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Antidiarrheal potential of *Distemonanthus benthamianus* Baillon. extracts *via* inhibiting voltage-dependent calcium channels and cholinergic receptors

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ABSTRACT

Objective: To evaluate spasmolytic mechanisms of aqueous and methanolic extracts from *Distemonanthus benthamianus* trunk-bark.

Methods: Spasmolytic activities of extracts were evaluated *in vitro* on spontaneous and potassium chloride-induced jejunum contractions, or against cholinergic [acetylcholine (0.3 μmol/L)] stimulations. High performance liquid chromatography analysis of both extracts was performed in reference to standard compounds.

Results: Extracts developed concentration-dependent inhibitory activities. The methanolic extract, which revealed better activity, produced spasmolytic and myorelaxant effects at concentrations of 0.01-0.30 mg/mL with EC_{50} of 0.06 and 0.09 mg/mL (95% *CI*: 0.03-0.3 mg/mL), respectively. Its anticholinergic effect was obtained at the same concentrations with EC_{50} of 0.11 mg/mL (95% *CI*: 0.03-0.3 mg/mL). Chromatograms showed the presence of gallic acid in both extracts, rutin being only detected in the aqueous extract.

Conclusions: *Distemonanthus benthamianus* extracts exhibit verapamil and atropine-like activities, thus highlighting calcium channels and muscarinic receptors blocking potentials, which may be conveyed by some phenolic compounds. These results confirm the antidiarrheal activity of *Distemonanthus benthamianus* extracts.

1. Introduction

Diarrhea is an alteration of intestinal peristaltic movement, characterized by an increase in volume, fluidity and stool frequency greater than 3 times per day[1,2]. The classification of different diarrhea types is essentially based on generally observed etiological and pathophysiological mechanisms. Thus, secretory diarrhea occurs when there is an electrolytes imbalance in the intestinal lumen, leading to body fluids loss. When unabsorbed osmolytes concentration increases in the bowel, fluids loss turns chronic and provokes excessive dehydration associated with hypermotility, characteristics that determine osmotic diarrhea[3,4].

There is a variety of medications available with different mechanisms of antidiarrheal action. Based on their chemical

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structure and their molecular targets, they are classified as antisecretory, adsorbents, motility inhibitors or combination drugs[5]. Although their beneficial effects, the main challenge faced is the management of their side effects[6,7]. Medicinal plants are empirically and popularly used as healing alternative, with large availability, lesser side effects and population acceptability[8].

Distemonanthus benthamianus (*D. benthamianus*) (Caesalpiniaceae) is a plant found in the tropical regions of West Africa; Western and Littoral regions of Cameroon. Its bark is traditionally used for the treatment of gastrointestinal and uro-genital infections, hematological disorders, dermatitis, pain and tumors[9,10]. Previous studies showed that flavonoids, sterols, triterpenes and alkaloids contents of *D. benthamianus* bark extract may determine its bactericidal activity[9,11,12]. Results obtained from same project have shown the antidiarrheal, cytokines inhibitory and epithelial regenerative effects of the aqueous and methanolic extracts *in vivo*. However, no scientific reports revealed the mechanisms that explain the antidiarrheal activity of the plants' trunk-bark. Therefore, this study was aimed to evaluate the calcium channels blockade and anticholinergic efficiencies of the aqueous and methanolic extracts from *D. benthamianus* trunk-bark.

2. Materials and methods

2.1. Chemicals

Potassium chloride (KCl) was purchased from Riedelde Haen (Seezle, Germany). Dimethyl sulfoxide, Tween 80, atropine sulphate and verapamil chlorhydrate were from Sigma-Aldrich (St. Louis, MO63103 USA). Chloroform, methyl alcohol and sodium chloride were obtained from Daejung Chemicals and Metals Co., Ltd (Gyeonggi-do, Korea). Gallic acid monohydrate was obtained from Sharlau Ltd (Sentmenat, Spain); quercetin dehydrate (97.0%) and acetylcholine chlorhydrate were gotten from Alfa Aesar (Kandel, Germany) and rutin trihydrate (≥95.0%) was from Solarbio Science and Technology Co., Ltd (Beijing, China). Calcium chloride dihydrate ($CaCl_2$•$2H_2O$) was purchased from E. Merck (Darmstadt, Germany).

2.2. Plant collection and extraction

The trunk-bark of *D. benthamianus* was collected in April 2014 (Souza Village, Littoral Region, Cameroon). Identification was conducted at Institute of Medical Research and Medicinal Plant Studies (IMPM) in Yaoundé, Cameroon. Sample was registered under voucher number TN 275. Trunk-bark was shade dried, powdered and hermetically kept until extraction. Aqueous extraction was performed by macerating 500 g of powder in distilled water (1.9 liters) within 48 h at 20-25 ℃, with regular stirring. Filtrations were successively conducted with nº3 and nº2 filter papers, the obtained filtrate was oven-dried (45 ℃), giving 16.94 g of aqueous extract (3.39% yield). The methanolic extract was obtained by soaking and regularly stirring 950 g of the same stored powder in 5 liters' methanol for 72 h under ambient condition. Similar filtration method as previously described was performed, the resulting filtrate was concentrated under reduced pressure (170 to 180 mbar) using a Rotary Evaporator at 40 ℃. Crude methanolic extract (92.74 g; 9.76% yield) was collected and both extracts were stored at + 4 ℃.

2.3. Animals

Twenty male BALB/c mice (24-30 g) were purchased from the National Health Institute, Islamabad, Pakistan. Prior to experiments, they were fed with a standard formulated rodents' pellets with free water access and acclimatized under ambient conditions (20-25 ℃, normal light/dark cycle) in the animal house of the Department of Pharmacy, COMSATS University Islamabad, Abbottabad Campus, Pakistan. Research Ethics Committee, Department of Pharmacy, approved experimental protocols involving animals and delivered an ethical clearance no Phm.Eth/FA17-CS-M10/18-010-72, according to U.K. Animals (Scientific Procedures) Act, 1986 guidelines and EU Directive 2010/63/EU for animal experiment.

2.4. High performance liquid chromatography fingerprint analysis (HPLC)

A Shimadzu Prominence preparative LC-20AP system (Shimadzu Corporation, Kyoto, Japan) was used to conduct the HPLC analysis of both trunk-bark extracts. The system was equipped with a Shimadzu HPLC quaternary pump, a Shim-Pack GIST C18 (150 mm × 4.6 mm i.d., 5 μm) column and a SPD- 20AV prominence UV/VIS detector. Samples were prepared by dissolving 5 mg extracts in 5 mL HPLC grade methanol and filtered (0.45 μm millipore filter) before injections. Three mobile phases made of 1% acetic acid in deionized water (A) and acetonitrile (B), as well as methanol (C), were supplied at a flow rate of 0.6 mL/min. Samples were injected at a volume of 20 μL, using an injection loop (Rheodyne, Cotati, CA, USA) and separated with the column (20-25 ℃). UV detection was carried out at 254 nm. Gallic acid, quercetin and rutin were standard compounds and samples were run within 40-55 min.

2.5. Preparation of jejunum fragments

Male mice, fasted for 18 h, were weighed and anesthetized with chloroform. The abdominal cavity was opened, and jejunum fragments (2-3 cm) were removed from the mesenteries and previously oxygenated in Tyrode solution (in mmol/L; 37 ℃): sodium chloride 136.9; KCl 2.7; $MgCl_2$ 1.1; NaH_2PO_4 0.4; $C_6H_{12}O_6$ 5.6; $NaHCO_3$ 11.9 et $CaCl_2$ 1.8; (pH 7.4). Fragments were further mounted in an isolated organ tissue bath containing the same physiological solution at 37 ℃, permanently aerated with carbogen (95% O_2, 5% CO_2). A preload (1 g) was applied on each fragment and spontaneous contractions were recorded with an isotonic

transducer (TRI201 AD, 2310016) coupled to a system PowerLab amplifier (AD Instruments, ML 846, Sydney, Australia). It was connected to a computer equipped with graphics software (version 5.3). Organs were equilibrated within 30 min prior to addition of any test substances. Therefore, they were stimulated with 0.3 μmol/L acetylcholine (ACh) to obtain responses considered as control. Tissue was stable when isotonic contractile responses were recorded. These experimental conditions made it possible to evaluate the spasmolytic and myorelaxant activities of the extracts in absence of any agonist[13].

2.6. Spasmolytic activity and KCl-induced calcium channels opening

The spasmolytic activity was directly evaluated by cumulative addition of extracts concentrations (3, 30 and 300 mg/mL stock solutions), or verapamil (10^{-3} to 10^{-6} mg/mL stock solutions) used as standard. However, myorelaxant effect was performed on jejunum fragments pre-contracted with a sub-maximal concentration of K^+ (80 mmol/L). Cumulative concentrations of each test substance

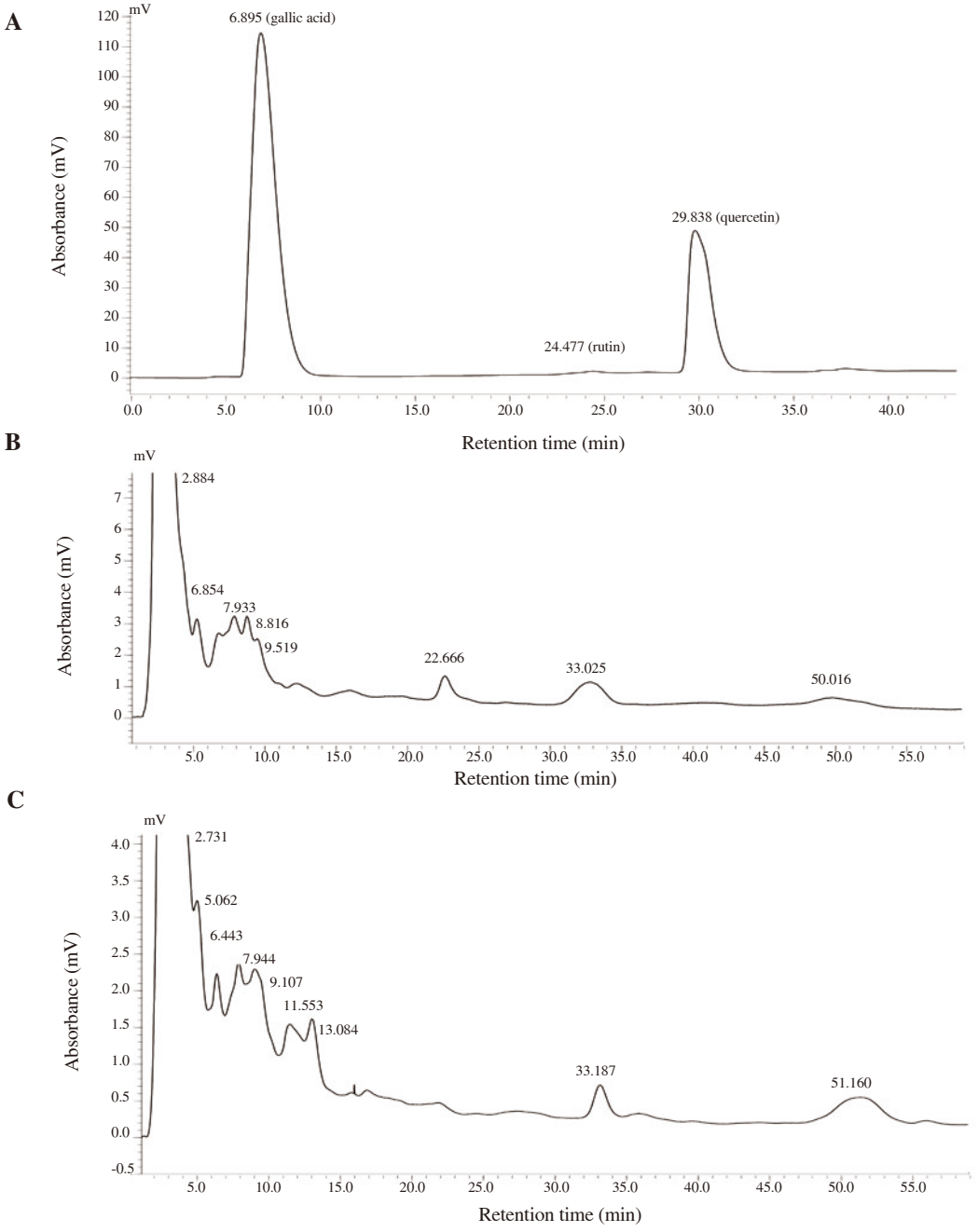

Figure 1. HPLC chromatograms of the aqueous (B) and methanolic (C) trunk-bark extracts of *Distemonanthus benthamianus*. Peaks of identified compounds are shown, in comparison with standard peaks (A) of gallic acid, rutin and quercetin.

were then added as a plateau was observed. Inhibition percentages were calculated from the recorded contraction load (g) in presence of different extracts and verapamil, relative to control contractions considered as 100%.

2.7. ACh antagonism activity

This experiment was performed on jejunum fragments previously isolated, prepared and mounted as described above. The antagonistic effects were evaluated by priorly incubating the jejunum fragments for 10-15 min, with individual concentrations of each extract or atropine used as standard substance. ACh (0.3 μmol/L) was added into the bath following each incubation and the contraction amplitude was recorded. Inhibition percentages were calculated from the contraction load (g) recorded with ACh (0.3 μmol/L), after pre-incubation of fragments with independent concentrations of extracts and atropine (antagonists), compared to control contractions considered as 100% according to the method of Gilani *et al*[14] with modifications. For each test, concentration-response curves were plotted and the median effective concentration (EC_{50}) was determined.

2.8. Statistical analysis

All data were expressed where applicable as mean ± standard error of mean (SEM). ANOVA two-way and Bonferroni post-test were used for statistical comparison. The software Graphpad prism 5.03 (GraphPad Software, Inc., San Diego, California, USA) allowed data calculations, analysis and graphing. Data were determined statistically significant at $P<0.05$.

3. Results

3.1. HPLC fingerprint

Chromatograms of aqueous and methanolic extracts of *D. benthamianus* trunk-bark and that of standard compounds are presented in Figure 1. When comparing the peaks of the aqueous (Figure 1B) and methanolic (Figure 1C) extracts with those of standard compounds (Figure 1A), gallic acid was identified in both

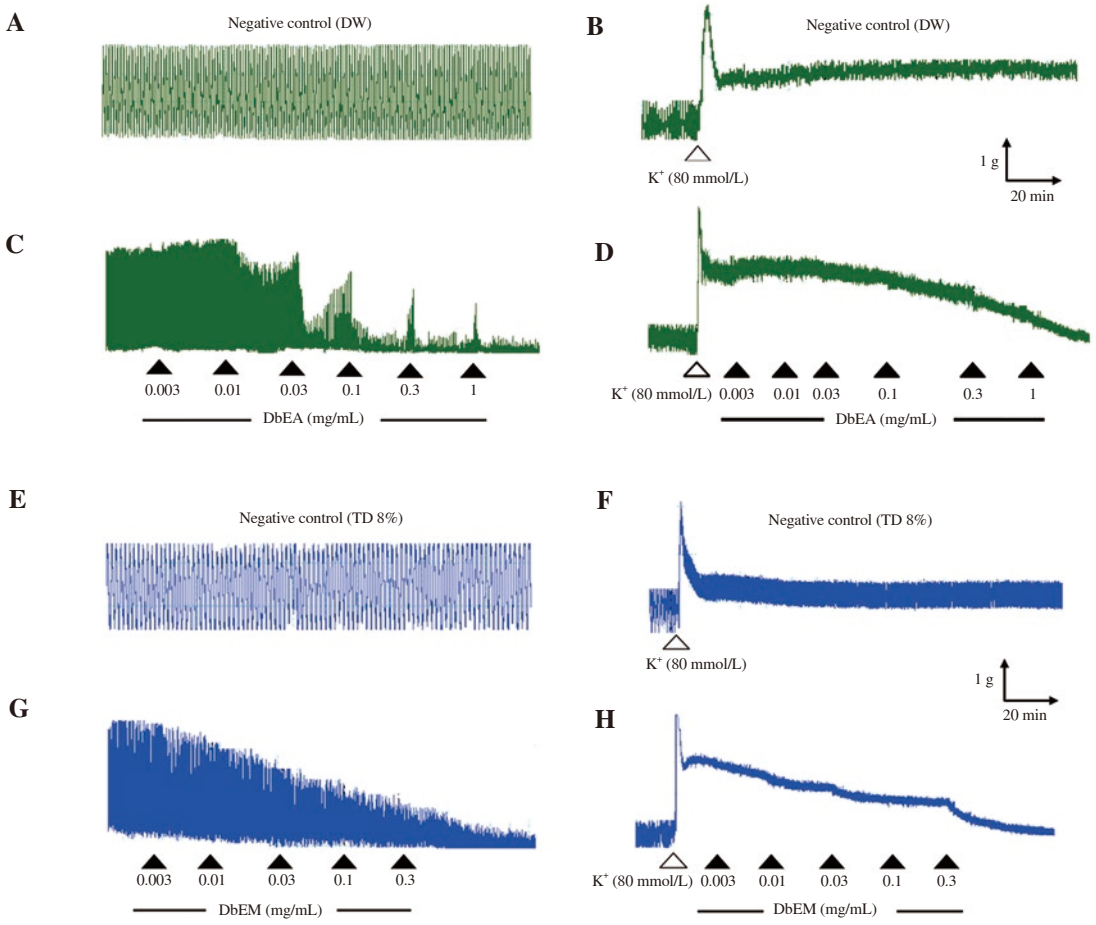

Figure 2. Original tracings showing spontaneous contractions (A, E), KCl-induced contractions (B, F), spasmolytic (C, G) and myorelaxant (D, H) effects of cumulative concentrations of aqueous (DbEA) and methanolic (DbEM) extract of *Distemonanthus benthamianus* on isolated mice jejunum preparation ($n = 5$); DW: deionized water; TD 8 %: Tween-dimethyl sulfoxide (8%).

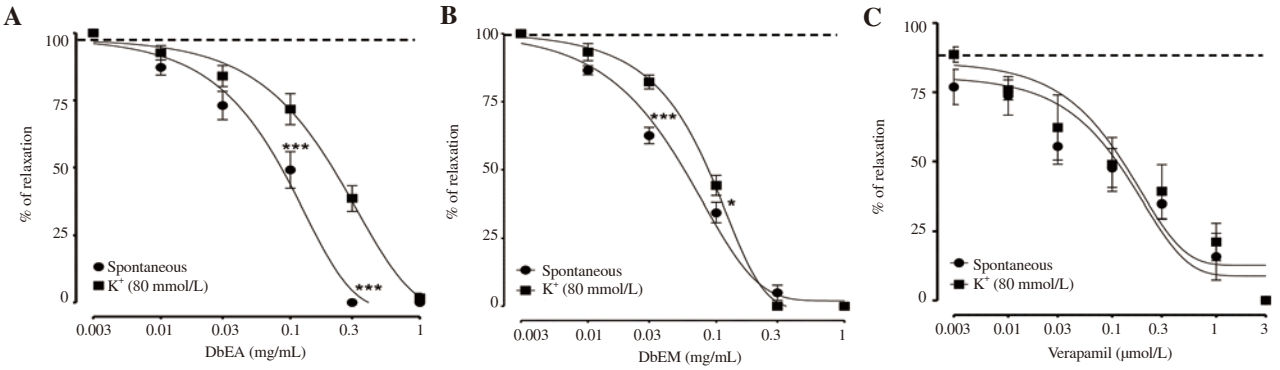

Figure 3. Inhibitory effects of (A) aqueous (DbEA) and (B) methanolic (DbEM) extracts, and (C) verapamil on spontaneous and KCl-induced contractions of isolated mice jejunum preparation. Values shown are mean ± SEM (n = 5). $^{*}P<0.05$; $^{***}P<0.001$: significant differences *vs.* K^+ (80 mmol/L).

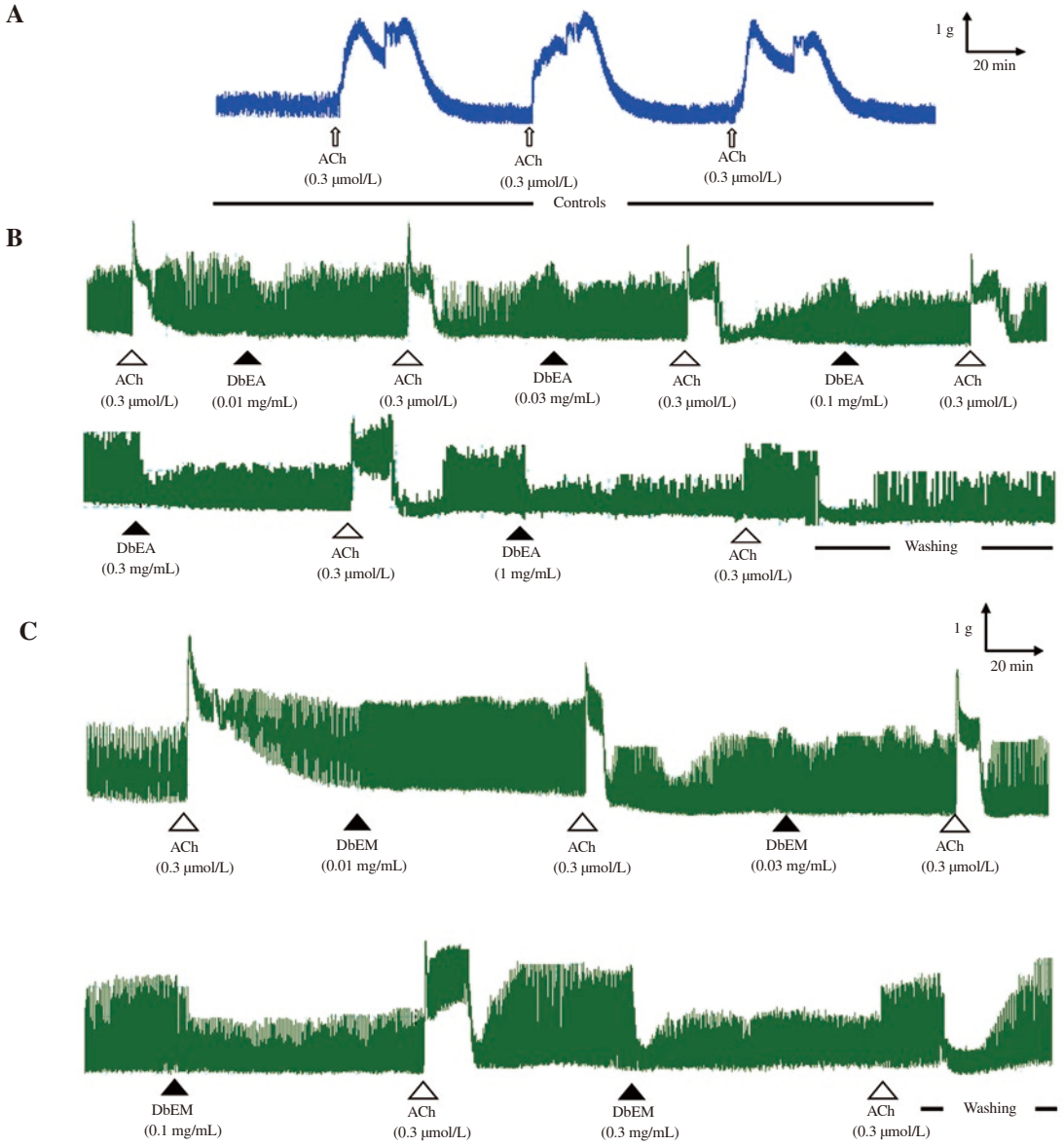

Figure 4. Original tracings showing (A) ACh (0.3 μmol/L)-stimulated high amplitude contractions and (B, C) antagonistic effects of cumulative concentrations of aqueous (DbEA) and methanolic extracts (DbEM) on isolated mice jejunum preparation (n = 5).

extracts at similar retention times (RT) compared to that of standard (RT = 6.895 min). It was detected in the highest concentration in the aqueous extract at RT = 6.854 min than in the methanolic extract (RT = 6.443 min). However, rutin was only identified in the aqueous extract (RT = 22.666 min). Many other unidentified compounds were also found.

3.2. Spasmolytic and calcium-channels blocking activities

No spasmolytic or myorelaxant activity was recorded with the different vehicles on jejunum preparations (Figures 2A, 2B and 2E, 2F). However, a concentration-dependent inhibitory effect was observed with both extracts. Thus, from 0.01 to 1 mg/mL of aqueous extract, an activity was detected with EC_{50} of 0.09 mg/mL (95% *CI*: 0.03-1 mg/mL) and of 0.21 mg/mL (95% *CI*: 0.1-1 mg/mL), respectively on spontaneous and KCl-induced contractions (Figures 2C, 2D; Figure 3A). The methanolic extract also produced spasmolytic and myorelaxant (Figures 2G, 2H; Figure 3B) effects at the concentrations ranging from 0.01 to 0.3 mg/mL and with EC_{50} of 0.06 and of 0.09 mg/mL (95% *CI*: 0.03-0.3 mg/mL), respectively. Extracts had verapamil-like activities.

3.3. Anticholinergic activities

Synchronic and isotonic contractions were obtained after stimulation of jejunum fragments with ACh (0.3 μmol/L) (Figure 4A). However, when the organ was incubated with cumulative concentrations of both extracts and atropine, a concentration-dependent inhibitory effect was obtained (Figure 5). The aqueous extract exhibited anticholinergic effect at the concentrations ranging between 0.01 and 1 mg/mL (Figure 4B), with EC_{50} of 0.32 mg/mL (95% *CI*: 0.1-1 mg/mL). Similar effects were obtained with 0.01 to 0.3 mg/mL of methanolic extract (Figure 4C); EC_{50} of 0.11 mg/mL (95% *CI*: 0.03-0.3 mg/mL). Extracts possessed greater activities than 0.003 to 3 μmol/L of standard atropine with the methanolic extract being the most active.

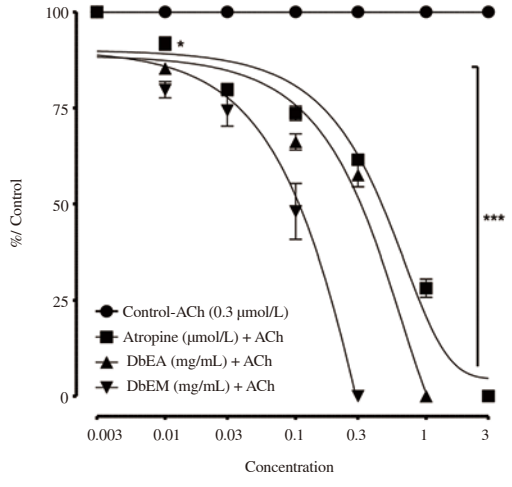

Figure 5. Concentration-dependent antagonistic effects of aqueous (DbEA) and methanolic (DbEM) extracts, and atropine on ACh-stimulated contractions of mice jejunum preparation. Values are mean ± SEM (n = 5). $^{*}P<0.05$; $^{***}P<0.001$: significant differences *vs*. ACh (0.3 μmol/L).

4. Discussion

D. benthamianus is found in Cameroon, where its bark is traditionally used to treat diarrhea. Previous study also reported that the plant material is used to treat hematological disorders, dermatitis, urogenital infections and gastric ulcers[15,10]. Adult patients suffering from diarrhea are recommended by Cameroonian practitioners to absorb 21 g of trunk bark powder in one cup of water within 3 d. In reference to that preparation, approximately equivalent to 300 mg/kg, the concentrations used in this study were selected. Results obtained in the same project showed that the aqueous and methanolic extracts of *D. benthamianus* have interestingly inhibited intestinal motility and stools' frequency in diarrheic rats, which suggested that extracts may have a tendency to further regulate intestinal smooth muscle functionality. Thus, the current study was undertaken *in vitro*, to determine the action mechanisms explaining the spasmolytic effects of extracts on isolated jejunum fragments.

It has been shown that when plant extracts are able to inhibit spontaneous rhythmic contractions of an isolated jejunum fragment, this would indicate that they possess spasmolytic activity[16]. But, since several pathways are involved, it is then necessary to look for the different mechanisms that can explain the activity. This could be performed through an evaluation of calcium channels inhibitory effects, potassium channels opening stimulatory effects, or evaluation of antimuscarinic activities[17]. Smooth muscles contraction depends on free cytoplasmic calcium, which promotes the activation of contractile elements of smooth muscle cells. Thus, an intracellular increase in free calcium would occur as a result of the L-type calcium channels opening, or the release from calcium stored in the sarcoplasmic reticulum[18,19]. It is known that high concentrations of extracellular potassium (K^+) induces smooth muscles contractions, by activating L-type calcium channels opening, thus causing extracellular calcium influx, followed by an activation of contractile elements[20,21].

The concentration-dependent myorelaxant activity obtained with extracts on KCl-induced sustained contractions clearly shows that they inhibited the L-type calcium channels, thus preventing calcium influx. Additionally, these myorelaxant effects were comparable to those expressed by verapamil, a known calcium channel inhibitor[22].

Moreover, ACh is a neurotransmitter released by the parasympathetic and intrinsic neurons of the enteric nervous system. This molecule, by binding to cholinergic receptors is able to transmit activation signals to the contractile elements in the intestinal wall[23]. Extracts antagonized the stimulatory effects of ACh (0.3 μmol/L) in an atropine-like manner, thus making it possible to confirm that in addition to inhibit the voltage-dependent calcium channels, they possessed an affinity with cholinergic receptors, specifically the M_3 muscarinic receptors, which would have potentiated the spasmolytic activity[24].

HPLC chromatograms revealed the presence of gallic acid in both extracts, but at low concentration in the methanolic extract. Previous studies have shown that gallic acid can mediate antidiarrheal activity in rats[25]. Therefore, the calcium channels blocking or anticholinergic activities produced by extracts may be attributed to their gallic acid content and/or the different compounds detected.

In conclusion, this study highlights that aqueous and methanolic extracts from *D. benthamianus* have spasmolytic and myorelaxant activities. These potentials are mediated through voltage-dependent calcium channels blockade and muscarinic receptors inhibition. Gallic acid and many other identified compounds may be responsible for the observed activities. *D. benthamianus* extracts can therefore be considered for further translational research.

Conflict of interest statement

No conflict of interest was declared by authors.

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Authors' contributions

WYN collected plant material, performed pharmacological assays, statistical analysis and drafted the manuscript. SK and HMR contributed in performing pharmacological assays and data analysis. MM and AA critically revised the manuscript and provided punctual assistance. GA, AJS, AK and TK designed, revised the manuscript and supervised the project. All authors approved the final version for submission.

References

[1] Sahoo HB, Sagar R, Kumar A, Bhaiji A, Bhattamishra SK. Antidiarrhoeal investigation of *Apium leptophyllum* (Pers.) by modulation of Na^+K^+ATPase, nitrous oxide and intestinal transit in rats. *Biomed J* 2016; **39**(6): 376-381.

[2] Rtibi K, Hammami I, Selmi S, Grami D, Sebai H, Amri M, et al. Phytochemical properties and pharmacological effects of *Quercus ilex* L. aqueous extract on gastrointestinal physiological parameters *in vitro* and *in vivo*. *Biomed Pharmacother* 2017; **94**: 787-793.

[3] Christian N. What is diarrhea? What causes diarrhea? The MNT Knowledge Center. [Online] Available from: https://www.medicalnewstoday.com/categories/gastrointestinal [Accessed on 15 April 2014].

[4] Leódido ACM, Costa LEC, Araújo TSL, Costa DS, Sousa NA, Souza LKM, et al. Anti-diarrhoeal therapeutic potential and safety assessment of sulphated polysaccharide fraction from *Gracilaria intermedia* seaweed in mice. *Int J Biol Macromol* 2017; **97**: 34-45.

[5] WHO. *The treatment of diarrhoea: A manual for physicians and other senior health workers*. 4th revision. World Health Organization; 2005, p. 1-50.

[6] Xiao L, Lin X, Cao J, Wang X, Wu L. MRI findings in 6 cases of children by inadvertent ingestion of diphenoxylate-atropine. *Eur J Radiol* 2011; **79**(3): 432-436.

[7] Wu PE, Juurlink DN. Clinical review: Loperamide toxicity. *Ann Emerg Med* 2017; **70**(2): 245-252.

[8] Akerele O. Nature's medicinal bounty: Don't throw it away. *World Health Forum* 1993; **14**(4): 390-395.

[9] Nguelefack EMP, Ngu KB, Atchade A, Dimo T, Tsabang N, Mbafor JT. Phytochemical composition and *in vitro* effects of the ethyl acetate bark extract of *Distemonanthus benthamianus* Baillon (Caesalpiniaceae) on *Staphylococcus aureus* and *Streptococcus agalactiae*. *Cameroon J Experiment Biol* 2005; **1**(1): 50-53.

[10]Saha JBT, Pétrissans A, Molina S, Ndikontar MK, Mounguengui S, Dumarçay S, et al. Study of the feasibility of a natural dye on cellulosic textile supports by red padouk (*Pterocarpus soyauxii*) and yellow movingui (*Distemonanthus benthamianus*) extracts. *Indust Crops Prod* 2014; **60**: 291-297.

[11]Evina JN, Bikobo DSN, Zintchem AAA, Nyemeck II NM, Ndedi EDFM, Diboué PHB, et al. *In vitro* antitubercular activity of extract and constituents from the stem bark of *Distemonanthus benthamianus*. *Brazilian J Pharmacogn* 2017; **27**(6): 739-743.

[12]Mebude OO, Adeniyi BA, Lawal O. *In vitro* antimicrobial activities of ethanol extract of *Distemonanthus benthamianus* (Aayan) Baillon (Fabaceae) on *Streptococcus mutans*. *British J Med Medic Res* 2017; **22**: 1-8.

[13]Khan T, Ali S, Qayyum R, Hussain I, Wahid F, Shah AJ. Intestinal and vascular smooth muscle relaxant effect of *Viscum album* explains its medicinal use in hyperactive gut disorders and hypertension. *BMC Complement Altern Med* 2016; **16**(251): 1-8.

[14]Gilani AH, Bashir S, Janbaz KH, Shah AJ. Presence of cholinergic and calcium channel blocking activities explains the traditional use of *Hibiscus rosasinensis* in constipation and diarrhoea. *J Ethnopharm* 2005; **102**: 289-294.

[15]Adeniyi BA, Obasi OJ, Lawal TO. *In–vitro* antifungal activity of *Distemonanthus benthamianus* stem. *Int J Pharm Pharm Sci* 2011; **3**(Suppl 4): 52-56.

[16]Hamid I, Janbaz KH. Ethnopharmacological basis for antispasmodic, antidiarrheal and anti-emetic activities of *Ceratonia siliqua* pods. *Bangladesh J Pharmacol* 2017; **12**(4): 384-392.

[17]Naghdi F, Gholamnezhad Z, Boskabady MH, Bakhshesh M. Muscarinic receptors, nitric oxide formation and cyclooxygenase pathway involved in tracheal smooth muscle relaxant effect of hydro-ethanolic extract of *Lavandula angustifolia* flowers. *Biomed Pharmacother* 2018; **102**: 1221-1228.

[18]Qayyum R, Qamar HM, Khan S, Salma U, Khan T, Shah AJ. Mechanisms underlying the antihypertensive properties of *Urtica dioica*. *J Transl Med* 2016; **15**(254): 1-13.

[19]Hafiz MA, Rahman KA, Muhammad FR, Imran I. Pharmacological evaluation of smooth muscle relaxant and cardiac-modulation potential of *Phyla nodiflora* in *ex–vivo* and *in–vivo* experiments. *Asian Pacific J Trop Med* 2017; **10**(12): 1146-1153.

[20]Rasheed HM, Khan T, Wahid F, Khan R, Shah AJ. Chemical composition and vascular and intestinal smooth muscle relaxant effects of the essential oil from *Psidium guajava* fruit. *Pharm Biol* 2016; **54**(11): 2679-2684.

[21]Cheng PC, Wang YC, Chen YS, Cheng RC, Yang JJ, Huang RC. Differential regulation of nimodipine-sensitive and –insensitive Ca^{2+} influx by the Na^+/Ca^{2+} exchanger and mitochondria in the rat suprachiasmatic nucleus neurons. *J Biomed Sci* 2018; **25**(44): 1-16.

[22]Ali N, Jamil A, Shah SWA, Shah A, Shah I, Ahmed G. Spasmogenic and spasmolytic activity of rind of *Punica granatum* Linn. *BMC Complement Altern Med* 2017; **17**(97): 1-7.

[23]Guo H, Zhang J, Gao W, Qu Z, Liu C. Anti-diarrhoeal activity of methanol extract of *Santalum album* L. in mice and gastrointestinal effect on the contraction of isolated jejunum in rats. *J Ethnopharm* 2014; **154**(3): 704-710.

[24]Shamim K, Taous K, Jabbar JS. Total phenolic and flavonoid contents and antihypertensive effect of the crude extract and fractions of *Calamintha vulgaris*. *Phytomedicine* 2018; **47**: 174-183.

[25]Sheng Z, Yan X, Zhang R, Ni H, Cui Y, Ge J, et al. Assessment of antidiarrhoeal properties of the aqueous extract and its soluble fractions of Chebulae Fructus (*Terminalia chebula* fruits). *Pharm Biol* 2016; **54**(9): 1847-1856. Doi: 10.3109/13880209.2015.1131993.