International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.1466.1473

Research Article
Gut Stimulatory Effect of *Terminalia chebula* in Experimental Animal Models

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Abstract

Background and Objective: Constipation is one of the universal gastrointestinal problems that affect people of all ages. Several medications are used to relieve constipation, but their use is associated with adverse effects. This study evaluated the gut stimulatory activity of *Terminalia chebula* powder extract using *in vitro* and *in vivo* experimental methods. **Materials and Methods:** The powder extract of *Terminalia chebula* (*T. Chebula*) was reconstituted in distilled water. *In vitro* gut motility studies were performed on isolated rat colon, Guinea pig ileum and rabbit jejunum, while *in vivo* study was conducted on mice to determine the charcoal transit time. The *in vitro* studies were conducted in the presence and absence of atropine. The results were statistically analysed and $p<0.05$ was considered to indicate significance. **Results:** The observations indicated that *T. chebula* extracts produced dose-dependent stimulation of the smooth muscles of the colon, ileum and jejunum and the magnitude was found to be less than acetylcholine (Ach). The dose-response curves of both *T. chebula* and Ach were found to be shifted to the right-hand side when tested in presence of atropine. The intestinal transit time for a charcoal meal in mice significantly increased ($p<0.05$) when compared with normal animals. **Conclusion:** The mild stimulatory action induced by *T. chebula* could be useful in relieving the defective gastric motility without affecting the functional integrity of the digestive system. However, more studies are needed to establish the safety and efficacy including the mechanism of action of *T. chebula*.

Key words: *Terminalia chebula*, gut stimulatory, gut motility, isolated tissues, intestinal transit time, laxative, constipation

Citation: Tandur, V.K., M.N. Inamdar, A. Alamri, M. Alhomrani, W.F. Alsanie, S.I. Rabbani and S.M.B. Asdaq, 2022. Gut stimulatory effect of *Terminalia chebula* in experimental animal models. Int. J. Pharmacol., 18: 1466-1473.

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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

Natural resources for ages are widely considered effective therapeutic agents in different parts of the world. Among them, medicinal plants have emerged as the main component of human society from the down to civilization to diseases[1]. Reports suggest that the presence of various phytoconstituents is responsible for the balanced biological effects, thus exhibiting minimum incidences of side effects on the living system. These are the of the major reasons for medicinal plants being components of a different system of healing diseases[2].

Terminalia chebula (Family: Combretaceae) is a large tree that grows in the sunny forest of several Asian countries. The leaves of the plant are alternate, elliptical, glabrous on both surfaces. The flowers have a slight fragrance and the fruits are pulpy[3]. The dried fruits are commercially marketed in several countries and are used medicinally as astringent, expectorant, anti-inflammatory, cardiotonic, laxative, antiseptic, anti-ulcer, antiemetic, diuretic and anti-cancer agent[4]. The major phytoconstituents reported in the plant are sterols, triterpenoids, phenolic compounds and glycosides. Some of them are characterized as chebulic acid, punicalagia, chebulanin, neochebulinic acid, chebulagic acid and terchebulin[5]. Due to the wide spectrum of pharmacological activities, the plant is sometimes also referred to as the 'King of medicine'[4].

Irregular bowel movement is one of the Universal problems and constipation is reported to be the common gastrointestinal disorder among the habitats of both developed as well developing countries[6]. In the United States, more than 2 million people visit the doctor with chief complaints about less than normal bowel clearance. Constipation is the passage of a small amount of hard and dry stools, usually fewer than three times a week. In most cases, the defect is imaginary and associated with lifestyle modification, poor eating habits, decreased fluid intake and lack of sufficient exercise[7]. In chronic conditions, constipation can cause abdominal discomfort and severe pain. Medications are reported to be the option if the condition accompanies severe acute abdominal pain[6].

Several classes of drugs have been approved for treating constipation. The major among them are bulk-forming agents, osmotic laxatives, emollient laxatives and stimulant laxatives[8]. Although, these medications were found to be effective in relieving constipation, however, the treatment is accompanied by several adverse effects such as hydro-electrolytic alternation, bacterial fermentation and cramping[9]. Several substances derived from natural resources such as Senna, Cascara, Psyllium, Rhubarb and Licorice have been tested as a therapeutic option for constipation. Depending on the availability, native medicinal plants of the region could become vital sources of drug[10]. An earlier study indicated that aqueous extract of *T. chebula* seeds relieved morphine-induced constipation, possibly through activation of calcium channels[11]. Another study suggested that administration of *T. chebula* at 550 mg kg^{-1} was found to shorten the intestinal transit time in time[12].

The present study was planned to evaluate the gut stimulatory activity of *T. chebula* extract using isolated rat colon, Guinea pig ileum and rabbit jejunum preparations in the presence/absence of atropine. The study also determined the intestinal transit time of charcoal movement in the intestine of mice after the administration of *T. chebula*.

MATERIALS AND METHODS

Study area: This research project was conducted from September, 2020-June, 2021 simultaneously at Al-Ameen College of Pharmacy, India, AlMaarefa University, Riyadh, Saudi Arabia and Taif University, Taif, Saudi Arabia.

Animals: The study used laboratory-bred animals, including 8 Wistar Albino rats, 7 Guinea pigs, 6 rabbits and 36 mice. The animals were housed in the standard animal facilities and were provided with food and water *ad labitum*. A 48 hrs acclimatization was done in the lab before animals were prepared for the experiment. The ethics committee of the college approved carrying out research following established guidelines.

Preparation of reagents and drugs: The chemicals and reagents used in the study were of analytical grade. These agents and the pure drugs such as acetylcholine and atropine were procured from regular suppliers of the institute. The powder extract of *T. chebula* was obtained as a gift sample from Natural Remedies (P) Ltd., Bangalore. The extract and drugs were reconstituted freshly in distilled water as the dosage requirements. The physiological salt solutions such as Tyrode solution and modified Ringer solution were prepared as per the procedure described in the literature[13].

Experimental protocol: The *in vivo* studies were conducted using isolated tissues such as rat colon, Guinea pig ileum and rabbit jejunum. The *in vitro* study was conducted on mice to determine the propulsive gut motility. The detailed procedure of each experiment is described in the following sections.

Isolated rat colon preparation: Eight Wistar rats of either sex weighing 150-200 gm were selected and kept for overnight fasting with free access to water. After sacrificing the animals by cervical dislocation under light ether anaesthesia, the abdomen was open and the colon was removed and mounted in the organ bath perfused under modified Ringer's solution. The concentration-dependent responses of Terminalia extract, as well as acetylcholine, were recorded in the absence and presence of Atropine sulphate (1 ng mL^{-1}) using 4 channels Polyrite (Recorders and Medicare systems (P) Ltd. Ambala)[14].

Isolated Guinea pig ileum preparation: Seven Guinea pigs of either sex weighing 500-750 gm were selected and fasted overnight, with free access to water. After sacrificing the animal with light ether anaesthesia, the abdomen was cut open and the caecum was lifted to trace the ileocaecal junction, about 15 cm proximal to the ileocaecal portion removed. One segment of ileum was tied with the thread to the top and bottom ends without closing the lumen and mounted in the organ bath containing Tyrode solution. The concentration-dependent responses to acetylcholine and *T. chebula* were recorded using 4 channel polyrite. Contact times of 30 sec and 3 min time cycle were kept for the recording of responses. Atropine sulphate (1 ng mL^{-1}) was added to the tissue bath and the concentration-response curve of Ach as well the powder extract of *T. chebula* was repeated in the presence of atropine sulphate[15].

Isolated rabbit jejunum preparation: Six rabbits of either sex weighing 1-3 kg were selected and starved for 24 hrs with free access to water. The rabbit was sacrificed under light ether anaesthesia. The abdomen was cut open and the caecum was lifted to trace the ileocaecal junction. The jejunum was identified, cut and immediately placed in the watch glass containing Tyrode solution. One segment of jejunum was taken and tied with the thread to the top and bottom ends without closing the lumen and mounted in the organ bath maintained at 32-35°C and bubbled with carbogen. The responses due to acetylcholine and extract of *T. chebula* were recorded using a 4-channel polyrite. Contact time of 30 sec, 3 min time cycle are kept for the recording of responses. The concentration-response curve of acetylcholine and *T. chebula* extract was repeated in the presence of Atropine sulphate 2 µg[16].

***In vivo* intestinal transit (IT) using mice:** Passage of charcoal meal through the gastrointestinal tract in mice was used as the parameter for evaluating intestinal motility (17). Female swiss Albino mice (20-30 gm) were randomly divided into 6 groups containing 6 mice in each group (total of 36 mice). The animals fasted for 24 hrs before the experiment with free access to water. Group-I (Control) animals were given 20 mL kg^{-1} of normal saline orally and Group-II (Standard) received Dulcolax (Bisacodyl) 10 mg kg^{-1}. Group-III animals received the extract of *T. chebula* at 86 mg kg^{-1}. After 15 min of the drug administration (oral route), 0.3 mL of a charcoal meal (distilled water suspension containing 10% gum acacia, 10% vegetable charcoal and 20% starch) was administered orally to each animal. About 20 min later animals were sacrificed under light ether anaesthesia and the abdomen was cut open. The distance travelled by the charcoal plug in comparison to the total length of the small intestine was recorded.

Statistical analysis: The data obtained from the study were statistically analyzed and given as Mean±Standard error of the mean. The comparison was done using a One-Way Analysis of Variance (ANOVA) subsequently Tukey multiple comparison tests were applied. The probability value of less than 0.05 was indicative of a significant difference.

RESULTS

Isolated rat colon preparation: The dose-response curve (DRC) recorded for acetylcholine (Ach) and Terminalia extract using the isolated rat colon preparation was shown in Fig. 1. The responses were recorded in the presence and absence of atropine. The response of Ach was recorded from the concentration of 1 µg mL^{-1} onwards and a saturation effect was observed from 0.03 mg mL^{-1}. On the other hand, the DRC of Terminalia extract was tested from 0.2-200 mg mL^{-1}. The observation indicated saturation in the contractile response from 60 mg mL^{-1}. Both the test drugs exhibited dose-dependent responses at lower concentrations and ceiling effect at higher doses. When both Ach and Terminalia extract was tested in the presence of atropine (1 ng mL^{-1}). The DRC of Ach, as well as Terminalia extract, was found to be shifted towards the right side. Further, a diminution in height was observed when Ach and Terminalia extract responses were recorded in the presence of atropine (Fig. 2).

Isolated Guinea pig ileum preparation: The standard drug Ach was tested at doses of 1 ng to 300 µg mL^{-1} using isolated Guinea pig ileum preparation. Observations indicated a dose-dependent increase in the contraction of smooth muscle from

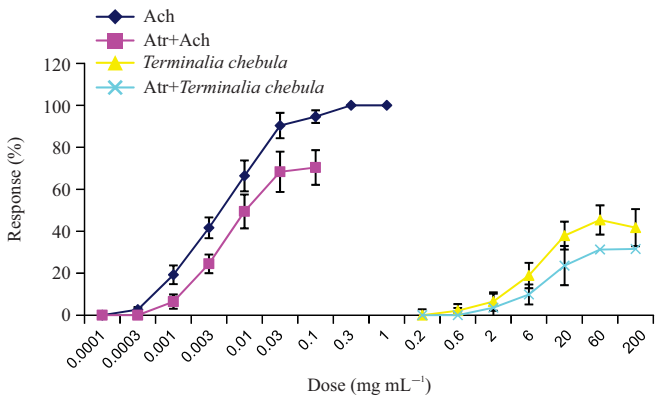

Fig. 1: Comparative DRC of Ach and *Terminalia chebula* on isolated rat colon preparation in the presence and absence of Atropine sulphate
Values are represented as Mean±SEM

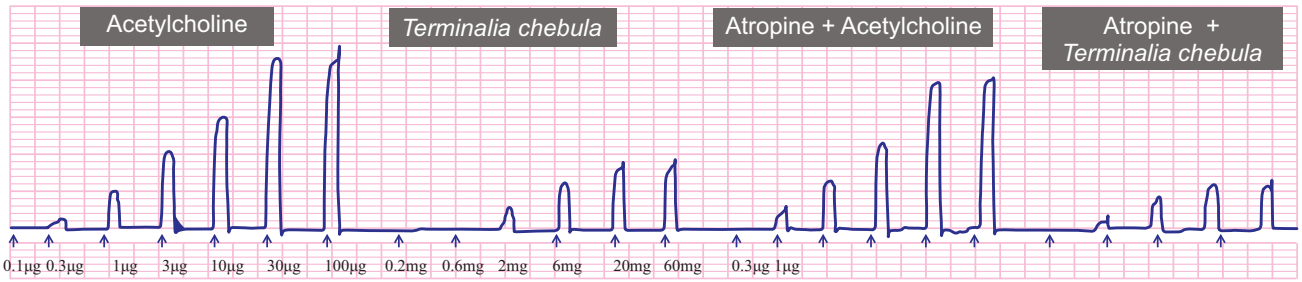

Fig. 2: Comparative DRC of Ach and *Terminalia chebula* on isolated rat colon preparation in the presence and absence of Atropine sulphate

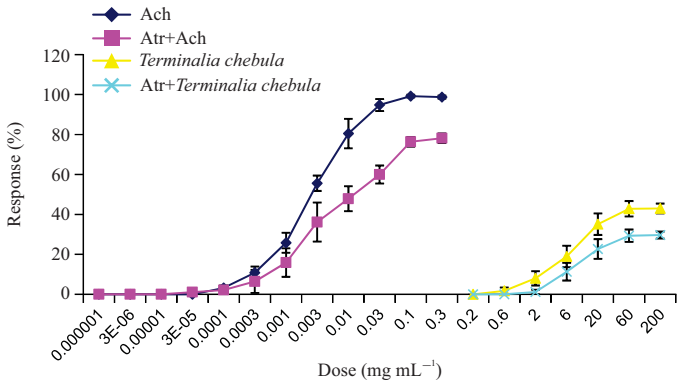

Fig. 3: Comparative DRC of Ach and *Terminalia chebula* on isolated Guinea pig Ileum preparation in the presence and absence of Atropine sulphate
Values are represented as Mean±SEM

3-30 µg mL^{-1} concentration. The saturation effect in the contraction response was recorded beyond 30 µg mL^{-1}. Similarly, when Terminalia extract was tested at concentrations of 0.2-200 mg mL^{-1}, a dose-dependent increase in contraction was observed to 60 mg kg^{-1} and a further increase in concentration produced a saturation effect. Upon atropinization of the tissue, both responses of Ach and the Terminalia extract in the tested doses were found to get shifted towards the right side. A ceiling effect was observed for Ach from 10 µg mL^{-1} and for Terminalia extract from 60 mg mL^{-1} onwards (Fig. 3 and 4).

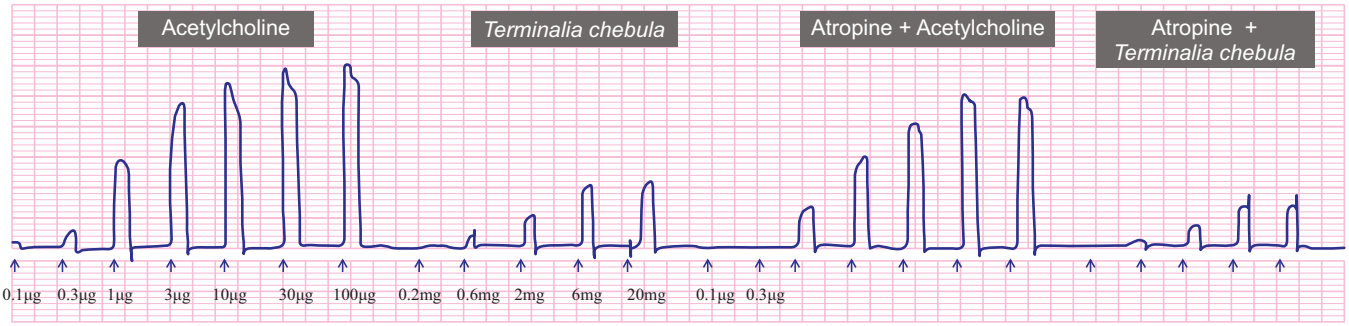

Fig. 4: Comparative DRC of Ach and *Terminalia chebula* on isolated Guinea pig ileum in the presence and absence of Atropine sulphate

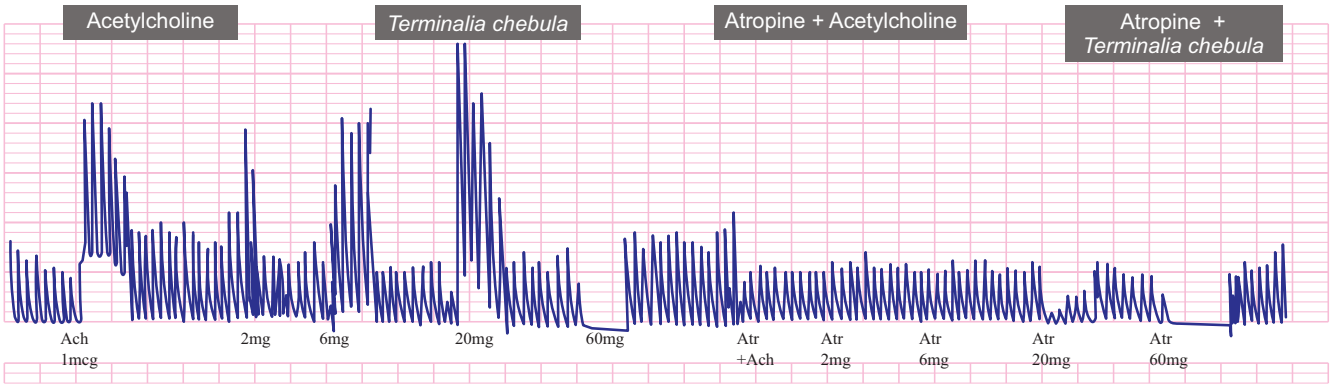

Fig. 5: Effect of Ach and *Terminalia chebula* on isolated rabbit jejunum preparation

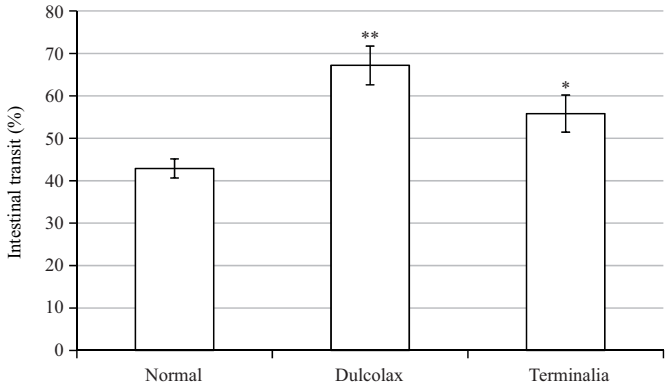

Fig. 6: Effect of Terminalia extract on the intestinal transit(IT) in mice
*p<0.05 when compared to normal and **p<0.01 when compared to normal

Isolated rabbit jejunum preparation: The spontaneous contracting response from the smooth muscles of rabbit jejunum was recorded in Fig. 5. Ach tested as a standard agent produced a spasmogenic effect at concentrations of 1-3 ng mL^{-1}. The doses of Ach produced increased tonicity of muscle at these doses. On the other hand, Terminalia extract produced increased pendular movement with an increase in tonicity when tested at lower concentrations (2-20 mg mL^{-1}). However, from the concentration of 60 mg mL^{-1}. The responses were found to be spasmolytic since the tonicity was found to get reduced at these doses. When the responses of Ach and Terminalia extract were repeated in the presence of atropine (2 µg mL^{-1}), a complete abolishing of the spasmogenic effect was observed for both the agents.

Intestinal transit (IT) in mice: An *in vivo* study to determine the intestinal transit of charcoal meal was done in mice and the data was represented in Fig. 6. The transit was recorded as a percentage millimetre and the observation indicated that the transit in normal, standard drug (Dulcolax-10 mg kg^{-1}) treated and Terminalia extract (86 mg kg^{-1}) treated animals were found to be 42.90, 67.19 and 55.82 mm, respectively. The comparison of data indicated a significant increase in transit for standard drug ($p<0.01$) and Terminalia extract-treated ($p<0.05$) groups compared to normal animals.

DISCUSSION

The present study evaluated the gut stimulatory activity of *T. chebula* powder extract using *in vitro* and *in vivo* experimental models. Administration of acetylcholine produced a dose-dependent contraction in the smooth muscles of the rat colon. The gut stimulatory response showed a ceiling effect when Ach was tested at a higher concentration. Similarly, when *T. chebula* powder extract solution was tested, a dose-dependent smooth muscle contraction was observed at lower doses and a ceiling effect at higher concentrations. The responses of both Ach and Terminalia extract were found to be diminished in the presence of atropine. The right-side shift of the DRC with both the drugs suggested that the gut-stimulatory action of *T. chebula* extract could have been blocked by atropine much like Ach (Fig. 1).

The observation recorded with the isolated Guinea pig ileum presented similarity with rat colon observations. Besides, both Ach and *T. chebula* extract exhibited a dose-dependent effect at lower concentrations and a ceiling effect at higher concentrations. Atropinization of the tissue diminished the dose-dependent responses of both Ach and Terminalia extract. The magnitude of *T. chebula* response was found to be less than Ach, though a higher concentration was used (Fig. 3). The observation indicated that Ach might have a strong gut stimulatory activity while *T. chebula* might produce a milder effect and these responses could have been mediated through cholinergic innervations[11].

Ach administration to the isolated rabbit jejunum in the present study increased the tonicity and produced forceful contractions. These effects appear to contribute to the spasmogenic of muscles. On the other hand, *T. chebula* extract at the tested doses showed mild contractions which were of the nature of pendular movements. Such to and fro movements of smooth muscles of the intestine are useful in churning and mixing gastrointestinal contents and are reported to assist in the digestion of food[15]. The actions of Ach as well the *T. chebula* extract on rabbit jejunum appear to get abolished in the presence of atropine (Fig. 5). The observations suggested that mild stimulation induced by *T. chebula* extract on rabbit jejunum could be mediated through parasympathetic innervations.

The *in vivo* study conducted in mice indicated that administration of *T. chebula* extracts significantly ($p<0.05$) increased the intestinal transit time of charcoal meal when compared to normal animals. The standard drug (Dulcolax) on the other hand, also increased the transit time but more significantly ($p<0.01$) when compared with the normal group (Fig. 6).

Cholinergic transmission plays an important role in the regulation of gut motility. Ach is the stimulatory neurotransmitter that is synthesized in several organs[17]. The synthesis of Ach is catalyzed by choline acetyltransferase which caused catalytic conversion of acetyl CoA and choline into Ach. The synthesized Ach is stored in the vesicles and is released from the nerve terminals upon stimulation[18]. The gut wall is found to have rich cholinergic innervations and when cholinergic motor neurons are activated by the enteric nervous system, Ach is released into the junction. The released Ach binds to the muscarinic receptors located in the epithelial cells producing contraction of smooth muscle fibers[19]. Several medicinal plants such as *Asparagus cochinchinensis* in the earlier studies have been reported to exhibit their gut stimulatory effect through cholinergic innervation[20].

Studies also indicated that activation of muscarinic receptors caused mucosal hydration and stimulates mucus secretion from goblet cells. The cholinergic action on the intestine not only increases the gut motility but also provides surface lubrication for smooth propulsion of luminal contents[21]. Mucus secretion and its beneficial effect in relieving the experimental model of constipation in mice has been reported with an extract of rhubarb[22]. Ach is also reported to be synthesized and stored in a wide variety of non-neuronal cells located in different parts of the body. These Ach molecules are not stored in the vesicles and are released by organic cation transporters. Their release is considered to produce slow and sustained gut stimulatory action[23]. In an earlier study, the role of calcium has been suggested for the *T. chebula* mediated stimulation in the gut smooth muscles and the action can be linked to the cholinergic innervations[11].

The responses observed in the present study suggested that Ach has a strong stimulatory action on gut motility while the actions of *T. chebula* are mild. The actions of *T. chebula* could benefit patients suffering from defective gut motility associated due to several origins. The stronger action on the gut muscles is not preferred due to complications such as spasms and interference in the physiology of digestion[24]. More studies are suggested to determine the actual potential of *T. chebula* in the treatment of constipation and to understand the precise mechanism of its action on gut muscles.

CONCLUSION

The present study evaluated the gut stimulatory action of *T. chebula* powder extract using *in vitro* and *in vivo* experimental models. The studies were conducted along with acetylcholine to record the contractile responses in the presence and absence of atropine. *T. chebula* exhibited a mild gut stimulatory action that could benefit in relieving constipation without affecting the physiology of the gastrointestinal system. The actions of *T. chebula* appear to be mediated through cholinergic innervations. The observations indicated that *T. chebula* could be one of the useful naturally derived medicine for improving gastric motility. However, more studies are needed to establish the precise mechanism of action of *T. chebula*.

SIGNIFICANCE STATEMENT

Constipation is a persistent gastrointestinal issue that must be managed to enhance the quality of life and avoid complications. Although there are several medications available to treat it, most of them have intolerable side effects, necessitating the development of a more effective and safe therapeutic approach. Most natural therapies are relatively safe and effective when utilized over a long period. The effectiveness of *Terminalia chebula* powder extract was investigated using recognized experimental models. The findings reveal that *T. chebula* has a mild stimulatory effect in the gut, suggesting that it could be used to treat faulty gastric motility without compromising the digestive system's functional integrity.

ACKNOWLEDGMENT

Majid Alhomrani would like to acknowledge Taif University for support No. TURSP (2020/257). Syed Mohammed Basheeruddin Asdaq wishes to express his gratitude to AlMaarefa University in Riyadh, Saudi Arabia, for providing funding (TUMA-2021-1) for this study.

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