Pharmacogn. Res.
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ORIGINAL ARTICLE

Phytochemical Screening, Acute and Sub-Acute Toxicity of Aqueous Extract from a Mixture of Some Recipe of *Herniaria glabra* L., *Opuntia ficus-indica*, *Zea mays* L. and *Zizyphus lotus* L. Used Traditionally against Renal Lithiases

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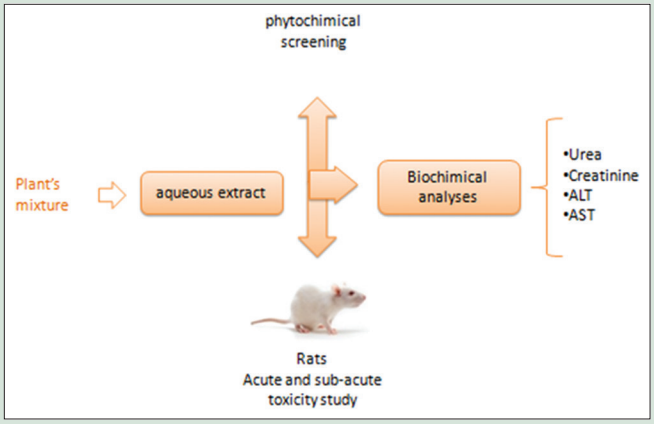
ABSTRACT

Introduction: Antiurolithiatic plants are used since ancient times, in the form of decoction, infusion, or juice, to eliminate kidney stones and to prevent their recurrence. On the basis of the available ethnopharmacological information, more scientific studies are needed to explore natural and safe antiurolithiatic compounds. **Materials and Methods:** The present work showed phytochemical screening, acute and sub-acute toxicity of aqueous extract of four plant's mixture: Aerial part (branches, flowers and leaves) of *Herniaria glabra*, flowers of *Opuntia ficus-indica*, *Zea mays* styles and fruits of *Zizyphus lotus* used traditionally against renal lithiases. Phytochemical screening was performed using qualitative methods. To measure acute toxicity, rats were administered orally by single doses of 0.1, 0.2, 0.5 and 2.0 g/kg body weight (b. w.) of extract of plant's mixture. General behavior adverse effects and mortality were determined during 15 days. For sub-acute study, the aqueous extract was administered at 100 mg/kg b. w. for 28 days to Wistar rats. Animals were monitored daily after an oral administration of aqueous extract of the mixture to detect any changes in b. w., behavior, autonomic profiles, or mortality. Calculation of relative organ weight (ROW) and biochemical analysis were carried out. **Results:** The acute oral toxicity study showed no mortality and no statistically significant decrease in b. w. and ROW of the treated groups of rats when compared to the control group was observed. In biochemical analysis, there was a significant increase in aspartate aminotransferase, creatinine, urea, and uric acid. **Conclusion:** This study found that aqueous extract of traditional recipe used against renal lithiasis in Fes-Meknes region containing: Flavonoids, tannins, catechic tannins, coumarins and the glycosides. The results of the acute and sub-acute toxicity studies indicated that the recipe extracts induce a slight hepatotoxic effects in rats treated orally with 100 mg/kg (b. w.).

Key words: Acute toxicity, biochemical parameters, four plant's mixture, phytochemical screening, sub-acute toxicity, toxicological evaluation

SUMMARY

- Mixture of some recipe of *Herniaria glabra* L., *Opuntia ficus-indica*, *Zea mays* L. and *Zizyphus lotus* L. used traditionally against renal lithiases
- The phytochemical results showed that our mixture was rich in flavonoids, tannins, saponins, alkaloids, cardiac glycosides derived from each plant studied
- The results of the present study showed that oral treatment of rats with 100 mg/kg for 28 days did not change the biochemical parameters of kidney function which illustrated normal architecture of kidney
- The results of the acute and sub-acute toxicity studies indicated that the recipe extracts induce a slight hepatotoxic effects in rats treated orally with 100 mg/kg (b. w.).



Abbreviations Used: ALT: Alanine aminotransferase; AST: Aspartate transminase; b. w.: Body weight; H: *Herniaria;* ip: Intra-peritoneal; ROW: Relative organ weight.

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DOI: 10.4103/pr.pr_89_19

Access this article online
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INTRODUCTION

Renal lithiasis is a multifactorial and a complex disease that results from a combination of factors related to both urine composition and kidney morphoanatomy.[1,2] In spite of substantial progress in the study of the biological and physical manifestations of kidney stones, there is no satisfactory drug to use in clinical therapy. Data from *in vitro*, *in vivo* and clinical trials reveal that phytotherapeutic agents could be useful as either an alternative or an adjunctive therapy in the management of nephrolithiasis.[3] In Morocco, the use of phytotherapy goes back several centuries and some medicinal plants are used in a traditional way for the treatment of renal lithiasis.[4] Among the most famous antilithiasis plants

Cite this article as: Touiti N, Bousta D, Boukhira S, Chebaibi M, Achour S. Phytochemical screening, acute and sub-acute toxicity of aqueous extract from a mixture of some recipe of *Herniaria glabra* L., *Opuntia ficus-indica*, *Zea mays* L. and *Zizyphus lotus* L. used traditionally against renal lithiases. Phcog Res 2020;12:60-4.

are: *Herniaria glabra L.* (*Caryophyllaceae*), *Opuntia ficus-indica* (cactaceae) flowers, *Zea mays* (fabaceae) and *Zizyphus lotus* fruits. The results of an *in vitro* dissolution study of cystine stones demonstrated the efficacy of *Herniaria hirsuta* extracts, flowers of *O. ficus-indica*, and corn styles. Probably resulting from the formation of complexes between cystine and polyhydroxylated molecules present in the extracts. These extracts could therefore constitute an interesting curative and/or prophylactic treatment for cystinuric patients.[5] The composition and toxicity of each separate plant has been studied *in vivo*. The genus *Herniaria* (*Caryophyllaceae*) contains several species which are widely distributed in Europe, Asia and North Africa. Beside the fact that an infusion of either *H. glabra L.*, *H. hirsuta L.* (hairy rupturewort) or *Herniaria fontanesii* J. Gay is well known in Moroccon folk medicine for the treatment of biliary dyskinesia, (uro) lithiasis or as a diuretic.[6] An infusion of *H. hirsuta L.* has a proven efficacy against urolithiasis and cholelithiasis.[7] Some phytochemical research on these species revealed the presence of several saponins and flavonoids.[8,9] The saponins from *H. glabra* are claimed to be responsible for the diuretic activity of the aerial parts.[9] Several toxicity of *Herniaria* genus has been shown in many scientific literatures. *H. glabra* appears to be relatively non-toxic at the doses consumed empirically in traditional Moroccan medicine. However, at higher doses it can cause liver and kidney toxicity.[10] The toxic effect of the butanolic extract of *Herniaria cinerea* has been tested by oral administration on Wistar male rats. The extract causes bloody diarrhea and respiratory troubles. The study of the histopathological lesions in the stomach, intestine, lung and kidney revealed an ulcerous effect on the digestive tract and alveolar destruction. In the kidney, they observed total tubular necrosis with hemorrhage.[11]

The dried stigmata from *Z. mays* L. are used traditionally for the treatment of uncomplicated urinary tract infections. A recent screening has indicated that existence of the unusual C-glycosidic flavones derhamnosylmaysin (6), 3'-deoxyrhamnosylmaysin (4), 3'-O-methylderhamnosylmaysin (3), apiferol (2) and alternanthin (8) might be related to the antiadhesive activity of this subfraction against uropathogenic *Escherichia coli*.[12]

The small fruits of *Z. lotus* L. have been studied. The study was realized on the pulp, crude protein, crude fat, crude fiber, ash, carbohydrate, pectin, moisture contents and calorific values were in the pulp, the pulp has a hard consistency, with a sweet taste and very specific flavor. The thin layer chromatography showed the presence of glucose, fructose and sucrose like sugars.[13]

A study demonstrated that the water extract from the bark of *Ziziphus attopensis* did not produce any toxic signs and symptoms of acute and chronic oral toxicity tests. Moreover, it did not cause any lethality or produced any remarkable hematological and blood chemical adverse effects both in chronic toxicity studies in Sprague Dawley rats.[14]

The qualitative and quantitative analysis of flavonoids from *O. ficus-indica* flowers methanol extract from the Mediterranean area is described. Seven compounds have been identified as kaempferol, quercetin and isorhamnetin glycosylated derivatives.[15] *O. ficus indica* aqueous extract did not show any biological toxicity on serum and lipid parameters in white male rabbits.[16] However, the toxicity of the mixture of these plants has not been studied.

Therefore, the present study was designed to investigate the acute and sub-acute toxicity effects of aqueous extract of mixture of aerial parts of *H. glabra*, flowers of *O. ficus-indica*, *Z. mays* styles and pulp of of *Z. lotus* fruits, using rats.

MATERIALS AND METHODS

Plant collection

Based on ethnopharmacological survey conducted in Fes-Meknes region. We selected a recipe containing four plants used against renal lithiases: Aerial part (branches, flowers and leaves) of *H. glabra L.*, flowers of *O. ficus-indica*, *Z. mays* styles and pulp of *Z. lotus* fruits. The plants were authenticated by Professor Bari Amina of Biotechnology and Natural Resources Preservation Laboratory, Sidi Mohamed Ben Abdellah University, Fez, Morocco, where a voucher specimen (references numbers *Z. lotus* L. BPRN09, *O. ficus-indica* BPRN24, *H. glabra L.* BPRN67, *Z. mays L.* BPRN68) was deposited.

Extract preparation

Powder of plant's mixture in equal parts was extracted using aqueous infusion. Plants bought from an herbalist were crushed. Indeed, 10 g of powder was added to 100 ml of boiled distilled water. Then have been macerated for 4 h with continuous agitation. The macerate was filtered through a Whatman No. 1 and then concentrated in a rotary vacuum evaporator. Until obtained a dry residue with a yield of 19.6%. Aqueous solutions were prepared daily for administration to rats.

Phytochemical screening

The dry extract is screened for phytochemical constituents (coumarins, leucoanthocyans, flavonoids, mucilags, tannins, quinones and cardiac glycosids) using a simple qualitative methods as described in the studies of.[17-19]

Human equivalent dose

According to the traditional use of the mixture, taking the mixture of almost 15 g of each plant in equal parts and the preparation was undertaking by infusion (300 mg/kg) twice per day for an adult who weights about 60 kg.

Human equivalent dose (HED) =10 mg of powder/kg body weight (b. w.) for an adult.

Conversion of human dose to animal dose

The animal dose should not be extrapolated to a HED by a simple conversion based on b. w. For more appropriate conversion of drug doses from animal studies to human studies, we suggest using the body surface area normalization method.

HED (mg/kg) = Animal dose (mg/kg) multiplied by $\frac{Animal\ km}{Human\ km}$ [20]

So animal dose = HED (mg/kg) multiplied by $\frac{Human\ km}{Animal\ km}$; with Human km = 37; Rat km = 6

Animal dose = 62 mg/kg ≅100 mg/kg

Experimental animals

For the acute toxicity study, male and female rats, with average age 6 weeks old (weighting ~75 ± 11 g), were devised into five groups, each group containing five animals. The animals were kept in well-ventilated environment, had a free access to water and food and were housed in a quiet room under a "12-h light: 12-h dark" cycle for 2 weeks before experimentation.

For the sub-acute study, Wister rats (70–95 g) were randomly divided into two groups of five animals each (two females and three males). The animals were separated by gender and housed five in each cage under the same conditions as mentioned above for the acute toxicity.

The care and handling of the animals were in accordance with the internationally accepted standard guidelines for the use of animals, and the protocol was approved by the Institutional Committee on animal care following the French Technical Specifications for the Production, Care and Use of the Laboratory Animals.

Toxicological evaluation of the recipe

Acute toxicity

This experimental study has been adapted to that described in guideline 425 (OCDE, 2008).[21] The aqueous extract was administered orally to rats (n = 5 in each group) with different doses of extract 100, 200, 500, 2000 mg/kg (b. w.). The animals were observed for 2 h for any behavioral changes, neurological and autonomic profiles or cases of death after 24 h, 72 h and 2 weeks. The animals were observed for obvious toxic symptoms and mortality in each group during 15 days by studying a single administration of four doses of aqueous extract: the general behavior of the animal, the weight, the morphological appearance of organs (liver, spleen and kidneys) and the relative organ weights (ROWs) in comparison with the control group, calculated by the following formula:

ROW = (organ weight/b. w.) × 1000).[22]

Sub-acute toxicity

It was determined from OECD Guideline 407.[23] Rats in the treated groups received extract daily by gavage at doses of 100 mg/kg (b. w.) during 28 days. Toxic manifestations and mortality were monitored daily and the b. w. changes were recorded every day.

Biochemical parameters

After the period of toxicity study, the rats were anesthetized by "ip" with sodium pentobarbital at the dose of 30 mg/kg and a blood sample was taken by cardiac puncture. Biochemical parameters were assayed on serum, all serum analysis was collected in heparin tubes for the determination of different biochemical parameters like creatinine, urea, asparate aminotransferase (AST), alanine aminotransferase (ALT). All parameters were studied by an auto-analyzer "Olympus AU 640."

Statistical analysis

Data were expressed as mean ± standard error of mean Comparisons of means were performed by using Student's t-test of. The level of statistical significance was set at $P < 0.05$.

RESULTS

Phytochemical screening

As shown in Table 1, phytochemical screening of aqueous extract of plant's mixture studied revealed an abundance of flavonoids, saponosids, tannins, mucilages, anthraquinones. However, coumarins, alcaloides, and free quinones were not detected.

Acute toxicity

Effects on body weight and weight of organs

Figure 1 showed the evolution of the mean b. w. in the treated and control rat groups.

During the experiment, the rats did not show any observable signs of toxicity or morbidity. Furthermore, no mortality was recorded. There was no significant difference in the ROW of liver, kidney and spleen of the treated groups of rats when compared to the control group [Table 2].

At the acute trial, rats from all groups did not show any clinical signs of toxicity. Serum AST activities was significantly increased and ALT activity was significantly decreased in rats treated with 2.0 g/kg dose. The treatment with plant's mixture: Aerial part (branches, flowers and leaves) of *H. glabra*, flowers of *O. ficus-indica*, styles of *Z. mays* and pulp of *Z. lotus* fruit did not cause any significant changes in urea and serum glucose levels [Table 3]. The serum levels of creatinine were significantly reduced in rats that were administered the 0.2 g/kg dose.

Sub-acute toxicity

Effects on body weight and relative organ weight

Results illustrated in [Figure 2 and Table 4] showed no significant difference in b. ws and ROW of rats treated by 100 mg/kg (b. w.) from the start until the end of treatment.

Biochemical parameters

The results shown in Table 5 showed significant reduction ($P < 0.001$) in ALT of treated rats when compared with the control group.

DISCUSSION

Despite considerable progress in medical therapy, there are no satisfactory drugs to treat kidney stones. Since ancient times, various herbal preparations have been used in renal lithiasis therapy, but conclusive scientific data on their therapeutic effects and efficacy are

Table 1: Phytochemical screening of the recipe

Chemical compound	Extract of four plants
Flavonoids (flavonols, flavanonols)	+
Saponins	+
Total tannins	+
Gallic tannins	+
catechic tannins	+
Leucoanthocyans	+
Cardiac glycosides	+
Mucilages	+
Alcaloïds	+
Free quinones	–
Coumarins	–

Presence of chemical compounds is: +Presence, –Absence

Table 2: Relative organ weights of rats after 15 days of observation, acute toxicity

Groups	Dose (mg/kg b. w.)	Relative organ weights (g)		
		Kidney	Liver	Spleen
Control	0	10.5±0.79	40.48±1.27	2.37±0.23
Treated	0.1	9.33±0.52	47.65±0.55	5.27±0.95
	0.2	10.9±0.25	39.37±1.35	2.45±0.17
	0.5	10.43±0.71	39.53±0.54	2.06±0.36
	2	10.76±0.56	37.78±1.52	2.69±0.03
Significance		NS	NS	NS

NS: Not significant

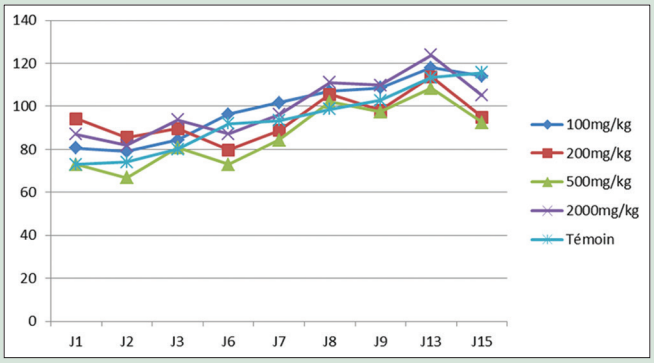

Figure 1: Effects of plant's recipe on rat body weight during 15 days. Values are expressed as mean ± standard deviation. Significantly different from control rats group are determined by student's test, $P < 0.01$, $n = 5$ for each group

Table 3: Serum levels of urea, creatinine, and serum activities of alanine aminotransferase and aspartate aminotransferase in rats after a single dose of aqueous extract of mixture of four plants

Parameter	Control	100 (mg/kg)	200 (mg/kg)	500 (mg/kg)	2000 (mg/kg)
Liver profile					
ALT (U/L)	72.33±21.2	69±1.41	55.5±9.2	55±15.71	58±11.31*
AST (U/L)	242±52.8	262±121.54	201±106.06	284.6±114.78	316.5±13.43*
Renal profile					
Urea (g/l)	0.34±0.02	0.36±0.07	0.47±0.15	0.37±0.17	0.28±0.13
Creatinine (mg/l)	3.6±0.57	3.66±0.57	3.33±0.57*	3±0.0	3±1

*Statistically significant as compared with control (P<0.05). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase

Table 4: Relative organ weights of rats after 28 days of treatment, sub-acute toxicity

Groups	Dose (mg/kg p.c)	Body weight (g)	Relative organ weights (g)		
			Kidney	Liver	Spleen
Control	0	142.66±3.78	9.11±0.45	42.12±3.02	4.73±1.18
Treated	100	103.33±8.38*	9.62±1.09*	47.91±6.87*	5.44±1.84*

Values are expressed as mean±SEM, n=5; significant. *P<0.1. SEM: Standard error of mean

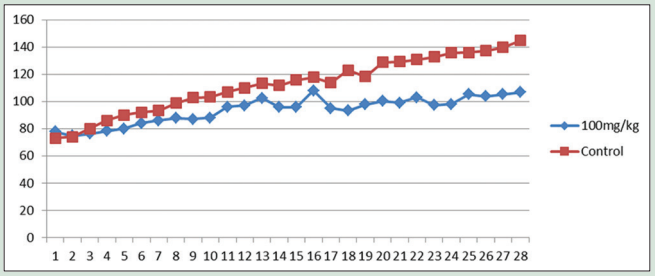

Figure 2: Effects of plant's recipe on rat body weight during 28 days of treatment. Values are expressed as mean ± standard deviation. Significantly different from control rats group are determined by student's test, P < 0.01, n = 5 for each group

Table 5: Serum levels of urea, creatinine, serum activities of alanine aminotransferase and aspartate aminotransferase in rats after 28 days of treatment with aqueous extract of plant's recipe

Parameters	Control	100 (mg/kg)
Liver profile		
ALT (U/L)	72±21.22	69.5±3.53***
AST (U/L)	242.66±52.88	361.66±192.79*
Renal profile		
Urea (g/l)	0.34±0.02	0.23±0.02*
Creatinine (mg/l)	3.6±0.57	4±1

Values are expressed as mean±SEM, n=3; significant. ***P<0.001, *P<0.1. AST: Aspartate transaminase; ALT: Alanine aminotransferase; SEM: Standard error of mean

not available.[24] To address this issue, the present study evaluated the phytochemical, acute and sub-acute toxicity of plants recipe used traditionally against renal lithiasis. The phytochemical results showed that our mixture was rich in flavonoids, tannins, saponins, alkaloids, cardiac glycosides derived from each plant studied, *H. glabra* is rich in flavonoids and saponins 8,9, *O. ficus* flowers contains phenolic acid and flavonoids.[25] Polyphenols possessing diuretic property has been often reported. Extracts of *O. ficus indica* fruit have been traditionally used as a diuretic. The diuretic action increases the quantity of fluid passing through the kidneys as a result of flushing out the salt deposits. Therefore, the increment in urine volume decreases the saturation of the salts and thus prevents the precipitation of the crystals at physiological pH.[26] Phytochemicals screening of Malaysian *Z. mays* hair showed the presence of flavonoids, saponins, tannins, phlobatannins, phenols, alkaloids and cardiac glycosides.[27] The pulp of *Z. lotus* is rich in polyphenol and flavonoids.[28] This shows that there is a synergistic effect between the components of each plant's recipe. The present work showed that infusion of plant's mixture did not induce any significant changes to the b. w. and ROW of rats (liver, kidneys, and spleen). The results showed a slight significant decrease in serum creatinine. Furthermore, the lowered serum ALT activities in rats treated with 2.0 g/kg of aqueous extract might be attributed to an initial liver damage. In fact, previous studies demonstrated reduced serum ALT activity associated to exposure to hepatotoxins.[29] Indeed, ALT is the more specific marker of liver cell damage, because it occurs more exclusively in the liver while AST is also found in heart, skeletal muscle, kidneys, brain, pancreas and blood (cells 39). In the liver, ALT is confined to cytoplasm, while AST is found in both mitochondria (80%) and cytoplasm (20%).[30] In the sub-acute toxicity study the infusion did not induce any significant changes in the b. ws and ROW of rats treated. The results showed a slight decrease of urea was noted that does not reflect any toxicity. Urea is a byproduct from protein breakdown. About 90% of urea produced is excreted through the kidney.[31] Meanwhile, the creatinine is a waste product from a muscle creatinine, which is used during muscle contraction. Creatinine is commonly measured as an index of glomerular function.[32] It is excreted exclusively through the kidney. Therefore, damage to the kidney will make the kidney inefficient to excrete both urea and creatinine and causes their accumulation in the blood. Therefore, the high level of blood urea and creatinine will indicate kidney damage.[33] In our study, there was no reduction in serum creatinine in the rats treated with 100 mg/kg (b. w.). The results of the present study showed that oral treatment of rats with 100 mg/kg for 28 days did not change the biochemical parameters of kidney function which illustrated normal architecture of kidney. It's proved by a non-significant change of serum urea. With the evidence of normal urea and creatinine level in blood for treatment group, it is suggested that there are no toxic effect on kidney function in treated group with 100 mg/kg (b. w) for 4 weeks.

From the biochemical study, it showed that the treatment dose (100 mg/kg b. w.) reduced significantly the liver enzymes (ALT) level, which is more liver-specific than AST,[34] in treated rats compared to control group of rats. The loss of b. w. in treated rats might be related to the liver enzyme. The same finding was reported by another work which evaluated the effect of *Nigella sativa* on the liver function of rats.[34]

CONCLUSION

This study highlighted that aqueous extract of traditional recipe used against renal lithiasis in Fes-Meknes region containing: Flavonoids, tannins, catechic tannins, coumarins and the glycosides. The results of the acute and sub-acute toxicity studies indicated that the recipe extracts induce a slight hepatotoxic effects in rats treated orally with 100 mg/kg (b. w.). These low levels of toxicity may not be significant in healthy individuals, but they may exacerbate pre-existing hepatic and renal disease.

Acknowledgements

The authors would like to thank to the National Center for Scientific and Technical Research from Morocco for the support of the doctoral grant of Touiti Noufissa.

Special thanks

Special thanks to H2020-MSCA-RISE-2016 project, Research and Innovation Staff Exchange (RISE), VAHVISTUS– Project Number: 734759, funded by the European H2020 program. The project title is "Integrative development of smart drug-vector nanostructures for adaptive drug delivery into target cells."

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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