Hygeia:: journal for drugs and medicines

April 2013 - September 2013
OPEN ACCESS
A half yearly scientific, international, open access journal for drugs and medicines
Research article section: Medicinal Natural products
Researcher ID: K-5130-2012, Article ID- Hygeia.J.D.Med/98/13

Evaluation of *Phoenix dactylifera* fruits for Antiurolithiatic activity

Challa Srinivas Reddy*, Pradeep Vardhaman

Vaagdevi College of Pharmacy, Hanamkonda, Warangal, Andhra Pradesh., India.

Article history: Received: 5 January 2013, revised: 20 January 2013, accepted: 15 February 2013, Available online: 3 April 2013

Abstract:

Plan: *Phoenix dactylifera L. of Arecaceae (Palmae) family is consumed worldwide as an edible fruit. Reviews indicated the use of fruit as antioxidant, anticancer, antilipaemic and antidiabetic in many traditional treatments. In the present study the antiurolithiatic activity of P. dactylifera fruits are reported.*

Materials and Methods: *The n-Butanol and Aqueous extract preparations were made and administered as test compounds, Cystone as reference standard in five groups. Ethylene glycol induced hyperoxaluria is selected as a screening method for induction of kidney stones and the levels of creatinine, urea and uric acid are analysed.*

Outcome: *The n-butanol and aqueous extracts of P. dactylifera fruits in the doses of 200mg/kg reduced creatinine, urea, uric acid levels, significantly in comparison with the control. But both the extracts showed results less than the standard drug 'Cystone' and the n-butanol extract was more effective than aqueous extract to decrease urea and uric acid levels. Among these two extracts the n-butanol extract exhibited more antiurolithiatic activity.*

Keywords*: Phoenix dactylifera, Ethylene Glycol, Urea, Uric Acid, Antiurolithiatic Activity.*

1. Introduction:

Urolithiasis (Urinary Calculi, Urinary Stones, Kidney Stones, Renal Stones and Renal Calculi) pertain to growth of hard, nonmetallic mineral calcifications that form in the urinary system, primarily in the kidney or ureter and may also migrate into the lower urinary system[1.] The clinical significance of the urolithiasis was first described in *Aphorisms of Hippocrates*[2]. Women are less likely to be affected than men in the ratio of 1:3.Among the several types of kidney stones formed, most common are the calcium oxalate stones. The process of formation of kidney stones may be due to nucleation, aggregation and crystal growth phenomena[3].Almost 40% of first time stone formers have a chance of forming stones within 3 years .The reason for the formation of the kidney stones for the second time is the incomplete treatment of the stones formed for the first time[4].Dietary factors (low intake of purine containing food),hereditary(cystinuria), diseased tissue(tuberculosis),less intake of Vit A are some of the contributing factors for formation of kidney stones[5,6].Some of the common symptoms associated with urolithiatic patients are Flank pain, Nausea, Blood In Urine, Fever and Chills.

For Correspondence:challa_cnu@yahoo.com , Contact: +91-9885108060
Hygeia.J.D.Med. Vol.5 (1), April 2013

Researcher ID: K-5130-2012 A

A member of the claudin gene family CLDN14 is identified as a risk locus for urolithiasis[7]. Recommended dietary life style which is vital for treatment of urolithiatic patients include increased water intake , limited tea, intake of less meat, limited dietary sodium and calcium, avoiding of certain antacids with calcium base .Various therapies include thiazide diuretics and alkali-citrate are used in attempt for treatment but scientific evidence is less influential[8].

Due to the presence of extensive dietary composition the fruits of *Phoenix dactylifera* are used as food supplement and in preparation of many ailments[9].Considering its rich source for various amino acids, minerals and ions , the fruits have been selected for the activity[10].

2. Materials and Methods:

2.1. Collection and identification of Plant Material

The fresh fruits of *Phoenix dactylifera* were obtained from a local date manufacturing company and the fruit pulp was dried in sun shade. The plant specimen was authenticated by Dr. Vatsavaya S. Raju, Retired Professor, Department of Botany, Kakatiya University, Warangal. A voucher specimen of the plant was deposited was deposited in the VCP herbarium under the specimen number VCP/PG/2012/2706.

2.2. Preparation of extract and administration

The fruit pulp of *Phoenix dactylifera* was dried at room temperature. The dried fruit pulp was then crushed by mechanical grinding and stored in a dry place until use. n-butanol extract (n-BE) was prepared by soxhlation. The concentrated extracts were obtained by evaporating the solvent, under reduced pressure in a rotary evaporator at 42-45^{0}C. The Aqueous extract (AqE) was obtained by maceration for 7 days. The solid extracts were scraped before complete drying, and then dried to a constant weight. The percentage yield of these solid extracts were 5.5% w/w and 5.1% w/w respectively .The samples were kept in an air tight container until use.

2.3. Experimental Animals

Wistar Albino rats of male sex weighing 150-200g were used in the study. They had free access to food and water and were maintained under standard laboratory conditions with alternating light and dark cycles of 12 h each. They were acclimatized to laboratory conditions for 2 days before behavioral studies. The Institution Animal Ethics Committee (IAEC) had approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare, and Government of India with a registration no.177/99/CPCSEA. All the readings were taken during the same time of the day i.e. between 10 a.m. and 2 p.m.

2.4. Phytochemical screening[11]

Preliminary phytochemical analysis for the extracts was performed by simple chemical tests.

2.5. Ethylene glycol induced urolithiasis model

Ethylene glycol induced hyperoxaluria method was used for the assessment of antiurolithiatic activity. Animals were divided into five groups of six animals each. Group I served as normal and received regular water and food *ad libitum*. Group II served as control and was given ethylene glycol (0.75%) in drinking water for induction of kidney stones until 28th day. Group III received reference standard Cystone (750mg/kg) from day 15 to 28 as it served as curative regimen. Group IV and group V received n-Butanol and Aqueous extracts with a dose level of (200mg/kg) respectively. The reference standard and the extracts were given once daily by oral route.

2.6. Assessment of anti urolithiatic activity:

2.6.1. Serum analysis:

Serum analysis was done at the end of the day .The blood was collected through retro-orbital plexus under anaesthetic conditions and biochemical investigations for Creatinine, Urea and Uric Acid were estimated.

2.7. Statistical analysis

The results were expressed as the Mean $\pm$ SD and analysed using one-way ANOVA followed by Dunnett's multiple comparison tests. Data were computed for statistical analysis using Graph Pad Prism Software and $p<0.05$ was considered to be statistically significant

3. Results:

3.1. Phytochemical investigations:

The photochemical investigations showed the presence of Carbohydrates, Steroids, Alkaloids, Phenolic Compounds and Flavonoids.

3.2. Biochemical investigations:

The serum levels of creatinine, urea and uric acid are reported in Table 1, and Table 2

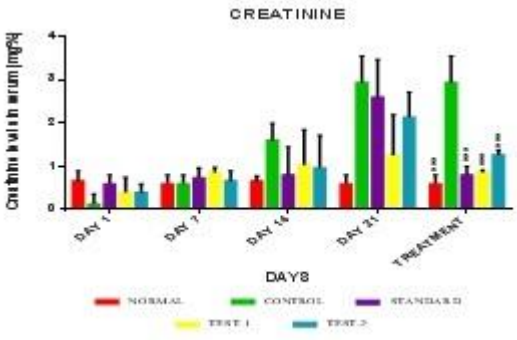

Fig 1: Creatinine levels in different groups in different days.

Table 1: Creatinine levels in serum on different days. (Mean ± SD, n = 6).

Days		*Normal*	*Control*	*Reference standard*	*Test 1*	*Test 2*
DAY 1	Mean±SD	0.666±0.230	0.133±0.230	0.6±0.2	0.4±0.346	0.4±0.2
DAY 7	Mean±SD	0.6±0.2	0.6±0.2	0.733±0.230	0.866±0.115	0.666±0 .230
DAY 14	Mean±SD	0.666±0.115	1.6±0.4	0.8±0.655	1.033±0.814	0.966±0.750
DAY 21	Mean±SD	0.6±0.2	2.933±0.611	2.6±0.871	1.26±0.935	2.13±0.57
TRTT	Mean±SD	0.6±0.2	2.933±0.611	0.8±0.2	0.866±0.057	1.26±0.11

All the values are expressed in Mean±SD of decrease in levels of Creatinine-value $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$, ns-non significant. Comparisions are done by one way ANNOVA using Dunnet's test. Comparisions are done between Disease control groups and the remaining groups.

Table 2: Uric Acid levels in serum on different days. (Mean ± SD, n = 6).

Days		*Normal*	*Control*	*Reference standard*	*Test 1*	*Test 2*
DAY 1	Mean +SD	1.153±1.017	0.384±0.384	0.897±0.967	0.036±0.063	0.666±0.763
DAY 7	Mean+ SD	1.149±1.012	1.41±0.587	1.666±0.222	2.050±0.800	1.922±0.384
DAY 14	Mean+ SD	1.153±1.017	3.205±0.222	2.435±0.222	2.820±0.222	2.691±0.666
DAY 21	Mean+ SD	1.12 ±0.999	3.717±0.222	3.461±0.384	3.205±0.222	3.717±0.22
TRTT	Mean+ SD	1.12 ±0.999	3.717±0.222	1.794±0.443	2.402±0.256	1.922±0.384

All the values are expressed in Mean±SD of decrease in levels of Uric Acid. P-value $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$, ns-non significant. Comparisions are done by one way ANNOVA using Dunnett's test. Comparisions are done between Disease control groups and the remaining groups.

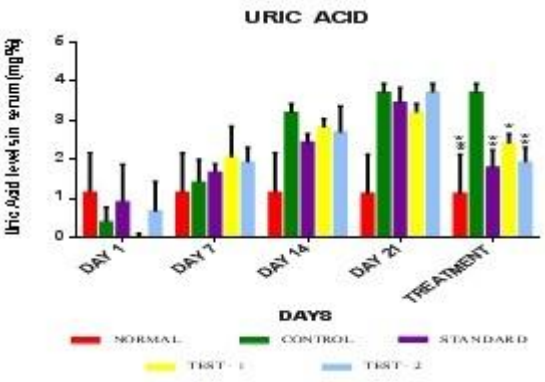

Fig 2: Uric Acid levels in different groups in different days.

Table 3: Urea levels in serum on different days. (Mean ± SD, n = 6).

Days		Normal	Control	Reference standard	Test 1	Test 2
DAY 1	Mean ±SD	39.53±5.57	35.862±2.389	36.78±2.106	32.38±2.438	33.103±6.011
DAY 7	Mean± SD	39.08±6.219	36.78±2.106	40.45±8.082	39.74±5.304	37.24±2.75
DAY 14	Mean± SD	39.08±4.844	48.73±3.471	47.356±5.222	49.655±2.758	51.49±2.10
DAY 21	Mean± SD	39.12±5.866	68.04±2.1069	72.643±6.804	66.66±5.22	62.988±4.21
TRTT	Mean ±SD	39.127±5.86	68.0453±2.106	22.988±2.106	31.005±4.483	24.82±1.37

All the values are expressed in Mean±SD of decrease in levels of Urea. P-value $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$, ns-non significant. Comparisions are done by one way ANNOVA using Dunnett's test. Comparisions are done between Disease control groups and the remaining groups.

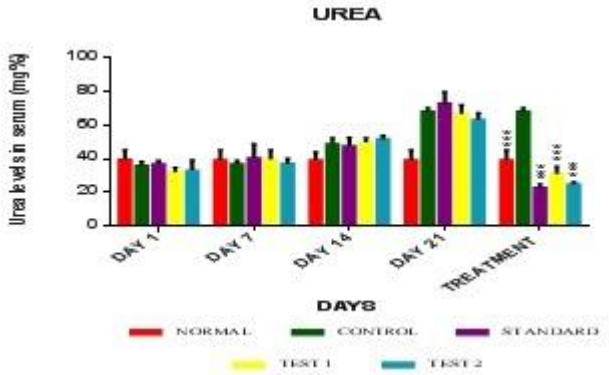

Fig 3: Urea levels in different groups in different days.

4. Discussion

Aqueous and n-Butnaol extracts of *Phoenix dactylifera* exhibited strong antiurolithiatic activity. These extracts were showing antiurolithiatic activity significantly in the dose level of 200 mg/kg.Various experimental procedures result in basically two type of hyperoxaluria: 1) Acute, when the rat is challenged by a single, large dose of lithogen. 2) Chronic, when the rat is continuously challenged with generally small doses of lithogen for a period of time.

In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea and creatinine get accumulated in blood. In this context, the suggested pharmacological action is that oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in cell membrane.

From the obtained results it was evident that extract was able to reduce the formed calcium oxalate stones *in-vivo*. And also it confirms that the biochemical parameters creatinine, urea, uric acid are increased during the 21 day induction of ethylene glycol and are confirmed by the biochemical tests.

After 21 days of treatment with ethylene glycol a 7 day treatment with n-Butanol and Aqueous extracts in the doses of 200mg/kg reduced creatinine ,urea, uric acid levels are significant ($p<0.001$) in comparison with the control. But both the extracts showed results less than standard and n-Butanol extract was more effective than aqueous to decrease urea and uric acid levels. Among these two extracts the n-Butanol extract exhibited more antiurolithiatic activity.

5. Conclusion

Form the above in-*vivo* preclinical experiments it was found that *Phoenix dactylifera* has good antiurolithiatic activity. It should be notified that a thorough evaluation is necessary to elucidate full pharmacological profile of the *Phoenix dactylifera* to develop as a good therapeutic molecule.

Acknowledgements

We wish to thank the Management, Vaagdevi College of Pharmacy, Ram Nagar, Hanamkonda, Warangal, AP, India, for financial grant on this project. Also thankful to Viswambhara Educational Society, Warangal and Principal, Vaagdevi College of Pharmacy, Warangal for providing necessary facilities.

References

1. Guyton AC, Hall JE.Textbook of Medical Physiology, Philadelphia: Saunders, 11th Ed.**2006**, 307-325.
2. Panagiotis Kallidonis, Despoina Liourdi, Evangelos Liatsikos. Medical Treatment for Renal Colic And Stone Expulsion. *European Association Of Urology* **2011**; 10:415-422.
3. Purnima Ashok, Basavaraj CK, Vishwanathswamy AHM. Antiurolithiatic and Antioxidant Activity Of *Mimusops Elengi* On Ethylene Glycol –Induced Urolithiasis In Rats. *Indian journal of pharmacology* **2010**; 42(6): 380-383.
4. Nikhil Johri, Bruce Cooper, William Robertson, Simon Choong, David Ricards, Robert Unwin. An Update and Practical Guide to Renal Stone Management, *Nephron Clinical Practice* **2010**; 116:c159-c171.
5. Grases F, Garcia-Gonzalez R, Genester C, Torres JJ, March JG. Vitamin A and Urolithiasis. *Clinica Chimica Acta* **1998**; 269: 147-157.
6. Ben Thomas, James Hall. Urolithiasis. *Renal and Urology* **2005**; 129-133.
7. Carla GM, Dawn SM. Genetic Determinants of Urolithiasis. *Nature Reviews: Nephrology* 2012; 8:151 -162.
8. Kalyani Divakar, Pawar AT, Chandrasekhar SB, Dighe SB, Goli Divakar. Protective Effect Of Hydro-Alcoholic Extract Of *Rubia cordifolia* Roots Against Ethylene Glycol Induced Urolithiasis In Rats. *Food and Chemical Toxicology* **2010**; 48:1013-1018.
9. The Ayurvedic Pharmacopoeia of India. Ministry Of Health And Family Welfare , India, Ed 1, Part- I, Vol – IV. 50-53.
10. Manjeshwar Shrinath Baliga, Bantwal Raghavendra Vittaldas Baliga, Shaun Mathew Kandathil, Harshith P.Bhat, Praveen Kumar Vayalil. A Review of the Chemistry and Pharmacology of the Date Fruits (*Phoenix dacylifera*).*Food Research International* **2011**; 44:1812-1822.
11. Ajayi IA, Ajibade O,Oderinde RA. Preliminary Phytochemical Analysis of some Plant Seeds. *Research Journal of Chemical Sciences* **2011**; Vol. 1(3):58-62.