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Effects of Aqueous Extract of *Hibiscus sabdariffa* L. (Malvaceae) on the Cardiac Contraction of Rat and Research of the Mechanism of Action

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Abstract: ***The modification of the ionic concentrations in physiological medium of reference (Mac Ewen), constitutes a model of study of the mechanisms of action for the transmembrane transport of a type of ion. Here, it is the influence of aqueous extract of Hibiscus sabdariffa on the transmembrane transport of the calcium which is required. A series of experiments highlighting calcium ion transport is thus undertaken. Aqueous extract of Hibiscus sabdariffa (AEHS) increases dose-dependently the amplitude of contractile activity of rabbit isolated heart while the frequency decreases concomitantly. Doses ranging from 10^{-6} mg / ml to 10^{-1} mg / ml increase the amplitude of the mechanical contractions from 13.5% to 54.28% for the respective doses of 10^{-6} mg / ml and 10^{-1} mg / ml. The frequency is reduced from 15% to 57%. However, reducing the frequency of rhythmic contractions is accentuated with the high concentrations (from 10^{-1} mg / ml) which induce arrhythmia beginning. In hyposodic medium to which AEHS is added, the contractile force increases from 08 (T1), 24.13 (T2), 43.33 (T3) and to 50% (T4), the heart rate decreases concomitantly from 11.11 (T1), 25 (T2), 33.33 (T3) and to 50% (T4). In hypopotassic medium added with AEHS, the contractile force increases by 6.89 (T1), 17.85 (T2), 26.92 (T3) and to 37.5% (T4) the frequency drops by 14.28 (T2), 33.33 (T3) and to 57.42% (T4). In hypocalcic medium to which one brings various concentrations of AEHS, the initially reduced contractile force is then raised successively to 06 (T3) and 14.70% (T4). In hypercalcic medium, the addition of AEHS increases by advantage the contractile force and the frequency. One can thus say that AEHS activates the extracellular calcium entry by an increase in the contractile force while lowering the frequency.***

Keywords: isolated hearth, modified physiological medium

1. Introduction

The aqueous extract of *Hibiscus sabdariffa* (AEHS) usually called `Bissap' in West Africa is very snuffed by our population. Our investigations have enabled to us to know that this drink is advised by certain doctors like food complement about anemias following paludism. What would have beneficial effects because of the enormous quantities of iron and vitamin C that it contains. On the other hand, Pousset (1989) showed that this substance was a diuretic.

The work undertaken by Fardji and Haji (2007) then checked by us even, showed the effect of AEHS on essential hypertension.

On the intestinal muscle smoothes, Ali and Mohamed (1991) showed a beneficial effect of AEHS on the gastro intestinal tract.

Calcium is an essential component in the muscular contraction. Its impulse and efflux make it possible to determine the mechanism of action of the products in pharmacology.

In this work we check the effect of AEHS on the transmembrane calcium which is the principal activator of the cardiac muscle.

2. Material and Methods

2.1 Material vegetable

The vegetable material is primarily composed of dried calyces of *Hibiscus sabdariffa*. The dried calyces came from the north region of Côte d'Ivoire, some time from Burkina faso or Mali (werstern Africa) and sold at the market of Adjame a central municipality of Abidjan.

Fifty gram of broyat of dried petals is put to boil with one (1) liter of water distilled in a pyrex bottle during one hour. The decoction obtained is cooled then filtered successively on absorbent cotton to retain the impurities of important size; then on filter paper Wattman for the low-size impurities according to the method of Abo et al., (2016). The aqueous filtrate is dehydrated and the base put at the drying oven to 40°c to accelerate dehydration.

An absorbent fine powder of red color crimson is obtained. A solution mother with a quantity of this powder is prepared. From this solution mother, the experimental solutions with various concentrations are carried out.

2.2 Animal Material

The rats of species *Ratus norvegecus* (Muridae), Wistar strain (Musa) are elevated in the animalery of the Laboratory of Animal Physiology of the University Felix Houphouët Boigny of Cote d'Ivoire (UFR Biosciences). They are milked according to the standards and International Conventions of animal experimentation and the guiding principles in the car and use of animals as approved by the National Institutes of Health. All rats were provided food and water ad.libitum and placed on a 12:12 hour-dark cycle, with the light cycle occurring during the day time at 24±3°c and 50% humidity. They weigh between 100 and 120 g.

2.3 Perfusion of the working heart

The rat wistar is anaesthetized using an injection of ethylurethane 20% at a rate of 1g/kg b.w. Isolated working heart performance was measured by use of the Langendorff-Neely technique as described in detail previously by Neely et al. (1967) and preformed by Barbato et al. (2002). Briefly sodium heparin (300 IU IP) was injected on the ventricle through the aorta, after extirpation. The heart was rapidly placed to the outlet of the valve of the survival system and was perfused by the physiological reference solution.

2.4 Various modified physiological mediums

Mac Ewen hyposodic (- 10%), Mac Ewen hypopotassic (- 10%), Mac Ewen hypocalcic (- 10%), Mac Ewen hypercalcic (+10%).

2.5 Statistical analysis

GraphPad Instat (San Diego CA, USA) program was used for statistical analysis of results. Values are expressed as mean followed by the standart error of the mean (MEAN±SEM). The difference between values was determined by Student-Newman-Keuls comparison test. It was considered significant for $p< 0.05$. GraphPad prism 6 (San Diego CA) was used for plotting graphs. Sigmoid curve was draw after transformation of values of x-axis as decimal logarithm and as a percentage for the y-axis.

3. Results

3.1 Dose-response effect of AEHS

The first series of experiment is to highlight the effect of increasing doses of AEHS on the isolated heart.

AEHS concentrations ranging from 10^{-6} mg / ml to 10^{-1} mg / ml allowed record types shown in figure 1. Qualitatively, the amplitude of heart contractions increased in the presence of AEHS, while the frequency decreases concomitantly. The amplitude of the mechanical contractions believes from 10.50, 28.45, 50.50, 75.50, 85.00 and 95.00 to 100% for the respective doses of 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} and 1 mg / ml. The frequency is reduced from 8.25, 10.50, 20.50, 25.50, 85.00 to 100% for the respective doses of 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} mg/ml.

However, reducing the frequency of rhythmic contractions is accentuated with the high concentrations (10^{-1} mg / ml) which induce arrhythmia beginning.

The curve of evolution of the amplitude of spontaneous contractions in cardiac function of the logarithm of the concentrations of AEHS has a sigmoid shape with a CE100 around 10^{-1} mg / ml. Equal to the CE0 around 10^{-7}mg / ml, and the EC50, around 10^{-4} mg / ml figure 2.

The curve of evolution of the frequency of spontaneous heart contractions in the logarithm of the concentration of AEHS has a sigmoid shape with an equal CE100 around 10^{-1} mg / ml, a CE0 around 10^{-6} mg / ml and a EC50 around $3x10^{-2}$ mg / ml (Fig. 3).

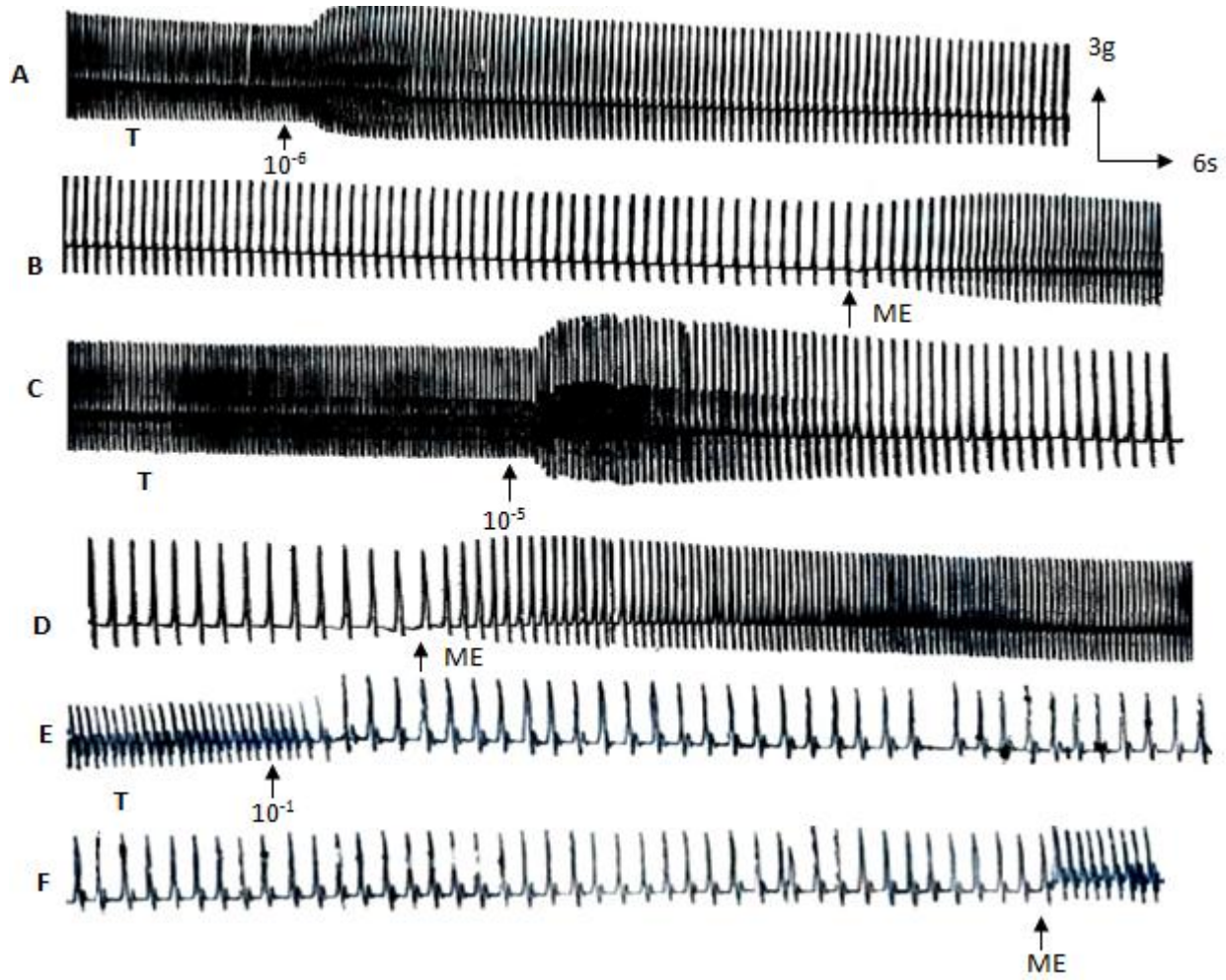

Figure 1: AEHS effect on mechanical activity of isolated rat heart

A: Recording control (T) monitoring the effect of AEHS 10^{-6} mg / ml
B: following (a) and back to Mac Ewen (ME).
C: Recording control (T) monitoring the effect of AEHS 10^{-5} mg / ml
D: Result of (c) and back to Mac Ewen (ME).
E:Recordingcontrol(T) monitoring the effect of AEHS to 10^{-1}mg / ml
F: Following (e) and back to Mac Ewen (ME).

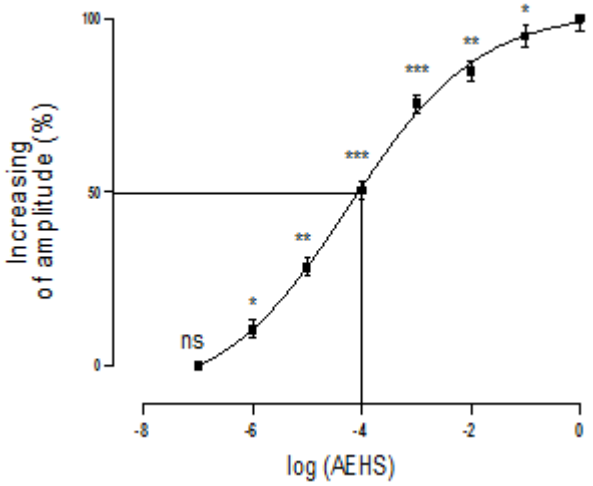

Figure 2: Curve of increasing the amplitude of the mechanical activity of isolated rat heart according to the concentration of AEHS. * p<0.05; ** p<0.01; *** p <0.001 and n = 5.

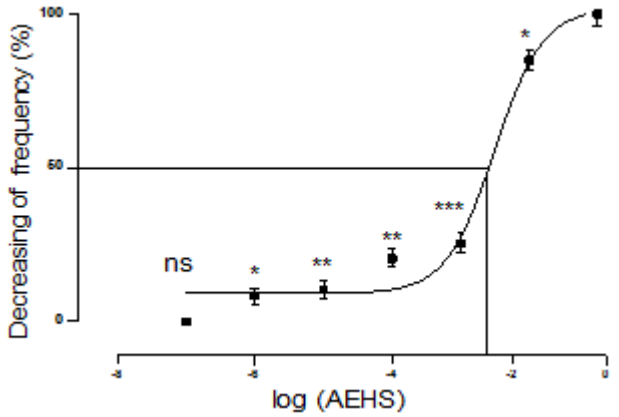

Figure 3: Curve of decrease in the frequency of heart contractions as a function of the concentration of AEHS. * p<0.05; ** p<0.01; *** p <0.001 and n = 5.

3.2 Effect of EAHS on the mechanical activity of the heart isolated from rat in medium hyposodic

In physiological medium of reference the concentration of salt ion is 130 mM. This quantity in this study is reduced to 117 mM (of 10%).

One records a transitory increase in the amplitude and heart rate in this condition. When AEHS is added to 10^{-8} mg/ml no change in amplitude and the frequency is recorded.

From 10^{-7} to 10^{-5} mg/ml the amplitude increase from 10 to 25%, concomitantly the frequency decrease from 11 to 24% significant manner ($p<0.05$).

From 10^{-2} to 10^{-1} mg/ml the amplitude increase from 40 to 50%, concomitantly the frequency decrease from 33 to 50% very significant manner ($p<0.01$).

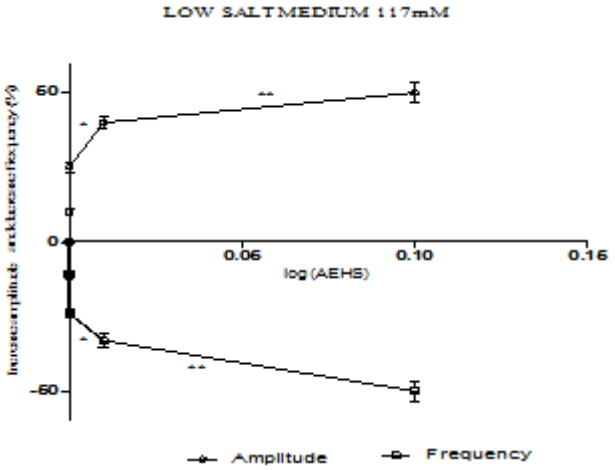

Figure 4: Curve of evolution of the amplitude and the frequency of cardiac contraction in low salt medium. *P<0.05; **P<0.01. n = 5.

3.3 Effect of AEHS on the mechanical activity of the heart isolated from rat in medium hypopotassic

In a physiological medium whose concentration in ion potassium was reduced of 10% of potassium ion, the amplitude of the cardiac contractions increases transitorily (+40%), then decreases gradually. The frequency of the cardiac contractions also decreases by approximately 8%. So in this same physiological medium hypopotassic, when AEHS with 10^{-2} mg/ml is added, the amplitude of the cardiac contractions increases more. The contractile force of the heart increases to 30%. The frequency of the cardiac contractions on the other hand decreases gradually to 25%.

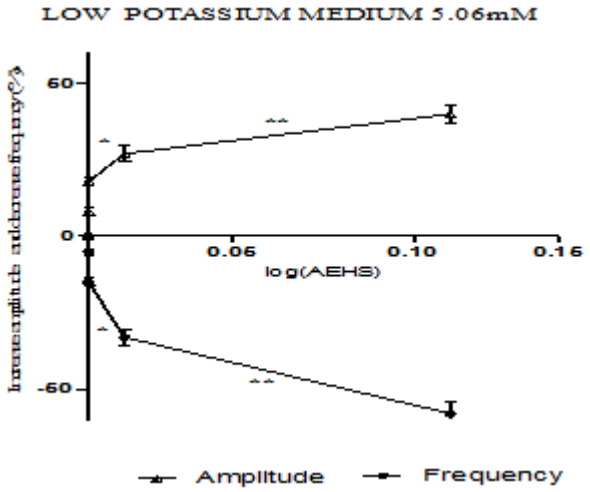

Figure 5: curve of evolution of the amplitude and the frequency of cardiac contraction in low potassium medium. *p<0.05; **p<0.01; n= 5

3.4 Effect of AEHS on the contraction of the rat isolated heart in hypocalcic medium

In a physiological solution whose concentration in ion calcium is low of 5 % of the concentration of reference, the cardiac contractions decrease regularly and gradually. After one minute the amplitude of contractions falls of 75%. The frequency of the cardiac contractions also decreases by 25%.

Added AEHS with 10^{-2} mg/ml to the physiological medium increases the amplitude of the cardiac contractions gradually. The contractile force of the isolated heart increases of 20%. The frequency of the cardiac contractions on the other hand, decreases at the same time of approximately 30%.

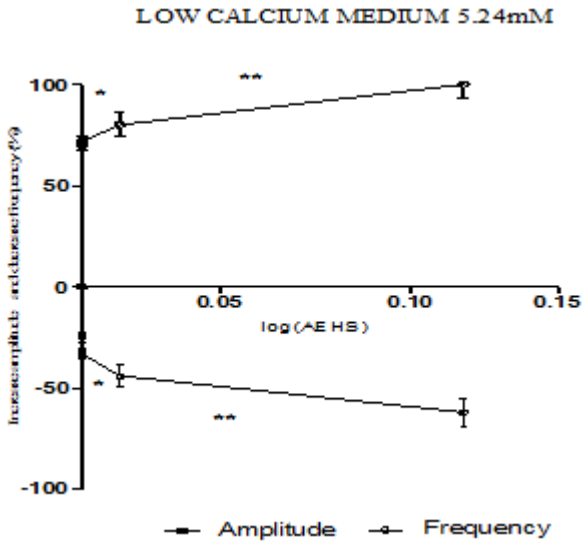

Figure 6: Curve of evolution of the amplitude and the frequency of cardiac contraction in low calcium medium. *p<0.05; **p<0.01; n= 5

3.5 Effect of AEHS on the contractions of the rat isolated heart in hypercalcic medium

In a physiological medium whose concentration in calcium ion was high (125%), the cardiac contractions increase in amplitude and frequency. The contractile force raises to27%. In this medium hypercalcic added AEHS with 10^{-5} mg/ml (low dose in order to reduce the extent of the calcium effect), the amplitude of the cardiac contractions increase more. The amplitude raise approximately to 20% compared to the medium hypercalcic only after 30 seconds. The frequency of the cardiac contractions are not signifcant variated compared to the hypercalcic medium only.

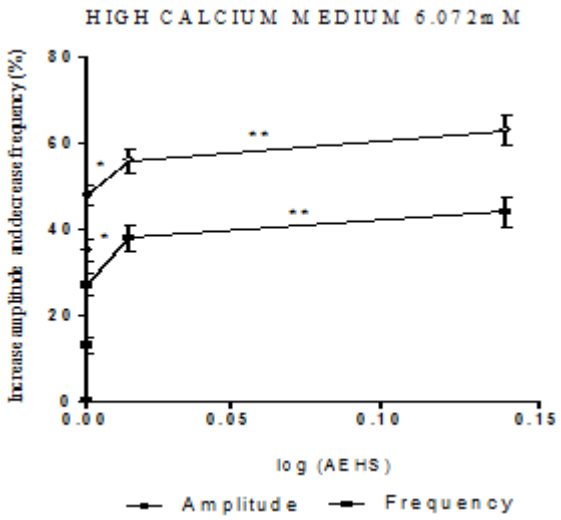

Figure 7: Curve of evolution of the amplitude and the frequency of cardiac contraction in high calcium medium. *p<0.05; **p<0.01; n= 5

4. Discussion

AEHS increases the contractile force and concomitantly decreases the frequency as Mansonia altissima (Sterculaceae) described by Aka (1980) as Salvia amplexicaulis (Lamiaceae) described by Ufuk et al. (2001) on the cardiovascular parameters of mammalians.

The element responsible for the increasing of the amplitude of the cardiac contractions is the variation of the calcium influx (Shannon et al. 2003). The models of study (variation of the ionic concentrations) were thus successively created to highlight the activation or the inhibition of calcic flow entering through the membrane of myocardic fibers.

Thus in medium hypocalcic, where one records a reduction in the amplitude and frequency of the cardiac contractions, EAHS causes an increase in the amplitude and a reduction of the frequency of the cardiac contractions. These effects are similar to those of the digitaliques ones (Gralinski et al. 2013). Here, calcium cytosolic thus increased in spite of a reduction of the calcic impulse.

In medium hypercalcic the stressing of inotropes the positive effect of AEHS, would come from the prompt increase in the cytosolic concentration of calcium.

The model of the medium hyposodic rests on the consequent fall of the depolarization of the cellular membrane which induces a reduction of the calcic impulse. The positive inotropic effect armature by AEHS, is thus probably due, under our experimental conditions, with the accumulation of the Ca2+ ions in the medium cytosolic following the blocking of the pump Na+/K+, person in charge of the opposite movement of the Na+/Ca2+ exchanger (Levi et al. 1997). This explanation also relates to the effects observed in hypo and hypercalcic mediums.

With regard to the marked negative chronotropic action of AEHS observed under our experimental conditions, it could come at the same time from the reduction of the sodic gradient and the inhibition of the pump Na+/K+ (Vanheed and Hemptine 1992), both persons in charge of the reduction in the exitability of the membrane of the cardiac cell. These two phenomena also cause a deactivation of the potassic current leaving responsible for the negative chronotropy (Stinner et al. 1989). It has been discovered that circulating endogenous cardiotonic steroid bind to Na+/K+ Atpase, activate cell signaling pathways and regulate diverse cellular functions (Silva and Soares 2012).

Admittedly, in medium hypopotassic, the positive inotrope effect recorded would be due to a calcic impulse supported by the depolarization of the membrane.

The decreased of frequency can be resulted by the relationship between iK, cK and the glycosides. The glycosides need not change alpha iK to effect positive inotropy in ventricular muscle (Browning et al. 1981).

Ca2+ thus allotted to the inversion of the activity of the Na+/Ca2+ exchanger with an increase in intracellular Ca2+. The resting membrane potential is continuously depolarized over the entire range of K+ concentration. It had been showed the antagonism between the glycoside and K+ (Bachmaier et al. 1985). Due to the presence of a Na+/Ca2+ antiport, a rise in intracellular Na+ also results in a rise in a consequent rise intracellular Ca2+. Most of this rise in Ca2+ is taken up into the sarcoplasmic reticulum and then released into the cytoplasm upon stimulation by an action potential (Foster and Coetze 2016). This larger Ca-release from the sarcoplasmic reticulum results in a stronger force of contraction (Ahmed et al. 2009; Katzung 2012).

5. Conclusion

These observations would confirm the glycoside type effect of AEHS. Previous work has shown the presence of aglycone compounds such as flavonoids glycosides in the plant. Our work confirms the effects of these compounds on the isolated heart of rats.

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Abbreviations

AEHS : aqueous extract of Hibiscus sabdariffa
mg/ml : milligram per milliliter
n : number of experiment