EFFECT OF CRUDE EXTRACT OF SECURIDACA LONGIPEDUNCULATA ON ISOLATED RABBIT HEART

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ABSTRACT
The plant securidaca longipedunculata is used in folklore medicine to treat cardiovascular disorder evaluated. The effect of ethanolic extract of the root bark of securidaca longipedunculata (EESL) on isolated rabbit heart (New Zealand weighing between 2-3kg) were assessed in Langendorff isolated rabbit heart. EESL showed a dual effect on heart rates and coronary flow, low dose caused significant (P<0.001) increase in coronary flow rate and heart rate while the opposite effect was observed at high dose. A gradual increase (P<0.001) in coronary perfusion pressure was observed with increasing concentration of EESL. With interventricular root infusion the extract evolved a depressant effect on heart rate and cardiac output, while ventricular pressure was significantly (P<0.001) high at high dose, than at the base line level. Co-administration of nifedipine with the extract antagonized the effect of extract on coronary circulation.

KEYWORDS: Isolated Rabbit heart, securidaca longipedunculata, coronary artery and nifedipin.

INTRODUCTION
Crude extract of Securidaca longipedunculata is one of the commonly used for controlling high blood pressure traditionally. Securidaca longipedunculata belongs to the family polygalaceae and it is a tropical tree, growing widely in Africa(Iwu 1986). Photochemical screening of the hydroalcoholic extract of the back root of Securidaca longipedunculata showed the presence of alkaloid glycosides (Jayaselcara et al 2002). The study of the root extract showed increase sodium ion current and enhanced the contractile response elicited by stable depolarization suggesting the possibility of one or more of the constituents acting on tight voltage-sensor of excitation contraction coupling in rat skeletal muscle (Mouzo et al 1999), whereas the depressor effect of the root extract on the cardiovascular parameters showed that the plant dose-dependently inhibited the pressure effect of noradrenaline on the arterial blood pressure and heart rate (Ojewale at al 2000). Its conceivable fact that regulation of blood pressure should involve either both heart and vessel or either of them depending on etiology. Regulation of cardiovascular system has a diverse approach and some of the mechanisms proposed by some studies are Nitric oxide and pros acyclic (Paul 2003), changes in the endothelia calcium ion concentration (Jiaxean 2005) and inhibition of contractile element (Pfizer et al 1986).

In the present study, we tested the hypothesis that receptor mediated increase or decrease in Ca2+ entry via operated calcium channel in coronary vessel or inhibition of contractile element of heart muscle could be a mechanism by which Securidaca longipedunculata regulate cardiac function in an attempt to regulate blood pressure.

The study also determine whether prevention of Ca2+ entry by nefidipine, a dihydropyridine, at a high dose can suggest the mechanism of relaxant effect of Securidaca longipedunculata on blood pressure regulation. Prevention of calcium level from increasing as much in cells when stimulated, leading to less muscle contraction. In the heart a decrease in calcium available for each beat result in a decrease in cardiac contractility. In blood vessel, a decrease in calcium result, in less contraction of the vascular smooth muscle and therefore an increase in blood vessel diameter, a phenomenon called vasodillation (Scultery and Tannanskovits 1991).

MATERIALS AND METHOD
A. preparation of crude extract of Securidaca longipedunculata
The crude extract was prepared according to the method of Eno et al (2004). In brief, fresh back root of Securidaca longipedunculata was purched from
herbalist. The plant was by verified Prof Akiniyi and found to be identical with the specimen sample deposited at the herbarium of the laboratory of vegetal biology, University of Maiduguri. The root of the plant was first washed free of sand, debris, and the back removed and air-dried at room temperature. The dried powder (200gm) of the root back was extracted in a soxhlet apparatus with 60%v/v ethanol. The final extract was evaporated in vacuo and the dry residue was kept at 4°C until used.

Acute toxicity study
The study was conducted on male and female Wistar rats, weighing between 150 gm to 250 gm and randomly divided into 7 groups (6 animal per group). Each group received intraperitoneal injection of each of the following doses; 20, 30, 50, 69, or 70 mg/kg of the crude extract. The control group was injected with isotonic saline. The maximal volume given was 0.5 ml. The animals were given free access to food and water ad libitum. After 24 hour the mortality in each cage was assessed. The LD₅₀. Was calculated using arithmetic method of Kaber as described by Aliu and Nwude (1982).

Experimental Animals
Adult male and female New Zealand White rabbits (2—3) Kg were used in this study. The animals were bred in the animal house, College of Medical Sciences, University of Maiduguri. The rabbits were chosen for the purpose of this experiment because their hearts and cardiac circulation closely resemble those of humans. The animals were acclimatized under standard laboratory conditions and normal photoperiod (12-hour-light-dark-cycle) for seven days. Commercial pellets and water were provided ad libitum. Two groups of rabbit (n = 5 rabbit group) were tested for coronary and cardiac functions following administration of extract of Securidaca longipedunculata. Coronary flow rate, cardiac output, perfusion pressure and heart rate were all determined.

Perfusion of coronary artery and ventricles
The studies on the coronary vascular bed were performed on the whole hearts using the langendorff preparation for perfused isolated heart. Briefly, using a Langendorff apparatus, rabbits were injected subcutaneously with heparine (100units/kg) 5min before anesthesized by a sharp blow on the back of the neck and hearts were immediately removed and rinsed in an ice-cold Krebs Hensilet solution containing 119 mM NaCL, 4.5 mM KCL, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 24.8 mM NaHCO₃, 10.3 mM glucose. Anesthetic agents were not used for fear of depressant effect on heart. Aortic artery was trimmed and the heart was mounted and cannulated in the retrograde mode according to langendorff method (Langedorff 1985) and perfused at a constant flows with Krebs Hensilet solution. The cannular was inserted into the ascending aorta and tied with a 3.0 polyester polyfilament to allow coronary perfusion via the aorta. The initial perfusion pressure was recorded and kreb's Hensilet buffer was gassed with 95% O₂ and 5% CO₂ mixture at a controlled pressure of 100 mmHg to give a pH of 7.4. Coronary perfusion pressure (CPP) was monitored with a TPS-2 Statham transducer connected to a sidearm of aortic perfusion catheter. Hearts was then allowed to equilibrate for 20 minutes and initial cardiac parameters of heart rate, coronary flow rate were recorded. The effects of different doses of the extract Securidaca longipedunculata were assessed by in bolus administration through other sidearm just above the attached aorta to the perfusion catheter, on the cardiac parameters and effect of 0.2mg nifedipine against 2mg extract were also tested. In the second heart experiment, the cannulae was inserted into the left ventricle through the left atrium and the cardiac output, heart rate and ventricular pressure were also recorded with extract of the same dose as above.

Statistical analysis
Values were expressed as mean± SEM. Data were subjected to one-way analysis of variance (ANOVA). Values of P<0.05 were considered significant.

RESULTS
EFFECT OF ADMINISTRATION of ETHANOLIC EXTRACT of securidaca longipedunculata THROUGH CORONARY ARTERY
In rabbits isolated heart, EESL showed a dual effect on coronary flow rate and heart rate; Low doses (0.1mg and 1mg) caused significant (P<0.05) decrease in coronary flow rate, while opposite effect was observed at high dose (2mg). The heart rate showed no significant increase with low doses, while a significant (P<0.05) decrease was recorded at high dose (Figure 1a,b and c) a significant gradual increase from 9.08± 0.40 mmHg to 11.24±0.94 mmHg in coronary pressure was also observed with gradual increase in concentrations of EESL.

Cardiac Function Following the Administration of Ethanol Extract of Securidaca longipedunculata into the Left Ventricle on Cardiac Output, Ventricle Pressure and Heart Rates
The extract evoked a depressant effect on the parameter of cardiac function (Figure 2a, b and c), determined by the heart rate, cardiac output and pressure of the ventricle. The base line values (before in fusion of EESL) of the heart rate, cardiac output and ventricular pressure were 39.0±5.33 beat/min, 11.4±1.01ml/min and 8.08±1.75mmHg, respectively. After perfusion of the heart with a solution containing the extract at a concentration of 2mg; heart rate and cardiac output significantly (P<0.01) decrease (31.8±4.76 beat/min and 5.34±0.82ml/min respectively), compared to the base line value (39.0±5.33 beat/min and 11.4±1.01 ml/min). The perfusion pressure value (11.62±0.39 mmHg) was however significantly higher (P<0.05) than that of the base line (8.08±0.75 mmHg).
The Effect of Co-Administration of Nifedipine and Extract of Securidaca longipedunculata On Coronary Flow Rate, Heart Rate and Perfusion Pressure

In the presence of nifedipine and the extract, the coronary flow rate of 20.5±1.36 ml/min was higher (p<0.01) than that of the base line (14.5±1.12 ml/min) while the heart rate of 34.1±1.89 beat/min was significantly (P<0.001) lower than the base line value 80.0±6.12 beat/min. The perfusion pressure showed no significant (P>0.05) difference with nifedipine (8.3±0.20 mmHg), while a significant (P<0.01) increase was recorded with EESL (11.24±0.49 mmHg, (Figure 3)
Fig. 2a. Effect of serial doses of Securidaca longipendunculata administered into the ventricle through left atrium on heart rate

Fig. 2b. Effect of serial doses of Securidaca longipendunculata administered into the ventricle through left atrium on flow rate

Fig. 2c. Effect of serial doses of Securidaca longipendunculata administered into the ventricle through left atrium on perfusion pressure
DISCUSSION

ACUTE TOXICITY
The observed physical signs of toxicity including a decrease in motor activity, palpitation, irregular heartbeat, cardiac arrest and death were indicative of both the peripheral and central effect of the extract of Securidaca longipedunculata. The obtained median lethal dose (LD_{50}) of 43mg/kg showed slight variation compared with the LD_{50} of 41.2mg/kg obtained in the earlier report by Olaleye et al (1998), indicating that the extract is safer and has a narrow therapeutic ratio which confers on it a narrow safety margin. However knowledge of pharmacokinetics of the extracts apart from the dose is also important in determining the degree of toxicity (Uguru 2006). Further toxicological studies of this plant are required.

CARDIAC EFFECT OF THE EXTRACT
It is conceivable that cardiovascular responses are controlled by two main systems; that is, the heart and the blood vessel. It was in this light that the experiment was first carried out on isolated rabbit heart to determine the regulatory role of EESL on the heart and our main finding of this study showed that EESL exerted a dual effect on the heart; at low dose it increase the coronary flow rate significantly, while the opposite effect was observed at high dose. A gradual increase in coronary pressure was also observed with increasing concentration of EESL. High dose (2 mg) induces negative chronotropic effect. However, the low and the high dose cause positive inotropic effect. It is likely that the in-vitro negative chronotropic effect of EESL was due to its direct effect on the heart, since it is only at high concentration that this effect was manifested. In other to investigate whether calcium channel blockage played a role in the regulation of coronary flow and pressure at high dose of the extract, nifedipine was co administer with EESL. Indeed the co-administration of nifedipine produces a significant increase in coronary flow rate. Coronary pressure was also sharply reduced suggesting that the EESL decrease coronary flow rate by increasing intra cellular calcium ion that eventually lead to vasoconstriction and increase coronary pressure. This finding is consistent with the report of Bruce et al (1986) that calcium ion blockers play a major role in regulating vascular smooth cell contraction, blood vessel diameter and blood pressure. The capacity of nifedipine to prevent EESL-induce coronary constriction and decrease coronary flow may be mediated by inactivated calcium channel blockers. Previous studies have demonstrated that calcium channel blockers improve oxygen supply by coronary vasodilation and also cause negative chronotropic and inotropic effect (Triggle, 1992).

The main clinical usage of calcium blockers is to decrease blood pressure, therefore, they are used in individuals with hypertension. Calcium channel blockers manifest their actions by blocking voltage-gate calcium channel in muscle cell of the heart and blood vessel (Sanguinetti and Kass 1984). It has been shown that in the heart, a decrease in calcium available for each beat result in a decrease in cardiac contractility, while in the blood vessel such a decrease in calcium results in less contraction of the smooth muscle, causing increase in blood vessel diameter (Calteral et al. 2005). The increase flow rate at low doses of extract maybe attributed to coronary dilation induced through one or more of the endothelium-derived relaxation factors (nitric oxide, prostacyclin, and endothelium hyperpolarization factor). Besides, NO may be implicated because studies have shown that endothelial calcium ion triggers nitric NO release by activation of NO synthase. High dose may have increase intracellular calcium ion concentration, leading to positive inotropic and pressure effect observed in the heart and coronary blood vessel, respectively. This view is supported by the result of the present study which showed that the administration of nifedipine antagonized high dose effect of extract. It has also been reported that operative sodium-potassium ATPase in intracellular accumulation of calcium ion could also be taken into consideration as a possible mechanism. The sodium-potassium ATPase was described by some studies as pathway for intracellular calcium ion accumulation in cardiac myocytes, thus resulting in
increased cardiac concentration. This intracellular calcium accumulation and binding, serve as the basis for using digitalis drug to treat congestive heart failure (Jana and Xie, 2008).

The depressant effect of EESL on the heart may also be as a result of high increase in intracellular calcium ion concentration and stoppage of contraction of the ventricle. The weak and irregular contraction observed in the atrium maybe due to calcium overload that triggered the opening of calcium-activated potassium channel of small and intermediate conductance and the release reactive oxygen species (ROS), thus indicating hyperpolarization of the endothelial cells(Michel and Paul, 2006).

It could be suggested, therefore, that EESL acted directly on inactivated calcium channel. This is because nifedipine which is an inactivated calcium channel blocker was capable of significantly attenuating the effect of nifedipine which is an inactivated calcium channel blocker; phenylalkylamines and dihydropyridine effect. I

REFERENCES