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Malformations Induced in Pregnant Rats and their Fetuses Treated with Fluconazole and / or Gamma Radiation

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ABSTRACT

The purpose of the present work is to study the synergistic effect of antifungal (fluconazole) treatment and / or g-radiation stress on pregnant mothers and their developing embryos by evaluating the maternal biochemical changes, embryological and histopathological lesions. Fluconazole is a broad-spectrum azole antifungal medication used for the treatment of several types of fungal infections including common forms such as vaginal candidiasis.

Fluconazole (50 mg/kg b.wt.) was daily administered by oral gavage to pregnant rats from the 4^{th} to the 13^{th} gestational days during which they were subjected to g-radiation at a dose level of 1Gy given at the 6^{th} day (post implantation period) and 1Gy on the 12^{th} day (organogenesis period) of gestation. The animals were dissected and examined on the 20^{th} day of gestation (one day prior to praturation). Fluconazole and radiation dual treatment resulted in increased maternal serum of lactate dehydrogenase (LDH), creatine phosphokinase (CPK), aspartate transaminase (AST) activities and sodium (Na^+) level accompanied with a decline in potassium (K^+) concentration.

The results showed that there was an elevation in the lipid peroxidation end product malondialdehyde (MDA) as well as nitric oxide (NO) in the brain and heart tissues of pregnant rats. Meantime, the developing embryos in the uteri showed various teratological, skeletal and histological impairments. Moreover, the fluconazole treatment and / or g-radiation harm effects were detected as growth retardation, malformations, intrauterine death and embryonic resorption. The examination of the endoskeletal system of fetuses showed retardation in the ossification of the skull bones and lack of ossification at the center of vertebrae and appendages. In addition, the embryonic histological examinations revealed heart loss of normal architecture, the interstitial tissues were oedematous and containing necrotic cellular debris together with fibrosis of nerve cells in the brain of fetuses.

Key words: Fluconazole, radiation, pregnancy, heart, brain, embryos, malformations, skeletal, histology.

INTRODUCTION

Fluconazole, a synthetic azole antifungal agent, was identified recently as a possible human teratogen [(1)]. During pregnancy women are more susceptible to fungal infection [(2)]. Studies have shown a higher prevalence of vulvovaginal candidiasis among pregnant than nonpregnant women and that this prevalence tends to increase as gestational age advances [(3)]. The use of fluconazole during the gestation of animals and human has been largely investigated.

It seems feasible that fluconazole becomes teratogenically operative only under high levels of exposure because no increment in congenital malformations have been reported after exposure to a single dose or multiple doses of 5-20 mg/day [4]. Several congenital anomalies were observed in children delivered from mother treated with fluconazole at doses of 400-800 mg/d and these anomalies were similar to those observed in animal studies. These anomalies including craniofacial, limb, brachycephaly, cleft palate, skeletal thin ribs, long bones, ossification defects, kidney and cardiac defects [5-6]. In addition, the teratogenicity of fluconazole in animals might be dose-dependent. **Tachibana,** [7] stated that when pregnant rats were treated with 25 or 125 mg/Kg during days 6-17 of gestation an increased occurrence of fetal anatomical variants including renal pelvis dilation and cardiac deformation (at 125 mg/Kg) and supernumeraty ribs (at 25 and 125 mg/Kg) were noted. Moreover, doses ranging from 80 to 320 mg/Kg on gestational day 6 to 17 resulted in increased in embryoletality, high incidence fetal resorption as well as significant number of stillbirth and fetal abnormalities including wavey ribs and abnormal limbs and craniofacial ossifications [8]. Maternal weight loss was impaired and placental weights were increased after exposure to fluconazole at doses of 25 to 50 mg/Kg and higher [9]. Furthermore, at doses of 50µg/ml of fluconazole and 75µg/ml morphogenesis was impaired as demonstrated by skeletal anomalies that developed from the second branchial arch [10]. The branchial arches are transitional embryonic structure involved in the development of several components of the head and neck [11]. Moreover, oxidative stress associated with fluconazole induced organ injury [12]. Lipid peroxidation is one of the most investigated consequences of reactive oxygen substances on membrane structure and function, it is also involved in the development of tissue injury in various biosystems [13]. **Hua,** [14] stated that fluconazole penetrated the central nervous system to the brain when rats treated with it at doses 10-20 mg/Kg and induced impairment in the brain tissues. In addition, fluconazole have been reported to affect the muscle membrane through Na^+/K^+ pump and membrane electrical properties and fluidity [15].

On the other hand, the steadily increasing use of nuclear and radiation technology extended to different fields, which have paralled by increasing potential risk for radiation exposure [16]. The deletrious effects of ionizing radiation on biological system are mainly mediated through the generation of reactive oxygen species (ROS) in cells as a result of water radiolysis [17]. ROS and oxidative stress may contribute to metabolic and morphologic changes in human and animals [18]. The uncontrolled ROS production could induce modification of lipids which play a role in the development of cardiovascular [19] and neurodegenerative damage [20]. Maternal exposure to γ-radiation at the dose 3 Gy on the 6^{th} and 12^{th} days of gestation induced pre-implantation death, increased incidence of intrauterine death, reduced the rate of growth as well as uterine retardation [21]. **Ramadan** [22] recorded that pregnant rats exposed to 3 Gy γ-irradiation on the 7^{th}, 11^{th} and 15^{th} days of gestation induced deformations in the skeletal system. Also, exposure to ionizing radiation induced oxidative stress in various organs, altering the cell membrane potential and these alterations may influence the biochemical parameters, enzyme activities [23], K^+, and Na^+ levels [23], lipid peroxidation [24] and histopathological disorders mainly in the heart [25] and brain cells [26].

In view of this consideration, the current study has been designed to investigate the adverse effect of fluconazole administration with radiation exposure to pregnant rats and the development of their fetuses. This was assessed biochemically by estimating malondialdehyde (MDA) and NO in the tissues of heart and brain, LDH, CKP and AST in the serum of pregnant rats, structural changes in the heart and brain of their fetuses as well as selected skeletal and morphological defects in fetuses.

MATERIAL AND METHODS

Experimental animals:

Twenty female albino rats weighting 130-150g (obtained from the Breeding unit of Atomic energy Authority, Egypt) were used in this study. The animals were maintained on standard laboratory diet and water *ad-libilum.* Mating was occurred in the oestrus stage of female and the detection of pregnancy was carried out using vaginal smear, where the spermatozoa were seen in the vaginal smear denoting day zero of gestation.

Radiation facility:

Whole body gamma irradiation at a dose level of 2Gy was performed using an indoor shielded Cs^{137} Gamma Cell-40 (at the National Center for Radiation Research and Technology, NCRRT) Atomic Energy Authority, Cairo, Egypt, at a dose rate of 0.61 Gy/min.

Fluconazole:

Antifungal (Flucoral) was supplied as capsules each contained 150mg of fluconazole purchased from Sedico Company in Egypt. The capsule content was dissolved in saline and administrated orally to pregnant rats at a dose of 50mg/Kg body weight according to **Vanessa and Guilhermino [27].**

Experimental design:

Pregnant rats were assigned into four groups each of five animals:

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

Group 2 (treated): Pregnant rats were treated orally with fluconazole at a dose of 50 mg/Kg b.wt/day from the 4^{th} to the 13^{th} day of gestation.

Group 3 (irradiated): Pregnant rats were exposed to whole body gamma rays delivered as 1 Gy on the 6^{th} day (post implantation period) and 1 Gy on the 12^{th} (organogenesis period) day of gestation (i.e. cumulative dose of 2 Gy).

Group 4 (treated and irradiated): Pregnant rats were treated orally with fluconazole at dose 50 mg/Kg b.wt. from the 4^{th} to the 13^{th} gestational day and irradiated on the 6^{th} and 12^{th} days of gestation.

Animals of each group were sacrificed on day 20 of gestation (1 day prior to delivery).

Morphological studies:

Animals of each group were daily weighed to monitor their body weight then sacrificed at the day 20 of gestation (1 day prior to parturition). The uteri were removed, weighed and photographed instantly. The fetuses were removed from the uteri and examined to determine both the number of live and dead fetuses. The abnormalities in the uterine horns and fetuses were studied. The weight of dams, fetuses and placenta were recorded using rough Metller Balance.

Skeletal preparation of fetuses:

The fetuses were preserved in 95% ethyl alcohol and cleared with KOH in order to study the skeletal abnormalities. The cartilage and bones were stained with alizarin red and alcian blue according to the method of **Macleod [28].**

Biochemical analysis:

Animals were sacrified at day 20 of pregnancy. Heparinized blood was withdrown by heart puncture under light ether anaesthesia and collected into sterile tubes. Serum was separated by centrifuging the blood at 2500 rpm for 15 min.

The activities of serum lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and aspartate transaminase (AST) were estimated according to the methods of **Bergmeyer and Brent** [29], **Minami and Yoshikawa** [30] **and Reitman and Frankel** [31], respectively. Serum potassium was measured according to the method of **Sundurman and Sundurman** [32] and sodium was estimated colourmetrically using commercial kit (Diamond company).

Heart and brain from pregnant rats were dissected out, washed, dried and homogenized in ice-cold saline to yield 10% homogenates then centrifugate at 3000 rpm for 15 min. The supernant was used for the estimation of malondialdehyde (MDA) and nitric oxide (NO) level according to the method of **Yoshioka *et al.*** [33] and **Green *et al.*** [34], respectively.

Histological studies:

The brain and heart of the fetuses were immediately excised, fixed in buffered formalin, dehydrated, cleared and embedded in paraffin wax. Sections were cut at 5-6 µm thickness and stained with Haematoxylin and Eosin for the demonstration of general histopathological changes.

Statistical analysis:

Data were analyzed by paired Student's t-test. Values were expressed as mean ± SE according to **Snedecor and Cochern** [35].

RESULTS AND DISCUSSION

Reproductive outcome:

As shown in table (1) the reproductive parameters have been affected by fluconazole and / or radiation exposure.

Table (1): Reproductive parameters in pregnant rats treated with fluconazole (50 mg/kg b.wt.) and / or radiation exposure (2 Gy).

Parameters	Animal groups			
	control	fluconazole	g-radiation	Fluconazole + g-radiation
Maternal weight loss during pregnancy (g) fromthe gestational days 4 to 20	45.64±0.238	44.44±0.293**	34.52±0.646***	27.32±0.260***
Uterus weight (g)	48.66±4.548	29.02±3.123***	24.96±3.333***	11.8±0.616***
Placental weight (g)	0.63±0.070	0.94±0.043**	0.36±0.052**	0.798±0.046*
Foetal body weight (g)	3.98±0.198	3.11±0.886**	2.88±0.159***	2.04±0.229***

- Each value represents the mean ±SE of 5 observations

-Significantly different when compared with the corresponding value of control rats at

***P < 0.05, **P < 0.01, *** P< 0.001.**

The results showed a highly significant reduction in the weight of fetuses, the uteri weight of the mother rats subjected to combined treatment with fluconazole and radiation ($P < 0.001$) as compared to control group. Moreover, the maternal weight loss during pregnancy has shown its highest value in dual treatment. Furthermore, there was a significant increase in the placental weight in the group of pregnant rats treated with fluconazole while the radiation group recorded highly significant decrease ($P < 0.01$) as compared to control group.

Morphological observations:

Morphological observations of the control uterus obtained from pregnant rats on day 20 of gestation showed normal distribution of the implanted fetuses between the two horns (Figs. 1 and 2).

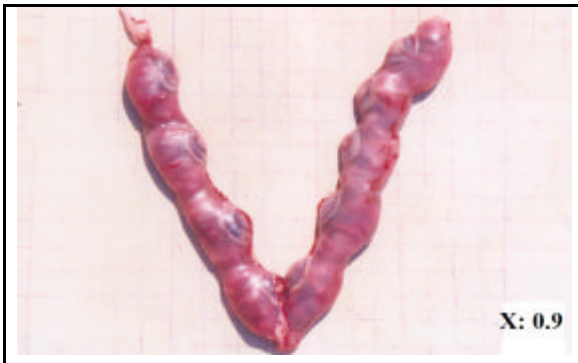

Figure (1): Uterus of a control pregnant rat excised on the 20th gestational day showing normal distribution of 9 implanted fetuses distributed between the 2 horns. (X: 0.9)

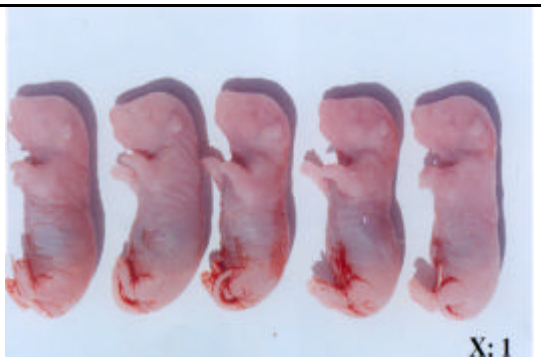

Figure (2): Extruded fetuses of a control mother rat exhibiting normal morphology and normal length. (X: 1)

The uterus of pregnant rats treated with fluconazole showed abnormal shortening and shrinkage of both horns and reduced number of fetuses (Fig. 3). The fetuses of this group exhibited congested blood vessels, microtia, conjoined legs, kypophysis and paralysis of hind limbs (Figs. 4 & 5).

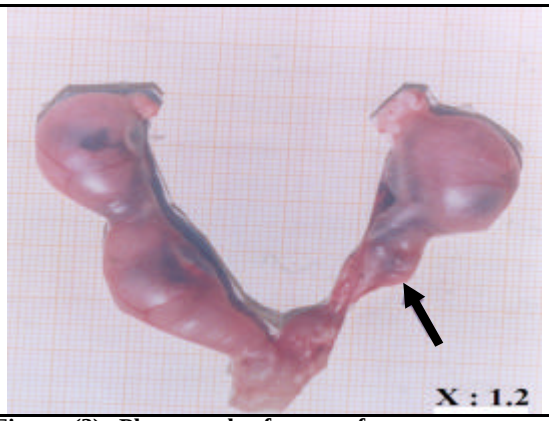

Figure (3): Photograph of uterus from pregnant rat treated with fluconazole showing abnormal shortening and shrinkage of both horns and diminution in the number and size of implanted fetuses in addition to clearly visible embryonic resorption site (-). (X: 1.2)

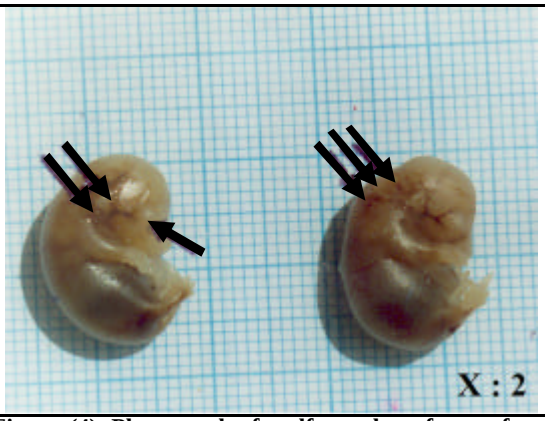

Figure (4): Photograph of malformed rat fetuses from fluconazole treated mother showing anophthalmia (-), shortness of neck, absence of ear (microtia) (- -), congested blood vessels, decrease body length, diminution of size, very thin skin, and abnormal bending of the body kypophysis (- - -). (X: 2)

The uterus and fetuses of the pregnant rats subjected to (2Gy) fractionated as (1Gy) on the 6th day and (1Gy) on the 12th day of gestation showed reduced number of implanted sites and high incidence of prenatal mortality (Fig. 6). Furthermore, the fetuses of this group revealed severe malformations as excencephaly, bending of the body (protrusion), micromelia and spina bifida of total vertebral column (Figs. 7 & 8). The pregnant rats treated with fluconazole and exposed to γ-radiation showed high incidence of foetal mortality in the uterus (Fig.9). Otherwise, the fetuses showed morphological lesions included microcephaly, adactyl, excencephaly and micromelia (Fig. 10).

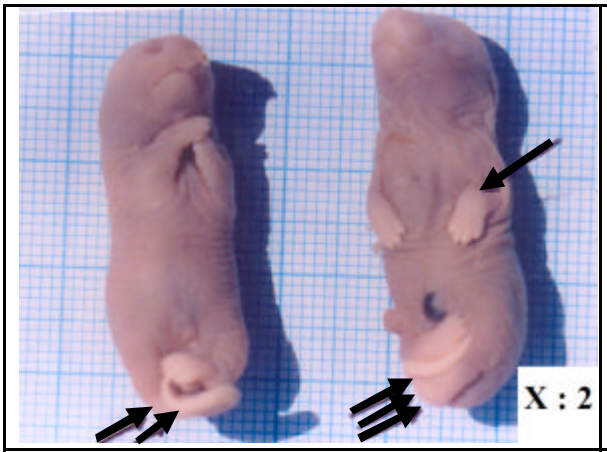

Figure (5): Photograph of malformed rat fetuses from fluconazole treated mother showing anophthalmia, microtia, clubbed limbs, micromelia, small limbs (-), bent tail, paralysis of hind limb (- -) and conjoined legs (- - -). (X: 2)

Figure (6): Photograph of uterus from pregnant rat exposed to gamma irradiation on days 6 and 12 of gestation illustrating reduced number of implantation sites, high incidence of prenatal mortality. (X: 0.9)

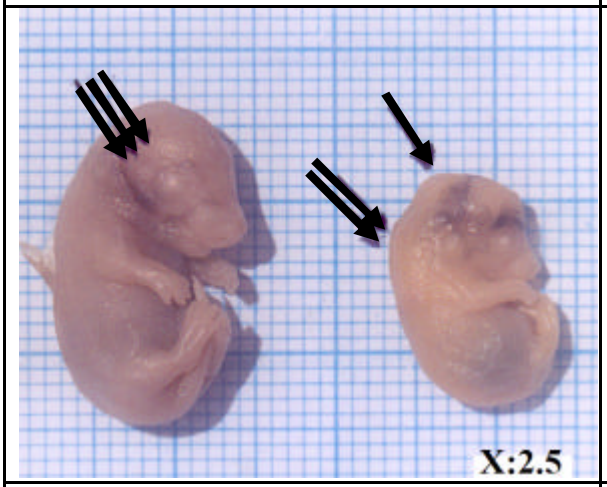

Figure (7): Photograph of fetuses from mother exposed to gamma irradiation on the 6^{th} and 12^{th} days of gestation showing excencephally (-), subcutaneous haem-orrhage, short neck, anophthalmia, abnormal bending of the body (protrusion) (- -), decrease body length, diminution in size, absence of ear (microtia) (- - -) and inverted tail. (X 2.5)

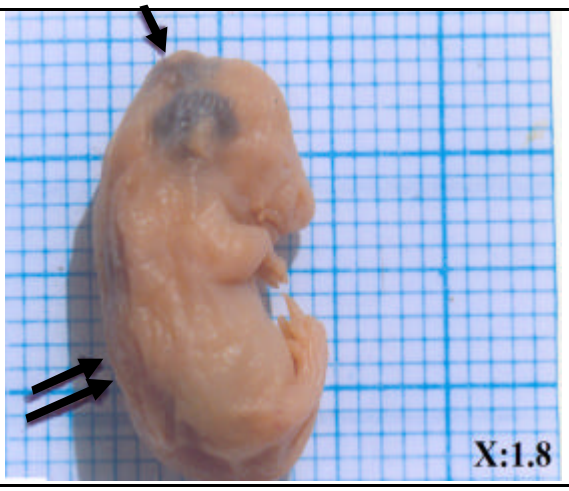

Figure (8): Fetuses maternally exposed to gamma irradiation showing generally malformations: anophthalmia, excencephaly (-), short neck, subcutaneous haermorrhage, small limbs (micromelia) and spina bifide of total vertebral column (- -). (X:1.8)

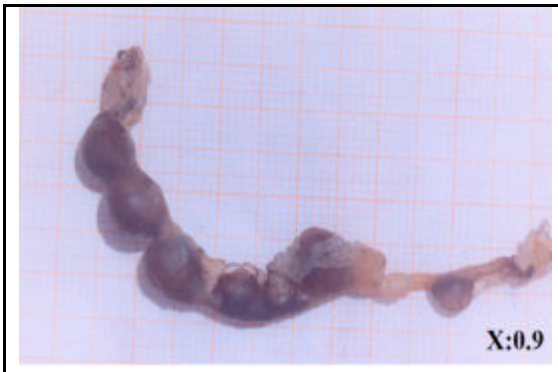

Figure (9): Photograph of uterus from pregnant rat treated with fluconazole and exposed to g-irradiation showing shortening of one horn with resorbed and reduced number of fetuses. (X 0.9)

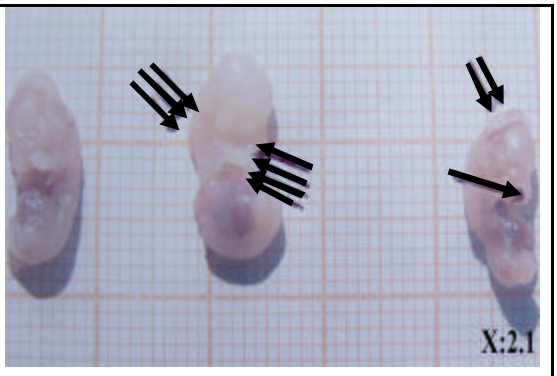

Figure (10): Photograph of malformed fetuses maternally exposed to g-irradiation during fluconazole treatment showing very thin skin, micrognathia (-), undifferentiated neck, adactyl, anophthalmia, microcephaly, microtia, tail is very short or completely absent, exencephaly (- -), kypophysis (- - -), and small limbs micromelia (- - - -). (X 2.1)

Endoskeleton studies:

Examination of the skeletal system of control litters on day 20 of gestation revealed deep stainability with alizarin. The bones were clearly demarcated indicating complete ossification of the skull and the other bones of the skeleton (Fig. 11).

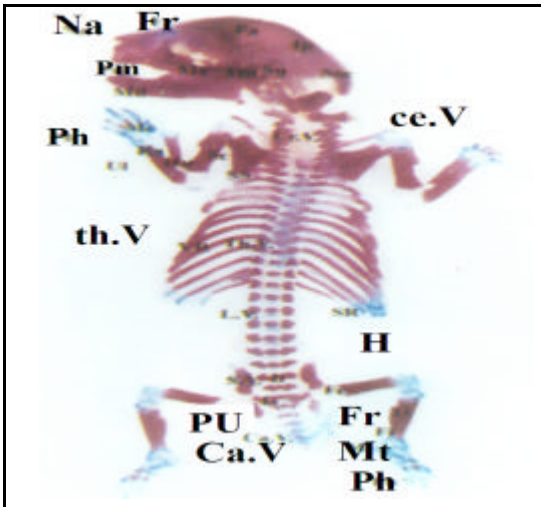

Figure (11): Dorsal view of control fetus endoskeleton on day 20 of gestation illustrating the normal ossification of thoracic vertebral (Th.v.) cervical (Ce.V.), lumbar (L.V.), sacral (Sa. v.), caudal (Ca. V.), pelvic girdle, frontal (Fr), metatarsals (Mt), femur (F), pubis (Pu), humer (H), phalangus (Ph), nasal (Na) and sternebrae (STR.) vertebrae.

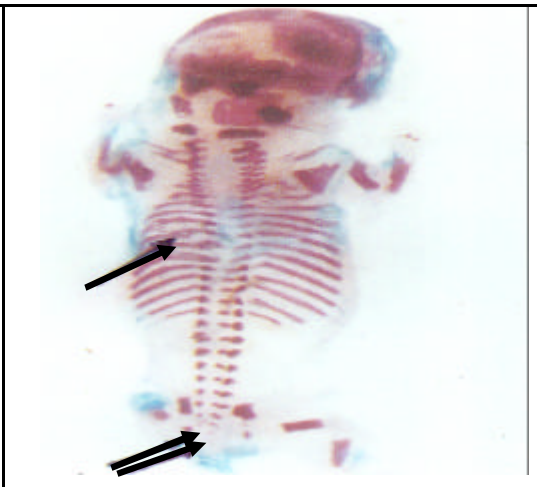

Figure (12): Dorsal view of fetus endoskeleton obtained from treated mother with fluconazole illustrating deformities of ribs (-) and the sacral vertebrae number 4, 5 (- -) and caudal vertebrae were non ossified or completely absent.

Following the treatment with fluconazole illustrated deformities of ribs and the sacral vertebra number of 4 & 5 and the cauda vertebra were non ossified or completely absent (Fig. 12).

Examination of the skeletal system of the animal exposed to γ-irradiation revealed that fetuses exhibited weak ossification of the nasal, frontal and parietal bones. The bones of the skull, sternebrae, metacarpals and metatarsals were not ossified (Fig. 13). The skeleton of fetuses of group 4 treated with fluconazole and γ-radiation showed severe non-ossified skull, lack of ossification of cervical, sacral and caudal vertebrae. Moreover, the thoracic bones showed that the ribs were malformed and reduced in number (Fig. 14).

Figure (13): Lateral view of the fetus endoskeleton maternally irradiated on 6th and 12th days of gestation showing weak ossification of the nasal, frontal and parietal bones. The bones of the skull (-), sternebrae (- -), metacarpals and metatarsals were non ossified.

Figure (14): Lateral view of fetus endoskeleton from irradiated mother during fluconazole treatment showing absence of lower jaw (-), non ossification of the skull bones, lack of ossification of cervical, sacral and caudal vertebrae. For thoracic bones the ribs were malformed and reduced in number.

Biochemical analysis:

In the present study, the serum activities of LDH, CPK and AST are significantly elevated in all treated groups as compared with control group (table 2).

Table (2): Effect of fluconazole (50 mg/kg b.wt) and / or g-radiation (2 Gy) on serum LDH, CPK and AST activities of pregnant rats.

parameters	Animal groups			
	control	fluconazole	g-radiation	Fluconazole + g-radiation
LDH (U/L)	624.254±9.813	696.674±7.339***	739.92±17.211***	775.95±16.234***
CPK (U/L)	250.77±5.44	319.022±21.085**	362.78±5.398***	388.496±3.637***
AST (U/L)	35.68±3.399	50.04±3.349**	63.56±2.579***	68.22±2.489***

Legend as table (1)

Table (3) showed that fluconazole treatment showed significant increase in serum sodium (Na^+) concentration, while whole body gamma irradiation of pregnant rats resulted in a highly significant increase while the dual treatment showed very highly significant increase in (Na^+) concentration ($P < 0.001$) as compared with control group. On the other hand, potassium concentration recorded very highly significant decrease in all treated group as compared with control group.

Table (3): Effect of fluconazole (50 mg/kg b.wt) and / or g-radiation (2 Gy) on serum Na^+ and K^+ levels of pregnant rats.

Animals groups	Parameters	
	Na^+ (m mol/L)	K^+ (m mol/L)
Control	159.08±16.898	6.04±0.122
Fluconazole	199.18±9.252*	5.02±0.053***
γ-radiation	217.218±5.342**	4.972±0.151***
Fluconazole + γ-radiation	249.20±13.829***	4.430±0.181***

Legend as table (1)

The results represented in table (4) indicated that fluconazole alone and / or exposure to gamma irradiation induced obvious brain and heart injury reflected by the index of lipid peroxidation (MDA) and nitric oxide (NO) which showed significant elevation in all experimental groups when compared to control group.

Table (4): Effect of fluconazole (50 mg/kg b.wt) and / or g-radiation (2 Gy) on MDA and NO (n mol/g tissue) level in the brain and heart tissues of pre gnant rat in different groups.

Animal groups	Parameters			
	MDA (n mol/g tissue)		NO (n mol/g tissue)	
	Brain	Heart	Brain	Heart
Control	51.752±4.252	32.218±2.556	2.828±0.0987	5.99±0.245
Fluconazole	70.206±4.417**	41.282±2.171**	3.646±0.183**	6.918±0.0787**
g-radiation	73.684±3.133***	45.570±1.676***	5.734±0.562***	10.83±0.377***
Fluconazole + g-radiation	76.892±2.869***	52.01±2.593***	6.018±0.304**	12.514±0.515***

Legend as table (1)

Histological study:

A-Brain:

Histological observations on brain tissue of control rat fetuses through light microscope showed normal nerve cells, pyramidal cells, and myelinated nerve fibres (Fig. 15). Treatment of pregnant rats with fluconazole showed necrotic, degenerated of pyramidal cells, fibrotic and vacuolated nerve cells (Fig. 16). Exposure of experimental animals to γ-radiation revealed severe necrotic pyramidal and nerve cells, hydropic degeneration and necrotic of nerve cells were observed in the brain tissue of rat fetuses (Fig. 17).

Brain section of embryo from pregnant mother exposed to γ-irradiation during fluconazole treatment showed necrotic and degenerated pyramidal cells, inflammatory cells, proliferating, fibrosis and vacuolation of nerve cells (Fig. 18).

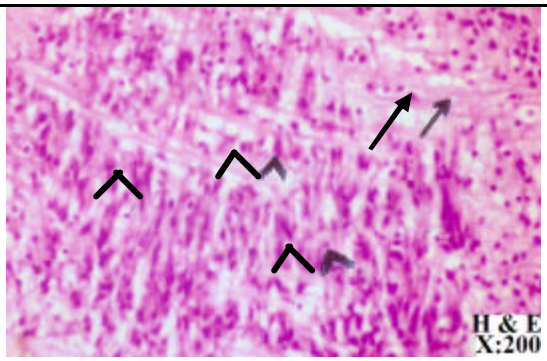

Figure (15): Photomicrograph of section in control brain hemisephere fetus rat showing normal pyramidal cells (Ù) and mylinated nerve fiber (-). (H & E X200)

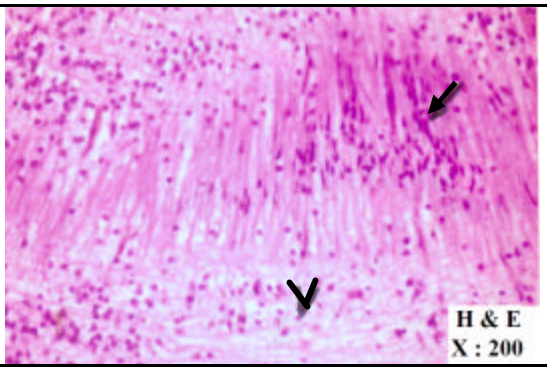

Figure (16): Photomicrograph of section in brain hemisephere of fetus from pregnant rat treated with fluconazole showing necrotic, degenerated of pyramidal cells (-), fibrotic and vacuolated nerve cells (V). (H & E x200)

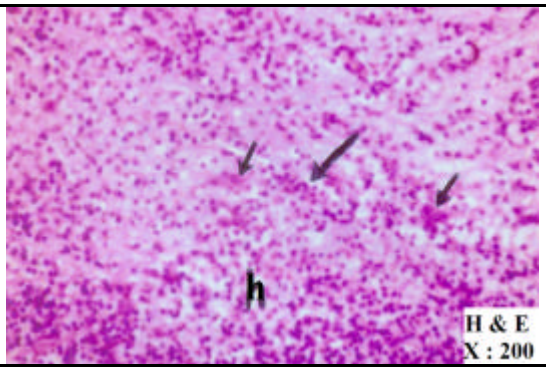

Figure (17): Photomicrographs of section in the brain hemisephere of fetus from pregnant mother exposed to g-irradiation showing severe necrotic pyramidal and nerve cells (long arrow ( ), hydropic degeneration (h) and sticking necrotic of nerve cells (small arrow (-). (H & E X 200)

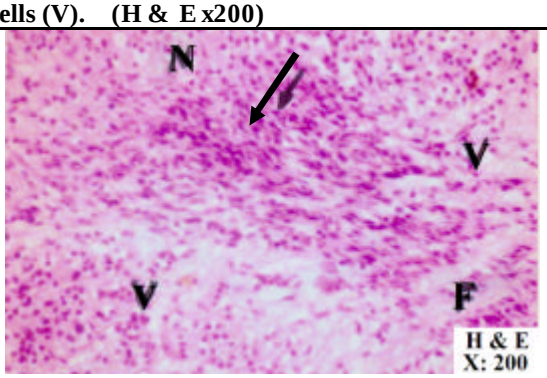

Figure (18): Photomicrograph of section in the brain hemisephere of fetus from pregnant rats treated with fluconazole and exposed to g-irradiation showing necrotic (N) and degenerated pyramidal cells, inflammatory cells (-), proliferating, fibrosis (F) and vacuolated (V) nerve cells. (H & E X200)

B-Heart:

Histological examination of the heart of control rat fetuses showed normal architecture of mSyocardial fibers (Fig. 19). Heart cells as depicted in Fig. (20) showed loss of striation, necrotic debris in the interstitial tissue and oedematous area post treatment with fluconazole.

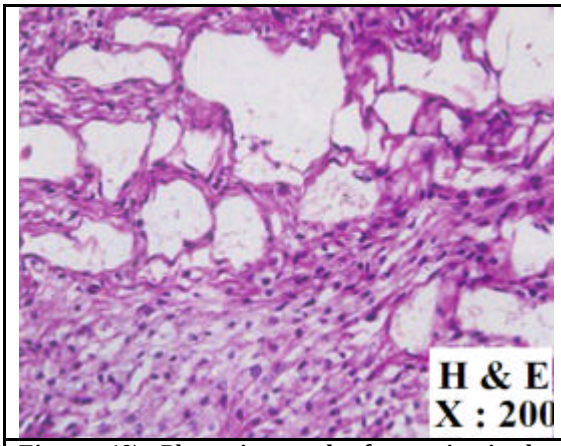

Figure (19): Photomicrograph of a section in the heart of control rat fetus showing normal histological myocardial fibers. (H & E 200)

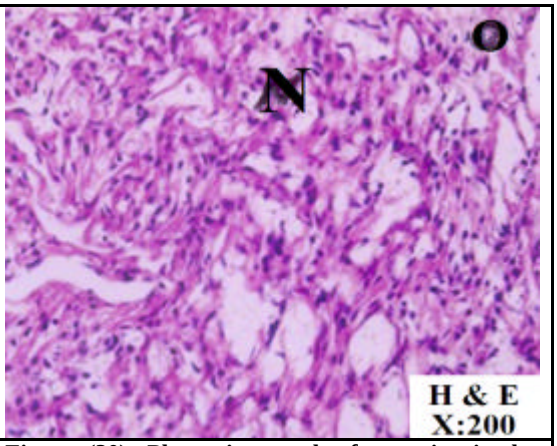

Figure (20): Photomicrograph of a section in the heart of fetus from pregnant rat treated with fluconazole showing loss of striation, necrotic debris (N) in the interstitial tissue and oedematous area (O). (H & E X 200)

Furthermore, exposure of the experimental animals to γ-radiation exhibited severe degenerated myocardium with pyknotic nuclei, dilatation between muscle fibers with vacuolated cells and interstitial oedema (Fig. 21). Figure 22 illustrated a section in the heart of rat fetuses tissue subjected to irradiation and treated with fluconazole showing loss of striation, haemorrhagic infiltration, increased fibroses tissues in between myocardium and vacuolar degenerative changes with increase number of pyknotic nuclei.

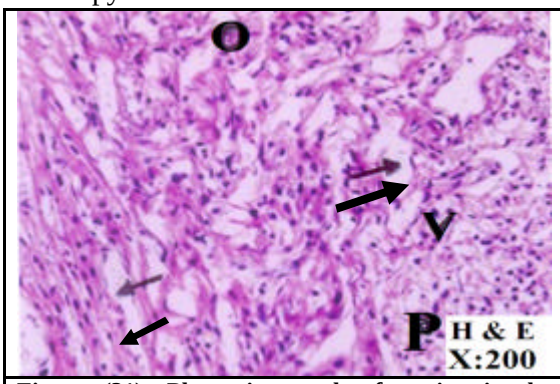

Figure (21): Photomicrograph of section in the heart of fetus from pregnant rat exposed to g-irradiation showing severe degenerated myocardium with pyknotic nuclei (P), dilatation between muscles fibers (-) with vacuolated cells (V) and interstitial oedema (O). (H & E X200)

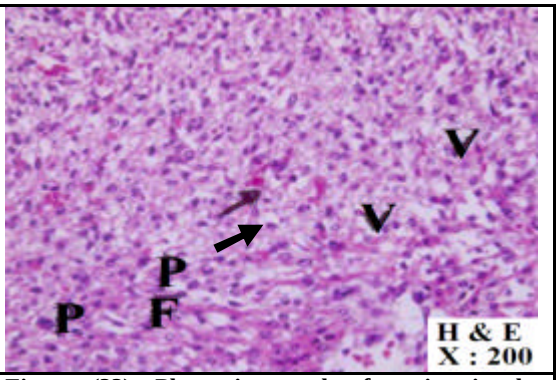

Figure (22): Photomicrograph of section in the heart of fetus from pregnant rat treated with fluconazole and exposed to g-irradiation illustrated loss of striation, haemorrhagic infiltration (-), increased fibrouses tissues (F) in between myocardium and vacuolar (V) degenerative changes with increase number of phyknotic nuclei (P). (H & E X200)

The results of the present study showed that fluconazole administration at the dose 50 mg/kg b. wt. from the 4th to 13th day of gestation and exposure to gamma irradiation 1 Gy on the 6th day (post implantation period) and 1 Gy on the 12th day (organogensis period) of gestation resulted in significant biochemical, histological skeletal disorders associated with embryological teratogenicity. The current study, showed that the dose of fluconazole 50mg/kg caused teratogenic effects that include head, ear, and limbs defects as well as loss in maternal weight. These results agree with the findings of **Norgaard,**[36] who found increased risk of congenital malformations after exposure to short-course treatment with fluconazole at a dose of 300 mg/dl in early pregnancy.

In addition, **Tiboni and Scott,** [37] observed that oral administration of fluconazole to pregnant rats during gestational days 6-17 were associated with several developmental disorders including limbs defects, ear, rib and heart anomalies. Moreover, a loss of maternal weight could be a symptom of ill health. This is related to the investigation of **Jick,** [38] who discovered that rats administration with 40mg/kg of fluconazole was related to systemic toxicity which led to loss in weight which is an index of ill health. Fluconazole reaches to the embryonic compartment through placental transfere [39]**.** Hence, **Khera,** [40]**,** reported that embryo toxicity is known to result in high resorption, fetal death, fetal body reduction and skeletal deformation when fluconazole is administered at high dose (80-320 mg/kg) to pregnant rats. The embryotoxicity induced could be related either to physiological or maternal homeostasis alterations. Furthermore, the impairment in both maternal gestation and fetal anomalies development could be a consequence of inhibition of maternal steroidogenesis. [41]

Research studies indicated that the correlation of fetotoxicity induced by fluconazole treatment resulted in increased abnormal craniofacial, limbs ossification. These abnormalities may be due to alterations of the pathogenetic pathway and interference with cellular and molecular mechanisms that control neural crest cell migration and may be causal in the elicitation of teratogenic effects [42].

In the present study, data denoted an increase in (MDA) content in the heart and brain tissues of pregnant rats. This observation is correlated to the findings of **Timboni and Giampier** [43] who stated that fluconazole has lipophilicity characteristics when combined with low protein bindings which are responsible for its higher tissue penetration. Brain tissues are very susceptible to oxidative injury induced by fluconazole treatment. Oxidative stress has been implicated in the pathogenesis of ischemic cerebral injury for increasing capacity of oxygen consumption and high level of unsaturated fatty acid [44].

Nitric oxide (NO) is a highly reactive molecule which regulates blood flow, augments regional blood flow and vasodilators, and improves cerebral circulation. An excess of lipid peroxide release react with NO, disrupting its physiological signalling and potentially led to the production of other toxic and reactive molecules notably peroxy-nitrite [45]. These led to increase in electrolyte (Na^+ - K^+) imbalance (cystosolic – calcium levels) resulting in membrane lysis. The relative importance of these effects contributing to clinical myotoxicity and elevated lipid peroxidation which led to block ion channels [46].

LDH, CPK and AST activities are considered as indicators of myocardial damage. The present data showed that LDH, CPK and AST activities have been found increased in the serum of pregnant rats. These results are in agreement with **Magda and Micheal** [47] who concluded that the increase in these enzymes was due to the damage of cellular membrane and cardiac toxicity.

Radiation exposure produces different lesions in both pregnant mother and their fetuses. The degree of lesion depends upon the dose rate of radiation, stage of pregnancy and age of animals [48]. Irradiation of mother with 1 Gy on the 6^{th} day and 1 Gy on the 12^{th} day of gestation as practiced in the present study was found to induce foetal intrauterine death and stillbirth together with serious teratogenic effects in the head, eye, skeletal ossification and extremities of surviving fetuses. Similar results were observed by **Walash** [49] **and Ramadan** [22] who reported that total body irradiation of pregnant rats induced many malformations in their fetuses during organogenesis period as exencephaly, haemorrhage and low birth weight. This can be due to the direct action of radiation on the embryo and placental dysfunction. This view is supported by the work of **Gaber,** [21] who cited that exposure of pregnant rats to gamma irradiation (3 Gy) on the 6^{th} and 12^{th} days of gestation increase the incidence of intrauterine fetal death as well as induce uterine growth retardation.

Moreover, the induction of retarded ossification in the skull and limbs was correlated with the reduction in weight of fetuses and the defect in the eyes, skull and nervous system as a result of the sensitivity of cells to chromosomal damage which resulted in delays in cell division [50]. The present

findings showed that the skeletal malformations in embryo obtained from mother exposed to gamma irradiation fractionated dose 2 Gy (1 Gy on the 6th day and 1 Gy on the 12th day of gestation) showed no ossification of skull bones and limbs bones. The observed deformities found in skeletal growth may be attributed to transplacental passage of parent component and adversely affected the morphogenesis of tissues during the active period of growth [(51)].

Lipid peroxidation is a feature of damage of various tissues induced by ionizing radiation. The current data revealed significant elevation in MDA in heart and brain tissues of pregnant rats after exposure to γ-irradiation. The elevation recorded can possibly be due to the radiation production of free radicals which are responsible for the deleterious effect in biological membranes [(52)]. Moreover, ROS may act as mediators of cells during their normal turnover in neurons during the development of nervous system and induced neurodegenerative disorders [(53)]. Also, they contribute to cerebrovascular complications, reduction in cerebral blood brain barrier and cerebral edema. In the current study, a significant increase of NO levels was observed in irradiated groups which might be due to changes of vasculature and more specifically of the endothelial cells which is characterized by an increase in biological active NO release from the endothelium following ionizing radiation exposure [(54)]. All these neurohistological and neurophysiological changes ultimately contribute to the complications associated with radiation exposure including morphological abnormalities [(55)].

Oxidative modification of lipids by ROS plays a role in cardiovascular disorders. So, **Nagaswa** [(56)] observed that muscle fibers showed varying degrees of damage ranging from fibrosis to necrosis in the heart of both mother and their fetuses after exposure to gamma irradiation with 6 Gy. These observation support the results of the present study. In addition, elevated lipid peroxides in irradiated rats were associated with disturbances in cell membrane permeability as exhibited by changes in ionic content [(57)]. Hence, the disturbance in Na^+ and K^+ ions induced by irradiation can be attributed to the stress exerted upon this pumping mechanism and in turn led to membrane permeability imbalance and hypoxia of blood which reduces the K^+ effect from tissue cells [(58)].

In the present study, oxidative stress in heart tissues was associated with a significant increase in the activity of serum LDH and CPK as common characteristics of cardio toxicity, released to the blood stream from damaged heart tissue [(59)]. Also, oxidative stress in heart tissues was associated with significant increase in the activity of serum AST (present in a large quantities in the heart) as a result of damage in cell membrane where it may cause increase in membrane permeability and or cell necrosis [(23)].

CONCLUSION

The results of the present investigation showed that administration of fluconazole combined to exposure to gamma irradiation have aggravation the obvious deleterious effects on pregnant rats and their fetuses development than exposure to fluconazole or irradiation alone.

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