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Research Article

ACUTE TOXIC EFFECT OF ETHYLENEDIAMINE DIHYDROCHLORIDE (EDA-2HCl) ON LIVER AND KIDNEY

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ABSTRACT. Ethylenediamine dihydrochloride (EDA-2HCl, 50 mg/kg/day) was given to Wistar albino rats for ten days and the histopathologically effects on liver and kidney were examined by electron microscope. Besides the effects of EDA-2HCl on superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) activities and amount of total sialic acid (TSA) and malondialdehyde (MDA) in plasma and tissues, amount of total protein in tissues were determined. Degeneration of membrane in hepatocytes and in tubule cell in kidney; dysmorphism, loosing of Krista and increasing of count of mitochondria in hepatocytes and hypertrophy in kidney; reduction of intensity of nuclear matrix, loosing of cytoplasm, swelling of smooth endoplasmic reticulum (SER) in hepatocyte and tubule cell; dilatation and decreasing of microvillus of bile canaliculi in hepatocytes, untidiness of basal membrane and derangement of basal infoldings in kidney were observed. Amount of TSA was increased significantly ($p<0.05$) only in plasma and decreased significantly ($p<0.001$) only in kidney. Amount of MDA was increased in plasma, liver and kidney significantly ($p<0.001$). SOD activity was decreased significantly ($p<0.001$) only in liver. GPX and CAT activities were not changed in liver and kidney. Amount of total protein was decreased only in kidney significantly ($p<0.001$). These findings indicate that EDA-HCl has toxic effect on ultrastructural morphology and also on these biochemical parameters even in this lowest dose untested before in liver and kidney.

Keywords: *Ethylenediamine dihydrochloride, antioxidant enzymes, rat, ultrastructure*

INTRODUCTION

Ethylenediamine (EDA) is a fundamental industrial chemical with a wide variety of applications [1-3]. It is used in petroleum production, photography, ore beneficiation, metal processing, water and stream treatment, and in the manufacture of adhesives, agricultural chemicals, asphalt additives, chelating agents, cleaners, polishes, coating corrosion inhibitors, elastomers, resins, lubricants, processing of paper and textiles, also in veterinary medicine as a urine acidifier and in human medicine as part of the formulation in aminophylline and mycolog cream [1, 2, 4-7]. EDA is the lowest molecular weight member of alkyleneamines [4]. It was reported that LD50 (oral) dose of EDA is 637-1850 mg/kg and LC50 dose is >29 mg/L in rats and LC50 dose (dermal) is 560 mg/kg in rabbits were reported [8].

It was reported that skin irritation and eye injury were occurred by the effect of EDA-2HCl in laboratory animals [9]. Irritation, allergic signs and changes in central nervous

system were also declared as biological effects of EDA [10]. Bronchial asthma due to EDA in chemical workers [11] and pulmonary edema, liver injury and rhabdomyolysis were noted [12].

It was shown that EDA-2HCl was not carcinogenic in rats but also had chronic toxic effect by increased mortality with high dose group (0,35 g/kg/day) and the significantly increase of incidence of chronic nephropathy was considered to have contributed to death in the high dose group rats of both sexes [1].

It was determined that EDA-2HCl was not mutagenic by ovary gene mutation assay, the sister-chromatid exchange test and unscheduled DNA synthesis assay [13] and Ames/Salmonella test [4]. In addition any reproductive toxicity in a two-generation reproduction study in rats [14] or teratogenicity of EDA-2HCl in rats and rabbits were reported [2, 15].

It was shown that principal metabolite of EDA was N-acetylethylenediamin in urine by liquid scintillation spectrometry [6], mass spectrometer and infra-red spectrometry [16]. It was also declared that about 4-13 and 8% of the dose (50 mg/kg iv or oral gavage 5, 50 and 500 mg14C-derivative/kg) was eliminated in the feces and as expired CO2, respectively and excretion was quite rapid, with over 70% of the applied dose eliminated within 24 hour in mice. It was determined rapid absorption of EDA as its concentration in plasma reached a maximum at about 1 hour after dosing and, liver and kidney were major organs with their highest concentration of EDA in mice [6].

It was reported that body and some organ weight gains were decreased in short and long-term effects of EDA-2HCl (7 days, 2.70 g/kg/day and 3 month 0, 0.05, 0.25, 1.00 g/kg/day [9] in mice and rats. Also in 0.5, 0.15, 0.05, 0 g/kg/day doses of EDA-2HCl reduction of body weight gain changes in liver (decrease) and kidney (increase) weights but no reproductive toxicity were observed in rats [14]. In long term effects of EDA-2HCl (2 years) at 0, 0.02, 0.10, 0.35 g/kg/day doses decreasing in body weight gain and increasing in liver and kidney weights were determined [1]. Besides, changing of clinical and hematological parameters, values of regarding to urine, hepatocellular pleomorphism [1, 9], enlarged nuclei, an increased degree of variation in nuclear shape were observed as characteristics of hepatocellular cytologic alterations, also rhinitis and tracheitis were occurred in high dose group by the effect of EDA-2HCl [1, 9]. We also observed hypertrophic hepatocytes and membrane degeneration, vacuolization in cytoplasm and pyknotic nuclei in hepatocytes and endothelial degeneration of vein as necrotic alterations and mono nuclear cell infiltration as inflammatory response in liver in our laboratory [17]. Besides, increasing of distance between parietal and visceral layer of Bowman capsule, atrophy in Glomerulus, loosing of Glomerulus, degeneration of membrane and hypertrophy in epithelia of tubules, loosing of nucleus and cytoplasm in epithelial cell, vacuolization in cytoplasm, pyknotic nucleus, degenerative nucleus and cytoplasmic residues in tubule lumen were observed in kidney. In addition, thickening of basal membrane of hepatocytes in liver and epithelia of tubules in kidney were determined previously [17].

SOD (EC.1.15.1.1), GPX (EC.1.11.1.9) and CAT (EC.1.11.1.6) are the most important enzymes in the investigation of tissue damage from oxidative stress and chancing of these enzyme's activities are considered as a marker of oxidant stress in related studies [18-20]. SOD is enzyme that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. GPX and CAT catalyze the decomposition of H_2O_2 to water and oxygen [19].

Malondialdehyde (MDA), the end product of lipid peroxidation is also another oxidative stress marker [21, 22]. It was reported that increased MDA levels in oxidative

stress induced by ischemia/reperfusion and decreased level of MDA by reason of antioxidant treatment [23-25].

Sialic acids (N-acetylneuraminic acids, SA) are negatively charged monosaccharides that are common constituents in the oligosaccharides of vertebrates and some invertebrate species. The majority of sialic acid in higher animals is bound up in glyco-conjugates. SA is possibly the most biologically important monosaccharide units of glyco-conjugates. SA often occurs as the terminal monosaccharide of oligosaccharide chains of glycoproteins, glycosphingolipids and GPI anchors. Both its negative charge and its terminal position make it critical in numerous biological processes. SA imparts a net negative charge to the cell surface and is important in cell-to-cell and cell-to-matrix interactions [26-28].

Quantitative and qualitative differences in SA are seen in health and disease, as well as at different stages of cell growth, differentiation, aging and malignant transformation [26, 27, 29-32]. In recent years, it has been reported that levels of SA are increased in certain types of cancer [33-37], and it has been proposed that SA may be a useful tumor marker for some cancer types [38].

However, to the best of our knowledge, there are no reports concerning the toxic effects of EDA-2HCl (50 mg/kg/day) on ultrastructural morphology and on these biochemical parameters previously. This is the first study evaluating the toxic effect of EDA-2HCl (50 mg/kg/day) on ultrastructure of liver and kidney. Also we intended to determine of alterations of antioxidant enzyme activities (SOD, GPX, and CAT), amount of MDA and TSA by the effect of EDA-2HCl in liver and kidney in rats. The hypothesis of this study is that EDA-2HCl is toxic in liver and kidney in rat in this tested lowest dose.

MATERIALS AND METHODS

Chemicals and Dose Preparation

EDA-2HCl, ($C_2H_8N_2.2HCl$; 1,2-diaminoethane dihydrochloride, MW; 133,02, Cat No:195804, 98% pure, Aldrich, St. Louis, US) was obtained from Sigma-Aldrich Company. Dose was prepared to give 50 mg/kg body weight (SIDS, 2001) and applied intramuscular injection (i.m.) for ten days.

Animals and Treatments

Thirty female Wistar albino rats weighing 165±10 g were obtained from Department of Experimental Animals of Trakya University, Edirne, Turkey. Animals were kept at 24-26 ºC, with a relative humidity of 50-70%, on a 12-h light/dark cycle, with complete air change of ten times per hour. They feed a standard diet include 21% protein and tap water ad libidum. They were divided randomly one control and one EDA-2HCl-treated groups (n=10 each). The EDA-2HCl-treated group was given 50 mg/kg body weight of EDA-2HCl intramuscular injection for ten days.

All animals were checked out before injection for ten days. It was observed no injury or no deterioration in general condition. Anyone was died during experiment period and animal count was remained.

Limitations of the study are the number of rats used in the study was the minimum number sufficient for statistical analysis and the lowest single dose of EDA was administered.

This study was approved by the Animal Experiments Local Ethics Committee of Trakya University.

Histopathological Procedures

Tissue samples were dissected out under anesthesia with 5-10 mg/kg Rompun (%2) (Xylazine hydrochloride) from Bayer (Istanbul, Turkey) and 50-70 mg/kg Ketasol (%10) from Richterpharma (Wels, Austria). Liver and kidney pieces of 1-2 mm in size were fixed in 4% glutaraldehyde in 0.1 M of phosphate buffer (pH 7.2–7.3) and, after primary fixations tissues were washed in phosphate buffer overnight. The tissues were post-fixed with 1% osmium tetra oxide in phosphate buffer for 1h at +4 ºC. The post-fixed tissues were then washed in phosphate buffer and dehydrated using graded ethyl alcohol. Dehydrated tissues were processed to make araldite-epon 812 blocks. Ultrathin sections were obtained by ultramicrotome (RMC-MTX Ultramicrotome-USA) and collected on copper grids for double staining (uranyl acetate and Reynold's lead citrate) [39-41]. Stained sections were observed under a Jeol-JEM 1010 transmission electron microscope.

Biochemical Assay

Tissue samples from liver and kidney were washed with cold 0,9% saline and kept frozen at -80 ºC until homogenization. Tissue samples (0,5 g) were homogenized with 5 volume per weight, 0,05 M phosphate buffer pH 7.0 containing 1% (w/v) Triton X-100 by glass-glass homogenizer for 5 minutes. Extract was centrifuged for 20 min. at 10 000 g, +4 ºC. The supernatant (S1) was used in the assay for GPX. CAT was measured in the same fraction (S1) after addition 0,2 volume ethanol (1% v/v) and incubation in the cold for 15 min. An aliquot of the S1 supernatant was precipitated on ice with 0,3 volume chloroform/ethanol (3:5 v/v), stirred on ice for 15 minute and centrifuged at 10 000 g for 15 minute. The supernatant from this (S2) was used in the assay of SOD activity [42].

SOD and GPX activities were measured by using Ransod kit (Randox Cat. No; SD 125, United Kingdom BT29 4QY) [43-46] and Ransel kit (Randox Cat. No; RS 505, United Kingdom BT29 4QY) respectively [47-49], with a spectrophotometer (Shimadzu-1240, Japan). One unit SOD activity was defined as that the amount of enzyme that causes a %50 inhibition of the rate of reduction of I.N.T. (2-(4-iodophenyl)-3-(nitrophenol)-5-phenyltetrazolium chloride) under the conditions of assay (at 37 ºC, pH 7,0). One unit GPX activity was defined as the amount of enzyme that oxidized 1 µmol NADPH to NADP in one minute at 37 ºC. One unit CAT was defined as the amount of enzyme, that decomposed 1 µmol H_2O_2 per minute at 30 ºC and pH 7,0 [50]. Total protein contents of liver and kidney were determined by the method of Lowry, using bovine serum albumin as a standard [51].

Tissue samples (0,5 gr) were homogenized with 1,5% KCl in five volumes (w/v) by glass-glass homogenizator for MDA determination. Homogenate added to reaction mixture included 8,1% sodium dodecyl sulphate (SDS), 0,82% thiobarbutiric acid and acetate buffer (3 M, pH: 3,5). Then reaction was followed in 532 nm by spectrophotometer [52, 53]. Values were expressed as µmol/L by using MDA standard curve prepared 1,1,3,3 tetramethoxypropan. Blood samples were taken from the heart for MDA and plasma was obtained after centrifuge for 10 min. at 5000 rpm. Serum MDA levels was determined by following color compound produced with thiobarbutiric acid in 532 nm by spectrophotometer [52, 53].

Tissue and serum samples were frozen at -80 ºC until use for determination of total sialic acid. After melting, tissues were homogenized in phosphate buffer (0,1 M, pH 7,0) by glass-glass homogenizator. TSA was liberated with 5% perchloric acid hydrolysis [54, 55]. Spectrophotometric determination was carried out at 525 nm.

Statistics

We used SPSS 19 software from the Biostatistics Scientific Department, Trakya University, for statistical analyses. The significant differences in the data were tested by using the Mann Whitney's *U* test. Mean is given with standard deviation (mean ± Std. Deviation). In the analyses, the $p<0.05$ and $p<0.001$ as significance level were used.

RESULTS AND DISCUSSION

All animals were checked out before injection for ten days. It was observed no injury or no deterioration in general condition. Anyone was died during experiment period and animal count was remained.

Histopathological Effect of EDA-2HCl on Liver and Kidney

Liver was observed by normal ultrastructural morphology in control group (Fig. 1a) and some degenerative changes were observed in 50 mg/kg EDA-2HCl. It was observed degeneration of nucleus membrane in Kupffer cell and increasing of distance between inner and outer membrane of nucleus of Kupffer cell, dysmorphism and electron dense areas at outer membrane of mitochondria in hepatocyte (Fig. 1b), loosing of Krista of mitochondria of hepatocytes, loosing of cytoplasm of hepatocyte, reduction of intensity of nuclear matrix (Fig. 1c), outer membrane degeneration of mitochondria, membrane degeneration of Disse side of hepatocyte, increasing of count of mitochondria (Fig. 1d). Enclosing of degenerated mitochondria in hepatocyte, swelling smooth endoplasmic reticulum, dilatation and decreasing of microvillus in bile canaliculi of hepatocyte (Fig. 1e) were observed in dose group.

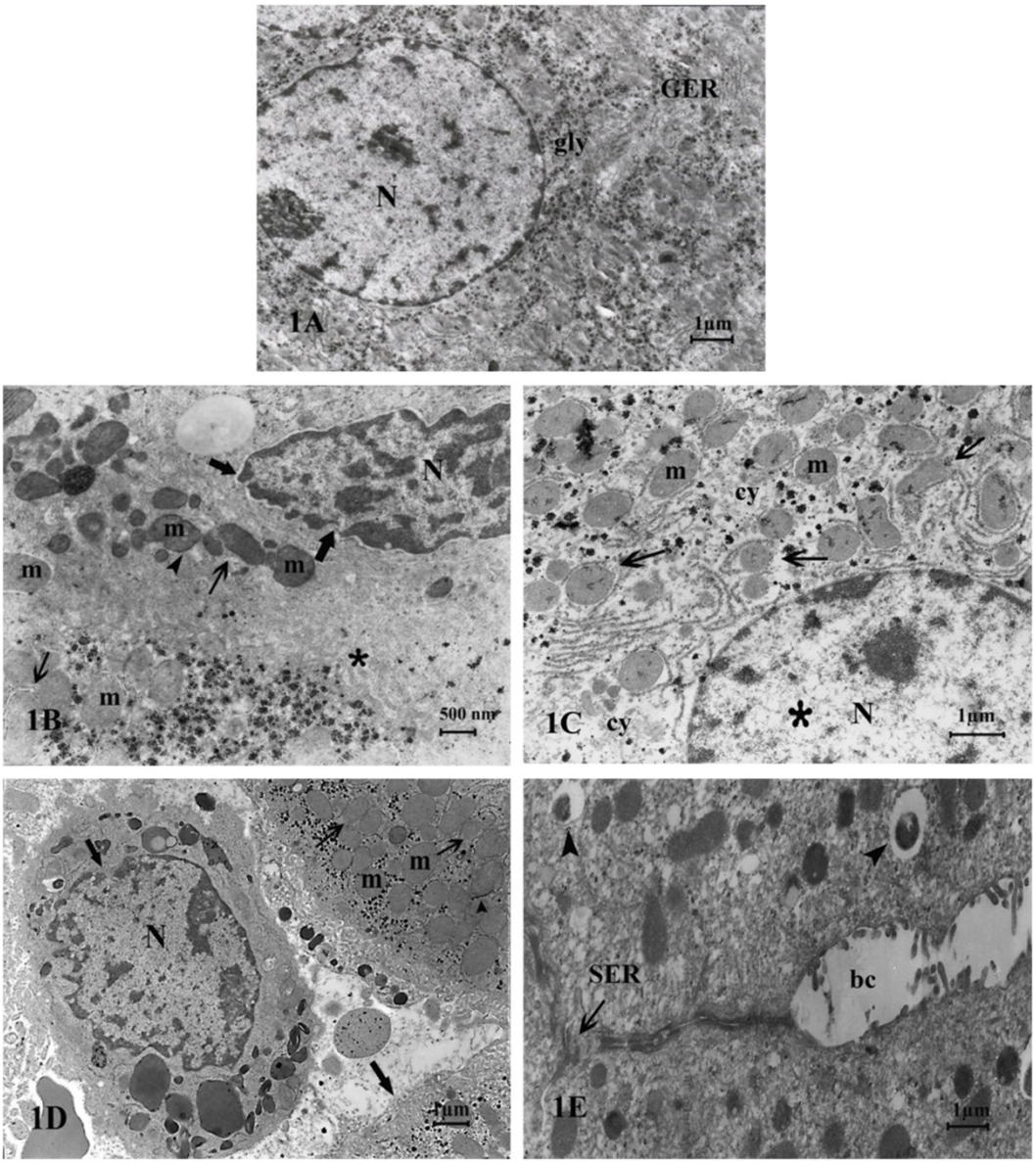

Fig. 1. *A) Liver from control group, nucleus (N), granular endoplasmic reticulum (GER), glycogen (gly), B) Degeneration of nucleus membrane in Kupffer cell (thick arrow), increasing of distance between inner and outer membrane of nucleus of Kupffer cell (thick arrow), dysmorphism (thin arrow) and electron dense areas at outer membrane of mitochondria (m) (arrow head) in hepatocyte C) Loosing of cytoplasm of hepatocyte (cy), outer membrane degeneration (thin arrow) and loosing of Krista of mitochondria (m), reduction of intensity of nuclear matrix (aster), D) Membrane degeneration of mitochondria (thin arrow), degeneration of nucleus membrane of Kupffer cell (thick arrow), membrane degeneration of Disse side of hepatocyte (thick arrow), electron dense areas around of mitochondria (arrow head), increasing of count of mitochondria (m), E) Dilatation and decreasing of microvillus of bile canaliculi (bc), swelling of smooth ER (SER), enclosed mitochondria by vesicles (arrow head), cell membrane degeneration (thin arrow)*

In control group, normal ultrastructural morphology of kidney was seen (Fig. 2a) but some changes were defined in dose group (50 mg/kg). Degeneration of nucleus membrane

and separation of nucleus between cytoplasm, reduction of intensity of nuclear matrix, cytoplasmic vacuolization (Fig. 2b), untidiness of basal membrane, dilatation in smooth ER, collagen fibers in connective tissue, derangement of basal infoldings of tubule cells, degeneration of cytoplasm and myelin figures in cytoplasm in tubule cells (Fig. 2c), loosing of Krista of mitochondria, membrane degeneration of mitochondria, loosing of membrane at basal area in tubule cells especially at basal infoldings (Fig. 2d), hypertrophy in mitochondrion and separation between inner and outer membrane of nucleus in tubule cells of kidney (Fig. 2e) were observed in dose group.

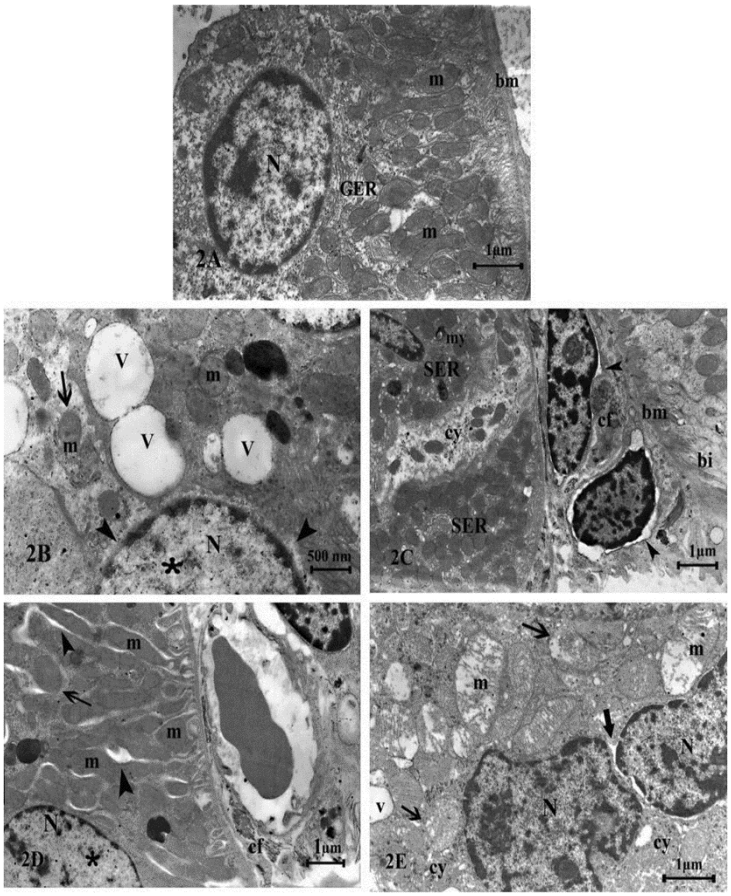

Fig. 2. *A) Kidney from control group B) Degeneration of nucleus membrane and separation of nucleus between cytoplasm (arrow head), disorganization of Krista of mitochondria (m), membrane degeneration of mitochondria (thin arrow), cytoplasmic vacuolization (v), reduction of intensity of nuclear matrix (aster) C) Degeneration of nucleus membrane in connective tissue cells (arrow head), untidiness of basal membrane (bm), dilatation in smooth ER (SER), collagen fibers in connective tissue (cf), derangement of basal infoldings of tubule cells, degeneration of cytoplasm (cy), myelin figure (my) D) Collagen fibers in extra cellular areas (cf), loosing of Krista of mitochondria (m), membrane degeneration of mitochondria (thin arrow), reduction of intensity of nuclear matrix (aster), loosing of membrane of basal infoldings (arrow head) E) Disappearing of Krista and hypertrophy in mitochondrion (m), membrane degeneration of mitochondria (thin arrow), degeneration of nucleus membrane (thick arrow), degeneration of cytoplasm (cy)*

Biochemical effect of EDA-2HCl on liver and kidney

Effect of EDA-2HCl on SOD, GPX and CAT activities in Tissues

SOD activity was decreased significantly (p<0,001) in liver but were not changed in kidney. GPX and CAT activity were not changed significantly in liver and kidney as shown in Table 1.

Table 1. *The effect of EDA-2HCl on SOD (U/ml), GPX (U/ml) and CAT(U/ml) activities. Mean is given with standard deviation (mean±Std. Deviation).* [b] *presents p<0,001 as significance level*

	SOD (U/ml)		**GPX (U/ml)**		**CAT (U/ml)**	
	liver	**kidney**	**liver**	**kidney**	**liver**	**kidney**
Control	192,242±0,6	200,514±0,4	3364,8±1590,1	5863,16±1700	68,90±22,9	96,04±17,7
EDA	188,983±2,0[b]	200,413±0,4	3785,4±1635,4	6724,61±1891	56,30±19,7	82,42±20,2

Effect of EDA-2HCl on MDA and TSA in Serum and Tissues

MDA was increased in plasma (p<0,001) and also increased in liver (p<0,001) and kidney (p<0,001) significantly. TSA in plasma was increased significantly (p<0,05) by the effect of EDA-2HCl. TSA was decreased in kidney significantly (p<0,001) but not changed significantly in liver. Amount of total protein was decreased in kidney significantly (p<0,001) but not changed significantly in liver as shown in Table 2.

Table 2. *The effect of EDA-2HCl on amount of TSA (mg/ml), MDA (µmol/L) and total protein (mg/ml). Mean is given with standard deviation (mean±Std. Deviation).* [a] *presents p<0,05 and* [b] *presents p<0,001 as significance level*

	TSA (mg/ml)		
	plasma	**liver**	**kidney**
Control	1,202±0,107	375,5±71,224	149,5±17,778
EDA	1,511±0,191 [a]	305,5±126,325	97,833±20,638 [b]
	MDA (µmol/L)		
	plasma	**liver**	**kidney**
Control	1,84±0,282	21,405±3,101	10,151±1,601
EDA	3,145±0,758 [b]	30,679±4,063 [b]	14,683±2,497 [b]
	Total protein (mg/ml)		
	plasma	**liver**	**kidney**
Control	10,151±1,601	55,56±7,62	6,537±0,37
EDA	14,683±2,497 [b]	54,21±5,52	5,364±0,64 [b]

Environmental pollution is increasing by progressive stage with advancement of industry in the present days. Alkyleneamines are a large chemical family which used several industrial applications such as photography, petroleum production, manufacture of adhesives, processing of paper and textiles [1, 2, 6]. In this study we examined toxic effects of EDA-2HCl (50 mg/kg/day) treatment by i.m. to Wistar albino rats for ten days.

Ultrastructural changes of liver and kidney and also alterations of antioxidant enzyme activities (SOD, GPX, and CAT), amount of MDA and TSA by the effect of EDA-2HCl in liver and kidney were observed.

Degenerative changes by the effect of EDA-2HCl (50 mg/kg) were determined in ultrastructural level in liver. Nucleus membrane of Kupffer cell was degenerated. Increase of distance between inner and outer membrane of Kupffer cell's nucleus was observed. Also reduction of intensity of nuclear matrix of hepatocytes was determent. These alterations of nucleus were point out to failure of nucleus metabolism in general. Besides, loosing of cytoplasm also indicate that impotence of relationship between nucleus and cytoplasm. We observed a sign of degeneration of nucleus and cytoplasm by the effect of EDA in our previous study by light microscope [17]. Also moderate degeneration in hepatocytes, changing in form and volume of nucleus, amount of multinuclear hepatocytes by the effect of EDA-2HCl were reported before [9].

Membrane degeneration especially of Disse side of hepatocyte were seen, outer membrane degeneration of mitochondria, loosing of Krista in hepatocytes and decreasing of microvillus in bile canaliculi of hepatocyte were represent the negative effect of EDA on membrane. In addition, we observed dysmorphism and electron dense areas at outer membrane of mitochondria, enclosing of degenerated mitochondria and increasing of count of mitochondria in hepatocytes. These changes could be considered an adaptive response against mitochondrial degeneration and hepatocytes may try to maintain their energy sources. Swelling smooth endoplasmic reticulum in hepatocyte and dilatation in bile canaliculi also indicate hypertrophy as we mentioned in our previous study [17]. It is possible that intracellular membrane degeneration may lead to lysis of organelles especially lysosomes and mitochondrion than degeneration processes would be progress as necrosis. We declared before that EDA could make necrotic degeneration and we have also confirmative explain by our findings of EDA's toxic effect by this ultrastructural evaluation. It was published that inflammation, necrosis, hepatomegaly and hyperplasia in liver in report of National Cancer Institute-NCI [55]. Inflammation (tracheitis and rhinitis) in upper respiratory tract beside inflammation in liver were represented before [1]. Observation of excessive smooth ER than granular ER comparatively in hepatocytes bring to mind that pH of cytoplasm declined and ribosomes left from ER. Membrane degeneration of mitochondria and loosing of Krista may indicate that failure of mitochondrial function, partial interruption of oxidative respiration and decreasing of ATP. Thereby, some of protein synthesis could be reduced and transport across from cell membrane may be disrupted. It may lead to chaos in metabolism in general meaning.

In control group, normal ultrastructural morphology of kidney was seen but some changes were defined in dose group (50 mg/kg). Degeneration of nucleus membrane, separation of nucleus between cytoplasm, reduction of intensity of nuclear matrix, cytoplasmic vacuolization, separation between inner and outer membrane of nucleus in tubule cells and reduction of intensity of nuclear matrix of kidney cell were cause to disorganization of cell metabolism. Also, loosing of cytoplasm is noted to insufficiency of completeness between nucleus and cytoplasm. Untidiness of basal membrane, loosing of membrane at basal area in tubule cells and derangement of basal infoldings are evocated that disruption transport of tubule cell. We observed that loosing of Krista of mitochondria, membrane degeneration of mitochondria and hypertrophy in mitochondrion. These findings indicated that failure of mitochondrial function, partial interruption of oxidative respiration and decreasing of ATP as we mentioned before in liver. Either degeneration of cytoplasm and myelin figures in tubule cells or

mitochondrial degeneration also slightly increasing of collagen fibers in connective tissue are consider that necrotic changing in kidney. Dilatation in smooth ER in tubular cell point out that occur adaptive response against to EDA for detoxification. Similarly we also mentioned that necrotic change in kidney in our previous study by observing membrane degeneration, glomerular atrophy, increasing of distance between parietal and visceral side of Bowman capsule [17]. Our findings from electron microscopic evaluation are supported by previous study. Chronic nephropathy was observed in kidney and cause of death was identified with this chronic nephropathy in high dose group by Hermansky et al., (1999) [1]. We also observed findings which indicated failure of kidney function by acute effect of EDA-2HCl associated with decreasing in amount of total protein in kidney. In addition, tubular epithelial hyperplasia because of EDA-2HCl in kidney was presented by NCI Report (1979). Histopathologic lesions in eyes, uterus were observed and explained that it were characteristic with necrosis and tubular epithelial degeneration and regeneration by the effect of EDA-2HCl [56]. Dilation of tubules and necrosis in kidney were reported in 200 mg/kg and its upper doses but not any negative effect in 100 mg/kg of EDA-2HCl in a long term study (90 days) which was tested 100-1600 mg/kg in rats and 25-400 mg/kg in mice by National Toxicology Program (NTP) [57]. Degeneration in kidney in 100 mg/kg but not harmful effect in 50 mg/kg of EDA-2HCl were observed in a short term study (12 days) [57].

We found significantly decreasing in SOD activity in liver but not changing in kidney. GPX and CAT activity were not changed significantly in liver and kidney. MDA was increased in plasma liver and kidney significantly. It may consider that enzymatic antioxidant defense system could have weakness by decreasing SOD activity and superoxide radicals lead to lipid peroxidation especially in cell membrane which confirmed by increased MDA levels. The degeneration of membrane, the first target of free radicals because of its lipid content, could be supported with our microscopic findings.

We observed an increased level of TSA in plasma and decreased significantly in kidney by the effect of EDA-2HCl. It may be occur as a result of membrane degeneration in kidney and TSA could be included in blood stream. Besides, EDA-2HCl may be cause to increase of sialisaiton in blood cell. The cause of membrane degeneration of tubular cell in kidney could be decreasing of SA level or changing of SA types. It was reported that changing of glycosylation was important in cell to cell adhesion before [26, 58]. In our study, alterations of TSA may be remarkable because of its importance in aging and malignant transformation process [59, 60].

CONCLUSION

Our findings indicate that EDA-2HCl has toxic effect on liver and kidney in rats at 50 mg/kg/day (i.m.) dose and this is the first time to reveal ultrastructural evaluation of EDA-2HCl. Also it was not detected any study associated with TSA, antioxidant enzymes and EDA-2HCl. We think that it has to be needed investigate in detail because of it's a wide variety of applications as fundamental industrial chemical.

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