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Impact of oxidative stress on pregnancy outcome in albino rats

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Abstract

Accumulative reports documented that oxidative stress is implicated in many human and animal diseases. However, the reports concerning the effect of oxidative stress on pregnancy outcome are limited and scarce. The objective of this study was to determine the impact of oxidative stress on pregnancy outcome and to assess the antioxidant effect of vitamin C and E on oxidative stress parameters in blood and placental tissue samples in experimental pregnant animals model exposed to oxidative stress. *Wister Albino* rats were used in this work to investigate the effects of oxidative stress exposure (addition of H_2O_2 to the drinking water) on pregnancy outcome. Rats were divided into 5 groups, as follows: Group I (included 7 normal pregnant rats which served as control group). Group II (exposed to 1 % H_2O_2) included 7 pregnant rats, the rats were allowed to become pregnant and received (1% H_2O_2) in drinking water from day 7^{th} till the day 19^{th} of pregnancy. Group III (exposed to 3% H_2O_2) included 8 pregnant rats. Same as group 2, but the rats were exposed to a higher concentration of H_2O_2 (3%) in drinking water. Group IV (included 8 pregnant rats). Pregnant rats received vitamins C and E without induction of oxidative stress. Group V (included 8 pregnant rats).induction of oxidative stress by 1% H_2O_2 with vitamins supplementation in the pregnant rats. Serum total antioxidants capacity (TAC), serum and placental tissue oxidative stress biomarker; 8-iso prostaglandin $F_2\alpha$ (8-Isoprostane) were measured using specific ELISA kits. Also placental tissues of pregnant rats were isolated and put directly in 10% formalin prepared for histopathological examination. Results revealed a significant decrease in the median values of the body weight and total serum antioxidants capacity (TAC) in groups II and III of rats compared with the control group. A significant higher median value of TAC obtained in the groups IV and V when compared with the control group. Significant higher levels of serum and tissue Isoprostane observed in both groups II and III compared with control group. Histopathological, oxidative stress induced macroscopically degenerative with microscopical appearance of vasculitis and hemorrhage within decidua. Data of the present study demonstrated that imbalance oxidative stress status in pregnant rats occurred due to exposure to oxidant, which played an important role in the pathogenesis of abnormal pregnancy outcome. In addition antioxidants supplementation (vitamins E and C) were valuable in reducing this stress.

Keywords: Oxidative stress; Antioxidants; Pregnancy outcome; 8-Isoprostane.
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تأثير الإجهاد التاكسدي في ناتج الحمل في الجرذان البيض

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الخلاصة

أثبتت التقارير المتراكمة المختلفة أن الإجهاد التاكسدي له دور في الكثير من أمراض الإنسان والحيوان، ولكن التقارير التي تثبت تلك العلاقة مع نواتج الحمل قليلة ونادرة.وهدفت الدراسة الى تحديد دور الإجهاد التاكسدي على نواتج الحمل وتقييم تأثير فيتامين C و E كمضادات للأكسدة على متغيرات الإجهاد التاكسدي في الدم وأنسجة المشيمة للجرذان الحبلى التي أعدت كموديل تجريبي للحيوانات المعرضة للإجهاد التاكسدي. استخدمت جرذان من نوع ألبينو للتحقق من تأثير التعرض للإجهاد ألتأكسدي في الجرذان الحبلى. اجريت الاختبارات على خمس مجاميع؛ المجموعة الأولى (المجموعة الضابطة) و شملت سبع جرذان حبلى و تم تخديرهن في اليوم التاسع عشر

من حملهن و أخذت عينات من الدم و من النسيج المشيمي. المجموعة الثانية (مجموعة معطاة ١% بيرو كسيد الهيدروجين مع ماء الشرب) شملت ٧ جرذان حبلى ومن اليوم السابع من الحمل تم تعريضهم للإجهاد التاكسدي (١% من بيرو كسيد الهيدروجين مع ماء الشرب) وفي اليوم التاسع عشر تم اخذ عينات الدم و من النسيج المشيمي. المجموعة الثالثة (مجموعة التعرض إلى ٣% بيرو كسيد الهيدروجين مع ماء الشرب) شملت ٨ جرذان حبلى نفس اجراءات المجموعة الثانية ماعدا اعطائهم تركيز أعلى (٣%) من بيرو كسيد الهيدروجين مع ماء الشرب. المجموعة الرابعة (مجموعة معطاة فيتامين C وE مع ماء الشرب) شملت ثمانية جرذان حبلى أعطيت لهن فيتامين C و E فقط مع ماء الشرب بمقدار ٥٠٠ ملغم/لتر ماء، و٤٠٠ وحدة عالمية / لتر على التوالي من اليوم الأول الى اليوم التاسع عشر من الحمل حيث تم اخذ نماذج الدم والمشيمة. واعتبرت هذه المجموعة، كمجموعة ضابطة ايجابية. المجموعة الخامسة (مجموعة معطاة ١% بيرو كسيد الهيدروجين وفيتامين C وE) شملت ثمانية جرذان حوامل من اليوم الاول الى السابع (حرية تناول الماء والطعام) ثم من اليوم السابع إلى نهاية اليوم الرابع عشر (لمدة سبعة أيام) تم اعطاءهم ١% من بيرو كسيد الهيدروجين مع ماء الشرب وفي اليوم الخامس عشر أعطيت لهن فيتامينا (C بمقدار ٥٠٠ ملغم/لتر ماء وE٤٠٠ وحدة عالمية / لتر) على التوالي وحتى اليوم التاسع عشر من الحمل حيث أخذ نماذج الدم والمشيمة وتم قياس قدرة مجموع مضادات الأكسدة (Total antioxidants capacity) والمؤشر الحيوي للأكسدة (8-iso prostaglandin F_2alpha) في مصل الدم والمؤشر الحيوي للأكسدة (8-iso prostaglandin F_2alpha) في مستخلص النسيج المشيمي. تم أخذ عينات من النسيج المشيمي (السخدي) للجرذان المختبرية ثم تم حفظ العينات في محلول الفورمالين المخفف بتركيز (١٠%) ثم تم اعداد وفحص النماذج المرضية النسيجية في مستشفى أزادي التعليمي في مدينة دهوك. بينت النتائج وجود انخفاض معنوي لمتوسطا وزن الجسم وقدرة مجموع مضادات الأكسدة في المجموعة II و المجموعة III مقارنة مع المجموعة I الضابطة. بينما وجد ارتفاع معنوي لمتوسطا قدرة مجموع مضادات الأكسدة في المجموعة IV و المجموعة V مقارنة مع المجموعة I الضابطة. كذلك وجد ارتفاع معنوي لمتوسط المؤشر الحيوي للأكسدة في مصل الدم ومستخلص النسيج المشيمي لمجموعة الجرذان في المجموعة II و المجموعة III مقارنة مع المجموعة I الضابطة. الفحص النسيجي للمشيمة أوضح وجود تغيرات مرئية ومجهريه ناتجة عن التهاب الأوعية الدموية ونزيفها في أنسجة المشيمة المتساقطة. الاستنتاجات: استنادا إلى متغيرات البحث المقاسة التي أظهرت وجود حالة عدم الاتزان بين المؤشر الحيوي للأكسدة وقدرة مجموع مضادات الأكسدة والتي لعبت دورا مهما في نواتج الحمل الغير طبيعية وتغيراتها المرضية المختلفة (من منع حدوث الحمل عند استخدامها من اليوم الاول الافتراضي للحمل كذلك ادت الى تقليل عدد المواليد وتنكس أنسجة المشيمة) كذلك وجد أن إضافة مضادات الأكسدة كان له دور في إعادة التوازن المذكور وتقليل حالة الإجهاد التاكسدي.

Introduction

A considerable amount of clinical and experimental evidences now exists suggesting the involvement of free radical-mediated oxidative processes in the pathogenesis of many human and animal diseases. Early pregnancy loss is unfortunately the most common complication of human gestation, occurring in as many as 75% of all women trying to conceive (1**).**

There are many causes of early pregnancy failure, but it now appears that oxidative stress may play a role. An emerging confluence of opinion suggests that oxidative stress is one of the main underlying mechanisms in the pathogenesis of disease processes continuum such as spontaneous abortion, hydatidiform mole and preeclampsia (2). Imbalance oxidative stress status occur due to high oxidants produced and defective antioxidants mechanisms (3). The antioxidant defenses act as a coordinated system when deficiencies in one component may affect the efficiency of the other (4). The most representative product that may reflect oxidative damage to the cells are F_2-isoprostanes, which are specific products of lipid peroxidation and stable compounds present in detectable volumes in all normal biological fluids and tissues (5,6). The measurement of F_2 α Isoprostanes may represent an important development in the assessment of free radical generation and oxidative stress *in vivo* (7). Correlation of *in vivo* and *in vitro* data suggests that overwhelming oxidative stress of the placental tissues represents a common pathophysiological mechanism for the different etiologies threatening pregnancy and its outcomes (8). However, limited informations and research work is present about the possible role of oxidative challenge on pregnancy outcome in animals. The present work aims to investigate the role of oxidative stress on pregnancy occurrence, outcome and efficacy of antioxidant challenge in experimental rat model.

Materials and Methods

This experimental study was performed on *Wistar Albino* rats, their weight ranged from (250 – 300gm). The rats were kept in the animal house with free access to food and drinking water and in a suitable room temperature (22-25 °C) with regular light cycles of 12/12 hours light/dark. In this study, the polygamous mating system was used for breeding (3 females and 1 males) were placed in a large cage together for 2 days only, and then the male was separated from the cage and this is regarded as the zero day of pregnancy (if any), frequent abdominal palpation was performed and body weight measurement in order to isolate the pregnant rat. Exploratory experimental animal study was done on two groups of female rats (each composed of 3

animals) by giving the 1st group 1% H_2O_2 and the 2nd 3% H_2O_2, started at day one of the expected pregnancy occurrence. Effective oxidant exposure (H_2O_2 water addition in drinking) in both groups was prevented pregnancy occurrence (0%), although the experiment was repeated for 3 times on 18 rats within 45 days. Therefore, the design of our experimental study was changed. At day 7 of pregnancy (pregnancy conformation was done by direct abdominal palpation and marked weight increase) addition of H_2O_2 to the drinking water was started**.** Thirty eight pregnant rats were chosen and divided into 5 groups; Group I (Control rat group included 7 pregnant rats), the female rats were allowed to become pregnant, till the day 19th of pregnancy they were anesthetized, blood and placental tissues were isolated. Group II (included 7 pregnant rats) taking 1 % H_2O_2 in drinking water, the rats were allowed to become pregnant. On day 7th of pregnancy, induction of oxidative stress was started by giving them 1% H_2O_2 in drinking water till the day 19th of pregnancy, they were anesthetized and blood and placental tissue samples isolated. Group III (included 8 pregnant rats) taking 3% H_2O_2, same as group 2, but the rats were given to higher concentration of H_2O_2 (3%) in drinking water. Group IV (included 8 pregnant rats), the rats were allowed to become pregnant and vitamins C and E supplement were started from the 1st day till the 19th day of pregnancy, when the blood and placental tissue samples were isolated, this group served as a positive control group. Group V (included 8 pregnant rats), the rats were allowed to become pregnant. Then 1% H_2O_2, was added to the drinking water started from the 7th day and end at the 14th day of pregnancy, then in the 15th day of pregnancy, only vitamin C and E (in a dose of 500 mg / L H_2O, 400 I.U /L of drinking water respectively) supplement were added to the drinking water till the 19th day of pregnancy, then sampling done.

From anesthetized rats by Chloroform, blood samples were obtained directly from the heart ventricle, 7– 9ml of blood was collected from each rat,sera were separated using cool centrifuge (4 ^{0}C) and stored at -28 ^{0}C until the time of analysis.

After blood sampling, using scissors, a U-shaped incision made from the lower abdomen to the bottom of the rib cage (the bottom of the U was near the pelvis) to expose the abdominal cavity (9) ; uterus was opened and conceptuses (fetuses, fetal membranes and placentas) obtained in order, then the number of pups recorded. Each placenta was carefully dissected free from the placental membranes and umbilical cord. One gm of placental tissue was obtained and homogenized in 4ml of physiologic buffer solution using ground glass electrical homogenizer (IKA-WERK, ULTRA - TURRAX) (10). Then homogenized tissue is centrifuged at 4C^o,the supernatant was taken and stored frozen at -28 ^{0}C till time of analysis (11). Moreover, placental tissues were prepared for histopathological examination. Serum total antioxidants capacity was measured by ELIZA method, using Cayman's Antioxidants Assay Kit (Cayman Chemical Company, USA). Serum and tissue levels of 8- isoprostaglandin $F_2\alpha$ (Isoprostane) were measured using direct 8-iso-Prostaglandin F2α Enzyme Immunoassay Kit manufactured by Assay Designs Company (USA).

Results

As shown in table 1, induction of oxidative stress in female rats (1% H_2O_2 and 3% H_2O_2) significantly affected all selected parameters; there were significant decreases in body weight, TAC (Fig.1) and count of Pups, associated with significant increases in serum and placental Isoprostane levels (Fig. 2). Increasing dose of H_2O_2 from 1% to 3% was significantly decreased ($P\leq0.015$) rats body weight when body weight compared between the 1% and 3% exposure groups, however, serum TAC, serum Isoprostane, tissue Isoprostane were not appreciably affected (Table 2). Induction of oxidative stress by 1% H_2O_2 increased serum Isoprostane ($P\leq0.028$), tissue Isoprostane significantly ($P\leq0.002$) and decreased count of pups ($P\leq0.001$) compared to control. Testing of antioxidant effect of vitamins E and C after induction of oxidative stress by 1% H_2O_2 (group v) decreased tissue Isoprostane level significantly ($P\leq0.028$). However, serum Isoprostane level remained significantly higher ($P\leq0.035$) compared to control group. Comparison of selected outcome (significant results) after induction of oxidative stress with 1% H_2O_2 (with and without antioxidant supplementation) showed that antioxidant supplementation decreased tissue Isoprostane significantly ($P\leq0.001$) and increased serum TAC ($P\leq0.004$). Tissue Isoprostane level was significantly negative correlated with serum TAC ($r= -0.521$, $P\leq0.00I$) and positively correlated serum Isoprostane ($r=0.44$, $P\leq0.006$) (Table 3). However, Body weight was significantly positively correlated with serum TAC ($r=0.372$, $P\leq0.021$) and negatively correlated with serum Isoprostane ($r= -527$, $P\leq0.001$) and tissue Isoprostane ($r=-0.521$, $P\leq0.001$). Interestingly, significant negative correlations were observed between the pups count with serum Isoprostane ($r= -0.613$, $P< -.001$) and tissue Isoprostane ($r=-0.364$, $P\leq0.025$).

Figure (3-A) shows placentas of normal pregnant rats at 19th day of pregnancy with normal number, size and appearance. However, figure (3-B) shows placentas of pregnant rats after induction of oxidative stress with a degenerated pup and small sized shrunk placental tissue.

Microscopically, histopathological appearance of placental tissue of control pregnant rat at 19th day pregnancy (Fig. 4), showing normal decidual cells separated by proliferating capillary with congested stroma. Changes observed in placental tissue of rat after induction

of oxidative stress are shown in figures 5 and 6. These changes included obliteration of blood vessels due to vasculitis, hemorrhage within decidua and hugely enlarged trophoblast cells.

Table 1. The difference in median of selected outcome parameters between the five studied groups.

Parameters	Control N=7	1% H_2O_2 N=7	3% H_20_2 N=8	Vit E&C N=8	Combined (1%H_2O_2+ Vit E&C) N=8	P -value (Kruskal-Wallis)
Body Wt (g)						≤ 0.001
Median	300	289	266	300	293	
Interquartile range	289-314	272-290	235-283	291-340	285-302	
S. TAC (µmol/L)						≤ 0.001
Median	709.2	650.3	460.9	785.9	1323.3	
Interquartile range	659.9-1255.9	425.6-844.5	281.9-562.8	561.4-1016.5	1053-1742.1	
S. Isoprostane (pg/ml)						≤ 0.002
Median	3311.3	26328.2	38022.4	3668.2	8357.6	
Interquartile range	2759.4-26510	13456.5-135357.5	26524.7-394299.2	1409.2-15613.9	4445.8-84972.7	
Pl. Isoprostane (pg/ml)						≤ 0.001
Median	2810.8	8639.5	7412	1792.2	1516	
Interquartile range	2034.5-3130.5	5014.4-12658.8	6332.2-10071.8	1137.5-2605.8	1469.7-1992.9	
Count of pups						≤ 0.001
Median	10	7	3	10	7	
Interquartile range	9-11	7-8	3-8	8-12	6-7	

Table 2. Test of significance for difference in median between each 2 groups.

Comparison between groups	Body weight	Serum TAC	Serum Isoprostane	Tissue Isoprostane	Count of Pups
Increasing dose of oxidative stress (1-3% H_2O_2)	0.015*	0.08	0.21	0.82	0.13
Oxidative stress (1% H_2O_2) compared with control	0.07	0.28	0.028*	0.002*	0.001*
Antioxidant effects of Vitamins combined with oxidative stress compared to oxidative stress alone (1% H_2O_2)	0.16	0.004*	0.73	0.001*	0.24

*statistically significant

Table 3. Linear Correlation Coefficient between Selected Parameters.

	Body Wt	Serum total anti oxidant	Serum Isoprostane	Tissue Isoprostane
Serum TAC	r=0.372 P≤0.05			
Serum Isoprostane	r=-0.527 P≤0.001	r=-0.258 P≤0.12[NS]		
Tissue Isoprostane	r=-0.521 P≤0.001	r=-0.555 P≤0.001	r=0.44 P≤0.006	
Count of pops	r=0.512 P≤0.001	r=0.255 P≤0.12[NS]	r=-0.613 P≤0.001	r=-0.364 P≤0.05

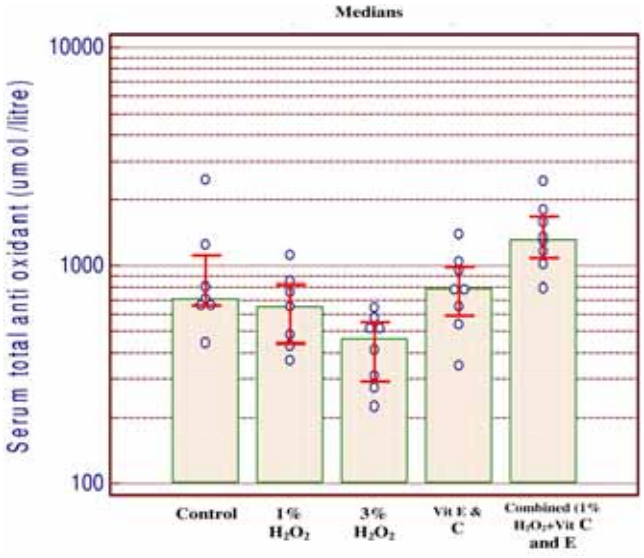

Fig. 1: Comparison of the median of serum total antioxidants in the studied groups.

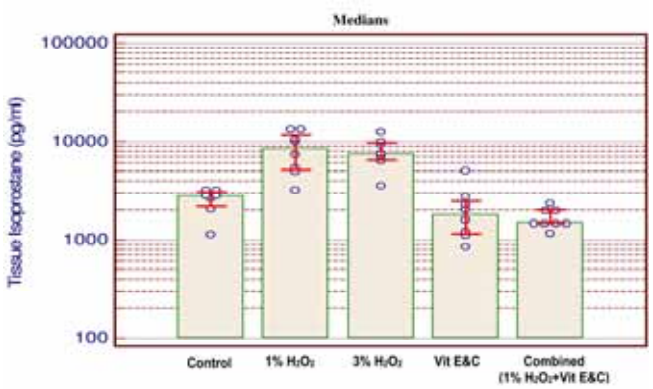

Fig. 2: Comparison of the median of placental tissue Isoprostane levels in the studied groups.

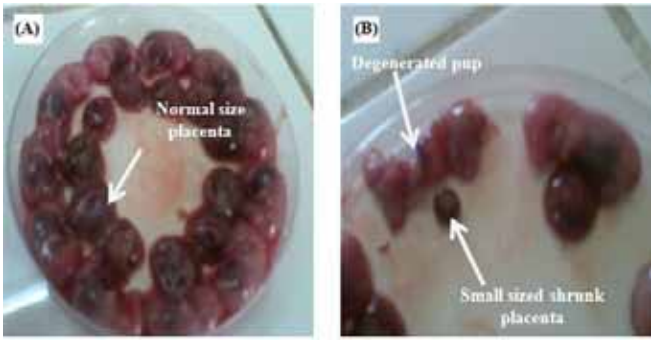

Fig. 3: Placentas and pups of a control rat with normal pregnancy (A), decrease number of pups, degenerated pups and placentas after induction of oxidative stress (B).

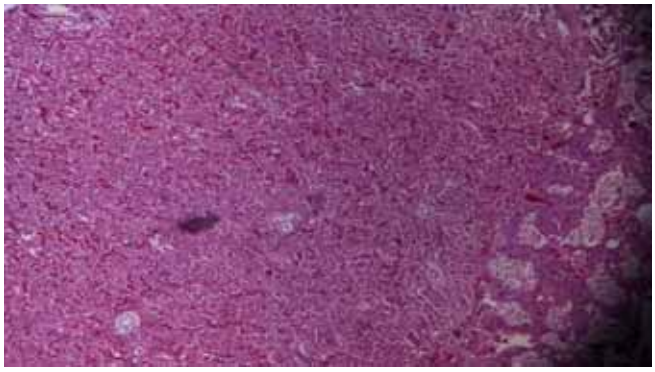

Fig. 4: Normal placental tissue of a control pregnant rat at 19th day pregnancy showing decidual cells separated by proliferating capillaries with congested stroma (X 40).

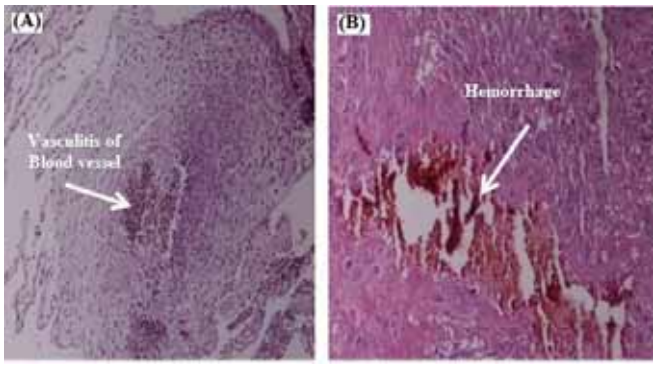

Fig. 5: Placental tissue of pregnant rats after induction of oxidative stress, the lumen of the blood vessel is obliterated due to vaculitis (A), and hemorrhage within decidua (B) (X40 and X100, respectively).

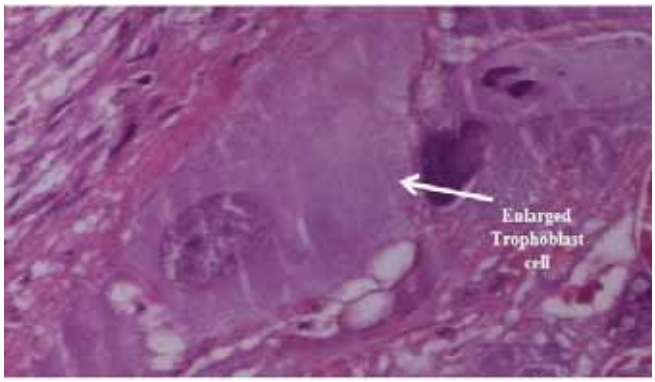

Fig. 6: Placental tissue of pregnant rat after induction of oxidative stress showing hugely enlarged trophoblast cells (X 400).

Discussion

Induction of oxidative stress by 3% H_2O_2 in rats, significantly decreased the body weight ($P \leq 0.001$) when compared with other groups. This finding is consistent with previous studies (12-14). Moreover, both serum and tissue isoprostane levels were significantly negatively correlated with body weight (table 3). However, the extent of oxidative stress did not correlate with the weight reduction (15). In addition to that, apart from a significant decrease in body weight, when H_2O_2 concentration was increased from 1% to 3% other selected parameters were not changed appreciably.

Exposure of pregnant rats to $NiCl_2$ (an oxidant substance) was investigated by Adjroud and Mouffok in 2009 (12), they reported a progressive diminution of the number of live fetuses in comparison with the control. The results of the present study showed significantly less number of pups (median =3) in the group exposed to 3% H_2O_2 compared with the control (median =10).

Induction of oxidative stress by 1% and 3% H_2O_2 significantly increased serum and placental tissue isoprostane levels. Excess formation of isoprostane causes damage to cell membranes and oxidative modification of plasma lipoproteins (16). Moreover, induction of oxidative stress was associated with decreased total antioxidant capacity (table 1). Similarly, oxidative stress increased formation of reactive oxygen species in rats lead to elevated oxidative stress and decreased total antioxidant capacity (14,17).

For the benefit used of antioxidants treatment, which last for 4 days only, a combined treatment of vitamins E and C was used. The antioxidant effect of combined treatment of vitamins E and C were evaluated after induction of oxidative stress by 1% H_2O_2, supplementation of these vitamins caused appreciable decreases in placental tissue and serum isoprostane levels, associated with significant increases in serum TAC; indicating that vitamins C and E are effective antioxidants for reducing oxidative stress burden during pregnancy. However, Thomson ***et al.*** (12) reported no protective effect of dietary carotenoid. It seems from the results of the present study that combination of antioxidants is very effective in reducing oxidative stress damage; this has been supported by others (19,20). Vitamin C donates a hydrogen atom to vitamin E derived phenolate radical thus regenerating its activity. Ascorbic acid is considered to be the most important antioxidant as well. Therefore, ascorbic acid can protect membranes against lipid peroxidation, in addition to that ascorbic acid enhances the activity of a- tocopherol, the chief lipid soluble and chain breaking antioxidant (21).

Supplementation of vitamins C and E increased serum TAC in pregnant rats; interestingly this rise was more obvious in the presence of oxidative stress. This finding probably indicates that supplementation of vitamins C and E during exposure to oxidative stress; increase the enzymatic and / or non enzymatic antioxidant activity in the body. Chronic reduction of uterine perfusion (blood flow) in pregnant rat is associated with increased oxidative stress as indicated by increased placental isoprostane and malondiadehyde levels (12,22). In this work, oxidative stress caused decrease in placental size with shrinkage and degenerative changes (figure 3-B). Moreover, reduction in blood flow was further confirmed by microscopical examination of placental tissue; oxidative stress caused obliteration of placental blood vessels due to vasculitis with hemorrhage within dicidua (figure 5).

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