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Research Article

MYOSTIMULATING EFFECT AND MECHANISM OF ACTION OF AN AQUEOUS EXTRACT OF *HIBISCUS SABDARIFFA* (MALVACEAE)) ON THE INTESTINAL MUSCLE OF THE RABBIT

***Méa Arsene, Abo, K.JC. and Kassi, Y.T.**

Laboratory of Animal Physiology, UFR Biosciences, Fellix Houphouet Boigny Univrsity, Abidjan, Côte d'Ivoire

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ABSTRACT

We undertook this work to determine the effect of a total aqueous extract of *Hibiscus sabdariffa* (Malvaceae) on the intestinal muscle of the rabbit to determine its mechanism of action. So the intestinal muscle is isolated and put in physiological medium. The amplitude and the frequency of the contractions are recorded on paper. The total aqueous extract of *Hibiscus sabdariffa* (AEHS) increases the amplitude of the rhythmic contractions of rabbit duodenum in a dose-dependent manner. The contractile force in reference medium is 0.44 ± 0.108 g/f. Doses ranging from 4.10^{-4} mg / ml to 8.10^{-2} mg / ml increases the amplitude of the contractions and basal tone ($p < 0.001$). AEHS for successive doses of 2.10^{-4}, 2.10^{-3} and 8.10^{-2} mg / ml increases the contractile force of 2.12 ± 0.247 g / f, 3.945 ± 0.162 g / f and 5.13 ± 0.290 g / f ($p < 0.001$). These increases are respectively of 381, 796 and 1065 %. Atropine has no effect on the effects induced by AEHS. Adding AEHS in the physiological medium containing adrenaline, increases rhythmic contractions abolished by adrenaline. These effects are identical to those of the propranolol in medium containing adrenaline. In a physiological medium containing nifedipine, calcium chelator and a calcium-free medium, the muscle is completely relaxed. In this case the addition of AEHS in these environments has no effect. But when the calcium is present in the physiological medium as reference, AEHS gradually increases the amplitude and the basal tone of the intestinal smooth muscle. AEHS therefore contains substances acting as propranolol (beta-blocker). This drink increases the influx of calcium ions. These facts justify the use of traditional medicine in AEHS to treat constipation and also all research undertaken for its hypotensive and antihypertensive effects.

INTRODUCTION

Hibiscus sabdariffa (Malvaceae) calyces infusion is called Sour Tea, Karkade, or Roselle (Red Sorrel) and is known everywhere. This decoction of the calyces of *Hibiscus sabdariffa* is called 'Bissap' in Sub-Saharan Africa and is usually consumed by many people. On the markets, cantiers, schools and during various ceremonies 'Bissap' is distributed as refreshment. Furthermore, due to its high consumption by the public, it is interesting to highlight the activity of this drink on basic physiological functions such as digestion, respiration and regulation. Adriana *et al.,* (2013) reported that grounded Hibiscus sabdariffa calyx increases the polyphenols bioaccesibility in gut that facilitate fibers digestion. The *Hibiscus sabdariffa* exerted important antihypertensive activity and demonstrated a tendency to reduce serum sodium concentration without modifying potassium levels (Herrera *et al.,* 2007; Ajay *et al.,* 2007). Mozaffari *et al.,* (2008) showed that consuming the infusion had positive effect on blood pressure in type II diabetic patients with mild hypertension. The objective of this series of experiments is shown its activity in the intestinal smooth muscle, comparing its effect with those substances known to determine its mechanism of action.

****Corresponding author: Méa Arsene,***
Laboratory of Animal Physiology, UFR Biosciences, Fellix Houphouet Boigny Univrsity, Abidjan, Côte d'Ivoire

Materials and methods

Biological material

Plant material

The calyces come from Seguela a city to the north of the Côte d'Ivoire located to 450 km of Abidjan. They are sold on the market of Adjame a central municipality of Abidjan. Fifty grams of crushed dried calyces are boiled with one (1) liter of distilled water in a Pyrex jar for ten minutes. The decoction is cooled and filtered successively on cotton wool to retain the important dimensions of impurities; then on Whatman filter paper to the impurities of small dimensions by the method revised by Irie *et al.,* (2016). The aqueous filtrate is dehydrated in the oven at 40 ° c. A hydrophilic fine powder purple red

color is obtained. A stock solution with a quantity of this powder is prepared. From this stock solution, test solutions of different concentrations are made.

Animal material

The rabbit of the specie *Oryctolagus cuniculus* (Leporideae) were brought from farms near Abidjan. They were acclimated to laboratory conditions in the Biological and Sciences United Formation (laboratory of animals physiology) for one week to regulated and harmonize their physiologicals states. They were kept at constant temperature (24±3°c) with 50-55% of humidity and a photoperiod of 12 hours of daylight and 12 hour of darkness. They are fed ad libitum with pellet and water. They were carefully screened and confirmed to be healthy during the procedures were conducted in accordance with the guiderlines for Care and Use of Laboratoratory Animals published by the National Institutes of Health.

Organ preparation

The equipment that allows the recording of contractions of the intestine is composed of a tank with a bain-marie in which a smaller tank contains the isolated intestine fragment. This last tank is connected by coils embedded in the water bath a flasks, one of which contains the physiological solution of reference. Each coil communicates with a tap has multiple selection paths. The physiological solution in which the intestine bath is oxygenated to an air bubble generator and its temperature is maintained at 38 ° C as described by Bleu *et al.,* (2011). Other wells contain different concentrations of the test substance. A permanent return to reference physiological solution cleans the organ (back to normal) before moving to the next test concentration. The mechanical contractions are recorded on paper.

Pharmacodynamic substances

Adrenaline (L-adrenaline) is from Biochemika, Atropine from Sigma, Acetylcholine from Sigma, Propranolol (Avlocardyl) from AstraZeneca, Nifedipine (Adalate) from Sigma.

Statistical analysis of results

Results are analyzed by analysis of variance ANOVA multiple comparison test of Tukey-Kramer. $P< 0.05$ is considered significant. The values are presented as average (mean), followed by the standard error of the mean (SEM). The curves are drawn through GraphPadprism5 (San Diego CA USA) biological software.

RESULTS

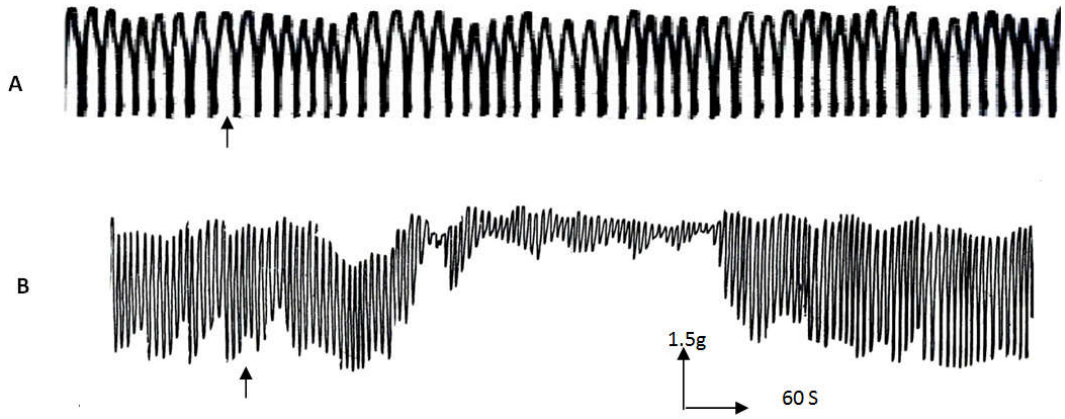
Dose-response effects of AEHS on the rhythmic spontaneous contractions of the intestine isolated from rabbit

During this study, spontaneous rhythmic contractions of the intestinal muscle were recorded in the reference physiological medium. Then increasing doses of AEHS are added to the physiological medium cumulatively. The effect of each dose is recorded and an interval of 15 minutes is observed between the different registrations. The recordings of the spontaneous and rhythmic contractions of a fragment of duodenum isolated from rabbit are modified in the presence of going concentrations of AEHS from $2x10^{-4}$ g/ml to $8x10^{-2}$ g/ml AEHS in amplitude. The reference force is recorded to 0.44±0.108 g/f. The dose lower than $2x10^{-4}$ g/ml do not have an effect on the rhythmic spontaneous contractions recorded in physiological medium of reference. From $2x10^{-4}$ to $8x10^{-2}$ g/ml after the pilot recording, the addition of AEHS increases the amplitude of the rhythmic spontaneous contractions (figure 1). Then successively doses to 4.10^{-4}, 8.10^{-4}, 2.10^{-3}, 4.10^{-3}, 4.10^{-2} and 8.10^{-2} g/ml increase the contractile force at 1.125±0.129, 2.12±0.300, 3.945±0.1630, 4.735±0246, 5.13±0.289 g/f. These increases are respectively of 155, 381, 796, 979 and 1065 %. The frequency of these spontaneous contractions is not modified. In addition, for doses raised starting from $4x10^{-2}$ g/ml, basic tonicity increases in a dose-dependent manner concomitantly to the reduction on the amplitude on the contractions. However at the end of 2 to 5 minutes of observation, tonicity goes down again whereas the spontaneous contractions increase and exceed the value of reference (figure 1E). The histogram of figure 2 presents the evolution of the contractile force according to the concentrations of AEHS. The graphics of figure 3 express the percentage of increase in the contractile force according to the logarithm of the concentration of AEHS. This curve takes a sigmoid form. The graphic determination of the effective concentration with 50% efficacy (CE50) gives a value which is around $4x10^{-3}$ g/ml.

Effect of AEHS on the intestine isolated from rabbit in the presence of the atropine

The aim of this experiment is to check if atropine can inhibit the observed effect of AEHS on the intestinal muscle. A single concentration of atropine having no inherent effect is used. After each recording, the organ is perfused with the reference solution before the addition of the following test dose. The figures 4A, 4B and 4C show the effect of interaction AEHS - atropine on the rhythmic spontaneous contractions of the intestinal muscle. With the physiological solution of reference, the amplitude of the spontaneous contractions is of 0.65±0.06 g/f. The addition of AEHS with $4x10^{-4}$ g/ml in this medium, involves a rise in the amplitude of the rhythmic spontaneous contractions which reach to 1.05±0.08 g/f. Then it is a variation of 61%.

The addition of the atropine with $5x10^{-4}$ g/ml six (6) minutes after the beginning of the effect of AEHS brings the contractile force to 1.03±0.08 g/f (figure 4A). It is a variation of 58 %; so the atropine did not modify significantly the effect induced by AEHS. The resumption of the experimentation with a fairly high concentration of AEHS is $4x10^{-3}$ g/ml, makes it possible to record an increase in the rhythmic contractions of 66% compared to the reference level (figure 4B). Basic tonicity is slightly high. The addition of the atropine to $5x10^{-4}$ g/ml brings back basic tonicity to its initial level at the end of 5 minutes. On the other hand, the increase in the rhythmic spontaneous contractions induced by AEHS is not modified. With a high concentration of AEHS which is $6x10^{-2}$ g/ml, the spontaneous contractions and basic tonicity increases considerably. The increase is of 140%. The addition of the atropine 5 minutes after the effect of AEHS brings back basic tonicity to its initial value after 15 minutes whereas, the increase in the rhythmic spontaneous contractions remain unchanged (figure 4C).



A: Pilot Recording followed by the effect of $4x10^{-4}$ g/ml
B: Pilot recording follow-up of the effect of $8x10^{-2}$ g/ml

Figure 1. Dose-response effect of the aqueous extract of AEHS on the contractions of the rabbit intestine

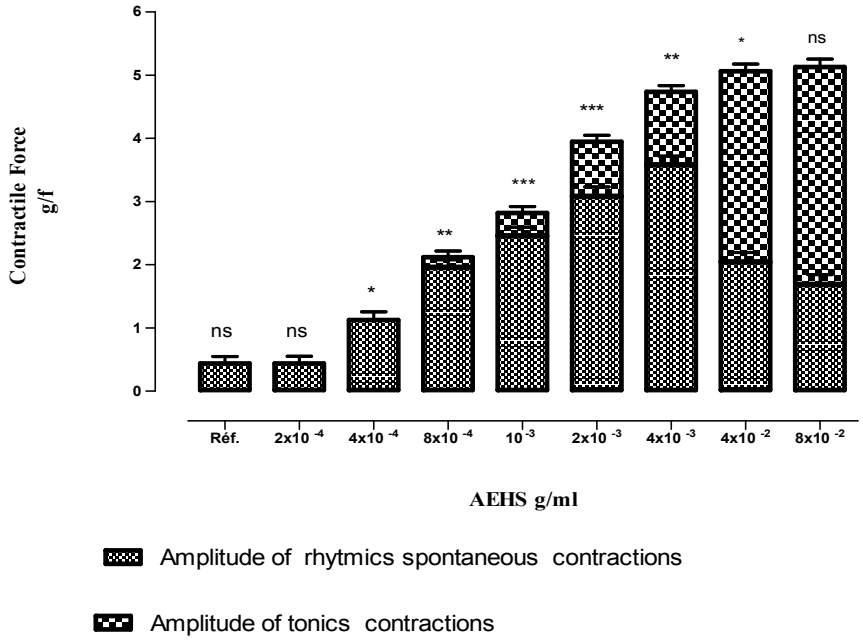


Figure 2. Histograms of evolution of the spontaneous contractions and the basic tonicity of the intestine isolated from rabbit according to various amounts of AEHS on average + SEM. NS; *P<0.05; ** P<0.01; ***P<0.001; (n=5)

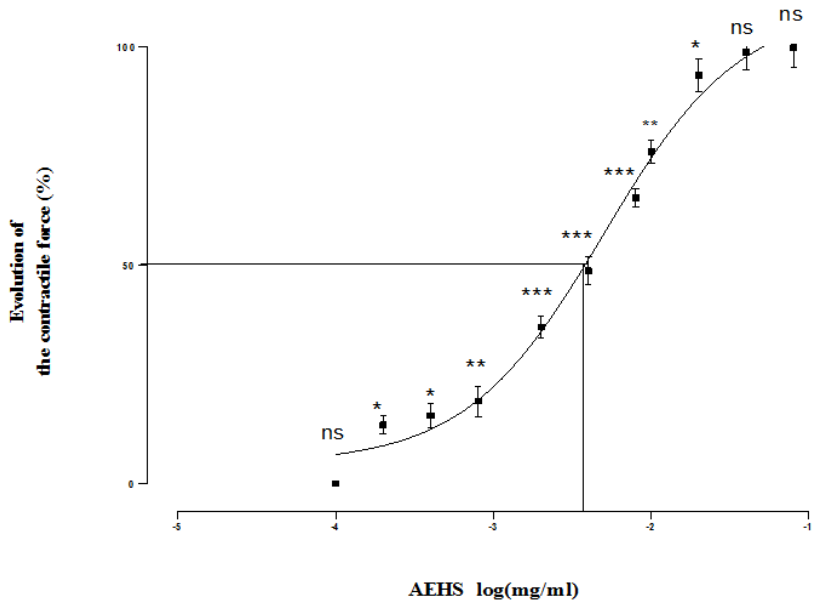


Figure 3. Curve dose- effect of the amplitude of the contractile force according to the logarithm of the concentration of AEHS (n=5)

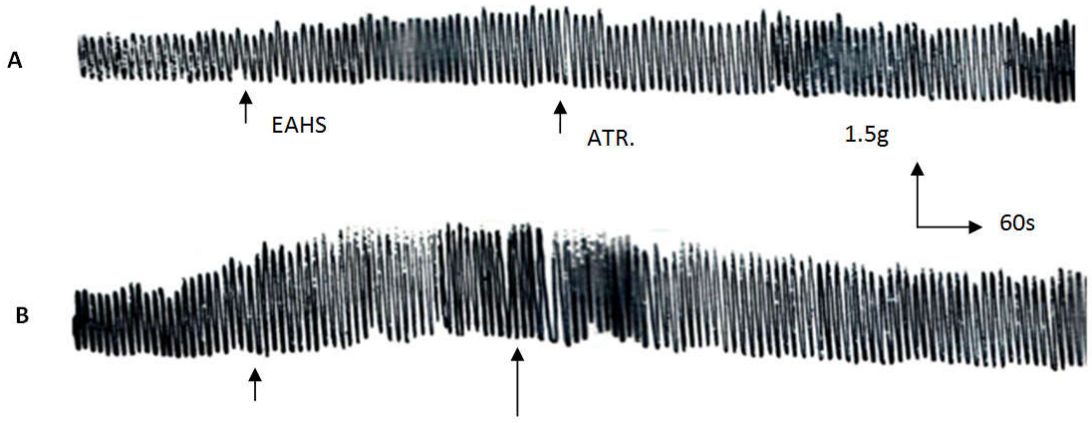
Comparative curves of the effect of AEHS alone and that of AEHS associated with the atropine with $5x10^{-4}$ g/ml (figure 5) watch which the action of the atropine is not very sensitive on the activity induced by AEHS, no significant (p 005)

Effect of EAHS on the intestine muscle in the presence of adrenaline

Adrenaline decreases the contractile force of the intestine muscle. This experimentation is to measure the effect of AEHS on the inhibition induced by adrenaline. The recording of figure 6A watches the inhibiting effect of adrenalin with $4x10^{-4}$ g/ml, on the spontaneous and rhythmic contractions of the intestine isolated from rabbit.

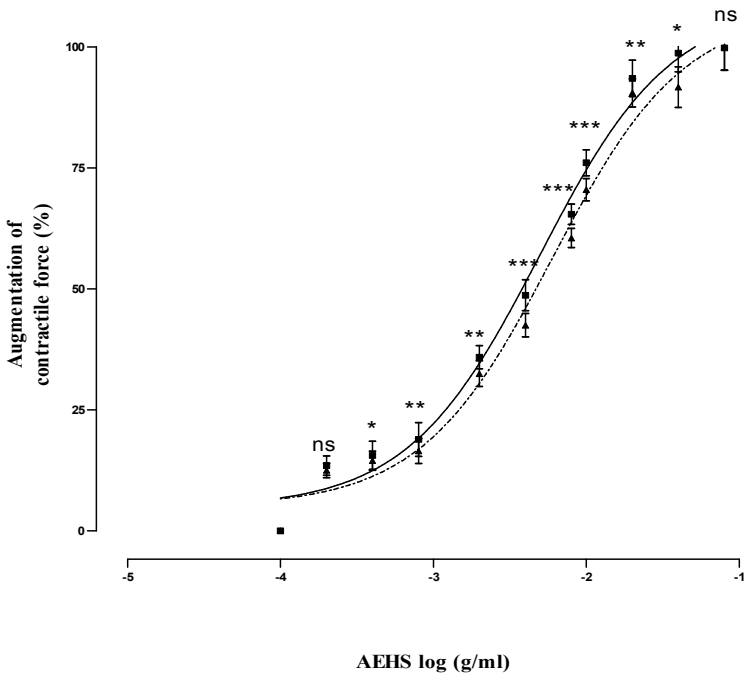
The contractions disappear completely with this concentration from adrenalin. If, following this effect, one adds AEHS to $4x10^{-2}$ g/ml in the physiological medium, the contractions reappear gradually. They reach an amplitude equalizes with 1.25±0.06 g/f. What represents an increase of 83.3% of the amplitude of reference (figure 6B).

With a concentration of AEHS twice higher, with $8x10^{-2}$ g/ml, the recovery is more fugacious. The amplitude of the spontaneous contractions increases considerably before returning to the reference level (figure 6C). The contractile force, reached here 2.15±0.12 g/f, is a rise in 143% compared to the amplitude of reference.



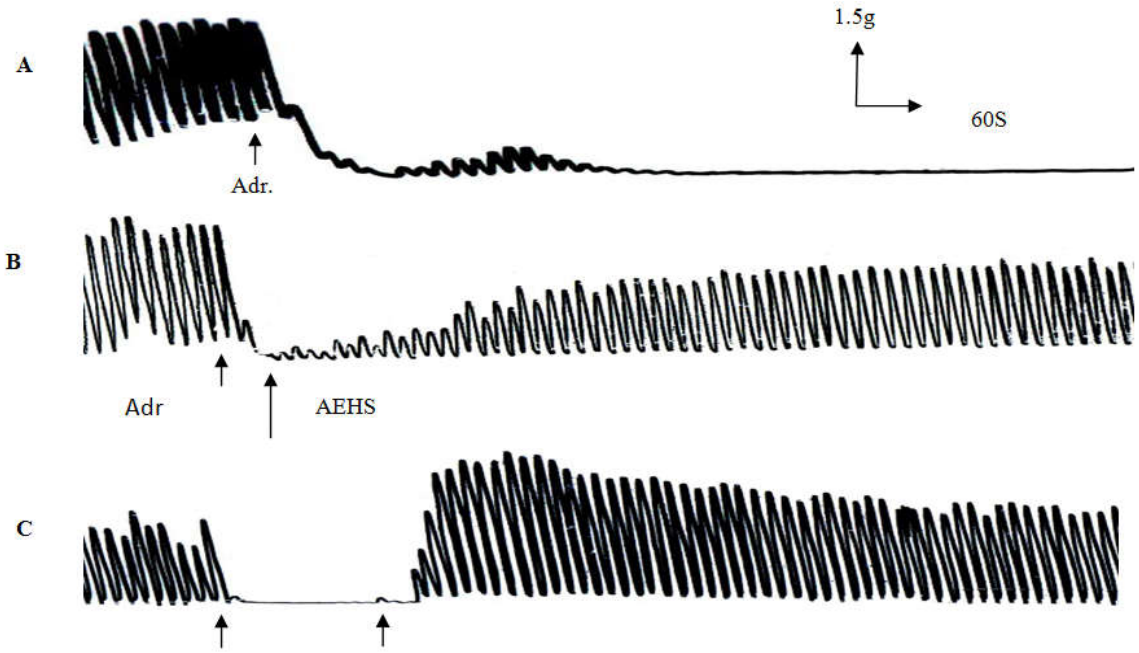
A: AEHS with $4x10^{-4}$ g/ml and atropine with $5x10^{-4}$ mg/ml
B: AEHS with $4x10^{-3}$ g/ml and atropine with $5x10^{-4}$ mg/ml (n=5)

Figure 4. Effects of interaction AEHS - atropine on the intestine isolated from rabbit



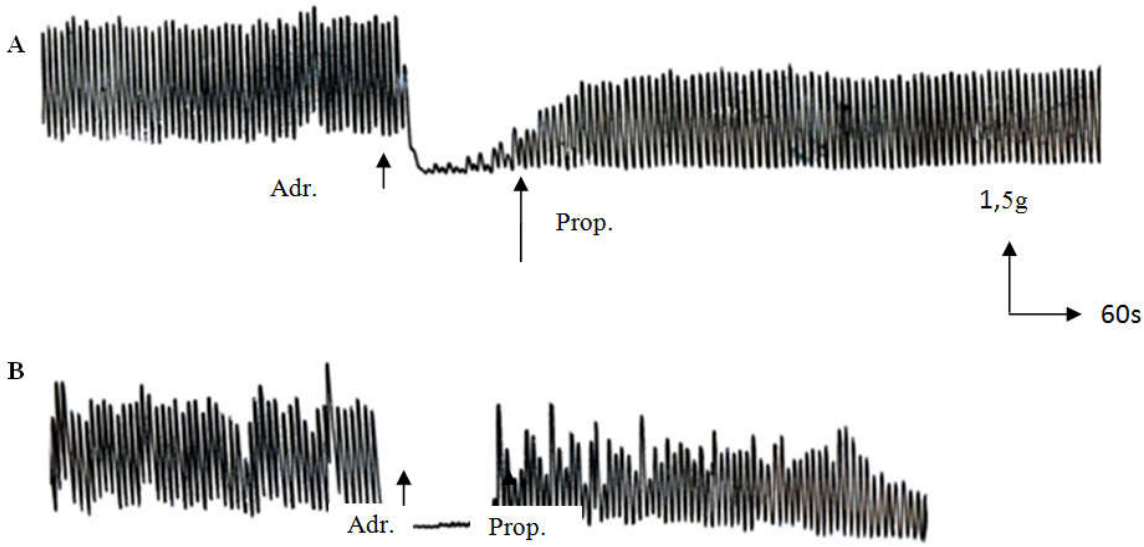
*p< 0.05; ** p<0.01; *** p<0.001

Figure 5. Curves of increase in the amplitude of the spontaneous contractile force of the intestine according to the concentration of AEHS and of AEHS + the atropine



A: effect of adrenaline with $4x10^{-4}$ g/ml on the intestinal smooth muscle
B: normal recording followed by the effect of adrenaline to $4x10^{-4}$ g/ml then of AEHS to $4x10^{-2}$ g/ml.
C: normal recording follow-up of the effect of adrenaline with $4x10^{-2}$ g/ml then of AEHS with $8x10^{-2}$ g/ml.

Figure 6. Effect of the interaction adrenaline - AEHS on the contractile activity of the intestine isolated from rabbit (n=5)



A: normal recording before the first arrow followed by the effect of adrenalin to $4x10^{-4}$ mg/ml, then of that of the propranolol with $4x10^{-4}$ mg/ml
B: normal recording before the first arrow followed by the effect of adrenalin to $4x10^{-4}$ mg/ml then of that of the propranolol with $4x10^{-3}$ mg/ml

Figure 7. Effect of the interaction adrenaline- propranolol on the intestine isolated from rabbit (n=5)

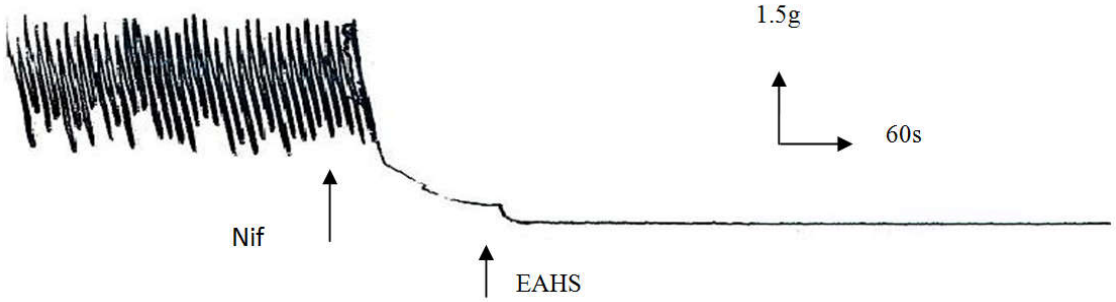


Figure 8. Effect of AEHS on the contractile activity of the intestine in the presence of the nifedipine (n=5).

Effect of propranolol on the rabbit duodenum in the presence of adrenaline

Propranolol is a blocker for the β-receptor on the intestine muscle. His effect inhibited those of adrenaline on the smooth muscle. So, in this experiment the aim is to show the effect of propranolol on the inhibition induced by adrenaline. The recordings of figure 7 represent the effect of the interaction adrenaline-propranolol on the rhythmic spontaneous contractions of the intestinal rabbit muscle. As in the series of preceding experiments, the level of amplitude of the contractions obtained in physiological solution of reference is of 0.65±0.08 g/f. The introduction into this physiological medium of adrenalin to $4x10^{-4}$ mg/ml decreases the contractile activity of the intestinal muscle (figure 7A). The rhythmic contractions disappear and basic tonicity is slightly with the lower part of its reference level. When the propranolol is added in the physiological medium containing adrenaline, hardly 10 s after the beginning of the effect of adrenalin, the rhythmic spontaneous contractions reappear. The contractile force reached 1.17±0.10 g/f, is a resumption of 80% of the initial amplitude. The continuation of the experimentation with an amount ten times higher of propranolol ($4x10^{-3}$ mg/ml), approximately 1 minute after the effect of adrenalin with $4x10^{-4}$ mg/ml, raises of advantage the spontaneous contractions which reach 1.25±0.16 g/f; what is equivalent to a resumption of 92% of the amplitude of reference (figure 7B).

Effect of AEHS on the intestine muscle of rabbit in the presence of nifedipine

Nifedipine is a blocker of calcium receptor on smooth muscle. In presence of nifedipine, the contractile force of the muscle is abolished. The study aims to know in this case the effect of AEHS when it is added in the medium containing nifedipine. The recording of figure 8 highlights the effect of AEHS on the contractile activity of the intestine muscle of rabbit in the presence of nifedipine. The contractile force in reference medium is evaluated with 0.65±0.06 g/f. The addition of nifedipine in the physiological medium involves a fast fall of the activity of the intestinal smooth muscle. The spontaneous and rhythmic contractions disappear and basic tonicity passes to the lower part of the reference level. The addition of a high concentration of AEHS with $8x10^{-2}$ g/ml, one minute after, does not modify of anything the effect induced by nifedipine.

DISCUSSION

The first experiments showed a dose-dependent increase in the amplitude of spontaneous contractions in a range of relatively low concentrations. In high concentrations, the basic tone is high until the intestinal muscle contracture. These observations highlight the myostimulant character of AEHS. These results are consistent with those of Ali *et al.,* (1991) on the isolated intestinal muscle of the guinea-pig. AEHS for them eaten stimulates intestinal muscle. At first sight, the effect is similar to that of acetylcholine on intestinal muscle. Indeed, AEHS mimics the action of acetylcholine and natural substances such as Emodin (active principle of *Rheum palmatum*) shown by Jin *et al.,* (1994) on isolated intestinal muscle of guinea-pigs, as *Citrus aurantifolia* on taenia coli of guinea-pig (Souza *et al.,* 2002). However, the use of atropine, parasympathicolytic for excellence, does not remove the effect induced by AEHS. This indicates that AEHS is not acting by cholinergic muscarinic (Mita *et al.,* 1993) is not enabled by AEHS. AEHS is not a substance that contains the cholinergic active ingredients. But, AEHS reverse the action of adrenaline by increasing the spontaneous contractions which are completely suppressed by the latter. A similar effect on intestinal muscle is induced by propranolol in the presence of adrenaline.

Propranolol antagonizes adrenaline, an inhibitor of beta-adrenergic receptors. What makes that AEHS may contain the type of active substances β-blockers. It is known that the action of beta-blockers (beta blockers) opposes the effects induced by adrenaline or beta receptors agonists (Hamid and Saeid 2006; Vrydag and Martin 2007). Beta blockers induce vasoconstriction, constriction of the uterus and of the intestinal musculature. The action of AEHS on the intestinal muscle could be beta-blockers such as propranolol action. Adrenaline causes dilation of vascular smooth muscle and bronchial structures (Annane *et al.,* 2007; Levy *et al.,* 2011; Moynihan *et al.,* 2008). However, β2-receptors are predominant in the intestinal smooth muscle. It is through the activation of ß2 catecholamines induce vasodilation, relaxation of intestinal muscles and uterus (Casteels *et al.,* 1977; Fabrice *et al.,* 2005). AEHS blocking β2-receptors causes a constriction of the smooth muscle of the intestine, as we have seen. It is well known that increased calcium influx is responsible for the potentiating of rhythmic contractions (Karaki *et al.,* 1972; Nargeot and Bourinet. 2005). Nifedipine is a calcium antagonist belonging to the dihydropyridine family. Nifedipine selectively inhibits the entry of calcium ions at the L-type channels depending on the cell membrane (Moynihan *et al.,* 2008). This action is mainly observed on vascular smooth muscle and to a lesser extent in heart muscle (Economy and Abuhamad. 2005). L-type channels are sensitive to calcium antagonists dihydropyridines such as nifedipine leading to their inactivation (closing) (Catterall *et al.,* 2005; Cens *et al.,* 2006). On the muscle relaxed by nifedipine, AEHS has no significant effect. In a medium free of calcium the intestinal smooth muscle is completely relaxed (Hurwitz *et al.,* 1967). In these circumstances AEHS has no effect. But in a environment where calcium is present AEHS increases rhythmic spontaneous contractions. According to Durbins and Jenkinson (1961), Meissner *et al.,* (1986) and Webb (2003) the potentiation of intestinal smooth muscle contractions was resulted from increased calcium influx.

Conclusion

These two observations support the conclusion that AEHS acts on calcium influx. Then AEHS contains the active ingredients which activating the entry of calcium ion in intracellular medium. .

REFERENCES

Adriana V.C., Luz R.B.M. , Margarita T.G.G., Aurelio D.L. 2013. In vitro gastrointestinal digestion of Hibiscus sabdariffa L.: the use of its natural matrix to improve the concentration of phenolic compounds in gut. *LWT-Food Sci. Technol.,* Vol. 1): 260-265

Ajay M., Chai H.J., Mustafa A.M., Gilani A.H., Mustafa M.R. 2007. Mechanisms of the anti-hypertensive effect of

Hibiscus sabdariffa L. calyces. *Journ. Ethnopharmacol.,* Vol. 109(3) : 388-393

Ali M.B., and Mohamed A.H. 1991. Effect of an aqueous extract of Hibiscus sabdariffa calyces on the gastro-intestinal tract. *Fitoterapia* 62 (6) : 475-479

Annane D., Vignon P., Renault A. 2007. Norepinephrine plus dopamine versus epinephrine alone for management of septick shock a randomised trial. *Lancet*., 370 : 676-684

Bleu G.M., Traore F., Coulibaly S., Nene-Bi S.A. 2011. Effets pharmacodynamiques d'un extrait hydroalcoolique de Curcuma longa L. Zingiberaceae) sur le système cardiovasculaire, la respiration et l'activité mécanique intestinale de mammifères. *Phytothérapie*., 9(1) : 7-17

Casteels R.K., Kuriyama H. and Suzuky H. 1977. Excitation-contration coupling in smooth muscle cells of rabbit main pulmonary artery. *J. Physiol. London.,* 271 : 63-79

Catterall W.A., Perez-Reyes E., Snutch T.P., Striessnig J. 2005. International union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. *Pharmacol. Rev.,* 57 (4) : 411-425

Cens T., Roussel M., Leyiris J.P., Fesquet P., Charmet P. 2006. Voltage and calcium-dependent inactivation in high voltage-gated Ca2+ channels. Progress in biophysic and molecular biology. 90 (1-3) : 104-117

Economy K.E., and Abuhamad A.Z. 2001. Calcium channel blockers as tocolytics. *Semin. Pernatol*., 25 (5) : 264-271

Fabrice P., Veronique L., Nadege B., Roger M., Bernard M. 2005. Rôle of β2-adrenoceptors (β-AR), but not β1-, β3-AR and endothelial nitric oxide in β-AR-mediated relaxation of rat intrapulmonary artery. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 372 (1) : 14-23.

Hamid N. and Saeid N. 2006. Alteration in α- and β-adrenoceptors profile of rabbit knee-joint blood vessels due chronic inflammation. *Cardiovascular System*, 453 (1) : 23-32

Herrera A.A., Miranda S.J., Vila-Castro P., Herrera A.S., Jimenez F.J.E., Zamilpa A., Ruben R.R., Hector P.M., Jaime T. 2007. Clinical effect produced by a standardized herbal medicinal product of Hibiscus sabdariffa on patients with hypertension. A randomized, double-blind, lisinopril-controled clinical trial. *Planta Med*., 73(1) : 6-12

Hurwitz L., Joiner P.D. and Vonhagen S. 1967. Mechanical response of intestinal smooth muscle in calcium-free medium. *Soc. Exptl. Med*., Vol. 125 : 518-522

Irie Bi J.S., Abo K.J.C., Kahou Bi G.P. 2016. Diuretic and salidiuretics activity of an aqueous extract of leafy branches of Mimosa invisa Mart. ex cola (Fabaceae) in rats. *Intern. J. Sci. Res.,* (IJSR. Vol. 5(4): 1475-1479

Jin Z.H., Ma D.L., Lin X.Z. 1994. Study on effect of emodin on the isolatd intestinal smooth muscle of guinea-pigs. Zhonqquo Zhong Xi Yi Jie He Za Zhi. 14(7): 429-431

Karaki H., Nasu T., Urakawa N. 1972. [Drug-induced contraction of smooth muscle and changes in calcium movement]. Nihon Yakurigaku Zasshi. 68(2):129-41.

Levy B., Perez J., Thivillier C. Gerard A. 2011. Comparaison of norepinephrine-dopamine to epinephrine for hemodynamic lactate randomised pilot. *Crit. Care Med.,* 39 : 450-455

Meissner G., Darling E. and Eveleth J. 1986. Kinetics of rapid Ca2+ release by sarcoplasmic reticulum. Effect of Ca2+, Mg2+, and adenine nucleotides. *Biochemistry*., Vol. 25 : 236-244

Mita M, Oishi K, Hashimoto T, Uchida MK. 1993. All-or-none response of isolated smooth muscle cells from guinea pig taenia caecum to acetylcholine. Nihon Yakurigaku Zasshi. 101(3):123-31.

Moynihan A.T., Smith T.J., Morrisson J.J. 2008. The relax act effect of Nifedipine in human uterine smooth muscle and the BK (Ca) channel. *Am. J ; Obstet. Gynecol*., 198 (2) : p 237

Mozaffari K.H., Jalali K.B.A., Afkhami A.M., Fatehi F., NooriS.M. 2008. The effects of sour tea (Hibiscus sabdariffa) on hypertension in patient with type II diabetes. *Jour. Hum. Hypert*., 23(1) : 48-54

Nargeot J. and Bourinet E. 2005. Voltage-dependent calcium channels as targets for analgesics. La Lettre du Pharmacologue. Vol. 19 (4) : 110-116

Souza A., Aka K.J., Datte Y.J., Abo J.C. 2002. Effet myostimulant de l'extrait aqueux de Citrus aurantifolia sur l'activité contractile de taenia coli de cobaye. *Revue Méd. Pharm. Afr.,* (16) : 103-112

Vrydag W. and Martin C.M. 2007. Tools to study β3-adrenoceptors. Naunyn-Schmiedeberg's Archives of Pharmacology. 374 (5) : 385-398

Webb C.R. 2003. Smooth muscle contraction and relaxation. *Adv. Physiol. Educ. Pub*. 1. Vol. 27(4): 201-206

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