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ACUTE TOXICITY AND DIURETIC ACTIVITY OF *MIMUSOPS ELENGI* EXTRACTS

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ABSTRACT

Kidney, as excretory organ of our body serves important function of excretion of waste products, regulation of fluid volume and electrolyte content etc. Damage to kidney can lead to severe life threatening complications. Diuretics are drugs capable of increasing levels of urine. The ethyl acetate, ethanol and water extract of *Mimusops elengi* was evaluated for diuretic activity. *Mimusops elengi* an herb traditionally claimed to diuretic potential and being used for kidney complications from centuries. Diuretic study was carried out in rodents by measuring the urine volume at 1, 2, 4, 6 and 24 hrs. The extracts were administered orally at the dose of 250 mg/kg b.w. Na^+/ K^+ ratio was higher in aqueous extract and followed by ethanol and ethylacetate extracts. The aqueous extracts showed a significant diuretic activity when compared with other extracts. From results it can be concluded that all the extracts tested have a diuretic potential.

KEY WORDS

Diuretics, Furosemide, Mannitol, Proximal tubule.

INTRODUCTION

The diuretics are drugs that act on the kidney and are able to increase the volume of urine excreted, so are being used in cardiac failure, acute oedema of the lung, nephritic edema syndrome, arterial hypertension, diseases related with the retenton of fluids etc.[1,2] The diuretics act primarily by inhibiting tubular reabsorption and the drugs, which cause a net loss of Na^+ and water in urine. Furosemide , a sulphamoyl derivative with a high efficiency and has 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.

Mimusops elengi (L) commonly known as Bakul is a small to large evergreen tree found all over the different parts of India. It is cultivated in gardens as an ornamental tree. It is cultivated in north and peninsular India, Andaman Island, Indomalaysia, Pakistan etc. A large glabrous evergreen tree 12-15 m. high, with a compact leafy head and short erect trunk, bark smooth and scaly.[3,4] It has been used in the indigenous

system of medicine for the treatment of various ailments. The bark is acrid, sweet, cooling, cardiotonic, alexipharmic, stomachic, anthelmintic, astringent, cures biliousness also used in fever, ulcers, diseases of the gums and teeth.[3] Charaka prescribed extract of the bark and root with honey or in a medicated ghee in helminthiasis and fevers. Sushruta gave flowers internally in coughs and bilious derangement, as an ingredient of medicinal liquor in diseases of the urinary tract. The bark powder is an ingredient in a number of commercial tooth powders, prescribed in disease of gums and teeth (ododentopathy).An infusion of the bark, in folk medicine, is prescribed internally in diseases of the bladder and urethra. Leaves may be boiled and applied to the head as a cold compress for headache and juice of the leaves squeezed into the eye for sore eyes.[3] The *M. elengi* have been evaluated for its antiulcer, anthelmintic, antihypertensive, antibacterial, antihyperlipidemic activities. [6-10] Thus, the medicinal attributes of *M. elengi* have been known since long time but the diuretic activity has not been experimentally proved. So, the present investigation was undertaken to confirm the diuretic activity of different extracts of *M. elengi* stem bark.

MATERIALS AND METHODS

Plant material The bark of *M. elengi* locally called as 'Bakul' were collected from near by pune region, Maharashtra, during May 2009 and was authenticated by Botanical Survey of India and deposited with a voucher specimen No. MI -2), BSI, Pune.

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.[11]

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 4000 mg/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.

Assessment of diuretic activity: Male Wistar rats (175-200g) were maintained under standard conditions of temperature and humidity. The method of Lipschitz et *al., 1943* 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.[12] On the day of experiment, normal group of animals were given normal saline orally (25 ml/kg b.w.) and the treated groups were given 250mg/kg b.w. of extracts of ethyl acetate, ethanol and water. The standard groups were given furosemide (20mg/kg) i.p. and mannitol (100mg/kg) intravenously. The rats were placed in metabolic cages specially designed to separate fecal matter and urine. The urine volume was registered at 1, 2, 4, 6 and 24 hrs 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 are found in the extracts of *M. elengi*. In the acute toxicity study, the single administration of these extracts up to 2 g/kg b.w. did not produce any mortality or adverse reaction after the administration 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 hrs.

Table 1

Qualitative phytochemical evaluation of the M. elengi Extracts

Phytoconstituents	Observation		
	Ethyl acetate	Ethanol	Aqueous
Saponins	-	-	+
Tannins	-	-	+
Alkaloids	+	-	+
Sterols	+	+	+
Glycoside	+	+	+
Flavonoids	-	+	+
Rducing suger	-	+	+
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. elengi* 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.

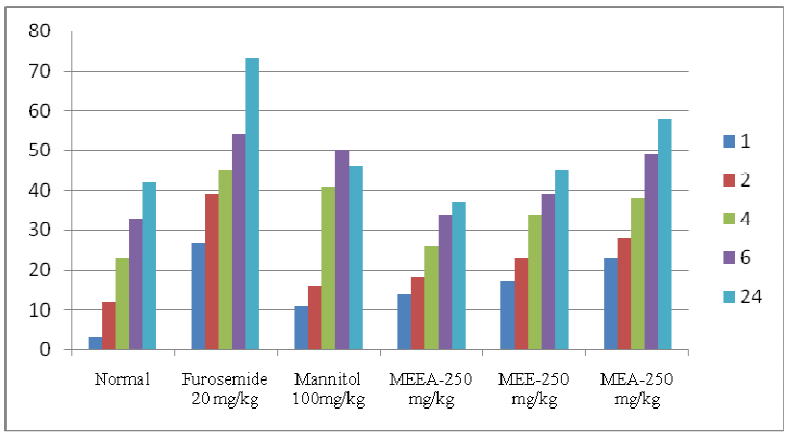

Figure 1 *Volume of urine in the experimental groups at 1, 2, 4, 6 and 24 hrs*

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.

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 hrs. 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. elengi* 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[13] by a combination of three processes: active transport, paracellular diffusion and solvent drag [14]. 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 corticomedullary 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 .[15] The chloride ion excretion was not elevated significantly by the lower dose and the results are indicating that the extract is a potent natriuretic.[16] The presence of phytoconstituents like terpenoids, saponins, flavonoids has been reported previously to be responsible for the diuretic activity in plants.[17,18] The maximum volume of urine at the end of 24 hrs was for aqueous extract may be due to the presence of flavonoids, saponins, tannins etc.[19] 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.[20,21] 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 b.w. 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. In conclusion, all the tested extract of *M. indica* showed a significant diuretic potential at 250mg/kg b.w. and can be recommended to treat the kidney complications as per traditional claim.

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