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MANAGEMENT OF INFLAMMATION AND LIVER DAMAGES WITH ALLIGATOR PEPPER FRUIT SUPPLEMENTS IN RATS FED HIGH-FAT DIET

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ABSTRACT

Fat makes food attractive and easily addicted to as it adds to its sensory values. The research investigated anti-inflammatory and hepatoprotective potentials of alligator pepper supplements in Wistar rats feeding on normal or high-fat diets (HFD). Thirty-six (36) Wistar rats were divided into groups 1-6 and randomly assigned diets. Groups 1, 2, 3 and 4 received standard diet, high fat diet, 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 their respective diets *ad libitum* for 8 weeks. Serum inflammatory biomarkers, liver enzymes and proteins were evaluated. There were no significant ($p>0.05$) differences in serum concentrations of IL-6 and TNF-α, ALT, AST, ALP, GGT, total protein, albumin and globulin between the group fed alligator pepper-supplemented standard diets and the group fed the standard diet. The groups fed alligator pepper-supplemented high-fat diets had significantly ($p<0.05$) lower levels of the studied parameters in comparison with the group fed high-fat diet but higher when compared with the group fed standard diet with the exception of GGT. CRP was significantly lower in the groups fed alligator pepper-supplemented standard diets compared with the control. It could be concluded from the findings that including the pepper in standard/normal diets may provide protection against inflammation and may have no beneficial or adverse effects on liver functions. However, adding the pepper in HFD provides fortification against HFD-causing inflammation and consequent liver degeneration.

Keywords: Inflammation, Liver damage, High-fat diets, Alligator pepper supplements

INTRODUCTION

Inflammation is a response of the immune system against attacks by potential dangers that could include toxic compounds, pathogens and damaged bodies. This response helps the organism survive when there is damage to the tissues or when infections occur (Furman *et al*., 2019; Chen *et al*., 2017). Once the danger is eliminated, the inflammatory response is resolved in somewhat rapid manner. Else, prolonged inflammation, otherwise known as systemic chronic inflammation results. The shift from short-lived (acute) to long-livid (chronic) inflammation is harmful to organs, alters cellular physiology and mediates non-communicable diseases such as type 2 diabetes and cardiovascular diseases (Schilrreff and Alexiev, 2022; Sugimoto *et al*., 2016). Some biological and environmental factors do prevent resolution of inflammation and promote chronic inflammation. One of such is diet high in fat, particularly saturated fat (Bachmann *et al*., 2020).

Excess consumption of diet high in fat leads to accumulation of fat and consequent secretion of proinflammatory factors by the white adipose tissues (Muñoz and Costa, 2013). For instance, tumour necrosis factor alpha (TNF-α), whose secretion increases during high-fat consumption, promotes chronic inflammation (Maurizi *et al*., 2018). Further, free fatty acid (FFA) level increases when high-fat diet is constantly consumed, and elicits increased secretion of TNF-α and IL-6 (Nicholas *et al*., 2024; Bradley *et al*., 2008), a condition that fuels chronic inflammation (Antonioli *et al*., 2017). This is the leading cause of other diseases such as liver and kidney damages, atherosclerosis and cardiovascular diseases (Duan *et al*., 2018).

Avoiding diets high in fat is the best option. However, the palatability and other sensory appeal of high fat diets get many people addicted to it. The problem is further worsened by life style that encourages patronage of eateries which usually contain disproportionately high amount of fat. It becomes worthwhile searching for easy way of mitigating the possible damaging effects of such diet. Inclusion of spices in the diet had been reported by Idoko *et al*. (2019) to suppress fat accumulation, and as such could prevent inflammation.

Some researchers have revealed alligator pepper (*Aframomum melegueta*) to be having anti-inflammatory properties (Ogwu *et al*., 2024). Whether the use of the pepper as a supplement could prevent inflammation and its consequences in rats consuming high-fat diet remains interesting subject of discussion. This research was therefore carried out to ascertain if inflammation and liver damage, two of the consequences of long-term consumption of high-fat diet could be prevented by supplementing the high-fat diet with alligator pepper fruit.

MATERIALS AND METHODS

Materials

All the materials; chemicals, reagents and equipment used met the research standard. Standard rat chow was bought from Dutsinma Central market, while the fat used was prepared by heating animal adipose tissue until fat was extracted. The fat was kept in Bama bottle until needed.

Identification and preparation of the plant material

Alligator pepper was purchased at Abuja Road in Dutsinma Local Government of Katsina State, identified at Herbarium at the Department of Plant Science and Biotechnology in Federal University of Dutsinma (*Aframomum melegueta* FUDMA/PSB/00305). The fruit of the alligator pepper was washed with clean water, air dried and grinded together with the seeds into a fine powder. The powder was stored in clean Bama bottle.

Experimental rats

Thirty-six Wistar rats weighing 135±5.3 g were bought from Vom Research Institute, Nigeria and transported in

aluminium cages to the experimental site; Department of Biochemistry and Molecular Biology of Federal University Dutsinma where they were kept in wooden cages and allowed for a 7-day acclimatization period.

Formulation of diets and experimental design

The formulation followed the previous procedure of Idoko and Imam (2024). Here, high-fat diet was formulated by mixing the rat chow with the animal fat in a ratio of 2:1 (wt/wt). Then, 99 g of the high-fat and standard diets were separately mixed with 1 g of the powdered alligator pepper to formulate 1% supplementation with the pepper. Further, 98 g of the high-fat and standard diets were separately mixed with 2 g of the powdered alligator pepper to formulate 2% supplementation with the pepper. Thereafter, 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.

Handling of blood sample

At the end of the 8 weeks of unrestricted feeding, the rats were sacrificed under anaesthesia brought about by putting the rats in a transparent bucket containing cotton wool soaked in chloroform. Blood collected into plain tubes were centrifuged at 1500g for 15min to obtain serum (Idoko *et al*., 2022).

Determination of biomarkers of inflammation in rats fed high-fat diet with alligator pepper supplement

Concentrations of serum inflammation biomarkers: tumour necrosis factor- alfa (TNF-α) interleukin-6 (IL-6) and C-reactive protein (CRP) were measured by multiplexed array assay following the instructions of the manufacturer.

Evaluation of liver function in rats fed high-fat diet with alligator pepper supplement

The liver function tests; serum AST, ALT, ALP, GGT, total protein, albumin and globulin were determined using Randox assay kits following the manufacturer's protocol.

RESULTS AND DISCUSSION

Serum concentration of inflammation biomarkers in rats fed high-fat diet with alligator pepper supplement

There were no significant ($p>0.05$) differences in serum concentrations of IL-6 and TNF-α between the group fed alligator pepper-supplemented standard diets and the group fed the standard diet with no supplementations. The groups fed alligator pepper-supplemented high-fat diets had significantly ($p<0.05$) lower levels of the biomarkers in comparison with the group fed high-fat diet but higher when compared with the group fed standard diet. CRP was significantly lower in the groups fed alligator pepper-supplemented standard diets compared with the control. The groups fed alligator pepper-supplemented high-fat diets had significantly ($p<0.05$) lower levels of CRP compared with the group fed high-fat diet but higher when compared with the control (Table 1).

Table 1: Serum concentration of inflammation biomarkers in rats fed high-fat diet with alligator pepper supplement

	IL-6 (pg/ml)	CRP (mg/ml)	TNF-α (pg/ml)
1	76.10±1.71^{a}	278.47±2.60^{a}	106.60±4.41^{a}
2	128.77±0.46^{b}	446.47±4.41^{b}	211.90±1.64^{c}
3	76.07±0.55^{a}	259.00±1.90^{d}	105.17±7.34^{a}
4	74.76±0.88^{a}	248.07±0.64^{d}	105.33±5.35^{a}
5	101.33±1.53^{c}	343.50±3.15^{c}	211.00±0.31^{c}
6	88.57±1.03^{d}	341.70±1.46^{c}	189.63±1.71^{b}

Results are means of 3 determinations± SEM. Comparisons made along the column where values with different superscripts are significantly different.

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.

TNF-α – tumour necrosis factor-alfa; IL-6 - interleukin-6; CRP- C-reactive protein.

Although the inclusion of alligator pepper in standard diet did not affect the concentrations of IL-6 and TNF-α, the results show that the inclusion of the pepper in standard diets may protect against inflammatory attacks as seen in lower level of CRP in the groups fed standard diet-supplemented diets. CRP is a general marker of systemic inflammation (Sproston and Ashworth, 2018; Darren *et al*., 2014) and a strong predictor of cardiometabolic diseases (Georgakis *et al*., 2021; Teixeira *et al*., 2014). In the cases of groups fed the high-fat diets, it is seen that the inclusion of the pepper, particularly at 2% concentration, improved the inflammatory status as indicated by the lowering of the inflammation biomarkers. The observed anti-inflammatory activities may not be unconnected with the abundance of certain phytonutrients that include polyphenols and essential oils as reported by Chiejina and Ukeh (2012). Components of the pepper such as 6-gingerol, 6-shogaol and 6-dehydroshogaol as reported by Sugita *et al*. (2013) had been demonstrated to have anti-inflammatory properties as they decrease inflammatory mediators such as TNF- α and IL-6 (Unuofin *et al*., 2021; Rondanelli *et al*., 2020; Azimi *et al*., 2016). From the results, it could be seen that the whole fruit used as a supplement in high-fat diet could still exert its anti-inflammatory activities as is the case with its extracts. Since these inflammatory factors are implicated in the pathophysiological processes of cardiometabolic disorders (Frederik *et al*., 2022), decreasing their concentrations could therefore be helpful in management of the diseases.

Serum activities of selected liver enzymes in rats fed high-fat diet with alligator pepper supplement

The concentrations of AST, ALT and ALP were not significantly ($p>0.05$) affected by inclusion of alligator pepper in standard diet, but significantly ($p<0.05$) decreased the enzymes in the groups fed high-fat diets with the supplement when compared with the group fed high-fat diet without the supplement. The activities of GGT were not significantly ($p>0.05$) affected (Table 2).

Table 2: Serum activities of selected liver enzymes in rats fed high-fat diet with alligator pepper supplement

	AST(µ/l)	ALT(µ/l)	ALP(µ/l)	GGT(µ/l)
1	35.50±0.93[a]	14.82±0.12[a]	55.77±1.48[a]	30.00±2.52 [a]
2	66.27±2.74[c]	41.96±1.50[b]	100.30±1.83[b]	28.00±1.00 [a]
3	35.40±0.95[a]	14.46±0.34[a]	54.36±1.32[a]	27.43±0.23 [a]
4	36.87±1.65[a]	14.14±0.31[a]	56.75±1.65[a]	27.15±0.80 [a]
5	54.87±1.85[b]	39.90±2.49[b]	76.97±0.17[c]	29.57±2.89 [a]
6	55.78±2.83[b]	29.12±1.77[c]	85.53±2.77[d]	30.43±0.90 [a]

Results are means of 3 determinations± SEM. Comparisons made along the column where values with different superscripts are significantly different.

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.

AST: Aspartate transaminase
ALT: Alanine transaminase
ALP: Alkaline phosphatise
GGT: Gamma glutamyl transferase

Serum concentrations of proteins in rats fed high-fat diet with alligator pepper supplement

Apart from the group that received high-fat diet which had significantly ($p<0.05$) the highest concentration of total protein, there were no significant ($p>0.05$) variation among the other groups. Inclusion of the pepper in standard diet did not significantly ($p>0.05$) affect the serum concentration of albumin and globulin. However, the inclusion in HFD significantly ($p<0.05$) lowered the globulin concentration but didn't affect the albumin concentration when compared with group fed HFD without supplementation (Table 3).

Table 3: Serum concentrations of proteins in rats fed high-fat diet with alligator pepper supplement

	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)
1	36.53±1.07[a]	27.93±1.16[a]	8.60±1.40[a]
2	43.80±2.13[b]	18.03±0.26[b]	25.77±1.94[c]
3	36.33±0.33[a]	28.90±0.49[a]	7.43±0.30[a]
4	36.07±0.69[a]	29.37±1.05[a]	6.70±0.57[a]
5	37.83±1.18[a]	18.33±0.88[b]	19.50±0.47[b]
6	36.33±0.68[a]	19.07±1.03[b]	17.27±1.44[b]

Results are means of 3 determinations± SEM. Comparisons made along the column where values with different superscripts are significantly different.

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.

Considering the fact that inflammation has very important physiologic functions that include toxin detoxification, repair of damaged tissues and defending the body against pathogenic attacks (Soliman & Barreda, 2022), antagonizing inflammatory process could be detrimental to the overall wellbeing of individuals (Frederik *et al*., 2022). Our results show that the anti-inflammatory property of the pepper, at the concentrations used, may not constitute any danger to the liver as the inclusion of the pepper in standard diet did not affect the activities of studied liver enzymes. AST, ALT, GDT and ALP are predominantly liver enzymes and their activities in the serum increase if the liver cells are injured and are, as such, routinely used in monitoring the health of the liver (Jain *et al*., 2022). Furthermore, the elevation of these enzymes in the serum is associated with other cardiometabolic diseases that include cardiovascular diseases and even cancer (Kunutsor *et al*., 2015).

It is further demonstrated in our finding that inclusion of the pepper in high-fat diets could give some protection against liver damage which is usually associated with consumption of such diet as could be inferred from the lower activities of these enzymes in comparison with the group fed HFD without the pepper. Inflammation plays a causal role in liver damage (Buzzetti *et al*., 2016). Therefore, the hepatoprotection seen in the groups fed alligator pepper-supplemented high-fat diet is partly attributable to the anti-inflammatory properties of the pepper as earlier seen. The non significant variations in the activities of GGT among all the groups remains unclear. However, it may not be unconnected with varied possible sources of the enzyme. In addition to liver, GGT is found in many tissues of the body such as spleen and kidney (Jolles *et al*., 2014).

Determination of the concentrations of serum proteins (total protein, albumin and globulin) is also a vital tool in monitoring the health conditions of the liver. Synthesized in the liver, the level of albumin is affected by factors such as nutrition and diseases including liver disease. Globulin comprises of proteins such as enzymes and carrier proteins (Jolles *et al*., 2014). Increased serum globulin is a common feature in liver cirrhosis due to spontaneous B cell immunoglobulin synthesis, and has been used in evaluation of hepatic fibrosis (El Balkhi *et al*., 2024). It is evident from our results that adding the pepper to the standard diet may have no effects on the synthesis and utilization of the proteins (albumin and globulin). The pepper could however improve the synthetic function of the liver in rats fed on HFD and decrease the leakage of proteins out of the cells. This is consistent with the earlier results on liver enzymes.

CONCLUSION

The research studied the use of alligator pepper fruit powder as a supplement in preventing inflammation and its

consequences in subjects consuming high-fat diet. It could be inferred and concluded from the findings that including the pepper in standard/normal diets may provide protection against inflammation and may have no beneficial or adverse effects on liver functions. However, adding the pepper in HFD provides fortification against HFD-causing inflammation and consequent liver degeneration.

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