

EFFECT OF BEET ROOT (*Beta vulgaris*), WHITE AND RED RADISH (*Raphanussativus* and *Raphanuslongipinnatus*) JUICE EXTRACT, ON SALT INDUCED HYPERTENSIVE WISTAR RATS

ABSTRACT

Background: Hypertension is one of the major global health challenges with high prevalence and undesirable complications, and is a major cause of premature death worldwide.

Objective: This study was conducted to evaluate the effect of juice extracted from beetroot (BR), red radish (RR) and white radish (WR) tubers on experimentally induced hypertension in 10-week-old Wistar rats.

Methodology: Total of 35 rats was randomly divided into seven (7) experimental groups A - G with 5 rats each. Rats in group A were not induced (normotensive group). All the rats in groups B-G were induced with hypertensive agent. Group B was not treated (negative control), groups C-G were treated simultaneously with a dose of 800 mg/kg body weight (orally) of BR, RR, WR, BR+RR combination, and standard drug (5mg Amlodipine) respectively. The study lasted for a period of 28 days during which the blood pressure and heart rate (HR) of each of the experimental rats were measured at 0, 7, 14, 21 and 28th day using Stethoscope technique. The interpretation of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were made from pulse tracings using Aneroid Sphygmomanometer. The mean blood pressure (MBP) was calculated from SBP and DBP.

Results: The BR, RR, WR and combination of BR+RR, did decrease the number of heart beats/minute from 192.00/minute to 208.80/minute which is within the normal base line, whereas the untreated rats recorded increase from 192.00/minute to 246.80/minute. The result showed that the hypertensive rats recorded higher ($P < 0.05$) mean values of SBP, the treated groups altered the rise from 110.40 mmHg to 100.40mmHg with the standard drugs, followed by the white radish treatment group, the combination of BR+RR, before RR, lastly the BR group. For DBP, at week 4, same impact reflected again, but week 2 and week 3 had WR to have the best effect, followed by BR +RR, then RR before the standard drug and then Beetroot. The BR + RR juice performed better than the individual RR and BR juice groups, suggesting possible synergistic effect.

Conclusion: The 3 juice extracts had demonstrated varying degrees of protection, provided scientific evidence of their anti- hypertensive activities in an experimentally induced hypertension. Further studies are hereby recommended to isolate the specific biomolecule active agent(s) and investigate the mechanism of action.

Keywords: Blood Pressure, Beet root, White radish, Red radish, Juice extract, Hypertensive, Wistar Rats

INTRODUCTION

Hypertension is a chronic medical condition and is a major cause of premature death worldwide (1). It is said to be in an individual when the systolic blood pressure of ≥ 140 mmHg and diastolic blood pressure of ≥ 90 mmHg, while in hypertensive wistar rats, systolic blood pressure of ≥ 115 mmHg and diastolic blood pressure of ≥ 105 (2,3). In Nigeria, the incidence of hypertension constitutes major non communicable diseases of public health importance which is said to have huge effect on the quality of life of individuals and the economy (1). The number of adults with high blood pressure increased from 594 million in 1975 to 13 billion in 2015 globally and this increase occurred in low- and middle-income countries (4). According to the last report of the World Health Organization (WHO) (5), hypertension was the cause of approximately 45% of deaths from heart disease and 51% of deaths from stroke, which represents a total of 9.4 million deaths per year.

Among the many risk factors that are implicated in the epidemiology of hypertension, behavioral and physiological risk factors have been more prevalent (6). The behavioral risk factors include smoking, alcohol consumption, while some factors related to diets include excessive sodium intake, high consumption of alcoholic drinks, low intake of fruits and vegetables while the concerned physiological risk factors are obesity, increased waist to hip ratio, hyperglycemia, and increased cholesterol level and sedentary lifestyle, could increase the prevalence of HBP(6).

It has also been stated that deficiency of some vitamins, such as folic acid, riboflavin, and vitamins C and D, can be considered risk factors in the development of this non-communicable disease (7). Faced with this situation, scientific organizations, such as the American Heart Association (AHA), have recommended dietary approaches to stop hypertension (DASH), as

effective nutritional strategies included in the treatment of HBP (8). Research interest has been directed at the possibility of using locally available foods such as fruits, vegetables and medicinal plants in prevention and treatment of hypertension. In line with the current research interest, therefore this work focused on the effect of three food items which are; beet root and two varieties of radish (7).

Beetroot (*Beta vulgaris* L.) is a crop belonging to the *Chenopodiaceae* family having, bright crimson colour. It is famous for its juice value and medicinal properties and known by several common names like beet, chard, spinach beet (9). It makes an excellent dietary supplement being not only rich in minerals, nutrients and vitamins but also has unique phyto constituents, which have several medicinal properties. Several parts of this plant are used in medicinal system such as anti-oxidant, anti-depressant, anti-microbial, anti-fungal, anti-inflammatory, diuretic, expectorant and carminative (10).

Radish (White radish- *Raphanus sativus* var *longipinnatus* L. and red radish- *Raphanus sativus* var. *sativus* S.) belongs to the Brassicaceae family, which includes white and red cabbage, broccoli, brussels sprouts, and cauliflower and contains glucosinolates, their derivatives, including isothiocyanates and nitriles, essential oils, flavonoids, and other polyphenol compounds (11). In particular, glucosinolates in the family Brassicaceae (syn. Cruciferae) have been found to have antioxidant effects and anti-cancer properties (12). Glucosinolates suppress lipid peroxide production and protect against DNA damage (13). In addition, they might exert an anti-hypertensive effect by mechanisms associated with anti-oxidation. Kerksick and colleagues (14) reported that beetroot juice serves as a strategy that could not only increase exercise performance but also favor the blood pressure parameters control in healthy subjects and hypertensive patients (in any of their categories with or without

pharmacological treatment), possibly through a higher synthesis of nitric oxide (NO). It was reported that the extract of radish (*Raphanus sativus* L.) increased the concentration of NO in serum and the activities of antioxidant enzymes such as glutathione peroxidase and catalase in red blood cells (RBCs) thus had antihypertensive effects in spontaneously hypertensive rats by increasing the serum concentration of NO and fecal concentration of Na⁺ and enhancing antioxidant activities (11).

In Nigeria, the prevalence of hypertension has been on the increase affecting a significant number of highly productive population (1). Some factors related to diets, such as excessive sodium intake, high consumption of alcoholic drinks, low intake of fruits and vegetables, and a sedentary lifestyle, rapid urbanization and uptake of western lifestyles, including high consumption of processed foods (with high salts and fats), could increase the prevalence of HBP (15). It has also been stated that deficiency of some vitamins, such as folic acid, riboflavin, and vitamins C and D, can be considered risk factors to develop this non-communicable disease (7). With an increasing adult population and changing lifestyle of Nigerians, the burden of hypertension may continue to increase as time unfolds (16). Additionally, there is also a high economic burden as a consequence of hypertension and associated cardiovascular complications in Nigeria which also reduce life expectancy (17) demonstrated by direct costs, for example: the cost of antihypertensive medications, administrative fees, laboratory fees and other out-of-pocket health expenditures, and indirect costs, such as: loss of savings from repeated healthcare expenses, hospital waiting times and work absenteeism (18). It is estimated that there were about 20.8 million cases of hypertension in Nigeria among people aged at least 20 years, with a prevalence of 28.0% and projected increase to 39.1 million cases with a prevalence of 30.8% by 2030 (19).

Monotherapy is the initial approach for reducing blood pressure (overall expected mean reduction: 10–15 mm Hg in systolic blood pressure (SBP) and 8–10 mmHg in diastolic blood pressure (DBP), although the use of combination therapy has shown the highest mean reductions(overall expected mean reduction: 20–25 mm Hg in SBP and 10–15 mmHg in DBP) (20, 21). The search for a healthy diet with minimal side effects, easily available, accessible and also affordable has been significantly emphasized especially in developing countries such as Nigeria. Several complementary and alternative medicine therapies, including dietary supplements, functional foods, vegetables, traditional herbs, and meditation, are regularly used especially in rural areas for treating hypertension due to their constituents, mainly bioactive compounds, fibers, minerals and vitamins. Epidemiological studies have shown that vegetables such as beetroot and radish are useful protective foods against coronary heart disease (15).

Therefore, these plants having high content of inorganic Nitrate NO_3^- (11), can be considered as an alternative remedy to conventional drugs in the treatment of hypertension. However, there is no evidence regarding the regular consumption of beetroot among hypertensive patients, this study investigated the efficacy of beetroot and radish juice on induced hypertension in rats with the aim of introducing a new approach to handling hypertension. In view of this, this study was aimed at investigating the ameliorative effect of the juice extracted from beetroot and radish in the reduction of high blood pressure caused by excess salt ingestion.

MATERIALS AND METHODS

STUDY LOCATION

The study was carried out at the Laboratory and Animal House in the Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

COLLECTION AND IDENTIFICATION OF THE ROOT VEGETABLES

Freshly matured Beetroot and radish root vegetables were purchased from food garden market, D-line, Port Harcourt located in Phalga Local Government Area in Rivers State. The tubers were taken to the Department of Forestry, College of Natural and Environmental Management, Michael Okpara University of Agriculture, Umudike for identification and authentication by the herbarium technologist.

JUICE EXTRACTION (PROCESSING)

Freshly matured purchased roots of beet root and two radish varieties were sorted and washed under a running tap to get rid of the dirt and then allowed to drain. The stalk was cut off and the roots chopped with kitchen knife into cube size, a cube of the vegetable sample weighed 1gram. Beetroot was cut into 90 cubes which yielded 78mls, meaning each cube of beetroot yield 1.2mls. Red radish was 114 cubes, it yielded 182 mills, meaning each cube yield 0.63 mills and for white radish, 126 cubes yielded 232 mills, meaning each cube yield 0.54 mills. The sample was poured into an electric blender (Electric Blender Juice Extractor – Hjb-33c made by Home Boss Industrial Group Limited, Guangdong, China), and blended without water for 2 minutes. Wet weight was used; the samples were weighed with core balance electric weight machine, Q T60/ max: 600g × 0.1g, 22 ADAM@. The juice that was obtained was filtered with a mesh of size 0.1 microns to obtain clear juice extracts, and were refrigerated till when they were used at 41°F (5°C).

Determination of juice concentration

According to the procedure adopted by Tafesse and colleagues (22), to determine the concentration of each of the juice extracts, three sets each of crucibles were used. Each crucible was weighed empty and their initial weights recorded. About 5 ml of each filtered extracts was

added into each crucible and oven-dried at 105°C to obtain dried particles. This procedure was replicated into 4 places. At the end of oven-drying, each of the crucibles containing the dried particles were reweighed to obtain the final weights. The values obtained by subtracting the initial weights from final weights were used to calculate the concentration (mg/ml) of the individual juice extract.

Mean= $\frac{(B-A) \text{ g} \times 1000 \text{ mg}}{5 \text{ ml}}$ = the concentration of each sample (mg/ml)

5 ml

Where;

A= initial weight of the sample, and

B= final weight of the sample.

ANIMAL STUDY

Procurement of experimental rats

The animals were procured from the Animal House of the Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. They were kept at the Laboratory Unit, Department of Veterinary Pharmacology for acclimatization for a period of seven days.

Experimental animals

Total of eighty (80) wistar rats were used in this study. Thirty-five wistar rats were used for the main study; while forty-five rats were used for the determination of median lethal doses of the three species of the test plants (acute toxicity testing). They were purchased from the Animal Laboratory House, Department of Veterinary Physiology and Pharmacology, College of

Veterinary Medicine, MOUAU and were housed in separate stainless-steel cages, given water and feed (Vital Feedgrower's mash) *ad libitum* daily. All the experimental animals were kept under ambient atmospheric pressure and 12-hour light/dark conditions. All procedures were carried out in strict compliance to the institution's ethical instructions for animal study, as well as adequate consultations to the Experimental Ethic Committee (EEC) guidelines to laboratory animal care and use (23).

ACUTE TOXICITY TEST (LD₅₀)

Each of the juice extract was tested for acute toxicity effect in wistar rats. The up and down method described by Rispin and colleagues (24) and modified by Saganuwa(25) was adopted for this study. A total of forty-five rats were used for the study. The forty-five rats were divided into three groups (A, B and C) of fifteen rats each. Groups A, B and C represented the white, red radish, and beetroot, respectively. Each of the extract group was further divided into three sub-groups (I, II, and III) with five rats each, which received the juice at 5000 mg/kg body weight (highest dose) with the use of oral gavages, 500 mg/kg (lowest dose), and distilled water at 5 ml/kg body weight (which served as normal control), respectively, for each group of the juice.

An initial stock solution of 100 mg/ml (w/v) concentration of the individual juice in distilled water was prepared and used for the study. The rats were allowed free access to feed and water *ad libitum* and observed for both acute and delayed signs of toxicity and possibly death over a 72-hour period.

The median lethal dose (LD₅₀) of the crude extract was then calculated using the formular:

$$LD_{50} = \sqrt{(\text{least dose with mortality} \times \text{Highest dose without mortality})} \quad (26).$$

INDUCTION OF HYPERTENSION

Thirty-five rats were used in this study. The method described by Yohannes and colleagues(27) was adopted. The rats were divided into 7 experimental groups (n = 5) designated as A, B, C, D, E, F, and G.

Table 1: Induction of hypertension

Experimental group	Number of rat	Treatment	Dose (mg/kg)	Remark
A	5	Distilled water	5 ml/kg	Normotensive
B	5	No treatment	No treatment	Hypertensive group
C	5	White radish	Single dose based on the result of acute toxicity	
D	5	Red radish		
E	5	Beet root		
F	5	Beetroot and red radish		
G	5	Standard drug	5 mg/kg of Amilodipin	treated groups

As preventive study, all the rats in group A (normotensive rats) received distilled water and served as the control while groups B-G received 16 % salt solution (induction group) and B was treated with only distilled water (negative control group), G was treated with standard anti-hypertensive drug (Amlodipine) the positive control group, and group C to F were treated simultaneously with a single dose of white radish, red radish, beet root extract and a

combination of beetroot and red radish respectively. The study lasted for a period of 28 days during which the blood pressure and heart rate of each of the experimental rats were measured at 0, 7, 14, 21 and 28th day using Non-Invasive Blood Pressure Technique (NIBPT) (28). The interpretation of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were made from pulse tracings, the mean blood pressure (MBP) was calculated from SBP and DBP using equation described by (28) and (27) as follows:

$$MBP = \frac{1}{4} (3DBP + SBP)$$

The 16% Salt solution was prepared by weighing out 16g of sodium chloride or common salt into 100ml measuring cylinder and distilled water added to make it up to 100ml. For each day a freshly prepared 16% salt solution was used, 1ml of 16% salt solution was administered to all the induction groups using Gavage (intragastric tube). After inducing the rats with 1 ml of salt solution, they were treated after 30 minutes interval or more with 2 ml per time, till the required dose had been administered.

EXPERIMENTAL DESIGN

Total of 35 rats were randomly distributed into seven (7) experimental groups A - G with 5 rats each. Rats in group A were not induced (normotensive group). All the rats in groups B-G were induced with a hypertensive agent. Group B was not treated (will serve as negative control), groups C-G were treated simultaneously with a single dose of white radish, red radish, beet root juice, combination of beetroot and red radish and standard anti-hypertensive drug (Amlodipine), respectively with the three juice extract samples at 800 mg/kg body weight (single dose). The treatment lasted for a period of 28 days.

DATA ANALYSIS

All data analyses were done using Statistical Package for Social Sciences (SPSS) version 23.0 (IBM Statistics, UK). Data values were reported as mean \pm standard deviation. One way another was used to determine the differences between the means. Posthoc test was done to ascertain the differences between the groups. Statistical significance was set at 99% confidence interval ($P < 0.01$).

RESULTS

4.1 showed the ameliorative effect of the different extracts and the standard drug on the heart rate following 16% salt inducement.

There was no statistically significant difference between the groups at baseline ($p: 0.561$). Similarly, at week one (that is, after 7 days of concurrent administration of the salt solution and the different juice extract), there were slight variation in the heart rate reading between the different treatment groups, however, these changes were not significant ($p: 0.782$). At week 2, the heart rate between the group was statistically significant ($p: 0.031$). A post-hoc test was further conducted to identify where the significance lies. Specifically, there was a statistically significant difference between the heart rate of the hypertensive group (224.00 ± 18.9) and white radish-treated group (199.60 ± 5.46 ; $p: 0.043$). Also, the heart rate of the hypertensive group differed from that of combination of beet root and red radish group (224.00 ± 18.9 vs 199.00 ± 9.87 ; $p: 0.036$) at 2 weeks.

After 21 days of concurrent administration of the salt solution and the different juice extract across the treatment groups, there was a significant difference in heart rate ($p: < 0.001$). The mean heart rate for the hypertensive group (235.40 ± 18.9) was significantly higher than that of normotensive group (208.80 ± 7.95 ; $p: 0.022$), beet root group (202.40 ± 13.9 ; $p: 0.003$), red radish (197.10 ± 8.4 ; $p: < 0.001$), combination of beet root and red radish (196.50 ± 9.9 ; $p: < 0.001$), white

radish (193.10 ± 5.5 ; $p < 0.001$), and Amlodipine (209.30 ± 13.6 ; $p: 0.026$). The white radish group had the lowest heart rate. A similar observation was noted at week 4, the heart rate showed significant different across all the groups ($p < 0.001$). The mean heart rate for the hypertensive groups (246.80 ± 39.8) was significantly higher than that of normotensive group (208.80 ± 6.4 ; $p: 0.023$), beet root group (202.40 ± 13.9 ; $p: 0.005$), red radish (194.00 ± 12.8 ; $p < 0.001$), white radish (186.60 ± 2.2 ; $p < 0.001$), combination of beet root and red radish (194.00 ± 2.0 ; $p < 0.001$), and Amlodipine (210.80 ± 7.9 ; $p: 0.036$). The white radish group had the lowest heart rate still at day 28, while the red radish, and the combination of beet root and red radish had similar value (194.00 ± 12.8 and 194.00 ± 2.0) they were next to the white radish in controlling the heart rate, then followed by beetroot (202.40 ± 13.9 ; $p: 0.005$) before the standard drug Amlodipine which could not even bring the heart rate to that of the normotensive (210.80 ± 7.9).

In summary, the insignificant different at the baseline and week 1, simply imply that the effect of the three-juice extract on the heart rate compared with the untreated hypertensive rat could be that the suspected antihypertensive effect of the three juice extracts has not set in. However, from week two (2), the increase in the heart rate of the hypertensive untreated group was higher, this suggest that the suspected antihypertensive activity of the three-juice extract started having effect at week two, following the concurrent administration better than the 5mg Amlodipine drug. At week three and four, the increase in the number of heart beats per minute in the hypertensive untreated rats became obviously higher compare with the salt induced and treated groups, where the mean heart rate beats per minute were returned to mean values lower than the normotensive groups. The untreated rat recorded increase in the heart rate from 192.00/minute to an exponential value of 246.80minute, whereas treated groups hindered the hypertensive effect of

16% salt solution (Ameliorative effect) from rising but were maintained within the normal (baseline) heart rate (192.00 – 208.80/minute).

UNDER PEER REVIEW

Table 2: Anti-hypertensive effects of beet root (*Beta vulgaris*) and two varieties of radish (*R. sativus* and *R. longipinnatus*) on heart rate of salt induced hypertensive wistar rats

Treatment groups	Baseline Mean ± SD (beats/minute)	Week 1 Mean ± SD (beats/minute)	Week 2 Mean ± SD (beats/minute)	Week 3 Mean ± SD (beats/minute)	Week 4 Mean ± SD (beats/minute)
Normotensive	200.00±5.51	204.40±5.51	208.80±7.95	208.80±7.95 ^a	208.80±6.4
Hypertensive	192.00±5.21	208.00±5.21	224.00±18.9 ^b	235.40±18.9 ^b	246.80±39.8
Beet root juice	193.20±7.88	197.80±7.88	202.40±13.9	202.40±13.9 ^c	202.40±13.9
Red radish	206.40±4.06	209.50±4.06	200.20±8.44	197.10±8.4	194.00±12.8
White radish	203.20±3.20	205.00±3.20	199.60±5.46 ^e	193.10±5.5	186.60±2.2
BR + RR	199.60±6.76	199.90±6.76	199.00±9.87 ^f	196.50±9.9	194.00±2.0
Amlodipine	201.20±5.88	204.50±5.88	207.80±13.6	209.80±13.6	210.80±7.9
p-value	0.561	0.782	0.031	<0.001	< 0.001
Post-hoc test	--	--	b&e: 0.043	a&b: 0.022	a&b: 0.023
p-value			b&f: 0.036	b&c: 0.003	b&c: 0.005
				b&d: 0.001	b&d: 0.001
				b&e: 0.001	b&e: 0.001
				b&f: 0.001	b&f: 0.001
				b&g: 0.026	b&g: 0.036

Note: Values are presented as means ± standard deviation; BR: beet root; RR: red radish; WR: white radish

4.2 showed anti-hypertensive effects of beetroot (*Beta vulgaris*) and two varieties of radish (*Raphanussativus* and *Raphanus Longipinnatus*) on the systolic blood pressure of salt induced hypertensive wistar rats

The study displays the ameliorative effect of the different extracts and the standard drug on the systolic blood pressure following 16% salt solution inducement. There was no statistically significant difference between the groups at baseline and week one (1). At week two (that is, after 14 days of concurrent administration of the salt solution and the different juice extract), there were slight variation in the systolic blood pressure between the different treatment groups, however, these changes were not significant (p: 0.006). A post-hoc test was further conducted to identify where the variation lies. Specifically, there was a statistically significant difference between the systolic blood pressure of the hypertensive group (117.80±5.4), red radish-treated

group (108.80 ± 2.9 ; $p: 0.011$), white radish group (108.60 ± 2.1 ; $p: 0.009$) and combination of Beet root and red radish group (108.60 ± 3.6 ; $p: 0.009$) Also, the systolic blood pressure of the hypertensive group differed from that of beet root extract treated group (109.40 ± 5.0 ; $p: 0.020$) and the standard drug treated group (110.60 ± 3.8 ; $p: 0.064$) which have similar systolic blood pressure result like the normotensive group (110.20 ± 1.3 ; $p: 0.044$) at 2 weeks.

At week three (21 days) of concurrent administration of the salt solution and the different juice extract across the treatment groups, there was a significant difference in the systolic blood pressure ($p: <0.001$). This difference was observed across all the group, the systolic blood pressure for the hypertensive group (121.40 ± 5.9) was significantly higher than that of normotensive group (110.20 ± 1.3 ; $p: 0.001$), beet root group shows little bit variation, lower compared to that of the normotensive (108.80 ± 3.8 ; $p: 0.001$), red radish, white radish and the combination of Beet root plus red radish shows lower value in their systolic blood pressure readings compared to the normotensive and the hypertensive group above, (107.80 ± 2.9 ; $p: 0.001$), (106.60 ± 2.1 ; $p: 0.001$) and (107.60 ± 3.6 ; $p: 0.001$) respectively, also the standard drug systolic blood shows lower reading compared to even the normotensive group (108.20 ± 3.2 ; $p: 0.001$).

A similar observation was noted at week 4, the systolic blood pressure showed significant different across all the groups ($p: <0.001$). The systolic blood pressure for the hypertensive groups (128.80 ± 11.6 ; $p: 0.001$) was significantly higher than that of normotensive group (110.60 ± 1.1 ; $p: 0.001$), beet root group (109.40 ± 5.0 ; $p: 0.001$), red radish (106.80 ± 4.8 ; $p: 0.001$), white radish (104.40 ± 0.89 ; $p: 0.001$), beet root and red radish combination (106.60 ± 5.5 ; $p: <0.001$), and Amlodipine (100.40 ± 6.7 ; $p: 0.036$). The standard drug had the list value (100.40 ± 2.97 ; $p: 0.001$) of systolic blood pressure, followed by white radish group with second

lowest systolic blood pressure at day 28 (104.40 ± 0.89). It was observed that the red radish and combination of beet root and red radish had similar values, which was next to white radish (106.80 ± 4.8 and 106.60 ± 5.5).

In summary, the untreated rat recorded increase in their systolic blood pressure from 111.00 minute to an exponential value of 128.80 minute, whereas the treated groups hindered the hypertensive effect of 16% salt solution (Ameliorative effect) from rising but were maintained within the normal (baseline) systolic blood pressure (110.40 – 100.40 minute) with the standard drug, which yielded the best result for the systolic blood pressure. Among the extract white radish still appear to lower the systolic blood pressure more compared to red radish, and combination of beet root plus red radish, before beetroot.

Table 3: Anti-hypertensive effects of beetroot (*Beta vulgaris*) and two varieties of radish (*Raphanussativus* and *Raphanus Longippinnatus*) on the systolic blood pressure (mmHg) of salt induced hypertensive wistar rats

Treatment groups	Baseline Mean \pm SD (mmHg)	Week 1 Mean \pm SD (mmHg)	Week 2 Mean \pm SD (mmHg)	Week 3 Mean \pm SD (mmHg)	Week 4 Mean \pm SD (mmHg)
Normotensive	111.40 \pm 0.81	109.40 \pm 1.12	110.20 \pm 1.3 ^a	110.20 \pm 1.3 ^a	110.60 \pm 1.1 ^a
Hypertensive	111.00 \pm 1.09	111.60 \pm 2.55	117.80 \pm 5.4 ^b	121.40 \pm 5.9 ^b	128.80 \pm 11.6 ^b
Beet root juice	110.00 \pm 0.63	110.20 \pm 2.70	109.40 \pm 5.0 ^c	108.80 \pm 3.8 ^c	109.40 \pm 5.0 ^c
Red radish	111.40 \pm 1.40	112.00 \pm 1.48	108.80 \pm 2.9 ^d	107.80 \pm 2.9 ^d	106.80 \pm 4.8 ^d
White radish	111.20 \pm 0.91	110.80 \pm 2.00	108.60 \pm 2.1	106.60 \pm 2.1 ^e	104.40 \pm 0.89 ^e
BR + RR	111.40 \pm 1.02	108.60 \pm 1.77	108.60 \pm 3.6	107.60 \pm 3.6 ^f	106.60 \pm 5.5 ^f
Amlodipine	110.40 \pm 0.67	110.60 \pm 2.15	110.60 \pm 3.8	108.20 \pm 3.2 ^g	100.40 \pm 6.7 ^g
p- value	0.914	0.093	0.006	<0.001	<0.001
Post-hoc test p- value			a&b : <0.044 b&c : <0.020 b&d : <0.011	a&b: <0.001 b&c : <0.001 b&d : <0.001 b&e : <0.001 b&f : <0.001 b&g : <0.001	a&b: <0.001 b&c : <0.001 b&d : <0.001 b&e : <0.001 b&f : <0.001 b&g : <0.001

Note: Values are presented as means \pm standard deviation; BR: beet root; RR: red radish

4.3 Showed anti-hypertensive effects of beetroot (*Beta vulgaris*) and two varieties of radish (*Raphanussativus* and *Raphanus Longipinnatus*) on the Diastolic blood pressure of salt induced hypertensive wistar rats.

This table presented the ameliorative effect of the different extracts and the standard drug on the diastolic blood pressure following 16% salt inducement. There was no statistically significant difference between the groups at baseline (p: 0.483). Similarly, at week one of concurrent administration of the salt solution and the different juice extract, there were slight variation in the diastolic blood pressure values between the different treatment groups, however, these changes were not significant (p: 0.013). A post-hoc test was further conducted to identify where the changes lie. Specifically, there was a statistically significant difference between the diastolic blood pressure of the hypertensive group (110.80 ± 6.9) and white radish-treated group (101.80 ± 1.9 ; p: 0.033). Also, the diastolic blood pressure of the hypertensive group differed from that of combination of beet root and red radish group (99.80 ± 2.8 ; p: 0.005) at week one. At week two (2), the diastolic blood pressure between the group was statistically significant (p: 0.005), the statistically significant lies between the normotensive and hypertensive group (110.60 ± 5.0 ; p: 0.046), the diastolic blood pressure for the hypertensive group (110.60 ± 5.0) was significantly higher than that of normotensive group (103.60 ± 0.89 ; p: 0.046), beet root group (103.00 ± 4.7 ; p: 0.024), red radish (102.00 ± 2.6 ; p: 0.008), combination of beet root and red radish (101.80 ± 3.3 ; p: 0.006), white radish (101.80 ± 1.8 ; p: 0.006), and Amlodipine (103.60 ± 3.8 ; p: 0.046). The white radish group and combination of beetroot plus red radish had similar value, also the lowest diastolic blood pressure (101.80 ± 1.8 and 101.80 ± 3.3), followed by red radish (102.00 ± 2.6), the standard drug and beet root extract were able to bring down the diastolic blood pressure to the normal range (103.60 ± 3.8 ; 103.00 ± 4.7 ; vs 103.60 ± 0.89).

Week three showed significant difference across all the groups ($p < 0.001$). The systolic blood pressure for the hypertensive groups (114.00 ± 5.6) was significantly higher than that of the normotensive group (103.60 ± 0.89 ; $p < 0.001$), the diastolic blood pressure of the white radish extract group was lower than even that of the normotensive (99.80 ± 1.8) against (103.60 ± 0.89), but red radish and the standard drug had similar impact as their systolic blood pressure had almost the same value (101.00 ± 2.6 and 101.60 ± 2.9), also the combination of beet root and red radish had an antihypertensive effect on the systolic blood pressure more than the standard drug group (100.80 ± 3.3 ; $p < 0.001$ vs 101.60 ± 2.9 ; $p < 0.001$), beetroot extract group had the least impact, though the impact was still significant (102.40 ± 3.4 ; $p < 0.001$).

The concurrent administration of the salt solution and the different juice extract across the treatment groups at week four displayed a statistically significant difference across all the groups ($p < 0.001$). The untreated group has the highest diastolic blood pressure compared to the normotensive group (120.60 ± 11.1 ; $p < 0.001$ vs 103.31 ± 5.4 ; $p < 0.001$), among the treated groups the standard drug had the best impact on the diastolic blood pressure compared to the extract-treated groups at week 4 (94.00 ± 6.2 ; $p < 0.001$), white radish-treated group had the second best diastolic blood pressure outcome compared with other extract-treated groups, with the value (97.40 ± 0.89 ; $p < 0.001$), the next was red radish and the combination of beet root plus red radish with similar output (100.20 ± 4.3 ; $p < 0.001$: 100.00 ± 1.0 ; $p < 0.001$), at week 4, beetroot extract was only able to bring down the diastolic blood pressure to almost the same level as that of the normotensive (103.00 ± 4.7 ; $p < 0.001$ and 103.31 ± 5.4 ; $p < 0.001$).

Table 4.: Anti-hypertensive effects of beetroot (*Beta vulgaris*) and two varieties of radish (*Raphanussativus* and *RaphanusLongippinnatus*) on the Diastolic blood pressure of salt induced hypertensive wistar rats.

Treatment groups	Baseline Mean ± SD (mmHg)	Week 1 Mean ± SD (mmHg)	Week 2 Mean ± SD (mmHg)	Week 3 Mean ± SD (mmHg)	Week 4 Mean ± SD (mmHg)
Normotensive	103.40±0.24	102.60±2.5	103.60±0.89 ^a	103.60±0.89 ^a	103.60±5.4 ^a
Hypertensive	103.60±0.40	110.80±6.9 ^b	110.60±5.0 ^b	114.00±5.6 ^b	120.60±11.1 ^b
Beet root juice	104.00±0.44	103.40±5.7	103.00±4.7	102.40±3.4 ^c	103.00±4.7 ^c
Red radish	104.20±0.37	105.20±3.0	102.00±2.6	101.00±2.6 ^d	100.20±4.3 ^d
White radish	103.60±0.50	101.80±1.9 ^c	101.80±1.8	99.80±1.8 ^c	97.40±0.89 ^c
BR + RR	104.40±0.24	99.80±2.8 ^f	101.80±3.3	100.80±3.3 ^f	100.00±1.0 ^f
Amlodipine	104.00±0.31	103.80±4.4	103.60±3.8 ^g	101.60±2.9 ^g	94.00±6.2 ^g
P-value	0.483	0.013	0.005	<0.001	<0.001
Pos hoc test p-value		b&e :0.033 b&f :0.005	a&b :0.046 b&c :0.024 b&d :0.008 b&e :0.006 b&f :0.006 b&g :0.006	a&b :<0.001 b&c :<0.001 b&d :<0.001 b&e :<0.001 b&f :<0.001 b&g :<0.001	a&b:<0.001 b&c :<0.001 b&d :<0.001 b&e :<0.001 b&f :<0.001 b&g:<0.001

Note: Values are presented as means ± standard deviation; BR: beet root; RR: red radish

4.4 showed anti-hypertensive effects of beetroot (*Beta vulgaris*) and two varieties of radish (*Raphanussativus* and *RaphanusLongippinnatus*) on the mean blood pressure of salt induced hypertensive wistar rats

The result presented showed that the same trend was maintained within and across the treatment groups. The mean value of mean blood pressure (MBP) was the same induction (baseline), but at week one, the mean value in the untreated group was not the same with the treated group, but this impact was not statistically significant from the post hoc test, the p value is (0.351). At week two, the combination of beetroot plus red radish extract treated group had better ability to ameliorate the potential rise in the MBP value compared to the white radish group and red radish

group alone (103.80 ± 3.3 ; $p: 0.008$; 104.20 ± 1.6 ; $p: 0.013$; 104.40 ± 3.0 ; $p: 0.016$) respectively, this could be attributed to probable synergistic effect of the BR + RR. The mean blood pressure value of the hypertensive untreated group was elevated as the induction continued just as that of other parameters early discussed. The beetroot treated group ameliorated the hypertensive effect better than the standard drug but this difference was not significant (105.20 ± 4.6 ; $p: 0.037$ and 106.20 ± 4.2).

After 21 days of concurrent administration of the extract treatment and the standard drug to the rats, the untreated hypertensive group kept on increasing (116.60 ± 5.7 ; $p: < 0.001$) while the normotensive was lower (106.00 ± 1.2 ; $p: < 0.001$). The white radish has the best antihypertensive effect, as it could prevent the mean blood pressure from increasing (101.80 ± 1.8 ; $p: < 0.001$), followed by the combination of BR + RR with the mean blood pressure value of (102.80 ± 3.3 ; $p: < 0.001$). The RR and the 5mg standard amlodipine drug had similar impact in by preventing a rise in the mean blood pressure beyond that of the normotensive group (103.40 ± 3.1 ; $p: 0.001$; 103.80 ± 2.8 ; $p: < 0.001$ and 106.00 ± 1.2 ; $p: < 0.001$) and this impact was strongly significant. Beetroot has the least antihypertensive effect among all the extract (104.60 ± 3.4 ; $p: < 0.001$).

Finally, at week four there was a statistically significant across all the groups and the p-value ($p: < 0.001$), the hypertensive group remain higher compared to the normotensive (123.60 ± 11.1 ; $p: < 0.001$; 105.80 ± 1.1 ; $p: < 0.001$). The 5mg amlodipine standard drug had the highest antihypertensive effect compared to the extract treated groups (96.20 ± 6.3 ; $p: < 0.001$), while white radish among other extract had the highest antihypertensive effect on the 16% salt induced wistar rats (99.80 ± 0.45 ; $p: < 0.001$), followed by the following (Red radish, combination of beetroot plus red radish, and beetroot (102.40 ± 4.2 ; $p: < 0.001$; 102.40 ± 0.55 ; $p: < 0.001$ and 105.20 ± 4.6) respectively.

In summary, white radish and the amlodipine standard drug were simultaneously effective in controlling hypertension among the 16% salt induced wistar rats, also the combination of the extract also was effective in almost similar action like the red radish, beetroot had the list impact, but it was still effective and with strong significant difference when compared to the untreated hypertensive rats' group.

Table 5: Anti-hypertensive effects of beetroot (*Beta vulgaris*) and two varieties of radish (*Raphanussativus* and *RaphanusLongippinnatus*) on the mean blood pressure (mmHg)of salt induced hypertensive wistar rats

Treatment groups	Baseline Mean ± SD (mmHg)	Week 1 Mean ± SD (mmHg)	Week 2 Mean ± SD (mmHg)	Week 3 Mean ± SD (mmHg)	Week 4 Mean ± SD (mmHg)
Normotensive	105.80±0.37	105.20±0.96	106.00±1.2	106.00±1.2 ^a	105.80±1.1 ^a
Hypertensive	105.40±0.24	109.80±2.43	112.80±5.4 ^b	116.60±5.7 ^b	123.60±11.1 ^b
Beet root juice	105.80±0.37	106.00±2.62	105.20±4.6 ^c	104.60±3.4 ^c	105.20±4.6 ^c
Red radish	105.80±0.37	107.60±1.36	104.40±3.0 ^d	103.40±3.1 ^d	102.40±4.2 ^d
White radish	105.60±0.50	105.00±0.89	104.20±1.6 ^e	101.80±1.8 ^e	99.80±0.45 ^e
BR + RR	105.80±0.37	103.60±1.86	103.80±3.3 ^f	102.80±3.3 ^f	102.40±0.55 ^f
Amlodipine	106.00±0.31	106.20±2.05	106.20±4.2	103.80±2.8 ^g	96.20±6.3 ^g
p-value	0.952	0.351	0.009	<0.001	<0.001
Post hoc test value			b&c:0.037	a&b:0.001	a&b:0.001
			b&d:0.016	b&c:0.001	b&c:0.001
			b&e:0.013	b&d:0.001	b&d:0.001
			b&f:0.008	b&e:0.001	b&e:0.001
				b&f:0.001	b&f:0.001
				b&g:0.001	b&g:0.001

Note: Values are presented as means ± standard deviation; BR: beet root; RR: red radish

DISCUSSION

Hypertension is one of the major global health challenges with high prevalence and undesirable complications, and is a major cause of premature death worldwide. Therefore, the study was conducted to evaluate the effect of juice extracted from beetroot (BR), red radish (RR) and white

radish (WR) tubers on experimentally induced hypertension in 10-week-old Wistar rats. The study showed the ameliorative effect of the different extracts and the standard drug on the heart rate following 16% salt inducement. There was no statistically significant difference between the groups at baseline. Similarly, at week one (that is, after 7 days of concurrent administration of the salt solution and the different juice extract), there were slight variation in the heart rate reading between the different treatment groups, however, these changes were not significant. This shows that the extracts under study have little effect on blood pressure of the rats in the first seven days. Rather, at week 2, the heart rate between the group was statistically significant. Specifically, there was a statistically significant difference between the heart rate of the hypertensive group and white radish-treated group. Also, the heart rate of the hypertensive group differed from that of combination of beet root and red radish group at 2 weeks. The result is an indication that the extracts have antihypertensive effects.

After 21 days or week three (3) of concurrent administration of the salt solution and the different juice extract across the treatment groups, there was a significant decrease in heart rate of the treated groups compared with the hypertensive group. The white radish group had the lowest heart rate. A similar observation was noted at week 4, with decrease in the heart rate. Furthermore, the white radish group had the lowest heart rate still at day 28, while the red radish, and the combination of beet root and red radish had similar value they were next to the white radish in controlling the heart rate, then followed by beetroot before the standard drug Amlodipine which could not even bring the heart rate to that of the normotensive.

Furthermore, the insignificant different at the baseline and week 1, simply imply that the effect of the three-juice extract on the heart rate compared with the untreated hypertensive rat could be that the suspected antihypertensive effect of the three juice extracts has not set in. However, from

week two (2), the increase in the heart rate of the hypertensive untreated group was higher, this suggest that the suspected antihypertensive activity of the three-juice extract started having effect at week two, following the concurrent administration better than the 5mg Amlodipine drug. At week three and four, the increase in the number of heart beats per minute in the hypertensive untreated rats became obviously higher compare with the salt induced and treated groups, where the mean heart rate beats per minute were returned to mean values lower than the normotensive groups. The untreated rat recorded increase in the heart rate from 192.00/minute to an exponential value of 246.80minute, whereas treated groups hindered the hypertensive effect of 16% salt solution (Ameliorative effect) from rising but were maintained within the normal (baseline) heart rate (192.00 – 208.80/minute). The results recorded here are good indications that the extracts have antihypertensive effects. And for effective result treatment should last up to three to four weeks.

Beetroot juice favors the blood pressure parameters control in healthy subjects and hypertensive patients (in any of their categories with, or without pharmacological treatment), possibly through a higher synthesis of nitric oxide (NO). Due to its high NO_3^- content, beetroots have been used as a dietary source of this anion for the production of NO, aiming at blood pressure lowering effects (29). Radish extracts have also been reported to enhance NO production and increase antioxidant levels (30) through vaso-relaxant effects, which are thought to be mediated by NO production (31). Webb and collaegues (32) reported a significant increase in NO synthesis (Plasma NO_3^- and NO_2^-), stabilizing of the endothelial function evaluated by the dilation of the brachial artery – mediated flow (DILA) and significant decrease of the systolic blood pressure (SBP) by up to 10 mmHg and diastolic blood pressure (DBP by up to 8 mmHg after 4 weeks of beetroot juice ingestion, whereas. In this study, beetroot juice decreases the SBP and DSP by 2 mmHg each.

According to some researchers such as (33), (34) and (35), the decreases in heart rate correlated with NO synthesis. This variation could be attributed to different soil type, climatic variations, soil nitrification and location where these tubers were grown. Ashor and colleagues (36) reported no significant effect of beetroot juice in reducing blood pressure after beetroot juice supplementation during a week in hypertensive patients with medical treatment. This is in line with the 7-day data, where no significant effect was observed in the reduction of HR, mean values, suggesting that interventions over two weeks generated better results (37, 36), compared to those with a duration of one week (36).

This study showed the ameliorative effect of the different extracts and the standard drug on the systolic blood pressure following 16% salt solution inducement. There was no statistically significant difference between the groups at baseline and week one. At week two (that is, after 14 days of concurrent administration of the salt solution and the different juice extract), there were slight decrease in the systolic blood pressure between the different treatment groups, however, these changes were not significant. At this point, the extracts have started having effects on the systolic blood pressure but longer treatment time is required to have a desired effect. There was a statistically significant decrease in systolic blood pressure of the red radish-treated group, white radish group and combination of Beet root and red radish group when compared with the hypertensive groups. Also, the systolic blood pressure of the hypertensive group was higher from that of beet root extract treated group and the standard drug treated group which have similar systolic blood pressure result like the normotensive group at 2 weeks.

At week three (21 days) of concurrent administration of the salt solution and the different juice extract across the treatment groups, there was a significant reduction in the systolic blood pressure. This reduction was observed across all the group, the systolic blood pressure for the

hypertensive group was significantly higher than that of normotensive group, beet root group shows little bit variation, lower compared to that of the normotensive, red radish, white radish and the combination of Beet root plus red radish shows lower value in their systolic blood pressure readings compared to the normotensive and the hypertensive group above. Also, with the standard drug, systolic blood pressure shows lower reading compared to even the normotensive group.

A similar observation was noted at week 4, the systolic blood pressure showed significantly lower values across all the groups when compared with hypertensive group. The systolic blood pressure for the hypertensive groups was significantly higher than that of normotensive group, beet root group, red radish, white radish, beet root and red radish combination, and Amlodipine. The standard drug had the list value of systolic blood pressure, followed by white radish group with second lowest systolic blood pressure at day 28. It was observed that the red radish and combination of beet root and red radish had similar values, which was next to white radish.

Therefore, the untreated rat recorded increase in their systolic blood pressure from 111.00 mmHg to an exponential value of 128.80mmHg, whereas the treated groups hindered the hypertensive effect of 16% salt solution (Ameliorative effect) from rising but were maintained within the normal (baseline) systolic blood pressure with the standard drug, which yielded the best result for the systolic blood pressure. Among the juice extract white radish still appear to lower the systolic blood pressure more compared to red radish, and combination of beet root plus red radish, before beetroot.

According to some researchers such as (33, 34, 35), the decreases in SBP and DBP were correlated with NO synthesis, implying that the beetroot juice used in this study, which recorded low mean values in SBP could mean there was lesser yield of NO synthesis. This variation could

be attributed to different soil type, climatic variations, soil nitrification and location where these tubers were grown. Previous study reported no significant effect of beetroot juice in reducing blood pressure after beetroot juice supplementation during a week in hypertensive patients with medical treatment(36). This is in line with the 7-day data, where no significant effect was observed in the reduction of, SBP mean values, suggesting that interventions over two weeks generated better results (37; 36), compared to those with a duration of one week (36).

According to previous report, the main bioactive compounds found in *Raphanus sativa* are water soluble alkaloids has been found to have anti-hypertensive effects (38). An in vitro study by Suh and colleagues (39) demonstrated that white radish taproot extract inhibited the abnormal proliferation of vascular smooth muscle cells. Castro-Torres and colleagues (40) revealed that white radish had the potential to diminish cholesterol level and reduce cholesterol gallstones in mice. Extracts of radish have been reported to induce blood pressure-lowering effect in rats (41), while the seeds of radish (0.1 -3mg/kg) lowered blood pressure as well as the heart rate (42). Previous study has shown that radish leaf powder suppressed hypertension in spontaneously hypertensive rats and inhibited ACE activity (41). The finding of this study showed the magnitude of the decrease in blood pressure indices were equivalent to what was achieved with anti-hypertensive (5mg Amlodipine) drug; hence it can suggest that the consumption of beetroot juice and red and white radish juices could have better effects on the stabilization of blood pressure values. This finding however, partially agreed with the report of Bahadoran and colleagues (41) who concluded that the individuals with high SBP values present a more significant decrease in the levels of variables after supplementation with beetroot juice while no significant changes was seen in SBP.

Mechanically, the increasing NO concentration promotes vasodilation through different cellular mechanisms (e.g cyclic guanosin monophosphate (cGMP)/cGMP-dependent protein kinase (PKG) pathway and hyperpolarization/relaxation after activation of K⁺ channels), and it is associated with significant decrease in blood pressure to muscle relaxation in the endothelium (32). It has been established that there is a positive correlation between the concentration of inorganic NO₃⁻ and the hypertensive effect (42). However, this study could not ascertain if the anti-hypotensive effect observed with the juice was actually due to synthesis of NO₃⁻ by the juices or whether it was due to their bioactive components, such as betalins, oxalic acid, phenol, hydroxycinnamic acid, among others, that mediated this ameliorative physiological response. The anti-proliferative and anti-inflammatory capacities of radish may also be partial contributors to its overall anti-hypertensive effects, as it inhibits proliferation of vascular smooth muscle cell in G1 phase of the cell cycle (39). Also, it was reported that radish contains glucosinolates, their derivatives, including isothiocyanates, essential oils, flavonoids, and other polyphenol compounds which have powerful antioxidant effects, which might have exerted the anti-hypertensive activities observed in this study (11).

This study presented the ameliorative effect of the different extracts and the standard drug on the diastolic blood pressure following 16% salt inducement. There was no statistically significant difference between the groups at baseline. Similarly, at week one of concurrent administration of the salt solution and the different juice extracts, there were slight variation in the diastolic blood pressure values between the different treatment groups, however, these changes were not significant. This is in line with the 7 day data, where no significant effect was observed in the reduction of blood pressure suggesting that interventions over two weeks generated better results (37,36) when compared to those with a duration of one week (36).

Specifically, there was a statistically significant difference between the diastolic blood pressure of the hypertensive group and white radish-treated group at week one. Also, the diastolic blood pressure of the hypertensive group differed from that of combination of beet root and red radish group at week one. At week two, the diastolic blood pressure between the group was statistically significant, the significant decrease between the normotensive and hypertensive group, the diastolic blood pressure for the hypertensive group was significantly higher than that of normotensive group, beet root group, red radish, combination of beet root and red radish, white radish and Amlodipine. The white radish group and combination of beetroot plus red radish had similar value, also the lowest diastolic blood pressure, followed by red radish, the standard drug and beet root extract were able to bring down the diastolic blood pressure to the normal range. This is support of previous studies which reported that radish is low in cholesterol and high in potassium, which makes it beneficial for cardiovascular health. Potassium helps regulate blood pressure levels, while the absence of cholesterol helps reduce the risk of heart disease (43). This variation could be attributed to different soil type, climatic variations, soil nitrification and location where these tubers were grown (36).

The pathophysiology of various forms of hypertension has been linked with formation of reactive oxygen species (ROS) which are upregulated in many animal models of hypertension. In hypertensive patients, increased ROS occurs as a result of oxidation of genomic and mitochondrial DNA, biomarkers of increased oxidation stress have been found to be increased in human hypertension (44). Therefore, the reduction in the diastolic levels of the Wistar rats treated with different types of juice extract shows that the extracts have antihypertensive effects and possibly ameliorate the effect of ROS as result of hypertension.

Week three showed significant difference across all the groups. The diastolic blood pressure for the hypertensive groups was significantly higher than that of the normotensive group. The diastolic blood pressure of the white radish extract group was lower than even that of the normotensive group, but red radish and the standard drug had similar impact as their systolic blood pressure had almost the same value, also the combination of beet root and red radish had an antihypertensive effect on the systolic blood pressure more than the standard drug group, the beetroot extract group had the least impact. The findings of this study showed the levels of the decrease in blood pressure indices were equivalent to what was achieved with anti-hypertensive (5mg Amlodipine) drug; hence it can suggest that the consumption of beetroot juice and red and white radish juices could have better effects on the stabilization of blood pressure values. This finding however, agreed with the report (41), who concluded that beetroot juice supplementation can cause a decline in blood pressure indices when there is prolonged treatment. Therefore, a better alternative to commercial drugs with several adverse effects.

The concurrent administration of the salt solution and the different juice extract across the treatment groups at week four displayed a statistically significant difference across all the groups. The untreated group has the highest diastolic blood pressure compared to the normotensive group. Among the treated groups the standard drug had the best impact on the diastolic blood pressure compared to the extract treated groups at week 4, the white radish treated group had the second best diastolic blood pressure outcome compared with other extract treated groups, with the value, the next was red radish and the combination of beet root plus red radish with similar output at week 4, beetroot extract was only able to bring down the diastolic blood pressure to almost the same level as that of the normotensive. This finding however, partially agreed with Bahadoran and colleagues (41), who concluded that beetroot juice has an antihypertensive effect.

and interventions over two weeks generate will generate better results (37,36). This further supports previous studies that reported that dietary nitrate, abundant in green leafy vegetables and beetroot, is reduced in vivo to nitrite and subsequently nitric oxide, and has been demonstrated to lower blood pressure, improve vascular compliance and enhance blood flow in non-pregnant humans and animals(44).

The study showed that the same trend was maintained within and across the treatment groups. The mean value of mean blood pressure (MBP) was the same at (baseline), but at week one, the mean value in the untreated group was not the same with the treated group, but this impact was not statistically significant from the post hoc test. At week two, the combination of beetroot plus red radish extract treated group had better ability to ameliorate the potential rise in the MBP value compared to the white radish group and red radish group alone, this could be attributed to probable synergistic effect of the BR + RR. And moreover, emphasis the need of combination therapy in management of HBP and HTN. The mean blood pressure value of the hypertensive untreated group was elevated as the induction continued just as that of other parameters early discussed.

At week 3, the white radish has the best antihypertensive effect, as it could prevent the mean blood pressure from increasing, followed by the combination of BR +RR. The RR and the 5mg standard amlodipine drug had similar impact in by preventing a rise in the mean blood pressure beyond that of the normotensive group and this impact was strongly significant. Beetroot has the least antihypertensive effect among all the extract.

Finally at week 4, the hypertensive group remained higher compared to the normotensive. The 5mg amlodipine standard drug had the highest antihypertensive effect compared to the extract treated group, while white radish among other extract had the highest antihypertensive effect on

the 16% salt induced wistar rats ($p < 0.001$), followed by red radish, combination of beetroot plus red radish and beetroot.

Therefore, the white radish and the amlodipine standard drug were simultaneously effective in controlling hypertension among the 16% salt induced wistar rats, also the combination of the extract also was effective in almost similar action like the red radish, beetroot had the list impact, but it was still effective and with strong significant difference when compared to the untreated hypertensive rats' group.

Prolonged use of excess salt (sodium chloride) has been linked with various side effects including hypertension, via depleting the supply of Nitric oxide (NO) and subsequently causing vasoconstriction through the following mechanisms: upregulation of ROS level, reaction with NO, reduction in its bioavailability and inhibition of expression of reaction with NO synthase at the transcription level (44). Increased formation of ROS is known to cause tissue damage (45). This is demonstrated in our study where the induction with 16% salt solution caused disruptive changes to the visceral organs histologically, antioxidants have been found to be potent in mopping up these ROS produced during oxidation processes. Based on the result of this study, the beetroot and the two variety of radish juices extract, which contain appreciable amount of vitamins A, C, E and other antioxidant properties could be said to have protective role on the integrity of visceral organs as against the destructive effects found in salt induced hypertensive rats. Several studies (11, 46, 47, 48), have reported the antioxidant effects of beetroot, red and white radish.

CONCLUSION

The LD₅₀ of the three juice extracts showed wide safety margin shows that the extracts are safe for consumption. The results of this study had shown that heart rate, systolic blood pressure,

diastolic blood pressure, and mean blood pressure parameters in the induced and treated wistar rats' groups were prevented or slowed down from the progression ($P < 0.05$) of a steady rise caused by 16% sodium chloride solution induced, as against the steady and progressive rise in the induced but untreated rats. The number of beats of the heart rate of the untreated rats recorded increase from 192.00/minute to an exponential value of 246.80/minute, whereas the treated groups hindered the hypertensive effect, but maintained a heart beats within the normotensive rat's baseline from 192.00/minute to 208.80/ minute.

The result of the systolic blood pressure shows that, the untreated rats recorded increase in their SBP 111.00mmHg to 128.80mmHg. The treated group with standard drug known as anti-hypertensive (5mg Amlodipine) recorded decrease from 110.40mmHg to 100.40mmHg, followed by the group treated with WR, BR +RR, RR, lastly BR treated group. For the DBP, at week 1 the hypertensive group recorded increase 110.60 ± 5.0 , compared with the normotensive which recorded 103.60 ± 0.89 . For the treatment group, at week 2 and week 3, the WR group recorded the best DBP result compared to BR +RR, RR, before the standard drug, BR was still the list among all the treatment groups, but at week 4, the treatment group with standard drug did better, compared to WR, BB+RR combination, RR, then BR treated groups.

ETHICAL APPROVAL

Ethical clearance for the animal study was obtained from the Research Ethics Committee of College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike.

RECOMMENDATIONS

In line with the findings of this study, the following recommendations were made;

- i) The consumption of the juice extracted from beetroot, red and white radish for the prevention of physiological hypertension has been shown to be very safe at $\leq 5000\text{mg/kg}$ dose.

- ii) For better anti-hypertensive activity, white radish or the combination of Beetroot plus red radish and red radish juice is recommended.
- iii) Further studies are recommended on the synergistic effect of red radish plus White radish on hypertension
- iv) Further studies are recommended on other health benefit and potentials of these White radish, red radish and beetroot juice/extract or these vegetables
- v) Further studies are hereby recommended to isolate the possible biomolecule active agent(s) and investigate the actual molecular putative mechanism of action involved in this antihypertensive effect.

REFERENCES

1. Odeyinka, O. T. and Ajayi, I. O. (2017). Prevalence of Hypertension and Diabetes and Their Determinants among Commercial Drivers in Ibadan Metropolis, South-Western Nigeria. *Nigerian. Journal of Cardiology*, **14(2)**: 75-83.
2. Kristek, F., Drobna, M. and Cacanyiova, S. (2017). Different Structural Alterations in Individual Conduit Arteries of SHRs Compared to Wistar Rats from the Prehypertensive Period to Late Adulthood. *Institute of Normal and Pathological Physiology of the Slovak Academy of Sciences*, Bratislava, Slovak. Pp. 345-678.
3. Luo, Y., Owens, D., Mulder, G., McVey, A. and Fisher, T. (2018). Blood Pressure Characterization of Hypertensive and Control Rat for Cardiovascular Studies. Charles River Laboratories, Wilmington, MA USA. Pp. 6598.
4. Zhou, B., Bentham, J., Di Cesare, M., Bixby, H., Danaei, G. and Cowan, M. J. (2017). Worldwide trends in blood pressure from 1975 to 2015: A pooled analysis of 1479 population-based measurement studies with 19.1 million participants. *Lancet*, **389**: 37–55.
5. World Health Organization. (2017). Global Health Risks Global Health Risks. Mortality and Burden of Disease Attributable to Selected Major Risks. No. 4.
6. World Health Organization (WHO) (2011). Global status report on non-communicable diseases. Geneva: WHO.

7. Eckel, R. H., Jakicic, J. M., Ard, J. D., De Jesus, J. M., Houston Miller, N., Hubbard, V. S., Lee, I. M., Lichtenstein, A. H., Loria, C. M. and Millen, B. E. (2014). AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: A report of the American college of cardiology/American heart association task force on practice guidelines. *Journal of American College of Cardiology*, **63**: 2960–2984.
8. McCartney, D. M. A., Byrne, D. G. and Turner, M. J. (2015). Dietary contributors to hypertension in adults reviewed. *Iranian Journal of Medical Sciences*, **184**: 81–90.
9. Yashwantkumar, B. (2015). Beetroot: A Super Food. *International Journal of Engineering Studies and Technical Approach*, **01(3)**: 1-15.
10. Kale, R. G., Sawate, A. R., Kshirsagar, R. B., Patil, B. M. and Mane, R. P. (2018). Studies on Evaluation of Physical and Chemical Composition of Beetroot (*Beta vulgaris* L.). *International Journal of Chemical Studies*, **6(2)**: 2977-2979.
11. Da-Hee, C., Sun-Hee, K., Nahye, M., Kang, J. C. and Moon-Jeong, C. (2012). The antihypertensive effect of radish leaves in spontaneously hypertensive rats. *Nutrition Research and Practice*, **6(4)**: 315-318.
12. Chuanphongpanich, S., Phanichphant, S., Bhuddasukh, D., Suttajit, M. and Sirithunyalug, B. (2006). Bioactive glucosinolates and antioxidant properties of broccoli seeds cultivated in Thailand. *WarasanSongkhlaNakharin*, **28**: 55-61.
13. Ippoushi, K., Takeuchi, A., Ito, H., Horie, H. and Azuma, K. (2007). Antioxidative Effects of Daikon Sprout (*Raphanus sativus* L.) and ginger (*Zingiber officinale* Roscoe) in Rats. *Food Chemistry*, **102**: 237-242.
14. Kerksick, C. M., Wilborn, C. D., Roberts, M. D., Smith-Ryan A., Kleiner, S. M., Jäger R., Collins R., Cooke M., Davis, J. N. and Galvan E. (2018). Exercise & sports nutrition review update: Research & recommendations. *Journal of the International Society of Sports Nutrition*, **15**: 38.
15. Diego, A. B. O., Andrés, F., Estevan, M., Salvador, V., Jorge, L. P. and Alexandra, P. (2018). Dietary Nitrate from Beetroot Juice for Hypertension: A Systematic Review. *Biomolecules*, **8(134)**: 1-12.

16. Kayima, J., Wanyenze, R. K., Katamba, A., Leontsini, E. and Nuwaha, F. (2013). Hypertension Awareness, Treatment and Control in Africa: A Systematic review. *BMC Cardiovascular Disorder*, **13**:54.
17. Abegunde, D. O., Mathers, C. D., Adam, T., Ortegon, M. and Strong, K. (2007). Chronic Diseases 1: The Burden and Costs of Chronic Diseases in Low-Income and Middle-Income Countries. *Lancet*, **370**: 1929–1938.
18. Ukwaja, K. N. and Onyedum, C. C. (2013). How to managed hypertension: the costs of blood pressure control in a Nigerian town. *Pan African Medical Journal*, **14**: 85.
19. Adeloye, D., Basquill, C., Aderemi, A. V., Thompson, J. Y. and Obi, F. A. (2015). An Estimate of the Prevalence of Hypertension in Nigeria: A Systematic Review and Meta-Analysis. *Journal of Hypertension*, **33**(2): 230-242.
20. Bronsert, M. R., Henderson, W. G., Valuck, R., Hosokawa, P. and Hammermeister, K. (2013). Comparative effectiveness of antihypertensive therapeutic classes and treatment strategies in initiation of therapy in primary care patients: a Distributed Ambulatory Research in Therapeutics Network (DARTNet) Study. *Journal of American Board of Family Medicine*, **26**: 529–538.
21. Paz, M. A., de-La-Sierra, A., Sáez, M., Barceló, M. A., Rodríguez, J. J., Castro, S., Lagarón, C., Garrido, J. M., Vera, P. and Coll-de-Tuero, G. (2016). Treatment Efficacy of Anti-Hypertensive Drugs in Monotherapy or Combination: ATOM Systematic Review and Meta-Analysis of Randomized Clinical Trials According to PRISMA statement. *Medicine*, **95**: e4071.
22. Tafesse, T. B., Hymete, A., Mekonnen, Y. and Mohammed, M. T. (2017). Antidiabetic Activity and Phytochemical Screening of Extracts of the Leaves of *Ajugaremotabenth* on Alloxan-Induced Diabetic Mice. *BMC Complementary and Alternative Medicine*, **17**(1): 243.
23. Louhimies, S. (2002). Directive 86/609/EEC on the Protection of Animals Used For Experimental and other Scientific Purposes. *Alternatives to Laboratory Animals (ATLA)*, **30**: 217 – 219.
24. Rispin, A., Farrar, D., Margosches, E., Gupta, K., Stitzel, K., Carr, G., Greene, M., Meyer, W. and McCall, D. (2002). Alternative Methods for the Median Lethal Dose (LD50) Test: The Up-And-Down Procedure for Acute Oral Toxicity. *Institute for Laboratory Animal Research Journal*, **43**(4): 233 – 243.

25. Saganuwa, S. A. (2015) Arithmetic-Geometric Harmonic (AGH) Method of Rough Estimation of Median Lethal Dose Using Up-And-Down Procedure. *Journal Drug Metabolism. Toxicology*, **6**: 180.
26. Khan, I. N., Jahan, S., Bhuiya, M. A. M, Mazumder, K. and Saha, B. K. (2013). Anti-Diarrheal Potential of Ethanol and Water Extracts of *Euphorbia Hirta* Whole Plant on Experimental Animals: A Comparative Study Scholars. *Journal of Applied Medical Sciences*, **1(3)**:199-204.
27. Yohannes, A. M., Willgoss, T. G., Baldwin, R. C. and Connolly, M. J. (2010). Depression and anxiety in chronic heart failure and chronic obstructive pulmonary disease: prevalence, relevance, clinical implications and management principles. *The International Journal of Geriatric Psychiatry*; **25**: 1209-1221.
28. Saleem, R., Ahmed, S. I., Azeem, M., Khan, R. A., Rasool, N., Saleem, H., Noor, F. and Faizi, S. (2005). Hypotensive Activity and Toxicology of Constituents from Root Bark of *Polyalthialongifolia* var. pendula. *Phytothermal Research*, **19**: 881 -884.
29. Clifford, T., Stevenson, E. J., Howatson, G. and West, D. J. (2015). The Potential Benefits of Red Beetroot Supplementation in Health and Disease. *Journal of Nutrients*, **7**: 2801-2822.
30. Chung, D., Kim, S., Myung, N., Cho, K. J. and Chang, M. (2012). The Antihypertensive Effect of Ethyl Acetate Extract of Radish Leaves in Spontaneously Hypertensive Rats. *Nutrition Research and Practice (Nutrition Research Practice)*, **6(4)**: 308-314.
31. Ghayur, M. N. and Gilani, A. H. (2006). Radish Seed Extract Mediates its Cardiovascular Inhibitory Effects via Muscarinic Receptor Activation. *Fundamental and Clinical Pharmacology*, **20(1)**: 57–63.
32. Webb, A. J., Patel, N., Loukogeorgakis, S., Okorie, M., Aboud, Z., Misra, S., Rashid, R., Miall, P., Deanfield, J. and Benjamin, N. (2008). Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension*, **51**: 784–790.
33. Kapil, V., Milsom, A. B., Okorie, M., Maleki-Toyserkani, S., Akram, F., Rehman, F., Arghandawi, S., Pearl, V., Benjamin, N. and Loukogeorgakis, S. (2010). Inorganic Nitrate Supplementation Lowers Blood Pressure In Humans: Role for Nitrite-derived NO. *Hypertension*, **56**: 274–281.

34. Gilchrist, M., Winyard, P. G., Aizawa, K., Anning, C., Shore, A. and Benjamin, N. (2013). Effect of Dietary Nitrate on Blood Pressure, Endothelial Function, and Insulin Sensitivity in Type 2 Diabetes. *Free Radical and Biological Medicine*, **60**: 89–97.
35. Kapil, V., Khambata, R. S., Robertson, A., Caulfield, M. J. and Ahluwalia, A. (2015). Dietary Nitrate Provides Sustained Blood Pressure Lowering in Hypertensive Patients: A Randomized, Phase 2, Double-Blind, Placebo-Controlled Study. *Hypertension*, **65**: 320–7
36. Ashor, A. W., Lara, J. and Siervo, M. (2017). Medium-Term Effects of Dietary Nitrate Supplementation on Systolic and Diastolic Blood Pressure in Adults: A Systematic Review and Meta-Analysis. *Journal of Hypertension*, **35**: 1353–1359.
37. Jajja, A., Sutyarjoko, A., Lara, J., Rennie, K., Brandt, K., Qadir, O. and Siervo, M. (2014). Beetroot Supplementation Lowers Daily Systolic Blood Pressure in Older, Overweight Subjects. *Nutrition Research*, **34**: 868–875.
38. Li, Y. B. and Jing, S. (2010). Determination of the Total Alkaloids in Semen Raphani by Acid Dye Colorimetry. *Information on Traditional Chinese Medicine*, **27**: 8–10.
39. Suh, S. J., Moon, S. K. and Kim, C. H. (2006). *Raphanus sativus* and its isothiocyanates inhibit vascular smooth muscle cells proliferation and induce G (1) cell cycle arrest. *Int. Immunopharmacology*, **6**: 854–861.
40. Castro-Torres, I. G., De la O-Arciniega, M., Gallegos-Estudillo, J., Naranjo-Rodriguez, E. B. and Dominguez-Ortiz, M. A. (2014). *Raphanus sativus* L. varniger as a source of phytochemicals for the prevention of cholesterol gallstones. *Phytotherapy Research*, **28**: 167–171.
41. Bahadoran, Z., Parvin, M., Ali, K., Fereidoun, A. and Asghar, G. (2017). The Nitrate-Independent Blood Pressure-Lowering Effect of Beetroot Juice: A Systematic Review and Meta-Analysis. *Advanced Nutrition*, **8(6)**: 830–838.
42. Siervo, M., Lara, J., Ogbonmwan, I. and Mathers, J. C. (2013). Inorganic Nitrate and Beetroot Juice Supplementation Reduces Blood Pressure in Adults: A Systematic Review and Meta-analysis. *Journal of Nutrition*, **b** 818–826.
43. Gamba, M., Asllanaj, E., Raguindin, P. F., Glisic, M., Franco, O. H., Minder, B., Bussler, W., Metzger, B., Kern, H. and Muka, T. (2021). Nutritional and phytochemical characterization of radish (*Raphanus sativus*): A systematic review. *Trends in Food Science and Technology*, **113**: 205-218.

- 44 Chhikara, N., Kushwaha, K., Sharma, P., Gat, Y. and Panghal, A. (2019). Bioactive compounds of beetroot and utilization in food processing industry: A critical review. *Food Chemistry*, (272): 192-200.
- 45 de Groot, H. (1994). Reactive Oxygen Species in Tissue Injury. *Hepatogastroenterology*. 41(4): 328-332.
- 46 Ravichandran, K., Thaw-Saw, N. M. M., Mohdaly, A. A. A., Gabr, A. M. M. and Kastell, A. (2013). Impact of processing of red beet on betalain content and antioxidant activity. *Food Research International*, 50: 670-675
- 47 Dambalkar, V. S., Rudrawar, B. D. and Poojari, V. R. (2015). Study of physico-chemical properties and sensory attributes of beetroot-orange RTS drink. *International Journal of Science and Research*, 4(10): 589-594.
- 48 Ingle, M., Thorat, S. S., Kotecha, P. M. and Nimbalkar, C. A. (2017). Nutritional Assessment of Beetroot (*Beta vulgaris* L.) Powder Cookies. *Asian Journal of Dairy and Food Research*, 36(3): 222-228.