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Original Research

The effects of apomorphine on paracetamol-induced hepatotoxicity in rats

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Abstract: It is becoming progressively more understandable that overdose of paracetamol in both humans and animals causes severe hepatotoxicity. Apomorphine is known as a neuroprotective agent. Due to the protective effect, apomorphine had been tested in experimental studies on different models. Findings obtained through series of expriments suggested that apomorphine may also be useful in liver toxicity. The aim of this study is to investigate the relationship among the hepatoprotective mechanism of apomorphine and to determine the possible role of apomorphine on paracetamol-induced hepatotoxicity in rats. 30 Sprague Dawley rats (adult male) were distributed into 5 groups. Group 1 was the control group and did not receive any medication. Group 2 received only paracetamol 2 g/kg by intragastric gavage to induce hepatotoxicity. Groups 3 and 4 were given apomorphine 1 mg/kg and 2 mg/kg by intraperitoneal injection, respectively. Groups 3 and 4 were given 2g/kg of Paracetamol. In Group 5, rats were treated with 2 mg/kg of apomorphine. Drug-treated rats were given food for the next 24 h until they were sacrified. Moreover, we also performed AST, ALT measurements in serum, MDA and SOD levels in liver tissues and histopathological analysis of the liver in all groups. Apomorphine had positive effects on both liver enzymes, oxidative stress markers and histopathological results in paracetamol-induced hepatotoxicity. Additionally, apomorphine at 2 mg/kg dose was significantly more protective as compared to 1 mg/kg as evidenced by the histopathological examination results. It was thought that apomorphine was found hepatoprotective on paracetamol-induced hepatotoxicity, especially at higher doses such as 2 mg/kg.

Key words: Apomorphine; Hepatotoxicity; Paracetamol; Rat.

Introduction

Paracetamol and NSAIDs, particularly ibuprofen and acetylsalicylic acid are among the most frequently used pharmaceutical drugs.

Paracetamol is considered to be safe at therapeutic doses and is a widely used antipyretic and analgesic drug in clinical practice. However, overdose of paracetamol in both humans and animals causes severe hepatotoxicity and necrosis (1). Paracetamol-induced hepatotoxicity is a toxic-metabolic damage that consequently results in death of hepatocytes as a result of activation of necrosis, apoptosis and inflammatory cytokines (2).

However, biochemical mechanisms of cell necrosis are still inscompletely explored and it is considered to be caused by simultaneous involvement of non-covalent bonding, the lipid peroxidation and oxidative stress (3,4). Oxidative capacity-mediated oxidative stress of N-acetyl-p-benzoquinone (NAPQI), which is a product of xenobiotic metabolism of paracetamol, is regarded as the main cause of paracetamol hepatotoxicity (5). When NAPQI is formed, it reacts with hepatic glutathione (GSH) easily. As a result it is excreted in the bile with multidrug resistance protein 2 (Mrp2) proteins, the adduct of GSH (6). NAPQI irreversibly binds with a number of intracellular target protein that causes mitochondrial oxidative stress in circumstances of the reduction of hepatic GSH levels occurring with paracetamol overdose, chemotherapy, antiretroviral treatment or drug-induced liver injury (7). The metabolic role of paracetamol results in degradation of membranes, depletion of ATP, formation of DNA damage and the necrosis of liver cells (8). At the same time, the formation of various reactive oxygen species (ROS) occurring as a result of oxidative stress are associated with paracetamol-induced hepatic necrosis. High malondialdehyde (MDA) level is an indicator of oxidative stress dependent on lipid peroxidation in paracetamol toxicity. ROS occuring in hepatocytes due to the overdosage of paracetamol, are detoxified with specific enzymes such as glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase that convert superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2) (9). Investigation of these enzyme systems in tissues is very important in order to reduce the effects of ROS result in hepatotoxicity.

Apomorphine, a potential antioxidant and powerful

D1 and D2 dopamine receptor antagonist that reaches to the brain quickly and accumulates in striatum, is proposed as a neuroprotective agent in the treatment of Parkinson's disease (10-12). Due to the emetic properties of apomorphine, in addition to the use in medicine it is used as a therapeutic for excretion of toxic substances in veterinary (13). Apomorphine has copper and iron chelating properties and is capable of readily penetrate from biological membranes into cells (14, 15). It reduces the risk of cell death depends on hydroxyl radicals and prevents the formation of H_2O_2 (16). Apomorphine, also inhibits membrane lipid peroxidation as *in vivo* and *in vitro* (11).

We investigated the relationship among the hepatoprotective mechanism of apomorphine, antioxidant enzymes and oxidative stress parameters and to investigate the role of apomorphine in paracetamol-induced hepatotoxicity in rats.

Materials and Methods

Chemicals

All chemicals were purchased from Sigma Chemical (Germany). Paracetamol was purchased from Dogal Ilac Hammaddeleri Tic. Ltd. Sti. (Turkey). Thiopental sodium was purchased from IE Ulagay A.S., Istanbul, Turkey and apomorphine (D043-5mg) was purchased from Sigma-Aldrich. Detection kits for superoxide dismutase activity (SOD) and malondialdehyde (MDA) were purchased from Cayman and CellBiolabs (USA). Reagents for alanine amino transferase and aspartate amino transferase were purchased from USCN Life Science (China).

Animals

Thirty adult male Sprague Dawley rats weighing about 250-275 g were obtained from Medicinal and Experimental Application and Research Center (ATADEM). Rat groups were given standard pellet based feed and tap water. All animals were kept under standard laboratory conditions in stainless steel cages (light period 07.00 a.m. to 8.00 p.m., 21±2 °C, and relative humidity 55%) throughout the experimental procedure. Experimental protocols and animal care had previously been approved by the Experimental Animal Ethics Committee, Atatürk University.

Experimental design

Animals fasted over night and were divided into five equal groups (n=6). The first group (Group 1) served as a control group and therefore did not receive any medication. Group 2 received only paracetamol, which was suspended in 1% carboxymethyl cellulose (CMC) in 1 x phosphate buffer solution (PBS) 2 ml (per rat and administered orally at a dosage of 2 g/kg by intragastric gavage). Group 3 and 4 were given apomorphine 1 mg/kg and apomorphine 2 mg/kg by intraperitoneal (ip) injection after 24 h fasted, respectively. One hour after administration of pretreatment drugs, Group 3 and 4 were given paracetamol (2 g/kg). In Group 5, rats were intraperitoneally injected with only apomorphine (2 mg/kg). The rats were given food after drug administration for the next 24 h until they were sacrified.

The experimental groups can be summarized as follows:

Group 1: No drug administration
Group 2: Paracetamol (2 g/kg)
Group 3: Apomorphine (1 mg/kg) + Paracetamol (2 g/kg)
Group 4: Apomorphine (2 mg/kg) + Paracetamol (2 g/kg)
Group 5: Apomorphine (2 mg/kg)

Following the experimental period, all rats were killed on the 24th hour after the administration of paracetamol by an overdosage of a general anesthetic (50 mg/kg thiopentalsodium). Blood samples were collected into heparinized bottles by heart puncture. There was an immediate removal of liver after sacrifice.

Biochemical analyses

ALT, AST and total protein measurements

Serum samples obtained from rats were separated from blood by centrifugation at 4000 rpm for 10 min at 4 °C and analyzed, immediately. ALT and AST activities of each liver sample were measured by ELISA kits. Concentration of proteins was determined by the Lowry method.

Biochemical investigation of liver tissues

Rat tissues were kept at -80 °C. Tissues for biochemical analysis were homogenized in the appropriate buffer on ice by Qiagen Tissue Lyser II after grinding in liquid nitrogen for each method separately and supernatants were removed. For biochemical investigation, SOD activities and MDA levels were studied by use of ELISA spectrophotometry (17, 18). Data was represented as the mean ± standard deviation per mg protein.

SOD activity

SOD activities of rat liver tissues were measured based colorimetric method under 450 nm with SOD assay kit (Biovision-K335-100; Milpitas, CA95035, USA) spectrophotometrically. The data was presented as the IC_{50} values and the data was represented as U/mL SOD per milligram protein.

MDA levels

Malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) and forms a colorimetric product at 532 nm. We determined the MDA levels of rat liver tissues by using the Lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit. The results were expressed as nmole MDA per milligram tissues (nmole/mg tissue).

Histopathological analysis

Liver tissue samples were fixed in 10% neutral buffered formalin after an immediate removal, dehydrated in graded concentrations of ethanol, cleared in xylol, and embedded in paraffin. Sections of 5 µm thickness were obtained and then stained with hematoxylin-eosin (H&E). Slides were examined by an Olympus CX31 light microscope and photographed. The sections of the liver were studied and scored, and the degree of necrosis and inflammation was determined as previously defined by Yaman et al. (19). Degree of necrosis was represented as the mean of 20 high power fields (HPFs),

Table 1. Serum levels of ALT and AST in all groups (U/L).

Groups	ALT (mean±SD)	AST (mean±SD)
Group 1	51.00 ± 9.68	88.35 ± 16.03
Group 2	185.13 ± 20.75[a]	249.00 ± 32.50[a]
Group 3	104.63 ± 29.40[b]	111.68 ± 28.96[b]
Group 4	84.13 ± 25.19[b]	106.95 ± 15.82[b]
Group 5	59.75 ± 4.46	90.83 ± 24.02

ALT: alanine aminotransferase, **AST:** aspartate aminotransferase. [a]**:** p<0.05 when compared to Group 1, [b]**:** p<0.05 when compared to Group 2.

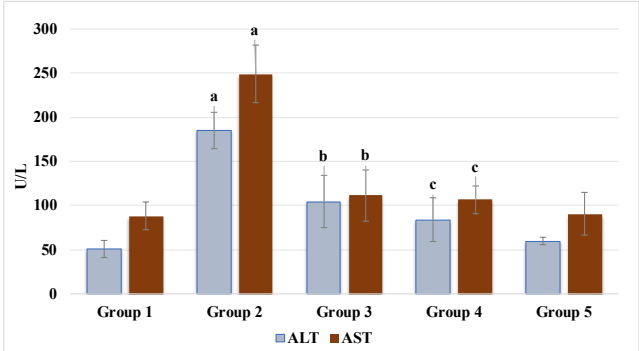

Figure 1. ALT and AST levels of rat serum in all groups (U/L).

and categorized on a scale of 0–3.

Statistical analysis

Data was analysed using SPSS 19.0 statistical software package for Windows (SPSS Inc, Chicago, USA). The results were reported as the Mean ± Standard Deviation. Groups were compared using the Duncan's multiple range test option and were considered to be significant at p<0,05. p<0.001 was considered as statistically significant and Rank–Sum test was used for the histopathological scores.

Results

Effects of apomorphine on levels of serum ALT and AST

Levels of Serum ALT and AST of the control and experimental groups were shown in Figure 1 and Table 1. Statistically significant (p<0.05) increase in the serum levels of AST and ALT was recorded in the paracetamol treated group (Group 2) as compared with Group 1. Pretreatment with apomorphine 1 and 2 g in Groups 3 and 4, ALT and AST levels were significantly reduced when compared to Group 2 (p<0.05).

Effects of apomorphine on liver antioxidant levels

The SOD activity and MDA levels of all groups were shown in Figure 2, Figure 3 and Table 2. The hepatic SOD activities were markedly (p<0.05) reduced in paracetamol administration group (Group 2), whereas the MDA levels were increased when compared to Group 1. MDA and SOD levels of Groups 3 and 4 were significantly different in comparison with Group 2 (p<0.05).

Table 2. The SOD activity (U/mg protein) and MDA levels (nmol / mg protein) of all groups.

Groups	MDA (mean±SD)	SOD (mean±SD)
Group 1	1,43 ± 0,31	25,59 ± 5,26
Group 2	3,12 ± 0,32[a]	10,94 ± 2,40[a]
Group 3	1,73 ± 0,90[b]	17,15 ± 7,64[b]
Group 4	1,46 ± 0,32[b]	21,49 ± 7,01[b]
Group 5	1,83 ± 0,49	23,97 ± 5,32

MDA: malondialdehyde, **SOD:** superoxide dismutase. [a]**:** p<0.05 when compared to Group 1, [b]**:** p<0.05 when compared to Group 2.

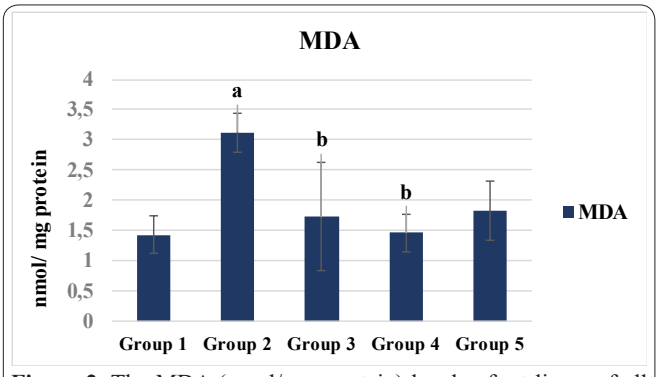

Figure 2. The MDA (nmol/ mg protein) levels of rat livers of all groups.

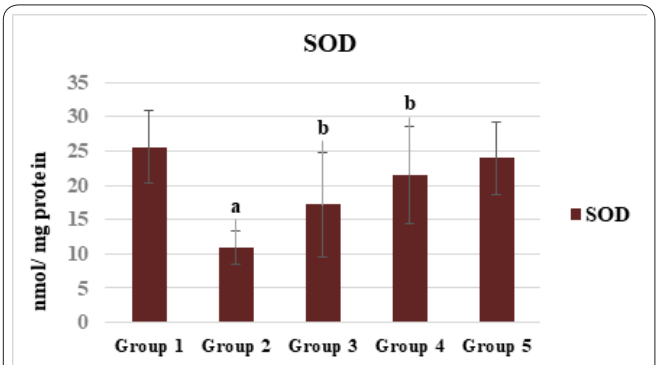

Figure 3. The SOD activity (U/mg protein) of rat livers of all groups.

Histological findings

The scoring of liver necrosis in all groups are shown in Table 3. İt is relevant to mention that In group 1 and 5, liver morphology was normal with radiating hepatic cords and centralized veins that is 0 staging score. However, Paracetamol caused severe hepatic pathological changes such as inflammation, significantly enhanced hepatic necrosis and excessive deposition of collagen in Group 2. The liver sections in Groups 3 and 4 showed lesser hepatic necrosis, lesser deposition of collagen and a markedly reduced staging score (grade 2) when compared to Group 2. Moreover, the liver necrosis levels were lower in Group 4 than that of Group 3 (Figure 4, Table 3).

Discussion

Paracetamol is commonly used with analgesic and

Table 3. The scoring of liver necrosis in all groups.

Groups	Score of liver necrosis			
	0	1	2	3
Group 1	20(100)	0(0)	0(0)	0(0)
Group 2	0(0)	1(5)	3(15)	16(80)
Group 3	0(0)	2(10)	8(40)	10(50)[a]
Group 4	0(0)	6(30)	11(55)	3(15)[a,b]
Group 5	20(100)	0(0)	0(0)	0(0)

data were expressed as number (%).[a]**:** p<0.001 when compared to Group 2, [b]**:** p<0.001 when compared to Group 3.

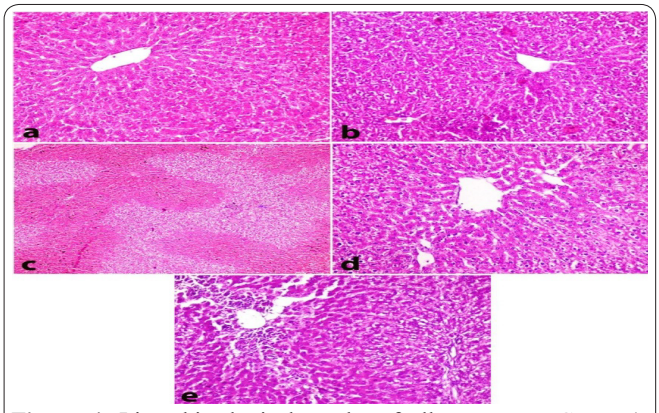

Figure 4. Liver histological results of all groups. **a.** Group 1: Normal hepatic parenchyma (H&E, 200x). **b.** Group 5: Normal hepatic parenchyma (H&E, 200x). **c.** Group 2: Diffuse necrosis in hepatic parenchyma (H&E, 100x). **d** and **e.** Paracetamol induced histopathologic changes in rat liver reduced by apomorphine treatment in Groups 3 and 4, respectively (H&E, 200x).

antipyretic properties for a long time. It has a safety profile at therapeutic doses. At overdoses, paracetamol has devastating effects on liver, kidney and other organs. It is mostly metabolized in the liver by conjugation with glucuronic acid and sulfates. However, smaller amounts of paracetamol are also metabolized to NAPQI in the liver, which leads to the formation of ROS, initiates lipid peroxidation that consequently results in apoptosis or necrosis of hepatocytes in increased levels (20). As known, SOD has protective effects against ROS and MDA is an important marker of lipid peroxidation (21, 22). Paracetamol-induced hepatotoxicity causes oxidative stress and increase the generation of ROS (23). In the liver, paracetamol overdosage produced a centrilobular hepatic necrosis that can prove to be lethal and might result into fulminant liver failure (23). Paracetamol-induced hepatotoxicity accounts for nearly 50% of all cases of acute liver failure in North American and European countries (24). Considering that rats developed only minor injuries in the liver even at higher doses of 1 g/kg paracetamol, we decided to administrate 2 g/kg in this experimental study.

Apomorphine is a dopamine receptor agonist with antioxidant properties proven by *in vivo* and *in vitro* studies. Additionally, it has inhibitor effects on membrane lipid peroxidation (25). Although its' neuroprotective effects are reported in various studies, there is no data about whether apomorphine has hepatoprotective effects. Therefore, we decided to investigate apomorphine's hepatoprotective effects in an experimental rat model.

Decrease in SOD activity indicates oxidative stress (26). In Groups 1 and 5, SOD activities were similar as expected. In Group 2, paracetamol-induced hepatotoxicity was occured and SOD levels in this group was significantly lower than Groups 1 and 5. The SOD levels of apomorphine treatment groups (Groups 3 and 4) revealed that apomorphine provided advantage in SOD levels in paracetamol-induced hepatotoxicity. The same situation was seen regarding to MDA levels in all groups. So it was thought that apomorphine has antioxidant properties in paracetamol-induced hepatotoxicity.

The level of ALT activity reflects hepatocyte damage and is considered to be a highly sensitive biomarker of hepatotoxicity. Damaged hepatocytes release ALT and AST into the extracellularly located space and these enzymes consequently entered into circulation (27). In Group 2, ALT and AST levels were significantly enhanced as compared to Group 1, due to the paracetamol-induced hepatotoxicity. However, apomorphine significantly reduced of these enzymes in Groups 3 and 4 in comparison with Group 2. This was an evidence of decreased hepatocyte damage with apomorphine treatment.

Paracetamol overdose cause liver cell necrose and induce apoptosis (28). In our study, liver necrose was occured in Group 2 as expected. However, liver necrose scores were significantly lower when compared to Group 2 in both apomorphine administration groups. Additionally, 2 mg/kg apomorphine was found as significantly more protective on liver necrose than 1 mg/kg. Histopathological investigation results revealed that apomorphine, specially at higher dose had positive effects in paracetamol-induced hepatotoxicity.

Currently, *in vivo* and *in vitro* models of drug-induced hepatotoxicity are crucial for identification of novel drug targets and new pharmacolgically efficient drugs. The most extensively studied experimental models related to hepatotoxicity and acute liver failure are the paracetamol-based models. Pathophysiology in mice reflects very closely what is observed in humans for various factors such as reactive metabolite formation, oxidative stress and necrosis. In comparison with mice, rats had lower susceptibility to paracetamol-induced damaging effects and developed minor injuries in liver even at higher doses of 1 g/kg (24). Therefore, we administrated 2 g/kg paracetamol to induce hepatotoxicity. We used Sprague Dawley rats in this experimental study and this is a limitation for us.

In conclusion, it was found that apomorphine had hepatoprotective effects on paracetamol-induced hepatotoxicity. Its' hepatoprotective property was increasing especially when used at higher doses such as 2 mg/kg. There is a need for further experimental studies to detect useful and appropriate apomorphine dose in hepatotoxicity.

References

1. Jaeschke H, Williams CD, McGill MR, Farhood A. Herbal extracts as hepatoprotectants against acetaminophen hepatotoxicity. World J Gastroenterol 2010; 16(19): 2448-2450.

2. Galal RM, Zaki HF, Seif El-Nasr MM, Agha AM. Potential protective effect of honey against paracetamol-induced hepatotoxicity. Arch Iran Med 2012; 15(11): 674-680.

3. Bessems JG, Vermeulen NP. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Crit Rev Toxicol 2001; 31(1): 55-138.

4. Randle LE, Sathish JG, Kitteringham NR, Macdonald I, Williams DP, Park BK. Alpha(1)-adrenoceptor antagonists prevent paracetamol-induced hepatotoxicity in mice. Br J Pharmacol 2008; 153(4): 820-830.

5. Graham GG, Davies MJ, Day RO, Mohamudally A, Scott KF. The modern pharmacology of paracetamol; therapeutic actions, mechanism of actions,metabolism, toxicity and recent pharmacological findings. Inflammopharmacology 2013; 21(3): 201-232.

6. Chen C, Hennig GE, Manautou JE. Hepatobiliary excretion of acetaminophen glutathione conjugate and its derivatives in transport-

deficient (TR^-) hyperbilirubinemic rats. Drug Metab Dispos 2003; 31(6): 798-804.
7. Knight TR, Kurtz A, Bajt ML, Hinson JA, Jaeschke H. Vascular and hepatocellular peroxynitrite formation during acetaminophen toxicity: role of mitochondrial oxidant stress. Toxicol Sci 2001; 62(2): 212-220.
8. Jaeschke H, McGill MR, Williams CD, Ramachandran A. Current issues with acetaminophen hepatotoxicity-a clinically relevant model to test the efficacy of natural products. Life Sci 2011; 88(17-18): 737-745.
9. Ferret PJ, Hammoud R, Tulliez M, Tran A, Trebeden H, Jaffray P, Malassagne B, Calmus Y, Weill B, Batteux F. Detoxification of reactive oxygen species by a nonpeptidyl mimic of superoxide dismutase cures acetaminophen-induced acute liver failure in the mouse. Hepatology 2001; 33(5): 1173-1180.
10. Di Chiara G, Gessa GL. Pharmacology and neurochemistry of apomorphine. Adv Pharmacol Chemother 1978; 15: 87-160.
11. Gassen M, Glinka Y, Pinchasi B, Youdim MB. Apomorphine is a highly potent free radical scavenger in rat brain mitochondrial fraction. Eur J Pharmacol 1996; 308(2): 219-225.
12. Bianchi G, Landi M. Determination of apomorphine in rat plasma and brain by high-performance liquid chromatography with electrochemical detection. J Chromatogr 1985; 338(1): 230-235.
13. Taba P, Lees A, Stern G. Erich Harnack (1852–1915) and a short history of apomorphine. Eur Neurol 2013; 69(6): 321-324.
14. Khaliulin I, Borman JB, Chevion M, Schwalb H. Cardioprotective and antioxidant effects of apomorphine. Free Radic Res 2003; 37(7): 721-730.
15. Gancher ST, Woodward WR, Boucher B, Nutt JG. Peripheral pharmacokinetics of apomorphine in humans. Ann Neurol 1989; 26(2): 232-238.
16. Youdim MB, Gassen M, Gross A, Mandel S, Grünblatt E. Iron chelating, antioxidant and cytoprotective properties of dopamine receptor agonist; apomorphine. J Neural Transm Suppl 2000; 58: 83-96.
17. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34(3): 497-500.
18. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxide in animal tissues by thiobarbutiric acid reaction, Anal Biochem 1979; 95: 351-358.
19. Yaman H, Cakir E, Akgul EO, Aydin I, Onguru O, Cayci T, Kurt YG, Agilli M, Aydin FN, Gulec M, Altinel O, Isbilir S, Ersoz N, Yasar M, Turker T, Bilgi C, Erbil KM. Pentraxin 3 as a potential biomarker of acetaminophen-induced liver injury. Exp Toxicol Pathol 2013; 65(1-2): 147-151.
20. Yayla M, Halici Z, Unal B, Bayir Y, Akpinar E, Gocer F. Protective effect of Et-1 receptor antagonist bosentan on paracetamol induced acute liver toxicity in rats. Eur J Pharmacol 2014; 726: 87-95.
21. Glantzounis GK, Salacinski HJ, Yang W, Davidson BR, Seifalian AM. The contemporary role of antioxidant therapy in attenuating liver ischemia-reperfusion injury: a review. Liver Transpl 2005; 11(9): 1031-1047.
22. Halladin NL. Oxidative and inflammatory biomarkers of ischemia and reperfusion injuries. Dan Med J 2015; 62(4): B5054.
23. Mobasher MA, Valverde ÁM. Signalling pathways involved in paracetamol-induced hepatotoxicity: new insights on the role of protein tyrosine phosphatase 1B. Arch Physiol Biochem 2014; 120(2): 51-63.
24. Maes M, Vinken M, Jaeschke H. Experimental models of hepatotoxicity related to acute liver failure. Toxicol Appl Pharmacol 2016; 290: 86-97.
25. Picada JN, Roesler R, Henriques JAP. Genotoxic, neurotoxic and neuroprotective activities of apomorphine and its oxidized derivative 8-oxo-apomorphine. Braz J Med Biol Res 2005; 38(4): 477-486.
26. Czerska M, Mikołajewska K, Zieliński M, Gromadzińska J, Wąsowicz W. Today's oxidative stress markers. Medycyna Pracy 2015; 66(3): 393-405.
27. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. Toxicology 2008; 245(3): 194-205.
28. Manov I, Motanis H, Frumin I, Iancu TC. Hepatotoxicity of anti-inflammatory and analgesic drugs: ultrastructural aspects. Acta Pharmacol Sin 2006; 27(3): 259-272.