


Original Article



Combination effect of ecstasy and curcumin on hematological parameters and serum immunoglobulin levels in early and late phase in male rats

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Abstract

Introduction: Consumption of ecstasy (3,4-methylenedioxymethamphetamine, MDMA), a derivative of amphetamine, can result in various undesirable side effects including hematological and serological parameters. This study is intended to examine the effects of curcumin along with MDMA in the early and late phase of consumption on hematological parameters and serum immunoglobulins (IgM, IgG and IgA) levels.

Methods: We used 56 male rats that are divided into 7 groups: group1 (control), group2 (MDMA+vehicle1), group3 (curcumin), group4 (MDMA+early curcumin), group5 (MDMA+vehicle2), group6 (MDMA+late curcumin) and group7 (MDMA+early&late curcumin). The groups were received MDMA (20mg/kg) orally and curcumin (20µM/kg) intra-peritoneally for 2 and 4 weeks (n=8). After 24h of final dose, rats were anesthetized and blood samples were collected and used for determination of hematological parameters and IgM, IgG and IgA levels using a Coulter Automated Cell Counter and ELISA kit.

Results: Our data indicated that either MDMA alone or in combination with curcumin in both early and late phases decreased lymphocytes, platelet, total leukocyte count and RBC, MCHC, RDW, immunoglobulin levels, as well as hemoglobin content in comparison with the control group. In contrast, the amount of neutrophils, eosinophils, monocytes, mean cell volume and HCT increased. Furthermore, we observed blood parasites of red blood cells in the MDMA groups with curcumin.

Conclusion: In conclusion, MDMA alone and in combination of curcumin altered the hematological parameters. Furthermore, their combination therapy induces toxic effects on hematological parameters and serum immunoglobulin levels. This is a serious consequence for recreational drug users.

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Keywords:

Ecstasy;
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Introduction

Ecstasy (3, 4 methylenedioxymethamphetamine,

MDMA), is a popular drug of abuse (Connor et al., 1998), which releases serotonin and dopamine in brain synaptic cleft that causing particular effects

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(Kankaanpää et al., 1998). As a results of repeated consumption, tolerance develops in the serotonergic neuronal system, consequently the more consumption of higher quantities of MDMA the more criminal convictions and personality disorders can occur (Jaehne et al., 2008). In addition, there are other consequences including acute toxic effects such as cardiac arrhythmias, renal failure, hepatotoxicity, rhabdomyolysis, seizures and intracranial hemorrhage that might be fatal in some cases (Hall and Henry, 2006). Studies over the last decade have indicated that the number of drugs of abuse such as MDMA has a potent immunosuppressive effect. (Friedman et al., 2003; Boyle and Connor, 2010). Thus, consumption of MDMA could have a deleterious effects because of its immune compromised properties that result in many infection due to the diminished host immune system (Cabral and Marciano-Cabral, 2004). It is a fact that the immune system is a defense system including the innate immune system and the adaptive immune system. This complex system maintains the integrity of the body and protects it against disease. (Arshad et al., 2017). The innate immune system is a non-specific immunity and serves as a primary defense for the body. Neutrophils, macrophages and dendritic cells have a major role in this process (Arshad et al., 2017). The adaptive immune cells (T cells, B cells and natural killer cells) produce immunological memory after the first encounter to a specific antigen (Lee et al., 2015). The immunosuppressive effects of MDMA were maximal 3-6h following drug administration, and in some cases were evident 24h later up to 48h. However there are a little information about the long time period of MDMD administration and drug withdrawal alterations.

Curcumin is a polyphenolic compound derived from the rhizome of the plant *Curcuma longa*. It is a natural antioxidant, antimicrobial and anti-inflammatory product and possesses expanded therapeutic properties in various chronic disease including cardiovascular, neurodegenerative, autoimmune, pulmonary, metabolic, gastrointestinal, psychotropic disorders and chronic wound healing (Hussain et al., 2017). Also, It was claimed that curcumin analogs exert immunomodulatory role (Lal et al., 2012; Bukhari et al., 2013). As it is known, curcumin commonly use in numerous food ingredients and co-

administration of this traditional natural medicine and MDMA on blood cells and serum immunoglobulin have not yet been studied. In order to investigate the deleterious effects of MDMA consumption, we investigate some of the hematological parameters and serum immunoglobulins levels in presence and without curcumin.

Materials and methods

Animals

Male Wistar rats weighing 230 ± 30 g were housed four per cage and maintained on a 12h light–dark cycle in an air-conditioned constant temperature ($22\pm 1^\circ\text{C}$) room. Food and water were available *ad libitum* at all times. The Ethic Committee for Animal Experiments of Urmia University of Medical Sciences approved the study plan, and all experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (IR.UMSU.REC.1395.281).

Drug preparation and administration

MDMA [The National Institute for Drug Abuse (NIDA), United States] and curcumin (Sigma-Aldrich, Ireland) were dissolved in 0.9% NaCl and ethyl oleate respectively. MDMA (20mg/kg) was administered orally and curcumin ($20\mu\text{M}/\text{kg}$) injected intra-peritoneally in rats.

Experimental design

Fifty-six rats were randomly divided into seven experimental groups ($n = 8$) (Shirpoor et al., 2019; Sadeghzadeh et al., 2019; amini et al., 2020) as follows: group1 (control), 2ml distilled water orally+ 0.2ml ethyl oleat intra-peritoneally for 2weeks; group2 (MDMA+vehicle1), 20mg/kg (connor et al., 1998) MDMA orally+ 0.2ml ethyl oleate intra-peritoneally for 2weeks; group3 (curcumin), $20\mu\text{M}$ curcumin intra-peritoneally for 2weeks; group4 (MDMA+early curcumin), 20mg/kg MDMA orally+ $20\mu\text{M}$ curcumin intra-peritoneally for 2weeks; group5 (MDMA+vehicle2), 20mg/kg MDMA orally+ 0.2ml ethyl oleate intra-peritoneally for 2weeks and after that, 2ml distilled water orally+ 0.2ml ethyl oleate intra-peritoneally for another 2weeks; group6 (MDMA+late curcumin), 20mg/kg MDMA orally+ 0.2ml ethyl oleate intra-peritoneally for 2weeks and after that, 2ml distilled water orally+ $20\mu\text{M}$ curcumin intra-peritoneally for another 2weeks; group7

(MDMA+early&late curcumin), 20mg/kg MDMA orally+ 20µM curcumin intra-peritoneally for 2weeks and after that, 2ml distilled water orally+ 20µM curcumin intra-peritoneally for another 2weeks.

Sample collection

Blood samples from anesthetized rats (pentobarbital sodium, 35mg/kg, ip) (Naderi et al., 2019) were obtained by cardiac puncture into EDTA and cloth vial respectively for hematological analyses and immunoglobulin's measurement under sterile condition (Gholizadeh-Ghaleh Aziz et al., 2019).

Hematological analysis

Complete blood counts (total and differential) and estimation of hematological indices, including total hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW) and blood platelet count (PLT) were performed using a Coulter Automated Cell Counter (LH500) (Ghosh et al., 2017).

Immunoglobins measurements

Enzyme-linked immunosorbent assay (ELISA)

In order to measure the serum immunoglobins including IgM, IgG and IgA we used sandwich ELISA and followed the manufactures instruction. Whole blood was clotted and centrifuged at 2325g for 5min at room temperature. Serum was obtained from each sample and kept at -80°C until used. Serum IgM, IgG

and IgA were measured using an ELISA kit (Shanghai Sangon Biotech, Inc., Shanghai, China). First, 40µl samples or standards were added to wells coated with anti-rat IgM, IgG and IgA antibodies, followed by addition of 10µl of anti-IgM, anti-IgG and antiIgA antibodies and streptavidin-horseradish peroxidase to each well and incubation at 37°C for 60min. Plates were washed three times with washing liquid and 100µl substrate solution was added to each well prior to incubation in the dark at 37°C (Shao et al., 2017).

Statistical analysis

Normality was checked by the Kolmogorov-Smirnov test. Data were statistically analyzed using SPSS version 16 by one-way analysis of variance (ANOVA) followed by Tukey's test. The significant level was set at $P<0.05$. Results are expressed as mean±SD.

Results

Results of the hematological examination including WBC (total leukocyte count), lymphocytes, platelet, neutrophils, eosinophils and monocytes are presented in Table 1. MDMA alone and in combination with curcumin in early and late phases decreased total leucocyte count, lymphocytes, platelet and increased neutrophils, eosinophils and monocytes compared to control and MDMA groups respectively ($P<0.001$). However, these parameters were not significant in MDMA treated rats alone.

There was no statistical significant differences in

Table 1: Effects of curcumin administration on white blood cell parameters after MDMA treatment. Data expressed as mean±SD (n=8).

| | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 |
|-----------------------------------|------------|------------|------------|------------------------------|------------|------------------------------|------------------------------|
| WBC 10³/µL | 8.78±0.14 | 8.70± 0.11 | 8.74±0.12 | 8.48±0.16 | 8.58±0.16 | 8.35±0.35 | 7.48±0.24 ^{\$\$\$} |
| N% | 20.13±1.16 | 21.25±0.89 | 20.1±1.17 | 22.25±0.46 | 21.50±1.31 | 23.75±0.89 ^{\$} | 29.13±1.02 ^{\$\$\$} |
| E% | 0.50±0.53 | 0.25±0.46 | 0.45±0.73 | 4.25±0.89 ^{\$\$\$} | 0.25±0.46 | 6.25±1.16 ^{\$\$\$} | 11.50±0.53 ^{\$\$\$} |
| M% | 0.75±0.66 | 0.75±0.89 | 0.73±0.76 | 0.50±0.53 | 0.75±0.89 | 0.50±0.53 | 3.75±0.89 ^{\$\$\$} |
| L% | 78.38±0.79 | 78.00±0.93 | 78.11±0.74 | 73.25±0.46 ^{\$\$\$} | 78.00±1.20 | 69.50±1.51 ^{\$\$\$} | 55.88±2.25 ^{\$\$\$} |
| Platelet 10³/µL | 849±1.69 | 852± 2.39 | 834±1.45 | 852±0.89 | 844±4.04 | 827±0.79 ^{\$\$\$} | 813±1.31 ^{\$\$\$} |

^{\$} $P<0.05$ and ^{\$\$\$} $P<0.001$ vs MDMA (vehicle1 and vehicle 2) groups (groups 2 and 5). Group1: control; group2: MDMA+vehicle1; group 3: curcumin; group4: MDMA+early curcumin; group5: MDMA+vehicle2; group6: MDMA+late curcumin and group7: MDMA+early&late curcumin.

WBC: white blood cell (total leucocyte count); N: neutrophils; E: eosinophils; M: monocyte, and L: lymphocyte.

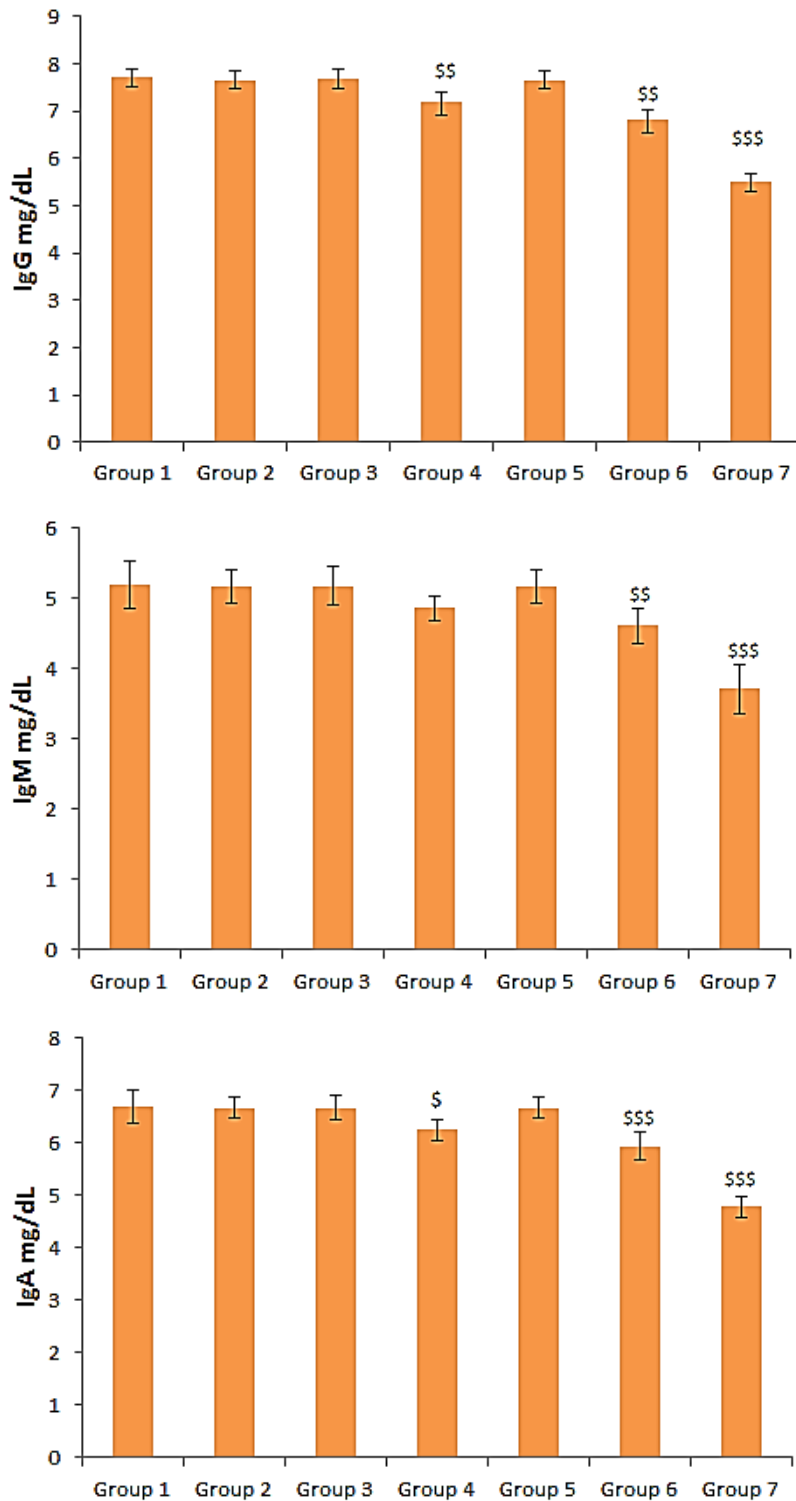


Fig.1. Serum immunoglobulin levels in different groups. Serum IgG, IgM and IgA levels were detected by sandwich ELISA. Data expressed as means±SD (n=8). Group1: control; group2: MDMA+vehicle1; group3: curcumin; group4: MDMA+early curcumin; group5: MDMA+vehicle2; group6: MDMA+late curcumin and group7: MDMA+early&late curcumin. \$ P<0.05, \$\$ P<0.01 and \$\$\$ P<0.001 vs MDMA+vehicle1 and MDMA+vehicle 2 groups.

basophils among groups (data are not shown). As shown in Table 2, MDMA alone and in combination with curcumin in the early and late phases caused a significant decrease in RBC, Hb, RDW ($P<0.001$) and MCHC ($P<0.05$) compared to control and MDMA

groups. However except RBC, they have not significant changes in MDMA treated rats alone. In addition, we observed that MCV and HCT increased in groups treated with both MDMA and curcumin together in comparison with the MDMA groups

Table 2: Effects of curcumin administration on hematological parameters after MDMA treatment. Data expressed as mean±SD (n=8).

| | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 |
|------------------------|-----------|------------------------|------------|---------------------------|------------|------------------------------|------------------------------|
| RBC10 ³ /μL | 7.8±0.07 | 7.6± 0.16 [*] | 7.74±0.12 | 7.37±0.05 ^{\$\$} | 7.3±0.15 | 7.2±0.1 ^{\$} | 5.82±0.08 ^{\$\$\$} |
| Hb | 14.5±0.31 | 14.4±0.31 | 14.43±1.17 | 14.2±0.1 | 14.3±0.13 | 13.8±0.2 ^{\$\$} | 13.22±0.15 ^{\$\$\$} |
| HCT | 43±0.75 | 43±1.06 | 43±1.01 | 45.25±2.05 ^{\$} | 42.25±2.05 | 42.5±2.87 | 44±0.75 |
| MCV | 48.9±0.32 | 48.9±0.07 | 48.8±0.33 | 49.1±0.1 | 48.75±0.11 | 52.87±0.15 ^{\$\$\$} | 56.47±1.62 ^{\$\$\$} |
| MCHC | 38.9±0.15 | 38.7±0.15 | 38.8±0.16 | 37.6±1.4 | 39±1.3 | 35.4±2.2 ^{\$\$} | 34.7±3.05 ^{\$} |
| RDW | 14.5±0.16 | 14.4±0.26 | 14.4±0.75 | 14.3±0.16 | 14.3±0.31 | 13.82±0.08 ^{\$\$\$} | 13.30±0.16 ^{\$\$\$} |

^{*}*P*<0.05 vs control, ^{\$}*P*<0.05, ^{\$\$}*P*<0.01 and ^{\$\$\$}*P*<0.001 vs MDMA (vehicle1 and vehicle 2) groups (groups 2 and 5). Group1: control, group2: MDMA+vehicle1; group3: curcumin; group4: MDMA+early curcumin; group5: MDMA+vehicle2; group6: MDMA+late curcumin and group7: MDMA+early&late curcumin.

RBC: red blood cell; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration and RDW: red blood cell distribution width.

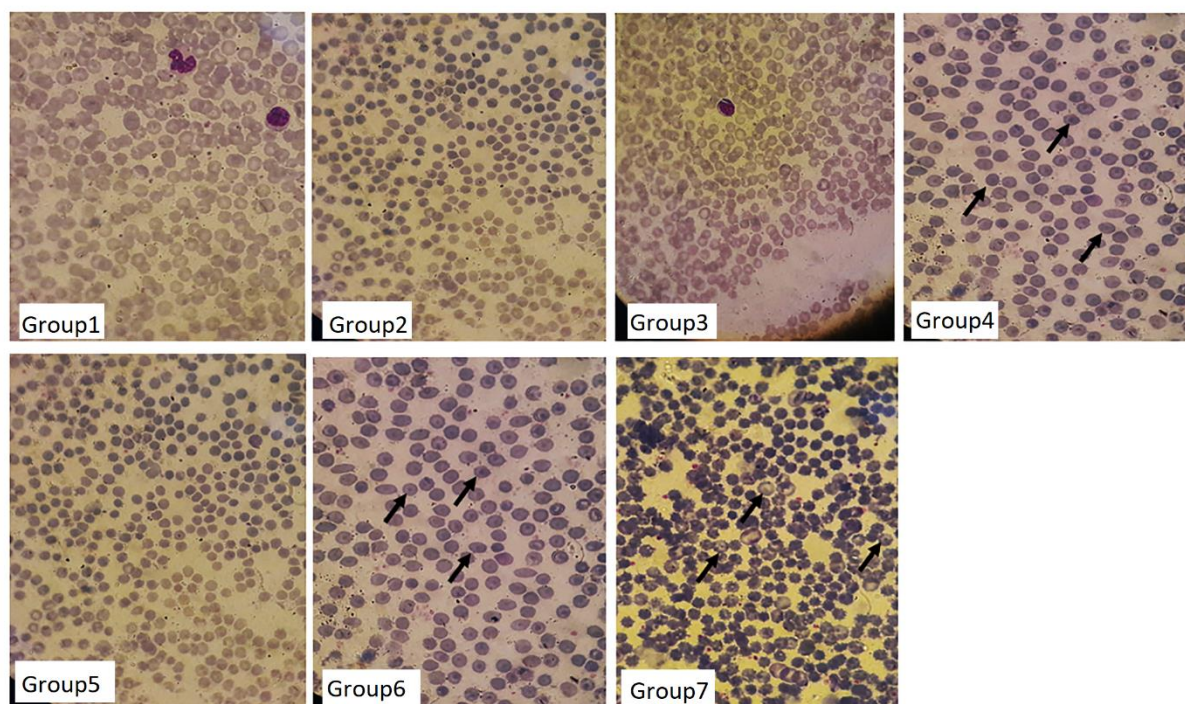


Fig.2. Blood smear and gimsa staining. Effect of curcumin and MDMA administration on Erythrocytes parasitic infection. Group 4, 6 and 7 showed parasites on blood cells (x 1000).

(*P*<0.001). The other important issue that was interestingly higher in RBCs of MDMA treated group that were received curcumin, was the presence of parasitic infection called Babesia with distribution of 10% of red blood cells in that groups (Fig. 2).

As shown in Figure 1, IgG, IgA and IgM levels were not different in MDMA treated groups. However, there

are significant reductions in IgG, IgA and IgM levels in MDMA+curcumin treated rats in early and late phases compared to MDMA+vehicle1 and MDMA+vehicle2 groups respectively (*P*<0.001).

Discussion

The present study showed that MDMA alone and in

combination with curcumin altered hematological parameters including significant decrease in total leukocyte count, lymphocytes, platelet, RBC, Hb, MCHC, RDW, Igs and increase in neutrophils, eosinophils, monocytes, HCT and MCV. However, in our study MDMA alone have not a significant effect in these cells and parameters. Since a proper function of immune system is important in host defense, any agent and concomitant materials with the potential for human use (or abuse), should be tested for its potential immunocompromised properties due to their profound adverse effects (House et al., 1994). Some previous studies *in vivo* and *in vitro* have demonstrated that MDMA acute administration leads to suppression of lymphocyte proliferation (Connor et al., 1998), B-cell proliferation (House et al., 1994) neutrophil oxidative burst (de Paula et al., 2009) and macrophagic monocytes distribution (Cerretani et al., 2008). These effects can be attributed to noradrenergic neurons and/or hypothalamic-pituitary-adrenal axis activations (de Paula et al., 2009) in which, cause leucocyte dissemination and produces a suppression of immune function and distribution (Dhabhar et al., 1995; Connor et al., 1998; Boyle and Connor, 2010). Consequently, the host resistance to various infections decrease and that can result in increased disease susceptibility (Boyle and Connor, 2010). However, it is possible that neutrophils accumulation may lead to release of reactive oxygen species (ROS) and elicit inflammatory responses (Wu et al., 2006), which may, in turn, recruit neutrophils (Cadet et al., 2003). MDMA increases oxidative stress, produce mitochondrial dysfunction and increase inflammation that culminates some toxic effects in individual (Yamamoto and Raudensky, 2008). Monocytes are responding to an accumulation of dead material and maturing to promote tolerance (Harms et al., 2012), It has been indicated that MDMA has an ability to induce apoptosis (Mobaraki et al., 2018). Eosinophils, with immune-modulating functions, are comparatively low in the blood. However, in several conditions such as allergic inflammation, parasite infections or even cancer, their number increase (Reichman et al., 2017). It has been shown that drug abuse severely suppresses the immune system and thereby, increases the risk of opportunistic infection (Roy et al., 2011). This is in line with our study to justify parasites on erythrocytes following MDMA and curcumin treatment.

Furthermore, the use of abuse drugs, such as ecstasy could induce severe aplastic anemia (Marsh et al., 1994; Clark and Butt, 1997) and MDMA leads to DNA damage (Alvarenga et al., 2010; Alvarenga et al., 2011). So, patients with aplastic anemia have lower hemoglobin values, higher reticulocyte counts, lower granulocyte and platelet values, and a higher MCV and fetal hemoglobin than normal controls (Tichelli et al., 1992). Dehydration maybe happened after consumption of MDMA and lead to elevation of HCT (Rigg and Lawental, 2018).

MDMA causes a biphasic effect on the production of cytokines and immunoglobulins. It was reported that MDMA prevented the conversion of IgM to IgG2a possibly by reducing interferon gamma (Connor et al., 2001). In another study, no significant effect on proliferation of B-cells and cytokines which is an essential physiological response following T-cell activation and proliferation (House et al., 1995). In our investigation, we measured hematological parameters after 24h treatment. Our data showed slightly alterations in hematological parameters, although they are not significant. However, we showed that MDMA in combination with curcumin altered mentioned factors. These results are in contradict with previous studies which are may be due to the duration of treatment or adaptation response which occurred after 2 or 4 weeks of treatment. Therefore, it seems that this effect is probably time-dependent. In addition, there may be a species-related differential sensitivity to this drug (Boyle and Connor, 2010).

According to the literatures, co-administration of MDMA with other chemical substances may greatly exacerbate the toxicity of MDMA whether this use is intentional or via impurities in the tablets (McNamara et al., 2007). After all, this is the first report that shows the synergistic exacerbating effect of curcumin with MDMA on hematological parameters. This effect is evident for acute curcumin administration and also consecutive exposures with a delay of 15 days after the first one exposure. Several investigations have shown that, curcumin can inhibit the innate immune system in autoimmune diseases, increased T helper1 cells in lung cancer, decreased the number of CD8+ T cells and reduced ROS production and inflammatory cytokines (Zou et al., 2018; Srivastava, 1989; Aggarwal and Harikumar, 2009; Arshad et al., 2017). However, in combination with MDMA, the

effect was complicated. To justify this, it was declared that, gastric mediated acid secretion reduced in curcumin-treated, therefore, gastric pH leads to a higher rate (Zhou et al., 2017). In addition, curcumin enhanced secretion of bile salts which increase PH in the lumen (Prakash and Srinivasan, 2012). Since MDMA uptake is significant pH dependence, alkalinization of extracellular pH increases the uptake rate, that is, an increase in the gradient of the outward proton concentration (Kuwayama et al., 2008).

Conclusion

While curcumin is generally regarded as safe and freely available in foodstuffs, ingestion with MDMA may have a potential to exacerbate the acute toxicity of the amphetamines and by modifying hematological parameters, it has remarkable healthy risks for human. So, it may well offer to provide insight to assess through multiple iterations of idiosyncratic reactions to MDMA. However, we did not study the molecular mechanisms of these interventions. More investigations may be needed to provide more information in this regard.

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Conflict of interest

The authors declare no conflict of interest.

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