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Effects of *Afromomum melegueta, Zingiber officinale* and *Piper nigrum* on Some Biochemical and Haematological Parameters in Rats Fed with High Lipid Diet

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ABSTRACT

Overweight and obesity are two conditions which are preventable. Their fundamental causes are energy imbalance between calories consumed and calories expended. In this study, thirty two albino rats of average weight 165±15 g were assigned into eight groups and the effects of three spices; Afromomum melegueta (AM), Zingiber officinale (ZO) and Piper nigrum (PN),on rats fed with High Lipid Diet (HLD) were investigated. Rats fed with HLD without treatment showed significant increase ($p<0.05$) in body weight and levels of Total Cholesterol (CHOL), Low Density Lipoprotein (LDL) and Triglyceride (TG) when compared to the control rats. Whereas, these significant increases were not observed in rats fed with HLD treated with aqueous extracts of AM, ZO and PN at 400 mg/kg b.wt. for 21 days. Extracts also improved the lipid profile of Normal Diet (ND)-fedcompared to the untreated HLD-fed rats. All extracts had no significant effects ($p>0.05$) on T-protein and albumin while a significant decrease was observed in % Packed Cell Volume (PCV) and White Blood Cell (WBC) count of all groups when compared to the control except for HLD-fed rats treated with ZO which showed no significant change in its level of PCV. All extracts improved the Atherogenic index (AI) of treated rats. The study therefore revel that aqueous extracts of AM, ZO and PN can be used in weight management as well as in improvement of lipid profile.

Keywords: *Spices, High Lipid Diet, Lipid profile, Overweight*

INTRODUCTION

Afromomum melegueta (AM), *Piper nigrum* (PN) and *Zingiber officinale* (ZO) are common spices with the later arguably the most studied among them. AM and ZO belong to the family Zingerberaceae while PN belongs to Piperaceae.

Afromomum melegueta, AM also known as alligator pepper, is a North African spice which is used in preparation of pepper soup. In traditional meet and greets, alligator pepper is given to people and new-born in Yoruba culture (Nigeria) as a baby welcoming process[1]. It is also used as flavouring in alcoholic beverages such as beer, ale, wine and gin[2]. Its aqueous extract has been shown to reduce gestational weight in pregnant rats[3]. Its essential oil includeshunulene, caryophyllene, their oxides and non-terpenoids[4].

Zingiber officinale, ZO simply known as ginger is generally safe for consumption[5]. Ginger possesses anticancer, anticlotting, anti-inflammatory, antihypertensive and analgesic activities[6]. Ginger oil has been shown to prevent skin cancer in mice[7].

Piper nigrum, PN is considered king of spices throughout the world due to its pungent principle 'Piperine'[8]. Black pepper is use for different purpose which includes, but not limited to, human dietaries, preservative, bio-control agent, and as human medicine[8,9,10].

The present study is aimed at evaluating the effects of the aqueous extracts of these widely used species (AM, ZO and PN) on some biochemical and haematological parameter in rats fed with high lipid diet.

MATERIAL AND METHODS

Plant material

The plants; *Afromomum melegueta* (Alligator pepper), *Zingiber officinale* (Ginger) and *Piper nigrum* (Black pepper) used for this study were purchased from Terminus market Jos, Plateau state Nigeria, and identified at the Department of Botany, University of Jos, Jos, Nigeria.

Experimental animals

Thirty two albino rats of average weight 165±15 g were obtained from the small animal holding unit, Department of Biochemistry, University of Jos. They were randomly assigned into eight groups, of four rats each, and acclimatized for 7 days. The animals were maintained under standard conditions, had free access to food (Grand Cereal, Oils and Mills Products, Jos, Nigeria) and water *ad libitum*. High Lipid Diet was formulated by weighing a known amount of Margarine (Fat) into the mixture of feed (1:4) and mixing it uniformly.

Experimental Design

A = Normal Control (NC)

B = High Lipid Diet (HLD)

C = Normal Diet + AM (NDAM)

D = High Lipid Diet + AM (HLDAM)

E = Normal Diet + ZO (NDZO)

F = High Lipid Diet + ZO (HLDZO)

G = Normal Diet + PN (NDPN)

H = High Lipid Diet + PN (HLDPN)

Each group consist of four animals, n = 4.

Group A served as control, were fed with normal diet and received 0.5 ml distilled water, orally per day. Group B were fed High Lipid Diet and 0.5 ml of distilled water per day. Groups C, E and G were fed Normal Diet with 0.5 ml oral administration of aqueous extracts of AM, ZO and PN respectively. Groups D, F and H were fed High Lipid Diet with 0.5 ml oral administration of aqueous extracts of AM, ZO and PN respectively. All extracts were at a concentration of 400 mg/kg bwt.for 21 days.

Preparation of extracts

The plant materials were dried under shade and grinded to powdered form using a blender. 150 g of the powdered forms were separately weighed and dissolved in 300 ml of distilled water and boiled for 45 minutes, allowed to cool and then filtered using Whatman Filter paper Grade No. 42. The filtrate was then allowed to dry in a hot air oven (40^{o}C). The extract was stored in an air tight container and was later reconstituted in distilled water to give the required dose of 400 mg kg^{-1}bwt.which was administered.

Sample collection

The rats were anesthetised with diethyl ether at 22nd day, the neck area was quickly cleared of fur and skin to expose the jugular veins. Blood samples were collected from the animals in batches. 2 animals' blood samples were separately collected into a clean, dry tube and allowed to clot for 45 minutes and spun at 3000 rpm for 5 minutes before the serum was collected for biochemical assay (CHOL, High Density Lipoprotein HDL, LDL,TG, Total protein and Albumin). Blood sample from the last 2 were separately collected into an anti-coagulant (EDTA) bottle and were used for Haematological assay (PCV and WBC).

Biochemical parameters

Biochemical parameters assayed include Total cholesterol concentration[11], serum HDL-cholesterol[12] and triacylglyceride[13]. LDL-cholesterol was estimated using Friedewaldformula[14]whileatherogenic index was calculated by finding the ratio of the serum Total cholesterol concentration to serum HDL-cholesterol concentration. Total proteins[15]and albumin[16] were also assayed.

Haematological parameters

Haematological parameters were determined using Mindray Haematology Analyser (Mindray BC-2300, Guangzhou Shihai Medical Equipment Co., Ltd, China).

Statistical analysis

Data were presented as Mean ± Standard Deviation (SD) of 2 replicates and were analyzed using DMRT following one-way analysis of variance (ANOVA) using SPSS 16.0 computer software package (SPSS Inc., Chicago, U.S.A). Differences at $p<0.05$ were considered significant.

RESULTS

Body weight

At the end of the period of administration, significant increase in final body weight to initial body weight of experimental animals was observed in Control, HLD, NDZO and NDPN treated animals. The highest % weight gain was seen in NDZO while the least was in NDAM (Table I).

Lipid profile

Total cholesterol was significantly increased ($p<0.05$) in HLD and significantly reduced ($p<0.05$) in HLDPN. Significant decrease ($p<0.05$) was seen in High Density Lipoprotein (HDL) of HLD and NDAM, while significant increase ($p<0.05$) was observed in Low Density Lipoprotein (LDL) and Triacylglyceride (TG) of HLD. Computed AI was significantly increased ($p<0.05$) in HLD, NDAM, NDZO, NDPN and HLDPN when compared to the control rats (Table II).

Selected Biomolecules

No significant changes ($p>0.05$) were observed in Total protein and Albumin of all groups when analysed (Table III).

Selected Haematological parameter

Aqueous extracts of the plants had varying effects on PCV of rats fed with Normal Diet and High Lipid Diet. A significant decrease ($p<0.05$) in PCV was observed for all extracts-treated groups except for HLDZO with no significant change ($p>0.05$). Comparison within groups show that the PCV of rats in HLD and NDAM are statistically similar, while those in HLDAM, NDZO and HLDPN are statistically similar as grouped but NDPN was not statistically different from both groups.

WBC was also significantly decreased ($p<0.05$) in all treated groups and HLD-fed rats (Table IV).

Table I: Effect of Extracts on Body Weight

Groups	Tag	Initial (g)	Final (g)	% Weight gain
A	NC	195.00±25.98[a]	246.00±18.03[b]	26.15
B	HLD	188.33±10.41[a]	258.33±07.64[b]	37.17
C	NDAM	161.67±02.89[a]	170.00±09.22[a]	05.15
D	HLDAM	150.00±10.00[a]	163.33±12.58[a]	08.89
E	NDZO	150.00±10.10[a]	195.00±22.91[b]	30.00
F	HLDZO	181.36±22.55[a]	198.33±18.93[a]	09.36
G	NDPN	168.33±10.41[a]	208.33±11.55[b]	23.76
H	HLDPN	176.67±30.55[a]	208.33±25.66[a]	17.92

NOTE: Values are Mean of 4 replicates ± SD except for % Weight gain

Values with different superscript are significantly different ($p<0.05$) across the row

Table II: Effects of Extracts on Lipid Profile

Groups	Tag	CHOL (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	TG (mmol/L)	AI
A	NC	2.57±0.21^{a}	0.93±0.06^{a}	1.91±0.13^{a}	0.83±0.06^{ac}	2.76±0.11^{a}
B	HLD	5.33±0.31^{c}	0.83±0.03^{b}	3.79±0.47^{b}	1.13±0.45^{b}	6.42±0.10^{b}
C	NDAM	2.57±0.25^{a}	0.80±0.01^{b}	1.41±0.20^{c}	0.80±0.30^{ac}	3.21±0.09^{c}
D	HLDAM	2.60±0.44^{ab}	0.93±0.15^{abc}	1.06±0.28^{c}	0.77±0.31^{a}	2.79±0.08^{a}
E	NDZO	3.10±0.74^{ab}	1.00±0.10^{ac}	1.74±0.03^{a}	0.80±0.30^{a}	3.10±0.11^{c}
F	HLDZO	2.93±0.75^{ab}	1.03±0.15^{ac}	1.34±0.75^{a}	0.80±0.30^{a}	2.84±0.07^{a}
G	NDPN	3.33±0.87^{ab}	0.97±0.06^{ac}	2.20±0.80^{a}	1.03±0.49^{a}	3.43±0.09^{d}
H	HLDPN	2.97±0.32^{b}	0.83±0.06^{ab}	1.91±0.29^{a}	0.50±0.00^{d}	3.58±0.10^{d}

NOTE: Values are Mean of 2 replicates ± SD
Values with different superscript are significantly different ($p<0.05$) down the column

Table III: Effects of Extracts on T-protein and Albumin

Groups	Tag	T-Protein (g/L)	Albumin (g/L)
A	NC	74.00±05.57^{a}	38.33±3.06^{a}
B	HLD	82.00±08.66^{a}	38.00±8.19^{a}
C	NDAM	70.00±12.12^{a}	34.67±4.73^{a}
D	HLDAM	80.33±07.23^{a}	43.00±7.21^{a}
E	NDZO	73.33±10.12^{a}	40.00±4.36^{a}
F	HLDZO	77.33±17.62^{a}	40.33±4.04^{a}
G	NDPN	65.33±21.83^{a}	34.67±1.51^{a}
H	HLDPN	69.00±15.62^{a}	34.67±1.53^{a}

NOTE: Values are Mean of 2 replicates ± SD
Values with different superscript are significantly different ($p<0.05$) down the column

Table IV: Effects of Extracts on PCV and WBC

Groups	Tag	PCV (%)	WBC (mm^3)
A	NC	44.33±0.58^{a}	8383.33±332^{a}
B	HLD	39.67±2.08^{b}	6700.00±249^{b}
C	NDAM	39.33±1.53^{b}	4516.69±267^{c}
D	HLDAM	37.00±4.00^{c}	1366.67±208^{d}
E	NDZO	36.00±2.00^{c}	1300.00±409^{d}
F	HLDZO	43.00±6.24^{ab}	0833.33±325^{d}
G	NDPN	39.67±3.51^{bc}	1283.33±530^{d}
H	HLDPN	35.67±1.15^{c}	1733.31±451^{d}

NOTE: Values are Mean of 2 replicates ± SD
Values with different superscript are significantly different ($p<0.05$) down the column

DISCUSSION

The *zingiberaceae* are important family in traditional medicine for treatment of many infections[17]while the *piperceae* family has *Piper nigrum* as the most well-known due to its economic importance[18]. In the present study, the phytochemical screening of the spices was not carried out due to their availability in open literatures. Extracts from all three spices (AM, ZO and PN) have been reported to contain several

phytochemicals which could be responsible for their therapeutic importance. Chiejina and Ukeh[17] reported the presence of tannins, phlabotannins, steroids, terpenes, saponins, flavonoids and alkaloids in methanolic extracts of both AM seed and ZO. Shamsuddeen*et al*. [19]reported the absence of alkaloids and steroids in aqueous extracts and saponins, steroids and tannins in ethanolic extracts of PN. Notable phytochemicals which can improve lipid profile, reduce weight and affects % PCV in experimental animals include flavonoids[20,21], saponins[22] and alkaloids[23].

At the end of the administration period, significant increases ($p<0.05$) were observed in the weight of NC, HLD, NDZO and NDPN when compared to the initial weight of rats. Consistent feeding of animals with HLD increases their weight which may lead to obesity. The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended. An increased intake of energy-dense foods that are high in fat and an increase in physical inactivity due to the increasingly sedentary nature of many forms of work, changing modes of transportation, and increasing urbanization are factors which can also lead to obesity[24]. The highest % weight gain (37.17%) recorded was in group of rats fed with HLD. Major complications associated with obesity include hypertension, atherosclerosis, diabetes and endocrine abnormalities[25], AM was able to prevent the increase in weight, followed by ZO and PN respectively in rats fed with HLD. AM also expresses its potential of reducing weights in ND treated rats.

Coronary Heart/Artery Disease (CHD/CAD) or atherosclerosis is a condition characterize deposits of lipids on the inner walls of an artery, the deposition slows the flow of blood through the arteries by narrowing them. Low Fat diets (LFD) as opposed to High Fat Diet (HFD) are prescribed for the management of atherosclerosis as there are no specific treatments for the ailment[26]. Extracts significantly reduce ($p<0.05$) the risk of atherosclerosis in all treated groups when compared to HLD rats as indicated in the computed AI. AM and ZO showed a greater reduction in this indexes. Ethanolic extract of ginger at 400 mg/kgbwt.has been shown to effectively reduce Triacylglyceride (TG) and Total cholesterol (CHOL) in livers of HLD-fed rat model[27]. In this study, significant reduction ($p<0.05$) was observed in CHOL, LDL and TG of ZO treated rats at the same dosage of 400 mg/kgbwt. When compared to the HLDuntreated rats. The mechanism of homeostasis was reported to be a decrease in CHOL biosynthesis and enhancing hepatic uptake of circulation LDL cholesterol[27].

No significant effects ($P>0.05$) were observed in T-protein and albumin of animals in all treated groups. No significant effects were also recorded within each pair of treatments i.e. ND and HLD, meaning the protein metabolism was not adversely affected. Total serum protein and albumin are generally influenced by total protein intake[28], it was however expected that no significant effect should be observed in the protein profile of the treated animals since the feed contain the same amount of protein per kg.

Effects of aqueous extract of AM, ZO and PN on PCV and WBC is presented on table IV. Significant reductions ($P<0.05$) were observed in all groups except the group fed HLDadministered ZO while in WBC significant decrease was observed in all groups. Cholesterols are essential components of cell membranes including White Blood Cells. They are needed for their shapes and specific functions. Increase in WBC levels may raise suspicion of infection or contamination of feed during administration.

CONCLUSION

It's evident from the study that aqueous extracts of AM, ZO and PN at 400 mg/kg bwt. were able to prevent significant increase in body weight of rats fed with High Lipid Diet. Extracts were also able to significantly prevent increase in total CHOL, LDL and TG when compared to the rats fed with HLD alone. All extracts were also able to prevent the risk of atherosclerosis in rats, they had no significant effect on T-protein and albumin when compared to control. Extracts can therefore be applied in weight management.

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