

**THE EFFECTS OF A NIGERIAN SPECIE OF *VISCUM ALBUM*
(MISTLETOE) LEAF EXTRACT ON THE BLOOD PRESSURE OF
NORMOTENSIVE AND DOCA-INDUCED HYPERTENSIVE RATS.**

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Summary: Fresh leaves from *V. album* plant was collected and extracted using standard methods. Acute toxicity studies showed that the crude extract had an LD₅₀ value of 417.5 mg/kg. mice, i.p. Based on this value, test doses (5-160mg/kg) below the LD₅₀ value were selected and used to determine the effects of each dose of extract on the arterial blood pressure (BP) and heart rate of normotensive and hypertensive rats. Some pharmacological agents like propranolol, noradrenaline, acetylcholine and atropine sulphate were also used to assess the mechanism of action of the extract on blood pressure. From the results, the extract produced a dose-dependent depression of blood pressure and heart rate in both normo- and hypertensive rats. At doses of 5mg/kg and 160mg/kg, the extracts produced about $8.99 \pm 3.2\%$ and $54 \pm 7.4\%$ depression of BP respectively, in normotensive rats while the corresponding values for the hypertensives were $4.8 \pm 2.3\%$ and $43.9 \pm 5.5\%$ respectively. The duration of action of the extract was also found to be dose-dependent. Noradrenaline (1.5 µg/kg) blocked the action of the extract. Both propranolol (1.0µg/kg) and atropine (1.5µg/kg) failed to block or abolish the action of the extract on rat BP. We suggest a non-adrenergic, non-cholinergic mechanism for the action of the extract on blood pressure.

Key Words: *Mistletoe leaf (Vicum album), blood pressure, non-adrenergic, non-cholinergic.*

Introduction

In a vast majority of people suffering from hypertension the major cause or predisposing factor is not clearly defined but has been attributed to genetic factors, high dietary salt intake and possibly, psychological factors (Sofowara, 1982; Khosh and Khosh, 2001; Obatomi, *et al*, 1994). In few cases, an underlying disease condition involving body organs (Kidney, heart, adrenal gland and the brain) is the major cause. Hypertension is the commonest cardiovascular disease of black Africans (Lawal and Falase, 1988) and a major cause of morbidity and mortality among adult Nigerians (Balogun and Ladipo, 1988). It is one of the leading causes of death and disability due to complications such as coronary heart disease, stroke, congestive heart failure, end-stage renal disease and peripheral vascular disease (Khosh and Khosh, 2001). Although great successes have been achieved in the detection and management of the disease using conventional methods, many patients still resort to the orthodox methods since many

of which are reputed to offer a complete cure (personal comm.). Herbs have for centuries been used to treat and manage various ailments (Khosh and Khosh, 2001); (Sofowara, 1982). Infact, herbal medicine still remains the first line of medication amongst a vast majority of Africans (Barbara and Theiss, 1992). The mistletoe leaf extract is said to possess antidiabetic (Obatomi, *et al*, 1994), immunomodulatory (Solar, *et al*, 1998), bacteriostatic (Fulder, 1998) and therapeutic values for many other ailments. Investigations aimed at providing the scientific basis to the hypotensive property of mistletoe leaf extract have already been provided (Obatomi, *et al*, 1994; Hajto *et al*, 1999; Lavastre, *et al*, 2002).

We have observed that most of the reports about mistletoe therapy in hypertension and some other ailments are related to the English species of mistletoe. Little literature is however available about the antihypertensive activity of Nigerian species of mistletoe although many of our traditional healers have widely claimed success with mistletoe therapy.

The present study is therefore aimed at providing a scientific justification for the use of this Nigerian species of the herb in the treatment of hypertension and also to elucidate the possible mechanism of action employed by this extract in producing blood pressure depression.

Materials and Methods

(a) Preparation of the Crude Extract

The crude extract was prepared according to the method of Parry, *et al*, 1987; and Eno, *et al*, 2000. In brief, fresh leaves of *V. album* were collected from the host plant (citrus). They were first washed free of sand and debris. Wash water was blotted off and the leaves ground to paste using an electric grinder/blender. A quantity of ground sample (100g) was weighed and Soxhlet-extracted with 400ml. distilled water for 10 hr. at 100°C. The extract was then slowly evaporated to dryness in an electric oven at 35-40°C. A starting sample of fresh material gave a mean yield of $5.1 \pm 2.3\text{g}$ ($n = 5$) of extract which was stored at -4°C until use. Weighed samples of the extract were then used to make up test solutions of desired concentrations.

(b) Acute Toxicity Test

Male white albino mice (20-25g) were randomly assigned to 7 groups of 10 animals per group. Each group was injected intraperitoneally with one of the following doses: 50, 100, 200, 400, 800 or 1600mg/kg of the crude extract. The control group was injected with isotonic saline. The maximum volume given to all groups was 0.5ml. The groups were returned to their home cages, and provided with food and water *ad libitum*. After 24 hr., the mortality in each cage was assessed. The percentages mortalities were converted to probit units (probability unit) using a standard probit table (13,14) and plotted against the \log_{10} of the dose of the extract. Regression lines were fitted by the least squares method and confidence limits for the LD_{50} values were calculated by the method of Litchfield and Wilcoxon (1949).

(c) Measurement of Arterial Blood Pressure and Heart Rate

Male albino white Wistar rats (200-250g) were obtained from the animal house of the Department of Physiology, University of Calabar (Calabar, Nigeria). Approval for the study was given by the Ethical Committee of the College of Medical Sciences. The rats were randomly selected and divided into two groups of 5 animals per group. These groups, 1, and 2, were the normotensive, (or sham-

operated group) and the hypertensive group. In the group 2 rats, (hypertensives) silicone rubber moulds containing deoxycosterone acetate (DOCA, 15mg/100g) were implanted subcutaneously to induce hypertension. This group was given normal saline (0.9% NaCl₂), in place of drinking water. The first of the groups (ie. Group 1 rats) were sham-operated and given drinking water. The operated rats received 100,000 IU penicillin (i.m.) to prevent postoperative infection. All rats were fed with rat cubes (Pfizer products, Nigeria) *ad libitum*. Rats were considered hypertensive when their mean arterial pressure (MAP) values were 140mmHg or above.

At the end of 6 weeks each rat was anaesthetized with 6% pentobarbitone (0.1ml/100g. body wt.). The trachea was intubated and femoral vein and carotid artery cannulated (Portex cannulae, external diameter 1.02mm; internal diameter 0.75mm). The cannulation of the femoral vein and carotid artery were for drug administration and blood pressure measurements respectively. The pressure transducer (M/N MB5049/41-6A) was connected to a recording polygraph (S/N 000237, M/N 30) for the blood pressure measurements. The temperature was maintained at $37 \pm 1^\circ\text{C}$ by means of a rat table heater. Graded doses (ie. 5, 10, 20, 40, 80, and 160mg/Kg) of the extract or drugs were injected (i.v.) via the femoral vein. The maximum volume of injected fluid was 0.2ml. Each injected dose was followed by a flushing injection of 0.2ml saline. Blood pressure responses were measured as change in mean arterial pressure (MAP in mmHg), from the pretreated levels. The MAP was calculated by using the formula: $\text{MAP} = \text{DP} + \frac{1}{3}(\text{SP} - \text{DP})$, where DP and SP are the diastolic and systolic pressures, respectively.

For simultaneous heart rate counting, the transducer signal was fed into a biotachometer in one of the polygraph channels.

In another group of normotensive rats, attempts were made to elucidate the possible mechanism of action of the extract on blood pressure. Some drugs like propranolol (1.0µg/Kg), noradrenaline (1.5µg/Kg), acetylcholine and atropine sulphates (1.5µg/Kg) were used to influence the action of the extract on blood pressure.

Statistical Analysis

The results are expressed as mean \pm standard error of mean (SEM). Paired student's t-test was used for statistical

analysis of data. Differences between group means were considered to be significant at $P < 0.05$.

Drugs and Chemicals

Propranolol, noradrenaline, DOCA-salt, were purchased from Sigma (U.S.A). Atropine sulphate acetylcholine and pentobarbitone from CIBA (U.S.A).

Results

(a) Acute Toxicity Test

Lethality studies showed that the crude extract from the leaves of *V. album* (mistletoe) had an LD_{50} value of 417.5mg/kg. mice, i.p. (Fig.1). The high dose recipients were immobile and were lying on their abdomen. They were cold to touch with piloerection.

(b) Responses of Arterial Blood Pressure and Heart Rate

The mean control arterial blood pressure (MAP) of the normotensive rats was about 95.31 ± 6.2 mmHg. Following slow intravenous administration of the crude extract (5-160/mg/kg. body wt.), there were significant reductions in blood pressure (BP) in a dose-dependent fashion (Fig. 2). In the normotensive group, the low doses of the extract (5 and 10mg/kg) produced about 8.99% and 26.82% reduction in BP respectively, while at the high doses (80 and 160mg/kg) tested produced about 45.2% and 54.1% reduction respectively. Apart from the dose-dependent nature of the extract in reducing BP, observation also showed that the extract's duration of action (BP depression) was also dose-dependent. (Table 1). The basal heart rate levels in both the normotensive and hypertensive animals were remarkably low. However, the crude extract (5-160mg/kg) dose-dependently reduced the rate of heartbeat in the normotensive rats (Fig.3). The low doses tested (5 and 10mg/kg) produced about 3.23% and 7.26% depression of heart rate (HR) respectively. The corresponding values for the high doses

(80 and 160mg/kg) were 27.4% and 37.1% respectively (% control). These depressions of HR by the crude extract (5-160mg/kg) were significant. $P < 0.05$ in all cases.

In the DOCA-induced hypertensive group, the mean control arterial blood pressure (MAP) was about 164.3 ± 5.7 mmHg (S.E.M; n =5). (Fig. 2). Following the administration of the crude extract (5 – 160mg/kg), the BP levels were dose – dependently depressed, and the duration of BP depression was also dose-dependent. The low dose extracts tested (5 and 10mg/kg), caused about 4.8% and 12.2% lowering of BP respectively (% control), while the high dose extracts (80 and 160mg/kg) produced about 31.3% and 43.9% depression respectively (% control).

The crude extract (5-160mg/kg) caused a dose-dependent depression of heart rate (Table 2). About 8.2% and 16.2% depression of HR was caused by the 5 and 10mg/kg extract respectively. The 80 and 160 mg/kg extracts produced about 31.8% and 37.4% depression of the hypertensive HR respectively.

The effect of some pharmacological agents on the extract-induced depression of BP was also studied in the normotensive rats (Table 3). From the results (Table 3), noradrenaline (1.5 μ g/kg) opposed the extract-induced depression of BP with a resultant high blood pressure (Fig. 4a). The figure (Fig. 4a) also showed that the extract (20 mg/kg) also blocked NA-induced increase in BP, resulting in hypotension. Administration of acetylcholine (1.5 μ g/kg) produce a depression of BP and this condition was aggravated by the injection of the extract (20mg/kg) (Fig. 4 b). The actions of propranolol (a β_2 -adrenoceptor blocker) and atropine sulphate (a muscarinic cholinceptor blocker) on blood pressure were both antagonized or prevented by the administration of the extract. (Figs. 4c and 4d).

Table 1. The degree of MAP depression and Duration of action

Extract Conc. (mg/Kg)	% Depression of MAP		Duration of BP Depression (sec.)	
	Normotensives	Hypertensives	Normotensives	Hypertensives
5	4.52 ± 2.3	8.75 ± 1.6	6.3 ± 1.4	8.1 ± 2.2
10	13.48 ± 3.4	25.29 ± 3.8	10.2 ± 3.2	12.2 ± 3.4
20	19.76 ± 3.8	35.68 ± 4.3	29.6 ± 6.2	25.4 ± 4.7
40	26.17 ± 4.3	44.25 ± 3.6	39.4 ± 3.8	36.7 ± 3.6
80	32.85 ± 3.7	48.19 ± 2.8	67.5 ± 4.9	63.8 ± 5.2
160	44.17 ± 4.3	57.86 ± 3.9	88.7 ± 5.4	85.5 ± 4.8

Effect graded of the crude extract (5-160mg/Kg) on the depression of MAP (%) and the duration of depression (sec.). Values represent means ± SEM, n=5.

Table 2.: The degree of heart rate depression and the duration of action.

Extract Concentration (mg/Kg)	Percentage Depression of Heart Rate		Duration of Heart rate depression (sec.)	
	Normotensives	Hypertensives	Normotensives	Hypertensives
5	1.66 ± 0.3	128.50 ± 6.2	5.88 ± 1.5	10.25 ± 3.2
10	3.34 ± 0.6	117.32 ± 8.1	10.41 ± 2.3	12.82 ± 2.8
20	15.01 ± 3.4	110.53 ± 5.7	30.32 ± 3.4	23.74 ± 4.5
40	22.16 ± 4.5	103.74 ± 7.2	38.62 ± 5.2	35.52 ± 6.4
80	30.82 ± 2.3	95.48 ± 4.8	68.28 ± 3.5	62.33 ± 5.8
160	35.83 ± 3.2	87.64 ± 5.5	90.35 ± 4.8	86.25 ± 6.6

Effect of graded doses (5-160 mg/Kg) of the crude extract on the percentage depression of Heart rate and the duration of depression (sec.). Data represents the mean Values ± SEM. (n = 5).

Table 3.:Effect of some pharmacological agents on the extract-induced depression of arterial BP.

Agents Injected (i.v.)	Mean Arterial Pressure of Normotensives (mmHg)		BP Response (% Control)
	Pre-injection (control) value (mmHg)	Post-injection (Treated) value (mmHg)	
Extract (20mg/Kg)	94.8 ± 4.6 (8)	64.2 ± 5.3	32.3% Decrease
Extract + NA(1.5µg/Kg)	65.2 ± 7.5 (5)	136.3 ± 8.6	109.1% Increase
ACh (1.5µg/Kg)	93.6 ± 5.2 (5)	67.2 ± 5.8	28.2% Decrease
ACh + Extract	86.6 ± 6.6 (5)	55.1 ± 4.6	36.4% Decrease
Prop. (1.0µg/Kg)	96.4 ± 4.4 (6)	80.5 ± 6.2	16.5% Decrease
Prop.+ Extract	92.1 ± 5.8 (6)	66.7 ± 5.4	27.6% Decrease
Atro. (1.5µg/Kg)	94.6 ± 3.8 (5)	145.3 ± 8.2	53.6% Increase
Atro. + Extract	145.3 ± 9.7 (6)	659.5 ± 5.7	34.3% Decrease

Effect of the crude extract (20mg/Kg i.v.) from *Viscum album* leaves and in combination with some pharmacological agents (Noradrenaline, (NA) 1.5µg/Kg; Acetylcholine (ACh), 1.5µg/Kg; Propranolol (Prop), 1.0µg/Kg and Atropine sulphate (Atro), 1.5µg/Kg on the arterial pressure (MAP) of normotensive rats. Data represent the mean values ± SEM and the number of experiments in parentheses.

Fig. 1: Lethality studies showing the effect of administering graded doses (50-1600mg/kg. i.p. mice) of the crude extract against the percentage mortality (converted to probits).

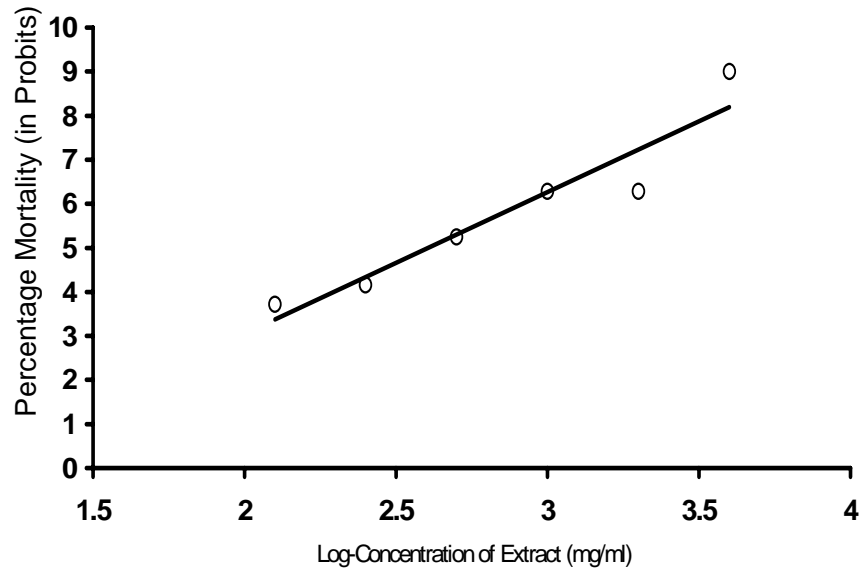


Fig. 2. Histogram showing the effect of crude extract (5-160mg/kg. i.v.) on the mean arterial blood pressure (MAP) in normotensive (□) and DOCA-induced hypertensive rat (▨) . Data represents mean values \pm S. E. M. (n=5).

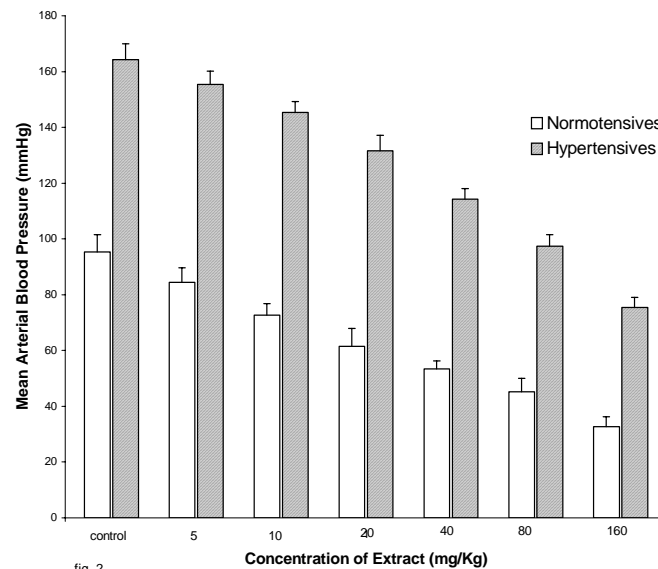


fig. 2

Fig. 3. Histogram showing the effect of crude extract (5-160 mg/kg.iv.) on the rate of heart beat (HR) in normotensive (□), and DOCA-induced hypertensive rats (▨). Data represents mean values \pm S.E.M. (n=5).

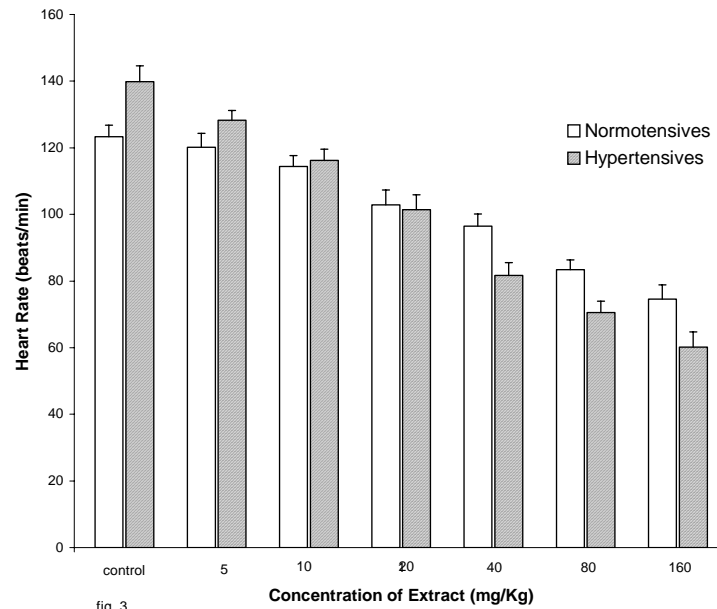
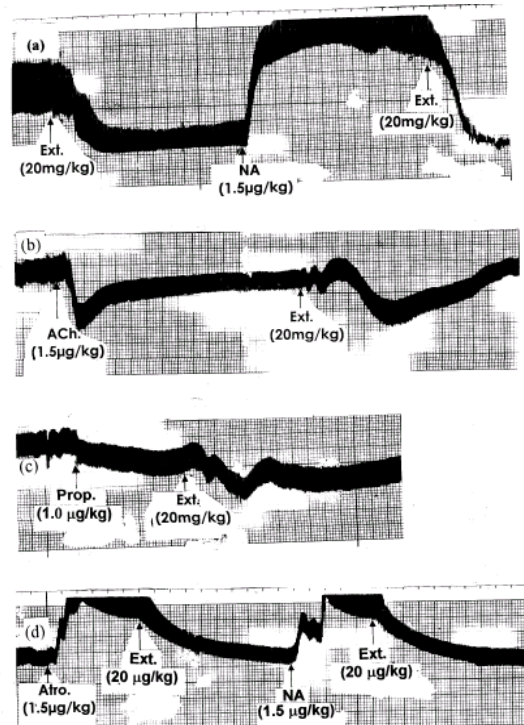


fig. 3

Fig. 4. Typical mechanical recordings showing the effect of (a) Noradrenaline (NA) on extract-induced depression of BP, and the effects of the crude extract (20mg/kg) on acetylcholine (Ach) (b); and propranolol (Prop) (c);-induced depression of BP. The effect of the extract (20mg/kg) on atropine (Atro) and noradrenaline (NA)-induced increase in BP is shown in (d).



Discussion

It now appears much clearer why many patients who undergo mistletoe therapy and even those who take it as tea for preventive purposes rarely complain of any adverse effect of the extract. From the present study, the extract has probably a very wide safety margin and is probably non-toxic as indicated by the high LD₅₀ value.

Blood pressure is the product of the cardiac output and the peripheral resistance of the vessels (Bowman and Rand, 1980). Therefore, the main organs concerned with its maintenance are the heart and the blood vessels, although, these are under the influence of the central nervous system. Blood pressure measurement therefore reflects the integrity of the cardiovascular system. In this study, the blood pressure of normotensive and hypertensive rats was measured. There is sufficient evidence that suggests that the crude extract from *V. album* leaves cause a depression of blood pressure in both groups (normotensives and hypertensives) in a dose-dependent manner. This result is consistent with earlier views that hypertension could be managed successfully using mistletoe therapy (Duke, 1985; Khosh and Khosh, 2001; Thompson, 2003). The duration of action of a drug is of a major clinical importance. This study shows that the duration of the extract was more prolonged with increased extract concentration. The extract probably decreased the blood pressure in both normotensive and hypertensive rats by decreasing the heart rate which is a major determinant of the cardiac output (Guyton and Hall, 2000). We are however unable to provide an explanation for the observed low basal heart rate in both normotensive and the hypertensive groups. Probably, we can only explain this in terms of agents in the food consumed (rat chow) or to reduced sensitivity of the measuring instrument.

However, blood pressure depression by the extract appeared to be more effective in the hypertensive than in the normotensive rats. It is probable that the more elevated pressure in the hypertensives is an indication that the activity of the crude extract is more in the hypertensives than the normotensives. It is very difficult to elucidate the possible mechanism of action of this crude extract. While it is quite evident that the extract may be reducing blood pressure by a reduction in heart rate, it is also quite clear from this study that the extract utilizes neither the adrenergic nor the cholinergic mechanism. This is

suggested because, blocking the adrenergic mechanism with propranolol (a β_2 -adrenoceptor antagonist), did not prevent the action of the extract suggesting that the extract is acting at a different site (i.e. non-adrenergic). Also, blocking the cholinergic mechanism with atropine failed to prevent the action of the extract, suggesting a non-cholinergic involvement. Since the innervation of the heart is both adrenergic and cholinergic, it is very likely that the depression of heart rate by the extract is via a non-neural mechanism. Therefore, moderation of calcium availability to the myocardial cells is strongly suspected.

In conclusion, the crude mistletoe extract reduces the mean arterial blood pressure of both the normotensive and hypertensive rats. It is very likely that this action is achieved by a reduction in heart rate and probably other mechanisms as well. This is consistent with other reports about the activity of the extract on blood pressure. However, that the extract employs the autonomic pathways (adrenergic and cholinergic) in depressing the heart rate is very unlikely.

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