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Original Article

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Abstract

The present study was undertaken to investigate the cardiovascular and the *in vitro* antioxidant activities of the methanol extract of the stem bark of *Erythrina senegalensis* (EMES), a plant used traditionally in Cameroon against arterial hypertension. The cardiovascular effects of EMES (11-44 mg/kg) were evaluated *in vivo* on normotensive rat arterial blood pressure and on rabbit's electrocardiogram (5.5- 55 mg/kg) while the *in vitro* study (10^{-10} - 1 mg/ml) on myocardial contractile performance was evaluated on rat isolated heart. The antioxidant activities of EMES (1-1000 µg/ml) were investigated on 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2)-induced haemolysis, nitric oxide (NO) production, as well as its reducing power. The intravenous injection of EMES provoked in anaesthetized normotensive rats, a dose dependent immediate reduction in arterial hypertension. The mean arterial blood pressure dropped by 11.29, 19.84 and 24.42 % at respective doses of 11, 22 and 44 mg/kg. When administered intravenously in rabbit EMES significantly reduced the heart rate, amplitudes of P wave and QRS complex while significantly increasing the duration of the QT interval. EMES exhibited a concentration dependent reducing power, antiradical activity on DPPH (EC_{50} of 46.9 µg/ml) and a significant ($p<0.001$) antioxidant effect on H_2O_2-induced haemolysis. EMES also substantially increase NO production. In conclusion, EMES exert immediate hypotensive effect which might be attributed to its negative inotropic and chronotropic effects. Its *in vitro* antioxidant activities could be beneficial for the treatment of cardiovascular ailments.

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Introduction

Erythrina senegalensis DC (Fabaceae) also known as "coral tree" is a thorny shrub small tree with bright red flowers arranged in panicles at the ends of the branches, found in Sudanese savannah regions (Malgras, 1992). Coral tree is widely used in the tropics and subtropics as street and park tree, especially in drier areas. It has long been used as a traditional medicine for the management of amenorrhea, dysmenorrheal, malaria, infection, wound and body pain, headaches and body weakness, gastric ulcer, diarrhea and constipation (Togala *et al*., 2008). The decoction of its stem bark is traditionally used by Bamoun population for the treatment of hepatitis and jaundice (Moundipa *et al*., 2002). In the Western region of Cameroon, the plant is also use to treat cardiovascular disease such as hypertension. In addition, decoctions of E. senegalensis materials are used in Mali as diuretic (Togola et al., 2005). Chemicals elucidated from *E. senegalensis* include: prenylflavonoids, 8-prenylleutone, auriculatin, erysenegalensein O, erysenegalensein D, erysenegalensein N, derrone, alpunumisoflavone, and 6,8- diprenylgenistein (Fomum *et al*.,1987; Oh *et al*., 1999). Many pharmacological works have demonstrated the antimicrobial (Koné *et al*., 2004; Doughari, 2010), antiplasmodial, analgesic and anti-inflammatory (Saidu *et al.*, 2000), hepatoprotective (Donfack *et al*., 2008), spasmolytic and anti-diarrhea (Otimenyin, 2010) activities of various extracts from this plant. The acute and subchronic oral toxicity of the aqueous extract from the stem bark of *E. senegalensis*, recently assessed, demonstrated no sign of toxycity (Atsamo *et al*., 2011). Presently, cardiovascular disease is one of the most important public health concerns, accountable for morbidity and mortality. Increased vascular oxidative stress is involved in the pathogenesis of hypertension mainly by mediating aberrant contractility (Touyz and Schiffrin, 2004; Wichitsranol *et al*., 2011). Hence, substances susceptible to neutralize free radicals are now regards as a supplementary therapy of many diseases including cardiovascular illness. To the best of our knowledge, there is no pharmacological study on the effects of *E. senegalensis* stem bark extract on the cardiovascular system. The present study therefore aimed at determining the cardiovascular and the *in vitro* antioxidant effects of the methanol stem bark extract of *Erythrina senegalensis.*

Materials and Methods

Plant material and extract preparation

The stem barks of *Erythrina senegalensis* were collected in Dschang (West region of Cameroon) in February 2007. The botanical identification was done at the National Herbarium in Yaoundé; where a voucher specimen is conserved under the reference number 35259Y. The plant material was shade dried and ground into a fine powder. The methanol extract was prepared by macerating 500 g of the powder in 3 l of methanol for 48 hours. The mixture was filtrated on Whatman No 1. The residue plant material was re-extracted in methanol for 24 hours. The two filtrates obtained were pooled and concentrated in a rotary evaporator under low pressure conditions to give 25 g.

Animals

Three months old Swiss mice of either sex weighing between 20 and 30 g were used for acute toxicity test while Wistar rats of the same age but weighing 200 to 280 g were used for isolated heart tests and for blood pressure recording. Both species were obtained from the animal house of the Faculty of Science, University of Dschang, Cameroon. Rabbits of either sex, aged between three and four months and weighing about 2 kg, were obtained from the animal house of the Laboratory of Nutrition and Pharmacology of the Felix Houphouet Boigny University, Abidjan, Ivory Coast. They were used for electrocardiogram studies. All animals were maintained under laboratory conditions (23 ± 2 °C, 12 h: 12 h light/ dark cycle, frequent air change) and had free access to tap water and food. All procedures used in the present study followed the "Principles of Laboratory Animal Care" from NIH Publication No. 85-23 and were approved by the Animal Ethics Committee of our University.

Acute toxicity of EMES

Mice were divided in 7 groups of 10 animals each (5 males and 5 females). They were fasted for 14 h and treated as follow: The first group (control group) was treated with distilled water while the remaining groups (2-7) received orally in a single administration, EMES at the doses of 1.25, 2.5, 5, 7.5, 10 and 12.5 g/kg respectively. Animals were observed for general behavioural change and mortality for a period of 14 days post treatment (Atsamo *et al*., 2011).

Effects of EMES on mean arterial blood pressure

Normotensive rats were prepared for acute blood pressure studies using a method previously described by Nguelefack *et al*. (2008). Briefly, animals were anaesthetized by intraperitoneal injection of urethane at the dose of 1 g/kg. Arterial blood pressure was measured directly from the left carotid artery via an arterial cannula connected to a pressure transducer (Ugo Basile PRC 21K-10). Blood pressure variations were detected and recorded on Ugo Basile unirecord model 7050. After a stabilization period of 30 minutes, a given dose of the test substance was injected into the right femoral vein. The effects of EMES (11, 22 and 44 mg/kg) was followed for an hour after injection and the mean arterial blood pressure (MABP) was calculated.

Assessment of the inotropic and chronotropic effects of EMES on isolated heart preparations

The experimental procedure used to evaluate the isolated heart activity was as previously described by Kouakou *et al*. (2007). Briefly, rat was anesthetized by an intraperitoneal injection of urethane at the dose of 1 g/kg and injected with heparinized physiological solution (2500 UI, 0.2 ml/100 g body weight) through the abdominal vein. After 2-3 minutes, the heart was rapidly removed and placed in another heparinized physiological solution.

The isolated heart was perfused with Mac Ewen solution of the following composition (mM): NaCl 147; KCl 5.6; $CaCl_2$ 2.6; NaH_2PO_4 0.66; CO_3NaH 11.9; $MgCl_2$ 0.24; $C_6H_{12}O_6$ 11 with a pH adjusted to 7.4. The solution was continuously bubbled with air (95% O_2, 5% CO_2). EMES was dissolved in Mac Ewen solution. The apex of the heart was fixed by a fine clip and linked to the needle of the cardiograph for recording. EMES was used at the concentrations of 10^{-10}, 10^{-8}, 10^{-6}, 10^{-4}, 10^{-2} and 1 mg/ml. After each treatment, the heart was washed by perfusion fluid for 10 min, time within which the baseline recording is achieved, and the second dose was then given. The recording before the direct perfusion of extract was considered as baseline reading for each dose (control).

Effect of EMES on global electrical activity (ECG) of the heart

The rabbit was anesthetized by intraperitoneal injection of urethane at the dose of 1 g/kg body weight. The saphenous vein was dissected and intubated with the help of a catheter joint to a syringe containing heparinized Mac Ewen solution. The plant extract (5.5-55 mg/kg) were injected to the animal by this way as previously described by Traoré *et al*. (2004). The armpits of the anterior limbs and the groin of the posterior limbs were shaved. After cleaning the shaved parts of the four limbs of the animal destined to receive the electrodes of registration with 90 % ethanol, electrolytic dough was applied. The four electrodes were put and bound to the four sockets of the registration cable connected to the electrocardiograph (CARDIOFAX EKG-6851K).

The ECG of the rabbit was recorded one minute after drug injection from the DIII derivation of the standards or bipolar Einthoven derivations as previously described by Traoré *et al*. 2004. The registration of the ECG was done on thermo sensitive paper, at constant speed (25 mm/s).

Evaluation of the antioxidant activities of EMES

DPPH radical scavenging test

The radical scavenging potential of the extracts was evaluated by using DPPH solution as described by Nguelefack-Mbuyo *et al*. (2010). Ascorbic acid was used as reference drug. Briefly, 150 µl of varying concentration (1–300 µg/ml) of extracts or ascorbic acid was added to 850 µl of methanol and

the absorbance read at 517 nm against a blank made up of methanol (A_1). Then 500 µl of DPPH (0.063 mg/ml in methanol) was added to the medium, the whole was incubated in dark for 20 minutes and the absorbance (A_2) measured spectrophotometrically at 517 nm. The experiment was done in triplicate and antioxidant activity (%) was calculated using the following equation:

Antioxidant activity = $[(A_2\text{-}A_1)_{control} - (A_2\text{-}A_1)_{sample}] \times 100/ (A_2\text{-}A_1)_{control}$

Where control tubes are those containing DPPH without any antioxidant substance. The EC_{50} of each substance was calculated using Graphpad Prism 5.01.

Effect of Erythrina senegalensis methanol extract against H2O2-Induced Haemolysis

The effects of *Erythrina senegalensis* on H_2O_2-induced haemolysis were assessed as described by Ko et al. (1997). Briefly, heparinized blood samples were collected from healthy anesthetized rats via the abdominal aorta and centrifuged at 1000 rpm for 10 minutes. Red blood cells (RBC) were isolated and washed three times in PBS (NaCl: 125 mM and NaH_2PO_4: 10 mM, pH 7.4). These cells were then suspended at 2% hematocrit in PBS. 800 µL of the RBC suspension were incubated in absence or in presence of plant extracts or ascorbic acid (1 – 300 µg/mL) for 15 min at 37 °C. Then, 100 µL of H_2O_2 solution was added in the medium at the final concentration of 7.5 mM. After a 4 h incubation period, aliquots of the mixture was taken out, diluted with 20 volumes of saline, and centrifuged at 3500 rpm for 10 min. The degree of haemolysis (A) was determined spectrophotometrically at 540 nm with a Helios Epsilon spectrophotometer. To yield the absorption (B) of a complete haemolysis, an aliquot of the RBC suspension was treated with 20 volumes of ice-cold distilled water and after centrifugation, the absorption was measured at the same wavelength. The percentage of haemolysis was then calculated: Haemolysis (%) = (A/B) × 100.

Reducing power (RP)

The reducing power was determined by the method of Athukorala *et al.* (2006) with a little modification. 0.2 ml extract was mixed with 0.5 ml of phosphate buffer (200 mM, pH 6.6) and 0.5 ml of potassium ferricyanide (30 mM) and incubated at 50°C for 20 min. Thereafter, 0.5 ml of trichloroacetic acid (600 mM) was added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The supernatant (0.5 ml) was mixed with 0.5 ml of distilled water and 0.1 ml of $FeCl_3$ (6 mM) and the absorbance was measured at 700 nm. Butylated hydroxytoluene (BHT) was used as positive control. Tested substances were used at the concentration range of 1 – 1000 µg/ml. High absorbance indicates a high reducing power.

Nitric oxide production test

In vitro NO generated from sodium nitroprusside at physiological pH interacts with oxygen to produce nitrite ions that can be measured by Greiss reaction (Ashokkumar *et al*. 2008). A sodium nitroprusside solution (10 mM) was freshly prepared in 0.1 mM phosphate buffer (pH 7.4) and 1520 µl of it were introduced in test tubes in presence of 180 µl distilled water or test substances (plant extracts or ascorbic acid) in the range concentration stated above. The mixture was incubated at 25°C for 2h 30 min. Then 500 µl of the incubated mixture was removed for nitrite measurement using Greiss reaction. Briefly, 500 µl of each experimental sample was incubated for 5 min with 500 µl 1 % sulphanilamide prepared in 5 % phosphoric acid. Then 500 µl of 0.1 % naphtyletylene diamine in distilled water was added into the reaction milieu and incubated for another 5 min. The absorbency of the chromophore formed was red at 530 nm. The quantity of nitrite in each sample was determined using the standard sodium nitrite curve.

Statistical Analysis

Statistical analysis was performed using Graph Pad Prism (version 5.01 for Windows). All data are expressed as Mean ± S.E.M. and were analyzed by one-way or two-way (only MAPB and reducing power) analysis of variance (ANOVA) using Tukey and Bonferroni as post-tests respectively. In all cases, differences were considered statistically significant at $p<0.05$.

Results

Acute toxicity

Oral administration of EMES at the doses lower than 7.5 g/kg produced no toxicity symptoms. However, doses equal or greater than 7.5 g/kg don't cause grossly negative behavioral changes but slow movement of animals, decrease in aggressiveness, touch and pain sensibility. All the observed signs appeared at least 2 h post treatment and disappeared depending to the dose of extract. No mortality was recorded up to 14 days. The median lethal dose (LD_{50}) of EMES was then greater than 12.5 g/kg body weight (Table 1).

Table 1: Behavioral acute toxicity in mice administered orally with the methanol extract of *Erythrina senegalensis*

Doses (g/kg)	D/T	Latency	Symptoms
0	0/10	-	None
1,25	0/10	-	None
2,5	0/10	-	None
5	0/10	-	None
7,5	0/10	> 2h< 4h	Locomotion↓, exploration↓ aggressiveness↓ touch sensibility ↓, pain sensibility↓
10	0/10	>2h< 8h	Locomotion↓, exploration↓, aggressiveness ↓, touch sensibility ↓, pain sensibility↓
12,5	0/10	>2h< 18h	Locomotion↓, exploration↓, aggressiveness ↓, touch sensibility ↓, pain sensibility↓

The stem bark methanol extract of *Erythrina senegalensis*, dissolved in distilled water, was administrated orally; each dose was administrated to group of 10 mice (5 males and 5 females). All the treated animals were examined for 14 days for any sign of toxicity (behavioural changes and mortality). D/T: dead/treated mice; none: no toxic symptoms were seen during the observation period; latency: time at which appear toxic signs after the dose; ↓ decrease

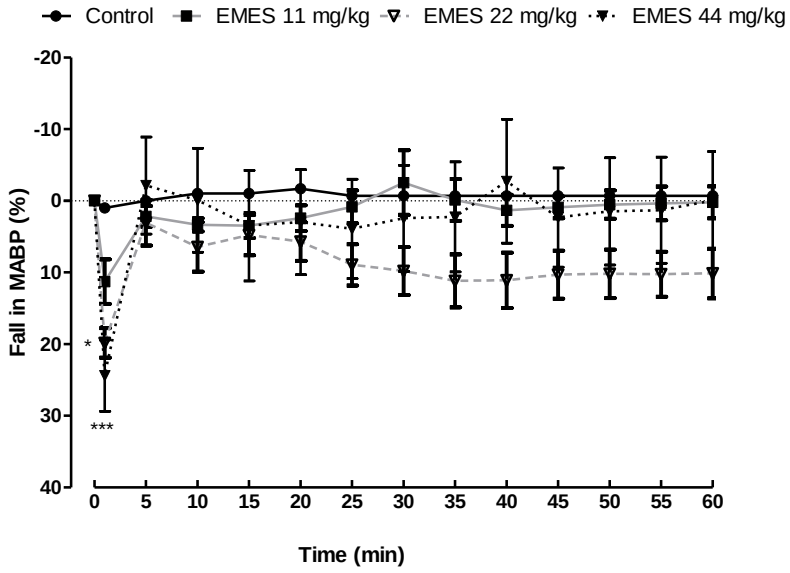

Fig. 1: Effect of the methanol extract of the stem bark of *Erythrina senegalensis* (EMES) on the mean arterial blood pressure (MABP) in normotensive rats. Each point represents the mean ± SEM (n=5). $^{*}p < 0.05$ and $^{***}p < 0.001$ significantly different compared to control (Two way ANOVA followed by Bonferroni test).

Effect of EMES on mean arterial blood pressure (MABP)

The intravenous injection of EMES provoked in anaesthetized normotensive rats, a dose dependent immediate fall in blood pressure. The mean arterial blood pressure (MABP) dropped by

11.29, 19.84 and 24.42 % respectively at the doses of 11 mg/kg, 22 mg/kg and 44 mg/kg just after extract injection. The immediate fall in blood pressure induced by EMES was followed by a return to the normal value 5 min after administration except at the dose 22 mg/kg (Fig.1).

Inotropic and chronotropic effects of EMES on rat isolated heart preparation

The perfusion of rat isolated heart with different concentrations of EMES induced significant ($p<0.01$- 0.001) negative inotropic and chronotropic effects. The force of contraction dropped from 100 % (control) to 91.59, 84.57, 82.53, 87.46, 85.82 respectively at the concentrations of 10^{-10}, 10^{-8}, 10^{-6}, 10^{-4}, 10^{-2} mg/ml. The most important effect was observed with the concentration 10^{-6} mg/ml which reduced the force of contraction by 17.47 %. The heart frequency dropped from 100 % (control) to 85.70, 88.85, 82.27, 81.10, 93.71 and 86.94 % respectively at the concentration ranging from 10-^{10} to 1 mg/ml. The most important chronotropic effect was recorded with the concentration 10^{-4} mg/ml (Fig. 2).

Effect of EMES on rabbit ECG

The intravenous administration of EMES through the saphenous vein provoked in anaesthetized rabbit some modifications of ECG parameters. The amplitude of P wave dropped from 0.10 ± 0.01 mV (control) to 0.06 ± 0.01 mV at the dose 55 mg/kg, representing a significantly ($p<0.05$) drop of 40 %. The QRS complex and T wave amplitude decreased significantly ($p<0.05$-0.001) in a dose dependent manner, with respective reduction of 34.09% and 64.28 % at the dose of 55 mg/kg. At this same dose, EMES significantly increased the QT interval by 44.82% while significantly ($p<0.05$) reducing the heart rate by 21.5 %. The PQ ant ST intervals were not affected by the treatment (Table 2).

Antioxidant assessment of EMES

Effect of EMES on DPPH radical

As shown in figure 3, EMES and ascorbic acid induced a concentration dependent radical scavenging activity on DPPH with respective IC_{50} of 46.93 and 3.20 µg/ml.

Effect of EMES on reducing capability

The reductive capability of EMES was compared to BHT by measuring their ability to transform the Fe^{3+} to Fe^{2+}. The reducing power of EMES increased with increasing amount of extract and was significant ($p<0.001$) higher than that of BHT at the concentration of 1000 µg/ml (Fig. 4).

Effect of EMES on NO production

As depicted in figure 5, EMES similar to ascorbic acid acted as prooxidant agents. Instead of inhibiting NO production, EMES at all concentrations used increased the generation of NO from sodium nitroprusside. The most potent prooxidant activity was observed at the highest concentration used with 1600 % increase in NO production.

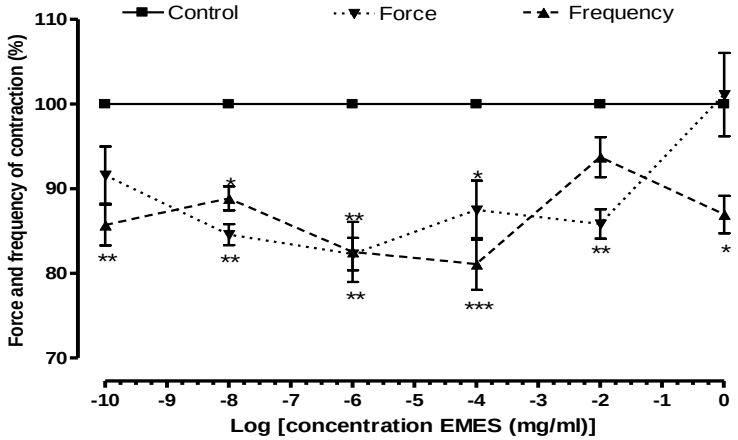

Fig. 2: Effects of the methanol extract of the stem bark of *Erythrina senegalensis* on force and frequency of contractions of rat isolated heart. Each point represents the mean ± SEM (n=5). [a]$p<0.05$; [b]$p<0.01$ and [c]$p<0.001$ significantly different compared to control (Two Way ANOVA followed by Bonferroni test).

Table 2: Electrocardiogram parameters in rabbits administrated intravenously with the methanol extract of the stem bark of *Erythrina senegalensis* (EMES)

Dose EMES (mg/kg)	Parameters						
	Wave (mV)			Intervals (ms)			HR (cycles/min)
	P	QRS	T	PQ	QT	ST	
Control	0.10 ± 0.00	0.44 ± 0.01	0.14 ± 0.01	80.00 ± 0.00	145.00 ± 5.00	80.00 ± 0.00	285.00 ± 10.60
5,5	0.10 ± 0.00	0.42 ± 0.01	0.10 ± 0.00	80.00 ± 0.00	152.50 ± 9.50	80.00 ± 0.00	270.00 ± 13.70
11	0.10 ± 0.00	0.39 ± 0.00	0.10 ± 0.01	80.00 ± 0.00	155.00 ± 8.60	80.00 ± 0.00	266.25 ± 11.25
22	0.10 ± 0.00	0.35 ± 0.01*	0.09 ± 0.00*	80.00 ± 0.00	163.75 ± 12.80	80.00 ± 0.00	262.50 ± 9.70
33	0.08 ± 0.00	0.33 ± 0.01**	0.08 ± 0.00**	80.00 ± 0.00	170.00 ± 10.00	80.00 ± 0.00	262.50 ± 9.70
44	0.07 ± 0.00	0.30 ± 0.02***	0.06 ± 0.00***	80.00 ± 0.00	185.00 ± 9.60	80.00 ± 0.00	240.00 ± 10.60
55	0.06 ± 0.01*	0.29 ± 0.02***	0.05 ± 0.00***	80.00 ± 0.00	210.00 ± 0.80***	80.00 ± 0.00	225.00 ± 0.00*

Values are mean ± SEM of 5 animals. * p<0.05, ** P< 0.01, *** P< 0.001 significantly different from the control using One Way ANOVA, followed by Tukey's multiple comparison tests. These registrations were done 1 minute after extract administration. HR: Heart rate.

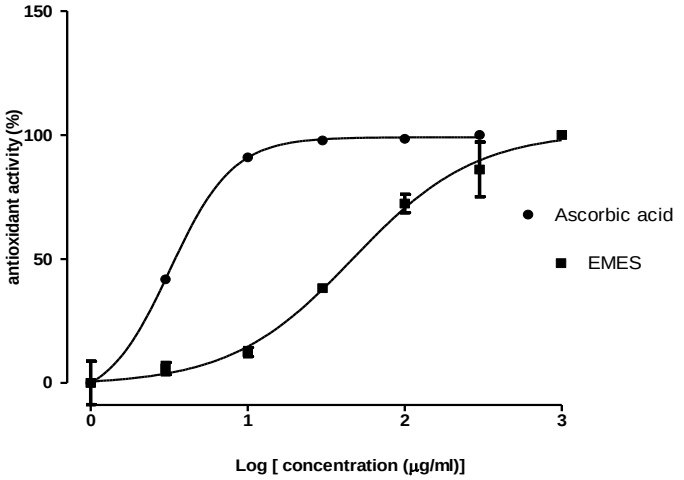

Fig. 3: Radical scavenging effect of *Erythrina senegalensis* methanol stem bark extract (EMES) and ascorbic acid on DPPH. Values are expressed as mean ± SEM. Experiments carried out in triplicate.

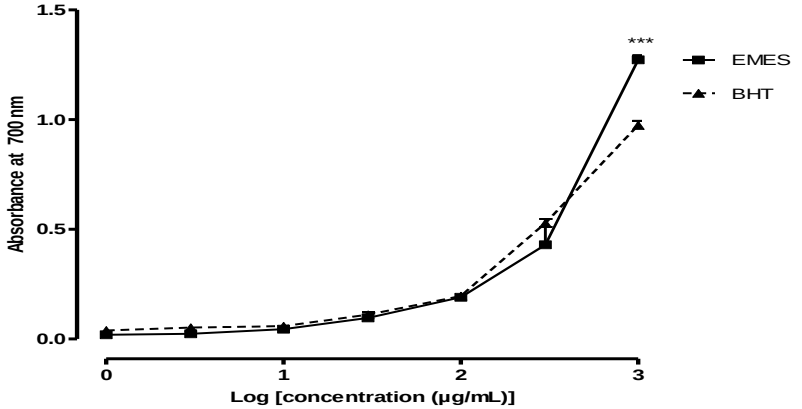

Fig. 4: Reducing capacity of *Erythrina senegalensis* methanol stem bark extract (EMES). Values are expressed as mean ± SEM of 8 different experiments carried out in duplicate; ***P<0.001 compared to Butylated hydroxytoluene (BHT).

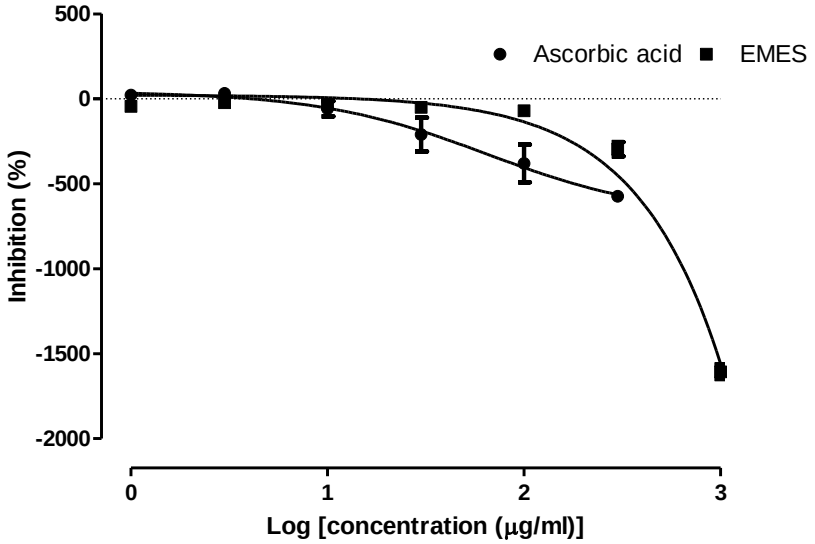

Fig. 5: Effects of ascorbic acid and *Erythrina senegalensis* methanol stem bark extract (EMES) on NO production. Each point represents the mean of three replications.

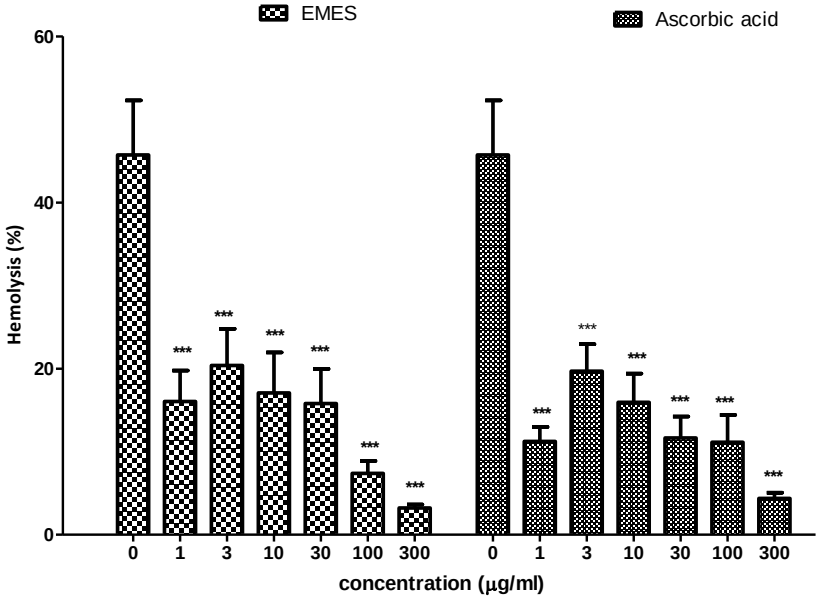

Fig. 6: Effects of *Erythrina senegalensis* stem bark methanol extract (EMES) and ascorbic acid on H_2O_2 – induced rat red blood cells haemolysis. Values are expressed as mean ± SEM of 9 different experiments. All points are significantly different (P<0.001) compared to the reference (0) which is haemolysis without any treatment.

Effect of EMES on H2O2 – induced – haemolysis

All the concentrations of the substances tested (EMES and ascorbic acid) strongly (p<0.001) inhibited H_2O_2-induced haemolysis of red blood cells. The effect of the extract was similar to that of ascorbic acid used here as the reference drug. In fact, at the concentration of 300 µg/ml, EMES and ascorbic acid reduced the haemolysis from 45.73 % in control to 3.22 % and 4.48 %, respectively (Fig. 6).

Discussion

As part of the pharmacological evaluation of EMES, we investigated its acute toxicity, cardiovascular and antioxidant effects. In the acute toxicity study, no adverse effect was observed up to the dose 5 g/kg of EMES. Mice receiving this extract at the dose comprised between 7.5 and 12.5 g/kg showed hypoactivity marked by reduction in aggressiveness, locomotion and pain sensitivity. It can be thought that this extract possess depressive effect on the nervous system. Similar results were obtained with the decoction of the stem bark of *Erythrina senegalensis* (Atsamo *et al*., 2011). All animals treated with the EMES survived beyond the 14 days observation period. The median acute toxicity value (DL_{50}) of EMES is then above 12.5 g/kg body weight. Loomis and Hayes (1996) described the classification of some chemical agents into categories of toxicity in which the dose of 5 g/kg was categorized as practically nontoxic. Accordingly, this result indicates that *Erythrina senegalensis* methanol stem bark extract has no acute toxic effect when administrated orally.

Intravenous administration of EMES induced a sudden fall in blood pressure follow by a rapid return to the initial level, suggesting the action of this extract on the cardiac pump as previously indicated by Nyadjeu *et al*. (2011), while working on *Cinnamomum zeylanicum* extracts. The rapid return of blood pressure to initial values might be explained by a reflex regulatory mechanism through baroreceptors which increase sympathic activity and thereby, the peripheral resistance. In order to test the hypothesis of EMES acting on the heart, its cardiac effect was evaluated on rat isolated heart and rabbit electrocardiogram.

EMES induced a negative inotropic and chronotropic effects on rat isolated heart, indicating that this extract has a cardiodepressive effect therefore, justifying the rapid fall observed in blood pressure after its intravenous administration.

To further understand the mechanism of action of EMES, it was evaluated on the heart global electrical activity through electrocardiogram. EMES significantly reduced the amplitude of P wave, QRS complex and heart rate while T wave was not affected. Similarly, QT interval increases while PR and ST intervals were not affected. These results are in accordance with those obtained by Traoré *et al.* (2004) who observed a diminution of the amplitude of P wave and QRS complex without any effect on PR and ST interval after intravenous injection of ethanol extract from the roots of *E. senegalensis* in rabbits. Depressive effect of EMES on P wave and QRS complex amplitude suggest that the extract may affect both atrial and ventricular depolarization. The fact that EMES increased the QT interval but failed to affect the ST interval indicates that only the QRS complex duration is modified. Thus, EMES is able to increase the time of the ventricular depolarization. EMES reduced the heart frequency without affecting the PR interval, suggesting a sinusal bradycardy and therefore, a depressive effect of EMES on the sinusal node. Machida et al. (2011) have demonstrated that the activation of selective acetylcholine-activated-K+ channels present in cardiac tissues induces the hyperpolarization of nodal and myocardic tissues thereby, reducing the SA node firing rate and the global depolarization of other cardiac tissues. This may clearly explain the reduction in QRS complex amplitude, and the negative chronotropic effect of the extract. Reducing the amplitude of depolarization may also reduce the calcium influx in cardiac cells and subsequently their force of contraction.

Oxidative stress has been implicated in the pathophysiology of various diseases including cardiovascular diseases (Sabuncuoğlu and Şöhretoğlu, 2012). It was therefore thought that the use of antioxidant substances might prevent the development of cardiovascular diseases and their related complications. Hence, the beneficial effects of plant extracts and natural products as antioxidant agents have become the focus of interest.

The antioxidant effects of EMES was evaluated on DPPH free radicals, NO production, oxidative stress induced hemolysis and its reducing power. EMES showed a potent DPPH scavenging activity, suggesting that the plant extract contains compounds that are capable of donating hydrogen or electron to a free radical in order to stabilise the odd electron which is responsible for radical's reactivity (Aiyegoro *et al*., 2010). To confirm this

hydrogen or electron donating capacity, EMES reducing power was evaluated. This extract exhibited concentration dependent reducing power which was significantly higher than that of BHT at the concentration of 1000 µg/ml, suggesting that its antioxidant activity is at least partially due to its capacity to release electrons.

NO is an important cell mediator with many regulatory functions but when produced in excess or in an oxidative stressed environment, it can become harmful to the organism. The present results showed that EMES was not able to reduce the production of NO from sodium nitropruside but instead increases this production, suggesting that it is not efficient in scavenging NO radicals. This result suggests that EMES may be helpful in NO deficient conditions such as essential hypertension. Oxydative stress generally leads to the modification of biological molecules such as nucleic acids, proteins and lipids. These modifications induce alteration of cell biophysic parameters, cell functions or even cell death. In the present study, hydrogen peroxide induced haemolysis that was significantly reduced by the plant extract. It is well known that red blood cells are powerful hydrogen peroxide scavenger due to their high content in catalase, which converts H_2O_2 into H_2O and O_2 (Mates *et al*., 1999; Powers and Lennon, 1999). The above result suggests that *E. senegalensis* stem bark methanol extract might prevent red blood cells haemolysis either by inhibiting the peroxidation of membrane lipids or by boosting endogenous catalase activity.

Conclusion

The results of the present study clearly indicated that EMES is devoided of acute toxicity. This extract induced immediate hypotension that may result from its negative inotropic and chronotropic effects on the heart. The antioxidant activities of EMES against various antioxidant systems might be beneficial for the treatment of cardiovascular ailments.

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