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Evaluating the effects of fermented cabbage juice on the gastrointestinal microbiome of wistar rats

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Abstract

This study is to evaluate the effects of fermented cabbage juice on the intestinal microbiota load, weight and some selected hematological parameters of animals using Wistar rats as test organism. A total of fifteen Wistar rats were placed in three different treatment groups comprising of five animals each. One of the groups was treated with fermented cabbage juice containing sodium chloride and the second group was treated with fermented cabbage juice without salt for seven days. The third group served as control. Animals were sacrifice after seven days of cabbage juice admiration, thereafter bacteriological and hematological tests were carried out using standard methods. Bacteriological results showed obvious reduction in the bacterial load of the two tests groups, with the control group showing no such microbial load reductions after the gut and fecal matter cultured and plated indicating potent antimicrobial activity of the cabbage juice. Comparison of the pretreatment and post treatment weights of the test groups showed a slight increase in mean weight of animals while the control group had slight mean decrease in weight. Hematological data showed appreciable increase in mean white blood cell count of treatment groups with the control having the least mean count. Lipid profiles of treated animals had low density lipoproteins and high density lipoproteins levels within healthy ranges. The findings of this result suggest that *Brassica oleracacae* possess strong antimicrobial activity and immune system boosting activity by enhancing normal gut microflora. The potential health benefits associated with consumption of fermented juice of cabbage appears rewarding.

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Introduction

Our ancestors in years past consumed a lot of food products that were unknowingly to them, subjected to microbial fermentation. Though they had no sordid knowledge of microbes and their roles in fermentation of food, they recognized over time that some of these fermented foods had improved palatability and also improved their health overall when consumed (Steinkraus, 2002). Thus, the stage was set for the purposeful application of fermentation to provide value in the areas of human nutrition, traditional medicine, and culture (ceremonies, and so on) (Henderson *et al.*, 2007, Mc Govern *et al.*, 2010). It is difficult to say with certainty when intentional fermentation began. However, sophisticated measurements of the chemical content within ancient Neolithic vessels suggest intentional fermentation of fruit, rice, or honey beverages has been in common practice for close to 10,000 years (Mc Govern *et al.*, 2004). As agriculture expanded, so too did intentional food fermentation techniques. Beyond the established use of fermentation for alcohol production, it is now obvious that household fermentation of cereals, dairy products, vegetables, fish, seafood and meat were a significant part of ancestral dietary practices. (Caplice and Fitzgerald 1999). These technological advances did not result in the abandonment of the consumption of fermented foods, in sharp contrast however, the consumption of fermented foods and beverages remain widespread in traditional dietary practices across the world, currently accounting for approximately one third of the human diet globally (Borresen *et al,.* 2012).

A micro biome which may as well be referred to as 'micro biota' is "an ecological community of commensal, symbiotic and pathogenic microorganisms that literally share our body space". This term was first used by Joshua Lederberg in 2001 (Lederberg and McCray 2001) while he was emphasizing the importance of the microorganisms inhabiting the human body in health and disease. Thus, the gut microbiota is the community of microorganisms that inhabit the gastrointestinal tract of animals. These microorganisms have been known to show several host-microbe relationships such as commensalism, mutualism and even disease causing relationships such as parasitism.

The skin, nasal passages and gastrointestinal tract of all animals are inhabited by a very broad number of microorganisms. Since these organisms are natural inhabitants of the above mentioned parts of the body, they are nonpathogenic and play crucial protective roles. The most studied of these are the microorganisms inhabiting the gut. These organisms are charged with a primary function of aiding food digestion and metabolism. Beyond digestion, these microbes contribute to the overall health status of the animal by educating and regulating the host's immune system and preventing colonization of the gut by other pathogenic or harmful microorganisms. Significant progress has also been made over the past decade in recognizing the important ways in which gut micro biota relate to brain function (Foster and Neufeld 2013).With the current rise in prevalence of conditions such as diabetes, irritable bowel syndrome and obesity, lots of research has been conducted in developing new drugs to tackle some of these conditions. However, because of the unwanted side effects and costs of development, production and purchase costs of these drugs, scientists are turning their attention to less conventional and more traditional methods of treatment used by our parents of old. Prominent among these is the consumption of fermented food (yogurt, "kimchi" "saukraut" etc), a practice that over time has been recognized to improve food palatability and also caused an improvement in overall health when consumed (Steinkraus, 2002). Several studies have implicated some otherwise "healthy" organisms that live within the human gut in some of these disease conditions. Such include studies carried out by Ley (Ley *et al.,* 2006) where results showed that the guts of obese rats contained more populations of formicates, thus implicating the phyla formicates in obesity. A lot of literatures have been published, all pointing to the beneficial physiological effects of fermented foods on enteral nutrient absorption and on the digestive tract health (Wang *et al.,* 2011, Lee *et al.,* 2012). It is in this

light that this study was carried out to investigate the effects of one of such fermented food (fermented cabbage juice) on the gut micro biota of Wistar rats and also the general effects a consumption of this food has on the blood.

Materials and methods

Plant collection and sample preparation

A ball of *Brassica oleracae* (wild cabbage) was purchased from the main market of Abraka town in Delta state, Nigeria. The ball was properly washed and cut into two halves. Each half was diced into small bits. The first portion weighing 400g was placed inside a well washed container, soaked with 500ml of sterile water and to this, 10 grams of table salt (sodium chloride) was added and the container was covered. This was labeled as T_1. The second portion, an equal weight of 400g was likewise placed in another rubber and equally 500ml of water was added, the container was covered and labeled T_2. The samples were left to ferment for 7 days while the pH was checked at a regular two days interval.

Plant sample collection

The liquid extract from the fermenting cabbage plant was collected by means of filtration, using sterile sieves. This liquid was then transferred into two separate sterile bottles, properly sealed, labeled appropriately and stored in a refrigerator.

Experimental animals

A total of Fifteen (15) wistar rats were used for the research work. These animals were purchased from the animal house of the Department of Pharmacology, Delta State University, Abraka. These animals were placed in a condition of optimum care with food and clean water for a period of one week to acclimatize. The animals were thereafter weighed and randomly assigned into three (3) groups of five (5) animals each and labeled 'T_1', 'T_2' and 'Control'.

Treatment of animals

Route Used (Oral): Treatment of animals with collected sample was carried out for duration of 7 days. The route of treatment was oral using a cannula. A corresponding 2ml volume of the labeled fermented cabbage juice was administered three times daily to all mice in their respective groups. Group T_1 animals were treated with cabbage juice extract labeled as T_1 while the T_2 animals were also treated with cabbage juice extract labeled as T_2. The "Control" group was fed normally with broilers marsh and water for a period of 7 days.

Collection of animal samples

Samples were collected from the faeces and gastrointestinal tract of animals in each group by swabbing with a sterile swab stick. The animals were also weighed again on day 7 and the new weights recorded.

Fecal matter from cages in each treatment group was randomly collected on days 0 and day 7. This was then cultured in a broth medium and incubated for 24 hours at 37^{o}C. Cultured organisms were then plated on a nutrient medium to assay bacterial load.

On day 7 of treatment, the animals were sacrificed and their blood samples collected according to the method described by Peggy *et al* 2012 into EDTA and heparinized bottles. These blood samples were then processed for different hematological parameters.

The stomach of each sacrificed animal was dissected and the gastrointestinal tract excised.

The colonic contents in the dissected intestines were swabbed with a sterile swab stick, labeled and cultured in a broth medium.

Serial dilution

A serial dilution is the step wise dilution of a substance wherein a dilution factor at every step is kept constant. A seven fold 1 in 10 serial dilutions was used. Exactly 9ml of Peptone water was transferred aseptically into 7 different sterile test-tubes. A 1ml volume of the broth was transferred into the first tube and d tube was rocked slowly, and from this another 1ml was taken and transferred to the second tube and likewise rocked for proper mixing. This process was

repeated for every other test tube until the final tube where a 1ml volume was equally taken and discarded. Thereafter 0.1ml of this mixture was taken from the last test-tube and poured into plates containing already solidified nutrient media and then spread with a sterilie spreading rod. The plates were then incubated at 37°C for 24hours and the colonies counted.

Bacteriology

The fecal and colonic samples collected were used to prepare an overnight culture broth of the organism. Culture broth was prepared using sterile peptone water. A 5ml volume each was transferred into McCartney bottles and this was inoculated by aseptically dipping the swab sticks into the bottles and incubating for 24 hours at 37°C.

A slant was prepared from the overnight culture broth using prepared nutrient agar. From the slants of organisms prepared, overnight broth cultures were made using peptone water prior to plating for colony count. From this overnight broth, a seven fold 1 in 10 serial dilution was made and 0.1 ml from the final dilution tube volume was plated on a solidified Nutrient agar media and a solidified McCartney agar by the spread plate method.

The plates were incubated for 24 hours at 37°C and bacterial colonies counted

Biochemical tests

Catalase Test, Indole Test, Hydrogen Sulphide Test, Fermentation Test, Oxidase Test, Citrate Utilization Test, Urease Test and Gram Staining were all carried out following standard microbiological procedure as described by Monica 2004

Hematological parameters

Hematological parameters for collected blood samples from the sacrificed animals were assessed by running the blood samples in an Abacus Junior Hematology Analyzer (S/N 111325).

Results

Microbial colony counts of fecal matter of the animals prior to the administration of the fermented cabbage juice showed a huge amount of fecal microbial load of the organisms that were randomly selected from the three groups.

Table 1. Body weight of animals before and after treatment.

	Control	T_1	T_2
Body Weight (g)			
Initial	147.4±22.7	144.8±22.5	99.4±26.4
Day 7	140.0±17.1	143.8±19.91	107.6±28.2

Results are presented as mean ± SDs.

T_1; fermented cabbage juice with sodium chloride.

T_2; fermented cabbage juice without sodium chloride.

In contrast, Post treatment colony counts for the animal groups treated with the fermented cabbage juice showed very marked reductions in the fecal microbial load of the animals as the colonies obtained on both Nutrient agar and Mac Conkey agar plates appeared distinct and countable, indicating a strong antimicrobial activity of fermented cabbage juice in concordance with studies carried out by Kyung and Fleming, 2004 on the antimicrobial properties of cabbage.

Key:WBC = white blood cell; RBC = red blood cells; HGB= haemoglobin; PCV= Pack cell volume; HDL= high density lipoproteins; LDL= low density lipoproteins; Na^{++}=sodium ions; K^{+} = potassium ions; Cl^{-} = chloride ions; Urea= Blood Urea.

Blood lipid profile analysis of the tests animals showed that the juice extract had minor to no effects on LDL and HDL levels. All test animals presented HDL levels that were significantly within the normal

range prescribed for healthy HDL levels (>35 mg/dl). Mean HDL levels for T1, T2 and Control groups are 26.6±2.30mg/dl,31.0±6.63mg/dl and 27.0±2.0mg/dl respectively. High density lipoprotein (HDL) levels were also significantly low and within normal healthy range (<130mg/dl) in all animals with T1 treatment group having mean value of 57.88±16.05mg/dl, T2 having mean values of 70.20±36.35mg/dl and control having mean LDL values of 57.2±5.89mg/dl. Other hematological parameters as presented in table remained fairly constant and within normal ranges in both treatment and control groups, and are statistically not significant to this study.

Table 2. Fecal sample microbial colony count.

Culture plate number	Pre-treatment colony count	Post treatment colony count
	Control	
1	TNTC	46
2	TNTC	TNTC
3	TNTC	TNTC
4	TNTC	TNTC
	Treatment group T_1	
1	131	71
2	TNTC	85
3	TNTC	92
4	TNTC	146
	Treatment group T_2	
1	TNTC	41
2	TNTC	59
3	TNTC	67
4	TNTC	83

Key:

TNTC; too numerous to count

Table 3. Biochemical test results for organisms isolated from fecal matter of rats at day 0.

Culture plate number	INDOLE	CATALASE	OXIDASE	UREASE	CITRATE	H_2S	GLUCOSE	LACTOSE	SUCROSE	MOTILITY	GRAM STAIN	SHAPE	IDENTIFIED ORGANISM
CONTROL GROUP													
1	+	+	_	+	+	_	A/G	-/ -	A/G	+	-	Rods	*Providentia spp*
2	+	+	_	+	+	_	A/G	-/ -	A/G	+	_	Rods	*Providentia spp*
3	+	+	_	+	+	_	A/G	-/ -	A/G	+	_	Rods	*Providentia spp*
TREATMENT GROUP T_1													
1	+	+	_	+	+	_	A/G	-/-	A/G	+	_	Rods	*Providentia spp*
TREATMENT GROUP T_2													
1	_	+	_	+	+	+	A/G	A/G	A/G	+	_	Rods	*Citrobacter spp*
2	_	+	_	+	+	+	A/G	A/G	A/G	+	_	Rods	*Citrobacter spp*
3	_	+	_	+	+	+	A/G	A/G	A/G	+	_	Rods	*Citrobacter spp*

Key**:** (+) Positive result ;(–) Negative result; A/G -Acid and GaS.

Discussion

A comparison of the mean post treatment weight of the three groups showed that animals in control group reduced slightly in body mass (140.0±17.1 as against 147.4±22.7 initial weight). Pre and post treatment weight measurements for the animals treated with the fermented cabbage juice showed a slight increase in mean body weight, this is in concordance with research carried out by Meizi *et al.,* 2015 where similar initial weight gains were recorded after a four week administration of fermented cabbage extract (Korean "kimchi"). This slight increase in weight may be due to an enhanced digestion and nutrient absorption and utilization caused by the introduction of healthy microbes present in the fermented juice extract.

Table 4. Biochemical test results for organisms isolated from fecal matter of rats at day 7.

Culture plate number	Indole	Catalase	Oxidase	Urease	Citrate	H_2S	Glucose	Lactose	Sucrose	Motility	GRAM stain	Shape	Identified organism
Control group													
1	_	+	_	+	+	+	A/G	-/-	A/G	+	_	Rods	*Proteus mirabilis*
2	_	+	_	+	+	+	A/G	-/ -	A/G	+	_	Rods	*Proteus mirabilis*
3	_	+	_	+	+	+	A/G	-/ -	A/G	+	_	Rods	*Proteus mirabilis*
4	_	+	_	+	+	+	A/G	-/ -	A/G	+	_	Rods	*Proteus mirabilis*
Treatment group T_1													
1	_	+	_	+	+	+	A/G	-/ -	A/G	+	_	Rods	*Proteus mirabilis*
2	_	+	_	+	+	+	A/G	-/ -	A/G	+	_	Rods	*Proteus mirabilis*
3	_	+	_	+	+	+	A/G	-/ -	A/G	+	_	Rods	*Proteus mirabilis*
4	_	+	_	_	+	+	A/G	A/G	A/G	+	_	Rods	*Citrobacter spp*
Treatment group T_2													
1	+	+	_	+	+	+	A/G	-/ -	A/G	+	_	Rods	*Proteus vulgaris*
2	+	+	_	+	+	+	A/G	-/ -	A/G	+	_	Rods	*Proteus vulgaris*
3	+	+	+	_	_	+	A/G	-/ -	A/G	+	_	Rods	*Aeromonas spp.*
4	+	+	+	_	+	+	A/G	A/G	A/G	+	_	Rods	*Aeromonas spp.*

Key: (+) Positive result ;(–) Negative result; A/G -Acid and GaS; C-Control; T1-Fermented Cabbage Juice with brine; T2-Fermented Cabbage Juice without brine.

A fecal and colonic content culture, and biochemical identification tests carried out on organisms isolated from the resultant culture of fecal and colonic material of the rats implicated the presence of Proteus species *(P. Vulgaris and P. Mirabilis*), Citrobacter species, Aeromonas species and Providentia specie, which are all part of normal flora found in the faeces of animals.

Prior to treatment, organisms isolated from fecal matter of the three treatment groups were mainly Providentia and Citrobacter species. Biochemical identification tests for organism isolated from the fecal matter of rats after treatment with cabbage juice extract indicated the introduction of Proteus species, specifically *Proteus mirabilis* as it was the most prevalent organism in both the control groups and in the group of animals treated with fermented cabbage brine juice (T1), while the group of animals treated with the ordinary fermented cabbage (T2) presented Aeromonas species in addition to Proteus mirabilis. Organisms isolated from the colon of the test animals in the various treatment groups indicated the major presence of Providentia, *Proteus vulgaris*, and

Citrobacter with animals in treatment group T1 presenting a more diverse array of the organisms. The control group, as well as treatment group T2 presented mostly Proteus *spp*, specifically. *Proteus vulgaris*.

Hematologic analysis of blood samples collected from the test animals showed mean increase in white blood cell levels within high normal ranges in the treatment groups (normal range is 4.5-11x 10^12/L).

Animals that were treated with fermented cabbage juice with brine (T1) had the highest mean white blood cell counts (WBC) of 11.21±2.8 x 10^12/L.

This was followed by animals treated with the ordinary fermented cabbage juice (9.84±6.21 x 10^12/L) while animals in the control groups had the least levels of white blood cell counts 9.76±4.29 x 10^12/L. The elevated white blood cell levels in the treatment groups could be attributed to the an "immune priming" effect of the host's immune system mediated by the introduction of non pathogenic bacteria present in the fermented cabbage juice.

Table 5. Biochemical test results for organisms isolated from colon of rats at day 7.

Culture plate number	Catalase	Indole	Citrate	Oxidase	H_2S	Urease	Glucose	Lactose	Sucrose	Motility	Gram stain	Shape	Name of Organism
CONTROL GROUP													
1	+	+	_	+	+	_	A/G	A/G	A/G	+	-	Rods	*Providentia*
2	+	+	_	+	+	+	A/G	A/G	A/G	+	-	Rods	*Proteus vulgaris*
3	+	+	_	+	+	+	A/G	A/G	A/G	+	-	Rods	*Proteus vulgaris*
4	+	+	_	+	+	_	A/G	-/-	-/-	+	-	Rods	*Providentia*
5	+	+	_	+	+	+	A/G	A/-	A/G	+	-	Rods	*Proteus vulgaris*
TREATMENT GROUP T_1													
1	_	+	_	+	+	+	A/G	A/G	A/G	+	-	Rods	*Citrobacter spp*
2	+	+	_	+	+	_	A/G	A/G	A/G	+	-	Rods	*Providentia*
3	+	+	_	+	+	_	A/G	A/G	A/G	+	-	Rods	*Providentia*
4	+	+	+	-	+	+	A/G	A/G	A/G	+	-	Rods	*Proteus vulgaris*
TREATMENT GROUP T_2													
1	+	+	_	+	+	_	A/-	A/G	A/G	+	-	Rods	*Providentia*
2	+	+	+	-	+	+	A/G	A/G	A/G	+	-	Rods	*Proteus vulgaris*
3	+	+	+	-	+	+	A/G	A/G	A/G	+	-	Rods	*Proteus vulgaris*

Key: (+) Positive result ;(–) Negative result; A/G -Acid and GaS; C-Control; T1-Fermented Cabbage Juice with brine; T2-Fermented Cabbage Juice without brine.

The pack cell volume (PCV) levels increased significantly above normal ranges in animals treated with the fermented cabbage juice with salt (52.98± 2.94%; normal range is 35-45%).

This increase in PCV may be due to the presence of salt as results obtained from studies carried out by of em *et al.*, 2012 on assessing variations in blood parameters of high salt loaded rats following the administration of Moringa oleifera leaf extract showed that salt markedly caused an increase in PCV.

Animals in treated with ordinary fermented cabbage juice and those in the control group had lower values of 43.72±7.74% and 45.92±7.8% respectively and these fell within normal (normal range is 35-45%).

Table 6. Hematological parameters rats.

WBC	RBC	HGB	PCV	HDL	LDL	Na++	K+	Cl-	Urea
10^9/L	10^12/L	g/Dl	%	mg/Dl	mg/Dl	mmol/L	mmol/L	mmol/L	mg/Dl
CONTROL									
9.75 ±4.8	8.09 ±0.9	12.26 ±1.2	45.92 ±7.8	27.0 ±2.0	57.2 ±5.9	134.8 ±3.9	3.44 ±0.2	92.0 ±3.4	11.0 ±0.7
T_1									
11.22 ±2.8	8.51 ±0.4	13.36 ±0.3	52.97 ±2.9	26.6 ±2.3	57.8 ±16.0	136.8 ±3.8	3.84 ±0.2	98.6 ±3.6	10.0 ±1.2
T_2									
9.84 ±6.2	7.42 ±1.5	12.06 ±1.4	43.7 ±7.7	31.0 ±6.6	70.2 ±36.4	137.0 ±3.3	3.84 ±0.4	95.6 ±2.3	11.48 ±0.6

Results are presented as mean ± SDs.

Key:WBC = white blood cell; RBC = red blood cells; HGB= haemoglobin; PCV= Pack cell volume; HDL= high density lipoproteins; LDL= low density lipoproteins; Na^{++}=sodium ions; K^{+} = potassium ions; Cl^{-} = chloride ions; Urea= Blood Urea.

Conclusion

The study revealed the consumption of fermented cabbage juice has the potential of reducing bad gut micro biota and also influencing different hematologic parameters, significantly the white blood cell count as it helps to prime the white blood cells, pending the infiltration of pathogenic organisms.

It is however, recommend that a safe concentration of salt should be added to help preserve the probiotic integrity of the juice as salt favors the growth of salt tolerant lactic acid bacteria, enhancing the fermentation process while inhibiting the contamination and growth of unwanted spoilage bacteria and fungi present.

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