Assessment of the Potential Genotoxicity and Cardiac Teratogenicity of Venlafaxine on Embryos of Pregnant Black Mice

Original Article

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ABSTRACT

Background: Depression during pregnancy is a very common problem worldwide, so the possibility of intake antidepressant medications during pregnancy is high. In Egypt, about 60% of pregnant women experience someforms of antenatal depression. The most widely prescribed antidepressants worldwide are Selective serotonin and serotonin–norepinephrine reuptake inhibitors (SSRIs and SNRIs).The mechanism of action is poorly understood but in general, these drugs act by blocking the serotonin and/or norepinephrine transporters.

Aim: The aim of this study is to investigate the effect of venlafaxine- one of the most widely prescribed SSRI (Selective serotonin reuptake inhibitors) on DNA, and also study its possible teratogenic effect on cardiac development.

Methods: The present study was carried out on fifty pregnant black mice (C57BL/6).The mice were randomly assigned to one of two groups: a control and venlafaxine-treated groups receiving (3mg, 10mg, 30mg and 100mg/kg/day). The fetuses were dissected for the evaluation of their cardiac structure. The micronucleus test was used to detect the ability of venlafaxine to induce DNA damage.

Results: The present work showed that administration of increasing concentrations of venlafaxine resulted in significant increase in the incidence of embryo heart anomalies in black mice as VSD, pulmonary trunk dilatation and right ventricle enlargement as compared to the control group. Also, this study showed an increase in the frequency of micronuclei in the blood of the adult mice after exposure to increasing dose of venlafaxine.

Conclusions: Physicians should make a proper decision regarding prescription of SSRI in general and venlafaxine, in particular, to treat depression during pregnancy weighing the risks and benefits for both mother and fetus.

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Key Words: Cardiac anomalies; genotoxicity; micronucleus; pregnancy depression; venlafaxine.

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INTRODUCTION

Serotonin is one of the neurotransmitters that is involved in transmission of nerve impulses in the central and peripheral nervous system[1]. It is considered as an important signaling molecule during embryo and fetal development. During craniofacial development, serotonin stimulates dental development[2]. It is also involved in shaping of the tempromandibular joint and in retinogenesis[3].

During early stages of embryogenesis, in cardiac development, serotonin is involved in the establishment of right/left laterality via the stimulation of fibroblast growth factor 8 (FGF8)/Nodal/ pituitary homeobox 2 (PITX2) pathways[4].

Studies have shown that disruption of the laterality pathway causes many different types of heart anomalies such as ventricular septal defect (VSD), atrial septal defect (ASD), dextrocardia, outflow tract defect (OTD) and pulmonary stenosis (PS)[5].

Sites of early serotonin biosynthesis during embryogenesis have not been detected, it has been suggested that serotonin may be probably of maternal origin[6].

Depression during pregnancy is a very common problem worldwide, so the rate of the exposure to antidepressant medications during pregnancy is very high. Studies revealed that it is marvelously increasing; for example 3-folds in the United States from 1998 to 2005[7], and 16-fold in Europe from 1997 to 2010[8]. In Egypt, about 60% of pregnant women experience antenatal depression[9].

The most widely prescribed antidepressants worldwide are Selective serotonin and serotonin–norepinephrine reuptake inhibitors (SSRIs and SNRIs).The mechanism of action is poorly understood but in general, these drugs act by blocking the serotonin and/or norepinephrine transporters[10].

The FDA considered venlafaxine as category C with regarded pregnancy risk which means that venlafaxine is

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suspected to cause harmful effects on the humen fetuses or neonates[11].

During the past decade, studies showed that treatment with SSRI/SNRIs during pregnancy has been associated with an increased risk of miscarriage, preeclampsia, preterm birth, decreased fetal body weight and head growth, congenital malformations, and behavioral disorders in offspring[12].

Recent work showed great debates in the increase of possibility of congenital heart defects associated with SSRI/SNRIS treatment. Also the available studies are not enough with respect to an association between treatments with SSRI especially venlafaxine and the increase in the incidence of congenital heart defects[13].

Micronucleus Test and Genotoxicity

The mammalian in vivo micronucleus assay is a mutagenicity test system used for the detection of chemicals that induce the formation of small membrane-bound DNA fragments known as micronuclei in the cytoplasm of interphase cells as a measure of genotoxicity. The test evaluates the formation of micronucleus in erythrocytes sampled either in the bone marrow or peripheral blood cells of animals, usually rodents[14].

Micronuclei are small, extranuclear chromatin bodies surrounded by a nuclear envelope. When a bone marrow erythroblast develops into a polychromatic erythrocyte (PCE) or reticulocyte, the main nucleus is extruded; any micronucleus that has been formed may remain behind in the cytoplasm. Visualization of micronuclei is facilitated in these cells because they lack the main nucleus. An increase in the frequency of micronucleated immature erythrocytes in treated animals is an indication of induced chromosomal damage[15].

Recently the micronucleus test – especially the direct assay- has become the most widely used test for genotoxicity as it is capable of assessing clastogenic, aneugenic, and epigenetic effects. It is simple, accurate and applicable. It can be done on any dividing cell population and its end point which is the frequency of micronucleated immature erythrocytes is easy to identify, so accurate data can be obtained[16].

A Test Chemical is Considered Positive if[17]

I. At least one of the doses exhibits a statistically significant increase in the frequency of micronucleated immature erythrocytes compared with the concurrent control.

II. The increase in the frequency of micronucleated immature erythrocytes is dose-related.

An increase in the frequency of micronucleated immature erythrocyte in treated animals reflects chromosomal damage induced by the test substance.

The available studies are not enough with respect to an association between treatments with SSRI especially venlafaxine and the increase in the incidence of chromosomal aberrations and congenital heart defects.

AIM OF THE WORK

The aim of the present work was to study the possible effects of venlafaxine treatment on cardiac development and DNA in black mice.

METHODS

The present study was carried out on fifty pregnant black mice (C57BL/6), obtained from the Animal House Center of Anatomy Department, Faculty of Medicine, Alexandria University. Mice were housed two per cage in a room temperature maintained at twenty degrees (20◦C) on a 12:12 hour light: dark cycle, average weight 18-22 gram.

Diet was administrated following the Egyptian Institute of Nutrition (EIN) recommendations. The animals were given food and water ad libitum during the experimental period.

All adult female mice (50) were mated with adult male mice (2:1) in standard caging conditions. The female mice were examined twice daily for the presence of vaginal plug.

Appearance of vaginal plug was the sure sign of mating and the day considered gestational day one (GD1)[18]. The pregnant females were isolated with daily follow up for miscarriage or any other conditions. The mice were left caged in appropriate circumstances from expected GD 1 to GD 8.

Efexor XR 75 mg (Venlafaxine hydrochloride) (- extended release capsules manufactured, packed and released by Pfizer Ireland Pharmaceuticals, Little Connell, Newbridge, Co. Kildare, Ireland. Obtained from Dawaee pharmacies, Alexandria, Egypt.

Grouping and start of treatment at GD8 as following

- **Group I:** (Control group) Included ten pregnant black mice received saline by gavage daily from GD8 to GD18.
- **Group II:** (Experimental group) Included forty pregnant black mice divided into:
  - **Subgroup IIa:** Included ten pregnant black mice received Venlafaxine hydrochloride from GD8 to GD18 by gavage daily at dose of 3 mg/kg/day in saline.
  - **Subgroup IIb:** Included ten pregnant black mice received Venlafaxine hydrochloride from GD8 to GD18 by gavage daily at dose of 10 mg/kg/day in saline.
  - **Subgroup IIc:** Included ten pregnant black mice received Venlafaxine hydrochloride from GD8 to GD18 by gavage daily at dose of 30 mg/kg/day in saline.
  - **Subgroup IId:** Included ten pregnant black mice received Venlafaxine hydrochloride from GD8 to GD18 by gavage daily at dose of 100 mg/kg/day in saline.

Sacrifaction and removal of uterine horns were carried on GD18 (Full term pregnancy) to exclude effects associated with delivery complications such as prolonged gestation and stillbirth.

1. Morphological study[19]

-]The uterine horns were dissected and the number of pups and resorptions were recorded.
-]The pups were separated from the placenta and evaluated for their sex, body weight and external morphology and After preservation in a preservative solution for one day, the pups were dissected for evaluation of cardiac structures under the Olympus SZ Dissecting Stereo Microscope:

The heart was separated from the lung and to get it out of the thoracic cage and divided into an anterior and a posterior halves using sharp scalpel followed by careful examination of its internal structure. The thickness of the right ventricular wall and pulmonary trunk diameter were measured using the Stereo Microscope.

The morphological study was done in Experimental embryology lab of the human Anatomy and Embryology department, Faculty of medicine, Alexandria University

2. Genetic study[20]

• DNA assessment with direct micronucleus test

o The tissues assessed for frequency of micronuclei were bone marrow and peripheral blood.

1. Two blood samples were obtained from adult pregnant black mice of the control and venlafaxine treated groups at GD 14 from tail vein and at scarification day (GD18) by cardiac puncture.
2. The blood samples were collected in EDTA treated tubes. Blood film stained with giemsa stain was done. The slides were examined under the Olympus CX41 Binocular LED – Sample Microscope.

o Bone marrow assay

1. The samples were collected from the femur of the animal at scarification day was preserved in EDTA tube then fixed by immersing them immediately in 4F1G (4% Formaldehyde and 1% Glutaraldehyde) in phosphate buffer solution (PH=7.2) at 4°C for 3 hours. Slides were made and Stained by uranyl acetate for 5 minutes then lead citrate for 2 minutes. Finally the sections were examined under the Olympus CX41 Binocular LED – Sample Microscope.

The genetic study was done at Experimental embryology lab of the human Anatomy and Embryology department, Faculty of medicine, Alexandria University

2. Statistical analysis of the data:

Data were fed to the computer using IBM SPSS software package version 20.0.For normally distributed data; comparison between more than two populations was analyzed by F-test (ANOVA).Significance test results were quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level, where values ≤ 0.05 are significant.

RESULTS

A. Resorption Sites

The present work revealed an increase in the frequency of spontaneous abortion manifested as resorption sites marked in subgroups IIc and IId. The mean number of resorption sites in subgroup II c was 1.60±1.647 and in subgroup II d was 1.00±1.333 which was significantly different ($p < 0.05$) from the control group, subgroup IIa and subgroup IIb. There was no significant difference between subgroup IIc and subgroup IId. In subgroup IIa and subgroup IIb, the mean value of the number of resorption sites was non-significant compared to the control group ($p > 0.05$). (Table 1, Figure 1)

B. Cardiac Anomalies

The results of the present work showed that venlafaxine has teratogenic effect on the heart of the pups of black mice. The number of pups of the control group was 120; the pups of venlafaxine treated groups were 375. Subgroup IIa consisted of 110 pups, subgroup IIb consisted of 100 pups, subgroup IIc consisted of 80 pups and subgroup IId consisted of 85 pups. The cardiac anomalies were significantly increased ($p < 0.05$) in venlafaxine treated groups in comparison with control group. The observed cardiac anomalies were: VSD, enlargement of the right ventricle and dilatation of pulmonary trunk. The percentages of cardiac anomalies were higher in subgroup IId than other venlafaxine treated groups and control group which means that the dose of 100mg/kg/day was highly toxic. (Table 2; Figures 2-6)

VSD was significantly higher ($p < 0.05$) in venlafaxine treated groups in comparison with control group. It was noted that the percentage of VSD anomaly among animals of subgroup IId was significantly lower than subgroups IIa, IIb and IIc. (Table 2, Figure 2)

Right ventricular hypertrophy was observed in zero pups out of 120 (0%) in the control group. In subgroup IIb, it was observed in 5 pups out of 100 (5%). In subgroup IIc, it was observed in 10 pups out of 80 (12.50%). In subgroup IId, it was observed in 12 pups out of 85 (14.12%). (Table 2, Figures 3, 4)

The mean thickness of the right ventricular wall in the control group was 112.5±10.85µm. while the mean thickness of the hypertrophied right ventricular wall was 280.5±26.92µm. (Table 2, Figures 3, 4)

Right ventricular wall enlargement was observed in subgroups IIb, IIc and IId and significantly higher ($p < 0.05$) in subgroups IIc and IId in comparison with the control group. There was no significant difference between subgroups IIc and IId. (Table 2, Figures 3, 4)

Pulmonary trunk dilatation was observed in 1.67% in the control group. In subgroup IIa, it was observed

in 13.64%. In subgroup IIc, it was observed in 6.25% and in subgroup IId, it was observed in 23.53%. (Table 2, Figures 5, 6)

The mean of normal pulmonary trunk diameter in the control group was 134.2±12.5µm. while the mean of dilated pulmonary trunk was 198.5±20.04µm. (Table 2, Figures 5, 6)

Pulmonary trunk dilatation was significantly higher ($p < 0.05$) in subgroups IIa and IId in comparison with the control group. It was significantly higher in subgroup IId than IIc. (Table 2, Figures 5, 6)

D. Other Defects

The present work showed a significant decrease ($p < 0.05$) in the placental weight at low doses of venlafaxine in subgroup IIa (mean: 0.101±0.047 mg) and subgroup IIb (mean: 0.078±0.057mg) in comparison with the control group and a consequent increase in the placental index (fetal weight / placental weight) in the same groups. (Table 3).

The external morphology of the placenta did not differ in the control group rather than the venlafaxine treated groups.

The present work also revealed that pups' body weights were significantly reduced ($p < 0.05$) in subgroup IId (Table 4) in comparison with the control group. In subgroups IIa, IIb and IIc, the pups' body weights were non-significantly different ($p > 0.05$) in comparison with the control group.

Major anomalies as anophthalmia (10.58%, Figure 7) and anencephaly (14.11%, Figure 8) were observed in subgroup IId.

2. Genotoxicity of Venlafaxine

The present work showed that administration of venlafaxine treatment induces DNA damage in black mice using the direct micronucleus test. The damaging effect of venlafaxine on DNA of the black mice increased with increasing the dose and the duration.

a) Peripheral blood assay: (Tables 5, 6, 8; Figures 9-10)

There was significant increase ($p < 0.05$) in the number of micronucleated erythrocytes in venlafaxine treated groups in comparison with control group.

There was significant increase ($p < 0.05$) in the number of micronucleated erythrocytes with increasing the dose of venlafaxine.

The number of micronucleated erythrocytes was significantly higher ($p < 0.05$) in subgroup IId than subgroup IIc which means that the dose of 100 mg/kg/day was highly toxic.

There was significant increase ($p < 0.05$) in the number of micronucleated erythrocytes in blood samples collected at GD 18 in comparison to blood samples collected at GD 14 (longer duration).

There was no significant difference ($p > 0.05$) in the number of micronucleated erythrocytes in blood samples collected at GD 18 in comparison to bone marrow samples collected at the same day.

b) Bone marrow assay: (Tables 7-8; Figures 11-13)

The bone marrow samples were collected at GD 18. There was significant increase ($p < 0.05$) in the number of micronucleated immature erythrocytes in venlafaxine treated groups in comparison with the control group.

There was significant increase ($p < 0.05$) in the number of micronucleated immature erythrocytes with increasing dose of venlafaxine.

The number of micronucleated immature erythrocytes was significantly higher ($p < 0.05$) in subgroup IId than subgroup IIc, which means that the dose of 100 mg/kg/day was highly toxic.

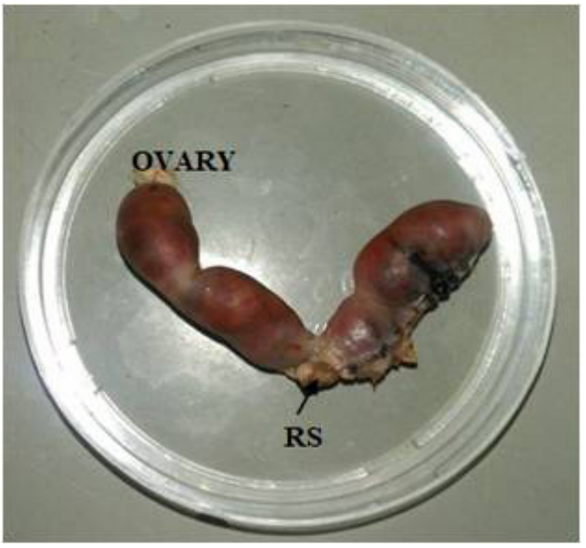

Fig. 1: A photograph of uterus of adult female mouse from subgroup IId. The photo shows one ovary and resorption site (RS).

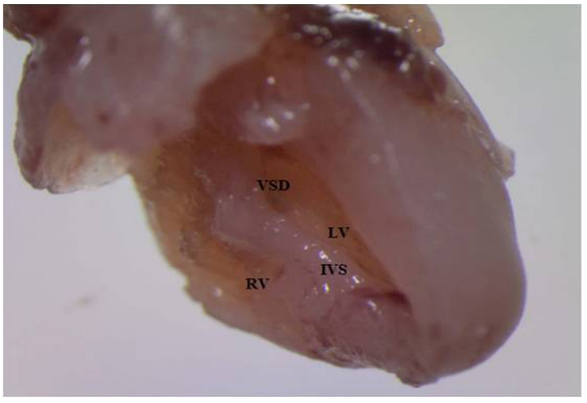

Fig. 2: A photograph of dissected heart of 18 days pup of black mouse of subgroup II d (anterolateral view) showing VSD. (x 16)IVS: Interventricular septum.VSD: Ventricular septal defect.LV: Left ventricle. RV: Right ventricle.

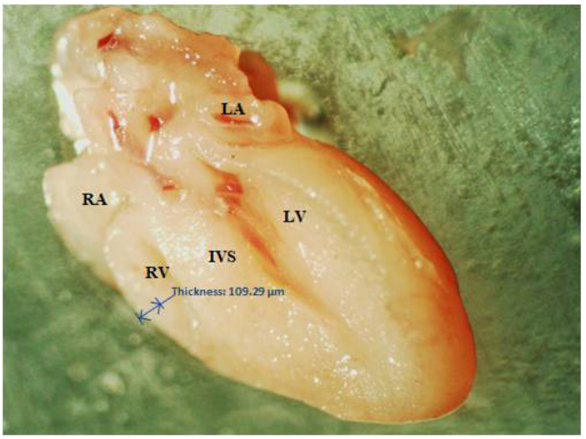

Fig. 3: A photograph of dissected heart of 18 days pup of black mouse of the control group (anterior view) showing the thickness of right ventricular wall measured using the Olympus SZ Dissecting Stereo Microscope. (x 16)IVS: Interventricular septum.LV: Left ventricle.RV: Right ventricle. LA: Left atrium.RA: Right atrium.

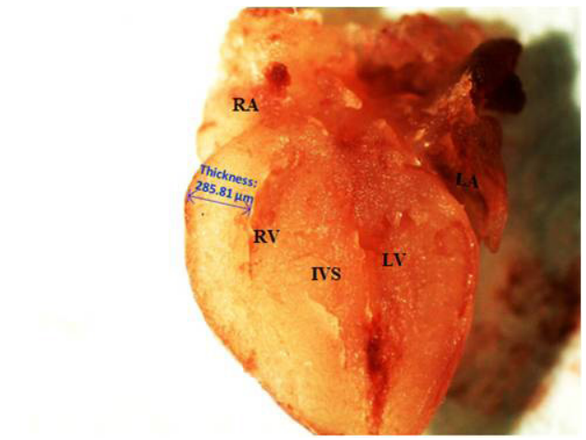

Fig. 4: A photograph of dissected heart of 18 days pup of black mouse of subgroup IIc (anterior view) showing the thickness of right ventricular wall measured using the Olympus SZ Dissecting Stereo Microscope. (x 12.5) IVS: Interventricular septum.LV: Left ventricle.RV: Right ventricle.LA: Left atrium.RA: Right atrium.

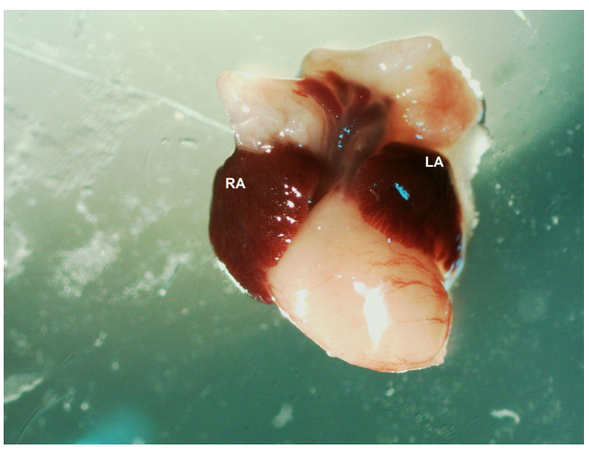

Fig. 5a: A photograph of dissected heart of 18 days pup of black mouse of the control group showing a normal shaped heart with the atria brownish in color. Right atrium (RA). Left atrium (LA).

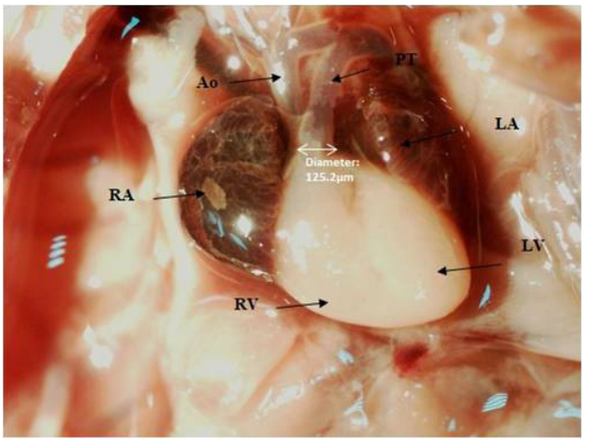

Fig. 5b: A photograph of dissected heart of 18 days pup of black mouse of the control group (anterior view) showing pulmonary trunk diameter measured using the Olympus SZ Dissecting Stereo Microscope. (x 16) PT: Pulmonary trunk. Ao: Aorta. LV: Left ventricle. RV: Right ventricle. LA: Left atrium. RA: Right atrium

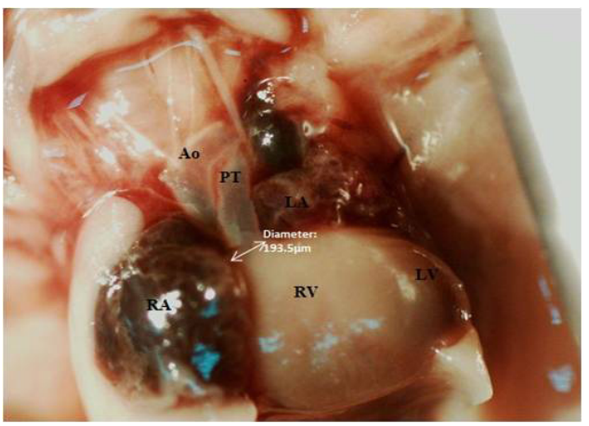

Fig. 6: A photograph of dissected heart of 18 days pup of black mouse of subgroup IId (anterior view) showing pulmonary trunk diameter measured using the Olympus SZ Dissecting Stereo Microscope. (x 16) PT: Pulmonary trunk.Ao: Aorta.LV: Left ventricle.RV: Right ventricle. LA: Left atrium.RA: Right atrium.

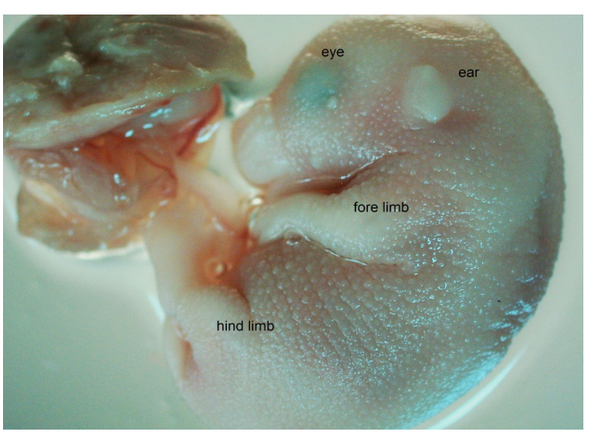

Fig. 7a: A photograph of 18 days pup of black mouse of the control group showing normal morphology with the eyes, ear, fore limb and hind limb are well seen. The photo was taken using Olympus SZ Dissecting Stereo Microscope (x 8).

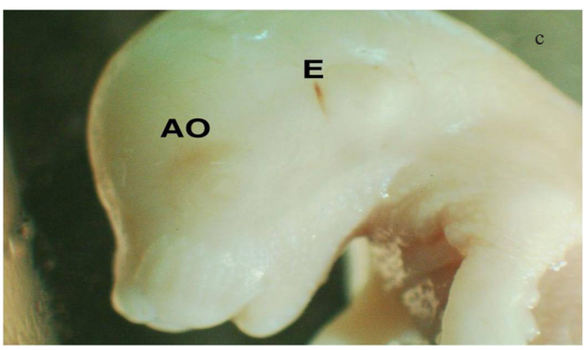

Fig. 7b and c: two photographs showing anophthalmia (Congenital absence of the eye) in 18 days pup of black mouse of subgroup II d. The photos was taken using Olympus SZ Dissecting Stereo Microscope (x 8).

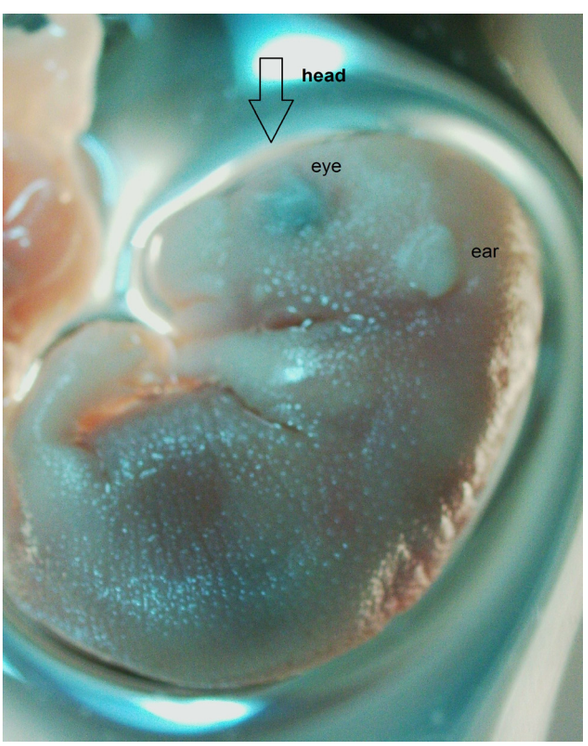

Fig. 8a: A photograph of 18 days pup of black mouse of the control group showing normal morphology of head containing the eyes anears. The photo was taken using Olympus SZ Dissecting Stereo Microscope (x 8).

Fig. 8b: A photograph showing anencephaly (congenital absence of cranial vault with part of neural tissues due to neural tube defect) in 18 days pup of black mouse of subgroup II d. The photo was taken using Olympus SZ Dissecting Stereo Microscope (x 5).

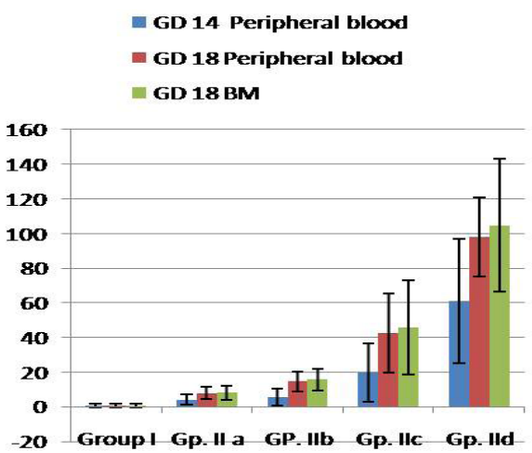

Fig. 9: Bar chart comparing different studied groups: the control group and venlafaxine treated groups regarding the number of micronucleated erythrocytes in peripheral blood samples at GD 14 and GD 18 and the number of immature micronucleated erythrocytes in bone marrow samples at GD 18.

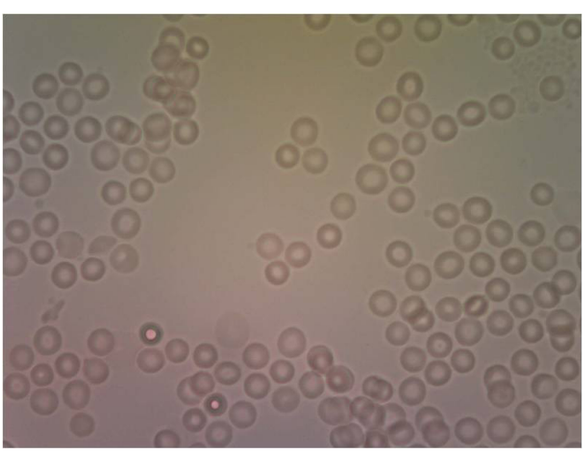

Fig. 10a: A photograph of a peripheral blood sample stained with Giemsa from adult pregnant black mouse of the control group showing normal RBC`s. (x 1000)

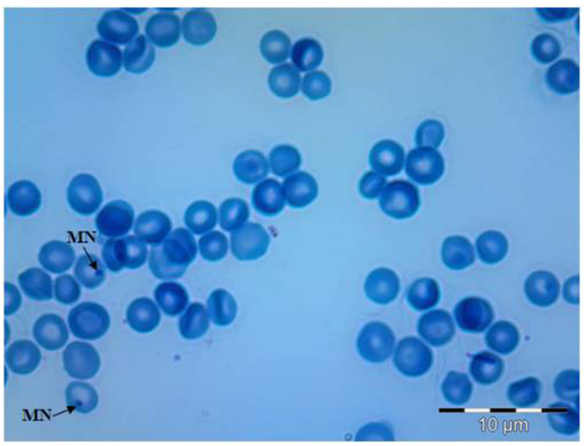

Fig. 10b: A photograph of a peripheral blood sample stained with Giemsa from adult pregnant black mouse of subgroup IId at GD18 showing RBCs containing micronucleus (MN) indicating genotoxicity of venlafaxine. (x 1000)

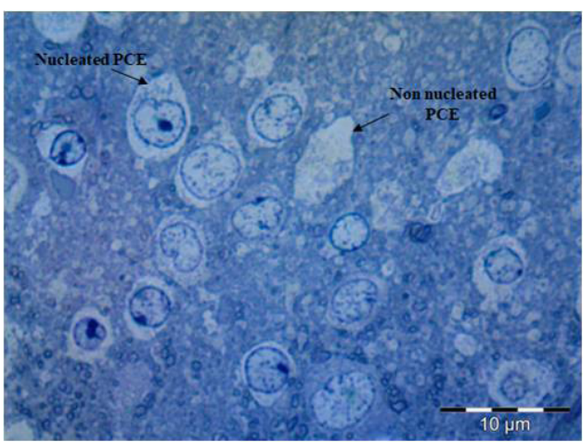

Fig. 11: A photograph of a bone marrow sample stained with uranyl acetate then lead citrate from the femur of adult pregnant black mouse of the control group at GD 18 showing nucleated polychromatic erythrocyte (PCE) and non-nucleated PCE. (x 1000)

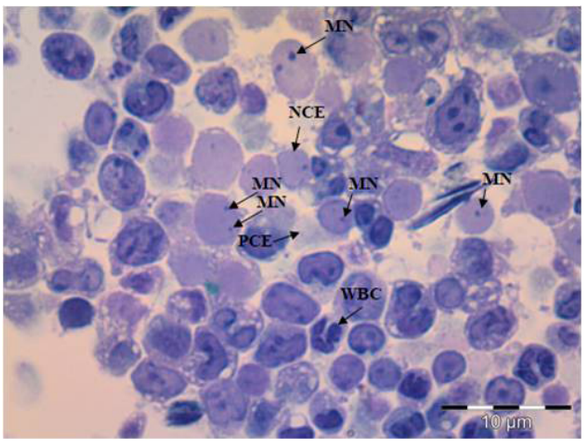

Fig. 12: A photograph of a bone marrow sample stained with uranyl acetate then lead citrate from the femur of adult pregnant black mouse of subgroup IIc at GD18 showing polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) containing micronucleus (MN) indicating genotoxicity of venlafaxine. (x 1000)

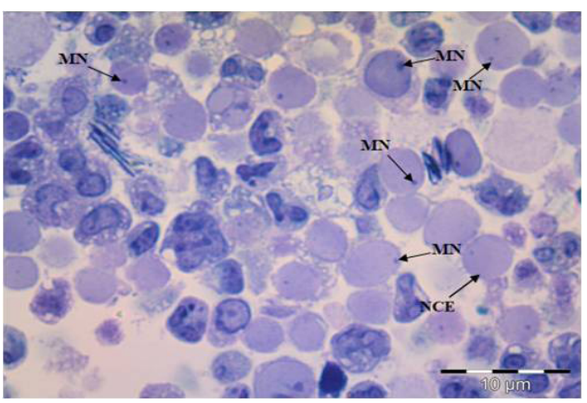

Fig. 13: A photograph of a bone marrow sample stained with uranyl acetate then lead citrate from the femur of adult pregnant black mouse of subgroup IId at GD18 showing normochromatic erythrocytes (NCEs) containing micronucleus (MN) indicating genotoxicity of venlafaxine. (x 1000)

Table 1: Comparison between different studied groups; the control group and venlafaxine treated groups regarding the number of resorption sites

Resorptions site No.	Gp. I Control group	Gp. II[a]	G. II[b]	Gp. II[c]	Gp. II[d]
Range	0-0	0-0	0-0	0-5	0-4
Mean± S.D.	0.00 ±0.000	0.00 ±0.000	0.00 ±0.000	1.60±1.647	1.00±1.333
F			16.05		
P			0.018*		
p1		-			
p2			-		
p3				0.001*	
p4					0.001*
p5					0.365

p1 comparison between the control group and group II a. p2 comparison between the control group and group II b.

p3 Comparison between the control group and group II c. p4 Comparison between the control group and group II d.

p5 Comparison between group II c and group II d. F: ANOVA test

*= statistically significant at $p \leq 0.05$

Table 2: Comparison between different studied groups: the control group and venlafaxine treated groups regarding the percentages of cardiac congenital anomalies (Pulmonary trunk dilatation, VSD, and right ventricular hypertrophy)

	Gp. I Control group "n=120"		Gp. II a "n=110"		G. II b "n=100"		Gp II c "n=80"		Gp. IId "n=85"	
	No.	%	No.	%	No.	%	No.	%	No.	%
Pul. Trunk Dilation	2	1.67	15	13.64	0	0.00	5	6.25	20	23.53
X^2						22.3				
p						0.001*				
p1				0.013*						
p2						0.078				
p3								0.065		
p4										0.002*
p5										0.0158*
VSD	2	1.67	12	10.9	15	15.0	15	18.75	6	7.06
X^2						15.6				
p						0.002*				
p1				0.039*						
p2						0.021*				
p3								0.011*		
p4										0.045*
p5										0.025*
Rt. Ventricle hypertrophy	0	0.0	0	0.00	5	5.00	10	12.50	12	14.12
X^2						6.98				
p						0.025*				
p1										
p2						0.465				
p3								0.042*		
p4										0.0314*
p5										0.522

p1 Comparison between the control group and group II a. p2 Comparison between the control group and group II b.
p3 Comparison between the control group and group II c. p4 Comparison between the control group and group II d.
p5 Comparison between group II c and group II d. X2: Chi-Square test
*= statistically significant at $p \leq 0.05$

Table 3: Comparison between different studied groups: the control group and venlafaxine treated groups regarding placental weight

Placenta wt. (gm)	Gp. I Control group	Gp. II[a]	G. II[b]	Gp. II[c]	Gp. II[d]
Range	0.11-0.29	0.03-0.20	0.02-0.23	0.087-0.22	0.11-0.31
Mean± S.D.	0.221±0.061	0.101 ±0.047	0.078±0.057	0.163± 0.042	0.188 ±0.036
F			16.54		
P			0.001*		
p1		0.013*			
p2			0.006*		
p3				0.098	
p4					0.103

p1 comparison between the control group and group II a. p2 comparison between the control group and group II b.
p3 Comparison between the control group and group II c. p4 Comparison between the control group and group II d.
p5 Comparison between group II c and group II d. F: ANOVA test
*= statistically significant at $p \leq 0.05$

Table 4: Comparison between different studied groups: the control group and venlafaxine treated groups regarding the pups' weight

Embryo wt. (gm)	Gp. I Control group	Gp. II[a]	G. II[b]	Gp. II[c]	Gp. II[d]
Range	0.78-1.6	0.65-1.4	0.63-1.5	0.54-1.6	0.53-1.08
Mean± S.D.	1.116 ±0.402	0.944±0.211	1.054± 0.226	1.014± 0.251	0.754±0.183
F			15.33		
P			0.004*		
p1		0.426			
p2			0.527		
p3				0.455	
p4					0.043*

p1 comparison between the control group and group II a. p2 comparison between the control group and group II b.

p3 Comparison between the control group and group II c. p4 Comparison between the control group and group II d.

p5 Comparison between group II c and group II d. F: ANOVA test

*= statistically significant at $p \leq 0.05$

Table 5: Comparison between different studied groups: the control group and venlafaxine treated groups regarding the pups' weight

Peripheral blood assay at GD 14	Gp. I Control group	Gp. II[a]	G. II[b]	Gp. II[c]	Gp. II[d]
Range	0-4	0-10	0-17	0-52	20-156
Mean± S.D.	0.60±1.188	4.10±3.007	5.60±4.935	19.70±17.045	61.05±35.782
F			38.5		
P			0.0001*		
p1		0.001*			
p2			0.001*		
p3				0.0001*	
p4					0.0001*
p5					0.001*
p6			0.106		
p7				0.003*	
p8					0.0001*
p9				0.005*	
p10					0.001*

p1 Comparison between the control group and group II a. p2 Comparison between the control group and group II b.

p3 Comparison between the control group and group II c. p4 Comparison between the control group and group II d.

p5 Comparison between group II c and group II d. p6 Comparison between group II a and group II b.

p7 Comparison between group II a and group II c. p8 Comparison between group II a and group II d.

p9 Comparison between group II b and group II c. p10 Comparison between group II b and group II d.

F: ANOVA test *= statistically significant at $p \leq 0.05$

Table 6: Comparison between different studied groups: the control group and venlafaxine treated groups regarding the number of micronucleated erythrocytes in peripheral blood samples at GD 18

Peripheral blood assay at GD 18	Gp. I Control group	Gp. II[a]	G. II[b]	Gp. II[c]	Gp. II[d]
Range	0.0-4.2	0.0-10.0	0.0-20.3	5.0-65.0	30.0-172.0
Mean± S.D.	0.61±0.901	7.98±3.65	14.6±5.65	42.6±22.8	98.0±22.8
F			42.65		
P			0.001		
p1		0.0021*			
p2			0.001*		
p3				0.0001*	
p4					0.0001*
p5					0.001*
p6			0.062		
p7				0.0014*	
p8					0.0001*
p9				0.005*	
p10					0.001*

p1 Comparison between the control group and group II a. p2 Comparison between the control group and group II b.

p3 Comparison between the control group and group II c. p4 Comparison between the control group and group II d.

p5 Comparison between group II c and group II d. p6 Comparison between group II a and group II b.

p7 Comparison between group II a and group II c. p8 Comparison between group II a and group II d.

p9 Comparison between group II b and group II c. p10 Comparison between group II b and group II d.

F: ANOVA test *= statistically significant at $p \leq 0.05$

Table 7: Comparison between different studied groups: the control group and venlafaxine treated groups regarding the number of micronucleated erythrocytes in bone marrow samples at GD 18

BM	Gp. I Control group	Gp. II[a]	G. II[b]	Gp. II[c]	Gp. II[d]
Range	0-3	3-14	9-30	10-100	70-200
Mean± S.D.	0.60±0.966	8.10±4.067	15.70±6.343	45.80±27.300	104.80±38.476
F			34.65		
P			0.0001*		
p1		0.001*			
p2			0.001*		
p3				0.0001*	
p4					0.0001*
p5					0.001*
p6			0.021*		
p7				0.001*	
p8					0.0001*
p9				0.0021*	
p10					0.0001*

p1 Comparison between the control group and group II a. p2 Comparison between the control group and group II b.

p3 Comparison between the control group and group II c. p4 Comparison between the control group and group II d.

p5 Comparison between group II c and group II d. p6 Comparison between group II a and group II b.

p7 Comparison between group II a and group II c. p8 Comparison between group II a and group II d.

p9 Comparison between group II b and group II c. p10 Comparison between group II b and group II d.

F: ANOVA test *= statistically significant at $p \leq 0.05$

Table 8: Comparison between different studied groups: the control group and venlafaxine treated groups regarding the number of micronucleated erythrocytes in peripheral blood samples at GD 14 and GD 18 and the number of immature micronucleated erythrocytes in bone marrow (BM) samples at GD 18

	Peripheral blood		P1	BM	P2
	GD 14	GD 18		GD 18	
Group I Control group	0.60±1.188	0.61±0.901	0.656	0.60±0.966	0.628
Gp. II [a]	4.10±3.007	7.98±3.65	0.003*	8.10±4.06	0.425
GP. II[b]	5.60±4.93	14.6±5.65	0.001*	15.70±6.34	0.226
Gp. II[c]	19.7±17.04	42.6±22.8	0.001*	45.8±27.3	0.301
Gp. II[d]	61.05±35.75	98.0±22.8	0.001*	104.8±38.47	0.211

P1 comparison between number of micronucleated erythrocytes at peripheral blood sapmles at GD 14 and GD 18.

P2 comparison between number of micronucleated erythrocytes at BM and peripheral blood at GD 18.

*= statistically significant at $p \leq 0.05$

DISCUSSION

The doses of venlafaxine used in the present work (3, 10, 30 and 100 mg/kg/day) were in the range of the therapeutic doses of this drug used in humans (i.e., 37.5, 75, 150 and 225 mg/day), and they were scaled to adjust for the difference in body surface area between human and black mice according to the study done by Nair AB et al.,[21] which demonstrated the equation of animal equivalent dose (AED).

The present work revealed an increase in the frequency of spontaneous abortion at venlafaxine doses of 30mg and 100mg /kg/day treated groups. This could be explained by either the serotonin theory which suggests that serotonin has abortive properties, or the adrenergic/noradrenergic theory, which suggests that catecholamines are associated with tocolytic properties. Since venlafaxine inhibits reuptake of both serotonin and noradrenaline, so both theories are acceptable[22].

A review by Broy P. and Berard A.,[23] including reviews, published from 1975 through 2009 for studies that examined the relation between poor pregnancy outcomes and in utero exposure to antidepressants with data on spontaneous abortions, agreed with the result of the present work and suggested that in utero exposure to antidepressants in general, paroxetine and venlafaxine in particular, increase the risk of spontaneous abortion.

The present work showed that administration of increasing concentrations of venlafaxine resulted in significant increase in the incidence of congenital heart anomalies in pups of black mice as ventricular septal defect (VSD), pulmonary trunk dilatation and right ventricular enlargement as compared to the control group.

VSD anomaly was significantly higher in subgroups IIa, IIb, IIc and IId in comparison with the control group. It was noted that the percentage of VSD anomaly among animals of subgroup IId was significantly lower than subgroups IIa, IIb and IIc. This may be due to genetic constitution of the tested animals of subgroup IId.

Pulmonary trunk dilatation was significantly higher in subgroups IIa and IId in comparison with the control group. This anomaly was not observed in subgroup IIb and non-significant in comparison to the control group in subgroup IIc may be due to genetic constitution of the tested animals of subgroups IIb and IIc.

Right ventricular wall enlargement was observed in subgroups IIb, IIc and IId and significantly high in subgroups IIc and IId in comparison with the contro group. This anomaly was not observed in subgroup IIa and non-significant in subgroups IIb may be due to the low dose of venlafaxine (3, 10 mg/kg/day) which was given to subgroups IIa and IIb. There was no significant difference between subgroups IIc and IId which means that this anomaly appeared with high doses and the dose of 30 mg/kg/day is toxic. It was noted that it appeared in the animals that had another anomaly as VSD or pulmonary trunk dilatation or animals had both VSD and pulmonary trunk dilatation.

Pulmonary trunk dilatation is a very rare congenital anomaly that may be explained as follows: in presence of other anomalies as interventricular septal defect which may cause left to right shunting. This shunting will lead to increase in pulmonary artery blood flow and shear stress leading to pulmonary artery dilatation[24].

Pulmonary trunk dilatation is significant clinically as it can predict pulmonary hypertension[24]. Based on this information, if pulmonary trunk dilatation is a sign of pulmonary hypertension, we can consider that the cause of right ventricle hypertrophy might be due to pulmonary hypertension.

A study done by Laurent et al.,[25] was in total agreement with the findings in the present work and stated that prenatal exposure to venlafaxine leads to an increase in cardiac anomalies including VSD, pulmonary trunk dilatation, absence of the innominate artery, and enlargement of one of the atria or the right ventricle, in 506 fetuses of rats which were examined under stereomicroscope.

A recent population-based cohort study included over 2.3 million births from Denmark, Finland, Iceland, Norway, and Sweden between 1996 and 2010 also agreed with the findings of the present work, stated that in utero exposure to antidepressants with effects on serotonin reuptake is associated with a 13% increased prevalence of major birth defects and a 15% increased prevalence of cardiac defects in a covariate controlled analysis[26].

Furthermore, a population based cohort study done by Pedersen et al.,[27] agreed with the findings of the present work. There was an increased prevalence of septal heart defects among children who were exposed in ureto to SSRI.

Another population based study by Colvin et al.,[28] included 123,405 pregnancies from 2002 to 2005. The study results showed an increased risk of cardiovascular defects and confirmed that in utero exposure to SSRI leads to increase in the prevalence of cardiovascular defects.

A previous cohort study by Vasilakis-Scaramozza[29] disagreed with the result of the present work and suggested that the exposure to tricyclic antidepressants (TCAs) and SSRIs during the first trimester of pregnancy was not associated with a significant increase of the risk of congenital anomalies in the offspring of mothers exposed to these drugs. The lack of association between in utero exposure to SSRI and congenital malformations in that study may be due to the small sample size.

The results of the present work revealed major anomalies as anophthalmia and anencephaly in subgroup IId.

Anencephaly can be explained as follows: serotonin influences neurogenesis and plays an important role in brain development. Serotonergic neurons appear as early as 5 weeks and by 15 weeks, the raphe nuclei already contain a typical arrangement of serotonin neurons. Disturbance of serotonin level by SSRI can lead to defective brain development as anencephaly[30].

Anophthalmia can be explained as follows: serotonin has an undeniable role in retinogenesis and in development of periocular mesenchyme. SSRIs affect serotonin level and may lead to anophthalmia[3].

A study done by Laurent et al.,[25] stated that prenatal exposure to venlafaxine was assossciated with increase in the incidence of major anomalies as anophthalmia and anencephaly.

Polen KN et al.,[31] in an earlier study suggested a relation between prenatal venlafaxine exposure and major congenital anomalies as anencephaly which agreed with the findings in the present work.

The findings in the present work differ from an earlier study done by Adrienne Einarson et al.,[32] on 150 women who were exposed to venlafaxine during pregnancy. They stated that there were no significant differences between the women of the control group and the women who took venlafaxine during their pregnancy in the comparison group. The study suggested that the use of venlafaxine during pregnancy does not increase the rates of major malformations above the baseline rate of 1%–3%.

The present work showed a decrease in the placental weight at low doses of venlafaxine (3, 10 mg/kg/day) and a consequent increase in the placental index in the same groups. This can be explained as serotonin is important for placenta development, acts as a vasoconstrictor in the placenta, and increases placental chorionic vein and umbilical artery vasoconstriction[33].

Previous studies demonstrated that activation of the serotonin receptor 5-HT2A in trophoblast cells activates intracellular signals that are known to regulate survival, differentiation, migration and invasion, suggesting a role for serotonin in placentation[34]. As a result, SSRI can affect the development of the placenta. SSRI treatment of pregnant women also alters gene expression in the placenta which could lead to an altered placental function, which in turn could affect the development of the fetus[35].

It was noted that the placental weight did not decrease at high doses of venlafaxine (30, 100 mg/kg/day). The explanation is unknown; this might need further study with large number of animals.

A study by Barak Y et al.,[36] discovered the existence of a functional link between the placenta and the developing heart. They were able to achieve a complete reversal of the cardiac phenotype of PPARγ of embryos through selective replacement of their trophoblast lineage by tetraploid chimera methodology. This may link the alteration of the placenta and congenital cardiomyopathies.

The present work showed a decrease in the fetal body weight at high doses of venlafaxine (100 mg/kg/day). The decrease in the fetal body weight can be explained by either alteration of placental function or by the possible toxic effect of venlafaxine[37].

Da Silva et al.,[38] detected decreased fetal and neonatal weight upon using venlafaxine and fluoxetine during gestation period in rodents at 40 and 80 mg/kg of venlafaxine. They explained these results by the potential toxic effects of SSRI on the rodents' embryos when administered during pregnancy.

A study by Singh M. and Singh KP.,[39] reported that the fetal body weights of prenatally exposed rats to venlafaxine were significantly reduced especially in high doses treated group. Their results showed fetal body weight reduction by 18.13, 25.31 and 30.94 % at respective doses of 25, 40 and 50 mg venlafaxine.

Another study by Gregory E. Simon et al.,[40] stated that in utero exposure to SSRI was associated with earlier delivery and consequent lower birth weight. Exposure to SSRIs was associated with a 0.9 week decrease in mean gestational age, a 175g decrease in mean birth weight. They also demonstrated that third-trimester SSRI exposure was also associated with lower Apgar scores.

A study done by Laurent et al.,[25] stated that prenatal exposure to venlafaxine alters the placenta with a consequent effect on fetal body weight and length. As a result, the placental index (fetal weight / placental weight) was increased.

In contrast, a cohort study by Nordeng et al.,[41]revealed that in utero exposure to antidepressant was not associated with low birth weight. The study included 63,395 women from the Norwegian Mother and Child Cohort Study. Of the 63,395 women, 699 (1.1%) reported using antidepressants during pregnancy, most frequently SSRIs (0.9%). There were 2887 infants born preterm (4.6%) and 1652 infants with low birth weight (2.6%), so the assossciation between SSRI use throughout pregnancy and preterm and low birth weight was non-significant.

The present work showed that administration of increasing concentration of venlafaxine treatment induces DNA damage using the direct micronucleus test. The direct micronucleus test is simple, accurate and applicable and its end point which is the frequency of micronucleated immature erythrocytes is easy to identify, so accurate data can be obtained.

In both peripheral blood and bone marrow assays, there was significant increase in the number of micronucleated erythrocytes in venlafaxine treated groups in comparison with control group.

There was significant increase in the number of micronucleated erythrocytes with increasing the dose of venlafaxine.

The number of micronucleated erythrocytes was significantly higher in subgroup IId than subgroup IIc which means that the dose of 100 mg/kg/day is highly toxic.

There was significant increase in the number of micronucleated erythrocytes in blood samples collected at GD 18 in comparison to blood samples collected at GD 14.

The DNA damage can be explained by the theory that serotonin is capable of inducing DNA cleavage in the presence of copper ions- which is a normal component of chromatin- through the generation of reactive oxygen species as hydroxyl radical[42].

The significant increase in the number of micronucleated immature erythrocytes in peripheral blood sample at GD 18 more than GD 14 is due to longer duration and exposure to venlafaxine.

The non-significant difference between the number of micronucleated immature erythrocytes in the bone marrow and the number of micronucleated erythrocytes in peripheral blood sample at GD 18 is explained by the theory stating that the spleen in mice does not remove micronucleated erythrocytes[43].

A previous study by Safarinejad MR.[44] agreed with the findings of the present work and stated that SSRI can impair semen quality and damage sperm DNA integrity.

A study by Kálmán J et al.,[45] was parallel with the findings of the present work. They studied the effect of venlafaxine on gene expression in human lymphocytes. They concluded that venlafaxine caused alterations in the expression of genes responsible for rapid adaptations to both external and internal stimuli, which is an important for neuronal plasticity.

In contrast, a study by Abdel-Wahab BA and Salama RH.,[46] claimed that Long-term venlafaxine treatment in low therapeutic doses (5, 10, 20 mg/kg/day) can protect against stress-induced oxidative cellular and DNA damage by attenuation of oxidative stress and lipid peroxidation.

CONCLUSION

The present work revealed that in utero exposure to venlafaxine alters the heart development with an increase in the incidence of the cardiac anomalies as VSD, right ventricle hypertrophy and dilatation of pulmonary trunk.

It also induces major anomalies such as anophthalmia and anencephaly, especially in high doses.

A decrease in the placental weight and consequent decrease in the fetal body weight also have been associated with venlafaxine.

Venlafaxine is implicated to cause spontaneous abortion in high doses.

This study also demonstrates that venlafaxine induces DNA damage with increasing the dose and duration of treatment.

CONFLICTS OF INTEREST

There are no conflicts of interest

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الملخص العربى

تقييم السمية الجينية المحتملة و تشوه القلب الناتج عن عقار الفينلافاكسين علي أجنة الفئران السوداء الحوامل

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المقدمه: اكتئاب الحمل مشكلة منتشرة عالميا و لذلك فإن معدل تعاطي الأدوية المضادة للاكتئاب خلال الحمل ازداد ، حيث أن في مصر ٦٠٪ تقريبا من النساء الحوامل يتعرضن لاكتئاب خلال فترة حملهن. إن مثبطات إعادة امتصاص السيرتونين الانتقائية و مثبطات إعادة امتصاص النورابينيفرين الانتقائية هما الأدوية الأكثر وصفا عالميا لعلاج اكتئاب الحمل . ان طريقة عمل هذه الادوية غير مفهومة تماما و لكن بصفة عامة فهى تعمل عن طريق منع نواقل السيرتونين و النورابينيفرين.

الهدف من البحث: وحيث أن الدراسات المتاحة الآن غير كافية للربط بين العلاج بمثبطات إعادة امتصاص السيروتونين الانتقائية و خصوصا عقار الفينلافاكسين و الزيادة في معدل حدوث خلل في الكروموسومات و التشوهات الخلقية في القلب. لذا كان الهدف من هذه الدراسة هو بحث تأثير الفينلافاكسين علي الحمض النووي و كذلك تأثيره المحتمل علي تطور القلب في الجنين.

مواد وطرق البحث: تم اجراء الدراسة على خمسين من الفئران السوداء الحوامل (٦/C٥٧BL).

تم توزيع الفئران عشوائيا على احدى المجموعتين:

١. مجموعة تحكم

٢. مجموعة تم اعطائها عقار الفينلافكسين بتركيزات (٣ مجم , ١٠ مجم , ٣٠ مجم , ١٠٠ مجم /كجم/يوم).

تم تشريح الاجنة و تقييم تركيب القلب.

و قد تم استخدام اختبار النوية الجيني للكشف عن قدرة الفينلافاكسين في إحداث تلف للحمض النووي.

النتائج: أظهرت الدراسة الحالية ان مع زيادة تركيز عقار الفينلافاكسين و زيادة الجرعة يزيد بشكل ذو دلالة احصائية معدل التشوهات الخلقية في القلب في أجنة الفئران السوداء كوجود ثقب في الحاجز بين البطينين، زيادة قطر الشريان الرئوي و تضخم في عضلة البطين الأيمن بالمقارنة مع مجموعة التحكم. كما اظهرت الدراسة الحالية أيضا زيادة ذو دلالة احصائية في معدل تكون النويات الناتجة عن تلف الحمض النووي كنتيجة لتناول عقار الفينلافاكسين بطريقة تعتمد علي الجرعة المأخوذة مما يثبت ان عقار الفينلافاكسين يؤدي إلي سمية جينية خصوصا في الجرعات الكبيرة.

الاستنتاج: لذا وجب علي الأطباء أن يتخذوا قرارا صائبا بخصوص وصف مثبطات إعادة امتصاص السيرتونين الانتقائية بشكل عام و عقار الفينلافاكسين بشكل خاص لعلاج الاكتئاب خلال فترة الحمل مع الموازنة بين المخاطر و المنافع العائدة علي الأم و الجنين.