

# Therapeutic and Immunomodulatory Effects of Raw Maize “OGI” on Rats Infected with *Escherichia coli* 0157:H7

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**Abstract:** *Escherichia coli* O157:H7 is known to cause food borne illness globally. Treatment of infections caused by this organism is difficult because the administration of antibiotics might precipitate kidney complications; therefore there is the need to search for alternative therapy. In this study, the therapeutic and immunomodulatory effects of raw maize “ogi” was investigated on rats infected with *Escherichia coli* 0157:H7. Infected rats treated with maize “ogi” slurry 1.0 mL once or twice daily and maize “ogi” liquor, 1.0 mL twice daily recovered 72 h while those that were treated with less than 1.0 mL recovered by 96 h. Without treatment with “ogi” however, the rats started recovering by 120 h. The treatment caused the white blood cells which had already gone up as a result of the infection to reduce significantly ( $P \leq 0.05$ ) by 24 h of administration of raw fermented maize “ogi” components to the infected rats. It also caused a significant decrease in the lymphocyte counts of the infected and treated rats by 24 h. On the other hand, there was an increase in the neutrophil count irrespective of the different volumes and different components of raw “ogi” used by 24 h but by the 72 h of treatment, it started to decrease and by 120 h reduced to normal levels. Since the administration of raw maize “ogi” either slurry or liquor caused the duration of infection in rats infected with *Escherichia coli* 0157:H7 to reduce from 120 h to 72 h, it is therefore suggested that people having diarrhoea caused by this organism could drink fermented raw maize “ogi” slurry or liquor to treat the infection.

**Key words:** Maize “ogi”, *E. coli* 0157:H7, therapeutic, immunomodulatory.

## 1. Introduction

Diarrhoea is a leading cause of morbidity and mortality among children in developing countries [1-3]. The main etiology of the diarrhoea is related to a wide range of bacteria (such as *Campylobacter jejuni*, *Escherichia coli*, *Salmonella* spp., *Vibrio cholerae*, *Yersinia enterocolitica* and *Aeromonas* spp.), enteroparasites (*Giardia* spp., *Cryptosporidium* spp., and *Entamoeba histolytica*), and viruses (Adenovirus, Norwalk virus, and Rotavirus) [4]. In southwest Nigeria, in a study that was carried out by Adebolu and Babafemi [5], *Escherichia coli* was found to be the most prevalent cause of infantile diarrhoea in the

geopolitical zone. Poor hygiene, lack of pipe borne water, consumption of contaminated food and close proximity to animals all contribute to easy and frequent acquisition of pathogens that cause this condition [6]. Children less than five years of age have 3.3 diarrhoeagenic episodes per year, and more than one-third of the deaths in this age group are associated with diarrhoea. Therefore, there are approximately 1.5 billion diarrhoeic episodes per annum and 4 million deaths in children less than five years of age (most from 6 months to 12 years) that are caused by the disease [7, 8]. In the investigation carried out by Black [9], he reported that as many as two million children die annually from this infection.

Diarrhoea, although self-limiting may sometimes require antibiotic therapy. However, most of the

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etiological agents especially bacteria have already developed resistance to most of the commonly employed antibiotics [10]. In addition, some of these antibiotics can also induce diarrhoea known as “antibiotics induce diarrhoea” [2, 11]. There is therefore the need to search for more effective treatment of this illness, more so that it is one of the major killer diseases of infants.

In most rural communities, where they do not have access to orthodox medicine, all kinds of plants or raw materials are exploited to take care of the different health problems they encounter. For example in some communities in the southwest Nigeria, uncooked “ogi” slurry, which is a Nigerian fermented food made from cereal grains such as maize (*Zea mays* Linn.) is used traditionally for the relieve of stomach discomfort and diarrhoea by the rural people. Olukoya et al., [12] when carrying out research on this assertion observed that “ogi” has antibacterial activity against common diarrhoeagenic bacteria and that the presence of *Lactobacilli* in the slurry was responsible for its effect. Adebolu [13] in her own contribution however reported that not only the slurry but the liquor, that is, the waste water of “ogi” that is normally poured away to scoop the slurry also has antibacterial activity against diarrhoeagenic bacteria and that the growth inhibitory activity is superior to that of slurry on most of the organisms tested. Moreover, the duration of fermentation of the “ogi” also play significant role on its antibacterial activity. Although there are a lot of reports on antibacterial activity of “ogi”, there is none on the effect of “ogi” on the haematological parameters in the course of treating established diarrhoea caused by *Escherichia coli* 0157:H7 in albino rats.

In this present study therefore, the effects of raw maize “ogi” on the heamatological parameters of rats during the course of treatment against diarrhoea caused by *Escherichia coli* 0157:H7 was investigated.

## 2. Materials and Methods

### 2.1 Materials Collection

Bacteria: *Escherichia coli* 0157:H7 used in this study was obtained from the Microbiology Department, University College Hospital, Ibadan, Oyo State, Nigeria.

Grains: White maize grains (*Zea mays* Linn.) was purchased at a local market in Akure, Ondo State, Nigeria.

Animals: Wister albino rats, aged 5-6 weeks with weight averaging 45 g were used. They were bought from the Department of APH (Animal Production and Health), Federal University of Technology, Akure, Ondo State, Nigeria.

### 2.2 Preparation of Maize “OGI” Slurry and Liquor

This was prepared according to the method of Odunfa and Adeleye [14] with slight modification. One kilogram of the grains was cleaned and steeped in cold water for 72 h at  $30 \pm 2$  °C. After steeping, the water was removed and the grains washed in two changes of water before wet milling using a properly washed local grinding machine. The resulting paste was filtered using a sterile muslin cloth, the pomace was discarded while the filtrate was collected into another sterile container and allowed to settle for 3 days during which time fermentation took place by natural flora of the grains. After 72 h fermentation, the liquor on top of the fermented “ogi” slurry was collected into a sterile container and tested for antibacterial activity on the test organism, so also the slurry using agar diffusion assay.

### 2.3 In-vivo Determination of Infectious Dose of *Escherichia coli* 0157:H7 in Rats Used

A total of 24 albino rats were used for this assay, different concentration of 18 h stock culture ranging from  $2.22 \times 10^3$  CFU/mL to  $2.22 \times 10^9$  CFU/mL of test organisms were orally administered into the rats that were grouped in threes, and different groups were

given different dose. A pre-ingestion period of two weeks was earlier observed for acclimatization to the new environment before this assay. During this period, rats were kept on broiler's starter and sterile distilled water. The rats were observed for any sign of illness and nature of stool. The determined dose was used to challenge another set of albino rats to induce infection in them.

#### *2.4 Infection of Albino Rats with the Calculated Infectious Dose of *Escherichia coli* 0157:H7*

A total of 60 rats were orogastically infected with the calculated ID (infectious dose) of *Escherichia coli* 0157:H7. Prior infection, the rats were observed for 14 days for any sign of illness before infecting them. During this period, the rats were kept on broiler starter and sterile distilled water. After infection, the rats were also daily examined for signs of illness such as weakness, loss of appetite, and watery stool.

#### *2.5 Treatment of Infected Rats with Fermented Raw Maize “OGI” Slurry and Liquor*

After infection had set in, the rats were divided into 10 groups (6 rats per group). The first group was given fermented raw maize “ogi” slurry, 1 mL per day; the second group was given 1 mL of maize “ogi” slurry two times daily (bd); the third group was given 0.5 mL maize “ogi” slurry per day; the fourth group was given 0.5 mL maize “ogi” slurry two times daily; the fifth group was given 1 mL of the liquor of the fermented maize “ogi” daily; the sixth group was given 1 mL of the liquor twice daily; the seventh group was given 0.5 mL of the liquor daily; the eighth group was given 0.5 mL of the liquor twice daily; the ninth group was infected and not treated while the last group was neither infected nor treated. These last two served as a control.

#### *2.6 Haematological Analysis—WBC (White Blood Cell)*

One millilitre of blood was collected from each rat

used into EDTA bottles. The blood was diluted to 1:20 with Turk's solution. The diluted sample was mixed and examined under the microscope using the improved Neubaer's counting chamber.

#### *2.7 Differential Count*

A thin blood film was made on a slide. When completely dried, the blood film was stained using Leishman stain. Buffered water was added for 10 minutes using plastic pipe. The stain was washed off with tap water. The slide was air-dried; a drop of immersion oil was placed on the lower third of the blood film. The blood film was examined microscopically ( $\times 100$  objective lens) and the different white cells were counted and expressed in percentage.

#### *2.8 Morphological Examination of the Faeces of Infected Rats before, during and after Treatment with Maize “OGI”*

The physical appearance of the faeces of the infected rats, infected and treated rats, infected and not treated rats and the uninfected rats were daily observed throughout the investigation for signs of infection.

### **3. Results**

Infected rats treated with maize “ogi” slurry 1.0 mL, once or twice daily and maize “ogi” liquor 1.0 mL twice daily recovered 72 h after the commencement of the treatment while those that were treated with less than 1.0 mL recovered by 96 h. Without treatment with “ogi” however, the rats started recovering by 120 h (Table 1).

The treatment caused the WBC which had already gone up from  $2,433.000 \pm 0.000^e \text{ mm}^3$  to  $3,900.000 \pm 529.1503^a \text{ mm}^3$  as a result of the infection to reduce significantly ( $P \leq 0.05$ ) by 24 h of administration of the different volumes of raw fermented maize “ogi” components to the infected rats. Although, the WBC increased to reach maximal levels by 72 h in all the

**Table 1 Morphological examination of the feces of infected rats before and during treatment with fermented maize “ogi”.**

Rats	Part of maize “ogi” used	24 h	48 h	72 h	96 h	120 h
A	Slurry	Semi formed stool	Semiformed stool	Normal	Normal	Normal
B	Slurry	Semi formed stool	Semiformed stool	Normal	Normal	Normal
C	Slurry	Semi formed stool	Semiformed stool	Big & formed stool	Normal	Normal
D	Slurry	Semi formed stool	Semiformed stool	Semiformed, big stool	Normal	Normal
E	Liquor	Semi formed stool	Semiformed stool	Long, black & formed.	Normal	Normal
F	Liquor	Semi formed stool	Semiformed stool	Normal	Normal	Normal
G	Liquor	Semi formed stool	Semiformed stool	Long & black	Normal	Normal
H	Liquor	Semi formed stool	Semiformed stool	Verylong & formed.	Normal	Normal
I	Control (I)	Unformed stool	Watery stool	Puffy, big & black stool	Very long stool	Normal
J	Control (II)	Normal	Normal	Normal	Normal	Normal

A = 1 mL of slurry per day; B = 1 mL of slurry two times per day; C = 0.5 mL of slurry per day; D = 0.5 mL of slurry two times per day; E = 1 mL of liquor per day; F = 1 mL of liquor two times per day; G = 0.5 mL of liquor per day; H = 0.5 mL of liquor two times per day; I = Infected and not treated; J = Not infected and not treated.

The notes of the following tables are the same with Table 1.

treated and untreated rats, the values started decreasing and reached minimum levels by 120 h at which time all the infected rats whether treated or not had recovered (Table 2). The administration of fermented maize “ogi” also caused a significant ( $P \leq 0.05$ ) decrease in the lymphocyte counts of the infected rats that were treated with the “ogi” by 24 h through 72 h and by 120 h, there was a significant ( $P \leq 0.05$ ) increase in lymphocyte counts in almost all the treatment regimens (Table 3). On the other hand, there was an increase in the neutrophil count irrespective of the different volumes and different components of raw “ogi” administered the infected rats by 24 h. However by the 72 h of treatment, it started to decrease and by

120 h reduced to normal levels (Table 4). The treatment also caused a significant ( $P \leq 0.05$ ) increase in monocyte count of the infected and treated rats to normal levels. This became apparent by 72 h of the administration of the fermented maize “ogi” (Table 5). Fig. 1 shows the anus of an infected rat with unformed stool sticking to it after infection with the ID of *Escherichia coli* 0157:H7 while Fig. 2 shows the unformed stool passed out by the rat.

#### 4. Discussion

The infected rats treated with fermented maize “ogi” slurry 1.0 mL once or twice daily and those that were treated with 1.0 mL liquor twice daily recovered 72 h.

**Table 2 Effect of fermented maize “ogi” on the white blood cell count of albino rats infected with *Escherichia coli* 0157:H7.**

Treatment	(mm <sup>3</sup> ) in 24 h	(mm <sup>3</sup> ) in 72 h	(mm <sup>3</sup> ) in 120 h
A	2,800.000 ± 100.000 <sup>d</sup>	8,200.000 ± 200.000 <sup>c</sup>	4,100.000 ± 0.000 <sup>c</sup>
B	3,100.000 ± 100.000 <sup>abc</sup>	7,300.000 ± 300.000 <sup>d</sup>	5,000.000 ± 100.000 <sup>b</sup>
C	3,300.000 ± 100.000 <sup>ab</sup>	9,100.000 ± 100.000 <sup>b</sup>	3,200.000 ± 300.000 <sup>e</sup>
D	3,100.000 ± 0.000 <sup>abc</sup>	7,300.000 ± 0.000 <sup>d</sup>	3,800.000 ± 0.000 <sup>d</sup>
E	3,000.000 ± 100.000 <sup>cd</sup>	8,100.000 ± 100.000 <sup>c</sup>	6,100.000 ± 100.000 <sup>a</sup>
F	3,200.000 ± 200.000 <sup>ab</sup>	9,000.000 ± 100.000 <sup>b</sup>	3,800.000 ± 0.000 <sup>b</sup>
G	3,300.000 ± 100.000 <sup>ab</sup>	8,500.000 ± 200.000 <sup>bc</sup>	3,900.000 ± 0.000 <sup>cd</sup>
H	3,400.000 ± 200.000 <sup>b</sup>	10,000.000 ± 100.000 <sup>a</sup>	4,000.000 ± 200.000 <sup>cd</sup>
I	3,900.000 ± 529.1503 <sup>a</sup>	10,100.000 ± 1212.436 <sup>a</sup>	4,033.333 ± 208.167 <sup>cd</sup>
J	2,433.000 ± 0.000 <sup>e</sup>	2,433.000 ± 0.000 <sup>e</sup>	2,433.000 ± 0.000 <sup>f</sup>

Since the significant value is 0.000, therefore, the values followed by similar alphabets along the same column are not significantly different at  $P \leq 0.05$ .

The same with the following tables.

**Therapeutic and Immunomodulatory Effects of Raw Maize “OGI” on Rats  
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**Table 3** Effect of fermented maize “ogi” on the lymphocyte count of albino rats infected with *Escherichia coli* 0157:H7.

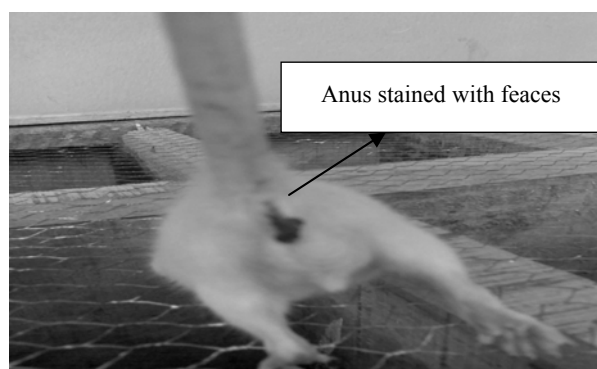
Treatment	(%) in 24 h	(%) in 72 h	(%) in 120 h
A	45.000 ± 1.000 <sup>e</sup>	40.000 ± 0.000 <sup>de</sup>	51.000 ± 1.000 <sup>d</sup>
B	53.000 ± 3.000 <sup>ab</sup>	43.000 ± 1.000 <sup>bcd</sup>	53.000 ± 1.000 <sup>c</sup>
C	50.000 ± 0.000 <sup>cd</sup>	39.000 ± 1.000 <sup>de</sup>	61.000 ± 1.000 <sup>a</sup>
D	51.000 ± 1.000 <sup>bcd</sup>	47.000 ± 1.000 <sup>b</sup>	59.000 ± 1.000 <sup>b</sup>
E	50.000 ± 2.000 <sup>cd</sup>	45.000 ± 0.000 <sup>bc</sup>	48.000 ± 0.000 <sup>e</sup>
F	53.000 ± 0.000 <sup>ab</sup>	42.000 ± 2.000 <sup>bcd</sup>	49.000 ± 1.000 <sup>e</sup>
G	51.000 ± 1.000 <sup>bcd</sup>	45.000 ± 2.000 <sup>f</sup>	58.000 ± 1.000 <sup>b</sup>
H	49.000 ± 1.000 <sup>d</sup>	33.000 ± 3.000 <sup>f</sup>	51.000 ± 0.000 <sup>d</sup>
I	52.000 ± 1.000 <sup>abc</sup>	36.667 ± 7.638 <sup>ef</sup>	49.000 ± 1.000 <sup>e</sup>
J	54.000 ± 0.000 <sup>a</sup>	54.000 ± 0.000 <sup>a</sup>	54.000 ± 0.000 <sup>c</sup>

**Table 4** Effect of fermented maize “ogi” on the neutrophil count of albino rats infected with *Escherichia coli* 0157:H7.

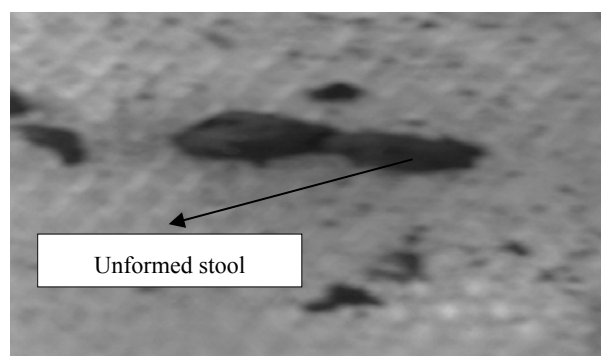
Treatment	24 h	72 h	120 h
A	53.000 ± 1.000 <sup>a</sup>	58.000 ± 2.000 <sup>abc</sup>	48.000 ± 2.000 <sup>abc</sup>
B	45.000 ± 1.000 <sup>de</sup>	56.000 ± 1.000 <sup>bcd</sup>	45.000 ± 0.000 <sup>de</sup>
C	47.000 ± 0.000 <sup>c</sup>	59.000 ± 0.000 <sup>ab</sup>	47.000 ± 2.000 <sup>bcd</sup>
D	47.000 ± 1.000 <sup>c</sup>	51.000 ± 1.000 <sup>d</sup>	38.000 ± 0.000 <sup>f</sup>
E	49.000 ± 0.000 <sup>b</sup>	53.000 ± 1.000 <sup>cd</sup>	50.000 ± 2.000 <sup>a</sup>
F	45.000 ± 0.000 <sup>de</sup>	55.000 ± 0.000 <sup>bcd</sup>	49.000 ± 2.000 <sup>ab</sup>
G	46.000 ± 1.000 <sup>cd</sup>	52.000 ± 2.000 <sup>d</sup>	40.000 ± 2.000 <sup>f</sup>
H	49.000 ± 1.000 <sup>b</sup>	62.000 ± 2.000 <sup>a</sup>	46.000 ± 1.000 <sup>cde</sup>
I	46.667 ± 1.528 <sup>c</sup>	60.333 ± 8.622 <sup>ab</sup>	48.333 ± 0.577 <sup>abc</sup>
J	43.600 ± 0.000 <sup>e</sup>	43.600 ± 0.000 <sup>e</sup>	43.600 ± 0.000 <sup>e</sup>

**Table 5** Effect of fermented maize “ogi” on monocyte count of albino rats infected with *Escherichia coli* 0157:H7.

Treatment	(%) in 24 h	(%) in 72 h	(%) in 120 h
A	1.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>b</sup>	0.000 ± 0.000 <sup>b</sup>
B	0.000 ± 0.000 <sup>b</sup>	1.000 ± 0.000 <sup>a</sup>	1.000 ± 0.000 <sup>a</sup>
C	1.000 ± 0.000 <sup>a</sup>	1.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>b</sup>
D	1.000 ± 0.000 <sup>a</sup>	1.000 ± 0.000 <sup>a</sup>	1.000 ± 0.000 <sup>a</sup>
E	0.000 ± 0.000 <sup>b</sup>	0.000 ± 0.000 <sup>b</sup>	0.333 ± 0.577 <sup>ab</sup>
F	1.000 ± 0.000 <sup>a</sup>	1.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>b</sup>
G	1.000 ± 0.000 <sup>a</sup>	1.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>b</sup>
H	0.000 ± 0.000 <sup>b</sup>	1.000 ± 0.000 <sup>a</sup>	1.000 ± 0.000 <sup>a</sup>
I	0.000 ± 0.000 <sup>b</sup>	0.667 ± 0.577 <sup>a</sup>	1.000 ± 1.000 <sup>a</sup>
J	0.700 ± 0.000 <sup>a</sup>	0.700 ± 0.000 <sup>a</sup>	0.700 ± 0.000 <sup>a</sup>



**Fig. 1** Rats showing symptom of diarrhoea.



**Fig. 2** Unformed stool of rats infected with *Escherichia coli* 0157:H7.

All the others recovered by 96 h. However, those that were not treated did not recover until 120 h. The recovery of the treated rats might be as a result of the presence of the bio-metabolites produced by the microflora of maize “ogi” which include *Lactobacillus plantarium*, *Saccharomyces cerevisiae* and *Candida krusei*, Adebolu et al. [15]. The reduction of WBC in rats treated with maize “ogi” is an indication of recovery from infection. The increase in neutrophil count by 24 h after infection sets in shows that the test organism stimulated the immune system leading to the proliferation of neutrophils showing serious infection. This observation agrees with the documentation of Cheesbrough [16] that the number of neutrophils increases significantly during infection. Furthermore, Aboderin and Oyetayo [17] and Oladunmoye [18, 19] documented that neutrophils are usually higher during active infection. The reduction of neutrophil counts observed by 72 h of administration of “ogi” show that it was able to modulate the immune system. This is as a result of the participation of neutrophil in phagocytising the invading bacteria. Neutrophils are majorly responsible for phagocytosis of pathogenic microorganisms during the first few hours after their entrance into tissues. During the course of digesting off the invading microorganisms however they die resulting in a decrease of their number [20]. The increase in lymphocyte count after treatment of the rats with maize “ogi” also attests to this. The administration of fermented maize “ogi” caused a significant decreased in the lymphocyte count by 24 h after treatment of the infected rats. By 72 h however, it started increasing, and this was probably because the lymphocytes specifically T-lymphocytes which are the most abundant lymphocyte in blood circulation and which play significant role in cell mediated immunity had transformed into lymphoblasts. This lymphoblast then participates directly in cytotoxic destruction of the invading bacteria. By 120 h after infection, the value returned to normal levels in most of the treated animals (Table 3). The monocyte count

of the rats before infecting them with test organism was 0.7%. After infection sets in, the monocyte count reduced to 0%. This may be as a result of the monocytes moving to the site of infection transforming into macrophages to phagocytose the organism. This agrees with the finding of Onifade and Audu [21] that lymphocytes and macrophages play significant role in conferring immunity to mice against *E. coli* infection.

## 5. Conclusion

From this study, administration of raw fermented maize “ogi” slurry (1.0 mL) once or twice daily or its liquor (1.0 mL) twice daily caused the recovery of rats infected with *E. coli* 0157:7 within 72 h while the untreated rats did not start recovering until after 120 h. It is conceivable that the administration of raw fermented maize “ogi” slurry once or twice daily or its liquor twice daily administered to the infected individuals would cause the recovery within 72 hours. Moreover, in addition to the antidiarrhoeal properties of fermented raw maize “ogi”, it seems that maize “ogi” also has immunomodulatory properties. It is therefore suggested that people having diarrhoea could drink fermented raw maize “ogi” slurry when treating the infection especially in rural areas where they might not have quick access to medical attention; and for those who could not drink the slurry, the liquor can be used as alternative. This will save many lives especially infants that are prone to the infection.

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