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COMPARATIVE ANTIHYPERTENSIVE PROPERTIES OF AQUEOUS EXTRACTS OF LEAVES, STEM BARK AND ROOTS OF *HURA CREPITANS* (L) IN ADRENALINE INDUCED HYPERTENSIVE ALBINO RATS.

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Abstract— **The objective of this study was to investigate and compare the antihypertensive properties of the different parts (leaves, stem bark and roots) of *Hura crepitans* with a known standard antihypertensive drug-Propanolol. There were six animal groups of five animals each and their body weights were measured before, after induction and after treatment. All the groups were induced with hypertension with 0.1ml of adrenaline for 7consecutive days except group 1, which acted as the normal control normotensive rats (NR). Group 2 consisted of hypertensive untreated rats (HUR); Group 3 consisted of propanolol treated hypertensive rats (PTHR); Group 4 consisted of aqueous leaf extract treated hypertensive rats (LTHR); Group 5 consisted of stem bark treated hypertensive rats (BTHR); and Group 6 consisted of aqueous root treated hypertensive rats (RTHA). All the treated groups were administered with equal doses of 500mg/kg of the different plant extracts through oral gavage, except group 3 that was administered with 0.65mg/kg of propanolol. Water and commercial rat feed were given *ad libitum*. The treatment lasted for 14days. Effects of the different extracts on different biochemical parameters such an body weight (BW), pulse rate (PR), serum creatine phosphokinase activity (CPA), Lactate Dehydrogenase Activity (LDHA), some liver enzymes (ALT & AST) and lipid profile (total Cholesterol (TC), Triglycerides (TG) Low Density Lipoprotein-Cholesterol (LDLC),High Density Lipoprotein (HDL) & Very Low Density Lipoprotein-Cholesterol (VLDLC). They were evaluated at various stages: pre-induction, post-induction and post-treatment. All the animals gained weight significantly (P<0.05). A significant decrease in pulse rate after treatment was recorded in all the treated animal groups when compared with UHA. All the treated animals showed significant decrease (P<0.05) in both ALT and AST activities. There was also significant decrease in LDHA in all the treated animal groups. In the LTHR, CPA increased significantly, while the other groups recorded non-significantly decrease in CPA. A significant decrease was recorded in TC in PTHR, LTHR and RTHR. TG and HDL decreased significantly in the treated animal groups. LDLC increased significantly in all the animal groups. There was also a significant decrease in VLDLC. These results suggest that the aqueous extracts of the different parts *Hura crepitans* possess hypertensive properties.**

Index terms- ***Hura crepitans*, anti-hypertension, pulse rate, body weight, lipid profile, creatine phosphokinase, lactate dehydrogenase, Alanine Amino Transaminase, Aspartate Amino Transaminases**

I. INTRODUCTION

Hypertension or high blood pressure is a chronic medical condition resulting to elevated blood pressure in the arteries assessed by systolic and diastolic measurements (Lifton *et al*, 2001). The European society of Cardiology has defined hypertension as systolic values above 140mmHg and diastolic values above 90mmHg. However, these values are affected by age, sex and size (Guiseppe and Robert, 2013).

Hypertension is classified as primary/essential and secondary. Primary hypertension results from no obvious underlying medical cause and accounts for 90-95% of all cases of hypertension but results from a complex interaction of genes and environmental factors (Carretero, *et al*, 2000). Secondary hypertension results from identifiable causes such as: renal disease, endocrine condition, obesity, pregnancy, etc. (Beevars, 1998; Grossman ,*et al*,2012).

Hypertension is referred to as a silent killer because in most cases, it does not manifest any symptoms or signs until complications develop in vital organs. However, the following symptoms may be associated with hypertension; dizziness, flushed facies, headache, fatigue, epistaxis, nervousness, cardiovascular and neurologic anomalies.

There are many treatment and management strategies of hypertension which include: lifestyle modifications (salt restriction, moderation of alcohol consumption, dietary changes, weight reduction, regular physical exercise, smoking cessation) and pharmacological therapy (Beta blockers, Diuretics, calcium antagonists, etc). (Elmer, *et al*, 2006;Graudal *et al*, 2014; Mente, *et al*, 2009, Fagard, 2011; WHO,2003; Mancia,*et al*, 2009).

The use of medicinal plants in developing countries for the treatment of various ailments has reached an estimated 3.5 million people (Balick and Cox, 1996). The non-availability and high cost of orthodox drugs have enhanced the reliance on medicinal plants in primary health care. The high incidence and morbidity of hypertension have led to various drugs and regimes being advocated for its control. Many new drugs have been introduced with better efficacy but with side effects. Recently, attention has been drawn towards herbal and mineral preparations, which are traditionally used as potential therapeutic agents in the prevention and management of cardiovascular diseases (Omale, *et al*, 2011).

Hura crepitans (L) is classified taxonomically as follows:

Kingdom: Plantae
Phylum: Angiosperms
Sub-Phylum: Eudicots
Class: Rosids
Order: Malpighiales
Family: Euphorbiaceae
Genus: Hura
Species: crepitans

Hura crepitans is also known as the sand box tree, possum wood or jabillo. Its igbo native name is "Ububu". In the wild, the tree grows above 40m and the stem and main branches are densely spiny. In ancient times, Hura crepitans' bark extract was used to treat leprosy. It is also known to be used for centuries against eczema. Moreover, the yellowish milky juice secreted by the plant was an important ingredient used by Amerindians to poison their darts. The juice is rich in lectins, which display powerful haemagglutinating activity hence inhibiting protein synthesis (Barbieri, *et al*, 1983).

Modern clinical trials have discovered a wide range of therapeutic actions of remedies derived from the bark, leaves and seeds of this plant. While still green, the seeds have a strong purgative effect and so does the oil extracted from them when dried (Poswal and Akpa, 1991).

Certain substances included in the complex chemical structure of Hura crepitans qualifies this plant, thanks to its combined astringent and emollient actions, for dermatological use against various skin diseases (David, *et al*, 2014).

It has been shown that Hura crepitans' seed oil has the potential for protection of cowpea seeds from *callosobruchus maculates* fabricius infestation (Adedire and Ajayi, 2003). Recently, the work of David, *et al*, (2014) has equally shown the antimicrobial activities of essential oils from Hura crepitans. This showcases their essential oils as potential raw materials for the development of antimicrobial drugs by pharmaceutical industries against various microbial infections and or diseases.

II. MATERIALS AND METHODS

A. Plant Material Collection

The plant materials used in this study were leaves, barks and roots of Hura crepitans (Ububu, Possum wood, Jabillo, etc.) Fresh samples were harvested from the premises of University of Nigeria, Nsukka and sent to the plant science and Biotechnology Department of the same University for Identification.

B. Animal Model/ Induction/Treatment

About thirty (30) male and female Wister albino rats of between 8-10 weeks of age were randomly assigned to six (6) groups of five (5) animals per group and housed in stainless steel cage. The animals were exposed to the normal 12 hours light and dark cycle under tropical weather conditions. The animals were fed on commercial rat feed and water *ad libitum.*

All the groups were allowed to acclimatize for a period of one week. After the period of acclimatization, all the rats were anaesthetized with diethyl ether and 0.1ml of adrenaline was injected into the rats through intra-peritoneal injection using a 1ml disposable syringe daily for seven consecutive days in order to induce hypertension except control group. All the plant extract treated groups were treated with dose of the extract (500Mg/Kg).

The six groups were labelled as follows:

Group 1: Normal control rats that received normal saline

Group 2: Untreated adrenaline induced hypertensive rats

Group 3: Positive Control group treated adrenaline induced hypertensive rats with Propanolol.

Group 4: 500mg/kg leaf extract of *Hura crepitans* treated adrenaline induced hypertensive rats

Group 5: 500mg/kg bark extract of Hura crepitans treated adrenaline induced hypertensive rats.

Group 6: 500mg/kg root extract of Hura crepitans treated adrenaline induced hypertensive rats.

The extracts were administered by oral gavage while the standard commercial drug (Propanolol) was administered through intra peritoneal (I.P) route once daily for two weeks.

C. Dose preparation of standard Antihypertensive Drug

The daily dose of propanolol for human is 45mg/70kg (Omale, *et al*, 2011). Therefore, a daily dose of 0.65mg/kg body weights of rats was administered through intra- peritoneal injection.

D. PLANT DOSE PREPEARATION

The different plant parts were pulverized into a fine powder using a pulverizer. 100g of the extract was put into 500ml of distilled water and vigorously shaken and allowed to settle for 24 hours. It was then filtered and dried in an oven in order to obtain the dry sample of the extract. 1g of the dry extract was dissolved in 10ml of distilled water in order to obtain 100mg/ml or 0.1g/ml.

E. Determination of Pulse Rate

The pulse rates of the animals were measured using the method described by Rod (2008). The femoral artery in the

groin on the medial aspect of the femur of the hind leg was used. The rats were restrained and once settled, the pulses were taken by placing finger over the femoral artery. The pulse rate was counted for one minute using a stop watch.

F. Body Weight Determination

The body weights of the rats were measured before induction of hypertension, after induction and after treatment using Kerro-BL20001 electronic weighing balance.

G. Determination of Serum Creatine Phosphokinase Activity

The activity of creatine phosphokinase was determined after 14 days of treatment according to the method described by Szasz *et al*, 1976 (Randox C.K. 110 kit was utilized for the quantitative *in vitro* determination of the enzyme in serum). 20ml of the sample was pipetted into a test tube and mixed with 1ml of the reagent (Randox C.K 110 kit). The mixture was left to incubate at 25^{o}c for 3 minutes and the initial absorbance was read. The absorbance was taken again after 1, 2 and 3 minutes simultaneously at 340nm using an Agilent 8453 spectrophotometer. The creatine phosphokinase activity was calculated using the formular U/L = 4127 x DA340nm/min Where DA=Change in absorbance.

H. Determination of Serum Lactate Dehydrogenase Activity

The serum lactate dehydrogenase activity was determined after two weeks of treatment according to the method described by Weishaar, 1975. A portion (2ml) of the sample was pipetted into a test tube and mixed with 1.0 ml of reagent (Randox kit). After mixing, the initial absorbance was read after 30 seconds and again after 1, 2, 3 minutes simultaneously at 340nm using Agilent 8453 spectrophotometer. LDH activity was calculated using the following formula:

U/L= 9683 x DA 340nm/min; Where DA=change in absorbance.

I. Lipid Profile

1) Total Cholesterol

This was determined using Randox Kit procedure as expressed by Akomas *et al., (2014).* In this method, 0.1ml of the serum was pipette into a test tube and 1000ηl of the Randox Cholesterol Reagent was added to it. The mixture was incubated for 10 minutes at 20 to 25^{o}c and absorbance at wave-length 540nm was then read using a spectrophotometer against the reagent blank. The standard absorbance of 0.119 was equivalent to 200.65mg of cholesterol per dL of blood as prescribed by the producer and was used to obtain the test result.

2) Triglycerides

This was determined using Randox Kit procedures as expressed by Ijioma *et al., (2014).* In this method, 0.1ml of the serum was pipette into test tube and 1000ηl of the Randox triglyceride reagent added to it. The mixture was incubated for 10 minutes at 20-25^{o}c. The absorbance of the sample and standard were measured using a spectrophotometer at wavelength 540nm against the reagent blank. The standard absorbance of 0.100 was equivalent to 196mg of triglycerides per dL of blood and was used to evaluate the test results.

3) High Density Lipoprotein cholesterol

This was determined Using Randox Kit procedures as expressed by Ijioma *et al., (2014).* In this method, O. I. of the serum was pipette into a test tube and 1000ηl of the Randox HDL-C reagent was added to it. The mixture was incubated for 10 minutes 1t 20-25^{o}c. The absorbance of both the sample and standard were measured using spectrophotometer at wavelength 540nm against the reagent blank. A standard absorbance stipulated by the Kit producer of 0.307 = 202.65mg of HDL-C per dL of blood was used to evaluate the test results.

4) Low Density Lipoprotein cholesterol and very low Density

Lipoprotein Cholesterol

Both were obtained by calculations using the formulae;

- LDL - C = Cholesterol – $\left[\frac{\text{Triglycerides}}{5} + \text{HDL}\right]$
- VLDL – C = $\frac{\text{Triglycerides}}{5}$

5) Determination of Alanine Amino Transaminase

The kit provides a rapid, simple, sensitive and reliable test suitable for high through put activity assay of ALT with a detection limit of 10mU per well.

kit contents:.

Components	**Cap code**	**100 assays**
ALT Assay Buffer	Wm	25ml
OxiRed^{TM} in(DMSO)	Red	200ul
ALT enzyme mix (lyophilized)	Green	1 vial
ALT substrate lyophilized	Orange	1 vial
Pyruvate standard (100nmol/ul)	Yellow	100ul
ALT positive control (lyophilized)	Blue	1 vial

Reagent preparation

ALT enzyme mixed and reconstituted with 220ul dH_2O.

Aliquot was stored at -20^{0} C.

ALT substrate reconstituted with1:1ml assay buffer.

Aliquot was stored at -20^{0}C.

ALT positive control: Reconstitute with 100ul dH_2O.

Aliquot was stored at -20^{0}C. 5-10ul was added as positive control and adjusted to the final volume to 20ul/well ALT assay buffer.

Standard Curve Preparation

Colorimeter assay:

The pyruvate standard was diluted to 1nmol/ul by adding 10ul of the standard to 990ul of ALT assay buffer, and mixed well. 0-, 2, 4, 6, 8, 10ul were added into a series of standard wells. Volume was adjusted to 20ul/well with ALT assay

buffer to generate 0, 2. 4, 6, 8, 10nmol/well of the pyruvate standard for the colorimeter assay.

Sample preparation

Serum samples were diluted in assay buffer. Test samples of up to 20ul/well were prepared with assay buffer in a 96-well plate.

Reaction Mix: Enough reagents were mixed for the number of the number of assays to be performed. For each well, a total 100ul reaction mix was prepared.

ALT Assay Buffer	86ul
Oxi Red Probe	2ul
ALT Enzyme mix	2ul
ALT Substrate	10ul

100ul of the sample reaction mix was added to each well containing the samples, standard and positive control (optional) and mixed well.

Measurement

OD $_{570nm}$ (A_1) at T_1 (T_1>10_{min}) was read, then again (A_2) at T_2 after incubating the reaction at 37^0C for 60min (or longer if the ALT activity is low). It was protected from light. The OD of the colour generated by oxidation of pyruvate is $\Delta A570nm=A_2-A_1$

Calculation

The pyruvate standard curve was plotted and used; $\Delta A570nm$ to obtain B (nmol) of pyruvate (amount of pyruvate generated T_1 and T_2 in the reaction wells)

ALT activity in the test sample was then be calculated:

ALT Activity= $\frac{B}{(T1-T2)XV}$ = nmol/min/ml = mU/ml

Where B is the pyruvate standard curve (in nmol)

T_1 is the time of the first reading) (A_1) (in min)

T_2 is the time of the second reading (A_2) (in min)

V is the original sample volume added into the reaction well (in ml).

One unit of ALT is defined as the amount of ALT which generates 1.0ul of pyruvate per minutes at 37^0C.

6) Determination of Aspartate Transaminase

Kit was developed based on Bergmeyer (1974) method

Kit Components

Assay Buffer	24ml
Co-factors	120µl
Enzyme mix	120µl
NADH	500µl

Materials

Pipetting devices and accessories

Spectrophotometer and cuvettes for measuring OD_{340nm}

Assay Procedure

All components were equilibrated to room temperature and thawed enzymes were kept in ice. For each assay, one standard and one blank control were included. For each sample and standard, working reagent was prepared by mixing 1000µl assay buffer, 5µl cofactor, 5µl enzyme mix and 20µl NADH. Transfer 990µl working reagent to each sample cuvette and standard cuvette and warm to desired temperature (37ºC). To blank control cuvette, 960µl assay buffer, 5µl cofactor, 5µl enzyme were added and mixed with 20µl H_2O; then warmed to desired temperature (37^0C). Samples were pre-warmed to the desired temperature. 100 µl sample was added to the sample cuvette. 100µl H_2O was transferred to the standard cuvette and blank control cuvette respectively. Mix immediately and read OD_{340nm} and 10 minutes.

Calculation

Determination of AST activity using the following equation:

$$AST= \frac{388x\Delta OD_S-\Delta\Delta O_{NADH}}{OD_{STD}-OD_{BLK}} (U/L)$$

OD_{STD} and OD_{BLK} are the OD_{340nm} values of NADH standard and blank at 5 min. respectively. The factor 388 is derived from:

Factor =$\frac{10\ Mm\ NADH}{206µl\ vol.\ WR}$ x $\frac{4µl\ vol.NADH}{220µl\ vol.Total}$ x $\frac{200µl\ vol.WR}{11(sample)\ density}$ 5min.

=388µM/min.

If the calculated AST activity is higher than 100µl dilute sample in assay buffer and repeat assay. Multiply results by the dilution factor.

7) Statistical Analysis

Data were expressed as mean ± SEM . One way analysis of Variance (ANOVA) followed by Benferonis multiple comparison test was applied. A probability value of 0.05 ($P<0.05$) was considered significant.

III. Results and Discussion

Medicinal plants have gained much ground due to the following reasons: wide availability, cheap and affordability, little side effects, wide range of therapeutic applications as well as inadequate trained medical personnel's and high cost of orthodox medicine (Bako, *et al*, 2010).

Table 1 shows the comparative body weights of non-hypertensive, untreated hypertensive rats and treated hypertensive rats. All the animals gained significant ($P<0.05$) weight throughout the experimental period. This increase in weight may be due to the effect of the extract and its nutritive value. This is because the proximate, elemental and fatty acid profile of *H.crepitans* have been shown to be rich in proteins, fatty acids and carbohydrates, which are necessary for energy, growth and repair (Oyeleke, *et al*, 2012; Alabi, *et al*, 2013). Also, the relative non-toxicity of the extract may have also contributed to weight gain as organ or tissue damage may result to weight loss. However, the balance commercial rat ration and water given *ad libitum* may be the principal contributor to weight gain.

Table 1: BODY WEIGHTS OF THE UNTREATED HYPERTENSIVE RATS IN COMPARISON WITH THE TREATED RATS

Groups	Treatment	Pre Induction Body Weight	Post Induction Body Weight	Post Treatment Body Weight
1	Normal	92.68 ± 8.69	114.94 ± 7.07	137.06 ± 9.79
2	Hypertensive Untreated	99.56 ± 3.72*	106.08 ± 2.80*	102.10 ± 5.39*
2	Hypertensive Untreated	99.56 ± 3.72	106.08 ± 2.80	102.10 ± 5.40
3	Propanolol Treated	94.98 ± 5.52*	109.42 ± 5.79*	138.00 ± 3.63*
2	Hypertensive Untreated	99.56 ± 3.72	106.08 ± 2.80	102.10 ± 5.39
4	500Mg/Kg *H.crepitans* leaf extract	85.23 ± 4.00*	94.44 ± 5.33*	118.2 ± 6.24*
2	Hypertensive Untreated	99.56 ± 3.72	106.08 ± 2.80	102.10 ± 5.40
5	500Mg/Kg *H.crepitans* bark extract	75.08 ± 6.80*	94.44 ± 5.97*	121.04 ± 9.96*
2	Hypertensive Untreated	99.56 ± 3.72*	106.08 ± 2.80	102.10 ± 5.40
6	500Mg/Kg *H.crepitans* root extract	78.78 ± 5.09	99.78 ± 5.52*	125.8 ± 4.17*

* The mean difference is significant at P< 0.05 level.

The values in the column were are expressed as mean ± S.E.M., n=5

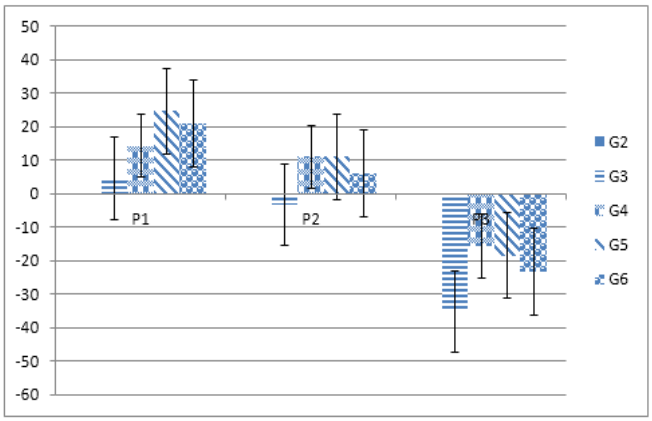

Fig 1: % Activity Series of Body Weight

P1= Pre-Induction ;P2= Post-Induction ;P3= Post-Treatment

Table 2 shows the pulse rates of all the animal groups at pre-induction and post treatment stages. The results show significant increase in pulse rate in the post induction stage and significant decrease in the post treatment stage. Increase in pulse rate may be due to heart disease or increased metabolic rate, (Pagana and Pagana, 2006). Adrenaline increases metabolic and pulse rates. This result suggests the hypotensive properties of the different parts of *H.crepitans* in comparison with the standard anti-hypertensive drug (Propanolol).

Table 2:PULSE RATES OF TREATED HYPERTENSIVE RATS

Groups	Pre Induction	Post Induction	Post Treatment
1	233 ± 4.17	226.4 ± 3.66	221.60 ± 2.14
2	227.20 ± 5.95	286.00 ± 6.78*	276.80 ± 6.22*
2	227.20 ± 5.95	286.00 ± 6.78	276.80 ± 6.22
3	224.00 ± 2.10	294.20 ± 4.08*	230.80 ± 2.65*
2	227.20 ± 5.95	286.00 ± 6.78	276.80 ± 6.22
4	234.6 ± 2.48	294.00 ± 2.61*	225.60 ± 11.30*
2	227.20 ± 5.95	286.00 ± 6.78	276.80 ± 6.22
5	234.4 ± 4.17	284.80 ± 3.14	237.80 ± 3.63*
2	227.20 ± 5.95	286.00 ± 6.78	276.80 ± 6.22
6	224.4 ± 2.48	286.80 ± 3.14	232.40 ± 4.12*

* The mean difference is significant at P< 0.05 level.

The values in the column were are expressed as mean ± S.E.M., n=5

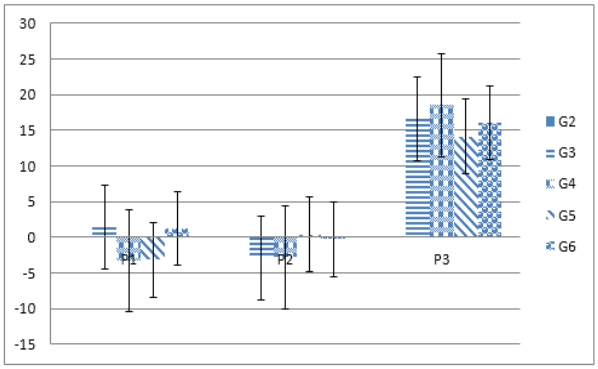

Fig 2:% Activity Effects of Pulse Rates

P1= Pre-Induction; P2= Post-Induction; P3= Post-Treatment

Table 3 shows the activities of Alanine Amino Transaminase (ALT) and Aspartate Amino Transaminase (AST) at the pre-induction, post-induction and post-treatment stages. At the post-induction stages, the activities of both ALT and AST increased significantly (P<0.05) when compared with the normal control animals. However, a significant decrease (P<0.05) was recorded in all the treated animals (propanolol and plant extract treated) when compared with the untreated hypertensive animals. Increased level of ALT and AST suggests heart or liver damage. This decrease recorded suggests possible amelioration of cardiovascular risk by the different aqueous extracts.

Table 3: ACTIVITIES OF SOME LIVER ENZYMES (ALT&AST) ON EXPERIMENTAL ANIMALS

Group	Pre Induction		Post Induction		Post Treatment	
	ALT1	AST1	ALT2	AST2	ALT3	AST3
1	30.80±2.33	81.80±1.07	29.60±2.04	82.20±2.22	26.40±1.72	80.80±11.12
2	33.00±1.41	80.20±0.80	41.60±8.26*	100.60±14.59*	50.60±6.94*	129±11.41*
2	33.00±1.41	80.20±0.80	41.60±8.26	100.60±14.59	50.60±6.94	129.20±11.12
3	30.40±2.32	80.40±1.80	39.80±7.17	95.00±2.61*	30.00±2.35*	84.80±2.24*
2	33.00±1.41	80.20±0.80	41.60±8.26	100.60±14.59	50.60±6.94	129.20±11.12
4	29.20±1.59	83.20±1.24	54.40±12.19*	110.2±12.44*	27.60±1.21*	87.40±7.26*
2	33.00±1.41	80.20±0.80	41.60±8.26	100.60±14.59	50.60±6.94	129.20±11.12
5	32±2.47	81.20±3.51	74.20±10.31*	89.4±4.27*	24.4±1.03*	81.40±5.05*
2	33.00±1.41	80.20±0.80	41.60±8.26	100.60±14.59	50.60±6.94	129.20±11.12
6	28.60±1.94*	81.60±2.94	53.40±4.02*	87.8±6.34*	27.40±2.25*	118.80±5.24*

* The mean difference is significant at P< 0.05 level.
The values in the column were are expressed as mean ± S.E.M., n=5
AST – ASPARTATE AMINO TRANSAMINASE

ALT- ALANINE AMINO TRANSAMINASE

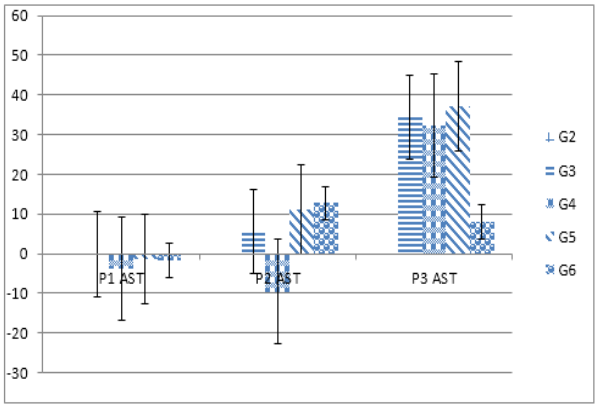

Fig 3: % Activity for AST
P1= Pre-Induction; P2= Post-Induction; P3= Post-Treatment

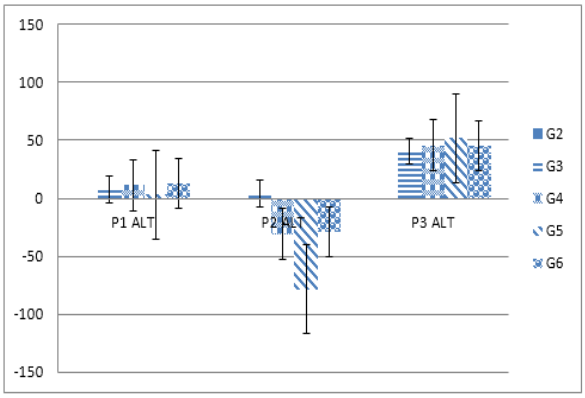

Fig 4: % Activity for ALT

P1= Pre-Induction; P2= Post-Induction; P3= Post-Treatment

The activities of lactate dehydrogenase (LDHA) and creatine phosphokinase (CPA) are shown in Table 4. The results show a significant increase in the activities of both LDH and CP at the post-induction stage when compared with the normal control groups. At the post treatment stage, LDH activity decreased in all the groups, when compared with the untreated hypertensive rats. Activities of CP decreased in all the treated groups except in the group treated with aqueous leaf extract. However, this decrease was non-significant (P<0.05). In the group treated with aqueous leaf extract, CP activity increased significantly (P<0.05). LDH is found in the cells of almost all body tissues. Cellular injury in tissues containing LDH can result in its release has been associated with cardiac tissue damage and higher concentration of LDH could be a symptom of heart damage. Therefore, the decrease in LDH activity of the different aqueous extracts of *Hura crepitans* suggests that the extracts may protect against cardiac tissue damage.

Table 4: ACTIVITIES OF CREATINE PHOSPHOKINASE (CPA) AND LACTATE DEHYDROGENASE (LDH) IN THE EXPERIMENTAL ANIMALS

Group	Pre Induction		Post Induction		Post Treatment	
	CPA	LDHA	CPA	LDHA	CPA	LDHA
1	16.34±0.49	102.26±0.88	16.14±0.37	106.96±1.46	15.40±1.08	100.12±2.26
2	16.66±0.33	109.44±2.81*	22.08±1.60*	122.16±0.63*	20.10±1.21*	120.20±1.50*
2	16.66±0.33	109.44±2.81	22.08±1.60	122.16±0.63	20.10±1.21	120.20±1.50
3	16.96±0.38	106.28±1.87	19.50±0.59	122.58±0.59	18.24±0.72	103.60±6.99*
2	16.66±0.33	109.44±2.81	22.08±1.60	122.16±0.63	20.10±1.21	120.20±1.50
4	17.08±0.70	107.26±1.58	34.78±13.81*	121.84±1.07	48.80±30.31*	111.60±1.63*
2	16.66±0.33	109.44±2.81	22.08±1.60	122.16±0.63	20.10±1.21	120.20±1.50
5	16.36±0.98	108.20±2.08	20.80±0.83	122.34±0.67	17.60±0.81	108.60±3.25*
2	16.66±0.33	109.44±2.81	22.08±1.60	122.16±0.63	20.10±1.21	120.20±1.50
6	16.54±0.15	106.40±1.86	20.12±0.62	120.98±0.83	18.16±1.32	114.40±0.33*

* The mean difference is significant at P< 0.05 level.
The values in the column were are expressed as mean ± S.E.M., n=5

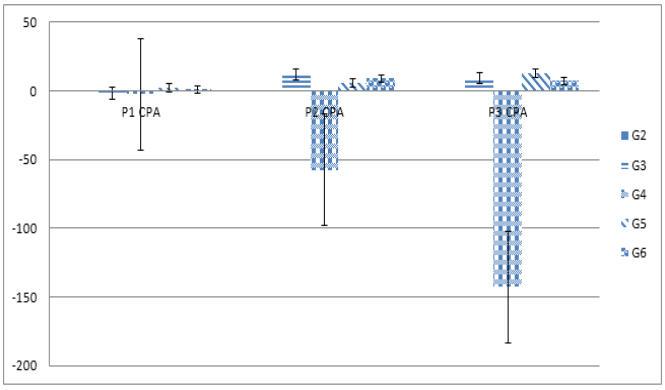

Fig 5: % Activity for CPA

P1= Pre-Induction; P2= Post-Induction; P3= Post-Treatment

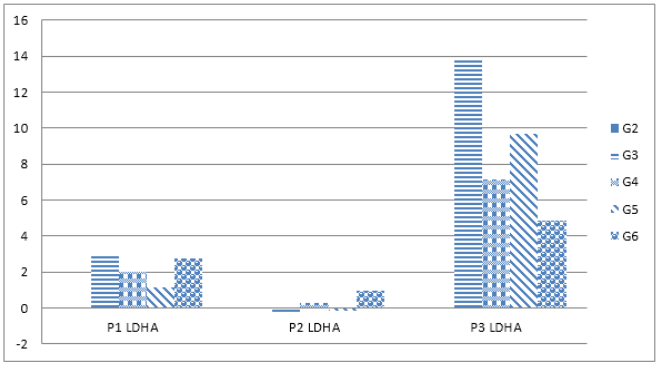

Fig 6: % Activity for LDHA

P1= Pre-Induction; P2= Post-Induction; P3= Post-Treatment

Table 5 shows the lipid profile of the different animal groups at the pre-induction stage Total Cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein-cholesterol (LDLC) and High Density Lipoprotein Cholesterol(HDL) values indicate starting values in order to assess the effects of induction of hypertension and treatment.

Table 5: LEVELS OF TOTAL CHOLESTEROL (Chol), TRIGLYCERIDES (TG), HIGH DENSITY LIPOPROTEIN (HDL), LOW DENSITY LIPOPROTEIN-CHOLESTEROL (LDLC) AND VERY LOW DENSITY LIPOPROTEIN- CHOLESTEROL (VLDLC) AT THE PRE- INDUCTION STAGE.

Group	Pre-Induction					
	Chol	TG	HDL	LDLC		VLDLC
1	154.2±6.87	154.4±2.79	51.4±2.89	71.92		30.88
2	171.2±3.54*	138.4±4.39*	47.0±7.20*	96.52*		27.68
2	171.2±3.54	138.4±4.39	47.0±7.20	96.52		27.68
3	172.4±7.10	161.2±7.68*	44.8±10.16*	95.36		32.24*
2	171.2±3.54	138.4±4.39	47.0±7.20	96.52		27.68
4	168.0±6.00*	143.6±4.41*	50.6±2.95	88.68*		28.72
2	171.2±3.54	138.4±4.39	47.0±7.20	96.5*		27.68
5	172.4±3.33	143.00±5.78*	44.4±3.72	99.40		28.60
2	171.2±3.54	138.4±4.39	47.0±7.20	96.52		27.68
6	161.2±7.97*	147.8±9.85*	44.2±3.06	87.44*		29.56

* The mean difference is significant at P< 0.05 level.
The values in the column were are expressed as mean ± S.E.M., n=5

Table 6 shows lipid profile after induction of hypertension. All the hypertensive induced treated group showed a significant increase in TC, TG, HDL, VLDLC and LDLC except the group treated with aqueous root that showed a non-significant increase in LDLC when compared with the normal control. This increase confirms the relationship between the elevated values of these biomolecules and hypertension (Omale, *et al*, 2011;Schneider and Agranoff,1991;Kennedy,1997; Stryer, 2000). Elevated levels of these lipid biomolecules constitute risk factors in the development of cardiovascular diseases (Austin, 1991).

TABLE 6: LEVELS OF TOTAL CHOLESTEROL (Chol), TRIGLYCERIDES (TG), HIGH DENSITY LIPOPROTEIN (HDL), LOW DENSITY LIPOPROTEIN-CHOLESTEROL (LDLC) AND VERY LOW DENSITY LIPOPROTEIN- CHOLESTEROL (VLDLC) AT THE POST-INDUCTION STAGE.

Groups	Post Induction				
	Chol	TG	LDLC	VLDLC	HDL
1	151.2±10.98	200.4±9.29	57.22	40.08	53.60±2.06
2	188.20±2.62*	217.0±6.16*	77.20*	43.40	67.6±6.21*
1	151.2±10.98	200.4±9.29	57.22	40.08	53.60±2.06
3	183.0±4.34*	237.0±2.92*	69.00*	47.40*	66.6±3.22*
1	151.2±10.98	200.4±9.29	57.22	40.08	53.60±2.06
4	187.2±2.42	237.4±4.59*	66.92*	47.48*	72.8±3.51*
1	151.2±10.98	200.4±9.29	57.22	40.08	53.60±2.06
5	183.2±3.99*	254.8±10.42*	63.04*	50.96*	69.2±6.45*
1	151.2±10.98	200.4±9.29	57.22	40.08	53.60±2.06
6	183.4±3.14*	259.6±11.22*	60.28*	51.92*	71.2±9.81*

* The mean difference is significant at P< 0.05 level.

The values in the column were are expressed as mean ± S.E.M., n=5

The levels of these lipid biomolecules after treatment with the various aqueous plant extracts and propanolol are shown in Table 7.All the treated groups, showed a significant decrease in TG, VLDLC, and HDL when compared with the untreated hypertensive group. A significant decrease in TC was also recorded except in the group treated with aqueous bark extract whose decrease was non-significant when compared with the untreated hypertensive groups. This reduction in these hypertension bio indicators could be due to several factors. According to the work done by Adindu *et al* (2015), *H.crepitans* parts (leaves, stem bark and roots) are very rich in phytochemicals such as flavoniods, carotenoids, alkaloids, lignans, etc. These phytochemicals such as Quercetin Isoflavoids, etc. have been shown to lower blood cholesterol level (Gebhardt, 1993; Kennedy, 1997;Hassel, 1998; Cook and Samman,1996; Yan *et al*,1995). The post-treatment values of these lipid biomolecules do not suggest very high risk conditions rather show hypotensive potentials (American Heart Association, 2009).

Post –Treatment				
Groups	Chol	TG	LDLC	VLDLC
1	77.6±13.51	107.4±8.84	3.72	21.48
2	85.8±8.77*	152.4±4.52*	-6.88*	30.48*
2	85.8±8.77	152.4±4.52	-6.88	30.48
3	69.0±6.58*	107.0±2.02*	-8.00	21.40*
2	85.8±8.77	152.4±4.52	-6.88	30.48
4	79.8±6.73*	101.4±3.35*	10.92*	20.28*
2	85.8±8.77	152.4±4.52	-6.88	30.48
5	83.6±7.46	76.2±10.32*	29.96*	15.24*
2	85.8±8.77	152.4±4.52	-6.88	30.48
6	76.6±0.51*	83.6±2.54*	12.68*	16.72*

* The mean difference is significant at P< 0.05 level.
The values in the column were are expressed as mean ± S.E.M., n=5

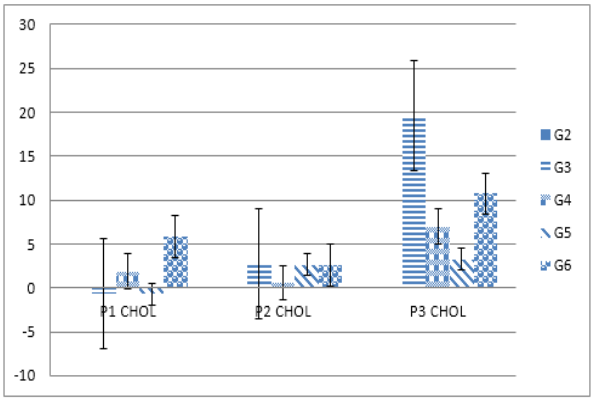

Fig 7: % Activity for Chol.

P1= Pre-Induction; P2= Post-Induction; P3= Post-Treatment

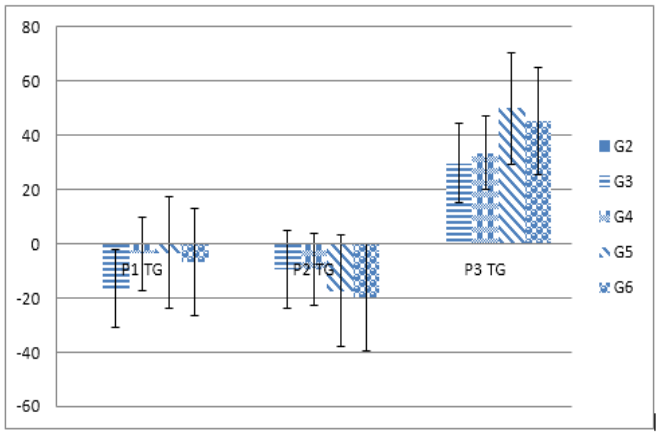

Fig 8: % Activity for TG

P1= Pre-Induction; P2= Post-Induction; P3= Post-Treatment

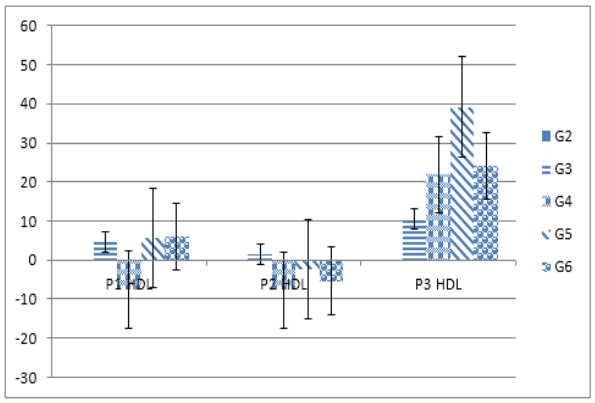

Fig 9: % Activity for HDL

P1= Pre-Induction; P2= Post-Induction; P3= Post-Treatment

IV. CONCLUSION

Plants have proved to have wide range of applications such as: food, source of raw materials, aesthetic value, environmentally friendly and for therapeutic purposes. In most developing countries, the use of plants as an alternative source of medicine has gained much ground due to the following reasons:

- Wide availability
- Cheap and affordable
- Little side effects
- Wide range of therapeutic applications
- Inadequate trained medical personnel and high cost of orthodox medicine

This study suggests that the various aqueous extracts of *Hura crepitans* have got profound hypotensive activity and this study has correlation with previously reported investigations using other plants. The mechanism by which *Hura crepitans* lowers blood is not yet fully established. However, it may be suggested that the hypotensive effect may be due to the stimulation of muscarinic receptors of the parasympathetic nerves by the compounds or the actions as an antagonist of α_2 – adrenergic receptors or a beta – blocker or Ca^{2+} channel blocker (Ameran, *et al*, 2004). The rich phytochemicals present in *Hura crepitans* suggests that the use of *Hura crepitans* as an antihypertensive agent may be useful.

There is the need to study the effect of dose on the efficacy of this herbal plant in the treatment of hypertension. This area is recommended for further study.

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