Original Research Article

Effects of *Moringa oleifera lam.* leaf powder on *Bifidobacteria* and *Escherichia coli* in the gut of albino rats

ABSTRACT – make as aim, location, duration of study, design of study, method, result and conclusion; Leave the space before giving units like % or g/kg

The *Moringa oleifera* leaf alters the microbiota in the gut, a situation which sends impulses to the brain. This study was carried out to determine the effect of dried *Moringa oleifera* leaves on *Bifidobacteria* and *Escherichia coli* in albino rats at 2.5%, 5% and 10%. In this experimental analytic study, there were five Groups in all. The 5-6 weeks old rats were habituated under laboratory conditions at the Animal house of the Department of Zoology, Faculty of Science, University of Ibadan, for two weeks in other to adapt to the environmental conditions during the experiment before exposing them to the moringa feed for four weeks. Each group had 5 animals. No supplement of Moringa feed was administered to Group A while Group B received streptomycin antibiotics. Group C, D and E received dried leaf supplement of *Moringa oleifera* (DMO) 1.25g/kg body weight, 2.5g/kg body weight and 5.0g/kg body weight respectively. *E.coli* counts increased only by 13% in group E, 26% in group B; but reduced by 9.7% in group D and 27.7% in group C between day 20 and day 28. The rate of *Bifidobacteria* viable counts increase in group E is expressed as $P = .05$ at the beginning of the experiment unlike *E.coli* counts where there was a decrease. Thus, the *Moringa oleifera* leaf powder is a potential prebiotic for probiotics like *Bifidobacteria*, and as well induce changes in the gut-brain axis.

Keywords: *Moringa oleifera*, *Escherichia coli*, *Bifidobacteria*, prebiotic, gut.

INTRODUCTION

In the sub-Himalayan areas of Afghanistan, India, Pakistan, Bangladesh, *Moringa oleifera* is known to be widely grown, likewise in the tropics. The leaves, bark, flowers, fruit, seeds, and root are used to make medicine. “Tired blood”, also known as anemia, is treated using *Moringa oleifera*. Other diseases like arthritis and rheumatism are also treated using *Moringa oleifera* [19, 20].

Myriads of infections, ailments and diseases are also treated by the use of *Moringa oleifera*; these ailments and diseases include constipation, epilepsy, stomach pain, stomach and intestinal ulcers, asthma. Others are cancer, intestinal spasms, headache, heart problems, high blood pressure, diabetes, diarrhea, kidney stones, fluid retention, thyroid disorders. Furthermore, bacterial, fungal, viral, and parasitic infections are not excluded [12, 13].

Moringa oleifera contains various nutrients and important classes of food such as proteins, vitamins, and minerals. A very good characteristic of *Moringa oleifera* is its antioxidant ability because it protects cells from damage. Interestingly, all parts of the *Moringa oleifera* tree are edible. Over the years, humans of been consuming all parts of the *Moringa oleifera* tree. Various chemicals like alkaloids, proanthocyanidins, cinnamates, flavonoids and anthocyanins have been reported to be found in the *Moringa oleifera* tree [8, 14].

Thus, the experimental effect of *Moringa oleifera* leaf powder on albino rats could involve actions against oxidants and inflammations with probable mechanisms of action which will be evaluated in this study. *Moringa oleifera* is potentially active against free radicals [7].

A laboratory albino rat is a rat of the species *Rattus norvegicus* (brown rat) which is bred and kept for laboratory analysis and research in numerous fields across the medical and health sciences [17]. Arguably, Wistar rats were the first set of rats to be developed for the purpose of research and stand as model organisms. Distinct characteristics such as high activity rate, long ears for hearing sensitivity, makes it preferable than other type of rats for research.

Bifidobacterium is a genus of gram-positive, non-motile, often branched anaerobicbacteria. They are ubiquitous and inhabit major areas in the gut and tissues of humans and animals. Some of the major areas they inhabit are the gastrointestinal tract, vagina, mouth of mammals, including humans; in an endo-symbiotic relationship. *Bifidobacteria* are one of the common probiotics and major genera of bacteria that constitute a good fraction the colon flora in mammals [6, 18]. **Remove hyperlinks looks as if copied from website**

In the gut of humans, there exists a microbiota of organisms which include beneficial organisms like a few strains of *Escherichia coli* (*E.coli*), a type of coliform bacteria which has a few species that act in the synthesis of some vitamins. However, some strains of *E.coli* produce toxins and cause diarrhea in humans. An example is the O157:H7 strain.

The gastrointestinal micro-ecosystem is always fluctuating leading to an altered microbiota which disrupts the intestinal microbial balance exposing the compromised host to opportunistic infections [17].
The *Moringa oleifera* leaf has since become an important food supplement worldwide because it possesses anti-inflammatory and antioxidant properties [2]. The *Moringa oleifera* leaf powder's beneficial and bacteriocidal effect on organisms in the gut representative of *Bifidobacteria*, a Gram positive anaerobe as well as a probiotic and *Escherichia coli*, a Gram negative facultative aerobe as well as a prominent coliform respectively explains the need for this study.
The aim of this study is to examine and analyze the effect of *Moringa oleifera* leaf powder on the population of *Bifidobacteria* spp. and *Escherichia coli* in the gut of Wistar Albino rats.

METHODS – please mention abount ethical approval with reference number; Leave the space before giving units like % or g/kg

Moringa leaf powder preparation:
Approximately 500g of fresh tender leaves of *Moringa oleifera* were washed in water to remove dirt and later washed in 1% saline solution to remove microbes and washed again with fresh water [4]. Water was allowed to drain for about 15 minutes. The *Moringa oleifera* leaves were dried in air at 25-28^{o}C, turned over at intervals with gloves and kept away from sun rays for 7 days. The *Moringa oleifera* dried leaves were processed into powder form and kept in well-covered containers to prevent air [9]. One hundred grams of the dry powder was obtained

Animal Grouping:
Five groups of five (n=5) *Rattus norvegicus* albino rats of weight range 170–230g were used and named as follows:

- Group A (normal control) – fed with normal feed diet (50g/kg body weight per day per rat)
- Group B (experimental control) – received streptomycin 40mg/kg body weight/day per rat
- Group C – received Dried leaf supplement of *Moringa oleifera* (DMO) 1.25g/ kg body weight/day per rat (2.5% moringa feed)
- Group D – received Dried leaf supplement of *Moringa oleifera* (DMO)2.5g/kg body weight/day per rat (5% moringa feed)
- Group E- received Dried leaf supplement of *Moringa oleifera* (DMO)5.0g/kg body weight/day per rat (10% moringa feed).

Exposure to these treatments was done after the acclimatization period.
Thorough close physical examination as well as temperature reading was done to ensure the rats were healthy.

Bacteria counts:
The determination of bacteria counts in the faeces was performed. Viable fecal bacteria counts were determined before exposure and determined at 4-day intervals up to the 28th day of exposure. Faeces were collected in sterile containers, weighed and suspended in 10ml of 0.9% saline solution. This was shaken vigorously for 10-20 minutes to allow the larger particles to settle below. 1ml of the suspensions were serially diluted 10-fold and appropriate dilutions were plated in duplicates on Nutrient agar and incubated at 37^{o}C for 24-48hrs both aerobically and anaerobically.
On the 28th day of exposure, the gastrointestinal tract of animals from each group was cut open and samples were taken from the duodenum and ileum of the small intestine, as well as the ascending colon and descending colon of the large intestine including the control groups to analyze the tissue samples from the duodenum and ileum of the small intestine and from the ascending and descending colon of the large intestine in order to know the quantity of *Bifidobacteria* spp. and *E. coli* strains in the different intestinal parts from animals of each group in comparison with the control group(s). Swab of the intestinal parts were taken after they were cut open.

Animal housing and feeding:
The *Rattus norvegicus* rats (aged between 5-7 weeks) were housed five per cage. Cages (24x18x12cm) were made of plastic and metal guaze cover. The animals were habituated under laboratory conditions at the Animal house of the Department of Zoology, Faculty of Science, University of Ibadan, for two weeks in other to adapt to the environmental conditions during the experiment. They were fed with standard diet 50g/kg body weight per day per rat, and water was provided *ad libitum* (without measurement) [15].

Isolation of *E. coli*:
Thirty-seven grams of Eosin Methylene Blue (EMB) Agar (HMK ltd), fifty-two grams of MacConkey agar (Biotec ltd) and twenty-eight grams of Nutrient agar (HMK Ltd) were dissolved in one liter of distilled water, swirled and sterilised by autoclaving for 15 minutes at 121^{o}C. The prepared media was allowed to cool to about 45^{o}C and 20 ml volumes of the liquid medium was poured aseptically into sterilized petri dishes and allowed to cool before inoculation with suspected colonies of *E. coli*.

Isolation of *Bifidobacteria*
Three selectively modified Bifidobacteria media (BFM), selective media recommended for the isolation of the *Bifidobacterium* spp. from tissues, faeces or stool specimens were used for the isolation and identification of *Bifidobacteria* spp.: BFM 1, BFM 2 and BFM 3.

***Bifidobacterium* Medium (BFM 1)** was specially composed with the following ingredients in Grams per Litre: Peptone special (23.0), Sodium chloride (5.0), Glucose (5.0), L-Cysteine hydrochloride (0.3), Starch soluble (1.0), Agar (15.0), with a final pH of5.5±0.2 (at 25°C).
[6].
***Bifidobacterium* Medium (BFM 2)** was specially composed with the following ingredients in Grams per Litre: MRS Agar (25.0), L-Cysteine hydrochloride (5.0) [6]
***Bifidobacterium* Medium (BFM 3)** was specially composed with the following ingredients in Grams per Litre: Peptone (5.0), Sodium chloride (5.0), Lactulose (5.0), L-Cysteine hydrochloride (0.5), Starch soluble (2.0),Tryptone (15.0), Meat Extract (2.0), Yeast Extract (7.0), Peptone(5.0), Riboflavin (0.001), Thiamine Chloride HCl(0.001), Methylene blue (0.016), Lithium chloride (2.0), Propionic acid (5ml) (added after sterilization at 55^{o}C) at a final pH (at 25°C) of 5.5 (with 10N NaOH).
NB: Lactulose is the main carbon source.
Methylene blue, propionic acid and lithium chloride are inhibitors of other bacteria.
The low pH inhibits *Enterobacter*. **BFM 3 is a novel media composition specifically used for this study**.
Sub-culturing:
The distinct colonies from agar plates were cultured on freshly prepared agar plates using proper streaking techniques. Pure isolates were sub-cultured on prepared nutrient agar slants in McCartney bottles at 37^{o}C overnight
Cultural characteristics of organisms
Distinct colonies from the plates were observed and classified based on the cultural characteristics such as shape, surface, elevation, colour, opacity, consistency and edges on the agar plate.
Biochemical tests
This involved the use of the indole, methyl red, VogesProskauer and citrate (IMViC) tests as well as catalase, urease and sugar fermentation tests.
The test required to identify *Bifidobacteria* is the Fructose-6-phosphatephospho-ketolase (F6PPK). The F6PPK detection was used for the identification of *Bifidobacteria* were the isolates were grown anaerobically for 42 hours in a 20 ml broth culture at 37°C. The broth was centrifuged for 3 minutes to harvest the cells at 14 000 × g. The centrifuged broth formed a pellet of harvested cells which was washed twice with a phosphate buffer (0.05M, pH 6.5, cysteine 500 mg/l) and ruptured in ice for 2 minutes and mixed with 0.25 ml each of Sodium flouro-iodoacetate and 7 fructose-6-phosphate solution. The reaction was incubated for 30 minutes at 37°C and 1.5 ml of Hydroxymine Chloride (pH 6.5) was added. At room temperature, 1 ml each of 15% Tricarboxylic Acid, 4M Hydrochloric Acid and Iron III Chloride Hexahydrate was added.
The tube was shaken vigorously after the addition of each solution. A reddish-violet colour indicated the presence of fructose-6- phosphate phosphoketolase characteristic of *Bifidobacteria* spp. The result was negative if the colour remained yellow [6].

RESULTS – combine discussion with this result; Leave the space before giving units like % or g/kg

The Gram-staining reaction revealed short Gram-negative rods which were further identified as *E.coli.* Indole, Methyl-red, citrate (IMViC) biochemical and sugar-fermentation tests results confirmed the presence of *E. coli*.
The Gram-staining reaction also revealed Gram-positive rods of various sizes and shapes, in single and chains further identified as *Bifidobacteria* of characteristic V-shape and Y-shape otherwise known as 'palisade' arrangements.
Fructoso-6-phosphate phospho-ketolase test results for *Bifidobacteria*
The F6PPK test result for *Bifidobacteria* was positive if there is a reddish-violet colour immediately after shaking the tube which indicates the presence of the fructoso-6-phosphate phospho-ketolase enzyme characteristic of *Bifidobacteria* spp. The F6PPK result was negative if the colour does not change from yellow to reddish-violet [6].
Bifidobacteria strains were not really affected by the streptomycin but were resistant to it unlike the *E. coli* strains that were susceptible to streptomycin.
Variation in counts of *E. coli* and *Bifidobacteria* in faecal samples in group B shows that *E. coli* was susceptible to streptomycin. In group C, D and E, the rats were fed with 2.5%, 5%, and 10% *Moringa oleifera* rat feed respectively (i.e 1.25g/kg body weight, 2.5g/kg body weight and 5.0g/kg BW respectively) between days 1 to 28.
Table 1 (one) shows the proximate analysis of the moringa oleifera leaf powder indicating the protein content, ash content and other nutrients.

Table 1: Proximate analyses of *Moringa oleifera* leaf powder:

Parameter	Calculated nutrients values
Dry matter, DM (%)	90.92
Crude protein, CP (% DM)	31.5
Ether extract, EE (% DM)	14.8
Crude fiber, CF (% DM)	37.4
Lysine (% DM)	0.94
Methionine (% DM)	0.42
Ash Content(% DM)	9.0
Calcium, Ca (% DM)	1.05
Phosphorus, P (% DM)	0.69
Vitamin B_1	0.09
Vitamin B_2	0.05
Vitamin B_3	0.8
pH	6.27

- Phytochemical analysis also showed the presence of flavonoids and saponins

The colony counts of both *E. coli* and *Bifidobacteria* in intestinal samples of the albino rat reveal a relatively high level of *Bifidobacteria* counts in the ascending colon of Group E rats shown in Table 2 (two).
Table 2 (two) shows antimicrobial activity of *Moringa oleifera* and streptomycin on intestinal isolates of *E.coli* and *Bifidobacteria* in Groups A-E, while Table 3 (three) shows that of the fecal isolates.

Table 2: Antimicrobial activity of *Moringa oleifera* and streptomycin on intestinal isolates of *E.coli* and *Bifidobacteria* in Groups A-E.

		ZONES OF INHIBITION (mm)			
		Bifidobacteria		***E. coli***	
Group	Intestinal part	M(50mg/l)	S(0.64mg/l)	M(12.5mg/l)	S(0.16mg/l)
A	Du	N	N	N	20
	I	N	N	N	16
	Ac	N	N	N	20
	Dc	N	N	N	20
B	Du	N	R	N	10
	I	N	R	N	16
	Ac	N	R	N	8
	Dc	N	R	N	12
C	Du	R	R	R	14
	I	R	R	R	18
	Ac	R	R	R	20
	Dc	R	R	R	18
D	Du	R	R	R	20
	I	R	R	R	16
	Ac	R	R	R	18
	Dc	R	R	R	20
E	Du	R	R	R	16
	I	R	R	R	18
	Ac	R	R	R	16
	Dc	R	R	R	20

N-Negligible<6, R-Resistant,Du-Duodenum, I-Ileum,Ac-Ascending colon,Dc-Descending colon.

However, Figures 1 (one) and 2 (two) shows the comparative variation in counts of *Bifidobacteria* and *E. coli* respectively in faecal samples between days 1 to 28 which reveals a decrease in the *E. coli* and *Bifidobacteria* viable counts up to day 8 in groups C, D and E. *Bifidobacteria* counts increased by more than 50% between day 12 and day 16 with group E having the highest percentage increase but became relatively stable between day 20 and 28. *E.coli* counts increased only by 13% in group E, by 26% in group B; but reduced by 9.7% in group D and 27.7% in group C between day 20 and day 28.

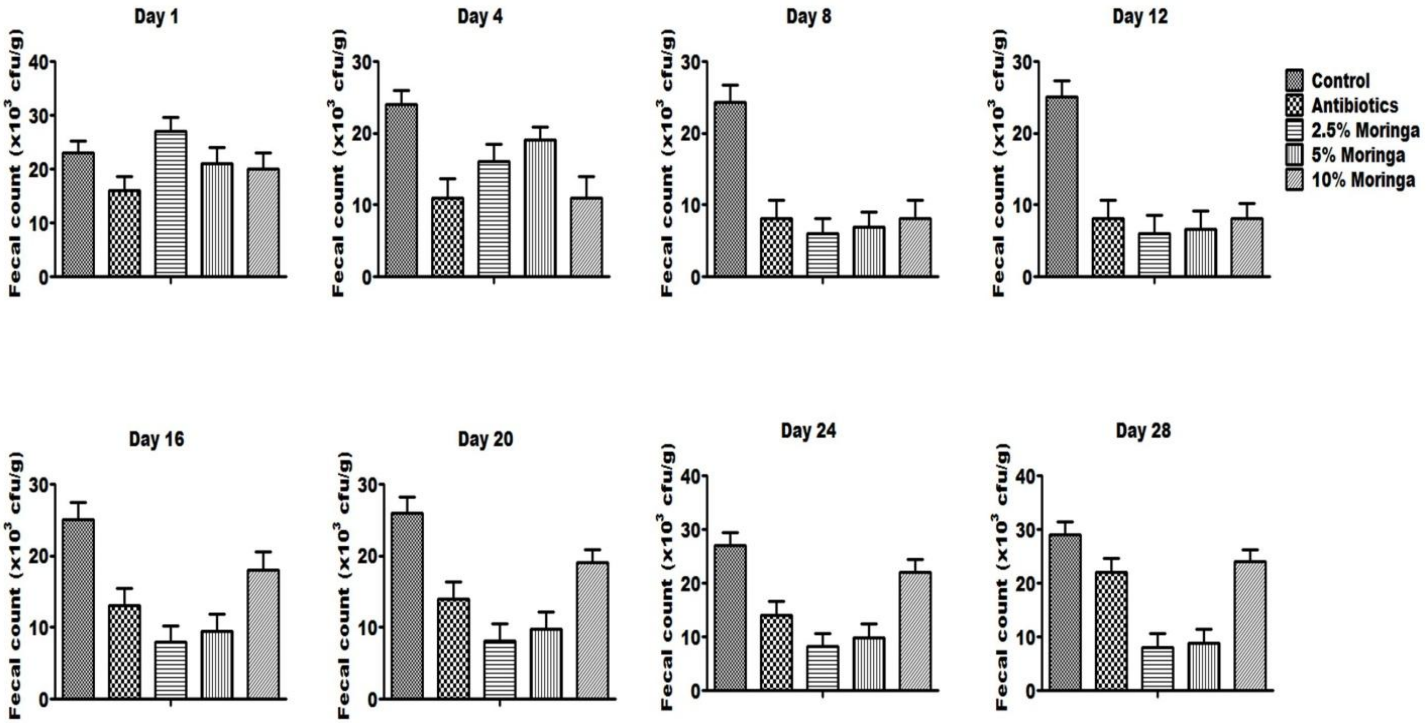

Figure 1: *Bifidobacteria* fecal counts in all groups between days 1 and 28.

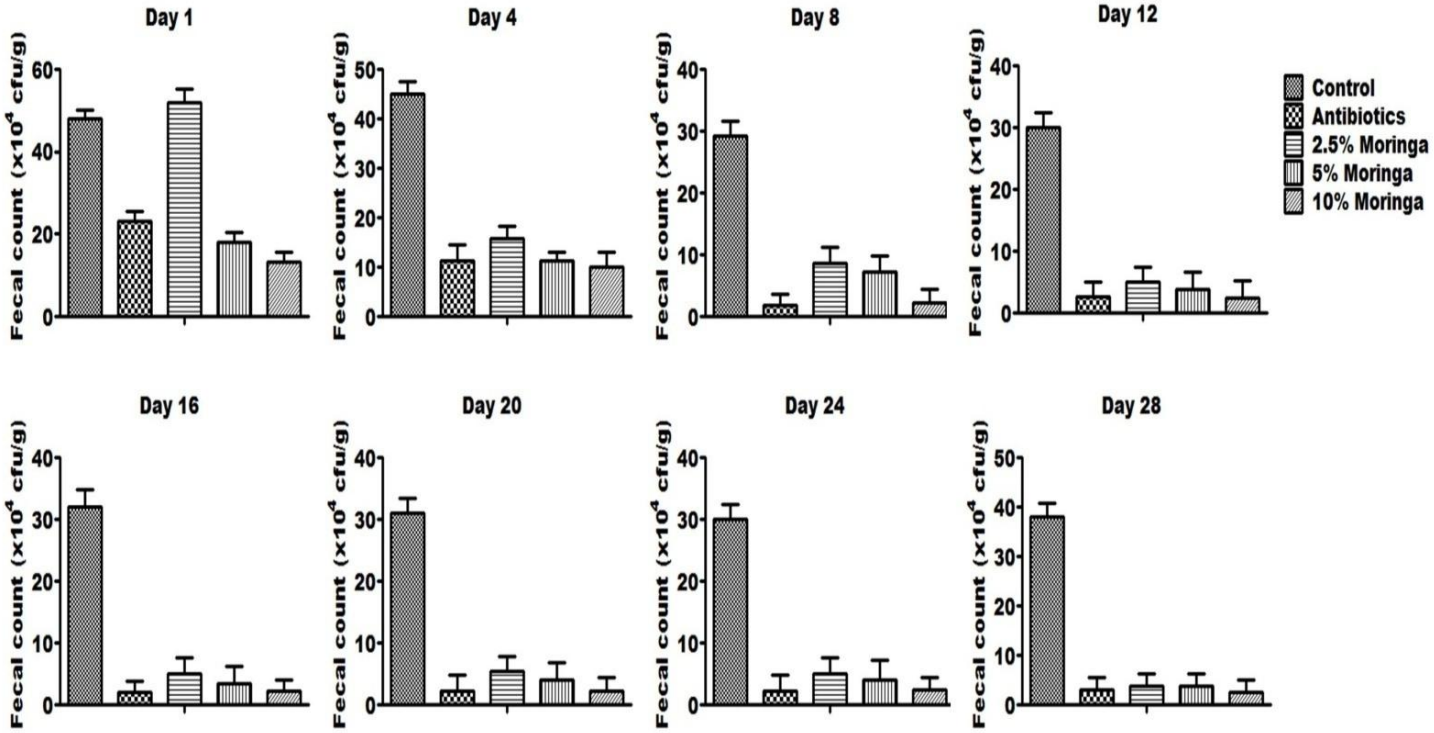

Figure 2: *E. coli* fecal counts in all groups between days 1 and 28.

The *E. coli* and *Bifidobacteria* counts in faecal samples in all groups between days 1 to 28 is shown in Table 3 (three). Group A without moringa feed supplement is the control group. Group B were given with normal rat feed and streptomycin 40mg/kg body weight.

Table 3: Antimicrobial activity of *Moringa oleifera* and streptomycin on faecal isolates of *E.coli* and *Bifidobacteria*.

	ZONES OF INHIBITION (mm)																			
	Group A (Control)				Group B (Antibiotics)				Group C (2.5% Moringa)				Group D (5% Moringa)				Group E (10% Moringa)			
	E. coli		Bfd		*E.coli*		Bfd		*E.coli*		Bfd		*E. coli*		Bfd		*E. coli*		Bfd	
	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M
Day 1	14	8	6	6	14	8	6	6	14	10	8	6	16	10	6	6	16	8	6	6
Day 4	14	8	8	6	16	8	6	R	14	6	6	R	16	6	6	R	18	6	6	6
Day 8	20	8	6	R	18	18	R	R	16	R	R	R	18	6	R	R	18	6	R	R
Day 12	20	6	6	6	20	R	R	6	20	R	R	R	18	R	R	R	24	R	R	R
Day 16	18	8	6	6	12	R	R	R	14	R	R	R	14	R	R	R	14	R	R	R
Day 20	18	6	6	6	12	R	R	R	14	R	R	R	20	R	R	R	14	R	R	R
Day 24	20	6	N	N	12	R	R	R	16	R	R	R	20	R	R	R	20	R	R	R

Day 28	20	N	N	N	10	R	R	R	14	R	R	R	20	R	R	R	16	R	R	R

E.coli-Escherichia coli, Bfd-*Bifidobacteria*, M-*Moringa oleifera*, S-Streptomycin, R-Resistant, N-Negligible(< 6)

Table 4 (four) reveals that at 50 mg/l for *Moringa oleifera* and 0.64 mg/l for streptomycin, *E. coli* is strongly inhibited while *Bifidobacteria* is slightly inhibited.

Table 4: Determination of minimum inhibitory concentration of *Moringa oleifera* and streptomycin

	Moringa oleifera(mg/l)			
	50	25	12.5	6.25
E. coli	24mm	16mm	8mm	0mm
Bifidobacteria	6mm	0mm	0mm	0mm
	Streptomycin(mg/l)			
	0.64	0.32	0.16	0.08
E. coli	32mm	20mm	12mm	0mm
Bifidobacteria	6mm	0mm	0mm	0mm

Figure 3 (three) shows the effect of the *Moringa oleifera* leaf powder on *Bifidobacteria* and *E. coli* in mean and standard deviation form.

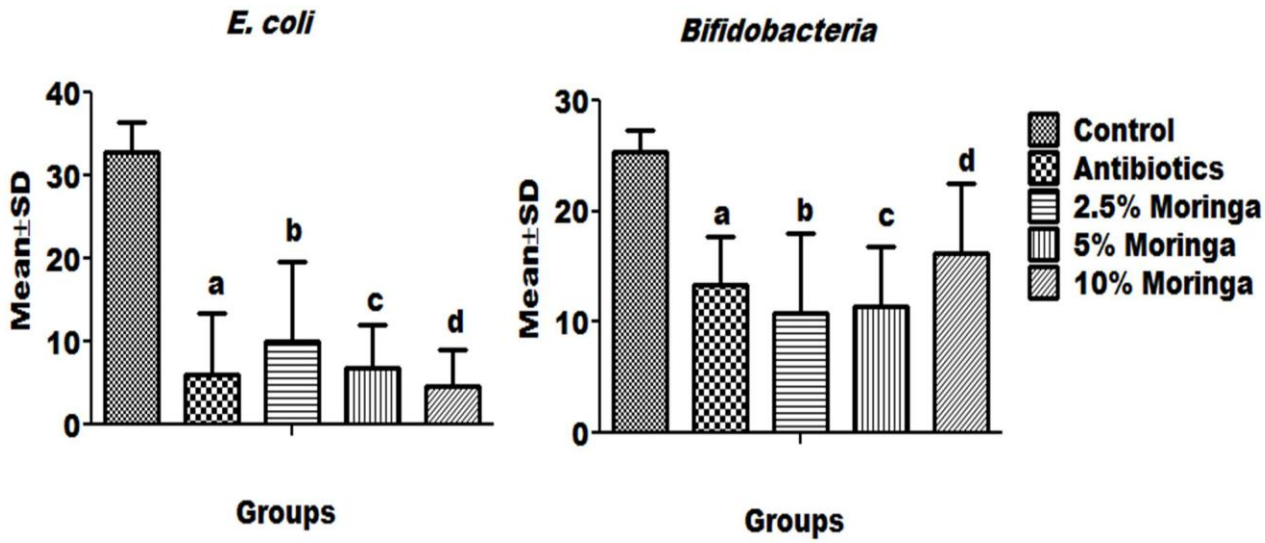

Figure 3: Effect Of *Moringa Oleifera* Leaf Powder On *Bifidobacteria* And *E. Coli* Counts In All Groups In "Mean±Sd" Form

a= Statistically significant compared to the control group.
b= Statistically significant compared to the control and antibiotics group.
c= Statistically significant compared to the control, antibiotics and 2.5% Moringa group.
d= Statistically significant compared to the control, antibiotics, 2.5% and 5% Moringa group

Table 5 (five) reveals a significant increase in both *Bifidobacteria* in group E and *E. coli* in group C with high maximum limits of the organisms in the respective groups using the Dunnet's test.

Table 5: Comparison ratio of *Bifidobacteria* and *E. coli* counts after various treatments of *Moringa oleifera* leaf powder using Dunnett's test.

	Bifidobacteria		***E. coli***	
Group	Min. limit	Max. limit	Min. limit	Max. limit
C	-8.4	3.6	-3.17	11.27
D	-7.85	4.15	-6.28	8.16
E	-3.02	8.98	-8.58	5.86

NB: Groups A and B are not included because they are negative and positive controls respectively.

DISCUSSION – combine this section with result at relevant places; Leave the space before giving units like % or g/kg

The *Moringa oleifera* tree plant is a very important source of micro and macro nutrients, including dietary fibre. It has respectable antioxidant and anti-inflammatory potency. As a single plant it contains almost all nutrients from all other herbs combined. It is a potential source of tocopherols, pro-vitamin A, vitamin C, calcium, protein and minerals. The *Moringa oleifera* plant leaf is the most nutritious part of the plant containing significant quantities of crude protein, vitamins and minerals. The percentage dry weight of crude protein of the *Moringa oleifera* leaf powder used in this study (31.5% Dry Matter) is higher than that of other vegetable leaves. Abiodun *et al*. [2] reported that the *Moringa oleifera* leaf powder has 25.29% protein dry weight of which no other vegetable has close to such amount.

Over the years, people have made use of plants and herbs to cure several illnesses and diseases like fever, wounds and bruises, constipation, weight management, cardiovascular diseases and many others.

The effect of *Moringa oleifera* leaves extract at different concentrations on growth of studied probiotic bacteria (i.e *Bifidobacteria* spp.) showed that the growth of all studied probiotic bacteria was affected by the *Moringa oleifera* leaf extract [3]. Furthermore, Abeer, *et al*. [3] reported that increasing the concentration of *Moringa oleifera* leaves extract from 0 to 8% led to increase in the probiotic bacterial growth at 37°C for 24hours of incubation time. The statement above is in tandem with the result obtained in this research work where much increase in *Bifidobacteria* counts were observed at 10% *Moringa oleifera* concentration (in group E) than all other groups between days 12 and 16. Abeer *et al*. [3] also stated that optimum growth was recorded for all probiotic bacteria at 8% concentration of the *Moringa oleifera* leaves extract at 37°C for 24 hours incubation time. *L. jonsonii* and *B. adolescentis* exhibited a higher growth than those of *L. casei* and *B. lactis*, respectively. The presence of essential amino acids in the *Moringa oleifera* leaves improved the growth of the organisms [3]. The antibacterial activity exhibited on some bacterial isolates by *Moringa oleifera* could be as a result of the presence of flavonoids and tannins, since these phytochemicals are reported to confer antibacterial activity.

Furthermore, this study reveals the *Moringa oleifera* leaf powder is being used as a modulatory tool against probable disruption of gut microbiota by certain factors causing intestinal microbial imbalance which exposes the compromised host to opportunistic infections. All animals in the groups fed with the *Moringa oleifera* leaf powder supplement were found to be healthier and stronger throughout the days of the experiment. The animals in groups C, D, and E appeared to be more active especially animals in group E with 10% *Moringa oleifera* leaf supplement.

The hyperactivity in these groups of animals exposed to the *Moringa oleifera* leaf powder could be linked to the biochemical signals between the gastrointestinal tract (GIT) and the central nervous system (CNS) apparently described as the gut-brain axis. This explains that changes in the microbiota of the GIT immediately triggers neurotransmitters to the parts of the nervous system connected with the gut which includes the vagus nerve, CNS, enteric nervous system, autonomic nervous system and hypothalamic-pituitary-adrenal (HPA) axis. Drugs and food always cause microbiota changes which further cause changes in the levels of cytokines, which further affect the brain functions [11].

CONCLUSION AND RECOMMENDATION

The addition of *Moringa oleifera* leaf supplement to animal food could help increase beneficial bacteria as well as inhibit harmful bacteria in the gut. However, the results of the present study could suggest *Moringa oleifera* mechanism of action in *E. coli* involves anti-propulsive and antisecretory effects. *Moringa oleifera* can be used to create high activated carbons

which are able to sequester and remove cyanobacterial microcystin-LR quite effectively and the leaf extract also appears to be capable of suppressing cyanobacterial growth as 20-160mg of *Moringa oleifera* extract per liter of water is able to suppress growth of *Microcystis aeruginosa* and cause the colony count to decline [7].

Therefore, moringa dried leaf supplement is suitable for the growth of beneficial organisms like *Bifidobacteria* in the gastrointestinal tract. Beneficial bacteria of intrinsic antibiotic resistance could also be boosted by *Moringa oleifera* plant leaf to restore the gut microbiota after antibiotic treatment. *Moringa oleifera* leaf supplement is suitable for improving the intestinal microbial balance thus could serve as a modulating tool for the immune system [4, 10].

Bifidobacteria has been known to prevent the incidence of inflammatory bowel diseases (IBD) like ulcerative colitis and Crohn's disease in the gut of humans and animals over the years [1, 16]. Thus, the increase in the load of *Bifidobacteria* in the gastrointestinal tract tends to reduce the rate of IBD occurrences [6].

Therefore, 10% moringa leaf in the powder form is recommended as a prebiotic for adults per meal per day for a substantial boost in the load of *Bifidobacteria* and other probiotics in the gastrointestinal tract and for an increase in activity as observed in this study.

Only at 10% Moringa powder concentration did *Bifidobacteria* grow maximally, while *E. coli* counts were low; but at 2.5% concentration, there were low *Bifidobacteria* counts but high *E. coli* counts. This typically reveals the importance of dosage and concentration in herbal therapy and medicinal plant intake as it were in pharmaceutical drugs administration. It also shows that the 2.5% moringa powder concentration was not effective enough in inhibiting the growth of *E. coli* and exhibiting the growth of *Bifidobacteria,* while the 10% moringa leaf powder concentration had optimum inhibitory effect on *E. coli* and served as a boost for probiotic *Bifidobacteria.* The powder form of the moringa leaf mixes well with food and quickly starts to break down as the food chyme undergoes an enzymatic process in the mouth.

In addition, the noticeable hyperactivity signs in the albino rats and the bidirectional neurohumoral communication system between the brain and the gut suggest the use of moringa leaf powder as a potential modulator of the gut flora connected with the correlating effect on the vagus nerves which later sends the information about the intestines to the brain.

Further studies can be done on how the moringa leaf supplement induces changes in the gut and the systematic effects it has on the brain. The correlation between the microbiota and human emotions could be checked in further studies putting into consideration possible feedback loops in the gut-brain axis.

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