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EFFECT OF *IRVINGIA GABONENSIS* ALMONDS POWDER SUPPLEMENTATION ON RAT BIOCHEMICAL AND PHARMACOLOGICAL PARAMETERS

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ABSTRACT

Irvingia gabonensis (Irvingiaceae) is a Non-Timber Forest Product (NTFP), known for its edible fruits. Its ethnobotanical aspects are widely discussed in the literature. This study investigates the effect of supplementation with *Irvingia gabonensis* almond powder on biochemical and pharmacological parameters in rats. Different feeding formulations were prepared by incorporating varying percentages (75%-25%, 50%-50%, and 25%-75%) of dried and ground *Irvingia gabonensis* almonds into Ivograin pellets, a basic rat food. For this purpose, 20 growing rats were separated into four groups of five each. These rats were fed ad libitum every day for 28 days with the formulated foods. This study shows that there was no significant difference ($p > 0.05$) in blood glucose levels between rats on supplemented diets and the control group. However, the effect of *Irvingia gabonensis* almonds powder was significantly weaker ($p < 0.05$) on lipid parameters (triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol), liver markers (AST, ALT), kidney markers (urea, creatinine) and atherogenic indices (RR, CA, AIP, and RC) compared to the control group. These results suggest that the almonds of *Irvingia gabonensis* fruit could be a good dietary supplement. Indeed, its regular consumption has beneficial effects for the prevention of overweight, cardiovascular disease, and diabetes.

INTRODUCTION

Rural populations lifestyle in tropical regions traditionally places great importance on the use of diverse forest resources. For populations living near and within forests, forest resources, particularly non-timber forest products, play an important role in well-being. These products are biological goods other than wood, originating from forests, wooded areas or trees located outside forests (Awono and Manirakiza, 2007). Because of their importance, their enhancement and sustainable management could effectively contribute to the achievement of the Sustainable Development Goals (SDGs), which aim, among other things, to eradicate poverty and ensure food security worldwide (Fandohan et al, 2015). Indeed, for rural populations, non-timber forest products are crucial as they contribute to health care, nutrition, monetary income and the improvement of various aspects of their daily lives (Cavendish, 2000). Although many of these plant resources have been and continue to be the subject of studies, many others remain insufficiently known and documented in tropical countries. This is the case of *Irvingia gabonensis*, also known as "wild mango" or "wild apple", which is a tropical plant native to Central and West Africa. It is particularly used for its fruit and almonds in traditional food and medicine. In Côte d'Ivoire, especially in the Man region, this fruit plays a vital role in traditional culture. Its almond is commonly consumed there in various forms: fresh, dried, or ground into powder to be incorporated into sauces and other culinary preparations.

In addition to its nutritional value, the almonds of *Irvingia gabonensis* is recognized in traditional medicine for its therapeutic properties, notably its antihyperglycemic, anti-inflammatory, and antioxidant effects (Ngondi et al, 2005). Several studies have been dedicated to this Irvingiaceae, due to its numerous beneficial properties confirmed by preliminary studies. However, few studies have focused on the biochemical

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and pharmacological analysis of its almond, particularly in the Ivorian context.

This study aims to analyze the biochemical and pharmacological properties of *Irvingia gabonensis* almonds and their effects on human health. More specifically, it seeks to (1) identify the nutritional composition of *Irvingia gabonensis* pulp and (2) determine the anthropometric parameters and digestibility index in rats after administration of a diet supplemented with *Irvingia gabonensis* almonds powder.

MATERIAL

Plant material

Irvingia gabonensis almonds

The plant material used was *Irvingia gabonensis* almonds purchased at the large market in Man (figure 1).

Figure 1: Dried *Irvingia gabonensis* almonds

Ivograin Pelleted Feed

The pelleted feed used as a control and support is a standard commercial formulation produced by "Ivograin" (Yopougon industrial zone, Ivory Coast) (figure 2). Its nutritional composition is shown in Table I.

Figure 2: Pelleted feed by "Ivograin"

Table I: Chemical composition of the Pelleted feed by "Ivograin"

Constituents	Nutritional value
Raw protein (%)	15.1
Raw fat (%)	3.8
Cellulosic matter (%)	14.8
Mineral matter (%)	9
Raw calcium (%)	1
Phosphorus (%)	0.9
Sodium (%)	0.3
Vitamin A (U.I/Kg)	50000
Vitamin D3 (U.I/Kg)	10000
Vitamin E (mg/Kg)	120

Animal material

The animal material consisted of 20 male and female albino rats (*Rattus norvegicus*) of the Wistar strain. They were growing, with an average weight between 36 and 42 g, and approximately 4 to 5 weeks old. These rats were provided by the Central Laboratory for Analysis at the University of Man (LCAU-M). They were fed ad libitum and subjected to a 12-hour day/night cycle. Their handling was carried out in accordance with good laboratory practices. The various experimental protocols were followed in accordance with European Directive 2012/707 on the protection of animals used for therapeutic purposes (figure 3).

Figure 3: Growing albino rats

Technical equipment

The technical equipment initially included polystyrene cages, used for raising rats. A biochemical analyser (RAYTO CHEM-RAY 120) was used to determine biochemical parameters. In addition, a modern electronic analyser (Culter, Mindray BC30 S) was used to determine the rat's haematological parameters.

A centrifuge (80-2B) was used to obtain serum and homogenization solutions. Two digital balances (SF-400) with a precision of 0.1 g were used to weigh the food and the rats. Finally, a scanning electron microscope with variable pressure (SEM, FEG Supra 40 VP Zeiss), equipped with an X-ray detector (OXFORD Instruments) and connected to a microanalysis platform (Inca Dry Cool, without liquid nitrogen), was used to determine the mineral content of the different samples.

Rat Feeding Method

The test was carried out as described by Pawlak and Pion (1968) and modified by Bouafou et al. (2007and 2008). The animals were divided into four groups of five, housed individually in cages. Each group was given a specific diet. Feed was provided ad libitum daily to determine the amount ingested.

The animal was held by the scruff of the neck with one hand, between the thumb and forefinger. Pressure from the thumb on the neck, behind the angle of the jaw, compressed the jugular vein, causing venous stasis towards the head and thus allowing the retro-orbital sinus to fill. Gentle traction on the upper eyelid with the forefinger induced exophthalmos, facilitating blood collection. The tip of the tube or pipette was slowly inserted into the inner corner of the eye. Progression into the tissues was facilitated by a slight rotation of the pipette. The vessel walls are very fragile, and as soon as the venous plexus is reached, blood spurts into the periorbital space and rises by capillary action into the pipette, which was held horizontally during filling. It was sometimes necessary to slightly withdraw the tip to initiate the flow or achieve faster filling (Zouaghi, 2011). Determination of physicochemical parameters

Figure 4: Devices used for dosages at the CNTS

The water in the drinking troughs was renewed regularly. The animals are weighed weekly to assess their weight gain.

Diet formulation

Dried *Irvingia gabonensis* kernels were prepared according to the method of Pawlak and Pion (1968), modified by Bouafou et al. (2007 and 2008). They were ground into flour which was then mixed with Ivograin growth pellets, according to the experimental formulations. Four formulations were prepared: the first group of rats received 75% Ivograin pellets and 25% *Irvingia gabonensis* flour; the second group, 50% of each; the third, 25% Ivograin pellets and 75% flour; and the last group, 100% pellets. Conducting the experiments

Animal experimentation was conducted according to the method of Adrian et al. (1991), modified by Bouafou et al. (2007 and 2008). Food was provided ad libitum, once a day (from 6:30 a.m. to 7:30 a.m.), in pellet form. Water was available ad libitum and replenished every three days. The experiment lasted 28 days, during which the rats, their faeces, and food remains were weighed daily to assess the digestibility index. At the end of the experiment, after a 16-hour fast, the rats were anesthetized, and samples were taken for biological analysis. Blood was collected by puncture of the retro-orbital sinus using Pasteur pipettes. Blood samples were taken in purple tubes containing EDTA (anticoagulant), in dry tubes and in GIS tubes (Zouaghi, 2011). Technique for puncturing the retro-orbital sinus

pH

The pH was measured on 10 ml of suspension according to the AOAC (2012) method using a HANNA brand pH meter.

Total ash content

The ashes were determined by the ACC08-01(2012) method.

Protein dosage

The Kjeldahl method is the referenced method used for determining the protein content in almond samples.

Protein content (%)

Lipid dosage

The crude lipid content of *Irvingia gabonensis* almonds was determined using the Soxhlet extraction method, according to the standardized AOAC procedure. Five grams of the sample, previously ground and homogenized, were placed in a cartridge sealed with degreased cotton to prevent particle dispersion. The cartridge was placed in the extractor, and extraction was performed using 200 mL of hexane under reflux for 8 hours at a controlled temperature of 60 °C. The solvent was then recovered in a flask containing two pumice granules, and then distilled using a rotary evaporator under vacuum in a water bath at 60 °C. The flask containing the lipid extract was dried in an oven at 105 °C for 30 minutes, cooled in a desiccator, then weighed in order to determine the lipid content.

Vitamin content dosage

The vitamin C content was determined by titration with a 2,6 dichlorophenol indophenol solution according to the AOAC method (2012).

Mineral dosage: Ca, Mg, K, Fe, and N.

The mineral extraction method used in this study is based on the technique described by Alpert (1998). It consists of dry calcination at 550 °C until complete mineralization, followed by mild acid digestion using nitric acid (HNO_3). After calcination, the resulting ash is transferred to a beaker containing acid and then heated to a moderate boil for approximately 30 minutes. This ensures complete solubilization of the minerals.

Statistical analyses

The results are presented as means ± standard error (M ± ESM). Statistical analysis was performed using GraphPad Prism 8.01 software (San Diego, California, USA). Student's t-test and one-way ANOVA1, followed by Tukey's comparison test were used to identify differences between the treated and control groups. Differences were considered statistically significant for $p < 0.05$.

RESULTS

Biochemical composition of *Irvingia gabonensis* flour

The amounts of protein, lipids and carbohydrates are recorded in Table II. These amounts are respectively 10.0 ± 0.12 mg/g, 1.54 ± 0.06 mg/g and 17.50 ± 0.19 mg/g per 100 g of dry matter.

Table II: Biochemical composition of *Irvingia gabonensis* flour

	Flour from Irvingia gabonensis
Protéin (mg/g)/100g	10.0 ± 0.12
Lipid (mg/g)/100g	1.54 ± 0.06
carbohydrate (mg/g)/100g	17.50 ± 0.19

Mineral composition of *Irvingia gabonensis* flour.

The results show that this flour is particularly rich in potassium, phosphorus, calcium, magnesium, and sodium. *Irvingia gabonensis* flour is particularly rich in potassium (15.09 mg/g), followed by phosphorus (8.01 mg/g), calcium (7.11 mg/g), magnesium (7.03 mg/g), iron (1.91 mg/g) and sodium (2.07 mg/g) (Table III).

Table III: Mineral composition of Irvingia gabonensis flour

Mineral	Irvingia gabonensis flour
P (mg/g)/100g	8.01
K (mg/g)/100g	15.09
Ca (mg/g)/100g	7.11
Fe (mg/g)/100g	1.91
Mg (mg/g)/100g	7.03
Na (mg/g)/100g	2.07

Effect of *Irvingia Gabonensis* flour on anthropometric parameters

1. Weight variations in rats after supplementation with *Irvingia gabonensis* flour

Before supplementation with *Irvingia gabonensis* flour, the initial mean weight of the rats did not vary significantly ($p > 0.05$) and ranged from 39.00 ± 3.85 to 53.50 ± 2.32 g. One week after supplementation, the weight of rats in groups 1, 2 and 3 increased by 31.56 to 51.49% compared to the control group. However, a significant difference ($p < 0.05$) was observed for the weight of the rats in group 2 (51.49% increase compared to the control group). During the second week, a significant increase in body weight was observed in rats in group 2 ($p < 0.05$). In contrast, the body weight of rats in group 3 decreased significantly compared to groups 1 and 2 ($p < 0.05$ and $p < 0.01$, respectively). At the end of the third week, the weight of rats in groups 1 and 2 had significantly increased compared to the control group ($p < 0.05$ and $p < 0.01$, respectively). The weight of rats in group 3 had significantly decreased compared to groups 1 and 2 ($p < 0.01$ and $p < 0.001$, respectively). At the end of the fourth week, the weight of rats in group 3 was significantly lower than that of rats in groups 1 and 2 ($p < 0.05$, $p < 0.01$). At the same time, the weight of rats in group 2 was significantly higher than that of the control ($p < 0.05$) (Table IV).

Table IV: Variation in the weight of rats after supplementation with *Irvingia gabonensis* flour

	Variation in animal weight (g)/Percentage (%) increase or decrease in parenthess				
Lot	Initial weight	Week 1	Week2	Week 3	Week 4
Witness	40.75 ± 4.46	55.8 ± 2.78	63.00 ± 5.40	81.5 ± 3.59	107 ± 1.94
Batch 1 (25% FIg)	45.00 ± 3.8	67.3 ± 6.36 (+46.17)	78.50 ± 2.32$^{\#}$ (+48.61)	108 ± 8.09*$^{\#\#}$ (59.45)	143 ± 7.12$^{\#}$ (37.25)
Batch 2 (50% FIg)	53.50 ± 2.32	76.3 ± 1.93* (51.49)	87.50 ± 4.44*$^{\#\#}$ (55.55)	118 ± 3.67**$^{\#\#\#}$ (64.86)	160 ± 3.84*$^{\#\#}$ (64.70)
Batch 3 (75% FIg)	39.00 ± 3.85	58.8 ± 6.20 (31.56)	59.25 ± 4.49 (-93.75)	74.5 ± 3.43 (-17.56)	89.0 ± 9.43 (-43.13)

#p < 0.05, ## p < 0.01: significant difference compared to the weight of rats in batches 1 and 2 compared to group 3. *p < 0.05; **p < 0.01; ***p < 0.001: significant difference compared to the control group. Fig: *Irvingia gabonensis* flour; n = 5, n ± esm.

2. Average daily food consumption per group after supplementation with *Irvingia gabonensis* flour

After the first week, the amount of food consumed by rats in groups 1, 2, and 3 increased significantly ($p < 0.05$ and $p < 0.001$). This increase represented between 89.34% and 179.18% of the consumption of rats in the control group. Concurrently, the amount consumed by rats in group 1 decreased significantly compared to that of rats in group 3 ($p < 0.05$). By the end of the second week, a significant increase in food consumption, ranging from 45.84% to 117.31%, was observed in all three

groups (1, 2, and 3) compared to the control group ($p < 0.05$ and $p < 0.001$). However, the amount of food consumed by the rats in group 1 decreased significantly ($p < 0.01$) compared to those in groups 2 and 3. During the third and fourth weeks, a significant increase in food consumption was observed in the rats of groups 1, 2 and 3 compared to the control group ($p < 0.01$, $p < 0.001$) (Table V).

Table V: Average amount of food consumed per day for each group following supplementation with *Irvingia gabonensis* flour

	Amount of food consumed (g) / Percentage (%) increase or decrease in parentheses			
Lot	Week 1	Week 2	Week3	Week4
Witness	39.4 ± 8.83	58.9 ± 2.23	54.7 ± 8.10	81.1 ± 8.78
Batch 1 (25% FIg)	74.6 ± 8.17*# (89.34)	85.9 ± 8.62*## (45.84)	115 ± 9.02*** (110.23)	108 ± 10.4** (33.16)
Batch 2 (50% FIg)	97.1 ± 9.02*** (146.44)	115 ± 3.83*** (95.24)	126 ± 9.52*** (130.34)	140 ± 7.17*** (72.62)
Batch 3 (75% FIg)	110 ± 6.58*** (179.18)	128 ± 10.8*** (117.31)	133 ±7.04*** (143.14)	128 ± 10.7*** (57.82)

DISCUSSION

Analysis of the biochemical composition of *Irvingia gabonensis* flour reveals interesting characteristics that suggest potential uses in nutrition. Its protein content (10.0%) is relatively high for a vegetable flour (FAO/WHO 1991). This high protein content could thus contribute to improving the nutritional balance of local diets (Ngondi et al., 2005). The lipid content, estimated at 1.54%, is relatively low. This low proportion of lipids in the flour suggests that it cannot raise cholesterol levels. This could meet the specific nutritional needs of populations requiring a reduction in their fat intake, particularly in the context of cardiovascular disease prevention (Ngondi et al., 2005). Regarding carbohydrates, their moderate content of 17.5% indicates that the flour can also serve as an energy source (Ngondi et al., 2005), although it is less concentrated in carbohydrates than other more conventional flours such as wheat or maize (FAO/WHO, 1991). Mineral analysis of *Irvingia gabonensis* flour reveals significant nutritional richness, particularly in minerals. Potassium (15.09 mg/g) is the predominant element. This flour could therefore contribute to the regulation of blood pressure and the maintenance of the body's fluid balance (FAO/WHO, 1991). A high potassium intake is often recommended in the prevention of cardiovascular diseases (Omoti and Okyi, 1987). Phosphorus (8.01 mg/g) is also present in significant quantities. It plays a fundamental role in the formation of bone structures (in the form of calcium phosphate) and in the production of cellular energy (ATP) (FAO/WHO, 1991). The presence of calcium (7.11 mg/g) reinforces the potential of using this flour for the prevention of bone disorders, such as osteoporosis (Ngondi et al., 2005). Furthermore, the phosphorus/calcium ratio appears to be well-balanced. This is beneficial to overall mineral metabolism (Omoti and Okyi, 1987). Magnesium (7.03 mg/g) is also noteworthy for its significant contribution. It is essential for numerous biological functions, such as enzyme activation, nerve transmission, and the regulation of muscle tone (Ngondi et al., 2005). Its high concentration makes *Irvingia gabonensis* flour particularly beneficial for improving metabolic health (Omoti et al., 1987). The sodium content (2.07 mg/g) remains moderate, which is a significant nutritional advantage (FAO/WHO, 1991). Controlled sodium ingested is crucial for limiting the risk of hypertension, especially when combined with high potassium intake (Omoti et al., 1987). Finally, the iron content (1.91 mg/g) is significant. Iron is essential for haemoglobin synthesis and the prevention of iron-deficiency anaemia (FAO/WHO, 1991). Therefore, this flour could be useful in programs to combat malnutrition, particularly in Africa where iron deficiencies remain common (Omoti et al., 1987). Our results corroborate those of Kouadio et al. (2021). These authors showed that the presence of potassium, phosphorus, and iron influenced the different PCF and PCC diets. Regarding anthropometric parameters, the body weight of rats in groups 1 and 2, whose diets were supplemented with 25% and 50% *Irvingia gabonensis* flour, respectively, increased each week of the experiment. Weaned rats were used directly for the test. Their basic diet consisted of Ivograin growth pellets, a balanced rat food. Rats in the control group, fed exclusively with these pellets, gained 40.75 ± 4.46 g in weight. Concurrently, rats in groups 1 and 2 showed a significant increase in body weight compared to the control group. This suggests the presence of molecules in *Irvingia gabonensis* flour responsible for this phenomenon. Weight gain in rats fed a diet supplemented with 25% and 50% *Irvingia gabonensis* flour decreased significantly ($p < 0.05$) compared to groups 1 and 2, starting from the second week. This decrease in body weight in rats indicates that *Irvingia gabonensis* flour contains hypocholesterolemic, hypolipidemic and hypoglycaemic molecules (Dhanya et al., 2017; Tony et al., 2023). Similar results with a 75% proportion of *Irvingia gabonensis* flour were reported by Kouadio et al. (2021). These authors showed that the ingestion of cocoa powder reduced the animals' body weight during treatment. Furthermore, the amount of food consumed by rats in groups 1, 2, and 3 was significantly higher than that consumed by the control group throughout the experiment ($p < 0.05$ and $p < 0.001$). This suggests that *Irvingia gabonensis* flour contains molecules that act on the appetite center. The diets of groups 1 and 2, richer in pellets (a balanced diet containing more carbohydrates and lipids, a source of energy), were superior to those of group 3. These results are consistent with those of Dally et al. (2014). They show that formulated foods containing Moringa are very palatable to rats and can serve as a reference food for the refeeding of malnourished children in Ivory Coast.

CONCLUSION

Results obtained in rats demonstrate that *Irvingia gabonensis* flour possesses pharmacological and biochemical potential. Its administration led to significant improvements in metabolic parameters, including a reduction in blood glucose, total cholesterol, and triglycerides, as well as an increase in HDL cholesterol. Furthermore, liver and kidney markers indicate good tolerability and a possible protective effect of this flour on vital organs. These beneficial effects could be attributed to the flour's high fibre, phenolic compound, and bioactive nutrient content. These results suggest that *Irvingia gabonensis* could represent a promising natural alternative in the management of

certain metabolic disorders.

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