Fırat Tıp Dergisi 2008;13(2):92-97

Experimental Research

www.firattipdergisi.com

The Effect of Melatonin Hormone on Formaldehyde-Induced Liver Injury: A Light Microscopic and Biochemical Study

Hıdır PEKMEZ[1], Neriman ÇOLAKOĞLU CAMCI[2], İsmail ZARARSIZ[3], İlter KUŞ[4], Murat ÖGETÜRK[4], Hacı Ramazan YILMAZ[5], Mustafa SARSILMAZ[a4]

[1] *Fırat University, Elazig School of Health Sciences*

[2] *Fırat University, Faculty of Medicine, Department of Histology and Embryology, ELAZIĞ*

[3] *Mustafa Kemal University, Tayfur Ata Sökmen Medical School, Department of Anatomy, HATAY*

[4] *Fırat University, Faculty of Medicine, Department of Anatomy, ELAZIĞ*

[5] *Süleyman Demirel University, Faculty of Medicine, Department of Medical Biology and Genetics, ISPARTA*

ABSTRACT

Objectives: This study aimed to investigate histological and biochemical changes in the livers of formaldehyde exposed rats and possible effects of melatonin hormone on these changes.

Materials and Methods: A total of 21 male Wistar-albino rats were divided into three equal groups. Control rats in Group I received 0.9% NaCl alone intraperitoneally (ip), rats in Group II were injected with 10% formaldehyde (10 mg/kg, ip) every other day and rats in Group III received melatonin (25 mg/kg, ip) plus formaldehyde. At the end of 14-day experimental period, all animals were decapitated. The liver tissue samples were processed histologically and analyzed under light microscope. Additionally, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), xanthine oxidase (XO), and malondialdehyde (MDA) levels in the liver tissue samples were determined.

Results: SOD, GSH-Px, CAT, XO and MDA levels of formaldehyde exposed rats were significantly higher than those of the control group rats. However, these biochemical values of rats treated with melatonin plus formaldehyde were lower than the non-melatonin group. Light microscopic evaluation of liver tissue samples of formaldehyde-exposed rats revealed enlarged sinusoids filled with blood and mononuclear cell infiltration in the portal areas and around the central veins. In addition, some of the hepatocytes showed vacuolar degeneration, and some had a hyperchromatic nucleus. In PAS staining, the hepatocytes around the portal areas were PAS negative. The rats treated with melatonin plus formaldehyde had somewhat fewer histological changes induced by formaldehyde exposure.

Conclusion: The liver damage caused by formaldehyde may be partially prevented by melatonin administration. *©2008, Firat University, Medical Faculty*

Key words: Liver, Formaldehyde, Melatonin, Oxidative Stress, Light Microscope

ÖZET

Melatonin Hormonunun Formaldehite Bağlı Karaciğer Hasarı Üzerine Etkisi: Işık Mikroskobik ve Biyokimyasal Bir Çalışma

Amaç: Bu çalışmada, formaldehite maruz bırakılan sıçanların karaciğerlerinde oluşan histolojik ve biyokimyasal değişiklikler ile melatonin hormonunun bu değişiklikler üzerine olası etkilerinin incelenmesi amaçlandı.

Gereç ve Yöntem: Toplam 21 adet erkek Wistar-albino cinsi sıçan üç eşit gruba ayrıldı. Grup I'deki kontrol sıçanlara sadece % 0.9'luk NaCl intraperitoneal (ip) uygulanırken, grup II'deki sıçanlara % 10 formaldehit (10 mg/kg, ip) ve grup III'teki sıçanlara ise formaldehitin yanı sıra melatonin (25 mg/kg, ip) günaşırı olarak enjekte edildi. 14 günlük bir uygulama sürecinden sonra bütün hayvanlar dekapite edildi. Karaciğer doku örnekleri histolojik işlemlerden geçirilerek ışık mikroskop altında incelendi. Ek olarak, karaciğer doku örneklerinde süperoksit dismutaz (SOD), glutatyon peroksidaz (GSH-Px), katalaz (CAT), ksantin oksidaz (XO) ve malondialdehit (MDA) seviyeleri belirlendi.

Bulgular: Formaldehite maruz bırakılan sıçanların SOD, GSH-Px, CAT, XO ve MDA seviyeleri kontrol değerlerinden anlamlı derecede yüksekti. Buna karşın, formaldehitle birlikte melatonin uygulanan sıçanların bu biyokimyasal değerleri melatonin uygulanmayan gruba nazaran düşüktü. Formaldehite maruz kalan sıçanların karaciğer doku örneklerinde gerçekleştirilen ışık mikroskobik değerlendirme, içi kan dolu sinüzoidler yanı sıra portal alanlar ve v.centralis civarında mononükleer hücre infiltrasyonunu açığa çıkardı. Ayrıca, hepatositlerin bazıları vakuoler dejenerasyon gösterirken, bazıları da hiperkromatik çekirdeğe sahipti. PAS boyamada, portal alanlara yakın hepatositler PAS negatifti. Melatonin ve formaldehitin birlikte uygulandığı sıçanlarda formaldehite bağlı histolojik değişiklikler nispeten daha az gözlendi.

Sonuç: Formaldehitin neden olduğu karaciğer hasarı melatonin uygulamasıyla kısmen önlenebilir. *©2008, Fırat Üniversitesi, Tıp Fakültesi*

Anahtar kelimeler: Karaciğer, Formaldehit, Melatonin, Oksidatif Stres, Işık Mikroskop

Formaldehyde (HCHO) is a highly water soluble, colorless agent with sharp odor and exists in the natural structure of the organism (1). HCHO taken in an organism is metabolized into formic acid in the liver and erythrocytes and excreted by urine,

[a] Yazışma Adresi: Dr. Mustafa Sarsılmaz, Fırat Üniversitesi, Anatomi Anabilim Dalı, ELAZIĞ
**4th Asian-Pasific International Congress of Anatomists (APICA), 7-10 September 2005, Kuşadası-Turkey
Tel: +90 424 2370000 e-mail: msarsilmaz@firat.edu.tr

feces, or expiration (2). It is used in paint, plastic, textile and leather industries, sanitary and cosmetic products, construction materials, paper, ink, confectionery productions and is used as a preserving additive in drugs (3,4). In medicine, it is also used for cadaver embalming, organ and tissue fixation, and disinfection and sterilization procedures (1,2,5,6). Various studies have reported the structural and functional disorders of the respiratory, gastrointestinal, reproductive and nervous systems associated with toxic effects of HCHO (7-10). Allergic effects of HCHO have also been reported (1).

HCHO has adverse effects on the histological structure and functions of the liver (11). When received through respiration, it has been found to decrease the liver weight and triglyceride level (12,13). Furthermore, the toxic effects of HCHO have been reported to cause structural changes in the epithelial biliary cells and damage intrahepatic and extrahepatic biliary ducts (14). HCHO exposure has led to disorders of oxidant and oxidant-antioxidant systems of the liver tissue and inflicted oxidative damage (15).

Melatonin is released from the pineal gland in the dark (16,17). In addition to the pineal gland, melatonin has been shown in the retina, intestines, erythrocytes, leucocytes, and many other tissues. The organs and tissues exposed to oxygen radical formation such as the liver, lungs, brain, and skin produce intracellular melatonin at lower levels (18-21). Melatonin hormone (N-acetyl-5-methoxytryptamine) acts in the regulation of many physiological functions such as endocrine rhythm, antigonatropic effects, protecting the nervous system, stimulation of the immune system, and the protection of free radicals (17,22-24). Recent studies have shown melatonin to be an antioxidant substance (25). Melatonin, which is both water and oil soluble, is available to each organelle of the cell (26).

This study aimed to investigate histological and biochemical changes in the liver of formaldehyde exposed rats and the effects of melatonin hormone on these changes.

MATERIALS AND METHODS

Animals and Treatments

The subjects of the study were 21 male Wistar-albino rats (weighing 310-320 g). The animals were divided into three equal groups. All animals received humane care in compliance with guidelines of Firat University Research Council's criteria. Control rats in Group I were injected intraperitoneal (ip) injection of 0.9% NaCl alone every other day. The rats in Group II received ip 10 mg/kg HCHO diluted 1/10 in 0.9% NaCl every other day. In addition to receiving HCHO, the rats in Group III received ip 25 mg/kg melatonin (Sigma Chemical Co.) diluted 1/10 in 0.9% NaCl every other day. To regulate endogenous melatonin secretion, all rats were kept in 12-hour light and 12-hour dark conditions throughout the experimental procedures. At the end of the 14-day experimental period, all animals were sacrificed.

Microscopic examination of liver tissue specimens

The liver tissue specimens were fixed in formaldehyde solution (10%). Tissue specimens were embedded in paraffin wax and sectioned (thickness, 5 µm). Paraffin sections were stained with hematoxylin-eosin (H&E), Masson's trichrome, and PAS and examined with an Olympus BH2 photomicroscope.

Biochemical analysis of liver tissues

For biochemical evaluations, rapidly harvested liver biopsy samples were washed in 0.15 M cold (+4°C) potassium chloride (KCl) and dried with blotting paper. The tissues were homogenized in KCl (0.15 M) at 16000 rpm for three minutes. The homogenate was centrifuged at 5000xg for one hour at +4°C and the supernatant was obtained and stored at –40°C for one week until analysis. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), xanthine oxidase (XO) and malondialdehyde (MDA) levels were measured in the supernatant by spectrophotometric methods.

Determination of superoxide dismutase activity

It was measured by the method of Sun et al. (27), in which SOD enzyme values are based on the reduction of nitroblue tetrazolium (NBT) by superoxide produced by the xanthine-xanthineoxidase system.

Determination of glutathione peroxidase activity

Glutathione peroxidase (GSH-Px, EC 1.6.4.2) activity was measured by the method of Paglia and Valentine (28). In a medium containing hydrogen peroxide, GSH-Px catalyzes the amplification of glutathione reductase (GSH) to oxidized form of glutathione. The oxidized glutathione is reduced to GSH by glutathione reductase and NADPH. GSH-Px activity was detected by decreased absorbance at 340nm during the conversion of NADPH to NADP+.

Determination of catalase activity

Catalase activity was measured according to the Aebi method (29), through the observation of hydrogen peroxide (H2O2) destroyed by the enzyme at 240 nm wavelength in a spectrophotometer.

Determination of xanthine oxidase activity

Tissue xanthine oxidase activity was studied according to the method of Prajda and Weber (30), by the observation of uric acid absorbance formed by xanthine at the level of 293 nm wavelength on spectrophotometer.

Determination of malondialdehyde level

MDA was measured by the Esterbauer and Cheeseman method (31). Malondialdehyde and thiobarbituric acid react at 90-100°C and form a pink-colored compound. The absorbances of the samples obtained were read at 532 nm.

Statistical analyses

"SPSS 11.0 for Windows" statistical program was used. The distribution of the groups was evaluated through a one-sample Kolmogorov-Smirnov Test, a nonparametric test. The group comparisons were performed by the use of parametric tests: one-way ANOVA test and LSD, a Post Hoc test. $P<0.05$ was considered statistically significant.

RESULTS

Light microscopic findings

In light microscopic evaluations, all three zones of hepatic acinus in the liver sections of the control group were normal. With PAS staining, the hepatocytes in all three zones were intensely glycogen stained (Figure 1).

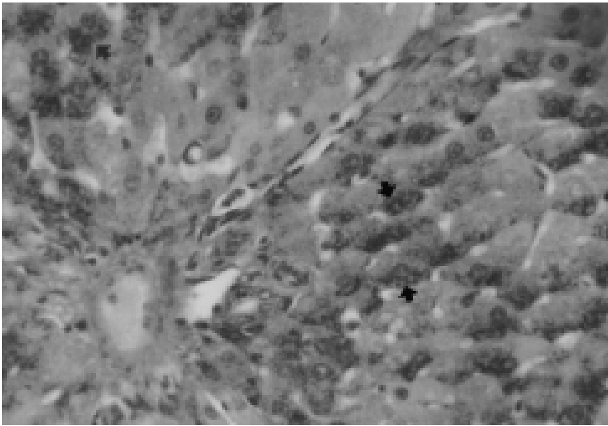

Figure 1. Group I. The glycogen content (arrow) in the hepatocytes around the portal area. PAS X240.

Upon evaluation of the liver tissue preparations of the rats exposed to excessive HCHO for 14 days, enlarged sinusoids were blood filled, and there was mononuclear cell infiltration in the portal area and around the vena centralis (Figures 2 and 3).

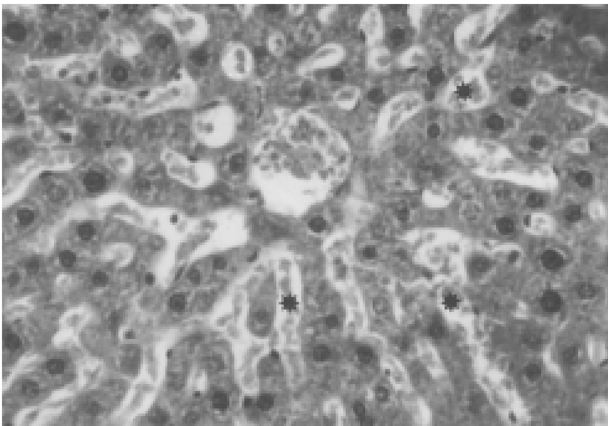

Figure 2. Group II. Dilatation and congestion (*) in the sinusoids. H&E X240.

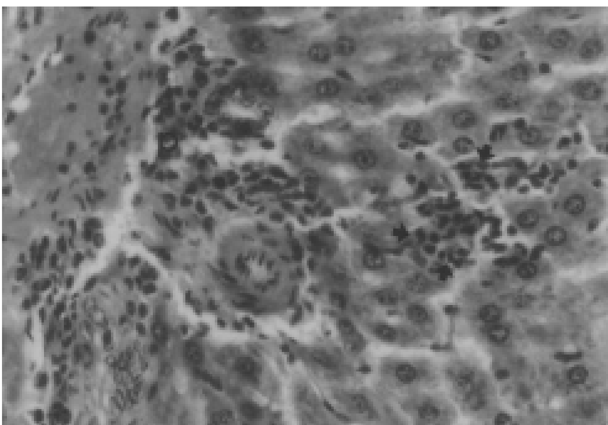

Figure 3. Group II. Mononuclear cell infiltration (arrow) around the portal area (p). H&E X240.

Furthermore, some hepatocytes had vacuolated cytoplasm (Figure 4) and some had a hyperchromatic nucleus (Figure 5). With PAS staining, the hepatocytes around the portal area were PAS negative; thus, there was no glycogen content (Figure 6).

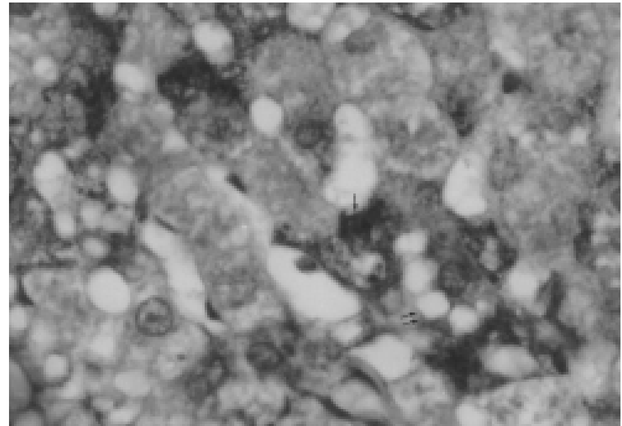

Figure 4. Group II. Glycogen content of hepatocytes (arrow) and vacuolization (double arrow) are visible. PAS X480.

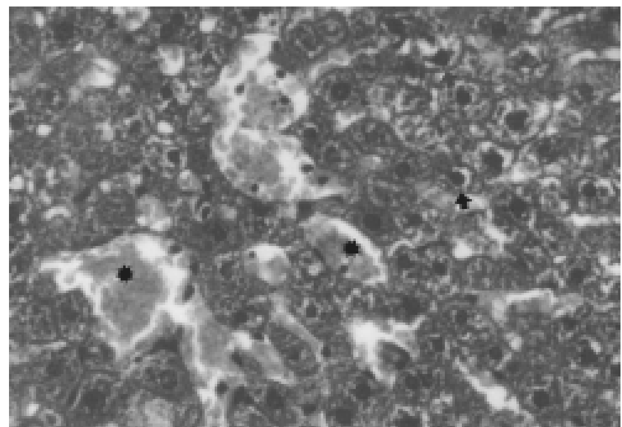

Figure 5. Group II. Hepatocytes with hyperchromatic nucleus (arrow) and vascular congestion (*) were evident. Masson's trichrome X240.

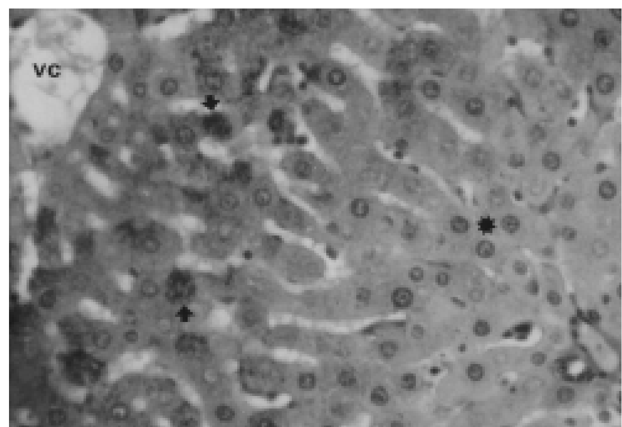

Figure 6. Group II. The hepatocytes around the portal area were PAS negative (*) and the glycogen containing hepatocytes around vena centralis (vc) were PAS positive (arrow). PAS X240.

The evaluation of the liver tissue preparations of the rats exposed to both HCHO and melatonin showed that enlarged sinusoids were filled with blood, and there were hepatocytes with hyperchromatic nuclei at various locations (Figure 7). PAS staining indicated glycogen presence in hepatocytes of all three zones as seen in the control group (Figure 8). In this group also, sinusoidal enlargements were not as common as

those in the HCHO group. Moreover, there was no cellular infiltration.

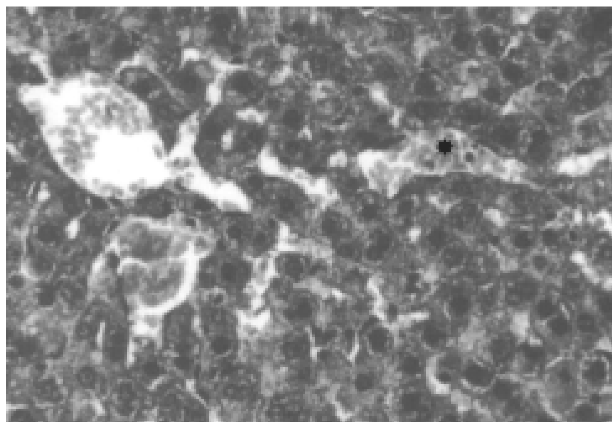

Figure 7. Group III. Enlargement and congestion of the sinusoids (*) are visible. Masson's trichrome X240.

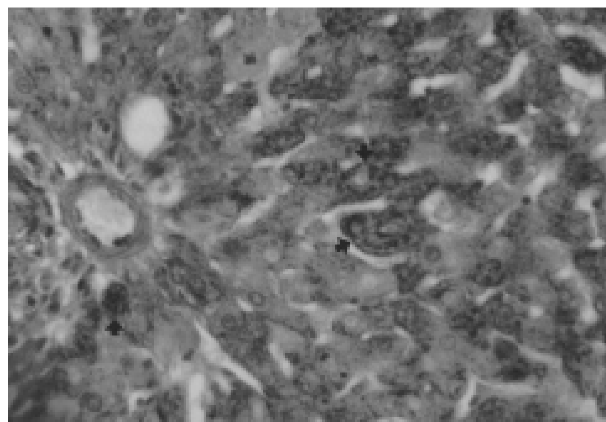

Figure 8. Group III. The hepatocytes with glycogen content around the portal area (arrow) are noticeable. PAS X240.

Biochemical findings

In HCHO-exposed rats, oxidative antioxidant enzymes SOD, GSH-Px, CAT values were significantly higher than those of the control group ($p<0.05$). MDA and XO levels of the same group were also statistically higher than the control group ($p<0.05$). In the HCHO and melatonin-exposed group, SOD, GSH-Px, CAT, XO and MDA levels were lower than the HCHO alone injected group ($p<0.05$). The biochemical findings are summarized in Table 1.

Table 1. MDA, XO, GSH-Px, CAT and SOD values of the study groups presented in mean ± SD (for each group n=7).

Groups	MDA (nmol/g)	XO (U/g)	GSH-Px (U/g)	CAT (k/g)	SOD (U/mg)
Control (I)	2.36±0.15	0.29±0.01	23.60±0.98	5.16±0.11	0.07±0.01
HCHO (II)	6.13±0.47*	0.52±0.01*	39.23±1.47*	10.21±0.21*	0.14±0.02*
HCHO+Melatonin (III)	2.67±0.22**	0.30±0.02**	34.11±0.78**	5.27±0.13**	0.12±0.02**

*p<0.05 versus controls, **p<0.05 versus formaldehyde (HCHO) group.

DISCUSSION

Previous studies have shown the toxic effects of formaldehyde (HCHO) on the skin and eyes, and on the respiratory, gastrointestinal, nervous, and reproductive systems (7-10). In addition to its deleterious effects on histological structure and functions of the liver, HCHO has been reported to cause a decrease in liver weight (12,13) and damage to the biliary ducts (14).

In our study, the light microscopic evaluation of the liver tissue sections revealed enlarged sinusoids filled with blood and cellular infiltration in the portal area and around vena centralis. Furthermore, some hepatocytes had cytoplasmic vacuolizations, while some had hyperchromatic nuclei. Strubelt et al. (15) found that HCHO exposure leads to mitochondrial destruction and damages rough endoplasmic reticulum. Beall and Ulsamer (32) reported centrilobular vacuolization and local cellular necrosis in the liver associated with HCHO exposure. In their study on rats, Dumont et al. (14) detected structural changes in the biliary epithelial cells after HCHO administration.

Free oxygen radicals are a result of metabolic intracellular process forming under natural conditions. They inflict oxidative damage on the cells by affecting membrane lipids, proteins, and nucleic acids. These potentially harmful effects are regulated by antioxidant defense mechanism. The antioxidant enzymes such as SOD and GSH-Px are needed for the maintenance of cellular balance and scavenging the free radicals away (33). Malondialdehyde (MDA) is one of the products of lipid peroxidation and is commonly used parameter to indicate oxidative stress (34).

HCHO disturbs the oxidant-antioxidant balance in various tissues and cause oxidative stress in parallel with tissue damage. In previous studies, increased MDA levels in the lung, liver, and testicular tissues of the rats exposed to HCHO were reported (34-36). In accordance with our findings, Strubelt et al. (15) have reported increased MDA levels in the liver tissues of HCHO-exposed animals. Similarly, Teng et al. (37) in their experimental study on isolated rat hepatocytes showed that HCHO at low concentrations leads to oxidative stress.

Skrzydlewska and Farbiszewski (38-40) noted methanol metabolized into HCHO and formic acid for the increased levels of SOD and GSH-Px levels in rat liver tissues. However, Datta and Namasivayam (41) found that methanol decreases SOD levels and increases CAT and MDA levels in rat hepatocytes. In our study, SOD, GSH-Px, and CAT values increased in the HCHO-exposed rats. Increased SOD activity may be a response of increased oxidative stress in the liver tissue. CAT increase, however, may be indicative of high degree oxidative stress due to elevated endogenous H_2O_2. It may also be an adaptive response to oxidative stress induced by HCHO. GSH-Px is an important antioxidant enzyme acting in

H_2O_2 elimination and lipid peroxidation. Increased GSH-Px activity suggests increased H_2O_2 products.

Melatonin is known to be involved in a variety of physiological processes including the regulation of endocrine rhythm, antigonadotropic effects, neuroprotective effects, stimulation of the immune system, and free radical scavenging action (17,22-24). In addition to these properties of melatonin, it is a potent antioxidant agent and exerts a protective effect against oxidative stress (25,42,43). In our study, melatonin was found to partially prevent the liver damage against HCHO intoxication. The exact mechanism of melatonin-provided prevention of hepatic damage induced by formaldehyde is not completely clear. Considering the distinctive properties of melatonin and the results of the present study, it is plausible that both its radical-scavenging and antioxidant actions are involved in preventing tissue damage.

We concluded that chronic exposure of formaldehyde causes structural degeneration and oxidative damage in the liver of rats. We also concluded that melatonin exerts a beneficial effect against formaldehyde toxicity in the liver appeared to be due to its antioxidant and free radical scavenger activity.

REFERENCES

1. Smith AE. Formaldehyde. Occup Med 1992: 42: 83-88.
2. Usanmaz SE, Akarsu ES, Vural N. Neurotoxic effects of acute and subacute formaldehyde exposures in mice. Envir Toxicol Pharmacol 2002: 11: 93-100.
3. Feron VJ, Till HP, de Vriger F, et al. Aldehydes: Occurrence carcinogenic potential, mechanism of action and risk assessment. Mutat Res 1991: 259: 363-385.
4. Heck HD, Casanova M, Starr TB. Formaldehyde toxicity: New understanding. Crit Rev Toxicol 1990: 20: 397-426.
5. Sarnak MJ, Long J, King A. Intravesicular formaldehyde instillation and renal complications. Clin Nephrol 1999: 51: 122-125.
6. Cohen BI, Pagnillo MK, Musikant BL, Deutsch AS. Formaldehyde evaluation from endodontic materials. Oral Health 1998: 88: 37-39.
7. Khamgaonkar MB, Fulare MB. Pulmonary effects of formaldehyde exposure: an environmental-epidemiological study. Indian J Chest Dis Allied Sci 1991: 33: 9-13.
8. Restani P, Galli CL. Oral toxicity of formaldehyde and its derivates. Crit Rev Toxicol 1991: 21: 315-328.
9. Taskinen H, Kyyrönen P, Hemminki K, et al. Laboratory work and pregnancy outcome. J Occup Med 1994: 36: 311-319.
10. Kilburn KH. Neurobehavioral impairment and seizures from formaldehyde. Arch of Environ Health 1994: 49: 37- 44.
11. Shizumi K, Sugita M, Yokote R, et al. Intestinal edema caused by ingested formalin. Chudoku Kenkyu 2003: 16: 447-451.
12. Kamata E, Nakadate M, Uchida O, et al. Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fisher-344 rats. J Toxicol Sci 1997: 22: 239-254.
13. Rusch GM, Clary JJ, Rinehart WE, Bolte HF. A 26-week inhalation toxicity study with formaldehyde in the monkey, rat and hamster. Toxicol Appl Pharmacol 1983: 68: 329-343.
14. Dumont M, D'Hont C, Moreau A, et al. Retrograde injections of formaldehyde into the biliary tree induce alterations of biliary epithelial function in rats. Hepatology 1996: 24: 1217-1223.
15. Strubelt O, Younes M, Pentz R, Kuhnel W. Mechanistic study on formaldehyde-induced hepatotoxicity. J Toxicol Environ Health 1989: 27: 351-366.
16. Susko I, Mornjakovic Z, Alicelebic S, Cosovic E, Beganovic A. Retinal and pineal melatonin-from a circadian signal to therapeutic use. Med Arh 2004: 58: 61-64.
17. Vijayalaxmi, Reiter RJ, Tan DX, Herman TS, Thomas CR. Melatonin as a radioprotective agent: a review. Int J Radiat Oncol Biol Phys 2004: 59: 639- 653.
18. Hardeland R, Reiter RJ, Poeggeler B, Tan DX. The significance of the metabolism of the neurohormone melatonin: antioxidative protection and formation of bioactive substances. Neurosci Biobehav Rev 1993: 17: 347-357.
19. Lee PP, Pang SF. Melatonin and its receptors in the gastrointestinal tract. Biol Signals 1993: 2: 181-193.
20. Pang SF, Allen AE. Extra-pineal melatonin in the retina: Its regulation and physiological function. In: Reiter RJ (Editor). Pineal Research Review. New York, NY: Alan R. Liss., 1986: 55-95.
21. Stefulj J, Hortner M, Ghosh M, et al. Gene expression of the key enzymes of melatonin synthesis in extrapineal tissues of the rat. J Pineal Res 2001: 30: 243-247.
22. Csernus V, Mess B. Biorhythms and pineal gland. Neuro Endocrinol Lett 2003: 24: 404-411.
23. Kus I, Sarsilmaz M, Ogeturk M, et al. Ultrastructural interrelationship between the pineal gland and the testis in the male rat. Arch Androl 2000: 45: 119-124.
24. Guerrero JM, Reiter RJ. A brief survey of pineal gland-immune system interrelationships. Endocr Res 1992: 18: 91-113.
25. Longoni B, Salgo MG, Pryor WA, Marchiafava PL. Effects of melatonin on lipid peroxidation induced by oxygen radicals. Life Sci 1998: 62: 853-859.
26. Arendt J. Melatonin. Clin Endocrinol 1988: 29: 205-229.
27. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988: 34: 497-500.
28. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967: 70: 158-169.
29. Aebi H. Catalase. In: Bergmeyer U (Editor). Methods of Enzymatic Analysis. New York and London: Academic Press, 1974: 673-677.
30. Prajda N, Weber G. Malignant transformation-linked imbalance: decreased xanthine oxidase activity in hepatomas. FEBS Lett 1975: 59: 245-249.
31. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hidroxynonenal. Methods Enzymol 1990: 186: 407-421.
32. Beall JR, Ulsamer AG. Formaldehyde and hepatotoxicity: a review. J Toxicol Environ Health 1984: 14: 1-21.
33. Husain K, Scott BR, Reddy SK, Somani SM. Chronic ethanol and nicotine interaction on rat tissue antioxidant defense system. Alcohol 2001: 25: 89-97.

34. Kamal AA, Gomaa A, el Khafif M, Hammad AS. Plasma lipid peroxides among workers exposed to silica or asbestos dusts. Environ Res 1990: 49: 173-180.

35. 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.

36. Tang M, Xie Y, Yi Y, Wang W. Effects of formaldehyde on germ cells of male mice. Wei Sheng Yan Jiu 2003: 32: 544-548.

37. Teng S, Beard K, Pourahmad J, et al. The formaldehyde metabolic detoxification enzyme systems and molecular cytotoxic mechanism in isolated rat hepatocytes. Chem Biol Interact 2001: 130-132: 285-296.

38. Skrzydlewska E, Farbiszewski R. Lipid peroxidation and antioxidant status in the liver, erythrocytes, and serum of rats after methanol intoxication. J Toxicol Environ Health A 1998: 53: 637-649.

39. Skrzydlewska E, Farbiszewski R. Liver and serum antioxidant status after methanol intoxication in rats. Acta Biochim Pol 1997: 44: 139-145.

40. Skrzydlewska E, Farbiszewski R. Decreased antioxidant defense mechanisms in rat liver after methanol intoxication. Free Radic Res 1997: 27: 369-375.

41. Datta NJ, Namasivayam A. In vitro effect of methanol on folate-deficient rat hepatocytes. Drug Alcohol Depend 2003: 71: 87-91.

42. Gupta YK, Gupta M, Kohli K. Neuroprotective role of melatonin in oxidative stress vulnerable brain. Indian J Physiol Pharmacol 2003: 47: 373-386.

43. Reiter RJ, Tan DX, Manchester LC, et al. Melatonin: detoxification of oxygen and nitrogen-based toxic reactants. Adv Exp Med Biol 2003: 527: 539-548.

Kabul Tarihi:23.01.2008