/kg WF-EE was devoid of any effect on inflammation at initial phase ($P>0.05$) but was significantly effective in later phases ($P<0.01$). Furthermore, anti-inflammatory effect of the WF-EE was studied in egg albumin induced edema model. Earlier studies have indicated the use of egg-albumin as a phlogistic agent and can be used to screen anti-inflammatory activity^{28,29}. At lower dose WF-EE (100 mg/kg, p.o.) failed to exhibit anti-inflammatory effect at time interval 20 and 40 min ($P>0.05$) but showed significant inhibition of inflammation at 60, 80, 100 and 120 min after egg albumin administration as compared to control. Where as, at higher dose WF-EE (200 mg/kg, p.o.) significantly inhibited ($P<0.05$) egg albumin induced inflammation at selected time interval. The anti-inflammatory effect of WF-EE was comparable to the reference standard i.e. indomethacin, against both carrageenan and egg albumin inflammation (Fig 2 and 3). The plausible mechanisms for the observed anti-inflammatory activity of WF-EE might be attributed to inhibition of synthesis, release or action of major inflammatory mediators like histamine, serotonin, bradykinins and prostaglandins.

CONCLUSION

Present investigation suggests the bronchoprotective and bronchodilatory potential of WF-EE via antihistaminic or anticholinergic mechanism, while anti-inflammatory effect probably through the attenuation of synthesis or release of major inflammatory mediators. Thus, the present study projects the importance of *Woodfordia fruticosa* flower ethanolic extract in the treatment and management of asthma related complications, but warrants further investigations with pertinent pharmacological screening of bioactive phytoconstituent(s) to ascertain its therapeutic potential and mechanism of action.

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