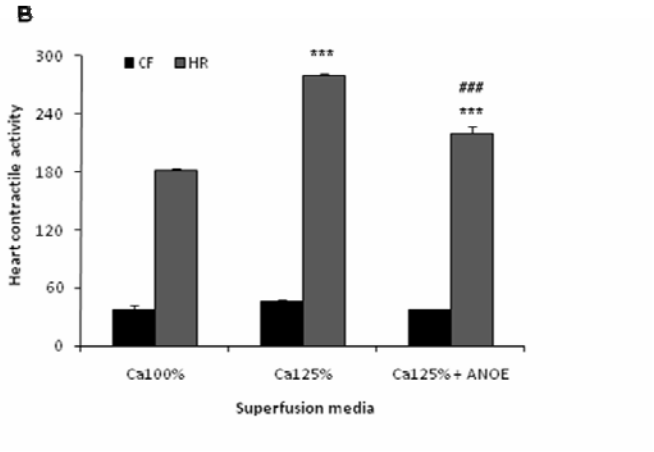

Figure 3B: The effects of ANOE on the heart contractile activity in Ca-enriched medium.

The effects of ANOE on the contractile activity of the heart seen in sodium-depleted medium (Na 50%)

The control recording revealed the CF of 35± 3.14 mm and the HR of 182± 2.44 c/min. After a transient increase, CF measured in Na 50% dropped down in a drastic manner before stabilizing at 13±1.31 mm (which represents a 63%-drop, $P < 0.001$ vs control), while HR increased considerably to reach 493±2.63 c/min (which represents a 271%-increase, $P < 0.001$ vs control). With Na 50% containing ANOE, CF kept dropping down, while after a substantial increase HR also dropped down in a quick and steep manner; both parameters were eventually brought down to the cardiac arrest level (Figure 4).

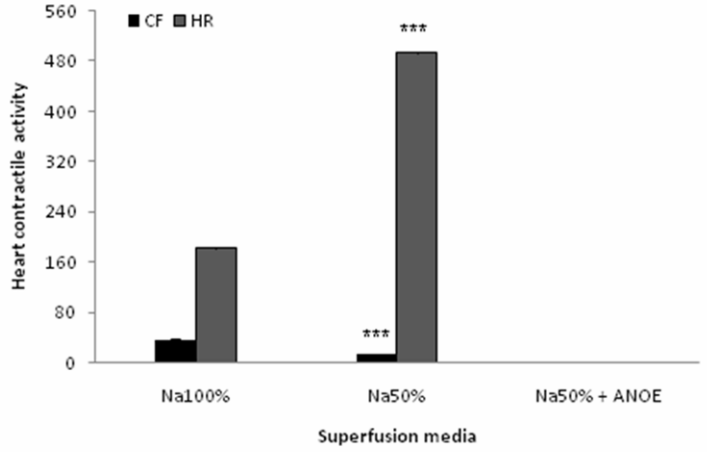

Figure 4: The effects of ANOE on the heart contractile activity in Na-depleted medium.
The control (Na 100%) represents the contractile force (CF) and heart rate (HR) recorded in normal Mac-Ewen physiological solution. In this experiment ANOE was used in the single concentration of 0.1µg/ml. Ordinate: CF is expressed in millimetres, while HR is expressed as the number of contractions/minute. Abscissa: super-fusion media. Data are expressed as means ± SEM ($n = 15$). ***, $P < 0.001$ vs. control for both CF and HR.

The effects of ANOE on the cardiac contractile activity in potassium-depleted medium (K 50%)

The control recording revealed the CF of 30±2.9 mm and the HR of 186±1.6 c/min. With K 50%, CF and HR increased by 30% ($P < 0.01$ vs control) and 106%, respectively ($P < 0.001$ vs control). With K 50% containing ANOE, CF and HR dropped down by 36% and 37%, respectively, as compared to the preceding increase ($P < 0.001$) (Figure 5).

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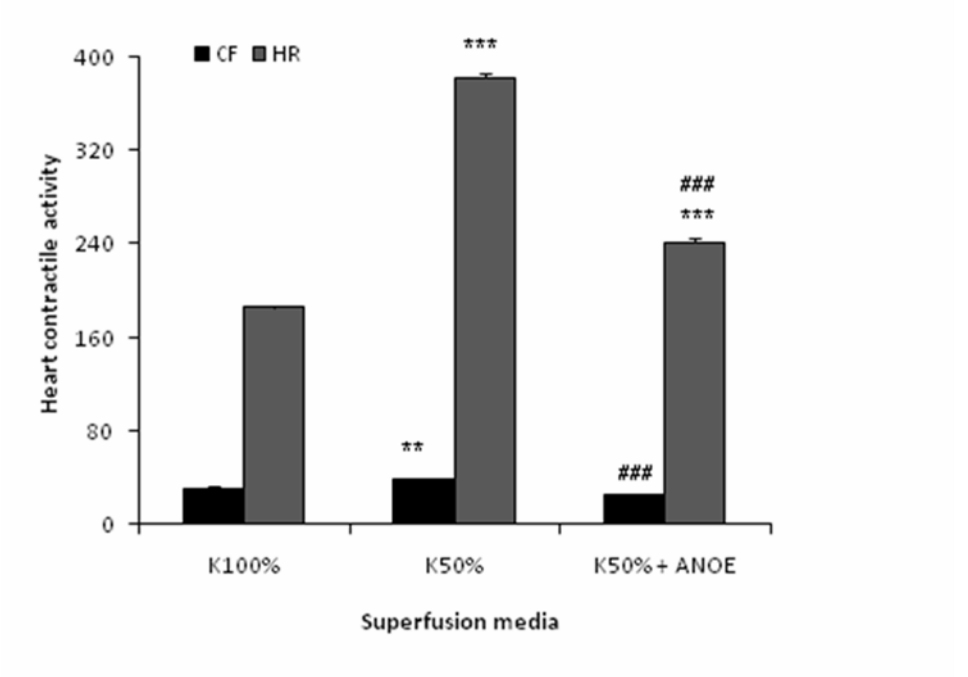

Figure 5: The effects of ANOE on the heart contractile activity in K-depleted medium.
The control (K 100%) represents the contractile force (CF) and heart rate (HR) recorded in normal Mac-Ewen physiological solution. In this experiment ANOE was used in the single concentration of 0.1µg/ml. Ordinate: CF is expressed in millimetres, while HR is given as the number of contractions/minute. Abscissa: super-fusion media. Data are expressed as means ± SEM (n = 15). For CF: **, $P < 0.01$ for K 50% vs control; and ###, $p < 0.001$ for K 50% vs K50% + ANOE. For HR: ***, $P < 0.001$ for K 50% and K 50% + ANOE vs control; and ###, $P < 0.001$ for K 50% vs K 50% + ANOE

Discussion

The overall objective of this study was to determine whether the traditional use of *Anacardium occidentale* in hypertension treatment is based on actual pharmacological effects of the plant that could be demonstrated in animals and, if so, to try to understand the mode of action of this plant using the tools at our disposal. Our results showed that ANOE exerts a concentration-related hypotensive effect on rabbits' arterial blood pressure. With doses ranging from 40 to 167 mg/kg b.w., this hypotension can be described as striking. The development of this hypotension showed that ANOE could have either a direct inhibiting effect on the cardiac activity or a vasodilating effect on vascular muscles. These effects might be coupled or associated with the influence of ANOE on the central nervous system (Cooper, 2001; Wang and Wang, 2007). ANOE's effects on animals *in vivo* were acetylcholine-like; the latter are known to be mediated by five receptors (M1-M5). Muscarinic acetylcholine receptors are involved into the regulation of many fundamental central and peripheral functions (Levine et al, 1999 and 2001). Besides M2 subtype widely present in mammalian hearts, some studies showed the presence of M1 subtype in rat heart and gave a strong indication of co-existence of functional M2 and M3 subtypes in rabbit heart (Kan et al, 1996; Wang et al, 2004). A recent study done in mice lacking M2 and M3 subtypes, clearly showed the important role of M2 (not M1 or M3) receptors in regulating heart contractile activity *in vivo*, while M3 subtype had a more significant role in peripheral regulation, thus controlling the modifications in blood pressure (Fisher et al, 2004). Since atropine failed to prevent the development of ANOE effects *in vivo*, it is reasonable to assume that ANOE did not act via M2 and M3 muscarinic receptor subtypes.

The action of ANOE on an isolated rat heart resulted in a transient positive inotropic effect followed by negative chronotropic and inotropic effects. This result led us to suggest that ANOE might contain both cardio-activating and cardio-inhibitory components, the effects of the latter components being seemingly stronger or more prominent. It is known that acetylcholine inhibits heart activity by decreasing the auricular contractile force (Prokopczuk et al, 1981; Groschner et al, 1986). Acetylcholine challenges muscarinic receptors within the heart (Webb and Hollander, 1956), whose activation induces negative inotropic effects akin to those of ANOE. The fact that atropine failed to prevent the development of ANOE-induced heart effects suggests that ANOE does not act via M1 and M2 muscarinic receptor subtypes.

Cardiac activity is normally regulated by a certain number of ion flows (Langer, 1965). Lüttgau and Niedergerke (1958) showed the cardiac activity to be dependent on extra-cellular ionic conditions. Studies of ANOE effects in modified physiological media were thus necessary to better understand the specific mode of action of this plant. It was shown by Verrijk et al. (1990) that

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calcium-enriched medium will support an increase in the cardiac contractile force, whereas a calcium-depleted medium will diminish that force. At 0.1µg/ml, ANOE, which normally induces a major reduction in both contractile force and heart rate, induced moderate negative inotropic and chronotropic effects in calcium-enriched medium. In contrast, negative inotropic and positive chronotropic effects obtained in calcium-depleted medium, were potentiated by ANOE. It thus seems that ANOE behaves like a calcium channel antagonist (Kohlhardt et al, 1978; Romero et al, 2003); it could act either through calcium channels' blockage which would prevent calcium from entering into cells (Fleckenstein, 1981 and 1983) or through the hindrance of calcium release from its internal storages, as seemingly suggested by the results obtained with calcium-depleted medium. Thus the two effects combined could give an account for cardio-depression.

While examining the effects of internal and external ions on calcium ion flow in mammalian cardiac muscle, Glitsh et al (1970) showed that the decrease in extra-cellular sodium ion concentration increases calcium influx, whereas the increase in extra-cellular sodium ion concentration decreases it. It is actually known that there exists a competition between these two ions which tend to pass through the same Na-Ca exchange channel. This could explain the temporary increase in cardiac contractile force seen in sodium-depleted medium prior to its eventual decrease. In sodium-depleted medium containing ANOE, this decrease in contractile force is even more prominent. The inhibiting action of ANOE seen in sodium-depleted medium would go in favour of the reduction in calcium influx attained via Na-Ca exchange channel inhibition, this channel thereby being responsible for the transition of calcium through the cell membrane (Wettwer and Ravens, 1991).

In potassium-depleted medium, positive inotropic and chronotropic effects were observed. Eisner and Lederer (1979a and b), who worked with mammalian ventricular fibres, reported about the blockage of Na+/K+ pump induced by a hypopotassic medium, leading to the intracellular impoverishment in potassium ions and fall in potassic conductance. The inhibition of Na+/K+ pump could cause an impoverishment in extracellular sodium ions (Laughlin, 2001; Scheiner-Bobis, 2002; Faller, 2008), which would go in favour of an increase in calcium influx triggered by setting Na+/Ca++ exchange mechanism in motion. Lepeschkin et al (1957) reported that, owing to the prolongation of the cardiac action potential plateau underpinned by the sustained calcium influx, a medium poor in potassium ions delays the repolarisation phase of the heart cycle causing the intracellular accumulation of calcium ions responsible for the positive inotropic effect observed by the authors. The reduction in heart contractile force confirms the assumption that ANOE works as a calcium channel antagonist. Thus ANOE probably also inhibits the release of intracellular calcium causing cardio-depression, the paramount role of calcium ions in contractile activity of muscular structures thereby being well-known (Katz et al, 1966; Domeier et al, 2008; Shenkman and Nemirovskaia, 2009; Piétri-Rouxel et al, 2010).

Conclusion

The bark stem extract of *Anacardium occidentale* showed hypotensive activities *in vivo* and cardio-inhibitory activities *in vitro*. In the modern world being under massive attack of cardiovascular diseases (CVDs) that pose as one of the greatest threats to humanity, these results can be exploited for further research in order to eventually come up with a natural cure for hypertension and CVD. Therefore, further physiological and pharmacological investigations both *in vivo* and *in vitro* will surely improve our knowledge of natural substances used to treat hypertension, especially that of *Anacardium occidentale* which displays characteristics justifying its use in traditional medicine.

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