PROTECTIVE EFFECTS OF FEIJOA EXTRACT ON MDMA TREATED MICE

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Summary

Feijoa sellowiana is an evergreen bush widespread in the Southern part of Iran. Because of its good antioxidant activity and its high total phenol and flavonoid contents, its hepatoprotective effect was determined against MDMA treated mice. A single intraperitoneal injection of 5 mg/kg MDMA was employed for inducing liver toxicity. Mice received treatment with a single dose of 10, 20, 40, 50 or 100 mg/kg of methanol extract of fruit peel. Plasma samples were used for biochemical analysis. Aspartate aminotransferase and Alanine aminotransferase activity and reduced glutathione level in blood samples were assayed. Extract produced a significant decrease in aminotransferase enzymes in a dose-dependent manner (P<0.001) during 24th hour, in comparison with positive control at a single dose. Level of GSH in blood samples treated by extract was increased in comparison with the positive control group (P<0.001). Extract showed a significantly decrease in the hepatotoxicity of MDMA in mice. The antioxidant activity of Feijoa fruit peel may be a possible mechanism for its hepatoprotective effect.

Key words: Aminotransferase, Feijoa sellowiana, Glutathione, Hepatoprotection, MDMA

Introduction

Feijoa sellowiana (Myrtaceae) is an evergreen bush widespread in the Southern part of Iran. Owing to its easy adaptability in subtropical regions, nowadays it is being extensively cultivated in many countries (1) and also in Iran where the fruit are widely popular. Although the chemical composition of Feijoa has been clearly reported pharmacological studies of its constituents have barely been carried out (2-5). Feijoa showed potent antimicrobial and antifungal activity and a sensible activity against H. pylori (6-8). Some anti-cancer activities of the full Feijoa extract have been reported (9,10). Moreover, antioxidant activities of an aqueous extract on oxidative burst of human whole blood phagocytes have been reported (4,10). Yet, limited information is available concerning application of Feijoa anti-oxidative activity (11). 3,4-Methylenedioxymethamphetamine (MDMA/ ecstasy) is a ring-substituted amphetamine derivative that was synthesized in year 1912 by the Merck chemical company. It has also attracted a great deal of media attention in recent years due to its widespread abuse as a recreational drug, by the younger generation (12-14).
Clinical evidence has been shown that the kidney is a target for MDMA toxicity. In this sense, MDMA is metabolized by cytochromes P<sub>450</sub> 2D, 2B and 3A and reactive metabolites are readily oxidized to the corresponding o-quinones and to the reactive oxygen species (12,13,15). Because of good antioxidant activity of *Feijoa* peel (11) and its high total phenolic and flavonoids contents, we examined protective effect of methanol extract of *Feijoa* fruit peel against MDMA treated mice, in order to understand the usefulness of this plant in medicine.

### Materials and methods

**Animals**

Male albino mice (6 to 8 weeks), weighing 25-30g were used for all experiments. They were housed individually in standard rat cages in a room on a 12-hour light-dark cycle at 22°C (22 ± 2) and 50 ± 5% relative humidity, including food and water *ad libitum*. The animals were adapted to the condition for 7 days prior to the beginning of the experiment. The experiments were performed during the day time (08:00-16:00 hours). Each animal was used only once. A research proposal was prepared according to the guidelines of the Committee, for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Institutional Animal Ethical Committee (IAEC) of Mazandaran University of Medical Sciences also approved the proposal.

**Plant material and preparation of extract**

*F. sellowiana* fruit were collected from Fajr Citrus Experimental Institute in autumn, 2009 and confirmed by Dr B. Eslami. Fruit peels were dried at room temperature and coarsely grounded prior to extraction. Sample was extracted at room temperature by percolation with methanol (400 ml × 3 times). The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained (22%). The extract was prepared in phosphate buffer (pH=7.4) for pharmacological studies.

**Experimental Design**

Mice were divided into treatment and control groups (negative/positive). Each group contained five male mice, which received treatment with a single dose of 10, 20, 40, 50 or 100 mg/kg of methanol extract of fruit peel. Solvent was injected to the negative control group (10 ml/kg, ip). The positive control groups have received an acute intraperitoneal dose of MDMA (5 mg/kg) without *Feijoa* extract (12).

**Biochemical Determinations**

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activity in blood samples were assayed by using a commercial Kit of Zist Chimie (Tehran, Iran). Reduced glutathione was estimated according to the method described by Ellman et al (16,17). A 1 ml supernatant was precipitated with 1 ml of 4% sulphosalicylic acid and cold digested for 1 h at 4°C. The samples were then centrifuged at 1200 × g for 15 min at 4°C. To 1 ml of the supernatant obtained, 2.7 ml of phosphate buffer (0.1 mmol/L, pH 8) and 0.2 ml of 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) was added. The yellow color developed was measured at 412 nm (UV- Visible EZ201, Perkin Elmer: USA).

**Analysis of Data**

Statistical analysis was performed using SPSS for Windows (Ver.10, SPSS, Inc., Chicago, USA). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison post test and expressed as Mean ± SD in the mean of 5 mice. *P*<0.05 was considered to be significant.
Results and discussion

Activity of serum amino-transferees enzymes changes

The results of present study showed that methanol extract of *Feijoa* fruit produced a significant decrease in aminotransferase enzymes in a dose-dependent manner (P<0.001) during 24th hour, in comparison with positive control at a single dose of 10, 20, 40, 50 and 100 mg/kg extract (Fig.1).

![Fig.1. Effect of *Feijoa* fruit methanol extract on plasma aminotransferase activities in MDMA treated mice AST, aspartate aminotransferase and ALT, alanine aminotransferase. Values are presented as Mean ± SD (N = 5), ***P < 0.001 with respect to control; (ANOVA followed by Tukey multiple comparison post test).](image-url)

Level of GSH in blood samples treated by methanol extract of *Feijoa* fruit peel was increased in comparison with the positive control group (P<0.001). The antioxidant activity of *Feijoa* fruit peel caused an inhibition in MDMA hepatotoxicity effect (p < 0.001 in respect to control group) (Fig.2).

The liver has been identified as the most important target for MDMA in mice (18). In order to elucidate the MDMA- induced hepatotoxicity, the effects of MDMA on plasma aminotransferase activities and Glutathione (GSH) level were determined.

In MDMA-treated mice, activity of aminotransferase enzymes were increased significantly as compared to normal control animals (542% for AST and 565% for ALT, P<0.001), which was correlated to the decrease of glutathione level from 77.1 µM in controls to 18.3 in treated mice (P<0.001). Methanol extract of *Feijoa* fruit peels reduced aminotransferase enzymes dose dependently. At the highest dose, 100 mg/kg, extract reduced AST and ALT levels in MDMA-treated mice to 104 and 105 % respectively (P<0.001). It also increased glutathione level to 42.1 µM vs. 18.3 in MDMA-treated mice (P<0.001). This increase was also dose dependent.
MDMA is believed the primary toxic constituent, present in ecstasy. Other toxic constituents have been also identified including MDA (3,4 methylenedioxy amphetamine) and DOM (4-methyl-2,5-dimethoxy amphetamine). The study showed that MDMA induced formation of reactive oxygen species and an oxidative stress, resulting in lipid peroxidation (18,19). More studies, however, are needed to further elucidation of the exact mechanism by which MDMA induces hepatotoxicity. Moreover, MDMA also showed an inhibitory effect on glutathione peroxidase, which catalyzes the destruction of H$_2$O$_2$ produced by lipid hydroperoxidase. With inhibition of glutathione peroxidase a reduction in GSH will occurred, which results in accelerated lipid peroxidation (20,21). Antioxidants, such as vitamin E been proposed to prevent membrane damage of lipid peroxidation, not only through glutathione peroxidase, but also by allowing hydrogen to be abstracted from their own structure rather than from the allylic hydrogen of an unsaturated lipid, thus, interrupting the free radical chain reaction (22). *Feijoa sellowiana* fruit peel extract showed a significantly decrease in the toxicity of MDMA. This may be happened through the above mentioned mechanism, as well as a good reductive capability of the extracts for reducing Fe$^{3+}$ to Fe$^{2+}$ by donating an electron, Fe$^{2+}$ chelating activity, along with anti-lipid peroxidation activity (11,23). The need for further investigation of individual compounds, coupled with their in- vivo antioxidant activities and figuring out the different antioxidant mechanisms seems necessary.

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References


