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RAT KIDNEY TISSUES: OXIDATIVE MODIFICATION OF PROTEINS UNDER WATER AND SALT LOADS ASSOCIATED WITH $HgCl_2$ NEPHROPATHY

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Abstract*: Some changes in the main indexes of the pro-oxidation system of rat's kidney tissues exposed to water and salt load on background of the nephropathy caused by mercury chloride were investigated. Conditions of the free-radical oxidation of kidney tissues' proteins under water and salt load alone or complicated by the nephropathy have been analyzed. It is found that water and salt load leads to a higher content of the thiobarbiturate reaction products (TBA-RP) in kidney tissues in comparison with the control experiments while the content of the protein oxidative modification products remains unchanged. Thus, water and salt load causes a higher activation level of free radical oxidation of lipids. A histochemical investigation has been performed to evaluate a degree of the oxidative modification of proteins in rat kidney tissues under water and salt load complicated by the mercuric nephropathy and a higher level of the oxidative modification of proteins has been determined by the Red/Blue coefficient in rat kidneys irrespectively of the load type in case of intoxication with mercury chloride.*

Keywords: *water and salt load, oxidative protein modification, kidney, mercury chloride, TBA-reaction products, environmental contamination*

1. Introduction

Heavy metals are known as one of the most dangerous environmental pollutants while soluble salts of mercury are among those, which cause very tough and long-lasting consequences. Mercury (II) chloride is a well soluble salt, which is still in use in some branches of chemical industry, pharmaceutical and agricultural applications such as disinfection, seeds sterilization and so on. Due to its extreme toxicity, ability to cumulate in tissues and aggravate along the food chains, this compound can cause devastating effect on the human and other organisms and misbalance the whole ecosystem for years.
Mercury chloride is a xenobiotic that exhibits rather wide toxicity effects affecting various organs and system but kidney is among those, which suffer the most of this kind of intoxication. That is why it is important to carry out wide investigations of its toxicity mechanism, effect on different organs and tissues, and changes caused by this toxic agent alone or in combination with other aggravating or mitigating conditions.
Kidney is a leading efferent organ of human and animals, which is responsible for maintenance of the water-electrolyte balance, acid-basic and osmotic homeostasis [1]. This organ is capable to provide highly adjustable and selective excretion of water and various ions to maintain stability of the inner biological liquids [2]. There is a dynamic equilibrium between pro- and antioxidants in the

tissues and organs of living organism, which can be disturbed by oxidative stresses and shifted towards more active formation of the free radicals. They facilitate oxidation of the macromolecules resulting in some disorders in the cell membranes structure and functioning [3].

A wide spectrum of issues related to disorders in the kidney function is being extensively investigated in various directions in the context of environmental protection, effect of various toxicants on biosystems and their mitigation, etc. That is why many efforts are targeted on experimental researches dealing with the oxidative, salt/water and intoxication stresses alone or on the background of other intoxications. Morphological and functional characteristics of the nephropathy caused by mercury chloride facilitate its using as an adequate model for the kidney pathology in comparison to both clinical and experimental puromicin or adriamicin nephrites [4, 5]. On the other hand, it is important to understand what kind of changes can be caused to the kidney tissues suffering of the mercury-type intoxication together with salt and/or water load. Glomerotubular disorders have been reported for pathogenesis of the kidney functionality affected by the experimental nephrites. These disorders are related to injuries to the proximal tubules epithelium in comparison to more resistive epithelium of the distal tubules [6-9].

The disorder in normal rats' kidney functioning is still noticeable, even one to three weeks after mercury chloride intoxication. It is interesting that the comparison between the results obtained after simultaneous water or salt load and the intoxication proves that the one-week-disorder is more significant than the three-week one in the former combination while total intoxication effects become worse in three weeks at the latter combination [10]. Proteinuria and reduced excretion of creatinine are the main signs of this disorder.

An increasing proteinuria on the background of acceleration of nitrates and deceleration of nitrites excretion has been reported for the combined intoxication by $HgCl_2$ and CCl_4 [11].

An interaction between $HgCl_2$ intoxication and oxidative stress induced by angiotensin II proved that even specific anti-angiotensin treatment is capable to diminish the content of peroxide radicals in the rat kidney tissues and reduce tubular damage caused by the intoxication [12]. In general, any heavy metal intoxication (including mercury chloride) leads to significant changes in antioxidant activities and lipid peroxidation values [13].

Therefore, it is obvious that various issues related to the heavy metals nephropathy alone or in combination with water or salt load remain interesting in the context of nephrology, toxicology and/or kidney tissues histology. Our investigation has been aimed onto biochemical evaluation of influence of the water and salt load on content of the oxidate modified proteins and lipids in the rat kidney under experimental nephropathy followed by additional histological analysis of the kidney tissues.

2. Materials and methods

This investigation has been carried out with the white nonlinear male adult rats with weight 180±10 g. The animals were kept in the vivarium at stable temperature and illumination and subdivided into eight groups:

Group 1 (6 individuals) – control, no load.

Group 2 (6 individuals) – water load induced (5 ml per 100 g of live weight).

Group 3 (6 individuals) – the 0.75 % salt load induced (0.75 % solution of NaCl was given as 0.65 mmol (14.8 mg of Na) per 100 g of live weight).
Group 4 (6 individuals) – the 3 % salt load induced (3 % solution of NaCl was given as 2.56 mmol (59 mg of Na) per 100 g of live weight).
Group 5 (6 individuals) – the 0.1 % solution of mercury chloride was injected subcutaneously 5 mg per 1 kg of live weight.
Group 6 (6 individuals) – the 0.1 % solution of mercury chloride was injected subcutaneously 5 mg per 1 kg of live weight followed by the water load (same as for the Group 2) induced in 72 hours.
Group 7 (6 individuals) – the 0.1 % solution of mercury chloride was injected subcutaneously 5 mg per 1 kg of live weight followed by the salt load (same as for the Group 3) induced in 72 hours.
Group 8 (6 individuals) – the 0.1 % solution of mercury chloride was injected subcutaneously 5 mg per 1 kg of live weight followed by the salt load (same as for the Group 4) induced in 72 hours.
The water and salt loads were injected by the metal endogastric probe 2 hours before euthanasia. The euthanasia was realized by decapitation under the light ether narcosis according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETC 123) [14]. The kidneys were taken out of the decapitated rats as soon as possible, dried by the filter paper and separated into three parts: cortex, medullar and papilla. Then the 5 % supernatant of the rat kidneys was prepared using the 50 mM tris-HCl buffer solution (pH=7.4) containing 0.1 % solution of trilon B by centrifugation at 900 g for 10 min. All these operations were performed at the temperature below 4 ^{0}C. Afterwards, the free radical oxidation conditions for lipids and proteins were determined in the post-nuclear supernatants by the content of TBA-RP and the oxide-modified proteins products (OMP-P) [15, 16]. A Mikel Calvo bromophenol blue staining method [17] was used for histochemical evaluation of the OMP-P samples and the "ColorPic" software was employed for the computer spectrometry of the histological micro sections. The R/B coefficient representing a ratio between the red (R, acidic proteins) and blue (B, basic proteins) staining of the cytoplasm was used to characterize a degree of the oxidative modification of proteins. All the pathological processes were recorded by taking digital photos of the micro samples parts by the camera OLYMPUS C740UZ and the microscope LUMAM-P8 equipped with various interchangeable lenses.

3. Results and discussion

It has been found that both salt and water load can cause changes in characteristics of the free radical oxidation of macromolecules within various kidney layers.
A rise for 35.7 % in comparison to the control samples value 44.3±4.26 μmole/l has been registered for the TBA-RP content in the kidney cortex tissue (see Table 1). A rise for 48.1% has been found for the same content in the medullar tissue while the rise in papilla tissue was only for 17.3 %.
Same values for the 3 % salt load case were 66.4 % for the cortex samples, 42.5 % - for the papilla and 24 % - for the medullar tissues.
Finally, the 0.75 % salt load gave rise for 58.4 % in the papilla, 55 % - cortex and 45.7 % - medullar layers in comparison to the corresponding control samples.
The photometry investigation at 370 and 430 nm showed no reliable changes in the

oxide proteins modification products contents after 5 % water, 3 % or 0.75 % salt loads (Table 1).

On the other hand, both 3 % and 0.75 % salt loads result in approx. 25 % rise in the TBA-RP content in various kidney tissues comparing to those after the water load. This may be caused by a deeper stress imposed by the salt load followed by more intense activation of the free-radical oxidation of lipids.

Table 1

Normalized optical densities (NOD) for the "blue" and "red" spectral zones (370 and 430 nm respectively)

Parameters / Groups	TBA-RP, μmole/g (tissues)	OMP-P (370), NOD (tissues)	OMP-P (430), NOD (tissues)
Control group			
Cortex kidney tissues	43.3±4.26	10.9±0.46	20.2±1.55
Medullar kidney tissues	60.8±4.51	13.3±1.95	20.1±0.60
Papilla kidney tissues	57.0±1.15	10.3±0.45	18.5±1.25
5 % water load			
Cortex kidney tissues	60.1±7.56*	11.4±0.41	18.5±1.10
Medullar kidney tissues	90.1±5.88*	11.2±1.36	18.0±1.55
Papilla kidney tissues	66.9±3.98*	10.1±0.87	18.2±0.26
3% salt load			
Cortex kidney tissues	73.7±2.86*	11.8±0.30	19.9±0.56
Medullar kidney tissues	75.4±2.09*	10.9±0.10	18.7±0.45
Papilla kidney tissues	81.1±3.59*	10.1±0.36	17.7±1.20
0.75% salt load			
Cortex kidney tissues	68.8±4.00*	11.9±0.85	18.5±0.21
Medullar kidney tissues	88.6±9.88*	11.7±0.55	16.9±0.85
Papilla kidney tissues	90.3±3.99*	10.5±0.7	17.9±1.58

Note: $p \leq 0.05$ comparing to the corresponding kidney tissues in the control series

A measuring method for the ratio between the basic and acidic protein groups is based on comparison between optical densities measured at 370 nm ("blue" spectral zone, related to the neutral aldehydo- and ketone-derivatives of the proteins) and 430 nm ("red" zone, the basic derivatives) [17]. All optical densities obtained in the experiments were normalized per 1 g of the tissue.

Then the R/B coefficient was calculated as a quotient of division the normalized "red" zone optical density by the "blue" zone optical density [17]. It is known [3, 18] that the free radical oxidation of proteins causes formation of new carboxyl groups and other acidic functions that results in shifting the R/B coefficient towards more acidic values [19].

That is why a value of R/B can be used as a characteristic of the oxidative modification of proteins. When its value is equal to 1, the content of the acidic and basic product is the same and it is greater than 1, the content of the acidic products is greater than the basic ones [17].

Data of Table 2 prove various depth of the oxide proteins modification in the epithelium of tissues of the kidney cortex sinuous tubules.

As seen from the data, no clear changes were registered in the cytoplasm of

epitheliocytes in the kidney sinuous tubules after 0.75 % salt load. However, both 5 % water and 3 % salt load caused shifts in the amino vs carboxyl groups in the proteins towards the latter. This can be considered as a sign of more intense oxidative modification of the proteins. It is interesting that for the 3 % salt load the above changes are more distinct for the samples taken at 8 pm than that for the 8 am samples.

The value of R/B becomes three times greater after injection of mercury chloride and again the 8 pm coefficients are some greater than the 8 am ones. On the other hand, the water load cause some decrease in the R/B values but they remain far above the control group. No significant changes in the R/B values were registered at the simultaneous mercury chloride intoxication and 0.75 % salt load while these parameters showed the highest level after same intoxication combined with 3 % salt load. It should also be stressed that R/B for the hyaline and granular cylinders of the sinuous tubules lumen was higher than that in the control group, which evidences deeper oxidative modification in the cell remainders.

Table 2

R/B coefficients for the epithelium of sinuous tubules of the rat kidney cortex tissue samples taken under various experimental conditions

Group	Sampling time		Deviation probability
	8.00	20.00	
Control	1.02±0.004	1.03±0.005	P>0.05
Water load	1.12±0.006	1.14±0.008	P>0.05
0.75 % salt load	1.03±0.006	1.04±0.007	P>0.05
3 % salt load	1.14±0.008	1.17±0.009	P=0.032
$HgCl_2$	2.94±0.014	3.21±0.017	P<0.001
$HgCl_2$ and water load	2.46±0.015	2.71±0.017	P<0.001
$HgCl_2$ and 0.75 % salt load	2.90±0.019	3.23±0.018	P<0.001
$HgCl_2$ and 3 % salt load	3.26±0.018	3.43±0.020	P<0.001

Note: $p \leq 0,05$ comparing to the corresponding kidney tissues in the control series

The above mentioned changes can also be proved by the series of microphotographs of the kidney core layer tissues. All the samples were stained with bromphenol blue by Mikel-Calvo method (see Figs. 1-8).

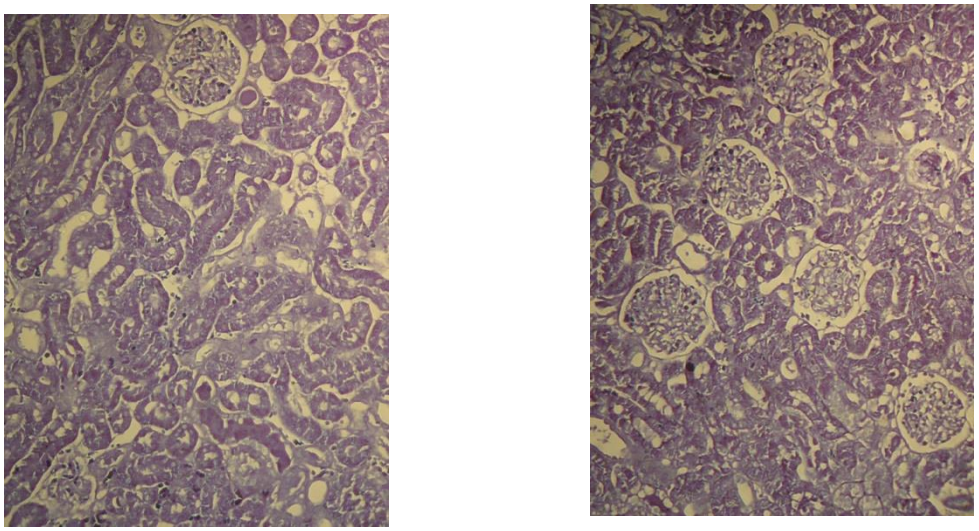
As seen from the comparison between the intact animals (Fig 1) and the animals exposed to 5 % water load (Fig. 2), an average percentage of the cells affected by granular degeneration has increased in the latter case. This situation can be caused by a higher functional load on epitheliocytes leading to more intense running of physiological processes, which knocks out the weakest cells and results in the changes represented in Fig. 2. It should also be noted that some signs of plethora can be seen in the venules of interstitium for the animals exposed to water load both for the 8 am and 8 pm euthanasia. However, no statistically relevant increase in the total interstitium volume was determined.

Application of mercury chloride causes some deep morphological changes of the kidney tissues. The severest affection can be noted for epithelium of the proximal tubules of core kidney tissues. Coagulation necrosis has stricken 40 % of the proximal tubules for the 8 am euthanasia (Fig. 5A) and 70 % - for the 8 pm euthanasia (Fig 5B). Besides, significant destruction of the cell nuclei (karyolysis) can be noted for both series of euthanasia (Fig 5 A and B). Therefore, the total affection of epitheliocytes with the alteration process can be stated. This process implies first reversible changes in the tissues structure followed by irreversible effects after a

longer exposition to the pathological processes.

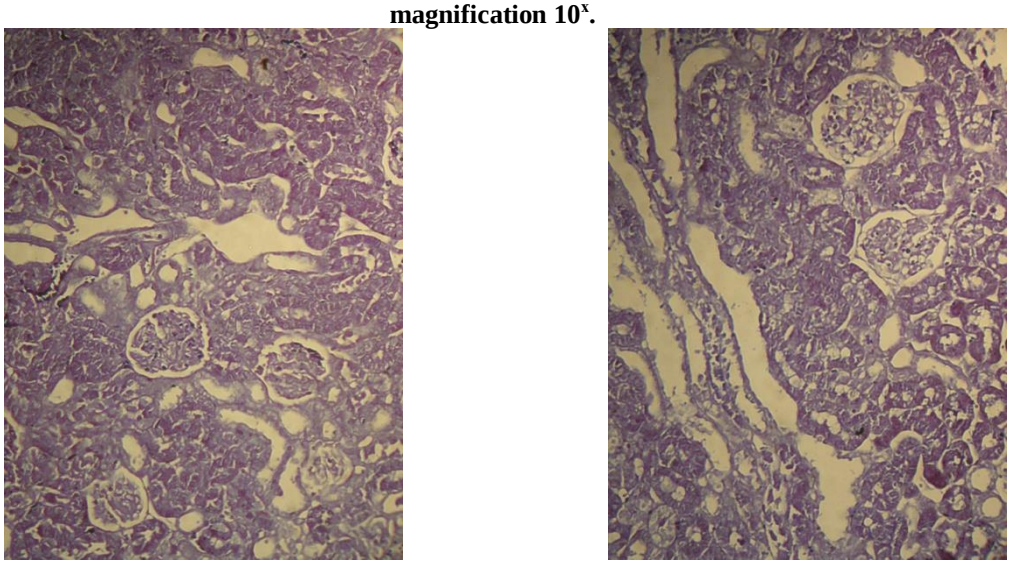
The tubules lumen is almost completely clogged with fragments of necrotized or desquamated cells.

It can be concluded that the mercury chloride intoxication (caused, for example, by environmental contamination or some other reasons) results in deep histological and morphological changes in the kidney tissues. That is why further experiments were aimed onto investigation of combined actions of the mercury chloride intoxication on the background of the salt and/or water load.



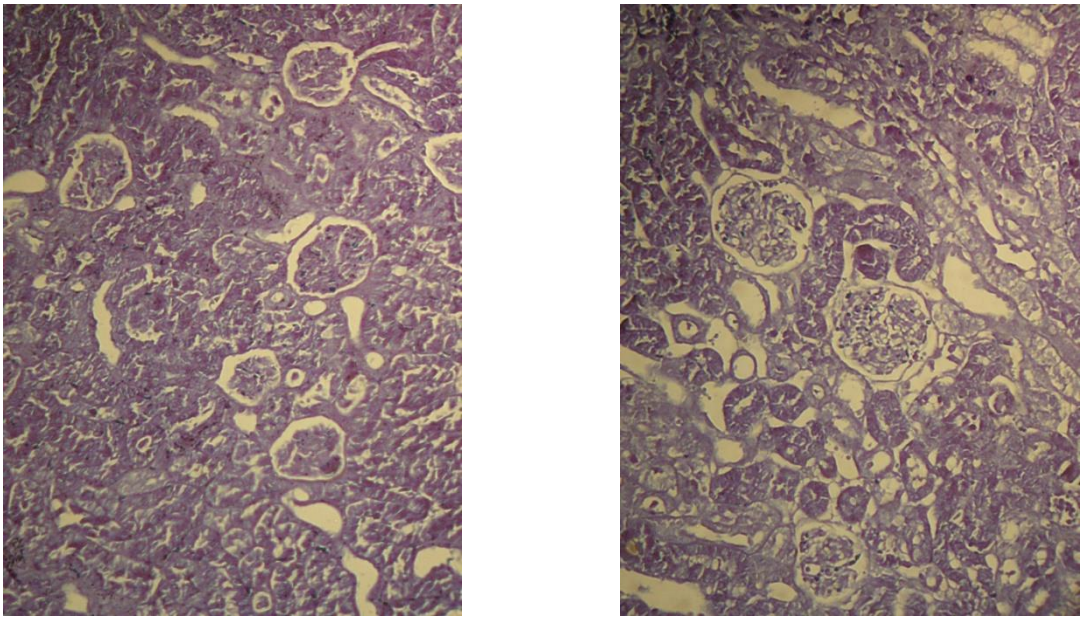
A B

Fig. 1. Core tissues of the intact animals. A) euthanasia at 8 am; B) euthanasia at 8 pm. Bromophenol blue staining by Mikel-Calvo method for the acidic and basic proteins. Lens magnification 10^x. Ocular magnification 10^x.



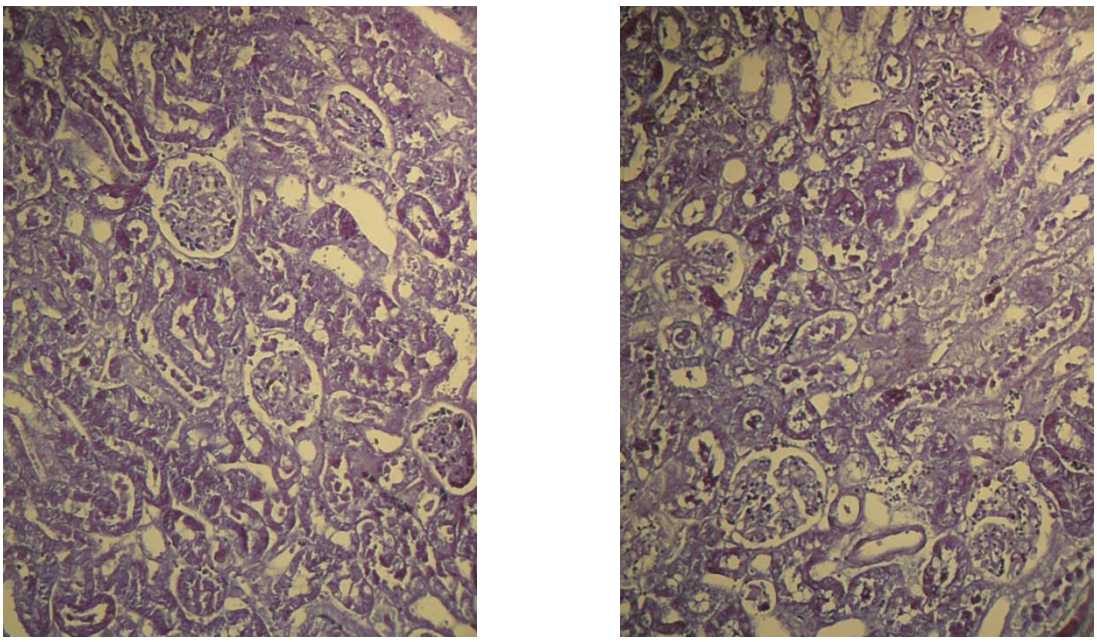
A B

Fig. 2. Core tissues of the rats exposed to 5 % water load. A) euthanasia at 8 am; B) euthanasia at 8 pm. Bromophenol blue staining by Mikel-Calvo method for the acidic and basic proteins. Lens magnification 10^x. Ocular magnification 10^x.



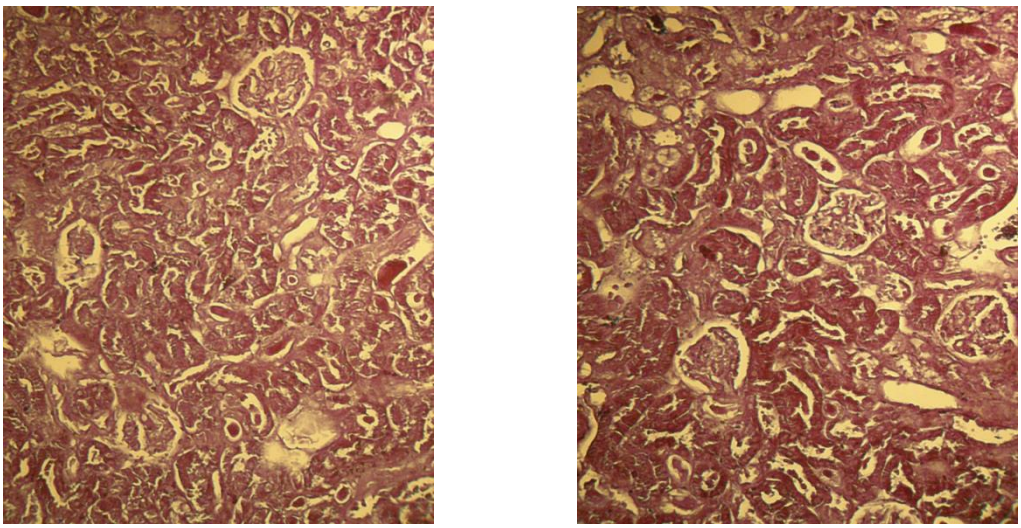
A B

Fig. 3. Core tissues of the rats exposed to 0.75 % salt load. A) euthanasia at 8 am; B) euthanasia at 8 pm. Bromophenol blue staining by Mikel-Calvo method for the acidic and basic proteins. Lens magnification 10^x. Ocular magnification 10^x.



A B

Fig. 4. Core tissues of the rats exposed to 3 % salt load. A) euthanasia at 8 am; B) euthanasia at 8 pm. Bromophenol blue staining by Mikel-Calvo method for the acidic and basic proteins. Lens magnification 10^x. Ocular magnification 10^x.

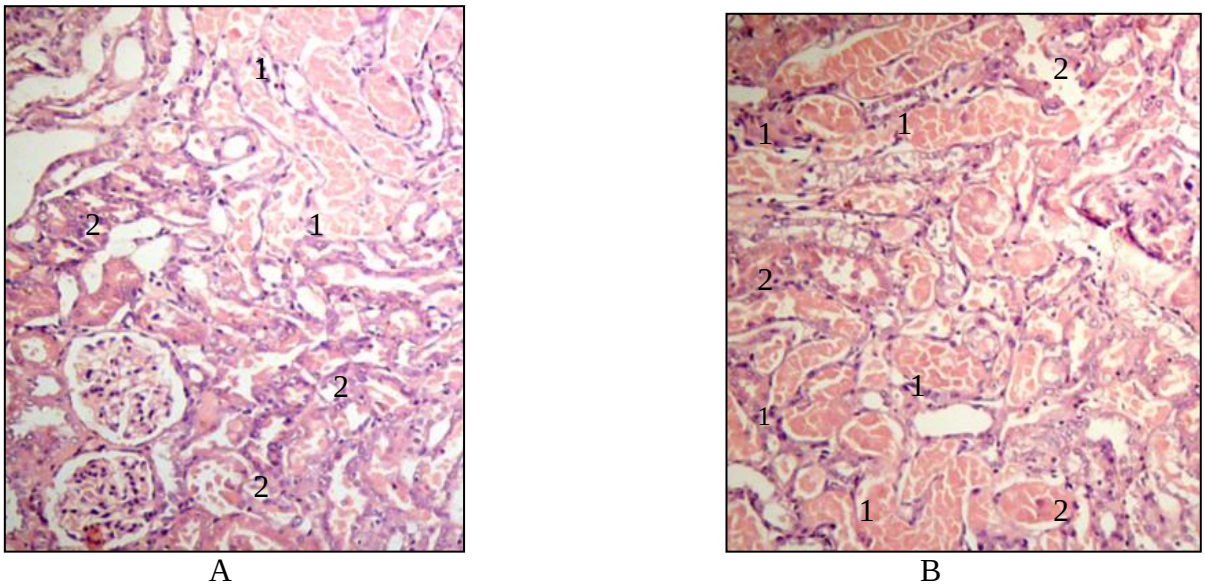


A B

Fig. 5. Core tissues of the rats exposed to mercury chloride. A) euthanasia at 8 am; B) euthanasia at 8 pm. Bromophenol blue staining by Mikel-Calvo method for the acidic and basic proteins. Lens magnification 10^x. Ocular magnification 10^x.

As seen from the Fig. 6, water load causes some relief in the kidney core tissues affected by the mercury chloride nephropathy. Although almost all epitheliocytes were involved in the process of alteration, its depth was lower and the process manifested itself mostly through the granular and hydropic degeneration.

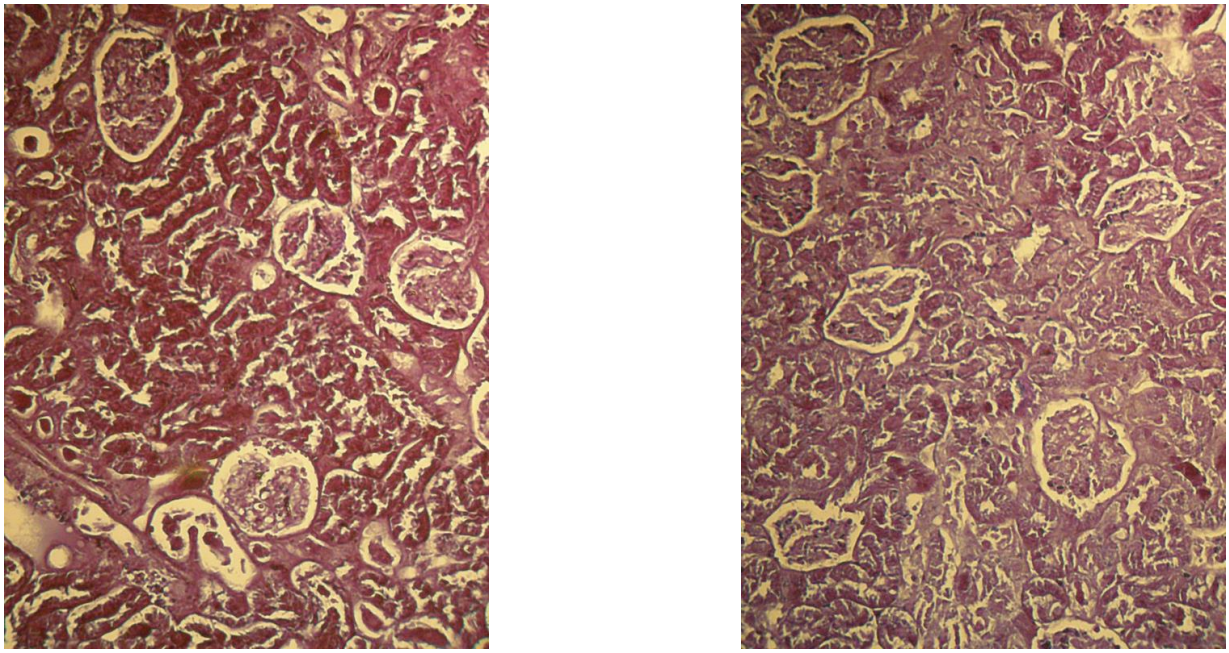
For example, comparison between Fig. 6A and B proves that only 9 % of epitheliocytes were necrotized for the 8 am euthanasia while for 8 pm that parameter was 16 %. No interstitium edema can be notice for any of the above cases.



A B

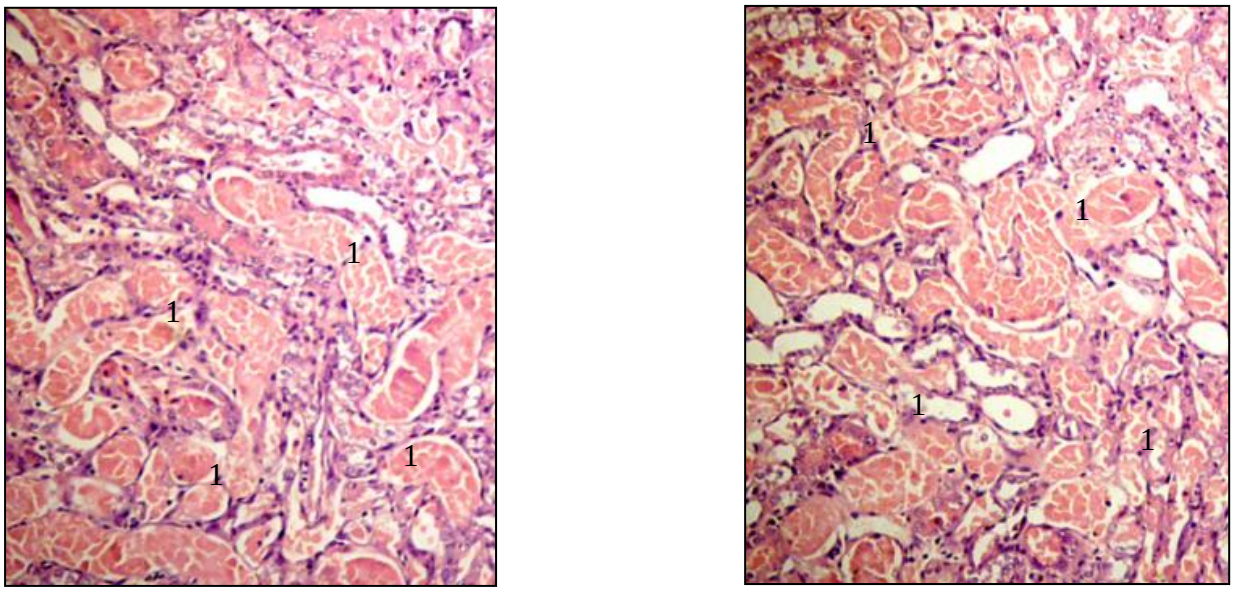
Fig. 6. Core tissues of the rats exposed to 5 % water load combined with the mercury chloride intoxication. A) euthanasia at 8 am; B) euthanasia at 8 pm. Necrosis spots (1) and granular or hydropic degeneration spots (2) are indicated. Bromophenol blue staining by Mikel-Calvo method for the acidic and basic proteins. Lens magnification 10^x. Ocular magnification 10^x.

Obvious positive changes can be seen from comparison between Fig 5 (mercury chloride nephropathy alone) and Fig 6 (same as in Fig. 5 but on background of the extra 5% water load): the kidney core tissue status is improving and the necrosis affects a lesser area of the tissue.



A B

Fig. 7. Core tissues of the rats exposed to 0.75 % salt load combined with the mercury chloride intoxication. A) euthanasia at 8 am; B) euthanasia at 8 pm. Bromophenol blue staining by Mikel-Calvo method for the acidic and basic proteins. Lens magnification 10^x. Ocular magnification 10^x.



A B

Fig. 8. Core tissues of the rats exposed to 3 % salt load combined with the mercury chloride intoxication. A) euthanasia at 8 am; B) euthanasia at 8 pm. Necrosis spots are indicated (1). Bromophenol blue staining by Mikel-Calvo method for the acidic and basic proteins. Lens magnification 10^x. Ocular magnification 10^x.

In contrary to the water load, serious deterioration in the kidney core tissue status was caused by the 3 % salt load exposed together with the mercury chloride intoxication. Massive necrosis has covered more than 90 % of the tubules in the juxtamedullary area of kidney. The rest of epitheliocytes were affected by degeneration, mostly of the hydropic type, which is more threatening than the granular one. The comparison between Fig. 8 A and B proves that about 70 % of the tubules were affected by deep necrosis for the 8 am euthanasia while this parameter reached 73 % for the 8 pm experiment. All these processes took place on the background of total involvement of the tubules epithelium in the process of alteration.

4. Conclusion

A content of TBA-RP in kidney tissues samples changes under both water and salt loads while contents of OMP-P remain almost unchanged.

Regardless of the sampling time, both types of the loads cause moderate changes in the depth of oxidative modification of proteins.

The injection of mercury chloride followed by water and/or salt load results in the activation of free-radical oxidation of proteins because of the damage of cell membranes.

A value of the oxide proteins modification index can bring important information related to pathogenesis and histology of the kidney tissues.

In general, it can be concluded that only moderate and reversible morphological changes were found in the kidney tissues underwent 5 % water and 3 % salt load while no morphological changes were found in the tissues after 0.75 % salt load. These morphological changes are in good agreement with histochemical data of the oxidative modification of proteins.

A classical necrotic nephrosis has been found in the animals after the mercury chloride intoxication. The nephrosis symptoms were more severe at 8 pm comparing to those at 8 am. Besides, the nephrosis symptoms were relieved partially by the water load while 3 % salt load caused worsening of the kidney tissues injury especially in case of the 8 pm results. No significant changes in the nephrosis symptoms were found after additional 0.75 % salt load. These results are also in good agreement with the histochemical data related to oxidative modification of proteins. Therefore, it can be concluded that water load can provide some relieving effect on the mercury chloride nephrosis while salt load results in further aggravation of its symptoms.

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