African Scientist Vol. 25, No. 2 June 30, 2024
Printed in Nigeria

1595-6881/2023 $80.00 + 0.00

https://africansciientistjournal.org

afs2024032/25208

Effect of Paxherbal Bitters on Glucose and Insulin Levels in Male *Wistar* Rats Fed a High Fructose Diet

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(Received June 17, 2024; Accepted in revised form June 20, 2024)

ABSTRACT: A diet high in fructose, leads to a metabolic syndrome, characterized by elevated blood sugar and insulin levels, amongst other metabolic disorders. This study sought to investigate if supplementation with Paxherbal bitters, could counteract the high-fructose diet-induced elevation of blood sugar and insulin levels. Twenty male *Wistar* rats, each weighing about 200 g, were divided into four groups of 5 rats each: the control, the high-fructose diet with fructose water (HFD+FW), the HFD+FW with Atorvastatin, and the HFD+FW with Paxherbal bitters, groups. After 28 days and an overnight fast, blood samples were collected for analysis of fasting blood sugar and insulin levels using the glucose oxidase and ELISA techniques, respectively. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated and statistical analysis was performed. The HFD+FW group exhibited significantly higher ($P<0.05$) blood sugar (108.60 ± 1.9 mg/dL), insulin (10.58 ± 0.40 µU/mL), and HOMA-IR (2.83 ± 0.06), compared with the control group. Paxherbal bitters significantly prevented ($P<0.05$) the rise in blood sugar (49.60 ± 6.36 mg/dL), insulin (5.13 ± 0.07 µU/mL), and HOMA-IR (0.63 ± 0.08) compared with the HFD+FW group. Paxherbal bitters supplementation demonstrated efficacy in preventing elevated blood sugar and insulin levels associated with a high-fructose diet in *Wistar* rats, suggesting it has a preventive therapeutic benefit.

Keywords: High fructose diet, Paxherbal bitters, Blood sugar, Insulin levels, HOMA-IR.

Introduction

The prevalence of metabolic disorders has escalated globally, drawing attention to dietary factors contributing to conditions like hyperglycemia and insulin deregulation. High fructose consumption, prevalent in modern diets, is linked to metabolic disruptions, including altered glucose homeostasis and increased insulin resistance. Amidst therapeutic endeavors, herbal remedies have gained traction due to their potentials in mitigating such metabolic anomalies. Herbal formulations, such as Paxherbal bitters, have garnered interest for their purported anti-diabetic properties owing to their bioactive constituents.

Previous research underscores the adverse impact of excessive fructose intake on metabolic health. Studies have demonstrated that high fructose consumption contributes to hyperglycemia by stimulating gluconeogenesis and impairing insulin sensitivity (Johnson *et al.,* 2007). An increase in free fatty acid and a dyslipidaemia that stimulates gluconeogenesis along with hyperglycaemia, following the precipitation of an insulin resistance, characterized by reduced cellular response to insulin, often ensues from prolonged high fructose consumption (Stanhope *et al.,* 2009). These disturbances contribute to the onset of metabolic syndrome, a cluster of conditions predisposing individuals to cardiovascular ailments and type 2 diabetes (Johnson *et al.,* 2007).

Herbal remedies have been a subject of scientific exploration for their potential therapeutic benefits. Paxherbal bitters, containing a blend of plant-derived compounds, may hold promise in counteracting the adverse effects of a high-fructose diet. Plants such as bitter leaf (*Vernonia amygdalina*) and *Aloe vera*, common constituents of Paxherbal bitters, have demonstrated anti-diabetic properties attributed to their bioactive compounds, including alkaloids, flavonoids, and tannins (Ojewole, 2005; Anionye *et al.*, 2015; Anionye and Onyeneke, 2016a).

The manufacturers of Paxherbal bitters claim that its uses include that "it promotes blood circulation, prevents kidney stones, helps in digestion and activates bile flow". It is said to act on both the pancreas and liver/gall bladder, helping to promote blood glucose regulation and 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" (Anionye *et al.*, 2015; Anionye and Onyeneke, 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 *et al.*, (2015), Anionye and Onyeneke, (2016a), Anionye and Onyeneke, (2020), 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. From their study it was revealed also that Paxherbal bitters derives its therapeutic potency and uniqueness from its 40 herbal constituents and a diverse array of phytochemical constituents sourced from these 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 antidiabetic, 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 the hyperglycaemia and insulin resistance 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 seeks to contribute to the understanding of Paxherbal bitters' potential in modulating glucose and insulin levels in rats consuming a high-fructose diet. By investigating the impact of Paxherbal bitters on these metabolic parameters, this research aims to elucidate its preventive or ameliorative effects against fructose-induced hyperglycemia and insulin deregulation, paving the way for potential therapeutic applications.

Materials and methods

Chemicals, drugs, and kits: Paxherbal bitters, the polyherbal supplement of interest in this study, was acquired from the producers at the Benedictine Monastery at Ewu-Ishan in Edo State, while Atorvastatin, a known ant-dyslipidaemic drug and reference drug for this study, was procured from a reputable pharmaceutical store situated opposite the University of Benin Teaching Hospital (UBTH) on Ugbowo-Lagos Road, Benin City, Edo State, Nigeria. The glucose kit utilized for the experiment to determine the fasting blood glucose was from Randox Lab UK, contained various reagents including phosphate buffer (0.1 mol/l, pH 7.0), phenol (11 mmol), and GOD-PAP reagent (4-aminophenazone (0.77 mmol/l), glucose oxidase (> 1.5 kU/l), peroxidase (> 1.5 kU/l)). The standard included in the glucose kit had a concentration of 5.49 mmol/l, equivalent to 99 mg/dl of glucose. These kits were purchased through the manufacturer's representative in Nigeria. Additionally, the Insulin Enzyme linked immunosorbent assay (ELISA) kit, acquired from MyBioSource UK, encompassed materials like microwells coated with Insulin Monoclonal Antibody, Insulin Standard Solutions, Insulin Enzyme Conjugate, Assay Diluent, TMB (Tetramethylbenzidine) substrate, stop solution, and wash concentrate. This kit was also purchased through the manufacturer's representative in Nigeria.

Experimental animals: Male *Wistar* rats (n = 20) were sourced from the Anatomy Department, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. These rats were acclimatized for two weeks, adhering to international guidelines (Canadian Council on Animal Care, 1984), and ethical approval was obtained from the Research Ethics Committee (REC) of the College of Medical Sciences, University of Benin, with approval number: CMS/REC/2023/485.

Experimental diets: The experimental procedure involved the administration of a basal diet, comprising standard pelleted grower's mash (Table 1) and a high-fructose diet (HFD) engineered to induce hyperglycemia and hyperinsulinaemia (Table 2). The metabolic syndrome-inducing diet was formulated by supplementing a proportional quantity of the basal diet with white crystalline powdered D (-) fructose, resulting in a diet with a 60 % fructose content (Abdelrahman *et al.,* 2018). This diet was supported by allowing the rats to drink *ad libitum*, 10 % fructose water (FW) (Lirio *et al.,* 2016).

Table 1: Composition of the basal diet (g/1000 g) based on of the standard pelleted growers mash of Jerrison Agro Allied Services, Benin City, Nigeria (Anionye *et al.*, 2018).

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) (Abdelrahman *et al.*, 2018).

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

Experimental design: For this study, twenty male *Wistar* rats, weighing between 180 g to 220 g were used. They were randomly divided into four groups, each serving a specific purpose:

- Group 1: Received a basal diet with clean tap water (normal control group).
- Group 2: Given a 60 % fructose diet (HFD) along with 10 % fructose-water (FW) (negative control group).
- Group 3: Given a 60 % fructose diet, 10 % fructose-water, and Atorvastatin (0.57 mg/kg-b.w) (positive control group)
- Group 4: Given a 60 % fructose diet, 10 % fructose-water, and Paxherbal bitters (600 mg/kg-b.w) (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 (Anionye and Onyeneke, 2016b; Anionye *et al.*, 2017). The dosage calculations aimed to ensure equivalence to the effective human dose.

For Paxherbal bitters: If a 70,000 g man (70 kg) consumes 40 mL, the expected consumption for a 200 g rat would be: X mL = 40 mL x 200 g / 70,000 g = 0.114 mL (~ 0.11mL)

Thus, the dosage for a 200 g rat was determined to be 0.6 $x10^{-3}$ mL/g of rat or equivalent to 0.6 mL/kg of rat body weight (b.w) or 0.6 g/kg-b.w or 600 mg/kg-b.w.

For Atorvastatin (EMDEX, 2007): If a 70,000 g man consumes 40 mg, a 200 g rat would be expected to consume:

X mg = 40 mg x 200 g / 70,000 g = 0.114 mg (requiring dissolving 0.114 mg of the drug in 1 mL of distilled water)

Therefore, the dosage for a 200 g rat would be approximately 0.57 x 10^{-3} mg/g of rat or 0.57 mg/kg of rat body weight.

Choice of Atorvastatin as the Reference Drug: An anti-hyperlipidaemic drug (Atorvastatin) was chosen as the reference drug for this study, instead of an antidiabetic drug, because the study was a preventive study in the context of inducing a metabolic syndrome involving an agent (fructose) whose aetiopathogenesis involves a lipid pathway. Atorvastatin was chosen, because it has been established that the pathogenesis of the anomalies of this study (hyperglycaemia and hyperinsulinaemia) caused by a high fructose diet is majorly the direct consequence of the dyslipidaemia (increased in blood free fatty acids, hypercholesterolaemia and hypertriglyceridaemia), which the high fructose diet causes in the first place (Sabir *et al.*, 2016). Fructose consumed in excess, ultimately affects the functions of the pancreas and the liver, via its activation of a dyslipidaemia that causes inflammation and oxidative stress (Basaranoglu *et al.*, 2015; Sabir *et al.*, 2016). Atorvastatin, in attempting to prevent the onset of dyslipidaemia in a scenario classical to metabolic syndrome induced by a high fructose diet, will prevent the onset of inflammatory and oxidative stress processes, and the resulting hyperinsulinaemia and hyperglycaemia, which would have caused further damage to the body system.

Considering all these, it was therefore chosen as the reference drug of this study, as it was the same thing the experimental supplement (Paxherbal bitters) was supposed to do.

Atorvastatin has been recommended by WHO, as an effective agent in the prevention of cardiovascular and metabolic diseases (Anionye and Onyeneke, 2020). Apart from its known anti-hyperlipidaemic effect which confers on it the ability to prevent cardiovascular and metabolic diseases, it has been shown to also have the "ancillary effect" of preventing the onset of diabetes mellitus, by its prevention of dyslipidaemia (which a high fructose diet also causes), which can precipitate insulin resistance and diabetes mellitus (Anionye and Onyeneke, 2020). Since this study was done within the context of metabolic syndrome, induced by a high fructose diet, a drug like Atorvastatin that could prevent both hyperlipidaemia, hyperglycaemia, insulin resistance, and other metabolic or physiological changes associated with metabolic syndrome, was therefore more relevant to this study.

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. Cages were managed within a 12-hour light-dark cycle, and regular cleaning and disinfection procedures were implemented to maintain a hygienic environment. The Paxherbal bitters and the Atorvastatin drug were administered using an oro-gastric gavage.

Blood sample collection: On the 29th day, after an overnight fast, the animals were anaesthetized using chloroform and blood samples were obtained via cardiac puncture. The samples were put into fluoride oxalate bottles for the evaluation of fasting blood glucose and lithium heparin bottles for the evaluation of plasma insulin levels (Aniagu *et al.*, 2015).

- *Determination of fasting blood glucose (FBG):* The Randox glucose kit was utilized, employing the glucose oxidase method as reported by Barham and Trinder (1972). Standard and sample solutions underwent preparation, treatment with Randox Glucose Reagent, incubation, and subsequent measurement of absorbance at 500nm. The concentration of glucose was computed using absorbance ratios alongside known standard concentrations.
- *Determination of insulin level:* The insulin level was determined by the ELISA method described by Engvall and Perlman (1971). The process involved a solid-phase two-site enzyme immunoassay, encompassing incubation, washing, substrate reaction, and culminating in a colorimetric endpoint measurement. Insulin activity was determined from a standard calibration curve.
- *Determination of insulin resistance and insulin sensitivity:* Insulin Sensitivity Measurement: Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was computed based on fasting glucose and insulin levels (Matthews *et al.*, 1985). The formula, HOMA-IR = (Fasting Glucose (mg/dL) × Fasting Insulin (µU/mL)/405, was used in the determination. Usually a HOMA-IR < 2.5, indicates normal insulin sensitivity, a HOMA-IR between 2.5 and 3.8, suggests borderline insulin resistance and a HOMA-IR > 3.8, indicates the likelihood of insulin resistance.

Statistical Analysis: The assessment of statistical significance was conducted using ANOVA at a 95 % confidence level, employing the SPSS v20 software statistical package. Results with $p < 0.05$ were considered significant.

Results

As shown in Table 3, the high fructose diet + 10 % fructose water (negative control group) induced a significant increase ($p < 0.05$) in FBG (108.60 ± 1.9 mg/dL) in the rats of Group 2, compared to its level in the normal control rats (53.40 ± 1.54 mg/dL) of Group 1. Atorvastatin significantly prevented this escalation ($p < 0.05$) by keeping the FBG level at 54.00 ± 1.90 mg/dL in the rats of Group 3. Interestingly, the Paxherbal bitters, with the same efficacy ($p > 0.05$) as atorvastatin, significantly prevented ($p < 0.05$) the elevation in fasting blood glucose by keeping it at 49.60 ± 6.36 mg/dL in the rats of Group 4.

The result shown in Table 4, reveal that the high fructose diet + 10 % fructose water, also induced a significant increase ($p < 0.05$) insulin level (10.58 ± 0.40 µU/mL), in the rats of Group 2, compared to its level in the normal control rats (5.70 ± 0.36 µU/mL) in Group 1. Atorvastatin significantly prevented this escalation ($p < 0.05$) by keeping the insulin levels at 5.40 ± 0.11 µU/mL in the rats of Group 3. Interestingly, the Paxherbal bitters with the same efficacy ($p > 0.05$) as Atorvastatin, significantly prevented ($p < 0.05$) the elevation in the insulin levels, by keeping it at 5.13 ± 0.07 µU/mL in the rats of Group 4.

The results in Table 5 reveal that the high fructose diet + 10 % fructose water, induced a significant increase ($p < 0.05$) in the HOMA-IR index (2.83 ± 0.06) in the rats of Group 2, compared to the its index in the normal control rats (0.75 ± 0.05) in Group 1. Atorvastatin significantly prevented ($p < 0.05$) this escalation by keeping the HOMA-IR index at 0.72 ± 0.02 in the rats of Group 3. Interestingly, the Paxherbal bitters with the same

efficacy ($p > 0.05$) as Atorvastatin, also significantly prevented ($p < 0.05$) the elevation in the HOMA-IR index, by keeping it at 0.63 ± 0.08 in the rats of Group 4.

Table 3: Fasting blood glucose levels of the rats fed high-fructose diet

Groups	Fasting Blood Glucose (mg/dL)
Group 1 (Control)	53.40 ± 1.54^{a}
Group 2 (HFD+FW)	$108.60 \pm 1.9^{b,c}$
Group 3 (HFD+FW + Atorvastatin)	$54.00 \pm 1.90^{a,d,e}$
Group 4 (HFD+FW + Pax)	$49.60 \pm 6.36^{a,d,e}$

All results are expressed as mean ± SEM (n=5). Means in the same column with different superscript on the same position, differ significantly at 95 % level of significance ($p < 0.05$).
HFD+FW: 60% High fructose diet + 10% fructose water; Pax**:** Paxherbal bitters,
HOMA-IR**:** Homeostatic Model Assessment of Insulin Resistance

Table 4: Insulin levels of the rats fed high-fructose diet and 10% fructose water

Groups	Insulin (µU/mL)
Group 1 (Control)	5.70 ± 0.36^{a}
Group 2 (HFD+FW)	$10.58 \pm 0.40^{b,c}$
Group 3 (HFD+FW + Atorvastatin)	$5.40 \pm 0.11^{a,d,e}$
Group 4 (HFD+FW + Pax)	$5.13 \pm 0.07^{a.d.e.}$

All results are expressed as mean ± SEM (n=5). Means in the same column with different superscript on the same position, differ significantly at 95 % level of significance ($p < 0.05$).
HFD+FW: 60% High fructose diet + 10% fructose water; Pax**:** Paxherbal bitters,
HOMA-IR**:** Homeostatic Model Assessment of Insulin Resistance

Table 5: HOMA-IR index of the rats fed high-fructose diet

Groups	HOMA-IR
Group 1 (Control)	0.75 ± 0.05^{a}
Group 2 (HFD+FW)	$2.83 \pm 0.06^{b,c}$
Group 3 (HFD+FW + Atorvastatin)	$0.72 \pm 0.02^{a,d,e,}$
Group 4 (HFD+FW + Pax)	$0.63 \pm 0.08^{a,d,e}$

All results are expressed as mean ± SEM (n=5). Means in the same column with different superscript on the same position, differ significantly at 95 % level of significance ($p < 0.05$).
HFD+FW: 60% High fructose diet + 10% fructose water; Pax**:** Paxherbal bitters,
HOMA-IR**:** Homeostatic Model Assessment of Insulin Resistance

Discussion

High fructose consumption, particularly in the form of a high fructose diet and fructose-rich beverages, has been linked to metabolic abnormalities such as hyperglycemia and hyperinsulinaemia. Metabolic syndrome, characterized by increased blood sugar levels, insulin resistance and altered lipid profiles, amongst other physiological and biochemical derangements, is a known consequence of excessive fructose intake (Sabir *et al.,* 2016; Softic *et al.,* 2020). The present study delved into the impact of Paxherbal bitters on glucose and insulin levels in male *Wistar* rats fed a high fructose diet, aiming to explore its potential as a possible therapeutic intervention against fructose-induced metabolic disturbances. Atorvastatin, a known anti-hyperlipidaemic drug, with ancillary effect of preventing hyperglycaemia and hyperinsulinaemia, served as the standard reference drug.

The results of this study shows that consumption of a high fructose diet (HFD) in conjunction with 10 % fructose water (FW) by the rats resulted in a substantial elevation ($p < 0.05$) in FBG, insulin and HOMA-IR compared to the results of the normal control rats (Group 1). This is because the excess fructose consumed by the rats in this study leads to denovo lipogenesis in their liver which causes a hepatic insulin resistance that results in the hyperinsulinaemia, hyperglycaemia and increase in the HOMA-IR index, seen in this study (Johnson *et al.,* 2007). The fructose consumed undergoes primary metabolism, predominantly facilitated by

KHK (ketohexokinase), leading to the formation of fructose-1-phosphate, a process favoured by KHK's lower Km (Michaelis constant) for fructose compared to hexokinase. The bypassing of the regulatory steps that controls glucose metabolism, by fructose, means that fructose is not as regulated as glucose is. So the excess fructose consumption enters a pathway that is largely unregulated. The Fructose-1-phosphate formed undergoes further metabolism facilitated by aldolase B and triokinase, ultimately resulting in the formation of glyceraldehyde-3-phosphate. Because there is little or no regulation of this pathway, excess fructose consumption results in excess glyceraldehyde-3-phosphate being produced, which is in turn converted to excess triglycerides in the liver. Insulin exerts its metabolic effects through the activation of phosphatidylinositol (PI) 3-kinase, so the impairment of this PI 3-kinase in the liver by the hepatic hypertriglyceridaemia, leads to hepatic insulin resistance and insulin being underutilized. In the presence of this hepatic insulin resistance, there is rebound increase in serum insulin (hyperinsulinaemia), reduction in liver glucose uptake (causing hyperglycaemia) and an increase in the HOMA-IR index as seen in this study. This aligns with previous studies that have consistently shown the adverse metabolic effects of high fructose intake, promoting insulin resistance and elevated blood glucose levels (Stanhope *et al.,* 2009; Softic *et al.,* 2020).

Atorvastatin, a commonly prescribed medication for managing cholesterol levels, exhibited a significant protective effect against the effect of the high fructose diet by maintaining the levels of FBG and insulin, and the HOMA-IR index, at levels comparable to the normal control rats. It was able to achieve this because by preventing the dyslipidaemia induced by a high fructose diet, it prevented the hepatic insulin resistance that would have caused an increase in blood levels of glucose and insulin. This is in line with studies indicating the potential of statins in improving insulin sensitivity and reducing insulin resistance in metabolic disorders. (Sathyapalan *et al.,* 2010; Anionye and Onyeneke, 2020).

Paxherbal bitters used in the study, showcased potential in preventing the drastic increase in FBG, insulin, and HOMA-IR levels induced by the high fructose diet. The ability of the Paxherbal bitters of this study to achieve this preventive role, is not farfetched considering the fact that it contains plants/herbs, such as *Aloe vera, Vernonia amygdalina and Gangronema latifolium,* with plant extracts (phytochemical and mineral constituents), such as complex carbohydrates, alkaloids, flavonoids, tannins, glycopeptides, peptides, amines and terpenoids, which are known to have hypoglycaemic effects, as well as effects that improve insulin sensitivity, which can also be referred to as their anti-diabetic effects (Adodo, 2002; Saidu *et al.,* 2007, Anionye *et al*., 2015; Anionye and Onyeneke 2016a; Etim *et al*., 2016; Anionye and Onyeneke 2016b). They all act synergistically to bring down or prevent a rise in the blood glucose level (Anionye *et al*., 2015). These results align with earlier research which revealed that herbal remedies like this have anti-diabetic and metabolic regulatory effects, due to their phytochemical constituent and bioactive compounds (Eidi *et al.,* 2006; Anionye and Onyeneke, 2016a; Omodanisi *et al.,* 2017).

The comparative effectiveness between Atorvastatin and Paxherbal bitters in preventing the rise in FBG, insulin, and HOMA-IR levels induced by the high fructose diet is noteworthy. Though their possible major mechanism of action is via the anti-hyperlipidaemic pathway, considering the fact that Paxherbal bitters has a myriad of phytochemicals, it may also be exhibiting its effect via other possible mechanisms of action. This indicates there may be diverse avenues for addressing metabolic disturbances associated with excessive fructose intake.

Conclusion

This study revealed that the co-administration of Paxherbal bitters as well as the drug Atorvastatin, in the situation were a high fructose diet is commonly consumed can result in positive healthy outcomes. The co-administration of Paxherbal bitters effectively countered the significant increase in FBG and insulin levels, and the HOMA-IR index, observed in groups of rats not given this polyherbal supplement, but given only a high fructose diet. This indicates that Paxherbal bitters has the potential in mitigating the adverse effects induced by a high fructose diet. Further investigations and clinical trials are therefore recommended to elucidate their mechanism of action and underlying efficacy in humans, paving the way for their potential integration into therapeutic strategies aimed at preventing or ameliorating metabolic abnormalities associated with diet-induced metabolic disturbances, especially from excessive fructose intake. Overall, this study underscores the promising role of Paxherbal bitters as a potential natural supplement in preventing the adverse effects of high fructose diets on metabolic health.

Acknowledgments

Our appreciation goes to the staff of Medical Biochemistry Department, University of Benin, who made the period of the research conducive for us and the staff of Quality Immunodiagnostic Pathology, Laboratory and Research Services for their assistance, in some of the laboratory tests undertaken during this research.

Institutional Review Board/Ethical Clearance

The study was conducted according to the guidelines of the international protocols on care of animals promoted by the Canadian Council on Animal Care, (1984), and was approved and given ethical clearance by the Research Ethics Committee (REC) of the College of Medical Sciences, University of Benin. The REC Approval Number being: CMS/REC/2023/485.

Funding Statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. It was funded by the authors.

Conflict of Interest

The authors declare no conflict of interest.

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