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Original Article

Acute Diuretic Activity of the Aqueous Ethanol Root Extract of *Corrigiola telephiifolia* Pourr. In Rats

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ABSTRACT

Objective: This study was undertaken to justify and validate a very frequent traditional use of a very well-known and widely used plant by a large part of the Moroccan population. It's about *Corrigiola telephiifolia,* and the activity in question is the diuretic activity. **Methods:** The aqueous ethanol root extract of *corrigiola telephiifolia* (200 mg/kg, 400 mg/kg, and 700 mg/kg) was orally administered to rats. The urinary excretion rate and pH, and electrolyte excretion were measured in the urine of saline-loaded rats. Negative control group received only an equivalent volume of distilled water, while the positive control groups received the diuretic drugs hydrochlorothiazide at dose 10 mg/kg. **Results:** The results showed that hydrochlorothiazide induced significant diuresis and electrolytes excretion at 1, 6 and 24 h after the treatment. Both the higher doses of the extract produced a significant increase in urine volume than the control from the first hour until the end of observation. However, the lowest dose increased significantly only at 24 h after the treatment. With regard to the electrolyte excretion, the tested doses of CTRE which have shown a significant increase in Na^+, K^+ and Cl^- excretion in comparison to normal control rats, are 400 and 700 mg/kg bw. **Conclusion:** These findings collectively indicate that the extracts of *C. telephiifolia* have a potential to induce diuresis markedly, and providing evidence, for its traditional use.

Key Words: Diuretic activity; *Corrigiola telephiifolia*; Diuretic plants; Urine electrolytes; Kaliuresis.

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INTRODUCTION

Since the beginning of time, humans have used plants: first to feed themselves, then to heal themselves. In the writings of all ancient civilizations and in all continents there are traces of this use. India, the Middle East, especially the Arabo-Muslim world, Egypt, Rome, and Greece represent civilizations in which aromatic and medicinal plants had an important role.[1]

During the last years, medication by plants is currently experiencing a veritable revival, in both the developed and developing countries, particularly in countries like Morocco, which is well known for its great wealth of plants (nearly 42 000 species, including nearly 600 used in traditional medicine).[2] It is one of the Mediterranean countries that have a long medical tradition and a traditional know-how based on medicinal plants.[3] By its geographic location, Morocco constitutes a very original natural crossroads offering a complete range of Mediterranean bioclimates and very variable pluviometry levels, favouring a rich and varied flora, with a very remarkable rate of vegetal endemism.[4] Some of the medicinal plants have been described in older Moroccan pharmacopoeia, and in several recent but limited ethnobotanical surveys.[5] Among these plants, *Corrigiola telephiifolia* Pourr (Caryophyllaceae) a species used in folk medicine in Morocco, and locally known as "serghina". It is characterised by alternate leaves that are spaced along the stem and by little white flowers, grouped in cymose inflorescence relatively dense. The root, which is used for various medicinal and cosmetic purposes, is a perennial taproot.[6] This small plant is found in Southern Europe and North Africa.[7] In Morocco, it commonly found in sandy or stony soils. Its roots are harvested in the west of the Kingdom, and annually a part of the harvest is exported.[8] When burned, the root of this aromatic plant releases an aromatic fume, which is used to treat flu and migraines.[7,8] The plant is also used for the treatment of dermatological diseases, inflammation, ulcer, cough and jaundice.[9-11] It is also used as an antispasmodic, aphrodisiac and an antiasthenic given to parturient women.[7] Root decoction has been reported to be used traditionally in the treatment of cancer digestive.[1] and in wound treatment.[12] Fresh roots pulverized and mixed with flour to prepare the bread are indicated often as a treatment for diabetes.[13] Several ethnobotanical studies carried out in several regions of Morocco have revealed the traditional use of corrigolia roots as a diuretic, this activity has also been mentioned in the Moroccan traditional pharmacopoeia.[6,8,12,14,15] Some of these activities have been evaluated by *in vitro* and *in vivo* studies; however no studies have been conducted before to evaluate its diuretic activity.

Indeed, medicinal plants are always considered as rich resources of ingredients which can be used in drug developments, not only when the constituents of the plants are used directly as therapeutic

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agents, but also as raw materials for drug synthesis or as models for pharmacologically active compounds.[16] All these make the knowledge of chemical, biological and therapeutic activities of medicinal plants become necessary.[9] In this perspective, this study was therefore undertaken to justify and validate a very frequent traditional use of a very well-known and widely used plant by a large part of the Moroccan population. It's about *Corrigiola telephiifolia,* and the activity in question is the diuretic activity.

MATERIALS AND METHODS

Plant material

The whole plant of *Corrigiola telephiifolia Pourr.* was harvested, in Mars 2018, based on ethnopharmacological information and traditional uses, from villages around Romany region, while respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner.

This plant has been authenticated by an expert in Botany from the National Scientific Institute in Rabat- Morocco. (Pr. Hamid KHAMAR), and also by another botanist expert (Pr Abdelhak CHERGUI) from the faculty of medicine and pharmacy in Rabat- Morocco. A voucher specimen is preserved in the Herbarium of Scientific Institute, under the code (RAB108763)

Preparation of extract

The plant material (roots) was separated from the aerial part. Thereafter it was carefully air-dried in an oven at a temperature of 40°C for three days, to a constant weight, and then the dry plant was cut and ground to fine powder mechanically in a mill. A quantity of the powder (500 g) was exhaustively extracted with ethanol water mixture (75: 25) in a Soxhlet extractor. The organic solvent was removed from the extract by evaporation under reduced pressure using rotatory evaporator (Rotavapor R-200, Buchi, Switzerland) at 40 ^{0}C and 50 mm Hg. The remaining extract was then dried with freeze–drying using lyophilizer (Free Zone® Dry 4.5, USA). Final extract was a yellow powder; it was stored in a refrigerator at 4°C until the time of drug administration.

Reference drug

Hydrochlorothiazide (ESIDREX* 25 mg cp), a diuretic drug which acts on the cortical diluting segment, was used as the reference drug. It was dissolved in distilled water just before to administration.

Pharmacological studies

Experimental Animals

The experiments were conducted in female rats Wistar, weighing between 200 and 230 grams. They were bred in the animal house of the faculty of science (Rabat-MOROCCO). Before the experiment itself, the animals (unanesthetized) were maintained under standard laboratory conditions (25 ± 2 °C) with dark and light cycle (12/12 h) and they were fed *ad libitum* with Cicalim pellets and barley. All animals had free access to tap water. Experiments were performed in accordance to the Guidelines for experiments involving animals.

Evaluation of the diuretic activity

Diuretic activity was determined by the method described by Kau et al. with slight modifications.[17] In the first step of experiments, female rats were randomly assigned five groups of six each (N=6). 18 h before treatment, animals were fasted overnight, with free access to tap water only. Then, all animals received isotonic saline solution (0.9% NaCl) at an oral dose of 25ml/kg body weight (bw) to impose a uni-form water and salt load. The rest of experimentation was conducted as follows:

- Groups (1) of rats were orally administered 5 ml/kg bw of *Corrigiola telephiifolia* roots extract (CTRE) at concentration 200 mg/Kg (bw) dissolved in distilled water;
- Groups (2) of rats were orally administered 5 ml/kg bw of CTRE at concentration 400 mg/Kg (bw) dissolved in distilled water;
- Groups (3) of rats were orally administered 5 ml/kg bw of CTRE at concentration 700 mg/Kg(bw) dissolved in distilled water;
- Groups (4) of rats were orally administered 5 ml/kg bw of Hydrochlorothiazide 10 mg/kg (HCTZ 10), dissolved in distilled water, as a reference drug;
- Groups (5) of rats were orally administered 5 ml/kg bw of distilled water (negative control group)

Immediately after administration, each of these rats was individually placed in a standard metabolic cage, especially designed to separate urine and feces, and kept at a controlled temperature of 22-25°C. During this period, no food and water was available to the animals. The color of urine was also followed. Cumulative urine output was recorded at 1, 6, and 24 h after dosing, and was used to determine the urinary volumetric excretion (UVE) calculated to the following formula.[19]

Urinary volumetric excretion (UVE) = volume of urine excreted (expressed as ml/100g) / volume of fluid overloaded (25ml/kg) X100.

Diuretic index and activity

The ratio of urinary excretion in test group to urinary excretion in the control group was used as a measure of diuretic index of a given dose of a drug. However, diuretic activity was derived from the ratio of urine volume in the test group and that in the Hydrochlorothiazide groups.

Analytical procedures

At the end of the 24 h period, urine samples were collected and aliquoted into eppendorf tubes. Urinary concentrations of Electrolytes (Na^+, K^+ and Cl^- ions) were evalued using fully automated analyzer (Architect c16000, Clinical Chemistry analyzer, USA) following standard protocols. In addition pH was directly determined on fresh urine samples using a pH meter at the end of experiments. Saluretic activity (Na + Cl) and natriuretic effect (Na/K) were also calculated.

Statistical analyses

The results of the experiment was analyzed using statistical package for social science (SPSS) and Data are given as means ± SEM (standard error of mean) of different experiments. Statistical analyses were assessed using two-way analysis of variance (ANOVA) followed by Bonferroni's post- test. Significant differences were set at P values lower than 0.05.

RESULTS

Effect of CTRE on urinary excretion

The animals were observed with no signs of dehydration at 24 h intervals. The details of urine volume, urinary volumetric excretion of the oral administration of CTRE (200, 400 and 700 mg/kg bw), reference diuretic (hydrochlorothiazide) and negative control are presented in Table 1. Oral administration of the aqueous ethanol plant extracts increased the urinary flow in a dose-dependent manner. The dose of 200 mg/kg of CTRE started to increase urine volume from the first hour, but changes do not reach statistical significant values until 24h (132%, p< 0, 05).

However, rats treated with the other dose of the CTRE produced a significant increase in urine volume, compared to control, at the 1st hour (34, 56%, p<0.05 for 400 mg/kg and 36, 48%, p<0.01for 700 mg/

kg), at 6th hour (74,8%, p<0.05 for 400 mg/kg and 89,44%.<0.01% for 700 mg/kg), and at 24th hour (144,72%, p<0.01 for 400 mg/kg and 156,16%, p<0.001 for 700 mg/kg). Data showed that the reference diuretic (hydrochlorothiazide) significantly increased urine output compared to the control at all-time points (36, 4%, p<0.01 at 1h; 75, 6%, p<0.05 at 6h; and 137, 36%, p<0.05 at 24h).

Comparing the different doses of the CTRE among each other, the dose of 700 mg/kg bw of the CTRE showed significantly better diuretic activity at 1h, 6h and at 24h than CTRE 400 mg/kg bw and 200 mg/kg bw.

Diuretic index and Diuretic activity

The diuretic index for these tree doses (200; 400 and 700 mg/kg bw) was 1.45 and 1.59 and 1.71 respectively, compared to 1.50 found for hydrochlorothiazide (**Table 1**). As regards diuretic activity the values found are: 0.96 for the dose of 200 mg /kg; 1.05 for the dose of 400 mg / kg and 1.13 for the dose of 700 mg /kg (Table 1). These two parameters are calculated using the 24 h urine collected at the end of the test.

Effects on urine pH

Urinary pH was measured at 24 h (Figure 1). The pH for rats treated with CTRE was 7.26 ± 0.04; 7.57 ± 0.68 and 9.06 ±0.11 for CTRE 200; CTRE400 and CTRE700 respectively. The urinary pH of the negative control group was 6.38 ±0.41, while the standard drug increased the urine pH 7.67 ±1.02, thus making the urine more alkaline.

Effects on electrolyte excretion

Table 2 shows the results of electrolyte excretion after oral administration of CTRE. All these results showed a significant increase in the excretion of sodium, potassium and chloride in a dose dependent manner. The tested doses of CTRE which have shown a significant increase in Na^+, K^+ and Cl^- excretion in comparison to normal control rats, are 400 and 700 mg/kg bw.

Furthermore, a dose dependent increase in the Na^+/K^+ ratio was also found (Table 2).

We could juste observed that how the saluretic index of the urinary Na+, K+, Cl– excretion of CTRE700 and HCTZ 10 were very much closer to each other (1,87 ; 1,93 ; 2,67 vs. 1,72 ; 1,82 ; 2,71). HCTZ 10 and CTRE400 and CTRE700 showed potent saluretic activity as compared to normal control (Table 2).

DISCUSION AND CONCLUSION

Diuretics are drugs that increase urine production in the kidneys, promoting the removal of salt and fluid from the body. They are

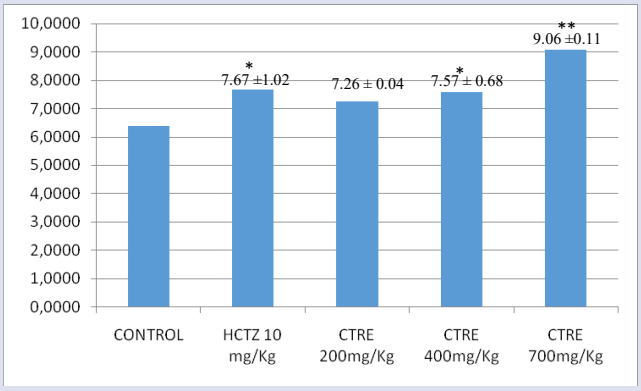

Figure 1. Effects of oral administration of CTRE on urinary pH. Values are mean ±SEM. *P < 0.05, **P< 0.001, significant against the control group.

Table 1: Effects of oral administration of CTRE and Hydrochlorothiazide on urinary excretion, diuretic index and diuretic activity.

Group	Urine volume (mL/100 g)			Urinary volumetric excretion (%)			[a]Diuretic index	[b]Diuretic activity
	1h	6h	24h	1h	6h	24h	(24 h interval)	
CONTROL	0,396 ± 0,230	0,808 ± 0,753	2,28 ± 0,252	15,84 ± 0,921	32,32 ± 0,920	91,2 ± 10,115	1	
HCTZ 10 mg/Kg	0,91 ± 0,307**	1,89 ± 0,470*	3,434 ± 0,305*	36,4 ± 12,299**	75,6 ± 12,299*	137,36 ± 12,210*	1,5	1
CTRE 200mg/Kg	0,6 ± 0,731	1,2 ± 0,214	3,3 ± 0,223*	24 ± 2,925	48 ± 2,925	132 ± 8,926*	1,45	0,96
CTRE 400mg/Kg	0,864 ± 0,141*	1,87 ± 0,656*	3,618 ± 0,629**	34,56 ± 5,639*	74,8 ± 5,639*	144,72 ± 25,181**	1,59	1,05
CTRE 700mg/Kg	0,912 ± 0,282**	2,236 ± 0,764**	3,904 ± 0,738***	36,48 ± 11,286**	89,44 ± 11,286**	156,16 ± 29,534***	1,71	1,13

The urine volumes expressed as mL/100 g were calculated based on body weight of rats and urinary volumetric excretion values are expressed as a percentage of the initial hydric overload (25 mL/kg). Values are expressed as the mean SEM. Statistical analyses were assessed using two-way analysis of variance (ANOVA) followed by Bonferroni's post- test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *versus* control.

a Diuretic index = volume of test group/volume of control group.

b Diuretic activity=urine volume of test group/urine volume of Hydrochlorothiazide group.

Table 2: Effect of aqueous ethanol extract of the root of *Corrigiola telephiifolia* on 24 h urinary electrolyte excretion in rats.

GROUP	Na^+ mmol/l	K^+ mmol/l	Cl^- mmol/l	Saluretic index[a]			Na/K	Na + Cl
				Na+	K^+	Cl^-		
CONTROL	60,2 ± 9,731	41,8 ± 8,584	42,8 ± 10,940				1,45	103
HCTZ 10 mg/Kg	103,8 ± 12,774**	76,00 ± 16,309**	116,00 ± 9,924***	1,72	1,82	2,71	1,43	219,8***
CTRE 200mg/Kg	77,4 ± 10,853	62,00 ± 9,219	58,8 ± 14,515	1,29	1,48	1,37	1,26	136,2
CTRE 400mg/Kg	92,2 ± 17,852*	72,6 ± 17,067*	94,0 ± 16,340***	1,53	1,74	2,20	1,32	186,2***
CTRE 700mg/Kg	112,8 ± 16,932***	80,8 ± 10,709**	114,2 ± 16,315***	1,87	1,93	2,67	1,41	227***

The results show the mean values and standard errors. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *versus* control group

[a]Saluretic index = mmol of electrolyte of test group / mmol of electrolyte of control group

prescribed mainly, either alone or in combination with other drugs, for: high blood pressure, kidney disease, congestive heart failure and pulmonary edema.[20,21] They are also used to treat cirrhosis of the liver and pregnancy-induced toxemia.[21] As was the case with the other medicines, diuretics also cause some side effects with different levels of severity. Examples of side effects include: electrolyte imbalance, metabolic alterations, headaches, dizziness and dehydration.[21] Currently, several molecules are used in therapeutic as diuretic agents, which differ in their mechanisms and sites of action. The scientific research is currently being continued for discovering of new molecules, with fewer side effects and more effective. The diuretic activity of a number of herbs used commonly as diuretic agents in traditional medicine has been confirmed in experimental animals.[22] In this study the diuretic effect of the orally administered aqueous ethanol root extract of *Corrigiola telephiifolia*, Moroccan species, was evaluated in normal adult female rats Wistar and compared with that produced by negative control and by Hydrochlorothiazide. Our extract was administered orally, because it is the main route of utilisation of our plant by the Moroccan population in traditional medicine. The choice of the plant is justified by its diuretic property documented in several ethnobotany surveys. According to a study led by Lakmichi and al.[7], the oral medium lethal dose value (LD50) of the hydroethanol root extract of *C. telephiifolia* is greater than 14000 mg/kg bw, which is significantly higher than the tree doses (200 mg/Kg; 400 mg/Kg and 700 mg/Kg) of CTRE, used for evaluation of a possible diuretic effect.

In order to demonstrate the diuretic activity of the extract, ie its ability to treat the edema, we have stimulated the occurrence of this state by administering, a saline solution (NaCl 0.9 %,) by oral gavage, to all the rats of the tests.[20]

The acute treatment of rats by the plant extracts showed a clear and significant diuresis, which appeared to be a function of dose and time, indicating the possibility of intrinsic and causal action, possibly receptor-mediated.[21] The comparison of the average values of the UVE obtained with the three doses tested, was made firstly with those of the negative control then with those obtained with HCTZ 10 mg/Kg, dose at which it exerts its maximum diuretic effect.[23] In the first case, all doses of the CTRE produced a better diuresis than the controls, however just the doses of 400 mg/Kg and 700 mg/Kg, were able to produce a significant increase in urine volume than the control from the first hour until the end of observation, as observed with clinically used loop diuretic.[24] Stimulation of diuretic activity of these two doses of plant extracts continued for at least 24 h after their administration to animals. This was not the case for the lowest dose, this could be due certainly to the low levels of the concentrations of the active component(s) responsible for the diuretic action.[25] By comparing the average value of UVE obtained with the reference drug and that obtained with our extract, we reveal that there is no significant difference, at all-time points, between urine output obtained in animals treated with HCTZ 10 and that obtained in animals treated with the middle and the higher doses of the CTRE, which could be indicated by the close values of the diuretic index of the HCTZ 10, CTRE 400 mg/Kg and CTRE 700 mg/Kg being 1.50; 1.59 and 1.71 respectively. We can even notice that the urinary excretion of the CTRE 700 mg/Kg was slightly greater than that induced by standard drug at all-time points, specially at 6 H (89,44% vs 75,60%) and at 24H (156,16% vs 137,36%). Thus, these observations indicate that the extract has a potential to induce diuresis markedly as those of known synthetic diuretics like HCTZ.

Diuretic activity is considered to be good if it is more than 1.50, moderate if it is 1.00-1.50, little if it is between 0.72-1.00 and nil if it less than 0.72.[26] On the basis of these data, it can be concluded, that the extract of *C. telephiifolia* at it higher dose exhibits moderate diuretic activity (1.13), however it was mild for the other doses, since their values were 0.96 and 1.05 for CTRE 200 mg/Kg and CTRE 400 mg/kg, respectively.

Diuresis has two aspects: an increase in water secretion and a net loss of electrolytes in the urine.[25] These processes may result from suppression of renal tubular reabsorption of water and electrolytes into the blood stream.[25] Therefore it is necessary, for evaluate the diuretic effect of *C. telephiifolia* roots, to measure on the one hand the urine volume, and the urinary concentrations of electrolytes on the other hand. The *C. telephiifolia* extract showed an electrolyte excretion clearly in proportion to the water excretion, in a dose dependent manner. Compared to normal control group, both the higher doses of the extract increased significantly ($P<0.05$) the urinary excretion of Na^+; cl^- and K^+. This effect may be due to the synergistic mechanism of the [$HCO3^-$ / Cl-], [$HCO3^+/H^+$] and the [Na^+/H^+] antiporter, leading to diuresis.[22,27] Moreover, the results of urinary electrolyte excretion, obtained with the three doses of our extract, were comparable to the HCTZ group. We therefore suggest that the diuretic effect of *C. telephiifolia* was again concluded to be saluretic type and similar to that produced by HCTZ,[23] rather than aquaretic type, which is a typical feature of most phytodiuretic agents.[20]

The sum of Na^+ and Cl^- urinary excretion was calculated as a parameter of saluretic activity,[22] this parameter was significantly increased by diuretic drug ($P<0.001$) and by the extract ($P<0.001$ at the highest and the middle doses tested), suggesting a similar mechanism of action in both cases.[21] This finding seems to be related to inhibition of the Na^+/Cl^- symporter (co-transporter system) in the distal convoluted tubule, by competing for the Cl^- binding site, and increasing the excretion of Na^+ and Cl^-.[28]

Concerning to K+ excretion, the lower dose of the CTRE did not produce a significant kaliuresis contrary the other doses compared to the control. In the same hand it is worth noting that there is no significant difference between the middle and higher doses of CTRE and the standard drug. In this regard, the plant extracts appeared to have active component(s) with a mechanism of kaliuresis similar to that of the standard drug (hydrochlorothiazide).[25]

With regard The Na/K ratio, it used as an indicator of natriuretic activity and resulted in values of 1.43; 1.26; 1.32 and 1.41 for HCTZ 10, CTRE 200, CTRE 400 and CTRE 700 respectively. Thus these data demonstrate that the extracts showed a greater natriuretic than kaluretic effect.[26]

In determination of urinary pH, the extracts at all the doses, showed a relative increase in pH values as compared to controls. This increase can be justified by the presence of components capable of causing metabolic changes, and consequently increasing the pH.[29]

In the light of the results of the pharmacological study, we can clearly infer that the extract of *C. telephiifolia* have a potential to induce dieresis markedly. Which correlate well with the popular use of this plant as a diuretic agent. That is why it would be interesting to fractionate the aqueous ethanol root extract of *corrigiola telephiifolia*, to determine the chemical structures of the active compounds and consequently understand the action mechanism involved in the diuretic effect of this medicinal plant. Such studies are needed to be carried out in the future.

COMPETING INTEREST

The author(s) declare that they have no competing interests.

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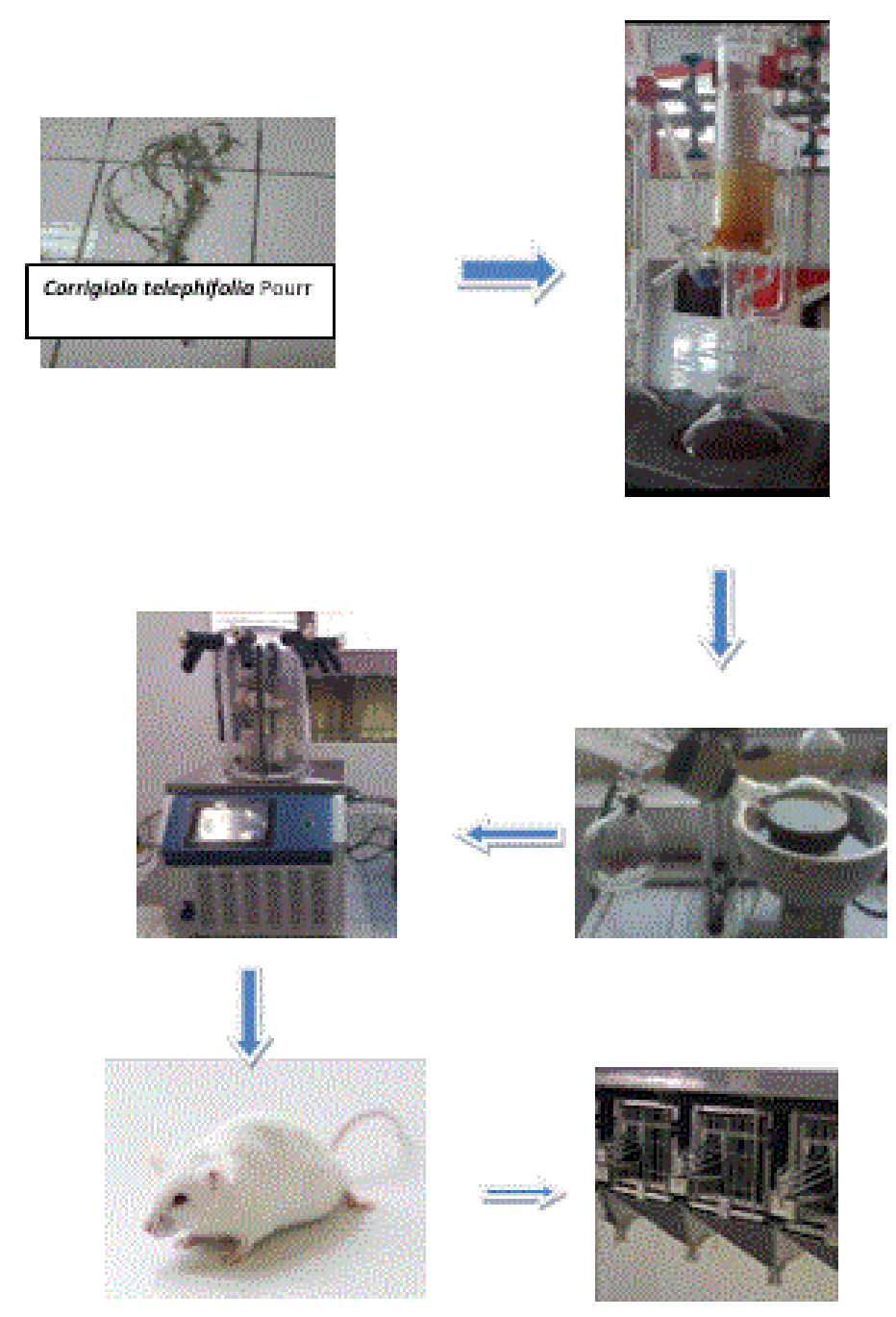
GRAPHICAL ABSTRACT



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