

Original Article

Effects of Ecstasy on Mouse Cardiac Histopathology, Electrocardiogram and Blood Cell Counts

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ABSTRACT

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Keywords

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Background and Aims: Ecstasy or 3-4-methylenedioxyamphetamine (MDMA) is a brain stimulant and a hallucinogenic material prepared by chemical changes in amphetamine. The aim of this study was to evaluate the changes induced by this drug in mouse cardiac histopathology, electrocardiogram (ECG) and blood cell counts.

Materials and Methods: In this experiment, 3 groups (n=10) of mice were enrolled. Group 1, as control, received placebo. Group 2 mice were given single daily low dose (20 mg/kg/d for 28 days) of intraperitoneal MDMA, and group 3 were given single daily high dose (40 mg/kg/d for 28 days) of intraperitoneal MDMA. An AVF lead ECG record was obtained, a blood sample was taken for complete blood counts, and the heart was removed for microscopic study of tissue sections with routine staining.

Results: The group 3 showed significant decrease in erythrocyte indices, myocarditis in 7 cases and monocyte infiltration around cardiac myocytes in 6 cases. In group 2, lower degree of myocardial injury was observed, but significant increase in QT and QTc durations was observed in ECG. In high dose group, red blood count, hematocrit, mean cell volume and mean corpuscular hemoglobin concentration showed significant changes in comparison with the control group.

Conclusion: Ecstasy can affect red blood cell index and lead to anemia. Many monocytes may be seen around cardiac cells, and increased ventricular depolarization and repolarization can lead to increase in QRS-QT interval. Combination of myocarditis, arrhythmia and sinus tachycardia reflect change in cardiac function and myocardial structure. Cardiac injury due to hypoxia and ischemia may cause myocardial infarction.

Introduction

Synthetic drugs such as ecstasy are currently under wide and growing abuse in the world. The young consume them for many reasons: curiosity, escape from psychic stress, social motivations and others. Many of these drugs, beside their direct effects on the nervous system, can have deleterious effects on body systems including heart, kidney, liver etc. Endocrine system is also affected by these drugs, which may lead to increased body temperature by actions on hypothalamic-pituitary-thyroid axis [1]. Their effects on hypothalamic-pituitary-adrenal axis can cause increased concentration of cortisol [2]. One of the nervous system stimulants is 3-4-methylenedioxyamphetamine (MDMA) or ecstasy, which was first introduced in 1985 by chemical changes made in amphetamine for therapeutic purposes [3]. This hallucinogenic agent is illegally distributed in the form of powder, capsule or tablet in various colors, and is usually shows its effects within 20-60 minutes of use. Its intestinal absorption is slow, leading to peak serum concentration after 2 hours of ingestion [4]. MDMA, like other psychotropic drugs, has fast stimulating impact as well as long-term side effects. Its short-term effects include tachycardia, hypertension, cardiac arrhythmias, headache, agitation, blurred vision, panic attacks, hepatic toxicity, convulsion, immunosuppression,

hypermenorrhea and hyperthermia. There are reports of disseminated intravascular coagulation, atrial fibrillation, subarachnoid hemorrhage, and acute liver failure as well. Long-term consequences of their abuse include damage to serotonergic neurons resulting in decreased memory functions [5,6]. Cardiac manifestations of its abuse may be associated with increased number of mitochondria in cardiomyocytes. Dilated cardiomyopathy after consumption of ecstasy has an incidence of 1 in 2500 users [7]. Beta blockers are frequent causes of toxicity in users of these drugs, which is due to induced hypertension and coronary artery spasm. A double blind study on sixteen healthy persons consuming MDMA concluded that beta blockers can prevent tachycardia, but are unable to combat hypertension and other side effects [8]. Other drugs of abuse may also cause severe cardiac diseases. For example, myocardial hypertrophy is a known side effect of cocaine, and a risk factor for myocardial infarction, congestive heart failure and sudden death [9].

Since there is little knowledge about the mechanism of actions of MDMA on the heart, the aim of this study was to evaluate the changes induced by this drug in mouse cardiac histopathology, electrocardiogram (ECG) and complete blood counts (CBC).

Materials and Methods

In this experiment, 30 adult (6 weeks) male Balb/c mice weighing 25 grams on average were included, which were randomly divided into 3 groups (n=10): the control group (which received placebo), low dose (LD) group receiving 20 mg/kg/day as single injection, and high dose (HD) group receiving toxic 40 mg/kg/day. Five mg of MDMA (Mehrदारو Co, Iran) was dissolved in 10 ml of distilled water and was drawn into insulin syringes to be injected intraperitoneally, 1 ml/day for the LD group. For HD group, 10 mg of MDMA was used in the same manner. After 28 days, the mice were anesthetized by 100 mg/kg sodium thiopental, and subcutaneous needles on the limbs were then connected to ECG electrodes for an AVF tracing to be recorded. Then 1 ml of blood was withdrawn from the ophthalmic vein to be mixed with 1.5 mg of K₂EDTA for the CBC test, using hematology analyzer Sysmex KX-21 (Sysmex, Japan). We determined white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), mean cell volume (MCV), mean

corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet values. Thoracic cage of the mice was then opened, and the heart was removed to be fixed in phosphate-buffered 10% formalin at 25°C. Two sections were prepared from the right and left ventricles of each heart (totally 60 sections), paraffin embedded, and stained with hematoxylin and eosin (H&E). Histopathologic evaluation of cardiac muscle was performed, looking specifically for tissue necrosis, atrophy/hypertrophy, myocarditis, endocarditis, rhabdomyolysis, monocytic infiltration around cardiomyocytes, fibrosis and other visible changes. The Ethics Committee of Shahid Sadoughi University of Medical Sciences (Yazd, Iran) approved this research.

Statistical Analysis

All of the data were analyzed by the SPSS 16 using mean, chi-square, ANOVA and Bonferroni tests. Any P. value less than 0.05 was considered as a significant difference.

Results

Table 1. shows the effects of two doses of ecstasy on CBC parameters.

Table 1. CBC parameters in the three studied groups.

Variable	Control	Low dose	High dose	P. value
WBC ($\times 10^9/L$)	3.40 \pm 1.25	3.62 \pm 1.24	3.98 \pm 1.56	0.634
RBC ($\times 10^{12}/L$)	8.90 \pm 0.52	7.57 \pm 0.63	7.53 \pm 1.61	0.010*
Hb (g/dL)	13.39 \pm 0.53	12.87 \pm 0.9	12.78 \pm 1.95	0.524
Hct (L/L)	48.26 \pm 2.59	41.36 \pm 3.2	34.26 \pm 13.11	0.002*
MCV (fL)	54.21 \pm 0.76	53.51 \pm 1.68	50.68 \pm 1.16	0.00*
MCH (pg)	15.16 \pm 0.51	17.01 \pm 0.42	17.56 \pm 4.5	0.12
MCHC (g/dL)	27.78 \pm 0.64	31.71 \pm 0.97	34.55 \pm 8.42	0.017*
Platelet ($\times 10^9/L$)	990.4 \pm 248.97	912.8 \pm 394.11	687.2 \pm 315.11	0.115

All data are presented as Mean \pm SD

*Significant difference

It shows that the means of RBC count, Hct and MCV are significantly lower in the high dose ecstasy groups compared with the control group. The means of RBC count are significantly lower in the low dose ecstasy group than in the control group. Moreover, the

mean of MCHC is significantly higher in the high dose ecstasy group. The Bonferroni test compared the P values of parameters between the two groups, as is shown in Table 2.

Table 2. P values of CBC parameters compared between the two groups by Bonferroni test

Groups compared	WBC	RBC	Hb	Hct	MCV	MCH	MCHC	Plt
LD vs. HD	1.000	1.000	1.000	0.167	0.000*	1.000	0.620	0.396
Control vs. HD	1.000	0.020*	0.889	0.002*	0.000*	0.153	0.014*	0.139
Control vs. LD	1.000	0.025*	1.000	0.187	0.677	0.381	0.254	1.000

*Significant difference

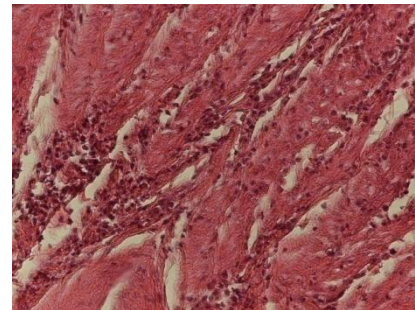
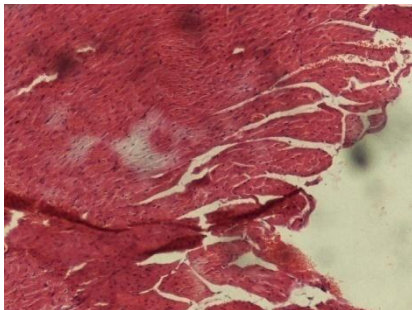
LD=low dose; HD=High dose

In Table 3, microscopic examination of cardiac tissue is displayed, which indicates a significant difference between the HD and the control groups regarding the presence of inflammation and the number of monocytes around cardiomyocytes. The table shows no significant difference between the LD and the

control group. We studied myocardial tissue for tissue necrosis, myocardial hypertrophy, atrophy, myocarditis, endocarditis, rhabdomyolysis, but did not find these changes in any of our study groups. No other pathologic change was found.

Table 3. Microscopic findings in the heart of mice in 3 groups.

Pathologic findings	High dose	Low dose	Control	P. value
Myocarditis	7	4	0	0.0049
Monocytes around myocardial cells	6	4	0	0.0149



a) Normal myocardial tissue in the control group

b) Myocardium, infiltration by mononuclear cells

Fig. 1. Myocardium: normal (a) and infiltration by mononuclear cells (b), H&E stain, ×100

Fig. 1 shows the normal myocardial tissue in one of the mice in the group control (a) and myocarditis in one of the mice in HD (b).

Table 4 shows the duration and height of waves and intervals in the ECG of the three study groups.

Table 4. ECG parameters in the three groups of mice.

Parameter	High dose	Low dose	Control
RR interval	0.118±0.004	0.13±0.015	0.128±0.08
Heart Rate	510.4±16.61	432.2±24.99	485.3±25.3
PR interval	0.034±0.001	0.034±0.001	0.032±0.00
P duration	0.01±0.001	0.01±0.00	0.01±0.00
QRS duration	0.012±0.00	0.01±0.00	0.01±0.00
QT interval	0.025±0.001	0.03±0.007*	0.02±0.00
QT c interval	0.071±0.0034	0.09±0.02*	0.06±0.001
P amplitude	0.013±0.002	0.01±0.002	0.01±0.001
Q amplitude	0.0007±0.0004	0.0016±0.00	0.0002±0.00
R amplitude	0.1198±0.0078	0.13±0.008	0.14±0.02
S amplitude	-0.097±0.0127	-0.11±0.009	-0.097±0.013
T amplitude	0.038±0.0076	0.025±0.005	0.04±0.003

*Significant difference Data are presented as mean±SD

Duration of the QT and QTc intervals are meaningfully more in the LD group than in the control group ($p < 0.05$), which could theoretically result in arrhythmias in the ecstasy abusers. Mean of the heart rate was higher in the HD group compared with the other groups, but the difference was not significant.

Discussion

This study compared CBC parameters, cardiac histopathology and ECG between 10 control mice, 10 mice receiving 20 mg/kg/d of intraperitoneal MDMA injection for 28 days, and 10 mice receiving 40 mg/kg/d of the drug. For the first time this study showed anemia by CBC and also kind of arrhythmia (QT - interval) after MDMA use. The observed dose-dependent significant fall in RBC count, Hct and MCV in the ecstasy groups shows that

this drug can cause anemia, the exact mechanism of which requires more studies. This effect of ecstasy was not mentioned before. Cardiac tissue damage due to amphetamines has attracted much attention during the last years. A study similar to ours conducted on rats after administration of MDMA indicated contraction bands in cardiomyocytes after 6 hours, and monocyte/macrophage accumulation around these cells after 16 hours [10]. Acute toxicity from ecstasy can be manifested by cardiac myocytolysis and hypertrophy in mice [11]. Another study on rats, following methamphetamine use for one week, showed cellular degeneration in subendocardial tissue. After 8 weeks, it resulted in myocytolysis and contraction band necrosis, associated with hypertrophy, cellular vacuolization, fibrosis, and mitochondrial injury [12]. A case report of

death in a 39-years old female after oral intake of ecstasy claimed that oral MDMA can induce cardiotoxicity, arrhythmia and cardiovascular collapse. Brain tissue necrosis, severe bronchopneumonia, hepatic injury and rhabdomyolysis with resulting myoglobinuria were also noted in that case [13]. In a forensic pathology study on 169 corpses with amphetamines in blood, it was proved that cerebral hemorrhage was the cause of death in 6 cases, and serotonin syndrome was found in 3 others. Heart disease was detected in 19 of them [14]. A comparison of 60 control subjects and 60 methamphetamine abusers demonstrated that methamphetamine can induce lipid peroxidation, inflammatory response, transaminitis, leukocytosis, thrombocytosis and anemia [15]. Binge administration of ecstasy may cause left ventricular dilatation in rats [16]. Yu et al. (2002) found vasoconstriction, myocardial hypertrophy, fibrosis and dilated cardiomyopathy following MDMA usage, all attributed to increased catecholamines acting on the mouse heart [17]. Also, Cerretani et al. (2008) described contraction band necrosis, macrophage accumulation around necrotic cardiomyocytes, and then calcium precipitation in the rat heart, all ascribed to oxidative stress and elevation of intracellular calcium [18].

A number of studies have tried to understand the mechanism of MDMA cytotoxicity, including measurement of cytokines in the rat heart after its intraperitoneal administration, thus showing high levels of cardioinhibitory cytokines after 3 and 6 hours of injection and

also its presence in plasma as early as 6 hours. Those rats died after 4 hours had high cardiac IL-6 and IL-1 β in western blot analysis [19]. In another study, ecstasy was identified as the cause of two consecutive myocardial infarctions during 3 months, which is believed to be due to increased levels of serotonin, dopamine and epinephrine in brain, leading to adverse cardiovascular effects [20]. Still others have tried to correlate neural and cardiac effects of MDMA toxicity based on changes in serotonin concentration [21]. It has been shown that ecstasy may increase mitogenic response in cardiac valves through a 5-hydroxytryptamine related mechanism, and can cause tricuspid regurgitation [22]. Electrocardiographic abnormalities due to MDMA in rabbits have been attributed to inhibition of nitric oxide synthase [23]. Methamphetamine increases catecholamine levels, which has detrimental effects on heart function through vasoconstriction, myocardial hypertrophy, and fibrosis [24].

One of the limitations of our study was lack of electron microscopic facilities, which would ideally focus on mitochondria, cell membranes and other intracellular components. So, we suggest more studies on the subject, with the final goal of development of both diagnostic and therapeutic measures for ecstasy abusers.

Conclusion

Ecstasy can cause decrease in RBC, Hb, and Hct, leading to anemia. Many monocytes were seen surrounding cardiac cells (myocarditis). Ventricular depolarization and repolarization

can lead to prolongation of QRS-QT interval. Combination of myocarditis, arrhythmia and sinus tachycardia reflects change in cardiac function and myocardial structure. Cardiac injury due to hypoxia and ischemia can cause myocardial infarction.

Conflict of Interest

There is no conflict of interests.

References

- [1]. Sprague JE, Banks ML, Cook VJ, Mills EM. Hypothalamic-pituitary-thyroid axis and sympathetic nervous system involvement in hyperthermia induced by 3, 4-methylenedioxymethamphetamine (Ecstasy). *J Pharmacol Exp Ther* 2003; 305(1): 159-66.
- [2]. Gerra G, Bassignana S, Zaimovic A, Moi G, Bussandri M, Caccavari R, et al. Hypothalamic-pituitary-adrenal axis responses to stress in subjects with 3,4 -methylenedioxy-methamphetamine ('ecstasy') use history: correlation with dopamine receptor sensitivity. *Psychiatry Res* 2003 Sep 30; 120(2): 115-24.
- [3]. Faria R, Magalhães A, Monteiro PR, Gomes-Da-Silva J, Amélia Tavares M, Summavielle T. MDMA in adolescent male rats: decreased serotonin in the amygdala and behavioral effects in the elevated plus-maze test. *Ann N Y Acad Sci* 2006; 1074: 643-9.
- [4]. Mas M, Farre M, de la Torre R, Roset PN, Ortuno J, Segura J, et al. Cardiovascular and neuroendocrine effects and pharmacokinetics of 3, 4-methylenedioxymethamphetamine in humans. *J Pharmacol Exp Ther* 1999; 290(1): 136-45.
- [5]. Koesters SC, Rogers PD, Rajasingham CR. MDMA ('ecstasy') and other 'club drugs'. The new epidemic. *Pediatr Clin North Am* 2002 Apr; 49(2): 415-33.
- [6]. Beebe DK, Walley E. Smokable methamphetamine (ice): an old drug in a different form. *Am Fam Phys* 1995; 51: 449-53.
- [7]. Mizia-Stec K, Gasior Z, Wojnicz R, Haberka M, Mielczarek M, Wierzbicki A, et al. Severe dilated cardiomyopathy as a consequence of Ecstasy intake. *Cardiovasc pathol* 2008; 17(4): 250-53.
- [8]. Hysek CM, Vollen Weider Fx, Liechti ME. Effects of a beta-blocker on the cardiovascular response to MDMA (Ecstasy). *Emerg med J* 2010; 27(8): 586 - 89. Epub 2010 April 8.
- [9]. Patel MM, Belson MG, Wright D, Lu H, Heninger M, Miller MA. Methylenedioxymethamphetamine (ecstasy)-related myocardial hypertrophy: an autopsy study. *Resuscitation* 2005; 66(2): 197-202.
- [10]. Shenouda Sk, Carvalho F, Varner KJ. The cardiovascular and cardiac actions of MDMA ecstasy and its metabolites. *Curr Pharm Biotechnol* 2010; 11(5): 470-75.
- [11]. Gesi M, Lenzi P, Soldani P, Ferrucci M, Giusiani A, Fornai F, et al. Morphological effects in the mouse myocardium after methylenedioxymethamphetamine administration combined with loud noise exposure. *Anat Rec* 2002; 267(1): 37-46.
- [12]. Yi SH, Ren L, Yang TT, Liu L. Myocardial lesions after long term administration of methamphetamine in rats. *Chin Med Sci J* 2008; 23(4): 239-43.
- [13]. Sano R, Hasuike T, Nakano M, Kominato Y, Itoh H. A fatal case of myocardial damage due to misuse of the "designer drug" MDMA. *Leg Med (Tokyo)* 2009 Nov; 11(6): 294-97.
- [14]. Pilgrim JL, Gerostamoulos D, Drummer OH. Involvement of amphetamines in sudden and unexpected death. *J Forensic* 2009; 54(2): 478-85.
- [15]. Suriyaprom K, Tanateerabunjong R, Tungtrongchitr. Alterations in malondialdehyde levels and laboratory parameters among methamphetamine abusers. *J Med Assoc Thai* 2011; 94 (12): 1533-39.
- [16]. Shenouda SK, Lord KC, McIlwain E. Ecstasy produces left ventricular

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- dysfunction and oxidative stress in rats. *Cardiovasc Res* 2008; 79(4): 662-70.
- [17]. Yu Q, Montes S, Larson DF, Watson RR. Effects of chronic methamphetamine exposure on heart function in uninfected and retrovirus-infected mice. *Life Sciences* 2002; 71: 953-65.
- [18]. Cerretani D, Riezzo I, Fiaschi AI, Centini F, Giorgi G, D'Errico S, et al. Cardiac oxidative stress determination and myocardial morphology after a single ecstasy (MDMA) administration in a rat model. *Int J Legal Med* 2008; 122(6): 461-69.
- [19]. Neri M, Bello S, Bonsignore A, Centini F, Fiore C, Földes-Papp Z, et al. Myocardial expression of TNF-alpha, IL-1beta, IL-6, IL-8, IL-10 and MCP-1 after a single MDMA dose administered in a rat model. *Curr pharm biotechnol* 2010; 11(5): 413-20.
- [20]. Sadeghian S, Darvish S, Shahbazi S. Two ecstasy induced myocardial infarctions during a three month period in a young man. *Arch Iran Med* 2007; 10(3): 409-12.
- [21]. Baumann MH, Rothman RB. Neural and cardiac toxicities associated with 3,4-methylenedioxymethamphetamine (MDMA). *Int Rev Neurobiol* 2009; 88: 257-96.
- [22]. Setola V, Hufeisen SJ, Grande-Allen KJ, Vesely I, Glennon RA, Blough B, et al. 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. *Mol pharmacol* 2003; 63(6): 1223-29.
- [23]. Tiangco DA, Halcomb S, Lattanzio FA, Hargrave BY. 3,4-Methylenedioxymethamphetamine alters left ventricular function and activates nuclear factor-kappa B (NF-κB) in a time and dose dependent manner. *Int J Mol Sci* 2010; 11(12): 4843-63.
- [24]. Qianli Yu, Sergiomontes, Douglas F. Effects of chronic methamphetamine exposure on heart function in uninfected and retrovirus-infected mice. *Health promotion science division* 2002; 953-65.