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INCLUSION OF SELECTED SPICES IN WISTAR RATS DIETS: EFFECTS ON THEIR HEART FUNCTIONS

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ABSTRACT: *The effects of extracts of Aframomum danielli, rough skin plum and country onion on the heart functions of albino wistar rats were evaluated. The rats' diets were substituted with these spices at 5%, 10% and 15% inclusions, respectively; feeding duration was 28 days. At intervals of 7 days, their blood was taken and analysed for triglycerides, total serum cholesterol, low density lipoproteins and high density lipoprotein cholesterol to ascertain the heart functions of the wistar rats. The values obtained from these tests showed that the triglyceride, total serum cholesterol and low density cholesterol in the blood of test animals reduced from 180.4 mg/dl to 163.87 mg/100g, 136.93 mg/dl to 130.00 mg/dl, and 77.92 mg/dl to 75.74 mg/dl, respectively, while the high density lipoproteins cholesterol in the blood of the test animals increased from 24.10 mg/dl to 28.73 mg/dl. Despite the increase in the HDL in the blood of the test animals, all the mean concentrations of high density lipoprotein (HDL) obtained in this study were found to be very low as MedlinePlus (2007) reported that 60 mg/dl or above HDL helps to protect the heart against diseases. Hence, constant use of these spices is recommended to help boost the HDL level and help curb any possible means of heart disease.*

KEYWORDS: Spices, wistar rats, heart functions, lipoproteins, cholesterol, triglycerides.

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INTRODUCTION

Spices such as *Aframomum danielli*, rough skin plum, and country onion are essential culinary components that are used in all cultures on a global scale. Nigeria's populace, both rural and urban, has turned to natural dietary ingredients because they are widely accessible, safe, and, more importantly, because of the high incidence of ailments and the myriad adverse effects of synthetic medications. A wide range of naturally occurring materials, including leaves, roots, bark, berries, rhizomes, buds, seeds, and the stigma of a plant or flower used in cooking, are referred to as spices and herbs. Their use is mostly linked to improving the flavour of dishes, such as meats, sauces, vegetables, and desserts (Tapsell et al., 2006). Preservatives, medications, and enhancing the organoleptic qualities of food have all made extensive use of spices. The nutritional value of these dietary plants has hitherto been considered insignificant, beyond their ability to replace salt. This is likely due to the comparatively tiny, albeit rising, amounts ingested (Carlsenet al., 2011; Pérez-Jiménez et al., 2011). Different works of literature, however, have suggested that throughout the past ten years, there has been a shift in this perspective as more research has been done on their contribution to dietary polyphenols, which are known to have certain characteristics linked to a lower risk of developing chronic non-communicable diseases. Since these meals are typically ingested in small amounts and in combination with other foods, it is yet unknown how beneficial they actually are to health.

Due to the strong antioxidant activity of several spices and their positive benefits on human health, spices have been the subject of substantial research in many different nations (Baselga–Escudero *et al.,* 2017). In addition to fruits and vegetables, spices and herbs can offer us additional sources of natural antioxidant compounds, sulphur-containing compounds, tannins, alkaloids, phenolic diterpenes, vitamins, and other nutrients (Srinivasan, 2014). These substances have various antioxidant properties. Flavonoid, for instance, can scavenge catalytic metal ions, making them inert.

Owing to the dearth of knowledge regarding the health advantages of spices, which have been used for centuries as flavouring agents in numerous traditional recipes, certain Nigerian spices, like *Aframomum danielli*, rough skin plum, and country onion, which are popular in South East Nigeria, need to be studied to determine how they affect the heart functions. The knowledge from this work will elucidate the usefulness of these spices and further confirm their effects on the heart functions of the consumers.

MATERIALS AND METHOD

Materials

The spices (*Aframonum danielli*, rough skin plum and country onion) were purchased from relief market Owerri, Imo State, Nigeria while the adult albino wistar rats of comparable sizes and weights ranging from 150-200 g were purchased from the animal farm of Ceslab Global Service, kilometre 7, Ikot Ekpene road, Umuahia, Abia state, Nigeria.

Preparation of Samples

The test spices purchased were carefully poured on a clean dry plastic container. From this container, they were sorted, cleaned, measured, milled, sieved and packaged in small plastic

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containers and then stored pending usage. The test animals were allowed to acclimatize for 1 week in a wire mesh cage.

Table 1: Formulation of Feed Indicating Percentage of the Spices in the Wistar Rat Diet

Sample	growers mash (%)	A. danielli (%)	Plum %0	Onion (%)
A (Control)	100	-	-	-
B1	95	5	-	-
B2	90	10	-	-
B3	85	15	-	-
C1	95	-	5	-
C2	90	-	10	-
C3	85	-	15	-
D1	95	-	-	5
D2	90	-	-	10
D3	85	-	-	15

The feed formulation process was done with maximum care in order to avoid any form of contamination.

Animal Grouping

The adult albino wistar rats of comparable sizes and weight ranging from 150-200 g were divided into ten (10) groups of six rats each and allowed to acclimatize for one (1) week. They were in a wooden-wire gauze cage during this period of acclimatization; the rats were fed with growers' mash and water was provided ad libitum. The animals were maintained and utilized in accordance with the standard guide for the care and use of laboratory animals. Group A served as the control while B1, B2 and B3 served as the groups fed with feed spiced with 5%, 10% and 15% concentration of *Aframomun danielli* respectively.

C1, C2 and C3 served as groups fed with spiced feed of 5%, 10% and 15% concentration of rough skin plum and D1, D2 and D3 served as groups fed with spiced feed of 5%, 10% and 15% country onion concentrations respectively. The animals were starved for a period of 12 hours before being fed with formulated feed commenced. The respective spices were prepared by mixing with feed at 5%, 10% and 15% respectively. Group A received normal feed and water only. The feeding period lasted for a period of 28 days (4 weeks).

Blood Sample Collection

The blood samples (5 ml) of each rat were obtained by sacrificing the rat at the end of every 7 days for a period of 28 days and dispensed into a plain container labelled appropriately A, B1, B2, B3, C1, C2, C3, D1, D2 and D3. The blood samples were collected and frozen until the time of analysis. Laboratory analysis was carried out for HDL (High density lipoprotein) cholesterol, LDL (Low density lipoprotein), Serum Cholesterol and TG (Triglyceride).

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Determination of Heart Function Test

The HDL determination was done spectrophotometrically as described in the bio-system cholesterol analytical kit (based on the Tietz, 1990 principles) while LDL, serum cholesterol and triglyceride were determined by colorimetric method of the NCEP (2001).

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS version 20. Data obtained were expressed as mean ± standard deviation and subjected to analysis of variance (ANOVA) to test the difference among means. Significant values were placed at $p < 0.05$.

RESULTS

Table 2: Triglyceride (TG) Content of Wistar Rats Fed with Spiced Diet

Samples	Days of Feeding 0	7	14	21	28
A.danielli.					
C1	$180.4^{a}\pm1.91$	$176.10^{b}\pm0.69$	$172.97^{b}\pm0.47$	$171.80^{b}\pm1.04$	$167.93^{b}\pm0.99$
C2	$180.4^{a}\pm1.91$	$175.73^{b}\pm0.61$	$171.87^{c}\pm0.50$	$169.10^{d}\pm1.14$	$164.23^{de}\pm0.40$
C3	$180.4^{a}\pm1.91$	$173.97^{cd}\pm0.29$	$169.90^{d}\pm0.46$	$166.60^{e}\pm1.61$	$163.87^{e}\pm1.10$
Plum					
C1	$177.2^{b}\pm0.69$	$174.69^{b}\pm0.90$	$173.43^{b}\pm0.65$	$169.27^{d}\pm1.33$	$165.17^{c}\pm1.00$
C2	$178.4^{ab}\pm0.35$	$172.73^{e}\pm0.31$	$171.47^{c}\pm0.65$	$167.23^{e}\pm1.02$	$165.07^{c}\pm0.15$
C3	$178.4^{ab}\pm0.35$	$171.10^{f}\pm0.36$	$169.40^{d}\pm0.56$	$165.50^{f}\pm0.87$	$164.67^{c}\pm0.12$
Onion					
C1	$179.77^{ab}\pm0.45$	$175.07^{bc}\pm0.23$	$172.93^{b}\pm0.43$	$170.73^{c}\pm0.42$	$169.20^{b}\pm1.56$
C2	$179.77^{ab}\pm0.45$	$173.4^{de}\pm0.69$	$171.07^{c}\pm0.67$	$168.30^{d}\pm0.17$	$165.93^{c}\pm0.98$
C3	$179.77^{ab}\pm0.40$	$171.73^{ef}\pm0.31$	$168.93^{d}\pm0.60$	$166.33^{ef}\pm0.50$	$165.20^{cd}\pm0.69$
Control	$178.87^{ab}\pm2.35$	$179.36^{a}\pm1.71$	$179.53^{a}\pm1.92$	$178.73^{a}\pm2.72$	$178.67^{a}\pm2.76$
LSD	.71	1.19	1.01	0.98	1.33

Values show mean values of duplicate analysis ± standard deviation. Figures with difficult superscripts in the column are significantly different ($p < 0.05$).

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Table 3: Total Serum Cholesterol of Wistar Rats Fed with Spiced Diet

Samples	Days of Feeding 0	7	14	21	28
A.danielli					
C1	136.30^{a}±2.05	135.07^{b}±0.23	134.20^{b}±0.40	133.57^{b}±0.31	133.13^{b}±0.64
C2	136.30^{a}±2.05	134.93^{b}±1.29	133.90^{b}±0.17	133.07^{b}±0.46	131.37^{d}±0.51
C3	136.30^{a}±2.05	134.03^{c}±0.15	133.50^{c}±0.00	132.23^{cd}±0.35	130.80^{e}±0.20
Plum					
C1	135.90^{a}±0.75	135.43^{a}±0.40	133.23^{c}±0.35	132.53^{c}±0.31	132.00^{c}±0.34
C2	135.97^{a}±0.85	135.00^{b}±0.20	132.83^{d}±0.35	131.23^{e}±0.35	130.13^{e}±0.40
C3	135.97^{a}±0.85	132.27^{d}±0.46	132.27^{e}±0.46	130.67^{e}±0.81	130.00^{e}±0.17
Onion					
C1	136.93^{a}±1.40	135.00^{b}±0.35	134.07^{b}±0.15	133.10^{b}±0.30	132.93^{b}±0.42
C2	136.03^{a}±0.40	135.00^{b}±0.69	133.50^{c}±0.61	132.47^{c}±0.12	131.67^{cd}±0.23
C3	136.03^{a}±0.40	134.00^{c}±0.35	132.27^{e}±0.91	131.81^{d}±0.00	131.40^{d}±0.80
Control	135.53^{a}±0.60	135.53^{a}±0.60	136.23^{a}±0.12	136.43^{a}±0.12	136.50^{a}±0.30
LSD	2.01	0.22	0.36	0.62	0.49

Values show mean values of duplicate analysis ± standard deviation. Figures with difficult superscripts in the column are significantly different ($p < 0.05$).

Table 4: Low Density Lipoprotein (LDL) Content in Wistar Rats Fed with Spiced Diet

Samples	Days of Feeding 0	7	14	21	28
A.danielli					
C1	77.76^{a}±0.72	77.92^{a}±0.62	76.89^{c}±0.03	76.65^{c}±0.08	75.74^{b}±0.08
C2	77.76^{a}±0.72	77.90^{a}±0.35	76.26^{d}±0.08	76.10^{d}±0.02	75.79^{b}±0.13
C3	77.76^{a}±0.72	77.80^{a}±0.01	76.22^{d}±0.04	76.04^{d}±0.08	75.85^{b}±0.13
Plum					
C1	76.98^{a}±0.18	77.12^{c}±0.01	77.19^{abc}±0.08	77.44^{a}±0.10	76.78^{a}±0.50
C2	76.98^{a}±0.18	77.27^{bc}±0.05	77.35^{ab}±0.03	77.50^{a}±0.14	76.65^{a}±0.13
C3	76.98^{a}±0.18	77.23^{c}±0.06	77.31^{abc}±0.03	77.42^{a}±0.13	76.49^{a}±0.08
Onion					
C1	76.93^{a}±0.71	77.04^{c}±0.93	77.56^{a}±0.04	76.99^{b}±0.11	75.98^{b}±0.13
C2	76.93^{a}±0.71	77.78^{ab}±0.64	77.27^{abc}±0.09	77.00^{b}±0.09	75.82^{b}±0.12
C3	76.93^{a}±0.71	76.50^{d}±0.16	77.00^{b}±0.10	76.82^{bc}±0.00	75.79^{b}±0.20
Control	76.79^{a}±0.16	76.84^{cd}±0.21	76.89^{c}±0.11	77.01^{b}±0.15	77.12^{a}±0.08
LSD	1.09	0.52	0.44	0.29	0.70

Values show mean values of triplicate analysis ± standard deviation. Figures with different superscripts in the column are significantly different ($p < 0.05$).

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Table 5: High Density Lipoprotein (HDL) Content in Wistar Rats Fed with Spiced Diet

	Days of Feeding				
Sample	0	7	14	21	28
A.danielli					
C1	$24.10^{f}\pm0.2$	$24.27^{d}\pm0.05$	$24.63^{e}\pm0.03$	$25.17^{g}\pm0.21$	$26.47^{e}\pm0.12$
C2	$24.67^{ef}\pm0.1$	$224.87^{b}\pm0.12$	$25.57^{c}\pm0.06$	$25.80^{f}\pm0.00$	$26.90^{d}\pm0.17$
C3	$24.93^{de}\pm0.23$	$25.27^{b}\pm0.12$	$25.73^{cd}\pm0.12$	$26.03^{e}\pm0.06$	$27.07^{d}\pm0.12$
Plum					
C1	$25.33^{cd}\pm0.10$	$25.10^{b}\pm0.17$	$26.00^{bc}\pm0.20$	$26.83^{d}\pm0.29$	$27.97^{c}\pm0.40$
C2	$26.20^{ab}\pm0.53$	$26.00^{a}\pm0.20$	$26.17^{ab}\pm0.12$	$27.20^{c}\pm0.35$	$28.57^{b}\pm0.06$
C3	$26.53^{a}\pm0.45$	$25.20^{b}\pm0.20$	$26.23^{ab}\pm0.06$	$27.40^{bc}\pm0.20$	$28.73^{a}\pm0.12$
Onion					
C1	$24.50^{ef}\pm0.17$	$24.97^{bc}\pm0.15$	$25.67^{d}\pm0.31$	$27.33^{bc}\pm0.12$	$28.50^{a}\pm0.00$
C2	$25.00^{ab}\pm0.20$	$25.37^{b}\pm0.40$	$26.03^{b}\pm0.06$	$27.53^{ab}\pm0.25$	$28.53^{a}\pm0.12$
C3	$25.77^{bc}\pm0.71$	$25.27^{b}\pm0.12$	$26.33^{a}\pm0.12$	$28.13^{a}\pm0.15$	$28.60^{a}\pm0.17$
Control	$24.49^{ef}\pm0.21$	$24.61^{cd}\pm0.33$	$24.84^{e}\pm0.21$	$25.14^{g}\pm0.41$	$25.58^{f}\pm0.71$
LSD	0.64	0.55	0.29	0.21	0.37

Values below mean values of triplicate analysis ± standard deviation. Figures with different superscripts in the column are significantly different ($p < 0.05$).

DISCUSSIONS

The heart function tests (TG - Triglyceride, HDL - High density lipoprotein cholesterol, LDL - Low density lipoproteins and serum cholesterol test) are groups of blood tests that give information about the state of a patient's heart and these tests were carried out on wistar rats fed with *Aframomum danielli*, rough skin plum and country onion spiced feed in order to ascertain the effects of these spices on the heart. The results obtained on the individual parameters analysed were presented in Tables 2 to 5.

Triglyceride (TG) Content

Table 2 shows the TG contained in the blood of rats fed with feed partially substituted with 5%, 10% and 15% concentrations of *Aframomum danielli*, rough skin plum and country onion spices. The values obtained for rats fed with *Aframomum danielli* spiced diets were in the range of 167.93 mg/dl to 180.4 mg/dl (C1), 164.23 mg/dl to 180.4 mg/dl (C2) and 163.87 mg/dl to 180.4 mg/dl (C3); that obtained from rough skin plum ranged from 165.17 mg/dl to 177.2 mg/dl (C1), 165.07 mg/dl to 178.4 mg/dl (C2) and 164.67 mg/dl to 178.4 mg/dl (C3); while the TG values for country onion ranged from 169.20 mg/dl to 179.77 mg/dl (C1), 165.93 mg/dl to 179.77 mg/dl (C2) and 165.20 mg/dl to 179.77 mg/dl (C3). The triglyceride contents of the control ranged from 178.67 mg/dl to 179.53 mg/dl and were found to be significantly different ($p > 0.05$) from the triglyceride content of wistar rats fed with spiced feed from Week 1 to Week 4.

The TG contained in the blood of rats fed with control feed increased significantly ($p < 0.05$) as the feeding period progressed, while the TG of rats fed with *A. danielli* supplemented diet

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decreased from the initial value of 180.40 mg/dl to 167.93 mg/dl, 164.23 mg/dl and 163.87 mg/dl for C1, C2 and C3 concentrations respectively. Similarly, the TG contained in the blood of wistar rats fed with rough skin plum spice decreased from the initial TG of 177.2mg/dl to 165.17mg/dl (C1) and from 178.4mg/dl of C2 and C3 to 165.07mg/dl and 164.67mg/dl, respectively, the decreases being dose-dependent. The TG values with the country onion supplemented diets were from 179.77mg/dl to 169.20mg/dl, 165.93mg/dl and 165.20mg/dl for the C1, C2 and C3 country onion spice concentrations. Triglyceride content of the control increased from 178.87mg/dl (0 day) to 179.36mg/dl and 179.53mg/dl on day 7 and 14 respectively. It later decreased from the initial 178.87mg/dl (day 0) to 178.73mg/dl and 178.67mg/dl on day 21 and 28 respectively.

The TG values in this study decreased significantly in the wistar rats fed with spiced diets for 28 days and such effect is considered beneficial to man in relation to risk factor for coronary heart disease (Yarnell *et al.*, 2001). Similar decreases had been reported by Kamisiah *et al.* (2005). When consumed calories are greater than the requirement of the body, the liver forms triglycerides from the excess energy and these are stored as fat (El Malik & Sabahelkhier, 2019). Thus, the increase in the TG of the control could be attributed to over feeding of the wistar during the early stage as they were starved at some point.

Total Serum Cholesterol Content

The total serum cholesterol levels in the albino rats treated with the test spices are shown in Table 3. The result shows changes in the blood cholesterol levels during the 28 day feeding period. At the onset of the experiment, the total serum cholesterol were within the range of 135.53 mg/L to 136.93 mg/L and there were no significant differences ($p > 0.05$) between the cholesterol levels of all the test rats and the control. The cholesterol levels changed with feeding time and spice type, all of which showed significant differences ($p < 0.05$) at the end of the feeding period (28 days).

At the lowest concentration (C1), the cholesterol level in the rats' bloods decreased from 136.30 mg/dL to 133.13 mg/dL in the *A. danielli* fed rats, 135.97 mg/dL to 132.00 mg/dL and 136.93 mg/dL to 132.93 mg/dL in the plum and country onion fed rats respectively. These translate to 2.35%, 2.87% and 2.92% decrease values in total serum cholesterol of test rats fed with *A. danielli*, rough skin plum and country onions respectively, indicating a progressive reduction of the total serum cholesterol in the blood of the rats. Also from the results, a slight decrease, but significantly ($p > 0.05$) different, was recorded for all the test spices at different inclusion levels in a more or less dose dependent trend.

At the highest level of inclusion of the spices, (C3), total serum cholesterol percentage decrease values were 4.04% (136.30-130.80 mg/dL), 4.39% (135.97-130.00 mg/dL) and 3.40% (136.03-131.04 mg/dL) for the rats fed with *A. danielli*, rough skin plum and country onion respectively. The feeding experiment therefore suggests that the spices have the ability to keep the blood cholesterol of the rats down by 2.28% to 4.39% within 28 days, depending on the spice and concentration of inclusion. The results of this present study thus suggest that the consumption of foods spiced with these three spices could cause reduction in the risk of cardiovascular disease such as coronary heart disease and stroke.

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Low Density Lipoprotein (LDL) Content

The mean concentration of LDL in the blood of wistar rats fed with diets spiced with *Aframomum danielli* at different concentrations reduced in the range of 77.76 to 75.74 mg/dl (5%), 77.76 to 75.79 mg/dl (10%) and 77.76 to 75.85 mg/dl (15%). Those fed with rough skin plum spiced diets had their LDL concentration reduced in the range of 76.98 to 76.78 to mg/dl (5%), 76.98 to 76.65 mg/dl (10%) and 76.98 to 76.49 mg/dl (15%), while that of country onion reduced from 76.93 to 75.98 mg/dl (5%), 76.93 to 75.82 mg/dl (10%) and 76.93 to 75.79 mg/dl (15%). The LDL concentration in the blood of wistar rats fed the normal feed (control) increased from 76.79 mg/dl (0 day) to 77.12 mg/dl (28 days).

There was no significant difference ($p > 0.05$) in the LDL concentration of rats fed with spiced and control feed on the first day of the feeding experiment, while significant differences ($p < 0.05$) based on spice type and spice concentration existed among all the samples from Day 7 to 28. It was observed that the LDL of rats fed with *Aframomum danielli* spiced diets subsequently reduced from Day 14 throughout the 28 days of feeding experiment, while that of the other spiced diets fluctuated. Furthermore, it was observed that the LDL of all the test rats fed with spiced feeds reduced at Day 28 while the control increased.

The decreases in LDL at the end of the feeding experiment agree with the work of Karaji-Bani *et al.* (2006) but disagree with the work of Mutalib *et al.* (2002). El Malik and Sabahelkhier (2019) stated that if the LDL concentration is high in blood, then it precipitates in the artery (arterial disease) and that is why LDL is known as bad cholesterol. Nevertheless, the mean concentrations of LDL in the bloods of all the test rats were found to be below 100 mg/dl optimal level of LDL-C in blood (Lee & Siddiqui, 2023).

High Density Lipoprotein Content

The mean concentration of HDL in the bloods of wistar rats fed with diets spiced with 5%, 10% and 15% *Aframomum danielli* increased from 24.10 mg/dl (Day 0) to 26.47 mg/dl (Day 28), 24.67 mg/dl (Day 0) to 26.90 mg/dl (Day 28) and 24.93 mg/dl (Day 0) to 27.07 mg/dl (Day 28) respectively. The HDL of test rats fed with rough skin plum spiced diets from Day 0 to 28 also increased from 25.33 mg/dl to 27.97 mg/dl (5%), 26.20 mg/dl to 28.57 mg/dl (10%) and 26.53 mg/dl to 28.73 mg/dl (15%), while that of country onion increased from 24.50 mg/dl to 28.50 mg/dl (5%), 25.00 mg/dl to 28.53 mg/dl (10%) and 25.77 to 28.60 mg/dl (15%).

There were significant differences ($p > 0.05$) in the HDL concentrations in the blood of the test rats based on spice types and concentration. All the mean concentrations of high density lipoprotein (HDL) obtained in this study were found to be low as MedlinePlus (2007) reported that 60 mg/dl or above HDL helps to protect the heart against diseases. They further stated that low HDL (< 40 mg/dl) levels may indicate an increased risk of atherosclerotic heart disease (Medline Plus, 2007). Rats have normal total cholesterol levels with a value of 10-54 mg/dl (Harini & Okid, 2009).

The total cholesterol levels in this study were found to have little increase and could lead to the decrease in the high density lipoprotein. Nurhidajah *et al.* (2019) reported that hypercholesterolemia (high cholesterol) in animals occurs when the total cholesterol level in the blood exceeds the normal range and hypercholesterolemia also causes HDL levels in the blood to decrease. However, the increment in the total cholesterol level has no adverse

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interference with cardio health as it falls within the healthy cholesterol level of 0-200 mg/dl (WHO, 2007).

CONCLUSION

This study showed that test spices (*Aframomum danielli*, rough skin plum and country onion) have hypolipidemic effects as it was observed that the triglyceride, total serum cholesterol and low density lipoproteins cholesterol in the bloods of test animals was reduced. The feeding experiment therefore suggests that the spices have the ability to keep blood cholesterol of the rats down by 2.28% to 4.39% depending on the spice and concentration. It could also be concluded that the consumption of foods spiced with these three spices could cause reduction in the risk of cardiovascular disease such as coronary heart disease and stroke. Due to the low HDL level in the blood of the test animals, this study recommends that these three test spices should be used often in food preparation in order to help increase the HDL in the blood and reduce any risk of atherosclerotic heart disease.

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