ANTIHISTAMINIC ACTIVITY MODELS

PRIYA GUPTA, VANITA G KANASE*, SHALAKA KADAM, SALMAN KAPADIA, FALAK BAMNE

Department of Pharmacology, Oriental College of Pharmacy, Sanpada West, Navi Mumbai, Maharashtra, India.
Email: vanita.kanase@gmail.com

Received: 23 April 2020, Revised and Accepted: 05 June 2020

ABSTRACT

Histamine is referred to as common allergic reactions and symptoms. Most of them are compared to histamine intolerance. Some common responses involved with this intolerance may vary but include headaches or migraines, nasal congestion or sinus problems, fatigue, hives, digestive problems, irregular menstrual cycle, nausea, and vomiting. Histamine is derived from a natural amino acid, S-histidine, through the histidine decarboxylase/ aromatic decarboxylase catalysis. Histamine is the compound that the mast cell generates for the immune response. Histamine promotes gastrointestinal secretion and induces capillary dilation, bronchial smooth muscle constriction, and reduced blood pressure. Antihistamines are medicinal products to treat allergic rhinitis and allergies. This includes the in vitro animal model and in-vivo tissue preparation antihistaminic activity. Animal models are significant instruments for understanding the pathological process of human illnesses in experimental medical science. Medicines associated with antihistamine include antiallergy, antivertigo, antimigraine, sedatives, antiemetic, etc. Elderly people are much more likely than youthful people to develop sleepiness from the use of antihistamines. The most common drugs used are cetirizine, levocetirizine, chlorpheniramine, diphenhydramine, loratadine, cimetidine, and fexofenadine. Animal models include histamine-induced bronchoconstriction, passive paw anaphylaxis, milk-induced leukocytosis and eosinophilia, clonidine, and haloperidol-induced catalepsy. While tissue models include isolated goat, and guinea-pig trachea chain preparations, as well as an isolated guinea pig, rat, mice ileum tissue preparation, and the dose-response curve of histamine, were plotted. The focus of the study had been on herbal plants and medicinal products, as they can effectively boost a variety of circumstances without significant adverse side effects. We can assess antihistaminic activity by using plant extracts or any synthetic drug.

Keywords: Histamine, Antihistaminic activity, Allergic rhinitis, Asthma, bronchoconstriction, Leukocytosis, Eosinophilia.

INTRODUCTION

Asthma is a common recurrent airway disease that is characterized by complex and persistent signs, reversible fluid restriction, and spasms. Allergy is one of the growing illnesses with various symptoms that influence humanity. Histamine is available in the mucosa of human nasal turbinates and turges which is in charge of bronchial constriction reactions [1-4]. Histamine causes the manifestation of hypersensitive responses, that for the most part, include intense irritation by the H1 histamine receptor [5, 6].

In every cell, there are four types of histamine receptors, that is, H1, H2, H3, and H4 [7]. Antihistamines (H1) structure a noteworthy helpful class of medications utilized in the treatment of an assortment of hypersensitive conditions such as rhinitis, urticaria, roughage fever, and even asthma [8,9]. Since their revelation and early improvement during the 1940s, histamine H1 receptor foils (antihistamines) have turned out to be one of the most broadly utilized classes of drugs for unfavorably susceptible issues [10]. More established original antihistamines show high restricting fondness for H1 receptors, yet a large number of these medications display restricting partiality for different classes of cell receptors, for example, the muscarinic cholinergic subtypes (M1M5) [11]. More up to this point, second-age antihistamines were created as moderately more particular histamine H1 receptor opponents than the initial operators, with some extent of limiting midway intervened impacts, as an example, sedation. Nonetheless, doubtlessly a portion of the fresher antihistamines is appropriate official to muscarinic receptors, just as to histamine H1 receptors in the cerebrum [12].

Another trademark highlight of the more established antihistamines is that they access the mind and tie to cell receptors within the central nervous system (CNS), causing sedation and disabled psychomotor execution [13]. The old-style antihistamines are linked to localized infection symptoms [14,15]. Histamine is one of the most prevalent inflammatory mediators; it triggers symptoms of allergic reactions, most of which involve severe H1 histamine-mediated inflammation [16,17]. The histamine H1 receptor is predominantly found in endothelial cells, soft muscle tissue, and neuro and adds to dilatation of blood vessels, enhanced capillary permeability, and cellular level pressure, thus causing increased intracellular calcium (Ca2+) and nitric oxide (NO) output at the molecular level [18-20].

MODELS FOR SCREENING OF ANTIHISTAMINIC ACTIVITY

In-vivo model/animal model
1. Histamine-induced bronchoconstriction in guinea pigs/mice/rats.
2. Passive paw anaphylaxis in rats/guinea pig/mice.
3. Milk-induced leukocytosis in mice.
4. Milk-induced eosinophilia in mice.
5. Clonidine-induced catalepsy in mice.
6. Haloperidol-induced catalepsy in mice.

In-vitro model/tissue model
1. Isolated goat trachea chain preparation.
2. Isolated guinea pig trachea chain preparation.
3. Guinea pig ileum tissue preparation.
4. Rat/mice ileum tissue preparation.

In-VIVO MODEL/ANIMAL MODEL
1. Histamine-induced bronchoconstriction in guinea pigs/mice/rats Animals were split into eight groups (n=6), the control group provided distilled water and a single extract dose was offered to other groups (75, 150, 200, 300, 600, and 1200 mg/kg p.o.). chlorpheniramine maleate (2 mg/kg) serves as a positive control. Pre- and post-medication treatment, each animal was kept within the histamine chamber and subjected to 0.2% histamine aerosol. The pre-convulsive...
Period (PCT) was calculated from the moment of initiation to the start of dyspnoea, contributing to the appearance of pre-convulsive dyspnoea within a min.

The percentage of protection offered by PCT drugs was determined for each dosage and positive control. The percentage protection was calculated using the formula below [21-31].

Percentage protection = (1 – T1/T2) × 100

Where T1 = PCT average before test drug administration and T2 = PCT average after test drug administration

2. Passive paw anaphylaxis in rats/guinea pig/mice
On days 1, 3, and 5, animals got subcutaneously 100 μg of egg white. Blood was gathered from the retro-orbital plexus and centrifuged to isolate serum on the 10th day of sensitization. It was entitled to clot the gathered blood and at 1500 rpm the serum was divided by centrifugation. Animals in eight groups (n=6) were divided. The saline solution got by the control group and other groups were given a singular concentrate portion of 85, 175, 250, 350, 700, and 1400 mg/kg p.o. Dexamethasone (0.27 mg/kg p.o.) was utilized as a standard. Animals were sensitized with serum into the left hind paw before medication therapy. The right hind paw got the same normal saline solution quantity. The animals were examined with 10 μg of egg white in 0.1 ml of normal saline solution in left paw 1-h post-administration of the study drug and the paw expansion using a plethysmometer was assessed. Following 24 h, the level of edema restraint was determined to utilize the equation underneath [32-34].

Inhibition rate = [1 – (T/C)] 100

T – Average relative difference in paw volume (test group).
C – Average relative difference in paw volume (control group).

3. Milk-induced leukocytosis in mice
Mice (Swiss Albino) were split into six categories with each category containing six mice. Blood samples were obtained using pentobarbital sodium (i.p.), using RO (retro-orbital) vein under sedation. Class 1 served as normal control, Class 2 that received Milk served as an intoxicant, Class 3 received standard as dexamethasone, and Class 4 to Class 6 received extract dose in low, moderate, and high dose. All classes got boiled and cooled milk infusion in the dose of 4 ml/kg s.c. after 30 min of drug treatment, excluding the normal control group.

Calculate the change in total leukocytes count pre and post 24 h drug administration (Table 1) [35-39].

4. Milk-induced eosinophilia in mice
Mice (Swiss Albino) were split into six categories with each category containing six mice. Blood samples were obtained using pentobarbital sodium (i.p.), using RO (retro-orbital) vein under sedation. Class 1 served as normal control, Class 2 that received Milk served as an intoxicant, Class 3 received standard as dexamethasone, and Class 4 to Class 6 received extract dose in low, moderate, and high dose. All classes got boiled and cooled milk infusion in the dose of 4 ml/kg s.c. after 30 min of drug treatment, excluding the normal control group.

Calculate the change in total eosinophils count pre and post 24 h drug administration (Table 1) [37,40-43].

5. Clonidine-induced catalepsy in mice
Mice (Swiss Albino) were split into five classes containing 5 mice each. Normal control (Class 1) provided saline solution (10 ml/kg) and other Class 3 to Class 5 received a single dosage of extract (100, 200, and 400 mg/kg body weight). Mice of Class 2 got standard dose chlorpheniramine maleate (antihistamine) (10 mg/kg, i.p.). One hour after intake of the drug, all the classes got clonidine (1 mg/kg s.c.), and the catalepsy period was determined at 15 min, 30 min, 60 min, 90 min, 120 min, 150 min, and 180 min (Table 2) [44,45].

6. Haloperidol-induced catalepsy in mice
Mice (Swiss Albino) were split into five classes containing five mice each. Normal control (Class 1) provided saline solution (10 ml/kg) and other Class 3 to Class 5 received a single dosage of extract (100, 200, and 400 mg/kg body weight). Mice of Class 2 got standard dose chlorpheniramine maleate (antihistamine) (10 mg/kg, i.p.). One hour after intake of the drug, all the classes got haloperidol (1 mg/kg s.c.), and the catalepsy period was determined at 15 min, 30 min, 60 min, 90 min, 120 min, 150 min, and 180 min (Table 3) [22,46].

Bar test scoring
The bar test was used for catalepsy measurements. In the bar test, the animal’s front paw was placed alternatively on a horizontal bar 3 cm above and 5 cm parallel to the foundation. The moment the mice removes its front paw from the bar were observed.

| Class | Test substance | Albino mice per group | Dose as required | Total |
|-------|----------------|-----------------------|------------------|-------|
| 1     | Normal saline  | 6                     | ml/kg            | 6     |
| 2     | Milk           | 6                     | mg/kg            | 6     |
| 3     | Milk+Dexamethasone | 6              | mg/kg            | 6     |
| 4     | Milk+Extract (Low dose) | 6          | mg/kg            | 6     |
| 5     | Milk+Extract (Moderate dose) | 6        | mg/kg            | 6     |
| 6     | Milk+Extract (High dose) | 6          | mg/kg            | 6     |
| Total animals required |              |                      |                  | 36    |

| Class | Test substance | Albino mice per group | Dose as required | Total |
|-------|----------------|-----------------------|------------------|-------|
| 1     | Normal saline+clonidine | 5              | ml/kg            | 5     |
| 2     | Standard+clonidine (chlorpheniramine maleate) | 5          | mg/kg            | 5     |
| 3     | Extract+clonidine (LOW DOSE) | 5          | mg/kg            | 5     |
| 4     | Extract+clonidine (medium dose) | 5          | mg/kg            | 5     |
| 5     | Extract+clonidine (high Dose) | 5          | mg/kg            | 5     |
| Total animal required |              |                      |                  | 25    |
### Table 3: Animal grouping for haloperidol-induced catalepsy study [22,46]

| Class | Test substance | Albino mice per group | Dose as required | Total |
|-------|-----------------|-----------------------|------------------|-------|
| 1     | Normal saline+haloperidol | 5 | ml/kg | 5 |
| 2     | Standard+haloperidol (chlorpheniramine maleate) | 5 | mg/kg | 5 |
| 3     | Extract+haloperidol (low dose) | 5 | mg/kg | 5 |
| 4     | Extract+haloperidol (medium dose) | 5 | mg/kg | 5 |
| 5     | Extract+haloperidol (high dose) | 5 | mg/kg | 5 |
|       | **Total animal required** | | | **25** |

**Catalepsy valuing was given as follows**

- **Step 1:** The mice were removed from the house cage and placed on the table. If the mice did not move, a value of 0.5 was allocated when touched or softly pushed back.
- **Step 2:** The mice’s front paws were alternatively put on a 3 cm long bar. If the mice did not correct the posture within 15 s, for each paw a value of 0.5 was added to the value of Step 1.
- **Step 3:** The mice’s front paws were alternatively put on a 5 cm long bar, if the mice did not correct the posture within 15 s, on each paw a value of 1 was added to the value of Step 1 and 2.

#### Formula to calculate catalepsy value

\[
\text{Total value} = 0.5 + [0.5 \times \text{time (in s) of front right paw on 3 cm long bar}] + [0.5 \times \text{time (in s) of front left paw on 3 cm long bar}] + [1 \times \text{time (in s) of front right paw on 5 cm long bar}] + [1 \times \text{time (in s) of front left paw on 5 cm long bar}] 
\]

### IN-VITRO MODEL

1. **Isolated goat trachea chain preparation**
   
   From the adult goat, isolated tracheal tissue was acquired instantly after the animals were slaughtered. Tracheal was cut into separate pieces and sequentially connected to form a chain. In Krebs bathwater, trachea was suspended and constantly aerated at 37±0.5°C. At one end of the prepared tracheal chain was connected to the s-shaped aerator pipe and another end connected to an isotonic frontal writing lever. The histamine dose-response curve (DRC) was traced on the kymographic sheet that is mounted on a revolving drum.

   A graphic of the largest percentage of contractile responses to histamine ordinate and abscissa concentration was considered for the screening of histamine DRC in the lack and existence of drug extract [47-55].

2. **Isolated guinea pig trachea chain preparation**
   
   From the guinea pig, isolated tracheal tissue was acquired instantly after the animals were slaughtered. The tracheal was cut into separate pieces and sequentially connected to form a chain. In Krebs bathwater, trachea was suspended and constantly aerated at 37±0.5°C. At one end of the prepared tracheal chain was connected to the s-shaped aerator pipe and another end connected to an isotonic frontal writing lever. The histamine dose-response curve (DRC) was traced on the kymographic sheet that is mounted on a revolving drum.

   A graphic of the largest percentage of contractile responses to histamine ordinate and abscissa concentration was considered for the screening of histamine DRC in the lack and existence of drug extract [55-58].

3. **Guinea pig/rabbit ileum tissue preparation**
   
   The guinea pigs/rabbit which were fasted overnight were sacrificed and the ileum was put in an organ bath with a Tyrode solvent that was constantly aerated at 37±0.5°C. The histamine dose-response curve was carried out in the plain Tyrode solvent and in the extract-containing Tyrode solvent. The largest percentage of contractile responses in the lack and existence of the extract was intended to generate the histamine dose-response curve [59-61].

4. **Rat/mice ileum tissue preparation**
   
   The rat/mice which were fasted overnight were sacrificed and the ileum was put in an organ bath with a Tyrode solvent that was constantly aerated at 37±0.5°C. The histamine dose-response curve was carried out in the plain Tyrode solvent and in the extract-containing Tyrode solvent. The largest percentage of contractile responses in the lack and existence of the extract was intended to generate the histamine dose-response curve [59,62-66].

### Tissue preparation

Before 1 day of the start of the study, the animals were dierted over night with free water exposure. Animals had been killed humanly by ether under sedation. The cervical dislocation slaughtered animals were used. About 1 cm from the ileocaecal junction, a 3 cm portion of the ileum was surgically removed. As described earlier, the transverse tissue sheet had been removed. Nearly 1.5 cm long strips were set in 5 ml organ water containing Krebs-Henseleit arrangement with 95% O2 and 5% CO2 and held at 37°C.

Tissue was fixed, with the aid of two tight loops. At one end of the prepared tracheal chain was connected to the s-shaped aerator pipe and another end connected to an isotonic frontal writing lever. Before the procedures began, the tissue strips were balanced for 45 min under resting stress of 1 g. Tissue responses, that is, histamine dose-response curve, had been traced on kymographic paper [67].

### CONCLUSION

Asthma is an immune-involved inflammatory disease. Treatment includes many factors that are capable of managing asthma. The current research was scheduled to assess the effect of the extract on different elements of asthma, such as bronchoconstriction, eosinophilia, and inflammation-related allergy using different animal models in vitro and in vivo.

Recently, as innovative clinical strategies for the research of anti-histaminic disease and its related disorders, many herbal plants and medicinal items have received research interest because they can effectively improve a variety of circumstances without severe adverse side effects.

### ACKNOWLEDGMENT

We are thankful for their guidance and help to our Principal, Dr. (Mrs.) Sudha Rathod, Dr. (Mrs.) Vanita G. Kanase, Mr. Imtiyaz Ansari, and Mrs. Pushplata Chougale as well as to the Department of Pharmacology, Oriental College of Pharmacy, Navi Mumbai.

### AUTHORS’ CONTRIBUTIONS

We declare that this work was done by the authors named in this article and all liabilities about claims relating to the content of this article will be borne by the authors. Ms. Priya Gupta, Ms. Shalaka Kadam, Mr. Salmon Kapadia, and Ms. Fakih Bamne collected the data and analyzed the data. Dr. (Mrs.) Vanita Kanase proofread the whole manuscript and suggested the necessary changes, and helps in designing the manuscript.

### CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.
AUTHORS' FUNDING
We thank Oriental College of Pharmacy for funding the project.

REFERENCES
1. Casale T, Rodbard D, Kaliner M. Characterization of histamine H1-receptors on human peripheral lung. Biochem Pharmacol 1985;34:3285-92.
2. Herxheimer H. Antihistamines in bronchial asthma. Br Med J 1949;2:901-5.
3. Wood-Baker R, Holgate S. The comparative actions and adverse effect profile of single doses of H1-receptor antihistamines in the airways and skin of subjects with asthma. J Allergy Clin Immunol 1993;91:1005-14.
4. Schmidt D, Ruelmann E, Branscheid D, Magnussen H, Rabe KF. Passive sensitization of human airways increases responsiveness to leukotriene C4. Eur Respir J 1999;14:319-23.
5. Orense GM, Carselie DS, Valencia CA. Comparative antihistaminic activities of 10 histamine H1 receptor antagonists in two functional models. Eur J Pharmacol 2005;506:257-64.
6. Slater JW, Zeichnich AD, Haxby DG. Second-generation antihistamines: A comparative review. Drugs 1999;57:31-47.
7. Zaneu M, Imny J, Vanci D. Evaluation of antihistaminic activity. World J Pharm Res 2018;7:278-88.
8. Saxena AK, Saxena M. Developments in antihistaminics (H1). In: Progress in Drug Research. Basel: Ernst Jucker; 1992. p. 35-126.
9. Shishoo CJ, Shraddha VS, Rathod IS, Vikas D. Design, synthesis and antihistaminic (H1) activity of some condensed, 3-amino-1,2-oxazin-4(3H)-ones. Yande Eur J Med Chem 2000;35:351-8.
10. Kudo N, Shrikawata O, Kuno T, Tanaka C. Antimuscarinic effect of antihistamines: Quantitative evaluation by receptor binding assay. Jpn J Pharmacol 1987;43:277-82.
11. Laak AM, den Kelder GM, Bast A, Timmerman H. Is there a profile of single doses of H1-receptor antagonist for CNS and peripheral receptors? An in vitro study. Eur J Pharm Col 1993;232:199-205.
12. Hindmarsh J, Shanki Z. Antihistamines: Models to assess sedative properties, assessment of sedation, safety and other side-effects. Clin Exp Allergy 1999;29:133-42.
13. Garrison JC. Histamine, bradykinine, 5-hydroxytryptamine and their antagonists. In: Gilman AG, Ral TE, Nies AS, Taylor P, editors. The Pharmacological Basis of Therapeutics. 6th ed. New York: Pergamon Press; 1990. p. 575.
14. Green JP, Weinstein H. Psychopharmacology: A Generation of Progress. New York: Raven Press; 1978. p. 319.
15. Thurmond RL, Gelfand EW, Dunford PJ. Review: The role of histamine H1 and H4 receptors in allergic inflammation: The search for new antihistaminics. Nat Rev Drug Discov 2008;7:41-53.
16. Yong YK, Zakaria ZA, Kadir AA, Somchit MN, Lian GE, Ahmad Z. Chemical constituents and antihistamine activity of Vahl (Moraceae) mother tincture. Homeopathy 2006;58:164.
17. Taur DJ, Nirimal SA, Patil RS, Karma MA. Antidistress and antiallergic effect of Ficus bengalensis bark in asthma. Nat Prod Rev 2007;21:1266-70.
18. Malik RG, Dha SK. Evaluation of effects of Bauhinia variegata stem bark extracts against milk-induced eosinophilia in mice. J Adv Pharm Technol Res 2011;2:132.
19. Rajasekaran S. Evaluation of effects of Parthenium hysterophorus l, leaves extracts against, milk-induced eosinophilia in mice. J Pharm Sci 2014;5:1303-06.
20. Dusser D. Role of eosinophils in asthma. Rev Mal Respir 2000;17:195-201.
21. Kumar D, Bhujbal SS, Patil PS, Buge PV. In-vivo and in-vivo activities of stem bark of methanolic extract of Alianthus excelsa Roxb. In the management of asthma. Int J Pharm 2012;6:284-9.
22. Ferré S, Guix T, Prat G, Jane F, Casas M. Is experimental catalepsy properly measured? Pharmacol Biochem Behav 1996;53:753-7.
23. Kumar D, Bhat ZA, Singh P, Bhujbal SS, Deosa RS. Antihistaminic activity of aqueous extract of stem bark of Alianthus excelsa Roxb. Pharmacogn Res 2011;3:220.
24. Saxena AK, Saxena M. Developments in antihistaminics (H1). In: Progress in Drug Research. Basel: Ernst Jucker; 1992. p. 35-126.
25. Mohrakhe JI, Sakurada S, Katsuyma S, Kutsuwa M, Kuriyama S, Lin ZY, et al. Role of histamine H1 receptor in pain perception: A study of the receptor gene knockout mouse. Eur J Pharmacol 2000;391:89-96.
26. Jutel M, Aksamis M, Aksis CA. Histamine, histamine receptors and their role in immune pathology. Clin Exp Allergy 2000;39:1786-800.
27. Singh S, Agrawal SS. Broncho-relaxant activity of Belamcanda chinensis. J Sci Res 1999;20:107-09.
28. Tripathi RM, Das PK. Studies on anti-asthmatic and anti-anaphylactic effect of Bixa orellana. Phytomedicine 1995;2:250-1.
29. Gokhale AB, Saraf MN. Broncho-protective effect of ethanolic extract of Tephrosia purpurea in-vivo. Indian J Drugs 1996;37:346-7.
30. Evans WC, Trease EV. Pharmacognosy. 14th ed. London: W.B. Sanders Company; 1997. p. 250-1.
31. Nimgukar CC, Patil SD, Kumar BD. Anti-asthmatic and anti-anaphylactic activities of Blatta orientalis mother tincture. Homeopathy 2011;100:138-43.
32. Okpo SO, Eze GI, Ajaonwu IH, Ijei OL, Uwaya DO. Ologe V. Evaluation of the anti-asthma activity of aqueous root, bark extract of Ficus exasperata Vahl (Moraceae). Int J Health Res 2012;5:5-12.
56. Kaley G, Weiner R. Prostaglandin ‘E’a potential mediator. Ann N Y Acad Sci 1971;180:347-8.
57. Sagar R, Sahoo HB. Evaluation of antiasthmatic activity of ethanolic extract of Elephantopus scaber L. leaves. Indian J Pharmacol 2012;44:398.
58. Ghosh MN. Fundamentals of Experimental Pharmacology. Kolkata, India: Scientific Book Agency; 1984. p. 60-3.
59. Singh N, Nath R, Gupta ML, Kohli RP. An experimental evaluation of anti-asthmatic potentialities of Inula racemosa (Puskar Mul). Quart J Crude Drug Res 1980;18:89-96.
60. Pandit P, Singh A, Bafna AR, Kadam PV, Patil MJ. Evaluation of antiasthmatic activity of Curculigo orchioides Gaertn. Rhizomes. Indian J Pharm Sci 2008;70:440.
61. Nagarajan K, Sharan G, Jeshvanth A, Nightingale T, Dheeneswari P, Sumanthi A. Evaluation of anti-histaminic activity of leaves of Acalypha canescana in isolated ileum of rabbit. Int J Pharm Pharm Sci 2010;2:19-20.
62. Afreen A, Kashyap P, Sawarkar H, Deshmukh V, Upadhyay A, Pal S. In-vitro and In-vivo models for evaluation of anti-asthmatic activity: A review. Int J Herbal Drug Res 2011;1:19-27.
63. Adusumalli SU, Ranjit PM, Harish MS. Antiasthmatic activity of aqueous extract of Pistacia integerrima galls. Int J Pharm Pharm Sci 2013;5 Suppl 2:116-21.
64. Taur DJ, Patil RN, Patil RY. Antiasthmatic related properties of Abrus precatorius leaves on various models. J Tradit Complement Med 2017;7:428-32.
65. Vogel GH, Vogel WH. Drug Discovery and Evaluation. Berlin: Springer Verlag; 1998.
66. Butler A, Elswood CJ, Burridge J, Ireland SJ, Bunce KT, Kilpatrick GJ, et al. The pharmacological characterization of 5-HT3 receptors in three isolated preparations derived from guinea-pig tissues. Br J Pharmacol 1990;101:591-8.
67. Saikia B, Barua CC, Haloi P, Patowary P. Anticholinergic, antihistaminic, and antiserotonergic activity of n-hexane extract of Zanthoxylum alatum seeds on isolated tissue preparations: An ex vivo study. Indian J Pharmacol 2017;49:42.