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Hepatoprotective Effects of Pentoxifylline against Acrolein-Induced Mitochondrial Dysfunction in Rat Liver

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A B S T R A C T

Acrolein (ACR) is α, β unsaturated aldehyde that exists extensively in the environment and (thermally processed) foods. It can also be generated through endogenous metabolism. The aim of this study was to investigate the possible protective role of Pentoxifylline (PTX) as a non-selective phosphodiesterase (PDE) inhibitor on toxicity of ACR. In this study, oxidative damages were measured by markers liver mitochondrial, such as, glutathione peroxidase (GPx), superoxide dismutase (SOD), lipid peroxidation (LPO) and total glutathione (GSH) in rats. Effective doses of ACR (2mg/kg/day) and PTX (50mg/kg/day) and vitamin E (15mg/kg/day) were administered alone or in combination for 14 days by intraperitoneal injection. At the end of the experiment, the liver mitochondria of the animals were separated. PTX ameliorated LPO, SOD and GPx in liver mitochondria of ACR-induced changes. Co-administration of PTX with ACR improved LPO in liver mitochondria. In conclusion, intracellular cAMP-elevating agents like PTX, may be considered beneficial for the protection or recovery of ACR-induced toxic damage in liver mitochondria.

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Introduction

Acrolein (ACR), a highly reactive α,β unsaturated aldehyde, is an environmental and industrial pollutant and toxin present in automobile exhaust, wood smoke, and overheated fat-containing foods. ACR also exists naturally in vegetables, fruits, and herbs [1] and is produced during the processing of fat-containing foods and meats. Also, ACR is also produced endogenously by normal cellular metabolism. ACR can be formed in various tissues via lipid peroxidation (LPO), metabolism of α-hydroxyamino acids, polyamines oxidation and via metabolism of drugs, such as the anticancer drug cyclophosphamide [4-7]. In addition, ACR is also a product of lipid peroxidation [7-9] , therefore continuously generated in biological systems under oxidative stress. Pentoxifylline (PTX), a methylxanthine derivative and nonspecific type 5 phosphodiesterase (PDE) inhibitor, is a drug widely used in the management of peripheral arterial disease. PTX can enhance the chemotactic response of neutrophils but inhibits phagocytosis and production of superoxide by neutrophils and monocytes [10] Recent studies have shown additional therapeutic potential for PTX as antioxidant, anti-inflammatory, and immunomodulator [11, 12]. Because PTX has antioxidative properties, there is a possibility that PTX would protect against the toxicity of ACR, an elevated level of PTX in body may act as a prophylactic against hepatic damage. Thus, the aim of this study was to determine if PTX have hepatoprotective effects. This property of PTX could very well play a critical role in the induction of hepatic dysfunctions mediated by oxidative damage to livermitochondria.

Materials and Methods

Reagents and Chemicals

Tetraethoxypropane (MDA), 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), n-butanol, sucrose , ethylenediamine tetraacetic acid (EDTA), comassie blue, bovine serum album in (BSA), 4,5(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), GPx and SOD (Ransel kit, Randox Laboratories Ltd, Crumlin, UK),bioxytech GSH kit (Oxis Research, USA), were used in this study. All other chemicals were obtained from the Sigma Company.

Animals and treatments

Adult male Wistar rats weighing 180–250 g maintained on a 12-hour light/dark cycle with free access to tap water and standard laboratory chow were used. Animals were randomly divided into six groups of five animals and treated for 2 weeks intraperitoneally (IP**).** The groups were as follows: control group, ACR group, PTX group, PTX & ACR group, alpha-tocopherol acetate (AT) group, and ACR & AT group.

ACR was administered (2mg/kg/day, IP) alone or in combination with PTX (50mg/kg /day, IP), and AT as (15 mg/kg/day). One group of animals received only normal saline and was assigned as control. At the end of the treatment, 24 hours past the end of treatment, animals were killed, liver tissue was separated and stored in liquid nitrogen, and then their mitochondria were isolated quickly and kept frozen at –80 C.

Experimental Protocols

Preparation of liver mitochondria

The liver was removed and minced with small scissors in a cold manitol solution containing 0.225 M D-manitol, 75 mM sucrose, and 0.2 mM ethylenediaminetetraacetic acid (EDTA). The minced liver (30 g) was gently homogenized in a glass homogenizer with a Teflon pestle and then centrifuged at 700 × *g* for 10 min at 4◦C .The supernatants were centrifuged at 7,000 × *g* for 20 min. These second supernatants were pooled as the crude microsomal fraction and the pale loose upper layer, which was rich in swollen or broken mitochondria, lysosomes, and some microsomes, of sediments were washed away.

The dark packed lower layer (heavy mitochondrial fraction) was resuspended in the manitol solution and recentrifuged twice at 7,000×*g* for 20 min. The heavy mitochondrial sediments were suspended in Tris solution containing 0.05 M Tris-HCl buffer (pH 7.4), 0.25 M sucrose, 20 mM KCl, 2.0 mM $MgCl^2$, and 1.0 mM Na2HPO4 at 4◦C before assay [13, 14].

Measurement of Cu/Zn- SOD activity

The activity of Cu/Zn- SOD was measured using a commercial kit (Ransod kit, Randox Laboratories Ltd, Crumlin, UK). Measurement of the enzyme was

based on the generation of superoxide radicals produced by xanthine and xanthine oxidase and reacted with 2-(4-iodophenyl)-3-(4-nitrofenol) 5-phenyltetrazolium chloride (INT) to form a red formazan dye. The formazan was read at 505 nm. One unit of Cu/Zn- SOD was defined as the amount of enzyme necessary to produce 50% inhibition in the INT reduction rate.

Measurement of GPx activity

The amount of GPx was determined using a commercially available kit (Ransel kit, Randox Laboratories Ltd, Crumlin, UK) by measuring the rate of oxidation of NADPH at 340 nm. A unit of enzyme was expressed as the amount of enzyme needed to oxidize 1 nmol of NADPH oxidase/minute.

Measurement of total GSH

Level of total glutathione (GSH) was measured using colorimetric assay kit. The kit uses 5, 50-dithiobis-2-nitrobenzoic acid (DTNB) and glutathione reductase. The procedure was carried out according to manufacturer's instruction and the levels were quantitated as micromolar GSH based on standard supplied along with the kit.

Measurement of the lipid peroxidation (LPO)

The lipid peroxidation (LPO) product in tissues was determined by TBA reagent, expressed as the extent of malondialdehyde (MDA) productions during an acid heating reaction. Briefly, the diluted samples by 1.5 ml TCA (20% w/v) were added to 250 µl of these samples and centrifuged in 3000 g for 10 min. Then, the precipitation was dissolved in sulfuric acid and 1.5 ml of the mixture was added to 1.5 ml of TBA (0.2% w/v). The mixture was then incubated for 1 h in a boiling water bath. Following incubation, 2 m l of n-butanol was added, the solution centrifuged, cooled and the absorption of the supernatant was recorded in 532 nm. The calibration curve of tetraethoxypropane standard solutions was used to determine the concentrations of TBA+MDA adducts in samples [15].

Total Protein

The protein content was quantified by the method of Bradford. Concentrated Coomassie blue (G250) was diluted in 250 µl distilled water, and then 750 µl of this diluted dye was added to 50 µl of sample. The mixture was incubated at room temperature for 10 min and an absorbance measurement was taken at 595 nm by a spectrophotometer. A standard curve was constructed by using bovine serum albumin ranging between 0.25 and 1 mg/ml [16].

Measurement of mitochondrial viability

This assay is a quantitative colorimetric method to determine cell viability. It utilizes the yellow tetrazolium salt (MTT), which is metabolized by mitochondrial dehydrogenase enzyme from viable cells to yield a purple formazan reaction product that was determined spectrophotometrically at wavelength of 570 nm. The percentage of mitochondrial viability of each test sample was calculated [17].

Statistical analysis

Mean and standard error values were determined for all the parameters and the results were expressed as Mean + SEM. All data were analyzed with SPSS Version 18 employing one-way ANOVA followed by Tukey post hoc test. Differences between groups was considered significant when $P < 0.05$.

Results

Lipid peroxidation (LPO)

In liver mitochondria: ACR caused a significant increase in LPO when compared to control (p =003). PTX caused a significant decrease in LPO when compared to ACR group (p =027). AT reduced LPO when compared to ACR (p =001). Coadministration of AT with ACR significantly reduced ACR induced LPO (p =035); Figure 1.

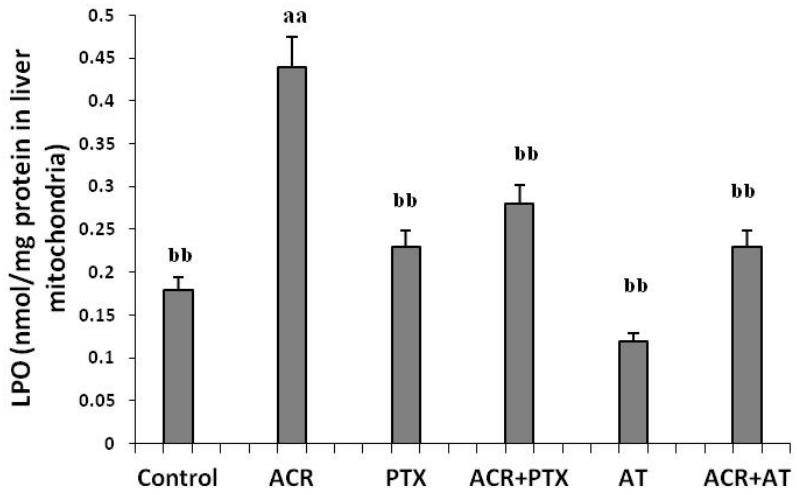

Fig. 1. Lipid peroxidation (LPO) in liver mitochondria of rats. [aa]Significantly different from control group at $p < .05$. [bb]Significantly different from ACR group at $p < .05$. ACR, acrolein; PTX, Pentoxifyllin; AT, (alpha-tocopherol; vitamin E).

Superoxide dismutase

In liver mitochondria: AT reduced SOD activity when compared to ACR (p =003). Coadministration of ACR with AT significantly reduced ACR induced SOD activity (p =008); Figure 2.

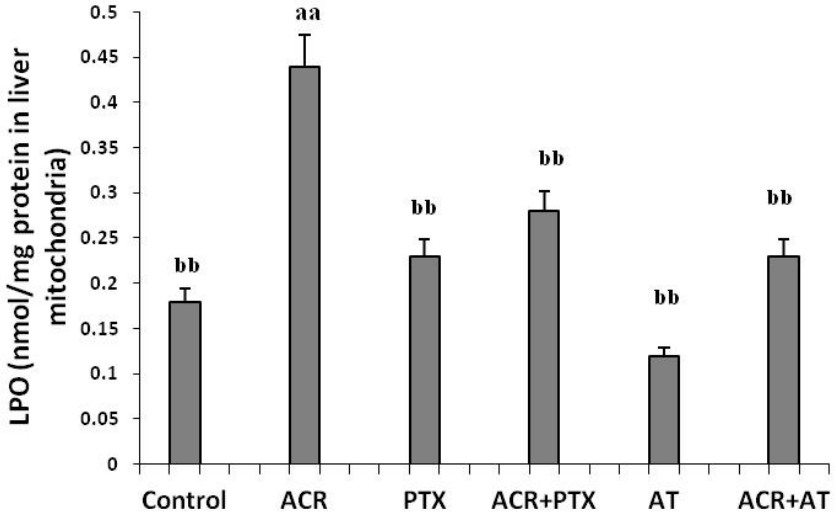

Fig. 2. Superoxide dismutase (SOD) activity in liver mitochondria of rats. [aa]Significantly different from control group at $p < .05$. [bb]Significantly different from ACR group at $p < .05$. ACR, acrolein; PTX, Pentoxifyllin; AT,(alpha-tocopherol; vitamin E).

Glutathione peroxidase

In liver mitochondria: AT reduced GPx activity when compared to ACR group (p =006); Figure 3.

Total glutathione

In liver mitochondria: Treatment with ACR decreased GSH as compared to control (p =085).PTX increased GSH as compared to ACR group (p =010). AT induced GSH when compared to ACR group (p =001); Figure 4.

Mitochondria viability

In liver mitochondria: Administration of ACR decreased viability of cells in comparison to PTX and AT group respectively (p =001); Figure 5.

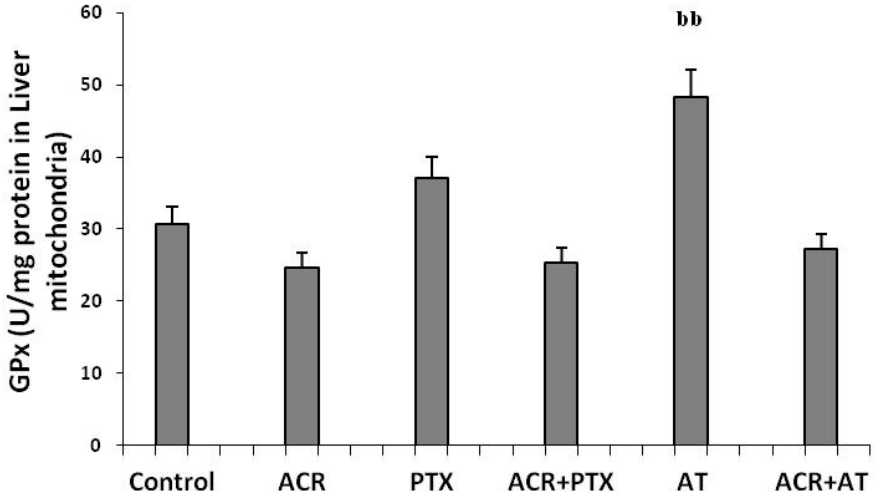

Fig. 3. Glutathione peroxidase (GPx) activity in liver mitochondria of rats. [aa]Significantly different from control group at p < .05. [bb]Significantly different from ACR group at p < .05. ACR, acrolein; PTX, Pentoxifyllin; AT,(alpha-tocopherol; vitamin E).

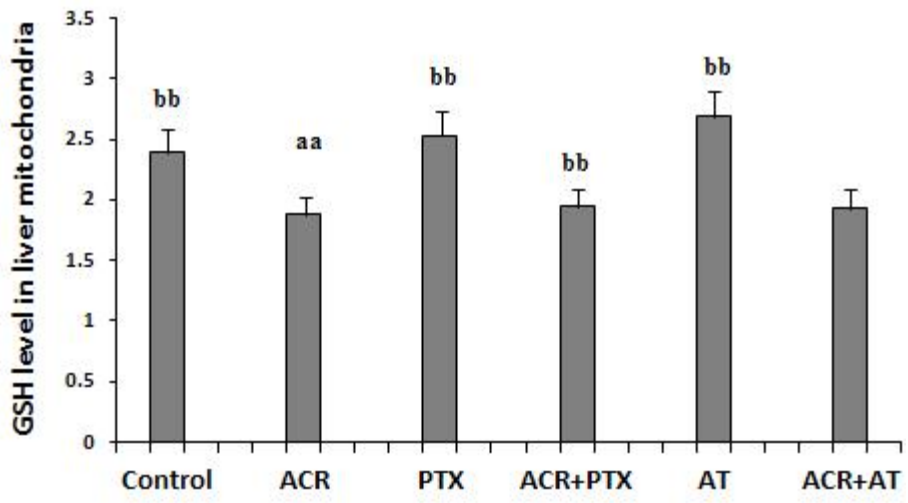

Fig. 4. Total glutathione (GSH) in liver mitochondria of rats. [aa]Significantly different from control group at p < .05. [bb]Significantly different from ACR group at p < .05. ACR, acrolein; PTX, Pentoxifyllin; AT,(alpha-tocopherol; vitamin E).

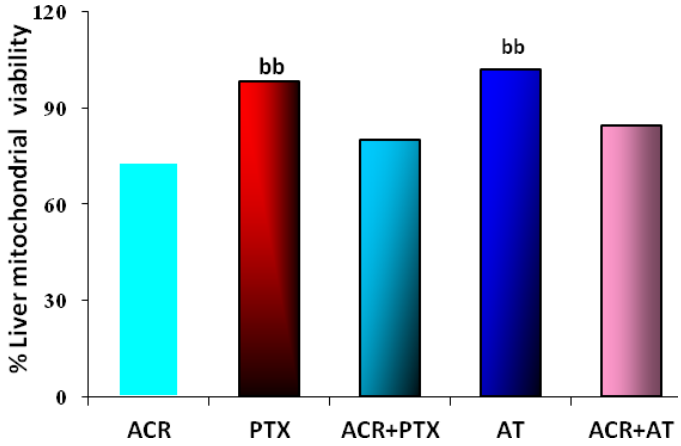

Fig. 5. Effects of PTX and ACR on mitochondrial viability (percent of control) in rat liver. [bb]Significantly different from ACR group at $p < .05$. ACR, acrolein; PTX, Pentoxifyllin; AT, (alpha-tocopherol; vitamin E).

Discussion

In fact, in the present study, our attentions were to address a novel link between the protective roles of PTX towards oxidative stress caused by ACR in liver mitochondria. Collectively, the main findings of the present study demonstrated an impairment of the enzymatic antioxidant defenses, mitochondrial toxicity and induction of oxidative stress by ACR that were restored by PTX as a potent selective PDE-5 inhibitor.

In the biological system, ACR exists naturally in foods and is formed during the combustion of organic materials. Thus, ACR is found in all types of smoke (including cigarette smoke) in the exhaust from internal combustion engines and in the vapors of over heated cooking oil where severe human toxic exposures have occurred [18]. In addition, both in vitro and in vivo studies showed that ACR toxicity is mediated by increased oxidants and oxidative damage [19], suggesting that ACR, acts not only as a direct oxidant, but also as a generator of oxidants [20].

GSH is a critical endogenous antioxidant involved in cell defense [21, 22]. The conjugation of ACR to GSH is regarded as a major pathway for the detoxification of ACR [9]. ACR may lead to the depletion of cellular GSH in a concentration dependent manner, which may cause harmful effects to human beings. It is documented that once the GSH level has reached a certain threshold, protein thiol groups could be progressively modified while a series of molecular effects may emerge such as cell proliferation, apoptosis and changes in gene/protein expression [23, 24]. Our results showed that ACR reduced GSH concentration in liver mitochondria. But it was significantly improved with PTX in both samples. Toxicity of ACR on respiratory system, kidney and in cell cultures has been widely reported that ACR effects are due to GST catalyzed GSH depletion [25]. Once target cells are depleted of a threshold cytosolic GSH level, ACR can directly react with other nucleophilic groups including proteins and alter their function [26].

ACR has been identified as both a product and initiator of lipid peroxidation, therefore continuously generated in biological systems under oxidative stress [5, 6, 27]. LPO of the membranous system initiates and propagates endogenous toxicants that can readily react with adjacent molecules like membrane proteins or diffuse to more distant molecules like DNA rendering adduct formation. In the present study, ACR treatment caused significant increase in LPO in liver mitochondrial cell.

Both SOD and GPx are recognized scavengers of ROS [28]. Antioxidant enzymes work in a corresponding way to prevent the oxidative stress [29]. This result indicated increased activities of SOD and GPx in ACR group compared with control. PTX is a xanthine derivative which has inhibitory effects on xanthine oxidase [30]. Xanthine oxidase is considered as a candidate for oxygen free radical formation in cells [31]. PTX down regulates production of tumor

necrosis factor-alpha (TNF-a). This cytokine provokes a rise in hydrogen peroxide production from mitochondria [32].

Regarding the positive effects of PTX in the present study, there are supports demonstrating that intracellular cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are able to ameliorate cellular oxidative stress [33, 34]. There are additional evidences indicating the benefit of PTX in reduction of oxidative stress in diseases like diabetes [35] and colitis [36]. Results of the present study reveal that PTX normalizes oxidant/antioxidant balance in liver mitochondria and is able to recover ACR-induced changes. Mitochondrial dysfunction may be a key mechanism for ACR toxicity. Mitochondria are the main generation sites of oxidants and are the targets of oxidants because they are particularly sensitive to oxidative insults [37]. ACR is a cytotoxic and genotoxic environmental pollutant [9]. The mechanisms of the toxicity have been suggested to be linked to mitochondrial dysfunction and tested in mitochondria isolated from rat brain [38, 39] and heart [40]. In agreement, there are evidences that cyclic nucleotides have potential to prevent free radical-induced LPO in different cells and animal models [41]. In this study ACR induced a significant decrease in the mitochondrial function because of its toxicity is showed on decreasing viable mitochondria. The above-mentioned points let us to conclude that beneficial affects of PTX in our previous study [12] and present results from inhibition of ACR-induced oxidative injuries (Fig 6).

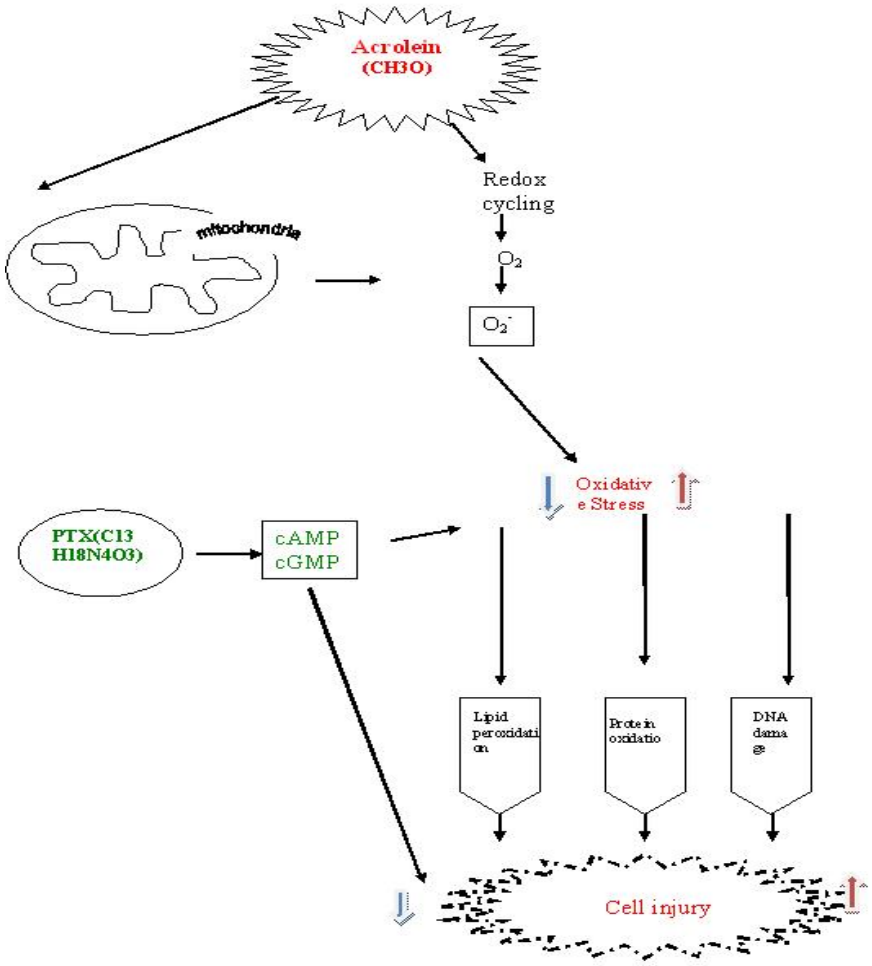

Fig. 6. Schematic showing oxidative-mediated ACR generated via free radicals and oxidative intermediates. PTX can be reduced oxidative injury.

In conclusion, our results demonstrated that PTX protected oxidative damage and mitochondrial dysfunction by ACR. The effects on reducing oxidative damage and improving mitochondrial function suggest that PTX is a powerful mitochondrial nutrient like AT. The dietary administration of antioxidant materials may be an effective strategy for reducing and/or preventing cigarette smoke-induced many disorders. PTX reduced oxidative biomarkers and mitochondrial toxicity. Therefore, we conclude that PTX, administered alone or combination have hepatoprotective effects on ACR intoxication, through different physiological pathways in liver.

Conclusions

These results demonstrated that PTX protected oxidative damage and mitochondrial dysfunction by ACR. The effects on reducing oxidative damage and improving mitochondrial function suggest that PTX is a powerful mitochondrial nutrient like AT. The dietary administration of antioxidant materials may be an effective strategy for reducing and/or preventing cigarette smoke-induced many disorders. PTX reduced oxidative biomarkers and mitochondrial toxicity. Therefore, we conclude that PTX, administered alone or combination have hepatoprotective effects on ACR intoxication, through different physiological pathways in liver.

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Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

References

[1] Feron V, Til H, De Vrijer F, Woutersen R, Cassee F, Van Bladeren P. Aldehydes: occurrence, carcinogenic potential, mechanism of action and risk assessment. Mutation Research/Genetic Toxicology. 1991;259:363–385.

[2] O'Brien PJ, Siraki AG, Shangari N. Aldehyde sources, metabolism, molecular toxicity mechanisms, and possible effects on human health. CRC Critical Reviews in Toxicology. 2005;35:609–662.

[3] Cannon J, Links CA, Cos LR. Cyclophospha- mide-associated carcinomaof urothelium: Modalities for prevention. Urology. 1991;38:413–416.

[4] Pan X, Kaneko H, Ushio H, Ohshima T. Oxidation of all cis 7, 10, 13, 16, 19 docosapentaenoic acid ethyl ester. Hydroperoxide distribution and volatile characterization. European journal of lipid science and technology. 2005;107:228–238.

[5] Uchida K, Kanematsu M, Morimitsu Y, Osawa T, Noguchi N, Niki E. Acrolein is a product of lipid peroxidation reaction. Journal of Biological Chemistry. 1998;273:16058–16066.

[6] Uchida K, Kanematsu M, Sakai K, Matsuda T, Hattori N, Mizuno Y, et al. Protein-bound acrolein: potential markers for oxidative stress. Proceedings of the National Academy of Sciences. 1998;95:4882–4887.

[7] Uchida K. Current status of acrolein as a lipid peroxidation product. Trends in cardiovascular medicine. 1999;9:109–113.

[8] Ghilarducci DP, Tjeerdema RS. Fate and effects of acrolein. Reviews of environmental contamination and toxicology. 1995;144:95–95.

[9] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free radical biology & medicine. 1991;11:81–81.

[10] Sener G, Akgun U, Satiroglu H, Topaloglu U, Keyer-Uysal M. The effect of pentoxifylline on intestinal ischemia/reperfusion injury. Fundam Clin Pharmacol. 2001;15:19–22.

[11] Noyan T, Önem Ö, Ramazan Şekeroğlu M, Köseoğlu B, Dülger H, Bayram İ, et al. Effects of erythropoietin and pentoxifylline on the oxidant and antioxidant systems in the experimental short bowel syndrome. Cell biochemistry and function. 2003;21:49–54.

[12] Ranjbar A, Ghahremani MH, Sharifzadeh M, Golestani A, Ghazi-Khansari M, Baeeri M, et al. Protection by pentoxifylline of malathion-induced toxic stress and mitochondrial damage in rat brain. Human & experimental toxicology. 2010;29:851–864.

[13] Ramasarma T. Generation of H2O2 in biomembranes. Biochimica et Biophysica Acta (BBA) 1982;694(1):69–93.

[14] Ghazi Khansari M, Mohammadi Bardbori A, Hossini MJ. Using Janus Green B to Study Paraquat Toxicity in Rat Liver Mitochondria. Annals of the New York Academy of Sciences. 2007;1090:98–107.

[15] Ernster L, Nordenbrand K. [92a] Microsomal lipid peroxidation. Methods in enzymology. 1967;10:574–580.

[16] Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing

the principle of protein-dye binding. Anal Biochem. 1976;72:248–254.
[17] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol methods. 1983;65:55–63.
[18] Beauchamp RO, Andjelkovich DA, Kligerman AD, Morgan KT, Heck HA, Feron V. A critical review of the literature on acrolein toxicity. CRC critical reviews in toxicology. 1985;14:309–380.
[19] Luo J, Shi R. Acrolein induces axolemmal disruption, oxidative stress, and mitochondrial impairment in spinal cord tissue. Neurochemistry international. 2004;44:475–486.
[20] Adams JD, Klaidman LK. Acrolein-induced oxygen radical formation. Free Radical Biology and Medicine. 1993;15:187–193.
[21] Zhu Q, Sun Z, Jiang Y, Chen F, Wang M. Acrolein scavengers: Reactivity, mechanism and impact on health. Molecular nutrition & food research. 2011;55(9):1375–90.
[22] Dickinson DA, Forman HJ. Cellular glutathione and thiols metabolism. Biochemical pharmacology. 2002;64:1019–1026.
[23] Kehrer JP, Biswal SS. The molecular effects of acrolein. Toxicological Sciences. 2000;57:6–15.
[24] Grafström RC, Dypbukt JM, Willey JC, Sundqvist K, Edman C, Atzori L, et al. Pathobiological effects of acrolein in cultured human bronchial epithelial cells. Cancer research. 1988;48:1717–1721.
[25] Cao Z, Hardej D, Trombetta LD, Trush MA, Li Y. Induction of cellular glutathione and glutathione S-transferase by 3H-1, 2-dithiole-3-thione in rat aortic smooth muscle A10 cells: protection against acrolein-induced toxicity. Atherosclerosis. 2003;166:291–291.
[26] Yousefipour Z, Ranganna K, Newaz M, Milton S. Mechanism of acrolein-induced vascular toxicity. Journal of physiology and pharmacology. 2005;56:337–337.
[27] Mohammad MK, Avila D, Zhang J, Barve S, Arteel G, McClain C, et al. Acrolein cytotoxicity in hepatocytes involves endoplasmic reticulum stress, mitochondrial dysfunction and oxidative stress. Toxicology and Applied Pharmacology. 2012.
[28] Griveau J, Dumont E, Renard P, Callegari J, Le Lannou D. Reactive oxygen species, lipid peroxidation and enzymatic defence systems in human spermatozoa. Journal of reproduction and fertility. 1995;103:17–26.
[29] Gupta S, Wen JJ, Garg NJ. Oxidative stress in Chagas disease. Interdisciplinary perspectives on infectious diseases. 2009.
[30] Hammerman C, Goldschmidt D, Caplan MS, Kaplan M, Schimmel MS, Eidelman AI, et al. Amelioration of ischemia-reperfusion injury in rat intestine by pentoxifylline-mediated inhibition of xanthine oxidase. Journal of pediatric gastroenterology and nutrition. 1999;29:69–74.
[31] Cross A, Jones O. Enzymic mechanisms of superoxide production. Biochimica et biophysica acta Bioenergetics. 1991;1057:281–298.
[32] Jackson MJ, Papa S, Bolaños J, Bruckdorfer R, Carlsen H, Elliott RM, et al. Antioxidants, reactive oxygen and nitrogen species, gene induction and mitochondrial function. Molecular aspects of medicine. 2002;23:209–209.
[33] Milani E, Nikfar S, Khorasani R, Zamani MJ. Abdollahi M. Reduction of diabetes-induced oxidative stress by phosphodiesterase inhibitors in rats. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2005;140:251–255.
[34] Abdollahi M, Chan TS, Subrahmanyam V, O'Brien PJ. Effects of phosphodiesterase 3, 4, 5 inhibitors on hepatocyte cAMP levels, glycogenolysis, gluconeogenesis and suceptibility to a mitochondrial toxin. Molecular and cellular biochemistry. 2003;252:205–211.
[35] Radfar M, Larijani B, Hadjibabaie M, Rajabipour B, Mojtahedi A, Abdollahi M. Effects of pentoxifylline on oxidative stress and levels of EGF and NO in blood of diabetic type-2 patients; a randomized, double-blind placebo-controlled clinical trial. Biomedicine & pharmacotherapy. 2005;59:302–306.
[36] Khoshakhlagh P, Bahrololoumi-Shapourabadi M, Mohammadirad A, Ashtaral-Nakhai L, Minaie B, Abdollahi M. Beneficial effect of phosphodiesterase-5 inhibitor in experimental inflammatory bowel disease; molecular evidence for involvement of oxidative stress. Toxicology Mechanisms and Methods. 2007;17:281–288.
[37] Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. Proceedings of the National Academy of Sciences. 1994;91:10771–1078.
[38] Luo J, Shi R. Acrolein induces oxidative stress in brain mitochondria. Neurochemistry international. 2005;46:243–252.
[39] Picklo MJ, Montine TJ. Acrolein inhibits respiration in isolated brain mitochondria. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2001;1535:145–152.
[40] Biagini RE, Toraason MA, Lynch DW, Winston GW. Inhibition of rat heart mitochondrial electron transport in vitro: implications for the cardiotoxic action of allylamine or its primary metabolite, acrolein. Toxicology. 1990;62:95–106.
[41] Aghababaeian R, Ghazi-Khansari M, Abdi K, Taghadosinejad F, Abdollahi M. Protective effects of sildenafil and dipyridamol from lead-induced lipid peroxidation in perfused rat liver. Int J Pharmacol. 2005;1:157–160.

