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ANTIHISTAMINIC AND ANTICHOLINERGIC STUDIES ON THE STEM EXTRACTS OF EUPHORBIA HETEROPHYLLA L.

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ABSTRACT: The present investigation has been carried out to evaluate the *in vitro* and *in vivo* antihistaminic and anticholinergic activities for the stem extracts of *Euphorbia heterophylla L.* Preliminary phytochemical screening has been carried out on the hydroalcoholic and acetone extracts of the plant. The antihistaminic activity was studied *in vivo* by histamine-induced bronchospasm and *in vitro* by histamine-induced guinea pig ileum contractions. The anticholinergic activity was studied by acetylcholine-induced bronchospasm and *in vitro* by acetylcholine-induced guinea pig ileum contractions. Pre convulsion time and percentage inhibition of contractions were calculated. Preliminary phytochemical screening showed the presence of flavonoids, tannins, alkaloids, glycosides and steroids. In histamine-induced bronchospasm studies acetone extracts of the plant have significantly increased PCT 4.10 and in acetylcholine-induced bronchospasm studies it was 10.23 for hydroalcoholic extract by Tukey's test (*$p<0.05$), compared with control. In histamine-induced ileum contraction studies, the hydroalcoholic extract exhibited response 4.3 with 18.2% inhibition. In acetylcholine-induced ileum contraction studies, the hydroalcoholic extract showed 4.2 with 18.2% inhibition by Dunnett's test. ($p<*0.05$). The results of present study indicate that plant hydroalcoholic extract showed better anticholinergic activity. Therefore stem extracts of *Euphorbia heterophylla* can be used as antihistaminic and anticholinergic agents which suggest their usage for various therapeutic ailments such as asthma, liver damage, inflammation, and ulcer *etc.* The activity may be due to the phytochemicals which need to be further explored out.

Key words: *Euphorbia heterophylla*, bronchospasm, ileum contractions, therapeutic ailments, phytochemicals

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INTRODUCTION

Plants have been an inbuilt and vital aspect of India's health protection system. The plant kingdom has long served as a bountiful source of useful drugs. The potential established therapeutic properties of the plants that have been scientifically validated in recent times. *Euphorbia heterophylla L.* is branched shrub belonging to the family *Euphorbiaceae*, widely distributed in South Asian countries. The plant was reported for activities like wound healing (Omale James et. al., 2010*),* anti-inflammatory (Falodun et. al., 2006), antimicrobial and anticancer activity (Meenakshi Sundaram et.al., 2010), hepatoprotective activity (Apiamu Augustine et.al., 2013). The leaves were reported to contain quercetin (Falodun et.al., 2004), antidiabetic activity (Annapurna, et.al., 2014), effect of plant extraction kidney, liver and pancreatic functions was reported (Okolie Ngozi Paulinus et.al., 2015), stigmasterol and 4-hydroxy benzoic acid were isolated from the leaf extracts showed good activity against xanthine oxidase enzymes (Abiodun fa lo dun et.al., 2008) diterpenoids were isolated from roots (Rowan et.al., 2001). The therapeutic benefits of the plant have been the major cause of number of chemical and pharmacological studies. Traditional uses of the plant include, purgative, extract of the decoction of leaves is used in the treatment of respiratory tract infections and asthma (Erden 1999). The enteric nervous system is considered to be an independent nervous system that controls and coordinates gastrointestinal motility. This motility is regulated by number of mediators, mainly acetylcholine (ACh), histamine, 5hydroxytryptamine (5HT), bradykinins, prostaglandins, substance P, and cholecystokinins which produce their contractile effects through an increase in cytosolic Ca 2+(Goyal et.al., 1996; Gilani et.al., 2008).On the basis of its traditional use in gastric disorder or respiratory diseases, the present study was undertaken to elucidate the possible underlying mechanism and the effect of the hydroalcoholic and acetone extracts of the plant on histamine and acetylcholine-induced smooth muscle contraction.

MATERIALS AND METHODS

Chemicals and reagents:

Histamine hydrochloride, acetylcholine, chlorpheniraminemaleate, atropine sulfate were purchased from Sigma-Aldrich chemical Co.

Experimental animals:

Guinea pigs (400–600 g) of either sex were purchased from Mahaveer enterprises, Hyderabad, Telangana, India, housed in standard conditions of temperature (22 ± 2°C), relative humidity (55 ± 5%), and light (12 h light/dark cycles). They were fed with standard pellet diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Nirmala College of Pharmacy, Atmakur, Mangalagiri, Guntur district, Andhra Pradesh, India, approval no 012/IAEC/NCPA/PhD/2016-17, as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Collection of plant material

The plant material was collected from local grounds of Prasadampadu and Enikepadu coordinates 16°32′45″N 80°34′12″E of Vijayawada rural region, Krishna district, Andhra Pradesh, India. The plant specimen was identified and authenticated by Dr. P. Satya Narayana Raju, plant taxonomist, Dept. of Botany & Microbiology, Acharya Nagarjuna University (ANU), Guntur (Dt), Andhra Pradesh, India. A voucher specimen 003/VIPW was deposited in Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada, A.P., India and Nirmala College of Pharmacy, Atmakur, Mangalagiri, A.P., India for future reference.

Preparation of the extract

The stems were dried under the sun, powdered coarsely using a mechanical grinder. Then extraction was carried out using 50:50 methanol-water and acetone as solvents by Soxhlet apparatus (JSGW).The extracts obtained were dried using Vacuum evaporator (Biotech). The percentage yield of extracts on air dried basis was obtained to be 30.33% w/w for hydroalcoholic extract and 27.21% w/w for acetone extract respectively.The extracts were preserved in refrigerator till use.

Phytochemical screening

The preliminary phytochemical screening was carried out on the hydroalcoholic and acetone extracts to reveal the presence of phytochemicals present in the extracts (Evans 2005).

Acute toxicity testing:

The animals were overnight fasted prior to the experiment. Different doses (50–2000 mg/kg, orally) of the hydroalcoholic and acetone extracts were administered to groups of guinea pigs. The animals were observed continuously for 1 hr, next half-hourly intervals for 4 hrs for any gross changes in their behavior and then up to 24 hrs for any mortality as per the Organization for Economic Co-Operation and Development (OECD) guidelines 425 (OECD guidelines 2008).

Histamine- induced bronchospasm in guinea pigs

Guinea pigs of either sex were divided into four groups. Each group comprised of four animals, Group-1: Control group animals received distilled water

Group-2: Standard group animals received chlorpheniraminemaleate

Group-3: Test-1 group animals received hydroalcoholic extract of *Euphorbia heterophylla* (EHHA)

Group-4: Test-2 group animals received acetone extract of *Euphorbia heterophylla* (EHAE)

Animals were exposed to 0.1% w/v of histamine dihydrochloride aerosol in a histamine chamber (Sigma Scientific). Progressive dyspnoea was observed in animals when exposed to histamine aerosol. Pre convulsion time (PCT) was determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsions on day 0 (T_1). As soon as dyspnoea commenced, the animals were removed from the chamber and placed in fresh air. Animals were given TPHA and TPAE at a dose of 400 mg/kg orally (*p.o.*) once a day for 7 days. On the seventh day, 2 hrs after the last dose, PCT was recorded (T_2).

Acetylcholine- induced bronchospasm in guinea pigs

Guinea pigs of either sex were divided into four groups. Each group comprised of four animals, Group-1: Control group animals received distilled water

Group-2: Standard group animals received atropine

sulfate Group-3: Test-1 group animals received EHHA

Group-4: Test-2 group animals received EHAE

Animals were exposed to 0.5% acetylcholine chloride aerosol. The experimental procedure was followed as above (Chandrakant Nimgulkar et.al., 2011).

The percentage increase in time of PCT was calculated using the following

formula: Percentage increased in time of $PCT = \left(1 - \frac{T1}{T2}\right) \times 100$

Where T_1 is PCT on day 0 and T_2 is PCT on day 7.

Statistical analysis

Results of the study were expressed as a mean ± Standard error of the mean (SEM) and analyzed statistically using One-way analysis of variance, followed by Tukey test for multiple group comparison with a control to find out the level of significance. Data were considered statistically significant at $^{*}p<0.05$ and **p<0.01 respectively.

Histamine- induced guinea pig ileum contraction

Guinea pigs of body weight 200–500 g were selected and allowed to starve overnight with free access to water. The animals were killed by a blow on the head and exsanguinated. The ileum was isolated, cut into individual sections of 1cm, and then divided into four groups; each group consisted of four ileums.

Group 1: Control group animals received histamine

Group 2: Standard group animals received chlorpheniramine

Group 3: Test-1 group animals received EHHA

Group 4: Test-2 group animals received EHAE

The isolated ileum was mounted in a 30 ml Organ bath (Lab Tree India) containing a tyrode solution, maintained at 37 ± 1 ^{0}C, and gassed with air. The tissue was equilibrated for 45 min during which the bath solution was replaced every 10 min. A drug tissue contact time of 1 min was maintained and 15 min time cycle was followed by recording the response of histamine. After obtaining a dose response curve of histamine on ileum, the extracts (0.5 mg) were added to the reservoir and same doses of histamine were repeated in presences of extracts.

Acetylcholine- induced guinea pig ileum contraction

Group 1: Control group animals received acetylcholine

Group 2: Standard group animals received atropine

sulfate Group 3: Test-1 group animals received EHHA

Group 4: Test-2 group animals received EHAE

The same above experimental procedure was carried out for the study (Savita et.al., 2011).

Statistical analysis

The results of the study were expressed as mean ± SEM and analyzed statistically using One-way Analysis of Variance (ANOVA) followed by Dunnett's test for individual comparison of groups with control. Data were considered statistically significant at *P<0.05 and **P<0.01 respectively.

RESULTS

Phytochemical screening:

Preliminary phytochemical screening of hydroalcoholic and acetone extracts of *Euphorbia heterophylla* showed the presence of alkaloids, glycosides, terpenoids, tannins, flavonoids, steroids, amino acids, and proteins.

Acute toxicity testing:

The hydroalcoholic and acetone extracts of the plant were administered orally to guinea pigs up to a dose of 2000 mg/kg body weight. After 24 hrs, the animals were found to be well tolerated, safe with no signs of mortality and toxicity. Hence a safe and therapeutically effective dose of 400 mg/kg of body weight was selected for the present study.

Effect of EHHA and EHAE on histamine- induced bronchospasm in guinea pigs

The plant extracts displayed spasmolytic effect, EHHA exhibited PCT 4.56 and EHAE 4.19 at 400 mg/Kg compared to control. Both extracts manifested complimentary effects comparable with standard drug chlorpheniramine which showed significant PCT 8.77 at 2 mg/Kg (**p<0.01) (Table 1 & Figure 1).

Table-1: Effect of EHHA and EHAE on histamine-induced guinea pig bronchial contraction

S.No	Groups	Drug and Dose	PCT Mean ± SEM
1	Control	Distilled water p.o.	2.22±0.24
2	Standard	Chlorpheniramine 2mg/kg	8.77±0.43**
3	Test-1	EHHA 400mg/Kg	4.56 ±1.06
4	Test-2	EHAE 400 mg/Kg	4.10±0.82

Each value was expressed as mean ± SEM, where n=4 in each group;*p<0.05, **p<0.01 compared with control by one-way ANOVA, Tukey's test.

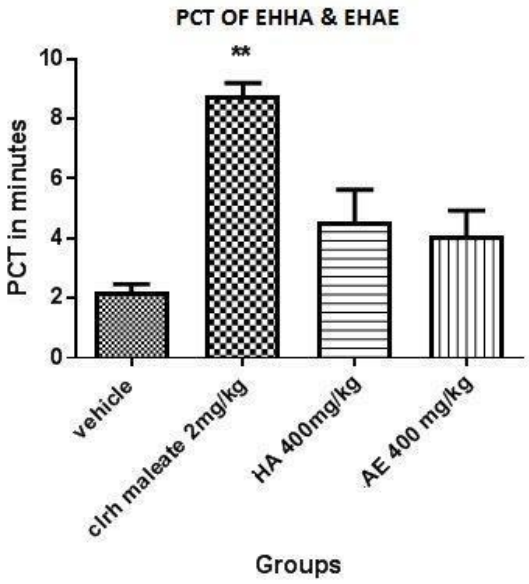

Figure-1: Effect of EHHA and EHAE on histamine-induced guinea pig bronchial contraction (clrh- chlorpheniramine)

Effect of EHHA and EHAE on acetylcholine-induced bronchospasm in guinea pigs

The PCT of EHHA at 400 mg/Kg was 10.23 (*p<0.05), and EHAE 5.44, which indicate that EHHA manifested superior spasmolytic activity than EHAE. The results of anticholinergic activity were comparable to standard drug atropine sulfate (Table 2 and figure 2).

Table- 2: Effect of EHHA and EHAE on acetylcholine-induced guinea pig bronchial contraction

S.no	Groups	Drug and Dose	PCT Mean ± SEM
1	Control	Distilled water p.o.	3.22±0.60
2	Standard	Atropine sulphate 2mg/kg	11.60±1.24**
3	Test-1	EHHA 400mg/Kg	10.23 ± 1.73*
4	Test-2	EHAE 400mg/Kg	5.44 ± 0.91

Each value was expressed as mean ± SEM, where n=4 in each group at *p<0.05 **p<0.01 compared with control by one-way ANOVA, Tukey's test.

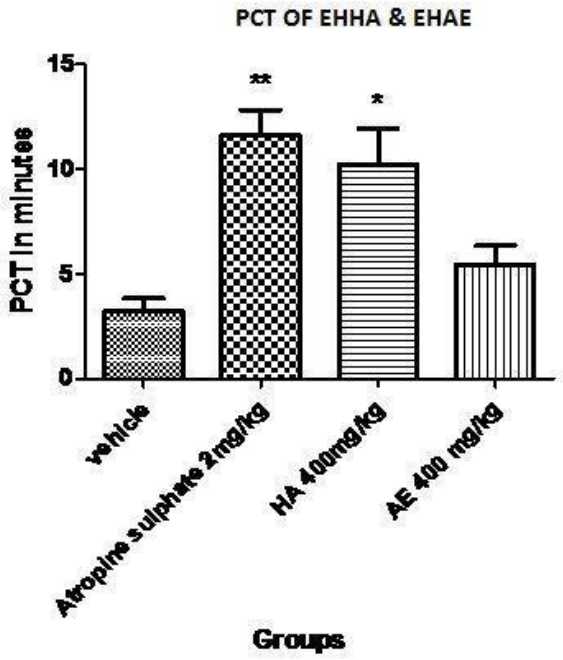

Figure-2: Effect of EHHA and EHAE on acetylcholine-induced guinea pig bronchial contraction

Effect of EHHA and EHAE on histamine -induced guinea pig ileum contractions

The plant extracts exhibited meaningful antihistaminic activity compared to control. EHHA at 0.5 mg exhibited response 4.3 with 18.2% inhibition (p<0.05), and EHAE 4.4 with 17% inhibition of ileum contractions. The standard drug chlorpheniramine produced response 1.8 with 63.3% inhibition (p<0.05) (Table 3 & Figure 3).

Table-3: Effect of EHHA and EHAE on histamine-induced guinea pig contractions on ileum

S.No	Groups	Drug and Dose	Response Mean ± SEM	% Inhibition
1	Control	Histamine 0.5 mg	4.9±0.08	0%
2	Standard	Chlorpheniramine 0.5mg	1.8±0.91*	63.3%
3	Test-1	EHHA 0.5 mg	4.3 ± 0.29*	18.2 %
4	Test-2	EHAE 0.5 mg	4.4 ± 0.56*	17 %

Each value was expressed as mean±SEM, where n=4 in each group at *p<0.05 compared to control by one-way ANOVA, Dunnett's test.

Effect of EHHA and EHAE on acetylcholine-induced guinea pig ileum contractions

The results indicate that EHHA showed response 4.2 with 24% inhibition (p<0.05) and EHAE 4.5 with 18.2 % (p<0.05) inhibition respectively compared to control. The standard drug atropine sulfate exhibited 2.2 with 60% inhibition (p<0.05). The % inhibition of EHAE was more when compared to EHHA (Table 4 and Figure 4).

Table-4: Effect of EHHA and EHAE on acetylcholine-induced guinea pig contractions on ileum

S.No	Groups	Drug and Dose	Response Mean ± SEM	% Inhibition
1	Control	Acetylcholine 0.1mg	5.5±0.27	0%
2	Standard	Atropine sulphate 0.5mg	2.2±0.81*	60%
3	Test-1	EHHA 0.5 mg	4.2 ± 0.37*	24 %
4	Test-2	EHAE 0.5 mg	4.5 ± 0.64*	18.2 %

Each value was expressed as mean ± SEM, where n=4 in each group at *p<0.05 compared with control by one-way ANOVA, Dunnett's test.

DISCUSSION

The results of the current study indicate that plant extracts possess antihistaminic and anticholinergic activities. The distinctive finding in the current study was that plant extracts possess spasmolytic effect which may be attributed to antagonizing histamine and acetylcholine-induced contractions in the guinea pig bronchi and ileum tissues. The effects were similar to antihistaminic drug atropine, the 5HT antagonist and muscarinic blocker, chlorpheniramine maleate. The support for the fact that plant extracts possess antihistaminic and anticholinergic activity comes from the findings of the literature review. Analogous to our study the aqueous extract of the leaves of the plant has shown significant anti-inflammatory activity, which may be due to the presence of high amount of flavonoids in the aqueous leaf extract indicating plant can be used in the treatment of inflammatory disorders like asthma (Falodun et.al., 2006). Further *E.heterophylla* has anthelmintic potential, as both ethanolic and aqueous crude extracts were 100% effective in inhibiting worm motility. The worm motility decreased with increasing extract concentration, the paralysis could be linked to the action of flavonoids, tannins, and alkaloids that were richly present in the plant. It was suggested that tannins bind to free proteins in the gastrointestinal tract of host animals (Nalule et.al., 2013). EHHA at 0.5 mg concentration showed 24% inhibition in acetylcholine-induced ileum contraction study.

Laxative, anticoagulant and abortifacient studies on the aqueous extract of leaves were studied (Unekwe et.al., 2006). Phorbols were ascribed to laxative effect which were present in plant extract (Falodun et.al., 2004). As an herbal laxative, sometimes life-threatening side effects may be associated with its use. The study on the effect of leaf extract on kidney, liver and pancreatic functions and plasma electrolytes in rabbits found that, elevation of plasma ALT, AST indicate hepatic damage, elevation of blood amylase indicates pancreatitis, an increase in kidney urea indicates impairment of kidney function, suggesting toxic effects on vital organs (Okolie et.al., 2015). *E. heterophylla* is listed as one of the toxic species (Okeniyi et.al., 2012).

The effect of leaf aqueous extract on liver hepatocytes was studied (Apiamu et.al., 2013), found that there was no significant effect on plasma protein, serum albumin, and blood urea nitrogen which forms an index of healthy biochemical status of liver and moreover there was no significant effect on the activities of ALT (Alanine aminotransferase and AST (Aspartate aminotransferase), indicating that plant may not be hepatotoxic and other tissue injuries relative to AST, however, caution should be taken in its use for medicinal and grazing purposes. The plant may be toxic in higher doses. Therefore dosage could be a criterion which can be standardized in minimizing the toxic effects. The ethanol extract of the plant was studied for wound healing activity, found that due to the presence of tannins, there was wound contraction (Omale James et. al., 2010).

The plant extracts were showing better anticholinergic activity, EHHA PCT 10.23 when compared to standard atropine 11.60 in the acetylcholine-induced bronchospasm study. In histamine-induced bronchospasm study, PCT for EHHA was 4.56 when compared to standard drug chlorpheniramine 8.77. Further in the acetylcholine-induced ileum contraction study also EHHA exhibited 24% inhibition than EHHA 18.2% in histamine-induced ileum contraction study. EHHA manifested better antihistaminic and anticholinergic activity than EHAE in both models of study.

The guinea pig bronchial and ileum smooth muscles have H1 receptors. The stimulation of H1 receptors causes contraction of bronchi and ileum (Goodman 2001). On smooth muscle, histamine produced membrane depolarization and increased excitability (Hemming et.al., 2000; Matsumoto et.al., 2009) One of the possible mechanisms for the spasmolytic activity of the extract could be mediated through the inhibition of histaminic receptors. In this study, EHHA and EHAE inhibited histamine and acetylcholine-induced contractions of guinea pig bronchi and ileum.

Acetylcholine, a neurotransmitter, is released by the parasympathetic nervous system and plays an important physiological role in the regulation of gut movements (Gilani et.al., 1997). The ileum is supplied with cholinergic nerves that produce contractions through muscarinic receptors, and the cholinergic nerve plays an important role in the regulation of gastrointestinal motility (Makhlouf et.al., 2006). Receptor-operated channels are activated by Ach (acetylcholine) through binding with muscarinic receptors. There are mainly two mechanisms related to Ach-induced contractions through binding with muscarinic receptors. One of the mechanism involves contraction through IP3 induced Ca 2+ release (Komori et.al., 1991), whereas, the other mechanism involves membrane depolarization by the activation of nonselective cation channels to stimulate the voltage-dependent Ca 2+ channels (Sims 1992).

Various studies support the involvement of 5HT in the regulation of gastrointestinal motility. 5HT3 antagonists have shown to possess gastrokinetic and antiemetic properties (Leibundgut et.al., 1987). In animals, 5HT produces contraction of smooth muscles through the 5HT2 receptors. 5HT releases the peripheral 5HT3 receptors on the vagal afferent fibers and causes relaxation of the stomach possibly leading to delay in gastric emptying (Andrews et.al., 1990). Other explanation could include an additional action of the antagonists at a site beyond the receptor, for instance, a direct blocking of the cation channels which mediate the Na+ fluxes carrying 5HT3induced depolarization (Cotrim et.al., 2008). The contractile effects of histamine on the isolated guinea pig ileum are known to be mediated through H1 histamine receptors (Black et.al., 1972). EHHE and EHAE inhibited histamine-induced contraction of guinea pig ileum comparable to the standard antihistaminic chlorpheniramine. Basing on this The antagonist activity of EHHE and EHAE against histamine-induced contraction supports the traditional use of *E. heterophylla* in asthma and respiratory tract infections *etc* (Erden et.al., 1999).

Additional support to augment the antihistaminic and anticholinergic nature of the plant extracts was ascertained to the presence of phytochemical constituents. Ascorbic acid was isolated from the aqueous extract of the plant (Keerthana Kesavan et.al., 2014) which was found to be antihistaminic (Kompauer et.al., 2007) and anticholinergic (Wawrzeńska M 1987). Stigmasterol, β- Stigmasterol glucoside was isolated from chloroform and ethyl acetate fractions of *E.heterophylla* leaf (Abiodun Falodun et.al., 2008). Stigmasterol has been studied for antihistaminic activity (Kumar SS et.al., 2011). Anti-inflammatory and anticholinergic properties of stigmasterol were recently reported (Najmeh Mokhber-Dezfuli et.al., 2014).

One of the most numerous and widespread groups of phenolics in higher plants is flavonoids, which inhibit intestinal motility *in vitro* and role of phenolic compounds as spasmolytic is already reported (Bigovic et.al., 2010). Flavonoid quercetin was reported (Falodun et.al., 2004) from the aqueous extract of leaves of the plant which is a known anti-inflammatory, antiallergic, antihistaminic, immunomodulatory drug (Shaik et.al., 2006) acts by various mechanisms like anti-allergic properties characterized by stimulation of immune system, antiviral activity, inhibition of histamine release, decrease in pro-inflammatory cytokines, leukotrienes creation, and suppresses interleukin IL-4 production. It can improve the Th1/Th2 balance and restraint antigen-specific IgE antibody formation. It is also effective in the inhibition of enzymes such as lipoxygenase, eosinophil and peroxidase and the suppression of inflammatory mediators (Mlcek et.al., 2016). Based on this report, the spasmolytic activity of EHHE and EHAE in this study could be attributed to flavonoids and other phenolic compounds present therein.

Interestingly, most of the H1 antagonists are also reported to inhibit the ACh responses, mediated by muscarinic receptors; it could be possible that one component of the extract is responsible for both antihistaminic and anticholinergic effects of extract. Since the specific components are not distinguished, and perhaps more than one component from the extract can inhibit ACh and histamine response. The evident antihistaminic and anticholinergic activity of EHHE and EHAE is in agreement with reported anticholinergic and antihistaminic activity of n-hexane extract of *Zanthoxylum alatum* seeds (Khosrokhavar Beenita Saikia et.al., 2017).

CONCLUSION

The present study revealed that *E.heterophylla* plant extracts exhibit antihistaminic and anticholinergic activities by *in vivo* histamine and acetylcholine-induced bronchospasm study as well as *in vitro* histamine and acetylcholine-induced ileum contraction studies in guinea pigs. Therefore the study directs the utility of plant in various inflammatory conditions like asthma and organ protective studies. The individual components present in the plant extracts need to be further explored out to enhance the therapeutic utilities of the plant.

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Conflicts of interest

There are no conflicts of interest

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