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Original Research Article

The Effect of Onions, Carrot and Garlic on Total Cholesterol Levels in Wistar Rats

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ABSRTACT

Vegetables are a primary source of fibre and antioxidants, contributing to their preventive benefits against cardiovascular illnesses. This study examined the impact of onions, carrots, and garlic on total blood cholesterol levels in adult Wistar rats. Thirty (30) Wistar rats, weighing between 43.6 g and 54.2 g, were randomly allocated into six (6) groups of five (5) rats each: one control group and five test groups. The control group had only plain water and rat chow (grower's mash). Test groups labelled A, B, C, D, and E were administered plain water and formulated mash incorporating 10% (w/w) ground onions, 10% (w/w) ground carrots, 10% (w/w) ground garlic, 5% (w/w) ground onions and garlic, and 5% (w/w) ground onions and carrots, respectively, over a three-week experimental duration. Blood samples were obtained for total cholesterol assessment, and statistical analysis was conducted using one-way ANOVA to identify significant differences. Total blood cholesterol levels in mmol/L ranged from 3.0 to 7.3 in the control group and 0.3 to 0.7 in the carrot group. The total blood cholesterol level variations among the rats were statistically significant, $p < 0.05$. The findings indicate that onions, carrots, and garlic exhibit hypocholesterolemic properties; however, caution is advised in their intake to mitigate potential harmful effects linked to excessive consumption.

Keywords: Carrot, Onions, Garlic, Cholesterol, Antioxidant, carotenoids.

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Introduction

Africa, particularly Nigeria, possesses a significant biodiversity of native green leafy vegetables.[1] These plants possess significant nutritional, economic, and medical (nutraceutical) benefits that remain unexamined. A substantial segment of the global population resorts to traditional or folk medicine due to the restricted availability, high cost, and unpleasant side effects of contemporary medication.[2] Carrot (*Daucus carota)*, onions (*Allium cepa)*, and garlic (*Allium sativum)* are consumed for their nutritional and health benefits. Although the health functionality of garlic has been extensively reported, very little is known about the specific benefits of onions.[3,4] However, modern studies have shown that the pharmacologically active constituents of *A. cepa* display broad-spectrum activities, which include antioxidant, anti-cancer,[5,6] anti-scar, hepatoprotective, antiplatelet, antithrombotic, immunoprotective, anti-inflammatory,[7] neuroprotective, antibacterial, and antifungal properties.

Also, carotenoids widely distributed in carrots are potent antioxidants that can neutralise the effects of free radicals. Flavonoids and phenolic derivatives present in carrot roots exert anti-carcinogenic activity, reduce inflammation, and modulate the immune response.[8]

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The bioactive constituents of carrots concentrated primarily on the external side of the root (cortex), together with its vitamins, bioactive components, and trace elements, make it one of the top ten vegetables and fruits on a nutritional basis.[9]
Likewise, garlic has been used as a vegetable and medicine in many cultures for thousands of years, dating back to when the Giza pyramids were built. Its therapeutic effects are due to the impressive activity of its bioactive compounds, such as organic sulphides, saponins,[10] phenolics, and polysaccharides.[11] *In vitro* and *in vivo* studies have shown that garlic compounds can modulate various signalling pathways.[12]
Although the nutritional and therapeutic significance of carrots, onions, and garlic have been examined, less scientific research has been conducted on the efficacy of these supplements or foods in reducing total blood cholesterol levels. This research study examined the efficacy of onions, carrots, and garlic in reducing total blood cholesterol levels.

Materials and methods

Collection of plant materials (onions, carrots and garlic)
Onions, carrots, and garlic were bought fresh from a local market in Ekpoma (located on a latitude of 6.743 and a longitude of 6.14029), Edo State, in March 2023. They were dried in the oven, milled, and kept in dry containers. After this, they were used to prepare the diet for the experiment.

Location of investigation
The study was conducted in the animal house of the Biochemistry Department of Ambrose Alli University, Ekpoma, Nigeria. The investigation followed the procedures described by the National Institute of Health Guidelines (USA) for the Care and Use of Laboratory Animals published by [13].

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Equipments and Reagents

Beakers, measuring cylinders, conical flasks, test tubes, racks, micropipette, spatula, Petri dishes, cuvette, poultry cages, syringes with needles, feeding and water troughs, UV-Vis spectrophotometer (Spectrum Lab TM 21A, England), weighing balance (Havard Trip, OHAU, Flaram, NJ 07932, USA), centrifuge (Hettich Universal, D7200, Tutteigen), acetone (Sigma Aldrich), and total cholesterol assay kit (Randox Laboratories Ltd, UK),.

Animals

Thirty (30) adult male Wistar rats weighing between 43.6 g and 54.2 g were bought from the animal house in the Anatomy Department, College of Medicine, Ambrose Alli University, Ekpoma, Edo State. They were randomly divided into six (6) groups based on their weight and designated control, A, B, C, D, and E representing control, onion, carrot, garlic, onion-carrot, and garlic-onion groups, respectively, and kept in well-ventilated cages disinfected using a local disinfectant (Izal). They were all allowed to acclimatise to rat chow (grower's mash) for one week before test feeding with prepared mash using dried and milled onions, carrots, and garlic, except for the control group. Tables 1 – 5 show the composition of prepared rat mash for the various groups.

Table 1: Composition of rat's mash for group A (Onion group)

Components	Quantity (g)	Percent content
Corn meal	624	62.4
Fish meal	76	7.6
Palm oil	100	10
Non-nutritive cellulose	80	8
Vitamin and mineral premix	21	2
Onion meal	100	10

Table 2: Composition of rat's mash for group B (Carrot group)

Components	Quantity (g)	Percent content
Corn meal	624	62.4
Fish meal	76	7.6
Palm oil	100	10
Non-nutritive cellulose	80	8
Vitamin and mineral premix	21	2
Carrot meal	100	10

Table 3: Composition of rat's mash for group C (Garlic group)

Components	Quantity (g)	Percent content
Corn meal	624	62.4
Fish meal	76	7.6
Palm oil	100	10
Non-nutritive cellulose	80	8
Vitamin and mineral premix	21	2
Garlic meal	100	10

Collection of serum samples for analysis

Following a three-week study, 5 mL of blood samples were obtained from the rats' earlobes, placed in centrifuge tubes, and spun at 1500 rpm for 10 minutes. Subsequently, serum was taken for analysis.

Determination of β-carotene

A previously reported method was used in this determination.[14] The extraction of *β*-carotene in the samples (onions, carrots, and garlic) was done with some modifications. The *β*-carotene was extracted by soaking 0.5 g of samples (onions, carrots, and garlic) in separate glasses of acetone at room temperature under dark conditions. The mixtures were magnetically stirred for 30 minutes. The extracts were centrifuged to separate the supernatants; these operations were repeated until the pulp was completely colourless. The volume of each was made up to 50 mL with acetone. The absorbance of the extracts was measured using an ultraviolet-visible spectrophotometer at 453 nm. *β*-carotene content in the samples was calculated using a calibration curve.

Table 4: Composition of rat's mash for group D (Onion and Carrot group)

Components	Quantity (g)	Percent content
Corn meal	624	62.4
Fish meal	76	7.6
Palm oil	100	10
Non-nutritive cellulose	80	8
Vitamin and mineral premix	21	2
Onion	50	5
Carrot	50	5

Table 5: Composition of rat's mash for group E (Onion and Garlic group)

Components	Quantity (g)	Percent content
Corn meal	624	62.4
Fish meal	76	7.6
Palm oil	100	10
Non-nutritive cellulose	80	8
Vitamin and mineral premix	21	2
Onion	50	5
Garlic	50	5

Total cholesterol analysis

The serum total cholesterol concentration was determined using the Randox (R) total cholesterol assay kit, which contained assay reagents, blanks, and cholesterol standards. Three test tubes were prepared and labelled sample, standard, and reagent blank. 10 µL of serum, standard cholesterol, and 10 mL of deionised water were pipetted into the respectively labelled test tubes, followed by adding 1000 µL of cholesterol reagent to all the test tubes. The test tubes were incubated for 15 minutes at 37℃, after which the absorbance was read with a UV-Vis spectrophotometer at 500 nm against blank. Total cholesterol was recorded as mmol/l.

Statistical examination

All measures were conducted in triplicate, and the results of each group were presented as mean ± standard deviation. Statistical analyses were performed using the one-way ANOVA feature of the SAS (version 9.1) software program, released in 2001, to compare the total cholesterol levels in the blood of the rats. A significance level of $p < 0.05$ was employed to ascertain differences among the rats.

Result and discussion

The β-carotene levels in onions, carrots, and garlic are presented in Table VI. Carrots contained 65.7 µg of β-carotene per 100 g, but onions and garlic had values of 8.6 µg and 3.4 µg per 100 g, respectively. Significant differences (p<0.05) were observed in the β-carotene concentration of the spices. The β-carotene concentration was reported as 123.0 mg/100 g on a dry basis by [15]; however, another study found a lower content value of 115.42 mg/100 g in carrot powder[16], and a higher value of 254.0 mg/100 g on a dry basis was reported by.[17] β-carotene safeguards cells from free radicals, which are unstable molecules that cause oxidative stress associated with chronic diseases such as cancer, cardiovascular and Alzheimer's diseases. It is metabolised into vitamin A in the body, which promotes optimal vision, skin health, immune function, and cognitive performance. The study also evaluated the average weight of the organs, as shown in Table 7, which presents the mean weight of different organs from experimental rats. The weight in grams of the kidney, heart, liver, spleen, lungs, and testes in the control group was as stated (Table 7). Comparing the weights of the organs in the various groups, there was no significant difference (p>0.05). The weight values in grams of the gastrointestinal tracts were 26.7, 19.6, 14.2, 16.0, 17.4, and 21.9 in the control, A, B, C, D, and E groups, respectively, demonstrating significant differences (p<0.05).

Table 6*:* β-carotene content of onions, garlic and carrot (µg/100g)

Spices	β-carotene content
Garlic	3.4^c
Onions	8.6^b
Carrot	65.7^a

Different superscripts on the same column indicate a significant difference (p<0.05).

Table 7: Mean weights (g) of various organs

Organ	Control	A	B	C	D	E
Kidney	0.8^a	0.9^a	0.8^a	0.9^a	1.0^a	0.8^a
Heart	0.6^a	0.6^a	0.5^a	0.5^a	0.4^a	0.4^a
Liver	6.6^a	5.8^a	5.6^a	4.8^a	5.1^a	4.7^a
Spleen	0.8^a	0.7^a	0.2^a	0.6^a	0.6^a	0.5^a
Lungs	1.5^a	0.9^a	1.0^a	1.2^a	1.6^a	1.5^a
GIT	26.7^a	19.6^b	14.2^b	16.0^b	17.4^b	21.9^a
Testes	2.9^a	3.5^a	2.7^a	2.5^a	2.3^a	2.9^a

Different letters within the same row indicate significant differences (p<0.05)

The results of the total cholesterol levels in the blood of the experimental animals are presented in Table 8. The table shows the total cholesterol levels in the blood of rats across different groups. Rats in the control group fed on plain water and rat chow (grower's mash) only exhibited the highest cholesterol levels of 3.0 - 7.3 mmol/L. Rats in group B (carrot group) had the lowest values of 0.3 - 0.7 mmol/L. Cholesterol is a waxy, fat-like substance found in the body. It is needed to make hormones, vitamin D, and other substances that aid food digestion. However, a high amount of cholesterol or low-density lipoprotein in the blood, a condition known as hypercholesterolemia, is a potential risk factor for cardiovascular diseases,[18] such as coronary artery disease, angina, aortic disease, and stroke. Different research has proven that these disease conditions have increased over the past twenty years. Recently, many cholesterol-lowering drugs have shown promising results, but inconvenient dosing schedules, high cost and side effects limit their use.[19] Due to these reasons, additional study approaches are surfacing. One is the use of dietary supplements due to their phytochemicals, which have been reported to possess various biological activities, including hypercholesterolaemia. As a result, researchers worldwide have gained substantial interest in garlic due to its effect on lipid levels.[20] Blood total cholesterol levels of 1.9 - 2.1

mmol/L (Table 8) obtained for rats in group C (garlic group) were lower than those in the control group. Thus, these results agree with findings from other researchers that have demonstrated that garlic and its extracts can lower the level of total cholesterol, low-density lipoprotein, and triglycerides in humans and rodents.[21] Similarly, it has been revealed that the level of plasma total cholesterol and low-density lipoprotein can be decreased by the addition of 8% raw garlic to the rat diet. [22] It has also been reported that serum total cholesterol and low-density lipoprotein can be reduced in humans and rodents with a combination of lemon juice and garlic. [23] Garlic possesses the potential for decreasing the absorption and synthesis of cholesterol and fatty acids, thus reducing cholesterol levels.[24] In a similar vein, various randomised controlled trials have investigated the benefits of a diet rich in onions on blood lipid levels.[25] Results of 1.2 - 2.2 mmol/L revealed that rats in group A (onions group) also had lower blood total cholesterol levels when compared to the control group, corroborating other scientific findings. It was reported that onion extracts have been proven to stimulate the removal of bile acids from the body while at the same time inhibiting cholesterol absorption, thus reducing plasma cholesterol levels.[26] Onions can activate the enzyme lecithin-cholesterol acyltransferase, facilitating the conversion of LDL cholesterol to HDL cholesterol. Likewise, recent studies have shown that carrot consumption moderately lowers cholesterol. The study result showed that rats in the control group had higher total cholesterol levels than rats in group B (carrot group), which agrees with other scientific findings. A significant 11% reduction of cholesterolemia has been observed in human subjects on consumption of carrots by [27], whereas an absence of effect was reported.[28] In this study, carrot usage in preparing rat's mash had a lowering effect on total cholesterol levels. Carrots may also lower cardiac risk, boost the body's immune system, help control diabetes, and maintain eye vision. Rats in group D (onion-carrot group) had cholesterol values of 1.5 -2.4 mmol/L in group E (garlic-onion group), indicating reduced cholesterol levels.

One limitation of this study is that the nutritional and beneficial components of garlic, onions, and carrots that are important for lowering serum cholesterol levels can vary with seasonal and environmental factors, which were not considered in this study. Future research will investigate how these factors impact nutrient and beneficial component levels to understand better their potential in managing hypercholesterolaemia.

Table 8: Total cholesterol level (mmol/L)

S/N of rats	Control	A	B	C	D	E
1	7.3^{a}	1.3^{c}	0.7^{d}	1.9^{b}	2.0^{b}	1.5^{c}
2	6.4^{a}	1.2^{c}	0.3^{d}	2.1^{b}	2.0^{b}	1.6^{c}
3	3.4^{a}	1.4^{c}	0.5^{d}	2.0^{b}	1.6^{c}	1.5^{c}
4	3.0^{a}	1.5^{c}	0.3^{d}	1.9^{c}	2.4^{b}	1.5^{c}
5	3.1^{a}	2.2^{b}	0.6^{d}	2.1^{b}	1.6^{c}	1.6^{c}
Mean	4.6	1.5	0.5	2.0	1.9	1.5
± SD	± 1.8	± 0.4	± 0.2	± 0.09	± 0.3	± 0.04

Result of total cholesterol levels of rats alongside Mean ± SD values of the groups. Different letters within the same row indicate significant differences ($p<0.05$)

Conclusion

This study demonstrates that three spices (carrots, onions, and garlic) can reduce total blood cholesterol levels. Consequently, it is proposed that these spices could address the prevalent hypercholesterolaemia problem in humans and animals, particularly in resource-poor settings, as consuming and incorporating them into the diet could offer a cost-effective and sustainable solution to reducing high blood total cholesterol levels. Further studies should, however, be conducted to ascertain the daily quantity necessary for dietary inclusion.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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