The Hepatoprotective Effects of Corn Silk against Dose-induced Injury of Ecstasy (MDMA) Using Isolated Rat Liver Perfusion System

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ABSTRACT

Background: Corn silk (CS) is widely used in Iranian traditional medicine. The aim of this study was to investigate hepatoprotective activity of CS by Isolated Rat Liver Perfusion System (IRLP).

Methods: Hydro-alcoholic extract of corn silk (10, 20, 40, and 100 mg kg-1) was evaluated for its hepatoprotective activity by IRLP. Phenol and flavonoid contents of the extract were determined as gallic acid and quercetin equivalents from a calibration curve, respectively.

IRLP system is ideal for studying biochemical alterations of chemicals with minimum neuro-hormonal effects. In this study, the liver was perfused with Kerbs-Henseleit buffer, containing different concentration of hydro-alcoholic extract of corn silk (10, 20, 40, 50,100mg/kg), added to the buffer, and perfused for 2 hours. During the perfusion, many factors, including amino-transferees activities and the level of GSH, were assessed as indicators of liver viability. Consequently, sections of liver tissues were examined for any histopathological changes.

Results: Histopathological changes in liver tissues were related to hydro-alcoholic extract of corn silk concentrations in a dose-dependent manner. Also, 50 and 100mg/kg doses caused significant ($P<0.05$) histopathological changes. Level of GSH in samples perfused with hydro-alcoholic extract increased compared to the control group.

Conclusion: Hepatoprotective effect of CS is due to decreased lipid peroxidation, although other mechanisms might also be involved.

Keywords: Glutathione, Krebs-Henseleit, Liver Injury, N-Methyl-3, Zea Mays, 4-Methylenedioxyamphetamine.

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INTRODUCTION

In recent years, considerable attention has been directed towards the identification of plants with antioxidant ability that may be used for human consumption. Diuretic, antilithiasic, uricosuric, and antiseptic properties are traditionally attributed to corn silk (CS), stigma/style of Zea Mays Linne (Poaceae/Gramineae), which, in many parts of the world, has been applied to the treatment of edema as well as cystitis, gout, kidney stones, nephritis, and prostatitis (1,2). CS contains proteins, vitamins, carbohydrates, Ca2+, K+, Mg2+ and Na+ salts, volatile oils, and steroids, such as sitosterol and stigmasterol, alkaloids, saponins, tannins, and flavonoids (2). Phenolic compounds present in CS are anthocyanins, p-coumaric acid, vanillic acid, protocatechuic acid, derivatives of hesperidin and quercetin, and bound hydroxycinnamic acid forms composed of p-coumaric and ferulic acid (3). There are also reports about antioxidant activity of CS (2,4). The constituents in the volatile

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extract and petroleum ether, ethanol, and water extract of CS exhibited clear antioxidant activities (1). On the other hand3,4-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 (5,6). Liver is a target for MDMA toxicity where it is metabolized by cytochromes P4502D, 2B, and 3A and reactive metabolites are readily oxidized to the corresponding o-qiuinones which leads to formation of reactive oxygen species (ROS) (7,8).

To the best of the authors' knowledge, hepatoprotective activity of CS has not been reported to date and nothing was found about the mechanism and/or hepatoprotective activity of CS. Therefore, the aim of the present study is to determine the hepatoprotective activity and antioxidant activities of CS against oxidants induced by MDMA by isolated rat liver perfusion system in order to understand the usefulness of this plant as a foodstuff and in medicine. Moreover, IRLP was employed to evaluate corn silk hepato-protective and its correlation to biochemical changes.

MATERIALS AND METHODS

Animals

Male albino Wistar rats (6 to 8 weeks), weighing 200-250g, were used for all experiments. They were housed individually in standard rat cages in a room on a 12- hour light- dark cycle with controlled temperature (22 ± 1°C) and a relative humidity of 50 ± 5%, with food and water ad libitum. The animals were adapted to the condition for 7 days prior to the beginning of the experiments (9). The experiments were performed during daytime (08:00 a.m. to 04:00 p.m.). Each animal was used only once. 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

CS (dried cut stigmata of Zea mays L, Poaceae flowers) used for this investigation were collected (km 5, Sari Road, Ghaemshahr, Iran) and were identified and confirmed in January 2008 and authenticated by Dr. Bahman Eslami (Department of Biology, Islamic Azad University of Qhaemshahr, Iran) and the voucher specimen was deposited in the herbarium of Sari School of Pharmacy (No. HS280). CS was dried at room temperature and an ethanol-water (1:1) extraction was performed using maceration method by soaking in the solvent mixture. The extract was collected after removing the solvent and lyophilization. The extract was prepared in phosphate buffer (pH=7.4) for the antioxidant studies (2).

Determination of total phenolic and flavonoid content

Total phenolic compound content was determined by the Folin-Ciocalteau method (10-12). The extract sample (0.5 ml of different dilutions) was mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent for 5 minutes and 2.0 ml of 75 g/l sodium carbonate were, then, added. The absorbance of reaction was measured at 760 nm after 2 hours of incubation at room temperature. The results were expressed as gallic acid equivalents. Total flavonoid was estimated according to the method proposed in our recent paper (4,13,14). Briefly, 0.5 ml solutions of the extract in methanol were mixed with 1.5 ml of methanol, 0.1 ml of 10% AlCl3, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double-beam spectrophotometer (Perkin Elmer). Total flavonoid contents

were calculated as quercetin from a calibration curve.

Experimental design

Rates were divided into five treatment groups and two control groups (positive and negative controls). Each group contained four male rats, and their livers were perfused by single doses of 10, 20, 40, 50, and 100mg/kg of the hydro-alcoholic extract of corn silk. To assess oxidant activity of MDMA as positive control, a high dose (20 mg/kg) of it was added to the perfusion medium, 30 minutes before administering the perfusion doses of the extract. Negative control livers were perfused with the perfusion buffer only (Total= 7groups). Following a preliminary study, a 50mg/kg dose was chosen for the remainder of the study in order to evaluate the hepatoprotectivity of corn silk (15).

Buffer

Perfusion fluid was made from Krebs-Henseleit buffer. The perfusion medium consisted of 118.9 mM NaCL, 4.76 mM KCL, 1.19 mM KH2PO4, 2.55 mM CaCL2, and 24.8 mM NaHCO3 at 37˚C. Glucose (1%W/V) was added as well (16,17). The perfusion medium was gassed continuously with carbogen (95% O2, 5%CO2) (Figure 1).

Perfusion conditions and parameters of liver viability

Temperature, perfusion pressure, flow rate, and perfusion fluid pH were closely monitored during the perfusion, particularly, during the first 30 minutes of equilibration. These parameters were initially checked every 10 to 15 minutes and the experiment did not begin until they had reached constant and acceptable values. The temperature in the perfusion system was also set and maintained at 37˚C. Perfusion pressure was below 10-15 cm of water with a flow rate of approximately 2 ml/min/g liver weight, to provide adequate oxygenation. The perfusion fluid pH was always set between 7.2 and 7.4 by adjusting the CO2 content. As soon as perfusion began, the liver developed an even, light-brown colored and soft and which was kept moistened. Serum amino-transferases activity (ALT&AST) served as indicators of liver viability during perfusion which was determined in the samples of perfusion medium (18).

Biochemical determinations

The activities of reduced glutathione (GSH) were estimated by Ellman's method (19).

Histological studies

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

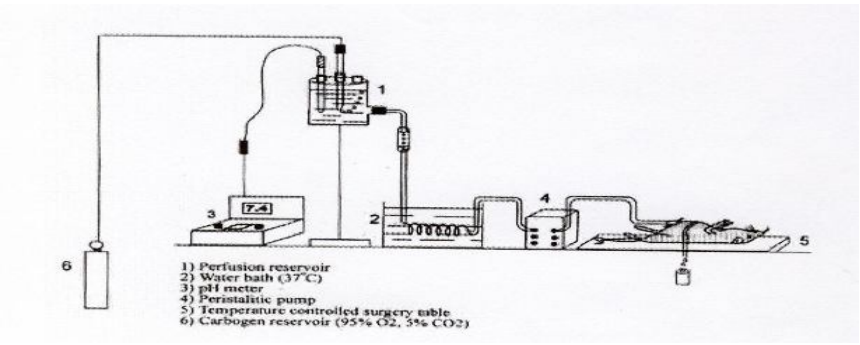

Figure 1. Schematic diagram of a rat liver perfusion system.

Surgery

The rats were anesthetized with ether. Heparin (500 unit; I.P.) was used to prevent blood coagulation prior to anesthesia (20). An incision was made along the length of the abdomen to expose the liver. Sutures were placed loosely around the common bile duct, which was, then, cannulated with PE-10 tubing and secured. Sutures were, then, placed loosely around the inferior vena cava, above and below the renal veins. The distal suture around the vena cava was tightened and an 18g polyethylene catheter was inserted and placed above the renal vein.The diaphragm was incised and the inferior vena cava ligated suprahepatically. Following the attachment of the perfusion tubing to the cannula, the liver was perfused in situ through the portal vein (20).

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 ± standard error of the mean (SEM). Student-Newman-Keuls test was used to evaluate the significance of the obtained results and $P<0.05$ was considered significant.

RESULTS

Total phenol and flavonoid contents

Total phenol compounds, as determined by Folin Ciocalteu method, were reported as gallic acid equivalents with reference to the standard curve ($y = 0.0063x$, $r2 = 0.987$). The total phenolic content of CS was 118.94 ± 2.78 mg gallic acid equivalent/g of the extract. The total flavonoid content was 58.22± 1.34 mg quercetin equivalent/g of extract, with reference to the standard curve ($y = 0.0067x+0.0132$, $r2= 0.999$).

Hepatic Glutathione reductase(GSH) level changes

Compared with the positive control group, the level of GSH in samples of perfused hydro-alcoholic extract of corn silk increased. Antioxidant effect CS has been shown to inhibit the hepatotoxic effect of MDMA ($P<0.05$) (Figure 2).

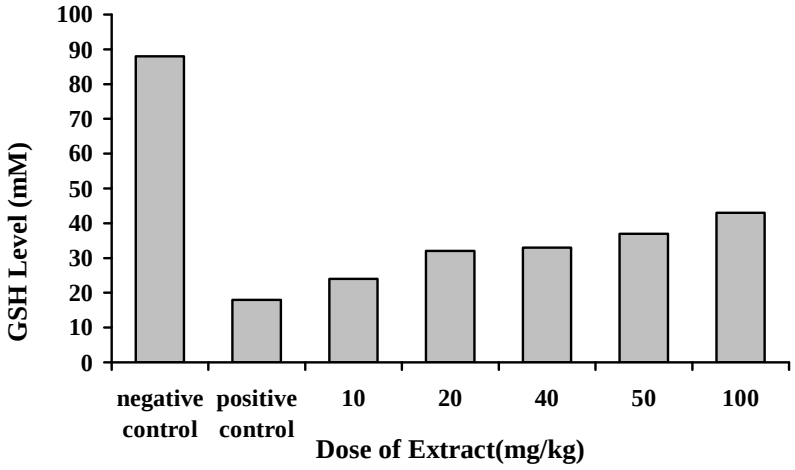

Figure 2. Level of glutathione (GSH) of the hydro-alcoholic extract of corn silk at different concentrations; Values are presented as mean ± SEM (N = 5) ***P<0.001 with respect to control, (ANOVA followed by Newman–Keuls multiple comparisons test).

Table 1. Histo-pathological effects of hydro alcoholic extract of corn silk at 40, 50, and 100mg/kg concentrations following isolated rat liver perfusion system.

Histopathological Parameters	Negative Control (Perfusion buffer)	Corn silk (mg/kg) 40 50 100 (mg/kg)	Positive Control(MDMA 20mg/kg)
Kupffer cells	+	+3 +2** +2**	+ 3*
Edematous cells	+	+1 +2* +2**	+4*
Mononuclear cells	+	+1 +2 +2	+2
Hemorrhage	_	+1 +3* +3**	+4
Necrosis	_	+1 +2* +3**	+ 5**

Light microscope observation

Histopathological studies using a light microscope showed significant hepatocellular damage, including necrosis and infiltration, resulting from hydro-alcoholic extract of corn silk (Figure 3.b) compared to the control group (Figure 3.a). In addition, other histopathological parameters, including the number of Kupffer and mononuclear cells, and edematous cells, and cell degeneration changed significantly with hydro-alcoholic extract of corn silk (Table 1).

DISCUSSION

Total phenol compounds were determined as gallic acid and the total flavonoid contents as quercetin equivalent/g of the extract. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities. Studies have shown that increasing levels of flavonoids in the diet can reduce occurrence of certain human diseases (21). CS extract contains high levels of total phenol and flavonoids. On the other hand, liver has been identified as the most important tissue target for MDMA in rats (22). In order to elucidate the MDMA–induced hepatotoxicity, the effects of MDMA on total glutathione in rat liver were determined. Moreover, the antioxidant effect of CS extract on MDMA–induced hepatotoxicity was determined. MDMA decreases glutathione activity significantly (23). Glutathione depletion has been shown to correlate with lipid peroxidation in the liver. CS extract reduced hepatotoxic effects of MDMA to almost 40-50% of controls.

MDMA is believed to be the primary toxic constituent in ecstasy. Other toxic constituents, including the 3,4-Methylenedioxyamphetamine (MDA) and 2,5-dimethoxy-4-methylamphetamine (DOM) have also been identified. In this study, MDMA induced the formation of reactive oxygen species and oxidative stress, resulting in lipid peroxidation (24,25). More studies; however, are required to elucidate the exact mechanism by which MDMA induces hepatotoxicity. Furthermore, MDMA has also been shown to be an inhibitor of glutathione peroxidase, which catalyzes the destruction of H2O2 produced by lipid hydroperoxidase through reduced glutathione. With inhibition of glutathione peroxidase, there is a reduction in GSH which results in accelerated lipid peroxidation (22,26). Antioxidants such as vitamin E and selenium have been proposed to prevent membrane damage from lipid peroxidation not only through glutathione peroxidase but also by allowing hydrogen to be abstracted

from their own structures rather than from the allylic hydrogen of unsaturated lipids, thus interrupting the free radical chain reaction (24). Treatment with CS extract has been shown to significantly decrease MDMA toxicity (Table 1). This may be through the mechanism mentioned above as well as extracts having a good reductive capability for reducing Fe+3 to Fe+2 by donating an electron Fe+2 chelating activity and anti-lipid peroxidation activity (25). Further investigation of individual compounds, their in vivo antioxidant activities, and different antioxidant mechanisms is mandatory. Our data showed that the administration of CS extract causes edema that can be assessed by histopathological examination (Table 1). Our findings are in agreement with the fact that oncotic agents can cause increases in pressure (27). In addition, isolated organs have a time-dependent tendency to absorb water, as with a relatively protein-free medium, water gradually escapes from the vascular space which results in the development of interstitial edema (27). Histopathological examination also revealed significant hemolysis as assessed by the hemolytic index (Figure 3.b).

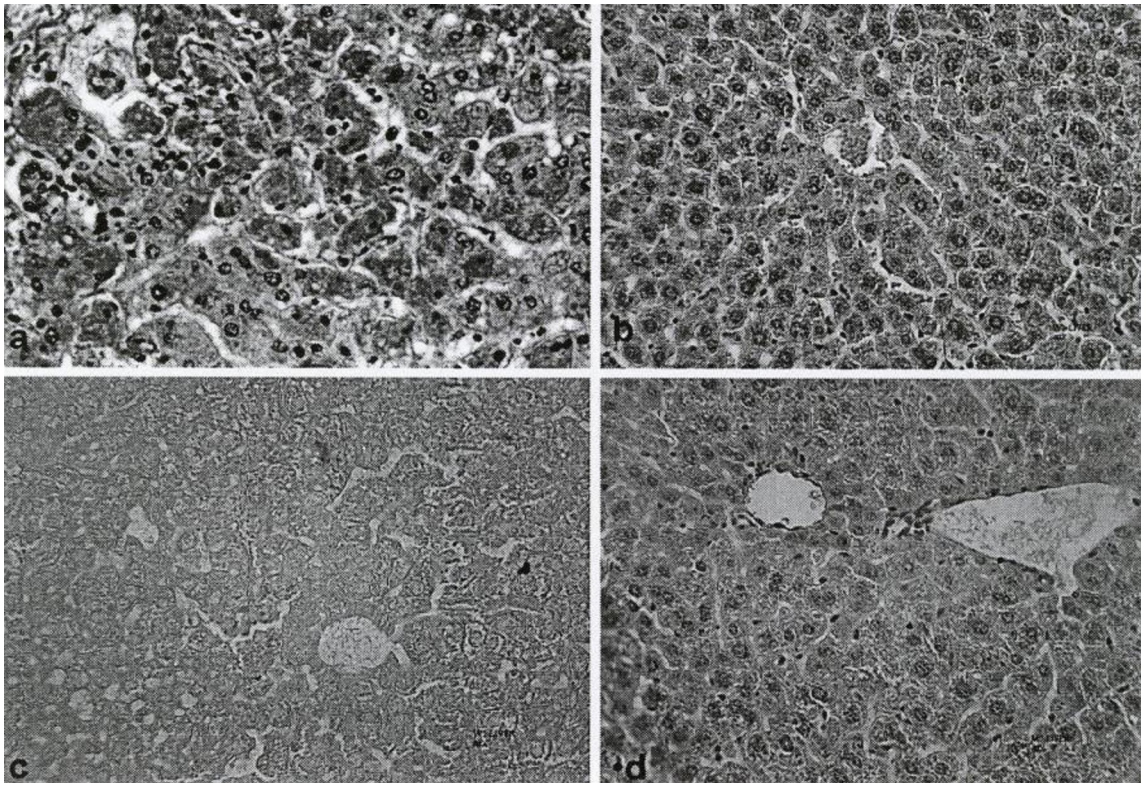

Figure 3. Photomicrograph of lobules from control and corn silk – treated liver goups: Controls showed red blood cells (RBC), hepatocytes (H) and central veins (CV) with acidophilic cytoplasms, surrounded by a bright basophilic nucleus (a). MDMA (20mg/kg) and positive controls demonstared limited changes in lobules of liver and hepatocellular necrosis, with infiltration of mononuclear cells and accumulation of necrotic Kupffer cells with pyknotic nuclei **(b).** Histopathological changes of corn silk at single doses of (50mg/kg) **(a)** and (100 mg/kg) **(d).**

This can be due to altered calcium homeostasis concomitant with a significant increase in cytosolic calcium, which has been previously reported with *Phytolacca Americana*'s effect on liver (28). Moreover, intracellular calcium homeostasis disturbances have been shown to be associated with a variety of toxicological and pathological processes. Karami *et al*. reported that accumulation of CS extract in liver provided protection against injury (29). In a similar manner, the results of the present study also demonstrated liver protection (Table 1). This could be a result of CS extract receptor binding, which is sufficient to affect different cells. In the present study, significant necrosis was also observed in the liver with 20 mg/kg of MDMA. CS extract decreases formation of reactive oxygen species and oxidative stress, resulting in lipid peroxidation (30). This may explain the observed necrosis (Figure 3.b). Therefore, the results of our study, in agreement with others, demonstrated that Isolated Rat Liver Perfusion System is a suitable model to study the hepatoprotective effects of chemicals such as MDMA. Further studies, however, are necessary to elucidate the exact mechanism by which CS extract induces hepatoprotectivity (30).

CONCLUSION

Liver perfusion is a suitable model to study the hepatoprotective effects of natural products (CS). The hepatoprotective effect of CS is due to decreased lipid peroxidation, although other mechanisms may also be involved.

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