Protective effects of atorvastatin and rosuvastatin on 3,4-methylenedioxymethamphetamine (MDMA)-induced spatial learning and memory impairment

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA) or “Ecstasy”, which has been used for recreational purposes, is shown to impair memory and brain functions. Statins, beyond their efficient cholesterol-lowering impact through inhibition of HMG-CoA reductase enzyme, possess multiple actions referred to as pleiotropic effects. In this regard, we aimed to investigate the neuroprotective effects of atorvastatin and rosuvastatin on MDMA-induced neurotoxicity. Adult male Wistar rats received atorvastatin (5, 10, and 20 mg/kg; orally) and rosuvastatin (5, 10, 20 mg/kg; orally) for 21 consecutive days. Then, spatial memory and learning were evaluated by Morris water maze (MWM) test. Rats were intraperitoneally injected with MDMA (2.5, 5, and 10 mg/kg) 30 min before the first training session in 4 training days of MWM task. Afterward, rats were euthanized and their hippocampuses were dissected to evaluate reactive oxygen species (ROS) production, lipid peroxidation (LPO), and caspase-3 and -9 activities. Our findings showed that MDMA (5 and 10 mg/kg) significantly impaired spatial memory functions and dramatically increased ROS production, LPO, and caspase-3 and -9 activities compared to control. Also, atorvastatin (5, 10, and 20 mg/kg) and rosuvastatin (20 mg/kg) significantly improved memory performances and inhibited the elevation of ROS, LPO, and caspase-3 and -9 activities induced by MDMA. In conclusion, the results indicated that MDMA-induced cognitive impairment is followed by oxidative stress and activation of apoptotic pathways in the hippocampus. However, atorvastatin and rosuvastatin suppressed these deleterious consequences of MDMA and revealed protective effects against activation of pathways leading to cell damage.

Keywords Spatial learning and memory · Neuroprotection · MDMA · Oxidative stress · Ecstasy · Statin
Introduction

The ring-substituted amphetamine derivate, 3,4-methylenedioxyamphetamine (MDMA), known as "Ecstasy," is a popular drug used for recreational purposes (Mead and Parrott 2020). The use of MDMA still falls behind cannabis, opioids, and other stimulant amphetamines worldwide (UNODC 2020, https://wdr.unodc.org/wdr2020/en/drug-use-health.html). Its consumption affects cognitive domains associated with robust deleterious effects on memory and learning, which has become a matter of great concern (McCardle et al. 2004). Several studies have been conducted to reveal the adverse effects of MDMA, especially neurotoxicity. Asl et al. (2013) found that MDMA administration in rats led to impairment of both acquisition and retention of spatial memory. Additionally, a relevant in vitro model has displayed the apoptotic cell death of cultured cortical neurons treated with either MDMA or its metabolites in a time and concentration manner (Capela et al. 2006). MDMA has been linked to the impaired cognitive performance and memory in regular MDMA users (Mead and Parrott 2020). However, all parts of the central nervous system are vulnerable to the neurotoxic effects of MDMA, hippocampus, and cerebral cortex have been found to be more susceptible (Costa et al. 2014).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are oxygen-containing and nitrogen-containing chemically reactive species. When the balance between the antioxidant system and ROS production disappears, oxidative stress develops, a phenomenon related to many pathological conditions (Di Meo et al. 2016). RNS, like ROS, can be either harmful or beneficial to the living system. Nitric oxide (NO), early recognized as an endothelium-derived relaxing factor in the blood vessels, is now identified as a mediator of many physiological processes, cellular toxicity, and tissue injury (Di Meo et al. 2016).

Various studies documented that MDMA administration perturbs the equilibrium between production and scavenging of ROS and RNS, which leads to a rise in the intracellular levels of free radicals and consequently causes oxidative/nitrosative stress (Cadet et al. 2001). Moreover, elevation of inflammatory mediators and immunological responses in the brain following MDMA administration could lead to neuronal death (Barbosa et al. 2012). There is a large body of literature on various neurodegenerative disorders and their connection with oxidative stress (Salim 2017) and neuroinflammation (Chen et al. 2016). The majority of ROS are derived from mitochondria, and it is postulated that mitochondrial disruption represents the common theme in neurodegenerative diseases (Mattson et al. 2008). For example, Taghizadeh et al. (2016) showed that MDMA administration disrupted mitochondrial electron transport chain activity and its oxidative defense integrity, causing cognitive deterioration. Therefore, it is important to investigate agents having mainly antioxidant, anti-inflammatory, and anti-excitotoxic properties to interfere with the MDMA cascade of events.

Statins are efficient and well-tolerated class of lipid-modifying agents that specifically and reversibly inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis. Statins are considered the first-line approach for managing dyslipidemia and preventing coronary artery diseases (Stone et al. 2014). Beyond their potent cardioprotective effects, they can exhibit neuroprotective strategies that may underlie their clinical benefits in neurodegenerative conditions (Kelley and Glasser 2014). Statins manifest multiple actions referred to as pleiotropic effects such as antioxidant activity against free radical production and effects on antioxidant enzymes (Stoll et al. 2004), inhibition of inflammatory responses through immunomodulating effects (Bu et al. 2011), anti-excitotoxic properties against overstimulation of glutamate receptors (Ramirez et al. 2011), and enhancing neurogenesis (Robin et al. 2014). However, the use of statins in neurodegenerative diseases is still controversial.

The various effects of statins on cognition and brain functions are related to their lipophilicity property (Saheki et al. 1994). Water-soluble or hydrophilic statins (e.g., rosuvastatin, fluvastatin, and pravastatin) do not readily pass the blood–brain barrier (BBB). In contrast, lipid-soluble or lipophilic statins (e.g., atorvastatin, simvastatin, and lovastatin) can easily penetrate the BBB. These findings elucidate that lipophilicity may influence statins’ properties on brain functions.

Based on the assumption that statins may have beneficial effects on cognitive abilities and brain functions, we aimed to investigate the impacts of two different statins, lipophilic atorvastatin and hydrophilic rosuvastatin, on MDMA-induced memory impairment and evaluate oxidative stress markers, as well as caspase-3 and -9 activities.

Materials and methods

Materials

MDMA was synthesized by the Department of Medical Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences according to the previous method (Pizarro et al. 2002). It showed an acceptable purity value (>95%), and its structure was fully confirmed with H-NMR, C-NMR, and mass spectrometry methodologies. The following chemicals were used in this study: KCl, MgCl₂, Tris-HCl, dimethylsulfoxide (DMSO), sodium succinate, Na₄HPO₄, sucrose, ethylene glycol-bis (2-aminoethyl ether)-N, N', N'-tetraacetic.
acid (EGTA), 4-2-hydroxyethyl piperazine ethanesulfonic acid (HEPES), Rhodamine 123 (Rh 123), 2’, 7’-dichloro-fluorescein diacetate (DCFH-DA), rotenone (Rot), Coomassie blue, ketamine, xylazine, and carboxymethylcellulose sodium (CMC). All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) except for atorvastatin (Darou Pakhsh Pharmaceutical Co., Tehran, Iran) and rosuvastatin (Abidi Pharmaceutical Co., Tehran, Iran).

Atorvastatin and rosuvastatin were suspended in an aqueous solution of CMC 1%, and MDMA was dissolved in saline. Solutions and suspensions of chemicals were always prepared on the day of the experiment.

Animals

In this study, adult male Wistar rats weighing 180–200 g were purchased from the Faculty of Pharmacy, Tehran University of Medical Sciences. Animals were housed in separate standard polypropylene cages under a standard temperature-controlled environment (23 ± 1 °C), 12-h light/12-h dark cycle schedule, humidity (55% ± 10%), and had free access to tap water and food. All ethical considerations and experimental protocols were performed in accordance with the AJA University of Medical Sciences ethics committee with the code number of IR.AJAUMS.REC.1400.026.

Spatial learning and memory assessment

Spatial memory and learning were evaluated by the Morris water maze (MWM) training protocol, the same as described previously (Taghizadeh et al. 2016; Eftekharzadeh et al. 2012). Briefly, the MWM test consisted of four trials per day for 4 consecutive days and one test session (probe test). In each trial, animals were randomly placed in one of the four quadrants of the MWM pool (north, east, south, and west). Then, they were allowed to find the hidden target platform, which was located 1 cm beneath the water’s surface, within 90 s. MDMA (2.5, 5, and 10 mg/ kg) or saline was administered intraperitoneally (I.P) to rats 30 min before the first trial in each 4 training days. Probe test was performed 24 h after the last training trial by removing the platform from the tank. A video camera was installed above the MWM pool to record the animal’s behavior. Different parameters of the MWM test, including escape latency (time spent to reach the platform), swimming speed, traveled distance (path length to find the hidden platform), and the time spent in the target quadrant (in the probe test), were calculated by the Etho-vision 7 tracking system (Noldus Information Technology, Wageningen, The Netherlands).

After the probe test of MWM, each group (n = 4) was anesthetized by injection of ketamine/xylazine (100 and 10 mg/kg, I.P, respectively) until loss of consciousness and loss of any response, and then euthanized under general anesthesia, and their hippocampuses were dissected on the ice-cold surface and stored at −80 °C until analysis.

Experimental groups

The animals were allocated to 12 groups. Each group contained eight rats.

Group 1: Control group was treated orally with a single daily dose of vehicle (CMC 1%) for 21 consecutive days, followed by injection of saline (I.P) 30 min before training sessions in MWM.

Groups 2 and 3: Atorvastatin or rosuvastatin + saline groups, which received atorvastatin or rosuvastatin (20 mg/kg, orally) once daily for 21 consecutive days, and then were injected (I.P) saline 30 min before the MWM task.

Groups 4, 5, and 6: MDMA groups received CMC 1% w/v orally once daily for 21 consecutive days. After this period, they were administered different doses of MDMA (2.5, 5, and 10 mg/kg; I.P) 30 min before the first training trial during 4 training days in the MWM task.

Groups 7, 8, and 9: Atorvastatin + MDMA groups, which received different oral doses of atorvastatin (5, 10, and 20 mg/kg) for 21 days, followed by MDMA (5 mg/kg, I.P) 30 min before the first training session in 4 training days of MWM.

Groups 10, 11, and 12: Rosuvastatin + MDMA groups, which received different oral doses of rosuvastatin (5, 10, and 20 mg/kg) for 21 days, followed by MDMA (5 mg/kg, I.P) 30 min before the first training session in 4 training days of MWM.

Different experimental groups are summarized in Table 1.

ROS assay

Production of ROS in hippocampal cells was measured by a fluorometric assay based on the conversion of 2’,7’-dichlorofluorescein diacetate (DCFH-DA), a non-fluorescent chemical to a fluorescent compound named 2’,7’-dichlorofluorescein (DCF). Samples were homogenized, 50 μl supernatant was added to 10 μl DCFH-DA and 162 μl assay buffer, and then solutions were incubated at 37 °C for 15 min. The fluorescence of DCF was examined spectrometrically by an ELISA fluorometer (Biotec, Tecan U.S.) with maximum excitation of 488 nm and maximum emission of 525 nm in the spectra within 60 min. Finally, the results were reported as the percentage of control, which was considered 100% (Baeeri et al. 2019).

Lipid peroxidation (LPO) assay

The end product of LPO is malondialdehyde (MDA) which reacts with Thiobarbituric acid (TBA) and produces a new chemical complex named TBA reactive substances.
(TBARS). Briefly, the samples were homogenized and mixed with 800 μL trichloroacetic acid 20% (TCA) followed by centrifugation at 3500 g for 30 min. Then, 600 μL of supernatant was mixed with 150 μL TBA (1% w/v). Finally, the cocktail was heated in a steaming water bath for 30 min followed by the addition of 400 μL n-butanol to extract TBRAS adducts. After cooling down, the absorbance was recorded at 532 nm by an ELISA reader (Baeeri et al. 2017).

Measurement of caspase-3 and -9 activity

Caspase-3 and -9 activities were evaluated using colorimetric assays based on the distinctive identification of specific amino acid sequences in these enzymes. A tetrapeptide substrate, containing a particular caspase recognition sequence, was labeled with the chromophore r-nitroaniline (ρNA). Then, ρNA was released from the substrate through cleavage by caspase and produced a yellow color, which its intensity was recorded using an ELISA reader at 405 nm. The amount of caspase activity is directly proportional to the production of yellow color upon cleavage. Concisely, the treated cells were lysed in the supplied lysis buffer and were incubated on ice for 10 min. Therefore, samples were incubated in caspase buffer (pH 7.4, 20% glycerol, 100 mM HEPES, 5 mM dithiothreitol, 0.5 mM ethylenediaminetetraacetic acid [EDTA]) containing 100 mM of caspase-3 and caspase-9-specific substrates (Ac-DEVD-ρNA and Ac-LEHD-ρNA, respectively) for 4 h at 37 °C. Then, absorbance was measured at 405 nm. Caspase-3 and -9 activities in different groups were reported as the percentage of control, which was assumed as 100% (Shiri et al. 2016).

Protein content measurement

To normalize the test results, 10 μL Bradford reagent was added to 100 μL of homogenized and diluted samples. Then, 5 min later, the absorbance was recorded at 595 nm by spectrophotometer. BSA was used as the standard (Bradford 1976).

Statistical analysis

Spatial memory performance parameters (escape latency, swimming speed, traveled distance, and the spent time in the target quadrant in probe test), oxidative stress markers, and caspase-3 and -9 were recorded for each group. Data were expressed as a mean ± SEM and analyzed by one-way analysis of variance (ANOVA) followed by the post hoc Tukey test to illustrate the significant differences between groups. A level of $P < 0.05$ was considered statistically significant.

Results

Memory performance in MWM

Figure 1a shows the latency to reach the hidden platform. The results on ANOVA revealed a significant difference between groups [$F (11, 372) = 22.49, P < 0.0001$]. Tukey post hoc showed that MDMA 5 mg/kg (63.19 ± 3.63 s, $P < 0.0001$) and MDMA 10 mg/kg (78.96 ± 2.36 s, $P < 0.0001$) significantly increased the escape latency compared to control (21.64 ± 2.24 s). Furthermore, the results shows that atorvastatin 5 mg/kg ± MDMA 5 mg/kg (44.60 ± 3.48 s, $P = 0.0210$), atorvastatin 10 mg/kg ± MDMA 5 mg/kg (39.12 ± 4.32 s, $P = 0.0003$),
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atorvastatin 20 mg/kg ± MDMA 5 mg/kg (39.95 ± 3.06 s, \(P = 0.0004\)), and rosuvastatin 20 mg/kg ± MDMA 5 mg/kg (45.96 ± 5.08 s, \(P = 0.0447\)) reduced the increase of latency compared to MDMA 5 mg/kg (63.19 ± 3.63 s) in 4 training days of MWM.

Figure 1b shows the traveled distance to reach the platform in different groups. Comparison between MDMA receiving groups and MDMA + atorvastatin/ rosuvastatin groups demonstrated that MDMA 5 mg/kg (2104.23 ± 123.66 cm, \(P < 0.0001\)) and MDMA 10 mg/kg (2486.68 ± 97.46 cm, \(P < 0.0001\)) significantly increased compared to control (615.45 ± 64.28 cm). ATV 5 mg/kg ± MDMA 5 mg/kg (1368.44 ± 124.06 cm, \(P = 0.0021\)), ATV 10 mg/kg ± MDMA 5 mg/kg (1242.69 ± 163.43 cm, \(P < 0.0001\)), ATV 20 mg/kg ± MDMA 5 mg/kg (1212.57 ± 113.75 cm, \(P < 0.0001\)), and RSV 20 mg/kg ± MDMA 5 mg/kg (1388.84 ± 175.20 cm, \(P = 0.0034\)) significantly decreased compared to compared with MDMA 5 mg/kg (2104.23 ± 123.66 cm).
(2486.68 ± 97.46 cm, P < 0.0001) significantly prolonged the traveled distance comparing to control (615.45 ± 64.28 cm), while groups receiving atorvastatin 5 mg/kg ± MDMA 5 mg/kg (1368.44 ± 124.06 cm, P = 0.0021), atorvastatin 10 mg/kg ± MDMA 5 mg/kg (1242.69 ± 163.43 cm, P < 0.0001), atorvastatin 20 mg/kg ± MDMA 5 mg/kg (1212.57 ± 113.75 cm, P < 0.0001), and rosuvastatin 20 mg/kg ± MDMA 5 mg/kg (1388.84 ± 175.20 cm, P = 0.0034) traveled significantly less distance to find the platform as compared with MDMA 5 mg/kg (2104.23 ± 123.66 cm) [F (11, 372) = 24.10, P < 0.0001].

In our study, rats’ locomotor activity in each group was evaluated by calculating the swimming speeds (velocity) in 4 training days of MWM (Fig. 1c). This parameter did not differ among the groups [F (11, 372) = 1.516, P = 0.1232], suggesting that the locomotor activity was unchanged with respect to different treatments.

The differences among the mean value of spending time in the target quadrant between the groups were greater than the level expected by chance, and this difference was statistically significant [F (11, 83) = 4.360, P < 0.0001]. This time was significantly lower in the MDMA 5 mg/kg (20.40 ± 1.27 s, P = 0.0022) and MDMA 10 mg/kg (20.15 ± 1.01 s, P = 0.0015) groups compared to the control (30.80 ± 1.08 s) (Fig. 1d). Our results indicated that the spent time in the target guardant in atorvastatin 20 mg/kg + MDMA 5 mg/kg (29.22 ± 1.55 s, P = 0.0280) was significantly increased compared to MDMA 5 mg/kg (20.40 ± 1.27 s).

Hippocampal ROS formation

As shown in Fig. 2, the result of multiple comparisons represents that there is a significant difference in the amount of ROS production among groups [F (11, 35) = 22.43, P < 0.0001]. Considering the obtained result of ROS generation in hippocampus, it was highly elevated in MDMA 2.5 mg/kg (213.74 ± 21.04%, P = 0.0107), MDMA 5 mg/kg (312.98 ± 24.90%, P < 0.0001), and MDMA 10 mg/kg (385.85 ± 28.51%, P < 0.0001) groups compared to control (100%). It was observed that the amount of ROS were dramatically reduced in pre-treatment with atorvastatin 5 mg/kg (213.37 ± 13.48%, P = 0.0389), atorvastatin 10 mg/kg (173.52 ± 9.14%, P = 0.0008), atorvastatin 20 mg/kg (159.55 ± 9.85%, P = 0.0006), and rosuvastatin 20 mg/kg (159.59 ± 16.76%, P = 0.0002) compared to MDMA 5 mg/kg (312.98 ± 24.90%).

LPO assay

Measurement of TBARS concentration gives a clue of LPO. As depicted in Fig. 3, the mean value of LPO in MDMA receiving groups and pre-treatment groups was significantly different [F (11, 35) = 7.376, P < 0.0001]. A considerable increase in LPO was observed in groups of MDMA 5 mg/kg (233.84 ± 12.28 μM/mg protein, P = 0.0007) and MDMA 10 mg/kg (247.41 ± 11.02 μM/mg protein, P < 0.0001) compared to control (146.40 ± 11.08 μM/mg protein). Likewise, this elevation was reduced significantly in atorvastatin 5 mg/kg ± MDMA 5 mg/kg (171.51 ± 9.55 μM/mg protein, P = 0.0384), atorvastatin 10 mg/kg ± MDMA 5 mg/kg (161.24 ± 10.18 μM/mg protein, P = 0.0082), atorvastatin 20 mg/kg ± MDMA 5 mg/kg (157.31 ± 13.10 μM/mg protein, P = 0.0044), rosuvastatin 10 mg/kg ± MDMA 5 mg/kg (172.28 ± 6.69 μM/mg protein, P = 0.0428), and rosuvastatin 20 mg/kg ± MDMA 5 mg/kg (152.13 ± 15.01 μM/mg protein, P = 0.0051) groups in comparison with of MDMA 5 mg/kg (233.84 ± 12.28 μM/mg protein).

Caspase-3 and -9 activities

As presented in Fig. 4a and b, there was a significant difference in the level of caspase-3 and -9 activities between...
groups \( F(11, 35) = 7.893, P < 0.0001 \), and \( F(11, 35) = 4.771, P = 0.0002 \), respectively. Our results show that there was a noticeable rise in the activities of both caspase-3 and caspase-9 following administration of MDMA at the doses of 5 mg/kg (170.07 ± 6.52%, \( P = 0.0004 \); 151.86 ± 5.21%, \( P = 0.0058 \)) and 10 mg/kg (172.44 ± 7.76%, \( P = 0.0002 \); 152.87 ± 17.15%, \( P = 0.0046 \)), respectively, as compared to the control (100%). A more noticeable activity was observed with the caspase-3. On the other hand, there was a remarkable decline in the caspase-3 and -9 activities in groups receiving atorvastatin 5 mg/kg ± MDMA 5 mg/kg (119.88 ± 8.46%, \( P = 0.0326 \); 104.61% ± 7.80, \( P = 0.0172 \)), atorvastatin 10 mg/kg ± MDMA 5 mg/kg (105.43 ± 8.14%, \( P = 0.0012 \), 103.66 ± 7.34%, \( P = 0.0300 \)), atorvastatin 20 mg/kg ± MDMA 5 mg/kg (107.13 ± 8.71%, \( P = 0.0054 \); 107.45 ± 4.56%, \( P = 0.0314 \)), and rosuvastatin 20 mg/kg ± MDMA 5 mg/kg (109.26 ± 8.29%, \( P = 0.0030 \), 103.05 ± 2.86%, \( P = 0.0122 \)), respectively, in comparison with MDMA 5 mg/kg group (170.07 ± 6.52%; 151.86 ± 5.21%). Data are presented as the mean ± SEM. ***\( P < 0.001 \) and ****\( P < 0.0001 \) compared to control; #\( P < 0.05 \) and ##\( P < 0.01 \) compared to MDMA 5 mg/kg; ATV atorvastatin, RSV rosuvastatin.

**Fig. 3** Plot of interaction effect of different MDMA doses by atorvastatin and rosuvastatin on mean of LPO in the hippocampus. Different experimental groups received ATV/RSV or vehicle (CMC 1%) for 21 consecutive days. Then, MWM was conducted to evaluate spatial memory performance. Animals were administered with MDMA/saline 30 min before training sessions in 4 training days of MWM task. Afterward, rats were killed and their hippocampuses were dissected to assess LPO. MDMA 5 mg/kg (233.84 ± 12.28 μM/mg protein, \( P = 0.0007 \)) and MDMA 10 mg/kg (247.41 ± 11.02 μM/mg protein, \( P < 0.0001 \)) significantly increased compared to control (146.40 ± 11.08 μM/mg protein). ATV 5 mg/kg ± MDMA 5 mg/kg (171.51 ± 9.55 μM/mg protein, \( P = 0.0384 \)), ATV 10 mg/kg ± MDMA 5 mg/kg (161.24 ± 10.18 μM/mg protein, \( P = 0.0082 \)), ATV 5 mg/kg ± MDMA 5 mg/kg (157.31 ± 13.10 μM/mg protein, \( P = 0.0044 \)), RSV 10 mg/kg ± MDMA 5 mg/kg (172.28 ± 6.69 μM/mg protein, \( P = 0.0428 \)), and RSV 20 mg/kg ± MDMA 5 mg/kg (152.13 ± 15.01 μM/mg protein, \( P = 0.0051 \)) significantly decreased in comparison with MDMA 5 mg/kg (233.84 ± 12.28 μM/mg protein). Data are presented as the mean ± SEM. ***\( P < 0.001 \) and ****\( P < 0.0001 \) compared to control; \( ^5P < 0.05 \) and \( ^{**}P < 0.01 \) compared to MDMA 5 mg/kg; ATV atorvastatin, RSV rosuvastatin.

**Fig. 4** Plot of interaction effect of different MDMA doses by atorvastatin and rosuvastatin on mean of caspase-3 (a) and caspase-9 (b) activities in the hippocampus. Different experimental groups received ATV/RSV or vehicle (CMC 1%) for 21 consecutive days. Then, MWM was conducted to evaluate spatial memory performance. Animals were administered with MDMA/saline 30 min before training sessions in 4 training days of MWM task. Afterward, rats were killed and their hippocampuses were dissected to assess caspase-3 and -9 activities. Caspase-3 and caspase-9 activities were increased in MDMA 5 mg/kg (170.07 ± 6.52%, \( P = 0.0004 \); 151.86 ± 5.21%, \( P = 0.0058 \)) and MDMA 10 mg/kg (172.44 ± 7.76%, \( P = 0.0002 \); 152.87 ± 17.15%, \( P = 0.0046 \)), respectively, as compared to the control (100%). A more noticeable activity was observed with the caspase-3. On the other hand, there was a remarkable decline in the caspase-3 and -9 activities in groups receiving atorvastatin 5 mg/kg ± MDMA 5 mg/kg (119.88 ± 8.46%, \( P = 0.0326 \); 104.61% ± 7.80, \( P = 0.0172 \)), atorvastatin 10 mg/kg ± MDMA 5 mg/kg (105.43 ± 8.14%, \( P = 0.0012 \), 103.66 ± 7.34%, \( P = 0.0300 \)), atorvastatin 20 mg/kg ± MDMA 5 mg/kg (107.13 ± 8.71%, \( P = 0.0054 \); 107.45 ± 4.56%, \( P = 0.0314 \)), and rosuvastatin 20 mg/kg ± MDMA 5 mg/kg (109.26 ± 8.29%, \( P = 0.0030 \), 103.05 ± 2.86%, \( P = 0.0122 \)), respectively, in comparison with MDMA 5 mg/kg group (170.07 ± 6.52%; 151.86 ± 5.21%). Data are presented as the mean ± SEM. **\( P < 0.01 \) and ***\( P < 0.001 \) compared to control; \( ^5P < 0.05 \) and \( ^{**}P < 0.01 \) compared to MDMA 5 mg/kg; ATV atorvastatin, RSV rosuvastatin.
Comparison with MDMA 5 mg/kg group (170.07 ± 6.52%; 151.86 ± 5.21%).

Discussion

The major finding within this series of experiments was that atorvastatin (5, 10, and 20 mg/kg) and rosuvastatin (20 mg/kg) markedly attenuated the MDMA-induced memory impairment, as well as oxidative stress and caspases activities in the hippocampus. To the best of our knowledge, this is the first study that reveals the protective effects of atorvastatin and rosuvastatin against MDMA-induced memory impairment through oxidative stress and apoptotic markers. Figure 5 represents a scheme of the proposed protective mechanisms of atorvastatin and rosuvastatin against MDMA-induced neurotoxicity in the brain.

The results indicated that intraperitoneally administration of MDMA in 4 consecutive days of MWM led to spatial memory and learning impairment in a dose-dependent manner. This finding is supported by previous studies, which showed that MDMA (5, 10, and 20 mg/kg) caused neurotoxicity and impaired memory performance (Taghizadeh et al. 2016; Gudelsky and Yamamoto 2008). However, the results of Able et al. (2006) and Sprague et al. (2003) studies differ from the current finding, reporting that no significant difference was observed between the MDMA-treated group and control group. A possible explanation for this might be the differences in the protocols compared to our study. In Sprague et al.’s (2003) study, MDMA was administered twice (20 mg/kg, subcutaneously, 12 h) on day 1, and MWM test was conducted 1 week later. Also, Able et al. (2006) performed MWM days 12–16 following injection of MDMA (4 × 15 mg/kg, I.P, 2 h apart) on day 1. It could be hypothesized that learning and memory impairments, as a consequence of MDMA administration, are probably more duration-dependent than dose-dependent. Also, different time intervals between MDMA administration and MWM task could be another reason, which in Sprague et al.’s (2003) and Able et al.’s (2006) studies were 7 and 12 days, respectively, compared to simultaneous administration of MDMA and MWM task in our study. This finding is in agreement with Arias-Cavieres et al. (2010) that reported even non-toxic doses of MDMA (2 × 0.2 and 2 mg/kg for 6

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Fig. 5 The proposed scheme of the protective effects of atorvastatin and rosuvastatin against MDMA-induced neurotoxicity in the hippocampus. ATV atorvastatin, RSV rosuvastatin, ROS reactive oxygen species, LPO lipid peroxidation, GSH glutathione, SOD superoxide dismutase, CAT catalase, MDA malondialdehyde.
training studies, MDMA induced neurotoxicity in cholinergic, serotonergic, and dopaminergic systems in the hippocampus and other brain regions involved in memory and learning (Sprague et al. 2003; Gudelsky and Yamamoto 2008).

ROS are naturally produced in mammalian cells during cellular respiration. The majority of generated ROS are superoxide anion, hydroxyl radical, and hydrogen peroxide (\(O_2^{-}\), \(OH\), and \(H_2O_2\), respectively) (Zorov et al. 2014). As ROS are cytotoxic molecules, there are natural neutralizing defense systems such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) (Zorov et al. 2014). When ROS production is imbalanced with antioxidants' capacity, the cell becomes vulnerable to oxidative stress; subsequently, ROS cause protein oxidation, LPO, and DNA damage (Zorov et al. 2014). Activation of cascades of caspases and apoptotic pathways through excess ROS production have been demonstrated (Redza-Dutordoir and Averill-Bates 2016). Release of cytochrome c from mitochondria, triggering caspase activation and apoptosis, appears to be largely mediated through direct or indirect ROS production (Redza-Dutordoir and Averill-Bates 2016). It is worthy to note that the nervous system is very susceptible to ROS damage due to its higher oxygen consumption, higher ratio of membrane surface area to cytoplasmic volume, higher polyunsaturated lipids level, and modest antioxidant defense mechanisms (Friedman 2011).

Previous studies have demonstrated that excess ROS formation can lead to LPO (Su et al. 2019). Activation of LPO can stimulate both extrinsic and intrinsic apoptotic signaling pathways (Su et al. 2019). It was also reported that ROS might lead to cardiolipin peroxidation, a specific phospholipid of mitochondrial inner membrane, and subsequently activate caspase-3 and -9, the main components of the intrinsic apoptosis pathway (Zhong et al. 2017). The nuclear factor kappa B (NF-κB) protein family is extensively involved in inflammation, stress responses, and cell death (Hoesel and Schmid 2013). It has been proved that LPO enhances NF-κB activity (Yin et al. 2015). Also, it was shown that anti-apoptotic Bcl-2 became inactivated through the NF-κB pathway upon lipid peroxidation (Bodur et al. 2012), implying that LPO activation induces apoptosis upon caspases activities through various pathways.

The current study results indicated that three doses of MDMA (2.5, 5, and 10 mg/kg) in a dose-dependent manner dramatically increased ROS production in the hippocampal neurons. Following MDMA administration at the doses of 5 and 10 mg/kg, the escape latency, the traveled distance, LPO, and caspase-3 and -9 activities were remarkably increased. However, the dose of 2.5 mg/kg, despite its elevation in ROS formation, could not lead to LPO and caspases activation, representing that maybe ROS formation is probably the primary consequence of MDMA-induced neurotoxicity. It seems that MDMA, through oxidative stress induced by ROS generation, LPO, and caspases activities, resulted in memory impairment. It is hypothesized that activation of these markers might recruit other damaging pathways leading to apoptosis and cell death.

There are several pathways linked to the neurotoxicity of amphetamine derivatives. It is suggested that MDMA neurotoxicity is mediated through glutamate receptors. A study supporting this hypothesis has shown that glutamate-induced neurotoxicity after MDMA administration was attenuated following pre-treatment with MK-801, as a glutamate-receptor antagonist (Finnegan and Taraska 1996). In contrast, Colado et al. (1998) did not find a particular role for glutamate receptors in the neurotoxic mechanism of MDMA. However, further studies on this argument are required. Recent studies show that when ROS production is increased, cytochrome c is released from mitochondria into the cytoplasm, and with other apoptogenic factors, ultimately activates cascades of caspases, promoting neuronal death (Jiménez et al. 2004). Moreover, activation of caspase-3 and -9 following cytochrome c release in cultured cerebellar granule cells were reported (Jiménez et al. 2004). However, in a study, treatment of hippocampal neurons with MDMA increased caspase-3 and -8 activities, but the cytosolic and mitochondrial cytochrome c contents remained unchanged (Capela et al. 2013). Barbosa et al. (2014) reported that independent of the mitochondrial pathway, the activity of caspase-3 was increased following MDMA exposure, highlighting the fact that MDMA activates cascades of caspases through other pathways in addition to the mitochondrial-dependent mechanism.

Our study suggests that atorvastatin 20 and 10 mg/kg and, to a lesser extent, 5 mg/kg improved memory performance in MDMA-induced neurotoxicity. Its potential protective effects in attenuation of ROS formation, LPO, and caspase-3 and -9 activities were recorded. Consistent with our findings, previous studies exhibit the neuroprotective activity of atorvastatin in scopolamine-induced (Javadi-Paydar et al. 2011), amyloid-β1–42 (Zhang et al. 2013), and benzodiazepine (Georgieva-Kotetarova and Kostadinova 2013) induced memory impairment. In confirmation of our study, it has been reported that pre-treatment with atorvastatin considerably reduced ROS, LPO, and caspase-3 activation and increased activity of amphetamine-receptor antagonist (Finnegan and Taraska 1996). It is suggested that MDMA inhibition of the upregulation of IL-1β, IL-6, and TNF-α in the hippocampus following amyloid-β1–42 administration (Zhang et al. 2013).

The current study results showed that pre-treatment with rosuvastatin 20 mg/kg inhibited ROS generation, LPO, and caspase-3 and -9 activity following MDMA-induced neurotoxicity, while doses of 5 and 10 mg/kg failed to be effective. Research on the neuroprotective effects of rosuvastatin
is carried out in several experimental models of CNS disorders. Georgieva et al. (2013) showed that rosuvastatin improved cognitive functions in diazepam-induced amnesia and preserved long-term memory. Rech et al. (2010) found that rosuvastatin improved memory defect in an experimental model of neurodegeneration induced by neonatal iron loading. The ability of rosuvastatin against oxidative damage in the cortex and hippocampus displays its possible neuroprotective mechanisms (Rech et al. 2010). These results are in line with the study describing the protective and anti-apoptotic effects of rosuvastatin in cardiac arrest-induced hippocampal damage (Qiu et al. 2017).

Interestingly, our findings showed that rosuvastatin 10 mg/kg ± MDMA 5 mg/kg decreased LPO but not ROS formation. Possible explanations for this result might be that rosuvastatin may possess a particular inhibitory effect through other pathways leading to LPO, rather than ROS; or maybe rosuvastatin has a special inhibitory or stabilizing property on LPO processes. As far as we know RNS, like ROS, play a crucial role in the induction of LPO (Dedon and Tannenbaum 2004). As a suggested possible pathway, rosuvastatin might probably ameliorate LPO through inhibition of MDMA-induced RNS formation, since NO level is increased following MDMA administration (Costa et al. 2018), \( \text{O}_2^\cdot \) reacts with NO through nitric oxide synthase (NOS) enzymes and results in a potent RNS known as peroxynitrite (ONOO\(^-\)), causing lipid and protein nitration. These are in line with the previous study, revealing that MDMA induced NO and RNS formation (Darvesh et al. 2005). Also, rosuvastatin displayed antioxidant activity in oxidative/nitrosative stress (Ajith et al. 2008; Maheshwari et al. 2015) through modulatory effects on NOS (Di Napoli et al. 2005) and specifically inhibited LPO (Ajith et al. 2008; Maheshwari et al. 2015). This result is in line with El-Aal et al. (2017) and Maheshwari et al. (2015) that rosuvastatin reduced LPO in the hippocampus and colon, and also elevated the capacity of GSH and SOD. However, further studies are required to fully identify its neuroprotective mechanisms.

Neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) have been announced to support neuron survival. In many neurological disorders, statins have illustrated neuroprotective effects through increasing hippocampal NGF and BDNF levels (Yang et al. 2012). Although numerous studies have revealed the neuroprotective effects of statins, some studies report that statins might impair cognitive abilities. For example, Okudan et al. (2020) showed that both high doses of rosuvastatin and simvastatin administration impaired memory and brain functions. These differences might be associated with differences in the types of the behavioral tests, the dosage of the statins, and the type of statins (hydrophilic vs. lipophilic). In our study, administration of atorvastatin or rosuvastatin alone produced no effect in MWM paradigm. The present finding seems to be consistent with the other research, which noted that statins have no impact on normal brain functions but exert neuroprotective effects against brain damages (van der Most et al. 2009). Moreover, this study demonstrated that despite the higher potency of rosuvastatin as a cholesterol-lowering agent (Jones et al. 2003), atorvastatin illustrated more efficient neuroprotective effects in MDMA-induced memory impairment. Maybe, this is because atorvastatin can penetrate BBB to a much greater magnitude due to its higher lipophilicity. Likewise, an in vitro study of statins revealed that lipophilic simvastatin presented the best characteristics in neurodegenerative conditions (Sierra et al. 2011). On the other hand, Haag et al. (2009) showed that statins are correlated with a reduced risk of Alzheimer’s disease regardless of lipophilicity. Nevertheless, no clinical differences in the neuroprotective properties of lipophobic and lipophilic statins have been documented (van der Most et al. 2009).

For future studies, it is recommended to evaluate the post-treatment effects of statins and other underlying involved mechanisms in MDMA-induced spatial memory impairment. Also, it is appreciated to examine whether these neuroprotective effects of statins are cholesterol-dependent or not.

In conclusion, we have demonstrated that administration of MDMA caused learning and memory impairment, induction of ROS production, LPO, and subsequently caspase-3 and -9 activities, which in turn promotes dysfunction and damage to hippocampal neurons. Pretreatment with both atorvastatin and rosuvastatin prevented neurotoxicity of MDMA, oxidative stress markers, and activation of caspases, while atorvastatin exhibited a more efficient protective effect.

Declarations

Conflict of interest The authors declare no conflict of interest.

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