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# EFFECT OF CAMPYLOBACTER COLI LPS ON PHAGOCYTOSIS IN VITRO AND IN VIVO

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## ABSTRACT

*Campylobacter coli* usually live in the intestinal tract of animals and cause inflammation of the intestine and diarrhea. In this study, *C. coli* were isolated from patients having diarrhea and identified based on morphological and biochemical characteristics. Lipopolysaccharide (LPS) is major outer membrane structural component of gram negative bacteria which induces a broad range of biological response. Here, LPS was isolated with hot phenol-water extraction and purified by gel filtration using Sephadex G-200. Phagocytosis is essential for fighting infections in immune responses to protect organisms from bacteria invasion hence we studied the effect of purified LPS on phagocytosis both *in vitro* and *in vivo*. The results reported in this study may prove to be valuable in analyzing the LPS effect on host immune response.

**KEYWORDS:** *C. coli*, LPS, Phagocytosis**.**

## INTRODUCTION

Campylobacter species are among the most commonly reported bacterial cause of acute gastroenteritis disease worldwide and especially in the industrialized countries.[1, 2] Approximately nine million human campylobacteriosis cases are reported annually in 27 European countries.[3] Although most of the human campylobacterios is caused by *C. jejuni*, the importance of *C. coli* study is being recognized due to an increasing resistance of this species to number of antimicrobials.[4] Most Campylobacter research focuses upon *C. jejuni* but still campylobacteriosis caused by *C. coli* infection results in millions dollars of annual cost.[5, 6]

Livestock wastes from intensive animal production such as manure or slurry contain pathogenic microorganisms including *Campylobacter* species.[7] It has been reported that drinking water may be the source of *C. coli* infection in grandparent breeder farms.[8] This has increased concern about effect of pathogens in animal manure on human and animal health. [9] For *Campylobacter* motility is important for intestinal colonization and invasion of epithelial cells. In *Campylobacter* species flagella function as secretory organelles and recognized as a major virulence factor for this pathogen.[10, 11] It is reported earlier that the flagellar structural protein (FlaA) is immunoprotective antigen,[12, 13] flagellins are heavily glycosylated proteins and are highly immunogenic.[14, 15]

Macrophages play an important role in recognition, uptake and killing of parasites during pathogen infections.[16] It has been reported that LPS activates several signal transduction pathways in macrophages leading to various gene activation and cytokine production.[17, 18] In this study we have isolated LPS from *C. coli* and detected its effect on Phagocytosis using human macrophages: both *in vivo* and *in vitro.*

## MATERIALS AND METHODS

### Isolation of *C. coli*

*C. coli* were isolated from diarrhea samples collected from Al-Yarmok Hospital Baghdad, Iraq. The serially diluted samples were inoculated on blood agar containing 5 % SRBCs and other supplement and incubated for 48 hours at 4 $^{0}$C under micro aerobic conditions. The single isolated colonies were identified by morphological characteristics, gram Staining and biochemical tests.

### Extraction of LPS from *C. coli*

The LPS was extracted from *C. coli* as described previously by Preston and Penner.[19] Briefly digestive enzymes were used to isolate LPS and hot phenol method was used for extraction process. The extracted LPS were then purified by gel filtration chromatography using Sephadex G-200 as described by manufacturer.

### Blood sample preparation

Two milliliter of blood samples was collected from twenty healthy persons in heparinized (50 IU/ml) silicon test tube to prevent the absorption of cells on glass tubes (according to WHO guideline). These Blood samples were directly used to evaluate phagocytosis.

### *In vitro* phagocytosis assay

The *in vitro* phagocytosis was determined by procedure described by Sun.[20] Briefly, monocytes were added into the wells and after 6 h of incubation the floating cells were removed and monocytes were washed twice with PBS. Under the phase microscope, about 300 macrophages per sample including LPS engulfed were counted and the percentage ratio was calculated as the relative number of cells that engulfed LPS per total cells.

**_In_ vivo phagocytosis assay**

The *in vivo* phagocytosis was estimated as described by Tanaka.[21] Briefly LPS was diluted 10-fold with PBS and injected into the mouse peritoneal cavity by 10 µl/g body weight. After overnight starvation the mice were sacrificed and macrophages were recovered from Peritoneal, Liver and Spleen. For each sample 400 cells were counted, and the percentage ratio was calculated as the relative number of cells that engulfed LPS per total cells.

## RESULTS AND DISCUSSION

*C. coli* were isolated from patients having diarrhea. The morphological characteristics such as colony morphology and motility were observed and gram staining with biochemical tests such as oxidase and hiturate were performed for identification of *C. coli*. The observed results were summarized in Table 1.

**Table 1: Biochemical test and characterization of *C. coli***

| Test | Result |
|---|---|
| Colony on blood agar | Appear small convert grey white colony |
| Gram staining | Negative (Gram negative bacteria) |
| Oxidase test | Positive |
| Hiturate test | Hydrolysis negative |
| Motility | Motile |
| Shape | Spiral shape |

LPS are heat stable endotoxins and long been known to induce variety of pathophysiologic responses, including fever, coagulant activity, septic shock, and death. [22] Here, we have used hot phenol method to extract LPS from the isolated *C. coli*. To remove the impurities such as protein and RNA, extracted samples were further purified by Size exclusion chromatography using Sephadex G 200. After purification absorbance was measured at 490 nm for collected fractions (Figure 1). The fraction showing maximum absorbance was used for further phagocytosis study.

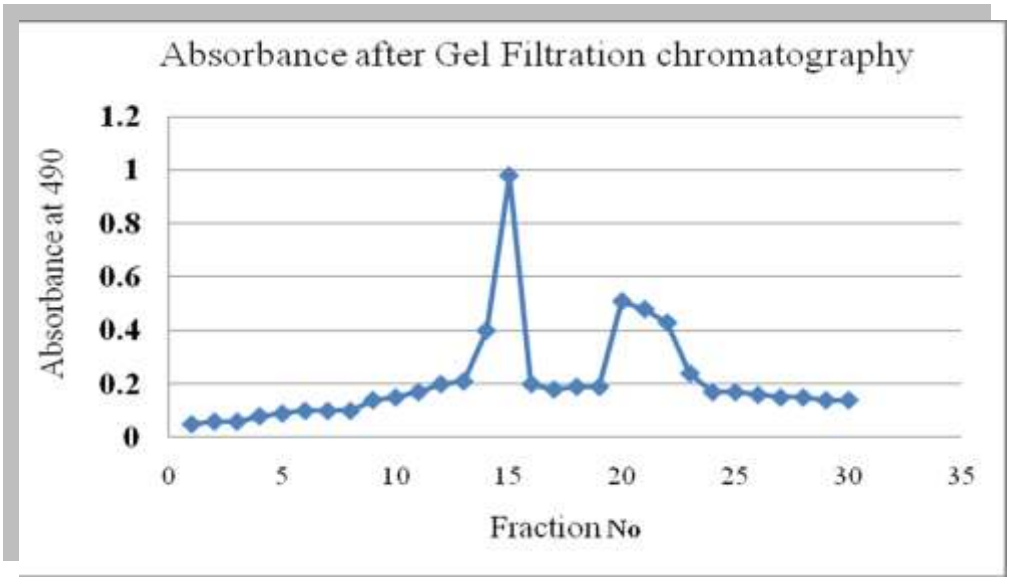


**Figure 1: Purification of LPS by gel filtration (Sephadex G-200)**

Phagocytosis is controlled by a complex signals located on the target cell surface and their respective receptors which determine phagocytosis process. [23] Many approaches have been designed to study these processes using macrophages as prototypic inflammatory cells, since these cells are potently activated by LPS. [24] In present investigation we studied phagocytosis both *in vivo* and *in vitro.* The percentage ratio was determined for phagocytosed LPS and presented in figure 2 for in *vitro* study and in figure 3 for *in vivo* study.

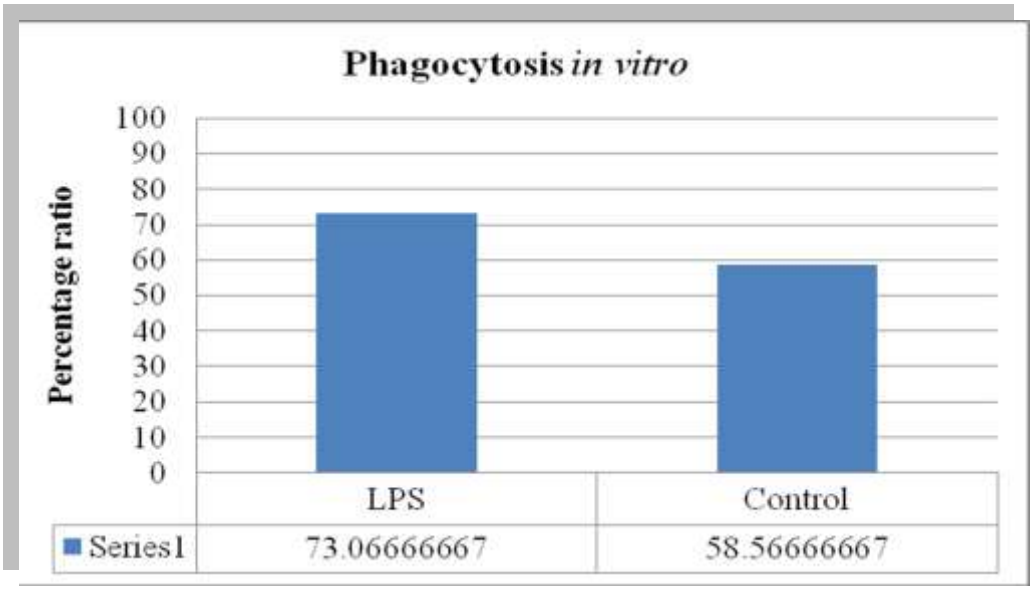


**Figure 2: Phagocytosis *in vitro***

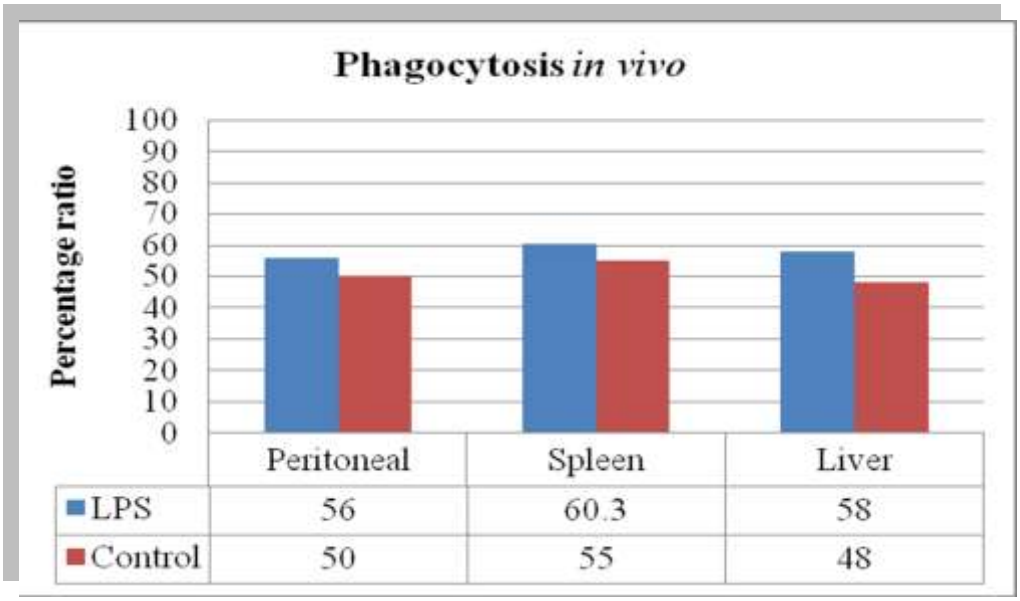


**Figure 3: Phagocytosis *in vivo***

In presence of LPS increased phagocytosis was observed in both *in vitro* and *in vivo* studies as compared to control which indicates that LPS exposure required for phagocytosis progress. The increased phagocytosis reported in this study may prove to be valuable in analyzing the alterations induced by LPS on macrophage involved in the host response.

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