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$_2$O$_2$ 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$_2$O$_2$) included 7 pregnant rats, the rats were allowed to become pregnant and received (1% H$_2$O$_2$) in drinking water from day 7th till the day 19th of pregnancy. Group III (exposed to 3% H$_2$O$_2$) included 8 pregnant rats. Same as group 2, but the rats were exposed to a higher concentration of H$_2$O$_2$ (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$_2$O$_2$ with vitamins supplementation in the pregnant rats. Serum total antioxidants capacity (TAC), serum and placental tissue oxidative stress biomarker; 8-iso prostaglandin F$_2$α (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.

Available online at [http://www.vetmedmosul.org/ijvs](http://www.vetmedmosul.org/ijvs)
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% H2O2 and the 2nd 3% H2O2, started at day one of the expected pregnancy occurrence. Effective oxidant exposure (H2O2 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 H2O2 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 % H2O2 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% H2O2 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% H2O2, same as group 2, but the rats were given to higher concentration of H2O2 (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% H2O2, 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 H2O2, 400 IU /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~ 9 ml of blood was collected from each rat, sera were separated using cool centrifuge (4 °C) and stored at -28 °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 4°C, the supernatant was taken and stored frozen at -28 °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 F2α (Isoprostane) were measured using direct 8-Isoprostaglandin F2α Enzyme Immunoassay Kit manufactured by Assay Designs Company (USA).

**Results**

As shown in table 1, induction of oxidative stress in female rats (1% H2O2 and 3% H2O2) 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 H2O2 from 1% to 3% was significantly decreased (P≤0.015) 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% H2O2 increased serum Isoprostane (P<0.028), tissue Isoprostane significantly (P≤0.002) and decreased count of pups (P≤0.001) compared to control. Testing of antioxidant effect of vitamins E and C after induction of oxidative stress by 1% H2O2 (group v) decreased tissue Isoprostane level significantly (P<0.028). However, serum Isoprostane level remained significantly higher (P≤0.035) compared to control group. Comparison of selected outcome (significant results) after induction of oxidative stress with 1% H2O2 (with and without antioxidant supplementation) showed that antioxidant supplementation decreased tissue Isoprostane significantly (P≤0.001) and increased serum TAC (P≤0.004). Tissue Isoprostane level was significantly negative correlated with serum TAC (r= -0.521, P≤0.001) and positively correlated serum Isoprostane (r=0.44, P≤0.006) (Table 3). However, Body weight was significantly positively correlated with serum TAC (r=0.372, P≤0.021) and negatively correlated with serum Isoprostane (r= -0.527, P≤0.001) and tissue Isoprostane (r= -0.521, P≤0.001). 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≤0.025).

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 of 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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control N=7</th>
<th>1% H₂O₂ N=7</th>
<th>3% H₂O₂ N=8</th>
<th>Vit E&amp;C N=8</th>
<th>Combined (1%H₂O₂+ Vit E&amp;C) N=8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Wt (g)</td>
<td>300</td>
<td>289</td>
<td>266</td>
<td>300</td>
<td>293</td>
</tr>
<tr>
<td>S. TAC (μmol/L)</td>
<td>709.2</td>
<td>650.3</td>
<td>460.9</td>
<td>785.9</td>
<td>1323.3</td>
</tr>
<tr>
<td>Median Interquartile range</td>
<td>659.9-1255.9</td>
<td>425.6-844.5</td>
<td>281.9-562.8</td>
<td>561.4-1016.5</td>
<td>1053-1742.1</td>
</tr>
<tr>
<td>S. Isoprostane (pg/ml)</td>
<td>3311.3</td>
<td>26328.2</td>
<td>38022.4</td>
<td>3668.2</td>
<td>8357.6</td>
</tr>
<tr>
<td>Median Interquartile range</td>
<td>2759.4-26510</td>
<td>13456.5-135357.5</td>
<td>26524.7-394299.2</td>
<td>1409.2-15613.9</td>
<td>4445.8-84972.7</td>
</tr>
<tr>
<td>Pl. Isoprostane (pg/ml)</td>
<td>2810.8</td>
<td>8639.5</td>
<td>7412</td>
<td>1792.2</td>
<td>1516</td>
</tr>
<tr>
<td>Median Interquartile range</td>
<td>2034.5-3130.5</td>
<td>5014.4-12658.8</td>
<td>6332.2-10071.8</td>
<td>1137.5-2605.8</td>
<td>1469.7-1992.9</td>
</tr>
<tr>
<td>Count of pups</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>9-11</td>
<td>7-8</td>
<td>3-8</td>
<td>8-12</td>
<td>6-7</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Comparison between groups</th>
<th>Body weight</th>
<th>Serum TAC</th>
<th>Serum Isoprostane</th>
<th>Tissue Isoprostane</th>
<th>Count of Pups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increasing dose of oxidative stress (1-3% H₂O₂)</td>
<td>0.015*</td>
<td>0.08</td>
<td>0.21</td>
<td>0.82</td>
<td>0.13</td>
</tr>
<tr>
<td>Oxidative stress (1% H₂O₂) compared with control</td>
<td>0.07</td>
<td>0.28</td>
<td>0.028*</td>
<td>0.002*</td>
<td>0.001*</td>
</tr>
<tr>
<td>Antioxidant effects of Vitamins combined with oxidative stress compared to oxidative stress alone (1% H₂O₂)</td>
<td>0.16</td>
<td>0.004*</td>
<td>0.73</td>
<td>0.001*</td>
<td>0.24</td>
</tr>
</tbody>
</table>

*statistically significant

Table 3. Linear Correlation Coefficient between Selected Parameters.

<table>
<thead>
<tr>
<th></th>
<th>Body Wt</th>
<th>Serum total anti oxidant</th>
<th>Serum Isoprostane</th>
<th>Tissue Isoprostane</th>
<th>Count of pops</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum TAC</td>
<td>r=0.372</td>
<td>r=-0.258</td>
<td>P&lt;0.05</td>
<td>P&lt;0.12[NS]</td>
<td></td>
</tr>
<tr>
<td>Serum Isoprostane</td>
<td>r=-0.527</td>
<td>r=-0.555</td>
<td>r=0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue Isoprostane</td>
<td>r=-0.521</td>
<td>r=-0.255</td>
<td></td>
<td>P&lt;0.006</td>
<td></td>
</tr>
<tr>
<td>Count of pops</td>
<td>r=0.512</td>
<td>r=0.613</td>
<td>r=-0.364</td>
<td>P&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>
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% H2O2 in rats, significantly decreased the body weight (P<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 H2O2 concentration was increased from 1% to 3% other selected parameters were not changed appreciably.

Exposure of pregnant rats to NiCl2 (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% H2O2 compared with the control (median =10).

Induction of oxidative stress by 1% and 3% H2O2 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% H2O2, 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).

Acknowledgments

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References


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