Original Article

A comparative diuretic evaluation of fruit and root of *Gokshura* (*Tribulus terrestris* Linn.) in albino rats

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Abstract

Background: *Gokshura Moola* (root of *Tribulus terrestris* Linn.) is one among the ingredients of *Dashamoola,* a group of ten medicinal plants principally comprising roots as the useful part. In practice instead of root, fruit of *Gokshura* is widely used in most of the preparations of *Dashamoola* in Kerala. *Dashamoola* occupies a significant role in a wide range of Ayurvedic formulations and holds a major share in the drug manufacturing industry. This high demand of *Dashamoola,* leads the use of fruit instead of its root and implies the need to compare the efficacy of root and fruit of *Gokshura*. **Aim:** This study is planned to assess whether fruit of *Gokshura* can be substituted for its root using the parameter of diuretic activity in Wistar albino rats. **Materials and methods:** Wistar albino rats were divided in to four groups. The group I control group and group II standard group was orally administered with carboxymethyl cellulose 2% in normal saline and furosemide (20 mg/kg) respectively. Group III was administered orally with decoction of *Gokshura* root and groups IV with *Gokshura* fruit decoction, with a dose of 8.64 ml/kg. The diuretic effect was evaluated by measuring urine volume, Na^+, K^+ and Cl^- ion content in urine. The results were analyzed by applying one-way ANOVA and LSD Post hoc pairwise comparison test. **Results:** Both test drugs in group III and group IV provided significant increase in urine output when compared to the control group ($P < 0.001$). Decoction of *Gokshura* root provided a significant increase in comparison to decoction of *Gokshura* fruit in regards of sodium ($P < 0.01$), potassium ($P < 0.001$), and chloride ion ($P < 0.05$) excretion. **Conclusion:** Diuretic action of both root and fruit of *Gokshura* is similar in terms of urine volume, but root is more effective in the basis of ionic excretion. Hence, while treating patients suffering from ionic imbalance, it is better to use fruit of *Gokshura* for protecting the ionic balance during diuresis. In all other conditions, root can be used for a better diuretic activity.

Keywords: *Dashamoola*, diuretic effect, *Gokshura*, *Tribulus terrestris* Linn.

Introduction

Dashamoola (group of ten medicinal plant roots) is a combination of five roots of herbs/shrubs (*Laghu Panchamoola*) and five roots of trees (*Brihata Panchamoola*). Among them, *Gokshura* (*Tribulus terrestris* Linn) is a herb of which the useful part is its root and fruit but the selection of plant part for formulation is still a controversy. In Kerala instead of roots, the fruits of *Gokshura* are being used in the preparations of *Dashamoola*.[1]

Dashamoola is a major ingredient of Ayurvedic formulations and has a demand of about 100 tons above annually. Even though the use of fruit is a wise decision to avoid the extinction of the plant by the abundant use of root, therapeutic efficacy needs to be verified. Hence, here comes the need to compare the root and fruit of *Gokshura* pharmacologically and to clarify whether there is any difference or which is more potent or to provide a scientific support about the usage of fruit and root of *Gokshura* for *Dashamoola* preparation.

The source plant of *Gokshura* is *T. terrestris* Linn. of Zygophyllaceae family. In Ayurvedic Pharmacopoeia of India, both root and fruit and the whole plant of *Gokshura* are indicated for medicinal preparations.[2] An ancient medical text *Charaka Samhita* has identified it as the best drug for

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"*Mootrakricha-Anilaharaanaam*" (obstructive uropathy).[3] *Charaka Samhita* has included this drug under the group of ten herbs viz. *Shwayathuhara* (anti-inflammatory), *Mootra Virechaneeya* (diuretic), and *Krimighna Mahakashaya* (anthelmintic).[4,5] *Sushruta Samhita* included *Gokshura* under, the plant groups such as *Dashamoola, Laghu Panchamoola* and *Kantaka Panchamoola*.[6] In Ayurveda classics, *Gokshura* is said to be useful in the treatment of *Mutrakrichha (*dysuria), *Madhumeha (*diabetes*), Ashmari (*renal calculi*), Hridroga (*cardiac diseases*),* etc.[7]

Since the drug *Gokshura* has its main action on *Mootravaha Srotasa* (urinary system*)*, for the comparative evaluation of the efficacy between the root and fruit, the best choice will be the analysis of diuretic action.

Materials and methods

Experimental animals

Wistar strain albino rats (150–300 g) of either sex were obtained from the pharmacology laboratory of Government Ayurveda College, Thiruvananthapuram. They were grouped and housed in polyacrylic cages (three animals per cage) and maintained under standard laboratory conditions. They were fed commercial rat feed and water *ad libitum*. The animals were acclimatized one week before experimentation period. The animals were exposed to 12-h light and 12-h dark cycle with the relative humidity at 50%–70% and the ambient temperature 22 ± 3°C during the experimental period. All animals were kept in the same environmental conditions. The experimental protocols were approved by the Institutional Animal Ethical Committee (Order No.: IAEC NO27/IAEC/AVC/2015) in accordance with the guidelines formulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals, India.

Procurement and preparation of test drug

The mature root and fruit of *T. terrestris* Linn. were collected during summer season, by the mid of May 2016 from Shanghumugham beachside in Thiruvananthapuram district, Kerala. The root and fruit of *T. terrestris* Linn. were cut into small pieces, washed, shade dried, and coarsely powdered. Forty-eight grams of coarsely powdered drug was mixed with 768-ml water and reduced to 96 ml according to the *Kashaya* (decoction) preparation procedure mentioned in *Sharangadhara Samhita.*[8]

Dose fixation

The dose for the experimental study was calculated by extrapolating the human dose to animal dose based on the body surface area ratio using the table of Paget and Barnes:[9]

Human dose of *Kashaya* = 96 ml/day

Rat dose = 96 × 0.018 (Paget and Barnes conversion factor)

=1.728 ml/200 g rat weight (8.64 ml/kg).

Preparation of standard drug

The standard drug furosemide (Lasix 40 mg batch no. 6NA0082) was taken as 20 mg/kg human dose.[10] The standard drug was dissolved (mg/ml/kg) in 2% carboxymethyl cellulose (CMC) solution which was prepared with normal saline.

Root of administration

Oral route using feeding cannula sleeved to the disposable syringe.

Grouping of animals

The acclimatized 24 Wistar strains of albino rats were weighed and grouped into four with six animals in each group. Selection was done randomly so as to assure equal distribution of gender, body weight, etc., in each group. Then, the animals were marked for proper identification and each group was kept in separate cages. Each cage was labeled separately for group identification. Group I was control group (CMC 2% in normal saline), group II – standard group, furosemide (20 mg/kg), group III – test group, *T. terrestris* Linn. root *Kashaya* effective dose (TTRED) and group IV – test group, *T. terrestris* Linn. fruit *Kashaya* effective dose (TTFED).

Diuretic activity

The diuretic effect was carried out as per the modified method of Lipschitz Test.[11] All the rats were withheld for food and water 18 h before the experiment. They were hydrated with 5 ml/kg of distilled water before drug administration to ensure uniform hydration. Immediately after oral drug administration, the animals were placed in metabolic cages provided with a wire mesh bottom and a funnel to collect the urine. [Figure 1] Stainless steel sieves were placed in the funnel to retain feces and to allow the urine to pass. The tip of funnel was put into a graduated beaker for proper measurement of urine. The urine was collected in measuring jar up to 5 h after the dosing. In this method, diuretic activity was evaluated by a single-dose drug administration. Volume of urine was measured and sample was analyzed for ions Na^+, K^+, and Cl^-.

Statistical analysis

The data on urine volume, Na^+, K^+, and Cl were collected for the control group, standard group, and two test groups.

Figure 1: Metabolic cages for urine collection

Descriptive statistics such as mean and standard deviation were calculated and statistically tested using one-way analysis of variance (ANOVA). Least significant difference *post hoc* pairwise comparison was carried out for mutual comparison of groups. A calculated "*P*" < 0.05 was considered to be statistically significant.

Results

Results [Table 1] showed a significant difference in the urine volume, sodium, potassium, and chloride ion concentration at the end of 5th h ($P < 0.001$) with respect to each group. On mutual comparison between groups, [Table 2] The root and fruit of *Gokshura* showed a significant increase in urine output when compared to the control group ($P < 0.001$). The comparison of the test groups (root and fruit *Kashaya*) did not show any significant difference in excreted urine volume.

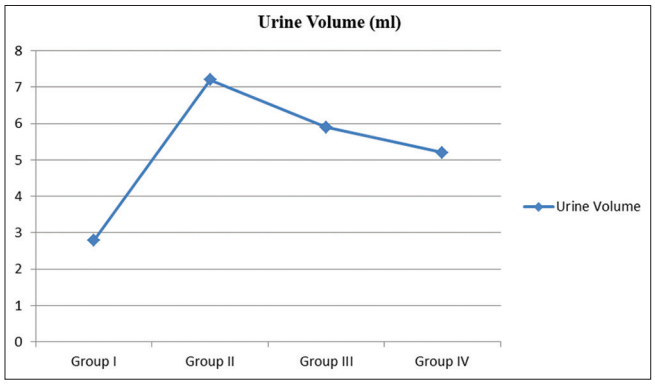
The concentration of excreted sodium ion level in urine was almost equal in the control group and TTRED. However, on comparison of the test groups, there showed a significant increase in sodium-ion excretion in TTRED was found ($P < 0.01$).

The group TTRED showed a significant increase in potassium ion excretion when compared to the control group ($P < 0.001$). In the case of chloride ion excretion, TTRED showed statistically high significance ($P < 0.001$) when compared to the control group than TTFED ($P < 0.05$). On comparison, TTRED showed a significant increase ($P < 0.001$) in comparison to TTFED in potassium and chloride ion excretion. Graph 1 and Graph 2 displays a comparative line diagram showing amount of excreted urine volume(ml) in four groups and Na+, K+, and Cl$^-$ in four groups respectively.

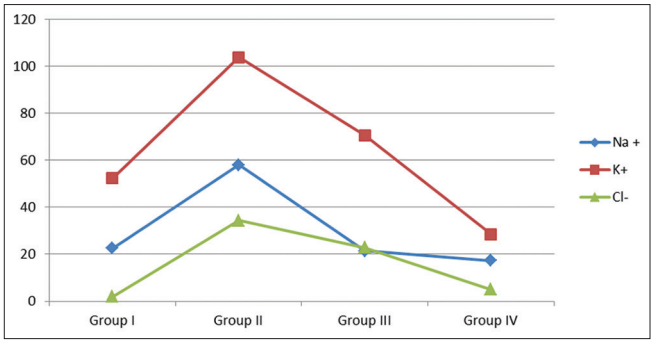
Discussion

Gokshura (*T. terrestris* Linn.) is a known diuretic herbal drug that is used in many Ayurvedic preparations and is a component of *Dashamoola,* an Ayurvedic combination used in the treatment of various diseases. Even though root is a useful part in *Dashamoola* as per classical references, only dried fruits are used for the manufacturing of formulations of *Gokshura*. However, only the fruits of *Gokshura* are available in the raw drug market, and none of the samples examined contain roots of the plant.

On considering *Rasapanchaka* (five principles of drug action), *Gokshura* possess *Madhura Rasa* (sweet taste), *Snigdha* and *Guru Guna* (unctuous and heavy attributes), *Shita Veerya* (cold potency), and *Madhura Vipaka* (sweet



Graph 1: Comparative line diagram showing urine volume in four groups. X-axis – Four study groups; Y-axis – Measurement of urine volume in ml. Group I – Control group (CMC 2% in normal saline). Group II – Standard group, Furosemide (20 mg/kg). Group III – Test group, TTRED. Group IV – Test group, TTFED. TTRED: *Tribulus terrestris* root *Kashaya* effective dose, TTFED: *Tribulus terrestris* fruit *Kashaya* effective dose



Graph 2: Comparative line diagram showing concentration of Na$^+$, K$^+$, and Cl$^-$ in four groups. Na, K, and Cl ion (mEq/ml). X-axis – Four study groups; Y-axis – Measurement of excreted ions in mEq/ml. Group I – Control group (CMC 2% in normal saline). Group II – Standard group, Furosemide (20 mg/kg). Group III – Test group, TTRED. Group IV – Test group, TTFED. TTRED: *Tribulus terrestris* root *Kashaya* effective dose, TTFED: *Tribulus terrestris* fruit *Kashaya* effective dose, CMC: Carboxymethyl cellulose

Table 1: Descriptive statistics and test of significance (ANOVA) for the comparison of urine volume sodium, potassium, and chloride

Group	Urine volume (ml/5 h)	Na$^+$ (mEq/L)	K$^+$ (mEq/L)	Cl$^-$ (mEq/L)
I. CMC 2% in normal saline	2.80±0.57*	22.5±0.56*	52.4±0.70*	2±0.70*
II. Furosemide (20 mg/kg)	7.20±0.28*	58±0.84*	103.9±1.27*	34.3±1.27*
III. TTRED	5.90±0.14*	21.4±0.42*	70.6±1.83*	22.9±0.14*
IV. TTFED	5.20±0.28*	17.3±0.98*	28.5±1.13*	5±0.14*

All the values are compared with control group and considered significant at *P<0.001. Values given are the AM±SD for all the observations. Na$^+$: Sodium, K$^+$: Potassium, Cl$^-$: Chloride, SD: Standard deviation, CMC 2%: 2% carboxymethyl cellulose, TTRED: *Tribulus terrestris* Linn. root *Kashaya* effective dose, TTFED: *Tribulus terrestris* Linn. fruit *Kashaya* effective dose, AM: Arithmetic Mean

Table 2: Comparison of urine volume, sodium, potassium, and chloride ion among each group

Groups (comparison)	*P*			
	Urine volume	Na^+	K^+	Cl^-
I (control) versus II (standard)	***	***	***	***
I (control) versus III (TTRED)	***	NS	***	***
I (control) versus IV (TTFED)	***	***	***	*
II (standard) versus III (TTRED)	**	***	***	***
II (standard) versus IV (TTFED)	***	***	***	***
III (TTRED) versus IV (TTFED)	NS	**	***	***

*Significant at 5% level ($P < 0.05$), **Significant at 1% level ($P < 0.01$), ***Significant at 0.1% level ($P < 0.001$), NS ($P > 0.05$). NS: Not significant. TTRED: *Tribulus terrestris* Linn. root *Kashaya* effective dose, TTFED: *Tribulus terrestris* Linn. fruit *Kashaya* effective dose

post-digestive effect).[2] *Madhura Rasa* and *Snigdha*, *Guru Guna*, are having *Bhouthika* (element) configuration of *Prithvi* (earth) and *Jala* (water). The *Gokshura* possess *Madhura Vipaka* which is having *Srushta Vit-Mutra* (potent excretion) action. These pharmacological properties, in turn, increase *Snigdha Guna* and *Kleda* (mucoid fluid metabolites) in human body. To reduce the *Kleda,* body will resort to a feedback mechanism, i.e., *Kleda* (fluid) excretion by *Mutra,* and urine output will increase accordingly to maintain the homeostasis. *Rakta Prasadana* (blood purification) is the function of *Shita Veerya*. The main function of kidney is maintaining the homeostasis by filtering *Rakta* and thus keeping a normal electrolyte balance in the body. Hence, administration of *Gokshura* with *Shita Veerya* will augment the function of kidney to accomplish its function. Diuretics are capable of increasing urine output and are useful in the treatment of diseases related with the retention of fluids. In this study, pharmacological evaluation of diuretic action of *Kashaya* of root and fruit of *Gokshura* was evaluated by modified Lipschitz test method using furosemide as a standard drug under controlled laboratory conditions. The diuretic effect was assessed by measuring the urine volume and amount of Na^+, K^+, and Cl^- ion concentration in excreted urine.

When compared with the control group, the standard drug and two test groups (TTRED and TTFED) showed a significant difference ($P < 0.001$) in amount of urine volume excreted. On considering the mutual comparison of root and fruit test groups, they did not show any significant difference, i.e., both TTRED and TTFED showed an almost equal diuretic effect in terms of urine volume.

As the standard drug furosemide is a loop diuretic, it shows increased sodium, potassium and chloride ion excretion through urine. The group TTRED did not show any particular action in sodium-ion excretion as the value was observed similar to that of the control group, but it showed effective potassium and chloride ion excretion. The group TTFED decreased the sodium and potassium output but showed only a mild increase than the control group in chloride ion excretion.

On mutual comparison, the group TTRED showed a significant difference ($P < 0.001$) with TTFED in sodium, potassium, and chloride ion excretion.

The results showed that the root *Kashaya* of *Gokshura* cause an increased amount of excreted urine volume and Na^+, K^+, and Cl^- excretion as compared to fruit *Kashaya* of *Gokshura*. However, it will not lead to electrolyte imbalance as it does not produce Na^+, K^+, and Cl^- ion excretion much like standard drug furosemide (a loop diuretic). In a sense, for simple diuresis, i.e., in patients who are suffering hypokalemia and hyponatremia or those patients who does not want much ionic urine excreton, can choose fruit of *Gokshura* as diuretic agent can be given. In all other conditions, root can be used for a better diuretic activity

Thus, the study revealed that both the root and fruit of *T. terrestris* Linn. possess diuretic action. On comparing both, the root shows more significance than fruit. Hence, *Dashamoola* can be said is better to use root of *Gokshura* at least in formulations containing root groups such as *Dashamoola, Laghu Panchamoola,* and *Kantaka Panchamoola.*

Since *Gokshura* is one among easily cultivable plant in dry sandy coastal lands, the manufactures can easily adopt the usage of *Gokshura* root in classical preparations mentions *Gokshura* in root groups.

Conclusion

The decoction of root and fruit of *Gokshura* showed an increased amount of excreted urine volume and decoction of *Gokshura* root showed more Na^+, K^+, and Cl^- excretion as compared to decoction of *Gokshura* fruit. Diuretic action of both root and fruit of *Gokshura* is similar in terms of urine volume, but root is more effective in the basis of ionic excretion. Hence, while treating patients suffering from ionic imbalance, it is better to use fruit of *Gokshura* for protecting the ionic balance during diuresis. In all other conditions, root can be used for diuretic activity.

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Conflicts of interest

There are no conflicts of interest.

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