ORIGINAL ARTICLE

GMJ.2016;5(3):131-138
www.gmj.ir

Received 2016-07-03
Revised 2016-07-15
Accepted 2016-07-27

Effect of Pentoxifylline on Apoptosis of Kidney's Cells Following Acute Methamphetamine Administration in Male Wistar Rats

Shabnam Movassaghi[1], Ali Yousefi Oudarji[1], Zahra Nadia Sharifi[1]✉

[1]Department of Anatomy, School of Medicine, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran

Abstract

Background: Methylenedioxymethamphetamine (MDMA) is a hallucinogenic drug of abuse which is the most popular drugs in the world and has been shown to induce apoptosis in kidney cells. As Pentoxifylline (PTX) increases cAMP and reduces tumor necrosis factor-α, the present study aimed to investigate the effect of PTX on kidney damage induced by acute administration of MDMA in the male rat. **Materials and Methods:** Thirty adult male Wistar rats were randomly divided into five groups: control group (without any intervention), MDMA as sham group (the group received 7.5 mg/kg MDMA three times at every two hours for one day), E1 (received 100 mg/kg PTX a week before MDMA administration), E2 (received 100 mg/kg PTX Just in the time of the third injection of MDMA) and E3 (received 100 mg/kg PTX followed by one dose of MDMA) groups. At the end of experiment period, kidneys were removed and prepared for H&E staining, TUNEL and western blot techniques. **Results:** Histopathological studies showed significantly decrease in the kidney cells damage, in the E1 group compared to MDMA group. The number of TUNEL-positive cells was increased significantly in MDMA group. A significant difference was revealed in the mean number of TUNEL-positive cells between the rats treated with PTX before MDMA administration and MDMA group. Expression of active caspase-3 was significantly increased in the MDMA group. While PTX treatment when administrated before MDMA injections could significantly decrease the caspase-3 activity. **Conclusion:** The PTX can substantially reduce the severity of lesions in the kidney following administration of MDMA. **[GMJ.2016;5(3):131-138]**

Keywords: Methylenedioxymethamphetamine; Protection; kidney; Pentoxifylline

Introduction

The 3,4-methylenedioxymethamphetamine (MDMA) as know ecstasy, is one of the most popular used drugs as a recreational drug by young people [1].There are increasing evidence of its toxicity, although MDMA has been considered as a safe drug. The MDMA can affect human organs such as brain, liver, kidney and heart. These effects seem to be dose- related and leading to apoptosis [2]. In the past few years, clinical documents have shown that the kidney is a target for MDMA toxicity. In this sense, Ecstasy is metabolized, and reactive metabolites are readily oxidized to the 5- Quinones and the reactive oxygen

GMJ

Tel/Fax: +98 71 36474503
PO Box 7193616563
Email:info@gmj.ir

✉ **Correspondence to:**
Zahra Nadia Sharifi. Department of Anatomy, School of Medicine, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran.
Telephone Number: +989122834627
E-mail:zsharifi@iautmu.ac.ir

species (ROSs) [3]. It has also been shown that MDMA induces cell death trough an apoptotic pathway by releasing of cytochrome C and caspase cascade activation [4].Cell deaths have been classified by consensus of opinions such as apoptosis, necrosis, necroptosis, autophagy and cornification [5]. Apoptosis is a specific morphological appearance of cell death specified by blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and global mRNA decay [6]. Pro- and anti-apoptotic genes interact with each other to control the completeness of the cell during apoptosis and in the last stage of this process, the Bcl-2 family regulates mitochondrial dysfunction [7]. Pentoxifylline (PTX) has been classified as a vasodilator and a phosphodiesterase inhibitor drug. The PTX is used for treating of intermittent claudication in the peripheral artery disease. This medication significantly decreases blood viscosity in the peripheral arterial disorders and enhancement erythrocytes deformability in healthy patients with peripheral vascular disease [8]. The mechanisms of its effects seem to be related to change in cellular functions and improve microcirculatory perfusion in peripheral and cerebral vascular beds [2]. Recently, PTX, with anti-inflammatory and antifibrogenic attributes, has been found to be useful in patients with nephropathy [9]. The objectives of the present study were to investigate the possible protective effects of PTX the kidney' cells apoptosis due to acute administration of MDMA.

Materials and Methods

Experimental Groups and Drug

Male Wistar rats aged 8 weeks were maintained at 22±1°C on a 12 h light/12 h dark cycle, with free access to water and food ad libitum. The rats were equally distributed into five experimental groups (6 rats per each group) including (1) Control group (without any intervention); (2) MDMA (sham) group: the group received 7.5 mg/kg MDMA three times at every two hours for one day) [10]; (3) Experimental group1(E1): received 100 mg/kg PTX a week before MDMA administration; (4) Experimental group (E2): received 100 mg/kg PTX just in the time of the third injection of MDMA; (5) Experimental group (E3): received 100 mg/kg PTX followed by one dose of MDMA.The rats were sacrificed after two weeks from the beginning of all experiments. All experimental procedures used in the current study were performed in accordance with the ARRIVE ethical guidelines. Chemicals were purchased from Sigma except PTX powder, which was gifted kindly by the Amin Pharmaceutical Co. (Esfahan-Iran). However, pure MDMA was gifted by Dr. Foroumadi, faculty of pharmacy and pharmaceutical sciences research center, Tehran University of Medical Sciences.

Histopathological Studies

Rats were sacrificed and their kidneys were removed for histopathological examination. The kidneys were completely excised and any extraneous tissue was omitted. Kidney samples were fixed in 10% buffered formalin and embedded in paraffin. After dewaxing 5 µm sections were stained with hematoxylin and eosin (H&E). All slides were evaluated by light microscopy (Olympus BX45) by one pathologist who assessed kidney morphology. A minimum of 20 fields was examined in each sample (magnification ×100).In Situ Apoptosis Assay by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining Kidneys were perfused with phosphate-buffered saline (PBS) (50 ml) using the transcardiac approach, followed by 4% phosphate-buffered formalin. Perfusion-fixed kidney tissues were further fixed overnight in a solution of 4% paraformaldehyde in PBS, embedded in paraffin and cut into 3 µm serial sections. The TUNEL staining was performed using an In Situ Cell Death Detection Kit (Roche, Mannheim, Germany) according to the manufacturer's protocol. Briefly, the sections were dewaxed in xylol, rehydrated by successive series of alcohol, washed with PBS and deproteinized (or permeabilized) by proteinase K (20 µg/ml) for 30 min at room temperature. The sections were rinsed and incubated with 3% H2O2 in methanol for 10 min in the dark to block endogenous peroxidase (POD) and the sections incubated with the TUNEL reaction mixture for 60 min in

37°C at humidified atmosphere and rinsed with PBS. Sections were visualized using converter POD for 30 min in 37°C at humidified atmosphere in the dark and rinsed with PBS, and 50-100 µl diaminobenzidine (DAB) substrate was added and rinsed with PBS. Four slide fields were randomly examined using a defined rectangular field area at 100× magnification.

Western Blotting

Protein expression in kidney was detected by western blotting. Briefly , part of kidney tissue was homogenized in ice-cold lysis buffer containing tris-HCl (50 mM, pH 8.0), NaCl (150 mM), Nonidet P-40 (1%), glycerol (10%), phenylmethylsulfonyl fluoride (10 ml/ml), sodium deoxycholate (0.5%) and aprotinin (30 ml/ml), in addition to a protease inhibitor cocktail (Roche Applied Science). The homogenized testes were subjected to centrifugation at 12000 g for 20 min at 4ºC, and the supernatant collected. A total of 100 µg of the total protein of the supernatant was loaded onto each lane and electrophoresed on SDS-PAGE gels (10%). Proteins were transferred onto nitrocellulose membranes for 1 h at room temperature and blocked with PBS that contained non-fat dried milk powder (5%) for 2h. Membranes were washed with Tris buffer that contained Tween 20 (1%), then probed with a monoclonal anti-caspase-3 antibody (1:1000; Abcam, St. Louis, MO, USA) overnight after which a secondary anti-rabbit akp-linked antibody (1:10000; Abcam,) was added for 1 h at room temperature, then the membranes were stained with BCIP/NBT. The β-actin served as a positive control for protein loading, and a high range molecular weight standard was used to determine protein sizes. Results were evaluated by the UVIdoc program (UVIdoc version 12.6 for Windows, Copyright 2004).

Statistical Analysis

Statistical analysis was performed using SPSS for Windows (Ver.19, SPSS, Inc., Chicago, USA). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison post test and expressed as mean ± SD. $P< 0.05$ was considered to be significant.

Results

Light Microscope Observation

Light microscope histopathological studies showed significantly decrease the kidney's cellular damage, including necrosis, hemorrhage, and tissue edema in E1 as compared to MDMA-treated, E2, and E3. Atrophied glomerulus, increased spaces of Bowman's capsules, congestion of interstitial space, and tissue edema were seen in MDMA-treated, E2, and E3 groups (Figure-1).

Detection of dead renal tubular epithelial cells using TUNEL staining To further evaluate the mechanisms of cell death, we examined the remnant kidney tissue cells for the presence of fragmented DNA using TUNEL staining assays. A higher level of TUNEL-positive cells was observed in renal tissue in MDMA group. There were significant differences in the number of apoptotic cells between control and MDMA groups (P=0.045). The proportion of TUNEL-positive cells in the kidneys from the E1 group was lower than in the MDMA group. Treatment with PTX before the MDMA injections significantly reduced the total number of TUNEL-positive cells in the E1 group. There were no significant differences between control group and E1 group (P= 0.994). The differences between the other two experimental groups and the control group were significant) Figure-2).

Western Blotting

As shown in the Western blotting analysis, compared to the control kidney, injection of MDMA could significantly increase apoptotic related protein, caspase-3. However, PTX administration at 100 mg/kg before MDMA exposure effectively suppressed the up-regulation of cleaved-caspase-3 protein, while PTX injection after MDMA administration did not affect the activity of caspase-3 compared with MDMA group and could not inhibit the up-regulation of cleaved-caspase-3 (Figure-3).

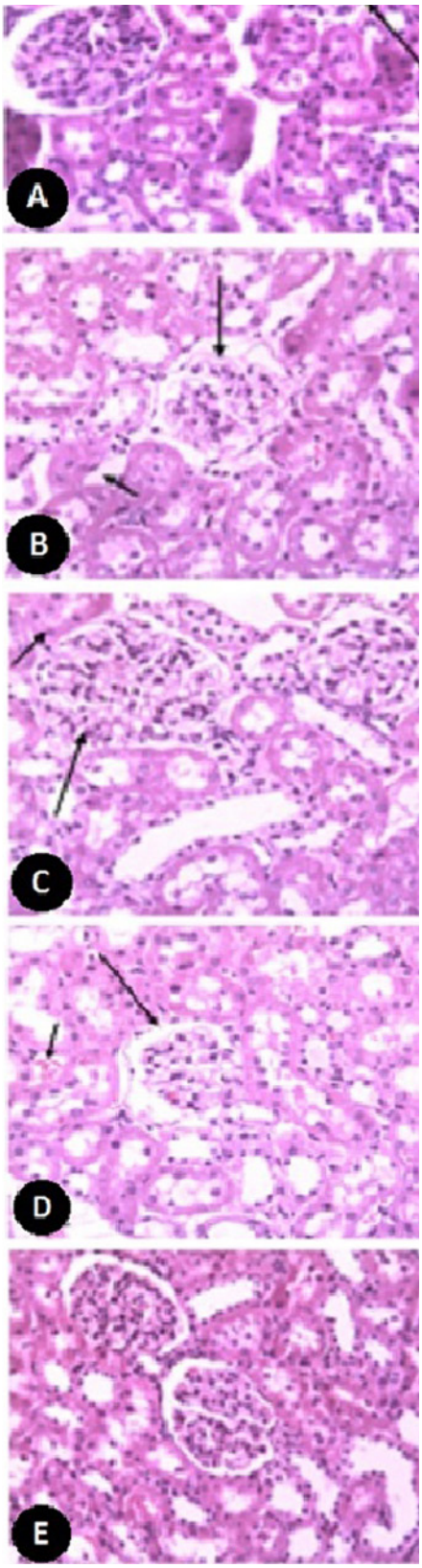

Figure 1. Photomicrograph of lobules from control (A), MDMA (B), E1(C), E2 (D) and E3 (E) groups. Long arrow shows glomerular structure and short arrow shows congestion in interstitial space. (Hematoxylin and eosin, ×40).

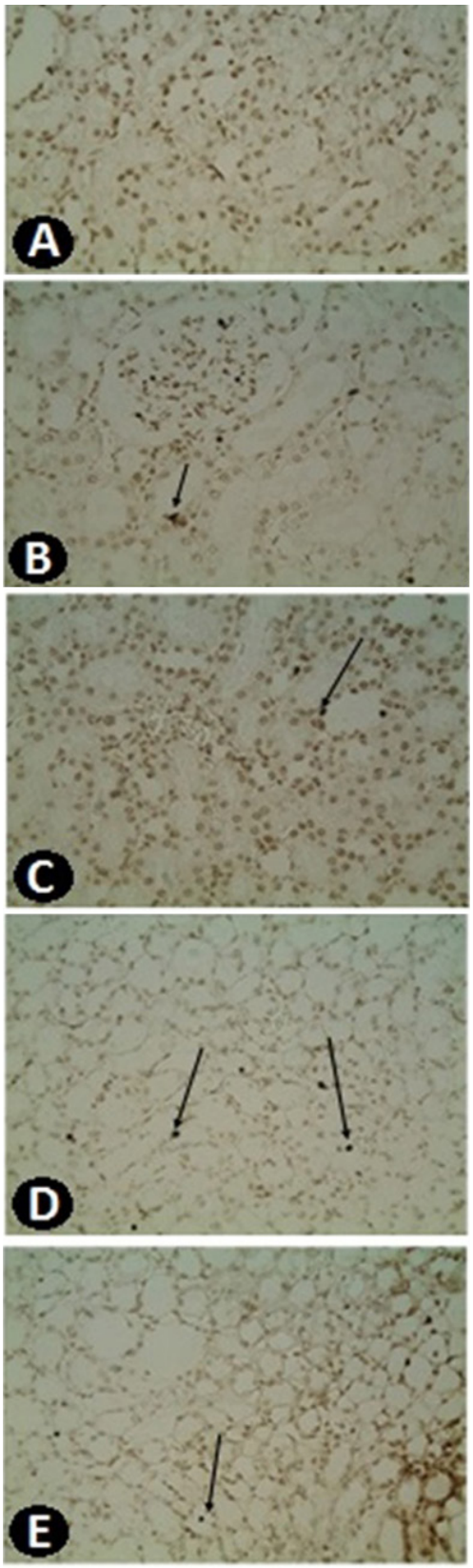

Figure 2. The TUNEL assay for apoptotic cells in the renal tissue. (A): Control group; (B): MDMA group; (C): E1 group; (D): E 2 group; (E): E3 group. The arrows point to an apoptotic cell. (TUNEL staining, ×40)

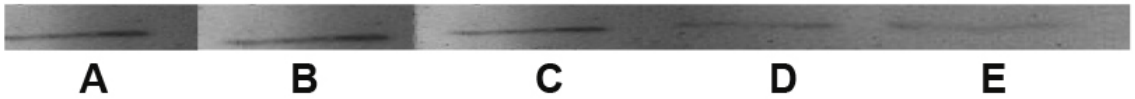

Figure3. Effect of PTX on MDMA-Induced renal cell apoptosis. The Western blotting analysis in experimental groups indicated that the PTX treatment could decrease the expression of caspase-3 after MDMA exposure. According to the weight of protein (caspase-3) and protein marker, the blocking is done within the target band (**A:** E3 group; **B:** E2 group; **C:** MDMA group; **D:** E1 group; **E:** Control)

Discussion

Several studies showed that MDMA could damage many organs such as brain, heart, liver, kidney, and testes. Over the past years, ecstasy has been associated with an aspect of nephrotoxic effects, such as acute kidney injury and hyponatremia [11].

The MDMA and its metabolites damage kidney during their excretion through this organ. Also, MDMA can cause rhabdomyolysis, which can lead to myoglobin deposition in the kidney, along with dehydration and electrolyte inconsistency may also contribute to acute and chronic renal failure [12]. Symptomatic hyponatremia is one of the most complications of ecstasy [11]. It has been shown that MDMA can induce oxidative stress leading cell damage in many tissues such as liver, kidney, heart, and retina [13].

Many other studies demonstrated that generation of MDMA metabolites in the kidney may be responsible for renal toxicity [14]. Our findings along with others corroborated that kidney is a potential target organ for ecstasy-induced toxicity. In this study, we showed the tubulointerstitial injury and a significant decrease was observed in the number of proximal tubule cells in MDMA-treated rats.

Increased production of ROS and toxic oxidation products may be responsible for damaging [15]. Increased oxidative stress can cause organ damage and apoptosis. Mitochondria, fat, and antioxidant defense are shown to be a major target of increased oxidative/nitrosative stress when exposure to toxic compounds and/or in pathological conditions [16]. It is possible that MDMA and/or its metabolites restrain the mitochondrial function by interacting with mitochondrial proteins [17]. Also, MDMA and its metabolites can cause indirectly mitochondrial dysfunction by increasing oxidative/nitrosative stress [18].

Apoptosis is a regulated process which involved in physiological and pathological responses, including cell damage followed by exposing toxic substances. Since MDMA had been characterized as a pro-apoptotic agent for neurons [19], we attempt to find if renal cells apoptosis could contribute to the nephrotoxic effect of this drug, focusing on the activation of caspases-3, which plays a key role in the executive phase of cell apoptosis. Our data showed that a high dose administration of MDMA has a toxic effect on the kidney tissue with a stimulating apoptotic pathway for normal renal cells. The MDMA treatment caused the release of caspase activation, thus suggesting for other apoptotic agents changing of mitochondrial membranes through alternating in Bcl-2-like proteins, cytochrome c releasing and activation of effector caspases. The formerly reported role of MDMA as an inductor of cellular oxidative stress [20], could clarify apoptosis caused by redox disruption has been shown to happen in many cases by changes in mitochondria [21]. Our data also demonstrated that MDMA injection would increase caspase-3 protein activity in renal tissue.

The PTX is a methylxanthine phosphodiesterase inhibitor, which can reduce platelet aggregation, enhancement red cell deformability, and lower blood viscosity [22]. Also, it can weaken the release of pro-inflammatory cytokines and inhibits the production of tumor necrotic factor-alpha (TNF-α) by blocking intracellular phosphodiesterase [23].

The TNF-α is an important cytokine participating in damage and inflammation in animal and human renal lesions [24]. The TNF-α expression, synthesis, and excretion can be decreased by PTX in chronic renal disease and diabetic patients [25]. This makes PTX a good candidate for the treatment of these cases.

All of our findings confirmed the results of other studies in this field. Since PTX can block inflammatory cytokine production (interleukin-1 and tumor necrosis factor); so, it can decrease the inflammatory reaction and also can inhibit cell damage indirectly, which have been shown in the results of microscopic studies the cell death process in treatment group [26].The helpful effects of PTX on gentamycin-induced alteration in glomerular basement membrane was proved [27]. Another study demonstrated that PTX could decrease apoptosis by reduction of TNF-α and oxygen free-radical concentrations so this drug could be protective against acute kidney injury Gentamycin-induced [28]. The protective effects of PTX also have been studied on Adriamycin-induced nephrotoxicity in rats. In this study, PTX could prevent tubular and interstitial apoptosis in renal tissue [29]. Ozkurkcugil et al., showed that PTX treatment is effective in preventing the negative effects of cigarette smoking on kidneys by inhibiting cell damage with its antioxidant properties [30].

The result of present study documented that MDMA abuse significantly reduced tubulointerstitial damage in affected kidneys when they were protected in rats by PTX treatment after MDMA administration. A significant decrease was observed in the number of cells in MDMA administered rats that did not receive PTX.

In our model, the PTX (100mg/Kg, i.p.) administrated after MDMA injections could not reduce either the number of affected renal cells and apoptotic bodies nor the caspase-3 expression significantly. On the other hand, when PTX was administrated before MDMA substantially improved renal injury in the number of affected renal cells and apoptotic bodies. Furthermore, the caspase-3 expression showed significantly decrease when administrated before MDMA injections.

Conclusion

The administration of PTX was shown to have the potential to prevent the negative effects of Ecstasy on kidney glomerulus by inhibiting cell damage. Further studies are needed to elucidate the exact mechanism by which PTX induces protection in the kidney.

Acknowledgement

We would like to offer our special thanks to Dr. Foroumadi and Amin pharmaceutical company for giving MDMA and PTX as gifts. The authors also would like to express their appreciations from Azad University of Medical Sciences Research Center for their contributions to this study.

Conflict of Interest

The authors declare no conflicts of interest and have approved the final article

References

1. Yamamoto BK, Moszczynska A. Amphetamine toxicities: classical and emerging mechanisms. Ann N Y Acad Sci. 2010;1187:101-21.
2. Movassaghi S, Nadia Sharifi Z, Mohammadzadeh F, Soleimani M. Pentoxifylline Protects the Rat Liver Against Fibrosis and Apoptosis Induced by Acute Administration of 3,4-Methylenedioxymethamphetamine (MDMA or Ecstasy).Iran J Basic Med Sci. 2013; 16(8):922-7.
3. Karami M, Saeidnia S, Nosrati A. Study of the Hepatoprotective Activity of Methanolic Extract of Feijoa sellowiana Fruits Against MDMA using the Isolated Rat Liver Perfusion System.Iran J Pharm Res. 2013 12(1):85-91.
4. Asl SS, Pourheydar B, Dabaghian F, Nezhadi A, Roointan A, Mehdizadeh M. Ecstasy-induced caspase expression alters following ginger treatment. Basic Clin Neurosci. 2013; 4(4):329-33.
5. Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Alnemri ES, Baehrecke EH, et al. Classification of cell death: recommendations

of the Nomenclature Committee on Cell Death. Cell Death Differ. 2009; 16(1):3–11.
6. Malhi H, Guicciardi ME, Gores GJ. Hepatocyte death: a clear and present danger. Physiol Rev. 2010; 90(3):1165–94.
7. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol. 2008; 9(1):47–59.
8. Tjon JA, Riemann LE .Treatment of intermittent claudication with pentoxifylline and cilostazol. Am J Health Syst Pharm. 2001 15; 58(6):485-93.
9. Kang JY, Shin HS. Effects of 1, 7-substituted methylxanthine derivatives on LPS-stimulated expression of cytokines and chemokines in Raw 264.7 and HK-2 cells.J Microbiol Biotechnol. 2015; 25(2):296-301.
10. Wang X, Baumann MH, Xu H, Morales M, Rothman RB. 3,4-Methylenedioxymethamphetamine administration to rats does not decrease levels of the serotonin transporter protein or alter its distribution between endosomes and the plasma membrane. J Pharmacol Exp Ther. 2005; 314(3):1002-12.
11. Campbell GA, Rosner MH. The agony of ecstasy: MDMA (3, 4 methylenedioxymethamphetamine) and the kidney. Clin J Am Soc Nephrol . 2008;3:1852-60.
12. Mas M, Farré M, de la Torre R, Roset PN, Ortuño J, Segura J, et al. Cardiovascular and neuroendocrine effects and pharmacokinetics of 3, 4-methylenedioxymethamphetamine in humans. J Pharmacol Exp Ther .1999; 290:136-45.
13. Miranda M, Bosch-Morell F, Johnsen-Soriano S, Barcia J, Almansa I, Asensio S, et al. Oxidative stress in rat retina and hippocampus after chronic MDMA ('ecstasy') administration. Neurochem Res. 2007;32:1156-62.
14. Carvalho M, Hawksworth G, Milhazes N, Borges F, Monks TJ, Fernandes E, et al. Role ofmetabolites in MDMA (ecstasy)-induced nephrotoxicity: an in vitro study using rat and human renal proximal tubular cells. Arch Toxicol. 2002;76:581-88.
15. Montiel-Duarte C, Ansorena E, Lopez-Zabalza MJ, Cenarruzabeitia E, Iraburu MJ,Cenarruzabeitia E, et al. Role of reactive oxygen species, glutathione and NF-kappaB in apoptosis induced by 3,4methylenedioxy methamphetamine ("Ecstasy") on hepatic stellate cells. Biochem Pharmacol. 2004; 67:1025–1033.
16. Milhazes N, Cunha-Oliveira T, Martins P, Garrido J, Oliveira C, Rego AC, et al. Synthesis and cytotoxic profile of 3,4-methylenedioxymethamphetamine ("ecstasy") and its metabolites on undifferentiated PC12 cells: A putative structure-toxicity relationship. Chem Res Toxicol. 2006; 19:1294–1304.
17. Fisher AA, Labenski MT, Malladi S, Gokhale V, Bowen ME, Milleron RS, et al. Quinone electrophiles selectively adduct "electrophile binding motifs" within cytochrome c. Biochemistry. 2007; 46:11090– 11100.
18. Moon KH, Abdelmegeed MA, Song BJ. Inactivation of cytosolic aldehyde dehydrogenase via S-nitro sylation in ethanol-exposed rat liver. FEBS Lett. 2007; 581:3967–72.
19. Stumm G, Schlegel J, Schäfer T, Würz C, Mennel HD, Krieg JC, et al. Amphetamines induce apoptosis and regulation of bcl-x splice variants in neocortical neurons. FASEB J. 1999; 13(9):1065-72.
20. Cadet JL, Thiriet N, Jayanthi S.Involvement of free radicals in MDMA-induced neurotoxicity in mice. Ann Med Interne (Paris). 2001; 152 Suppl 3:IS57-9.
21. Chandra J, Samali A, Orrenius S.Triggering and modulation of apoptosis by oxidative stress. Free Radic Biol Med. 2000; 29(3-4):323-33.
22. Mollitt DL, Poulos ND. The role of pentoxifylline in endotoxin-induced alterations of red blood cell deformability and whole blood viscosity in the neonate. J Pediatr Surg. 1991; 26:572–574.
23. Deree J, Martins JO, Melbostad H, Loomis WH, Coimbra R. Insights into the regulation of TNF-α production in human mononuclear cells: the effects of non-specific phosphodiesterase inhibition. Clinics. 2008; 63:321–28.
24. Ozen S, Saatci U, Tinaztepe K, Bakkaloglu A, Barut A. Urinary tumor necrosis factor levels in primary glomerulopathies. Nephron. 1994; 66:291–4.
25. Rodriguez-Morán M, González-González G, Bermúdez-Barba MV, Medina de la Garza CE, Tamez-Pérez HE, Martínez-Martínez FJ, et al. Effects of pentoxifylline on the urinary protein excretion profile of type 2 diabetic patients with microproteinuria: A double-blind, placebo-controlled randomized trial. Clin Nephrol. 2006; 66:3–10.
26. Hashemi M.The Study of Pentoxifylline

Drug Effects on Renal Apoptosis and BCL-2 Gene Expression Changes Following Ischemic Reperfusion Injury in Rat. Iran J Pharm Res. 2014; 13(1):181-9.
27. Stojiljkovic N, Veljkovic S, Mihailovic D, Stoiljkovic M, Radenkovic M, Rankovic G, et al. Protective effects of pentoxifylline treatment on gentamicin-induced nephrotoxicity in rats. Ren Fail. 2009; 31:54-61.
28. Stojiljkovic N, Veljkovic S, Mihailovic D, Stoiljković M, Ranković G, Jovanović I, et al. Pentoxifylline ameliorates glomerular basement membrane ultrastructural changes caused by gentamicin administration in rats. Bosn J Basic Med Sci. 2009; 9:239.
29. Usta Y, Ismailoglu UB, Bakkaloglu A, Orhan D, Besbas N, Sahin-Erdemli I, et al. Effects of pentoxifylline in adriamycin-induced renal disease in rats. Pediatr Nephrol. 2004; 19:840-43.
30. Ozkurkcugil C, Yilmaz MY, Ozkan L, Kokturk S, Isken T. Protective effects of pentoxifylline on cigarette smoking-induced renal tissue damage in rats. Toxicol Ind Health. 2011; 27:335-40.