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Morphological, Biochemical and Ultrastructural Changes in the Pregnant Rat Placenta and the Liver of their Fetuses Treated with Folic Acid and / or Gamma Radiation

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Abstract

Backgrounds: The efficacy of antioxidant supplementation and oxidative stress of gamma irradiation for and during pregnancy is poorly established. The present study aimed to detect the toxic effects of high dose of folic acid and / or gamma radiation on the placenta of pregnant rat and the liver of their fetuses.

Material and Methods: Pregnant albino rats were divided into four groups. The first group served as a control, the second group received oral intake of folic acid (5 mg/kg) from the 5^{th} to 20^{th} day of gestation, the third group was irradiated with gamma radiation (3Gy, as fractionated doses (1Gy/ 3 times) on each 5^{th}, 10^{th} and 15^{th} days of gestation, the fourth group was imanaged with combined treatment.

The pregnant rats were sacrified after 20 days of pregnancy and samples were taken from the blood, placenta and the fetal liver for the morphological, biochemical and electron microscopic studies.

Results: The present results showed a significant elevation in serum gamma glutamyltransferase (γGT), lactate dehydrogenase (LDH) in placental tissue of pregnant rats associated with an increase of phosphorus content in liver of fetuses. Fetal malformations including: protrusion, anotia, short neck dactylomegaly, subcutaneous haemorrhage, paralysis in the fore limbs and congested blood vessels.

The ultrastructural changes revealed sever damage in the placenta following folic acid administration and / or exposure to whole body gamma radiation.

Also the fetal liver showed an appearent signs of damage under the combined treatment. The obtained changes were represented by: dilatation of the blood sinsoids, swollen mitochondria, fragmented rough endoplasmic reticulum and necrosis.

Conclusion: It could be concluded that administration of folic acid and/or exposure to gamma radiation during pregnancy induced morphological, biochemical and ultrastructural changes in both placenta of the pregnant rats and liver of their fetuses.

Key words: Folic acid, radiation, Pregnancy, placenta, liver, fetus, γ-GT, LDH, phosphorus, Teratology, electron microscop.

Introduction

Folic acid (B vitamin) needed for cell replication and growth. Folic acid helps from building blocks DNA baby's genetic information and building blocks of RNA and needed for protein synthesis in all cells.

The growing tissues such as those of the fetus and rapidly regenerating cells like red blood cells have a high need for folic acid (Health notes, 2005). The requirement for folic acid increases considerably during pregnancy (Truswell, 1985). Vitamin B also helps the body to convert carbohydrates into glucose which is burned to produce energy.

B complex vitamins are essential in the breakdown of fats and proteins. B complex vitamins also play an important role in maintaining muscle tone along the lining of the digestive tract and promoting the health of the nervous system, skin, hair, eyes, mouth and liver (Living Naturally, 2006). Hathcock (1997) stated that supplemental folic acid should not exceed 1.000 mcg for adult men and women and a

800 mcg for pregnant and lactating women less than 18 years of age to prevent folic acid from masking symptoms of vitamin B_{12} deficiency.

In association with a continual increase in the environmental radiation load, mainly as a consequence of long-lasting radioactive fall out coming from the testing of nuclear weapons and accidents of nuclear fittings in different parts of the world, the question of the transmission of radiation-induced genetic damage to the next generation becomes very real. Different types of radiation cause damage to different organs and production of genetic changes which affecting future generation (Omran and Abu-Zied 2006). In addition, Rezk and Ibrahim (2006) observed that folic acid at a dose 4mg/kg b.wt was found to offer protection during pregnancy and suppressing the embryonic mortality rates and serious fetal malformations when pregnant rat were exposed to gamma rays at dose 3 Gy on day 10 of gestation.

Aldeen and Konermann (1978), studied the effect of acute X-irradiation during pre and post-implantation stage on mouse embryos by exposing pregnant mice to a single dose of 3 Gy. They found that direct effect of irradiation on mother must lead to embryonic death. Moreover, Walash *et al.* (1989) stated that whole body γ-irradiation of pregnant rats at dose of 2 Gy in organogenesis stage caused malformations in the skull, anophthalmia and defects in the central nervous system. Moreover, Gaber (1990) found that exposed pregnant rats to gamma irradiation showed an increase in the incidence of intrauterine foetal death, as well as induced uterine growth retardation. Placenta is a complex mammalian tissue that performs many metabolic function as it is the major transporting system of nutrients necessary for foetal growth (Dorothy, 1983). Abu Gabal *et al.* (1994) noted severe degeneration in maternal and foetal rat placenta and marked loss of DNA in different layers. Ashry (1997) stated that gamma irradiation with 1 Gy induced degenerative signs in placenta represented by pyknotic nuclei, fibrosis and vacuolation of the cytoplasm. While fractionated dose of 2Gy showed progressive degenerative features including: haemorrhage and congestion of blood vessels. Walash *et al.* (1988) and Abu Gabal *et al.* (1995) studied the effect of gamma irradiation on liver of pregnant rats during organogenesis period. Their results showed degenerative and necrotic lesions in hepatic lobules. In addition Katarina *et al.* (2002) reported that irradiation with dose of 3 Gy γ-irradiation caused latent cytogentic damage to the liver.

The present study was performed to identify the toxic effects of folic acid (5mg/kg b.w) and / or gamma irradiation at dose of 3 Gy on the placenta of pregnant rats and the liver of their fetuses.

Material and Methods

Experimental animals

A total number of 40 adult pregnant albino rats weighing (150-170g) were used. They were housed at room temperature and allowed food and water.

Experiment was performed according to the international guidelines of animal handling and care.

Irradiation processing:

Whole body gamma irradiation at a dose level of 3 Gy was performed using an indoor shielded Cs-137 irradiator (Gamma cell-40 installed in the National Center for Radiation Research and Technology (NCRRT) Atomic Energy Authority, Cairo, Egypt, which emitting a dose of gamma radiation at the rate of 1.26 Gy/min

Folic Acid:

Folic acid was purchased from the Nile Company Pharmaceutical and Chemical Industries. It was dissolved in distilled water and administered to pregnant rats at a dose level of 5 mg/kg b.wt using an oral stomach tube.

Experimental design:

Pregnant rats were classified into 4 groups each of five animals:

Group 1 (Control): Pregnant rats served as a control untreated group.

Group 2 (treated): Pregnant rats treated orally with folic acid at a dose of 5 mg/kg b.wt /day from 5^{th} to 20^{th} day of gestation.

Group 3 (irradiated): Pregnant rats

exposed to whole body gamma rays delivered as 1 Gy increament on 5th, 10th and 15th days of gestation up to total cumulative dose of 3 Gy.

Group 4 (Irradiated and treated): Pregnant rats treated orally with folic acid at dose 5mg/kg b.wt from 5th to 20th gestational day and irradiated on the 5th, 10th and 15th days of pregnancy.

Experimental parameters:

Five rats of each group were scarified at the end of day 20 of gestation and blood samples were collected in heparnized tube by heart puncture. Placenta and liver were dissected out, and homogenized in 10% sucrose buffer 0.25µ. The activity of gamma glatamyel transferase in serum was measured calorimetrically **(**Tietz, 1986), whereas phosphorus is measured using commercial kits (Henry, 1974), and kits were used for colorimetric determination of lactate dehydrogenase activity (Diamond-Diagnostic, Egypt).

Morphological studies:

Pregnant rats of each experimental group, were dissected then the embryos were observed externally and the morphologically prominent abnormalities or deformities were photographed for detailed evaluation.

For electron microscopy, the specimens from liver fetal and placenta of adult rats were washed with cacodylate buffer and then immersed for 2h in 4% glutraldhyde fixative (pH 7.2) which, contained 4% glutrldehyde. In a 0.2 M caccodylate buffer. They were washed 3 times (5 min each time) and post-fixation was done in 2% osmium tetroxide with 0.3M caccodylate buffer for 2h. After dehydration in ascending grades of ethyl alcohol, the specimens were embedded in Epon 812. Semi-thin sections were performed for purpose of orientation. Ultra-thin sections were examined under the (Transmission JE M100 CX) electron microscope at the National Center for Radiation Research and Technology (NCRRT) after staining with uranyl acetate and lead citrate (Hayut, 1986).

Statistical analysis:

Student t-test was applied for the statistical analysis of the results obtained according to Snedecor and Cochron (1978).

Results

Biochemical observations:

The results of the present investigations showed that folic acid administration at dose 5 mg/kg b.wt./day on gestational days 5th to 20th and / or γ-irradiation at a fractionated dose rate 1 Gy up to 3 on each days 5th, 10th and 15th of gestation affected the metabolic activates in the placenta of pregnant rats and this in turn affected the metabolism of liver embryo. These metabolic disorders were manifested by elevation of the level gamma glutamyl transferase in serum and lactate dehydrogenase in placenta tissues of pregnant rats ($P < 0.01$) when compared to control groups. The same results were obtained due to elevated level of the folic acid administration either alone or after irradiation ($P < 0.05$, $P < 0.001$) respectively (table 1).

Fetuses exposed to gamma irradiation on gestational days 5th, 10th and 15th showed elevation in phosphorus content in liver tissue ($P < 0.01$).

Moreover, treatments with folic acid discerned a significant change ($P < 0.05$) as compared with the control groups (table 1).

Morphological observations:

The mortality rate in the fetuses in the rats treated with folic acid (5mg/kg b.wt.) and irradiated accumulative dose 3 Gy reached zero percentage. There was no data reported in this group.

Morphological observations of the uterus of the control pregnant rats revealed healthy bright the appearance and normal distribution of the implanted fetuses between the two horns and normal development of fetuses (Figs. 1 and 2).

In figures (3) and (4a, b) the uterus of group (2) showed frequent implantation sites. The fetuses of the same group showed subcutaneous haemorrhage, abnormal bending of body (protrusion), anotia, short nect and dactylomegaly.

The uterus of the group (3) displayed shorting of one horn of uterus than the other one Fig (5) with decreased fetal number.

However the number of surviving

fetuses of irradiated mothers showed a number of malformations including: congested blood vessels, paralysis of fore limb, thin skin and decreased body length (Fig. 6a, b).

On the other hand, gamma irradiation on days 5^{th}, 10^{th} and 15^{th} of gestation during folic acid treatment showed high incidence of prenatal mortality which appeared as residual bodies Fig (7).

Furthermore, the fetuses of this group showed pre-natal death and embryos replaced by residual bodies Fig (8).

Ultrastructural studies

1- Control group:

Electron microscopic examination of the liver of control rat foetuses showed the normal ultrastructure. The cytoplasm of the hepatocytes contains mitochondria, rough endoplasmic reticulum and small amount of glycogen. Also, the nuclei are rounded and central in position and have a coarse pattern of heterochromatin (Figs. 9, 10).

2- Folic acid -treated group:

A remarkable alteration could be observed on examined sections of treated group with folic acid at a dose of 5mg/kg b.wt/day revealing loss of normal architecture with marked degeneration of hepatocytes. (Figs. 11, 12).

3- Irradiated group:

At the ultrasturtural level the irradiated animals an accumulative dose 1 Gy on 5th, 10th and 15th days up to 3 Gy during pregnancy showed severe damage in the fetal hepatocytes with hydropic degeneration (Fig. 13). In such cells, the cytoplasmic matrix became lytic and the cytoplasmic organelles are highly degenerated. The mitochondria were swollen with ruptured outer membranes and cristae. The rough endoplasmic reticulum was broken into fragments. The nuclei contained translucent fragmented and marginal chromatin and the nucleolus was prominent at the periphery. The blood sinusoids were damaged and contained phagocytic kupffer cells, and the nuclei of the endothelial cells were mostly pyknotic with disturbed erythrocytes (Fig. 14).

Placenta

1- Control group:

The placenta is a temporary organ, consisting of a maternal portion and a fetal portion. (Fig. 15) showed the general ultrastructural of the trophobalast comprising, cytotrophoblastic cell and syncytotrophoblastic cell.

2- Folic acid-treated group:

Administration of folic acid at a dose of 5mg/kg b.wt from days 5th to 20^{th} induced marked ultrastructural abnormalities in the placental cells. They appeared with degenerated cytoplasmic matrix. Whereas many lysosomal bodies were represented (Fig. 16). Also, the degenerated cytoplasm organoids were occupied by numerous heterogenous vesicles and vacuoles with variable sizes (Fig. 17).

3- Irradiated group:

The electron microscopic examination of the placental sections of the irradiated animals at accumulative dose 1 Gy on 5th, 10th and 15th days up to 3 Gy showed the placental cells which were greatly injuried, the nuclei contained fragmented chromatin with corrugated nuclear membrane .The cytoplasm was disorganized with increased amount and different sizes and shapes of lysosomal bodies (Fig. 18). In some of the placental cells the cytoplasm became lytic and the cytoplasmic organelles are highly degenerated with lots of lysosomes. The mitochondria were small and electron dense. The endoplasmic reticulum was broken into fragments. Cell nuclei showed chromatin disintegration (Fig. 19).

4-Folic acid - irradiated group:

This group was given folic acid at a dose of 5mg/kg and then irradiated at accumulative dose 3 Gy revealed severely effected placental cells. As represented in figs. (20 & 21) degenerated cyncytiotrophoblast and highly atrophied and karyolytic nuclei with corrugated outer and inner membranes were well marked. Furthermore, fibroid areas and increased collagen fibers were observed.

Table (1): effect of folic acid and/or gamma irradiation exposure on serum level of gamma glutamyl transferase; placenta tissues content of lactate dehydrogenase of pregnant rats and phosphorus content in the liver tissue of feuses.

parameters	Goup 1 (control)	Group 2 (folic treated)	Group 3 (Irradiated)	Group 4 (irradiated and folic treated)
Gamma glutamyl transferase (U/l)	4.324±0.769	6.858±0.206a	8.540±0.447c	11.026±0.425c
Lactate dehydrogenase (U/dI)	0.138±0.006	0.144±0.018c	0.302±0.005c	0.380±0.015c
Phosphorus content (mg/dI)	47.77±1.312	51.482±0.746a	55.506±2.110b	

Each value represents the mean of five rats ± S.E
a-Significant changes from control at P < 0.05
b-Significant changes from control at P < 0.01
c-Significant change from control at P < 0.001

Fig.1: Uterus of a control pregnant rat excised on the 20th gestational day showing normal distribution of 9 implanted embryos distributed between the 2 hours (X 0.7).

Fig.2: Embryos of a control mother rat illustrating normal development (X 0.7).

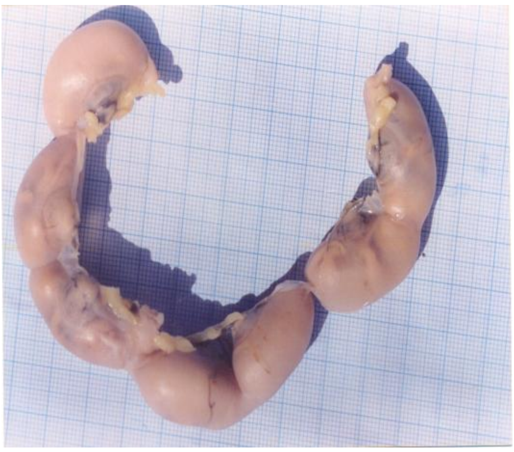


Fig.3: The uterus of animal received folic acid during gestation period showing frequent implantation sites (X 1).

Figs.4 (a, b): (a): Fetuses of rat treated with folic acid note: Subcutaneous haemorrhage abnormal bending of the body (protrusion), absence of ear (anotia), short neck and abnormally large size of finger (dactylomegaly) (↑). (X 2.2).

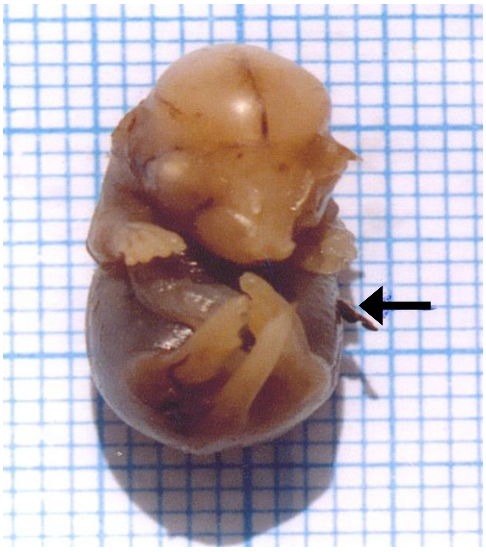


Fig.4 (b): high magnification for previous picture (x 4.8).

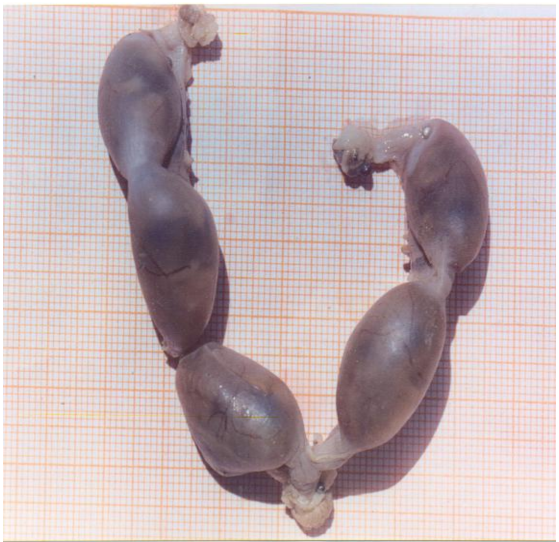
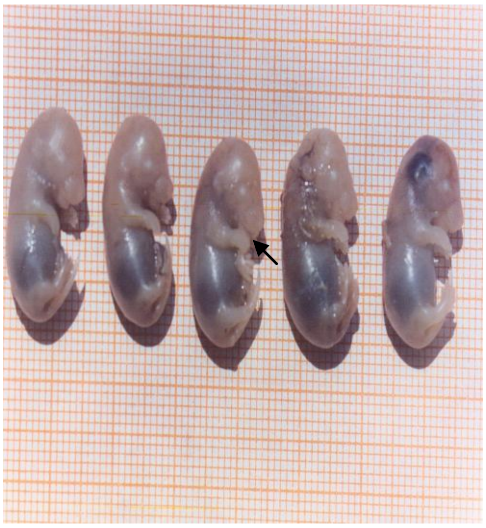


Fig.5: Uterus of pregnant female rats irradiated on 5th, 10th and 15th gestational days and excised on 20th day showing shortness of one horn of uterus than the other one. (X 1.3).



Figs.6 (a, b): (a): Extended fetuses of a mother exposed to γ-irradiation showing the decrease of body length, diminution in size, thin skin, subcutaneous haemorrhage, congested blood vessels and paralysis of fore limb (↑). (X 1.6).

Fig.6 (b): High magnification for previous picture (x 4.8).

Fig.7: Uterus of pregnant rats exposed to γ-irradiation on days 5th, 10th and 15th of gestation during folic acid treatment exhibiting high incidence of prenatal mortality which appeared as residual bodies.
(X 1.6).

Fig.8: Extended rat fetuses of mother exposed to γ-irradiation during folic acid treatment showing pre-natal death and embryos replaced by residual bodies. (X. 2).

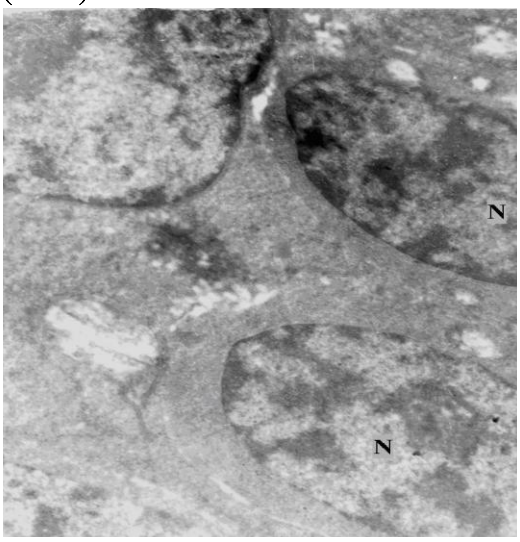


Fig.9: Electron micrograph of the liver of a fetus showing a normal architecture of hepatocytes. Notice large nuclei (N) with dense matrix. (X-8000).

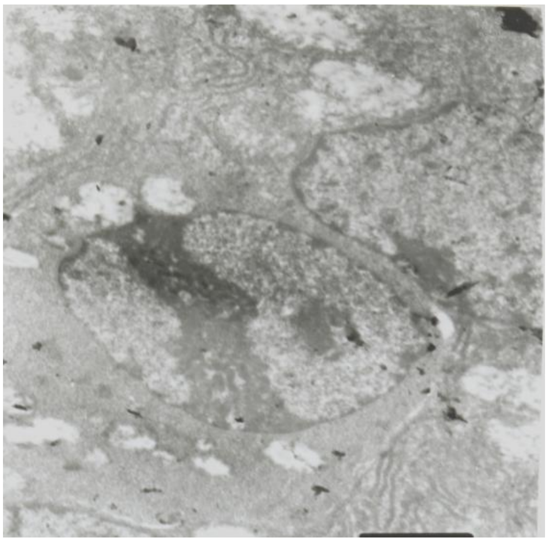


Fig.10: Electron micrograph of the liver of a fetus showing mitotic figure. (X -6000).

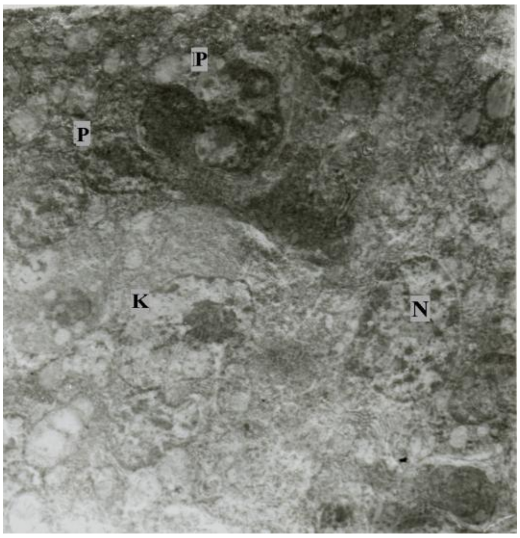


Fig.11: Electron micrograph of the liver of a fetus whose mother which received folic acid during pregnancy showing the cytoplasm is severely degenerated . Note pyknotic (p) and karyolytic (k) nuclei,degenerated cristae of mitochondria (M) and dilated cisternae of rough endoplasmic retculium. (X-2800)

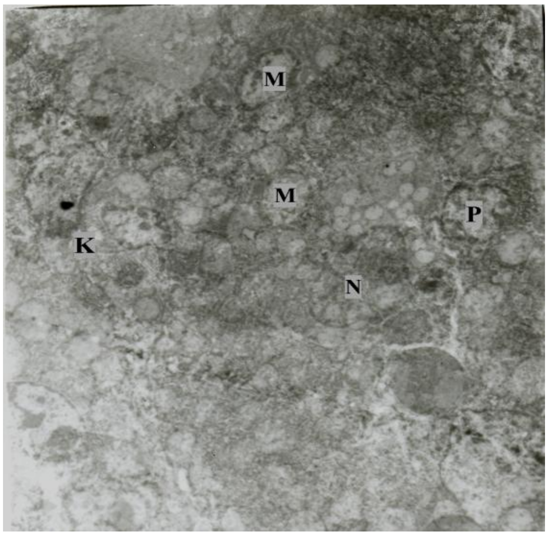


Fig.12: Electron micrograph of the liver of fetus whose mother received folic acid during pregnancy showing irregular architechteur of hepatocytes. Note swollen mitochondria (M) with degenerated cristae pyknotic (p) and karyolytic nuclei (k) (X-2800).

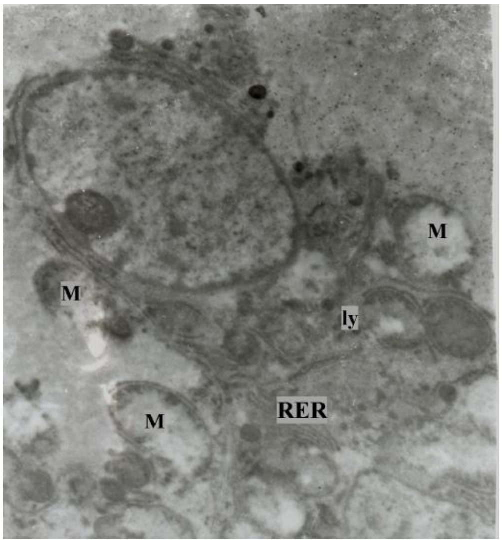


Fig.13: Electron micrograph of the liver of fetus whose mother exposed to γ-radiation during pregnancy showing hydropic degeneration of the cytoplasm. Swollen and degenerated mitochondria (M) and fragmented rough endoplasmic retieulum (RER). Note lysosomes (ly) (X-6000).

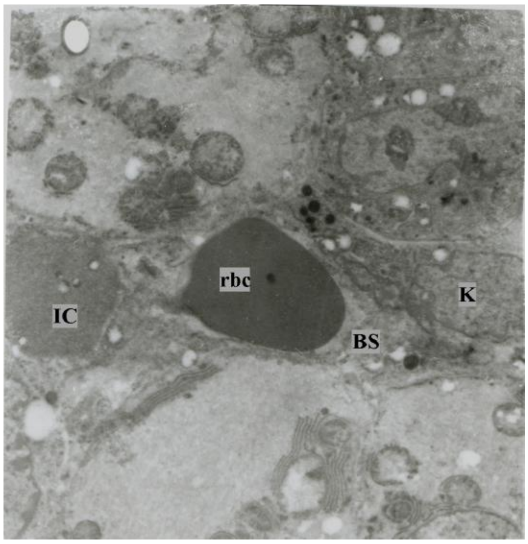


Fig.14: Electron micrograph of the liver of fetus whose mother exposed to γ-radiation during pregnancy showing blood sinusoid (BS) is dilated and contains kupffer cell (K) and inflammatory cells (IC) (X-4600).

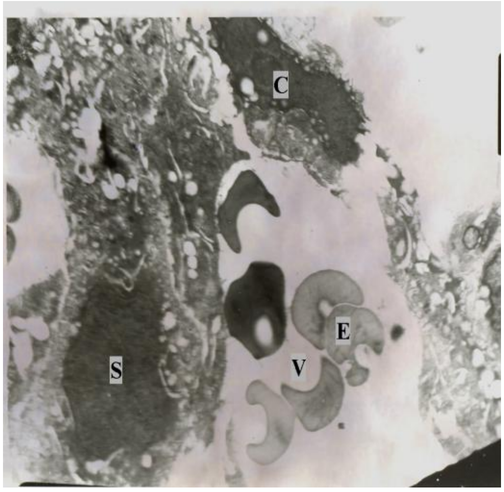


Fig.15: Electron micrograph of control rat Placenta showing the ultrastructure of the tropoblastic components. The syncytiotrophoblast (S) has irregular microvilli (v) and the cytotrophoblast (C). blood vessel (V) which contain erythrocytes (E). (X- 2800).

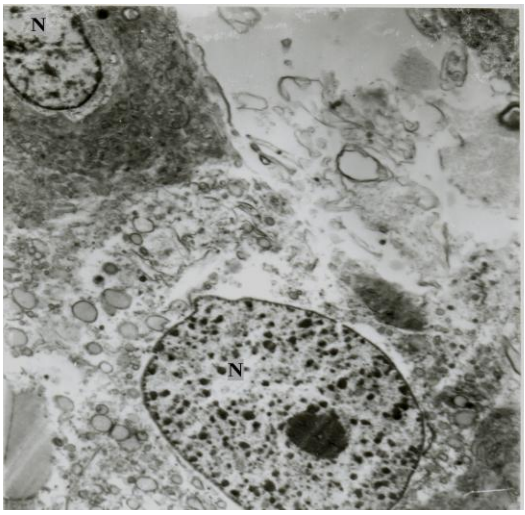


Fig.16: Electron micrograph of the placenta of an adult rat treated with folic acid showing disorganization of the cytoplasmic structure and it contained the debris of degenerated organoids and lots of lysosomes (y) . The nucleus (N) contained fragmented chromatin. (X 2800).

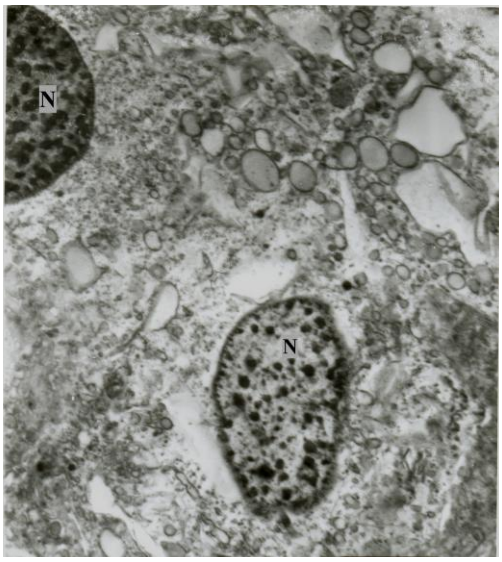


Fig.17: Electron micrograph of the placenta of an adult rat treated with folic acid showing the cytoplasm which contained lots of irregular vacuoles indicating degenerated organoids . The nucleus (N) had fragment chromatin. (X 3600).

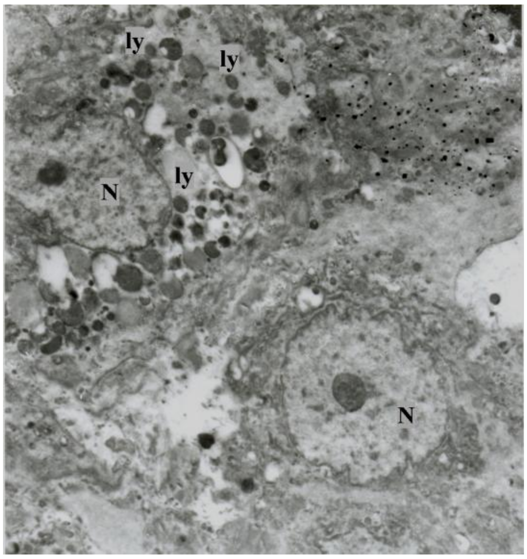


Fig.18: Electron micrograph of the placenta of an adult rat exposed to γ-radiation showing highly degenerated cytoplasmic organelles. Note increased amount of lysosomal bodies (Ly). Karyolytic nucleus (N) had undulating membranes. (X 3600).

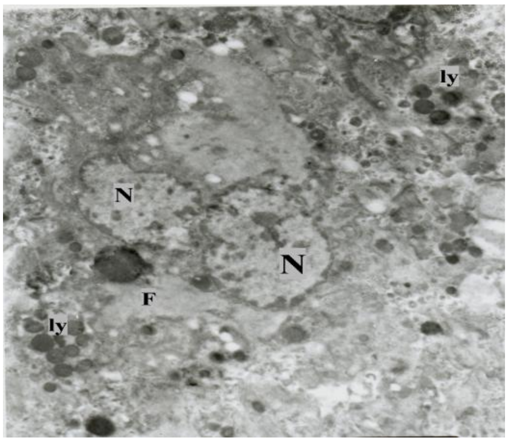


Fig.19: Electron micrograph of the placenta of an adult rat exposed to γ-radiation showing fibroid areas and collagen fiber (F), increased of lysosomal bodies (Ly) and atrophied and karyolytic nuclei (N) with irregular nuclear membrane. (X 4600).

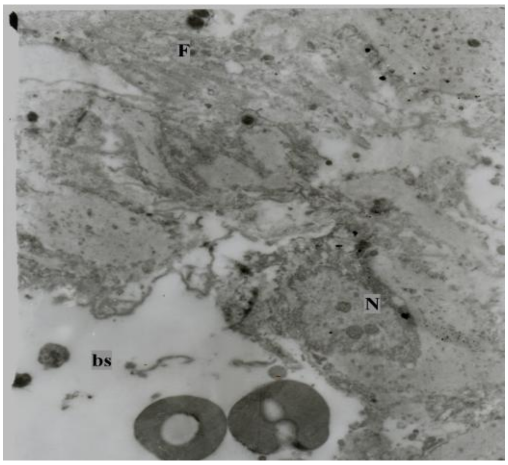


Fig. 20: Electron micrograph of the placenta of an adult rat treated with folic acid perior to irradiation showing degenerated syncytiotrophoblast with highly atrophied and karyolytic nuclei (N) and increased collagen fibers (F). Note the maternal blood spaces (bs) which contain deformed RBC (X 2800).

Discussion

The results of the current study revealed that whole body gamma irradiation of pregnant rats at an accumulative dose 1 Gy/3 times on both 5th, 10th and 15th days of gestation resulted in a significant biochemical disturbance with considerable ultrastructural damage in the placenta of the pregnant rats and liver of their fetuses associated with embryonic disorders.

Data obtained from the present study revealed a significant elevation in maternal serum of γ-GT and LDH in the placental tissue together with an elevation in phosphorus content in the liver of fetuses as compared to those of control. This was a correlation to Omran and Abu-zied (2006) who stated that the increase in γ-GT has been considered as an indicator for tissue injury. The synthesis of γ-GT is regulated by presence of reactive oxygen species since the enzyme is essential for the metabolism of antioxidant glutathione.

It was clamid that glutathione depletion led to increased γ-GT level which indicated hepatocellular necrosis (Whilfield, 2000).

On the other hand, Said and Hanafy (2006), demonstrated decreased cellular glutathione altering protein thiols which disordering the transport and storage of Ca^{+2} in mitochondria, endoplasmic reticulum and cell membrane of liver so the content of Ca^{+2} increasing and ultimately causing cell death and γ-GT was released into the blood.

Radiation exposure induced marked elevation in LDH in placental tissue of pregnant female rats. This is usually used as marker to placental dysfunction and also cytotoxic effect of exposure to irradiation (Gharib, 2007).

In this respect, Yoshid *et al.* (1995) attributed the disorders in the placenta circulation to involvement of endocrine mechanisms and decreased blood follow in the placenta which affect the pituitary function. In addition to the possible pathological changes which can observed in the structure and function of the epithelial cells of renal tubules with subsequent alterations in the active transport of calcium and phosphorus through these organs. These findings one supported by Abdel Gawad *et al.* (2005), who recorded that under effect of irradiation the maternal calcium and phosphorus content were not sufficient to satisfy the fetuses with its requirement for normal growth. The various degrees of damage observed in the liver of rat embryos in the present study are most probably due to the direct effect of radiation on the mitochondria and endoplasmic reticulum which leading to the release of enzymes from cellular organelles like lysosomes, microsomes and others.

It is possible to assume that severe malformations observed in the fetuses were due to vascular disturbance, amino acid abnormalities and hormone inbalance (Ramadan and Rezk, 2006) Otherwise , the excessive production of oxygen free radicals in fetuses of irradiated mother might increase the risk of fetal abnormalities (Rezk and Ibrahim, 2006).

Diamant (1981) noticed decreased total protein in the placenta post-exposure to γ-rays and this may be due to the destructive effect of these rays. The significant decrease in fetal size related to radiation which induced depression in the cellular differentiation process taking place in the embryonic cells were noticed by Rezk *et al.* (2005).

These observation were similar to those described by Amvrsene *et al.* (1994) who reported that total body irradiation of rat embryos at the pre-implantation and organogenesis stages produced increased incidence of malform- ations due to irradia-tion which caused death to the zygote while during organogenesis the cell death may be due to disruption in the morphog-enetic movements of differentiation and caused pre and neonatal mortality. Furthermore, Suitor and Bailey (2000) found that the vertebra of vertebral column did not close properly after fertilization and exposure to gamma irradiation so the spinal fluid bulge and cause paralysis this observation are correlated to our present results.

Results of the present study suggest that susceptibility of tissue and embryonic development have been influenced by folic acid administration. The current study despicted that folic acid administration at dose 5 mg/kg b. wt / day revealed a significant elevation in serum of γ-GT level, LDH in the placental tissues of pregnant female rats and also recorded an elevation in phosphours content in the liver tissue of fetuses.

Abdel Gawad *et al.* (2005) reported that degenerated cells of placenta taken from exposed pregnant rats may be responsible for reduced size of fetuses and may be due to the disturbance in transport of nutrients to the fetuses and resulted in fetal disorders.

Accordingly, Rezk and Ibrahim (2006) reported that after absorption of the drug from the gut caused teratogensis which associated with reproductive and developmental toxicity as a result of exposure liver to damage

The present results which indicated the dystrophic changes in placenta and fetal liver exposed to γ-rays or treated with folic acid are in agreement with many authors. Rowell and Clark (1982), Khera, (1991) and Ubbink (1995) stated that irradiation caused fetal growth and embryo lethality due to inhibition of protein synthesis or placental dysfunction On the other hand, Hartridge, *et al.*(1999) noticed that administration of folic acid at dose 10mg/100g b.wt can precipitated in the nervous system and caused degeneration of the spinal cord which linked to the increased risk of neural tube defect because folic acid at this dose caused blocked permeability which did not allow easier transfer of mineral and protein from blood to the bone and cells. This view is supported by the work of Baggot *et al.* (1992) who reported that high blood supply levels were associated with spontaneous abortion and developmental abnormalities. Whether administration of folic acid and exposure to γ-irradiation inhibit the synthesis of nucleic acids and cell can not complete its mitosis and also radiation inhibit the conversion of folic acid to folinic acid (Kamen, 1997). In this respect increase of malformation following folic acid administration and radiation exposure as result of decreased hepatic metabolism (Chevias *et al.* 1987). The severity of these changes might be attributed to synergistic effect of folic acid and gamma rays exposure.

At the ultrastructural level, folic acid caused variable degrees of damage in the fetuses liver. The commen lesions which were observed are: hydropic degeneration, deformation of the cytoplasmic organelles and nuclear damage. The cytoplasmic organelles which were markedly affected by folic acid treatment in the fetal liver and placenta cells are the mitochondria and the endoplasmic reticulum. The mitochondria are the most sensitive structures to any affecting chemical factor. Stevens and lowe (1995) considered the mitochondrial damage as an early event for the cell injury. Also mitochondria are the main sites of the energy production in the cells, their damage may results in lowed energy output. This in turn may be a factor in inducing other changes observed in the cells.

Moreover, mitochondria are known to contain fatty acid oxidases, these enzymes are necessary for the metabolism of triglycerides. This leads to another suggestion that the mitochondrial damage observed in the present study may be involved in the lipid changes.

The observed alterations of the rough endoplasmic reticulum constitute the main adverse effect of γ-ray and folic acid on the fetal liver cells and placental cells due to its important role in protein synthesis. In addition smooth endoplasmic reticulum represents the main site of metabolism of various drugs (Davis, 1984), so it was markedly affected following folic acid administration. Rubin (2000), attributed the damage of cell membranes and organelles following drug treatment to the O_2 metabolites which react with the unsaturated fatty acids in phospholipids (lipid peroxidation). He also attributed it to the deficiency of the protective enzymes, glutathione peroxidase and superoxide dismutase, from the liver following drug treatment.

The present study indicates that folic acid has an dystrophic effect on the nuclei of hepatocytes and placental cells, which showed variable changes in their amount of chromatin and others showed signs of shrinkage pyknosis and karyolysis. These changes may be due to the interference of folic acid with nuclear DNA in liver cells and placenta cells. On the contrary Kamen (1997), reported that folic acid is necessary for the production and maintenance of new cells. This is especially important during periods of rapid cell divion and growth such as infancy and pregnancy.

Also, Frenech *et al.* (1998) recorded that folate is needed to make DNA and RNA, the building blocks of cells. It also helps prevent changes to DNA that may lead to cancer. Several authors suggested that a deficiency in folate might predispose people to develop cancer of the cervix, colon, lung; breast and mouth (Butterworth *et al.*, 1992; Heimburger *et al.*, 1992; Kim and Mason 1995 and Zhang *et al.*, 1999). Whereas, some observations suggested that folate supplements may prevent colon cancer, especially when taken for many years (Ginovannucci *et al.*, 1998). Moreover, high-dose folate (10 mg/daily) might be helpful for normalizing abnormalities in the appearance of the cervix in women taking oral contraceptive, but it dose not appear to reverse actual cervical dysplasia (Burrerworth, *et al.*, 1982, Burrerworth 1992 and Butterworth *et al.*, 1992). Very high dosages of folate may be helpful for gout (Oster, 1973), although some authorities suggested that these was actually a contaminant of folate that caused the benefit seen in some studies (Boss *et al.*, 1980). Furthermore, other studies have found no benefit at all (Boss *et al.*, 1980).

The present findings revealed that when pregnant rats were exposed to gamma-radiation, the fetal liver and placenta cells induced ultrastructural changes in the hepatocytes and placenta cells.

The results obtained in the present study pointed to prominent cellular changes which were observed in the distortion of the nuclei, swollen mitochondria, increased lysosomes and fragmentation of endoplasmic reticulum.

Exposure to ionizing radiation is characterized by excessive production of ROS associated with an increase in the process of lipid peroxidation (Saada *et al.*, 2001 & 2003). ROS such as hydroxyl radicals (OH), superoxide anion (O_2) and hydrogen peroxide (H_2O_2) produced in the living cell during normal metabolic functions or as a consequence of response to abnormal stress symbolized a great threat to biomembranes and interact with vital molecule causing their alteration and destruction (Benderitter *et al.*, 1995). The polyunsaturated fatty acids of cell membrane phospholipids are major targets of the highly reactive (OH) attack (Haliwell and Gutteridge, 1989). This is associated with a decrease in the activity of antioxidant enzymes of the body with consequent damage of cellular biomemb-ranes (El Habit *et al.*, 2000 and Saade and Azab, 2001). Radiation induced damage to membrane of the subcellular organelles which may be attributed to peroxidation of membrane lipid protion monitored by the increase in the thiobarbituric acid reactive substances (TBARS) concentration (Azab, 2007).

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التغيرات المورفولوجية والبيوكيميائية والنسيجية الدقيقة فى مشيمة الجرذان الحوامل وكبد أجنتها المعاملة بحمض الفوليك و / أو آشعة جاما

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فاعلية مضادات الأكسدة والعبء التأكسدى لآشعة جاما ما زالت فى حاجة إلى المزيد من الأبحاث. يهدف هذا البحث إلى تقييم التأثيرات الضارة للجرعة الكبيرة من حمض الفوليك و / أو آشعة جاما على مشيمة الجرذان الحوامل وكبد أجنتهم.

تم تقسيم إناث الجرذان الحوامل إلى أربعة مجموعات. المجموعة الأولى هى المجموعة الضابطة وأعطيت المجموعة الثانية حمض الفوليك بجرعة 5مجم/كجم من وزن الجسم من اليوم الخامس إلى اليوم العشرين من الحمل. المجموعة الثالثة تعرضت للتشعيع الجامى بجرعة 3 جراى كجرعة مجزئة (1 جراى / ثلاث مرات) فى اليوم الخامس والعاشر والخامس عشر من الحمل. المجموعة الرابعة عوملت بحمض الفوليك وتعرضت لآشعة جاما معا بنفس الجرعات التى عوملت بها المجموعتين الثانية والثالثة.

تم ذبح الجرذان فى اليوم العشرين من الحمل ، أخذت عينات الدم لتعيين التغيرات البيوكيميائية فيه ، أخذت أيضا عينات من مشيمة الإناث البالغة وكذلك كبد الأجنة وتم تجهيزها للدراسة بالميكروسكوب الإلكترونى.

أوضحت النتائج أن الأمهات المعاملة بحمض الفوليك أو المعرضة للإشعاع الجامى قد ظهرت بها زيادة فى نشاط إنزيم الجاما جلوتاميل ترانسفيريز فى السيرم مع إرتفاع ملحوظ فى اللاكتيت ديهيدروجينيز فى أنسجة المشيمة للأم الحامل مصحوبا بإرتفاع فى محتوى الفوسفور فى أنسجة الكبد للجنين. ووجد أن المعاملة بحمض الفوليك أو التعرض للإشعاع قد أدت إلى العديد من التشوهات منها إنحناء الظهر وإختفاء الأذن وقصر الرقبة ، نزيف تحت الجلد وشلل فى الأطراف الأمامية وكذلك إحتقان فى الأوعية الدموية.

كما أوضحت النتائج أضرارا هستولوجية بالغة فى نسيج مشيمة إناث الجرذان وكذلك نسيج الكبد للأجنة نتيجة تعاطى حمض الفوليك أو تعريض أجسامها للتشعيع الجامى. بينما تسبب التعرض المشترك فى المزيد من الأضرار والتشوهات شملت إتساع الجيوب الدموية ووجود خلايا التهابية وتحلل سيتوبلازم الكثير من الخلايا وحدوث ضرر لكل من الميتوكوندريا والشبكة الإندوبلازمية كما شمل حدوث تغيرات فى بعض الأنوية فى نسيج المشيمية.

ونستنتج من هذه الدراسة أن إعطاء الفئران الحوامل جرعة مقدارها 5مجم/كجم من حمض الفوليك أو التعرض للإشعاع أو الإثنين معا قد أدى إلى ظهور تغيرات ضارة سواء فى مورفولوجية الأجنة أو التغيرات التركيبية وكذلك القياسات البيوكيميائية التى تم قياسها.