

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 1.0ml once or twice daily and maize “ogi” liquor, 1.0ml twice daily recovered 72h while those that were treated with less than 1.0ml recovered by 96h. Without treatment with ‘ogi’ however, the rats started recovering by 120h. 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 24h 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 24h. 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 24h but by the 72h of treatment, it started to decrease and by 120h 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 120h to 72h, 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.

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KEYWORDS

Maize “ogi”;
E.coli 0157:H7;
Therapeutic;
Immunomodulatory.

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*, *Escheri-*

chia 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], *Es-*

cherichia 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 aetiological 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.

MATERIALS AND METHODS

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 45g were used. They were bought from the Department of Animal Production and Health (APH), Federal University of Technology, Akure, Ondo State, Nigeria.

Preparation of maize “ogi” slurry and liquor

This was prepared according to the method of Odunfa and Adeleye^[4] with slight modification. One kilogram of the grains was cleaned and steeped in cold water for 72h at 30±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.

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 18h stock culture ranging from 2.22x10³cfu/ml to 2.22x10⁹cfu/ml of test organisms

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were orally administered into the rats that were grouped in threes, different groups were given different dose. A pre-ingestion period of 2 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.

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 infectious dose (ID) 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, watery stool.

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, 1ml per day, the second group was given 1ml of maize "ogi" slurry two times daily (bd), the third group was given 0.5ml maize "ogi" slurry per day, the fourth group was given 0.5ml maize "ogi" slurry two times daily, the fifth group was given 1ml of the liquor of the fermented maize "ogi" daily, the sixth group was given 1ml of the liquor twice daily, the seventh group was given 0.5ml of the liquor daily, the eighth group was given 0.5ml of the liquor twice daily, the ninth group was infected and not treated while the last group was neither infected and nor treated. These last two served as a control.

HAEMATOLOGICAL ANALYSIS

White blood cell (WBC)

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.

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 (X 100 objective lens) and the different white cells were counted and expressed in percentage.

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.

RESULTS

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

The treatment caused the WBC which had already gone up from $2433.000 \pm 0.000 \text{ } \mu\text{m}^3$ to $3900.000 \pm 529.1503^a \text{ } \mu\text{m}^3$ as a result of the infection to reduce significantly ($p \leq 0.05$) by 24h 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 72h in all the treated and untreated rats, the values started decreasing and reached minimum levels by 120h 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 24h through 72h and by 120h, 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 24h. However by the 72h of treatment, it

started to decrease and by 120h 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 72h of the administration of the fermented maize "ogi" (TABLE 5). Figure. 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 figure. 2 shows the unformed stool passed out by the rat.

DISCUSSION

The infected rats treated with fermented maize 'ogi' slurry 1.0ml once or twice daily and those that were treated with 1.0ml liquor twice daily recovered 72h. All the others recovered by 96h. However, those that were not treated did not recover until 120h. 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 24h after infection

TABLE 1 : Morphological examination of the faeces of infected rats before and during treatment with fermented maize "ogi"

Rats	Part of maize "ogi" used	24h	48h	72h	96h	120h
A	Slurry	Semi formed Stool	Semi formed Stool	Normal	Normal	Normal
B	Slurry	Semi formed Stool	Semi formed Stool	Normal	Normal	Normal
C	Slurry	Semi formed Stool	Semi formed Stool	Big & formed stool	Normal	Normal
D	Slurry	Semi formed Stool	Semi formed Stool	Semi formed, big stool	Normal	Normal
E	Liquor	Semi formed Stool	Semi formed Stool	Long, black & ormed.	Normal	Normal
F	Liquor	Semi formed Stool	Semi formed Stool	Normal	Normal	Normal
G	Liquor	Semi formed Stool	Semi formed Stool	Long & black	Normal	Normal
H	Liquor	Semi formed Stool	Semi formed Stool	Very long & 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

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

TABLE 2 : Effect of fermented maize "ogi" on the white blood cell count of albino rats infected with *Escherichia coli* 0157:H7

Treatment	(mm ³) in 24h	(mm ³) in 72h	(mm ³) in 120h
A	2800.000±100.000 ^d	8200.000±200.000 ^c	4100.000±0.000 ^e
B	3100.000±100.000 ^{abc}	7300.000±300.000 ^d	5000.000±100.000 ^b
C	3300.000±100.000 ^{ab}	9100.000±100.000 ^b	3200.000±300.000 ^c
D	3100.000±0.000 ^{abc}	7300.000±0.000 ^d	3800.000±0.000 ^d
E	3000.000±100.000 ^{cd}	8100.000±100.000 ^c	6100.000±100.000 ^a
F	3200.000±200.000 ^{ab}	9000.000±100.000 ^b	3800.000±0.000 ^b
G	3300.000±100.000 ^{ab}	8500.000±200.000 ^{bc}	3900.000±0.000 ^{cd}
H	3400.000±200.000 ^b	10000.000±100.000 ^a	4000.000±200.000 ^{cd}
I	3900.000±529.1503 ^a	10100.000±1212.436 ^a	4033.333±208.167 ^{cd}
J	2433.000±0.000 ^e	2433.000±0.000 ^e	2433.000±0.000 ^f

Since the significant value is 0.000, therefore, the values followed by similar alphabets along the same column are not significantly different at $P \leq 0.05$. **KEY:** A= 1.0ml of slurry per day, B = 1.0ml of slurry two times per day, C= 0.5ml of slurry per day, D= 0.5ml of slurry two times per day, E= 1.0ml of liquor per day, F= 1.0ml of liquor two times per day, G= 0.5ml of liquor per day, H= 0.5ml of liquor two times per day, I= Infected and not treated, J= Not infected and not treated.

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 increase significantly during infection. Furthermore, Aboderin and Oyeta^[17] and Oladunmoye^[18,19], documented that neutrophils are usually higher during active infection. The reduction of neutrophil counts observed by 72h 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

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

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

KEY: A= 1.0ml of slurry per day, B = 1.0ml of slurry two times per day, C= 0.5ml of slurry per day, D= 0.5ml of slurry two times per day, E= 1.0ml of liquor per day, F= 1.0ml of liquor two times per day, G= 0.5ml of liquor per day, H= 0.5ml of liquor two times per day, I=Infected and not treated, J= Not infected and not treated.

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

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

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

KEY: A= 1.0ml of slurry per day, B = 1.0ml of slurry two times per day, C= 0.5ml of slurry per day, D= 0.5ml of slurry two times per day, E= 1.0ml of liquor per day, F= 1.0ml of liquor two times per day, G= 0.5ml of liquor per day, H= 0.5ml of liquor two times per day, I=Infected and not treated, J= Not infected and not treated.

maize “ogi” caused a significant decreased in the lymphocyte count by 24h after treatment of the infected rats. By 72h however, it started increasing, this was probably because the lymphocytes specifically T- lymphocytes which are the most abundant lymphocyte in

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

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

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

KEY: A= 1.0ml of slurry per day, B = 1.0ml of slurry two times per day, C= 0.5ml of slurry per day, D= 0.5ml of slurry two times per day, E= 1.0ml of liquor per day, F= 1.0ml of liquor two times per day, G= 0.5ml of liquor per day, H= 0.5ml of liquor two times per day, I=Infected and not treated, J= Not infected and not treated.



Figure 1 : Rats showing symptom of diarrhoea

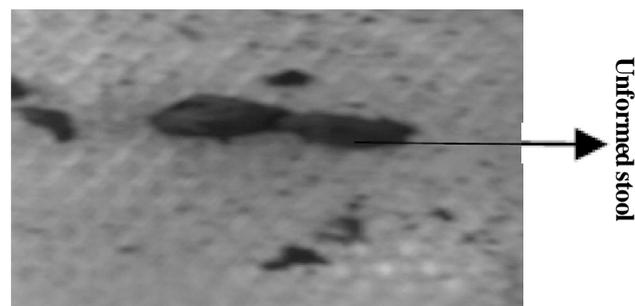


Figure 2 : Unformed stool of rats infected with *Escherichia coli* 0157:H7

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 120h 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.

CONCLUSION

From this study, administration of raw fermented maize “ogi” slurry (1.0ml) once or twice daily or its liquor (1.0ml) twice daily caused the recovery of rats infected with *E. coli* 0157:7 within 72h while the untreated rats did not start recovering until after 120h. 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 anti-diarrhoeal 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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