Available online at www.sciencedirect.com

ScienceDirect

Journal of the Chinese Medical Association 77 (2014) 463–468

www.jcma-online.com

Original Article

Damage induced by paracetamol compared with *N*-acetylcysteine

Abdullah Kisaoglu [a], Bunyami Ozogul [a], Mehmet Ibrahim Turan [b,*], Ismayil Yilmaz [c], Ismail Demiryilmaz [d], Sabri Selcuk Atamanalp [a], Ebubekir Bakan [e], Halis Suleyman [f]

[a] *Department of General Surgery, Ataturk University, Erzurum, Turkey*
[b] *Department of Pediatric Neurology, Diyarbakir Research and Educational Hospital, Diyarbakir, Turkey*
[c] *Department of General Surgery, Erzincan University, Erzincan, Turkey*
[d] *Department of General Surgery, Ibni Sina Hospital, Kayseri, Turkey*
[e] *Department of Biochemistry, Ataturk University, Erzurum, Turkey*
[f] *Department of Pharmacology, Recep Tayyip Erdogan University, Rize, Turkey*

Received July 29, 2013; accepted January 29, 2014

Abstract

Background: This study investigated the effect of thiamine pyrophosphate (TPP) on oxidative liver damage induced in rats with high-dose paracetamol.
Methods: Rats for this experiment were divided into the following groups: healthy control, paracetamol control, thiamine + paracetamol, TPP + paracetamol, and *N*-acetylcysteine + paracetamol. Oxidant and antioxidant parameters and liver function test levels were compared between the groups.
Results: The results show that TPP and *N*-acetylcysteine with paracetamol equally prevented a rise in oxidants such as malondialdehyde and nitric oxide. They also prevented a decrease in enzymatic and nonenzymatic antioxidants such as glutathione, glutathione peroxidase, glutaredoxin, glutathione S-transferase, superoxide dismutase, and catalase in the rat liver.
Conclusion: Thiamine pyrophosphate and *N*-acetylcysteine had a similar positive effect on oxidative damage caused by paracetamol hepatotoxicity. These findings show that TPP may be beneficial in paracetamol hepatotoxicity.

Keywords: hepatotoxicity; paracetamol; rat; thiamine; thiamine pyrophosphate

1. Introduction

Paracetamol is an effective *para*-aminophenol type analgesic and antipyretic drug.[1] A 150 mg/kg dose of paracetamol has a high probability of toxic effects.[2] According to United States statistics, more than 100,000 cases of paracetamol poisoning occur annually.[3] Paracetamol poisoning occurs in the context of suicide and in doses administered for therapeutic purposes.[4] Studies have reported that use of paracetamol can lead to severe damage in the liver and to liver failure.[5] Because of this serious impact on the liver, the agent is tested directly for the extent of hepatotoxicity.[6] The hepatotoxic effect of paracetamol is associated with the toxic metabolite *N*-acetyl-*p*-benzoquinoneimine (NAPB), which forms in the liver.

This metabolite is detoxified by endogenous glutathione (GSH). However, if paracetamol is taken in high doses, it depletes GSH stores and leads to an inability to detoxify NAPB sufficiently, which results in hepatic toxicity.[7,8] A rise in lipid peroxidation in paracetamol toxicity is reportedly associated with a decrease in the antioxidant GSH.[9] Nitric oxide (NO) increases in paracetamol-associated hepatotoxicity, thereby

Conflicts of interest: The authors declare that there are no conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Dr. Mehmet Ibrahim Turan, Department of Pediatric Neurology, Diyarbakir Research and Educational Hospital, 21070 Uckuyular-Diyarbakir, Turkey.
E-mail address: turan78tr@hotmail.com (M.I. Turan).

http://dx.doi.org/10.1016/j.jcma.2014.01.011

intensifying oxidative stress.[10] This suggests that oxidative stress is an important factor in liver toxicity caused by paracetamol.[11] *N*-acetyl cysteine (NAC) is used in cases of paracetamol toxicity, although it does not represent a definitive solution, and the condition may even require liver transplantation.[1] These data from the literature suggest that other stress factors, as well as GSH, have a role in paracetamol toxicity. Thiamine pyrophosphate (TPP) that we tested in this study is an active metabolite of thiamine (TA). Thiamine pyrophosphate is also a cofactor of the enzymes that have a role in maintaining cell redox by synthesizing nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione.[12] Several drugs and substances reportedly prevent the conversion of TA into TPP in the body.[13–15] This suggests that the toxic effects of drugs may be associated with the inhibition of TPP formation.

Our review of the literature revealed no data concerning the use of TPP to inhibit paracetamol-induced hepatic damage. The purpose of this study was therefore to investigate the effect of TPP on oxidative liver damage induced by paracetamol in rats, and to compare this with the effects of TA and NAC.

2. Methods

2.1. Animals

The animals in the experiment (male albino Wistar rats) were obtained from the Ataturk University Medical Experimental Practice and Research Center (Erzurum, Turkey). Thirty rats weighing 225–240 g were randomly divided into five groups before the experiment. They were housed and fed in a normal laboratory environment (22°C).

2.2. Chemical substances

Regarding the chemical substances used for the experiments, thiopental sodium was provided by I.E. Ulagay (Istanbul, Turkey). Thiamine and TPP were obtained from Biopharma (Moscow, Russia). *N*-acetylcysteine was obtained from the Husnu Arsan Drug Company (Istanbul, Turkey) and paracetamol was obtained from the Atabay Drug Company (Istanbul, Turkey).

2.3. Experimental protocol

Experimental animals were divided into the healthy group (HG), paracetamol control group (PAG), thiamine group (TAG), thiamine pyrophosphate + paracetamol group (TPPG), and *N*-acetylcysteine + paracetamol group (NACG). The TAG group was injected intraperitoneally with 20 mg/kg TA ($n = 6$); the TPPG group, 20 mg/kg TPP ($n = 6$); and the NACG group ($n = 6$), 300 mg/kg NAC. The HG ($n = 6$) and PAG ($n = 6$) were administered distilled water by the same route. One hour before the administration of drugs and distilled water, paracetamol at a dose of 1000 mg/kg was administered orally to all rat groups (excluding the HG). The doses used in this study were selected on the basis of our previous studies.[16,17] Twenty-four hours after the administration of paracetamol, all animals were sacrificed under high-dose anesthesia. Their livers were removed and biochemical investigations were performed. The biochemical results from the TAG, TPPG, and NACG were compared to those of the PAG and HG.

2.4. Biochemical analyses

2.4.1. Specimen preparation

From each extracted liver, 0.2 g was weighed. The livers were homogenized in 0.5% hexadecyltrimethylammonium bromide containing 1.15% potassium chloride solution for the malondialdehyde (MDA) assay and 7.5 pH phosphate buffer for other measurements. This totaled 2 mL in an iced environment. They were subsequently centrifuged at 15,320*g* for 15 minutes at 4°C. The supernatant part was used as a specimen for analysis. For all measurements, tissue-protein estimation was performed using Bradford's method.[18]

2.4.2. Malondialdehyde assay

This assay was based on spectrophotometric measurement at an emission wavelength of 532 nm of the absorbance of the pink complex formed at a high temperature (95°C) by thiobarbituric acid and MDA.[19]

2.4.3. Nitric oxide assay

Nitric oxide levels were measured using the Griess reaction,[20,21] which is based on a two-step process. In the first step, nitrate is converted into nitrite by nitrate reductase. In the second step, nitrite reacts with the Griess reagent. At the end of this reaction, a deep purple azo compound forms. The absorbance of this azo compound was measured photometrically at the 540 nm wavelength. This azo chromophore accurately determines nitrite concentrations as a marker of NO.

2.4.4. Total glutathione assay

5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) is a disulfide chromogen easily reduced by sulfhydryl group compounds. The resulting yellow color is measured spectrophometrically at 412 nm.[22]

2.4.5. Glutathione peroxidase assay

Glutathione peroxidase (GPO) activity was determined by the method described by Lawrence and Burk.[23] After tissue homogenization, the supernatant was used for GPO measurement. The mixture was incubated after adding monopotassium phosphate, EDTA, GSH, beta-NADPH, sodium azide, and GRx. As soon as hydrogen peroxide (H_2O_2) was added, the chronometer was turned on and the absorbance at 340 nm was recorded every 15 seconds for 5 minutes.

2.4.6. Glutathione reductase assay

Glutathione reductase (GRx) activity was determined spectrophotometrically by measuring the rate of NADPH oxidation at 340 nm, based on Carlberg and Mannervik's method.[24] After tissue homogenization, the supernatant was

used for GRx measurement. After adding NADPH and glutathione disulfide (GSSG), the chronometer was turned on and the absorbance was measured spectrophotometrically at 30-second intervals for 5 minutes at 340 nm.

2.4.7. Glutathione S-transferase activity assay

Glutathione *S*-transferase (GST) activity was determined using the method described by Habig and Jakoby.[25] In brief, enzyme activity was assayed spectrophotometrically at 340 nm in a 4-mL cuvette containing 0.1 M phosphate buffered saline (pH 6.5), 30 mM GSH, 30 mM 1-chloro-2,6-dinitrobenzene, and tissue homogenate.

2.4.8. Superoxide dismutase assay

Measurements were performed by the method described by Sun et al.[26] Superoxide dismutase (SOD) forms when xanthine oxidase converts xanthine into uric acid. If nitro blue tetrazolium (NBT) is added to this reaction, SOD reacts with NBT and a purple formazan dye occurs. The absorbance of the purple formazan dye was measured at 560 nm.

2.4.9. Catalase activity assay

Decomposition of H_2O_2 in the presence of catalase (CAT) was measured at 240 nm.[27] The CAT activity was defined as the amount of enzyme required to process 1 nM H_2O_2 per minute at 26°C and pH 7.8.

2.5. Analysis of liver function tests

Venous blood samples were collected into tubes without an anticoagulant. After clotting, the serum was separated by centrifugation and stored at −80°C until assay. Using a Cobas 8000 autoanalyzer (Roche Diagnostics GmBH, Mannheim, Germany) with commercially available kits (Roche Diagnostics), serum aspartate aminotransferase (AST) and alanine transaminase (ALT) activities were measured spectrophotometrically for the liver function tests and lactate dehydrogenase (LDH) activity was measured as a marker of tissue injury.

2.6. Statistical analysis

All data were subjected to the Kruskal-Wallis test using SPSS version 18.0 software (IBM Corporation, Armonk, NY, USA). Differences between groups were obtained using Wilcoxon rank sum tests with Bonferroni corrections. Significance was declared at $p \leq 0.05$. The results are expressed as the mean ± the standard error of the mean (SEM).

3. Results

3.1. MDA, NO, GSH, GPO, GRx, GST, SOD, and CAT analyses

Significant differences between the groups were determined in the parameters MDA, NO, GSH, GPO, GRx, GST, SOD, and CAT ($p < 0.05$). As Fig. 1 shows, MDA concentrations in the liver tissue in the HG, TAG, TPPG, NACG, and PAG were

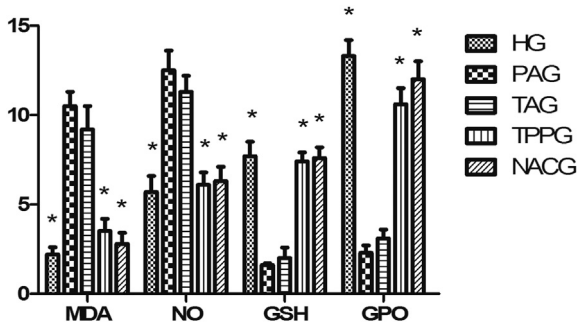

Fig. 1. A comparison of the groups, based on the level of MDA (μmol/g protein), NO (μmol/g protein), GSH (nmol/g protein), and GPO (U/g protein). The bars are the mean ± standard error of the mean. GPO = glutathione peroxidase; GSH = glutathione; HG = healthy group; MDA = malondialdehyde; NACG = *N*-acetylcysteine + paracetamol group; NO = nitric oxide; PAG = paracetamol control group; TAG = thiamine + paracetamol group; TPPG = thiamine pyrophosphate + paracetamol group. * A value of $p \leq 0.05$ is significant.

2.2 ± 0.4 μmol/g protein ($p < 0.05$), 9.2 ± 1.3 μmol/g protein ($p > 0.05$), 3.5 ± 0.7 μmol/g protein ($p < 0.05$), 2.8 ± 0.6 μmol/g protein ($p < 0.05$), and 10.5 ± 0.8 μmol/g protein, respectively. The NO levels were 5.7 ± 0.9 μmol/g protein ($p < 0.05$), 11.3 ± 0.9 μmol/g protein ($p > 0.05$), 6.1 ± 0.7 μmol/g protein ($p < 0.05$), 6.3 ± 0.8 μmol/g protein ($p < 0.05$), and 12.5 ± 1.1 μmol/g protein, respectively (Fig. 1). The GSH levels for these groups were 7.7 ± 0.8 nmol/g protein for the HG ($p < 0.05$), 2.0 ± 0.6 nmol/g protein for the TAG, ($p > 0.05$), 7.4 ± 0.5 nmol/g protein for the TPPG ($p < 0.05$), 7.6 ± 0.6 nmol/g protein for the NACG ($p < 0.05$), and 1.6 ± 0.1 nmol/g protein for the PAG (Fig. 1). Glutathione peroxidase activity in the HG and in the PAG was 13.3 ± 0.9 U/g protein ($p < 0.05$) and 2.3 ± 0.4 U/g protein, respectively. This activity in the liver tissues of the TAG, TPPG, and NACG was 3.1 ± 0.5 U/g protein ($p > 0.05$), 10.6 ± 0.9 U/g protein ($p < 0.05$), and 12 ± 1 U/g protein ($p < 0.05$), respectively (Fig. 1). As Table 1 shows, the GRx and GST activity in the liver tissue was significantly higher in the HG, TPPG, and NACG than in the TAG and PAG ($p < 0.05$). The SOD and CAT activities were also significantly higher in the HG, TPPG, and NACG than in the TAG and PAG ($p < 0.05$).

3.2. AST, ALT, and LDH analyses

As Fig. 2 shows, the blood AST values in the HG, TAG, TPPG, NACG, and PAG were 36.8 ± 4.8 U/L ($p < 0.05$), 265.6 ± 9.8 U/L ($p > 0.05$), 64 ± 4.5 U/L ($p < 0.05$), 109.5 ± 2.8 U/L ($p < 0.05$), and 274.1 ± 8.2 U/L, respectively. The ALT levels in the blood specimens of the HG, TAG, TPPG, NACG, and PAG were 15.1 ± 3.5 U/L ($p < 0.05$), 150.8 ± 10.7 U/L ($p > 0.05$), 32.6 ± 2.8 U/L ($p < 0.05$), 55.6 ± 2.3 U/L ($p < 0.05$), and 157.3 ± 13.3 U/L, respectively (Fig. 2). As Fig. 3 shows, the LDH levels in the HG, TAG, TPPG, NACG, and PAG were 157.3 ± 11 U/L ($p < 0.05$), 543.3 ± 5.8 U/L ($p > 0.05$), 199 ± 5.6 U/L ($p < 0.05$), 285.2 ± 8.7 U/L ($p < 0.05$), and 600 ± 12.6 U/L, respectively.

Table 1
Comparisons of groups, based on GRx, GST, SOD, and CAT activities.[a]

	GRx (U/g protein)	GST (U/g protein)	SOD (mmol/min/mg tissue)	CAT (mmol/min/mg tissue)
PAG	3.3 ± 0.6	6.6 ± 0.5	7.8 ± 0.6	9.5 ± 0.9
$n = 6$				
HG	16.8 ± 0.9	9.5 ± 0.9	17.3 ± 0.8	21 ± 1.3
$n = 6$	$p < 0.05$*	$p < 0.05$*	$p < 0.05$*	$p < 0.05$*
TAG	5.5 ± 1.6	5.8 ± 0.8	9.3 ± 0.9	11.8 ± 1.3
$n = 6$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
TPPG	14.6 ± 0.9	8.5 ± 0.7	14.6 ± 0.7	18.1 ± 1.2
$n = 6$	$p < 0.05$*	$p < 0.05$*	$p < 0.05$*	$p < 0.05$*
NACG	15.6 ± 0.9	9 ± 1.1	16 ± 0.9	20.5 ± 1.9
$n = 6$	$p < 0.05$*	$p < 0.05$*	$p < 0.05$*	$p < 0.05$*

All values are expressed as the mean ± standard error of the mean.
CAT = catalase; GRx = glutathione reductase; GST = glutathione S-transpherase; HG = healthy group; n = number of animals; NACG = *N*-acetylcysteine + paracetamol group; PAG = paracetamol control group; SOD = superoxide dismutase; TAG = thiamine + paracetamol group; TPPG = thiamine pyrophosphate + paracetamol group.
*A $p \leq 0.05$ was significant.
[a] A comparison of the activities of GRx, GST, SOD, CAT of each group versus the control group, based on Wilcoxon rank sum tests with Bonferroni corrections.

4. Discussion

This study investigated the effect of TPP on oxidative liver damage induced in rats with high-dose paracetamol. This study also evaluated TPP in comparison with TA and NAC. Mammalian peroxisomes contain TPP; however, no pyrophosphorylation of thiamine occurs in these organelles, which suggests that TPP is already pyrophosphorylated when it enters the peroxisome.[28,29] The entry of TPP into the cell is not fully understood. However, it may depend on a specific transport system or (in a bound form) on 2-hydroxyacyl-CoA lyase 1 translocation.[28] We therefore examined the effect of TPP and of TZ, and compared this with NAC. The results showed that paracetamol administered orally at a dose of 1000 mg/kg led to pronounced oxidative stress in the liver. Under physiological conditions, the oxidant/antioxidant balance is maintained in favor of antioxidants. A compromise of this balance leads to tissue damage (i.e., oxidative stress). Tissue damage is evaluated through the oxidant/antioxidant balance. This balance alters in favor of antioxidants in various models of damage in living tissues: a rise in oxidant levels and a decrease in antioxidant levels occurs.[30] Our results are compatible with these data from the literature. In the experiment, the level of MDA (an oxidant parameter) in the rat groups receiving TPP and NAC decreased, compared to the level in the PAG. A rise in MDA levels in a tissue shows that free oxygen radicals have increased in that tissue. A rise in free oxygen radicals increases lipid peroxidation. Malondialdehyde is a product of lipid peroxidation and leads to cell damage by causing cell membrane compounds to cross-link.[31,32] Studies show that the level of MDA rises in hepatic oxidative damage.[33]

Nitric oxide is another factor leading to lipid peroxidation. It also reportedly initiates lipid peroxidation in an environment containing superoxide.[34] Excess NO production in the liver therefore leads to hepatic damage.[35] The finding that the NO levels in the rat liver tissue is lower in the TPPG and the NACG than in the PAG indicates that paracetamol causes oxidative stress in the rat liver, which is inactivated and prevented to a significant extent by TPP and NAC.

Excessive doses of paracetamol reportedly increase lipid peroxidation by raising GSH.[9] The hepatotoxic effect of paracetamol may be via its metabolite NAPB. This toxic metabolite is detoxified by endogenous GSH. However, when paracetamol is administered in toxic doses, excessive NAPB forms and cannot be sufficiently detoxified by GSH. The production of NAPB in amounts that exceed the detoxifying

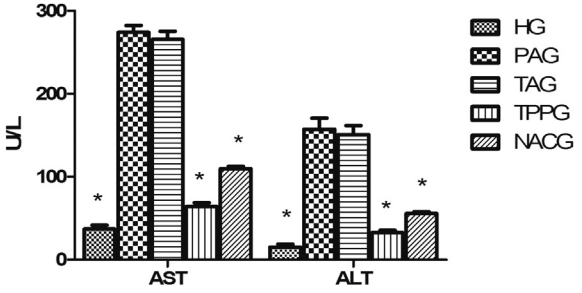

Fig. 2. A comparison of groups, based on AST and ALT activity. The bars are the mean ± standard error of the mean. ALT = alanine transaminase; AST = aspartate aminotransferase; HG = healthy group; NACG = *N*-acetylcysteine + paracetamol group; PAG = paracetamol control; TAG = thiamine + paracetamol; TPPG = thiamine pyrophosphate + paracetamol. * A value of $p \leq 0.05$ was significant.

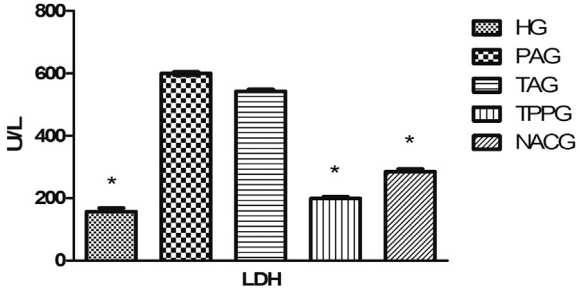

Fig. 3. A comparison of the groups, based on LDH activity. The bars are the mean ± standard error of the mean. HG = healthy group; LDH = lactate dehydrogenase; NACG = *N*-acetylcysteine + paracetamol group; PAG = paracetamol control group; TAG = thiamine + paracetamol group; TPPG = thiamine pyrophosphate + paracetamol group. * A value of $p \leq 0.05$ is significant.

capacity of GSH also results in liver toxicity.[36,37] These data from the literature agree with our own findings. Glutathione is an endogenous antioxidant molecule. In addition to GSH, enzymatic antioxidants such as GPO, GRx, GST, SOD, and CAT protect cells against free oxygen radical damage.[38] A decrease in GPO activity that catalyzes the reduction of lipid peroxides will result in severe cell damage.[39] Glutathione is reduced by GRx to maintain antioxidant activity.[40] Therefore, GRx activity has to be maintained at a specific level to protect the integrity of the cell against oxidative damage. A mechanism that functions against oxidative stress is GST. This molecule inactivates hyperperoxides and hydroxy alkalines, malondialdeydes, and propenals.[41] Enzymes such as SOD and CAT are natural antioxidants that slow the rate of lipid peroxidation. Superoxide dismutase protects cells against the harmful effects of superoxide radicals, whereas CAT directly neutralizes the H_2O_2 that increases during oxidative stress.[42] Catalase is more effective in environments where H_2O_2 is present in high concentrations.[43] In liver tissue, the activities of GSH, GPO, GRx, GST, SOD, and CAT were higher and the levels of MDA and NO were lower in the TPPG and NACG than in the PAG in our study. The data from the literature and our experiment results indicate that significant oxidative stress develops in the liver of rats that are administered paracetamol. The activities of AST, ALT, and LDH in the PAG blood specimens were significantly higher than in the HG, TPPG, and NACG blood specimens. In this study, no statistically significant difference existed between the oxidant and antioxidant parameters in the TPPG and NACG at the doses used. However, compared to NAC, TPP more effectively reduced the activity of AST, ALT, and LDH. The most widely accepted tests used for evaluating liver function are AST, ALT, and LDH activity assays.[44] The activities of these enzymes increase in liver damage.[44] This stems particularly from an increase in aminotransferase levels or an excessive increase in cell membrane permeability.[45] Thiamine pyrophosphate reduces the severe increase in LDH, AST, ALT, and oxidant parameters in liver damage caused by methotrexate.[33]

In conclusion, high-dose paracetamol (i.e., 1000 mg/kg) altered the oxidant/antioxidant balance in liver tissue in favor of oxidants. Paracetamol significantly raised the levels of AST, ALT, and LDH in the rat liver tissue. Thiamine pyrophosphate and NAC equally prevented paracetamol from changing the oxidant/antioxidant balance in favor of oxidants. However, compared to NAC, TPP at a dose of 20 mg/kg more significantly reduced the activity of AST, ALT, and LDH. By contrast, TA failed to protect the liver against oxidative liver damage induced by paracetamol. These findings show that TPP can be useful in paracetamol hepatotoxicity.

References

1. Katzung BG. *Basic and clinical pharmacology*. 10th ed. New York: McGraw Hill Companies Inc; 2007.
2. Dart RC, Erdman AR, Olson KR, Christianson G, Manoguerra AS, Chyka PA, et al. Acetaminophen poisoning: an evidence-based consensus guideline for out-of-hospital management. *Clin Toxicol (Phila)* 2006;**44**:1–18.
3. Oliver LH, Lewis SN. *Acetaminophen*. 7th ed. New York: McGraw-Hill; 2010.
4. Black M. Acetaminophen hepatotoxicity. *Annu Rev Med* 1984;**35**:577–93.
5. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, et al. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. *Hepatology* 2005;**42**:1364–72.
6. Zimmerman HJ. *Classification of hepatotoxins and mechanisms of toxicity*. New York: Appleton-Century-Crofts; 1978.
7. Rumack BH, Matthew H. Acetaminophen poisoning and toxicity. *Pediatrics* 1975;**55**:871–6.
8. Klein-Schwartz W, Doyon S. Intravenous acetylcysteine for the treatment of acetaminophen overdose. *Expert Opin Pharmacother* 2011;**12**:119–30.
9. Horton AA, Fairhurst S. Lipid peroxidation and mechanisms of toxicity. *Crit Rev Toxicol* 1987;**18**:27–79.
10. Gardner CR, Heck DE, Yang CS, Thomas PE, Zhang XJ, DeGeorge GL, et al. Role of nitric oxide in acetaminophen-induced hepatotoxicity in the rat. *Hepatology* 1998;**27**:748–54.
11. Gibson JD, Pumford NR, Samokyszyn VM, Hinson JA. Mechanism of acetaminophen-induced hepatotoxicity: covalent binding versus oxidative stress. *Chem Res Toxicol* 1996;**9**:580–5.
12. Gangolf M, Czerniecki J, Radermecker M, Detry O, Nisolle M, Jouan C, et al. Thiamine status in humans and content of phosphorylated thiamine derivatives in biopsies and cultured cells. *PLoS One* 2010;**5**:e13616.
13. Subramanian VS, Subramanya SB, Tsukamoto H, Said HM. Effect of chronic alcohol feeding on physiological and molecular parameters of renal thiamin transport. *Am J Physiol Renal Physiol* 2010;**299**:F28–34.
14. Hanninen SA, Darling PB, Sole MJ, Barr A, Keith ME. The prevalence of thiamin deficiency in hospitalized patients with congestive heart failure. *J Am Coll Cardiol* 2006;**47**:354–61.
15. Gastaldi G, Casirola D, Patrini C, Ricci V, Laforenza U, Ferrari G, et al. Intestinal transport of thiamin and thiamin monophosphate in rat everted jejunal sacs: a comparative study using some potential inhibitors. *Arch Int Physiol Biochim* 1988;**96**:223–30.
16. Turan MI, Siltelioglu Turan I, Mammadov R, Altunkaynak K, Kisaoglu A. The effect of thiamine and thiamine pyrophosphate on oxidative liver damage induced in rats with cisplatin. *BioMed Res Int* 2013;**2013**:783809.
17. Turan M, Cayir A, Cetin N, Suleyman H, Turan IS, Tan H. An investigation of the effect of thiamine pyrophosphate on cisplatin-induced oxidative stress and DNA damage in rat brain tissue compared with thiamine: thiamine and thiamine pyrophosphate effects on cisplatin neurotoxicity. *Hum Exp Toxicol* 2014;**33**:14–21.
18. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;**72**:248–54.
19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;**95**:351–8.
20. Bories PN, Bories C. Nitrate determination in biological fluids by an enzymatic one-step assay with nitrate reductase. *Clin Chem* 1995;**41**:904–7.
21. Moshage H, Kok B, Huizenga JR, Jansen PL. Nitrite and nitrate determinations in plasma: a critical evaluation. *Clin Chem* 1995;**41**:892–6.
22. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 1968;**25**:192–205.
23. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun* 1976;**71**:952–8.
24. Carlberg I, Mannervik B. Glutathione reductase. *Methods Enzymol* 1985;**113**:484–90.
25. Habig WH, Jakoby WB. Assays for differentiation of glutathione S-transferases. *Methods Enzymol* 1981;**77**:398–405.
26. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem* 1988;**34**:497–500.
27. Aebi H. Catalase in vitro. *Methods Enzymol* 1984;**105**:121–6.
28. Fraccascia P, Sniekers M, Casteels M, Van Veldhoven PP. Presence of thiamine pyrophosphate in mammalian peroxisomes. *BMC Biochem* 2007;**8**:10.
29. Sniekers M, Foulon V, Mannaerts GP, Van Maldergem L, Mandel H, Gelb BD, et al. Thiamine pyrophosphate: an essential cofactor for the

alpha-oxidation in mammals—implications for thiamine deficiencies? *Cell Mol Life Sci* 2006;**63**:1553−63.
30. Kisaoglu A, Borekci B, Yapca OE, Bilen H, Suleyman H. Tissue damage and oxidant/antioxidant balance. *Eurasian J Med* 2013;**45**:47−9.
31. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007;**39**:44−84.
32. Karihtala P, Soini Y. Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. *APMIS* 2007;**115**:81−103.
33. Demiryilmaz I, Sener E, Cetin N, Altuner D, Suleyman B, Albayrak F, et al. Biochemically and histopathologically comparative review of thiamine's and thiamine pyrophosphate's oxidative stress effects generated with methotrexate in rat liver. *Med Sci Monit* 2012;**18**:BR475−81.
34. Hogg N, Kalyanaraman B. Nitric oxide and lipid peroxidation. *Biochim Biophys Acta* 1999;**1411**:378−84.
35. Wang JF, Greenberg SS, Spitzer JJ. Chronic alcohol administration stimulates nitric oxide formation in the rat liver with or without pretreatment by lipopolysaccharide. *Alcohol Clin Exp Res* 1995;**19**:387−93.
36. Goldfrank LR, Flomenbaum NE, Lewin NA, Weisman RS, Howland MA. *Goldfrank's toxicologic emergencies*. 4th international ed. East Norwalk, CT: Appleton and Lange; 1990. pp. 183−5. 251−7.
37. Goodman LS, Gilman A. *The pharmacological basis of theurapeutics*. 8th ed. New York, NY: Permagon Press; 1991.
38. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. *Nutrition* 2002;**18**:872−9.
39. Rambabu JP, Rao MB. Effect of an organochlorine and three organophosphate pesticides on glucose, glycogen, lipid, and protein contents in tissues of the freshwater snail *Bellamya dissimilis* (Muller). *Bull Environ Contam Toxicol* 1994;**53**:142−8.
40. Young IS, Woodside JV. Antioxidants in health and disease. *J Clin Pathol* 2001;**54**:176−86.
41. Cnubben NH, Rietjens IM, Wortelboer H, van Zanden J, van Bladeren PJ. The interplay of glutathione-related processes in antioxidant defense. *Environ Toxicol Pharmacol* 2001;**10**:141−52.
42. Niwa Y, Ishimoto K, Kanoh T. Induction of superoxide dismutase in leukocytes by paraquat: correlation with age and possible predictor of longevity. *Blood* 1990;**76**:835−41.
43. Halliwell B. Superoxide dismutase, catalase and glutathione peroxidase: solutions to the problem of lung with oxygen. *New Phyto* 1974;**73**:1075−86.
44. Yabe Y, Kobayashi N, Nishihashi T, Takahashi R, Nishikawa M, Takakura Y, et al. Prevention of neutrophil-mediated hepatic ischemia/reperfusion injury by superoxide dismutase and catalase derivatives. *J Pharmacol Exp Ther* 2001;**298**:894−9.
45. Lott JA, Wolf PL. Alanine and aspartate aminotransferase (ALT and AST). In: Lott JA, Wolf PL, editors. *Clinical enzymology: a case-oriented approach*. Chicago, IL: Year Book Medical Publishers; 1986. pp. 111−38.