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PHARMACOLOGICAL EFFECTS OF AN AQUEOUS EXTRACT OF LEAVES OF *CASSIA OCCIDENTALIS* (CAESALPINIACEAE) ON *IN VITRO* CONTRACTIONS OF ISOLATED RABBIT DUODENUM

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SUMMARY

This study aims to evaluate the potential pharmacological effects of *Cassia occidentalis* (Caesalpiniaceae) on duodenal contractions, to justify its use in traditional medicine to treat constipation. Thus, EACo contains both myostimulant and myorelaxant compounds whose effects are expressed as a function of the dose. These pharmacological effects would be due to the presence of sterols and polyterpenes, polyphenols, flavonoids, catechic tannins, alkaloids and saponins highlighted in EACo by the phytochemical screening carried out. The aqueous extract of *Cassia occidentalis leaves* causes between 10^{-2} mg/ mL and 3 mg/ mL an increase in rhythmic contractions of intestinal smooth muscle. These positive inotropic effects antagonized by Atropine (10^{-3} mg/ mL) are totally abolished in poor and calcium-free medium ($0Ca^{2+}$, $0Ca^{2+}$ + EDTA (10^{-5} M) and $0Ca^{2+}$ + EGTA (10^{-5} M)). These results suggest the presence of cholinomimetic substances in the crude extract. These cholinomimetic substances would promote the entry of calcium into the smooth muscle cell by stimulating muscarinic receptors. These substances would justify the traditional use of this plant as a laxative or purgative.

KEYWORDS: *Cassia occidentalis*, myostimulants, choliomimetics.

INTRODUCTION

Plants have long been a very important source of medicines for several diseases (**Méité, 2010**). *Cassia occidentalis* is well known in traditional medical practice. This plant is native to the tropical and subtropical areas of America and is widespread throughout the tropics (**CFP, 2015**). In West Africa and particularly in Senegal and Ivory Coast, it is found around villages and in fields where it can be a formidable weed of crops. According to **Pousset (2006)**, *Cassia occidentalis* (Caesalpiniaceae) is very commonly used in Senegal, in rural areas for its purgative properties. This plant is widely used in traditional medicine, particularly for its analgesic, antifungal, anti-inflammatory and antipyretic properties **(Aké-Assi, 2011).** In Côte d'Ivoire, *Cassia occidentalis* leaves are used in the treatment of high blood pressure and diabetes **(Tra-Bi *et al.*, 2008)**. According to **Fassassi (2010)**, this plant is widely used in traditional medicine, particularly for its antispasmodic properties. It is also used in traditional medicine to treat constipation (**Bekro *et al.*, 2013**). The macerate of *Cassia occidentalis leaves* is given to asthma patients to drink **(Kanté, 1982, Kouakou *et al.*, 2009 and Irié-N'guessan *et al.*, 2010)**. This plant is one of the stimulants of smooth muscle. It would cause a pronounced stimulation that would promote contractions at the time of childbirth; hence its contraindication in pregnant women in modern medicine **(Kerahoro, 1974; Fassassi, 2010).** The objective of this study is to highlight the potential pharmacological effects of *Cassia occidentalis* (Caesalpiniaceae), a plant of the Ivorian pharmacopoeia, in the treatment of constipation.

I- MATERIAL AND METHODS

I.1-Material

I.1-1-Animal material

Rabbits, of the *Oryctolagus species cuniculus* (Leporidae), weighing between 1.8 and 2.5 kg, are used to study isolated intestinal contractions. These rabbits come from breeding farms located in the commune of Bingerville (Côte d'Ivoire).

I.2-Physiological solutions and pharmacodynamic substances

I.2.1- Mac Ewen's physiological solutions

The physiological solution used is Mac Ewen glucose solution, at pH = 7.4. It is made up of NaCl, KCl, $CaCl_2$, PO_4HNa, CO_3HNa, $MgCl_2$.

Glucose (2 g/l) is dissolved in Mac Ewen's solution, just before its use for the study of intestinal contractions, in order to avoid fermentation.

I.2.2- Pharmacodynamic substances

The pharmacodynamic substances used are

- Atropine (PROLABO, France), a competitive inhibitor of acetylcholine and cholinergic agonists. It is used to block muscarinic cholinergic receptors.
- EDTA and EGTA (SIGMA, USA), calcium chelators.

I.3- Preparation of the aqueous extract of *Cassia occidentalis leaves* (Caesalpiniaceae)

The method of preparation of the aqueous extract of *Cassia occidentalis leaves* is that described by certain traditional practitioners from central Ivory Coast and by **Abdoul (2022)**.

Cassia *occidentalis* leaves are dried for 15 days, in a room, at room temperature of 30 °C, then crushed using a grinder (RETSCHGM-300, Germany). One hundred and twenty grams (120 g) of leaf powder are used in decoction for 20 minutes in 2 liters of distilled water. The decoction obtained is filtered twice on cotton wool and once on Whatman filter paper N°2. The collected filtrates are dried in an oven (Vacutherm Vacuum Oven, France) at 40 °C for 72 hours. After evaporation, a dry pellet is collected, then crushed in a porcelain mortar to obtain a fine water-soluble powder which is the aqueous extract of *Cassia occidentalis leaves* (EACo).

This extract (EACo) is used for toxicity studies, for phytochemical screening and for pharmacological studies.

I.4- Characterization of the main chemical constituents of *Cassia occidentalis* (Caesalpiniaceae)

Detection of secondary metabolites in EACo **is** performed by tube tests, using appropriate reagents (**Wagner and Bladt, 2001**), according to the techniques described by **Abo (2013)** and **N'guessan (2019)**.

The detection of sterols and terpenes was carried out using the Liebermann reaction. The characterization of compounds belonging to the polyphenol group is carried out using the ferric chloride reaction. Compounds belonging to the flavonoid group are detected by the cyanidin reaction. Compounds belonging to the tannin group are detected by the Stiasny reaction. The search for saponosides is based on the property of aqueous solutions containing saponosides to foam after stirring. The search for alkaloids is carried out using general reagents for the characterization of alkaloids. Two reagents are used, namely the Dragendorff reagent (potassium iodo -bismuthate reagent) and the Bouchardat reagent (iodo-iodide reagent).

I.5- Recording of pendulum movements of the isolated intestine

I.5.1- Experimental device

The experimental device consists of an isolated organ tank containing the physiological liquid (glucose Mac Ewen) which is immersed in a thermostated water bath, maintained at a constant temperature of 38 °C, and oxygenated using an aquarium bubbler. The perfusion liquid bottle is placed at a height of 40 cm above the apparatus and is used to supply the isolated organ tank with glucose Mac Ewen.

Before reaching the organ tank, the reference liquid passes through a polyvinyl catheter, then a coil immersed in a thermostatically controlled water bath which allows this liquid to warm up and maintain the same temperature of 38 °C. This arrival of liquid is controlled by a central tap. A lever system carrying a recording stylus, balanced by a counterweight, allows the recording of the contractile movements of the isolated intestine on recording paper fixed to the cylinder, coated with soot, driven in rotary motion, at constant speed, by a motor.

I.5.2- Collection and placement of the duodenal fragment

After a 24-hour fasting period, the rabbit is sacrificed by cervical dislocation. Following a laparotomy, fragments of duodenum approximately 3 cm in length are removed and stored in a petri dish containing glucose-rich, oxygenated Mac Ewen solution maintained at a temperature of 38 °C. Using a thread passed through the wall of a fragment of duodenum, a knot is made at one end, allowing the fragment to be fixed to the bottom of the isolated organ tank containing the glucose-rich, oxygenated Mac Ewen solution maintained at a temperature of 38 °C.

II.5.3- Experimental protocol

Using a graduated syringe, a volume of the dilutions made from the EACo is introduced into the isolated organ tank, a cup containing 250 ml of glucose Mac Ewen. To move from one test to the next, the preparation is washed carefully with Mac Ewen (750 ml) in order to avoid the cumulative effect of the test substances.

We performed interactions between EACo and atropine at increasing concentrations in order to see the antagonistic effect or not of atropine on the extract. Thus, 30 s after injection of atropine at a given concentration, the tested product is injected.

EDTA is used to chelate residual calcium from the normal physiological medium devoid of calcium ($0Ca^{+2}$) while EGTA to chelate intracellular calcium. These substances used, are directly added to the organ survival solution using a graduated syringe.

II RESULTS AND DISCUSSION

II.- Dose-response effects of aqueous extract of *Cassia occidentalis leaves* (EACo) on contractions of isolated rabbit duodenum

Figures **1** and **2** show, respectively, the increases in the amplitude of rhythmic contractions of the isolated rabbit duodenum (**Figures 1)** and in the basal tone **(Figures 2)** induced by EACo.

Figure 1 shows typical recordings of the effects of aqueous extract of *Cassia occidentalis leaves* (EACo) on contractions of isolated rabbit duodenum, for concentrations ranging from 10^{-2} to 3 mg/ mL.

The aqueous extract of *Cassia occidentalis leaves*, for concentrations ranging from 10^{-2} to 2 mg/ mL, causes a dose-dependent increase in the amplitude of rhythmic contractions of the duodenum. These increases in rhythmic contraction amplitude for EACo concentrations of 10^{-2} mg/ mL, 10^{-1} mg/ mL, 1 mg/ mL, and 2 mg/ mL were 0.70 ± 0.05 gF to 0.93 ± 0.06 gF ($p < 0.05$), 0.70 ± 0.04 gF to 1.04 ± 0.05 gF ($p < 0.05$), 0.70 ± 0.05 gF to 1.64 ± 0.06 gF ($p < 0.01$), and 0.71 ± 0.03 gF to 1.64 ± 0.05 gF ($p < 0.01$), respectively. For the EACo dose of 3 mg/ml, the increase in the amplitude of contractions increased from 0.79 ± 0.03 gF to 1.14 ± 0.05 gF ($p < 0.05$).

Figure 2 shows recordings of EACo concentrations ranging from 10^{-2} to 3 mg/ mL, the basic tonus also increases in a dose-dependent manner. These tone increases are 1.25 ± 0.15 mN, 1.43 ± 0.12 mN, 5.36 ± 0.18 mN, 8.51 ± 0.15 mN and 12.14 ± 0.24 mN for EACo concentrations of 10^{-2} mg/ mL, 10^{-1} mg/ mL, 1 mg/ mL**,** 2 mg/ mL and 3 mg/ mL respectively.

For these two parameters, the 50% effective concentrations (EC_{50}) determined for EACo are 0.1 mg/ mL and 1.29 mg/ mL respectively.

EACo concentrations below 10^{-2} mg/ mL have no significant effects ($p > 0.05$) on duodenal contractions.

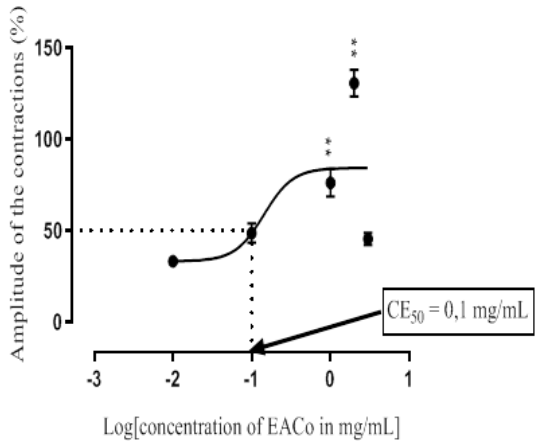

Figure 1: Increases in the amplitude of rabbit intestinal contractions as a function of EACo concentration.
n = 3; *p < 0.05; **p < 0.01 compared to control recordings.

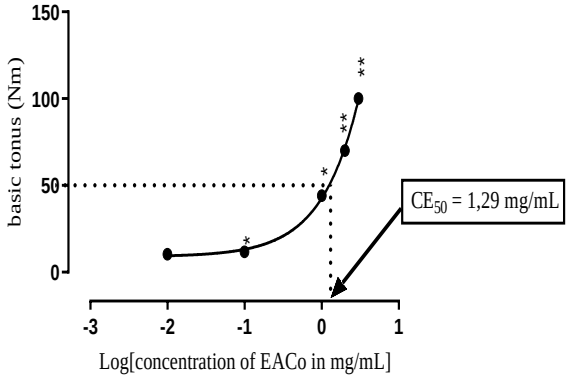

Figure 2: Increases in baseline tone of rabbit intestinal contractions as a function of EACo concentration.
n = 3; * p < 0.05; **p < 0.01 compared to control recordings

III- Interaction of aqueous extract of *Cassia occidentalis leaves* (EACo)-atropine on contractions of isolated rabbit duodenum

Cholinomimetic substances in EACo, the effects of this extract are evaluated in the presence of atropine, a specific inhibitor of muscarinic cholinergic receptors.

Figures **3 and 4** show, respectively, the effects of atropine at different concentrations on the increase in amplitude of rhythmic contractions and on the increase in basal tone induced by EACo at 3 mg/ mL.

EACo, at a concentration of 3 mg/ mL, induced an increase in baseline tone of 11.17 ± 0.23 mN and an

increase in the amplitude of spontaneous rhythmic contractions of the duodenum of 44.44 ± 8.1% (**Figures 3 and 4)**.

When EACo at a dose of 3 mg/ mL was added 1 minute later in the physiological medium containing 10^{-6} mg / mL of atropine, the basic tone decreased from 11.17 ± 0.23 mN to 5.67 ± 0.12 mN; a significant decrease ($p < 0.01$) of 49.24 ± 4.84% compared to the effect of EACo alone , and the amplitude of the contractions increased by 37.5 ± 5.21%, which represents a decrease in the effect of EACo on the amplitude of 15.62 ± 1.84% ($p < 0.05$) compared to that of this extract alone. **(Figure 3)**.

Atropine at 10^{-5} mg/ mL and 10^{-4} mg/ mL, when added to the physiological medium before EACo at 3 mg/ mL, resulted in highly significant decreases ($p < 0.001$) in the effects of this extract on the basic tone of 64.19 ± 4.15% and 79.14 ± 4.60% respectively and insignificant increases ($p < 0.05$) in the amplitude of contractions of 33 ± 5.51% and 25 ± 5.21% respectively. Indeed, in the presence of atropine at 10^{-5} mg/ mL and 10^{-4} mg/ mL, EACo resulted in increases in baseline tone of 4 ± 0.15 mN and 2.33 ± 0.13 mN, respectively, accompanied by increases in contraction amplitude from 0.50 ± 0.02 gF to 6.67 ± 0.05 gF and from 5.67 ± 0.03 gF to 6.67 ± 0.04 gF, respectively. **(Figure 4)**.

When EACo at a concentration of 3 mg/ mL is added 1 min later in the physiological medium containing atropine at 10^{-3} mg/ mL, the basic tonus increases very slightly and transiently (about 10 s) to the value of 1.33 ± 0.15 mN; i.e. 88.09 ± 2.66% reduction in the increase in tone induced by EACo added alone, then a return of tone to normal. On the other hand, the amplitude of rhythmic contractions increases in a non-significant manner ($p > 0.05$) by 2.78 ± 0.36%, then returns to normal **(Figure 4)**.

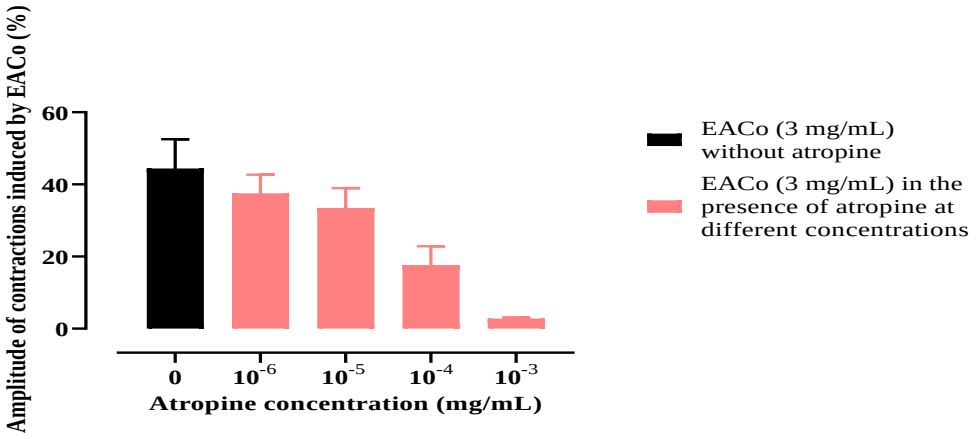

Figure 3: Effects of atropine on the increase in the amplitude of rhythmic contractions of isolated rabbit duodenum induced by EACo at 3 mg/ mL.

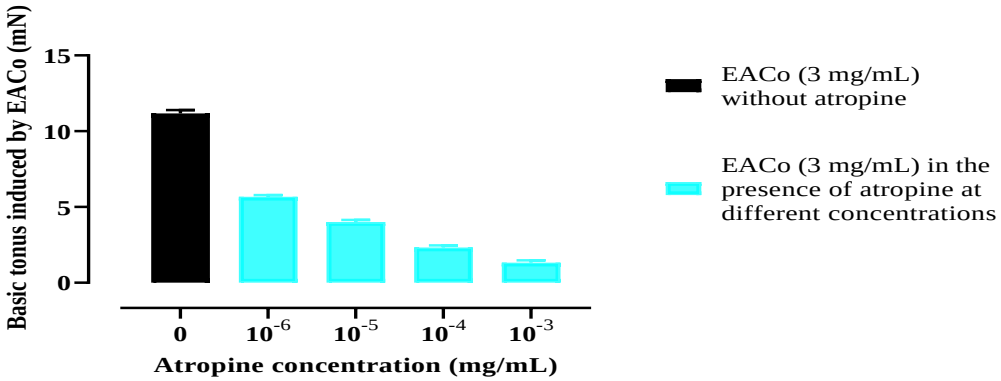

Figure 4: Effects of atropine on the increase in basal tone of isolated rabbit duodenum induced by EACo at 3 mg/ mL.

IV- Effect of EACo on contractions of the isolated rabbit duodenum in calcium-free medium (0 Ca^{2+}) and containing or not EDTA and/or EGTA

To better appreciate the effect of calcium on myostimulation induced by EACo, this extract is tested on the duodenum in a physiological environment devoid of calcium (Ca^{2+}).

Figure 5A is a typical recording of the effects of EACo at 3 mg/ mL on contractions of isolated rabbit duodenum. At this concentration, the effects of EACo

are reflected by an increase in the amplitude of spontaneous rhythmic contractions of 45 ± 2.8% and in the basal tone of 11.67 ± 0.72 mN.

In a physiological medium devoid of calcium, the amplitude of rhythmic contractions of the intestine decreased very significantly ($p < 0.001$) by 75.03 ± 6.85%, from 8.33 ± 0.12 gF to 2.08 ± 0.08 gF. After the addition of EACo (3 mg/ mL) in this medium, rhythmic contractions of the intestine increased significantly ($p < 0.05$) and increased from 2.08 ± 0.08 gF to 6.67 ± 0.14 gF; or 220.67 ± 15.58% increase, and the basic tonus increases to 8.33 ± 0.28 mN, then decreases to maintain with an increase of 1.67 ± 0.18 mN (**Figure 5B**).

Figure 5C shows a typical recording of the effect of EACo (3 mg/ mL) on rhythmic contractions of the intestine in a physiological medium devoid of calcium (0 Ca^{2+}), EDTA at 10^{-5} mg / mL has been added. In 0 Ca^{2+} EDTA (10^{-5} mg/ mL), rhythmic contractions of the intestine are completely abolished. One (1) minute after the addition of EACo (3 mg/ mL) to this medium, rhythmic contractions resume, the amplitude of which gradually increases to stabilize at a value equal to 3.75 ± 0.05 gF, but the tone remains stable ($p > 0.05$).

Figure 5D represents the typical recording of the effect of EACo (3 mg/mL) on rhythmic contractions of the intestine in normal physiological medium devoid of calcium (0 Ca^{2+}) to which EGTA at 10^{-5} mg/mL has been added. In 0 Ca^{2+} medium containing EGTA (10^{-5} mg/mL), rhythmic contractions of the intestine are completely inhibited. Two (2) minutes after the addition of EACo (3 mg/mL) in this medium, there is a resumption of rhythmic contractions whose amplitude increases progressively to stabilize at a value equal to 8.33 ± 0.16 gF. The resumption of rhythmic contractions of the intestine, in the physiological medium devoid of calcium (0 Ca^{2+}) and containing EGTA (10^{-5} mg/mL) with EACo (3 mg/mL), is later and the increase in the amplitude of the contractions is greater compared to those of the 0 Ca^{2+} medium containing EDTA (10^{-5} mg/mL) with EACo (3 mg/mL).

In 0 Ca^{2+} medium containing EDTA (10^{-5} mg/mL) and EGTA (10^{-5} mg/mL), rhythmic contractions of the intestine remain totally inhibited when EACo (3 mg/mL) is added (**Figure 5E)**.

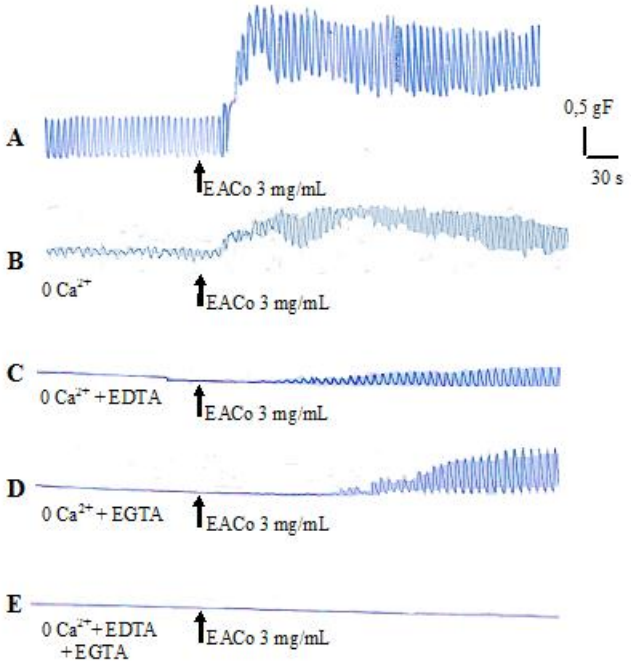

Figure 5: Effects of EACo on contraction of the rabbit duodenum in a physiological medium devoid of calcium (0 Ca^{2+}) and containing or not EDTA and/or EGTA.

A: Control recording (before the arrow) and effects of EACo at 3 mg/ mL on intestinal contractions.
B to **E:** Control recordings (before arrows) and effects of EACo at 3 mg/ mL on intestinal contractions (after arrows) in 0 Ca^{2+} (**B**), 0 Ca^{2+} + EDTA 10^{-5} mg / mL (**C**), 0 Ca^{2+} + EGTA 10^{-5} mg / mL (**D**) and 0 $Ca2^{+}$ + EDTA 10-5 mg/ mL + EGTA 10^{-5} mg/ mL (**E**).

V- DISCUSSION

The pharmacological study allowed to evaluate the effects of the aqueous extract of *Cassia occidentalis* (EACo) on the "*in vitro*" contractions of rabbit duodenal smooth muscle.

The results of the dose-response study show that, for low concentrations (10^{-2} to 10^{-1} mg/ mL), EACo induces a dose-dependent increase in the amplitude of rhythmic

contractions of the duodenum. The 50% effective concentration (EC_{50}) is equal to 0.1 mg/ mL. For doses of EACo from 10^{-1} to 3 mg/mL, a dose-dependent increase in basal tone also appears, and the EC_{50} is equal to 1.29 mg/ mL in this case.

This positive inotropic effect induced by EACo is comparable to the effects of aqueous extracts of *Swartzia madagarensis* (Cesalpiniaceae) and *Mareya micrantha* (Euphorbiaceae) on rabbit intestinal contractile activity (**Traoré *et al.*, 2004)**. A similar result was also highlighted on the contractions of guinea pig Taenia coli with *Bridelia ferrugina* (Euphorbiaceae) by **Néné Bi *et al.* (2009)**, with *Sesamum radiatum* (Pedaliaceae) by **Konan *et al.* (2011)** and with *Morinda morindoides* (Rubiaceae) by **Gboko (2014**).

Dose-response curves showing the effects of EACo on duodenal contractions as a function of the logarithm of the concentration of this extract have sigmoidal shapes. This, on the one hand, confirms that the effect of EACo is dose-dependent and, on the other hand, suggests that its activity could be mediated by the activation of cholinergic receptors **(Miller and Tainter, 1944)**.

The effects of EACo are similar to those of acetylcholine and substances containing cholinergic-like compounds **(Bolton, 1981; Ochillo *et al.*, 1981)**. Indeed, **Dosso *et al.* (2012)** showed the presence of cholinergic-type substances in the aqueous extract of *Mareya micrantha* (Euphorbiaceae) which induces myostimulation of the isolated rabbit duodenum.

Cholinomimetic substances in EACo, the effects of this extract are evaluated in the presence of atropine, a specific inhibitor of muscarinic cholinergic receptors. Indeed, atropine is a non-selective antagonist that blocks all subtypes of muscarinic receptors **(Bolton, 1981; Peralta *et al*. 1988)**.

EACo -Atropine antagonism indicates that the increase in rhythmic and tonic contractions of the intestine induced by EACo at 3 mg/ mL is reduced in a dose-dependent manner by atropine (10^{-6} to 10^{-4} mol / mL) and, for the dose of 10^{-3} mol / mL of this antagonist, the effects of EACo are almost totally inhibited (on tone) or inhibited (on amplitude). This result shows that EACo indeed contains muscarinic cholinomimetic substances responsible for the increase in contractions of rabbit intestinal smooth muscle.

Acetylcholine is the major excitatory neurotransmitter in the enteric nervous system **(Waterman et *al.*, 1994)**. Thus, the presence of cholinomimetic constituents in EACo may explain the usefulness of this plant in the treatment of constipation.

The cholinomimetic substances of EACo would act by stimulating the M3 receptors linked to G $proteins_{q/11}$ and would activate phospholipase C (PLC) which releases two molecules: diacyl glycerol (DAG) and inositol phosphate (IP3). IP3 causes the exit of calcium from the endoplasmic reticulum and DAG induces the opening of non-selective ion channels, two modifications which would be at the origin of the entry of calcium into the smooth muscle cell and/or the release of calcium from intracellular reserves and therefore an increase in the cytosolic calcium level responsible for the positive inotropic and tonotropic effects of the intestinal smooth muscle (**Bolton, 1981; Hurwitz, 1986; Ehile *et al.*, 1990; Dosso, 2014**). Indeed, the increase in cytosolic calcium is followed by the binding of Ca^{2+} to calmodulin (Cam) which associates with myosin light chain kinase (MLK) to increase its activity in the hydrolysis of adenosine triphosphate (ATP). Phosphorylation of the heads of the MLK leads to the sliding of actin filaments (contraction) accompanied by an increase in ATP hydrolysis **(Bolton, 1981; Ehile *et al.*, 1990; N' guessan, 2019)** responsible for increasing the contraction of the intestinal muscle.

Similar results are indicated by **Goueh *et al.* (2009)** with the aqueous extract of *Trema leaves guineensis* (Umaceae) and by **Méité *et al.* (2010)** and **Dosso (2014)** with aqueous extracts of leaves of *Mareya micrantha* (Euphorbiaceae), from tests carried out on rabbit duodenum.

To better appreciate the effect of calcium on myostimulation induced by EACo, this extract is tested on the duodenum in a physiological medium devoid of calcium (Ca^{2+}). In an extracellular medium devoid of calcium (0 Ca^{2+}), the rhythmic contractions of the duodenum are strongly reduced. In such a physiological medium 0 Ca^{2+}, EACo has myostimulant effects which result in an increase in rhythmic contractions and the basic tone of the duodenum. EACo would therefore act on extracellular and/or intracellular Ca^{2+}.

To verify the probable action of EACo on extracellular Ca^{2+}, a study of the effects of this extract is carried out in a medium devoid of calcium (0 Ca^{2+}) and containing ethylene diamine tetra-acetic acid (EDTA). In medium 0 Ca^{2+} + EDTA, the rhythmic contractions of the duodenum are totally inhibited. EDTA is a calcium chelator and therefore renders extracellular calcium ineffective **(Perry, 1974; Godfraind *et al.*, 1986)**. It prevents the entry of calcium into the cell by blocking calcium channels **(Fleckenstein, 1983; Godfraind *et al.*, 1986)**, which results in a complete inhibition of intestinal contractions. The addition of EACo in such a medium (0 Ca^{2+} + EDTA) causes a resumption of rhythmic intestinal contractions.

The same experiment was repeated with ethylene glycol tetraacetic acid (EGTA), a substance that chelates divalent cations **(Godfraind *et al.*, 1986; Fleckenstein, 1993)**, leading to a decrease in the amount of intracellular calcium **(Smith *et al.*, 1984)**, also induces the abolition of rhythmic contractions of the intestine

when this substance is tested alone in calcium-free medium (0 Ca^{2+}). The addition of EACo to this medium (0 Ca^{2+} + EGTA), leads to a resumption of rhythmic contractions with greater contraction amplitudes than in the study with EDTA, but with a later effect.

When the calcium-free medium contains EDTA and EGTA, contractions are completely inhibited, even after the addition of EACo.

These results indicate that EACo stimulates rabbit duodenal smooth muscle by mobilizing both extracellular and intracellular calcium to increase the amplitude of duodenal smooth muscle contractions.

VI- CONCLUSION

The study of the effects of the aqueous extract of *Cassia occidentalis leaves* reveals that this extract induces a dose-dependent increase in rhythmic and tonic contractions of the isolated rabbit duodenum. These positive inotropic and tonotropic effects would be related to the presence of muscarinic cholinomimetic substance in this extract. EACo would also act on the rabbit duodenal smooth muscle by mobilizing both extracellular calcium and intracellular calcium.

The myostimulant properties of *Cassia occidentalis* on duodenal smooth muscle explain its excitatory effects on gastrointestinal motility and therefore justify its use in traditional medicine against gastrointestinal problems, particularly against constipation.

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