ORIGINAL ARTICLE

Beneficial effects of a fermented maize product with its supernatant, *Lactobacillus fermentum* and *Lactobacillus brevis* in rat model of colitis

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Abstract

Background: African fermented foods such as maize cereal (*Ogi*) and its supernatant (*Omidun*) are reservoirs of beneficial lactobacilli and carboxylic acids. Based on their constituents, their beneficial effect in a rat model of 2,4,6-trinitrobenzene sulphonic (TNBS) acid-induced colitis was assessed in comparison with two lactobacilli in a rat model of colitis. **Methods:** Female Wistar rats were distributed into seven groups of 5 rats each; the rats were pretreated for 14 days prior to colitis induction with *Ogi*, *Omidun*, *L. fermentum*, *L. brevis* and 7 days post colitic induction. Colitis was induced by an intracolonic administration of TNBS. The response of the rats to treatment was assessed macroscopically and biochemically. **Results:** Treatment with the Dexamethasone, *Ogi* and *Omidun* resulted in a significant reduction in colonic damage score and weight/length ratio ($p<0.05$). Treatment with *Ogi*, *Omidun*, *L. brevis*, and dexamethasone significantly prevented depletion of colonic glutathione and superoxide dismutase. The up-regulation of myeloperoxidase activity was inhibited in all treated colitic rats ($p<0.05$). However, *Ogi* appears to produce a better protective effect than the other treatment groups. **Conclusions:** This study reports that *Ogi* protects Wistar rats against the deleterious effect of trinitrobenzene sulphonic acid better than pure lactobacilli strains.

Keywords: Fermented food, lactic acid bacteria, inflammation, oxidative stress.

1 Introduction

Inflammatory Bowel Disease (IBD) is a chronic, idiopathic inflammatory disease of the gastrointestinal tract [1]. In healthy individuals, immunological tolerance to the gut microbiome is maintained, whereas in those with IBD, these homeostatic mechanisms remain disrupted [2]. Furthermore, the composition of the intestinal flora in patients with IBD is altered when compared with healthy individuals, resulting in a general loss of diversity [2]. It has been shown that dysbiosis can lead to a qualitative and quantitative decrease in the mucosal barrier and consequently, translocation of pro-inflammatory substances into the colon, which could be responsible for inflammation and subsequently colitis [3]. Therefore, there is currently a strong interest in exploring beneficial bacteria in the treatment of colitis.

Lactic acid bacteria (LAB) are found in food and contribute to the healthy microbiota of human intestinal mucosal surfaces. Traditional fermented products are one of the primary sources of LAB [4-6]. In developing countries where probiotic products are not readily available, the next best option is fermented foods. Fermented cereal is consumed worldwide and provided different names in different countries. In Nigeria, *Ogi* is the name attributed to fermented cereal e.g.

maize, sorghum, etc. This fermented cereal is frequently consumed as a meal for breakfast by many tribes. Occasionally, Raw *Ogi* (the slurry) is administered as a local fermentation product remedy against diarrhea, especially in rural areas with little access to proper health care [7]. *Omidun*, the supernatant obtained from the raw *Ogi* slurry, is locally utilized as a solvent to soak the bark or root of some plants and for treating fever and malaria [8]. *Ogi* has been shown to contain eleven carboxylic acids with butyric acid, lactic acid, and acetic acid as the major constituents [9]. In addition, *Ogi* and *Omidun* contain several species of beneficial LAB. We have previously reported isolation and identification of *Weissella paramesenteroides*, *L. brevis*, *L. rossiae*, *L. fermentum*, *L. plantarum*, *Acetobacter pasteurianus* and *Paenibacillus sp.* in different varieties of *Ogi* and *Omidun* with interesting antibacterial properties [10].

To the best of our knowledge, no published data on beneficial effects of *Ogi* and *Omidun* in comparison to lactobacilli strains on colitis are available. Thus, this study assessed the beneficial effect of *Ogi*, *Omidun* and two pure lactobacilli strain in a rat model of colitis.

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2   Methods

2.1 Bacterial strains

Lactobacillus brevis FA021 and *Lactobacillus fermentum* FA020 has been previously isolated and selected because of their substantial antibacterial properties. Those target bacteria were used in this study because the source of isolation was human and displaying antibacterial properties. For this *in vivo* rat study, they were subcultured on de Man, Rogosa Sharpe broth (Oxoid, UK) and incubated at 37ºC for 24-48hrs under microaerophilic conditions after which they were centrifuged at 4000 rpm for 6 minutes. The supernatant was decanted off and the bacterial pellets were washed with normal saline and re-suspended in 0.5 ml of normal saline.

2.2 Collection of ogi slurry and its supernatant (*Omidun*)

Maize used for the production of *Ogi* slurry and *Omidun* was purchased from a local vendor at Agbowo, Ibadan, Oyo State, Nigeria. The vendor made *Ogi* by soaking maize grains in water for 48 hours and then wet milling before fermentation at room temperature for 24 hours; thereafter, appropriate volumes of the supernatant (*Omidun*) and *Ogi* were collected and used for the analysis. Samples were handled within 72 h after which another fresh batch was collected to maintain the 72-h interval use. The colony-forming units/ml for every fresh sample was analyzed by plating out 1 ml of Omidun and 1 g *Ogi* in MRS agar (Oxoid, UK) after appropriate dilutions were made in saline solution.

2.3 Ethical consideration

Experimental procedures and protocols used in the current study conform to the "Guide to the care and use of animals in research and teaching" (NIH publications number 85-93 revised in 1985).

2.4 Rat weight analysis

The body weight of experimental animals was measured weekly. After two weeks of experimental feeding, colitis was induced and the final weight measured at the third week.

2.5 Experimental animals

Thirty-five healthy female Wistar albino rats were obtained at 4 weeks from the Animal House, Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan. The rats were housed in Makrolon cages in a well-ventilated animal house facility at room temperature (28 + 2ºC). They were fed with standard rat pellets (Ace Feeds Nigeria Limited) and water *ad libtum* until they reached the target weight for the study (160-180 g). Experimental rats were randomly distributed into seven groups of five rats each. Four of the seven groups were pre-treated with *L. brevis* (0.5ml of 1.21 x 10^8 CFU/ml), *L. fermentum* (0.5ml of 3.8 x 10^7 CFU/ml), *Ogi* (1.25 mg/ml of 8.6 x 10^8 CFU/ml) and *Omidun* (0.5ml of 1.25 x 10^7cfu/ml) for 13 days (D0-D12), before induction of colitis on day 13 (D13) and for seven days post colitic induction. The remaining three groups served as controls: dexamethasone-treated colitic rats (4mg/ml), untreated colitic rats and healthy non-colitic rats.

2.6 Induction of Colitis

Colitis was induced in 30 out of 35 rats using a previously reported method [11]. Momentarily, food was withdrawn 18h prior to the induction of colitis. Rats were anesthetized with ketamine (50 mg/kg) and diazepam (2.5 mg/kg). Colitis was induced by a single intracolonic administration of 0.25 mL of 40 mg/mL trinitrobenzenesulfonic acid dissolved in 50% ethanol) into the distal colon by means of a soft pediatric catheter introduced 8 cm into the anus. The animals were maintained in a head-down position for 5 minutes to recover from anesthesia and return back to their cages.

2.7 Assessment of colonic damage and response to treatment

Rats were euthanized by an overdose of ether anesthesia at the seventh day post-colitic induction. The distal colon was excised, luminal contents flushed with cold normal saline and colon placed on ice. Thereafter, each colon was opened by an incision along the mesenteric border, the body weight and length measured, and the disease severity scored. Disease severity was assessed using a standard scoring system [12], scored on a scale of 0 – 10 depending on the severity of the inflammation and ulcer.

Table 1: The weight of Wistar rats before and after the induction of colitis and treatment Weight

WEEK	Weight (g)						
	L. fermentum	*L. brevis*	*Omidun*	*Ogi*	Dexa.	Neg.control	Non-colitic
1	172 ± 3.0	174.4±3.3	170.4±2.9	164.1±8.6	164.3±5.3	159.4±6.7	161±2.9
2	178.6 ±7.5	178.6±7.4	178.8±4.1	168.4±7.3	169 ± 5.1	169.9±6.6	165.8±3.0
3	173.4±9.43	163.6±6.5	166.9±5.1	159.9±1.6	162.9±4.0	157.2±3.9	175.4±4.81
% W.L	2.9*#	8.4#	6.6#	4.8*#	4.1*#	7.5**	0

Dexa – dexamenthasone, Neg.control - untreated colitic rats, * $p<0.05$ (treatment groups vs. TNBS control group). # $p<0.05$ (Healthy group vs. treatment groups), ** TNBS control group vs healthy group, WL.: weight loss.

Thereafter, for biochemical assays, samples were cut from the entire colon.

2.8 Sample preparation for biochemical assays

A known weight of freshly excised tissue was homogenized in HTAB buffer (50 mg/ml) and centrifuged at 12,000 rpm for 10 minutes at 4ºC. Two hundred microliter of the supernatant was collected and the remaining homogenized samples was stored at -20ºC. The supernatant was used to estimate the total glutathione content (GSH), and Superoxide Dismutase (SOD). GSH level was estimated according to the method previously described [13] and the results were expressed as nanomoles per gram of wet tissue. A method described by Mistra & Fridovich [14] was used to evaluate the superoxide dismutase (SOD) content of the colon samples. The remaining frozen homogenized colon sample was allowed to undergo two cycles of freezing and thawing. Thereafter myeloperoxidase activity (MPO) was quantified by a method previously described [15]. The results were expressed as MPO units per gram of wet tissue; one unit of MPO activity was defined as that degrading 1 mmol hydrogen peroxide/min at 25°C.

2.9 Statistical analysis

Data obtained were expressed as the mean ± SEM. Differences between means were tested for statistical significance using a one-way analysis of variance (ANOVA) with the Tukey post hoc test. P-value <0.05 was considered significant.

3 Results

There was an increase in the body weight of the rats, during the first two weeks of the experiment prior to colitis induction (Table 1). All colitis induced rats lost weight immediately after induction, gaining weight gradually. At the end of the third week, the colitic rats treated with the *L. brevis* group had the highest weight loss (8.9%) while those treated with *L. fermentum* group had the least weight loss (2.9 %). Colonic damage in experimental animals evidenced by the size of lesions, number of ulcers as well as hyperemia, were scored on a scale of 0-10 as presented in Table 2. Treatment with the standard drug (dexamethasone), *Ogi* and *Omidun* respectively resulted in a significant reduction in colonic damage score (4.0 ± 0.71, 4.15 ± 0.29 and 4.4 ± 0.31) in comparison with untreated colitic group (6.0 ± 0.41, p≤0.04). Similarly, the colonic weight/length ratio increased significantly in untreated colitic rats (170.7 ± 6.9 mg/cm) in comparison with non colitic rats (113.3 ± 2.8 mg/cm, p<0.0001). Treatment with Dexamethasone, *Ogi* and *Omidun* resulted in a significant reduction in colonic weight/length ratio (105.5 ± 6.9, 117.15 ± 9.1 and 129.3 ± 10.54 mg/cm) in comparison with untreated colitic group (170.7 ± 6.9 mg/cm p≤0.011). Furthermore, a significant reduction of myeloperoxidase

activity (MPO) was observed in all treated colitic rats (3.05 ± 0.15 - 3.8 ± 0.08 U/mg tissue) with *Ogi* producing the best effect when compared with untreated colitic rat (4.51 ± 0.10 U/mg tissue, p≤0.007 (cf. Table 3). In addition, about 4-fold depletion of colonic glutathione level was observed in untreated colitic rats (56.18±5.49 nmol/mg tissue) in comparison with non-colitic rats (202.3 ± 3.97 nmol/mg tissue, p<0.0001 (cf. Table 3). However, treatment with, *Ogi*, *L. brevis*, *Omidun*, and dexamethasone significantly prevented depletion of colonic glutathione in colitic rats in comparison to untreated colitic rats (119.4 ± 2.65 vs 203.6 ± 16.83 nmol/mg tissue p≤0.0001). Interestingly, the GSH level in colitic rats treated with *Ogi* was identical to the non-colitic healthy rats (p=0.94; Table 3).

Table 2: Effects of treatment on macroscopic colon damage score and colonic weight/ length ratio in colitic rats

Treatment Group	damage score (0–10)	weight/length (mg/cm)
Healthy (non-colitic)	0.0 ± 0.0	113.3 ± 2.8
Colitic control	6.0 ± 0.41**	170.7 ± 6.90**
Dexamethasone	4.0 ± 0.71#	105.5± 6.90*
L. fermentum	5.25 ± 0.25#	175.3 ± 5.70#
L. brevis	5.00 ± 0.41	150.8 ± 15.57#
Omidun	4.43 ± 0.31*#	129.3 ± 10.54*
Ogi	4.15 ± 0.29*#	117.1 ± 9.10*

Macroscopic damage (Mean ± SEM). *p<0.05 (treatment groups vs. TNBS control group), #p<0.05 (healthy group vs. treatment groups), ** TNBS control group vs healthy group.

Similarly, a significant 6-fold depletion of colonic SOD activity was observed in untreated colitic rats (0.013 ± 0.001 U/g tissue) in comparison with non colitic rats (0.08 ± 0.002 U/mg tissue, p<0.0001). In dexamethasone, *Omidun* and *Ogi* treated groups, a significant reduction in the depletion of SOD was observed (0.031 ± 0.002 vs 0.035 ± 0.004 U/g tissue p≤0.0007) with *Ogi* showing the highest reduction in the depletion of SOD.

Table 3: Biochemical Analysis of Rats Samples

Treatment Group	MPO (U/mg tissue)	GSH (nmol/mg tissue)	SOD (nmol/mg tissue)
Healthy (non-colitic)	0.98±0.07	202.3±3.97	0.079 ± 0.002
Colitic control	4.51±0.10**	56.18±5.49**	0.013 ± 0.001**
Dexamethasone	3.76±0.07	131.1±7.24*#	0.031 ± 0.002*#
L. fermentum	3.35±0.31*#	66.21±3.3#	0.022 ± 0.009#
L. brevis	3.46±0.12*#	119.4±2.65*#	0.012 ± 0.001#
Omidun	3.8±0.075*#	152.2±11.76*#	0.031±0.003*#
Ogi	3.05±0.15*#	203.6±16.83*	0.035± 0.0024*#

Data are expressed as Mean ± SEM. * p<0.05 (treatment groups vs. TNBS control group), # p<0.05 (Healthy group vs. treatment groups), ** TNBS control group vs healthy group.

Assessment of difference between *Omidun*/*Ogi* in comparison to *L. brevis* and *L. fermentum* in MPO, GHS, SOD, damage score, weight length ratio revealed that activities of *Ogi* or *Omidun* is significantly better in all tested parameter (GSH, p=0.0001, 0.03. SOD, p=0.005, 0.0003, damage score p=0.02, Weight/length ratio, p=0.005, 0.0006) except MPO where *L. brevis* was better than *Omidun* (p=0.04).

4   Discussion

In the present study, dexamethasone, *Ogi* and *Omidun* were able to ameliorate the macroscopic colonic damage caused by intracolonic delivery of TNBS in colitic rats. In contrast, treatment with *L. brevis* and *L. fermentum* showed non-significant protection against macroscopic damage to the colon. An increase in colon weight/length ratio is an indication of intestinal edema, a symptom of inflammation [16]. Significant reduction in colonic weight/length ratio as observed in colitic rats treated with dexamethasone, *Ogi* and *Omidun*, signified resolution of intestinal edema, a symptom of inflammation. Furthermore, the up-regulation of MPO, an indicator of neutrophil infiltration, was moderately inhibited in colon tissues in the treatment groups. Prevention of access of neutrophils to the inflamed areas of colon could prevent the release of proteases [17]. The inhibition of the up-regulation of myeloperoxidase enzyme could also be an indication of successful gut microbiota repopulation by *Ogi*, *Omidun*, *L. brevis,* and *L. fermentum*. It has also been shown that proteases produced by members of the gut microbiota could prevent the activation of an immune response and consequently inflammation by degrading the antigenic structure of most antigens [18]. However, dysbiosis of the gut microbiota eliminates much of this function, allowing the successful penetration of antigens and antigenic products and subsequently inflammation.

Ogi and its constituents are reportedly dominated by LAB with the identification of *L. fermentum*, *L. plantarum*, *L. pantheris,* and *L. vaccinostercus* [10,19]. Consumption of *Ogi* might be beneficial because of the ability of LAB to modulate intestinal microbiota. The better activities of *Ogi* and *Omidun*, in comparison to single cultures of LAB used in our study, may be due to the synergistic effects of different lactobacilli species in *Ogi* and *Omidun* [6,10]. Moreover, different beneficial bacteria have been reported to decrease the intestinal microbiota imbalance induced by IBD when used simultaneously. VSL#3, a probiotic mixture of *Streptococcus thermophiles*, *L. plantarum*, *Bifidobacterium brevis*, *B. longum*, *B. infantis*, *Lactobacillus acidophilus*, *L. paracaseiand*, *L. bulgaricus*, and has been successfully used as adjunctive treatment to treat ulcerative colitis [20].
Our findings suggest that *Ogi*, *Omidun*, *L. brevis* and to a lesser extent *L. fermentum* prevent the depletion of GSH and might

be useful to improve antioxidant status in colitis. It is known that some lactobacilli are able to produce antioxidants that help eliminate oxidative stress produced by reactive oxygen species [21]. *Lactobacillus fermentum* has also been previously shown to contain a notable level of reduced glutathione [22]. The increased colonic superoxide dismutase activity observed, is indicative that the individual lactic acid bacteria, as well as their presence in combination in Ogi and Omidun, reduced oxidative stress thereby preventing exacerbation of inflammation. A previous report has demonstrated the ability of *Lactobacillus lactis* and *Lactobacillus plantarum* to produce superoxide dismutase enzyme and ameliorate experimental colitis [23].
The anti-inflammatory and antioxidant activities shown by *Ogi*, *Omidun*, and to a lesser extent by *L. brevis* and *L. fermentum* may be due to their ability to prevent the adhesion of harmful bacteria to the intestinal epithelium by competing for nutrients. Members of the genus *Lactobacillus* have been shown to be able to adhere to intestinal mucus and extracellular matrix through various adherence factors, such as those with mucin binding domains [24]. An altered mucus layer, which is a prominent feature in colitis, could interfere with the binding ability of LAB and consequently be responsible for the dysbiosis of the intestine LAB population.

5   Conclusion

This study suggests that *Ogi*, *Omidun* and to a lesser extent, *L. fermentum* and *L. brevis* produce beneficial effects against TNBS induced colitis. The activities of fermented food products are better than single lactobacilli cultures. The limitations of the study are not ascertaining the mechanism of action of *Omidun* against the colitic rats and also, the study investigated only two LAB strains.

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