ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

A Study on Nephroprotective and Antiurolithiasis activities of Ethanolic Extract of *Asarum europaeum* Leaves against Gentamicin Induced Nephrotoxicity in *Wistar* Rats

Srilakshmi Mamillapalli[1], Pavan Chand Akkiraju[2]

1. Department of Pharmacology, Sarada College of Pharmaceutical Sciences, Kondakavuru, Narasarao pet, Guntur, A. P., India.

2. Assistant Professor, Department of Biotechnology, P.V.P. College of Arts, Science & Commerce, Pravaranagar, Loni – 413713, Ahmednagar (Dt.), Maharashtra.

Manuscript Info

Manuscript History:

Received: 15 May 2015
Final Accepted: 22 June 2015
Published Online: July 2015

Key words:

Asarum europaeum, nephrotoxicity, antiurolithiasis, nephroprotective activity, ethanolic extract

Corresponding Author

Pavan Chand Akkiraju

Abstract

The drugs like gentamicin can show nephrotoxicity in the animals, after their administration. The normal functions of the cells are disturbed or completely lost due to the action of the drugs. Nephrolithiasis refers to calculi in the kidneys, which induces renal injury through intratubular obstruction and promote cell swelling and finally leads to chronic renal failure and papillary necrosis. The incidence of nephrolithiasis (kidney) and Ureterolithiasis (Ureter) is of significant importance in research, worldwide. The medicinal properties of plants have been investigated during recent years, due to their potent pharmacological activities, economic viability and low toxicity. *Asarum europaeum* is a traditional plant of India and used as anti-inflammatory, antioxidant, antitumorigenic, antiulcerogenic, hepatoprotective, antipyretic, diuretic and nephroprotective agent. The current study focused on the nephroprotective and antiurolithiatic activities of *Asarum europaeum*.

INTRODUCTION

Nephrotoxicity denotes the disruption of normal cellular functions of mitochondria and membrane integrity in the epithelial cells lining glomerulus and renal tubules. It induces renal injury through intratubular obstruction such as crystal disposition and promotes cellular swelling and tubular luminal occlusion (osmotic effects). Medications can also cause chronic renal failure leading to chronic interstitial injury and papillary necrosis (Preminger, 2007; Wolf, 2011). A kidney stone is also known as renal calculus is a solid concentration or crystal aggregation formed in the kidneys from dietary minerals in the urine. Kidney stone typically leaves the body by passage in the urine system. Ureteral obstruction causes post renal azotemia and hydronephrosis which leads to pain in the flank, lower abdomen and groin (a condition called renal colic). It can be associated with nausea, vomiting, fever, blood in the urine, pus in the urine and painful urination (Pearle *et al*., 2007; Cavendish, 2008).

Urolithiasis is a significant morbidity, affecting all geographical, cultural, and racial groups. The life time risk is about 10-15% in the developed world, but can be as high as 20-25% in Middle East. The increased risk of dehydration in hot climates, coupled with a diet that is 50% lower in calcium and 250% higher in oxalates compared to Western diets, accounts for the higher net the Middle East (Lieske and Segura, 2004).

Eighty percent (80%) of those with kidney stones are men; Men most commonly experience their first episode between 30-40 years. Most stones in women are due to either metabolic defects (such as cystinuria) or infection (Weiss *et al*., 2007). The total cost for treating urolithiasis was US$ 2 billion in 2003 (Pietrow and Karellas, 2006).

In North America and Europe, the annual incidence (number of new cases per year) of kidney stones is roughly 0.5%. In the United States, the prevalence (frequency in the population) of Urolithiasis has increased from 3.2% to 5.2% from the mid-1970 to the mid-1990s (Moe, 2006). The age of onset shows a bimodal distribution in women, with episodes peaking at 35 and 55 years (Lieske and Segura, 2004). Recurrence rates are estimated at 50% over a 10 year period and 75% over 20years (Moe, 2006), with some people experiencing ten or more episodes over the course of lifetime (Weiss *et al*., 2007).

Nephrolithiasis is caused by risk factors such as preexisting azotemia, diabetes, struvite calculi, hereditary diseases such as primary hyperoxaluria, Dent disease, cystinuria and polycystic kidney disease, spinal cord injuries and functional or anatomical Urological anomalies predisposes patients with kidney stones to an increased risk of renal failure. These nephrolithiatic risk factors are the major causes of morbidity worldwide. Significant efforts are needed to identify novel drug treatments (Pearle *et al*, 2005). The most morbid and potentially dangerous aspect of stone disease is the combination of Urinary tract obstruction, Upper urinary tract infection, Pyelonephritis, Pronephrosis and Urosepsis. Metabolic evaluation, Dietary therapy and Medical therapy are generally effective at delaying the tendency for stone formation. The most important dietary therapy is maintaining a high fluid intake and reducing dietary intake of calcium (Mariappan and Loong, 2004).

Treatment of Nephrolithiasis involves emergency management of Renal Colic by medical therapy such as drugs like NSAIDs, Antiemetics and Oral narcotics in combination form with Acetaminophen, Antidiuretics, Antibiotics, Uricosuric agents, alpha adrenergic antagonists are also used (Lezin *et al*. 1992). Surgical therapy includes Ureteral Stent placement, Percutaneous nephrostomy (Preminger *et al*, 2005). Shock Wave Lithotripsy (SWL) and Ureteroscopy are both recognized as first-line treatments of Ureteral stones (Preminger *et al*, 2007). Through these drugs are effective in the treatment of Nephrolithiasis, they will contribute sufficiently for enough side effects, hence the usage of herbal medicines has increased because of lesser side effects.

Herbalism, a traditional medicinal or folk medicinal practice based on the use of plants and plant extracts. The applications of herbal medicine are extended as a way to learn about potential future medicines (Acharya *et al*., 2008). Herbal products are often perceived as safe because they are natural. Natural products drug discovery will be more personalized and include a wide use of ancient and modern therapeutic skill in a complementary manner. Herbal showing nephroprotective and antiurolithiasis activity have been studied and some significant observations have been reported. The following are the list of medicinal plants proved to be effective in the treatment of Nephrlithiasis: *Rubia cordifolia*, *Moringa oleifera*, *Didymocarpus pedicellata*, *Aerva lanata*, *Helianthus annus Linn.*, *Arachis hypogeal Linn.*, and *Hemidesmus indicus*.

The medical properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. Asarum europeum is well known plant traditionally it has been used as Anti-Inflammatory, Antioxidant, Antitumorigenic, Antiulcerogenic, Hepatoprotective, Antipyretic, Diuretic and Nephroprotective. But Nephroprotective activity is not explored scientifically, so the present study is to evaluate the Nephroprotective and Antiurolithiasis activity of *Asarum europeum* leaves in *Wistar* rats.

The current study also focus to develop the pharmacological activity of the herbal drug using ethanolic extract of *Asarum europeum* leaves, which is traditionally claimed for its effectiveness in the treatment of aliments with low toxicity and economic viability.

MATERIALS & METHODS

The authenticated plants of *Asarum europaeum* were collected from Sri Venkateswara University, Tirupati and brought to the laboratory of Pharmacology at Sarada College of Pharmaceutical Sciences, Narasaraopet. The leaves were dried in shade and powdered by mechanical grinder. The powder of the leaves were initially defatted with Petroleum ether (60-80^{o}C) followed by 1000 mL of ethanol (95%) by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of solvent. The extract was filtered using Whattman filter paper (No. 1) and then concentrated in vacuum and dried at 45^{o}C for ethanol elimination and the extract was kept in a sterile bottle under refrigeration condition of about 2-7^{o}C. The ethanolic extracts were analyzed for the presence of alkaloids, terpenoids, Cardiac glycosides (Keller-Killani Test), reducing sugars, saponins, carbonyls, tannins, Phlobatannis, flavonoids and steroids, by using the standard protocols.

Albino *Wistar* rats of either sex weighing between 150-200g were procured from registered breeders (GENTOX Bio Services Private Limited, Hyderabad). The animals were housed under standard conditions of temperature (25 ± 2^{o}C) and relative humidity (30-70%) with a 12:12 light and dark cycle. They were fed with standard pellet diet and water ad *libitum*. The Urea, creatinine and total protein were estimated by commercially available kits. Male *Wistar*

rats weighing 150-200g were administered with Gentamicin subcutaneously with a single dose of 40mg/kg body weight and calculi producing diet (CPD) in rat feed for 30 days to induce Nephrolithiasis.
The experimental design includes, 5 groups of rats indicated in Roman numbers (I to V), and six rats from each group are used as sample collecting individuals. The groups are administered with gentamicin, CPD, EEAE and standard drug (Cystone). After acclimatization of animals, Group-I was administered with vehicle for 30 days of experimental animal period. Group-II Animals were administered with 40mg/kg/day of Gentamicin subcutaneously along with CPD and vehicle for 30 days. Group-III animals were administered with inducing agents along with EEAE (200 mg/kg) for 30 days. Group-IV animals were administered with inducing along with EEAE (400 mg/kg) for 30 days. Group-V animals were administered with inducing agents along with standard drug (Cystone-200 mg/Kg) for 30 days. After experimental period, the blood and urine samples were collected to study serum creatinine and urea (blood), total protein and pH (Urine) were evaluated.
After experimental period, the animals were sacrificed and kidneys were excised. The wet weights of the kidneys were evaluated (Prasad *et al*., 2010). The homogenates were prepared and further used for estimation of antioxidant parameters, i.e. estimation of lipid peroxidation (n.mol/g protein), Super Oxidase Dismutase (SOD) (Units/mg protein) and catalase (Units/mg protein). All the values are expressed as mean ± S.E.M. All the data was analyzed using one way analysis of variance (ANOVA) followed by Bonferroni Multiple Comparison Test.

RESULTS

Percentage yield of EEAE

The shade dried leaves of *Asarum europaeum*, weighing about 300g was extracted by Soxlet extraction method using 95% ethanol and the extract was evaporated to dryness using Rotatory Vacuum Evaporator. The weight of ethanolic extract obtained was 24.77g. Its percentage yield is calculated by the following formula: Percentage yield = weight of extract obtained / Weight of crude powder * 100. The percentage yield of ethanolic extract of *Asarum europaeum* (EEAE) is 8.3% w/w.

Preliminary Phytochemical Investigation

The results of preliminary Phytochemical screening of the ethanolic extract of leaves of *Asarum europaeum* were shown in the table I. The ethanolic extract gave positive results for alkaloids, reducing sugars, tannins, steroids and phlobatatanins.

Effect of EEAE on Urinary Parameters in rats

The effect of EEAE on urinary parameters showed a significant decrease in the levels of total protein, increase in pH of urine and a decrease in the kidney weight, when compared to the negative control group (Table II; Figure 1).

Effect of EEAE on serum biochemical parameters

A Significant reversion of elevated levels of serum urea and creatinine in groups treated with EEAE as compared to negative control group was tabulated in table III and figure 2**.**

Effect of EEAE on antioxidant enzyme levels in renal tissue

The groups which are treated with extract showed a significant increase in the levels of antioxidant enzymes such as SOD and catalase when compared to negative control group and decreasing LPO indicated by decreased levels of Malondialdehyde (Table IV; Figure 3).

Figure 1: Effect of EEAE on Urinary Parameters in rats

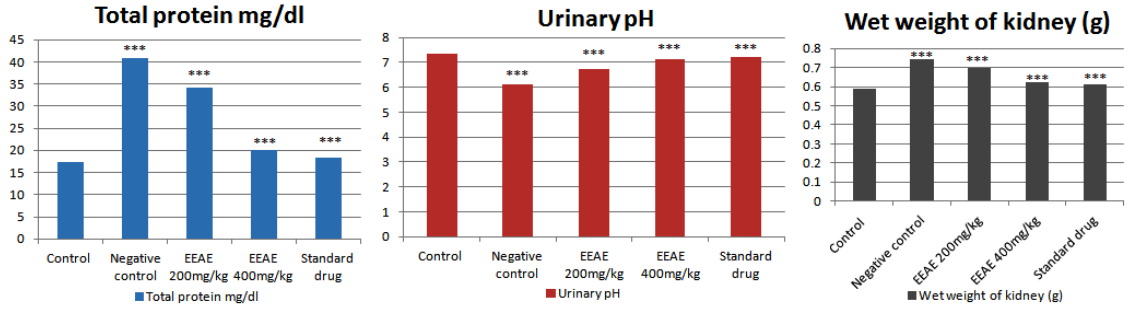

Figure 2: Effect of EEAE on serum biochemical parameters

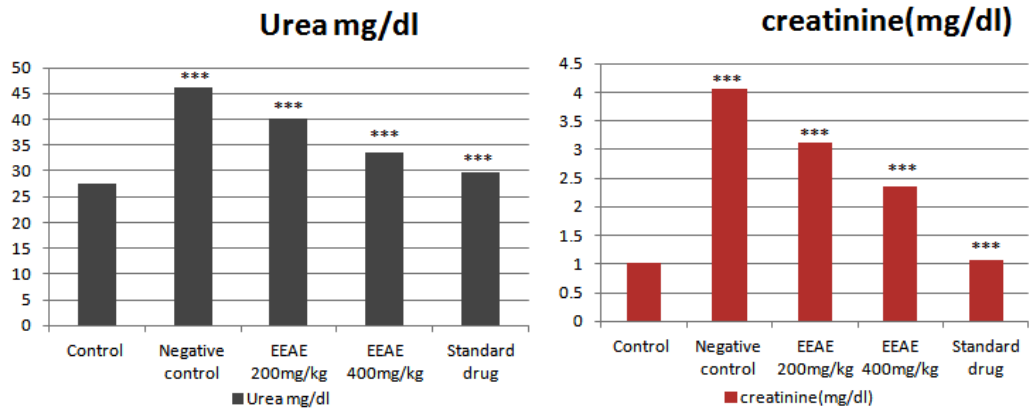

Figure 3: Effect of EEAE on antioxidant enzyme levels in renal tissue

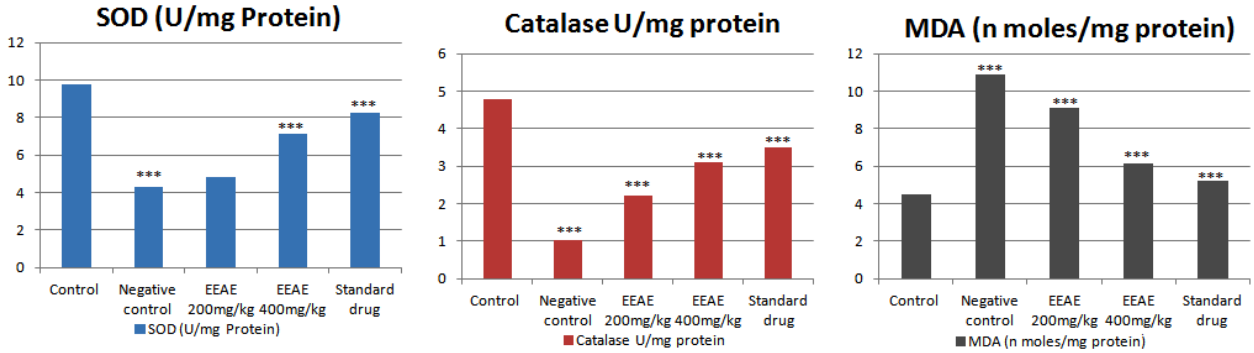

Table I: preliminary phytochemical screening of EEAE

S. No.	Phytochemical tests	Results
1	Alkaloids	Present
2	Reducing sugars	Present
3	Saponins	Absent
4	Tannins	Present
5	Flavonoids	Present
6	Steroids	Present
7	Phlobatannis	Present
8	Carbonyl compounds	Absent
9	Terpenoids	Absent
10	Cardiac glycosides	Absent

Table II: Effect of EEAE on Urinary Parameters in rats

S. No.	Treatment	Total protein mg/dl	Urinary pH	Wet weight of kidney (g)
1	Control	17.26 ± 0.03	7.34 ± 0.03	0.59 ± 0.008
2	Negative control	40.79 ± 0.05^{a***}	6.11 ± 0.006^{a***}	0.76 ± 0.006 ^{a***}
3	EEAE 200mg/kg	34.21 ± 0.04^{b***}	6.73 ± 0.01^{b***}	0.70 ± 0.009 ^{b***}
4	EEAE 400mg/kg	20.09 ± 0.02^{b***}	7.11 ± 0.008^{b***}	0.64 ± 0.007 ^{b***}
5	Standard drug	18.40 ± 0.15^{b***}	7.23 ± 0.008^{b***}	0.61 ± 0.006 ^{b***}

All the values are expressed as mean ± SEM (n=6); Data were analyzed by ANOVA followed by Bonferroni Multiple Comparison Test. Compared Control *vs* negative Control: $^{a***}p<0.05$; Compared Control *vs* negative Control *vs* Group III, IV and V: $^{b***}p<0.05$; ns- Statistically not significant.

Table III: Effect of EEAE on serum biochemical parameters

S. No.	Treatment	Urea mg/dl	creatinine(mg/dl)
1	Control	27.42 ± 0.04	0.9 4 ± 0.01
2	Negative control	46.08 ± 0.05[a]***	4.05 ± 0.006[a]***
3	EEAE 200mg/kg	40.10 ± 0.30[b]***	3.12 ± 0.01[b]***
4	EEAE 400mg/kg	33.58 ± 0.13[b]***	2.36 ± 0.03[b]***
5	Standard drug	29.72 ± 0.11[b]***	1.07 ± 0.01[b]***

All the values are expressed as mean ± SEM (n=6); Data were analyzed by ANOVA followed by Bonferroni Multiple Comparison Test. Compared Control *vs* negative Control: [a]*** $p<0.05$; Compared Control *vs* negative Control *vs* Group III, IV and V: [b]*** $p<0.05$; ns- Statistically not significant.

Table IV: Effect of EEAE on antioxidant enzyme levels in renal tissue

S. No.	Treatment	SOD(U/mg protein)	Catalase (U/mg protein)	MDA (n moles/mg protein)
1	Control	9.75 ± 0.16	4.79 ±0.08	4.49 ± 0.17
2	Negative control	4.33 ± 0.07[a]***	1.02 ± 0.03[a]***	10.86 ± 0.28[a]***
3	EEAE 200mg/kg	4.84 ± 0.07 [b ns]	2.23 ± 0.07[b]***	9.08 ± 0.17[b]***
4	EEAE 400mg/kg	7.11 ± 0.10[b]***	3.11 ± 0.07[b]***	6.12 ± 0.12[b]***
5	Standard drug	8.24 ± 0.21[b]***	3.49 ± 0.09[b]***	5.30 ± 0.21[b]***

All the values are expressed as mean ± SEM (n=6); Data were analyzed by ANOVA followed by Bonferroni Multiple Comparison Test. Compared Control vs negative Control: [a]*** $p<0.05$; Compared Control vs negative Control vs Group III, IV and V: [b]*** $p<0.05$; ns- Statistically not significant.

DISCUSSION

Gentamicin is a widely used amino glycoside antibiotic for the treatment of Gram negative bacterial infections. Although the drug has been proved for its usefulness, its nephrotoxic action limits the extent of its use. Evidence suggests that reactive oxygen species (ROS) may be involved for GM: induced renal tubular damage, which finally leads to nephrotoxicity. GM has also been shown to inhibit calcium reabsorption in proximal tubules, which leads to the deposition of calcium oxalate crystals, leading to a condition Hyperoxaluria. In the present study, 5% ammonium oxalate is used along with GM (40mg/Kg) to induce nephrotoxicity and urolithiasis in rats.

Several approaches using different mechanisms have been attempted to reduce GM and CPD induced nephrotoxicity and urolithiasis. Many medicinal herbs possessing nephroprotective and antiurolithiatic property have been reported, however, studies on beneficial effects of *Asarum europaeum* on drug induced or chemical induced nephrotoxicity are very limited.

In the present study, GM and CPD induced hyperoxaluria and also caused papillary damage in the kidney. The supersaturation of the urine with calcium oxalate is the most important factor in nucleation, growth, aggregation and crystallization. It has been reported that oxalate has about 15 folds greater effect on calcium oxalate crystallization than urinary calcium alone.

Treatment with EEAE caused a significant reduction in urinary excretion of calcium and oxalate, therefore, reducing the supersaturation of urine. This might be responsible for dissolving and also in preventing the formation of stones. This is supported by the wet kidney weight. On histological examination, the negative control group (II) showed calcium oxalate crystals in majority of tubules and weight of kidneys when compared to the control group. Group III and IV showed a very few crystals, indicating the ability of EEAE in dissolving the calculi and comparatively decrease in kidney weight. Group V administered with standard showed less stone formation, when compared to negative control group.

The pH of the urine can be predicted by the type of stones formed in the kidneys. If the pH is acidic (5.0 or 0), the stones likely to form are uric acid type, If 5.0 to 6.5, calcium oxalate type and if alkaline, i.e. 7.2 or above indicates magnesium or ammonium phosphate type.

In the present work, the pH of the negative control (group 2) is decreased to 5.0 to 6.0, when compared to control group I (pH 6.0 to 7.0). EEAE treated groups III and IV reversed the acidic pH to normal. This increase in urinary pH might be responsible for dissolution of complexes, which contributes to their significant antiurolytiatic activity. In the current work, antiurolythiatic activity of *Asarum europaeum* may be due to its diuretic activity, which is attributed to the presence of glycoproteins and flavonoids.

Parameters such as total protein content in urine are used to study the extent of renal damage induced by GM and CPD. In group II, there is significant increase in urinary excretion of total protein when compared to group I, which could be associated with necrosis of proximal tubules, the primary state of drug accumulation. In groups treated with EEAE (Group III, IV) and Group V there is reduction in levels of total protein in urine as compared to group II which could be due to the ability of EEAE to partially ameliorate the tubular necrosis. In group II, negative control, there is significant increase in the levels of N-compounds such as urea and creatinine in blood, condition called azotemia, which is due to insufficient filtration of blood by the kidneys, when compared to group I. In group III, IV and V, there is significant reduction in blood levels of urea and creatinine when compared to group II.

In the present study, we evaluate the protective effects of EEAE against GM and CPD induced nephrotoxicity in rats. In our study, administration of GM resulted in oxidative damage to the lipids and proteins of the kidney in the rats. There was a significant decrease in SOD and catalase activities in rats treated with GM and CPD (Group II). However, rats treated with extract (group III, IV) and group IV could restore the antioxidant capacity when compared to group II.

The levels of TBARS were significantly increased negative control group II when compared to group I. In group III and IV, there is a significant decrease in TBARS levels as compared to group II. In the current work, the antioxidant property of *Asarum europaeum* is may be owed to the presence of poly phenolic compounds, glycoproteins and flavonoids.

The plant extract of doses 200mg/Kg and 400mg/kg along with standard group V showed significant decrease in total protein, wet weight of kidney, urea, creatinine and lipid peroxidation and significant increase in urinary pH, SOD and catalase in a dose dependent manner at $p<0.05$, when compared to negative control group. The extract dose at 400mg/Kg showed more significant results when compared to 200mg/Kg and showed similar results as compared to standard group.

CONCLUSION

The preliminary Phytochemical screening of the ethanolic extract of *Asarum europaeum* showed the presence of alkaloids, tannins, flavonoids, steroids, phlobatanin. The EEAE administered at doses 200mg/Kg and 400mg/Kg showed significant alterations on the increased levels of urinary parameters such as total protein, weight of kidney and pH. It is also showed a significant reversal of increased biochemical parameters such as urea and creatinine in extract treated groups. The extract showed protective effect by altering the decreased levels of antioxidant enzymes (SOD and catalase) in renal tissue and inhibited lipid peroxidation.

In an attempt to provide scientific validation of medicinal herbs for their glycoproteins, polyphenolic compounds and flavonoids in the ethanolic extract of leaves of *Asarum europaeum*. Since the crude form of the drug is only used, it would be worthwhile to carry out the study after isolation of individual constituents.

BIBLIOGRAPHY

1. Acharya, D., Shrivastava, A. (2008): Indigenous Herbal Medicines: Tribal formulations and traditional herbal practices, Avishkar Publishers, India, pp. 440.
2. Cavendish, M. (2008): Kidney disorders. “Diseases and Disorders”, Tarrytown, New York: Marshall Cavendish Corporation (1st Edition), pp. 490-3.
3. Lezin, St M., Hofmann, R., Stoller, ML. (1992): Pyonephrosis: diagnosis and treatment. Br J Urol., Oct: 70 (4): 306-3.
4. Lieske, JC., Segura, JW. (2004): "Chapter 7: Evaluation and Medical Management of Kidney Stones". In Potts, JM. Essential Urology: A Guide to Clinical Practice (1st Ed.). Totowa, New Jersey: Humana Press. pp. 117–52. ISBN 978-1-58829-109-7
5. Mariappan, P., Loong, CW. (2004): Midstream urine culture and sensitivity test is a poor predictor of infected urine proximal to the obstructing ureteral stone or infected stones: A prospective clinical study. J Urol.; 171: 2142-5
6. Moe, OW. (2006): Kidney stones: Pathophysiology and medical management. Lancet; 367: 333-344.
7. Pearle, MS., Calhoun, EA., Curhan, GC. (2005): Urologic Diseases of America Project: Urolithiasis. J Urol.; 173: 848–857.
8. Pearle, MS., Calhoun, EA., Curhan, GC. (2007): "Chapter 8: Urolithiasis". In Litwin, MS; Saigal, CS. Urologic Diseases in America (NIH Publication No. 07–5512). Bethesda, Maryland: US Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. pp. 283–319.

9. Pietrow, PK., Karellas, ME. (2006): Medical management of common urinary calculi, *Am Fam Physician.* Jul 1; 74 (1):86-94.
10. Preminger, GM. (2007): "Chapter 148: Stones in the Urinary Tract". In Cutler, RE. The Merck Manual of Medical Information Home Edition (3rd ed.). Whitehouse Station, New Jersey: Merck Sharp and Dohme Corporation.
11. Preminger, GM., Assimos, DG., Lingeman, JE., Nakada, SY., Pearle, MS., Wolf JS Jr. (2005): Chapter 1: AUA guideline on management of staghorn calculi: diagnosis and treatment recommendations. J Urol. Jun; 173(6):1991-2000.
12. Preminger, GM., Tiselius, HG., Assimos, DG., Alken, P. (2007): "2007 Guideline for the management of ureteral calculi". The Journal of Urology **178** (6): 2418–34.
13. Weiss, M., Liapis, H., Tomaszewski, JE., Arend LJ. (2007): "Chapter 22: Pyelonephritis and other infections, reflux nephropathy, hydronephrosis, and nephrolithiasis". In Jennette, JC; Olson, JL; Schwartz, MM et al. Heptinstall's Pathology of the Kidney **2** (6th Ed.). Philadelphia: Lippincott Williams & Wilkins. pp. 991–1082.
14. Wolf, Jr. JS. (2011): "Background". Nephrolithiasis. New York: Web MD.