Therapeutic effect of P-coumaric acid in the experimentally infected rats with *Salmonella typhi*

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Abstract. *Salmonella enterica* subspecies *enteric* serotype typhi which infect the human and cause typhoid fevers (systemic infection). Experimental infection with *Salmonella typhi* are very important because they explains the pathogenicity of *Salmonella typhi* through a histopathological findings. The herbal inhibitors like P-Coumaric acid explain anti-inflammatory effect and have a selective effect against type III secretion system formation of *Salmonella typhi*. our study includes 15 adult male albino rats were divided into three groups each group composed from 5 rats. A group: were orally challenged with 1ml of $3 \times 10^6$ CFU of *Salmonella typhi* for one day. Group B: were also orally administered with 1ml of $3 \times 10^6$ CFU of *Salmonella typhi*, additionally, they also orally received 5ml of 100mg/kg of P-Coumaric acid for three consecutive days. Group C: serving as control group, they orally administered with 5ml of normal saline. All the animals are sacrificed after five days post infection, liver, small intestine and colon specimens were taken for histopathological investigation. the histological sections of liver and small intestine of group A animals showed pathological changes like degeneration of hepatic cells and inflammatory cell infiltration of the lamina properia in small intestine, while the group B animals showed normal liver and small intestine sections like the control group.

1. Introductions

*Salmonella* species are the gram negative bacteria; they associated with some human and animal disease which begin Minor diarrhea toward general disease such as typhoid fever (Wester and Hensel., 2001).

*Salmonella* is divided into two species, *Salmonella bongori* and *Salmonella enteric*. *Salmonella enterica* subspecies *enterica* can be further divided into over 2500 serovars based on their flagella (H) antigen and LPS O antigen structure (Sabbagh et al., 2010). Many serovars are host-adapted and tend to cause life threatening systemic disease in their host. For example, *S. typhi* and *S. paratyphi* cause systemic infection within humans and specific primates (Zhang and Mosser, 2008).

*Salmonella* specie and other Gram- negative bacteria have altered difficult mechanisms to infect and colonize their hosts one such mechanism is called the virulence-related type III secretion system, which consists of specific organelles that carriage effectors proteins into the cytoplasm (Shanmugas-amy et al., 2011). Experimental studies of salmonellosis are very important because they explains many parameters such as pathogenicity of *Salmonella* which focused on histopathological finding additionally with...
macrophagic finding which exposed on the infection animals (Muna et al., 2016) Other salmonellosis experimental studies focused on host immunity against *Salmonella* infection by studying of some humoral immunity like, interleukins (Urfalıoğlu et al., 2017)

(Yousif and AL-Naqeeb, 2010, noted that the experimentally infected mice are able to induce humoral and cellular immune response which represented by producing antibody against *Salmonella*.

Infection of mice with *Salmonella typhimurium* is the best available model to study gastroenteritis, an important disease in many developing countries (Santors et al., 2001; Srinivasan et al., 2004). Srinivasan et al., 2004 demonstrated that variation in the initial dose of infection with *Salmonella* has a profound effect on the response of *Salmonella* flagellin-specific CD4 T cells in vivo, and that low-dose infection can evade *Salmonella* flagellin-specific T cell activation completely. The most important phenolic compound is P-Coumaric acid (P-CA) it blocks type III secretion system (Hutchins et al., 2015). (Yama zaki et al., 2012) showed that P-Coumaric acid inhibits type III secretion system of *Pseudomonas aeruginosa* through alteroxos genes that encode effector proteins

(Khokhani et al., 2013) Conducted that plant phenolic compound like 4-methoxycinnamic acid and benzoic acid altered type III secretion system expression of *Erwinia amylovora* weakened the hypersensitivity; additionally plant pathogenic bacteria such as *Erwinia amylovora* response in tobacco by suppression of type III secretion system of this bacterium. (Yama zaki et al., 2012)

Our current study aims to study of *Salmonella typhi* pathogenicity in rats and effect of P-Coumaric acid on *Salmonella* by investigation on histopathological changes of the infected animals organs.

2-Materials and Methods

2-1-Bacterial Inoculum preparation.

*Salmonella typhi* inoculum was achieved according to (Marcq et al., 2011) which includes the following steps:

1. *Salmonella typhi*: was grown on Tryptic Soy Agar for 24h at 37°C.
2. Single colony was chosen for inoculation 10ml of sterile cooled Tryptic Soy broth then incubated for 24h at 37°C.
3. Bacteria were precipitated at 3500rpm for 10min.
4. Bacterial pellet was suspended with Tryptic Soy broth to obtain bacterial concentration at $3 \times 10^6$ CFU according to the McFarland tube No 0.5 after dilution it.

Not: the *Salmonella typhi* was isolated from diarrheic stool and diagnosed by Enterosystem kit R18 (Italy) additionally it give positive result of virulence genes of type III secretion system (invA, sipB, sopB nd sscC by PCR technique

2-2-P-Coumaric Acid Solution Preparation

One gram of P-Coumaric acid was dissolved in a 5%dimethyl sulfoxide (DMSO) for obtaining 100mg/kg, which were orally administered of the rats. It was used to selectively inhibit for virulence factors for *Salmonella typhi* (Urfalioglu et al., 2017).

2-3-Experimental Design

The Fifteen white rats were divided into three groups:

Group A: contain 5, rats were orally have given dose with 1ml of $3 \times 10^6$ CFU of *Salmonella typhi* for one day.

Group B: contains 5, rats were also orally having given dose with 1ml of $3 \times 10^6$ CFU of *Salmonella typhi*. Additionally, they also orally received 5ml of 100mg/kg of P-Coumaric acid for three consecutive days.

The animals were orally administrated by using syringe with flexible attached tube as well as the animals of A and B groups were fasted for 24h before challenged with *Salmonella typhi*.
Group C: contains 5 rats which serving as control group, they orally administrated with 5ml of normal saline. All the animals are killed after five days postinoculation. Liver, small intestine and colon sections were collected for histopathological investigation.

2-4-Histological Study
The liver, small intestine and large intestine of the rats were removed after scarifying the animals and fixed in 10% formalin. Paraffin blocks were sectioned at 5 microns thickness by rotary microtome. The paraffin sections were prepared in the Educations Hilla Hospital then stained by Haematoxylin and Eosin stains for investigation histopathological changes and examined with the light microscope (Aughey and Frye, 2011).

3-Results and Discussion
This study show that liver of rats which inoculated with Salmonella typhi without treated with (P-Coumaric acid) explains: blood congestion inside central vein with infiltration of inflammatory cells in the lumen, degeneration of hepatocytes and enlargement of sinusoids. And the results agree with (Hartman et al., 2010; Malik et al., 2015). the data as below in the figure

![Figure 1: Photomicrograph for liver rat of A group showing: blood congestion inside central vein (A), degeneration of hepatocytes (D) and enlargement of sinusoids (E). (Haematoxylin & Eosin X400)](image)

While, the animals treated with Salmonella typhi and P-Coumaric acid explain normal liver tissue section like the liver section of the animal's control. The normal liver tissue of animals that treated with P-Coumaric acid (P-CA) after infection with Salmonella typhi gives improvement for the protective effect of (P-CA). These finding was supported by Urfaloğlu et al., (2017) who reported that anti-inflammatory and antioxidant effects of P-CA in experimental sepsis rats. Like worker by Zhao et al., (2016) who documented that anti-inflammatory effect of (P-CA) in immunized rats with LPS. The data as below in the figure
This study showed that small intestine of rat which inoculated with *Salmonella typhi* without treated with (P-Coumaric acid) explains: infiltration of inflammatory cells in the lamina propria. The result agree with (Everesti, 1999).

While, sections of small intestine rats that inoculated with *Salmonella typhi* and treated with P-Coumaric acid explains that normal tissue sections. The normal small intestine tissue of animals that treated with P-coumaric acid (P-CA) after infection with *Salmonella typhi* gives improvement for the protective effect of P-Coumaric acid. the data show below in the figure 4.
Figure 4: Photomicrograph for small intestine rat of B group showing: normal small intestine tissue section, improvement for the protective effect of P-CA (I.G) intestinal gland. (Haematoxylin & Eosin X400)

This study showed that Colon of rats explains: Deteriorated colonic wall due to inadequate fixation, apparently normal tissue. This study agrees with Singh. (2001) said that *Salmonella typhi* adapted and entry to M-cell and macrophage. The data as below in the figure

Figure 5: Photomicrograph for colon rats showing: normal colon tissue section.
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