

# Alteration of Blood Pressure Indices and Pulse Rates by an Aqueous Extract of the Leaves of *Chromolaena odorata* (L) King and Robinson (Asteraceae).

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## ABSTRACT

The ability of an aqueous extract of the leaves of *Chromolaena odorata* to alter blood pressure indices and pulse rates were investigated in normal and sub-chronic salt-loaded rats. The normal control and treatment control groups received a diet consisting 100% of the commercial feed, while the test control, reference treatment and test treatment groups received an 8% salt-loaded diet. The extract was orally administered daily at 100 and 200 mg/kg body weight; while the moduretics was administered at 1 mg/kg. The test control, reference and control groups received appropriate volumes of water by the same route.

On gas chromatographic analysis of the crude aqueous extract, thirty known flavonoids were detected, consisting mainly of kaempferol (52.294%), quercetin (34.901%) and apigenin (7.699%). Carotene (50.111%), lycopene (32.734%), and malvidin (11.692%) were the most abundant of the ten known carotenoids that were detected; while sitosterol (98.255%) was the most abundant of the seven known phytosterols detected. Of the six known hydroxycinnamic acid derivatives detected, p-coumaric (55.430%) and caffeic acids (44.505%) were the most abundant.

The extract dose dependently lowered the systolic, diastolic, pulse and mean arterial pressures of the treated rats, compared to test control and corresponding values on day 0. It produced a mixed effect on the pulse rates of the test groups compared to the test control. This result implies that the extract may probably manage hypertension by altering all the blood pressure indices; and in addition, supports the use of the leaves in traditional health care, for the management hypertension.

(Keywords: blood pressure indices, carotenoids, *Chromolaena odorata*, flavonoids, hydroxycinnamic acids, phytosterols, pulse rates, salt-loading)

## INTRODUCTION

Hypertension is one of the most important risk factors for cardiovascular diseases that are the leading cause of mortality worldwide (Jonas *et al.*, 2010). The relative impact of blood pressure components (systolic blood pressure, SBP; diastolic blood pressure, DBP; pulse pressure, PP and mean arterial pressure, MAP) on cardiovascular risk is currently under debate. Their contributions to cardiovascular disease change across the lifespan: from diastolic blood pressure to systolic blood pressure and ultimately to pulse pressure (Franklin *et al.*, 2001; Assmann *et al.*, 2005; Freitag *et al.*, 2006; Kengne *et al.*, 2009). Fast heart rate is a potent precursor of hypertension, atherosclerosis, and their sequelae (Gillman *et al.*, 1993; Palatini and Julius, 1997); and has been associated with an increased risk of death from cardiovascular and non-cardiovascular causes. This relationship has been found in general populations, in elderly individuals, and in hypertensive cohorts (Palatini, 1999).

The risk of hypertension-related morbidity and mortality is substantially reduced by antihypertensive drug therapy (ALLHAT, 2002; Gu *et al.*, 2010). Effective antihypertensive drugs currently in use include diuretics,  $\beta$ -blockers, calcium channel blockers, angiotensin converting enzyme inhibitors, and angiotensin receptor blockers (Gu *et al.*, 2010). All of these classes of drugs reduce cardiovascular disease mortality equally; though differ in the degree of protection they provide. Herbal products are becoming increasingly popular, for the management of

hypertension and other cardiovascular diseases, probably due to the fact that they are relatively cheaper. *Chromolaena odorata* is one of such plants with a potential for use in the management of hypertension.

*Chromolaena odorata* (L) King and Robinson belongs to the Asteraceae family. It is native to South and Central America, but is presently found throughout the tropics, Nigeria inclusive (Fosberg and Sacht, 1980; State of Queensland, 2007). It is commonly called "Awolowo", "Independence weed", siam weed, trifid weed, bitter bush or jack in the bush (Okon and Amalu, 2003). The Ibo people of South Eastern Nigeria call it "ahihia eliza" or "obiara kara". In traditional health care practice, it is popular for its antispasmodic, anti-protozoal, antifungal, anti-trypanosomal, anti-hypertensive and antibacterial activities (Phan *et al.*, 2001). It has also been reported to possess anti-inflammatory, astringent, diuretic, weight reducing, hepatotropic and hypolipidemic properties (Akinmoladun *et al.*, 2007; Ikewuchi, and Ikewuchi, 2011). In the southern part of Nigeria and in Vietnam, fresh leaves or a decoction of *Chromolaena odorata* are used for the treatment of leech bite, soft tissue wounds, burn wounds, skin infection, dento-alveolitis, and to stop bleeding (Phan *et al.*, 2001).

The phytochemical screening of the leaves revealed the presence of alkaloids, cyanogenic glycosides, flavonoids (aurone, chalcone, flavone and flavonol), phytates, saponins and tannins (Igboh *et al.*, 2009). Consequent upon the use of this plant in traditional health care as an anti-hypertensive and diuretic, and due to the scarcity of information in the literature regarding the evaluation of its hypotensive property, the present study was undertaken to investigate the effect of aqueous extract of the leaves of *Chromolaena odorata* on the blood pressure indices and pulse rates of salt-loaded rats.

## **MATERIALS AND METHODS**

### **Preparation of Plant Extract**

Wistar albino rats (weighing 180-210 g at the start of the study) were collected from the animal house of the Department of Physiology, University of Nigeria, Enugu Campus. Samples of fresh whole *Chromolaena odorata* plants were procured from within the Abuja campus of the University of Port Harcourt, Nigeria. After due identification at

the University of Port Harcourt's Herbarium, the identity was confirmed/ authenticated by Dr. Michael C. Dike of Taxonomy Unit, Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria; and Mr. John Ibe, the Herbarium Manager of the Forestry Department, National Root Crops Research Institute (NRCRI), Umuahia, Nigeria. The leaves were removed, cleaned, oven dried at 55 °C and ground into powder. The resultant powder was soaked in hot, boiled distilled water for 12 h, after which the resultant mixture was filtered and the filtrate was stored in the refrigerator for subsequent use. A known volume of this extract was evaporated to dryness, and the weight of the residue used to determine the concentration of the filtrate, which was in turn used to determine the dose of administration of the extract. The residue of the crude aqueous extract was used for the phytochemical study, to determine its composition.

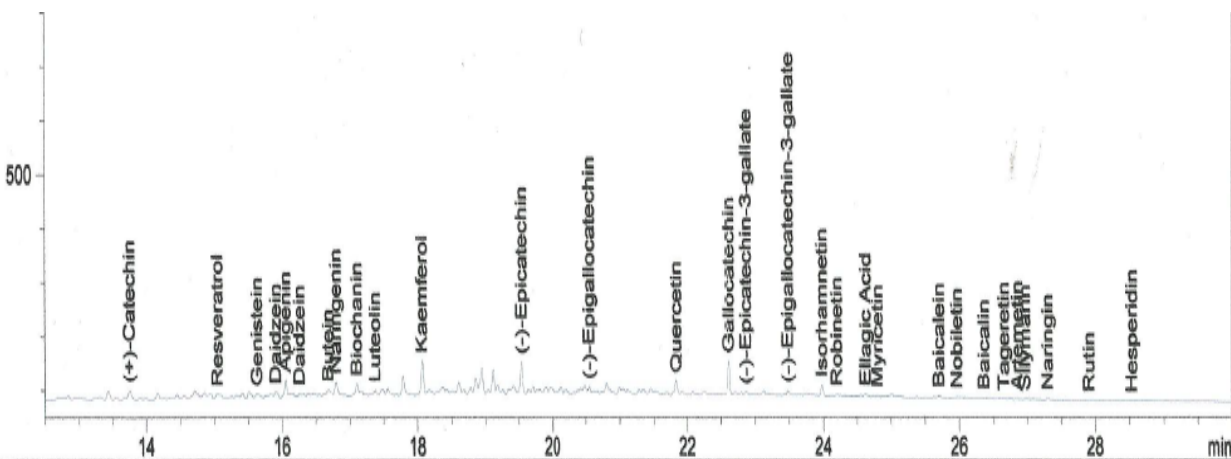
### **Determination of the Phytochemical Content of the Leaf Extract**

#### **Calibration, Identification and Quantification:**

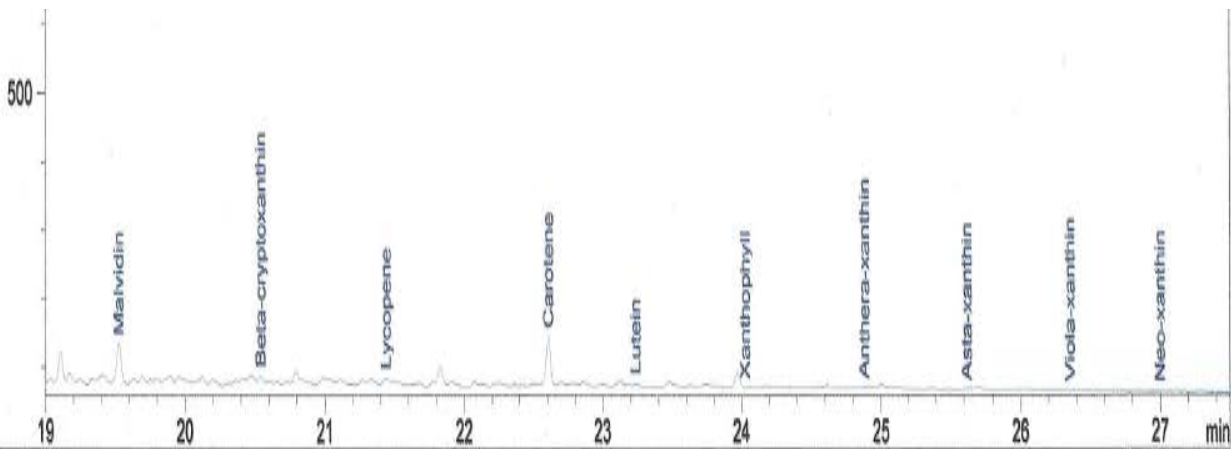
Standard solutions were prepared in methanol for flavonoids, acetone for carotenoids, methylene chloride for phytosterols, and ethanol for hydroxycinnamic acids. The linearity of the dependence of response on concentration was verified by regression analysis. Identification was based on comparison of retention times and spectral data with standards. Quantification was performed by establishing calibration curves for each compound determined, using the standards. Samples chromatograms of the extracts are shown in Figures 1-4.

### **Determination of carotenoid composition**

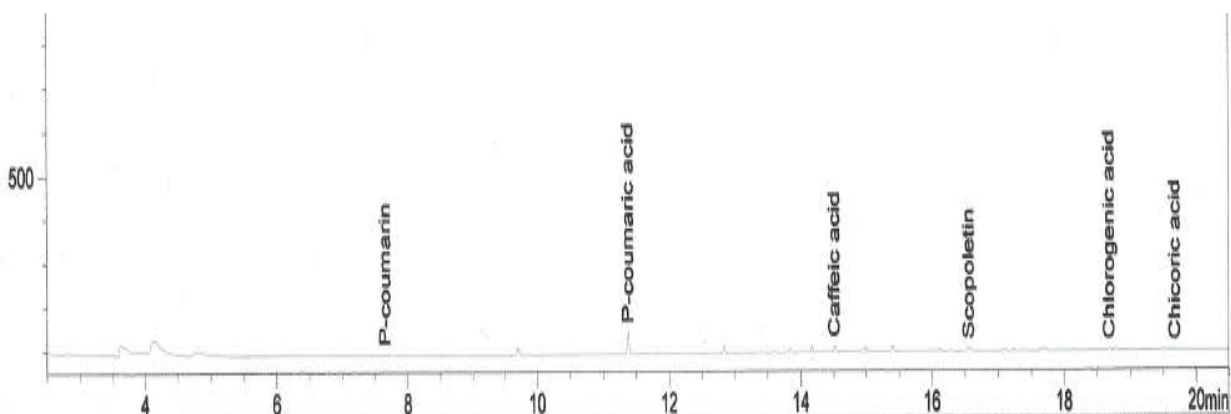
The extraction was carried out by a modification of the method of Rodriguez-Amaya and Kimura (2004). The carotenoids were successively extracted with acetone and a (1:1) mixture of diethyl ether and petroleum ether, were concentrated and re-dissolved in acetone before saponifying and re-extracting with a (1:1) mixture of diethyl ether and petroleum ether. The resultant extracts were dried and re-dissolved in petroleum ether and subjected to gas chromatography analysis.



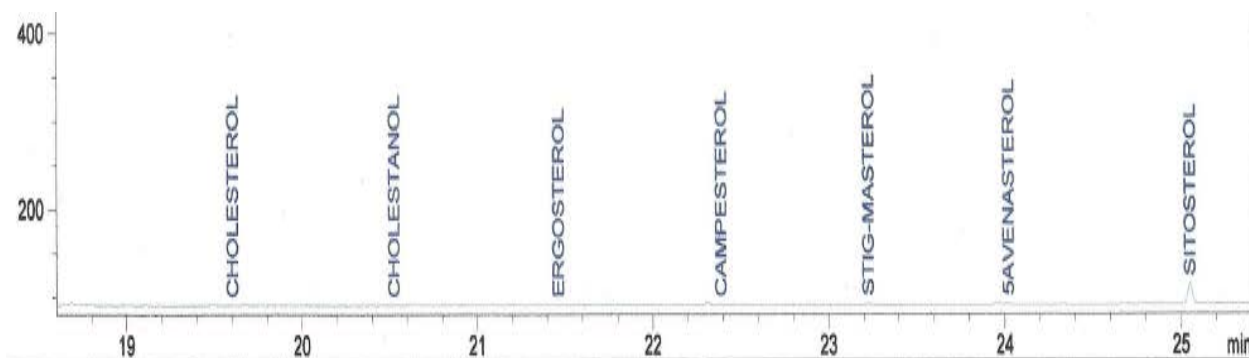
**Figure 1:** The Chromatogram of the Flavonoids Fraction of an Aqueous Extract of the Leaves of *Chromolaena odorata*.



**Figure 2:** The Chromatogram of the Carotenoids Fraction of an Aqueous Extract of the Leaves of *Chromolaena odorata*.



**Figure 3:** The Chromatogram of the Hydroxycinnamic Acid Derivatives Fraction of an Aqueous Extract of the Leaves of *Chromolaena odorata*.



**Figure 4:** The Chromatogram of the Phytosterols Fraction of an Aqueous Extract of the Leaves of *Chromolaena odorata*.

The carotenoid extracts were analyzed using a Hewlett-Packard HP6890 GC with flame ionization detector (FID; range scanned, 300 to 600 nm) and powered with HP Chemstation Rev A 09.01 (1206) software. The capillary column was a ZP-5 Column (30 m × 0.32 mm × 0.25 μm film thickness). Temperature program of 45 °C, held for 6 min, then temperature programmed at 38C/min to 250 °C. Initial column head pressure 3.47 psi. (Constant flow mode; 1 psi = 56894.76 Pa).

#### **Determination of Flavonoid Composition**

The extraction was carried out according to the method of Millogo-Kone *et al.* (2009). The sample was extracted with methanol and the resultant extract was subjected to gas chromatographic analysis.

Chromatographic analyses were carried out on an HP 6890 (Hewlett Packard, Wilmington, DE, USA), GC apparatus, fitted with a flame ionization detector (FID), and powered with HP Chemstation Rev. A 09.01 [1206] software, to quantify and identify compounds. The column was a capillary HP INNOWax Column (30 m × 0.25 mm × 0.25 μm film thickness). The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. Nitrogen was used as the carrier gas. The hydrogen and compressed air pressures were 22 psi and 35 psi. The oven was programmed as follows: initial temperature at 50 °C, first ramping at 8 °C/min for 20 min, maintained for 4 min, followed by a second ramping at 12 °C/min for 4 min, maintained for 4 min.

#### **Determination of Hydroxycinnamic Acid Composition**

The extraction was carried according to method of Ortan *et al.* (2009). The sample was extracted thrice with methanol, and the pooled extracts was concentrated and subjected to gas chromatographic analysis.

Gas chromatographic analysis was performed with a Hewlett-Packard (HP 6890) Series system, with a flame ionization detector (FID), and powered by HP Chemstation Rev A 09.01 (1206) software. The column was HP-5 (30 m × 0.32 mm × 0.25 μm film thickness). The samples were introduced via an all-glass injector working in the split mode, with nitrogen as the carrier gas, at a flow rate of 1mL/min. The injection and detector temperatures were 260 °C and 300 °C, respectively. The oven temperature was programmed at the start of the run from 170 °C to 250 °C at 5 °C/min.

#### **Determination of Phytosterol Composition**

Extraction of oil was carried out according to AOAC method 999.02 (Association of Official Analytical Chemists, 2002), while the analysis of sterols was carried out according to AOAC method 994.10 (Association of Official Analytical Chemists, 2000). This involved extraction of the lipid fraction from homogenized sample material, followed by alkaline hydrolysis (saponification), extraction of the non-saponifiables, clean-up of the extract, derivatization of the sterols, and separation and quantification of the sterol derivatives by gas chromatography (GC) using a capillary column. Chromatographic analyses were carried out on an HP 6890 (Hewlett

Packard, Wilmington, DE, USA), GC apparatus, fitted with a flame ionization detector (FID), and powered with HP Chemstation Rev. A 09.01 [1206] software, to quantify and identify compounds. The column was HP INNOWax Column (30 m × 0.25 mm × 0.25 µm film thickness). The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. Nitrogen was used as the carrier gas. The hydrogen and compressed air pressures were 22 psi and 35 psi. The oven was programmed as follows: initial temperature at 60 °C, first ramping at 10 °C/min for 20 min, maintained for 4 min, followed by a second ramping at 15 °C/min for 4 min, maintained for 10 min.

### **Experimental Design for the Antihypertensive Study**

Studies were conducted in compliance with the applicable laws and regulations for handling experimental animals. The rats were weighed and sorted into seven groups (Table 1) of five animals each, so that their average weights were approximately equal. The animals were housed in plastic cages at the animal house of the Department of Biochemistry, University of Port Harcourt. After a 1-week acclimatization period on guinea growers mash (Port Harcourt Flour Mills, Port Harcourt, Nigeria), they were weighed, and their baseline blood pressure indices were taken, before commencing the experiment.

Hypertension was induced by giving 8% salt-loaded feed for six weeks, to the appropriate rats. The 8% salt-loaded regimen was adapted from Ikewuchi *et al.* (2009, 2011a, b, c). At the end of six weeks, they were again weighed, and their blood pressures indices were taken, before commencing the administration of the extract. The Moditen™ (amloride hydrochloride-hydrochlorothiazide; product of Greenfield Pharmaceutical Co. Ltd, Jiang Su Province, China) and the extract were administered daily by intra-gastric gavages, for ten days. The dosages of administration of the extracts were adopted and modified from Ikewuchi and Ikewuchi (2011). The animals were allowed food and water *ad libitum*. The blood pressures and pulse rates were measured on days 4 and 8, after the commencement of the administration.

**Table 1:** Experimental Design for the Anti-Hypertensive Screening.

Group	Treatment
Normal control	Normal feed and water
Test control	8% salt-loaded feed and water
Reference treatment (reference)	8% salt-loaded feed and moduretic (1 mg/kg body weight)
Treatment 1	8% salt-loaded feed and aqueous leaf extract (100 mg/kg)
Treatment 2	8% salt-loaded feed and aqueous leaf extract (200 mg/kg)
Treatment control 1	Normal feed and aqueous leaf extract(100 mg/kg)
Treatment control 2	Normal feed and aqueous leaf extract (200 mg/kg)

### **Determination of Blood Pressure and Pulse Rate of the Rats**

The systolic and diastolic blood pressures and the pulse rate of the rats were measured via femoral pulse, using Omron RX Classic™ sphygmomanometer (OMRON Healthcare UK, LTD). The pulse pressure and mean arterial blood pressure were calculated mathematically from systolic and diastolic blood pressures as earlier reported by Ikewuchi *et al.* (2011a,b), using the following formulae.

$$\text{Pulse pressure} = \text{SBP} - \text{DBP} \quad (1)$$

$$\text{MABP} = \text{DBP} + \frac{1}{3}(\text{SBP} - \text{DBP}) \quad (2)$$

Where SBP = Systolic blood pressure  
 DBP = Diastolic blood pressure  
 MABP = Mean arterial blood pressure

### **Statistical Analysis of Data**

All values are reported as the mean ± S.D. (standard deviation). The values of the variables were analysed for statistically significant differences using the Student's *t*-test, with the help of SPSS Statistics 17.0 package (SPSS Inc., Chicago Ill). *P*<0.05 was assumed to be significant. Graphs were drawn using Microsoft Office Excel, 2010 software.

## RESULTS

The GC chromatogram of the flavonoid fraction of the aqueous extract of the leaves of *Chromolaena odorata* is given in Figure 1. Thirty known flavonoids were detected, consisting mainly of kaempferol (52.294%), quercetin (34.901%), apigenin (7.699%), isorhamnetin (3.705%) and luteolin (1.245%).

Figure 2 shows the GC chromatogram of the carotenoid fraction of the aqueous extract of the leaves of *C. odorata*. Ten known carotenoids were detected, consisting 50.111% carotene, 32.734% lycopene, 11.692% malvidin and 4.844% astaxanthin.

The GC chromatogram of the phytosterol fraction is shown in Figure 3. Seven known phytosterols were detected, consisting 98.255% of sitosterol.

Figure 4 shows the GC chromatogram of the hydroxycinnamic acid derivatives' fraction. Six known hydroxycinnamic acid derivatives were detected, consisting mainly of p-coumaric acid (55.430%) and caffeic acid (44.505%).

Figure 5 shows the time course of the effect of aqueous extract of the leaves of *Chromolaena odorata* on the systolic pressure of normal and salt-loaded rats. There were no significant differences in the systolic pressures of all the animals at baseline. The salt-loading caused significant increase ( $P<0.05$ ) in systolic pressure.

The systolic pressure of the test control group was significantly higher ( $P<0.05$ ) than those of the treated animals on days 4 and 8. Compared to values on day 0, the reference (on day 4 and 8), treatment 1 (on day 4) were significantly lower ( $P<0.05$ ) lower, while treatment control 2 was significantly higher ( $P<0.05$ ).

The time course of the effect of aqueous extract of the leaves of *Chromolaena odorata* on the diastolic pressures of normal and salt-loaded rats is shown in Figure 6. The diastolic pressure of the test control group was significantly higher ( $P<0.05$ ) than those of the treated animals on days 4 and 8. Compared to their corresponding values on day 0, the reference (on day 4), treatment 1 (on days 4 and 8) and treatment 2 (on day 4) were significantly lower ( $P<0.05$ ); while that of test control was significantly higher ( $P<0.05$ ) on day 4.

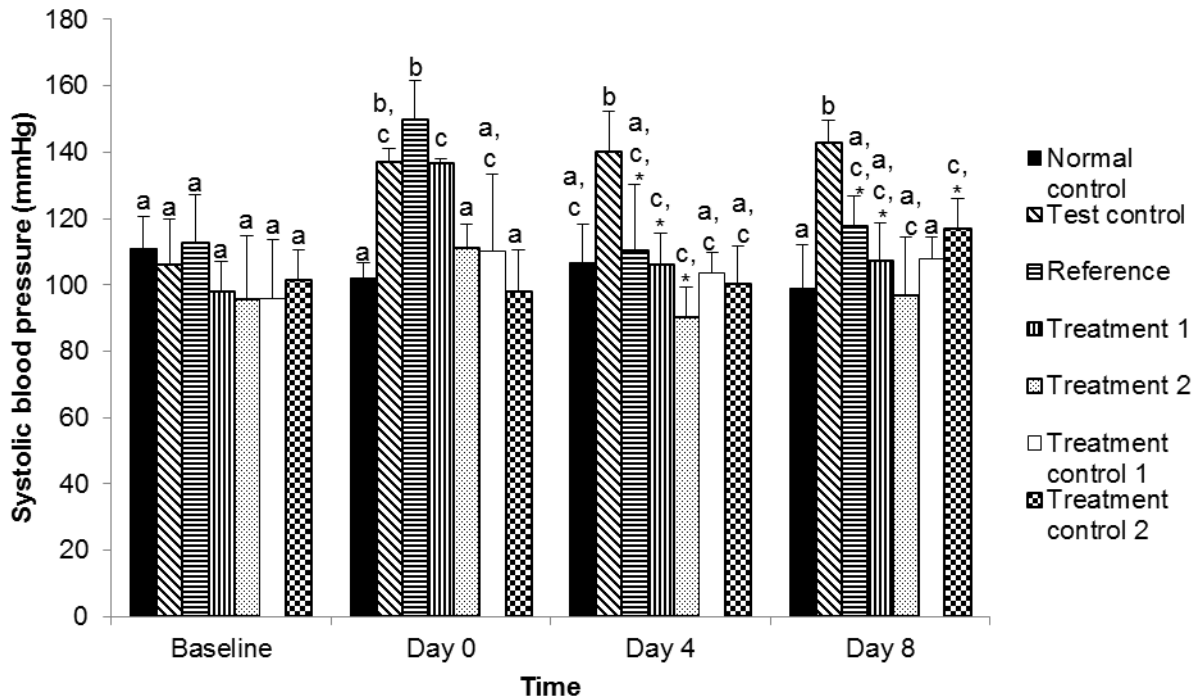
The time course of the effect of aqueous extract of the leaves of *Chromolaena odorata* on the pulse pressures of normal and salt-loaded rats is given in Figure 7. The pulse pressures of the test control group was not significantly different from that of the treated animals, except for that of treatment 2 on day 8, which was significantly lower ( $P<0.05$ ). Compared to corresponding values on day 0, the treatment 2 (on day 8) and treatment control 2 (on day 4) were significantly lower ( $P<0.05$ ).

Figure 8 shows the time course of the effect of aqueous extract of the leaves of *Chromolaena odorata* on the mean arterial pressures of normal and salt-loaded rats. The mean arterial pressures of the test control group was significantly higher ( $P<0.05$ ) than those of the treated animals on days 4 and 8. Compared to corresponding values on day 0, the mean arterial pressures of the reference (on day 4), treatment 1 (on day 4 and 8) and treatment 2 (on day 4) were significantly lower ( $P<0.05$ ), while that of the test control was significantly higher ( $P<0.05$ ).

The time course of the effect of aqueous extract of the leaves of *Chromolaena odorata* on the pulse rates of normal and salt-loaded rats is presented in Figure 9. The pulse rates of the test control group was significantly lower ( $P<0.05$ ) than those of the treated animals on day 4, but not significantly different from them on day 8. Compared to corresponding values on day 0, the pulse rates of the control group (on day 4), test control (on day 8), reference (on day 8) and treatment 1 (on day 8) were significantly higher ( $P<0.05$ ), while those of test control (on day 4), treatment 2 (on day 4), treatment control 1 (on day 4) and treatment control 2 (on days 4 and 8) were significantly lower ( $P<0.05$ ).

## DISCUSSION

The 200 mg/kg treatment produced significantly lower pulse pressure on day 8, compared to test control and corresponding value on day 0. The implication of this for the reduction of cardiovascular complications in the hypertensive cannot be over emphasized. According to Assmann *et al.* (2005), increased pulse pressure predicts cardiovascular and coronary artery disease, myocardial infarction (MI), and congestive heart failure, independent of diastolic blood pressure and systolic blood pressure, other risk markers, and "white coat" hypertension.

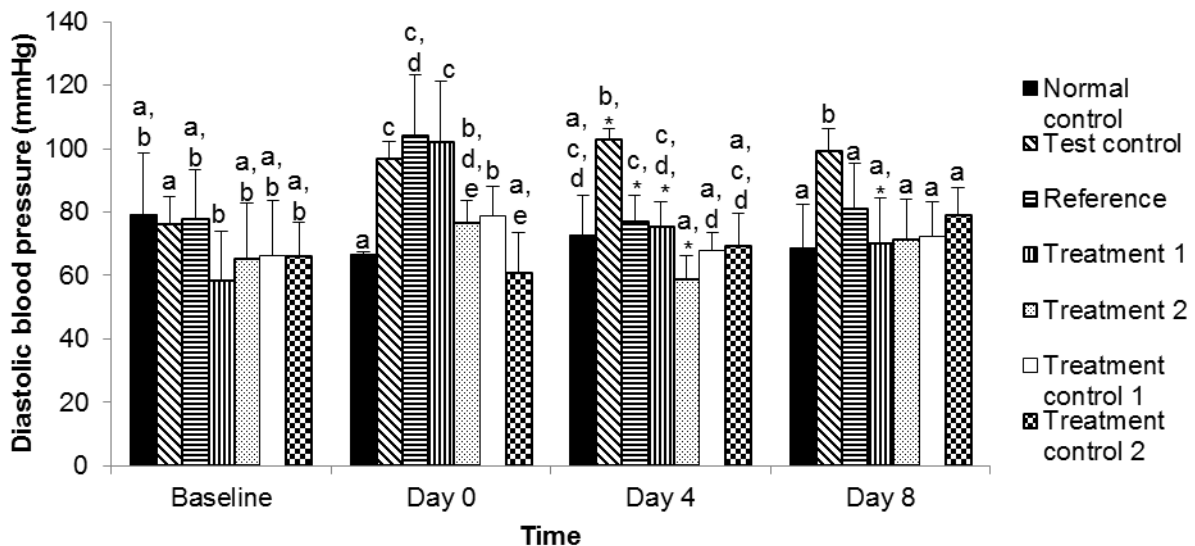


**Figure 5:** Time Course of the Effect of Aqueous Extract of the Leaves of *Chromolaena odorata* on the Systolic Blood Pressures of Normal and Salt-Loaded Rats.

Values are means  $\pm$  S.D., n=5 per group.

<sup>a,b,c</sup>Entries in the same blocks with different superscripts are significantly different at  $P < 0.05$ .

\* $P < 0.05$  compared to corresponding values on day 0.

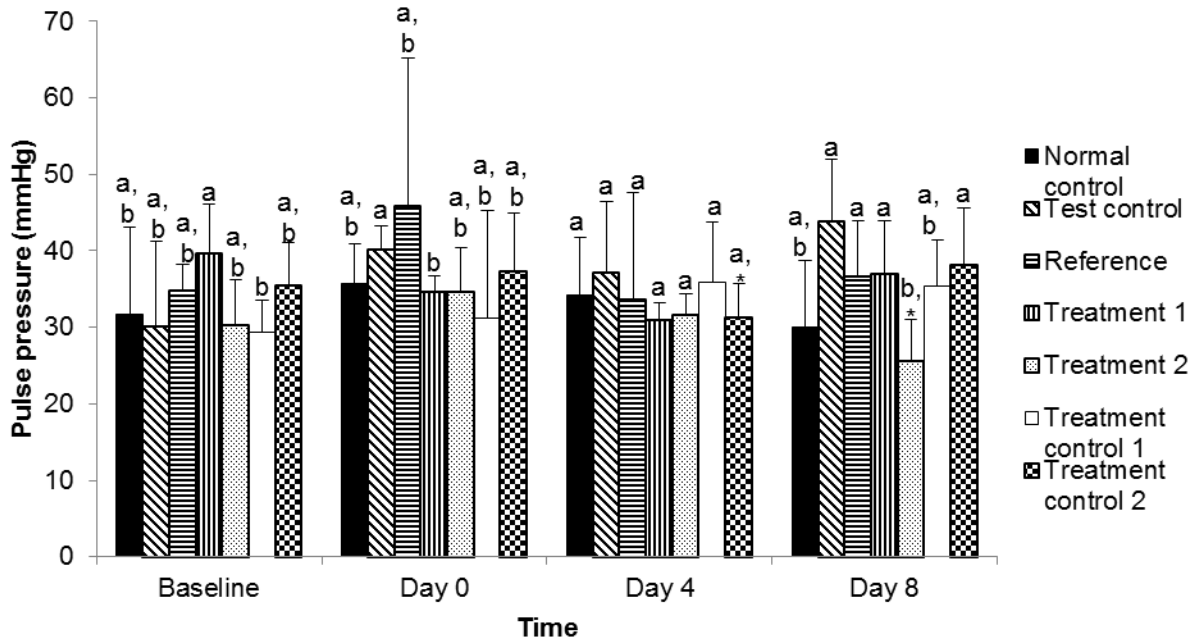


**Figure 6:** Time Course of the Effect of Aqueous Extract of the Leaves of *Chromolaena odorata* on the Diastolic Blood Pressures of Normal and Salt-Loaded Rats.

Values are means  $\pm$  S.D., n=5 per group.

<sup>a,b,c</sup>Entries in the same blocks with different superscripts are significantly different at  $P < 0.05$ .

\* $P < 0.05$  compared to corresponding values on day 0.

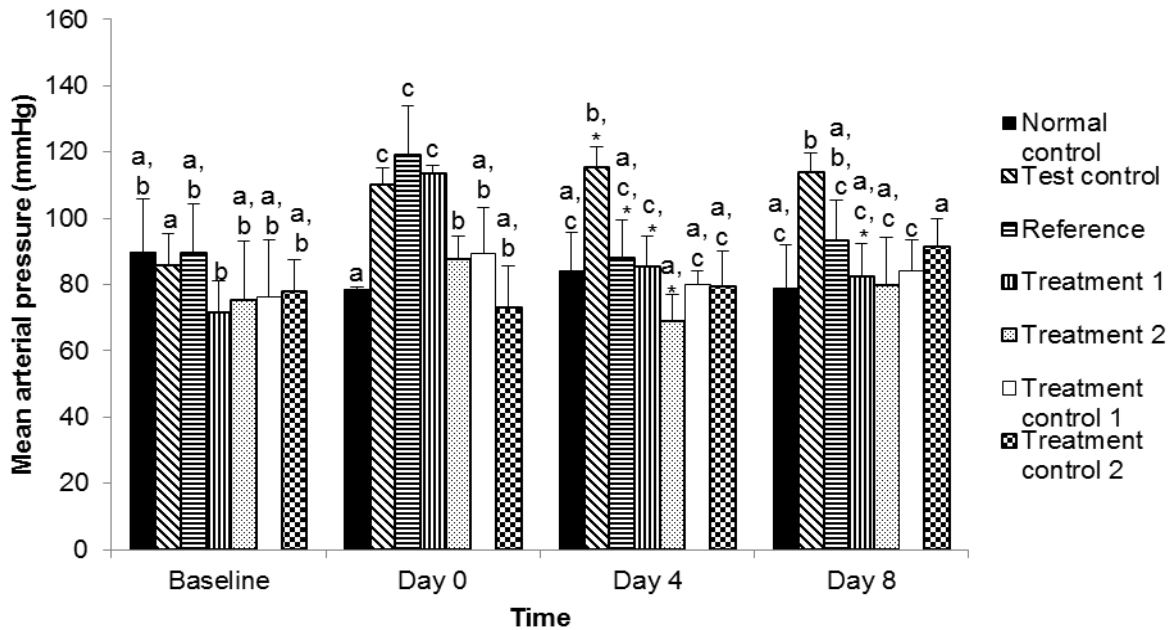


**Figure 7:** Time Course of the Effect of Aqueous Extract of the Leaves of *Chromolaena odorata* on the Pulse Pressure of Normal and Salt-Loaded Rats.

Values are means  $\pm$  S.D., n=5 per group.

<sup>a,b,c</sup> Entries in the same blocks with different superscripts are significantly different at  $P < 0.05$ .

\* $P < 0.05$  compared to corresponding values on day 0.



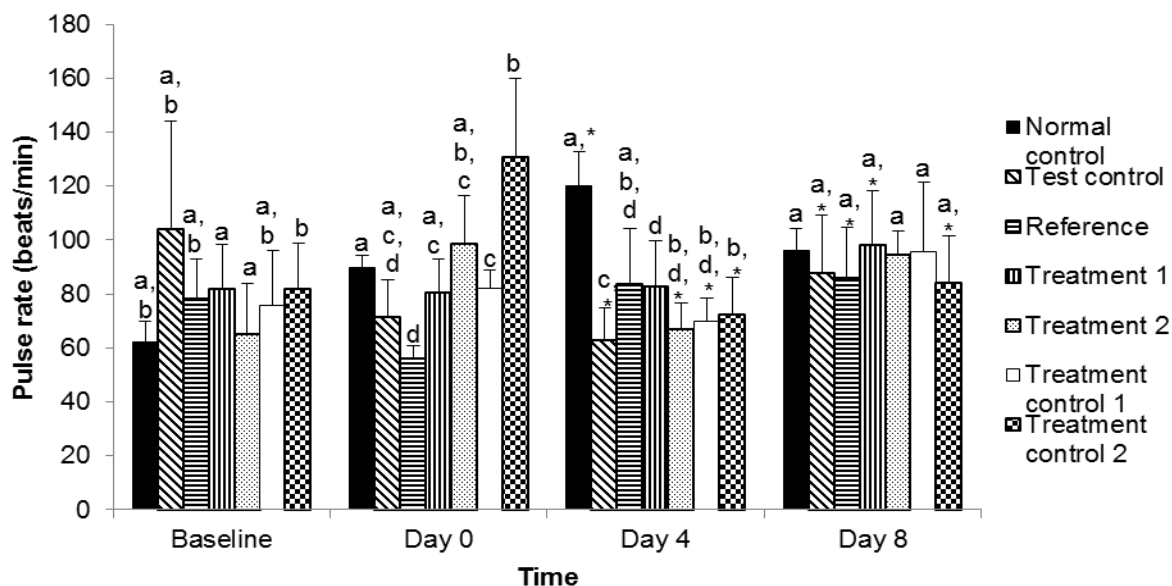
**Figure 8:** Time Course of the Effect of Aqueous Extract of the Leaves of *Chromolaena odorata* on the Mean Arterial Pressure of Normal and Salt-Loaded Rats.

Values are means  $\pm$  S.D., n=5 per group.

<sup>a,b,c</sup> Entries in the same blocks with different superscripts are significantly different at  $P < 0.05$ .

\* $P < 0.05$  compared to corresponding values on day 0.





**Figure 9:** Time Course of the Effect of Aqueous Extract of the Leaves of *Chromolaena odorata* on the Pulse Rate of Normal and Salt-Loaded Rats.

Values are means  $\pm$  S.D., n=5 per group.

<sup>a,b,c</sup>Entries in the same blocks with different superscripts are significantly different at  $P < 0.05$ .

\* $P < 0.05$  compared to corresponding values on day 0.

The treatment produced a mixed effect on the pulse rates of the treated animals. The effect seemed both dose and time dependent. Fast heart rate is associated with an increased risk of death from cardiovascular and non-cardiovascular causes (Palatini, 1999). So, a lowered heart rate signifies ability to reduce cardiovascular risk.

The GC analysis of the extract showed the presence of pharmacologically active compounds like kaempferol, quercetin, and sitosterol. These may probably have been responsible for the hypotensive effect of the extract. According to Ogundaini *et al.* (1983, cited in Ogundaini *et al.*, 2005),  $\beta$ -sitosterol has hypotensive properties with little effect on the heart rate. Quercetin had earlier been reported to reduce elevated blood pressure, cardiac and renal hypertrophy and functional vascular changes in spontaneously hypertensive rats (Duarte *et al.*, 2001). According to Ahmad *et al.* (1993), kaempferol-3-O-rutinoside causes remarkable decrease in systolic, diastolic, mean arterial blood pressure and heart rate.

## CONCLUSION

The implication of this result is that the extract may probably manage hypertension by altering the systolic, diastolic, pulse and mean arterial pressures. The result also supports the use of the leaves in traditional healthcare, for the management hypertension.

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