SPASMOGENIC ACTIVITY OF 1-HYDROXY-3,7,8– TRIMETHOXYXANTHONE ISOLATED FROM THE METHANOL EXTRACT OF THE STEM BARK OF *ANTHOCLEISTA VOGELII* PLANCH. (LOGANIACEAE) IN RATS.

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Summary

A novel xanthone, 1-hydroxy-3,7,8-trimethoxyxanthone (AV) isolated from the methanol extract of the stem bark of *Anthocleista vogelii* Planch. produced a dose-dependent effect on the tone and force of the spontaneous contraction of the rat ileal and stomach smooth muscle fragments at concentrations ranging from 2.50 x 10^{-2} to 1.60 µg/ml. A concentration of 8.00 x 10^{-1} µg/ml of AV produced maximal contractile effect in a cumulative as well as in a single concentration. 8.00 x 10^{-1} µg/ml, atropine, reduced this spasmogenic effect induced by the compound (AV) by 80.98% on ileal fragment and by 81.84% on stomach smooth muscle fragment. Similarly, the same concentration of atropine reduced the contractile effect of acétylcholine (8.00 x 10^{-1} µg/ml) by 78.08% on the ileum and 100 % on the stomach fragments. Nifedipine, (1.60 x 10^{-2} µg/ml) and pyrilamine maleate, (6.00 x 10^{-1} µg/ml) completely inhibited the spasmogenic effect of AV in both ileal and stomach smooth muscle fragments. These results point to a possible stimulation of these muscle fragments through muscarinic receptors which increase Ca^{2+} mobilisation from both extra and intramuscular medium.

***Key words*:** 1-hydroxy-3,7,8– trimethoxyxanthone (AV) – ileum – stomach – spasmogenic activity.

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Introduction

Anthocleista vogelii Planch. is a tree of the Loganiaceae family which commonly grows around river edges and banks or in marshy areas of the tropical humid forest of West Africa (Irvine 1961), with great concentration in Cameroon and Gabon (Leewenberg,1972). This plant is 6 to 20 meters high, usually with buttrous roots, with branches having spikes, which also have sessile leaves and short petals.

The stem bark of this plant is used in African traditional medicine for the treatment of gastro-intestinal disorders, to cure fever, stomach ache and as purgative while the combination of the stem bark and the leaves is used as anti-inflammatory and anti-diabetic agents and also in the treatment of wounds (Daziel 1937).

In Cameroon, the stem bark is reported to be used to treat abdominal pains (Adjanohoun et al., 1996). Recent findings in our laboratory (unpublished work) indicated the antiulcer properties of the xanthone 1-hydroxy-3,7,8-trimethoxyxanthone (AV) isolated from the methanol stem bark extract of *Anthocleista vogelii.* There is no report in literature yet on the direct effect of this xanthone on the intestinal smooth muscle. We are reporting in the present study, the spasmogenic properties of AV on isolated gastric smooth muscle fragments and postulating the possible mechanisms of action involved

Materials and methods

Animals:

The experiments were carried out on wistar strain male adult rats of 12 to 16 weeks old and weighing between 170 to 200 g. The rats were bred in the animal house of the Department of Animal Biology, Faculty of Science, University of Dschang, Cameroon and fed on normal laboratory rat diet. Food and water were supplied *ad libitum*. Prior to the experiment, the animals were subjected to fasting for 48 hours but allowed free access to water.

Collection and preparation of plant material:

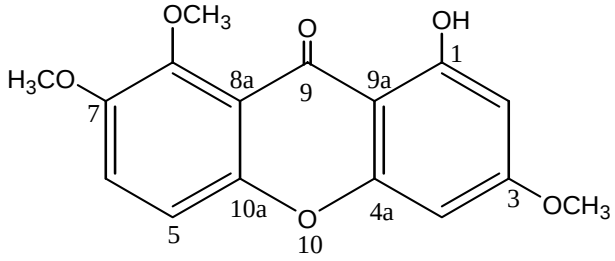
The stem bark of *Anthocleista vogelii* was collected in Bandjoun, in the West Province of Cameroon during the month of May 2002. Mr. Paul Mesili, now a retired Botanist of the Cameroon herbarium, Yaoundé**,** carried out the authentication of the plant material. A voucher specimen coded BUD 0636 was deposited at the Botany Department, University of Dschang for future reference. The collected fresh stem bark was air dried and ground into fine powder in a high speed grinding mill.

Extraction and isolation of 1-hydroxy-3,7,8–trimethoxyXanthone (AV) :

Three kilograms of the stem bark powder were soaked in 4 litters of hexane and allowed to mix up perfectly. This mixture was allowed to stand for 72 hours followed by filtration. The filtered portion was concentrated to dryness under reduced pressure in a rotary evaporator at a temperature of 70°C. This procedure produced a hexane extract of 31.6 g corresponding to an extract yield of 01.05%.

The residue hexane extract obtained was macerated in 3 litters of acetone for 72 hours and the filtrate obtained there of, was further concentrated at a temperature of 55°C to give 112.3 g of acetone extract corresponding to a 03.74% yield. The residue obtained from this second phase was further macerated in 2.5 litters of methanol for 72 hours and the filtrate obtained was concentrated at a temperature of 65°C to give 676 g of methanol extract corresponding also to 22.53% yield.

One hundred grams of the methanol extract was chromatographed on silica gel column elution performed with a gradient mixture of CH_2Cl_2-methanol. Sixty fractions of 250 ml each were collected and combined on the basis of their TLC profiles to give 10 majors fractions labelled F_1-F_{10}. These 10 fractions were tested for their antiulcerogenic properties. The ten fractions were purified on column chromatography, using hexane-CH_2Cl_2, CH_2Cl_2-acetone and acetone-methanol as eluent. Fraction 3 (F_3) afforded a yellow water soluble crystalline compound (24 mg) identified by spectroscopic means (RMN^{1}H, RMN^{13}C, Masse, IR, UV) as 1-hydroxy-3,7,8-trimethoxyxanthone (AV) which we used as the main pure isolated compound in these trials.



1-hydroxy-3,7,8-trimethoxyxanthone
($C_{16}H_{14}O_6$)

Experimental

The rats were killed by cervical dislocation. The abdomens were opened and 1 cm of ileal segments were cut and cleansed of adhering connective tissue. The segments were rinsed with warm (37 °C) tyrode solution composed as follows (mM): NaCl 137.93; KCl 2.70; $CaCl_2$ 1.83; $MgSO_4$ 0.83; $NaHPO_4$ 0.42; $NaHCO_3$ 11.90 and glucose 5.56. Stomach fragments were prepared by cutting a portion of stomach (0.5 x 1 cm) along the greater curvature. One end of the segment was attached to a hook at the bottom of an organ bath containing tyrode solution and the other end attached to a Ugo Basile isotonic transducer with the resting tension of 0,5g. Spontaneous contractile activities of these fragments were then recorded at 37 °C on a Ugo Basile Unirecorder Gemini model 7050. The organ bath was bubbled with air and the set–up allowed 45 minutes to stabilize. After a resting period of 45 minutes, with 15 minutes washout interval, four kinds of experiments were carried out on the tissues.

1- A single concentration-effect curve of the 1-hydroxy-3,7,8-trimethoxyxanthone (AV) on the spontaneous activity was established. This was done by adding, every 5 minutes, graded concentrations (2.50×10^{-2} - 1.60 µg/ml) of the AV to the tissue bath and observing their effects on the force of contraction. The effects of these concentrations on the baseline tone of the tissues were also observed.

2- Isolated rat ileums or stomach fragments were exposed to cumulative concentrations of AV (2.50×10^{-2} - 1.60 µg/ml) in order to obtain concentration-response curves.

3- In order to determine the possible mechanism of action of AV, the following protocol was used: control curves were obtained at the beginning of the experiment

when the tissues were contracted with 8.00 x 10^{-1} µg/ml of AV. This was followed by exposure to atropine (2.50 x 10^{-2} – 8.00 x 10^{-1} µg/ml), nifedipine (10^{-2} – 8.00 x 10^{-2} µg/ml) and pyrilamine maleate (7.50 x 10^{-2} – 6.00 x 10^{-1} µg/ml) for 5 minutes after the addition of AV. The response of AV on the contractions elicited by the three antagonists was recorded for 5 minutes and the corresponding effect was determined by comparing the average amplitude of ileum or gastric contractions with the amplitude of contraction obtained when the antagonists alone were added to the bath.

4- In the last set of experiments, the effect of AV was studied in a free calcium medium. This Ca^{2+}-free medium was obtained by omitting $CaCl_2$ from the reference tyrode solution. The results were expressed as force of contraction of the internal standard Ca^{2+}-contraction.

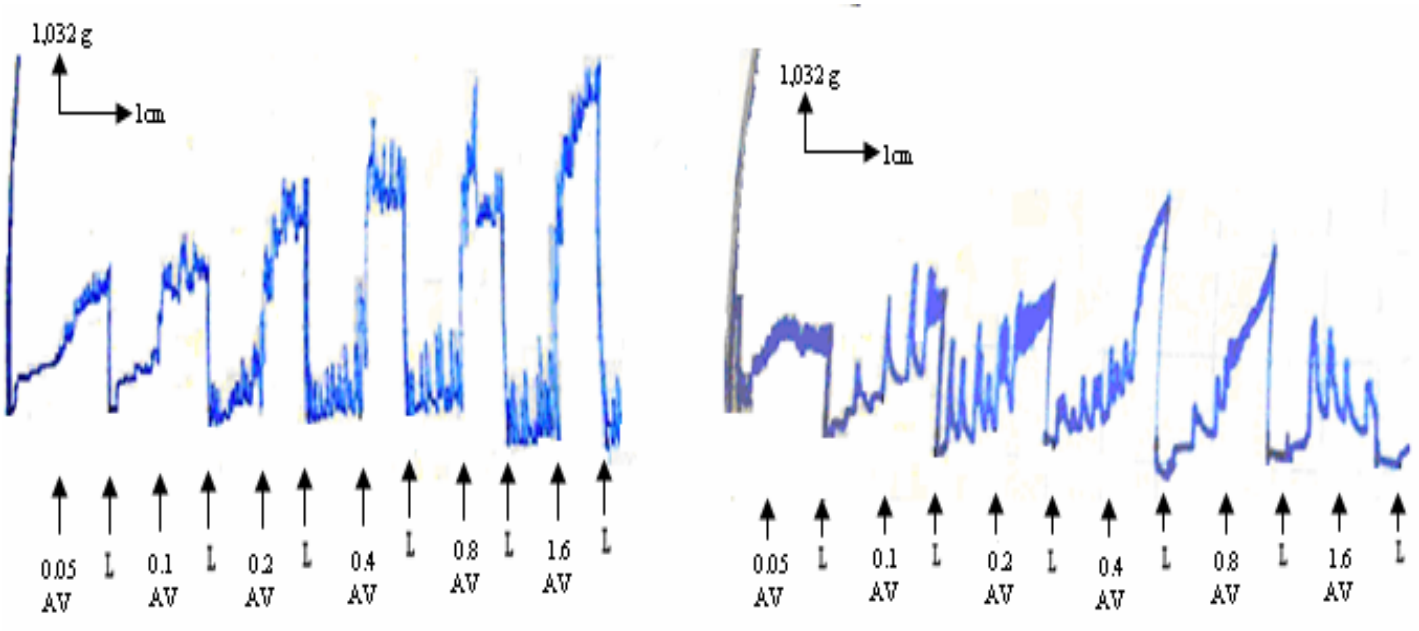
Statistical analysis:

Statistical analysis was performed using ANOVA and Duncan's test and significance of difference between treatment was accepted at $p<0,05$. Data were expressed as mean ± standard error on the mean.

Results

Effect of AV on rat ileum: 1-hydroxy-3,7,8–Trimethoxyxanthone (AV) (2.50 x 10^{-2} – 1.60 µg/ml) produced a significant ($p<0.001$) concentration – dependent increase of spontaneous rat ileal contractions (figure 1). The force of contraction increased from 1.35 ± 0.59 mN at 2.50 x 10^{-2} µg/ml to 3.85 ± 0.70 mN at 1.60 µg/ml. Cumulative addition of AV was observed which showed a significant ($p<0.001$) concentration – dependent increase with increases in the AV concentrations added (figure 2). Acetylcholine at concentrations similar to those of AV also produced significant ($p<0.001$) increase in ileal contractions. Pre-treatment of ileal fragments with increased concentrations of atropine (2.50 x 10^{-2} - 8.00 µg/ml) prevented the expression of the previously observed spasmogenic activity of AV (8.00 x 10^{-1} µg/ml) by over 80.98 % (figure 3). Nifedipine (8.00 x 10^{-2} µg/ml) and pyrilamine maleate (6.00 x 10^{-1} µg/ml) completely inhibited contractions induced by AV (8.00 x 10^{-1} µg/ml) on ileal fragments (figures 4 and 5). AV in the free Ca2+ medium produced a low contraction of the ileal fragment as compared to a normal tyrode medium.

Effect of AV on rat stomach strip: Figures 1 and 2 also show the spasmogenic activity of single and cumulative concentrations of the AV on rat stomach strips. Results similar to those for ileal fragments were obtained. Thus, concentrations of AV (0.2 – 0.8 µg/ml) at single concentrations and (2.50 x 10^{-2} - 1.60 µg/ml) at cumulative concentrations significantly ($p<0.05$) increased contraction forces. The spasmogenic activity of AV (8.00 x 10^{-1} µg/ml) was reduced by over 81.84 % by atropine at the same concentration and was abolished by pre-treatment with nifedipine (1.60 x 10^{-1} µg/ml) and pyrilamine maleate (6.00 x 10^{-1} µg/ml). In a free Ca^{2+} medium, AV produced a low contraction of gastric strip as compared to a normal medium.



A B

Figure 1_a: Original concentration-response curves of 1- hydroxyl-3,7,8-trimethoxyxanthone (AV) added to the rat intestinal (A) and gastric (B) smooth muscle fragments.

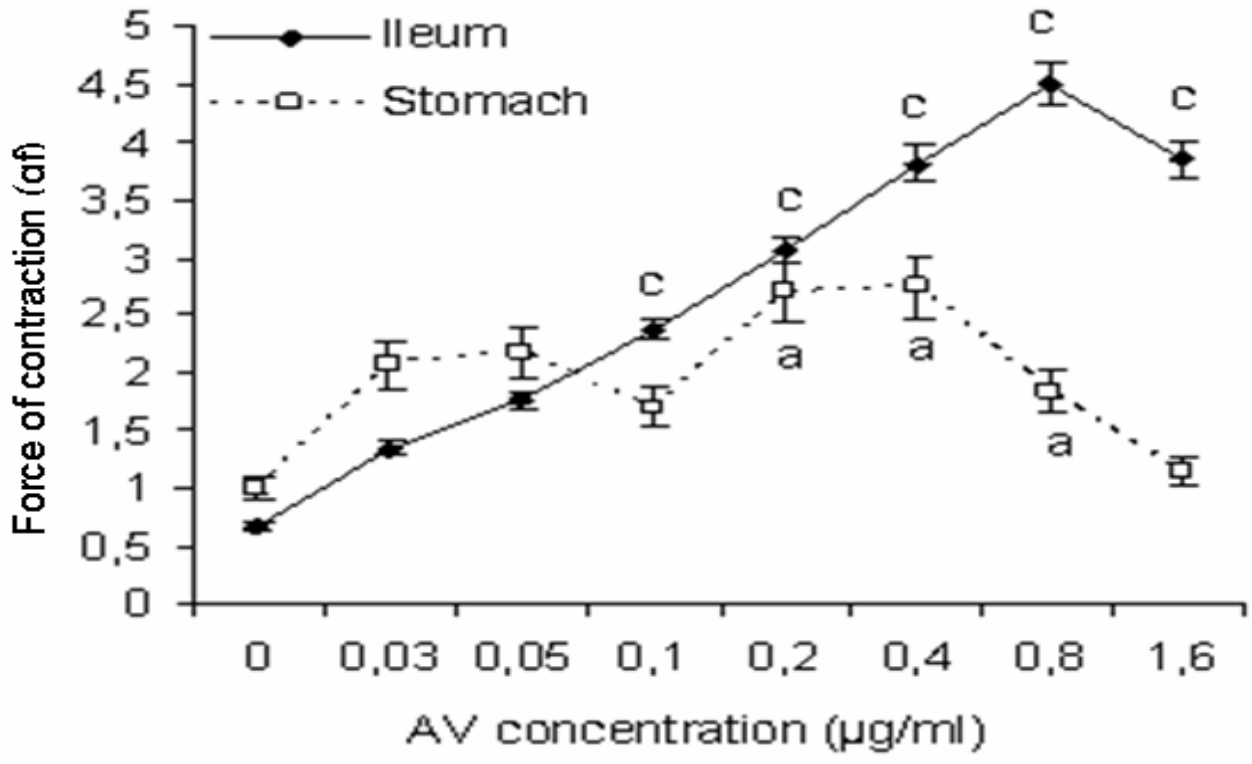


Figure 1_b: concentration-response curves of 1- hydroxyl-3,7,8-trimethoxyxanthone (AV) added to the rat intestinal and gastric smooth muscle strips. Each point represents the mean ± SE of 5 experiments. $^{a}p<0.05$ $^{c}p<0.001$ compared with initial concentration of AV (0 µg/ml). AV: 1- hydroxy 3,7,8 – Trimethoxyxanthone

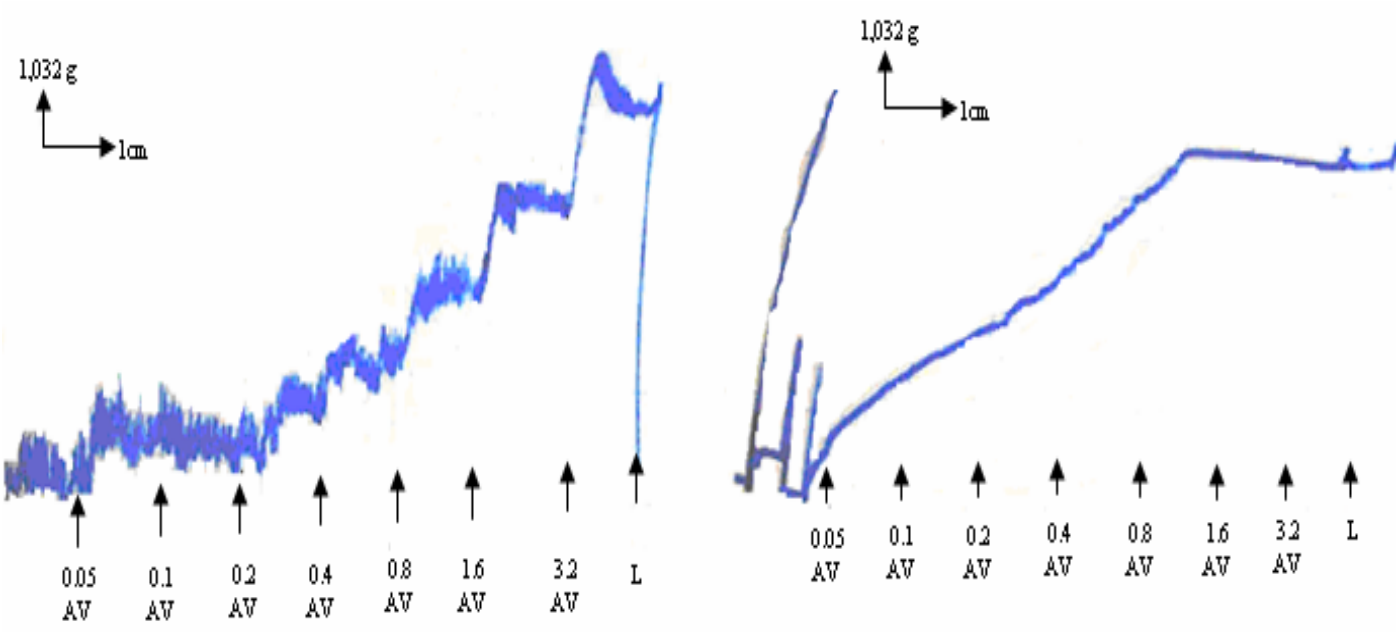


Figure 2_a**:** Original cumulative concentration-response curves of 1- hydroxyl-3,7,8-trimethoxyxanthone (AV) added to the rat intestinal (A) and gastric (B) smooth muscle fragments.

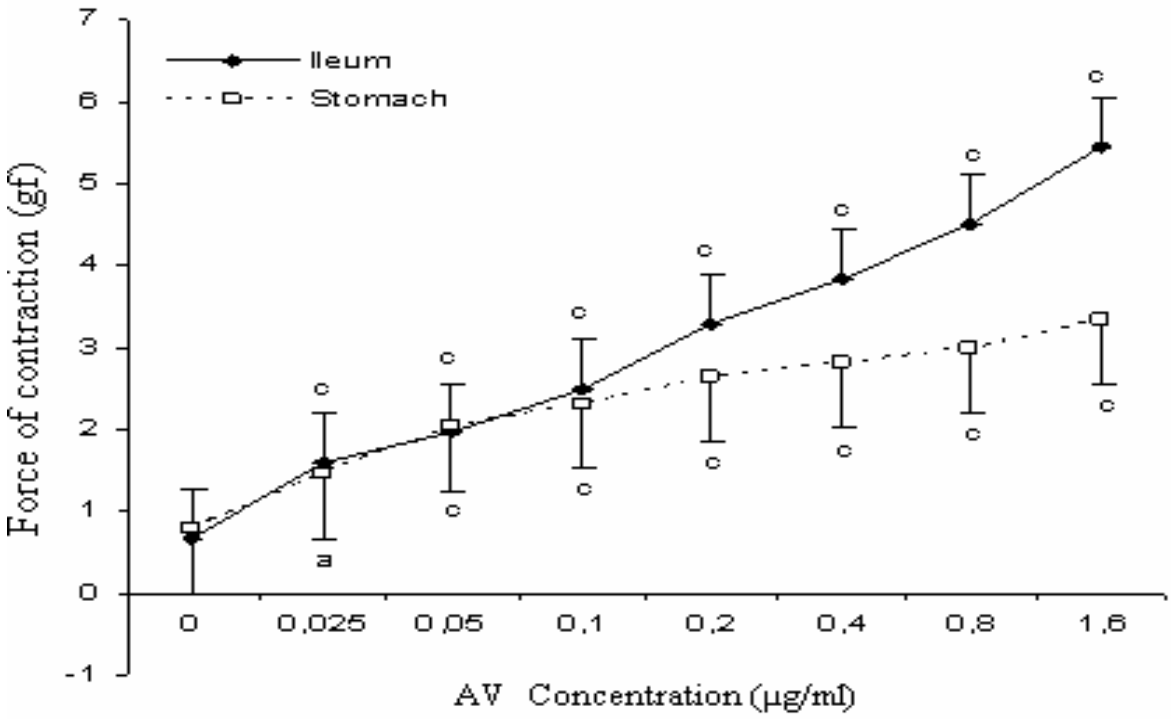


Figure 2_b: cumulative concentration-response curves of 1- hydroxyl-3,7,8-trimethoxyxanthone (AV) added to the rat intestinal and gastric smooth muscle fragments. Each point represents the mean ± SE of 5 experiments. $^{a}p<0.05$ $^{c}p<0.001$ compared with initial concentration of AV (0 µg/ml). AV: 1- hydroxy 3,7,8 – Trimethoxyxanthone.

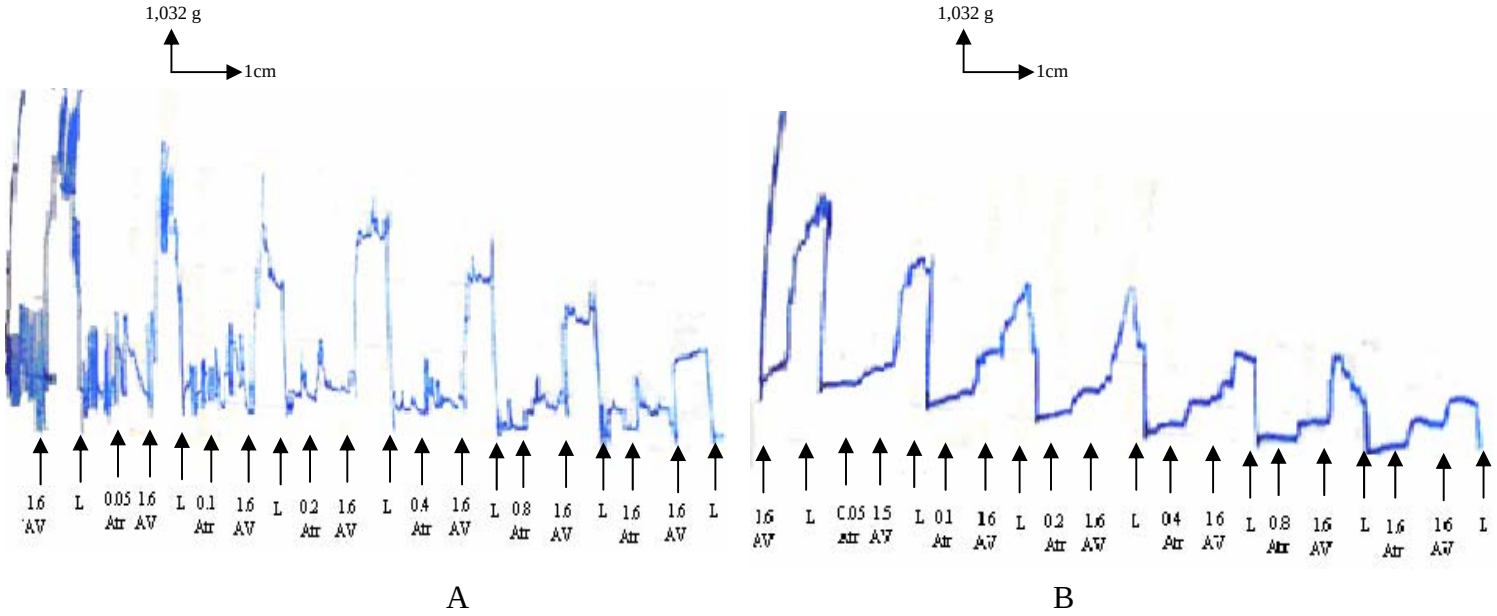


Figure 3$_a$: Original curves representing the Inhibitory effect of atropine on AV-induced contraction of rat ileal (A) and stomach (B) smooth muscle fragments**.**

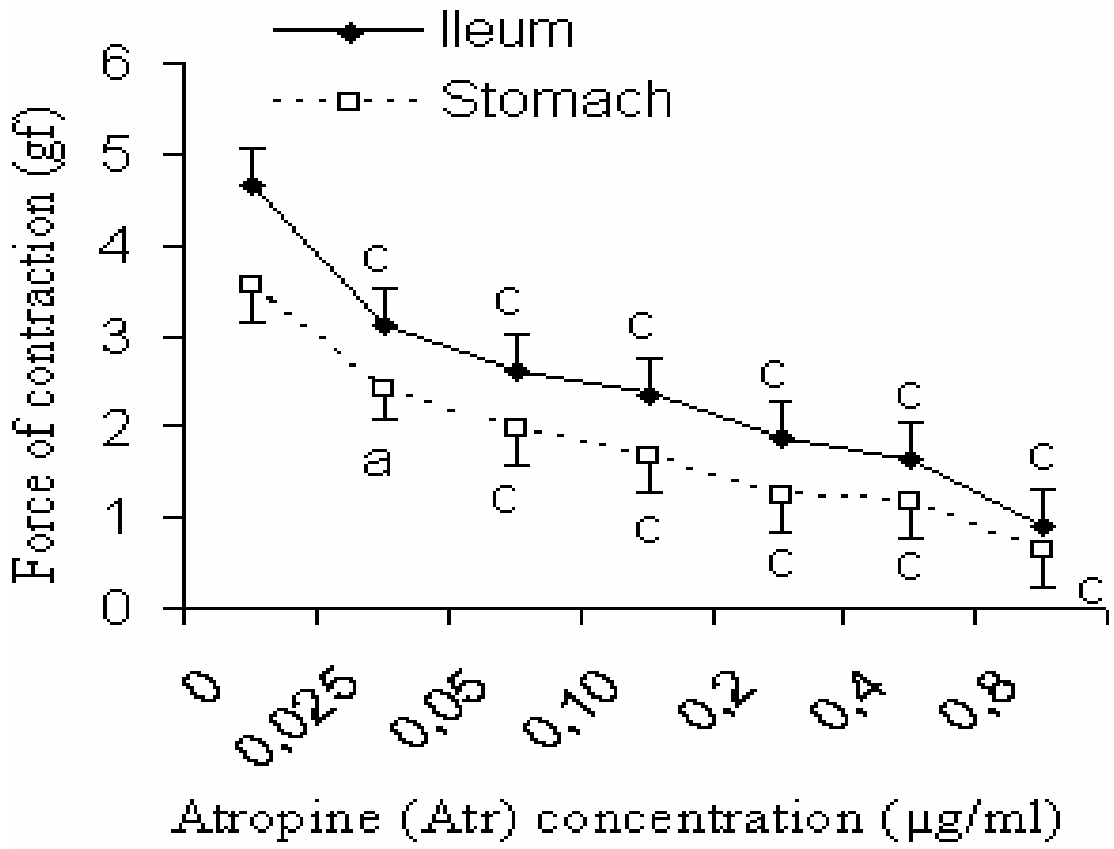


Figure 3$_b$: Inhibitory effect of atropine on AV-induced contraction of rat ileal and stomach smooth muscle fragments**.** Each point represents the mean ± SE of 5 experiments. $^a p<0.05$ $^c p<0.001$ compared with initial concentration of atropine (0 µg/ml).

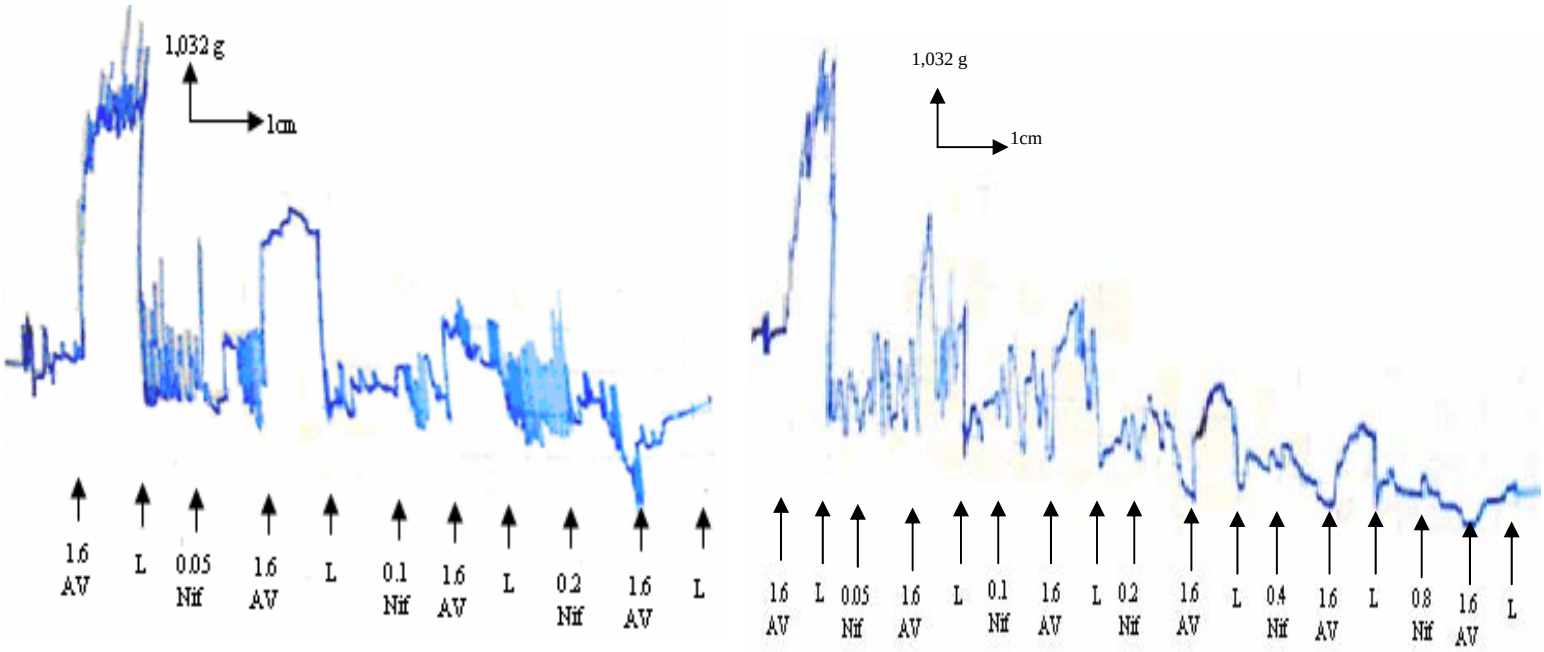


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Figure 4_a: Original curves representing the Inhibitory effect of Nifedipine on AV-induced contraction of rat ileal (A) and stomach (B) smooth muscle fragments.

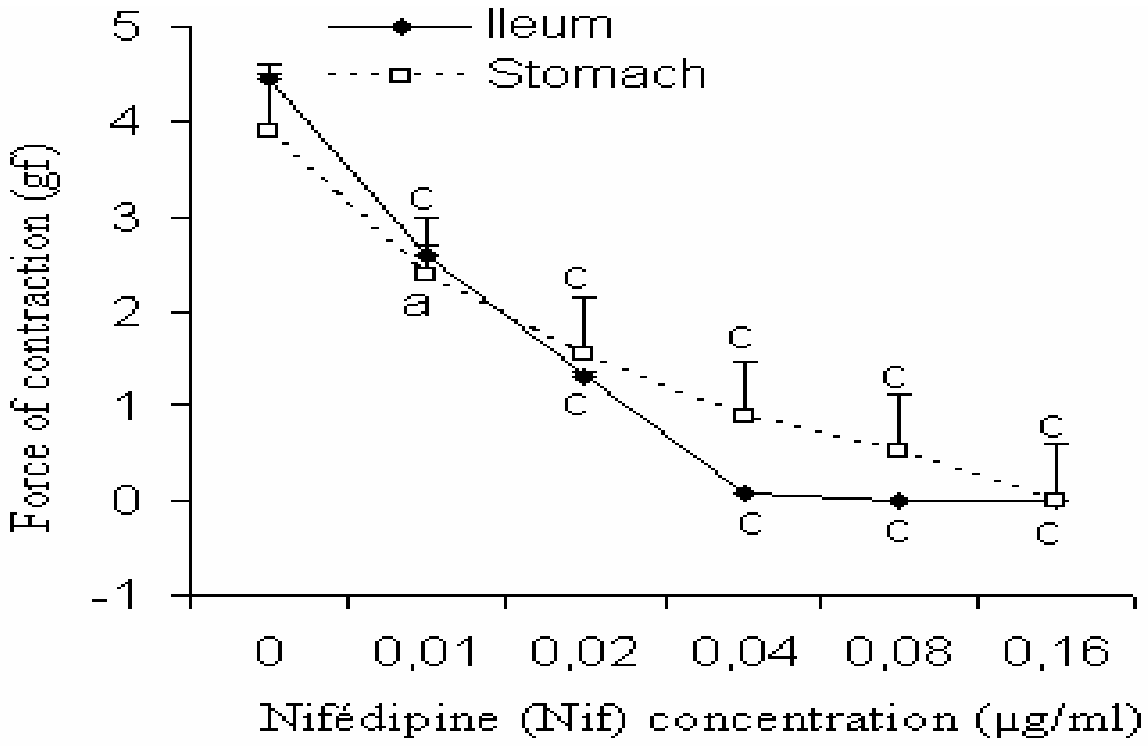

Figure 4_b: Inhibitory effect of nifedipine on AV-induced contraction of rat ileal and stomach smooth muscle fragments. Each point represents the mean ± SE of 5 experiments. $^{a}p<0.05$ $^{c}p<0.001$ compared with initial concentration of nifedipine (0 µg/ml).

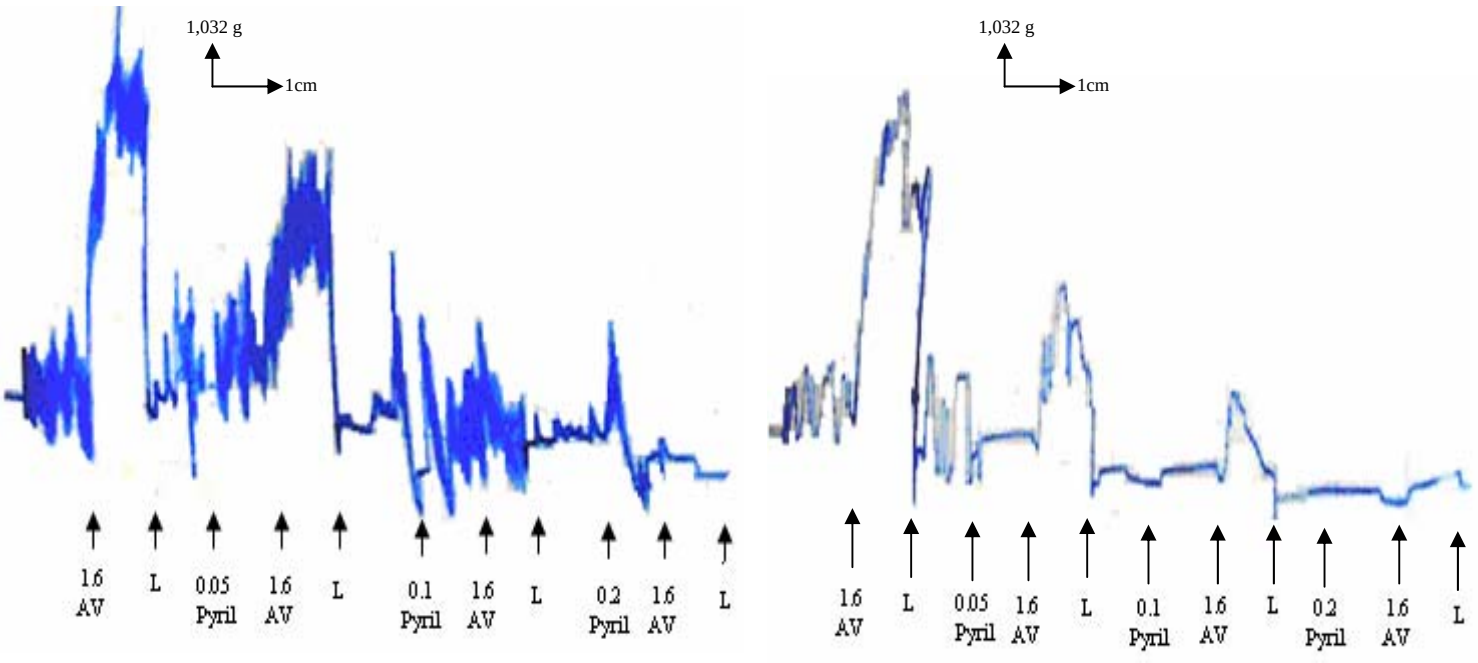

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Figure 5$_a$: Original curves representing the Inhibitory effect of Pyrilamine maleate on AV-induced contraction of rat ileal (A) and stomach (B) smooth muscle fragments**.**

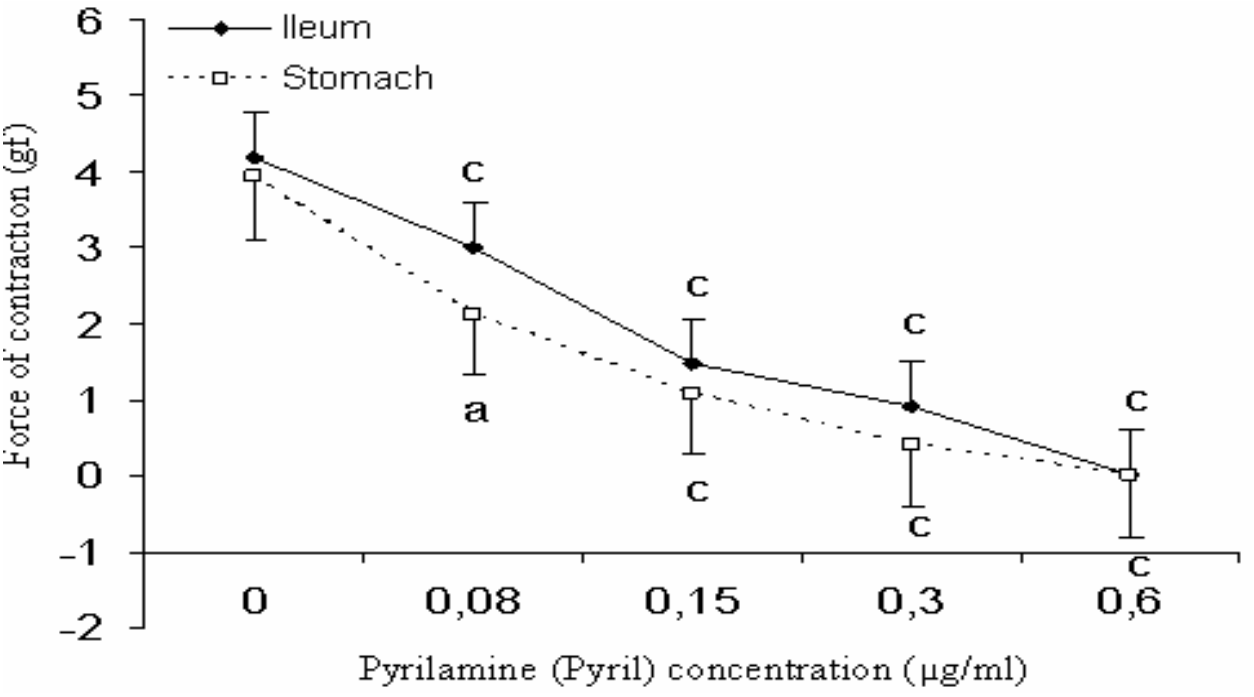

Figure 5$_b$: Inhibitory effect of pyrilamine maleate on AV-induced contraction of rat ileal and stomach smooth muscle fragments. Each point represents the mean ± SE of 5 experiments. $^a p<0.05$ $^c p<0.001$ compared with initial concentration of pyrilamine maleate (0 µg/ml).

Discussion

The results of this study clearly show that 1-hydroxy-3,7,8-trimethoxyxanthone possesses stimulant effect on rat intestinal and gastric smooth muscle preparations in vitro. Since AV is active in vitro, it is possible that the spasmogenic principle is active per se and not due to any metabolic transformation. Contractions of smooth muscle are dependent on an increase in the concentration of the cytosolic free Ca^{++} which activates the contractile elements. The source of Ca^{2+} may be intracellular or extra cellular, depending on the contractile agent and the type of smooth muscle (Mei Lu *et al*., 2001). Acetylcholine, a major excitatory transmitter, in both intestine and stomach by binding to muscarinic receptors, activates receptor-operated channels which become permeable to mono and bivalent cations such as Na^{+} and Ca^{2+} (Inoue *et al.*, 1993). Ca^{2+} can gain access to the cytoplasm also via the opening of voltage-operated channels (Inoue *et al*., 1990; Cloarec *et al*., 1989; Dimo *et al*., 1998). Atropine, a known anti cholinergic agent, inhibited by 80.98 and 78.08% the spasmogenic effect of AV on ileal and stomach smooth muscle respectively, suggesting that AV may be acting through muscarinic receptors (Dimo *et al.*, 2001). The effect of AV in the medium containing nifedipine (a calcium channel blocker) and pyrilamine maleate (a specific histamine channel inhibitor) was completely abolished suggesting that AV may exert his spasmogenic activity through calcium channel and histaminic receptors.

When the smooth muscle was exposed to a calcium free bath medium, contraction was low compared to what obtained in a normal medium due to the mobilisation of intracellular calcium only. This led us to think that the source of calcium is both intra and extra cellular. AV may thus be acting by mobilizing intracellular Ca^{++}.

In conclusion, the results obtained in the present investigation provide pharmacological support for the use of 1- hydroxy 3,7,8 – TrimethoxyXanthone (AV) as a spasmogenic agent. Pharmacological characterization of the compound suggests that the mechanism of action might be due to an interference with calcium metabolism in smooth muscle.

Acknowledgment

The authors acknowledge with thanks the financial contribution of the TWAS Trieste Italy and IPICS Uppsala Sweden through the CAM02 project.

References

Adjanohoun JE, Aboubakar N, Dramane K, Ebot ME, Ekpere JA, Enow-Orock EG, Focho D, Gbile ZO, Kamanyi A, Kamsu Kom J, Keita A, Mbenkum T, Mbi CN, Mbiele AL, Mbome IL, Mubiru NK, Nancy WL, Nkongmeneck B, Satabie B, Sofowora A, Tamze V, and Wirmum CK. Contribution to ethnobotanical and floristic studies in Cameroon. OAU/STRC 1986: P. 273.

Cloarec D, Bruley Des Varannes S and Galmiche JP. Modification de la motricité digestive. Des concepts fondamentaux aux applications thérapeutiques. Ed. Frison. Roche. Paris 1989 PP. 581-589.

Dalziel JM. The useful plant of West tropical Africa. Crown Agents for the Colonies, London. 1955 P. 361.

Dimo T, Rakotonirina VS, Kamgang R, Tan PV, Kamanyi A and Bopelet M. Effects of leaf aqueous extract of *Bidens pilosa* (Asteraceae)on KCl and nor epinephrine-induced contractions of rat aorta. *J. Ethnopharmacol*. 1998 60: 179-182.

Dimo T, Rakotonirina A, Kamgang R, Tankwa RT, Wanda K, Germain JM. Effets de l'extrait au cyclohexane de *Bidens pilosa* L. (Asteraceae) sur la motricité intestinale de rat. *Af. J. Sc. And Technol. Science and Engineering series* 2001 Vol. 2(1): 32-35.

Inoue R, Isemberg G. Effect of membrane potential and acétylcholine-induced inward current in guinea-pig ileum. *J. Physiol*.1990 424: 57-71.

Inoue R, Chen S, Siemen D, Hescheler J. Non selective cation channels: pharmacology, physiology and biophysics. Basel : Birkenhäuser Verlag 1993 pp. 261-268.

Irvine FR. Woody plant of Ghana. *Oxford Univ. Press, London* 1961 P. 878.

Mei-Lu, Lucia B, Claudia D, Cristiana M, Giuseppe S, Gabriela M. Muscle relaxing activity of *Hyssopus officinalis* essential oil on isolated intestinal preparations. *Planta Med*. 2002 68: 213-216.