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Preventive Effect of *Irvingia Wombolu* Pulp and Peel Extracts against High Fat-High Fructose Diet Induced Insulin Resistance in Rats

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ABSTRACT

Production of free radicals is a normal metabolic process which if uncontrolled can result in metabolic disturbances such as hyperglycaemia and insulin resistance. The management of metabolic syndrome as well as type II diabetes, therefore should take into account the existence of these conditions: free radical production, hyperglycemia and insulin resistance. Irvingia wombolu (IW) has been previously shown to have antioxidant and antihyperglycemic potential in streptozotocin induced diabetic rats. This study was conducted to establish the preventive effect of the aqueous pulp extracts (APE) and hydroethanolic peel extracts (HPE) of IW on a high fat-high fructose (HFHF) diet induced insulin resistance in rats. Chemical analysis and antioxidant capacity of the extracts were determined. The animals were divided randomly into four groups of five rats each; the control group (C), those receiving only HFHF diet; rats receiving HFHF diet + APE and the group receiving HFHF + HPE. After an experimentation period that lasted six weeks, fasting blood glucose levels, triglycerides and total cholesterol concentrations, body and liver weight were measured. A protective effect of extracts during induction of insulin resistance by diet was notified by less increase of: fasting plasma glucose and triglycerides, post-prandial glycaemic peak. Antioxidant status measured by FRAP antioxidant capacity; SOD, catalase, reduced glutathione and the level of reduced protein groups were also ameliorated. These results suggest that early APE and HPE administration to HFHF fed rats could prevent diabetes complications and modulate insulin resistance.

Keywords: High fat-high fructose diet, insulin resistance, *Irvingia wombolu* extracts

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INTRODUCTION

Metabolic syndrome is a pathophysiological state with insulin resistance as a major characteristic[1,2]. The macronutrient content of diets has been linked to the insulin resistance syndrome. Previous works have shown that, high-fat, particularly high saturated fat diets induced weight gain, insulin resistance, and hyperlipidemia in humans and animals[3,4]. In addition to the known effects of dietary fats, dietary fructose has been shown to produce weight gain and induce insulin resistance, hyperlipidemia, and hypertension in experimental animals[4]. The prevalence of metabolic syndrome has dramatically increased worldwide due to a modern lifestyle and an increased consumption of high sugar diets especially fructose[5]. The increase in fructose consumption is largely because of an augmentation in the consumption of soft drinks and many other beverages with high fructose levels, and the consumption of foods such as breakfast cereals, baked foods, condiments, and desserts sweetened with sucrose and high fructose corn syrups[6]. There is clinical and epidemiological evidence that suggests a progressive association between fructose consumption and metabolic syndrome[7,8]. A seven-day high fructose feeding period was followed by a reduction in insulin binding and insulin sensitivity[9]. Also diets containing 15% of energy from fructose caused detrimental changes in glucose metabolism in both normal and hyperinsulinemic males[10]. Animal studies have also suggested that a high-fructose diet induces insulin resistance in both skeletal muscle and liver[11], and also leads to hypertriglyceridemia[12] and hypertension[13].

Irvingia spp. (*Irvingia gabonensis* which has a sweet edible pulp, and *Irvingia wombolu* (IW) which has a bitter inedible pulp) are found growing wild in the humid lowland forests of tropical Africa in Angola, Cameroon, Central African Republic, Congo, Equatorial Guinea, Gabon and Zaire with IW additionally extending to Senegal[14]. *Irvingia spp*. are commonly known as the African mango, Dika nut, bush mango, wild mango, or Manguier sauvage in french. Studies have also shown that IW fruits have an antioxidant and anti-hyperglycemic potential in streptozotocin induced diabetic rats[15]. This study however investigates the potential properties of extracts of the pulp and peeling of IW to protect against insulin resistance and oxidative stress induced by a HFHF diet in rats.

MATERIALS AND METHOD

Composition of high fat high fructose diet

The High Fat High Fructose (HFHF) diet consisted of 25.3% fructose (administered orally by gavage at a dose of 5g/kg body weight), 25% lipids, 15% carbohydrate, 22.5% proteins, 1.2% vitamins, 5.5% minerals and 5.5% fiber. The control group received standard diet for 6 weeks.

Plant material:

Fruits of *Irvingia Wombolu* (IW) were harvested in March 2007 in the administrative department of Dja, Ebolowa, South region, Cameroon.

Extraction of Plant Material

After drying in an oven at 50^{o}C for 3 days, the pulp and peel were separated from the kernel and ground using an electric blender and store in a dessicator. Ground materials (100g each) were extracted by maceration for 48 h with 1 litre of solvent (aqueous or hydroethanolic (1:1)). The resulting supernatant was filtered and evaporated in an oven at 50°C until completely dried. The yield of extraction was 10% and 7.8% respectively for aqueous and hydroethanolic extracts of pulp and peel respectively. Dried extracts were ground into powder using an electrical blender and stored in a desiccator.

Animals and treatment Schedule

Twenty adult male albino wistar rats weighing 250-300g were obtained from the animal house of the Department of Biochemistry, University of Yaounde I, Cameroon. The animals were acclimatized for 6 days with a 12 h light and 12 h dark cycle before the start of experimentation. Standard feed and water was provided *ad libitum* to all experimental animals. After acclimatization, they were randomly divided into four groups (n=5 each): Group I was the normal control group, fed with a standard diet; Group II was the diet control group, fed with a HFHF diet, Group III was the HPE-treated group, fed with a HFHF diet plus hydroethanolic peel extract and group IV was the APE- treated group, fed with a HFHF diet plus aqueous pulp extract. Peel and pulp extracts were suspended in DMSO 10% (v/v) and administered by oral gavage at a dose of 400 mg/kg body weight/rat/day for 6 weeks. The body weight and fasting blood glucose levels of all rats were recorded at regular intervals during the experimental period. The schedules and procedures were performed in the animal house of the Laboratory of Biochemistry of the University of Yaounde I, Cameroon. The study protocol was approved by the institutional animal ethical committee.

Oral Glucose Tolerance Test (OGTT)

The OGTT was performed at the end of the experimental period. After a fasting period of 12h, each extract was administered at dose of 400mg/kg body weight and 30min after, an oral glucose load (2g/kg body weight) was administered. Blood samples were collected from the tail vein at 0, 30, 60, 90 and 120 min for determination of glucose levels using an Accu-Check Blood Glucose Monitor .

Blood and tissue collection

After 6 weeks of treatment, 12-hour-fasted animals were sacrificed by cervical decapitation. Blood was collected in EDTA tubes and centrifuged at 3000 rpm for 10 min to obtain plasma which was stored at -20°C for the measurement of biochemical parameters. Tissues (liver, kidney and heart) were collected, rinsed in 0.9% saline solution and homogenates prepared using in saline solution (0.9%). The obtained supernatants were stored at -20°C for further analysis. Erythrocyte suspension was prepared after washing pellets three times using 0.9% saline solution after which were they lysed by adding water and stored at -20°C for further use.

Biochemical analysis

Plasma glucose, triglycerides and cholesterol

Plasma glucose was estimated by the GOD-PAP method [16], triglycerides by the GOP-PAP method[17], total cholesterol by the CHOD-PAP method[18] and HDL cholesterol by the CHOD-PAP method[19].

Lipid oxidation

Plasma and tissue (heart, liver, and kidney) hydroperoxide and TBAR's concentrations were assessed as described respectively by. Jiang et *al*[20] and Gutteridge and Wilkins[21].

Superoxide dismutases (SOD)

Erythrocytes SOD activity was assessed as directed by Misra et Fridovish [22].

Thiol groups

Plasma, erythrocyte and tissue (heart, liver, kidney) SH groups were assayed as described by Ellman[23].

(*Ferric Reducing antioxidant Power*) *FRAP*

Plasma, erythrocyte and tissue (heart, liver, kidney) antioxidant status was evaluated using the FRAP assay as was described by Benzie and Strain[24].

Statistical analysis

All data were expressed as mean ± standard deviation. Significant differences among the groups were determined by the test of Kruskal-Wallis followed by the Bonferonni post hoc test using the Statistical Package for Social Sciences (SPSS) analysis program. Results were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

In addition to the variation of glycemia in post-prandial period, fasting blood glucose and glycemia after 120 min in post-prandial period were used as indirect markers of the insulinoresistance[26]. Figure 1 shows the changes in plasma glucose concentrations of rats fed

with HFHF diet with or without IW extracts after a glucose load. Results showed that blood glucose concentration of the HFHF group was significantly higher than that of the ND group at 30min and in all time intervals. Co-administration of HPE and APE with HFHF diet significantly lower blood glucose elevation 30 min after the glucose loading. The significant increase in glucose concentration after glucose loading in rats fed with HFHF diet alone indicates that the ability of insulin to stimulate glucose disposal is markedly impaired in peripheral tissues associated with insulin resistance by fructose feeding[27].

The figure 2 below shows the effects of sub-acute administration of *Irvingia wombulu* fruit pulp and peel extracts on body weight. Consumption of a HFHF diet over 6 weeks significantly increased body weight in all experimental groups although rats receiving HFHF diet supplemented with HPE showed a slight increase in body weight as compared to other groups (Figure 2).

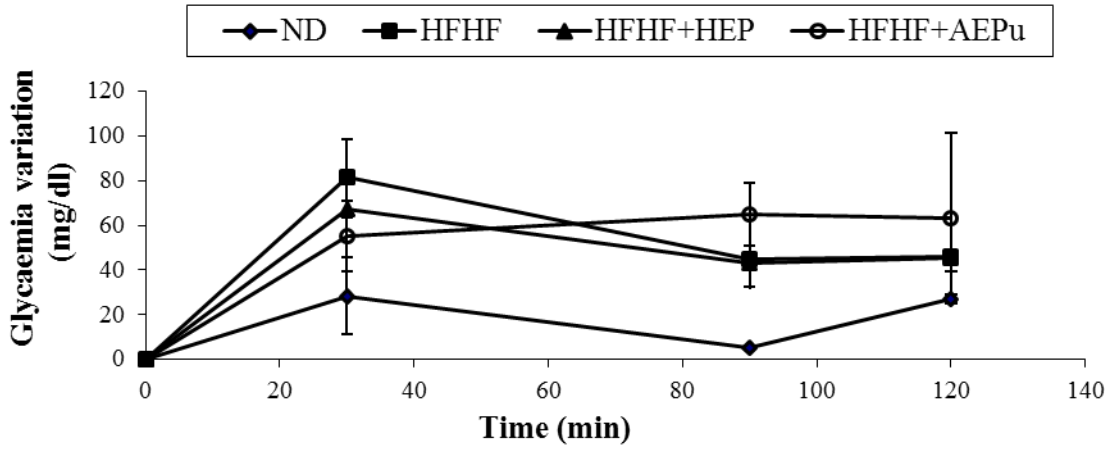

Figure 1: Effect of the extract of *Irvingia wombulu* fruit pulp and peel on postprandial glucose level after glucose loading in rats fed HFHF diet.

Results are expressed as mean ±SD, n=5. ND= normal diet, HFHF= high fat high fructose, HPE= hydroethanolic peel extract, APE= aqueous pulp extract.

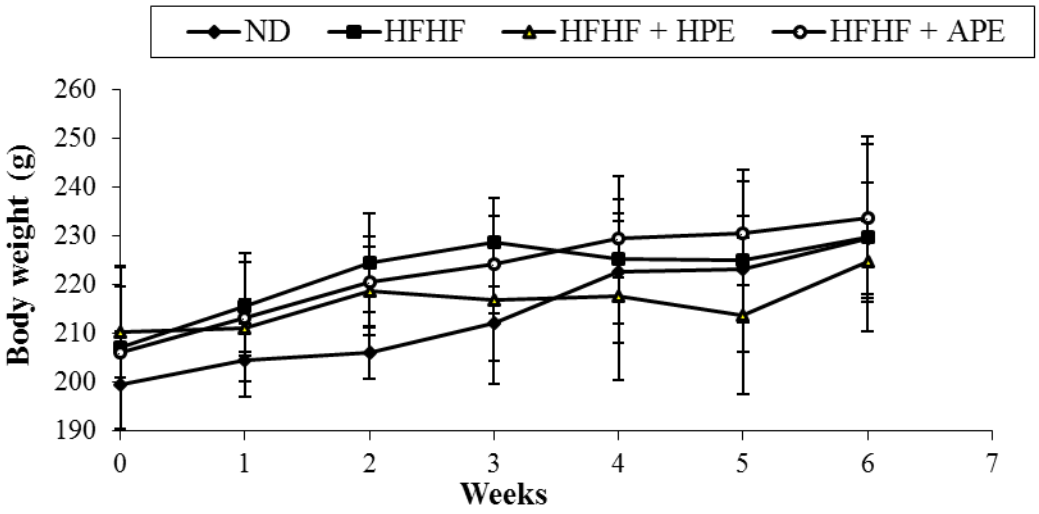

Figure 2: Effect of sub-acute administration of the extract of *Irvingia wombulu* fruit pulp and peel on body weight.

Results are expressed as 6 week treatment mean ±SD, n=5. ND= normal diet, HFHF= high fat high fructose, HPE= hydroethanolic peel extract, APE= aqueous pulp extract.

As shown in Table 1, high levels of fasting blood glucose, results revealed a significant increase in the blood glucose levels in group of rats fed HFHF diet alone compared to the negative control indicating that the diet brought about an increase in blood glucose. Co administration of HFHF diet with HPE and APE extracts at dose of 400mg/kg significantly prevented the rise in blood glucose levels by 33.65- 37.43 % ($p<0.05$). The literature suggested that plant extracts improved blood glucose levels through several mechanisms[28]. The effect of APE and HPE extracts can be explained by the ability of their bioactive components to decrease glyceamia by interfering with one or more of the processes involved in blood glucose homeostasis such stimulation of insulin synthesis and/or secretion from pancreatic beta-cells,improvement of insulin sensitivity[29], inhibition of key carbohydrates metabolism enzymes and reduction of the absorption of end products of carbohydrates digestion in small intestine.[28]

Results in table 1 indicated a significant elevation of triglycerides ($p<0.01$) and reduction of HDL cholesterol level ($P<0.01$) in rats receiving HFHF diet compare to the negative control. Previous studies had demonstrated that short-term consumption of a high-sucrose diet increased triglyceride levels in liver and plasma[30]. Fructose-induced hypertriglyceridemia is a result of enhanced lipogenesis, overproduction of VLDL-triglyceride and decreased TG clearance[31] . Co administration of HFHF diet with HPE and APE extracts at a dose of 400mg/kg significantly decreased the rise in blood glucose by 33.65- 37.43 % ($p<0.05$) and triglycerides levels by 47.19-50.71% ($p<0.05$) compared to rats fed HFHF diet alone. The extracts of *IW* may act by

inhibiting the hepatic production of triglycerides as does certain inhibitors of hepatic triglyceride secretion like Benfluorex[32] and also by stimulating peripheral uptake of glucose. These extracts could thus prevent diabetic pathological conditions induced by hyperlipidemia through the lowering of TG and TC in type 2 diabetes.

Fructose-induced hyperglycemia is one of the important factors leading to increased Reactive Oxygen species (ROS) and lipid peroxidation causing the reduction of the antioxidant defense status in various tissues[33]. A link between oxidative stress and insulin resistance has been demonstrated[34] and oxidative stress has been proposed as the root cause underlying the development of insulin resistance, beta-cell dysfunction and impaired glucose tolerance[35]. Tables 2 and 3 show the effects of sub-acute administration of *Irvingia wombulu* fruit pulp and peels extracts on antioxidant capacity and lipid peroxidation the extraction of marker levels in plasma, liver and the kidney.

Table 1: Effects of sub-acute administration of the extract of *Irvingia wombulu* fruit pulp and peels on blood glucose and lipid profile parameters after a 6 weeks treatment.

	Glucose (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
ND	65.47±4.33	76.85±10.32	51.58±7.67	25.08±6.77	41.45±15.48
HFHF	112.42±6.94[a]	85.85±10.34[a]	97.83±16.49[a]	16.91±0.39[a]	46.36±12.86
HFHF+HPE	74.59±11.59[*]	77.02 ±9.99[*]	48.22±4.63[*]	19.73±0.61[*]	47.64±9.39[*]
HFHF+AEP	70.33 ±4.68[*]	74.7 ±15.44[*]	51.66±1.40[*]	17.04±1.70[*]	47.32±15.76[*]

Results are expressed as means ± SD., n=5. ND: Normal Diet; HFHF: High Fat High fructose Diet; HPE: Hydroethanolic peel extract; AEPu: Aqueous pulp extract

*[a]Values are statistically significant at p<0.05 compared with ND. *Values are statistically significant at p<0.05 compared with HFHF*

Table 2: Effect of sub-acute administration of the extract of *Irvingia wombulu* fruit pulp and peels on antioxidant capacity markers

	FRAP (µmol equiv vitC/l)	*SOD (U/mg protein)*	*Plasma Thiol (µM)*	*Liver Thiol (µM)*	*kidney Thiol (µM)*
ND	122.22±13.94	0.63±0.11	44.30±7.65	19.91±1.66	14.89±0.33
HFHF	166.67±17.73[a]	0.42±0 .02[a]	72.40±5.47[a]	21.39±0.58[a]	17.38±0.83[a]
HFHF+HEP	174.44 ±0.79	0.25±0.02[*]	80.88±9.54[*]	18.88±1.18[*]	15.47±0.77[*]
HFHF+AEPu	170± 4.81	0.30±0.01[*]	75.94±9.10[*]	21.79±1.68	14.84±0.43[*]

Results are expressed as means ± SD., n=5. ND: Normal Diet; HFHF: High Fat High fructose Diet; HPE: Hydroethanolic peel extract; AEPu: Aqueous pulp extract

*[a]Values are statistically significant at p<0.05 compared with ND. *Values are statistically significant at p<0.05 compared with HFHF*

Table 3: Effect of sub-acute administration of the extract of *Irvingia wombulu* fruit pulp and peels on lipid peroxidation markers in plasma, liver and kidney

		Plasma		Liver		Kidney	
		ROOH	TBAR'S	ROOH	TBAR'S	ROOH	*TBAR'S*
Plasma	ND	4.30±0.21	0.49±0.20	1.43±0.009	0.75±0.09	0.41±0.02	1.24±0.09
	HFHF	4.16±0.14	0.48±0.19	1.83±0.03[a]	1.22±0.35	0.62±0.01[a]	1.03±0.014
	HFHF+HEP	3.52±0.42[*]	0.18±0.06	1.96±0.05[*]	0.92±0.20	0.48±0.07[*]	0.98±0.05
	HFHF+AEPu	3.35±0.10[*]	0.41±0.09	1.98±0.22[*]	0.78±0.05	0.62±0.14	1.07±0.02

ROOH: Hydroperoxydes (mM / 100g of tissue); TBAR'S (µM/100g of tissue Results are expressed as means ± SD., n=5. ND: Normal Diet; HFHF: High Fat High fructose Diet; HPE: Hydroethanolic peel extract; AEPu: Aqueous pulp extract

[a]Values are statistically significant at $p<0.05$ compared with ND. *Values are statistically significant at $p<0.05$ compared with HFHF

As shown in Table 2, we observed a decrease in plasma antioxidant capacity measured by FRAP method ($p<0.05$) and reduced thiols level ($p<0.05$) in groups receiving HFHF diet. It is known that over half of FRAP activity arises from the antioxidant activity of uric acid[36]. The observed changes in FRAP can thus be interpreted as a result of increased concentration of uric acid caused by fructose in all these groups. A significant reduction in the level of thiols ($p<0.01$) and SOD activity ($p<0.01$) was noticed in rats fed with HFHF diet without any supplementation. However, the administration of extracts did not prevent the problem suggesting that both extracts do not have any protective effect on the activity of this enzyme. It is well known that a prolonged exposure of rats to conditions of hyperglycemia reduces the activity of SOD and other antioxidant enzymes[37].

The preventive effect of extract against HFHF diet induced lipid peroxidation was more pronounced in plasma as the level of hydroperoxides was lower in groups supplemented with extracts. The extracts did not have any protective effect on the liver as hydroperoxide levels was not lowered in HPE and APE groups. However their administration limited the rate of formation of MDA which is a secondary products of lipid peroxidation.

It is well-known that decreasing lipid peroxidation is an important health challenge to avoid the oxidative damage of arterial walls. In the present work, the lowering effects of peel and pulp extract of *I.Wombolu* on lipid peroxidation are thus a predictive benefit for patients with type 2 diabetes. As suggested in a previous study[15], the presence of polyphenol in extract could be responsible of this effect. Moreover, the increase of plasma glutathione levels as well as the significant increase of plasma and kidney thiol groups, whose oxidation is an early determinant of oxidative stress[38] indicated protective activity of *I.Wombolu* extracts against free radicals and

glucose mediated protein damage. These effects could have significant clinical consequences if it is considered that oxidation has been reported as a risk factor of oxidative complications like nephropathies and glomerulopathies in diabetes[39].

CONCLUSION

The current study indicates that protein the administration of the extract of pulp and peels of *I.Wombolu* prevents hyperglycemia as well as reduces oxidative stress in rats fed with high-fat-high-fructose diet. These extracts can therefore find potential use in the management of type 2 diabetes and metabolic syndrome.

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