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(Research Article)

Diuretic Activity of Siddha Mineral Formulation *Ashta Gunma Thiraavagam* in Rats

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ABSTRACT

Ashta Gunma Thiraavagam (AGT) is a classical Siddha medicine indicated for many renal disorders. The present study was aimed to explore the diuretic activity of AGT in male Albino Wistar rats. The study was undertaken in four groups – control, AGT at two dose levels of 0.5 and 1 ml/kg with Frusemide (20mg/kg) as reference standard was administered orally and the investigation was carried out to evaluate the urine output at different time intervals of 15, 30, 45, 60, 120 minutes and to evaluate the concentration of electrolytes Na^+, K^+, Cl^- ions in the urine at 120 minutes. The results showed that AGT demonstrated with significant diuretic potential in animal model.

Key Words: Albino Wistar rats, *Ashta Gunma Thiraavagam*, Frusemide, Urine output, Electrolyte.

INTRODUCTION

A diuretic is a substance which increases urine and solute excretion. Diuretics are normally required to remove fluid, which is composed of water and solutes of which sodium is the most important [1]. Diuretics are the drug of choice in the treatment of oedema i.e., cardiac oedema, hepatic oedema, renal oedema, pulmonary oedema, cerebral oedema, hypertension, congestive cardiac failure, renal failure and renal calculi etc., They are also useful in the treatment of poisoning of drugs like barbiturates, salicylates with their forced diuresis action[2].

Generally, synthetic diuretics such as Loop diuretics and Thiazide like diuretics accomplish the demand. Prolonged usage of diuretics often result in hypokalaemia, hyponatraemia, hypovolaemia, hypotension, dehydration and some may cause hyperglycemia, hyperlipidaemia, GIT disturbances, allergic rashes [3].

The Siddha system of Medicine, with vast heritage is enriched with many formulations which enhance urine output and indicated in the treatment of renal disorders, hepatic disorders like ascites and jaundice. This traditional treasure has to be validated scientifically to approach the global community. Hence an attempt had been made to study the diuretic effect of *Ashta Gunma Thiraavagam (AGT)* in Wistar albino rats.

Thiraavagam is one among the thirty two types of internal medicines being used in the Siddha Medicine from time immemorial [4]. *Thiraavagam* refers to an acidic liquid preparation obtained by the process of destructive distillation of salts and alkalies with or without any addition of fluids in a peculiar distillation setup called *Vaalaiyanthiram* [5]. *Thiraavagam* has peculiar properties, where little dosage of the drug and its easy absorption will increase the curative aspect of the condition to a greater extent.

As per Siddha literature, *Anuboga Vaithya Navaneetham* - Volume 3, *Ashta Gunma Thiraavagam* is a kind of medicine made from combination of six salts namely Salt petre, Alum, Borax, Sal ammoniac, Alkaline earth salt and Sapo mollis[6]. *Ashta Gunma Thiraavagam* is a traditionally used Siddha preparation which enhances elimination of excess body fluids as in oedema, ascites, urinary disorders. This medicine, a combination of six salts made under traditional method of preparation and with varied indications is not yet studied scientifically. This study deals with the scientific approach of AGT, to prove its diuretic effect with bio-chemical parameters in animal model.

MATERIALS AND METHODS

Ashta Gunma Thiraavagam is a classical Siddha medicine, mentioned in *"Anuboga Vaithya Navaneetham"*. The raw drugs of *Ashta Gunma Thiraavagam* were obtained from the country drug shop at Chennai, Tamilnadu, authenticated at the Department of Gunapadam (Pharmacology), Government Siddha Medical College, Arumbakkam, Chennai. The raw drugs required for the medicine were *Vediuppu* (Potassium nitrate), *Padigaram* (Common Alum), *Vengaram* (Borax), *Navacharam* (Ammonium chloride), *Pooneeru* (Fuller's Earth) and *Savukkaram* (Sapo mollis).

Purification of Raw Drugs

The raw drugs were subjected to '*Suddhi*' (purification process) as per Classical Siddha text [7].

Salt petre was soaked in lemon juice and then dried in sunlight until the moisture content is lost. Common Alum was dissolved in pure water, filtered and allowed to boil in a pan. When it attains a thick molten consistency, it is allowed to cool. Borax was allowed to heat on a pan to complete dehydration. Sal ammoniac was ground with cow's urine and allowed to dry. Alkaline earth salt was dissolved in 4 times of fresh water, stirred well, allowed to remain for sometime decant the clear supernatant liquid in flat porcelain vessels, then it is allowed to boil till all the moisture evaporates. The earthy portions were discarded. The dried quintessence thus got above was subjected to the same process for ten times. Sapo mollis was steamed in cow's urine, and then allowed to dry. All these purified materials were kept in a glass container separately and these materials were used for the preparation of trial drug *Ashta Gunma Thiraavagam*.

Preparation of *Ashta Gunma Thiraavagam*

The procedure for the preparation of the drug as described in the classical text was strictly adhered. ***Ashta Gunma Thiraavagam*** was prepared by distillation process in the following way.

Purified Salt petre (280 gms), Purified common Alum (105 gm), Purified Borax (21 gm), Purified Alkaline earth salt (10.5 gm), Purified Sal ammoniac (10.5 gm), Purified Sapo mollis (8.4 gm) were ground well and transferred to the *Vaalaiyanthiram* made of earthen distillation set up and intensely heated . During the process of heating, the salts decompose completely releasing the acidic fumes and then they get condensed at the condenser submerged in cold water and collected at the receiver vessel kept adjacently. Then, it was stored in an air tight glass container.

PHARMACOLOGICAL ACTIVITY

Animals

Healthy male Wistar rats weighing between 180-220 g were obtained from the Vel's College of Pharmacy, Chennai. The animals were acclimatized to standard laboratory conditions (temperature: 25±2°C) and maintained on 12-hour light: 12-hour dark cycle. They were provided with regular rat chow and drinking water *ad libitum*. The study was conducted in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals) guidelines and the study was approved by IAEC (Institutional Animal Ethical Committee) with Approval number: XIII / VELS / PCOL 21 / 2000 / CPCSEA / IAEC / 08.08.2012.

Evaluation of Diuretic activity

Diuretic study was carried out by Lipchitz method [8]. In this method 24 male Albino Wistar rats, weighing about 180-220 gm were taken. The animals were divided into four groups each containing six rats (n = 6). Rats were kept for 18 hours fasting, before the study.

Frusemide (20 mg/kg) and *Ashta Gunma Thiraavagam* (0.5 and 1ml/kg) were used as reference standards and were dissolved in saline solution for administration, while normal saline (25 ml/kg) was used as a vehicle. The Group I was given normal saline, served as control. Group II and Group III received the trial drug AGT at the doses of 0.5ml/kg and 1ml/kg respectively. Group IV received Frusemide (20 mg/kg) and served as standard. *Ashta Gunma Thiraavagam* (0.5 and 1ml/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 doses of *Ashta Gunma Thiraavagam* 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. During the period, the animals were not provided with food or water. Further, care was taken to avoid the contamination of urine with the faeces. The urine was collected in measuring jars at different time intervals. The total volume of urine collected was measured for all the groups. The bodyweight (before and after test period), total urine volume, urine sodium and potassium concentrations were taken from individual rat and measured by using flame photometry [9, 10]. Chlorine concentration was estimated by titration of silver nitrate solution (N/50) by using one drop of 5% potassium chromate solution as indicator[11].

Statistical Analysis

All results were expressed as mean ± standard error. The data was analyzed statistically using ANOVA followed by Dunnet's Multiple Comparison Test. P value less than 0.05 was considered as statistically significant.

Table-1: Effect of *Ashta Gunma Thiraavagam* on urine output in rats

Group	Treatment	Urine volume at different time intervals (in ml)				
		15 min	30 min	45 min	60 min	120 min
Control	Normal saline (25 ml/ kg)	0.24±0.2	0.52±0.4	1.01±0.3	1.56±0.4	2.12±0.4
Test 1	AGT 0.5ml/kg	0.21±0.3	0.50±0.2	1.15±0.5	1.42±0.5	2.23±0.5
Test 2	AGT 1ml/kg	0.20±0.2	0.48±0.3	1.42±0.4	2.05±0.5	4.38±0.5*
Standard	Frusemide (20 mg/ kg)	0.22±0.2	0.64±0.2	2.28±0.5	3.55±0.6*	6.10±0.8**

*Values are expressed as mean ± SEM, * p< 0.05, ** p< 0.01 when compared to normal saline (control) (Dunnet's multiple comparison test)*

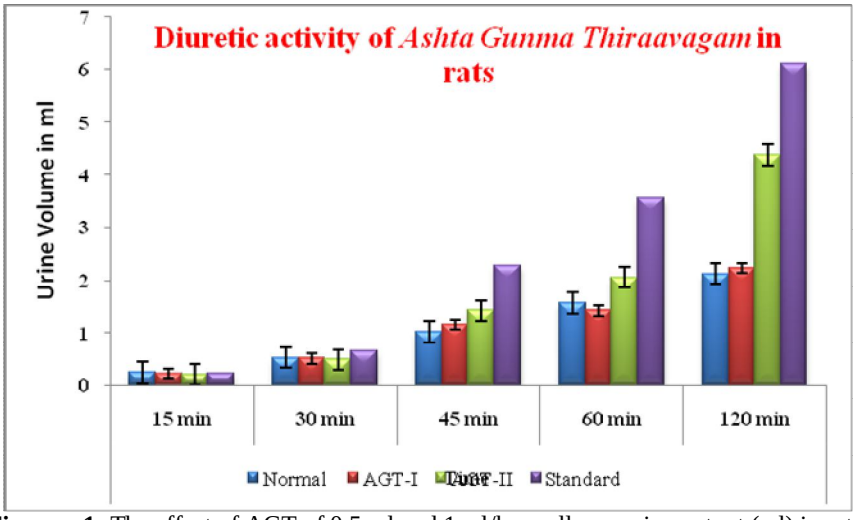

Figure – 1: The effect of AGT of 0.5 ml and 1 ml/kg orally on urine output (ml) in rats.

Table 2: Effect of *Ashta Gunma Thiraavagam* on electrolyte levels in urine

Group	Treatment	Sodium (mMol/l)	Potassium (mMol/l)	Chloride (mMol/l)
Control	Normal saline (25 ml/ kg)	52.41±1.69	92.11±2.30	10.24±1.14
Test 1	AGT 0.5ml/kg	92.40±3.02**	98.16±2.52	12.00±0.85
Test 2	AGT 1ml/kg	94.23±2.53**	100.22±3.50	12.17±1.00
Standard	Frusemide (20 mg/ kg)	104.61±3.40**	127.54=5.12**	14.31±1.22*

Values are mean ± SEM, * p< 0.05, ** p< 0.01 when compared to normal saline (control) (Dunnet's multiple comparison test)

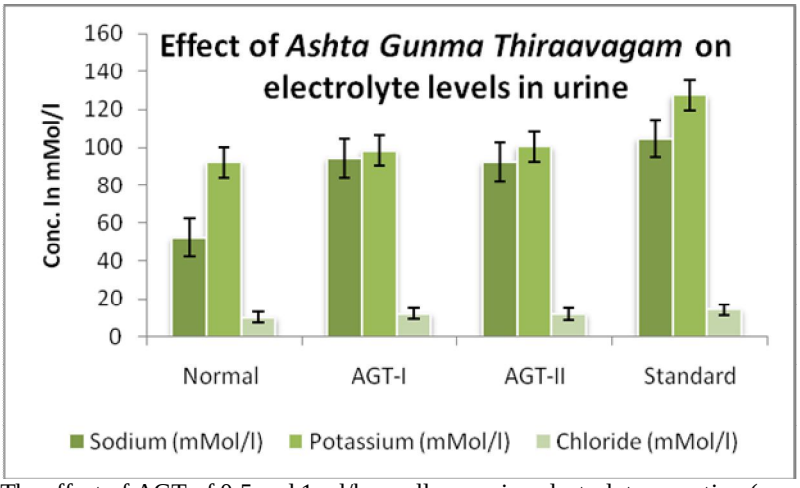

Figure – 2: The effect of AGT of 0.5 and 1 ml/kg orally on urine electrolyte excretion (mmol/L) in rats.

RESULTS AND DISCUSSION

As per acute toxicity study, AGT at the dose of 0.5 and 1ml per kg body weight was selected as median therapeutic dose for the study. The action of *Ashta Gunma Thiraavagam*, standard drug Frusemide and control group, on urine output and electrolyte levels were studied to exhibit the diuretic activity. The study was carried out in albino Wistar rats and the results were listed in Table 1 and Table 2.

Table 1, shows the urine volume collected at different time intervals 15, 30, 45, 60, 120 minutes in control group, trial drug AGT(treated orally at the dose levels of 0.5 and 1 ml/kg) and standard drug Frusemide. Table 2, shows the electrolyte excretion of Na^+, K^+ and Cl^- ion concentration in mMol/L, in the urine collected at 120 minutes.

Effect on Urine volume

The Frusemide treated rats showed a significant increase in volume of urine 6.10 ± 0.8 when compared to control group. The AGT treated rats at different dose levels of 0.5 and 1 ml/kg exhibited diuretic action as follows. AGT given rats at the dose of 0.5 ml/kg produce insignificant diuretic action, but the dose level of 1 ml/kg shown statistically significant increase in urine volume ($p<0.01$) which was found to be 4.38± 0.5 at 120 minutes. But, AGT at the dose of 1 ml/kg produced lesser diuretic effect when compared to the standard drug Frusemide (6.10± 0.8). The effect of drug was much pronounced at 120 minutes in all the groups. The drug AGT showed a dose-dependent increase in excretion of

urine. From the above results, it is inferred that AGT shown diuretic activity by increasing urine volume, and was found to be dose-dependent compared to control group and produced significant diuretic activity with the high dose of 1ml/kg than the lower dose.

Effect on Urinary electrolyte excretion

The effect of standard drug Frusemide and different doses of AGT on electrolyte excretion, Na^+, K^+ and Cl^- is shown in Table 2. The trial drug AGT shown moderate diuretic activity, by increasing Na^+, K^+, Cl^- excretion, compared with the standard drug Frusemide. The trial drug AGT at the dose level of 1ml/kg showed increase in electrolyte excretion compared to 0.5 ml/kg (Na^+ - 92.40 ± 3.02, K^+ - 100.22 ± 3.50, Cl^- - 12.00 ± 0.85). This was found to be relatively less when compared to standard drug (Na^+ -104.61 ± 34, K^+ - 127.54= 5.12, Cl^- - 14.31± 1.22).

However the study revealed that AGT at the dose level of 1 ml/kg excreted mild level of potassium (K^+ - 100.22 ± 3.50) when compared to the standard drug (127.54 – 5.12). The K^+ loss that occurs with many diuretics may lead to hypokalaemia. For this reason, generally potassium-sparing diuretics are recommended. In the present study, AGT showed less effect on K^+ in urine, which may decrease the risk of hypokalaemia. Further, AGT at the dose levels of 0.5 ml/kg and 1 ml/kg exhibited Cl^- excretion near to the standard drug Frusemide. AGT 1 (12.17 ± 1.00), AGT 2 (12.00± 0.85) and Frusemide (14.31 ± 1.22).

CONCLUSION

From the above study, it is observed that *Ashta Gunma Thiraavagam* is a moderate diuretic with dose- dependent action. Although the drug AGT produced moderate effect when compared to the standard, it proves the claim of the drug *Ashta Gunma Thiraavagam* as a potassium sparing diuretic. Further studies should be carried out to exhibit the mode of action of AGT on blood flow, loop permeability, anti- diuretic hormone secretion, tubular reabsorption which paves the way of new safe potassium sparing diuretic from the traditional medicine.

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