ISSN: 03875547
Volume 44, Issue 06, December, 2021

The Toxic Effects of Toluene on oxidative stress status in the Genital system of Female Mice

Maha Fadhel Mohammad[1], Luma Yaseen Mousa[2], Duaa Hammoud Idayyir[3], Zina Murshd Kadim[4]

Department of Medical Lab. Techniques, pharmacy College Al-Esraa University College[1,2,3,4]

Keywords:
Toluene, Uterus, Ovary, Oxidative Stress, GSH) and Peroxynitrite radical

ABSTRACT

Toluene is environmental pollutant and has many toxic effect on people in this experimental we study the toxic effect, twenty hundred and foure female divided into three groups as following: 1st groups administrated orally with distal water and olive oil as control group, 2nd group orally administration with (0.2 ml/kg B.W.) of toluene for one month from LD50, 3rd group administrated (0.22ml /kg B.W.) with toluene orally from LD50 for two month, we collected blood sample directly from heart at 0, 30 and 60 days of experimental for measurement Glutathione (GSH) and Peroxynitrite radical concentration related to toluene toxicity on reproductive organ. The results indicated toxic effects of toluene in reproductive organ sections show sever pathological lesion and decrease in GSH and Peroxynitrite.

1. INTRODUCTION

Toluene enhances the formation of reactive oxygen species (ROS). These occur in the cell when the synthesis of oxidants is just out of balance. The increase of oxidative free radicals that results might cause directly damage proteins, DNA, and lipids resulting in cellular malfunction and obstructing the body's natural defensive systems against These compounds have been suggested as a probable mode of action for a variety of dangerous substances due to a variety of processes [13]. Glutathione peroxidase (GPx) is a natural antioxidant enzyme present in a variety of cell, tissues having peroxidase activity that play a biological role in protecting cells from oxidative damage [10] DNA, proteins, and membrane lipids can all be damaged by these reactive species. Both in vivo and in vitro, it significantly enhances oxidative stress measures. It reduces the activity of antioxidant enzymes by a large amount [3].

2. Materials and methods

2.1 Experimental animals

One hundred and twenty female albino mice (Musmusculus), (35 to 40 g.) They were 4 to 6 months old and were taken from an animal shelter. Animals were kept in plastic cages in a temperature controlled environment (22–25°C). The experiment for this study was carried out in the animal house of the Pathology Department at Baghdad University's College of Veterinary Medicine. with regulated lighting utilizing an automated electrical timer that provides twelve hours of daily illumination (7 .00 Am to 19. 00 Pm) a twelve-

hour day cycle, and a twelve-hour night cycle Mice were given standard mice food (pellet diet) and distal water for two weeks to allow for adaptation.

3 - Experimental study for determination half lethal dose (LD50):
1- half lethal dose (LD50):
(114) female mice for determination the (LD50) and divided into:
A – Probit study:
(18) male and female mice were used for determination the ranges of the lethal dose of Toluene (16) administration with (2) control, this methods was repeated daily for seven days by dividing into two group contain (2) mice
B – Probit study: (6) (11)

Determination of serum Glutathione (GSH) concentration (µm/I)
(5)
Determination of serum peroxynitrate radical concentration (m/ I)
(21) .

Histopathological studies (14).

2.2 Statistical analysis
The SSP program was used to do the statistical analysis.

3. Results

Table (1): The effects of toluene administration on oxidative enzyme glutathione (GSH) of female albino mice after 30, 60 days of administration

Groups	Zero day	30 days	60 days
Control	6.5 ± 0.37^{Aa}	6.4 ± 0.28^{Aa}	6.45 ± 0.33^{Aa}
G1	6.3 ± 0.2^{Aa}	4.4 ± 0.2^{Bb}	2.0 ± 0.1^{Cb}
G2	6.36 ± 0.1^{Aa}	3.2 ± 0.5^{Bc}	1.3 ± 0.4^{Cc}

Values are expressed as mean ± S. E, n = 10, different small letters denoted that significant differences between treatment (administration), capital different letters denoted that significant differences between periods.

Table (1) at days (30) there is significant decrease ($p \leq 0,05$) in serum concentration of glutathione concentration mostly at G2 of administration comparing with G1 administration and control respectively (3.2 ± 0.5, 4.4 ± 0.2 and 6.4 ± 0.28), while at days (60) showed significant decrease ($p \leq 0,05$) mostly at G2 administration compared with G1 dose administration and control respectively (1.3 ± 0.4), (2.0 ± 0.1), (6.45± 0.33)

ISSN: 03875547
Volume 44, Issue 06, December, 2021

Table (2): The effects of toluene administration on antioxidant enzyme glutathione peroxidation (peroxynitrate radical concentration) of female albino mice after 30. 60 days of administration

Groups	Zero	30 days	60 days
Control	6.8 ± 0.14^{Aa}	6.73 ± 0.16^{Aa}	6.75 ± 0.18^{Aa}
G1	6.4 ± 0.24^{Aa}	$5.4 \pm 0.6Bb$	4.9 ± 0.5^{Bb}
G2	6.46 ± 0.20^{Aa}	4.5 ± 0.3^{Bb}	$3.96\ 0.4^{Bb}$

Values are expressed as mean ± S. E, n = 10, different small letters denoted that significant differences between treatment (administration), capital different letters denoted that significant differences between periods

Table (2) at days (30) showed there is no significant decrease ($p \leq 0,05$) in serum concentration of peroxynitrite radical concentration mostly at G2 of administration comparing with G1 administration and control respectively (1.13 ± 0.022), (1.36 ± 0.025), (1.70 ± 0.034), while at days (60) showed significant decrease ($p \leq 0,05$) mostly at G2 administration compared with G 1 dose administration and control respectively (1.02 ± 0.024), (1.29 ±0.027), (1.69 ± 0.02)

3.1 Toluene's effects on mouse ovaries and uterine tissue

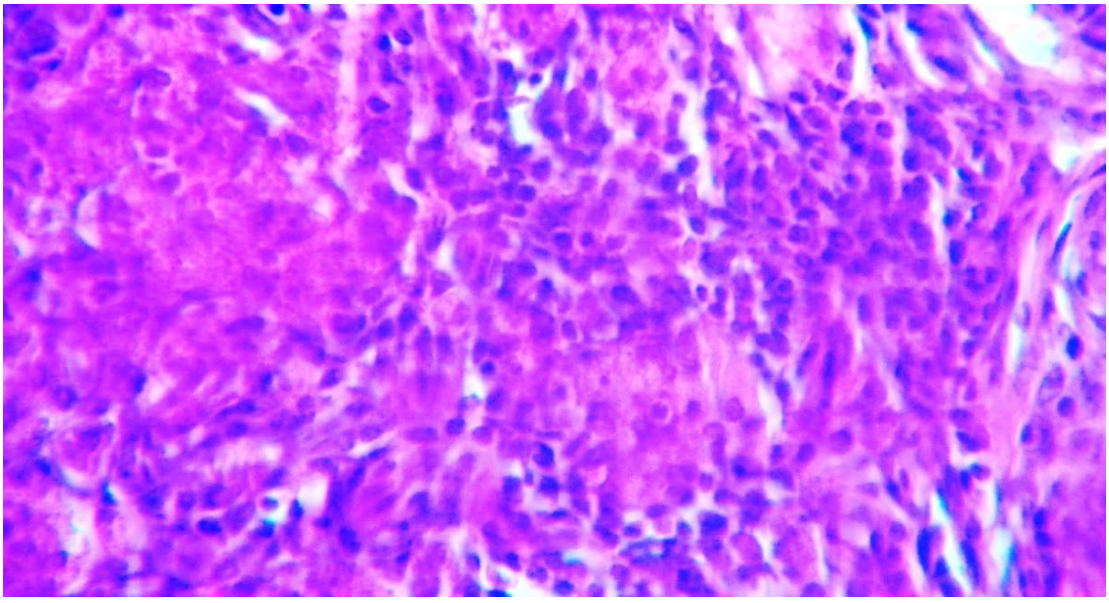


Figure (1): ovaries of mice 30 days after toluene (G1) administration: A - medulla with no hemosiderin pigment, B - necrotic region, C - macrophages engulfing hemosiderin (H &E stain 40 X)

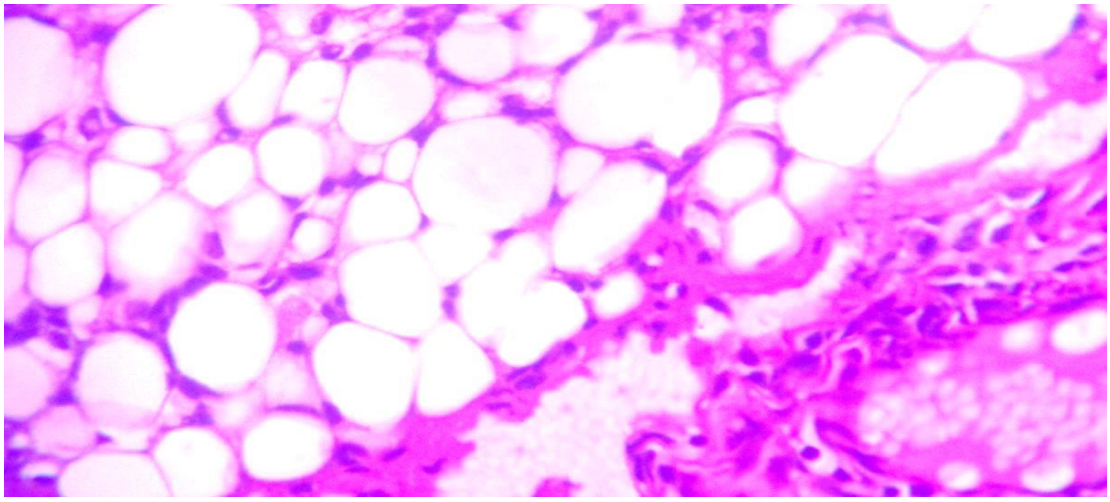


Figure 2: Mice ovary after 60 days of toluene treatment (G1): A – fibrin exudate, B – macrophage invasion of tissue (H &E stain40 X)

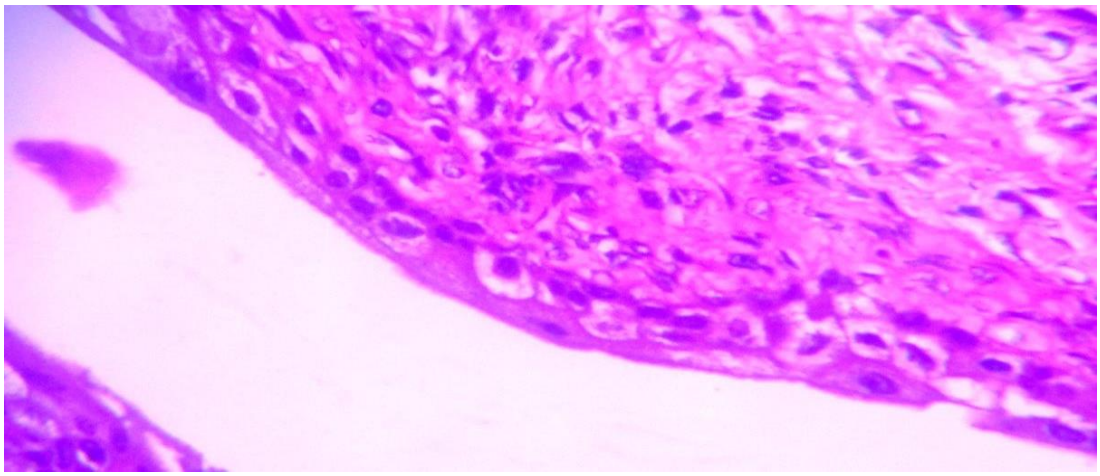


Figure 3: The uterus of mice after 30 days of toluene injection (G2): a- vacuolation and necrosis in various locations, b- sloughing of endothelial cells (H &E stain40 X)

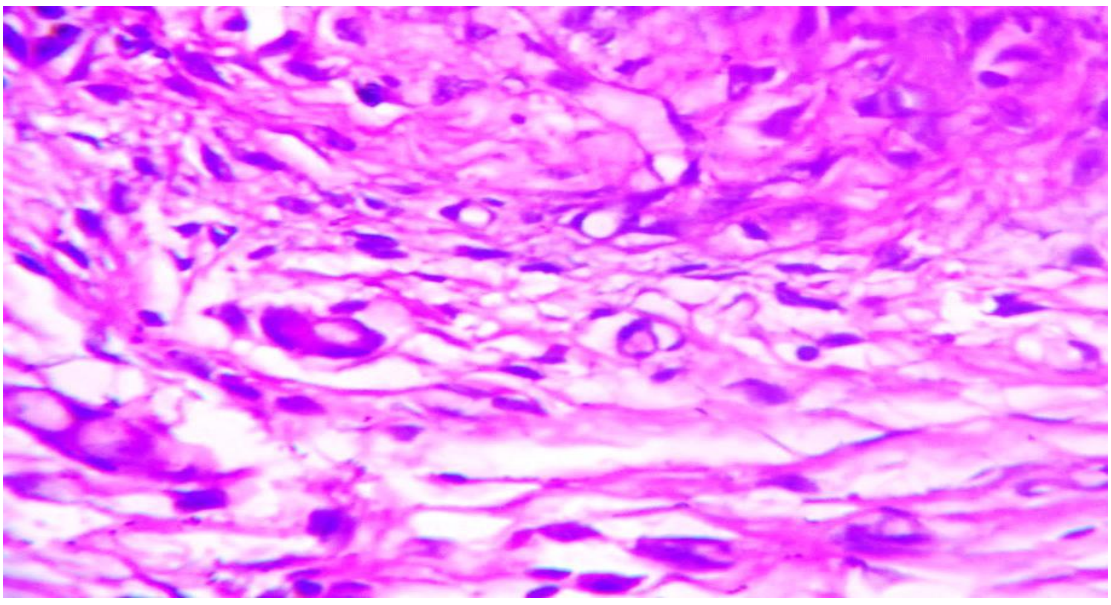


Figure 4: The uterus of mice after 60 days of toluene administration (G3): A - endothelial cell hyperplasia, B - endometrial vacuolation and hemorrhagic, C - fibrinous infiltration with mononuclear cells (H &E stain40 X)

4. Discussion

Determination of LD50

Acute toxicity:

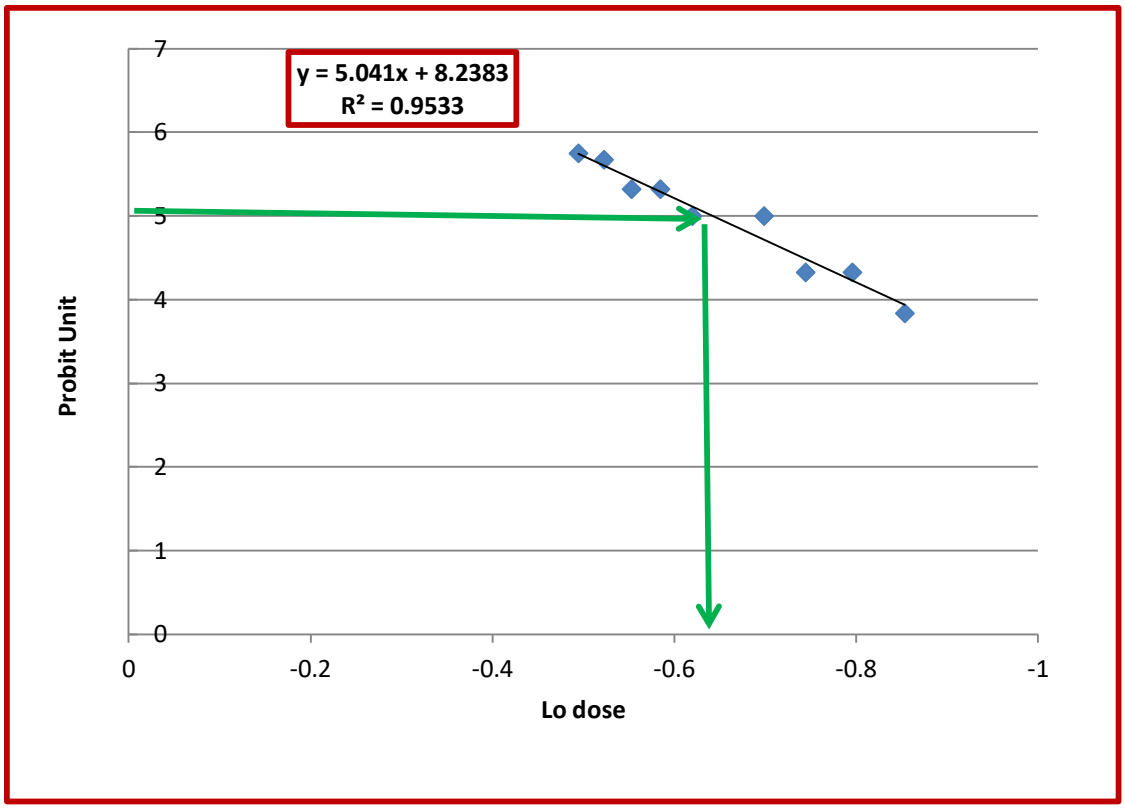
1 – Pilot study

The range of LD50 were mainly (0.1 ml/kg to 0.32 ml/kg B.W.) which cause the death of foure animals from eight animals

2 - Probit method:

The mortality percent and conversion to prob. No. according to acute toxic effects of toluene in G1 . G2 , G3 , G4 ,G5 ,G6 ,G6,G7,G8,G9 , G10, G11, G12 are listed in table :

Group	Dose	Log dose	N	LIVE	DEAD	DEAD %	CORRECTED	PROBIT UNIT
G1	0.1	-1	8	8	0	0	3	3.12
G2	0.14	-.0.853	8	7	1	13	13	3.88
G3	0.16	-0.795	8	6	2	25	25	4.35
G4	0.18	-0.744	8	6	2	25	25	4.35
G5	0.2	-0.698	8	4	4	50	50	5.00
G6	0.22	-0.657	8	4	4	50	50	5.00
G7	0.24	-0.619	8	3	5	63	63	5.34
G8	0.26	-0.585	8	3	5	63	63	5.34
G9	0.28	-0.552	8	2	6	75	75	5.68
G10	0.30	-0.522	8	1	7	87	87	6.18
G11	0.32	-0.494	8	0	8	100	98	6.89
G12	Control		8	8				



5=0.8519x +11.289
X= 5 – 8.2383/0.8519
X= -0.64239
Antilog =0.526 (LD_{50})
Corrected formula * :
For the 0% dead : 100 (0.25 /n) = 100 (0.25 / 8) = 3 ,
For the 100 % dead : 100 (n-0.25) = 100 ; (8-0.25) / 8 = 97 ,
N is the number of animals in the group

It was modified rations perdition 0 and 100 % as deemed percentage 0 % after adjustment of 3 % while consider the percentage of perdition 100 % adjusted ratio 97 % to the need for amendment of the ratios of 0 % and 100 % when using away link to estimate the value of catalytic dose lethal and that the lack of units probability landscaps to 0 or 100 % according to equation (4) LD50 was measured after we used pilot logarithm of dose was used against probit response from which LD50 was determined by vertical cross link from 5 probit response to the log no. dose (figure 2). LD50 was calculated as antilog no. depend on [19] According to toxicity rate Toluene's LD50 was to be given to mice by orally administrated via insulin needle Since the LD50 was classified as a toxicity rat. (0,2 – 0.22 ml / kg. b. w.) in mice and consider as highly toxic compound.

4.1 Effects of toluene on antioxidant status (GSH and peroxynitrite radical concentration)

The result indicated significant decrease ($p \leq 0.05$) in glutathione and in peroxynitrite radical concentration that indicated it is antioxidant effects, one of the important mechanisms of toxicity of the toluene is oxidative stress. Toluen-e promote the formation of reactive oxygen species (ROS), which are the primary cause of cellular damage and/or interfere with the body's natural defensive systems against toxic substances through a variety of processes, especially in the liver and brain [3]. Antioxidants are anti-oxidant compounds, and the relative relevance of these substrates varies depending on the kind, location, and target of (ROS)-induced

damage [7] revealed that injecting toluene into the brain and liver induced a considerable increase in the rate of ROS formation and a decrease in glutathione (GSH) levels. and these agreed with these results GSH is an antioxidant that participates in the catalytic cycles of various antioxidant enzymes, including GSH-Px and GSH reductase The inability of the main antioxidant system to combat free radicals was demonstrated by the decrease in GSH [1]. The lower GSH content showed an increase in the production of reactive oxygen species (ROS), which induce lipid peroxidation in the lungs. [16], The current study showed reduction in GSH level. The present study are in agreement with [20] that indicated reduction in the serum glutathione and these Antioxidant defense mechanisms may be to blame for the reduction. while are in disagreement with [1] who reported in albino rats increased in GSH due to The oxidant–antioxidant equilibrium was reached once the lipid peroxidation products were depleted as well as those that have been produced as a result of ongoing oxidative stress. Toluene induces a considerable drop in the activity of antioxidant enzymes both in vivo and in vitro, as well as an increase in oxidative stress indices [10] examined the toxic effects of chronic toluene inhalation in high concentration (3000 ppm, 8 hours a day, 6 days a week for 16 weeks on the sciatic nerves of rats and found that toluene decreased the antioxidant enzyme activities (GSH-Px) levels, According to a research conducted by [9] were Thinner in high dosage toluene increased GSH-Px activity in erythrocytes, according to researchers.

It was reported that erythrocytes GSH-Px levels were fairly low. The current study was not are in agreement with [19] reported that increase of serum GSH and lipid peroxidation (GSH – PX) and explain of that is possible due GSH-Px activity as antioxidant enzyme to minimized the toxic effects of long term exposure to toluene increase in GSH as result of it is antioxidant enzyme against ROS generation by toluene under long term exposure to the thinner (Toluene are the most components, 63 %) and increase in lipid peroxidation happens when polyunsaturated fatty acids react with reactive free radicals. radicals to decrease in damage to biological membranes of cells. The present study also are in agreement with evidence was supported the idea of [15]. The cytochrome P450 serious enzyme is engaged in the oxidation of a wide range of chemicals and uses molecular oxygen in its catabolism of toluene [7]. can contribution to enhancement of (ROS) formation and these trigger depletion in the GSH – PX as the defect mechanism against [13] indicated in studies in mice exposure to toluene decrease in serum GSH-Px. As a result, this enzyme shields the body against the extremely reactive hydroxyl radical (OH), which is produced by H2O2 [17]. As a result, the reduction in GSH-Px activity in the lungs enhanced the toxicity of free radicals produced by toluene effects.

5. Conclusion

Toxic Effects on the Genital System of Female Mice exposed to toluene caused oxidative damage. Despite the fact that increases in oxidative damage corresponded to changes in oxidative stress.

6. References

[1] Agacdiken, A., Basyigit, I., Özden, M., Yildiz, F., Ural, D., Maral, H., ... & Komsuoglu, B. (2004). The effects of antioxidants on exercise-induced lipid peroxidation in patients with COPD. Respirology, 9(1), 38-42.

[2] Aranda, J. F., Madrigal-Matute, J., Rotllan, N., & Fernández-Hernando, C. (2013). MicroRNA modulation of lipid metabolism and oxidative stress in cardiometabolic diseases. Free Radical Biology and Medicine, 64, 31-39.

[3] Baydas G, Ozveren F, Tuzcu M, (2005). Effects of thinner exposure on the expression pattern of neural cell adhesion molecules, level of lipid peroxidation in the brain and cognitive function in rats. Eur J Pharmacol 512(2-3):181-187.

[4] Brahmane, R. I., Wanmali, V. V., Pathak, S. S., & Salwe, K. J. (2010). Role of cinnarizine and nifedipine on anticonvulsant effect of sodium valproate and carbamazepine in maximal electroshock and pentylenetetrazole model of seizures in mice. Journal of pharmacology & pharmacotherapeutics, 1(2), 78..

[5] Burtis, C. A., & Ashwood, E. R. (1999).textbook of clinical chemistry. 3d ED . London . Vol.12 Chapter (33) : 1145- 1150

[6] Goldstein , A ; Aronw, L; S.M. and Wiley , J and sons . (1974) . Principle of drug actions , second edition . Newyork ,Sydeny , Torinto ., (2) ; 376-394.

[7] Halliwell, B., & Gutteridge, J. M. (1985). The importance of free radicals and catalytic metal ions in human diseases. Molecular aspects of medicine, 8(2), 89-193.

[8] Halliwell, B., Aeschbach, R., Löliger, J., & Aruoma, O. I. (1995). The characterization of antioxidants. Food and Chemical Toxicology, 33(7), 601-617.

[9] Ihsan Halifeoglu , Halit Canatan , Bilal Ustundag , Nevin Ilhan and Fatma Inanc ; (2000), Effect of thinner inhalation on lipid peroxidation and some antioxidant enzymes of people working in paint thinner . Department of Biochemistry and Clinical Biochemistry, Colle`e of Medicine and Firat Medical Center , Firat

[10] Karabulut, O. A., Ilhan, K., Arslan, U., & Vardar, C. (2009). Evaluation of the use of chlorine dioxide by fogging for decreasing postharvest decay of fig. Postharvest biology and technology, 52(3), 313-315.

[11] Klassen , C. D. (2001) . Casaret and Doull,s toxicology : the basis science of poisons . Mc .Graw – Hill . Medical Publishing division . USA

[12] Kodavanti, P. R. S., Royland, J. E., Richards, J. E., Besas, J., & MacPhail, R. C. (2011). Toluene effects on oxidative stress in brain regions of young-adult, middle-age, and senescent Brown Norway rats. Toxicology and applied pharmacology, 256(3), 386-398.

[13] Liu, X., Song, Q., Tang, Y., Li, W., Xu, J., Wu, J., ... & Brookes, P. C. (2013). Human health risk assessment of heavy metals in soil–vegetable system: a multi-medium analysis. Science of the Total Environment, 463, 530-540.

[14] Luna HT and Lee G (1968). Manual of histological staining method of the Armed Forces Institute of Pathology . 3rd Ed. The Blakiston Division. McGrawHill Book Co. New York. USA.

[15] Mattia, C. J., Adams Jr, J. D., & Bondy, S. C. (1993). Free radical induction in the brain and liver by products of toluene catabolism. Biochemical pharmacology, 46(1), 103-110.

[16] Nandi, S., Toliyat, H. A., & Li, X. (2005). Condition monitoring and fault diagnosis of electrical motors—A review. IEEE transactions on energy conversion, 20(4), 719-729.

[17] Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. Journal of functional foods, 18, 757-781.

[18] Taş, D., Dellaert, N., Van Woensel, T., & De Kok, T. (2013). Vehicle routing problem with stochastic

travel times including soft time windows and service costs. Computers & Operations Research, 40(1), 214-224.

[19] Tas, U., Ogeturk, M., Meydan, S., Kus, I., Kuloglu, T., Ilhan, N., ... & Sarsilmaz, M. (2011). Hepatotoxic activity of toluene inhalation and protective role of melatonin. Toxicology and Industrial Health, 27(5), 465-473.

[20] Ulakoğlu, e. Z., Saygi, a., Gümüştaş, m. K., Zor, e., Öztek, i., & Kökoğlu, e. (1998). Alterations in superoxide dismutase activities, lipid peroxidation and glutathione levels in thinner inhaled rat lungs: relationship between histopathological properties. Pharmacological research, 38(3), 209-214.

[21] Vanuffelen, e. B., Van der zee, j., De koster, m. B., Vansteveninck, j., & Elferink, G. J. (1998). Intracellular but not extracellular conversion of nitroxyl anion into nitric oxide leads to stimulation of human neutrophil migration. Biochemical Journal, 330(2), 719-722

[22] WHO, (2014). World Health Organization, Ambient Air Pollution Database - Update 2014