

## Anti-dysentery Effect of Milled Fermented Maize (“Ogi”) in *Escherichia coli* Infected Albino Rats

Osheke Shekins Okere<sup>a</sup>, Samuel Omolabi Adeyemo<sup>b</sup>, Moses Dele Adams<sup>a\*</sup>, Joshua Omoniyi Kolawole Abioye<sup>c</sup>

<sup>a</sup> Department of Biochemistry, Bingham University, Karu, Nasarawa State, Nigeria

<sup>b</sup> Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria

<sup>c</sup> Department of Biological Sciences, Bingham University, Karu, Nasarawa State, Nigeria

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### Abbreviations:

b.w: Body weight, *E. coli*: *Escherichia coli*, %: Percentage, mg/kg: Milligram/kilogram

### Corresponding Author:

Adams M.D.\*

M.Sc. (Ph.D In View)

Email: mdadamsonline@yahoo.com

Okere O.S.

B.Sc, M.Sc, PGDE, (Ph.D In View)

Adeyemo S.O.

B.Sc, M.Sc, Ph.D

Abioye J.O.K.

B.Sc, M.Sc, Ph.D

### Abstract

Anti-dysentery effects of uncooked milled, fermented maize (known as “ogi” in Southwestern Nigeria), were investigated in *Escherichia coli* infected albino rats. “Ogi” was prepared by

fermentation of white maize grains for 5 days; wet milled, sieved and allowed to settle for 24 hours. Dysentery was precipitated in albino rats by infection with the pure culture of *E. coli*. Frequency of blood stool was determined by counting fecal spots on absorbent paper before and after treatment with “ogi”. Physicochemical analysis of “ogi”, comprising titratable acidity, pH 0 and phytochemical screening, was also carried out. Result indicated that 100 mg/kg b.w., 200 mg/kg b.w. and 400 mg/kg b.w. of “ogi” reduced the number of fecal spots by 48.4%, 80.2% and 96.9%, respectively. Physicochemical analysis revealed that “ogi” had pH of 3.51; titratable acidity of 42.4% lactic acid and presence of alkaloids, saponins, cardiac glycosides and phlobatannins. Dose-dependent therapeutic potency against dysentery has been manifested by “ogi”, most likely aided by its high acidity, presence of lactic acid bacteria and their secondary metabolites, as well as the phytochemicals present in “ogi”. This could be a very simple and cheap solution to the ravaging problem of dysentery in poor families of many developing countries.

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## 1. Introduction

Diarrheal diseases represent a major health problem in developing countries and also a high risk to travelers who visit these countries. The global death toll from diarrheal diseases is placed at over two million deaths per year (1.7- 2.5 million deaths), ranking third among all causes of infectious disease deaths worldwide. Most of these deaths occur in children under five years of age (kosek et al., 2000; WHO, 2004; United Nations,

2005). Moreover, out breaks of cholera, shigellosis and typhoid fever most often occur in resource-poor countries, adding to the burden of disease among the most vulnerable such as refugees, internally displaced populations and groups living in shanty towns. The diversity of bacterial and viral infections that may cause diarrhea (Girard et al., 2006) complicates accurate surveillance and

diagnosis, especially in developing countries with little or no access to modern laboratory procedures. Among the principal bacterial agents of diarrheal diseases are *Vibrio cholera* (cholera), a variety of *Salmonella spp.*, including *S. typhi* (typhoid fever), and of *Shigella spp.*, the agents of *Shigellosis* (bacterial dysentery), *Campylobacter spp.*, (especially *C. jejuni*) and a variety of enteropathogenic *Escherichia coli* strains, including the travelers' diarrhea. The increased frequency of antibacterial drug resistance among these pathogens is a source of major concern (Rowe et al., 1997; Sur et al., 2003; krishma et al., 2006).

Fermented cereals are very widely utilized as food in African countries and in fact, cereals account for as much as 77% of total caloric consumption (Chavan and Kadan, 1989). Majority of traditional cereal based foods consumed in Africa are processed by natural fermentation, and are particularly important as weaning foods for infants, and as dietary staples for adults (Umoh and Fields, 1981; Akobundu, 1982). Ogi, being an important cereal product in the latest African sub-region, is believed to possess some biochemical properties (Osungbaro and Taiwo, 2009).

Previous studies by Aderiye and David (2013a) reported the effects of fermented maize Gruel (OGI) on the haemato-biochemical profile of Wistar albino rats challenged with *Shigella dysenteriae* JBA 010 while Komolafe et al. (2013) worked on therapeutic and immunomodulatory effects of raw maize "OGI" on rats infected with *Escherichia coli* 0157:H7.

Dubreuil (2013) evaluated the antibacterial and anti-diarrheal activities of plant products against enterotoxinogenic *Escherichia coli* while Ajanaku et al. (2012) saw the nutritive value of Sorghum ogi with groundnut seed (*Arachis hypogaea*) while Adebolu et al. (2007) worked on the evaluation of ogi liquor from different grains for antibacterial activities against some common diarrhoeal bacteria in South West Nigeria. Mbata et al. (2009) carried out studies on the microbiological, nutrient composition and antinutritional contents of fermented maize flour fortified with bambara groundnut (*Vigna subterranean L*) while Adebolu et al. (2007) worked on evaluation of ogi liquor from different grains for antibacterial activities against some common diarrhoeal bacteria in South West Nigeria.

Again, Aremu et al. (2011) worked on the biochemical evaluation of fermented white maize (*Zea Mays L.*) blended with Scarlet Runner Bean (*Phaseolus coccineus L.*) flour while Aderiye and David (2013b) studied the evaluation of

prophylactic and therapeutic properties of ogi in rabbits infected with *Salmonella typhi*. Banigo and Muller (1972) worked on Manufacture of "Ogi", Nigerian fermented cereal porridge, looking at the comparative evaluation of corn, sorghum and millet whereas Mbata et al. (2007) discovered the nutritional status of maize fermented meal by fortification with bambara-nut.

Furthermore, while Pikuda and Ielaboye (2013) worked on the proximate and chemical composition of OGI prepared from whole and powdered grains (Maize, Sorghum and Millet) Nwokoro and Chukwu (2012) carried out studies on Akamu, a traditional fermented maize food.

Adeyemi (2006) then worked on the Ogi quality of sorghum flour dry-milled from fermented sorghum grains. Despite these studies, the anti-dysentery potential of "Ogi" has not been elucidated. Therefore, this study was designed to evaluate the anti-dysentery effect of milled fermented maize ("Ogi") in *Escherichia coli* infected albino rats.

### 1.1 Objective of research

The main objective of this study is to evaluate the anti-dysentery effect of milled fermented maize ("Ogi") in *Escherichia coli* infected albino rats.

### 1.2 Justification of research

Milled fermented maize ("Ogi") has been claimed, in traditional medicine, to possess anti-dysentery activity without scientific evidence to either substantiate or refute the purported claim. Therefore, this research work was designed to evaluate the anti-dysentery effect of milled fermented maize ("Ogi") in *Escherichia coli* infected albino rats.

## 2. Materials and Methods

### 2.1 Materials

#### 2.1.1 Chemicals and reagents

All chemicals and reagents used are of analytical grade, obtained from British Drug House (BDH Chemicals Ltd, Poole, England). Nutrient agar was obtained from Oxoid Limited, Basingstoke, Hants, England.

#### 2.1.2 Raw materials

Raw white maize samples used in this study was obtained from Masaka Market, Karu, Nasarawa State, Nigeria. 700 grams of grain were soaked in water for five days, during which fermentation took place. It was wet-milled, sieved with fine cloth and the sieveage was allowed to settle for 24 hours, after which the clear water was removed. The "ogi" sample that was obtained was stored in a refrigerator at 5° C.

## 2.2 Methods

### 2.2.1 Experimental animals

Experiment rats were purchased from the Animal House of Bingham University, New Karu, Nasarawa State, Nigeria. The animals were housed in steel cages and kept at room temperature. All rats were fed with water and pelleted standard laboratory feed for fourteen (14) days prior to commencement of the experiment.

### 2.2.2 Test organisms

*Escherichia coli* strain that was used to induce dysentery was obtained from stock culture collections in the Department of Biological Sciences, Bingham University, New Karu, Nasarawa State, Nigeria. The organism was cultured into nutrient agar medium from a stock to obtain a pure distinct colony by streak plate method which was further subcultured into six (6) different plates at room temperature for 24 hours to multiply the organism. Thereafter, a loop-full of this test organism was transferred into 5 ml of normal saline.

### 2.2.3 Physicochemical analysis

Total titratable acidity and pH values were determined according to the methods described by AOAC (1998).

### 2.2.4 Phytochemical screening

Phytochemical screening of “Ogi” sample was carried out for alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, anthraquinones, cardiac glycosides and phlobatannins using the methods described by Sofowora (1982) and Trease and Evans (1986).

### 2.2.5 Experimental design

Thirty (30) albino rats of Wistar strain of various

body weight ranging between 100 g and 250 g were randomly distributed into five (5) groups (A-E) comprising 6 rats each as shown in Table 1.

**Table 1:** Animal Grouping

Group	Treatment
A	No induction, no treatment with “ogi”
B	<i>E. coli</i> induced, no treatment with “ogi”
C	<i>E. coli</i> induced and treated with 100 mg/kg BW of “ogi”
D	<i>E. coli</i> induced and treated with 200 mg/kg BW of “ogi”
E	<i>E. coli</i> induced and treated with 400 mg/kg BW of “ogi”

Dysentery was induced in the experimental rats by oral administration of 3 ml of the prepared *E. coli* strain once daily. Dysentery was confirmed some hours after induction by frequent stooling, measured by placing the rats of each group singly in cages with absorbent paper on the floor of their cages and counting the faecal spots.

### 2.2.6 Statistical analysis

Results were expressed as the mean  $\pm$  SEM of six determinations. The data were analyzed using Duncan Multiple Range Test and complemented with Student’s t-test. The differences were considered statistically significant at  $p < 0.05$ . All these analyses were done using SPSS 17.0 Software (Statistical Package for Social Sciences, Inc., Chicago, IL, USA).

## 3. Results and Discussion

The *E. coli* infected rats were observed to be sluggish, weak and inactive after about 2 hours of induction.

**Table 2:** Frequency of Stooling in Dysentery-induced Rats Before and After Treatment with Milled Fermented Maize “Ogi”

S/No.	Treatment	No. of fecal spots		Percentage Reduction (%)
		Before Treatment	After Treatment	
1.	No <i>E. coli</i> induction	0	-	-
2.	<i>E. coli</i> induction + 0 m/kg b. w	5.6 $\pm$ 2.01	Not applicable	Not applicable
3.	<i>E. coli</i> induction + 100 mg/kg b. w	6.2 $\pm$ 1.1	3.2 $\pm$ 0.8	48.4
4.	<i>E. coli</i> induction + 200 mg/kg b. w	5.8 $\pm$ 1.8	0.8 $\pm$ 0.3	86.2
5.	<i>E. coli</i> induction + 400 mg/kg b. w	6.5 $\pm$ 2.0	0.2 $\pm$ 0.1	96.9

pH value of “ogi” = 3.5  $\pm$  0.1, Titratable acidity of “ogi” = 42.2  $\pm$  0.5% lactic acid

**Table 3:** Phytochemical Constituents of Milled Fermented Maize “Ogi”

S/No.	Phytochemical Constituents	Test Result
1.	Alkaloids	+
2.	Flavonoids	-
3.	Tannins	-
4.	Saponins	+
5.	Steroids	-
6.	Terpenoids	-
7.	Anthraquinones	-
8.	Cardiac glycosides	+
9.	Phlobatannins	+

**Key:** + = Present, - = Absent

They started showing symptoms of bloody diarrhea after 12 hours of induction as shown in Table 2. Table 3 shows the phytochemical constituents of milled fermented maize “Ogi”.

The results of *E. coli* induction of the experimental rats as shown in Table 2 indicated that the *E. coli* strain used successfully precipitated dysentery in the experimental rats, as judged from the number of bloody fecal spots in the groups B, C, D, E,

compared to the control, where there was no single fecal spot. The *E. coli* strain could have caused damage in the epithelial cells of the intestinal wall of the rats, thereby causing diarrhea, as earlier reported by Gorden and Mall (1993) and Leyer et al (1995).

As the treatment of “ogi” was administered, however, there was reduction in frequency of stooling by the infected rats, as shown in Table 2. The reduction was dose dependent, ranging from 48.4% in the group treated with 100 mg/kg b. w. to 62% in the group treated with 200 mg/kg b.w.

The acidic nature of “ogi” (pH 3.5 and 42.4% lactic acid) could have contributed to the reduction of *E. coli* population and therefore reduction of bloody fecal spots. Most bacteria cannot grow at pH value that is less than 4 except the lactic acid bacteria (Adebolu et al., 2007). This low pH could be a significant factor in inhibiting the *E. coli* from growing, as *E. coli* has optimum growth pH of 6 to 7 (Bettleheim, 2000), although it can survive at pH levels as low as 2.5 (Miller and Kaspar, 1994; Conner and Kotrola, 1995). Raw “Ogi” actually contains lactic acid bacteria (Adeyemo et al., 1992). Lactic acid bacteria produce various antimicrobial substances during fermentation of their substrate such as organic acids, hydrogen peroxide, carbon dioxide, diacetyl and bacteriocins which act as biopreservatives in foods (De Vuyst and Vandamine, 1994; Wong et al., 2000). The acidic environment created by administration of “Ogi” could have made a way for ecological succession in which lactic acid bacteria eventually became predominant. This could be confirmed from the fact that the dysentery reduction in “Ogi” treated *E. coli* infected rats was dose dependent. Thus, *E. coli* was rendered ineffective as lactic acid bacteria became dominant.

Bacteriocinns are proteinaceous antimicrobial compounds that are also produced by lactic acid bacterial. They have been reported to be very effective in inhibiting Gram-negative bacteria such as *E. coli* (Von-Mollendorf et al., 1995; Montville and Chen, 1998; Todorov and Dicks, 2004). The dose dependent rapid reduction in fecal spots observed in this study could have been due to inhibitory action of such biomolecules as bacteriocins.

Table 3 presents the results of phytochemical screening of “Ogi”, indicating the presence of alkaloids, saponins, cardiac glycosides and phlobatannins. Generally, the antimicrobial properties of plant extracts are attributed to secondary metabolites like these phytochemicals (Okoli and Iroegbu, 2005). Saponins and alkaloids have been shown to be most efficient against Gram

positive and Gram negative bacteria (Iwu et al., 1999). These bioactive compounds could have synergistically contributed to the potency of “Ogi” against *E. coli* precipitated dysentery observed in this study.

## Conclusion

Available evidence from the present study indicated that the milled fermented maize (“Ogi”) possess anti-dysentery activity. In view of the high prevalence of diarrheal diseases in developing countries and its ravaging effect particularly on children, a very simple and cheap treatment in raw “Ogi” may be an effective solution. Its possible multi-action approach through production of acid environment and various antibacterial agents may also be promising in solving the problem of drug resistance which *E. coli* quickly acquires.

## Limitations

The present study only investigated the anti-dysentery activity of milled fermented maize (“Ogi”) in *Escherichia coli* infected albino rats. It did not determine the lethal dose (LD<sub>50</sub>) of “ogi” as well as its possible toxicological implications. The study could not isolate and characterize the bioactive anti-dysentery agent in “ogi” using column chromatography, gas chromatography/mass spectrometry (GC/MS) or High Performance Liquid Chromatography (HPLC). Histology of the tissues was not also carried out.

## Recommendations

For further studies, the milled fermented maize (“Ogi”) should be isolated and characterized to obtain the bioactive agents as well as provide its possible molecular mechanism of action. Toxicity study should be done on selected tissues of animals to determine functional toxicity. Further studies should carry out histology of tissues which would provide information on whether the extract has the structural toxicity or not.

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## Authors' Contribution

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

## Competing Interests

Authors have declared that no competing interests exist.

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