Saudi Pharmaceutical Journal 31 (2023) 101705

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Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Comparative evaluation of the potential anti-spasmodic activity of *Piper longum, Piper nigrum, Terminalia bellerica, Terminalia chebula,* and *Zingiber officinale* in experimental animals

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ARTICLE INFO

Article history:
Received 17 April 2023
Accepted 13 July 2023
Available online 20 July 2023

Keywords:
Piper longum
Piper nigrum
Terminalia bellerica
Terminalia chebula
Zingiber officinale
Natural products
Anti-spasmodic activity

ABSTRACT

Background: Spasm of muscle is one of the frequent complaints seen by most of the population worldwide. The present study evaluated the efficacy of some of the commonly used herbal extracts against known spasmogens, such as histamine and 5-hydroxytryptamine (5-HT).
Material and methods: The study was conducted on isolated guinea pig ileum and rat uterus preparations using histamine and 5-HT, respectively. Five herbal extracts such as *Piper longum (P.L)*, *Piper nigrum (P.N)*, *Terminalia bellerica (T.B)*, *Terminalia chebula (T.C),* and *Zingiber officinale (Z.O)* were tested. Herbal extracts at doses 50, 150, 500, 1500, and 5000 mcg/ml were pretreated to the isolated tissue preparation, and the contractile response of histamine and 5-HT was recorded. The efficacy and the inhibitory concentration (IC50) were calculated and statistically analyzed by one-way ANOVA.
Results: The study indicated that all five herbal extracts produced a concentration-dependent suppression of histamine and 5-HT-induced responses. A significant ($p < 0.05$) non-competitive antagonism was observed against the known spasmogen induced smooth muscle contraction for *P.L, P.N, T.B,* and *Z.O* in both guinea pigs and rat uterus preparation. Moreover, *P.L* and *P.N* completely abolished (100%) the contractile response induced by histamine and 5-HT. Although, T.C produced a concentration-dependent reduction in known spasmogen-induced contraction but the response was found to be statistically non-significant (p greater than 0.05).
Conclusion: The finding suggested that P.L. and P.N. have better activity in terms of reducing the spasmogenic contractions compared to other extracts. Additionally, T.B. and Z.O. can lessen the uterine and intestinal contractions brought on by spasmogens. Although P.L and P.N demonstrated better efficacy against the spasmogenic activity of histamine and 5-HT, more research, particularly on isolated phytochemicals of the extracts and involving different experimental models, is required before establishing the precise safety and efficacy against spasmogenic-induced disorders.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2023.101705
1319-0164/

1. Introduction

Abdominal cramps and pain are one of the frequently reported symptoms in adults. It is estimated that more than 30% of the population suffers from this sudden acute painful condition in their lifetime. The uterus is one of the common discomfortable states in almost 90% of women of reproductive age. If they exist for prolonged periods, these conditions can cause anxiety, mood swings, nausea, irritation, and depression (Tytgat, 2007). Several factors have been associated with smooth spasms, such as lack of nutrients, muscular tension, over-use of muscle, demand for increased blood flow, dehydration, electrolyte imbalance, and several underlying medical conditions (Murata et al., 2019).

According to the available data, most of the muscle spasms disappear on their own. Stopping the triggering factors and applying gentle heat ease the spasm (Maughan et al., 2019). However, if pain persists, the most suitable medical intervention is non-steroidal anti-inflammatory drugs (NSAIDs) (Blyton et al., 2012). Some commonly used NSAIDs are ibuprofen, diclofenac, naproxen, mefenamic acid, and indomethacin. An early study indicated that about 40% of elderly patients in the United States received at least one NSAID prescription annually for a chronic period (Patel et al., 2019). Regular use of NSAIDs has been reported to cause several complications, such as dyspepsia, peptic ulceration, hemorrhage, and perforation, that can even lead to death (Bindu et al., 2020).

Healing diseased states through natural resources has been practiced since ancient times. According to a report, an estimated 25% of modern medicines are derived from plants and practiced traditionally (Ziemska et al., 2019). Herbal medicines are still consumed by about 80% of the world's population. Some of the main advantages of this mode of treatment are that it is easy to access, economical, and devoid of major toxic manifestations (Jafari et al., 2014). Some traditionally used plant-based medicines for treating muscular spasms include *Piper longum (*Linn*), Piper nigrum (*Linn*), Terminalia bellerica* (Gaertn.*), Terminalia chebula* (Retz.*),* and *Zingiber officinale* (Rosc.) (Luo et al., 2020).

Piper longum (P.L) is a deciduous slender, aromatic climber belonging to the family *Piperaceae*. As folk medicine, the plant is popular for treating cough, asthma, constipation, stomachache and diseases of spleen. Previous studies indicated that the plant possesses several biological activities such as spasmolytic, anticancer, antidiabetic, antimicrobial, anti-inflammatory, cardioprotection, and hepatoprotection. Various active constituents like piperine, pipalestrol, pippalartin, pipperleguminin, piperlongumine, and piplartine have been identified and linked to the pharmacological properties of plants (Quijia et al., 2021). *Piper nigrum (P.N)* is another member of the family *Piperaceae*. Traditionally, the drug treats cold, dyspnea, intermittent fever, cough, and dysentery. The plant is a flowering woody perennial climber that grows in shades. Chemical constituents such as piperamide, piperamine, piperettine, pipericide, piperine, sarmentine have been identified for therapeutic activities. These include antimicrobial, antitumor, anti-inflammatory, antidepressant, antidiarrheal, anticonvulsant, anti-spasmodic, and analgesic (Takooree et al., 2019).

Terminalia bellerica (T.B) (Family: *Combretaceae*) is a deciduous tree with broadly elliptical leaves that has been traditionally used as hepatoprotective, antimicrobial, angiogenic, antidiabetic, spasmolytic, anti-thrombotic, wound healing, anti-cancer, antihypertension, and bronchodilators. The phytoconstituents responsible for these activities include belleric acid, chebulagic acid, bellericoside, bellricanin, and chebulinic acid (Gupta et al., 2020). *Terminalia chebula (T.C)* also belongs to the family *Combretaceae* which grows as a large deciduous tree having alternate leaves and used as folk-medicine in indigestion, constipation, liver diseases and anorexia. The plant's fruit is reported to possess several therapeutic activities such as anti-microbial, anti-spasmodic, anti-HIV, anticancer, antidiabetic, anti-inflammatory, anti-ulcer, and wound healing. Important active ingredients of the plant are ellagic acid, chebulinic acid, chebulagic acid, corilagin, chebulanin, terchebulin, and phloroglucinol (Zhang et al., 2016).

Zingiber officinale (Z.O) (Family: *Zingiberaceaa*) is an herbaceous rhizomatous plant having aromatic, thick-lobed rhizomes, and is popular among the folks as medicine in the treatment of headache, nausea, vomiting, hypertension, and hypercholesteremia. The rhizomes exhibit several biological activities such as anti-cancer, anti-spasmodic, anticoagulant, antiemetic, anti-inflammatory, antinociceptive, antimicrobial, and lipid-lowering. The vital phytoconstituents identified are gingerol, gingerdione, zingerone, shogaol, and zingiberene (Zhang et al., 2021). Although these herbal medicines were routinely used to treat spasms, the literature review lacked elaborate clinical and preclinical evidence. This research aims to validate the traditional claim and compare the anti-spasmodic potentials of *Piper longum, Piper nigrum, Terminalia bellerica, Terminalia chebula,* and *Zingiber officinale* in isolated smooth muscle preparations such as guinea pig ileum and rat uterus.

2. Materials and methods

2.1. Chemicals, botanicals, and drugs

The chemicals required for the study were procured from regular chemical suppliers of the institution. These were of analytical grade and specifically manufactured for research purposes. Herbal extracts in dry powder / semi-dried form were obtained as a 'gift sample' (only for research) from Natural Remedies Ltd, Bangalore, India. The identification and authentication of the botanicals were made by the scientists of Natural Remedies, where the samples are also stored in their herbarium. Histamine hydrochloride was purchased from Acros Organics, Belgium, and 5-hydroxytryptamine (5-HT) from Sigma-Aldrich, Bulgaria. Chlorpheniramine hydrochloride, cyproheptadine hydrochloride, and diethylstilbesterol were purchased from Wockhardt Limited, Mumbai, India.

2.2. Animals

Eighteen Wistar Albino rats Wistar weighing 150–200 gm, aged 4–5 months, were used in the study. The animals were in-bred at the central animal house, Al-Ameen College of Pharmacy (AACP), Bangalore. The animals were maintained with free access to food (standard pellet diet, Amrut feed, Bangalore) and at room temperature. Female rats were used for uterine preparation and treated for three days with diethylstilbesterol, I.P, before sacrificing. Twelve guinea pigs were procured from National Tuberculosis Institute, Bangalore. Animals of either sex weighing 500–900 gm were used for the study and were maintained in the Central animal house, AACP, Bangalore, one week before the experiment. A total number of animals were allocated depending on the number of test compounds, doses, and the number of trials. The experiment was conducted as per the guidelines of CPCSEA and after approval from the Institutional Animal Ethics Committee of Al-Ameen College of Pharmacy, Bangalore, India (AACP/M−112).

2.3. Physiological salt solution

Tyrode solution was used in the guinea pig ileum and was prepared as per the procedure mentioned in the literature (Donnerer et al., 2014). The composition of Tyrode solution in mM is NaCl 137, KCl 2.7, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, $MgCl_2$ 0.1–1.0, $CaCl_2$ 1.8, and glucose 11.1. De Jalon was used in the rat uterus preparation and prepared as per the procedure described in the literature

(Ngadjui et al., 2021). The composition of De Jalon solution in mM is NaCl 154, KCl 5.6, $NaHCO_3$ 6.0, $CaCl_2$ 0.55, and glucose 2.78.

2.4. Extractions and preparation of test compounds

The technique of extraction of active ingredients in the labs of Natural Remedies Ltd was done by maceration with either water or methanol. Crude botanicals were kept in respective solvents for 48 h at 25 °C, filtered and subjected to a spray drying technique to preserve the composition of the phytochemicals. The extraction of active constituents from *Terminalia chebula* and *Terminalia bellerica* was carried out using water. In contrast, other three botanicals (*Piper nigrum, Piper longum,* and *Zingiber officinale*) were carried out using methanol. The stock solution of *Terminalia chebula* (spray-dried powder), *Terminalia bellerica* (spray-dried powder), *Piper nigrum* (semi-dried methanolic paste), *Piper Longum* (dried methanolic paste) and a dried rhizome of *Zingiber officinale* (semi-dried methanolic paste) was freshly prepared at the time of the experiment. The test botanicals were dissolved in distilled water with the help of a vortex, and the supernatant of the stock solution was further diluted as per the required dosage (50 – 5000 mcg/ml) (Quijia et al., 2021; Takooree et al., 2019; Gupta et al., 2020; Zhang et al., 2016; Zhang et al., 2021).

A stock solution of histamine and 5-HT were prepared by dissolving 20 mg of the agents in 20 ml of distilled water. Further dilutions were made with distilled water to obtain the required concentration. Chlorpheniramine (CPM) and cyproheptadine (CYP) solutions were prepared by dissolving the drugs in distilled water to get the concentration of 1 mg/ml (stock solution), which were later reconstituted to obtain the required doses. The overview of the plan of study is represented in the following Fig. 1.

2.5. Isolated guinea pig ileum preparation

The animal was sacrificed (depending on a number of assays planned) by cervical dislocation under ether anesthesia, and the ileum was isolated. The terminal 10 cm near the ileocecal junction was discarded, mesentery was trimmed, and the 2–3 cm long ileum was mounted in a 20 ml organ bath containing Tyrode's solution. Experiments were conducted at 37 °C, tension 0.5 g, and the medium was aerated with air (Ventura-Martinez et al., 2020). The tissue was connected to a isometric force transducer, and the contractions were recorded on a polygraph (Recorders and Medicare Systems, Ambala, India). The tissue was given a 30-min equilibration period with medium changes every 5 min before the experimental procedures. Initially, a concentration–response curve was obtained for histamine, and the sub-maximal concentration was determined. Varying concentrations of test botanicals (0.1 ml) were added to the organ bath, and after a 2-min contact period, the agonist (histamine) was added, and contractions were recorded. Similarly, standard antagonist chlorpheniramine (CPM) responses were recorded on the same tissue. Experiments were repeated 3–4 times for each concentration of the test and CP.

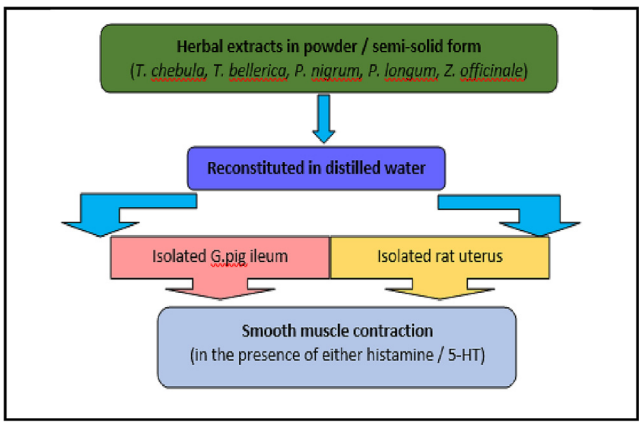

Fig. 1. An overview of the plan of the study.

2.6. Isolated rat uterus preparation

Young female Wistar rats were injected with diethylstilbesterol (0.25 mg / 100 g) intraperitoneally for three days (to prime the animals and to maintain the estrus phase) before sacrificing the animal. The animal was then sacrificed by cervical dislocation under ether anesthesia, and the abdomen was opened. The uterus's horns were dissected, separated from the surrounding fatty material, and transferred to a dish containing De Jalon solution. The tissue was mounted and connected to the transducer. Experiments were conducted at 37 °C, tension 0.5 g, and the medium was aerated with air (Monji et al., 2018). The contractions were recorded on a polygraph (Recorders and Medicare systems, Ambala, India). The tissue was given a 30-min equilibration period with medium changes every 5 min before the experimental procedures. Initially, a concentration–response curve was obtained for 5-hydroxytryptamine, and the sub-maximal concentration was determined. Different concentrations of test botanicals were added to the organ bath, and after a 2-min contact period, the agonist (5-HT) was added, and contractions were recorded. The test and standard antagonist cyproheptadine (CYP) responses were recorded on the tissue. The experiment was repeated 3–4 times for each concentration of the test and CYP.

2.7. Statistical analysis

Data obtained from the study are expressed as means ± standard error (SE). Statistical analysis was done using the statistical program IBM SPSS version 20.0 for Windows (SPSS Corporation, Chicago, IL, USA). The mean percentage of contractions induced by different concentrations of test /CYP was determined, taking the contraction induced by histamine / 5-hydroxytryptamine as 100%. One-way analysis of variance (ANOVA) and Dunnett's post hoc test were used to analyze the statistical significance for multiple groups. $P < 0.05$ indicates the statistical significance when comparing different groups.

3. Results

3.1. Guinea pig ileum preparation

The dose–response curve of histamine was recorded using isolated guinea pig ileum preparation. Different histamine concentrations (1 to 64 $\times$ 10^{-7} M) were tested, and the observations indicated a concentration-dependent smooth muscle contraction. The peak response was found at 3.2 $\times$ 10^{-7} M of histamine, and beyond this concentration, a ceiling effect was observed when the higher dose (3.2 $\times$ 10^{-7} M) was tested (Fig. 2).

The effect of different concentrations of P.N on the dose–response curve of histamine is represented in Fig. 3. Five concentrations such as 50, 150, 500, 1500, and 5000 mcg/ml were tested. The observations recorded suggested a concentration-dependent inhibition of the dose–response curve of histamine. With a lower concentration of P.N (50 mcg), the dose–response of histamine appears to be unaltered with a slight shift towards the right-hand side. However, as the concentration of the extract was increased, the extract reduced the amplitude of the dose–response curve of

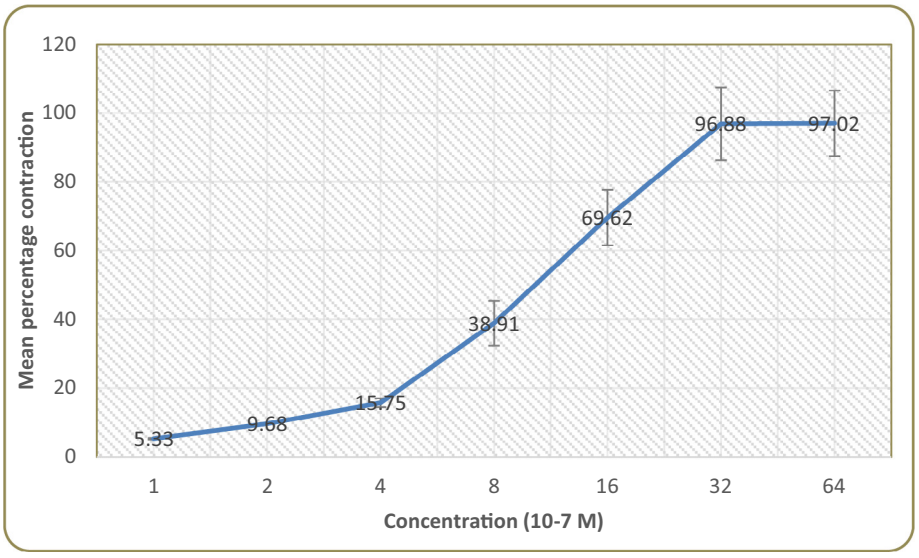

Fig. 2. Dose-response curve of histamine using guinea pig ileum.

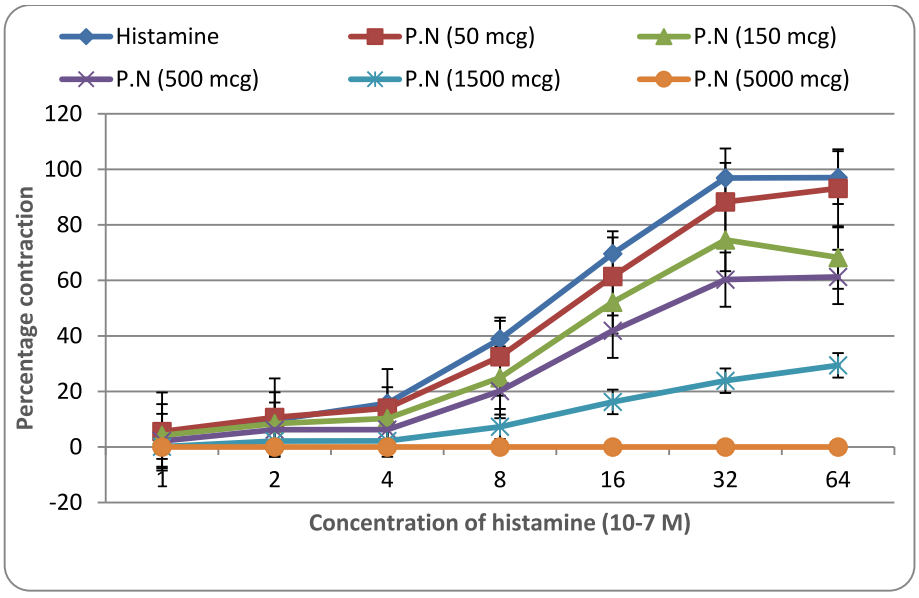

Fig. 3. Dose-response curve of histamine against different concentrations of *P.nigrum.*

histamine and the highest concentration of P.N (5000 mcg/ml) completely abolish the effect of histamine.

Fig. 4 represents the effect of different herbal extracts against a histamine-induced contraction in guinea pig ileum. *P.L.* at 50 mcg/ml induced smooth muscle contraction in the presence of histamine. Further, when the doses were increased, the extract suppressed the contractile response of the histamine and, at 5000 mcg/ml, produced complete abolition of the histamine effect. On the other hand, *P.N.* showed a concentration-dependent reduction in the histamine responses, and complete abolition of the histamine effect was observed at 5000 mcg/ml. Three other extracts, such as *T.B*, *T.C,* and *Z.O.* showed a concentration-dependent suppression in the contractile responses of histamine. None of these extracts were found to abolish the histamine responses completely. The mean percentage inhibition of these extracts at the highest tested dose (5000 mcg/ml) was found to be *T.B* (73.09 %), *T.C* (63.6 %), and *Z.O* (73.2 %). CPM was tested as a standard antihistamine drug at the same doses (50, 150, 500, 1500, and 5000 mcg/ml) and produced concentration-dependent suppression in the histamine contraction. At the highest tested dose (5000 mcg/ml), CPM was found to produce 16.6% of the contractile response. All the responses of test compound beyond 500 mg/ml were found to be significantly ($P < 0.01$) reduced compared to histamine.

Different doses of herbal extracts, such as 50, 150, 500, 1500, and 5000 mcg/ml were tested against a histamine-induced contraction in guinea pig ileum. *P.L.* at the initial doses (50, 150, and 500 mcg/ml) did not induce a significant effect in the inhibition of histamine-induced contraction. However, the higher tested doses such as 1500 and 5000 mcg/ml produced significant ($p < 0.05$) efficacy in the prevention of histamine-mediated spasmogenic response. Similar observations were recorded for *P.N.* at the doses tested, significant effectiveness was found at 1500 and 5000 mcg/ml. *T.B.* did not produce significant action at doses 50, 150, 500, and 1500 mcg/ml. The higher dose (5000 mcg/ml) was found to be significantly effective ($p < 0.05$) in preventing the

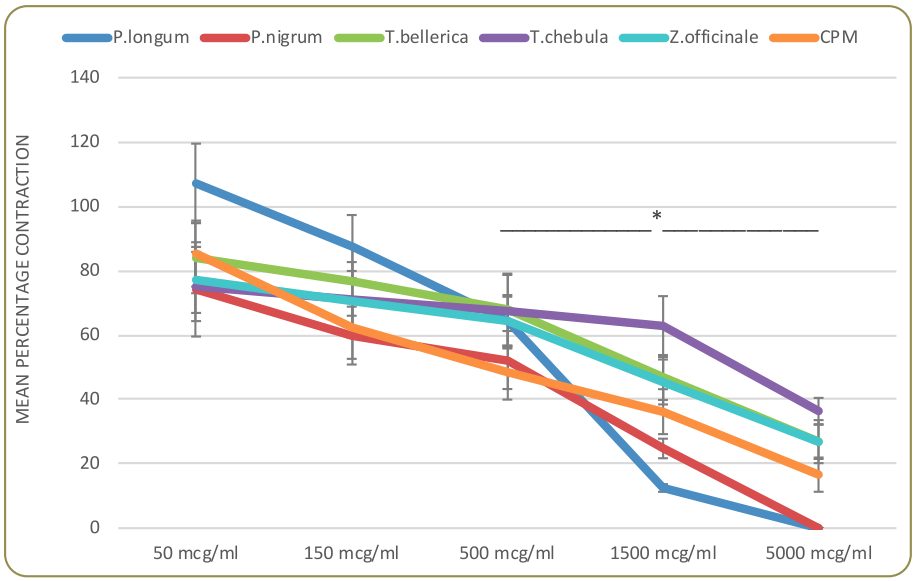

Fig. 4. Effect of different herbal extracts on the histamine-induced contractions in guinea pig ileum.

contraction produced by histamine. Similarly, *Z.O.* was also found to be effective only at the highest tested dose (5000 mcg/ml). However, *T.C.* in all tested doses did not produce a significant effect against histamine-induced smooth muscle contraction in guinea pig ileum. Further, CPM in this study significantly ($p < 0.05$) inhibited histamine-mediated contraction only at the highest dose (5000 mcg/ml) (Table 1).

The IC_{50} values tested for the inhibitory effect of histamine-induced contraction were highest for *T.C* (2237.5 mcg/ml), followed by *T.B* (1190 mcg/ml). Other extracts were found to have IC_{50} values such as *P.L* (400 mcg/ml), *P.N* (184 mcg/ml), *Z.O* (915 mcg/ml), and CPM (134.9 mcg/ml) (Fig. 5).

3.2. Rat uterus preparation

The dose–response curve of 5-hydroxytryptamine (5-HT) was tested at the doses such as 1 to 64 $\times$ 10^{-7} M using isolated rat uterus preparation. Administration of the doses showed

Table 1
Percentage inhibition of the different herbal extracts against histamine-induced contraction.

Doses of the extract	*P. longum*	*P. nigrum*	*T. bellerica*	*T. chebula*	*Z. officinale*	CPM
50 mcg/ml	−7.14 ± 1.26	25.78 ± 7.65	16.02 ± 2.41	24.89 ± 6.52	22.84 ± 4.23	14.68 ± 3.69
150 mcg/ml	12.5 ± 0.98	40.14 ± 11.36	23.31 ± 6.92	29.05 ± 8.39	29.60 ± 3.54	37.835 ± 8.22
500 mcg/ml	35.71 ± 8.82	47.74 ± 12.87	32.26 ± 7.65	32.37 ± 9.60	35.86 ± 7.09	51.74 ± 9.02
1500 mcg/ml	87.5 ± 15.94*	75.22 ± 14.26*	53.16 ± 13.52	37.33 ± 9.89	54.56 ± 8.91	63.79 ± 12.67
5000 mcg/ml	100 ± 19.62**	100 ± 19.82**	73.09 ± 19.02*	63.60 ± 13.62	73.17 ± 17.29*	83.36 ± 12.71*

Values are represented as mean ± SE. **Statistics**: One-way ANOVA. $P < 0.05$ and $P < 0.01$ compared with histamine response.

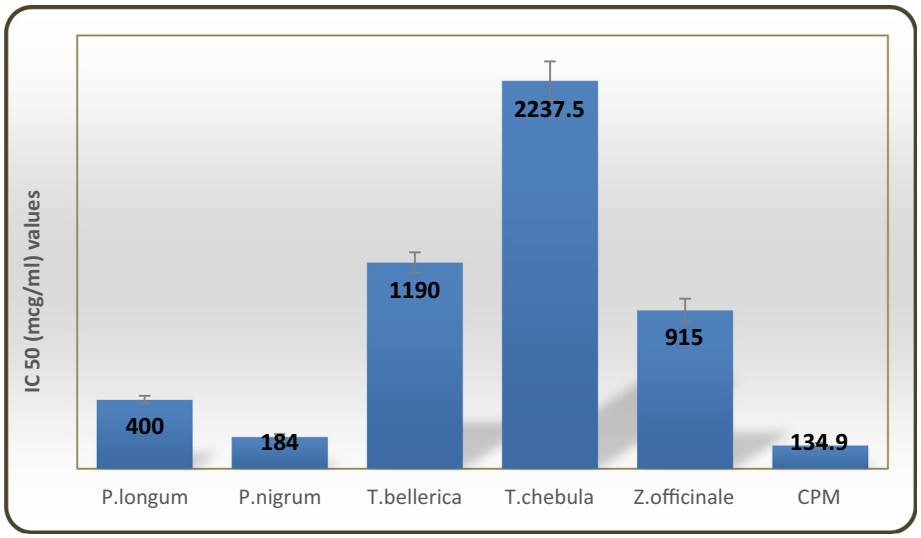

Fig. 5. IC_{50} values of different herbal extracts on histamine-induced contractions in guinea pig ileum.

concentration-dependent responses in the smooth muscle. The threshold value for the response was found to be 4×10^{-7} M, while the peak/ceiling response was recorded at 64×10^{-7} M (Fig. 6).

The observation represented in Fig. 7 indicate the responses of different concentrations of P.N tested against the dose–response curve of 5-HT. The lower tested concentration of P.N (50 mcg) reduced slightly the amplitude of the dose–response curve and when the concentration of the extract was increased, a gradual shift of the curve towards the right-hand side was observed. P.N. at the highest tested concentration (5000 mcg) completely abolish the effects of 5-HT.

Fig. 8 represents the effect of different herbal extracts against a 5-HT-induced contraction in a rat uterus. *P.L.* produced a concentration-dependent suppression of 5-HT-induced contraction and the highest dose (5000 mcg/ml) abolished completely the action of 5-HT. *P.N.* also showed a concentration-dependent inhibition of 5-HT-induced muscular contraction. This extract produced complete abolition of 5-HT action at both 1500 and 5000 mcg/ml. However, the other three extracts such as *T.B*, *T.C*, and *Z.O.* although showed a concentration-dependent suppression of 5-HT induced effect but did not abolish it completely. The highest tested dose (5000 mcg/ml) of these extracts was found to produce the mean percentage inhibition such as *T.B* (94.1 %), *T.C* (69.7 %), and *Z.O* (89.7 %). CYP tested as a standard antagonist for 5-HT produced concentration-dependent inhibition in the contractile response of 5-HT and the peak effect was observed at 5000 mcg/ml (Mean percentage inhibition = 88.6 %). The observation also indicated that beyond 150 mg/ml, all tested compounds (except *T.chebula*) produced a significant ($P < 0.01$) reduction in the contraction compared with 5-HT.

The data in Table 2 represents the percentage effectiveness of different doses of herbal extracts against 5-HT-induced contractions. None of the extracts at the lowest tested dose (50 mcg/ml) had a significant effect. The extracts of *P.L* and *P.N* have shown a significant ($p < 0.05$) effect from 150 mcg/ml, and the efficacy enhanced when the doses were increased. *T.B.* was observed to

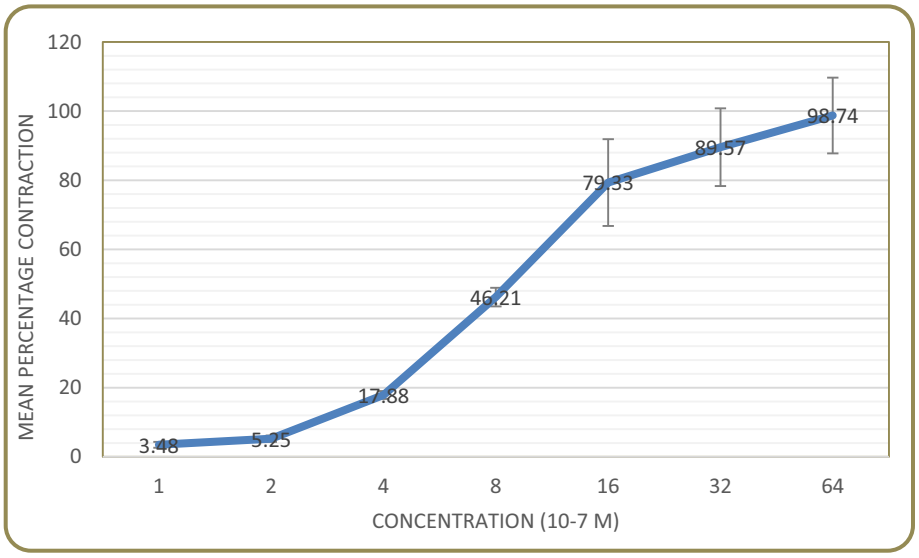

Fig. 6. Dose-response curve of 5-HT using rat uterus preparation.

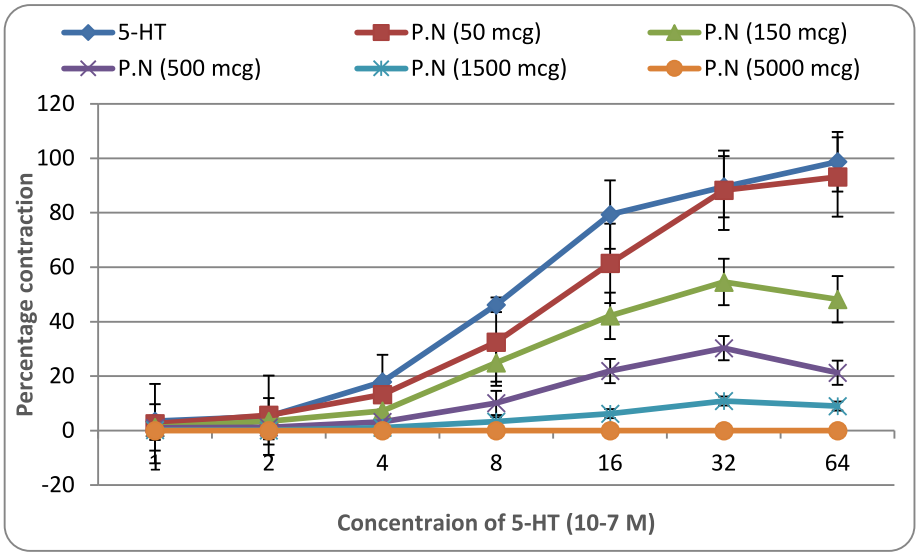

Fig. 7. Dose response curve of 5-HT against different concentration of P.nigrum.

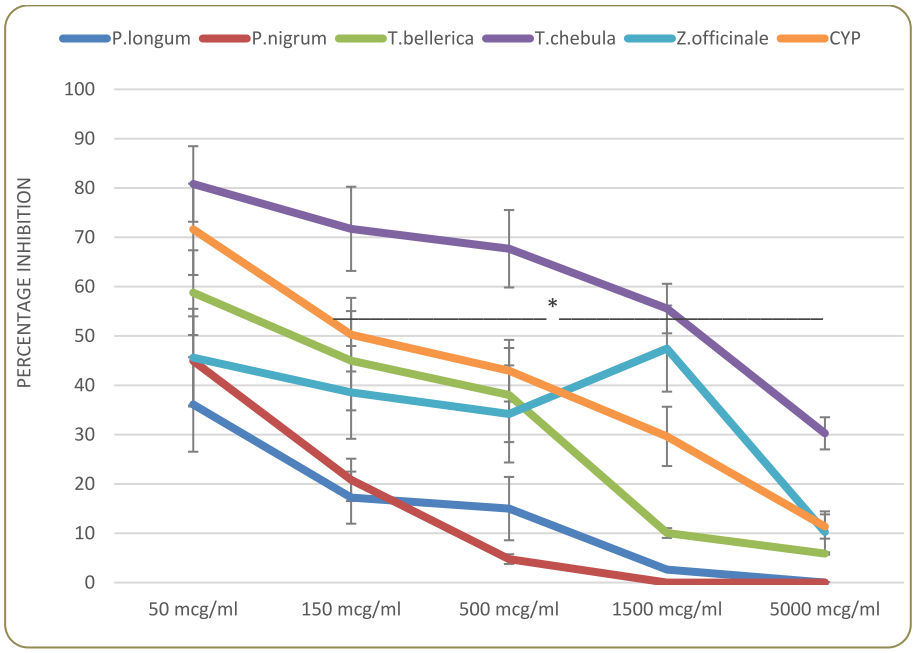

Fig. 8. Effect of different herbal extracts on 5-HT induced contractions in rat uterus preparation.

Table 2
Percentage inhibition of the different herbal extracts against 5-HT-induced contraction.

Doses of the extract	*P. longum*	*P. nigrum*	*T. bellerica*	*T. chebula*	*Z. officinale*	CYP
50 mcg/ml	63.88 ± 14.56	55.04 ± 8.54	41.21 ± 8.02	19.19 ± 2.03	54.41 ± 9.32	28.38 ± 6.20
150 mcg/ml	82.77 ± 18.54*	79.17 ± 11.80*	55.02 ± 10.26	28.28 ± 7.52	61.44 ± 10.23	49.74 ± 8.84
500 mcg/ml	85.01 ± 19.25*	95.23 ± 17.96**	61.97 ± 11.62	32.32 ± 8.62	65.81 ± 9.65	57.04 ± 10.29
1500 mcg/ml	97.20 ± 17.77**	100 ± 15.26**	89.95 ± 13.08*	44.44 ± 12.69	52.56 ± 8.49	70.36 ± 9.25
5000 mcg/ml	100 ± 18.01**	100 ± 19.52**	94.11 ± 16.43*	69.74 ± 11.17	89.74 ± 12.38*	88.63 ± 11.08*

Values are represented as mean ± SE. **Statistics**: One-way ANOVA. P < 0.05 and P < 0.01 compared with 5-HT response.

be significantly effective ($p < 0.05$) at 1500 and 5000 mcg/ml. However, *Z.O.* exhibited significant action only at the highest tested dose (5000 mcg/ml), while *T.C.* did not produce significant action at all the tested doses. On the other hand, CYP was found to be significantly ($p < 0.05$) efficacious at only the highest dose (5000 mcg/ml), and its other doses did not produce significant action on 5-HT-induced smooth muscle contraction.

The IC_{50} values in Fig. 7 suggest that *T.C.* has the highest value (1103 mcg/ml). The IC50 values found for other extracts such as *P.L, P.N, T.B, and Z.O* include 24.73, 15.29, 293.33, and 62.33 mcg/ml, respectively. The IC_{50} value for cyproheptadine was found to be 33.25 mcg/ml.

4. Discussion

The present study evaluated the anti-spasmodic properties of five herbal extracts, such as *P.L, P.N, T.B, T.C,* and *Z.O,* against histamine and 5-HT-induced contraction in isolated tissue preparations. Histamine produced smooth muscle contraction in isolated guinea pig ileum, and 5-HT produced contraction in rat uterus. Both the known spasmogens produced a concentration-dependent smooth muscle contraction in isolated tissues (Figs. 2 and 6).

Histamine is reported to induce smooth muscle contraction by binding to H1 receptors. The H_1 receptors are membrane-bound and are coupled with G-protein, especially Gq/11. The activation increases the level of phospholipase A_2 and D inside the cell, which in turn increases diacylglycerol and intracellular calcium levels, resulting in muscle contraction (Naganuma et al., 2018). On the other hand, 5-HT-induced smooth muscle contraction involves binding to $5HT_{2A}$ that is coupled with G-protein Gα. The interaction activates the phospholipase C that, causes the release of inositol triphosphate and diacylglycerol. The inositol triphosphate triggers calcium release from the sarcoplasmic reticulum, whereas diacylglycerol activates the phosphokinase C that in turn phosphorylates the L-type calcium channel thus triggering the smooth muscle contraction (Bhaskaran et al., 2014).

The present study indicated that the tested herbal extracts exhibited a concentration-dependent reduction in the smooth muscle contraction induced by histamine in guinea pig ileum. Among the tested extracts, only *P.L* and *P.N* completely abolished the histamine-induced contraction, and the effect was observed at 5000 mcg/ml (Fig. 4). Similarly, these two extracts, when tested in isolated rat uterus preparation, completely suppressed the contraction induced by 5-HT at 5000 mcg/ml (Fig. 8). The tested extracts in the earlier studies have shown anti-spasmolytic activity. *P.L.,* in a previous study, inhibited histamine as well as 5-HT-induced smooth muscle contraction (Kumar et al., 2011). Similarly, *P.N.* reduced potassium chloride-induced contraction in gastric and uterine muscles of experimental animals (Bui et al., 2017). *T.B.* tested in an isolated rabbit jejunum reduced the spontaneous contractions induced by carbachol and potassium ions (Gilani et al., 2008). *T.C.* inhibited the smooth muscle contraction induced by carbachol in an isolated tissue preparation of rat ileum (Mard et al., 2011). *Z.O.* in an isolated chicken intestinal preparation

exhibited spasmolytic activity induced by histamine and serotonin (Ghayur et al., 2005).

The percentage efficacy determined for different herbal extracts indicated that both *P.L* and *P.N* at a higher dose (5000 mcg/ml) exhibited 100% suppression ($p < 0.05$) on the smooth muscle contraction in guinea pig ileum. *T.B.* and *Z.O.* showed significant ($p < 0.05$) effectiveness (73%) at the highest tested dose (5000 mcg/ml), while *T.C.* did not produce significant action in any of the tested doses (Table 1). These observations showed quite a lot of similarity when herbal extracts were tested against a 5-HT-induced contraction in an isolated rat uterus. Only *P.L* and *P.N* at 5000 mcg/ml were found to produce significant ($p < 0.01$) and complete abolition of 5-HT induced contraction. *T.B.* and *Z.O.* produced significant ($p < 0.05$) activity at 5000 mcg/ml but did not completely abolish 5-HT-induced uterine contraction. On the other hand, *T.C.* at all tested doses did not produce significant effectiveness against 5-HT-induced contraction (Table 2).

Data from earlier studies indicated that calcium enhancement in the intracellular regions of smooth muscle plays an important role in the induction of contraction (Endo et al., 2006). The research on the tested herbal extracts suggests that these agents negatively affected the calcium concentration in the smooth muscles leading to the suppression of contraction (Kumar et al., 2011; Bui et al., 2017; Gilani et al., 2008; Mard et al., 2011; Ghayur et al., 2005). A previous study on *Zizyphus lotus* (L) suggested that the plant extract inhibited the contractions induced by acetylcholine, potassium chloride, and barium chloride in isolated rat duodenum. The study indicated that the active phytoconstituents present in the extract might have reduced the calcium levels in the smooth muscle to exhibit anti-spasmodic activity (Borgi et al., 2009). Among the tested extracts, *P.L* and *P.N* appear more potent in suppressing histamine and 5-HT-induced contraction. The role of other mechanisms involving neurotransmitters can be speculated for their potent action (Lai et al., 2019). The IC_{50} values obtained from different responses of the extracts on the dose–response curve of spasmogens also indicated that these extracts suppressed smooth muscle contraction at a lower concentration. The IC_{50} values for *P.L.* were 400 mcg/ml against histamine and 24.73 mcg/ml against 5-HT. In addition, the IC_{50} values for *P.N* were observed to be 184 mcg/ml against histamine and 15.29 mcg/ml against 5-HT (Figs. 5 and 9).

This study tested CPM and CYP as standard anti-histaminergic and anti-serotonergic agents, respectively. These agents also produced a concentration-dependent suppression of contractions induced by spasmogens (Histamine and 5-HT). However, both the standard drugs did not abolish the action of known spasmogen completely (Figs. 4 and 8), and the highest tested dose (5000 mcg/ml) produced the maximum efficacy of 80% in guinea pig ileum and 70% in rat uterus (Table-1 and 2). Being a known antagonist, these agents could have acted on either the H_1 receptor (CPM) or $5HT_{2A}$ receptor (CYP) in the smooth muscle to reduce all the related changes produced by histamine or 5-HT (Díaz Nebreda et al., 2019; Kim et al., 2019). The tested extracts might have also produced similar action on the H_1 and $5HT_{2A}$ receptors and blocked all molecular mechanisms in the smooth muscles to reduce the contraction.

Studies conducted in the past indicated that extracts of P.L and P.N contain several phytochemicals such as alkaloids, carotenoids, esters, flavonoids, steroids, and terpenoids (Afreen et al., 2021; Salehi et al., 2019). One of the active components present in the species of piparaceae called piperine has been isolated and identified. The structural elucidation suggested that piperine is acylpiperidine alkaloid having the chemical composition C17H19NO3 (Fig. 10) and named (IE,3E)-1-(1,3-benzo dioxol-5-yl)-5-oxopenta-1,3-dien-5-yl group at nitrogen atom (Singh and Choudhary, 2015). Further to establish the type of inhibition, P.N was tested in different concentration against the dose–response curves of histamine and 5-HT. The observations recorded indicated that the concentrations of P.N extracts shifted the dose–response curves towards right-hand side (Figs. 3 and 7). These responses suggested that the extracts might induce competitive antagonism

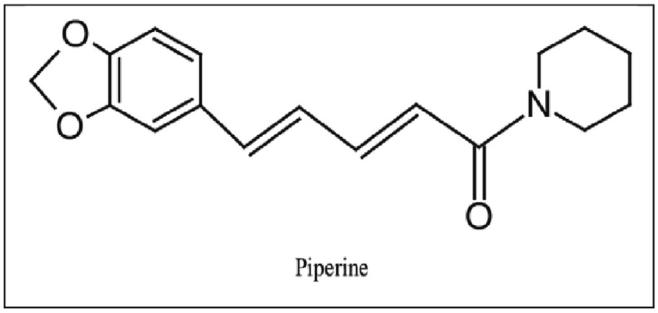

Fig. 10. Chemical structure of piperine isolated from *P.L* and *P.N.*

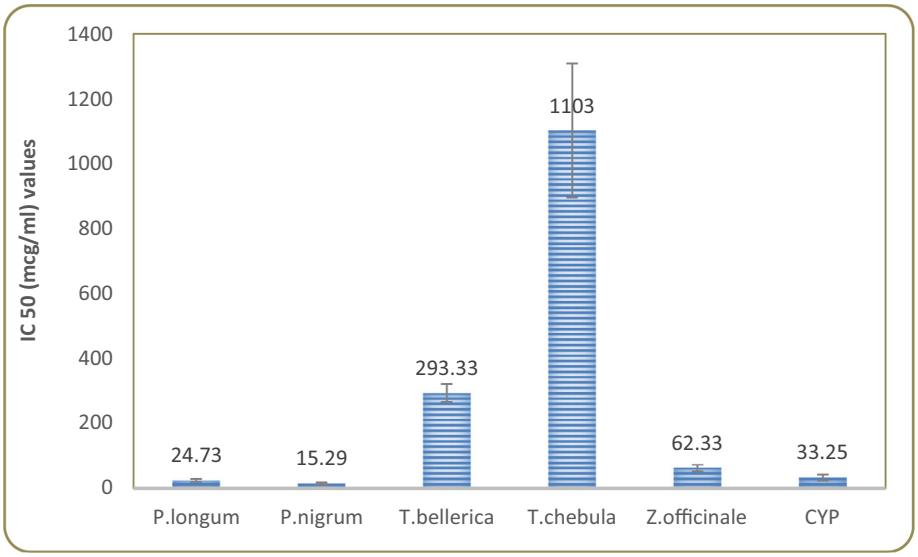

Fig. 9. IC_{50} values of different herbal extracts on 5-HT induced contractions in rat uterus.

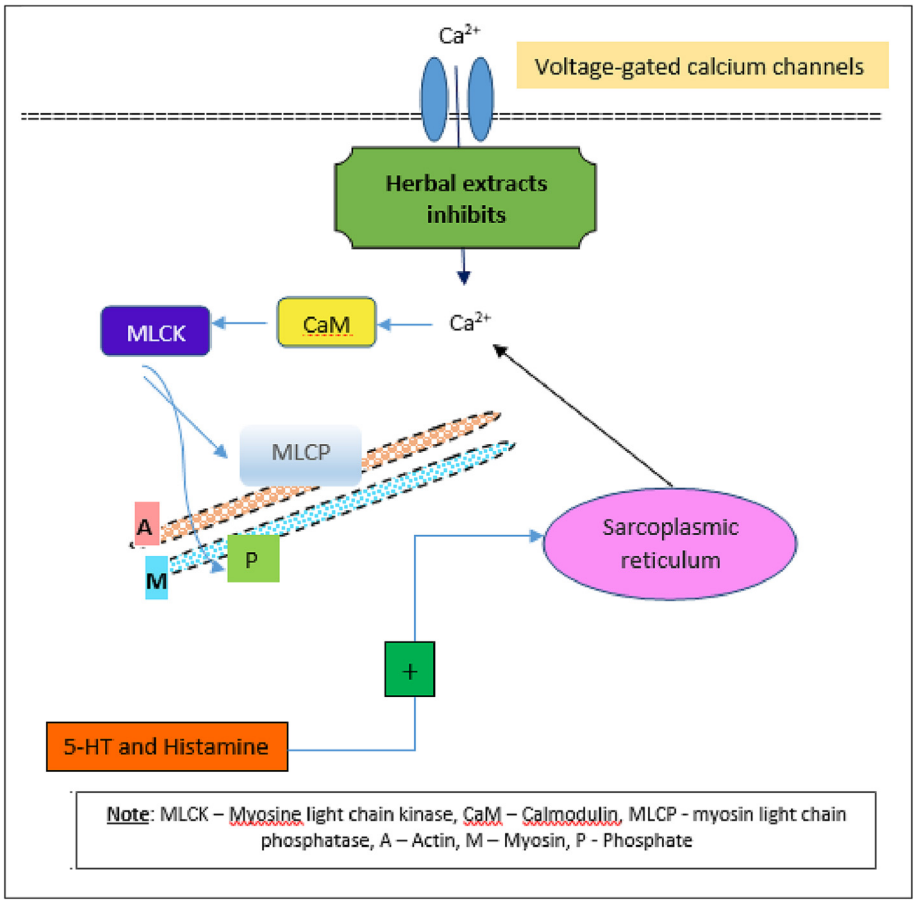

Fig. 11. Proposed anti-spasmodic mechanism of herbal extracts.

against the spasmogenic activity of histamine or 5-HT (Nunes-Neto PA, et al. 2017).

Considering these, in the present study also, piperine and other phytoconstituents present in the extracts might have exhibited anti-spasmodic activity against known spasmogens in isolated tissue preparations. Affecting calcium concentration in the smooth muscles appears to be one of the possibilities for the spasmolytic action of the herbal extracts (Bui et al., 2017; Ghayur et al., 2005; Borgi et al., 2009) and so is represented in Fig. 11. Although *P.L* and *P.N* showed more potency against the spasmogenic activity of histamine and 5-HT but more research, especially on isolated phytochemicals of the extracts and involving different experimental models, is essential before establishing the precise safety and efficacy against spasmogenic-induced disorders.

5. Conclusion

The observations from the study indicated that *Piper longum, Piper nigrum, Terminalia bellerica,* and *Zingiber officinale* exhibited concentration-dependent anti-spasmodic activity in isolated guinea pig ileum and rat uterus. The study validated the traditional claim that these medicinal plants possess spasmolytic activity and might provide an option for treating spasms with herbal medicines. *Piper longum* and *Piper nigrum* showed potent spasmolytic effects against histamine and 5-HT-induced smooth muscle contractions, probably by acting as competitive antagonists. One of the possible mechanisms suggested for the anti-spasmodic property include reduction of calcium concentration in the smooth muscle. The herbal extracts need further research before establishing their efficacy in treating intestinal and uterine painful contractions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors would like to acknowledge the Researchers Supporting Project number (RSP2023R431), King Saud University, Riyadh, Saudi Arabia, for extending financial support to do this research project.

Funding

The authors are thankful to AlMaarefa University for providing support to do this research.

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