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RESEARCH ARTICLE

INVESTIGATION AND MECHANISMS OF ACTION OF THE AQUEOUS EXTRACT OF *HIBISCUS SABDARIFFA* (AEHS) ON THE AORTA ISOLATED FROM GUINEA PIG AND ON BLOOD PRESSURE OF RABBIT

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ABSTRACT

Objective: *Hibicus sabdariffa* L. is traditionally used in Africa to treat several ailments and associated diseases. Despite the extensive use of these plants in traditional health care, literature provides little information regarding its pharmacological properties even if in the last three decades, a lot of concerted efforts have been channeled into researching this local plant with pharmacological values effects. This work was therefore designed to investigate the hypotensive and antihypertensive therapeutic values effects of aqueous extract of *Hibicus sabdariffa* L., in order to assess the efficiency of the traditional use of this Malvaceae from Ivory Coast.

Methods: The aorta of guinea pig is isolated and dose-effect tests of AEHS are recorded. Different doses of AEHS are injected to the rabbit and the effects recorded. Several drugs with a known pharmacological effect are injected in order to determine the mechanism of action of AEHS.

Results: Concentrations ranging from 10^{-6} mg/ml and 16 mg/ml induce a dose-dependent vasorelaxation. It was estimated that vasorelaxation becomes meaningful between 10^{-2} mg/ml and 1 mg/ml (25 to 48 %). Under, non-significant difference between 10^{-6} and 10^{-4} mg/ml (0 to 4% of relaxation). The vasorelaxation becomes highly significant between 2 mg/ml and 16 mg/ml (from 68 to 100 %). The maximum relaxation is reached from 8 mg/ml, concentration from which the resting tone of the muscle not changing significantly (3.9 to 4.10^{-2} mm). The effects of the aqueous extract of *Hibicus sabdariffa* were observed on blood pressure of the rabbit. The average normal blood pressure measured on animals was 78 ± 3.8 mmHg. The injection at doses from 10^{-5} g / kg b.w. to $5x10^{-1}$ g/kg b.w. shaped to a decrease of 1 to 100%, from 78 ± 3.6 to 38 ± 1.8mmHg, as drop from 1 to 100%. It was found that atropine has no effect on hypotension induced by AEHS. A dose of AEHS 6 x 10^{-6} g/kg b.w. that has no effect as single treatment, together with simultaneously injected acetylcholine in a dose of 10^{-6} mg / kg b.w., has been resulted the production of hypotension near 48.71%. Under these treatments blood pressure of the animal passes from 78 ± 3.6 mmHg to 40 ± 2.1 mmHg. Adrenaline injected at a dose of 10^{-4} mg / kg b.w. causes hypertension. Blood pressure goes from 78.0 ± 3.5 mmHg to 118.0 ± 3.5 mmHg. This pressure is suppressed by simultaneous injection of AEHS in dose of $8x10^{-3}$ g / kg b.w.

Conclusion: It was suggested that AEHS has an hypotensive effect in normal animals and antihypertensive effect to hypertensive animals.

INTRODUCTION

The aqueous extract of *Hibicus sabdariffa* (AEHS) calyx called in the West African sub-region 'Bissap or Bassap' is a very popular drink. It's differently named Karak or Karkadè the in north Africa, Wandjo or Omutete in central Africa.

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About 75 to 80% of the world population use herbal medicines, mainly in developing countries, for primary health care because of their better acceptability with human body and lesser side effects. In the last three decades, a lot of concerted efforts have been channeled into researching the local plants with hypotensive and antihypertensive therapeutic values (Tabassum, 2001). *Hibiscus sabdariffa* L. extracts contain hibiscus acid and flavonoids such as cyanidin 3-rutinoside, delphinidin 3-sambubioside, cyanidin 3-sambubioside, cyaniding-3-glucoside, delphinidin 3-glucoside (Sindi, 2014).

The bioactive compounds with phenolic nature ussualy have antioxidant, antihypertensive, antihyperglycemia and anti-inflammatory properties (Hopkins, 2013) and we suppose that *Hibiscus sabdariffa* L. flowers extracts can have it. The diuretic and urinary antiseptic effects of *Hibiscus sabdariffa* L. were recognized (Pousset, 1989). Authors such Tsen and Hsu (Tseng, 1998), argue that the substance induces apoptosis of leukemia cells in mice. In traditional medicine, the leaves of *Hibiscus sabdariffa* have long been used to heal the wounds and the gales (Sharaf et al., 1962 an d Watt, 1962). The mixture of *Olea europea* L. leaves and *Hibiscus sabdariffa* L. flower extracts has been exerted higher cytoprotective effects and antioxidant activities. A good potential of this mixture for pharmaceutical and nutraceutical application is connected with synergistic effects of the single phytochemicals in plant extracts (Micucci, 2015). In the hypertensive subjects the aqueous extract of *Hibicus sabdariffa* can show antihypertensive effect (Mohamed and Haji, 2007) described a draft of the mechanism of action of antihypertensive substance. Hypertension is a disease prevalent in Africa and many other countries in the world. The blood pressure is the result of force shrink sleeving of the court to the product of the resistance of the blood vessels. That is why any substance or medication able to vary this arterial blood pressure must be able to change one of the two settings see the two concomitantly. Using electrophysiological techniquess we would like to demonstrate the effect of this just on the isolated aortic from guinea pig and normo-tended rabbits.

MATERIALS AND METHODS

Extraction of AEHS

The flowers of the plant are harvested in Seguela located 450 km of Abidjan to the northwest of the Cote d'Ivoire. Fifty grams of crushed dried petals are added to a liter of water and leave to macerate for 24 hours under brand magnetic stirrer (IKA-Labortechnik) in a 2-liter Erlenmeyer flask. The solution is filtered successively on hydrophilic cotton then Wattman filter paper to remove impurities of small dimensions as the method of authors [11]. The aqueous filtrate is dehydrated in a rotary evaporator and the pellet BUCCHI put in the oven VENTICELL mark (Medcenter) at 40 ° C to accelerate dehydration. After evaporation, dry and compact base is obtained. The pellet is then scraped crashed into a porcelain mortar. At the end of this operation, the water-soluble powder that is harvested is Hibiscus *sabdariffa* aqueous extract (AEHS). Powder Bissap (Hibiscus *sabdariffa*), once weighed, diluted in saline to form AEHS stock solution of 10 g / ml and / or 10 mg /ml.

Recording of the variation of the basal tone of the guinea pig

Animal were brought from farms located in the sub of Abidjan (Cote d'Ivoire) and were acclimated to laboratory conditions in the Departement of Biosciences of the University Felix Houphouet Boigny for weeks to regulated and harmonize their physiological states, before the experiments. Animals are anesthesia using an injection of ethylurethane 40% by reason of 1g/kg of b.w. An incision at the level of the thoracic cage, allows to release the skin. The rib cage is split laterally for easy access to the court. The aorta is clear of muscle structures that surround is sectioned at the level of aortic arch and to its lower part to the entry of the thorax. The isolated body is deposited in a bucket, containing the physiological solution of glucose and hydrogen peroxide according to the method described by authors (Abo, 1996). The mechanism of survival and of registration includes a tank to isolated body to two buckets, a bath-marie circulating and a generator of air, a transducer, amplifier and a chart recorder. The mechanism of survival of the isolated body is constituted of two buckets with a capacity of 20 ml who immersed in a bain-marie irrigated continuously in tap water by circulating in the closed bath thermostat LaudaEcoline (E 100) in which air generator allows continuous circulation of the oxygen. The tatters of isolated body are cracked laterally and two nodes are facts to symmetrical ends. The nodes allow the fixation of lambeau to bracket of the strain gauge of the recorder by one end and the other end attached to the bottom of the tank to isolated body. The method is similar described by Toshiko et al. (Toshiro, 2002). The increasing doses of AEHS are thus introduced into the solution of survival contained in the tank on a cumulative basis.

Recording the rabbits arterial blood pressure

The rabbits used are raised on farms in Bingerville or in Abobo and put to acclimatize in the animalery of UFR Biosciences. They are of the species Oryctologus *cumuculus* family Leporidae and fed ad libitum pellet produced by a food company. Their weight varies between 1.5kg and 2kg, thereby ensuring their resistance to general anesthesia for the study of blood pressure. Thus, they were kept at constant temperature (24±2°c) with 50-55% of humidity and a photoperiod of 12 hours of daylight and 12 hours of darkness. All procedures were conducted in accordance with the published by the National Institutes of Health.

Electrophysiological technical equipment

The recording of arterial blood pressure of rabbits was performed with a Ludwig mercury manometer according to method described by Ao et al. (Abo, 2000). In accordance with the National Government rules of Cote d'Ivoire, rabbits was anesthetized through intraperitoneal injection with ethyl urethane dosed at 40% at 1 g / kg b.w. His carotid artery was exposed and intubated and connected to the heart through a polyvinyl tube and the manometer, this allowed us to obtain directly the intracarotid pressure which was recorded on a recording paper. The saphenous vein was intubated with a catheter connected to a syringe for administration of pharmacodynamics substances and the extract. Our method of recording the blood pressure is described by Abo et al. (Abo, 2016).

Pharmacodynamic substances

Atropine from Sigma- Aldrich (France); Acetylcholine from Sigma-Aldrich (France); Adrenaline (L-adrenaline) from Fluka (Suisse); Propranolol Sanofi-Avensis (France).

Measure of area under the curve and statistical analysis

The software GraphpadInstat (San Diego CA, USA) was used for statistical analysis of results. Values are given as mean

followed by the standard error of mean (MEAN+SEM). Student-Newmann-Keuls comparison test at the level of α= 0.05 evaluated the different between two means. The software Graph Pad prism 5 (San Diego CA, USA) was used for plotting graphs. Sigmoid curve was drawn after transformation of values of x-axis as decimal logarithm and as a percentage for the y-axis.

RESULTS

Dose-response effect of AEHS on the aorta guinea pig muscle

During this experiment, the aorta of the guinea-pig is isolated and the endothelium maintained intact. Increasing doses of AEHS were added to physiological solution. In physiological solution reference, the tonus of basis is determined to 800 mg (0.8 gf). The concentrations ranging from 10^{-6} mg/ml to 10^{-4} mg/ml do not alter the activity of shrink sleeving aortic muscle (EC0). The (EC0) defined the minimum concentration of the extract (AEHS) having no effect on the aortic muscle. For the doses ranging from 10^{-2} to 8 mg/ml a gradual decline of the tonus of basis in a dose-dependent manner is recorded. This is equivalent to the area of sigmoid curve. As well of 10^{-2} to 1 mg/ml, the percentage of relaxation is included between 25 and 48% (-0.2 gf to -0.384 gf) in relation to the tonus of reference database. The EC50, concentration giving 50 % of relaxation reached between 1 mg/ml) and 2 mg/ml, is determined graphically (Figure). Of 2 mg/ml to 8 mg/ml a significant evolution of the curve is recorded. The percentage of vasodilatation increased from 68 to 100% either of -0.544 gf to -0.8 gf by report to the tonus of reference. The muscle is then completely relaxed. The EC100, effective concentration giving the maximum vasorelaxation is reached at a dose of 8 mg/ml. Beyond the 8 mg/ml, (the double dose of 16 mg/ml), the vasorelaxation is evolved more significantly.

Table 1. Variation of the basal tone of the aortic muscle on average in percentage and as a function of the concentration of AEHS

Doses (mg/ml)	MEAN (percentage)	SEM
10^{-6}	0	0.00
10^{-4}	4	0.00
10^{-2}	25	1.8±0.1
1	48	2.9±0.05
2	68	3.1±0.08
4	99	3.5±0.10
8	100	3.9±0.15
16	100	4.0±0.25

AEHS effect dose -response on normal blood pressure

In this study, increasing doses of AEHS were injected to rabbits, in time intervals of 15 minutes after the effect of each dose. Recordings of Figure 1 show the effect of AEHS on blood pressure in rabbits. The average value of the blood pressure of rabbits registered under the experimental conditions is 78 ± 3.8 mmHg (P <0.05). Variations of this blood pressure based on different concentrations of AEHS injected directly into the vascular system, are recorded in the Table. The extract of AEHS, between 10^{-5} and 5.10^{-3} g/ kg b.w., induced hypotension ranging from 1 to 16 mmHg. Drop in blood pressure ranging from 1 to 33%.

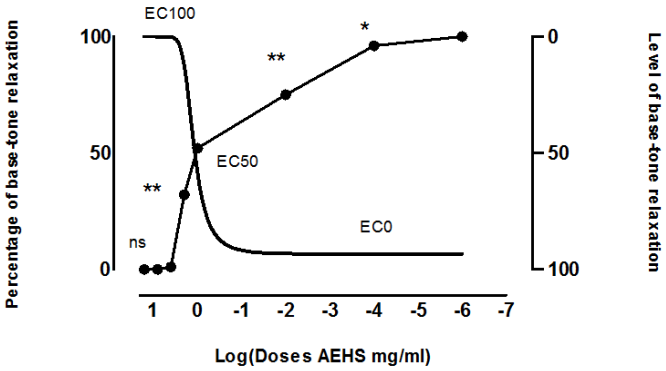

Figure 1. Curves of the variation of the percentage and the level of basal tone of the aortic muscle of guinea pig in a dose-dependent manner of AEHS

However, doses of 10^{-2} to 5.10^{-1} g/kg b.w., induced a hypotension ranging from 20 to 40 mmHg. Drop in blood pressure ranging from 52 to 100%. The maximum blood pressure is raised to 38 ± 1.8 mm Hg for the dose of $5x10^{-1}$ g / kg b.w. This is the highest concentration at which the effect is reversible. So it's the effect 100% efficiency (CE_{100}).The curve of Figure 2 shows the changes in blood pressure of rabbits against the logarithm of the concentration of AEHS. Sigmoid shape, it determines the range of ineffective concentrations (CE_0) for doses less or equal to 10^{-5} g / kg b.w. EC_{50} thus determined is 10^{-2} g / kg b.w.

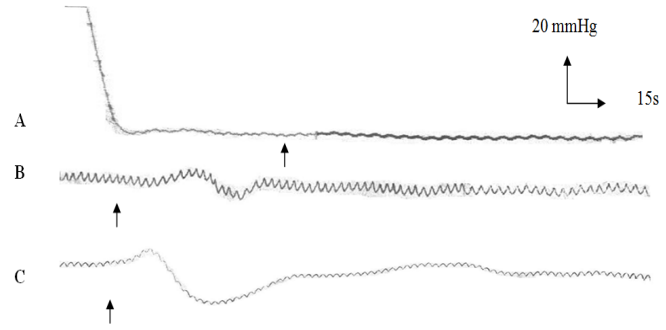

Figure 2. AEHS dose-response effect on the blood pressure of the rabbit. A: recording indicator monitoring the effect of EAHS 10^{-5} g / kg b.w. B: recording indicator monitoring the effect of EAHS to $5x10^{-5}$ g / kg b.w. C: recording indicator monitoring the effect of EAHS to $5x10^{-3}$ g / kg b.w. D: recording indicator monitoring the effect of EAHS to $5x10^{-1}$ g / kg b.w

Effect of interaction AEHS -atropine on rabbit blood pressure

In this series of experiments, AEHS at only dose 10^{-2} g/kg b.w. is injected and increasing doses of atropine in intervals of 10 s. Figure 3 shows the types of records the blood pressure of rabbits in the presence of a dose of AEHS and varying doses of atropine. At a dose of 10^{-2} g / Kg b.w. EAHS results in hypotension of approximately 20 mmHg. The blood pressure of the animal goes from 78 mmHg to 58 mmHg. Simultaneous injection of AEHS (10^{-2} g / kg b.w.) and varying concentrations of atropine ($5x10^{-4}$ and $5x10^{-3}$ mg / kg b.w.) with no specific effect does not change the hypotension induced by AEHS significantly. The curve of variation of the blood pressure of rabbits in the presence of single AEHS and that in the presence of the two substances are juxtaposed Figure 4.

Table 2. Changes in blood pressure in rabbits following injection of increasing doses of EAHS

Doses of EAHS	10^{-5}	5.10^{-5}	10^{-4}	5.10^{-4}	10^{-3}	5.10^{-3}	10^{-2}	5.10^{-2}	10^{-1}	5.10^{-1}
Pressure mmHg	78 ±3.8	73 ±3.6	72 ±3.2	70 ±3.0	66 ±2.8	62 ±2.6	58 ±2.4	46 ±2.2	42 ±2.0	38 ±1.8
Percentage increase	1	8	11	16	27	33	52	79	90	100

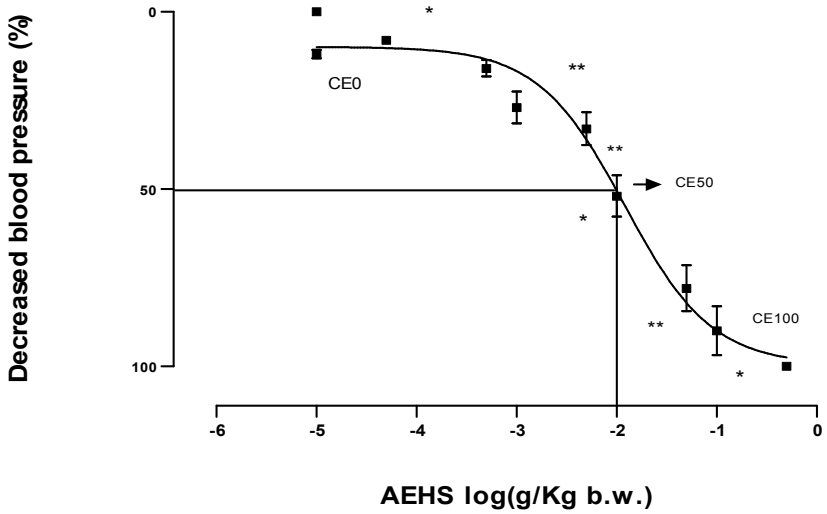

Figure 3. Curve of the variation of the percentage of decrease in blood pressure as a function of the logarithm of the EAHS concentration (n = 10 * P <0.05; ** P <0.001)

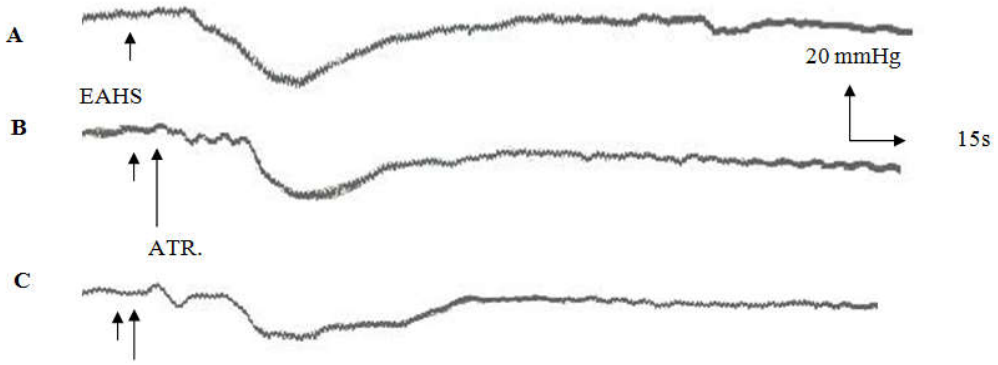

Figure 4. Interaction AEHS-atropine on the rabbit blood pressure
A: Check the witness before the arrow, monitoring the effect of AEHS 10^{-2} g / kg bw
B: Registration witness before the arrows monitors the effects of AEHS 10^{-2} g / Kg b.w. and atropine $5x10^{-4}$ mg / kg b.w
C: Control Register before the arrows monitors the effects of AEHS 10^{-2} in g / kg b.w. and atropine 5.10^{-3} mg / kg b.w

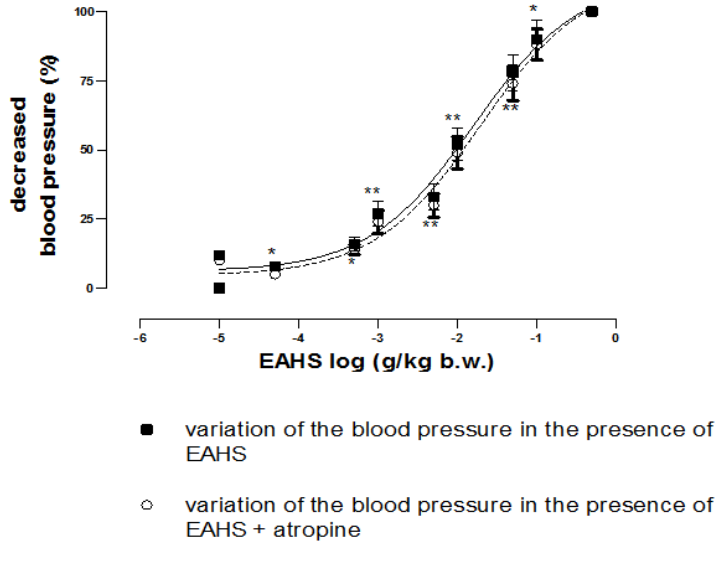

Figure 5. Curves of variation of the blood pressure according to increasing doses of AEHS in comparison with that of the simultaneous injection of AEHS and atropine ($5x10^{-3}$ g / kg b.w.); (n = 5 and * P <0.05; ** P <0.001).

Effect of the interaction AEHS- acetylcholine on rabbit blood pressure

In this series of experiments, AEHS is injected at ineffective dose of $6x10^{-6}$ g / kg b.w., which therefore does not cause low blood pressure (Figure 6A). Furthermore, in a second experiment, acetylcholine is injected at a concentration of 10^{-6} mg / kg bw, which also has no effect on the blood pressure of the rabbit (Figure 6B). By cons when the two substances at concentrations above, are injected sequentially over 10 s period, hypotension is developed which can reach 40 mmHg or a reduction of 48.71% of the normal blood pressure (Figure 6C) and this, whatever the order of administration of these substances.

Effect of AEHS on the hypertension induced by adrenaline

The purpose of this study was to measure the effect of AEHS on hypertension induced by adrenaline. Adrenaline was injected at a concentration of 10^{-4} mg / kg of body weight, causes an hypertension of 30 mmHg. Blood pressure goes from 78.0 ± 3.5 mmHg to 118.0 ± 3.5 mmHg (Figure 7A). When AEHS was injected at a dose of $6x10^{-3}$ g / kg b. w., 10 s intervals after adrenaline, the hypertension reduced. If, AEHS at $8x10^{-3}$ g / kg b.w. was injected before adrenaline (10^{-4} mg / kg b.w.) induced hypertension was almost abolished (Figure 7B; 7C).

Interaction propranolol-adrenaline effect on rabbit blood pressure

As in the previous case, the injection of adrenaline to 10^{-4} mg / kg body weight results in an increase in blood pressure of 40 mmHg (Figure 8A). If in the same experimental conditions, propranolol, which has no intrinsic specific activity (ISA), is injected at a dose of 10^{-4} mg / kg b.w. no change in blood pressure of the rabbit is recorded (Figure 8B). Successive injection of epinephrine and propranolol at the same concentrations as previously deletes the hypertension induced by epinephrine (Figure 8C).

DISCUSSION

This experimental work shows that AEHS lowers the blood pressure of the rabbit dose -dependent manner. The effect of AEHS is thus comparable to that of acetylcholine at the level of the blood pressure of mammals (Vanhoutte *et al*., 1975; Bolton *et al*., 1979; Peralta, 1987), and certain natural substances such as *Mareya micrantha* (Abo, 2000). The evolution of the change in blood pressure induced by AEHS shows that the mechanisms of action of this substance pass through a receptor activation. These receptors are not to type muscarinic cholinergic, since atropine does not inhibit induced hypotension.

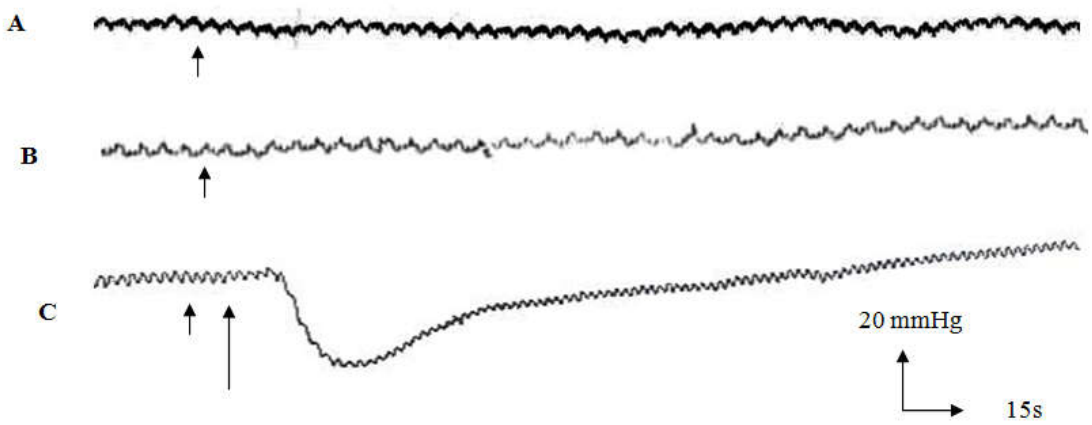

Figure 6. Effect of AEHS-acetylcholine interaction on rabbit blood pressure
A: Check the witness before the arrow, monitoring the effect of AeHS to $6x10^{-6}$ g / kg b.w.
B: Recording witness before the boom, followed by the effect of acetylcholine 10^{-6} g / kg b.w.
C: Recording witness before the arrow, monitoring the effect of acetylcholine effect to 10^{-6} g / kg b.w. and EAHS to $6x10^{-6}$ g / kg b.w.

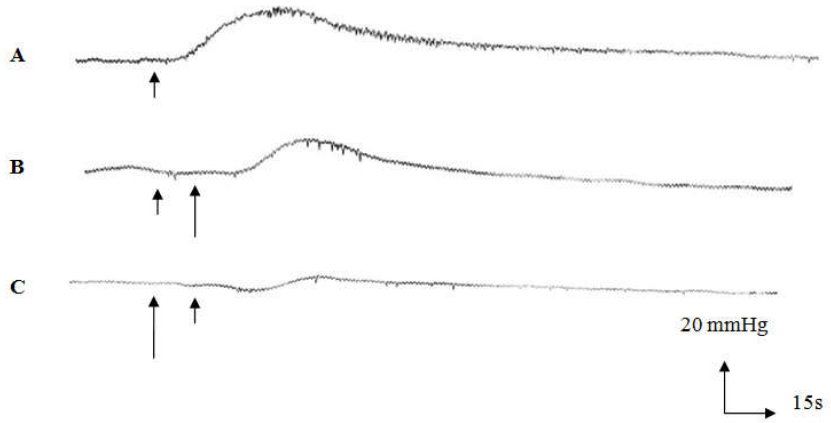

Figure 7. AEHS adrenaline interaction on blood pressure rabbit
A: recording lamp followed by injection of adrenaline to 10^{-4} mg / kg bw
B: recording lamp followed by injection of adrenalin to 10^{-4} mg / kg bw and the injection of AEHS to $6x10^{-3}$ g / kg bw
C: recording lamp followed by injection of EAHS to $8x10^{-3}$ g / kg bw and the injection of adrenaline to 10^{-4} mg / kg bw

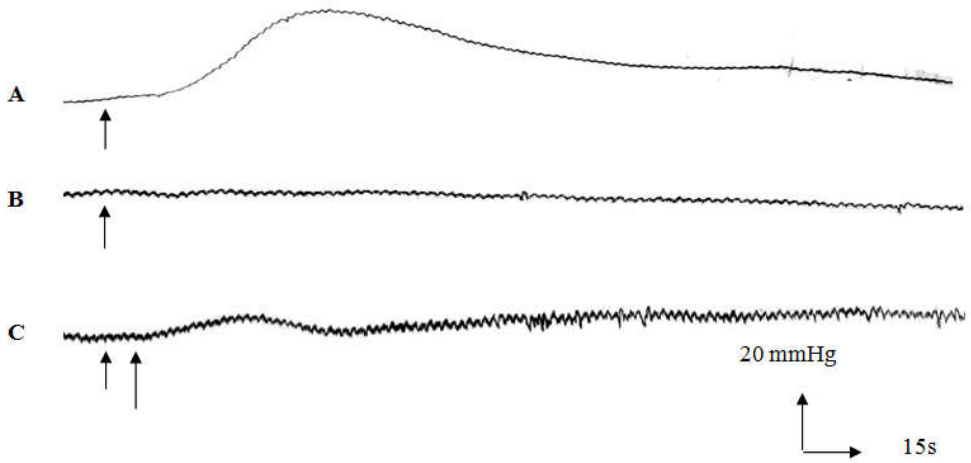

Figure 8. Propranolol-adrenaline interaction on the blood pressure of rabbits:
A: recording lamp followed by injection of adrenaline to 10^{-4} mg / kg bw
B: recording lamp followed by injection of propranolol to 10^{-4} mg / kg bw
C: recording indicator followed by injection of propranolol to 10^{-4} mg / kg bw and the injection of adrenaline to 10^{-4} mg / kg bw

The simultaneous action of AEHS and acetylcholine at none significant doses induced hypotension in the rabbit. These two products may have neighboring receptors or receptor nearby activities and synergistic actions. Indeed, adrenaline - AEHS interaction demonstrates the inhibition of the effect of adrenaline same effect as propranolol. The propranolol which is a β-blocker has similar effect with AEHS. It's known that AEHS contains the active ingredients of β-blocker type. The adrenergic β-receptors type are preponderant at the vascular level. The inhibition of these receptors explains the observed effects. According to authors, (Davis, 2009), phenolic compounds, hydrocarbons, unsaturated fatty acids, amino acids and many other bioactive substances can participate in the formation of many glycosides contained in the plant. The cardiac glycosides such as ouabain and digoxin increase the sodium and calcium content of smooth muscle cells, so inducing arterial vasoconstriction and a rise in blood pressure. A slight vasodilation of resistance vessels followed by a fall in diastolic blood pressure could be a contributing factor for the beneficial effects of cardiac glycosides in patients with congestive heart failure.

This vasodilation may be caused by central (neurohumoral) effects of digitalis glycosides (Kovinich, 2010). However, the effect of glycosides on the smooth muscle vascular is a vasorelaxation due to a production of the nitrogen oxide (NO). The endothelium inner layer of the blood vessels regulates the vasomotricity via controlling the production of NO. In effect, the eNOs produced by the endothelial cells catalyzed the synthesis of nitrogen oxide NO (or nitric oxide) which acts on the smooth muscle cells vascular as vasodilator. Therefore, it has been showed that treatment of endothelial cells bovine by the cyanidine-3-glucoside (Cy-3-gluc) during 8 hours reinforced the expression of the protein (enzyme) eNOs in a manner dependent on the dose (Kirch, 2001). The potential effects of NO are a decrease of the arterial pressure (antihypertensive medications), a barrier to fatty deposits on artery walls (anti-atherogenic) and an activity anti-thrombolytic. By measuring *in vitro* relaxation of the coronary arteries of pigs subjected to variable doses of extracts of pigments, a dose-dependent vasorelaxation under effects of aronia and blueberry extracts but not extract of elderberry have been observed (Xu, 2004).

The study also shows that the action resulted from the production of NO by the endothelium as described by authors (Bell, 2006). We suppose that vasorelaxation obtained with the AEHS would result from the production of NO by the endothelium. Thus, the β-blocker effect combined to vasorelaxation through the aorta explains the observed recordings.

Conclusion

This study shows that AEHS has a good therapeutic potential and we notice a relationship between vasorelaxation effect and antihypertensive effect. But its first utility is to supply the deficient of vitamin C and iron to patients. Further, we advise the continuation of work to isolate the beneficial active ingredients for hypertensive patients.

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