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Research Article

Control of Weıght, Insulın Resıstance and Oxıdatıve Stress in Rats Fed Hıgh-Fat Dıet Usıng *Aframomum melegueta* (Allıgator Pepper)

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ABSTRACT

Consumption of diets rich in fat is very predominant due to the sensational values it adds to the foods. If overconsumed, such foods lead to metabolic diseases. The inclusion of alligator pepper in the diets as a means of ameliorating such diseases was investigated. Thirty-six (36) Wistar rats were divided into 6 groups. Groups 1, 2, 3, and 4 received a standard diet, high-fat diet (HFD), 1%, and 2% alligator pepper-supplemented standard diet respectively. Groups 5 and 6 got 1% and 2% alligator pepper-supplemented HFD respectively. The groups were fed *ad libitum* for 8 weeks. Blood glucose level, body weights, activities of antioxidant enzymes, and serum concentrations of malondialdehyde (MDA) and insulin were determined. homeostasis model assessment of insulin resistance (HOMA-IR) was computed. The groups that received alligator pepper-supplemented HFD had significantly ($p<0.05$) higher final average weight, insulin level, and HOMA-IR in comparison with the control but lower in these parameters when compared with the HFD group. All the groups fed supplemented diets had significantly ($p<0.05$) higher serum catalase activities in comparison with the HFD group. Also, all, except the group fed 1% alligator pepper-supplemented HFD had significantly ($p<0.05$) higher superoxide dismutase activities than the HFD group. It is concluded that including pepper in the standard diet may have no special advantages with respect to the studied parameters, but could decrease weight gain, and improve insulin sensitivity and oxidative stress defense mechanism in rats fed HFD.

Keywords: Alligator Pepper, High-fat diet, Insulin Resistance, Oxidative Stress, Weight

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INTRODUCTION

The alligator pepper plant is a herbaceous and perennial one. Belonging to *Zingiberaceae* (ginger) family, the alligator fruit is a flavouring spice with many uses in ethnobotany (Adefegha *et al*., 2017). The alligator pepper fruit consists of pod that on exfoliation, releases seeds that are enclosed in alligator-like bumpy skins. The spice made from the pepper is usually made by pounding the whole pod, and often time mixing with other ingredients such as African black pepper.

Many health benefits such as anti-inflammatory and antioxidant properties have been attributed to the richness of the pepper in functional polyphenols (Okeke *et al*., 2018; Alaje *et al*., 2014) and other important phytochemicals including alkaloids, essential oils, sterols and flavonoids (Akinwumi, 2011). This plant could therefore be helpful to people prone and/or addicted to consuming unhealthy diets such as high-fat diets (HFD) since the harmful effects of these diets are mediated mostly through weight increase and consequent generation of excess free radicals and inflammation.

Consumption of diets high in saturated fats has been fingered as one of the leading causes of obesity, cardiovascular diseases, and some other metabolic disorders (Nettleton *et al*., 2016). Fat contributes a great deal to flavour perception and eating pleasure, feed intake, and satiation (Goldberg *et al*., 2018; Boesveldt & de Graaf, 2017), and are energy-rich. Therefore, consumption of diets rich in fat could always result in overweight and other metabolic dysregulation except measures are taken to mitigate against such dysregulations.

Although alligator pepper is reported variously to have anti-oxidant and anti-inflammatory properties, studies on the safety of its inclusion in the diets and efficacy in preventing the manifestation of the harmful effects of high-fat diet have not been encompassing. This research studied weight controlling effect, the status of insulin action, and oxidative stress in rat models fed high-fat diets with alligator pepper supplementation.

MATERIALS AND METHODS

Chemicals, Reagents, Equipment and Feed Ingredients

All the chemicals and reagents were of analytical grade and the equipment used were validated for functionality before use. Standard rat chow was bought from Dutsinma Central market, while fat was prepared by heating animal fat until the oil was extracted. The oil was kept in a 500 mL sealed bottle until needed.

Identification and Preparation of the Plant Material

Alligator pepper was purchased at Abuja Road marketplace in Dutsinma Local Government of Katsina State, identified by at Herbarium at the Department of Plant Science and Biotechnology in Federal University of Dutsinma and assigned voucher number *Aframomum melegueta* FUDMA/PSB/000305. The fruit of the alligator pepper was washed thoroughly with tap water, air dried and grinded together with the seeds into a fine powder. This was stored in clean Bama bottle.

Experimental Rats

Thirty-six 5-week-old Wistar rats were bought from Research Institute, Vom, Nigeria and transported in aluminium cages to the experimental site; Department of Biochemistry and Molecular Biology animal house, Federal University Dutsinma where they were transferred into wooden cages and allowed for a 7-day acclimatization period.

Formulation of high-fat and supplemented diets

The high-fat diet was formulated by mixing the rat chow with the animal fat in a ratio of 2:1 ($^{w}/_{w}$). Then, 99 g of the high-fat and standard diets were separately mixed with 1 g of powdered alligator pepper to formulate 1% supplementation with the alligator pepper. Further, 98 g of the high-fat and standard diets were separately mixed with 2 g of powdered alligator pepper to formulate 2% supplementation with the alligator pepper.

Experimental Design

The 36 rats were divided into six groups of 6, and the formulated diets were randomly assigned to the groups as follows; Groups 1 and 2 respectively received standard diet and high-fat diet, while Groups 3 and 4 got 1% and 2% alligator pepper-supplemented standard diet respectively. Groups 5 and 6 received 1% and 2% alligator pepper-supplemented HFD respectively. All the groups had unrestricted access to their respective diets for eight weeks.

Feed Consumption and Weight Gain

The daily feed consumption and weekly changes in weight were determined using mobile electronic weighing balance.

Handling of Blood Sample

At the end of the 8 weeks of unrestricted feeding, the rats were sacrificed under anaesthesia using chloroform. The Blood sample was collected into plain bottles of 5 mL and centrifuged at 1500 g for 15 min to obtain the serum (Idoko *et al*., 2018).

Estimation of Blood Glucose Level, Insulin Concentration and Computation of HOMA-IR

The initial fasting blood glucose level was measured after allowing the rats to fast overnight. The fasting blood sugar was measured on weekly bases using AkuChek glucometer. The Insulin was determined in the blood by reacting 3,3,5,5-tetramethylbenzidine (TMB) with insulin bound conjugate in accordance with the method reported by Shen (2019). Homeostasis model assessment was calculated using the formula described by Jinhua *et al*. (2013);

HOMA index = glucose (mmol/l) x insulin (µmol/l)/22.5

Determination of Antioxidant Status

The serum concentrations of catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined using commercial Randox assay kits. For MDA concentration, it was estimated by measuring TBARS as described by Satoh (1978).

Data Analysis

Analysis of variance (ANOVA) was performed for the variability of the means, while Duncan's multiple-range test was used to determine the significance of the variations. Version 16 of SPSS was the statistical package used.

RESULTS AND DISCUSSION

Weight control and feed intake in rats fed high-fat diet with alligator pepper supplement

At the commencement of the experiment, the rats were carefully divided into groups such that there was no significant ($p>0.05$) difference in their initial average weight. At the end of the experiment however, the groups that received 1% and 2% alligator pepper-supplemented HFD had significantly ($p<0.05$) higher final average weight in comparison with that of the control but lower average weight when compared with the HFD-group. Groups fed 1% and 2% alligator pepper-supplemented standard diets did not significantly ($p>0.05$) differ in final average weight from the control. Feed intakes were significantly lower in the groups fed with high-fat diets when compared with the control (Table 1).

Table1. Weight and feed-intake in rats fed high-fat diet with alligator pepper supplement

Group	Initial average weight (g)	Final average weight (g)	Percentage weight gain (%)	Average weekly feed intake
1	133.94 ± 4.71^a	213.72 ± 0.97^a	59.56	76.45 ± 1.00^a
2	134.32 ± 2.22^a	280.16 ± 2.44^b	108.56	63.22 ± 2.4^b
3	134.94 ± 4.09^a	215.24 ± 2.02^a	59.51	77.04 ± 3.17^a
4	136.72 ± 2.17^a	212.28 ± 3.09^a	55.27	77.27 ± 2.91^a
5	137.1 ± 1.71^a	233.28 ± 3.03^c	70.15	63.13 ± 1.19^b
6	137.14 ± 1.83^a	234.18 ± 2.36^c	70.76	64.07 ± 2.09^b

Same superscripts along the same column indicates absence of significant difference, while different superscripts along the same column signifies significant difference (P>0.05). Groups 1 and 2 respectively received standard diet and high fat diet, while Groups 3 and 4 got 1% and 2% alligator pepper-supplemented standard diet respectively. Groups 5 and 6 received 1% and 2% alligator pepper-supplemented HFD respectively.

The result of the weight and feed-intake in rats fed high-fat diet with alligator pepper supplement shows that 1% or 2% supplementation with alligator pepper may not affect feed intake. In other words, these levels of supplementation may not alter both the physical and biochemical factors influencing feed intake. Sensory as well as external stimulation of feed such as taste, smell, colour and texture coupled with need for energy balance influence feed intake (Martin, 2016). Furthermore, ghrelin is a hunger hormone with its circulation increasing during negative energy balance and decreasing when energy balance is positive while leptin, another hormone acts almost in an antagonistic manner to ghrelin (Eckstein, 2011).

Although the groups fed with HFDs had lower feed intake, the percentage weight gains were higher in them. This is understandable since HFDs contain much more energy values compared with the standard diets. However, it is interesting to note that supplementing HFD with alligator pepper could decrease weight gain by about 35%. Like ginger, alligator pepper is reach in 6-gingerol (Sugita *et al*., 2013; Umukoro and Ashorobi, 2007). This phytochemical is reported to be anti-obesogenic, a property exerted by decreasing the expression of lipogenic enzymes such acetyl CoA carboxylase (ACC) and fatty acid synthase (Suk *et al*., 2017; Brahmanaidu *et al*., 2015). Aqueous extracts of alligator pepper have been reported to be effective in weight management and prevention of dyslipidaemia (Bennett and Inengite, 2022). Furthermore, Tramontin *et al*., 2020 and Mao *et al*. (2019) had demonstrated these compounds to cause increased thermogenesis as well as increased breakdown of white adipose tissue. By decreasing weight gain particularly in the HFD-fed groups, the pepper may therefore be effective in controlling other complications associated with weight gain.

Measures of average weekly blood glucose levels and insulin resistance in rats fed high-fat diet with alligator pepper supplement

Figure 1 depicts the average weekly blood glucose levels. As seen in the figure, the blood glucose of the HFD group rose but decreases after the 3rd week of the experiment. The fluctuations in other groups were not significant (p>0.05) and all stabilizes towards the 8th week.

The results of final blood glucose, insulin and computed HOMA-IR are as presented in Table 2. The group fed 2% alligator pepper-supplemented standard diet had significantly (p<0.05) lower glucose level compared with the control. Both insulin level and HOMA-IR in the alligator pepper-supplemented standard diet groups did not differ significantly (p>0.05) from the control. The groups on alligator pepper-supplemented HFDs have significantly (p<0.05) higher insulin level and HOMA-IR when compared with the control but low when compared with the group fed with HFD.

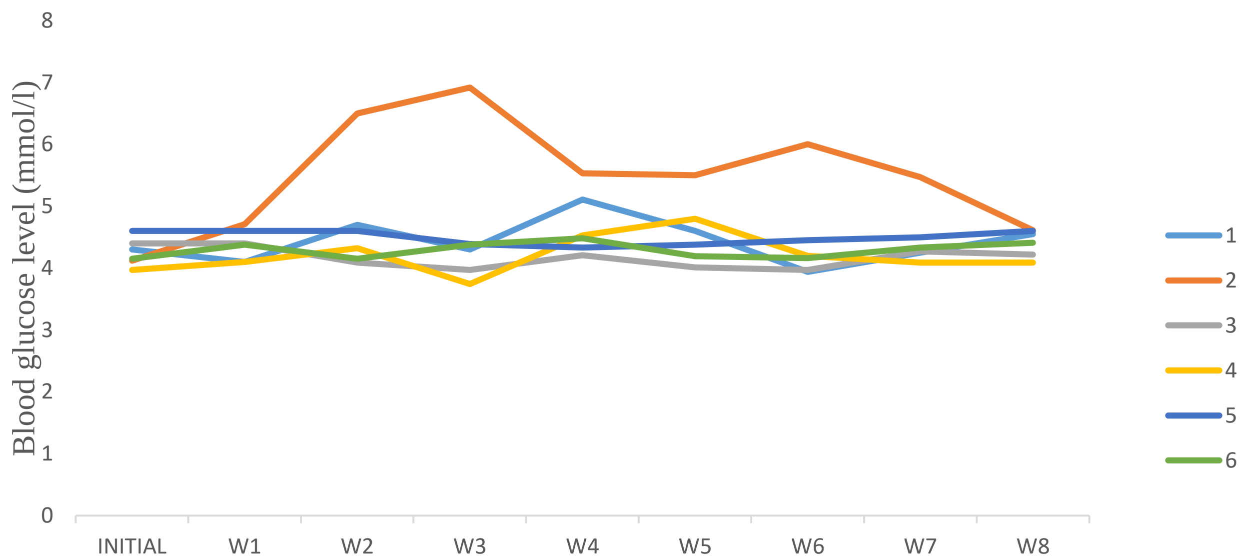

Figure 1. Average weekly blood glucose levels rats fed high-fat diet with alligator pepper supplement

Table 2: Control of insulin resistance in rats fed high-fat diet with alligator pepper supplement

Groups	BGL (mmol/l)	Insulin (µU/l)	HOMA-IR
1	4.50±0.07[a]	7.11±0.33[a]	1.42±0.07[a]
2	4.61±0.08[a]	23.50±0.74[b]	4.81±0.15[b]
3	4.22±0.03[ab]	6.86±0.16[a]	1.29±0.02[a]
4	4.09±0.29[b]	7.04±0.20[a]	1.29±0.11[a]
5	4.6±0.04[a]	17.24±0.21[c]	3.52±0.05[c]
6	4.41±0.03[ab]	13.34±0.07[d]	2.61±0.02[d]

Same superscripts along the same column indicates absence of significant difference at, while different superscripts along the same column signifies significant difference (P>0.05). Groups 1 and 2 respectively received standard diet and high fat diet, while Groups 3 and 4 got 1% and 2% alligator pepper-supplemented standard diet respectively. Groups 5 and 6 received 1% and 2% alligator pepper-supplemented HFD. BGL-Blood glucose level.

Only the group fed HFD diet had early significant rise in blood glucose level. The rise might have been met with increased synthesis and release of insulin as seen in table 2 leading to the lowering of glucose towards the end of the experimental period. This, also is an indication of insulin resistance as seen in the computed HOMA-IR in all the groups fed HFDs. Insulin resistance being a condition of irresponsiveness to insulin effects (Sarwar *et al*., 2022), is accompanied by high insulinemia (Janssen, 2021) as a compensatory mechanism to counter the effect of low responsiveness to insulin. Further, hyper insulinemia could also be triggered by dietary stimulation of beta cells (Astley *et al*., 2018).

Gingerenone-A and gingerol compounds contained in alligator pepper are reported to modulate fatty acid metabolism via activation of adenine monophosphate (AMP)-activated protein kinase

(AMPK) pathway, an action that could improve insulin sensitivity (Suk *et al*., 2017). It could be inferred from the results that dietary supplementation with alligator pepper could improve insulin sensitivity and ameliorate both insulin resistance and insulinemia in rats feeding on diets high in fat.

This supplementation being able to improve insulin sensitivity is very instructive since insulin resistance plays a causative role in many of the complications associated with the intake of diets high in fat such as disorders of blood glucose utilization, increased decomposition of lipids in adipocytes, and lipid accumulation. Specifically, type 2 diabetes and cardiovascular diseases which are major causes of both mortality and morbidity are strongly associated with impaired insulin signaling (Zhao *et al*., 2023; Saklayen, 2018).

Anti-oxidation effects of alligator pepper supplementation in rats fed a high-fat diet

The serum activities of GPx did not significantly ($p>0.05$) differ among all the groups while all the groups fed supplemented diets had significantly ($p<0.05$) higher serum catalase activities in comparison with the HFD group. All, except the group fed 1% alligator pepper-supplemented HFD had significantly ($p<0.05$) higher SOD activities than the HFD group with the group fed 2% alligator pepper-supplemented standard diet having the highest activity of serum SOD (Table 3).

Table 3: Anti-oxidation effects of **alligator pepper** supplementation in **rats fed high-fat diet**

	SOD (u/l)	Catalase (u/l)	GPx (u/l)	MDA (nmol/l)
1	4.27±0.12a	2.96±0.07a	12.91±0.06a	66.37±1.81a
2	1.00±0.08b	0.50±0.01b	12.83±0.17a	133.65±1.22e
3	4.28±0.09a	2.94±0.13a	12.82±0.03a	65.03±0.23a
4	6.09±0.15c	2.94±0.07a	12.86±0.13a	65.73±2.86a
5	1.08±0.06b	2.86±0.08a	12.96±0.04a	101.33±0.67c
6	2.67±0.27d	2.98±0.05a	12.97±0.04a	114.79±1.49d

Groups 1 and 2 respectively received a standard diet and high fat diet, while Groups 3 and 4 got 1% and 2% alligator pepper-supplemented standard diet respectively. Groups 5 and 6 received 1% and 2% alligator pepper-supplemented HFD. BGL-Blood glucose level.

SOD is an antioxidant enzyme that converts superoxide molecules into less toxic hydrogen peroxide and oxygen (Filip *et al*., 2014). Catalase, on the other hand, degrades H_2O_2 into water and oxygen thereby completing the detoxification of superoxide started by the SOD (Michele, 2021). Glutathione peroxidase catalyses the detoxification of lipid peroxides, H_2O_2 and other peroxides in the cell. These peroxides, if not removed can easily decompose into free radicals causing damages to the proteins, lipids and DNA (Muthukumar *et al*., 2011). Free radicals attack lipid, protein, carbohydrate and DNA components of the body, impair normal functioning of the cell and lead to diseased condition through cell death (Miral and Pawel, 2012). MDA is one of the by-products of lipid peroxidation and could directly destroy membrane structure or destroy DNA and RNA components of the cell (Iuchi *et al*., 2021). The results demonstrate the capacity of supplementation with alligator pepper to scavenge free radicals and prevent lipid peroxidation arising from consumption of HFDs as seen in higher activities of SOD, catalase and lower MDA concentration in groups fed HFD supplemented with the alligator pepper. However, supplementing standard diet with the alligator pepper may not affect the oxidative status. As stated earlier, alligator pepper is rich in 6-gingerol and 6-shogaol both of which have demonstrated antioxidant properties (Mohammed *et al*., 2018). This, they do by preventing the generation of free radicals, scavenging free radicals, and increasing the activities of antioxidant enzymes (Sueishi *et al*., 2019; Kiptiyah *et al*., 2017). In addition, genkwanin flavonoid found in alligator pepper has been reported to possess free radical scavenging activity (Porras *et al*., 2019). From the results of the reseach work, the whole fruit powder contained same phytochemicals and also posses same antioxidant activities as contained in some of its extract

CONCLUSIONS

Investigation was carried out on the weight controlling effect, the status of insulin action and oxidative stress in rats fed with high-fat diet and standard diet with alligator pepper supplementation. From the findings, it was concluded that the addition of the alligator pepper into the standard diet may have no special advantages in respects to the studied parameters. The inclusion of the HFD could decrease weight gain, improve insulin sensitivity, and oxidative stress. However, more researches are needed for its overall safety before recommendations could be made.

Conflict of Interest
The authors confirm that there are no known conflicts of interest.

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