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Modulatory Impact of Methanolic Extract of *Irvingia gabonensis* Leaves on Selected Biochemical Indices in Fat-Loaded Sprague-Dawley Rats.

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ABSTRACT

Obesity is among the health conditions that have challenged orthodox medicine. Over time, natural herbal medicines have been used to prevent and treat diseases. Therefore, this study evaluated the potential of the methanolic extract of Irvingia gabonensis (bush mango leaf) to ameliorate biochemical changes in Sprague-Dawley rats fed high-fat diets. The methanolic extract of I. gabonensis leaf was administered orally at a dose level of 400 mg/kg) body weight, and the reference anti-obesity drug Orlistat® (30 mg/kg body weight), respectively, for four weeks showed no significant difference ($p>0.05$) in serum blood glucose concentration across the groups. However, a significant decrease ($p<0.05$) in body weight, liver function markers (ALP, AST, ALT, albumin, total protein, total bilirubin and conjugated bilirubin), kidney function markers (urea and creatinine) and an increase in serum potassium, sodium and chloride were observed in the high-fat diet-induced treated groups when compared with the induced non-treated rats. This study also found that the high-fat diet altered systems in the liver, kidney, and heart. It caused histopathological changes in the architecture of these organs. Treatment with I. gabonensis extract, however, significantly ($p<0.05$) restored these alterations to near-normal values. The results suggest that I. gabonensis extract has ameliorative effects against biochemical changes associated with high-fat diet consumption in Sprague-Dawley rats. The plant extract may be explored for the management of Obesity.

Key words: *Irvingia gabonensis,* High-Fat, Obesity, Sprague-Dawley

INTRODUCTION

Obesity is a metabolic disorder of global health importance characterised by the accumulation of excess body fat and increased body weight gain (Spiegelman and Flier, 2001). Obesity is a major risk factor for other metabolic disorders. It is associated with a high risk of various kinds of chronic diseases, such as atherosclerosis, hyperlipidaemia, hypertension, insulin resistance, fatty liver and cancer (Wellen and Hotamisligil, 2005). The most highly recommended preventive and treatment strategy for high-fat diets is lifestyle modification, such as regular physical exercise and diet control (Chandrasekaran *et al.*, 2012). However, the sedentary lifestyle and busy schedules of most patients make this preventive and treatment strategy difficult to implement. Furthermore, several synthetic anti-obesity drugs, such as orlistat, rimonabant, and sibutramine, have been approved as a therapeutic approach to combat obesity (Van Gaal *et al.*, 2005). However, the loss of efficacy and side effects associated with these synthetic drugs have limited their overall usefulness (Brenot *et al.*, 2013).

Irvingia gabonensis (Family: Irvingiaceae), commonly known as African wild mango or African bush mango, is a polyphenolic-rich plant native to Western and Central Africa (Arogba, 2014). It is used in folkloric medicine to treat many diseases, including diabetes mellitus (Oloyede, 2005). Ethnomedicinal treatments use the bark, kernels, leaves, or roots to manage several ailments, including diarrhoea, hernias, yellow fever, and dysentery, as well as to reduce the effects of poison in French Equatorial Africa (Ainge and Brown, 2001; George and Zhao, 2007). In certain parts of Africa, the bark extract is ingested to produce an analgesic effect (George and Zhao, 2007). The hypoglycaemic and anti-diabetic effects of the plant extracts have been reported (Ngondi *et al.*, 2005; Ngondi *et al*, 2006). Studies have also shown that *I. gabonensis* possesses diuretic (Nosiri *et al.,*

2011), antioxidant (Ekpe *et al.,* 2019), anti-ulcer (Nosiri *et al.,* 2018), hypolipidemic, hypoglycaemic, hepatoprotective, and anti-obesity effects (Ngondi *et al.*, 2005; Omonkhua and Onoagbe, 2012a).

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats aged 6 weeks and weighing 150±20g were obtained from the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Lagos, Nigeria.

Collection of Plant Material

Irvingia gabonensis leaves were obtained from a private farm at Obazagbon Community, Oredo L.G.A, Benin City, Edo State, Nigeria. A botanist, Dr Timothy Odara of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria, validated the fresh leaves. A voucher specimen (voucher number: UBHi153) was thereafter deposited at the Department's herbarium.

Extraction of *Irvingia gabonensis* leaves.

The fresh leaves of *Irvingia gabonensis* were air-dried for two weeks in the shade, and the dried material was further pulverised into a coarse powder. 10000 g of dried, pulverised *Irvingia gabonensis* was soaked in 5.5 litres of methanol, periodically stirred for 72 hours, and filtered through a double-layered muslin cloth. The filtrate was then concentrated to dryness using a rotary evaporator at 60 °C. It was further dried using a freeze dryer and stored in sterile bottles for analysis.

Acute Oral Toxicity Study

Acute oral toxicity study of the methanol extract of *Irvingia gabonensis* was carried out on Sprague-Dawley rats according to the Organisation for Economic Co-operation and Development (OECD) 2001 Guideline 423. A total of fifteen (15) Sprague-Dawley rats (150± 20 g) were selected and fasted overnight before administration of different concentrations of the extracts, but water was not withheld. The rats were divided into five (5) groups, and varying doses of *Irvingia gabonensis* (100, 250, 500, 1000 and 2000 mg/kg body weight) were administered to the rats orally in a single dose by gavage. After administration of the extract, the Sprague-Dawley rats were observed closely for physical evidence of toxicity by monitoring changes in skin, somato-motor activity, mucous membrane, fur, eyes, behavioural patterns, tremors, salivation, convulsion, diarrhoea, sleep, lethargy, coma and mortality from the first minute of administration to 30minutes, 4 hours, then over a period of 24 h and thereafter daily for 14 days. Food consumption and weight changes were also recorded during the observation period.

Experimental Design

In this study, Sprague-Dawley rats were exposed to a high-fat diet (HFD) (Table 1) following the standard method of Bais *et al* (2014). Sixty male Sprague-Dawley rats weighing 160 ± 20 were divided into four groups of six rats each and were treated as follows:

Group 1: Normal control (ND)
Group 2: High-fat diet (HFD) + 1 % NaCl (in drinking water)
Group 3: HFD + *Irvingia gabonensis* (400 mg/kg) + 1 % NaCl (in drinking water)
Group 4: HFD + Orlistat (30 mg/kg) + 1 % NaCl (in drinking water)

All animals were initially acclimatised for two weeks before the start of the study and were maintained on a normal diet during this period. The animals were then exposed to the various diet combinations for eight weeks and treated with *Irvingia gabonensis* (400mg/kg) extract or the reference drug Orlistat® ug (30mg/kg), depending on the group, for another four weeks. Water and food were given to the animals *ad libitum* throughout the duration of the study; their body weight, BMI and blood glucose concentration were taken weekly.

Collection of Blood and other Tissue Samples

At the end of the study, the animals were fasted overnight and were sacrificed through cervical dislocation. The animals were dissected, and blood samples were collected through cardiac puncture and placed in plain tubes (for serum and other biochemical assays). To obtain serum, the blood samples collected in plain tubes were allowed to stand for 2 hours and then centrifuged at 3000 rpm for 10 min. The liver, heart and kidney tissues were carefully excised, and portions were taken for some biochemical assays and histological examinations.

Preparation of Tissue Homogenates

Liver, heart, and kidney tissues were harvested after sacrificing the animals and placed in plain containers containing 10 mL of normal saline, then stored on ice (4°C). Then, 1 g of each tissue was homogenised in 9 mL of ice-cold phosphate-buffered saline (0.9%). The resulting homogenates were centrifuged at 5000 rpm for 10 min, and the supernatant obtained was used for subsequent analysis. (Mohammed *et al.,* 2014)

Table 1. Composition of the Experimental Diets

Nutrient	ND	HFD
Corn starch	62.5 %	46.5 %
Sucrose	10 %	10 %
Fish meal	14 %	14 %
Lard	4 %	20 %
Vitamin mix	3.5 %	3.5 %
Mineral mix	1 %	1 %
Coconut chaff	5 %	5 %

Cha and Jones (1998)

RESULTS AND DIS

Metabolic syndrome as a result of high-fat diets is among the health conditions that have defied orthodox medicine; hence, it remains a task for scientists to continually explore alternative natural products with therapeutic benefits for their management. The present study was set out to evaluate the potential of *Irvingia gabonensis* leaf extracts in ameliorating possible biochemical and metabolic aberrations associated with consumption of a high-fat diet. Findings in this study showed that the *I. gabonensis* extract had no deleterious effect on Sprague-Dawley rats, suggesting that the animals can tolerate the plant extract and its bioactive components up to the highest dose (2000 mg/kg). This may justify its use in treating different diseases locally and for consumption as food.

Effect of methanol extract of *Irvingia gabonensis* leaf on mean body weight of Sprague-Dawley rats fed on high-fat diets.

Groups	Initial weight (g)	Final weight (g)	Weight gain (g)
ND	164.75 ± 7.23^{a}	229.5 ± 15.38^{a}	64.75 ± 10.85^{a}
HFD	210.00 ± 2.84^{c}	329.5 ± 10.01^{b}	98.75 ± 9.67^{d}
HFD+Ig	222.50 ± 7.54^{cd}	300.25 ± 2.49^{b}	77.75 ± 7.69^{e}
HFD+Orl	229.50 ± 2.50^{d}	324.25 ± 7.97^{b}	94.75 ± 5.80^{d}

Data are presented as mean ±SEM (n = 5). Values followed by different alphabet superscripts within a column are significantly different at p <0.05. ND = Normal control; HFD = High fat diet; HFD+Ig = High fat diet + *Irvingia gabonensis* (400mg/kg) extract; HFD+Orl = High fat diet + Orlistat (30 mg/kg)

Effect of methanol extract of *Irvingia gabonensis* leaf on organ weight of Sprague-Dawley rats fed on high-fat diets.

Group	Heart (g)	Liver (g)	Kidney (g)
ND	0.65 ± 0.02^{a}	6.10 ± 0.26^{a}	1.05 ± 0.06^{a}
HFD	0.80 ± 0.04^{a}	9.93 ± 0.36^{b}	1.48 ± 0.04^{ab}
HFD+Ig	0.77 ± 0.02^{a}	8.47 ± 0.33^{ab}	1.30 ± 0.05^{a}
HFD+Orl	0.82 ± 0.03^{a}	8.76 ± 0.25^{bc}	1.44 ± 0.07^{ab}

Data are presented as mean ±SEM (n = 5). Values followed by different alphabet superscripts within a column are significantly different at p <0.05. ND = Normal control; HFD = High fat diet; HFD+Ig = High fat diet + *Irvingia gabonensis* (400mg/kg) extract; HFD+Orl = High fat diet + Orlistat (30 mg/kg)

Several studies have established a direct link between high-fat diets and diabetes (Golay and Ybarra, 2005; Nguyen *et al.,* 2011; Wang *et al.,* 2016). Based on the above, this study also investigated the role of *I. gabonensis* in modulating blood sugar levels in rats fed high-fat diets. However, there were no significant differences (p > 0.05) across the groups studied. Weight gain is a major risk factor for

obesity (Jiang *et al.*, 2016; Anronow, 2017; Ebrahim *et al.,* 2020). A significant increase was observed in the weight of the High-Fat diet (HFD) untreated group compared to the treated groups (HFD+Ig and HFD+Orl). This significant reduction in the treated groups, compared with the untreated group, in relative organ and body weight is similar to that reported by Lazic *et al.* (2020).

Serum electrolytes, urea, and creatinine are well-established markers of proper renal function (Achuba, 2018). Also, several studies have previously reported that the incidence of high levels of intracellular electrolyte imbalance, as well as urea and creatinine, in high-fat diet-exposed patients often results from the malfunction of the Na^{+}/K^{+-} ATPase pump (Grobee, 1994; Matute *et al.*, 2017; Ebrahimi *et al.,* 2018; Iqbal *et al.*, 2019). The effectiveness of *I. gabonensis* extract in significantly reversing changes in electrolyte balance may be linked to several bioactive agents, such as flavonoids, alkaloids, and polyphenols (Arogba, 2014; Ewere *et al.,* 2016). Furthermore, the extract's ability to reverse the decrease in potassium and sodium concentrations is similar to previous work by Emejulu *et al.* (2016), where fruit extract juice increased serum potassium concentrations, implying that the plant extract may be a rich source of this cation. Elevated sodium, chloride, urea, and creatinine are indices of renal malfunction; thus, their rise often occurs when the kidney loses its ability to clear them from the circulation properly. As stated earlier, high-fat diets may have contributed to renal damage; hence the rise in serum sodium, chloride, urea, and creatinine levels in the HFD-fed groups.

Effect of methanol extract of *Irvingia gabonensis* leaf on relative organ – body weight of Sprague-Dawley rats fed on high-fat diets

Groups	Heart ($\times 10^{-3}$)	Liver ($\times 10^{-2}$)	Kidney ($\times 10^{-3}$)
ND	2.67	2.49	4.31
HFD	2.74	3.39	5.07
HFD+Ig	2.58	2.83	4.33
HFD+Orl	2.48	2.68	4.64

Data are presented as mean ±SEM (n = 5). Values followed by different alphabet superscripts within a column are significantly different at p <0.05. ND = Normal control; HFD = High-fat diet; HFD+Ig = High-fat diet + *Irvingia gabonensis* (400mg/kg) extract; HFD+Orl = High-fat diet + Orlistat (30 mg/kg).

Effect of Methanol Extract of *Irvingia gabonensis* Leaf on Serum Electrolyte Concentrations of Sprague-Dawley Rats Fed on High-Fat Diets

Groups	K (mEq/L)	Na (mg/l)	Cl (mEq/L)
ND	5.42 ± 0.08^{a}	25.35 ± 2.92^{a}	102.74 ± 0.66^{a}
HFD	4.82 ± 0.10^{ab}	33.05 ± 0.48^{c}	99.32 ± 2.38^{a}
HFD+Ig	4.71 ± 0.10^{ab}	30.73 ± 0.34^{c}	94.47 ± 0.43^{c}
HFD+Orl	4.64 ± 0.05^{ab}	31.00 ± 0.46^{c}	94.94 ± 0.94^{c}

Data are presented as mean ±SEM (n = 5). Values followed by different alphabet superscripts within a column are significantly different at p <0.05. ND = Normal control; HFD = High-fat diet; HFD+Ig = High-fat diet + *Irvingia gabonensis* (400mg/kg) extract; HFD+Orl = High-fat diet + Orlistat (30 mg/kg).

Effect of Methanol Extract of *Irvingia gabonensis* Leaf on Kidney Function of Sprague-Dawley Rats Fed on High Fat Diets

Groups	Creatinine (mg/dL)	Urea (mg/dL)
ND	0.36 ± 0.09^{a}	5.88 ± 0.04^{a}
HFD	0.54 ± 0.09^{e}	6.09 ± 0.09^{c}
HFD+Ig	0.42 ± 0.07^{a}	5.99 ± 0.12^{ac}
HFD+Orl	0.35 ± 0.11^{a}	5.66 ± 0.07^{a}

Data are presented as mean ±SEM (n = 5). Values followed by different alphabet superscript within column are significantly different at p <0.05. ND = Normal control; HFD = High-fat diet; HFD+Ig = High-fat diet + *Irvingia gabonensis* (400mg/kg) extract; HFD+Orl = High-fat diet + Orlistat (30 mg/kg).

The liver, as the body's metabolic hub, can be considered the engine room of metabolic functions. This is so because it is here that the breakdown and synthesis of several essential metabolites, including drugs, micro- and macro-molecules, are organised, mobilised and distributed to other parts of the body where they are needed (Kuntz and Kuntz, 2008; Rui, 2014; Chiang, 2014; Alamri, 2018).

In the present study, the observed significant elevation of serum liver function enzymes ALP, AST, and ALT in the untreated, experimentally induced high-fat diet (HFD) groups indicates a possible alteration in liver physiologic state and function. These enzymes, when elevated in serum, are often used as a first-line indicator of possible liver damage (Giannini *et al.,* 1999; Anahat and Randolph, 2012). On the other hand, the ability of the plant extract of *I. gabonensis* to significantly mediate the reversal of the reported rise in these enzymes in the HFD+Ig group, comparable to the normal control group, may be an indication of the ability of the plant leaf extracts to improve hepatic functions.

In considering the earlier assertion of a possible disarmament of the plasma membrane in the current study, the increased levels of serum albumin in only the untreated groups (HFD) may be a justification of the said assertion as albumin is a major component of the plasma membrane and often acts as a transporter for several lipoproteins (Cooper, 2000; Garcia-Martinez *et al.,* 2013 Mathew *et al.,* 2020). Also, rising serum albumin and protein concentrations have been increasingly implicated in renal malfunction linked to high-risk cardiovascular anomalies such as high-fat intake (Yang *et al.,* 1997). It is opined that with high-fat intake, there is a change in the rate of balance between muscle protein synthesis and degradation; thus, this imbalance often leads to increased availability of proteins in circulation, as the rate of absorption for tissue repair is often greatly impaired (Katsanos and Madarino, 2011; Beals *et al.,* 2019). In the present study, the elevation of serum conjugated bilirubin in the untreated groups (HFD) compared to the normal control further supports our earlier postulation of possible high-fat-mediated hepatic compromise. It is pertinent to state here that conjugated bilirubin is often water-soluble, which is usually compartmentalised alongside the bile and is often removed from circulation alongside the bile in the liver (Te, 2019).

Effect of Methanol Extract of *Irvingia gabonensis* Leaf on Some Liver Function Enzymes of Sprague-Dawley Rats Fed on High-Fat Diets

Groups	ALP (U/l)	AST (U/l)	ALT (U/l)
ND	25.85 ± 2.90^{a}	126.31 ± 12.51^{a}	28.66 ± 0.29^{a}
HFD	51.23 ± 7.55^{b}	184.21 ± 15.27^{d}	33.57 ± 1.08^{b}
HFD+Ig	29.59 ± 0.96^{c}	80.92 ± 12.68^{e}	30.30 ± 0.54^{ab}
HFD+Orl	31.77 ± 1.19^{c}	31.57 ± 1.18^{f}	28.92 ± 0.34^{a}

Data are presented as mean ±SEM (n = 5). Values followed by different alphabet superscript within column are significantly different at p <0.05. ND = Normal control; HFD = High-fat diet; HFD+Ig = High-fat diet + *Irvingia gabonensis* (400mg/kg) extract; HFD+Orl = High-fat diet + Orlistat (30 mg/kg).

Effect of Methanol Extract of *Irvingia gabonensis* Leaf on Some Liver Function Indices of Sprague-Dawley Rats Fed on High Fat Diets

Groups	Total Protein (g/dL)	Albumin (g/dL)	Total BIL. (mg/dL)	Conjugated BIL. (mg/dL)
ND	9.81 ± 0.06^{a}	22.88 ± 1.53^{a}	2.04 ± 0.05^{a}	3.76 ± 0.07^{a}
HFD	12.07 ± 0.59^{c}	21.91 ± 0.56^{a}	3.15 ± 0.17^{b}	5.22 ± 0.24^{b}
HFD+Ig	10.24 ± 0.35^{ab}	20.41 ± 0.89^{a}	2.43 ± 0.04^{a}	4.13 ± 0.06^{c}
HFD+Orl	10.20 ± 0.22^{ab}	21.60 ± 1.32^{a}	2.24 ± 0.02^{a}	4.00 ± 0.08^{ac}

Data are presented as mean ±SEM (n = 5). Values followed by different alphabet superscripts within a column are significantly different at p <0.05. ND = Normal control; HFD = High-fat diet; HFD+Ig = High-fat diet + *Irvingia gabonensis* (400mg/kg) extract; HFD+Orl = High-fat diet + Orlistat (30 mg/kg).

Serum lipid profiling is a well-known diagnostic marker for assessing, monitoring, and managing obese patients. In the current study, serum lipid profiles (total cholesterol, triacylglycerol, HDL-cholesterol, LDL-cholesterol, and VLDL-cholesterol) were monitored to provide insight into the health status of rats fed a high-fat diet, as well as how *I. gabonensis* extract treatment contributed to this response. Findings from the study revealed a significant rise in serum total cholesterol and triacylglycerol levels in the untreated groups (HFD) relative to the normal control. This observation aligns with previous reports in high-fat-induced animals (Shamekh *et al.,* 2011), where total cholesterol and triacylglycerol levels often increase. Also, as observed in this study, there was a

concomitant reduction in HDL-cholesterol levels, often referred to as the good cholesterol, in the untreated high-fat diet-fed groups (HFD) compared with the normal control. This finding corroborates earlier reports of high-fat-mediated dyslipidemia (Ames, 1991; Klop *et al.,* 2013; Chan *et al.,* 2016; Laaksonen *et al*., 2018). Treatment with *I. gabonensis* extracts reversed high-fat diet-mediated dyslipidemia by modulating lipid profile parameters, similar to the standard reference drug orlistat® (HFD+Orl) and was comparable to the normal control.

Effect of Methanol Extract of *Irvingia gabonensis* Leaf on Lipid Profile of Sprague-Dawley Rats Fed on High Fat Diets

Groups	Total CHOL (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
ND	109.69 ± 1.56^{ad}	143.67 ± 5.08^{a}	54.72 ± 0.35^{a}	80.50 ± 2.36^{a}	28.73 ± 1.01^{a}
HFD	219.05 ±39.20^{c}	179.16 ± 3.53^{c}	51.34 ± 0.20^{a}	182.79 ±38.76^{c}	35.83 ± 0.70^{c}
HFD+Ig	107.67 ± 0.73^{a}	139.53 ± 5.10^{a}	52.35 ±0.49^{ab}	79.33 ± 0.81^{a}	27.90 ± 1.02^{a}
HFD+Orl	111.21 ± 1.72^{ad}	133.89 ± 5.03^{d}	52.20 ±0.16^{ab}	84.00 ± 1.78^{a}	26.77 ± 1.00^{a}

Data are presented as mean ±SEM (n = 5). Values followed by different alphabet superscript within column are significantly different at p <0.05. ND = Normal control; HFD = High fat diet; HFD+Ig = High fat diet + *Irvingia gabonensis* (400mg/kg) extract; HFD+Orl = High fat diet + Orlistat (30 mg/kg).

CONCLUSION

Results from the studies demonstrated that *Rhizophora racemosa* and *Rhizophora harrisonii* leaf extracts are effective, environmentally friendly corrosion inhibitors for mild steel in nitric acid medium. Their high inhibition efficiencies, favourable adsorption characteristics, and environmentally benign nature highlight their potential as sustainable alternatives to conventional synthetic corrosion inhibitors in industrial applications.

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