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Research Article

Antiurolithiatic Activity of Aqueous Extract of *Ziziphus lotus* on Ethylene Glycol-Induced Lithiasis in Rats

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ABSTRACT

In Morocco, *Ziziphus lotus* is commonly used as an urolithiatic agent in the traditional medicine. To confirm this effect, an aqueous extract of *Ziziphus lotus* (AEZL) has been studied in ethylene glycol-induced urolithiasis model of rats.

30 male rats were randomly divided into five groups of six animals each. Group I served as a vehicle control and received distilled water (0.5 ml/100 g p.o.). All remaining groups received calculi inducing treatment for 28 days, comprised of 0.75% v/v ethylene glycol with 1% w/v ammonium chloride in drinking water ad libitum for 3 days followed by only 0.75% v/v ethylene glycol for 25 days. Group II served as lithiatic control and received distilled water (0.5 ml/100 g p.o.). Group III served as curative treatment group and received AEZL at doses of 150 mg/kg from 14th day to 28th day. Group VI served as preventive treatment group and received AEZL at doses of 150 mg/kg from 1st day to 28th day. Group V served as therapeutic and received a drug "Cystone" at dose of 750 mg/kg from 14th day to 28th day.

The extract treatment decreased the levels of oxalate and calcium in urine. Crystalluria analysis showed that untreated rats excreted large CaOx monohydrate and few dihydrate crystals while treated animals excreted mostly small CaOx dihydrate crystals. Significant similarity was observed between preventive and therapeutic anti-urolithiatic effect of AEZL and anti-urolithiatic effect of cystone ($P<0.001$).

These results demonstrated that AEZL have an anti-urolithiatic effect with preventive and therapeutic treatments in this experimental condition.

Key words: Urolithiasis, Ethylene glycol, *Ziziphus lotus*, Urinary parameter, Rats.

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INTRODUCTION

Urolithiasis (UL) is a common and complex disorder. Epidemiologic studies have quantified the burden of disease and have identified a variety of risk factors, which may help improve our understanding of the pathophysiology as well as lead to new approaches to reduce the risk of stone formation.

The incidence of UL, defined as the first stone event, varies by age, sex, and race. As with prevalence, white males have the highest incidence rates. In men, the incidence begins to rise after age 20, peaks between 40 and 60 years at ~3/1,000/year and then declines.[1] In women, the incidence is higher in their late twenties at 2.5/1,000/year and then decreases to 1/1,000/year by age 50, remaining at this rate for the next several decades.[2]

Stone formation is a complex process that results from a succession of several physicochemical events including supersaturation, nucleation, growth, aggregation and retention within the renal tubules.[3] The majority of stones, up to 80%, found very often both in humans and in rats[4] (Christina AJ *et al.* 2005) are composed mainly of calcium oxalate.[5,6]

Various therapies have been used to treat this illness, the extracorporeal shock wave lithotripsy (ESWL) and drug treatment which revolutionized urological practice almost become the standard procedure for eliminating kidney stones. However, in addition to traumatic effects of shock waves, residual stone fragments persist and infection could occur. Moreover, ESWL may cause acute renal injury, a decrease in renal function,[7] haemorrhage and hypertension.[8]

Medicinal plants remain an important source of new drugs. These plant products are reported to be effective in decreasing the recurrence rate of renal calculi with no side effects.[9,10]

Some medicinal plants such as *Herniaria hirsuta*,[11] *Phyllanthus niruri*, *Aerva lanata*, *Crataeva nurvala* and *Herniaria hirsuta* have been found to inhibit calcium oxalate crystallization.[11,12] The juices of lemon and orange are also widely used to inhibit crystallization.

Aromatic and medicinal plants possess a diversity of secondary metabolites with therapeutic properties.[13] Many studies have shown that medicinal and aromatic plants are rich source of phytochemicals with multiple biological effects such as antioxidant, antimicrobial, anti-inflammatory and pesticidal.[14] Phytobotanical and ethnobotanical research have focused mainly on the search for single 'active principle' in plants, based on the assumption that a plant has one or a few ingredients responsible of its therapeutic effects. Nevertheless, traditional systems of medicines generally assume that a synergy of all ingredients of the plants will bring about the maximum of therapeutic efficacy.[15]

Ziziphus lotus (ZL) is a medicinal plant largely used in the treatment of a variety of disease by Moroccan population, it is found mainly in the Mediterranean bioclimatic semi-arid and arid zones according to

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Emberger classification. Several studies have shown some properties as antidiabetic, antioxidant, antimicrobial, anti-inflammatory, immunomodulator and wound healing among many other effects.[16,17] The various parts of Z. lotus including seeds, fruits, root, shoots possess valuable phytochemical compounds. These phytochemicals such as polyphenol, flavonoids, tannins, saponins and alkaloids are believed to be responsible for many biological activities.[18,19]

The aim of the present study was to evaluate the anti-urolithiatic effects of *Ziziphus lotus* extract in an ethylene glycol induced urolithiatic model of rats.

MATERIALS AND METHODS

Collection and extraction of plant material

Ziziphus lotus, known as 'Nbeg or Sedra', is belonging to family of Rhamnaceae. It is an indigenous plant, Widespread in tropical and subtropical regions some parts of this plant have been used by the population of Kenitra (Northern of morocco): fruit, seeds.

The fruits of ZL were collected from the region of Sidi Slimane. The identification of species was done in our Laboratory, the fruits were dried in hot air oven at 50°C for 6 hours, a water decoction by suspending of one kilogram of ZL in fifteen liters of distilled water, brought to a strange boil, and then simmered at low heat for 3-4 hours. The filtrates were collected and evaporate in a rotary evaporator until concentrated, The ZA extract was stored at 4-5◦C after preparation.

Acute toxicity

Acute toxicity test of *Ziziphus lotus* was conducted by established method.[20] Briefly, the ZL aqueous extract was injected intraperitoneally to mice at various dose levels such as 0.5, 0.75, 1.5 and 2.5 g/kg of body weight. Five mice in each dose group were closely observed for 24 h for any mortality and next ten days for any delayed toxic effect.

Hence, the effective therapeutic dose was taken 150 mg/kg of body weight as one tenth of the approximate median lethal dose (LD 50 >1.5 g/kg).

Experimental animal

30 males of wistar rats were selected for this study, weighing between 220 and 280g and acclimated in the 24°C a 12 h dark-light cycle and allowed free access to drinking water and standard pellet diet.

The rats were randomly divided into five groups of six animals each. Group I served as a vehicle control and maintained on regular rat food and drinking water ad libitum and received distilled water (0.5 ml/100 g p.o.). All remaining groups received calculi inducing treatment for 28 days, comprised of 0.75% v/v ethylene glycol with 1% w/v ammonium chloride in drinking water ad libitum for 3 days to accelerate lithiasis followed by only 0.75% v/v ethylene glycol for 25 days. Group II served as lithiatic control and received distilled water (0.5 ml/100 g p.o.). Group III served as curative treatment group and received AEZL at doses of 150 mg/kg from 14th day to 28th day of calculi induction. Group VI served as preventive treatment group and received AEZL at doses of 150 mg/kg from 1st day to 28th day of calculi induction. Group V served as a Therapeutic and received a drug "Cystone" at dose of 750 mg/kg from 14h to 28. The aqueous extract of ZL was dissolved in distilled water and was given once daily by oral route (0.5 ml/100 g).

Collection and analysis of urine

All animals were kept in individual metabolic cages and 24 h urine samples were collected on 0, 14 and 28th day of calculi inducing treatment. After measurement of urine volume, all urine samples were analyzed for pH, calcium and oxalate content.

Calcium in urine was estimated using kit by Beacon Diagnostics Pvt. Ltd., India. The urine oxalate level was measured using the method of Hodgkinson.[20]

The urine of individual animals was observed under polarizing field microscopy (x400) for calcium oxalate crystals.

Statistical analysis

The results were expressed as mean ± standard deviation (SD) and were analyzed statistically using one-way analysis of variance (ANOVA) followed by Dunnett's comparison test. P-values were calculated against vehicle and lithiatic control groups and $P < 0.05$ was considered significant.

RESULTS

No acute toxicity was found to the dose up to 3.5 g/kg BW of *Ziziphus lotus* aqueous extract. The concentrations of urinary calcium and oxalate, for preventive groups at 14th day are shown in Table

Urinary volume

The values reported in Table 1 showed that urinary volume was similar in all groups at the beginning of the experiment. During the experiment, the values remained mostly constant for control group but increased for both groups receiving EG only and EG plus AEZL.

The urine output was found to increase significantly ($P < 0.05$) by stone-inducing treatment. Treatment of animals by both doses of ATC (100 and 200 mg/kg) in curative as well as preventive regimen decreased urine output significantly ($P < 0.05$) than that of calculi-induced rats but significantly higher than that of vehicle treated rats (Table 1).

Urinary calcium and oxalate

The levels of calcium and oxalate were significantly ($P <0.001$) increased in 24 h urine of ethylene glycol feeding lithiatec group compared to the control group in the urine sample of same duration. In contrast, the levels of calcium and oxalate were significantly decreased in 24 h urine in the in parallel ethylene glycol and *Ziziphus lotus* extract feeding preventive group compared to the ethylene glycol feeding group.

As similar to the preventive groups, the feeding ethylene glycol significantly increased the concentrations of above-mentioned urinary parameters in the curative group compared to the control group. On the other hand, feeding of *Ziziphus lotus* extract significantly decreased the levels of calcium, and oxalate in curative group compared to the ethylene glycol treated lithiatic group. Elevated actions were observed for the anti-lithiatic drug, cystone, feeding group compared to control and curative group.

Urinary calcium excretion was decreased significantly ($P < 0.05$) in EG-treated rats. Treatment with AEZL in only preventive regimen increased calcium excretion significantly ($P < 0.05$) than that of EG-treated rats (Table 2).

Urinary oxalate excretion was increased significantly ($P < 0.05$) in EG-treated rats. Treatment with AEZL in preventive as well as curative regimen decreased oxalate excretion significantly ($P < 0.05$) than that of EG-treated rats. (Table 2).

Crystalluria analysis

Qualitative crystalluria analysis demonstrated the absence of particles in control rats (Figure 1a). However, for all untreated nephrolithiasic rats (group II), calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) crystals were detected throughout the experiment (Figure 1b). For all nephrolithiasic rats treated with AEZL from 1st day, crystalluria was composed of a few COM particles (figure

Table 1: Effect of AEZL on urine volume (ml/24h).

Group	Treatment	Urine volume (ml/24h) (mean ± s.d.)		
		Day 0	Day 14	Day 28
I	Distilled water p.o.	3.45±0.10	3.47±0.12	3.43±0.03
II	0.75% v/v EG + 2% w/v AC p.o.	3.53±0.03	3.5±0.20	5.46±0.05^{a**}
III	0.75% v/v EG + 2% w/v AC +AEZL	3.6±0.60	3.97±0.47^{a*}	7.03±0.53$^{a**, b*}$
IV	0.75% v/v EG + 2% w/v AC +AEZL	3.77±0.10	3.9±0.05	6.42±0.10$^{a**, b*}$
V	0.75% v/v EG + 2% w/v AC + Cyctone	3.4±0.09	3.16±0.21	6.66±0.20$^{a**, b*}$

Signification: * p<0.05 ** p<0.01 n=6

[a]Comparisons are made with Group I (Vehicle control).

[b]Comparisons are made with Group II (Lithiatic control).

Table 2: Effects of aqueous extract of Ziziphus lotus on various urinary parameters in ethylene glycol + ammonium chloride induced urolithiasis.

Group	Treatment	Urine parameters (mean ± s.d.)		
		Oxalate (mg/24h)	Calcium (mg/24h)	pH
I	Distilled water p.o.	0.4±0.10	1.11±0.45	8.40±0.40
II	0.75% v/v EG + 2% w/v AC p.o.	1.4±0.17^{a**}	3.34±0.22^{a*}	8.46±0.30
III	0.75% v/v EG + 2% w/v AC +AEZL	0.5±0.05^{b**}	1.07±0.18^{b*}	8.30±0.26
IV	0.75% v/v EG + 2% w/v AC +AEZL	0.67±0.07^{b**}	1.09±0.15^{b*}	8.33±0.30
V	0.75% v/v EG + 2% w/v AC +Cyctone	0.57±0.06^{b**}	1.56±0.12^{b*}	8.26±0.30

Signification: * p<0.05 ** p<0.01 n=6

[a]Comparisons are made with Group I (Vehicle control).

[b]Comparisons are made with Group II (Lithiatic control).

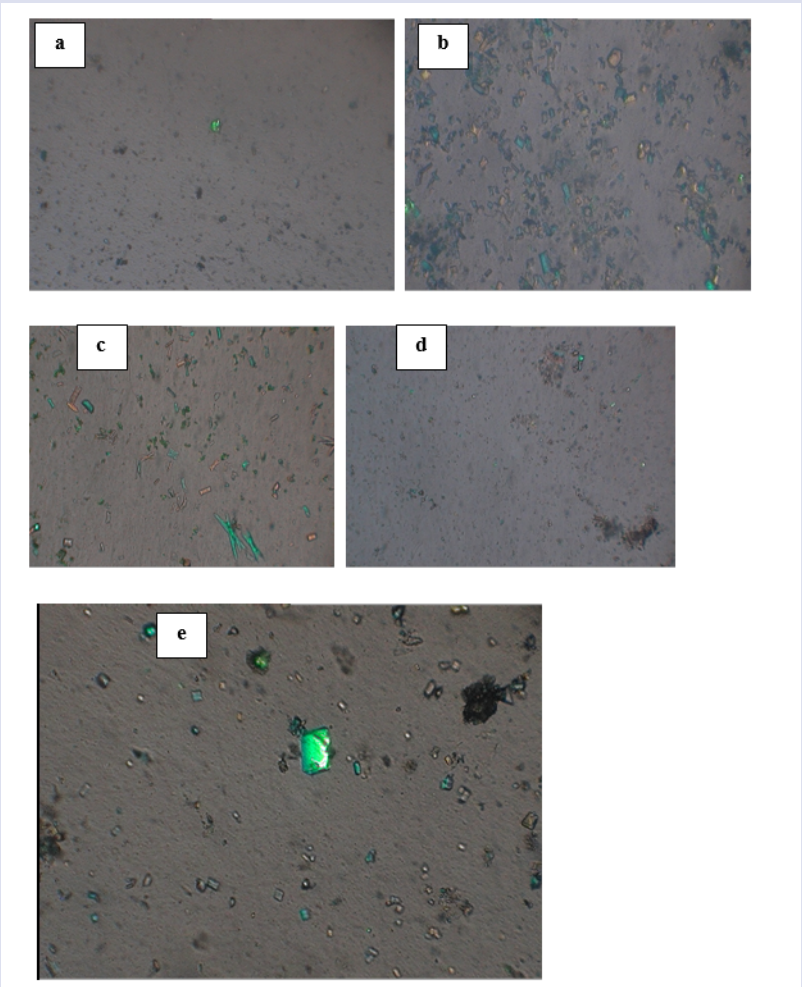

Figure 1: Microscopic examination of urinary crystals excreted by rats of different groups; vehicle (a) vehicle control, (b) Lithiatic control, (c) curative (d) Lithiatic rats receiving a preventive treatment (e) lithiatic rats receiving a drug treatment (cystone).

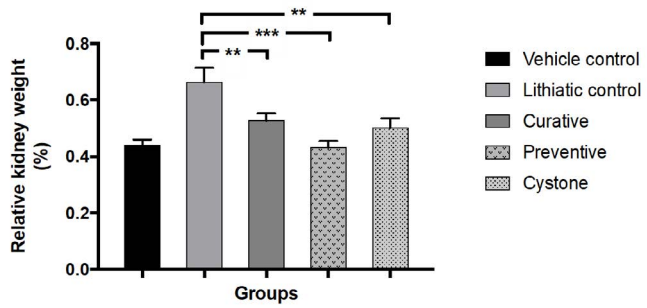

Figure 2: Relative kidney weight of control and treatment groups.

1c), compared with nephrolithiasic rats treated with AEZL from 14 days (curative treatment) which their urine was composed mostly of COD particles and only with few COM particles were present in their urine samples (Figure 3d). Interestingly, the size of both type of crystals was smaller when compared to untreated nephrolithiasic rats.

Kidney weight

The data of kidney weights of therapeutic groups at the end of 28 d experimental period is presented in figure 3. The mean weight of kidney of the ethylene glycol fed lithiatic group was significantly increased compared to the control group when this induction of curative group was similarly recovered by the feeding of ZL extract and antilithiatic drug, cystone, in curative and cystone groups, respectively.

Values are expressed as mean ± SEM; n=6. Animals treated with Z. lotus showed minimal relative kidney weight compared to non-treated animals.

DISCUSSION

The aim of the present study was to examine the anti-urolithiatic effects of the whole *Ziziphus lotus* extract in an ethylene glycol induced urolithiatic model of rats. The data of this study showed that ZL has an anti-urolithiatic effect at least in this experimental condition.

Urinary stone disease is mainly the result of supersaturation of urine with certain urinary salts such as CaOx, the most common constituent of kidney stones.[22]

Ethylene glycol is an intermediate in the synthesis of a number of commercial chemical products, including polyethylene terephthalate (PET) resins, unsaturated polyester resins and polyester fibers. It is also a constituent in antifreeze, surface coatings, heat transfer fluids and industrial coolants, surfactants and emulsifiers.[23] General populations, or consumer, exposure occurs primarily from the use of ethylene glycol in automotive antifreeze. There have been a number of acute human poisonings from accidental or intentional ingestion of antifreeze, when the kidneys are the most sensitive target organ. Regimens for the treatment of acute ethylene glycol poisoning are designed to prevent kidney damage and to prevent metabolism to the toxic acidic metabolites in several previous studies.[24,25] Ethylene glycol has in itself a low toxicity, but *in vivo* it is broken down to four organic acids: glycoaldehyde, glycolic acid, glyoxylic acid and oxalic acid. The metabolites are toxic to cells that cause depression in central nervous system and cardiopulmonary and renal failure.[26] That is why ethylene glycol has been chosen as an appropriate material for the induction of urolithiasis in rats like many other previously developed models.[27,28]

One of the ethylene glycol metabolites, oxalate is usually precipitated as calcium oxalate in the kidneys and other tissues when glycolic acid causes severe acidosis.[29]

Administration of 0.75% (v/v) ethylene glycol to young male albino rats for 14 d period forms renal calculi composed mainly of calcium oxalate. As mentioned above, the biochemical mechanisms for this process are related to an increase in the urinary concentration of oxalate.

Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which is basically of two types: (1) acute, when the rat is challenged by a single, large dose of lithogen, (2) chronic, when the rat is continuously challenged with generally small doses of lithogen for a period of time.

It has been reported that hyperoxaluria is usually measured by determining urinary oxalate, and crystal deposition and confirmed by microscopic examining of urine samples.[30] It has been also reported that hyperoxaluria causes increased renal retention and excretion of oxalate.[31]

In the present study, male Wistar albino rats were selected to induce urolithiasis because of the similarities of their urinary system with that of human. It has also been reported that the amount of stone deposition in female rats is significantly lower than that in male rats.[32]

In our study, the administration of ethylene glycol to rats significantly increased the excretion of calcium and oxalate in 24 h urine sample of the both preventive and therapeutic groups. After oral treatment with the aqueous extract of Zizphus lotus (500 mg/kg BW), the urinary excretion of calcium and oxalate were significantly decreased and Urine volume was significantly increased in both preventive and therapeutic intervention trials although the therapeutic group was found more potent than the preventive group. Cystone was found more effective than *Ziziphus lotus* extract. However, comparable anti-urolithiatic effects of the *Ziziphus lotus* extracts with proven anti-urolithiatic drug authenticated the beneficial effect ZL in preventing calculi formation by supersaturated lithogenic substances in kidneys and by a diuretic effect.

Hence, in the current investigation, the histopathological studies suggested that no microcrystalline deposition and kidney damage in the AEZL extract treated groups (Figure 1) were left and the successful prevention of crystal deposition was obtained at the dose of 500 mg/kg, which may be due to the active compounds present in aqueous extract of this plant. Additionally, the significantly lower kidney weights in the therapeutic AEZL fed group suggested the reduction and excretion of kidney stones in this group. All these findings enabled us to confirm the inhibitory and curative potential of ZL on ethylene glycol induced urolithiasis in rats.

EG has also been criticized because it induces metabolic acidosis, and some of its metabolites are nephrotoxic in nature.[33]

The increased weight of kidneys of the animals treated only with ethylene glycol + ammonium chloride was due to urinary super saturation and calculogenesis with respect to stone-forming constituents.[34] The low weight of kidney reflects the role of AEZL in the inhibition of stone formation.

Generally, urine has acidic pH, which is useful to prevent crystallization of stone-forming promoters by dissolving them into urine.[35] The measures of pH have shown alkaline urine in lithiatic control group caused by hyperoxaluric medium. Alkaline pH promotes the crystallization in urine and thus stone formation. The urinary pH in the treated lithiatic groups have been found significantly reduced compared to the lithitic control group, this suggests that the AEZL possesses a good anti-urolithiatic activity, the similar results have been recorded by Atmani F. with extract of *Herniaria hirsuta*.[36]

The presence of small crystals in the urine of preventive group suggest the possibility that the drug facilitates the excretion of the crystals in the urine, thereby preventing the occurrence of crystal deposition in the kidney may also play a role in the observed beneficial effect of the drug in this study. In addition, the tendency for alkalinization of the urine in the AEZL-treated animals probably plays a role in solubilization of the oxalate crystals. This may also be one of the mechanisms involved in the action of plant components.

In summary, the results of the present study showed that the administration of ethylene glycol caused statistically significant decreases in the levels of calcium and oxalate whereas increases in the levels of urine volume. In contrary, the administration of AEZL to rats significantly reduced and prevented the growth of kidney stones and significantly improved the renal impairment in the same ethylene glycol induced urolithiatic model of rats. From these results, it can be concluded that the supplementation of *Ziziphus lotus* extract has a potent beneficial effect on ethylene glycol induced urolithiasis model of rats. Further study is required to elucidate the chemical constituents of the extract and the mechanism(s) responsible for its pharmacological anti-urolithiatic activities.

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DECLARATION OF INTEREST

There are neither any conflicts of interests nor any financial interests in this study from the part of the contributing authors. No funding was received for this work.

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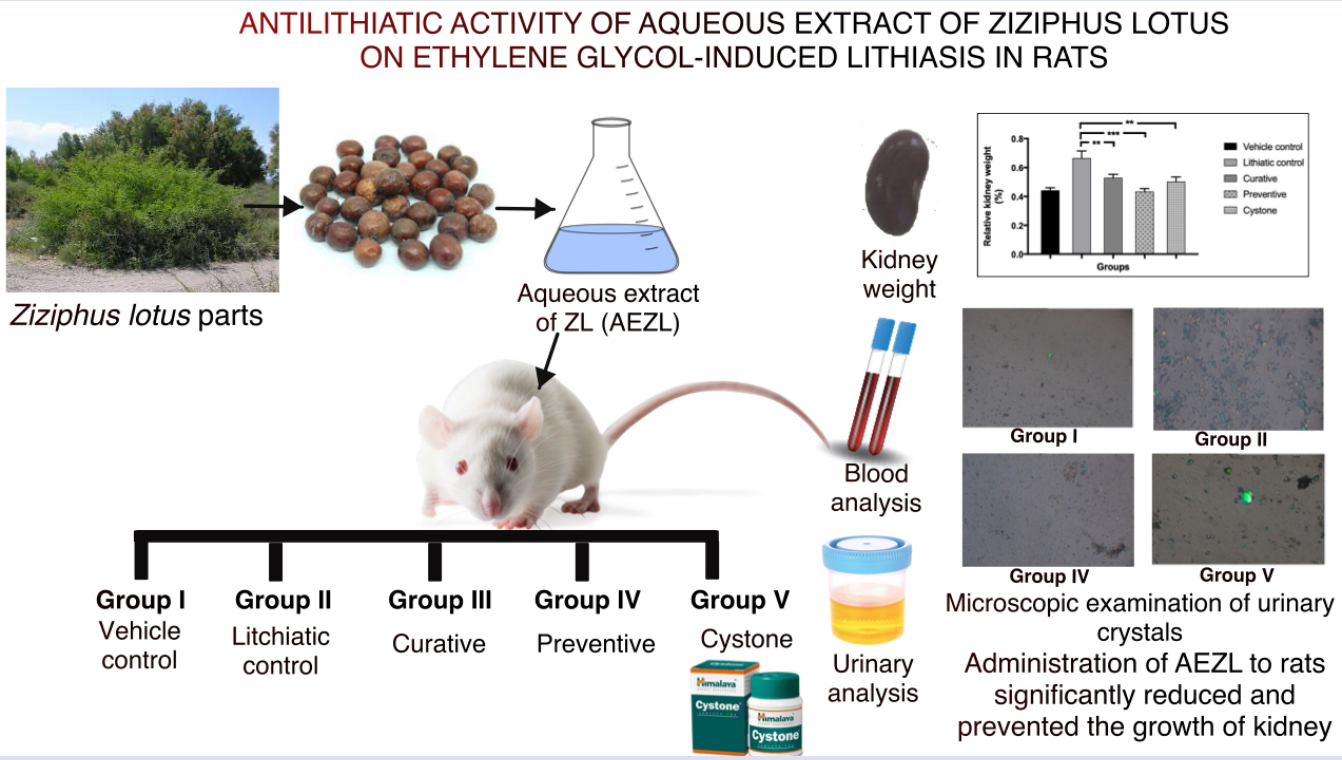
GRAPHICAL ABSTRACT



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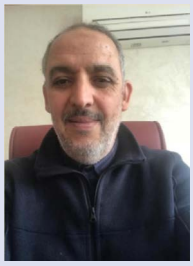

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