Pharmacological Study

Evaluation of anti-urolithiatic activity of Pashanabhedadi Ghrita against experimentally induced renal calculi in rats

Sanjay Kumar Gupta, Madhav Singh Baghel1, Chaturbhija Bhuyan2, B. Ravishankar3, Ashok B. K.4, Panchakshari D. Patil5

Associate Professor and I/C Head, Department of Shalya Tantra, 1Director, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, 2Director, Centre for Care of Ano-Rectal Research by Indian System of Medicine and Allied Science, Bhubaneswar, Orissa, 3Director, Research and Development, SDM Ayurveda College, Kuthpady, Udupi, 4Research Associate, Drug Discovery Group, Research and Development Himalaya Health Care, Bengaluru, Karnataka, 5Senior Research Fellow, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

Abstract

Population in an industrialized world is afflicted by urinary stone disease. Kidney stones are common in all kinds of urolithiasis. One distinguished formulation mentioned by Sushruta for management of Ashmari (urolithiasis) is Pashanabhedadi Ghrita (PBG), which is in clinical practice since centuries. Validation of drug is the requirement of time through the experimental study. In this study, trial of PBG has been made against ammonium oxalate rich diet and gentamicin injection induced renal calculi in albino rats. The calculi were induced by gentamicin injection and ammonium oxalate rich diet. Test drug was administered concomitantly in the dose of 900 mg/kg for 15 consecutive days. Rats were sacrificed on the 16th day. Parameters like kidney weight, serum biochemical, kidney tissue and histopathology of kidney were studied. Concomitant treatment of PBG attenuates blood biochemical parameters non-significantly, whereas it significantly attenuated lipid peroxidation and enhanced glutathione and glutathione peroxidase activities. It also decreased crystal deposition markedly into the renal tubules in number as well as size and prevented damage to the renal tubules. The findings showed that PBG is having significant anti-urolithiatic activities against ammonium oxalate rich diet plus gentamicine injection induced urolithiasis in rats.

Key words: Ammonium oxalate, Ashmari, gentamicin, Pashanabhedadi Ghrita, urolithiasis

Introduction

Mankind has been afflicted by urinary stones (Urolithiasis) since centuries, and it is proven to be an important cause of renal failure. Even in the 4th century B.C., Hippocrates is noted the presence of the renal stone together with renal abscess and he has mentioned that in his Hippocratic oath “... I will not cut, even for stone, but leave such procedures to the practitioners of the craft.” The specialty of urology branch has been recognized since ever. It is estimated that at least 10% of the population in the industrialized part of the world is afflicted by urinary tract stone disease. Among those, kidney stones are common in industrialized nations with an annual incidence of 0.5-1.9%. About 12% of the population of India is expected to have urinary stones and out of that about 50% of cases encounter loss of one or both kidneys with or without renal damage upto some extent. Nearly 15% of the population of northern India is also suffering from kidney stones. Upper as well as lower urinary tract stones occur frequently but the incidence shows wide variation on the regional basis in India.[3]

Ayurveda, a traditional system of Indian medicine, recommends several medicinal plants and compound medicinal preparations for the treatment of urolithiasis.[4,5] Herbs and herbal drugs have created interest among the people by its clinically proven effects. The overuse of synthetic drugs, results in higher incidence of adverse drug reactions, has motivated humans to return to nature for safe remedies. One such distinguished formulation mentioned by Sushruta in the management of Ashmari (urinary stones) is Pashanabhedadi Ghrita (PBG) which is more popular among Vaidyas (physicians). The name of the formulation itself suggests anti-urolithiatic action, i.e., Pashana means stone and Bheda means to crush. However, until date there is no experimental basis is available to prove clinical evidence on this formulation, hence the present study was designed to evaluate anti-urolithiatic activity of PBG in experimental animals.

Address for correspondence: Dr. Sanjay Kumar Gupta, Department of Shalya Tantra, IPGT and RA, Gujarat Ayurved University, Jamnagar- 361 008, Gujarat, India. E-mail: drskgupta17@gmail.com
Materials and Methods

Animals
Wistar strain albino rats of either sex; weighing 160-220 g were used for the study. The animals were obtained from the animal house attached to our institute. Six animals were housed in each cage made up of poly-propylene with stainless steel top grill. The dry wheat (post-hulled) waste was used as bedding material and was changed every morning. The animals were exposed to 12-h light and 12 h dark cycle with the relative humidity of 50-70% and the ambient temperature during the period of experimentation was 22 ± 0.3°C. Animals were fed with Anruth brand rat pellet feed supplied by Pranav Agro Mills Pvt. Limited. Tap water ad libitum was used for their drinking purpose. The experiments were carried out in conformity with the Institutional Animal Ethics Committee (IAEC) after obtaining its permission (IAEC03/08-11/PhD/01).

Test formulation
The raw materials [Table 1] of the test formulation were procured from pharmacy attached to Gujarat Ayurved University, Jamnagar and authenticated by pharmacognosist. From these raw drugs, “PBG” was prepared in the Department of Rasashastra and Bhaishajya Kalpana of I.P.G.T. and R.A. as per the classical reference of Ghrita Paka Kalpana.[7] The finished product was stored in air tight glass container and utilized for experimental study.

Dose fixation
The human dose of PBG mentioned in classical texts is 10 g per day. Considering this, the dose of the experimental animals was calculated by extrapolating the human dose to rat dose as 900 mg/kg based on the body surface area ratio by referring to the standard table of Paget and Barnes (1964).[8] The test drug was administered orally with the help of a gastric catheter of suitable size sleeved onto a disposable syringe.

Anti-urolithiatic activity
Hyperoxaluria and calcium oxalate deposition in the kidney was induced by using injection gentamicin (40 mg/kg/Intra Peritonial) and Calculi Producing Diet (CPD).[9] The diet is made by powdered standard rat pellet feed mixed with ammonium oxalate (5%), then made into pellet and dried up properly. The selected animals were weighed and randomly divided into four groups, consisting of six animals in each group. Group-I received only distilled water orally for 15 days, served as normal control. Group-II received gentamicin (40 mg/kg, Sub-cutaneous., day 1st-8th), CPD and

| Sanskrit name    | Botanical name             | Part used          |
|------------------|---------------------------|--------------------|
| Go Ghrita        | Cow ghee                  |                    |
| Ingredients for Kwatha (Decoction) |                  |
| Pasanabheda      | Bergenia ligulata (Wall.) Engl | Moola (Root)      |
| Shwetarka        | Calotropis gigantean (L.) W.T. Aiton | Panchanga (Whole plant) |
| Apamarga         | Achyranthus aspera Linn.  | Panchanga (Whole plant) |
| Changeri         | Oxalis comiculata Linn.   | Panchanga (Whole plant) |
| Shatawari        | Asparagus racemosus Wild  | Kanda (Tubers)     |
| Gokshura         | Tribulus terrestris Linn. | Phala (Fruit)      |
| Brihati          | Solanum indicum Linn.     | Phala (Fruit)      |
| Kantakari        | Solanum surattense Burm   | Panchanga (Whole plant) |
| Brahmi           | Bacopa monnieri L. Pennell| Panchanga (Whole plant) |
| Kokilaksha       | Barleria preonitis Linn.  | Panchanga (Whole plant) |
| Usheera          | Vetiveria zizanioidis (L.) Roberty | Moola (Root)      |
| Gunja            | Abrus precatorious Linn.  | Beeja (Seed), Moola (Root), Patra (Leaf) |
| Vikshadani       | Dendrophthoe falcate (L.f) Ettingsh | Panchanga (Whole plant) |
| Shyonaka         | Oroxylum indicum (L.) Benth. ex Kurz | Moola Twak (Root bark) |
| Varuna           | Crateva nurvala Buch. Ham | Twak (Bark)       |
| Shakaja Phala    | Tectona grandis Linn.     | Beeja (Seed)       |
| Yava             | Hordeum vulgare Linn.     | Panchanga (Whole plant) |
| Kulattha         | Dolichos biflourus Linn.  | Beeja (Seed)       |
| Maricha          | Piper nigrum Linn.        | Phala (Fruit)      |
| Nirmali          | Strychnos potatorum Linn. | Beeja (Seed)       |
| Drugs for Kalka (Paste) |                  |
| Ushaka (Ksharrittika) | -                      |                    |
| Saindhava        | Rock salt                |                    |
| Shilajatu        | Betumin                  |                    |
| Kaseesa          | FeSO₄                    |                    |
| Hingu            | Ferula foetida Linn.     |                    |
| Tuttha           | CuSO₄                    |                    |
water (from day 1st to 15th) and served as negative control (diet control). Group-III received gentamicin (40 mg/kg, SC., day 1st-8th); CPD and plain Goghrita (0.9 g/kg, orally, day 1st-15th) and served as Vehicle Control (VC). Group-IV received gentamicin (40 mg/kg, SC., day 1st-8th); CPD and Pashanabhedadi Ghirota (0.9 g/kg, orally, day 1st-15th) and coded as PBG. The gentamicin injection was given after 2 h of drug administration, and normal diet was replaced by CPD for 15 days.

Assessment of urinary parameters
On the 15th day after-drug administration, the rats of all the four groups were hydrated with distilled water (2 ml/100 g rat), housed in separate metabolic cages and urine samples were collected for 24 h and the urinary pH and specific gravity were determined by strip method.

Assessment of kidney parameters
On the 16th day the animals were weighed and anaesthetized by diethyl ether. Blood was collected from retro orbital plexus by capillary puncturing for estimation of serum biochemical parameters such as serum urea,[10] serum creatinine,[11] serum uric acid,[12] and serum calcium.[13] Then the animals were sacrificed by over dose of ether anesthesia. The abdomen was opened by midline incision and kidney was dissected out carefully and cleaned off the extraneous tissue. Kidney was weighed and one kidney of each animal was transferred to 10% formalin solution for the purpose of histopathological studies while the other kidney was utilized for estimation of biochemical parameters in tissue homogenate parameters. The pathogenesis of calcium oxalate stone formation is a multi-step process and in essence includes urinary saturation, urinary super saturation, nucleation, crystal growth, crystal aggregation, and crystal retention. Various substances

Statistical analysis
The obtained data have been presented as mean ± SEM, difference between the groups was statistically determined by Student t-test for unpaired data with the level of significance set at P < 0.05.

Results
An apparent and statistically significant increase in weight of kidney was occurred in the diet control group in comparison to the normal control group. Administration of Goghrita and PBG failed to attenuate the weight of kidney to a significant extent in comparison to diet control group [Table 2]. Feeding of ammonium oxalate diet leads to significant decrease in urinary pH in comparison to normal control. Administration of PBG non-significantly attenuated pH of urine.

| Groups       | Relative weight of kidney (g/100 g) | Specific gravity | Urine pH |
|--------------|-------------------------------------|-----------------|----------|
| Control      | 0.97±0.09                           | 1.007±0.004     | 7.6±0.35 |
| Diet control | 1.26±0.07*                          | 1.019±0.003     | 6.6±0.18*|
| VC           | 1.51±0.20                           | 1.016±0.002     | 7.0±0.25 |
| PBG          | 1.53±0.09                           | 1.021±0.002     | 7.2±0.20 |

*P<0.05 (Compared with normal control), VC: Vehicle control, PBG: Pashanabhedadi Ghirota

Microscopic examination of kidney sections from the normal control group showed normal cytoarchitecture [Figure 1]. Sections from gentamicin injection plus ammonium oxalate diet control group shows the presence of a large number of crystal material containing tubules, especially in the cortical region; dilatation of tubules (due to stones) along with necrosis of the tubular epithelium [Figure 2]. Sections from VC treated group shows a moderate decrease in the size and number of crystal containing tubules and mild tubular epithelial necrosis [Figure 3], while marked decrease of these features are observed in PBG treated group [Figure 4].

Table 3: Effect on serum biochemical parameters

| Groups       | Serum Blood urea (mg/dL) | Serum creatinine (mg/dL) | Serum uric acid (mg/dL) | Serum calcium (mg/dL) |
|--------------|--------------------------|--------------------------|------------------------|-----------------------|
| Control      | 46.40±3.30               | 0.52±0.03                | 1.16±0.13              | 8.50±0.21             |
| Diet control | 81.00±12.57*             | 0.92±0.12*               | 1.30±0.14              | 7.82±0.22             |
| VC           | 98.83±19.47              | 1.20±0.28                | 1.56±0.13              | 8.37±0.25             |
| PBG          | 102.80±21.95             | 0.92±0.36                | 2.30±0.61              | 7.76±0.20             |

*P < 0.05, **P < 0.05 (Compared with normal control), VC: Vehicle control, PBG: Pashanabhedadi Ghirota

Discussion
The pathogenesis of calcium oxalate (CaOx) stone formation is a multi-step process and in essence includes urinary saturation, urinary super saturation, nucleation, crystal growth, crystal aggregation, and crystal retention. Various substances
Gupta, et al.: Anti-urolithic activity of Pashanabhedadi Ghrita

in the body have an effect on one or more of the above stone forming processes and thereby influencing a person’s ability of the body to promote or prevent stone formation. Promoters of stone formation facilitate stone formation whilst inhibitors prevent it. Low urine volume, low urine pH, calcium, sodium, oxalate, and urate are known to promote stone formation. Many inorganic (e.g., citrate, magnesium) and organic (e.g., urinary prothrombin fragment 1, glycosaminoglycans, osteopontin) substances are known to inhibit stone formation. Organic inhibitory compounds adsorb to the surface of the crystals, thereby inhibiting crystal growth and nucleation.[19]

The incidence and nature of spontaneous urolithiasis are imperfectly known in the rats.[20] In the present study, 5% ammonium oxalate is used instead of 3% ammonium oxalate.

Table 4: Effect on tissue biochemical parameters of kidney tissue homogenate

| Groups          | Lipid peroxidation (μ mole MDA/g) | Nitric oxide (μ mole/g) | Glutathione (n mole/g) | Gluthathione peroxidase (μ mole/min/g) |
|-----------------|----------------------------------|-------------------------|------------------------|---------------------------------------|
| Control         | 22.23±0.60                       | 1.80±0.14               | 09.28±0.26             | 3.58±0.24                             |
| Diet control    | 37.11±5.22*                      | 2.64±0.31*              | 05.70±1.25*            | 2.41±0.44#                            |
| VC              | 37.23±22.35                      | 2.08±0.37               | 12.63±2.59*            | 14.47±5.47*                           |
| PBG             | 19.71±3.69*                      | 1.82±0.63               | 13.66±3.05*            | 14.78±3.46**                          |

*P<0.05, (Compared with normal control), **P<0.05, #P<0.05 (Compared with diet control), MDA: Malondialdehyde, VC: Vehicle control, PBG: Pashanabhedadi Ghrita
as reported by Sanjay Kumar et al., because the preliminary studies have shown less incidence of calculi deposition with 5% ammonium oxalate. This treatment schedule of gentamicin and ammonium oxalate rich diet increases calcium and oxalate super saturation, renal tubular injury and produce conditions conducive to the formation and growth of CaOx stones.\(^{23}\) The main causes of CaOx stone formation appear to be chronic hyperoxaluria.\(^{21}\) In the present study, gentamicin and ammonium oxalate rich diet-induced hyperoxaluria not only increased CaOx deposition in the kidney but also causes papillary damage and incrustations as reported earlier.\(^{25}\) This treatment schedule of gentamicin and ammonium oxalate increase calcium and oxalate super saturation, renal tubular injury and produce favourable condition to the formation and growth of CaOx stones.\(^{25}\)

In pondral changes, it is observed that significant increase in kidney weight of the negative control group. This increase supported the results of stone deposition in kidney, it was also supported by histopathological study in which plenty of stones were present in sections of kidney of this group (Figure 2). Further, extensive degenerative changes are also observed in kidneys of this group, but administration of PBG and VC in animals was failed to attenuate increased kidney weight in comparison to the negative control group.

The type of stones formed can be predicted from the pH of the fasting urine. Crystalluria is pH dependant. Dissolution of calculi can be achieved by alteration in urinary pH form. If the pH is acidic 5.5 or below, the stones are likely to be of acidic type (uric acid),\(^{26}\) if 5.0-6.5 CaOx\(^{25}\) type and if alkaline (7.2 or above) indicates magnesium ammonium phosphate type. In the present study a decrease in pH was observed on induction of ammonium oxalate type of stones in negative control. Treatment with PBG reversed acidic pH to slightly alkaline pH (7.2 ± 0.20), where as in VC group pH of urine was observed neutral (7.0 ± 0.25). This increase in urinary pH might be responsible for dissolving the complexes of ammonium and oxalate. Thus, some of the anti-urolithic effects of PBG and VC are possibly due to its effect on urinary pH.

Due to the presence of stones, there is obstruction to the out flow of urine and because of this, the Glomerular Filtration Rate (GFR) also decreases. Reduction in the GFR leads to accumulation of the waste products, particularly nitrogenous substances such as urea, creatinine, and uric acid in blood.\(^{26}\) Blood urea nitrogen level is considered as a good indicator of balance in the nitrogen metabolism. It tends to enhance with increase tissue catabolism. In the present study significant increase in blood urea level was observed in the negative control group where as in the treated group with PBG and VC failed to attenuate it. Enhanced serum creatinine indicates renal impairment due to hyperoxaluria. Treatment with PBG and VC failed to attenuate elevated serum creatinine level reflected impaired renal function. Other serum biochemical parameters (serum uric acid and calcium) were not affected to a significant extent by ammonium oxalate rich diet plus gentamicin administration in any group.

Studies show that oxalate, a major stone-forming constituent, has been reported to induce lipid peroxidation and causes tissue damage by reacting with polyunsaturated fatty acids in cell membranes.\(^{27}\) The polyunsaturated fatty acid content of kidney makes it prone to Reactive Oxygen Species (ROS) attack.\(^{28}\) Imbalance between oxidants and anti-oxidants level result in Oxidative Stress (OS). Low levels of renal cellular glutathione are reported to favor lipid peroxidation and retain calcium and oxalate in the kidney.\(^{29}\) In the present study, it is observed that ammonium oxalate rich diet, which was fed to rats with injection gentamicin leads to significant increase in lipid peroxidation level in kidney tissue of the negative control group in comparison to normal control. But in PBG group, it is observed that treatment with PBG significantly attenuated lipid peroxidation. Hence PBG may be considered to prevent the lipid peroxidation-induced renal damage caused by CaOx crystal deposition in the kidney tissue. Therefore, PBG can be attributed to check CaOx crystal attachment and stones formation.

The impaired anti-oxidant protection might be responsible for the accumulation and retention of oxalate and subsequent deposition of CaOx in the kidney. Treatment with anti-oxidants was reported to reduce hyperoxaluria and the resultant OS in rats.\(^{30}\) In present study ammonium oxalate rich diet with gentamicin administration significantly decreased glutathione and glutathione peroxidation activity and non-significantly increased nitric oxide level in negative control group. Treatment with PBG and VC decreased nitric oxide level non-significantly and enhanced anti-oxidant system like glutathione peroxidase and total glutathione content significantly. This shows anti-urolithic effects of PBG and VC, which might be due to quenching oxidant constituents in the kidney. It was reported that the protective effect of Berginia ligulata in hyperoxaluric OS and CaOx crystal deposition is due to their potential anti-oxidant activity. Achyranthis aspera, Quercus salieina, Anni visnaga, and Mimusops elengi, which are also ingredients of test formulation are also reported to be having the protective effect against oxalate-induced renal tubular epithelial cell injury in cell culture due to their anti-oxidant activity.\(^{31}\) Further one more ingredient of this formulation, Varuna (Crataeva nurvala) prevents stone formation due to the anti-lithogenic activity and the anti-crystallization property.\(^{32}\) It was also reported that Crataea nurvala, Tribulus terrestris, and Dolichos biflorus were found to be effective in preventing the deposition of the stones in experimental rats.\(^{33}\) The ethanolic extract of Asparagus racemosus Wild had an inhibitory potential on lithiasis induced by oral administration significantly reduced the elevated level of calculeogenic ions in urine and it elevated the urinary concentration of magnesium, which is considered as one of the inhibitors of crystallization.\(^{34}\) Thus, the observed anti-urolithic activity of test formulation in the present study may be attributed to collective effect of these drugs. The mechanism involved in observed activity profile may be,

- Improving the renal tissue anti-oxidant status and cell membrane integrity
- Inhibition of crystal nucleation, aggregation and growth,
- By increasing urine volume, pH and anti-calculifying activity,
- Regulation of oxalate metabolism.

**Conclusion**

PBG is having significant anti-urolithic activity besides it is having marked anti-oxidant activity. However, further detailed study is required to explore the active principle responsible for this and also to know the exact mechanism involved in observed activity profile.
References

1. Stamatelou KK, Francis ME, Jones CA, Nyberg LM, Curhan GC. Time trends in reported prevalence of kidney stones in the United States: 1976-1994. Kidney Int 2003;63:1817-23.
2. Lieske JC, Pena de la Vega LS, Slezak JM, Bergstralh EJ, Leibson CL, Ho KL, et al. Renal stone epidemiology in Rochester, Minnesota: An update. Kidney Int 2006;69:760-4.
3. Colorbawalla BN. Incidence of urolithiasis in India. ICMR Tech Rep 1971;8:42-5.
4. Agarwal S, Gupta SJ, Saxena AK, Gupta N, Agarwal S. Urolithic property of Varuna (Crataeva nurvala): An experimental study. AYU 2010;31:361-6.
5. Singh RG, Behura SK, Kumar R. Litholytic property of Kalutha (Dolichos biflorus) vs potassium citrate in rat renal calculus disease: A comparative study. J Assoc Physicians India 2010;58:286-9.
6. Susruta, Susruta Samhita, Kaviraj Ambikadatta Shastri editor. 12 ed. Chaukhambha Sanskrit Sansthan, Varanasi; 2001. Chikitsa Sthana, Ashmari Chikitsa Adhyaya, 7/5-8 p.
7. Sharanagatharcharya, Sharanagatha Samhita. Commentator: Dr. Brahmananda Tripathi. Chaukhambha Sanskrit Surabharathi Prakashana, Varanasi: 2003. Madhyam Khand, Snehalapalana 9/1-2 p.
8. Paget GE, Barnes JM. Toxicity Tests. In: Laurence DR, Bacharach AL, editors. Evaluation of drug activities pharmaceutmics. New York: Academic Press; 1956. p. 161.
9. Kumar S, Sigmon D, Miller T, Carpenter B, Khan S, Malhotra R, et al. A new model of nephrolithiasis involving tubular dysfunction/injury. J Urol 1991;146:1384-9.
10. Tiffany TO, Jansen JM, Burts CA, Overton JB, Scott CD. Enzymatic kinetic rate and end-point analyses of substrate, by use of a GeMSAEAC fast analyzer. Clin Chem 1970;16:829-40.
11. Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. Scand J Clin Lab Invest 1965;17:381-7.
12. Kabazakallan P, Kallinay S, Westcott A. Determination of uric acid in serum, with use of uricase and a tribromophenol-aminophtypine chromogen. Clin Chem 1973;19:522-4.
13. Tietz NW. Textbook of Clinical Chemistry. W. B. Saunders; 1986. p. 1350.
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
15. Slot C. Determination of peroxidase. Science 1973;179:588-90.
16. K, Vikas, P, Despande A, Mahor A, Bharti JS, Saxena AK. Animal models of kidney stone formation: An analysis. World J Urol 1997;15:236-43.
17. Nuvet RR, Phillips PH. Modification of the nitroprusside method of analysis for glutathione. Arch Biochem 1951;30:217-25.
18. Gupta, et al.: Anti-urolithic activity of Pashanabhedadi Ghrita

हिंदी सारांश
पापाथेभेदादिघृत का चूहों की वृक्षाश्मरी पर एक प्रायोगिक मूल्यांकन

संजय कुमार गुप्ता, माधवसिंह बचेल, चुतुर्जुष्ठ भुयाँ, विवेरकार, अशोक बी. के., पंचक्षरी दी. पाटेल

आयोगीक जगत में वृक्षाश्मरी रोग विशेषकर वृक्षाश्मरी का प्रचलन कुछ अधिक ही देखने को मिलता है। सूचना के द्वारा बताया गया एक विशेष योग्य ‘पापाथेभेदादिघृत’ का वर्गों से चिकित्सा में योग्य कहा जा रहा है, इसकी उपयोगिता निश्चित करना बंदम नक्षत्र की आवश्यकता है। चूहों पर किये गए इस प्रयोग में आमोनियम अक्सालेट प्रमुख आहार एवं जेटेन्द्रासिन इंजेक्शन के प्रयोग से उत्पन्न वृक्षाश्मरी पर पापाथेभेदादिघृत का मूल्यांकन किया गया है। अध्ययनान्तर आयुक्त का योग्यता के आरसे से ही कहा गया (90 मिनट./कि.ग्रा., 95 दिनों के लिए)। सर्वप्रथम चूहों को सोलहवें दिन विच्छेद कर उनके स्तर एवं वृक्षों का उपयुक्त प्रायोगिक अध्ययन किया गया। सर्वप्रथम मामलों में कई विशेष परिवर्तन देखने को नहीं मिला। जबकि लिपिद पेयमिसौलेशन में विशेष गैरसामान्य देखने को मिली। यही नूतनाधियों एवं नूतनाधियों पर–आक्सीडेंज क्रियाओं में बढ़ाती देखने को मिली। वृक्ष व नस्लियों में क्रिक्ट का उपरांत कम होने से बड़े उत्पन्न वृक्षाश्मरी की हानि लिया। अंततः यह सिद्ध हुआ कि ‘पापाथेभेदादिघृत’ में, प्रायोगिक चूहों में आमोनियम अक्सालेट उत्पन्न आहार एवं इंजेक्शन जेटेन्द्रासिन द्वारा उत्पन्न वृक्षाश्मरी के प्रति अधिक प्रतिक्रियाकार क्षमता दिखाई है।