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ESTIMATION OF LD_{50} AND ID_{50} DOSES OF *SALMONELLA TYPHIMURIUM* INOCULATED EXPERIMENTALLY IN MICE

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ABSTRACT

Salmonella is a significant foodborne pathogen worldwide. This study was studied in vivo to estimate the lethal dose and infective dose of *Salmonella typhimurium* isolated from a child suffering from diarrhea, using mice white (BALB/c) of both sexes with an age range from six to eight weeks old, which administrated orally. The mice were observed daily for thirty days, the rested forty-eight mice divided randomly into eight groups each with six mice. The seven groups of mice administrated orally with one of the calculated (CFU/ml) diluents by using needle gavage about (0.5ml) and the eight groups administrated phosphate buffer saline (PH=7.2) and deemed as a control group. The result evidenced the LD_{50} was ($1x10^8$ cells) and infective dose (ID_{50}) was ($1x10^6$ cells).
***Keywords*:** *Salmonella typhimurium*, lethal dose (LD_{50}), infective dose (ID_{50})

Introduction

Salmonella spp. are the most common causes of foodborne illness in humans and animals (Takele *et al.*, 2018). Nontyphoidal *Salmonella enterica* (NTS) diseases are a major load to global common health, as they lead to infections ranging from gastroenteritis to the systemic diseases and there is presently no vaccine available (Ferreira *et al.,* 2015), virulence of those facultative intracellular pathogens is subject on their ability to infest and proliferate within non-phagocytic cells (DeLeo and Otto, 2008). *Salmonella* spp. poses a threat to both human and animal health, with higher than 2600 serovars having been recorded to date (Gong *et al,* 2016). Systemic infections are serious manifestations for salmonellosis, to facilitate systemic infections, intracellular *Salmonella* existing in the immune cells like macrophages and dendritic cells (DCs) may be transported from the intestinal tracts to other regions of the body(Sundquist *et al*., 2004; Shallal, 2016). *Salmonella enterica* serovar *Typhimurium* is foodborne pathogens causing inflammatory diseases in the intestinal tract following diarrhea and is in charge of thousands of deaths worldwide (Schulte and Hensel, 2016). So this study was designed to evaluate the LD_{50} and ID_{50} by using white BALB/c mice as an animal experimental model.

Materials and Methods

Bacterial isolates:

Salmonella typhimurium isolate was provided by the public health Zoonotic Diseases/ College of Veterinary Medicine/ University of Baghdad. From 6 years old child suffered from diarrhea for more than 7 days.

Experimental mice:

The study was carried out in the animal house in the Veterinary college/ University of Baghdad in Iraq A total of 48 mice (BALB/c) of both sexes with age from 6 to 8 weeks old, were used in the present study were adapted for two weeks before started the experiment by reserved in separated clean and sterilized cages, they were fed on pellets and cleaned water. Then divided randomly into 8 groups each group with 6 mice. The seven groups of mice administrated orally with one of the calculated (CFU/ml) diluents by using a stomach tube about (0.5ml) and the eight groups administrated PBS (pH=7.2) and mediated as a control group. All groups were noted for 30 days to account for live and dead mice and determine the lethal dose according to (Reed and Muench, 1938).

Details concerning experiments as follow:
Estimation of lethal dose (LD_{50})

Each five colonies of *Samonella Typhimurium* was inoculated in the (10ml) of the Brain heart infusion broth situated at 37 ˚C for (18hrs) then centrifuged in the cooling centrifuge (8000rpm) round per minute for (15minutes) then the pellet later washing three times with phosphate buffer saline (PH=7.2) and suspending by using (1ml) of PBS (PH=7.2). The suspension was diluted by ten-fold dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 1111 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}). The viable count of bacteria in each diluent was formed according to the manner of (Miles & Misra, 1938).

Statistical analysis:

Chi square was administered to decide the statistical differences among tested groups by applying SPSS statistical program.

Results

The result of lethal dose (LD_{50}) and infective dose (ID_{50}) in mice was ($1x10^8$ CFU/ml) and ($1x10^6$ CFU/ml) respectively which estimated by observant the dead and live mice in each group during 30 days of the experiment showed in a table (1).

Table 1: Estimation of LD_{50} & ID_{50} of *Salmonella typhimurium* isolated from human child diarrhea in mice.

Groups	Dose (Cells)	Observed values		Accumulated values		Mortality (%)
		Live	Dead	Total live	Total dead	
1	$1x10^{11}$	0	6	0	21	100
2	$1x10^{10}$	0	6	0	15	100
3	$1x10^{9}$	2	4	2	9	81
4	$1x10^{8}$	3	3	5	5	50
5	$1x10^{7}$	4	2	9	2	18
6	$1x10^{6}$	6	0	15	0	0
7	$1x10^{5}$	6	0	21	0	0
8	PBS	6	0	-	-	0

No. of mice in each group=6 Total No. of mice=48

The percentage of deadness was calculated according to the manner of (Reed &Munch, 1938).

Discussion

The infective dose of *Salmonella typhimurium* was approached to that referred by (Blaster & Newman, 1982) which mentioned that the infective dose range between 10^5-10^{10} cells.

The result of the lethal dose (LD_{50}) in the experimentation of this study compatible with a study of (Al-saadi, 2013) who listed the LD_{50} of *Salmonella Hadar* in the mice was ($1x10^8$ CFU/ml). The LD50 of this study was high dose when equated with that referred by (Yousif, 2000) and with (Al-Hashimi, 2005) who recorded the LD_{50} of *S.enteritidis* in mice was ($1.4x10^6$ CFU/ml). Other studies listed high LD_{50} number such as (Al-Mansory, 2009) who establish that LD_{50} of *Salmonella enteritidis* in the rabbit was ($2x10^{10}$ CFU/ml) and with (Al-Naqeeb, 2009) who found that the LD_{50} of *Salmonella Hadar* in mice was ($1.5x10^9$ CFU/ml) and also with (Shallal, 2011) who recorded the LD_{50} of *Salmonella mbandaka* in mice was ($1.3x10^{9.5}$ CFU/ml).

Conclusion

It could be concluded those data showed that it requires a very low number of microorganisms to cause diseases in young children, the older and immune-compromised persons. As it is apparent from the result noted above, *Salmonella typhimurium* did not vary from other nontyphoidal *Salmonella* spp. for this study involved criteria, which means that *Salmonella typhimurium* have the like virulence for the mice administrated orally.

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