academicJournals

Vol. 12(1), pp. 11-18, 8 January, 2018
DOI: 10.5897/AJPP2017.4868
Article Number: 111281155633
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Antidiarrhoeal and antispasmodic activity of leaves of *Syzygium cumini* L. (Myrtaceae) mediated through calcium channel blockage

Fabio de Souza Monteiro*, Antonio Felipe Silva Carvalho, Elismar de Castro Marques, Rachel Melo Ribeiro, Antônio Carlos Romão Borges, and Marilene Oliveira da Rocha Borges

Pharmacology Research and Post-Graduate Laboratory, Department of Physiological Sciences, Federal University of Maranhão, Av. Portugueses, 1966, 65085-580, São Luís, Maranhão, Brazil.

Received 13 November, 2017; Accepted 18 December, 2017

Syzygium cumini **L. Skeels (Myrtaceae) commonly known as jambolan is used as traditional medicine to treat gastrointestinal disorders in children in Brazil. This work is one of the first to evaluate the antidiarrhoeal and antispasmodic activity of the standardized extract of** ***S. cumini*** **leaves (HESc) in experimental models** ***in vitro*** **and** ***in vivo*** **rodents. Mice pre-treated with HESc (100, 250 and 1000 mg/kg) and atropine (1.0 mg/kg) had reduced intestinal transit velocity of 11.0; 23.2 and19.1%, respectively compared to saline control (46.6±0.9). In isolated rats jejunum, HESc (50, 150 and 300 µg/mL) shifted to the right cumulative concentration-response curves to ACh with changing maximum effect (E_{max}), which is characteristic of non-competitive antagonism to ACh. HESc also promoted relaxation (E_{max} 90.2±5.8%) in preparations pre-contacted with KCl (75 mM). Additionally, it reduced the maximal $CaCl_2$-induced response in 15.4; 56.3 and 92.1% in a concentration-dependent manner. The study results show that HESc has an antidiarrhoeal and spasmolytic potential that can be partly explained by the reduction of intestinal transit velocity and blockage of the voltage-dependent calcium channels in the smooth intestinal muscle.**

Key words: *Syzygium cumini*, antidiarrhoeal activity, antispasmodic effect, leaves, rat jejunum.

INTRODUCTION

Diarrhoea can be defined as a symptom of the gastrointestinal disorder; it is characterized by increase in stool frequency and alteration in consistency. It results from the imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hypermotility, bringing about excess loss of body fluids and electrolytes in feces (Sharma et al., 2015; Fernández-Bañares et al., 2015; Nemeth and Pfleghaar, 2017).

Treatment of gastrointestinal disorders and the search for new therapeutic agents are still a challenge. A potential antidiarrhoeal agent may exhibit its effect by inhibiting the gut motility (spasmolytic) and/or electrolyte outflux in the form of wet droppings, for example. The World Health Organization (WHO) has approved the use of traditional (folklore) medicines for treating many

*Corresponding author. E-mail: fabiodesouza8@gmail.com.

diseases (Hasler, 2003; Achem, 2004; Bellini et al., 2014; Qi and Kelley, 2014; Megbo et al., 2017; Schiller, 2017).

Natural products continue to play a highly significant role in drug discovery and development process. When analyzing drugs approved by Food and Drug Administration (FDA) between 1981 and 2014, approximately half of the drugs were based on natural products or derivatives thereof (Newman, 2013; Lahlou, 2013; Harvey et al., 2015; Newman et al., 2016).

Medicinal plants till date are quite used to treat various diseases. For example, *Syzygium cumini* is extensively used for the treatment of different diseases, such as inflammation, constipation, diarrhoea, obesity, urinary disorders, diabetes and hypertension. Despite this extensive use in folk medicine, most of the time there are no scientific studies that prove people use them (Zahoor et al., 2017; Mukherjee et al., 2017; Baliga et al., 2011; Ayyanar and Subash-Babu 2012).

Syzygium cumini L. Skeels*, of* the Myrtaceae family, popularly known as jamun, is included in the List of Medicinal Plants of Interest to the Public Health System (Renisus), issued by the Ministry of Health, which includes plants with potential use for medicines (Souza, 2005; Brasil, 2009; Swami, 2012; Subash-Babu and Ayyanar et al., 2013). All parts of *S. cumini* are widely used as traditional medicine, for example, juice of tender leaves is given in goat milk to treat diarrhoea in children (Morton, 1963; Corrêa, 1974; Lainetti and Brito, 1979; Nadkarni, 1976).

Pharmacological studies show that *S. cumini* species have several functions, among them, antioxidants, antibacterial, antifungal, anti-allergic, anti-inflammatory, anti-hyperlipidemic, gastroprotective, cardioprotective, hepatoprotective, anticancer, radioprotective and antidiarrhoeal (Jagetia and Baliga, 2002; Barh and Viswanathan, 2008; Chaturvedi et al., 2009; Schoenfelder et al., 2010; Patel et al., 2010).

The antidiarrhoeal activity was investigated using extracts obtained from the stem bark and seeds of *S. cumini* (Mukherjee et al., 1998; Mazumder et al., 2006; Shamkuwar et al., 2012; Chandra, 2013). However, there is no report available on the hydro-alcoholic extract of the leaves of *S. cumini* on its anti-diarrhoeal or antispasmodic activity despite its medicinal use in diarrhoea.

The research group previously showed that a standardized hydroalcoholic extract prepared from the leaves of *S. cumini* (HESc) is safe, with no evidence of toxicity in rodents (Silva et al., 2012). We subsequently demonstrated the hypotensive and antihypertensive activity of HESc, causing a reduction in vascular reactivity associated with the inhibition of extracellular calcium influx, whose mechanism was attributed to the marked presence of flavonoids (Mahmoud et al., 2001; Ribeiro et al., 2014). This work is the first to evaluate HESc in experimental models *in vitro* and *in vivo* animals, analyzing the intestinal transit velocity in Swiss albino mice, and the contractile activity in isolated rat jejunum, respectively.

MATERIALS AND METHODS

Plant material

Leaves of *S. cumini* were collected from the Campus of the Federal University of Maranhão (2°33'11.7"S 44°18'22.7"W), São Luís, Brazil, in January 2014. A voucher specimen was identified and deposited in the herbarium of the “Profa. Dra Berta Lange de Morretes” Medicinal Plant Garden, UFMA (No. 01079/1079).

Preparation of crude extract

The leaves were mechanically ground to give 920 mg powder. This was added to 1 L of ethanol (70%) and mixed at 8 h each for 72 h. After this period the hydroalcoholic extract was filtered using a cotton funnel. After this process, the extract was concentrated using a rotatory evaporator under reduced pressure and filtered again. We obtained a concentrate of 150 mg/ml and a yield of 16.3% proportional to the 920 mg initially obtained. Such concentrate was denominated in a hydroalcoholic extract of *S. cumini* (HESc). Finally, the extract was lyophilized to obtain a powder and the dry residue obtained was solubilized in distilled water to a concentration of 10 mg/ml. It was re-diluted in distilled water as needed for each experimental protocol.

Experimental animals

Swiss albino mice (25 to 30 g) and Wistar albino rats (250 to 300 g) of either sex from the Universidade Federal de São Luis, Brazil were used. Animals were housed under controlled temperature (25±1°C) and lighting (lights on 06:00 to 18:00 h); they had free access to food and potable water. All procedures described in the present study were approved by the Animal Research Ethics Committee of the State University of Maranhão, Brazil (Protocol number 003584/2014- 97).

In vivo experiments

Small intestinal transit

Swiss albino mice were fasted for 6 h prior to the experiments, but were allowed free access to water. The animals were treated with HESc (100, 250 or 1000 mg/kg, *p.o*., respectively), atropine sulfate (1.0 mg/kg, *p.o*.) or saline (10 mL/kg, *p.o*. n=6), 60 min prior to the administration of a 5% charcoal suspension in 1% guar gum (0.1 mL/10 g body weight, *p.o.*). After 30 min, the animals were euthanized and their small intestines were removed. The distance traveled by the charcoal plug from the pylorus to the cecum was measured and expressed as a percentage of the total intestinal length (adapted from Freire et al., 2011).

In vitro experiments

Effect of HESc on ACh-induced cumulative dose-response curves in isolated rat jejunum

All rats were euthanized by decapitation with guillotine following the principles of laboratory animal care based on the guidelines of the bioethics committee. Segments of jejunum (1.5 cm long) were suspended in a 10 mL organ bath containing Tyrode’s solution (composition in mmol/L: NaCl, 137; KCl, 2.7, $MgCl_2 \cdot 6H_2O$, 0.5; $CaCl_2 \cdot 2H_2O$, 1.8; NaH_2PO_4, 0.4; $NaHCO_3$, 12; glucose, 5.5), aerated with 95% O_2, 5% CO_2 (pH 7.4) and maintained at 37°C. The preparations were set up under a tension of 1 g and responses were recorded on a smoked Kymograph paper through an isotonic frontal writing lever (magnification x 6). After 30 min equilibration period, cumulative concentration-response curves for ACh (10^{-9} to 10^{-4} M) were recorded in the absence and presence of HESc (50, 150 and 300 µg/mL). This curve was compared with those obtained in the absence of HESc and the results were expressed as percentages of the maximal response to ACh alone (Van Rossum, 1963).

Effect of HESc on KCl-induced tonic contractions in isolated rat jejunum

Isolated rat jejunum was obtained as described earlier. Segments of jejunum (1.0 cm long) were suspended in a 5 mL organ bath containing Tyrode's solution aerated with 95% O_2, 5% CO_2 (pH 7.4) and maintained at 37°C. The preparations were set up under a tension of 1 g. In addition, for the recording of the isometric tension, the thread from the muscle strips was attached to an isometric force transducer that was connected to a bridge amplifier (ADInstruments Ltd, Grove House, Hastings, U.K.). Isometric tension changes were digitized using either PowerLab/4SP (ADInstruments Ltd, Grove House, Hastings, U.K.) and stored on a personal computer for later analysis. After 30 min equilibration period, segments of jejunum were contracted with KCl (75 mM) and when a stable contraction was attained (15-20 min), HESc (9; 27; 81; 243 and 729 µg/mL) was cumulatively added in an attempt to obtain dose-relaxation curves. The relaxant effect induced by HESc was expressed as the reverse percentage of the initial contraction force elicited by KCl.

Effect of HESc on $CaCl_2$-induced cumulative dose-response curves in calcium-free solution in isolated rat jejunum

The jejunum was mounted as described earlier. The preparations were set up under a tension of 1 g, and responses were recorded on a smoked Kymograph paper through an isotonic frontal writing lever (magnification x 6). After the stabilization during 30 min in normal Tyrode's solution, the external calcium was eliminated with depolarizing Tyrode's solution (KCl, 70 mM; Ca^{2+}-free). Cumulative concentration-response curves of Ca^{2+} were obtained by cumulatively adding $CaCl_2$ (3 x 10^{-5} to 10^{-1} M) in the absence and presence of HESc (27, 81 and 243 µg/mL), which were added to the bath 10 min before addition of Ca^{2+}. This curve was compared with those obtained in the absence of HESc and the results were expressed as percentages of the maximal response to $CaCl_2$ alone (Van Rossum, 1963).

Statistical analysis

Values were expressed as mean ± S.E.M. Statistical analysis was performed using GraphPad Prism 5.01 software (GraphPad Software Inc., San Diego, CA, USA). Differences between means were compared using t-test (non-paired) and one-way ANOVA followed by Bonferroni's test as appropriate and p values < 0.05 were considered indicative of significance.

RESULTS AND DISCUSSION

In this study, it is shown for the first time the investigation of the antidiarrhoeal and antispasmodic activities of the HESc, analyzing the intestinal transit velocity in Swiss albino mice, and the contractile activity in isolated rat jejunum, respectively. It is demonstrated that HESc has antidiarrhoeal and antispasmodic effect by decreasing the speed of intestinal transit and blocking the influx of calcium, respectively.

Charcoal meal test in mice is a method used to study the effect of drugs on the motility of intestine (Misar, 2000). The transit velocity of the small intestine compared to the effect produced by saline control (46.6±0.9%), was reduced to 11.0; 23.2; 19.1 and 38.5%, respectively, after pre-treatment of mice with HESc (100, 250 and 1000 mg/kg) and atropine (1.0 mg/kg), administered orally, 60 min before administration of coal meal. Although HESc was less efficient in decreasing intestinal transit compared to atropine (standard drug), it reduced intestinal transit significantly in all doses tested compared to the control as shown in Figure 1.

Anti-diarrhoeal activity has already been demonstrated in studies carried out with the seeds and bark of the stem of *S. cumini*. In both studies, it was possible to show a significant reduction in gastrointestinal motility in tests of coal meal in rats. The underlying mechanism of action of the plant extract appeared to be antispasmodic whereby the extract produced relief from diarrhea (Mukherjee et al., 1998; Shamkuwar et al., 2012; Srivastava and Chandra, 2013).

It is well known that aqueous herbal medicines are traditionally used for their antispasmodic and antidiarrhoeal activity in various countries (Hajhashemi et al., 2000; Mujumdar et al., 2000; Sadraei et al., 2003). Non-specific anti-diarrhoeal drugs involve actions on intestinal transit that results in symptomatic improvement in a variety of conditions. Furthermore, the study on antispasmodic effect might help to deduce the possible mechanism of action (Schiller, 1995).

Antispasmodics may be classified, for example, into antimuscarinics, smooth muscle relaxants (that is, drugs that directly inhibit smooth muscle contractility, for example, by increasing cyclic AMP levels or by interfering with the intracellular calcium pool), similar agents papaverine and Ca^{2+} blocking channels (especially Ca^{2+} L-channel blockers) (Christen, 1990; Singh et al., 2003).

Muscarinic receptors of the M_3 subtype are present in the intestinal smooth muscle. These receptors are responsible for initiating contraction in response to acetylcholine (ACh) binding. This neurotransmitter is released by parasympathetic postganglionic neurons that innervate the digestive tract (Weiser et al., 1997). Upon binding with ACh, the M_3 receptor initiates a cellular signaling cascade. Briefly, the alpha subunit of the Gq/11 protein activates the effector phospholipase C (PLC), which increases the inositol triphosphate (IP_3) secondary messenger responsible for releasing calcium from the sarcoplasmic reticulum. This Ca^{2+} release activates voltage-gated Ca^{2+} (Ca_V) channels indirectly that leads to influx of Ca^{2+} from extracellular fluid (Caulfield, 1993;

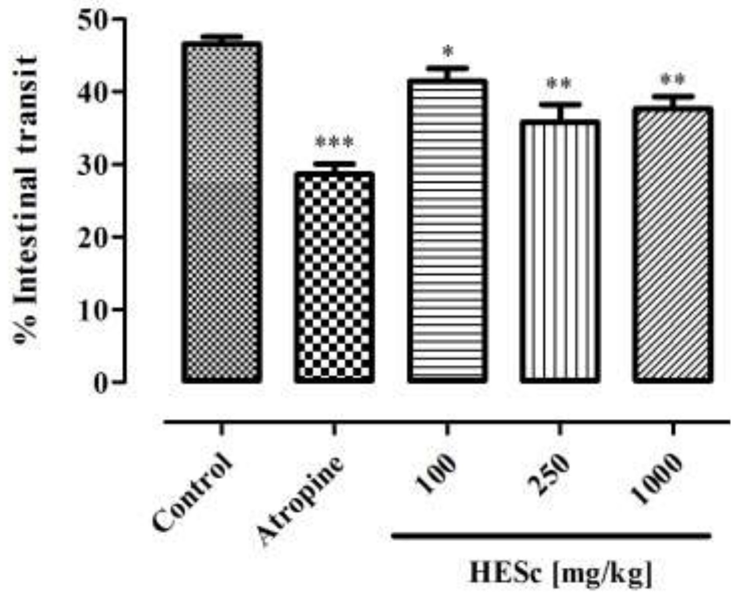

Figure 1. Effect of HESc on small intestinal transit in mice. Columns and bars represent means and S.E.M., t-test, **p* < 0.05, ***p* < 0.01 (Control vs. HESc), ****p* < 0.001 (Control vs. atropine) (n = 6).

Eglen et al., 1996; Honda et al.,1996; Catterall et al, 2005).

The isolated rat jejunum was used in this study to initially investigate whether the reduction of intestinal motility caused by HESc was mediated by competitive antagonism to the M_3 receptor, with consequent interference in the availability of intracellular Ca^{2+}. For this, the effect of HESc on cumulative concentration-response curves to the addition of ACh (10^{-9} to 10^{-4} M) was evaluated. The pD_2 value (pD_2 = -log EC_{50}, negative logarithm of molar concentration of agonist that caused half-maximal response) was 6.4±0.05 M. In the presence of HESc (50, 150 and 300 µg/mL), the pD_2 value was altered to 5.8±0.2, 6.2±0.2 and 5.1±0.1 M, respectively, and E_{max} to ACh was reduced in 17.8, 34.3 and 57.3% (Figure 2), suggesting a non-competitive type antagonism.

Spasm is characterized by a muscle contraction and in the smooth muscle this contraction occurs after the elevation of the intracellular calcium concentration ($[Ca^{2+}]_i$) due to the opening of the voltage-dependent calcium channels (Ca_V) present in the plasma membrane or due to its release of sarcoplasmic reticulum (RS) controlled by secondary messengers, for example, IP_3. The functional regulation of $[Ca^{2+}]_i$ to trigger a contractile response in smooth muscle is related to two stimuli that lead to two types of couplings: (1) electromechanical coupling, which is involved with the membrane potential change (Vm) and (2) drug-mechanical coupling when the contraction induced by an agonist is always greater than that observed only with the change of Vm (Al-Zuhair et al., 1996; Rembold, 1996; Bolton, 1979).

The contraction in the smooth muscle in response to several agents is often composed of two phases: a fast and unstained phasic component, followed by a slow and sustained tonic component (Breemen and Saida, 1989). The phasic component is due in part to the Ca^{2+} of the sarcoplasmic reticulum and the tonic component is mainly due to Ca^{2+} from the extracellular medium entering the cell through the Ca_V (Abdellatif, 1989; Kobayashi et al., 1989; Takano and Kamiya, 1996).

In order to verify whether HESc would promote relaxation of the pre-contracted jejunum, which would be suggestive, at a functional level, of blocking Ca^{2+} influx through the plasma membrane, its effects were evaluated on the tonic component of the contractile response induced by KCl 75 mM (electromechanical coupling). It was observed that HESc promotes relaxation of the jejunum (Figure 3B) in a concentration-dependent manner with a E_{max} of 90.2±5.8% (Figure 4); we hypothesized that HESc would prevent Ca^{2+} influx through Ca_V.

To confirm this hypothesis, the effect of HESc on the cumulative concentration-response curves to the $CaCl_2$ was evaluated. The jejunum was contracted with increasing concentrations of $CaCl_2$ (3 x 10^{-5}M to 10^{-1} M), with a pD_2 value of 2.07±0.09 M. In the presence of HESc, at 27, 81 and 243 µg/mL, the pD_2 values were lowered to 1.59±0.12, 1.83± 0.14 and 1.29±0.06 M, respectively. In addition, HESc displaced the cumulative

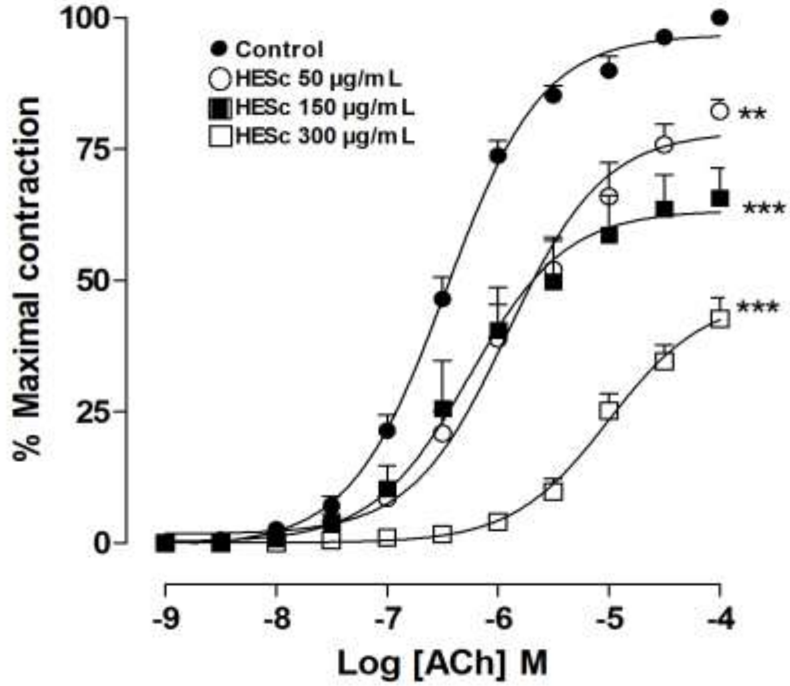

Figure 2. Effect of HESc on isolated rat jejunum contractile-response to ACh. Symbols and vertical lines indicate means ± SEM, respectively. One-way ANOVA followed by Bonferroni's test (Control vs HESc), ***p* < 0.01; ****p* < 0.001 (n = 4 – 6).

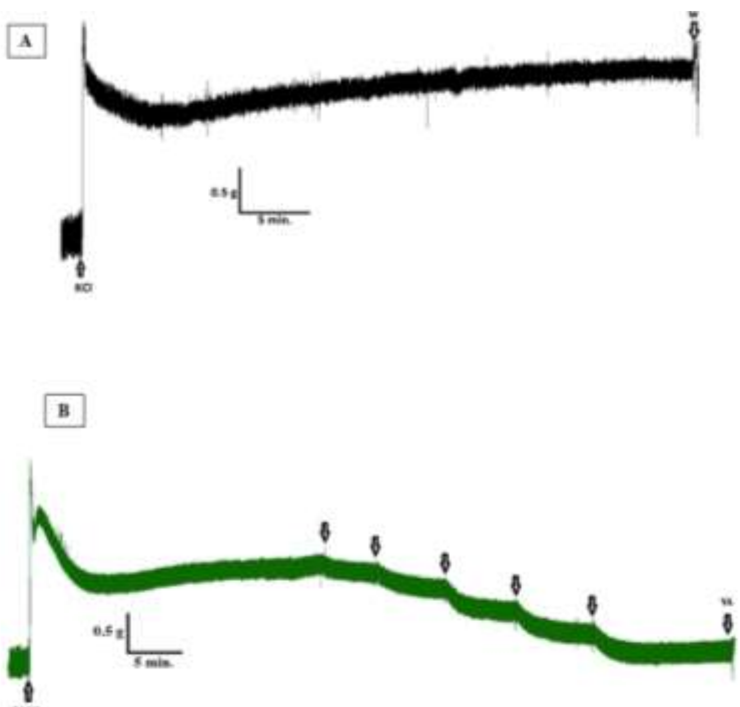

Figure 3. Representative originals records in the absence (A) and presence of HESc (B) on isolated rat jejunum contractile-response to KCl. Arrow represents concentration of HESc (9, 27, 81, 243, and 729 µg/mL). KCl: potassium chloride and W: washout.

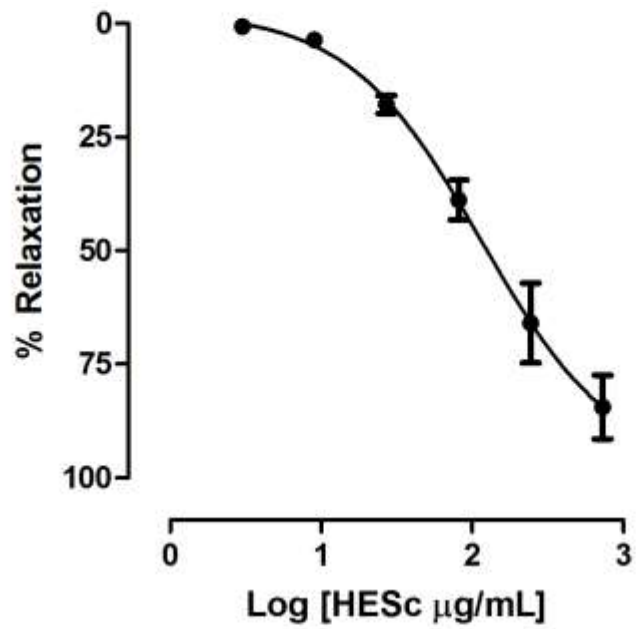

Figure 4. Effect of HESc on KCl-induced (75 mM) tonic contraction in isolated rat jejunum. Symbols and vertical lines indicate means ± SEM, respectively (n = 4).

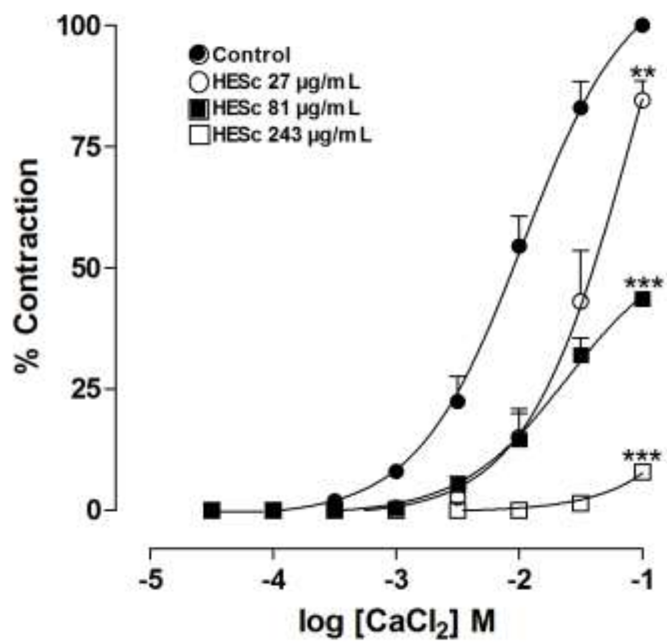

Figure 5. Effect of HESc on isolated rat jejunum contractile-response to $CaCl_2$. Symbols and vertical lines indicate means ± SEM, respectively. One-way ANOVA followed by Bonferroni's test (Control vs EHF-SC), $^{**}p < 0.01$; $^{***}p < 0.001$ (n = 3).

concentration-response curves of $CaCl_2$ to the right, in a non-parallel manner and the maximal response was reduced, in a concentration-dependent manner, by 15.4; 56.3 and 92.1%, respectively (Figure 5), suggesting that the antispasmodic effect is possibly mediated through the inhibition of Ca^{2+} influx probably through of Ca_V.

Other studies show that antispasmodic constituents present in various medicinal plants mediate their effect generally by blocking the calcium channel (Ghayur et al., 2006; Gilani et al., 2006; Shah et al., 2010). Similar results of inhibition of contractile responses to calcium were found in previous studies with the hydroalcoholic extract of *S. cumini* by our laboratory in preparation of vascular arteries rings isolated from normotensive and spontaneously hypertensive rats. The effects were attributed to the presence of flavonoids detected by phytochemical screening (Abreu et al., 2002; Ribeiro et al., 2014)

Conclusion

In this study, it can be concluded that the antidiarrhoeal effect of HESc, observed by the reduction of the intestinal transit, can be explained by the blockage of calcium influx through Ca_V responsible for the antispasmodic activity. These properties may explain the use of *S.* cumini as an antidiarrhoeal agent in traditional medicine and contribute to the future indication of *S. cumini* as a possible therapeutic alternative to treat gastrointestinal diseases. However, further studies are needed to explore the secondary metabolites responsible for the results obtained in this study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENT

This work was supported by grants from Federal University of Maranhão and Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão (FAPEMA).

REFERENCES

Abdel-Latif AA (1989). Calcium-mobilizing receptors, polyphosphoinositides, generation of second messengers and contraction in the mammalian iris smooth muscle: Historical perspectives and current status. Life Sci. 45(9):757-786.

Abreu IC, Silva SN, Ribeiro RM, Baima CFS, Olea RSG, Borges ACR, Borges MOR (2002). Efeito dos extratos de *Jatropha gossypiifolia* L., *Passiflora edulis* Sims. e *Syzygium jambolanum* D.C. na disponibilidade de íons cálcio. Rev. Cienc. Saúde. 4:41-46.

Achem SR (2004). Treatment of spastic esophageal motility disorders. Gastroenterol. Clin. North Am. 33(1):107-124.

Al-Zuhair H, El-Sayeh B, Ameen HA, Al-Shoora H (1996). Pharmacological studies of cardamom oil in animals. Pharmacol. Res. 34:79-82.

Ayyanar M, Subash-Babu P (2012). *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. Asian. Pac. J. Trop. Biomed. 2(3):240-246.

Ayyanar M, Subash-Babu P, Ignacimuthu S (2013). *Syzygium cumini* (L.) Skeels., a novel therapeutic agent for diabetes: folk medicinal and pharmacological evidences. Complement. Ther. Med. 21(3):232-243.

Baliga MS, Bhat HP, Baliga BRV, Wilson R, Palatty PL (2011). Phytochemistry, traditional uses and pharmacology of *Eugenia jambolana* Lam. (black plum): A review. Food Res. Int. 44(7):1776-1789.

Barh D, Viswanathan G (2008). *Syzygium cumini* inhibits growth and induces apoptosis in cervical cancer cell lines: a primary study. Ecancermedicalscience. 2:83.

Bellini M, Gambaccini D, Stasi C, Urbano MT, Marchi S, Usai-Satta P (2014). Irritable bowel syndrome: a disease still searching for pathogenesis, diagnosis and therapy. World J. Gastroenterol. 20(27):8807.

Bolton TB (1979). Mechanisms of action of transmitters and other substances on smooth muscle. Physiol. Rev. 59:606-718.

Brasil (2009). RENISUS – Relação nacional de plantas medicinais de interesse ao SUS. Secretaria de Vigilância em Saúde, Ministério da Saúde. Available at: http://portal.saude.gov.br/portal/arquivos/pdf/RENISUS.pdf

Breemen CV, Saida K (1989). Cellular mechanisms regulating $[Ca^{2+}]_i$ smooth muscle. Annu. Rev. Physiol. 51(1):315-329.

Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J (2005). International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. Pharmacol. Rev. 57(4):411-425.

Caulfield MP (1993). Muscarinic Receptors-Characterization, coupling and function. Pharmacol. Therapeut. 58:319-379.

Chaturvedi A, Bhawani G, Agarwal PK, Goel S, Singh A, Goel RK (2009). Antidiabetic and antiulcer effects of extract of *Eugenia jambolana* seed in mild diabetic Rats: study on gastric mucosal offensive acid-pepsin secretion. Indian J. Physiol. Pharmacol. 53:137-46.

Christen MO (1990). Action of pinaverium bromide, a calcium-antagonist, on gastrointestinal motility disorders. Gen. Pharmacol. 21:821-825

Coelho DL, Brandão EG, Rosas LV, Lima RA, Pinto MN, Pantoja TM (2016). The medical plant use in fighting parasitosis and intestinal worm's good neighborhood in the garden in the municipality Benjamin Constant-Am, Brazil. South Am. J. Basic Educ. Techn. Technol. 3(2):37-50.

Corrêa MP (1974). Dicionário das plantas úteis do Brasil, volumes. *VI, Ministério da Agricultura, Rio de Janeiro*. Available at: https://www.bdpa.cnptia.embrapa.br/consulta/?initQuery=t

Cragg GM, Newman DJ (2013). Natural products: a continuing source of novel drug leads. Biochim. Biophys. Acta. 1830(6):3670-3695.

Eglen RM, Hedge SS, Watson N (1996). Muscarinic receptor subtypes and smooth muscle function. Pharmacol. Rev. 48:531-565.

Fernández-Bañares F, Accarino A, Balboa A, Domènech E, Esteve M, Garcia-Planella E, Santos J (2016). Chronic diarrhoea: Definition, classification and diagnosis. Gastroenterol. Hepatolol. (English Edition). 39(8):535-559.

Freire SMF, Andrade KNS, Aragão GA Jr, Noronha EP, Silva SN, Cartágenes MSS, Borges MOR, Ribeiro MNS, Torres LMB Borges ACR (2011). Antiulcerogenic activity of the extracts of *Struthanthus marginatus*. Rev. Bras. Farmacogn. 21(6):1089-1095.

Ghayur MN, Gilani AH, Khan A, Amor EC, Villasenor IM, Choudhary MI (2006). Presence of calcium antagonist activity explains the use of *Syzygium samarangense* in diarrhoea. Phytother. Res. 20:49-52.

Gilani AU, Shah AJ, Ahmad M, Shaheen F (2006). Antispasmodic effect of *Acorus calamus* Linn. is mediated through calcium channel blockade. Phytother. Res. 20:1080-1084.

Hajhashemi V, Sadraei H, Ghannadi AR, Mohseni M (2000). Antispasmodic and Antidiarrhoeal effect of *Satureja hortensis* L. essential oil. J. Ethnopharmacol. 71:187-192.

Harvey AL, Edrada-Ebel R, Quinn RJ (2015). The re-emergence of natural products for drug discovery in the genomics era. Nat. Rev. Drug Discov. 14(2):111-129.

Hasler WL (2003). Pharmacotherapy for intestinal motor and sensory disorders. Gastroenterol. Clin. North Am. 32(2):707-732.

Honda K, Takano Y, Kamiya HO (1996). Involvement of protein kinase C in muscarinic agonist-induced contractions of guinea pig real longitudinal muscle. Gen. Pharmacol.: The Vascular System. 27(6):957-961.

Jagetia GC, Baliga MS (2002). *Syzygium cumini* (Jamun) reduces the radiation-induced DNA damage in the cultured human peripheral blood lymphocytes: a preliminary study. Toxicol. Lett. 132(1):19-25.

Kobayashi S, Kitazawa T, Somlyo AV, Somlyo AP (1989). Cytosolic heparin inhibits muscarinic and alpha-adrenergic Ca^{2+} release in smooth muscle. Physiological role of inositol 1, 4, 5-trisphosphate in pharmacomechanical coupling. J. Biol. Chem. 264(30):17997-18004.

Lahlou M (2013). The success of natural products in drug discovery. Pharmacol. Pharm. 4(3A):17-31.

Lainetti R, BRITO NS (1979). *A cura pelas ervas e plantas medicinais brasileiras*. Editora Tecnoprint Ltda, Rio de Janeiro.

Mahmoud II, Marzouk MS, Moharram FA, El-Gindi MR, Hassan AM (2001).Acylated flavonol glycosides from *Eugenia jambolana* leaves. Phytochemistry. 58:1239-1244.

Mazumder R, Bhattacharya S, Mazumder A, Pattnaik AK, Tiwary PM, Chaudhary S (2006). Antidiarrhoeal evaluation of *Aegle marmelos* (Correa) Linn. root extract. Phytother. Res. 20(1):82-84.

Megbo BC, Samuel AM, Dio DW (2017). *Phoenix dactylifera* fruit: a nutraceutical agent in the treatment of diarrhea. Innovat Int. J. Med. Pharm. Sci. 2(3).

Morton JF (1963). The jambolan (*Syzygium cumin* L. Skeels)-its food. Proc. Fla. State Hortic. Soc. 76:328-338.

Mujumdar AM, Upadhye AS, Misar AV (2000). Studies on antidiarrhoeal activity of *Jatropha curcus* root extract in albino mice. J. Ethnopharmacol. 70(2):183-187.

Mukherjee PK, Harwansh RK, Bahadur S, Banerjee S, Kar A, Chanda J, Katiyar CK (2017). Development of Ayurveda–Tradition to trend. J. Ethnopharmacol. 197:10-24.

Mukherjee PK, Saha K, Murugesan T, Mandal SC, Pal M, Saha BP (1998). Screening of anti-diarrhoeal profile of some plant extracts of a specific region of West Bengal, India. J. Ethnopharmacol. 60(1):85-89.

Nadkarni AK (1976). Nadkarni's Indian Materia Medica, Popular Prakashan, Bombay, 1:517-548. Available at: https://www.scopus.com/record/display.uri?eid=2-s2.0-84865955512&origin=inward&txGid=9ca90e4d9eee1d1cbdb86a637fe67662

Nemeth V, Pfleghaar N (2017). Diarrhea. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2017-.2017 Oct 9.

Newman DJ, Cragg GM (2016). Natural products as sources of new drugs from 1981 to 2014. J. Nat. Prod. 79(3):629-661.

Patel S, Shanmugarajan TS, Somasundaram I, Maity N (2010). Protective effect of *Syzygium cumini* seeds against doxorubicin induced cardiotoxicity in rats. Int. J. Pharm. Life Sci. 1(6):343-349.

Qi Z, Kelley E (2014). The WHO traditional medicine strategy 2014–2023: A perspective. Science 346(6216):S5-S6.

Rembold CM (1996). Electromechanical and pharmacomechanical coupling. In: Barany M, ed. Biochemistry of Smooth Muscle Contraction. Chicago, Ill: Academic Press. 18:227-239.

Ribeiro RM, Pinheiro Neto VF, Ribeiro KS, Vieira DA, Abreu IC, Silva SN, Borges MOR (2014). Antihypertensive effect of *Syzygium cumini* in spontaneously hypertensive rats. Evid-Based Complement. Altern. Med. 2014:605452.

Sadraei H, Ghannadi A, Malekshashi K (2003). Relaxant effects of essential oil of *Melissa officinalis* and citral on rat ileum contractions. Fitoterapia 74:445-452.

Sagrawat H, Mann AS, Kharya MD (2006). Pharmacological potential of *Eugenia jambolana*: A review. Pharmacogn. Mag. 2(6):96-105.

Schiller LR (1995). Review article: anti-diarrhoeal pharmacology and therapeutics. Aliment. Pharmacol. Ther. 9(2):87-106.

Schiller LR (2017). Antidiarrheal Drug Therapy. Curr. Gastroenterol. Rep. 19(5):18.

Schoenfelder T, Warmlin CZ, Manfredini MS, Pavei LL, Réus JV, Tristão TC, Costa-Campos L (2010). Hypoglycemic and hypolipidemic effect of leaves from *Syzygium cumini* (L.) Skeels, Myrtaceae. in diabetic rats. Rev. bras. Farmacogn. 20(2):222-227.

Shah AJ, Gowani SA, Zuberi AJ, Ghayur MN, Gilani AH (2010). Antidiarrhoeal and spasmolytic activities of the methanolic crude extract of *Alstonia scholaris* L. are mediated through calcium channel blockade. Phytother. Res. 24:28-32.

Shamkuwar PB, Pawar DP, Chauhan SS (2012). Antidiarrhoeal activity of seeds of *Syzygium cumini* L. J. Pharm. Res. 5(12): 5537-5539.

Sharma DK, Gupta VK, Kumar S, Joshi V, Mandal RSK, Prakash AGB, Singh M (2015). Evaluation of antidiarrhoeal activity of ethanolic extract of *Holarrhena antidysenterica* seeds in rats. Vet. World 8(12):1392-1395.

Silva SN, Abreu IC, Silva GFC, Ribeiro RM, Lopes AS, Cartágenes MSS, Borges MOR (2012). The toxicity evaluation of *Syzygium cumini* leaves in rodents. Rev. Bras. Farmacogn. 22(1):102-108.

Singh RK, Pandey HP, Singh RH (2003). Irritable Bowel Syndrome: Challenge ahead. Curr. Sci. 84(2):1525-1533.

Souza VC, Lorenzi H (2005). Botânica sistemática: guia ilustrado para identificação das famílias de Angiospermas da flora brasileira, baseado em APG II. Instituto Plantarum.

Srivastava S, Chandra D (2013). Pharmacological potentials of *Syzygium cumini*: a review. J. Sci. Food Agric. 93(9):2084-2093.

Swami SB, Thakor NSJ, Patil MM, Haldankar PM (2012). Jamun (*Syzygium cumini* (L.)): A review of its food and medicinal uses. Food Nutr. Sci. 3(8):1100.

Van Rossum JM (1963). Cumulative dose-response curves. II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. Arch. Int. Pharmacodyn. Ther. 143:299.

Weiser M, Mutschler E, Lambrecht G (1997). Characterization of postjunctional muscarinic receptors mediating contraction in rat anococcygeus muscle. Naunyn Schmiedebergs Arch. Pharmacol. 356(5):671-677.

Zahoor M, Yousaf Z, Aqsa T, Haroon M, Saleh N, Aftab A, Ramazan H (2017). An ethnopharmacological evaluation of Navapind and Shahpur Virkanin district Sheikupura, Pakistan for their herbal medicines. J. Ethnobiol. Ethnomed. 13(1):27.