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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

An experimental model for study of the renal protective activity of corn silk against dosage induced By MDMA using in situ rat renal system

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Abstract

Corn silk (CS) is widely used in Iranian traditional medicine. The aim of present study was to investigate renalprotective activity of CS by Isolated Rat Renal Perfusion System (IRRP) Phenol and flavonoid contents were measured by Folin Ciocalteu and $AlCl_3$ assays, respectively. Hydro alcoholic extract of Corn silk (10, 20, 40 and 100 mg/kg) was studied for its renal protective activity by IRRP. 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 neural-hormonal effects. In this study, the renal was perfused with Kerbs-Henseleit buffer, containing different concentration of hydro alcoholic extract of Corn silk (10, 20, 40, 50,100mg/kg) were added to the buffer and perfused for 2 hours. During the perfusion, many factors including Urea and creatinine levels and concentration of GSH was assessed as indicator of renal viability. Consequently, sections of renal tissue were examined for any histo-pathological changes. The results showed that histo-pathological changes in renal tissues were related in a dose-dependent manner to hydro alcoholic extract of Corn silk concentrations. Doses of 50, 100 mg/kg caused a significant ($P<0.05$) histo-pathological changes. Level of GSH of sampels of perfused of hydro alcoholic extract of CS were increased compared with the positive control group. We concluded that renal protective effect of CS may be due to the decreased lipid peroxidation, although other mechanisms may also be involved.

Keywords: Corn silk; Renal perfusion; Kerbs-Henseleit buffer; Histo-pathological changes; Glutathione

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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, as well as antilithiasic, uricosuric, and antiseptic, properties are traditionally attributed to Corn silk (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 for cystitis, gout, kidney stones, nephritis, and prostatitis (Elghorab et al., 2007; Ebrahimzadeh et al.,2008a). CS contains proteins, vitamins, carbohydrates, Ca^{2+}, K^{+}, Mg^{2+} and Na^{+} salts, volatile

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oils, and steroids such as sitosterol and stigmasterol, alkaloids, saponins, tannins, and flavonoids (Ebrahimzadeh et al., 2008b). 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 (Kaur et al., 2006). There are also reports about antioxidant activity of CS (Maksimovic et al., 2003). The constituents in the volatile extract and petroleum ether, ethanol, and water extract of CS exhibited clear antioxidant activities (Elghorab et al., 2007).

On the other hand 3, 4-methylenedioxyme thamphetamine (MDMA, or ecstasy) is a ring-substiuted 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 (Kalant., 2001; Carvalho et al., 2002a). The liver and renal is a targets for MDMA toxicity that MDMA is metabolized by cytochromes P4502D, 2B, 3A and reactive metabolites are readily oxidized to the corresponding o-qiuinones and form reactive oxygen species (Tucker et al., 1994; Carvalho et al ., 2002b).

Renalprotective activity of CS has not been reported to date and nothing was found about mechanism activity of CS. Therefore, the aim of the present work was to determine the renal protective activity or antioxidant activities of CS against oxidant induced by MDMA by isolated renal perfusion system. Moreover, IRRP was employed to evaluate CS renal protective and its correlation to biochemical changes.

Materials and Methods

Animals

Male albino Wistar rats (6 to 8weeks), 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 ± 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 (Ebrahimzadeh et al., 2009). 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

CS (dried cut stigmata of Zea mays L, Poaceae flowers) was used for this investigation were collected (Road sari–Ghaemshahr) and were identified and confirmed in January 2010 and authenticated in Department of Biology, Islamic Azad University of Qhaemshahr, Iran. 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 antioxidant studies (Ebrahimzadeh et al., 2008a).

Determination of total phenolic and flavonoid content

Total phenolic compound content was determined by the Folin-Ciocalteau method (Nabavi et al., 2009a; Nabavi et al., 2009b; Dehpour et al., 2009). The extract sample (0.5 ml of different dilutions) was mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagents 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 method of our recent paper (Ebrahimzadeh et al., 2009a; Ebrahimzadeh et al., 2009b; Ebrahimzadeh et al., 2009c). Briefly, 0.5 ml solution of the extract in methanol were mixed with 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 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

Rates were divided into five treatment group and two control groups (positive & negative control). Each group contained four male rates and their kidneys were perused by a single dose of 10, 20, 40, 50 and 100 mg/kg of hydro alcoholic extract of CS. To assess oxidant activity of MDMA, a high dose (20 mg/kg) 30 minute before perfusion doses of the extract was added to the perfusion medium. Negative and positive control kidneys were perfused with the perfusion buffer and MDMA (20 mg/kg) respectively. (Total =7groups). Negative control kidney were perused with the perfusion buffer only (Total = 7groups). Following the preliminary study, the dose of 50 mg/kg was chosen for the remaining of the study in order to evaluate the renal-protective of CS (Maksimovic et al., 2002).

Buffer

Perfusion fluid was made of Kerbs-Henseleit buffer. The perfusion medium consisted of 118.9 mM NaCl, 4.76 mM KCl, 1.19 mM KH_2PO_4, 2.55 mM $CaCL_2$ and 24.8 mM $NaHCO_3$, at 37˚C. Glucose (1%W/V) is usually added (Jeong et al., 2004; Wolkoff., 1987). The perfusion medium was gassed continuously with carbogen (95% O_2, 5%CO_2) as shown in Fig. 1.

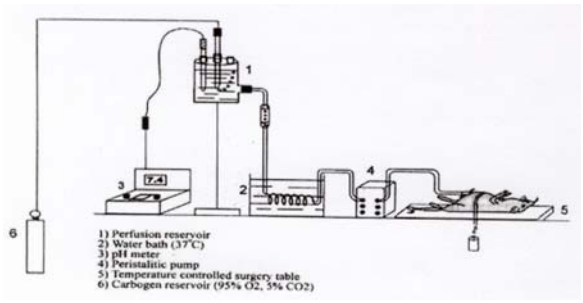

Fig. 1: Schematic diagram of a rat renal perfusion system

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 perfusion fluid pH was always set between 7.2 and 7.4 by adjusting the CO_2 gases. As soon as perfusion began, the renal developed an even, light-brown colour, was soft and kept moistened. Perused samples were carefully separated into new, well labelled, corresponding plain sample bottles at room temperature (Ghazi-Khansari et al., 2002).

Biochemical determination

The activities of reduced glutathione (GSH) were estimated by Ellman's method (Ellman, 1995).

Histological studies

The kidney was 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 renal was cut into small pieces. Sections were prepared and stained by Eosin-Hematoxylin and examined blind 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 (karami et al., 2010). 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 and 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 (karami et al., 2010).

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 in the mean of 4 rats (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.

Results

Total phenol and flavonoid contents

Total phenol compounds, as determined by Folin Ciocalteu method, are reported as gallic acid equivalents by reference to standard curve ($y = 0.0063x$, $r^2 = 0.987$). The total phenolic content of CS was 118.94 ± 2.78 mg gallic acid equivalent/g of extract. The total flavonoid contents was 58.22 ± 1.34 mg quercetin equivalent/g of extract, by reference to standard curve ($y = 0.0067x + 0.0132$, $r^2 = 0.999$).

Renal Glutathione reductase (GSH) level changes

Level of GSH of sampels of perfused hydro alcoholic extract of corn silk was increased compared with the positive control group. Antioxidant effect CS has shown to inhibit the renal toxic effect of MDMA. P value was less than 0.05 in respect to control group (Fig. 2).

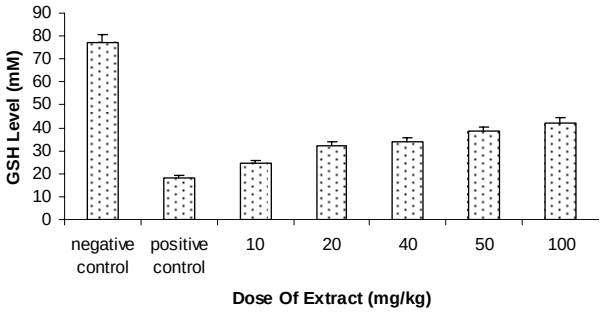

Fig. 2: Level of Glutathione (GSH) hydro alcoholic extract of Corn silk at difference concentrations. Values are presented as mean ± SEM (N = 5) ***P<0.001 with respect to control, (ANOVA followed by Newman–Keuls multiple comparisons test).

Light microscope observation

Histo-pathological studies using a light microscope showed significant renal damage including necrosis and infiltration, due to hydro alcoholic extract of corn silk (Fig. 3c) when compared to negative & positive control

groups (Fig. 3a&b). In addition, other histo-pathological parameters including mononuclear cells, edematous cells and cell degeneration changed significantly with hydro alcoholic extract of corn silk (Table 1).

Table 1: Histo-pathological effects of hydro alcoholic extract of corn silk at difference concentrations (10, 20, 40, 50, 100 mg/kg)

Samples	Edematous cells	hemorrhage	Mononuclear cells	Necrosis
10-HA-MDMA	3+	3+	2+	4+
20-HA-MDMA	3+	2+	2+	3+
40-HA-MDMA	2+	2+	2+	2+
50-HA-MDMA	2+	1+	1+	1+
100-HA-MDMA	1+	1+	1+	1+
Positive-Control	4+	4+	3+	5+
Negative-Control	1+	2+	1+	1+

-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 compounds were determined as gallic acid and the total flavonoid contents as quercetin equivalent/g of 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 could decrease certain human diseases (Nabavi et al., 2008). CS extract showed high level of total phenol and flavonoids contents.

The liver and kidney have been identified as the most important tissues target for MDMA in rat (Carvalho et al., 2001). In the present study, we sought to determine how we can prevent or decrease the renal toxic effect of MDMA. The activity of glutathione decreased significantly in response to MDMA (Hibayama, 1992). Glutathione depletion has been shown to correlate with lipid peroxidation in kidney. So when CS extract as antioxidan was used, renal toxicity effect was reduced almost by 40-50%.

MDMA is believed to be the primary toxic constituent which is present within ecstasy. Other toxic constituents have also been identified including the MDA and PMA (Paramethoxy amphetamine). In this study, MDMA induces formation of reactive oxygen species and an oxidative stress, resulting in lipid per oxidation (Torres et al., 1991; Khaled and Neil, 1999). More studies, however, are needed to further elucidate the exact mechanism by which MDMA induces renal toxicity. Moreover, MDMA also was shown to be an inhibitor of glutathione peroxidase, which catalyzed the

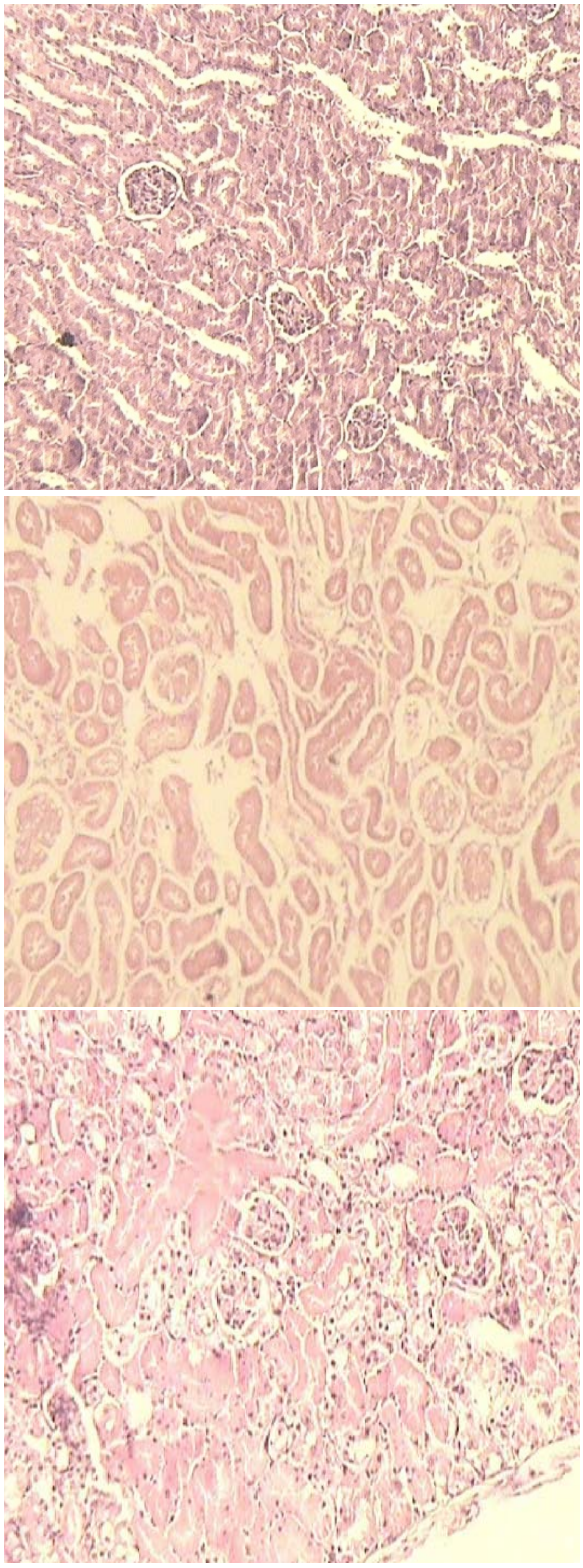

Fig. 3: A representative section of a normal renal (a), an acute 20 mg/kg of MDMA treated rat renal kidney (b) and a single dose of 50 mg/kg of hydro alcoholic extract of corn silk one hour before MDMA injection (c)

destruction of H2O2 of lipid hydroperoxidase by reduced glutathione. Therefore, with inhibition of glutathione peroxidase, there is a reduction in GSH which resulted in accelerated lipid peroxidation (Maddaiah, 1990; Carvalho et al., 2001).

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 (Khaled and Neil, 1999). Treatment with CS extract has been shown to significantly decrease the toxicity of MDMA (Table 1). This may be due to reducing of Fe^{+3} to Fe^{+2} by donating an electron or anti-lipid peroxidation activity (Torres et al., 1991). Further investigation of individual compounds, their *in vivo* antioxidant activities and in different antioxidant mechanisms is needed.

Our data showed that administration of CS extract causes oedema which can be assessed by histo-pathological examination (Table 1). Our findings are in agreement with the fact that an oncotic agent can cause increase in pressure (Chen and Cosgriff, 2000). In addition, isolated organs have a time-dependent tendency to absorb water, as with relatively protein-free medium water which gradually escapes from the vascular space and therefore interstitial edema develops (Chen and Cosgriff, 2000). Histo-pathological examination revealed significant hemolysis as assessed by the hemolytic index (Fig.3a). This can be due to altered calcium homeostasis concomitant with a significant increase in cytosolic calcium, which has been previously reported for *Phytolacca Americana* in liver (Rossini et al., 1976). Moreover, the disturbance of intracellular calcium homeostasis has been shown to be associated with a variety of toxicological and pathological processes. Accumulation of CS extract in the renal as the target organ has been shown to cause protection (Takahashi et al., 2001). In a similar manner the results of this study also showed renal protection (Table 1). This in fact, could be a result of CS extract receptor binding, which is sufficient to affect different cells. In this study, significantly high necrosis was also observed in the liver at MDMA dose of 20 mg/kg. CS extracts decreases formation of reactive oxygen species and an oxidative stress, resulting in lipid per oxidation (Karami et al., 2001). This may explain the observed necrosis (Fig. 3b).

Conclusion

The results of our study demonstrated that liver perfusion is a suitable model in order to study the renal protective effect against MDMA. More studies, however, are needed to further elucidate the exact mechanism by which CS extract induces renal protection.

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