ANTIUROLITHIATIC ACTIVITY OF AQUEOUS EXTRACTS OF *ASPARAGUS RACEMOSUS WILLD.* AND *TAMARINDUS INDICA* LINN. IN RATS

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Summary

In the present study, aqueous extract of *Asparagus racemosus* roots and aqueous extract of Tamarindus indica fruit pulp were evaluated for their antiurolithiatic potential in albino rats of Wistar strains. The stones were produced in this study by zinc disc foreign body insertion technique supplemented with 1%ethylene glycol, which is commonly used in these type of studies. The weight of the stones was used as criteria for assessing the preventive or curative effect of the two plants. Two doses of extract for prophylactic and curative groups were used. In both groups the administration of the drugs have resulted in reduction in the weight of stones compared to the control group, but neither was significantly reduced.

Keywords: *Asparagus racemosus Tamarindus indica*, antiurolithiatic activity

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Introduction

Urolithiasis is the third most common affliction of the urinary tract, exceeded only by urinary tract infections and pathologic condition of prostate[1]. It is a worldwide problem affecting all geographical, cultural or racial groups, the history of which can be traced to the earliest antiquity of human history[2]. Even though surgical treatment is the treatment of choice for urinary stones, medical interventions and lifestyle changes are equally important, as the recurrence rate is as high as 50% within 5 years after surgery without medical treatment[1]. Both *Asparagus* and *Tamarindus* have been mentioned in the traditional medicine to have antiurolithiatic activity[3]. However, there was no evidence in the literature of previous experimental studies on antiurolithiatic activity of *Asparagus racemosus* and *Tamarindus indica* this study was carried out.

Materials and methods

Plant materials

Both the plants *Asparagus racemosus* Willd and *Tamarindus indica* Linn available locally were collected and identified properly by botanical and pharmacognostic characteristics in the Dept of Botany, MGM College Udupi. The voucher specimens were preserved in the laboratory for future reference.

Method of extraction

Preparation of extract from roots of Asparagus racemosus :

The roots were cleaned, washed, dried in the shade and pulverized. The coarse powder was subjected to successive solvent extraction with water. A semi-solid extract was obtained after complete elimination of solvent under reduced pressure. The yield of the extract was 10%. The extract was stored in a desiccator and used for further experiment after dissolving it in distilled water.

Preparation of extract from fruits of Tamarindus indica*:*

Ripe dried pods of *Tamarindus indica* were peeled, deseeded, and air-dried in the shade for ten days. Dried fruit pulp is a gummy substance easily miscible in water. A semi solid extract was obtained from this dried fruit pulp after complete elimination of solvent under reduced pressure. The yield of extract was 15%. The extract was stored in a desiccator and used for further experiment after dissolving it in distilled water.

Animals

Inbred albino rats of Wistar strain of either sex were used. They were housed in standard conditions of temperature (25±2°C), relative humidity of 45-55%, in animal house of Kasturba Medical College, Manipal. They were fed standard pellet diet (Hindustan Lever rat pellets) and water *ad libitum*. The experiments were started after the approval of IAEC.

Antiurolithiatic study

Method of induction of urolithiasis by insertion of zinc disc [4,5].
Rats were anaesthetized with intraperitoneal ketamine(50mg/kg). A suprapubic incision was made and the abdomen was opened. The urinary bladder was then carefully exposed and the urine in the bladder was aspirated with a sterile syringe. A small nick was made at the apex end of urinary bladder and the sterile zinc disc (previously weighed) was carefully inserted into the bladder. Then the bladder was closed in a single stitch using chromic catgut (4-0). The abdomen was then closed in layers with chromic catgut and skin was closed with silk thread. The rats were allowed to recover from anaesthesia. Food and 1% ethylene glycol in water was given ad libitum. The stone was allowed to form and grow inside the bladder during the study period. After the study period the rats were sacrificed and zinc disc implants/stones were removed from the bladder and dried. Stones taken out were weighed. The difference between initial and final weights indicated the amount of stone formed.

Grouping of animals for different treatments and procedure of the study
Adult albino rats of Wistar strain, weighing between 150-200 g,
were selected for both the studies.
In the study using roots of *Asparagus racemosus* the rats were divided into 6 groups with 6 animals in each group receiving different treatments. Group I - *Prophylactic control* (1%ethylene glycol for 4 weeks), Group II - *Prophylactic treatment* (1%ethylene glycol + aqueous extract of A.racemosus,1600mg/kg orally for 4 weeks), Group III - *Prophylactic treatment* (1%ethylene glycol + aqueous extract of A.racemosus,3200mg/kg orally for 4 weeks), Group IV - *Curative control* (1%ethylene glycol for 4 weeks followed by water for 4 weeks), Group V - *Curative treatment* (1%ethylene glycol for 4 weeks followed by aqueous extract of A.racemosus 1600mg/kg for 4 weeks), Group VI - *Curative treatment* (1%ethylene glycol for 4 weeks followed by aqueous extract of A.racemosus 3200mg/kg for 4 weeks) .
In the study using fruits of T*amarindus indica* the rats were divided into 6 groups with 6 animals in each group receiving different treatments. Group I -*Prophylactic control* (1%ethylene glycol for 4 weeks), Group II - *Prophylactic treatment* (1%ethylene glycol + aqueous extract of T.indica,600mg/kg orally for 4 weeks), Group III - *Prophylactic treatment* (1%ethylene glycol + aqueous extract of T.indica,1200mg/kg orally for 4 weeks), Group IV - *Curative control* (1%ethylene glycol for 4 weeks followed by water for 4 weeks), Group V - *Curative treatment*(1%ethylene glycol for 4 weeks followed by aqueous extract of T.indica 600mg/kg for 4 weeks), Group VI - *Curative treatment* (1%ethylene glycol for 4 weeks followed by aqueous extract of A.racemosus 1200mg/kg for 4 weeks) .
Prophylactic activity against urolithiasis was tested using Groups I to III in both the studies and, after 4 weeks, animals were sacrificed and vesical calculi were collected, weighed and statistically evaluated.

Curative property was tested in using Groups IV to VI in both the studies and , at the end of eight weeks, animals were sacrificed and vesical calculi were collected, weighed and statistically evaluated.

Weight of stones

The difference between the weight of the implanted zinc discs at the time of implantation and final weight of the dried calculi taken out from the bladder at the end of the 4^{th} and 8^{th} week period indicated the weight of deposited stone.

***Statistical analysis*:**

Statistical evaluation was done by using ANOVA in SPSS version 10. Turkey test was used as a post hoc test.

Results

The results of different groups studied using aqueous extract of roots of *Asparagus racemosus* are shown in table 1, whereas those of aqueous extract of fruits of *Tamarindus indica* are shown in table 2. In these studies, even though the extracts from both the plants showed decrease in the weight of stones compared to control after the study period. The reduction was not statistically significant.

Table 1. Weight of stone material deposit on zinc discs in control and A. racemosus (1600 mg/kg, 3200 mg/kg, orally) treated rats.

Groups	No. of rats	Weight of stone (mg) (Mean± SEM)
Group I - prophylactic control	6	198.5 ± 118.10
Group II - prophylactic treatment (1600mg/kg)	6	185.5 ± 96.1
Group III – prophylactic treatment (3200mg/kg)	6	67.1 ± 11.5
Group IV – curative control	6	145.1 ± 42.69
Group V – curative treatment (1600mg/kg)	6	143 ± 103.4
Group VI – curative treatment (3200mg/kg)	6	96 ± 51.81

Table 2. Weight of stone material deposit on zinc discs in control and T. indica (600 mg/kg, 1200 mg/kg, orally) treated rats.

Groups	No. of rats	Weight of stone (mg) (Mean± SEM)
Group I - prophylactic control	6	198.5 ±118.10
Group II - prophylactic treatment (600mg/kg)	6	166.33±31.76
Group III – prophylactic treatment (1200mg/kg)	6	86.16±23.71
Group IV – curative control	6	145.1 ±42.69
Group V – curative treatment (600mg/kg)	6	109.66±31.39
Group VI – curative treatment (1200mg/kg)	6	133.5±68.74

Discussion

In the present study, aqueous extract of *Asparagus racemosus* roots and aqueous extract of Tamarindus indica fruit pulp were evaluated for their antiurolithiatic potential in albino rats of Wistar strains. The method used for induction of stones in this study was zinc disc foreign body insertion technique supplemented with 1%ethylene glycol, which is commonly used in these type of studies.

In this study, the weight of the stones was used as criteria for assessing the preventive or curative effect of the two plants. Two doses of extract for prophylactic and curative groups were used. In both groups the administration of the drugs have resulted in reduction in the weight of stones compared to the control group, but neither was significantly reduced.

The decrease in the bladder stone formation was inconsistent with the increase in the dose of the extract which could be due to variability in the response due to physiological variation. The stone formation in the control group itself was highly variable, ranging from 12mg to 750 mg in prophylactic control group and 11 mg to 310 mg in curative control group, which is indicative of normal physiological variation. Among the different strains of rats used for preclinical studies, Wistar rats are much less susceptible to persistent bladder infection and struvite stone formation in comparison to other strains. In a study where female Fischer 344 (F344), Lewis (LEW), Sprague-Dawley (SD), and Wistar (WIS) rats were inoculated with a host-adapted strain of *Ureaplasma parvum*, and necropsied at 2 weeks post inoculation; 100% of F344, 42% of SD, 10% of LEW, and 10% of WIS rats remained infected. Severe bladder lesions and struvite calculi were seen in 64% of F344 rats; in other rat strains, bladder lesions were mild or absent[6].

Conclusion

In conclusion we can not rule out the possibility of antiurolithiatic activity as there was reduction in size of the stones. Further studies are needed to prove the stone dissolving property of *Asparagus racemosus* and *Tamarindus indica* in other animal models .

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