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Cardioprotective Potential of *Beta vulgaris* (Beetroot) in Doxorubicin-Induced Cardiac Injury among Male Albino Wistar Rats

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Abstract

We investigated the effect of pretreatment of adult male albino rats with *Beta vulgaris* (beetroot) in doxorubicin-induced cardiac injury experimental model. Twenty-four (24) animals after acclimatization for two (2) weeks, were randomly distributed into four (4) groups, each containing six (6) and exposed to treatments for twenty (20) days as follows: Group 1 (normal control) received normal saline, group 3 received 200 mg/kg of beetroot and group 4 received 400 mg/kg of beetroot orally (from day 1 to 20). All the rats in groups 2 (positive control) to 4 received 1.25 mg/kg bodyweight of doxorubicin intraperitoneally on day 15 of study. Animals were sacrificed on day 21 and blood sample was collected through cardiac puncture, into plain tubes for biochemical analysis, while the heart was surgically removed for histological analysis. The results showed that bodyweight was significantly ($p < 0.05$) increased in beetroot-pretreated rats; group 3 (211.75 ± 11.56) and group 4 (221.75 ± 2.21) when compared to normal control (194.00 ± 11.43) and positive control (201.55 ± 21.95) groups. There was significant ($p < 0.05$) increase in serum level of Lactate dehydrogenase, LDH (16.62 ± 3.55), Aspartate Transferase, AST (64.50 ± 8.02) and Alanine aminotransferase, ALT (56.67 ± 3.83) in the positive control group (2) indicating Doxorubicin-induced cardiac injury. However, in rats pretreated with beetroot, we observed significantly ($p < 0.05$) decreased serum levels of LDH in group 3 (12.65 ± 2.67) and group 4 (10.70 ± 2.85), AST in group 3 (45.67 ± 1.97) and group 4 (43.00 ± 2.37), as well as ALT in group 3 (43.67 ± 8.38) and group 4 (36.50 ± 6.92) when compared to positive control. Also, the difference in serum levels of these biochemical parameters between group 3 and 4 is significant ($p < 0.05$). Furthermore, histological analysis showed distorted myofibrils, loss of myocyte nuclei and orientation resulting in overall loss of myocardial architecture in positive control group, meanwhile appearance of marked improvement in the heart tissues of rats pretreated with beetroot was observed when compared to the positive and normal control. In this study, *Beta vulgaris* (beetroot) seems to have role in healthy weight gain in the rats and a cardio protective potential which benefit on the rats is in a manner that appear to be dose-dependent.

Keywords: *Beetroot; Doxorubicin; Biochemical Parameters; Cardio Protective*

Introduction

Cardiovascular disease has been ranked high among the causes of mortality linked to morbidity around the globe, and has persisted as one of the leading cause of fatality with sudden death at several instances in the last decade [1,2].

Beta vulgaris (beet) is an herbaceous biennial or, rarely, perennial plant with heart-shaped leaves on stems and flowers on dense spikes; native to Mediterranean, the Atlantic coast of Europe, the Near East and India. [3]. Beets has numerous cultivated varieties, the best known of which is the root vegetable known as the beetroot or garden beet [4]. Beetroot is a vegetable plant which has been used over the years in traditional medicine to treat a wide variety of diseases. Some of these claims include therapeutic use of beetroot as anti-tumor, carminative, emmenagogue, hemostatic, renal protective, and as potential herb used in cardiovascular conditions [5].

Beetroot is reported among the highest nitrate-accumulating vegetables. Nitrate (NO_3^-) and nitrite (NO_2^-), present in beetroot and in other food sources, have been associated with cardiovascular benefits [5]. Nitrate itself is not considered to mediate any specific physiological function; rather, beneficial effects of nitrate are attributed to its *in vivo* reduction to nitric oxide (NO), which is a multifarious messenger molecule with important vascular and metabolic functions.

The limited NO bioavailability is the main mechanism involved in endothelial dysfunction, that is crucial for the development of cardiovascular diseases. Beetroot is known to be a powerful antioxidant [6]

Doxorubicin is a notable beneficial drug with anti-cancer activity employed in cancer therapy. But it also has enormous side effect, most dangerous of which is dilated cardiomyopathy leading to congestive heart failure in patients undergoing long term chemotherapy for the drug [7]. The rate of cardiomyopathy is dependent on its cumulative dose, with an incidence about 4% when the dose of doxorubicin is 500 - 550 mg/m^2, 18% when the dose is 551 - 600 mg/m^2 and 36% when the dose exceeds 600 mg/m^2 [8]. Doxorubicin is believed to cause cardiomyopathy in several ways including oxidative stress, down regulation of genes for contractile proteins, and p53 mediated apoptosis [9].

There are several biochemical parameters that can be used in the study of tissues as it relates to damages or disease conditions. For instance, Alanine aminotransferase (ALT) is an enzyme involved in amino acid metabolism and is, therefore, found in many tissues. The highest levels of Alanine aminotransferase (ALT) are found in the liver and kidney tissues [10]. Tissue destruction leads to the release of the intracellular enzyme into the circulating blood. ALT measurements are shown to be useful in the diagnosis and treatment of certain liver diseases (e.g. viral hepatitis and cirrhosis) and heart disease [10].

Aspartate Transferase is a pyridoxal phosphate (PLP)-dependent transaminase enzyme (EC2.6.1.1). Aspartate Transferase catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism. Aspartate Transferase (AST) is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells [11]. Serum AST level, serum ALT (Alanine Transaminase) level, and their ratio (AST/ALT ratio) are commonly measured clinically as biomarkers for liver health [11]. Aspartate transaminase catalyzes the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate [11]. Aspartate (Asp) + α-ketoglutarate ↔ oxaloacetate + glutamate (Glu).

Lactate dehydrogenase (also called lactic acid dehydrogenase, or LDH) is an enzyme found in almost all body tissues [12]. It plays an important role in cellular respiration, the process by which glucose (sugar) from food is converted into usable energy for our cells [12]. Although LDH is abundant in tissue cells, blood levels of the enzyme are normally low. However, when tissues are damaged by injury or disease, they release more LDH into the bloodstream. Serum levels of LDH are elevated in a wide variety of pathologic conditions, most notably cardiac and hepatic disease. LDH is a tetrameric enzyme consisting of two basic subunits. Five isoenzymes can be observed after electrophoresis. The relative ratios of the isoenzymes vary with the tissue source of the LDH [12].

Beetroot contain an array of nutrients such as folic acid, vitamins A and C, vitamin B6, niacin, and biotin, minerals content of iron, magnesium, selenium, potassium, calcium, zinc, phosphorus, and sodium [6]. It is a rich source of phytochemical compounds that include carotenoids, phenolic acids and flavonoids [5]. It is also one of the few vegetables that contain a group of highly bioactive pigments known as betalains. Betalain pigments (betacyanins and betaxanthins) have specifically been shown to possess various antioxidant functions. A

number of investigations have reported betalains to have high antioxidant and anti-inflammatory capabilities *in vitro* and a variety of *in vivo* animal models [13]. These have sparked curiosity on a possible role for beetroot in clinical pathologies characterized by oxidative stress and chronic inflammation such as liver disease, arthritis and cancer.

Aim of the Study

In this present study, the aim was to investigate whether beetroot has any protective effect on cardiac injury induced by doxorubicin. Specific objectives were to ascertain if pre-treatment of rats with beetroot can reduce cardio toxic injury inflicted on the heart of the rats and to examine whether beetroot has a dose-dependent activity in the study. The scope was limited to exploring analysis of biochemical parameters such as, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and then histopathological studies of the heart of the rats.

Materials and Methods

Experimental animals

Twenty-four (24) male albino rats weighing between 140 - 170g were obtained from animal house in the Department of biochemistry, university of Port-Harcourt, Rivers state. Nigeria and acclimatized for two weeks during which they were allowed free access to pelleted growers mash, distilled water and good ventilation.

Chemicals/reagents

AST kit, ALT kit, LDH kit, Chloroform, Sodium Chloride (Nacl), Sodium Hydroxide Solution (0.4M), 10% formalin, absolute ethanol, methylated spirit, normal saline, distilled water. Doxorubicin (Braithwaite Memorial Specialist, Hospital, BMH, 84 Forces Avenue, Orogbum, Port Harcourt, Rivers state, Nigeria). All other reagents/chemicals obtained from standard suppliers were of analytical grade.

Collection of extract /extraction procedure

The fresh leaves of *Beta vulgaris* were harvested from Nature Pure Laboratory Farm, Igboghene, Yenagoa, Bayelsa State, Nigeria and identified at Nature Pure Laboratory by an Agricultural Biochemist, in the Department of Agriculture, Niger Delta University: Voucher No: NDU/FA/CS/BRT.001. The fresh leaves of *Beta vulgaris* were washed severally with clean water to make it dust and debris free and then sun dried for three days. The dried leaves were homogenised to a powdered form with the aid of an electric blender. Two hundred grams (200g) of the powdered leaves were inoculated into a glass container and mixed with 1000 ml of ethanol (98%) and allowed to stand at room temperature for a period of three days (72 hours) with occasional agitation to ensure proper extraction. The mixture then is strained, the marc (damp solid material) is pressed, and the combined liquids are clarified by filtration using a double chess net after standing. The filtrate collected was then concentrated in a water bath (40ºC) for 72 hours to yield 30 gram of thick brownish red paste. 15g of the paste were mixed with 100 ml of normal saline water and preserved in an airtight container then stored in a refrigerator for further use.

Experimental design and procedures

After acclimatization for two weeks, the 24 male wistar albino rats were randomly distributed into four groups with 6 rats in each compartment and each rat was marked numerically according to their compartments and their weights were also recorded.

Group 1 (normal control) were fed with growers' mash, distilled water, and administered with normal saline throughout the experiment, group 2 (Positive control) were fed with growers' mash and distilled water. Group 3 were administered with 200 mg/kg body weight of *Beta vulgaris* (beet root) extract. Group 4 were administered with 400 mg/kg body weight of *Beta vulgaris* (beet root) extract orally.

Rats in group 2 to 4 were administered with 1.25 mg/kg bodyweight single dose of doxorubicin intraperitoneally on day 15. All the rats were sacrificed on day 21 after the weight of each rat was recorded.

Sample collection and biochemical analysis

The animals were subjected to euthanasia with general anaesthesia (chloroform), dissected and blood was collected through cardiac puncture and preserved in plain bottles. Blood was centrifuged at 2800 rpm for 10 minutes; the serum was separated and used for biochemical assay. The heart was surgically removed as specimen for histological analysis.

Determination of biochemical parameters

Alanine aminotransferase (ALT): ALT activity was determined when 0.1 ml of diluted sample was mixed with phosphate buffer (100 mmol/L, pH 7.4), L-alanine (100 mmol/L) and α-oxoglutarate (2 mmol/L) and the mixture incubated for exactly 30 minutes at 37°C. 0.5 ml of 2, 4-dinitrophenylhydrazine (2 mmol/L) was added to the reaction mixture and allowed to stand for exactly 20 minutes at 25^0C. Then 5.0 ml of NaOH (0.4 mol/L) was added and the absorbance read against the reagent blank after 5 minutes at 546 nm.

Aspartate transferase (AST): AST activity was determined following the principle described by Reitman and Frankel [14]. Aspartate aminotransferase was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. 0.1ml of diluted sample was mixed with phosphate buffer (100 mmol/L, pH 7.4), L-aspartate (100 mmol/L), and α-oxoglutarate (2 mmol/L) and the mixture incubated for exactly 30 min at 37°C. 0.5 ml of 2,4-dinitrphenylhydrazine (2 mmol/L) was added to the reaction mixture and allowed to stand for exactly 20min at 25^0C. Then 5.0 ml of NaOH (0.4 mol/L) was added and the absorbance read against the reagent blank after 5 minutes at 546 nm.

Lactate dehydrogenase (LDH): 0.01 ml of diluted sample was mixed with phosphate buffer (50 mmol/L, pH 7.4), Pyruvate (0.6 mmol/L), and NADH (0.18 mmol/L) and the mixture incubated for exactly 30 min at 30°C. The absorbance was read at 0.5 min (initial), 1, 2 and 3 min at 340 nm to determine LDH activity.

Statistical analysis

All measurements were expressed as Mean ± Standard deviation. The statistical significance was evaluated using GraphPad InStat, (One-way Analysis of Variance (ANOVA) under Tukey-Kramer Multiple Comparisons Test). Values were considered statistically significant when $P < 0.05$.

Results

The results of the study are presented in table 1, table 2 and figure 1. Table 1 shows the mean body weight of male albino rats administered with doxorubicin, following pre-treatment with beetroot (*Beta vulgaris* L.) extract for 20 days. Table 2 shows the mean concentration of LDH, AST and ALT in male albino rats administered with doxorubicin, following pre-treatment with beetroot (*Beta vulgaris* L.) extract.

	Body weight (g)
Normal control with normal saline	194.00 ± 11.43[a]
Positive control with 1.25 mg/kg	201.55 ± 21.95[a]
Test group1 with 200 mg/kg extract and1.25 mg/kgDOX	211.75 ± 11.56[a]
Test group 2 with 400 mg/kg extract and 1.25 mg/kgDOX	221.75 ± 2.21[b]

Table 1: *Effect of doxorubicin and beetroot (Beta vulgaris L.) on the mean body weight of male albino rats. Values are recorded as MEAN ± SD of triplicate determinations. Means with different superscript letters are statistically different at 95% confidence level (p ≤ 0.05).*

Treatment	ALT (u/l)	AST (u/l)	LDH (u/l)
Normal control with saline water	40.50 ± 9.26[a]	42.25 ± 3.86[a]	9.450 ± 3.28[a]
Positive control with 1.25 mg/kg Dox	56.67 ± 3.83[a]	64.50 ± 8.02[a]	16.62 ± 3.55[a]
Test group 1 with 200 mg/kg extract and 1.25 mg/kg DOX	43.67 ± 8.38[b]	45.67 ± 1.97[b]	12.65 ± 2.67[b]
Test group 2 with 400 mg/kg extract and 1.25 mg/kg DOX	36.50 ± 6.92[c]	43.00 ± 2.37[c]	10.70 ± 2.85[c]

Table 2: *Effect of doxorubicin and beetroot (Beta vulgaris L.) on biochemical parameters; ALT, AST, LDH in male albino rats. Data expressed as Mean ± SD (standard deviation), values with different superscript letters are significantly different at P < 0.05.*

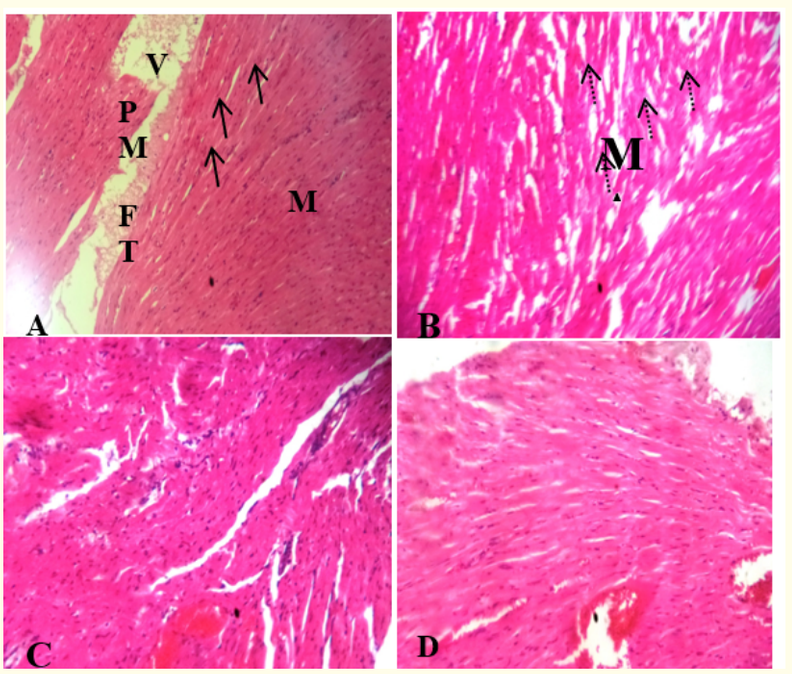

Figure 4*: Funnel plot of the comparison between depression and marital status in Ethiopia, 2021.*

The results in table 1 indicates that, there was increased mean body weight in the 4 groups, in ascending order from normal control - positive control -test group1 -test group 2. However, these difference in mean weight gain was only significant ($p < 0.05$) when comparing the test group 2 (221.75 ± 2.21) that was administered 400 mg/kg beetroot extract with normal and positive control groups (194.00 ± 11.43 and 201.55 ± 21.95) as well as with test group 1 (211.75 ± 11.56). This may imply beetroot extract involvement in weight gain activity.

The result in table 2 showed a significant ($p < 0.05$) increase in serum level of AST (64.50 ± 8.02), ALT (56.67 ± 3.83) and LDH (16.62 ± 3.55) in the positive control group administered with 1.25 mg/kg DOX alone, when compared with the serum levels of ALT (43.67 ± 8.38), AST (45.67 ± 1.97) and LDH (12.65 ± 2.67) in test group 1 administered with 1.25 mg/kg DOX and 200 mg/kg beetroot extract. Similarly, comparing the concentrations of these biochemical parameters in positive control group with the test group 2; ALT (36.50 ± 6.92), AST (43.00 ± 2.37) and LHD (10.70 ± 2.85), the difference was significant ($p < 0.05$) both suggestive of a possible attenuation of the effect of doxorubicin by beetroot extract.

Discussion and Conclusion

It is no longer news, the worrisome report on health statistics that more people die from cardiovascular diseases annually than any other cause, ranking cardiovascular diseases the number one (1) cause of death globally; with an estimated 17.9 million deaths in 2016, representing 31% of all global deaths out of which 85% are due to heart attack and stroke [15,16]. Concerted research efforts towards reducing cardiovascular related mortality will therefore continue to be of immense relevance.

In this study, we investigated the effect of beetroot on cardiac injury in an experimental rat model where 24 male albino rats weighing between 140 - 170g were placed in 4 groups of 6 each. Group 1 (Normal control) was administered normal saline, group 2 (Positive control) administered 1.25 mg/kg doxorubicin, group 3 (Test group 1) administered 200 mg/kg beetroot extract and 1.25 mg/kg doxorubicin, group 4 (Test group 2) administered 400 mg/kg beetroot extract and 1.25 mg/kg doxorubicin.

The results showed that the mean body weight of rats (in test group 2) pre-treated with higher dose (400 mg/kg) of beetroot extract before doxorubicin was administered and caused cardiac injury was significantly ($p < 0.05$) increased than the mean body weights of normal control and positive control rats. See table 1. In particular, this mean body weight of high dose beetroot-fed rats was also significantly higher than that of the low dose (200 mg/kg) beetroot-fed rats (in test group 1) that were equally administered doxorubicin to cause cardiac injury. The mean body weight of low dose beetroot-fed rats was not significantly different from the control rats. This observation seems to suggest that the administration of beetroot potentially increased weight gain in the rats. Concerns whether the gained weight is normal, healthy or not may be buttressed by the report of McDermott [17] that plain beet juice is low in calories and has virtually no fat, thus helping to maintain a healthy weight.

We carried out general investigation of some biochemical parameters such as ALT, AST and LDH whose serum level increases are accepted as standard clinical markers of some sort for general and or specific tissue damage (s), e.g. kidney, liver, muscles and heart. However, this phase of the research focuses on the heart which doxorubicin (a known myocardial toxic drug) was used to induce injury. Furthermore, to ascertain evidence of myocardial injury, the histopathological analysis was performed and these were considered sufficient for what perhaps, specific markers such as Creatine Kinase-MB (CK-MB) or Troponin I will do. See figure 1. The result indicates that among all four groups, serum level of ALT, AST and LDH were highest for the positive control rats given doxorubicin and no pre-treatment with beetroot extract. This increased biochemical parameters/clinical biomarkers of tissue (particularly of the heart which is under investigation) damage was not significant compared to its counterpart normal control group. However, when compared to the rats (in test group 1 and test group 2) that were equally given doxorubicin, but in addition pre-treated with beetroot extract doses (200 mg/kg and 400 mg/kg) respectively, this increase was significant. More so, the serum level of ALT, AST and LDH was decreased significantly ($P < 0.05$) for rats (in test group 2) pre-treated with higher dose (400 mg/kg) of beetroot extract than rats (in test group 1) pre-treated with lower dose (200 mg/kg) of beetroot extract.

The foregoing observations imply that the rats pre-treated with extract of beetroot before doxorubicin-induced myocardial injury was inflicted showed less severity of the toxic effect of doxorubicin. This may give credence to the report of Ali., *et al.* [5] regarding traditional

medicine, that beetroot is considered a potential herb used in cardiovascular conditions. Also, beetroot reportedly has rich content of nitrates which are believed to be important in cardiomyopathy onset and progression.

In this investigation, *Beta vulgaris* (beetroot) appears to have a cardio protective potential on rats (when they were pre-treated with it) against doxorubicin induced cardiac injury. Pre-treatment with beetroot seemed to have maintained the level of biochemical and histopathological parameters close to normal range. And this activity of beetroot followed a dose-dependent pattern. However, the exact mechanism of action of beetroot in this investigation may need to be elucidated in further research.

Limitation of the Study

Although this is a phase of an ongoing research, we admit some limitations. For instance, studies on the isolation, characterization and purification of the active constituent in beetroot were not done. Also, elucidating the possible mechanism of action was not included in the scope of the investigation. Moreover, if analysis of specific biomarkers of cardiomyopathy such as CK-MB and Troponin I was done, then the histopathological study corroborating these biomarkers would have been apt. Body mass index investigation to ascertain if the observed weight gains in beetroot treated rats was healthy will be necessary in further studies.

Bibliography

1. George AM., *et al.* "The Global Burden of Cardiovascular Diseases and Risk Factors: 2020 and beyond". *Journal of the American College of Cardiology* 74.20 (2019): 2529-2532.

2. Peter E., *et al.* "Analysis of Body Mass Index and Carotid Artery Thickness in Healthy Young Male Adults; Using Ultrasound Method". *EC Cardiology* 7.3 (2020): 01-04.

3. Kavitha K., *et al.* "Phytochemical screening and Evaluation of Analgesic activity of various extracts of beet root (Beta vulgaris)". *World Journal of Pharmacy and Biotechnology* 3.2 (2016): 60-64.

4. Jain SGV and Sharma PK. "Anti-inflammatory activity of aqueous extract of Beta vulgaris L". *Journal of Basic and Clinical Pharmacy* 2.3 (2011): 83-86.

5. Ali AE., *et al.* "Beetroot (Beta vulgaris L.) Extract Ameliorates Gentamicin-Induced Nephrotoxicity Associated Oxidative Stress, Inflammation, and Apoptosis in Rodent Model". *Hindawi Publishing Corporation Mediators of Inflammation* 98.3 (2014): 1-12.

6. Navnidhi C., *et al.* "Bioactive compounds of beetroot and utilization in food processing industry: A critical review". *Food Chemistry* 272.30 (2019): 192-200.

7. Alkuraishy HM., *et al.* "Doxorubicin-Induced Cardiotoxicity: Molecular Mechanism and Protection by Conventional Drugs and Natural Products". *International Journal of Clinical Oncology and Cancer Research* 2.2 (2017): 31-44.

8. Pal R., *et al.* "Hepatoprotective activity of Beta vulgaris against CCl 4 induced acute hepatotoxicity in rats". *Archives of Applied Science Reserach* 2.1 (2010): 14-18.

9. Rašković A., *et al.* "The Protective Effects of Silymarin against Doxorubicin-Induced Cardiotoxicity and Hepatotoxicity in Rats". *Molecules* 16.10 (2011): 8601-8613.

10. Stockham., *et al.* (2008).

11. Xing-Jiu H., *et al.* "Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) Detection Techniques". *Sensors* 6.6 (2016): 756-782.

12. Masterson JE and Schwartz SD. "The enzymatic reaction catalyzed by lactate dehydrogenase exhibits one dominant reaction path". *Chemical Physics* 442.17 (2014): 132-136.

13. Fidelis EO and Henrietta AO. "Antioxidant and Antioxidant capacity of raw and processed Nigerian Beetroot (Beta vulgaris)". *Nigerian Journal of Basic and Applied Science* 24.1 (2016): 35-40.

14. Reitman S and Frankel S. "A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases". *American Journal of Clinical Pathology* 28.1 (1957): 56-63.

15. WHO. World Health Organisation cardiovascular disease risk charts: revised models to estimate risk in 21 global regions". *The Lancet Global Health* 7.10 (2019): 1332-1345.

16. Roth GA., *et al.* "Global, regional and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017". *Lancet* 392 (2018): 1736-1788.

17. McDermott A. "11 Health Benefits of Beet Juice". Healthline (2020).

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