Dysbiosis in Obese Wister Rats fed with Pakala (*Phaseolus lunatus*) and Iru (*Parkia biglobosa*)

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Abstract

Introduction: Obesity triggers a vast number of comorbidities associated with non-communicable diseases such as hypertension and diabetes. It is widely known that obesity is affected by numerous factors, such as diet and gut microbiota. These have been implicated in energy homeostasis and metabolic functions. **Methodology:** This study was designed to assess anti-obesity potential of underutilized wild beans- *Phaseolus lunatus* and *Parkia biglobosa* on wistar rats fed with high fat diets. Control and test groups of animals were fed with their respective diets for five weeks with close observation of Microbiota and histopathological study of the liver.

Results: Obese rats fed with *Phaseolus lunatus* decreased in weights ($p \leq 0.05$) effectively than obese rats fed with *Parkia biglobosa*. The gut microbiota was found to contain *Proteus vulgaris*, *Escherichia coli*, *Bacillus cereus*, and *Enterobacter aerogenes* while histopathological study shows liver of obese rats treated with fermented *Parkia biglobosa* had better restorative changes compared to obese rats treated with *Phaseolus lunatus.*

Conclusion: This present study showed *Phaseolus lunatus* and *Parkia biglobosa* improved the health of obese animals by reducing the weights of the obese animals and also helps to have an insight to the level of effects of *Phaseolus lunatus* and *Parkia biglobosa* on liver organ in the treatment of obesity.

Introduction

Obesity is disequilibrium in energy balance and is currently a global health problem in Western societies, where its prevalence has increased considerably in recent years. Obesity triggers a vast number of comorbidities associated with hypertension, cardiovascular disease, and diabetes, as well as other conditions (1). It is widely known that obesity is affected by numerous factors, such as diet, lifestyle, and genetic background (2), and recently it has been shown to be related to gut microbiotas (3), which have been implicated in energy homeostasis and metabolic functions (4). Moreover, the same factors that affect obesity can modulate gut microbiota composition, and the function of the gut microbiota will be affected by factors involved in gut microbiota-host equilibrium (5).

The human gastrointestinal tract is a diverse and dynamic microbial ecosystem, comprising approximately 1014 bacterial cells and up to 1000 different species (6). An appropriate balance between harmful and health-promoting gut microbiota can support human health by maintaining host immune homeostasis; increasing the efficiency of energy production in the gut through fermentation of non-digestible dietary compounds. Synthesizing vitamins, such as B_{12} and K; controlling intestinal epithelial cell proliferation (7), stimulating immunological defense; creating a protective barrier; and inhibiting the growth of potential pathogens (8,9,10,11,12).

Dysbiosis indicate an imbalanced microbiota, including four types of imbalance: (i) loss of keystone species, (ii) reduced richness or diversity, (iii) increased pathogens or pathobionts, or (iv) modification or shift in metabolic capacities (13). Reports have shown that a state of dysbiosis is often associated with diseases, including inflammatory bowel disease, allergies, colorectal cancer, and liver diseases, as well as obesity, diabetes, and cardiovascular diseases (14). In the present study both condiment bean *Parkia biglobosa* and wild bean *Phaseolus lunatus* are comparatively under investigation with a view to determine the gut microbiomic changes on experimental rats when fed upon by these underutilized beans. The former also known as the 'African locust bean is a perennial deciduous tree of the family *Fabaceae* (15). It is found in a wide range of environments in Africa and is primarily grown for its pods that contain both a sweet pulp and valuable seeds (16,17). Well the latter *Phaseolus lunatus* also known as butter beans is an herbaceous species with an annual or short-cycle perennial lifecycle (18). Though edible it is yet to be fully explored due to the difficulty in cooking. Against this backdrop the present study is intended to explore on our previous work (19,20, 21) and ultimately report on these Nigerian wild underutilized beans in relation to their anti-obesity potentials.

Materials and methods

Collection and preparation of materials

Dry wild beans, *Phaseolus lunatus (Pakala*), were sourced from the bush of Ado Ekiti environment while *Parkia biglobosa* (African locust bean) were bought from the market. The *Pakala* beans was collected whole, dried and the beans removed from the pods, with diverse colors but the *P. biglobosa* were collected with the pods. The seeds of *P. lunatus* and *P. biglobosa* were identified and authenticated by the Chief Botanist of the Department of Plant Science, Ekiti State University and deposited in the University Herbarium with Voucher Number UHAE-1010066 and UHAE 2020063 respectively.

The *P. lunatus (*pakala) bean was selected and sun dried for some hours and was later blended using a blender into a powder form which was used in treatment of the experimental rats. For preparation of *P. biglobosa* the seeds were selected and rinsed with clean water before boiling. The first boiling was for 2 hours; it was then dehulled and washed with water. It was boiled again for 45mins, it was later drained using plastic sieve. For fermented locust bean starter culture i.e *Bacillus subtilis* was added to the locust beans after draining, it was then spread into a fermenting can and wrapped with cloth for 48hrs. After fermentation, the *P. biglobosa* was then dried in the oven at 55°C to 60°C for 5days and was powdered into feed for rat feeding treatment. For unfermented locust beans there was no addition of starter culture and it was dried immediately after cooking and draining.

Experimental animals

Fifty albino Wistar rats were obtained from the Animal House, College of Medicine, Ekiti State University Ado Ekiti. They were maintained in line with the directions and principles of the Experimental Animal Research Ethics Committee of Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria (Reference number: ORD/ETHICS/AD/043). The rats were separately grouped into experimental and control groups as indicated in Table 1a with ten rats in each group. The rats were fed for five weeks with beans and feed formation of high fat diet while the control group was fed with normal rat chow diet formulated by BioOrganic Feeds (Table1b). Daily food consumption, body weight, behavioral and physiological changes were observed for four weeks as shown in Table 1a.

Table 1a: EXPERIMENTAL DESIGN

s/n	Experimental group	Composition
1	Control	Chow
2	Fermented group	80g fermented locust beans + 20g fat
3	Unfermented	80g Unfermented locust beans + 20 g fat
4	*Pakala*	80g Pakala+20g fat

Table 1b: Animal diet compositions

Ingredient (g)	Normal Chow diet (ND)	High-fat diet (HFD)
Casein,	200	200
L-Cystine	3	3
Corn starch	285	0
Maltodextrin	35	125
Sucrose	325	80
Cellulose	50	50
Soybean oil	25	35
Lard	20	350
Mineral mix,	10	10
Dicalcium phosphate	13	13
Calcium carbonate	5.5	5.5
Potassium citrate,	16.5	16.5
Vitamin mix,	10	10
Choline bitartrate	2	2
Total	**1000**	**896**

Microbiota Analysis

This was to detect the microorganism present in the gastrointestinal tract of the rats before and after treatment. After the collection of the stool, 1g of the sample dissolved in 10ml of distilled water for some minutes before serial dilution was done using 10^3 dilution. A loopful of the inoculum was aseptically streaked on well labelled petri dishes (in duplicates) containing nutrient agar and incubated at temperature of 37°C for 24hours. Sub-culturing was done. Morphological features of the grown organisms were examined and recorded. The pure colonies transferred to a slant for further identification. Various biochemical tests such as- indole test, motility test, oxidase test, urease, Triple sugar iron Agar (TSIA), catalase, coagulase, lactose, mannose, glucose, and various sugar tests were carried out on the isolates to identify the isolated organisms.

Histopathological investigation

The sacrifice of the rats was done in the Biochemistry laboratory in Ekiti State University, Ado Ekiti. The rats were sacrificed by exposure to chloroform fumes in a container. Following a midline incision in the anterior abdominal wall, the liver tissues were excised and fixed in 10% formal saline by total immersion for 48hrs to prevent from bacteria reaction and decay. The organs were dehydrated using alcohol (ethanol) ranging from 50%, 70%, 90%, Absolute 1, Absolute 2; the alcohol was removed using xylene or benzene, after which they were processed using paraffin wax embedding method. Using a Rotary microtome, thin sections of about 4µm were cut and picked in a water bath using a glass slide. The sections were stained routinely with Hematoxylin and Eosin and viewed under an OMAX 40X-2000X light microscope. This was done at the Department of Anatomy, Ekiti State University, Ado-Ekiti.

Results and discussion

The results of the study show that fermented *Parkia biglobosa* (African locust beans) locally known as *iru*, *Phaseolus lunatus* (*pakala* lima beans), used in treatment of experimental rats fed with high fat diet in inducing obesity probably exhibited hypolipidemic activities to the treated rats as there was significant decrease in the daily weight of the test groups (fermented, unfermented and *pakala*) when compared to the control group.

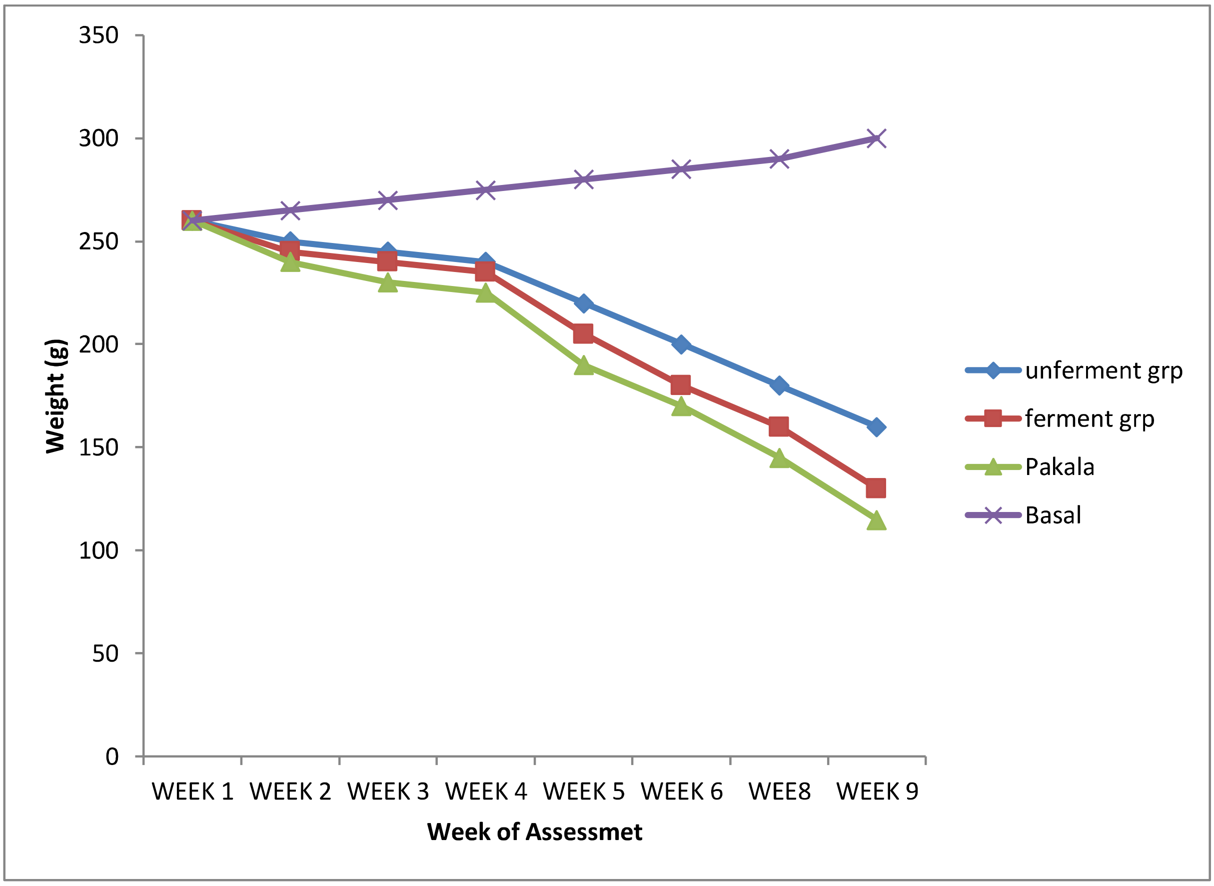

Figure 1: Chart showing average weight (g) of the obese rats against the period of the experiment

Table 2: Result of Microbiota Analysis

Grp	Nos of Colony	Edge	Color	Shape	Size	Surface	Organism Detected
Unfermented	275±0.1	Smooth	Cream	Round	Small	Smooth	*Bacillus cereus, Escherichia coli*
Fermented	32±0.2	Smooth	Pink	Irregular/ Round	Large	Smooth	*Escherichia coli, Proteus vulgaris Bacillus cereus*
Negative	46±0.01	Nill	Cream	Irregular/ Round	Large	Smooth	*Enteroobacter aerogenes*
Pakala + Fat	23±3.0	Nill	Greenish opaque	Round	Small	Smooth	*Escherichia coli, Enterobacter aerogenes*
Fermented+ Fat	25±0.01	Smooth	Cream	Irregular/ Round	Small	Smooth	*Bacillus cereus, Escherichia coli Proteus vulgaris*
Otili+ Fat	127±4	Smooth	Cream	Cream	Large	Smooth	*Escherichia coli Citrobacter freundii*

However, the decrease in their average daily weight within and among the groups could be attributed to the varied treatments given to the various groups and the period of treatments also. The weights of rats in the *pakala* group when the administration of treatment started are (as shown in figure 1) which significantly decreased when compared to the control group on the 7th day, treated group has significantly decreased (as shown in figure1) and the control significantly increased (as shown in figure 1). The treated group was observed to decrease significantly in weight till the end of the experiment. The decrease in the daily weight of the experimental rats in this study confirms the report on *P. lunatus* pakala beans as earlier described by Awoyinka *et al*., (20). Beans contain dietary fibre which when consumed decreases total and low- density lipoprotein (LDL) cholesterol, as well as decreasing the risk for developing coronary heart disease, metabolic syndrome, stroke, hypertension, diabetes, obesity and some gastrointestinal diseases (22). *Pakala* bean contains polyphenol which is a bioactive compound. Bioactive like polyphenolic compounds, prebiotics and probiotics are the best-known dietary strategies for regulating the composition of gut microbial populations or metabolic/immunological activities, which are called “Three “p” for gut health” (23). Polyphenols are a class of plant secondary metabolites which have been found to possess various antioxidant, anti-inflammatory or anticarcinogenic biological activities. It is known that many natural products contain a large amount of polyphenols that possess the functions of protecting cardiovascular system and reducing inflammatory responses (24, 25).

Although, polyphenols are thought to prevent development of chronic diet-related diseases, most of them could not be absorbed directly by the small intestine. Therefore, their bioavailability and impact on the host mostly depends on the function of the gut microbiota and their component's conversion (26). Intestinal microbes play a crucial role in converting dietary polyphenols into absorbable bioactive substances. Moreover, some intestinal metabolites derived from natural polyphenol products have more biological activities than their own fundamental biological functions (27). The human gastrointestinal tract is a diverse and dynamic microbial ecosystem, comprising approximately 1014 bacterial cells and up to 1000 different species (6). Firmicutes and Bacteroidetes are the two dominant phyla, representing more than 90% of all the phylotypes, followed by lower relative abundances of Actinobacteria, Proteobacteria, Fusobacterisa and Verrucomicrobia (28, 29, 30).

Those commonly considered as the most beneficial bacterial genera are *bifidobacteria, lactobacilli* and butyrate producers such as *Eubacterium rectale, Roseburia species* (28). An appropriate balance between harmful and health-promoting gut microbiota can support human health by: maintaining host immune homeostasis. It Increases the efficiency of energy production in the gut through fermentation of non-digestible dietary compounds. It also helps in synthesising vitamins, such as B_{12} and K; controlling intestinal epithelial cell proliferation (7), stimulating immunological defence; creating a protective barrier; and inhibiting the growth of potential pathogens (8,9,10,11,12). Conversely, an unfavourable gut microbiota composition and function (i.e. dysbiosis) could trigger the development of diseases through intestinal-derived endogenous endotoxins, such as lipopolysaccharides, indoxyl sulphate and L-carnitine (31). These metabolites may potentiate the development of acute diseases, such as diarrhoea and chronic diseases, including obesity, metabolic diseases, cancer and cardiovascular disease (CVD) (32, 33, 34). There has been a long understanding of the interaction between environmental factors and gut microbiota, including that of pH, peristalsis, redox potential and nutrient availability (35). Dietary changes are thought to be responsible for around 57% of the variation in the gut microbiota, compared with only 12% for genetics (36). For example, a study showed that the microbiota of mice fed a low-fat, polysaccharide-rich diet markedly increased in populations of *Firmicutes*, and decreased in levels of *Clostridium*, *Eubacterium*, *Enterococcus* and *Bacteroides spp* when the mice were switched to a high sugar/ high-fat diet (37, 38). On the other hand, a high-fibre diet can limit the growth of potentially pathogenic *E. coli*, (39). This has been observed in humans within 24 hours following a change from a high-fat/low-fibre diet to one which is low-fat/high-fibre (40). These agrees with the microbiotal study of the fecal sample of the treated groups, the results show the presence of microorganisms like *Proteus vulgaris* and *Bacillus cereus* found in the group treated with unfermented *Parkia* biglobosa. The pakala group was found to have *Enterobacter aerogens* in its gut microbiota, which when compared with the control group was absent, the gut of the control group was found to possess *Escherichia coli*, which is a normal fecal flora shown in Table 2.

The treatments administered which are *Parkia biglobosa* and *Phaseolus lunatus* (pakala lima beans) are examples of probiotics and prebiotics which are dietary substrates which induce change in the growth of favourable bacteria which pass largely unmetabolised in the upper gastrointestinal tract and are selectively utilized by host microorganisms conferring health benefits (41).

Histopathological investigation of the liver shows that obese rats treated with unfermented *Parkia biglobosa* had an essentially normal looking architecture with few areas of distortion of sinusoids (figure 2A). The liver of rats treated with fermented

Parkia biglobosa and fat shows evidence of restorative changes with slight engorgement of blood vessels (figure 2B); while the obese rats treated with *pakala* and fat shows slight derangement in the sinusoidal arrangement and presence of cellular infiltrates in few areas (figure 2C). However, the untreated group of obese rats showed evidence of distortion in the sinusoidal pattern of hepatocytes with inflammatory cells and engorgement of blood vessels.

Conclusion

In summary, fermented *Parkia biglobosa* and *Phaseolus lunatus* (pakala lima beans) played an important clinical role in human nutrition with reduced risk of obesity and could be an exceptionally cost effectual approach in improving health. The gut microbiota of obese rats can also be influenced by the diet they consume.

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Conflict of Interest

The authors declare no conflict of interest regarding the publication of this paper.

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