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In vivo effects of pentoxifylline on enzyme and non-enzyme antioxidant levels in rat liver after carrageenan-induced paw inflammation

Stefani Vircheva [1], Albena Alexandrova [1]*, Almira Georgieva [1], Polina Mateeva [1], Rositza Zamfirova [1], Marta Kubera [2] and Margarita Kirkova [1]

[1] *Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland*

The present study aimed to investigate the effects of pentoxifylline (PTX) on the carrageenan (CG)-induced paw oedema and on the endogenous levels of cell enzyme and non-enzyme antioxidants in rat liver, 4 and 24 h after CG injection. PTX (50 mg kg^{-1}, i.p.), administered 30 min before CG, decreased the paw oedema, 2–4 h after CG administration. The drug protected CG-induced decrease of glutathione (non-enzyme antioxidant) and had no effect on CG-unchanged activities of superoxide dismutase, glutathione peroxidase (enzyme antioxidants) and glucose-6-phosphate dehydrogenase (enzyme, important for the activity of GSH-conjugated antioxidant enzymes). The drug showed a good antioxidant capacity in chemical systems, generating reactive oxygen species. The present results suggest that the antioxidant activity of PTX might contribute to its beneficial effects in liver injuries. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS — antioxidant enzyme activities; carrageenan; glutathione level; pentoxyfilline

ABBREVIATIONS — CG, carrageenan; PTX, pentoxifylline; ROS, reactive oxygen species; OS, oxidative stress; GSH, glutathione; GPx, glutathione peroxidase; SOD, superoxide dismutase; Glu-6-P-DH, glucose-6-phosphate dehydrogenase; TFNα, tumour necrosis factor-alpha; i.p., intraperitoneal; TBAR, thiobarbituric acid reactive substance;

INTRODUCTION

Pentoxifylline (PTX), a phosphodiesterase inhibitor, is a methylxanthine compound that inhibits the tumour necrosis factor-alpha (TNFα) production.[1–3] The enhanced production of TNFα (one of the primary events in many types of liver injury) starts a formation of pro-inflammatory cytokines, which destroy hepatocytes and induce fibrogenesis.[4,5] The possible hepatoprotective role of PTX in several liver diseases, characterized with increased levels of TNFα[6,7] is widely studied. Beneficial effects of PTX have been found in the treatment of nonalcoholic steatohepatitis,[6,8–10] several alcoholic hepatitis and hepatorenal syndrome,[11,12] hepatic ischemic damage,[3,13] experimental hepatopulmonary syndrome,[14] etc.

PTX is gaining acceptance for conservative treatment of Peyronie's disease and neuropathic injuries. The drug shows an antihyperalgesic effect on experimental inflammatory pain[15] and possesses a therapeutic potential as anti-inflammatory agent.[16,17] The treatment with combined PTX-Vit.E plus clodronate has a significant effect on necrosis, by completely reversing severe progressive osteoradionecrosis (a late terminal sequel of irradiation that does not resolve spontaneously) and the associated radiation-induced fibrosis.[18] Administered in conjunction with vitamin E (a strong antioxidant), PTX reduces the extent of fibrotic lesions, induced by radiation therapy for breast cancer.[19]

There are many data that the oxidative stress (OS) is a key catalyst in development of liver diseases and that different substances, incl. drugs are able to reduce it. A few data concerning the antioxidant capacity of PTX were found in the literature. The findings in the both experimental models — D-galactosamine-induced hepatotoxicity[20] and carbon tetrachloride-induced liver toxicity[21] suggest that the hepatoprotective role of PTX might be determined to its antioxidant properties.

A model, often used in the experimental practice, is the acute peripheral inflammation, induced by carrageenan (CG). This inflammatory model is associated with a production of reactive oxygen species (ROS). Using this model, PTX has been studied in a view of its potential anti-

* Correspondence to: A. Alexandrova, Institute of Neurobiology, Bulgarian Academy of Sciences, 23, Acad. G. Bonchev St, 1113 Sofia, Bulgaria.
E-mail: a_alexandrova_bas@yahoo,com

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inflammatory activity[16] and its use as a cytokine modulating anti-inflammatory agent.[22] The drug is found to inhibit the mechanical hypernociception in rats induced by the intraplantar CG-injection, suggesting that the antinociceptive activity of PTX is associated with the inhibition of the release of both TNFα and IL-1β.[15]

CG-model is appropriate for testing the anti-inflammatory action and the antioxidant efficacy of different substances, including drugs.[23–28] However, we fail to find data regarding PTX antioxidant activity in this inflammatory model. The present study aimed to investigate the effects of PTX on both CG-induced paw oedema and levels of antioxidants (enzyme and non-enzyme) in rat liver, using the CG-model of acute peripheral inflammation. The antioxidant capacity of PTX in chemical systems, generating ROS was also tested.

MATERIALS AND METHODS

Materials

The chemicals (γ-CG, PTX, NADPH, glutathione (GSH), riboflavin, potassium phosphates, etc.) used in the present study were purchased from Sigma–Aldrich (Germany). Their solutions were freshly prepared with over-glass redistilled water.

Male 'Wistar' rats (130–150 g) were used in the present experiments. The animals were housed in a room with controlled temperature ($22 \pm 1\,^{\circ}C$), humidity ($60 \pm 10\%$) and light (12 h per day) for a week before being used. Food and water were available ad libitum.

All experiments were performed according to the 'Rules for care and experiments on laboratory animals' of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences.

Experimental design

The acute inflammation was induced by intraplantar injection (i.pl.) of 0.1 ml CG (1% w/v) into the right hind paw. PTX ($50\,mg\,kg^{-1}$) was administered 30 min before the induction of CG-inflammation. The following experimental groups were created: controls (injected with saline), CG-treated, PTX-treated and PTX/CG-treated. The paw volume was measured plethysmographically immediately before the injection of CG or saline (0-time), 2, 4 and 24 h after CG injection and just before euthanasia. For the biochemical experiments, the animals were sacrificed under light ether anaesthesia 4 and 24 h after CG or PTX administration.

Preparations

Liver and spleen were carefully removed and washed with cold 0.15 M KCl. Liver, perfused with cooled 0.15 M KCl and spleen were saved at low temperature ($-70\,^{\circ}C$) before the experiments. Both tissues were homogenized in cold 0.15 M KCl by a Potter-Elvehjem glass homogenizer with Teflon pestle. The biochemical parameters were measured in the supernatants obtained after centrifugation of tissue homogenates at 9000*g* for 20 min.

Analytical procedures

Protein content was measured by the method of Lowry *et al*.[29]

Total GSH level, measured according to Tietze[30] was expressed in $ng\,mg^{-1}$ protein, using GSSG as a reference standard.

Glutathione peroxidase (GPx) activity measured by the method of Gunzler *et al*.[31] was expressed in nmoles NADPH oxidized per min per mg protein, using a molar extinction coefficient of $6.22 \times 10^6\,M^{-1}\,cm^{-1}$.

Glucose-6-phosphate dehydrogenase (Glu-6-P-DH) activity determined after Cartier *et al*.[32] was expressed in nmoles $NADP^+$ reduced per min per mg protein, using a molar extinction coefficient of $6.22 \times 10^6\,M^{-1}\,cm^{-1}$.

Superoxide dismutase (SOD) activity determined according to Beauchamp and Fridovich[33] was expressed in $U\,mg^{-1}$ protein (one unit of SOD activity is the amount of the enzyme, producing 50% inhibition of nitro-blue tetrazolium-reduction).

Hydroxyl radicals (·OH) were generated in Fenton system (20 mM potassium phosphate uffer, pH 7.2; 0.1 mM $FeCl_3$; 0.1 mM EDTA; 0.1 mM ascorbic acid; 0.5 mM H_2O_2 and 3.4 mM deoxyribose). The degradation of deoxyribose (a detector of ·OH radicals) was measured in terms of the formation of thiobarbituric acid reactive substances (TBARs).[34] After 30-min incubation at $37\,^{\circ}C$, in the presence of increasing PTX concentrations, the absorbance at 532 nm was read against blank sample (without drug); A_{600}, a non-specific base-line drift, was subtracted from A_{532}.

Superoxide anion radicals (O_2^-) were generated photochemically in medium containing: 50 mM potassium phosphate buffer, pH 7.8; 1.17×10^{-6} M riboflavin; 0.2 mM methionine; 2×10^{-5} M KCN and 5.6×10^{-5} M nitro-blue tetrazolium (NBT). The O_2^--produced NBT reduction was measured in the presence of increasing PTX concentrations.[33]

Statistical analysis

The data were statistically analysed by one-way ANOVA (Dunnett post-test), $p < 0.05$ being accepted as the minimum level of statistical significance of the established differences.

RESULTS AND DISCUSSION

The intraplantar CG-injection in rat hind paw leads to oedema and produces an acute peripheral inflammation. The inflammation is accompanied with an increased formation of ROS, such as superoxide (O_2^-), hydroxyl (·OH) and peroxynitrite ($NOOO^-$) radicals.[35–37] O_2^- participates in the prostaglandin phase swelling (2–4 h) of CG-paw oedema[35] and, together with $NOOO^-$ and ·OH is of significance for the acute inflammation and inflammatory pain.[28,37,38]

According to Abdel-Salam *et al*.,[16] the intraperitoneal (i.p.) administration of PTX (36 and $72\,mg\,kg^{-1}$), 30 min

prior to CG, reduces the paw oedema response with 18.9 and 40.1%, respectively, at 2 h post-CG. The authors observe a more pronounced anti-oedema effect with higher doses of PTX (144–300 mg kg^{-1}, i.p.). The present findings were similar: (1) the intra-plantar CG-injection led to a time-dependent increase in the paw volume of the right hind paw, the maximum being at 2–4 h and (2) PTX (50 mg kg^{-1} i.p.), administered 30 min before CG decreased paw oedema (Fig. 1). Therefore, PTX (in appropriate high doses) would be useful as an anti-inflammatory agent.

Using CG-model of inflammation, we studied PTX effects on the levels of enzyme and non-enzyme antioxidants in rat liver. The results, presented on Table 1, showed that 4 and 24 h after CG administration, the activity of enzyme antioxidants (SOD and GPx) and the activity of Glu-6-P-DH (enzyme, important for the activity of GSH-conjugated antioxidant enzymes) were unchanged; PTX, alone had also no effect. By this reason, the finding that PTX/CG combination did not change the enzyme activities was expectable. In other experimental models—D-galactosamine-induced hepatotoxicity[20] and carbon tetrachloride-induced liver toxicity[21] however, a reduction of the enzyme antioxidants SOD and GPx is established; it is suggested that the pretreatment of animals with PTX (50 or 100 mg kg^{-1} for 7–21 days) might prevent the oxidative damage by increasing antioxidant enzyme levels.

The present study showed that, CG (4 and 24 h after its administration) led to a decreased level of non-enzyme antioxidant GSH in rat liver and that the pretreatment with PTX had a protective effect (Fig. 2). GSH is in excess in the cells and presents almost exclusively in its reduced form (GSH). Since GSH plays a key role in defending against

Table 1. Effects of PTX on antioxidant enzyme activities in rat liver and spleen after CG-induced paw inflammation: PTX (50 mg kg^{-1}, i.p.) was administered 30 min before the induction of CG-inflammation. Antioxidant enzyme activities were measured in 4 and 24 h. Values represent the mean ± SEM of five animals in each experimental group

	Enzyme activities		
	SOD	GPx	Glu-6-P-DH
Experimental groups	Rat liver		
I. Control animals			
Controls	69.1 ± 6.89	238 ± 19.1	31.1 ± 2.86
Pentoxifylline (50 mg kg^{-1})			
4 h after PTX	71.1 ± 6.60	238 ± 8.9	37.6 ± 2.37
24 h after PTX	62.4 ± 4.80	218 ± 16.9	37.3 ± 4.48
II. Carrageenan (CG)—treated animals			
4 h after CG	63.3 ± 2.69	209 ± 14.3	34.4 ± 6.16
24 h after CG	70.2 ± 6.87	197 ± 12.2	27.0 ± 1.14
Pentoxifylline (50 mg kg^{-1})			
4 h after CG	73.2 ± 4.05	197 ± 11.9	33.5 ± 2.17
24 h after CG	71.8 ± 7.13	200 ± 11.3	33.9 ± 2.53
	RAT SPLEEN		
I. Control animals			
Controls	9.6 ± 0.33	99 ± 5.8	26.3 ± 4.18
Pentoxifylline (50 mg kg^{-1})			
4 h after PTX	10.9 ± 0.49	90 ± 4.4	28.4 ± 1.75
24 h after PTX	10.8 ± 0.43	105 ± 6.6	31.1 ± 2.84
II. Carrageenan (CG)—treated animals			
4 h after CG	11.1 ± 0.60	86 ± 3.1	28.1 ± 4.25
24 h after CG	10.8 ± 0.51	84 ± 4.1	25.8 ± 3.49
Pentoxifylline (50 mg kg^{-1})			
4 h after CG	10.9 ± 0.56	93 ± 7.0	26.3 ± 1.54
24 h after CG	11.3 ± 0.80	98 ± 5.8	32.3 ± 1.06

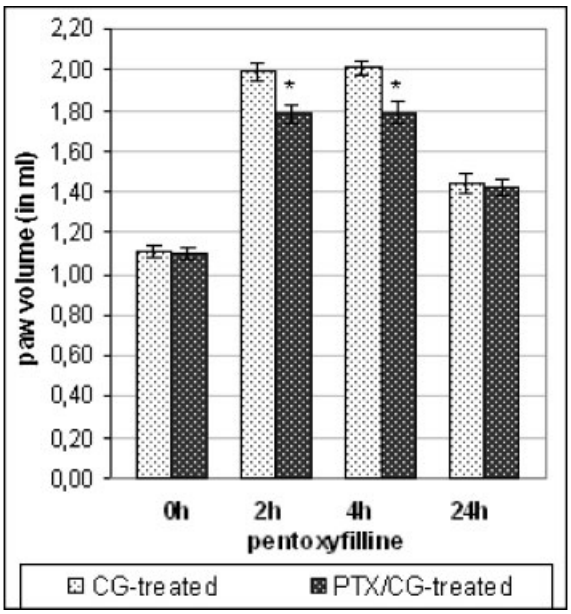

Figure 1. Effects of PTX on CG-induced acute inflammation: the edema volume was measured in 2, 4 and 24 h after CG administration. Values represent the mean ± SEM of 5–10 animals for each experimental group. Statistically significant differences versus controls at: $^{*}p < 0.05$

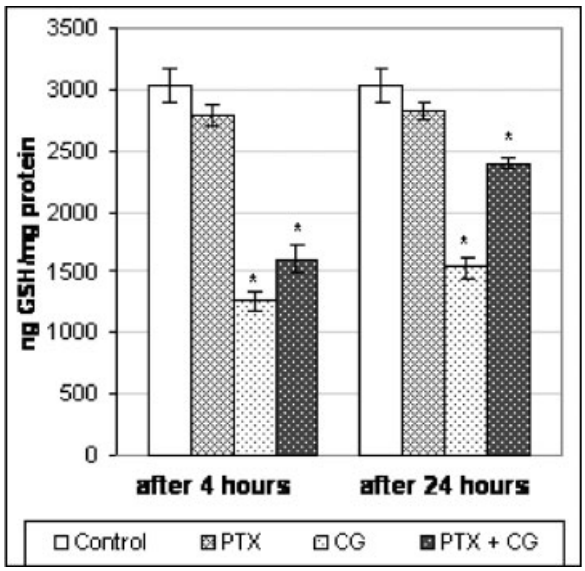

Figure 2. Effects of PTX on total GSH level in rat liver after CG-induced paw inflammation: PTX (50 mg kg^{-1}, i.p.) was administered 30 min before the induction of CG-inflammation. Total GSH level was measured in 4 and 24 h. Values represent the mean ± SEM of five animals in each experimental group. Statistically significant differences versus controls at $^{*}p < 0.05$

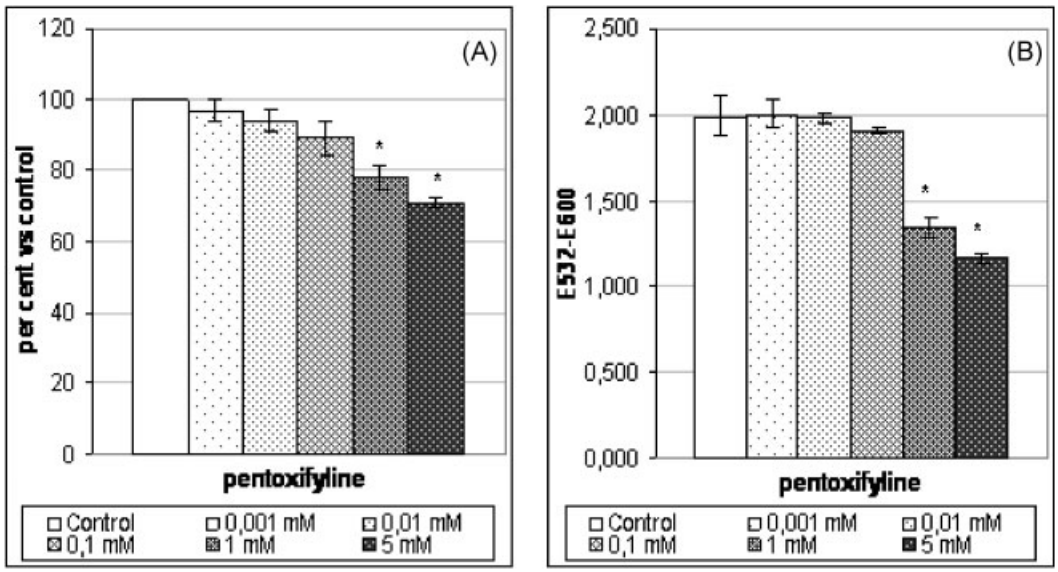

Figure 3. Effects of PTX in O_2^--generating (A) and $^{\cdot}OH$-generating (B) systems: values represent the mean ± SEM of nine independent experiments. Statistically significant differences versus controls at $^*p<0.05$

oxidative damage, the PTX protection on liver GSH level might be due to an antioxidant capacity of this drug. Acting as antioxidant, PTX, having no effects on the levels of the studied antioxidants, would have a protective effect only in the case of OS-induced changes in endogenous levels of the cell antioxidants. Thus, PTX protected CG-decreased GSH level, but not CG-unchanged enzyme activities, incl. GPx activity the cofactor of which is GSH.

Using CG-inflammatory model, we carried out additional experiments regarding the effects of PTX on spleen antioxidant status, but the levels of enzyme (Table 1) and non-enzyme antioxidants (data not shown) in CG-, PTX- and PTX/CG groups were unchanged compared to the controls.

In order to evaluate PTX efficacy as antioxidant, we studied its effects in simple chemical systems generating $^{\cdot}OH$ or O_2^- radicals where increasing drug concentrations (1×10^{-6}–5×10^{-3} M) were used. In PTX concentrations higher than 10^{-5} M, the O_2^--provoked NBT-reduction was decreased by about 30% (Fig. 3A) and the $^{\cdot}OH$-provoked DR degradation by about 40% (Fig. 3B). These results showed that PTX possesses a good antioxidant capacity.

In general, the present study shows that PTX decreases CG-induced paw oedema, showing anti-inflammatory action. The drug protects against CG-induced decrease of liver GSH level and has a good antioxidant activity in chemical systems, generating ROS (O_2^- and $^{\cdot}OH$). In conclusion, the present in vivo results suggest that the PTX antioxidant activity might contribute to its beneficial effects in liver injuries, especially inflammation-provoked.

CONFLICT OF INTEREST

None known.

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REFERENCES

1. Neuner P, Klosner G, Schauer E, *et al*. Pentoxifylline in vivo down-regulates the release of IL-1 beta, IL-6, IL-8 and tumour necrosis factor-alpha by human peripheral blood mononuclear cells. *Immunology* 1994; **83**: 262–267.
2. Chen YL, Le Vraux V, Giroud JP, Chauvelot-Moachon L. Anti-tumor necrosis factor properties of non-peptide drugs in acute-phase responses. *Eur J Pharmacol* 1994; **271**(2–3): 319–327.
3. El-Ghoneimi A, Cursio R, Schmid-Alliana A, *et al*. Pentoxifylline inhibits liver expression of tumor necrosis factor alpha mRNA following normothermic ischemia-reperfusion. *HPB (Oxford)* 2007; **9**(2): 112–119.
4. Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. *N Engl Med* 2000; **343**: 1467–1476.
5. Gutierrez-Reyes G, Lopez-Ortal P, Sixton S, *et al*. Effect of pentoxifylline on levels of pro-inflammatory cytokines during chronic hepatitis C. *Scand J Immunol* 2006; **63**(6): 461–467.
6. Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2001; **48**(2): 206–211.
7. Warne JP. Tumour necrosis factor alpha: a key regulator of adipose tissue mass. *J Endocrinol* 2003; **177**(3): 351–355.
8. Adams LA, Zein CO, Angulo P, Lindor AG. A pilot trial of pentoxifylline in nonalcoholic steatohepatitis. *Am J Gastroenterol* 2004; **99**(12): 2365–2368.
9. Satapathy SK, Sakhuja P, Malhotra V, Sharma BC, Sarin SK. Beneficial effects of pentoxifylline on hepatic steatosis, fibrosis and necroinflammation in patients with non-alcoholic steatohepatitis. *J Gastroenterol Hepatol* 2007; **22**(5): 634–638.

10. Mihaila RG, Nedelcu L, Fratila O, Rezi EC, Domnariu C, Deac M. Effects of lovastatin and pentoxyphyllin in nonalcoholic steatohepatitis. *Hepatogastroenterology* 2009; **56**(93): 1117–1121.
11. De BK, Gangopadhyay S, Dutta D, Baksi SD, Pani A, Ghosh P. Pentoxifylline versus prednisolone for severe alcoholic hepatitis: a randomized controlled trial. *World J Gastroenterol* 2009; **15**(13): 1613–1619.
12. Assimakopoulos SF, Thomopoulos KC, Labropoulou-Karatza C. Pentoxifylline: a first line treatment option for severe alcoholic hepatitis and hepatorenal syndrome? *World J Gastroenterol* 2009; **15**(25): 3194–3195.
13. Kaptanoglu L, Kapan M, Kapan S, Goksoy E. Oktar H.Effects of nimodipine and pentoxyfylline in prevention of hepatic ischemic damage in rats at normal and hypothermic conditions. *Eur J Pharmacol* 2008; **587**(1–3): 253–256.
14. Zhang J, Ling Y, Tang L, Luo B, Chacko BK, Patel RP. Fallon MB.Pentoxifylline attenuation of experimental hepatopulmonary syndrome. *J Appl Physiol* 2007; **102**(3): 949–955.
15. Vale ML, Benevides VM, Sachs D, *et al*. Antihyperalgesic effect of pentoxifylline on experimental inflammatory pain. *Br J Pharmacol* 2004; **143**(7): 833–844.
16. Abdel-Salam OM, Baiuomy AR, El-Shenawy SM, Arbid MS. The anti-inflammatory effects of the phosphodiesterase inhibitor pentoxifylline in the rat. *Pharmacol Res* 2003; **47**(4): 331–340.
17. Groesdonk HV, Heringlake M, Heinze H. Anti-inflammatory effects of pentoxifylline: importance in cardiac surgery. *Anaesthesist* 2009; **58**(11): 1136–1143 [Article in German].
18. Delanian S, Lefaix JL. Complete healing of severe osteoradionecrosis with treatment combining pentoxifylline, tocopherol and clodronate. *Br J Radiol* 2002; **75**(893): 467–469.
19. Delanian S, Porcher R, Rudant J, *et al*. Kinetics of response to long-term treatment combining pentoxifylline and tocopherol in patients with superficial in patients with superficial radiation-induced fibrosis. *J Clin Oncol* 2005; **23**(34): 8570–8579.
20. Taye A, El-Moselhy MA, Hassan MK, Ibrahim HM, Mohammed AF. Hepatoprotective effect of pentoxifylline against D-galactosamine-induced hepatotoxicity in rats. *Ann Hepatol* 2009; **8**(4): 364–370.
21. Delanian S, Noyan T, Kömüroğlu U, Bayram I. Sekeroğlu MRComparison of the effects of melatonin and pentoxifylline on carbon tetrachloride-induced liver toxicity in mice. *Cell Biol Toxicol* 2006; **22**(6): 381–391.
22. Vajja BN, Juluri S, Kumari M, Kole L, Chakrabarti R, Joshi VD. Lipopolysaccharide-induced paw oedema model for detection of cytokine modulating anti-inflammatory agents. *Int Immunopharmacol* 2004; **4**(7): 901–909.
23. Parola M, Curzio M, Negro F, Paradisi L, Torrielli MV. Lipid peroxidation in the rat liver after acute inflammation induced by CG.I. Influence of non-steroidal anti-inflammatory agents. *Boll Soc Ital Biol Sper* 1981; **57**, 638–644 (in Italian).
24. Bilici D, Akpinar E, Kiziltunc A. Protective effect of melatonin in CG-induced acute local inflammation. *Pharmacol Res* 2002; **46**, 133–139.
25. Cuzzocrea S, Mazzon E, Sautebin L, *et al*. Protective effects of Celecoxib on lung injury and red blood cells modification induced by CG in the rat. *Biochem Pharmacol* 2002; **63**: 785–795.
26. Leduc C, Gentili ME, Estebe JP, Le Corre P, Moulinoux JP, Ecoffey C. The effect of local anesthetics and amitriptyline on peroxidation in vivo in an inflammatory rat model: preliminary reports. *Anesth Analg* 2002; **95**: 992–996.
27. Wu Y, Zhou C, Li X, *et al*. Evaluation of antiinflammatory activity of the total flavonoids of Laggera pterodonta on acute and chronic inflammation models. *Phytother Res* 2006; **20**: 585–590.
28. Halici Z, Dengiz GO, Odabasoglu F, Suleyman H, Cadirci E, Halici M. Amiodarone has antiinflammatory and anti-oxidative properties: an experimental study in rats with CG induced paw oedema. *Eur J Pharmacol* 2007; **566**: 215–221.
29. Lowry OH, Rosenbrough NJ, Farr AL, Randal RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265–278.
30. Tietze F. Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal Biochem* 1969; **27**: 502–522.
31. Gunzler WA, Vergin H, Muller I, Flohe L. Glutathion peroxidase. VI. Die reaction der glutathion peroxidase mit Verschieden hydroperoxiden. *Hoppe-Seyler's Z Physiol Chem* 1972; **353**: 1001–1004.
32. Cartier P, Leroux JP, Marchand JCl. Techniques de dosage des enzymes glycocytiques tissulaires. *Ann Biol Clin* 1967; **25**: 109–136.
33. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and assay applicable to acrylamide gels. *Anal Biochem* 1971; **44**: 276–287.
34. Halliwell B, Grootveld M, Gutteridge J. Methods for the measurement of hydroxyl radicals in biochemical systems: deoxyribose and aromatic hydroxylation. *Methods Biochem Anal* 1988; **33**: 59–90.
35. Oyanagui Y. Inflammation and superoxide production by macrophages. *Agents Action Suppl* 1980; **7**: 174–179.
36. Salvemini D, Mazzon E, Dugo L, *et al*. Pharmacological manipulation of the inflammatory cascade by the superoxide dismutase mimetic, M40403. *Br J Pharmacol* 2001; **132**: 815–827.
37. Khattab MM. TEMPOL, a membrane-permeable radical scavenger, attenuates peroxynitrite- and superoxide anion-enhanced CG-induced paw oedema and hyperalgesia: a key role for superoxide anion. *Eur J Pharmacol* 2006; **548**: 167–173.
38. McCord JM, Roy RS. The pathophysiology of superoxide: roles in inflammation and ischemia. *Can J Physiol Pharmacol* 1982; **60**: 1346–1352.

