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Full Length Research Paper

Evaluation of the Lipopolysaccharide of *Escherichia coli* O157:H7 for prophylactic ability against diarrhoea caused by homologous organism in Wistar albino rats

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In searching for a prophylactic measure against diarrhoea caused by *E. coli* 0157:H7, the lipopolysaccharide (LPS) of the organism was investigated for possible protective effect in Wistar albino rats against the organism. The *E. coli* O157:H7 used in this study was isolated from diarrhoeic patients attending one of the Federal Medical Centers in South West, Nigeria using standard microbiological methods and its identity confirmed using both serological and polymerase chain reactions. The LPS of the organism was extracted using hot-phenol water method while antibodies to the LPS of the isolated *E. coli* O157:H7 was raised in New Zealand White rabbits by subcutaneous immunization method. The results showed that the LPS of *E. coli* O157:H7 raised in the rabbits elicited serum antibodies with high titre and bactericidal activity. Active and passive protection in Wistar albino rats against *E. coli* 0157:H7 with the LPS and the corresponding anti LPS conferred a 70% and 50% protection respectively in the rats used for the assays. This study has been able to show that lipopolysaccharide extracted from *E. coli* O157:H7 was able to confer protection in Wistar albino rats against diarrhoea caused by the organism. It is therefore conceivable that LPS can be used as a vaccine for the prevention of the infection in both animals and human beings.

Keywords: E. coli O157:H7, Lipopolysaccharide, prophylaxis

INTRODUCTION

Escherichia coli O157:H7 was first recognized as a human pathogen in 1983 (Edward *et al*. 1994). Diseases caused by this pathogen have subsequently been recognized worldwide (Ademokoya *et al.* 2014). Infection with *E. coli* O157:H7 causes a spectrum of illnesses with high morbidity and mortality, ranging from watery diarrhoea to hemorrhagic colitis and the extra-intestinal complication of haemolytic uremic syndrome (HUS) (Isibor *et al*. 2013). *E. coli* O157:H7 infections are mostly food or water borne and have been implicated in undercooked ground beef, raw milk, cold sandwiches, water, unpasteurized apple juice, sprouts and vegetable (Brazil *et al*. 2007). This organism has a unique cultural characteristic of inability to ferment sorbitol – a nutrient used for isolation of *E. coli* from stool specimen. This pathogen has become more significant than other well-recognized food borne pathogens for reasons including the severe consequences of infection that affect all age groups, their low infectious dose, their unusual acid tolerance and their apparent special but inexplicable

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Table 1. Antibody titre of rabbit`s sera against *E. coli* O157:H7 LPS determined by passive haemagglutination test

Rabbits	Initial exposure (µg/ml)	Titre	Booster dose (µg/ml)	Titre
A	300	1:1024	600	1:2048
B	300	1:1024	600	1:2048
C	300	1:1024	600	1:2048

Table 2. Antibody titre of rat's sera against *E. coli* O157:H7 LPS determined using passive haemagglutination test

Rat group	Primary immunization (LPS conc in µg/ml /Titre: O/I)			Secondary immunization (LPS conc in µg/ml /Titre: O/I)		
A	50	1:512	1:512	100	1:1024	1:1024
B	100	1:512	1:512	200	1:1024	1:1024
C	150	1:512	1:512	300	1:1024	1:1024
D	200	1:1024	1:1024	400	1:1024	1:1024
E	250	1:1024	1:1024	500	1:1024	1:1024

Key: O/I = Oral/Intraperitoneal; A, B, C, D, E= 10 rats per group. Each rat in the group was administered the LPS dose captured against each group.

association with ruminants that are used for food (Buchanan and Doyle, 1997).

E. coli O157:H7 is highly resistant to many antibiotics, and there is no evidence that antibiotics improve the course of disease besides, treatment with some antibiotics might precipitate kidney complications as documented by Walterpiel *et al.* (1992). In view of the foregoing, there is therefore the need to prevent the spread of the disease caused by this pathogen. This study is therefore designed to investigate the possibility of prophylactic strategy against the infection using lipopolysaccharide extracted from the organism or its corresponding antibody so that in case of epidemiological outbreak, it will be easy to control the infection.

MATERIALS AND METHODS

Stool samples were collected from diarrhoeic patients from the Federal Medical Centre, Owo in southwest, Nigeria after seeking ethical consent from appropriate authorities. The samples were immediately brought to the laboratory and cultured on eosin methylene blue agar and those positive for *E. coli* were sub-cultured on sorbitol MacConkey agar for presumptive identification of *E. coli* O157:H7. To confirm the organism, serological assay was carried out using Wellcolex Diagnostics *E. coli* O157:H7 kit (Oxoid, TSMX7825, US) and PCR assay for the detection of O157 and H7 flagella genes. The LPS of the organism was extracted using hot phenol – water technique method of Simin *et al.* (2011) and stored at 4^{o}C before used. The infective dose of the organism was determined by orogastically dosing the animals with increasing concentrations of the organism as described by Olorunfemi and Adebolu (2012).

For the active protection assay, 100 rats were grouped into two, one group was orogastically dosed with an increased concentration of the organism`s LPS at 7d interval using LPS concentrations that ranged from 50 to 500 µg/ml while the second group was dosed intraperitoneally with the same concentrations and time intervals. Seven days after the last injection, their bloods were collected through venepuncture, allowed to clot, the antibody titres were determined using passive haemagglutination test. For the passive protection assay, three rabbits were each subcutaneously injected with increasing concentration of the extracted LPS (300 to 600µg/ml) at 7 days interval for three consecutive weeks according to the method of Estela *et al*. (2013). Seven days after the last injection, their bloods were also collected through venepuncture, allowed to clot, the antibody titres were determined using passive haemagglutination test and the serum was used to immunize Wistar albino rats using the method of Mullan *et al.* (1974). The immunized rats (actively and passively immunized) were then challenged with the infective dose of the organism and were observed for symptoms of infection. The microbial load of *E. coli* O157:H7 in the special organs: liver, spleen, ileum and faeces of the infected rats were counted. Rats that were not immunized served as control. For data analysis, one way ANOVA was carried out using statistical software SPSS.

RESULTS

After the primary immunization of the animals with the LPS of *E. coli* O157:H7, the antibody titres of the rabbits to the LPS averaged 1:1024 (Table 1) while it averaged 1:512 for the rats (Table 2). However, after the administration of the booster dose, the titres increased as showed in both Tables. In the protection experiment, immunisation of the rats with the LPS of the organism whether actively or passively induced, caused a significant reduction ($p<0.05$) in the population of the organism in the organs of the rats and their faeces after challenge with the organism (Figures 1 and 2). Active immunization however exerted a greater effect. On the

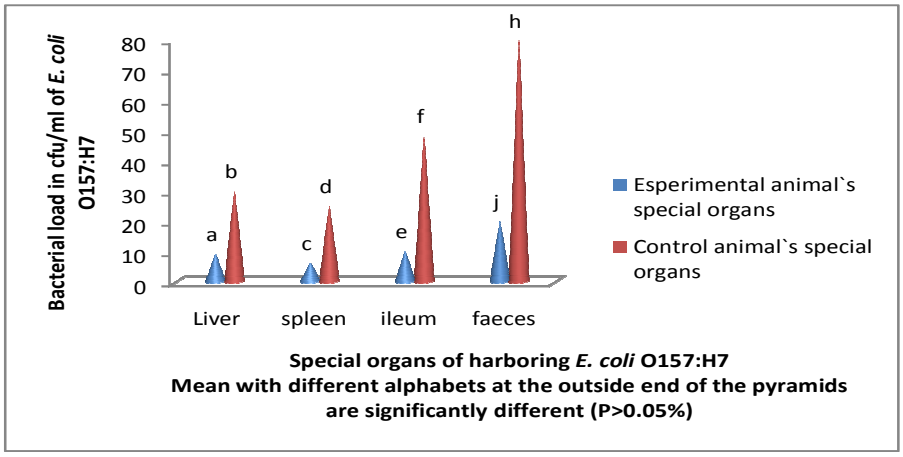

Figure 1. Effect of active immunization with the LPS of *E. coli* O157:H7 on the growth of *E.coli* O157:H7 in some organs of Wistar albino rats infected with the infective dose of the organism.

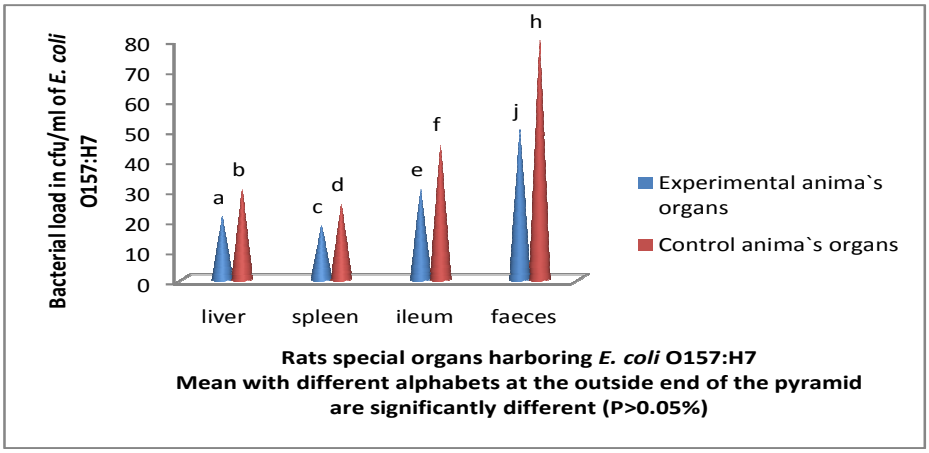

Figure 2. Effect of passive immunization with anti *E. coli* O157:H7 LPS serum on the growth of *E. coli* O157:H7 in some organs of Wistar albino rats infected with the infective dose of the organism.

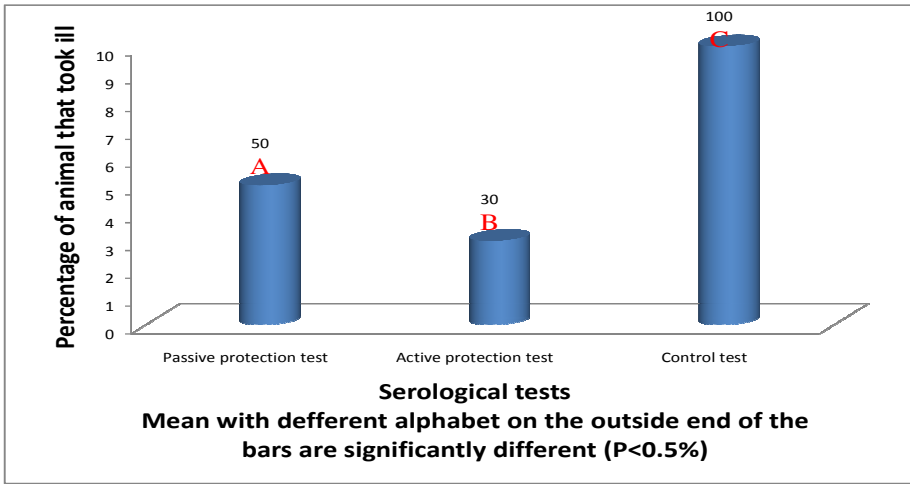

Figure 3. Comparison of percentage protection mediated by active and passive immunization with LPS of *E. coli* O157:H7 in Wistar albino rats against *E. coli* O157: H7 infection.

whole, active immunisation with the LPS of *E. coli* O157:H7 offered 70% protection on the rats while passive immunization offered only 50% protection (Figure 3).

DISCUSSION

The role of LPS of *E. coli* O157:H7 in conferring prophylactic immunity in Wistar albino rats against diarrhoea caused by *E. coli* O157: H7 was carried out in this study. The result of the active protection assay in this study (70%) however was lower than that obtained in our previous study (Ademokoya *et al.* , 2012) which was 100%. On the other hand, the result of the passive protection in this study (50%) was higher than the result of the passive protection recorded by the same authors. The discrepancy could be due to the antigen used in raising the antibody. For this study, *E. coli* O157:H7 LPS antigen was used while whole cells of *E. coli* O157:H7 antigen was used in the previous study. Moreover, in this investigation, the *E. coli* O157:H7 isolated from South West, Nigeria elicited similar immunogenicity and bactericidal activities to that reported by Edward *et al.* (1994).

The initial high antibody titres recorded in rabbit as compared with that of rats in Tables 1 and 2 could be due to the animal`s body weight and the route of inoculation. The average weight of the rabbits used in this assay was 855g and were inoculated subcutaneously, while the average weight of the rats used was 70g and were inoculated orogastically and intraperitoneally. The rise in antibody titres from 1:1024 for the rabbit and 1:512 for the rats to 1:2048 and 1:1024 respectively is an evidence of active antibodies response to the organism, that is, *E. coli* O157:H7. This is in accordance with the report of Betty *et al*. (2007). The higher protection capacity of active immunization assay with the LPS of *E. coli* O157:H7 as compared with passive immunization method in this study shows that antibody to LPS is not playing major role in protecting the animals from infection but that other factors such as the macrophages and T-cells on which LPS has mitogenic effect on, are involved in the successful protection of the rats from the infection.

CONCLUSION AND RECOMMENDATION

Lipopolysaccharide has been found to mediate effective protection against *E. coli* O157:H7 isolated from diarrhoeic patients in South West Nigeria. Effort therefore should be geared in producing LPS based-vaccines for prophylaxis against the infection caused by the organism in human and to prevent shedding and carriage in ruminants. Moreover, hand washing and working practices that prevent cross-contamination should be advocated among the people in the communities.

REFERENCES

Ademokoya AA, Adebolu TT, Oladunmoye MK (2012). The antibiogram and role of antibody in prophylaxis of albino rats against diarrhoea caused by *Escherichia coli* O157:H7. *J. Clin. Immunol. Immunopathol. Res.* 4(3): 29-33.

Ademokoya AA, Adebolu TT, Ogundare AO (2014). Antigenic and molecular properties of *Escherichia coli* O157:H7 isolated from diarrhoeic patients in Ondo State. *Int. J. Cur. Res. Biosci. Plant Biol.* 1 (5):75-82.

Betty AF, Daniel FS, Alice SW (2007). Balley and Scotf`s Diagnostic Microbiology. Twelfth Edition. Andrew Allen Publisher, pp. 120.

Buchanan RI, Doyle MP (1997). Food borne disease significance of *E. coli* O157:H7 and other enterohaemorrhagic *E. coli. Food Technol.* 51 (10):69-76.

Edward K, John BR, Joseph S, Dolores A, Bryla, Shousun CS (1994). Preparation characterization and immunological properties in mice of *E. coli* O157 O- specific polysaccharide protein conjugate vaccine. *Infection and Immunity. Am. Society for Microbiol.* pp. 5048-5054.

Dupont HI, Formal SB, Hornick RB, Snyder MJ, Kalas JP (1971). Pathogenesis of *Escherichia coli* diarrhoea. *New Engl. J. Med.* 285:1-9

Estela T, Susan J, Waheed P, Bryan PH, Bobby JC (2013). Role of antilipopolysaccharide antibodies in serum bactericidal activity against *Samonella enterica serovar typhimurium* in healthy adults and children in the United State. *J. Clin. Vaccine Immunol.* 20(10):1491-1498

Karch H, Tarr P, Bielaszewska M (2005). Current epidemiological status of enterohaemorrhagic *Escherichia coli* O157:H7 in Africa. *Chinese Med. J.* 119 (3): 217-222

Nataro JP, Kaper JB (1998). Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev*. **11**:142.

Mulian NA, Newsome PG, Palmer GH, Wilson ME (1974). Protection against gram negative infection with antiserum to lipid A from *Salmonella Minnesota* R595. *Infection and Immunity*, Washington. 10: 1195-1201.

Olorunfemi OB, Adebolu TT (2012). Immunomodulatory and therapeutic effects of whey on rats infected with diarrhoeagenic bacteria. *Biosci., Biotechnol. Res. Asia* 9(1): 173- 179.

Simi R, Noor A, Bahman T, Mahmoo JT, Omid Z, Reza A, Faramarz, M, Amir, HZ (2011). Extraction, Purification and characterization of lipopolysaccharide from *E. coli* and *Salmonella typhi*. *Avicenna Journal of Med. Biotechnology* 3(1):3-9.

Walterspiel JN, Ashkenazi S, Marrow AL, Leary TG (1992). Effect of sub- inhibitory concentrations of antibiotics on extracellular shiga – like toxin. *Med. J. Infect.* 20(1): 25-9.