**THERAPEUTIC POTENTIAL OF LESSER-KNOWN VEGETABLES IN MANAGING HYPERCHOLESTEROLEMIA IN RATS**

ABSTRACT

**Aim**: To identify some lesser-known vegetables and use their extracts to manage Hypercholesterolemia induced rats.

**Study design**: The study adopted experimental methods to evaluate the efficacy of lesser-known vegetable extracts in managing hypercholesterolemia in rats.

**Place and duration of study**: The study was conducted at the Small Animals experimental unit of the National Veterinary Research Institute, Vom, Plateau state, Nigeria and lasted for seven weeks.

**Methodology**: Four vegetables were identified namely: Petroselinum *crispum*, Bidens *pelosa*, Launacea *taraxacifolia* and Vitex. *doniana*. Extracts of the vegetables were derived using cold water extraction. Acute toxicity test LD50 was conducted for the four-leaf extracts. The rats were fed high fat diet comprising refined ground nut oil (13%) groundnut cake (3%) coconut oil (9%) and wheat flour (1%). One of the control groups was treated with the drug atorvastatin at 500mg/kg and 1000mg/kg body weight daily and the other group was fed the high fat diet but not treated. Their lipid profiles were determined.

**Results**: The percentage yield of the vegetables extracts ranged from 11-15%. The results showed that the four aqueous leaf extracts were of good nutrient quality and within the safe level. All the groups except the first controls (unhypercholesterolemic untreated) had post-induction elevated lipid profiles. The leaf extracts they showed anti-hypercholesterolemic properties by lowering the elevated lipid profile levels from a range of 96.45mg/dl - 88.78mg/dl to 26.40 – 20.68mg/dl and 97.80 – 90.85mg/dl to 26.78 – 16.70mg/dl,and elevating the high density lipoprotein cholesterol from 51.82 – 37.50 to 60.33 – 58.80mg/dl and 40.30 – 36.68 to 52.56 – 65.78mg/dl for 500 and 1000mg/kg body weights of the rats, respectively, in a dose-dependent manner. This indicates the efficacy of the leaves in halting and reversing the effect of induction and high fat diet.

**Conclusion**: The vegetables can serve as acceptable therapeutic agents of plant origin in hypercholesterolaemic and dyslipidaemic conditions with *B. pilosa* and *L. taraxacifolia*most likely to produce more improved effect at a dose-dependent rate.

**Key words**: Hypercholestrolamia; Vegetables; Lesser known; rats.

1.0**INTRODUCTION**

Hypercholesterolemia is defined as excessively high plasma cholesterol levels, and is a strong risk factor for many negative cardiovascular events such as peripheral vascular and coronary artery disease (Stapleton *et al*., 2014). Hypercholesterolemia is widely recognized for its very significant role in cardiovascular disease. Excessive amounts of cholesterol in the body may build up in the artery walls overtime, narrowing and hardening them until the blood flow is reduced. Cholesterol is harmful not only because it adheres to arterial walls to cause arteriosclerosis, but at a certain level of blood cholesterol, there is increased risk of arteriosclerosis and heart attack.

 Most of the cholesterol in the body is produced by the liver. The body makes all the cholesterol it needs. Any added cholesterol from food is harmful. This means that the level of cholesterol in the body is affected by the types of food consumed. Foods high in saturated fats, mostly of animal foods, elevate cholesterol levels. A great deal can be done about modifying its harmful effects (Lichtenstein and Horn 2019). In Nigeria, the prevalence in women was higher at 40% than estimated among men at 38%. Across the goe-political zones, the prevalence of hyopercholesterolemia was highest in the south-south at 53% followed by North-Central (36%) and South-East (34%). The South-West (3.2%) and the North-West (4%) had the lowest estimated prevalence rates. The distribution of the prevalence of hypercholesterolemia across study settings suggest a significantly higher rate in Urban settings at 52% compared to rural settings at 10% (Adeloye et al, 2020). Cardiovascular disease is one of the leading causes of deaths. There is an estimated 62 million people with cardiovascular disease globally.

Sedentary lifestyle, obesity, advanced age, unhealthy diets among others are risk factors for hypercholesterolemia. One’s state of health is a faithful reflection of the individual’s daily actions. Any excess in eating or drinking, any transgression of natural law sooner or later reflects on theindividual’shealth. Nutritional investigation has concentrated on the effect diet has on the prevention and treatment of many diseases. It has been demonstrated that the abundant use of fruits and vegetables prevent the initiation of cancers, diabetes, cardiovascular and other deadly diseases. Eating whole grain cereals and oil-bearing nuts reduce excessive levels of cholesterol in the blood and the risk of myocardial infarction. Conversely the consumption of much meat increases the risk of cardiovascular disease and some type of cancer (NIH 2022). The aim of this work is to use the extracts of selected vegetables to manage hypercholesterolemia in induced rats.

**2.0 MATERIALS AND METHODS**

**2.1. Determination of percentage yield of leaf extracts**

The vegetables extracts were obtained using cold water extraction method and the percentage yield calculated using the method of Kumar et al. (2020)

**2.2. Induction of hypercholesterolaemia in Wistar rats**

The rats were fed high fat diet comprising of standard rat chow (NVRI Dagwom Feeds, Vom) supplemented with unrefined groundnut oil, groundnut cake, coconut oil and wheat flour at 13%, 3%, 9% and 1%, respectively, of the feed. These feed ingredients were mixed, molded and baked in the oven at 550C to cake the feed and avoid wastage by the rats. All experimental rats were provided access to feed and water *ad libitum* for 6 weeks. During this period, the initial and final bodyweight of each rat was recorded, while three (3) rats each were randomly selected at day 0, 2 and 4 weeks to evaluate for lipid profiles. At week 6, the rats were randomly assigned to treatment groups and treated with extracts and astorvastatin orally daily for 7 days. The body weights of the rats were recorded at the end of the treatment and bled via jugular venesection to obtain the serum for lipid profile. The study lasted for 7 weeks.

**2.3. Determination of total cholesterol**

The approach used in this study was modified from that described by Rathod et al. (2020).

Hydrolysis and oxidative enzymatic reaction were adapted for cholesterol analysis. The indicator quinoneimine was produced in the presence of phenol peroxidase by the action of 4-amino antipyrine and hydrogen peroxidase.

Total cholesterol (mg/dl) = Absorbance of sample/Absorbance of standard × 202.65

**2.4 Low density lipoprotein (LDL)**

The following expression was adapted to calculate low density lipoprotein-cholesterol (LDL-C):

LDL-C conc (mg/dL) = total cholesterol (TC) x (HDL + triglycerides/5).

**2.5. High density lipoprotein (HDL)**

In the presence of divalent cations, a polysaccharide precipitated low and very low-density lipoprotein (LDL and VLDL) from serum. The amount of HDL in the supernatant was then measured.

The concentration of the cholesterol in the supernatant was analyzed as elucidated by Kim et al. (2022).

HDL-C (mg/dl) = Absorbance of sample/Absorbance of standard x 202.65

**2.6. Triacylglycerol**

Enzymatic hydrolysis with lipases was used to evaluate triacylglycerol. Quinoneimine, is an indicator produced from 4-aminophenazone, 4-chlorophenol and hydrogen peroxide under the influence of catalytic peroxidase.

**3.0 RESULTS AND DISCUSSION**

Table 1 shows that after induction of hypercholesterolaemia, there was increase in total cholesterol, triglyceride and LDL levels and a decrease in the HDL levels.

On treatment with the aqueous leaf extracts, however, the total cholesterol levels of the experimental rat groups were decreased as against the increase in the total cholesterol levels of the hypercholesterolaemic-induced untreated rats. A similar result was obtained by Dhandapani (2007), Okwari et al. (2013), Martial et al. (2021) as well as El-Bakry, Ibrahim and Mohaseb (2020) following the administration of aqueous leaf extracts of *Ecliptaprostrata*, *Moringa oleifera*, *Clerodendrumthomsoniae*and green tea extract, respectively in rats fed a high fat diet. A dose-dependent effect of the aqueous leaf extracts on total blood cholesterol was observed, however, *L*. *taraxacifolia* (500 mg/kg) and *V*. *doniana* (1000 mg/kg) extracts were more effective in decreasing the Total Cholesterol levels and also, compared favorably with the standard drug. Thus, the significant decreases in the total cholesterol level of the test group is indicative of the hypocholestrolaemic properties of the leaf extracts and this could be attributed to their content of saponins, flavonoids and steroids (Yepshak et al, 2024). The hypocholesterolaemic effect of saponins in particular has been proven and could be explained by their abilities to inhibit acyl-CoA cholesterol acyl transferase activity (Zhao et al., 2008), and also due to the inhibitory effect of saponins on cholesterol absorption.

**Table 1: Effect of the aqueous extracts of the leaves on the total cholesterol (mg/dL) of hypercholesterolaemic-induced rats**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Groups** | **Baseline** | **End-line** | **MD** | **T** | **%D** |
| *P*. *crispum*500 | 162.00±3.34 | 99.80 ± 5.40 | 62.20 ± 0.16 | 97.14\*\* | 38.40  |
| *B*. *pilosa500* | 150.80±4.54 | 82.68±4.68 | 68.12 ± 0.22 | 51.95\*\* | 45.17 |
| *L. taraxa*500 | 130.40±2.48 | 76.80 ±4.30 | 53.60 ± 0.29 | 45.78\*\* | 41.10 |
| *V. doniana*500 | 127.50±9.71 | 78.40±6.43 | 49.10 ± 0.54 | 22.41\*\* | 38.51 |
| *P*. *crispum1000* | 136.40±6.25 | 80.50±4.23 | 55.90 ± 0.67 | 20.34\*\* | 40.98 |
| *B*. *pilosa1000* | 157.60±8.82 | 97.60 ±5.30 | 60.00 ± 0.68 | 21.66\*\* | 38.07 |
| *L. taraxa*1000 | 144.60±6.35 | 102.20±8.68 | 42.40 ± 0.57 | 18.23\*\* | 29.32 |
| *V. doniana*1000 | 133.80±16.74 | 77.30± 6.40 | 56.50 ± 0.35 | 39.95\*\* | 42.23 |
| UN | 133.90±22.09 | 154.60±6.40 | -20.70 ± 0.22 | -23.38\*\* | 15.46 |
| HU | 160.20±2.39 | 201.20±7.30 | -41.00 ± 0.59 | -17.05\*\* | 25.59 |
| HT  | 140.60±7.94 | 79.38± 2.60 | 61.22 ± 0.38 | 39.77\*\* | 43.54 |

MD = mean difference; t = t-test value; %D = percentage difference; \* = (P < 0.05); \*\* = (P < 0.01); baseline = after induction; end-line = after treatment; increase = ; decrease = ; *L*. *taraxacifolia*; UN = untreated non-hypercholesterolaemic; HU=hypercholesterolaemic untreated; HT = hypercholesterolaemic treated with standard drug.

The triglyceride (TG) levels of all the hypercholesterolaemic rats in Table 2, showed significant decreases in triglyceride levels which compared well with the standard drug, as against a significant increase in TG level in the hypercholesterolaemic-induced untreated rats. More so, P. crispum and L taraxacifolia both at 500 mg/kg bodyweight were better than the standard drug (astrovastatin). This result is consistent with the works of Nnodim, Emejulu and Nwadike (2011), Okwari et al. (2013), Zetina-Esquivel (2015) and Martial et al. (2021) following the administration of aqueous leaf extracts of *Acalypha capitata*, *Moringa oleifera*, *Carica papaya* and *Clerodendrumthomsoniae*, respectivelyin hypercholesterolaemic rats. The significant decrease in TG level of the test group treated with the leaf extract, as against the increase in the hypercholesterolaemic untreated rat group signifies the triglyceride lowering effect of the test samples. At 500 mg/kg body weight, *L*. *taraxacifolia* showed the highest TG reducing effect, followed by *P*. *crispum* at the same dose.

**Table 2: Effect of the aqueous extracts of the leaves on the triglycerides (mg/dL) of hypercholesterolaemic-induced rats**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Groups** | **Baseline** | **End-line** | **MD** | **T** | **%D** |
| *P*. *crispum*500 | 124.20 ± 2.18 | 72.25± 87.80 | 51.95 ± 0.19 | 66.48\*\* | 41.83  |
| *B*. *pilosa500* | 150.40 ± 15.40 | 125.40±5.60 | 25.00 ± 0.27 | 22.61\*\* | 16.62 |
| *L. taraxa*500 | 153.30±2.44 | 80.90±3.70 | 72.40 ± 0.29 | 61.98\*\* | 47.23 |
| *V. doniana*500 | 136.30±2.20 | 90.38±6.50 | 45.92 ± 0.26 | 43.19\*\* | 33.69 |
| *P*. *crispum1000* | 167.60 ± 3.85 | 101.30±4.78 | 66.37 ± 0.28 | 59.09\*\* | 39.60 |
| *B*. *pilosa1000* | 164.08 ± 30.45 | 119.78±2.80 | 44.30 ± 0.11 | 98.89\*\* | 27.00 |
| *L. taraxa*1000 | 138.45±19.20 | 102.60±4.60 | 35.85 ± 0.18 | 47.76\*\* | 25.89 |
| *V. doniana*1000 | 132.40±12.0 | 96.40± 7.56 | 36.00 ± 0.30 | 29.53\*\* | 27.19 |
| UN | 80.60±4.18 | 80.56±5.25 | 0.04 ± 0.27 | 0.37 | 0.05 |
| HU | 131.40±10.31 | 201.30±5.60 | -69.91 ± 0.23 | -73.25\*\* | 53.20 |
| HT  | 161.04±2.02 | 120.40±10.02 | 40.64 ± 0.30 | 32.64\*\* | 25.24 |

MD = mean difference; t = t-test value; %D = percentage difference; \* = (P < 0.05); \*\* = (P < 0.01); baseline = after induction; end-line = after treatment; increase = ; decrease = ; *L*. *taraxacifolia*; UN = untreated non-hypercholesterolaemic; HU=hypercholesterolaemic untreated; HT = hypercholesterolaemic treated with standard drug.

The result in Table 3 showed that after treatment with the aqueous leaf extracts, the LDL levels of all the hypercholesterolaemic ratssignificantly decreased, contrary to the control (hypercholesterolaemic untreated) rat group which had significant increase in LDL-cholesterol. This result is consistent with that reported by several studies (Dhandapani, 2007; Okwari et al., 2013; Martial et al., 2021 and El-Bakry, Ibrahim & Mohaseb, 2020) following the administration of aqueous leaf extracts of *Ecliptaprostrata*, *Moringa oleifera*, *Clerodendrumthomsoniae*and green tea extract, respectively in hypercholesterolaemic-induced rats. In Table 3, it was also observed that the hypercholesterolaemic rat groups fed the leaf extracts had higher ameliorative effect on LDL levels than the standard drug. Thus, the significant reductions in LDL-cholesterol on treatment with the leaf extracts suggests that the leaf extracts reduced the hepatic triglyceride and favoured the redistribution of cholesterol among the lipoprotein molecules.

**Table 3: Effect of the aqueous extracts of the leaves on the low-density lipoprotein (mg/dL) of hypercholesterolaemic-induced rats**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Groups** | **Baseline** | **End-line** | **MD** | **T** | **%D** |
| *P*. *crispum*500 | 88.78±4.30 | 20.68± 4.25 | 68.10 ±0.08 | 206.58\*\* | 76.71  |
| *B*. *pilosa500* | 93.60±8.65 | 22.80±2.22 | 70.80 ± 0.16 | 104.92\*\* | 75.64 |
| *L. taraxa*500 | 96.45±4.35 | 26.40±7.86 | 70.05 ± 0.53 | 321.84\*\* | 72.63 |
| *V. doniana*500 | 89.78±4.55 | 20.90±4.52 | 68.88 ± 0.12 | 146.63\*\* | 76.72 |
| *P*. *crispum1000* | 95.60±7.36 | 16.70±3.25 | 78.90 ± 0.10 | 198.49\*\* | 82.53 |
| *B*. *pilosa1000* | 97.80±4.26 | 18.76±5.60 | 79.04 ± 0.21 | 917.58\*\* | 80.82 |
| *L. taraxa*1000 | 95.70±5.78 | 26.78±4.31 | 68.92 ± 0.15 | 114.13\*\* | 72.02 |
| *V. doniana*1000 | 90.85±3.68 | 19.20± 6.89 | 71.65 ± 0.16 | 109.69\*\* | 78.87 |
| UN | 23.40±3.80 | 24.00±2.40 | -0.60 ± 0.18 | -8.34 | 2.56 |
| HU | 92.34±4.40 | 102.80±3.60 | -10.46 ± 0.09 | -260.42\*\* | 11.33 |
| HT  | 78.80±2.50 | 30.60±5.50 | 48.20 ± 0.51 | 229.42\*\* | 61.17 |

MD = mean difference; t = t-test value; %D = percentage difference; \* = (P < 0.05); \*\* = (P < 0.01); baseline = after induction; end-line = after treatment; increase = ; decrease = ; *L*. *taraxacifolia*; UN = untreated non-hypercholesterolaemic; HU=hypercholesterolaemic untreated; HT = hypercholesterolaemic treated with standard drug.

The HDL levels of the hypercholesterolaemic rat groups increased after treatment with the aqueous leaf extracts against the decrease in HDL in the hypercholesterolaemic untreated control groups as shown in Table 4. The rat groups fed 1000mg/kg body weight of the aqueous leaf extract, had higher increase in HDL levels more than the standard drug. A similar observation was reported by Nnodim, Emejulu and Nwadike (2011), Kolawole et al. (2012), Okwari et al. (2013), Zetina-Esquive (2015) and Martial et al. (2021), where the administration of aqueous leaf extracts of *Acalyphacapitata*, *Perseaamericana*, *Moringa oleifera*, *Carica papaya* and *Clerodendrumthomsoniae*, respectively showed a significant increase in the HDL level of all hypercholesterolaemic rats on a dose-dependent rate. The significant increase observed in HDL levels across the experimental/treated groups in Table 4 could be as a result of the reduction in LDL cholesterol level due to the presence of anti-oxidant and anti-dyslipidaemic compounds such as flavonoids, saponins, alkaloids, tannins and steroids (Yepshak et al, 2024). Beneficially, HDL have the role of removing cholesterol from tissues (they bring cholesterol from peripheral tissues to the liver), and they have anti-atherosclerosis properties which includes inhibition of endothelial adhesion molecule expression and LDL oxidation, as well as promotion of reverse cholesterol transport (Sanossian et al., 2007).

**Table 4: Effect of the aqueous extracts of the leaves on the high-density lipoprotein (mg/dL) of hypercholesterolaemic-induced rats**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Groups** | **Baseline** | **End-line** | **MD** | **T** | **%D** |
| *P*. *crispum*500 | 40.4±3.00 | 60.33± 5.80 | -19.93 ±0.49 | -98.67\*\* | 49.33  |
| *B*. *pilosa500* | 37.50±4.70 | 50.68±3.33 | -13.18 ± 0.14 | -228.06\*\* | 35.15 |
| *L. taraxa*500 | 51.82±5.62 | 58.80±6.79 | -6.98 ± 0.16 | -109.19\*\* | 13.47 |
| *V. doniana*500 | 43.40±2.78 | 58.90±4.50 | -15.50 ± 0.06 | -591.51\*\* | 35.71 |
| *P*. *crispum1000* | 39.32±5.69 | 52.56±4.32 | -13.24 ± 0.42 | -76.64\*\* | 33.67 |
| *B*. *pilosa1000* | 37.90±3.80 | 56.70±5.60 | -18.80 ± 0.12 | -389.75\*\* | 49.60 |
| *L. taraxa*1000 | 36.68±3.81 | 65.78±7.00 | -29.20 ± 0.30 | -239.97\*\* | 79.61 |
| *V. doniana*1000 | 40.30±4.10 | 60.45± 3.80 | -20.17 ± 0.43 | -115.35\*\* | 50.05 |
| UN | 41.54±4.60 | 41.68±4.80 | -0.14 ± 0.17 | -2.04 | 0.34 |
| HU | 40.35±8.50 | 34.80±6.20 | 5.55 ± 0.12 | 109.41\*\* | 13.75 |
| HT  | 40.68±2.80 | 58.90±3.60 | -18.22 ± 0.15 | -294.02\*\* | 44.79 |

MD = mean difference; t = t-test value; %D = percentage difference; \* = (P < 0.05); \*\* = (P < 0.01); baseline = after induction; end-line = after treatment; increase = ; decrease = ; *L*. *taraxacifolia*; UN = untreated non-hypercholesterolaemic; HU=hypercholesterolaemic untreated; HT = hypercholesterolaemic treated with standard drug.

**4. Conclusion**

The study showed that aqueous leaf extracts of *P. crispum*, *B. pilosa*, *L. taraxacifolia* and *V. doniana* offer benefits such as ameliorating hypercholesterolaemia on a dose-dependent rate. The use of aqueous leaf extracts as a therapeutic agent in the treatment of hypercholesterolaemia was observed to produce improved hypoglycaemic, anti-dyslipidaemic, effects. Generally, aqueous leaf extract of *B. pilosa* and *L. taraxacifolia* showed greater ameliorative effects on lipid profile of the hypercholesterolaemic induced rats. The ameliorative effects of the extracts compared favorably with the standard drug in most of the parameters at a dose-dependent rate.

**5. Recommendations**

Further investigation in human subject is recommended to confirm the observed results. Further studies should also be carried out to investigate the ameliorative effect of the use of *P. crispum*, *B. pilosa*, *L. taraxacifolia* and *V. doniana*leaf extracts on haematological parameters of induced hypercholesterolaemic rats.Food industries should be encouraged to incorporate *P. crispum*, *B. pilosa*, *L. taraxacifolia* and *V. doniana*leaves in food products in the right proportion in a bid to diversify diets and promote its therapeutic function on hypercholesterolaemic conditions.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE**)

 Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

**AUTHORS’ CONTRIBUTIONS**

Nanyen B. Yepshak designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Godiya M. Saidu managed the analyses of the study. Afiniki E. Dapas managed the literature searches. All authors read and approved the final manuscript.

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