

Study of Histopathology and Antioxidant Activity of Methanolic Extract of *Feijoa Sellowiana* against Dosage Induced by MDMA in Mouse Liver

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Summary

Liver injury induced by viruses, Chemical and Drug and protect by different medicinal plants. *Feijoa sellowiana* (Myrtaceae) is an evergreen bush native to southern of South America, where it is widely distributed and also in Iran where the fruits are very popular. *Feijoa* showed potent antimicrobial, antifungal, anti-cancer and antioxidant activity of the full *Feijoa* extract have been reported.

3, 4-methylenedioxymethamphetamine (MDMA, or ecstasy) is a ring-substituted amphetamine derivative that has attracted a great deal of media attention in recent years due to its widespread abuse as a recreational drug by the young generation.

The liver is a target for MDMA toxicity that MDMA is metabolized by cytochromes P4502D, 2B and 3A and reactive metabolites are readily oxidized to the corresponding o-quinones and to formation of reactive oxygen species (ROS).

In this study we tried to find out the antioxidant activity or hepatoprotective effect of *Feijoa* with determination activity of aminotransferase enzymes (SGOT, SGPT) and glutathione reductase (GSH) level and liver histopathology in comparison with positive & negative controls. The untreated male mice (25-30g body weight) were used to investigate the hepatotoxicity induced by MDMA (5mg/kg) methanolic extract at doses (10, 20, 40, 50, 100 mg/kg) was used. Liver sections were also taken for histopathological examination. The results showed that the activity of SGOT & SGPT are significantly decreased by methanolic extract and level of GSH are significantly increased by methanolic extract. Necrosis and other cellular lesions in liver tissue were decreased. The finding show that *Feijoa Sellowiana* is a hepatoprotective plant.

Keywords: *Feijoa Sellowiana*, MDMA, SGOT & SGPT, GSH, Necrosis

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Introduction

Feijoa sellowiana (Myrtaceae) is an evergreen bush native to southern of South America, where it is widely distributed. Owing to its easy adaptability in subtropical regions, nowadays it is extensively cultivated in many countries (1) and also in Iran where the fruits are very popular. Although the chemical composition of *Feijoa* has been clearly reported (2-6) pharmaceutical studies of its constituents have barely been carried out. *Feijoa* showed potent antimicrobial and antifungal activity and a sensible activity against *H. pylori* (7-9). Some anti-cancer activities of the full *Feijoa* extract have been reported (10, 11). Moreover, antioxidant activities of an aqueous extract on oxidative burst of human whole blood phagocytes have been reported (5, 11). Yet little information is available about *Feijoa* antioxidative activity. In this study, we examined the antioxidant activity of methanolic extract of *Feijoa sellowiana* against dosage induced by MDMA in mouse liver, in order to understand the usefulness of this plant as a foodstuff as well as in medicine. 3,4-methylenedioxymethamphetamine (MDMA, or ecstasy) is a ring-substituted amphetamine derivative that were synthesized in year of 1912 by Merck chemical company and has attracted a great deal of media attention in recent years due to its widespread abuse as a recreational drug by the young generation(12, 13). clinical evidence has shown that the liver is a target for MDMA toxicity; in this sense, MDMA is metabolized by cytochromes P450 2D, 2B and 3A and reactive metabolites are readily oxidized to the corresponding o-quinones and to formation of reactive oxygen species(ROS)(14,15). In this study we tried to find out the hepatoprotective effect of *Feijoa Sellowiana* with determination activity of aminotransferase enzymes (SGOT& SGPT), Hepatic glutathione reductase(GSH)level and liver histopathology in comparison with positive &negative controls.

Materials and methods

Animals

Male albino mouse (6 to 8weeks), 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 ± 1°C) and 50±5% with relative humidity, including food and water ad libitum. The animals were adapted to the condition for 7 days prior to the beginning of the experiments. The experiments were performed during the day time (08:00-16:00 hours). Each animal was used once only. A research proposal was prepared according to the guidelines of 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 approved the proposal.

Plant

Feijoa fruits were collected from Fajr citrus experimental institute in autumn 2006. Fruit peels were dried at room temperature and coarsely ground before extraction. A known amount of each part was extracted at room temperature by percolation method using methanol and water methanol/ water (80:20, 400 ml×3 times). The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained, which was then freeze-dried for complete solvent removal (16). The extract was prepared in phosphate buffer (pH=7.4) for pharmacological studies. The fruit peels aqueous extract, fruit peels methanolic extract, FM (20.5%) were obtained, respectively.

Experimental design

Mice divided into five treatment groups and control group (negative & positive). Each group contained five male mice and treatment by a single dose of 10, 20, 40, 50 and 100 mg/kg of methanol extract of fruits of *Feijoa* respectively (Total = 7 groups). Solvent was injected to the negative group (10 ml/kg, ip). Following the preliminary study, the dose of 100 mg/kg was chosen for the remaining of the study in order to evaluate the hepatoprotective of *Feijoa* (17).

Biochemical determinations

The activities of Aspartate aminotransferase (AST) activity and Alanine aminotransferase (ALT) activity in samples of blood were assayed using a commercial kit of Zist himie (Tehran, Iran). Reduced glutathione (GSH) was estimated by Ellman's method (18).

Histological studies

The liver was completely excised and freed of any extraneous tissue. Multiple samples were then taken from each liver (mean 3 mm) and placed in 10% neutral buffered formalin. The liver was cut into small pieces, sections prepared and stained by Eosin-Hematoxylin and examined blind for histo-pathological changes.

Analyses of the data

Statistical analysis was performed using SPSS for Windows (Ver. 10, SPSS, Inc., Chicago, USA). All values were analyzed by one-way analysis of variance (ANOVA) and expressed as mean \pm Standard error in the mean of 5 mice (S.E.M). Student-Newman-Keuls test were used to evaluate the significance of the obtained results. $P < 0.05$ was considered to be significant (19).

Results

Activity of serum amino-transferases enzymes changes

The results of present study showed that the methanol extract of fruits of *Feijoa* produced statistically and it significantly decreased the activity of amino-transferases enzymes and dose-dependently ($P < 0.01$) at 24th hour, in comparison with positive control at single dose of 10, 20, 40, 50 and 100 mg/kg (Fig. 1).

Hepatic Glutathione reductase (GSH) level changes

Level of GSH of samples of blood of methanol extract of fruits of *Feijoa* were increased compared with the positive control group. Antioxidant effect *Feijoa* have shown to inhibit these hepatotoxic effect of MDMA. P value was less than 0.05 in respect to control group (Fig. 2).

Light microscope observation

Histo-pathological studies using a light microscope showed significantly decrease hepatocellular damage including necrosis and infiltration, due to methanol extract of fruits of *Feijoa* (fig. 3.d) when compared to control group (fig. 4.a). In addition, other histo-pathological parameters including the number of Kupffer and mononuclear cells, had changed significantly with methanol extract of fruits of *Feijoa* respectively (Table. 1.).

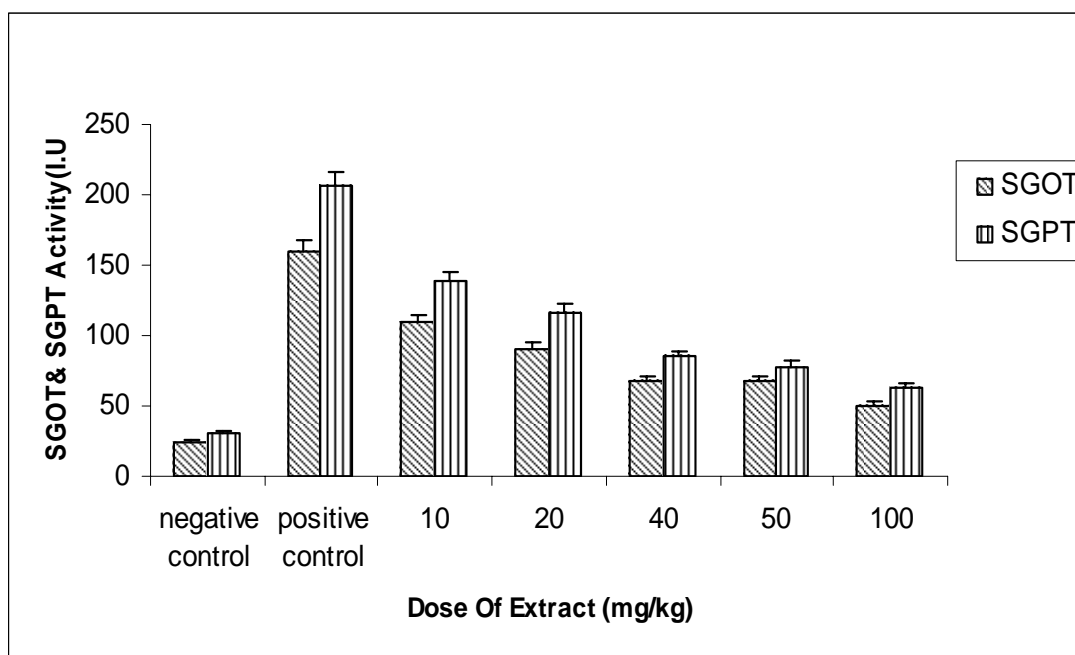


Fig.1. Activity of ALT(Alanine transferases) & AST(Aspartate transferases) enzymes methanolic extract of fruits of *Feijoa Sellowiana* at differences concentrations. Values are presented as mean \pm SEM (N = 5), ***P < 0.001 with respect to control, (ANOVA followed by Newman-Keuls multiple comparisons test).

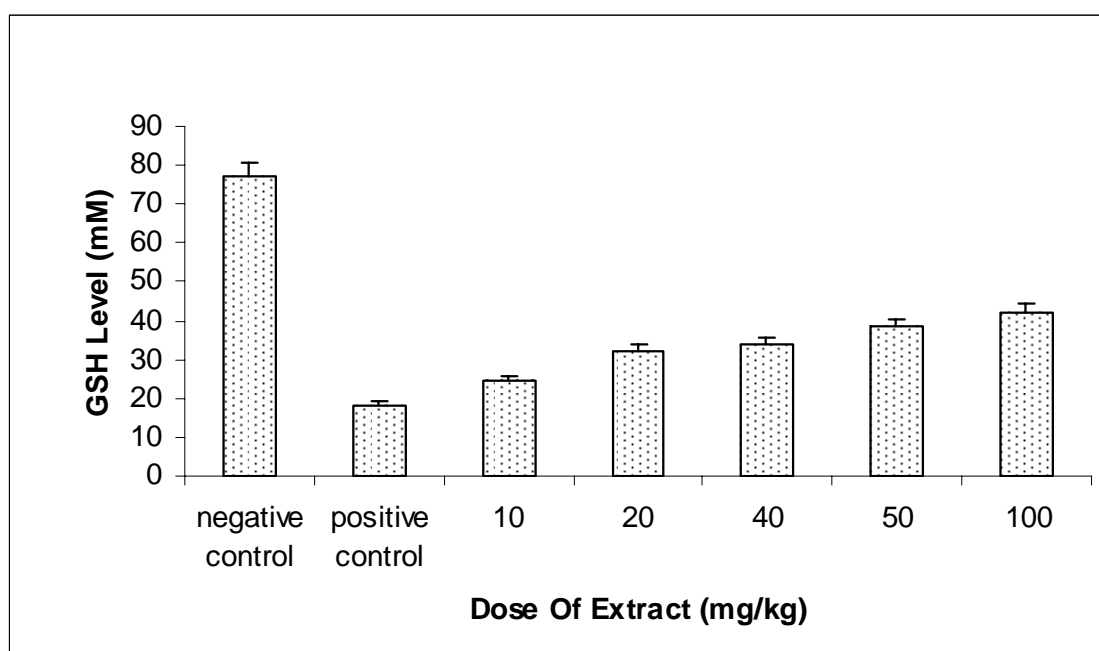


Fig.2. Level of Glutathione (GSH) methanolic extract of fruits of *Feijoa Sellowiana* at difference concentrations. Values are presented as mean \pm SEM (N = 5) ***P < 0.001 with respect to control, (ANOVA followed by Newman-Keuls multiple comparisons test).

Table.1. Histo-pathological effects of methanol extract of fruits of *Feijoa Sellowiana* concentrations of (10, 20, 40, 50, 100mg/kg).

Histopathological Parameters	Negative Control (10ml/Kg)	<i>Feijoa Sellowiana</i> (mg/kg)					Positive Control(5mg/kg)
		10 (mg/kg)	20 (mg/kg)	40 (mg/kg)	50 (mg/kg)	100 (mg/kg)	
Kupffer cells	+	+3	+3	+3	+2 ^{**}	+2 ^{**}	+3 [*]
Edematous cells	+	+1	+1	+1	+2 [*]	+2 ^{**}	+4 [*]
Mononuclear cells	+	-	+1	+1	+2	+2	+2
hemorrhage	-	+1	+1	+1	+2 [*]	+3 ^{**}	+4
Necrosis	-	-	+1	+2 [*]	+3 ^{**}	+3 ^{**}	+5 ^{**}

-no effect, +1Minor effect, +2Medium effect, +3Major effect, +4high effect, +5super effect. *P<0.05, **P<0.01, significantly different from control using Fisher exact test. Data are means of three replicates.

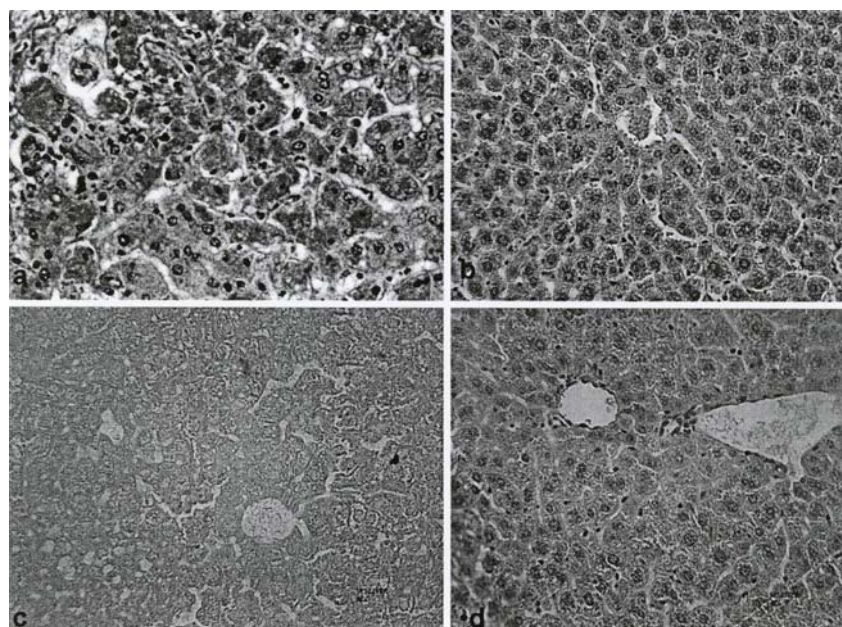


Fig.3. Photomicrograph of lobules from control groups and extract of fruits of *Feijoa Sellowiana* at differences concentration – treated liver. Staining of Neagative Control shows that cytoplasm was acidophilic and surround by a bright basophilic nucleus (a). MDMA (5mg/kg) or positive control showed limited changes in lobules of liver and hepatocellular necrosis, with infiltration of mononuclear cells and accumulation of necrotic Kupffer cells with Pyknotic nuclei (b). Histo-pathological changes of aqueous(for compare)& methanol extract of fruits of *Feijoa Sellowiana* at single dose of(100mg/kg) respectively.(c&d).

Discussion

The liver has been identified as the most important tissue target for MDMA in mice (20). In order to elucidate the MDMA – induced hepatotoxicity, the effects of MDMA on transaminase enzyme effect and total glutathione mouse liver were determined.

Moreover the effect of antioxidant of *Feijoa* on MDMA – induced hepatotoxicity was determined. In the present study, we sought to determine how we can prevent or decrease the hepatotoxic effect of MDMA. The activity of aminotransferase enzyme was increased significantly which was well correlated with decrease of glutathione (21). On the other hand when of aqueous & methanol extracts of fruits of *Feijoa* as antioxidant were used, this hepatotoxicity effect on enzyme was reduced almost to 40-50% of control. Glutathione depletion has been shown to correlate with lipid peroxidation in liver. MDMA, believed to be the primary toxic constituent, are present within ecstasy. Other toxic constituents have also been identified including the MDA and DOM. In this study, MDMA induces formation of reactive oxygen species and an oxidative stress, resulting in lipid peroxidation (20, 22). More studies, however, are needed to further elucidate the exact mechanism by which MDMA induces hepatotoxicity. Moreover, MDMA also was shown to be an inhibitor of glutathione peroxidase, which catalyzed the destruction of H_2O_2 of lipid hydroperoxidase by reduced glutathione. Therefore, with inhibition of glutathione peroxidase, there is a reduction in GSH which resulted in accelerated lipid peroxidation (23, 24). Antioxidants such as vitamin E and selenium have 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 on unsaturated lipid, thus interrupting the free radical chain reaction (25). Treatment with methanolic extract of *Feijoa sellowiana* fruits has been shown to significantly decrease the toxicity of MDMA (Table.1). This may be through the mechanism mentioned above as well as extracts had good reductive capability for reducing Fe^{+3} to Fe^{+2} by donating an electron Fe^{+2} chelating activity and anti-lipid peroxidation activity (26). Further investigation of individual compounds, their in vivo antioxidant activities and in different antioxidant mechanisms is needed.

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