Urol Res (2004) 32: 362–366
DOI 10.1007/s00240-004-0432-8

ORIGINAL PAPER

J. L. Nishiura · A. H. Campos · M. A. Boim
I. P. Heilberg · N. Schor

Phyllanthus niruri normalizes elevated urinary calcium levels in calcium stone forming (CSF) patients

Received: 24 June 2003 / Accepted: 26 April 2004 / Published online: 19 June 2004

Abstract *Phyllanthus niruri* is a plant used for years in Brazil to treat urinary calculi. We prospectively evaluated the effect of *P. niruri* intake on 24 h urinary biochemical parameters in an attempt to assess its in vivo effect in calcium stone forming (CSF) patients. A total of 69 CSF patients (39 males and 30 females, 38 ± 8 years old) were randomized to take either *P. niruri* ($n = 33$) (450 mg capsules, td) or placebo ($n = 36$) for 3 months. Blood calcium, uric acid, citrate, magnesium, oxalate, sodium and potassium were determined at baseline and at the end of the study. A subset analysis was made in patients classified according to the presence of metabolic abnormalities (hypercalciuria, hyperuricosuria, hyperoxaluria, hypocitraturia and hypomagnesiuria). Overall, there were no significant differences in the mean values of urinary parameters between the urine samples before and after *P. niruri* intake, except for a slight reduction in mean urinary magnesium after *P. niruri*, which was within the normal range. However, in the subset analysis, we observed that *P. niruri* induced a significant reduction in the mean urinary calcium in hypercalciuric patients (4.8 ± 1.0 vs 3.4 ± 1.1 mg/kg/24 h, $P < 0.05$). In this short-term follow-up, no significant differences in calculi voiding and/or pain relief between the groups taking *P. niruri* or the placebo were detected. Our data suggest that *P. niruri* intake reduces urinary calcium based on the analysis of a subset of patients presenting with hypercalciuria. Larger trials including primary hypercalciuric stone formers should be performed in order to confirm these findings and to determine the possible clinical consequences of urinary calcium reduction during *P. niruri* administration.

Keywords *Phyllanthus niruri* · Natural products · Nephrolithiasis · Hypercalciuria · Calcium stone formers · Urinary calculi

J. L. Nishiura · A. H. Campos · M. A. Boim · I. P. Heilberg
N. Schor (✉)
Nephrology Division,
Universidade Federal de São Paulo,
Escola Paulista de Medicina,
Rua Botucatu, 740 Vila Clementino São Paulo,
SP 04023-900, Brazil
E-mail: nestor@nefro.epm.br
Tel.: +55-11-55746300
Fax: +55-11-55739652

Introduction

In Brazil, a tea made from the plant *Phyllanthus niruri* (stone breaker or "quebra pedra") has been used in folk medicine to treat urinary calculi, among other conditions [1].

According to Calixto et al. [1], alkaloids from plants of the genus *Phyllanthus* present an antispasmodic activity leading to smooth muscle relaxation, mostly evidenced in the urinary tract, which would facilitate the elimination of urinary calculi. The analgesic activity of *P. niruri* has also been demonstrated by other investigators in Brazil [2, 3].

Previous studies by our laboratory have shown that the aqueous extract of *P. niruri* has an inhibitory effect on calcium oxalate (CaOx) crystal growth and aggregation in an in vitro model of crystallization of human urine [4]. In addition, the whole plant aqueous extract prevented an increase in the size of matrix bladder calculi as well as in the size and number of formed satellite crystals in a rat model [5]. Campos and Schor [6] also demonstrated that the aqueous extract of *P. niruri* reduced calcium oxalate crystal uptake by canine distal tubular cells, without evidence of cytotoxicity or biochemical alterations of the culture medium.

Taken together, these findings are consistent with the notion that the active constituents of *P. niruri* may have a beneficial effect in the treatment of urinary calculi.

Our laboratory also demonstrated that human volunteers who received large oral doses of *P. niruri* (20 g/day, in the form of tea) presented no detectable clinical or biochemical adverse effects, with excellent tolerability

[1]. Nevertheless, there is no report to date on the effects of *P. niruri* on the promoters or inhibitors of lithogenesis in humans.

Therefore, based on the promising experimental data, and also on an adequate safety profile, the aim of the present study was to evaluate, in a controlled manner, the effect of *P. niruri* on the urinary excretion of promoters and inhibitors of lithogenesis in CSF patients.

Patients and methods

A total of 69 CSF patients (39 males/30 females, 38 ± 8 years old) were randomly assigned to take a 450 mg capsule (three times a day) of lyophilized 2% aqueous extract of *P. niruri* ($n = 33$) or placebo (*Chicorium sativum*, $n = 36$) for a period of 3 months. As safety was one of our main concerns and no clinical or laboratory endpoint was available to define the *P. niruri* therapeutic range, we arbitrarily chose a total daily dose of *P. niruri* approximately 15 times lower than that employed in toxicological studies previously conducted in our laboratory (unpublished data). *C. sativum* was considered a good alternative for a placebo because its color and taste are similar to those of *P. niruri*. It is a green vegetable commonly consumed by our population and there has been no report of its having any toxic effect, even when consumed in quantities far higher than those administered to our patients. The protocol was submitted to the University Ethics Committee and approved.

All patients included presented with at least one renal stone. Diagnosis was based on the presence of stone(s) in both renal ultrasonography (US) and plain abdominal x-ray (thus, calcium-containing stones). Patients with diseases that might have led to secondary calcium stones, such as primary hyperparathyroidism, distal renal tubular acidosis, urinary tract infections, primary hyperoxaluria, etc., were not included in the study.

Both the *P. niruri* and placebo capsules were manufactured in the same way. *P. niruri* was grown at the experimental center of the Universidade Estadual de Campinas, CPQBA, Paulínia, São Paulo, Brazil. A voucher specimen (ref. 481) has been deposited in the herbarium of the same institution. A *P. niruri* crude extract was obtained from the whole plant, as is done in folk medicine. Plant samples were cut and dried at 50°C for 2 months in a ventilated room. After drying, the plants were ground in a mechanical mill and used for tea preparation. The tea was stirred for 30 min at 72°C and then vacuum filtered, concentrated, lyophilized and encapsulated.

A phytochemical analysis (fingerprint) of *P. niruri* was performed by high performance liquid chromatography (HPLC) assay (Fig. 1). The samples were prepared by dissolving the capsules in 20% ethanol. The resultant solution was partitioned with methylene chloride three times and the aqueous phase filtered through a 0.22 μm filter (Millipore, USA) and injected into the HPLC analyzer.

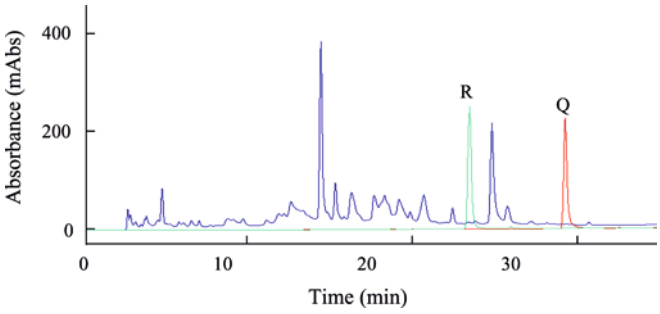

Fig. 1 Typical chromatogram from a *Phyllanthus niruri* phytochemical analysis. The tracing is representative of three experiments. Rutin (*R*) and quercetin (*Q*) (Sigma Chemical, USA) were used as reference compounds

Blood calcium, uric acid, sodium, potassium and creatinine, and 24 h urinary calcium, uric acid, citrate, magnesium, oxalate, sodium and potassium were determined at baseline and at the end of the study.

Urinary oxalate was measured with the Sigma Oxalate Diagnostic kit (Sigma, St Louis, Mo., USA). Calcium was determined by atomic absorption spectrophotometry (Perkin-Elmer Atomic Spectrophotometer 290B). Creatinine was measured by the alkaline picrate Jaffe reaction [7] and uric acid by the uricase method [8]. Urinary citrate was determined by the citrate-lyase enzymatic reaction [9]. Sodium and potassium were measured by flame emission spectrophotometry.

The criteria for metabolic disturbances, based on the literature [10], were defined as: hypercalciuria (urinary calcium ≥4 mg/kg/day), hyperoxaluria (urinary oxalate ≥45 mg/day), hyperuricosuria [urinary uric acid ≥750 mg/day (female) or ≥800 mg/day (male)], hypocitraturia (urinary citrate ≤ 320 mg/day) and hypomagnesiuria (urinary magnesium ≤ 60 mg/day).

A subset analysis was made in patients classified according to the presence of metabolic abnormalities (hypercalciuria, hyperuricosuria, hyperoxaluria, hypocitraturia and hypomagnesiuria).

Urinary tract US was performed before and at the end of the study in order to detect the number and size of calculi.

The non-parametric Mann-Whitney U-test was used to compare the results between groups. The Wilcoxon test was used to compare the results obtained for the same group before and after *P. niruri* administration. Statistical significance was defined as $P < 0.05$.

Results

As shown in Table 1, the groups were similar in terms of gender distribution, age and body weight. The relative distribution of metabolic abnormalities, such as hypercalciuria, hyperuricosuria, hypocitraturia, hyperoxaluria and hypomagnesiuria, was also similar between groups. Some patients presented more than one metabolic abnormality.

Table 1 Number and percentage (%) of patients with metabolic diagnoses in both groups. [a] mean ± SD

	Placebo ($n=36$)	*Phyllanthus niruri* ($n=33$)
Gender	22 male/14 female	17 male/16 female
Age (years)[a]	37 ± 8	39 ± 9
Body weight (kg)[a]	70 ± 11	68 ± 13
Hypercalciuria	12 (33%)	8 (24%)
Hyperuricosuria	3 (8%)	1 (3%)
Hypocitraturia	10 (28%)	15 (45%)
Hyperoxaluria	6 (17%)	6 (18%)
Hypomagnesiuria	7 (19%)	3 (9%)

Serum biochemical parameters were not significantly different following *P. niruri* or placebo administration (Table 2). The mean values of urinary promoters or inhibitors of lithogenesis are shown in Table 3. There was no significant difference in mean urinary volume, calcium, uric acid, creatinine, sodium, potassium, citrate or oxalate after *P. niruri* or placebo administration compared to the baseline. There was a slight but significant decrease in mean urinary magnesium after *P. niruri* administration (82 ± 15 vs 74 ± 19 mg/24 hs), which occurred within normal limits.

Despite of the lack of statistical differences between the mean parameters induced by *P.niruri*, when patients were classified according to their individual metabolic abnormality(ies), as shown in Table 4, a significant difference was found among the patients with hypercalciuria, who exhibited lower urinary calcium levels after *P. niruri* (4.8 ± 1.1 vs 3.4 ± 1.1 mg/kg/24 h, $P < 0.05$). Urinary calcium did not change in either the non-hypercalciuric patients or in those subjects who received the placebo. Individual values of urinary excretion of calcium (mg/kg/24h) in hypercalciuric and non-hypercalciuric patients are shown in Fig. 2. A separate analysis was made according to gender for metabolic disturbances, such as hypercalciuria and hyperuricosuria, the definitions criteria of which are different for males and females. When analyzing urinary calcium levels according to gender, we observed that both female and male hypercalciuric patients presented a significant reduction in mean urinary calcium after *P. niruri* intake (5.1 ± 1.3 vs 3.9 ± 1.4 and 4.5 ± 0.6 vs 3.0 ± 0.6 mg/kg/24h, respectively) but not after placebo intake (4.6 ± 0.1 vs 5.3 ± 2.0 and 5.0 ± 0.7 vs 4.2 ± 1.8 mg/kg/24h, respectively). Among the normocalciuric males, there were no differences in urinary calcium after *P. niruri* or the placebo. The normocalciuric females presented a slight but significant increase in mean urinary calcium, after administration of both *P. niruri* and the placebo (2.3 ± 0.7 vs 3.0 ± 1.4 and 2.2 ± 0.5 vs 2.9 ± 1.1 mg/kg/24h, respectively), but this increase occurred within the normal limits. Urinary uric acid levels were not different when separately analyzed for females or males with normo- or hyperuricosuria.

Finally, the number of calculi as well as calculus size observed by US were not modified by *P. niruri* or the placebo (Table 5). Four of 33 patients receiving *P. niruri* and five of 36 patients receiving the placebo passed calculi during the 3 months of the study (data not shown).

Table 2 Serum parameters pre- and post-placebo or *P. niruri* administration. Values are means ± SD

	Placebo		*P. niruri*	
	Pre	Post	Pre	Post
Calcium (mg/dl)	9.5 ± 0.6	9.5 ± 0.4	9.1 ± 1.4	9.5 ± 0.4
Uric acid (mg/dl)	4.9 ± 1.7	4.9 ± 1.6	5.2 ± 1.6	4.9 ± 1.6
Creatinine (mg/dl)	0.9 ± 0.1	0.8 ± 0.3	0.8 ± 0.1	0.8 ± 0.2
Sodium (mEq/l)	140 ± 2.5	139 ± 1.3	139 ± 2	138 ± 1.6
Potassium (mEq/l)	4.4 ± 0.4	4.3 ± 0.4	4.3 ± 0.3	4.3 ± 0.3

Discussion

Urolithiasis is a disease highly prevalent throughout the world, carrying significant morbidity and consequent costs. Although considerable efforts have been made to identify effective treatments for the disease, this is a goal yet to be achieved.

Herbal medicines have been used for a long time for the treatment of many different diseases, with variable and sometimes negative consequences. Thus, carefully conducted studies are essential to evaluate the potential use of a given plant or compound for treating a particular illness. Despite the widespread use of *P. niruri* as a folk remedy for urinary calculi in Brazil, no controlled study evaluating its effect on lithogenesis has been published.

The present study was designed to evaluate the potential therapeutic role of *P. niruri* in urolithiasis. In our clinical practice, there have been anecdotal reports of higher calculus voiding among patients who consumed *P. niruri* preparations. In the present series, however, neither the number nor the size of the calculi observed by US was modified after *P. niruri* administration. In addition, no effect of *P. niruri* was detected on painful crisis frequency or magnitude. As this study was not intended to identify the differences in clinical characteristics, we believe that the short-term nature of our observations and the relatively small number of patients might have accounted for these negative findings. Although we did not find any significant effect on calculus elimination following the short-term administration of *P. niruri*, a potentially important effect related to urolithiasis, namely control of hypercalciuria, was disclosed. Regardless of gender, patients presenting with hypercalciuria, one of the most prevalent biochemical abnormalities found in urolithiasis, had their urinary calcium levels significantly reduced following a 3 month period of *P. niruri* administration. Although this conclusion is drawn from a subgroup analysis, all patients were randomized to receive *P. niruri* or a placebo and treated in a double-blind fashion. While all hypercalciuric patients receiving *P. niruri* had their calcium levels normalized, the response of hypercalciuric patients receiving the

Table 3 Urinary parameters pre- and post-placebo or *P. niruri* administration. Values are means ± SD. * $P < 0.05$ pre vs post

	Placebo		*P. niruri*	
	Pre	Post	Pre	Post
Volume (ml)	1,965 ± 702	2,048 ± 951	1,786 ± 700	1,839 ± 874
Calcium (mg/24 h)	231 ± 108	230 ± 109	200 ± 86	206 ± 97
Uric acid (mg/24 h	555 ± 196	539 ± 267	504 ± 174	490 ± 157
Creatinine (mg/24 h)	1,497 ± 456	1,481 ± 565	1,385 ± 380	1,379 ± 452
Sodium (mEq/24 h)	243 ± 111	228 ± 103	219 ± 99	186 ± 72
Potassium (mEq/24 h)	53 ± 24	57 ± 23	48 ± 18	53 ± 16
Citrate (mg/24 h)	497 ± 266	477 ± 289	366 ± 215	427 ± 389
Magnesium (mg/24 h)	88 ± 30	81 ± 25	82 ± 15	74 ± 19*
Oxalate (mg/24 h)	31 ± 13	33 ± 15	31 ± 18	33 ± 13

Table 4 Urinary parameters pre- and post-placebo or *P. niruri* administration according to the metabolic disturbance. Values are means ± SD. * $P < 0.05$ pre vs post

	Placebo			*P. niruri*		
	n	Pre	Post	*n*	Pre	Post
Calcium (mg/kg/24 h)						
Hypercalciurics	12	4.9 ± 0.6	4.5 ± 1.8	8	4.8 ± 1.0	3.4 ± 1.1*
Non-hypercalciurics	24	2.4 ± 0.6	2.8 ± 1.0	25	2.4 ± 0.8	2.9 ± 1.5
Uric acid (mg/24 h)						
Hyperuricosurics	3	1,023 ± 193	1,179 ± 467	2	815 ± 52	732 ± 168
Non-hyperuricosurics	33	513 ± 132	481 ± 151	31	484 ± 159	474 ± 145
Citrate (mg/24 h)						
Hypocitraturics	10	242 ± 52	268 ± 124	16	223 ± 53	246 ± 62
Non-hypocitraturics	26	554 ± 177	447 ± 187	17	554 ± 177	447 ± 187
Oxalate (mg/24 h)						
Hyperoxalurics	6	53 ± 8	44 ± 23	6	61 ± 17	48 ± 12
Non-hyperoxalurics	30	27 ± 9	31 ± 12	27	25 ± 9	29 ± 11
Magnesium (mg/24 h)						
Hypomagnesiurics	7	42 ± 9	57 ± 14	3	53 ± 6	69 ± 6
Non-hypomagnesiurics	29	99 ± 21	87 ± 24	30	85 ± 13	75 ± 20

placebo was not significantly different from the baseline pre-treatment levels. In addition, urinary calcium levels in non-hypercalciuric patients remained unaffected by *P. niruri* administration.

We recognize that our results are preliminary and that we do not have the necessary elements to explain the intriguing effects of *P. niruri* or to conclude that it will be beneficial for the long-term treatment of urolithiasis. In analyzing our results, some points must be taken into consideration. We did not use a purified compound but rather a liophylized extract (containing a mixture of substances, including alkaloids, flavonoids, terpenes, lignans, tannins and coumarins). It has been reported that the aqueous extract of *P. niruri* contains more than 50 different chemical compounds. The phytochemical and pharmacological properties of the genus *P. niruri* have been accounted for by the action of different substances. Rutin, β amylin, β-sitosterol and caffeic acid posses anti-inflammatory and/or analgesic activities, geranin inhibits angiotensin-converting enzyme, quercetin inhibits phosphodiesterase, niruside inhibits HIV reverse transcriptase, and repandusinic acid A may act as a hepatoprotector[1]. To date, none of these compounds has been shown to have an effect on calciuria. Moreover, literature on well-conducted clinical trials with natural products is scarce, and only a handful of papers on *P. niruri* are available. Only a single clinical study has addressed the effects of *P niruri* on the urinary system [11], and experimental reports from our group focusing on the relationship between *P. niruri* and lithogenesis are available [4, 5, 6]. Thus, attempts to clearly interpret the present findings may lead to inaccuracies and would be speculative. In order to provide mechanistic insights into the effects of *P. niruri* on urinary calcium, additional studies should be carried out.

In summary, we have identified a potential beneficial effect of *P. niruri* on hypercalciuria, an important risk factor for stone formation. These findings will have to be confirmed in a larger trial involving only hypercalciuric patients. In addition, longer-term studies are necessary to define whether these biochemical modifications can be translated into clinical benefit.

Acknowledgements This research was supported by CEME (Central de Medicamentos), FOR (Fundação Oswaldo Ramos), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). We thank Dr. Antonio José Lapa and Mirtes Midori Tanae for providing the aqueous extract of *Phyllanthus niruri*.

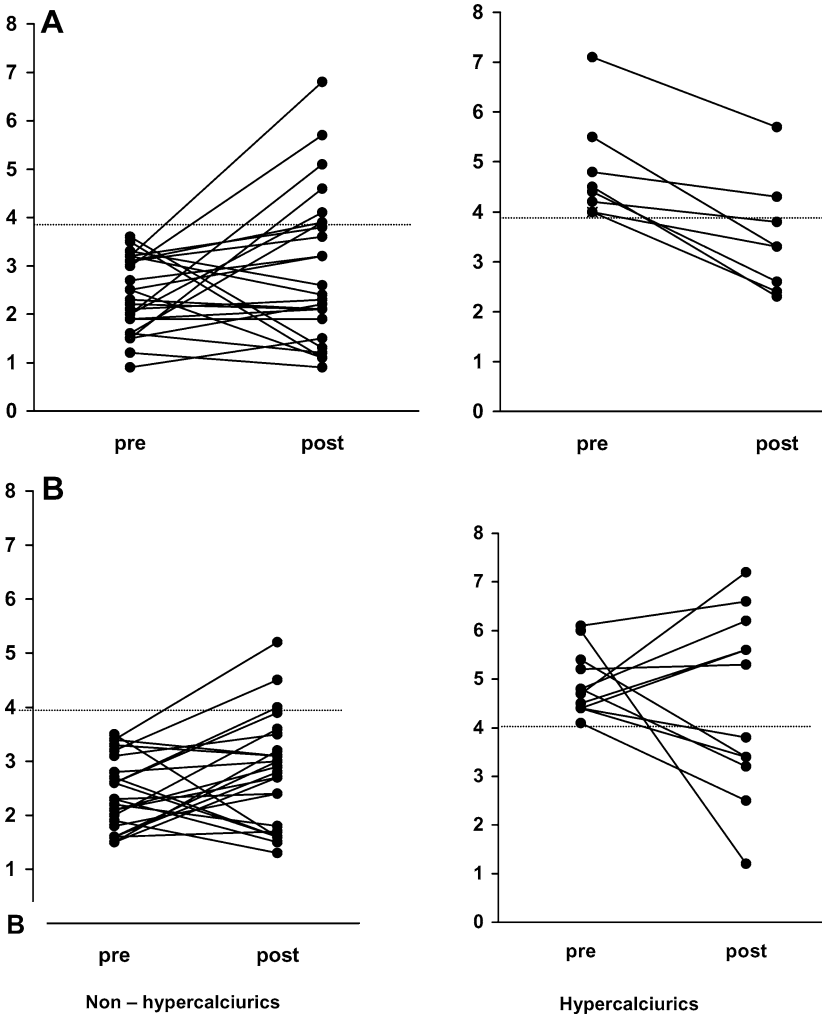

Fig. 2 Urinary calcium (mg/24h) pre and post *P. niruri* (**A**) or placebo (**B**) administration in hypercalciuric and non-hypercalciuric patients

Table 5 Number of calculi and calculus size pre- and post-placebo or *P. niruri* administration detected by ultrasonography. Values are means ± SD

	Placebo		*P. niruri*	
	Pre	Post	Pre	Post
Number of calculi	2.0 ± 1.2	1.6 ± 1.4	1.8 ± 0.9	1.5 ± 1.4
Calculi size (cm)	0.6 ± 0.2	0.6 ± 0.3	0.6 ± 0.2	0.6 ± 0.2

References

1. Calixto JB, Santos AR, Cechinel Filho V, Yunes RA (1998) A review of the genus *Phyllanthus*: their chemistry, pharmacology, and therapeutic potencial. Med Res Rev 18: 225
2. Santos AR (1994) Analgesic effects of callus culture extracts from selected species of *Phyllanthus* in mice. J Pharm Pharmacol 46: 755
3. Santos AR (1995) Analysis of the mechanisms underlying the antinoceptive effect of the extracts of plants from the genus *Phyllanthus*. Gen Pharmacol 26: 1499
4. Barros ME, Schor N, Boim MA (2003) Effects of an aqueous extract from *Phyllanthus niruri* on calcium oxalate crystallization in vitro. Urol Res 30: 374
5. Freitas AM, Schor N, Boim MA (2002) The effect of *Phyllanthus niruri* on urinary inhibitors of calcium oxalate crystallization and other factors associated with renal stone formation. BJU Int 89: 829
6. Campos AH, Schor N (1999) *Phyllanthus niruri* inhibitors calcium oxalate endocytosis by renal tubular cells: its role in urolithiasis. Nephron 81: 393
7. McFate RP, Cohn C, Eichelberger L, Cooper JA (1954) Symposium on azotemia.Am J Clin Pathol 24: 511
8. Fossati P, Prencipe L, Berti G (1980) Use of 3,4-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzyme assay of uric acid in serum and urine. Clin Chem 26: 227
9. Holt C, Cowley DM, Chalmers AH (1985) Rapid estimation of urinary citrate by use of a centrifugal analyzer. Clin Chem31: 779
10. Pak CYC, Skurla C, Harvey J (1985) Graphic display of urinary risk factors for renal stone formation. J Urol 134: 867
11. Srividya N, Periwal S (1995) Diuretic, hypotensive and hypoglycaemic effect of *Phyllanthus amarus*. Indian J Exp Biol 33: 861