

## Liver, Kidney Function Tests and Oxidative Damage During and after Treatment of *Salmonella typhimurium* Infection in Experimental Local Rabbits

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### ABSTRACT

This study aimed to evaluate the liver, kidney damage caused by *S. typhimurium* and to estimate the oxidative damage in association with this bacteria. A highly virulent isolates of *S. typhimurium* were obtained from the department of internal and preventive medicine/ College of Veterinary Medicine/ University of Baghdad. A twenty five local rabbits of both genders with age range (2-4 months) weeks old were used for this study, the rabbits were divided randomly into five groups each group contains 5 rabbits :- group 1: drenched orally with 5 ml of normal saline and consider as control group, group 2: were drenched orally with (5 ml) suspension which contain ( $5 \times 10^9$  CFU) of *Salmonella typhimurium* and regarded as infected group, group 3 were drenched orally with (5 ml) suspension which have ( $5 \times 10^9$  CFU) of *Salmonella typhimurium* then treated with a single dose of gentamicin alone at 0.05ml/kg (5mg/ml) orally after presence of signs (after 24hrs. post inoculation), group 4 were drenched (5 ml) suspension having ( $5 \times 10^9$  CFU) of *Salmonella typhimurium* then treated with a single dose of Ca-EDTA alone at 40mg/kg orally after presence of signs (after 24hrs. post inoculation) and group 5 were drenched (5 ml) suspension that contain ( $5 \times 10^9$  CFU) of *Salmonella typhimurium* then treated with a single dose of combined gentamicin at 0.05ml/kg (5mg/ml) orally after presence of signs (after 24hrs. post inoculation) and Ca-EDTA 40mg/kg after presence of signs (after 24hrs. post inoculation).

The results of biochemical profile showed a significant increase ( $p < 0.05$ ) in ALT, creatinine and urea levels in infected group as compared with control group, while, the treated groups especially group 5 showed a significant improvement in ALT, Urea and creatinine levels which returned to relative normal levels as compared with infected group after 96hrs. post treatment. Also, the results of oxidative stress showed a significant increase in the levels of MDA in G2, G3, G4 and G5 after 48 hrs. post treatment, while the level of GSH showed a significant decrease in the level at 48hrs., both were returned to relative normal levels after 96hrs. post treatment especially in group 5.

In conclusion, *S. typhimurium* can causing liver and kidney damage which is manifested by increase ALT, Urea and Creatinine. Also, MDA and GSH is increased due to salmonellosis.

**Keywords:** Salmonella, ALT, Urea, Creatinine, MDA, GSH.

### INTRODUCTION

*Salmonella spp.* is a kind of zero-tolerance foodborne pathogens, which poses a great threat to quality of food products and public health<sup>1</sup>. It has been recognized as intestinal pathogen in both humans and animals<sup>2</sup>. *Salmonella spp.* have the capacity to form biofilms on several surfaces, which can favour survival in hostile environments, such as slaughterhouses. *Salmonella* strains present differences in pathogenicity<sup>3</sup>. Many researches mentioned the importance of this bacteria as a pathogen by having a multidrug resistant bacteria due to the use of non-specific antibiotic and random

administration of antibiotic and this will make persistent strains have ability to resist a large number of antibiotics<sup>4</sup>. As a consequence of salmonellosis, Liver may show different abnormalities such as hepatomegaly, toxic hepatitis and jaundice<sup>5</sup>, also, acute kidney injury is one of the complications for salmonellosis<sup>6</sup>. The damaging of liver can be recognized by detection of specific enzyme (ALT), while the kidney damage were expressed by creatinine and urea<sup>7</sup>. Many significant bacterial pathogens have evolved virulence mechanisms to evade degradation and exposure to reactive oxygen (ROS) and reactive nitrogen species (RNS), allowing them to survive and

Table 1: levels of ALT, Urea and Creatinine in five groups at 48 and 96hrs.

Groups	ALT (U/l)		Urea (mmol/l)		Creatinine( $\mu$ mol/l)	
	48	96	48	96	48	96
Group 1	37.5 $\pm$ 1.842 B	45.75 $\pm$ 6.157 C	6.941 $\pm$ 0.0514 B	6.885 $\pm$ 0.151 C	59.187 $\pm$ 1.086 B	58.812 $\pm$ 0.931 C
Group 2	79.75 $\pm$ 1.25 A	93.75 $\pm$ 3.526 A	11.802 $\pm$ 0.091 A	12.764 $\pm$ 0.036 A	225.375 $\pm$ 3.404 A	261.825 $\pm$ 5.826 A
Group 3	71.5 $\pm$ 2.25462 A	61.75 $\pm$ 1.25 B	10.546 $\pm$ 0.533 A	8.397 $\pm$ 0.057 B	204.584 $\pm$ 11.148 A	153.296 $\pm$ 7.241 B
Group 4	72 $\pm$ 5.354 A	52.25 $\pm$ 2.754 B	10.494 $\pm$ 0.973 A	7.726 $\pm$ 0.048 B	220.520 $\pm$ 11.314 A	123.125 $\pm$ 7.508 B
Group 5	74.5 $\pm$ 5.239 A	49.75 $\pm$ 1.868 C	10.492 $\pm$ 0.887 A	7.528 $\pm$ 0.035 B	212.750 $\pm$ 9.572 A	105.150 $\pm$ 2.566 B

Capital letters denote significant ( $p < 0.05$ ) differences between groups

replicate inside their hosts. Due to the highly reactive and short-lived nature of ROS and RNS, combined with limitations of conventional detection agents, the mechanisms underlying these evasion strategies remain poorly understood<sup>8</sup>. So that, this study aimed to estimate the kidney and liver profiles after *S. typhimurium* inoculation and after treatment and to explore the oxidative damage caused by this bacteria.

## MATERIALS AND METHODS

A highly virulent isolate of *S. typhimurium* from domestic and street diarrheic and non-diarrheic dogs in Baghdad Province which obtained from Department of internal and preventive medicine/ College of Veterinary Medicine/University of Baghdad were previously diagnosed and confirmed by PCR as mentioned in previous study<sup>9-10</sup>. Infective dose of bacteria was prepared as described by Miles and Misra<sup>11</sup>, containing  $5 \times 10^9$  CFU as recommended by Habasha *et al.*<sup>12</sup>.

The experimental study was conducted on 25 local rabbits of both genders aged between 2-4 months old were adapted for two weeks before starting the experiment by rearing in clean and disinfected cages, fecal cultures for *S. typhimurium* were done before infection to exclude the possibility of natural infection and carrier animals. They were fed on ad libitum alpha alpha and clean water. These rabbits divided randomly into five groups as follows: Group 1 : five rabbits were used as negative control which drenched orally by plastic syringe with normal saline at dose 5 ml, Group 2: five rabbits were used as infected group which drenched 5 ml suspension which have ( $5 \times 10^9$  CFU) of *Salmonella typhimurium*, Group 3 : five rabbits were drenched 5 ml suspension having ( $5 \times 10^9$  CFU) of *Salmonella typhimurium* then treated with single dose of gentamicin (Vapco, Jordan) alone at 0.05ml/kg (5mg/ml) orally (according to manufacturer) after presence of signs (24hrs. post infection), Group 4 : five rabbits were drenched 5 ml suspension containing ( $5 \times 10^9$ ) CFU of *S. typhimurium* then treated with single dose of Ca-EDTA (GLS company, USA) alone at 40mg/kg orally (according to manufacturer) after presence of signs (24hrs. post infection), Group 5 : five rabbits were drenched 5 ml suspension containing ( $5 \times 10^9$ ) CFU of *S. typhimurium* then treated with combination of single dose of

gentamicin at 0.05ml/kg (5mg/ml) orally and Ca-EDTA 40mg/kg orally after presence of signs (24hrs. post infection). All rabbits were examined clinically and for the presence of bacteria in feces as previously mentioned by<sup>9</sup>.

### Blood Collection

Blood samples were collected from jugular vein from all groups for culturing and for serum collection, Plain tubes were centrifuged for 5-10 minutes at 3000 rpm, all samples collected after 48 and 96 hours after presence of signs.

### Serum biochemical profile

Measurement of serum ALT, Urea and Creatinine by using (Biolabo, France) kit according to<sup>13</sup>, these were measured at 48 and 96 hrs. after presence of signs.

### Determination of serum Malondialdehyde (MDA) and Glutathione (GSH) concentrations

Malondialdehyde and Glutathione concentrations measurement done by modified Thiobarbituric acid (TCA) interaction method according to<sup>14</sup>, these were measured at 48 and 96 hrs. after presence of signs.

### Statistical analysis

All data were analyzed statistically using the Microsoft program (SPSS), a nova one way, the level of statistical significance was set at ( $P < 0.05$ ) as described by<sup>15</sup>.

## RESULTS

The presented results of biochemical profile showed a significant increase ( $p < 0.05$ ) in ALT, Creatinine and urea levels in infected group as compared with control group, while, the treated groups especially group 5 showed a significant improvement in ALT, Urea and creatinine levels which returned to relative normal levels as compared with infected group especially after 96hrs. post treatment (Table 1).

Also, the results of this study showed a significant increase in the levels of MDA and decrease in GSH level after 48 hrs. post bacterial inoculation in G2, G3, G4 and G5 as compared with control group. After 96 hrs. post treatment, there was a significant decrease in the level of MDA and a significant increasing in the level of GSH appeared as compared with control group (Table 2).

## DISCUSSION

Table 2: Levels of MDA and GSH at 48 and 96hrs.

Groups	MDA (mg/ml)		GSH (mg/ml)	
	48	96	48	96
Group 1	2.235 ±0.358 B	2.280 ±0.218 C	16.087 ±0.098 A	16.130 ±0.135 A
Group 2	5.440 ±0.215 A	5.625 ±0.143 A	7.655 ±0.210 B	6.756 ±0.143 C
Group 3	5.265 ±0.609 A	4.472 ±0.196 B	7.783 ±0.305 B	10.084 ±0.322 B
Group 4	4.535 ±0.192 A	3.705 ±0.115 B	9.261 ±0.367 B	11.531 ±0.224 B
Group 5	4.535 ±0.095 A	3.437 ±0.017 B	8.794 ±0.303 B	12.528 ±0.245 B

Capital letters denote significant ( $p < 0.05$ ) differences between groups

As mentioned in introduction, salmonella can affect the liver<sup>5</sup> as well as, the kidney<sup>6</sup>, ALT enzyme is regarded as a good indicators for liver function, also, Creatinine and urea are considered the main markers of kidney damage associated with glomerular filtration and renal tubule dysfunctions as mentioned by<sup>16</sup>, So that, this idea supported and declared our results. The current results were resembles to the results of El-Jakee et al.<sup>17</sup> who found that ALT, creatinine and urea levels were significantly increase in group of mice inoculated with *Salmonella typhimurium*. Also, Srikanth and Kumar<sup>18</sup> concluded that there was an increasing in ALT levels in patients with typhoid.

A central mechanism of the innate immune response to defend against pathogens is the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) by specialized phagocytic immune cells, Macrophages and neutrophils generate ROS after detection of pathogen-associated molecular patterns (PAMPs) through the NADPH oxidase complex<sup>19</sup>. Endogenous antioxidants, including such non-enzymatic scavengers as glutathione (GSH) and such antioxidant enzymes as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), are the first lines of defense against oxidative stress and act by scavenging potentially damaging free radical moieties<sup>20</sup>.

*Salmonella enterica* subsp. typhimurium (*S. Typhimurium*), which is a major cause of gastroenteritis and some systemic diseases, remains inside a specific *Salmonella*-containing vacuole (SCV), where it injects bacterial effector proteins directly into the host cell through a type III secretion system (T3SS). A specific set of type III effectors associated with a *Salmonella* pathogenicity island (SPI), known as SPI-2 effectors, have been implicated in ROS and RNS evasion strategies<sup>21,22</sup>, however, the relationship between SPI-2 and oxidative stress evasion is a contentious topic after a recent study concluded that the contribution of SPI-2 to *Salmonella* pathogenesis is unrelated to its interaction with oxidative stress<sup>23</sup>. The current results were in compatible with results of Shukla et al.<sup>24</sup> who showed that the levels of malondialdehyde (MDA) were significantly ( $P < 0.05$ ) higher in *Salmonella*-infected mice compared with control groups, Also, they showed a decrease in GSH levels. While, the results were disagree with results of Barkaoui et al.<sup>25</sup> who mentioned that there was an increasing in the levels of GSH after salmonella infection.

## CONCLUSIONS

It have been concluded that *S. typhimurium* can causing liver and kidney damage which is manifested by increase ALT, Urea and Creatinine. Also, MDA and GSH is increased due to salmonellosis.

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