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Research Article

Evaluation Of The Diuretic Activity For The First Time Of Hyoscyamus Albus And Umbilicus Rupestris In Rats

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ABSTRACT

Medicinal plants have proven their important activity among time history in different diseases treatment and helping the production of novel drugs. The aim of the present study is to evaluate the diuretic activity of the methanolic extract HAMeOH and URMeOH extracted from leaves of two medicinal plants *Hyoscyamus albus* and *Umbilicus rupestris* in animal model. For diuretic activity, this latter is assessed using furosemide (40 mg / kg by weight) as a reference standard the used doses of HAMeOH and URMeOH have been administrated as (100 and 200 mg / kg by weight.) and were administered orally to experimental rats. Diuretic effect of the extracts HAMeOH and URMeOH was assessed by measuring the urine volume (ml / Kg), urine pH, Na + excretion, K^+ excretion and Cl^-. The results show that extracts HAMeOH and URMeOH also the furosemide increased significantly ($P \leq 0.05$) the urine volume and also increased the excretion of electrolytes Na +, K^+, Cl^- compared to the control group.

INTRODUCTION:

All drugs which increase the urinary volume and the excretion of electrolytes have more important effect in treatment of edema, acute and chronic renal failure, moderate hypertension.Umbilicus rupestris (Salisb.) Dandy is a perennial plant, belongs to the family of Crassulaceae**.** It presents on the rocks, the old walls and in mountains; only in North Africa. Leaves of this plant are used in traditional medicine against the ignitions of skin, wounds, burns, disinfectant, anti-parasitical. Infused (leaves) of this plant is used like an ophthalmic disinfectant [8]. Hyoscyamus albus is plant which appropriate to solonaceae family and have been used as medicinal traditional plant [11].

Amaranthus spinosus Linn , (Amaranthaceae) is known as mullu keerai in Siddha medicine, which is a traditional system of medicine of South India. This herb is commonly known as prickle amaranthus. It is a branched erect herb, armed with axillary spines and ovate-elliptic leaves up to 12 cm × 6 cm. Unisexual flowers are seen in axillary clusters and in terminal of the plant . Literature in Siddha medicine claims that the decoction of Amaranthus spinosus has potent diuretic activity and reduces edema secondary to cardiovascular diseases and kidney diseases [12]. Its antimalarial, anthelmintic [12], anti-diabetic and anti-hyperlipidemia activities have been confirmed by various studies. It also stimulates proliferation of B lymphocytes in vitro, it inhibits the spontaneous and dexamethasone induced apoptosis in murine primary [14] .

Our research aim is to investigate the diuretic activity, this study was done to evaluate the diuretic activity of methanolic extracts of *Hyoscyamus albus* and *Umbilicus rupestris* in rats.

MATERIALS AND METHOD

Chemical products

Methanol (Sigma-Aldrich), Formaldehyde CH2O (EDEN LABO) ,Carrageenan (Sigma- Aldrich), Indomethacin (Sigma-Aldrich) histamine (Sigma-Aldrich) , Serotonin (Sigma-Aldrich) have been used in this study.

Collection of the plant

The leaves of *Umbilicus rupestris* were harvested in March 2012 from the Wilaya of Batna region Tibhirine . Botanical species identification was realized by Dr. B. OUDJHIH, Botanical Laboratory, Department of agronomy, University of Batna 1. The leaves of these plants freshly harvested and were washed and then dried in the shade for 40 days in a dry and aerial place for the extraction of active compounds. They were coarsely ground and collected in clean sterilized bags.

Extraction by organic solvents

1 Kg of powdered leaves of each plant *H.albus* and *U.rupestris* was extracted with 5 L of petroleum ether for three times. Then, the marc was dried and extracted with 5 L of chloroform for three times and with 5 L of methanol for three times and the supernatants were filtered separately using cotton and Whatman filter paper. The solvents were then evaporated under reduced pressure (204 mbar) and controlled temperature (30 °C) using a vacuum rotary evaporator (Buchi Rotavapor).

Phytochemical screening

The phytochemical screening of HAMeOH and URMeOH was realized by using the method of qualitative and quantity assay. Phytochemical constituents such as: phenolic compounds, terpenoids , saponins , alkaloids , steroids and tannins were analyzed qualitatively.

Animals

Wister rats weighted (140-170 g) provided by the Pasteur Institute – Algiers. These rats were allowed a favorable condition before and during the experiment: Temperature (23 • ± 2) ° C, relative humidity 50 -55 % with 12 hours' light / 12 hours' night cycle respectively. The food and water were given.

Diuretic activity

The rats selected for the study were putted at fasting during (18h) before the treatment, but they have the access only to water, then received an oral load of solution normal saltworks (0,9%) of 0,05 ml \g b.w. immediately after the administration of the solution saltworks, the rats were placed in metabolic cages [14]. The rats were distributed according to weight's in six groups of six animals each one as follows: Group I (control) received 5 ml/kg b.w. of ionized water. Group II: received furosemide (40 mg/kg b.w) like standard of reference; Group III and IV: received HAMeOH (100 and 200 mg/kg b.w.) respectively; Group V and VI: received URMeOH (100 and 200 mg/kg b.w.) respectively .

5 hour of treatment, the urines of each rat were collected and measured.

RESULTS AND DISCUSSION

The cumulated urinary excretion was calculated compared to the body weight and was expressed in ml for 100 g of body weight. the concentrations of

electrolytes (Na + and K +) (as described in the table) were measured by using the urinary ionogram after 5 hours of experiment and expressed as a µEq/100 g of weight body [17]. The diuretics are employed to treatement of the congestions of the lungs, oedema, hypertension, volume more load and peripheral oedema . The diuretics are defined as chemicals which increase the speed of formation of urine. In fact substances act in the kidney and support the loss of sodium of organization [7].

Table 1. *Effect of the extracts HAMeOH and URMeOH on urinary volume.*

Groupes	Volume of urine (ml/kg b.w.)	Diuretic action	Diuretic activity
Control groupe	2.00±0.0	1	
1. Furosemide (40 mg/Kg b.w.)	14.80±1.70***	7.4	1
2. HAMeOH (100mg/kg b.w.)	3.50±0.70 *	1.95	0.26
3. HAMeOH (200mg/kg b.w.)	4.00±1.41**	2	0.27
4. URMeOH (100mg/kg b.w.)	3.85±1.06*	1.92	0.25
5. URMeOH (200mg/kg b.w.)	8.20±1.13***	4.1	0.55

**** (P<0.0001): highly significant difference, ** (P<0.001): very significant difference, *(P<0.05) significant difference. statistically significant compared to their respective control.(b.w) : by weight*

HAMeOH with the concentration 100 mg/Kg b.w; increased the volume of urine in a significant way (P≤0.05) compared to negative control. This effect could increase in a very significant way (P≤0.001) with the concentration 200mg/Kg b.w. Still, the concentration 100 mg/Kg b.w. of URMeOH made increased the volume of urine in a significant way (P≤0.05) compared to negative control. This effect could increase in a very significant way (P≤0.0001) with the concentration 200mg/Kg b.w. The furosemide which was used like a standard of reference had the best volume of urine in a highly significant way (P≤0.0001) compared to the negative control (Table 1). The diuretic activity is considered well if it is more than 1.50, moderate if it is entre1.00-1.50, weak if it is 0.72-1.00 [1]. Revealed that the compound which increase the urinary rupture and the excretion of electrolytes are called diuretic compounds. These compounds act mainly on the various parts of the nephron and increase the production of urine .The collected urines during five hours were subjected to an analysis electrolytes (Na+, K+, and Cl -) are presented on table 2.The diuretic activity of the extracts HAMeOH and URMeOH is respectively.The diuretic activity is considered well if it is more than 1.50, moderate if it is entre1.00-1.50, weak if it is 0.72-1.00 [18

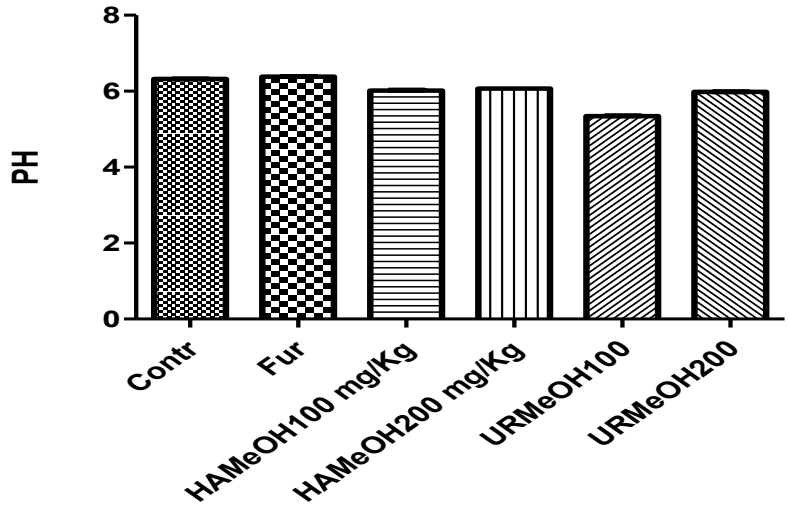
Table 2: Effets *de* HAMeOH et URMeOH sur l'excrétion des électrolytes chez les rats.

Groupe	Concentration des Électrolytes Urinaires (mmol /L			Na^+/K^+	$Cl^-/Na^+ + K^+$
	Na^+	K^+	Cl^-		
Groupe contrôle	114.3±2.058	75.48±2.121	92.06±2.121	1.51	0.485
Furosémide (40 mg/Kg)	149.9±2.843***	133.8±1.492 ***	196±3.330***	1.12	0.69
HAMeOH (100mg/kg p .c)	115.6±3.352	75.77±3.125	124.9±10.44 ***	1.52	0.652
HAMeOH (200mg/kg p .c)	114.1±3.458	78.48±4.950 ***	154.5±3.11***	1.45	0.802
URMeOH (100mg/kg p .c)	139.7±0.961***	125.9±2.850 ***	146.7±1.853 ***	1.1	0.552
URMeOH (200mg/kg p .c)	145.2±1.747***	146.7±1.605 ***	131.4±30.06 ***	0.99	0.45

**** (P<0.0001): highly significant difference, ** (P<0.001): very significant difference, *(P<0.05) significant difference. statistically significant compared to their respective control*

The proportioning of the electrolytes showed the increase of sodium in a highly significant way (P ≤ 0.0001) in groups treated with the extract URMeOH (100 and 200 mg/Kg b.w.) Still the furosemide increase the rate of the sodium in a highly significant way (P ≤ 0.0001). But the HAMeOH does not have any effect on the increase of sodium compared to the negative control (P>0.05). URMeOH (100 and 200 mg/Kg b.w) as well as the furosemide could increase the rate of potassium in a highly significant way (P≤0.0001). The concentration 200 mg/Kg b.w. of HAMeOH increased the excretion of potassium in a highly significant way (P≤0.0001).All groups treated by the furosemide ,HAMeOH and URMeOH increased the excretion of chlorine in a significant way (P≤0.0001) compared to negative control. The report Na+/K+ was calculated as being an indicator of the natriuretic activity gave the following results: 1.12, 1.52, 1.45, 1.10 for Furosemide, HAMeOH 100, HAMeOH 200 and URMeOH 100 respectively. The extracts increase the excretion of sodium more than the potassium which is regarded as products diuretic hypokaliémies and which is an effect similar to that of Furosemide [19]. The extract HAMeOH and URMeOH can show the negligence of the effect saving of potassium , they act like furosemide , the effect diuretic can be due to stimulation of regional blood circulation or the initial vasodilatation [2] or by the inhibition of the tubular reabsorption of water and anions [3]. Diuretics of the handle as furosemide increase the urinary flow and the urinary rate of excretion of sodium, of potassium and chloride by inhibiting Na +-K+ and Cl- symportor in the thick ascending loop and by inhibiting the carbonic enzyme anhydrase. The reports/ratios were found (Cl - / Na + + K + were found 0.65, 080, 0.55, 0.45 and 0.69 for HAMeOH 100, HAMeOH 200, URMeOH 100, URMeOH 200 and Furosemide respectively, these values show that the extracts and the standard have a carbonic activity of inhibition of anhydrase. The report/ratio was calculated to show the effectiveness to inhibit the enzyme the carbonic anhydrase and the carbonic inhibition of anhydrase can be excluded if the values of the report/ratio of (Cl -/ Na+ + K + is between 0,8 and 1,0 and the inhibition of the activity of the enzyme increases with the decreasing reports/ratios [19].

Figure . *Urinary pH of the rats treated with the extracts HAMeOH and URMeOH*



The pH of collected urinary is in the interval of 5.345±0.162 at 6.380±0.254, There is not a change in urinary of pH in a significant way (P ≤ 0.05) of the batches treated either with the extracts or with the furosemid comparing with the batch of control. Furosemide, diuretic of loop (loop dieuretic) known for its effect diuretic and saluretic [4]. It acts by preventing the reabsorption of the electrolytes in the handle of Henlé by preventing the symportor Na + / K + / 2Cl (system of Co - conveyor) on the level of the handle of Henlé [5]. The increase in the excretion of sodium and water also provides a strong base of its anti-hypertensive action [20]. Previous studies also showed that there are several phytochimical compounds, which can be responsible for diuretic effects such as the flavonoïds, saponins and alkaloids [2,3].

CONCLUSION

We conclude that our plants Hyoscyamus albus and Umbilicus rupestris have a significant effects on urinary excretion of electrolytes but the molecules responsible of this effect have to be determined and must targeted for further research to prove another activities which have been not proven before.

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