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Comparative studies on the effects of *Zinginber officinale* and *Cinnamomum zeylanicum* diets on the lipid profile, body weight, liver and kidney functions of male wistar rats.

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ABSTRACT

The study comparatively evaluated the effects of *Zinginber officinale (ginger)* and *Cinnamomum zeylanicum* (cinnamon) diets on the lipid profile, body weight, liver and kidney functions of male wistar rats. Lipid profile, body weight, liver and kidney function parameters were determined using standard biochemical methods. While for animal study, the male albino rats weighing 120 - 180g were procured from the animal house of Department of Zoology and Environmental Biology, University of Nigeria, Nsukka Campus. The rats were allowed to acclimatize for seven days with free access to normal feed and water before they were randomly distributed into four groups of six rats each as follows: Group A: rats fed on normal feed (control), Group B: rats fed on ginger supplemented diet, Group C: rats fed on cinnamon supplemented diet and Group D: rats fed on mixture of ginger and cinnamon diets. At the end of 30 days feeding experiment, the rats were bled and blood collected was used for biochemical assays, using standard biochemical methods. Results of liver function assays revealed that the ALP,AST and total bilirubin concentrations of rats fed on cinnamon diet (group C) and those fed on ginger diet (group B) were significantly higher than the control. This suggests possible liver toxicity. Result of kidney function assay revealed that BUN and creatinine concentrations were significantly higher than the control. This suggests that cinnamon diet could possibly be toxic to kidney. Results of lipid profile assays showed significant decrease in total cholesterol, triglyceride and LDC – cholesterol levels of rats fed on ginger diet (group B), rats fed on cinnamon diet (group C) and rats fed on mixture of ginger and cinnamon diet (group D) when compared to the control. Furthermore the results of the percentage body weight changes at weekly interval showed that while rats fed on normal feed (group A) were gaining weight at weekly intervals, rats fed on ginger diet (group B), rats fed on cinnamon diet (group C) and rats fed on mixture of ginger and cinnamon diet (group D) experienced weight reduction. However, highest weight reduction was observed in rats fed with cinnamon diet (group C). Finally the result of this research reveals that ginger and cinnamon have antihyperlipidemic effects and could be useful in weight control. Results of this study further suggests that cinnamon diet in combined state with ginger is safer since cinnamon diet alone at high dose taken over a long time could be toxic to both the liver and kidney.

Keywords: *Zinginber officinale*, *Cinnamomum zeylanicum* diets, lipid profile, body weight, liver and kidney.

INTRODUCTION

Recently, due to increasing cases of metabolic diseases, there is a renewed interest in understanding the nutritional and health benefits of spices supplemented in diets, based on the cellular and molecular modes of action of the active chemical components and their biological properties.

A spice could be a seed, fruit, root, bark, or other plant substance primarily used for flavoring, coloring or preserving food. There are many spices and most notable examples are turmeric, ginger, nutmeg, cinnamon, cayenne pepper, black pepper etc. In South-Eastern Nigeria, the use of spices such as ginger and cinnamon is common.

Zingiber officinale, a perennial plant from the family Zingiberaceae commonly known as ginger is one of the commonly used spices around the world. Ginger is reported to possess anti-allergic and anti-nauseant effects [1]. It is also reported to have anti-hepatotoxic effects [2], anti-inflammatory, anti-pyretic, anti-septic and hypoglycemic effects [3], anti-platelet effect [4] and hypolipidemic activities [5]. It has aromatic, stimulant and tonic activities [6]. It has also been reported that taking 1.2 grams of ginger powder before meal accelerates emptying of the stomach by 50%.

Plate 1: Freshly Harvested Ginger Source: Altmas and Marcussan (2001)

Plate 2: Cinnamon Bark (Solid & Grinded Form) Source: Jayaprakasha and Rao (2011)

While *Cinnamomum zeylanicum* (Cinnamon) from family Lauraceae is used in both European and Arabian countries as spice that is used to flavor most food [5]. Cinnamon is used in folk medicine for its anti-diabetic, anti-hyperlipidemic and anti-obesity properties [6], anti-oxidant and hepatoprotective activities [7]. In addition to being used as a spice and flavoring agent, cinnamon is also added to flavour chewing gums due to its mouth refreshing effects and ability to remove bad breath. Cinnamon has also been traditionally used as tooth powder and to treat toothaches, dental problems, and oral microbiota [8]. This work however examined the effects of *Zinginber officinale* and *Cinnamomum zeylanicum* diets on the lipid profile, body weight, liver and kidney functions of male wistar rats.

Aim of the Study

This study is aimed at comparatively evaluating the effects of *Zinginber officinale* and *Cinnamomum zeylanicum* diets on the lipid profile, body weight, liver and kidney functions of male wistar rats.

Specific Objectives

To determine comparatively the effects of ginger and cinnamon supplemented diets on some liver marker enzymes (AST, ALP, ALT) of male wistar albino rats.

To determine comparatively the effects of ginger and cinnamon supplemented diets on the kidney function (Urea and Creatinine) of male wistar albino rats.

To determine comparatively the effects of ginger and cinnamon diets on the lipid profile (Total cholesterol HDL, LDL, TG, VLDL) of male wistar albino rats.

To determine comparatively the effects of ginger and cinnamon supplemented diets on the body weight of the rats.

Significance of the Study

The results from this study will be particularly useful to nutritional biochemists, dieticians, foods scientist, food technologists, and medicinal chemists. Also the study will be very useful to health care practitioners, for providing advisory information to the populace regarding the health benefits of ginger and cinnamon.

Again, the study will be helpful to food regulatory agencies, government and non-governmental health organizations in decision making regarding setting standards which would be vital for local food and nutritional industries. Finally, students, researchers planning to work on this area will find the result very valuable.

MATERIALS AND METHODS

Plant Materials

Ginger and Cinnamon used in this study were procured from Relief market Onitsha, Anambra State, and were identified and authenticated by a botanist.

Animals

Twenty four (24) male Wistar albino rats weighing between 120-180g used for the study. The rats were obtained from the Animal House of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The rats were fed with rat pellets and water *ad libitum* before the commencement of the research.

Normal Rat Feed

The rat feed used in this study is grower's mash of Top feed, produced by Premier Mill company, Calabar, Nigeria.

Equipment

Standard equipment from reputable manufactures were used.

Reagents

All the reagents used in this study were of standard analytical grade.

METHODOLOGY

Ginger powder preparation

Ginger root were washed with water, sliced into small pieces and sun dried. It was then milled into powdered form using electric blender.

Cinnamon powder preparation

Dried cinnamon bark was sliced into small pieces and milled into powdered form using electric blender.

Ginger Diet

This was formulated by mixing homogenously 30% weight by volume (W/V) of ginger powder with 70% of normal rat feed.

Cinnamon Diet

This was formulated by mixing homogenously 30% weight by volume (W/V) of cinnamon powder with 70% of normal rat feed.

Experimental Design

Twenty four (24) mature male albino rats weighing 120-180g were procured from Animal House of the Department of Zoology and Environment Biology, University of Nigeria, Nsukka campus. They were allowed to acclimatize for seven days with free access to normal feed and water. After acclimatization, the rats were randomly distributed into four groups of six rats each in four aluminum cages as follows:

Group	Diet/Treatment
A	Rats fed with normal rat feed
B	Rats fed with ginger supplemented diet
C	Rats fed on cinnamon Supplemented diet.
D	Rats fed with mixture of 15% cinnamon powder, 15% ginger powder and 70% rat feed.

All the protocols as approved by Institutional Animal Ethics Committee (IAEC) were observed in this study. At the end of the 30 days feeding experiment, the rats were bled from the retro-bulbar plexus canthus of the eye. The blood collected was centrifuged at 30,000 revolution per minute for 5 minutes and the serum separated from the cells. Plasma blood sample was collected into an EDTA bottle.

METHODS

Liver Function Markers Assays

Determination of Aspartate Amino Transferase (AST)

Aspartate amino transferase (AST) was determined by the Reitman-Frankel colorimetric method for in-vitro determination of AST in serum or plasma [8] using a Quimica Clinica Applicada (QCA) test kit (Quimica Clinica Applicada, Spain).

Determination of the Serum Alanine Amino Transferase

About 0.5ml of ALT reagent was added to all the test tube and blank 0.1ml distilled water was added to the test blank, they were mixed and incubated for 30 minutes. After the incubation 0.5ml of ALT reagent (2,4-dinitrophenyl-hydrazine) was added to all the test tubes, mixed and incubated for 20 minutes at room temperature.

Determination of Serum Bilirubin

Jendrassik-Grof method for the *in vitro* determination of total bilirubin in serum or plasma [9]; [10], using the Quimica Clinica Applicada (QCA) Bilirubin test kit (QCA, Spain).

Direct conjugated bilirubin

Clean four test tubes were arranged and labelled for total bilirubin and another 4 test tubes were labelled for direct bilirubin. For direct bilirubin, 200ml was added to the both test tubes, a drop of reagent 2 (sodium nitrite) was added to mark the sample. A 200ml of sodium nitrite was added only on the direct bilirubin. A 1000ml of reagent 3(sodium benzoate) and 200mlof the same reagent were added both to sample and blank. All the test tubes were mixed and allowed to rest for 10minutes at room temperature. For direct conjugated bilirubin, the absorbance was read at 540nm

Total bilirubin

A 0.1ml of reagent 4(sodium hydroxide) was added to all the test tube,mixed and allowed to rest for 10minutes at room temperature.

Determination of Alkaline Phosphates(ALP)

Phenolphthalein monophosphate method for the determination of alkaline phosphatase in serum or plasma [11]; [12]; [13], using Quimica Clinica Applicada (QCA) test kit. (QCA, Spain) was used.

Kidney Function Markers Essays

Assay of Blood Urea Nitrogen (BUN)

To clean test tubes, 100µl of reagent 1 were added. Then, 10µl of standard sample were added to the standard test tube and 10µl of the serum sample was added to their respective sample test tubes,mixed and incubated at 37 ^{0}C for 10 minutes in water bath. Reagent 2 and reagent 3 was prepared by diluting the constituent of reagent 2 with 660mlof distilled water and the constituent of reagent 3 with 750ml of distilled water.2.5ml of working reagent 2 and of reagent 3 were added to all the test tubes, mixed and incubated for 15 minutes at 37 ^{0}C in water bath.Absorbance was read at 540nm against blank.

Calculation

$$Urea\ concentration = \frac{Absorbance\ of\ sample \times 13.31}{\text{Absorbance of standard} \times 1}$$

Determination of Serum Creatinine

A working reagent composed of equal volumes of reagent A and reagent B was prepared and mixed. 100µl of standard sample was added to the standard test tube and 100µl of the serum sample was added to their respective sample test tubes, 1ml of the working reagent was added to all the test tubes and mixed. Absorbance was read at 500nm against blank.

Calculation

$$Serum\ Creatinine\ concentration = \frac{change\ in\ absorbance\ of\ sample \times 180\mu mol/l}{\text{change in absorbance of standard} \times 1}$$

Determination of Serum Lipid Profile

Determination of serum total cholesterol (enzymatic colorimetric method)

All the reagents used were in the ready to use form. Six sets of test tubes were labelled blank, standard and those containing the samples. Then 10ul of CAL was pippeted into the standard test tube, 10ul of water into the blank and 10ul of the sample serum was pipetted into the sample test tube labeled A, B,C and D respectively. Then 1ml of the cholesterol reagent was added into all the test tube, mixed and incubated at 37^{0}C for five (5) minutes. The absorbance of the entire test was then read at 500nm wavelength.

Calculations

The cholesterol content of each of the serum sample was calculated using the following formula.

$$\frac{Absorbance\ of\ sample}{\text{Absorbance of standard}} \times \frac{Concentration\ of\ standard}{1}$$

The result is expressed in mg/dl.

Determination of serum triglyceride

The reagents used were in the ready to use form. Six sets of test tubes were labelled blank, standard and those containing the samples. Ten microlitre (10ul) of CAL (cal trigs randox) was pippeted into the standard test tube. For the blank, 10ul of water and 10ul of the serum were pipetted into the Calculations different test tubes. Then 1ml of the triglycerides reagent (randox trigs R1b) was added into all the test tubes including blank and standard, mixed and incubated for five (5) minutes. The spectrophotometer was zeroed and the absorbance of the entire test read at 500nm wavelength.

Triglyceride content of each of the sample was calculated using the following formula.

$$\frac{Absorbance\ of\ sample}{\text{Absorbance of standard}} \times \frac{Concentration\ of\ standard}{1}$$

Determination of serum high density lipoprotein

Dextran sulphate-Mg method of [14] for the in vitro determination of HDL- cholesterol in serum, using Biosystem HDL test kit (Biosystem, S.A. Spain).

A 200µl of the sample and 500µl of Reagent (A) was pipetted into labeled centrifuge test tube. The sample and reagent were thoroughly mixed and left to stand for 10 minutes at room temperature. It was centrifuged at a minimum of 4000 revolution per minute for 10 minutes. The supernatant was carefully collected and Reagent (B) was brought to room temperature, 1000µl of HDL cholesterol working reagent (B) was added to three set of clean test tubes labeled reagent blank,standard and sample respectively. A 50µl of distilled water was added to the test tube labeled reagent blank and mixed well, 50µl of standard was added to the test tube labeled standard and mixed well while 50µl of sample supernatant was added to the test tube labeled sample and mixed well. The mixture was incubated for 10 minutes at 37°C. The absorbance of the sample and standard against the reagent blank at 500nm was read. The HDL cholesterol content of each of the samples using the following formula was calculated.

$$\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times 200mg/dl$$

Determination of Body Weight of Rats

The rats were weighed at weekly interval using digital electronic compact weighing scale obtained from Department of Biochemistry Laboratory, Uli Campus. The weights were recorded in grams.

Statistical Analysis

The data collected from the variables were expressed in mean and standard deviation. The data collected from the variables were subjected to analysis of variance (ANOVA)

and using statistical package for social sciences (SPSS). Also ANOVA was used in comparing the mean differences in variables amongst all the groups involved in this study. The acceptable level of significance is $p < 0.05$.

RESULTS

Liver Function Profile For Different Groups Of Rats Used In The Study

Table 1: Concentrations of liver function parameters for different groups of rats used in the study

Groups	ALT (Iu/L)	ALP(IU/L)	AST(IU/L)	Total Bilirubin (Mg/Dl)
A	20.60 ±2.40	77.60±2.56	11.60±1.25	8.60±1.20
B	21.50±1.50	87.60±3.74*	13.00±1.74*	14.80±2.10*
C	22.50±2.00	118.00±5.86*	14.00±2.10*	16.85±2.50*
D	21.00±1.57	80.46±4,76	12.56±1.86	13.21±1.95

Result in Table 1 revealed that the concentrations of ALP and AST for rats fed on ginger diet (group B) and rats fed on cinnamon diet (group C)were significantly higher ($p<0.05$) than those of the control group. Table 1 also revealed that there were no significant difference ($p>0.05$) observed in the concentration of ALP and AST for rats fed on mixture of ginger and cinnamon (group D) compared to the control (Group A). The results in Table 1 also revealed significant increase in the bilirubin concentrations of rats fed on ginger diet (group B) and rats fed on cinnamon diet (group C) when compared to the control.

Kidney Function Profile

The results of blood urea nitrogen and creatinine for different group of rats used in the study is given in Table 2

Table 2: Blood urea nitrogen (BUN) and creatinine concentrations for different groups of rats used in the study.

Group	BUN (mg/dl)	Creatinine (mg/dl)
A	13.40±0.35	0.95±0.03
B	18.70±0.25*	1.21±0.04
C	22.40±0.55*	1.51±0.05*
D	16.50±0.45*	1.25±0.06

Results in Table 2 revealed that BUN for rats fed with ginger (group B) and rats fed with cinnamon (group C) were significantly higher ($P<0.05$) than that of the control (group A). The result in Table 2 also revealed that creatinine concentration of rats fed on cinnamon diet (group C) was significantly higher than that of the control group.

Lipid Profile

Result of the lipid profile assays for different groups of rats used in the study is shown on Table 3.

Table 3: Lipid profile concentrated of different group of rats used in the study

Group	Total cholesterol	Triglyceride	HDL-Chol.	LDL-Chol.
A	165.55± 1.45	121.8±1.22	27.04±0.05	65.37±0.25
B	150.10±0.85*	98.37±1.38*	26.01±0.26	52.16+ 0.40
C	125.04±0.68*	74.26±0.95*	28.08+ 0.45	48.14±0.50*
D	107.50±0.75*	66.15±0.1.15*	35.20±0.56*	29.20±0.35*

Result in Table 3 revealed significant decrease (P<0.05)in total cholesterol level of rats fed on ginger (group B), rats fed on cinnamon diet (group C) and rats fed on mixture of cinnamon and ginger diet (group D) when compared to that of control (group A).

However, highest decreases in total Cholesterol level, was observed on rats fed on cinnamon diet (group C). Result in Table 3 also revealed significant decrease (P<0.05) in triglyceride and LDL of rats fed on mixture of cinnamon and ginger (group D) when compared to control group.

Also significant increase in HDl-chol of rats fed with mixture of cinnamon and ginger diet were observed in this study (Table 3)

Percentage Body Weight Charge at Weekly Interval

Results in Table 4 indicate the percentage body weight changes at weekly intervals for different group of rats used in the study.

Table 4: Percentage body weight change at weekly interval for different group of rats used in the study

Time on Diet Mean Percentage Increase and Decrease in Body Weight (%) (Weekly)

	Group A	Group B	Group C	Group D
0				
1	+ 11.14± 0.75	-6.07± 0.56	-7.74±0.45	-5.94±0.35
2	+16.98±1.25	-8.03±0.78	-9.94±0.55	-7.71±0.45
3	+34±1.75	-10.20±1.25	-12.14±1.75	-9.25±0.55

NB: Minus (-) sign represent percentage decrease in body weight while plus (+)sign represent percentage increase in body weight.

Result in Table 4 revealed that while rats fed on normal feed (group A) were gaining weight at weekly intervals, rats fed on ginger diet (group B), rats fed on cinnamon diet (group C) and rats fed on mixture of ginger and cinnamon diets (group D) were experiencing weight reduction at weekly intervals. Results in Table 4 also revealed that rats fed on cinnamon diet (Group C) exhibited highest weight reduction at weekly intervals.

DISCUSSION

This study comparatively evaluated the effects of ginger and cinnamon supplemented diets and their effects on some biochemical markers of male wistar albino rats.

The result of liver function profile in Table 1 showed that the concentrations of ALP and AST for rats fed on ginger diet (group B) and rats fed on cinnamon diet (group C) were significantly higher (P<0.05) than those of the control (group A).

Increase in liver enzymes concentration suggests liver toxicity. Results in Table 1 further revealed that there was no significant difference (P > 0.05) observed in the concentrations of ALP and AST for rats fed on mixture of ginger and cinnamon (group D), when compared to the control group. This suggests positive synergistic effect of mixture of ginger and cinnamon. The results of this study therefore suggest that it is safer to use mixture of ginger and cinnamon in a combined form rather than taking any of them alone, especially when taking in large quantity and over a long period of time.

The results of kidney function profile (Table 2) revealed that blood urea nitrogen (BUN) and creatinine concentration of rats fed on cinnamon diet (group C) were significantly higher than that of control. This therefore suggests that high intake of cinnamon diet over a long period of time could be toxic to the kidney. However rats fed with mixture of ginger and cinnamon diet (group D) experience no significant difference (P > 0.05) in their BUN and creatinine concentrations. The result of this study therefore suggests that it is safer to take mixture of ginger and cinnamon diet in a combined form especially when taking over a long period of time.

The results of lipid profile assays for different groups of rats used in the study as

shown in Table 3 revealed that there were significant difference (P<0.05) in total cholesterol level of rats fed on ginger diet (group B), rats fed on cinnamon diet (group C) and rats fed on mixture of cinnamon and ginger (group D) when compared to that of control (group A). This observation is in agreement with [14] who also observed that oral administration of ginger and cinnamon powders at doses of 200mg/kg body weight brought about significant reduction in the total cholesterol of rats.

The results of this study is also in agreement with the works of [15], [16], [17], [18] [19] who also discovered antihyperlipidemic effects of administration of cinnamon and ginger diets to rats. This could be as a result of high phenolic contents possessed by cinnamon and ginger.

Results in Table 3 also showed significant decrease in triglyceride and LDL – cholesterol levels of rats fed on cinnamon (group C) and rats fed on mixture of cinnamon and ginger (group D) when compared to control group. This observation is also in agreement with the works of [20] who observed that combined administration of ginger and cinnamon on high fat diet-induced hyperlipidemia could bring about antihyperlipidemia effects in rats.

Furthermore, the results in Table 3 revealed significant increase in HDL-cholesterol level of rats fed with mixture of ginger and cinnamon diets. This suggests synergetic effects of ginger and cinnamon diets in increasing HDL-cholesterol levels in albino rats. This observation is also in agreement with [21] who also suggested that combined powder of green coffee, cinnamon and ginger had synergetic antihyperlipidemic activity. High density lipoprotein (HDL – cholesterol) is called good cholesterol because of its ability to scavenge and remove LDL – cholesterol from blood vessels and transport them to the liver where it could be reprocessed.

The results of percentage body weight changes at weekly intervals (Table 4) revealed that while rats fed on normal feed (group A) gained weight at weekly intervals, rats fed on ginger diet (group B), rats fed on cinnamon diet (group C) and rats fed on mixture of ginger and cinnamon diets (group D) were experiencing weight reduction at weekly intervals. This observation is in agreement with the work of [22] [23] who also showed that the combination of cinnamon and ginger could be effective as anti-obesity and antihyperlipidemic agent. They suggested that this could be due to the phenolic contents of these plants which are good antioxidants.

However in this study highest reduction in body weight was observed with the rats fed on cinnamon diet (group C), while in the work of [24] [25], highest reduction in body weight was observed in the group fed with mixture of ginger and cinnamon diets.

CONCLUSION

Finally the results of this study reveals that ginger and cinnamon have antihyperlipidemic effects and could be useful in weight control. Results of this study further suggests that cinnamon diet in combined state with ginger is safer since cinnamon diet alone at high dose taken over a long time could be toxic to both the liver and kidney.

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