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Original Article

Phytopharmacological evaluation of aerial parts of *Woodfordia fruticosa* (L.) Kurz in Cough Variant Asthma

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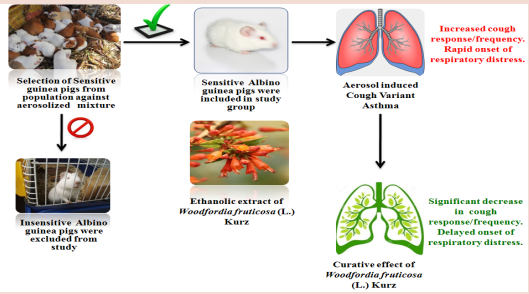
ABSTRACT

Background: Cough variant asthma (CVA) is characterized by prolonged non productive cough which responds to bronchodilator therapy. None of herbal drug is reported to possess pharmacological activity against CVA. **Objective:** To investigate the pharmacological potential of ethanolic extract of *Woodfordia fruticosa* (L.) Kurz (EEWF) against CVA as well as to develop an efficient screening model for CVA. **Material and Method:** Anti-tussive effect of EEWF was evaluated against nebulized aqueous solution of 0.1 g/ml of citric acid to determine the cough response. EEWF potential was finally accessed against aerosolic mixture of 0.3 g/ml of citric acid mixed with 0.1% histamine and 2% acetylcholine chloride to evaluate the convulsive latency, percentage protection and cough frequency against CVA. **Results:** EEWF at aerosolic dose of 6% w/v exhibit decrease in of the average coughs frequency (4.83 ± 0.30) which is quite significant effect as compared to standard drug codeine. EEWF against aerosol induced CVA was found to exhibit a significant bronchoprotection of 41.75% and decreases number of coughs (7.16 ± 0.47) at 200 mg/kg as compared to control (14.16 ± 0.60). **Conclusion:** EEWF at 200 mg/kg dose exhibited bronchoprotective and anti-tussive effects against aerosol induced CVA.

Key words: Acetylcholine, Anti-tussive, Bronchoprotection, Citric acid, Cough, Cough variant asthma (CVA), Ethanolic extract of *Woodfordia fruticosa* (L.) Kurz (EEWF), Histamine.

SUMMARY

- The present study investigated a potential curative effect of ethanolic extract of *Woodfordia fruticosa* (L.) Kurz in Aerosol induced Cough Variant Asthma.
- As none of any model exists to assess the activity of the drug for CVA, so our study also aimed to develop an effective pharmacological screening model for CVA.
- The extract not only protects the animals from bron¬choconstriction and bronchospasm but also suppress the cough frequency, which almost covers the basic etiological relevance for CVA, which pharmacologically corroborates its effective treatment against CVA.



PICTORIAL ABSTRACT

Abbreviations used: CVA: Cough Variant Asthma, EEWF: Ethanolic Extract of *Woodfordia Fruticosa (L.) Kurz*, OECD: Organization for Economic Cooperation and Development.

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INTRODUCTION

Cough variant asthma (CVA), one of the most common causes of chronic cough,[1-4] is considered a precursor and a variant form of classic asthma with typical symptoms of wheezing and dyspnea.[5-9] The etiological basis for effective management of asthma needs bronchiodialator action as reliever medication, anti-inflammatory as preventive medication and long acting β2 agonist as symptom controller.[10-11] Cough variant asthma (CVA) is one of the commonest forms of asthma characterized by an unproductive dry cough as the main or only symptoms and CVA patients has a more sensitive cough reflex. Complications like excessive mucous production and cough reflex may synergize the complication as bronchiolar chocking and asthmatic attack. [12]

Woodfordia fruticosa (L.) Kurz is a straggling leafy shrub of family Lythraceae, locally known as Dhatki or Dhai, having the bright red color flowers is distributed abundantly throughout the India, as well as in the majority of the South and East Asian countries.[13-14] Phytochemical studies of *Woodfordia fruticosa* can be ascribed for its important bioactive phytoconstituents such as flavonoids, sterols, anthraquinones, saponins and tannins.[15-17] Earlier studies indicates, that *Woodfordia fruticosa* possess important pharmacological activities including antipyretic, anti-inflammatory,[18] immunomodulatory,[19] etc. None of herbal origin drug posses combine effect as anti-asthmatic, and anti-tussive activity. So as ideal management aerial parts of *Woofordia fructucosa* could be explored against asthma specific cough reflex i.e. cough variant asthma.

MATERIAL AND METHODS

Plant material

The aerial parts of *Woodfordia fruticosa* (L.) Kurz for the present study were collected in the month of January locally from, Bhopal, Madhya Pradesh, India. The plant was identified and authenticated by Dr. Zia Ul Hasan, Head of Department, Department of Botany, Safia Science College, Bhopal, (M.P.) India, and a specimen voucher (334/Bot/Saifia/12), deposited in the Herbarium of the Department of Pharmacognosy, Truba Institute of Pharmacy, Bhopal, (M.P.), India, for future reference.

Extraction

The aerial parts of *Woodfordia fruticosa* were shade dried for 2 weeks, then pulverized to a coarse powder, passed through sieve No. 20 to maintain uniformity. Coarsely dried powder was first defatted with petroleum ether (60-80°C) for 72 hours to remove fatty materials and then extracted with ethanol (95%) using soxhlet apparatus for 36 hr., obtained orange, brown extract was collected and concentrated in vacuum under reduced pressure using a rotary flash evaporator and the dried crude extract was stored in airtight container at 4°C for further study. The yield of the extract was 13.72%.

Phytochemical screening

Ethanolic extract of *Woodfordia fruticosa* (EEWF) was subjected to various phytochemical screening tests for the identification of the phytoconstituents presents in the aerial parts of *Woodfordia fruticosa* using standard procedures.[20]

Animals

The experiment was carried out on Healthy albino guinea pigs (400-600 g). Animals were provided from the authorized animal house of Truba Institute of Pharmacy, Bhopal, Madhya Pradesh, India. The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25 ± 2°C relative humidity 44-56% and light and dark cycles of 12:12 hours, fed with standard pellet diet and water *ad libitum* during experiment. The experiment was approved by the institutional animal ethics committee (IAEC) as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines (Approval No. 1196/a/08/CPCSEA).

Acute oral toxicity study

The acute oral toxicity study was evaluated as per Organization for Economic Cooperation and Development (OECD) guidelines no. 425, on guinea pigs of either sex, weighing between 400-600 g. Before the experiment, animals were fasted overnight with water *ad libitum*. Three animals were selected which receives a dose of 2000 mg/kg. All three animals were received a single dose of 2000 mg/kg body weight of EEWF by oral gavage. Animals were observed individually for any sign of toxicity, behavioral changes, and mortality after dosing, with special attention given during the first 4 hours, and thereafter for 24 hours, for a total period of 7 days.

In-vivo citric acid induced anti-tussive evaluation[21]

Guinea pig (400-600 g) of either sex was selected and divided into four groups, i.e. control, standard (codeine, 0.03GM/ml) and test groups (EEWF, 3% w/v and 6% w/v, body weight, respectively) consisting of six animals in each group. Each unanaesthetized and unrestrained animal was individually placed in a transparent chamber of (dimensions, 30 cm x20 cm x20 cm) and exposed to nebulized aqueous solution of 0.1 g/ml of citric acid (nebulizing rate 0.7 ± 0.04 ml/min) for continuous 7 minutes. During the last 5 minutes of exposure each animal was observed continuously and closely to determine the number of cough responses. The above protocol was performed for every animal from each group for 10 minutes after exposing animals to aerosol solutions of normal saline (for baseline measurement), codeine solution (0.03 gm/ml, standard), EEWF (3% w/v and 6% w/v).

In-vivo aerosol induced cough variant asthma (CVA)

The effect of ethanolic extract of *Woodfordia fruticosa* against cough variant asthma was evaluated by a modified method. Guinea pigs (400-600 g) of either sex were screened out by challenging the animals by putting them one by one in an aerosolized chamber nebulized with mixture of 0.3 g/ml of citric acid mixed with an equal volume solution of 0.1% histamine and 2% acetylcholine chloride (nebulizing rate 0.7±0.04 ml/min) to determine the pre-convulsive time[22] as well as appropriate cough response and was observed for maximum 4 mins. Within that period of time the animal was regarded as insensitive or not suitable if they do not show any respiratory distress (convulsions) and tussive response. The sensitive guinea pigs after screening were selected and grouped into three groups, i.e. control and test groups (EEWF, 100 mg/kg and 200 mg/kg, body weight, p.o.) consisting of six animals in each group. Animals of test groups were treated with a single oral dose of EEWF extract (EEWF, 100 mg/kg and 200 mg/kg, body weight, p.o. respectively) daily for 15 days prior to bronchial challenge. On last day extract was administered 1 h before the bronchial challenge and after 30 minutes, animals were individually placed in a specified polystyrene transparent chamber (dimension, 30 cm x 20 cm x 20 cm) nebulized with an aerosolic mixture of 0.3 g/ml of citric acid mixed with an equal volume solution of 0.1% histamine and 2% acetylcholine chloride to evaluate the convulsive latency, percentage protection and cough frequency.

RESULTS

Preliminary phytochemical investigation of EEWF revealed the presence of alkaloids, glycosides, flavonoids, tannins, triterpenoids, polyphenols, carbohydrates and proteins. Acute toxicity studies of EEWF were performed in accordance with OECD 425 and extract found to exhibit a great margin of safety up to dose of 2000 mg/kg and there was no change in the behavioral pattern and not any sign of toxicity and mortality observed during the overall toxicity studies. Accordingly 1/10 of this dose was considered to be the experimentally safe dose. EEWF at 3% and 6% w/v aerosolic dose against citric acid induced tussive reaction was found to exhibit a significant ($P<0.001$) reduction in cough response as compared to control (Table 1). After accessing the anti-tussive activity separately, the EEWF potency was evaluated against aerosol (an aerosolic mixture of 0.3 g/ml of citric acid mixed with an equal volume solution of 0.1% histamine and 2% acetylcholine chloride) induced cough variant asthma (CVA) and was evaluated for convulsive latency, percentage protection and cough frequency. As no any drug was reported as standard treatment for CVA for pharmacological screening models, so here we are comparing the efficacy of the EEWF with the control group. EEWF at 100 and 200 mg/kg was found to exhibit a significant bronchoprotection of 36.77% and 41.75% respectively as compared to control and significant ($P<0.01$ and $P<0.001$) decreases in cough response at the dose level of 100 and 200 mg/kg respectively as compared to control (Table 2).

DISCUSSION

The present investigation was attempted to evaluate the bronchoprotective and anti-tussive effect of EEWF against aerosolized cough variant

Table 1: Anti-tussive effect of EEWF against citric acid induced tussive reaction

Treatment groups	Dose	No. of cough
Control	-	12.5 ± 0.56
Standard	0.03 gm/ml	3.33 ± 0.21***a
EEWF	3% w/v	7.16 ± 0.47***a, ***b
EEWF	6% w/v	4.83 ± 0.30***a

All values are represented as mean ± SEM, n = 6 animals in each group, Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test, a- Significant difference as compared to control group, b- Significant difference as compared to standard group, and *P<0.05, **P<0.01, ***P<0.001.

Table 2: Effect of EEWF against aerosolized induced CVA

Treatment groups	Dose (mg/kg)	Latency (in seconds)		% protection	No. of coughs
		Before treatment	After treatment		
Control	-	88.83 ± 0.83	89.66 ± 0.95	-	14.16 ± 0.60
EEWF	100	70.5 ± 1.05	111.5 ± 1.47***	36.77	9.66 ± 0.33***
EEWF	200	86 ± 1.29***	147.66 ± 0.66***	41.75	7.16 ± 0.47**

All values are represented as mean ± SEM, n=6 animals in each group, Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test. Results are considered significant as compared to control group and *P<0.05, **P<0.01, ***P<0.001.

asthma (CVA). Therefore evaluation was first undertaken for anti-tussive effect of EEWF against citric acid induced tussal response. Finally the ability of the extract against aerosolized (aerosolic mixture of 0.3 g/ml of citric acid mixed with an equal volume solution of 0.1% histamine and 2% acetylcholine chloride) induced CVA has been evaluated.

Histamine is an important mediator of bronchial muscle contraction and the obstruction of these may occur via H1 receptors. In addition, acetylcholine released from efferent nerve endings of the inner bronchus results in the excessive formation of inositol 1,4,5-triphosphate (IP3) in bronchial muscles that lead to the intracellular release of calcium and initiate bronchoconstriction. It has been reported that bronchial acetylcholine and H1 receptor blockade results in bronchodilation, which is considered as vital in the treatment of asthma.[23] A prominent effect caused by both leads to varied degree of bronchoconstriction that causes asphyxia and death. Bronchodilators can delay the occurrence of these symptoms.[24]

In animals, coughing has been elicited by number of methods.[25-28] In the present study, the anti-tussive activity of EEWF has been compared with that of standard drug codeine against coughing induced by chemical stimulation (citric acid). The extract showed significant inhibition of cough as compare to standard drug codeine in dose dependent manner. Thus the extract might be acting via the central nervous system cough suppressant action, but the exact mechanism of action cannot be withdrawn from the preliminary study. In the conclusive study against aerosolized induced CVA, we exposed the animals against acetylcholine, histamine and citric acid, and the oral dose of EEWF was found to possess significant bronchoprotective and anti-tussive activity that might suggested that the extract possess histaminic and cholinergic receptor antagonistic property along with the central cough suppressant, this observed activity may be correlated with the presence of saponins and polyphenols in EEWF.[29]

CONCLUSION

In conclusion, results suggested the potential role of EEWF for the treatment against CVA. The extract not only protects the animals from bronchoconstriction and bronchospasm but also suppress the cough frequency, which almost covers the basic etiological relevance for CVA, which pharmacologically corroborates its effective treatment against CVA.

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REFERENCES

1. Irwin RS, Curley FJ, French CL. Chronic cough: The spectrum and frequency of causes, key components of the diagnostic evaluation, and outcome of specific therapy. Am Rev Respir Dis. 1990; 141(3): 640-7.
2. Dicpinigaitis PV. Chronic cough due to asthma: ACCP evidence- based clinical practice guidelines. Chest 2006; 129(Suppl 1): 75S-9.
3. Niimi A. Geography and cough aetiology. Pulm Pharmacol Ther. 2007; 20(4): 383-7.
4. Matsumoto H, Niimi A, Takemura M, Ueda T, Yamaguchi M, Matsuoka H, *et al.* Prevalence and clinical manifestations of gastro-oesophageal reflux-associated chronic cough in the Japanese population. Cough 2007; 3(1):1.
5. Corrao WM, Braman SS, Irwin RS. Chronic cough as the sole presenting manifestation of bronchial asthma. N Engl J Med. 1979; 300(12): 633-7.
6. Johnson D, Osborn LM. Cough variant asthma: A review of the clinical literature. J Asthma. 1991; 28(2): 85-90.
7. Koh YY, Jeong JH, Park Y, Kim CK. Development of wheezing in patients with cough variant asthma during an increase in airway responsiveness. Eur Respir J. 1999; 14(2): 302-8.
8. Fujimura M, Ogawa H, Nishizawa Y, Nishi K. Comparison of atopic cough with cough variant asthma: is atopic cough a precursor of asthma? Thorax 2003; 58(1): 14-8.
9. Matsumoto H, Niimi A, Takemura M, Ueda T, Tabuena R, Yamaguchi M, *et al.* Prognosis of cough variant asthma: a retrospective analysis. J Asthma. 2006; 43(2): 131-5.
10. Chris Brown, Shane Brun, Jonathan Burdon H, John Fardy, Kerry Hancock, Chris Hogan, *et al.* Asthma: basic facts. Jenni Harman, Sue Markham, editors. Asthma management Hand book. South Melbourne: National asthma council Australia; 2006. p. 3-4.
11. The Global Asthma Report 2014, ISBN: 978-0-473-29125-9 (PRINT) | 978-0-473-29126-6 (ELECTRONIC)
12. Hisako Matsumoto, Akio Niimi, Masaya Takemura, Tetsuya Ueda, Masafumi Yamaguchi, Hirofumi Matsuoka, *et al.* Features of cough variant asthma and classic asthma during methacholine-induced brochoconstriction: a cross-sectional study. Cough 2009; 5(3): 1-6.
13. Kirtikar KR, Basu BD. Indian Medicinal Plants. India; LM. Basu publishers; 1935. Parts 1-3.
14. Khare CP. Encyclopedia of Indian Medicinal Plants. Rational Western Therapy, Ayurvedic and Other Traditional Usage, Botany. Berlin; Springer; 2004. p. 483–4.
15. Chauhan JS, Srivastava SK, Srivastava SD. Phytochemical investigation of the flowers of *Woodfordia fruticosa.* Planta medica. 1979; 36(2): 183-4.
16. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. India, CSIR; 1956. p. 259-61.
17. Das PK, Goswami S, Chinniah A, Panda N, Banerjee S, Sahu NP, *et al. Woodfordia fruticosa*: traditional uses and recent findings. J Ethnopharmacol. 2007; 110(2): 189-99.
18. Alam M, Susan T, Joy S, Ak SU, Kundu AB. Anti-inflammatory activity of Plectranthus urticifolius Hook. F. and *Woodfordia fruticosa* Kurz. in albino rats. Ind Drug 1990; 27(1): 559-62.
19. Kroes BH, van den Berg AJJ, Abeysekera AM, de Silva, KTD, Labadie RP. Fermentation in traditional medicine: the impact of *Woodfordia fructicosa* flowers on the immunomodulatory activity, and the alcohol and sugar contents of Nimba Aristha. J of Ethnopharmacol. 1993; 40(2): 117-25.
20. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. India: Nirali Prakashan; 2005. p. 50-198.
21. Sunita P, Jha S, Pattanayak SP. *In vivo* Antitussive Activity of *Cressa cretica* Linn. using Cough Model in Rodents. Phcog Res. 2009; 1(3): 15761.
22. Xiangping C, Zhaohui X, Dazheng W, *et al. In vitro* and *in vivo* evaluation of the anti-asthmatic activities of fractions from Pheretima. J Ethnopharmacol. 2007 111(3): 490–5.
23. Matsumoto T, Ashida Y, Tsukuda R. Pharmacological modulation of immediate and late airway response and leukocyte infiltration in the guinea pig. J Pharmacol Exper Ther. 1994; 269(3): 1236–44.
24. Kumar D, Bhujbal S, Deoda S, Mudgade C. *In vitro* and *in vivo* antiasthmatic studies of *Ailanthus excelsa* Roxb on guinea pigs. J Sci Res. 2010; 2(1): 196–202.
25. Tedeschi RE, Tedeschi DH, Hitchens JT, Cook L, Mattis PA, Fellows EJ. A new antitussive method involving mechanical stimulation in unanesthetized dogs. J

Pharmacol Exp Ther. 1959; 126(4): 338-44.
26. Turner RA. Screening Methods in Pharmacology. New York, Academic Press; 1968. p. 128.
27. Cavanagh RL, Gylys JA, Bierwagen ME. Antitussive properties of Butorphanol. Arch In Pharmacodyn. 1976; 220(2): 258 -68.
28. Pickering RW, James GWL. The antitussive activity of a novel compound RU 20201. Drug Res. 1979; 29(2): 2879.
29. Minky M, Ankush S. Phytoconstituents responsible for anti-inflammatory activity. J Nat Pharm. 2013; 4(1): 1–12.

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