

## EXPERIMENTAL PAPER

### Study of antiurolithiatic activity of a formulated herbal suspension

NISHI SAXENA, AMEETA ARGAL\*

Rajeev Gandhi College of Pharmacy  
Bhopal  
Madhya Pradesh  
India-462042

\*Corresponding author: phone: +91786 9801910, e-mail: ameetaargal@yahoo.com, nishi.saxena12@rediffmail.com

#### Summary

Urolithiasis is the process of formation of stone in the urinary tract by crystal nucleation, aggregation and retention in the urinary tract. Traditional medicinal plants have been successfully used to overcome urolithiasis. Hence, herbal formulation containing a mixture of plant extracts was prepared and evaluated for the antiurolithiatic activity. This formulation contained alcoholic extracts of fruit of *Tribulus terrestris*, root of *Boerhavia diffusa* and leaves of *Azadirachta indica*. Studies were performed in ethylene glycol-induced urolithiasis model using Cystone as a standard drug. Ethylene glycol increases the level of calcium, oxalate and phosphate which are responsible for urolithiasis. The herbal suspension decreased the level of calcium, oxalate and phosphate significantly at doses of 200 and 300 mg/kg when compared to the negative control group. Creatine, uric acid and urea were also decreased significantly at all dose levels. Histopathology has supported these results. The level of LD<sub>50</sub> was found to be higher than 2000 mg/kg. Therefore, the prepared formulation has appreciable significant antiurolithiatic activity and is safe for use.

**Key words:** antiurolithiatic activity, *Tribulus terrestris*, *Boerhavia diffusa*, *Azadirachta indica*, herbal suspension

## INTRODUCTION

Lithiasis is the process of formation of stone and urolithiasis are the solid nonmetallic minerals in the urinary tract [1]. Stones result due to phase change whereby dissolved salts condense into solids because of super saturation [2]. Among several

types of kidney stones, the most common are calcium oxalate. The formation of these stones occurs with crystal nucleation, aggregation and retention within urinary tract. If a stone blocks the flow of urine, excruciating pain may result [3]. Herbs have been proved to have significant analgesic activity [4]. Recurrent stone formation is a common part of the medical care of patients with stone disease [5]. Calcium containing stones, especially calcium oxalates and phosphate are the most commonly occurring ones [6]. In Ayurveda, the medicinal use of plants decrease the recurrence rate of urolithiasis without any potential side effects [7]. Surgery, lithotripsy, and local calculus disruption using a high power laser are also used to treat calculi [8]. However, these procedures are expensive and recurrence is quite common, requiring preventive treatment [9]. Phytotherapy can reduce the reoccurrence rate [10]. Antiuro lithiatic activity has been studied in plants [11, 12]. Validation of herbal drugs is the requirement of time through the experimental study [13].

Therefore, herbal suspension was formulated, evaluated and studied for antiuro lithiatic activity. It was prepared from fruits of *Tribulus terrestris* L. (*Zygophyllaceae*), roots of *Boerhavia diffusa* Linn. (*Nyctaginaceae*) and leaves of *Azadirachta indica* A. Juss (*Meliaceae*). All plant parts of *T. terrestris* have antimicrobial and antifungal activity but the activity of fruits was the best one [14]. Its diuretic activity is due to high concentration of potassium salts present in it. It is antiuro lithiatic and is reported to inhibit stone formation [15, 16]. It has immunomodulatory [17], analgesic [18] and anti-inflammatory activities [19]. It also relaxes spasm of smooth muscle [20]. The leaf of *B. diffusa* has analgesic, anti-inflammatory [21] and antibacterial [22] activities. Inhibition of crystals and defragmentation of some grown crystals was found in *in vitro* study [23]. *A. indica* has anti-inflammatory [24] antimicrobial [25] and antiuro lithiatic [26] activities.

## MATERIALS AND METHODS

### Plant material

The fruits of *T. terrestris* (TT), roots of *B. diffusa* (BD) and leaves of *A. indica* (AI) were collected locally and authenticated from the Department of Botany, Janata PG College, A.P.S. University, Rewa (M.P.). The voucher specimen number is JC/B/PAN/054c-e. They were cleaned, dried in shade and grinded to a coarse powder.

### Preparation of herbal suspension (TAB)

The powdered plant material was macerated individually in glass beakers with alcohol for 7 days. It was stirred occasionally. Then it was filtered and concentrated

at 78°C to get the extracts of TT, BD and AI. The extracts were mixed equally (1:1:1), triturated with Tween 80 (5% v/v), sorbitol (1% w/v), potassium sorbate (0.02% w/v), lemon oil (0.01% v/v) and distilled water. A uniformly dispersed suspension TAB was obtained.

## Animals

Male Wistar albino rats weighing between 140 to 200 g were taken for the study. They were subjected to standard laboratory conditions with 12 hours light and dark cycle, temperature  $25 \pm 2^\circ\text{C}$ , relative humidity  $60 \pm 5\%$  standard pellet diet and water *ad libitum*. The experiment was approved by the Institutional Animal Ethics Committee (1413/a/11/CPCSEA) as per CPCSEA guidelines (protocol approval No. SBRL/IAEC/2013/04).

## Acute toxicity

Acute toxicity studies were done as per OECD guidelines. A dose up to 2000 mg/kg was given to the rats orally. They were observed for any mortality, physiological or behavioural changes.

## Antiurolithiatic activity in ethylene glycol-induced urolithiasis

Ethylene glycol-induced hyperoxaluria model was used for the study. Six groups, each of six rats, were taken for the activity. Groups II to VI were given ethylene glycol EG (0.75%) in drinking water for 28 days, to induce renal calculi. The groups were assigned as:

Group I – normal control – administered with vehicle only

Group II – negative control – given EG only

Group III – standard – EG + Cystone 500 mg/kg (p. o.) from 15<sup>th</sup> to 28<sup>th</sup> day

Group IV – TAB 100 – EG + suspension 100 mg/kg (p. o.) from 15<sup>th</sup> to 28<sup>th</sup> day

Group V – TAB 200 – EG + suspension 200 mg/kg (p. o.) from 15<sup>th</sup> to 28<sup>th</sup> day

Group VI – TAB 300 – EG + suspension 300 mg/kg (p. o.) from 15<sup>th</sup> to 28<sup>th</sup> day

## Collection and analysis of urine

After completion of dosing the animals were transferred to metabolic cages. The urine was collected and estimated for calcium, oxalate and phosphate [29, 30, 31] (tab. 1).

Table 1.

Effect of herbal suspension on urinary parameters

S. no.	Group	Treatment	Urinary parameters [mg/dl]		
			Calcium	Oxalate	Phosphate
1	I	Normal control	2.46±0.09	0.77±0.06	3.18±0.14
2	II	Negative control	4.31±0.18a <sup>***</sup>	2.82±0.13a <sup>***</sup>	7.08±0.18a <sup>***</sup>
3	III	Cystone 500 mg/kg	2.93±0.12b <sup>***</sup>	1.67±0.09a <sup>***</sup> ,b <sup>***</sup>	4.23±0.16a <sup>**</sup> ,b <sup>***</sup>
4	IV	TAB 100 mg/kg	3.58±0.14 a <sup>**</sup>	1.98±0.08 a <sup>***</sup> ,b <sup>***</sup>	5.91±0.24a <sup>***</sup> ,b <sup>**</sup>
5	V	TAB 200 mg/kg	3.47±0.31 a <sup>**</sup> ,b <sup>*</sup>	2.23±0.13a <sup>***</sup> ,b <sup>**</sup>	5.32±0.23 a <sup>***</sup> ,b <sup>***</sup>
6	VI	TAB 300 mg/kg	3.0±0.1b <sup>***</sup>	1.84±0.11a <sup>***</sup> ,b <sup>***</sup>	5.37±0.22 a <sup>***</sup> ,b <sup>***</sup>

All values are mean ± SEM, n = 6, \*p&lt;0.05, \*\*p&lt;0.01, \*\*\*p&lt;0.001

a – significant difference as compared to normal control (Group-I)

b – significant difference as compared to negative control (Group-II)

## Collection of blood and serum analysis

The blood samples from rats were collected from retro-orbital puncture. The samples were kept aside for 30 minutes at a room temperature. They were centrifuged at 3000 rpm, 20°C for 15 minutes and analyzed for urea, uric acid and creatinine [32] (tab. 2). The rats were sacrificed, kidneys were removed and used for histopathological examination (fig. 1).

Table 2.

Effect of herbal suspension on serum parameters

Sample No.	Group	Treatment	Serum parameter [mg/dl]		
			Creatinine	Uric acid	Urea
1	I	Normal control	0.7±0.03	1.4±0.1	37.35±1.04
2	II	Negative control	1.58±0.09a <sup>***</sup>	3.6±0.08a <sup>***</sup>	70.22±2.12a <sup>***</sup>
3	III	Cystone 500 mg/kg	1.22±0.06 a <sup>***</sup> ,b <sup>**</sup>	2.15±0.09a <sup>**</sup> ,b <sup>***</sup>	52.22±1.85 a <sup>***</sup> ,b <sup>***</sup>
4	IV	TAB 100 mg/kg	1.49±0.05 a <sup>***</sup>	2.86±0.08 a <sup>***</sup> ,b <sup>***</sup>	61.42±1.95 a <sup>***</sup> ,b <sup>***</sup>
5	V	TAB 200 mg/kg	1.35±0.04 a <sup>***</sup>	2.56±0.1 a <sup>***</sup> ,b <sup>***</sup>	57.37±0.56 a <sup>***</sup> ,b <sup>***</sup>
6	VI	TAB 300 mg/kg	1.37±0.02 a <sup>**</sup>	2.37±0.06 a <sup>***</sup> ,b <sup>***</sup>	55.7±1.18 a <sup>***</sup> ,b <sup>***</sup>

All values are mean ± SEM, n=6, \*p&lt;0.05, \*\*p&lt;0.01, \*\*\*p&lt;0.001.

a – significant difference as compared to normal control (Group I)

b – significant difference as compared to negative control (Group II)

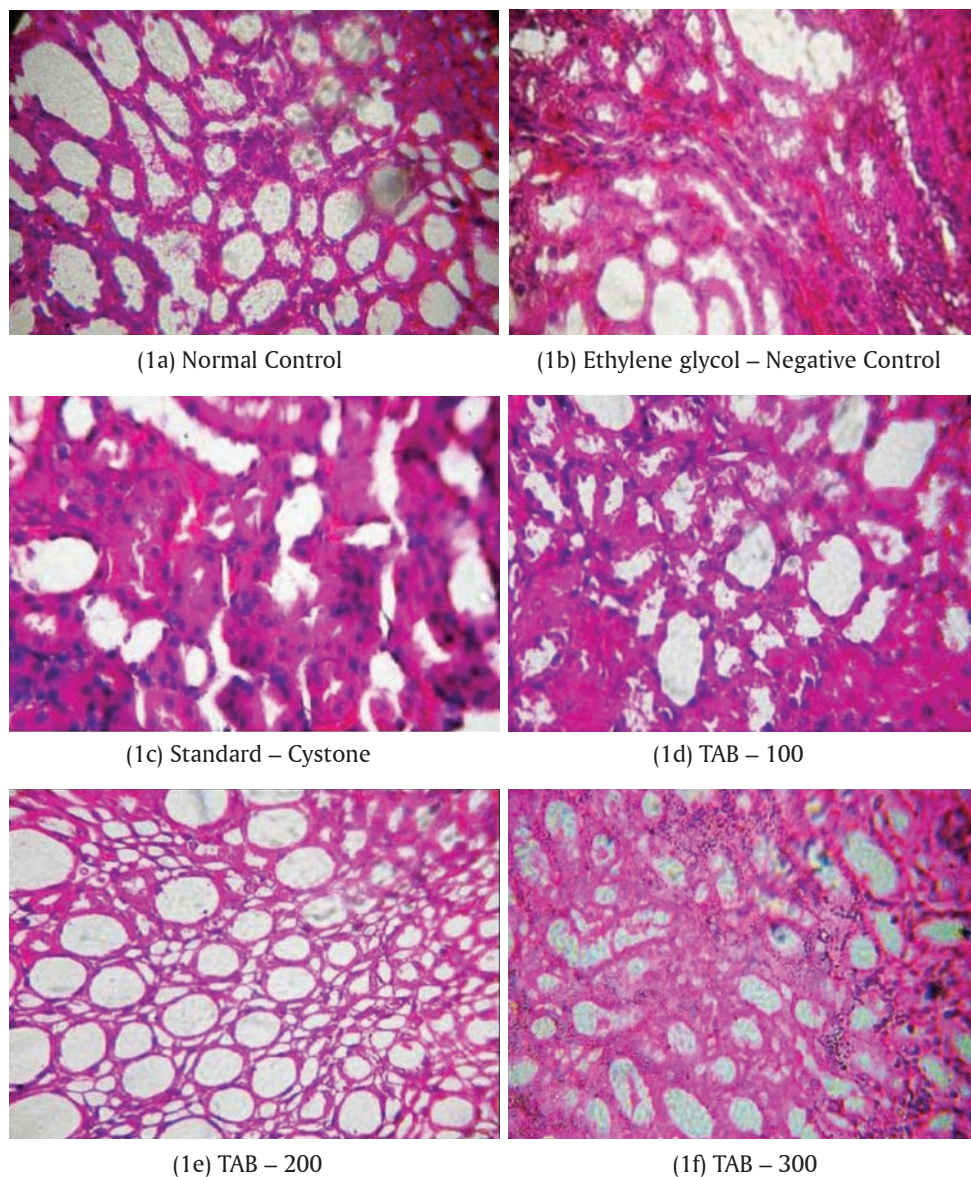


Figure 1.

Histology of sections of kidney for antiurolithiatic activity of TAB

### Statistical analysis

All the values are expressed as mean  $\pm$  standard error of mean (SEM) and analyzed for ANOVA and post-hoc Tukey-Kramer multiple comparisons test by

employing statistical software, GraphPad InStat 3. Differences between groups were considered significant at  $p < 0.05$  level.

## RESULTS

The results of acute toxicity studies show that no toxicity was observed till the dose of 2000 mg/kg. Hence,  $LD_{50}$  was considered to be higher than 2000 mg/kg. There was an increase in the excretion of calcium, oxalate and phosphate in all ethylene glycol-treated groups, as compared to normal control animals. The herbal suspension TAB decreased the excretion of calcium, oxalate and phosphate significantly at a dose of 200 and 300 mg/kg when compared to negative control group. The decrease in these levels by TAB 300 mg/kg was found to be similar to that of the standard drug Cystone at  $p < 0.001$  (tab. 1). This reduces the possibility of agglomeration and formation of stone. Creatinine, uric acid and urea were also decreased significantly in all doses of suspension-treated animals (tab. 2). Histology of kidney shows that when comparison of ethylene glycol-treated animals was done with the normal group, the tissues were degenerated, having vacuoles and obstructions. Crystals were seen deposited in the tubule. Cystone and formulation-treated groups had less damage than the negative control group. The regeneration of tissues was also seen much better in these groups (fig. 1).

## DISCUSSION AND CONCLUSION

Decrease in urinary output causes supersaturation of urine due to increase in calcium, oxalate and phosphate leading to formation of stones [33, 34]. The increase in urinary phosphorus leads to formation of calcium phosphate crystals [35]. High concentration of oxalates can lead to formation of calcium oxalate [12]. Ethylene glycol disturbs oxalate metabolism. The presence of polymorphic irregular crystal inside the tubules causes dilatation of the proximal tubules along with interstitial inflammation. This might be due to oxalate [36]. Usually patients with urolithiasis suffer from pain, therefore, plants having analgesic, antiurolithiatic and diuretic activity were chosen. *T. terrestris* extract has a potential to inhibit nucleation and growth of the CaOx crystals [37]. When compared to ethylene glycol-treated negative control group, the polyherbal suspension TAB reduced urinary calcium, oxalate, phosphate, creatine, uric acid and urea resulting in antiurolithiatic effect (tab. 1-2). It prevents supersaturation and formation of stones. Thus, it can be concluded that the prepared polyherbal suspension can be used as a safe and effective antiurolithiatic formulation. It can be a boon to people suffering from lithiasis.

## ACKNOWLEDGEMENTS

The authors are thankful to All India Council of Technical Education (Ref. No. 823/RID/RPS-34/ POLICY – IV PVT./2011-12) for funding our work. We also thank Sapiens Bioanalytical Research Lab, Bhopal for providing facilities.

## REFERENCES

1. Ashok P, Koti BC, Vishwanathswamy AH. Antiurolithiatic and antioxidant activity of *Mimusops elengi* on ethylene glycol-induced urolithiasis in rats. *Indian J Pharmacol* 2010; 42(6):380-383.
2. Coe FL, Evan A, Worcester E. Kidney stone disease. *J Clin Invest* 2005; 115(10): 2598-2608.
3. Atodariya U, Barad R, Upadhyay S, Upadhyay U. Anti-urolithiatic activity of *Dolichos biflorus* seeds. *J Pharmacogn Phytochem* 2013; 2(2):209-213.
4. Pathak AK, Argal A. Analgesic activity of *Calotropis gigantea* flower. *Fitoterapia* 2007; 78:40-42.
5. Yadav RD, Alok S, Jain SK, Verma A, Mahor A, Bharti JP et al. Herbal plants used in the treatment of urolithiasis: a review. *Int J Pharmaceutical Sci Res* 2011; 2(6):1412-1420.
6. Narayana SV, Ali VS. Pashanabheda. *J Res Indian Med* 1967; 1:24.
7. Baheti DG, Kadam SS. Antiurolithiatic activity of a polyherbal formulation against calcium oxalate induced urolithiasis in rats. *JAPER* 2013; 3(1):6-14.
8. Makasana A, Ranpariya V, Desai D, Mendpara J, Parekh V. Evaluation for the anti-urolithiatic activity of *Launaea procumbens* against ethylene glycol-induced renal calculi in rats. *Toxicol Rep* 2014; 1:46-52.
9. Prasad K, Sujatha D, Bharathi K. Herbal drugs in urolithiasis – a review. *Pharmacog Rev* 2007; 1:175-179.
10. Nagal A, Singla RK. Herbal resources with antiurolithiatic effects: A Review. *Indo Global J Pharm Sci* 2013; 3(1):6-14.
11. Kishore RN, Mangilal T, Anjaneyulu N, Abhinayani G, Sravya N. Investigation of anti-urolithiatic activity of *Brassica oleracea* gongyloides and *Desmostachya bipinnata* in experimentally induced urolithiasis in animal models. *Int J Pharm Pharm Sci* 2014; 6(6):602-604.
12. Khan A, Khan SR, Gilani AH. Studies on the *in vitro* and *in vivo* antiurolithic activity of *Holarrhena antidysenterica*. *Urol Res* 2012; 40(6):671-681.
13. Gupta SK, Baghel MS, Bhuyan C, Ravishankar B, Ashok BK, Patil PD. Evaluation of anti-urolithiatic activity of Pashanabhedadi Ghrita against experimentally induced renal calculi in rats. *Ayu* 2012; 33(3):429-434.
14. Al-Bayati FA, Al-Mola HF. Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. *J Zhejiang University Science B*. 2008; 9(2):154-159.
15. Chhatre S, Nesari T, Somani G, Kanchan D, Sathaye S. Phytopharmacological overview of *Tribulus terrestris*. *Phcog Rev* 2014; 8:45-51.
16. Sangeeta D, Sidhu H, Thind SK, Nath R. Effect of *Tribulus terrestris* on oxalate metabolism in rats. *J Ethnopharmacol* 1994; 44:61-66.
17. Tilwari A, Shukla NP, Uma Devi P. Effect of five medicinal plants used in Indian system of medicines on immune function in Wistar rats. *Afr J Biotechnol* 2011; 10:16637-16645.
18. Heidari MR, Mehrabani M, Pardakhty A, Khazaeli P, Zahedi MJ, Yakhchali M et al. The analgesic effect of *Tribulus terrestris* extract and comparison of gastric ulcerogenicity of the extract with indomethacin in animal experiments. *Ann N Y Acad Sci* 2007; 1095:418-427.
19. Baburao B, Rajyalakshmi G, Venkatesham A, Kiran G, Shyamsunder A, Gangarao B. Anti-inflammatory and antimicrobial activities of methanolic extract of *Tribulus terrestris* Linn plant. *Int J Chem Sci* 2009; 7:1867-1872.
20. Arcasoy HB, Erenmemisoglu A, Tekol Y, Kurucu S, Kartal M. Effect of *Tribulus terrestris* L. saponin mixture on some smooth muscle preparations: a preliminary study. *Boll Chim Farm* 1998; 137:473-475.
21. Hiruma-Lima CA, Gracioso JS, Bighetti EJB, Robineou GL, Souza Brito ARM. The juice of fresh leaves of *Boerhaavia diffusa* L. (Nyctaginaceae) markedly reduces pain in mice. *J Ethnopharmacol* 2000; 71:267-274.

22. Girish HV, Satish S. Antibacterial activity of important medicinal plants on human pathogenic bacteria - a comparative analysis. *World Appl Sci J* 2008; 5(3):267-271.
23. Chauhan CK, Joshi MJ, Vaidya AD. Growth inhibition of struvite crystals in the presence of herbal extract *Commiphora wightii*. *J Mater Sci Mater Med* 2009; 20(1):S85-92.
24. Biswas K, Chattopadhyay A, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (*Azadirachta indica*). *Curr Sci* 2002; 82(11):1336-1345.
25. Maragathavalli S, Brindha S, Kaviyarasi NS, Annadurai BB, Gangwar SK. Antimicrobial activity in leaf extract of neem (*Azadirachta indica* Linn.). *Intern J Sci Nat* 2012; 3(1):110.
26. Hwisa NT, Assaleh FH, Gindi S, Melad FE, Chandu BR, Katakam P. A study on antiurolithiatic activity of *Melia azadirachta* L. aqueous extract in rats. *Am J Pharm Sci* 2014; 2(1):27-31.
27. Atmani F, Slimani Y, Mimouni M, Hacht B. Prophylaxis of calcium oxalate stones by *Herniaria hirsuta* on experimentally induced nephrolithiasis in rats. *BJU Inter* 2003; 92:137-40.
28. Mitra SK, Gopumadhavan S, Venkataranganna MV, Sundaram R. Effect of Cystone, a herbal formulation, on glycolic acid-induced urolithiasis. *Phytother Res* 1998; 12:372-374.
29. Medeiros DM, Mustafa MA. Proximate composition, mineral content and fatty acids of cat fish (*Ictalurus punctatus* Rafinesque) for different seasons and cooking methods. *J Food Sci* 1985; 50:585-587.
30. Hodgkinson A, Williams A. An improved colorimetric procedure for urine oxalate. *Clin Chim Acta* 1972; 36:127-132.
31. Fiske CH, Subbarow Y. The colorimetric determination of phosphate. *J Biol Chem* 1925; 66:375-400.
32. Caraway WT. Uric acid. In: Seligson D (ed). *Standard methods of clinical chemistry*. 4<sup>th</sup> ed. London Academic Press 1963:239-247.
33. Michell AR. Urolithiasis-historical, comparative and pathophysiological aspects: a review. *J R Soc Med* 1989; 82:669-672.
34. Karadi RV, Gadge NB, Alagawadi KR, Savadi RV. Effect of *Moringa oleifera* Lam. root-wood on ethylene glycol induced urolithiasis in rats. *J Ethnopharmacol* 2006; 105: 306-311.
35. Soundararajan P, Mahesh R, Ramesh T, Begum VH. Effect of *Aerva lanata* on calcium oxalate urolithiasis in rats. *Indian J Exp Bio* 2006; 44:981-986.
36. Talekar YP, Gund KA, Kale SD, Apte KG, Parab PB. Antiurolithic activity of corn silk extract in rats. *Int J Univers Pharm Life Sci* 2013; 2(4):65-77.
37. Aggarwal A, Tandon S, Singla SK, Tandon C. A novel antilithiatic protein from *Tribulus terrestris* having cytoprotective potency. *Protein Pept Lett* 2012; 19:812-819.

## BADANIA NAD DZIAŁANIEM MIESZANKI ZIOŁOWEJ W KAMICY NERKOWEJ

NISHI SAXENA, AMEETA ARGAL\*

Rajeev Gandhi College of Pharmacy  
Bhopal  
Madhya Pradesh  
India-462042

\*autor, do którego należy kierować korespondencję: tel.: +91786 9801910,  
e-mail: ameetaargal@yahoo.com, nishi.saxena12@rediffmail.com



## Streszczenie

Kamica nerkowa to proces polegający na tworzeniu, agregacji i odkładaniu się kryształów w układzie moczowym. Do przeciwdziałania kamicy nerkowej z powodzeniem stosuje się tradycyjne zioła. W przedstawionej pracy przygotowano mieszankę ziołową zawierającą wyciągi roślinne i zbadano jej właściwości hamujące rozwój kamicy. W skład mieszanki wchodziły wyciągi alkoholowe z owoców *Tribulus terrestris*, korzeni *Boerhavia diffusa* oraz liści *Azadirachta indica*. Badanie przeprowadzono na modelu kamicy nerkowej indukowanej glikolem etylenowym, stosując jako kontrolę standardowy lek Cystone. Glikol etylenowy podnosi poziom wapnia, szczawianów i fosforanów, które są odpowiedzialne za rozwój kamicy. Zastosowanie mieszanki ziołowej (w dawce 200 i 300 mg/kg) istotnie obniżyło poziom wapnia, szczawianów i fosforanów w porównaniu z negatywną grupą kontrolną. Wszystkie dawki obniżyły też istotnie poziom kreatyny, kwasu moczowego i mocznika. Uzyskane wyniki zostały potwierdzone przez badanie histopatologiczne. Dawka LD<sub>50</sub> była wyższa niż 2000 mg/kg, przygotowana mieszanka ma zatem silne działanie przeciwkamicowe i jest bezpieczna w użyciu.

**Słowa kluczowe:** działanie przeciwkamicowe, *Tribulus terrestris*, *Boerhavia diffusa*, *Azadirachta indica*, mieszanka ziołowa