INDUCED OXIDATIVE STRESS BY DEFEROXAMINE (DESFERAL) DURING THE PREGNANCY: PRE AND POST-NATAL DEVELOPMENTAL STUDY

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ABSTRACT

The aim of the study is to evaluate the effect of induced oxidative stress by the deferoxamine (desferal) during the pregnancy on pre and post-natal developmen. One hundred twenty adult female albino rats, 3 months old, with an average weight of 175-200 g, were randomly distributed into two main groups (60 rats/group). The 1st (control group) was injected with 0.3 ml physiological salt solution / animal, and the 2nd group was injected with 100 mg deferral/kg B.W. which was given intraperitoneal once daily for three consecutive weeks (during the pregnancy period). At the end of the treatment, each group in turn was divided into two parts, each section comprising 10 animals, blood samples were collected for biochemical analysis, then uterine and fetal tissues collected to observation the studied parameters. Results showed decrease in the numbers of implantation and blastocysts. The number of alive embryos were significantly reduced with an increasing number of dead and reabsorbed embryos, malformation of fetus increase in uterine of the stressed female group compared with control group. Postnatal observations showed significant variations in postnatal development parameters. Our study suggests that the deferral injection has a pro-oxidant capacity on rats' model at dose of 100 mg/kg, and the oxidative stress plays an important role during pregnancy especially on prenatal development as well as persist up to postnatal life.

Keywords: Deferral, Oxidative Stress, Pre and Postnatal Developments.

INTRODUCTION

Oxidative stress occurs naturally in the body because of an imbalance between oxidants and antioxidants, and it has a vital and effective role in many metabolic processes necessary for the growth and development of various body systems as well as its physiological role in many body functions (Agarwal *et al*., 2005). Although reactive oxygen species (ROS) are necessary to maintain fetal development, over-production of the ROS during embryonic life has a detrimental effect due to their interaction with different important cellular processes (Amin *et al.,* 2014). The ROS attack large cellular molecules, and cell structures such as the mitochondria membrane and the endoplasmic reticulum causing oxidative damage leading to changes in the cellular activity through their effect on gene regulatory action on many cellular proteins (He *et al.,* 2017).Most of the biological processes in the uterine The ROS regulate many of the transcription pathway by direct interaction with proteins tissue and ovarian are naturally involved free radicals that act as secondary messengers to perform their cellular response (Torres-Osorio *et al*., 2019).

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and modification of transcription factors and gene expression (Allen and Tresini, 2000). During pregnancy, an increase of stress severity may lead to unwanted

Pregnancy outcome for instance spontaneous abortion, recurrent pregnancy loss, birth defects, pre-eclampsia, intrauterine growth restriction, minor congenital anomalies, and future diseases in adulthood (Pereira and Martel, 2014; Duhig *et al.*, 2016). It was found that there is a strong link between increased ROS and early pregnancy loss (Bertout *et al.*, 2004). In another study conducted on mice, defects in the mitochondrial respiratory chain resulted in impaired fertility and increased miscarriage rate due to increased oxidative stress (Takamasa *et al*., 2014). Hubel (1999) found that oxidative stress causes impairment the blood vessels in the placenta leading to decreased oxygen supply, causing abnormal differentiation of the Trophoblast. In addition to that, Min *et al.* (2009) recorded decreased blood flow in the placenta and damage to fetal tissue due to intrauterine stress. The placenta considered as a rich source of oxidants and antioxidants due to an increase in metabolic efficiency in the placental mitochondria (Agarwal *et al.,* 2012). The formation of an abnormal placenta causes damage to the syncytiotrophoblast that is sensitive to stress during the first trimester of pregnancy, this contributes to recurrent pregnancy loss (Agarwal *et al.,* 2012).

Desferal (deferoxamine mesylate) is an iron-chelating agent used to treat iron overload caused by blood transfusions in adults and children at least 3 years old (Brittenham, 2011). Desferal can lead to increase oxidative stress via activation of free radical production signaling pathway (Francisco *et al.,* 2010). In this research work, our aims are to prove that desferal has pro-oxidant activity during pregnancy and to figure out its effect on pre and postnatal life in rats' model.

MATERIALS AND METHODS
Animals
The study was conducted in the Laboratory of the Physiology, Biochemistry and Pharmacology department / Faculty of Veterinary Medicine, University of Mosul. One hundred twenty adult females albino rats at 3 months of age were used, with an average weight of 175-200 g. They were distributed randomly into two main groups, which are control and treatment by 60 rats / group according to the design of the experiment. The laboratory animals were placed in plastic cages for breeding for a period of two weeks, and for environment acclimatization and observing the health status of the rats. The diseased animals were excluded and replaced with healthy rats. The laboratory animals were placed under appropriate environmental conditions in terms of temperature ($24 \pm 1°C$), humidity ($60 \pm 10\%$), ventilation and adequate lighting, and water and feed were provided to them freely throughout the experiment period (3 weeks).

Experiment of induced oxidative stress
For inducing oxidative stress, 10 adult females were used in this experiment, were injected with desferal a dose of 100 mg /ml /kg BW, i.p once daily for three consecutive weeks. Blood samples were collected one day before the injection of desferal, and after the completion of the injection period for measuring the level of each of MDA and glutathione as evidence of induced oxidative stress. The prescribed dose of desferal was adopted after confirming the occurrence of oxidative stress.

Experimental design

The experimental animals were distributed into two equal groups: the control group, which included 60 adult females, were injected with a physiological salt solution of 0.3 ml / animal, and the treatment group that was injected with desferal (deferoxamine methane, NOVARTIS-Switzerland), it was given intraperitoneal once daily for three consecutive weeks throughout the pregnancy period. The day one of pregnancy was determined by viewing the vaginal plug, as well as the presence of sperm in the vaginal swab (Palmer and Post, 1995). Each group was distributed into three subgroups:

1. Treatment group from day 1-7 of pregnancy (20 animals / subgroup).
2. Treatment group from day 1-14 of pregnancy (20 animals / subgroup).
3. Treatment group from day 1-20 of pregnancy (20 animals / subgroup).

At the end of each trial, the rats were distributed into2 parts; part 1 were sacrificed for biochemical analysis and prenatal life assessment. While, part 2 of each subgroup were left until parturient for assessing postnatal development.

Blood samples were collected from the orbital eye using capillary tube (Van Herck *et al.,* 1998). Uterine samples collection were involved to investigate the prenatal development, In addition to use these samples for assessing oxidative stress biomarkers in both rat dams and their fetuses.

Biochemical analysis

MDA was measured as a bio marker of lipid oxidation in serum and tissue samples, according to the (Buege and Aust, 1978), where the measurement was based on the interaction between MDA with thiobarbituric acid (TBA), a component of the MDA-TBA2 complex, which absorbs in the wave length of 532nm.

The concentration of glutathione was determined by the modified Almann method (James et al., 1982). The basis for the estimation of glutathione depends on the interaction between the detector DTNB reagent with glutathione in the sample. The golden yellow color and the intensity of the color depends on the concentration of glutathione. This color is measured using a spectrophotometer at a wavelength of 412 nm.

Pre and postnatal development assessment

The number of implantation sites, the number of blastocysts in the first week of pregnancy, the number of live and dead embryos, and the number of absorbed embryos in the second week of pregnancy were used to assess the prenatal developments. In addition to that during the third week of pregnancy, the data of the fetuses were recorded in terms of their total number, live, dead and deformed number.

For postnatal development females of part 2 were left until delivery. The data were collected immediately after 24 hours of birth. Total number of birth, number, live births, death and a number of anomalies were involved. Weekly body weight gain was measured in pups from 24 hours after delivery, D7, D14, D21, and D28 individually. Pups were randomly chosen for studying the postnatal growth rate. The litter size was checked after 24 hours of birth up to 28 days for the following parameters; days for ears, hair and teeth appearance, eye-opening and weaning days were recorded as an indicator for normal growth parameters.

Statistical analysis

All data were expressed as mean ± S.E, independent t-test was employed for determining the significant between pre and post induction of oxidative stress. Whereas, paired t-test was employed for determining the significant between studied groups by using SPSS program (Allen *et al*., 2014). *P*-value 0.05 was considered significant.

RESULTS

Experiment of oxidative stress induction.
Effect of desferal injection on glutathione level.
The injection of 100 mg desferal/kg body weight led to significant decline in serum glutathion level after 14 and 20 days of injection between pre and post injection at P-value 0.001, 0.002 respectively. Whereas, injection of DFO up to 7 days showed non significant decline in glutting at P-value 0.055. (Figure 1).
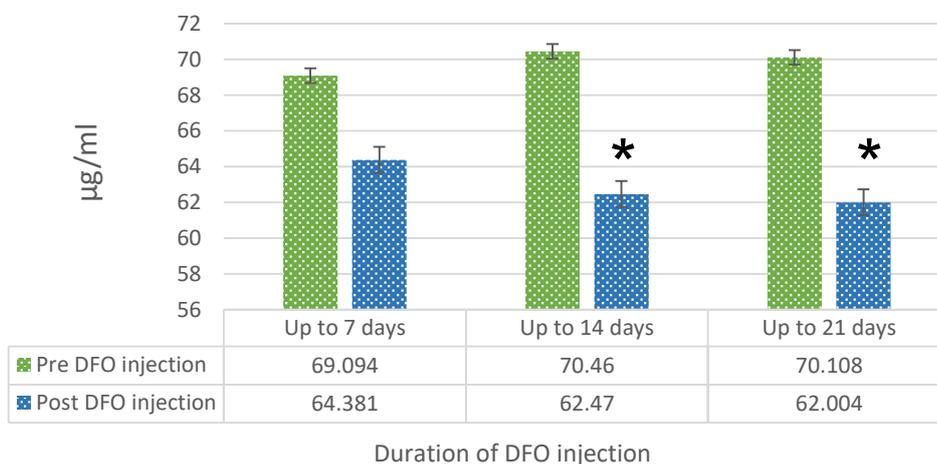

	Up to 7 days	Up to 14 days	Up to 21 days
Pre DFO injection	69.094	70.46	70.108
Post DFO injection	64.381	62.47	62.004

Duration of DFO injection

Figure (1): Effect of desferal injection on serum glutathione.

Effect of desferal injection on lipid peroxidation marker.

Lipid peroxidation marker (MDA) was significantly higher in post injection duration compare to pre injection period after 14 and 20 days of continuous desferal injection to induce oxidative stress at P-value 0.001, 0.004 respectively. In the time, the first 7 continuous days had no significant elevation in lipid peroxidation level at P-value 0.064. (Figure 2).
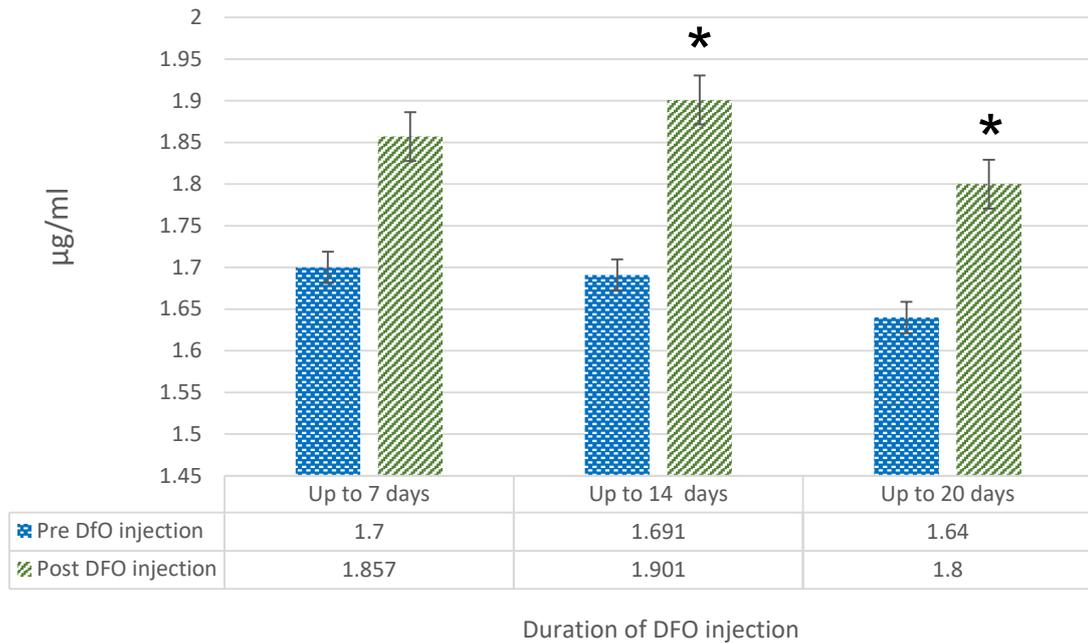
Figure (2): Effect of deferral injection on serum lipid peroxidation .

Experiment of effect of induced oxidative stress in both pregnant rats and their fetuses.
Effect of induced oxidative stress on glutathione level after 7, 14 and 20 days of pregnancy.

The statistical analysis showed no significant differences for the glutathione level in serum and uterine tissue after injecting female rats with desferal for 7 days at significant level the 0.064 and 0.18 for both serum and uterine tissue, respectively. (Figure 3).
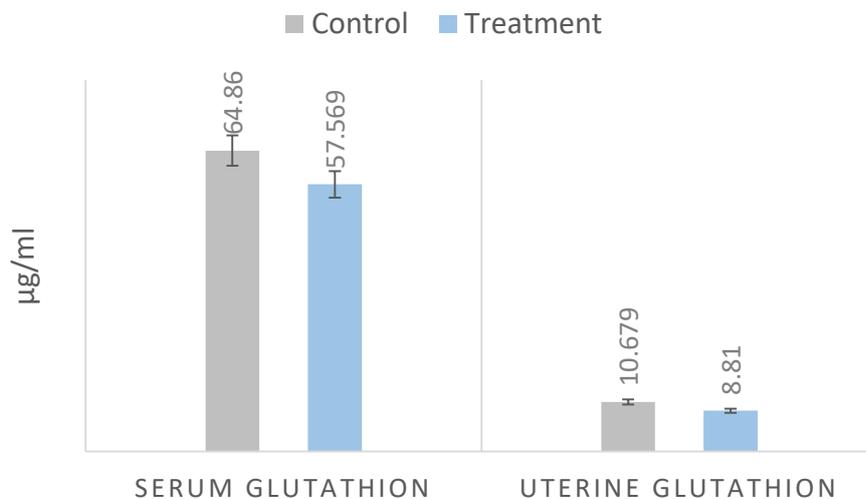

Figure (3): Effect of oxidative stress on both serum and uterine tissue glutathione level after 7-days of pregnancy.

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The induced oxidative stress showed significant changes represented by a decrease in the glutathione level in serum and embryonic tissues at the *P*-value was 0.032 and 0.001, respectively. (Figure 4)
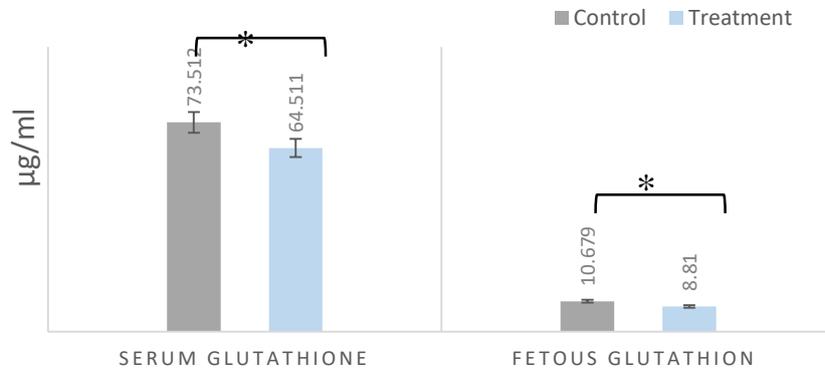

Figure (4): Effect of induced oxidative stress on both serum and uterine tissue glutathione level after 14-days of pregnancy.

Whereas, glutathione level in both pregnant rats and their fetuses after 20 days of injection with desferal led to a significant decrease in the serum glutathione level at P-value was 0.006 and 0.042, respectively. (Figure 5)
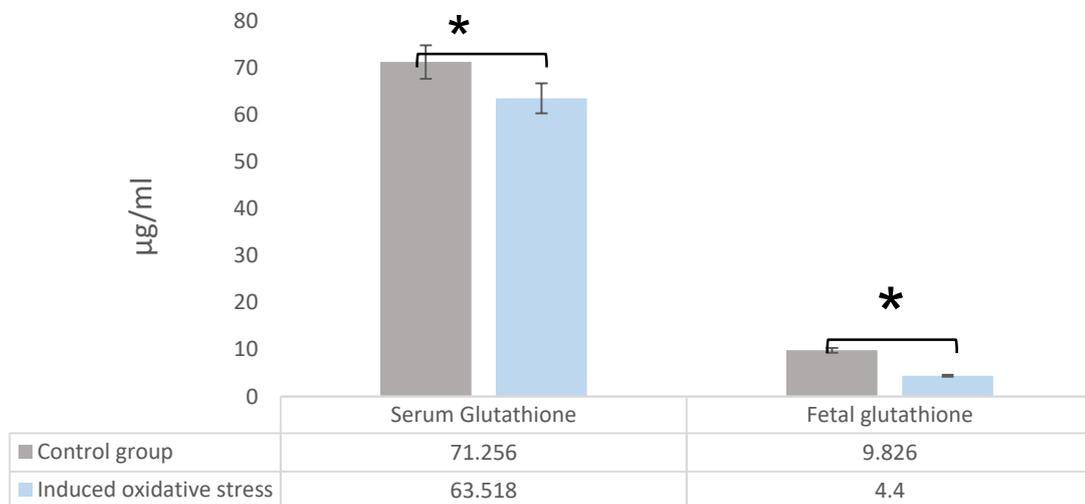

	Serum Glutathione	Fetal glutathione
Control group	71.256	9.826
Induced oxidative stress	63.518	4.4

Figure (5): Effect of induced oxidative stress on both serum and uterine tissue glutathione level after 20-days of pregnancy.

Effect of induced oxidative stress on MDA level after 7, 14 and 20 days of pregnancy.

The induced oxidative stress by desferal did not showed non-significant elevation in the MDA level of both serum and uterine tissue after ip injection for 7 days at the *P*-value was 0.076, 0.085 for both serum and uterus respectively. (Figure 6).

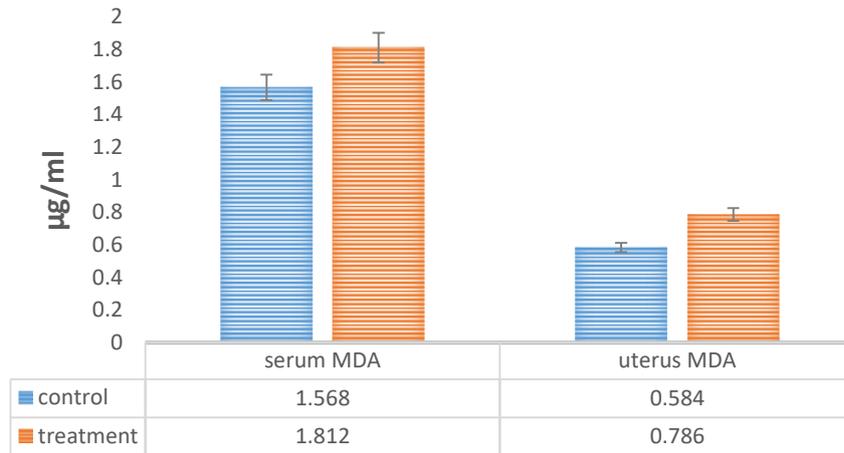|	serum MDA	uterus MDA
control	1.568	0.584
treatment	1.812	0.786

Figure (6): Effect of induced oxidative stress on both serum and uterine tissue MDA level after 7-days of pregnancy.

The MDA level in the serum of pregnant rats showed a significant increase compared to the control group at the level *P*-value 0.032, While, the mean value level of MDA of belongs to induced oxidative stress group was not significant compared to the its value of the control group P 0.084. (Figure 7)

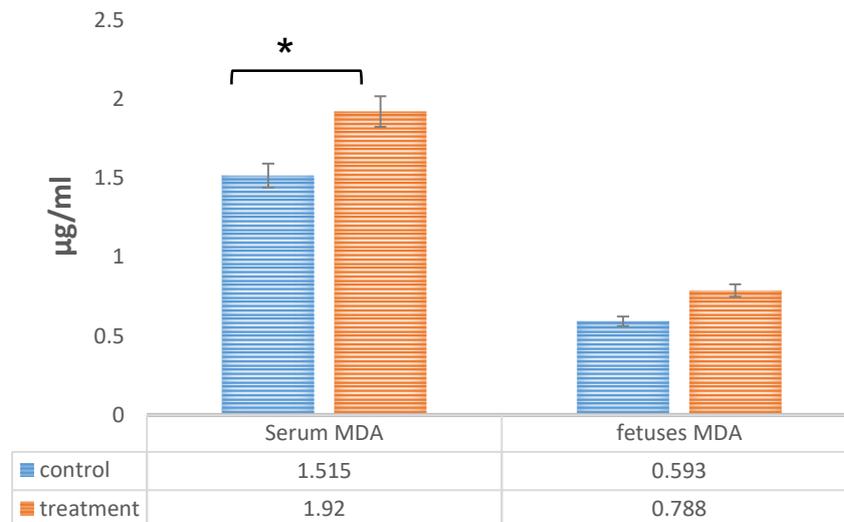|	Serum MDA	fetuses MDA
control	1.515	0.593
treatment	1.92	0.788

Figure (7): Effect of induced oxidative stress on both serum and fetus MDA level after 14-days of pregnancy.

Induced oxidative stress up to 20 days during pregnancy showed a significant increase in the MDA level in serum and fetal tissues between studied groups at the *P*-value was 0.006 and 0.001, respectively. (Figure 8).
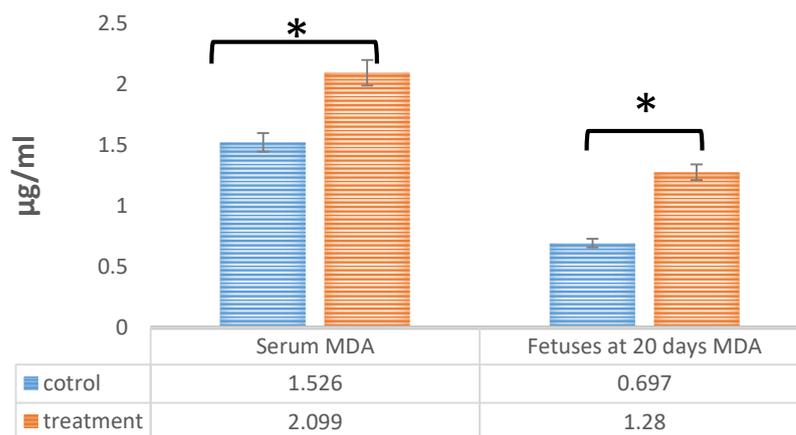
Figure (8): Effect of induced oxidative stress on both serum and fetus MDA level after 20-days of pregnancy.

Experiment of the effect of induced oxidative stress on prenatal development and weight of fetuses at day 20 of pregnancy.

The statistical analysis of the fetal development data of studied females' rats showed a non-significant decrease in the number of implantations (P 0.104) between control and stressed females. Whereas, the oxidative stress led to a significant decrease (P 0.043) in the average number of blastocysts in the uterus of the stressed females compared with the control females. (Figure 9).
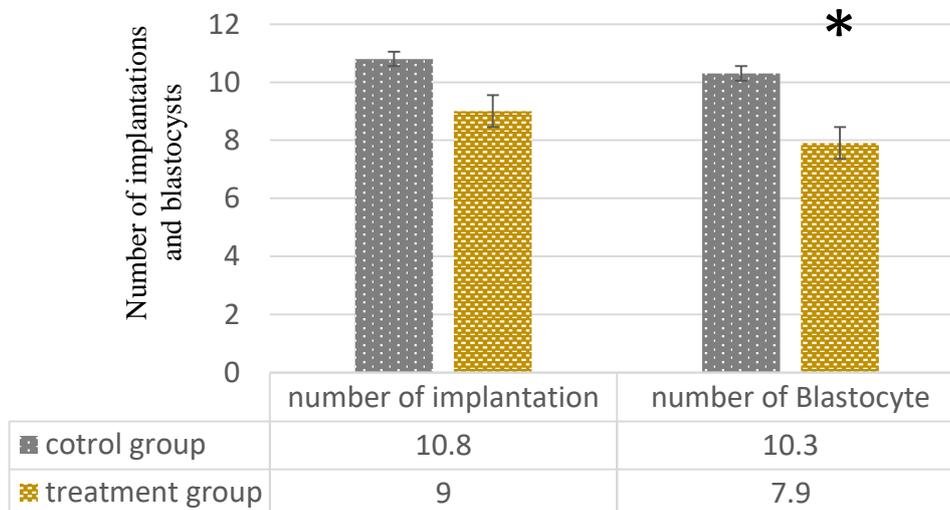

Figure (9): Effect of induced oxidative stress on embryonic development during the first 7 days of pregnancy.

The induced oxidative stress showed no significant decrease in both the total number of embryos and the number of alive embryos of stressed females compared to control females (P 0.105 and 0.051, respectively). Whereas, the induced oxidative stress resulted in a significant increase in the number of dead embryos and the number of absorbed embryos in the stressed females compared with the control females (P 0.037, 0.001). (Figure 10).

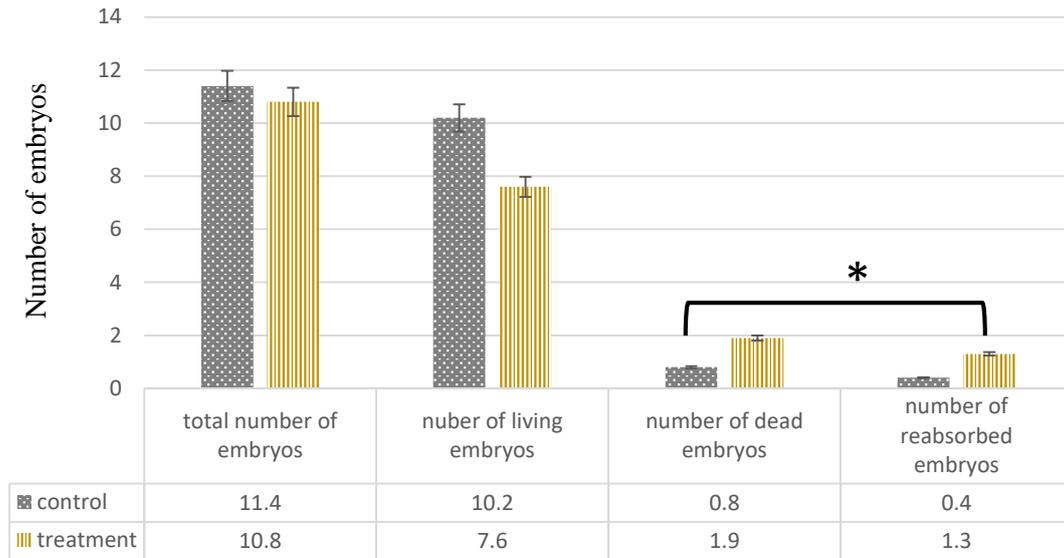|	total number of embryos	nuber of living embryos	number of dead embryos	number of reabsorbed embryos
control	11.4	10.2	0.8	0.4
treatment	10.8	7.6	1.9	1.3

Figure (10): Effect of induced oxidative stress on fetal development after 14 days of pregnancy.

       The induced oxidative stress in female rats led to a significant decrease in the total number of adult fetuses and the number of alive fetuses / female compared with the control group females (P 0.001). Whereas, the rate of the number of dead fetuses and the rate of the number of fetal anomalies / female showed a non-significant increase of the intrauterine stressed fetuses compared with the non-stressed fetuses (P 0.813, 0.264, respectively). The weight of the fetuses at day 20 of pregnancy did not show difference between the intrauterine stressed fetuses and non-stressed fetuses (P 0.061). (Figure 11 and 12).
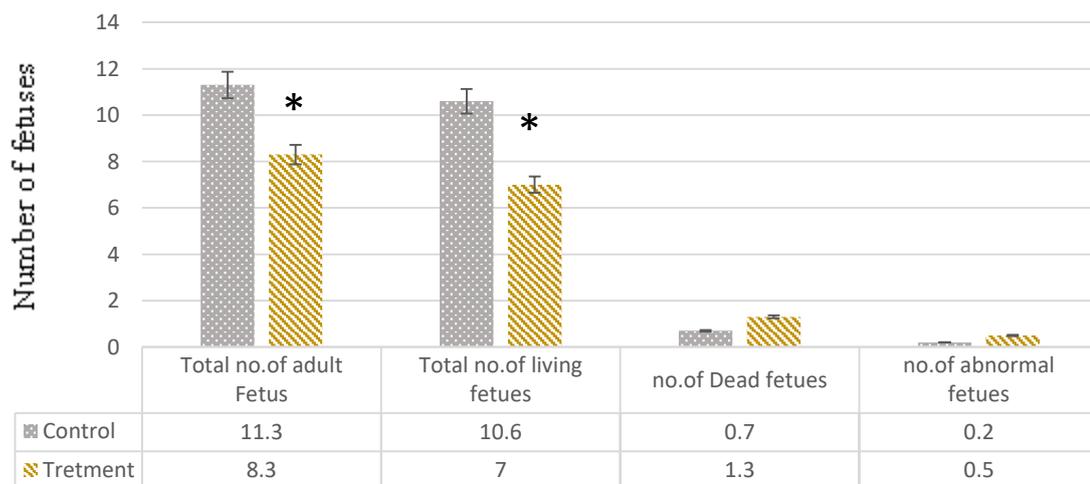

	Total no.of adult Fetus	Total no.of living fetues	no.of Dead fetues	no.of abnormal fetues
Control	11.3	10.6	0.7	0.2
Tretment	8.3	7	1.3	0.5

Figure (11): The effect of induced oxidative stress on fetal development after 20 days of pregnancy.

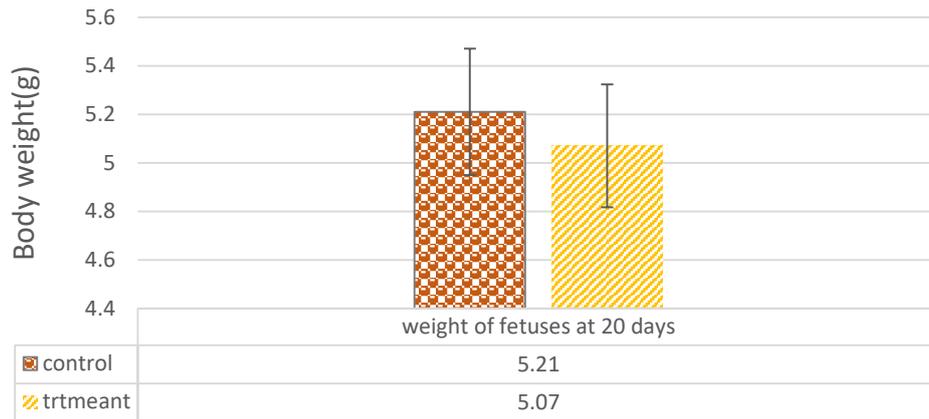|	weight of fetuses at 20 days
control	5.21
trtmeant	5.07

Figure (12): Effect of induced oxidative stress on fetuses' body weight at day20 of pregnancy.

Experiment of the effect of induced oxidative stress on certain postnatal development parameters and weaning day.

The postnatal development of the newborns of stress subjected rats during a period of 7 and 14 days of pregnancy showed a difference between the two studied groups of some studied criteria. For instance, there was a significant increase in the number of days required for the appearance of hair and teeth in the newborns from the stresses dams compared with the newborns from the normal dams (P 0.004 and 0.001 respectively). While, the other criteria did not show any significant differences between the two groups. (Figures 13 and 14).

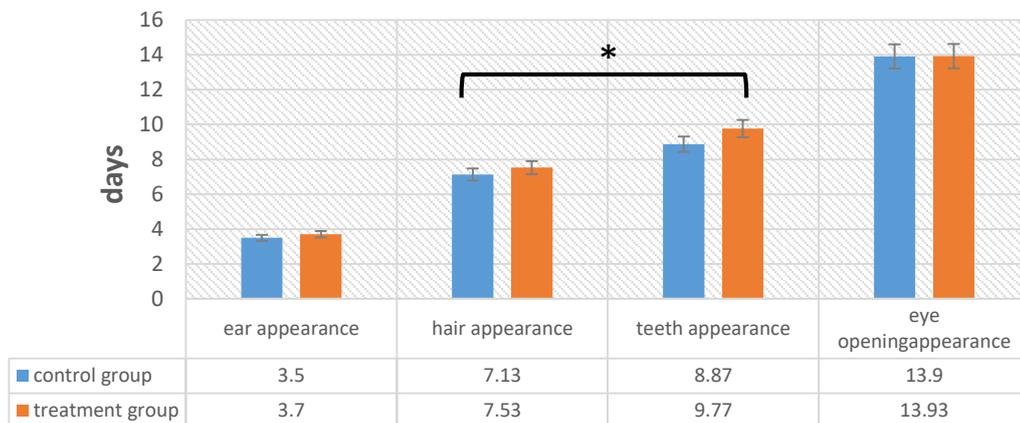|	ear appearance	hair appearance	teeth appearance	eye openingappearance
control group	3.5	7.13	8.87	13.9
treatment group	3.7	7.53	9.77	13.93

Figure (13): The effect of induced oxidative stress on postnatal development parameters of the experimental female infants during the first week of pregnancy.
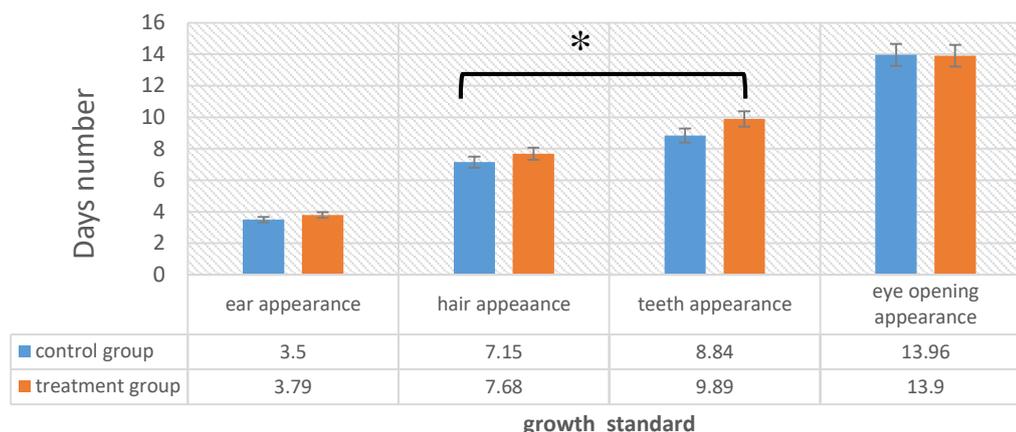
Figure (14): The effect of induced oxidative stress on postnatal development parameters of intrauterine stress subjected pups during the second week of pregnancy.

While, the induced oxidative stress up to 20 days of pregnancy lead to clear changes in some of the studied growth parameters. For instance, the appearance of hair, teeth and the appearance of the eye opening was delayed in the pups of mothers subjected to intrauterine stress up to 20 days compared with the same criteria for the pups of dams of the control group (P 0.045, 0.006, 0.03, respectively). (Figure 15).
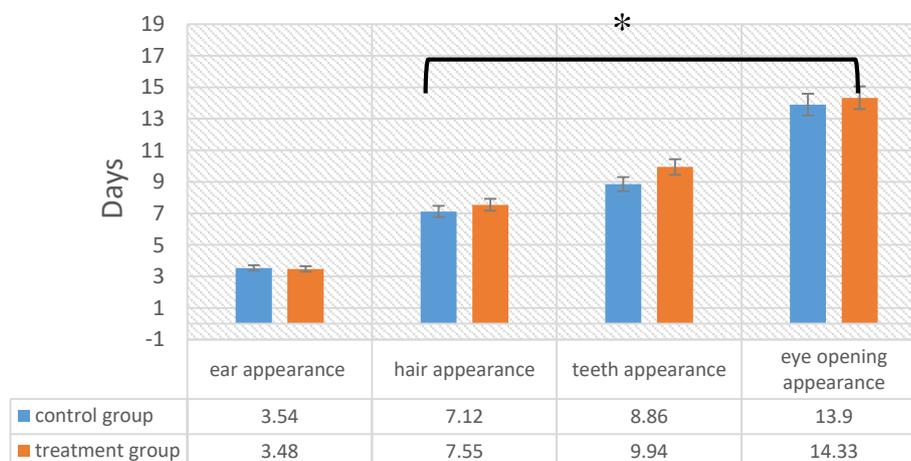

Figure (15): The effect of induced oxidative stress on postnatal development parameters of intrauterine stress subjected pups during the third week of pregnancy.

Statistical analysis using independent t-test for evaluating the data of the weaning age of the pups from both stressed and non-stressed dams resulted in a significant increase in the number of weaning days required for weaning in the intrauterine stressed pups group compared with the control group (P 0.001) (Figure 16).
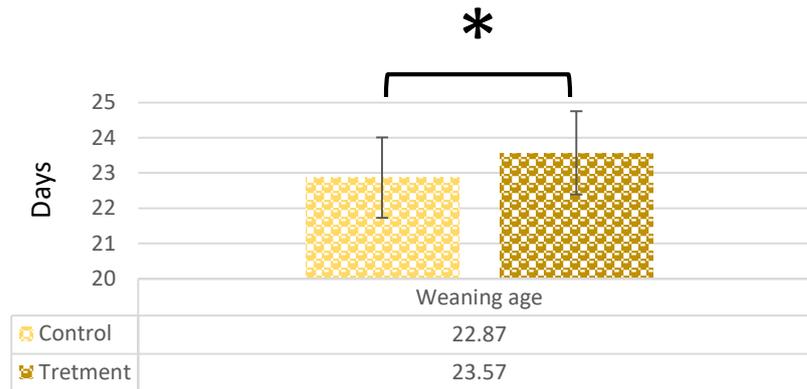
Figure (16): The effect of induced oxidative stress on the weaning age of the experimented pups.

DISCUSSION

Excessive oxidative stress as a result of an increase in the level of free radicals, especially ROS and hydroxyl, and a decrease in the activity and efficiency of antioxidants within the uterine tissue and the placenta. This gives an important explanation for many cases of pregnancy failure and defects in the growth and development of fetuses during and after pregnancy in a certain period (Silveira *et al.,* 2018; Wu *et al.*, 2017).

For the purpose of inducing oxidative stress in rats, Desferal was used in a high dose for two consecutive weeks. Injecting intraperitoneal resulted in oxidative stress induced through apparent changes in the levels of oxidative markers. desferal works to oxidize iron in cytosol and convert it from the Fe^{+2} to the Fe^{+3} by reacting with hydrogen peroxide because of the generation of free hydroxyl radicals in the cell. This indicates that Desferal, in addition to its role as an antioxidant, acts as a pro-oxidant when used in a high dose for a long time. The stimulation of the lipid peroxidation process resulted from Desferal may be with the presence or absence of iron through the production of highly reactive hydroxyl radicals that are toxic to cells, and this opinion is identical to what was reported by Mukosera *et al.* (2020); Tada *et al.* (2015); Kim *et al.* (2007); Poot *et al.* (1989); Morehouse *et al.* (1988) ; Morehouse *et al.* (1987).

The current results are in agreement with a previous study in which researchers demonstrated that 6 mmol of desferal when incubated for 2 hours with erythrocytes led to oxidative stress events by reducing the redox potential of NAD in erythrocytes (Niihara *et al.,* 2002). The other cause of the oxidative stress induced by desferal may be attributed to its role as an electron transport medium from ascorbic acid in cells to oxygen to produce the superoxide anion, which in turn leads to the production of the active nitroxide radicals, as well as the role of desferal in the Fenton reaction (Jiang *et al*., 2016).

The current study resulted in clear changes in the levels of oxidative stress parameters for pregnant rats and their fetuses at different stages of pregnancy. This was evident in the second week of pregnancy and continued until the end of the trial

period in both mothers and their fetuses at the level of blood and tissue. The reason for this is due to the insufficiency of the action or the low level of antioxidants, for example glutathione peroxidase, as well as the overproduction of free radicals of various kinds. Where, glutathione is a strong and effective reducing substance in the cell and has a distinct role in the regeneration and maintenance of moderate levels of the enzyme glutathione reductase very important in vital tissues and developing, including the pregnant uterus (Wickens, *et al.*, 1981; Yu, 1994). The current results showed an increase in the level of malondialdehyde and a decrease in the level of glutathione peroxidase, which is due to an increase in the level of free radicals and a decrease in the level of glutathione in the tissue and blood, and this corresponds to previous studies that illustrate the role of oxidative stress in pregnancy (Lu, *et al.*, 2018; Al-Gubory *et al.*, 2010; Patil *et al.*, 2007). Tissue glutathione depletion directly affects the level of antioxidants, making cells more vulnerable to oxidative stress even under physiological conditions (Sekhon *et al.*, 2010) .this mechanism is attributed to a series of interconnected enzymatic processes associated with cellular oxidative stress, which may result in cell death or failure in its basic function. Among these enzymatic processes is the stimulation of arachidonic acid by activating the enzyme phospholipase A2 associated with the release of arachidonic acid, which works to destroy cells that have depleted glutathione because of metabolism (Balsinde *et al.*, 2002). Arachidonic induction by the action of the enzyme lipoxygenase, which in turn stimulates the synthesis of the active superoxide radicals during the metabolism of arachidonic acid, which plays a dangerous toxic role as a result of depletion of glutathione leading to cell damage and death (Mytilineou *et al.*, 2002).

This cellular pathway is one of the most important pathways associated with glutathione depletion, which induces a series of cellular events that end with an increase in the level of free radicals and their attack on cellular molecules, causing sufficient oxidative damage to cell death or loss of its basic function (Katsuki *et al.*, 1995). The increase in malondialdehyde in the body is an important indicator of the severity of the activity of the free radicals produced and their effect on the lipid peroxidation resulted from the occurrence of oxidative stress (Ruder *et al.* 2009). The active aldehydes are the second messengers of the deleterious activity of the active oxygen species, which are also by-products of the lipid peroxidation of unsaturated fatty acids (Ishii, *et al.*, 2006). Malondialdehyde is also produced as a byproduct of the inactive thromboxane formation from its inactive form, inactive thromboxane TXA2, which is a vasoconstrictor agent (Bauer *et al.,* 1999). This in turn supports our hypothesis about the lack of oxygen supply to the placental tissue on the one hand and the uterine tissue in the areas of growth and development of fetuses due to the lack of blood flow in them. On the other hand, a second byproduct of lipid peroxidation is the proliferation of smooth muscle cells of the lining of blood vessels that contribute to vascular injury (Watanabe *et al.,* 2002).This results in early loss of embryos as well as impaired growth and development of embryos.

With regard to previous studies, it has been shown that oxidative stress is closely related to early miscarriage and many conditions related to pregnancy, which in turn occurs as a result of lack of oxygen in the uterine tissue during the early stages of pregnancy. Where the placenta are characterized by low antioxidant activity as

well as their low concentrations, especially in the first trimester of pregnancy (Schoots *et al*., 2018). The blastocysts are more susceptive to damage resulting from variation in oxygen levels and free radicals resulting from the biological processes accompanying the implantation of embryos and their growth (Aouache *et al.,* 2018; Mujica *et al.,* 2018). The current study demonstrated that early exposure to oxidative stress in pregnant rats led to many undesirable effects, represented by a decrease in the number of blastocysts in the first week, an increase in the number of dead and absorbed embryos in the second week, and a decrease in the number of live embryos in the third week of pregnancy. The reason for these changes may be attributed to the enzymatic activity associated with the growth and development of the embryos and its increased influence on the metabolic processes in the placenta due to the imbalance of oxygen levels in the placental tissue, which results in an excessive increase in the synthesis and production of free radicals (Schoots *et al*., 2018). Whereas, it is known that placental hypoxia is closely related to the conversion of xanthine dehydrogenase into xanthine oxidase, the main source and important in generating cytotoxic free superoxide radicals (Poston *et al.,* 2011). Burton *et al.* (2009) observed that lack of oxygen in the placental tissue causes stress of the endoplasmic reticulum stress and stimulates the formation and accumulation of misfolded proteins in the cell, leading to stimulation unfolded protein responses to try to restore the balance of the endoplasmic reticulum. This activity is mainly related to the functioning of placental cells in accordance with their levels of antioxidants and the level of oxygen in order to prevent tissue damage leading to premature death of the fetus or the inability of the uterine tissue to receive a fertilized egg (Torres-Cuevas *et al.,* 2017).

Conclusion

Our study suggest that the desferal injection has a pro-oxidant capacity on rats' model at dose of 100 mg/kg BW. An oxidative stress plays an important role during pregnancy especially on prenatal development as well as persist up to postnatal life.

Acknowledgement

الإجهاد التأكسدي الناجم عن الديفيروكسامين (الديسفيرال) أثناء الحمل: دراسة قبل الولادة وبعدها

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الخلاصة

الهدف من الدراسة هو تقييم تأثير الإجهاد التأكسدي الناجم عن الديفيروكسامين (الديسفيرال) أثناء الحمل على النمو قبل الولادة وبعدها. وزعت عشوائيا مائة وعشرون أنثى بالغة من الجرذان ألبينو بعمر 3 أشهر

بمتوسط وزن 175–200 غم في مجموعتين رئيسيتين هما مجموعة السيطرة والتجربة (60 جرذ/ مجموعة). المجموعة الأولى (مجموعة السيطرة) تم حقنها بمحلول الملح الفسيولوجي 0,3 مل / حيوان ، والمجموعة الثانية تم حقنها بمحلول الديسفيرال بجرعة 100 ملغم / كغم وزن الجسم داخل الغشاء البريتوني (IP) مرة واحدة يومياً ولمدة ثلاثة أسابيع متتالية (أثناء فترة الحمل). عند نهاية المعاملة، تم تقسيم كل مجموعة بدورها إلى قسمين، كل قسم يضم 10 حيوانات، جمعت عينات الدم للتحليلات الكيموحيوية، ثم جمعت أنسجة الرحم والأجنة لملاحظة المتغيرات المدروسة. تبين من النتائج انخفاضًا في عدد الزرع وعدد الكيسات الأريمية. إذ انخفض عدد الأجنة الحية بشكل ملحوظ مع زيادة عدد الأجنة الميتة والمعاد امتصاصها، وزيادة تشوه الأجنة في رحم مجموعة الإناث المجهدة مقارنة مع مجموعة السيطرة. ظهر من المشاهدات بعد الولادة اختلافات معنوية في معايير النمو بعد الولادة. تقترح دراستنا أن حقن الديسفيرال له قدرة إحداث الإجهاد التأكسدي في الجرذان بجرعة 100 مغم / كغم، وان الإجهاد التأكسدي يلعب دورًا مهمًا أثناء الحمل ولاسيما في مرحلة ما قبل الولادة ويستمر حتى بعد الولادة.

الكلمات المفتاحية: ديسفيرال، الإجهاد التأكسدي ، تطورات قبل الولادة وبعدها.

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