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Protective Effects of Edaravone against Methamphetamine-Induced cardiotoxicity

Motahareh Koohsari[1]; Fatemeh Shaki[1*], Daniel Jahani[1].
[1] Mazandaran University of Medical Sciences, Sari, Iran.

ABSTRACT

Methamphetamine (METH) is widely abused in worldwide. METH use could damage the dopaminergic system and induce cardiotoxicity via oxidative stress and mitochondrial dysfunction. Edaravone, a sedative-hypnotic agent, is known for it's antioxidant properties. In this study we used edaravone for attenuating of METH-induced cardiotoxicity in rats. The groups (six rats in each group) were as follows: control, METH (5 mg/kg IP) and edaravone (5, 10 and 20 mg/kg, IP) was administered 30 min before METH. After 24 hours, animals were killed, heart tissue was separated and mitochondrial fraction was isolated and oxidative stress markers were measured. Edaravone significantly ($p<0.05$) protected the heart against lipid peroxidation by inhibition of reactive oxygen species (ROS) formation. Edaravone also significantly ($p<0.05$) increased the levels of heart glutathione (GSH). METH administration significantly ($p<0.05$) disrupted mitochondrial function that edaravone pre-treatment significantly ($p<0.05$) inhibited METH-induced mitochondrial dysfunction. Protein carbonyl level also increased after METH exposure, but was significantly ($p<0.05$) decreased with edaravone pre-treatment. These results suggested that edaravone is able to inhibition of METH-induced oxidative stress and mitochondrial dysfunction and subsequently METH-induced cardiotoxicity. Therefore, the effectiveness of this antioxidant should be evaluated for the treatment of METH toxicity and cardio degenerative disease.

Key words: Edaravone, Methamphetamine, cardiotoxicity, Oxidative stress, Mitochondria

* Authors for correspondence: fshaki.tox@gmail.com

INTRODUCTION

Methamphetamine (METH) is a highly addictive stimulant which its illicit use becomes a serious issue in the worldwide especially in teenagers. It has been shown that both acute and chronic using of METH could lead to cardiomyopathy and cardiac failure (1, 2). Even previous studies reported that METH use accounted for at least 5% of all patients presenting to the emergency department with heart failure (3) . Therefore, in respect to increase in frequency of METH use, cardiotoxicity of METH can be considered as a medical concern that needs to effective treatment.

The main proposed mechanisms for METH cardiotoxicity is the induction of sympathomimetic effects on central and peripheral nervous system which leads to elevation of circulating catecholamine concentration (4-7). In fact, high concentration of catecholamine causes narrowing and spasm of the blood vessels, tachycardia, hypertension and probably, death of the cardiomyocyte (8) . Recently, several clinical and experimental studies revealed the evidences of the role of oxidative stress in cardiovascular disease such as heart failure. Excessive ROS production leads to oxidation of cellular proteins and lipids, and DNA damage and activation of intracellular signaling that finally could lead to initiation of cell death, which has been emerge as pathophysiological mechanism in a broad spectrum of cardiovascular diseases (9) .

So far, no antidotes exist for the treatment of cardiac toxicity of METH use in various countries, so because of known important role of oxidative damage in cardiotoxic effects of METH, using an antioxidant may be helpful.

Edaravone (3-methyl-1- phenyl-2-pyrazolin- 5-one) is a known potent and novel free radicals scavenger (10) that widely used as a treatment for acute ischemic stroke in Japan since 2001 (11).

Edaravone showed protective effects against oxidative damage to the heart, lung and brain tissue in various clinical and experimental models (10, 12, 13). The most proposed mechanism for free radicals scavenging effects of edaravone is quenching of hydroxyl radicals and following lipid peroxidation (11).

Thus, the aim of the current study was to test the effect of edaravone treatment against METH-induced cardiac toxicity, focusing on its inhibitory effects on oxidative stress and mitochondrial dysfunction.

MATERIAL AND METHODS

Animals treatment

Male Wistar rats (200-250 g) were kept in an air-conditioned room with controlled temperature of 22 ± 2 ◦C and maintained on a 12:12 h light cycle with free access to food and water. All experimental procedures were conducted according to the ethical standards and protocols approved by the Committee of Animal Experimentation of Mazandaran University of Medical Sciences, Sari, Iran. All efforts were made to minimize the number of animals and their suffering.

Animals were randomly divided into six groups of six rats per group animals and the groups were as follows: control group, METH group, METH plus different concentration of edaravone group, and vitamin E group (as positive control). All chemicals were dissolved in normal saline. One group of animals received only normal saline and was assigned as control. METH was administered (5 mg/kg IP) and edaravone (5, 10 and 20 mg/kg, IP) was administered 30 min before METH. After 24 hours, animals were killed, heart tissue and blood sample was separated and then heart was minced and homogenized with glass handheld homogenizer (10). The biochemical parameters determined included: total protein, ROS, lipid peroxidation, GSH and protein carbonyl.

Also, mitochondria were prepared from heart using differential centrifugation technique. The mitochondrial function was measured by determination of reduction of MTT (3-[4,5-dimethylthiazol-2-yl]- 2,5-diphenyltetrazolium bromide) in heart isolated mitochondria (10).

Cardiac enzymes assays: blood samples were collected from each animal and the serum obtained by centrifugation was used for determination of lactic acid dehydrogenase (LDH) and creatine phosphokinase (CPK) with commercial Kit (Pars Azmon, Iran).

Total protein assay:

Protein concentrations were determined through the Coomassie blue protein-binding method as explained by Bradford, 1976 (14).

Quantification of ROS level:

The ROS level measurement was performed using DCFH-DA as indicator. Briefly, DCFH-DA was added (final concentration, 10 μM) to samples (1mg

protein/ml) and then incubated for 10 min. The amount of ROS generation was determined through a Shimadzu RF5000U fluorescence spectrophotometer at 485-nm excitation and 520-nm emission wavelength The results were expressed as fluorescent intensity per 1mg protein (15).

Measurement of GSH content:
GSH content was determined using DTNB as the indicator and the developed yellow color was read at 412 nm on a spectrophotometer (UV-1601 PC, Shimadzu, Japan). GSH content was expressed as μM(16).

Measurement of Lipid peroxidation:
The content of malondialdehyde (MDA) was determined thiobarbituric acid reactive substances (TBARS) expressed as the extent MDA of productions during an acid-heating reaction. Briefly, 0.25ml sulfuric acid (0.05M) was added to 0.2mL samples (1mg protein/ml) afterwards, with the addition of 0.3 mL 0.2% TBA. All the microtubes were placed in a boiling water bath for 30 min. At the end, the tubes were shifted to an ice-bath and 0.4 ml n-butanol was added to each tube. Then, they were centrifuged at 3500×g for 10 min. The amount of MDA formed in each of the samples was assessed through measuring the absorbance of the supernatant at 532 nm with an ELISA reader (Tecan, Rainbow Thermo, Austria). Tetrametoxypropane (TEP) was used as standard and MDA content was expressed as nmol/mg protein (17).

Protein carbonyl:
Determination of protein carbonyl by spectrophotometric method, briefly 200μL of heart tissue is needed to hemogenate. Samples are extracted in 500 μL of 20% (w/v) TCA. Then, Samples placed at 4 ° C for 15 min. The precipitates are treated with 500 μL of 0.2% DNPH and 500 μL of 2 NHCl for control group, and Samples are incubated at room temperature for 1 h with vortexing at 5-min intervals. Then proteins are precipitated by adding 55 μL of 100% TCA. The micro-tubes are centrifuged and washed three times with 1000 μL of the ethanol-ethyl acetate mixture. And the micro-tubes are dissolved in 200 μL of 6 M guanidine hydrochloride. The carbonyl content is determined by reading the absorbance at 365 nm wavelength (18).

Statistical Analysis
Results are presented as mean±SEM. All statistical analyses were performed using the SPSS software, version 21. Statistical significance was determined using the one-way ANOVA test, followed by the post-hoc Tukey test. Statistical significance was set at $P < 0.05$.

RESULTS

Cardiac marker enzymes
As shown in Fig. 1, significant increase in the levels of cardiac injury markers (LDH and CPK levels) were observed in METH group compared to control group ($P<0.05$). Administration of ED (20 mg/kg) significantly ($p<0.05$) decreased the levels of LDH and CPK in METH-treated rats as compared with METH group (Fig.1). In group treated with vit E, no significant change in the cardiac marker enzymes was observed as compared with METH-treated group.

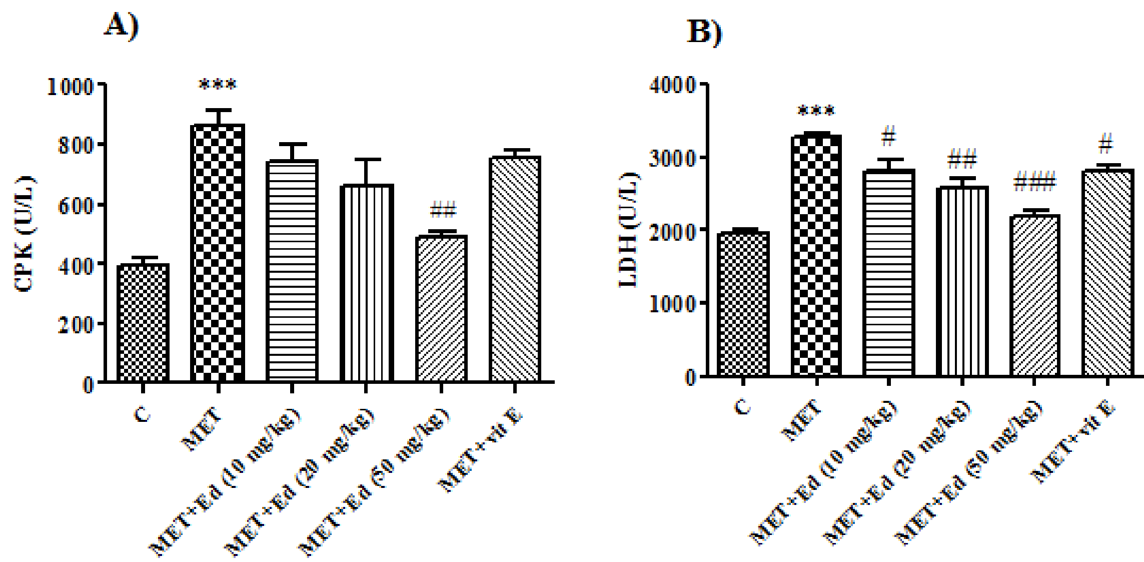

Figure1. Effects of Edaravone on cardiac marker enzymes. Data were expressed as mean±SEM (n = 6). ***Significantly different from control group (P < 0.001), #Significantly different from Methamphetamine group (P < 0.05),## Significantly different from Methamphetamine group (P < 0.01), ### Significantly different from Methamphetamine group (P < 0.001)

ROS formation:

As shown in Fig.2, METH administration significantly (p<0.05) increased ROS production as compared to control group, whereas ED pretreatment at low doses didn't have any significant effects in METH-induced ROS formation. Pretreatment with 20 mg/kg of ED resulted in significant reduction of METH-induced ROS production as compared to METH group (p<0.05). Also, vitamin E administration significantly (p<0.05) decreased METH induced-ROS formation.

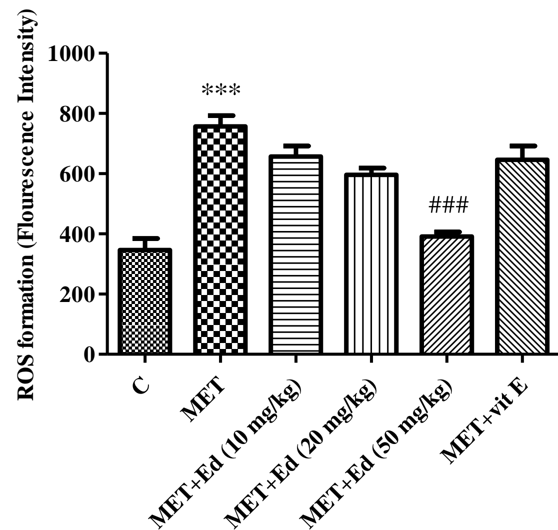

Figure 2. Effects of Edaravone on Methamphetamine - induced ROS production in heart tissue Data were expressed as mean±SEM (n = 6). ROS production was evaluated by DCF as indicator as described in Materials and Methods. ***Significantly different from control group (P < 0.001), ###Significantly different from Methamphetamine group (P < 0.001)

Lipid peroxidation:
Administration of METH significantly increased MDA level (a marker of LPO) in comparison with control group ($p<0.05$). Pre-treatment with ED decreased METH induced-LPO as dose dependent manner that was significant ($p<0.05$) at doses of 10 and 20 mg/kg. ED at doses of 5mg/kg did not have any effect on METH induced-LPO. The same effect was observed by administration of vitamin E (Fig. 3).

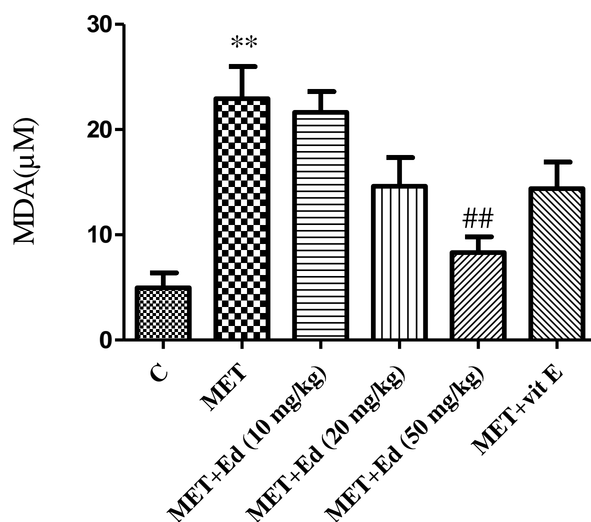

Figure 3. Effects of Edaravone on Methamphetamine - induced lipid peroxidation in heart tissue. Data were expressed as mean±SEM (n = 6). lipid peroxidation was evaluated by TBA as indicator as described in Materials and Methods. *Significantly different from control group ($P < 0.001$),###Significantly different from Methamphetamine group ($P < 0.001$)

GSH concentration:
METH administration caused significant decrease in GSH content compared to control group ($p<0.05$). Pre-treatment with ED reversed METH induced-GSH oxidation as dose dependent manner that was significant ($p<0.05$) at dose of 20 mg/kg. Administration of vitamin E did not significantly change GSH content when compared to METH group (Fig. 4).

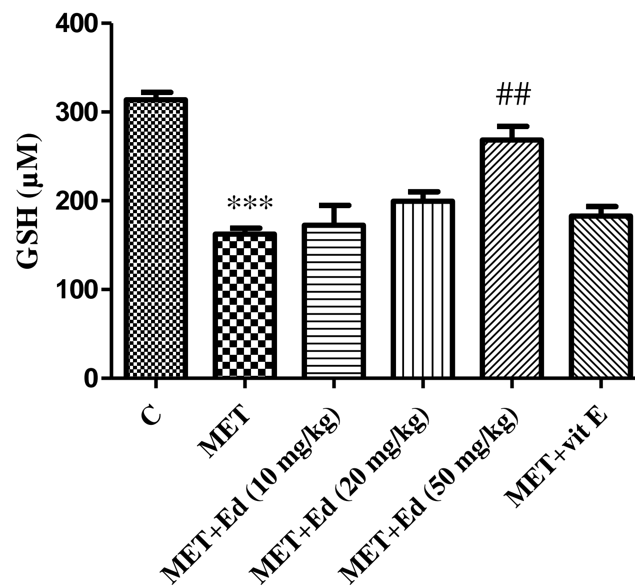

Figure4. Effects of Edaravone on Methamphetamine-induced GSH oxidation in heart tissue. Data were expressed as mean±SEM (n = 6). GSH content was evaluated by DTNB as indicator as described in Materials and Methods. ***Significantly different from control group ($P < 0.001$),##Significantly different from Methamphetamine group ($P < 0.01$)

Protein Carbonyl concentration:
As shown in Fig. 5, increased protein carbonyl concentration in METH-treated rats was observed as a consequence of oxidative damage compared with control group ($P<0.05$). Also, pretreatment of ED (20 mg/kg) significantly ($P<0.05$) reduced protein carbonyl level in heart tissue of METH treated rats.

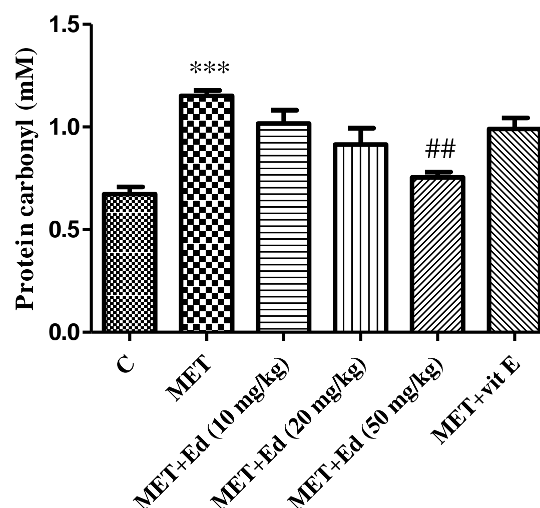

Figure5. Effects of Edaravone on Methamphetamine-induced protein carbonyl in heart tissue. Data were expressed as mean±SEM (n = 6). Protein carbonyl

content was evaluated by DNPH as described in Materials and Methods. ***Significantly different from control group ($P < 0.001$), ## Significantly different from Methamphetamine group ($P < 0.01$)

Mitochondrial function:

As shown in Fig. 6, decreased mitochondrial function in METH-treated rats was shown as a consequence of mitochondrial damage compared with control group (P<0.05). Also, pretreatment with ED (20 and 50 mg/kg) significantly (P<0.05) inhibited METH-induced mitochondrial function.

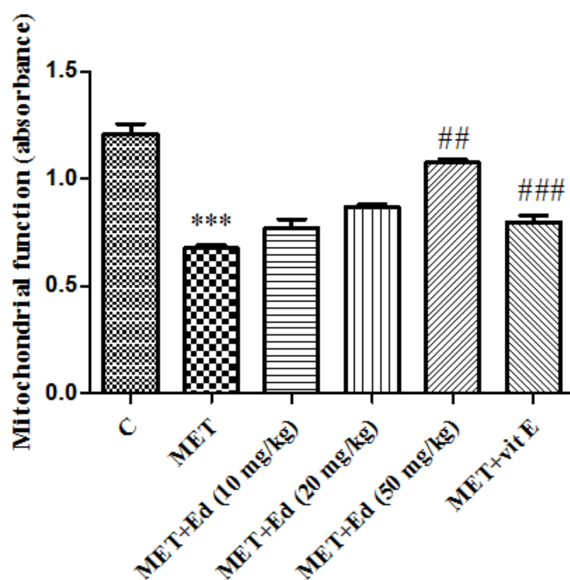

Figure6. Effects of Edaravone on Methamphetamine-induced mitochondrial dysfunction in heart tissue. Data were expressed as mean±SEM (n = 6). Mitochondrial function was evaluated by MTT as described in Materials and Methods. ***Significantly different from control group ($P < 0.001$), ## Significantly different from Methamphetamine group ($P < 0.01$), ### Significantly different from Methamphetamine group ($P < 0.001$)

DISCUSSION

Methamphetamine is a highly addictive abused drug (19) that was reported by the World Health Organization (WHO) as the second most popular illicit drug in the world (20).

Previous studies showed acute METH use could leads to cardiovascular problems such as the chest pain, rapid heart rate, high blood pressure and shortness of breath that can be followed by myocardial ischemia or infarction and sudden cardiac death. These effects of METH probably are due to an increase in dopamine and glutamate formation in the both central and peripheral branches of nervous system (21-23) .Then auto-oxidation of high concentration of dopamine can leads to increased production of ROS such as superoxide ($O_2^{\circ-}$) and hydroxyl radicals (·OH) in cell (24). Although previous data point toward oxidative damage as important contributors to a number of pathologic conditions associated with METH abuse especially its neurotoxicity but little is known about mechanism of METH-induced cardiotoxicity. In this study, in addition of determination of the oxidative stress parameters in the heart of rats which received METH, we also investigated the role of edaravone, a known antioxidant, in abrogating oxidative stress and maintaining the normal function of heart.

Our data indicated that METH impaired the antioxidant defense system in heart tissue (decreased GSH) in the METH group and increased oxidative damage to cellular macromoles (shown as lipid peroxidation and protein carbonyl) that corresponded to an increase in intracellular ROS concentration. In comparison with control rats, ROS production was increased in rats treated with METH. Our data confirmed the previous studies that showed METH decreased intracellular GSH, increased MDA levels and intracellular ROS production (19, 25) .

We demonstrated in this study that treatment with METH results in the damage to of heart cells that shown by an increase in cardiac marker enzymes (CPK and LDH).

In Fact, at physiological levels, ROS are involved in cellular signaling and maintain homeostasis(26) but excessive ROS cause , protein oxidation lipid peroxidation, and DNA damage .These events could lead to irreversible cell damage and death, which have been implicated in a wide range of pathological cardiovascular conditions (27-29).

On the other hand, there are many documented evidences for main role of oxidative stress plays in the pathogenesis and development of cardiovascular diseases such as hypertension, hypercholesterolemia, diabetes mellitus, atherosclerosis, myocardial infarction, angina pectoris, and heart failure (30-32).

Antioxidants are pivotal in maintaining redox balance by either preventing the formation of free radicals, detoxifying them, or by scavenging the reactive species or their precursors.

Edaravone is a drug that has been used in the treatment of acute ischemic stroke and showed ability for attenuation of oxidative mediated injury

in various organs (33, 34) through ROS scavenging, prevention of lipid peroxidation and mitochondrial protection.

On this basis, we evaluated the protective effects of edaravone against METH cardiotoxicity in rats after acute exposure to METH. ED decreased METH induced ROS production and also significant decreased in LPO and PC content compared with METH treated group. Our data was consistent with previous studies that reported antioxidant and radical scavenging effects of ED (35, 36).

High level of ROS in cell can damage cell and organelle membrane such as mitochondrial membrane which could triggering cell death signaling that finally could lead to several pathological conditions such as myocardial infarction (37, 38). Also, cardioprotective effects of edaravone were seen in several studies. Ikegami et al. showed that edaravone could protect against anthracycline-induced cardiotoxicity and prevent cardiac function deterioration (12). In an in vitro study, edaravone prevented doxorubicin-induced cardiotoxicity in H9c2 cardiac cells against via inhibition of ROS production and mitochondrial protection (12).

CONCLUSION

In this study, ED inhibited METH-induced cardiac cell death that shown by increases in cardiac – marker enzymes in serum. Also, pathological changes in heart tissue such as necrosis significantly attenuated by ED pretreatment.

In overall, we showed that edaravone has beneficial effects on myocardial and oxidative injury following METH administration in rats. Therefore, edaravone would be evaluated for treatment of cardiovascular diseases that oxidative stress is involved in their pathogenesis.

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