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SYMPOSIUM PAPER

Role of scanning electron microscopy in identifying drugs used in medical practice

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Abstract Several plant preparations are administered for treatment of stone disease without scientific basis. This paper presents the results of in vitro and animal experimental studies using scanning electron microscopy (SEM) in the identification of the therapeutic properties of trial drugs in medicine. In the first set of the study, urinary crystals namely calcium oxalate monohydrate and calcium oxalate dehydrate were grown in six sets of Hane's tubes in silica gel medium. Trial drugs namely scoparia dulcis Lynn, musa sapiens and dolicos biflorus were incorporated in the gel medium to identify the dopant effect of the trial drugs on the size and extent of crystal column growth. The changes in morphology of crystals were studied using SEM. In the second set, six male Wistar rats each were calculogenised by administering sodium oxalate and ethylene glycol and diabetised using streptozotocin. The SEM changes of calculogenisation were studied. The rats were administered trial drugs before calculogenisation or after. The kidneys of the rats studied under the scanning electron microscope showed changes in tissue morphology and crystal deposition produced by calculogenisation and alterations produced by addition of trial drugs. The trial drugs produced changes in the pattern of crystal growth and in the crystal morphology of both calcium oxalate monohydrate and calcium oxalate dihydrate grown in vitro. Elemental distribution analysis showed that the crystal purity was not altered by the trial drugs. Scoparia dulcis Lynn was found to be the most effective anticalculogenic agent. Musa sapiens and dolicos biflorus were found to have no significant effect in inhibiting crystal growth. The kidneys of rats on calculogenisation showed different grades of crystals in the glomerulus and interstitial tissues, extrusion of the crystals into the tubular lumen, collodisation and tissue inflammatory cell infiltration. Scoparia dulcis Lynn exhibited maximum protector effect against the changes of calculogenisation. Musa sapiens and dolicos biflorus had only minimal effect in preventing crystal deposition, inflammatory cell infiltration and other changes of calculogenisation. SEM was found to be effective in assessing the effect of drugs on crystal growth morphology and tissue histology.

Keywords Urinary stone · SEM · Crystal growth · Scoparia dulcis Lynn · Musa sapiens · Dolicos biflorus · Inhibition · Calculogenisation

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Introduction

Prevention of stone formation is primarily centered on effectively restricting the initial process of stone nucleation in the urinary tract. Randall's plaques were considered as the primary initiation of urinary stone forming pathology [1]. Intracellular calcium and oxalate levels in the renal tissues assist in fixing crystal particles in the process of this

initiation of stone formation [2]. Renal tubular injury produced by lack of protective effect in the kidney tubules and the injury produced by the harmful agents like oxalate are also reported [3]. It is thus obvious that stone formation is influenced by presence of increased amounts of calculogenic agents like oxalate and calcium, presence of unhealthy urothelial tissues as would happens in tissue damage produced by toxic agents and the absence of protective effect in the stone forming organ. The primary objective of deciding a treatment schedule for the urinary stone patient should take into consideration the correction of physicochemical abnormality in the urine, and seeing that the drugs themselves do not produce significant side effects [4]. Various ayurvedic medicines and plant extracts have been used for the treatment of urinary stone disease from time immemorial [5–7]. They are, however, given without scientific basis. Usefulness of any drugs will imply clinical improvement and metabolic correction in blood, urine, body fluids and tissues. Clinical experiments cannot be done without doing in vitro and animal experimental work. The present paper was worked up to identify the role of various plant extracts namely scoparia dulcis Lynn, musa sapiens and dolicos biflorus in inhibiting crystal nucleation and growth and in protecting the kidney tissues from the damages produced by the tissue destroying and calculogenising agents like oxalates. The studies were conducted in in vitro and animal models using male witsar rats. The objective of this paper was to identify the influence of the above three plant extracts in the process of urinary stone formation in the kidney. Scanning electron microscopy (SEM) was utilised for identifying the actual role of these plant extracts in the process of calculogenesis and prevention of crystal deposition and growth.

Materials and methods

Urinary crystals namely calcium oxalate monohydrate and calcium oxalate dihydrate were grown in six sets of Hane's tubes in silica gel medium (Fig. 1). Trial drugs namely scoparia dulcis Lynn (Fig. 2), musa sapiens (Fig. 3) and dolicos biflorus (Fig. 4) were incorporated in the gel medium to identify the dopant effect of the trial drugs on the size and extent of crystal column growth. The changes in morphology of crystals were studied using SEM. In the second set of studies, six male Wistar rats each were calculogenised by administering sodium oxalate and ethylene glycol and diabetised using streptozotocin. The SEM changes of calculogenisation were studied. The rats were administered trial drugs before calculogenisation/diabetisation or after. The kidneys of rats were collected and studied under the scanning electron microscope under different magnifications. Scanning electron microscopic changes and extent of crystallisation were assessed to reach final conclusions.

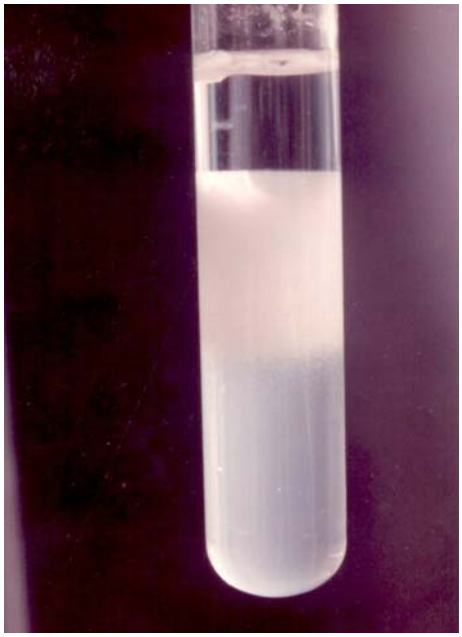

Fig. 1 COM crystals grown in Hane's tube

Fig. 2 Scoparia dulcis Lynn

Results and discussions

In the first set of experiments, COM (Fig. 5) and COD crystals were successfully harvested from the gel in the in vitro crystal growth medium. Addition of the trial drugs to the

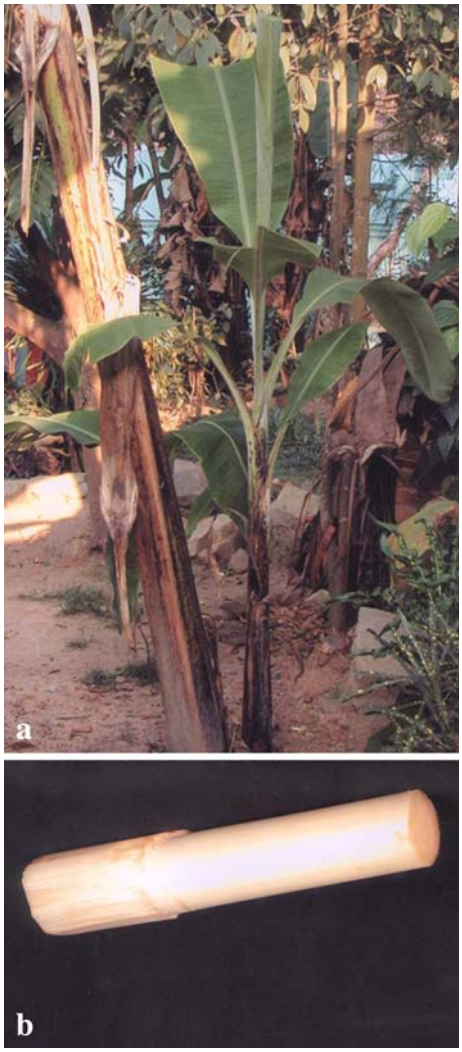

Fig. 3 **a** Musa sapientum, **b** Musapith

Fig. 4 Dolicos biflorus

silica gel medium produced changes in the pattern of crystal growth and in the crystal morphology of both calcium oxalate mono hydrate and calcium oxalate dihydrate. The morphological changes were pronounced in the SEM studies.

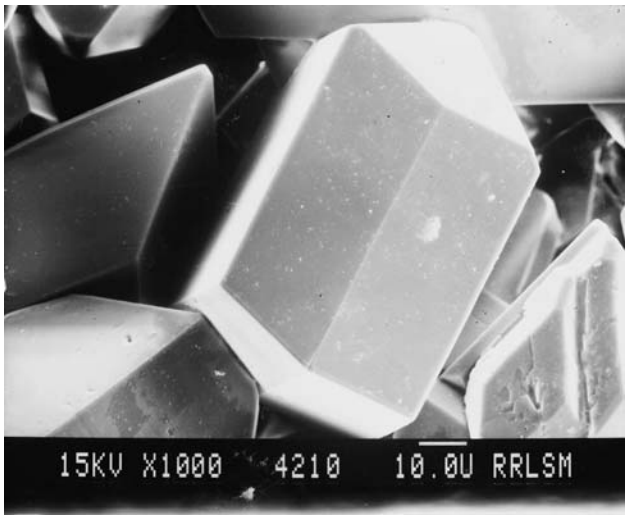

Fig. 5 SEM of COM crystals grown in control set in silica gel medium

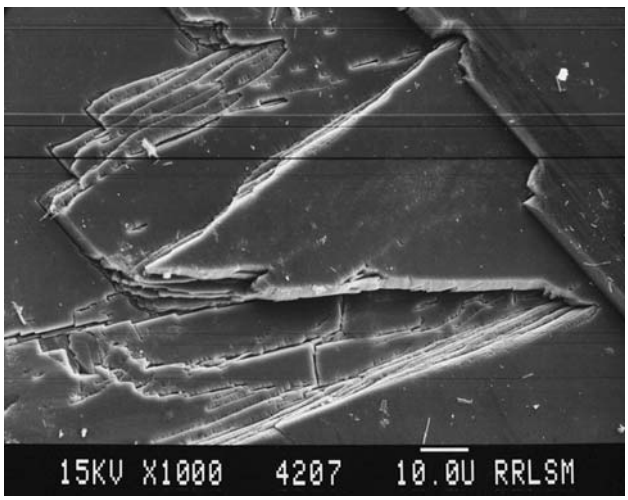

Fig. 6 SEM of COM crystals grown after adding extract of scoparia dulcis Lynn

Elemental distribution analysis showed that the crystal purity was not altered by the trial drugs. Scoparia dulcis Lynn was found to be the most effective anticalculogenic agent. The crystals were distorted and ill formed (Fig. 6). Musa sapiens and dolicos biflorus were found to have no significant effect in inhibiting crystal growth.

In the second set of experiments, the kidneys showed maximum changes of calculogenisation and hence the protective effects of trial drugs could be studied well in this experiment. Major components of calculogenisation included presence of different grades of crystals in the glomerulus and interstitial tissues, extrusion of the crystals into the tubular lumen, collodisation and tissue inflammatory cell infiltration (Fig. 7). Scoparia dulcis Lynn was found to exhibit maximum protector effect against the changes of calculogenisation (Fig. 8), when given before calculogenisation. Musa sapiens and dolicos biflorus were

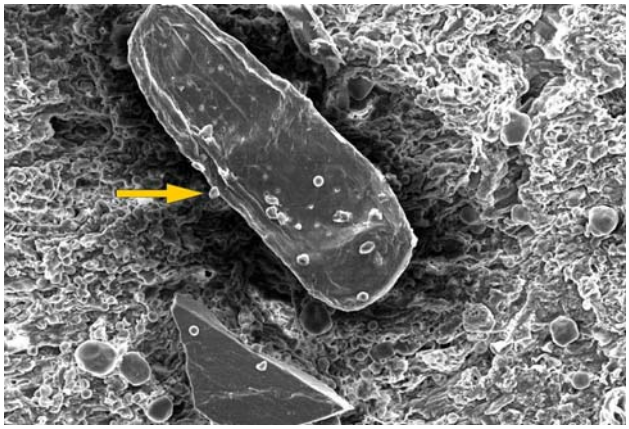

Fig. 7 Cut surface of kidney of rat in diabetised calculogenised group showing large crystals seen destroying the surrounding tissue

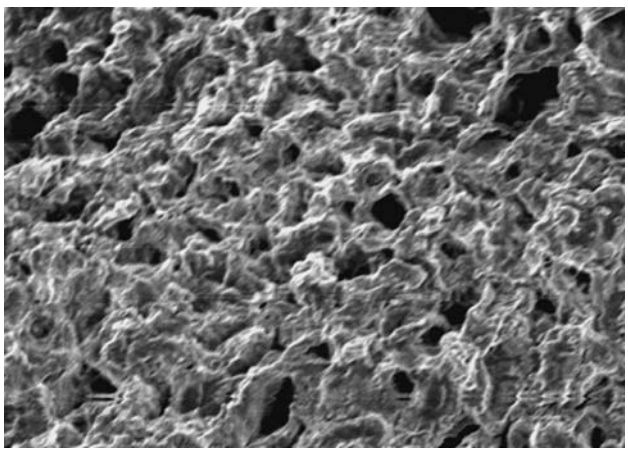

Fig. 8 Cut surface of rat's kidney in sweet broom weed administered after 1% oxalate showing normal architecture

found to have only minimal effect in preventing crystal deposition, inflammatory cell infiltration and other changes of calculogenisation. The SEM was found to be effective in assessing the effect of drugs on crystal growth morphology and tissue histology. After calculogenisation of the rats, administration of the scoparia dulcis Lynn produced significant reduction in the size of the crystals which were already formed in the tubular lumen. The injury to the urothelium also decreased significantly when scoparia dulcis Lynn was administrated after the calculogenisation of the rat tissue. There was no significant protective effect seen in the rats administered either musa sapiens or dolicos biflorus. Dolicos biflorus itself appeared to produce certain tissue injuries even before the calculogenisation occurred.

Discussion

It is well recognized that the nucleation of crystals in any crystal growth medium will be influenced by the presence of dopants in the medium. Dopant may be promoter or inhibitor. The SEM findings of the in vitro crystal growth study showed that the presence of scoparia dulcis Lynn in the environment reduced the size of crystals, and aggregation of crystals in the growth medium.

Stone formation in the kidney may be dictated by cellular and urinary macromolecules which increase the aggregation of the crystals and help them in getting attached to the renal cells and mature to form a stone [8]. Earlier investigators [9–11] have recognised that the early histological changes of calculogenisation are centered on renal tubules and papillae. Large crystal masses have been reported in the interstitium of corticomedullary junction in diabetised calculogenised rats [12]. The observations of the present study indicate that toxicity of the rat renal tissues can be significantly produced by calculogenising agents like oxalates and glyoxalates. Such calculogenisation will produce injury to the urothelium and presence of large crystals in the tubular lumen. When scoparia dulcis Lynn was administered to the rats before calculogenisation, injury to the renal tubular cells was significantly reduced. There was also almost total absence of crystals on calculogenisation.

Presence of free floating crystals in the tubular lumen may not be considered pathological [13]. The pathology arises when these crystals get adherent to the tubular epithelium. From this, it is clear that a drug which is to be effective for preventing stone formation should have the capacity to prevent adhesions between the forming crystals and the tubular lumen. Adhesions of the crystals to the urothelium may produce release of renal enzymes which may be helping the further adhesions between the crystals and the tubular lumen [14]. Scheid et al. [15] believe that oxalate itself at high concentrations can injure the tubular cells.

Conclusion

From these observations, it can be concluded that extract of plant scoparia dulcis Lynn can be beneficial in preventing stone formation. Dolicos biflorus and musa sapiens would not produce any significant positive effect in preventing stone formation. They might produce deleterious effects in the ultramicroscopic structure of the urinary tubules and urothelium. The therapeutic effects of various trial drugs can be scientifically evaluated by the use of scanning electron microscopic studies of the crystals grown in vitro and kidney tissues in animal experiments.

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