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ORIGINAL ARTICLE

High Fructose Diet-induced Inflammation and Oxidative Stress in Male *Wistar* Rats: Preventive Effects of Yoyo Cleanser Bitters

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ABSTRACT

Background: Modern diets high in fructose, have been associated with increased inflammation and oxidative stress, however, herbal remedies like Yoyo cleanser bitters (Yoyo) have been suggested as possible remedies. Accordingly, this study aimed to determine if co-administration of Yoyo cleanser bitters could prevent inflammation and oxidative stress in male *Wistar* rats fed with a high fructose diet

Methods: Twenty male *Wistar* rats, approximately 200g each, were divided into four groups namely: control, high fructose diet with fructose water (HFD+FW), HFD+FW with Atorvastatin (0.57 mg/kg), and HFD+FW with Yoyo (600 mg/kg), groups. After a 28-day experimental period, blood samples were assessed for highly sensitive C-reactive protein (Hs-CRP), malondialdehyde (MDA), and total antioxidant capacity (TAC), using standard methods.

Results: Rats on the high fructose diet only displayed significantly ($P<0.05$) elevated Hs-CRP (0.63±0.04 mg/dl) and MDA (0.32±0.01 µM) levels, compared to the control rats (0.27±0.01 mg/dl; 0.10±0.01 µM). Both Atorvastatin and Yoyo significantly ($P<0.05$) prevented the elevation of Hs-CRP (Atorvastatin: 0.28±0.00 mg/dl; Yoyo: 0.29±0.01 mg/dl), while only Yoyo Cleanser bitters significantly ($P<0.05$) prevented the increase in MDA level (0.08±0.02 µM) when compared to the HFD+FW group. Additionally, the high fructose diet significantly ($P<0.05$) reduced TAC, but both Atorvastatin (0.38±0.03 µmol/ml) and Yoyo (0.47±0.04 µmol/ml) prevented the reduction of TAC when compared to the HFD+FW group (0.21±0.06 µmol/ml).

Conclusion: Yoyo cleanser bitters attenuated inflammation and oxidative stress induced by high fructose intake, thus suggesting that its co-administration has some preventive therapeutic potential.

Keywords: High-fructose diet; Inflammation; Oxidative stress; Metabolic syndrome; Yoyo cleanser bitters.

INTRODUCTION

Modern dietary habits have witnessed a notable shift towards increased consumption of high-fructose diets, largely attributed to the widespread availability of processed foods and sugar-sweetened beverages. High intake of fructose, a simple sugar abundant in fruits and added sugars, has been implicated in the development of various metabolic disorders, including obesity, insulin resistance, cardiovascular diseases, and non-alcoholic fatty liver disease (NAFLD).[1,2]

Central to the problem of excessive fructose consumption, is the inflammatory and oxidative consequences associated with it. High-fructose diets induce increased production of free fatty acids and dyslipidemia, which has been associated with elevated levels of inflammatory markers, such as high-sensitive C-reactive protein (Hs-CRP), indicative of systemic inflammation.[3] Moreover, heightened fructose intake is known to exacerbate oxidative stress by disrupting the delicate balance between reactive oxygen species (ROS) production and antioxidant defenses, ultimately leading to lipid peroxidation and cellular damage.[4] The metabolism of the excess fructose in the liver results in increase breakdown of ATP, leading to increased production of AMP (adenosine monophosphate) which is broken down into uric acid through a series of enzymatic reactions.[5,6] Elevated uric acid levels contribute to increased production of reactive oxygen species (ROS) via the activation of xanthine oxidase, a key enzyme in purine metabolism.[7] These highly reactive molecules can damage cellular components, including lipids, proteins, and DNA, creating a state of oxidative stress within the liver and other tissues. Malondialdehyde (MDA) levels, a byproduct of lipid peroxidation, due to ROS attacks on polyunsaturated fatty acids in cell membranes, is usually increased in this process.[8] The elevated MDA causing the generation of MDA epitopes which are associated with the expression of pro-inflammatory genes and the activation of several downstream inflammatory signaling pathways.[9] and mitochondrial dysfunction are critical factors in the development of oxidative stress as it compromises

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cellular energy metabolism and exacerbates the production of ROS.[10]

The chronic oxidative stress induced by high fructose consumption depletes the body's antioxidant defenses.[11] Total Antioxidant Capacity (TAC) is a measure of the overall ability of the body to neutralize ROS. A high fructose diet reduces TAC by overwhelming the antioxidant defense systems, such as glutathione, superoxide dismutase (SOD), and catalase, leading to a diminished capacity to counteract oxidative stress and further promoting cellular damage and inflammation.[11] Fructose metabolism promotes de novo lipogenesis (DNL) in the liver, leading to the synthesis of triglycerides,[12] exported as very low-density lipoprotein (VLDL), increasing circulating triglyceride levels, ultimately resulting in dyslipidemia and insulin resistance, key components of metabolic syndrome.[13] The accumulation of lipids in the liver and adipose tissue causes hypertrophic adipose tissue to release pro-inflammatory cytokines such as TNF-α and IL-6, and chemokines that attract immune cells, particularly macrophages, with the resultant secretion of additional pro-inflammatory cytokines, stimulating the liver to produce CRP, and a characteristic sustained elevation of Hs-CRP. This creates a state of chronic low-grade inflammation, termed meta-flammation.[15,16] Oxidative stress and inflammation interfere with insulin signaling pathways, and is closely linked to the development of type 2 diabetes,[17] impaired endothelial function, and hypertension.[18]

In light of these concerns, there is a growing interest in exploring interventions capable of mitigating the adverse effects of inflammation and oxidative stress caused by high-fructose diets. Yoyo cleanser bitters, a herbal formulation produced by Abllat Nigeria Company Limited, Lagos, renowned for its diverse phytochemical composition, has shown promise in exerting anti-inflammatory and antioxidant effects.[19,20] It is composed of five herbal constituents, which includes *Aloe vera* (True aloe, Lily of the forest*), Acinos Arvensis* (Basil thyme), *Citrus aurantifolia* (Bitter orange), *Chenopodium murale* (Nettleleaf goosefoot), *and Cinamomum aromaticum* (Cassia). Additionally, water-soluble vitamins (B1, B2, B3, B6, and B12) and minerals (copper, zinc, iron) are incorporated to enhance the herbal mixture, as outlined by Anionye and colleagues, in 2017.[21] NAFDAC has declared Yoyo cleanser bitters safe for human consumption and preliminary toxicity studies also indicate that the recommended dose of 600 mg/kg body weight used in this study was effective and not toxic to living systems.[20,21] There remains a gap in understanding their specific impact on inflammation and antioxidant imbalance induced by high-fructose diets. Therefore, the primary objective of this study was to investigate the potential protective role of Yoyo cleanser bitters against inflammation and oxidative damage triggered by excessive fructose consumption via the assessment of an inflammatory marker (Hs-CRP) and oxidative stress indicators (MDA and TAC). It was also hoped that this study will contribute to laying the groundwork for the development of novel preventive and therapeutic strategies for metabolic disorders linked to modern dietary patterns.

MATERIALS AND METHODS

Chemicals, Drugs, and Kits

Yoyo cleanser bitters with batch number YYBL-2209, manufactured by Abllat company Nigeria limited, Ikeja-Lagos and Atorvastatin (Lot: 15033), manufactured by TEVA UK limited, were purchased from pharmaceutical stores opposite the University of Benin Teaching Hospital (UBTH), Ugbowo Lagos Road, Benin City, Edo State, Nigeria. The materials and reagents utilized for assessing malondialdehyde, such as 1% Thiobarbituric acid (TBA), 0.4% NaOH, Glacial acetic acid, and Distilled water, were of analytical grade. For evaluating the Total Antioxidant Capacity, materials included assay buffer (30 ml x 4 vials), reaction buffer (16 ml x 1 vial), substrate powder (x 1 vial), dye reagent powder, dye reagent diluent (2 ml x 1 vial), and standard powder (x 1 vial). A commercially available ELISA kit was used to evaluate the Hs-CRP activity (MyBioSource, UK).

Experimental Animals

The male *Wistar* rats (n=20) utilized in this investigation were procured from the Anatomy Department, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. Following international guidelines, a two-week acclimatization period was observed for these rats before the onset of experiments.[22] Additionally, ethical clearance and approval were obtained from the Research Ethics Committee (REC) of the College of Medical Sciences, University of Benin, with REC Approval No.: CMS/REC/2023/485, ensuring compliance with ethical standards for animal experimentation.

Experimental Diets

The experimental design involved assigning the rats to two distinct diets: a control diet composed of standard pelleted grower's mash (Table 1), and a specialized high-fructose diet, that is metabolic syndrome-inducing[23] and formulated to induce the conditions of oxidative stress and inflammation (Table 2). The formulation of the experimental diet entailed enriching the basal diet with a calculated

Table 1: Composition of the normal/basal diet (g/1000g) based on the standard pelleted growers mash of Jerrison Agro Allied Services, Benin City, Nigeria[42]

Ingredients	Basal diet (g)
Maize	280.0
Wheat Offal	280.0
Palm Kernel Cake	208.0
Soyabean Meal	48.0
Groundnut Cake	100.0
Fish Meal (65%)	12.0
Lysine	1.6
Bone Meal	12.0
Limestone	52.0
Methionine	0.8
Grower Premix	2.4
Salt	3.2
Total	**1000.0**

Table 2: Composition of the 60% high fructose diet (metabolic syndrome-inducing) diet (g/1000g)[23]

Ingredients	High-Fructose Diet (g)
Basal diet in Table 1	400.0
Pure white crystalline powdered fructose	600.0
Total	**1000.0**

proportion of white crystalline powdered D (-) Fructose, resulting in a diet composition with a 60% fructose content.[23] Furthermore, to support this dietary regimen, the rats were provided with drinking water containing 10% fructose.[24]

Dosage Determination

Yoyo cleanser bitters: The established dose for a 70kg man is 40ml,[21,25,26] the expected consumption for a 200g rat was therefore calculated as follows: Xml = 40ml x 200g / 70,000g = 0.114ml (approximately 0.11ml). Thus, the dosage for a 200g rat would be 0.6 x 10^{-3}ml/g of rat or equivalent to 0.6ml/kg of rat which is equivalent to 0.6 g/kg or 600 mg/kg of rat body weight.

Atorvastatin: A 70kg man consumes 40mg,[27] therefore a 200g rat would be expected to consume: Xmg = 40mg x 200g / 70,000g = 0.114mg (requiring dissolving 0.114mg of the drug in 1ml of distilled water). Therefore, the dosage for a 200g rat would be approximately 0.57 x 10^{-3}mg/g of rat or 0.57mg/kg of rat body weight.[27]

Experimental Protocol

Twenty male *Wistar* rats, weighing between 180g and 220g, were randomly assigned to four groups, each fulfilling a specific role in the study design:

I. Group 1: Received the basal diet along with clean tap water, serving as the normal control.

II. Group 2: Consumed a high fructose diet supplemented with 10% fructose-water

III. Group 3: Consumed a high fructose diet combined with 10% fructose-water and Atorvastatin (0.57 mg/kg-bw)

IV. Group 4: Consumed a high fructose diet alongside 10% fructose-water and Yoyo cleanser bitters (600 mg/kg-bw)

The feeding regimen consisted of providing the rats with ad-libitum access to food, while closely monitoring their daily intake throughout the four-week study period. Cages were maintained within a 12-hour light-dark cycle in the animal house of the Department of Medical Biochemistry, College of Medical Sciences, University of Benin and regular cleaning and disinfection procedures were implemented to ensure a sanitized and healthy environment. To assess potential signs of toxicity, physiological changes, and mortality among the animals, continuous observations were conducted, following methodologies outlined in previous studies.[28, 29]

Blood Sample Collection and Analysis

Blood samples were collected and put in a lithium heparin bottle on the morning of the 29th day, after an overnight fast following the 28-day study. The blood was collected under chloroform anesthesia, via cardiac puncture and used for the evaluation of Hs-CRP, MDA, and TAC.

a. Quantification of Malondialdehyde (MDA) Levels: MDA, was determined spectrophotometrically using the TBARS method.[30] This involved a reaction between MDA and thiobarbituric acid under acidic conditions, resulting in a pink-colored product with maximum absorbance at 532nm.[30]

b. Assessment of Plasma Total Antioxidant Capacity (TAC): This microplate assay kit measured total antioxidant capacity by evaluating the ability of antioxidants to reduce Fe^{3+}-TPTZ to Fe^{2+}-TPTZ complex, generating a colorimetric readout at 593nm. It assessed the remaining antioxidant capacity after oxidative stress.[31]

c. High Sensitive C - Reactive Protein (Hs-CRP) Analysis: Utilizing a Sandwich-ELISA approach, the MyBioSource rat ELISA kit UK, was used to determine the Rat Hs-CRP concentration by measuring the colorimetric reaction between antibodies and enzyme-conjugated compounds. The resulting color

intensity at 450nm was directly proportional to the concentration of rat Hs-CRP.[32]

Statistical Evaluation

Statistical significance was assessed using ANOVA at a 95% confidence level, followed by Tukey's multiple comparison tests, employing the SPSS-20 software statistical package. The results were expressed as mean ± SEM (= standard error of the mean) of five determinations (n=5) Results with $p<0.05$ were considered significant.

RESULTS

As shown in Table 3 and Table 4, the high fructose diet + 10% fructose water led to a significant increase ($p<0.05$) in Hs-CRP and MDA levels of rats in Group 2 when compared to the normal control group of rats (Group 1). Specifically, Group 2 (HFD+FW) demonstrated heightened levels of Hs-CRP at 0.63±0.04 mg/dl and MDA at 0.32±0.01 µM, contrasting with the Control group's Hs-CRP of 0.27±0.01 mg/dl and MDA of 0.10±0.01 µM. Both the Atorvastatin (Group 3) and the Yoyo cleanser bitters (Group 4) group of rats did not experience this increase in Hs-CRP, as the Atorvastatin (0.28±0.00 mg/dl) and Yoyo cleanser bitters (0.29±0.01 mg/dl) respectively effectively prevented ($p<0.05$) the increase in Hs-CRP, compared to Group 2 . Their levels were not significantly different ($P>0.05$) from the levels in the positive control group (Group 1).

Concerning the mitigation of the increase in MDA levels, only Yoyo cleanser bitters demonstrated a significant ($P<0.05$) preventive effect at 0.08±0.02 µM, while Atorvastatin was not significant ($P>0.05$) when compared with the level of MDA in Group 2 rats (Table 4).

Furthermore, as seen in Table 5, the Group 2 rats displayed a significant decrease ($P<0.05$) in TAC when compared to the control rats. Notably, both Atorvastatin and Yoyo cleanser bitters significantly ($P<0.05$) prevented this decline in their respective groups, with Yoyo cleanser bitters (Group 4) exhibiting a TAC level of 0.47±0.04 µmol/ml, notably significantly higher ($P<0.05$) than both Groups 1 (control) and 3 (Atorvastatin).

DISCUSSION

Atorvastatin, a statin drug that prevents dyslipidaemia, was chosen as the ideal reference "preventive drug" for this research because a high fructose diet relies largely on the "lipid pathway" that causes a dyslipidaemia that induces the inflammation and oxidative stress that characterizes the metabolic syndrome it causes.[12,13] Thus, Atorvastatin in preventing this dyslipidaemia, prevents the onset of the inflammation and oxidative stress experienced in the "metabolic syndrome" caused by a high fructose diet. Atorvastatin apart from it being an anti-dyslipidaemic drug, has also been documented to have the "ancillary effect" of preventing inflammation and oxidative stress by effectively reducing pro-inflammatory cytokines, enhancing antioxidant defenses, while lowering low density lipoprotein (LDL) cholesterol and triglycerides.[33] Atorvastatin also improves endothelial function and mitigates oxidative stress through mitochondrial protection.[33]

Table 3: High-sensitive C-reactive protein (Hs-CRP) levels of rats fed a high-fructose diet

Groups	Hs-CRP (mg/dl)
Group 1 (Control)	$0.27±0.01^{a}$
Group 2 (HFD+FW)	$0.63±0.04^{b,c}$
Group 3 (HFD+FW + Atorvastatin)	$0.28±0.00^{a,d,e}$
Group 4 (HFD+FW + Yoyo)	$0.29±0.01^{a,d,e}$

HFD+FW:*High fructose diet + fructose water.* ***Yoyo:*** *Yoyo cleanser bitters.* ***Hs-CRP:*** *High sensitive C-reactive protein.*

*******In each group, the 1st superscript alphabet, is position 1, 2nd superscript alphabet is position 2, 3rd superscript alphabet is position 3. Comparison of significance between groups, must be done taking cognizance of these positions. So comparing position 1 superscript alphabet (designated as "a") of group 1, with position 1 of group 2 (which is alphabet "b"), implies that they are different from each other, because they are different alphabets. Same reasoning goes for position 2 in group 2 (with alphabet "c") and position 2 in group 3 (with alphabet "d"), which indicates that group 3 differs from group 2. However position 3 of groups 3 and 4, both have the alphabet "e", meaning they do not differ from each other.*

Table 4: Malondialdehyde (MDA) levels of the rats fed a high-fructose diet

Groups	MDA (µM)
Group 1 (Control)	$0.10±0.01^{a}$
Group 2 (HFD+FW)	$0.32±0.01^{b,c}$
Group 3 (HFD+FW + Atorvastatin)	$0.17±0.10^{a,c,e}$
Group 4 (HFD+FW + Yoyo)	$0.08±0.02^{a,d,f}$

HFD+FW: *High fructose diet + fructose water.* ***Yoyo:*** *Yoyo cleanser bitters.* ***MDA:*** *Malondialdehyde.*
As explained in footnote of Table 3*.**

Table 5: Total antioxidant capacity (TAC) levels of the rats fed a high-fructose diet

Groups	TAC (µmol/ml)
Group 1 (Control)	$0.42±0.03^{a}$
Group 2 (HFD+FW)	$0.21±0.06^{b,c}$
Group 3 (HFD+FW + Atorvastatin)	$0.38±0.03^{a,d,e}$
Group 4 (HFD+FW + Yoyo)	$0.47±0.04^{a,d,f}$

HFD+FW:*High fructose diet + fructose water.* ***Yoyo:*** *Yoyo cleanser bitters.* ***TAC:*** *Total antioxidant capacity.*
As explained in footnote of Table 3*.**

Modern dietary trends, characterized by the increased consumption of processed foods and sweetened beverages, have led to a surge in high fructose intake, contributing to the growing prevalence of metabolic disorders such as obesity, insulin resistance, and cardiovascular diseases.[34, 35] High fructose diets have been associated with systemic inflammation and oxidative stress, both of which play pivotal roles in the pathogenesis of metabolic dysfunction.[36, 37] In this context, herbal remedies like Yoyo cleanser bitters (Yoyo) have gained attention for their potential anti-inflammatory and anti-oxidant properties.[20, 38] This study aimed to evaluate the preventive effects of Yoyo on inflammation and oxidative stress induced by a high fructose diet in rats, with a focus on inflammatory markers such as high sensitive C-reactive protein (Hs-CRP), oxidative stress indicators including malondialdehyde (MDA), and total antioxidant capacity (TAC).

High-sensitive C-reactive protein (Hs-CRP) is a sensitive biomarker of systemic inflammation and is often elevated in conditions associated with metabolic dysfunction.[39] In this study, rats fed a high fructose diet supplemented with fructose water exhibited a significant increase in Hs-CRP levels compared to the control group, indicating the presence of systemic inflammation induced by high fructose consumption. However, administration of Yoyo effectively prevented the rise in Hs-CRP levels as did Atorvastatin, suggesting its potential anti-inflammatory effects. The finding of this exhibited anti-inflammatory effect of Yoyo cleanser bitters is consistent with previous studies demonstrating the anti-inflammatory properties of herbal remedies.[40, 41] The effectiveness of this herbal bitters in reducing inflammation is likely attributed to its constituent plants known for their anti-inflammatory properties. Notable examples of such plants include *Aloe vera,* and *Cinnamonum cassia.*[20, 42]

The anti-inflammatory properties of these plants arise from their phytochemical constituents, which likely act synergistically to modulate or prevent an increase in blood levels of CRP.[43] Previous studies indicate that the herbal bitters of this study contain significant amounts of alkaloids, tannins, flavonoids, phenols, saponins, and cyanogenic glycosides, all of which are phytochemicals known for their anti-inflammatory properties.[21,44,45] The presence of flavonoids alone could explain their anti-inflammatory properties and their ability to prevent endothelial dysfunction. Examples of flavonoids derived from the constituents of these bitters include quercitrin, quercetin, and kaempferol.[46,47] Flavonoids, such as quercetin, are polyphenolic compounds that have been suggested to play a significant role in the treatment and prevention of hypertension.[48] They are known for their anti-inflammatory, anti-allergic, and antioxidant effects, as well as their strengthening and protective effects on fragile capillary and venous structures.[46,47] Moreover, flavonoids exhibit various therapeutic functions, including hepatoprotective, anti-spasmodic, hypo-cholesterolaemic, anti-inflammatory, and diuretic effects.[46,47]Yoyo cleanser bitters, from the study carried out by Onyeaghala and colleagues, was also identified to have biological compounds with anti-inflammatory properties, an example being, Phenol, 2, 4 – bis (1,1 dimethylethyl), an alkylated phenol which has been classified as a tannin, one of the major occurring phyto-compounds in most medicinal plants.[45] Tannins have been reported to possess other biological benefits such as accelerating blood clotting, reducing blood pressure, decreasing serum lipid level, reducing liver necrosis, decreasing inflammation and modulating immunerespon-ses.[45]

Malondialdehyde (MDA) is considered a reliable marker of oxidative stress.[49] Elevated MDA levels signify increased oxidative damage to cellular membranes and are implicated in the pathogenesis of various metabolic disorders.[50] In this study, rats fed a high fructose diet displayed significantly higher MDA levels compared to the control group, indicating enhanced lipid peroxidation and oxidative stress. Interestingly, while Atorvastatin was unable to significantly prevent this increase, administration of Yoyo cleanser biters effectively mitigated the increase in MDA levels, suggesting its potent antioxidant properties. Yoyo's ability to suppress lipid peroxidation and preserve cellular membrane integrity underscores its potential as a therapeutic agent for combating oxidative stress-induced cellular damage. This is in agreement with the findings of Anionye and colleagues demonstrating that Yoyo cleanser bitters attenuates MDA levels, thus highlighting its ability to prevent lipid peroxidation in tissues and cells.[21]

Total antioxidant capacity (TAC) reflects the cumulative antioxidant activity within a biological system and serves as a crucial defense mechanism against oxidative stress.[51] A decline in TAC levels indicates an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, predisposing cells to oxidative damage.[52] In this study, rats fed a high fructose diet exhibited a significant decrease in TAC levels compared to the control group, indicative of compromised antioxidant defenses. However, Atorvastatin was able to prevent this decline. In addition, the administration of Yoyo cleanser bitters not only prevented this decline, but effectively restored TAC levels to those comparable to the control group, suggesting its ability to enhance antioxidant capacity and protect against oxidative stress-induced cellular damage. This feature of Yoyo bitters is not

farfetched considering its rich phytochemical constituents, like its flavonoid content. Flavonoids, and by extension, the herbal bitters, often exert their therapeutic effects by inhibiting enzyme systems (e.g., lipoxygenase, cyclooxygenase, elastase, and aldose reductase), scavenging free radicals, and acting as cofactors for antioxidants like vitamin C.[20,48] Flavonoids like quercetin exert their antihypertensive effects, including reduction in oxidative stress, inhibition of angiotensin-converting enzyme (ACE) activity, reduction in inflammation, and improvement of endothelial function, ultimately leading to a decrease in the occurrence of metabolic syndrome.[21,42]

Conclusion: This study demonstrates that Yoyo cleanser bitters (Yoyo) can mitigate the harmful effects of high fructose diets in rats, possibly through the attenuation of inflammation, enhancement of antioxidant capacity and mitigation of oxidative stress. These findings suggest that Yoyo cleanser bitters could be a promising natural preventive therapeutic agent for the prevention of inflammation and oxidative stress linked to high fructose consumption, and other metabolic disorders such as obesity, insulin resistance, diabetes mellitus, and cardiovascular diseases. Further studies are therefore recommended to corroborate these findings.

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