American-Eurasian Journal of Toxicological Sciences 6 (2): 39-51, 2014
ISSN 2079-2050

DOI: 10.5829/idosi.aejts.2014.6.2.84210

Prenatal Exposure to *Salmonella typhimurium* LPS Effects on Neurodevelopment of Brain and Anxious Related Disorders

[1,2]*Behnam Rashidieh,* [1,3]*Jalal Solati and* [1]*Bahman Tabaraei*

[1]Department of Microbiology of Karaj Islamic Azad University, Iran
[2]Specialty Services Department of IBRC (Academic Center for Education, Culture and Research)
[3]Friedrich-Alexander, Universität Erlangen-Nürnberg, Germany

Abstract: Lipopolysaccharide (LPS) also known as endotoxin, is a component of the Gram-negative bacteria cell wall which is a mitogen and activate B-cells, causing the release of inflammatory cytokines such as IL- 1 and 2 Tumor Necrosis Factor- α (TNF- α) that followed by stimulation of immune system. Stimulation of immune system and the effects of these toxins are more intense and serious in uterus and during the formation of the nervous center. Nowadays, it has been demonstrated that some of the complications of nervous system diseases, even in old ages and behavioral disorders as well as diseases such as Parkinson are directly related to fetal conflict with infectious factors. Some studies also have investigated the embryonic loss of neurons due to bacterial toxins in infected mothers. In this study, the effects of *Salmonella typhimurium* LPS on the formation of nerve centers and behavior of NIH mice race were examined. *Salmonella typhimurium* LPS were extracted by hot phenol extraction method and then was injected intraperitoneally (IP) to different groups of mice at 30, 60 and 120 µg/Kg of body weight doses. After prove of onset mice pregnancy, LPS was injected to different groups of mice at the eighth and tenth day of pregnancy. After parturition, offspring's anxiety as a physiological behavior and intake of food and water tests were carried out. The results showed that maternal LPS injection could cause behavioral changes in offspring.

Key words: Bacterial Toxin · *Salmonella typhimurium* Lipopolysaccharides · The Mouse Brain · Anxiety and Physiological Behavior

INTRODUCTION

Lipopolysaccharides exert a variety of biological and endotoxic activities in humen including pathophysiological effects such as fever, tachycardia, tachypnea, leucopenia and hypotension, the hallmarks of sepsis and septic shock [1]. The molecular mechanisms of these effects are unknown and comprise the activation of various host cells, in particular monocytes and macrophages, leading to the secretion of nitric oxide, vasoactive lipids, cytokines such as interleukin (IL)-1, IL-6, IL-12 and Tumor Necrosis Factor (TNF) α[2].

Studies of the chemical prerequisites for endotoxic activity of LPS have revealed that the activation of monocytes/macrophages depends on a peculiar primary structure of lipid A present in enterobacterial genera. These observations were interpreted as indicating an influence of a variation in the primary structure of endotoxin molecules on their physicochemical behavior [3].

Prenatal bacterial infections impair short- and long-term behavior and central nervous system activity in animals [4]. Maternal immune activation can also induce neuropsychiatric disorders, including schizophrenia, autism and even greater disaster like Parkinson [5]. These results suggested that prenatal LPS exposure induced autism-like effects in offspring [6]. In addition to behavioral impairments, our model of prenatal LPS exposure resulted in striatal dopaminergic impairments in offspring, including reduced levels of tyrosine hydroxylase, dopamine and metabolites [7]. Moreover, in pregnant rats, LPS exposure induced sickness behavior, including reduced open-field general activity [8].

Corresponding Author: Behnam Rashidieh, Department of Microbiology of Karaj Islamic Azad University, Iran.

Nevertheless a considerable amount is known about the behavioral alterations and brain damage, little is known about how maternal exposure to LPS in influences by the immune systems of animals. Likewise, little is known about what LPS-induced changes occur in the pregnant dam on different days that subsequently affect the developing fetus [9]. Thus, in this experiment, we performed reproductive, anxiety and physiological evaluations, both in dams and in their offspring, to better understand what triggered the impairments in the offspring. For this purpose, we evaluated physiological behavior in dams that received LPS on GD=8 and GD=10. This behavior was also evaluated in the offspring with or without LPS challenge in adulthood. The present study investigated for persistent changes in adulthood, similar as occurs in some neurological disorders.

MATERIALS AND METHODS

Preparing the Toxins

Bacteria: Strain of *Salmonella typhimurium* (PTCC 1735) was grown in Luria-Bertani broth medium at 37°C in shaker incubator overnight. After centrifugation of culture media, bacterial sediment were harvested and used for LPS extraction and purification [10].

LPS Extraction and Purification: LPS was extracted by hot phenol-water method as described by Westphal [11]. This protocol was achieved from a study on the purification of lipopolysaccharides that has been done by Pakzad *et al*. [12] and recently by Rezania and his colleagues [12, 13]. The bacterial suspensions (10^8CFU/mL) were centrifuged at 10, 000×g for 5 min. The pellets were washed twice in PBS (pH = 7.2) (0.15 M) containing 0.15 mM $CaCl_2$ and 0.5 mM $MgCl_2$. Pellets were then re-suspended in 10 mL PBS and sonicated for 10 min on ice. To eliminate contaminating protein and nucleic acids, treatment with proteinase K, DNase and RNase was performed prior to extraction step. For this purpose, proteinase K (100 µg/mL) (Roche, Mannheim, Germany) was added to the cell mixture and the tubes were kept at 65°C for an additional hour. Mixture was subsequently treated with RNase (40 µg/mL) (Roche, Mannheim, Germany) and DNase (20 µg/mL) (Roche, Mannheim, Germany) in the presence of 1 µL/mL 20% MgSO4 and 4 µL/mL chloroform and incubation was continued at 37°C overnight. At the next step, an equal volume of hot (65–70°C) 90% phenol was added to the mixtures followed by vigorous shaking at 65-70°C for 15 min. Suspensions were then cooled on ice, transferred to 1.5 mL polypropylene tubes and centrifuged at 8500×g for 15 min. Supernatants were transferred to 15 mL conical centrifuge tubes and phenol phases were re-extracted by 300 µL distilled water. Sodium acetate at 0.5 M final concentration and 10 volumes of 95% ethanol were added to the extracts and samples were stored at -20°C overnight in order to precipitate LPS. Tubes were then centrifuged at 2000×g 4°C for 10 min and the pellets were re-suspended in 1 mL distilled water. Extensive dialysis against double distilled water at 4°C was carried out at the next step until the residual phenol in the aqueous phases was totally eliminated. Final purified LPS product was lyophilized and stored at 4°C [11, 13].

SDS-PAGE: Separation over SDS-PAGE gel followed by silver staining was used to detect and visually characterize the purified LPS. Sliver staining is a highly sensitive method capable of detecting as low as 1 *ng* LPS and is routinely used for visualization of the band pattern of purified LPS.

The purified LPS was solubilized in sample buffer to the desired concentration (1 mg/mL) and boiled for 5 min. 16 µL/well from each sample was separated on 15% SDS gel with a 4% stacking gel under reducing condition at 100 mA for 2 h using mini-PROTEAN electrophoresis instrument (Bio-Rad Laboratories, California, USA). Silver and coomassie blue staining of the gels was performed according to the standard protocol. Staining of agarose gel with ethidium bromide was done as well in order to show if any contamination with nucleic acids persist. To do this, 10 µL/well of reconstituted LPS from *S. typhimurim* and also 10 µL/well bacterial suspensions (10^8 colonyforming units/ mL), as positive control, were loaded on agarose gel and stained with ethidium bromide [13].

HPLC (High Performance Liquid Chromatography): The purity of LPS isolated from *S. typhimurium* was assessed by HPLC. The band profile of LPS extracted from *S. typhimurium* was analyzed and compared to that of extra pure commercial standard. HPLC separations were carried out by a Knauer Smartline 1000 pump equipped with a Smartline UV detector 2500 (Berlin, Germany) and a Rheodyne 7725 injection valve (Cotati, CA, USA). The method was optimized at the 0.8 ml/min flow rate and 210 nm wavelength UV detection. Separation was carried out over C_{18} column with 4.6 mm diameter and 250 mm length from Grace Company (Munich, Germany) with a mixture of water and acetonitrile (95:5) as mobile phase. Extra pure *E. coli* LPS (Sigma, Saint Louis, USA) was used as standard [13].

Limulus Amebocyte Lysate (LAL) Assay: The potency of LPS samples were determined by the limulus amebocyte assay gel clot method (LONZA, Walkersville, USA) which had a sensitivity of 0.06 endotoxin units per milliliter (UE/mL), according to the protocol published elsewhere [14].

Toxicity of *Salmonella typhimurium* LPS by comparing with its detoxified form in fever induction method was evaluated in mice. Samples should be stored in a place that does not have active bacterial and endotoxin levels do not degrade.

***In-vivo* Study Model**

Animals: Male and female NIH mice weighing approximately 25g were obtained from Razi Vaccine & Serum Research Institute. They were acclimatized according to standard protocols such as IACUS Protocol, Institutional Animal Care and Use Committees. Mice were maintained in groups of six in standard cages. The animals were allowed free access to food and water at all times and were maintained on a 12 h light/dark schedule in a controlled temperature (23 - 18°C).

For mating purposes, a female mouse was housed overnight with a male starting at 18:00 h. Each female mouse was visually inspected for the presence of a vaginal plug the next morning at 06:00 h. The presence of plug was designed as day 0 of gestation (GD= 0). The pregnant mice were caged individually.

The litters remained with their mothers until weaning (GD = 21) and were separated according to sex on Day 36. The male offspring maintained in groups of 4 in the above-mentioned conditions. The male offspring were distributed into control and experimental groups.

Injection: Purified LPS was dissolved in sterile pyrogen-free saline before use. The dams were randomly assigned to a saline control group and LPS groups. The dams in the LPS groups were administered a single intraperitoneal (i.p.) injection of 30, 60 and 120 µg/kg LPS on day 8 of pregnancy (GD= 8). The dams in the control group were administered a single i.p. injection of saline on GD= 8 [14]. The dosage of LPS we chose could induce systemic inflammation, resulting in a low percentage of fetal anomalies, but not abortion and possible intra-uterine fetal death (IUFD) [15]. This procedure repeated with similar dosage on day 10 (GD= 10). The rationale for choosing gestational day 10 was that this period corresponds to the first-to-second trimesters of human pregnancy, with respect to developmental biology and percentage of gestation from mice to human [16].

Other scientific literatures state that this time phase is the period of early fetal brain development [17], cerebral organogenesis in mice, especially neural-plate formation [18] and also embryonic stem cell formation which is one of the main periods of vulnerability of the immune system to environmental insults [19]. In addition, other investigators suggest that maternal infection from early to mid-pregnancy is more likely to be related to long-lasting developmental brain and behavioral abnormalities in the offspring [20].

Each dam was administered with appropriated dosage of saline or LPS solution. Following injection with LPS or saline, the dams continued to be housed in the above-mentioned conditions.

It is possible that high doses of LPS cross the placenta and go to fetal circulation system. Therefore, low doses of LPS were selected in order to prevent possible direct exposure of fetus to LPS. With this dose and route of application we observed no abortion in our endotoxin groups.

All injections were given between 13:00 and 14:00 h.

The Physiological Tests: The pregnant mice were caged individually. Physiological tests for anxiety level, sexual and feeding behaviors were carried out in fetuses born compared to control samples.

To test water and food, the consumption of these two factors in a week compared with the control group. Cross maze light/dark arm anxiety test was used.

After collection and recording data, these data were statistically analyzed by SPSS software.

Anxiety Test: One of the most popular tests of anxiety-like behavior in mice and rats is the elevated plus maze (EPM) test, in which the reduced number of entries or time spent in the open arms of the EPM suggests the operation of anxiety-like processes. This wooden, plus shaped apparatus was elevated to the height of 50 cm above the floor and consists of two open arms (30 cm x 5 cm), two enclosed arms (30 cm x 5 cm x 15 cm) and central platform (5 cm x 5 cm) each with an open roof. The maze was placed in the center of a quiet and dimly lit room. The mice behavior was directly observed using a mirror, suspended at an angle above the maze. Behavioral data were collected by a ‘‘blind’’ observer who quietly sat 1 m behind one of the closed arms of the maze, using a chronometer. The anxiety test of the offspring was carried out at postnatal Day61. It was repeated three times, three separate cohorts of male offspring were used

for tests and each mouse was only used on EPM once. We repeated this test three times due to our different results on EPM from other investigations and the results represent an average of the three test sessions. For testing purpose, male offspring were placed individually in the center portion of the plus-maze, facing one of the open arms.

The observer measured: (1) the time spent in the open arms, (2) the time spent in the closed arms, (3) the number of entries into the open arms and (4) the number of entries into the closed arms during the 5min test period. An entry was defined as all four paws in the arm. The elevated plus-maze was thoroughly cleaned with distilled water following the testing of each animal to avoid possible biasing effects due to odor clues left by previous mice. For the purpose of analysis, open-arm activity was quantified as the amount of time that the mice spent in the open arms (OAT) relative to the total amount of time spent in any arm (open/total x 100) and the number of entries into the open arms (OAE) was quantified relative to the total number of entries into any arm (open/total x 100). The total number of open arms entered, as well as the total number of closed arms entered were used as indexes of general locomotors activity (LMA) [21].

All testing was conducted between 13:00 and 16:00 h.

Food and water intake in order to find out whether i.p. exposure to LPS during pregnancy affects food/water intake in dams or in their male off spring under our experimental conditions, a feeding/drinking study was conducted. Mice had free access to food and water before the experiments began. Each cage contained 3 or 4mice which were given with the same amount of food and water. Their food intake was measured the following day by subtracting the uneaten food manually. The amount of water ingested in our experiment was measured with 0.2 mL graduated glass burettes adapted with a metal drinking spout.

Immediately after injection of LPS/saline in pregnant dams, each mouse was returned to its cage and we measured the cumulative water intake manually the following day. These measurements were done in 3 days after injection of the LPS/saline in respect to pregnant dams (N ¼ 10 in each group) and in 5 days in PND 56-60 in male offspring (N ¼ 10 in each group) and it was calculated as food (in grams 0.1 g)/water (in ml 0.2 ml) intake per mice per day. Measurements were performed by the same experimenter and we used separate cohorts of pregnant dams for food/water intake, but the ame groups of male offspring were used for food/water intake and anxiety test on EPM.

Statistical Analysis: Data were analyzed using SPSS. Since data displayed normal distribution and homogeneity of variance, one-way ANOVA was used for comparison between the effects of different doses of LPS with vehicle. Differences with $p < 0.05$between experimental groups at each point were considered statistically significant.

RESULTS

Anxiety Test: One-way analysis of variance was performed and results showed that in newborns, anxiety levels were significantly reduced compared to those in the controls, whereas in mothers, after LPS injection the anxiety level increased.

This study showed that intra-peritoneal injection of LPS to pregnant females on the eighth day of gestation resulted in a significant increase in the number of entries into the open arms in newborns at doses of 60 and 120, compared with that in the control group (which received saline only). It also caused a significant increase in the time spent in the open arms at all doses compared to the control group. None of the doses had significant influence on the newborn motor activity level.

LPS treatment in pregnant females on the eighth day of gestation caused a significant reduction in the number of entries into the open arms compared to that in the control group. LPS treatment in pregnant females on the eighth day of gestation resulted in a significant decrease of time spent in the open arms in doses of 60 and 120 compared to that in the control group.

None of the doses had significant influence on the mothers' motor activity level. LPS treatment in pregnant females on the tenth day of gestation also caused a significant increase in the number of entries into the open arms by newborns in doses of 60 and 120 compared to that in the control group. LPS treatment in pregnant females on the tenth day of gestation caused a significant increase in the time spent in the open arms by newborns in doses of 60 and 120 compared to that in the control group.

None of the doses had significant influence on the motor activity level of newborns.

LPS treatment in pregnant females on the tenth day of gestation resulted in a significant decrease in the number of entries into the open arms in dose of 120 compared to that in the control group.

LPS treatment in pregnant females on the tenth day of gestation caused a significant decrease in time spent in the open arms in doses of 120 compared to that in the control group.

Table 1: Comparison of food consumption between control group and LPS injected females

	Factor	N	Mean	Std. Deviation	Std. Error Mean
Food Female	Control	30	0.23690	0.016695	0.003048
	Test	30	0.21157	0.026204	0.004784

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Food Female	Equal variances assumed	9.158	0.004	4.466	58	0.000	0.025333	0.005673	0.013978	0.036688
	Equal variances not assumed			4.466	49.212	0.000	0.025333	0.005673	0.013935	0.036732

Table 2: Comparison of food consumption between control group and LPS injected Males both male and female was injected but male mice was chosen for food and water intake because the instability of female due to their physiological and biological circumstance especially their period time that cannot be determined, can cause some deficiencies in the result of this test.

	Factor	N	Mean	Std. Deviation	Std. Error Mean
Food Male	Control	30	0.24113	0.013323	0.002432
	Test	30	0.21653	0.021919	0.004002

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Food Male	Equal variances assumed	5.055	0.028	5.253	58	0.000	0.024600	0.004683	0.015226	0.033974
	Equal variances not assumed			5.253	47.854	0.000	0.024600	0.004683	0.015183	0.034017

Table 3: Comparison of water consumption between control group and LPS injected Females

	Factor	N	Mean	Std. Deviation	Std. Error Mean
Water Female	Control	30	0.56853	0.091360	0.016680
	Test	30	0.35900	0.079740	0.014558

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Water Female	Equal variances assumed	0.679	0.413	9.464	58	0.000	0.209533	0.022140	0.165216	0.253851
	Equal variances not assumed			9.464	56.959	0.000	0.209533	0.022140	0.165198	0.253868

Table 4: Comparison of water consumption between control group and LPS injected Males (No male injected in the experiment)

	Factor	N	Mean	Std. Deviation	Std. Error Mean
Water Male	Control	30	.62020	.094149	.017189
	Test	30	.35440	.116794	.021323

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Water Male	Equal variances assumed	0.000	0.990	9.705	58	0.000	0.265800	0.027389	0.210975	0.320625
	Equal variances not assumed			9.705	55.500	0.000	0.265800	0.027389	0.210922	0.320678

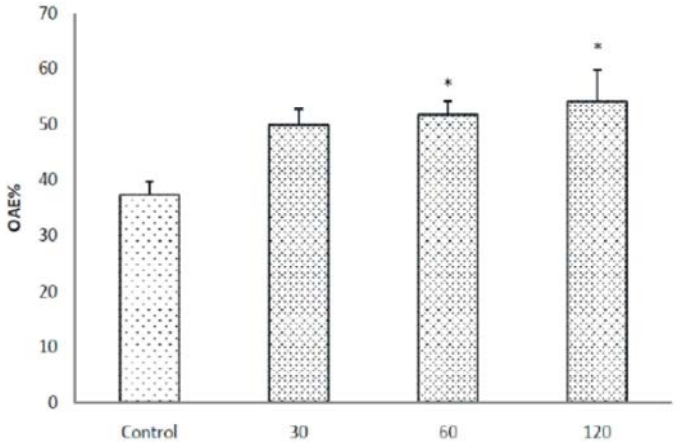

Fig. 1: The effects of injection of 30, 60 and 120 µg/kg LPS doses to mother on the eighth day of gestation on the adult newborn number entries into the open arms compared to the control group which received saline only. (p<./05 ** p <./01 *indicates difference from the control group)

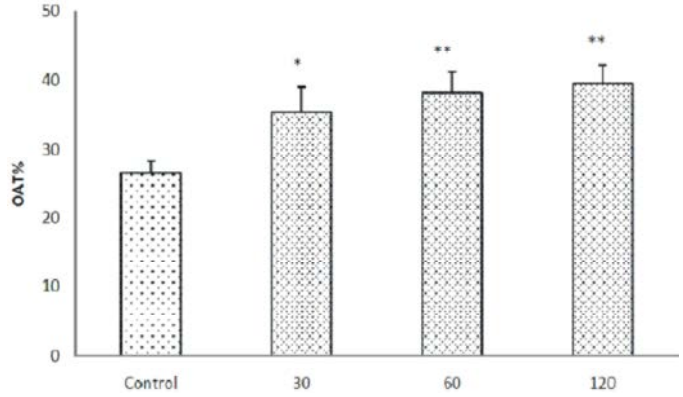

Fig. 2: The effects of injection of 30, 60 and 120 LPS doses to mother on the eighth day of gestation on the adult new born time spent in the open arms compared to the control group which received saline only. (p<./05 ** p <./01 *indicates difference from the control group)

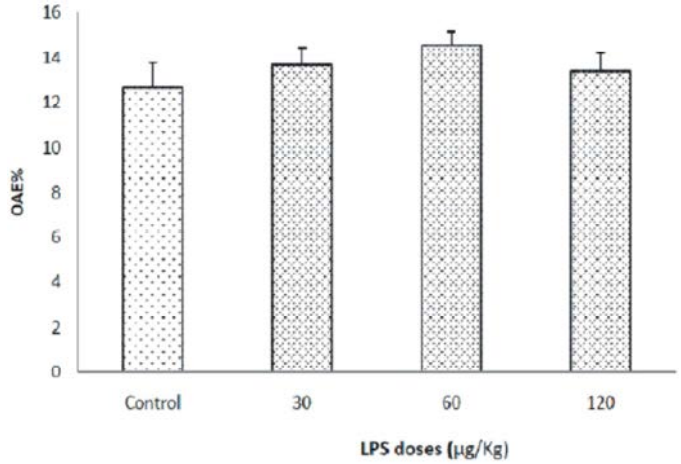

Fig. 3: The effects of injection of 30, 60 and 120 LPS doses to mother on the eighth day of gestation on motor activity of adult new born compared to the control group which received saline only. (p<./05 ** p <./01 *indicates difference from the control group)

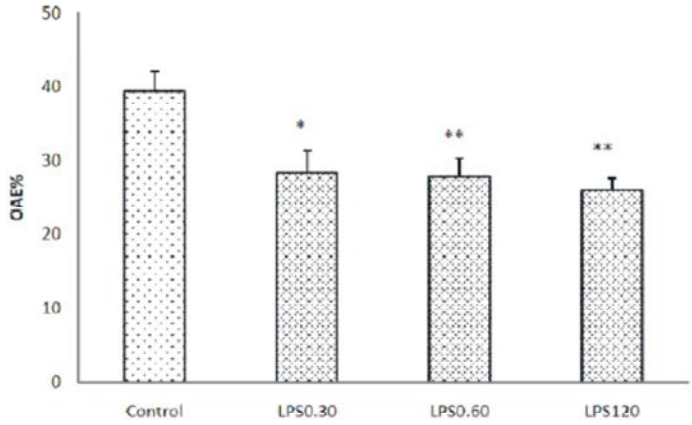

Fig. 4: The effects of injection of 30, 60 and 120 LPS doses to mother on the eighth day of gestation on the mother number of entries into the open arms compared to the control group which received saline only. (p <./05 ** p <./01 *indicates difference from the control group)

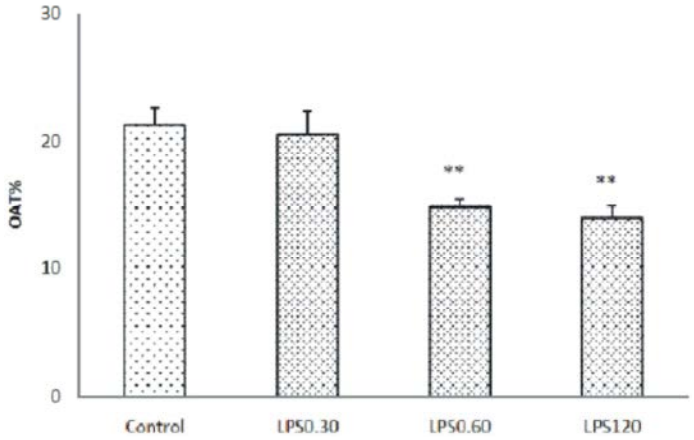

Fig. 5: The effects of injection of 30, 60 and 120 LPS doses to mother on the eighth day of gestation on mother time spent in the open arms compared to the control group which received saline only. (p <./05 ** p <./01 *indicates difference from the control group)

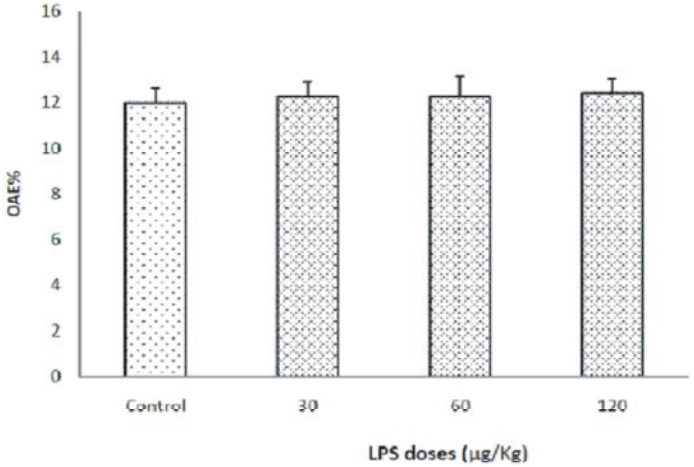

Fig. 6: The effects of injection of 30, 60 and 120 LPS doses to mother on the eighth day of gestation on mother motor activity compared to the control group which received saline only. (p <./05 ** p <./01 *indicates difference from the control group)

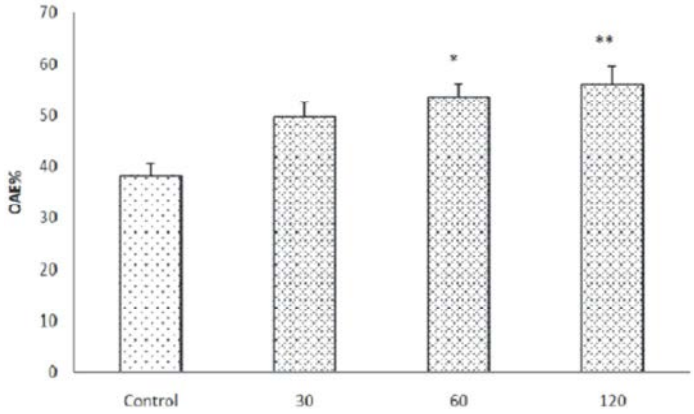

Fig. 7: The effects of injection of 30, 60 and 120 LPS doses to mother on the tenth day of gestation on adult newborn number of entries into the open arms compared to the control group which received saline only. (p <./05 ** p <./01 *indicates difference from the control group)

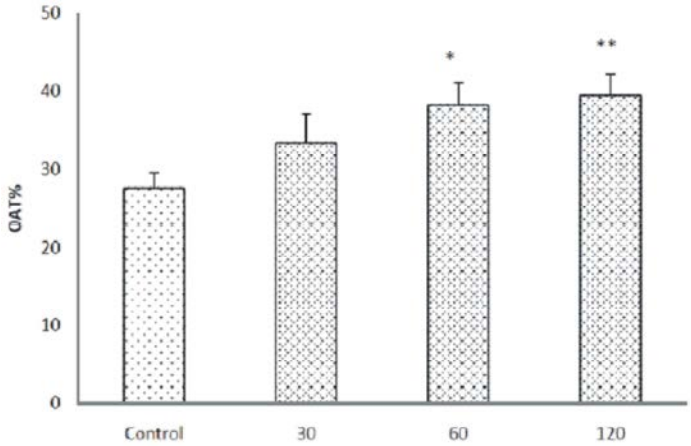

Fig. 8: The effects of injection of 30, 60 and 120 LPS doses to mother on the tenth day of gestation on adult newborn time spent in the open arms compared to the control group which received saline only. (p <./05 ** p <./01 *indicates difference from the control group)

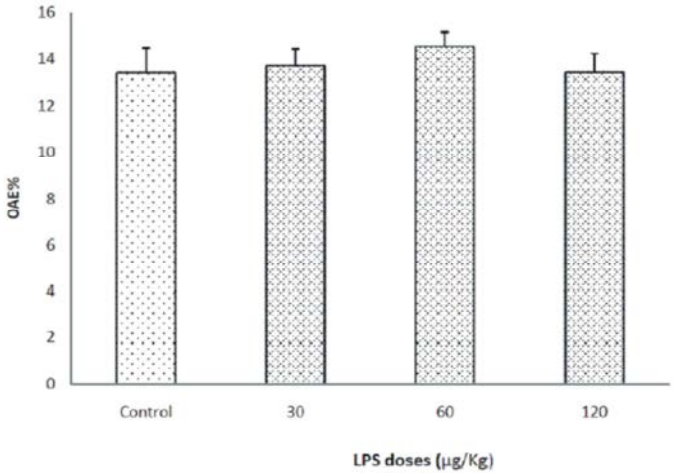

Fig. 9: The effects of injection of 30, 60 and 120 LPS doses to mother on the tenth day of gestation on motor activity of adult newborn compared to the control group which received saline only. (p <./05 ** p <./01 *indicates difference from the control group)

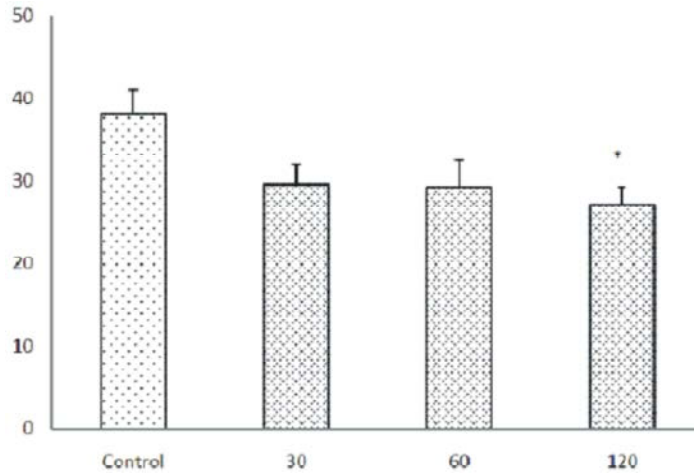

Fig. 10: The effects of injection of 30, 60 and 120 LPS doses to mother on the tenth day of gestation on their number of entries into the open arms compared to the control group which received saline only. (p <./05 ** p <./01 *indicates difference from the control group)

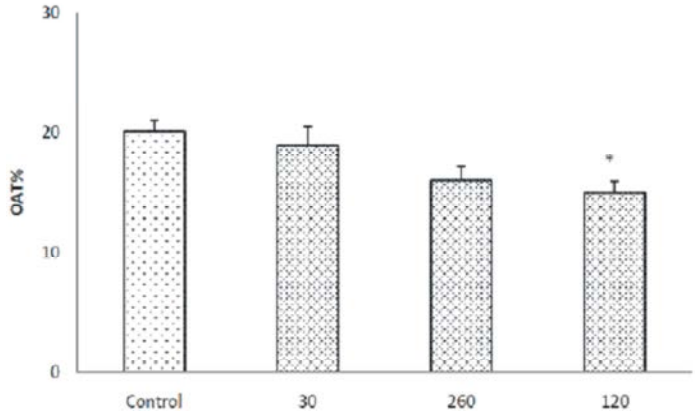

Fig. 11: The effects of injection of 30, 60 and 120 LPS doses to mother on the tenth day of gestation on their time spent in the open arms compared to the control group which received saline only. (p <./05 ** p <./01 *indicates difference from the control group)

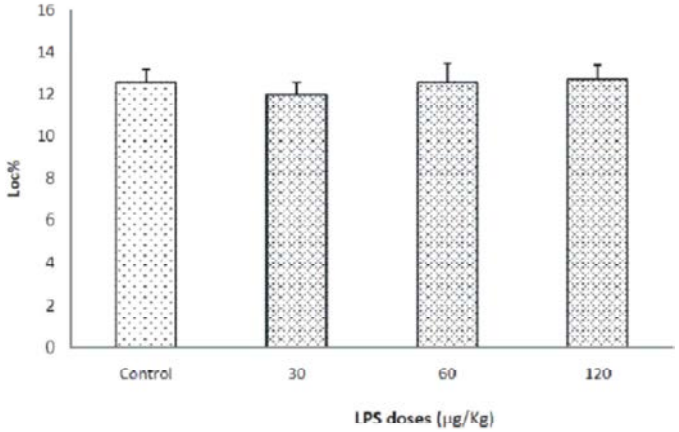

Fig. 12: The effects of injection of 30, 60 and 120 LPS doses to mother on the tenth day of gestation on their motor activity compared to the control group which received saline only. (p <./05 ** p <./01 * indicates difference from the control group)

None of the doses had significant effects on mothers' motor activity.

Water and Food Test: The T-test was used to analyze food and water data.

A separate comparison of the amounts of food and water consumption between control females and those which were affected by LPS at the embryonic stage and a separate comparison of the amounts of food and water consumption between control males and those which were affected by LPS at the embryonic stage indicated differences between four groups (two groups for food consumption and the other two for water consumption).

DISCUSSION & CONCLUSION

The results of previous researches on the effects of toxins on nervous cells development suggest that exposure of embryo to even small amounts of toxins in the embryonic period, especially during the formation of the nervous centers, may cause loss of cells or defects in them and then may cause some complications such as Parkinson's or Multiple sclerosis (MS) in future [29]. In addition, effects of stimulating the immune system by introducing toxins into the body could result in hyperthermia and releasing of inflammatory cytokines, cause changes in mothers' food consumption patterns, which in turn results in adverse effects on fetal growth and development. Hematological characteristics are important tools that can be used as an effective and sensitive index to monitor physiological and pathological changes [34].

Our study focused on the formation of the nervous centers, investigating the effects of LPS in different doses and on different days of pregnancy on the behavioral patterns of mothers and the fetuses. Obviously, there are too many variables to measure; in light of this we studied changes in anxiety levels and changes in water and food consumption in mothers and newborns. From among infectious agents, we selected lipopolysaccharide because it is a pyrogenic factor acting as an antigenic agent which can generate many changes into the immune system. Then, *Salmonella* was selected because it is a zoonotic microorganism and pathogenic in humans and mice and its pathogenicity in mice has been well documented [22]. NIH mice have many similarities to humans in terms of evolution and this is why most drug tests and experiments which cannot be carried out on humans is done on this animal.

Even this experiment can be extended to indicate what parts of the mouse brain are affected by the introduction of toxins throughout its brain development. Moreover, the findings can be generalized to the human brain.

Similar researches have studied the effects of other bacterial products on nervous system and suggested several solutions [23]. For example, Pacheco-López showed that *Staphylococcus aureus* toxin treatment caused significant differences in the endocrine neurobiological structure and behavior [24]. These observations were completed and approved in 1999. Nakagawa *et al*. also studied the inhibitory effects of anisodamine on super antigen [25].

According to other scientists, Cook in Albert Einstein College of Medicine as a case in point, have also done a research on this subject. Cook and his colleagues carried out a study on the measurement of Staphylococcal Enterotoxin B in Serum and Culture Supernatant with a Capture Enzyme-Linked Immuno sorbent Assay [30]. Furthermore, Boka G. worked on cytokines derived by entry of antigen to the body and its effects on Parkinson [31].

Entry of any toxins with infectious agents can cause many risks in the period after birth, most of which are unknown. The issue gets more complicated when the mother's body gets exposed to two or more antigens with synergistic interaction effects.

In 2001, study of the double effect of super antigen *Staphylococcal* TSST-1 with LPS on the mortality of mice showed that these toxins can reinforce each other and can synergistically cause death in doses that cannot lead to any death when applied alone[26].

Here, we only examined the effect of LPS on mice brain and offspring’s and choose anxiety as an index of physiological effects. Fear and anxiety warn people to be ready for facing the problems. People need postural control and maintaining balance in their activities of daily living. Nowadays, postural perturbations are used by clinicians to increase brain activities in patients with different mental and psychological problems [33]. LPS can induce multiple and complex effects *in vivo*, each of them can be a complicated factor in the body.LPS, by virtue of its effect on the immunity system and an increase in cytokines such as IL-1, IL-2, TNF-α, leads to hyperemia regardless of sex and pregnancy. Increasing anxiety level in pregnant females is contradicted with decreasing anxious level in those whom injected in embryo era and can only be defined by misevaluation of nervous system

caused by toxin entry prior to the birth. Neurotransmitters especially gamma amino butyric acid (GABA) and sensory receptors play a key role in control of anxiety related behavior. Hence, any damage to these centers or any changes in neurotransmitters secretion can lead to fluctuation in stress and anxious behavior. The GABA receptors are a class of receptors that respond to the neurotransmitter GABA, the chief inhibitory neurotransmitter in the vertebrate central nervous system. GABA, also known as stress index in physiological tests, may related to this pattern of decreasing anxiety level in cause of inhibitory effect of toxin and can be evaluated accurately in further studies as it was shown that entry of toxins in pregnancy period can lead to pathological disorders such as decline in neuron cells [27].

Results derived from comparing the offspring born from pregnant females and born from control group females suggest that injection on the eighth and tenth day affects the forming nerve centers, reduces the level of anxiety and increases the percentage of open arm entries (OAE) and seconds spent in the open arms (OAT) compared to the control group, which indicate a significant difference in anxiety level. However, the injection of pregnant females leads to reduction in their time spent in open arms and in the number of open arm entries, which is indicative of an increase in anxiety levels. Although, this increase in anxiety levels in the second group of mice was significant only in dose of 120 µg/kg. However, none of the doses have significant effects on motor activity, confirming the percentages of OAE and OAT. The increased anxiety levels in mothers with a decrease in level of anxiety in newborns which had been exposed to the toxin in their embryonic period is inconsistent and interoperate only by effect on formation of nerve centers in embryo. In addition to behavioral changes, changes in dietary pattern were registered by measuring the amount of water and food consumption. Toxin injections lead to reduced food and water consumption, especially in the first six hours after injection in which body temperature is high due to fever. Body weight is regarded as a non-specific indicator of general wellbeing of animals. Reduction in body weight is an indicator of decline in general health conditions [32]. This can also be true for body temperature. Body temperature and body weight assay were out of objection in our study. So, these parameters did not record but touching the mice, this change could be felt obviously. These changes in food and water consumption in rats that were injected with the toxin were significant in the week in which records were done. Overall, it can be concluded that toxin may affect the absorption of water and food centers or centers that cause perception of hunger. This effect is significant both in males and in females.

REFERENCES

1. Rietschel, E.T., H. Brade, O. Holst, L. Brade, S. Müller-Loennies, U. Mamat, U. Zähringer, F. Beckmann, U. Seydel, K. Brandenburg, A.J. Ulmer, T. Mattern, H. Heine, J. Schletter, S. Hauschildt, H. Loppnow, U. Schönbeck, H.D. Flad, S. Hauschildt U.F. Schade, F. Di Padova, S. Kusumoto and R.R. Schumann, 1996. Bacterial endotoxin: chemical constitution, biological recognition, host response and immunological detoxification. Curr. Top. Microbiol. Immunol., 216: 39-81.
2. Pugin, J., R.J. Ulevitch and P.S. Tobias, 1995. Mechanism of cellular activation by endotoxin. Prog. Surg., 20: 8-17.
3. Müller-Loennies, S., U. Zähringer, U. Seydel, S. Kusumoto, A.J. Ulmer and E.T. Rietschel, 1998. What we know and don't know about the chemical and physical structure of lipopolysaccharide in relation to biological activity. Prog. Clin. Biol. Res., 397: 51-72.
4. Meyer, U., J. Feldon and S.H. Fatemi, 2009. *In-vivo* rodent models for the experimental investigation of prenatal immune activation effects in neurodevelopmental brain disorders. Neurosci Biobehav., Rev., 33: 1061-1079.
5. Atladóttir, H.O., P. Thorsen, L. Østergaard, D.E. S chendel, S. Lemcke, M. Abdallah and E.T. Parner, 2010. Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. J. Autism Dev. Disord., 40: 1423-1430.
6. Kirsten, T.B., G.P. Chaves-Kirsten, L.M. Chaible, A.C. Silva, D.O. Martins, L.R. Dagli, A.S. Torrão, J. Palermo-Neto and M.M. Bernardi, 2012. Hypoactivity of the central dopaminergic system and autistic-like behavior induced by a single early prenatal exposure to lipopolysaccharide. J. Neuro Sci. Res., 90: 1903-1912.
7. Kirsten, T.B., M. Taricano, P.C. Maiorka, J. Palermo-Neto and M.M. Bernardi, 2010. Prenatal lipopolysaccharide reduces social behavior in male offspring. Neuroimmunomodulation, 17: 240-251.
8. Meyer, U., J. Feldon and K. Yee, 2009/ A Review of the Fetal Brain Cytokine Imbalance Hypothesis of Schizophrenia Schizophr Bull., 35(5): 959-972.

9. Kirsten, T.B., B.P. De Oliveira, A.P. De Oliveira, K. Kieling, W.T. De Lima, J. Palermo-Neto and M.M. Bernardi, 2011. Single early prenatal lipopolysaccharide exposure prevents subsequent airway inflammation response in an experimental model of asthma. Life Sci., 89: 15-19.
10. Sezonov, G., D. Joseleau-Petit and R. D'Ari, 2007. *Escherichia coli* Physiology in Luria-Bertani Broth. J. Bacteriol., 189: 8746-8749.
11. Westphal, O. and K. Jann, 1965. Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. In: Whistler RL, Wolfan ML, editors. Methods in Carbohydrate Chemistry. New York: Academic Press, pp: 83-91.
12. Pakzad, I., A. Rezaei, M.J. Rasaei, H.A. Zavaran, A. Kazeminejad, B. Tabaraei and S. Raivandi, 2004. Extraction, purification and detoxification of *Brucella abortus* LPS and biological activities evaluation Hakim, 6(4): 79-73.
13. Rezania, S., N. Amirmozaffari, B. Tabarraei, M. Jeddi-Tehrani, O. Zarei, R. Alizadeh, F. Masjedian and A.H. Zarnani, 2013. Extraction, purification and characterization of lipopolysaccharide from *Escherichia coli* and *Salmonella typhi*. Avicenna J. Med. Biotechnol., 3: 3-9.
14. Yin, E.T., C. Galanos, S. Kinsky, R.A. Bradshow, S. Wessler, O. Lüderitz and M.E. Sarmiento, 1972. Picogram-sensitive assay for endotoxin: gelation of Limulus polyphemus blood cell lysate induced by purified lipopolysaccharides and lipid A from Gram-negative bacteria. Biochem. Biophys. Acta, 261: 284-289.
15. Bell, M. and J. Hallenbeck, 2002. Effects of intrauterine inflammation on developing rat brain. Journal of Neuroscience Research, 70: 570-579.
16. Kaufman, M.H., 1992. The atlas of mouse development. pp: 6-26 UK: Academic Press, Edinburgh University.
17. Wei, Y.L., X.H. Li and J.Z. Zhou, 2007. Prenatal exposure to lipopolysaccharide results in increases in blood pressure and body weight in rats. Acta Pharmacologica Sinica, 28: 651-656.
18. Kirsten, T., M. Taricano, J.C. Flório, J. Palermo-Neto and M.M. Bernardi, 2010. Prenatal lipopolysaccharide reduces motor activity after an immune challenge in adult male offspring. Behavioural Brain Research, 211: 77-82.
19. Merlot, E., D. Couret and W. Otten, 2008. Prenatal stress, fetal imprinting and immunity. Brain, Behavior and Immunity, 22: 42-51.
20. Mednick, S.A., R.A. Machon, M.O. Huttunen and D. Bonett, 1988. Adult schizophrenia following prenatal exposure to an influenza epidemic. Archives of General Psychiatry, 45: 189-192.
21. Urakubo, A., L. Jarskog, J. Lieberman and J. Gilmore, 2001. Prenatal exposure to maternal infection alters cytokine expression in the placenta, amniotic fluid and fetal brain. Schizophrenia Research, 47: 27-36.
22. Solati, J., M.R. Zarrindast and A.A. Salari, 2010. Dorsal hippocampal opioidergic system modulates anxiety-like behaviors in adult male Wistar rats. Psychiatry and Clinical Neurosciences, 64: 634-641.
23. Curtiss, R., S.M. Kelly and J.O. Hassan, 1993. Live oralavirulent *Salmonella* vaccines. Vet. Microbiol., 37: 397-405.
24. Totowa, N.J. Sophie Vasseur, Albrecht Hoffmeister Andrés Garcia-Montero, Marc Barthet, S. Vasseur, A. Hoffmeister, A. Garcia-Montero, M. Barthet, L. Saint-Michel, P. Berthézène, F. Fiedler, D. Closa, J.C. Dagorn and J.L. Iovanna, 2003. Mice with targeted disruption of p8 gene show increased sensitivityto lipopolysaccharide and DNA microarray analysis of livers revealsan aberrant gene expression response. BMC Gastroenterology, 8: 25.
25. Pacheco-López, G., M.B. Niemi, W. Kou, M. Härting, A. Del Rey, H.O. Besedovsky and M. Schedlowski, 2004. Behavioural endocrine immune-conditioned response is induced by taste and superantigen pairing. Neuroscience, 129: 555-562.
26. Nakagawa, S., K. Kushiya, I. Taneike, K. Imanishi, T. Uchiyama and T. Yamamoto 2005: Specific inhibitory action of anisodamine against a staphylococcalsuperantigenic toxin, toxic shock syndrome toxin 1 (TSST-1), leading to down-regulation of cytokine production andblocking of TSST-1 toxicity in mice. Clinical and Diagnostic Laboratory Immunology, 12: 399-408.
27. Dinges, M.M. and P.M. Schlievert, 2001. Role of T cells and gamma interferon during induction ofhypersensitivity to lipopolysaccharide by toxic shocksyndrome toxin 1 in mice: Infection and Immunity, 69: 1256-1264.
28. Rounioja, S.J. Räsänen, M. Ojaniemi, V. Glumoff, H. Autio-Harmainen and M. Hallman, 2005. Mechanism of acute fetal cardiovasculardepression after maternal inflammatorychallenge in mouse. The American Journal of Pathology, 166: 1585-1592.

29. Oppenheim, R.W., 1991. Cell Death During Development of the Nervous System Annual Review of Neuroscience, 14: 453-501 (Volume publication date March 1991) DOI:10.1146/annurev.ne.14.030191.002321.
30. Cook, X. Wang, N. Robiou and B.C. Fries, 2007: Measurement of Staphylococcal Enterotoxin B in Serum and CultureSupernatant with a Capture Enzyme-Linked Immunosorbent Assay Albert Einstein College of Medicine.
31. Nagatsu, T., M. Mogi, H. Ichinose and A. Togari, (2000) Cytokines in Parkinson's disease Advances in Research on Neurodegeneration, pp: 143-151 DOI 10.1007/978-3-7091-6284-2_12PrintISBN978-3-7091-7246-9 Online ISBN 978-3-7091-6284-2 Publisher Springer Vienna Copyright Holder Springer-Verlag/ Wien.
32. Bhattacharya, S. and P. Kanti Haldar, 2013. Acute and Sub-Chronic Toxicity Study of Trichosanthes dioica Root in Mice American-Eurasian Journal of Toxicological Sciences, 5(1): 30-35.
33. Rahimi, A., 2014. Postural Perturbations. Toxic or Helpful for Brain? American-Eurasian Journal of Toxicological Sciences, 6(1): 09-14.
34. Erhunmwunse, N.O. and M.O. Ainerua, 2013. Characterization of Some Blood Parameters of African Catfish (*Clarias gariepinus*) American-Eurasian Journal of Toxicological Sciences, 5(3): 72-76, ISSN 2079-2050.