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Myorelaxant And Calcium Channel Blocker Properties Of An Aqueous Extract Of *Solanum Torvum* (Solanaceae) On Isolated Rabbit's Intestinal Smooth Muscle

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***ABSTRACT**: The aqueous extract of Solanum torvum (EASt), at concentrations ranging from 10^{-1} to 6.10^{-1} mg/ml, caused a dose-dependent decrease in the amplitude of spontaneous rhythmic contractions and basal tone of isolated rabbit's duodenum. This myorelaxation was dose dependently antagonized by propranolol (10^{-4} to 10^{-2} mg/ml). In a physiological solution devoid of calcium, EASt completely inhibits intestinal rhythmic contractions and causes a decrease in basal tone. A completeinhibition of intestinal rhythmic contractions was also observed by adding EASt to a 0 Ca^{2+}physiological solution+ EDTA. However, in a 0 Ca^{2+} solution+ EGTA, the crude extract of Solanum torvum did not exhibit any effect.*

EASt therefore has myorelaxant properties and might act by promoting the decrease of intracellular calcium concentration in the intestinal smooth muscleby stimulating adrenergic receptors and blocking the intracellular and extracellular calcium channels.

Detection of phytocompounds such as tannins and flavonoidsin the aqueous extract of Solanum torvum through phytochemical screening which antispasmodic, anti-diarrheal and myorelaxant effects have been demonstrated by other authors could justify its myorelaxant properties and traditional use as an anti-diarrheal.

***KEY WORDS:** Solanum torvum, isolated duodenum, myorelaxant, adrenergic receptors, calcium channel blocker*

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I. INTRODUCTION

Nowadays, medicinal plants play a key role in the treatment of several pathologies such as diabetes, high blood pressure and malaria or diarrhea (WHO, 2003).

Diarrhea has become a recurring affliction in the world today. This disease, in its acute form, has globally increased mortality rate (WHO, 2003). Despite WHO's efforts to control this endemic disease in developing countries, the cost of modern medicines is still high**.**Their undesirable side effects, lack of infrastructure, socio-cultural practices and easy access to plants coerce the population to increasingly rely on traditional medicine (Kamanyi*et al*., 1997). Several medicinal plants are used in the treatment of diarrhea. Indeed, N'guessan (2008) has identified about 1500 species of medicinal plants used in the treatment of various pathologies. Many of them are used in the treatment of diarrhea and colics (Ambe*et al*., 2015).*Solanum torvum* (Solanaceae) is a medicinal plant commonly used in Ivory Coast for its anti-diarrheal properties. However, its anti-diarrheal properties have not yet been investigated. Therefore, scientific studies need to be carried out to check its properties.

The aim of this study is to identify the main phytochemical compounds of *Solanum torvum*, assess their effects on the motility of isolated rabbit's duodenum and determine their mechanism of action.

II. MATERIAL AND METHODS

1-1-Material

1-1-1- Animal material

Rabbits of the species *Oryctolagus cuniculus* (Leporideae) were used for investigating the effect of EASt on isolated duodenum contractions (motility). These rabbits were taken from breeding farms located in the city of Bingerville (Abidjan, Ivory Coast). They were acclimated for a week in cages, at the animal husbandry of the faculty of Biosciences at the University of Felix Houphouet-Boigny (Abidjan, Ivory Coast), to regulate and harmonize their physiological states before experiments. In this animal husbandry the mean temperature was 26 ± 4 °C with a relative humidity of 60 % and a photoperiodicity of 12/24. These animals were fed with food pellets and given water *ad libitum*.

1-1-2- Plant material

Fresh leaves of *Solanum torvum* (Solanaceae) were collected from the district of Cocody (Abidjan, Ivory Coast). Plant material was identified and authenticated by the National Floristic Center of the University of Felix Houphouet-Boigny (Abidjan, Ivory Coast).

1-2- Methods

1-2-1- Preparation of the aqueous extract of *Solanum torvum* (EASt)

EASt was prepared using 250 g of fresh leaves of *Solanum torvum* (Solanaceae). Leaves were slashed into pieces and boiled for 15 minutes in 1.5 liters of distilled water. The decoction was filtered twice on hydrophilic cotton, then once on Wattman filter paper (No. 1). Filtrates were dried in an oven at 50 °C for 72 hours. After drying, a powder representing the aqueous extract of *Solanum torvum* (EASt) was obtained.

1-2-2-Identification of some secondary metabolites in EASt

The detection of secondary metabolites in EASt was carried out through tube tests, using appropriate reagents (Wagner and Bladt, 2001), according to the techniques described by Bekro *et al*., (2007) and Abo (2013).

Detection of sterols and polyterpenes was performed using Liebermann reaction. Characterization of compounds belonging to polyphenols was carried out by ferric chloride reaction. Those belonging to flavonoids were identified through cyanidin reaction. Identification of saponins was based on the property of aqueous solutions containing saponins to produce foam after being shaken. Quinonic compounds were revealed by Borntraeger reaction. Alkaloids identification was carried out using two reagents: Dragendorff's reagent (potassium iodobismuthate reagent) and Bouchardat's reagent (iodine-iodide reagent). Finally, compounds belonging to the group of tannins were identified by Stiasny reaction.

1-2-3- Recording technique of the contractile activity of isolated rabbit's duodenum

The experimental technique was similarto that described by Mea *et al*. (2017). The experimental device encompassed an isolated organ vessel containing Mac Ewen solution, oxygenated by an aquarium bubbler and immersed in a thermostatic water bath. A vial containing the physiological glucose solution, placed on top of the device, supplied the isolated organ vessel through a coil dipped into the thermostatic water Bath, allowing the physiological fluid to remain at the temperature of 38 °C. The arrival of the Mac Ewen solution to the organ vessel was controlled by a central valve.

Duodenum fragments were collected from a rabbit, after 24 hours fasting, after a laparotomy, and kept alive in a Mac Ewen solution, oxygenated and maintained at a constant temperature of 38 °C.

For the experiment, a fragment of duodenum (about 3 cm) was mounted in the isolated organ vessel at one end and connected to the other end to a lever system carrying a recordingstylus. This stylus will pattern the contractile movements of the isolated intestine on a recording paper, coated with black smoke and pasted on a cylinder driven in a rotary motion, at constant speed, by an engine. A calibration of the inscribing stylus indicates in our experimental conditions, a height difference of 24 mm equaling to a force of 1 g (10 mN), or 1 gram force (1 gF), exerted by the intestine.

1-2-4-Physiological solution and chemicals

The standard physiological solution was the Mac Ewen solution containing: NaCl (58.44 g/mol), KCl (74.56 g/mol), $CaCl_2$ (110.99 g/mol), Na_2HPO_4 (156.01 g/mol), $NaHCO_3$ (84.10 g/mol), $MgCl_2$ (203.31 g/mol), and glucose (180.16 g/mol). This solution was used at pH= 7.4.

The chemicals used were: adrenaline (Renaudin, France), a myorelaxant, propranolol (SIGMA, USA), a β-blocker, EDTA (Ethylene diamine tetra acetic acid, SIGMA, USA) and EGTA (Ethylene glycol-bis (β-aminoethylether) N, N, N ', N'-tetraacetic acid, SIGMA, USA) both of them were used to chelate the residual calcium of the physiological solution devoid of calcium (0 Ca^{2+}).

1-2-5- Statistical analyses

Data analyses and graphical representations were carried out using GraphPad Instat and GraphPad Prism 4 Softwares (Microsoft, San Diego, California, USA). Values were expressed as mean ± standard of deviation. Data analysis was carried out using one way analysis of variance (Anova one way), followed by Tukey-Kramer multiple comparison test and significance of difference was observed at $p < 0.05$. The Softwares Paint and Microsoft Picture were used to display the plot of intestinal contractions in black on a grey background.

III. RESULTS

2-1-Phytochemical screening of the aqueous extract of *Solanum torvum* (EASt)

The phytochemical screening of EASt revealed the presence of some chemical compounds in this extract. This study exhibited in EASt the presence of sterols and polyterpenes, polyphenols, flavonoids, saponins, alkaloids and catechin tannins (Table 1).

Table1: Phytochemical screening of the aqueous extract of *Solanum torvum* (Solanaceae)

Phytochemical compound		Reagents	Results
Sterols and polyterpenes		Liebermann	+
Polyphenols		**ferric chloride**	+
Flavonoïds		Cyanidin	+
Saponins		Vigorous shaking	+
Alkaloïds		Dragendorff	+
		Bouchardat	+
Tannins	catechin	Stiasny	+
	gallic	Chlorideacid	–
Quinonic compound		Borntraeger	–

+ : Presence of compound

– : Absence of compound

2-2. Dose-response effects of EASt on the contractile activity of isolated rabbit's intestine

Figure 1 is a typical record of the effects of EASt, at concentrations ranging from 10^{-1}mg/ml to 6.10^{-1} mg/ml, on intestinal contractions of rabbit. The aqueous extract of *Solanum torvum*, administered at increasing doses ranging from10^{-1} to 6.10^{-1} mg/ml, exhibited dose-dependent decrease in the amplitude of spontaneous rhythmic contractions and basal tone of isolated rabbit duodenum fragments.

Doses below 10^{-1}mg/ml have no significant effects on intestinal contractions. The maximum myorelaxant effects of EASt were obtained at the dose of 6.10^{-1}mg/ml, with a decrease in contractions amplitude to a level of 95.72 ± 0.31 % ($p < 0.001$) and a decrease in tone to 0.80 ± 0.01 gF ($n = 3$). These effects remained constant for larger doses.

Figure 2 shows the decrease in the amplitude of rhythmic contractions of duodenum according to EASt concentration. It was used to determine, a 50 % inhibitory concentration (IC_{50}) of intestinal rhythmic contractions, which was equaled to 0.30 mg/ml.

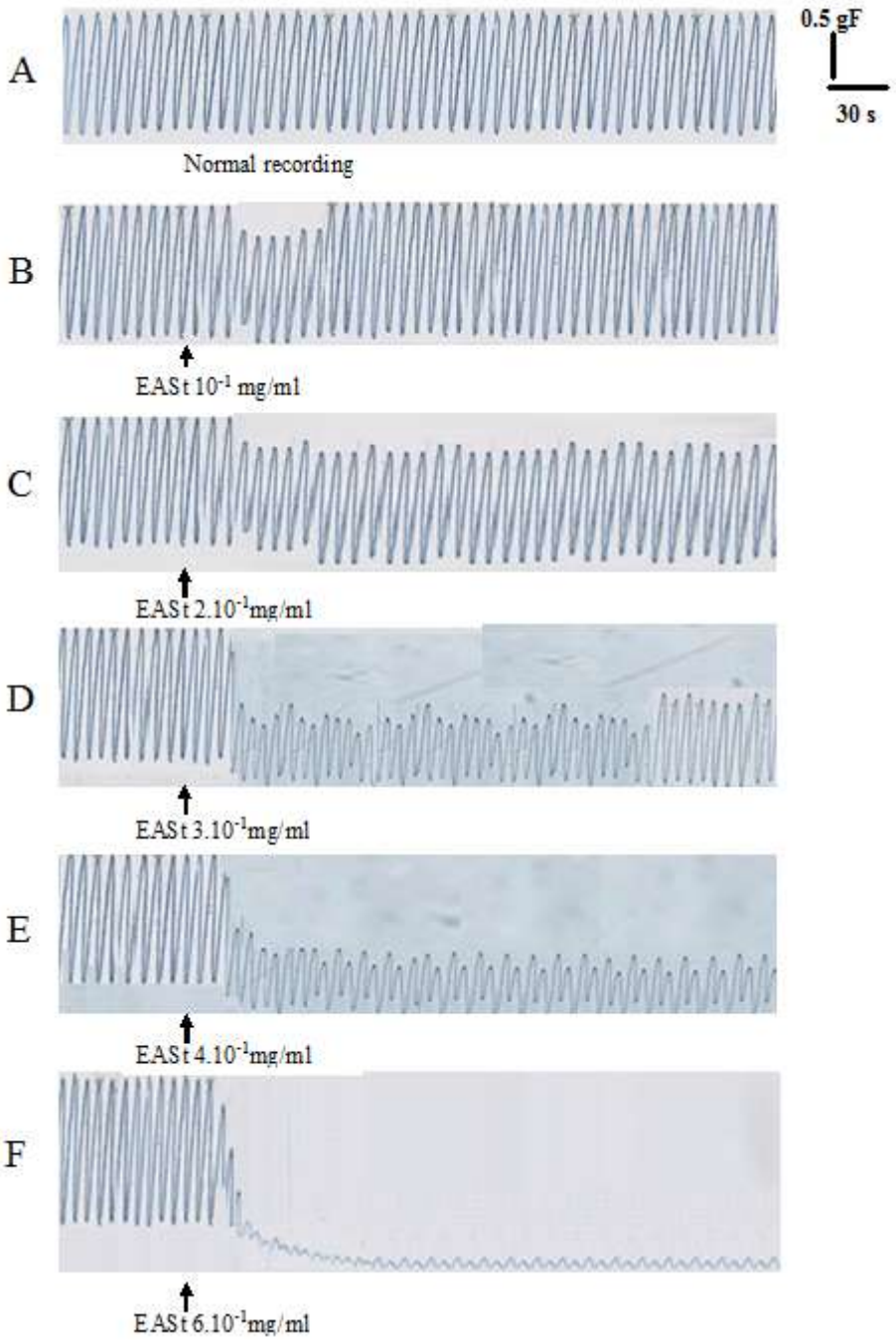

Figure1: Dose-response effect of the aqueous extract of *Solanum torvum* (EASt) on contractions of isolated rabbit's duodenum

A: Normal recording of intestinal contractions.
B to **F**: Normal recording (before arrows) and effects of EASt (after arrows) at 10^{-1} mg/ml (**B**), 2.10^{-1} mg/ml (**C**), 3.10^{-1} mg/ml (**D**), 4.10^{-1} mg/ml (**E**) and 6.10^{-1} mg/ml (**F**) on contractions of isolated rabbit's intestine.

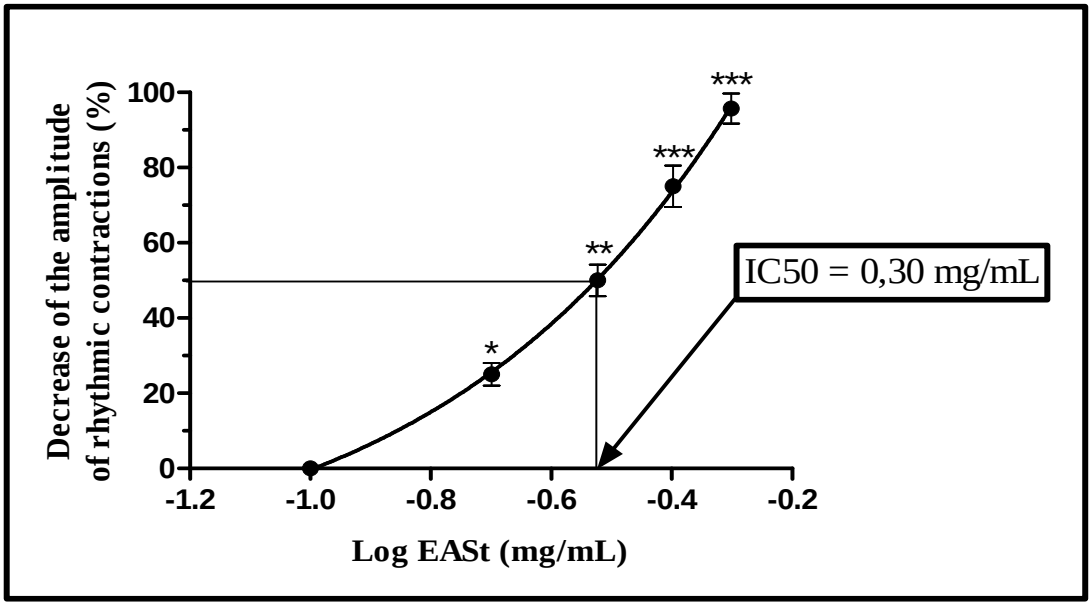

Figure 2: Decrease in the amplitude of rabbit's intestinal contractions according
To EASt concentration
n = 3; * p < 0.05; * * p < 0.01; p < 0.001 compared to control recordings.

2-3. EASt-Propranolol interaction on the contractile activity of isolated rabbit's intestine

Figure 3 is a typical record of the effects of EASt at a dose of 3.10^{-1} mg/ml on rabbit intestinal contractions, with or without propranolol (Prop). EASt, at a dose of 3.10^{-1} mg/ml, induced a decrease in the amplitude of spontaneous rhythmic contractions by 74.51 ± 4.25 % and lowered the base tone to 0.35 ± 0.04 gF (n = 3) (Figure 3A).

When EASt at a concentration of 3.10^{-1} mg/ml was added 30 s later to the physiological solution containing propranolol at a concentration of 10^{-4}mg/ml, the amplitude of the rhythmic contractions and the base tone respectively decreased by 53.33 ± 0.00 % and 0.27 ± 0.01 gF (n = 3) (Figure 3B);thus areduction of the effect of EASt on the amplitude of contractions to a level of 21.18 % ($p < 0.01$) and on the tone to 0.08 % ($p< 0.05$) when the intestine is pretreated with propranolol. When EASt at the concentration of 3.10^{-1} mg/ml was added 30 s later to the physiological solution containing propranolol at a concentration of 10^{-3} mg/ml, the amplitude of the rhythmic contractions decreased by13.33 ± 4.44 % (n = 3) (Figure 3C); areduction of the effect of EASt to reach a level of 61.20 % ($p < 0.001$). In this case, EASt has no significant effect ($p> 0.05$) on the basal tone of the isolated rabbit's intestine.

EASt at a concentration of 3.10^{-1} mg/ml, added 30 s later to the physiological solution containing propranolol at a concentration of 10^{-2} mg/ml, did not show any effect on the contractions of isolated rabbit's intestine (Figure 3D).

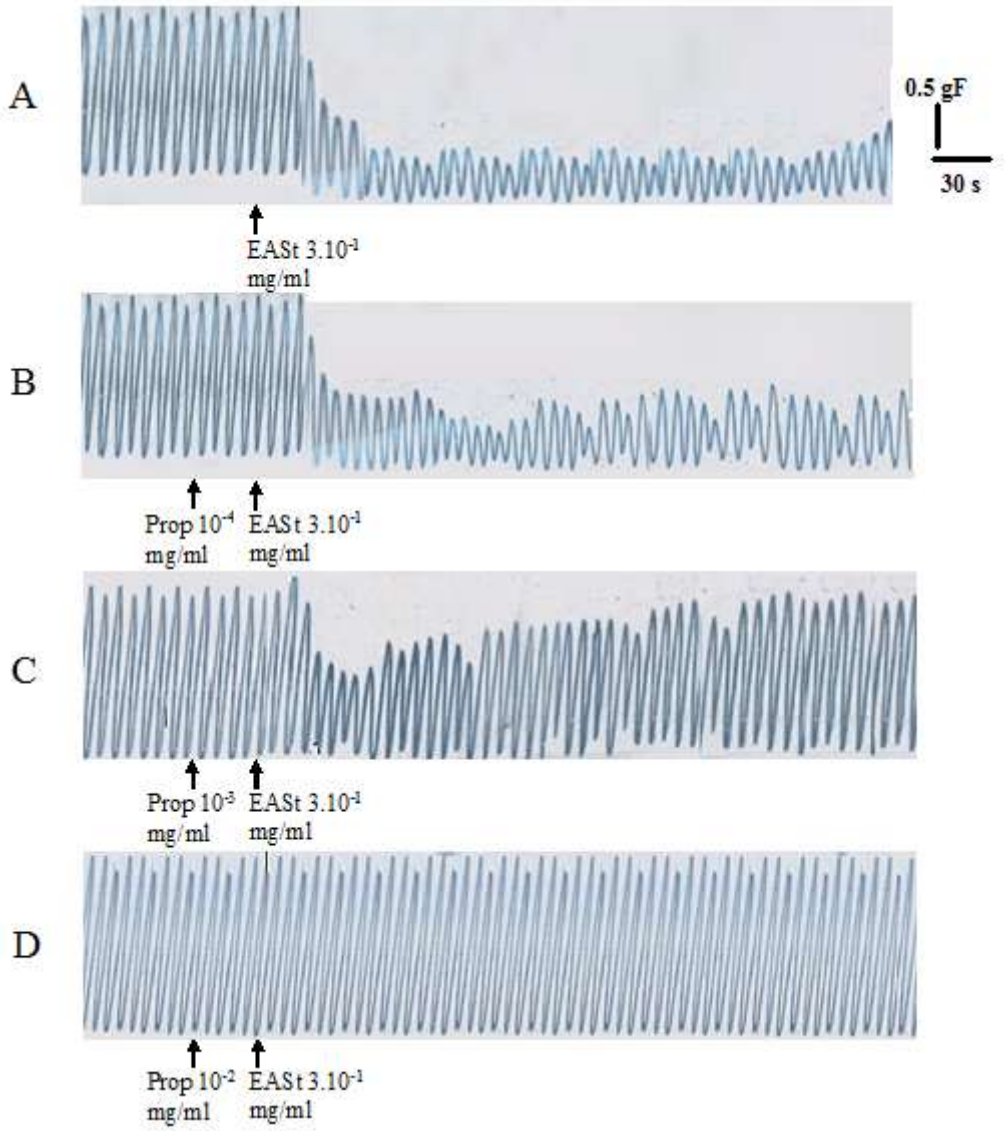

Figure3 : EASt-Propranolol interaction on contractions of isolated rabbit's duodenum

A: Normal recording (before arrow) and effects of EASt at a concentration of 3.10^{-1}mg/ml on intestinal contractions.
B to **D**: Normal recording (before the first arrow) and effect of EASt at a concentration of3.10^{-1}mg/ml (after the 2nd arrow) on contractions 30s after treatment of the intestine with propranolol (Prop) at concentrations of 10^{-4}mg/ml (**B**), 10^{-3}mg/ml (**C**) and 10^{-2} mg/ml (**D**).
Figure 4 shows the effects of propranolol on the reduction of the amplitude of rhythmic contractions and the decrease in the base tone of the intestine induced by EASt.

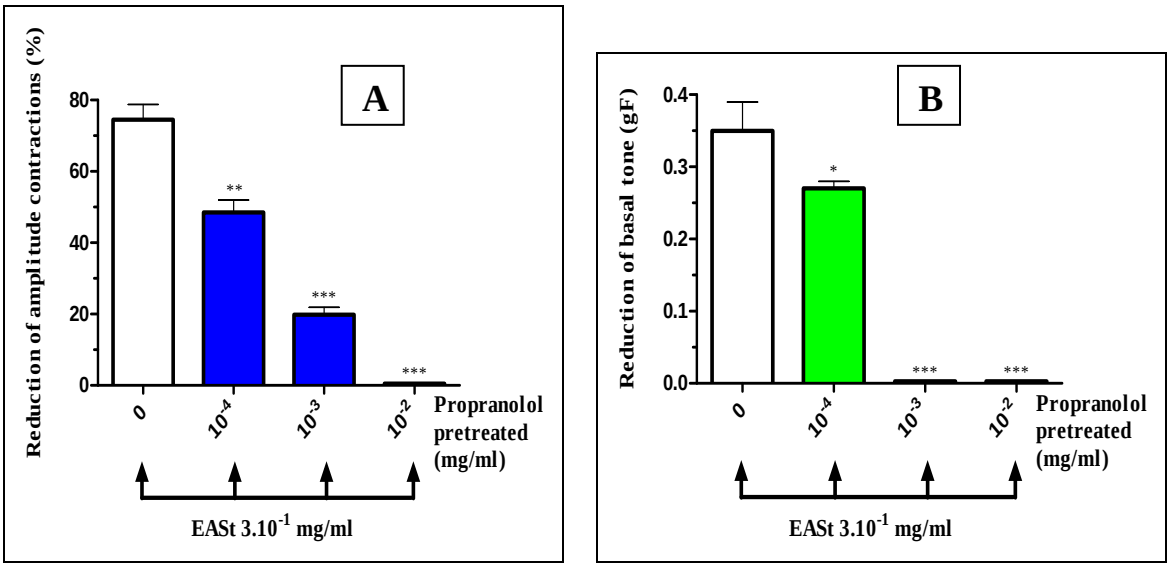

Figure 4: Effects of EASt on the amplitude of rhythmic contractions (A) and on the basal tone (B) of isolated rabbit's intestine pretreated with propranolol

n = 3; * p <0.05; ** p <0.01; *** p <0.001 compared to the effects of EASt alone.

2-4-Effect of EASt on the contractile activity of isolated rabbit duodenum in a physiological solution devoid of calcium (0 Ca^{2+}), with or without EDTA or EGTA

Figure 5A is a typical record of the effects of EASt at a concentration of 3.10^{-1} mg/ml on the contraction of isolated rabbit's duodenum. At this concentration, the effects of EASt showed a decrease in the amplitude of spontaneous rhythmic contractions and lowered the basal tone.

In a physiological solution devoid of calcium (0 Ca^{2+}), very weak rhythmic contractions of the intestine appear. After adding EASt at a concentration of 3.10^{-1} mg/ml to this 0 Ca^{2+}solution, the rhythmic contractions were totally inhibited and the basal tone decreased by 1.88 ± 0.02 gF (n = 3) (Figure 5B).

Figures 5C and 5D show typical records of the effect of EASt at 3.10^{-1} mg/ml on contractions of isolated rabbit's duodenum in physiological solutiondevoid of calcium (0 Ca^{2+}) and containing EDTA or EGTA at a concentration of 10^{-5} mg/ml. The solution 0 Ca^{2+} + EDTA virtually inhibits the rhythmic contractions of the duodenum. By adding EASt to this solution, a complete inhibition of intestinal rhythmic contractions was observed (Figure 5C). In 0 Ca^{2+} + EGTAsolution, duodenal rhythmic contractions completely disappeared. In this case, the addition of EASt has no effect (Figure 5D).

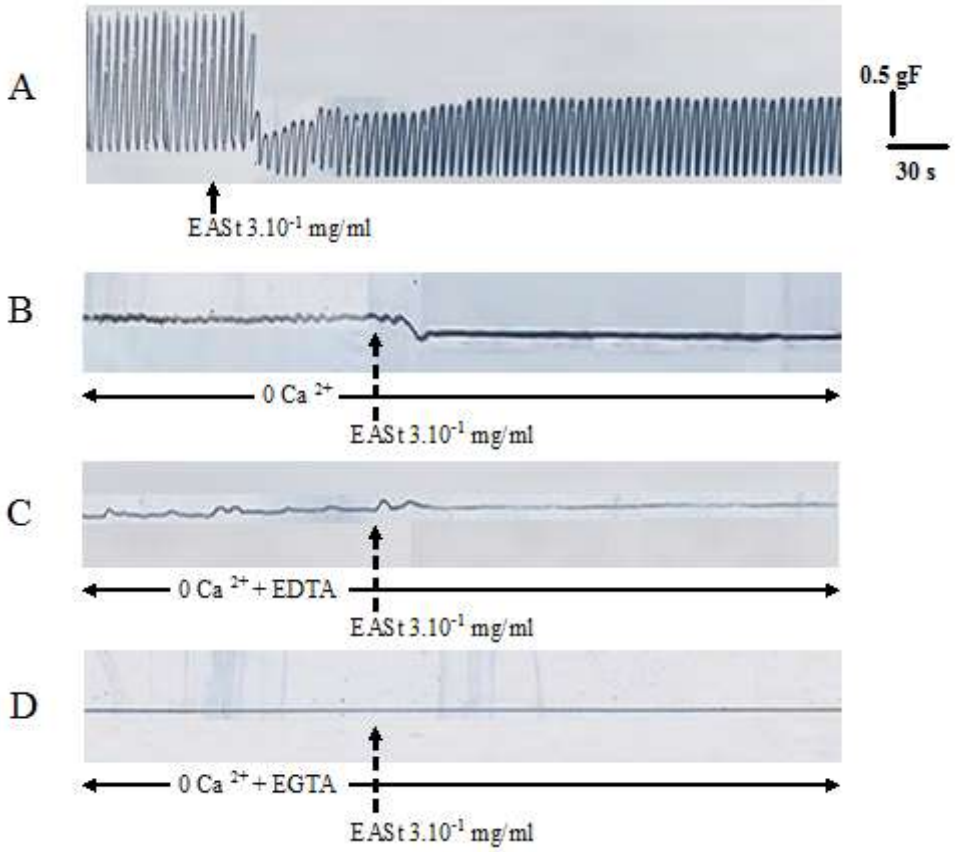

Figure 5: Effects of EASt on contractions of isolated rabbit's duodenum in physiological solutiondevoid of calcium, with or without EDTA or EGTA

A: Normal recording (before arrow) and effects of EASt at a concentration of 3.10^{-1} mg/ml on intestinal contractions.
B to **D**: Control recording (before arrows) and effects of EASt at a concentration of 3.10^{-1} mg/ml on intestinal contractions (after arrows) in 0 Ca^{2+} (B), 0 Ca^{2+} + EDTA 10^{-5} mg/ml (C) and 0 Ca^{2+} + EGTA 10^{-5} mg/ml (D) physiological solution.

IV. DISCUSSION

The pharmacological study helped to assess the effects of the aqueous extract of *Solanum torvum* (EASt) on the contractions of rabbit duodenal smooth muscle. The results showed that the significant decreased of intestinal contractions performed by EASt were dose-dependent. These effects indicate that this extract has myorelaxant properties.

The phytochemical screening of EASt showed the presence of sterols and polyterpenes, polyphenols, flavonoids, catechin tannins, alkaloids and saponins. Some of these secondary metabolites may be responsible for the pharmacological effects of this plant. Indeed, tannins, flavonoids and reducing compounds are generally responsible for the antidiarrheal and myorelaxant properties of medicinal plants (Tripathi 1994, Venkatesan *et al*.2005).Flavonoids have already shown their ability to inhibit spamogen-induced contractions (Capasso *et al*., 1988). Quercetin, a flavonoid isolated from leaves of *Conyza flaginoides* (Asteraceae), inhibited the spontaneous contractions of rat's ileum (Mata *et al*., 1997). According to these authors, quercetin increases the uptake of Na^+ and K^+ ions in the intestine. The antispasmodic effect of the crude aqueous extract of *Achille anobilis* (Asteraceae) on rat's duodenum was also attributed to some flavonoids found in this plant (Karamenderes and Aydin, 2003). Tripathi (1994) also reported that Quercetol, a flavonoid, decreases the amplitudes of intestine contractions by inhibiting the secretion of acetylcholine. According to Galvez *et al*. (1996) and Sanchez *et al*. (1997), flavonoids inhibited the increase of intestine contractions induced by prostaglandin E. The different flavonoids identified in the butanol and acetate extracts of *Morinda morindoides* (Rubiaceae) leaves have a spasmolytic action on guinea pig ileum (Chellaiah *et al*., 2010).

The myorelaxant effects observed with EASt are similar to those of adrenaline. This suggests the presence of possible bioactive compounds with adrenomimetic activities in this extract.

To confirm the presence of these adrenomimetic compounds, the effects of EASt were evaluated with propranolol, an inhibitor of α and β-adrenergic receptors (Marquez *et al*., 1981, Triggle 1985, Corallo *et al*., 1988). This study showed that EASt induced myorelaxant effects in a dose-dependent manner and even inhibited by propranolol. This indicates the presence of compounds in EASt that could act on the smooth muscles of the duodenum through adrenergic receptors. According to Guimaraes and Moura (2001), the activation of α and β-adrenergic receptors activate the Gi protein which inhibits the activity of adenyl cyclase, leading to a decrease in the production of cyclic adenosine monophosphate (cAMP). cAMP is therefore no longer able to activate protein kinases that phosphorylate regulatory proteins of the sarcoplasmic reticulum membrane and myofibrils. The absence of phosphorylation leads to the closure of membrane calcium channels, a deactivation of the sarcoplasmic reticulum calcium pump and a decrease in the sensitivity of myofibrils for calcium. The result is a decrease in contractions.Thus, the aqueous extract of *Solanum torvum* (Solanaceae) might contain adrenomimetic compounds, responsible for the intestinal contraction inhibition, which could act by activating the adrenergic receptors and induce the inhibition of membrane calcium channels.

Calcium is essential for the contractile activity of duodenal smooth muscle. Indeed, contraction of smooth muscle depends on calcium (Rodger 1985, Olmo *et al*., 1997); especially the increase of intracellular calcium. Thus in a physiological solutiondevoid of Ca^{2+} duodenum contractions were inhibited. This inhibition of contractions in 0 Ca^{2+}solutionwas reinforced by the addition of EASt. Indeed in 0 Ca^{2+}solution EASt causes a further decrease in tone and a complete inhibition of rhythmic contractions. This suggests that EASt has an inhibitory action on intracellular Ca^{2+}. To check this hypothesis, a study with EDTA was conducted in a solution devoid of calcium. EDTA is a calcium chelator and therefore inhibits the action of extracellular calcium (Ehile *et al*., 1990). It prevents calcium to enter into the cell by blocking calcium channel (Fleckenstein, 1983). The addition of EASt to 0 Ca^{2+}solution completely inhibits persistent weak rhythmic contractions. Similarly, when EASt was added to a 0 Ca^{2+}solution+EGTA, a substance that chelates divalent cations (Kpahé, 2013), there is a complete suppression of rhythmic contractions. When the same experiment was carried out with a Ca^{2+}solutioncontaining EGTA, a substance that decreases the amount of intracellular calcium (Smith *et al*., 1984), EASt has no effect on contractions. Based on these results, it appears that *Solanum torvum*extract relaxes rabbit's duodenal smooth muscle by blocking both extracellular and intracellular calcium.

The myorelaxant properties of *Solanum torvum* on duodenal smooth muscle, basically justify the traditional use of this plant against gastrointestinal problems such as diarrhea, probably through the inhibition of intestinal motility.

Conflict of interest

The authors declare no competing interests

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