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TOXIC EFFECTS OF COMBINED EXPOSURE TO CADMIUM AND NICKEL ON WHITE ALBINO RATS

(With 3 Tables and 13 Figures)

By

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التاثيرات السامة للتعرض لخليط من الكادميوم والنيكل على الجرذان البيضاء

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تتعرض الكائيات الحيه في أغلب الاحيان لأكثر من ملوث في نفس الوقت مما يؤدى إلى أخطار بيئيه أعظم فداحه مما لو كان بمفرده ولذلك تمت هذه الدراسة على ٦٠ من الجرذان البيضاء نصفهم من الأناث والنصف الآخر من الذكور لمدة ٦أسابيع. أربعون جرذا (٢٠ أناث + ٢٠ ذكور) تعرضوا لتركيز ٥٠ جزء في المليون من كلوريد الكادميوم + ٥٠ جزء في المليون من كلوريد النيكل في ماء الشرب وأما الباقى من الجرذان (١٠ إناث + ١٠ ذكور) فتم إستخدامهم كضابط للتجربه. وقد تم في هذه الدراسة قياس مستوى كل من النيكل والكادميوم في الدم وكذلك هرمونى التيستيسترون والأستروجين وكذلك الثيول الكلى واليوريا وحمض البوليك والكرياتنين. وكذلك تم دراسة التغيرات الباثوجيه لانسجة الحيوانات المتعرضه. وأظهرت نتائج هذه الدراسه وجود زياده معنويه في مستوى الكادميوم فيدم كل من الإناث والذكور المتعرضه. أما مستوى النيكل في الدم فقد سجلت النتائج إرتفاعا معنويا في الإناث المتعرضه على مدى الأسابيع السته للتجربه. أما الذكور فكان إرتفاع مستوى النيكل فيها معنويا فقط في الأسبوع السادس. وسجلت النتائج عدم حدوث تغير معنوى في مستوى هرمونى التيستيسترون والاستروجين الا أنه شوهد إنخفاضا معنويا في معدل التيستيسترون في الأسبوع الخامس فقط من التعرض. وشهدت النتائج إنخفاضا معنويا في معدلات الثيول الكلى في كلا الجنسين. أما مستويات كل من اليوريا وحامض البوليك والكرياتنين فقد سجلت زياده معنويه طوال فتره التجربه كما تم تسجيل التغيرات الباثولوجيه في كل من أنسجة الخصيه والمبيض والكبد والطحال والرئه والقلب والكلى.

SUMMARY

Exposure of biological organisms to more than one pollutant in the same time constitutes an additional environmental hazard. A toxicological investigation on 60 white albino rats (30 males and 30 females) was

carried out for 6 weeks. Forty rats (20 males and 20 females) were exposed to 50 ppm cadmium chloride plus 50 ppm nickel chloride in drinking water. The rest rats (10 males and 10 females) were used as control. Cadmium, nickel, testosterone, estrogen, total thiol, urea, uric acid and creatinine levels in the blood were estimated. A histopathological investigation on different body tissues was also carried out. The results revealed a significant increase in blood cadmium levels in both exposed male and female rats. Nickel blood levels were also elevated in female rats over the whole period of the investigation. A significant increase in nickel level in males was recorded at the 5 th and 6 th week. The levels of both testosterone and estrogen showed no significant changes except a significant decrease in testosterone at the 5 th week post exposure. The total thiols level were significantly decreased in both sexes. Urea, uric acid and creatinine levels were significantly increased. The most important histopathological changes were testicular degeneration, ovarian follicular atresia, spleen haemosidrosis and focal lytic lesions in the liver. Also, marked pathological changes were recorded in lungs, heart and kidneys.
***Key words**: Cadmium-nickel-toxicity-thiols- creatinine-testosterone-rats.*

INTRODUCTION

A synergistic or antagonistic action may occurs as a results to the biological organisms exposed to more than one toxicant at the same time. In our environment, the biological organisms are exposed to more than one pollutants especially in industrial pollutants, therefore the study of the combined effect of cadmium and nickel is our choice.

Cadmium has a variety of industrial uses and these have resulted in an increase in cadmium abundance in the environment worldwide during the last several decades. Cd is the major environmental pollutant that has aroused serious global concern. It is released during combustion of coal and mineral oils, smelting, alloy processing and mining operations. More than 50% of the total body burden of Cd accumulates in liver, kidneys and testes and is responsible for cellular and functional damage (Friberg et al., 1974).

Exposure to Cd may cause diminished fertility, increased intra-uterine death (due to congenital malformations), and insufficient fetal growth in hamsters, rats and mice (Carmichael et al., 1982; Elinder, 1986). Birth defects such as cleft palate, hydrocephalus and forelimb ectrodactyl have been observed after experimental exposure of rats to Cd

(Feuston and Scott, 1985; Holt and Webb. 1987). A reduced number of implantations was reported in mice (Yu et al., 1985). Acute exposure causes haemorrhagic necrosis of the testes and long term exposure reduces spermatogenesis in rats(Ragan and Mast, 1990).

The study of nickel toxicity in rats in levels of 250, 500 and 1000 ppm did not affect the growth rate or reproduction and no signs of toxicity were apparent even after 3-4 months of exposure (Phatak and Patwardhan, 1952). On the other hand nickel has been reported to enhance anaphylaxis, it also inhibits humoral immunity, natural killer activity and impairs resistance to pathogenic challenge (Burns et al., 1996).

Compounds of Ni and Cd are carcinogenic to human and/or experimental animals (Hartwig et al., 1998). In general, the carcinogenic potential of metals depends largely on oxidation and solubility which affect the bioavailability of the respective compound. Ni and Cd at low, biologically relevant concentrations have been shown to interfere with nucleotide excision repair, the major repair system involved in the removal of DNA induced environmental mutagens, leading to pronounced enhancement of cytotoxicity and mutagenicity in combination with other DNA damaging agents. They also inhibit the repair of oxidative DNA base modifications induced by reactive oxygen species, which are continuously generated during aerobic metabolism. Ni and Cd also inhibit the DNA-Protein interactions involved in DNA damage recognition (Hartwig et al., 1998). So the aim of our study is to investigate the effect of combined effect of Cd and Ni on albino rats.

MATERIALS and METHODS

Materials:

Cadmium chloride and nickel chloride were obtained in pure form from Sigma Chemical Co., USA.

Animals and treatments:

Sixty (three month old) albino rats (30 males and 30 females) were used in this study. They were housed in plastic cages with ad libitum access to Food and water. The light cycle was 12:12 hr (0700-1900 hr; light), and the room temperature was maintained at 22-24 °C. The rats were housed one week in this condition before start of the experiment (which extended 6 weeks). Forty rats (20 males and 20 females) were exposed to 50 ppm cadmium chloride plus 50 ppm nickel chloride in drinking water. The rest rats (10 males and 10 females) were used as control. Each five animals were taken from rats exposed to the

mixture of toxicants and sacrificed at intervals 3, 4, 5 and 6 weeks. At each intervals, blood samples were obtained for hormonal analysis (testosterone and estrogen), for biochemical determination (urea, uric acid, creatinine and total thiols) and for metal estimation (cadmium and nickel).

Estimation of testosterone and estrogen:

For both hormones (testosterone and estrogen), Gamma Counter Mini - Assay type 620 was used in their measuring. Testosterone was determined by using coat - A -Count Total Testosterone Kits (Catalogue number TKTT1, 100 tubes, DPC, Diagnostic products Corporation, Los Angeles, USA.) according to the method of Wilson and Foster (1992), while in case of estrogen Coat-A-Count Estradiol Kit (Catalogue number TKE 21 100 tubes, DPC, Diagnostic Products Corporation, Los Angeles, USA) was used according to the described method of Burtis and Ashwood (1994).

Estimation of total thiols:

Total thiols in serum were determined according to the method described by Ellman (1959).

Metals determination:

One ml of blood was digested by using a mixture of $HClO_4$-HNO_3, for determination of Cd and Ni. The previously mentioned metals Cd and Ni were measured by using atomic absorption spectrophotometer (GBC 906 AA).

Biochemical parameters:

Urea, uric acid and creatinine were measured by using commercial kits (Diamond Diagnostics) according to Patton and Crouch (1977) for urea, Fossati et al. (1980) for uric acid and creatinine were estimated after Henry (1974).

Histopathology:

Representative samples from heart, liver, kidneys, lungs, spleen, testes, and ovaries were fixed in 10% neutral buffered formalin. Tissue samples were processed routinely for paraffin embedding technique. Embedded tissues were then sectioned at 3 μ and stained with haematoxylin and eosin (H & E).

Electron microscopy:

Immediately after necropsy, 1 mm-cubes from all tissues were made and fixed by immersion in 3% buffered glutaraldehyde. Tissue samples were then post-fixed in 1% osmium tetroxide, dehydrated in ascending grades of ethanol and embedded in Epon-812. Semi-thin

sections were prepared and stained with 1% toluidine blue. After orientation of tissues, ultrathin sections were made and double stained with uranyl acetate and lead citrate. Stained sections were examined using transmission electron microscope (JEOL, 100 CXII) operated at 80 Kv.

Statistical analysis:

Student's "t" test was used to calculate the significance between control and investigated animals. Probability values <0.05 and <0.001 were considered statistically significant and this according to Kalton (1967).

RESULTS

Biochemical and metal changes:

The most prominent results recorded in our study for biomarkers changes were a limited effect in testosterone levels and no significant change was recorded in estrogen levels. The testosterone level was significantly decreased only in the 5 th week post-exposure (table 1). The only change observed in urea levels was a significant increase in the 5 th week for male rats and in the 6 th week in the female (table 2). A significant increase in uric acid and creatinine levels was recorded allover the whole period of the experiment in both sexes. Total thiols levels were diminished in both sexes during the whole period of exposure (table 2). Cadmium and nickel blood levels were significantly elevated post-exposure all over the period of investigation (table 3).

Histopathology:

Throughout the experimentation period, the cardiac histological changes were the most remarkable followed by the changes of the other tissues including liver, kidneys, spleen, ovaries and testes. The observed histological changes, were not sex-related. At the first two intervals post-exposure, the cardiac histological changes were represented by edema separating myocardial fibers, granular degeneration of myofibers, sarcoplasm paleness and scattered haemorrhages. At the last two intervals, similar and more severe changes were noticed. Myocardial haemorrhages were diffuse and more frequent (Fig.1). Edema noticeably separated myocardial fibers. Thin myofibers were frequent. Many of myofibers nuclei were pyknotic. Wavy appearance of myocardial fibers was observed in some cases (Fig. 2). Occasional focal myolysis was also seen. Edema, sinusoidal distention, cell swelling, focal cellular dissociation and degeneration of bile duct epithelium were the hepatic changes discerned at the first two intervals. Livers at the last two

intervals were more affected with apparent dissociation of hepatocytes and indistinction of cellular outlines. Focal vacuolar degeneration (hydropic degeneration) was frequent (Fig. 3) in some cases, focal lytic necrosis was detected (Fig. 4).

Gonadal changes were also detected. Testes examined at the first two intervals showed congestive edema with separation of the somniferous tubules. At the last two intervals, testicular tubular degeneration was evident. The degenerated seminefcrous tubules revealed incomplete stages of spermatogenesis with deterioration of spermatogonia cells (Fig. 5). Ovarian follicular atresia was noticed only at the last two weeks (Figs. 6). Atretic follicles showed disarrangement of granulosa cells, discontinuation of the previtteline membrane and degeneration of the thecal layers. Pulmonary congestion and edema was observed along the experimentation period. Also, at the last week, the significant renal changes were in the form of glomerular tuft swelling and tubular cell degeneration (Fig. 7).

Marked haemosiderosis was detected in the spleens examined at the last two weeks (Fig. 8). Occasional necrosis in the splenic follicles was also seen.

Electron microscopy:

Electron microscopy of the cardiac tissues revealed noticeable myofiber changes. Frequently, there were thin and thick myofibers (Fig. 9). Thin myofibers had indistinct Z-bands. Approximated and disarranged Z-bands were also found (Fig. 10). Many glycogen granules were detected between the myofibers. Mitochondria were swollen and many of them had deteriorated cristae. Elongated mitochondrial profiles were also observed. Fragments of disrupted myofibers with dissoluted myofibrils were noticed with the presence of swollen mitochondria filling the gaps left at the site of fragmentation (Fig. 11). Lamellar structures and glycogen rosettes were also detected at the site of lysed myofibrils. All the examined liver samples had relatively little glycogen content. Increased mitochondrial-RER association were frequent (Fig. 12). Some of the examined liver samples revealed the presence of hepatocytes with increased cytoplasmic lysosomal structures (Fig. 13).

DISCUSSION

Cadmium and nickel have been recognized as the most toxic industrial and environmental pollutants of which there is a continuing hazard to biological organisms especially animals and human exposure.

The effects of combined exposure for six weeks on male and female hormones were significant decrease in testosterone level at the fifth week of exposure and no significant change was recorded in estrogen level. At the last two weeks, testicular tubular degeneration was evident in the present intoxicated animals. with disruption of spermatogonia cells. Cadmium is known to induce a severe acute toxicity to rat testis at doses appreciably lower than those required for toxicity to other organs (Fahim and Khare, 1980). The observed testicular changes, mainly in the form of incomplete stages of spermatogenesis, may be ascribed to the effect of cadmium on the testicular vasculature (vascular endothelium). The cadmium-induced endothelial damage results in a remarkable reduction in blood of flow of testes to less than 10% of the control values due to increased capillary permeability (Phillips et al., 1985). Other testicular toxicants, regardless of their site of action, produce similar histological changes including generalized germ cell depletion (Creasy and Foster, 1991).

The only change in testosterone level recorded in our cases was that at the fifth week, which was probably attributed to the combined effect of both nickel and cadmium as exposure to cadmium chloride at the doses of 50 and 100 ppm via drinking water for 40 days in rats was found to induce no alteration in plasma testosterone (Caflisch, 1994). The demonstrated ovarian follicular atresia may be attributed to cadmium localization in the growing follicles. Some toxic agents interfere with granulosa cell proliferation and thecal cell differentiation (Clarkson et al., 1983). Cadmium is known to induce necrosis of the preovulatory follicles and considered as a female reproductive toxicant (Peerboom-Stegeman and Jongstra-Spaapen, 1979; Schrag and Dixan, 1985).

Histopathological changes in the heart of exposed animals were edema separating myocardial fibers, granular degeneration of myofibers, sarcoplasm paleness and scattered haemorrhages at the last two weeks. The present myocardial pathological changes (light and electron microscopic changes) which included myofibrillar lysis and myofiber fragmentation were probably related to direct cellular injury evoked by cadmium. Such direct cardiotoxic effect is proposed to be the of a selective or random interaction of the toxicant with some myocyte molecule (Van Stee, 1980). The described ultrastructural myofibrillar lysis is considered by some authors (Ferrans and Butany, 1983) as a distinctive sublethal injury of the cardiac myocytes. Disorganization of Z-bands in cardiac myofibers, as noticed here, was described by Van

Fleet et al. (1991) as necrosis with contraction bands which is characterised by myofibril hypercontraction which could be progressed to myocytelysis. Some previous studies correlated between cadmium toxicity and the resultant myocardial dysfunction. In this respect, Kopp et al. (1983) found that rats exposed to Cd in drinking water developed electrocardiographic and biochemical changes in the myocardium and impairment of the functional status. According to these authors, the effects were related to (1) decreased high-energy phosphate stored in the myocardium, (2) reduced myocardial contractility, and (3) diminished excitability of the cardiac conduction system. Jamal and Sprowls (1987) found that rats feed on diets supplemented with copper, selenium and Cd had a marked reduction in heart cytosolic-glutathione peroxidase, superoxide dismutase, and catalase. They suggested that heart mitochondria are the site of the Cd-induced biochemical changes in the myocardium.

Degeneration of the bile duct epithelium was the hepatic changes noticed at the first two intervals. Hepatocytes at the last two weeks of the investigation showed apparent dissociation and indistinction of cellular outlines. Focal vacuolar degeneration (hydropic degeneration) was frequent and in some cases, focal lytic necrosis was also seen. The observed light and electron microscopic hepatic changes were probably related to localization of cadmium in the liver. Cadmium was found to bind to the cytosolic metal-binding protein, metallothionein and the half-life of the resultant compound (cadmium-metallothionein) may be extended to months (Jeffery, 1991).

A significant inhibition of total thiols was observed over the whole period of the experiment. Following an oral exposure of cadmium chloride, Cd is thought to reach the kidneys as a Cd-metallothionein complex formed and released either from intestinal cells or hepatocytes. Inside the tubular cells it is thought that lysosomal degradation of cadmium metallothionein results in release of free Cd, which in turn induces metallothionein excretion (Smith et al., 1989; Beyermann et al., 1994). The significant renal changes were in the form of glomerular tuft swelling and tubular degeneration. In the same line creatinine, urea and uric acid levels were elevated in exposed animals.

Cadmium has a half-life of greater than 10 years in humans and thus accumulates in the body over time. Approximately 50 % of the body burden of Cd can be found in the kidney. Cd produces proximal tubular dysfunction (pars convoluta and transition between pars convoluta and pars recta) and injury characterized by increases in

urinary excretion of glucose, amino acids, calcium and cellular enzymes. This injury may progress to chronic interstitial nephritis (Goldstein and Schnellmann, 1996). The primary renal toxicity of Cd affects proximal renal tubular function and is manifested by increased Cd in the urine, proteinuria, aminoaciduria, glucosuria and decreased renal tubular reabsorption of phosphate (Goyer, 1996).

Concerning the low toxic effects induced by nickel, our discussion is directed to explain the recorded changes in reference to cadmium. In a study of nickel toxicity in rats, levels of 250, 500 and 1000 ppm Ni in different forms did not affect growth rate or reproduction, and no signs of toxicity were apparent even after 3-4 months of continuous feeding (Phatak and Patwardhan, 1952). A similar low toxicity for Ni is evident from long-term study of Schroeder et al. (1974) with rats. Rats of both sexes exposed to 5 ppm Ni as a soluble form via drinking water exhibited some increased growth, but there was no effect on survival, longevity, incidence of tumors, or specific lesions.

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LEGENDS FOR FIGURES

Fig. 1: Heart showing haemorrhages between myocordial fibers. Myofibers are separated by edema. Sarcoplasm is pale and vacuolated. Male rat 3 weeks post-exposure. HE.X 280.

Fig. 2: Heart showing wavy appearance of myofibers. The affected myofibers have sarcoplasm of granular appearrance. Male rat 3 weeks post-exposure. HE.X 280.

Fig. 3: Vacuolar degeneration (hydropic degeneration) in the liver of a female rat exposed for 4 weeks. HE.X 280.

Fig. 4: Liver of a male rat, exposed for 4 weeks, showing focal lytic necrosis (arrow).The lysed hepatocytes lost their outlines with disappearance of their nuclei. Most of other hepatocytes have cytoplasm of ground glass appearance.HE.X 280.

Fig. 5: Testicular degeneration in a rat exposed for 4 weeks. Stages of spermatogenesis in the degenerated tubules are incomplete and spermatogonia cells (s) are dissociated. HE.X 280.

Fig. 6: Ovary of a rat, exposed for 4 weeks, showing follicular atresia. The atretic follicles have lysed oocytes (o). Granulosa cells are disarranged and thecal layers are degenerated. HE.X 280.

Fig. 7: Kidney showing swelling of the glomerular tufts (t) which fill the glomerular spaces. Tubular cells are degenerated. Male rat exposed for 3 weeks. HE.X 280.

Fig. 8: Marked haemosiderosis in spleen of a male rat exposed for 4 weeks. HE. X 280.

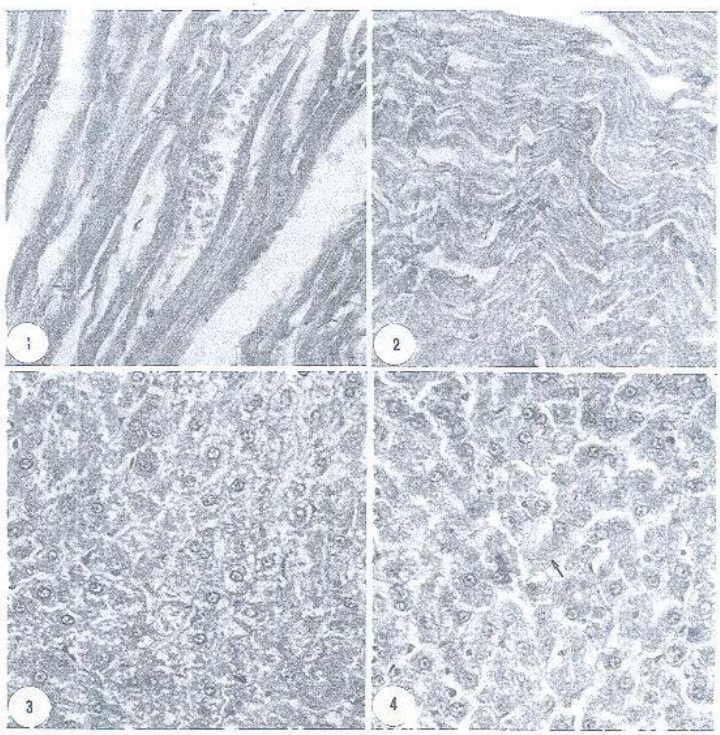
Fig. 9: Transmission electron micrograph showing thin (arrow) and thick (arrowhead) myofibers. Mitochondria (m) are swollen and have deteriorated cristae. Lamellar structures (L) and glycogen rossettes are seen at the site of disrupted myofibers. Male rat exposed for 4 weeks. X 7200.

Fig. 10: Approximated and disarranged Z-bands (arrowhead) in myofibers which have disarranged myofibrils. A- and I- bands are indistinct. Numerous glycogen rossettes (r) are seen inbetween myofibers. Swollen profiles of mitochondria (m) are also observed. male rats exposed for 4 weeks. Transmission electron micrograph. X 7200.

Fig. 11: Fragmented myofibers (arrow) having dissoluted myofibrils in the heart of a male rat exposed for 4 weeks. Swollen mitochondria (m) are seen at the site of fragmentation. Transmission electron micrograph. X 7200.

Fig. 12: Liver showing increased mitochondrial-RER associations (arrowhead. Glycogen content in sparse. male rats exposed for 4 weeks. Transmission electron micrograph.X 7200.

Fig. 13: Hepatocyte containing lysosomal structures (arrowhead) in the liver of a female rat exposed for 4 weeks. Transmission electron micrograph. X 7200.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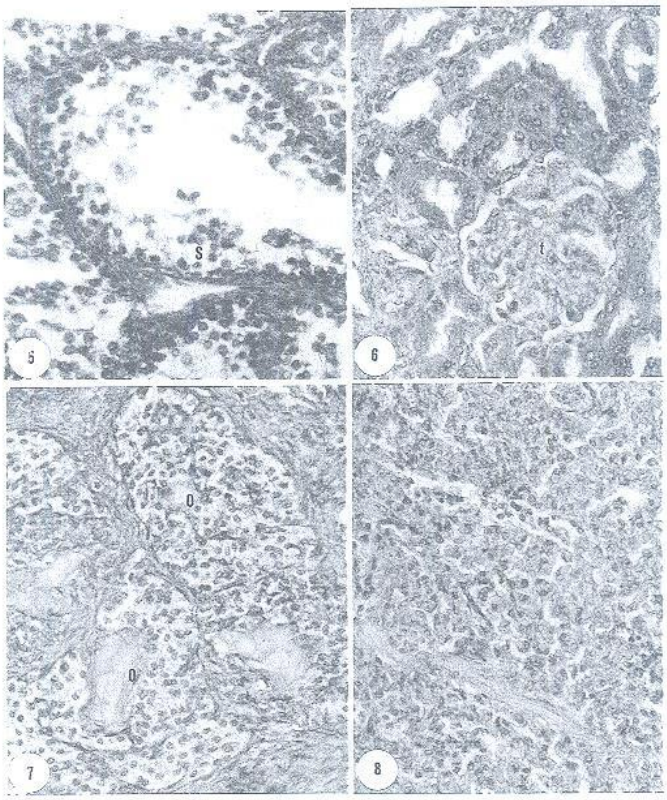
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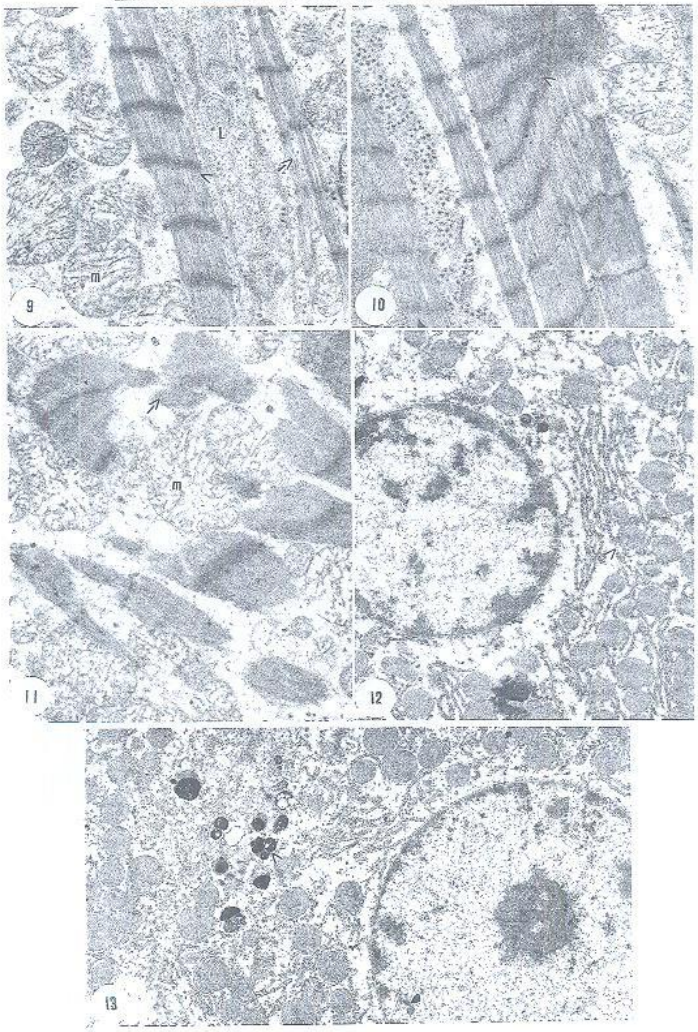
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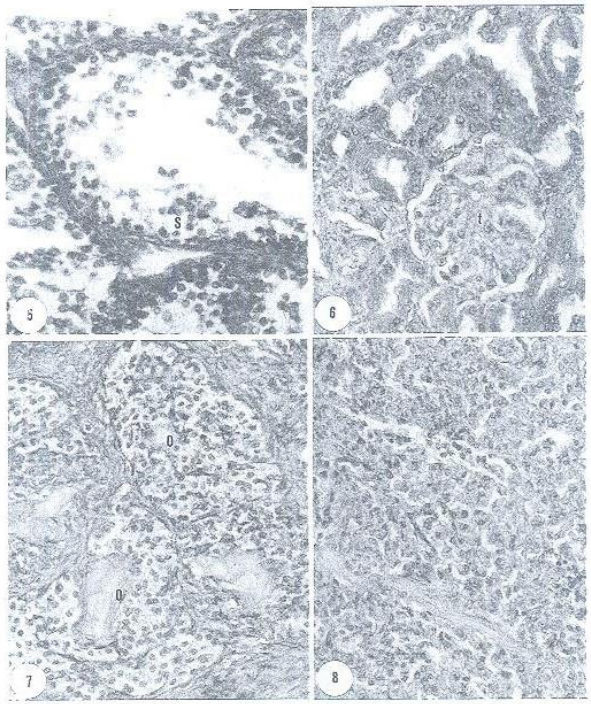
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Table 2. Some biochemical changes in the blood of investigated rats.

Male group	Total thiols (mmol/L)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Female group	Total thiols (mmol/L)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
3 weeks P.E.	0.371 ± 0.022*	43.749 ± 2.228	7.017 ± 0.306**	0.568 ± 0.016*	3 weeks P.E.	0.288 ± 0.036*	43.823 ± 2.177	6.299 ± 0.261**	0.600 ± 0.028**
4 weeks P.E.	0.451 ± 0.031*	46.764 ± 4.146	9.728 ± 0.598**	0.580 ± 0.013*	4 weeks P.E.	0.108 ± 0.010*	45.587 ± 1.610	8.056 ± 0.692**	0.595 ± 0.070*
5 weeks P.E.	0.306 ± 0.006*	47.058 ± 1.833*	9.785 ± 0.793**	0.566 ± 0.034*	5 weeks P.E. -	0.132 ± 0.018*	47.352 ± 2.439	10.113 ± 0.497**	0.570 ± 0.019*
6 weeks P.E.	0.301 ± 0.064*	49.705 ± 3.888	10.499 ± 0.406**	0.570 ± 0.014**	6 weeks P.E.	0.149 ± 0.015*	50.293 ± 2.474*	10.456 ± 0.370**	0.580 ± 0.021*
Control	2.52 ± 0.542	42.125 ± 1.081	1.99 ± 0.25	0.272 ± 0.060	Control	1.305 ± 0.256	41.982 ± 1.009	1.79 ± 0.24	0.278 ± 0.061

- The values are mean ± S.E.M.
- P.E. means post-exposure
*: Significant at $p < 0.05$.
**: Significant at $p < 0.001$.

Table 3. Cadmium and nickel blood levels in exposed rats.

Male group	Cadmium (PPM)	Nickel (PPM)	Female group	Cadmium (PPM)	Nickel (PPM)
3 weeks P.E.	0.133 ± 0.027**	0.066 ± 0.036	3 weeks P.E.	0.050 ± 0.023	0.250 ± 0.084*
4 weeks P.E.	0.216 ± 0.013**	0.070 ± 0.033	4 weeks P.E.	0.150 ± 0.021**	0.483 ± 0.036**
5 weeks P.E.	0.320 ± 0.035**	0.156 ± 0.038*	5 weeks P.E.	0.383 ± 0.075**	0.666 ± 0.153*
6 weeks P.E.	0.476 ± 0.011**	0.296 ± 0.025**	6 weeks P.E.	0.633 ± 0.151*	0.883 ± 0.082**
Control	0.0025 ± 0.0001	0.0028 ± 0.0001	Control	0.0024 ± 0.0001	0.0029 ± 0.0002

- The values are mean ± S.E.M.
- P.E. mean post-exposure
*: Significant at $p < 0.05$.
**: Significant at $p < 0.001$.

Table 1. Hormonal blood levels in investigated rats.

Investigated male rats			Investigated female rats		
Group of male rats	Testosterone (nmole/L)	% of control	Group of female rats	Estrogen (nmole/L)	% of control
Control males	5.275 ± 0.613	100 ± 11.620	Control females	1.720 ± 0.641	100 ± 37.267
3 week post-exposure	5.140 ± 0.888	97.440 ± 16.833	3 week post-exposure	0.988 ± 0.036	57.441 ± 2.092
4 week post-exposure	4.920 ± 0.658	93.270 ± 12.473	4 week post-exposure	1.238 ± 0.099	71.976 ± 5.755
5 week post-exposure	2.866 ± 0.392*	54.330 ± 7.431	5 week post-exposure	1.313 ± 0.088	76.337 ± 4.825
6 week post-exposure	3.800 ± 1.838	72.038 ± 34.843	6 week post-exposure	1.575 ± 0.314	91.569 ± 18.255

- The values are mean ± S.E.M.
*: Significant at $p < 0.05$.