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*Section A-Research paper*

# Physiological and Biochemical Study of Modulation of Pituitary-Adrenal Axis, Pituitary-Gonadal Axis and Reproductive Efficiency Responses to Stress Induced by Exposure to Toluene in Rabbits

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## Abstract

Toluene is a widely abused inhaled solvent. The current study has been designed to conclude whether toluene abuse affect the pituitary-adrenal axis(PAA), reproductive functions of both sex of rabbits. Five–month–old male and female rabbits were exposed to toluene vapor inhalation (8000ppm; 2h/day) daily for 30 days resulted in a dose-dependent salivation and lacrimation were observed during exposure periods and intensified with recurrent contact. Suppression of total body weight gain and food utilization were experiential in toluene group. Females were exposed from 30 days before mating until day 7 of gestation. The serum and ovary of the rabbits were collected; ELISA was applied to determine the sex hormone(Estradiol (E2) and Progestron (P4), Testosterone(T), corticosterone ) in serum; the pituitary, adrenal, ovary samples were stained with haematoxilyne and eosin and the number of follicles was calculated. The sex hormone of the low concentration group treated with tolune was significantly lower than that of the control group($P \leq 0.05$);no significant difference was found in P4 and the number of follicles between different groups.

Testicular and reproductive toxicity was evaluated after males were exposed for 30 days, including the mating time. While mating behavior and fertility were not affected, there was a rise in fetal mortality and the frequency of dams with stillborn offspring in the toluene group. Microscopic examination of the epididymal region of the toluene group revealed a considerable decrease in sperm counts, sperm motility, and sperm quality, but no changes in testicular weight or intratesticular spermatogenesis were seen. Rabbits with ovaries that had been altered by exposure to 8000 ppm toluene produced sperm without tails. The weights of

the spleen, thymus, testes, and ovaries of rabbits exposed to 8000 ppm toluene were substantially lower than those of the control group, whereas the weight of the adrenal gland was significantly larger. Degenerative alterations in the testis, decreased density and motility of epididymal sperm, and a rise in the incidence of aberrant sperm were all found. Animals' fertility was reduced because the average fertility rate was altered. These results reveal reproductive failure in both sexes of rabbits exposed to inhaled toluene levels of 8000 ppm, which cause detectable testicular degeneration and affect the morphology of sperm in the epididymis and disturb sperm maturation. After being exposed to 8000 ppm toluene for 1 month, no discernible changes were seen in blood levels of luteinizing hormone, follicle-stimulating hormone, or testosterone. The medulla showed no significant changes, whereas the cortex became hypertrophied in those who inhaled toluene. Furthermore, adrenocortical cell size was dramatically increased in treated-rabbits compared to the control group.

The sex hormone in the serum of female rabbits is inhibited after exposure to a particular level of toluene.

Key word: Tolune, Pituitry gland, Reproductive, Stress.

## Introduction

A necessary shock on fundamental biological processes may be triggered when a living thing is exposed to external variables like poisons or stressors. The toxicological interaction of numerous stimi may cause new and different biological reactions, either qualitatively or quantitatively, when they are encountered simultaneously[1]. Due to the presence of additive and synergistic toxicological interactions, it is possible that harmful consequences may occur at exposure levels that would be regarded safe in the absence of the modulating factor[2].

Toluene, also known as toluol, methylbenzene, and phenyl methane, is an aromatic hydrocarbon that is utilized primarily as a component of gasoline (92%). Toluene is a byproduct of styrene manufacturing and coke-oven operations [4,5] and is used in the manufacturing of a wide variety of industrial chemicals (benzene, toluene diisocyanate, phenol, benzoy, benzoic acid, toluene sulfonates, nitrotoluene, vinyl toluene, and saccharin). Toluene is a common solvent for a variety of different materials, including paints, lacquers, and adhesives. Inhaling the vapors might be harmful. The majority of human exposure to toluene occurs through accidental or intentional inhalation of airborne toluene[8], which can occur during the manufacturing, shipping, and refueling processes for gasoline and toluene. Workers in the chemical and petrochemical industries, as well as those who apply paint and

dye, are at the greatest risk of accidental exposure to toluene. Toluene can be ingested through the use of gasoline, cosmetics, and rubber cement, among other products. Adhesives, inks, dyes, fabric paintbrush cleaners, stain removers, nail polish and cigarette smoke. Female rabbit sex hormone serum is suppressed after exposure to a threshold amount of toluene [9–15].

The aim of the study is to effect of inhalant abusing toluene on hypothalamus-pituitary-adrenal axis(HPA), hypothalamus-pituitary-gonads axis(HPG) and reproductive index in both sex rabbits.

## Material and Methods

**Experimental Materials:-**The Toluene that used in this experiment were obtained from the chemistry. Department, College of Science, Basrah University.

**Experimental Animals:-** forty local mature both sex of rabbits.

A total of 24 healthy domestic rabbits (*Lepus cuniculus domastica*) were briught from Iraqi local markers in Basra city. These rabbits were included both sex with 1500 to 2000 gm of weight and nine months age. During one month, rabbits were acclimated to their new environment at the animal house of Veterinary Medical College at University of Basrah. The animals in the experiment were housed in a climate-controlled room (24°C) with a twelve hours light/dark cycle, fed a feed consisted of hay in addition to green alfalfa (*Medicago sativa*), and given a prophylactic medicine against coccidiosis (Amprollium 1g/L of drinking water).

**Experimental Design:-**Rabbits were randomly classified into main 4 equal groups. The first group (6) male rabbits had been considered as controls. The second group (6) male rabbits treated with toluene was inhalated (8000ppm) to rabbits for1month. The third group (6) female rabbits had been regarded as control. The forth group (6) female rabbits treated with toluene was inhalated (8000ppm) to rabbits for 1month.

Male rabbits were housed in a 1:2 ratio with fertile females for two days as part of a fertility study. According to [14], the first day of pregnancy is considered to be the day that a vaginal smear is examined after the joining period. Male rabbits were separated into groups and given the extract to test orally beginning 30 days after mating.

**Sampling:-**

**Blood samples:-**

Samples of blood were obtained from each animal fasted rabbits (treated animals and controls), from the heart at the end of the experiment period, and permitted for clotting at

temperature of room to separate the serum for hormonal assay such as estrogen, progesterone, testesterone and corticosteron level by using ELSA Kits performed as previously described [16].

**Sacrifice and Preservation of Tissues: -**

Six animals/group were sacrificed (treated animals and controls). The adrenals glands, pituitary, ovaries, uterus and testes were eliminated directly, adhering tissues clean and weighed were reserved in 10% formaline for fixation to be ready for histopathological examination using the light microscopy [17].

**Evaluation of Fertility: -**

Using a microscope (40X), spermatozoa were evaluated for the following characteristics after being collected via mincing the cauda epididmus in a known volume of physiological saline (w/v) at 37 C:

**Implantation Site**

According to [18], pregnant rabbits were slaughtered immediately after giving birth to observe the impact of treatments on the implantation location of blastocytes. After being slaughtered, the animals' uteri were opened by making a longitudinal incision, revealing the blue implantation sites, which were counted. It was decided to measure the uterus and both ovaries for weight. Each animal's ovaries were removed, put in a plate, and counted for the corpa lutea number. The percentage of implantation success was considered as:

$$\text{Percentage of success (Ps)} = \frac{\text{No. of implants}}{\text{No. of corpa lutea}} \times 100$$

$$\text{Mutagenic Index} = \frac{\textbf{\textit{Number of dead implants}}}{\textbf{\textit{Total number of implants}}} \times \mathbf{100}$$

**Sperm Concentration(Count):-**

After diluting the sperm postponement with water (1:20), mixing the contents, and delivering a drop of the mixture into the Neubaure haemocytometer on opposite sides of the chamber of counting, the spermatozoa concentration was determined. After letting the haemocytometer settle for 5 minutes, sperm were calculated in the huge five squares and the result was represented as concentration of sperm in million. This procedure was taken from [19].

**Sperm Motility:-**

Microscopical evaluation of sperm motility followed the mowing down of sperm production. The amount of immotile sperm was first calculated, then the total sperm count was taken. According to [20], motility of sperm was reported as the proportion of active sperms to the total number of sperms.

**Viability of Sperm via Negrosin-Eosin Stain:-**

This method is utilized for determination of which sperm are viable and which are not. Drop some Negrosin-Eosin stain into the sperm solution on the slide, let it sit at 37 degrees Celsius for 5 minutes, and then have a look at it under the microscope. Red staining may be seen on the sperm cell's head after it has died. Yet, the Negrosin-Eosin-free spermatozoa were able to survive. sperm percentage of total sperm count, as determined by [21].

**Maturation of Sperm via Aniline-Blue:-**

Nuclear maturation was assessed via aniline-blue stain, depend on [22]. Blue-staining sperm nuclei were deemed to be of a less developed stage. Nevertheless, aniline-blue did not stain nuclear mature sperm. One hundred sperm preparations from each group were analyzed to determine the proportion of immature sperm.

**Sperm Morphology:-**

At the end of the five minutes at 37 degrees Celsius, a drop of Negrosin-Eosin stain was placed into the sperm deferment. Next, a clean slide was deposited with a drop of sperm suspension and the liquid was spread out to create a thin film. Air drying the film followed by microscopic examination for morphological alterations in sperm followed the protocol described in [19]. Amorphous, pin, and small head were selected as indicators of cranial abnormalities. Coiled flagellum, bent flagellum, and a bent flagellum tip were all noted as anomalies in the tail. The findings reveal the total prevalence of aberrant forms as a percentage.

**Hormonal assay:-**Determination of serum testosterone, estradiol(E2), progesterone(P4) and corticosteroid by,(ELISA assay).

**Statistical analysis:**

Data were analysed by one way analysis of varians (*t* test)[23].

## Results

**1-Changes in Weight of Body and Consumption of Food.**

There were abnormal clinical signs observed in animals treated with toluene by inhalation. Visible signs were noted during experiment period emaciation and rough hair. Body weight was registered in males and females in (Table1). There were statistically significant decrease ($p \leq 0.05$) in body weight related to exposure to toluene than in the control group. The food consumption in the treated group was decrease than that in the control group.

**Table (1) Effect of Toluene on Changes in Body weight in Males and Females Rabbits (Mean ± SD) (n=6)**

| Parameters / Treatments | Body Weight (g) | | | |
|---|---|---|---|---|
| | Males | | Females | |
| | Initial weight (g) | Final weight (g) | Initial weight (g) | Final weight (g) |
| Control (normal saline) | 1840 ±125.4 NS | 1985±93.54 A | 1550±108.01NS | 1700±101.49 A |
| Toluene | 1850±123.13 NS | 1475±169.55 B | 1516±85.08NS | 1100±114.01B |

**A,B,C= differences between groups, N=number of animals, P≤0.05 vs. control.**

**2-Steriods Hormones in Female Rabbits.**

The results in (Table2) described change in reproductive hormones in females treated with toluene by inhalation. There were statistically significant raise (p≤0.05) in progesterone, testesterone and corticosterone and significant decline (p≤0.05) in estrogen while in females rabbits related to exposure to toluene than in the control group.

**Table (2) Effect of Toluene on Steroids Hormones in Female Rabbits (Mean ± SD)(n=6)**

| Parameters / Treatments | Estrogen (µg/dl) | Progesterone (ng/ml) | Testesterone (ng/ml) | Corticosteron (µg/dl |
|---|---|---|---|---|
| Control (normal saline) | 49.95±4.32 A | 0.79±0.014 B | 0.70± 0.013 B | 4.93±0.013 B |
| Toluene | 36.21±4.18 B | 1.28±0.025 A | 0.91±0.011 A | 9.89±0.021 A |

**A,B,C= differences between groups, P≤0.05 vs. control, N=number of animals.**

**3-Effect of Toluene on Relative Organs Weight in Female Rabbits.**

The result in Table (3) described relative weight in females treated with toluene by inhalation. There were statistically significant increase (p≤0.05) in weight of adrenal glands and uterus while significant decrease (p≤0.05) in ovaries, thymus and spleen in females rabbits related to exposure to toluene than in the controls.

**Table (3) Toluene impact on Relative Organs Weight in Female Rabbits (Mean± SD) (n=6)**

| Weight Of organs (g) / Treatments | Adrenal Glands (g) | Ovaries (g) | | Thymus (g) | Spleen (g) | Uterus (g) |
|---|---|---|---|---|---|---|
| | | Right | Left | | | |
| Control (normal saline) | 0.028±0.0015 B | 0.030±0.0011 A | | 0.72±0.034 A | 0.70±0.001 A | 0.25±0.011 B |

| | | | | | |
|---|---|---|---|---|---|
| **Toluene** | **0.055±0.0001 A** | **0.014±0.0011 B** | **0.34±0.031 B** | **0.27±0.001 B** | **0.33±0.016 A** |

**A,B = differences between groups, P≤0.05 vs. control, N=number of animals.**

**4-Effect of Successful Implantation %, Corpora Luteum Number and Toluene on Implantation Site in Sacrificed Female Rabbits**

Implantation site, corpora luteum number and successful implantation % are illustrated in the Table (4).

The results displayed a significant (P≤0.05) reduction of implantation site in toluene-treated female rabbits in comparison with controls.

Number of corpora luteum a non-significant (P≤0.05) decrease in toluene-treated female rabbits in comparison with controls.

Successful implantation % low in toluene-treated female rabbits is 41.66% in comparison with controls 79.87%.

**Table (4) Effect of Toluene on Successful Implantation %, Number of Corpora Luteum and Implantation Site in Sacrificed Female Rabbits (Mean ±SD) (n=6)**

| Parameters / Treatments | Implantation Site | Corpora Luteum Number | Successful Implantation % | Fetuses Resorption |
|---|---|---|---|---|
| **Control Normal saline** | **5.12± 1.05 A** | **6.41±1.03 Ns** | **79.87%** | **0 B** |
| **Toluene** | **2.5±1.53 B** | **6.00±0.66 Ns** | **41.66 %** | **2±1.03 A** |

**A,B,C= differences between groups, P≤0.05 vs. control, N=number of animals.**

**5-Effect of Toluene on Steroids Hormones in Male Rabbits**

The results in (Table5) described change in reproductive hormones in males treated with toluene by inhalation. There were statistically significant increase (p≤0.05) in estrogen while significant decrease (p≤0.05) in FSH, LH, progesterone, testesterone and corticosterone in females rabbits related to exposure to toluene than in the control group.

**Table (5) Effect of Toluene on Steroids Hormones in Male Rabbits (Mean±SD) (n=6)**

| Parameters / Treatments | FSH (µlU/ml) | LH (ng/ml) | Estrogen (µg/dl) | Progesteron (ng/ml) | Testesterone (ng/ml) | Corticosteron (µg/dl |
|---|---|---|---|---|---|---|
| **Control (normal saline)** | **2.86±0.011 A** | **5.30±0.006 A** | **48.58±0.63 B** | **1.18±0.027 A** | **0.6±0.024 A** | **5.91±0.032 A** |
| **Toluene** | **2.10±0.017 B** | **4.40±0.0034 B** | **90.74±0.36 A** | **0.64±0.021 B** | **0.1±0.015 B** | **3.39±0.038 B** |

**A,B= differences between groups, P≤0.05 vs. control, N=number of animals.**

**6-Effect of Toluene on Relative Weight of Adrenal, Testicular, Thymus, Spleen weights in Female Rabbits**.

The influence of inhalation of toluene on weights of adrenal glands, testes, thymus and spleen are demonstrated in Table (6). The results showed that the toluene had high significant decrease ($p<0.01$) on weights of both testes, thymus and spleen of male rabbits while the toluene had high significant increase ($p<0.01$) on weights adrenal glands in comparison with controls.

**Table (6) Effect of Toluene on Relative Weight of Adrenal, Testicular, Thymus, Spleen weights in Female Rabbits (Mean ±SD) (n=6)**

| Parameters / Treatments | Weight Of organs(g) | | | | |
|---|---|---|---|---|---|
| | Adrenal glands | Testes | | Thymus | Spleen |
| | | right | left | | |
| Control (normal saline) | 0.028±0.0015 B | 1.90±0.01 A | 1.95±0.02 A | 0.82±0.034 A | 0.70±0.001 A |
| Toluene | 0.065±0.0001 A | 0.34±0.01 B | 0.41±0.02 B | 0.47±0.031 B | 0.27±0.001 B |

**A,B= differences between groups, P≤0.05 vs. control, N=number of animals.**

**7- Effect of Toluene on Epididymal Spermatozoa Characteristics.**

The results in Table(7) described characteristics of epididymal spermatozoa in male rabbits treated with tolu caused significant decrease ($p<0.05$) in all characters of epididymal spermatozoa in comparison with controls.

**Table (7):Effect of Toluene on Epididymal Spermatozoa Characteristics.(Mean±SD) (n=6)**

| Treatments | Studied characters | | | | | |
|---|---|---|---|---|---|---|
| | Viability% | Sperm count×$10^6$ | Intact sperms(%) | Live sperms(%) | dead sperms(%) | abnormal sperms(%) |
| Control (normal saline) | 90.00±2.24 A | 210.30±1.67 A | 80.65±1.47 A | 75.11±3.74 A | 27.04±3.21 B | 15.34±1.41 B |
| Toluene | 22.16±2.38 B | 60.50±0.42 B | 63.82±2.80 B | 18.50±2.12 B | 87.40±4.12 A | 60.01±2.78 A |

**A,B= differences between groups, P≤0.05 vs. control, N=number of animals.**

## 8-Histopathological Changes:-

**1-Pituitary gland:- Section of pituitary gland of control female. Showing pituicytes with dense nuclei deeply stained normally distributed within nervous tissue**

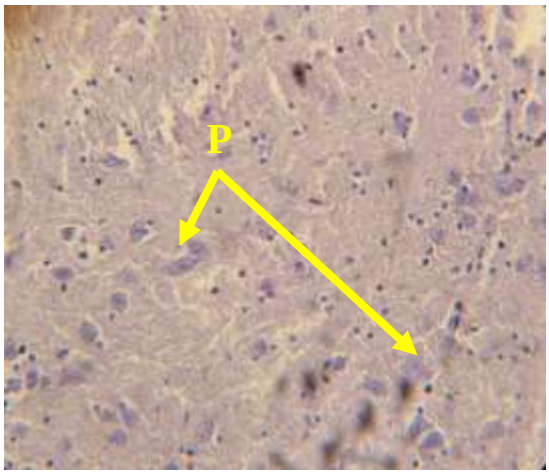


**Fig.1 :Section of pituitary gland of control female. Showing pituicytes (P) with dense nuclei deeply stained normally distributed within nervous tissue stain (H&E) 400X.**

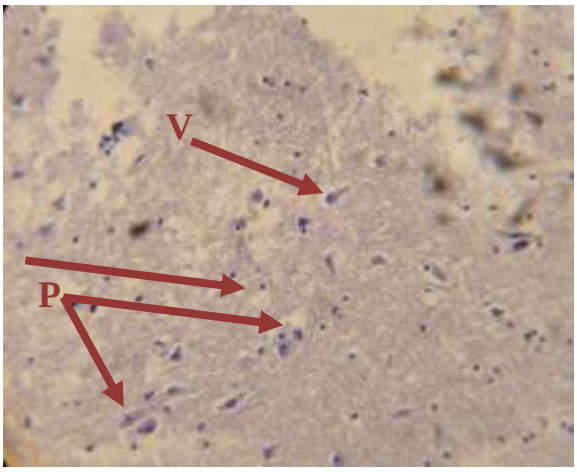


**Fig.2: Section of pituitary gland of toluene-treated female rabbits display few numbers of pituicytes (P) with enlarge nuclei, vacuolation of the cells (V), few numbers of herring bodies (HB), stain (H&E) 400X.**

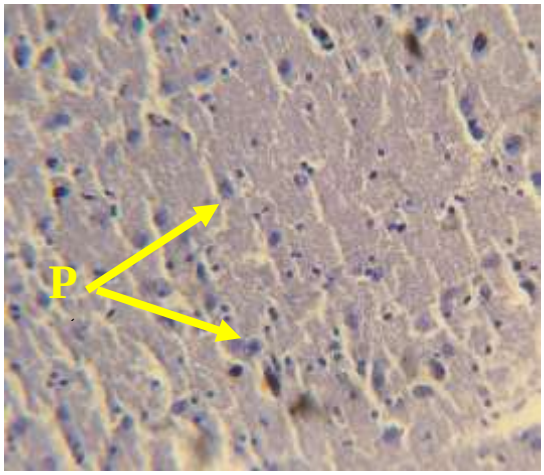


**Fig.3: Section of pituitary gland of control male. Display pituicytes (P) with dense nuclei deeply stained normally distributed within nervous tissue stain (H&E) 400X.**

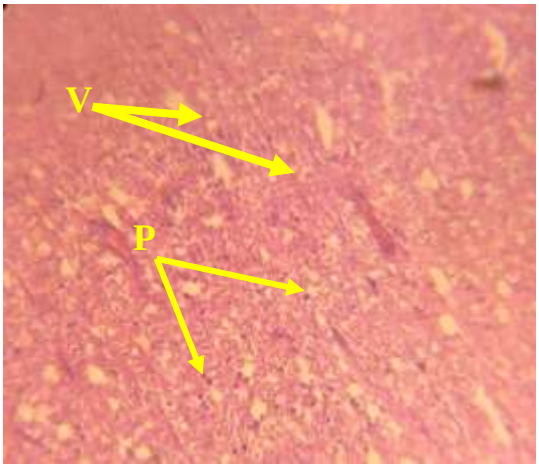


**Fig. 4: Section of pituitary gland of toluene-treated male rabbits. Display few numbers of pituicytes (P) with enlarge nuclei, vacuolation of the cells (V), few numbers of herring bodies (HB), stain (H&E) 400X.**

**2-Adrenal gland**

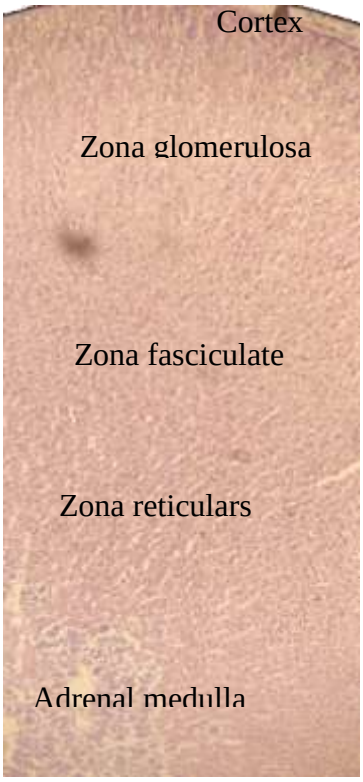


**Fig.5 :Section of adrenal gland of control female stain with (H&E), 100X.**

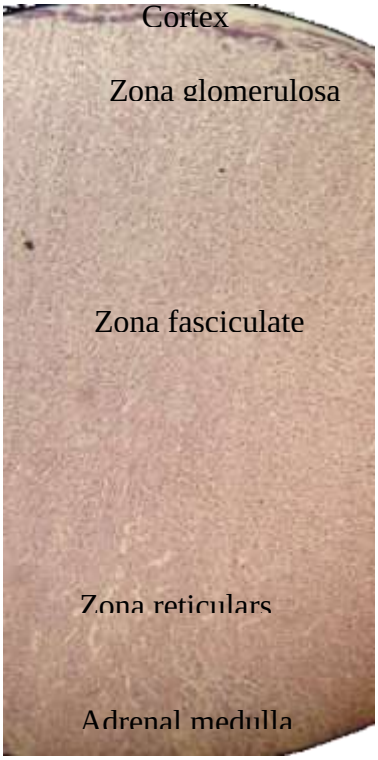


**Fig. 6:Section of adrenal gland of female rabbits treated with toluene stain with (H&E), 100X.**

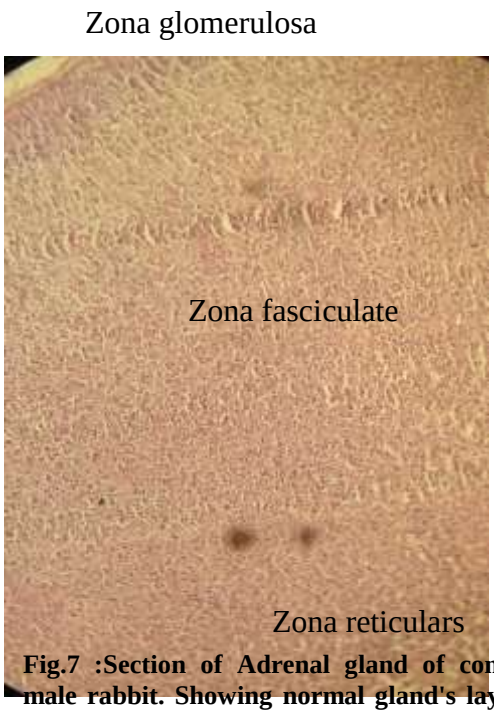


**Fig.7 :Section of Adrenal gland of control male rabbit. Showing normal gland's layers, surrounded by medulla(M), zona reticularis (ZR), zona fasciculate (ZF), zona glomerulosa (ZG), capsule(C) and stain**

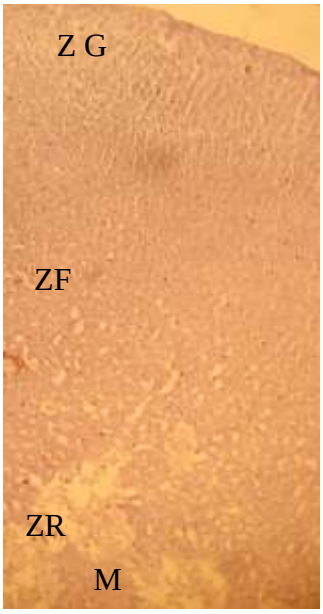


**Fig. 8: Section of Adrenal gland of male rabbits treated with toluene. Showing minimal to modulate fibrosis of capsule(C), medulla (M), normal zona reticularis (ZR), enlarged zona fasciculate (ZF), prominent zona glomerulosa (ZG)**

## Ovaries

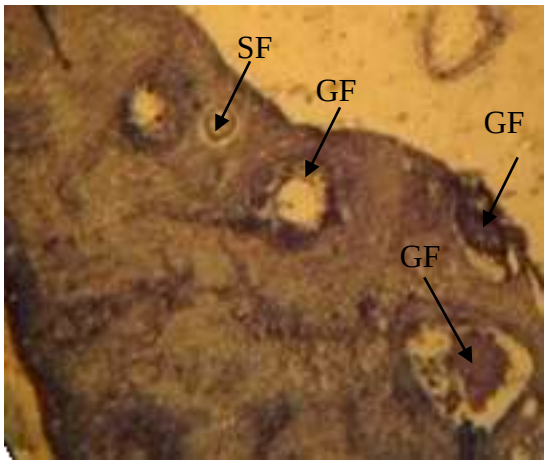


**Fig.9:Section of ovary of control rabbit. Showing normal ovarian cellular tissue with secondary follicles(SF), normal primary follicles (PF), normal Graafine follicles (GF), and stain (H&E) 100X.**

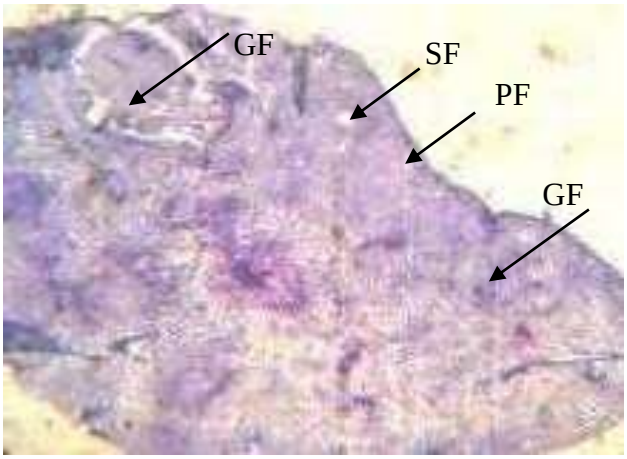


**Fig.10:Section of ovary of rabbit treated with Toluene. Disturbed ovarain tissue, primary follicles (P F),secondary follicles (SF), growing follicles (gf)and absence of Graafine follicle and large number thickned wall blood vessels(BV)with less blood encorgement, stain (H&E) 100X.**

## 4-Uterus

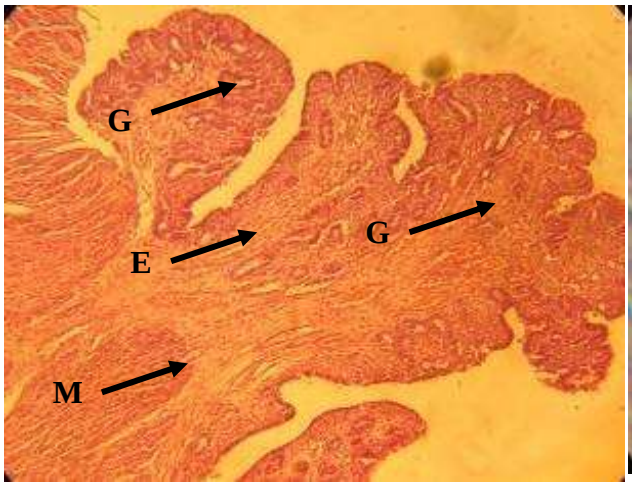


**Fig.9 :Section of uterus control female shows, uterine gland (arrows), muscular layer (M) and endometrial laver(E), 100X.**

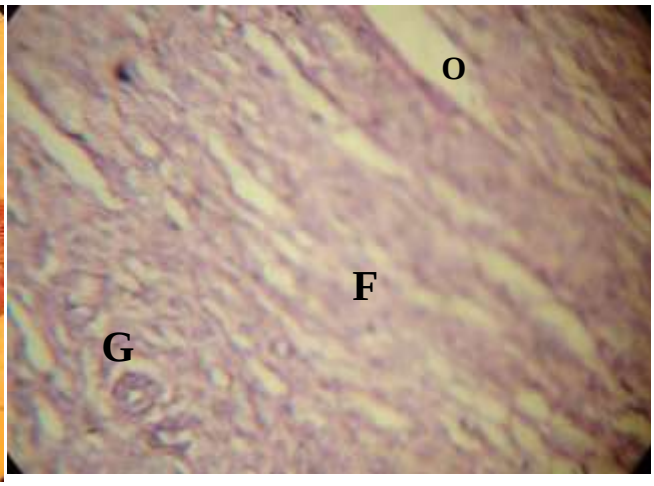


**Fig.10:Section of uterus in female treated with tolueneshows, oedema(O) (arrows), fibrosis (F), uterine gland (G), 400X**

## 5-Epididymal Sperm Count

It is a highly sensitive spermatogenesis test, sperm count is also closely linked with fertility. Treatment of rabbits with toluene dramatically decreased total sperm count (Table7), and testicular histology corroborated this finding, showing degeneration and atrophy in certain seminiferous tubules in conjunction with a low luminal spermatozoa concentration.

## 6- Viability and Motility of Sperm

Testing the motility of sperm is a crucial part of semen analysis, since it allows researchers to tell the difference between dead and alive sperm in samples when there are a lot of them. The findings showed that after being exposed to toluene, the proportion of alive and moving organisms dropped.

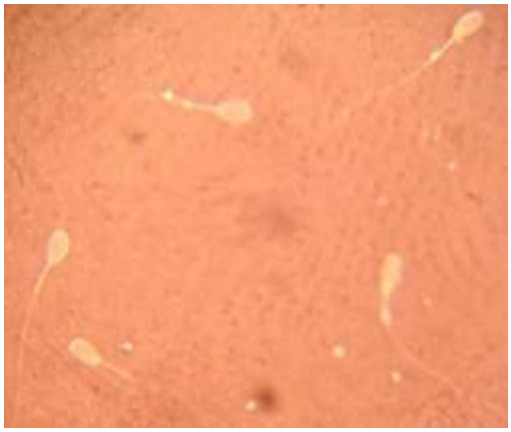

**Fig.11: Showing live sperm in male rabbits control. Stained with E&N 400X.**

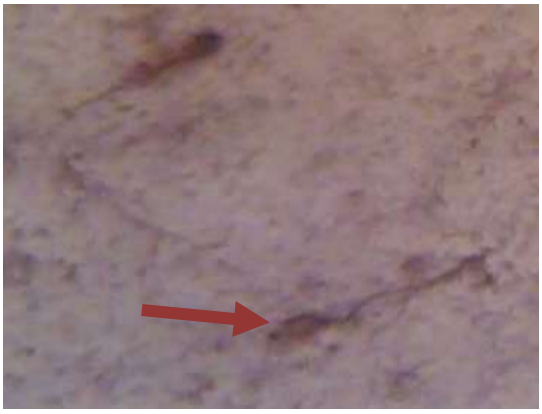

**Fig.12:Showing dead sperm in male rabbits treated with toluene. Stained with E&N 400X.**

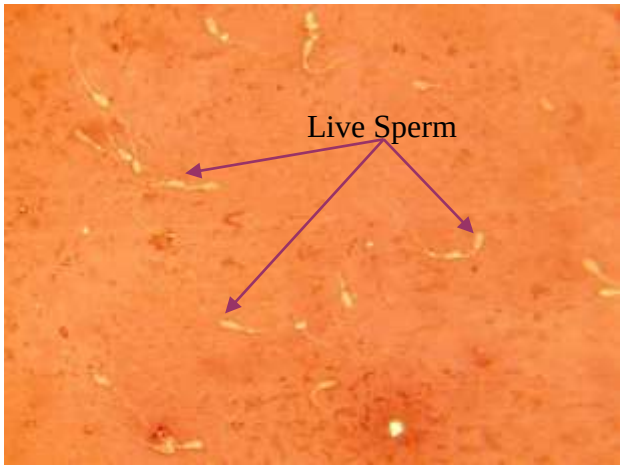


**Fig.13: Showing live sperm in male rabbits control. Stained with E&N 100X.**


**Fig.14:Showing dead sperm in male rabbits treated with toluene. Stained with E&N 100X.**

**7-Testes**

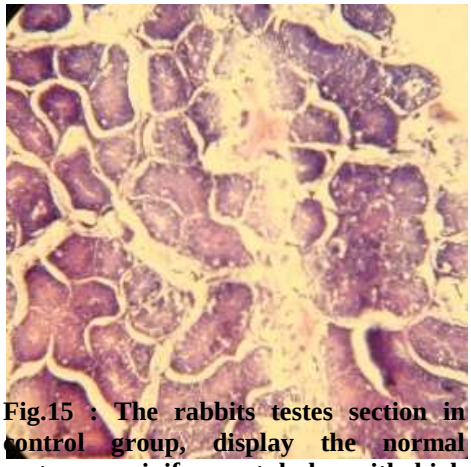

**Fig.15 : The rabbits testes section in control group, display the normal mature seminiferous tubules with high concentration of spermatozoa in lumen and complete spermatogenesis**

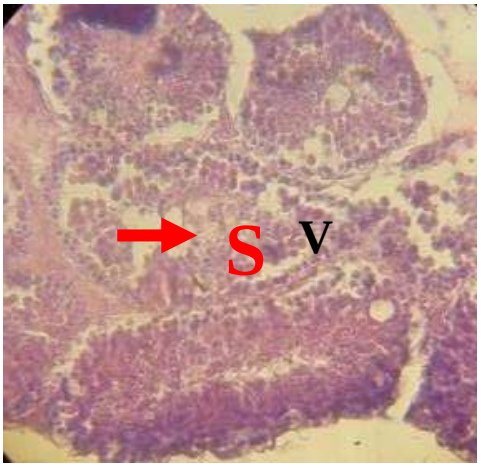


**Fig.16:Section of the testes of toluene treated-rabbits, display the degeneration of seminiferous tubules (S) with depression in lumen spermatozoa concentration. Intraepithieal vacuoles (V) in tubules (H& E 100X).**

**8-Epididemus**

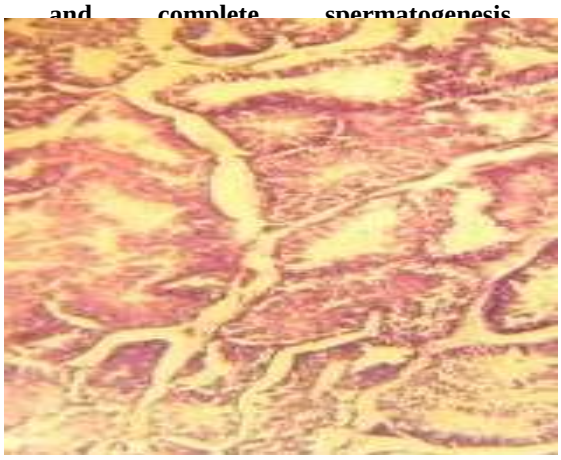

**Fig.17: Display epididimus in male rabbits treated with toluene. Stained with E&H 400X.**

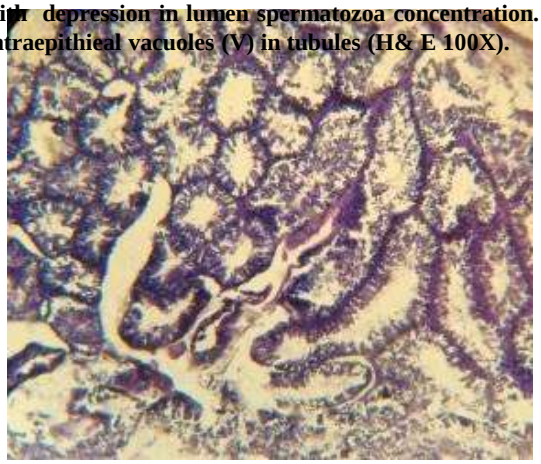

**Fig.18:Section of the epididymus of rabbits in toluene group, showing depression in concentration of lumen spermatozoa and the seminiferous tubules (S) degeneration (H&E 100X).**

## Discussion

With the direct conclusion potentially occupying the future generation [24,25], exposure to dangerous chemicals is close to the top industrial illness and the top clinical issue. Toluene, among these toxicants, has been found to produce a wide variety of injuries and damage to many bodily tissues[3].

Salivation and lacrimation, perhaps due to CNS gloominess, were reported in females of the 8000 ppm-treated group beginning 30 days after contact and exacerbated with frequent exposure[26].

Toluene considerably increased plasma ACTH concentration [27]. It was shown that the region positive for corticosterone, an indicator of cortical hypertrophy, expanded across the brains of the tolune group. Thus, it is believed that the stimulation of cortical cells was

responsible for the growth of these cells, which leads to cortical hypertrophy. The HPA axis, which includes the hypothalamus, pituitary gland, and adrenal gland, may be activated in response to exposure to toluene[28].

Time course and dosage response of toluene-induced species of reactive oxygen (ROS) production in the rabbit brain have been determined. The probe 2',7'-dichlorofluorescin diacetate was used for determination of the rate of oxygen radical production. Exposure of rat kidney and lung mitochondrial and cerebellar synaptosomal crude fractions to 1.5, 1.0 and 0.5 g/kg ip toluene induced a dose- dependent increase in ROS production. At low concentrations of toluene, crude synaptosomal fractions from striatum and crude mitochondrial fractions from liver and hippocampus produced the highest levels of reactive oxygen species (ROS). The hippocampus showed the greatest amounts of generated ROS among brain regions. Toluene-induced reactive oxygen species (ROS) peaked after 2 hours of exposure in vivo to a single dosage of toluene (8000ppm), and this peak matched well with toluene blood levels assessed at the same time[29]. Even when toluene levels in the blood dropped to undetectable levels during the following 24 hours, the increased oxidative activity remained stable. Our findings show that toluene exposure raises the typical rate of oxygen radical formation throughout the body, and that this impact persists in the tissues even after blood toluene levels have dropped rapidly[30]. Some of the clinical findings in long-term toluene abusers could be explained by the fact that even brief exposure to the substance causes long-lasting alterations associated to reactive oxygen species (ROS) [31].

The sex hormone of the low concentration group was considerably lesser than that of the controls ($P \leq 0.05$); significant difference was found in P4 and the number of follicles between different groups. Exposure to a certain amount of toluene has an inhibiting effect on the sex hormone in the serum of female rats [32-35]. Unfortunately, there is a lack of data on its impact on ovarian function. Previous in vitro investigations showed that toluene reduced the survival, proliferation, and estrogen secretion of ovarian cells [36,37]. This research used female rabbits to examine the *in vivo* effects of toluene. Low-dose exposure (8000 ppm) dramatically reduced body weight, our study revealed. The research is mixed on whether or not animals exposed to toluene gain weight. In response to toluene exposure, some studies have observed a drop in weight of body, while others have described a rise in weight of body [38]. The route of delivery, such as gavage, injection, or inhalation, may have a role in this difference [39]. We first confirmed that the reproductive toxicity of toluene exposure was

caused by the disruption of structure of ovarian, the folliculogenesis inhibition and markers related with steroidogenesis, the apoptosis induction, autophagy, and the subsequent loss of ovarian function and follicle development potential in the present study. At a level of 8000 ppm, we found that ovarian weight in rabbits was decreased. Although the developing follicles number was decreased at 8000 ppm, a rise in aberrant follicles was seen alongside this trend. Our findings corroborate those of prior research showing that exposure to toluene disrupts the follicular development process in adult female rabbits and causes changes in the histological structure of the ovaries [40,41]. According to the knowledge that hydrocarbons are endocrine disruptors, it has been looked at how they affect the function of the ovaries directly. It has been found that the hormones progesterone and testosterone were much more secreted in the toluene group than in the control group. Increased testosterone levels are consistent with the large rise in levels of Cyp17a mRNA reported by RT-PCR, which is consistent with the knowledge that Cyp17a, the steroidogenic factor, has vital role in androgen synthesis, such as testosterone [42,43]. Previous research using cultured ovarian granulosa cells revealed that the toluene addition led to inhibition of secretion of progesterone [44], therefore the increase in progesterone liberate is at odds with those findings. While progesterone reduces reactive oxygen species (ROS) generation in the benzene-treated rats ovaries [45] as well as defends against bropirimine-mediated embryolethality [46], it seems to have antioxidative and protective properties. Since the corpus luteum's primary function is the secretion of progesterone, rising progesterone levels with increasing toluene dosages may not be due to an increase in corpus lutea but rather to the ovary's strategy of defence against exposure to toluene. The corpora lutea are not only controlled by the ovary but also by the uterine, pituitary, and the brain [47], it is possible that other causes are responsible for the large rise in progesterone. The feedback links between ovarian and gonadotropic hormones suggest that toluene's effects on FSH, the hormone responsible for the formation of ovarian follicles, may lead to an increase in progesterone. The large amount of progesterone released after toluene exposure may protect ovarian cells by activating autophagy, since this process has previously been found to be activated by progesterone as a strategic neuroprotective strategy [49].

Autophagy has been around for a long time (evolutionarily speaking, that is) because it helps protect cells from a wide range of damaging factors, both within and outside the body [50]. It's a crucial mechanism that may promote cell survival or initiate pathways of cell death

[51,52]. Yet, exposure to toluene induced apoptosis even at maximally effective dosing (8000 ppm). These findings suggest that autophagy technology, which served for protection of ovarian cells from the harm by preventing apoptosis, may be triggered by a very high dosage of toluene exposure. Nevertheless, increased autophagy after extreme dosing was linked to apoptotic promotion rather than suppression. Our findings corroborate the involvement of environmental pollutants, especially industrial chemicals, in inducing autophagy [53]. These contaminants may increase autophagy as defensive responses to cellular damages, or they can change autophagy's protective role into that of pro-cell death mechanisms. It has been established that apoptosis and autophagy are two closely related processes that may happen at the same time and distribute various components in responses to different stimuli [54, 55]. Autophagy activation seems to precede apoptosis inhibition in certain instances of low-dose toxins [56,57], protecting cells from harm via self-repair.

Animal testes are particularly vulnerable to harm caused by genetic abnormalities, toxic exposure in the workplace, and other factors. There is a list of specific causes of testicular damage[58]. The group treated with testesin had a substantial decrease in weight after inhaling toluene (Table 2). These adjustments received higher marks.

The inhalation group had increased CRF immunoreactivity in the paraventricular nucleus (PVN). Testicular weight was found to be considerably lesser in the toluene-treated group in comparison with the controls. These shifts seemed muddier with prolonged exposure to toluene. Meanwhile, there was a discernible decrease in epididymal mass. Histopathological findings from the current investigation corroborated the decrease in testicular mass suspected from clinical examination alone, as seen in Fig (16,18).

## References

**1-Rodriguez-Mateos, A.; Vauzour D.; Krueger, C.G.; Shanmuganayagam, D.; Reed, J.; Calani, L.; Mena, P.; Del Rio, D. and Crozier, A.( 2014).** Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: An update. *Arch. Toxicol*;88:1803–1853.

**2-Benayoun, B.A.;Pollina E.A. and Brunet, A.(2015).** Epigenetic regulation of ageing: Linking environmental inputs to genomic stability. *Nat. Rev. Mol. Cell Biol.* ;16:593–610.

**3-Hu, Z.; Brooks, S.A.; Dormoy, V.; Hsu, C.-W.; Hsu, H.-Y.;Lin, L.-T.; Massfelder, T.; Rathmell, W.K.; Xia, M. and Al-Mulla, F.(2015).** Assessing the carcinogenic potential of

low-dose exposures to chemical mixtures in the environment: Focus on the cancer hallmark of tumor angiogenesis. *Carcinogenesis.* 36:S184–S202.

**4-Izzotti, A. and Pulliero, A.(2014).** The effects of environmental chemical carcinogens on the microRNA machinery. *Int. J. Hyg. Environ. Health.* ;217:601–627.

**5-Büntgen, U.(2011).** 2500 years of European climate variability and human susceptibility. Science **331**, 578–582.

**6-Metcalfe, J. D.; Le Quesne, W. J. F.; Cheung, W. W. L. and Righton, D. A. (2012)**. Conservation physiology for applied management of marine fish: an overview with perspectives on the role and value of telemetry. *Phil. Trans. R. Soc. B* **367**, 1746–1756.

**7-Eastman,A.(2013).** Eastman solvents-performance sheet. Suggested replacements for toluene.

**8-Ellermann, T.; Nøjgaard, J.K.; Nordstrøm, C.; Brandt, J.; Christensen, J.; Ketzel, M. and Jensen, S. S. (2012).** The Danish Air Quality Monitoring Programme. Annual Summary for 2011. Scientific Report from DCE – Danish Centre for Environment and Energy, Aarhus University.

**9-Oshiro, W.M.; Kenyon, E.M.; Gordon, C.J.; Bishop, B.; Krantz, Q.T.; Ford, J. and Bushnell, P.J. (2011).** Extrapolating the acute behavioral effects of toluene from 1-to 24-hour exposures in rats: Roles of dose metric and metabolic and behavioral tolerance. *Toxicol Sci*. 123(1):180-192.

**10-Samuel-Herter, S.R.; Slaght, S.L. and McKay, B.E. (2014).** Age-dependent time courses of recovery for motor functions following acute toluene intoxication in rats. *Develop Psychobiol*. 56(4): 657-673.

**11-Riegel, A.C.; Ali, S.F, Torinese, S. and French, E.D. (2004).** Repeated exposure to the abused inhalant toluene alters levels of neurotransmitters and generates peroxynitrite in nigrostriatal and mesolimbic nuclei in rat. *Ann N Y Acad Sci.* 1025: 543-51.

**12-Rumchev, K.; Spickett, J.; Bulsara, M.; Philips, M. and Stick, S.(2004).** Association of domestic exposure to volatile organic compounds with asthma in young children. *Thorax* 59(9): 746-751.

**13-Schaper, M.; Demes, P.; Kiesswetter, E.; Zupanic, M. and Seeber, A. (2004).** Colour vision and occupational toluene exposure: results of repeated examinations. *Toxicol Lett* 151: 193-202.

**14-Schaper, M.; Seeber, A. and van Thriel, C.(2008).** The effects of toluene plus noise on hearing thresholds: an evaluation based on repeated measurements in the German printing industry. *Int J Occup Med Environ Health*. 21: 191-200.

**15-Seeber, A.; Demes, P.; Kiesswetter, E.; Schaper, M.; van Thriel, C. and Zupanic, M. (2005).** Changes of neurobehavioral and sensory functions due to toluene exposure below 50 ppm? *Environ Toxicol Pharmacol.* 19:635-643.

**16- Mohammad – Reza, P.; Farzaneh, D.; Taherch, T.K. and Zoherb, P.P.(2005).** The effects of hydroalcholic extract of *Actinidia chinensis* on sperm count and motility, and blood levels of estradiol and testosterone in male rats. *Achieves of Iranina Medicin*e, 8(3): 211-216.

**17-Luna,L.G.(1993).** Manual of histology staining methods of the Armed Forces Institute of Pathology.3rd ed., New York, Mc grawHill.

**18-El-Tahar,K.E.H.; El-Nasser,M.A.; Ageel,A.M.; El-Obeid,H.A. and Al-Rashood, K.A.(1991).** Effects of N-methyl and N-isobutyl-1,2-diphenyl ethanol amine on the

**19-Laing ,J.A. (1979).** Fertility and infertility in domestic animals. 3rd edition *Bailliere Tindall*, a division of Cassell Lt.

**20-Morel, B.S.; Mercier, S. and Roux, C. (1998).** Inter individual variations in the disomy frequencies of human spermatozoa and their correlation with nuclear maturity as evaluated by aniline blue staining. *Fertil. Steril*,; 69 : 1122-1127.

**21-Wang, A.; Nie, W.; Li, H.; Hou, Y.: Yu, Z. and Fan, Q. (2014).** Epigenetic upregulation of corticotrophin-releasing hormone mediates postnatal maternal separation-induced memory deficiency. *PLoS ONE* 9:e94394.

**22- Bancroft,J.D.; Stevens,A. and Turner,D.R.(1990).** Theory and practice of histological techniques .3rd Ed. Churchill Livingstone. 221-6.

**23- SPSS Statistical Packages for the Social Sciences. (2007).** Statistical soft ware for windows version 13.0 Microsoft. SPSS®, Chicago, IL,USA..

**24-Trujillo, V.; Durando, P. E. and Suarez, M. M. (2016).** Maternal separation in early life modifies anxious behavior and Fos and glucocorticoid receptor expression in limbic neurons after chronic stress in rats: effects of tianeptine. *Stress* 19, 91–103.

**25-Shwe, T-T-W.; Yamamoto, S.; Nakajima, D.; Furuyama, A.; Fukushima, A.; Ahmed, S.; Goto, S. and Fujimaki, S.(2007).** Modulation of neurological related allergic reaction in mice exposed to lowlevel toluene. *Toxicol Appl Pharmacol* 222:17–24.

**26-Tang, H.L.; Chu, K.H.; Cheuk, A.; Tsang, W.K.; Chan, H.W. and Tong, K.L. (2005).** Renal tubular acidosis and severe hypophosphataemia due to toluene inhalation. Hong Kong Med J. 11(1):50-53.

**27-XU You-qiong,J.I.A.; Hai-mei,Y.A.N.G and Jin-song, (2015).** Effect of toluene on the ovary and sex hormone of female rabbits.

**28-Sandi, C. and Haller, J. (2015).** Stress and the social brain: behavioural effects and neurobiological mechanisms. *Nat. Rev. Neurosci.* 16, 290–304.

**29-Santarelli, S.; Lesuis, S. L.; Wang, X. D.; Wagner, K. V.; Hartmann, J.; Labermaier, C.(2014).** Evidence supporting the match/mismatch hypothesis of psychiatric disorders. *Eur. Neuropsychopharmacol.* 24, 907–918.

**30-Ryan S. J., Ehrlich D. E., Rainnie D. G. (2016).** Morphology and dendritic maturation of developing principal neurons in the rat basolateral amygdala. *Brain Struct. Funct.* 221, 839–854.

**31- Romano-López, A.; Mendez-Diaz, M.; Garcia, F. G.; Regalado-Santiago, C.; Ruiz-Contreras, A. E. and Prospero-Garcia, O. (2016).** Maternal separation and early stress cause long-lasting effects on dopaminergic and endocannabinergic systems and alters dendritic morphology in the nucleus accumbens and frontal cortex in rats. *Dev. Neurobiol.* 76, 819–831.

**32 Rana, S.; Pugh, P. C.; Jackson, N.; Clinton, S. M. and Kerman, I. A. (2015).** Inborn stress reactivity shapes adult behavioral consequences of early-life maternal separation stress. *Neurosci. Lett.* 584, 146–150.

**33-Irles, C.; Nava-Kopp, A. T.; Morán, J. and Zhang L. (2014).** Neonatal maternal separation up-regulates protein signalling for cell survival in rat hypothalamus. *Stress* 17, 275–284.

**34- Grimm, S.; Pestke, K.; Feeser, M.; Aust, S.; Weigand, A. and Wang, J. (2014).** Early life stress modulates oxytocin effects on limbic system during acute psychosocial stress. *Soc. Cogn. Affect. Neurosci.* 9, 1828–1835.

**35-Geoffroy, M.; Pinto, C.; Pereira, S.; Li, L. and Power, C. (2016).** Child neglect and maltreatment and childhood-to-adulthood cognition and mental health in a prospective birth cohort. *J. Am. Acad. Child Adolesc. Psychiatry* 55, 33.e33–40.e33.

**36-Fine, R.; Zhang, J. and Stevens, H. E. (2014).** Prenatal stress and inhibitory neuron systems: implications for neuropsychiatric disorders. *Mol. Psychiatry* 19, 641–651.

**37-Fan, Y.; Herrera-Melendez, A. L.; Pestke, K.; Feeser, M.; Aust, S. and Otte, C. (2014).** Early life stress modulates amygdala-prefrontal functional connectivity: implications for oxytocin effects. *Hum. Brain Mapp.* 35, 5328–5339.

**38-Alteba, S.; Korem, N. and Akirav, I. (2016).** Cannabinoids reverse the effects of early stress on neurocognitive performance in adulthood. *Learn. Mem.* 23, 349–358

**39-Arnett, M. G.; Pan, M. S.; Doak, W.; Cyr, P. E.; Muglia, L. M. and Muglia, L. J. (2015).** The role of glucocorticoid receptor-dependent activity in the amygdala central nucleus and reversibility of early-life stress programmed behavior. *Transl. Psychiatry* 5:e542.

**40-Arp, J. M.; Ter Horst, J. P.; Loi, M.; den Blaauwen, J.;Bangert, E. and Fernández, G. (2016).** Blocking glucocorticoid receptors at adolescent age prevents enhanced freezing between repeated cue-exposures after conditioned fear in adult mice raised under chronic early life stress. *Neurobiol. Learn. Mem.* 133, 30–38.

**41-Zhang, X.; Wang, B.; Jin, J.; An, S.; Zeng, Q. and Duan, Y. (2014).** Early deprivation reduced anxiety and enhanced memory in adult male rats. *Brain Res. Bull.* 108, 44–50.

**42- Zelena, D.; Stocker, B.; Barna, I.; Tóth, Z. E. and Makara, G. B. (2015).** Vasopressin deficiency diminishes acute and long-term consequences of maternal deprivation in male rat pups. *Psychoneuroendocrinology* 51, 378–391.

**43- Zalosnik, M. I.; Pollano, A.; Trujillo, V.; Suarez, M. M. and Durando, P. E. (2014).** Effect of maternal separation and chronic stress on hippocampal-dependent memory in young adult rats: evidence for the match-mismatch hypothesis. *Stress* 17, 445–450.

**44-Xu, L.; Sun, Y.; Gao, L.; Cai, Y.-Y. and Shi, S.-X. (2014).** Prenatal restraint stress is associated with demethylation of Corticotrophin Releasing Hormone (CRH) promoter and enhances CRH transcriptional responses to stress in adolescent rats. *Neurochem. Res.* 39, 1193–1198.

**45-Xiong, G. J.; Yang, Y.; Wang, L. P.; Xu, L. and Mao, R. R. (2014).** Maternal separation exaggerates spontaneous recovery of extinguished contextual fear in adult female rats. *Behav. Brain Res.* 269, 75–80.

**46-Wu, Y.; Patchev, A. V.; Daniel, G.; Almeida, O. F. and Spengler, D. (2014).** Early-life stress reduces DNA methylation of the Pomc gene in male mice. *Endocrino.*155, 1751–1762.

**47-van der Doelen, R. H.; Arnoldussen, I. A.; Ghareh, H.; van Och, L.; Homberg, J. R. and Kozicz, T. (2015).** Early life adversity and serotonin transporter gene variation interact to affect DNA methylation of the corticotropin-releasing factor gene promoter region in the adult rat brain. *Dev. Psychopathol.* 27, 123–135.

**48-Uribe-Marino, A.; Gassen, N. C.; Wiesbeck, M. F.; Balsevich, G.; Santarelli, S. and Solfrank, B.(2016).** Prefrontal cortex corticotropin-releasing factor receptor 1 conveys acute stress-induced executive dysfunction. *Biol. Psychiatry* 80, 743–753.

**49-St-Cyr, S. and McGowan, P. O.(2015).** Programming of stress-related behavior and epigenetic neural gene regulation in mice offspring through maternal exposure to predator odor. *Front. Behav. Neurosci.* 9:145.

**50-Sowa, J.; Bobula, B.; Glombik, K.; Slusarczyk, J.; Basta-Kaim. A. and Hess, G. (2015).** Prenatal stress enhances excitatory synaptic transmission and impairs long-term potentiation in the frontal cortex of adult offspring rats. *PLoS ONE* 10:e0119407.

**51-Sotnikov, S.; Wittmann, A.; Bunck, M.; Bauer, S.; Deussing, J. and Schmidt, M. (2014).** Blunted HPA axis reactivity reveals glucocorticoid system dysbalance in a mouse model of high anxiety-related behavior. *Psychoneuroendocrinology* 48, 41–51.

**52-Silberman, D. M.; Acosta, G. B. and Zorrilla Zubilete M. A. (2016).** Long-term effects of early life stress exposure: role of epigenetic mechanisms. *Pharmacol. Res.* 109, 64–73.

**53-Abdulkarem, A.; Nouf, A.; Lamjed, M.; Mukhtar, A.; Alexander, V. S.; Saleh, A.; and Abdel Halim, H.(2021).**Toluene can disrupt rat ovarian follicullogenesis and steroidogenesis and induce both autophagy and apoptosis. *Biology*. 10, 1153.

**54-Callan, S.; Kott, J.; Cleary, J.; McCarthy, M.K.; Baltes, B.B. and Bowen, S.E.(2016).** Changes in developmental body weight as a function of toluene exposure: A meta-analysis of animal studies. *Hum. Exp. Toxicol*. 35, 341–352.

**55-Kareem, L.A.; Banna, H.B. and Naoom, K.M.(2014).** Effects of toluene and formaldehyde on oogenesis in adult female mice. *Diyala J. Med.* 6, 33–40.

**56- Ivell, R.; An and-Ivell, R.(2018).** Insulin-like peptide 3 (INSL3) is a major regulator of female reproductive physiology. *Hum. Reprod. Update*. 24, 639–651.

**57-Veerakumar, U.; Al Shaali, S.J. and Abbas, A.(2016).** Subduing Harmful Exposure of BTEX by Improving Facility and Practices. In Proceedings of the Abu Dhabi International Petroleum Exhibition & Conference, Abu Dhabi, United Arab Emirates.

**58- Roberts, L.; Nicolich, M. and Schreiner, C.(2007).** Developmental and reproductive toxicity evaluation of toluene vapor in the rat: II. Developmental toxicity. *Reprod. Toxicol*., 23, 521–531.