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RESEARCH

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Antiurolithiatic effect of a polyherbal formulation against sodium oxalate-induced urolithiasis in rats

Amany A. Sayed*, Amel M. Soliman, Sohair R. Fahmy and Rasha Hosny

Abstract

Background The present study assesses the role of polyherbal formula (LACTN) against sodium oxalate (NaOx)—stimulated urolithiasis prophylactic and curative. Forty-eight rats were separated into the following regimen's groups: Groups I, II, III, and IV (prophylactic groups). Group I (control group) received saline as vehicle. Group II (urolithiatic group) received 70 mg NaOx / kg b.wt, i.p. Groups III and IV received LACTN and cystone prophylactic (500 and 750 mg/kg b.wt, respectively) from 1st day to 7th day of urolithiasis induction. Groups V, VI, VII, and VIII (curative groups): these were divided into the prophylactic regimes, but LACTN and cystone were administered from 7th day to 14th day of urolithiasis induction.

Results Urolithiatic rats co-/post-treated with LACTN and cystone elevated the urinary volume significantly ($P < 0.05$). Also, a significant decrease ($P < 0.05$) was observed in the serum and urine calcium and phosphate concentrations beside the increased magnesium value in urolithiatic rats co-/post-treated with LACTN. LACTN significantly ($P < 0.05$) ameliorates serum and urine creatinine, urea, and uric acid concentration. Moreover, urolithiatic rats co-/post-treated with LACTN significantly ($P < 0.05$) diminished MDA and increased GSH, GST, SOD, and CAT compared with urolithiatic rats.

Conclusions LACTN has anti-lithogenesis effect that may be due to its ability to remove the already presenting stone and/or to prevent the generation of extra calcium oxalate crystals. This action may be due to its components' synergetic action that may provide encouraging combined formula to prevent/treat urolithiasis.

Keywords Urolithiasis, Polyherbal formula, Calcium oxalate, Oxidative stress

Background

Urolithiasis is a hurting ailment generally known as kidney and/or urinary stone that prescribed as the third disorder in the urinary diseases causing a major impact on public health that may progressed to renal calcification (Ahmed et al., 2020; Patel & Acharya, 2020; Saleem et al., 2020). It affects both genders, but according to the survey of National Health and Nutrition Examination men are more susceptible to urolithiasis (Pandhare et al., 2021). Mainly, calcium oxalate and/or calcium phosphate are the most common renal stones and increased the risk by overproduction of reactive oxygen species (ROS) (Ahmed et al., 2020; Pandhare et al., 2021). The lithogenesis of calcium oxalate begins with nidus formation that developed finally to form stone (Akila et al., 2011). Many stone inhibitors are used in urolithiasis cases to avoid recurrence, but they have some pharmacological restrictions on long term of use. So, they did not considered the final effective treatment for removing stone (Patel & Acharya, 2020). Recently, local calculus damage by using a high-powered laser is the recent strategy to remove the

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calculi. Yet, these treatments are of high cost, and repetition rate is very high in the absence of preventive care (Pandhare et al., 2021). Thereby, for effective treatment firstly the preventive as well as curative therapies are the proper strategies for better relief.

World Health Organization (WHO) recommends the consumption of herbal agents in health care plans (Shirfule et al., 2009). Traditional medicine prescribe a combination of various herbal products for synergistic action; as the formed complex exhibit many pharmacological actions like diuretic, antimicrobial, antioxidant, anti-inflammatory, pain-relieving, antispasmodic, litholytic activities (Akila et al., 2011; Olayeriju et al., 2020). Thus, the current study aimed to evaluate the antiurolithiatic efficacy of new formula named LACTN consisting from five different components (Table 1); each one has a unique effect that aid to prevent/treat urolithiasis.

Lens culinaris (lentil) belongs to family Leguminoceae and is categorized as one of the best important traditional ingredient according to FAO (1998). Lentil contains many dietary vitamins such as folate and vitamin B2 beside its magnesium, selenium, zinc, iron, and potassium content (Abdel-Rahman & Abdel-Baky, 2021; Rico et al., 2021). Also, lentil has the greatest antioxidant potency due to its flavonols, hydroxycinnamic acid, isoflavones, and anthocyanins (Rico et al., 2021). It has a potent activity against renal disorders (Sreedevi, 2018). *Allium cepa* (onion) belong to Liliaceae family. It used in a folk medicine as analgesic in abdominal and rheumatic pains and in treating urinary stones (Vahdani et al., 2013). Bulb onion enrich with quercetin and flavonoids contents that increase its antioxidant activity (Rampalli et al., 2013). *Cucumis melo* named as Musk melon has diuretic activity (Patel et al., 2021) and can inhibit nephrolithiasis (Afzal et al., 2021). *Triticum aestivum* (wheat bran) is a cereal grass belonging to the family Gramineae. It contains a many minerals including calcium, phosphorus, selenium, zinc, iodine, magnesium, boron, chromium, potassium, iron, and molybdenum (Sundaresan et al., 2015). *T. aestivum* extract has the ability to inhibit calcium oxalate crystal development and aggregation with above 70% degree (Sekkoum et al., 2011). *Nasturtium officinale* (watercress) belongs to Liliaceae family and is traditionally used in salads and soups. It has renoprotective effect that may be returned to its anti-inflammatory and antioxidant effects (Karami et al., 2018; Shahani et al., 2017). In addition, watercress has diuretic influence and can be used to prevent renal stone (Karami et al., 2018; Mehrabi et al., 2016).

Although using each of these agents individually by native people as a diuretic or in treatment of renal stones, no scientific research has been conducted on their combination effect on renal stones. Therefore, the present study is just a preliminary assessment to demonstrate the role of LACTN as anti-lithogenesis (preventive) and anti-urolithiasis (curative) when administered orally to male urolithiatic rats induced by sodium oxalate (NaOx). To assess the effectiveness of polyherbal LACTN, this study compares its effect with cystone which is also polyherbal formula listed in Table 2.

Table 1 Plants components of polyherbal formula (LACTN)

No.	Biological name	Vernacular name	Family	Part used
1	*Lens culinaris*	Lentils	Fabaceae	Seeds
2	*Allium cepa*	Onion	Liliaceae	Bulbs
3	*Cucumis melo*	Snake melon	Cucurbitaceae	Fruit
4	*Triticum aestivum*	Wheat	Gramineae	Straws
5	*Nastarium officinale*	Watercress	Brassicaceae	Arial parts

Methods

Plants collection and authentication

All plants components of polyherbal formulation were purchased from local vegetable markets of Giza district, Egypt, except for *Triticum aestivum* that was collected from one village near Mansoura, Daqahlia, Egypt. Each component was submitted for authentication at Faculty of Science, Cairo University, Botany Department, Egypt.

Preparation of polyherbal formula (LACTN)

LACTN formula consists of five components with various morphological parts as given in Table 1. Each one was prepared separately and then mixed to obtain the final formula.

Preparation of *Lens culinaris* extract

Lentil seeds were ground and percolated through petroleum ether to eliminate fatty content. Then, the sample

Table 2 Plants components of standard polyherbal formula (cystone)

No.	Biological name
1	*Achyranthes aspera*
2	*Onosma bracteatum*
3	*Rubia cordifolia*
4	*Cyperus scariosus*
5	*Saxifrage ligulata*
6	*Vernonia cinerea*
7	Shilajeet
8	*Didymocarpus pedicellata*

was extracted with chilled ethanol for 3 h (ethanol/water, 80:20 v/v) at room temperature. The sample was then centrifuged at 3000 g for 25 min, and then the supernatant was collected. Extraction was prepared trice; the supernatants were combined and then evaporated at 40℃ (Talukdar, 2012).

Preparation of *Allium cepa* extract

Allium cepa bulbs (onion bulbs) were subjected to maceration using 95% ethanol for 24 h, followed by reflux over a water bath for 3 h. Then, the obtained extract will be concentrated under vacuum (Sindu et al., 2012).

Preparation of *Cucumis melo* extract

Cucumis melo mature fruits were sliced to small pieces, dark-dried, and crudely powdered. The powdered material was soaked in 60% ethanol at 60℃ for 18 h, and the extract obtained was evaporated to dryness (Janapareddi et al., 2013).

Preparation of *Triticum aestivum* extract

Triticum aestivum was grinded with automatic grinder and fine powder of *T. aestivum* was extracted with ethanol at room temperature (400 g/600 ml). Then, it was filtered after 24 h, and the resultant *Triticum aestivum* ethanolic extract (TAEE) was concentrated by rotary evaporator to get a crude extract.

Preparation of *Nastarium officinale* extract

Nastarium officinale (watercress) aerial parts were shade-dehydrated and milled. Then, about 700 mg of plant powder was extracted thrice with 80% ethanol/water at room temperature overnight. The solvent was subtracted by rotary evaporation (50℃) (Zargari et al., 2015).

Preparation of LACTN

The resultant extracts of the previous five constituents were placed individually in sterilized containers and refrigerated at 4℃. LACTN was freshly prepared at the time of administration during the experiment by mixing equal proportions of 1 g of each component.

Free radical scavenging activity of LACTN

The free radical scavenging action of LACTN was estimated by DPPH assay (Fonseca-Silva et al., 2011) and compared with ascorbic acid as reference standard. Diverse concentrations (10–70 mg/ ml) of LACTN and ascorbic acid were prepared. A 1.0 ml of the each, at final concentrations of 10–70 mg/ ml, was mixed with 2 ml of 0.3 mM DPPH solution in methanol. The absorbance was estimated at 517 nm after 20 min of dark incubation at room temperature. The experiment was performed trice.

The percentage of scavenging was calculated according to the following equation:

$$\%\ \text{of radical scavenging activity} = \left[\text{Abs (control)} - \text{Abs (sample)} / \text{Abs (control)}\right] \times 100.$$

Experimental animals

Male albino rats (*Rattus norvegicus*) weighing 150–180 g were obtained from the animal house of National Research Center, Cairo, Egypt. The animals were housed in polypropylene cages for one week before initiation of the experiments for acclimatization. Rats were kept at temperature 23 ± 2 °C and the humidity 50% with a 12 h light: 12 h dark series. They were fed a standard diet and tap water ad libitum. The protocol for conducting the acute toxicity studies and in vivo studies was performed according to the guidelines of the care and use of laboratory animals (8^{th} edition) and the study was approved by the Institutional Animal Care and Use Committee (IACUC), (CUFS/S/PHY/25/14) of the Faculty of Science, Cairo University, Egypt.

Acute toxicity study

Acute toxicity study were carried out according to OECD guidelines (No: 423) using Wistar rats to investigate the safety of LACTN.

Experimental design and protocol

Forty-eight male albino rats were divided into two main groups for prophylactic (Prophylactic regimen) and therapeutic regimens (Curative regimen). The rats of each regimen were then subdivided into four groups as follows:

Prophylactic regimen (1–7d)

Group I—Control: rats were given 1 ml of saline (vehicle).

Group II—*Urolithiatic: rats were intraperitoneally injected with sodium oxalate (NaOx) (70 mg/kg dissolved in saline) for 7 days.*

Group III—*LACTN-treated: rats were administered LACTN (500 mg/kg b.wt) prophylactic with NaOx from 1 to 7 days of urolithiatic induction.*

Group IV—Cystone-treated: rats were administered with cystone (750 mg/kg b.wt) prophylactic with NaOx from 1 to 7 days of urolithiatic induction.

Curative regimen (7–15)

Group I—Control: rats were given 1 ml of saline.

Group II—Urolithiatic: rats were injected with NaOx (70 mg/kg dissolved in 0.5 ml of saline) as intraperitoneal injection (i.p).

Group III—LACTN: rats were first injected with NaOx from day 1 to the 7th day, then LACTN was administered from day 8 till the 15th day.

Group IV—Cystone-treated: rats were first injected with NaOx from day 1 till the 7th day, then cystone was administered from day 8 till the 15th day.

Urinalysis

Twenty-four hours prior the end of the experiment period of each studying regimen, the rats were weighed and positioned alone in the metabolic cage. Urine samples were collected for 24 h. Urine was collected in a 50-ml beaker maintained in an ice bath. For analysis, the volume of the collected urine was quantified using measuring cylinder, urinary pH and specific gravity were determined by strip method. Other part of urine specimen were centrifuge (3000 rpm, 10 min.) for microscopic examination and the urine supernatant was used for investigation of creatinine, urea, uric acid, calcium, phosphorus, and magnesium.

Evaluation of urine and serum kidney function markers

At the termination of the experiment of each regimen, the rats were euthanized by over dose of sodium pentobarbital. Blood was collected by heart puncture in centrifuge tube for analysis of creatinine, urea, and uric acid (Bartles et al., 1972; Tietz et al., 1990), and other elements (calcium, phosphorus, and magnesium) according to Gindler and King (1972), El-Merzabani et al. (1977), and Grindler and Heth (1971), respectively, using Biodiagnostic kits (Dokki, Giza, Egypt). Kidney were excised, washed with saline, and immediately blotted using filter paper to remove traces of blood, weighed relative to its body weight and then divided into two parts. The first part homogenized in ice-cold 0.1 M Tris–HCl buffer (pH 7.4, 10% w/v). The homogenate was centrifuged at 3000 rpm for 15 min at 4℃ and the resulting supernatant was stored at – 20ºC for determination of oxidative/antioxidative stress markers (MDA, GSH, GST, SOD, and CAT) according to Ohkawa et al. (1979), Beutler et al. (1963), Habig et al. (1974), Nishikimi et al. (1972), and Aebi (1984), respectively, while the other part was put in 10% formal saline for fixation preparative to histological examination of crystals.

Statistical analysis

Results were displayed as mean ± SEM ($n = 6$). The statistical significance was analyzed by ANOVA (one-way analysis of variance) followed by Duncan's test. Values of $p < 0.05$ were considered as statistically significant. Data analysis was performed using SPSS version 15.0 software.

Results

Figure 1 shows that LACTN formula revealed a great antioxidant activity at concentrations from 10 to 70 mg/ml. It exhibited a maximum antioxidant effect at 70 mg/ml. In this concentration, the extract scavenges 98.8% of DPPH free radical, as compared with ascorbic acid which showed 100% DPPH inhibition at this concentration.

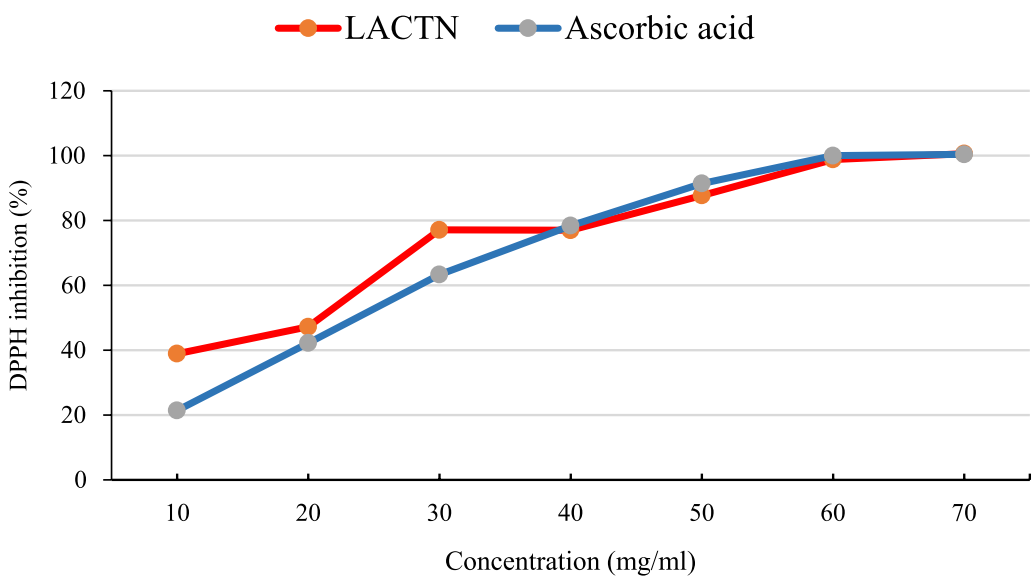

Fig. 1 Percentage inhibitions of DPPH by LACTN and ascorbic acid

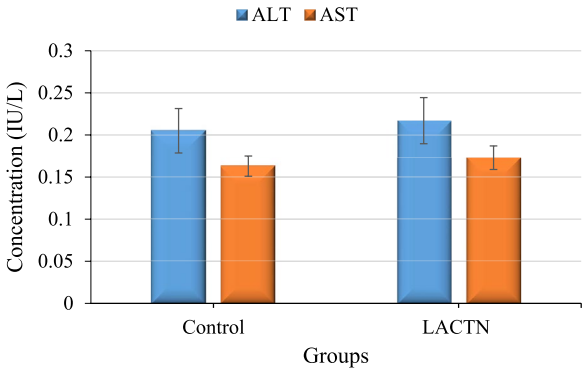

Fig. 2 Effect of LACTN on AST and ALT activities at an acute limit dose (5000 mg/kg b.wt).

Acute toxicity study

The limit test (432) was performed at 5 g LACTN/kg b.wt. The rats were orally treated and examined for 14 days. LACTN was found to be safe as no rat died up to 5 g/kg b.wt and no gross behavioral changes occurred. Further, there is no significant change between control and LACTN group in the AST and ALT activities (Fig. 2).

Effect of LACTN on kidney weight ratio, pH and urinary output of urolithiatic rats

Urolithiatic groups of prophylactic or curative regimens have significant ($P<0.05$) increase in kidney weight ratio showed by weighting and macroscopically, as compared with control rats (Fig. 3). Also, urolithiatic groups excrete significant ($P<0.05$) low urine volume as compared to corresponding control groups. However, a significant ($P<0.05$) increase in urine excretion was observed in both treatments (LACTN and cystone) in the prophylactic and curative regimens (Table 3). Urinary pH of control rats is neutral. However, urolithiatic group exhibit alkaline pH when compared with the control rats. LACTN and cystone returned urinary pH near to control values in both regimens (Table 3).

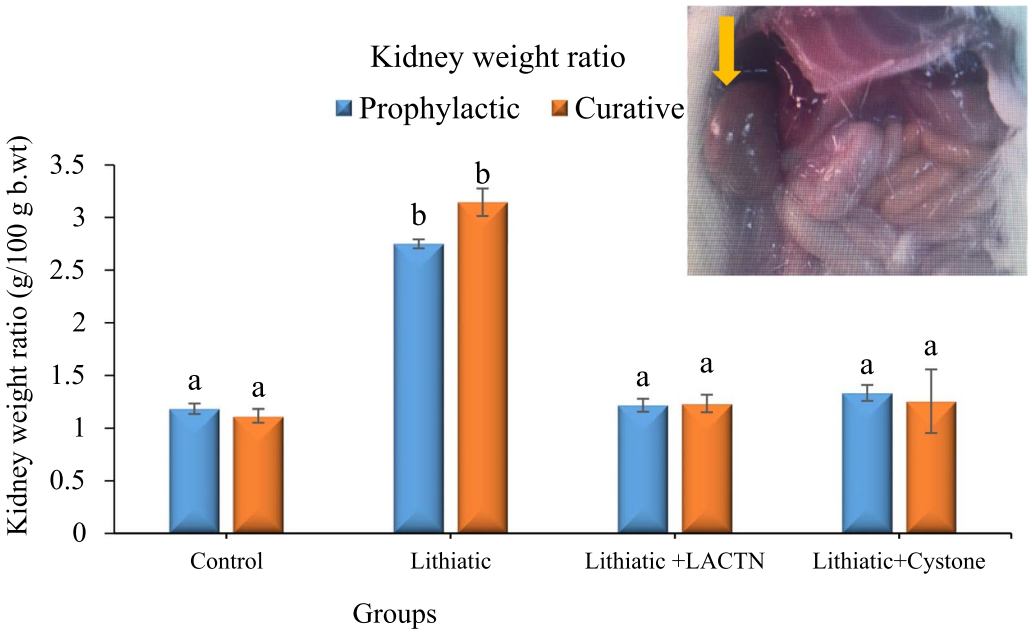

Fig. 3 Effect of LACTN on kidney weight of urolithiasis

Table 3 Effect of polyherbal formula (LACTN) on urinary output and pH of urolithiatic rats

Parameters	Groups							
	Prophylactic				Curative			
	Control	Urolithiatic	Urolithiatic + LACTN	Urolithiatic + Cystone	Control	Urolithiatic	Urolithiatic + LACTN	Urolithiatic + Cystone
u. Output (ml/24 h)	8.583 ± 0.300^{a}	2.400 ± 0.306^{b}	11.017 ± 0.277^{c}	9.700 ± 0.493^{d}	6.643 ± 0.084^{a}	2.983 ± 0.489^{b}	6.581 ± 0.185^{a}	6.416 ± 0.234^{a}
u. pH	6.567 ± 0.136^{a}	12.833 ± 0.088^{b}	9.467 ± 0.186^{a}	9.900 ± 0.169^{a}	7.165 ± 0.307^{a}	13.205 ± 0.197^{b}	7.850 ± 0.322^{a}	7.336 ± 0.211^{a}

Values are expressed as mean ± SEM ($n=6$). ANOVA post hoc with Duncan's test was performed to compare between groups. Values with different row superscript letters of the same regimen are significantly different ($P<0.05$)

Effect of LACTN on urinary kidney function markers and some urinary elements

Tables 4 and 5 reveal that urine creatinine, urea, uric acid, calcium, and phosphorus levels increased significantly ($P<0.05$) in both regimens of urolithiatic rats in comparison with control groups, while creatinine clearance and magnesium levels were decreased significantly ($P<0.05$). On the other hand, rats treated with LACTN exhibit a significant ($P<0.05$) decrease in the urinary kidney functions, calcium, and phosphorus levels in comparison with the untreated urolithiatic groups. But, urolithiatic rats treated with LACTN have a significant ($P<0.05$) increase in creatinine clearance and urine magnesium levels, in comparison with untreated one. Similarly, administration of cystone caused a significant ($P<0.05$) decrease in the urine creatinine, urea, and uric acid concentrations, as compared to urolithiatic groups.

Table 4 Effect of polyherbal formula (LACTN) on some urinary kidney function markers of urolithiatic rats

Parameters (mg/dl)	Prophylactic							
	Groups				Curative			
	Control	Urolithiatic	Urolithiatic + LACTN	Urolithiatic + Cystone	Control	Urolithiatic	Urolithiatic + LACTN	Urolithiatic + Cystone
u. Creatinine	17.917 ± 0.327^{a}	26.400 ± 0.248^{b}	18.683 ± 0.276^{ac}	19.183 ± 0.237^{c}	36.083 ± 0.506^{a}	57.604 ± 2.496^{b}	34.117 ± 1.495^{a}	34.901 ± 1.433^{a}
u. Urea	2.600 ± 0.148^{a}	6.183 ± 0.221^{b}	2.717 ± 0.201^{a}	2.317 ± 0.111^{a}	12.403 ± 0.475^{a}	17.302 ± 0.249^{b}	12.383 ± 0.409^{a}	12.647 ± 0.606^{a}
u. Uric acid	7.217 ± 0.083^{a}	13.100 ± 0.191^{b}	7.283 ± 0.108^{a}	7.850 ± 0.138^{c}	55.701 ± 1.258^{a}	81.050 ± 1.181^{b}	58.283 ± 0.851^{a}	56.984 ± 1.071^{a}

Values are expressed as mean ± SEM ($n=6$). ANOVA post hoc with Duncan's test was performed to compare between groups. Values with different row superscript letters of the same regimen are significantly different ($P<0.05$)

Table 5 Effect of polyherbal formula (LACTN) on some urinary elements of urolithiatic rats

Parameters (mg/dl)	Groups							
	Prophylactic				Curative			
	Control	Urolithiatic	Urolithiatic + LACTN	Urolithiatic + Cystone	Control	Urolithiatic	Urolithiatic + LACTN	Urolithiatic + Cystone
u. Calcium	52.717 ± 1.043^{a}	98.317 ± 3.020^{b}	55.500 ± 1.601^{a}	59.865 ± 2.812^{a}	81.500 ± 4.678^{a}	145.165 ± 6.395^{b}	85.223 ± 3.609^{a}	88.735 ± 3.691^{a}
u. Phosphorous	37.083 ± 0.588^{a}	47.700 ± 0.771^{b}	37.417 ± 0.935^{a}	37.502 ± 0.945^{a}	55.151 ± 1.205^{a}	81.022 ± 2.158^{b}	57.833 ± 1.404^{a}	55.206 ± 1.533^{a}
u. Magnesium	1.1667 ± 0.033^{a}	0.6317 ± 0.018^{b}	1.4333 ± 0.080^{c}	1.4667 ± 0.056^{c}	2.117 ± 0.130^{a}	0.745 ± 0.068^{b}	2.167 ± 0.102^{a}	2.533 ± 0.099^{c}

Values are expressed as mean ± SEM ($n=6$). ANOVA post hoc with Duncan's test was performed to compare between groups. Values with different row superscript letters of the same regimen are significantly different ($P<0.05$)

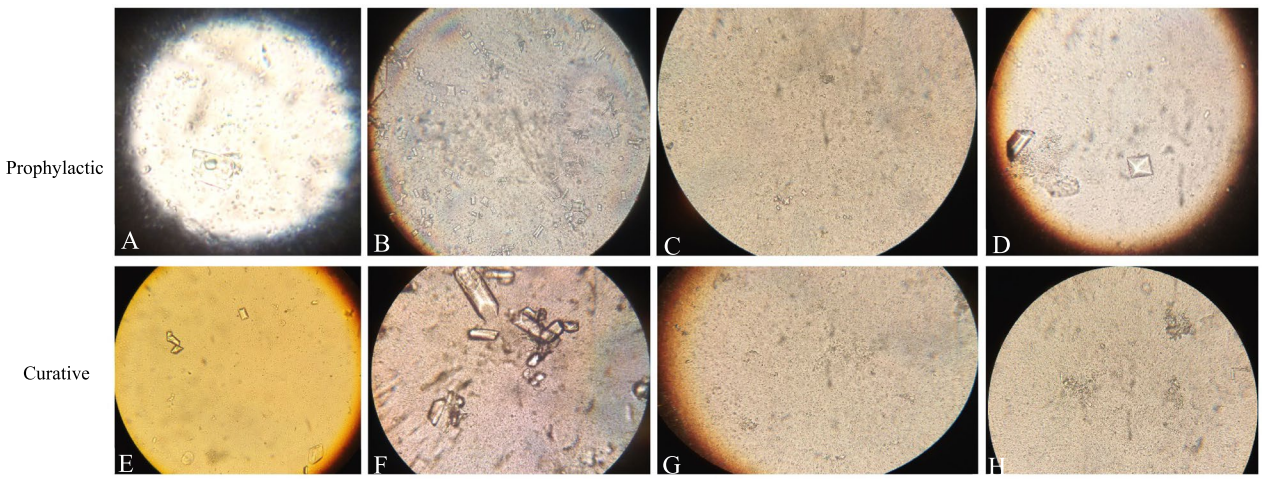

Fig. 4 Light microscopy of calcium oxalate crystals in urine. Prophylactic groups (**A**–**D**), curative groups (**E**–**H**) represent control, urolithiasis, LACTN, and cystone, respectively

Microscopic examination of the collected urine

Microscopic examination of urine of the control groups (prophylactic and curative, respectively) was observed to be lacking of crystals (Fig. 4A, E). In contrast, urine of urolithiatic rats shows abundant, large crystals and crystal aggregates with characteristic shape of CaOx (Fig. 4B, F). However, urine of LACTN- (Fig. 4C, G) and cystone-treated shows less abundant smaller and discrete crystal fragments with obviously reduced size and numbers (Fig. 4D, H).

Effect of LACTN on serum kidney function markers and some elements

Statistically, there is a significant ($P<0.05$) rise in creatinine, urea, and uric acid as well as calcium, and phosphorus concentrations in the urolithiatic groups of both regimens compared with the control groups (Tables 6 and 7). These increases mostly reversed significantly ($P<0.05$) due to LACTN or cystone administration in both cases, in comparison with urolithiatic groups. While a significant ($P<0.05$) decrease in magnesium level was observed in the urolithiatic groups and returned to rise significantly ($P<0.05$) by the administration of LACTN or cystone when compared with untreated urolithiatic groups.

Effect of LACTN on kidney oxidative and antioxidative stress markers

Significant ($P<0.05$) elevation of MDA was observed in the urolithiatic groups (prophylactic and curative) when compared to control groups; this elevation was reserved significantly ($P<0.05$) in groups treated with LACTN or cystone. In contrast, significant ($P<0.05$) diminution in GSH, GST, SOD, and CAT was observed in the urolithiatic groups as compared with corresponding control groups. Notably, levels of these antioxidant molecules replenished significantly ($P<0.05$) upon the LACTN or cystone treatment of both studied regimens relative to urolithiatic groups (Table 8).

Effect of LACTN on renal calculi deposition using histological evaluation

In prophylactic and curative regimens (Figs. 5, 6, respectively), control groups exhibit intact renal tissue architecture (Figs. 5A, 6A). In contrast, CaOx crystals deposits between renal tubules were detected in the renal tissues of urolithiatic rats (Figs. 5B, 6B, C). Histological analysis of LACTN shows partial prevention of the CaOx crystals deposition in renal tissue. No/few crystals observed in comparison with the urolithiatic group in prophylactic regimen (Fig. 5C, D) also in curative regimen (Fig. 6D).

Table 6 Effect of polyherbal formula (LACTN) on some serum kidney function markers of urolithiatic rats

Parameters	Groups							
	Prophylactic				Curative			
	Control	Urolithiatic	Urolithiatic + LACTN	Urolithiatic + Cystone	Control	Urolithiatic	Urolithiatic + LACTN	Urolithiatic + Cystone
s. Creatinine	1.417 ± 0.114^{a}	4.833 ± 0.220^{b}	1.717 ± 0.224^{ac}	2.033 ± 0.180^{c}	2.267 ± 0.161^{a}	4.933 ± 0.228^{b}	2.133 ± 0.123^{a}	2.233 ± 0.173^{a}
s. Urea	12.167 ± 0.348^{a}	15.333 ± 0.235^{b}	12.117 ± 0.335^{a}	12.157 ± 0.462^{a}	15.183 ± 0.327^{a}	26.817 ± 1.131^{b}	15.433 ± 0.408^{a}	13.950 ± 0.571^{a}
s. Uric acid	3.967 ± 0.225^{a}	6.467 ± 0.260^{b}	4.083 ± 0.210^{a}	4.117 ± 0.371^{a}	4.050 ± 0.198^{ac}	8.367 ± 0.236^{b}	3.767 ± 0.154^{a}	4.417 ± 0.232^{c}

Values are expressed as mean ± SEM ($n=6$). ANOVA post hoc with Duncan's test was performed to compare between groups. Values with different row superscript letters of the same regimen are significantly different ($P<0.05$)

Table 7 Effect of polyherbal formula (LACTN) on some serum elements of urolithiatic rats

Parameters (mg/dl)	Groups							
	Prophylactic				Curative			
	Control	Urolithiatic	Urolithiatic + LACTN	Urolithiatic + Cystone	Control	Urolithiatic	Urolithiatic + LACTN	Urolithiatic + Cystone
s. Calcium	1.250 ± 0.283^{a}	5.417 ± 0.167^{b}	1.600 ± 0.186^{ac}	1.933 ± 0.362^{c}	6.567 ± 0.362^{a}	10.150 ± 0.312^{b}	5.883 ± 0.283^{a}	6.200 ± 0.393^{a}
s. Phosphorous	3.083 ± 0.241^{a}	5.933 ± 0.330^{b}	3.433 ± 0.173^{a}	3.167 ± 0.246^{a}	3.867 ± 0.156^{a}	6.600 ± 0.299^{b}	4.017 ± 0.294^{a}	3.767 ± 0.176^{a}
s. Magnesium	2.733 ± 0.076^{a}	1.050 ± 0.121^{b}	3.000 ± 0.137^{a}	2.300 ± 0.166^{c}	3.250 ± 0.245^{a}	1.105 ± 0.238^{b}	3.167 ± 0.165^{ac}	2.617 ± 0.125^{c}

Values are expressed as mean ± SEM ($n=6$). ANOVA post hoc with Duncan's test was performed to compare between groups. Values with different row superscript letters of the same regimen are significantly different ($P<0.05$)

Table 8 Effect of polyherbal formula (LACTN) on some oxidative stress markers of urolithiatic rats

Parameters	Groups							
	Prophylactic				Curative			
	Control	Urolithiatic	Urolithiatic + LACTN	Urolithiatic + Cystone	Control	Urolithiatic	Urolithiatic + LACTN	Urolithiatic + Cystone
MDA (nM/g. tissue)	18.475 ± 1.111^{a}	37.872 ± 1.633^{b}	27.351 ± 1.824^{c}	21.242 ± 0.954^{c}	22.888 ± 0.861^{a}	41.778 ± 1.892^{b}	24.975 ± 0.873^{a}	25.323 ± 0.782^{a}
GSH (mg/g. tissue)	4.833 ± 0.439^{a}	1.817 ± 0.079^{b}	4.667 ± 0.246^{a}	5.000 ± 0.208^{a}	8.084 ± 0.170^{a}	4.717 ± 0.244^{b}	7.501 ± 0.227^{ac}	7.132 ± 0.2186^{c}
GST (U/g. tissue)	53.333 ± 2.445^{a}	21.833 ± 1.470^{b}	52.483 ± 1.804^{a}	52.733 ± 0.884^{a}	49.804 ± 3.272^{a}	29.263 ± 0.887^{b}	47.105 ± 0.957^{a}	47.583 ± 0.546^{a}
SOD (U/g. tissue)	742.333 ± 9.636^{ac}	458.833 ± 10.928^{b}	765.643 ± 14.582^{c}	699.167 ± 24.844^{a}	666.500 ± 19.056^{a}	392.000 ± 27.995^{b}	700.220 ± 15.338^{a}	703.500 ± 23.906^{a}
CAT (U/g. tissue)	0.530 ± 0.017^{a}	0.290 ± 0.024^{b}	0.510 ± 0.018^{a}	0.498 ± 0.011^{a}	0.916 ± 0.107^{a}	0.398 ± 0.017^{b}	0.980 ± 0.029^{a}	0.654 ± 0.049^{c}

Values are expressed as mean ± SEM ($n = 6$). ANOVA post hoc with Duncan's test was performed to compare between groups. Values with different row superscript letters of the same regimen are significantly different ($P < 0.05$)

Similarly, the kidney sections of urolithiatic rats received cystone showed no to few crystal deposition either in prophylactic regimen (Fig. 5E, F) or in curative one (Fig. 6E). These findings suggest the efficacy of LACTN as antilithiatic agent like the standard cystone drug.

Discussion

Polyherbal formula (LACTN) was evaluated against urolithiasis in rat model as is the most rapid screening for new antiurolithiatic prescription to the most common prevalent stone (Pandhare et al., 2021).. While the individual component of LACTN has no serious toxic effect, the present study investigates the toxic study of LACTN safety to ascertain its safety before its usage. According to the acute oral toxicity test (OECD guidelines, 423), LACTN has no serious symptoms or behavioral change and no mortality was recorded up to the dosage of 5000 mg/kg b.wt.

The present study observes the formation of calculi in diverse fields of the renal tissues of urolithiatic rats. This may be due to many factors: firstly, the kidney weight relative to the body weight of urolithiatic rats shows a noticeable increase when compared with control rats. This may be indicating the accumulation of fluid and/or crystal formation in the renal tissues supported by the histological examination and this is in line with the data of Saleem et al. (2020). Secondly, excess calcium concentration in urine; as the hypercalciuria is a risk aspect that may enhance nucleation of the renal calculi formation (Pandhare et al., 2021). Thirdly, increased phosphate level in urine may provide a good environment for formation of calcium phosphate crystals, which further induces CaOx deposition in kidney (Patel & Acharya, 2020). Fourthly, the relationship between urinary volume and CaOx formation, as lowering of urine volume increases the saturation of CaOx crystals components, leading to its formation (Patel & Acharya, 2020). On the vice versa, the development of CaOx crystals and their preservation resulted in a decline in urine volume in urolithiatic rats (Pandhare et al., 2021). Fifthly, the pH of urine is highly important aspect in urolithiasis. Alkaline pH in urolithiatic rats promotes the formation of CaOx crystals in the current alkaline lithiasis group, as diminished urinary volume provokes oxalate's supersaturation (Patel & Acharya, 2020). Sixthly, the increased urinary uric acid may lower CaOx solubility besides its binding with the normally present stone inhibitors and hence inhibit their action (Pandhare et al., 2021). Seventhly, creatinine clearance and urinary magnesium concentrations are reducing, signifying that they are storing in the blood, increasing the hazard possibility of urolithiasis (Pandhare et al., 2021). Finally, the formation of renal calculi in the urolithiatic rats may be due to increased glycolic acid oxidase activity by NaOx, resulting in the generation of glycolate and oxalate, which increases free radicals and lipid peroxidation, finally leading to tubular necrosis (Pandhare et al., 2021).

Administration of LACTN repressed the creation of CaOx crystals in several sections of the renal tubules. Each component of LACTN has ameliorative role on urolithiasis and/or nephroprotective activity. *Lens culinaris* and *Allium cepa* have renoprotective effects (Chinnala et al., 2017; Rico et al., 2021); while *Cucumis melo, Triticum aestivum, Nasturtium officinale* have both nephroprotective and antiurolithiatic activities (Afzal et al., 2021). Collectively, the present study revealed that LACTN play a great action to minimize the stone formation and/or dissolve the already formed by many

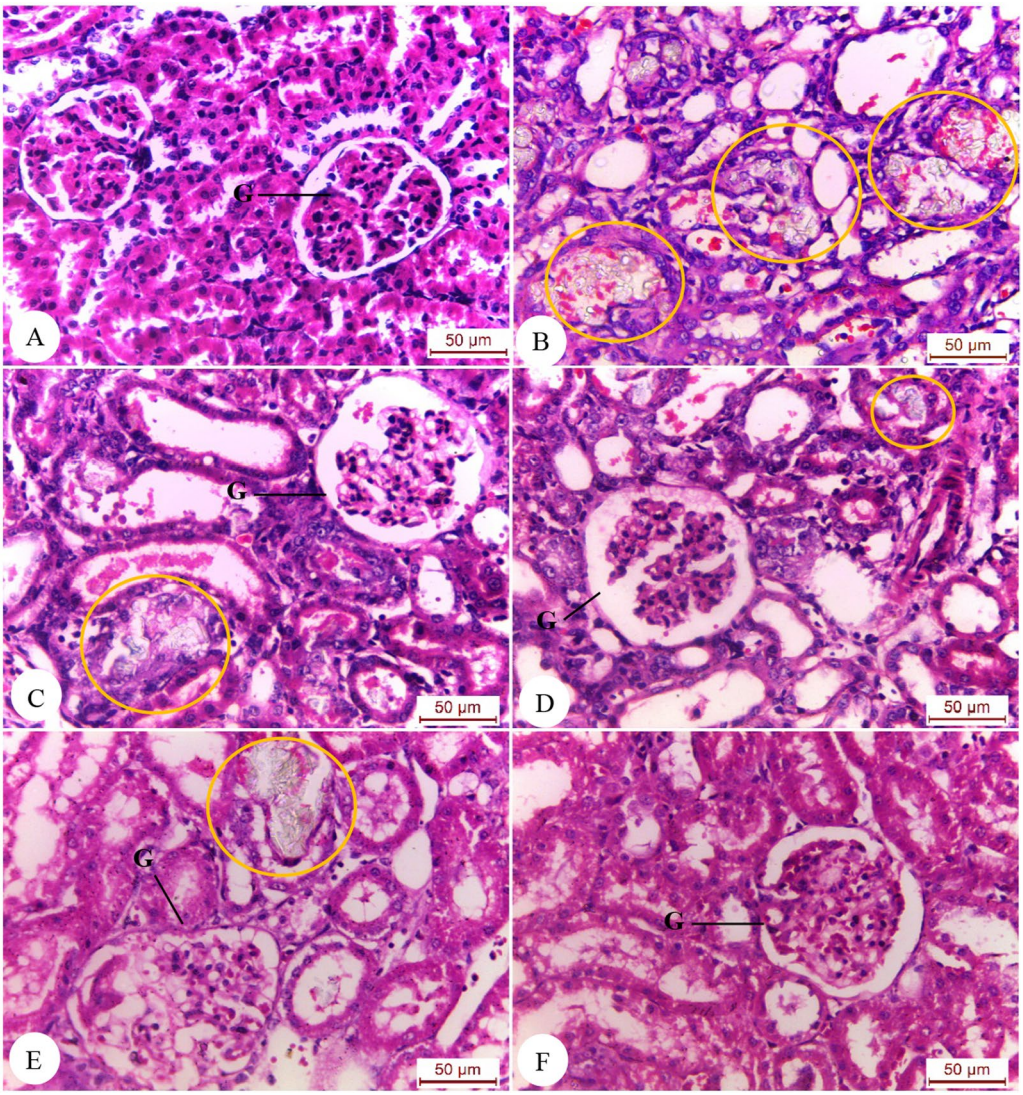

Fig. 5 Photomicrograph of kidney sections of rats in prophylactic regimen (H&E). **A** Control rats showing normal glomeruli (G) and tubular renal structure. **B** Urolithiatic rats showing multiple crystals deposition (yellow circle). **C**, **D** Urolithiatic rats treated with LACTN (500 mg/kg b.wt) showing low crystal deposits. **E**, **F** Urolithiatic rats treated with cystone (750 mg/kg b.wt) showing more or less drop in crystal deposition accumulation

properties. Some components of LACTN (*Allium cepa, Cucumis melo, Lens culinaris*) contain magnesium and citrate which considered a good inhibitor for urolithiasis (Mallek-Ayadi et al., 2017; Patel & Acharya, 2020; Tosin et al., 2017). Magnesium complexes with oxalate, so lessens the supersaturation of CaOx resulting in decreasing the rate of growth and nucleation of CaOx crystals. On the other hand, citrate complexes with calcium making soluble complex that lessened the supersaturation, and crystal aggregation and formation (Patel & Acharya, 2020). They added that potassium and citrate that present in *Lens culinaris, Allium cepa*, *Cucumis melon,* and *Triticum aestivum* (Abd El-Sattar, 2018; Albuquerque et al., 2006; Galdon et al., 2008; Khalid et al., 2021; Ruan et al., 2018) considered alkali therapy that increasing urinary citrate that complexed with urinary calcium and prevent urolithiasis. In addition, saponin that present in *Allium cepa*, *Triticum aestivum*, *Cucumis melon* (Afzal et al., 2021; Lanzotti et al., 2012; Rajoria et al., 2017) has diuretic property which can leak the excessive calcium and oxalate ions through expulsion (Patel & Acharya, 2020). Also, presence of stone inhibitors like phytate in *Triticum aestivum* and *Lens culinaris* (Sekkoum et al., 2011; Thavarajah et al., 2009) aid to inhibit CaOx crystal formation due to formation of insoluble Ca-phytate complex in the gastrointestinal tract (Nirumand et al., 2018).

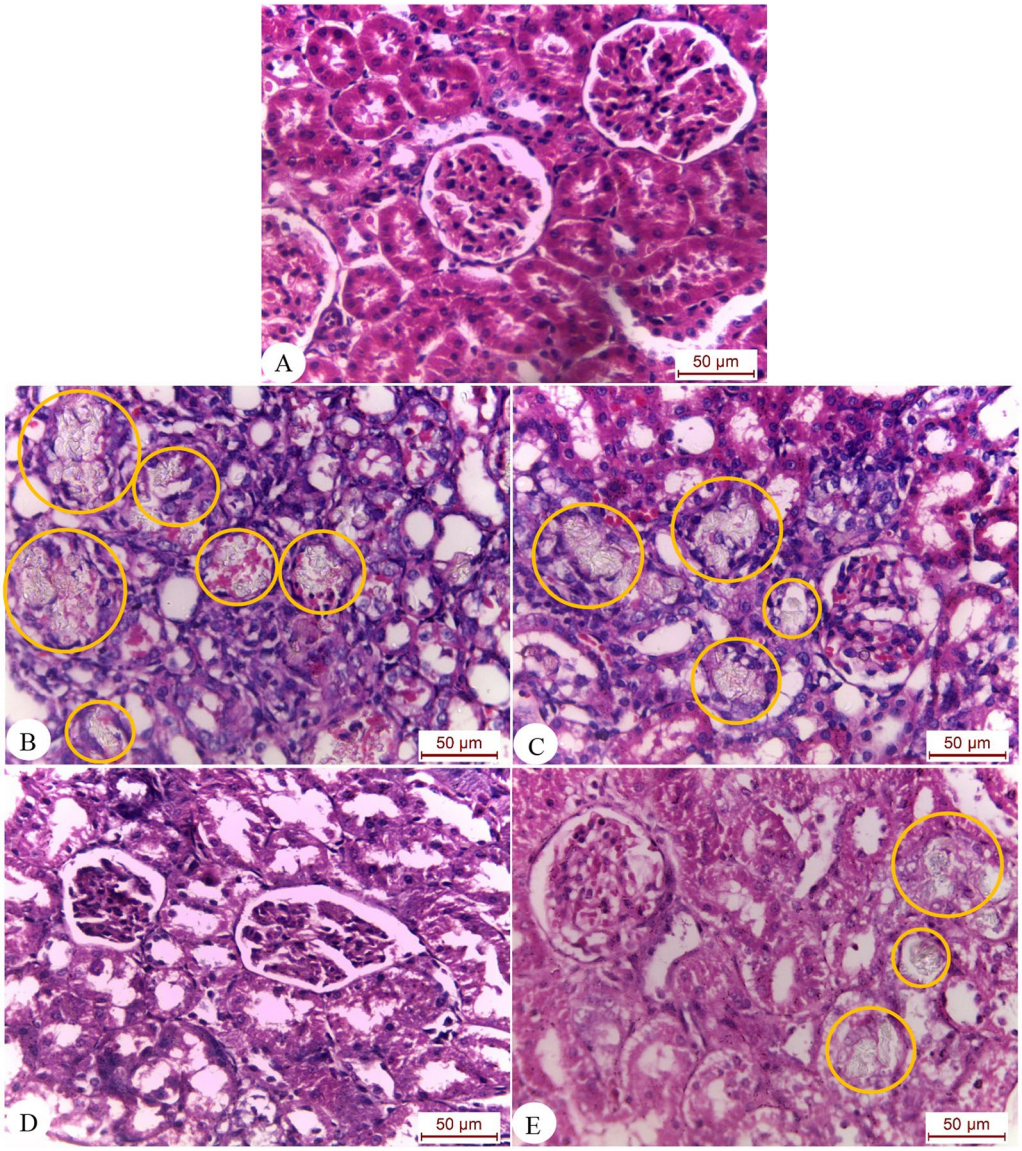

Fig. 6 Photomicrograph of kidney sections of rats in curative regimen (H&E). **A** Control rats showing normal glomeruli (G) and tubular renal structure. **B**, **C** Urolithiatic rats showing multiple crystals deposition (yellow circle). **D** Urolithiatic rats treated with LACTN (500 mg/kg b.wt) showing less crystal deposits. **E** Urolithiatic rats treated with cystone (750 mg/kg b.wt) showing more or less drop in crystal deposition numbers

Moreover, *C. melo* resemble *Coccinia indica* and *Benincasa hispida* (Cucurbitaceae), so it contribute in alleviating urolithiasis by diuresis effect, lowering the excretion of uric acid, lowering of concentrations of stone forming components, and preventing renal peroxidative that finally reduce CaOx attachment to renal tubules (Kumar et al., 2014; Patel et al., 2011, 2021). Afzal et al. (2021) added that *C. melo* treatment decrease serum and urinary phosphate levels by inhibiting tissue non-specific alkaline phosphatase (TNAP) enzyme. Recently, Patel et al. (2021) reported that *Cucumis melo* has antiurolithiasis effect due to its antimicrobial effect and its mucoproteins content that may act as anti-adherence and defensive barrier to the renal tubular layer. *Triticum aestivum* has an inhibitory effect on crystal development and aggregation (Sekkoum et al., 2011). This effect may be due to its phytate content (the phosphate form in natural agents) which chelate calcium and hence reduce its content in serum and urine. Also, *T. aestivum* contain tannins that has anticrystallization role that limit formation of CaOx crystal (Bawari et al., 2020). *Nasturtium officinale* (Watercress) has nephroprotective and some antilithiatic effects

(Karami et al., 2018; Mehrabi et al., 2016; Shahani et al., 2017). They detect high phenolic and flavonoid contents in *N. officinale*, which have diuresis and antioxidant activities (Patel & Acharya, 2020). Lastly, LACTN has in vitro DPPH free radical scavenging activity and in vivo lipid peroxidation inhibitory as well as antioxidant potency which may accompanied with lithiasis treatment (Olayeriju et al., 2020). This effects may be due to its components; since all LACTN constituents (*Lens culinaris, Allium cepa, Cucumis melo, Triticum aestivum, Nasturtium officinale*) rich with polyphenols, saponins, and flavonoids that have antiurolithiatic effect (Brandolini et al., 2013; Patel et al., 2016; Rico et al., 2021; Rolim et al., 2018; Zeb, 2015; Zou et al., 2011).

Conclusions

LACTN increases the urinary volume, decreases the calcium excretion, increases the magnesium level in blood and urine, improves the kidney functions markers, lowers the lipid peroxidation, and replenishes the antioxidant status. All these properties enabled LACTN to increased urination, subsequently decreased saturation of CaOx crystals components, and prohibited stone development salts from becoming supersaturated in urinary tract. This study suggests that the presence of all components in one formula may boost the antiurolithiatic action than the effect of each individual component.

Abbreviations

CaOx	Calcium oxalate
CAT	Catalase
GSH	Glutathione reduced
GST	Glutathione-s-transferase
MDA	Malondialdehyde
SOD	Superoxide dismutase

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Author contributions

AAS, AMS, and SRF constructed the scientific idea. AAS and RH carried out the practical part of the current study. AAS carried out the formal analysis, methodology, and writing of the original draft. All authors have read and approved the manuscript.

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Availability of data and materials

On request.

Declarations

Ethics approval and consent to participate

The protocol for conducting the acute toxicity studies and in vivo studies was performed according to the guidelines of the care and use of laboratory animals (8th edition), and the study was approved by the Institutional Animal Care and Use Committee (IACUC) (CUFS/S/PHY/25/14) of the Faculty of Science, Cairo University, Egypt.

Consent for publication

Not applicable.

Competing interests

There is no any potential conflict of interest.

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