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The effects of *Escherichia coli* 0157: H7 lipopolysaccharide(LPS) from human, cattle and poultry isolates on haematological parameters of neonatal albino rats

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ABSTRACT

The response of some haematological parameters in neonatal albino rats to different filtrate dosages of cell free extracts of Escherichia coli 0157:H7 obtained from human, cattle and chicken sources were investigated. At higher levels of extracts administration [1.0ml and 2.0ml], Packed cell volume [PCV], Haemoglobin count [HBC] and Red Blood cell Count [RBC] had highest decrease with human faeces isolates [IHF] from 49.9 to 48.0%, 12.0-10.4g dl^{-1} and 7.7 to 7.6 x 10^9 ml^{-1} respectively. White Blood count [WBC], Erythrocytes sedimentation Rate [ESR), Lymphocytes and Neutrophils counts on the other hand had highest increase with cattle isolates [ICT] ranging from 4.0 to 8.2 x 10^6 ml^{-1}, 3.0 to 6.0x10^6 ml^{-1}, 2.20 to 3.80 x 10^6 ml^{-1} and 1.60 to 3.90 x 10^6 ml^{-1} respectively. Statistical analysis showed significant difference [P<0.05] in the effects of the filtrate dosages on neutrophils and PCV in all the isolates. Significant difference [p<0.05] on the effects of the various dosages of human isolates [IHF] poultry [IPB] and cattle [ICT] isolates on both WBC and ESR were also observed . However, no significant difference was observed [P>0.05] with regards to the general effects of isolates on the haematological parameters studied. Cell free extracts of E. coli 0157:H7 used were found to have an overall negative effect on the haematological indices. Albino rats can therefore be recommended for use as a reliable small animal model to study host early responses as well as the role of bacterial virulence factors in the induction of haematological diseases

Key words: *E. coli 0157:H7, Lipopolysaccharide, haematological parameters, disease, neonatal rats*

INTRODUCTION

Escherichia coli 0157:H7 is a notable pathogen that causes food borne illness [7]. Infection often leads to haemorrhagic colitis and occasionally to kidney failure, especially in children and elderly. Most illness has been associated with eating under cooked contaminated ground beef, drinking unpasteurized milk, fruit juice [5] or drinking contaminated water [10].

E. coli 0157:H7 infection was first recognized as a human pathogen in 1982, when it was associated with two outbreaks that occurred in Oregon and Michigan and involved the consumption of hamburgers from a fast food restaurant [9].

Symptoms of *E. coli* 0157:H7 infection include; asymptomatic faecal shedding of the organism, bloody diarrhea accompanied by abdominal cramps, vomiting and occasionally fever, haemolytic uremic syndrome [HUS] and thrombotic thrombocytopenic purpura [TTP] [3].

Laboratory rat of the species *Rattus norvegicus* have served as an important animal model for research in psychology, medicine, pharmacology and other fields. A variety of animal species including mice, rabbits, gnotobiotic piglets and albino rats have been used as animal models to study the pathogenesis of enterohaemorhagic *E. coli* infections [8]. Although researches have been carried out on the histopathological effects of *E. coli* 0157:H7 on albino rats, there are few documented information on the *in vivo* effects of the lipoplysaccharide on haematological indices despite the haemorrhagic potential of this pathogen. This work investigates the effects of cell free extracts of *E coli* 0157:H7 on some hematological indices of neonatal rats.

MATERIALS AND METHODS

Resuscitation of *Escherichia coli* 0157: H7 stock culture.
Escherichia. coli 0157: H7 stock culture isolated from faeces of cattle, chicken droppings and human faeces coded ICT, IPB and IHF respectively were used for this study. The stock cultures were inoculated in peptone water [LAB M], incubated at 35^0C for 24hrs.

Confirmatory testing of *E. coli* 0157:H7
Approximately 0.lml of the 24h broth cultures were subcultured on Sorbitol MacConkey agar [BIOMARK] supplemented with cefixime [0.5mg l^{-1}] and tellurite [2.5mg l^{-1}] and incubated at 37^0C for 24h. Presence of colourless to grey Colonies confirms *E. coli* 0157:H7.

Preparation of cell free extracts of *Escherichia coli* 0157:H7.
Pure reactivated Colonies of *E. coli* 0157:H7 were grown separately in 20ml portions of Brain Heart infusion [BHI] broth [Fluka Biochemika] and incubated for 72 hours at 35^0C in a shaker water bath. The broth cultures were centrifuged at 4000rpm for 45 minutes and the supernatants carefully dispensed into sterile universal bottles while the residual cells were discarded. The supernatants were sterilized by membrane filtration using Millipore membrane of 0.22μm pore size and filtrate preserved at 4^0C in a refrigerator

Maintenance of neonatal rats
Fifteen albino Wistar rats from the University of Calabar, Nigeria were used for the study after obtaining all necessary clearance. The rats were sustained with their normal commercially available feed [growers mash] and water *ad libitum* for 2 weeks. Rats weighing between 150 and 280g were used for the experiment.

Fecal examination of neonatal rats
Confirmation of the infection status of the rats was ascertained through rectal swabs enriched in phosphate buffered saline [Fluka Biochemical] supplemented with cefixime [0.5mg l^{-1}] and vancomycin [8.0mg l^{-1}] for 24hours at 37^0C. The enriched samples were cultured on Sorbitol MacConkey agar [BIOMARK Ltd] supplemented with cefixime [0.5mg l^{-1}] and tellurite [2.5mg ml^{-1}]. Absence of gray to white colonies indicated that the rats were not infected with *E. coli* 0157:H7.

Inoculation of neonatal rats with cell free extracts.
Three groups of 4 animals each were housed in plastic cages according to their inoculated isolates namely: ICT, IPB and IHF. Approximately 0.2, 0.5, 1.0 and 2.0ml of the extracts were administered intra-muscularly to each rat in the various groups using 21G and 25G syringes, the fourth group comprising 3 uninoculated rats served as control. After 24h of administration of the extracts, the rats were sacrificed and blood samples collected by cardiac puncture into K_3 EDTA bottles.

Analytical procedures

The haematological analysis for Packed Cell Volume [PCV], Total White Blood Count [WBC], Haemoglobin Count [HBC], Red Blood Count [RBC] and Differential Leucocytes Count were undertaken using Sysmex Kx-21N Haematology Automated Analyzer.

Erythrocytes Sedimentation Rate analysis

This was prepared using the Westergren method [4]. Approximately 0.4ml of sodium citrate anticoagulant was pipetted into a small container and 1.6ml of EDTA anticoagulated blood added and mixed. A Westergren pipette was inserted and positioned vertically using the safe suction method. The blood was sucked to the "O" mark of the Westergren pipette, avoiding air bubbles as possible. After 1h, the level at which the plasma meets the red cells was calculated in mm.

Chi-Square, [X^2] and ANOVA was used for the statistical analysis at probability level of 0.05.

RESULTS

The result of some hematological indices of neonatal rats injected with different doses of cell free extracts of *E. coli* 0157:H7 is presented in Table 1. Significant variations were observed amongst the parameters studied at different filtrate dosages. The highest PCV value of 70.5% was recorded in 0.5ml of IPB isolate and the least PCV of 21.0% was recorded in 1.0ml of IPB isolate.

Statistical analysis showed significant difference in the effects of the filtrate dosage on PCV in the three isolates at $P<0.05$. Total white blood cell count increased with increase in filtrate dosages on the three isolates. The highest WBC value of 3.5 x 10^6 ml^{-1} was recorded in 0.5ml of

Table 1: Some haematological parameters in neonatal rats injected with different volumes of *E.coli* 0157:H7 cell-free extracts

	Haematological Parameters					
Source of Extracts	Volume of Extract [ml]	PVC [%]	WBC [x10^6 ml^{-1}]	Hbc [g dl^{-1}]	RBC [10^9 ml^{-1}]	ESR [mm hr^{-1}]
IPB	0.2	41.0	6.5	14.0	8.5	2.0
	0.5	70.5	3.5	12.0	10.5	2.5
	1.0	21.0	4.2	7.8	4.9	8.5
	2.0	49.4	11.4	11.8	7.5	6.0
ICT	0.2	32.9	2.7	7.9	4.9	9.0
	0.5	48.8	8.2	12.3	8.1	4.0
	1.0	24.0	4.0	8.2	7.4	3.0
	2.0	32.0	8.2	7.7	5.0	6.0
IHF	0.2	47.5	6.0	11.6	7.7	2.5
	0.5	51.8	12.1	12.7	7.3	3.8
	1.0	49.9	12.8	12.0	7.7	3.0
	2.0	48.0	4.3	10.4	7.6	2.8
Control	-	62.1	6.9	13.3	9.4	2.0

PCV – Packed cell volume; ESR – Erythrocytes sedimentation rates; BC – White blood count IPB – Poultry bird faeces isolates
Hbc – Haemoglobin count ICT – Cattle faeces isolates; RBC – Red blood count IHF – Human faeces

Table 2: Differential leucocytes count in neonatal rats injected with different doses of *E. coli* O157:H7 cell-free extracts

		Differential leucocytes count [X10^6 ml^{-1}]			
Source of Extracts	Volume of Extract [ml]	Lymphocytes	Neutrophils	Monocytes	Eosinophils
IPB	0.2	4.60	1.80	0.20	0.10
	0.5	2.80	0.50	0.14	0.04
	1.0	3.20	0.80	0.20	0.04
	2.0	8.10	2.70	0.50	0.11
ICT	0.2	1.51	1.03	0.14	0.03
	0.5	6.30	1.20	0.60	0.10
	1.0	2.20	1.60	1.16	0.08
	2.0	3.80	3.90	0.40	0.10
IHF	0.2	3.70	2.10	0.20	0.10
	0.5	7.50	4.00	0.40	0.20
	1.0	7.30	5.00	0.40	0.30
	2.0	2.80	1.30	0.20	0.04
Control	-	6.10	0.60	0.20	0.10

IPB – Poultry bird faeces isolates; ICT – Cattle faeces isolate; IHF – Human faeces isolate

IPB isolate. There was no significant effect of the filtrate dosages on WBC in IPB and ICT isolates at P>0.05. However, there was significant effects of the filtrate dosages on IHF at P<0.05. Red blood cell count and Hemoglobin count decreased with different filtrate dosages. Moreover, there was no significant effect of the isolates at P>0.05. ESR increased with increase filtrate dosages in IPB isolate but decreased with increased filtrate dosages in ICT and IHF isolates. There was a significant effect of the filtrate dosages on ESR in IPB in ICF isolates at p<0.05 but no significant in IHF at p>0.05.

The results of differential leucocytes count in neonatal rats challenged with different doses of cell free extracts from *E. coli* 0157: H7 is presented in Table 2. There was significant increase in the number of monocytes and neutrophils as filtrate dosages increased in all the isolates. There was significant effects of the isolates at p<0.05. Lymphocytes increased as filtrate dosages increased in ICT and IPB isolates. There were no significant effect of the filtrate dosages on lymphocytes monocytes and eosinophils in all the isolates at p>0.05. Also, no significant difference was observed on the effects of cell free extracts of the three isolates on all the haematological parameters

DISCUSSION

The cell extracts from *E. coli* 0157:H7 had profound effects on the hematological indices of neonatal rats. There was significant reduction in the values of packed cell volume [PCV], when compared with the control. This reduction may be attributed to the destruction of the red blood cells by the extracts resulting in haemolytic anaemia. This agrees with the findings of Cheesbrough [4], who reported that PCV is usually reduced in anemic conditions. However, there were insignificant effects of the filtrate dosages on RBC and HBC which might be because injection of lipopolysaccharide [LPS] into the experimental animals causes a wide spectrum of nonspecific pathophysiological reactions. Injection of fairly small doses of LPS may cause death in most animals; however, small doses of extracts administered were enough to elicit a wide range of biological effects on RBC and WBC as confirmed by Brooks and Antai [1]. Corresponding increase in WBC as filtrate dosages increase indicates that the extracts triggered the release of host immune defensive mechanisms thus increasing the number of leucocytes in circulation on the activation of immune and inflammatory responses by the antigen [endotoxin]. There was significant increase in erythrocyte sedimentation rate [ESR], as filtrates dosages increase in IPB probably because the extracts might have triggered high body temperature leading to raised ESR. This result agrees with the report by Cheesbrough [4] and Brook *et al*. [2] who highlighted factors that contributed to raised ESR to include acute and chronic inflammatory conditions, high body temperatures above 15^{o}C and presence of endotoxins

In ICT and IHF isolates, ESR decreased with increase in filtrate dosages probably because of the suppression of the immune response to the high dosages of the extracts. Significant increases in neutrophils counts were not unexpected because neutrophils play a key role in cell mediated immunity. Neutrophils and monocytes are the first leucocytes attracted to an infection sites by chemotactic factors [11]. Increase in lymphocytes as filtrate dosages increase in ICT and IPB might be because large lymphocytes predominate in children [4], accounting for numerous lymphocyte counts.

Insignificant difference observed in all the parameters in the three isolates suggests that *E. coli* 0157:H7 pathogenicity profile is identical no matter the source. The significant difference observed in the hematological parameters treated with different filtrate dosages of the extracts is probably because the extracts elicited a wide range of biological effects on the parameters studied. It is therefore possible that higher doses of extracts administration may exert higher toxicity effects. Lipopolysaccharide therefore plays a major role in the pathogenesis of *Escherichia coli* O157:H7 infections.

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