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

Amit Kumar Srivastava¹, Srivastava Abhinav Siddharth², Nagar Hemant³, Srivastava Rajnish³, Deepa⁴ and Shukla Gaurav⁵

¹Department of Pharmacology, Sapience Bio-analytical Research Lab, Indrapuri, Bhopal (M.P) India.
²Department of Pharmacology, Truba Institute of Pharmacy, Karond, Gandhi Nagar Bypass Road, Bhopal (M.P) India.
³Faculty of Pharmacy, Moradabad Educational Trust, Ram Ganga vihar, Civil lines, Moradabad (U.P) India.
⁴Department of Pharmacology, Truba Institute of Pharmacy, Karond, Gandhi Nagar Bypass Road, Bhopal (M.P) India.
⁵Department of Pharmacology, NRI Institute of Pharmaceutical Sciences, SajjanSingh Nagar, Raisen Road, Bhopal (M.P) India.

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 assessed against aerosolic mixture of 0.3 g/ml of citric acid mixed with 0.1% histamine and 2% acetylsalicylic acid 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:** Acetylsalicylic acid, 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 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.

PICTORIAL ABSTRACT

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

**Correspondence:**
Mr. Amit Kumar Srivastava, Department of Pharmacology, Sapience Bio-analytical research Lab. Indrapuri, Bhopal (M.P) India-462021.
E-mail: amitpharmacy21@gmail.com
DOI : 10.5530/pj.2015.5.8

INTRODUCTION

Cough variant asthma (CVA), one of the most common causes of chronic cough,¹⁴ is considered a precursor and a variant form of classic asthma with typical symptoms of wheezing and dyspnea.¹⁻⁹ The etiological basis for effective management of asthma needs bronchodilator 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 bronchial 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 *Woodfordia fruticosa* 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/Safia/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.

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

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 unanesthetized and unrestrained animal was individually placed in a transparent chamber of (dimensions, 30 cm x 20 cm x 20 cm) and was observed for maximum 4 mins. Within that period the animal was regarded as insensitive or not suitable if they do not show any respiratory distress (convulsions) and tussive response. 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 3% and 6% w/v and was found to exhibit a significant bronchoprotection against citric acid induced tussive reaction was 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 asthma (CVA) was 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).

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

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

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.
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 (aerosol mixture of 0.3 g/ml of citric acid mixed with an equal volume solution of 0.1% histamine and 2% acetycholine 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, acetycholine released from effenter 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 bronchodilatation, which is considered as vital in the treatment of asthma. 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. In animals, coughing has been elicited by number of methods. 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 compared 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.

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.

ACKNOWLEDGEMENTS

I would like to express my special thanks of gratitude to Dr. Zia-Ul-Hasan, Head of Department, Department of Botany, Saifia Science College Bhopal for his valuable support in authentification of drug. I am highly indebted to the institutional committee of Truba Institute of Pharmacy for providing laboratory and animal facility.

REFERENCES

Pharmacol Exp Ther. 1959; 126(4): 338-44.


**ABOUT AUTHORS**

**Mr. Amit Kumar Srivastava:** Presently working as Senior Research Officer in the Department of Pharmacology, Sapience Bio-analytical Research Lab, Bhopal, Madhya Pradesh. He has two years of research experience. He has published 12 papers (both original and review articles) in reputed peer reviewed international and national journals and presented various research papers at national and international conferences. His area of expertise and interest includes preclinical pharmacological screening model development aimed at mechanistic studies, safety pharmacology and regulatory toxicological studies, and Bio-analytical method development of new chemical entities in the preclinical discovery phase.

**Mr. Hemant Nagar:** Currently working as Assistant Professor in the Department of Pharmacology, Truba Institute of Pharmacy, Bhopal, Madhya Pradesh. He has more than six years of teaching and research experience. He has supervised more than 50 undergraduate and 30 postgraduate students for their research projects. He has to his credit over 16 papers (both original and review articles) in reputed peer reviewed international and national journals. His area of expertise includes standardization and development of novel and herbal formulations, peptic ulcer, diabetes, Neuropharmacology and dermatology. He is also a reviewer for several international and national research journals.