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PHARMACOLOGICAL EVALUATION OF ANTIASTHMATIC ACTIVITY OF *FUMARIA OFFICINALIS* EXTRACTS

R. Dutta[1], M. K. Sharma[1], and M. Jha[2*]

[1] Maharaj Vinayak Global University, Jaipur, Rajasthan, India

[2*]Pinnacle Biomedical Research Institute, Near, Bharat Scout and Guides Campus, Shanti Marg, Shyamla Hills Road, Bhopal, M.P., India

Correspondence Author : Dr. Megha Jha

Pinnacle Biomedical Research Institute, Near, Bharat Scout and Guides Campus, Shanti Marg, Shyamla Hills Road, Bhopal, M.P., India

Email:meghajhabtbpl@gmail.com

Abstract

Fumaria officinalis (*F. officinalis*) belongs to family papaveraceae and is traditionally used to treat hypertension, hepatitis diabetes, many inflammatory and painful-ailments. In the present study, *in vitro* antioxidant effect of extracts of *F. officinalis* leaves was assessed against superoxide radical scavenging assay and was evaluated for antiasthmatic activity by using various *in vitro* and *in vivo* animal models. *In vitro* models like isolated guinea pig tracheal chain preparation and isolated guinea pig ileum preparation were studied to know basic mechanism by which herbal extract shows relaxant activity. Decrease in contractile tone of tracheal chains was considered as the relaxant effect. *In vivo* Bronchial hyper reactivity was studied by using Wistar rats of either sex. The Broncho alveolar lavage fluid (BALF) was examined by sensitizing the animals with ovalbumin (OVA).Phytochemical analysis revealed the presence of carbohydrates, glycosides, alkaloids, flavonoids, triterpenoids and steroids. The effects of *F. officinalis* extract on the contractile responses of the isolated guinea-pig ileum were investigated. Contraction changes in the guinea pig ileum were monitored using a force displacement transducer amplifier connected to a physiograph. *F. officinalis* extract inhibited the contractile responses in a dose-dependent manner and also decreased the amplitude of peristaltic waves. Isolated tracheal smooth muscles contractions induced by acetylcholine was significantly ($p<0.05$) reduced by the extract, so also that induced by acetylcholine at concentrations of 20 mg/ml. The findings were similar to that of 10µg/ml chlorpheniramine maleate. In case of *in vivo* model, there was significant decrease in inflammatory cell count in BALF with the treatment of methanolic extract of *F. officinalis* leaves at the dose of 200 and 400 mg/kg, p.o. Extract of *F. officinalis* also restored the level of lung oxidative markers (LPO, GSH, and SOD). Histopathological examination of lung tissue showed that extract of *F. officinalis* protected the lungs from severe changes caused by OVA. These observed effects can be attributed to the presence of phytochemicals such as flavonoids, alkaloids and steroids in the extract. The study concluded that methanol extract of *F. officinalis* possess antiasthmatic activity.

Keywords: *Fumaria officinalis,* Broncho alveolar, Antioxidant, Antiasthmatic activity.

Introduction

Asthma is a chronic inflammatory disease of the lungs, characterized by airway hyper-responsiveness to both inhaled allergens and nonspecific stimuli (Takeda *et al.,* 2012). The airway hyper-responsiveness results from epithelial injury caused by the accumulation of activated eosinophils and mast cells within the respiratory tract (Holgate, 2008). There is also convincing evidence that increased immunoglobulin E (IgE) levels and goblet-cell hyperplasia are observed in asthma (Chu *et al.,* 2012). The prevalence of asthma is increasing worldwide, and it has become a significant cause of health challenge especially in developed countries (Inam *et al.,* 2017). Type 2 helper T (Th2) cells seem to play a pivotal role in immune dysfunction, which contributes to the development of asthma (Liu *et al.,* 2016; Liang *et al.,* 2017; Venturini *et al.,* 2018). In addition, it has been observed that the Th-2-associated cytokines (e.g., interleukin-4 (IL-4), IL-5, and IL-13) were released by the airway epithelial cells. IL-4 has important roles in allergic inflammation and airway remodeling (Jie *et al.,* 2009) and promotes the differentiation of B-lymphocytes, which lead to IgE generation (Deo *et al.,* 2010). IL-5 is the most specific to eosinophils and is responsible for eosinophil growth, differentiation, mobilization, activation, recruitment, and survival (Simon *et al.,* 2004). Eosinophils differentiate within tissues undergoing an allergic response, including asthma (Cameron *et al.,* 2000). Thus, regulating the IL-4 or IL-5 is a useful therapeutic approach in allergic asthma (Rana *et al.,* 2016). Ovalbumin- (OVA-) induced asthma rats are a representative asthma animal model resembling human asthma and dexamethasone (DEXA) is a well-known glucocorticoid and is widely used as an anti-inflammatory control drug for development of new antiasthmatic agents (Ku *et al.,* 2012; Andre *et al.,* 2018; Kwon *et al.,* 2008).In India, in various traditional systems like Ayurveda, Unani and Siddha, numerous herbs were mentioned for therapeutic use in asthma. The plant, *F. officinalis* belongs to family Papaveraceae. It is widely found in Pakistan, Turkey and India (Latif *et al.,* 2011). It is used traditionally as a potent hepatoprotective, hypotensive, blood purifier, diuretic, antidiabetic and antioxidant plant (Gupta *et al.,* 2015). It is also helpful in rheumatism, abdominal cramps, fever, conjunctivitis and diarrhoea (Ivanov *et al*., 2014). Recent studies carried out on *F. officinalis* demonstrated its pharmacological use in the treatment of hyperglycemia, hyperthermia, helminthic infections and as antimicrobial agent against Methicillin resistant *Staphylococcus aureus* (MRSA) (Latif *et al.,* 2011). *F. officinalis* comprises of tannins, potassium salts and microsomal enzyme inducer alkaloids such as protopine and allocryptopine (Vrba *et al.,* 2011). Although, there is no scientific proof of the efficacy of plant extracts for antiasthmatic activity, the aim of this study was to evaluate antiasthmatic effect of *F. officinalis*

Materials and Methods

Plant Material and Chemical

The leaves of *F. officinalis* were collected from local area of Bhopal (M.P.) in the month of July, 2019. The identification and authentication of plant was done by Dr. Saba Naaz, Botanist, from the Department of Botany, Safia College of Arts and Science, peer gate Bhopal. A voucher specimen number 203/Saif./Sci./Clg/Bpl. was kept in Department of Botany, Safia College of Arts and Science, peer gate Bhopal, for future reference. All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). Chlorpheniramine maleate and dexamethasone (Alkem, Mumbai), All the chemicals used in this study were of analytical grade.

Extraction

Cold Maceration Method

In present study, plant material was extracted by using cold maceration method; the leaves of *F. officinalis* were collected, washed and rinsed properly. About 3kg of the powder was extracted with different organic solvent petroleum ether, ethyl acetate, chloroform and methanol and allow standing for 4-5days each. The extract was filtered through Whattman no.1 filter paper to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. Extract was transferred to beaker and evaporated & excessive moisture was removed and extract was collected in air tight container. Dimethyl sulfoxide (DMSO) was used to dissolve each extracts and sterilized using 0.22µm syringe filters (Axiva, Scichem Biotech) for further use(Nayak*et al.,* 2019).

Preliminary Phytochemical Screening

After obtaining of dry extract, qualitative preliminary phytochemical screening was performed to find out the presence of various phytochemicals such as steroids, saponins, alkaloids, flavonoids, tannins, phenolic compounds, and glycosides(Jain *et al.,* 2019).

Antioxidant Activity

Superoxide Radical Scavenging Activity

The principal behind this assay was the capability to inhibit reduction of nitro blue tetrazolium (NBT) in the NBT system (Beauchamp & Fridovich, 1971). For determination of superoxide dismutase activity, a method developed by *Martinez et al.* was used with a slight modification(Martinez *et al.,* 2001). Each 3 ml reaction mixture comprised of 50 mM sodium phosphate buffer, pH 7.8, 13 mM methionine, 2 mM riboflavin, 100 mM EDTA, NBT (75 mM) and 1 ml sample solution. The formation of blue colourformazan was followed by perceptive the rise in absorbance after 10 min lighting from a fluorescent lamp at 560 nm. The whole reaction assembly was surrounded in a box, covered with aluminum foil. Tubes with reaction mixture were kept in the dark and served as blanks. Super oxide scavenging activity (%)

$$\%\text{Inhibition} = \frac{\left(\text{Absorbance}_{\text{Blank}} - \text{Sample Absorbance}\right)}{\text{Absorbance}_{\text{Blank}}} \times 100$$

In Vitro Anti Asthmatic Activity

Isolated Guinea Pig Ileum Preparation

The guinea pig (overnight fasted) was sacrificed and 2 cm length of ileum was mounted in an organ bath containing tyrode solution. The tyrode solution was continuously aerated and maintained at 37 ± 0.5ºC. The tissue was allowed to equilibrate for 30 min. under a load of 500 mg, contact time of 30 sec. and the response of histamine was recorded by 5 min time cycle. After obtaining a dose response curve of histamine (10µg/ml) on ileum at the doses of (0.1, 0.2, 0.4, 0.8 and 1.6 ml), methanolic FOLE (5, 10 and 20µg/ml) was added to the reservoir and maximum contraction dose of histamine were repeated in presence of plant extract. Same procedure was repeated for standard drug Chlorpheniramine maleate (CPM 10µg/ml) as that of extract. The responses of spasmogens were seen and data represent in % relaxation (Pandit *et al.,* 2008; Lazuhiro *et al.,* 2006).

Isolated Guinea Pig Tracheal Chain Preparation

Guinea pigs were sacrificed and the trachea was rapidly dissected free of surrounding tissues and placed in Petri dishes containing oxygenated Kreb's solution (NaCl 110 mM, KCl 4.80, $MgSO_4$ 0.5 mM, $CaCl_2$ 2.35 mM, KH_2PO_4 1.2 Mm, $NaHCO_3$ 25mM, and dextrose 11.0 mM). After clearing from adhering fat and connective tissues, trachea was cut transversely into 8-10 segments each containing 3-4 cartilage rings. All rings were cut open opposite the tracheal is muscle and sutured together to form a tracheal chain. Tracheal preparation was suspended between two metal hooks in 10 ml organ bath containing Krebs solution at 37°C, pH 7 and gassed with 5% CO_2 in O_2. A resting tension of 1 g was applied and tissues were allowed to equilibrate for 30 min with frequent washings at 10 min interval. Responses were recorded on a Kymograph fitted to a rotating drum (Sherrington Rotating Drum MODEL NO- RLPE-15). After the equilibrium period, contraction was induced by adding acetylcholine. Thereafter, standard CPM (10 µg/ml) and the FOLE at 5, 10, 20 mg/ml were added. The effect of extract of treatment drug and its interaction with contractile response was recorded (Vogel & Vogel, 1998; Schachter *et al.,* 2003; Holroyde, 1986).

Animals

All ethical and handling guidelines were followed as set by Indian Legislation and approved by Institutional Animal Ethics Committee. All animals were procured and housed in animal house maintained under standard hygienic conditions. Animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No. 1824/PO/ERe/S/15/ PCSEA). Protocol Approval Reference No. PBRI/IAEC/PN-18032.

Acute Oral Toxicity

Wistar ratsof either sex weighing 200 ± 20 gm were used in the study. Acute oral toxicity study was performed as per Organization for Economic Co-operation and Development (OECD)-423 guidelines. The animals were divided in 3 groups (n=3) and were fasted overnight prior to drug administration. Following the period of fasting, the animals were weighed and the test substance was administered. The animals were given methanolic extract of *F. officinalis* in the doses of 5, 50, 300 and 2000 mg/kg body

weight orally. The animals were observed for 5 min every 30 min till 2 h and then at 4, 8 and 24 h after treatment for any behavioral changes/mortality. They were further observed daily for 7 days for mortality. No mortality up to 7 days after treatment was observed with methanolic extract of *F. officinalis* and therefore was found safe up to dose of 2000 mg/kg. Doses were selected based on acute oral toxicity study. Therefore the regime for the *F. officinalis* dose was 200 and 400mg/kg.Acute toxicity was determined as per reported method (Jonsson *et al.,* 2013).

Ovalbumin-Induced Airway Inflammation

Sensitization and Challenge with Antigen

Animals were divided into five groups (n=6) viz. All the animals except in the nonsensitized group (NS), were sensitized by an intraperitoneal injection of 1ml alum precipitate antigen containing 20µg of ovalbumin and 8mg of alum suspended in 0.9% sodium chloride solution.

A booster injection of this alum-ovalbumin mixture was given 7 days later. Non sensitized animals were injected with alum only. Seven days after (15 day) second injection, animals were exposed to aerosolized ovalbumin (1%) for 30 min. which was performed by placing the rats into a closed plexiglass chamber and filling the chamber with aerosolized ovalbumin which was generated by a nebulizer. Standard and test groups received dexamethasone (1 mg/kg i.p.) as standard and FOLE (200 and 400 mg/kg) as test drug, 5 h before antigen challenge.The rats were sacrificed at the end of study (24 hr after sensitization) and catheter was inserted in trachea. Bronchoalveolar lavage fluid was collected by lavaging the lung with 2 aquilots of 5 ml of 0.9% sodium chloride solution total recovery volume per rat was approximately 8 ml (Chapman *et al.,* 2007).

Grouping of Animals

G-I Control= Non-sensitized received 8mg alum in 1ml (i.p.)
G-II Ovalbumin induced= Sensitized received Ova lbumin 20µg + 8mg alum in 1ml (i.p.);
G-III Ovlabumin 20µg + 8mg alum in 1ml (i.p.) + Dexamethasone (1mg/kg, i.p.)
G-VI Ovlabumin 20µg + 8mg alum in 1ml (i.p.) + FOLE (D2) (200 mg/kg)
G-V Ovlabumin 20µg + 8mg alum in 1ml (i.p.) + FOLE (D2) (400 mg/kg)

Total leukocytes, eosinophils and neutrophils were counted under microscope and histopathological evaluation of lung tissue was carried out. Lung wet to dry weight ratio was taken. Estimation of biochemical parameters: Superoxide dismutase, lipid peroxidase and glutathione reductase was done in lung homogenate.

Statistical Analysis

The results were expressed as Mean ± SEM and statistically analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni test, with level of significance set at $p<0.05$ and <0.01.

Results

Preliminary phytochemical investigation of methanolic extract of *F. officinalis* showed presence of carbohydrates, glycosides, alkaloids, flavonoids, triterpenoids and steroids. The animals were showed no mortality and safe up to the dose 2000 mg/kg body weight. Dose was selected by using acute toxicity study (OECD, 423). The present study was performed at two dose levels of methanolic extract of *F. officinalis* at 200 and 400 mg/kg of body weight. The ability to reduce NBT by PMS-NADH coupling can measure the superoxide radicals generated from dissolved oxygen. The decrease in absorbance at 560 nm with the *F. officinalis* and the reference compound ascorbic acid indicates their abilities to quench superoxide radicals in the reaction mixture. Superoxide free radicals showed maximum inhibition of 82.65 at concentration of 100 µg/ml ascorbic acid with IC50 value of 16.45 and methanolic extract showed maximum inhibition of 65.867 with IC50 value of 24.40 proving better antioxidant activity as compared to other solvent extracts as shown in Table 1.In the present study, we have used histamine and acetylcholine as spasmogens in the form of aerosols to cause immediate bronchoconstriction in guinea pigs. The histamine (10µg/ml) produced dose dependent contraction of guinea pig ileum as indicated in the Table 2&3. The FOLEA and FOLME significantly inhibited ($p<0.05$) the contractile effect of histamine on isolated guinea pig ileum preparation, inhibition of contractile response produced by FOLME is less in comparison of FOLEA. The percentage relaxation of FOLEA on histamine induced contraction was found to be 54.20%. Similarly, the percentage relaxation of FOLME on histamine induced contraction was found to be 90.35% in comparison with Chlorpheniramine (10µg/ml) 96.26%. Therefore, the FOLME showed almost similar antihistaminic effect that of chlorpheniramine maleate. As shown in Table 4 of isolated guinea pig tracheal chain preparations, FOLME was found to possess antiasthmatic effects. The FOLME extract at concentrations of 10 mg/ml was able to significantly inhibit contractions induced by both spasmogen (acetylcholine) on the tracheal chains Table 5. The action of the extract was found to be similar to that of the standard drug. OVA has been used as an allergen in asthma animal models and the utility of OVA-induced asthma model has been well established and this model has been widely used to evaluate anti-asthmatic effects and immunological mechanisms involved in the pathogenesis of asthma. Therefore, the present study focused on the inhibitory effect of FOLME on the cell numbers. To distinguish the inflammatory cells and count the cell numbers. Changes in total cell levels in the BALF were examined after the last OVA challenge. In the OVA-sensitised mice, infiltration of inflammatory cells was observed. OVA challenge significantly ($p<0.05$) induced influx of total leukocytes, eosinophils, neutrophils, lymphocytes into BALF Table 6. Suppression of cellular infiltration was observed in OVA-challenged mice. OVA-sensitised mice that were treated with FOLME at 400 mg/kg showed reduced eosinophil, leukocytes, lymphocytes and neutrophil counts versus mice treated with lower doses of FOLME 200 mg/kg. The drug control dexamethasone also exerted similar suppressive effects on cellular influx into BALF after OVA challenge. Wet/dry weight ratio was higher in sensitized group when compared with non-sensitized group. Treatment of Dexamethasoneat a dose of 1 mg/kg reduced the wet/dry weight ratio which was gained during the OVA induced asthma. Pre-treatment with FOLME at the dose of 200 and 400mg/kg reduced the wet/dry weight in a dose-dependent manner table 7. Ova albumin Induced group increased LPO and reduced SOD and GSH in lunghomogenate. *F. Officinalis* extracts significantly reversed the effect of inducer on LPO level table 8. The tissues were stained with hematoxylin and eosin. The airways

in asthma undergo significant structural remodelling. In the present study maximum pathological changes were observed in sensitized group. Dexamethasone and the methanolic extract of *F. officinalis* protected the lungs from pathological changes induced by OVA. Also Animals which were not sensitized with Ovalbumin but only treated with *F. officinalis* at the dose of 400 mg/kg protected the lungs from pathological changes induced by OVA (Fig 1).

Discussion and Conclusion

The present study was carried out to scientifically apprise some of the folklore and ethnopharmacological uses of *F. officinalis.* In this study, an attempt has been made to evaluate preliminary phytochemical, antioxidant and antiasthmatic activity of *F.officinalis.* Preliminary phytochemical investigation of *F. officinalis* revealed the presence ofcarbohydrates in all solvent extracts, glycoside, alkaloids, flavanoids and steroids in ethyl acetate extract, flavanoids and steroids in chloroform extract, glycoside, alkaloids, flavanoids and steroids in methanolic extract. Natural extracts contain number of antioxidant components derived from plants, such as coumarin, benzoic acid, tannic acid, cinnamic acid, and flavonoids. Sakuranetin is a flavonoid used in treatment which can attenuate airway hyper-responsivenessAHR, while decreasing 8-isoprostane, Th2 pro-inflammatory cytokines, IgE, and vascular endothelial growth factor levels, as well as remodeling airways by inhibiting NF-κB activation (Sakoda *et al.,* 2016; Toledo *et al.,* 2013). Astragalin, another flavonoid, suppresses eosinaphilia infiltration stimulated by LPS and H_2O_2via the Toll-like receptor 4-PKCβ-NADPH signaling pathway (Cho *et al.,* 2014). Previous studies have shown that cells involved in an asthmatic inflammatory process have a capacity for producing reactive oxygen species (ROS). Activated eosinophils, neutrophils, monocytes, and macrophages generate superoxides (O_2-) via a membrane-associated NADPH-dependent complex. The subsequent dismutation of O_2- can result in the formation of hydrogen peroxide (H_2O_2). O_2- and H_2O_2 are moderate oxidants and both are critical in the formation of potent cytotoxic free radicals in biological systems through their interactions with other molecules (Ramos *et al.,* 1992). This process is involved in asthmatic inflammation; moreover, the concentration of nitric oxide (NO) is increased in airways of asthmatic subjects (Kharitonoy *et al.,* 1994). Many studies have reported increased indices of oxidative stress in the blood and airways of asthmatic subjects (Denny *et al.,* 2003). Airway epithelial cells are in close contact with the external environment in humans. When asthmatic patients are exposed to exogenous ROS-such as environmental tobacco smoke (Kobayashi *et al.,* 2014), airborne pollution (Delfino *et al.,* 2013), home dust mites (Setiawan *et al.,* 2016) or sulfar mustard (Nobakht *et al.,* 2016) in the air, which may trigger symptoms of asthma. Increases in ROS levels are strongly related to the severity of asthma in patients (Dozor, 2010). There are higher amounts of ROS and RNS in asthmatic patients, which lead to airway inflammation (Zuo *et al.,* 2013). Some of the commonly used medicinal plants in asthma were *Ephera sinica*, *Allium sativum*, *Trifolium pratense*, *Coleus barbatus*, *Echinacea angustifolia*, *Scutellaria lateriflora*. These plants contain steroids, flavanoids, alkaloids and glycosides. In present study, as per the screening presence of flavanoids may be responsible for the antiasthmatic effect of *F. officinalis.* Leaves part of the plant was screened for antiasthmatic activity against histamine and acetylcholine induced contraction on isolated guinea pig ileum and isolated guinea pig tracheal chain models.Antioxidant activity of *F. officinalis* was performed including superoxide scavenging activity. The inhibitory concentration (IC_{50}) of *F. officinalis* (FOLME) against Superoxide scavenging assays found to be 24.40 in comparison with ascorbic acid used as standard 16.45. These various antioxidant activities were compared with standard antioxidants such as ascorbic acid. Guinea pigs have been the most commonly used small animal species in preclinical studies related to airway diseases. These animals show physiological analogy to human bronchi and asthma syndrome. Histamine when inhaled causes hypoxia and leads to convulsion in the guinea pigs and causes very strong smooth muscle contraction, profound hypotension, and capillary dilation in the cardiovascular system. A prominent effect caused by histamine is severe bronchoconstriction in the guinea pigs that causes asphyxia and death (Goodman & Gilman 1980). Bronchodilators can delay the occurrence of these symptoms. In the present study, we have used histamine and acetylcholine as spasmogens in the form of aerosols to cause immediate bronchoconstriction in guinea pigs. The histamine (10µg/ml) produced dose dependent contraction of guinea pig ileum as indicated in the graph (Table 2). The FOLEA and FOLME significantly inhibited ($p<0.05$) the contractile effect of histamine on isolated guinea pig ileum preparation, inhibition of contractile response produced by FOLME is less in comparison of FOLEA. The percentage relaxation of FOLEA on histamine induced contraction was found to be 54.20%. Similarly, the percentage relaxation of FOLME on histamine induced contraction was found to be 90.35% in comparison with Chlorpheniramine (10µg/ml) 96.26%. Therefore, the FOLME showed almost similar antihistaminic effect that of Chlorpheniramine maleate. As shown in table 3 of isolated guinea pig tracheal chain preparations, FOLME was found to possess antiasthmatic effects. The FOLME extract at concentrations of 20 mg/ml was able to significantly inhibit contractions induced by both spasmogen (acetylcholine) on the tracheal chains. The action of the extract was found to be similar to that of the standard drug. OVA has been used as an allergen in asthma animal models and the utility of OVA-induced asthma model has been well established and this model has been widely used to evaluate anti-asthmatic effects and immunological mechanisms involved in the pathogenesis of asthma. Therefore, the present study focused on the inhibitory effect of FOLME on the cell numbers. To distinguish the inflammatory cells and count the cell numbers. Changes in total cell levels in the BALF were examined after the last OVA challenge. In the OVA-sensitised mice, infiltration of inflammatory cells was observed. OVA challenge significantly ($p < 0.05$) induced influx of eosinophils and neurophils into BALF Table 4 Suppression of cellular infiltration was observed in OVA-challenged mice. OVA-sensitised mice that were treated with FOLME at 400 mg/kg showed reduced eosinophil and neutrophil counts versus mice treated with lower doses of FOLME 200 mg/kg. The drug control Dexamethasone also exerted similar suppressive effects on cellular influx into BALF after OVA challenge. From the results of both *in-vitro* and *in-vivo* models, it was concluded that, FOLEA and FOLME possess antiasthmatic activity. *F.officinalis* contains the phytoconstituents like flavonoids and phenolioc

compounds which may be responsible for its antiasthmatic activity.The broad pharmacological aspects of *F.officinalis* already summarized in our previus review article (Dutta *et al.,* 2019).Additionally the antioxidant property of *F. officinalis* might also be one of the reasons for its above said activity. These findings scientifically validated the traditional claim of *F. officinalis* for treating asthma in the folk medicine. Further, the study may be extended to isolate and validate the active principal which is responsible for the antiasthmatic activity.

Table 1 : % Inhibition of ascorbic acid, petroleum ether, ethyl acetate, chloroform and methanolic extract using superoxide radical scavenging method

S. No.	Conc. (µg/ml)	Ascorbic acid % Inhibition	Pet. ether % Inhibition	Ethyl acetate % Inhibition	Chloroform % Inhibition	Methanol % Inhibition
1	20	52.327	23.836	39.210	39.633	47.813
2	40	58.815	32.863	43.864	42.736	54.866
3	60	64.456	40.338	50.493	44.569	57.968
4	80	70.380	42.313	61.071	46.544	62.482
5	100	82.651	45.557	63.610	49.788	65.867
	IC 50	**16.45**	**109.43**	**55.03**	**104.91**	**24.40**

Table 2 : Histamine (10µg/ml) induced contraction on guinea pig ileum

Dose (ml)	Height of Response (mm)			
	Control	Standard	FOLEA	FOLME
0.1 ml	12.33±1.366	11.83±0.983	11.83±1.940	11.66±1.505
0.2 ml	22.83±1.602	23.16±1.940	24.5±2.428	23.5±1.974
0.4 ml	34.5±2.588	33.00±3.521	35.5±2.345	33.83±1.602
0.8 ml	52.66±2.658	51.33±3.559	52.50±1.870	51.33±1.211
1.6 ml	44.50±1.870	44.66±0.816	44.83±2.136	42.33±1.632

Table 3 : Effect of FOLEA and FOLME on histamine(10 µg/ml)induced contraction on guinea pig ileum

S. No.	Treatment	Dose (ml)	Height of Response (mm)	% Relaxation
1	Normal Saline + Histamine	0.2 ml + 0.8 ml	53.5±2.428	-
2	Standard + Histamine	0.2 ml + 0.8 ml	2.0±0.632	96.26
3	FOLEA (5 mg/ml)+ Histamine	0.2 ml + 0.8 ml	38.66±2.581	27.73
4	FOLEA (10 mg/ml)+ Histamine	0.2 ml + 0.8 ml	30.66±2.658	42.69
5	FOLEA (20 mg/ml)+ Histamine	0.2 ml + 0.8 ml	24.5±2.664	54.20
6	FOLME (5 mg/ml)+ Histamine	0.2 ml + 0.8 ml	15.5±1.378	71.25
7	FOLME (10 mg/ml)+ Histamine	0.2 ml + 0.8 ml	11.83±0.752	77.88
8	FOLME (20 mg/ml)+ Histamine	0.2 ml + 0.8 ml	5.16±0.752	90.35

Table 4 : Acetylcholine (10µg/ml) induced contraction on guinea pig trachea

Dose (ml)	Height of Response (mm)			
	Control	Standard	FOLEA	FOLME
0.1 ml	14.5±1.378	13.83±0.752	14.16±2.136	14.50±1.760
0.2 ml	25.00±1.673	23.33±1.632	26.66±2.732	25.66±2.338
0.4 ml	36.66±2.581	33.16±3.311	37.33±2.338	35.83±1.602
0.8 ml	54.83±2.401	51.50±3.449	54.16±1.722	53.50±1.870
1.6 ml	47.00±1.6	45.00±1.00	46.00±1.700	45.00±1.600

Table 5 : Effect of FOLEA and FOLME on acetylcholine (10µg/ml) induced contraction on guinea pig trachea

S. No.	Treatment	Dose (ml)	Height of Response (mm)	% Relaxation
1	Normal Saline + Ach	0.2 ml + 0.8 ml	52.0±3.405	-
2	Standard + Ach	0.2 ml + 0.8 ml	2.33±0.516	95.51
3	FOLEA (5 mg/ml)+ Ach	0.2 ml + 0.8 ml	40.83±2.483	21.48
4	FOLEA (10 mg/ml)+ Ach	0.2 ml + 0.8 ml	33.33±2.732	35.90
5	FOLEA (20 mg/ml)+ Ach	0.2 ml + 0.8 ml	25.66±2.338	50.65
6	FOLME (5 mg/ml)+ Ach	0.2 ml + 0.8 ml	17.16±1.169	67.00
7	FOLME (10 mg/ml)+ Ach	0.2 ml + 0.8 ml	14.00±1.095	73.07
8	FOLME (20 mg/ml)+ Ach	0.2 ml + 0.8 ml	7.66±0.816	85.26

Table 6 : Effects of ethyl acetate and methanolic extract of *F. officinalis* on OVA-induced BAL inflammatory cells

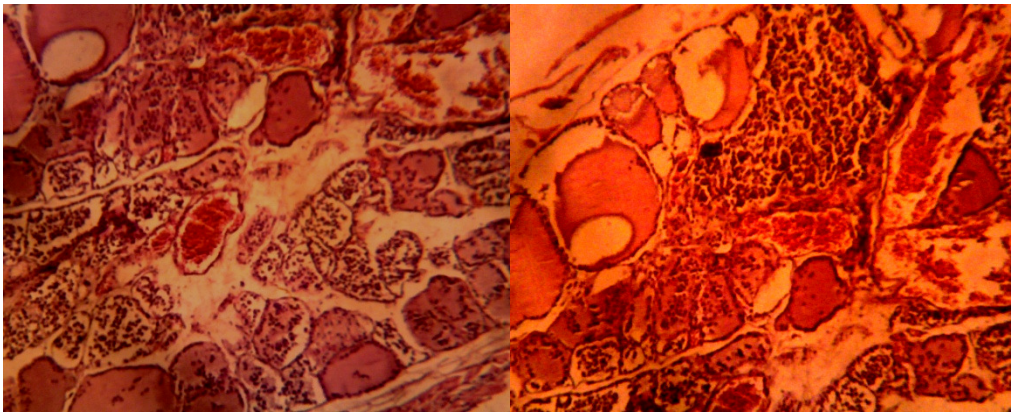
Group	Treatment	Neutrophiles	Lymphocytes	Eosinophiles	Leucocytes
I	Control	2.90±0.163	6.93±0.078	0.08±0.015	7.80± 0.060
II	Ova Albumin	7.14±0.894	15.31±0.630	1.03±0.052	16.29±1.021
III	Ova + Dexa (STD	3.10±0.132	7.92± 0.159	0.11±0.013	8.93±0.204
IV	Ova+ FOLME (200 mg/kg)	6.64±0.082	11.12±0.265	0.42±0.085	12.86±0.388
V	Ova+ FOLME (400mg/kg)	4.90±0.130	9.73±0.106	0.16±0.020	9.94±0.120

Table 7 : Effect of *F. officinalis* on lung wet-to-dry weight ratio in asthma and chronic lung inflammation

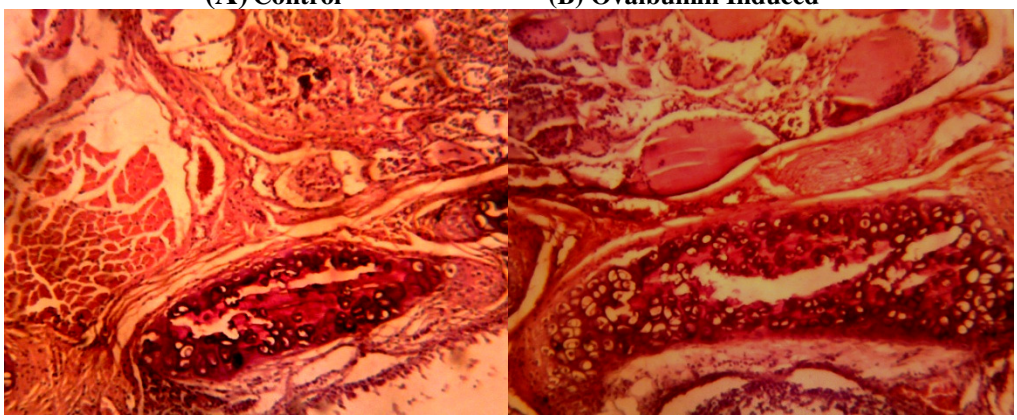
Group (n=5)	Treatment	Lung weight ratio(wet/dry) (Mean ± SEM)
I	Control	2.73±0.710
II	Ova Albumin	3.58±0.675
III	Ova+Dexa (STD)	2.79±0.141
IV	Ova+ FOLME (200 mg/kg)	2.77±0.516
V	Ova+ FOLME (400mg/kg)	2.71±0.039

Table 8 : Effect of *F. officinalis* extract on lung antioxidant status

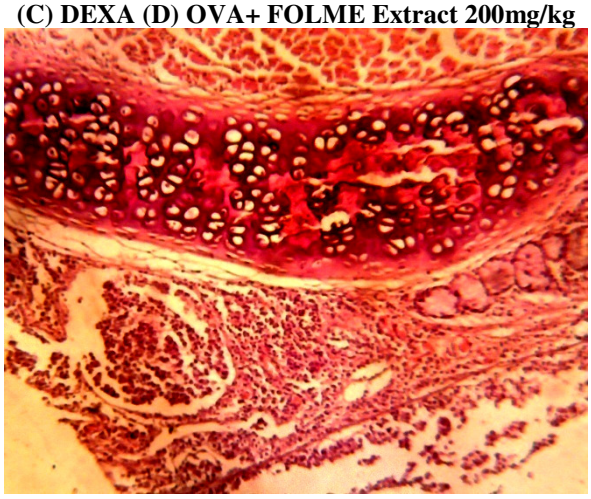
Group	Treatment	MDA level	SOD level	GSH level
I	Control	67.35±7.960	91.37±13.52	2.73±0.710
II	Ova Albumin	126.95±11.202	19.284±9.981	3.58±0.675
III	Ova+Dexa (STD)	75.45±8.306	75.801±7.832	2.79±0.141
IV	Ova+ FOLME (200 mg/kg)	115.5±9.076	58.14±3.845	2.77±0.516
V	Ova+ FOLME (400mg/kg)	95.15±9.36	63.63±6.058	2.71±0.039



(A) Control **(B) Ovalbumin Induced**



(C) DEXA (D) OVA+ FOLME Extract 200mg/kg



(E)OVA+ FOLME Extract 400mg/kg

Fig. 1 : (A) Control group showing medium sized airway normal structure, epithelium showing mucous cells; (B) Negative control group showing cellular debris in bronchial, lumen, infiltration of eosinophils and constricted bronchioles; (C,D,E) Treated groups (Dexa, FOLME 200 mg/kg and FOLME 400 mg/kg showing mild vascular congestion and thickness in nasal septum, other areas showing normal structure.

Ethics approval and consent to participate
All animal experiments were performed with prior permission from Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal, India (approval no. PBRI/IAEC/PN-18032)

Human and animal rights
No human were used in this study. Animal care and experiments were performed in accordance with OECD guidelines no. 423

Consent for publication
Not applicable

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Not applicable

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Conflict of interest
The authors declare no conflict of interest, financial or otherwise.

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