

Effect of melatonin and n-acetylcysteine on hepatic injury in rat induced by methanol intoxication: a comparative study

M. KOKSAL¹, Z. KURCER², D. ERDOGAN³, M. IRAZ⁴, M. TAS⁵,
M.A. EREN⁶, T. AYDOGAN⁶, T. ULAS⁶

¹Department of Histology and Embryology, Faculty of Medicine, Harran University, Sanliurfa (Turkey)

²Department of Pharmacology, Faculty of Medicine, Zonguldak Karaelmas University, Zonguldak (Turkey)

³Department of Histology and Embryology, Faculty of Medicine, Gazi University, Ankara (Turkey)

⁴Department of Pharmacology, Faculty of Medicine, Bezmialem Vakif University, Istanbul (Turkey)

⁵Andrology Unit, Kanuni Sultan Süleyman Research and Education Hospital, Istanbul (Turkey)

⁶Department of Internal Medicine, Faculty of Medicine, Harran University, Sanliurfa (Turkey)

Abstract. – Background: Methanol intoxication leads liver injury; in contrast melatonin and n-acetyl cysteine (NAC) are known to have protective effects on liver.

Aim: We aimed to investigate the ultrastructural effects of melatonin and NAC on livers of methanol intoxicated rats and compare potential protective effects of melatonin and NAC on their liver ultrastructure.

Materials and Methods: Fifty-six adult male Wistar rats were carried out and were randomized to eight groups that have seven rats each: Control groups (C 6h, C 24h), treated with intragastric (i.g.) 1.0 ml saline; Methanol groups (M 6h, M 24h), treated with a dose of 3 g/kg i.g. methanol; Melatonin plus methanol groups (MEL+M 6h, MEL+M 24h), treated with dose of 10 mg/kg i.p melatonin immediately, following with a dose of 3 g/kg i.g. methanol; NAC plus methanol groups (NAC+M 6h, NAC+M 24h), treated with dose of 150 mg/kg, following with a dose of 3 g/kg i.g. methanol. 24 h group rats were given the same dose of melatonin and NAC 12 h after intoxication. Electron microscopy was used to evaluate histological changes in liver tissue at both 6th and 24th hour.

Results: Histopathological damage was found to be higher in methanol-induced intoxicated rats compared with the controls. Extensive tubules of smooth endoplasmic reticulum, increased mitochondria, increased primary lysosomes and some marked openings of bile canaliculus were distinguished. Melatonin administration prevents liver injury especially in early hours and although not as effective as melatonin, NAC also prevents liver injury.

Conclusions: Melatonin is much more efficient than NAC, as well as significantly greater hepatoprotective effect against the liver injury secondary to the methanol intoxication.

Key Words:

Methanol intoxication, Melatonin, N-acetyl cysteine, Hepatic injury, Rat, Electron microscopy

Introduction

Methanol is extensively used as an industrial solvent and cleaner¹. It is rapidly absorbed following inhalation, ingestion or cutaneous exposure. It is oxidized in the liver to formaldehyde, then to formic acid, which contributes to the profound metabolic acidosis, obtundation, visual disturbance and sometimes death². It was hypothesized that because of acidosis, the generation of oxygen radicals might be enhanced, leading to membrane damage, lipid peroxidation, and mitochondrial damage^{3,4}.

Melatonin, the chief indolamine produced by the pineal gland, has been shown to be an effective antioxidant and free-radical scavenger, which is capable of stimulating GSH synthesis⁵⁻⁷. It is clearly a lipid soluble agent; it seems also capable of entering the aqueous environments of the cell⁸. Administration of melatonin at pharmacological doses has been shown to decrease free radical formation and lead to a substantial recovery of the major antioxidant enzymes, thus limiting oxidative damage to the liver⁹. Melatonin is effective against pathologi-

cal states characterized by an increase in basal rate of reactive oxygen species (ROS) production, and protects liver from oxidative damage in multiple conditions¹⁰⁻¹⁶.

N-acetyl cysteine (NAC), a small molecule containing a thiol group, has antioxidant properties and is freely filterable with a ready access to intracellular compartments and may directly react with electrophilic compounds such as formaldehyde and free radicals¹⁷⁻¹⁹. Consequently, NAC was widely used in clinical practice as an antioxidant and had been investigated whether it was beneficial for reducing liver ischemia reperfusion (IR) injury in animal models²⁰⁻²². The results suggested that NAC was an effective antioxidant in methanol intoxication. It may have efficacy in protecting free radical damage to liver cells following methanol intoxication. A simultaneous administration of methanol and NAC resulted in a lower degree of parenchymal damage^{23,24}. The results indicate that methanol intoxication causes pronounced morphological changes in the examined organ

Previous reports have indicated that antioxidant agents, particularly melatonin and NAC, have beneficial effects in the treatment of methanol-induced liver injury²³⁻²⁷. Beyond these studies, we aimed to investigate and compare potential protective effects of melatonin and NAC on liver histopathological alterations by methanol intoxication in rats. To our knowledge, both melatonin and NAC have not been performed and compared yet to prevent the toxic effects of methanol on liver injury. Moreover, we used electron microscopy in our investigation.

Materials and Methods

Animals and Experimental Groups

Fifty-six adult male Wistar rats, weighing 180-200 g, were used. Animals were housed under continuous observation in appropriate cages in a quiet temperature ($21\pm 2^\circ\text{C}$) and humidity ($60\pm 5\%$)-controlled room in which a 12-12 h light-dark cycle was maintained. The animals were housed four or three per cage, and fed with commercial standard diet and water ad libitum, but were made to fast overnight before surgery. All experiments in this study were performed in accordance with the national Institutes of Health guidelines and were approved by the Committee on Animal Research at Inonu University, Malatya, Turkey.

Rats were randomly assigned to eight groups each containing 7 rats as follows:

- Control groups (C 6h, C 24h) treated with intragastric (i.g.) 1.0 ml saline.
- Methanol groups (M 6h, M 24h) treated with a dose of 3 g/kg i.g. methanol.
- Melatonin plus methanol groups (MEL+M 6h, MEL+M 24h), treated with dose of 10 mg/kg i.p. melatonin immediately, following with a dose of 3 g/kg i.g. methanol.
- NAC plus methanol groups (NAC+M 6h, NAC+M 24h) treated with dose of 150 mg/kg, following with a dose of 3 g/kg i.g. methanol. The rats in 24 h groups were given the same dose of melatonin and NAC 12 h after intoxication. All rats were sacrificed after 6 and 24 hours.

The dose of methanol causing 50% lethality in rats is 9.5 g/kg. Methanol was administered intraperitoneally to avoid uncertainties of gastrointestinal absorption. The methanol dosage used for this study was 3 g/kg (one-fourth of the 50% lethality dose) mixed with saline (1:1, vol/vol). Melatonin and methanol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Melatonin was dissolved in ethanol and further diluted in saline (0.09% NaCl w/v) to give a final concentration of 1%. Methanol was administered as a 50% solution in isotonic saline through a plastic tube with a syringe. NAC was purchased from Hüsnü Arsan laçları Co. (Istanbul, Turkey).

Tissue Preparation

After 6 and 24 h all drug received and intoxicated rats were killed under anesthesia (75 mg/kg ketamine hydrochloride and 8 mg/kg xylazine). Their livers were removed quickly. Tissues were placed in 2.5% gluteraldehyde in Sorenson's phosphate buffered saline solution for routine histopathologic examination by electron microscope.

Electron Microscopic Examination

All liver tissues were cut into small pieces (1 mm³) and fixed with were fixed in 2.5% gluteraldehyde buffered with 0.2 M NaH₂PO₄ + NaHPO₄ (pH: 7.2-7.3) and post fixed in 2% OsO₄. After dehydrated with acetone tissues were embedded in 00 gelatin capsules containing araldite, dodecylsuccinic anhydride (DDSA), benzyl-dimethylamine (BDMA) and dibutyl ftalate embedding material. Semi thin sections (1 micron)

of blocks were taken and stained with toluidine blue. After marking the regions that desired to examine, thin sections were taken, stained with uranyl acetate and lead citrate, and examined under Carl Zeiss EM 900 (Carl Zeiss SMT AG Company, Oberkochen, Germany) and photographed.

Results

Control Group.

Both in 6th and 24th hours, animals from the control group presented no histological alterations. Cytoplasm and nucleus were found to be normal, and also, rough endoplasmic reticulum (RER), tubules of smooth endoplasmic reticulum (SER) and mitochondria were found to be normal (Figure 1A, 1B).

Methanol Group.

Sixth hour in methanol group, significant ultra-structural changes were observed in parenchymal cells. Besides RER, extensive tubules of SER, increased mitochondria, increased primary lysosomes and some marked openings of bile canaliculus were distinguished

(Figure 2A). However in 24th hour, very large autophagic vacuoles and change of the nucleus shape were observed in liver parenchyma cells. In larger magnification, degeneration of mitochondria marked as cristolysis, and there were marked collagen fiber accumulation around the sinusoids (Figure 2B, 2C, 2D).

Methanol plus Melatonin Group

Sixth hour in methanol plus melatonin group, structure of liver parenchyma were found to be quite normal. RER and tubules of SER and the other structures were normal, however matrix of mitochondria was found to be little intense and cristolysis were seen in some areas (Figure 3A). However in 24th hour group, structure was protected lesser than 6th hour melatonin group. Prevalent cristolysis of mitochondria and increased primary lysosomes were observed (Figure 3B, 3C).

Methanol plus NAC Group

Although findings were not bad as methanol group, in NAC+M 6h group, structure of liver parenchyma were less protected than in MEL+M 6h group. Cristolysis of mitochondria and prevalent increased of primary lysosomes in cytoplasm were realized (Figure 4A). In 24th hour, some

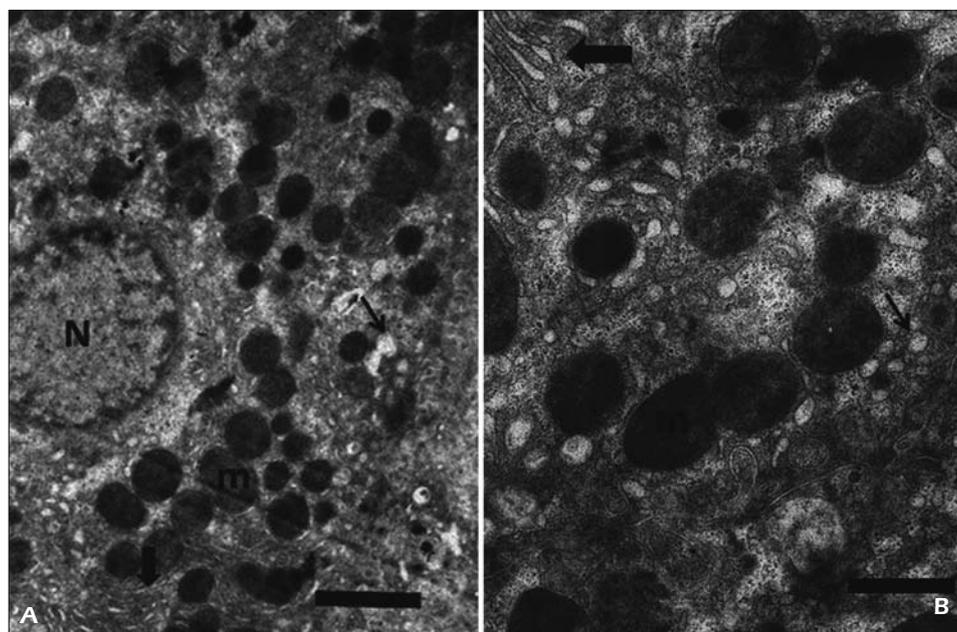


Figure 1. Both in 6th (A) and 24th (B) hours in control group, animals from the control group presented normal histology. Cytoplasm and nucleus (N) were found to be normal (A). Also, in large magnification granular endoplasmic reticulum: GER (thick arrow), tubules of aGER (thin arrow) and mitochondria (m) were found to be normal (B), (A: Uranyl acetate – Lead citrate × 7500 bar indicates 2.5 µm, B: Uranyl acetate – Lead citrate × 17500 bar indicates 1.1 µm).

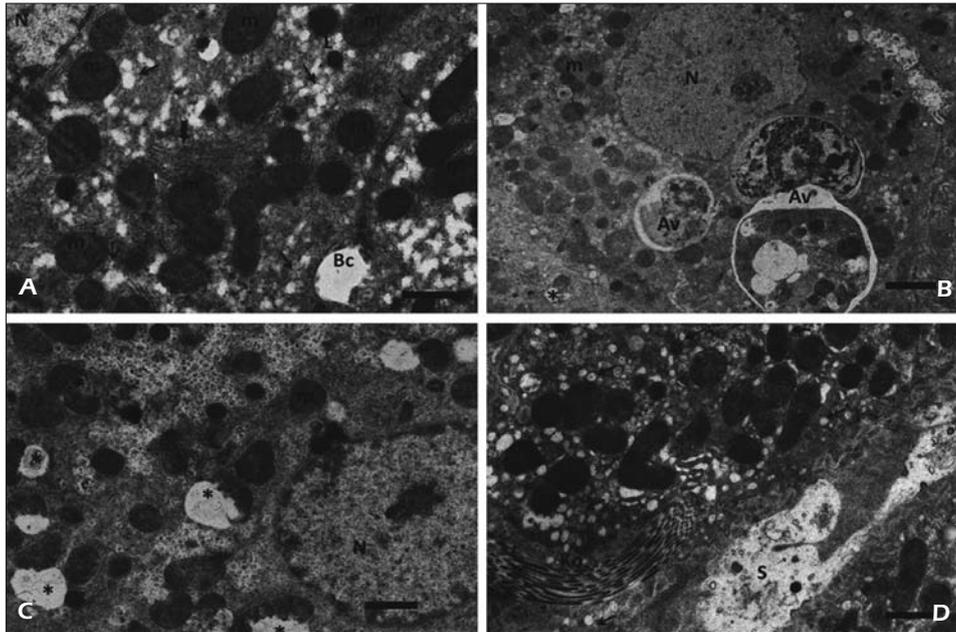


Figure 2. Sixth hour in methanol group, significant ultra-structural changes were observed in parenchymal cells. GER (thick arrow), tubules of aGER (thin arrow), mitochondria (m), primary lysosomes (pl) and some marked openings of bile canaliculus (Bc) were observed (A). In 24th hour in methanol group, very large autophagic vacuoles and change of the nucleus shape were observed in liver parenchyma cells (B). In larger magnifications besides the normal mitochondria (m), cristolysis of mitochondria (*) and marked collagen fiber (Ko) accumulation around the sinusoids were observed (C-D). (A-C-D: Uranyl acetate – Lead citrate × 17500 bar indicates 1.1 µm, 2B: Uranyl acetate – Lead citrate × 7500 bar indicates 1.1 µm.)

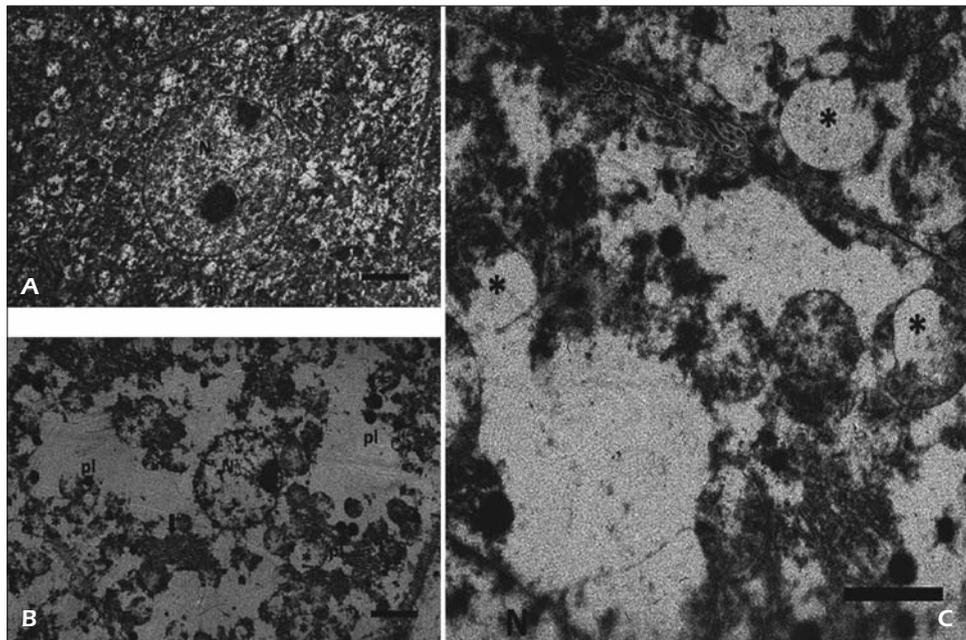


Figure 3. Sixth hour in methanol plus melatonin group, structure of liver parenchyma were found to be quietly normal. GER (thick arrow) and tubules of aGER (thin arrow) and the other structures were normal, however matrix of mitochondria (m) was found to be little intense and cristolysis (*) were seen in some areas (A). In 24th hour in methanol+melatonin group, structure was not protected quietly by melatonin. Prevalent cristolysis of mitochondria (*), (B-C) and increased primary lysosomes (pl), (C) were observed (A-B: Uranyl acetate – Lead citrate × 7500 bar indicates 2.5 µm, C: Uranyl acetate – Lead citrate × 17500 bar indicates 1.1 µm.)

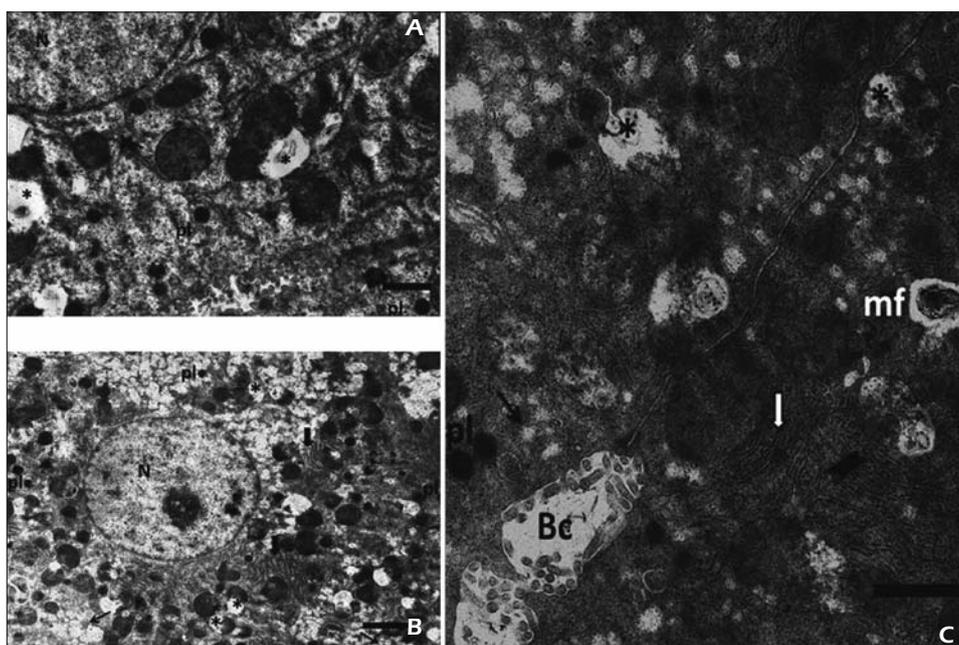


Figure 4. Sixth hour in methanol plus NAC group, structure of liver parenchyma were less protected than in methanol plus melatonin group. Cristolysis of mitochondria (*) and prevalent increased of primary lysosomes (pl) in cytoplasm were observed (A). In 24th hour methanol+NAC group, some openings of GER (thick arrow) and the tubules of aGER (thin arrow), cristolysis of mitochondria (*) and a marked prevalent increased of primary lysosomes (pl) were observed (B-C). Also in larger magnification an extensive bile canalicule (Bc) and vacuoles containing myelin figures (mf) could be seen. (A-C: Uranyl acetate – Lead citrate \times 17500 bar indicates 1.1 μ m, B: Uranyl acetate – Lead citrate \times 7500 bar indicates 2.5 μ m.)

openings of RER and the tubules of SER, cristolysis of mitochondria and a prevalent increase of primary lysosomes were also distinguished (Figures 4B, 4C).

Discussion

To the best of our knowledge, evidenced by electron microscopy findings, this is the first report to investigate the melatonin and NAC administration on liver injury in a rat model caused by methanol intoxication. The main findings of this study were that, (1) although kidney damage is usually expected in methanol intoxication, some ultra-structural degeneration could be found in other organs such as liver (2) melatonin administration prevents liver injury and (3) NAC, although not as effective as melatonin, also prevents liver injury caused by methanol intoxication.

Melatonin protects DNA, proteins, and biological membrane lipids from the deleterious effects of free radicals, without the need for a specific receptor on the cells^{28,29}. While it is clearly a lipid

soluble agent, it seems also capable of entering the aqueous environments of the cell. This apparently amphiphilic nature allows melatonin to be protective of membranes, cytosolic molecules and nuclear DNA from free radical damage⁸. Melatonin displayed the effects as antioxidants and free-radical scavengers, especially the hepatoprotective effects revealed by some researchers³⁰⁻³⁴. It is shown that melatonin administration reduces histopathological signs of liver injury such as focal necrosis and apoptosis^{35,36}. Kurcer et al evaluated the effect of melatonin on methanol induced liver injury by light microscopy and found that melatonin has protective effects against methanol induced liver injury²⁷. They observed that liver MDA levels, an end product of lipid peroxidation, and liver protein carbonyl (PC) levels, a marker of protein oxidation were increased and that liver antioxidant enzyme activities such as catalase (CAT), super oxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were decreased after methanol intoxication. They also observed that pretreatment with melatonin (10 mg/kg) decreased the MDA levels significantly, restored the PC levels to the control and prevented the reduction in all of the antioxi-

dant enzyme activities. In our study, we firstly demonstrated that acute methanol intoxication deteriorates hepatic ultra-structural changes and melatonin pretreatment reversed these both at 6 and 24h. Our results indicate that melatonin improves the ultra-structural features of the liver damage especially in 6th hour.

Modulation of cellular thiols has been used to protect the liver from attack of reactive oxygen intermediates and is currently being investigated as a novel therapeutic strategy in various forms of liver failure²⁵. One of the most extensively studied agents is NAC⁹. NAC may also exert its antioxidant effect indirectly by facilitating reduced glutathione (GSH) biosynthesis and supplying GSH for glutathione peroxidase-catalyzed reactions³⁴. NAC possesses the capacity to react directly with electrophiles such as free radicals and formaldehyde. Skrzydlewska et al showed that NAC is effective against methanol induced liver injury, and reported that it should be considered as adjunctive compound in therapy after methanol intoxication^{25,26}. As well as, Kasacka and Skrzydlewska²³ and Dobrzynska et al²⁴ reported in their studies that NAC has protective effects on liver injury induced by methanol intoxication. In summary, the results suggest that NAC may prevent methanol-induced liver damage. These protective effects may be connected with its antioxidant and free radical scavenging actions. After administering 3 g/kg of methanol to rats, Dobrzynska et al²⁴ measured lipid peroxidation, liver surface charge density and fine structure of liver. According to their study ingestion of NAC with methanol partially prevented the methanol-induced changes. In our study, NAC improves the ultra-structural features of the liver damage, although not as effective as melatonin, in methanol induced liver damage.

The effects of both melatonin and NAC on methanol induced liver damage have not been studied earlier. The protective effects of them on liver injury have been shown in some studies; these were acetaminophen toxicity, cardiopulmonary bypass model and hepatic ischemia/reperfusion induced liver injury and melatonin appeared to be significantly more potent than NAC^{19,34,37}. Like these studies, in our investigation liver injury due to methanol intoxication was improved after melatonin and NAC administration. Also our findings supported that melatonin has been more potent than NAC. This may be due to the higher efficacy of melatonin in scavenging various free radicals and also to its

ability in stimulating the antioxidant enzymes, which is not observed with NAC⁵⁻⁷.

Although we have not encountered earlier studies investigating fine structure of liver after methanol intoxication, there were some researches mentioning the effects of ethanol, using the same metabolic pathway with methanol, on ultra-structure of liver. Romert et al³⁸ administered 100-300 mg ethanol to pregnant mini-pigs and their half-term fetuses and examined under electron microscope. They pointed out that, the ultra-structural changes in the hepatocytes of the pregnant mini-pig seem to indicate an adaptation of these cells to ethanol by development of a microsomal or catalase ethanol-oxidizing system, while the hepatocytes of the mini-pig fetus in contrast show obvious signs of cellular injury. While the hepatocytes of the pregnant mini-pig developed an extensive SER, and showed an increased number of mitochondria, micro bodies and autophagic vacuoles, extensive Golgi complexes with accumulation of secretion, and a reduction of glycogen, the hepatocytes of the half-term fetuses exhibited profound changes of mitochondria and endoplasmic reticulum after alcohol exposure. Many mitochondria showed abnormal shape and increased size, disorientation of cristae and accumulation of para crystalline material. An increased number of autophagic vacuoles containing remnants of mitochondria were observed. The granular endoplasmic reticulum (GER) exhibited aggregations of endoplasmic cisternae which were well defined and not bounded by a membrane. Le Bail et al³⁹ stated that in liver diseases such as alcoholic liver diseases, they demonstrated some major pathological entities including peri-sinusoidal fibrosis. Also in our study, sixth hour methanol group demonstrated extensive tubules of SER, increased mitochondria, increased primary lysosomes and some marked openings of bile canaliculus, and in 24th h hour, very large autophagic vacuoles and change of the nucleus shape were observed in liver parenchyma cells. In larger magnification, degeneration of mitochondria marked as cristolysis, and marked collagen fiber accumulation around the sinusoids, that might be the initiation of fibrosis, were observed.

After administering 1.5 g/kg of methanol into the stomach of 36 rats, through a gastric tube, Kasacka and Skrzyolewska⁴⁰ evaluated methanol influence on the liver of rats. The liver was taken from rats under the ether anesthesia after 6, 12, and 24 hours as well as after 2, 5, and 7 days of

methanol administration. They stated that methanol caused morphological changes in the rat liver and that intensification of these changes depended on the time after intoxication. Their results showed that methanol intoxication caused visible changes in liver. Only 6 h after intoxication, lobular peripheral hepatocytes presented characteristic features of vacuolar degradation persisting up to 48 h. Since the second day of intoxication, many cells with double nuclei were found more frequently than in controls. Single hepatocytes or small hepatocyte clusters with the features of deliquescent necrosis could be seen after 5 and 7 days of examination. Also in our both of MEL+M 24h and NAC+M 24h group, in spite of the treatment ultrastructure of liver was not so preserved as in MEL+M 6h and NAC+M 6h groups. In our study, the rats in 24 h groups were given the same dose of melatonin and NAC at 12 h after intoxication. Both melatonin's and NAC's half-life is short, so if we had administered therapeutic doses in earlier hours and had repeated doses in short time intervals, we might have seen better ultrastructure in liver tissue^{41,42}.

Conclusions

Our findings demonstrate that methanol causes hepatic degeneration in rats and treatment with melatonin and NAC prevents histological damages in liver. Considering the doses used melatonin appears to be more efficient than NAC in affording protection against liver injury induced by methanol. This also indicates that these compounds may have clinical relevance and may be considered as adjunctive compounds in therapy during methanol intoxication.

Acknowledgements

This study was supported by a grant from the Scientific Research Institution of Harran University (HUBAK No 615).

References

- 1) SCHIAVONC, F.M., CENTOR FD, BROGON GX. Ethylene glycol, methanol and isopropyl alcohol P V, editor. Boston/Toronto/London: Little Brown and Company; 1993.
- 2) KUTEIFAN K, OESTERLE H, TAJAHMADY T, GUTBUB AM, LAPLATTE G. Necrosis and haemorrhage of the putamen in methanol poisoning shown on MRI. *Neuroradiology* 1998; 40: 158-160.
- 3) BRALET J, BOUVIER C, SCHREIBER L, BOQUILLON M. Effect of acidosis on lipid peroxidation in brain slices. *Brain Res* 1991; 539: 175-177.
- 4) CHACON E, ACOSTA D. Mitochondrial regulation of superoxide by Ca²⁺: an alternate mechanism for the cardiotoxicity of doxorubicin. *Toxicol Appl Pharmacol* 1991; 107: 117-128.
- 5) TAN DX, HARDELAND R, MANCHESTER LC, POEGGELER B, LOPEZ-BURILLO S, MAYO JC, SAINZ RM, REITER RJ. Mechanistic and comparative studies of melatonin and classic antioxidants in terms of their interactions with the ABTS cation radical. *J Pineal Res* 2003; 34: 249-259.
- 6) ALLEGRA M, REITER RJ, TAN DX, GENTILE C, TESORIERE L, LIVREA MA. The chemistry of melatonin's interaction with reactive species. *J Pineal Res* 2003; 34: 1-10.
- 7) HARDELAND R, POEGGELER B, NIEBERGALL R, ZELOSKO V. Oxidation of melatonin by carbonate radicals and chemiluminescence emitted during pyrrole ring cleavage. *J Pineal Res* 2003; 34: 17-25.
- 8) REITER RJ, TAN DX, GITTO E, SAINZ RM, MAYO JC, LEON J, MANCHESTER LC, VJAYALAXMI, KILIC E, KILIC U. Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage. *Polish J Pharmacol* 2004; 56: 159-170.
- 9) PASTOR A, COLLADO PS, ALMAR M, GONZALEZ-GALLEGO J. Antioxidant enzyme status in biliary obstructed rats: effects of N-acetylcysteine. *J Hepatol* 1997; 27: 363-370.
- 10) DOBSAK P, SIEGELOVA J, EICHER JC, JANCIK J, SVACINOVA H, VASKU J, KUCHTICKOVA S, HORKY M, WOLF JE. Melatonin protects against ischemia-reperfusion injury and inhibits apoptosis in isolated working rat heart. *Pathophysiology* 2003; 9: 179-187.
- 11) KANEKO S, OKUMURA K, NUMAGUCHI Y, MATSUI H, MURASE K, MOKUNO S, MORISHIMA I, HIRA K, TOKI Y, ITO T, HAYAKAWA T. Melatonin scavenges hydroxyl radical and protects isolated rat hearts from ischemic reperfusion injury. *Life Sci* 2000; 67: 101-112.
- 12) LAGNEUX C, JOYEUX M, DEMENGE P, RIBUOT C, GODIN-RIBUOT D. Protective effects of melatonin against ischemia-reperfusion injury in the isolated rat heart. *Life Sci* 2000; 66: 503-509.
- 13) LEON J, ACUNA-CASTROVIEJO D, ESCAMES G, TAN DX, REITER RJ. Melatonin mitigates mitochondrial malfunction. *J Pineal Res* 2005; 38: 1-9.
- 14) MALDONADO MD, MURILLO-CABEZAS F, TERRON MP, FLORES LJ, TAN DX, MANCHESTER LC, REITER RJ. The potential of melatonin in reducing morbidity-mortality after craniocerebral trauma. *J Pineal Res* 2007; 42: 1-11.
- 15) CATALA A, ZVARA A, PUSKAS LG, KITAJKA K. Melatonin-induced gene expression changes and its preventive effects on adriamycin-induced lipid peroxidation in rat liver. *J Pineal Res* 2007; 42: 43-49.

- 16) XU J, SUN S, WEI W, FU J, QI W, MANCHESTER LC, TAN DX, REITER RJ. Melatonin reduces mortality and oxidatively mediated hepatic and renal damage due to diquat treatment. *J Pineal Res* 2007; 42: 166-171.
- 17) SOCHMAN J. Regional ischaemic and reperfusion injury, free oxygen radicals and the possible role of N-acetylcysteine in acute myocardial infarction. *Cor Vasa* 1994; 36: 269-279.
- 18) HARRISON PM, KEAYS R, BRAY GP, ALEXANDER GJ, WILLIAMS R. Improved outcome of paracetamol-induced fulminant hepatic failure by late administration of acetylcysteine. *Lancet* 1990; 335: 1572-1573.
- 19) HUANG H, YIN R, ZHU J, FENG X, WANG C, SHENG Y, DONG G, LI D, JING H. Protective effects of melatonin and N-acetylcysteine on hepatic injury in a rat cardiopulmonary bypass model. *J Surg Res* 2007; 142:153-161.
- 20) DEMIR S, INAL-ERDEN M. Pentoxifylline and N-acetylcysteine in hepatic ischemia/reperfusion injury. *Clin Chimica Acta* 1998; 275: 127-135.
- 21) FUSAI G, GLANTZOUNIS GK, HAFEZ T, YANG W, QUAGLIA A, SHETH H, KANORIA S, PARKES H, SEIFALIAN A, DAVIDSON BR. N-Acetylcysteine ameliorates the late phase of liver ischaemia/reperfusion injury in the rabbit with hepatic steatosis. *Clin Sci* 2005; 109: 465-473.
- 22) GLANTZOUNIS GK, YANG W, KOTI RS, MIKHAILIDIS DP, SEIFALIAN AM, DAVIDSON BR. Continuous infusion of N-acetylcysteine reduces liver warm ischaemia-reperfusion injury. *Brit J Surg* 2004; 91: 1330-1339.
- 23) KASACKA I, SKRZYDLEWSKA E. Ethanol and N-acetylcysteine influence on the development of liver changes in experimental methanol intoxication. *Roczniki Akademii Medycznej w Białymstoku* 2001; 46: 133-144.
- 24) DOBRZYNSKA I, SKRZYDLEWSKA E, KASACKA I, FIGASZEWSKI Z. Protective effect of N-acetylcysteine on rat liver cell membrane during methanol intoxication. *J Pharm Pharmacol* 2000; 52: 547-552.
- 25) SKRZYDLEWSKA E, FARBISZEWSKI R. Protective effect of N-acetylcysteine on reduced glutathione, reduced glutathione-related enzymes and lipid peroxidation in methanol intoxication. *Drug Alcohol Depend* 1999; 57: 61-67.
- 26) SKRZYDLEWSKA E, ELAS M, OSTROWSKA J. Protective effects of N-acetylcysteine and vitamin E derivative U83836E on proteins modifications induced by methanol intoxication. *Toxicol Mech Methods* 2005; 15: 263-270.
- 27) KURCER Z, OGUZ E, IRAZ M, FADILIOGLU E, BABA F, KOKSAL M, OLMEZ E. Melatonin improves methanol intoxication-induced oxidative liver injury in rats. *J Pineal Res* 2007; 43: 42-49.
- 28) REITER RJ. Oxidative damage in the central nervous system: protection by melatonin. *Progr Neurobiol*. 1998; 56: 359-84. Epub 1998/10/14.
- 29) REITER RJ, POEGGELER B, TAN DX, CHEN LD, MANCHESTER LC, GUERRERO JM. Antioxidant capacity of melatonin: A novel action not requiring a receptor. *Neuroendocrinol Lett* 1993;15:103-116.
- 30) REITER RJ, TAN DX. Melatonin: a novel protective agent against oxidative injury of the ischemic/reperfused heart. *Cardiovasc Res* 2003; 58: 10-19.
- 31) REITER RJ, TAN DX, OSUNA C, GITTO E. Actions of melatonin in the reduction of oxidative stress. A review. *J Biomed Sci* 2000; 7: 444-458.
- 32) RODRIGUEZ-REYNOSO S, LEAL C, PORTILLA E, OLIVARES N, MUNIZ J. Effect of exogenous melatonin on hepatic energetic status during ischemia/reperfusion: possible role of tumor necrosis factor-alpha and nitric oxide. *J Surg Res* 2001; 100: 141-149.
- 33) SIGALA F, THEOCHARIS S, SIGALAS K, MARKANTONIS-KYROUDIS S, PAPALABROS E, TRIANTAFYLLOU A, KOSTOPANAGIOTOU G, ANDREADOU I. Therapeutic value of melatonin in an experimental model of liver injury and regeneration. *J Pineal Res* 2006; 40: 270-279.
- 34) SENER G, TOSUN O, SEHIRLI AO, KACMAZ A, ARBAK S, ERSOY Y, AYANOGLU-DÜLGER G. Melatonin and N-acetylcysteine have beneficial effects during hepatic ischemia and reperfusion. *Life Sci* 2003; 72: 2707-2718.
- 35) BULBULLER N, AKKUS MA, CETINKAYA Z, ILHAN YS, OZERCAN I, KIRKIL C, DOĞRU O. Effects of melatonin and lactulose on the liver and kidneys in rats with obstructive jaundice. *Pediatr Surg Int* 2002; 18: 677-680.
- 36) PADILLO FJ, CRUZ A, NAVARRETE C, BUJALANCE I, BRICENO J, GALLARDO JI, MARCHAL T, CABALLERO R, TÚNEZ I, MUNTANÉ J, MONTILLA P, PERA-MADRAZO C. Melatonin prevents oxidative stress and hepatocyte cell death induced by experimental cholestasis. *Free Radical Res* 2004; 38: 697-704.
- 37) SENER G, SEHIRLI AO, AYANOGLU-DULGER G. Protective effects of melatonin, vitamin E and N-acetylcysteine against acetaminophen toxicity in mice: a comparative study. *J Pineal Res* 2003; 35: 61-68.
- 38) ROMERT P, MATTHIESSEN ME. Fine structure of hepatocytes from mini-pigs and mini-pig fetuses exposed to alcohol (ethanol) in vivo. *Acta Anat* 1984; 120: 190-195.
- 39) LE BAIL B, BIOULACSAGE P, SENUITA R, QUINTON A, SARIC J, BALABAUD C. Fine-structure of hepatic sinusoids and sinusoidal cells in disease. *J Electron Microscop* 1990; 14: 257-282.
- 40) KASACKA I, SKRZYDLEWSKA E. Morphological changes in the liver of rats intoxicated with methanol. *Roczniki Akademii Medycznej w Białymstoku* 1999; 44: 89-101.
- 41) MISTRALETTI G, SABBATINI G, TAVERNA M, FIGINI MA, UMBRELLO M, MAGNI P, RUSCICA M, DOZIO E, ESPOSTI R, DeMARTINI G, FRASCHINI F, REZZANI R, REITER RJ, IAPICHINO G. Pharmacokinetics of orally administered melatonin in critically ill patients. *J Pineal Res* 2010; 48: 142-147.
- 42) HOLDINESS MR. Clinical pharmacokinetics of N-acetylcysteine. *Clin Pharmacokinet* 1991; 20: 123-134.