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Immunomodulatory and Therapeutic Effects of Whey on Rats Infected with Diarrhoeagenic Bacteria

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The effect of the consumption of fermented whey in treating diarrhoea caused by Enterotoxigenic *Escherichia coli*, *Salmonella typhi* and *Shigella dysenteriae* in albino rats and on their hematological parameters was investigated in this study. Prior the therapeutic experiment, the antibacterial activity of fermented whey on the selected bacteria was first assayed using agar diffusion method. Fermented whey exerted growth inhibitory activity on all the test organisms with diameter of zone of inhibition ranging from 8.0mm – 13.0mm. In the therapeutic experiment, infected rats treated with fermented whey (2.0 and 2.5ml respectively) recovered by 72h while the ones that were not treated did not recover until after 120h. The administration of fermented whey also caused a significant ($p<0.05$) decrease in white blood cells (WBC) and neutrophils of the infected rats which went up as a result of infection to reduce to normal levels faster than in the infected and not treated rats. It however caused an increase in both lymphocytes and monocytes, cells which play significant role in maintaining the integrity of the host system. Since the consumption of fermented whey has therapeutic, immunostimulatory and immunomodulatory effects on the infected rats, it is conceivable that fermented whey can be used to treat diarrhoea caused by these organisms in humans.

Keywords: Fermented Whey, Therapeutic Effect, Immunostimulatory, Immunomodulatory, Bacterial Diarrhoea.

Diarrhoea is an illness characterized by an increase in frequency and fluidity of stools. It is one of the most common diseases causing infant death in many developing countries (Walderman, 1998; Kosek *et al.*, 2003; Black, 2004; Cheesbrough, 2006; Willey *et al.*, 2008).

Although diarrhoea is self-limiting however when it's as a result of bacterial infections, antibiotics therapy may be required. However, most bacteria have developed resistance to most of the commonly employed antibiotics (Ashebir and Ashenafi, 1999). Moreover, some of these antibiotics can also induce diarrhoea known as "antibiotic induced diarrhoea" (Marteau *et al.*, 2002; Cheesbrough, 2006). Therefore it is necessary to find alternative means of treatment of this disease.

In searching for alternatives to antibiotics in treating bacterial diarrhoea, Adebolu and Ademulegun (2005) observed that fermented whey, a byproduct of cheese-making has antibacterial activities against common diarrhoeagenic bacteria. Olorunfemi *et al*. (2010) in their investigation observed that the antibacterial activity of fermented

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whey is as a result of the presence of lactic acid bacteria such *Micrococcus lactis*, *Bacillus brevis*, *Lactobacillus acidophilus* and *Pediococcus cerevisiae* in the fermented whey. In all these investigations however, whey was not used to treat established diarrhoeal infection. This present study therefore was designed to investigate whether fermented whey can be exploited in treating diarrhoea caused by selected diarrhoeagenic bacteria and also to know the effect of its consumption on the hematological parameters of the treated rats.

MATERIALS AND METHODS

Bacteria used

Enterotoxigenic *Escherichia coli*, *Salmonella typhi* and *Shigella dysenteriae* used in this study was obtained from the Microbiology laboratory, Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.

Source of whey

Fresh whey was collected from Fulani women who produce local cheese in a suburb village in Akure, Ondo State, Nigeria.

Animals

Wister albino rats aged 5 – 6 weeks with weight averaging 85g were used. They were bought from the Animal House, Faculty of Health Sciences, Obafemi Awolowo University, Ile – Ife, Osun State, Nigeria.

Determination of antibacterial activity of whey on test organisms

This was done using agar diffusion method of Adebolu *et al*., (2007). Each organism was grown in nutrient broth at 37^0C for 18h. The cells were harvested at 3000rpm for 5min into 20ml sterile distilled water. One milliliter of the harvested bacterial cells containing approximately 10^6 cfu/ml in different universal bottles, one organism per bottle, was separately taken into different sterile petridishes, one organism per plate. Each plate was then overlaid with 20ml nutrient agar, carefully swirled to allow even distribution of the organisms within the agar and was allowed to gel before 2 wells (8mm in diameter) were bored in the agar with the aid of a sterile cork borer. Whey was introduced into one of the wells while 0.1ml of sterile distilled water was put into the other well, to serve as control. The plates were incubated at 37^0C for 24h. The diameter of the zones of inhibition around the wells was measured and recorded. This assay was repeated every 24h for 5d, using fermented whey at 30 ± 2^0C in order to evaluate the effect of fermentation on the antibacterial potency of whey.

Antibiotic sensitivity pattern of the test organism

This was done as above except that instead of making wells on the already seeded plates and pouring in whey, commercial antibiotics disc was placed on the seeded agar plates before the plates were incubated at 37^oC for 24h.

Screening of fermented whey for factors responsible for antibacterial activity on the test bacteria

This was done according to the method of Jin *et al.* (1996).

Infection of rats with the test organisms

Prior infection of the rats, the infectious dose of each of the test organisms was first determined. Healthy albino rats were grouped in three's and different groups were orogastrically challenged with different doses of a particular organism. The rats were daily observed for symptoms of diarrhoea. The dose that was able to cause diarrhoea in all the rats in a particular group was taken as the infectious dose. This dose was then administered to another group of healthy rats and the rats were daily observed for signs of infection.

Treatment of infected rats with fermented whey

After infection sets in, the infected rats were grouped into six groups. Group one was administered 2.5ml whey daily, the second group was administered 2.0ml whey daily, the third group was administered 1.5ml whey daily, the fourth group was administered 1.0ml whey daily, the fifth group was administered 0.5ml whey daily. The sixth group on the other hand was not treated with whey. This served as control. The rats were daily observed for signs of recovery.

Determination of the effects of whey on the hematological parameters of apparently healthy rats and infected rats

This was done according to the method of (Aning *et al*., 1998)

RESULTS

The whey used exerted varying degree of growth inhibitory activity on the test organisms

Table 1. Diameter of zones of inhibition of the selected diarrhoeagenic bacteria using fermented local whey.

Fermentation Duration (h)	Diameter of zones of inhibition (mm) A	B	C
0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
24	2.0 ± 0.02	2.0 ± 0.11	0.0 ± 0.00
48	4.0 ± 0.02	4.0 ± 0.12	1.0 ± 0.12
72	12.0 ± 0.13	13.0 ± 0.11	8.0 ± 0.02
96	0.0 ± 0.00	2.0 ± 0.02	2.0 ± 0.11

Values are mean ±SD (n=4)
Key:A- ETEC *E.coli,* B- Shigella dysenteriae, *C- Salmonella typhi*

Table 2. Comparative growth inhibition of the test organisms using 72h fermented whey and some antibiotics.

Treatment	Diameter of zones of inhibition (mm) A	B	C
Fermented whey	12.0	13.0	8.0
Oflomed	36.0	19.0	2.0
Cephorex	31.0	17.0	10.0
Ampiclox	10.0	0.0	1.0
Gentamycin	9.0	3.0	9.0
Tetracycline	7.0	3.0	0.0
Nalidixic acid	6.0	0.0	1.0
Nitrofurantoin	2.0	2.0	1.0
Colistin	1.0	0.0	0.0
Cotrimoxazole	0.0	0.0	1.0
Augmentin	0.0	0.0	1.0

A- ETEC *E.coli,* B- Shigella dysenteriae,
C- Salmonella typhi

Table 3. Effects of the blockage of the inhibitory substances in 72h fermented whey on its antibacterial potency on the test bacteria.

Treatment	Diameter of zones of inhibition (mm) A	B	C
UFW	13.0	3.0	14.0
TTW	13.0	3.0	14.0
NPW	3.0	1.0	6.0
CTW	11.0	2.0	11.0

A- ETEC *E. coli,* B- Shigella dysenteriae, *C- Salmonella typhi,* UFW- Untreated fermented whey, TTW- Trypsin treated whey, NPW- Whey neutralized with 1M NaOH, CTW- Catalase treated whey

based on the extent of fermentation of the whey sample. Whey fermented at 30 ± 2^{0}C for 72h exerted the greatest growth inhibitory effect on all the test organisms (Table 1). The growth inhibition mediated by this whey was higher than that of the commercial antibiotics used except oflomed and cephorex which had growth inhibitory effect of 36.0mm and 31.0mm respectively on *Escherichia coli* and 19.0mm and 17.0mm respectively on *Shigella dysenteriae*. For *Salmonella typhi* however, gentamycin (9.0mm) and cephorex (10.0mm) were the only antibiotics that exerted greater inhibition than that of the fermented whey on the organism (Table 2). In determining the factor present in fermented whey responsible for the inhibition, it was observed that organic acid present in whey exerted the highest growth inhibitory activity on the test organisms (Table 3).

In the therapeutic assay, rats infected with Enterotoxigenic *Escherichia coli* and treated with whey (dose ranging from 2.0 to 2.5ml daily) recovered by the 72h of treatment while the infected rats that were not treated on other hand did not recover until after 120h (Table 4). The same result was also seen in rats infected with *Shigella dysenteriae,* the treated rats recovered by the 72h of treatment with whey (Table 5). There was however a great deviation from this observation in

Table 4. Physical examination of rats infected with Enterotoxigenic *Escherichia coli* and their stool for signs of recovery after treatment with fermented whey.

Treatment	Duration of treatment (h)				
Dose (ml)	24	48	72	96	120
W (0.0)	B,D,F	B,D,F	B,D,F	B,D,F	B,C,F
W (0.5)	B,D,F	B,D,F	B,D,F	A,C,E	A,C,E
W (1.0)	B,D,F	B,D,F	B,C,F	A,C,E	A,C,E
W (1.5)	B,D,F	B,D,F	A,C,F	A,C,E	A,C,E
W (2.0)	B,D,F	B,D,F	A,C,E	A,C,E	A,C,E
W (2.5)	B,D,F	B,D,F	A,C,E	A,C,E	A,C,E

A-Active rats, B-Dull rats/ reduced activity, C-Eat well, D- Loss of appetite, E- Formed stool, F- Unformed stool, W- Treated with whey

Table 5. Physical examination of rats infected with *Shigella dysenteriae* and their stool for signs of recovery after treatment with fermented whey.

Treatment	Duration of treatment (h)				
Dose (ml)	24	48	72	96	120
W (0.0)	B,D,F	B,D,F	B,D,F	B,D,F	B,D,F
W (0.5)	B,D,F	B,D,F	B,D,F	B,D,F	B,D,F
W (1.0)	B,D,F	B,D,F	B,D,F	B,D,F	B,D,F
W (1.5)	B,D,F	B,D,F	A,C,E	A,C,E	A,C,E
W (2.0)	B,D,F	B,D,F	A,C,E	A,C,E	A,C,E
W (2.5)	B,D,F	B,D,F	A,C,E	A,C,E	A,C,E

Key: A-Active rats, B-Dull rats/ reduced activity, C-Eat well, D- Loss of appetite, E- Formed stool, F- Unformed stool, W- Treated with whey

Table 6. Physical examination of rats infected with *Salmonella typhi* and their stool for signs of recovery after treatment with fermented whey.

Treatment	Duration of treatment (h)				
Dose (ml)	24	48	72	96	120
W (0.0)	B,D,F	B,D,F	B,D,E	B,D,E	B,C,E
W (0.5)	B,D,F	B,D,F	B,D,E	B,D,E	B,C,E
W (1.0)	B,D,F	B,D,E	B,D,E	B,D,E	B,C,E
W (1.5)	B,D,F	B,D,E	B,D,E	B,D,E	B,C,E
W (2.0)	B,D,F	B,D,E	B,C,E	B,D,E	B,C,E
W (2.5)	B,D,F	B,D,E	B,D,E	B,D,E	B,C,E

Key: A-Active rats, B-Dull rats/ reduced activity, C-Eat well, D- Loss of appetite, E- Formed stool, F- Unformed stool, W- Treated with whey

Table 7. Effects of whey administration on the hematological parameters of albino rats infected with *Escherichia coli.*

Treatment Dose (ml)	A (x 10^9/ml)	B (%)	C (%)	D (%)	E (%)
W (0.0)	4.50±1.08	62.00±0.81	36.50±0.39	1.00±0.00	0.00±0.00
W (0.5)	3.40±0.12	59.50±0.39	40.00±1.01	0.50±0.00	0.00±0.00
W (1.0)	3.10±0.10	62.25±0.33	34.75±1.09	1.00±0.00	2.00±0.00
W (1.5)	3.20±1.09	65.75±0.53	30.25±0.28	2.00±0.81	2.00±0.00
W (2.0)	2.80±1.29	40.00±1.01	56.50±0.39	2.00±0.00	0.05±0.00
W (2.5)	2.60±1.70	41.50±0.39	56.25±0.28	2.00±0.00	0.25±0.00
CN	2.70±1.12	44.00±0.40	54.00±0.40	1.00±0.00	1.00±0.00

Values are mean ±SD (n=4)
Key: A- Total White Blood cell count, B- Neutrophils count, C- Lymphocytes count, D- Monocytes count, E- Eosinophils, W- Treated with whey, CN- Not infected, not treated

Table 8. Effects of whey administration on the hematological parameters of albino rats infected with *Shigella dysenteriae.*

Treatment Dose (ml)	A (x 10^9/ml)	B (%)	C (%)	D (%)	E (%)
W (0.0)	15.89±0.41	61.50±0.22	36.00±0.40	2.00±0.81	0.50±0.58
W (0.5)	14.00±0.12	61.00±0.40	39.00±0.00	0.00±0.00	0.00±0.00
W (1.0)	14.50±0.20	60.50±0.18	39.50±0.18	0.00±0.00	0.00±0.00
W (1.5)	12.75±0.25	64.00±0.00	35.50±0.33	0.00±0.00	0.50±0.59
W (2.0)	10.25±0.16	27.00±0.58	73.00±0.59	0.00±0.00	0.00±0.00
W (2.5)	10.00±0.43	22.25±0.21	76.75±0.41	1.00±0.00	0.00±0.00
CN	10.00±0.40	37.00±0.40	63.00±0.56	1.00±0.00	0.00±0.00

Values are mean ±SD (n=4)
Key: A- Total White Blood cell count, B- Neutrophils count, C- Lymphocytes count, D- Monocytes count, E- Eosinophils, W- Treated with whey, CN- Not infected, not treated

Table 9. Effects of whey administration on the hematological parameters of albino rats infected with *Salmonella typhi.*

Treatment Dose (ml)	A (x 10^9/ml)	B (%)	C (%)	D (%)	E (%)
W (0.0)	4.50±0.18	69.25±0.22	30.50±0.16	0.50±0.57	0.00±0.00
W (0.5)	4.83±0.19	63.75±0.25	36.25±0.21	0.00±0.00	0.00±0.00
W (1.0)	4.25±0.22	64.00±0.54	35.50±0.81	0.50±0.57	0.00±0.00
W (1.5)	3.05±0.21	62.00±0.00	36.75±0.25	1.00±0.81	0.25±0.50
W (2.0)	3.65±0.57	33.00±0.45	64.00±0.54	3.00±0.44	0.25±0.50
W (2.5)	3.80±0.16	32.25±0.22	64.25±0.22	3.00±0.00	0.25±0.50
CN	3.60±0.20	41.50±0.18	56.50±0.24	2.00±0.81	0.00±0.00

Values are mean ±SD (n=4)
Key: A- Total White Blood cell count, B- Neutrophils count, C- Lymphocytes count, D- Monocytes count, E- Eosinophils, W- Treated with whey, CN- Not infected, not treated

rats infected with *Salmonella typhi,* the infected rats did not start recovering until 120h of administration of the fermented whey (Table 6).

In the hematological assays, the administration of whey to rats infected with ETEC *Escherichia coli* caused their total white blood counts (WBC) and neutrophils counts which had gone up as a result of infection to reduce to normal levels as that of the control rats by the fifth day of treatment with fermented whey (Table 7). It however caused the lymphocytes counts and the monocytes count on the other hand to increase. Similar results were observed for *Shigella dysenteriae* (Table 8) and *Salmonella typhi* (Table 9).

DISCUSSION

In this study, locally prepared whey fermented for 48h and 72h respectively inhibited the growth of all the test bacteria (Table 1). The inhibition mediated by the latter however was superior to that exerted by the 48h fermented whey. The growth inhibition mediated by fermented whey shows that it has bioactive components, which had growth inhibitory activity on these organisms. These bioactive components according to the report of this investigation are organic acid and hydrogen peroxide because when they were selectively blocked, there was a reduction in the growth inhibitory activity of the fermented whey on the test organisms (Table 3). Out of these two metabolites however, organic acid was observed to exert the greatest growth inhibitory effect on the test bacteria in this study These metabolites have been reported by many researchers to have potent antibacterial activity (Tagg *et al*., 1976; Lindgren and Dobregosz, 1990; Brink *et al.*, 1994 and Ogunbanwo *et al*., 2003., Savadogo *et al.*, 2006)..

In the *in vivo* assay, the administration of fermented whey to the infected rats caused the duration of infection to reduce from 120h to 72h for those treated with 2.0ml and 2.5ml of the whey sample except in the case of infection with *Shigella dysenteriae* in which the infected rats did not start recovering until 120h after infection. This agrees with findings of Adebolu *et al*. (2010) that administration of the liquor of fermented maize “ogi” (another food product) to rats infected with diarrhoeagenic *Escherichia coli* caused the duration of infection to reduce from 9days to 72h.

The administration of fermented whey (2.0 and 2.5ml respectively) also caused a significant ($p \leq 0.05$) decrease in WBC and neutrophils of the infected rats which went up as a result of infection to reduce to normal levels faster than in the infected and not treated rats. It however caused an increase in both lymphocytes and monocytes counts. Lymphocytes are responsible for antibody production and cellular immunity while monocytes are highly phagocytic when they transform into macrophages (Wiley *et al.,* 2008). These immune cells are responsible for maintaining the integrity of the host system. The ability of fermented whey to stimulate and to modulate the immune system as observed in this study shows that it has both immunostimulatory and immunomodulatory properties.

In conclusion therefore, fermented whey has been shown to have antidiarrhoeal activity in addition to antibacterial activity against diarrhoaegenic bacteria such as Enterotoxigenic *Escherichia coli Salmonella typhi* and *Shigella dysenteriae* in rats. Moreover, it also has both immunostimulatory and immunomodulatory activities. It is therefore suggested that people having diarrhoea could drink whey fermented at 30 ± 2^{0}C for 72h to treat the infection especially in rural areas where they might not have access to quick medical attention.

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