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Phytotherapy and renal stones: the role of antioxidants. A pilot study in Wistar rats

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Abstract Since ancient times, various herbal preparations have been used in renal lithiasis therapy, but conclusive scientific data on their therapeutic effects and efficacy are not available. To address this issue, the present study evaluated the antilithiasic activity of a traditional Mallorcan herbal preparation, and compared its effects with those of the antioxidant flavonoids, catechin and epicatechin. Thirty-six male Wistar rats were assigned randomly to four groups ($n = 9$): a control group, a catechin (CAT) treatment group, an epicatechin (EPI) treatment group, and a group treated with a folk herbal extract (FHE). After 16 days of treatment, calcium oxalate lithiasis was induced in the rats using ethylene glycol. After 8 days (treatment + ethylene glycol), 24-h rat urine was collected, the animals were sacrificed and their kidneys were removed for histological and chemical analysis. The calcium concentration in kidney tissue was significantly lower in the CAT-treated (2.4 ± 0.3 mg/g), EPI-treated (1.8 ± 0.3 mg/g) and FHE-treated (2.1 ± 0.3 mg/g) groups, than in the control group (5.4 ± 1.4 mg/g). Examination of paraffin-embedded kidney sections showed that control group rats had the greatest amount of calcification. There were no significant differences between control and treated groups with respect to urinary calcium, magnesium, oxalate and citrate concentrations. These results demonstrate the ability of herbal preparations and antioxidants to prevent the development of papillary and intratubular calcification in the kidney.

Keywords Renal stones · Phytotherapy · Antioxidants · Rats

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Introduction

Since ancient times, a variety of herbal preparations have been successfully used in renal lithiasis therapy [1–3], although generally, there has been no indication of the type of calculus being treated. During recent decades, studies of the antilithiasic effects of herbal extracts have been reported, but in the majority of these reports, the effects did not seem to be mediated by urinary biochemical changes [3–5]. Infection can provide a focus for renal stone formation, so herbal extracts with antibacterial activity (such as cranberry and buchu) may show some antilithiasic activity [6]. However, there is some clinical evidence to suggest that cranberry may slightly increase the risk of oxalate stone formation [6]. Diuretic effects may also reduce stone development when total fluid intake and output increased, and such effects have been attributed to several herbal preparations. Thus, juniper is a well-known diuretic herb that probably has this property owing to its essential oil, although other compounds in the berries could enhance the diuretic effect [7]. The legume gahat (*Vigna unguiculata*) has been used for centuries in Nepal and Pakistan to treat symptoms associated with urinary calculi. In a recent study, it was reported that there were no significant differences in common urinary electrolytes in urine samples taken before and after gahat intake [5]. The authors concluded that if this legume is effective in preventing urinary calculi, it must act through a different mechanism. It may be that the high

phytate content of the seeds explains its activity, but this remains to be proven.

As mucoproteins are promoters of the crystallization processes, some prevention of stone formation may be due to saponin disaggregation of mucoprotein suspensions, but no conclusive experiments addressing this possibility have been reported. Thus, although phytotherapeutic extracts are reported in folk culture, conclusive scientific evidence of the therapeutic roles and efficacy of the herbs is not available. Recently, antioxidant activity has been attributed to *Trigonella foenum graecum* L. seeds, supporting anecdotal information on antilithiasic activity of the plant [2], and we have also observed some antioxidant activity in hyperoxaluric rats treated with lemon juice [8]. The aims of the present study were to evaluate the antilithiasic activity of a traditional herbal preparation, very popular in Spain during the 1970 decade (known as Fagolitos), and to compare its effects with those of the antioxidant flavonoids, catechin and epicatechin.

Methods

Animals and treatments

Thirty-six male Wistar rats, each weighing approximately 260 g, were acclimated for 7 days in cages prior to experimental treatment. The experiments were performed in accordance with internationally accepted standard guidelines for the use of animals. Rats had ad libitum access to standard food and tap water under a controlled 12-h light/dark cycle at 22 ± 2°C.

The animals were divided into four groups (nine animals per group): a control group, a catechin (CAT) treatment group, an epicatechin (EPI) treatment group, and a group treated with the folk herbal extract (FHE). For 16 days, the control group was supplied with drinking water without additives, the CAT and EPI groups were supplied drinking water supplemented with catechin 100 mg/L or epicatechin 100 mg/L (supplied by Sigma–Aldrich Quimica S.A., Madrid, Spain), respectively, and the FHE-treated group was supplied with drinking water supplemented with 7 mL/L of a herbal extract (prepared and supplied by Salva Trobat Chemist's shop, Palma de Mallorca, Spain) renowned in the Balearic Islands for its antilithiasic activity. The dose of FHE administered to the rats was equivalent to the human dose and was calculated considering that a dose of 30 mL/day of herbal preparation was recommended for humans. FHE contains fluid extracts of *Arctotaphylos uva-ursi* L. (2.16%), *Zea mays* L. (2.16%) and *Ricinus zanzibariensis* L. (46.48%), tincture of *Sabal serrulata* L. (21.5%), mother tincture of *Agathosma betulina* L. (17.5%), glycerin (10%), and anis essence (0.2%). After 16 days, the drinking water of rats in all groups (containing the corresponding additives in each case) was also supplemented for a further 8 days with 0.8% v/v ethylene glycol (EG) plus 1% w/v NH_4Cl.

Monitoring and sampling

Animal weights were monitored throughout the study. On the final day of the experiment, 24-h urine samples were collected from each group of rats following transfer to metabolic cages. The animals were then sacrificed, and their kidneys were removed for histological and chemical analysis.

Determination of calcium and other elements in kidney

The left kidneys were lyophilized and weighed, then digested in a sand bath using a 1:1 HNO_3:$HClO_4$ mixture until the solution was clear. For chemical analysis, the digested samples were diluted with distilled water, and the concentrations of calcium, phosphorus, magnesium, iron and zinc were determined using inductively coupled plasma atomic emission spectrometry (Perkin-Elmer SL, Optima 5300 DV Spectrometer) and the appropriate calibration curve.

Histological examination

The right kidneys were placed in 4% buffered formaldehyde at pH 7 (supplied by Panreac Quimica S.A., Barcelona, Spain), fixed for 24 h at room temperature, embedded in paraffin, sectioned (4 μm), and stained with hematoxylin-eosin, von Kossa (for phosphate detection) and the localization of crystals were observed by polarized light. Histological analysis and crystal localization was performed by an experienced pathologist.

Urine analysis

The urine of rats was analyzed after EG-induced urolithiasis. The urine samples were analyzed for calcium, magnesium and phosphorus using inductively coupled plasma atomic emission spectrometry, and oxalate and citrate were analyzed using Boehringer Mannheim kits (10755699035 and 10139076035, respectively).

Statistical analysis

Results are presented as mean ± standard error (SE). A one-way ANOVA was used to determine the significance of differences among groups. The Student's *t* test was used to assess differences among means. Conventional Windows software was used for statistical computations. A P value <0.05 was considered to indicate a significant difference.

Results

Body weight

No significant differences were found in body weight among control and treated rats (Table 1).

Calcium and other element levels in kidney

The calcium and other element concentrations determined from left kidney tissues are shown in Fig. 1. Calcium concentrations were significantly lower in the CAT, EPI and FHE treatment groups compared with the control group, but no significant differences were found among the three treatment groups. No significant differences were detected among all groups (treatment and control) in the concentrations of phosphorus, magnesium, iron and zinc in kidney tissues.

The calcium reference values in renal tissue of untreated animals was 0.25 ± 0.02 mg/g dry kidney.

Histological examination

Examination of kidney sections showed that rats of the control group had the greatest amount of crystallization, which was evident in all parts of the kidney. Intratubular crystals, compatible with calcium oxalate detected by polarized light, were observed in all groups (Fig. 2). The von Kossa tinction detected a very small phosphate salts deposition. Marked deposits were observed by polarized light on the surface of the papillary tips of control rats (Fig. 3), although fewer rats in the CAT, EPI and FHE treatment groups exhibited this histopathology (Fig. 4).

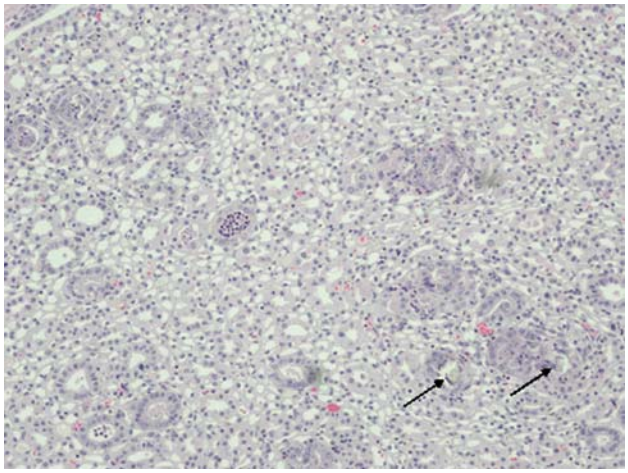

Fig. 2 Intratubular crystals in kidneys of ethylene glycol (EG)-treated rats, crystals observed by polarized light (H&E, ×100)

Table 1 Body weight of rats (g) before (day 0) and after (day 8) addition of ethylene glycol to drinking water

	Control group	CAT-treated group	EPI-treated group	FHE-treated group
Day 0	327 ± 4	323 ± 4	330 ± 6	304 ± 7
Day 8	240 ± 8	232 ± 8	235 ± 9	224 ± 8

Values represent mean ± SE of nine animals per group

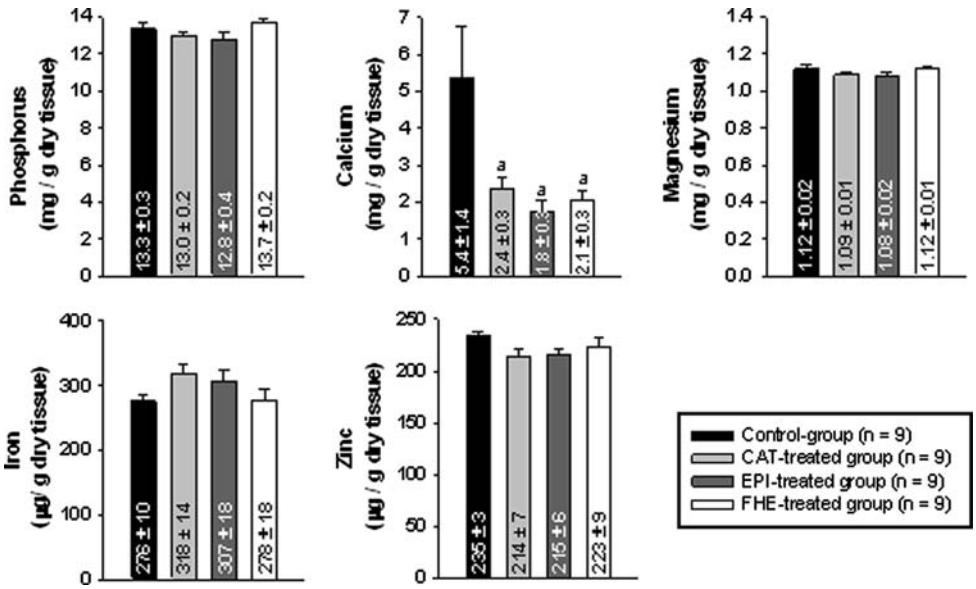

Fig. 1 Calcium and other element concentrations in rat kidneys. Values represent mean ± SE of nine animals per group. *a* values are significantly different to the control $P < 0.05$

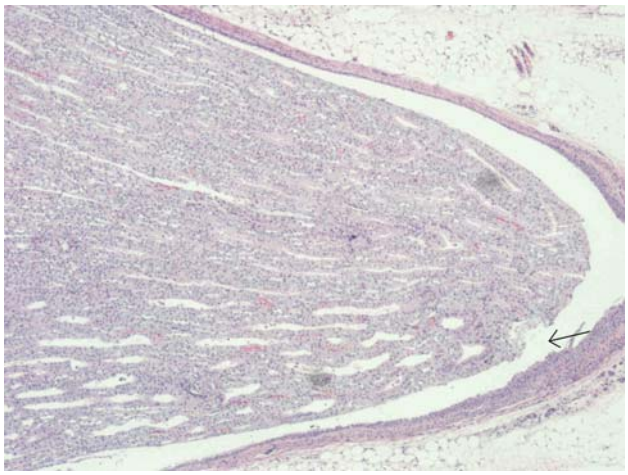

Fig. 3 Crystal deposits on the surface of the papillary tips in kidneys of ethylene glycol (EG)-treated rats, crystals observed by polarized light (H&E, ×40)

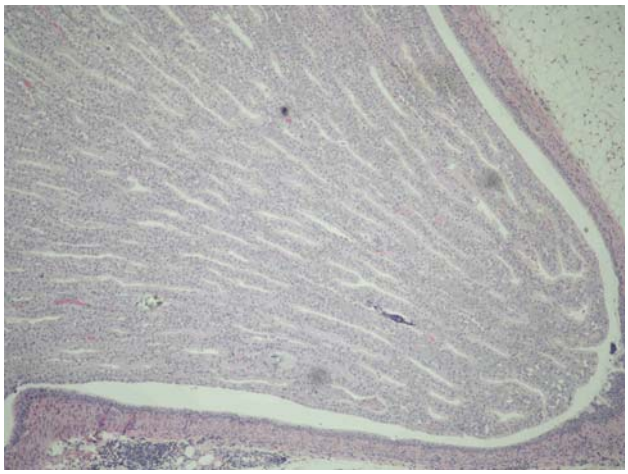

Fig. 4 Absence of crystal deposits on the surface of the papillary tips in kidneys of epicatechin (EPI)-treated rats, crystals observed by polarized light (H&E, ×40)

Urine analysis

The concentration of the main urinary biochemical parameters of rats in each group are given in Table 2. No major differences were evident among the control and treatment groups, although there was a significant increase in urinary phosphorous observed in the FHE-treated group.

The reference urinary values of untreated animals was pH = 7 ± 0.2, Ca = 3.21 ± 0.18 mmol/L, P = 21.64 ± 0.84 mmol/L and citrate = 9.96 ± 1.3 mmol/L.

Discussion

Renal calculi can be broadly classified into two groups: tissue attached and tissue unattached [9]. Attached renal calculi are mainly calcium oxalate monohydrate (COM) with a detectable site of attachment to the renal papilla. They consist of a core located near the attachment site (concave zone), and radially striated concentrically laminated peripheral layers. Unattached calculi have no detectable site of attachment to papilla. They develop in renal cavities of low or reduced urodynamic efficacy and can exhibit diverse composition and structure. Several reports about the genesis of COM papillary calculi [10–12] have been published since Randall's first description of papillary calcification. It seems clear that renal epithelial cell injuries have a decisive role in development of such renal calculi [13, 14], and the lithogenic effect of EG must be mainly attributed to oxidative damage caused by the high level of oxalate generated by EG. Thus, although the EG rat model can be questioned as a general model to study renal stone formation, it must be considered as an interesting model for evaluation of renal papillary stone development, at least for those stones where genesis is linked to oxidative cell damage. Thus, the first studies on experimental EG renal lithiasis appeared in the 1960s [15, 16], but the importance of oxidative damage

Table 2 Urinary biochemical data at the end of the experiment, following 8 days of ethylene glycol supplementation

	Control group	CAT-treated group	EPI-treated group	FHE-treated group
V (mL/24 h)	6.4 ± 0.9	8.8 ± 2.5	4.8 ± 0.9	7.1 ± 1.3
pH	8.3 ± 0.3	7.7 ± 0.4	8.9 ± 0.1[a]	7.8 ± 0.3[b]
Phosphorus (μmol/24 h)	415.3 ± 53.5	450.1 ± 153.9	502.7 ± 41.5	829.1 ± 78.1[a,b,c]
Calcium (μmol/24 h)	5.7 ± 0.9	4.8 ± 2.1	4.6 ± 1.2	5.0 ± 0.7
Magnesium (μmol/24 h)	32.8 ± 2.3	21.0 ± 8.2	21.2 ± 0.3[c]	34.4 ± 4.1[b]
Oxalic acid (μmol/24 h)	74.2 ± 18.6	56.0 ± 15.4	64.3 ± 13.0	52.2 ± 14.2
Citric acid (μmol/24 h)	6.8 ± 1.2	5.8 ± 2.1	5.0 ± 0.9	5.2 ± 1.8

Values represent mean ± SE of nine animals per group

[a] Values are significantly different to CAT-treated group $P < 0.05$

[b] Values are significantly different to EPI-treated group $P < 0.05$

[c] Values are significantly different to control group $P < 0.05$

caused by hyperoxaluria was not clearly proposed until the end of the twentieth century [17]. During this period, there were several reports of prophylactic treatment of EG-induced nephrolithiasis using herbal extracts and antioxidants [3, 18–23], but in none did the effect seem to be mediated by diuretic or other urinary biochemical changes, and the positive effects on calcium oxalate lithiasis are most likely due to antioxidative effects.

The present study investigated the effects of the well-recognized antioxidant herbal flavonoids CAT and EPI on EG-induced renal lithiasis in rats, and the results were compared with those obtained using FHE, a folk herbal extract that also contains recognized antioxidants. As in previous studies [3–5], the medicinal plant compounds used had little effect on urinary chemistry related to urolithiasis. In fact the consumption of CAT or EPI did not have any detectable effects on urine chemistry. When treated with FHE, calcium deposition in rat kidneys was significantly reduced to levels similar to those seen with CAT and EPI. These results clearly demonstrate the ability of antioxidants to prevent the development of papillary and intratubular calcification of the kidney, consequently preventing the development of papillary calculi.

Diverse plant extracts recommended to avoid renal stones, such as the FHE used in this study, have a high antioxidant capacity owing to the presence of flavonoids and vitamins [8, 24, 25]. These substances may prevent calcium oxalate crystal development in the kidney by avoiding hyperoxaluria-induced peroxidative damage to the renal tubular membrane surface (lipid peroxidation) [17, 26, 27] and the papillary tip epithelium, which can in turn prevent heterogeneous calcium oxalate crystal nucleation on damaged cells or cellular detritus, and subsequent development of kidney stones.

Consequently, the present study suggests that the antioxidant action of herbal extracts could be of importance in explaining their antilithiasic action, but this behavior must be restricted to effects on the development of COM papillary calculi. Thus, it can be concluded that the antioxidant activity of herbal extracts could have an important role in avoidance of COM papillary calculi formation, particularly if formation of these calculi is induced by lesions caused by cytotoxic substances with oxidative capacity.

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