

Cardiovascular studies of White Squill (*Urginea Maritima*) Extract

Kawa Dizaye *

Badr-Aldin K. Hamad**

ABSTRACT

Background and Objective: The White Squill (*Urginea maritima*) has been used as a medicinal plant through centuries over the world, believed to have certain traditional actions. The Squill bulb was used by herbalists traditionally for the treatment of cardiac failure, chronic bronchitis, rodenticides and asthma. Novel cardiac glycosides recently have been isolated from squill known as bufodienolides. In this study in vivo and in vitro pharmacological properties of extract of white squill were evaluated.

Methods: Eighteen local domestic rabbits (*Oryctolagus cuniculus*) were used for in vitro studies (effect of the plant extract on isolated pulmonary arteries and atrium) and in vivo studies (effect of the extract on renal function). While six male albino rats were used for studying the effects of the plant extract on blood pressure and heart rate.

Results: White squill extract induced a quite clear positive inotropic effect. The extract also produced significant increases in urine flow, total solute excretion, urinary Na^+ excretion rate and significant reduction in urinary K^+ excretion rate in rabbits. White squill extract produced a fall in blood pressure of the rat which was accompanied by a negative chronotropic effect.

Conclusions: The positive inotropic effect results mostly likely from blocking Na^+/K^+ -ATPase by glycoside constituent of the extract. The diuretic and natriuretic effects of the plant extract look like effects of potassium sparing diuretics. The hypotensive effect could be attributed to its diuretic property. The mechanism of bradycardia might be due to increased vagal tone, a reflex mechanism through baroreceptors.

Key words: *Urginea maritima*, positive inotropic effect, hypotensive, diuretic and natriuretic effect.

INTRODUCTION:

The number of medicinal plants has been estimated to be on the order of 40,000 to 70,000¹, which means that almost 25% of all plant species have some sort of medicinal use somewhere in the world. This heritage from our ancestors has continued to develop in Western medicine and has resulted in the isolation and production of pure active compounds (e.g. morphine, atropine and digoxin) and later in the development of novel synthetic compounds based on this knowledge (e.g. local anaesthetics based on cocaine, analgesics based on morphine).² To determine the chemical nature of such compounds, isolation of a substance in

separation techniques, chemical properties and spectral characteristics area prerequisite for establishing its correct structure. Thus, medicinal plants are used in crude or purified form in the preparation of drugs in different systems. White Squill is one of the traditionally used famous plants. It has two major species, *Urginea indica* (Indian squill) and *Urginea maritima* (white squill & Red squill) the bulb was used for the treatment of cardiac failure, chronic bronchitis, and asthma and also used as diuretic. White squill contains a number of glycosides of bufodienolide type. Most important one is scillaren A, about two third of total glycoside in quantity. It also contains

*Ass. Professor/College of Medicine, Hawler Medical University.

**College of Medicine, Hawler Medical University.

The aglycon is called as scillaridin A. The plant also contains other cardiac glycoside in small amounts like glucoscillaren A and proscillaridin A³. Other constituents found in squill include flavonoids sinistrin (a carbohydrate similar to inulin) and related carbohydrates⁴. *Urginea maritima* has been widely used by herbalists, mainly for its effect upon the heart and for its stimulating, expectorant and diuretic properties. The fresh bulb is slightly more active medicinally than the dried bulb⁵. Squill extract has been reported to exhibit peripheral vasodilatation in anesthetized rabbits⁶. It resembles digitalis in its cardiotoxic activities, but is less cumulative and acts rapidly. It is not a perfect substitute for digitalis, as it associated with irritant effect and is poorly absorbed³. It is used internally in the treatment of bronchitis, bronchial asthma, whooping cough and edema. Externally, the bulb has been used in the treatment of dandruff and seborrhea⁷. Squill has been used traditionally as a cancer remedy, and silliglaucosidin has shown activity in an experimental cancer cell line.⁸ The aim of this study was to observe some of the in vivo and in vitro pharmacological effects of a type of squill which is white squill (*Urginea maritima*).

MATERIALS AND METHODS:

Preparation of plant extract The fresh plant *Urginea maritima* was obtained from Iraqi Kurdistan region. It was identified in the department of biology (taxonomy branch) of college of education. The fresh bulb was cut into slices, dried and crushed into powder, 0.5gm of the powder was put in 50 ml solvent and left for 1 hour then filtered into a conical flask. An equivalent of 10mg dried material per ml of aqueous infusion was obtained.

Animals:

Rat In this study six male albino rats were used. Their weights ranged from 170-200 gm. They were kept in the animal house of college of Medicine/Hawler Medical

maintained at 25C°. A 12 hr light/ dark cycle was set. Rodent food rich in nutrient and tap water were supplied.

Rabbit

Eighteen male domestic rabbits (*Oryctolagus cuniculus*) were used for in vivo and in vitro studies. In the animal house the rabbits were kept in a suitable room temperature (25 C°) & were fed barley and vegetables. The weight of the rabbits used in this experiment ranged from 1-1.9 kg.

In vitro studies

Tension study

Isolated atrial muscle

The rabbits were sacrificed and the thorax was rapidly opened and the left atrium freed from ventricular and connective tissues. Preparation of left atrium was placed in a Petri dish containing aerated, freshly prepared Krebs solution (composition in g/L= NaCl 6.9, KCl 0.35, CaCl₂ 0.28, NaHCO₃ 2.1, MgSO₄.7H₂O 0.29., KH₂PO₄ 0.16 & glucose 2) .One end of the atrium is ligatured to a J shaped tube by monofilament nylon; the other end is ligatured to a fontal transducer for tension and magnification. The preparation is placed vertically in an organ bath containing Krebs solution which is aerated through the J shaped tube. The aeration with O₂ is for the sake of tissue survival and it also helps in better mixing of the added drugs and chemical substances with the Krebs solution. Contractions of left atrial preparations were obtained by square wave pulses (frequency 2-5 Hz, duration 5 ms) of twice threshold voltage (usually 5-10 V) delivered by student stimulator. The temperature of the organ bath was set and maintained at 37 C° to obtain optimal activity of the tissue. After the atrium was set in the organ bath, it was left for 30 min to equilibrate before any recordings were made. Meanwhile the physiological solution was washed and replaced every 15 minutes. The tension variations were recorded with a two channel oscilograph recorder& isometric

was recorded at a speed of 0.25mm/second. The studied drugs were added to the physiological solution in the organ bath followed by a wash whenever the effects were recorded.

Isolated pulmonary arteries

After the rabbit was sacrificed, the pulmonary arteries were excised and placed in a Petri dish containing Krebs solution. A spiral strip of 2-3cm long was set up in an organ bath containing aerated Krebs solution, and temperature set at 37C°. Contraction was recorded on a physiological recorder as previously described.

In Vivo studies

Anesthesia

The male rabbits were anesthetized by a combination of ketamine and xylazine. Both ketamine and xylazine were injected intraperitoneally together in a dose of 35mg/kg, 5mg/kg respectively. Surgical anesthesia was reached in about 15 minutes after injection¹⁰.

Collection of urine sample

The urine samples were collected by applying firm pressure on the lower abdomen and above the bladder by using the thumb and index finger. This maneuver is repeated until the bladder is emptied. Care should be taken to avoid injury to the bladder and hematuria¹¹. Na⁺ and K⁺ concentrations in urine were measured by flame photometry. Urine osmolality was measured by using semielectronic osmometer. The total solute excretion (TSE) was estimated by multiplying urine osmolality by urine flow rate.

Measurement of blood pressure and heart rate in conscious rats. Systolic blood pressure and heart rate were measured by a tail-cuff device that used a pneumatic sensor to detect the tail arterial pulse and an aneroid sphygmomanometer for measurement of the blood pressure. Animals were placed in a Plexiglas restraining cage at an ambient air temperature of 30-31C° for at least 20 minutes before each blood pressure determination. Animals were adapted to the

measurement procedure three times before the first pressure recording was made. Statistical analysis. All data are expressed as means \pm standard error of means (M \pm SEM) and statistical analysis was carried out using statistically available software (SPSS Version 11.5). Data analysis was made using one-way analysis of variables (ANOVA). Comparisons between groups were done using Duncan test and paired student t-test. P<0.05 was considered as statistically significant.

RESULTS:

In vitro studies:

Effects of white squill (*Urginea maritima*) extract on isolated pulmonary artery of rabbit: *Urginea maritima* extract caused vasoconstriction of isolated pulmonary artery of rabbit as shown in (Figure 1). Whereas, the plant extract could reverse the vasodilator activity of isosorbide dinitrate. The vasoconstrictor effect of the extract was not inhibited by doxazosin but it was blocked by amlodipine (Figure 2).

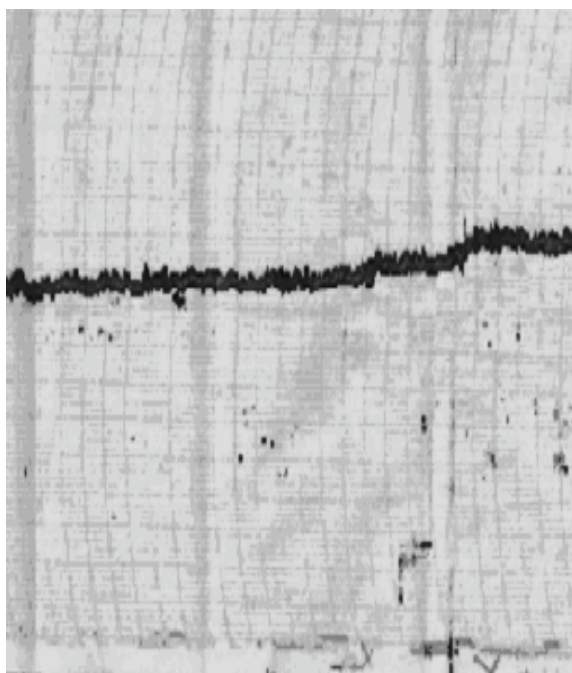


Figure 1: vasoconstrictor effect of isolated pulmonary artery caused by the extract of *Urginea maritima*.

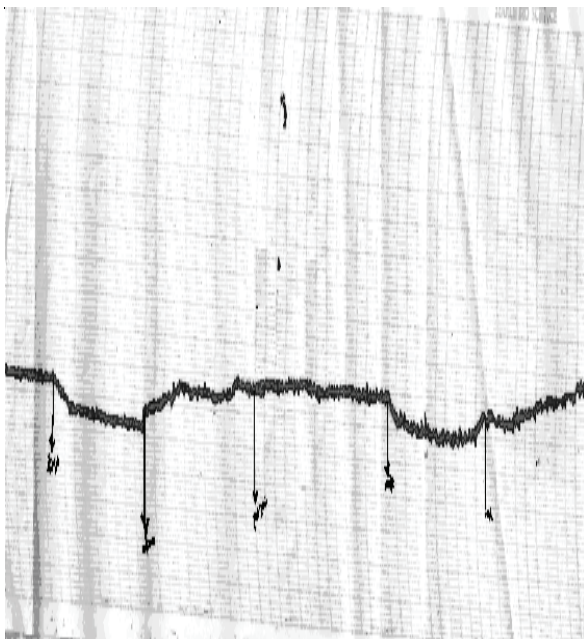


Figure 2: Effects of isosorbide dinitrate, *Urginea maritima* extract, doxazocin and amlodipine on isolated pulmonary artery of the rabbit.

Effects of *Urginea maritima* extract on isolated atrium of rabbit: The extract of *Urginea maritima* produced contraction of isolated atrium of rabbit as shown in (Figure 3)

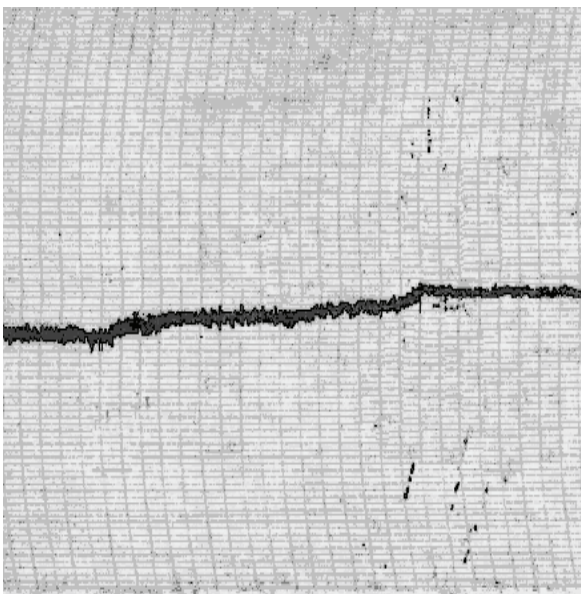


Figure 3: Cardiotoxic activity of white squill (*Urginea maritima*) extract on isolated atrium of rabbit.

Verapamil produced negative inotropic effect on isolated atrium which was antagonized by adding *Urginea maritima* extract. The extract showed positive inotropic activity which was not reversed by timolol (Figure 4).

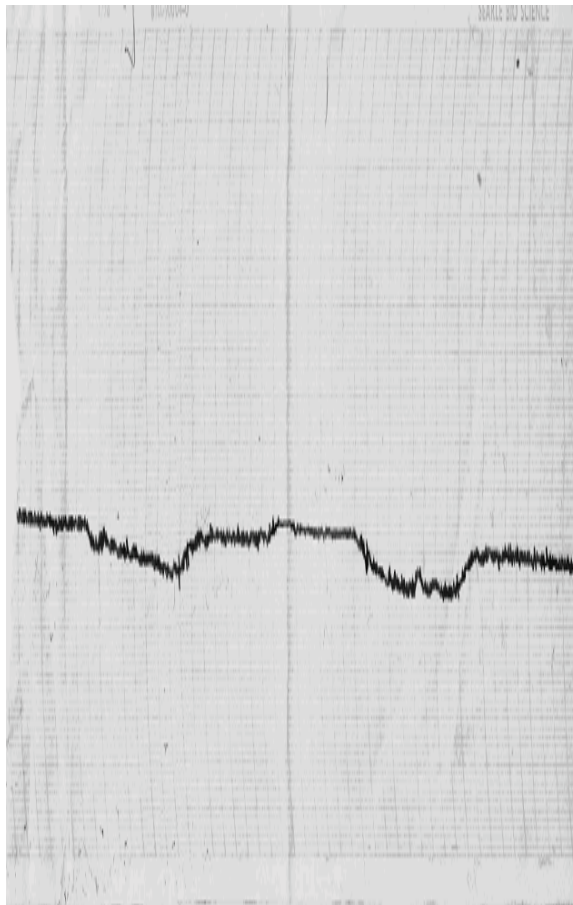


Figure 4: Effects of verapamil, *Urginea maritima* extract, and timolol on isolated atrium of the rabbit.

In vivo studies:

Effect of white squill (*Urginea maritima*) on renal function of rabbit:

The Intravenous injection of 10mg of *Urginea maritima* extract produced significant increases in urine volume, sodium excretion and total solute excretion, accompanied by a significant decrease in urinary K^+ excretion. Meanwhile, the injection of this dose of the plant extract caused no significant changes in osmolality as shown in (Table1).

Table 1: |The effects of intravenous injection of *Urginea maritime* Extract on the renal function of rabbit; n=6.

Parameters	Control	T1	T2	T3
Urine flow rate ml/hr/Kg	5.76±1 a	25.06±2.44 b	20.87±2.65 b	11.41±2.77 a
Na ⁺ Exc. Rate μEq/hr/Kg	134.28±19.24 a	391.58±100.9 b	466.1±122.2 b	252.35±100 ab
K ⁺ Exc. Rate μEq/hr/Kg	38.37±13.1 a	23.7±8.21 ab	10.66±3.06 b	9.53±2.16 b
Osmolality mOsmol/l	481.5±86.09 a	341.29±51.35 a	402.66±68 a	368.2±73.9 a
TSE mOsmol/hr/kg	2.571±0.428 a	6.00±0.902 b	8.294±1.499 b	3.827±0.911 a

The different letters mean there is a significant difference at P> 0.05

T1=measurements after 30 minutes, T2= measurements after 1 hour, T3= measurements after 1:30 hour.

Comparative effects of *Urginea maritime* extract and frusemide produced different effects on renal function of rabbit. Intravenous injection of *Urginea maritime* extract and frusemide on renal function of rabbit as shown in (Table 2).

Table 2: The comparative effects of intravenous injection of frusemide and *Urginea maritime* extract on the renal function of rabbit; n=6.

parameters	Control/plant	Control/frusemide	T1 /plant after 30 min.	T1/frusemide after 30 min
Urine flow rate ml/hr/Kg	5.76±1 a	8.4±1.86 a	25.06±2.44 b	26.76±4.61 b
Na ⁺ Exc. Rate μEq/hr/Kg	134.28±19.24 a	155±50.78 a	391.58±100.9 b	942.03±194.12 c
K ⁺ Exc. Rate μEq/hr/Kg	38.37±13.1 a	11.48±4.87 a	23.7±8.21 b	46.55±14.24 c
Osmolality mOsmol/l	481.5±86.09 a	405.28±84.49 a	341.29±51.3 a	272.48±25.17 a
TSE.mOsmol/hr/kg	2.570.7±0.428 a	3.236±1.013 a	6.00±0.902 b	6.971±0.802 b

T1=Treatment, The different letters mean there is a significant difference at P> 0.05

Table 3: the effect of subcutaneous injection of *Urginea maritima* extract on blood pressure and heart rate of rat, (n=6)

Parameters	Control	T1	T2
Blood Pressure mmHg	119± 1.65 ^a	101± 3.1 ^b	117± 0.96 ^a
Heart Rate Beat/min	367±3.85 ^a	327±2.99 ^b	349±2.39 ^a

T1=measurements after 30 minutes, T2=measurements after 1 hour

The different letters mean there is a significant difference at P< 0.05

DISCUSSION:

In vitro studies

Effect of *Urginea maritima* extract on isolated pulmonary artery of rabbit. *Urginea maritima* extract has vasoconstrictor activity and could reverse the vasodilator effect of isosorbide dinitrate in isolated pulmonary artery of rabbit. The vasoconstrictor effect of the extract was not inhibited by doxazocin (alpha -1 antagonist). This demonstrated that, the plant extract has no alpha -1 adrenoceptor agonist activity. Amlodipine reversed the vasotonic activity induced by the plant extract. The mechanism of vasoconstrictor effect of the extract could be attributed to increased calcium permeability of smooth muscle cell membrane of the blood vessel. Increased intracellular calcium is caused by inhibition of the membrane Na⁺ / Ca⁺² exchange system which is resulted from intracellular accumulation of Na⁺. These actions are related to inhibition of Na⁺/ K⁺-ATPase which might be induced by bufodienolide (cardiac glycoside) present in the plant extract.^{12, 13, 14} In addition, another suggested mechanism, blockade of Na⁺-K⁺-ATPase by the cardiac glycoside (bufodienolide) is known to inhibit the

nitric oxide whilst stimulating the release of the potent vasoconstrictor endothelin¹⁵. Our finding was in contrast to reports of many researchers who showed that *Urginea maritima* extracts caused peripheral vasodilatation in anesthetized rabbits^{16, 17}. This phenomenon was demonstrable for an in vivo study. This could be explained as that the plant caused direct vasoconstriction of peripheral blood vessels caused by the glycoside (bufodienolide) of the plant extract. Then the reflex withdrawal of the sympathetic tone to blood vessels due to the improved heart function was the overriding effect, so the net result was vasodilatation¹⁸. The reflex mechanism is explained as follow: when cardiac output increased by the plant glycoside, blood flow increases and exerts a force on the inner surface of the arterial wall (the endothelial cells) termed shear stress. In response to this shear stress, arterial endothelium releases PGI₂ which in turn increases amounts of nitric oxide resulting in vasodilatation¹⁹. Effect of *Urginea maritima* extract on isolated atrium of rabbit. From our results we found that white squill has quite clear positive inotropic effect (Figures 3 and 4). This effect is not due to activation of Beta -1 adrenoceptor because the effect was not blocked by timolol. This property is not resulted from the increase in the intracellular level of cAMP or inhibition of phosphodiesterase enzyme as the plant extract had negative chronotropic effect in rat (Table 4). The cardiotoxic activity of the plant extract was antagonized by verapamil. The increase in the contractile force of the heart by *Urginea maritima* is most probably resulted from inhibiting the enzyme Na⁺/ K⁺ ATPase by the cardiac glycoside (bufodienolide) which is responsible for the active extrusion of intercellular Na⁺ in exchange for extracellular K⁺ and finally intracellular calcium level is increased. This explanation is supported by reports of many research workers who showed that cardiac

pump.^{20, 21, 22}

In vivo studies

The effects of intravenous injection of *Urginea maritima* aqueous extract on the renal function of rabbit. Results of this study showed that intravenous infusion of 10mg of *Urginea maritima* extract induced significant increase in urine flow, total solute excretion and urinary sodium excretion rate which were accompanied by insignificant increase in osmolality and significant reduction in urinary potassium excretion rate. The results were more obvious after 30 minute which indicates that the plant extract has rapid and short duration of action with less cumulative effect³. These diuretic and natriuretic properties may be due to more polarity of the diuretic active ingredient of plant extract. Frusemide caused profound increase in urine flow, urinary sodium and potassium excretion rate. The extract and frusemide differ significantly in their natriuretic and kaliuretic effects. The renal effect of plant extract doesn't resemble the renal effect of the loop diuretic which is acting on Na⁺, K⁺,Cl⁻ transport system in the ascending limb of the loop of Henle, as, first the plant natriuretic effect was lower than that of frusemide and second, the plant extract decreased K⁺ excretion whereas frusemide increased it. The K⁺ retaining effect of the plant extract also indicates that the extract doesn't resemble thiazide diuretics which are acting on early distal convoluted tubule through inhibition of Na⁺, Cl⁻ symporter.^{23, 24}

The diuretic and natriuretic effects of the plant extract look like potassium sparing diuretics which are acting on late distal convoluted tubule and collecting duct. The most likely mechanism might be the inhibition of Na⁺/K⁺ ATPase activity which is enhanced by aldosterone, that's to say it may act similarly as the aldosterone antagonist (spironolactone). The most possible & reasonable explanation for this property is that the glycoside (bufodienolide) of *Urginea maritima* is Na⁺/K⁺ pump antagonist, furthermore

analogue.^{25, 26} Effects of *Urginea maritima* extract on systemic blood pressure and heart rate of the rat: The subcutaneous injection of 10mg of *Urginea maritima* extract produced a significant drop in systolic blood pressure and a decrease in heart rate of the experimental rats. The falls in blood pressure and heart rate were more obvious and significant after half hour while they were insignificant after 1 hour. This again supports the idea that the active constituent of plant extract has less cumulative and short duration of action³. As it was seen previously, this plant extract has vasoconstrictor activity. Therefore, the most likely mechanism of the hypotensive effect of the plant extract is through its diuretic effect. Beside the hypotensive effect of the plant extract, there was a reduction in heart rate. The mechanism of bradycardia might be due to increased vagal tone, a reflex mechanism through

CONCLUSIONS:

baroreceptors, and direct action on SA and AV nodes.^{27, 28}

1-*Urginea maritima* extract has a quite clear positive inotropic effect. This may result from blocking Na⁺/K⁺-ATPase by glycoside constituent (bufodienolide) of the extract.

2-The extract of the plant produced a significant increase in urine flow, total solute excretion, urinary Na⁺ excretion rate and a significant reduction in urinary K⁺ excretion rate in rabbits. These effects could be most likely explained by aldosterone antagonist activity on Na⁺/K⁺ pump. This property could be attributed to the inhibitory effect of glycoside (bufodienolide) of plant extract on Na⁺/K⁺ ATPase pump.

3-The plant extract produced a fall in blood pressure of the rat which was accompanied by negative chronotropic effect.

4-The hypotensive effect could be

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explained by diuretic property of the plant

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