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Evaluation of diuretic activity of ethanolic extract of *Stigma maydis* in rats

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Abstract
Stigma maydis (*Zea mays*) is yellowish thread-like strand found inside the husks of corn. Corn Stigma measure 4-8 in (10-20 cm) long and are collected for medicinal use before the plant is pollinated. The diuretic activity of ethanolic extract of *Stigma maydis* in albino rats was studied by the lipschitz Test. The diuresis activity was determined by administered the rats with different dose treatments of 200 mg/kg (low), 400 mg/kg (medium) and 500 mg/kg (high) of ethanolic extract of *Stigma maydis*. Cumulative urine volume was significantly increased with the dosage levels (200-500 mg/ kg). The urinary excretion of water and electrolytes exhibited by standard drugs were significantly, as compared to control group over a period of 24 hours. After 7 h furosemide treated group showed higher indices for diuresis (4.71), lipschitz value (2.09) and also After 24 hr also Furosemide treated group showed higher indices for diuresis diuresis (3.70), lipschitz value (3.37). Standard drugs showed higher values of saluretic index (Na^+ 1.95, k+1.72 Cl^- 1.71) and (Na^+ 1.46, k+1.16 Cl^- 1.26) were observed with different dosage of ethanolic extract of *stigma maydis* treated groups. the higher natriuretic values were observed with urea treated group (Na^+/ K^+ 3.23) where as ethanol extract of *stigma maydis* treated group shown (Na^+/ K^+ 3.26) moderately good natriuretic effect as compare to standard drug treatments. From the result it can be observed that ethanolic extract of *Stigma maydis* has shown a significant diuretic activity by increasing urinary output and increased excretion of sodium, potassium, chloride when compared to control.

Keywords: diuretic activity, saluresis, ethanol, lipschitz test, furosemide, *cornsilk*, poaceae

Introduction
The drug consists of the stigmas of the female flowers harvested during the flowering period. The pale yellowish or brownish stigmas are filamentous, 0.1-0.2 mm thick, and upto 20 cm long. It is native to Central America, but now a days cultivated worldwide. The drug is imported from the former USSR, Bulgaria, Albania, and former Yugoslavia and it is also obtained from the USA. Corn have sweetish taste and its odour is faint, used in cystitis, rheumatism and arthritis[1]. The plant is reported to possess antiviral, antifungal, urolithiatic and antitumour activity. It contains fixed oil, essential oil (containing carvecrol and other terpenes), flavonoids, saponins, bitter substances, tannin-like polyphenols, reducing sugars, mucilage. Its utilization by the Peruvian Indians as an intoxicant is supposed to be based on the presence of alkaloids, which after being inhaled, cause psychic stimulation.

Material and Methods
Plant material
2000 kg of *stigma maydis* were collected from local market in rainee session. The *stigma maydis* was dried at room temperature (24 to 27^0 c) or shade dried. The dried *Stigma maydis* was then ground to coarse powder. *Stigma maydis* was identified and authenticated by a botanist Dr. S.K. Mahajan, retired professor of P.G. College, Khargone [M.P., India].

Processing of Plant material
After authentication, *Stigma maydis* was dried at room temperature until they were free from the moisture.

Reagents
All the reagents were of Analytical grade and purchased from kasliwal brothers, Indore, India.

Preparation of plant extract
The *Stigma maydis* were shade dried and powdered. The crude plant extract was prepared by Soxhlet extraction method. 500 g of powdered plant material was extracted with 2000 ml of ethanol. The process of extraction was carried out up to 24 hr, till the solvent in siphon tube of an extractor became colorless. The extract were filtered and evaporated to dryness using rotary evaporator. Further the dried extracts were maintained in a refrigerator at 4 ºC for further diuretic activity.

Experimental Animals
Albino rats weighing between 140-200 g of either sex were used in the study and were obtained from the animal house of GRY institute of Pharmacy, borawan, M.P., India. The animals were maintained under standard husbandry conditions for an acclimatization period of 15 days before performing the experiments. All rats were housed in metallic cages and temperature maintained at 22+2 °C.

Experimental Design
The diuretic activity of ethanol extract of *Stigma maydis* in albino rats was studied by the lipschitz test. Male Albino rats were divided into 4 groups of 7 rats in each. The group I serves as normal control received normal saline 25 ml/kg b.wt, the group II received Furosemide (25 mg/kg, p.o) in vehicle, III group received Urea (1 g/kg, p.o) & IV group were treated with low (200mg/kg), medium (400mg/kg),

and high (500mg/kg) doses of ethanolic extract of *Stigma maydis* in vehicle and immediately after the extract treatment, all the rats were hydrated with saline (15 ml/kg) and placed in the metabolic cages, and kept at 21°C±0.5°C. Urine was collected 7 and 24 hours after dosing and following parameters were measured.

- Total urine excretion (ml/100 g b.w.).
- Urinary excretion of Na^+ and K^+ (in mEq/L/100 g body weight) measured using digital flame photometer.
- Urinary excretion of Cl^- (in mEq/L/100 g body weight) measured using argentometric titration.

Furthermore, important indices such as diuretic index. lipschitz value, Saluretic and natriuretic activity were determined.

Estimation of Urinary Electrolytes

An ethanolic extract were dissolved in distilled water & calibrated flame photometer was used to determine the concentration of urine electrolytes like sodium, potassium and chloride. Before estimation of electrolyte the samples were filtered to remove debris & shedding.

Statistical analysis

Results were expressed as mean ± SEM. Differences among data were determined using Student t-test (unpaired) using Graphpad Prism software (Graphpad Prism software Inc., Version 4.00.255). Differences between the data were considered significant at $P<0.05$.

Result and Discussion

The results obtained with evaluation of diuretic activity of ethanolic extract of *Stigma maydis* was shown in Table.1 & Fig. no.1. From the result it can be observed that a ethanolic extract of *Stigma maydis* has shown a significant diuretic activity by increasing urinary output and increased excretion of sodium, potassium, chloride when compared to control. The diuretic activity was assessed by determination of lipschitz value using urea (1 g/kg) as standard. The standard diuretic drugs, urea and furosemide exhibited a significant increase in the urinary excretion over 24 hours (Table no.1). The urinary excretion of water and electrolytes exhibited by these standard drugs were significantly, as compared to control group over a period of 24 hours (Table no.1). After 7 hr furosemide treated group showed higher indices for diuresis (4.71), lipschitz value (2.09) and after 24 hr also furosemide treated group showed higher indices for diuresis diuresis (3.70), lipschitz value (3.37). furosemide treated group showed maximum diuretic effect (diuretic index & lipschitz value) lasting over 24 h. the ethanolic extract (200mg/kg, 400mg/kg,500mg/kg) of *stigma maydis* treated groups, showed statistically significant ($P<0.001$) diuretic effects as compared to control group.

Saluretic and Natriuretic activity

The results obtained of saluretic index & natriuretic activity of ethanolic extract of *Stigma maydis* was shown in Table.2 & Fig. no.2**.** Although, the dose dependent rise in urinary excretion of water was observed (Table no.1). The increase in urinary electrolyte excretion was found to be independent of the dose administered (Table no.2). Standard drugs showed higher values of saluretic index (Na^+ 1.95, k+1.72 Cl^- 1.71) and the values of saluretic index (Na^+ 1.46, k+1.16 Cl^- 1.26) were observed with different dosage of ethanolic extract of *stigma maydis* treated groups. the higher natriuretic values were observed with urea treated group (Na^+/ K^+ 3.23) where as ethanol extract of *stigma maydis* treated group shown (Na^+/ K^+ 3.26) moderately good natriuretic effect as compare to standard drug treatments. With regard to different doses of ethanol extract of *stigma maydis* treatments, the electrolytic excretion was inferior as compared to standard diuretic drugs in both 7 and 24 h urine. However, the ethanolic extract of *stigma maydis* showed a significant saluretic index & Natriuretic activity over a period of 24 h. Medicinal plants and botanicals offer a natural safeguard against diseases and are a substantial treatment for certain diseases. Diuretics have proved to be extremely valuable in the treatment of mild to moderate hypertension and also in enhancing the effect of other antihypertensive agents. Diuretics relieve pulmonary congestion and peripheral oedema. These agents are useful in reducing volume over load and relieve orthopnea and paroxysmal nocturnal dyspnoea in CCF and acute left ventricular failure. They decrease plasma volume and subsequently venous return to the heart. This decreases the cardiac work load, oxygen demand and plasma volume and also decreases blood pressure. Thus diuretics play an important role in hypertensive patients. They are used to induce forced diuresis (forced alkaline dieresis and forced acidic diuresis) in cases of aspirin and morphine poisoning. Diuretics are also useful in prevention of recurrent calculi. The present study revealed that ethanol extract of *Stigma maydis* on different dosage (200mg/kg, 400mg/kg, 500 mg/kg) significantly increased the urinary output, as well as the elimination of urinary electrolytes in a dose dependant manner.

Table 1: Effect of different doses of Ethanolic Extract of *stigma maydis* on urinary volume

Groups	Dose (mg/kg)	After 7 hr			After 24 hr		
		Volume of urine (ml/100 gm	Diureti c index	Lipschti z value	Volume of urine (ml/100 gm	Diureti c index	Lipschti z value
Control (Normal saline)	25ml/kg	0.249±0.14	1.00	_	1.13±0.01	1.00	_
Urea	1000	0.561±0.1**	2.25	1.00	1.24±0.01*	1.09	1.00
Furosemide	25	1.174±0.9*	4.71	2.09	4.19±0.02***	3.70	3.37
Ethanolic Extract of *stigma maydis*	200	0.287±0.2*	1.15	0.51	1.87±0.04**	1.65	1.50
Ethanolic Extract of *stigma maydis*	400	0.317±0.1**	1.27	0.56	1.98±0.02**	1.75	1.59
Ethanolic Extract of *stigma maydis*	500	0.398±0.14*	1.59	0.61	2.02±0.04**	1.78	1.62

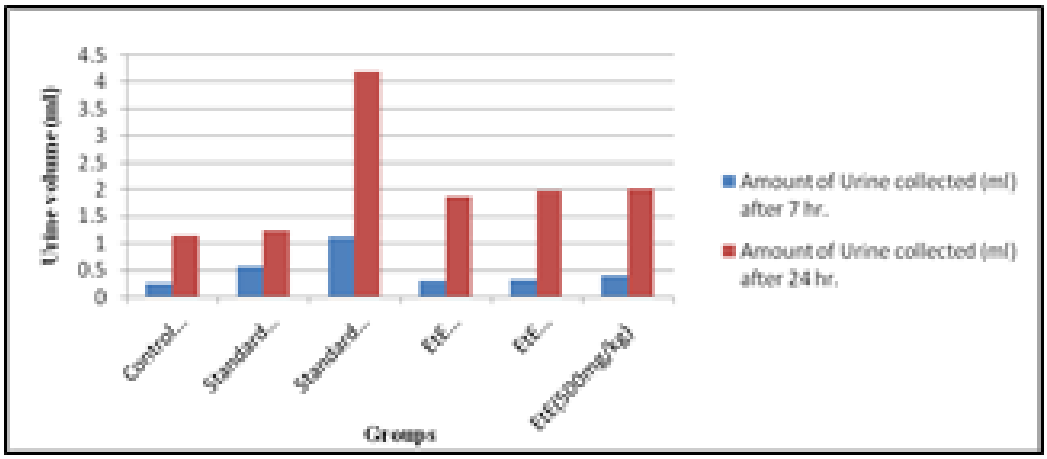

Fig 1: Effect of different doses of Ethanolic Extract of *stigma maydis* on urinary volume

Table 2: Effect of different doses of Ethanolic Extract of *stigma maydis* on Electrolyte concentration.

Group	Dose (mg/kg)	Concentration of ions mEq/l/100g			Saliuretic index			Natriuretic activity
		Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Na^+/K^+
Control (Normal saline)	25ml/kg	0.43±0.05*	0.18±0.02*	0.63±0.05	1.00	1.00	1.00	2.38
Urea	1000	0.84±0.08**	0.26±0.05*	1.08±0.06***	1.95	1.44	1.71	3.23
Furosemide	25	0.79±0.08**	0.31±0.04*	1.08±0.04***	1.83	1.72	1.71	2.54
Ethanolic Extract of *stigma maydis*	200.	0.57±0.03^{ns}	0.18±0.03^{ns}	0.78±0.2*	1.32	1.00	1.23	3.16
Ethanolic Extract of *stigma maydis*	400.	0.62±0.04*	0.19±0.04^{ns}	0.80±0.8^{ns}	1.44	1.05	1.26	3.26
Ethanolic Extract of *stigma maydis*	500.	0.63±0.02*	0.21±0.02**	0.80±0.4*	1.46	1.16	1.26	3.00

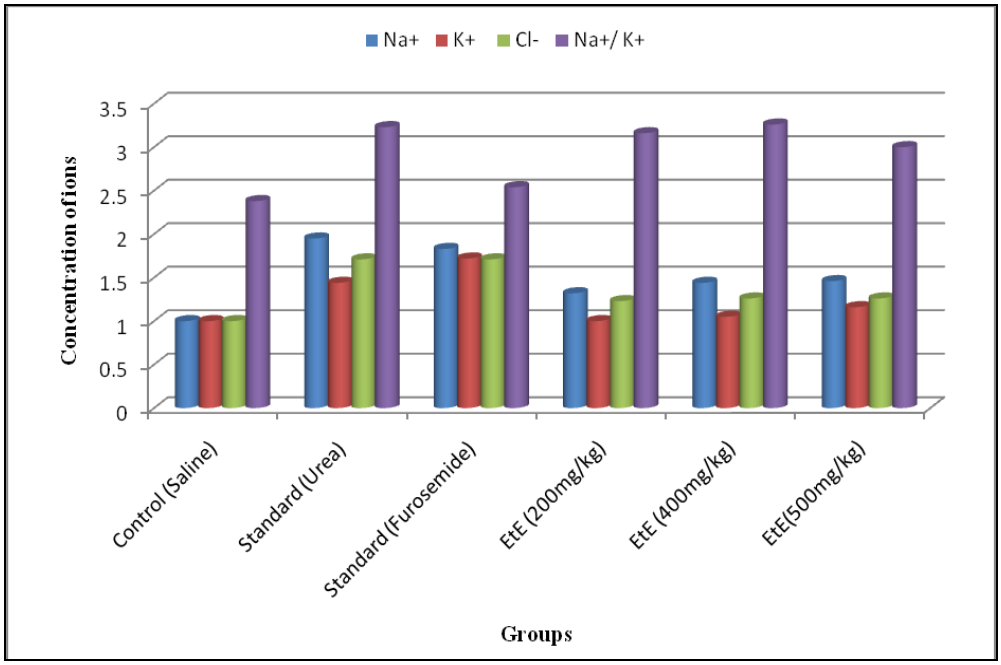

Fig 2: Saliuretic & Natriuretic Effect of different doses of ethanolic extract of *stigma maydis*

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