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SPASMOGENIC EFFECT OF THE AQUEOUS EXTRACT OF *TAMARINDUS INDICA L.*  
(CAESALPINIACEAE) ON THE CONTRACTILE ACTIVITY OF GUINEA-PIG TAENIA COLI.

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### Abstract

The effect of aqueous extract of *Tamarindus indica* (AETI) was studied on the guinea pig taenia coli, due to its use for treatment of constipation in traditional medicines. AETI, at concentrations ranging from 10<sup>-8</sup> mg/ml to 10<sup>-2</sup> mg/ml, increased the spontaneous contractile activity of guinea pig taenia coli in a dose-dependent manner (EC50= 4x10<sup>-6</sup> mg/ml). This activity was unaffected by atropine. In high K<sup>+</sup>, Ca<sup>2+</sup>-free solution containing EDTA, AETI as well as acetylcholine, used as a control, induced tonic contraction. These results suggest that the plant extract exert a spasmogenic effect that would not involve cholinergic mechanism of action. However, these active principles could mobilize both extra cellular calcium and intracellular calcium from internal stores.

**Key words:** Contractions, Smooth muscle, Taenia coli, *Tamarindus indica*

### Introduction

*Tamarindus indica* (Caesalpinaceae) is a plant widely used in traditional medicine in Africa for the treatment of many diseases such as fever, dysentery, jaundice, gonococci and gastrointestinal disorders (Kheraro and Adam, 1974; Kobayashi et al., 1996; Ferrara, 2005). Pharmacological investigations on *Tamarindus indica* extracts reported them to have antibacterial, antifungal (Pousset, 1989), hypoglycaemic, cholesterolemic (Nabawyia et al., 1997), cytotoxic (Kobayashi et al., 1996), anti-inflammatory (Rimbau et al., 1999), gastrointestinal (Coutino-Rodriguez et al., 2001), hypolipomic and antioxidant activities (Ferrara, 2005; Martinello et al., 2006).

Phytochemical investigations carried out on *Tamarindus indica* revealed the presence of many active constituents such as phenolic compounds, cardiac glycosides (Rasu et al., 1985); L(-) malic acid (Kobayashi et al., 1996), tartaric acid, pectin and mucilage (Ibrahim et al., 1995; Cuutino-Rodriguez et al., 2001). The fruits and leaves appear to be the plant's most used organs in folk medicine. However, in Ivory Coast, barks of stems are also used in the treatment of haemorrhoid and chronic constipation.

Based on this current usage, we examined the effects of an aqueous extract of *Tamarindus indica* and its mechanisms of action on the contractile activity of the guinea-pig taenia coli.

### Materials and methods

#### Plant material

The aqueous extract was prepared from the stem bark of *Tamarindus indica L.* (Caesalpinaceae) authenticated by Prof. A. L. Ake, an expert botanist (Department of Botany, University of Cocody, Abidjan). The material was collected in February 2002. Voucher specimen number 20834 has been preserved, catalogued and deposited in the "Centre National de floristique" of Cocody University, Abidjan, Côte d'Ivoire.

### Preparation of the aqueous extract

The bark was crushed into small pieces and macerated in n-hexane with a magnetic stirrer for 24 h. to remove substances soluble in hexane such as, chlorophyll, oils, terpenoids. The supernatant was then removed and the solid remainder was totally dried. The dried part was extracted with distilled water for 24 h. The extract obtained was filtered on cotton and Wattman paper. The solvent was evaporated using Rotavapor BUCCHI. The residue, soluble in water, was labelled "aqueous extract of *tamarindus indica*"(AETI) and hold at 5°C).

### Animals

Guinea-pigs (*Cavia porcellus*) of both sexes, weighing 300-500g, were obtained from the Laboratory of Physiology (University of Cocody). They were kept at 25 -30°C with free access to food and water. 24 h prior to the experiments, they were deprived of food.

All experiments were carried out in accordance with Ivory Coast government rules for biomedical research involving animals.

### Evaluation of pharmacological activity in guinea-pig taenia coli.

Animals were sacrificed by cervical dislocation. Taenia coli was removed and put in a Petri box containing the physiological solution of Mac Ewen [composition (mM): NaCl, 130; KCl, 5.6; CaCl<sub>2</sub>, 2.6; NaH<sub>2</sub>PO<sub>4</sub>, 0.91; NaCO<sub>3</sub>H, 11.9; MgCl<sub>2</sub>, 0.24; glucose, 11].

A portion of taenia coli of about 6 mm in length was suspended in 20ml tissue bath (organ bath LAUDA E 100) containing the Mac Ewen physiological solution continuously bubbled with air and maintained at 37°C. One end was attached to an organ holder in the bottom of the organ bath and the other one was attached to a transducer (F30 HSE 372) under a resting tension of 1g. Contractile responses were displayed on a Rikadenki two-channels diagram recorder. The organ was allowed to equilibrate at least 90min before the addition of the plant extract or any drugs.

Concentration–response curve was obtained by cumulatively adding increasing concentrations of the extract (10<sup>-8</sup>-10<sup>-2</sup>mg/ml) in the organ bath. The maximum effect obtained from the cumulative concentrations was taken as the 100% response value. Additional experiments using a single efficient concentration were performed to examine the effect of the plant extract.

### Drugs

The drugs used: Acetylcholine(ACh), Atropine(ATR) and Ethylene Diamine Tetraacetic Acid (EDTA), from Sigma Chemical Co.(St. Louis, MO, USA).

### Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) obtained from n separate experiments. Values were analysed using One-way Analysis of Variance (ANOVA) followed by the Dunnett Multiple Comparisons Test and were considered to differ significantly when P < 0.05.

### Results and Discussion

At rest, the isolated segments of the guinea-pig taenia coli contracted spontaneously ((732±33.19 mg wt.). Figure 1a shows that the aqueous extract of *tamarindus indica*, in ranging concentrations of 10<sup>-8</sup>-10<sup>-2</sup> mg/ml, exhibited a concentration-dependent spasmogenic effect, characterized mainly, by an increase of the spontaneous contractile amplitude, which were also associated with a small increase of basal tone as shown in Figure 1b. Indeed, the magnitude of spontaneous contractions evoked by the plant extract increased significantly from 29.45±4.72% (P<0.05, n=5) to 88.116±2.20% (P < 0.01) at concentrations of 10<sup>-7</sup>mg/ml and 10<sup>-6</sup>mg/ml respectively.

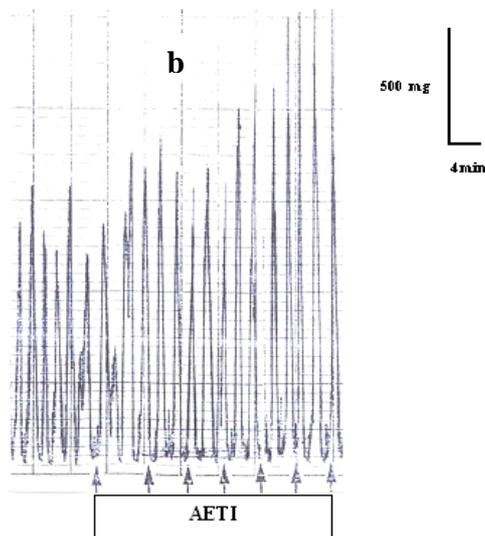
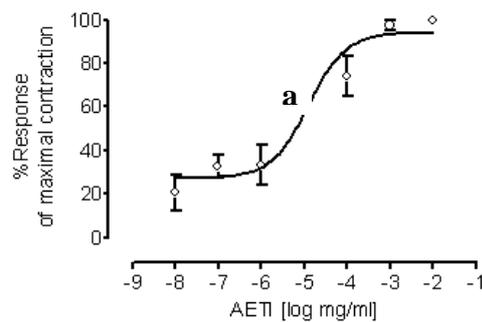
Acetylcholine, a well known neurotransmitter; regulates the peristaltic movements of the gut via contraction through muscarinic receptors (Brown and Taylor, 1996). Then, we examined whether the contractile responses of the plant extract were mediated by cholinergic mechanisms. With acetylcholine as a control drug, the plant extract was challenged in the presence and in the absence of atropine, a cholinergic receptors antagonist (Neal, 1997). Table 1 shows that the contractile responses induced by acetylcholine (10<sup>-6</sup>mg/ml) were

**Table 1:** Effects of acetylcholine ( $10^{-6}$  mg/ml) and the aqueous extract of *Tamarindus indica* ( $10^{-4}$  mg/ml) on the contractile activity of the guinea-pig taenia coli in the absence or in the presence of atropine ( $10^{-5}$  mg/ml). (n=4).

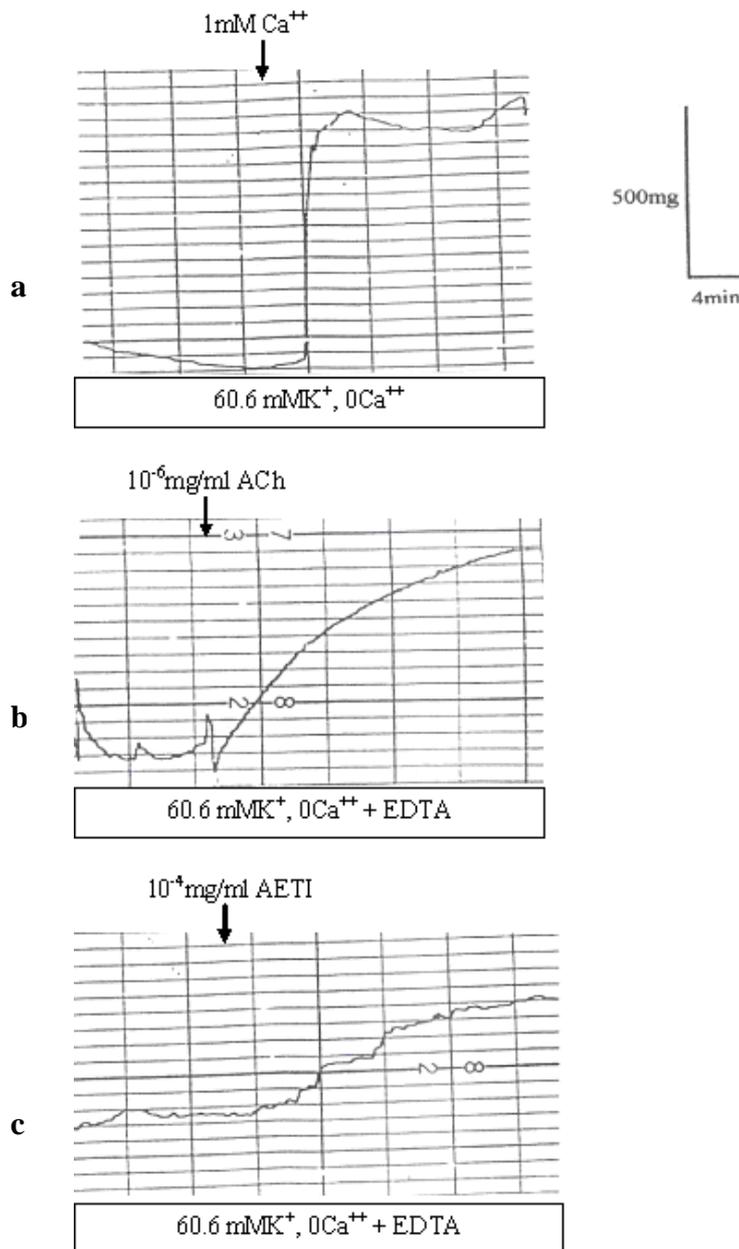
	Control	ACh	ACh-ATR	AETI	AETI-ATR
Contractile Force (mg, wt)	1076.2±25.2	1428.7±33 <sup>***</sup>	1204.7±41 <sup>*</sup>	-	-
Augmentation of contractions Amplitude (%)	850±26.40	32.74±5.31	12.74±3.80 <sup>††</sup>	40.05±1.50	38.4.1±1.00

<sup>\*</sup>P<0.05, <sup>\*\*</sup>P<0.01, <sup>\*\*\*</sup>P<0.001 vs. control.

<sup>††</sup>P<0.01 vs. effect of drug in the absence of atropine.



**Figure 1:** Effects of aqueous extract of *Tamarindus indica* (AETI) on the guinea-pig contractile activity. (a) concentration-contraction curve to AETI in smooth muscle of guinea-pig taenia coli. Each point value represents the mean of 6 different preparations and the vertical bars represent S.E.M. (b) recordings of contractions evoked by increasing concentrations of AETI ( $1 \times 10^{-8}$ ;  $1 \times 10^{-7}$ ;  $1 \times 10^{-6}$ ;  $1 \times 10^{-5}$ ;  $1 \times 10^{-4}$ ;  $1 \times 10^{-3}$ ;  $1 \times 10^{-2}$  mg/ml). Concentrations of the plant extract were applied every 4 minutes. Horizontal scale, 4min.; Vertical scale, 500mg.



**Figure.2:** Extract of *Tamarindus indica* (c) on the guinea-pig taenia coli in high K<sup>+</sup> calcium-free solution (60.6mMK<sup>+</sup>, 0Ca<sup>++</sup>, 10<sup>-5</sup> mM EDTA). Horizontal scale, 4min.; vertical scale, 500mg.

significantly reduced by atropine (10<sup>-5</sup>mg/ml). ACh-evoked contractions were in larger magnitude when atropine was not present (32.74±5.31 vs 12.380±3.80, p<0.01, n = 5). In contrast, atropine (10<sup>-5</sup> mg/ml) failed to modify the contractile responses elicited by the plant extract (10<sup>-4</sup> mg/ml). The extract still increased the magnitude of spontaneous contractions in the absence or in the presence of atropine (40.05±1.50 vs 38.4±1.00, p>0.05) suggesting that cholinergic receptors may not be involved.

To further investigate the mechanism of action of the aqueous extract of *Tamarindus indica*, experiments were carried out in depolarizing calcium-free medium. In the guinea-pig isolated taenia coli, the spontaneous contractile activity of the guinea-pig isolated taenia was totally abolished by adding EDTA (10<sup>-5</sup>

M). As shown in Figure 2a, in these conditions, the addition of calcium (1mM) exhibited a tonic contraction to maximal amplitude of  $600 \pm 23.41$ mg. In the same way, the plant extract as well as acetylcholine elicited tonic contraction to a maximal amplitude of  $275 \pm 25.31$ mg and  $210 \pm 17.32$  respectively, as shown in Figure 2b and Figure 2c.

The present data show that the aqueous extract of *Tamarindus indica* exerted a large spasmogenic effect on the contractile activity of the guinea-pig isolated taenia coli. Atropine did not inhibit the contractile responses elicited by this plant extract, indicating that the extract did not act through cholinergic mechanisms. It is well established that the augmentation of cytosolic calcium ions rate, which is the main factor of contractile protein activity (Oashi et al., 1974; Casteels et al. 1977), results from different sources: the influx of calcium from extracellular medium (Datte et al., 1996; Nasu and Magami, 2000; Nasu, et al., 2001) and the release of calcium from intracellular stores (Grosset and Mironneau, 1977; Karaki and Mitsui, 1988; Ehile et al., 1990; Endo and Nakajima, 1973; Nilsson and Hellstrand, 1993). Furthermore, acetylcholine stimulates the contractile activity of the smooth muscle by intensifying calcium influx across the membrane and by mobilizing calcium from internal stores such as sarcoplasmic reticulum and from calcisomes, in an inositol triphosphate (IP3) dependent way (Brading et al, 1980, Lalanne et al., 1984, Gálvez et al., 2005). Our results show that, in depolarizing calcium-free medium, a small amount of calcium could induce tonic contraction and that the plant extract, as well as acetylcholine, exhibited a tonic contraction under those conditions. This observation suggested that the extract could mobilize calcium from internal stores like acetylcholine. In that regard, contractions evoked by the plant extract would depend on both the transmembrane calcium influx and the mobilization of calcium from internal stores

Based on the chemical compounds of *Tamarindus indica*, like flavonoids and cardiac glycosides which have been reported to have spasmolytic activity in isolated tissues like ileum (Ko et al, 2003), and a stimulating activity in isolated tissues like duodenum (Ehile et al., 1990) respectively, contractile responses of the stem barks extract may be attributed to the presence of cardiac glycosides like pectin and tartaric acid in fruits extract (Ibrahim et al., 1995; Cuntino-Rodriguez et al., 2001). This hypothesis might be elucidated.

Overall, barks of Stems of *Tamarindus indica* have been shown to exert a spasmogenic effect on the isolated intestinal smooth muscle of the guinea-pig taenia coli. These results may provide a rational basis to support its use in local folk medicine as a laxative for the treatment of constipation and digestive disorders.

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