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Research Article
A Preclinical Study to Evaluate the Spasmolytic Activity of *Piper longum*, *Piper nigrum*, *Terminalia bellerica*, *Terminalia chebula* and *Zingiber officinale*

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Abstract

Background and Objective: Muscular spasms can occur for a variety of reasons and are quite common in the general population. In this work, the efficacy of certain commonly used herbal extracts for spasmolytic activity was investigated using isolated rat fundus and rabbit jejunum preparations. **Materials and Methods:** Five herbal extracts, including *Zingiber officinale* (ZO), *Piper longum* (PL), *Piper nigrum* (PN), *Terminalia bellerica* (TB) and *Terminalia chebula* (TC), were examined. In an isolated rat fundus, 5-Hydroxytryptamine (5-HT) was utilized as a spasmogen and the effects of extracts were assessed. After giving herbal extracts, the frequency and amplitude of contraction in the rabbit jejunum were measured. The efficacy and influence of the extracts on smooth muscle contraction were determined and statistically assessed using one-way ANOVA. **Results:** Data from the study showed that all five herbal extracts suppressed 5-HT-induced responses in a concentration-dependent manner. At the highest tested dose (5000 mcg mL^{-1}), all the extracts except ZO abolished the 5-HT induced contraction and TC exhibited this effect even at the initial dose (1500 mcg mL^{-1}). The lower doses (5-500 mcg mL^{-1}) significantly ($p<0.001$) increased the contraction amplitude while the highest dose (5000 mcg mL^{-1}) reduced the frequency and amplitude of contractions in rabbit jejunum. Overall, TC was more effective than other herbal extracts evaluated in the study at preventing 5-HT-mediated contraction in both rat fundus and rabbit jejunum preparations. **Conclusion:** This study demonstrated the potential role of herbal extracts in the functioning of gastrointestinal smooth muscles. Therefore, the studied herbal extracts could be taken up further for in-depth analysis to explore their possible role in the therapeutic management of gastrointestinal diseases.

Key words: Herbal extracts, isolated rat fundus, isolated rabbit jejunum, anti-spasmodic activity, *Zingiber officinale*, *Piper longum*, *Piper nigrum*, *Terminalia bellerica*, *Terminalia chebula*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plant-based medicine has been popular since ancient times and is reported to have contributed to the development of about 25% of medicines used nowadays[1]. According to reports, around 80% of the total population uses herbal medicine for treating diseases of various origins. *Piper longum*, *Piper nigrum*, *Terminalia bellerica*, *Terminalia chebula* and *Zingiber officinale* are traditionally used folk medicines for treating various ailments, including musculoskeletal disorders[2].

A deciduous, thin, aromatic climber of the Piperaceae family, *Piper longum* (PL) has been shown to have a variety of biological functions. It has been reported to work against several types of cancer and alleviate inflammation and breakdown spasms. Important substances that relate to the pharmacological activities of plants have been found, including piperine, pipalestrol, pippalartin, pipperleguminin and piplartine[3]. *Piper nigrum* (PN), a blooming woody perennial climber that grows in shady areas, is another member of the Piperaceae family. Piperamide, piperamine, piperettine, pipericide, piperine and serpentine are significant phytoconstituents known for their medicinal effects. It is reported to obviate pain, normalize mood and stabilize smooth muscle contractions[4].

A deciduous tree in the Combretaceae family called *Terminalia bellerica* (TB) is well known for managing diabetes, protecting the liver against toxic substances, promoting the healing of wounds, stabilizing smooth muscle contraction and reducing respiratory distress. Bellricanin, chebulinic acid, belleric acid and chebulagic acid are among the chemical components identified from the plant[5]. *Terminalia chebula* (TC) is another member of the Combretaceae family, growing as a large deciduous tree with alternate leaves. The fruits of this plant are known to possess medicinal values for several ailments including muscle rigidity. Several phytocomponents have been found in the plant, including ellagic acid, chebulinic acid, chebulagic acid, corilagin, chebulanin, terchebulin and phloroglucinol[6].

The Zingiberaceae family includes the herbaceous rhizomatous plant *Zingiber officinale* (ZO), which has aromatic, thick-lobed rhizomes. According to reports, the rhizomes exhibit a variety of biological properties. Some of the significant active components found in ZO are gingerol, gingerdione, zingerone, shogaol and zingiberene[7].

A muscular spasm is an involuntary, painful and sudden contraction that occurs for a few seconds and is relieved independently. According to a study, about 56% of patients reported to clinical in the United States suffered a spasm[8]. Dehydration, electrolyte imbalance, excessive usage of the muscles, muscular stress, a deficiency in nutrients, a need for increased blood flow and a number of underlying medical disorders are the main known causes of muscle spasms[9]. In most cases, the spasms resolve on their own and avoiding the triggering situations aids in faster recovery[10]. If the spasm persists, medical interventions such as Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are required[11]. However, chronic intake of these agents is known to produce several adverse effects like dyspepsia, hemorrhage, peptic ulcer and perforation[12]. The search for safe medicines to treat spasms has given the scope for testing herbal drugs[13].

According to the literature, the above-mentioned herbal drugs are effective traditionally for spasm disorders without detailed scientific evidence. Some are combined or separately in different formulations sold as over-the-counter herbal formulations. However, there is data available that compares the potential of one of them against another. The absence of information on individual potential will continue exposing many herbal products without scientific validation. Therefore, our interest was to compare the spasmolytic potential of these herbal products in established experimental animal models using isolated tissue preparations.

MATERIALS AND METHODS

Study area: The study was conducted in the Research Laboratories of Al-Ameen College of Pharmacy, Bangalore as per the guidelines of 'Good Laboratory Practices' and after approval from the Institutional Animal Ethics Committee. The study duration was between September, 2020 to June, 2021.

Chemical and drugs: The reagents and chemicals used in the study were of analytical grade meant for the research purpose. These were obtained after placing a purchase order from regular suppliers authorized by the Al-Ameen College of Pharmacy, Bangalore, India. Natural Remedies in Bangalore, India provided a gift sample of extracts for research purposes. As 5-Hydroxytryptamine (5-HT) was purchased from an authorized supplier of Sigma-Aldrich, Bulgaria. Cyproheptadine hydrochloride was procured from Wockhardt Limited, Mumbai, India.

Animals: The current investigation utilized eighteen Wistar rats (either sex), who were 4-5 months old and weighed 150-200 g. The experimental animals were raised in the Al-Ameen College of Pharmacy's Central Animal House in Bangalore under typical laboratory conditions, including free access to food and water and housing at room temperature.

Six rabbits of either sex and weighing one to three kilograms were also supplied from the main animal house. The experiment was carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and after approval from the Institutional Animal Ethics Committee of Al-Ameen College of Pharmacy, Bangalore, India (AACP/M112/2).

Preparation of tests and standard drugs: Freshly prepared solutions for TC, TB, PN, PL and ZO were used. The extracts were supplied either as a spray-dried powder (TC and TB), semi-dried methanolic paste (PN and ZO) or dried methanolic paste (PL). The solutions were freshly made by dissolving in distilled water using a blender and the supernatant was further diluted to provide the appropriate dosages (50-5000 mcg mL^{-1})[3-7].

Isolated rat fundus preparation: Under a mild ether anesthetic, the experimental rats were killed by cervical dislocation and their stomachs were then revealed. According to the procedure outlined in the literature, the fundus was located and cut along the pylorus, leaving a narrow strip of pyloric tissue adhering to the fundus and the tissue was then immediately placed in the dish with the Tyrode solution. Alternating zigzag cuts were performed to produce a fundus strip preparation in areas of the fundus that had less curvature. The fundus strip's ends were knotted together with thread and then mounted in an organ bath filled with Tyrode's solution that was kept at a temperature of 37°C with continuous aeration. The preparation was loaded with 1 g and given 30 min to equilibrate[14]. Using the fundus strip preparation, a concentration-dependent response curve for 5-hydroxytryptamine was obtained and the ceiling response was calculated. After two minutes of contact time with various concentrations of herbal extracts (5-5000 mcg mL^{-1}), an agonist (5-HT) was added (30 sec of contact time) and the smooth muscle contractions were measured. Using the same tissue, the responses to extracts and the conventional antagonist cyproheptadine (CYP) were recorded. For the CYP, experiments were repeated three to four times for each of the five tested concentrations of extract.

Isolated rabbit jejunum preparation: Using cervical dislocation while under a light ether anesthetic, an experimentally acquired rabbit was sacrificed and the sampling for each test agent was done 3-4 times. To locate the jejunum, the lower abdomen was made visible. The caecum and jejunum were separated from the stomach by about 15 and 10 cm, respectively. The separated tissue was then sliced into 2-3 cm long segments without mesenteric connection and immediately placed in a beaker containing Tyrode solution. The tissue strip's higher end was fastened to the force transducer, while its lower end was fastened to the tissue holder. The preparation was immediately placed in a 25 mL organ bath containing Tyrode's solution and kept at 37 with constant aeration. Finally, a 1 g of calibrated steel metal load was added to the tissue, which was allowed to stabilize for 30 min. The rhythmic contractions were amplified and recorded using two-channel pyrite[15]. By keeping a 1 min gap between individual doses, the herbal extracts' spasmolytic activity was measured at various concentrations such as 5, 150, 500, 1500 and 5000 mcg mL^{-1}. A normal reaction was recorded before assessing the response of extracts and the decline in response caused by the test agent was compared to the normal.

Statistical analysis: The contraction caused by the agonist 5-hydroxytryptamine was assumed to be 100% when calculating the mean percentage of contractions produced by various concentrations of extracts/CYP. The data were analyzed using one-way ANOVA and a *post hoc* comparison was made using Dunnett's Test. The p-value less than 0.5 was considered to indicate the significance when the data between groups were compared.

RESULTS

Rat fundus preparation: The concentration-dependent curve of 5-HT was obtained at different concentrations, the lowest tested dose was 1×10^{-5} M. A sigmoid curve was obtained and the threshold value for the 5-HT induced contraction was 4×10^{-5} M. The mean percentage contraction progressively increased when the doses were enhanced. The highest tested concentrations, 32 and 64×10^{-5} M produced 89.57% and 98.74% responses, respectively (Fig. 1).

Figure 2 shows the effect of different concentrations of extracts on the 5-HT (64×10^{-5} M) mediated contraction in isolated rat fundus. The PL at 50 mcg mL^{-1} produced 65% of 5-HT response and as the dose was increased, the 5-HT responses were suppressed. At 5000 mcg mL^{-1}, PL was observed to abolish the response of 5-HT completely. The PN at a low dose (50 mcg mL^{-1}) produced 54.05% of 5-HT response and this was further suppressed when the doses were increased, ultimately blocking the 5-HT-mediated contraction at the dose of 5000 mcg mL^{-1} completely. The TB also exhibited similar suppression on the 5-HT mediated

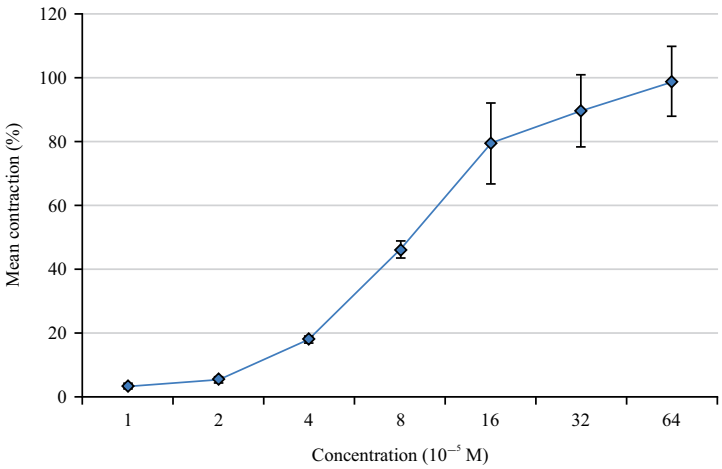

Fig. 1: Dose-response curve of 5-HT recorded as mean percentage contraction using rat fundus

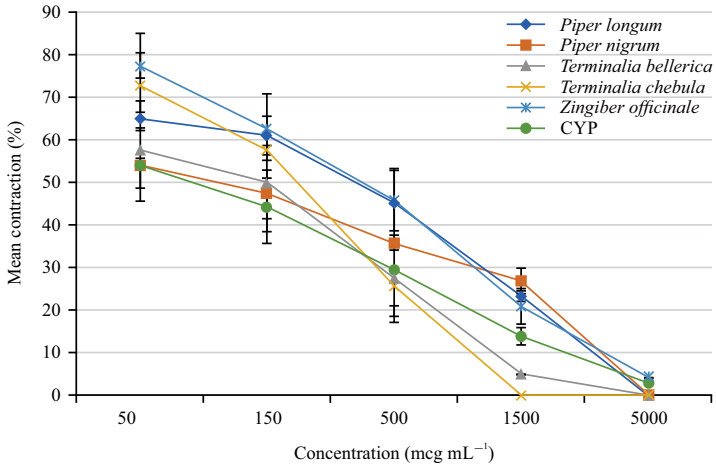

Fig. 2: Response of different herbal extracts on the 5-HT-induced contractions (mean percentage) in rat fundus
Values are expressed as mean percentage contraction±SD and Statistics: One-way ANOVA followed by Dunnett's Test

contraction and completely blocked the effect at 5000 mcg mL^{-1}. The TC at a lower dose (50 mcg mL^{-1}) produced 72.69% of 5-HT, but at 1500 and 5000 mcg mL^{-1} completely suppressed the contraction. The ZO also produced a dose-dependent reduction of the 5-HT-induced rat fundus contraction. However, none of the tested doses completely abolished the 5-HT response. The CYP tested in the study as a standard anti-serotonergic agent showed a dose-dependent suppression of 5-HT responses. Still, the drug at tested doses did not produce complete blockage of the 5-HT effect.

Table 1 lists the various doses of herbal extracts' percentage of effectiveness in preventing 5-HT-induced smooth muscle contraction. Lower tested dosages of PL (50, 150 and 500 mcg mL^{-1}) did not significantly inhibit 5-HT-mediated contraction. The larger doses (1500 and 5000 mcg mL^{-1}) however, demonstrated a substantial ($p<0.01$) efficacy against the 5-HT contraction. Similar findings were made for PN and ZO, where the higher tested dosages (1500 and 5000 mcg mL^{-1}) substantially ($p<0.01$) demonstrated spasmolytic effect against 5-HT-mediated contraction and PN was found to block the 5-HT-induced contraction at 5000 mcg mL^{-1}. Furthermore, at dosages beginning at 500 mcg mL^{-1}, TB, TC and CYP revealed significant effects ($p<0.05$). The TB caused a full blockade at 5000 mcg mL^{-1}, whereas TC blocked at 1500 mcg mL^{-1}. When CYP was tested at various doses, a full abolishment of the 5-HT action of this kind was not observed.

The IC_{50} values recorded for various herbal extracts suggested that the highest value (424.66 mcg mL^{-1}) was observed for PL, followed by ZO (341.5 mcg mL^{-1}). The IC_{50} values recorded for TB, TC and PN were 203.2 mcg, 173.1 mcg and 128.33 mcg mL^{-1}, respectively. The IC_{50} value for CYP against 5-HT-induced contraction was 26.14 mcg mL^{-1} (Fig. 3).

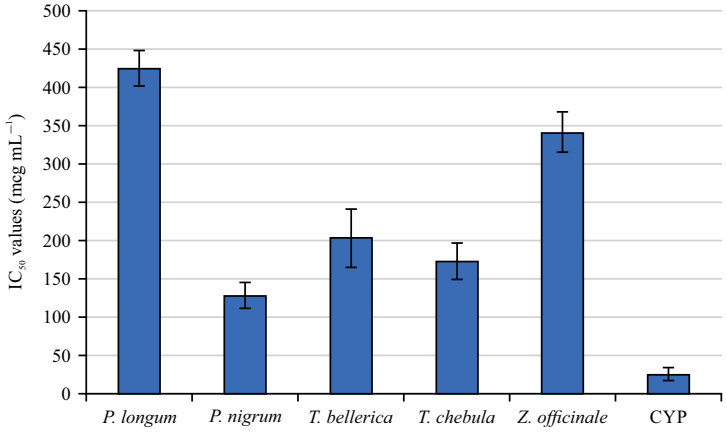

Fig. 3: IC_{50} values of different herbal extracts against 5-HT-induced contractions in rat fundus

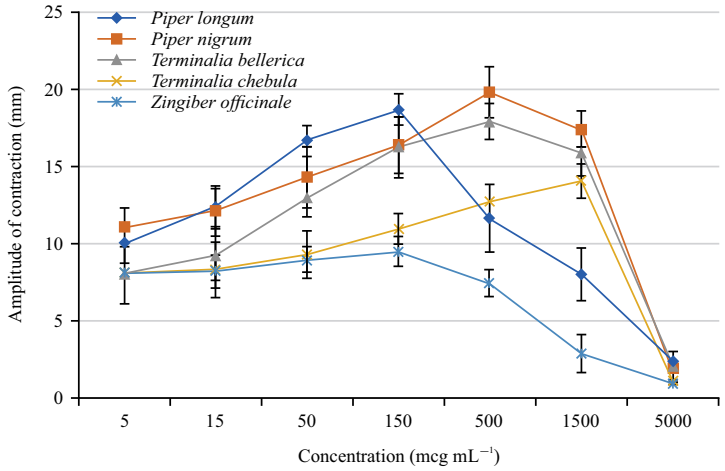

Fig 4: Effect of different herbal extracts on contraction amplitude in rabbit jejunum preparation

Values are expressed as mean percentage contraction±SD and Statistics: One-way ANOVA followed by Dunnett's Test

Table 1: Percentage inhibition of serotonin contractions by extracts

Doses of the extract	*P. longum*	*P. nigrum*	*T. bellerica*	*T. chebula*	*Z. officinale*	CYP
50 mcg mL^{-1}	35.03±6.32	45.94±7.09	42.51±8.08	27.30±5.23	22.91±4.29	36.07±5.46
150 mcg mL^{-1}	39.07±7.08	52.61±8.29	50.01±9.66	42.45±6.09	37.50±5.28	55.74±6.76
500 mcg mL^{-1}	54.81±9.52	64.41±7.99	72.5±9.83*	74.41±9.18*	54.16±6.27	70.61±8.13*
1500 mcg mL^{-1}	76.85±9.45*	73.19±10.06*	95.04±9.46**	100±8.64**	79.16±8.56*	86.22±8.90*
5000 mcg mL^{-1}	100±10.65**	100±9.25**	100±9.89**	100±7.46**	95.83±9.03**	91.23±10.09**

Values are expressed as mean percentage contraction±SD, Statistics: One-way ANOVA followed by Dunnett's Test, *p<0.05 and **p<0.01 compared between groups

Rabbit jejunum preparation: The analysis of the results indicated that PL showed a dose-dependent contraction when tested at a concentration of 5-150 mcg, but afterward, a contraction was reduced as the dose was increased. The PN at 5000 mcg mL^{-1} reduced the smooth muscle contraction beyond normal levels. Similarly, PN and TB induced dose-dependent smooth muscle contraction when tested at 5-500 mcg mL^{-1}, further, an increase in the dosage (1500 mcg mL^{-1}) reduced the contraction and the maximum tested dose (5000 mcg mL^{-1}) decreased the contraction beyond the normal level.

However, TC showed a slow increase in smooth muscle contraction when tested at doses 5-500 mg mL^{-1} and then the highest dose (5000 mcg mL^{-1}) spontaneously reduced the contraction beyond the normal level. The ZO produced a marginal increase in contraction amplitude when tested at 5-1500 and at 5000 mcg mL^{-1} showed a sudden reduction in contraction that went below normal levels (Fig. 4).

Table 2: Percentage change in the amplitude of contraction in rabbit jejunum

Doses of the extract	*P. longum*	*P. nigrum*	*T. bellerica*	*T. chebula*	*Z. officinale*
5 mcg mL^{-1}	19.86±1.26***	27.33±3.58***	0.12±0.01	0.37±0.01	0.49±0.01
15 mcg mL^{-1}	34.97±2.02***	33.58±2.86***	13.28±1.14**	3.13±0.22	1.71±0.06
50 mcg mL^{-1}	51.85±4.28***	43.80±4.85***	38.08±2.74***	13.37±1.08**	10.37±0.82**
150 mcg mL^{-1}	56.98±5.06***	51.00±4.59***	50.61±3.26***	26.73±2.67***	15.11±2.09***
500 mcg mL^{-1}	31.07±2.73***	59.54±6.49***	55.18±2.43***	37.01±2.93***	-8.22±0.01*
1500 mcg mL^{-1}	-0.12±0.04	53.85±5.91***	49.56±4.08***	42.88±2.95***	-51.75±0.01***
5000 mcg mL^{-1}	-40.25±0.02***	-18.22±0.01***	-89.80±0.002***	-23.42±0.001***	-182.41±0.001***

Values are expressed as mean percentage contraction±SD, Statistics: One-way ANOVA followed by Dunnett's Test, *p<0.05, **p<0.01 and ***p<0.001 compared between groups

Table 3: Percentage change in the frequency of contraction in rabbit jejunum

Doses of the extract	*P. longum*	*P. nigrum*	*T. bellerica*	*T. chebula*	*Z. officinale*
5 mcg mL^{-1}	9.12±1.26	8.23±1.33	8.16±1.66	7.68±1.39	8.46±1.39
15 mcg mL^{-1}	9.23±2.96	9.12±3.12	8.46±0.67	7.95±0.99	9.64±1.86
50 mcg mL^{-1}	12.23±3.62*	8.26±1.82	9.12±1.29	8.23±0.56	12.68±2.79*
150 mcg mL^{-1}	14.02±3.16**	8.12±0.96	9.46±1.74	8.49±1.16	15.93±3.67**
500 mcg mL^{-1}	13.20±2.99*	7.99±0.84	8.02±0.95	9.45±0.39	10.96±2.59
1500 mcg mL^{-1}	9.06±1.73	6.23±0.26	7.95±0.37	9.82±1.62	8.46±1.26
5000 mcg mL^{-1}	5.11±1.16*	6.19±0.67*	4.59±0.06*	2.13±0.33**	2.09±0.71**

Values are expressed as mean percentage contraction±SD, Statistics: One-way ANOVA followed by Dunnett's Test, *p<0.05 and **p<0.01 compared between groups

Table 2 represents the percentage change in contraction amplitude after administering different doses of herbal extracts. The PL and PN showed a dose-dependent and significant ($p<0.001$) alteration in the amplitude of smooth muscle contraction. On the other hand, the highest tested dose (5000 mcg mL^{-1}) reduced contraction beyond normal, as indicated by a negative sign. The TB at a lower tested dose (5 mcg mL^{-1}) did not significantly change the smooth muscle contraction. Still, as the dose increased, the contraction amplitude increased significantly ($p<0.001$) to 500 mcg mL^{-1}. Further, the increased dose reduced the amplitude and the maximum dose (5000 mcg mL^{-1}) decreased the contraction beyond normal levels. The TC at lower doses (5 and 15 mcg mL^{-1}) did not produce significant change. However, an increase in the amplitude of contraction (%) was seen at doses 50 to 500 mcg mL^{-1} and less than normal contraction at 5000 mcg mL^{-1}. The ZO at 50 and 150 mcg mL^{-1} showed an increase in the contraction amplitude and a further increase in dosage showed a significant ($p<0.05$) suppression in the contraction amplitude beyond normal levels.

The activity of various herbal extracts on the contraction frequency suggested that PL at 50, 150 and 500 mcg mL^{-1} significantly ($p<0.05$) increased the frequency compared to the control. However, the extract at the highest tested dose (5000 mcg mL^{-1}) significantly ($p<0.05$) reduced the incidences of contraction. Similarly, ZO at 50 and 150 mcg mL^{-1} produced a significant ($p<0.05$) augmentation in frequency and at a maximum dose (5000 mcg mL^{-1}), significantly inhibited ($p<0.05$) the frequency of contraction compared to control. However, PN, TB and TC showed a significant ($p<0.05$) reduction of frequency of contraction at 5000 mcg mL^{-1} compared to the control and other doses did not produce such alteration (Table 3).

DISCUSSION

This study suggested that the herbal extracts produced a concentration-dependent reduction in the smooth muscle contraction mediated by 5-HT in rat fundus. All the tested extracts completely inhibited the 5-HT-mediated contraction and the activity was observed at 5000 mcg mL^{-1}. Moreover, TC produced complete suppression at 1500 mcg mL^{-1} (Fig. 2). The percentage efficacy evaluation indicated that all the herbal extracts at higher doses (1500 and 5000 mcg mL^{-1}) significantly suppressed the 5-HT-mediated contraction. Besides, TB and TC also showed a significant reduction in 5-HT mediated response at 500 mcg mL^{-1} onwards (Table 1).

These herbal extracts in previous studies have produced anti-spasmolytic activity. According to Kumar *et al.*[15], extract prepared from an herbal agent was observed to inhibit histamine and 5-HT-induced smooth muscle contraction[15]. The PN and its active constituent, piperine, inhibited potassium chloride-mediated contraction in smooth muscles of gastric and uterine[16]. The TB reduced the spontaneous contractions brought on by carbachol and potassium ions when evaluated in an isolated rabbit jejunum[17]. In an isolated tissue preparation of the rat ileum, studies on TC revealed that carbachol-induced smooth muscle contraction was inhibited[18]. In addition, ZO was discovered to have a spasmolytic effect against histamine and serotonin when it was evaluated in an isolated chicken intestinal preparation[19].

The CYP in this study is used as a standard anti-serotonergic agent. The drug also produced a dose-dependent reduction in 5-HT-mediated smooth muscle contraction (Fig. 2). However, none of the doses of CYP tested in this study produced a complete abolition of the 5-HT responses (Table 1). According to earlier investigations, 5-HT-mediated smooth muscle contraction includes binding to 5HT2A in combination with G-protein Gα[20]. The inositol triphosphate and diacylglycerol are then released as a result of activating the phospholipase C enzyme. In addition to causing calcium to be released from the sarcoplasmic reticulum, inositol triphosphate also activates phosphokinase C, which leads to the contraction of smooth muscle[20]. The CYP is an antagonist of 5-HT and its action could have prevented all the cellular and sub-cellular changes that could ultimately lead to the inhibition of muscle contraction.

The IC_{50} values also indicate that CYP and the herbal extracts might have acted as an antagonist of 5-HT to prevent its physiological effects. Previous studies suggested that calcium concentration in the muscle cell plays a crucial role in contraction[21]. The studies conducted earlier suggested that the tested herbal extracts had negatively influenced the calcium concentration inside the smooth muscle[16-19,22]. Based on this information, the herbal extracts also might have prevented all the changes induced by 5-HT in the smooth muscles. Besides, the role of herbal extracts on other pathways, such as adrenergic neurotransmission, can be linked to their potent response[23].

The studies on the isolated rabbit jejunum indicated that the herbal extracts dose-dependently enhanced the contraction amplitude when tested at 5-500 mcg mL^{-1}. The amplitude of contraction decreased when the extract dosage was increased to 1500 mcg mL^{-1}, at 5000 mcg mL^{-1}, it reduced beyond normal levels (Fig. 4). The percentage change evaluation also indicated that the lower doses (5-500 mcg mL^{-1}) increased the amplitude and higher doses (1500 and 5000 mcg mL^{-1}) reduced it (Table 2). According to the literature, the mobilization of calcium ions in the smooth muscle cells is essential for impulsive contraction in the rabbit jejunum. The calcium level plays a role in frequencies and contraction amplitude[24]. The herbal extracts tested in the study have been reported earlier to limit the availability of calcium ions inside the smooth muscles [16-19,22]. This mechanism may lessen the intensity and frequency of contractions in the jejunum of rabbits[25,26].

The study to find out the influence of herbal extract on the contraction frequency suggested that PL and ZO showed increased frequency. However, all the extracts at a higher dose (5000 mcg mL^{-1}) minimized the frequency significantly ($p<0.05$) less than the control values (Table 3). The spasmogenic activity observed at lower doses and the reduction of spontaneous muscle contraction beyond the normal level at higher doses needs more research involving different *in vivo* and *in vitro* models.

FUTURE RECOMMENDATIONS

The observations of the present study indicated that the herbal extracts of *Piper longum*, *Piper nigrum*, *Terminalia chebula*, *Terminalia bellerica* and *Zingiber officinale* attenuated the smooth muscle contractions induced by 5-HT. The study suggested that the test compounds might possess the anti-spasmogenic property and could be an alternative safe medicine for treating spasms of the gastrointestinal tract since the agents derived from nature *per se* are devoid of major toxic manifestations. However, the observations of the present study were recorded from isolated animal tissues and the effect when tested on whole animals might not be the same. Therefore, more studies are needed involving different experimental models to precisely establish the safety and efficacy of plant-based products.

CONCLUSION

The results of the current study suggest that *Piper longum*, *Piper nigrum*, *Terminalia bellerica* and *Zingiber officinale* have dose-dependent inhibitory effects on 5-HT-induced smooth muscle contraction. The agents might have produced this action by antagonizing the 5-HT receptors and lowering the intracellular calcium levels. Being derived from nature, these agents could treat various spasmogenic disorders. However, the spasmogenic induced by lower doses of extracts and inhibition of normal spontaneous contraction in rabbit jejunum suggests the possibility of adverse effects. More research is needed on these herbal agents before ascertaining their safety and efficacy in managing muscular diseases.

SIGNIFICANCE STATEMENT

Five herbal extracts namely *Zingiber officinale* (ZO), *Piper longum* (PL), *Piper nigrum* (PN), *Terminalia bellerica* (TB) and *Terminalia chebula* (TC) were examined for spasmolytic activity using animal models. As 5-HT was used to induce the spasm and the effect of five extracts at 50-5000 mcg mL^{-1} was tested in isolated rat fundus and rabbit jejunum preparations. The extracts at the tested doses exhibited a dose-dependent reduction in smooth muscle contraction. Among the tested herbal extracts, TC was found to be more effective in decreasing the spasmogenic activity of 5-HT. The data of the study established the anti-spasmogenic properties of the herbal extracts.

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