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PHARMACOLOGICAL ASSESSMENT OF *ARJUNA GHRITA* FORMULATIONS FOR *MOOTRASANGRAHNIYA* (ANTI-DIURETIC) ACTIVITY IN RATS

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ABSTRACT

The formulations of *Terminalia arjuna* Roxb. Arn. & Wt. bark are highly recommended for cardiac patients. In classics Arjuna is also reported as mutrasangrahniya (Anti-diuretic). However, other properties and uses of Arjuna are not well-known. Therefore, the present study is aimed to assess anti-diuretic activity of Arjuna ghrita on acute and long-term administration in rats to support the traditional claim made on Arjuna. In acute study, the test formulations were administered once while in chronic study test formulations were administered for 7 consecutive days. Furesemide (10 mg/kg/day) was used as reference standard drug. Total urine volume at 6 and 24 hrs, pH, specific gravity and electrolytes excretions were evaluated in acute and chronic phase of study. The results revealed that there was decease in urine volume, pH, and specific gravity and significantly diminish electrolyte excretions in Arjuna ghrita treatment, which is totally reversal to furesemide, a standard diuretic drug denoted that Arjuna ghrita (4.3g/kg, po) was act as anti-diuretic or urinary astringent in albino rats and may be useful in the clinical conditions of nocturia.

KEYWORDS: *Terminalia arjuna* Roxb. Arn. & Wt. Arjuna ghrita, Urinary astringent, Furesemide, Anti-Diuretic activity.

INTRODUCTION

The occurrence of nocturia in older men is high and increases with age: a systematic review on this subject suggest that in men in their age of 70s and 80s are 68.9–93% reported at least 1 void per night and 29–59.3% reported at least 2 voids per night.[1] This high prevalence rate is seen in populations of various ethnicity and nationality throughout the world.[2] It also concerns with the sleeping, sexual activity, depression, mental function, and vitality of human being.[3] The degree of difficulty seems to be associated to sleep quality as it would interfere with slow-wave (deep) sleep.[4] Anti-diuretic therapy may be apt in patients whose nocturia is caused by nocturnal polyuria (NP). In some countries, anti-diuretic therapy with the synthetic analogue of arginine vasopressine, desmopressin, is the only pharmacological therapy which is indicated specifically for nocturia.[5] Considering the adverse effects of modern therapy, traditional system of medicine provide better alternative for such conditions. According to the present scenario it is estimated that 40% of the world populations depends directly on plant based medicine or formulation for their health care.[6]

The Arjuna (*Terminalia arjuna* Roxb. Arn. & Wt.) belonging to family Combrataceae is a well-known medicinal plant and bark is extensively used in Ayurvedic system, particularly as cardiac tonic. The bark is also prescribed in biliousness and sores and as an antidote to poison, and it is believed to have an ability to cure hepatic, congenital, venereal and viral diseases. A decoction of its bark with cane sugar and boiled cow's milk is highly recommended for endocarditis, pericarditis and angina.[7] *T. arjuna* is very flexible drug and used to prepare various Ayurvedic cardio-tonic formulations like Arjuna decoction, Arjuna ghrita, Arjuna kshirpaka, Arjunarista, Kakubhadi churna etc. Further, bark of *T. arjuna* has mootrasangrahniya (anti-diuretic) property.[8,9] In ancient period physician used drugs in crude as well as processed forms as per need. Acharya

charak has stated that Ghrita (Ghee) is a universal carrier and assimilation base of medicament.[10] *T. arjuna* bark extract in Ghrita has significant ability to enhance the antioxidant potential of ghrita and also improved the phytosterol content and self-life of Ghrita. Since, effect of Arjuna ghrita as mootrasangrahniya in experimental studies have not available after extensive literature search hence present study was planned to review the role of Arjuna ghrita on urine formation.

MATERIALS AND METHODS

Procurement of raw drug

The identification of *Terminalia arjuna* Roxb. Arn. & Wt. tree at village- Dhamora, District- Chhatarpur, Madhyapradesh was confirmed by comparing its characters mentioned in various floras. Bark of that tree were collected and coded as sample-A. Another sample of Arjuna bark were collected from raw drug department of Pharmacy, Gujarat Ayurved University, Jamnagar and coded as sample-B. The plant specimens were further identified by Botanical Survey of India, Howrah and certificate has been issued in this concern. Collection of pure cow ghrita (ghee) was personally done from the milkman at Dared, Jamnagar, Gujarat.

Preparation of the Arjuna Ghrita

Arjuna ghrita formulations AGA & AGB were prepared by Arjuna bark decoction and paste of respective bark samples of A & B along with ghrita as per standard procedures described in the texts.[11,12] The paste and decoction of Arjuna bark were mixed together. Cow ghrita was then added, boiled on mild fire and stirred well continuously so that the paste is not allowed to adhere to the vessel. Ghrita paka was done till it fulfills the pakapariksha (tests of medicated ghee). It was cooled, stored in sterile air-tight glass containers, for experimental study.

Animals

Wistar albino rats of either sex weighing 240±20 g were procured from the animal house attached to the Pharmacology laboratory, I.P.G.T. & R.A. Gujarat Ayurved university. The animals were maintained as per standard husbandry conditions in terms of temperature, relative humidity and light cycles. The experiment was carried out after obtaining permission from Institutional Animal Ethics Committee (IAEC/14/2013/28) as per guideline of CPCSEA, India.

Dose

Dose of the drug was fixed by extrapolating the human dose to laboratory animals on the basis of body surface area ratio as per the table of Paget and Barnes (1964).[13] Human dose for *Ghrita* is 1 *Pala* (48g)/day.[14] Rat dose of *Ghrita* was calculated as 4.3 g/kg body weight and administered orally with the help of feeding cannula.

Statistical analysis

The data are expressed as mean ± standard error of mean for six rats per experimental group. One way analysis of variance (ANOVA) was used to compare the mean values of quantitative variables among the groups followed by Dunnett's multiple 't' test and Students 't' test for unpaired data by using Sigma stat software to determine significant difference between groups at $P<0.05$.

Experimental Protocol

The experiment of anti-diuretic activity of Arjuna ghrita was designed as per method of Gillard et al**.** (1971)[15] with modification as per experimental need. The selected animals were divided into three groups, each comprising of three male and three female rats. The group (I) was kept as normal control received distilled water (10 ml/kg, po). Group (II) was kept as standard drug treated with Furesemide (10 mg/kg, po). Group (III) and (IV) were kept as drug treated groups and received Arjuna ghrita sample A (AGA) and sample B (AGB) (4.32g/kg, po) respectively.

The test drugs and distilled water were administered to the respective groups of overnight fasted rats. As normal urine output in the rats are very low (1 to 2 ml/rat per day) hence, to get the measurable quantity of urine, all the rats were administered with distilled water (5 ml/100 g) after 30 minutes of test drug administration and further (2.5 ml/100 g) after 6 hrs. The animals were placed individually in metabolic cages and urine was collected in conical flasks placed below the polythene funnel of the metabolic cages. The urine was collected after 6 hrs and then 24 hrs of drug administration. The collected urine samples were tested for leucocytes, nitrite, urobilinogen, protein, pH, blood, specific gravity, ketone, bilirubine and glucose using urine test kit (Bayer Diagnostics, UK). The urine samples were subjected to quantitative analysis of Na^+ and K^+ (cations) with the help of a flame photometer.[16] The concentration of urine chloride was determined titrimetrically by silver nitrite solution (0.1N), using one drop of 5% ferric alum solution as an indicator.[17]

RESULTS

Qualitative tests are very useful for the gross detection of chemical moiety in test drugs. Results of qualitative tests indicated the presence of alkaloids, steroids, tannins, flavonoids, saponins, carbohydrates, glycosides and triterpinoides in aqueous and methanolic fractions of *T. Arjuna* bark sample A & B. marked amount of alkaloids, tannins and glycosides were detected in sample A than sample B in respective fractions of *T. Arjuna*.

The urine collected at different intervals in test drug treated groups including control showed the absence of leucocytes, nitrite, urobilinogen, protein, blood, ketone, bilirubine and glucose. Effects of test drug on urine volume showed the decrease in urine output in AGA treated group while increase in AGB treated group during acute and chronic administration in rats however values not reach to significant level when compared to control group. Standard drug furesemide produced significant increase in urine volume in comparison to control group (Table 1).

Table-1. Effect of *Arjuna ghrita* on urine volume in rats.

Groups	Acute anti-diuretic study		Chronic anti-diuretic study	
	Urine volume (ml/100g/6hr)	Urine volume (ml/100g/24hr)	Urine volume (ml/100g/6hr)	Urine volume (ml/100g/24hr)
C	0.91±0.11	1.22±0.13	0.95±0.12	1.27±0.14
S	2.45±0.12**[a]	3.18±0.19**[a]	2.62±0.10**[a]	3.16±0.13*[a]
AGA	0.85±0.10	1.11±0.12	0.98±0.31	1.17±0.29
AGB	0.98±0.15	1.27±0.19	1.17±0.13	1.38±0.14

*Data: Mean ± SEM; *P<0.01, **P<0.001 compared with control group (Unpaired 't' test); [a]P<0.01 compared with control group (Anova followed by Dunnett's multiple 't' test).*

Table 2. Effect of *Arjuna ghrita* on specific gravity and pH of urine in rats.

Groups	Acute anti-diuretic study		Chronic anti-diuretic study	
	Specific gravity	pH	Specific gravity	pH
C	1.013±0.002	7.08±0.08	1.012±0.001	7.08±0.08
S	1.006±0.003*[a]	7.91±0.08***[a]	1.006±0.003	8.17±0.10***[a]
AGA	1.006±0.002**	6.91±0.23	1.006±0.002*	6.92±0.20
AGB	1.003±0.001***[a]	6.83±0.21	1.003±0.001***	6.83±0.21

*Data: Mean ± SEM; *P<0.05, **P<0.02, ***P<0.001 compared with control group (Unpaired 't' test); [a]P<0.01 compared with control group (Anova followed by Dunnett's multiple 't' test).*

Table 3. Effect of *Arjuna ghrita* on sodium, potassium and chloride content of urine in rats.

Groups	Acute anti-diuretic study			Chronic anti-diuretic study		
	Sodium (meq/lit)	Potassium (meq/lit)	Chloride (meq/lit)	Sodium (meq/lit)	Potassium (meq/lit)	Chloride (meq/lit)
C	36.81±4.91	176.92±19.89	285.64±08.51	36.81±4.90	176.92±19.89	285.64±08.51
S	57.91±8.98[a]	142.56±31.71	343.48±22.08*	45.36±8.15	280.34±32.69*[a]	330.49±16.73*
AGA	19.13±0.78**	77.78±9.68***[b]	308.07±04.75*	36.38±3.83	122.22±24.64	304.53±04.48
AGB	18.26±1.54**[a]	122.05±27.15	247.87±49.64	33.33±4.36	118.87±26.34	284.46±17.67

*Data: Mean ± SEM; *P<0.05, **P<0.01, ***P<0.001 compared with control group (Unpaired 't' test); [a]P<0.05, [b]P<0.01 compared with control group (Anova followed by Dunnett's multiple 't' test).*

Both samples of *Arjuna ghrita* and furesemide produced significant decrease in specific gravity and non-significant decrease in urine pH during acute and chronic administration in rats when compared to control group (Table 2). Standard drug furesemide produced significant increase in excretion of sodium, potassium and chloride in the urine during acute and chronic administration in rats. Both samples of Arjuna ghrita produced opposite effect as significant retention of sodium and potassium and excretion of chloride when compared to control group. Both samples produced almost similar magnitude of effects except AGA produced more retention of potassium and excretion of chloride during acute administration in rats (Table 3).

DISCUSSION

Bark of *T. arjuna* has mootrasangrahniya (anti-diuretic) properties hence in present study, the Arjuna ghrita an Ayurvedic formulations prepared from different samples of Arjuna evaluated for its anti-diuretic activity in rats. The ICS standardisation report also defines nocturnal polyuria (NP) and 24-h (or global) polyuria since these two types of polyuria are associated with nocturia in a significant number of cases. 24-h polyuria is defined as a urine output exceeding 40 ml/kg bodyweight per 24 h; NP is defined as a nocturnal urine output (including the first morning void) of >33% of the total 24-h voided volume in the elderly person.[18] The present study reveals that the Arjuna ghrita prepared with sample A collected from Dhamora produced non-significantly diminish in urine volume while Arjuna ghrita prepared from both sample A and B significantly shrink the urinary electrolyte excretion. Furosemide inhibits both solute-free water clearance (CH_2O) and the reabsorption of solute-free water, indicating a marked effect in the ascending limb of the loop of Henle.[19] Therefore, the electrolytes get excrete through it. Where the Arjuna ghrita treated group showed retention of electrolytes and ultimately effect of test drug exhibits contrary action of furosemide. The observed pattern is totally divergent to the pattern observed with thiazide diuretics, wherein, increase in sodium, potassium, and urine formation is observed.[20] Thiazides act by decreasing the re-absorption of sodium in the distal convoluted tubule. This occurs due to the inhibition of the Na^+/Cl^- co-transporter on the luminal membrane.[21]

Urine specific gravity decreased after administration of furosemide as well as Arjuna ghrita treated group. These findings indicate that fluid is redistributed from extra- to intravascular compartments after administration of furosemide.[22] Arjuna ghrita also showed similar effect as drug has some effect on the kidneys' ability to concentrate the urine for as long as 6 hours after administration. In general, there is a good correlation between the specific gravity and osmolality of a urine sample. In certain clinical conditions, such as uncontrolled diabetes mellitus, nephritic syndrome, after the administration of intravenous radio contrast material or saline diuresis, dependence upon specific gravity for determining the concentrating ability will result in over- or underestimation.[23]

The *T. arjuna* are rich in secondary metabolites and anti-diuretic activity can be attributed to their high steroids, tannins, terpenoids and saponins.[24,25] The major chemical components found positive in the Arjuna are tannins, alkaloids and glycoside, by qualitative tests.[26] Tannin has extremely water retention effect as it is powerful astringent. In justification of this statement the active principle in *Fuchsia magellanica* is related tannin which is reported as anti-diuretic.[27] Moreover, *Phyllanthus emblica* also contain good amount of hydrolysable tannins and also reported as anti-diuretic.[28,29] On other hand Arjuna contains anthraquinone derivates which is also reported as anti-diuretic.[30, 31] Therefore, some of these components present in the formulation may have played role as anti-diuretic or urinary astringent. This can be beneficial in such clinical conditions like poly urea, diabetes and excessive urine. It is also advisable with certain types of drugs as co therapy to retard adverse effect of frequent urination. In addition to that, As per Ayurveda theory Ghrita has rasayana property, which nourishes the body and repair the degenerative changes as it may immense help to restore the body functions and conditions like nocturia in elder people.[32]

Furthermore, both the formulations did not produce any toxic effect on renal system in stipulations of serum urea, creatinine, leucosites, nitrite, urobilogen, protein, pH, blood, specific gravity, ketone, bilirubine and glucose. These annotations suggest that Arjuna ghrita may be safe for regular consumption.

CONCLUSION

From the present study, it is concluded that Arjuna ghrita has produced anti-diuretic activity in rats which support the traditional claim made on Arjuna as mutrasangrahniya (Anti-diuretic) property. Hence, it can be suggests the use of Arjuna ghrita as anti-diuretic or urinary astringent and may be useful in the clinical conditions of nocturia.

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