Protective Effects of Atorvastatin and Rosuvastatin on 3,4-methylenedioxymethamphetamine (MDMA)-Induced Spatial Learning and Memory Impairment

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Research Article

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Abstract

3,4-methylenedioxymethamphetamine (MDMA) or "Ecstasy", which has been used for recreational purposes, is shown to cause learning and memory impairment. Statins, beyond their efficient cholesterol-lowering action through inhibition of 3-hydroxy-3-methylglutaryl-CoA (HMG-COA) reductase, possess multiple neuroprotective impacts referred to as pleiotropic effects. In this regard, we aimed to investigate the protective effect of atorvastatin and rosuvastatin in MDMA-induced neurotoxicity. Adult male Wistar rats received atorvastatin (5, 10, 20 mg/kg; orally) and rosuvastatin (5, 10, 20 mg/kg; orally) for 21 consecutive days. Then, Morris water maze (MWM) was performed to examine learning and memory functions. Rats were injected with MDMA (2.5, 5, and 10 mg/kg; I.P) 30 min before training sessions in 4 training days of MWM task. Afterward, rats were sacrificed under general anesthesia 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 impaired spatial memory functions and dramatically upregulated ROS production, LPO, and caspase-3 and 9 activities. Also, atorvastatin (5, 10, 20 mg/kg) and rosuvastatin (20 mg/kg) significantly improved memory performances and inhibited upregulation of ROS, LPO, and caspase-3 and -9 activities induced by MDMA. In conclusion, the amelioration of MDMA-induced memory impairment and hippocampal apoptosis through atorvastatin and rosuvastatin could be accredited to the observed suppression of ROS production, LPO, and caspase-3 and -9 activities, since excessive exposure of hippocampus to oxidative stress enhanced apoptotic caspases activities, promoted to neuronal apoptosis.

Introduction

The ring-substituted amphetamine derivate, 3,4-Methylenedioxymethylamphetamine (MDMA), known as "Ecstasy", is one of the most widespread drugs used for recreational purposes [1]. Its consumption affects cognitive domains, associated with robust deleterious effects on memory and learning, becoming a matter of great concern [2]. Several studies have been conducted to reveal the adverse effects of MDMA, especially neurotoxicity. Soleimani et al. found that MDMA administration in rats led to impairment of both acquisition and retention of spatial memory [3]. Additionally, a relevant in vitro model has established the apoptotic cell death of cultured cortical neurons treated with either MDMA or its metabolites in a time and concentration manner [4]. MDMA has been linked to the impaired cognitive performance and memory in regular MDMA users [5]. However, all parts of the central nervous system are vulnerable to the neurotoxic effects of MDMA, hippocampus, striatum, and cerebral cortex have been found to be more susceptible [6].

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 that is related to many pathological conditions [7]. RNS, like ROS, can be either harmful or beneficial for living system. Nitric oxide (NO), early recognized as an endothelium-derived relaxing factor in the blood vessel but now is identified as a mediator of physiological processes, as well as cellular toxicity and tissue injury [7].

Putative studies documented that MDMA administration perturbs the equilibrium between production and scavenging of ROS and RNS, which lead to a rise in the intracellular level of free radicals and consequently cause oxidative/nitrosative stress [8]. Moreover, the elevation of pro-inflammatory mediators and immunological responses in the brain could lead to neuronal death following MDMA administration [9]. There is a large body of literature on various neurodegenerative disorders and their determined connection with oxidative stress and neuroinflammation [10, 11]. The majority of ROS is derived from mitochondria, and it is postulated that mitochondrial disruption represents the common theme in neurodegenerative diseases [12]. For example, Taghizadeh et al. showed that MDMA administration disrupted mitochondrial electron transport chain activity and its oxidative defense integrity, which led to the cognitive deterioration [13]. 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 an efficacious and safe drug class that specifically and reversibly inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-COA) reductase, the rate-limiting enzyme in cholesterol biosynthesis in the mevalonate pathway. Statins are considered the first-line approach for managing dyslipidemia and preventing primary and secondary coronary artery disease [14]. Beyond their potent cardioprotective effects, they can exhibit neuroprotective strategies that may underlie their clinical benefits in neurodegenerative conditions [15]. Statins manifest multiple overlapping actions referred to as pleiotropic effects such as antioxidant activity, inhibition of inflammatory responses, anti-excitotoxic properties, and enhancing neurogenesis [16–19]. The neuroprotection debate is the ongoing controversy over the use of statins in neurodegenerative diseases.

It has been announced that the various effects of statins on cognition and brain functions have been related to their lipophilicity property [20]. Water-soluble or hydrophilic statins (rosuvastatin, fluvastatin, and pravastatin) do not readily pass the blood-brain barrier (BBB). In contrast, lipid-soluble or lipophilic statins (atorvastatin, simvastatin, and lovastatin) can easily pass the BBB. These findings elucidate that lipophilicity may influence their 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.

Material 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 [21]. It showed an acceptable purity value (> 95%), and its structure was fully confirmed with H-NMR, C-NMR, and Mass spectrometry methodologies. In our

study, the following chemicals were used: KCl, $MgCl_2$, Tris–HCl, dimethylsulfoxide (DMSO), sodium succinate, Na_2HPO_4, sucrose, Ethylene glycol-bis (2-aminoethyl ether)-N, N, N', N'-tetraacetic acid (EGTA), 4–2-hydroxyethyl piperazine ethanesulfonic acid (HEPES), Rhodamine 123 (Rh 123), 2', 7'-dichlorofluorescein 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 separately standard polypropylene cages under a standard temperature-controlled environment (23 ± 1°C), 12-h light/12-h dark cycle schedule, humidity (55% ± 10%), and they had free accessibility to tap water and food. Experimental protocols were performed in accordance with the Tehran University of Medical Sciences Animal Ethics Committee and designated to reduce animals suffering stress and decrease the number of rats used.

Spatial learning and memory assessment

Spatial memory and learning were evaluated by the Morris water maze (MWM) training protocol, the same as described previously [13, 22]. Concisely, the MWM test consisted of four trials per day for four 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 surface of the water within 90 seconds. MDMA (2.5, 5, and 10 mg/ kg) or saline was administered intraperitoneally to rats 30 minutes before the first trial in each four 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 Ethovision 7 tracking system (Noldus Information Technology, Wageningen, the Netherlands).

After the probe test of MWM, each group (n = 4) were anesthetized by intraperitoneal injection of ketamine/xylazine (100 and 10 mg/kg, respectively) until loss of consciousness and loss of any response, then sacrificed 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 the following groups. Each group contained eight rats.

CMC 1% + MDMA groups received CMC 1% w/v orally via a gastric metallic tube, once daily for 21 consecutive days. After this period, they were administered different doses of MDMA (2.5, 5, and 10 mg/kg) intraperitoneally 30 min before the first training trial during 4 training days in the MWM task.

Atorvastatin + MDMA groups, which were divided into three groups according to the different oral doses of atorvastatin (5,10 and 20 mg/kg) in CMC1% for 21 days, followed by MDMA (5 mg/kg, I.P) during acquisition trials (i.e., 4 days of training in MWM).

Rosuvastatin + MDMA groups, which were divided into three groups according to the different oral doses of rosuvastatin (5,10 and 20 mg/kg) in CMC1% for 21 days followed by MDMA (5 mg/kg, I.P) during acquisition trials (i.e., 4 days of training in MWM).

Atorvastatin/Rosuvastatin + saline groups, which received atorvastatin or rosuvastatin orally once daily for 21 consecutive days, then were injected saline rather than MDMA, 30 minutes before the MWM task.

Control group was treated orally with a single daily dose of vehicle (CMC 1%) for 21 successive days, followed by intrapersonal injection of saline 30 minutes before training sessions in MWM. Different experimental groups are explained in Table 1.

Table 1
Summarized the employed experimental protocol in our study.

Experimental groups	Treatments on days 1–21	MWM on days 22–26	
MDMA groups	CMC 1% orally	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	**Sacrificed after the probe test and hippocampuses were dissected to evaluate ROS, LPO, and caspase3 and − 9 activities**
Atorvastatin + MDMA groups	Different oral doses of atorvastatin (5,10 and 20 mg/kg) in CMC1%	MDMA 5 mg/kg, I.P, 30 min before the first training trial during 4 training days in the MWM task	
Rosuvastatin + MDMA groups	Different oral doses of rosuvastatin (5,10 and 20 mg/kg) in CMC1%	MDMA 5 mg/kg, I.P, 30 min before the first training trial during 4 training days in the MWM task	
Atorvastatin group	atorvastatin 20 mg/kg in CMC 1%, orally	Saline	
Rosuvastatin group	rosuvastatin 20 mg/kg in CMC 1%, orally	Saline	
Control	CMC 1% orally	Saline	

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, 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 is considered 100% [23].

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 of n-butanol to extract TBRAS adducts. After cooling down, the absorbance was evaluated at 532 nm by an ELISA reader [24].

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 minutes. 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 − 9-specific substrates (Ac-DEVD-ρNA and Ac-LEHD-ρNA, respectively) for 4 hours at 37°C. Then, absorbance was measured at 405 nm. The caspase-3 and − 9 activities in different groups were reported as the percentage of control, which was considered as 100% [25].

Protein Content Measurement

For normalizing data obtained from the mentioned tests, 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 [26].

Statistical analysis

Spatial memory performance parameters (escape latency, swimming speed, traveled distance, and the spent time in the target quadrant in probe test) and oxidative stress biomarkers were recorded for each group. Data were expressed as a mean ± SEM and were 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

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 5mg/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, $P = 0.0003$), atorvastatin 20 mg/kg ± MDMA 5 mg/kg (39.95 ± 3.06 s, $P = 0.0004$), rosuvastatin 20 mg/kg ± MDMA 5 mg/kg (45.96 ± 5.08 s, $P = 0.0447$) prevented 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 prolonged the traveled distance comparing to control (615.45 ± 64.28 cm), while groups receiving atorvastatin 5mg/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), 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 mean of swimming speed (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 was 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), 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 pretreatment groups was significantly different [F (6, 31) = 1.430, p = 0.2351]. 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 prohibited significantly in atorvastatin 5mg/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) 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-B and 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 5mg/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), 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%).

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 apoptosis 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 oxidative damage and apoptosis in hippocampal neurons.

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 researches, which showed that MDMA (5, 10, and 20 mg/kg) caused neurotoxicity and impaired memory performance [13, 27]. However, the results of Able et al. [28] and Sprague et al. [29] 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 could be the different conducted protocols. In Sprague et al. [29] 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. [28] 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 impairment, as a consequence of MDMA administration, is probably duration-dependent rather than dose-dependent. Also, different time intervals between MDMA administration and MWM task could

be another reason, which in Sprague et al. [29] and Able et al. [28] 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. [30] that reported even non-toxic doses of MDMA (2 × 0.2 and 2 mg/kg for 6 training days of MWM) caused memory impairment. In previous studies, MDMA induced neurotoxicity in cholinergic, serotonergic, and dopaminergic systems in the hippocampus and other brain regions involved in memory and learning [27, 29].

ROS are naturally produced in mammalian cells during cellular respiration. The majority of generated ROS are superoxide anion, hydroxyl radical, and hydrogen peroxide ($O_2^{\bullet}$ ▯, $OH^{\bullet}$, and H_2O_2, respectively) [31]. As ROS are cytotoxic molecules, there are natural neutralizing defense systems such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) [31]. When ROS production is imbalanced with antioxidants' capacity, the cell becomes vulnerable to oxidative stress; subsequently, ROS cause protein oxidation, LPO, and DNA damage [32]. Activation of cascades of caspases and apoptotic pathways through excess ROS production have been demonstrated [33]. Release of cytochrome c from mitochondria, known as the central event, triggering caspase activation and apoptosis, appears to be largely mediated through direct or indirect ROS production [34]. It is worthy to note that the nervous system is very susceptible to ROS damage due to its high polyunsaturated lipids levels [35].

Previous studies have demonstrated that excess ROS formation can lead to LPO [36]. Activation of LPO can stimulate both extrinsic and intrinsic apoptotic signaling pathways [36]. It was also reported that ROS might lead to cardiolipin peroxidation, a specific phospholipid of mitochondria inner membrane, that subsequent products of LPO activate caspase-3 and − 9, the main components of intrinsic apoptosis pathway [37]. The NF-κB protein family is extensively involved in inflammation, stress responses, and cell death [38]. It has been proved that LPO enhances NF-κB activity [39]. It was also shown that antiapoptotic Bcl-2 became inactivated through the NF-κB pathway upon lipid peroxidation and its products [40], 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 and LPO in the hippocampal neurons. Following MDMA administration at the doses of 5 and 10 mg/kg, the escape latency, the traveled distance, and caspase-3 and − 9 activities were remarkedly increased. It seems that MDMA, through oxidative stress induced by ROS generation and LPO, activated apoptosis and resulted in memory impairment. 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.

There are several pathways linked to the neurotoxicity of amphetamine derivates. It is suggested that MDMA-neurotoxicity is via glutamate receptor-dependent mechanism. This hypothesis has been supported by a study showing that glutamate-mediated neurotoxicity after MDMA administration was attenuated following pretreatment with MK-801, as a glutamate-receptor antagonist [41]. In contrast, Colado et al. didn't found a particular role for NMDA receptors in the neurotoxic mechanism of MDMA

[42]. However, further studies on this argument are warranted. Recent studies show that when ROS production is increased, cytochrome c is released from mitochondria to cytoplasm and, with other apoptogenic factors, ultimately activates cascades of caspases, which in turn promotes death in neurons [43]. Moreover, activation of caspase- 3 and caspase-9 in cultured cerebellar granule cells following cytochrome c release was reported [43]. However, in a study, treatment of hippocampal neurons with MDMA increased caspase-3 and − 8 activities, but the cytosolic and mitochondrial cytochrome c content did not show any change [44]. Barbosa et al. reported that independent of the mitochondrial pathway, the activity of caspase-3 was increased following MDMA exposure [45], Highlighting the fact that MDMA activates cascade of caspases through other pathways in addition to the mitochondrial-dependent pathway.

We showed 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 apoptosis via caspase-3 and − 9 activities were recorded. In consistent with our findings, previous studies exhibit the neuroprotective activity of atorvastatin in scopolamine-induced [46], amyloid-β1–40 [47], and benzodiazepine [48] induced memory impairment. In confirmation of our study, it was reported that pretreatment with atorvastatin considerably reduced ROS, LPO, and caspase-3 activation and increased the gene expression of antioxidant enzymes [49]. On the other hand, it was demonstrated that atorvastatin inhibited hippocampal upregulation of IL-1β, IL-6, and TNF-α following amyloid-β1–42 administration [47].

The current study results showed that pretreatment 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. [48] showed that rosuvastatin improved cognitive functions in diazepam-induced amnesia and preserved long-term memory. Rech et al. [50] found that rosuvastatin improved memory defect in an experimental model of neurodegeneration induced by neonatal iron loading. The ability of rosuvastatin against oxidative damage of the cortex and hippocampus displays its possible neuroprotective mechanisms [50]. These results are in line with studies describing the protective effect against oxidative stress and antiapoptotic impact of rosuvastatin in spinal cord-induced neuronal death [51] and cardiac arrest-induced hippocampal damage [52].

Interestingly, our findings showed that rosuvastatin 10 mg/kg ± MDMA 5 mg/kg decreased LPO but not ROS formation. Possible explanations for this 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 [53]. 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 [54], $O_2^{\bullet}$ 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 previous study revealed that MDMA induced NO and RNS formation [55]. Also, rosuvastatin

displayed antioxidant activity in oxidative/nitrosative stress through modulatory effects on NOS, and specifically inhibited LPO [56–58]. This result is in line with El-Al et al. [59] and Maheshwari et al. [57] that rosuvastatin reduced LPO in the hippocampus and colon, also elevated the capacity of GSH and SOD. However, further studies are required to fully identify its neuroprotective mechanisms.

In recent years there has been enormous interest in statins' potential to treat Alzheimer's disease since the incidence of the disease is significantly diminished in patients receiving statins therapy to treat dyslipidemia [60]. Neurotrophins such as nerve growth factor (NGF) and Brain-derived neurotrophic factor (BDNF) have been proved to support neuron survival. In many neurological disorders, statins have illustrated neuroprotective effects through increasing hippocampal NGF and BDNF levels [61]. Although numerous studies revealed the neuroprotective effects of statins, there are studies reporting that statins impair cognitive abilities. It was shown that both high-dose rosuvastatin and simvastatin administration impaired learning and memory functions [62]. These differences might be associated with differences in the types of the behavioral tests, the dosage of the statins, and type of (hydrophilic or lipophilic). In our study, administration of atorvastatin or rosuvastatin alone produced no effect in MWM paradigm. The present finding seems to be consistent with other research, which noted that statins have no impact on normal brain functions but exert neuroprotective effects against brain damages[63]. Moreover, this study demonstrated that despite the higher potency of rosuvastatin as a cholesterol-lowering agent [64], atorvastatin illustrated more efficient neuroprotective effects in MDMA-induced memory impairment. Maybe this is associated with its higher lipophilicity which can readily penetrate BBB to a greater extent. However, no clinical differences in the neuroprotective properties of lipophobic and lipophilic statins have been documented [63].

For future studies, it is recommended to evaluate the post-treatment effects of statins as well as 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 MDMA administration induced learning and memory impairment, as well as upregulation of ROS production and LPO and subsequently, caspase-3 and − 9 activity, which in turn promotes dysfunction and death in hippocampal neurons. Pretreatment with both atorvastatin and rosuvastatin prevented neurotoxicity of MDMA, oxidative stress markers, and cascade of caspases, while atorvastatin exhibited a more efficient protective effect.

Declarations

Due to technical limitations, Declarations section is not available for this version.

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Figures

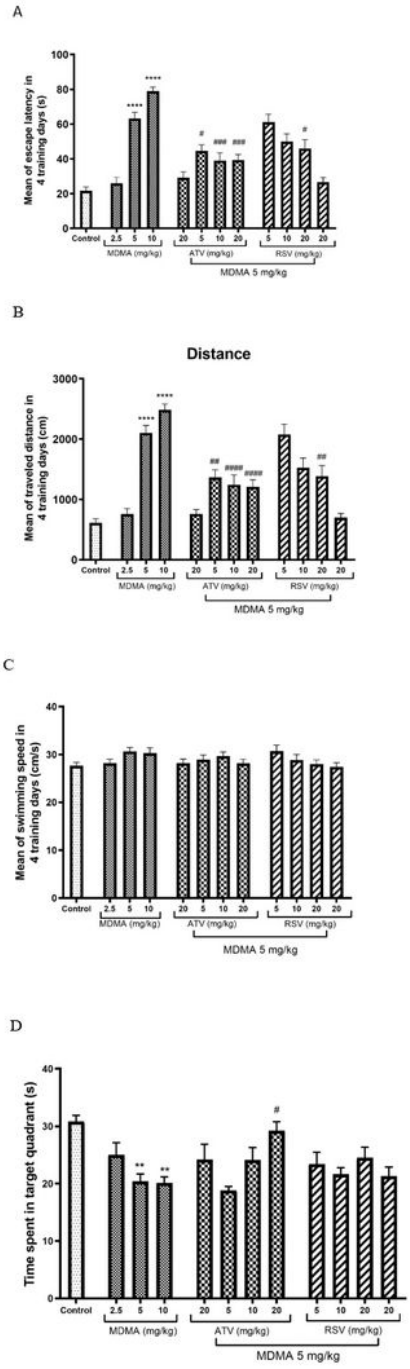

Figure 1

Plot of interaction effect of different MDMA doses by atorvastatin and rosuvastatin on mean value of latency (A), traveled distance (B), and swimming speed (C) of 4 training days as well as time spent in the quadrant in probe test (D) in MWM. Data are presented as the mean ± SEM. *P < 0.05, **P < 0.01, ***P <

0.001, ****P < 0.0001 compared to control; #P < 0.05, ##P < 0.01, ###P < 0.001, ####P < 0.0001 compared to MDMA 5 mg/kg; ATV: Atorvastatin, RSV: Rosuvastatin.

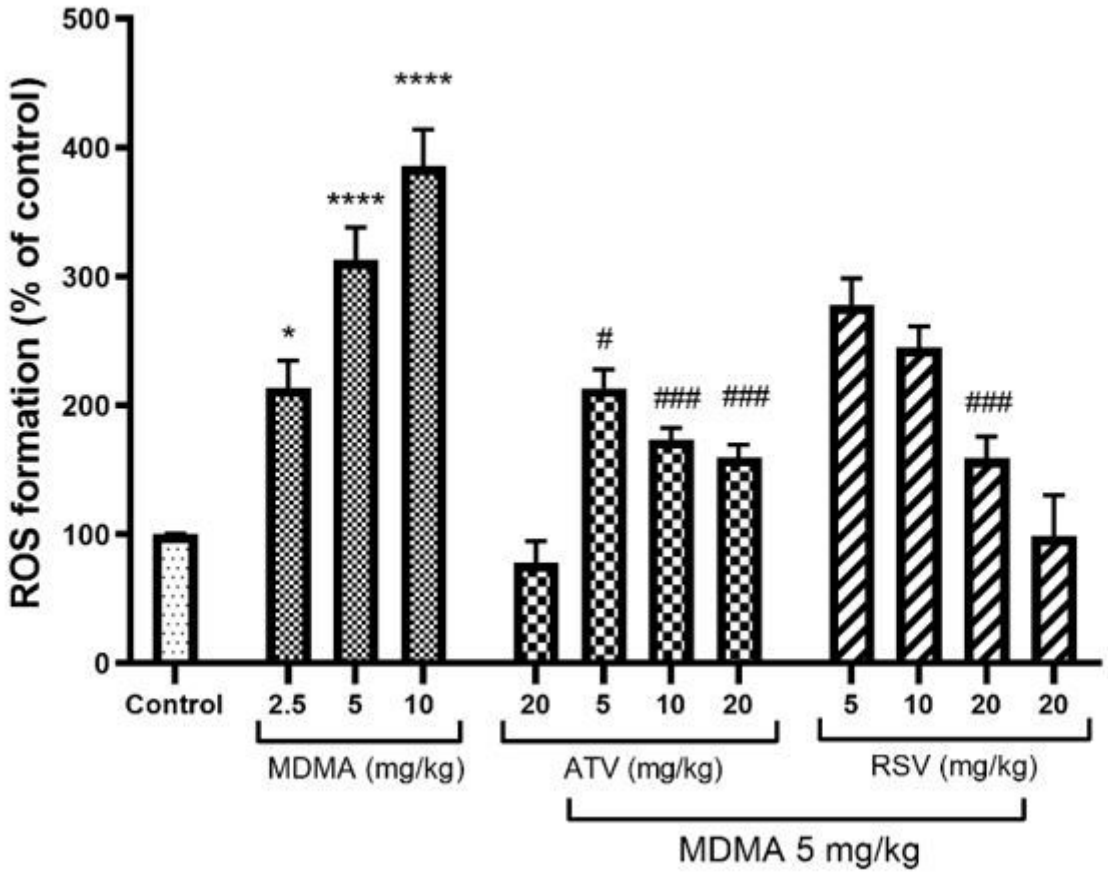

Figure 2

Plot of interaction effect of different MDMA doses by atorvastatin and rosuvastatin on mean of ROS production in the hippocampal neurons. Data are presented as the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 compared to control; #P < 0.05, ##P < 0.01, ###P < 0.001, ####P < 0.0001 compared to MDMA 5 mg/kg; ATV: Atorvastatin, RSV: Rosuvastatin.

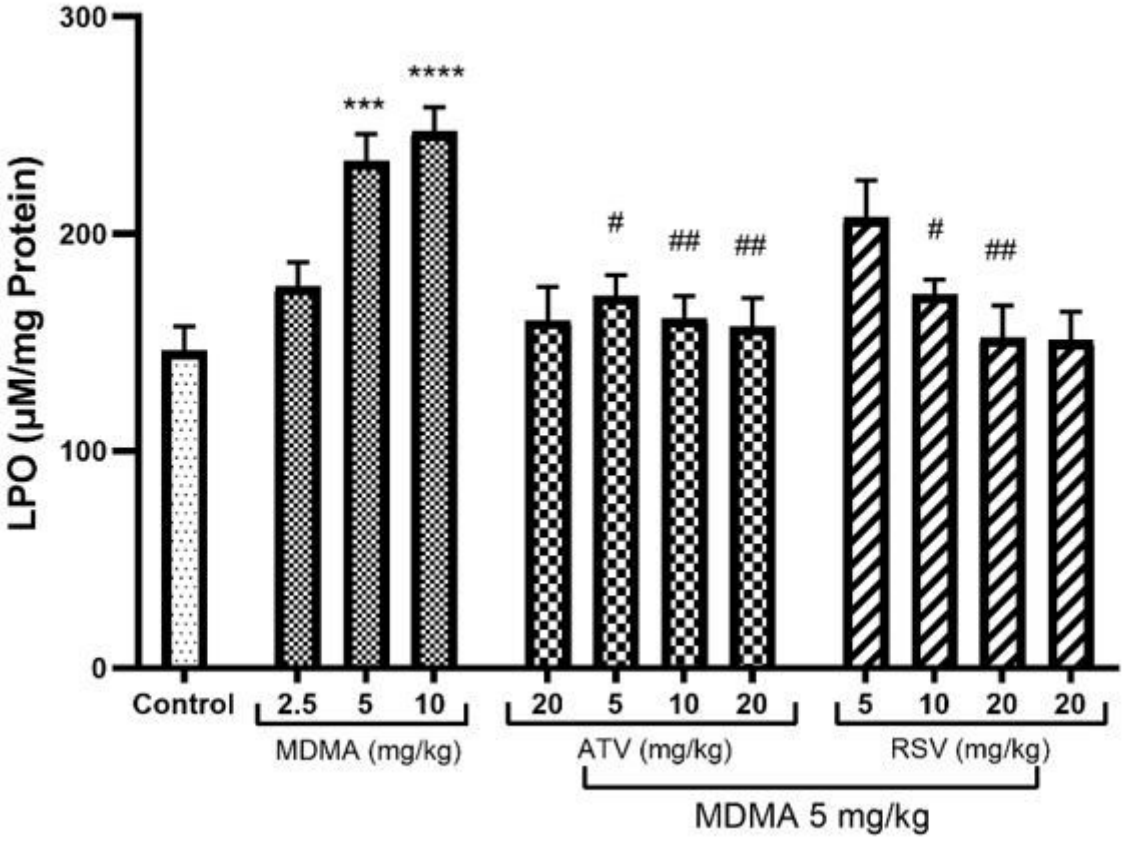

Figure 3

Plot of interaction effect of different MDMA doses by atorvastatin and rosuvastatin on mean of LPO in the hippocampal neurons. Data are presented as the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 compared to control; #P < 0.05, ##P < 0.01, ###P < 0.001, ####P < 0.0001 compared to MDMA 5 mg/kg; ATV: Atorvastatin, RSV: Rosuvastatin.

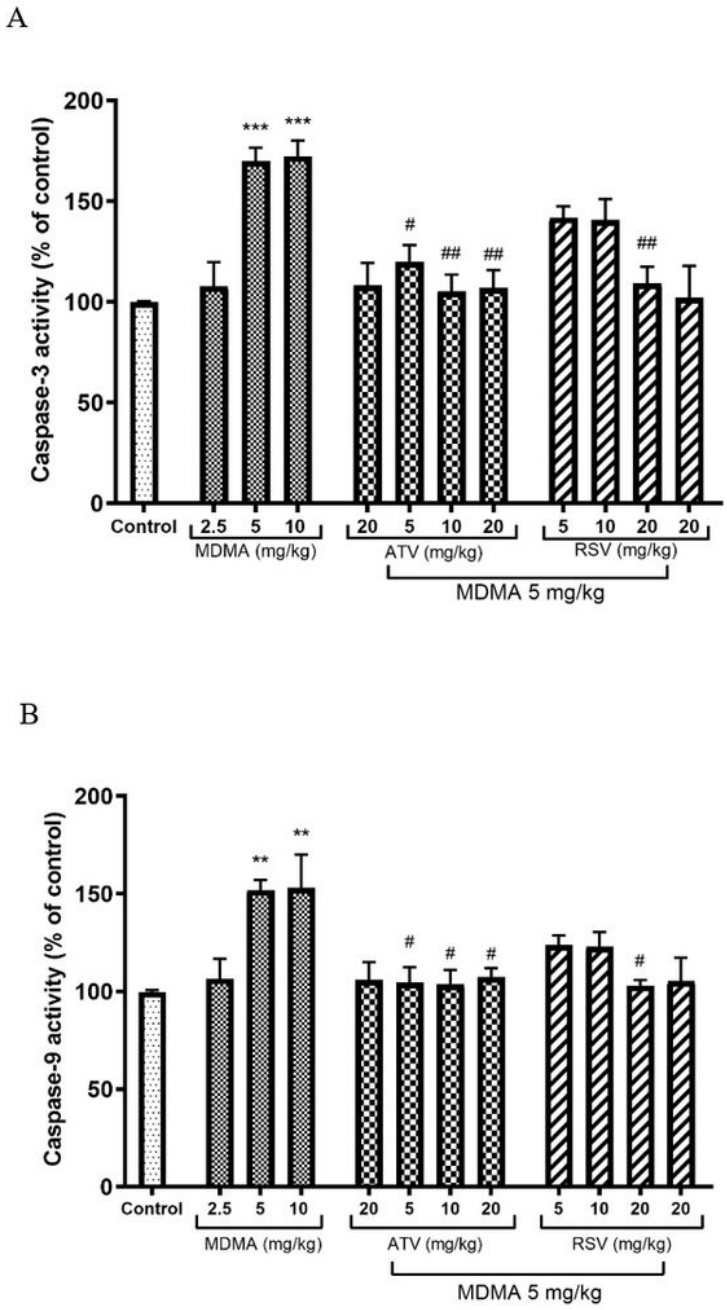

Figure 4

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 hippocampal neurons. Data are presented as the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 compared to control; #P < 0.05, ##P < 0.01, ###P < 0.001, ####P < 0.0001 compared to MDMA 5 mg/kg; ATV: Atorvastatin, RSV: Rosuvastatin.

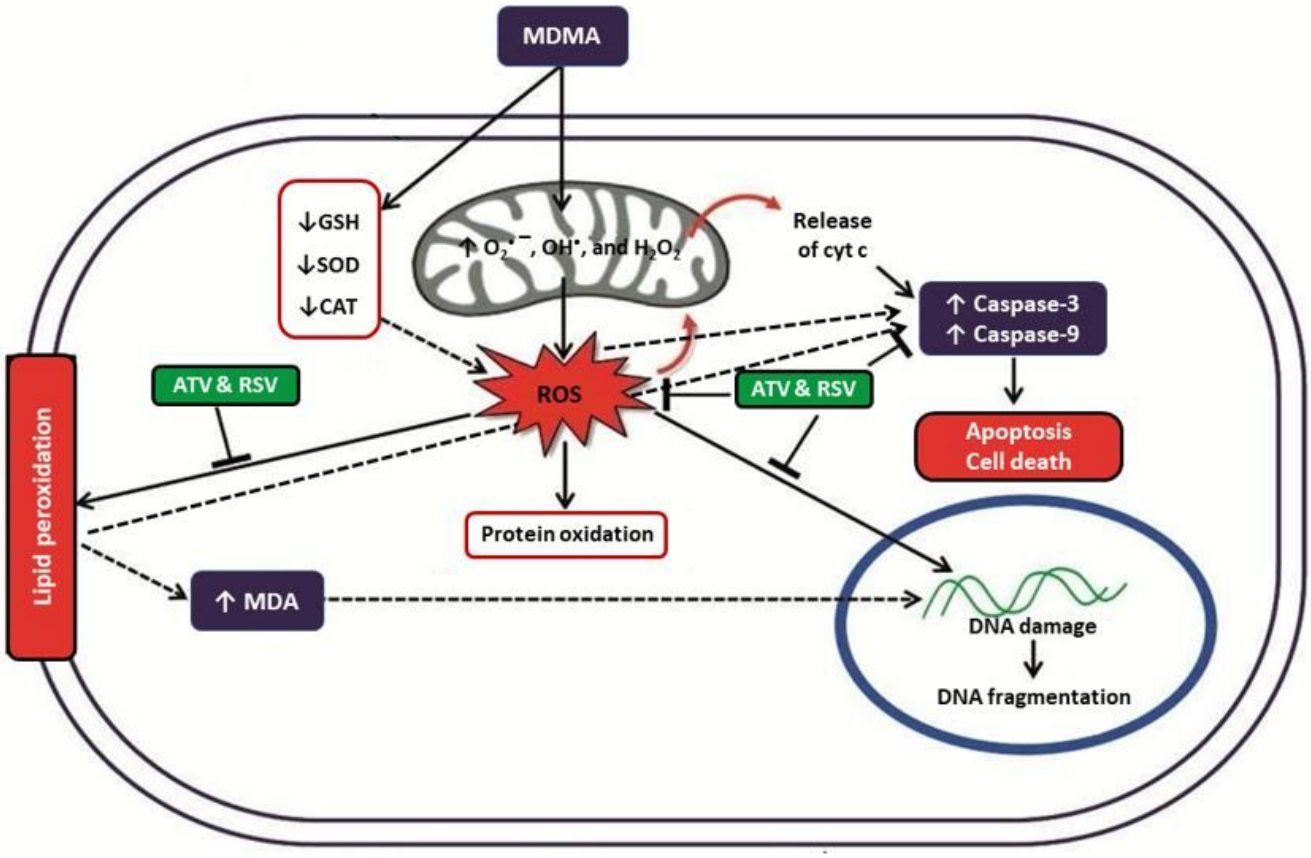

Figure 5

The proposed scheme of the protective effects of atorvastatin and rosuvastatin against MDMA-induced oxidative damage and apoptosis in hippocampal neurons. ATV: Atorvastatin, RSV: Rosuvastatin, ROS: Reactive oxygen species, LPO: Lipid peroxidation, GSH: Glutathione, SOD: superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde,