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The Possible Protective Role of Bone Marrow Transplantation against Alternations Induced by Gamma Radiations on Fetal Gastrointestinal Tract of Pregnant Albino Rats

Nahed Mohamed Mansour Emam

Biological and Geological Dept., Faculty of Education, Suez Canal University, Ismailia, Egypt.

Abstract

Aim of the work:It is well recognized that radiation can be carcinogenic for a wide variety of tumors, especially, in breast, thyroid, and bone marrow which appear to be radiosensitive.

Bone marrow cells (BM) suppress immune cell responses and have beneficial effects in various inflammatory-related immune disorders. The present study is designed to evaluate the possible role of bone marrow to restore certain histopathological and histochemical changes in the fetal gastrointestinal tract of pregnant rats which exposed to gamma rays. **Material and Methods**: The experimental animals were divided into five groups:

1-Control pregnant rats.

2- Group of pregnant rats irradiated with γ-rays on day 7 of gestation.

3- Group of pregnant rats irradiated with γ-rays on day 14 of gestation.

4- Group of pregnant rats irradiated with γ-rays on day 7 of gestation and treated with BM one hour post-exposure

. **5**-group of pregnant rats irradiated with γ-rays on day 14 of gestation and treated with BM one hour post-exposure. All the previous groups were sacrificed on day 20 of gestation (1 day prior delivery).

Results: Exposure of pregnant rats to γ-rays on day 7 or day 14 of gestation showed many pathological and histochemical changes in the fetal gastrointestinal tract. These changes were more pronounced on day 14 of gestation. The changes include increased proliferation in the mucosal layer, increased signs of lymphocytic infiltration and pyknotic nuclei in addition to highly distorted circular muscle fibers. Also, altered collagen, total protein, polysaccharides contents were noted post-irradiation. Bone marrow transplantation post-irradiation improved the tissue architecture which restored it's normal histological and histochemical pictures.

Conclusion: It can be concluded that bone marrow transplantation post-irradiation showed somewhat a considerable ability to overcome radiation injuries or damages from the histological and histochemical point of view on day 7 or 14 of gestation in the studied previous fetal tissues although the healing was incomplete on day 7 or 14 of gestation when compared with the control groups.

Key words: γ-rays- pregnant rats-fetuses and embryos-bone marrow transplantation.

Introduction

The effects induced by ionizing radiation during embryonic development and fetal growth are a very important subject. Mammalian embryos were more sensitive to ionizing radiation than adults. Several authors showed that radiation induced abnormalities in mammals are closely related to the period of development of which radiation was given **(Sharma and Saini, 2003;Kheifets *et al.*,2005; Maganha *et al.*, 2006).**

De Santis *et al.* (2005) suggested that ionizing radiation represented a possible teratogen for the fetus, but this risk has been found to be dependent on the dosage and the effects correlatable to the gestation age at exposure.

Ramadan (2007a) demonstrated a significant decrease in fetal numbers with numerous embryonic malformations in irradiated pregnant rats with gamma rays at a dose of 0.5Gy for 4 times on gestational days 9, 10, 11 and 12.

Qiu *et al.*(2011) concluded that exposure to electromagnetic waves altered the expression levels of tight junction protein, in the organs of adult male rats, which may induce changes in barrier structure and function. In addition, gamma radiation stimulates acute phase response. This response is essential for generation and limitation of inflammation. Individual acute phase reactants have different biochemical functions and hence regulatory mechanisms that are differentially observed within hours or days **(Campbell *et al*., 2005).**

Generally in the irradiated rats at two doses of 2 and 4Gy from cesium-137 source, a marked decrease in hepatic contents of DNA, and glutathione was realized. The level of cholesterol, triglyceride , low density lipoprotein , alkaline phosphatase and gamma-glutamyl transferase were significantly increased in sera of the irradiated rats. This was coupled with a decreased serum level of high-density lipoprotein and total serum protein by irradiation **(Makhlouf and Makhlouf, 2012).**

Stem cells are self-renewal and give rise to all differentiated cell types of the adult body. They are classified as toti-, pluri- or multi-potent stem cells based on the number of different cell types they can give rise to it. Recently it has become apparent that chromatin regulation plays a critical role in determining the fate of stem cells and their descendants **(Sang *et al.*, 2009).** Stem cell response can be influenced by the multitude of chemical, topological, mechanical and physiochemical factors **(Tay *et al.*, 2010).**

Iyer and Rojas (2008) found that bone marrow mesenchymal stromal cells attenuated a self-inflammatory reaction and enhanced the anti-inflammatory reaction by regulating the proliferation, differentiation, and delomorphous nature of immunocytes. Mesenchymal stem cells (MSCs) are multipotent stem cells and had strong immunoregulatory effects. MSCs also played a special role in inhibiting inflammatory reactions and promoting tissue repair (**Hanson *et al*., 2010).**

Tu *et al*. (2012) indicated that mesenchymal stem cells can reduce the expression of various inflammatory factors and promote the repair of various tissues and organ injury. bone marrow mesenchymal stromal cells can effectively relieve injury to pancreatic acinar cells and small intestinal epithelium, promote the proliferation of enteric epithelium and repair of the mucosa, attenuate systemic inflammation in rats with severe acute pancreatitis .Rats with a radioactive intestinal injury were injected with labeled bone marrow mesenchymal stromal cells and the intestinal chorioepithelium regeneration occurred in the injured intestinal mucosa and the radial related regions (e.g., kidney, spleen, stomach**) (Sémont *et al.*, 2006).**

After injecting bone marrow mesenchymal stromal cells into rats with an intestinal injury (ischemia/reperfusion), the permeability of the intestine was reduced and the injury to the intestinal villi was attenuated **(Jiang *et al*. ,2011).**

After intravenous injection of bone marrow mesenchymal stromal cells in experimental rats with spinal injury, bone marrow mesenchymal stromal cells assembled and survived in the host injury spinal cord and promoted the neural repair and recovery of nerve function **(Neuhuber *et al*., 2005).**

Yagi *et al*.(2010) demonstrated that transplantation of bone marrow mesenchymal stromal cells can attenuate the effects of systemic inflammation and organ injury in two different animal models of injury. This therapeutic effect was observed in three vital organs (liver, kidney and lung) in animals demonstrating the anti-inflammatory and anti-apoptotic effects of bone marrow mesenchymal stromal cells.

Kafafy *et al* .(2006) mentioned that exposure of pregnant rats to 3Gy γ-rays on day 7 and day 13 of gestation followed by BMT one hour post-irradiation showed insignificant decrease in RBCs and hemoglobin but not in WBCs as compared to the control group. Bone marrow transplantation after total body irradiation in mice demonstrated normal blood cell counts in 75% of recipients **(Moroz *et al* ., 2009).**

According to **Bakhit (2010)** BMT post-irradiation showed a marked ability to overcome radiation injuries or damages from the biochemical, biological, histological and histochemical point of view on day 7 of gestation in both maternal and fetal tissues with less extent on day 14 of gestation.

The present study has been involved to investigate the possible protective role of bone marrow transplantation from radiation hazards on the fetal gastrointestinal tract of pregnant rats

Material and Methods

1- Experimental animals:

Mature male and virgin female albino rats of pure strain (*Rattus albinus*) ranging from 120-150 gm body weight were used in this experiment.

Rats were obtained from the animal house belonging to the National Center for Radiation Research and Technology "NCRRT", Atomic Energy Authority, Egypt.

Animals were housed in especially designed cages, six females per cage. The males were kept separated from females until mating.

All rats were kept under normal conditions of light, temperature and relative humidity and provided with normal diet pellets and drinking tap water during the whole experimental period.

The diet was purchased from the Factory of Oil and Soap Company, Cairo as well as some vegetables as a source of vitamins. During mating females were housed with males 2:1. Care and cleaning were important for maintaining the rats in good healthy state.

2- Radiation source:

Irradiation was performed by Gamma cell-40 (Cesium 137) located at the National Center for Radiation Research and Technology "NCRRT", Atomic Energy Authority, Cairo, Egypt. The Gamma - cell 40 (Cesium 137) irradiation unit manufactured by using the Atomic Energy of Canada Limit.

The dose rate of the Cesium 137 unit was 0.48 Gray / min at the time of the experimentation.

Pregnant rats were whole body irradiated at a single dose of 2Gy gamma rays in the 7^{th} or 14^{th} day of gestation and sacrificed on day 20 of gestation (1 day prior delivery).

4- Mating:

The females were marked and housed six per cage. Vaginal smears were recorded daily. The female completed at least two consecutive estrus cycles before use.

Females of proestrus and estrus periods were placed with fertile males at the ratio 2:1. Next morning, pregnancy was assured by the presence of vaginal plug that persists for 18-24 hours after which it falls. In the absence of vaginal plug, a drop from vaginal contents was prepared and examined under the microscope for the presence of spermatozoa. The presence of spermatozoa in smears confirmed that mating had taken place and that day was taken as the first day of pregnancy **(Eda *et al.*, 2009).**

Also the pregnancy was ascertained by the daily examination of vaginal smears **(Ramadan, 2007b).** Confirmed pregnant females were randomly assigned to control and treated groups.

The gestation period in the pregnant rats was 21 day. Pregnancy was followed up by gradual increase in maternal body weights, a sudden decrease of their weight and /or presence of blood drops were considered as sings of abortion. The females that didn't mate within two estrus cycle were excluded from the study **(Wilkinson, 2000).**

5- Bone marrow transplantation:

Donors and recipients were chosen from the inbred strain. Sisters to sisters (isologous or syngenic transplantation). The donors were sacrificed by cervical dislocation.

Femur bones were dissected out, cleaned and both ends were chipped by bone nibbling forceps. Then the marrow was blown of the femur into saline solution under sterilized conditions by compressed air from a 10 ml syringe fitted at one end of the shaft (**Ashry *et al*., 2009).**

The bone marrow was collected into sterile containers surrounded by ice cubes, and mixed by drawing and expelling it several times from the syringe without needle in order to avoid mechanical damage to the cells.

6- Groups of animals:

Five groups of pregnant rats were used in the present experiment. Each group consisted of 6 animals

The animals were divided into the following groups:

1- A normal untreated control pregnant rats. The rats were sacrificed on day 20 of gestation.

2- A group of pregnant female rats exposed to 2 gray of gamma rays on day 7 of gestation (late implantation and early organogenesis) and sacrificed on day 20 of gestation (R_7).

3- A group of pregnant female rats exposed to 2 gray of gamma rays on day 7 of gestation and received bone marrow transplantation (BMT) 75 $\times 10^6 \pm 5$ cells by intraperitoneal injection one hour post-irradiation and sacrificed on day 20 of gestation (R_{7+BM}).

4- A group of pregnant female rats exposed to 2 Gray of gamma rays on day 14 of gestation (organogenesis) and sacrificed on day 20 of gestation (R_{14}).

5- A group of pregnant female rats exposed to 2Gy of gamma rays on day 14 of gestation and treated with bone marrow transplantation (BMT) one hour post- irradiation and sacrificed on day 20 of gestation (R_{14+BM}).

The Histological and histochemical studies :

Following pregnant rats sacrifice, small pieces of fetal oesophagus, stomach and ileum were quickly removed and fixed in 10% neutral buffer formol and Carnoy's fluid for the histological and histochemical studies. Specimens were washed and dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax.

Sections were then cut at 5µ thickness and stained by haematoxylin and eosin stain according to the method of **Drury and Wallington (1980)**, Periodic acid Schiff technique for demonstrating polysaccharides in tissues **(Pearse, 1977).** Mercuric bromophenol blue method for detecting total protein **(Mazia *et al*., 1953).** Mallory's trichrome stain for demonstrating collagen fibers **(Pearse, 1977).**

Image analysis: The optical density (pexil) of total protein and PAS+ve materials were analyzed by using image pro. Program .

Results

-Histopathological and histochemical observations of embryos oesophagus.

The oesophageal wall of the control fetal oesophagus (Fig. 1) is formed of the four layers mucosa, submucosa, submucosa, muscularis and serosa). The mucosa layer consists of a-epithelium (a layer of stratified squamous non- keratinized epithelium), b-corium (a layer of connective tissue containing blood vessels and mucous glands near the stomach) c -muscularis mucosa (formed of smooth fibers, which are arranged as inner circular and outer longitudinal layers). The Submucosa layer is a layer of loose connective tissue containing blood vessels, lymph vessels and scattered mucous glands. The musclaris layer consists of muscle fibers arranged in inner circular and outer longitudinal pattern. The serosa is the outer thin layer.

Increased proliferation in the mucosal layer in addition to signs of lymphocytic infiltration within

muscularis and submucosa layers were detected in the fetal oesophagus of gamma irradiated on 7 day of gestation group (R7) (Fig. 2).

The hypertrophied mucosal layer covered with horny layer was detected in some areas of the fetal esophagus of gamma irradiated and bone marrow transplantation group (R7+BM). The submucosa was occupied by some signs of lymphocytes and small hemorrhagic areas (Fig. 3). Also highly thickened and distorted longitudinal and circular muscle fibers were detected in the same figure.

Fetuses taken from gamma irradiated on day 14 of gestation (R14)showed many dystrophic changes in the oesophagus. These changes include: malformed oesophgeal tissue with loss of its normal architecture accompanied with delaminated mucosal layer, increased signs of lymphocytic infiltration,presence of numerous pyknotic nuclei and highly distorted circular muscle fibers (Fig.4).

Figure (5) illustrated incomplete healing of the fetal esophagus maternally irradiated with gamma rays on day 14 of gestation and received bone marrow (R14+BM). Obvious return to somewhat normal appearance ,but increased proliferation of mucosal layer, lymphocytic infiltrations and some degenerated small areas were still detected.

Thin collagen bundles are surrounding the mucosal layer and serosa of the fetal oesophagus of the control group (Fig.6). Some collagen bundles are distributed throughout the submucosa and muscularis layers. Increased collagen fibers were detected in the oesophagus of fetuses of all the treated groups (Figs.7-10).

Also some small hemorrhagic areas could be detected in the mucosa and muscularis layers in the previous figures.

Fig. (11) Showing normal distribution of PAS +ve materials in the fetal oesophagus of the control group with dense stain affinity in the mucosal cells and muscle fibers of muscularis.

The mucosa layer of the fetal oesophagus of group R7 showed poor stain affinity of PAS +ve materials (Fig.12).

Reduced stain affinity of PAS +ve materials was detected in the thickened mucosal layer of the fetal oesophagus of groups R7+BM and R 14+BM, while submucosa and muscularis showed moderate stain affinity (Figs.13; 15).

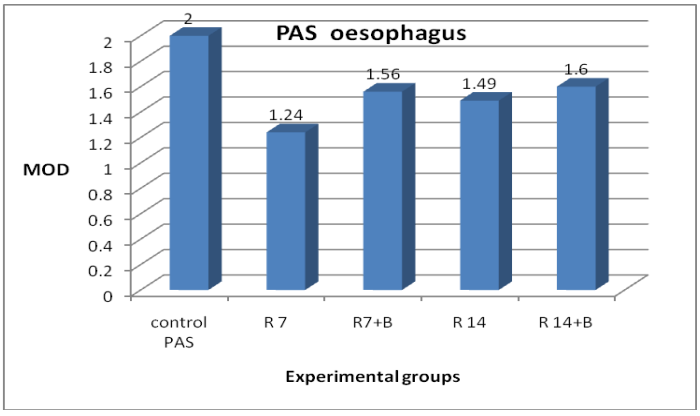
Figure (14) showing malformaed layers of the fetal oesophagus from group R14 with reduced stain affinity of PAS +ve materials. Decreased PAS +ve materials was also detected by the mean optical density (MOD), since MOD values reached (1.24± 0.08), (1.56±0.09), (1.49±0.21) and (1.60 ± 0.07) in R7, R7+BM, R14, R14+BM respectively compared with the control group (2.00 ± 0.19) as shown in table (1) & histogram (1).

Normal distribution of total protein in the fetal oesophagus of the control group was observed in fig. (16), Oesophagus of fetuses of all the treated groups showed increased stain affinity of total protein, this increase was also detected by increased MOD (Figs. 17-20). The MOD values reached (1.11± 0.06), (1.56±0.09), (1.28±0.27) and (1.41 ± 0.08) in R7, R7+BM, R14, R14+BM respectively compared with the control group (1.08 ± 0.13) as shown in table (1) & histogram (2).

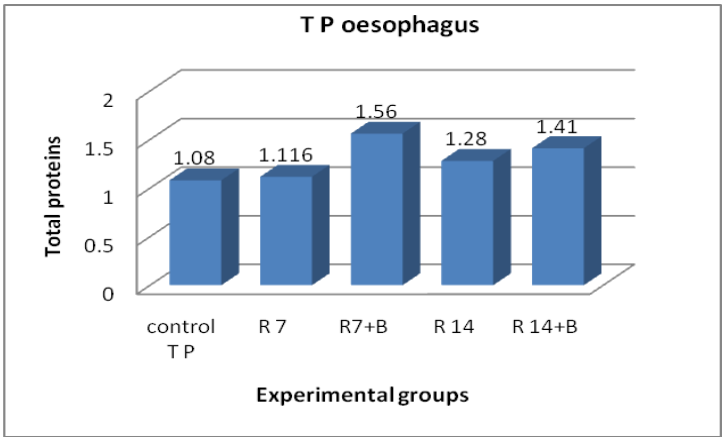
Table (1): Revealing MOD values of PAS +ve materials, total protein in oesophagus of the control and treated groups

	Esophagus Mean ±SD				
PAS +ve materials	**control**	**R7**	**R7+BM**	**R14**	**R14+BM**
	2.00 ± 0.19	1.24± 0.08	1.56±0.09	1.49±0.21	1.60 ± 0.07
Total protein	**control**	**R7**	**R7+BM**	**R14**	**R14+BM**
	1.08 ± 0.13	1.11±0.06	1.15±0.06	1.28±0.27	1.41 ± 0.08

Histogram (1): Revealing MOD values of PAS +ve materials in oesophagus of the control and treated groups



Histogram (2): Revealing MOD values of total protein in oesophagus of the control and treated groups



Stomach: Different layers of the control fetal stomach was detected in figure. (21). Control fetal stomach is consists of fundic and pyloric portions. The present study was carried on the fundic part which is consisting of: 1- Fundic mucosa (which contains: the surface epithelium, lamina propria and muscularis mucosa). 2- Submucosa (consists of loose connective tissue that contains blood vessels, nerve endings and lymphatics).3-Musculosa (which is formed of small muscle fibers arranged in three layers: oblique, middle circular and outer longitudinal).4-Serosa (contains loose connective tissue with blood vessels, nerves and it is covered by simple squamous epithelium).

Fetal stomach of group R7 showed many changes. Numerous lymphocytes with signs of hemorrhagic areas in the submucosa were detected (Fig.22) These changes were more pronounced in group R14 in addition to numerous pyknotic nuclei , highly degenerated and malformed fetal stomach layers were detected (Fig.24). The delaminated mucosal layer appeared highly corrugated and ruptured and contained numerous degenerated areas as shown in the same figure.

Figure (23) showing vacuolated columnar cells of the mucosal layer of fetal stomach of group R7+BM.,while figure (25) exhibiting incomplete healing of fetal stomach maternally irradiated with gamma rays on 14 day of gestation and received bone marrow (R14+BM), although some lymphocytes and degenerated areas were still detected. Concerning collagen fibers, the control fetal stomach contained thin collagen layer encircling the mucosal layer and small patches are distributed in the submucosa and muscularis layers (Fig.26). Increased collagen fibers were detected in the different layers of the fetal stomach of all the treated groups (Figs.27-30). These collagenous fibers were more pronounced in group R14 (Fig.29).

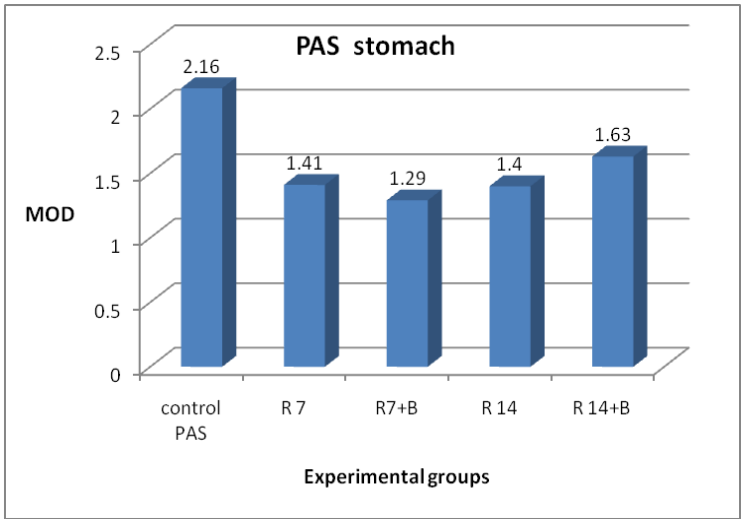
Figure (31) showing dense stain affinity of PAS +ve materials in numerous goblet cells of the mucosal layer of the control fetal stomach, also serosa accquired a dark stain affinity with moderate stain affinity in submucosa and muscularis layers. Decreased stain affinity of PAS +ve materials was noticed in the different layers of the fetal stomach of all the treated groups (Figs.28-33), but few deeply stained goblet cells where detected (Figs.32;35).This decrease was realized by MOD values,since the values of MOD (mean optical density) reached 1.41±0.10; 1.29±0.22; 1.40±0.35 and1.63 ± 0.09 in R7, R7+BM, R14, R14+BM groups respectively compared with the control group (2.16±0.16) as shown in table (2) & histogram (3).

Normal distribution of total protein in the stomach of a control fetus could be detected in figure. (36). Increased stain affinity of total protein was realized in the fetal stomach of all the treated groups (Figs.37-40). Degenerated areas were negatively stained (Fig.39) in group R14.These results were supported by MOD values. The values of MOD (mean optical density) reached 1.23±0.05; 1.05±0.09; 1.14±0.24and 1.37 ± 0.07 in R7, R7+BM, R14, R14+BM groups respectively compared with the control group (0.09±0.12) as shown in table (2) & histogram (4).

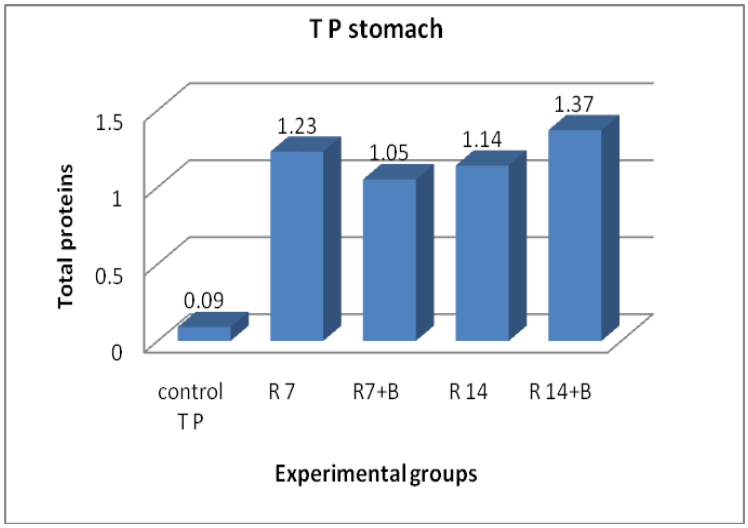
Table (2): Revealing MOD values of PAS +ve materials, total protein in stomach of the control and treated groups

	Stomach Mean ±SD				
PAS	**Control**	**R7**	**R7+BM**	**R14**	**R14+BM**
	2.16±0.16	1.41±0.10	1.29±0.22	1.40±0.35	1.63 ± 0.09
Proteins	**Control**	**R7**	**R7+BM**	**R14**	**R14+BM**
	0.09±0.12	1.23±0.05	1.05±0.09	1.14±0.24	1.37 ± 0.07

Histogram (3): Revealing MOD values of PAS +ve materials in stomach of the control and treated groups



Histogram (2): Revealing MOD values of total protein in oesophagus of the control and treated groups



Ileum: Normal histological structure of the control fetal ileum was observed in figure. (41).The wall of the small intestine is formed of four layers: 1-mucosa which consists of inner part which contains villi and outer part that contains folds surrounded by connective tissue which called crypts. 2-submucosa which contains lymphoid aggregations which are known as Peyer's patches. 3-musculosa which consists of double layers of smooth muscle fibers arranged as inner circular and outer longitudinal layers.4-serosa formed by the peritoneal covering of the small intestine loose connective tissue containing blood vessels, nerves and lymphatics. Fetuses of group R7 showed ileum with distorted and ruptured circular and longitudinal muscle fibers. Columnar epithelial cells of the mucosal layer are hardly detected and the lamina propria occupied by numerous lymphocytes and RBCs (Fig.42).

Fetuses of group R7+BM showed ileum with highly elongated villi or atrophied villi (Fig. 43). Also numerous degenerated areas were observed in the lamina propria as shown in the same figure. Intensive malformed architecture of the ileal tissue from fetus maternally irradiated with gamma rays on day 14 of gestation was observed (Fig.44). Also numerous lymphocytes and lots of deeply stained nuclei (pyknotic) were observed in the different layers. Distorted and ruptured circular and longitudinal muscle fibers of ileum muscularis were realized (Fig.44).

Fetuses of group R14+BM showed ileum with incomplete healing ,whereas some villi were enlarged with increased signs of exofoliation and some columnar cells were ruptured. Few villi appeared with erotid tips and other villi were highly atrophied (Fig.45).

Figure (46) showing normal distribution of collagen fibers in the control fetal ileum. Increased collagen bundles were detected in the fetal ileum tissue of all the treated groups (Figs.47-50) while group R14+BM showed somewhat moderate increase in the collagen fibers (Fig.50). Also numerous hemorrhagic areas were detected in the different layers of the ileum (Figs.47& 48).

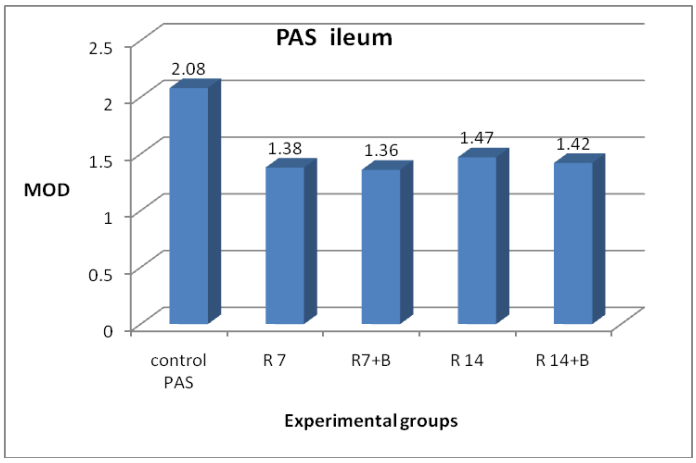
Well developed PAS + ve materials were detected in the ileum of a control fetus with dense stain affinity in the goblet cells (Fig. 51). Decreased stain affinity of PAS + ve materials was observed in the fetal ileum tissue of all the treated groups (Figs.52-55), but the goblet cells accquired deep stain affinity (Figs.52&53).These results are in accordance with MOD values which reached 1.38±0.10; 1.36±0.14; 1.47±0.26 and 1.42±0.09 in R7, R7+BM, R14, R14+BM groups respectively compared with the control group (2.08±0.06) as shown in table (3) & histogram (5).

Normal distribution of total protein in the control fetal ileum was detected in fig. (56). Increased in stain affinity of total protein was demonstrated in the fetal ileum tissue of all the treated groups (Figs.57-60), but the degenerated areas were faintly stained (Fig.59). The values of MOD (mean optical density) reached 1.40±0.12; 1.33±0.06; 1.26±0.06 and 1.28 ± 0.08 in R7, R7+BM, R14, R14+BM groups respectively compared with the control group 1.12±0.05 as shown in table (3) & histogram (6).

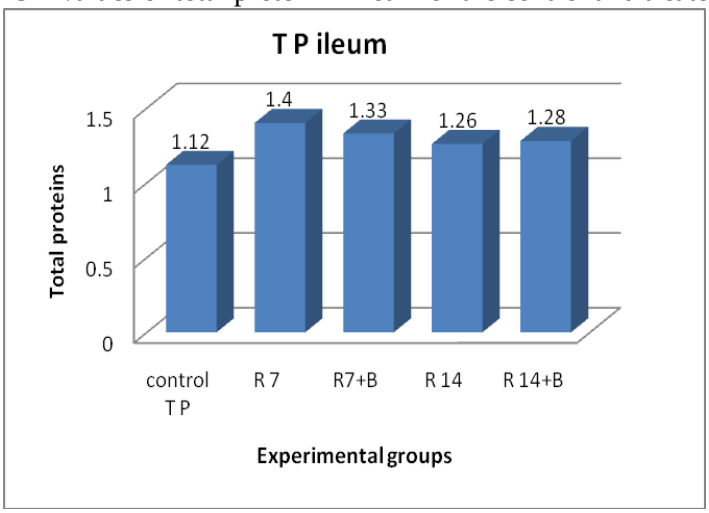
Table (3): Revealing MOD values of PAS +ve materials, total protein in ileum of the control and treated groups

	Ileum Mean ±SD				
PAS+ve materials	**control**	**R7**	**R7+BM**	**R14**	**R14+BM**
	2.08±0.06	1.38±0.10	1.36±0.14	1.47±0.26	1.42 ± 0.09
Total protein	**control**	**R7**	**R7+BM**	**R14**	**R14+BM**
	1.12±0.05	1.40±0.12	1.33±0.06	1.26±0.06	1.28 ± 0.08

Histogram (5): Revealing MOD values of PAS +ve materials in ileum of the control and treated groups



Histogram (6): Revealing MOD values of total protein in ileum of the control and treated groups



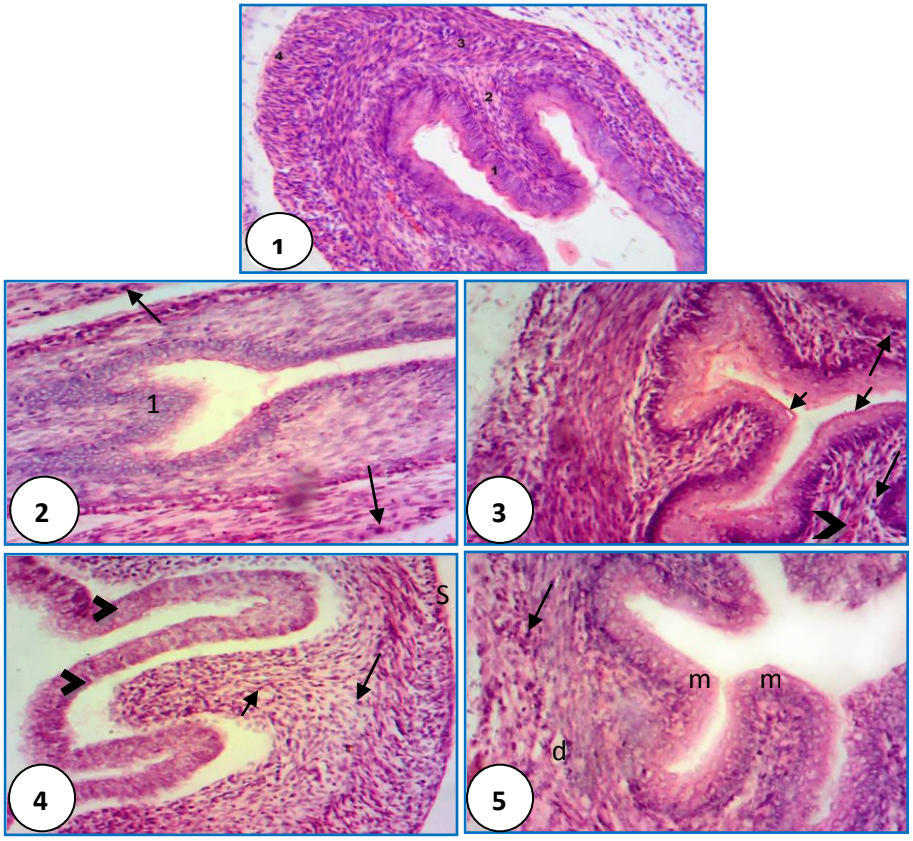


Fig. (1): A photomicrograph showing normal oesophageal structure of control fetus. Notice: 1-mucosa layer,2-Submucosa,3-muscularis,4-serosa. **(H&E×200)**

Fig.(2): Section of fetal esophagus maternally irradiated with gamma rays on 7 day of gestation showing increased proliferation in the mucosal layer(1) and signs of lymphocytic infiltration within muscularis and sub mucosa (arrows). **(H & E X 100).**

Fig.(3): A photomicrograph of fetal esophagus maternally irradiated with gamma rays on day 7 of gestation and received bone marrow showing hypertrophied mucosal layer . This layer covered with horny layer (short arrows) in some parts. The submucosa layer showed signs of lymphocytic infiltration (long arrows) and small hemorrhagic areas (arrow head). Note highly thickened and distorted longitudinal and circular muscle fibers. **(H & E X 100).**

Fig.(4): Transverse section of fetal esophagus maternally irradiated with gamma rays on day 14 of gestation showing malformed oesophgeal tissue with loss of its normal architecture , delaminated mucosal layer (arrow head), increased lymphocytic infiltrations in the submucosa (long arrow) ,highly thickened and disturbed muscularis and numerous pyknotic nuclei (short arrow). S: indicates serosa. **(H & E X 100).**

Fig.(5): A photomicrgraph showing incomplete healing of fetal esophagus maternally irradiated with gamma rays on day14 of gestation and received bone marrow. Obvious return to somewhat normal appearance, but increased proliferation of mucosal layer (m), lymphocytic infiltrations (arrow) and some small degenerated small areas (d) were still detected **(H & E X 100).**

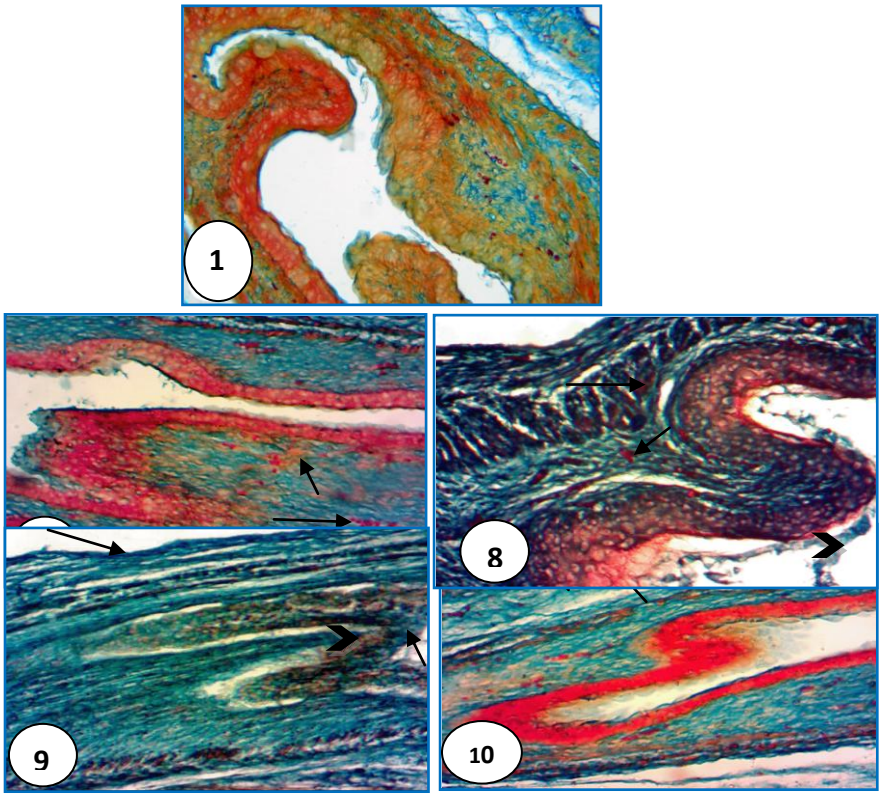


Fig.(6): A photomicrograph showing normal fetal oesophagus with thin collagen bundles surrounding the mucosal layer and serosa. Some of these bundles are distributed throughout the submucosa and muscularis layers. **(Mallory's trichorome stain X 100)**

Fig.(7): A photomicrograph of fetal esophagus maternally irradiated with gamma rays on day 7 of gestation showing increased collagen fibers of the illustrated esophageal layers. Notice numerous small hemorrhagic areas (long arrow with some necrotic areas (short arrow) in the mucosa.

(Mallory's trichorome stain X 100)

Fig. (8): Increased collagenous bundles are still detected throughout fetal esophageal tissue maternally irradiated with gamma rays on 7 day of gestation and received bone marrow .Collagen fibers are surrounding the highly proliferated mucosal layer (arrow head). Also numerous hemorrhagic areas could be detected in the mucosa (short arrows) and muscularis layer (long arrow).

(Mallory's trichorome stain X 100)

Fig.(9): A photomicrograph of fetal esophagus maternally irradiated with gamma rays on 14 day of gestation showing malformed oesophageal tissue showing small hemorrhagic areas (arrow head) which acquired red bright color with thick layers of collagen which are surrounding the mucosa (small arrow) and serosa layers (long arrow), while some bundles are distributed throughout the submucosa and muscularis. (**Mallory's trichorome stain X 100)**

Fig. (10): A photomicrograph showing incomplete healing of fetal esophagus maternally irradiated with gamma rays on 14 day of gestation and received bone marrow. Some collagenous bundles surrounding the mucosa and serosa layers, while thin bundles are randomly distributed throughout the submucosa. Notice: red brightly numerous small hemorrhagic areas in the mucosa (arrows).

(Mallory's trichorome stain X 100)

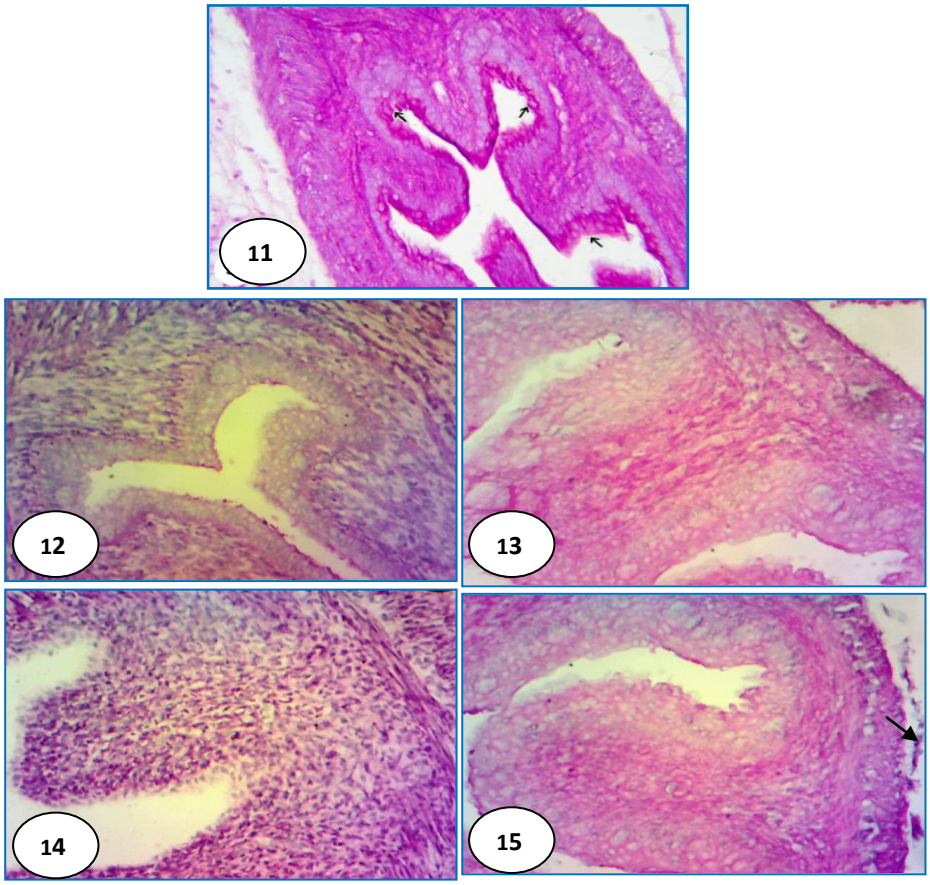


Fig.(11): A photomicrograph showing PAS distribution in the fetal oesophagus of the control group . Notice: the dense stain affinity in the mucous cells (arrows) and muscle fibers of muscularis. **(PAS×200)**

Fig.(12): Showing faintly stained PAS +ve materials in the stratified cells of the mucosa layer of fetal esophagus maternally irradiated with gamma rays on day 7 of gestation. **(PAS × 200)**

Fig.(13): A photograph of fetal esophagus maternally irradiated with gamma rays on day 7 of gestation and received bone marrow showing thickened mucosal layer with reduced stain ability of PAS +ve materials, while submucosa and muscularis showed moderate stain affinity. **(PAS × 200)**

Fig.(14): A photograph of fetal esophagus maternally irradiated with gamma rays on 14 day of gestation showing intensive malformed oesophageal tissue with reduced stain affinity of PAS +ve materials in the different layers. **(PAS × 200)**

Fig.(15): A photograph showing fetal esophagus maternally irradiated with gamma rays on 14 day of gestation and received bone marrow transplantation showing mucosal layer with reduced stain affinity of PAS +ve materials . Some aggregations of mucous were observed (arrow) beside the serosa. **(PAS × 200)**

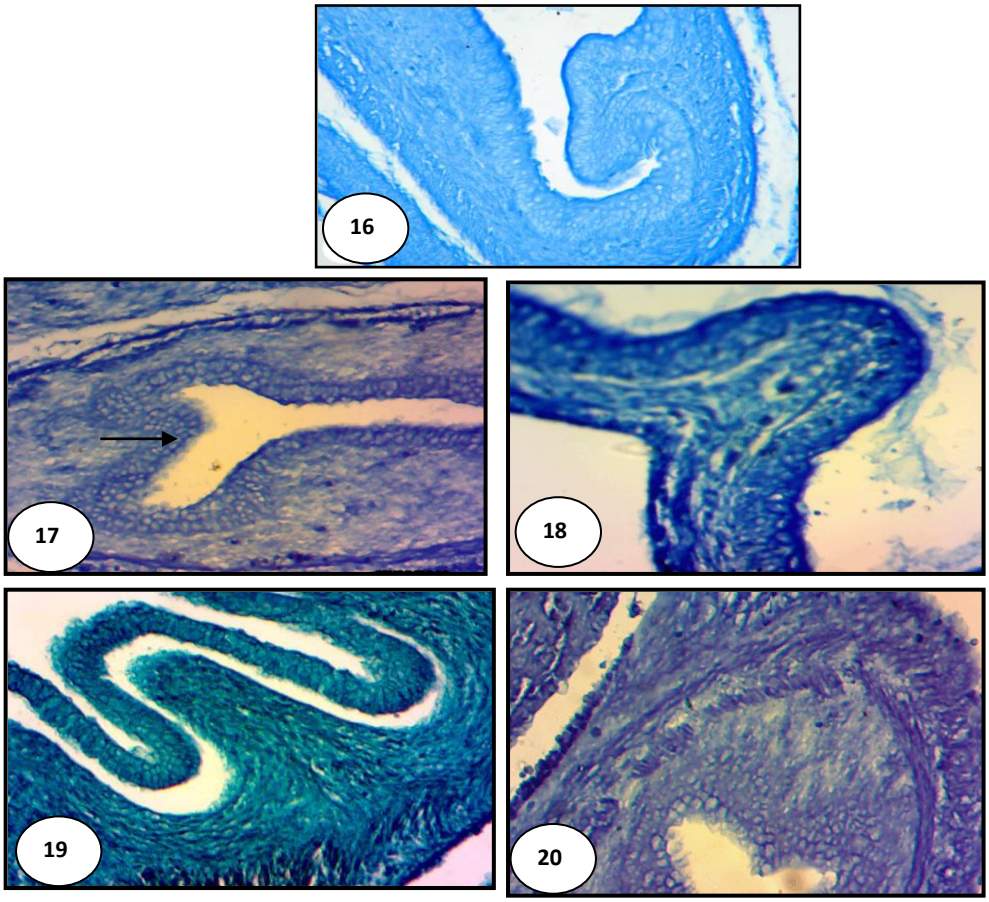


Fig.(16): A photomicrograph showing normal distribution of total protein in the fetal oesophagus of the control group. **(Mercuric bromophenol blue ×200)**

Fig.(17): Showing increased total protein in the different layers of fetal esophagus maternally irradiated with gamma rays on 7 day of gestation with increased proliferation in the mucosal layer (arrow) **(Mercuric bromophenol blue ×200)**

Fig.(18): A photomicrograph showing fetal esophagus maternally irradiated with gamma rays on 7 day of gestation and received bone marrow .Notice deeply stain affinity of total protein in the illustrated layers. **(Mercuric bromophenol blue ×200)**

Fig. (19): A photomicrograph of fetal esophagus maternally irradiated with gamma rays on 14 day of gestation showing delaminated mucosal layer of the oesophageal tissue with increased stain affinity of total protein. **(Mercuric bromophenol blue ×200)**

Fig.(20) : A photomicrograph of fetal esophagus maternally irradiated with gamma rays on 7 day of gestation and received bone marrow showing increased stain affinity of total protein in the submucosa and thickened mucosal layer,while expanded muscularis was deeply stained. **(Mercuric bromophenol blue ×200)**

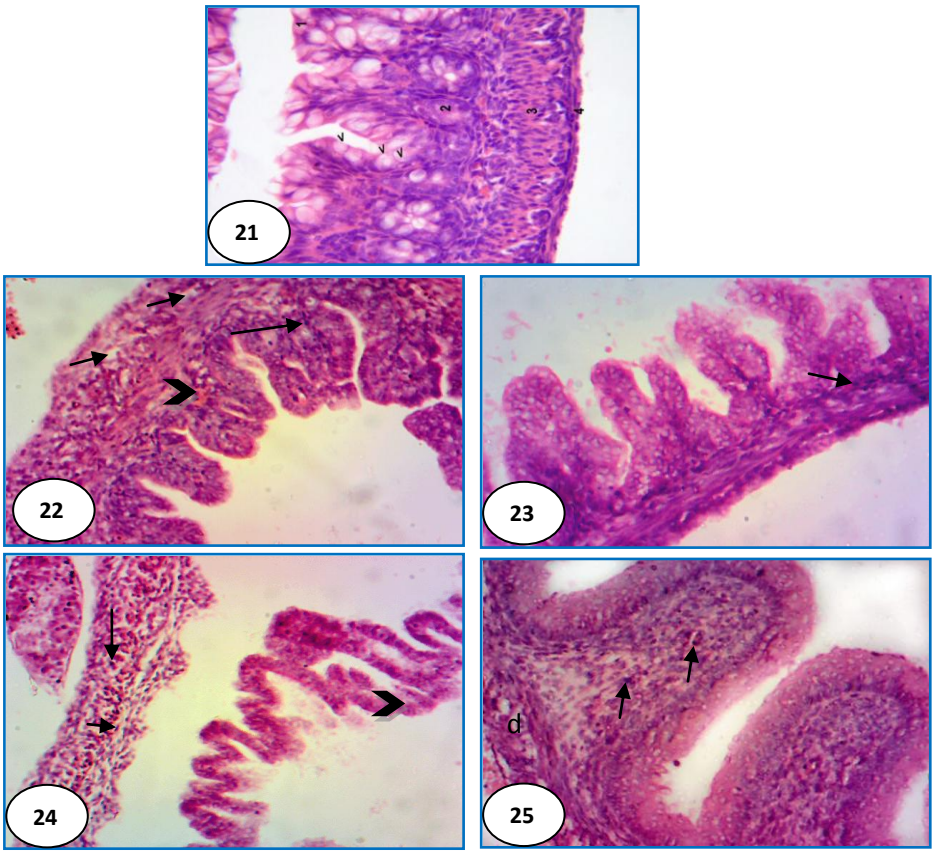


Fig. (21): A photomicrograph showing well developed stomach of control fetus with the different layers which including, 1-mucosa with well developed mucus cells (arrow head), 2-sub mucosa, 3- muscularis, 4-serosa. **(H&E×200)**

Fig.(22): A photograph of fetal stomach maternally irradiated with gamma rays on day7 of gestation showing degenerated muscularis (short arrows), numerous lymphocytes (long arrows) and some hemorrhagic areas in the submucosa (arrow heads). **(H&E×200)**

Fig.(23): A photomicrograph of fetal stomach maternally irradiated with gamma rays on day7 of gestation and received bone marrow showing vacuolated columnar cells of the mucosal layer . Arrow indicates lymphocytic infiltration. **(H&E×200)**

Fig.(24): A photomicrograph showing fetal stomach maternally irradiated with gamma rays on day 14 of gestation showing highly degenerated and malformed stomach layers. The delaminated mucosal layer appeared corrugated and ruptured (arrow heads). Note numerous lymphocytes (long arrows) and pyknotic nuclei (short arrows). **(H&E×200)**

Fig.(25): incomplete healing of fetal stomach maternally irradiated with gamma rays on 14 day of gestation and received bone marrow . Some lymphocytes (arrows) and degenerated areas (d) could be detected. **(H&E×200)**

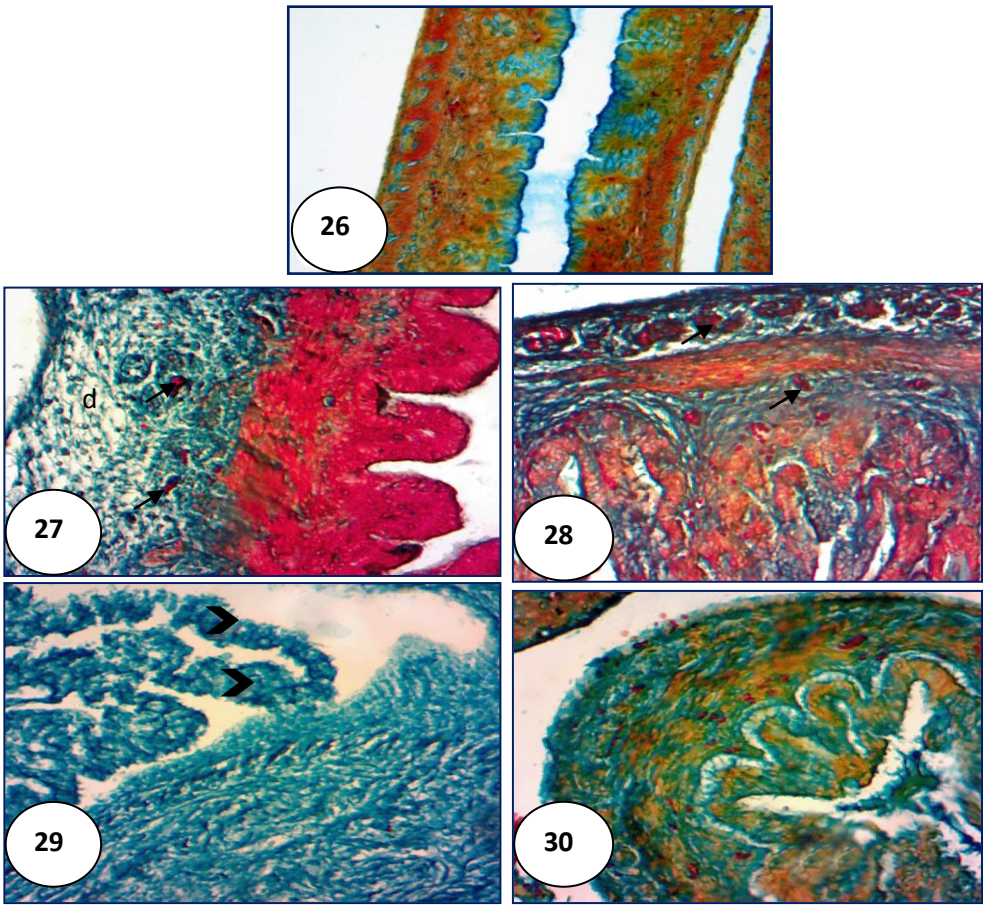


Fig.(26): A photomicrograph showing normal distribution of collagen fibers in the control fetal stomach. Notice: the collagen layer that encircles the mucosal layer with small patches disturbed throughout the submucosa and muscularis layers. **(Mallory's trichorome stain ×200)**

Fig.(27): Showing increased collagenous fibers in highly affected layers of the fetal stomach maternally irradiated with gamma rays on 7 day of gestation. Notice degenerated areas (d) in the muscularisa and some hemorrhagic areas in the submucosa (arrows). **(Mallory's trichorome stain ×200)**

Fig.(28): Showing increased collagenous fibers in the fetal stomach maternally irradiated with gamma rays on 7 day of gestation and received bone marrow. Notice: brightly red stained hemorrhagic areas (arrows) in most layers of the tomach.

(Mallory's trichorome stain ×200)

Fig.(29): Showing increased collagenous fibers in the malformed fetal stomach maternally irradiated with gamma rays on 14 day of gestation. Notice: highly corrugated and delaminated mucosal layer (arrow heads). **(Mallory's trichorome stain ×200)**

Fig.(30): Showing increased collagen fibers with numerous brightly red stained hemorrhagic areas in the fetal stomach maternally irradiated with gamma rays on 14 day of gestation and received bone marrow. **(Mallory's trichorome stain ×200)**

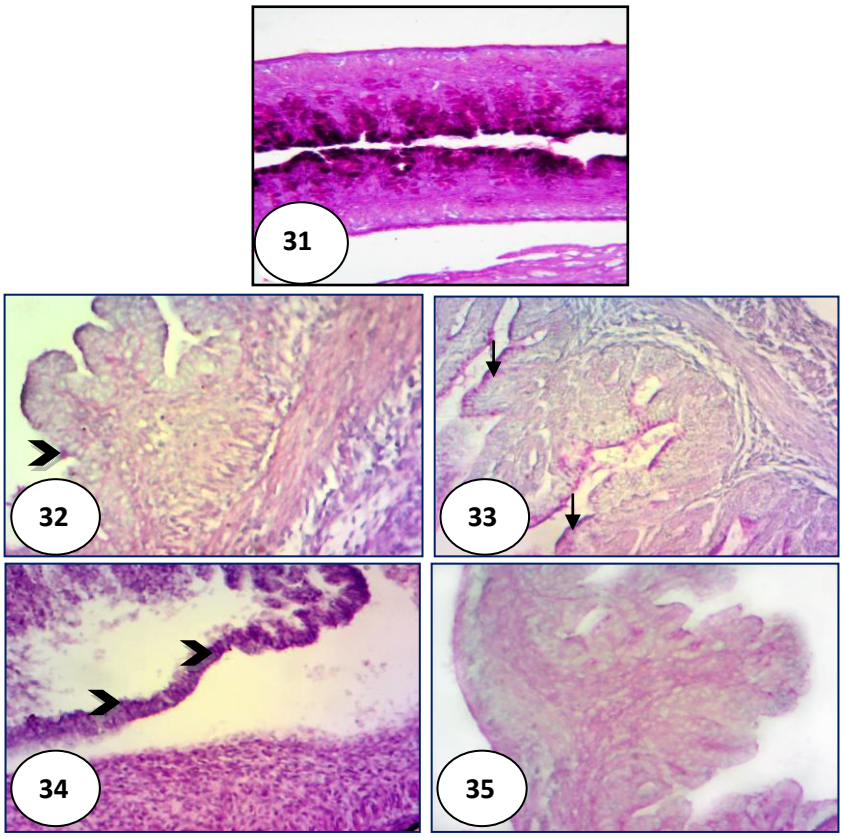


Fig.(31): Showing normal distribution of PAS +ve materials in the control stomach with dense stain affinity in the numerous goblet cells of the mucosal layer, also serosa acquired dark stain affinity**. (PAS×200)**

Fig.(32): Showing faintly stained PAS +ve materials in the stratified cells of the different layers of fetal stomach maternally irradiated with gamma rays on day 7 of gestation. Few goblet cells appeared deeply stained (arrow head). **(PAS×200)**

Fig.(33): Showing reduced stain affinity of PAS +ve materials in the fetal stomach layers maternally irradiated with gamma rays on day 7 of gestation and received bone marrow . Highly affected goblet cells appeared deeply stained (arrows). **(PAS×200)**

Fig.(34): A photomicrograph of fetal stomach maternally irradiated with gamma rays on day 14 of gestation showing intensive malformed stomach tissue with increased stain affinity of PAS +ve materials in the mucosal layer. Notice: highly corrugated and delaminated mucosal layer (arrow head). **(PAS × 200)**

Fig.(35): A photograph of fetal stomach maternally irradiated with gamma rays on 14 day of gestation and received bone marrow showing decreased stain affinity of PAS +ve materials in the different layers. **(PAS × 200).**

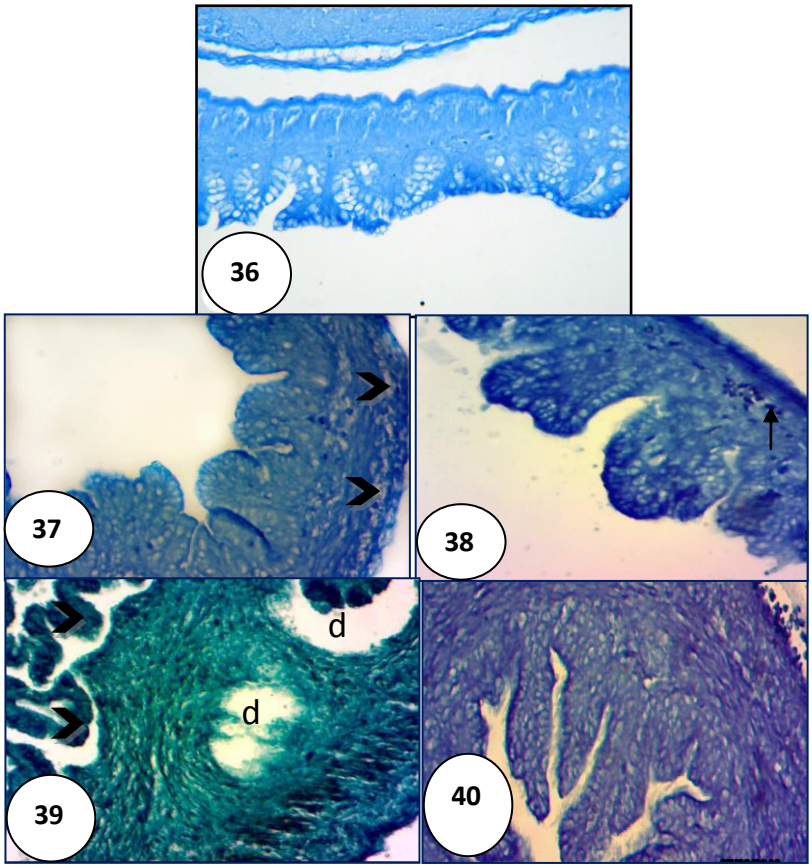


Fig.(36): A photomicrograph showing normal distribution of protein in the stomach of a control fetus.
(Mercuric bromophenol blue ×200)

Fig.(37): Showing increased total protein in the different layers of fetal stomach maternally irradiated with gamma rays on 7 day of gestation. Notice degenerated areas were negatively stained (arrow heads)

(Mercuric bromophenol blue ×200)

Fig.(38): A photomicrograph of fetal stomach maternally irradiated with gamma rays on day 7 of gestation and received bone marrow showing increased total protein in the different layers with few deeply stained RBCs (arrow).

(Mercuric bromophenol blue ×200)

Fig.(39): Showing increased total protein in the highly malformed layers of fetal stomach maternally irradiated with gamma rays on 14 day of gestation. Note degenerated areas were negatively stained (d). The delaminated mucosal layer (arrow heads) was deeply stained.

(Mercuric bromophenol blue ×200)

Fig.(40): Showing increased total protein in the different layers in the fetal stomach maternally irradiated with gamma rays on day 14of gestation and received bone marrow .

(Mercuric bromophenol blue ×200)

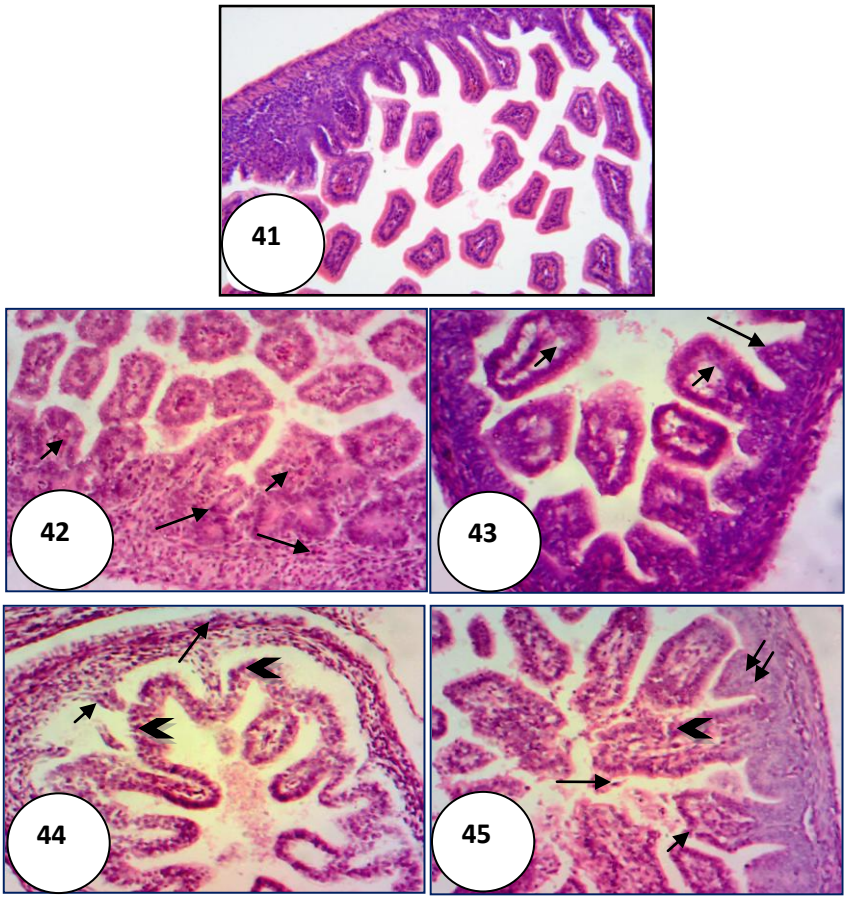


Fig. (41): A photomicrograph showing normal histological structure of the fetal ileum taken from the control group. Notice: Payer's patchs in the sub-mucosa layer. **(H&E×200)**

Fig.(42): A photomicrgraph of the fetal ileum maternally irradiated with gamma rays on 7 day of gestation showing highly distorted layers of the tissue especially circular and longitudinal muscle fibers of the muscularis layer. Columnar epithelial cells of the mucosal layer are hardly detected. The lamina propria occupied by numerous lymphocytes (long arrows) and RBCs (short arrows). **(H&E×200)**

Fig.(43): Showing highly enlarged abnormal villi with numerous goblet cells were detected in fetal ileum maternally irradiated with gamma rays on 7 day of gestation and received bone marrow transplantation. Some villi were highly atrophied (long arrow). Notice numerous degenerated areas were observed in the lamina propria(short arrow) and many deeply stained nuclei were observed . **(H&E×200)**

Fig.(44): Showing intensive malformed architecture of the ileal tissue from a fetus maternally irradiated with gamma rays on day 14 of gestation. Most cells appeared with degenerated cytoplasm, pyknotic nuclei (short arrow). Also, numerous lymphocytes (long arrow) were detected with highly corrugated and delaminated mucosal layer from the under lining lamina propria (arrow heads). **(H&E×200)**

Fig.(45): A photomicrograph showing incomplete healing of the fetal ileum maternally irradiated with gamma rays on 14 day of gestation and received bone marrow. Some villi were enlarged with increased signs of exofoliation (long arrow). Some columnar cells were ruptured (arrow head) and some villi appeared with erotid tips (short arrow). Other villi were highly atrophied (double arrows).

(H & EX 100).

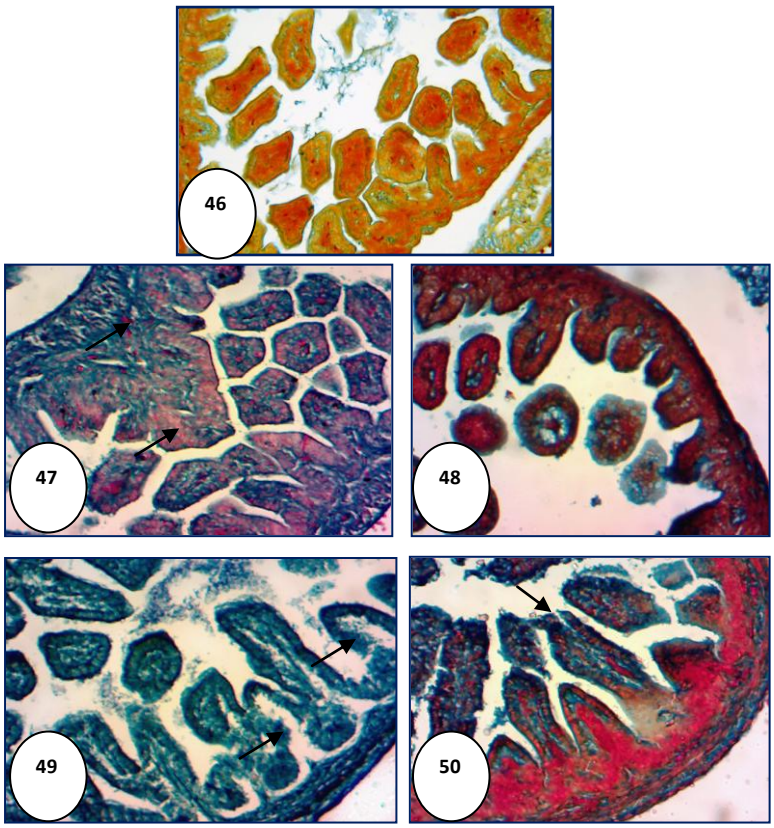


Fig.(46): A photomicrograph showing normal distribution of collagen fibers in the control fetal ileum. **(Mallory's trichorome stain ×200)**

Fig.(47): A photomicrograph of the fetal ileum maternally irradiated with gamma rays on day7 of gestation showing increased collagen fibers in all the different layers especially in the muscle layer. Notice: numerous hemorrhagic areas (arrows) in the different layers of the ileum. **(Mallory's trichorome stain ×200)**

Fig.(48): Showing moderate increase in the collagen bundles in the fetal ileum maternally irradiated with gamma rays on 7 day of gestation and received bone marrow. Notice numerous hemorrhagic areas in the different layers. **(Mallory's trichorome stain ×200)**

Fig.(49): A photograph of fetal ileum maternally irradiated with gamma rays on 14 day of gestation showing highly increased collagen fibers. Notice: highly degenerated villi and ruptured mucosal and submucosal layers with detachment of the epithelial structure from the underlining lamina propria (arrows). **(Mallory's trichorome stain ×200)**

Fig.(50): A photograph of the fetal ileum maternally irradiated with gamma rays on day 14of gestation and received bone marrow showing somewhat moderate increase in the collagen fibers. Notice highly elongated villi with signs of erosion (arrow). **(Mallory's trichorome stain ×200)**

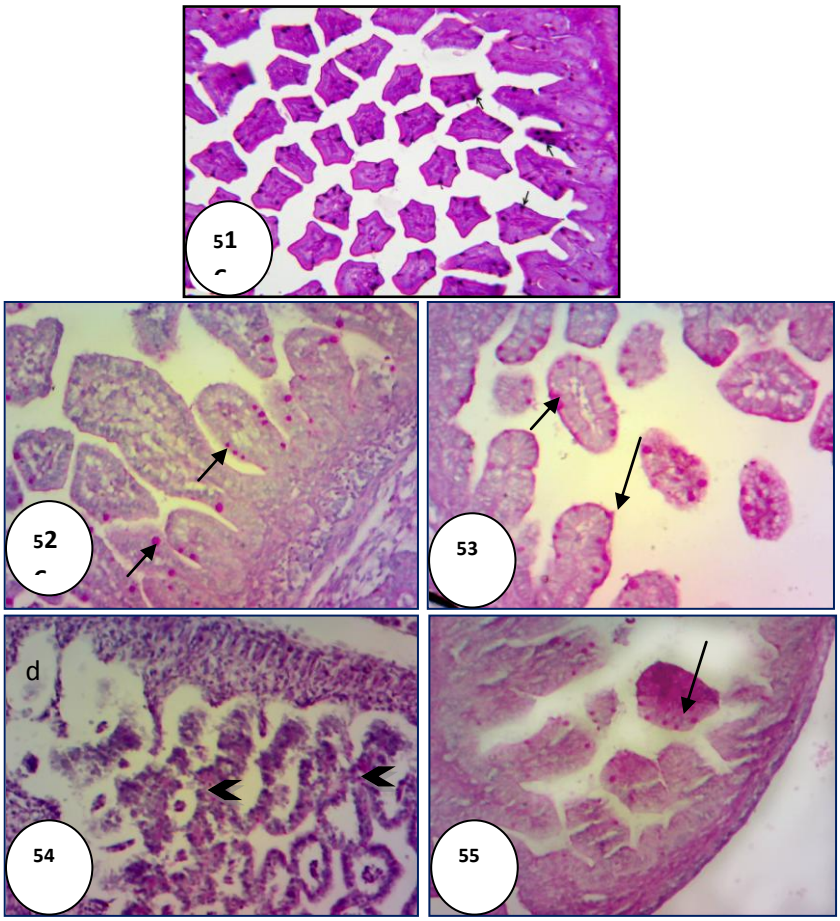


Fig.(51): A photomicrgraph showing normal distribution of PAS +ve materials in ileum of the control fetus. Notice: dense reaction in the goblet cells (arrows). **(PAS ×200)**

Fig.(52): Showing faintly stained PAS +ve materials in the different layers of the fetal ileum maternally irradiated with gamma rays on day7 of gestation. Notice: deeply stained goblet cells in some villi (arrows) **(PAS × 200)**

Fig.(53): Showing reduced stain affinity of PAS +ve materials in the fetal ileum maternally irradiated with gamma rays on day7 of gestation and received bone marrow. Notice dense reaction in the goblet cells of most villi (arrows). **(PAS × 200)**

Fig.(54): Showing somewhat reduced stain affinity of PAS +ve materials in the fetal ileum maternally irradiated with gamma rays on day 14 of gestation. Notice: highly malformed ileal tissue,negatively stained degenerated areas (d) and delaminated mucosal layer (arrow heads). **(PAS × 200)**

Fig.(55): Showing decreased stain affinity of PAS +ve materials in the fetal ileum maternally irradiated with gamma rays on day 14 of gestation and received bone marrow . Notice :dense reaction in some villi (arrow). **(PAS × 200)**

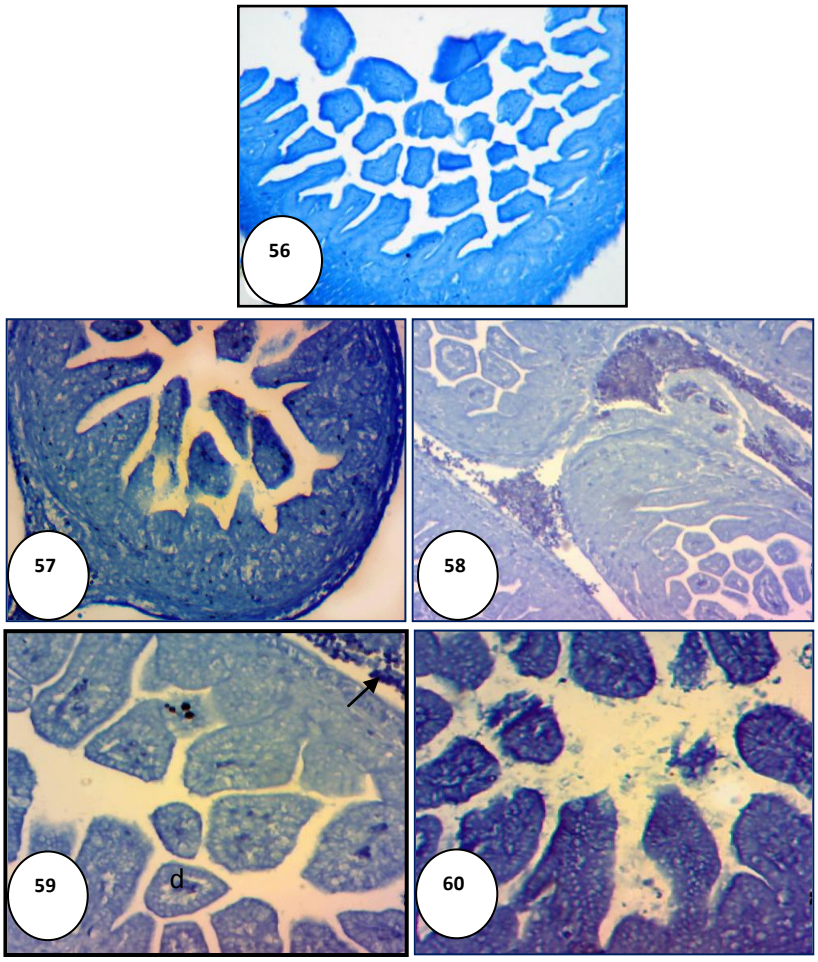


Fig.(56): A photomicrograph showing normal distribution of total protein in the control fetal ileum.
(Mercuric bromophenol blue ×200)

Fig.(57): Showing highly increased total protein in the different layers of the fetal ileum maternally irradiated with gamma rays on day 7 of gestation. (**Mercuric bromophenol blue ×200)**

Fig.(58): A photomicrograph of fetal ileum maternally irradiated with gamma rays on day 7 of gestation and received bone marrow showing somewhat increased total protein in the different layers.
(Mercuric bromophenol blue ×200)

Fig.(59): A photomicrograph of the fetal ileum maternally irradiated with gamma rays on day 14 of gestation showing a slight increase in stain affinity of total protein. Degenerated areas (d) appeared negatively stained and RBCs appeared deeply stained (arrows) . **(Mercuric bromophenol blue ×200)**

Fig.(60): Showing increased stain affinityof total protein in the different layers of the fetal ileum maternally irradiated with gamma rays on day 14 of gestation and received bone marrow .
(Mercuric bromophenol blue ×200)

Discussion

Humans on earth are exposed to many sources of ionizing radiation. The largest component of man-made background radiation relates to exposures associated with medical diagnosis and treatments. Clinical and pathological studies revealed that radiation therapy can produce significant tissue injury (**Malik *et al.*, 2010).** On the other hand, bone marrow cells suppress immune cell responses and have beneficial effects in various inflammatory-related immune disorders (**Yagi *et al.*, 2010)**. Therapeutic studies for bone marrow transplantation for treatment of radiation injuries and its consequences are not sufficient. Thus, this work investigates the role of bone marrow transplantation against alternations induced by gamma radiations on fetal gastrointestinal tract of pregnant rats.

Exposure to γ-rays during different periods of gestation induced significant developmental toxicity in pregnant female rats and their fetuses. This developmental toxicity included increased resorption and reduced fetal weight **(Ramadan, 2007b).** The degree of damage induced by irradiation depends on the degree of differentiation, state of the cell concerning its cycle, the dose rate and the age of the animal at the time of irradiation (**Salama, 2004).**

In the present study exposure of pregnant rats to 2Gy of γ-rays on day 7 or day 14 of gestation showed many histopathological and histochemical changes in the fetal oesophageal tissues. These changes were more pronounced on day 14 of gestation. The changes include increased proliferation in the mucosal layer, increased signs of lymphocytic infiltration, degenerated areas and pyknotic nuclei in addition to highly distorted circular and longitudinal muscle fibers.

In this respect, **Harikumar and kuttan (2004)** reported that exposure to radiation causes damage to normal mucosal cells lead to rapidly dividing cells of gastrointestinal tract. Also, **Coia *et al*. (1995)** described the late effects of radiation on three gastrointestinal sites, the esophagus, the stomach, and the bowel. Esophageal dysmotility and benign stricture following esophageal irradiation are predominantly a result of damage to the esophageal wall; although mucosal ulcerations were noticed following high-dose of radiation. The major late morbidity following gastric irradiation was gastric ulceration caused by mucosal destruction.

Moreover, the increased signs of lymphocytic infiltration in the tissues were observed post-irradiation were also recorded by several authors on several tissues (**Ali and Haggag, 2006; Abdel Mottaal and Abdel Maguid, 2007; Bakhit, 2010).**

It is clear that generally fetal tissues are more sensitive to γ-rays than the maternal ones and this sensitivity is more pronounced on day 14 of gestation. This sensitivity was discussed by several authors **(Oktem *et al*., 2005; Ittrich *et al*., 2007).**

Exposure to ionizing radiation whether occupational or during radiotherapy led to serious systemic damage to various cellular and sub cellular structures and affected seriously the biological cell membrane **(Salama *et al.*, 2007).**

Ionizing radiation induced delayed destabilization of the genome in the progenies of

surviving cells. This phenomenon, which is called radiation induced genomic instability, is manifested by delayed induction of radiation effects, such as cell death, chromosome aberration, and mutation in the progeny of cells surviving radiation exposure **(Suzuki *et al.*, 2009).**

In the present study bone marrow transplantation post-irradiation improved the fetal oesophageal tissue architecture taken from pregnant rats exposed to 2Gy on day 7 or especially on day 14 of gestation comparable to their severe effects with radiations, but increased proliferation of mucosal layer, some lymphocytic infiltrations and some degenerated small areas were still detected. **Tu *et al*. (2012)** indicated that mesenchymal stem cells can reduce the expression of various inflammatory factors and promote the repair of various tissues and organ injury.

Signs of improvement in many tissues of pregnant rats or their fetuses exposed to different doses of gamma rays and treated with bone marrow were detected by many authors **(Hussein, 2004; Hasan, 2007; Bakhit, 2010).**

In the present study highly increased collagen fibers were detected in fetal oesophageal tissue of the pregnant rats exposed to γ-rays on day 7 or 14 of gestation. In accordance to **Ahmadian *et al*. (2006)** who showed that radiation exposure of rats led to increased collagen in the skin. Moreover increased collagen post-irradiation exposure in the different tissues was detected by several authors in several tissues **(Eid and Al Dossary, 2007; El-Salkh, 2009). George *et al*. (2001)** suggested that decreased synthesis of collagenolytic enzymes might contribute to further accumulation of collagen.

In the present study, increased collagen fibers was noticed in the fetal oesophageal tissue of the pregnant rats exposed to γ-rays on day 7 or 14 of gestation followed by bone marrow transplantation. Similar findings were observed by **Bakhit (2010)** on collagen fibers of fetal hepatic or lung tissues of the pregnant rats exposed to γ-rays on day 14 of gestation followed by bone marrow transplantation.

Concerning polysaccharides, the fetal oesophageal tissue maternally exposed to 2Gy γ-rays on day 7 or day 14 of gestation showed decreased stain affinity. These results are not in accordance with those of **Moustafa and Hafez (1998)** who noticed increased glycogen content in cytoplasm of the fetal liver tissue taken from mothers exposed to 2Gy gamma rays on day 11 of gestation.

Decreased glycogen content in the fetal liver tissue post-irradiation was also noted by **Eid and Al Dossary (2007)**. They postulated this decrease to vacuolation and degeneration in hepatocytes or tissues**.** In the present study oesophageal tissue maternally treated with γ-rays on day 7 or day 14 of gestation followed by bone marrow appeared with reduced stain affinity of PAS +ve materials. In contrast to **Bakhit (2010)** fetal lung tissue taken from mothers exposed to γ-rays on day 7 or day 14 of gestation followed by bone marrow transplantation restored the normal polysaccharides content to the normal level.

In the present study increased stain affinity of total protein content was observed in the fetal oesophagus tissue exposed maternally to γ-rays on

day 7 of gestation. This increase in stain affinity of total protein may be due to increased RBCs , increased collagen fibers or may be due to appearance of the fibrous tissue, but reduced stain affinity of total protein may be due to damaged protein molecules by irradiation.

Bone marrow treatment post-radiation exposure on day 7 or 14 of gestation showed increased total protein content in the fetal eosophageal tissue and deeply stained RBCs. In contrast**,** somewhat normal total protein content was detected in the fetal lung tissue maternally treated with the bone marrow post-irradiation on day 7 of gestation (**Bakhit, 2010)**.

Fetal stomach tissue was more sensitive to γ-rays especially on day 14 of gestation. Numerous lymphocytes, many degenerated areas and numerous hemorrhagic areas were detected in the fetal stomach post-irradiation. Degenerated areas observed in the present study post-irradiation may be due to the direct or indirect effect of radiation. When gamma rays enter biological materials, energy is converted into chemical and heat energy **(Bakhit, 2010).** Also, the gastric irradiation caused gastric mucosal destruction and ulceration **(Coia *et al.* , 1995).** Moreover, the lymphocytic infiltration in the tissues were observed post-irradiation were also recorded by several authors (**Ali and Haggag, 2006; Abdel Mottaal and Abdel Maguid, 2007).** Also, **Al-Dossary (2007)** observed many hemorrhagic areas and many pyknotic nuclei in the epithelial cells in the fetal lung tissue maternally exposed to radiation.

In the present study bone marrow transplantation post-irradiation on day 7 or 14 of gestation showed moderate improvement in the architecture of the fetal stomach tissue, in spite of presence of some lymphocytes and degenerated areas.

Also, **Yagi *et al.* (2010)** reported that histological analysis demonstrated a significant reduction of tissue injury with less apoptotic cells in bone marrow mesenchymal stromal cells treated animals compared to controls and the tissue sections showed significantly less inflammatory cell infiltration compared to control animals

The present results revealed that in case of injury, the stem cells from bone marrow are responsible for tissue regeneration and these cells have unique properties that make them attractive candidates for the treatment of diseases and injuries. Also stem cells can be transplanted to replace non-functional or lost stem cells in tissues to accelerate tissue healing and restore the original function **(Burt *et al.*, 2008).** The regenerative potential of stem cells was studied by several authors **(Ferrari *et al.*, 1998; Pye and Watt , 2001; Kirsch *et al.*, 2010).**

In the present study highly increased collagen fibers were observed in the fetal stomach tissue maternally exposed to γ-rays on day 7 or day 14 of gestation. In accordance with **Bakhit (2010)** highly increased collagen fibers were observed in the fetal lung and hepatic tissues maternally exposed to 2Gy γ-rays on day 7 or day 14 of gestation.

Also increased collagen fibers in the fetal stomach tissue maternally treated with 2Gy γ-rays on day 7 or 14 of gestation followed by bone marrow treatment were observed in the present

study. Concerning polysaccharides, the fetal stomach tissue maternally exposed to γ-rays on day 7 or 14 of gestation showed decreased stain affinity. In contrast to the present results **Moustafa (2000)** noticed increased PAS +ve materials in the fetal tissues post exposure to 2Gy γ-rays.

Fetal stomach tissue taken from mothers exposed to 2Gy γ-rays on day 7 or 14 of gestation followed by bone marrow transplantation also appeared with reduced stain affinity of PAS +ve materials in the different layers. In contrast, **Bakhit (2010)** noticed increased content of PAS +ve materials in the fetal lung tissues post exposure to γ-rays followed by bone marrow transplantation.

In the present study increased stain affinity of total protein content was observed in the fetal stomach tissue exposed maternally to γ-rays on day 7 or 14 of gestation. This increase in stain affinity of total protein may be due to increased RBCs in the blood vessels or may be due to increased collagen fibers. Increased total protein in other tissue post exposure to different types of radiations was noticed by many authors **(Al Dossary, 2007; El-Salkh, 2009).**

In the present study increased total protein content was still detected in the fetal stomach tissue maternally treated with the bone marrow post-irradiation on day 7 or 14 of gestation. In contrast**, Bakhit (2010)** noticed somewhat normal total protein content in the fetal lung tissue maternally treated with the bone marrow post-irradiation on day 7 of gestation.

ILEUM

In the present study exposure of pregnant rats to 2Gy of γ-rays on day 7 or 14 of gestation led to many histopathological changes in the fetal ileum tissue. These changes were more drastic on day 14 of gestation. These changes include: highly elongated or atrophied villi, distorted and ruptured circular and longitudinal muscle fibers of ileum muscularis in addition to many degenerated areas, pyknotic nuclei and numerous hemorrhagic areas.

Irradiation of the gastrointestinal tract in mice caused a series of biological effects that begin with immediate nausea and vomiting, delayed malnutrition and diarrhea ;all these factors can progress to late fibrosis and necrosis **(Okunieff *et al*. , 2008).** Exposure to toxic agents such as radiation, increased risk of induction or progression of cancer during embryonic and childhood development (**Kheifets *et al*., 2005)**. Also exposure to radiation increased proliferation and regeneration of the small intestine mucosa (**Okunieff *et al*., 2008).**

According to **Shao and Sheng (2010)** the intestinal epithelium is one of the most rapidly proliferating tissues in the body and is able to replicate the total mass every 3–8 days. There is normally a dynamic equilibrium between cell proliferation at the base of the crypt and programmed cell death to preserve a functional cell steady state. The irradiation leads to atrophic changes in the gut epithelium.

In the present study bone marrow transplantation post-irradiation (2Gy) on day 7 or 14 of gestation caused moderate improvement in the architecture of the fetal ileum tissue, in spite of presence of some degenerated areas and deeply stained nuclei. In accordance with **Hui *et al*. (2012),**

bone marrow transplantation improved the recovery of the intestinal mucosa after whole body irradiation.

Also **Tu *et al*. (2012)** indicated that bone marrow mesenchymal stromal cells can effectively relieve injury of small intestinal epithelium, promote the proliferation of enteric epithelium and repair of the mucosa, attenuate systemic inflammation in rats with severe acute pancreatitis. Bone marrow mesenchymal stromal cells have the ability to inhibit inflammatory reactions and promote tissue repair. The improvement observed in the fetal ileum tissue maternally exposed to γ-rays and treated with bone marrow may be due to the ability of bone marrow cells to differentiate to mature, non-haematopoitic cells of multiple tissues **(Abedi *et al*., 2004**).

Among all these findings, **Okamato *et al*. (2002& 2006)** reported that bone marrow-derived cells (BMDCs) could also differentiate into epithelial cells to repopulate the damaged intestinal epithelium. In the gastrointestinal tract, BMDCs have also been observed to differentiate into subepithelial myofibroblasts, which are important to regulate intestinal stem cell microenvironment (**Brittan *et al*., 2002**). Moreover, In the gastrointestinal tract, bone marrow-derived cells have been reported to have the capacity to differentiate into epithelial cells **(Zhang *et al*., 2008 and Liu *et al*., 2010).**

Generally**, Yagi *et al*.(2010)** concluded that transplantation of mesenchymal stem cells has a therapeutic benefit in injured animals by providing anti-inflammatory and anti-apoptotic effects. In addition, these stem cells can genetically react to inflammatory conditions.

Results of the present study showed increased collagen fibers and brightly red stained hemorrhagic areas in the fetal ileum tissue maternally exposed to 2Gy of γ-rays on day 7 or 14 of gestation with moderate increase in the fetal ileum tissue taken from mothers exposed to 2Gy γ-rays on day 14 of gestation and treated with bone marrow. These results are in controversy with those of **Bakhit (2010)** who noticed normal distribution of collagen fibers in the fetal lung and liver tissues maternally treated with 2Gy γ-rays on day 7 or day 14 of gestation followed by bone marrow treatment, a slight increase of these fibers was realized in walls of the blood vessels and alveolar septae on day 14 of gestation.

Concerning polysaccharides, the fetal ileum tissue maternally exposed to 2Gy γ-rays on day 7 or 14 of gestation showed decreased stain affinity, but deeply stained goblet cells in some villi were detected. **Eid *et al.* (1994)** indicated that the frequency of changes in polysaccharides content was high in lung and ileum tissue of rat embryos exposed to 3Gy on day 6 and 12 of pregnancy.

Fetal ileum tissue taken from mothers exposed to 2Gy γ-rays on day 7 or 14 of gestation followed by bone marrow transplantation showed also reduced stain affinity of PAS +ve materials . In contrast fetal lung tissue taken from mothers exposed to 2Gy γ-rays on day 7 or day 14 of gestation followed by bone marrow transplantation restored the normal polysaccharides content with a

slight increase in stain affinity in walls of the bronchioles of the fetal lung tissue on day 14 of gestation **(Bakhit, 2010).**

In the present study increased stain affinity of total protein content was observed in the fetal ileum tissue exposed maternally to 2Gy γ-rays on day 7 of gestation, but on day 14 of gestation it showed less increased stain affinity.

Increased stain affinity of total protein was also observed in the fetal lung tissue exposed maternally to 2Gy γ-rays on day 7 of gestation **(Bakhit, 2010).** Highly affected protein post-irradiation exposure may be due to response of hydrogen bonds of these materials to radiation or may be due to the immune response to the shock given by injury **(Mansour, 2008).** This agrees with the histopathological lesions observed in the ileal tissues as well as the occurrence of numerous inflammatory cells in all tissues under investigation in the irradiated rats of the present study. Generally γ-rays cause lesions on template DNA strand which result in impaired gene transcription, therefore the synthesis of functional m-RNA is impaired and this may alter the pattern of protein synthesis either by stimulation or by inhibition **(Mahdy *et al*., 1997**; **El-Wakf *et al*., 1999** ; **Ali *et al*., 2007).**

Bone marrow treatment post-radiation exposure on day 7 of gestation nearly restored the total protein content in the fetal ileum tissue to somewhat less increased level, but the increased values were detected on day 14 of gestation.

However, **Bakhit (2010)** reported that somewhat normal total protein content was detected in the fetal lung tissue maternally treated with the bone marrow post-irradiation on day 7 of gestation, but on day 14 of gestation showed increased stain affinity of total protein was detected.

It is clear that pregnant rats and their fetuses which were exposed to 2Gy γ-rays on day 14 of gestation were more sensitive to γ-rays than those exposed on day 7 of gestation. Bone marrow transplantation cannot completely overcome radiation injury, but restore the normal histological pattern and normal content of collagen, polysaccharides and total protein in the tissues exposed on day 7 or day 14 of gestation.

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الملخص العربى

التأثير الوقائي المحتمل لزراعة نخاع العظام ضد التغييرات المستحدثة بآشعة جاما فى أنسجة القناة الهضمية المعوية الجنينية من أمهات الجرذان الحوامل

ناهد محمد منصور امام

قسم العلوم البيولوجية والجيولوجية -كلية التربية بالعريش - جامعة قناة السويس الاسماعيلية - مصر

يستخدم حديثا العديد من تفنيات زراعة نخاع العظام فى مجال الطب والعلاج للحد من العديد من المشاكل الطبية لذلك استهدفت هذه الدراسة التغيرات النسيجية والكيميانسيجية التي تحدث في الجهاز الهضمى لأجنة الجرذان التى عرضت أمهاتها لجرعة مقدارها 2 جراي من آشعة جاما. كما اشتملت الدراسة على الدور الوقائى لنخاع العظم باعتباره مضاد للأكسدة وعلى إمكانية تلافى الآثار الجانبية للإشعاع وذلك بحقنه في التجويف البطني في اليوم السابع والرابع عشر من الحمل بعد التشعيع.

ولقد استخدمت فى هذه الد راسة الجرذان البيضاء من النوع *Rattus albinus* وتم التشعيع باستخدام خلية جاما 40 وهى وحدة تشعيع تحتوى على عنصر السيزيوم 137المشع . وقد تضمن البحث خمس مجموعات من الجزذان البيضاء البالغة الحوامل كانت المجموعة الاولى منها ضابطة. ومجموعتان من الجرذان الحوامل عرضت للإشعاع الجامي بجرعة مقدارها 2 جراي (مجموعة عرضت فى اليوم السابع من الحمل وتم ذبحها فى اليوم العشرين و مجموعة عرضت فى اليوم الرابع عشر من الحمل وذبحت فى اليوم العشرين للحمل). و مجموعتان من الجرذان الحوامل عولجت بنخاع العظم (75×10 لكل جرذ) بعد تعرضها لنفس الجرعة الإشعاعية السابقة بساعة واحدة وفي نفس الأيام (فى اليوم السابع من الحمل وفى اليوم الرابع عشر من الحمل) وذبحت فى اليوم العشرين للحمل.

تشير نتائج هذا البحث أن التعرض لآشعة جاما بجرعة مقدارها 2 جراي قد أدت إلى ظهور العديد من التغيرات النسيجية في ا جنة الأمهات الحوامل وكانت حدة هذه التغيرات أكثر وضوحا في اليوم الرابع عشر للحمل عن تلك التي لوحظت في اليوم السابع للحمل. أظهرت نتائج هذا البحث أن انسجة مرىء الاجنة كان أكثر حساسية لآشعة جاما وخاصة في اليوم الرابع عشر للحمل حيث ظهرت أعداد كبيرة من الخلايا الإلتهامية وبقع عديدة من تجمعات دموية وعدد كبير من المناطق المتحطمة وكذلك ظهر نشاط كبير لانقسام الخلايا الطلائية وفقدان للتخطيط العضلى .

كذلك إتضحت الحساسية المفرطة لآشعة جاما في معدة اجنة الجرذان الحوامل وخاصة في اليوم الرابع عشر للحمل حيث ظهرت العديد من المناطق المتحطمة والعديد من الخلايا الالتهابية بالاضافة الى بقع عديدة من تجمعات دموية

ايضا إتضحت الحساسية الشديدة لأشعة جاما في لفائفى الأجنة التي عرضت أمهاتها لأشعة جاما وخاصة في اليوم الرابع عشر للحمل حيث كانت هناك العديد من الخلايا الالتهابية فى النسيج مع ظهور بعض مناطق النزف داخلية و وتأكل فى قمم بعض الخملات بالاضافة الى فقدان للتخطيط العضلى وصاحب كل ذلك زيادة الألياف الكولاجينية وتغير محتوى المواد عديدة التسكر والبروتينات الكلية في نسيج القناه الهضمية المعوية للأجنة . ولكن حقن نخاع العظم بعد الإشعاع بساعة واحدة قد سبب تحسنا ملحوظا في الشكل النسيجي للقناة الهضمية المعوية و مع استعادة محتوى الكولاجين و المواد عديدة التسكر والبروتينات الكلية في أنسجة الأجنة وخاصة في اليوم السابع للحمل.

ويتضح من ذلك أن أنسجة أجنة الأمهات الحوامل تكون أكثر حساسية للتشعيع في فترة تكوين الأعضاء (اليوم الرابع عشر للحمل) عن فترة غرس الأجنة (اليوم السابع للحمل) كما وضح أن حقن نخاع العظم في فترة إنغراس الأجنة يكون أكثر إيجابية لتلافى التأثير المدمر لآشعة جاما وتحسن الصورة النسيجية والكميانسيجية لأنسجة المرىء والمعدة واللفائفى لأجنة الجرذان الحوامل .

وتشير النتائج الى مدى فاعلية زراعة نخاع العظام للتغييرات النسيجية التى يحدثها الاشعاع ولكن ينصح بالعديد من الدراسات البيوكيمائية والنسيجية المرضية والخلوية عن زراعة نخاع العظام وأثرة فى علاج العديد من التغيرات والذى يعتبر علاجا جذريا للعديد من الامراض المزمنة المنتشرة وذلك لتفادى التأثيرات الجانبية الخطيرة طويلة المدى او قصيرة المدى خلال أو بعد عمليات زراعة النخاع .