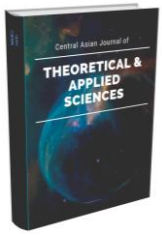

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Article

Determining the Levels of Few Inflammatory Cytokines in Lab Animals Exposed to Various Concentrations of *Salmonella typhi* Lipopolysaccharides

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Abstract: The current study aims to quantify the amounts of inflammatory cytokines exposed to different lipopolysaccharide concentrations isolated from Salmonella typhi. Using the chloroform-methanol procedure, LPS was isolated from *S. typhi* and then dried with a lyophilizer device. The purity of LPS was confirmed by gas chromatography-mass spectrometry (GC-MS). Doses of 0.625, 1.25, and 2.5 mg/100 g body weight (B.W) were used in this experiment. Four groups of white mice were injected with three doses of LPS, with each group receiving three replicates, aged (2–3) months injected intraperitoneally (IP) with three doses of LPS every 48 hours, and followed by a final booster dose one week later. Subsequently, blood samples were collected and serum levels of interleukin-1 (IL-1) and interleukin-7 (IL-7) were measured by enzyme-linked immunosorbent assay (ELISA) method. The results showed that IL-1 levels were (9.933 ± 0.513 , 9.033 ± 0.568 and 10.700 ± 0.101) pg/ml at the concentrations of 0.625, 1.25, 2.5) mg/100 g (b.w) respectively, compared to the control sample which was (5.933 ± 0.890). Furthermore, the levels of IL-7 were 304.000 ± 0.208, 301.866 ± 0.276 and 333.566 ± 0,164) pg/ml at 0.625, 1.25, and 2.5 mg/100 g (b.w) respectively, while the control sample was (290.700 ± 0.112) pg/ml. The gain of lipopolysaccharide from *S. Typhi* provoked the immune system inside the injected rats, leading to the secretion of cytokines like IL-1 and IL-7 in any respect tiers used in comparison to the manage. In addition, the lifestyles of IL-1 could limit the manufacturing of IL-7.

Keywords: Interleukins, Bacteria, LPS, Tumor necrosis factor

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1. Introduction

According to Kutsehera et al (2021) Lipopolysaccharide (LPS) is a major constituent of the cell walls of Gram-negative bacteria. LPS usually have three components. Known as O antigen (Somatic antigen), the outer content is composed of several oligosaccharide types (Grigoryan et al., 2021). The second part, commonly referred to as the core region, connects to the last partition, lipid A, which is a very toxic component (Kalambhe et al., 2012), and tends to promote the immune system and secretion multiple cytokines (Liu et al., 2018) among them Interleukins (IL-1, Il-8, IL-7, and IL-10) (Tanaka et al., 2014; Villar-Fincheria et al., 2021). Salmonella is a rod-gram-negative pathogenic bacterium responsible for many diseases including typhoid fever, intestinal diseases in both humans and animals and the decomposition of food (Ashurst et al., 2022; Patra et al., 2015). It includes a significant amount of LPS, up to one gramme of body weight (Sali et al., 2019; Daigle et al.,

2021). Furthermore, a small amount of LPS that passes through the body's bloodstream could lead to a response from the immune system (Liu et al., 2018; Paola et al., 2022). The current study aimed to extract the LPS from the cell wall of *S. typhi*, and determined the levels of some inflammatory cytokines at different concentrations of LPS in the white rats.

2. Materials and Methods

Preparation of Bacteria

Pure culture of *Salmonella typhi* was obtained from laboratories of the department of biology, college of science, University of Mosul. *S. typhi* was cultured in 4 liters of Brain heart infusion broth medium (BHI), incubated in 37 ˚c for 48-72 hours in a shaker incubator, cultures were centrifuged at 5000 rpm for 30 minutes, and bacterial sediments were washed thrice with 2 mL of ethyl alcohol (95%), shaken well, then centrifuged at 3000 rpm for 10 minutes. Cell pellets were kept in closed tubes in a refrigerator at 4-8 ℃ until used (Subhiy *et al*., 2016).

Extraction of Bacterial LPS

Bacterial pellet was resuspended in 10% EDTA and cell wall was disrupted by sonication (Ultrasound Omni international, UK) at 20000 vibration per minutes for 30 seconds under cooled conditions. Samples were subjected to cooled centrifugation and the supernatant was collected in sterile test tubes. 1 mL of chloroform/methanol (1:2 ratio) was added to bacterial disrupted solution and covered by parafine oil then shaken for two hours. Three layers were formed, the layer of chloroform and methanol was collected and air dried (Kalambhe *et al*., 2017).

Lyophilization of LPS

Granules of LPS were lyophilized using the devise (Alpha-1-2 Lb plus 19616, Germany). Samples were stored at 4℃. The purity of LPS was determined using gas chromatography-mass spectrometry (GC-MS) (5973 network mass selective detector, USA) (Jackie *et al*., 2021).

Animal Injection

Four groups of white rats with ages ranged between 1-2 months were grown under suitable conditions and used in the experiment. Animals were injected intra-peritoneal three times every 48-hour with LPS extract followed by a final booster dose one week after the final dose (Zhong *et al*., 2018).

Determination the levels of cytokines

Blood samples were collected from animals treated with LPS. Blood samples were centrifuged and serum was collected and used in ELISA experiments. Kits were used from Elab science Biotechnology Inc. Rat IL-1 and IL-7 levels were determined in blood samples of rats injected with three concentrations of LPS (0.625, 1.25, 2.5 mg/100gm for body weight) as mentioned elsewhere (Jianjian *et al*., 2017).

3. Results

Results of the current study showed that LPS extracted from *S. typhi* contained several compounds as analyzed by GC-MS. The compound top six compounds present were (2,4-Decadienal 2.23%, Hexacosyl trifluoroacetate 2.510%, Hexacosyl heptafluoro-

butyrate 4.37%, Octadecanoic acid 4.47%, n-Hexadecanoic acid 10.18%, and oxacycloheptadec-8-en-2-one 55.93%) as shown in Table 1 (Kintz *et al*., 2017).

Table 1. Major LPS components from *S. typhi*

	Name	Percentage
1	2-4 Decadienal	2.23%
2	Hexacosyl tri-fluoroacetate	2.56%
3	Hexacosyl hepta-fluoroacetate	4.37%
4	Octadecanoic acid	4.47%
5	n-hexadecenoic acid	10.18%
6	Oxacyclohepta-dec-8-en-2-one	55.93%

The current study also showed a clear effect of LPS isolated from *S. typhi* in all the three concentrations used in stimulating an increase in the production of cytokines IL-1 and IL-7. The current study also found that the concentration 2500 mg/100gm of body weight had a significant effect on the level of IL-1, where it was equal to 10.7000 ± 0.101 pg/mL when compared to the concentrations 625 and 1250 mg/100gm b.w. However, the levels were equal to 9.9333±0.511 and 9.0333±0.568 pg/mL respectively, compared to the control sample that was equal to 5.9333±0.8900 pg/mL as shown in Table 2. At the same time, we noticed that the three concentrations 625, 1250 and 2500 mg/100gm b.w. had a clear and significant effect on the level of IL-7 in the blood and were equal to 304.000±0.208, 301.866±0.276 and 333.566±0.146 pg/mL, respectively as compared to the control sample which was equal to 290.700±0.112 pg/mL as shown in Table 2. Results were statistically analyzed by ANOVA program.

Table 2. Levels of IL-1 and IL-7 in injected animals

Concentrations mg/100gm B.W.	**IL-1 pg/mL**	**(IL-7) pg/mL**
0,625	9.933 ab ± 0.513	304.000 a ± 0.208
1,25	9.033 b ± 0.568	301.866 a ± 0.276
2,5	10.700 a ± 0.101	333.566 a ± 0.164
control	5.933 c ± 0.890	290.700 b ± 0.112

Similar letters indicate no significant differences, while different letters indicate significant differences.

Levels of IL-7 and IL-1 and their activity in the body of an injected rats was compared, we noticed that the levels of IL-7was higher than IL-1 in all three concentrations as shown in Table 3.

Table 3. Comparison between IL-1 and IL-7

Cytokines	Concentrations of LPS Mg/100	Levels of cytokines
IL-1	0,625	9.933±0.513 ab
	1,25	9.033±0.568 b
	2,5	10.700±0.101 a
	control	5.933±0.890 c
(IL-7)	0,625	304.000±0.208 a
	1,25	301.866±0.276 a
	2,5	333.566±0.164 a
	control	290.700±0.112 b

Similar letters indicate no significant differences, while different letters indicate significant differences.

4. Discussion

Analysis of LPS from the cell wall of *S. typhi* showed the presence of different compounds via GC-MS. Several compounds were detected in different percentages. The compounds detected were (2-4 Decadienal 2.23%, Hexacosyl trifluorbarelate 2.56%, Hexacosyl heptafluovoacedate 44.37%, Octadecanoic acid 4.47%, n-hexadecanoic 10.18% and oxacyclo hepta-dec-8-en-2-one 55.93%), these results come in agreement with Jackie *et al*. (2021).

Our study also showed significant differences between levels of IL-1 in all three concentrations when compared with control samples as shown in Table 2. The value of IL-1 for (625, 1250, 25.00 mg/100gm b.w.) was equal to (9.933±0.513, 9.033±0.568, 10.700±0.101), respectively, and the control sample was equal to (5.755± 0.890), this result agree with the findings of Febriza *et al.* (2020) and Ruemmale *et al*. (2002). The presence of LPS in peripheral blood after infection is due to the production of IL-1 from monocytes, endothelial cells protoblasts (Rattanasrisomporn *et al*., 2022). IL-1 has the ability to induce the differentiation of T and B lymphocytes and promote the synthesis of specific proteins that stimulate the liver cells (Poko *et al*., 2010). LPS also stimulates the release of reactive oxygen and nitrogen particles, through TLR4-mediated carriers called NADPH oxidase which is activates the macrophages and leads to oxidative and nitrosative stress and damages the cells then causes failure of organs (Huang *et al*., 2020; Page *et al*., 2020; Yang *et al*., 2020).

The current study showed that the levels of (IL-7) raised in peripheral blood of an injected rat understudy compared with control samples, the levels were equal to (304.000±0.208, 301.866±0.276, 333.566±0.164) pg/mL for three concentration (0,625, 1,25, 2,5) mg/100 b.w, respectively, while the control was equal to (290.700±0.112) pg/mL. It was also noticed that there was no significant difference between the three concentrations understudy, as shown in Table 2. The current results explain the effect of LPS concentrations on the levels of (IL-7) regardless of the concentration, these findings agree to what was observed by Febriza *et al*., (2020) and Kang *et al*., (2019) whom mentioned that IL-7 is produced by macrophages and is activated by T cells lymphocyte when the body is exposed to bacteria causing disease especially those that have endotoxin like LPS (Chaiwut *et al*., 2022).

From Table 3, we notice an increase in the levels of IL-1 which was significantly affected by all concentrations of LPS, in comparison with IL-7 which kept its level non-significantly in all three concentrations. This result may be due to exhibited rapid expression of mRNA for IL-1 at pro infection by monocytes then was followed by cytokine synthesis. A given LPS induced either high or low expression of the battery of cytokines tested (Chaiwut *et al*., 2022) indicating that the expression of these pro-inflammatory cytokines may be regulated by a single or a cluster of genes (Agarwal *et al*., 1995). We can also mention that the increase in the level of IL-1 causes critical induction of IL-7 secretion in macrophages infected with LPS, this agrees with Guimaraes *et al.* (2020) when they used *Brucella abortus* to infect macrophages. Moreover, IL-7 is the central inflammatory cytokine produced by most infections, and recognition that it can induce tolerance made us consider the possibility that IL-7 is responsible for the induction of tolerance (Loosbrook *et al*., 2014).

5. Conclusion

We conclude from this study, that extracted lipopolysaccharide from *S. typhi* induced the immunity system in injected rats and caused the secretion of cytokines like IL-1 and IL-7 in all concentration in a high level compared with the control sample.

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