

Escherichia coli Generate Oxidative Stress and Enhance Lipid Peroxidation in the Kidney of the Rat

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Abstract

The urinary tract is generally infected by *Escherichia coli* bacteria. They commonly originate in the patient's own bowel, and infection occurs mostly via the ascending route. In this study, we have observed the influence of *E. coli* on oxidative stress generation and lipid peroxidation in the kidney of the rat. *E. coli* were taken from the soil, urine, buffalo intestine and goat intestine for this study. Rats were infected with isolated *E. coli* from different sources and Lipid peroxidation, glutathione assay was done to achieve the goal of this study. Percentage survival data showed that the *E. coli* isolated from urine had more lethargy phenomena because their survival present was 66.66% and the mortality rate was higher in this group. Although *E. coli* isolated from Goat intestine has also shown the same mortality, but the *E. coli* isolated from urine sample shown this mortality from the second day whereas the *E. coli* isolated from goat intestine from the third day. By and large data indicated that the *E. coli* isolated from urine sample generated high oxidative stress and damage rat kidney because the kidney is an organ frequently exposed to oxidative stress. Overall, free radical generated due to *E. coli* infection further enhances lipid peroxidation in the rat which is harmful to the physiology because it may cause a renal disorder in the rat.

Keywords: *E. coli*; Virulence; Rat; Kidney; Lipid peroxidation

Introduction

Our gastro-intestinal tract is a biologically diverse and complicated system which contains numerous bacterial species. Intestinal microbes harbor a diverse microbial community, often containing opportunistic bacteria with virulence potential [1]. Both the host and bacteria are thought to derive benefit from each other. While most of the activities of the normal flora benefit their host, some of the normal flora are parasitic (live at the expense of their host), and some are pathogenic (capable of producing disease) [2]. Diseases that are produced by the normal flora in their host are called endogenous diseases. Most endogenous bacteria are opportunistic in infection [1]. Among endogenous bacteria, *Escherichia coli* are generally harmless for humans but some of them are parasitic and may cause life dangerous complication like a severe kidney damage function or even sepsis. *E. coli* may live for months in water and ground but they get the opportunity they multiply very quickly and cause digestion problems [3,4]. We can describe *E. coli* as opportunistic bacteria only in the specific situation when it arrives from the intestine to other organs and tissues and causes illnesses by producing Shiga toxin [5,6]. The most frequent are urinary tract and sexual organs infections. *E. coli* outbreak has killed many persons in the USA in and other parts of the world [7,8]. Virulence factors of *E. coli* are studied and reported in different population by many research groups [9-13]. *E. coli* infection was also reported in domestic animals like rabbits, cats, dogs, goats and horses [14]. The main site of *E. coli* infection is kidney and their infection is spread through blood [15]. Therefore in this study kidney and serum burden was measured after total dosages regimen (infection time period) and data of their dilution series showed that kidney and blood burden was higher in buffalo having *E. coli* infection. The sources of *E. coli* were soil, urine, buffalo intestine and goat intestine. This study was designed to investigate the antibiotic resistance, serum analysis and virulence of *E. coli* (isolated from the different source) in the rat.

Materials and Methods

Concept design of the study

The present study was emerged from isolation of *E. coli* from soil sample, urine sample, goat (G) and buffalo (B) intestine. They were collected locally and stored at -20°C until use.

Isolation of *E. coli* bacteria

Samples were firstly inoculated in 1% tryptone broth for 24 hours for the isolation of *E. coli* from different sources mentioned above. Then the broth was again inoculated on Mac-konkey Agar media for 24 hrs. Then the pink colonies were selected and again transferred on EMB agar media for 24 hrs. The metallic shine colonies were selected and stored in LB agar media for at 4°C until use.

Biochemical tests for identification of bacteria

Urease test: Urea agar medium and urea broth were prepared and sterilized by autoclaving at 121°C. Media was poured into the tubes and allows them to solidify in a slanting position to form slopes. The tubes were labeled with the name of the inoculating organism. Inoculate the liquid and agar media with a transfer loop. Incubate the slants/broths for 24-48 hrs at 37°C.

Carbohydrate catabolism by microorganisms test: Glucose

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<i>E. coli</i> from different sources	Gentamycin	Erythromycin	Chloramphenicol
Soil	20 ± 1.81	20 ± 1.63	22.5 ± 1.92
Urine	23 ± 1.90	33.5 ± 2.72	26.5 ± 2.23
Buffalo intestine	22.5 ± 1.68	22 ± 1.69	21 ± 1.64
Goat intestine	24 ± 1.91	22.5 ± 1.79	23.5 ± 1.87

Table 1: *E. coli* isolated from different source showing the zone of inhibition (in mm) against few antibiotics.

Source of isolated <i>E. coli</i>	Dosing period	Infection of <i>E. coli</i>	Total no. of animals	Survived animals	Dead animals	% Survival
Control	3 days	PBS solution	6	6	0	100%
Soil	3 days	1 ml of <i>E. coli</i> broth	6	6	0	100%
Urine	3 days	1 ml of <i>E. coli</i> broth	6	4	2	66.66%
B. Intestine	3 days	1 ml of <i>E. coli</i> broth	6	6	0	100%
G. Intestine	3 days	1 ml of <i>E. coli</i> broth	6	5	1	88.88%

Table 2: Surveillance of infected rats with *E. coli* isolated from different sources.

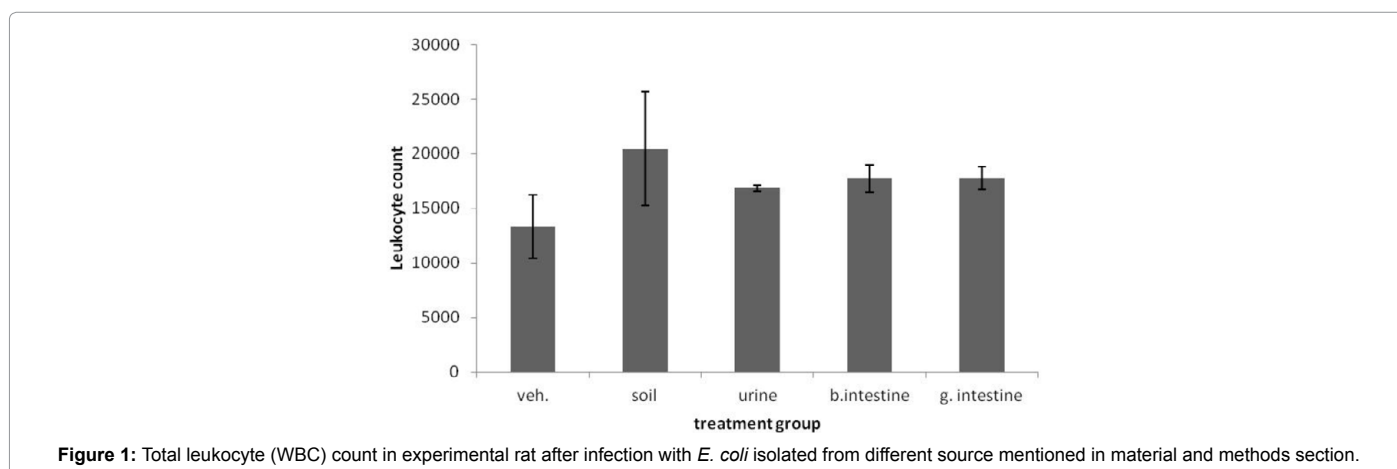


Figure 1: Total leukocyte (WBC) count in experimental rat after infection with *E. coli* isolated from different source mentioned in material and methods section.

media was used to identify the oxidative and fermentative abilities of microorganisms. Acid end products were generated, pH was lowered and the bromthymol blue indicator was turned yellow.

IMVIC tests: Indole methyl red Voges-Proskauer citrate (IMVIC) test was under taken to identify the *E. coli* in frequent form.

Animals study

Swiss albino rats of either sex weighing 100 ± 5 gm were selected randomly from the animal house of Madras Veterinary College, Chennai, India. They were housed in a controlled room with a 12 h light dark cycle at the room temperature of 22 ± 2°C, humidity 30% to 60%. They were kept on standard pellet diet (Lipton India Ltd.). Animal maintenance and handling were in accordance to the internationally accepted standard guidelines of Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). Experiments were conducted according to the guidelines and ethical norms, approved by ministry of social justice and empowerment, government of India. Six rats were taken for each *E. coli* sample isolated from different sources. 10⁹ CFU/ml broth cultures of *E. coli* strains isolated from different sources were given to experimental animals.

Leucocytes counting

It was done by using following formula:

$$WBC/\mu L = \frac{\text{Average of cells} \times \text{Correction for dilution}}{\text{No. of squares counted} \times \text{Volume of one square}}$$

Biochemical assays

Lipid per oxidation (LPO): MDA, an end product of LPO reacts

with TBA to form a colored substance. Measurement of MDA by TBA reactivity is the most widely used method for assessing LPO. Lipid peroxidation was measured via the amount of liberated malondialdehyde (MDA) in the system that assayed the pink product, as reported earlier [16]. The colored end product was read at 540 nm.

Glutathione (GSH) measurement: Cellular GSH activity was measured according to method of Paglia and Valentine et al. [17]. The rate of change of absorbance during the conversion of NADPH to NADP was recorded using Shimadzu spectrophotometer at 340 nm for 3 min.

Statistical analysis

Results were expressed as mean ± SD (standard deviation). Three sets of experiments were performed and the results were analyzed by One-Way ANOVA (analysis of variance) test. The upper level of significance was chosen as P<0.05 (Significant).

Results

Pure culture of isolated *E. coli* from different sources was tested with three antibiotics (Gentamycin, Erythromycin, and Chloramphenicol) to study the inhibition effects. The result is depicted in Table 1. Maximum inhibition was observed by Erythromycine (33.5 mm) followed by Chloramphenicol (26.5 mm) for urine isolates cultures. Gentamycine inhibited more to the goat intestine isolate (24.0 mm). Data surveillance of test animal (Table 2) relieved that out of six animals tests for each group of culture only two were found dead by urine isolate and only one were found dead by goat intestine isolated culture whereas other culture did not cause dead and showed 100% survival. Variation in

S. No.	Infection of <i>E. coli</i> isolated from different sources	Serum burden (number of <i>E. coli</i> ± SD)	
		First dilution	Second dilution
1	Control	1.00 ± 0.29	0.00 ± 0.00
2	Soil	5.00 ± 1.31	3.00 ± 0.96
3	Urine	2.00 ± 0.48	1.00 ± 0.33
4	B. intestine	693.00 ± 80.27	420.00 ± 74.09
5	G. Intestine	17.00 ± 5.24	09.00 ± 3.38

Table 3: *E. coli* burden in blood serum of rat.

S. No.	Infection of <i>E. coli</i> isolated from different sources	Serum burden (number of <i>E. coli</i> ± SD)	
		First dilution	Second dilution
1	Control	2.00 ± 0.61	0.00 ± 0.00
2	Soil	101.00 ± 29.64	33.00 ± 13.19
3	Urine	213.00 ± 54.79	122.00 ± 27.41
4	B. Intestine	240.00 ± 61.52	50.00 ± 17.13
5	G. Intestine	169.00 ± 36.74	140.00 ± 31.18

Table 4: *E. coli* burden in kidney of rat.

S. No.	Infection of <i>E. coli</i> isolated from different sources	LPO level (nM/mg protein ± SD)
1	Control	0.54 ± 0.23
2	Soil	1.36 ± 0.83
3	Urine	4.09 ± 0.38
4	B. Intestine	1.69 ± 0.79
5	G. Intestine	2.52 ± 0.30

Table 5: LPO level in rat infected by *E. coli* isolated from different sources.

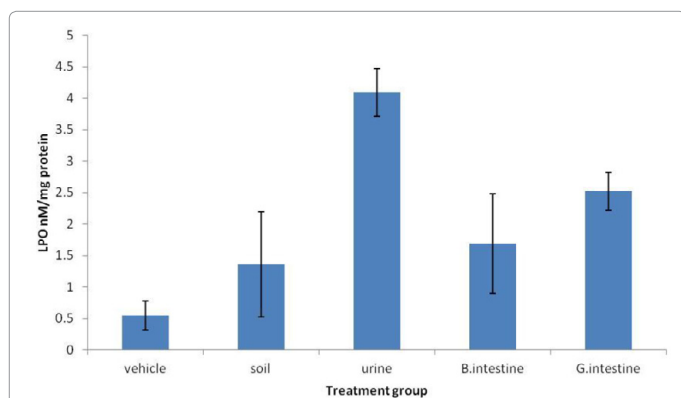


Figure 2: Lipid peroxidation in infected rat showed that there was increase in LPO in treating group and it was found significant from control at $P < 0.05$.

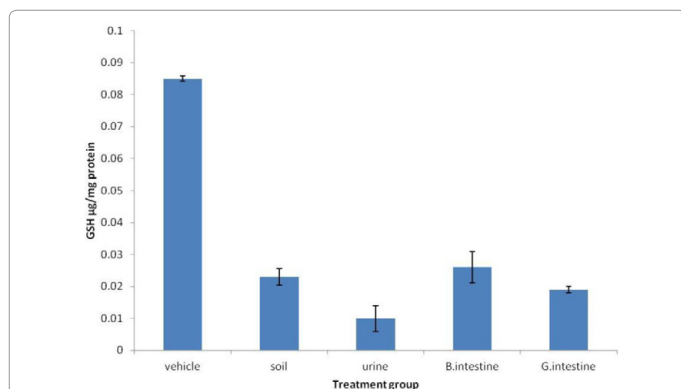


Figure 3: Glutathione measurement showed that there was decrease in GSH level in treated rat and significant from control at $P < 0.05$.

white blood cells (WBC) count is one of the important parameters to be assessed when body get infected by any type of microorganism. As immunity of body starts fighting against pathogens, WBC count starts varying. WBC counting of all groups has revealed that infection was higher in *E. coli* treated group in compare to control group means WBC counting increased phenomena showed infectious phenomena. Here data of WBC counting showed that isolated *E. coli* had a property to induce disease but these data were not sufficient to differentiate their mortality. Data on WBC count in test animal is depicted in Figure 1.

Kidney and blood burden of *E. coli* isolated from urine was very low but with the respect of lethargy phenomena it is comprehended that *E. coli* isolated from urine was more virulence because their few amount were sufficient to produce disease condition. Presences of *E. coli* in the blood (Table 3) has revealed that the maximum *E. coli* cells were observed in the sample isolated from the buffalo intestine (B) in both dilution followed by samples isolated from the goat intestine (G) and least from soil and urine samples. The burden of *E. coli* in the kidney due to the *E. coli* infection was estimated (Table 4). Here minimum *E. coli* burden was observed from samples isolated from soil and maximum was in buffalo intestine (B).

E. coli is considered as the normal bowel flora of different species of mammals and birds but some strains of *E. coli* possess pathogenic character due to the acquisition of virulent factors. Microbial characteristics associated with virulent *E. coli* include the production of Enterotoxin, Verotoxin, Colicins and Siderophores, type-1 pili and motility, resistance to the lytic action of the host complement and antibiotics. Lipopolysaccharide (LPS) or endotoxins are the principal components of gram-negative bacteria like *E. coli*. It has been extensively studied as a major factor contributing to the pathogenesis of Gram-negative bacterial infections. Endotoxin is a highly conserved cell wall component that is recognized by the immune system of higher vertebrates as a pathogen-associated molecular pattern (PAMP) and can elicit a systemic inflammatory response. The LPO and GSH estimation (Table 5) (Figures 2 and 3) were found significantly different from control at $P < 0.05$. Here maximum LPO was determined as 4.09 due to treatment with urine samples whereas maximum GSH level was found due to treatment with buffalo intestine samples among the other treatments. With the above-mentioned result, the kidney and serum burden was measured with regimens showed more virulence to cause infection. The increase in LPO and decrease in GSH in treating group cause increase oxidative stress in the kidney that may causes the renal disorder and physiological misbalance.

Discussion

Due to reactive oxygen species, lipid peroxidation occurred and free radical of PUFA (poly unsaturated fatty acid) generated and cell damage is continuing by the effect of the ROS. One approach to combat the bacterial induced oxidative stress is the use of antioxidant treatments through intraperitoneal, intravenous, or dietary administration. In this study, LPO level was increased in all treated group of rat and among them LPO level was higher in the group which was infected with *E. coli* isolated from the urine sample and decreased GSH content. Further the study indicated the increased oxidative stress in treated group and this type of oxidative stress was higher in that group which was infected with *E. coli* isolated from the urine sample. Serum examination was done after infection of *E. coli* isolated from the different sample of soil, urine, buffalo intestine and goat intestine and bacterial burden was high as compared to control (Tables 4 and 5). In all the groups, over one-fourth of the kidneys were infected. Bacteria persisted in high numbers in the infected kidneys. The pathological change observed

in the infected kidneys may be due to the infiltration of the pelvic epithelium and sub epithelial connective tissue by mononuclear cells and polymorphonuclear leukocytes. With the above mentioned data (result section), the kidney and serum burden of *E. coli* was measured with regimens showed more virulence to cause infection the antibiotic effect on *E. coli* are also measured. The increase of LPO and decrease in GSH in treating group may cause increase oxidative stress in the kidney (Figures 2 and 3).

Conclusion

This study showed the effect of *E. coli* infection in terms of perturbation of LPO and GSH in experimental animal. Peroxidation of lipids disturbs the integrity of cell membranes and leads to rearrangement of membrane structure. They produce adverse modifications to cell components, such as lipids, proteins, carbohydrates, DNA and alter physiological functions.

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