Research Article

EVALUATION OF DIURETIC, SALURETIC, NATRIURETIC AND CARBONIC ANHYDRASE INHIBITOR EFFECTS OF AQUEOUS EXTRACT OF MUTSANKAN CHOORANAM (*AZIMA TETRACANTHA*) IN WISTAR ALBINO RATS

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ABSTRACT

Objective: The present work was carried out to validate the diuretic effect of Mutsankan chooranam (*Azima tetracantha*) including electrolyte excretion in animal model. **Methods:** In this study, the aqueous extract of Mutsankan chooranam at the doses of 100 mg/kg and 200 mg/kg were evaluated in wistar albino rats by measuring urine volume, urine pH, urinary excretions of electrolytes, saluretic, natriuretic and carbonic anhydrase inhibitor effects. Furosemide was used as a reference drug. **Results:** The urine output and urinary electrolyte excretion were found to be significantly higher in rats treated with MSC100 and MSC 200 as compared to control in a dose dependent manner. MSC 200 has shown more activity than MSC 100. **Discussion:** The findings of this study indicated that MSC possesses significant diuretic, saluretic and natriuretic activity. The diuretic activity observed with *Mutsankan Chooranam* may be due to the flavonoids and alkaloid it contains. **Conclusion:** Thus, the present study supports the traditional uses of *Azima tetracantha* for its diuretic potential. Individual separation of chemical properties of this plant and undergo detailed scientific study will make it possible to understand the exact diuretic mechanism.

Keywords: Siddha medicine, diuretic, Saluretic, Natriuretic, *Azima tetracantha*, Mutsankan Chooranam,

INTRODUCTION

Diuretics are one of the group of medicines that make the kidneys permit the passage of excess water and salt, thereby increasing the elimination of salt and water by stimulating the formation of urine. This vital process prevents excess accumulation of water and concentration of Sodium in tissues. Due to this mechanism, diuretics are essential drugs in the initial management of hypertension by decrease afterload thereby reducing plasma volume, thus decreasing blood pressure ¹, oedema associated heart failure, portal hypertension and some renal pathologies. Most diuretics, in addition to promoting the elimination of water and sodium (salt), also lead to the elimination of potassium. If the amount of serum potassium level is too low it may lead to some effects such as leg cramps, dry mouth and even cardiac arrhythmias. So research still continues to find out more potent and less or no side effects diuretics in herbal medicines ².

There are so many single herb, poly herbal formulations in Siddha system of medicine that were traditionally used as diuretics. These preparations promote urinary secretions, the elimination of toxins and prevents fluid retention in oedema and helps to lower the blood pressure. However, only a relatively few herbs have been evaluated for its possible diuretic effects. One of the potent diuretic plant mentioned in Siddha classical literature is '*Azima tetracantha*'. It is an ornamental plant belonging to the family Salvadoraceae. It is known as Needle Bush or Mistletoe Berry

thorn in English; Mutsangan in Tamil. It is a rambling shrub with spines. The margin of the leaves is thickened and found throughout the east coastal region of south India and Sri Lanka. Leaves, root, root bark and latex from root bark are used to treat oedema, eczema and rheumatoid arthritis ³.

Even though this plant is used in Siddha system of medicine for its diuretic effect, there is no scientific validation done so far. So, the present work was carried out to validate the diuretic effect of Mutsankan chooranam (*Azima tetracantha*) including electrolyte excretion in animal model.

MATERIALS AND METHODS

Collection and Identification of Plant materials

Fresh leaves of *Azima tetracantha* was collected from different localities of Chennai and Chengalpeta and was identified taxonomically comparing it with specimens of the herbarium by botanist of Government Siddha Medical College and the specimen kept in the Gunapadam (Pharmacology) department for future reference.

Preparation of Aqueous extract of Mutsankan Chooranam

After identification and authentication, leaves were cleaned well and dried in the shade for a week. After that the plant material was made into a chooranam (fine powder) form by using stone

mortar. And then it was filtered by thin white cloth to facilitate extraction and labelled as MSC.

20 g powdered material of MSC mixed with 100 ml of sterile distilled water in a flask. The contents of the flask were shaken thoroughly to get homogenous mixture and kept for four days. After filtering and concentrating the aqueous extract in lyophilizer, a residue of 1.96 g was obtained (yield of 9.8%)⁴. The extract was dissolved in distilled water and were given orally to animals.

Pharmacological activity

Animals

The diuretic activity of the lyophilized aqueous extract of MSC were assayed using 24 Male Wistar albino rats procured from C. L. Baid Metha College of Pharmacy, Chennai-97 weighing between 240 to 300 grams. All animals were maintained for the duration of the experiment in a room with controlled water and fed at a natural light regime. The study was conducted in accordance with CPCSEA guidelines and the study was approved by the IAEC (Approval no: IAEC/XLIV/48/CLBMCP/2016).

Evaluation of Diuretic Activity

Diuretic study was carried out by Lipchitz method⁵. All experimental animals were fasted and deprived of water for eighteen hours prior to the experiment. They were randomly divided into 4 groups with 6 animals each. They were housed in polyethylene cages with metal grill at ratio of 6 animals per cage. On the day of experiment 4 Groups of 6 animals each randomly selected. Group I received 25ml/kg of 0.9% Sodium Chloride solution and served as negative control. Group II received Furosemide at the dose of 10 mg/kg body weight as a positive control. Group III and Group IV treated with the trial drug Mutsankan Chooranam (MSC) at the doses of 100mg/kg and 200mg/kg of body weight respectively⁶. Both the standard and trial groups, the drugs were suspended in 2ml/kg normal saline. All solutions were administered orally using intra gastric tube. During the period food or water was not provided to the animals. After administration of saline, standard and test drugs, all the rats were placed in individual metabolic cages (six animals in each cage) at the room temperature of 25± 0.5°C.

Measurement of Urine parameters

The diuretic effect of the trial drugs MSC100 and MSC200 was evaluated by collecting the total urine volume up to 5 hours after dosing. Urine pH of each urine samples were measured by using a pH-meter. Urinary excretion of sodium and potassium were measured by flame photometry^{7,8} and Chlorine concentration was estimated by titration with Silver nitrate solution (N/50) using 1 drop of 5% potassium chromate solution as indicator⁹.

Diuretic index of the standard and test groups were calculated by the following formula.

Diuretic index = Urine volume of treated group / Urine volume of the control group

Saluretic index and Na⁺/K⁺ Ratio were calculated with standard formula.

Saluretic, natriuretic and carbonic anhydrase inhibition

The sum of Na⁺ and Cl⁻ urinary excretion was calculated as a parameter of saluretic effect. The ratio Na⁺/K⁺ was calculated for natriuretic effect. Carbonic anhydrase inhibition effect was calculated by the estimation of ratio Cl⁻ / (Na⁺ + K⁺)¹⁰.

STATISTICAL ANALYSIS

The results were expressed as arithmetic mean ± SEM. Statistical analysis was performed using the one-way analysis of variance and subsequently followed by Dunnet's multiple comparison test. P values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

The diuretic effects of aqueous extract of MSC were evaluated in wistar albino rats as per Lipchitz method as shown in Table and Table 2. Results of Table 1 showed the urine volume up to 5 hours and other diuretic parameters include pH and diuretic index of all tested groups. Table 2 showed the effect of aqueous extract of MSC on electrolyte excretions such as concentration of sodium, potassium and chloride, saluretic index and Na⁺/K⁺ ratio of all the test groups and control group. Table 3 Showed effects on natriuretic, saluretic and carbonic anhydrase inhibition of Mutsankan Chooranam.

Effect on urine volume, pH and diuretic index

Results showed that aqueous extracts of Mutsankan Chooranam (MSC) increased the total volume of urine measured at 5 hours. The diuretic action of MSC extracts at doses of 100 and 200 mg / kg is found in a range that varies between 3.91 and 5.39, evidencing that the highest value is obtained at a higher dose which indicates dose-dependent activity. Though the result with 200 mg / kg of MSC extract is slightly lower than the reference diuretic drug furosemide (6.68), the diuretic effect of MSC 200 showed highly significant diuretic action.

Urinary pH was measured after 5 hours of experiment. The urinary pH of control rats was 6.32 ± 0.11. The urine pH of MSC100 and MSC 200 treated group was 6.92±0.17 and 7.10±0.05 respectively at 5 h urine sample. The standard drug (Furosemide) increased the pH (7.16±0.13). Both MSC 200 group and standard group making the urine slightly alkaline.

The diuretic index, which indicates the diuretic potential of any substance, is considered to be good if the values are greater than 1.50, moderate if the values are between 1.00 and 1.50, mild if the values lie between 0.72 and 1.00, and nil if the value is <0.72.^{11,12}

The diuretic index of MSC 100 and MSC 200 was 1.40 and 1.93 respectively which indicates that the trial drug Mutsankan Chooranam possess moderate and good diuretic potential with dose-dependent relationship. The standard drug furosemide also indicates its good diuretic potential (2.39).

Effects on electrolyte excretion

The urinary excretion of electrolytes after 5 h administration of MSC 100 and MSC 200 significantly increased when compared to the control group which is shown in (Table.2). However, when analyzing the dose of 100mg and 200 mg / kg of MSC, it is evident that the higher dose of MSC produced greater elimination of electrolytes. The elimination of all the above electrolytes produced by the standard drug furosemide is slightly higher than that of the trial drug.

The Na⁺ / K⁺ ratio for all the experimental groups treated with aqueous extracts of MSC 100 and 200, reference drug furosemide showed values similar to that of control group suggesting that the trial drug tested increase the excretion of potassium similar to that of furosemide which allows to infer that the mechanism through which MSC extracts can exert their action, is similar to the way loop diuretics work.

Table 1. Effect of aqueous extract of MSC on urine output, pH and diuretic index in rats.

Groups	Urine volume	pH	Diuretic index (Test/Control)
Control	2.80±0.09	6.32±0.11	--
Standard	6.68±0.13**	7.16±0.13	2.39
MSC 100mg/kg	3.91±0.08*	6.92±0.17	1.40
MSC 200mg/kg	5.39±0.23**	7.10±0.05	1.93

The data is expressed as Mean ± SEM.; ANOVA followed by Dunnet's Multiple comparison test. *P<0.05, **P<0.01, ***P<0.001 vs control; n=6

Table 2. Effect on urinary electrolyte excretion, Saluretic index and Na⁺/K⁺ ratio

Groups	Electrolyte excretion (mEq/L)			Electrolyte index		
	Na ⁺	K ⁺	Cl ⁻	Na ⁺	K ⁺	Cl ⁻
Control	82±1.5	50.5±1.8	95.7±2.1	1.00	1.00	1.00
Standard.	142.3±3.2**	85.7±1.9**	176.7±2.5***	1.74	1.70	1.85
MSC 100	116.8±2.4*	71.7±2.0*	124.7±1.9*	1.42	1.42	1.30
MSC200	129±1.5*	80.2±1.9**	159.5±2.5**	1.57	1.59	1.67

The data is expressed as Mean ± SEM.; ANOVA followed by Dunnet's Multiple comparison test. *P<0.05, **P<0.01, ***P<0.001 vs control; n=6

Table 3. Effect of Aqueous extract of Mutsankan Chooranam on natriuretic effect, saluretic effect and carbonic anhydrase inhibition

Groups	Saluretic effect	Natriuretic effect	CAI effect	Saluretic index	Natriuretic index	CAI index
Control	177.7±3.02	1.63±0.06	0.72±0.021	1.00	1.00	1.00
Standard	318.2±1.74**	1.66±0.03	0.78±0.03	1.79	1.01	1.08
MSC 100	241.5±0.81*	1.63±0.05	0.66±0.02	1.36	1.00	0.91
MSC200	288.5±3.10**	1.61±0.03	0.76±0.01	1.62	0.98	1.05

The data is expressed as Mean ± SEM.; ANOVA followed by Dunnet's Multiple comparison test. *P<0.05, **P<0.01, ***P<0.001 vs control; n=6

Effect on urine volume, pH and Diuretic index **Fig.1. Showing effect of MSC on urine volume, pH and diuretic index on experimental animals****Effect on urinary electrolyte excretion** **Fig.2. Showing effect of MSC on urinary excretion of Na⁺, K⁺ and Cl⁻ on experimental animals****Electrolytes index** **Fig.3. Showing effect of MSC on electrolytic index on experimental animals**

Effects on natriuretic, saluretic and carbonic anhydrase inhibition

Table 3 showed the results for natriuretic, saluretic and carbonic anhydrase inhibition effects by the administration of trial drug MSC 100, MSC 200 and standard drug Furosemide. Aqueous extract of Mutsankan Chooranam at doses of 100 mg and 200 mg showed marked natriuretic, saluretic and carbonic anhydrase inhibition effect as compared to normal control. The standard

drug furosemide (10 mg/kg) showed potent natriuretic, saluretic and carbonic anhydrase inhibition activity as compared to normal control.

According to literature, it is reported that the trial drug Mutsankan Chooranam is a powerful diuretic given in rheumatism, dropsy, dyspepsia and chronic diarrhoea and as a stimulant tonic after confinement¹³. In herbal therapy, wide ranges of

phytoconstituents were responsible for diuretic activity includes alkaloids, glycosides, tannins, phenolics coumarins, triterpenoids etc. These phytoconstituents present in plant extract desired pharmacological effect on body and thus act as natural diuretic¹⁴. The leaves are found to contain azimine, azcarpine, carpine, narcissoside, friedelin, lupeol, glutinol, β -sitosterol and isorhamnitine-3-O-rutinoside etc^{15, 16}. The seeds contain a complex mixture of 26 flavonoids predominantly as glycosides and acyl-glycosides, with traces of aglycones¹⁷.

Active ingredients present in *Azima tetraacantha* that could all be together or partly responsible for the increase in diuresis, saluretic and natriuretic activity. The diuretic activity observed with *Mutsankan Chooranam* may be due to the flavonoids and alkaloid it contains. Individual separation of chemical properties of this plant and undergo detailed scientific study will make it possible to understand the exact diuretic mechanism which favours the new pathway to explore potent diuretic therapy with *Mutsankan Chooranam*.

CONCLUSION

From this present study it can be concluded that the aqueous extract of *Mutsankan Chooranam* at the dose of 100 mg and 200 mg moderate and marked diuretic effect respectively when compared to the control group. Sodium, Potassium and Chloride concentrations in excreted urine are increased and Na + / K ratio - allowed to compare its action with loop diuretic furosemide, showed significant hypernatraemic, hyperchloraemic and hyperkalaemic diuretic potential. However, it was found that the diuretic effect is lower than that of the standard drug furosemide. There is a clear dose-response relationship based on urinary excretion after administration of the aqueous extract of *Mutsankan Chooranam*. In this experimental model, these actions are most likely due to the presence of alkaloids and flavonoid compounds. So, it is interpreted that this result would validate the diuretic activity of the herb *Azima tetraacantha* in the form of *Chooranam* used in Siddha system of medicine. It is necessary to carry out future detailed phytochemical and pharmacological studies which is required to determine the exact mechanism.

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