ABSTRACT: An experimental work was carried out in wistar rats to study the histopathological changes caused by different diets on the kidney and urinary bladder and to understand the risk of calcium oxalate crystallization. Both non-diabetic non-calculogenic and diabetic non-calculogenic rats were used for this study. The rats were fed on standard laboratory diet / diet low in vitamin A and diet rich in vitamin C for three months. At the end of the experiment the kidney and urinary bladder were excised and the tissues were processed for the histopathological study. The histopathological changes such as epithelial desquamation, interstitial inflammation and crystalline deposits of grade + were observed in the kidneys of rats on low vitamin A diet indicating the tendency of low vitamin A diet to promote lithogenesis. Amorphous debris and the birefringent crystalline deposits seen in the tubules along with metaplasia in the urinary bladder of rats on high vitamin C diet which indicate the tendency of high vitamin C diet to aggravate the calculogenic propensity.

Key words: Non-diabetic non-calculogenic (NDNC), Diabetic non-calculogenic (DNC), Lithogenesis

INTRODUCTION

Urolithiasis is a condition in which crystals in the urine combine to form stones or uroliths. The calculi are formed by deposits of polycrystalline aggregates composed of varied amounts of crystalloid and organic matrix which vary in size and may be found anywhere in the urinary tract from the kidney to the bladder. This causes irritation and secondary infection. Kidney stone formation or urolithiasis is a succession of several physicochemical events including super saturation, nucleation, growth, aggregation and retention within the kidneys (Yadav, et al, 2011). It is understood that among the various stone problems encountered in the human body, urinary stone disease is the most complicated.

The indispensable role of vitamins in nutrition and the various disorders due to vitamin deficiency have attained great attention in relation to clinical problems. It is seen that the dietetic pattern among the humans is diverse and rarely selective and hence the possibility for single extreme vitamin deficiency is rare; but avitaminosis is observed among them. It is known that vitamin A is necessary to keep the epithelial tissues intact. Vitamin A signs of deficiency include corneal keratinization and ulceration, respiratory and skin infections, salivary gland enlargement, and urinary tract disease (Rogers, 1979, John S Munday et al, 2009). Vitamin C supplementation is not recommended because ascorbate is a metabolic precursor of oxalate. Increased intake of vitamin C has been associated with increased incidence of urolithiasis (Anderson, 1973, Mayak Mohan Agarwal et al, 2011). The aim was to study the histopathological changes if any on the kidney and urinary bladder of wistar rats being fed on different diets to understand the risk of calcium oxalate crystallization.

MATERIALS AND METHODS

The experimental work was done in male rats of wistar species each weighing 200-250 gm. The control and experimental rats were maintained in cages and were fed on standard laboratory diet / diets low in vitamin A, rich in vitamin C and oxalate and water / drugs ad libitum. Six rats each were included in the control and experimental group and the experiment was conducted for a period of three months.
Diabetes was induced in the experimental group of rats by injecting 3% aqueous solution of alloxan monohydrate in a dose of 150 mg/kg body weight prepared by weighing the requisite dose of the drug and dissolving it immediately in distilled water and administering to the rats. The urine and blood samples of these rats were collected and the presence of sugar was noted.

The low vitamin A diet was prepared for the experimental rats by mixing 5 kilogram of ground raw rice with one kilogram of casein and 25 milliliter of kidicare syrup and ten numbers of powdered B complex tablets, the food stuffs known to be deficient in vitamin A. This low vitamin A diet was given to the experimental rats for three months to see whether this diet has any role in calculogenesis. The high vitamin C diet was prepared by mixing 5 kilogram of rat feed with 10 grams of vitamin C. This high vitamin C diet was given to the experimental rats for three months to see whether this diet aggravated the calculogenic propensity.

**Observation**

The histopathological lesions if any and presence of crystals in the kidneys and the urinary bladder of the rats were studied. The kidneys of the non-diabetic non-calculogenic rats showed normal histology (x200) (Figure 1). In the kidneys of diabetic non-calculogenic rats, sclerosis of some glomeruli (x200) (Figure 2), and interstitial inflammation was observed. No crystals were seen. Urinary bladder showed no significant changes.

The kidneys of non-diabetic non-calculogenic rats on low vitamin A diet showed epithelial desquamation. The bladder did not show any significant change. The kidneys of diabetic non-calculogenic rats on low vitamin A diet showed interstitial inflammation and epithelial desquamation (x450) (Figure 3). The tubules were dilated and had debris and crystal deposits of grade+ (crystals in 1-3 tubules in each cross section). The bladder showed squamous metaplasia (x450) (Figure 4).

![Figure -1ND NC: Normal histology of kidney](image1)

![Figure -2- D NC: Glomerulosclerosis indicate diabetic changes](image2)
The kidneys tubules of non-diabetic non-calculogenic rats, on high vitamin C diet showed amorphous debris in some tubules when viewed under high magnification (x450) and some crystalline deposits in certain tubules. Bladder showed focal squamous metaplasia. In the diabetic non-calculogenic rats, there were occasional tubules in the kidneys showing birefringent crystals of grade+ and amorphous debris (Figure.5). Under polarizing light the crystals were clearly seen (x450) (Figure.6). The bladder showed no significant change.

Figure -3 - Interstitial inflammation & epithelial desquamation in kidney

Figure-4-Chronic inflammation, ulceration & squamous metaplasia in bladder

Figure -5- Amorphous debris in kidney
DISCUSSION
The normal glomeruli and absence of debris and crystalline structures in the kidney and the normal histology of the urinary bladder of the non-diabetic non-calculogenic rats indicate that these rats don’t exhibit changes of either diabetes or calculogenesis. The sclerosis of some glomeruli of the kidneys and interstitial inflammation seen under higher magnification in the diabetic non-calculogenic rats indicate the diabetic changes produced on administration of diabetogenic drug. The epithelial desquamation, interstitial inflammation and crystalline deposits of grade + seen in the kidneys of rats on low vitamin A diet indicates the tendency of low vitamin A diet to promote lithogenesis. The mechanisms by which vitamin A deficiency causes calculus formation in rats are unclear. Vitamin A deficiency alters the composition of urine (Grases et al, 1998, John S Munday et al, 2009). In addition, squamous metaplasia of the urinary tract can result in keratin debris which promotes calculus formation (Kankesan et al, 2003).

The amorphous debris and the birefringent crystalline deposits seen in the kidney tubules along with metaplasia in the urinary bladder of rats on high vitamin C diet indicate the tendency of high vitamin C diet to aggravate the calculogenic propensity. Khan et al (1996) feels that calcium oxalate crystals are always found intermingled with cellular degradation products.

CONCLUSION
The experimental studies on animals and clinical studies have showed that vitamin A deficiency enhanced the severity of urinary calculi disease. But no systematic study till date is available to correlate the vitamin A-deficient status with their predisposition to urinary calculi disease. Similarly, till date no clear evidence or reports have been put forth to show that there is no correlation between vitamin C and kidney stones. So the present investigation was carried out to find out whether vitamin A-deficient state and hypervitaminosis C can enhance the risk of urolithiasis in the diabetics. The epithelial desquamation, interstitial inflammation and crystalline deposits of grade + seen in the kidneys of rats on low vitamin A diet indicates the tendency of low vitamin A diet to promote lithogenesis. The amorphous debris and the birefringent crystalline deposits seen in the kidney tubules along with metaplasia in the urinary bladder of rats on high vitamin C diet indicate the tendency of high vitamin C diet to aggravate the calculogenic propensity. In fact the basic objective of the study was to understand the relationship between diabetics and urinary stone disease in human beings. The uncertainties in the knowledge about the relationship between diabetics and urinary stone formation prompted this study.

ACKNOWLEDGEMENT
I am extremely thankful to my guide Dr.Y.M.Fazil Marickar, Prof& H.O.D., (Rtd), Department of Surgery, Medical College Hospital, Trivandrum and co-guide Dr.K.O.John, Professor of Zoology, Department of Zoology, Mar, Ivanios College, Trivandrum for their constant sincere support and encouragement rendered to me throughout the work. I am also thankful to the Principal of Medical College for providing all the required facilities in the Animal House associated with the Medical College for doing the experimental work. I am also thankful to Dr.Harikrishnan, the Pathologist, Medical College, Trivandrum for the sincere help rendered to me in the completion of the histopathological work. I also thank the University of Kerala for granting JRF for three years which helped me in completing the work successfully.
REFERENCES


