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# The Role of Carnosine in Protection Against the Damaging Effect of Maternal Nicotine Exposure During Gestation and Lactation on the Lung of Albino Rat Offspring

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The aims of this study were (1) to investigate the effect of maternal nicotine exposure, during gestation and lactation, on the lung histological structures of rat offspring and its reversibility and (2) to establish whether carnosine would protect the neonatal rat lung against the adverse effects of maternal nicotine exposure. After mating, the pregnant albino rats were divided into three groups; group I (control group) received a daily subcutaneous injection of normal saline, group II received a daily a subcutaneous injection of nicotine (1 mg $kg^{-1}$ body weight) and group III received daily both of subcutaneous injection of nicotine (1 mg $kg^{-1}$ body weight) and intramuscular injection of carnosine (10 mg $kg^{-1}$ body weight). The lung tissue of the rat pups was collected for histological and histomorphometric study on postnatal days 7, 21 and 49. The 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. Loss of normal lung architecture and rupture septa with coalescence of alveoli giving picture of microscopical emphysema were also noticed. There was also significant decrease in the alveolar count $mm^{-2}$ and the percentage of elastic tissue fibers with significant increase in the percentage of collagen fibers in the lung parenchyma of these rat pups, compared with age-matched controls. These changes were irreversible as they progressed even after withdrawal of nicotine following weaning, implying that these changes could be induced at gene level. The treatment with carnosine limited the deleterious effects of nicotine on the histological structure of lung parenchyma of rat pups especially the alveolar count, which did not show significant changes compared with the age-matched controls, as time laps. However, carnosine did not prevent completely the induction of microscopic emphysema resulted from maternal nicotine exposure.

**Key words:** Histomorphology, alveolar count, maternal nicotine exposure, microscopical emphysema, cellularity, carnosine

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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.

It has been found that during maternal smoking, nicotine is metabolized by the mother's cells producing nicotine-n-oxide, which has oxidant properties and is carried to the developing fetus interfering with normal fetal and eventually neonatal lung development (Maritz and Thomas, 1994; Maritz and van Wyk, 1997). Whether injury was indeed due to oxidants released under influence of nicotine is not certain. However, studies by Gillespie *et al*. (1987) showed that in vitro exposure to nicotine augments polymorphonuclear leucocyte superoxide anion generation. Moreover, it was found that smoking nicotine caused marked decrease in lung ascorbic acid, thereby rendering the lung more vulnerable to the effect of oxidants (Maritz, 1993; Maritz and van Wyk, 1997).

Carnosine is a naturally occurring dipeptide, beta-alanyl-L-histidine, found in brain, innervated tissues and the lens, at concentrations up to 20 mM in humans, where it represents an appreciable fraction of the total water-soluble nitrogen-containing compounds (Boldyrev, 2000; Nagasawa *et al*., 2001; Fontana *et al*., 2002). Numerous studies have demonstrated that both at the tissue and organelle level, carnosine possesses strong and specific antioxidant properties (Nagasawa *et al*., 2001; Fontana *et al*., 2002; Kang *et al*., 2002). The mechanisms of such protection are explained in terms of proton buffering, heavy metal chelating, as well as free radical and active sugar molecule scavenging, preventing modification of biomacromolecules and keeping their native functional activity under oxidative stress (Boldyrev, 2000; Fontana *et al*., 2002; Kang *et al*., 2002).

The present study investigate the effect of maternal nicotine exposure during gestation and lactation on neonatal rat lung structure and its reversibility and determine whether treating the mothers with carnosine could protect the neonatal rat lung against the possible adverse effects resulted from maternal nicotine exposure.

## MATERIALS AND METHODS

Twelve 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±2°C) with optimal illumination cycle and free access to drinking water and a pellet diet. The animals were mated overnight and were afterwards divided into three groups as follows:

- Group I (control group; n = 4): the maternal rats received subcutaneous injection of normal saline.
- Group II (group of nicotine-treated mothers; n = 4): the maternal rats of this group received a daily dose of subcutaneous injection of nicotine (1 mg $kg^{-1}$ body weight) up to weaning on postnatal day 21.
- Group III (group of carnosine- and nicotine-treated mothers; n = 4): the mothers received daily doses of both of subcutaneous injection of nicotine (1 mg $kg^{-1}$ body weight) and intramuscular injection of carnosine (10 mg $kg^{-1}$ 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 occurs in 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.

Four 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 was 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 (g)/ mean body weight (g)×100. This percentage represented the relative lung weight. The results were subjected to statistical analysis.

**Light microscopical study:** The lung specimens were fixed in 10% formol saline and processed for paraffin blocks. Sections of 3 μm 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.

**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 $\mu m^2$, the following parameters were estimated:

- Alveolar count $mm^{-2}$ = (number of alveoli in the field ÷ total area of the field in $\mu m^2$) × $10^6$
- The percentage of elastic fibers in the field = (Area of elastic fibers ÷ total area of the field) × 100.
- The percentage of collagen fibers in the field = (Area of collagen fibers ÷ total area of the field) × 100.
- 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 $mm^{-1}$ of septum = (total number of nuclei ÷ length of septum in $\mu m$ ) × 1000.

These data were measured in 10 randomly selected non-overlapping fields from each section, using objective lens × 20 and the mean values were obtained. The results were subjected to statistical analysis and represented in histograms (Fig. 1-4).

**Statistical analysis:** The Statistical Package for the Social Sciences (SPSS version 7.5) was used in data analysis. Data were expressed as mean±SE. One-way analysis of variance (ANOVA) was used.

## RESULTS

There were no significant differences in the mean body weight and the relative lung weight between the different experimental groups at any time interval.

**Statistical results of the histomorphometric study:** Estimation of the number of alveoli in the respiratory unit ($mm^{-2}$) (Fig. 1) as well as the percentage of elastic tissue content of the lung parenchyma (Fig. 2) demonstrated marked reduction in the neonatal rats of nicotine exposed mothers (group II) from the first week of their age and this reduction was statistically significant compared with the age-matched controls. The drop in the value of these parameters continued till the postnatal age of three weeks (weaning age) and maintained at this low level till the age of 7 weeks (4 weeks after removal of nicotine). The

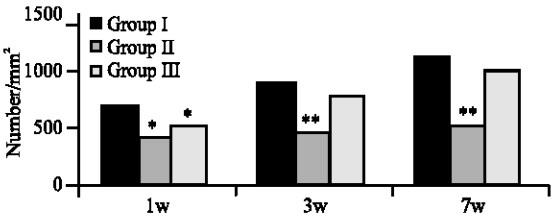



Fig. 1: The mean number of alveoli/$mm^{-2}$ of lung tissue. All measurements are demonstrated in the lung parenchyma of the different experimental groups at the postnatal ages of 1, 3 and 7 weeks. *: significant with respect to the control group ($p<0.05$). **: highly significant with respect to the control group ($p<0.01$)

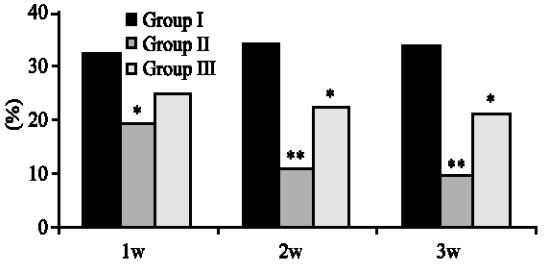



Fig. 2: The mean area percentage of the elastic fibers. All measurements are demonstrated in the lung parenchyma of the different experimental groups at the postnatal ages of 1, 3 and 7 weeks. *: significant with respect to the control group ($p<0.05$). **: highly significant with respect to the control group ($p<0.01$)

reduction at the postnatal ages of 3 and 7 weeks was highly significant compared with the age-matched controls. Carnosine treatment of nicotine-exposed mothers led to statistically insignificant changes in the number of alveoli in the respiratory unit ($mm^2$) but showed statistical significant reduction in the percentage of elastic tissue content of the lung parenchyma, compared with age-matched controls, at the postnatal ages of 3 and 7 weeks.

Measurements of the percentage of collagen fibers in the lung parenchyma (Fig. 3) showed the highest values in rat pups of nicotine-exposed mothers and such increase become statistically significant and highly significant at the postnatal ages of 3 and 7 weeks, respectively, compared with the age-matched controls. In the rat pups of mothers treated with nicotine and carnosine (group III), the values of these measurement were lower than those of group II at all experimental intervals. However, they showed significant increase compared with the age-matched controls at the postnatal ages of 3 and 7 weeks.

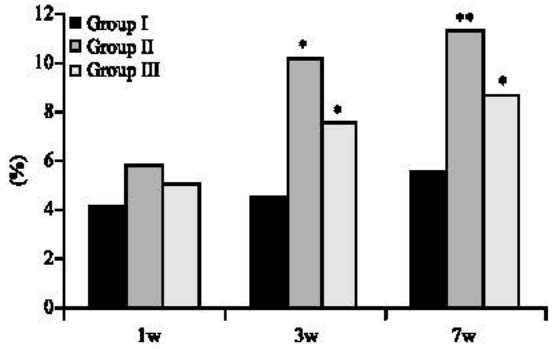



Fig. 3: The mean area percentage of the collagen fibers. All measurements are demonstrated in the lung parenchyma of the different experimental groups at the postnatal ages of 1, 3 and 7 weeks. *: significant with respect to the control group ($p<0.05$). **: highly significant with respect to the control group ($p<0.01$)

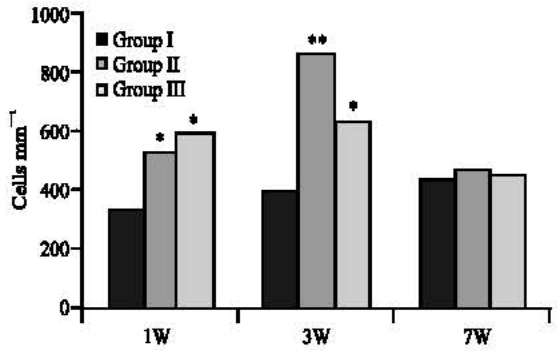
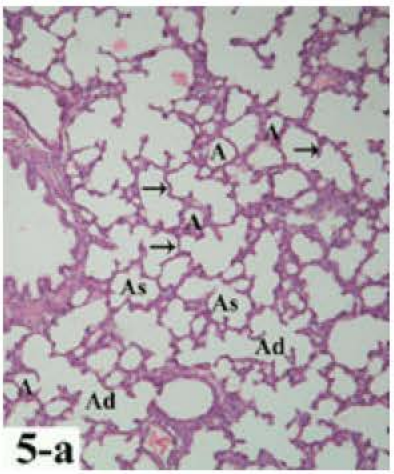



Fig. 4: The cellularity of alveolar septa. All measurements are demonstrated in the lung parenchyma of the different experimental groups at the postnatal ages of 1, 3 and 7 weeks. *: significant with respect to the control group ($p<0.05$). **: highly significant with respect to the control group ($p<0.01$)

Septal cellularity of lung tissue (Fig. 4) of the rat pups of mothers treated with both of carnosine and nicotine (group III) showed the highest increase of all groups at the postnatal age of one week and such increase was statistically significant compared with the age-matched control. At the postnatal age of 3 weeks, the measurements of this parameter demonstrated its highest value in the rat pups of nicotine-exposed mothers (group II), which was statistically highly significant compared with the age-matched control. At the postnatal age of 7 weeks, no significant differences in the septal cellularity of both of groups II and III compared with the controls.



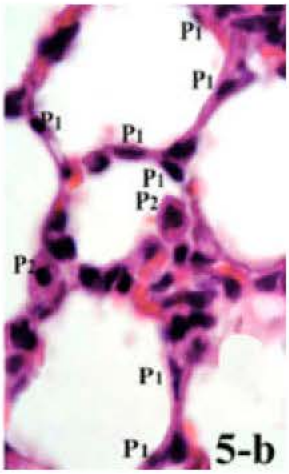



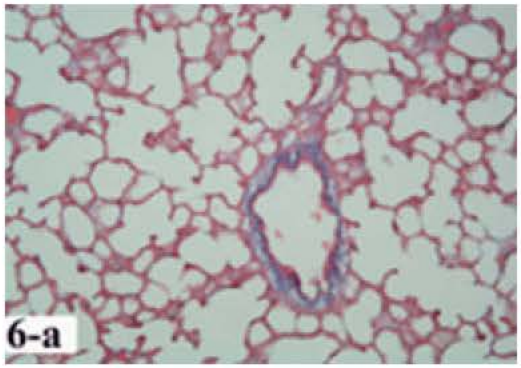

Fig. 5: Photomicrographs of cross sections of lung specimens of rat pups of the control group showing. a) Normal alveolar ducts (Ad) with numerous alveolar sacs (As) and alveoli (A). Note the thin alveolar septa between adjacent alveoli (arrows). (Hx. and E. x100). b)Alveolar walls lined with many Type I pneumocytes with flattened nuclei (P1) and few large Type II pneumocytes with rounded nuclei (P2). (HX. and E. x1000)

**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. 5a). The alveolar walls were lined with many Type I pneumocytes with flattened nuclei and few large Type II pneumocytes with rounded

nuclei (Fig. 5b) and vesicular cytoplasm. The inter-alveolar septa showed mini amount of collagen fibers (Fig. 6a) and considerable amount of intact elastic fibers (Fig. 6b).

**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 exudates with collapsed alveoli and thickened septa (Fig. 11) while other areas showed expanded alveoli with ruptured septa (Fig. 11). 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.



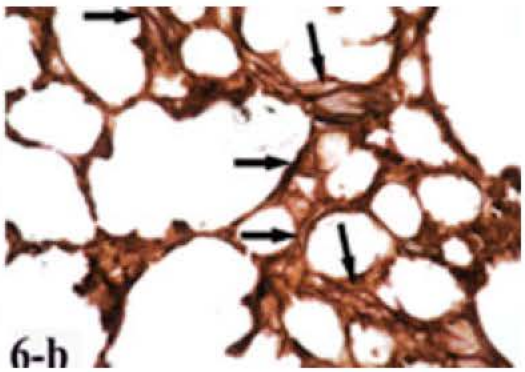




Fig. 6: Photomicrographs of cross sections of lung specimens of rat pups of the control group showing. a) Minimal amount of collagen fibers in the lung parenchyma. (Masson's trichrome; X200). b) Considerable amount of intact elastic fibers in the inter-alveolar septa (arrows). (Modified Taenzer-Unna orcein; x400)

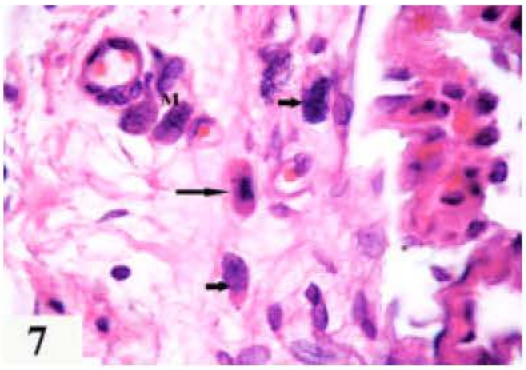



Fig. 7: A photomicrograph of a cross section of a lung specimen of a rat pup of nicotine-exposed mother (group II), 1 week after birth, showing aggregation of considerable number of mast cells in the pleura (M); some of these cells are partially (short arrows) or completely (long arrow) degranulated. (Hx. and E. x1000)

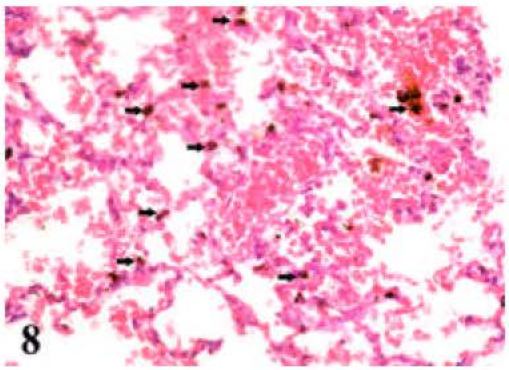



Fig. 8: A photomicrograph of a cross section of a lung specimen of a rat pup of a nicotine-exposed mother (group II), 1 week after birth, showing congestion and extravasation of RBCs into the alveolar lumens with many hemosedrin-containing macrophages (arrows). (Hx. and E.; x400)

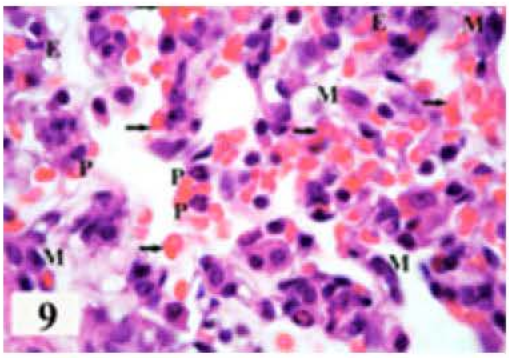



Fig. 9: A photomicrograph of a cross section of a lung specimen of a rat pup of a nicotine-exposed mother (group II), 1 week after birth, showing marked inflammatory cell infiltration including numerous eosinophils (E), neutrophils (P) and macrophages (M). Note the intra-alveolar extravasated RBCs (arrows). (Hx. and E. x400)

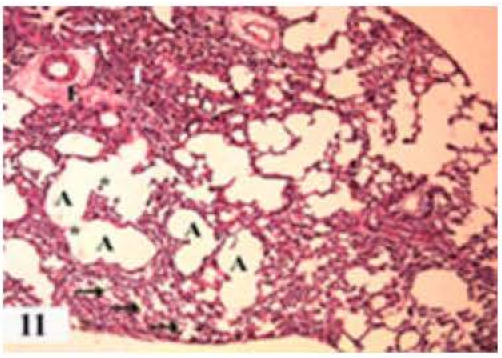



Fig. 11: A photomicrograph of cross section of lung specimen of rat pup of group II, 3 weeks after birth, showing areas of alveolar collapse (arrows) and thickened inter-alveolar septa with cellular exudates (E) and cellular infiltration (I). Note the area of expanded alveoli (A) with ruptured septa (*). (Hx. and E. x400)

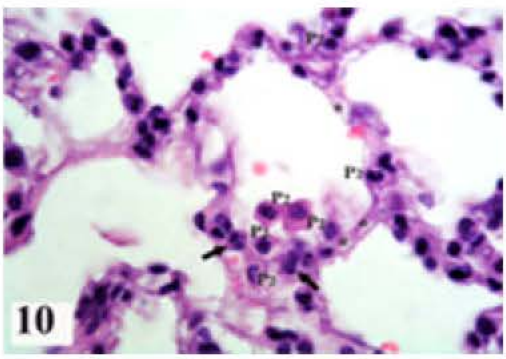



Fig. 10: A photomicrograph of a cross section of a lung specimen of a rat pup of a nicotine-exposed mother (group II), 1 week after birth, showing alveolar walls with sites of degenerated Type I pneumocytes (*) and increased number of the Type II pneumocytes (P2); some of them are dividing (arrows). (Hx. and E.; x1000)

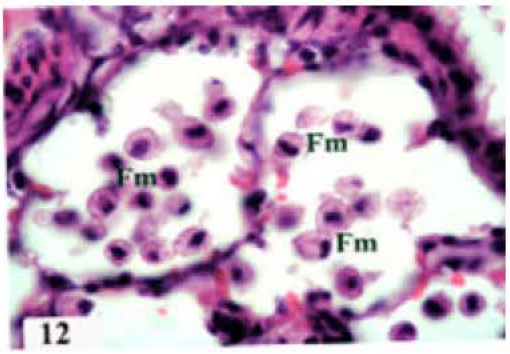



Fig. 12: A photomicrograph of a cross section of a lung specimen of a rat pup of a nicotine-exposed mother (group II), 3 weeks after birth, showing alveolar lumens infiltrated with large number of foamy macrophages (Fm). (Hx. and E. x1000)

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. 13). Other areas showed marked expansion of the alveoli with ruptured septa leading to alveolar coalescence (emphysema) (Fig. 14). 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 (Fig. 15a and b).

**Group III (group of carnosine- and nicotine-treated mothers):** At the age of one week, the specimens of the animals of this group showed areas of normally appeared alveoli and other areas of corrugated septa (collapsed alveoli) with extravasation of RBCs into the alveolar lumen as well as inflammatory cellular infiltration. There was marked proliferation and aggregation of large number of Type II pneumocytes (Fig. 16a and b). At the age of three weeks, the lung specimens showed areas of collapsed alveoli with considerable thickening of the alveolar septa (Fig. 17). Aggregation of Type II pneumocytes had still been demonstrated in some alveoli.

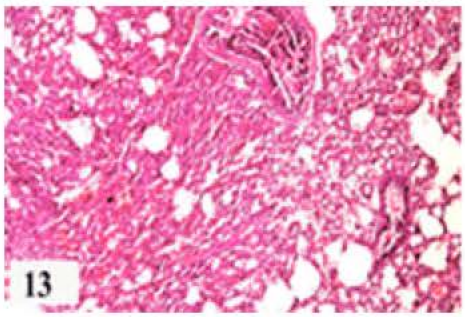



Fig. 13: A photomicrograph of a cross section of a lung specimen of a rat pup of a nicotine-exposed mother (group II), 7 weeks after birth, showing massive alveolar collapse with loss of normal lung architecture. (Hx. and E. x100)

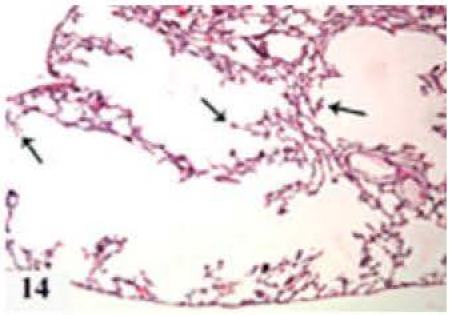
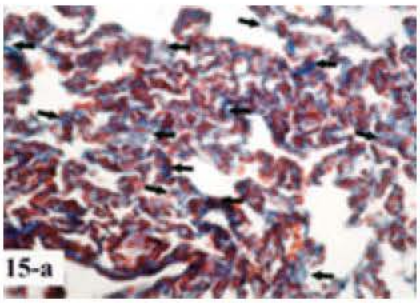



Fig. 14: A photomicrograph of a cross section of a lung specimen of a rat pup of a nicotine-exposed mother (group II), 7 weeks after birth, showing marked expansion of the alveoli with ruptured septa (arrows) leading to alveolar coalescence. (Hx. and E. x100)



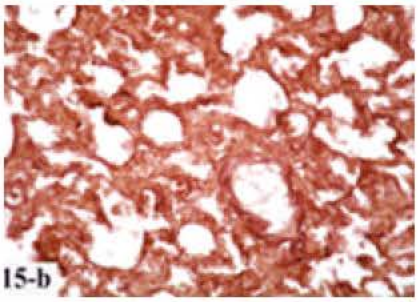
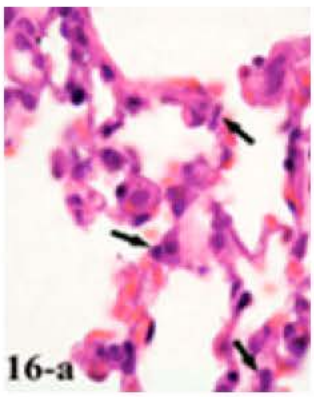




Fig. 15: Photomicrographs of cross sections of lung parenchyma of rat pups of nicotine-exposed mothers (group II), 7 weeks after birth, showing: (a): Considerable increase in fibrous tissue formation (arrows). (Masson's trichrome; x400) (b): Marked reduction of the elastic tissue content. (Modified Taenzer-Unna orcein; x 400)

The elastic tissue content of the lung parenchyma was considerable in some areas and deficient in others.



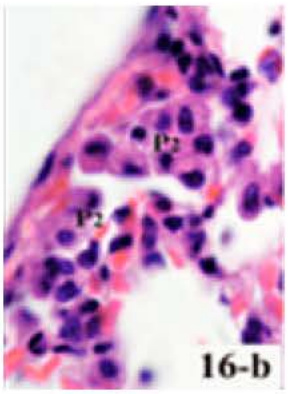




Fig. 16: Photomicrographs of different fields of a cross section of a lung specimen of a rat pup of a carnosine- and nicotine-exposed mother (group III), 1 week after birth, showing: (a): Alveolar walls with many dividing Type II pneumocytes (arrows). (Hx. An d E. x1000), (b): Intra-alveolar aggregation of a large number of Type II pneumocytes (P2). (Hx. and E. x1000)

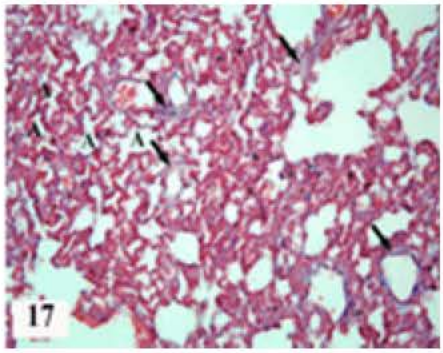



Fig. 17: A photomicrograph of a cross section of a lung specimen of a rat pup of a carnosine- and nicotine-exposed mother (group III), 3 weeks after birth, showing collapsed alveoli (A) with considerable thickening of the alveolar septa (*). Note the areas of the lung parenchyma of increased fibrous tissue formation (arrows). (Masson's trichrome; x200)

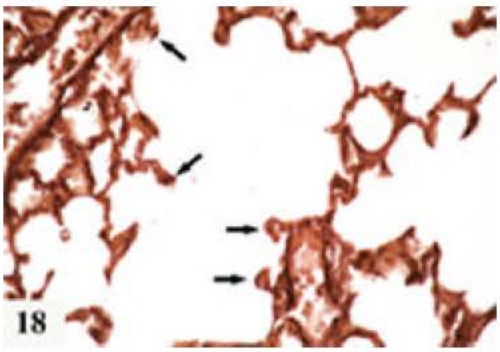



Fig. 18: A photomicrograph of a cross section of a lung specimen of a rat pup of a carnosine- and nicotine-exposed mother (group III), 7 weeks after birth, showing a decreased elastic tissue content with expanded alveoli and ruptured septa (arrows). (Modified Taenzer-Unna orcein; x400)

Increased fibrous tissue formation in localized areas of the parynchyma (Fig. 17) could be seen.

At the age of seven weeks, no further changes in the lung parenchyma of the animals of this group was detected, apart from subsidence of the inflammatory reaction and disappearance of the aggregated pattern of Type II pneumocytes. Areas of decreased elastic tissue content with ruptured septa leading to expanded alveoli (emphysematous changes) could be seen (Fig. 18).

## 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 controls. 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 as well as body weight and lung volume of the neonates at birth and up to maturity. On contrary, 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 their lung parenchyma with large number of inflammatory cells. It is well known that mast cells can 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 total leucocyte, neutrophil and eosinophil blood counts were all higher in smokers than non smokers and they showed a dose dependent.

In the present study, 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. Perel'man *et al*. (1989) concluded that in lung injuries, carnosine treatment induced marked proliferation of Type II pneumocytes and massive excretion of the surfactant leading to rapid restoration of lung airness within the wound. This conclusion is compatible with the present morphometric and histological results, which revealed that lungs of rat pups of mothers treated with both nicotine and carnosine showed proliferation and aggregation of large number of type II pneumocytes with more increase in the septal cellularity, during the first week after birth, as compared with those of mothers exposed to nicotine only. On the other hand, the decrease in the septal cellularity of the former group of rat pups, on the third week after birth, indicates an earlier completion of the healing process in this group of animals than those of mothers treated with nicotine only.

The present histological and histomorphometric studies showed that maternal nicotine exposure caused marked reduction of the elastic tissue of neonatal rat lung, which was statistically significant, compared with the controls, since the first week of their postnatal life. Identical observations were reported by Maritz and van Wyk (1997). Moreover, 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 of the lung 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 control. In contrast, Sekhon *et al*. (2001) and Sandberg *et al*. (2004) demonstrated that although prenatal nicotine exposure of rhesus monkeys and lambs altered their lung development, it did not affect lung elasticity. This can lead to suggestion that elastic tissue affection as results of prenatal nicotine exposure is dependant on species sensitivity.

It is suggested 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, 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. The association of progressive elastic tissue destruction with marked neutrophil and eosinophil infiltration of the lung parenchyma as well as the aggregation of foamy macrophages in alveolar lumens of prenatally nicotine-exposed rat pups of the current experiment are fully consistent with the above-mentioned postulations. In addition, the role of the inflammatory cells, neutrophils and foamy macrophages, in elastic tissue destruction can explain the prevention of marked destruction of the lung parenchymal elastic tissue, in the current work, of the rat pups of nicotine-exposed mothers treated with carnosine that was proved to be an anti-inflammatory and antihistaminic agent (Ermakova *et al*., 1988).

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 controls 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 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 (emphysema) give another explanation for the reduction of the alveolar count in the rat pups of nicotine-exposed mothers and are supported by findings from other studies (Maritz and van Wyk, 1997; Maritz and Windvogel, 2003). Moreover, 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 and Dennis (1998) and Maritz and Windvogel (2003) stated that as the animals aged, suppression of alveolarisation in the lungs of prenatally nicotine-exposed offspring as well as shortening and destruction of alveolar walls, resulted in bigger alveoli and a reduced internal surface area available for gas exchange giving a condition resembling panlobular emphysema. Thereby, 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 present 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 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 lung changes of the rat pups of nicotine-exposed mothers to the 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, where its destruction resulted in gradual development of pulmonary emphysema as the animals aged. On the other hand, Maritz and Windvogel (2003) described 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.

The present study demonstrated that treatment of the nicotine-exposed mothers with carnosine resulted in the partial improvement of the lung parameters especially the alveolar count, which did not show significant changes compared with the controls, with time laps, thereby potentiating alveolarisation after birth and restoring the alveolar surface necessary for gas exchange. Thus, the hypothesis that maternal nicotine permanently affects the process of alveolisation in the neonates by induction of changes at gene level (Maritz, 2002), if it is applicable, may lead to the assumption that carnosine could have a role in protection of the concerned gene. Furthermore, the role of carnosine in potentiating alveolarisation is compatible with the conclusion reached by Perel'man *et al.* (1989) who stated that carnosine nearly twice accelerates reparative process and alveolus formation in the wound lips after lung injury, as compared with controls. On the other hand, the present study showed that although carnosine minimized the effect of nicotine on the percentages of elastic tissue content and the fibrous tissue formation of the lung parenchyma, yet these parameters still showed significant changes compared with the age-matched controls. In addition, treatment with carnosine did not prevent completely the induction of microscopic emphysema in lungs of rat pups of nicotine-exposed mothers.

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. Further evidence in support of this hypothesis is the data obtained from the studies of Maritz (1993) and Maritz and Thomas (1994) who revealed that some of the lung changes as result of maternal nicotine exposure, corresponded with the changes induced by oxidants including injury to the alveolar-capillary membrane and interference with lung growth and development. 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). Numerous studies have demonstrated that both at the tissue and organelle level, carnosine possesses strong and specific antioxidant properties protecting cell membranes from oxidative damage (Quinn *et al*., 1992), retarding senescence and rejuvenating senescent cultures thus preserving cellular integrity (McFarland and Holliday, 1994) and scavenging active sugar molecule and oxygen free-radicals (Boldyrev, 2000; Kang *et al*., 2002). Therefore, limitation of the deleterious effects of maternal nicotine-exposure on offspring's lung structures as result of maternal treatment either with carnosine, as shown in the present work, with antioxidant oils isolated from garlic and onion (Helen *et al*., 1999) or with ascorbic acid (Maritz and van Wyk, 1997), supports the hypothesis that nicotine may induce damage of neonatal lung structures via the generation of oxidants.

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. Carnosine supplementation dose not prevent completely these adverse effects but it appears to retard their progression with considerable improvement of alveolar count.

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