Anticholinergic, antihistaminic, and antiserotonergic activity of n-hexane extract of Zanthoxylum alatum seeds on isolated tissue preparations: An ex vivo study

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Abstract:
Objectives: The aim of this study was to evaluate anticholinergic, antihistaminic, and antiserotonergic activity of the n-hexane extract of the seeds of Zanthoxylum alatum (ZAHE) on isolated ileum of rat and guinea pig and fundus of rat.

Materials and Methods: ZAHE was prepared using soxhlet extraction and cumulative concentration response curves were constructed using various doses on the tissues for acetylcholine (ACh), 5-hydroxytryptamine (5-HT), and histamine with or without n-hexane extract. Atropine, ketanserin, and pheniramine maleate were used as antagonists for ACh, serotonin, and histamine, respectively.

Results: ZAHE-induced concentration-dependent inhibition of isolated ileum and fundus in rat and ileum of guinea pig. The half maximal effective concentration (EC\textsubscript{50}) of ACh in the presence of atropine (10\textsuperscript{-6} M; P < 0.05) and ZAHE (1000 \mu g/ml; P < 0.01) was significantly higher than EC\textsubscript{50} of ACh alone. The EC\textsubscript{50} of 5-HT in the presence of ketanserin (10\textsuperscript{-5} M; P < 0.01) and ZAHE (1000 \mu g/ml; P < 0.05) was higher than EC\textsubscript{50} of 5-HT alone. Similarly, the EC\textsubscript{50} of histamine in the presence of pheniramine maleate (10\textsuperscript{-6} M; P < 0.01) and ZAHE (300 \mu g/ml; P < 0.01 and 1000 \mu g/ml; P < 0.05) was also significantly higher than EC\textsubscript{50} of histamine alone.

Conclusion: From the study, it was observed that ZAHE shows significant anticholinergic, antiserotonergic, and antihistaminic activity. The study provides sufficient evidence that the seeds can be used in gastric disorders, cough, chest infection, etc., as per folklore claims.

Key words: Fundus, guinea pig, ileum, ketanserin, Wistar rat, Zanthoxylum alatum

Plants have been an innate and vital aspect of India’s healthcare system. In recent times, numerous scientific studies are conducted to validate or establish the potential effect of the plants in different disorders.

Zanthoxylum alatum (ZA) is an evergreen small xerophytic important medicinal tree or shrub of the family Rutaceae, native to Himalayan regions in India, which is locally known as Tejphal (Hindi), Tejowati (Sanskrit), Mukthrubi (Manipur), Timur (Nepal). Common names of this plant are Indian Prickly Ash, Nepal pepper, or toothache tree. The ethnomedical importance of bark, fruits, and seeds of ZA are well known since long in indigenous system of medicine as carminative, stomachic, and antihelmintic.[1] The fruit and seeds are used as an aromatic tonic in fever and dyspepsia. Fruits extract is effective in expelling roundworms. In Nepalese folk medicine, ZA is used in cold and cough, tonsillitis, headache, fever, vertigo, diarrhea, and dysentery.[2] ZA is used in Indian folk medicine for treatment of fever, dyspepsia, and cholera.[3] Powdered fruit is mixed with Mentha species, and table salt is eaten with boiled egg for chest infection and digestive problems.[4]

The enteric nervous system is considered to be an independent nervous system that controls and coordinates gastrointestinal motility. This motility is regulated by numerous mediators, mainly acetylcholine (ACh), histamine, 5-hydroxytryptamine (5-HT), bradykinins,
prostaglandins, substance P, and cholecystokinin which achieve their contractile effects through an increase in cytosolic Ca\(^{2+}\).\(^{1,6}\) 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 seeds of ZA n-hexane extract (ZAHE) on ACh, 5-HT, and histamine-induced smooth muscle contraction.

### Materials and Methods

**Drugs and Reagents**

The drugs used were ACh chloride (Sigma-Aldrich, USA), atropine sulfate, (Sigma-Aldrich, USA), serotonin hydrochloride (Sigma-Aldrich, USA), ketanserin (+) tartrate (Sigma-Aldrich, USA), histamine dihydrochloride (Hi-Media, Mumbai, Maharashtra, India), pheniramine maleate (Sigma-Aldrich, USA). Other chemicals used in the preparation of physiological solutions were of analytical grade.

**Plant Material and Preparation of Extract**

Fresh seeds of ZA were collected, identified, weighed, dried in shade, and powdered. Preparation of n-hexane extract was done as per standard methods in soxhlet extractor using rotary evaporator (Buchi, Rotavapor R210, Switzerland). Percentage yield of powder with respect to dry powder was 8.36\% w/w.

**Animals**

Male Wistar albino rats (150–200 g) and guinea pigs (300–400 g) were selected for the study and housed in standard environmental conditions, fed with rodent pellet diet, and drinking water was given _ad libitum_. The study was approved by the Institutional Animal Ethical Committee (770/ac/CPCSEA/FVSC, AAU/IAEC/15-16/367).

**Tissue Preparation**

The rats and guinea pigs were fasted overnight with free access to water before 1 day of the experiment. Animals were sacrificed humanely under ether anesthesia. Ileum of rat and guinea pig were placed in Tyrode’s solution, whereas fundus of rat was placed in Kreb’s solution. Tissue was mounted with the help of two fine hooks. One hook was inserted at one end of the tissue and fixed to the tip of aeration tube while the other was pierced through the opposite end of the tissue and connected to the isometric force transducer (PowerLab, 4/35, 4-Channel Data Acquisition System, Model: ML866/P, ADInstruments, Australia). Before starting the experiments, the tissue strips were equilibrated for 45 min under a resting tension of 1 g. Responses of the tissues were recorded in LabChart 7 software (AD Instruments, Australia) program.

**Ileum**

Ileum of either rat or guinea pig was cleaned off from extraneous tissue, and the lumen was cleaned with gentle care by flushing the Tyrode’s solution into it. A terminal segment of ileum about 1–1.5 cm was mounted in the organ bath maintained at 37°C and continuously bubbled with 95\% O\(_2\) and 5\% CO\(_2\). Then, using various concentration of ACh (10\(^{-10}\)–10\(^{-9}\) M) and ZAHE (30, 100, 300, 1000 μg/ml) alone, ACh in the presence of atropine (10\(^{-4}\) M) and ZAHE (30, 100, 300, 1000 μg/ml), concentration response curve (CRC) of each of them were recorded separately. In case of guinea pig ileum, CRC of histamine (10\(^{-9}\)–10\(^{-3}\) M) and ZAHE (30, 100, 300, and 1000 μg/ml) alone; histamine in the presence of pheniramine maleate (10\(^{-6}\) M) and ZAHE (30, 100, 300, and 1000 μg/ml) were also recorded.

**Fundus**

The fundus portion of the stomach of rat was cut and opened along the lesser curvature into a sheet, and 1 cm long strip was prepared by cutting along the longitudinal fibers. The strip was mounted and allowed to equilibrate in Kreb’s solution maintained at 37°C and continuously bubbled with 95\% O\(_2\) and 5\% CO\(_2\). Thereafter, following addition of 5-HT (10\(^{-9}\)–10\(^{-3}\) M) and ZAHE (30, 100, 300, 1000 μg/ml) alone, 5-HT in the presence of ketanserin (10\(^{-5}\) M), and ZAHE (30, 100, 300, 1000 μg/ml), CRC was recorded.

### Results

ACh (10\(^{-9}\)–10\(^{-3}\) M) produced concentration-dependent contraction on isolated rat ileum [Figure 1a]. ZAHE in cumulative concentrations (30, 100, 300, and 1000 μg/ml) produced concentration-dependent inhibition of the spontaneous contractions on rat ileum [Figure 1b]. The EC\(_{50}\) of ACh in the presence of atropine (1.189 ± 0.121 × 10\(^{-5}\) M; *P < 0.05*) was significantly higher than EC\(_{50}\) of ACh alone (3.368 ± 0.018 × 10\(^{-7}\) M) [Table 1]. Similarly, the EC\(_{50}\) of ACh in the presence of ZAHE (300 μg/ml; 6.293 ± 2.647 × 10\(^{-7}\) M and 1000 μg/ml; 3.860 ± 0.204 × 10\(^{-7}\) M; *P < 0.01*) was significantly higher than EC\(_{50}\) of ACh alone [Table 1]. In the presence of atropine, and ZAHE, a rightward shift in the CRC of ACh was recorded [Figure 1c]. Thus, ZAHE showed atropine-like activity in the ileum of rat.

The effect of 5-HT (10\(^{-9}\)–10\(^{-3}\) M) on isolated fundus of rat showed an increase in spasmodic activity [Figure 2a]. ZAHE in cumulative concentrations (30, 100, 300, and 1000 μg/ml) produced concentration-dependent inhibition of the spontaneous contractions of rat fundus [Figure 2b]. A rightward shift was observed in the dose–response curve to acetylcholine in the absence and presence of antagonist atropine and _Zanthoxylum alatum_ n-hexane extract on isolated ileum of rat.

| Table 1: Half maximal effective concentration values (half maximal effective concentration values) obtained from the cumulative dose–response curves to acetylcholine in the absence and presence of antagonist atropine and _Zanthoxylum alatum_ n-hexane extract on isolated ileum of rat |
| --- |
| **Drug** | **EC\(_{50}\) (mean±SEM)** |
| Acetylcholine (10\(^{-9}\)–10\(^{-3}\) M) | 3.368±0.018±10\(^{-7}\) M |
| Atropine (10\(^{-6}\) M) + acetylcholine | 1.189±0.121±10\(^{-5}\) M* |
| ZAHE (300 1-g/ml) + acetylcholine | 6.293±2.647±10\(^{-7}\) M |
| ZAHE (1000 1-g/ml) + acetylcholine | 3.860±0.204±10\(^{-6}\) M* |

Results are expressed as mean ± SEM (n = 4). *P<0.05, **P<0.01 - significantly different when compared to acetylcholine group. SEM = Standard error of mean.
Histamine ($10^{-9}$–$10^{-3}$ M) produced a concentration-dependent contraction of guinea pig ileum [Figure 3a]. While ZAHE (30, 100, 300, 1000 µg/ml) induced a concentration-dependent inhibition of the spontaneous contractions of guinea pig ileum [Figure 3b]. The $EC_{50}$ of histamine alone was $1.957 \pm 0.058 \times 10^{-7}$ M. ZAHE shifted the CRC of histamine, thereby increasing the $EC_{50}$ of histamine significantly (300 µg/ml, $1.189 \pm 0.045 \times 10^{-6}$ M; $P < 0.01$ and 1000 µg/ml, $1.407 \pm 0.209 \times 10^{-6}$ M; $P < 0.05$) as did pheniramine maleate ($1.852 \pm 0.134 \times 10^{-5}$ M; $P < 0.01$) [Figure 3c and Table 3]. Therefore, the antihistaminic effect of ZAHE on guinea pig ileum could be attributed to a potentiation of the antihistaminic properties of histamine.

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The distinctive finding in this study was that ZAHE has spasmolytic effects on isolated preparation of rat ileum, fundus, and guinea pig ileum. It could antagonize ACh, 5-HT, and histamine-induced contraction in the isolated tissues of rat and guinea pig. The effects were similar to muscarinic blocker atropine, ketanserin, the 5-HT antagonist, and/or pheniramine maleate, the antihistaminic drug.

Discussion

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ACh, a neurotransmitter, is released by the parasympathetic nervous system and plays an important physiological role in the regulation of gut movements. ZAHE produced significant relaxant effect on the CRC for ACh on isolated rat ileum. Pretreatment with atropine abolished the contractile effect of ACh. 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. Receptor-operated channels are activated by ACh through binding with muscarinic receptors. There are mainly two mechanisms related to ACh-induced contractions through binding with muscarinic receptors. One

Figure 2: (a) Typical tracing showing contractile effect of 5-hydroxytryptamine (10⁻⁸–10⁻³ M) on the spontaneous contractions in isolated rat fundus. (b) Typical tracing showing inhibitory effect of Zanthoxylum alatum n-hexane extract (30, 100, 300, and 1000 μg/ml) on the spontaneous contractions in isolated rat fundus. (c) Comparison of dose–response curves of 5-hydroxytryptamine in the absence and presence of antagonist ketanserin and Zanthoxylum alatum n-hexane extract on isolated rat fundus. Values are mean ± standard error mean (n = 4)
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of the mechanism involves contraction through IP_3‑induced Ca^{2+} release,[9] whereas, the other mechanism involves membrane depolarization by the activation of nonselective cation channels to stimulate the voltage‑dependent Ca^{2+} channels.[10] It may be possible that ZAHE binds on muscarinic receptors or affects at least one of these mechanisms. The contractile activity of ACh on ileum was significantly inhibited by ZAHE confirming the presence of anticholinergic components (atropine‑like) in it. The antagonistic action of ZAHE on cholinergic receptor may explain the medicinal use of ZAHE as anti‑diarrheal and other gastric disorder. Antispasmodic activity of leaves, barks, and fruits of ZA was also reported in rabbit jejunum.[11]

Various studies support the involvement of 5-HT in the regulation of gastrointestinal motility. 5-HT_3 antagonists have shown to possess gastrokinetic and antiemetic properties.[12] In animals, 5-HT produces contraction of smooth muscles through the 5-HT_2 receptors. The pronounced inhibitory activity of ZAHE against 5-HT‑induced contraction in the fundus strip of rats indicates that the extract may be helpful in dyspepsia and stomach ulcer by inhibiting the gastric emptying rate.[13] 5-HT releases the peripheral 5-HT_3 receptors on the vagal afferent fibers and causes relaxation of the stomach possibly leading to delay in gastric emptying.[14] The extract also antagonizes 5-HT‑induced contractions of fundus strip such as ketanserin.
Table 2: Half maximal effective concentration values (half maximal effective concentration values) obtained from the cumulative dose–response curves to 5-hydroxytryptamine in absence and presence of antagonist ketanserin and Zanthoxylum alatum n-hexane extract on isolated fundus of rat

| Drug                       | EC so (mean±SEM)       |
|----------------------------|------------------------|
| 5-HT (10^{-6}-10^{-3} M)   | 4.172±0.993x10^{-7} M  |
| Ketanserin (10^{-6} M)+5-HT | 2.114±0.166x10^{-6} M**|
| ZAHE (300 g/ml)+5-HT       | 4.682±1.069x10^{-7} M  |
| ZAHE (1000 g/ml)+5-HT      | 1.708±0.207x10^{-6} M  |

Results are expressed as mean ± SEM (n = 4) *P<0.05, **P<0.01 - significantly different when compared to 5-HT group. SEM = Standard error of mean, 5-HT = 5-hydroxytryptamine

Table 3: Half maximal effective concentration values (half maximal effective concentration values) obtained from the cumulative dose–response curves to histamine in the absence and presence of antagonist pheniramine maleate and Zanthoxylum alatum n-hexane extract on isolated ileum of guinea pig

| Drug                       | EC so (mean±SEM)       |
|----------------------------|------------------------|
| Histamine (10^{-6}-10^{-3} M) | 1.957±0.058x10^{-7} M  |
| Pheniramine maleate (10^{-6} M)+histamine | 1.852±0.134x10^{-6} M**|
| ZAHE (300 g/ml)+histamine  | 1.187±0.045x10^{-6} M**|
| ZAHE (1000 g/ml)+histamine | 1.407±0.209x10^{-6} M* |

Results are expressed as mean ± SEM (n = 4). *P<0.05, **P<0.01 - significantly different when compared to histamine group. SEM = Standard error of mean

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 5-HT-induced depolarization. Seeds of ZA are used in gastric dyspepsia; hence, our study shows that it can be of use in such conditions due to stress or emotional unrest by inhibiting the secretion of 5-HT.

One of the possible mechanisms for the spasmylic activity of the extract could be mediated through the inhibition of histaminic receptors. In this study, ZAHE inhibited histamine-induced contraction of guinea pig ileum in concentration-dependent manner. On smooth muscle, histamine produced a concentration-dependent membrane depolarization and increased excitability. The contractile effects of histamine on the isolated guinea pig ileum are known to be mediated through H1 histamine receptors. ZAHE inhibited histamine-induced contraction of guinea pig ileum comparable to the standard antihistaminic pheniramine maleate. The antagonist activity of ZAHE against histamine-induced contraction supports the traditional use of ZA in cough and chest infection etc.

EC50 values in our study indicated that extract has anticholinergic, antiserotonergic, and antihistaminic activity. Moreover, the parallel rightward shift of the dose-response curves of ACh, 5-HT, and histamine in the presence of increasing concentrations of the extract is comparable to various standard antagonists such as atropine, ketanserin, and pheniramine maleate-like inhibition. Hence, the present study provides adequate evidence for its traditional use in gastrointestinal disorders, cough, and chest infection etc.

Analogous to our studies, it was reported that the methanolic-aqueous extract of the aerial part of ZA was studied for muscle relaxation effect in gut, air passageway, and in cardiovascular system.

Various phytochemical constituents such as alkaloids, sterols, phenolics, lignins, coumarins, terpenoids, flavonoids and their glycosides and benzenoids, fatty acids, alkenic acid, and amino acid have been isolated from ZA. In our phytochemical studies, the hexane extract of the seeds contain phenolics, flavonoids, and terpenoids. The spasmylic effect of the extract may perhaps be associated with the phenolic compounds present in the seeds of the plant. 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 spasmylic is already reported. Based on this report, the spasmylic activity of ZAHE 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, is in agreement with Khosrokharav et al.,[22] who also reported anticholinergic and antihistaminic activity of methanolic extract of barberry fruit in the guinea pig ileum.

Conclusion

The present study showed ZA seeds have anticholinergic, antiserotonergic, and antihistaminic activity in the ileum and fundus of rats and ileum of guinea pig. The seeds have long history of folklore use in gastrointestinal disorders, vomiting, chest infection, and worm infestation, etc.; thus, it can be a good contestant for an alternate herbal drug for the above ailments.

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Conflicts of Interest

There are no conflicts of interest.

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