ACUTE TOXICITY AND DIURETIC ACTIVITY OF *MANGIFERA INDICA* L. BARK EXTRACTS

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Summary

Introduction: Kidney, the excretory organ of our body serves the important function of excretion of waste products, regulation of fluid volume and electrolyte content of the extracellular fluid. Diuretics are drugs capable of increasing levels of urine. Materials and methods: The Ethyl acetate, ethanol and water extract of *Mangifera indica* was evaluated for diuretic activity. Diuretic effect was carried out in rats (175 – 200 kg bwt) by measuring the urine volume by 1, 2, 4, 6 hours and later at 24 hours. Positive control was furosemide (20mg/kg) intraperetoneally and mannitol (100mg/kg) intravenously given. The extracts were administered orally at the dose of 250 mg/kg b.wt. Preliminary phytochemical tests were carried out in different extracts. Results: Na^+, K^+ and Cl^- concentrations were determined. Na^+/ K^+ ratio was higher in aqueous extract and followed by ethanol and ethylacetate extracts. The aqueous extracts show best diuretic effect when compared with other extracts.Conclusion: It was determined that all the extracts showed diuretic effect and cation excretion outstandingly.

Key words: Diuretics, Furosemide, Mannitol, intraperitoneally, proximal tubule

Introduction

Mangifera indica L (Family-Anacardiaceae) is commonly used in folk medicine for a variety of remedies (1,2). The diuretics are drugs that act on the kidney and are able to increase the volume of urine excreted, reason why are used in cardiac failure, chronic and moderate cardiac insufficiencies, acute oedema of the lung, nephritic edema syndrome, arterial hypertension, diseases related with the retention of fluids etc (3,4). The diuretics act primarily by inhibiting tubular reabsorption and the drugs, which cause a net loss of Na^+ and water in urine, are called diuretics. Furosemide is a sulphonamyl derivative which is a high efficacy diuretic which has its primary action on medullary ascending limb of loop of Henle and can produce substantial effect because of limited capacity for salt absorption in distal tubule and collecting duct. Mannitol is an osmotic diuretic, which is a sugar, when administered intravenously, is not metabolized and rapidly filtered by glomeruli but not reabsorbed. It causes water to be retained in the proximal tubule and descending limb of Henle. The medicinal attributes of *M. indica* have been known since long time. A number of pharmacological properties have been reported where the diuretic activity has not been experimentally proved. The present investigation was undertaken to confirm the diuretic activity of different extracts of *M. indica* stem bark.

Materials and methods

Plant material

The bark of *M. indica* locally called as 'Komanga' (in Malayalam) were collected from herbal garden of Amala Ayurvedic Hospital, Thrissur, Kerala, during May 2004 and it was authenticated by Dr. K. Sunil, Department of Botany S.N.M.College, Maliankara. A voucher specimen was kept at Fr.Gabriel Herbarium (No MI -2), Thrissur, Kerala.

Preparation of plant extract

Shade dried bark were powdered, defatted with petroleum ether and extracted with ethylacetate, ethanol, methanol and water in a soxhlet apparatus, concentrated to get the residue. The yields of the extracts were 8.0, 11.2, 3.4 and 17.47% respectively.

Animals:

Male Wistar rats (175-200g) Male Balb/C mice (25-30g) were used for the experiments. They were housed in environmental conditions and fed with standard rodent diet and water *ad libitum.* All animal experiments conducted during the present study got prior permission from Institutional Animal Ethics Committee (IAEC) and followed the guidelines of IAEC.

Phytochemical analysis:

Phytochemical analysis of the major phytoconstituents of the plant extracts was undertaken using standard qualitative colour tests as described earlier (5).

Acute toxicity:

Mice were divided into eight groups of six animals each. The control group received normal saline (2ml/kg, p.o.) The other groups received 50, 100, 200, 400, 800, 1000, 2000 and 4000mg/kg of the extracts, respectively. Immediately after dosing the animals were observed for their behavior continuously for the first four hours. They were kept under observation up to 14 days after extract administration to find out the mortality and body weight was observed. (6).

Assessment of diuretic activity:

Male Wistar rats (175-200g) were purchased from Small Animals Breeding Station, Mannuthy, Thrissur. They were maintained under standard conditions of temperature and humidity. The method of Lipschitz et *al* (7) was employed for the assessment of diuretic activity. Six groups of six rats each were fasted and deprived of water for eighteen hours prior to the experiment. On the day of experiment, normal group of animals were given normal saline orally (25 ml/kg body weight.) and the treated groups were given 250mg/kg bodyweight of extracts of ethyl acetate, ethanol and water. The standard groups were given furosemide (20mg/kg) intraperitoneally and mannitol (100mg/kg) intravenously**.** The rats were placed in metabolic cages specially designed to separate feacal matter and urine. The urine volume was registered at 1, 2, 4, 6 and 24 hours post administration. During this period no food or water was given to the animals. The total urine volume was measured for both control and treated animals. The sodium, potassium and chloride ion concentration in the urine samples were determined.

Results

The preliminary phytochemical characterization showed that flavonoids, polyphenols, sterols, tannins, saponins, polysaccharides, terpenes etc are found in the extracts of *M. indica*. In the acute toxicity study, the single administration of these extracts up to 4.0g/kg body weight did not produce any mortality or adverse reaction after the administration of of a single limit dose. There was no statistically significant body weight change in animals treated with the extracts (data not given). It was found that the ethyl acetate, ethanol and aqueous extract showed diuretic activity when compared with the standard furosemide and mannitol. In the normal rats the diuresis began passed one hour of the administration, showing low volumes of urine excreted until completing 34.5 mL at 24 hours.

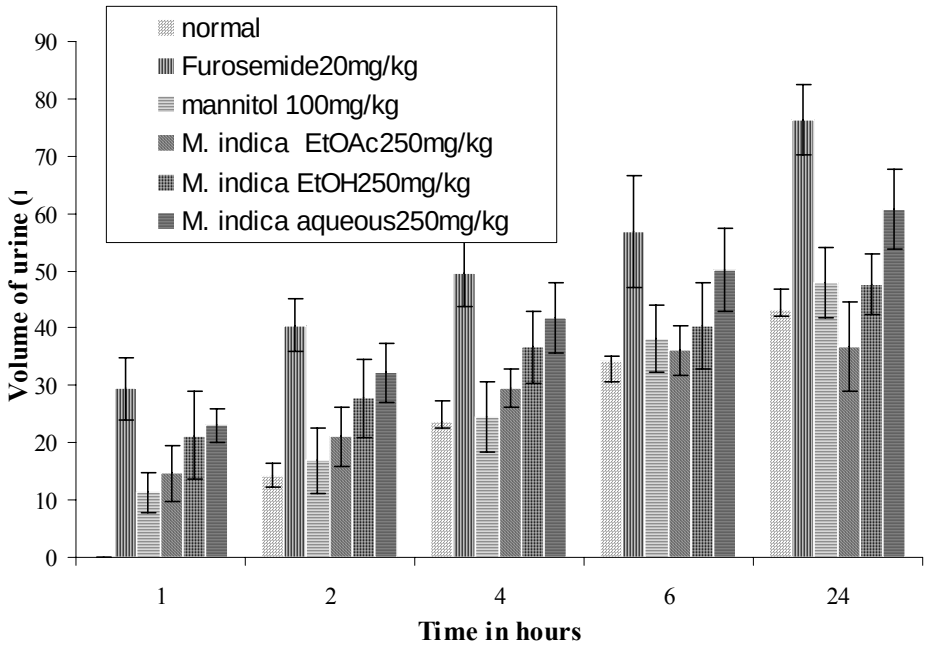
Table 1. Qualitative phytochemical evaluation of the *Mangifera indica* extracts

Phyto Constituents	**Observations**			
	Ethyl acetate	**Ethanol**	**Methanol**	**Water**
Saponins	–	–	–	+
Tannins	–	+	+	+
Alkaloids	–	–	–	+
Sterols	+	–	+	+
Glycosides	–	+	–	+
Flavonoids	–	+	+	+
Reducing sugars	+	–	+	+
Polysaccharides	–	+	+	+

The values of excreted Na^+ and K^+ in urine were equally low. On the contrary, in the group dealt with furosemide (positive control), the beginning of the diuretic action was at 60 minutes.

A final volume of 64.7 mL was reached being significantly different from the obtained in the negative control group ($p< 0.05$). In-group III dealing with mannitol, does not show significant increase in urine volume. The beginning of urine for the watery extract of the *M. indica* was also at 60 minutes post administration, but the volume was smaller (2.8mL), differing significantly from the values obtained with furosemide ($p<0.001$) being reached a total volume of 50.8 mL.

Figure 1. Volume of urine in the experimental groups at 1, 2, 4, 6 and 24 hours



The order of activity of increase of urine output was greater for aqueous extract than that of ethanol extract which is greater than that of ethyl acetate extract. Similarly it was obtained an increase of the excretion of Na^+ in the urine significantly superior to the one registered in the negative control group ($p<0.001$) and very highly significantly superior compared with furosemide group. Mannitol administration did not increase the Na^+ concentration when compared with water extract treated group. The K^+ concentration in urine, was very high significantly superior compared with negative control, furosemide and mannitol groups ($p<0.001$). The aqueous extract administration increases the Na^+ concentration than other extract treated group. Where as the ethyl acetate fraction showed lesser urinary excretion when compared with other extracts. The Na^+/ K^+ ratio of the aqueous extract treated group showed higher level than other extract treated group.

Group	**Dose mg/kg**	**Electrolyte concentration in m eq/l**			
		Na^+	**K^+**	**Cl^-**	**Na^+/ K^+**
Normal	Saline 5ml/kg	178.25± 12.76	87.20 ± 10.5	113.2± 8.9	2.04
Furosemide	20mg/kg	323.61± 12.42**	123.56± 10.8 **	215.7± 11.4**	2.61
Mannitol	100mg/kg	261.53± 10.51**	112.32± 6.21**	123.6± 9.1^{ns}	2.32
EtOAc extract	250mg/kg	189.60± 7.98^{ns}	93.61± 9.4	121.8± 9.6	2.02
EtOH extract	250 mg/kg	206.11± 13.5**	109.78± 17.1^{ns}	187.43± 8.9**	2.24
Aqueous extract	250 mg/kg	234.19± 9.02	156.34± 6.3**	176.34± 10.5 **	2.38

Table 2. Electrolyte excretions of successive extracts of *M. indica* extracts in rats. ** indicates P<0.01, 'ns' indicates not significant

Discussion

Diuretics are drugs capable of increasing levels of urine, so they are useful in the treatment of diseases related with the retention of fluids. Many herbal diuretics exert their action by directly effecting electrolyte balance of minerals.

In the normal rats diuresis began with low volumes of urine excreted until completing 24 hours. The level of excreted Na^+ and K^+ in urine was equally low. The furosemide (positive control) treated group, the diuretic action start at 60 minutes and increased significantly ($p< 0.05$) from normal rats. In the mannitol administered group showed lesser urine volume when compared with furosemide. The beginning of urine for the watery extract of the *M. indica* was also at 60 minutes post administration, but the volume was smaller than furosemide.

The ethyl acetate fraction did not increase urinary excretion when compared with other extracts. All extracts did not increase the Na^+ concentration when compared with the positive controls. Thirty to seventy percentage of K^+ filtered by the glomerulus is known to be reabsorbed by the proximal convoluted tubule (8) by a combination of three processes: active transport, paracellular diffusion and solvent drag (9). The mechanism of action are complex and involve a variety of energy dependent trans membrane pumps as well as Channels in between the loose fitting cells of the proximal tubule (PT). About 80% of the nephrons lie in outer cortex, having short loops of Henle and low Na^+ reabsorptive capacity where as 20% are juxta medullary possessing long loops of Henle and are responsible for creating the cortico medullary osmotic gradient. The redistribution of blood flow between these two types of nephrons can alter salt and water excretion. The increase in the ratio of concentration of excreted sodium and potassium ions indicates that the extract increases sodium ion excretion to a greater extent than potassium, which is a very essential quality of a good diuretic with lesser hyperkalaemic side effect (10). The chloride ion excretion was not elevated significantly by the lower dose and the results are indicating that the extract is a potent natriuretic (11).

The presence of phytoconstituents like terpenoids, saponins, flavonoids has been reported previously to be responsible for the diuretic activity in plants (12,13). The maximum volume of urine at the end of 24 hours was for aqueous extract may be due to the presence of flavonoids, saponins, tannins (14) etc.The best diuretic effects could be associated to the flavonoid content, also it promote high levels of Na^+ and K^+ in urine. There are correspondence between the volume of urine and the concentration of Na^+, this aspect is logical because the mechanism of action of diuretic drugs is to decrease the tubular reabsorption of this ion, it produces the dragging of the osmotic equivalent of water, other explanation that can support this, is the high ion concentrations in this medicinal plants. (15-16). However, the contribution of polyphenolic compounds to diuretic effect cannot be ruled out. Further studies like isolation and characterization of diuretic principle from the bark of the plant is needed to understand and confirm the exact mechanism of action.

The extracts showed diuretic effects after the administration of 250mg/kg body weight dose Out of these extracts water extract showed better diuretic properties and also superior urine excretions of ions compared to the positive and negative controls.

Conclusions

All the extracts of *M. indica* showed diuretic effects after the administration of 250mg/kg body wt. The aqueous extracts show the best diuretic properties and also superior urine excretions of $Na^+$and K^+.

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