The Egyptian Journal of Hospital Medicine (Oct. 2015) Vol. 61, Page 670- 684

# Ameliorative Effect of Olive Leaf Extract on the Fetal Lung Tissue of Diabetic Pregnant Rats

**Mervat A. Abd Rabou [(1)] , Fawzya A. Al-Ghamdi [(2)]and Nehal A. Abu Elnaga[(3)]**

(1) Biology Department, Faculty of Science, Al-Jouf University, Saudi Arabia.

(2) Zoology Department, Faculty of Science, Abdul-Aziz University, Saudi Arabia.

(3) Zoology Department, Faculty of Science, Al-Azhar University, Cairo.

## ABSTRACT

**Aim of the work-** Diabetes mellitus is a common metabolic disease not only affecting the individual, but also imposes adverse effects on the offsprings. Besides increasing congenital malformations, maternal diabetes is suggested to be associated with early pregnancy loss, altered sex ratio in the offspring and long-term postnatal diseases. Antidiabetic plants are used as supportive therapy in the treatment of diabetes during pregnancy, so the present study aims to investigate the protective effect of olive leaf extract on the fetal lung of the diabetic pregnant rats.

**Material and methods -** Forty pregnant albino rats were used and categorized after mating into four groups; group 1: control group(**C**), group 2: rats treated with olive leaf extract during the period of pregnancy (**O**) (1 ml/100gm. b .wt), group 3: streptozotocin induced diabetic rats (**D**)(STZ 35 mg/kg b.wt), group 4: diabetic rats treated with olive leaf extract (**D+O**) (as in groups 2&3). The pregnant females of different groups were dissected during the 19 $^{th}$ day of pregnancy. Lung samples of fetuses were taken for the histological and histochemical studies.

**Results**- Histopathological and histochemical observations of fetal lung tissue showed that the olive leaf extract succeeded to minimize the drastic changes which were observed in the fetal lung of diabetic rats. **Conclusion**- It is recommended that the use of the olive leaf extract has the ability to minimize the adverse effects in the fetal lung tissue of diabetic rats.

**Key words-Pregnant diabetic rats, olive leaf extract, fetus, lung, hyperglycemia.**

## INTRODUCTION

Diabetic mellitus is a common metabolic disorder characterized by hyperglycemia and other symptoms due to impairment of insulin production and/or insulin resistance. [1] Diabetes can cause many serious complications. Acute complications such as ketoacidosis and nonketotic coma can be developed. Long term complications include retinal, micro vascular, cardiac and neural damage. [2] The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves and arteries .[3,4] The hyperglycemic maternal environment has also been associated with neonates that are at greater risk for future development of negative health outcomes such as future obesity, insulin resistance, type 2 diabetes mellitus and metabolic syndrome. [5]

Islet hyperplasia and B-cell degranulation were found in the fetuses of the third generation from mothers (second generation) born to a diabetic mother in the first generation.[6]

Perinatal morbidity and mortality, congenital malformations, abnormal fetal growth, spontaneous preterm birth, hypoxic complications and trauma during delivery are increased in diabetic pregnancies. [7] Histopathologic evidence of lung involvement in subjects with diabetes mellitus has included thickened alveolar epithelial, pulmonary microangiopathy and abnormal pulmonary function.[8]

Olive tree (*Oleaeuropea)* leaves have been widely used in traditional remedies in European and Mediterranean countries as extract herbal teas and powder. They contain several potentially bioactive compounds that may have hypoglycemic properties. [9] Olive leaf extract was used by the ancient Egyptian and Mediterranean people to treat a variety of health conditions, including infections, fever and pain. [10]

The active medical constituents find in unprocessed olive leaf are oleuropein, oleuropeoside and hydroxytyrosol, as well as several other polyphenols and flavenoids including oleocanthal. [11] They added that the olive fruit, its oil and the leaves of the olive tree have a rich history of nutritional and medicinal usesOleuropeosits (oleuropein), flavones, flavonols and substituted phenols (tyrosol, hydroxytyrosol) are phenolic



Received:15/9/2015
Accepted:22/9/2015 DOI: 10.12816/0018769

compounds in the olive leaf extract [12]**.** It has been reported by many researchers that the olive leaf extract has an antimicrobial activity because of its high phenolic content. [13,14, 15, 16] Also, olive leaf extract had antimicrobial activity. [17, 18]

Oleuropein has some benefits such as: antioxidant activity [19], anti-inflammatory effect [20], anti-atherogenic effect [21], anti-cancer effect [22,23], antimicrobial effect[24], antiviral effect [25] , skin protectant [26], anti-aging [27], neuro protective activity [28]**,** anti-platelet aggregation [29]**,**antipyretic effects [30], hypotensive [31] and prevention of free radical formation.[32] Oleuropein was reported to have an anti-hyperglycemic effect in the diabetic rats.[ 33, 34, 35]

## MATERIALS AND METHODS

The present work was carried out on forty mature pregnant albino rats [200 ± 20 gm). They were obtained from El Rammed Medical Hospital, Cairo. The rats were stayed for 2 weeks for adaptation then the experiment was started. They fed on rodent diet, some vegetables and provided with milk and tap water *ad libitum*.

Streptozotocin ( STZ ) was purchased from Sigma, St .Louis, MO, USA. Diabetes mellitus was induced in fasted animals of **D** and **D+O** groups (12 hours) by a single intraperitoneal injection of Streptozotocin (35 mg/kg b.wt.). It was dissolved in 0.01 mole/1citrate buffer (pH 4.5) then animals were orally injected with 2 ml of glucose solution. After 48 hours of STZ injection, blood glucose levels were measured by glucometer. Rats with fasting blood glucose level more than 250mg /dl are considered diabetic [36]. 5.5 grams of olive leaf powder were soaked in 100 ml boiled distilled water and covered for ten minutes, then cooled to room temperature and filtered. It was given orally with a dose of 1ml/100 gm of b. wt. (using the stomach tube) every day till the 19 $^{th}$ day of pregnancy in **O** and **D+O** groups. This dose is equivalent to the therapeutic human dose (500mg) [9].

Rat's estrus cycle usually begins at 6 – 7 weeks of age; the estrus cycle repeats itself every 4 - 5 days. The stage of estrus cycle was determined by the vaginal smear technique as determined by **Taylor.** [37]

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 this is considered as zero day of pregnancy.[38] The pregnant rats were randomly divided into four groups, control [**C**), olive (**O**), diabetic (**D**) and diabetic + olive (**D+O**).

In 19$^{th}$ day of pregnancy, the pregnant rats were anesthetized by ether then sacrificed and specimens of the lung were taken from the fetuses of pregnant rats of all groups. The specimens were 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 .The paraffin blocks were sectioned at 5 micron thick and mounted on clean glass slides. The sections were stained by hematoxylin and eosin according to the method of Drury and Wallington [39] , Mallory's trichrome stain for demonstrating collagen fibers[40], periodic acid Schiff's reaction for demonstrating polysaccharides [40] and mercuric bromophenol blue method for detecting total proteins [41] . Beta amyloid was detected by Congo red technique. [42]

## RESULTS

### Histopathological observations of the lung:

Well developed architecture of fetal lung tissue of the **control** and **O** group are shown in **figs. 1, 2** with normal distribution of collagen fibres in both groups **(Figs. 5, 6)**. These collagen fibres support walls of the bronchioles, blood vessels and interalveolar septa. Fetal lung tissue of group **D** showed congested interalveolar septa and numerous hemorrhagic areas **(Fig. 3).** Highly increased collagen fibres were demonstrated around walls of bronchioles and the arterial walls, but they were decreased in the interalveolar septa in group **D (Fig. 7).** In group **D+O** somewhat normal architecture of fetal lung tissue was demonstrated **(Fig.4)** with somewhat normal distribution of collagen fibers **(Fig.8)**.

### Histochemical observations of the lung:

### DNA

Moderately stained DNA materials were detected in groups **C** and **O (Figs. 9, 10)**. In group **D** increased DNA materials were observed in some thickened interalveolar septa, but some of them contained faintly stained nuclei. Nuclei of WBCs in the hemorrhagic area beside the lung tissue were moderately stained, also some interstitial cells (fibroblasts and mast cells) are moderately stained, but degenerated areas were negatively

stained **(Fig.11)**. Somewhat normal appearance of DNA materials was detected in group **O+D (Fig.12).** The MOD values reached 133.69 ± 10.82, 110.82 ± 7.53 and 123.87 ±5.46 in groups **O** , **D** and **O+D** respectively compared to the control group 134.85 ± 6.25. The percentage of change reached -0.899% , -17.066% and -7.96% respectively in groups **O**, **D** and **O+D** as shown in table 1 and histogram 1.**Amyloid protein**

Fetal lung tissue of the **control** and **O** group showed faintly stained amyloid protein **(Figs. 13, 14)** in both groups. Increased amyloid protein was realized in the highly thickened interalveolar septa of lung tissue of group **D (Fig. 15),** but slightly increased amyloid protein were detected in some interalveolar septa of group **D+O (Fig.16)**.

**Polysaccharides**

Normal distribution of PAS +ve materials was detected in the fetal lung tissue of the **control** and **O** groups **(Figs.17.18)**. Deep staining affinity of PAS +ve materials was observed in the fetal lung tissue of group **D (Fig.19)**. Lung tissue of group **D+O** showed somewhat normal appearance of PAS +ve materials **(Fig.20)**. The MOD values reached 103.9 ± 12.6, 153.4 ± 11.25 and 110 ±12.27 in groups **O**, **D** and **O+D** respectively compared to the **control** group (104.6 ± 8.43). The percentage of change reached -0.669%, 46.65%) and -5.16 % respectively in groups **O**, **D** and **O+D** as shown in table 2 and histogram 2.

**Total protein**

**N**ormal distribution of total protein was detected in the fetal lung tissue of **control** group **(Fig.21).** Somewhat normal appearance of total protein was noticed in the fetal lung tissue of group **O (Fig.22)**. Reduced staining affinity of total protein was demonstrated in the interalveolar septa of the fetal lung tissue of group **D (Fig.23)**, but large hemorrhagic area beside the lung tissue acquired deep blue staining affinity. Somewhat normal distribution of total protein was detected in the fetal lung tissue of group **D+O (Fig.24).** The MOD values reached 158.78 ± 5.43, 144.97 ± 6.55 and 150.90 ±7.565 in groups **O** , **D** and **O+D** respectively compared to the **control** group (156.88 ± 5.27). The percentage of change reached 1.212, - 7.588% and -3.81% respectively in groups **O**, **D** and **O+D** as shown in table 3 and histogram 3.

## DISCUSSION

Diabetes mellitus is characterized by a series of complications that affect blood and tissues .[43] It is well recognized that the combined stress of insulin-dependent diabetes mellitus and pregnancy causes a metabolic environment that is often life threatening to both the mother and fetus. [44] This is primarily due to difficulty in control of diabetes in the mother because of the natural diabetogenic state of pregnancy. Congenital anomalies occur in 6–8% of the fetuses of diabetic mother compared to 2% in non-diabetics. [45]

Clinical and experimental study have clearly demonstrated that hyperglycemia is the major teratogenic factor for embryonic malformations, although other associated factors, such as ketone bodies, branched amino acids, and triglycerides have also been shown to exert adverse effects on the developing embryos [46].The mechanisms by which maternal hyperglycemia causes embryonic malformations remain to be fully delineated. Hyperglycemia occurring in diabetic pregnancy is one of the important factors responsible for the development of oxidative stress and reactive oxygen species [ROS). [47]

In diabetes, protein glycosylation and glucose auto-oxidation can lead to formation of free radicals and this can induce lipid peroxidation (LPO). [48]The principal free radicals are superoxide ($O^2$), hydroxyl ($OH^-$) and peroxyl ($ROO^-$) radicals. Free radicals may play a role in DNA damage, protein modification , glycosylation and lipid oxidative modification reactions in diabetes[47]**.** Oxidized low density lipoprotein may be more toxic to cells. A variety of antioxidants scavenges ROS and prevents the occurrence of oxidative damage to biological structures. Evidence of ROS involvement in hyperglycemia-induced embryo death was obtained in several studies.[45,47] Enzymatically produced ROS can disturb embryo development *in vitro* similar to the effect of high glucose .[49]

The diabetic patients need alternative therapies to control all the pathological aspects of the diabetes. [50] Herbal medicines are better and safer than conventional medicines**.** Some medical plants are rich sources of antidiabetic, antihyperlipidemic and antioxidant agents such as flavonoids, gallotannins, amino acids and other related polyphenols. [51, 52] Some of these plants have a greater consumption during pregnancy as

they are considered safe and are also reported to have beneficial effects in the treatment of intrauterine growth retardation.[53] In this respect, the antioxidant properties of oleuropein and hydroxytyrosol in olive leaves extract allow them to be efficient in the protection against diabetes. [54] So, this study is a step to evaluate the effects of water extract of olive leaves as an antidiabetic agent during gestation period.

**Damasceno *et al*.** [55] demonstrated that severely diabetic rats presented higher DNA damage confirming the interaction between hyperglycemia-induced genotoxicity and teratogenesis. **Damasceno *et al*.** [56] found that oxygen and nitrogen species, the products of free radicals, which are dependent on fatty acid oxidation, can induce chromosome breaks in streptozotocin- induced diabetes models. **Zabihi and Loeken** [57] used a similar diabetes model, recovered embryos from the diabetic rats at day 10 or 11 of pregnancy.

Diabetes during pregnancy causes an abnormal intrauterine metabolic and hormonal milieu that result in congenital malformations and neonatal hyperglycemia.[58] It also enhances the risk of short and long-term postnatal disease, including macrosomia [59], glucose intolerance, insulin resistant [60], type 2 diabetes later in life [61, 62] and obesity. [63]

Diabetes during pregnancy is associated with increased morbidity (hypoglycemia, hypocalcemia, polycythemia, hyperbilirubinemia) and fetal mortality[64]. Maternal diabetes constitutes an unfavorable environment for fetal-placental and embryonic development.[65]

A significant decrease in blood glucose level occurred in the STZ-diabetic group treated with *Oleaeuropaea* aqueous extract. Islets of Langerhans were hypertrophied in the STZ-diabetic group and this hypertrophy showed a significant increase in the average of islets size at the last week, while the treatment with oleaeuropaea aqueous extract showed a reduction of the islet size compared to the islets of the STZ –diabetic mice.[66]

The olive oils and thyme leaves have strong capability of enhancing hormonal functions by subsequently the fertility of females. Consumption of these oils reduced risk of infertility in females. Traditionally, thyme is reported to the effect the menstrual cycle and, therefore, large amounts could not be ingested .[67]

Histopathological results of this study showed normal structure of fetal lung tissue of the control pregnant rats and those of group **O** with normal distribution of collagen fibres, amyloid protein and DNA materials. The microscopic appearance of fetal lung of the pregnant diabetic rats in the present study showed severe histopathological changes. These changes include congested interalveolar septa and numerous hemorrhagic areas. These results agree with results of **Eid *et al.*** [67] They noticed lots of histopathological changes in different organs in pregnant diabetic rats and their fetuses.

Diabetics can be attributed to increased glycosylation of connective tissues and other proteins in the lungs, leading to a decrease in elasticity, flexibility and recoiling capacity ultimately producing stiff lung i.e. restrictive lung pathology [68]**.** Reduced elastic recoil of the lungs because of increased glycosylation of connective tissues is one of the long term effects of diabetes on the respiratory system .[69]

Highly increased collagen fibres were demonstrated around walls of bronchioles and the arterial walls, but they were decreased in the interalveolar septa. These results agree with those of **Eid *et al*.** [67] In spite of increased DNA materials in some thickened interalveolar septa others showed reduced staining affinity and around the arterial walls. Slightly increased amyloid protein in the highly thickened interalveolar septa was also detected in this group.The restrictive pathology is due to overall effect of glycosylation on the collagen and elastic framework of the respiratory system. These tissues being present everywhere such as in skin, muscles, fascia, joints, lung parenchyma and pleura; there is overall damage to the whole respiratory system. These micro damages produce less effective "negative-pressure-pump" and a less compliant lung. [70]

In the present work diabetic rats treated with olive leaf extract showed no inflammatory infiltration in the fetal lung sections. The anti-inflammatory role of medicinal plants was also noticed by several authors. [67, 71, 72] **Ozbek *et al*.** [71] proposed a biological mechanism that may explain these anti-inflammatory and anticancer effects. This mechanism involves the shutting down of an intercellular signaling system called tumor necrosis factor [or TNF]-mediated signaling. The hypoglycemic and

antioxidant effects of oleuropein have been reported in alloxan –diabetic rabbits. [34]

Somewhat normal distribution of collagen fibres was demonstrated in fetal lung tissue of group **D+O**. **Horn *et al.*** [73] declared that the presence of collagen in the peri-sinusoidal spaces might affect the blood supply to liver cells and would reduce the exchange of metabolites, perhaps causing hepatocellular dysfunction and necrosis. In this group (**D+O**) somewhat normal content of DNA materials was observed with slightly increased amyloid protein. Olive leaf extract is more effective than glibenclamide (Synthetic drug) and may be use as an antidiabetic agent **.** [74]

Increased amyloid protein in the highly thickened interalveolar septa of group **D** and increased DNA materials were observed in some thickened interalveolar septa, but some of them contained faintly stained nuclei. Nuclei of WBCs in the hemorrhagic area beside the lung tissue are moderately stained, also some interstitial cells (fibroblasts and mast cells) are moderately stained, but degenerated areas were negatively stained in the same group. Somewhat normal appearance of DNA materials was detected in group **O+D**. The decrease of DNA content was associated with a decrease in protein content in fetal lung cells of the diabetic rats. These results go in agreement with those of **Blasiak *et al*.** [82]**,** they reported that alloxan can damage DNA in normal cells, operating therefore as a genotoxic compound. The observed DNA damage might be due to the induction of DNA strand breaks and/or the formation of alkali labile sites, which can be transformed into strand breaks in the alkaline comet assay. Also, **El-Nabarawy** [83] reported that diabetes can generate oxidative stress and DNA damage to embryo and placenta and this can be ameliorated by oral doses of olive leaves extract by using alkaline comet assay. She also added that the ability of streptozotocin to generate free radicals in the presence of suitable reducing agents, like reduced glutathione and oxygen is well known. Streptozotocin exerts its DNA-damaging action, at least in part, by the production of free radicals and this action can be modulated by common antioxidants. **Shaffie *et al*.** [78] found marked diminution of protein content in alloxan diabetic rats .They added that alloxan exerts its DNA-damaging action, at least in part, by the production of free radicals and this action can be modulated by common antioxidants, which can easily supplement the diet .

In the present study, the fetal lung tissue of diabetic rats showed deep staining affinity of PAS +ve materials. These results did not agree with those of **El-missiry and El-Gindy** [75] and **Eid *et al.*** [67] They observed a decrease in glycogen content in sections of fetal tissues of diabetic mothers. They mentioned that diminution of carbohydrates may be due to degeneration and inflammation or may be due to damaging effect of streptozotocin on the cytoplasmic organelles especially Golgi apparatus and the associated enzymes. Decrease in mucopolysaccharides content in the kidney of diabetic rats has been explained by **Tunez *et al*.** [76]**,** they postulated that the decrease of glycogen content of rats treated with streptozotocin might be due to express of glycogenolysis. Increased PAS+ve materials in this study may be due to congestion observed in the interalveolar septa , since RBCs contain 10% of their weight carbohydrates [67,77] **.** These results are in agreement with those of **Shaffie *et al*.** [78] However, **Poop and Cattley** [79] and **Al Dossary**[80] reported that the decrease in mucopolysaccharides content in tissue made by several factors may be due to the disturbed role of Golgi apparatus, which is responsible for synthesis of polysaccharides .

In the present work, treatment of the diabetic rats with olive leaf extract (Group **D+O**) showed an improvement in polysaccharides content when compared to the diabetic group, but still more than the normal content. These effects may be due to antioxidant nature of this plant. According to **Poop and Cattley** [79]**, Tavafi *et al*.** [81] and **Eid *et al*.** [67] it seems clear that the increase of polysaccharides deposition is a sign of glycogenesis.

In the present work, the diabetic rats showed reduced staining affinity of total protein in the interalveolar septa of the fetal lung tissue. This result agrees with those **Eid *et al*.** [82] They noticed decreased total protein in different organs of diabetic rats and their fetuses. This decrease may be due to the decrease in ribosomal granules of rough endoplasmic reticulum or due to the decrease in DNA content.

Increased protein content indicating that olive leaf extract is more effective in improving lung cell dysfunction induced by

streptozotocin. It may also cause increased amount of ribosomes in the rough endoplasmic reticulum in cells, reflecting their ability to stimulate protein synthesis. Olive leaf extract is an effective antioxidant which can protect proteins against oxidation. **Sierens *et al.*** [84] stated that the antioxidant species may act *in vivo* to decrease damage of protein content in tissues. Increased protein content in group **D+O** indicating that olive leaf extract is more effective in improving kidney cell dysfunction induced by streptozotocin .

## CONCLUSION

It is clear that use of olive leaf extract has the ability to minimize the damage of hyperglycemia in the lung of fetuses.

## REFERENCES


1) **Arita R (2011):** Mechanism of diabetes-induced microvascular damage and therapeutic potential of ROCK inhibition. Nihon Ganka Gakkai Zassh, 115:985-997.

2) **Soler M J, Riera M and Batlle D (2012):** New experimental models of diabetic nephropathy in mice models of type 2 diabetes: efforts to replicate human nephropathy. Exp. Diabetes Res., 5:115-125.

3) **Lovell H G(2000):** Angiotensin converting enzyme inhibitors in diabetic patients with normotensive microalbuminuria. Cochrane Database Syst. Rev., 2:1-10

4) **Morrish N J, Wang S L, Stevens L K, Fuller J H and Keen H( 2001):** Mortality and cause of death in the multinational study of vascular disease in diabetes. Diabetologia, 44(12):12-21.

5) **Calkins K and Devaskar S (2011):** Fetal origins of adult disease. Health Care, 3: 158–176.

6) **Van Assche F and Aerts L(1985):** Long term effect of diabetes and pregnancy in the rat. Diabetes, 34 ( 2) :116-118.

7) **Kari T M (2014):** Diabetic pregnancy and fetal consequences. New Reviews, 15 (3): 83 -90.

8) **American diabetes Association (ADA) (2014):** Standers of medical care in diabetes. Diabetes Care, 37: 14-80.

9) **Wainstein J, Ganz T, Boaz M, Bar Dayan Y, Dolev E and Kerem Z (2012):** Olive leaf extract as hypoglycemic agent in both human diabetic subjects and in rats. J. Med. Food, 15(7): 605-610.

10) **Omar S H (2010):** Oleuropein in olive and its pharmacological effects. Sci.Pharm., 78:133-154.

11) **Soni M G, Burdock M S, Christian C M and Crea R (2006):** Safety assessment of aqueous olive pulp extract as an antioxidant or antimicrobial agent in foods. Food Chem. Toxicol., 44: 903-915.

12) **Benavente-Garcia O J, Castillo J, Ortuno L and Del Rio J(2000):** Antioxidant activity of phenolics extracted from *Oleaeuropaea* leaves. Food Chem., 68: 457-462.

13) **Markin D L, Duek I and Berdicevsky N (2003):** *In vitro* antimicrobial activity of olive leaves. Mycoses, 46: 132-136.

14) **Pereira A P, Ferreiara F, Marcelino P, Valentao P and Andrade *et al*. (2007):** Phenolic compounds and antimicrobial activity of olive (*Oleaeuropaea*) leaves. Molecules, 12: 1153-1163.

15) **Sudjana A N, C, D'Orazio V, Ryan N, Rasool J *et al.* (2009):** Antimicrobial activity of commercial *Oleaeuropaea* (olive) leaf extract. Int. J. Antimicrob. Agents, 33: 461-463.

16) **Aytul K K ( 2010):** Antimicrobial and antioxidant activities of olive leaf extract and its food applications. M.Sc.Thesis, Graduate School of Engineering and Sciences of Izmir Institute of Technology, Turkey.

17) **Ahmed A, Rabii N, Garbaj A and Abolghait C (2014):** Antibacterial effect of olive (*Oleaeuropaea* ) leaves extract in raw peeled shrimp (*Penaeussemisulcatus)*. International Journal of Veterinary Science and Medicine, 2(1):53-56.

18) **Malik S N (2015):** Antibacterial activity of olive (*Oleaeuropaea*) leaves and arugula (*Eruca sativa*) seeds extract. International Journal of Pharmacognosy and Phytochemical Research, 7(2): 307-310.

19) **Andreadou I, Iliodromitis E K, Mikros E, Constantinou M, Agalias A, Magiatis P, Skaltsounis A L, Kamber E, Tsantili-Kakoulidou A and Kremastinos D T (2006):** The olive constituent oleuropein exhibits anti-ischemic, antioxidative and hypolipidemic effects in anesthetized rabbits. J. Nutr., 136: 2213–2219.

20) **Visioli F, Bellosta S and Galli C (1998):** Oleuropein the bitter principles of olives enhances nitric oxide production by mouse macrophages. Life Sci., 62: 541–546 .

21) **Carluccio M A, Siculella L , Ancora M A Massaro M Scoditti E, Storelli C, Visioli F, Distante A and De Caterina R (2003):** Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediterranean diet phytochemicals. Arterioscler. Thromb. Vasc. Biol., 23(4):622-629.

22) **Hamdi H K and Castellon R (2005):** Oleuropein, a non-toxic olive iridoid, is an anti-tumor agent and cytoskeleton disruptor. Biochem. Biophys. Res. Commun., 334: 769–778.

23) **Menendez J A, Vazquez-Martin A, Colomer R, Brunet J, Carrasco-Pancorbo A, Garcia-Villalba R, Fernandez-Gutierrez A** and **Segura-Carretero A (2007) :** Olive oil's bitter principle reverses acquired auto-resistance to trastuzumab (Herceptin™) in HER2-over expressing breast cancer cells. B.M.C. Cancer, 7: 80-87.

24) **Caturla N, Perez Fons L, Estepa A and Micol V (2005):** Differential effects of oleuropein, a biophenol from *Oleaeuropaea*, on anionic and zwiter ionic phospholipid model membranes. Chem. Phys. Lipid, 137:2-17.

**25) Ma S C, He Z D, Deng X L, But P P, Ooi V E, Xu H X, Lee S H and Lee S F (2001):** *In vitro* evaluation of secoiridoidglucosides from the fruits of Ligustrumlucidum as antiviral agents. Chem. Pharm. Bull., 49: 1471–1473.

**26) Ancora C, Roma C and Vettor M (2004):** Evaluation of cosmetic efficacy of oleuropein. Int. J. Cosmet. Sci., 2:4–6.

**27) Katsiki M, Chondrogianni N, Chinou I, Rivett A J and Gonos E S (2007):** The olive constituent oleuropein exhibits proteasome stimulatory properties *in vitro* and confers life span extension of human embryonic fibroblasts. Rejuvenation Res., 10: 157–172.

**28) German J B and Walzem R L (2000):** The health benefits of wine. Annu. Rev. Nutr., 20: 561–593.

**29) Petroni A, Blasevich M, Salami M, Papini N, Montedoro G F and Galli C (1995):** Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. Thromb. Res., 78: 151–160.

**30) Visioli F, Bellomo G, Montedoro G F and Galli C (1995):** Low-density lipoprotein oxidation is inhibited *in vitro* by olive oil constituents. Atherosclerosis, 117: 25–32.

**31) Khayyal M T, El-Ghazaly M A, Abdallah D M, Nassar N N, Okpanyi S N and Kreuter H (2002):** Blood pressure lowering effect of an olive leaf extract (*Oleaeuropaea*) induced hypertension in rats. Arzneimittelforschung, 52: 797–802.

**32) Andrikopoulos N K, Kaliora A C, Assimopoulou A N and Papageorgiou V P (2002):** Inhibitory activity of minor polyphenolic and nonpolyphenolic constituents of olive oil against *in vitro* low-density lipoprotein oxidation. J. Med. Food, 5: 1–7.

**33) Al-Azzawie H F and Alhamdani M S (2006):** Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. Life Sci., 78: 1371–1377.

**34) Mohamed A A (2014):** Physiological studies of some traditional medicine efficacy on diabetic male albino rats. Ph. D. dgree, Zoology Department, Faculty of Science, Al-Azhar University, Cairo.

**35) Salem A A (2015):** Effect of feeding on olive oil and thyme on pregnancy and lactation periods. International Journal of Nutrition and Food Sciences, 4(1): 19-28.

**36) Waer H F and Helmy S A (2012):** Cytological and histochemical studies on rat liver and pancreas during progression of streptozotocin induced diabetes and possible protection using certain natural antioxidants. The Egyptian Journal of Hospital Medicine, 48: 452– 471.

**37) Taylor P (1986):** Handling the reproductive cycle and mating. In: Practical Teratology. Academic Press. Inc. London . PP: 3-9.

**38) Eda K, Buyuknacar H, Gocmen C, Evruke I and Onder S (2009):** Differential effect of neocuproine, a copper(I) chelator, on contractile activity in isolated ovariectomized non-pregnant rat, pregnant rat and pregnant human uterus. European Journal of Pharmacology, 605: 158-163.

**39) Drury R and Wallington E (1980):** Carleton's Histological Technique, 4th Ed. Oxford. Univ. Press, New York, Toronto.

**40) Pearse A (1977):** Histochemistry, Theoretical and Applied. 3th ed., vol. 1. Churchill livingstone, London.

**41) Mazia D, Brewer P and Alfert M (1953):** The cytochemical staining and measurement of protein with mercuric bromophenol blue. Biol. Bull., 104: 57-67.

**42) Valle, S. (1986):** Special stains in microwave oven. J. Histotechnol., 9:237-248.

**43) Naziroglu M. and Cay M (2001):** Protective role of intraperitoneally administered vitamin E and selenium on the antioxidative defense mechanism in rats with diabetes induced streptozotocin. Biol. Trace. Elem. Res., 79:149–159.

**44) Vanheest J and Rodgers C (1997)**: Effects of exercise in diabetic rats before and during gestation on maternal and neo-natal outcomes. Am. J. Physiol., 273(7):27–33

**45) Damasceno D, Volpato G, Calderon I and Rudge M (2002):** Oxidative stress and diabetes in pregnant rats. Anim. Reprod. Sci., 72:235–244

**46) Zhao Z and Reece E (2005):** Experimental mechanisms of diabetic embryopathy and strategies for developing therapeutic interventions. J. Pathol., 189: 12-19.

**47) Naziroglu M and Butterworth P (2005)**: Protective effects of moderate exercise with dietary vitamin C and E on blood antioxidative defense mechanism in rats with streptozotocin-induced diabetes. Can. J. Appl. Physiol. 30(2):172–185.

**48) Simsek M, Naziroglu M and Erdinc A (2005)**: Moderate exercise with a dietary vitamin C and E combination protects against streptozotocin-induced oxidative damage to the kidney and lens in pregnant rats. Exp.Clin.Endocrinol. Diabetes, 113(1):53–59 .

**49) Cederberg J . Basu S and Eriksson U (2001):** Increased rate of lipid peroxidation and protein carbonylation in experimental diabetic pregnancy. Diabetologia, 44:744–766

**50) Meral I, Yener Z, Kahraman T and Mert N (2001):** Effect of *Nigella sativa* on glucose concentration, lipid peroxidation, anti-oxidant defense system and liver damage in experimentally induced diabetic rabbits. Physiol. Pathol. Clin. Med., 48: (10)593-599.

**51) Muruganandan S, Srinivasa K, Gupta S, Gupta P. K. and Lal J (2005):** Effect of maniferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats. J. Ethnopharamcol., 97:497–501.

**52) Bansal P, Paul P, Mudgal N, Nayak P, Pannakal S, Priyadarsini S and Unnikrishnan S (2012):** Antidiabetic, antihyperlipidemic and antioxidant effects of the flavonoid rich fraction of *Pilea microphylla* (L.) in

high fat diet/streptozotocin-induced diabetes in mice. Experimental and Toxicologic Pathology**,** 64 (6): 651–658

**53) Mostafa A M, Serwah A H, Mohamed W S and Mohamed K M (2013):** Effects of some antidiabetic medicinal plants on pancreas and liver of diabetic Albino rats. The Egyptian Journal of Hospital Medicine, 48: 452– 461.

**54) Jemai H, El Feki A and Sayadi S (2009):** Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from olive leaves in alloxan-diabetic rats. J. Agric. Food Chem., 57: 8798–8804.

**55) Damasceno D C,Volpato G T and Sinzato Y K (2011):** Genotoxicity and fetal abnormality in streptozotocin-induced diabetic rats exposed to cigarette smoke prior to and during pregnancy. Experimental and Clinical Endocrinology and Diabetes, 119: 549–553.

**56) Damasceno D C, Netto A O, Lessi IL, Gallego F Q, Corvino S B, Dallaqua B, Sinzato Y K, Bueno A, Calderon I M and Rudge V (2014):** Streptozotocin-induced diabetes models: pathophysiological mechanisms and fetal outcomes. Bio. Med. Research International, 2014: 819065-819076.

**57) Zabihi S and Loeken M. (2010):** Understanding diabetic teratogenesis: where are we now and where are we going?: birth defects research. Clinical and Molecular Teratology, 88: 779-790.

**58) Martin F I, Heath P and Mountain K (1987)**: Pregnancy in women with diabetes mellitus. Med. J. Aust., 146: 187–190.

**59) Small M, Cameron A, Lunan C B and MacCuish A C(1987):** Macrosomia in pregnancy complicated by insulin dependent diabetes mellitus. Diabetes Care, 10: 594–599.

**60) Martin M E, Garcia A M, Blanco L, Herrera E and Salinas M (1995):** Effect of streptozotocin diabetes on polysomal aggregation and protein synthesis rate in the liver of pregnant rats and their offspring. Biosci. Rep., 15: 15–20.

**61) Vohr B B, Lipsitt L P and Oh W (1980):** Somatic growth of children of diabetic mothers and reference to birth size. J. Pediatr., 97: 196–199.

**62) Pettitt D J, Nelson R G, Saad M F, Bennett P H and Knowler W C (1993):** Diabetes and obesity in the offspring of Pima Indian women with diabetes during pregnancy. Diabetes Care, 16: 310–314.

**63) Portha B, Chavey A and Movassat J (2011):** Early-life origins of type 2 diabetes: fetal programming of the beta-cell mass. Experimental Diabetes Research, 2011: 105076-105092.

**64) Griz L, Rodrigues R and Rodrigues . (2003):** Endocrinologia Diabetes. Edited by Bandeira, F., Macedo, G., De Janeiro .pp:883-890.

**65) Rudgy A V, Picula F, Marini G, Damasceno D C, Calderon, I M and Barbosa A P (2013):** Translational research in gestational diabetes mellitus and mild gestational hyperglycemia. Arquivos Brasileiros de Endocrinologia and Metabologia, 57: 497-508.

**66) Sanarya T, Al-Badri1 M, Al-Ani K and Al-Jashamy A (2011):**The effect of aqueous olive leaves extract on the pancreatic islets of streptozotocin induced diabetes mellitus in mice. Annals of Microscopy, 11:4-11.

**67) Eid F Shoman H, Abu Elnaga N and Abd El-Halim H (2014):** Effect of Olive Leaf Extract on the Kidney of Pregnant Diabetic Rats and Their Fetuses. International Journal of Advanced Research (2014), Volume 2, Issue 11, 740-76**.**

**68) Gajbhiye R N and Tambe A S (2014):** Pulmonary function test in type II diabetics. Global J. of Biology, 4(1):20-22.

**69) Jhanwar R, Gupta B S, Sharma S K, Singh V, Ganesh H K, Bhardwaj V, Choudhary R, Khan T and Joshi A (2002):** Ventilatory pulmonary dysfunction in type 2 diabetes mellitus. JAPI., 50: 1473-1483.

**70) Strojek K, Ziora D, Scroczynski J and Okle K (1992):** Pulmonary complications of type1 (insulin dependent) diabetic patients. Diabetologia, 35: 1173 – 1176.

**71) Ruberto G, Baratta M T Deans S . and Dorman H J (2000):** Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. Planta Med., 66(8):687-693.

**72) Ozbek H, Ugras and Dulger H (2003):** Hepatoprotective effect of *Foeniculum vulgare* essential oil. Fitoterapia, 74(3):317-319.

**73) Horn T, Jung J and Christoffersen P (1985):** Alcoholic liver injury: early changes of the Disse space in acinar zone. Liver, 6: 301-310.

**74) Eidi M and Darzi R (2009):** Antidiabetic effect of *Oleaeuropaea* in normal and diabetic rats. Phytother. Res., 23:347-350.

**75) El-Missiry M . and El Gindy A M (2000):** Amelioration of alloxan induced diabetes mellitus and oxidative stress in rats by oil of *Eruca sativa* seeds. Annals of Nutrition and Metabolism, 44:97-100.

**76) Tunez L, Munoz M C, Ferjoo-Lopez L, Valdvira E, Bujalance-Arenas L and Montilla P (2003):** Effect of melatonin on hyper lipidemic nephropathology under constant light exposure. J. Physiol. Biochem., 55(2): 104 -114.

**77)AbdRabu M (2011):** Modulation of radiation injury in pregnant rats by bone marrow transplantation. M.Sc. Zoology Department, Faculty of Science, Al-Azhar University, Cairo.

**78) Shaffie N M, Mors, A, Ali A G and Sharaf H A (2010):** Effect of Craway, Coriander and Fennel on the structure of kidney and islets of Langerhan in alloxan-induced diabetic rats: histological and histochemical study. Researcher, 2(7): 27-40.

**79) Poop, J A and Cattley, R C (1991):** Hepto-biliary system. In: Handbook of Toxicologic Pathology. Haschek WM and Rousseaux CG eds , San Diego, Academic Press. pp: 279 – 314.

**80) Al Dossary A (2007):** Histological and histochemical response to low frequency electromagnetic field on liver, kidney and spleen of adult female's albino rats and their fetuses. M.Sc. Thesis, King Faisal Univ. K.S.A.

**81) Tavafi M, Ahmadvand H and Toolabi P (2012):** Inhibitory effect of olive leaf extract on gentamicin-induced nephrotoxicity in rats. Iranian Journal of Kidney Diseases, 6: 25-32.

**82) Blasiak J, Sikora A, Czechowska A and Drzewoski J (2003):** Free radical scavengers can modulate the DNA-damaging action of alloxan. Acta. Biochimica. Polonica, 50 (1): 205–210.

**83) El- Nabarawy S K (2014):** Oxidative damage in embryo and placenta of streptozotocin-induced diabetic rats. The Egyptian Journal of Hospital Medicine, 55: 218–227.

**84) Sierens J, Hartley J A and Campbell M J (2001):** Effect of phytoestrogen and antioxidant supplementation on oxidative DNA damage assed using the comet assay. Mutat. Res., 7: 169 –176.

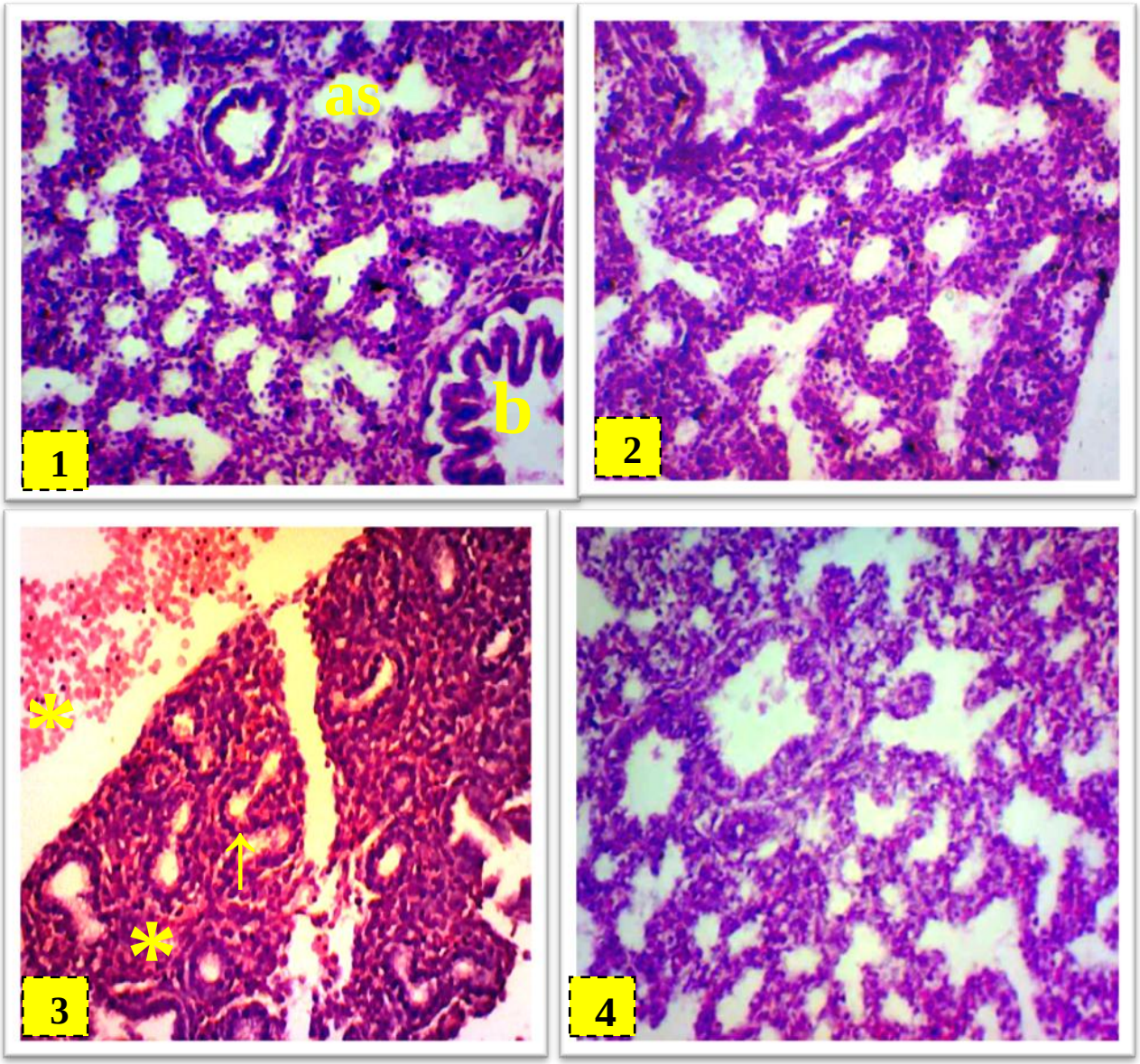



**Fig. 1-** A Photomicrograph of the fetal lung tissue of the control group. Notice: the bronchiole (b), alveolar sacs (as) and the interalveolar septum ( ⬆ ).

**Fig. 2-** A photomicrograph showing well developed architecture of fetal lung tissue of group **O**.

**Fig. 3-** A Photomicrograph of the fetal lung tissue of group **D** showing congested interalveolar septa (↑), the numerous hemorrhagic areas in and around the lung tissue (*)

**Fig. 4 -**A photomicrograph showing somewhat normal architecture of fetal lung tissue of group **D+ O.**

**(HX&E X200).**

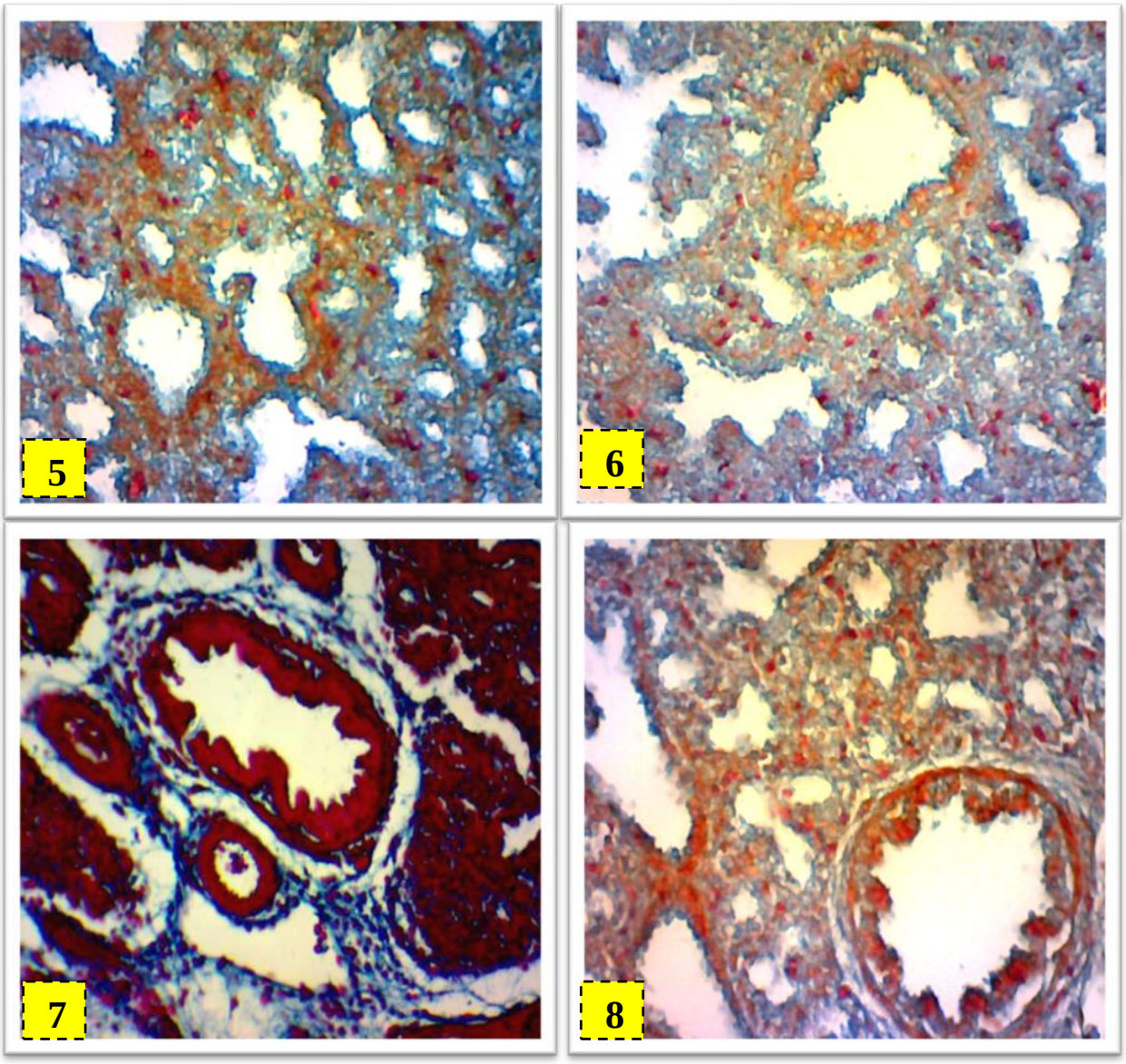



**Fig. 5-** A photomicrograph of a section of fetal lung tissue of the control group showing thin collagen fibres which they support walls of the bronchioles, interalveolar septa and blood vessels.
**Fig. 6-** A photomicrograph of fetal lung tissue of group **O** showing normal distribution of collagen fibres.
**Fig. 7-** A photomicrograph showing highly increased collagen fibres in the fetal lung tissue of group **D** especially around walls of bronchioles, the arterial wall, but they are decreased in the interalveolar septa.
**Fig. 8-** A photomicrograph showing somewhat normal distribution of collagen fibres in the fetal lung of group **D+O.** **(Mallory's trichrome stain X200).**

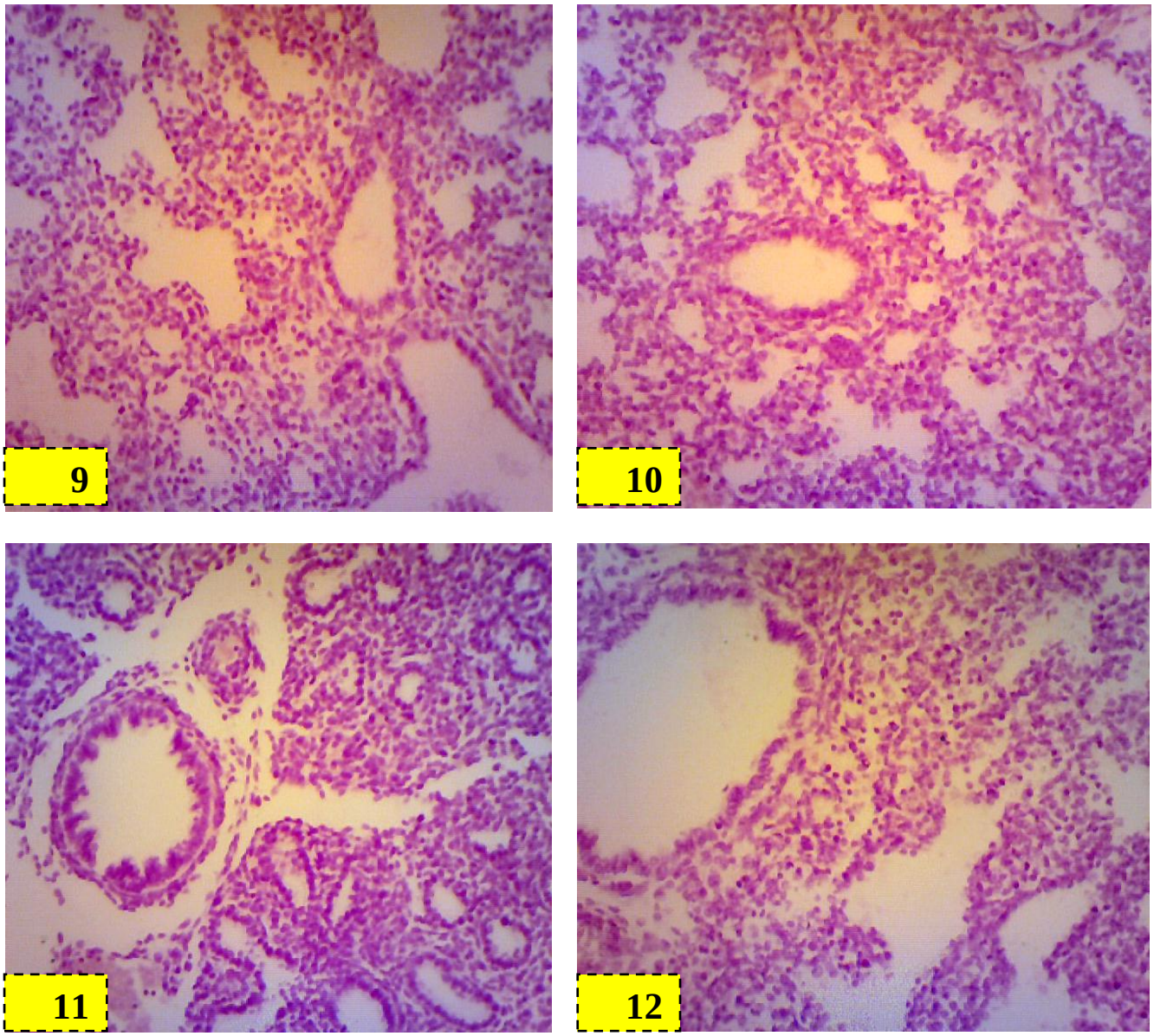



**Fig. 9-** A photomicrograph showing moderately stained DNA materials in the fetal lung tissue of the control group.

**Fig. 10-** A photomicrograph showing moderately stained DNA materials in fetal lung tissue of group **O**.

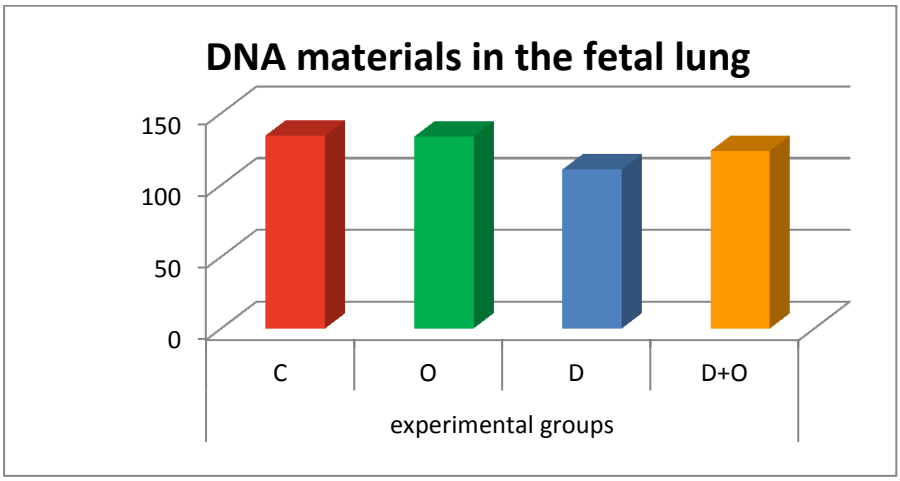
**Fig. 11-** A photomicrograph showing fetal lung tissue of group **D**. Notice: increased DNA materials in some thickened walls of the alveolar sacs, but some of them contain faintly stained nuclei. WBCs in the hemorrhagic area beside the lung tissue are moderately stained, but degenerated areas are negatively stained.

**Fig. 12-** A photomicrograph showing somewhat normal appearance of DNA materials in the fetal lung tissue of group **D+O**. **(Feulgen reaction x 200)**

**Table 1 - Revealing MOD values** of **DNA materials in the fetal lung tissue of the control and treated groups.**

| **Groups** | **C** | **O** | **D** | **D + O** |
|---|---|---|---|---|
| **Average ± SD** | 134.58±6.25 | 133.69±10.82 | 110.82±7.53** | 123.87±5.46 |
| **% of change** | | - 0.899 | -17.66 | -7.96* |

* Significant ** Highly significant (P<0.01)




**Histogram 1- Revealing MOD values of DNA in the fetal lung tissue of the control and treated groups.**

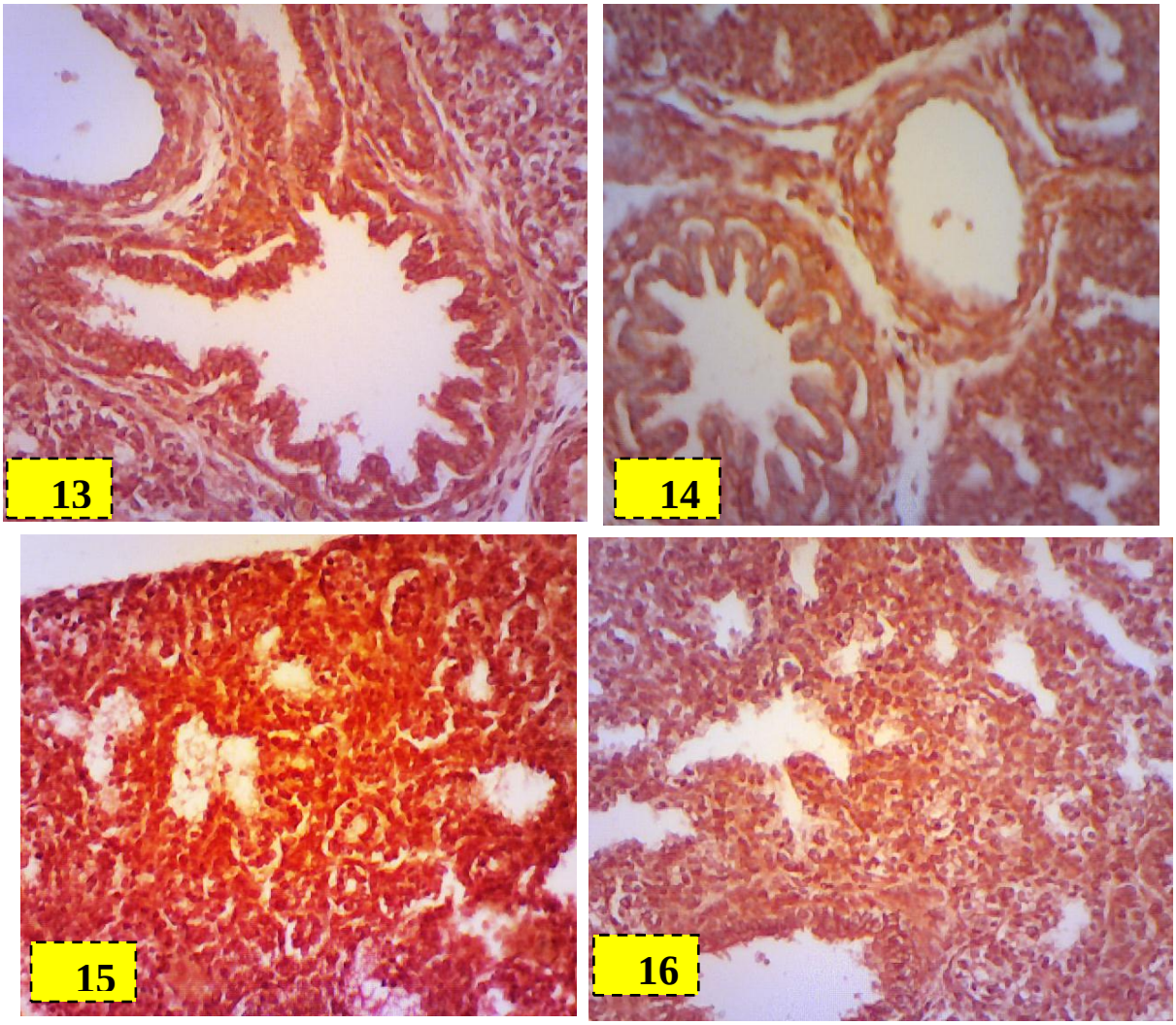



**Fig. 13-** A photomicrograph showing faintly stained amyloid protein in the fetal lung tissue of the control group.
**Fig. 14-** A photomicrograph faintly stained amyloid protein in the fetal lung tissue of group **O**.
**Fig. 15-** A photomicrograph showing increased amyloid protein in the highly thickened interalveolar septa of fetal lung tissue of group **D**.
**Fig.16-** A photomicrograph showing slightly increased amyloid protein in wall of some interalveolar septa in fetal lung tissue of group **D+O**. (Congo red X 200)

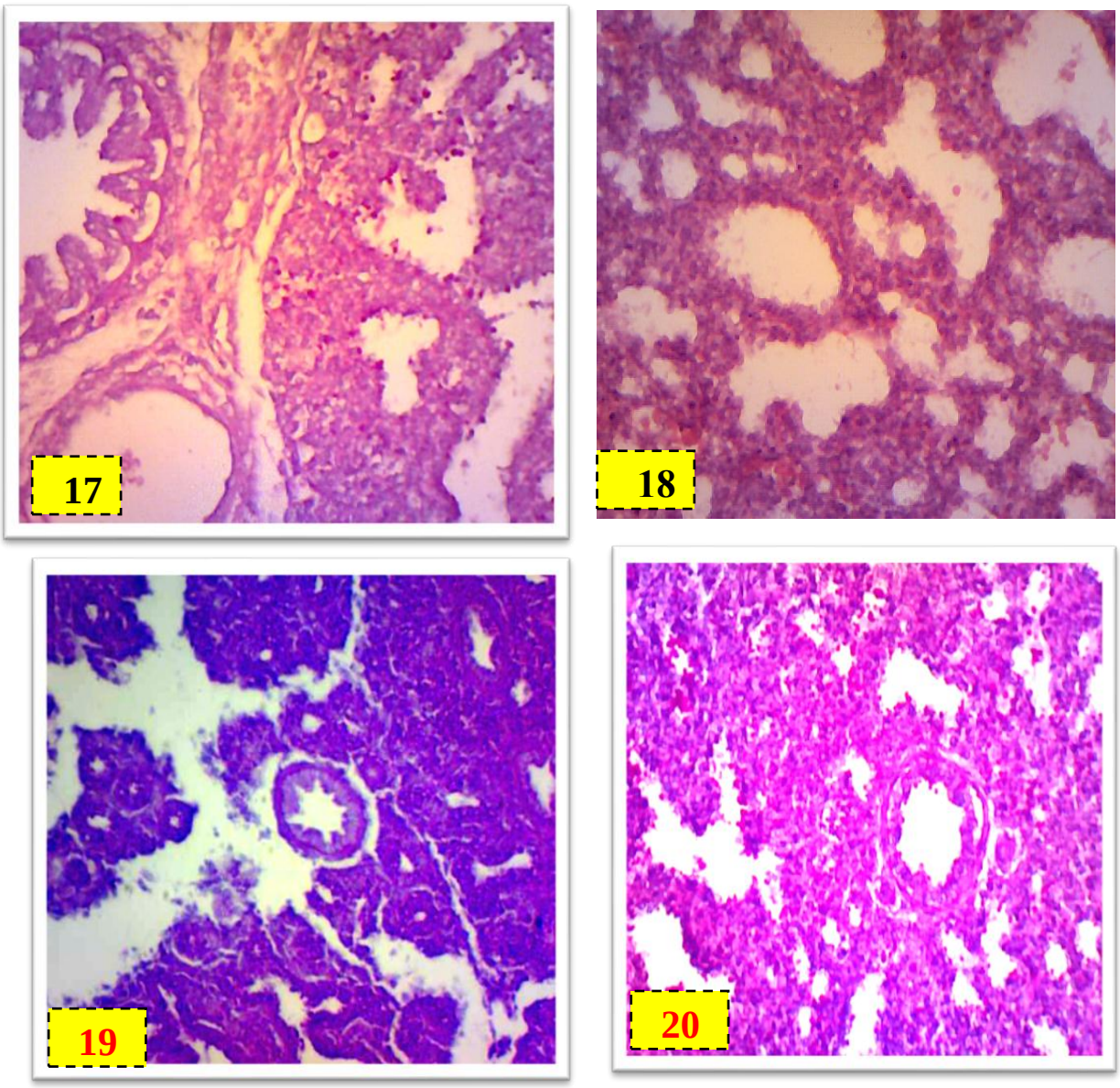



**Fig. 17-** A photomicrograph showing normal distribution of PAS +ve materials in the fetal lung tissue of the control group.

**Fig. 18-** A photomicrograph showing normal distribution of polysaccharides in the fetal lung tissue of group **O**.

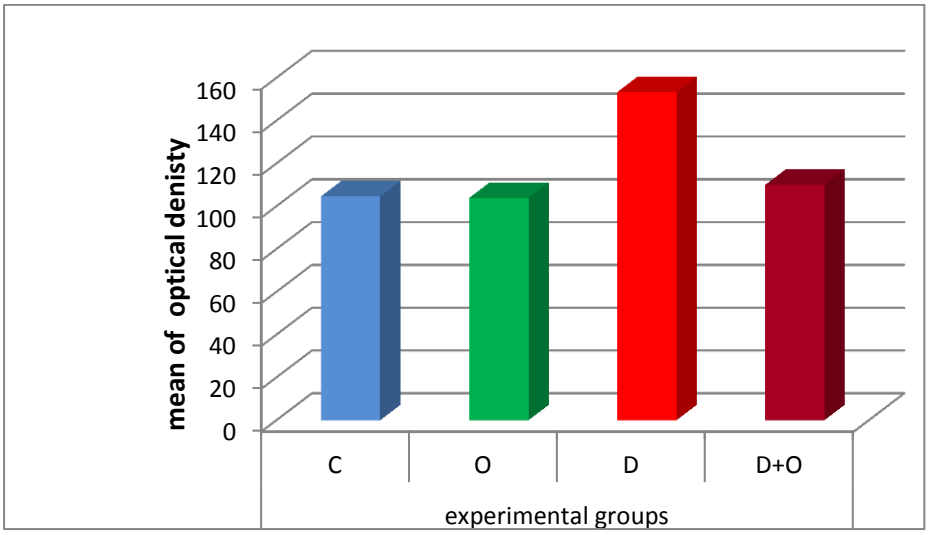
**Fig.19-** A photomicrograph showing deeply stained PAS+ve materials in the bronchioles, walls of the blood vessels and interalveolar septa of the fetal lung tissue of group **D.**

**Fig. 20-** A photomicrograph showing somewhat normal distribution of PAS +ve materials in the fetal lung tissue of group **D+O .**

**Table 2 - Revealing MOD values** of **PAS + ve materials in the lung of the control and treated groups.**

* Significant (P< 0.05) ** Highly significant (P<0.01)

| **Experimental groups** | C | O | D | D+O |
|---|---|---|---|---|
| Average±SD | 104.6±8.43 | 103.9±12.6 | 153.4±11.25** | 110±12.27** |
| % | | -0.669 | 46.65 | 5.162 |




**Histogram 2- Revealing MOD values of PAS +ve materials in the lung of the control and treated groups**

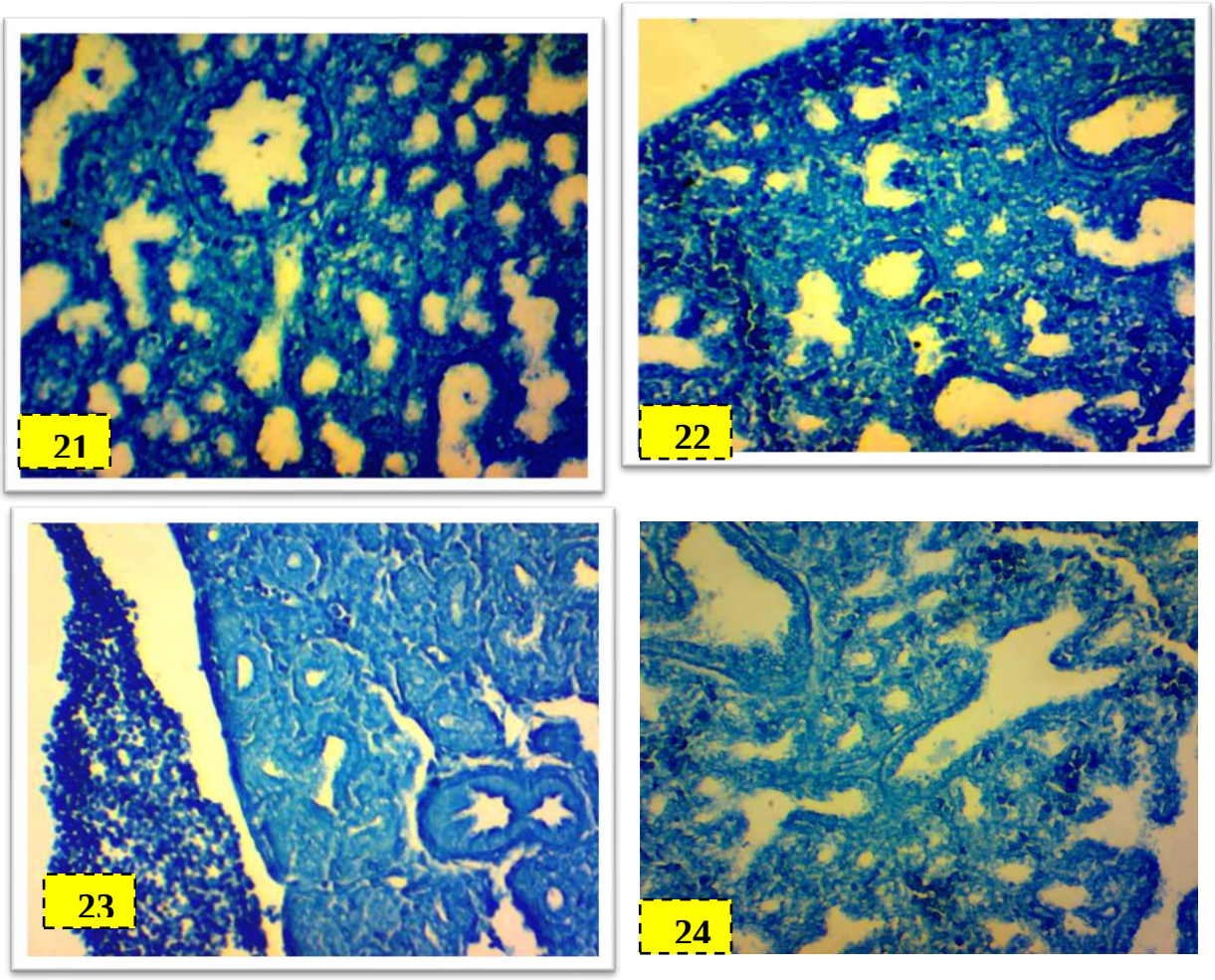



**Fig. 21-** A photomicrograph showing normal distribution of total protein in the fetal lung tissue of the control group.

**Fig. 22-** A photomicrograph showing somewhat normal distribution of total protein in the fetal lung tissue of group O.

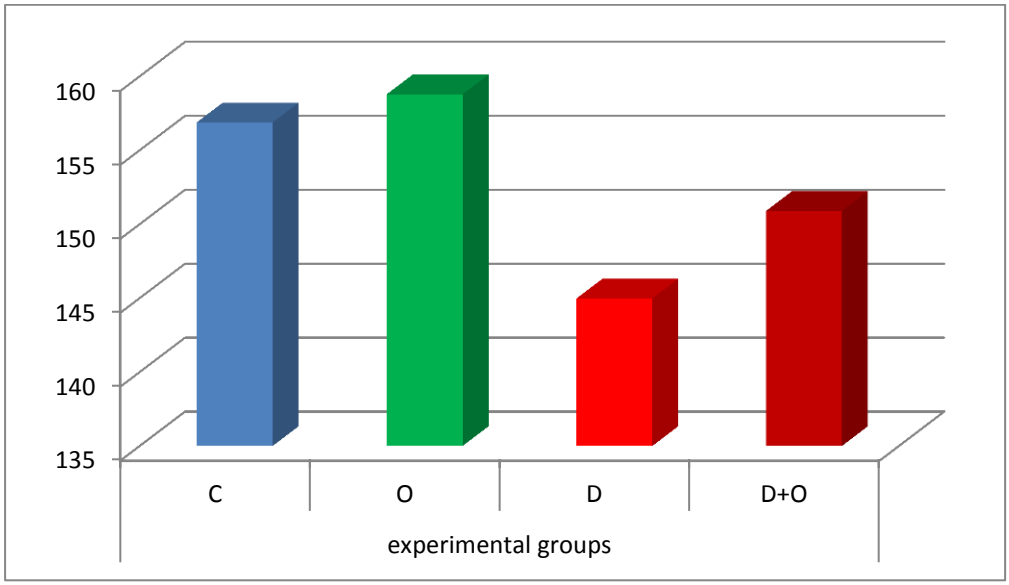
**Fig. 23-** A photomicrograph showing reduced staining affinity of total protein in the interalveolar septa of the fetal lung tissue of group **D**, but large hemorrhagic area beside the lung tissue acquired deep blue staining affinity.

**Fig. 24-** A photomicrograph showing somewhat normal distribution of total protein in the fetal lung tissue of group **D+O .(Mercuric bromophenol blue X 200)**

**Table 3 - Revealing MOD values of protein materials in lung tissue of the control and treated groups**

| **Experimental groups** | C | O | D | D+O |
|---|---|---|---|---|
| Average±SD | 156.88±8.27 | 158.78±5.43** | 144.97±6.55** | 150.90±7.565** |
| % | | 1.212 | -7.58841 | - 3.81 |

* Significant (P< 0.05) ** Highly significant (P<0 ).




**Histogram 3 –Revealing MOD values of total protein in lung of the control and treated groups.**