Effects of Toluene and Formaldehyde on Oogenesis in Adult Female Mice

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Abstract

Background: The present study was designed to investigate the effects of toluene and formaldehyde inhalation on the ovaries of adult female mice at room temperature.

Material & Methods: Mice were divided into three groups: control group, toluene exposed and formaldehyde exposed groups. The female mice were exposed to (3 ml = 300 ppm toluene) and (2.5 ml=300 ppm formaldehyde) 3 hours daily for 21 days. In this study a haemotoxlyin and eosin stains as well as the periodic acid Schiff (PAS) were used in order to illustrate the histological structures of the ovaries.

Results: Female exposed to both solvents showed a significant decrease in their weights. Histological examination of the ovaries of the exposed mice (either to toluene or formaldehyde) revealed an increase in thickness of zona pellucida of ovarian follicles, significant increase in the number of primary, secondary and Graffian follicles with decrease in the number of primordial follicles after inhalation comparisons to the normal groups.

Discussions: The results indicate that exposure to these organic solvents may suppress the central nervous system that contains vital centers which leads to reduction of mice weight.

Conclusion: Both solvents may cause injuries to the ovaries followed by changes of the histological structure for them. This might be due to disruption of endocrine function which leads to suppression of the releasing of gonadotropins hormones.

Key word: Toluene, Formaldehyde, Oogenesis.

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Introduction

None polar organic Solvents are chemically heterogeneous compounds that all share the properties of dissolving fats, oils, resin, cellulose, acetate and cellulose nitrate. This common feature make them widely use in industry, such as paint, lacquer pesticides, plastics, explosive, rubber, cellulose and in pharmaceutical and leather industry [1]. The most important toxicological properties of none organic solvents are their ability to evaporate and to dissolve fats. By dissolving fats, organic solvents can damage haematopoietic tissues, reproductive system, skin and parenchymatous organs rich fats [2]. None polar organic solvents such as toluene and formaldehyde are probably the most widely used substances in the workplace (laboratories) and among the most dangerous. Also the risks are multiple and varied, some are important even before conception. Moreover, documentation is available on the risks for fetuses brought by male parent, before and after conception. For example, it has been reported that exposure of male to anaesthetic gases can lead to adverse pregnancy in their unexposed wives [3]. Anderson et al 1994 suggested that the male

can contribute to the incidence of malformation in fetuses if substances observed in his body are secreted into the seminal fluid, subsequently absorbed in the vaginal mucosa during intercourse, after which they can affect a developing embryo or fetus [4].

Exposure to organic solvents may occur in a variety of setting, including occupational exposure in the workplace, non-occupational or incidental exposure to the home (using solvents to clean floor, extra) or exposure as the result of solvent abuse. Human reproductive and embryogenesis involves multiple processes such as proliferation, migration, differentiation and organogenesis, precisely timed events that may be susceptible to environmental insult at different stages. Environmental factors interfering with specific biological process may consequently induce the range of different developmental effects. These effects may be manifested during pregnancy as pregnancy loss, at the birth time as adverse pregnancy outcome, or in postnatal life as functional defects [5].

There are numerous organic solvents, therefore, this issue of risk will focus on two common organic solvents which found in the workplace or home, or are frequently abused such as toluene and formaldehyde. However, from our knowledge using internet and scientific journals, no adequate studies were found about the effect of toluene and formaldehyde on the morphological and histological changes of the ovaries of the female reproductive system. Therefore the first goal of this study is to assist the effects of toluene and formaldehyde on the ovaries of the adult mice.

Materials and Methods

175 albino female mice, approximately (8 – 16) weeks and weighting (25 - 30) gm were used. The mice were produced and housed in the animal house facilities of the department of anatomy/ College of Medicine/ Haweler Medical University. They were maintained under a controlled light cycle (12h light, 12h dark), at (22 ± 2C°) and with free access to food and water.

Pilot study:

Pilot studies were conducted in order to examine the lethal and affected dose, time and duration of inhalation for each solvent. In order to achieve these measurements, a special box was manufactured of iron and glass with (30 x 30 x 30) cm dimension with operable slide for keeping mice during inhalation. This preliminary experiment includes 50 female adult mice, divided into two groups. Each group includes 25 mice subdivided into 5 groups (each subgroup includes 5 mice). The first group assigned for toluene and the second group assigned for formaldehyde.. The animal weight was measured before and after inhalation of each solvent to examine the solvents inhalation effect on animal weight. Inhalation of toluene or formaldehyde started from (20ml descending to 15, 10, 5, 3, 1) ml.

All animals died when exposed to (20 ml) after 20 minutes, to (15 ml) after 2 hour, to (10 ml) after 2 days, 3 hours daily. Most animals died when exposed to (5 ml) after 7days, 3 hours daily. While some were survived when exposed to (3ml) of toluene, 3 hours daily for 21 days. So this dose was selected as affected dose. At the end of each inhalation animals' weight were recorded, killed by cervical dislocation and then the ovaries were rapidly removed cleared from fat and mesentery using forceps and scissors. For operations, each organ was fixed in bouin's fixative for histological studies.

Exposure of female mice to Toluene and formaldehyde:

One hundred healthy Albino female mice were selected to study the effects of toluene and formaldehyde on the histological appearance of the ovaries (50 mice for each solvent). Before starting inhalation, the mice

weight was recorded. 10 gm of cotton immersed in (3 ml = 300 ppm) of toluene and in (2.5 ml=300 ppm) of formaldehyde, and placed at one corner of the box. Almost 10 mice were inserted each time in the box for each solvent. The prisoned animals were allowed to eat and drink freely during the 3 hours of inhalation time.

Toluene or formaldehyde inhalation was repeated for 21 days, 3 hours daily. At the end of inhalation duration, the mice weight was recorded again to examine the effects of toluene or formaldehyde inhalation on weight, then the animals were killed by cervical dislocation and the ovaries were rapidly removed. For processing, each organ was fixed overnight in Bouin's fixative for histological studies. Each inhalation repeated 5 times.

Control animals:

Twenty-five normal healthy female albino mice were also inserted in cage for same period, killed by cervical dislocation and ovaries removed immediately from the sacrificed animals, fixed in Bouins fixative for histological studies. These sections compared with that of exposed animals either to toluene or formaldehyde.

Preparation of tissue sections: Each fixed ovary was subjected to the Bancrof"s procedure [6].

Haematoxylin and eosin staining (HE):

In order to illustrate the histological structures of the ovaries, representative sections were taken for routine histology using HE stains. Five µm paraffin sections were prepared according to Bancrof"s protocol [6]. The number and type of different ovarian follicles such as primordial, primary, secondary and Graffian follicles were measured.

Periodic Acid Schiff stain (PAS):

This experiment was designed to detect the carbohydrate localization in the ovaries sections before and after inhalation and thus determine whether these carbohydrate undergo considerable various of glycohistochemical changes during oocyte development or ovarian follicle maturation . The representative sections were subjected to the protocol of Humason [7]. Using PAS stain of the thickness of the zona pellucida of each oocyte was examined.

Either For HE or PAS, a minimum of 4 sections, prepared on at least 10 separate occasions, from different female mice, were scored for each ovary.

Statistical analysis:

Statistical analysis was performed by SPSS (Statistical package for social sciences) software program using complete randomized design (CRD) and analysis of variance (ANOVA) for comparison among the means. All the data are expressed as (mean ± S.E.) and Duncan's test was used as a multiple comparison test.

Results

Female mice exposed inhaled toluene for 21 days 3 hours daily showed a significant decrease in their weight at ($p<=0.05$) with average vale (25.39) grams ± S.E in comparison with the average value of weight before toluene inhalation (27.50) ± S.E (Tab. 1). Female mice inhaled formaldehyde for 21 days 3 hours daily showed a significant decrease in their body weight at ($p<=0.05$) with mean vale (24.63) gm ± S.E in comparison with the average value of body weight before FA inhalation (27.04)gm ± S.E (Tab. 2). A significant decrease in the number of primordial follicles was detected in the ovaries of mice exposed to toluene by inhalation at ($p<=0.05$) with average value (36.63) ± S.E in comparison to the control mice with mean value (47.33) ± S.E (Tab. 2 and Fig. 1). The number of primary follicles within the ovaries of mice exposed to toluene by inhalation was significantly increased at ($p<=0.05$) with average value (13.29) ± S.E in comparison with control group with average value (9.56) ± S.E (Tab. 2 and Fig.

2). A significant increase in the number of secondary follicles was detected in the ovary of mice exposed to toluene by inhalation at ($p<=0.05$) with average value (6.46) ± S.E compared with control group with average value (4.03) ± S.E (Tab. 2 and Fig. 3). Regarding the number of Graffian follicles, the results showed that there is a significant increase in the number of Graffian follicles in the ovaries of mice exposed to toluene by inhalation at ($p<=0.05$) with average value (3.1) ± S.E compared with control group with average value (0.4) ± S.E (Tab. 2 and Fig. 4). The study showed that there is a marked thickness in the zona pellucida of ovarian follicles of ovaries sections from mice exposed to toluene by inhalation (plate 2) if compared with (plate 1) of control group.

A significant decrease in the number of primordial follicles was detected in the ovaries of mice exposed to formaldehyde by inhalation at ($p<=0.05$) with average value (36.16) ± S.E in comparison to the control mice with average value (47.33) ± S.E (Tab. 2 and Fig. 1). The number of primary follicles within the ovaries of mice exposed to formaldehyde by inhalation was significantly increased at ($p<=0.05$) with average value (18.56) ± S.E in comparison with that of control group with average value (9.56) ± S.E (Tab. 2 and Fig. 2). A significant increase in the number of secondary follicles was detected in the ovary of mice exposed to formaldehyde by inhalation at ($p<=0.05$) with average value (9.16) ± S.E compared with control group with average value (4.03) (Tab. 2 and Fig. 3). Regarding the number of Graffian follicles, the results showed that there was a significant increase in the number of Graffian follicles in the ovaries of mice exposed to formaldehyde by inhalation at ($p<=0.05$) with average value (1.83) ± S.E compared with control group with average value (0.4) ± S.E (Tab. 2 and Fig. 4). Concerning to the Sections of ovaries stained with Periodic Acid Schiff (PAS), the results showed that there is a marked thickness in the zona pellucida of ovarian follicles of ovaries sections from mice exposed to formaldehyde by inhalation (plate 4) if compared with (plate 3) of control group

Discussion

The study showed the weight decreased significantly after exposure to toluene. This is in agreement with what reported by Akiko [8, 9, 10]. This decrease in the weights may be as a result that the central nervous system CNS is the primary target organ for toluene toxicity in both humans and animals for acute and chronic exposures.

The current study revealed that the average number of primary, secondary and graffian follicles are significantly higher in toluene-exposed group than the control group, while the mean number of primordial follicles is lower in exposed group compared to control group. The ovaries are target organs for injury caused by many chemicals [11]. The female reproductive system is complex and variety of factors, both endogenous and exogenous hormones can affect the functioning of this system. The ovary is the central component of the hypothalamic pituitary-ovarian axis. It functions cyclically to produce a single oocyte. The follicle is the basic functional unit of the ovary and consists of an immature oocyte surrounded by multiple layers of specialized follicular cells and the theca cells [12]. There are many solvents (including toluene) which cause damage to ovaries, causing disruption in follicular growth process by affecting the luteal function [13].

Chemicals exposure leads to significant alterations in the levels of the reproductive hormones in the animals. The levels of estrogen and progesterone were significantly suppressed after exposure to chemicals this suppression was also indicated by the arrest of the estrous cycling of the female rats at

diestrus [14]. The effects of solvents on the levels of these hormones in the rats show significant suppression of the level of luteinizing hormone LH of the female tested animals. Low level of LH may negatively affect other LH – dependent physiologic functions including ovulation. On the other hand, the elevation of estradiol and progesterone levels with a concomitant significant suppression of FSH in the female animals is consistent with a compensatory response arising from reduced negative feedback on the hypothalamus-pituitary levels by the lowered estrogen and progesterone levels. This suggests that the effect of the chemicals was at the level of the gonads [15].

Concerning the effect of FA, also a significant decrease in the body weight of both gender in formaldehyde exposed group was found, these results were in agreement with that of **[**16]**.** Predominant signs of short-term exposure to formaldehyde in humans are irritate the eyes, nose and throat, together with concentration-dependent discomfort, lachrymator, sneezing, coughing, nausea, salivation, vomiting, spasm and dyspnoea. Long -term exposure signs include focal ulceration in the stomach [17]. These effects lead to decreased food and water consumption, therefore reduced weight gain.

The results about the histological structure of ovaries showed that the average number of primary, secondary and graffian follicles is significantly higher in formaldehyde-exposed group than control group, while the mean number of primordial follicles is lower in exposed mice compared to control mice.

The exact mechanism of formaldehyde action toxicity is not clear, but it is known that it can interact with molecules on cell membranes (e.g., proteins and DNA) and disrupt cellular functions. High concentrations cause precipitation of proteins, which results in cell death. Once absorbed, formaldehyde is oxidized to formic acid and Co2, which may cause acid-base imbalance and a number of other systemic defects [18].

It is hypothesized that FA (and possibly many other substances) affect ovarian follicles of rats (differentiation of follicle somatic cells), leading to a disruption in ovum maturation (final stages of gametogenesis), and delaying the ovulation and may cause menstrual disorders in women occupationally exposed to formaldehyde [18].

Regarding the Periodic Acid Schiff (PAS) staining of zona pellucida of ovarian follicles, the results showed that mice exposed to either toluene or formaldehyde caused an increase in the thickness of zona pellucida of ovarian follicles. The zona pellucida is a thick extracellular coat that surrounds all mammalian ova and preimplantation embryos. The zona pellucida supports communication between oocytes and follicle cells during oogenesis; protects oocytes, eggs, and embryos during development and regulates interactions between ovulated eggs and free-swimming sperm during and following fertilization. [19].The environmental exposure to such as organic solvents and smoke increase the ZP thickness. The zona pellucida thickness of oocytes and embryos of non-smoking women was significantly thinner than those of active and passive smokers [20**].**

Formaldehyde is metabolized to formate and Co2 by formaldehyde hydrogenise (FDH). The toxicity of the latter and formaldeyde in humans as well as animals includes metabolic acidosis. Alcohol toxicity generates free oxygen free radicals which can initiate auto oxidation of lipids and in turn stimulate glycation of proteins. These reactions by free radical may accumulate with time and through alterations in protein structure and function, these oxidation products may contribute to the development of these changes. [21]. Presence of these free

radicals might produce these changes followed by increases in the thickness of the zona pellucida after exposure to toluene and formaldehyde.

In conclusion, the results of this study indicate that exposure to toluene and formaldehyde organic solvents may suppress the central nervous system that contains vital centers and this lead to reduction in the mice weight. On the other hand, inhalation of these solvents may cause injuries to the ovaries followed by changes in the histological structure of them due to disruption of endocrine function.

Table (1): (Mean ± S.E) of body weight in female mice before and after toluene and formaldehyde inhalation.

Groups	Body Weight (gm)			
	Toluene		Formaldehyde	
	Before exposure Mean ± S.E.	After exposur Mean ± S.E.	Before exposure Mean ± S.E.	After exposure Mean ± S.E.
Female	27.514 ±O.225 [a]	25.396 ±0.202 [b]	27.040 ± 0.252 [a]	24.638±0.240 [b]

Mean with same letters has non-significant differences **Mean with different letters has significant differences**

Table (2): Mean ± S.E. of the effects of toluene and formaldehyde exposure on the number of different follicles in ovary compared with Control group.

Groups	Ovarian follicles			
	Primordial follicles Mean ± S.E.	Primary follicles Mean ± S.E.	Secondary follicles Mean ± S.E.	Graffian follicles Mean ± S.E.
Control	47.332 ± 2.523 [a]	9.566 ± 1.99 [a]	4.033 ± 0.531[a]	0.4 ± 0.221 [a]
Toluene	36.633 ± 2.996	13.299 ± 1.598 [a]	6.466 ± 0.834	3.1 ± 0.481 [b]
Formaldehyde	36.166 ±1.066 [b]	18.232 ± 0.711 [b]	9.166 ± 1.018 [c]	1.833 ± 0.223 [c]

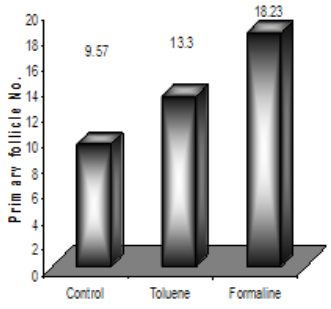


Figure (1): Effect of toluene and formaldehyde inhalation on the number of primordial follicles of ovaries compared with control group

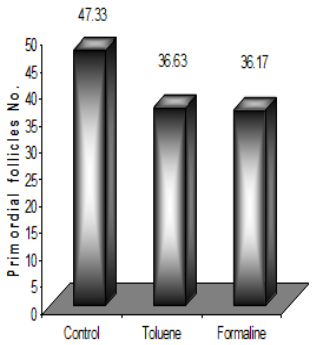


Figure (2): Effect of toluene and formaldehyde inhalation on the number of primary follicles of ovaries compared with control group

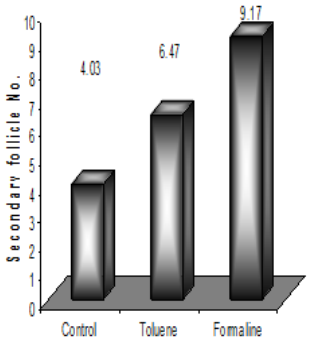


Figure (3): Effect of toluene and formaldehyde inhalation on the number of secondary follicles of ovaries compared with control group.

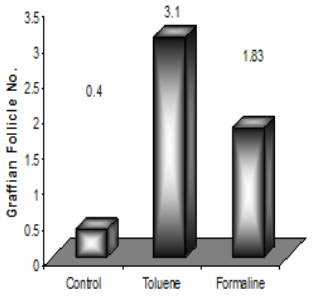


Figure (4) ; Effect of toluene and formaldehyde inhalation on the number of Graffian follicles of ovaries compared with control group.

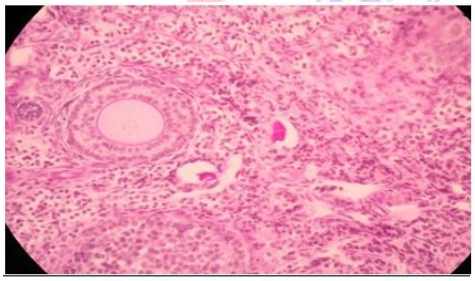


Plate (1): Section from normal mouse ovaries showing normal thickness of zona pellucida of ovarian follicle (ZP) (arrow). (PAS X200).

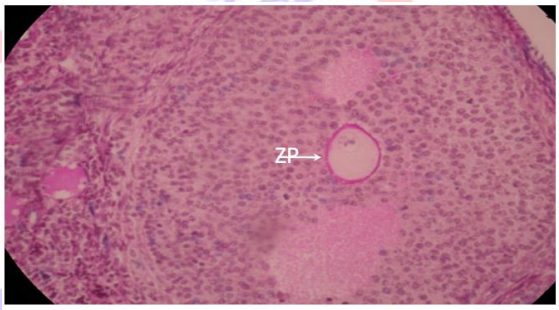
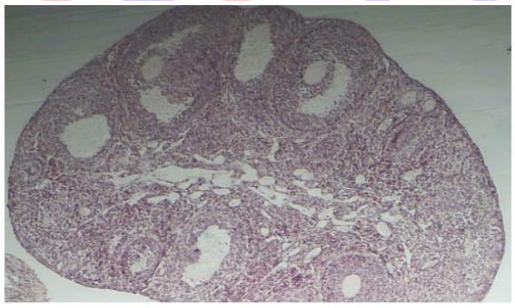


Plate (2): Section from mouse ovary after toluene inhalation showing moderate increase in the thickness of zona pellucida of ovarian follicle (ZP) (arrow). (PAS X200)



Plate(3):- section from ovary of female mouse exposed to formaldehyde showing ovarian follicles stained with H&E. (X200). MPOF= multilaminar primary ovarian follicle , SOF= secondary ovarian follicle ,GOF= graffian ovarian follicle

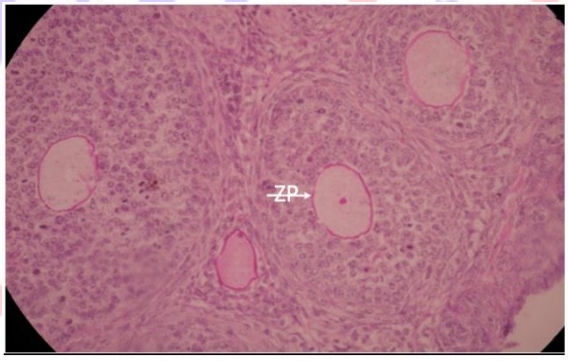


Plate (4): Section from mouse ovary after formaldehyde inhalation showing marked increase in the thickness of zona pellucida of ovarian follicles (ZP)(arrow). (PAS X200)

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