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RESEARCH ARTICLE

ESTIMATION LETHAL DOSE OF SALMONELLA MBANDAKA INOCULATED EXPERIMENTALLY IN MICE.

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Abstract

Salmonella is an important food borne pathogen worldwide. This study was intended for in *vivo* to estimation lethal dose of *Salmonella mbandaka* isolated from human infantile diarrhea by calculating the lethal dose (LD_{50}) , using mice (BALB/c) of both gender with age range from 6 to 8 weeks old, which drenched orally . The mice were monitored daily for a maximum of 30 day , the rested fourty two mice were divided randomly into seven groups each of its have 6 mice. The six groups of mice inoculated orally with one of the calculated (CFU/ml) diluents by using polyethylene tubes about (0.5) ml and the seven group inoculated Phosphate Buffer Saline (pH=7.2) and considered as a control group. The lethal dose (LD_{50}) of *S. mbandaka* in mice was ($1.3 \times 10^{9.5}$ cells / ml).

Introduction:-

Nontyphoidal *Salmonella* enterica (NTS) infections are a major burden to global public health, as they lead to diseases ranging from gastroenteritis to systemic infections and there is currently no vaccine available (Ferreira *et al* ; 2015) . *Salmonella* spp. pose a threat to both human and animal health, with more than 2600 serovars having been reported to date (Gong *et. al*, 2016). *Salmonella enterica* serovars cause a variety of diseases ranging from self-limiting gastroenteritis to severe systemic infections. ,virulence of these facultative intracellular pathogens is dependent on their ability to invade and replicate within non-phagocytic cells (DeLeo and Otto, 2008). In vivo Boyle *et . al* ,(2016) referred *Salmonella typhimurium* cause system accurately modeled key aspects associated with *Salmonella enteritis* perfused rat small intestinal model, as well as dynamic changes to smooth muscle activity, metabolic competence, and luminal fluid accumulation during short-term infection with the enteropathogenic bacteria . Systemic infections are severe manifestations of salmonellosis; to facilitate systemic infection, intracellular *Salmonella* present in immune cells such as macrophages and dendritic cells (DC) may be carried from the intestinal tract to other areas of the body(Sundquist *et al*., 2004). Moreover, the histopathological changes in experimentally in mice inoculated orally with (1.3×10^{7} cells / ml) of *Salmonella mbandaka* have been reported previously (Shallal *et. al*, 2013). However , clinical signs and gross pathological changes haven't presented. Therefore, this study was designed to study the clinical, bacteriological and gross pathological aspects (Shallal *et. al*, 2015). The intraperitoneal route was better than oral route in inducing infection , this may interpret by presence of several barriers in the gastrointestinal tract such as intestinal acidity, competitive by normal flora, secretory IgA and other barriers but in intraperitoneal route, there were fewer barriers, so large numbers of bacteria must be inoculate orally to induce both infection and death in mice (AL-Qaisi , 2004) .

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Material and methods:-

Bacterial isolates:-

Salmonella mbandaka strain from diarrheic child was used for inducing infection, the isolates was obtained from Zoonosis unit in Veterinary College /University of Baghdad (Shallal *et. al* , 2015) and isolated according to the standard method according to (Quinn *et. al*, 2004). This isolate was serotyped in the Central Public Health Laboratories (National Center of *Salmonellae* in Baghdad).

Experimental mice:-

The study was carried in the experimental house in the science college of wasit university in Iraq A total of 42 mice (BALB/c) of both gender with age range from 6 to 8 weeks old, were used in this study. The mice were obtained from the (National Centre of Researches and Drugs Monitor in Baghdad) and adapted for two weeks before experiments. Bacteriological examination showed that the mice were ngative for *Salmonella* at the beginning of the study. Then divided randomly into 7 groups each with 6 mice. The six groups of mice drenched orally with one of the calculated (CFU/ml) diluents by using polyethylene tubes about (0.5) ml and the seven group drenched PBS (pH=7.2) and considered as a control group. . All groups were observed for 30 days to calculate the live and dead mice and determine lethal dose according to (Reed and Muench, 1938) .

Details regarding the experiments are as follow:-

Determination of lethal dose (LD_{50})

Each five colonies of *S. mbandaka* was inoculated in (10 ml) of Brain heart infusion broth at 37 ˚C for (18) hours then centrifuged in cooling centrifuge (8000) rpm (round per minute) for (15) minutes then the sediment (pellet) after washing three times with PBS (pH=7.2) was suspending by using (1) ml of PBS (pH=7.2) and ten fold dilution (10^{-1},10^{-2}.10^{-3},10^{-4},10^{-5},10^{-6},10^{-7},10^{-8},10^{-9} and 10^{-10}) were done. The viable count of the bacteria in each diluent was made according to method of Miles and Misra ,(1938) and dilution which contain ($1.3\times10^{9.5}$ cells /ml) was consider as lethal dose .

Ethical improvement:-

This study was approved by the ethical and research committee of Veterinary Medicine College/University of Baghdad and Science College /University of Wasit .

Statistical analysis:-

Chi square was conducted to determine the statistical differences among the tested groups by using SPSS statistical program (ready–made statistical design).

Result and discussion:-

The results of this study revealed that the lethal dose (**LD_{50}**) of *Salmonella mbandaka* in mice was ($1.3\times10^{9.5}$ cells). The LD_{50} was estimated by calculating the dead and alive mice in each group during (30) days of the experiment (Table 1).

Table 1:- Calculation of LD_{50} of *Salmonella mbandaka* isolated from human in mice .

Groups	Dilution of bacteria	Dose (cells)	Observed values		Accumulated values		Rates	
			Dead	Live	Total dead	Total live	Fractional ratio	Percent ratio
1	10^{-2}	1.3×10^{11}	6/6	0/6	14	0	14/14	100%
2	10^{-3}	1.3×10^{10}	4/6	2/6	8	2	8/10	80%
3	10^{-4}	1.3×10^{9}	3/6	3/6	4	5	4/9	44%
4	10^{-5}	1.3×10^{8}	1/6	5/6	1	10	1/11	10%
5	10^{-6}	1.3×10^{7}	0/6	6/6	0	16	0/16	0%
6	10^{-7}	1.3×10^{6}	0/6	6/6	0	22	0/16	0%
7	PBS	-	0/6	6/6	0	28	0/16	0%

No. of mice in each group = 6 **Total No. of mice = 42**

The percentage of mortility was calculate according to the method of Reed and Munch, (1938).
Proportional distance = % mortality above 50% - 50% / % mortality above 50% - mortality below 50% .

The lethal dose of *Salmonella mbandaka* was ($1.3\times10^{9.5}$ cells / ml) obtained in this work , indicated that the strong of virulence of this isolate as it cause localized infections . The rout of infection in the present experiment was inoculated orally . The result was very large dose when compared with that mentioned by Yousif, (2000) and Al-Hashimi, (2005) which referred that the LD_{50} of *S. typhimurium* and *S. enteritidis* in mice were (2×10^6 CFU/ml) , (1.4×10^6 CFU/ ml) respectively . Mikula *et al*., (1988) found that post oral infection of calves with *S. typhimurium* 4/5 strain at a dose (1×10^6 C.F.U./ml), there was discontinuous and irregular of the brush border membrane of jejunal enterocyte. In contrast, this result with Shallal , (2013) who injected an intra-peritoneal (I.P) BALB /c mice for typical (*eae A+, bfp+)* and atypical (*eae A+, bfp-)* enteropathogenic *Esherichia coli* reported LD_{50} of them were ($1\times10^{8.6}$ CFU /ml) and (1×10^7 CFU/ml) respectively , and with Benedict and Flamiano , (2004) were used mice models to determine the minimum lethal dosage (MLD) of *E. coli* found to be an intra-peritoneal (I.P) injection of 0.5 ml of 10^7CFU/ ml as it induced fatality in all replicates within 24 hrs. These results are approximate with the study by Yousif and Al-Naqeeb, (2010) reported that the LD_{50} of *Salmonella hadar* drenched orally in mice was (1.5×10^9 CFU/ml) , also with Al-Mansory , (2009) determined the lethal dose of *Salmonella enteritidis* in rabbits was (2×10^{10} CFU/ml) .

Conclusion:-

It could be concluded these data shows that it takes a very low number of microorganisms to cause illness in young children , the elderly and immune – compromised people . As it is obvious from the results mentioned above , *Salmonella mbandaka* did not differ significantly from other nontyphoid *Salmonella* spp. for all this study included criteria , which means that *S. mbandaka* have the same virulence for mice inoculated orally .

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