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THE EFFECT OF MATERNAL NICOTINE EXPOSURE DURING GESTATION AND LACTATION ON THE LUNG OF ALBINO RAT OFFSPRING

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INTRODUCTION

Environmental tobacco smoke is a severe health problem, not only for the whole pediatric population but also for the fetus in utero (**Sekhon et al., 2001; Sandberg et al., 2004**). There is compelling evidence that maternal smoking is associated with premature birth, low birth weight and increased fetal and neonatal morbidity and mortality (**Cliver et al., 1995**). Epidemiological studies have shown that environmental tobacco smoke is associated with a significantly increased incidence of wheezing, bronchitis, lower respiratory illness and increased number of hospital admissions during infancy and childhood (**Lam et al., 2001**). These studies further suggest that there is a stronger correlation between prenatal, rather than postnatal, exposure and lower respiratory illness in the offspring of smoking mothers. Altered mechanical properties of the lungs have also been found in these infants and children including signs of airway obstruction with reduced forced expiratory flow rates (**Brown et al., 1995; Jones et al.,2000**).

The relationship between prenatal nicotine exposure and altered lung development and function has been studied in several animal models (**Sekhon et al., 2001; Elliot et al., 2001., Sekhon et al., 2002**). These studies have shown lung hypoplasia and changes in the airways resulting in increased pulmonary resistance as well as damage of alveolar structure resulting in a condition that resembled panlobular emphysema as the animals aged. In addition, studies of **Maritz (2002)** revealed that the alveolar structure changes of the lungs of rat pups, induced by maternal nicotine exposure, were irreversible and even became progressively worse after nicotine withdrawal. It was recently illustrated that *Klotho* gene expression was essential to maintain pulmonary integrity during postnatal life and its destruction during early lung development might give rise to lung lesions, similar to emphysema, as the lung aged (**Suga et al., 2000**). Therefore, **Maritz (2002)** ascribed the permanent lung changes of the rat pups as result of maternal nicotine exposure to the induction of changes at gene level.

The aim of this work was to investigate the effect of nicotine exposure to the mothers during gestation and lactation on the histological structures

of the lungs of the albino rat offspring and the possible reversibility of this effect after weaning.

MATERIALS AND METHODS

Ten virgin (Sprague-Dawley) adult female albino rats were used in this study. The animals were kept in plastic cages in an air-conditioned animal house (temperature 22 ± 2Ċ) with optimal illumination cycle and free access to drinking water and a pellet diet. The animals were mated overnight and were afterwards divided into two groups as follows:

*Group I (control group; n = 5): the mothers of this group received subcutaneous injection of normal saline.

*Group II (group of nicotine-treated mothers; n=5): the mothers of this group received a daily dose of subcutaneous injection of nicotine (1mg /kg body weight) up to weaning on postnatal day 21.

The daily dose of 1mg nicotine/kg body weight used in this study lies within the range of nicotine levels obtained by the habitual smokers, smoking more than 10 and less than 20 cigarettes/day **(Hafstrom et al., 2002; Sandberg et al., 2004).** Because nicotine readily crosses the placenta and passes into the milk of the mothers **(Maritz and Windvogel, 2003)**, the fetal and neonatal rats would be expected to receive nicotine via placenta and mother's milk up to weaning on postnatal day 21.

Five rat pups of each group were killed by overdose of ether, at postnatal ages of 1, 3 and 7 weeks. The lungs were removed *en bloc* and the total body weight as well as the lung weights of each animal were calculated. The mean body weight and the mean lung weight of the animals of each group, at the same interval, were measured and the percentage of the mean lung weight to the mean body weight was determined as follows: **% = Mean lung weight (gm)/ mean body weight (gm) X 100**. This percentage represented the relative lung weight. The results were subjected to statistical analysis.

A- Light microscopical study

Lung specimens were fixed in 10% formol saline and processed for paraffin blocks. Sections of 3µm in thickness were cut and stained with hematoxylin and eosin, modified Taenzer-Unna orcein **(Drury and Wallington, 1980)** and Masson's trichrome **(Masson, 1924)** for light microscopical study.

B- Histomorphometric quantification

Using the image-analyzer computer assisted by the software Leica Qwin 500 and its binary image with a standard measuring frame of 119616.7 μm^2, the following parameters were estimated:

1. Alveolar count/mm^2 = (number of alveoli in the field ÷ total area of the field in μm^2) x 10^6

2. The percentage of elastic fibers in the field = (Area of elastic fibers ÷ total area of the field) x 100.

3. The percentage of collagen fibers in the field = (Area of collagen fibers ÷ total area of the field) x 100.

4. The cellularity of alveolar septa using the method of **Maritz and van Wyk (1997)**: The length of the septum visualized in the center of the microscopic field was measured and the total number of nuclei, taken as representative of cells lying within the septum, was counted. The cellularity of the septum was measured as the number of cells/mm of septum = (total number of nuclei ÷ length of septum in µm) x 1000.

These data were measured in 10 randomly selected non-overlapping fields from each section and the mean values were obtained. The results were subjected to statistical analysis and represented in tables (Tables 1,2,3,4) and histograms (Figs. 1 ,2,3,4).

C- Statistical analysis

Comparison of significance between group I (control group) and group II was done using Student's "t" test. Data were expressed as mean ± SE.

RESULTS

There were no significant differences in the mean body weight and the relative lung weight between the control group and group II at any time interval.

A-Statistical results of the histomorphometric study

* The measurements of both of the number of alveoli in the respiratory unit (mm2) (Table 1; Fig. 1) and the percentage of elastic tissue content of the lung parenchyma (Table 2; Fig. 2) of the rat pups of nicotine exposed mothers (group II) demonstrated statistically significant reduction, compared with the age-matched control, from the first week of their age. The drop in the

values of the measurements of these parameters, compared with the control, continued until the postnatal age of 7 weeks (4 weeks after removal of nicotine). The reduction at the postnatal ages of 3weeks and 7 weeks was highly significant compared with the age-matched control.

* Measurement of the percentage of collagen fibers in the lung parenchyma of rat pups of nicotine–exposed mothers (Table 3; Fig. 3) showed statistically significant and highly significant increase of their values at the postnatal ages of 3 and 7 weeks, respectively, compared with the age-matched control.
* Septal cellularity of lung tissue (Table 4; Fig. 4) of the rat pups of mothers treated with nicotine (group II) showed statistically significant increase, compared with the age-matched control, at the postnatal ages of one week and 3 weeks. However, at the postnatal age of 7weeks, the septal cellularity of groups II did not show significant changes, compared with the age-matched control.

Table 1: The mean number of alveoli / mm2 of the lung tissue of group I and II

Age	Group I	Group II
	Number / mm2 ± SE	Number / mm2 ± SE
1 week	687.7 ± 15.76	419.16 ± 16.16*
3 weeks	897.2 ± 18.88	457.17 ± 13.49**
7 weeks	1102.11 ± 17.01	494.27 ± 11.98**

*: significant with respect to the control group (P< 0.05)
SE= Standard Error
**: highly significant with respect to the control group (P< 0.01)

Table 2: The mean area percentage of the elastic fibers in the lung parenchyma of the group I and II.

Age	Group I	Group II
	% ± SE	% ± SE
1 week	32.11 ± 2.23	19.11 ± 1.09*
3 weeks	33.61 ± 1.78	11.19 ± 0.90**
7 weeks	34.01 ± 3.30	9.30 ± 0.72**

*: significant with respect to the control group (P< 0.05)
SE= Standard Error
**: highly significant with respect to the control group (P< 0.01)

Table 3: The mean area percentage of the collagen fibers in the lung parenchyma of the group I and II.

Age	Group I	Group II
	% ± SE	% ± SE
1 week	4.1 ± 0.29	5.97 ± .91
3 weeks	4.56 ± 0.23	10.18 ± 0.77*
7 weeks	4.59 ± 0.19	11.36 ± 0.51**

*: significant with respect to the control group (P< 0.05)
SE = Standard Error
**: highly significant with respect to the control group (P< 0.01)

Table 4: The cellularity of alveolar septa in group I and group II

Age	Control	Nicotine
	Cells/mm ± SE	Cells/mm ± SE
1 week	327.04 ± 12.76	522.73 ± 12.95*
3 weeks	400.43 ± 11.22	863.47 ± 17.09**
7 weeks	436.41 ± 12.30	470.65 ± 17.77

*: significant with respect to the control group (P< 0.05)
SE= Standard Error
**: highly significant with respect to the control group (P< 0.01)

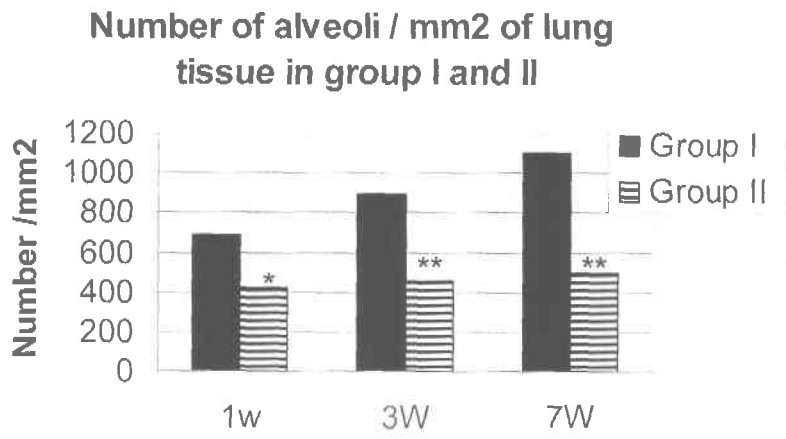


Fig. (1): A histogram showing the mean number of alveoli / mm2 of lung tissue of group I and II.
*: significant with respect to the control group (P< 0.05).
**: highly significant with respect to the control group (P< 0.01). W = week

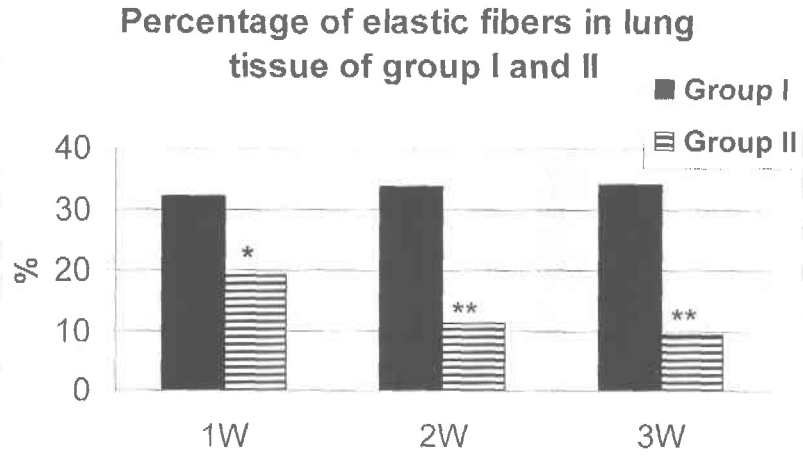


Fig. (2): A histogram showing the mean area percentage of the elastic fibers in the lung parenchyma of the group I and II.
*: significant with respect to the control group (P< 0.05).
**: highly significant with respect to the control group (P< 0.01). W = week

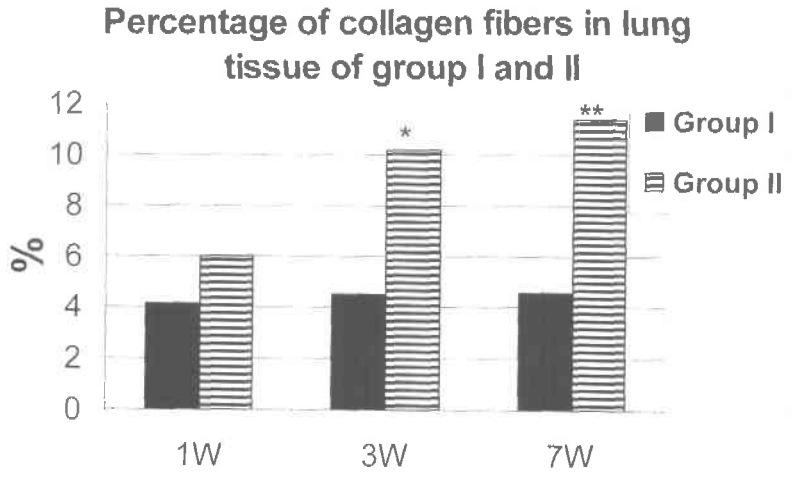


Fig. (3): A histogram showing the mean area percentage of the collagen fibers in the lung parenchyma of group I and II.
*: significant with respect to the control group (P< 0.05).
**: highly significant with respect to the control group (P< 0.01). W = week

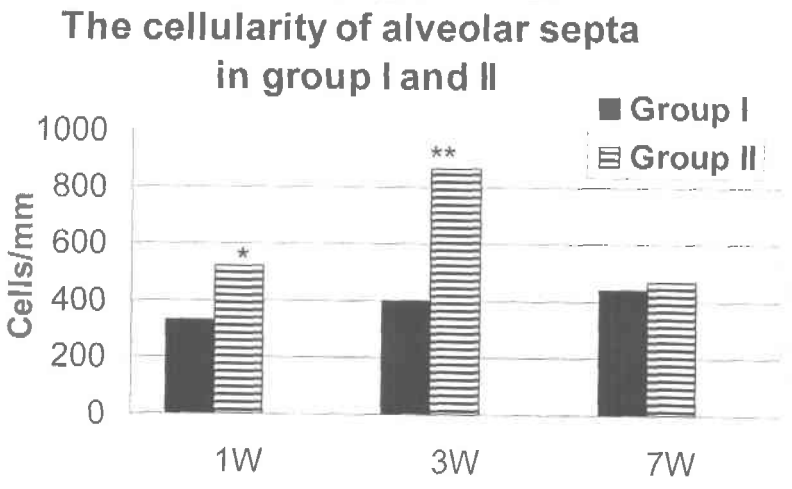


Fig. (4): A histogram showing the cellularity of alveolar septa in group I and group II
*: significant with respect to the control group (P< 0.05).
**: highly significant with respect to the control group (P< 0.01). W = week

Histological study

Control group (group I)

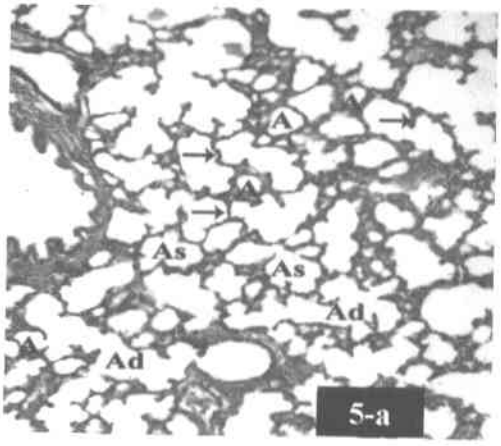
The histological study of specimens of the control group showed several long passages, alveolar ducts, which opened along their length into numerous alveolar sacs and alveoli with normal thickness of alveolar septa (Fig.5-a). The alveolar walls were lined with many Type I pneumocytes with flattened nuclei and few large Type II pneumocytes with rounded nuclei (Fig.5-b) and vesicular cytoplasm. The inter-alveolar septa showed minimal amount of collagen fibers (Fig.6-a) and considerable amount of intact elastic fibers (Fig.6-b).

Group II (group of nicotine-exposed mothers)

At the postnatal age of one week, the lung specimens from the newly born animals of this group showed aggregation of considerable number of mast cells in the pleura; some of these cells were completely or partially degranulated (Fig.7). The alveolar tissues showed congestion and extravasation of red blood cells (RBCs) into the alveolar lumens with many hemosedrin-containing macrophages (Fig. 8). There was marked inflammatory cell infiltration of the lung parenchyma including numerous eosinophils, neutrophils and macrophages (Fig. 9). The alveolar walls showed sites of damaged Type I pneumocytes and increased number of the Type II pneumocytes; some of them were dividing (Fig. 10).

At the age of three weeks, the lung specimens of rat pups showed areas of cellular infiltration and cellular exudate with collapsed alveoli and thickened septa (Figs. 11-a,b) while other areas showed expanded alveoli with ruptured septa (Fig. 11-a). The alveolar lumens were infiltrated with large number of foamy macrophages (Fig. 12). Proliferation and migration of Type II pneumocytes and deposition of fibroblasts in the inter-alveolar septa were also detected (Figs. 13-a,b). There was considerable reduction of the elastic fibers (Fig. 14) as well as increased fibrous tissue deposition (Figs.11-b) in the lung parenchyma compared with the control.

At the age of seven weeks (4 weeks after weaning) the lung specimens of the animals of this group showed areas of massive alveolar collapse with loss of normal lung architecture (Fig. 15). Other areas showed marked expansion of the alveoli with ruptured septa leading to alveolar coalescence (microscopical emphysema) (Fig. 16). Considerable increase in fibrous tissue formation with marked reduction in the elastic tissue content of the lung parenchyma, compared with those of the control, could be clearly seen (Figs. 17-a,b).



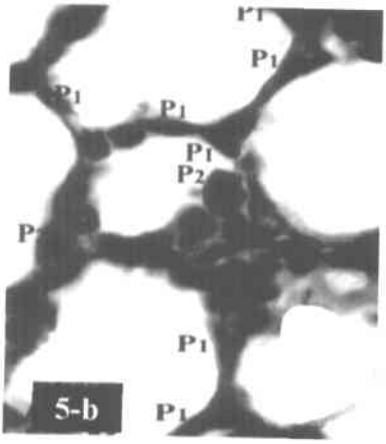
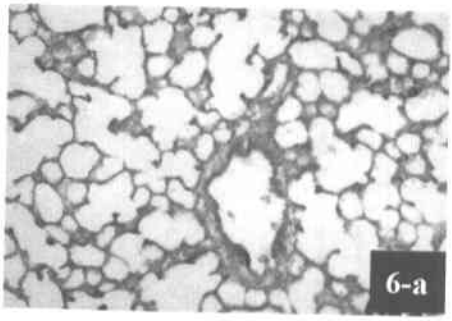


Fig. (5-a,b): Photomicrographs of cross sections of lung specimens from rat pups of the control group (group I) showing:
(a): Normal alveolar ducts (Ad) with numerous alveolar sacs (As) and alveoli (A). Note the thin alveolar septa between adjacent alveoli (arrows). (Hx.& E.; x 100)
(b): Alveolar walls lined with many Type I pneumocytes with flattened nuclei (P1) and few large Type II pneumocytes with rounded nuclei (P2). (Hx.& E.; x 1000)



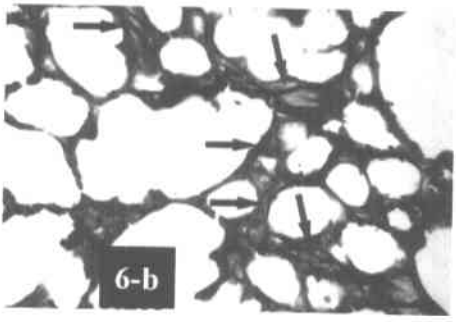


Fig. (6-a,b): Photomicrographs of cross sections of lung specimens from rat pups of the control group (group I) showing:
(a): Minimal amount of collagen fibers in the lung parenchyma. (Masson's trichrome; x 200)
(b): Considerable amount of intact elastic fibers in the inter-alveolar septa (arrows). (Modified Taenzer-Unna orcein; x 400)

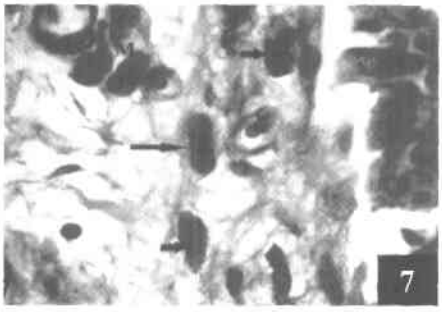


Fig. (7): A photomicrograph of a cross section of a lung specimen from a rat pup of nicotine-exposed mother (group II), 1 week after birth, showing aggregation of considerable number of mast cells in the pleura (M), some of which are partially (short arrows) or completely (long arrow) degranulated. (Hx.& E.; x 1000)

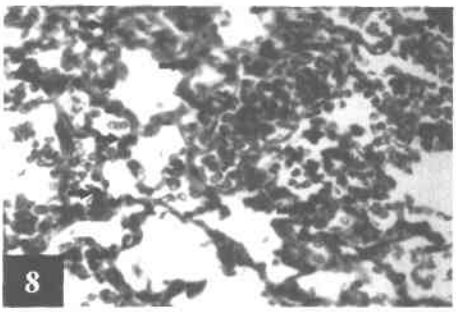


Fig. (8): A photomicrograph of a cross section of a lung specimen from a rat pup of nicotine-exposed mother (group II), 1 week after birth, showing congestion and extravasation of RBCs into the alveolar lumens with many hemosedrin-containing macrophages (arrows). (Hx.& E.; x 400)

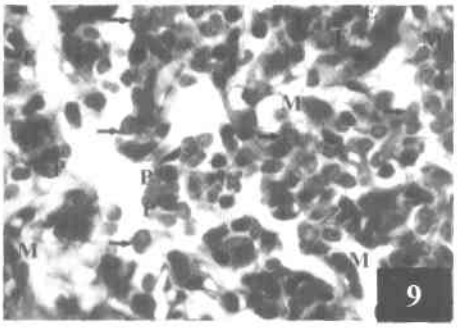


Fig. (9): A photomicrograph of a cross section of a lung specimen from a rat pup of nicotine-exposed mother (group II), 1 week after birth, showing marked inflammatory cell infiltration including numerous eosinophils (E), neutrophils (P) and macrophages (M). Note the extravasated RBCs (arrows). (Hx.& E.; x 1000)

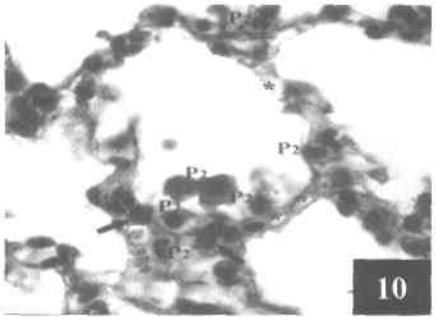


Fig. (10): A photomicrograph of a cross section of a lung specimen from a rat pup of nicotine-exposed mother (group II), 1 week after birth, showing alveolar walls with sites of degenerated Type I pneumocytes (*) and increased number of the Type II pneumocytes (P2), some of which are dividing (arrows). (Hx.& E.; x 1000)

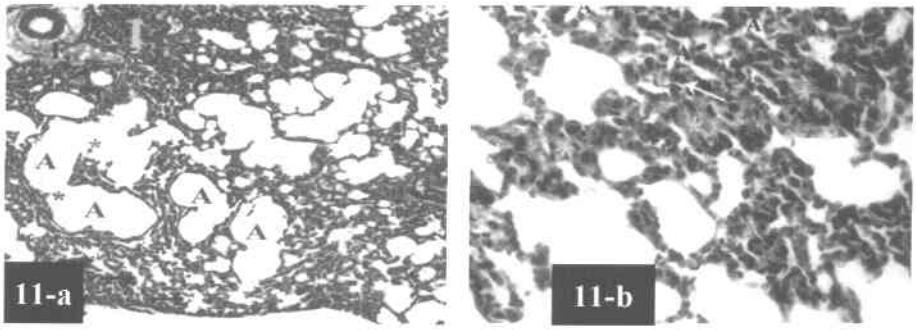


Fig. (11-a, b): Photomicrographs of cross sections of lung specimens from rat pups of nicotine-exposed mothers (group II), 3 weeks after birth, showing:
(a): Areas of alveolar collapse (arrows) and thickened inter-alveolar septa with cellular exudate (E) and cellular infiltration (I). Note the area of expanded alveoli (A) with ruptured septa (*). (Hx.& E.; x 400)
(b): Collapsed alveoli (A) with thickened septa (arrow) and increased fibrous tissue formation (*).(Masson's trichrome; x 400)

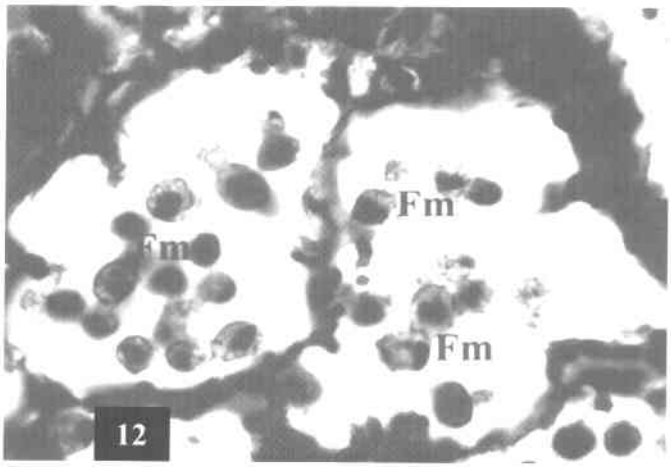


Fig. (12): A photomicrograph of a cross section of a lung specimen from a rat pup of nicotine-exposed mother (group II), 3 weeks after birth, showing alveolar lumens infiltrated with large number of foamy macrophages (Fm). (Hx.& E.; x 1000)

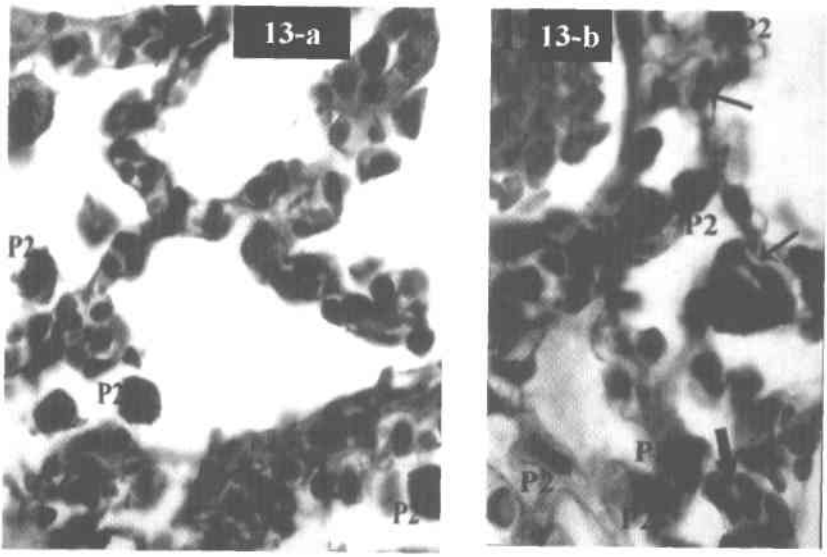


Fig. (13-a,b): Photomicrographs of cross sections of lung specimens from rat pups of nicotine-exposed mothers (group II), 3 weeks after birth, showing:
(a): Migration of Type II pneumocytes (P2). (Hx.& E.; x 1000)
(b): Many Type II pneumocytes (P2), some are in a dividing stage (thin arrows). Not the deposited fibroblasts (thick arrow) in the inter-alveolar septa. (Hx.& E.; x 1000)

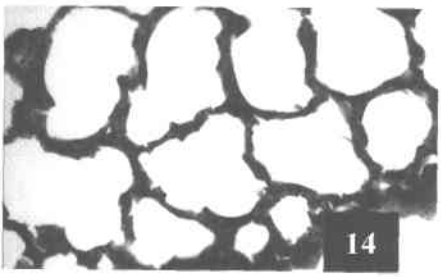


Fig. (14): A photomicrograph of a cross section of a lung specimen from a rat pup of nicotine-exposed mother (group II), 3 weeks after birth, showing reduction of the elastic tissue content of the lung parenchyma. (Modified Taenzer-Unna orcein; x 400)

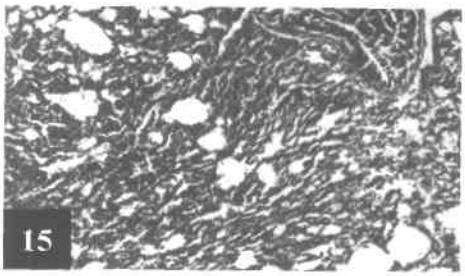


Fig. (15): A photomicrograph of a cross section of a lung specimen of a rat pup of a nicotine-exposed mother (group II), 7 weeks after birth, showing massive alveolar collapse with loss of normal lung architecture. (Hx.& E.; x 100)

Fig. (16): A photomicrograph of a cross section of a lung specimen from a rat pup of nicotine-exposed mother (group II), 7 weeks after birth, showing marked expansion of the alveoli with ruptured septa (arrows) leading to alveolar coalescence. (Hx.& E.; x 100)

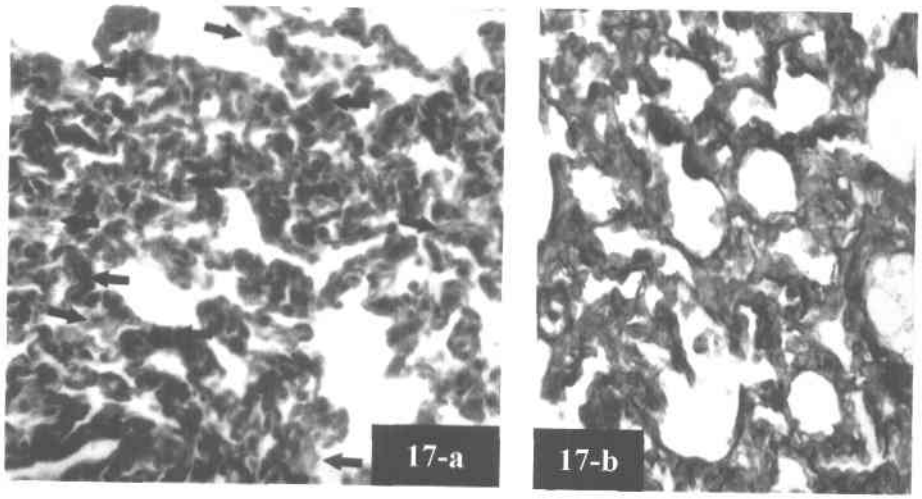


Fig. (17-a,b): Photomicrographs of cross sections of lung specimens from rat pups of nicotine-exposed mothers (group II), 7 weeks after birth, showing:

(a): Considerable increase in fibrous tissue formation (arrows). (Masson's trichrome; x 400)

(b): Marked reduction of the elastic tissue content. (Modified Taenzer-Unna orcein; x 400)

DISCUSSION

Maternal smoking, even passive smoking are recognized as resulting in an increased incidence of respiratory disease of the offspring with damage to their normal alveolar structure **(Gamieldin and Maritz, 2004; Sandberg et al., 2004).** The present study showed that maternal nicotine exposure during gestation and lactation exerted adverse effects on the histological structure of the lungs of the neonatal rats, without significant differences in their body weight or the relative lung weight compared with the control. Similar findings were demonstrated by **Maritz and Windvogel (2003)** and **Sandberg et al. (2004)** who were working on rat and lamb pups and emphasized that maternal nicotine exposure had no effect on length of gestation, body weight and lung volume of the neonates at birth and up to maturity. On contrary, **Teasdale and Ghislaine (1989), Cliver et al. (1995) and SeKhon et al. (2002),** working on human and monkey neonates, claimed that maternal smoking was associated with premature birth, low birth weight and lung hypoplasia as result of adverse effects of nicotine on the utero-placental circulation and thus the nutrient supply to the fetuses. In addition, **Bardy et al. (1993)** demonstrated a positive correlation between the concentration of nicotine in the human maternal blood and fetal growth retardation. However, this contradiction could be contributed to species sensitivity differences.

The present study demonstrated aggregation of large number of mast cells in the lung pleura of the prenatally nicotine-exposed rat pups as early as the first week of their life with degranulation of some of these cells. This was accompanied with massive infiltration of the lung parenchyma with large number of inflammatory cells. It is well known that mast cells form and store histamine in their granules, which is related to allergy and antigen antibody reaction **(Williams et al., 1995)**. This leads to suggestion that the maternal nicotine was irritant to the lung tissue of the rat pups resulting in activation of the mast cells, which in turn initiated the inflammatory reaction and its sequences. This suggestion is supported by the findings of **Jensen et al. (1998)** who revealed that the total leucocytic count as well as, neutrophil and eosinophil blood counts were all higher in smokers than non smokers and they showed a dose dependent.

In the present work, maternal nicotine exposure interfered with the morphometric and morphologic characteristics of the alveolar septa of the offspring leading to its thickening and an increase in its cellularity with considerable proliferation and aggregation of Type II pneumocytes and degeneration of Type I pneumocytes. Similar findings were reported by **Maritz and Thomas (1994) and Sekhon et al. (1999**). Moreover, **Maritz and van Wyk (1997)** attributed the decrease in the ratio of Type I to Type II pneumocytes and the increase in the septal cellularity to Type II cell proliferation and differentiation in response to Type I cell damage induced by maternal

nicotine exposure. This is compatible with the present histological and histomorphometric results, which revealed that lungs of rat pups, of nicotine-exposed mothers, showed proliferation and aggregation of large number of type II pneumocytes with more increase in the septal cellularity, compared with the age matched control, up to the postnatal age of 3 weeks (end of period of nicotine exposure).

The current histological and histomorphometric studies showed that maternal nicotine exposure caused progressive and statistically significant reduction of the elastic tissue content of the lung of rat pups, compared with the control, up to the postnatal age of 7 weeks. Identical observations were reported by **Maritz and Woolword (1992)** and **Maritz and van Wyk (1997)**. Furthermore, the statement of **Dolley (1995)** that most of the elastic tissue of neonatal lung was deposited before birth and that the maternal nicotine prenatally interfered with the process of elastogenesis in the lung of rat pups, matches perfectly the present findings that revealed early significant reduction of the lung elastic tissue content of the neonates of nicotine-exposed mothers, as compared with the age matched control. In contrast, **Sekhon et al. (2001) and Sandberg et al. (2004)** demonstrated that prenatal nicotine exposure of rhesus monkeys and lambs did not affect their lung elasticity, although it altered their lung development. This can lead to suggestion that elastic tissue affection as results of prenatal nicotine exposure is dependant on species sensitivity.

It is postulated that progressive destruction of the elastic tissue of alveolar walls of prenatally nicotine-exposed pups is caused by endogenously released enzymes that may degrade reticulin, elastin and ground substance of lung parenchyma **(Maritz, 2002).** In agreement, **Rcilly and Chapman, (1988)** showed that in smokers' lungs, there were increased levels of neutrophil elastase activity as well as accumulation of macrophages in the lung parenchyma, which had the potential to participate in connective tissue turnover and lung destruction. Moreover, **Sukura et al. (1995)** and **Yuasa and Kanazawa (1995)** postulated that in lung diseases, elastase activity was correlated with neutrophils and foamy alveolar macrophages, which might be, in part, derived from these cells. In the current experiment, the association of progressive elastic tissue destruction with marked neutrophil and eosinophil infiltration of the lung parenchyma and the aggregation of foamy macrophages in alveolar lumens of prenatally nicotine-exposed rat pups are fully consistent with the above-mentioned postulations.

The current histomorphometric study revealed that the alveolar count of the rat pups of nicotine-exposed mothers showed marked reduction, which was statistically significant as compared with the age-matched control, during the whole period of the experiment (up to 4 weeks after weaning). Equivalent observation was reported by **Maritz and Windvogel**

(2003) who attributed that reduction to the role of maternal nicotine on suppression of the process of alveolarisation and retardation of secondary septa formation. This explanation is supported by a previous study of **Vidic et al. (1989)** who revealed that chronic exposure of rats to whole cigarette smoke during pregnancy induced a slower pace of septal growth and thus of alveolarisation in the lungs of the offspring. The histological findings of the present work that showed destruction of the alveolar septa resulting in the fusion of adjacent alveoli with formation of abnormally large ones (microscopical emphysema) give another explanation for the reduction of the alveolar count in the rat pups of nicotine-exposed mothers and are supported by similar findings deduced from other studies **(Maritz and van Wyk, 1997; Maritz and Windvogel, 2003)**. Moreover, **Maritz and Woolword (1992)** and **Maritz and van Wyk (1997)** added that since the elastic tissue is a part of lung connective tissue structure involved in the formation of alveoli, therefore the low alveolar count at the onset of the period of rapid alveolarisation was due to the adverse effect of maternal nicotine exposure on elastogenesis in neonatal lung.

Maritz (2002) explained two mechanisms for the development of emphysema; one was the coalescence of the alveoli as result of ruptured septa and the second mechanism was alveolar dilatation with retraction of the alveolar septa that became progressively shorter until completely effaced. The histological findings of the present work are in favor of the former mechanism for the development the emphysematous changes in the lungs of rat pups of nicotine-exposed mothers and are supported by identical observations represented by **Maritz and Dennis (1998)** and **Maritz and Windvogel (2003)**.

The current histological and histomorphometric studies showed that despite the fact that the animals were not exposed to nicotine after weaning, there was progressive reduction of both of the alveolar number and the elastic fibers content, leading to emphysematous changes, as well as an increase in the fibrous tissue formation in the lung parenchyma of these animals, as compared with the age-matched control. This can lead to the suggestion that the maternal nicotine-exposure resulted in permanent structural damage of the offspring's lungs. Such suggestion is in full agreement with the conclusions deduced from the previous studies **(Martiz and Dennis, 1998; Maritz and Windvogel, 2003; Gamieldin and Maritz, 2004; Sandberg et al., 2004). Maritz (2002)** attributed the permanent and progressive lung changes of the rat pups of nicotine-exposed mothers to induction of changes at gene level during early lung development, which rendered the lung of the offspring more susceptible to emphysematous changes. Moreover, **Finlay et al. (1997)** and **Suga et al. (2000)** determined the blamed gene, named *Klotho* gene, which was essential for maintaining the pulmonary integrity during postnatal life. They added that mutation of this gene during pregnancy and early lung development might result in

gradual development of pulmonary emphysema as the animals aged. On the other hand, **Maritz and Windvogel (2003)** ascribed the irreversible lung changes to the retardation of secondary septal formation, which is essential for alveolar formation resulting in reduction of alveolar count despite of nicotine withdrawal after weaning.

Maritz and van Wyk (1997) assumed that nicotine exerted its effect on fetal lung through its metabolic product nicotine-n-oxide, which had oxidant properties. Inhibition of glycolysis and chemoattraction of neutrophils, enhancing their superoxide anion generation to which type I pneumocytes were sensitive (**Maritz and Thomas, 1994**) as well as induction of lipid peroxidation in rat lung **(Helen et al., 1999; Kalpana and menon, 2004)** were among the oxidative activities of nicotine demonstrated in previous studies. In addition, other studies showed that smoking of nicotine caused a marked reduction of ascorbic acid of adult lung, thereby, rendering the lung more vulnerable to the effect of oxidants **(Maritz, 1993).** In the present work, the infiltration of large number polymorphonuclear leucocytes in the lung parenchyma of rat pups of nicotine-exposed mothers with damage of the alveolar septa and type I pneumocytes matches the observations represented by **Maritz and Thomas (1994).** Those authors revealed that these lung changes, induced by maternal nicotine exposure, corresponded well with the changes induced by oxidants. Furthermore, limitation of the deleterious effects of maternal nicotine-exposure on offspring's lung structures as result of maternal treatment with antioxidant oils isolated from garlic and onion (**Helen et al., 1999**) or with ascorbic acid **(Maritz and van Wyk, 1997),** adds further evidence in support of the hypothesis that nicotine may induce its damaging effects via generation of oxidants. However, the prophylactic role of antioxidants needs further study.

From the above data, it can be concluded that maternal nicotine exposure during gestation and lactation has deleterious effects on the lung histological structures of the rat offspring. Although no direct evidence is available, the persistence and the progression of the lung changes after nicotine withdrawal imply that these changes could be induced at gene level. Whether maternal antioxidants administration has a prophylactic role against these nicotine-induced damaging effects needs further studies.

SUMMARY

The aim of this work was to investigate the effect of maternal nicotine exposure, during gestation and lactation, on the lung structure of the albino rat offspring, and its reversibility after nicotine withdrawal. Ten adult female albino rats were used in this study and after mating, they were divided into two groups; group I (control group) received daily a subcutaneous injection of normal saline and group II received daily a subcutaneous injection of nicotine (1mg /kg body weight). The above-mentioned treatment was continued up to weaning on postnatal day 21. Five rat pups of each group were

killed at postnatal ages of 1, 3 and 7 weeks. The Body weight and the lung weights of each killed animal were calculated, and then the lung specimens were processed for histomorphometric and histological studies.

The histomorphometric study revealed that the percentage of elastic tissue and the number of the alveoli/ mm2 of the lung parenchyma of the rat pups of nicotine-exposed mothers showed a statistically highly significant reduction, compared with the control, as the animals aged. The percentage of collagen fibers in the lung parenchyma of this group of animals showed progressive and statistically significant increase compared with the control.

The histological study showed that maternal nicotine exposure resulted in marked affection of the lung parenchyma of the rat pups including massive cellular infiltration, thickening of the alveolar septa with increase of their cellularity, proliferation and migration of Type II pneumocytes, damage of the elastic tissue and increased fibroblast deposition. Alveolar collapse with loss of normal lung architecture as well as rupture septa and coalescence of alveoli giving picture of microscopical emphysema were also noticed. These changes were irreversible as they maintained and progressed even after withdrawal of nicotine following weaning.

In conclusion, maternal nicotine exposure during gestation and lactation has permanent deleterious effects on the lung histological structures of the rat offspring and the progression of these changes after nicotine withdrawal implies that these changes could be induced at gene level.

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الملخص العربى

تأثير تعرض الأم للنيكوتين أثناء الحمل و الرضاعة على رئة الفئران البيضاء المولودة

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الهدف من هذا العمل هو بحث تأثير تعرض الأم للنيكوتين أثناء الحمل والرضاعة على بنية رئة الفئران البيضاء المولودة ومدى انعكاسه بعد توقف التعرض للنيكوتين. وقد استخدم في هذا البحث عشر فئران من الاناث البالغة وتم تقسيمهم بعد التزاوج الى مجموعتين؛ المجموعة الأولى (المجموعة الضابطة) وقد حصلت يوميا على حقن تحت الجلد بمحلول ملحي والمجموعة الثانية وقد حصلت يوميا على حقن تحت الجلد بمادة النيكوتين (١ مجم/كم من وزن الجسم) وقد استمر العلاج السابق حتى موعد الفطام في اليوم الواحد والعشرين بعد الولادة. وتم التضحية بخمسة من صغار الفئران من كل مجموعة عند عمر أسبوع وثلاثة أسابيع وسبعة أسابيع من تاريخ الولادة. وأحتسب وزن الجسم ووزن الرئة لكل فأر ثم تم تجهيزعينات الرئة للدراسة الهيستومورفومترية والهيستولوجية.

وقد كشفت الدراسة الهيستومورفومترية تناقصا ذو دلالة احصائية عالية في نسبة الأنسجة المطاطية وكذلك في عدد الحويصلات الهوائية في كل مليمتر المربع من برنشيمة رئة صغار الفئران للأمهات المعرضة للنيكوتين بالمقارنة بالمجموعة الضابطة مع تقدم عمر الحيوانات. وكذلك أظهرت نسبة ألياف الكولاجين ببرنشيمة رئة هذه الفئران زيادة متصاعدة وذو دلالة احصائية بالمقارنة بالمجموعة الضابطة.

وقد أظهرت الدراسة الهيستولوجية أن التعرض للنيكوتين عن طريق الأم الحامل نتج عنه أصابة واضحة في برنشيمة رئة صغار الفئران والتي شملت ارتشاح خلوي شديد وزيادة في سمك حواجز الحويصلات الهوائية مع زيادة خلويتها وتكاثر ونزوح النوع الثاني للخلايا الرئوية وإصابة الأنسجة المطاطية وكذلك زيادة في ترسيب الخلايا الليفية. وقد لوحظ أيضا اضمحلال الحويصلات هوائية وكذلك تمزق الحواجز فيما بينها

مما نتج عنه انتفاخ الرئة الميكروسكوبي. وقد استمرت هذه التغيرات في الحدوث حتى بعد توقف التعرض للنيكوتين بعد الفطام.

ويستخلص من هذا البحث أن تعرض الأم الحامل للنيكوتين أثناء الحمل والرضاعة له تأثيرات ضارة ودائمة على التركيب الهيستولوجي لرئة الفئران المولودة وأن استمرار حدوث تلك التغيرات بعد توقف التعرض للنيكوتين قد يعطي انطباعا بأن تلك التغيرات قد حدثت على مستوى الجينات.