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EFFECT OF PAXHERBAL BITTERS ON INFLAMMATION AND ANTIOXIDANT STATUS IN MALE *WISTAR* RATS FED A HIGH FRUCTOSE DIET

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ABSTRACT

Modern diets are often high in fructose, and this has been associated with increased inflammation and oxidative stress. Herbal remedies like Paxherbal bitters have been suggested as possible remedies. This study aims at determining if the co-administration of Paxherbal bitters could prevent the inflammation and oxidative stress, which occurs when male *Wistar* rats are fed a high fructose diet. Twenty male *Wistar* rats, approximately 200g each, were divided into four groups: the control, high fructose diet with fructose water (HFD+FW), HFD+FW with Atorvastatin, and HFD+FW with Paxherbal bitters, groups. After a 28-day experimental period, blood samples were assessed for high-sensitive C-reactive protein (HsCRP), malondialdehyde (MDA), and total antioxidant capacity (TAC), using standard methods. Statistical analysis was done using the SPSS-20 package. Results has shown that rats on the high fructose diet, displayed significantly ($P<0.05$) elevated HsCRP (0.63±0.04 mg/dl) and MDA (0.32±0.01 µM) levels, compared to control levels (0.27±0.01 mg/dl; 0.10±0.01 µM). Both Atorvastatin and Paxherbal bitters significantly ($P<0.05$) prevented the elevation of HsCRP, while only Paxherbal bitters significantly ($P<0.05$) prevented the increase in MDA level (0.13±0.02 µM) compared to the level seen in the HFD+FW group. The high fructose diet significantly ($P<0.05$) reduced the TAC in the rats, but Atorvastatin and Paxherbal bitters preserved the TAC (0.38±0.03 µmol/ml; 0.49±0.04 µmol/ml) of their respective groups compared to that of the negative control group (0.21±0.06 µmol/ml). This study shows that Paxherbal bitters prevented the inflammation and oxidative stress induced by a high fructose intake, suggesting its co-administration, has some preventive therapeutic role.

Keywords: High-fructose diet, Inflammation, Oxidative stress, Metabolic syndrome, Paxherbal bitters

INTRODUCTION

Modern dietary patterns have witnessed a surge in high fructose consumption, predominantly due to the increased availability of processed foods and sweetened beverages. High intake of fructose, a monosaccharide present commonly in fruits, has been associated with various metabolic complications, including obesity, insulin resistance, cardiovascular diseases, and non-alcoholic fatty liver disease (NAFLD) (Bray *et al.*, 2004; Malik *et al.*, 2010; Jegatheesan, and De Bandt, 2017).

Of particular concern are the pro-inflammatory and pro-oxidative effects associated with high fructose intake which has been implicated in causing a metabolic syndrome that ultimately result in cardiovascular and metabolic diseases (Bray *et al.*, 2004; Malik *et al.*, 2010; Jegatheesan, and De Bandt, 2017). High fructose diets have been linked to increased levels of markers of inflammation such as high sensitive C-reactive protein (HsCRP), indicating it causes systemic inflammation. Moreover, excessive fructose consumption have also been shown to lead to enhanced oxidative stress, disrupting the balance between reactive oxygen species (ROS) production and antioxidant defenses, subsequently triggering lipid peroxidation and cellular damage (Farah *et al.*, 2006; Basaranoglu, *et al.*, 2013). "High fructose diets contribute to oxidative stress through several interconnected mechanisms, thereby also promoting inflammation. Firstly, fructose metabolism in the liver generates reactive oxygen species (ROS) via pathways like mitochondrial dysfunction and NADPH oxidase activation, initiating oxidative damage to cellular components. Mitochondrial dysfunction can result from increased flux (due to excess fructose) through the glycerol phosphate shuttle, leading to elevated electron flow to the mitochondrial electron transport chain and subsequent ROS production" (Farah *et al.*, 2006; Basaranoglu, *et al.*, 2013). Additionally, fructose metabolism can lead to the accumulation of intermediates such as fructose-1-phosphate, which can disrupt mitochondrial respiration and contribute to oxidative stress. This includes lipid peroxidation, where ROS attack polyunsaturated fatty acids in cell membranes, yielding reactive lipid peroxides like malondialdehyde (MDA) and perpetuating oxidative injury (Farah *et al.*, 2006; Basaranoglu, *et al.*, 2013). Concurrently, high fructose intake may deplete antioxidant reserves, such as glutathione, or hinder antioxidant enzyme activity, compromising the cell's ability to counter oxidative stress. Moreover, oxidative stress-induced activation of pro-inflammatory pathways exacerbates ROS generation and impairs antioxidant defenses, fostering a cycle of damage and inflammation. Additionally, high fructose diets can induce endoplasmic reticulum (ER) stress, triggering the unfolded protein response (UPR) and further contributing to ROS production and cellular dysfunction. This intricate interplay underscores the role of oxidative stress in the pathogenesis of metabolic disorders associated with high fructose consumption, including non-alcoholic fatty liver disease (NAFLD) and cardiovascular diseases (Bray *et al.*, 2004; Malik *et al.*, 2010; Jegatheesan, and De Bandt, 2017).

Several studies have underscored the need to explore interventions capable of mitigating the detrimental effects of high fructose intake. Herbal remedies have garnered attention for their potential therapeutic properties, particularly in managing serious medical conditions (Tukur *et al.*, 2024). Paxherbal bitters, a herbal formulation known for its diverse phytochemical composition, has shown promise in exerting anti-inflammatory and antioxidant effects (Anionye *et al.*, 2015). Indications or claims of the uses of Paxherbal bitters include that "it promotes blood circulation, prevents kidney stones, helps in digestion and activates bile flow. It increases immunity of the body against bacterial and fungal infections. It acts on both the pancreas and liver/gall bladder, helping to

promote the production and release of the pancreatic enzyme lipase and bile, which ensure good digestion of fats and oils (preventing hyperlipidaemia/dyslipidaemia) and proper functioning of the excretory functions of the liver. It is also claimed to help in the prevention of diabetes and acceleration of body repairs, healing of wounds and toothaches" (Anionye and Onyeneke, 2015; Anionye *et al.*, 2016a). These claims have not been evaluated by NAFDAC and there is paucity of scientific literature with research findings in respect of Paxherbal bitters. In recent years, Anionye and his colleagues have conducted a series of investigations into Paxherbal bitters. They have meticulously elucidated its effects, composition, potential antioxidant capabilities, and its ability to potentially prevent cardiovascular diseases. Furthermore, they have underscored its safety for consumption, emphasizing that it poses no toxicity to the blood or organs of the body. (Anionye *et al.*, 2015; Anionye and Onyeneke, 2016a, Anionye and Onyeneke, 2020). Paxherbal bitters uniqueness is seen in the synergism of its 40 herbal constituents (Anionye *et al.*, 2015; Anionye and Onyeneke, 2016a; Anionye and Onyeneke, 2020):

- *Cymbogon citratus* (Lemon grass)
- *Aloe vera* (True aloe, Lily of the desert)
- *Rawolfia vomitoria* (Swizzle stick)
- *Sida acuta* (Broom weed)
- *Gongronema latifolium* (Utazi)
- *Zingiber officinale* (Ginger)
- *Xylopia aethiopica* (Uda)
- *Vernonia amygdalina* (Bitter leaf)
- *Tridax procumbens* (Tridax daisy, coat buttons)
- *Capsicum annuum* (Green pepper)
- *Carica papaya* seed (Pawpaw seed)
- *Glycine max* (Soya bean leaves)
- *Garcinia kola* (Bitter kola)
- *Ageratum conyzoides* (Goat weed)
- *Cajanus cajans* (Pigeon peas)
- Cocoa leaves
- Cocoa Powder
- *Bellis perennis* (Wild daisy)
- *Morinda citrifolia* (Noni)
- *Aspilia africana* (Haemorrhage plant)
- *Capsicum Spp.* (Pepper plant)
- *Citrus aurantium* (Bitter orange)
- *Alstonia boneei* (Stool wood)
- *Ocimum sanctum* (Holy basil)
- *Pennisetum pupureum* (Napier grass)
- *Tagetes erecta* (African marigold)
- *Daucus carota* peels (Carrot peels)
- *Ananas comosus* root (Pineapple root)
- *Jatropha curcas* (Physics nut)
- *Citrus limon* leaves (Lemon leaves)
- *Uraria pita* (Prishnaparni)
- *Viscum album* (Mistletoe)
- *Aloe barteri* (African aloe)
- *Citrus aurantifolia* (Lime)
- *Cassia alata* (Candlestick senna)
- *Cochlospermum religiosum* (L.) (Cotton tree/Cotton seed leaves)
- *Persea americana* (Avocado pear)
- *Eucalyptus officinalis* (Thunder protector/fever tree)
- *Musa paradisiaca* (French plantain)
- *Morinda Lucida* (Brimstone tree)

Paxherbal bitters derives its therapeutic potency from a diverse array of phytochemical constituents sourced from indigenous botanicals. These constituents, carefully selected for their pharmacological properties, is what most likely converge in a synergistic manner to imbue Paxherbal bitters with its characteristic efficacy and potency (Anionye *et al.*, 2015; Anionye and Onyeneke, 2016a; Anionye and Onyeneke, 2020).

Among the myriad phytochemicals found within Paxherbal bitters are flavonoids, alkaloids, terpenoids, tannins, saponins, phenolic compounds, and glycosides. And of these phytochemicals, flavonoids abundant in botanicals like Lemon grass (Cymbopogon citratus) and Bitter leaf (Vernonia amygdalina), (which make up some of its herbal constituents) contribute potent antioxidant and anti-inflammatory properties (Anionye *et al.*, 2015; Anionye and Onyeneke, 2020).

However, while some studies have explored the potential of herbal interventions in addressing metabolic disorders, there remains a gap in understanding their specific impact on inflammation and oxidative stress induced by high fructose diets. Investigating the effects of Paxherbal bitters in this context could offer valuable insights into its potential as an adjunctive therapy in mitigating the adverse effects associated with high fructose intake.

This study therefore was aimed at assessing the impact of Paxherbal bitters on preventing the rise in inflammatory markers (HsCRP) and oxidative stress indicators (malondialdehyde - MDA) in male *Wistar* rats fed a high fructose diet. The evaluation of total antioxidant capacity (TAC) alongside these markers will provide a comprehensive understanding of Paxherbal bitters' potential in counteracting inflammation and oxidative damage triggered by high fructose consumption.

MATERIALS AND METHOD

Chemicals, Drugs, and Kits

Paxherbal bitters were directly sourced from its manufacturers at the Benedictine Monastery at Ewu-Ishan in Edo State. Atorvastatin was obtained from pharmaceutical stores located opposite the University of Benin Teaching Hospital (UBTH) on Ugbowo Lagos Road, Benin City, Edo State, Nigeria. Materials and reagents used for malondialdehyde assessment, including 1% Thiobarbituric acid (TBA), 0.4% NaOH, Glacial acetic acid, and Distilled water, all of analytical grade, were procured from an accredited dealer - Pyrex Laboratories in Benin, Nigeria. For the Total Antioxidant Capacity assessment, the following materials were obtained from Pyrex Laboratories: 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). The High Sensitive C-reactive protein (HsCRP) ELISA kit by MyBioSource, UK, was purchased from the manufacturer's representative in Nigeria. The kit included a Microtiter plate (96 well plate) and eight vials of standards (S1: 0ng/ml, S2: 0.156ng/ml, S3: 0.312ng/ml, S4: 0.625ng/ml, S5: 1.25ng/ml, S6: 2.5ng/ml, S7: 5.0ng/ml, S8: 10ng/ml).

Experimental Animals

The male Wistar rats (n=20) utilized in this study were procured from the Anatomy Department, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. Adhering to international guidelines (Canadian Council on Animal Care, 1984), these rats underwent a two-week acclimatization period before the commencement of the experiments. Furthermore, 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 protocol encompassed the allocation of two distinct diets: a control diet consisting of standard pelleted grower's mash as described by Anionye *et al.,* (2018) and a specialized high-fructose diet designed to trigger inflammation and oxidative stress due to the resultant metabolic syndrome it causes. The formulation of the experimental diet involved augmenting the basal diet with a calculated proportion of white crystalline powdered D (-) Fructose, thereby achieving a diet composition with a 60% fructose content (Abdelrahman *et al.,* 2018). Additionally, to support this diet, the rats were provided with drinking water *ad libitum,* containing 10% fructose (Lírio *et al.,* 2016).

Experimental Design

Twenty male Wistar rats, with weights ranging from 180g to 220g, were randomly divided into four groups, each fulfilling a specific role in the study design:

i. Group 1: Consumed the basal diet along with clean tap water. It served as the normal control.
ii. Group 2: Received a high fructose diet supplemented with 10% fructose-water. It served as the negative control
iii. Group 3: Administered a high fructose diet combined with 10% fructose-water and Atorvastatin (0.57 mg/kg-bw). It served as the positive control.
iv. Group 4: Given a high fructose diet alongside 10% fructose-water and Yoyo bitters (600 mg/kg-bw). It served as the experimental group.

Dosage Regimen

Dosage determinations for Paxherbal bitters and Atorvastatin were established based on established human doses and then adjusted according to the weight of the rats (Mendie, 2009; Anionye *et al.,* 2017a; Anionye and Onyeneke, 2016b). The dosage calculations was aimed at ensuring equivalence to the established effective human dose.

For Paxherbal bitters: If a 70,000g man (70kg) consumes 40ml, the expected consumption for a 200g rat would be: Xml = 40ml x 200g / 70,000g = 0.114ml (approximately 0.11ml) Thus, the dosage for a 200g rat would be 0.6 $x10^{-3}$ml/g of rat or equivalent to 0.6ml/kg of rat or 0.6 g/kg or 600 mg/kg of rat body weight (b.w).

For Atorvastatin: If a 70,000g man consumes 40mg, 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$x10^{-3}$mg/g of rat or 0.57mg/kg of rat body weight (EMDEX., 2007).

The Feeding Protocol

The feeding protocol involved providing the rats with ad-libitum access to food, and their daily intake was closely monitored throughout the four-week study period. The rats were managed within a 12-hour light-dark cycle, and regular cleaning and disinfection procedures for the cages, were implemented to maintain a hygienic environment.

Observations and Toxicity Evaluation

To evaluate any potential signs of toxicity, physiological alterations, and mortality among the animals, constant observations were conducted, aligning with methodologies outlined in prior studies (Fielding and Metheron, 1991; Vijayalakshmi *et al.*, 2000).

Blood Sample Collection and Biochemical Assays

Blood samples were obtained from the rats under chloroform anesthesia, on the 29th day, following an overnight fast from the evening of 28th day of the experimental period. The blood was collected through cardiac puncture into plain bottles for the assessment of the inflammatory marker: (high sensitive C-reactive protein - HsCRP) and oxidative stress indicators: malondialdehyde (MDA) and total antioxidant capacity (TAC).

High Sensitive C-reactive Protein (HsCRP) Assay

Utilizing a Sandwich-ELISA approach, this kit measured Rat HsCRP concentration by assessing the colorimetric reaction between antibodies and enzyme-conjugated compounds. The resulting color intensity at 450nm was directly proportional to the concentration of rat HsCRP (MyBioSource, 2019a).

Assay of Malondialdehyde (MDA) Level

MDA, a lipid peroxidation byproduct, was quantified spectrophotometrically using the TBARS method. This involved a reaction between MDA and thiobarbituric acid under acidic conditions, forming a pink-colored product with maximum absorbance at 532nm (Varshney and Kale, 1990).

Determination 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 remaining antioxidant capacity after oxidative stress (MyBioSource, 2019b).

Statistical Analysis

The assessment of statistical significance was conducted using ANOVA at a 95% confidence level as well as the tukey's multiple comparison test, employing the SPSS-20 software statistical package. Results with $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Result

From the results shown in in Tables 1 and 2, the high fructose diet + 10% fructose water group (negative control group) there was a significant increase ($p<0.05$) in Hs-CRP and MDA levels in the rats of this group, compared with these same parameters in the rats of the normal control group (Group 1). Specifically, Group 2 (HFD+FW) exhibited elevated levels of Hs-CRP at 0.63±0.04 mg/dl and MDA at 0.32±0.01 µM, in contrast to the control group's Hs-CRP of 0.27±0.01 mg/dl and MDA of 0.10±0.01 µM (Table 1 and 2). Generally, Atorvastatin (Group 3: HFD+FW + Atorvastatin) and Paxherbal bitters (Group 4: HFD+FW + Pax) demonstrated significant prevention ($p<0.05$) of Hs-CRP increase in the rats of their respective groups when compared to the rats of the negative control group, mirroring levels similar to that of the normal control group: Atorvastatin - Hs-CRP being at 0.28±0.00 mg/dl and Paxherbal bitters - Hs-CRP being at 0.30±0.03 mg/dl. However, in mitigating MDA levels, only Paxherbal bitters showcased a significant preventive ($P<0.05$) effect at 0.13±0.02 µM, while Atorvastatin though it prevented an increase in MDA, its effects was not statistically significant ($p>0.05$) within the period of the experiment, when compared to the level of MDA in the negative control group fed solely the high fructose diet. The negative control group exhibited a significant decrease ($P<0.05$) in total antioxidant capacity (TAC) compared to the normal control rats (Table 3). Atorvastatin and Paxherbal bitters however, significantly prevented this decrease ($p<0.05$) in their respective groups.

Table 1: High sensitive C-reactive protein (Hs-CRP) levels of the rats fed high-fructose diet

Groups	Hs-CRP (mg/dl)
Group 1 (Control)	0.27 ± 0.01^{a}
Group 2 (HFD+FW)	$0.63\pm0.04^{b,c}$
Group 3 (HFD+FW + Atorvastatin)	$0.28\pm0.00^{a,d,e}$
Group 4 (HFD+FW + Pax)	$0.30\pm0.03^{a,d,e}$

The results were expressed as mean ± SEM (= standard error of the mean) of five determinations (n=5) and subjected to one-way analysis of variance (ANOVA). Means in the same column with different superscript alphabets on the same position, differ significantly at 95% level of significance ($p < 0.05$) using Turkey's test of significance.
HFD+FW: High fructose diet + fructose water.
Pax: Paxherbal bitters.
Hs-CRP: High sensitive C-reactive protein.

Table 2: Malondialdehyde (MDA) levels of the rats fed high-fructose diet

Groups	MDA (µM)
Group 1 (Control)	0.10 ± 0.01^{a}
Group 2 (HFD+FW)	$0.32\pm0.01^{b,c}$
Group 3 (HFD+FW + Atorvastatin)	$0.17\pm0.10^{a,c,e}$
Group 4 (HFD+FW + Pax)	$0.13\pm0.02^{a,d,e}$

The results were expressed as mean ± SEM (= standard error of the mean) of five determinations (n=5) and subjected to one-way analysis of variance (ANOVA). Means in the same column with different superscript alphabets on the same position, differ significantly at 95% level of significance ($p < 0.05$) using Tukey's test of significance.
MDA: Malondialdehyde.

Table 3: Total antioxidant capacity (TAC) levels of the rats fed high-fructose diet

Groups	TAC (µmol/ml)
Group 1 (Control)	0.42 ± 0.03^{a}
Group 2 (HFD+FW)	$0.21\pm0.06^{b,c}$
Group 3 (HFD+FW + Atorvastatin)	$0.38\pm0.03^{a,d,e}$
Group 4 (HFD+FW + Pax)	$0.49\pm0.04^{a,d,e}$

The research results were expressed as mean ± SEM (= standard error of the mean) of five determinations (n=5) and subjected to one-way analysis of variance (ANOVA). Means in the same column with different superscript alphabets on the same position, differ significantly at 95% level of significance ($p < 0.05$) using Tukey's test of significance.
TAC: Total antioxidant capacity.

Discussion

High fructose consumption has been implicated in the genesis of metabolic disorders, fostered by the systemic inflammation and oxidative stress they cause, which are both pivotal in the development of metabolic dysregulation (Johnson *et al*., 2007). Such disorders significantly contribute to the rising burden of metabolic syndrome, typified by insulin resistance, obesity, and cardiovascular complications. Inflammation, marked by elevated levels of High-sensitive C-reactive protein (Hs-CRP), serves as a key player in these pathophysiological mechanisms, often exacerbated by the oxidative stress resulting from excessive fructose intake (Ballatori *et al.,* 2009). This interplay between inflammation and oxidative stress instigates a vicious cycle, intensifying the impact of a high fructose diet on overall metabolic health. The present study investigates the potential therapeutic effects of herbal bitters (Paxherbal bitters) on inflammation and oxidative stress markers in rats subjected to a high fructose diet. Understanding their impact may offer insights into novel strategies mitigating fructose-induced metabolic disturbances. The investigation revealed a noteworthy increase in High sensitive C-reactive protein (Hs-CRP) and Malondialdehyde (MDA) levels in rats subjected to a high fructose diet and 10% fructose water in comparison to these same parameters in normal control rats. Hs-CRP, an indicator of systemic inflammation, substantially rose ($P<0.05$) in the HFD+FW group compared to the Control group, indicating heightened inflammatory responses due to the high fructose diet (Johnson *et al*., 2007; Rippe and Angelopoulos, 2013; Stanhope *et al.,* 2009). This was in keeping with the study by Tandiari *et al*. (2021), which demonstrated that a high fructose diet increased inflammatory markers such as high sensitive C-reactive protein (CRP), in animal models. Likewise, Consumption of high fructose diets has been associated with the activation of inflammatory pathways, leading to tissue damage and inflammatory responses as was highlighted by Gersch *et al*., (2007).

It is noteworthy that Atorvastatin and Paxherbal bitters, displayed significant preventive effects ($P<0.05$) against the elevation of Hs-CRP. Paxherbal bitters demonstrated similar efficacy to Atorvastatin in mitigating the increase in Hs-CRP. These findings confirms the earlier claims of the possible anti-inflammatory properties of herbal bitters (Lin and Lin, 2011; Anionye *et al.,* 2015). Paxherbal was able to achieve this likely because of the plants it contains which have anti-inflammatory properties. Example of such plants are: *Aloe vera*, *Vernonia amygdalina*, *Gangronema latifolium*, *Cymbogon citratus*, *Garcinia kola*, *Chenobium murale* and *Cinnamonum cassia* (Anionye and Onyeneke, 2016a), just to mention but a few. The anti-inflammatory properties of these plants, stem from their phytochemical constituents which likely act synergistically to bring down or prevent a rise in the blood levels of HsCRP (Vivekananthan, *et al*., 2003, Anionye *et al.,* 2015). Earlier studies indicate that the bitters of this study have appreciable amounts of alkaloids, tannins, flavonoids, phenols, saponins and cyanogenic glycosides which are phytochemicals known to have anti-inflammatory properties (Anionye, and Onyeneke, 2016a; Anionye *et al.,* 2015)

Similarly, Malondialdehyde (MDA), a marker of lipid peroxidation and oxidative stress, exhibited a significant increase in the HFD+FW group, reflecting increased oxidative damage attributed to the high fructose diet (Ballatori *et al*., 2009; Stanhope *et al.,* 2009). And this corroborated the findings of Muriel *et al*. in 2021, which revealed that excessive fructose consumption is linked to an increase in the production of reactive oxygen species (ROS), leading to oxidative stress. Paxherbal bitters exhibited significantly pronounced effects ($P<0.05$) in preventing lipid peroxidation, indicating it has antioxidant properties as already postulated by Anionye *et al.,* 2015, capable of combating oxidative stress induced by the high fructose diet (Ballatori *et al.,* 2009; Sathyapalan *et al.,* 2013).

Furthermore, the high fructose diet + 10% fructose water resulted in a significant decrease in total antioxidant capacity (TAC) compared to the normal control, which is in keeping with prior research results which revealed that high fructose intake decreased antioxidant levels and antioxidant capacities, contributing to elevated oxidative stress levels (Jarukamjorn *et al.,* 2016). However, both Atorvastatin and the herbal bitters showed significant preventive effects ($P<0.05$) against this decrease in TAC levels, indicating their potential in enhancing antioxidant defense mechanisms (Johnson *et al.,* 2007; Sathyapalan *et al.,* 2013; Anionye *et al.,* 2015). Oxidative stress leads to the generation of MDA *in-vivo* via the peroxidation of polyunsaturated fatty acids (Ho *et al.,* 2013). MDA interacts with proteins and is itself potentially atherogenic. Hence its elevation can precipitate a negative cardiovascular event (Ho *et al.,* 2013). MDA's reaction with lysine residues generates lysine – lysine cross-links which have been identified in apolipoprotein B (apoB) fractions of oxidized low density lipoprotein (OxLDL), and have been postulated to impair the interaction between OxLDL and macrophages and thereby to promote atherosclerosis (Ho *et al.,* 2013) and indirectly causing hypertension which is a major hallmark of metabolic syndrome (Jegatheesan, and De Bandt, 2017; Elufioye and Mada, 2018). Antioxidants are major key players in mitigating the adverse effect of oxidative stress by continuous eradication of excess free radicals, and its depletion equals an increased oxidative stress induced cellular and systemic destruction. It therefore is a positive finding of this study that Paxherbal bitters was able to prevent the high fructose diet from causing the oxidative stress that would have led to the rise of MDA and a decrease in the total antioxidant capacity (TAC) of the system. The bitters were likely able to achieve their effect because of its constituent antioxidant plants and antioxidant phytochemical constituents. Previous studies have shown that Paxherbal bitters contain herbaceous plants and species with their constituent phytochemicals or biological compounds that are harmless sources for obtaining the natural antioxidants (Anionye *et al.,* 2015; Anionye and Onyeneke, 2016a). Its plant constituents known to possess antioxidant properties include: *Xylopia aethiopica, Aloe vera, Vernonia amygdalina, Gangronema latifolium , Cymbogon citratus*, *Garcinia kola, Chenobium murale, Zingiber officinale, Capsicum annum, Garlic allium, Theobroma cacao* (Anionye *et al.,* 2015; Anionye and Onyeneke, 2016a). Examples of constituent phytochemicals in Paxherbal bitters with antioxidant and other related properties include:

i. Isoborneol (0.61%, known to be antioxidant and neuroprotective),
ii. 2,4-Ditertbutylphenol (4.61%, known to be antioxidant and antifungal), and
iii. Shogaol (15.16%, known to be anti-tussive nd antioxidant (Elufioye and Mada, 2018; Anionye and Onyeneke, 2020).

The results of this study showed that the detrimental impact of a high fructose diet causing inflammation and oxidative stress, as evidenced by the elevation of Hs-CRP and MDA levels and the reduction in TAC, were successfully ameliorating by Paxherbal bitters. This indicates that the co-administration of Paxherbal bitters had a positive therapeutic effect in preventing high fructose diet induced inflammation and oxidative stress.

CONCLUSION

The co-administration of Paxherbal bitters in the setting of increased fructose consumption, to male *Wistar* rats as seen in this study, showed Paxherbal bitters as a supplement that can mitigate the inflammatory cascade and oxidative stress triggered by a high fructose diet. This was evidenced by the way the Paxherbal bitters prevented a significant rise in the inflammatory marker—high sensitive C-reactive protein (HsCRP), as well its ability to prevent lipid peroxidation and antioxidant imbalance, by its respectively preventing a rise in MDA levels and improving the total antioxidant capacity of the rats fed with the high fructose diet. These findings substantiate the therapeutic potential of Paxherbal bitters as a preventive or adjunctive supplement against a high fructose diet-induced inflammation and oxidative stress that more often than not mediate other disorders. These promising findings advocate for further exploration and clinical investigations to harness the potential health benefits offered by Paxherbal bitters as a supplement in preventing metabolic diseases, especially those associated with the deleterious effects of excessive fructose consumption.

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