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Study of antacid and diuretic activity of ash and extracts of *Musa sapientum* L. fruit peel.

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ABSTRACT

The antacid and diuretic activity of different extracts and ash of peels of *Musa sapientum* L. (*Musaceae)* in rat was studied. The study suggested that the ash has good antacid activity. Diuretic study was carried out as per Lipschitz et al., (1943), where successive aqueous, ethanolic and petroleum ether extracts and ash of peel of fruit of *Musa sapientum* L. were studied for diuretic activity. The 6 hrs acute study of successive aqueous, ethanolic extracts and ash of the peel showed increase in urine volume and K^+ ion excretion as compared to normal saline. Urinary levels of sodium, potassium (by flame photometry) and chloride (by titrimetry) were estimated. On the basis of observed results it is concluded that ash might have good antacid activity while successive ethanolic extract and ash exhibited evidence of diuretic potentials in normal rat in our experimental model. The histopathological examination and toxic effects on vital organs remained to be studied before their recommendation for use as diuretic agents.

KEY WORDS - Antacid; Diuretic; Flame photometry; *Musa sapientum; Musaceae*.

INTRODUCTION

Musa sapientum Linn (Musaceae) commonly called 'Kela' in Hindi (English: Banana) is extensively cultivated in northern part of Maharashtra. It is one of the most popular fruit crop in India and possesses many curative properties and prevent many kinds of illnesses and conditions. Different parts of plant are used very frequently in different worship ceremonies by the Indians every part of the tree is being used for some purpose like food, fuel or timber (1).

It is perennial herbaceous plant, grows to 5-9 m in height. It has tuberous subterranean rhizome, from which the leaves are folded within each other producing false stem, from which the long, narrow blades protrude and spread out. In the center of the folded leaf sheaths, a growing point forms the top of rhizomes, grows up and emerges as an overhanging inflorescence with a succession of reddish brown bracts. The bracts unfold from the base to the tip and fall off. Within the lower 1-12 bracts arise 14-18 female flowers in double rows these develop into fruits (2). The roots are adventitious. The leaves are large from 1.5-3.5 meters and 0.6 meter wide (3).

In India hot water extract of dried fruits, flowers and roots is used orally for diabetes, the dried flower along with dried fruits of *Coccinia indica* is used to prevent conception orally. The roots are used as anthelmimtic, aphrodisiac, laxative and tonic. The fresh fruit is used for peptic and duodenal ulcers. The leaf ash is mixed with honey and taken orally for cough. Ripe plantain is emollient, demulcent and nutrient. Unripe plantain is cooling and astringent. Fully ripe fruit have laxative effect while flowers are used as astringent. Root is anthelmintic and a valuable alternative (4, 2). The peels of *Musa sapientum* plant are used in Jalgaon and its environment as antacid and diuretic agent. But adequate characterization of its antacid and diuretic activity has not been yet performed. Also such reports are not available in the literature though the activity is reported. The present study was undertaken for scientific evaluation of antacid (in vitro) activity using Acid-neutralizing capacity test USP29 and diuretic (in vivo) activity in normal healthy rats.

MATERIALS AND METHODS

Plant material

The fresh banana fruits were procured from local farmers in the Pune region and identified correctly by Pharmacognosy Department of MAEER'S Maharashtra Institute of Pharmacy, Paud Road, Pune-411038, India. The peels of the fruits were dried and powdered using laboratory grinder.

Drugs

Frusemide and urea were procured from Himedia Laboratories, Mumbai, while, Gokharu khada (Diuretic preparation, as described in Ayurveda) was purchased

from market (manufacturer: Ayurved Rasashala, Pune, India). All other reagents were analytical grade.

Preparation of the extracts

Fresh banana peels were extracted with decreasing polarity of solvents to obtain successive extracts using pet ether, benzene, chloroform, ethanol and water. The extracts were prepared using soxhlet apparatus as one part of the material with three parts of solvent for 3 hrs. This was repeated twice with fresh solvent. The extracts from both washes were pooled and concentrated under vacuum at 60^0 C to obtain a dry extract. The marc obtained following the pet ether extraction was later extracted with benzene and dried in same manner as above. The same procedures were followed using chloroform, ethanol (95%) and water to obtain their successive extracts. All the extracts were stored at room temperature in tightly closed containers.

Preparation of ash

The ash was prepared by incineration of the peels into the furnace at 800^0 C for 1.5 hrs and cooling at room temperature. Fine powder was prepared using laboratory grinder and passed through sieve no. 120.

Animals

The male Wistar rats weighing 160-200 gm were used to study the diuretic activity. The animals were housed under standard environmental conditions (22 ± 3^0 C, 55±5 % humidity and a 12 h light/ dark cycle) and fed with standard rodent diet and water *ad libitum* .The institutional Animal Ethical Committee approved all the experimental protocols.

Toxicological study

The male albino rats of Wistar strain weighing 160-200 gm were divided into different groups comprising of six animals each. The control group received normal saline 25ml/kg i.p. The other groups received 100, 200,400,600,800,1000,2000,3000 and 4000 mg/kg of test extracts. The animals were observed continuously for the behavioural changes for the first 4 hours and then observed for mortality if any for 24 hours. (5)

Acid-Neutralizing Capacity USP

The Acid-Neutralizing capacity was carried out as per USP29. In short, all tests were conducted at temperature 37 ± 3^0 C. A pH meter was standardized using the 0.05M potassium biphthalate and 0.05M potassium tetraoxalate standardized buffers. Magnetic stirrer was used to produce stirring rate 300± 30rpm. 0.5 gm of each ash, pet ether, successive ethanolic and successive water extracts were transferred to 250 ml beaker and 70 ml distilled water was added. It was mixed with magnetic stirrer for 1 min. Then 30 ml 1.0N HCl was added to the test solutions with continuous stirring for 15 min. Excess HCl was titrated with 0.5 N NaOH to attain a stable pH of 3.5. The number of mEq of acid consumed was calculated by formula:

$$\text{Total mEq} = (30\times N_{HCl}) - (V_{NaOH}\times N_{NaOH})$$

Where N_{HCl} and N_{NaOH} are normalities of hydrochloric acid and sodium hydroxide respectively and V_{NaOH} is volume of sodium hydroxide and the result were expressed as total mEq per gm of substance(6).

Diuretic study (Lipschitz method)

Diuretic activity was carried out as per the method of Lipschitz et.al. (1943)(7, 8). In brief, ash, pet ether, successive ethanol (95%) and successive aqueous extracts were subjected to diuretic study. The screening was performed on healthy rats (160-200 gm). Frusemide (20 mg/kg), urea (500 mg/kg) and Gokharu khada (500 mg/kg) were used as reference standards and were dissolved in saline solution for administration while normal saline (25 ml/kg) was used as vehicle. The rats were divided in 12 groups each containing 6 rats (n = 6). Rats were kept for fasting for 18 hrs before the study. The control group received normal saline and test groups received different extracts (500 and 1000 mg/kg) and ash (500 and 1000 mg/kg) dissolved in normal saline. The doses of extracts were decided on the basis of acute toxicity study. The doses were given by oral route and rats were kept in specially designed metabolic cages for the collection of urine for 6 hrs. The urine volume during 6 hrs is measured and urine electrolyte estimation was carried out for Na^+, K^+ using flame photometer (9) and Cl^- was estimated by titration (8,10,11).

Statistical analysis

All results are expressed as mean ± standard error. The data was analyzed statistically using ANOVA followed by Dunnett's Multiple Comparison Test.

RESULTS

Extraction

The florescence study showed characteristic color changes with solvents like pet ether, benzene and ethanol (95%). The obtained yields are given in Table 1. Since the yields of successive extract of benzene and chloroform were insignificant so further studies for these extracts were discontinued and only pet ether, successive ethanol (95%) and successive aqueous extracts were subjected to further studies.

Acid-Neutralizing Capacity Test USP29:

The ash complied with the Acid-Neutralizing Capacity Test and pH after 10 min was 7.8. Ash neutralized 10.6 mEq of acid whereas pet ether, ethanol and aqueous extracts were unable to neutralize acid.

TABLE 1: Estimated yields % using different solvents.

Sr. No.	Solvent	Estimated yields % (w/w)
1	Pet ether	2.41
2	Benzene	0.03
3	Chloroform	0.02
4	Ethanol (95%)	1.36
5	Water	0.36

Toxicological study

Neither mortality nor any gross behavioural changes were observed during and after the treatment. The ash, ethanolic and aqueous extracts were found to be safe up to 2000 mg/kg.

Diuretic activity

Frusemide treated rats showed a significant increase in volume of urine and urinary excretion of sodium, potassium and chloride ($p< 0.01$) as compared to control while urea treated rats did not show any significant increase in urine volume but has high electrolyte excretion potential ($p< 0.01$). Higher electrolyte excretion ($p< 0.01$) was observed in ayurvedic diuretic preparation, Gokharu Kadha but not significant increase in urine volume. The successive aqueous extract was unable to produce significant actions in dose of 500 mg/kg but at high dose of 1000 mg/kg successive aqueous extract showed significant increase in volume of urine and also urinary excretion of sodium, potassium and chloride. The successive ethanolic extracts has shown diuretic activity ($p<0.01$) wherein significant increase in K^+ but not in Na^+ excretion when compared to control was observed. Pet. ether extract did not show remarkable increase in volume of urine, urinary sodium, potassium or chloride. It was observed that ash also increased diuresis and urinary excretion of electrolyte ($p< 0.01$). The results are summarized in Table 2.

DISCUSSION

Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea. They decrease plasma volume and subsequently venous return to the heart (preload). This decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure (12). Thus, diuretics play an important role in hypertensive patients. In present study, we can demonstrate that ash and successive aqueous and ethanol extract may produce diuretic effect by increasing the excretion of Na^+, K^+ and Cl^-.

The control of plasma sodium is important in the regulation of blood volume and pressure; the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles (13). The regulation of Na^+/ K^+ balance is also intimately related to renal control of acid-base balance. The K^+ loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally potassium-sparing diuretics are recommended (14). In present study, ash and successive aqueous and ethanol extract showed elevated levels of K^+ in urine, which may increase risk of hypokalemia, and hence its potassium sparing capacity has to be investigated.

Active phytoprinciples such as flavonoids, saponins and terpenoids are known to be responsible for diuretic activity (15,16,17,). These active principles in successive aqueous and ethanolic extract may be responsible for diuretic activity. Isolation of these active principles and study of their exact mechanism of action needs to be investigated.

Results of present investigation showed that ash is most effective in increasing urinary electrolyte concentration of all the ions i.e. Na^+, K^+ and Cl^- followed by successive aqueous and ethanol extracts while petroleum ether extract did not show significant increase in urinary electrolyte concentration.

In the *in vitro* studies simulated to compare the antacid effects, ash showed good antacid activity while others did not. Antacids are weak bases that react with gastric acid to form water and salt thereby diminishing gastric acidity. Antacid products vary widely in their chemical composition, acid neutralizing capacity, sodium content and palatability. Acid neutralizing capacity depends upon capacity to neutralize gastric HCl and the extent of food contents in stomach (12). It was observed that 0.5 gm of ash neutralized 10.6 mEq of HCl acid in Acid Neutralizing Capacity Test USP29. These beneficial effects observed under in vitro simulations remains to be investigated in vivo as well as at the biochemical level to understand the mechanisms involved.

Table 2: Diuretic activity of successive aqueous, ethanolic and pet. ether extracts of Musa sapientum.

Group	Treatment (n= 6)	Volume of Urine (ml/ 6 hrs)	Sodium (mMol/l)	Potassium (mMol/l)	Chloride (mMol/l)
I	Normal saline (25 ml/ kg)	0.74±0.19	98.3±11	52.3±8.9	101.2±8.85
II	Frusemide (20 mg/ kg)	3±0.34*	137±7.6*	96.64±10.2*	153±13.2*
III	Urea (500 mg/ kg)	0.85±0.13	112.1±11.9*	78.7±7.9*	121.3±14.1*
IV	Gokharu Kadha (500 mg/kg)	0.71±0.18	118.3±10.4*	87.3±8.4*	131.2±9.3*
V	SAE (500 mg/ kg)	1±0.17	99.7±8.4	114.7±9.8*	122±14.2*
VI	SAE (1000 mg/kg)	1.5±0.25*	110.7±2.3**	133±7.3*	157.7±12.6*
VII	SEE (500 mg/ kg)	1.09±0.18*	98.6±5.3	110.7±6.9*	115.2±10.3**
VIII	SEE (1000 mg/ kg)	1.42±0.10*	100.2±1.3	120.8±3.5*	131.1±13.2*
IX	Ash (500 mg/ kg)	1.12±0.20*	100.8±0.3	90.2±6.8*	120.3±7.6*
X	Ash (1000 mg/ kg)	2.44±0.39*	119.1±13.3*	104.4±7.5*	133.4±9.7*
XI	PEE (500 mg/kg)	0.69±0.12	90.8±9	52.7±8.2	98.7±7.7
XII	PEE (1000 mg/ kg)	0.73±0.17	92.1±8.3	51.9±7.6	97.7±7.2

*Values are mean ± SEM, * p< 0.01, ** p< 0.05 when compared to normal saline (control)*
Note: SAE: Successive Aqueous Extract, SEE: Successive Ethanolic Extract, PEE: Pet Ether Extract.

CONCLUSION

On the basis of the results of present investigation, we can conclude that ash and successive ethanolic extracts might be good diuretics. In present study, no lethality was observed at least for the dose and duration used. However, advanced toxicological studies remain to be performed in mice and rats. It remains necessary to study eventual adverse effect(s) of this plant such as alteration of some neural, metabolic and hormonal parameters. Future studies play an important role before its recommendation to clinical use. The precise site(s) and the molecular and cellular mechanism(s) of this ash and successive ethanolic extract action remain to be elucidated. Also, the selective isolation or activity-guided isolation along with deciphering the chemical nature of the compounds will help further to elucidate the exact mechanism(s).

REFERENCES

1. T.K. Bose, *Fruits of Indian Tropical and subtropical*, (Naya Prakash, Calcutta, 1985) pp. 346.
2. Ivan A Ross, *Medicinal plants of the world*, *Vol .2.*, (Humana Press Inc. Totowa. NJ. 2003) pp. 319-326.
3. K.M. Nadkarni, *Indian Materia Medica*,(Popular Prakashan, Bombay, 2000) pp. 822
4. S. R. Jain and S.R.Sharma. Hypoglycemic drugs of Indian indigenous origin. *Planta Med.* **15(4)**: 439-442 (1967).
5. R. A. Turner, The Organisation of Screening. In: *Screening Methods in Pharmacology*, Vol. I, New York and London, Academic Press; pp. 21(1965).
6. United States Pharmacopoeia (USP29), (The United States Pharmacopeial Convention, Rockville) pp.2561 (2006).
7. W.L. Lipschitz, Z.Haddian and A.Kerpscar. Bioassay of diuretics. *J. Pharmacol. Exp.Ther.* 79: 97-110 (1943).
8. T. Murugesan, L. Manikandan, K.B. Suresh, M. Pal and B.P. Saha. Evaluation of diuretic potential of *Jussiaea suffruticosa* Linn.

extract in rats. *Indian J.Pharm.Sci*. **62(2)**: 150-151(2000).

9. G.H. Jeffery, J. Bassett, J. Mendham, R.C. Denny, *Vogel's Textbook of Quantitative Chemical Analysis*, 5th ed. (AddisonWestley Longman Ltd., England, 1989) pp. 801.
10. A.H. Beckette, J.B. Stenlake, *Practical Pharmaceutical Chemistry*, Part I.1st ed. (CBS Publishers and Distributors, New Delhi,1997) pp. 197
11. T. Vetrichelvan, M. Jegadeesan, M.S. Palaniappan, N.P. Murali and K. Sasikumar. Diuretic and anti-inflammatory activities of *Aerva lanata* in rats. *Indian J. Pharm. Sci*.**62 (4)**: 300-302 (2000).
12. R.D. Hoeland and M.J. Mycek, *Lippincott's Illustrated Reviews: Pharmacology*, (Lippincott Williams and Wilkins, Philadelphia, 2000) pp. 157-58; 240-41.
13. A.C .Guyton and J.E. Hall, The body fluid compartments: extracellular and intracellular fluids; interstitial fluid and edema .In: *Textbook of medical physiology*, ninth edition. Singapore, PA: W. B. Saunders Company; 306-308 (1998).
14. I. F.Stuart, *Human Physiology*, (Wm. C. Brown publishers, Dubuque, Iowa Second edition) pp. 500-503, 508.
15. A. R. Sood, A. Bajpai and M. Digits. Pharmacological and biological studies on saponins. *Indian J Pharmacol*. **17(3)**: 178-179 (1985).
16. S.H. Rizvi, A. Shoeb, R.S. Kapil and Satya P. Popli. Two diuretic triterpenoids from *Antiderma menasu*. Phytochemistry. **19(11)**: 2409-2410 (1980)
17. A.Chodera, K. Dabrowska, A. Sloderbach, L. Skrzypczak and J. Budzianowski. Effect of flavanoid fractions of *Solidago virgaurea* L. on diuresis and levels of electrolytes. *Acta Pol Pharm*.**48**: 35-37 (1991).