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Original Article

Effect of Pentoxifylline on Histomorphological Changes of Kidney after Ecstasy (MDMA) Administration in Male Wistar Rat.

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Abstract

Methylenedioxymethamphetamine (MDMA),the Ecstasy brand, leads to cell death in many tissues such as kidney because of its oxidative stress properties. The present study aimed to investigate the possible effects of vasodilators such as pentoxifylline as a vasodilators on ecstasy induced renal damage caused by ecstasy abuse. This experimental study was carried out in four groups of six male wWistar rats weighing 250-300 g (n=24). The control group was kept under standard laboratory conditions. In Ecstasy group, this substance was injected (as 7.5 mg/kg, q 2 h × 3 doses) intraperitoneally (IP)in the treatment group . In pentoxyfyllinePTX(PTX) treated group, 200 mg/kg of pentoxifylline PTX was concurrently administered with the third dose of ecstasy injection and in vehicle group, normal saline was injected. Animals' The kidneys were evaluated for histomorphological changes by H&E and PAS stainings. Then the degrees of renal damage were measured scored by Image Tools Version 2 software. Although There were no significant differences in diameter and number of glomerular cells between control and pentoxifyllinePTX treated groups was detected. However, a significant difference was seen with between vehicle and Ecstasy groups ($P < 0.05$). Tubulointerstitial injury was seen in Ecstasy and vehicle groups but it had been decreased in pentoxyfyllinePTX treated group. Findings of this study suggest that pentoxifylline PTX can excertexert a positivesignificant effect on improving the ecstasy induced renal tissue injury injuriescaused by ecstasy abuse.

Keywords: Ecstasy, Kidney, pentoxifylline, Rat, histomorphological changes

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Introduction

The consumption of Ecstasy (3,4-methylene-dioxymethamphetamine, MDMA) one of the mostcommonillicit synthetic drugs (Khajeamiri et al. 2011), known on the street as 'meth', 'crystal' and 'speed'increases thrill-seeking/risk-taking behavior and has become more popular among younger people (Kalant 2001). Ecstasy is a methamphetamine derivative that possesses serious toxic effects including hyperpyrexia, rhabdomyolysis, cardiac arrhythmias, cerebrovascular lesions, intravascular coagulopathy and hepatic necrosis (Malpass et al. 1999; Gowing et al. 2002).

Psychoactive drugs can induce adverse effects on various organs such as heart, kidney and liver. They also stimulate the body's endocrine system, Hypothalamic-Pituitary-Thyroid Axis and the adrenal glands. The body temperature elevation , adrenocorticotropic hormone (ACTH) and cortisol secretion were also reported following the use of Ecstasy (Gerra et al. 2003; Sprague et al. 2003).

MDMA is readily absorbed from the intestinal tract and reaches its peak concentration in the plasma in 2 hours after oral administration (Kalant 2001). MDMA is primarily excreted via the kidney (Baumann et al. 2007). Several factors may contribute to the adverse renal effects of ecstasy, including disseminated intravascular coagulation (DIC), vasoconstriction, rhabdomyolysis, hyperthermia and metabolism (Carvalho et al. 2002). Ecstasy has been associated with acute kidney injury (AKI) that is most commonly secondary to nontraumatic rhabdomyolysis. More common, ecstasy has led to serious hyponatremia and hyponatremia-associated deaths. Hyponatremia in these cases is due to a "perfect storm" of ecstasy-induced effects on water balance (Campbell and Rosner 2008).

Renal damage was also seen in rats following ecstasy administration (Ninkovic et al. 2008) and polyuria. In addition renal glycosuria and low tubular reabsorbtion of phosphorous have been found in patients who ingested ecstasy (Kwon et al. 2003).

Increasing evidence indicated that the drug is metabolized by cytochrom P450 enzyme system (K.-P. Kreth et al. 2000; Fonsart et al. 2008; Meyer et al. 2009). It was also shown that exposure of rat and human renal proximal tubular cells (PTC) to MDMA produced cell necrosis (Carvalho et al. 2002). There is some evidence suggesting that oxidative stress plays a part in the initial stages of MDMA-induced tissue damage. Such effects have been also found in the liver (Ninković et al. 2004).

Pentoxifylline (PTX), a xantine derivation and a nonspecific phosphodiesterase inhibitor, was first considered in the treatment of peripheral vascular diseases (Samlaska and Winfield 1994). PTX exerts several pharmacologic effects, including improvement in microcirculation, increase in erythrocyte deformability, reduction in blood viscosity, inhibition of platelet aggregation, endothelium-dependent vascular relaxation (Samlaska and Winfield 1994), immunomodulatory (Noel et al. 2000; S. Kreth et al. 2010), anti-inflammatory, and antiproliferative effects (Savic et al. 2002). PTX downregulates several proinflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) and IL-6 (Samlaska and Winfield 1994; Heystek et al. 2003). Some possible pharmacologic mechanisms of PTX that indicate it as a candidate to ameliorate AKI include interaction at the level of the adenosine receptors (Berens and Luke 1990), increase in erythrocyte deformability (Samlaska and Winfield 1994; Albornoz et al. 1997), stimulation of vasodilatory prostaglandin production and prevention of vascular congestion (Krysztopik et al. 2000; Stojiljkovic et al. 2009), and suppression of TNF-α and nitrite production, proteinuria, and apoptosis (Nasiri-Toosi et al. 2013).

PTX has also gained considerable interest as a reactive oxygen species (ROS) scavenger, and several studies have shown its potential antioxidant effects (Dousa 1998; Prasad and Lee

2007). Moreover, many studies indicate beneficial effects of PTX in diabetic patients. Badri et al. concluded that PTX ameliorates microalbuminuria and proteinuria in patients with diabetic and nondiabetic kidney diseases (Badri et al. 2011).
In this study, we investigated the protective effects of PTX treatment on ecstasy-induced histological changes in the kidney sections of rats.

Methodology

Animal

All procedures used in this study were approved by the ethics committee of National Institutes of Health (NIH). Twenty-four male wistar rats weighing 250-300 g were used in this study. Animals were housed under standard conditions (12 h light / dark cycles), at temperature 22±2 °C with free access to water and food ad libitum. Animals were divided into four equal groups as follows:
Control group: animals were kept under standard laboratory conditions.
Ecstasy group: Ecstasy was injected (7.5 mg/kg, q 2 h × 3 doses) intraperitoneally (IP) to rats (Baumann et al. 2007; Khazaei Koohpar et al. 2013).
Experimental group: Ecstasy was injected (7.5 mg/kg, q 2 h × 3 doses) and concurrent with the third dose of ecstasy injection, 200 mg/kg of PTX was injected IP to rats.
Vehicle group: Ecstasy was injected (7.5 mg/kg, q 2 h × 3 doses) and concurrent with the third dose of ecstasy injection, normal saline (NS; 0.9% NaCl) was injected IP to rats.
The rats in each group were sacrificed 2 weeks after the start of the experiment and their kidneys were excised for histopathological analysis.

Histomorphological study

Kidneys were removed and fixed in formalin %10. The organs were embedded in paraffin. Serial paraffin sections (5 μm) of the kidneys were cut and stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). Sections were used to calculate the glomerular cell numbers (GCN), proximal tubule cell numbers (PTCN), glomerular diameters (GD) and tubulointerstitial injuries.
Three slides from each sample were randomly selected and photomicrographs were prepared and examined from five fields per slide under a light microscope at a magnification of ×400. The nuclei of all cells in all groups were counted and the diameters of glomerular cells were also measured. Image Tools Version 2 software was used to perform these investigations. A semiquantitative scale was designed to evaluate the degree of tubulointerstitial injuries (Sunami et al. 2004). Score ranging from 0 to 4 was determined as follows: 0= normal kidney; 1= mild change: tubulointerstitial space dilation; 2= moderate change: tubulointerstitial space dilation and infiltration of inflammatory cells; 3= severe change: tubulointerstitial space dilation, infiltration of inflammatory cells and damage to cells such as tubular atrophy; and 4= very severe change: extensive damage to the whole kidney tissue which was not observed in this study (Sunami et al. 2004).

Statistical analysis

Data were statistically analyzed using the SPSS Software package. One-way ANOVA and Tukey's Honestly Significant Difference (HSD) tests were used to determine the significant differences between group means. Data were expressed as mean ± standard error of the mean (SEM). The statistical significance was determined as $P < 0.05$.

Results

The findings showed a significant decrease in the mean glomerular diameter of ecstasy group in comparison with other groups except vehicle group (Figure 4). Mean glomerular diameters of experimental and control groups were similar to each other and both were significantly higher than those of the ecstasy group (Figure 1).

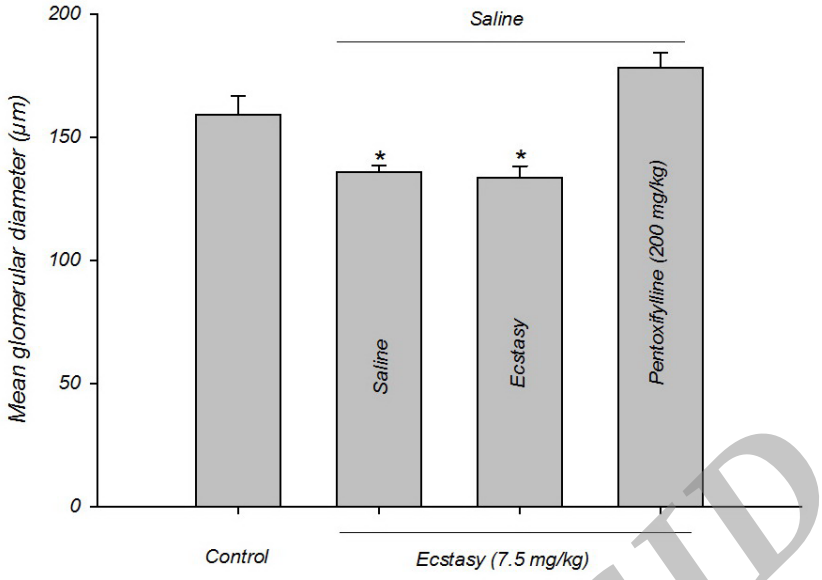

Figure 1: Comparison of mean glumerular diameter between groups. *= P < 0.05 in comparison with other groups.

Comparing the mean number of glomerular cells between groups, indicated reduced number of glomerular cell in ecstasy and vehicle groups. Pentoxifylline can prevent these negative effects. There are significant differences between the experimental and the ecstasy group, but there was no significant difference between control and experimental groups (Figure 2).

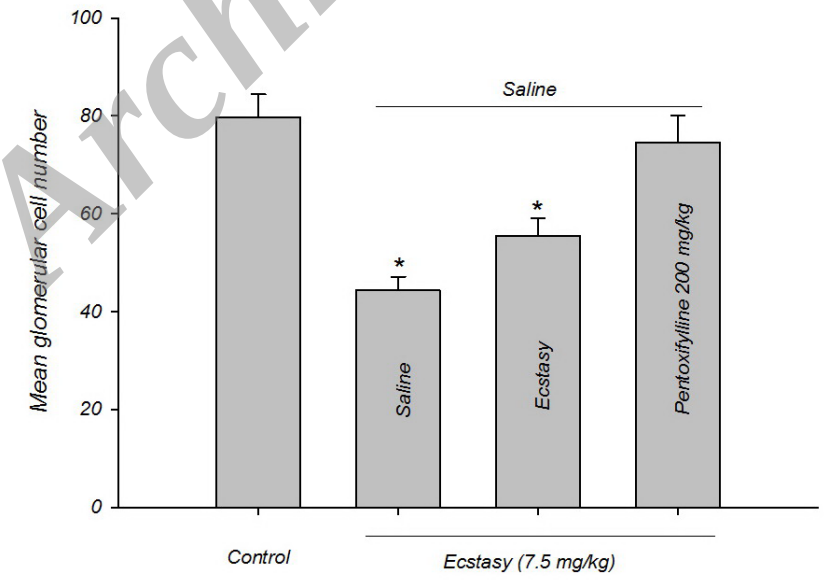

Figure 2: Comparison of mean glumerular cell number between groups. *= P < 0.05 in comparison with other groups

According to our findings, there was a significant difference in mean number of proximal tubule cells between control group and other groups. Pentoxifylline has a positive effect on the number of proximal tubular cells but no significant difference was seen between the experimental group and the ecstasy group; Pentoxifylline failed to prevent the reduction in proximal tubule cells caused by ecstasy (Figure 3).

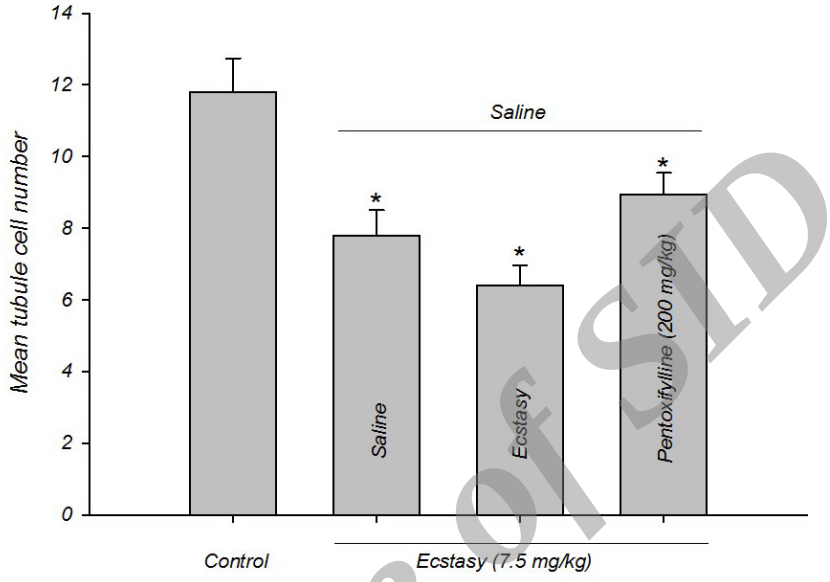

Figure 3: Comparison of mean tubule cell number between groups. *= P < 0.05 in comparison with the control group.

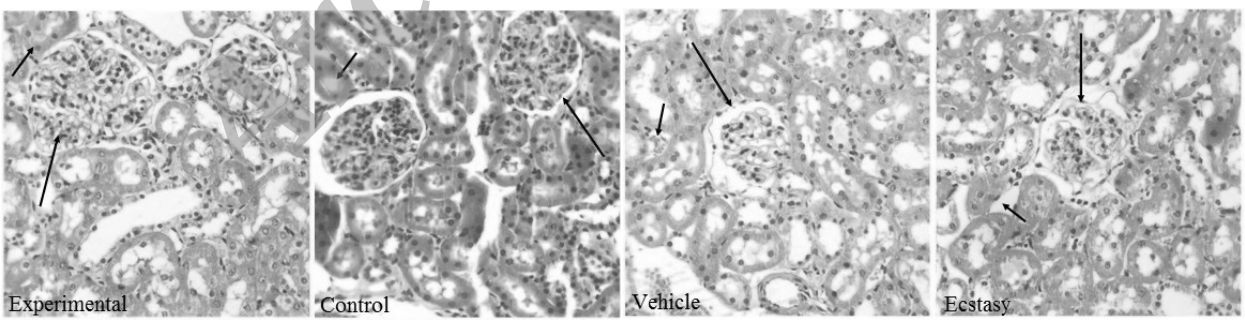

Figure 4: Comparison of histological changes between groups. Specific histological changes were not observed in control and experimental groups. Glomerular structure (long arrow) and proximal tubule (short arrow) are normal. Glomeruls were atrophied in ecstasy and vehicle groups and the spaces of Bowman's capsules were increased in them (long arrow). Congestion of interstitial space is seen in vehicle group (short arrows) and elevation of interstitial spacean indicative of tissue edema (short arrows) is observed in ecstasy group (H&E ×40).

Evaluation of tubulointerstitial injury in different groups

According to our analysis, the severity of tubulointerstitial injuries was more in ecstasy and vehicle groups in comparison with other groups. PTX administration led to less damage in PTX treated group and 60% of specimens were categorized in normal score (Figure 4). Overall there was no significant difference in normal scores between experimental and control groups (Figure 5).

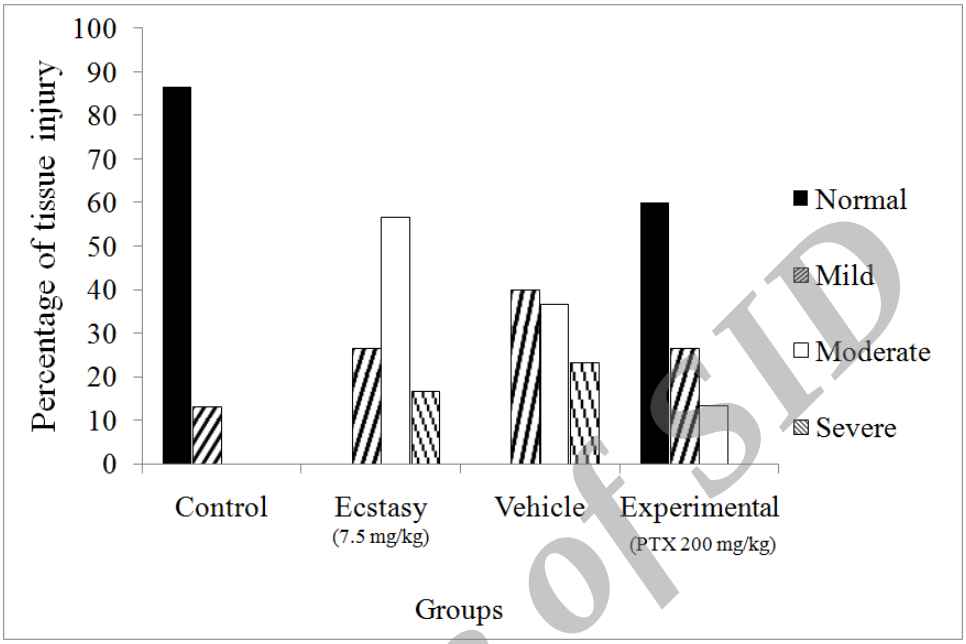

Figure 5: Comparison of the tubulointerestitial injuries in different groups.

Discussion

The present study was conducted to evaluate the protective effects of PTX on kidneys of rats that were exposed to Ecstasy. Our results revealed that PTX had a significant protective effect on GD, GCN and reduction of severity of tubulointerstitial injury.

Several studies showed that MDMA can damage many tissues such as brain, heart, liver, kidney, and testes. MDMA and its metabolites are known to damage kidney during their excretion through this organ. MDMA-induced rhabdomyolysis, which can lead to myoglobin deposition in the kidney, along with extreme dehydration and electrolyte imbalances may also contribute to acute and chronic renal failure (Mas et al. 1999).

The first case of AKI associated with ecstasy use was reported in 1992. The patient developed AKI after ingesting three doses of ecstasy and developed disseminated intravascular coagulation, which was likely contributory in the development of AKI (Fahal et al. 1992). Isolated proximal tubule dysfunction after ecstasy use has also been described in a single case report of an 18-yr-old who developed transient glycosuria, phosphaturia, and a solute dieresis (Kwon et al. 2003). One of the most serious medical complications of ecstasy abuse is related to symptomatic hyponatremia (Campbell and Rosner 2008). An increase in level of blood urea nitrogen (BUN) and creatinine was also reported in dose dependent manner in MDMA-treated rats (Ahmadizadeh et al. 2010). It has been demonstrated that MDMA can induce oxidative stress leading cell injury on various

tissues such as, liver, kidney, heart and retina (Miranda et al. 2007). Other studies showed that generation of MDMA metabolites in kidney may be responsible for kidney toxicity (Carvalho et al. 2002). Our findings along with others' confirmed that kidney is a potential target organ for ecstasy-induced toxicity.

To date, the effect of PTX on renal damage caused by Ecstasy has not been reported. However, there are many reports of nephroprotective effects of PTX (Nasiri-Toosi et al. 2013).

PTX reduced histological changes, such as congestion and tubular vacuolization in Cyclosporine-induced renal toxicity in ischemic kidney (Ates et al. 1996). The beneficial effects of PTX on gentamycin-induced alteration in glomerular basement membrane (GBM) was demonstrated (Stojiljković et al. 2009). Another study showed that PTX can reduce apoptosis by declining TNF-α and oxygen free-radical concentrations and it's vasodilatory and decongestant effects could induce a significant protective effect against Gentamycin-induced AKI (Stojiljkovic et al. 2009). After PTX treatment, minimal levels of hydropic epithelial cell degeneration, tubular dilatation, and vascular congestion were observed in Amikacin-induced nephropathy in male wistar rats. This observation supports the hypothesis that PTX can protect kidney tissue against oxidative damage caused by Amikacin treatment (Ozer et al. 2009).

Antioxidant effects of PTX on Cisplatin-induced nephrotoxicity in male Wistar rats were examined. PTX alleviated Cisplatin-induced morphologic changes (Saad et al. 2004). The protective effects of PTX also have been studied on Adriamycin-induced nephrotoxicity in rats. PTX prevented tubular and interstitial apoptosis in renal tissue (Usta et al. 2004). The positive effects of PTX on cigarette smoking-induced renal tissue damage in rats was reported. It was found that PTX prevents the negative effects of smoking on glomerular diameter, glomerular cell number and proximal tubule cell number. Their study shows that PTX treatment is effective in preventing the negative effects of cigarette smoking on kidneys by inhibiting cell damage with its antioxidant properties (Ozkurkcugil et al. 2011).

The result of present study showed that the mean GD of experimental and control groups were similar to each other. A similar effect was observed in the evaluation of mean GCN. Ecstacy abuse significantly reduced GCN and GD in affected kidneys while they were protected in rats under PTX treatment. A significant decrease was observed in number of proximal tubule cells in ecstasy administered rats that did not receive PTX. In contrast, mean PTCN was not similar between experimental and control groups. These results showed that PTX treatment can not prevent the negative effects of Ecstasy on PTCN. In tubulointerstitial injury evaluation, PTX administration led to less damage in PTX treated group.

Conclusion

The results of present study suggest that Pentoxyfilline have the potential to prevent the negative effects of Ecstasy on kidney glomerulus by inhibiting cell damage. Further studies are needed to more elucidate the exact mechanism by which Pentoxyfilline induces renal protection.

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