International Journal of Animal Sciences and Veterinary Advances 2(1): 5-8, 2010
ISSN: 2041-2908

Submitted Date: July 21, 2009 Accepted Date: August 19, 2009 Published Date: February 10, 2010

Hypotensive Effect of Aqueous Seed Extract of *Hibiscus sabdariffa linn* (*Malvaceae*) on Normotensive Cat

[1]I.G. Bako, [2]M.A. Mabrouk, [3]I.M. Maje, [4]A.A. Buraimoh and [5]M.S. Abubakar
[1]Department of Human Physiology, Faculty of Medicine,
Ahmadu Bello University Zaria, Nigeria
[2]Department of Medical Physiology, Faculty of Medicine, Al- Azhar University Cairo, Egypt
[3]Department of Pharmacology, Faculty of Pharmaceutical Sciences,
Ahmadu Bello University Zaria, Nigeria
[4]Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria, Nigeria.
[5]Department of Pharmacognosy and drug Development, Faculty of Pharmaceutical Sciences,
Ahmadu Bello University Zaria, Nigeria

Abstract: The hypotensive effects of aqueous seed extract of *Hibiscus sabdariffa l.* were evaluated in normotensive cat. The effects of the aqueous extract were compared with normal basal rhythm and Acetylcholine. The drugs and various doses of the extracts were injected through a cannula inserted in the femoral artery. The extract produced a significant reduction in cat blood pressure. The potency of the extract seems to be high because it has activity at 500µg/ml. The 1mg/ml of the extract exhibited more effective response, however the standard drug Acetylcholine showed a greater potency than the extract.

Key words: Acetylcholine, blood pressure, *Hibiscus sabdariffa* and hypertension

INTRODUCTION

Hibiscus sabdariffa Linn is a herb belonging to the malvaceae family. It is grown in all parts of the world and cultivated for its leaf, fleshy calyx, seed or fibre (Dalziel, 1973). Hibiscus *sabdariffa l.* is taken as a common local drink popularly known as zobo in Nigeria. It is a medicinal herb, used in folk medicine in treatment of hypertension (Wang *et al*., 2000; Odigie *et al*., 2003). *Hibiscus anthocyanin*, a group of phenolic natural pigments present in the dried flower of *Hibiscus sabdariffa* and *Hibiscus rosasinensis,* have been found to have cardioprotective (Jonadet, 1990; Olatunji *et al*., 2005), hypocholesterolemic (Chen *et al*., 2003), anti-oxidative and hepatoprotective (Wang *et al*., 2000; Amin and Hamza, 2005) effects in animals.

Delphinidin 3-sambubioside, a *Hibiscus anthocyanin,* induces apoptosis in human leukemia cells through oxygen reactive species-mediated mitochondrial pathway (Ali *et al*., 2005). Polysaccharides from *Hibiscus sabdariffa* flowers stimulate proliferation and differentiation of human keratinocytes (brunold *et al.,* 2004). *Hibiscus protocatechuic* acid has inhibitory and inductive effect on tumour promotion in mouse skin and in human leukemia cells respectively. *Hibiscus sabdariffa* has been reported to be antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, refrigerant, sedative, stomachic and tonic (Morton, 1987; Olalelye, 2007). In Nigeria, a decoction of the seeds is given to augment or induce lactation in cases of poor milk production, poor letdown and maternal mortality (Okasha *et al*., 2008). However, there is dearth of literature supporting the use of seeds. In light of this, the study is designed to evaluate the hypotensive effect of *Hibiscus sabdariffa* seed extract.

MATERIALS AND METHODS

Chemicals and drugs: All chemicals and drugs used were of analytical grade. Heparin, sodium Thiopentane, Atropine, Acetylcholine, Adrenaline (Aldrich Chemical company, Gillingham England) were obtained from Department of pharmacology Ahmadu Bello University Zaria, Nigeria.

Plant materials: The samples of *Hibiscus sabdariffa l.* seed were collected in Gaya Hong Local Government in Adamawa state of Nigeria. The plant was identified in the Department of Biological Sciences, Ahmadu Bello University, Zaria by Mallam Musa Ibrahim (Taxonomist) and authenticated voucher samples were deposited in the Herbarium section (code number 1056).

Extract preparation: The *Hibiscus sabdariffa l.* seeds were washed thoroughly, sun dried and ground into powder. The extraction of *Hibiscus sabdariffa l.* seed was done in Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria. Maceration method was used for aqueous extraction. The mixtures were then shaken for ten hours with mechanical

Corresponding Author: I.G. Bako, Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University Zaria, Nigeria, Tell: +2348036982739

shaker. The supernatant liquid (extract) was filtered through a plug of cotton or glass wool. The process was repeated for complete extraction. The extracts were then poured into evaporating dish to evaporate the solvent in the extract over the water bath at the temperature of 40ºC - 45ºC (Abdul, 1990).

Experimental design: The experiment was an in vivo and two cats were used. The cat was anesthetized by injecting Thiopentane sodium via intraperitoneal route. The animal lost consciousness after 20 minutes of administration, after which it was properly secured to the dissecting table by using twine to tie its limbs, so as to prevent unwanted movement of the animal during the experiment. The left femoral vein was exposed and tied with peripheral ligature. Also, the right carotid artery was exposed and cannulated for blood pressure measurement. The basal blood pressure was recorded on a filter paper of the micro-dynamometer after the administration of the heparinized normal saline to prevent blood clothing. The drugs and extract were administered through the cannula inserted through the left femoral vein. The speed and sensitivity of the machine were 95mm/minute and 1 respectively. The first set of drugs recorded were the standard drugs (Ach and Adrenaline), followed by graded dose response for each extract, and the dose which caused maximum effect was chosen as experimental dose. Flushing was properly done after every administration of extract and drugs till it was brought back to normal.

Phytochemical Analysis: The aqueous seed extract of *Hibiscus sabdariffa l.* were subjected to preliminary phytochemical screening, to identify the chemical constituents. The methods of analysis employed were those described by Brain and Turner (1975).

Acute toxicity study: The lethal dose (LD50) of the plant extract was determined by the method of Lorke (1983) using 13 rats. In the first phase rats were divided into 3 groups of 3 rats each and were treated with the aqueous extract of the seed at doses of 10, 100 and 1000 mg/kg body weight intraperitoneal. They were observed for 24 h for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat each and were also treated with the aqueous extract at doses of 1000, 1600, 2900 and 5000 mg/kg bodyweight *(i.p)*. The median lethal dose (LD50) was calculated using the second phase.

Statistical Analysis: All data are expressed as Mean ± S.E.M. The data obtained were analyzed using one way analysis of variance (ANOVA) and Turkey-Kramer *post hoc* test for multiple comparisons. The ($P<0.05$) will be accepted as significant (Betty and Jonathan, 2003).

RESULTS

Acute toxicity study (LD_{50}): The seed extracts are characterized by a very low degree of toxicity. The acute

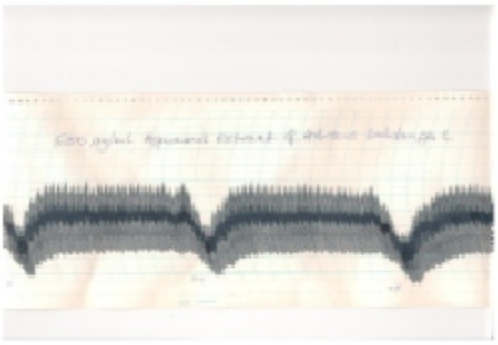

Fig 1: Tracing of 500µg/ml of aqueous seed extract of *Hibiscus sabdariffa l.* with volumes of 0.2, 0.4 and 0.8mls toxicity LD_{50} of *Hibiscus sabdariffa l.* seed extract in albino rats was found to be above 5000 $mgkg^{-1}$ according to the method of Lorke (1983).

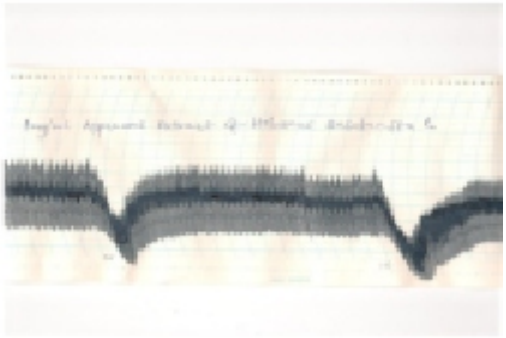

Fig. 2: Tracing of 1mg/ml of aqueous seed extract of *Hibiscus sabdariffa l.* with volumes of 0.8 and 1.6mls.

Phytochemical Analysis: The preliminary phytochemical screening of the aqueous seed extract of *Hibiscus sabdariffa l.* found the presence of alkaloids, saponins, Cardenoledes, Deoxy sugar tannins, anthraquinones, steroidal ring, cardiac glycosides, flavonoids and phlobatanins.

Blood pressure values: The blood pressure values of the cat for normal saline, the injected standard drugs and aqueous extract of *Hibiscus sabdariffa l.* as obtained from the micro-dynamometer are shown in the Table 1-3 and Fig. 1-5.. One centimeter (1cm) of the standard ruler used in measuring the systolic and diastolic blood pressure change corresponds to 10mmHg pressure change in glass sphygmomanometer. On the tracing the values from the baseline to the lowest border of the tracing represent the diastolic pressure while from the baseline to upper border represent the systolic pressure. The results of blood pressure values were analyzed by comparing the mean ± SEM of normal basal rhythm, acetylcholine and aqueous seed extract of *Hibiscus sabdariffa l.* using one way analysis of variance as shown in the tables. The blood pressure (systolic and diastolic) pulse pressure and mean arterial pressure in all the three doses (0.5, 1.0 and

Table 1: Comparison between Normal Basal Rhythm, Acetylcholine and Aqueous Extract of *Hibiscus sabdariffa l.*

Blood pressure [mm Hg]	Basal Rhythm	Acetyl- Choline	Aqueous extract of *Hibiscus sabdariffa linn*		
			0.5 mg/ml	1.0 mg/ml	5.0 mg/ml
Systolic pressure	48.0±1.4	39.0±3.8^{S}	35.0±0.4^{S}	35.5±2.4^{S}	44.0±3.1^{S}
Diastolic pressure	32.5±1.6	16.3±3.4^{S}	26.5±0.5^{S}	26.5±0.7^{S}	26.5±0.8^{S}
Pulse pressure	15.5±2.5	19.5±0.9^{S}	8.5±0.6^{S}	6.8±10^{S}	17.8±5.6^{NS}
MAB pressure	36.5±2.4	35.3±1.1^{NS}	29.3±0.5^{S}	31.3±0.6^{S}	33.8±1.1^{S}

Table 2: Comparison between Acetylcholine and aqueous extract of *Hibiscus sabdariffa l*

Blood pressure [mm Hg]	Acetylcholine	Aqueous extract of *Hibiscus sabdariffa linn*		
		0.5 mg/ml	1.0 mg/ml	5.0 mg/ml
Systolic pressure	39.0±3.8^{S}	35.0±0.4^{S}	35.5±2.4^{S}	44.0±3.1^{S}
Diastolic pressure	16.3±3.4^{S}	26.5±0.5^{S}	26.5±0.7^{S}	26.5±0.8^{S}
Pulse pressure	19.5±0.9^{S}	8.5±0.6^{S}	6.8±10^{S}	17.8±5.6^{NS}
MAB pressure	35.3±1.1^{NS}	29.3±0.5^{S}	31.3±0.6^{S}	33.8±1.1^{S}

Table 3: Drug Antagonistic Studies

Drug + Aqueous Extract	0.2 ml Atropine [100µg/ml] + 0.4ml Aq. extract [1mg/ml]			0.1ml Ach [10µg/ml] + 0.4ml aq. Extract [1mg/ml]		
Blood pressure parameters [mm Hg]	Atropine	Atropine+aq. ext	% change	Ach	Ach + aq. Ext	% change
Systolic pressure	39	40	2.5	40	35	12.5
Diastolic pressure	30	30	0.0	30	27	10.0
Pulse pressure	9	10	2.5	10	8	20.0
MAB pressure	33	33.3	1.0	33.3	29.5	11.5

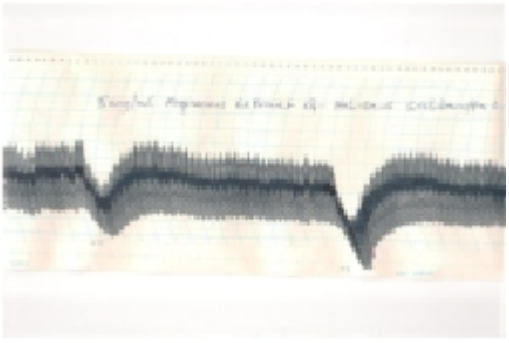

Fig. 3: Tracing of 5mg/ml of aqueous seed extract of *Hibiscus sabdariffa l.* with volumes of 0.8 and 1.6mls.

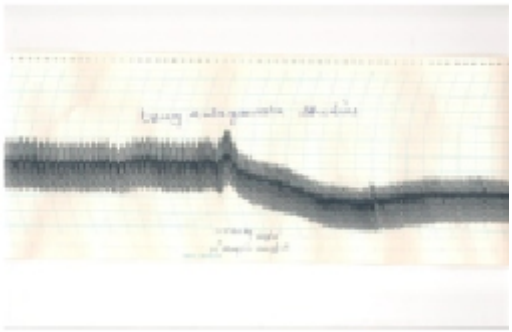

Fig. 4: Tracing of drug antagonist studies of 0.4ml of 5mg/ml aqueous seed extract of *Hibiscus sabdariffa l.* blocked by 0.1ml of 100µg/ml of Atropine.

5.0mg/ml) of seed extract decreased significantly (P<0.05) when compare to normal basal rhythm, except in 5mg/ml of the pulse pressure there was insignificant

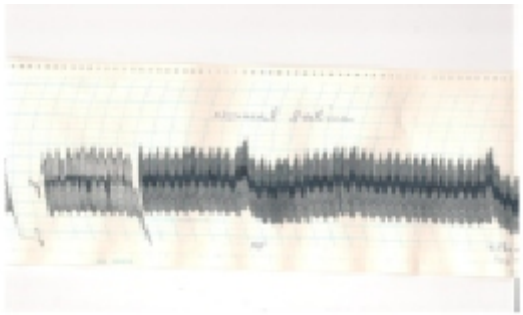

Fig 5: tracking of normal saline

decrease (P>0.05) when compare to normal basal rhythm. The tracings of 500µg/ml in Fig. 1, 1mg/ml in Fig. 2 and 5mg/ml in Fig. 3 showed hypotensive effect *Hibiscus sabdariffa l.*

The standard drug Acetylcholine also when compared to the normal basal rhythm, the blood pressure (systolic and diastolic), pulse pressure and mean arterial pressure in all the doses of Acetylcholine decreased significantly (P<0.05) as shown in Table 2 and Fig 2.

In the drug antagonist studies in Table 3, both Atropine and Acetylcholine blocked the effect of the seed extract. Although the percentage change of Atropine was less than that of Acetylcholine, this indicates that Atropine has stronger blocking effect as seen in Fig. 4.

DISCUSSION

The results of the present study reported that, the aqueous seed extract *Hibiscus sabdariffa l.* showed blood pressure lowering effect in normotensive cat with

significant statistical difference ($P<0.05$). The blood pressure (systolic and diastolic), pulse pressure and mean arterial pressure in all the three doses (0.5, 1.0 and 5.0mg/ml) of seed extract decreased significantly ($P<0.05$) when compare to normal basal rhythm, except in 5mg/ml of the pulse pressure there was insignificant decrease ($P>0.05$) when compare to normal basal rhythm. Intravenous injection of aqueous extracts of *Hibiscus sabdariffa* calyx to anaesthetized cats (Ali *et al.*, 1991) and anaesthetized rats (Adegunloye *et al.*, 1996) lowered blood pressure in a dose-dependent manner. The standard drug Acetylcholine also when compared to the normal basal rhythm, the blood pressure (systolic and diastolic), pulse pressure and mean arterial pressure in all the doses Acetylcholine decreased significantly ($P<0.05$). These effect was completely blocked by Atropine and Acetylcholine in seed extract while the calyx effect was resistant to a number of standard receptor blocking agents, but the hypotensive effect was partially blocked by atropine (Ali *et al.*, 1991), and atropine and antihistamine (H_1 blockers). Therefore, the hypotensive action of *Hibiscus sabdariffa l.* may be mediated through cholinoceptors as cholinergic antagonist, because Atropine blocked the effect of seed extract. From this study it can be inferred that, *Hibiscus sabdariffa l.* seed has an appreciable blood pressure lowering effect.

ACKNOWLEDGMENT

The authors are grateful to the following; All the technical staff in the Department of pharmacology Ahmadu Bello University, Zaria for the role they played in the laboratory work during this research particularly Mr. John and Mallam. Ibrahim Adamu. We also acknowledge University Board of Research of Ahmadu Bello University, Zaria for supporting this research work.

REFERENCES

Abdul, G., 1990. Introduction to Pharmacognosy. University press, 1st Edn., pp: 175-200.

Adegunloye, B.J., J.O. Omoniyi, and O.P. Ajabonna, 1996. Mechanisms of the blood pressure lowering effects of the calyx extract of *Hibiscus sabdariffa* in rats. Afr. J. Med. Med Sci., 25: 235-238.

Ali, B.H., N. Al-Wabel and G. Blunden, 2005. Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa l.* a review, Phytother. Res., 19(5): 369-375.

Ali, M.S., W.M. Salih, A.H. Mohammed, and AM. Homeida, 1991. Investigation on the antispasmodic potential of *Hibiscus sabdariffa* calyces J. Ethnopharmacol., 31: 249-257.

Amin, A. and A.A. Hamza, 2005. Hepatoprotective effect of *Hibiscus, Rosmarinus* and Salvia on azathioprine-induced toxicity in rats. Life Sci., 77(3): 266-278.

Brain, K.R. and T.D. Turner, 1975. The practical evaluation of phytopharmaceuticals, wright scientechnica, Bristol. pp:57-58.

Betty, R.K. and A.C. Jonathan, 2003. Essential Medical Statistics. 2nd Edn., Blackwell science USA, pp: 15-409.

Brunold, C., A. Deter, F. Knoepfel-Sidler, J. Hafner, B. Muller, and A. Hensel, 2004. Polysaccharides from Hibiscus sabdariffa flowers stimulate proliferation and differentiation of human keratinocytes. Planta Med., 70(4): 370-373.

Chen, C.C., J.D. Hsu, S.F. Wang,, H.C. Chang, M.Y. Yang, E.S. Kao,, Y.O. Ho and C.J. Wang, 2003. *Hibiscus sabdariffa l.* extract inhibit the development of atherosclerosis in cholesterol-fed rabbits. J. Agr. Food Chem., 51(18): 5472-5477.

Dalziel, J.M., 1973. The useful plants of west Tropical Africa. The Crown Agents, London, pp: 314-315.

Jonadet, M., J. Bastide, P. Bastide, B. Boyer, A.P. Carnat and J.L. Lamaison, 1990. *In vitro* enzyme inhibitory and In vivo cardio-protective activities of *Hibiscus sabdariffa l.* J. Pharmacol., Belgium, 45(2): 120-124.

Lorke, D. 1983. A new approach to practical acute toxicity testing. Arch. Toxicol., 54: 275-287.

Morton, J.F., 1987. Roselle. In: Fruits of Warm Climate. C.F. Dowling, (Ed.), Media, Inc. Greensboro, NCP. pp: 281-286.

Odigie, I.P., R.R. Ettarh and S.A. Adigun, 2003. Chronic administration of aqueous extract of Hibiscus sabdariffa attenuates hypertension and reverses cardiac hypertrophy in 2K-1 C hypertensive rats. J Ethnopharmacol., 86: 181-185.

Olaleye, M.T., 2007. Cytotoxicity and antibacterial activity of methanolic extract of Hibiscus sabdariffa. J. Med. Plant. Res., 1(1): 009-013.

Olatunji, L.A., J.O. Adebayo, A.A. Adesokan, A.O. Olatunji and O.A. Soladote, 2005. Chronic administration of aqueous extract of *Hibiscus sabdariffa* enhances Na^+- K^+ ATPase and Ca^{2+}-Mg^{2+} ATPase activities of rats' heart. Pharm. Biol., 44(3): 213-216.

Okasha, M.A.M., M.S. Abubakar and I.G. Bako, 2008. Study of the effect aqueous *Hibiscus sabdariffa l.* seed extract on serum prolactin level in lactating albino rats. Eur. J. Sci. Res., 22(4): 575-583.

Wang, C.J., J.M. Wang, W.L. Lin, C.Y. Chu, F.P. Chou and T.H. Tseng, 2000. Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. Food Chem. Toxicol., 38(5): 411-416.