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REVIEW ARTICLE ON EXPERIMENTAL RAT MODELS FOR RENAL STONE DISEASE

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ABSTRACT

Urolithiasis or renal calculus disease is most common disorder of human excretory system in which solid concretion of mineral salts are found in any part of the urinary tract from kidney to urethra. Various theories are proposed to explain the mechanism behind development of the renal stone disease but still the exact aetiology of the disease remains unclear. Although major technological advances have been made in the last few decades for the surgical management of the urolithiasis, yet little progress has been seen for conservative management and prevention of recurrence of disease. Traditionally the disease has been studied in human beings through assessment of urine, serum and adoption of imaging modalities to scan the body for detection of stone. In order to understand its aetiology in better way and develop modalities for its conservative management and prevention of recurrence, an urgent requirement was felt to develop valid and reliable animal experimental models. Variety of animal models have been developed to understand the pathophysiology of the disease and assess the efficacy of various treatment modalities for the management and prevention of the disease. Among these animal models, rodents are more commonly used in animal experiments, particularly rats and mice due to their availability, size, low cost, ease of handling and fast reproduction rate. As these rat models are hope of developing breakthrough intervention for the prevention and management of renal stone disease, this review article attempts to summarise all types of experimental rat models along with methods of their handling and induction of renal stones.

Key Words: Urolithiasis, Experimental Rat Models, Renal Stone Induction

INTRODUCTION: Urolithiasis or renal stone disease is one of the most common disease of human excretory system. Urolithiasis affects all ages, sexes, and races but occurs more frequently in men than in women within the age of 20–49 years. Urolithiasis affects about 12% of the world population at some stage in their lifetime. In the United States, kidney stone affects 1 in 11 people while in Indian population, about 12% of them are expected to have urinary stones.[i] Approximate 2 million people in India are affected with urolithiasis every year and some parts of country has name denoted as a 'Stone Belt' that is, Gujarat, Maharashtra, Punjab, Rajasthan, Delhi, Haryana and part of states on North East India.[ii] There have been significant advances in the field of urology regarding management of urolithiasis but challenge of preventing disease from the recurrence is still open as aetiology and pathogenesis behind development of renal stone still remain poorly understood. Various animal models have been developed and are being used by researcher worldwide to understand the exact etio-pathogenesis of renal stone disease. As anatomical and physiological differences of these

experimental animals with human body limit generalising the findings from these models into humans. Hence no single experimental model is considered perfect but Rat remains the most preferably used animal for this purpose because of its anatomic and physiological similarities with humans.

In order to study the etio-pathogenesis and progress of disease, renal stones are induced in urinary system of these animal models by subjecting them to various stone inducing methods. These animals are observed during this course and samples of blood, urine and kidney are collected to analyse the process of stone formation and effect of any therapeutic intervention for prevention and treatment of renal stone disease.

In this review article we attempted to summarise experimental rat models, their types, method of handling, various means of inducing renal stones and methods of collections samples of blood, urine and kidney specimens for investigation purpose.

Animal Models

Use of animals for experimental study is quite common in biomedical research. Rodents particularly rat and mice are more commonly used in animal experiments, particularly rats and mice due to their availability, size, low cost, ease of handling and fast reproduction rate. Other animals includes rabbits, fly, porcine, ferrets, guinea pigs, and rhesus macaque.

Types of animal models[iii]

1. **Inbred or Strains** are laboratory animals which are bred to maintain genetic homogeneity. It is defined as a strain that has been through at least 20 generations of sib-mating making animals from the same inbred strain effectively genetically identical.
2. **Outbred or Stocks** are laboratory animals which are bred to maintain genetic heterozygosity. It is defined as a closed population for at least four generations of genetically variable animal that are bred to maintain maximal genetic variability.
3. **Congenic strains-** It is developed by inbreeding in between strain in which part of the genome of one animal strain is transferred to another, most often by backcrossing the donor strain to the receiver strain with appropriate selection. Congenic strain introduces a particular trait or mutation into a predominantly inbred background.
4. **Transgenic strains-** It is developed by inserting foreign genes into their genome. Transgenic animals with particular mutations of interest are relatively simple to produce with modern genetic engineering methods like the injection of targeting vectors or CRISPR technology. Once produced these mutations of interest frequently must be bred into a congenic line. For example **Oncomice** is with an activated oncogene so as to significantly increase the incidence of cancer. **Doogie mice** is with enhanced N-methyl-D-aspartate receptor (NMDA) receptor function, resulting in improved memory and learning.
5. **Gene knockout strains-** Gene knockout is a genetic procedure in which one of an organism's genes is rendered inactive, or knocked out. Gene function is studied using knockout species, which are frequently employed to investigate the effects of gene deletion. A knockout rat is a genetically modified rat in which a single gene has been turned off by a targeted mutation. Knockout rats are useful tools for investigating gene function and drug discovery and development since they can imitate human disorders. Obese mice, for

example, are prone to obesity due to a lack of Carboxypeptidase E. Because the Myostatin gene is deactivated, Mighty mice are muscular and robust. Obese mice, for example, are prone to obesity due to a lack of Carboxypeptidase E. Because the Myostatin gene is deactivated, Mighty mice are muscular and robust.

Inbred, Transgenic, and Congenic mice with inbred backgrounds are the most extensively used mouse models among all of these categories.

Rat

The domestic rat (Rattus norvegicus) is the second most commonly used animal model in biomedical research. It is a member of the Rodentia order and the Muridae family. The most commonly used rat models are Wistar rats, Sprague-Dawley rats, and Long Evans rats[iv].

Rat vs Mice[v]

The mouse is a member of the Muridae family, subfamily Murinae. The majority of pet mice are descended from the wild house mouse (Mus musculus). Rats are larger, more aggressive, more resistant to a variety of diseases as compared to mice. Inbred strains of laboratory mice are most usually employed, while outbred stocks of laboratory rats are most commonly used. Most popular laboratory mice and laboratory rat models are albinos, just like the majority of laboratory rodents, due to a common mutation in the Tyrosinase gene, which is the rate-limiting enzyme in the formation of melanin pigment. Because many of the earliest established strains were albino, and albinism was an easy selection marker in the early days, albinism is common among laboratory mice.

Types of Rat[vi]

1. Wistar Albino Rat

The most frequent hybrid albino model animal is the Wistar rat. It holds the distinction of being the first rat stock to be created specifically for use as a model animal. Wistar rats get their name from the Wistar Institute in Pennsylvania, where they were created. Because of its small body size, it is easy to handle. Wistar rats were used to create the Sprague Dawley Rat and the Long Evans Rat.

Figure 1. Wistar Albino Rat

2. Sprague Dawley Rat

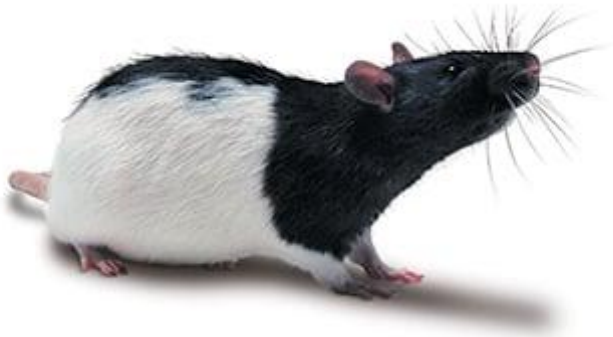
The Sprague Dawley rat is a hybrid albino strain with a long narrow head and a high reproductive rate. It also has a low frequency of spontaneous tumours. It can be distinguished by its larger tail in comparison to its body length. Because of its placid disposition and ease of handling, it is widely employed in neurological research. It was created in the Sprague Dawley farm in Wisconsin, USA.

Figure 2. Sprague Dawley Rat

3. Long Evans Rat

The Long Evans rat was developed through crossbreeding between Wistar albino and wild grey rats and is named after scientists Dr. Long and Dr. Evans. It can be identified by its white body and black hood. It is most commonly used in behavioural and obesity studies.

Figure 3. Long Evans Rat



Lifespan & Breeding[vii]

Rats have an average lifespan of 12-18 months. They are born without hair and open their eyes after 10-12 days. After 21 days, the young are weaned. Puberty occurs between the ages of 7-9 weeks. After 9 weeks, breeding begins, and the breeding life span is 9-12 months. It's possible that replacing breeders when they're 6-9 months old is ideal.

Genome[viii]

The C57BL/6 strain was used to complete the sequencing of the laboratory mouse genome in late 2002. After humans, this was only the second mammalian genome to be sequenced. The haploid genome is approximately three billion base pairs long (3,000 Mb dispersed over 19 autosomal chromosomes plus 1 or 2 sex chromosomes), making it comparable to the human genome in size. The laboratory mouse currently has 23,139 primary coding genes, compared to an estimated 20,774 in humans.

Housing

Animals are normally kept at a relative humidity of 30-70 percent and a temperature of 18-26 degrees Celsius, with at least 10 room air changes each hour. Standard shoebox cages with or without filter tops are available. Paper, wood shavings, wood chips, or corncob can all be used as bedding. Rats are only kept on wire flooring in the most extreme cases.

Handling

Laboratory mice have traditionally been picked up near the base of the tail. Recent study, however, has found that this form of treatment increases anxiety and unpleasant behaviour. Instead, utilising a tunnel or

cupped hands to handle mice is recommended. In behavioural tests, tail-handled mice are less motivated to examine and explore test stimuli than tunnel-handled mice, who readily investigate and respond to test stimuli.

Nutrition

Food consumption is roughly 15 g per 100 g of body weight per day, whereas water consumption is roughly 15 ml per 100 g of body weight per day. In most laboratory experiments, it is vital to prevent biological variation, and laboratory mice are nearly always given only commercial pelleted mouse feed to achieve this goal. Wheat bran, sunflower seed extract, wheat, barley meal, alfalfa meal, grass, corn, sorghum red, oats shelled, wheat flakes, carob, striped sunflower, black sunflower, carrots, flaxseed, peanut, raisins, peas, rye bran, and beet pulp are among the ingredients in Commercial pelleted mouse feed. Raw protein 11.2 percent, crude oils and fats 2.9 percent, raw fibre 8.8 percent, raw ash 4.67 percent, and humidity 12 percent should be the analytical constituents of commercial pelleted mouse feed.

Vital Parameters

Table 1. Vital Parameters

Sr. No.	Parameter	Value
1.	Weight	150-200gm/rat
2.	Blood volume	50ml/kg BW or 10ml/rat
3.	Heart Rate (HR)	330-480 beats per minute
4.	Respiratory Rate (RR)	85 breaths per minute
5.	Body Temperature	35.9-37.5ºC
6.	Urine output	3ml/100gmBW/24 hours or 5 ml/24 hours

Identification of Sex

Males have a greater (1.5-2 times) ano-genital distance than females as well as a larger genital papilla. Absence of testicles is not a useful criterion for sexing as the testis is retractable throughout life into the open inguinal canal.

Marking of Animals

Picric acid is used to stain the animals for their identification. Animals are marked with Picric Acid on head (H), body (B), tail (T), head and body (HB), Body and tail (BT), head and tail (HT), head, body and tail (HBT).

Identification of Pain

Table 2. Identification of Pain

Sr. No.	Acute Pain	Chronic Pain
1.	Anorexia (No faecal pellets)	Decreased body weight
2.	Decrease in appetite (few faecal pellets)	Reluctance to move
3.	Rubbing or scratching surgical site	Change in behaviour
4.	Biting or shaking affected body part	Poor grooming
5.	Vocalization	Change in bowel or urinary activity
6.	Restlessness	Rough hair coat
7.	Porphyrin discharge	-

	(Red-brown pigment from eyes/nose)	
8.	Increased respiration	-

Pain relief Medication

Non-Steriodal Anti-Inflammatory Drugs (NSAIDs) and Opiods are used as per estimation of severity and chronicity of pain.

Drug administration[ix]

Oral drug administration is done through metallic Oro-gastric tube which is inserted through the mouth. Length of the tube to be inserted is equal to the distance from the nose to the first rib of the rat. The tube should be marked with the distance that needs to be inserted into the rat. Others routes of drug administration in laboratory rat are mainly subcutaneous, Intra-peritoneal and intravenous. To facilitate intravenous injection into the tail, laboratory mice can be carefully warmed under heat lamps to dilate the vessels. Intramuscular administration is not recommended due to small muscle mass. Intra-cerebral administration is also possible. Each route of drug administration has a recommended injection site, approximate needle gauge and recommended maximum injected volume at a single time at one site, as given in the table below.

Table 3. Route of drug administration in rats

Sr. No.	Route	Recommended site	Needle gauge	Maximum volume
1.	Subcutaneous	Dorsum, between scapula	25-26 G	2-3 ml
2.	Intra-peritoneal	Left lower quadrant	25-27 G	2-3 ml
3.	Intravenous	Lateral tail vein	27-28 G	0.2 ml
4.	Intramuscular	Hind limb or caudal thigh	26-27 G	0.05 ml
5.	Intra-cerebral	Cranium	27 G	0.01 ml

Anaesthesia[x]

Anaesthesia is required for collection of blood sample from intra-orbital sinus, creation of wound and harvesting specimen for histopathological examination. Usually Isoflurane or Halothane or Ether are used as Inhalational Anaesthesia to collect blood samples while Ketamine or Xylazine are used as Intra-peritoneal Anaesthesia to create wound and harvest specimen for histopathological examination. A common regimen used for general anaesthesia for the house mouse is Ketamine (100 mg/kg body weight) plus Xylazine (5–10 mg/kg body weight) injected by the intra-peritoneal route. Xylazine can also be used as sole anaesthetic agent for sedation or anaesthesia or muscle relaxation in animals or non-human mammals with the average duration of effect of about 30 minutes.

Blood Sample Collection[xi]

Most common site of blood sample collection is peri-orbital or retro-orbital sinus which requires General anaesthesia with Ether. Next most common site of blood collection is tail vein followed by Saphenous vein, Juglar vein, Dorsal Pedal vein.

Frequency of blood collection should not be more than once in two weeks and total blood Volume drawn in single sitting should not exceed 50ml/kg BW. In other words maximum 1 ml blood can be drawn from a Mice while 10 ml blood can be drawn from a Rat. 10% of total blood volume of Rat can be drawn in one attempt

and can be repeated after 3-4weeks. If more than 10% blood is drawn, then fluid replacement with Lactated Ringer's Solution (LRS) should be done to maintain adequate fluid volume.

The animal is scuffed with thumb and forefinger of the non-dominant hand and the skin around the eye is pulled taut. A capillary is inserted into the medial canthus of the eye at the 30 degree angle to the nose. Slight thumb pressure is enough to puncture the tissue and enter the plexus or sinus. Once the plexus or sinus is punctured, blood will come through the capillary tube. Once the required volume of blood is collected from plexus, the capillary tube is gently removed and wiped with sterile cotton. Bleeding can be stopped by applying gentle finger pressure.

Urine Sample Collections[xii]

Housing of Rat in the Metabolic Cage is most commonly used method of urine sample collection. Metabolic Cage is attached with pot which collect 24 hours urine volume. Catheterisation and Vesical Puncture can also be used for urine sample collection. Nowadays collection of urine sample through hydrophobic sand is also being done although it cause significant discomfort to Rat which results in significant lifestyle fluctuation.

Harvesting of Kidney[xiii]

Rat has unipapillary kidneys with average weight of 0.75–1.2 g and dimension of 1.6 × 1.0 × 0.9 cm. It has kidney with overall smaller collecting system, with fewer urinary tubules and on average 30,000 nephrons. The cortex-medulla ratio of the kidney of rat is 2:1 which is similar to that of humans.

Euthanasia

According to the American Veterinary Medical Association (AVMA), compressed CO2 gas is the finest euthanasia agent for animals, with a flow rate of 10% to 30% volume/min being ideal for euthanizing laboratory mice. Experimental animals can also be sacrificed using injectable anaesthetics like barbiturates and inhalable anaesthetics like Halothane in overdose. Cervical dislocation and decapitation are two physical techniques of euthanasia. Decapitation refers to the removal of a person's or animal's head. Applying pressure to the neck and dislocating the spinal column from the skull or brain to euthanize tiny animals. The goal is to immediately remove the spinal cord from the brain so the animal can die promptly and painlessly. Firm pressure is given to the base of the skull, with the thumb and fingers sharply pinching and twisting. The tail is being dragged rearward at the same time. The spinal cord is severed either at the base of the brain or in the cervical spine area. It is a morally acceptable way of putting small rodents like rats, mice, and squirrels to sleep.

Renal Stone Induction Methods

Because hypercalciuria and hyperoxaluria are two of the most common pathophysiological alterations associated with renal stone disease, they are employed to produce stones in rat models. The following are four techniques for inducing urolithiasis in rat models:

Hypercalciuria

GHS Rats are a strain of multi-generation inbred Sprague-Dawley rats with higher calcium absorption capacity due to an increased number of Vitamin D receptors in the gut, kidney, and bones, leading in hypercalciuria. To cause hypercalciuria, calcium chloride can also be given orally by combining it with water.

1. **Hyperoxaluria**

Exogenous lithogenic food in the form of Sodium oxalate, Glycolic acid, and Ethylene Glycol is used in this approach. These lithogenic substances can be given in a variety of ways, but the most typical method is to mix them with drinking water.

Table 4. Showing various modes of interventions for induction of hyperoxaluria[xiv]

Sr. No.	Intervention	Dosage	Route	Duration	Toxicity
1.	Glycolic Acid[xv]	3% w/v	Orally mixing with drinking water	3-4 Weeks	More
2.	Ethylene Glycol	0.75% v/v			
3.	Ethylene Glycol with Ammonium Chloride, Calcium Chloride, Vitamin D	0.75% v/v		1 Week	
4.	Sodium Oxalate	10mg/kg	Interperitoneal	15 minutes to 6 hours	-
5.	Hydroxy L Prolene (HLP)	2.5mg/kg	Interperitoneal	4 weeks	Less

Ethylene Glycol is the most common lithogenic diet used to promote kidney stone formation. Ethylene Glycol, on the other hand, has been established in several tests to be a hazardous toxin that can induce multi-organ failure. Ammonium chloride promotes stone formation by lowering urine pH, while Vitamin D promotes stone formation by inducing hypercalcemia.

2. Dietary method

Table 5. Lithogenic diet for induction of renal stones[xvi]

Sr. No.	Lithogenic Diet	Mechanism	Duration	Toxicity
1.	Potassium oxalate supplementation	Calcium Oxalate Deposition	4 Weeks	Very Low
2.	Mg deficiency diet in hyperoxaluric rats	Calcium Phosphate Deposition	4 Weeks	Very Low
3.	Pyridoxine deficiency diet	Calcium Phosphate Deposition	4 Weeks	Very Low
4.	Low citrate diet	Reduction in urine pH	4 Weeks	Very Low

By counteracting the effect of hypocitraturia, magnesium supplementation in Pyridoxine deficient rats successfully inhibits calcium oxalate crystal formation.[xvii]

3. Surgical methods

Intestinal resection is another approach for inducing renal stones in rats, according to some studies. Resection of the terminal ileum causes hyperoxaluria, hypocitraturia, and crystal development in the renal parenchyma, according to some studies.[xviii] Because some studies have revealed an increased incidence of urolithiasis after Roux-en-Y gastric bypass surgery in humans, gastric bypass surgery could become another surgical technique to produce renal stone in rat models.[xix]

DISCUSSION

Although urolithiasis is a disease which is known since ancient times but still today its management remains quite challenging. Conservative management is confined only to certain dietary and fluid modifications such as maintaining adequate fluid intake and avoiding of variety of dietary food which might precipitate calculi formation in urinary tract along with few medications such as Potassium Citrate which reduce the chances of stone formation by decreasing the acidity of urine.

In current scenario more and more renal stones are being treated by minimally invasive procedures such as ESWL, URS, PCNL and open surgical approaches like Nephrolithotomy, Pyelolithotomy, or Ureterolithotomy are also not uncommon when multiple, large or complicated stones restrict the usefulness of these minimally invasive procedures. Not only these procedures have their own limitations and complications in the way of treating urolithiasis but also are costly and need expertise. After surgical removal of stone, prevention of recurrence of urolithiasis is still remained a major challenge as till today no medicine can be claimed as effective for prevention of renal stone from recurrence.

Considering all these factors, more research resources should be allocated to explore the various interventions for prevention and treatment of Urolithiasis. Studying the intervention for prevention is not easy in clinical trials involving humans. So animal experimentation involving rats and mice has huge potential to play a significant role in finding out solution not only for prevention but also treatment of urolithiasis.

Currently there are ongoing efforts to develop and employ various animal experimental models to study variety of disease condition and their therapeutic and prophylactic solutions. Rats are most commonly used animal due to their availability, size, low cost, ease of handling and fast reproduction rate. Wistar rat is most common hybrid albino model animal with distinct honour of being the first rat stock developed to serve as a model animal.[xx]

There are various methods to induce urolithiasis in rat models by producing hypercalciuria or hyperoxaluria through lithogenic diet or pharmacological intervention of such as ethylene glycol. Surgical methods produce hypercalciuria or hyperoxaluria by resection of part of intestine or gastric bypass surgery. Genetically Hypercalciuric Stone forming (GHS) Rats are genetically modified rats with excess Vitamin D receptors resulting in increased calcium absorption leading to hypercalciuria. Lithogenic diet along with pharmacological intervention for induction of renal stone is easiest, tolerable method which is being used most commonly in animal experimental studies for renal stone disease.[xxi]

CONCLUSION

Urolithiasis or renal stone disease is one of the most common disease of human excretory system. Despite significant advances in the field of urology regarding management of urolithiasis, there are big challenge of preventing disease from the recurrence as aetiology and pathogenesis behind development of renal stone still remain poorly understood. Future research is required to study the subject more thoroughly and deeply to develop innovative solutions for this disease. Understanding of various animal experimental models and familiarity with their handling methods among scientific

community can play a significant role to further understand the mechanism behind aetio-pathogenesis the disease and develop intervention for prevention and treatment of Urolithiasis by adopting to more and more animal experimentation studies.

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