Comparative effects of fermented and unfermented *Parkia biglobosa* bean seeds in diabetic induced experimental rats

ABSTRACT

Aims: The present work investigates the hypoglycemic effect of fermented seeds of *Parkia biglobosa* (PB, African locust beans) in alloxan-induced diabetic rats. Its effect was compared with that of unfermented *Parkia biglobosa*.

Methodology: In order to assessthe hypoglycemic effects of the fermented seeds on experimental animals, fasting blood sugar (FBS), total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined. The microbiota analysis of the fecal sample was also determined.

Result: Alloxanadministered to the rats significantly increased the FBS of the test animals. However, dietary supplementation with fermented PB for threeweeks ameliorated the FBS of the rats like that of the unfermented group. The animals fed with fermented *Parkia biglobosa* gained weight likethose fed with unfermented *Parkia biglobosa*. High levels of HDL and low levels of LDL were observed in animals treated with fermented Parkia biglobosa compared with low levels of HDL and high levels of LDL observed in animals treated with unfermented *Parkia biglobosa*.

Conclusion: The results of this present study demonstrate that both fermented and unfermented seeds of *Parkia biglobosa* exert a hypoglycemic effect. Hence, *Parkia biglobosa* has an anti-diabetic property with fermented form seemingly better.

Key Words: Alloxan; Diadebtics; Fermented; *Escherichia. coli*; Hypoglycemic; Hyperglycemic; *Parkia biglobosa.*

INTRODUCTION:

Diabetes mellitus is characterized by elevated blood glucose levels. It is a condition that affects the pancreatic B-cells and is brought on by either insufficient insulin secretion or cellular resistance to insulin. Diabetes mellitus comes in two different forms. Diabetes types I (Insulin-Dependent Diabetes Mellitus IDDM) and II include (Non-Insulin-Dependent Diabetes Mellitus, NIDDM). The difference in their onset, intensity, and complications determines how they are classified (ADA, 2013; Alberti &Krall, 1991; Haffner *et al*., 1996). Because of the hepatic overproduction of glucose by glycogenolysis and gluconeogenesis and a decrease in the clearance of glucose from the circulation into adipose tissues and muscles in type I diabetes, the insulin released by the B-Cells of the pancreas is insufficient to metabolize glucose (Murray *et al*., 1996). Type II diabetes is caused by insulin insensitivity, which means that although the B-cells produce normal or even high insulin levels, they are not insulin-responsive due to a lack of insulin receptors. There are, however, some types of diabetes that are recognized and categorized as secondary diabetes. They include diabetes brought on by medicines, illnesses, or surgery that destroys the pancreas, as well as diabetes brought on by hormonal insufficiencies (ADA, 2013;

Haffner *et al*., 1998). Additionally, it has been established that diabetes is a hereditary condition; some members of families with known diabetics have been discovered to have a variety of peculiar symptoms, even though their blood sugar levels may be normal at the time of examination but develop the condition years later (Granner,1996). The prevalence of diabetes poses a serious threat to public health worldwide (WHO, 1985). More than 170 million people worldwide are thought to have diabetes, and by 2030, that number is projected to quadruple. The trend of a sedentary lifestyle is followed by the biggest growth in prevalence (Lipscombe &Hux, 2007).

The management of diabetes mellitus involves the use of substances with hypoglycemic properties. Insulin is the most widely used hypoglycemic medication in clinical diabetes management (injection). The cost of the medicine and other considerations, such as its ease of availability, prevent this form of treatment from being accessible to the rural populace and poor urban residents. Therefore, alternative treatments that could stop the disease at a reasonable cost and with ease of access are urgently needed. Plants that have one or more organs that can be utilized to treat certain illnesses or act as building blocks for the manufacture of effective medications are considered medicinal plants (Sukrankultur, 2007). According to (Eddouks*et al*., 2002), diseases were treated and prevented using plants before synthetic medications were developed. Also, according to Balunas and Kinghorn (2005), plants were the very first and only medications utilized by humans throughout history to treat illnesses. In addition to using medicinal plants found in their natural habitats to cure diabetes, many Nigerians do so. The African locust bean is one of the leguminous plants used by humans, especially in several African nations (*Parkia biglobosa*). The seeds are famous for being used to make a regional condiment called dawadawa (Hausa) or iru (Yoruba). Also, while considering the plant's protein and amino acid composition, *Parkia biglobosa*has an exceptional protein quality (Hassan &Umar, 2005; Cook *et al*., 2000; Lockeet*et al*., 2000). The plant is frequently found in Nigeria's northern and western regions. Dawadawa is a common ingredient in soups and sorghum- or millet-based porridge throughout the West African savannah. It is also utilized similarly to cheese or stock cubes in North American and European cooking. The locust bean plant is economically significant because it fixes atmospheric nitrogen through the root nodules of leguminous plants, stabilizes soil in agricultural areas, cycles nutrients from deeper solid layers, and provides shade (Simonyan, 2012; Campbell-Platt, 1980). (Vietmeyer, 2006; Cobley and Steele, 1976). The red tannin found in the bark is used to color leather. The pods and bark are used in traditional medicines. The yellow pulp around the seeds is a high-energy snack that is also rich in vitamin C. An extract from the pods is used to harden beaten earth floors and as a glaze for pottery.

In order to determine the hypoglycemic activity of fermented locust bean and, subsequently, its prospective application as a blood glucose lowering medication, its hypoglycemic qualities were studied in this study in Wistaralbino rats. Its impact was compared with that of locust beans without fermentation.

METHOD AND MATERIALS

Materials Collection and Preparation

The *Parkia biglobosa* seeds werebought from King's Market (Oja Oba), Ado Ekiti, Ekiti State, Nigeria. The Plant Science and Biotechnology Department at Ekiti State University in Ado-Ekiti conducted the plant authentication. To get rid of spoiled seeds, dirt, and other impurities that were mixed in with the seeds during harvesting and processing were manually separated and washed. 600 g of seeds were cooked under pressure for 2 hours to soften the cotyledon. This was followed by cooling down at normal temperature. The method used to separate the seeds from the cotyledons was rubbing thembetween palms. Water was used to wash the cotyledons thoroughly and repeatedly. The seeds' shaft was taken out using a locally manufactured basket sieve. The cleaned, sterilized seeds were then boiled again for an additional one hour and drained. The boiled seeds were divided into two portions. One portion (300g) was oven dried at 50°C, and the second portion (300g) was inoculated and fermented at 35°C for 48 hours. Afterward, both samples were oven-dried at 50^{o}C and ground into powders.

Preparation of Starter Culture

Preparation of strains of *Bacillus subtilis* for 'iru' production

The inocula were prepared by growing the strains of *Bacillus subtilis* in 50ml Nutrient Broth (NB) in 250ml conical flasks for 24 hours under agitation (200rpm) at 35^{o}C. The turbid cultures were centrifuged at 10,000rpm, 4^{o}C for 10mins. The supernatant was decanted, and the cell pellet was re-suspended in 5 mlof sterile distilled water. The cell population was determined by measuring broth cultures' optical densities (OD) at 540nm with Pye Unicam SP6-250 visible spectrophotometer. The inoculum volume required to inoculate 300g of substrate to give a final inoculation ratio of 10^{4}cells per gram of substrate was calculated (Omodara&Aderibigbe, 2014).

Process of Fermentation

The method of Omodara and Aderibigbe(2018) was modified to prepare the dried African locust bean seeds. After being manually cleaned of dirt, the dried seeds were cooked under pressure for four hours. The boiled seeds were thoroughly washed and dehulled to remove the testa. Then, 300 grams (300g) of the boiled substrate was weighed separately into twenty sterile fermenting cans. Each substrate was inoculated with one milliliter(1.0 ml) of starter cultures. The sterile spatula was used to mix the starter culture substrate and incubated at 35 C for 36 hours.

Animal Preparation and Alloxan Administration

Twenty male albino rats (Wistar strain), weighing an average of 125g, were used. 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 animals were randomly divided into five groups of four after a week of acclimatization, and each group received grower's mash as food. The control group, Group 2, receivednormal saline only (no treatment). A single injection of freshly made alloxan solution at a dose of 120 mg/kg was administered to Groups 1, 3, 4, and 5 using saline (0.9% w/v

NaCl). Only sixrats were successfully put into a diabetic state, which was defined as fasting blood glucose exceeding 80 mg/dl. The diabetic control group, Group 1, received no treatment; Group 3, the reference anti-diabetic drug Glibenchamide, was administered orally with a syringe at a dose of 0.01 mg/150 g bodyweight; and Groups 4 and 5, a diet of milled fermented and unfermented *Parkia biglobosa* seeds, respectively, were given treatment. For three weeks, these animals were fed fermented and unfermented locust beans at a rate of 60 grams per cage, along with 40 grams of grower's mash per cage. Group 3 consisted of control animals that had no diabetes at all.

Assessing Fasting Blood Sugar

After the acclimatization period, animals used in this study were allowed to fast for twelve (12) hours before Parkia biglobosa was administered. The blood glucose levels were taken by sterilizing their tails with 10% alcohol, cutting the tails using scissors, and then allowing the blood to touch the test strip, which was inserted into a calibrated glucose meter. This gave direct reading after five (5) seconds in mg/dl. This was done to determine the effect of *Parkia biglobosa* on blood glucose levelscompared to their initial glucose level.

Analysis of Microbiota

The dilution streak plate method was used to examine the microbiota analysis. 1g of the fecal sample from each group was weighed and kept in sterile test tubes. Ten milliliters(10ml) of steriledistilled water was added, and the feces were given time to dissolve. In sterile test tubes with 9ml of sterile distilled water, 1ml of the suspension was pipetted and shaken. This was repeated until the dilution factor was 10^3. This was streaked on already prepared nutrient agar and incubated for 24 hours at 37°C. For the purpose of pure isolation of microorganisms, the morphological traits and numbers of the colonies were observed and then sub-cultured on new plates containing nutrient agar. The pure colonies were transferred and kept on a slant for further identification.

Serum Collection and Animal Sacrifice

Rats in each study group were fasted the night before being slaughtered by cervical dislocation while under anesthesia at the end of the 14-day treatment period. After the rats were killed, 2-4 ml of each animal's blood was taken and put in particular sterile vials (plain bottles for enzyme analysis and EDTA bottles for hematological indices). The blood was centrifuged at 3000 RPM for 30 minutes after standing for 30 minutes to allow the blood to coagulate in preparation for enzyme analysis. The serum, or supernatant, was carefully decanted and stored at four °C for further investigation. For biochemical analysis, the liver, kidney, and heart were removed, and the remaining portion was fixed with 10% formalin.

Analysis of the Lipid Profile

Using the Random Kit, the lipid profile of the serum was examined for total cholesterol, low-density lipoprotein, high-density lipoprotein, and triglycerides.

Liver and Serum Enzyme Activities are Determined

Random Kit was used to measure the aspartate aminotransferase and alanine aminotransferase activities in the liver and serum.

RESULT AND DISCUSSION

Parkia biglobosa seeds are naturally underdeveloped and unappealing in their raw form, but when they are fermented into a condiment, their physical, chemical, and nutritional properties alter (Amoa-Awua*et al*., 2005). These seeds are fermented to increase protein and fat enrichment and decrease total carbohydrate content (Bourdichon*et al*., 2012). According to Hassan and Umar (2005), *Parkia biglobosa* seeds are a good source of protein and essential amino acids, especially amino acids containing sulfur. Its regular use as a condiment may be a beneficial dietary option for managing hypoglycemia. Fermented foods' high nutritional qualities have been widely established (Steinkraus, 1997; Gadaga*et al.*, 2004). African locust beans, or *Parkia biglobosa*, efficiently reduce the effects of diabetes problems on the body's organs, such as the pancreas, liver, and kidneys. Poorly managed diabetes could harm the small blood vessels in the eyes, kidneys, feet, and nerves, as well as the big blood vessels in the heart, brain, and legs (macrovascular issues, microvascular complications).

In both clinical and experimental forms of diabetes, hyperlipidemiais linked to an abnormal rise in glucose, mainlyas it occurs in diabetes mellitus, one of the most prevalent chronic diseases in the world (Bierman *et al*., 1975). This was demonstrated in the current study by the hyperglycemia, hypercholesterolemia, and hypertriglyceridemia that the diabetic rats displayed. Both fermented and unfermented *Parkia biglobosa* seeds dramatically lowered the glucose level (Fig. 1) to a level that was comparable to that of diabetic rats receiving glibenclamide treatment. The current finding shows that these seeds may have an extrapancreaticantihyperglycaemic mode of action because alloxan is known to kill pancreatic beta-cells. This concurs with Jackson and Bressler's earlier suggestion (1981). There have also been reports of other plants and extracts having antihyperglycemic and insulin-stimulating properties (Prince *et al*., 1998; Venkateswaran *et al*., 2002; Latha & Pari, 2003; Akinmoladun&Akinloye, 2004).

Metabolites such as glycosides, alkaloids, and flavonoids have been discovered to be present in most plants with hypoglycaemic characteristics (Loew &Kaszkin, 2002). In the current study, Alloxan-induced diabetic rats were used as test subjects to compare the effects of fermented and unfermented seeds of *Parkia biglobosa* (African locust bean). This natural, nutritious condiment is often used as a spice in some African diets. *Parkia biglobosa's* effects were contrasted with those of unfermented Parkia. Test animals' fasting blood sugar (FBS) levels significantly increased after rats were given alloxan. However, oral *Parkia biglobosa* supplementation for three weeks reduced alloxan-induced diabetes in the fermented group in a way equivalent to the unfermented group. Weight loss and dehydration have been linked to diabetes mellitus (Pupim*et al*., 2005). In contrast to the unfermented group, which lost weight, it was found that the rats' weight loss was mitigated by the fermented *Parkia biglobosa* seeds in the group. The high protein and amino acid content of *Parkia biglobosa's* fermented seeds may have contributed to the current study's finding that they inhibited weight loss (Fetuga*et al.,* 1974).

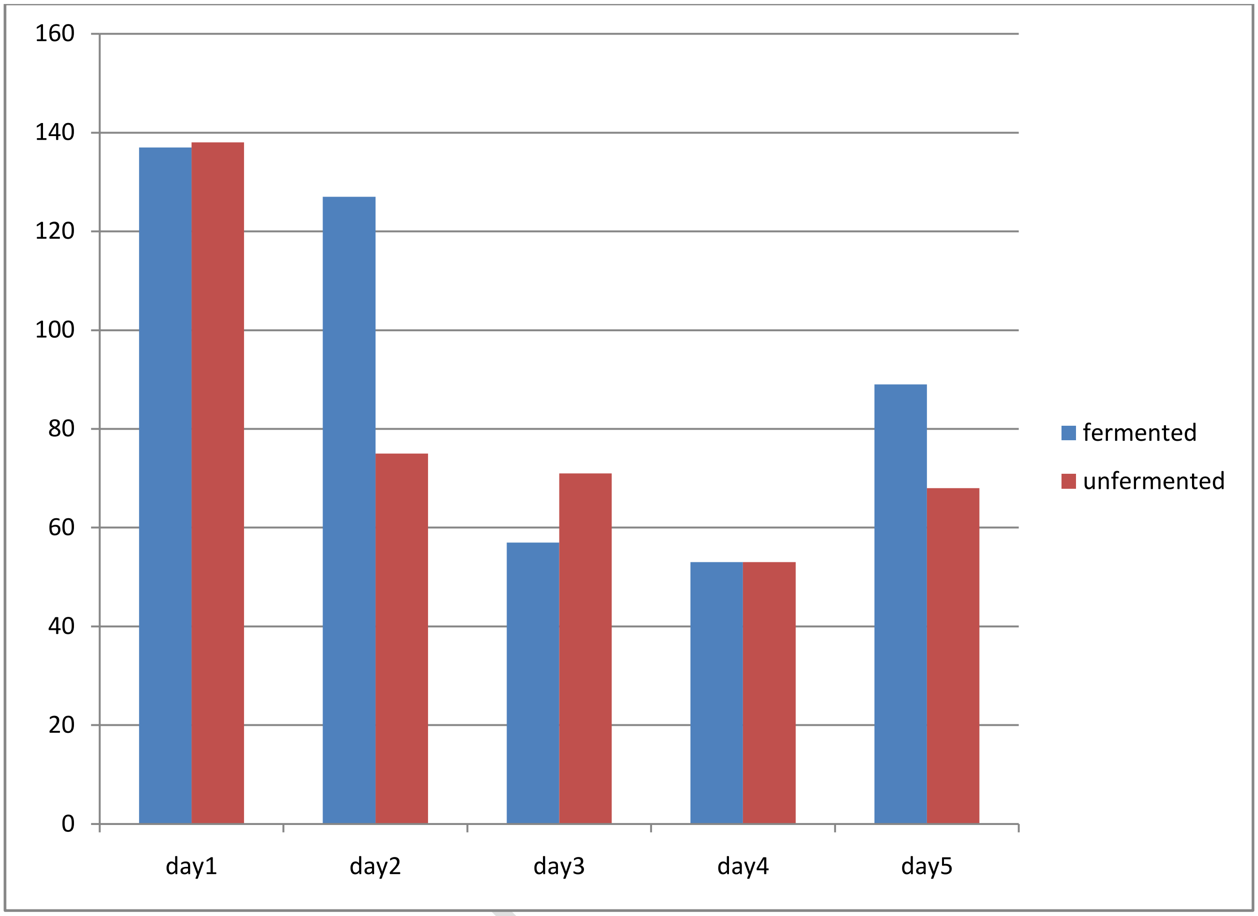

Fig. 1: Graph showing Fasting Blood Sugar level in mg/dL in rats fed with fermented and non-fermented *Iru*.over 5 Day period

Insulin controls lipid metabolism in addition to controlling carbohydrate metabolism. Hypercholesterolaemia and hypertriglyceridemiaare linked to insulin insufficiency, just like in diabetes mellitus, and have been observed in experimental diabetic rats (Loci *et al*., 1994;

Ahardh*et al*., 1999; 1983; Bopanna *et al.,* 1997). Relative molecular ordering of the remaining phospholipids due to hypercholesterolemiamay reduce membrane fluidity (Bopanna *et al.,* 1997). One of the main risk factors for coronary heart disease is triglyceride buildup (CHD), the elevated levels of triglycerides and cholesterol. In addition, animals given the fermented African locust bean had high levels of HDL and low levels of LDL compared to unfermented animals, which had low levels of HDL and high levels of LDL. Animals fed unfermented locust beans had considerably higher total cholesterol and triglyceride levels than the fermented group, which had lower cholesterol and triglyceride levels **(Table 1).** Compared to the fermented group with low albumin levels, animals treated with unfermented locust beans likewise showed increased albumin levels. However, therapy with both fermented and unfermented *Parkia biglobosa* considerably lowered both hypercholesterolemia and hypertriglyceridemia, with the effect of fermentation being more evident. Therefore, there is an indication that PB may prevent the progression of CHD in diabetics.

Table 1: Result of Albumin level and lipid profile of diabetic rats fed with fermented and unfermented *iru*

GROUP	ALBUMIN (mg/dL)	TRIGLYCERIDE mmol/L	HDL mmol/L	LDL mmol/L	TOTAL CHOLESTEROL mmol/L
FERMENTED	36.3±0.01	0.84±0.01	0.22±0.01	2.32±0.01	2.25±0.01
UNFERMENTED	42.0±0.01	0.79±0.01	0.16±0.01	2.42±0.01	2.93±0.01

Despite the existence of well-known anti-diabetic medications, treatments made from medicinal plants are being utilized more and more successfully to treat this condition and better manage its complications (Bhattaram*et al*., 2002). Also, it has been claimed that herbal remedies and plant medicines are less harmful and devoid of side effects than synthetic drugs, which is causing people to favor natural plants over synthetic medicines (Saravanan &Pari, 2005). The WHO's interest in the hypoglycaemic drugs of plant origin used in the conventional treatment of diabetes may have been driven by growing evidence of the therapeutic efficacy of herbal remedies (WHO, 1980). It isinteresting to note that fermented *Parkia biglobosa* seeds significantly raised the serum level of HDL-C, also known as the "good cholesterol" (Agerholm-Larsen, 2000). An elevated HDL-C/LDL-C ratio was observed in the test animals as a result of the combined effects of elevated HDL-C (also known as "good cholesterol") and lowered LDL-C (also known as "bad cholesterol"). Given that a high HDL-C/LDL ratio has been demonstrated to be advantageous and is suggestive of a lower risk of CHD, this strongly supports the idea that dietary supplementation with fermented *Parkia biglobosa* can reduce the risk of developing heart disorders (Castelli, 1984). In the present investigation, treatment with unfermented *Parkia biglobosa* led to an HDL-C/LDL-C ratio that was much lower than the value in the rats treated with fermented *Parkia biglobosa*, which is a significantadvantage over the unfermented *Parkia* *biglobosa*. According to the results of the current study, both fermented and unfermented *Parkia biglobosa* seeds can lower blood sugar levels in rats that have been given alloxan to cause diabetes. In particular, weight loss and hyperlipidemia were revealed to be two consequences of diabetes that the fermented Parkia biglobosa may well manage. In order to stop the development of arteriosclerosis and potential concomitant cardiovascular pathology in diabetes, fermented *Parkia biglobosa* offers therapeutic promise. The unfermented *Parkia biglobosa*, on the other hand, raises blood LDL levels, which lowers the HDL-C/LDL-C ratio. As a result, the unfermented *Parkia biglobosa* exhibits a potential contraindication in cardiovascular illnesses because this is not conducive to cardiovascular pathogenesis.

In clinical diagnostics, the hepatic serum enzymes are an important instrument that can reveal the extent and kind of pathological damage to any tissue (Daisy &Sapriya, 2012). When tissues have been exposed to a particular pharmacological substance, the integrity of the plasma membrane and tissues is frequently evaluated using the AST, ALT, and ALP biomarkers. Aspartate transaminase (AST) and alkaline phosphatase (ALP) activity levels in the fermented *Parkia biglobosa* treated groups were significantly higher when compared to the unfermented treated groups, while ALT activity levels were significantly lower in the fermented treated diabetes group when compared to the unfermented group of the blood (Table 2). Compared to the unfermented group, the fermented group had considerably higher AST and ALP levels, and the liver levels of ALP were higher (Table 3). The well-known transaminases ALT and AST are utilized as biomarkers to identify potential toxicity in ill animals' blood. According to research by Kim *et al*. (2002) and Lee *et al.* (2018), a high concentration of these enzymes can cause hepatocellular damage or necrosis. According to Pratt and Kaplan's research, high ALT levels are frequently utilized as indicators of liver injury. In their studies, Westerbacka et al. (2004) and Lee et al. (2018) found that increased ALT is linked to the buildup of living fat, which can result in insulin resistance.

Table 2: ALP, AST, and ALT in the Serum of diabetic rats fed with fermented and unfermented *Iru*.

GROUP	ALP *(IU/L)*	AST *(IU/L)*	ALT *(IU/L)*
FERMENTED	333.6±0.15	32.6±0.15	42.3±0.15
UNFERMENTED	91±0.15	24.2±0.15	58.2±0.15

Table 3: ALP, AST, and ALT in the Liver of diabetic rats fed with fermented and unfermented *Iru*.

GROUP	ALP *(IU/L)*	AST *(IU/L)*	ALT *(IU/L)*
FERMENTED	606.5±0.05	218.4±0.05	583±0.05
UNFERMENTED	626±0.05	84.7±0.05	525±0.05

The observation in this study's Table 4 also demonstrates the biochemical analysis of the feacal sample. The organisms in the unfermented group were identified as both gram-negative bacilli, catalase positive, motile, glucose positive, and triple sugar iron producers, but they differed in their reactivity to indole and utilization of citrate, being Citrobacter freundii and Proteus vulgaris, while the organisms in the fermented group were Citrobacter freundii, Proteus vulgaris, and Enterobacter aerogenes were found in the feces of the glibenclamide-treated rats. These organisms have been identified as gut microorganisms present throughout the human body, with E. coli being the most common in the gastrointestinal tract (GI tract). The fermentation of locust bean seeds using a starting culture of Bacillus subtilis and the subsequent eating of its microbial cells by the rats during treatment are the sources of the organism *Bacillus subtilis* in the fermented group. A significant role for *Bacillus subtilis* in the gut may be possible, given their high metabolic activity. It might help the gut's digestive system produce necessary enzymes (Sharma &Satyanarayana, 2013). In a study, they successfully decomposed cholesterol in vitro following oral treatment in animal trials (Kim et al., 2002) and dramatically decreased plasma low-density lipoprotein cholesterol, hepatic total cholesterol, and triglycerides (Gaggia et al., 2011). Consequently, the results of this study demonstrate that fermented *Parkia biglobosa's* overall hypoglycemic effect and the lowering of rats' blood glucose levels may have been influenced by the presence of Bacillus subtilis in the gut microbiota. Indole and tryptamine, as well as endogenes (serotonin, melatonin, and kynurine) produced by these gut microorganisms, have an impact on microbial metabolism, microbial composition, and the host immune system (Gao *et al.*, 2018). Indole, which can control bacterial motility, antibiotic resistance, pathogenicity, and the development of intestinal biofilms, can be produced by Proteus vulgaris and *E. coli* (Gao *et al.*, 2018). Short-chain fatty acids, such as vitamins K and B, are produced by intestinal microorganisms (SCFAs). There is evidence that these short-chain fatty acids (SCFAs), produced by bacteria through the fermentation of carbohydrates, may impact blood pressure (Ufnal&Nowinski, 2018).

Table 4: Result of the colony count and organism morphology from the Microbiota study.

Group	No of colony	Edge	Color	Shape	Size	Surface	ORGANISM IDENTIFIED
Fermented	35± 0.01	Serrated	Cream	Spherical	Small	Flat/Elevated	*Bacillus subtilis* *Escherichia coli*
Unfermented	7±0.1	Serrated	Yellow	Round	Small	Rough/smooth	*Citrobacter freundii* *Proteus vulgaris*

Conclusion

The African locust bean significantly altered and destabilized fasting lipid and blood sugar profiles in this study. As a result, it can be used as a preventative measure and a treatment for several disorders brought on by hyperglycemia and hyperlipidemia. Those with diabetes should specifically eat more African locust beansto lower their blood sugar levels.

Ethical Approval: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).

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