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Diuretic activity of *Maydis stigma* extract in rats

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Maydis stigma (corn silk) is a herbal drug reputed for the treatment of urinary ailments in various traditional medicine systems. To determine its influence on urinary volume and the excretion of sodium, potassium and chloride, 5% and 10% decoctions were administered daily to adult male Wistar rats for eight days. The concentration of electrolytes and urea in plasma, the influence of treatment on urinary pH value as well as creatinine clearance were also investigated. Daily oral administration of 5% decoction at the dose of 10 ml/kg led to a significant and acute diuresis in rats, reaching the peak value in the first 24 h of treatment. Over a similar period, application of 10% decoction did not affect urinary excretion of water, but significantly increased the pH value of excreted urine. A significant decrease in sodium and chloride plasma levels was observed in both treated groups. The creatinine clearance was markedly increased after the treatment with both extracts. Our findings indicate that the diuretic effect of 5% aqueous *Maydis stigma* extract is in accordance with the increase in glomerular filtration rate and inhibition of sodium and chloride tubular reabsorption, caused a by still unidentified intrinsic factor, but not the salt-loading effect.

1. Introduction

The herbal drug *Maydis stigma* (dried cut stigmata of maize, *Zea mays* L. ssp. *mays*, Poaceae female flowers) is recognized, both in traditional and official medicine, as a mild diuretic, urinary demulcent, to pass stones and gravel from kidneys and urinary bladder, against benign prostatic hyperplasia, cystitis, gout, chronical nephritis and similar ailments (Tucakov 1990; BHP 1996; Czygan 1997). Similar findings were published in ethnopharmacological studies (Bastien 1983; Cáceres 1987; Yeşilada 1995). However, even though *M. stigma* has been extensively used in diuretic therapy, there is no published experimental pharmacological data demonstrating the possible mechanism of diuretic action of this herbal drug. In this paper, we report on the influence of *M. stigma* aqueous extracts on the urinary excretion of water and electrolytes in rats. Their effects on plasma creatinine, urea and electrolyte homeostasis were also measured, in order to assess the efficacy of this herbal drug as a diurectic agent.

Table 1: Average diuretic effect of *Maydis stigma* aqueous extracts, during 8 days of the treatment

Group	Relative diuretic effect (%)[a]	N[b]
Control	118.9 ± 8.2	8
5% Decoction	$209.5 \pm 16.3^{*}$	7
10% Decoction	115.5 ± 7.6	7

a Day zero = 100 % (basal value)
b Number of subjects
*$p < 0.001$

2. Investigations and results

2.1. Diuretic activity

An almost twofold increase in urinary output was observed in the group of animals treated with a 5% decoction, compared to the control group. The application of a 10% decoction showed no influence on the urinary excretion of water (Table 1). Diuresis effectuated within the first three days of treatment and reached the peak level in

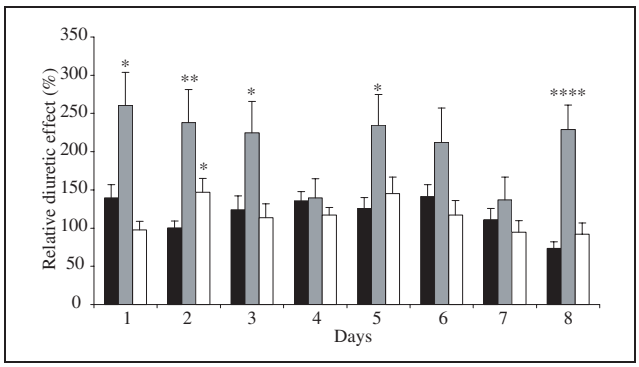

Fig. 1: Average urinary volume excretion in rats treated by oral administration of aqueous extracts of *Maydis stigma.* Values are mean ± SEM (n = 8 for control group – black bar, n = 7 for 5% decoction – grey bar and n = 7 for 10% decoction – white bar), given in % of basal values, taken as 100%.
*$p < 0.05$
**$p < 0.01$
***$p < 0.005$
****$p < 0.001$

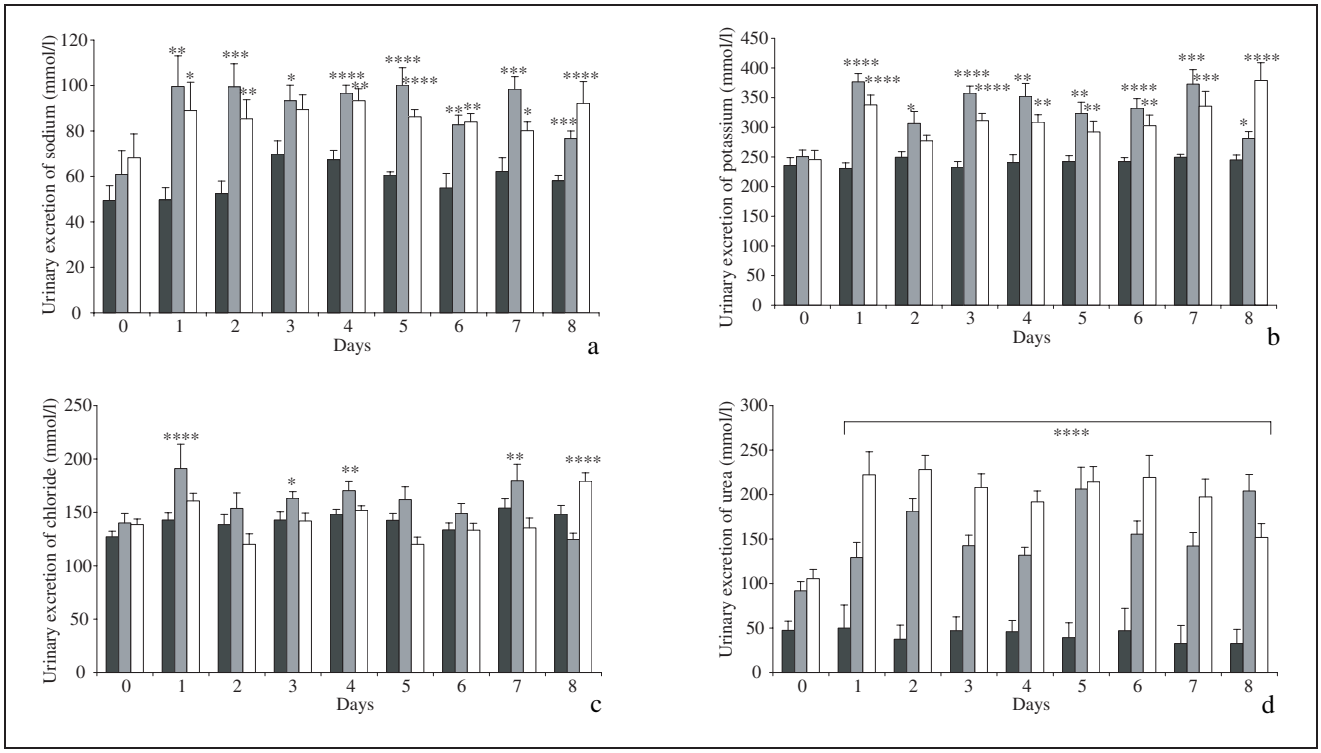

Fig. 2: Effects of oral administration of aqueous extracts of *Maydis stigma* on urinary excretion of sodium (a), potassium (b), chloride (c) and urea (d). Values are mean ± SEM (n = 8 for control group – black bar, n = 7 for 5% decoction – grey bar and n = 7 for 10% decoction – white bar).
*p < 0.05
**p < 0.01
***p < 0.005
****p < 0.001

the first 24 h, but the significance of the difference between control and treated group clearly decreased during the fourth day (Fig. 1). From the fifth day, another increase in urinary output was detected in rats treated with 5% decoction.

2.2. *Effects on excretion of electrolytes and urea*

Oral administration of both extracts caused a distinct increase of urinary excretion of all relevant osmotically active electrolytes (Table 2). A significant increase in urinary excretion of sodium (Na^+), potassium (K^+) and chloride (Cl^-) ions occured in the first 24 h, remaining stable at high values during the whole experiment (Fig. 2a–c).

Oral administration of *M. stigma* aqueous extracts in both concentrations induced a manifold increase of urea levels in excreted urine, particularly when the 10% decoction was applied (Table 2). The observed effect occured immediately and remained stable during the whole experiment (Fig. 2d).

Table 2: Influence of oral administration of *Maydis stigma* aqueous extracts on average urinary excretion of electrolytes and urea during 8 days of the treatment

Group	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	Urea (mmol/l)	N[a]
Control	58.4 ± 2.2	242.1 ± 4.0	144.0 ± 4.7	42.0 ± 1.3	8
5% Decoction	93.7 ± 2.8**	338.1 ± 7.9**	161.8 ± 5.0*	166.1 ± 6.2**	7
10% Decoction	86.2 ± 2.5**	316.3 ± 8.3**	142.9 ± 4.0	205.1 ± 6.5**	7

[a]Number of subjects
*p < 0.05
**p < 0.0001

Table 3: Influence of oral administration of *Maydis stigma* aqueous extracts on levels of osmotically active electrolytes, urea and creatinine in plasma after 8 days of the treatment

Group	Na^+	K^+ (mmol/l)	Cl^-	Urea	Creatinine (μmol/l)	N[a]
Control	156.9 ± 1.0	7.5 ± 0.1	114.3 ± 1.6	7.9 ± 0.6	37.3 ± 1.6	8
5 % Decoction	147.1 ± 0.9***	7.6 ± 0.1	107.8 ± 0.9**	7.3 ± 0.3	39.3 ± 1.9	7
10 % Decoction	148.6 ± 1.2***	7.6 ± 0.1	108.9 ± 0.7*	7.1 ± 0.1	35.9 ± 1.8	7

[a]Number of subjects
*p < 0.05
**p < 0.005
***p < 0.001

Table 6: Body weight and average food/water intake during the experiment

Parameters	Control	5% decoction	10% decoction
Body weight (g)[a]			
at the beginning	264.4 ± 12.4	260.0 ± 18.3	233.6 ± 16.5
at the end	285.6 ± 35.5	303.8 ± 27.7	290.0 ± 18.9
Average increase (g/day)	2.4	4.9	6.3
Water intake (ml/day)			
at the beginning	41.3 ± 6.1	44.0 ± 3.5	35.4 ± 3.9
at the end	30.5 ± 7.2	34.8 ± 4.1	31.1 ± 4.5
Food intake (g/day)			
at the beginning	46.1 ± 4.1	45.8 ± 3.6	40.9 ± 4.2
at the end	34.0 ± 7.8	34.4 ± 4.8	30.4 ± 2.6

[a]All results are presented as mean ± SEM.

2.3. Effects on the levels of osmotically active electrolytes, urea and creatinine levels in plasma

After eight days of treatment, *M. stigma* aqueous extracts in both concentrations caused a significant decrease in Na^+ and Cl^- levels in plasma (Table 3). However, the levels of K^+, urea and creatinine in plasma have not been influenced significantly.

2.4. Effect on creatinine clearance

The influence of oral administration of *M. stigma* aqueous extracts on creatinine clearance is presented in Table 4. Our results demonstrate that both decoctions significantly increased glomerular filtration rate in rats.

2.5. Effect on urinary pH value

Oral administration of aqueous *M. stigma* extracts induced a significant increase in overall urine alkalinity (Table 5). On the contrary, a trend towards increased urine acidity was observed in the control group, receiving distilled water. An increase of urinary pH value in the rats treated with *M. stigma* aqueous extracts was observed from the fourth day onwards, when the difference between the control and treatment groups became significant (Fig. 3).

2.6. Electrolyte content and pH of extracts

The mean of pH in tested aqueous extracts was 6.3 ± 0.1, as mean ± SEM. No measurable levels of electrolytes were recorded in the investigated decoctions.

Table 4: Influence of oral administration of *Maydis stigma* aqueous extracts on creatinine clearance

Group	Creatinine clearance ($mg \cdot min^{-1} kg^{-1}$)	N[a]
Control	3.73 ± 0.43	8
5% Decoction	5.02 ± 0.39*	7
10% Decoction	5.47 ± 0.26**	7

[a]Number of subjects
*$p < 0.05$
**$p < 0.01$

Table 5: Effects of oral administration of *Maydis stigma* aqueous extracts on average urinary pH value during 8 days of the treatment

Group	pH value	N° of subjects
Control	6.2 ± 0.1	8
5 % Decoction	6.5 ± 0.1*	7
10 % Decoction	6.7 ± 0.1*	7

*$p < 0.05$

2.7. Influence of treatment on body weight, daily water and food intake

Influence of treatment on body weight of laboratory animals and daily water and food intake is presented in Table 6. A tendency towards decrease in daily food and water consumption was observed, probably due to vehicle loading of animals. No clear correlation could be established between the primary effects of treatment (diuresis) and daily changes in body weight or water and food intake, indicating that the study design did not affect the overall condition of rats.

2.8. Results of preliminary chemical investigations

It was established that the extracts of *M. stigma* contained predominantly polyphenolic constituents, such as flavonoids, phenolic acids, coumarins and condensed tannins. The results of the quantification of total polyphenols, flavonoids, coumarins and tannins are listed in Table 7.

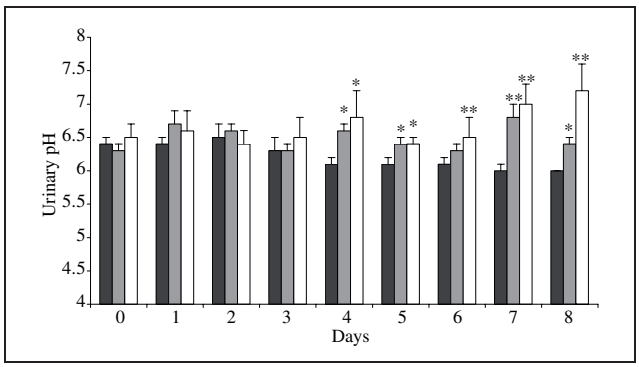

Fig. 3: Effects of oral administration of aqueous extracts of *Maydis stigma* on urinary pH value. Values are mean ± SEM (n = 8 for control group – black bar, n = 7 for 5% decoction – grey bar and n = 7 for 10% decoction – white bar).
*$p < 0.01$
**$p < 0.05$

Table 7: Contents of total polyphenols, tannins, coumarins and flavonoids in *Maydis stigma*

Constituent	Content
Total polyphenols	1271.3 ± 38.8[a]
Tannins	1106.3 ± 56.3[a]
Total coumarins	550.0 ± 20.2[b]
Total flavonoids	135.0 ± 35.0[c]

[a]As pyrogallol equivalents per 100 g of plant material.
[b]As coumarin equivalents per 100 g of plant material.
[c]As hyperoside equivalents per 100 g of plant material.

3. Discussion

Our results demonstrate that oral administration of aqueous *Maydis stigma* extract in a concentration of 5% induced a significant increase in urinary excretion of water. A diuretic effect after the administration of 10% decoction did not occure. A similar event was described by Haloui et al., who reported the absence of a diuretic effect after the administration of centaury and rosemary extracts in higher doses (Haloui et al. 2000). Diuresis started immediately after the administration of the 5% decoction, reaching the peak value at the first day of treatment and decreasing quickly, most probably after activation of certain compensatory mechanisms involved in electrolyte homeostasis. During the experiment, another equivalent cycle was observed. Also, a significant increase in urinary excretion of osmotically active electrolytes was seen, along with a distinct decrease in Na^+ and Cl^- plasma levels. However, although a pronounced increase in urinary excretion of K^+ was also recorded, its plasma levels remained unchanged possibly due to mobilization of this electrolyte from intracelular pools that balanced the loss in circulation. Oral administration of *Maydis stigma* 10% decoction led to a significant increase in urinary pH value, probably due to pronounced influence of the applied extracts on urinary excretion of urea. The influence of the 5% decoction on the urinary excretion of urea was also statistically significant, but less pronounced if compared to that of the 10% decoction.

Essentially, herbal drugs with diuretic activity are not considered diuretics, but aquaretics. Usually containing essential oils, flavonoids, saponins and/or tannins, herbal drugs increase the blood flow in renal glomerules and, thus, glomerular filtration rate. On the other hand, aromatic or saponin-containing herbal drugs irritate the renal epithelium, stimulating the glomerular filtration (Tomić 1984; Robbers 2000). Unlike synthetic diuretics, herbal drug preparations are considered not affecting reabsorbtion of sodium and chloride in renal tubules, leaving their concentration in plasma undisturbed (Tomić 1984; Robbers 2000). However, the results of our study strongly suggest that direct influence on glomerular filtration rate (indicated by significant increase of creatinine clearance) along with simultaneous inhibition of sodium and chloride reabsorption, should be considered the basic mechanism of diuretic activity of *Maydis stigma* aqueous extracts. An opinion that the increase of urine output after the administration of *Maydis stigma* preparations might result from a high potassium content in plant material, prevails in references available (Tucakov 1990; Czygan 1997). Our results, however, indicate that diuretic activity of aqueous *Maydis stigma* extracts is intrinsic, and not a result of the salt-loading effect, as no measurable levels of sodium and potassium were detected in plant extracts. Unfortunately, we can only speculate about the chemical constituents of *Maydis stigma* responsible for observed diuretic effect, but it seems reasonable to consider polyphenols as potentially active agents, since their presence was confirmed and their content determined in our preliminary chemical investigations.

Diuretic activity of *Maydis stigma* was one of the subjects of an investigation conducted under standardized conditions in a placebo controlled double-blind crossover study in healthy young volunteers in Vietnam (Dat et al., 1999). However, the study recorded no influence of *Maydis stigma* or the other investigated extracts to urine output and the sodium excretion, which is opposite to our results. We are aware of the fact that some limit to the rat model exists and the sensitivity of the rat kidney to diuretics is different from that of the human kidney. This makes the simple extrapolation of our experimental data to human model impossible. The absence of a clear diuretic effect described by Dat et al. (1999) might also be in consequence to one, usually overlooked, but very important factor that can impact the results of any pharmacological investigation: the question of the biological source of the herbal drug *Maydis stigma*. It is generally accepted that maize as a plant species could be considered an artifact, bred by a long-lasting, specific and systematic process to meet some of our needs. For that reason, one can find a number of maize hybrids, adapted to fit extremely different environments on corn fields all over the world and botanically systematized in several varieties (such as dent corn, flint corn, pop corn etc) (Jevtić 1986). Evidently, a high genetic biodiversity in the available maize pool exists, implying that substantial chemical differences between maize hybrids could not only be expected, but designated as the major source of possible discrepancies in the results of pharmacological investigations on this herbal drug. In other terms, pharmacological properties of *M. stigma* might greatly depend on the appropriate choice of plant material. Bearing in mind that the biological source of *M. stigma* is not homogeneous, we believe that our prospective investigations will support this hypothesis and help us find the best candidates among numerous maize hybrids in Serbia for future production of a standardized, chemically defined and, after all, effective herbal drug *Maydis stigma.*

4. Experimental

4.1. Plant material and extraction procedure

Dried cut stigmata of *Z. mays,* commercially available in Serbian herbal apothecaries under the pharmacopoeial name *Maydis Stigma* and/or the common name "kukuruzna svila", were supplied by the Institute for Medicinal Plant Research "Dr. Josif Pančit" (Belgrade, Serbia).

Prior to extraction, the plant material was reduced to a coarse powder. The extracts were prepared by boiling 5.0 and 10.0 g of plant material in 100 ml of distilled water for 5 min to prepare 5 and 10% (w/V) decoctions. After 10 min of infusion, the extracts were allowed to cool down at room temperature and filtered. Extracted plant material was washed with distilled water and the volume of the prepared decoctions was adjusted to 100 ml using these washings. All extracts were prepared *ex tempore*.

4.2. Study design

This research was conducted in accordance with the internationally accepted principles for laboratory animal use and care. For this purpose, we adapted the method according to Haloui et al. (2000). Diuretic activity of prepared *M. stigma* decoctions was studied in adult normotensive male Wistar rats, weighing 200–250 g at the beginning of the experiment. Twenty-four animals, individually housed in metabolic cages and maintained on standard pellet diet and water *ad libitum*, were randomly allocated in three groups. Control group received vehicle (distilled water), while two treated groups received drug decoctions at the dose of 5 and 10%, respectively. The number of animals in each group was at least seven. The rats were given the drug extracts or vehicle by single oral administration at a volume of 10 ml/kg per day. On the day zero of this study, established to determine basal levels of urine excretion in all investigated groups, animals received no treatment. Duration of the experiment was nine days (day zero plus eight days of treatment). The animals were kept on a 12 h light-dark cycle, at the average room temperature of 22 °C.

4.3. Urine and blood sampling

Urine samples were collected daily, at 8 : 00 h, before administration of the next drug/vehicle dose, and their volumes were measured in graduated cyllinders. Urine samples were kept in individual airtight plastic containers at −80 °C until analyzed. At the end of the experiment, the blood samples were obtained from all animals under anaesthesia immediately before killing. Blood samples were centrifuged at 4000 rev/min and kept at −80 °C until analyzed.

4.4. Parameters of investigation

The volume of excreted urine was monitored daily. The Na^+, K^+, Cl^-, creatinine and urea levels were analyzed in both urine and plasma, using DADE Dimension Clinical Chemistry System (Dade Behring Inc., Newark, DE, USA). Prior to analysis, urine samples were diluted (1 : 1, v/v) with distilled water. The pH value of urine was measured using a pHel-1 tester (Hanna Instruments Deutschland GmbH, Germany). Electrolyte levels in investigated plants extracts, as well as their pH value, were determined using the same methodology and instrumentation. In addition, certain parameters such as body weight of animals, as well as daily water and food consumption were monitored during the whole study.

4.5. Preliminary chemical investigations

The chemical composition of investigated extracts was monitored by TLC, according to Wagner and Bladt (Wagner 1996). The contents of polyphenols (flavonoids, coumarins and tannins) in plant material were determined by spectrophotometric methods, according to procedures described in pharmacopoeias (total polyphenols and tannin contents: Ph. Jug. 2000, flavonoids and coumarins: DAB 1996). All determinations were performed using a Specord M40 UV-VIS spectrophotometer (Carl Zeiss, Jena, Germany) in triplicate.

4.6. Statistical analysis

All data were expressed as mean ± SEM. Mean values were considered significantly different if $P < 0.05$. The experimental design allowed us to apply a factorial ANOVA (time, treatment and interaction) for comparison. The Tukey-Snedecor test was applied to perform comparisons between groups, at the probability level of 0.05.

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