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Animal Models For Urolithiasis – A Short Review

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ABSTRACT

Urolithiasis is a major health problem in south East Asia. This disease in India is characterized by endemic occurrence in a distinct geographical pattern the reasons for this is unclear. It has been suggested that dietary patterns of the population may play an important role in development of urolithiasis. The most painful urologic disorder is formation of calculi or stones in the kidneys and urinary bladder due to imbalance between promoters and inhibitors of crystallization in urine. Stone formation is documented from traditional periods and is considered as a medical challenge due to its multifactorial etiology. Stone formation commonly occur due to inadequate urinary drainage, foreign bodies in urinary tract, microbial infections, diet with excess oxalates and calcium, vitamin abnormalities like vitamin A deficiencies, excess vitamin D and metabolic diseases like hyperthyroidism, cystinuria, gout, intestinal dysfunction etc. Methods for the induction of experimental urolithiasis in animals are necessary for the understanding of the etiology of this disease as well as for the development of drugs which may prevent the growth of urolithiasis or may help in the resolution of the renal calculi.

Keywords: Urolithiasis, Hyperthyroidism, Gout, Cystinuria, Ethylene Glycol.

INTRODUCTION

Urinary calculi are the third prevalent disorder in the urinary system. Approximately 80% of these calculi are composed of Ca $(COO)_2$ (calcium oxalate) and Ca_3 $(PO_4)_2$(calcium phosphate). Urinary calculi may cause obstruction, hydronephrosis, infection and hemorrhage in the urinary tract system. [1] Kidney stone formation is a complex process that results from a succession of several physicochemical events including supersaturation, nucleation, growth aggregation and retention within the renal tubules. [2] Among the used treatments, there are Extracorporeal Shock Wave Lithotripsy (ESWL) and drug treatment which revolutionized urological practice and almost become the standard procedure for eliminating kidney stones.[3] However, these procedures are highly costly and these procedures have a disadvantage of recurrence. [4]The recurrence rate without preventive treatment is approximately 10% at 1 year, 33% at 5 year and 50% at 10 years.[5] Therefore; it is worthwhile to look for an alternative to these means by using medicinal plants or Phytotherapy. A number of vegetable drugs have been used in many parts of the world for the treatment of urolithiasis. [6] Calcium oxalate kidney stones in both humans and mildly hyperoxaluria rats are located on renal papillary surfaces and consist of an organic matrix and crystals of calcium oxalate.

The rate of occurrence of stone formation is three times higher in men than women, so male albino Wistar rats were used in this study because of enhancing capacity of testosterone and inhibiting capacity of estrogens in stone formation. Many *in vivo* models have been developed to investigate the mechanisms involved in the formation of urinary stones, and to ascertain the effect of various therapeutic agents on the development and progression of the disease. Rats are the most frequently used animals in models of CaOx deposition in the kidneys, a process that mimics the etiology of kidney stone formation in human

ANIMAL MODELS FOR UROLITHIASIS

In vivo animal models for urolithiasis are commonly divided into three categories.

These are in the following

I. Chemical Induced Urolithiasis:

Chemicals Along With Drinking Water by Oral Administration

II. Diet Induced Urolithiasis:

1. Induced by oxalate calculi producing diet (CPD)
2. Urinary calculi formation in rats by high calcium diet

III. Minor surgical Methods:

1. Induction of urolithiasis by insertion of zinc disc into the urinary tract
2. Calcium oxalate seed model

I.CHEMICAL INDUCED UROLITHIASIS

ETHYLENE GLYCOL INDUCED UROLITHIASIS MODEL

Kidney stones in humans are most commonly composed of Ca $(COO)_2$ (calcium oxalate) & $Ca_3(PO4)_2$(calcium phosphate). [7] $Ca(COO)_2$ (calcium oxalate) urolithiasis experimental models in rats, induced by Ethylene Glycol ($C_2H_6O_2$) alone, or in combination with other drugs such as Ammonium Chloride (AC), are often used to study the pathogenesis of kidney crystal deposition.[8, 9] A metabolic precursor of oxalate is Ethylene Glycol ($C_2H_6O_2$). Hyperoxaluria, CaOx crystalluria, and occasional deposition of Ca $(COO)_2$ (calcium oxalate) crystals in the kidney were resulted by the administration of ethylene glycol. However, with Ethylene Glycol ($C_2H_6O_2$) various crystal deposition rates have been reported. None of the rats developed renal crystal deposition when treated with 0.8% Ethylene Glycol ($C_2H_6O_2$) for up to 24 days is found by Boevé's group. [8] A crystal deposition rate of 16.6% and 50% when rats were treated with 0.5% and 0.75% Ethylene Glycol (C2H6O2), respectively, for 24 days is reported by Khan.[10] A 71.4% kidney crystal deposition rate when rats were treated with 0.5% Ethylene Glycol ($C_2H_6O_2$) of for four weeks is found by Lee.[11] induction of metabolic acidosis, has been used in conjunction with Ethylene Glycol ($C_2H_6O_2$) ingestion to promote the deposition of Ca $(COO)_2$ (calcium oxalate) crystals in rat kidneys by the Ammonium chloride ingestion. Within 4 to 7 days with a dose of 1% or 2% AC in all rats' combination with EG the kidney stones were deposited. [8, 12]

EXPERIMENTAL DESIGN

Thirty male Sprague-Dawley adult rats weighing 250-300 gm were randomly divided into three groups. Each group consists of 10 rats.

Table 1: Treatment Groups

Group no*	Treatment	Treatment period
I	Standard rat chow diet & tap water	Fed for 4 weeks
II	Lab Diet lactose rich diet containing 3.68% sucrose, 30% lactose, 23.4% protein, 10% fat, 5.3% crude fiber, 6.9% ash minerals (calcium 0.95%, phosphorus 0.67%, magnesium 0.21%), vitamin A 22 IU/gm, vitamin D 4.5 IU/gm, vitamin E 49 IU/gm & tap water.	Fed for 4 weeks
III	Lactose rich diet & 1% Ethylene Glycol in drinking water	Fed for 4 weeks

*All rats were housed individually in metabolism cages and maintained at 22± 2 °C

URINE AND BLOOD SAMPLING

Urine was collected over a 24-hour interval and stored on 1% 2M HCL. The daily urine volume, urine pH and total excretion of oxalate, citrate, creatinine, uric acid, calcium, magnesium, phosphate, sodium, potassium and chloride were measured in the fourth week. On the day of sacrifice, rats were anesthetized with sodium pentobarbital (50 mg/kg BW, i.p.) and five ml. venous blood was withdrawn from the inferior vena cava for determination of serum creatinine, calcium, magnesium, phosphorus, sodium, potassium and chloride.

VERIFICATION OF CRYSTALLURIA AND URINARY STONE FORMATION

The collected urine was centrifuged at 3000 *g* for 10 minutes and then examined under light microscopy (×40) for determination of quantitative crystalluria. On the day of sacrifice, the kidneys were removed, cut longitudinally, and examined grossly to verify stone formation. The right kidney tissue was fixed with formaldehyde for hematoxylene eosin stain for grading of CaOx crystal deposition.

ASSAYS

Urinary oxalate was determined by the oxalate oxidase enzymatic method with an oxalate assay kit (Sigma). Urinary citrate was assayed by the enzymatic method (by using citrate assay kit). Serum and urine creatinine concentrations were measured by the Rate Gaffe reaction. The serum and urinary sodium, potassium, chloride and phosphate were measured by auto analyzer.

EVALUATION

The results are expressed as means± S.D. The differences of body and kidney weight, serum and urinary parameters among 3 different groups were tested using the Kruskal-Wallis test. The incidence of stone formation and crystal deposit in rats fed with different diet was evaluated by Fisher's exact test. A probability value of less than 0.05 was considered significant.

II. DIET INDUCED UROLITHIASIS

1.UROLITHIASIS INDUCED BY OXALATE CALCULI PRODUCING DIET (CPD)

The addition of 30% glycolic acid to the standard chow diet can lead to production of renal calculi within four weeks. [13] This is one of the easiest and least time consuming methods of producing urolithiasis in rats which has been used for studying the preventive effect of test compounds.

EXPERIMENTAL DESIGN

Male Wistar rats weighing 180-200g are acclimatized to the laboratory conditions for one week and then placed randomly into groups of ten. The control diet is the standard Purina laboratory chow (PLC) and the oxalate calculi producing diet (CPD) was containing 3% glycolic acid. The compounds to be tested for anti urolithiasis activity are usually added to the CPD in well defined concentrations. All diets are fed in powder form ad libitum.

Food and water consumption are measured during the 2nd & 4^{th} week of the experiment. Rats are weighed weekly. Pooled 24h urine samples from each group are collected using metabolic cages .In order to avoid feed contamination of urine, only water is provided during urine collection .therefore five rats from each group are placed in metabolic cages from 8:00a.m to 8:00p.m and the remaining five from each group complete the 24h collection from 8:00p.m. to 8:00a.m .Urinary p^H is measured and acidified urinary samples are stored at -20^0c until they are analyzed .At The end of the 4^{th} week on their respective diets ,all rats are euthanized. The urinary tracts are examined grossly and kidneys are preserved in phosphate buffered 10% formalin and stained with Mayer's Hemotoxylin and eosin for histological studies. Kidneys, heart, femur and a section of skeletal muscle were collected from each rat for oxalate and /or glycolate determination. Oxalate is determined by permanganate titration. Glycolate is determined by colorimetric method using 2, 7-dihydronapthalene after urine and tissue homogenates are prepared according to the procedure described by chow were treated with cation and anion exchange resins. [14, 15, 16]

Calculi are analyzed qualitatively as follows: oxalate – the fine crystals or pulverized calculi are immersed in 10% HCL and then covered with a small amount of MnO_2 (manganese dioxide). The release of small gas bubbles (CO_2) indicate the presence of oxalate, calcium-to the acid solution of the calculi, an excess amount of sodium hydroxide is added. The formation

of a white fine precipitate or film indicates the presence of calcium.

2. URINARY CALCULI FORMATION IN RATS BY HIGH CALCIUM DIET

A diet containing high fluoride and high calcium with calcium: phosphorous ratio 4:5:1 has been found to produce calcium oxalate crystalluria and promote the formation of bladder stones in rats. [17]

EXPERIMENTAL DESIGN

Weaning male rats with an average body weight of 150-200g are distributed in the groups of 12 each. The control rats received a basal diet containing 10% casein, 80% sucrose and adequate amount of minerals and vitamins. The experimental group received the diet containing high calcium and high fluoride. The composition of these diets are given tables 2& 3

Table 2: Composition of normal and experimental diets

Ingredients	Normal diet	Experimental diet
Casein	100	100
Sucrose	800	800
Refined peanut oil	50	50
Vitamin mixture[1]	10	10
Mineral (salt) mixture	40(mixture b)	40(mixture a)
Sodium fluoride	0	0.05

Vitamin mixture

1g of vitamin mixture contains Vitamin A 1000IU(International *Unit)*, Vitamin D 100IU, Vitamin E 10IU (International *Unit)*, Vitamin K 0.5mg, Thiamine 0.5Mg, Riboflavin 1.0mg, Pyridoxine 0.4mg, Pantothenic Acid 4.0mg, Niacin 4.0mg, Choline 200mg, Inositol 25mg, P-Amino benzoic Acid 10mg, Cyanocobalamine 2µg, Biotin 0.02mg, Folic Acid 0.2mg sufficient starch is added to make 1g.

Mineral salt mixture is prepared according to composition described by Hubbal [18]. The experimental groups of rats receive the diet with mineral (salt) mixture containing higher calcium concentration (a) where as the control groups receive the normal mineral (salt) mixture (b) as given in

Table 3: composition of mineral (salt) mixture

Ingredients	Mixture (a) g	Mixture (b) g
Potassium Aluminium Sulphate	0.18	0.18
Calcium carbonate	560	312
Potassium phosphate	230	230
Potassium chloride	112	112
Sodium chloride	69	69
Magnesium carbonate	35	35
Magnesium sulphate	20	20
Ferric phosphate	21	21
Cupric sulphate	0.9	0.9
Magnesium sulphate	0.4	0.4
Potassium iodide	0.008	0.008
Sucrose	0	250

In both the groups the rats are housed individually in stainless steel metabolic cages and the diets are fed ad libitum for 10 weeks. De Ionized, glass distilled water is provided for drinking. The Temperature of room housing the rat colony is maintained 24 ± 1^0C .The rats are weighed regularly and the daily intake of food recorded. Urine is collected over toluene for 2 consecutive days at the beginning of the experiment period. Volume & ph are recorded immediately. By using the standard procedure fresh urine is examined microscopically for crystalluria. [19] Urine is also analyzed for creatinine and oxalic acid.

After 10 weeks the rats are killed and the whole urinary tract is dissected out and examined for crystal deposits. All Urinary concentrations are removed, weighed and freeze dried to constant weight. The procedures for quantitative estimations of calculi components are described by Leonard and Butt. [20] The calculi are analyzed for proteins by the method of Lowry for calcium and magnesium by atomic absorption spectrometry for phosphorous by the method of Fiske and Subba Row.[21,22] Fluoride can be estimated by using a fluoride ion electrode after acing the calculi at low temperature according to the method of Singer and Armstrong .[23]

IV.MINOR SURGICAL METHODS

1. METHOD OF INDUCTION OF UROLITHIASIS BY INSERTION OF ZINC DISC

Rats were anaesthetized with intraperitoneal ketamine (50 mg/kg). A suprapubic incision was made and the abdomen was opened. The urinary bladder was then carefully exposed and the urine in the bladder was aspirated with a sterile syringe. A small nick was made at the apex end of urinary bladder and the sterile zinc disc (previously weighed) was carefully inserted into the bladder. Then the bladder was closed in a single stitch using chromic catgut (4-0). The abdomen was then closed in layers with chromic catgut and skin was closed with silk thread. The rats were allowed to recover from anesthesia. Food and 1% ethylene glycol in water was given ad libitum. The stone was allowed to form and grow inside the bladder during the study period. After the study period the rats were sacrificed and zinc disc implants/stones were removed from the bladder and dried. Stones taken out were weighed. The difference between initial and final weights of stones indicated the amount of kidney stone formed. [24]

WEIGHT OF STONES

The difference between the weight of the implanted zinc discs at the time of implantation and final weight of the dried calculi taken out from the bladder at the end of the 4th and 8th week period indicated the weight of deposited stone.

EVALUATION

The data obtained from the study was statistically evaluated using a parametric test ANOVA (Analysis of Variance) and Turkey as post hoc test. This was done with SPSS (Statistical package for social science) software.

2. CaOx SEED MODEL

Rats under ether anesthesia had their bladder exposed through a suprapubic incision and a CaOx crystal (seed) of < 3 mm diameter was maintained into the bladder. After suturing the bladder, muscle and skin, the animals were maintained in individual cages for 24 h for observation. To prepare the CaOx crystal, small discs of CaOx were maintained in individual cages for 24 h for observation. To prepare the CaOx were obtained by a supersaturation reaction 100 ml of calcium chloride (0.4 mol/L) and 100 ml of potassium oxalate (0.4 mol/L) were mixed together by constant drop wise addition to 300 ml of distilled water for 2 h with shaking at 75µC (micro coulomb).The mixture was maintained under agitation at 75µC(micro coulomb) for an additional 5 h. Crystals were washed and maintained in a stove at 37µC (micro coulomb) for 2 weeks to allow aggregation and growth of the seed. The resultant material was transferred to a template containing cylinders of 3 mm diameter to obtain small discs of Calcium oxalate discs were weighed and sterilized before use.

CONCLUSION

The current review conveys information about different in vivo animal models for urolithiasis. Stone

formation is more in male rats when compared to female rats. Any ideal animal model should possess the characteristics of rapid induction, reproducibility & prevent / reduce symptoms of urolithiasis on treatment with drugs. Commonly used animal models for urolithiasis are CPD (glycolic acid 3% with normal feed) to induce calcium oxalate stones Gentamicin (40mg/kg, sc) + ammonium oxalate (3%) in feed, foreign body insertion method to induce struvite stones, EG + AC in drinking water.so we conclude that these methods are showing better inducing methods for urothiasis.

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REFERENCES

[1] Hadjzadeh, M., Khoei, A., Hadjzadeh, Z., Parizady, M., 2007. Ethanolic extract of Nigella sativa L. seeds on ethylene glycol-induced kidney calculi in rats. Urol. J. 4, 86–90

[2] Khan, S.R., Finlayson, B., Hackett, R.L., 1982. Experimental calcium oxalate nephrolithiasis in the rat. Role of the renal papilla. American Journal of Pathology 107, 59–69.

[3] Kishimoto, T., Yamamoto, K., Sugimoto, T., Yoshihara, H., Maekawa, M., 1986. Side effects of extracorporeal shock-wave exposure in patients treated by extracorporeal shock-wave lithotripsy upper urinary tract. European Urology 12, 308–313.

[4] Prasad, K., Sujatha, D., Bharathi, K., 2007. Herbal drugs in urolithiasis – a review. Phcog. Rev. 1, 175–179.

[5] Doddametikurke, R.B., Biyani, C.S., Browning, A.J., Cartledge, J.J., 2007. The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. EAU-EBU Update Series, vol. 5, pp. 126–136.

[6] Grases, F., Ramis, M., Costa-Bauzá, A., March, J.G., 1995. Effect of Herniaria hirsute and Agropyron repens on calcium oxalate urolithiasis risk in rats. Journal of Ethnopharmacology 45, 211–214

[7] Asper R (1984) Epidemiology and socioeconomic aspects of urolithiasis. Urol Res **12**: 1-5.

[8] Boevé ER, Ketelaars GAM, Vermeij M, Cao LC, Schroder FH, de Bruijn WC (1993) An ultra structural study of experimental induced microliths in rat proximal and distal tubules. J Urol 149: 893-899.

[9] Khan SR, Shevock PN, Hackett RL (1989) Urinary enzymes and calcium oxalate urolithiasis. J Urol **142**: 846849.

[10] Khan SR (1991) Pathogenesis of oxalate urolithiasis: Lesson from experimental studies with rats. Am J Kid Dis **4**: 398-401.

[11] Khan SR, Glenton PA (1995) Deposition of calcium phosphate and calcium oxalate crystals in the kidneys. J Urol **153**: 811-817.

[12] Lee YH, Huang WC, Chiang H, Chen MT, Huang JK, Chang LS (1992) determination role of testosterone in the pathogenesis of urolithiasis in rats. J Urol **147**: 11341138.

[13] Chow FHC, Hamar DW, Boulay JP, Lewis LD(1978).Prevention of oxalate urolithiasis by some compounds .Invest Urol.15:493-495

[14] Chow FHC, Hamar DW, Udall RH (1974) Prevention of oxalate and phosphate lithiasis by alanine. Invest Urol.12:50-54

[15] Clark LC ,Thompson HL (1949).Determination of creatinine and creatinine in urine Anal Chem.21:1218-1221

[16] Colkins VP (1943) .Micro determination of glycolic and oxalic acids .Analyt Chem.15:762-766

[17] Anasuya A (1982) .Role of fluoride in formation of urinary calculi .studies in rats.J Nutr.112:1787-1795

[18] Hubbell RB ,mendel LB ,Wakeman AJ(1937).A salt mixture for use in experimental diets.J Nutr.14:273-285

[19] Mc Carrisson R (1931).The causation of stone in india.Br Med J.1:1009 pp.1015

[20] Leonard RH ,Butt AJ (1955).Quantitative identification of urinary calculi.Clin Chem.1:241-248

[21] Lowry OH ,Roseburgh NJ ,Farr AL,Randall RJ(1951).Protein measurement with the Folin phenol reagent .J Biol Chem.193:265-275

[22] Fiske CH,Subba Row YJ (1925).The colorimetric determination of phosphorus.J Biol Chem.66:375-400

[23] Singer L, Armstrong WD (1968).Determination of fluoride in bone with the fluoride electrode.Anal Chem.40:613-614

[24] Jameel Fahad, Vijayalakshmi, M. C. Satish Kumar, Sanjeeva, G. Prabhath Kodancha, Benegal Adarsh, A.L.Udupa, U.P.Ratnakar (2010). Antiurolithiatic activity of aqueous extract of bark of moringa oleifera (lam.) in rats. Health .2:352-355