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EVALUATION FOR THE FIRST TIME OF THE ACUTE DIURETIC ACTIVITY OF *RUTA CHALEPENSIS L* AQUEOUS EXTRACT AERIAL PART FROM MOROCCO IN WISTAR 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. *Ruta chalepensis L*. (family: Rutaceae), commonly named Awermi and Fijel in Morocco. It is used, in the traditional medicine for the treatment of various disorders, as analgesic and antipyretic and for the treatment of rheumatism and mental disorders. Also it has emmenagogue, abortificient, antihelmintic and spasmolytic effects as well as its potency as anti-inflammatory, antihelminthic, antifungal, antifertility, anticonvulsant and sedative. In children, infused *Ruta chalepensis L* leave extract has been used for treatment of convulsion and other nervous disorders. In Africa, the aqueous decoction of the leaves is used for the treatment of fever. The purpose of this study was to examine the acute diuretic potential and effect on urinary electrolytes of the aqueous extract aerial parts of *Ruta chalepensis L* at a dose of 3.5g/kg, 4.5g/kg and 10g/kg in normal wistar male and female rats. The aqueous extract was administered intraperitoneally and the diuresis was followed in the first hour, second hour, fourth hour and 24 h. The administration of the aqueous *Ruta chalepensis L* extract produced a significant increment on diuresis from the first hour to the 24 hour. Furosemide at a dose of 20 mg/kg, adminestred intraperitoneally had a similar effect when compared to *Ruta Chalepensis L* administration suggesting a similar mechanism of action. The mechanism of action of furosemide is by inducing a loss of water through the inhibition of NaCl reabsorption. The results suggest that this receptor-mediated mechanism may account for the diuretic effect of *Ruta chalepensis L* as well. We concluded that aqueous extract aerial parts of *Ruta Chalepensis L* administered, particularly, at the dose of 10 g/kg induce significant effect on urinary output of water and electrolytes in normal wistar rats.

KEYWORDS: *Ruta Chalepensis L*; Diuretic activity; Aqueous extract; Normal wistar rat.

1. INTRODUCTION

The medicinal use of plants is an ancient tradition, much older than the contemporary sciences of medicine, pharmacology and chemistry. The World Health Organization (WHO) estimated that more than 75% of the world's populations still rely on herbal medicines, usually obtained from healers for basic medical care (Herrera et al., 2008).

Among these medicinal plants we find diuretic plants, they are very useful when there is less urination. These plants are valuable for accelerating the purification of the body, some remove chlorides and are used when there is eodema and swelling, other they have the property to lower the rate of urea in the blood. Some others, they act as a sedative for urinary tract pain and antiseptics and are therefore effective against microbes (Wright et al., 2007).

Diuretic drugs are generally used clinically in eodematous conditions, such as, congestive heart failure, liver cirrhosis, syndromesphrotic and hypertension. Of these drugs some of them come from plants (Sadki et al., 2010).

In view of the side effects of diuretic drugs such as metabolic alkalosis and hypokalemia, the use of herbal medicine is common in the traditional treatment of certain kidney diseases. Indeed, many plants have diuretic activity and used in ethnomedicine has been confirmed in experimental animals. Recent years have seen a dramatic expansion in the knowledge of the molecular mechanism of the phyto-therapeutic agents used to treat urolithiasis. The discovery and elucidation of the mechanism of action, particularly the clinical role of these herbal remedies, has made an important

contribution to a treatment for urinary stone disease as an alternative therapy or supplement.

Ruta chalepensis L. (family: Rutaceae), commonly named Awermi and Fijel in Morocco is a perennial herb (ca. 80 cm tall), Its flowers are cymes with 4-5 sepals, 4-5 petals, 8-10 stames and a superior ovary. *Ruta chalepensis L* is a perennial herb that widely distributed in the Mediterranean area (Iauk L., et al 2004) and also was introduced in America after the Spanish conquest (Zeichen R., et al 2000). It is one of the most frequently used plants for medicinal purposes (Arenas P., G.P. Savitry. 1994) (AlSaid M.S., et al 1990). Oil glands, that are principally present in leaves, give its strong deterrent odors (Cabrera A., and Zardini E. 1978) (Trease G.E. and Evans W.Ch. 1980). *Ruta chalepensis L* pharmacological properties, attributed to the high content of alkaloids (Günaydin K. and Savci S. 2005), such as furocoumarins, coumarins and furoquinolone alkaloids (HnatyszynO,. et al 1974), flavonoids, phenols, amino acids and saponins found in the leaves and stems of the plant (Di-Stasi L.C., et al 1994). *Ruta Chalepensis L* is used, in the traditional medicine for the treatment of various disorders, as analgesic and antipyretic and for the treatment of rheumatism and mental disorders (Iauk L., et al 2004). Also it has emmenagogue, abortificient, antihelmintic and spasmolytic effects (Atta A. H., et al 1998) as well as its potency as anti-inflamatory (Calzada F., et al 2006), antihelminthic (Ali-Shtayeh M.S., et al 1999), antifungal (Ulubenlen A., et al 1993), antifertility, anticonvulsant and sedative (Aguilar-Santamaria, L. and Tortoriello 1996) (Rustaiyan A., et al 2002). In children, infused *Ruta chalepensis L* leave extract has been used for treatment of convulsion and other nervous disorders. In Africa, the aqueous decoction of the leaves is used for the treatment of fever (AlSaid M.S., et al 1990). More than fifty chemical compositions of *Ruta chalepensis* essential oil were studied by many research teams in Iran (Rustaiyan A., et al 2002), Greece (Tzakou O., et al 2001), Turkey (Baser, K.H.C., et al 1996) and India (Bagchi G.D. Dwivedi P.D., et al 2003) (Mbiantcha, M., et al 2011).

The purpose of this study was to examine the acute diuretic potential and effect on urinary electrolytes of the aqueous extract aerial parts of *Ruta chalepensis L* from morocco.

2. MATERIALS AND METHODS

2.1. Preparation of plant material extracts

The plant was harvested, dried and stored in the sun at room temperature for about 6 weeks it has been identified by RAHOU Abdelilah Botanist of the department of Biology of the Faculty of Sciences of Meknès.

In order to be in accordance with the traditional form of use of the *Ruta chalepensis L* as recommended by traditional therapists, we proceeded to an aqueous decoction to obtain study extracts. 80 g of dry matter (areal parts plant blossoms and leaves) were decocted in a flask containing 2000ml of distilled water, the decoction was kept under continuous reflux for two hours, at the end of this operation, the decoction obtained after cooling was filtered through a funnel containing a cotton wool and centrifuged 2500tours/min. The solution obtained was adjusted with distilled water to a final concentration of 3.5g/kg, 4.5g/kg and 10g/kg.

2.2. Evaluation of acute toxicity

To determine the acute toxicity parameters of the aqueous extract of *Ruta chalepensis L* on white males mice divided into several batches treated with different doses of the extract. The results obtained at the end of the experiment give a Lethal Dose 100 (LD100), 30g/kg/vi body weight, a lethal dose 50 (LD50) of 19.6g/kg/vi body weight and a Maximum Tolerated Dosage (DMT) of 4.5g/Kg/vi of body weight.

The value of the Lethal Dose 50 obtained makes it possible to deduce that the total aqueous extract of *Ruta chalepensis L* is relatively harmless in Swiss albino white mice (Bellahmar et al., 2019).

2.3. Reference drug

Furosemide (LASILIX 20mg, SANOFI, Morocco), a high-ceiling loop diuretic, was used as the reference drug (positive control). It was dissolved in distilled water prior to administration.

2.4. Experimental animals

Adult wistar rats male and female weighing 210–250 g bred in the animal house of the Department of Biology, Faculty of Sciences, Meknes, Morocco, were housed under standard environmental conditions (22±2 ◦C) with a 12 h light–dark cycle. The experiments were conducted in accordance to internationally accepted standard procedure for animal use.

The rats were deprived of water but not the food for 18 h. Their urinary bladders were emptied by gentle compression of the pelvic area and by pulling their tails.

Each of these rats was then intraperitonial administered 15mL of isotonic saline solution (NaCl, 0.9%) to impose uniform water load (Ratnasooriya et al., 2004). Forty-five minutes later, the rats were divided into five groups (N= 5) and treated intraperitonally in the following manner (Table 1):

Table 1: Corresponding injected doses of aqueous extract of *Ruta chalepensis L* for the treatment of each groups.

Group	Dose
1	1 ml of distilled water;
2	1 ml of furosemide at 0.02 g / kg;
3	1 ml of extract of ruta chalepensis at 3.5 g / kg;
4	1 ml of extract of ruta chalepensis at 4.5 g / kg;
5	1 ml of extract of ruta chalepensis at10 g / kg.

Each of these rats was individually placed in a metabolic cage and cumulative urine output was recorded at 1, 2, 4, and 24 h. The color of urine was also followed.

2.5. Sample analysis and statistical calculations

The osmolarity of urine samples is measured by cryometry using an osmometer (KNAUER).

The concentration of urinary sodium is determined by flame photometry.

Each experimental value is given with its arithmetic mean ± standard error of mean (ESM).

The comparison of two means is made according to the Student's test.

2.6. Phytochemical screening

The extracts of the dry powdered leaves of *Ruta chalpensis L* were analyzed for the presence of various phytochemical constituents like carbohydrates, reducing sugars, monosaccharide, Tannins, Saponnins, Flavonoids, Terpenes/steroids (Liebermann-Burchard's Test), Alkaloids, Anthraquinones (Borntrager's test), were identified using standard phytochemical procedures (Damoda k et al., 2011) (Evans WC et al., 1996).

3. RESULTS

The administration of aqueous plant extracts increased urine output in a dose-dependent manner (Figure 1). The dose of 3.5 g / kg of *Ruta chalepensis L* resulted in no increase in urinary output. However, the dose of 4.5g / kg significantly increased diuresis at 1 and 4 h. Urine production continued to be stimulated throughout the study period, so that urinary excretion was significantly higher 24 h after treatment with a dose of 4.5 g / kg compared to controls. The most apparent and significant effect of the extract was obtained at a dose of 10 g / kg and was observed at 1, 2 and 4 h, and the maximum effect was at 24 h. Although the dose effect of the reference diuretic drug was faster and greater than that of the extracts, the 24-hour urine output was no different than the control, but a significant difference between furosemide-treated animals and those treated with aqueous extract solutions.

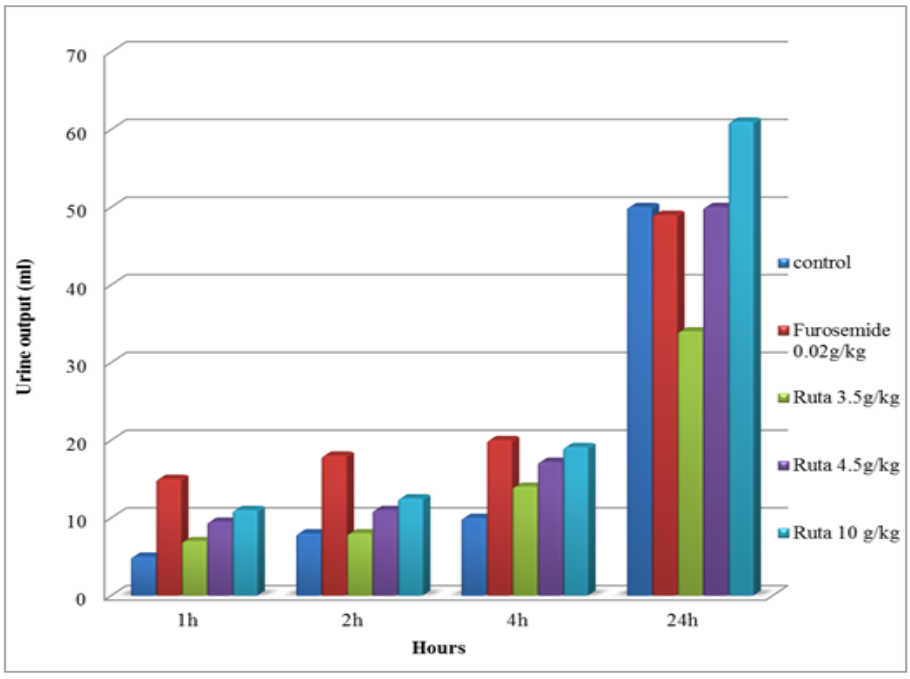

Figure 1: Time course of diuresis in rats treated with different doses of *Ruta Chalepensis L* aqueous extract aerial part.

The color of the urine samples obtained from treated rats appeared to be identical to that of the group control. The pH slightly changes to acid values (figure 2). These characteristics suggest that the plant extract acts in a similar way like furosemide.

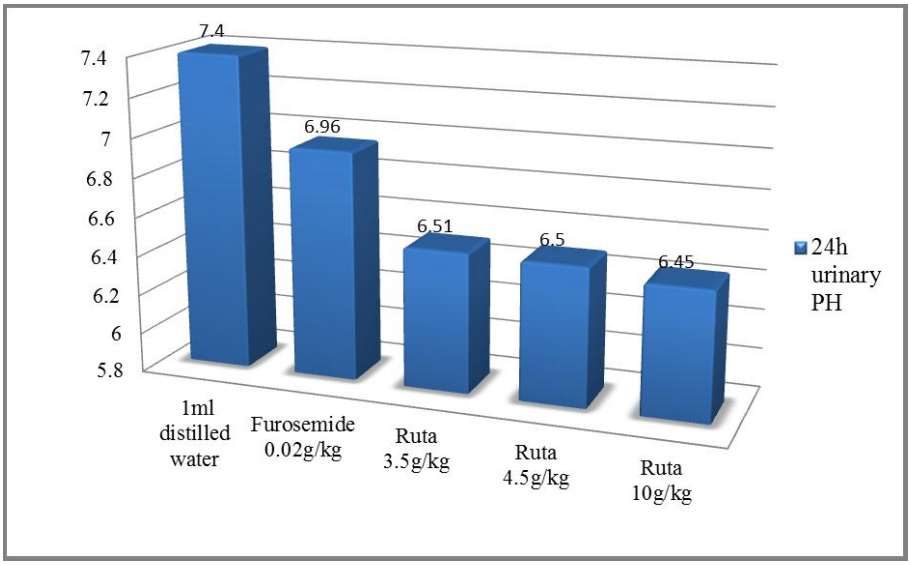

Figure 2: 24 hour urinary PH wistar rats treated with different doses of *Ruta Chalepensis L* aqueous extract aerial part.

Table 2: Effect of *Ruta Chalepensis L* aqueous extract aerial part administered intraperitoneally on urinary electrolyte excretion.

Treatment	Total Na^+ (µmoles/kg)	Total K^+ (µmoles/kg	Total Cl^- (µmoles/kg)	Na^+/K^+ ratio
Normal saline 25 ml/kg	1555 ± 14.02	704±7.34	579 ±15.01	2.20
Furosemide 20 mg/kg	3445± 4.80**	1245 ± 11.06**	2032± 15.74**	2.76
***R.Chalepensis L* 3.5g/kg**	2050 ± 4.42*	999±10.11*	2002 ±10.20*	2.05
***R.Chalepensis L* 4.5g/kg**	2070 ± 4.50*	1000±10.12*	2003±11.00*	2.07
***R.Chalepensis L* 10g/kg**	3200 ± 0.80*	1190 ± 6.23	2095± 13.20*	2.69

Values are expressed as the mean± SEM. *p < 0.001 compared to the control group, **p < 0.001 compared to Furosemide group (ANOVA followed by student test).

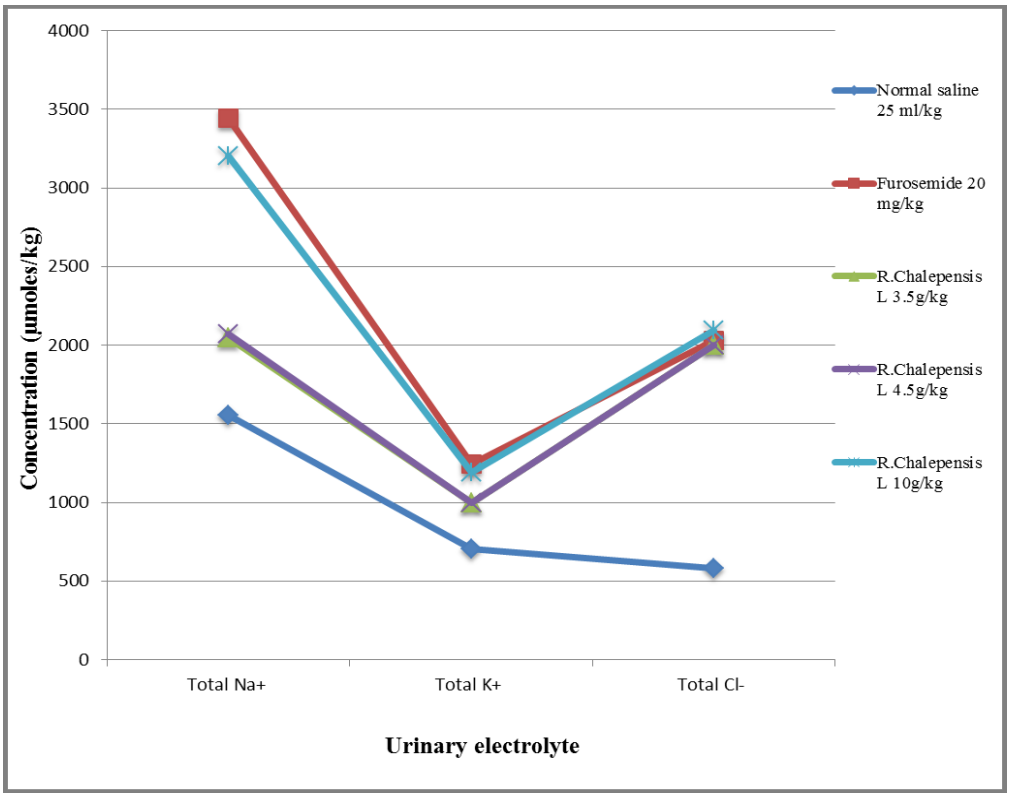

Figure 3: Effect of *Ruta Chalepensis L* aqueous extract aerial part administered intraperitoneally on urinary electrolyte Total Na^+ (µmoles/kg), Total K+ (µmoles/kg) and Total Cl^- (µmoles/kg).

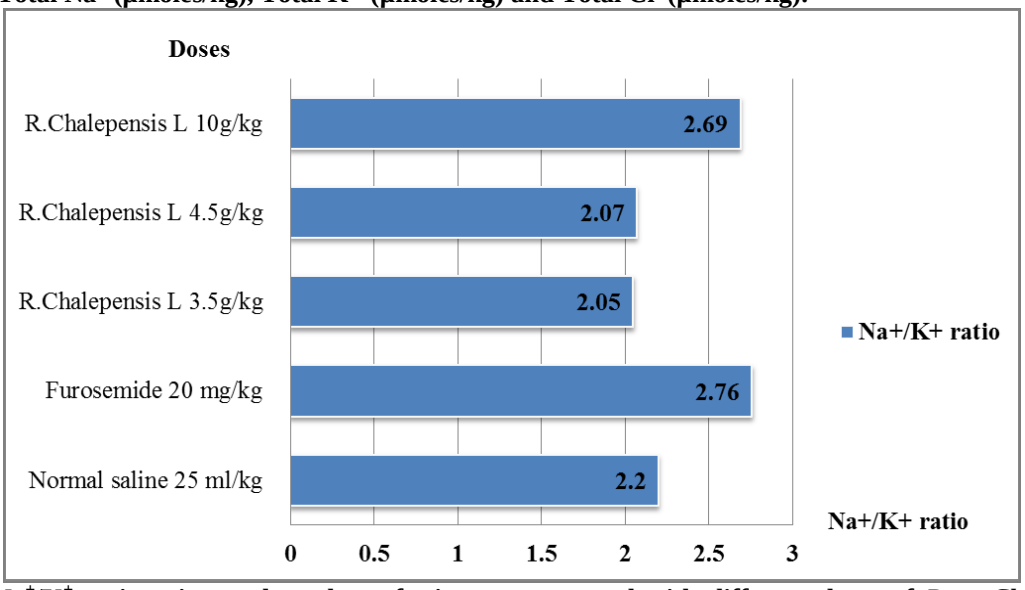

Figure 4: Na^+/K^+ ratio urinary electrolyte of wistar rats treated with different doses of *Ruta Chalepensis L* aqueous extract aerial part.

Ruta Chalepensis L aqueous extract aerial part administered intraperitoneally. Showed a significant increase in the excretion of sodium, potassium and chloride in a dose dependent manner (Table 2, Figure 3). Moreover, a dose dependent increase in the Na^+/K^+ ratio was also found ,the ratio can define the nature of the diuretic mechanism (Figure 4).

Table 3: Results of the phytochemical screening of the aerial part of *Ruta chalepensis L.*

	Compound species	**Compound species**	***Ruta chalepensis L***
Nitrogen compounds	Alcaloïdes	Alcaloïdes	+++
Phenolic compunds	Tanins	tanins catéchiques tanins galliques	++ **0**
	Flavone compounds	Anthocyanes Flavones flavanones Flavanols and flavanonols Leucoanthocyanes catéchols	**0** **0** ++ **0** **0** ++
	Anthracene derivatives	Anthracene free Anthracene combined : O-glycosides O-glycosides with reduced genome C-glycosides	**0** ++ **0** ++ +
Steroids and Terpenoids	Terpenoids :	Sterols et triterpenes Saponosides	++ **0**
	Reducing compounds	Mucilages Oses and holosides Cyanogenic compounds	+++ +++ **0**

4. DISCUSSION

Our current study examined the diuretic potential of *Ruta chalepensis L* extracts in rats. The mechanism of action by diuresis was induced by the aqueous plant extracts was also investigated and compared with a standard reference drug, furosemide (Diezi, 1992; Francine and Andrew, 1995) and control group (distilled water). The acute treatment of rats by the plant extracts drove a significant diuretic activity in a dose-dependent manner (figure 1). Diuretic activity also caused mild acidification of the urine (Osorio and Teitelbaum, 1997) (figure 2).

Our results showed also that urinary Na^+ and K^+ concentrations increased under current conditions (table 2 and figure 3).

The Na^+/K^+ ratio can define the nature of the diuretic mechanism .When Na^+/K^+ ratio is approximately 1, meaning that it eliminates the two electrolytes equally. On the other hand, with tiacids this ratio is less than one (with a greater excretion of K^+ than Na+), and with spironolactone it is greater than one (with a lower excretion of K^+ than Na^+) the case in our study (Boffil M et al., 2006) (table 2 and figure 4).

The extract of *Ruta chalepensis L* caused a significant increase in urine output from the first hour at the concentration of 4.5g/kg and 10g/kg as observed with clinically used loop diuretic (Ratnasooriya et al., 2004), while at the concentration of 3.5g/kg extract took 4 h after the administration to increase significantly urinary output.

These features suggest that the plant extract is acting in a similar way as furosemide (diuretics Henle).

The loop diuretics act at the luminal (urinary) pole of the Henle wide ascending loop cells by specifically blocking the cotransport Na^+- K^+- $2Cl^-$(Bleich M and Greger R, 1997). The inhibition of Na^+- K^+- $2Cl^-$ reabsorption causes a decrease in cytosolic concentration of Cl^- and Na^+ leading to a cellular hyperpolarization responsible for a decrease in cationic reabsorption. A considerable quantity of sodium chloride (NaCl) not reabsorbed by the cells of the loop of Henle is found in the light of the distal convoluted tube and then in that of the collecting tube whose cells will reabsorb some of it in exchange for potassium and potassium H^+ ion.

The renal effect of the extracts could result from a glomerular effect modulating the filtration rate and / or from a tubular effect where the reabsorption activity of water and electrolytes would be decreased. These actions could be directly or secondarily related to the modulation of one or more endocrine systems acting on renal function.

It has been reported by (Nilveses et al., 1989) that an increase in urine production in the rat may result from a high potassium content of plant infusion. The diuretic effect may be due to this excess potassium which acts individually or in synergy with natural products such as flavonoids, saponins and organic acids. That these effects could be due to electrolytes, present in many quantities in the plant extract, which exert their action on the kidneys (Table 3).

The theory that the majority of plant medications have a diuretic effect only because of the presence of potassium seems somewhat dubious (Ribeiro et al., 1988). In fact, pharmacodynamic studies on medicinal plants have focused on: no correlation exists between the diuretic and effects observed on the K^+ content of the extract (Jouad et al., 2001).

It is also possible that the aqueous extract exhibits cumulative effects. Effect due to the presence of one or more secondary active metabolites (Tanira et al., 1988).

This could be because the aqueous extract may contain several substances acting synergistically or antagonistically to produce a resultant effect. This hypothesis was confirmed by (Kanias et al., 1979) who demonstrated that manganese and potassium are partially transferred into prepared solutions and, therefore, the diuretic action of the drugs examined should not be attributed exclusively to the presence of potassium and chlorine content, but also to other constituents.

5. CONCLUSION AND PERSPECTIVE

The present study demonstrates the diuretic activity of the aqueous extract of *Ruta chalepensis L*, which increased urinary volume and electrolyte (sodium, potassium and chloride) excretion. The diuretic of the aqueous extract was similar to that of the reference drug (furosemide), suggesting a similar mechanism of action. Further study of *Ruta chalepensis L* is necessary in order to isolate the compounds present in this species, as well as identify which compounds are responsible for the diuretic effect shown by the aqueous extract. And for elucidate the chronic diuretic effect and Histological study.

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