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The effect of cellulose on crystal formation in the kidneys of guinea pigs fed diet rich in calcium and sodium

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ABSTRACT

Our earlier report showed that diet rich in calcium and sodium fed to guinea pigs can be urolithogenic. In this study, we investigated the effect of adding 3% or 15% cellulose as a source of fiber into similar diet to asses the formation of kidney calculus. From the result, animals fed diet with 3% cellulose showed a relatively high concentration of urinary oxalate, urate and large number of crystals in the kidneys. When the cellulose level was raised to 15%, the concentrations of urinary calcium, urate and the number of crystals formed in the kidneys were lowered. We therefore, conclude that high fiber diet may be beneficial in reducing the incidence of kidney stones of calcium origin.

INTRODUCTION

Over the years, substantial efforts were made to explain why some individuals form kidney stones and others do not. In Malaysia (Syed *et al.,* 1990) and other Industrialized nations (Smith, 1976), majority of patients with kidney stones were classified as having the syndrome of indiopathic calcium urolithiasis. These patients formed predominantly calcium oxalate stones, frequently mixed with calcium phosphate (Syed *et al*., 1988; Prien & Prien, 1968). A number of factors had been investigated and an association of its pathogenicity with diet had been postulated (Enderson, 1973). High incidence was seen in individuals with high sucrose and low fibre diet (Haber *et al*., 1977) whilst Rao et al. (1982) showed low incidence in diet with less meat, fat and sugar but more dietary fiber. Over the years cellulose, a component of dietary fiber has been found to have blood cholesterol lowering effect (Nomani *et al*., 1982), but its application to kidney stone etiology is unknown. In this paper, we summarise the evidence that the inclusion of cellulose in the diet may be beneficial in reducing crystals accumulation in the kidneys and subsequently may reduce the incidence of kidney stones in individuals.

MATERIAL AND METHOD

Animals

Twenty male guinea pigs averaging 250-400g were obtained from the animal unit, Institute for Medical Research (IMR), Kuala Lumpur. They were assigned at random, 10 animal per treatment of 2 different treatments. Each animal was kept in a separated cage.

Diet

The basal diet was semipurified in nature and formulated to be isofibrous and isonitrogenous. It contained corn starch, corn oil, casein supplemented with DL-methionine. The diet also contained the USP salt mixture supplemented with copper sulphate and zinc carbonate. The diet were urolithiatic in nature (Coburn & Packett, 1962) and were prepared by adding to the basal diet 3% calcium and 0.7% phosphorus. In addition, the diet also contained 48% sodium and 1.6% potassium to potentiate the development of crystals in the kidneys fed with urolithiatic diet (Syed *et al*., 1993). Cellulose obtained from Sigma was added to the diet at 3% and 15% @ levels respectively. The detailed composition of the experimental diets are shown in the Table 1.

Table 1. Composition of experimental diets

Composition	Diet	
	Low Fiber (%)	High Fiber (%)
Casein	25.0	25.0
DL methionine	0.3	0.3
Corn oil	4.0	4.0
Corn starch	53.77	41.77
Cholesterol	0.21	0.2
Vitamin mix	3.0	3.0
Basal Mineral @ Add	0.630	0.630
1. $CaCO_3$	3.0	3.0
NaH_2PO_4	0.7	0.7
2. NaCl	4.8	4.8
Potassium acetate	1.6	1.6
Cellulose	3.0	15.0
Total	100	100

@ Basal mineral contained $MgSO_4$ 0.3%; MnSO4 0.062; $CuSO_4$ 0.02%; KIO_2, 0.0020%; $ZnCO_3$ 0.01%; Ferric citrate 0.036% & Chloride 0.2%, respectively.

Experimental design

The animals were provided with about 30g feed and tap water *ad libitum*. The duration of the experiment was 60 days including 14 days acclimatization.

Body weight per week and daily feed consumption records were maintained on each animal. Urine was collected each morning at 7 am from the individual animal. Two ml toluene was added to each bottle and kept at 40º C in cold room at the Department of Biochemistry, Medical Faculty, UKM. At the end of the week the urine were pooled together as weekly urine collection. At the end of the experiment each animal was fasted for 12 hour prior to killing by cervical dislocation. Each animal was incised and the visceral organs exposed. The kidneys were removed kept frozen in a plastic bag until analysis.

Analysis

The concentration of calcium and oxalate in the urine were determined by atomic absorption spectrophotometry using model Shimadzu model AA-670 using the method described by Hurst (1969), Kramlm (1966) and Yanagawa *et al*. (1983), respectively. Uric acid concentration was determined by colorimetry as described by Caraway (1963) and sodium was estimated by flame photometry. Concentration of calcium, oxalate and sodium were expressed as mmol/L^{-1} and uric acid as μg/ml^{-1}.

Kidneys

A method developed by the unit to determine the presence of crystals was used (Syed *et al*., 1992). Briefly, a fragment of kidney medulla and pelvis each 5 mm was placed in a petri dish containing 5 ml distilled water. Next, the tissues were gently squashed to free any crystal present. About 1 ml homogen was placed onto a glass slide and the presence of crystals were examined under 10 times magnification. Ten or more crystals per night power field indicate the development of calculi.

Statistics

Student's T test was used to detect differences in all parameters in the 2 groups of animals studied. All values were expressed as mean ±1 standard error. Correlation was also done on the mean volume of the urine collected over the weeks duration.

RESULTS

The urinary fluid volume for the group fed urolithiatic diet with 15% cellulose from 1st week to 6 week were 318.67±107.65m1, 297.67±79.38m1, 226. 17±62.82m1 150.5±10.09m1, 156.17±26.96m1 and 287.5±102. 50m1, respectively. The collection for each given week was high and significantly different $p<0.05$ from the other group that was fed urolithiatic diet incorporated with 3% cellulose (Figure 1).

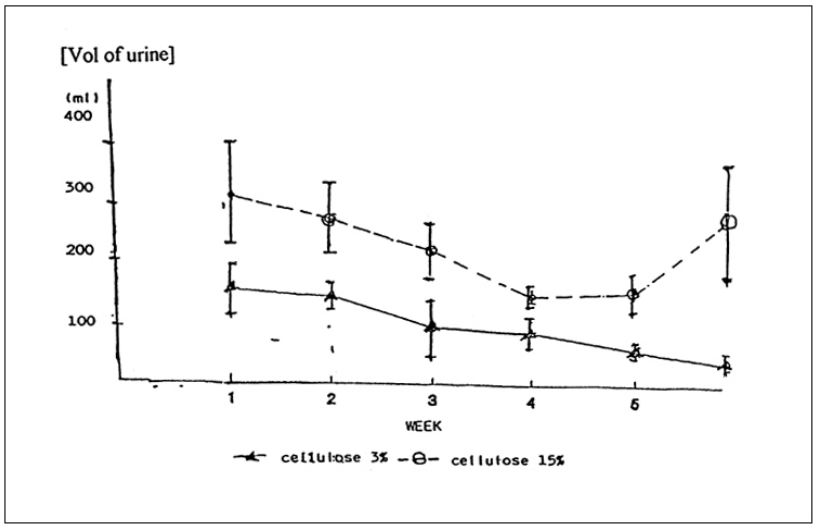

Figure 1. Volume of urine (ml) collected versus week

The amount of urine (ml) for the later group was 160.83±43.03m1, 147.50±36.25m1, 97±47.53m1, 89.50±39.89m1, 58.5±1.Sml and 36±9m1, respectively. The weekly collection showed a progressive reduction as the experiment progress with r =-0.98.

Next, the concentration of urinary calcium oxalate, uric acid and sodium were also studied. As expected, the addition of 15% cellulose to the urolithiatic diet reduces the concentration of these urinary constituents.

A shown in Figure 2, the concentration of calcium was significantly lower at $p<0.05$ for any given week.

Similarly with that of oxalate (Figure 3). significant differences at $p<0.05$ was observed in urine from the 3rd week onward. Urine values for the 3rd week for uric acid and sodium are shown in Figure 4 and Figure 5. the animals fed with 15% cellulose showed lower concentration of uric acid as early as the 3rd week and sodium from the start of the experiment.

As shown in Table 2, the presence of crystals in the kidneys was observed in animals fed 3% cellulose. No crystals were formed with the groups fed 15% cellulose.

Table 2. Appearance of crystals in the kidneys

Type of urolithiatic diet	Cellulose	No. of animals (N = 10)	Percentage
High ratio of Ca/P & Na/K	15%	-	-
	3%	7	70%
High ratio of Ca/P & Low ratio of Na/K	3%	1	10%

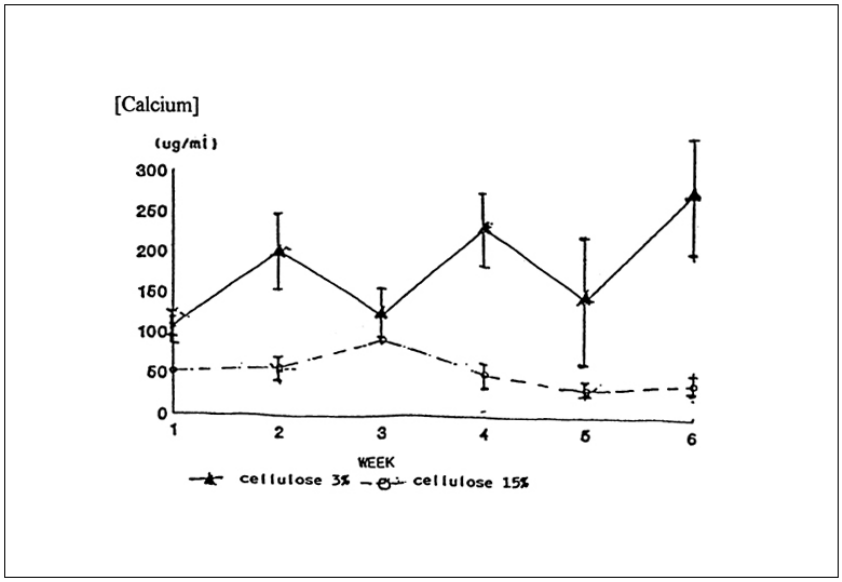

Figure 2. Concentration of calcium ion in urine versus week

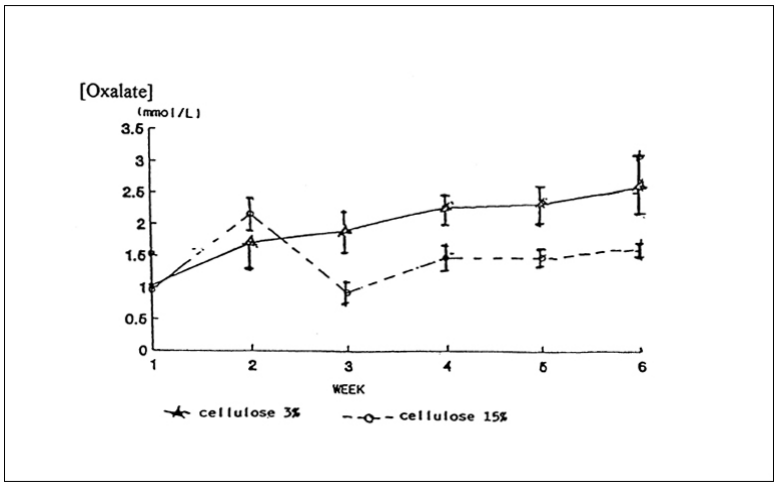

Figure 3. Concentration of oxalate in urine versus week

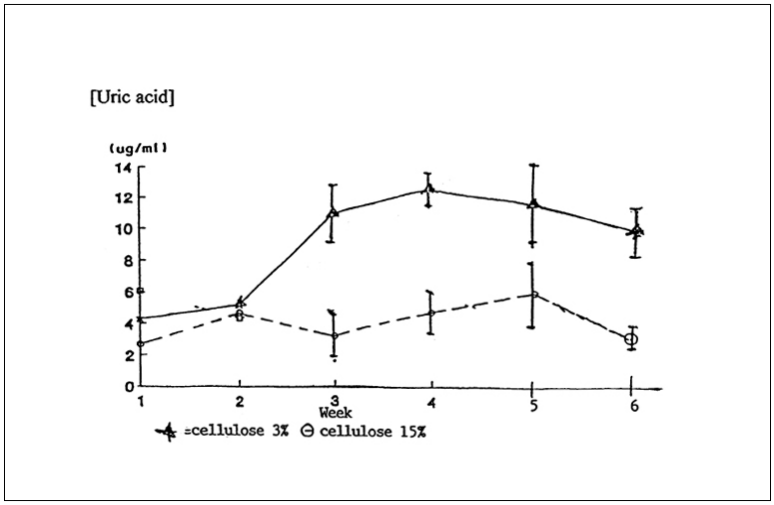

Figure 4. Concentration of uric acid in urine versus week

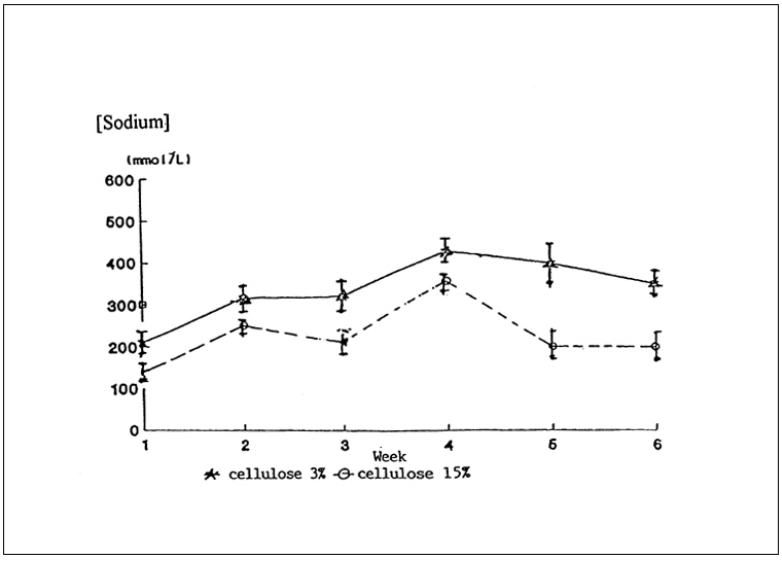

Figure 5. Concentration of sodium ion in urine versus week

DISCUSSION

Inclusion of high cellulose to the diet reduces the incidence of urolithiasis. The presence of high cellulose in the diet could reduce formation of calcium, oxalate, uric acid and sodium in the urine. Robertson (1969) have suggested that the saturation of these materials are essential for spontaneous precipitation of nucleus formation, aggregation of crystals during calculi development. How cellulose was able to reduce the development of crystals is still debatable. It could be related to the retention of minerals in the gastrointestinal tract.

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