

## The Renal Protective Effects of Corn Silk and Feijoa by using in situ Rat Renal System

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### ABSTRACT

**Background:** Corn silk (CS) is widely used in Iranian traditional medicine. *Feijoa sellowiana* (FS), on the other hand, is a non-native plant widespread in the southern part of Iran. The aim of the present study was to examine the renal protective activity of CS and FS against dosage-induced ecstasy (MDMA) by in situ rat renal perfusion (IRRP) system.

**Methods:** Hydro-alcoholic extracts of CS and FS (10, 20, 40 and 100 mg/ kg) were studied for their renal protective activities by IRRP system. In this study, the kidneys were perfused with Kerbs-Henseleit buffer, containing different concentrations of hydro-alcoholic (HA) extracts of CS and FS (10, 20, 40, 50, and 100mg/kg) added to the buffer and perfused for two hours. During the perfusion, many factors, including urea, creatinine and GSH levels assessed as indicator of renal viability. Consequently, sections of renal tissue were examined for any histopathological changes.

**Results:** The results showed that histopathological changes in renal tissue related to HA extract of CS AND FS concentrations dose-dependently. Doses of 50, 100 mg/kg caused significant histopathological changes ( $P<0.05$ ). Glutathione (GSH) levels of samples perfused by HA extract of CS and FS increased compared with the positive control group.

**Conclusion:** Renal protective effects of CS and FS decrease lipid peroxidation, although other mechanisms may also be involved.

**Keywords:** Corn Silk, Feijoa Sellowiana, GSH, Renal Perfusion.

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### INTRODUCTION

In recent years, a great deal of attention has been directed towards the identification of plants with antioxidant ability that can be used for human consumption. Diuretic, as well as antilithiasic, uricosuric, and antiseptic properties are traditionally attributed to CS, stigma/style of *Zea mays* Linne (Poaceae/ Gramineae), which has been used in many parts of the world for the treatment of edema as well as cystitis, gout, kidney stones, nephritis, and prostatitis [1,2]. CS contains proteins, vitamins, carbohydrates,  $\text{Ca}^{2+}$ ,  $\text{K}^{+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^{+}$  salts, volatile oils, steroids, such as sitosterol and stigmasterol, alkaloids, saponins, tannins, and flavonoids [2].

Although the chemical composition of FS (*Myrtaceae*) has been clearly reported,

pharmacological studies of its constituents have barely been carried out [3]. Some anti-cancer activities of the full FS extract have been reported [4]. However, limited information is available on the application of FS antioxidative activity [5].

Phenolic compounds present in CS are anthocyanin, p-coumaric acid, vanillic acid, protocatechuic acid, derivatives of hesperidin and quercetin, and bounded hydroxycinnamic acid components composed of p-coumaric and ferulic acid [6]. There are also reports on the antioxidant activity of CS [2, 7]. The constituents in the volatile petroleum ether and the hydro-alcoholic extract of CS exhibited clear antioxidant activities [1].

On the other hand, 3, 4-methylenedioxymethamphetamine (MDMA

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or ecstasy) is a ring-substitute 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 [8,9]. The liver and kidney are targets for MDMA toxicity because MDMA is metabolized by cytochromes P450 2B and 3A. Then reactive metabolites are readily oxidized to the corresponding o-quinones and to the formation of reactive oxygen species (ROS) [10,11].

Renal protective activities of CS and FS have not been reported yet and no studies are available on the mechanism of CS and FS. Therefore, the aim of the present study is to determine the renal protective activity or antioxidant activities of CS and FS against harmful oxidizing property of MDMA by isolated renal perfusion system in order to understand the usefulness of these plants as a foodstuff as well as in medicine. Moreover, IRRP was employed to evaluate CS and FA renal protective effects 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 at 22°C ( $22 \pm 1^\circ\text{C}$ ) and  $50 \pm 5\%$  relative humidity with food and water ad libitum. The animals were adapted to the condition for seven days prior to the beginning of the experiment [12]. 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 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 approved the proposal.

### *Plant material and preparation of the extract*

CS (dried cut stigmata of *Zea mays* L, *Poaceae* flowers) used for this investigation was collected (at km 5, Sari-Ghaemshahr

Road), identified and confirmed in January 2011 and authenticated by Dr. Bahman Eslami (Department of Biology, Islamic Azad University of Qhaemshahr, Iran) and the voucher specimen was deposited in Sari School of Pharmacy Herbarium (no. HS280).

FS leaves were collected from Fajr Citrus Experimental Institute in autumn, 2011. The leaves were dried at room temperature (RT) and coarsely grounded prior to extraction.

Samples of CS and FS were extracted at RT by percolation with methanol and water (80:20) (400 ml \* 3 times) separately. The resulting extracts were concentrated over a rotary vacuum until a crude solid extract was obtained. Extracts of CS and FS were prepared in normal saline for pharmacological studies.

### *Determination of total phenolic and flavonoid content*

Total phenolic compound content was determined by the Folin-Ciocalteu method [12-14]. The extract sample (0.5 ml of different dilutions) was mixed with 2.5 ml of 0.2 N Folin-Ciocalteu reagent for 5 min and 2.0 ml of 75 g/l sodium carbonate were then added. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. Results were expressed as Gallic acid equivalents. Total flavonoid was estimated according to the method of our recent paper [13-15]. Briefly, 0.5 ml solution of extract in methanol was mixed with 1.5 ml of methanol, 0.1 ml of 10%  $\text{AlCl}_3$ , 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.

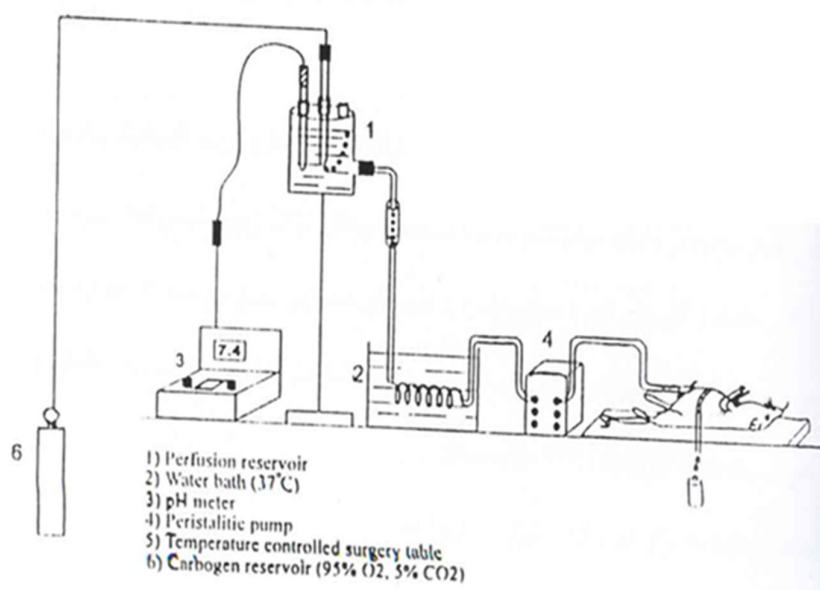
### *Experimental design*

The rats were divided into ten treatment groups and two control groups (positive and negative controls). Each group contained five male rats and their renal were perfused by a single dose of 10, 20, 40, 50, and 100mg/kg of hydro alcoholic extracts of CS and FS. To

assess oxidant activity of MDMA as positive control, 30 minutes before perfusion doses, a high dose (20 mg/kg) of the extract was added to the perfusion medium. Negative control kidneys were perfused with the perfusion buffer only (Total =12 groups). Following the preliminary study, 50mg/kg dose was chosen for the rest of the study in order to evaluate the renal-protective of CS and FS by IRRP. IRRP system is ideal for studying biochemical alterations of chemicals with minimum neural-hormonal effects [16].

### Buffer

Perfusion fluid was made of Kerbs-Henseleit buffer. The perfusion medium comprised of 118.9mM Na CL, 4.76 m M KCL, 1.19mM KH<sub>2</sub>PO<sub>4</sub>, 2.55 mM CaCL<sub>2</sub>, and 24.8 mM NaHCO<sub>3</sub>, at 37°C. Glucose (1%W/V) was added [17,7]. The perfusion medium was gassed continuously with carbogen (95% O<sub>2</sub>, 5% CO<sub>2</sub>) (Figure 1).



**Figure 1.** Schematic diagram of Isolated Rat Renal.

### *Perfusion conditions and parameters of renal 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 not raised above 10-15 cm of water with a flow rate of approximately 2 ml/min/g liver weight, to provide adequate oxygenation. The pH of perfusion fluid was always set between 7.2 and 7.4 by adjusting the CO<sub>2</sub> gas. As soon as perfusion began, the kidneys developed an even light-brown color and grew soft. The

kidneys were maintained moistened. The perfused samples were carefully separated into new well-labeled corresponding plain sample bottles at RT [18].

### *Biochemical determinations*

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

### *Histological studies*

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

## Surgery

The rats were anesthetized with ether. Heparin (500 unit; I.P.) was used to prevent blood clotting prior to anesthesia (22). An incision was made along the length of the abdomen to expose the renal. The renal artery was cannulated with PE-10 tubing and secured. Then the distal suture around the vena cava was tightened and an 18g polyethylene catheter was inserted. The diaphragm was incised and the inferior vena cava ligated suprahepatically. Following attachment of the perfusion tubing to the cannulate, the renal was perfused in situ through the vena cava [20].

## Data analysis

Statistical analysis was performed using SPSS for Windows (Version 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 five rats (S.E.M). Student-Newman-Keuls test was used to evaluate the significance of the obtained results.  $P < 0.05$  was considered significant.

## RESULTS

### Total phenol and flavonoid contents

Total phenol compounds, as determined by Folin-Ciocalteu method, reported as Gallic acid equivalents by reference to the standard

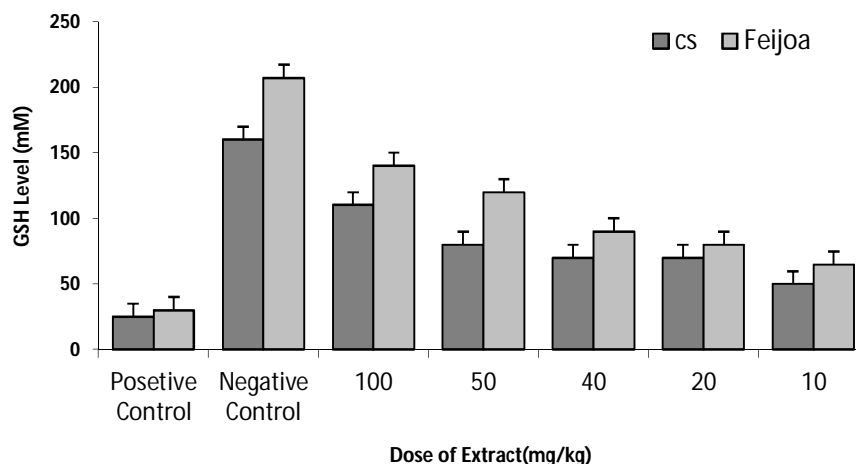
curve ( $y = 0.0063x$ ,  $r^2 = 0.987$ ). The total phenolic contents of CS and FS were  $118.94 \pm 2.78$  and  $95.54 \pm 1.25$  mg Gallic acid equivalent/g of extracts and the total flavonoid contents were  $58.22 \pm 1.34$ ,  $48.42 \pm 1.22$ mg quercetin equivalent/g of extracts respectively, with respect to the standard curve ( $y = 0.0067x + 0.0132$ ,  $r^2 = 0.999$ ).

### Renal glutathione reductase (GSH) level changes

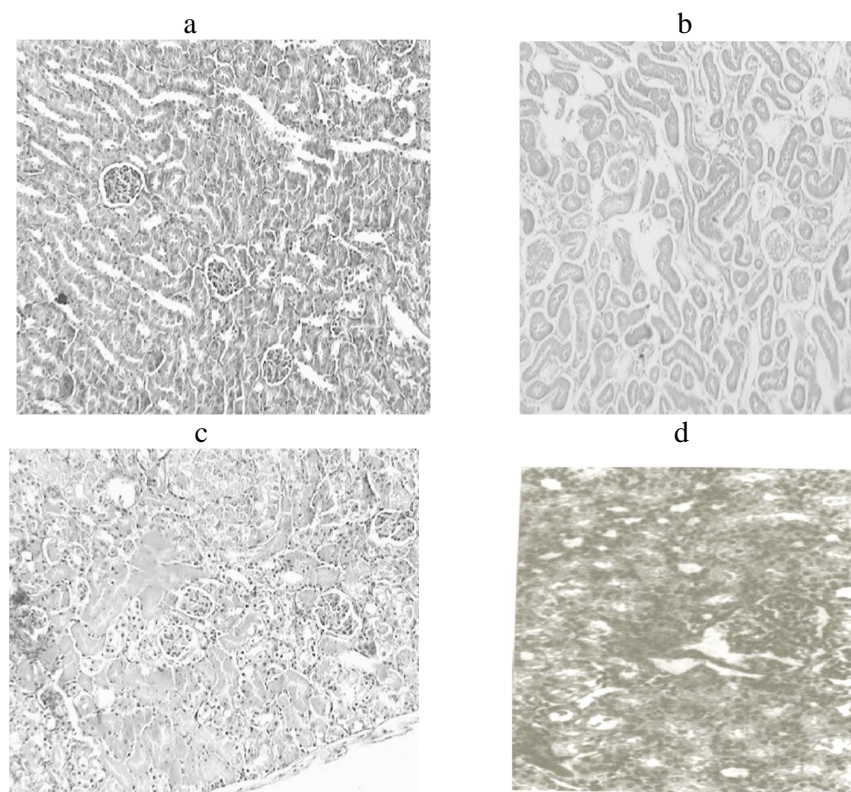
GSH levels of perfused samples by hydro alcoholic extract of CS and FS increased compared with the positive control group. Antioxidant effects of CS and FS have been shown to inhibit the renal toxic effect of MDMA. P-value was less than 0.05 with respect to the control group (Figure 2).

### Light microscope observation

Histo-pathological studies using a light microscope showed significant renal damage including necrosis and infiltration, due to hydro alcoholic extract of CS and FS (Figures 3.c, d) when compared to negative and positive control groups (Figures 3 a, b). In addition, other histo-pathological parameters including mononuclear cells infiltration, edematous cells, and cell degeneration changed significantly with hydro-alcoholic extracts of CS and FS (Table 1).



**Figure 2.** Level of Glutathione (GSH) hydro alcoholic extract of Corn silk & *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).



**Figure 3.** Left is a representative section of a normal renal (a), Middle ,an acute 20 mg/kg, i.p. of MDMA treated rat renal (b) and right a single dose of 50 mg/kg of hydro alcoholic extract of Corn silk & *Feijoa sellowiana* one hour before MDMA injection (c&d).

**Table 1.** Histo-pathological effects of hydro alcoholic extract of Corn silk & *Feijoa sellowiana* at difference concentrations of (10, 20, 40, 50, 100mg/kg) separately.

Samples	Edematous cells	hemorrhage	Mononuclear cells	Necrosis
10-HA-MDMA	33&	33&	22&	44&
20-HA-MDMA	3&2	22&	22&	23&
40-HA-MDMA	22&	22&	22&	22&
50-HA-MDMA	22&	11&	1*1&	21&
100-HA-MDMA	11&	21&	21&	2**1&
Positive-Contorol	44&	44&	33&	55&
Nagative-Control	11&	22&	11&	11&

-no effect, +1Minor effect, +2Medium effect, +3Major effect, +4high effect, +5super effect, HA(hydro alcoholic), Posetive control(.20mg/kg of NMDA)Nagative control(10ml/kg of buffer) \*P<0.05, \*\*P<0.01, significantly different from control using Fisher' exact test. Data are means of three replicates.

## DISCUSSION

Total phenol and the flavonoid compounds were determined as gallic acid contents and quercetin equivalent/g of extract respectively. 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 could prevent certain human diseases [21]. CS and FS extracts have high level of total phenol and flavonoids contents .

The liver and kidney have been identified as the most important target tissues for MDMA in rats [22]. In order to elucidate MDMA-induced renal toxicity, we assessed the effects of MDMA on total glutathione in

kidneys of rats. Moreover, the antioxidant effects of CS and FS extracts on MDMA-induced renal toxicity were assessed.

In the present study, we sought to determine how we can prevent or reduce the renal toxic effects of MDMA. We found that the activity of glutathione was decreased significantly [23]. Glutathione depletion has been shown to correlate with lipid peroxidation in the kidneys. Hence, when CS and FS extract were used as antioxidants, renal toxicity MDMA reduced to 40-50% of the controls.

MDMA has been believed to be the primary toxic constituent within ecstasy. In this study, MDMA induced formation of reactive oxygen species and oxidative stress, resulting in lipid peroxidation [24, 25]. Moreover, MDMA was also shown to be an inhibitor of glutathione peroxidase, which catalyzes the destruction of  $H_2O_2$  of lipid hydro peroxidase by reduced glutathione. Consequently, inhibition of glutathione peroxidase brings about 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 caused by lipid peroxidation, not only through glutathione peroxidase but also by allowing hydrogen to be removed from their own structures rather than from the allylic hydrogen on unsaturated lipids, consequently this interrupts the free radical chain reaction [24]. Treatment with CS extract has been shown to significantly reduce the toxicity of MDMA (Table 1). This may be through the mechanisms mentioned above as well as the extracts having good reductive capability for reducing  $Fe^{+3}$  to  $Fe^{+2}$  by donating an electron and the  $Fe^{+2}$  chelating and anti-lipid peroxidation activities [26]. Further investigation of individual compounds and their in vivo antioxidant activities (with different antioxidant mechanisms) is needed.

Our data showed that administration of CS and FS extracts cause edema which can be assessed by histopathological examination (Table 1). Our findings are in agreement with the fact that an oncotic agent can increase tissue pressure [27]. In addition, isolated

organs have a time-dependent tendency to absorb water, as in a relatively protein-free medium water gradually escapes from the vascular space, and therefore, interstitial edema develops [27]. Histopathological examination revealed significant hemolysis as assessed by hemolytic index (Figure 3a). This can be due to altered calcium homeostasis concomitant with a significant increase in cytosolic calcium, which has been previously reported for the effects of *Phytolacca Americana* on liver [28]. Moreover, the disturbances of intracellular calcium homeostasis have been demonstrated to be associated with a variety of toxicological and pathological processes. Accumulation of CS extract in the renal tissue as the target organ has been shown to be protective [29]. In a similar manner, the results of our study have also confirmed its renal-protective effects (Table 1), which in fact could be an influence of CS and Feijoa extract on different cells. In this study, significant renal necrosis was also observed at MDMA dose of 20 mg/kg. CS and FS extract decrease formation of reactive oxygen species, oxidative stress, and resulting lipid peroxidation. That this may explain the observed significant changes renal damage including necrosis and infiltration (Figure 3c & d).

## CONCLUSION

To sum up, the results of our study, in agreement with others, demonstrate that renal perfusion is a suitable model in order to study the renal protective activity of MDMA. More studies, however, are needed to further elucidate the exact mechanism by which CS and FS extract induces renal protection.

## ACKNOWLEDGEMENTS

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