

Effect of Aqueous Extract of the Leaves of *Tridax procumbens* Linn on Blood Pressure Components and Pulse Rates of Sub Chronic Salt-Loaded Rats.

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ABSTRACT

The effect of aqueous leaf extract of *Tridax procumbens* on the blood pressure components and pulse rates of sub chronic salt-loaded rats was investigated. The control group received a diet consisting 100% of commercial feed, while the four test groups received a diet consisting of 8% salt and 92% commercial feed all through, except for the reference treatment group that had its salt-loading discontinued after six weeks. The extract was orally administered daily at 150 and 200 mg/kg body weight; while the test control, reference and control groups received appropriate volumes of water by the same route.

The extract at 200 mg/kg dose, immediately lowered the systolic and mean arterial pressures of the rats, an effect that continued to the 72nd hr when it rose before finally falling by the 192nd hr. However, the 150 mg/kg dose produced an immediate increase which continued to the 24th hr, before falling. The 200 mg/kg dose immediately lowered the diastolic pressure of the rats, an effect that continued through the 24th hr (when it was significant) to 72nd hr when it rose before finally falling by the 192nd hr. The extract prevented the salt-loading induced upsurge in pulse pressure. The pulse rates of the test groups were lower and more stable compared to the test control. This result implies that the extract may probably manage hypertension by alteration of the systolic and pulse pressures; and confirms the use of the leaves in traditional medicine for the management of hypertension.

(Keywords: blood pressure components, pulse rates, salt-loading, *Tridax procumbens*)

INTRODUCTION

One of the world's leading causes of death is cardiovascular disease [Jonas *et al.*, 2010]. Hypertension is one of its most important risk factors. The relative contributions of the various blood pressure components (systolic blood pressure, SBP; diastolic blood pressure, DBP; pulse pressure, PP and mean arterial pressure, MAP) to cardiovascular risk changes across the lifespan: from DBP to SBP and ultimately to PP [Franklin *et al.*, 2001; Assmann *et al.*, 2005; Freitag *et al.*, 2006; Kengne *et al.*, 2009].

Fast heart rate, a potent precursor of hypertension, atherosclerosis, and their sequel [Gillman *et al.*, 1993; Palatini and Julius, 1997], is associated with an increased risk of death from cardiovascular and noncardiovascular causes. This relationship has been found in general populations, in elderly individuals, and in hypertensive cohorts [Palatini, 1999]. Therefore, any pharmacologic intervention that improves or normalizes abnormal blood pressure may be useful for reducing the risk of cardiovascular diseases. Several drugs are at present, available for the management of hypertension [ALLHAT, 2002; Gu *et al.*, 2010]. Antihypertensive drugs differ in the degree of protection they provide against the risk of cardiovascular mortality. Presently, there is renewed interest in the use of herbal products.

Tridax procumbens Linn (family Compositae or alt Asteraceae) is a native of Central America and tropical South America, though now widespread in the tropical and subtropical parts of the world [Jahangir, 2001]. Traditionally, it is used for the treatment of bronchial catarrh, dysentery, epilepsy, hypertension, malaria, hemorrhage and stomachache.

It has antioxidant, antiseptic, anti-protozoal, anti-inflammatory, hepatoprotective, immunomodulatory, insecticidal, and parasitocidal properties, and marked depressant action on respiration [Salahdeen *et al.*, 2004; Ravikumar *et al.*, 2005; Saxena and Albert, 2005; Bhagwat *et al.*, 2008; Hemalatha, 2008; Pareek *et al.*, 2008; Prajapati *et al.*, 2008; Martín-Quintal *et al.*, 2009; Wani *et al.*, 2010].

We had earlier investigated the nutritional potential of the leaves [Ikewuchi *et al.*, 2009; Ikewuchi and Ikewuchi, 2009a,b], as well as the protective effect of aqueous extracts of the leaves against cholesterol and salt loading in Wistar albino rats [Ikewuchi and Ikewuchi 2009c; Ikewuchi, *et al.*, 2010]. In the present study, the effect of aqueous extract of the leaves of *Tridax procumbens* on the blood pressure components and pulse rates of sub-chronic salt-loaded rats was investigated.

MATERIAL AND METHODS

Collection of Animals and Preparation of Plant Extract

Albino rats were collected from the animal house of the Department of Physiology, University of Nigeria, Enugu Campus, Enugu, Nigeria. Samples of the fresh *Tridax procumbens* plants were collected from within the Choba and Abuja Campuses of University of Port Harcourt, Port Harcourt, Nigeria. After due identification at the University of Port Harcourt Herbarium, Port Harcourt, Nigeria, they were rid of dirt and the leaves were removed, oven dried at 55°C and ground into powder. The resultant powder was soaked in boiled distilled water for 12 hr, after which the resultant mixture was filtered and the filtrate, hereinafter referred to as the aqueous extract was stored 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 to the test animals.

Experimental Design

Studies were conducted in compliance with applicable laws and regulations. The rats were randomly sorted into five groups of five animals each, so that the average weight difference was

±1.8 g. The animals were housed in plastic cages. After a one-week acclimatization period on guinea growers mash (Port Harcourt Flour Mills, Port Harcourt, Nigeria), the treatment commenced and lasted for seven weeks.

The control group received a diet consisting 100% of the commercial feed, while the four test groups received a diet consisting 8% salt and 92% commercial feed. The 8% dietary salt-loading was adapted from Obiefuna *et al.* [1991]. At the end of the sixth week, the rats were weighed and their blood pressure and pulse rate determined, before commencing the administration of the extract, while the reference treatment group had its salt-loading discontinued. The first test group (TP 1) received daily by intra-gastric gavage 150 mg/kg body weight of the *Tridax procumbens* extract; the second group (TP 2) received 200 mg/kg body weight of the *Tridax procumbens* extract; while the other three groups, test control, reference treatment (reference) and control groups received appropriate volumes of water by the same route. The dosage of administration of the extract was adapted from Bhagwat *et al.* [2008]. The animals were allowed food and water *ad libitum*. The blood pressures and pulse rates were measured at 3, 24, 72 and 192 hrs.

Determination of Blood Pressure and Pulse Rate of the Rats

The systolic (SBP) and diastolic (DBP) 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 (PP) and mean arterial pressure (MAP) were calculated mathematically from Systolic (SBP) and diastolic (DBP) blood pressures according Freitag *et al.* [2006] and [Nworgu *et al.*, 2008], using the following formulae:

i. Pulse pressure

= Systolic pressure - Diastolic pressure

ii. Mean arterial pressure (MAP)

$$= DBP + \frac{SBP - DBP}{3}$$

Statistical Analysis of Data

All values are quoted as the mean ± SEM. The values of the various parameters for all the groups were analyzed for statistical significant

differences using the student's t-test, with the help of SPSS Statistics 17.0 package. $P < 0.05$ was assumed to be significant.

RESULTS

Figure 1 shows the time course of the effect of aqueous extract of *Tridax procumbens* on the systolic pressure of sub chronic salt-loaded rats. There were no significant differences in the systolic pressure of all the groups at 0, 3 and 72 hrs. The systolic pressure of TP 2 at 24 hr was significantly ($P < 0.05$) lower than test control and

reference, but not different from control and TP 1. At 192 hr, the systolic pressure of TP 2 was significantly ($P < 0.05$) lower than test control, but not significantly lower than control, reference and TP 1.

The time course of the effect of aqueous extract of *Tridax procumbens* on the diastolic pressure of sub chronic salt-loaded rats is given in Figure 2. At 0 hr, the diastolic pressures of the test groups were significantly ($P < 0.05$) higher than reference, but not significantly higher than control and test control.

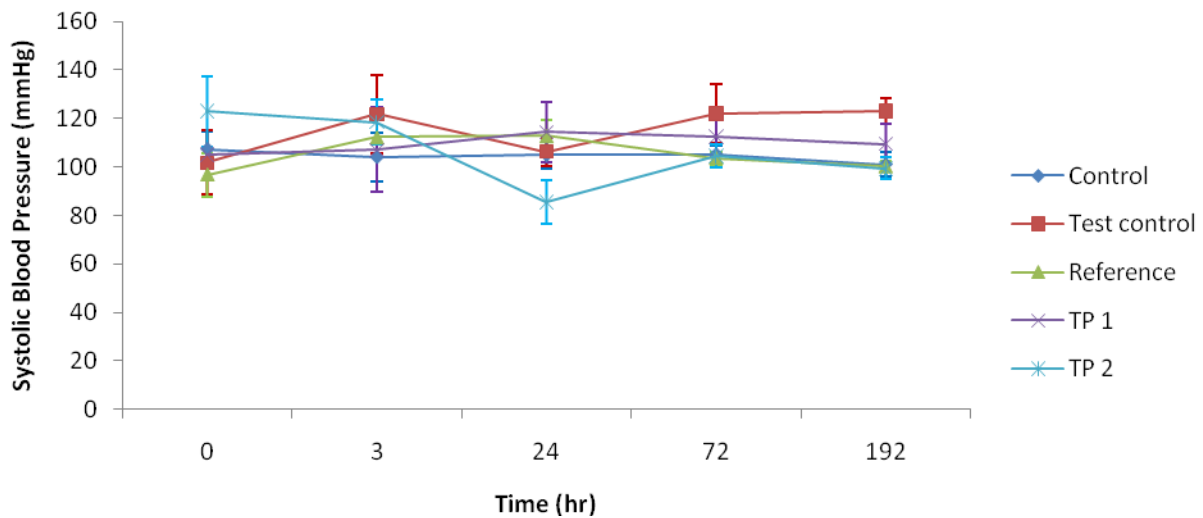


Figure 1: The Time Course of the Effect of Aqueous Extract of the Leaves of *Tridax procumbens* on the Systolic Blood Pressure of Sub Chronic Salt-Loaded Rats.

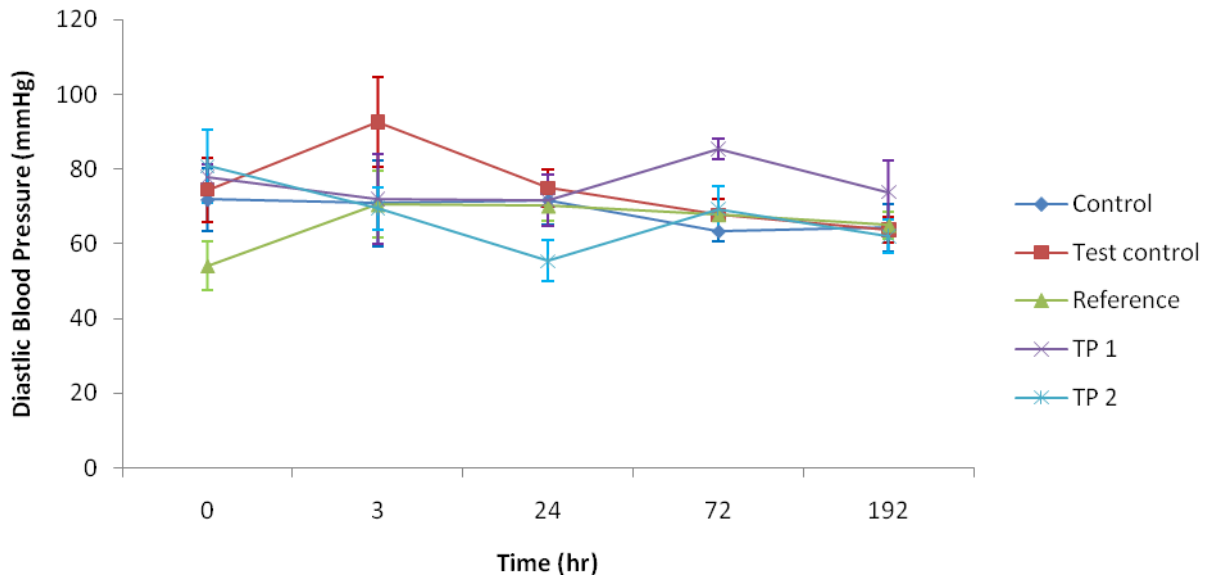


Figure 2: The Time Course of the Effect of Aqueous Extract of the Leaves of *Tridax procumbens* on the Diastolic Blood Pressure of Sub Chronic Salt-Loaded Rats.

There were no significant differences in the diastolic pressure of all the groups at 3, 72 and 192 hrs. The diastolic pressure of TP 2 at 24 hr, was significantly ($P<0.05$) lower than control and test control, but not significantly lower than reference and TP 1. Compared to corresponding 0hr values, only the diastolic pressures of reference (at 24 hr) and TP 2 (at 24 hr) were significantly higher and lower, respectively.

Figure 3 shows the time course of the effect of aqueous extract of *Tridax procumbens* on the pulse pressure of sub chronic salt-loaded rats. The pulse pressure of test control at 0 hr was significantly ($P<0.05$) lower than reference, but not significantly lower than control and the test groups. At 3 and 24 hrs, the pulse pressure of test control was not significantly lower than the other groups. At 24 hr, the pulse pressures of the test groups were lower than the other groups, with that of TP 1 being significantly ($P<0.05$) lower than test control and reference. At 192 hr, the pulse pressure of the test groups were significantly ($P<0.05$) lower than test control, and comparable to control and reference. Compared to corresponding values at 0 hr, test control (at 192 hr) and TP 1 (at 192 hr) were significantly ($P<0.05$) higher.

The time course of the effect of aqueous extract of *Tridax procumbens* on the mean arterial pressure of salt-loaded rats is given in Figure 4. At 0 hr, the mean arterial pressures of the test

groups were not significantly higher than the other groups. At 3 hr, the mean arterial pressure of test control was not significantly higher than the other groups. At 24 hr, the mean arterial pressure of TP 2 was significantly ($P<0.05$) lower than control, test control and reference, but not different from TP 1. There were no significant differences in the mean arterial pressure of all the groups at 72 and 192 hrs; with TP 2 being lower than test control. Figure 5 shows the time course of the effect of aqueous extract of *Tridax procumbens* on the pulse rate of sub chronic salt-loaded rats.

The pulse rate of test control was higher (though not significantly) than all the other groups at 0 hr. At 3 hr, the pulse rates of the test groups were significantly ($P<0.05$) lower than reference, but not different from control and test control. At 24 hr, the pulse rate of test control was significantly ($P<0.05$) higher than control, but not significantly higher than reference and the test groups. At 72 hr, the pulse rate of TP 1 was significantly ($P<0.05$) lower than test control, but not significantly lower than control, reference and TP 2 (the highest). At 192 hr, the pulse rates of the test groups were lower than the other groups, though TP 1 was significantly ($P<0.05$) lower than reference and TP 2. Compared to corresponding values at 0 hr, reference (at 192 hr) was significantly ($P<0.05$) higher, while TP 1 (at 3 hr) and TP 2 (at 72 hr) were significantly ($P<0.05$) lower.

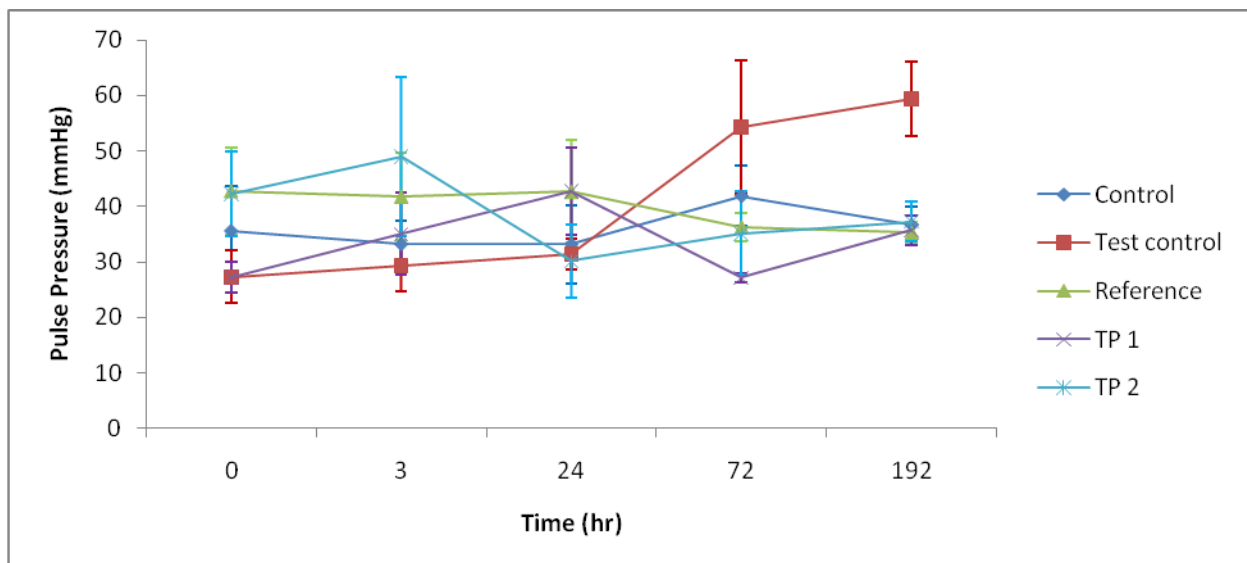


Figure 3: The Time Course of the Effect of Aqueous Extract of the Leaves of *Tridax procumbens* on the Pulse Pressure of Sub Chronic Salt-Loaded Rats.

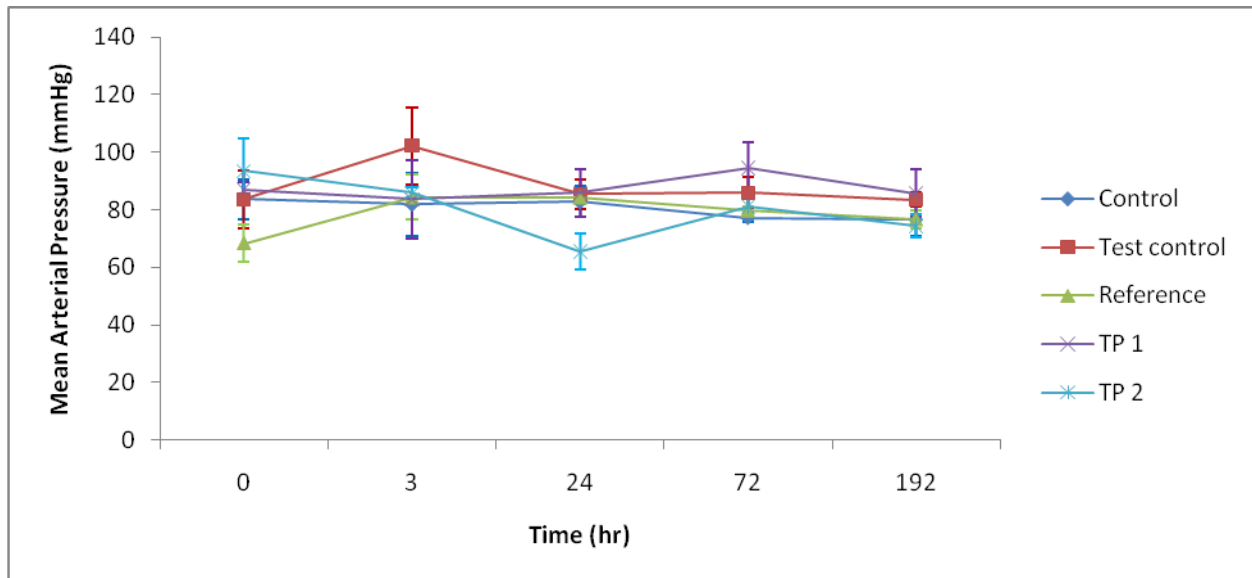


Figure 4: The Time Course of the Effect of Aqueous Extract of the Leaves of *Tridax procumbens* on the Mean Arterial Pressure of Sub Chronic Salt-Loaded Rats.

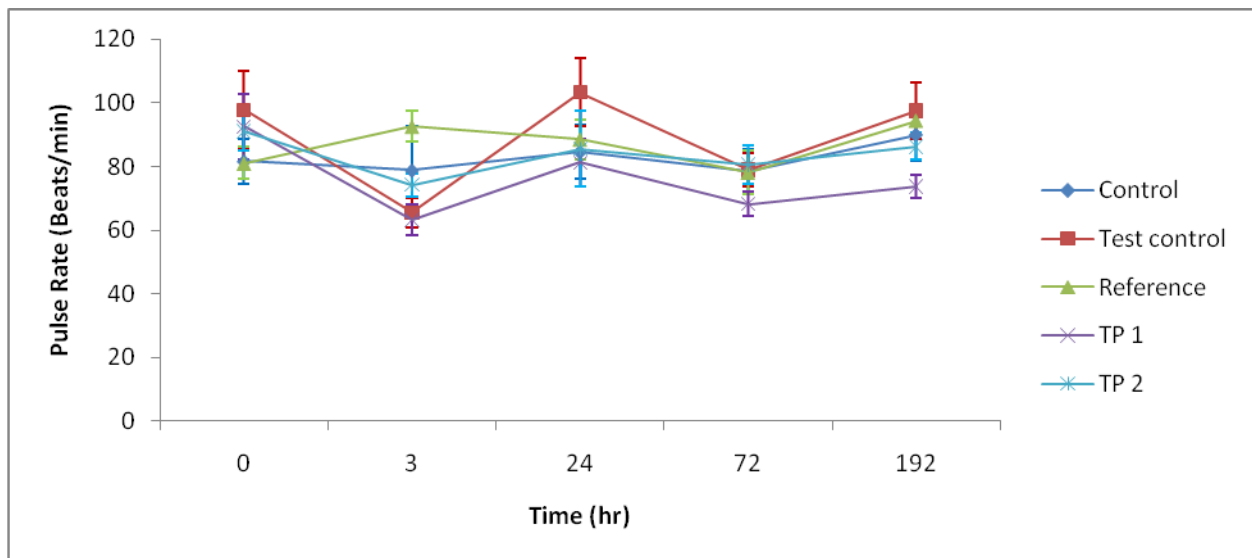


Figure 5: The Time Course of the Effect of Aqueous Extract of the Leaves of *Tridax procumbens* on the Pulse Rate of Sub Chronic Salt-Loaded Rats.

The effect of aqueous extract of *Tridax procumbens* on the percentage decreases in blood pressure and pulse rates of sub chronic salt-loaded rats is given in Table 1. At 3 and 24 hrs, the percentage reductions in systolic pressure of TP 2 were significantly ($P < 0.05$) higher than test control and reference, but not significantly higher than control and TP 1. At 72

and 192 hrs, the percentage reduction in systolic pressure of TP 2 was not significantly higher than control, test control, reference and TP 1. The percentage decrease in diastolic pressures of TP 2 at 3 hr, was significantly ($p < .05$) higher than reference, but not significantly higher than control, test control and TP 1.

Table 1: Effect of Aqueous Extract of *Tridax procumbens* on the Percentage Decreases* in Blood Pressure and Pulse Rates of Sub Chronic Salt-Loaded Rats.

Parameter	Control	Test control	Magnitude (%) Reference	TP 1	TP 2
Systolic Blood Pressure					
3hr	6.816±7.548 ^{a,b}	-30.392±23.844 ^{a,b}	-22.568±19.277 ^a	-1.948±16.846 ^{a,b}	-2.110±13.830 ^{a,b}
24 hr	1.466±4.807 ^{a,b}	-11.938±16.209 ^{a,b}	-22.346±16.785 ^a	-9.011±12.098 ^{a,b}	22.405±18.482 ^b
72 hr	0.802±5.266 ^{a,c}	-31.406±24.302 ^{a,b}	-11.152±11.168 ^{a,b,c}	-7.460±8.498 ^{a,c}	10.246±10.600 ^{a,b,c}
192 hr	5.065±4.735 ^{a,b}	-27.762±14.137 ^a	-7.456±10.950 ^{a,b}	-4.766±8.579 ^{a,c}	13.121±14.903 ^{a,b}
Diastolic Blood Pressure					
3hr	1.414±10.801 ^{a,b}	-32.212±21.592 ^{a,b}	-37.328±24.691 ^a	8.670±12.900 ^{a,b}	-8.324±16.425 ^{a,b}
24 hr	-1.556±6.495 ^{a,c}	-6.306±15.594 ^{a,b,e}	-33.398±8.094 ^{e,t}	6.628±11.029 ^{a,b,c}	28.220±8.928 ^{b,d}
72 hr	7.452±9.584 ^{a,b}	2.588±15.367 ^a	-31.846±13.171 ^a	-9.968±11.180 ^a	10.531±9.862 ^{a,b}
192 hr	7.804±9.924 ^{a,b}	10.322±11.382 ^{a,b}	-27.018±15.995 ^a	3.140±13.359 ^{a,b}	14.465±19.528 ^{a,b}
Pulse Pressure					
3hr	-7.066±21.040 ^a	-27.892±32.395 ^a	-14.074±28.611 ^a	-38.212±34.244 ^a	-38.475±54.572 ^a
24 hr	-5.378±27.647 ^{a,b}	-31.368±26.030 ^a	-11.390±31.202 ^{a,b}	-84.018±40.321 ^{a,b}	5.249±37.803 ^{a,b}
72 hr	-35.136±25.249 ^{a,c}	-141.728±78.232 ^{a,b}	6.986±13.142 ^{b,d}	-2.620±8.345 ^{a,b,c}	9.396±18.073 ^{a,b,c}
192 hr	-27.31±68.39 ^a	-143.47±112.14 ^b	7.45±32.51 ^a	-27.64±39.21 ^{a,b}	-23.80±120.58 ^{a,b}
Mean Arterial Pressure					
3hr	2.492±9.067 ^{a,b}	-31.430±22.449 ^{a,b}	-29.748±19.903 ^a	7.668±16.248 ^{a,b}	-7.960±14.736 ^b
24hr	0.322±3.426 ^{a,b}	-8.554±15.672 ^{a,b}	-27.444±11.156 ^a	0.610±10.406 ^{a,b}	24.890±12.927 ^b
72hr	5.780±6.907 ^a	-10.902±17.758 ^{a,b}	-21.120±11.233 ^a	-8.876±10.047 ^a	9.100±9.483 ^{a,b}
192hr	7.790±5.123 ^{a,b}	-5.482±11.850 ^{a,b}	-16.602±12.301 ^a	0.086±11.433 ^{a,b}	12.602±17.631 ^{a,b}
Pulse Rate					
3hr	3.002±14.124 ^{a,b,c}	27.164±13.512 ^{a,d}	-15.592±8.524 ^b	29.960±5.222 ^{c,d,e}	16.226±9.510 ^{a,c}
24 hr	-3.732±4.724 ^a	-12.436±15.311 ^a	-10.768±9.976 ^a	9.847±8.652 ^a	2.705±16.708 ^a
72 hr	1.868±8.312 ^a	12.558±11.429 ^{a,c}	1.336±12.456 ^{a,b,c}	23.381±7.661 ^c	11.293±3.572 ^{a,c}
192 hr	-6.573±7.899 ^{a,b}	-14.215±31.256 ^{a,b}	-16.945±3.271 ^a	17.055±8.578 ^b	2.548±11.372 ^{a,b}

Values are mean ± SEM, n=5, per group. Values in the same row with the different superscripts are significantly different at P<0.05. *Percentage decrease = percentage decrease from corresponding 0 hr value.

The percentage decrease in diastolic pressure of TP 2 at 24 hr, was significantly (P<0.05) higher than control and reference, but not test control and TP 1. The percentage decrease in diastolic pressure of TP 2 at 72 and 192 hrs were not significantly higher than control, test control, reference, and TP 1. At 72 hr, the percentage decrease in pulse pressure of TP 2 was the highest and that of test control was the least, although only that of reference was significantly (P<0.05) higher than control. At 192 hr, the percentage decrease in pulse pressure of the test groups were higher (though not significantly) than test control, lower than reference and comparable to control. The percentage reduction in mean arterial pressure of the test groups at 3 hr, were higher than test control and reference and comparable to control; with TP 2 being

significantly (P<0.05) higher than reference. The percentage decrease in mean arterial pressure at 24 hr, for the test groups were higher than the other groups, with that of TP 2 being significantly (P<0.05) higher than reference. The percentage decreases in mean arterial pressures of TP 2 at 72 and 192 hrs, were not significantly higher than all the other groups. At 3 hr, the percentage decrease in pulse rates of the test groups were significantly (P<0.05) higher than reference, but not different from control and test control. The percentage decreases in pulse rates of the test groups at 24 hr, were not significantly higher than the other groups, although test control was the least. The percentage decrease in pulse rate of TP 1 at 72 hr, was significantly (P<0.05) higher than control, but not significantly higher than test control, reference and TP 2. The percentage

decrease in pulse rate of TP 1 at 192 hr, was significantly ($P < 0.05$) higher than reference, but not significantly higher than control, test control and TP 2.

DISCUSSION AND CONCLUSION

The extract at 200 mg/kg dose, immediately lowered the systolic pressure of the rats, an effect that continued to 72nd hr when it rose before finally falling by the 192nd hr. However, the 150 mg/kg dose produced an immediate increase which continued to the 24th hr, before falling. The 200 mg/kg dose immediately lowered the diastolic pressure of the rats, an effect that continued through the 24th hr (when it was significant) to 72nd hr when it rose before finally falling by the 192nd hr.

Given the fact that the salt loading continued during the treatment, Figure 3 shows that the extract prevented the salt-loading induced upsurge in pulse pressure. 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 [Assmann *et al.*, 2005]. The 200 mg/kg dose produced immediately lowered mean arterial pressure in the rats, an effect that continued to 72nd hr when it rose before finally falling by the 192nd hr. The extract immediately lowered the pulse rates, and had an undulating effect on the pulse rates of the rats. The effects were relatively stable compared to the test control and were on the average, lower than the test control.

Fast heart rate is a potent precursor of hypertension, atherosclerosis, and their sequelae [Palatini and Julius, 1997]. In addition, many leading epidemiological studies have shown that high heart rate (tachycardia) is associated with an increased risk of death from cardiovascular and non-cardiovascular causes [Palatini, 1999]. Studies of young adults who were followed to middle age suggest that an elevated pulse rate may be an important indicator of cardiovascular risk in children and youth [Gillum, 1991]. Therefore, the lowering of pulse pressure by the extract indicates its ability to lower cardiovascular risk.

The implication of these is that the extract may probably manage hypertension by alteration of the

systolic and pulse pressures. This result confirms the use of the leaves in traditional medicine for the management of hypertension.

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SUGGESTED CITATION

Ikwuchi, J.C., E.N. Onyeike, A.A. Uwakwe, and C.C. Ikwuchi. 2011. "Effect of Aqueous Extract of the Leaves of *Tridax procumbens* Linn on Blood Pressure Components and Pulse Rates of Sub Chronic Salt-Loaded Rats". *Pacific Journal of Science and Technology*. 12(1):381-389.

