Toxicological Studies on the Sinai Plant *Urginea maritima* (Scilla) in Wistar Albino Rats

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Abstract

The varied toxicity of *Urginea maritima* (a plant that grows in north Sinai) on Wistar albino rats was investigated. Twenty-four rats weighing 100±20 gm. were divided into 4 groups, the first group, which included six male rats and the second group, which included six female rats, were given di-methyl sulfoxide orally for 45 consecutive days, and were regarded as the control groups. The third group included six male rats who received 36 mg/kg B.W (1/20 LD_{50} from the alcoholic extract of *Urginea maritima*) for 45 days in succession. The fourth group was made up of six female rats who received a daily dose of 26 mg/kg B.W (1/20 LD_{50} of the alcoholic *Urginea maritima* extract) for 45 successive days. Serum samples were taken for biochemical study after the end of the experiment, and sciatic nerve, heart, and brain tissues were also taken for histopathological analysis. The findings demonstrated significant changes in the exposed animals' serum levels of calcium (Ca^{++}), sodium (Na^{+}), and potassium (K^{+}) ions. While serum K^{+} levels dramatically increased, serum Ca^{++} and Na^{+} levels significantly dropped. Lactic acid dehydrogenase (LDH) and serum creatine kinase (CK-MB) activities also significantly increased. In addition, pathological alterations in the heart, brain, and sciatic nerve were discovered when compared to control groups.

Keywords: *Urginea maritima*, CK-MB, LDH, Ions, LD_{50}, Cardiotoxicity

Introduction:

Herbal medicine is one of the earliest kinds of healthcare that humans have ever used. Throughout history, herbs and plants have been employed by all societies. It was a

critical stage in the growth of contemporary civilization. The ape saw and admired the wide variety of plants in the area. Plants provide us with medicine, food, clothes, and shelter.

Through experimentation and observation of wild animals, medicinal plant uses have been established ***(Ijaz et al., 2017)***. Since ancient times, different portions of medicinal plants have been used to treat various diseases; however, some of these plants are poisonous to both humans and animals. It is crucial to be aware of dangerous plants because they might work as powerful medicinal agents when administered in a precise, directed dosage ***(El-Seedi et al., 2013)***.

Egypt has large expanses of desert and tropical environments, which creates favorable habitat for the growth of wild plants tolerant of the harsh circumstances there. One of the main locations for medicinal plants is Sinai, which is a region of Egypt, particularly in north Sinai. Typically, it sustains 600–700 plant species ***(Kamel et al., 2008)*.** Of the 700 plant species, 229 species are found in the Mediterranean coastal region ***(Gibali, 1988)***, 250 species are located in anticlines ***(Gazar et al., 2000)***, and 119 species are found in the interior region ***(Gibali, 2000)***.

Numerous elements, including topography, soil salinity, and physical characteristics, regulate the distribution of medicinal plants in north Sinai. The primary threats to north Sinai's medicinal plants are ongoing urbanization, land reclamation initiatives, and quarrying ***(Moustafa et al., 2001)***. The most widely utilized species in Asia, Europe, and Egypt is *Urginea maritima* Fig. (1).

The Ebers papyrus, which dates to 1500 BC, has the earliest information that is now known about this plant in Egypt. Ancient scholars including Hippocrates, Theophrastus, Dioscorides, Pliny, and Pseudo-Apuleius all took it into consideration. Dioscorides claimed that this plant may be processed in various ways for various purposes. Iranian traditional healers have acknowledged Greek material, but there were very tiny variations in the prescriptions and ways of processing ***(Bozorgi et al., 2017)***. According to reports, the primary phytochemical components of *Urginea maritima* bulbs are bufadienolide-type glycosides (cardiac glycosides) ***(Mulholland et al., 2013)***.

Additionally, this plant contained phytosterols, phenolic compounds, and other phytochemical components ***(Kameshwari, 2013)***. The glycosides Scillaren-A and Scillaren-B are also present in fresh bulbs of *Urginea maritima*. The primary components of *Urginea maritima* bulbs are glycosides, which are often solid, crystalline, colorless, or pigmented compounds with a bitter flavor; some have a distinctive taste.

There is optical activity in glycosides. Most are insoluble in ether and soluble in water, alcohol, and acetone. They are effortlessly hydrolyzed by enzyme activity, acid, or base. Due to their physiological effects on living organisms—either beneficial or harmful—many glycosides are utilized as drugs or poisons ***(Bălăşoiu et al., 2013)***. Cardiac glycosides are steroidal substances having a C-24 or C-23 ring that have chronotropic and inotropic properties.

The primary component of cardiac glycosides is a steroidal nucleus, which cell receptors have been shown to identify. All of these elements are different in the lactone moiety at the steroidal nucleus' C-17 location. These compounds are categorized as cardenolides and bufadienolides, respectively, based on a lactone ring that contains five or six carbon atoms ***(Vaklavas et al., 2011)***.

From this plant, numerous researchers discovered chemicals called bufadienolides. The majority of researchers employ nuclear magnetic resonance spectroscopy (NMRS) to identify bufadienolides; however, other studies used high-performance liquid chromatography (HPLC) to identify these molecules Fig. (2).

The Sugar moiety has an impact on the pharmaco-kinetic and pharmaco-dynamic properties of these drugs ***(Bozorgi et al., 2017)***. When handled, the lengthy, acicular crystal bundles of calcium oxalate that are also present in the bulbs easily enter the skin and produce significant discomfort. It also contains many flavonoids, including as kaempferol and quercetin ***(Kameshwari, 2013)***.

The extract of *Urginea maritima* did not exhibit any discernible antiviral activity, although one of the active components, scillarenin, possesses antimicrobial activities ***(Soltan and Zaki, 2009)***. According to ***(Mammadov et al., 2010)***, the maximum antioxidant and free radical-scavenging capacity, is found in the ethanolic and methanolic extracts of *Urginea maritima* bulbs and leaves respectively Fig. (3).

According to ***Mulholland et al. (2013)*** and ***Kayiran and Özkan (2017)***, *Urginea maritima* has been used in medicine for many centuries due to its potent, digitalis-like cardiac impact. It is also used as a diuretic and to treat illnesses of the bladder and ureter. In cases of cancer, amenorrhea, dysmenorrhea, itching, ascites, chronic rhinitis, chronic cough, cardiac problems, leprosy, chronic pulmonary diseases, and skin conditions, it has anti-inflammatory properties.

According to ***Kameshwari (2013)***, all secretory organs are stimulated by *Urginea* species in a small dosage, which also soothes irritated mucous surfaces and controls overproduction of secretions. It alleviates dropsies that have cardiac origins. When there is no

inflammation, it can be applied in every situation. When there is dry skin, a parched tongue, fevered lips, and facial contractions, it works better. When the heart's activity and pulse are both weak and fast, it is used to treat cardiac dropsy.

L-azatidine-2-carboxylic acid, bufadienolides, scillirosidin, and proscillaridin A were among the active substances with insecticidal properties that ***Bozorgi et al., (2017)*** reported have been identified from Urginea species. Compared to ovabin and digoxin, the anti-proliferative properties of proscillaridin A from *Urginea maritima* are extremely powerful against human breast cancer cells ***(Elghuol et al., 2012)***.

Proscillaridin was one of 1120 substances evaluated against human multiple myeloma cell lines. It blocked more than 90% of various cytokines released by these cells and reduced cell survival by more than 50% when compared to other cardiac glycosides with the same ability ***(Feng et al., 2010)***.

The inhibitory effects of *Urginea maritima* extracts on mushroom tyrosinase were studied by ***Namjoyan et al., 2016***. They discovered that the extract of *Urginea maritima* exhibits anti-tyrosinase action. This finding suggests that *Urginea maritima* may be used to prevent or cure pigmentation disorders. Tyrosinase inhibitors have been utilized more and more in pharmaceutical and cosmetic products since it is necessary for the production of melanin from tyrosine. Since 1920, it has been widely recognized that *Urginea maritima* is an effective and particular rodenticide.

Spanish fishermen have traditionally employed the bulb as an ichthyotoxic ***(Mulholland et al., 2013)***. The red type of *Urginea maritima* was frequently employed for derivatization because it had a higher concentration of deadly components than the white kind ***(Bozorgi et al., 2017)***. All parts of the plant, including the leaves, flowers, stalks, scales, and primarily the roots and bulb core, contain scilliroside, the primary dangerous substance.

The oral LD_{50} of *Urginea* species varies between 400 and 3000 mg/kg B.W in different animals as a result of the great diversity in their toxicological profiles ***(Bayazi and Konar, 2010; Bozorgi, et al., 2017)***. The effects of intravenous injection of *Urginea maritima* extract on rabbit renal function were examined by ***Dizaye and Hamed, 2010***. The findings showed that an intravenous infusion of 10 mg. of *Urginea maritima* extracts significantly increased urine volume, total solute excretion, and urinary sodium excretion rate. Osmolarity increased insignificantly and urinary potassium excretion rate significantly decreased as a result. The findings became more obvious after 30 minutes, demonstrating that the plant extract acts quickly and briefly with no cumulative effect.

The fact that the active component in plant extract is more polar may be the cause of these diuretic and natriuretic actions.

Systolic blood pressure and heart rate both significantly decreased after receiving a subcutaneous injection of 10 mg. of *Urginea maritima* extract. Like digitalis, *Urginea maritima* possesses cardiotonic qualities. However, its constituents are less powerful than digitalis and poorly absorbed from the digestive tract. In order to stop gastric acid from degrading it, its medical oral preparations are enteric-coated.

The current study was therefore created to investigate the cardiotoxicity of *Urginea maritima*, one of the poisonous and therapeutic plants, through the oral administration of its alcoholic extract to male and female Wistar albino rats for 45 repeated days. The measurement of the serum activities of LDH, CK-MB, total and ionized Ca^{++}, Na^{+}, and K^{+} ions has been successful in achieving this goal. For the purpose of evaluating the histo-pathologic impact, the heart, brain, and sciatic nerve underwent histo-pathological investigation.

Materials and methods:

Materials

The investigated plant *Urginea maritima*

Urginea maritima was collected from El Arish, north Sinai, Egypt in September 2016.

Chemicals

Reagents for testing blood total and ionized calcium Ca^{++}, Na^{+}, and K^{+} levels with ready-made kits acquired from Biodiagnostic Co. and for quantitatively determining serum CK-MB and LDH activities. From ALDRICH Company, DEMSO 5% were bought. We bought ethanol 95% from Biodiagnostic Co.

Animals

For this study, a total of 48 healthy Wistar albino rats were employed. The Laboratory Animal Resource Center Faculty of Veterinary Medicine at Suez Canal University, Ismailia, Egypt, provided 24 males and 24 females, each weighing about 100±20 gm. In order to determine the median lethal dosage (LD_{50}), 24 rats were employed, and 24 more were used in the other toxicological studies. Prior to the experiment, they were held for two weeks to allow for acclimatization. The animals were housed in stainless steel cages with free access to food and water at a constant temperature of 27 °C±5, along with good ventilation and a typical diet of 72% corn, 27% soy beans, and 1% fish meal. The Suez Canal University Faculty of Veterinary Medicine's animal ethical committee gave its approval to the study's plan.

Methods:

Preparation of plant extract

Small portions of plant samples (bulbs) were chopped up and dried in a hot air oven at 40 °C for an

entire night. The dried material (50-100 gm.) was crushed to a coarse powder and extracted with ethanol 95% with sporadic stirring at room temperature over four days, three times consecutively. The extract was filtered before it was evaporated at 40 °C in a hot air oven and dissolved in DEMSO 5%. We subsequently used 1/20 of the acute oral LD_{50} for our toxicological research after determining the acute oral LD_{50} in both male and female rats.

Determination of acute oral (LD_{50})

Animal grouping in the determination of acute oral LD_{50} in both male and female rats

To test the LD_{50} in both sexes, twenty-four rats (12 males and 12 females) weighing 100±20 gm. were employed. A substance's fatal dose is the dosage that, when administered to rats, will result in the death of 50% of the rats. An indicator of a poison's strength is its LD_{50} value (the lower the LD_{50} dose, the more toxic the substance). This experiment used graded concentrations starting at 40 mg. of *Urginea maritima* extract/kg B.W to calculate the LD_{50} of the alcoholic extract. The multiplying factor changed the dose for the second rat either up or down is (1.3). In another word, the dose for each successive animal was adjusted up or down depending on the previous outcome. The oral LD_{50} in both males and females was then calculated according to ***Bruce, (1985)***.

Animal grouping in the toxicological investigation

Twenty-four animals were divided into four groups:

- The first and second groups of 6 males and 6 females respectively received (DEMSO 5%) orally and kept as control.
- The third group of 6 males was given orally 1/20 LD_{50} of the alcoholic extract daily for 45 consecutive days (the dilution of 1/20 according to pilot investigation).
- The fourth group of 6 females was given orally 1/20 LD_{50} of the alcoholic extract daily for 45 consecutive days (the dilution of 1/20 according to pilot investigation).

Serum and tissue sample collection

Rats were put to death under anesthetics on the 46^{th} day of the experiment, and blood was collected in standard polystyrene microtubes for biochemical evaluation. At room temperature, blood samples were allowed to coagulate before being centrifuged at 1,000 x g for 10 minutes; at 4 ºC, serum was then collected and kept for analysis. Samples of the brain, heart, and sciatic nerve were quickly obtained, washed in 0.9% sodium chloride in distilled water, and then put in formalin-filled tubes for histological analysis.

Biochemical analysis of serum

The serum was analyzed for LDH, CK-MB activities, and total, and ionized calcium Ca^{++}, Na^{+}, and K^{+} ions levels.

Measurement of serum CK-MB:

CK-MB, also known as creatine phosphokinase, is an enzyme expressed in skeletal muscle, smooth muscle, and the brain. The CK-MB enzyme was measured spectrophotometrically according to ***Steen et al., (2010).***

Estimation of serum LDH

LDH is an oxide- reductase, which catalyzes the interconversion of lactate and pyruvate. This enzyme was measured in serum spectrophotometrically according to ***Mittal et al., (2009)***.

Estimation of serum total calcium

Serum total calcium (Ca^{++}) is determined by the direct calorimetric complexometric test (Arsenazo III) according to the method described by ***Bauer, (1981).***

Estimation of serum ionized calcium (Ca^{++})

The Ca^{++} ion concentration was determined by the chromogenic complex formed between calcium ions and o - cresolphthalein, which was measured at 575 nm and was proportional to the concentration of calcium ions present. The linear range of detection for this kit is between 0.4–2.0 mg **(*Caprita et al., 2013).***

Estimation of sodium (Na^{+}) and potassium (K^{+})

Serum Na^{+} and K^{+} determination was done by emission flame photometry after suitable dilutions described by ***Cooper, (1963)***.

Histopathological examination:

The obtained heart, brain, and sciatic nerve samples were fixed in 10% formalin, dehydrated in progressively stronger alcohol, cleaned in xylol, and then embedded in paraffin. Sections of 5-7 were made in the paraffin blocks. For histological analysis, the acquired slices were stained with hematoxylin and eosin ***(Bancroft and Gamble, 2008)***.

The Tucsen® ISH1000 digital microscope camera, which has a resolution of 10 MP (megapixels), was used to calibrate a regular digital microscope camera to acquire all of the images (3656 x 2740 pixels each image). For image improvement and capture, "IS Capture" software was utilized. The UIS optical system (Universal Infinity System, Olympus®, Japan) was used to collect all images at a 400x magnification (objective lens, 40x).

Statistical analysis

Preliminary data obtained in this study were statistically analyzed using SPSS program version 17.0 (SPSS Inc., Chicago, Illinois) according to ***Steel and Torrie, (1981)***. Multiple comparisons were carried out using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test for post hoc analysis. Data were expressed as means ± SE. The probability level is 5% (a P value of ≤ 0.05 was considered to judge the statistical significance).

Results:

Acute oral LD_{50}:

The oral LD_{50} of *Urginea maritima* alcoholic extract was found to be 716.14 and 517.61 mg/kg B.W in male and female rats respectively as shown in Fig. (4, 5).

Results of the toxicological investigation:

The effect of daily oral administration of 1/20 LD_{50} of alcoholic extract of *Urginea maritima* to male and female albino rats for 45 successive days on serum enzymatic activities (LDH and CK-MB), serum electrolytes levels (total and ionized Ca^{++}, Na^{+}, K^{+}) were recorded. In addition, heart, brain, and sciatic, histopathological investigations were illustrated.

Effect on serum enzymatic activity:

Effect on serum (LDH and CK-MB) activities:

The serum activities of LDH and CK-MB enzymes of control and treated rats were determined and statistically analyzed. Their mean values with their standard errors are demonstrated in Fig. (6).

Effect on serum electrolytes:

(Total and ionized Ca^{++}, Na^{+}, k^{+}) of male and female Wistar albino rats:

The serum level of ionized and total and ionized Ca^{++}, Na^{+} and k^{+} of control and treated rats were determined and statistically analyzed. Their mean values with their standard errors were demonstrated in Fig. (7).

Histopathological finding:

Histopathological finding of heart:

The muscle fibers in the first and second groups' myocardium (control males and female groups got DEMSO 5%) were arranged normally into fascicles made of myocytes, and their myofibril-rich eosinophilic cytoplasm was in good supply. The nuclei were vesicular and oval. Thin-walled capillaries and sparse, loose connective tissue were visible in the interstitial tissue Fig. (8).

The heart of the third and fourth groups (treated males and females with 1/20 LD_{50}) (716.14 and 517.61 mg/kg, respectively) of *Urginea maritima* alcoholic extract daily for 45 days showed degenerated myocytes with the disappearance of fascicular arrangement, degenerated myofibrils, lost striations, pyknotic nuclei with shrinkage and condensation of chromatin, congested thick-walled stromal vessels with areas of marked edema Fig. (9).

Histopathological finding of the brain:

Normal neurons with stellate cytoplasmic processes and basophilic cell bodies were found in the brains of the first and second groups (control males and females received DEMSO 5%), as well as a scattering of tiny glial cells and thin-walled capillaries Fig. (10).

The third and fourth groups' brains (treated males and females with 1/20 LD_{50}) (716.14 and 517.61 mg/kg, respectively) revealed

vacuolar degeneration of neurons with minor cell body shrinkage, dilated and congested arteries, and significant edema in the background Fig. (11).

Histopathological finding of the sciatic nerve:

The first and second groups' sciatic nerves (control males and female groups received DEMSO 5%) displayed a typical, regular distribution of axons into fascicles and dispersed spindle-wavy Schwann cells. The lipid-rich myelin heath that wrapped the axons had a dot in the center and appeared to be a gap. Vessels with thin walls could be seen here and there Fig. (12).

The sciatic nerve of the third and fourth groups (treated males and females with 1/20 LD_{50}) (716.14 and 517.61 mg/kg respectively) of *Urginea maritima* ethanolic extract daily for 45 consecutive days) showed disruption of fascicles in the nerve trunk by the marked interstitial edema and scattered congested vessels, degenerated axons with partial loss of myelin sheath. The deteriorated Schwann cells had areas of enlargement and contraction together with strongly discolored nuclei Fig. (13).

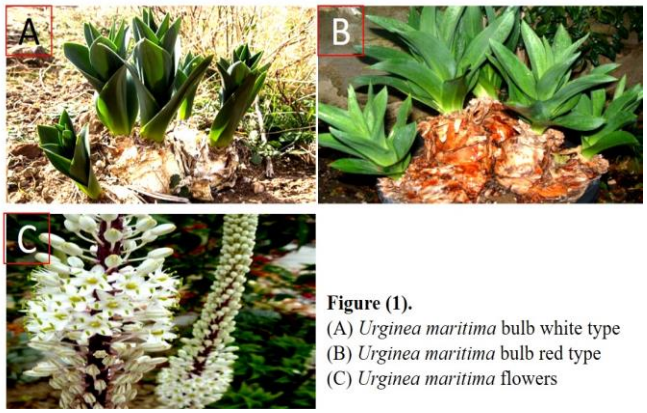

Figure (1).
(A) *Urginea maritima* bulb white type
(B) *Urginea maritima* bulb red type
(C) *Urginea maritima* flowers

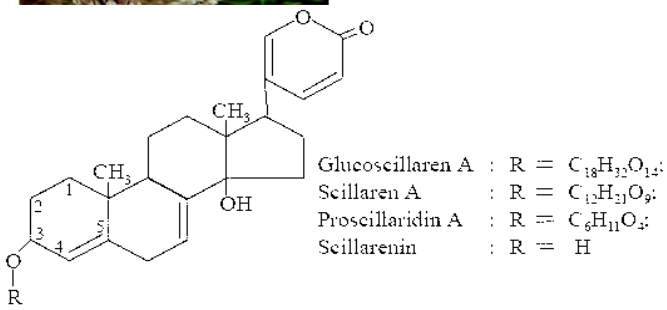

Figure (2):

Chemical structure of bufadienolides isolated from *Urginea* species (Glucoscillaren A = Scillarenin + Rhamnose + Glucose + Glucose; Scillaren A = Scillarenin + Rhamnose + Glucose; Proscillaridin A = Scillarenin + Rhamnose)

Figure (3). *Urginea maritima* ethanolic extract

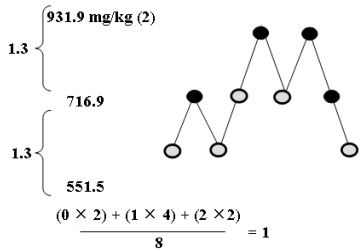

Figure (4). Schematic drawing showing up-and-down method for LD_{50} determination of *Urginea maritima* in male rats.
Log LD_{50} = log 551.5 + [1 × (log 716.9 – log 551.5)] = 2.742 + 1 (2.855 – 2.742) = 2.855
Anti-log 2.855 = 716.14
LD_{50} = 716.14 mg/kg body weight

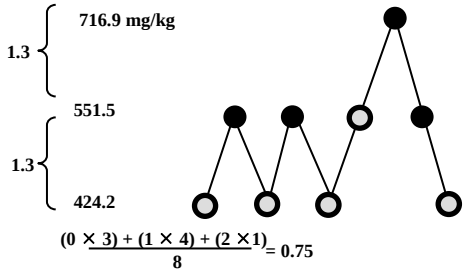

Figure (5). Schematic drawing showing up-and-down method for LD_{50} determination of *Urginea maritima* in female rats.
Log LD_{50} = log 424.2 + [0.75 × (log 551.5– log 424.2)]
= 2.628 + 0.75 (2.742– 2.628) = 2.714
Anti-log 2.714 = 517.61
LD_{50} = 517.61 mg/kg body weight

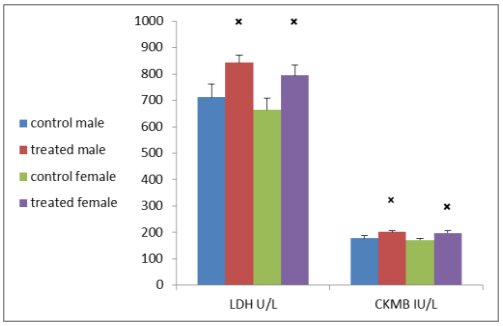

Figure (6). LDH and CK-MB activities of control and treated male and female Wistar albino rats
Data are presented as mean ± SE (n=6 animal /group)
* Significant difference between treated and control groups at P≤ 0.05

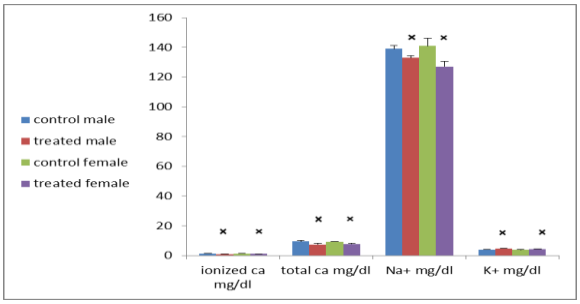

Figure (7). Serum ionized and total Ca^{++}, Na^{+} and K^{+} levels of control and treated male and female Wistar albino rats
Data are represented as mean ± SE (n=6 animal /group)
* Significant difference between treated and control groups at P≤ 0.05

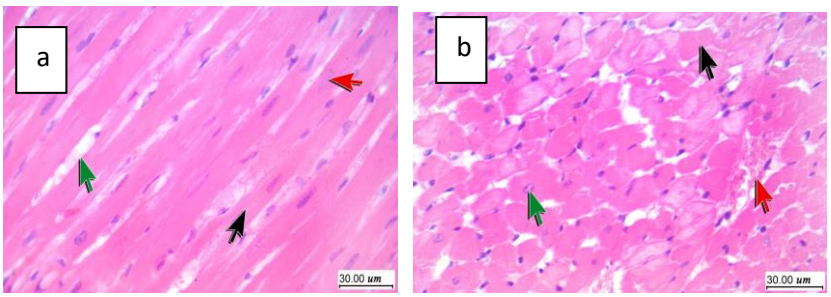

Figure (8). A control male (a) and female (b) rat myocardial histopathological sample reveals a normal appearance of muscle fibers with cytoplasmic striations (black arrow), nuclei designated as (red arrow). Vascular interstitial tissue revealed thin walls (green arrow). (H&E X400).

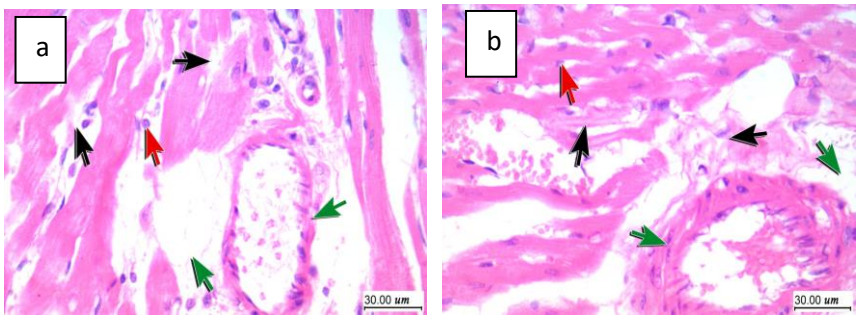

Figure (9). An intoxicated male (a) and female (b) rat myocardial histopathological sample demonstrates deteriorated myocytes with the loss of fascicular organization and striations (black arrow). The pyknotic nuclei were smaller and had condensed chromatin (red arrow). Edematous, thick-walled stromal vessels with congestion (green arrow). (H&E X400)

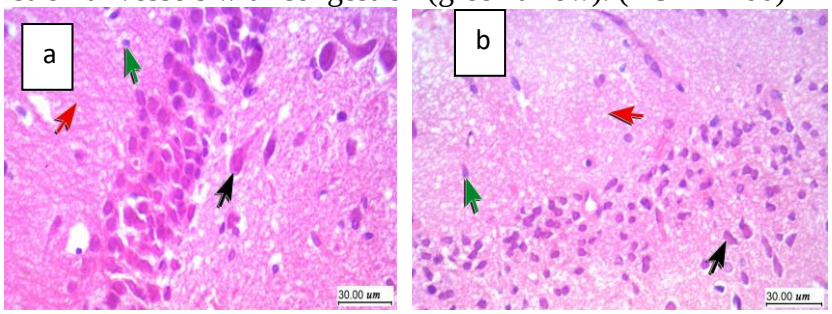

Figure (10). Brain images of control male (a) and female (b) rats show normal neurons with stellate cytoplasmic processes and basophilic cell bodies (black arrow) contained within an eosinophilic fibrillary backdrop (red arrow), with sporadic tiny glial cells (green arrow) generated by the cytoplasmic processes (green arrow). (H&E X400)

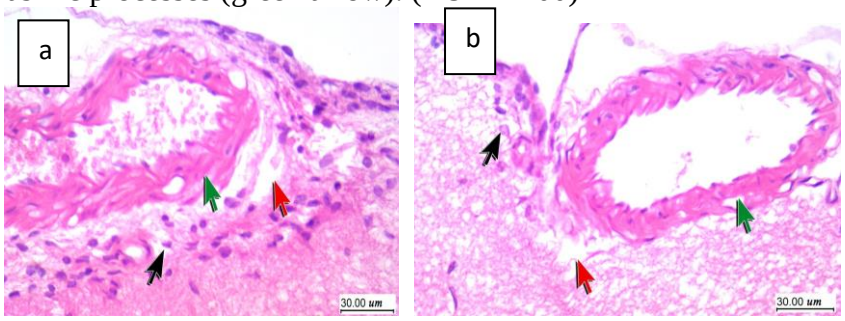

Figure (11). An image of the brains of treated male (a) and female (b) rats shows vacuolar degeneration of neurons (black arrow), modest cell body shrinkage, dilated and congested arteries (green arrow), and considerable edema in the background (red arrow). (H&E X400)

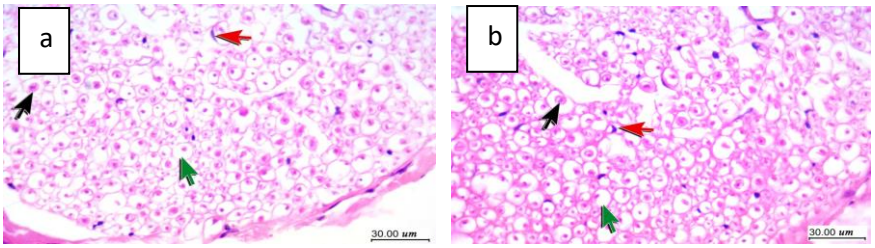

Figure (12). Sciatic nerve scans of the nerve trunks of control male (a) and female (b) rats show that the axons are normally arranged into fascicles and that there are a few scattered spindle-wavy Schwann cells (red arrow). In cross section, the axons with lipid-rich myelin sheaths appeared as a dot in the center and as empty space (green arrow) (black arrow). Between them are numerous thin-walled containers. (H&E X400)

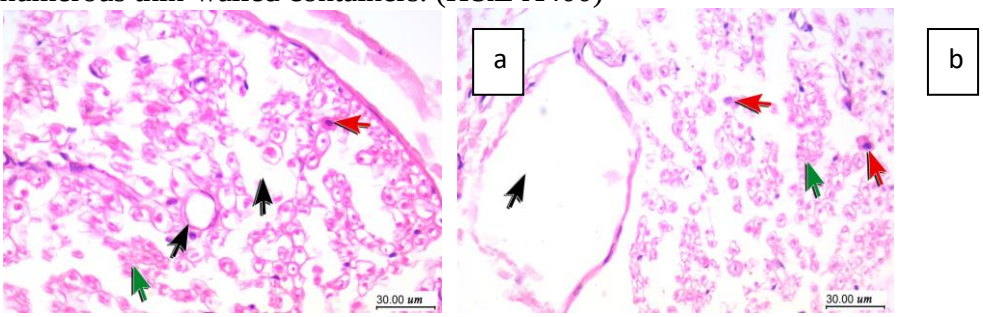

Figure (13). Male and female treated rats' sciatic nerves in (a) and (b) reveal deteriorated axons with partial loss of myelin sheath, significant interstitial edema (black arrow) in the nerve trunk, and disruption of fascicles (green arrow). The degraded Schwann cells have strongly pigmented nuclei and foci of swelling and shrinking (red arrow). (H&E X400)

Discussion:

For the past 400 years, plants have been used extensively in the treatment of numerous ailments. The creation of drugs had previously relied mostly on natural products. The results of the current study, which examined the toxic effects and serum biochemical changes in experimental rats orally intoxicated with *Urginea maritima* alcoholic extract at (1/20 LD_{50}) daily for 45 days, revealed different effects between male and female rats, coming in at 716.14 and 517.61 mg/kg B.W, respectively. This suggests that females are more susceptible to the acute toxicity of *Urginea maritima*. These findings are consistent with those of ***Bozorgi et al., (2017)*** who showed that sex influences the toxicity of red *Urginea* since scilliroside affects female rats more severely than male rats and is less toxic in the presence of testosterone.

Our results indicated significance increase in serum LDH enzyme as shown in Fig. (6), in correlation with the histo-pathological findings

as there is cardiac myocytes degeneration Fig. (9), vacuolar degeneration of brain neurons as shown in Fig. (11)**,** these results were in agreement with ***Mittal et al., (2009)*** who reported that, LDH is an oxido-reductase which catalyzes the interconversion of lactate and pyruvate. When disease or injury affects tissues containing LDH, the cells release LDH into the blood, where it is identified in higher than normal levels. Therefore, LDH is most often measured to evaluate the presence of tissue or cell damage**.**

According to ***Borrayo-Sánchez et al., (2006)***, the skeletal muscle, smooth muscle, and brain are where CK-MB is primarily expressed. By absorbing adenosine triphosphate (ATP), creating adenosine diphosphate (ADP), then reversing the process, this enzyme aids in the conversion of creatine to phosphocreatine. In the current investigation, serum CK-MB levels in all treated animals showed a considerable rise, as depicted in Fig (6). The heart of treated rats got (1/20 of LD_{50} of *Urginea maritima* alcoholic extract) showed degraded myocytes with lost fascicular organization, degeneration of myofibrils, and disappearing striations, which is also consistent with our histological findings as seen in Fig. (9), the pyknotic nuclei displayed chromatin condensation and shrinkage. Elevated CK-MB is a sign of muscle injury and has been linked to myocardial infarction, myositis, ***Brancaccio et al., (2007)***.

In line with ***Dizaye and Hamed's (2010)*** assertion that *Urginea maritima* has the effect of inhibiting Na^+/K^+ -ATPase due to the active principle bufadienolide (cardiac glycoside) present in extract, our results demonstrated a significant increase in serum K^+ and a significant decrease in total, ionized Ca^{++} and Na^+ as shown in Fig. (7). Inhibiting the membrane Na^+/Ca^{++} exchange mechanism leads to an increase in intracellular Na^+, which in turn causes an increase in intracellular calcium. According to ***Repke et al., (1998)***, the steroid cardiac glycosides of *Urginea maritima* have a specific inotropic effect by inhibiting the Na^+/K^+-ATPase enzyme in the heart muscle. This inhibition caused an increase in intracellular Na^+ and Ca^{++} levels, which in turn made cardiac contraction stronger.

According to ***Sundaragiri and Tandur (2016)***, *Urginea maritima* includes the Na^+/K^+-ATPase blockers bufadienolide, Glucoscillarene A, proscillaridine A, scillarene A, scilliglaucoside, and scilliphaeoside. Due to its toxicity, it causes full atrio-ventricular block and bradycardia. Additionally, according to ***Winnicka et al., (2006)***, *Urginea maritima* includes cardiac glycosides that enhance intracellular Ca^{++} by inhibiting Na^+/K^+-ATPase function. A sustained increase in intracellular Ca^{++} may also activate

several hydrolytic enzymes, including proteases, nucleases, and lipases. Dysregulation of these ions activates a number of intracellular pathways. These modifications might significantly contribute to its cellular toxicity. In addition to impairing energy generation, activated hydrolytic enzymes can also cause a cytoskeletal breakdown, diminish phospholipid levels, disrupt membrane and cytoskeletal proteins, as well as nuclear chromatin, and eventually induce apoptosis.

As the cardiotonic action of *Urginea maritima* depends on the cardiotrophic steroidal bufadienolide glycosides, which exert their action primarily by inhibiting membranous adenosine triphosphates of myocardial tissues, we noticed nervous signs in rats during the determination of LD_{50} as frequent convulsions with shallow rapid breathing. The force of cardiac muscular contractions (contractility) is increased as a result of the inhibition of membranous adenosine triphosphates, which causes an increase in intracellular Ca^{++} and Na^{+} and a decrease in intracellular K^{+}. This has a net positive inotropic effect. Cardiac glycosides interact as negative chronotropic (causing peripheral vasodilation and bradycardia) and positive inotropic agents.

Also rats and humans have both indicated that *Urginea maritima's* ethanolic extract has a diuretic effect. *Urginea* is harmful to humans, causing symptoms like seizures, nausea, and vomiting, according to ***Sundaragiri and Tandur (2016) and Tuncok et al., (1995)***.

The impact of *Urginea* species on the brain and sciatic nerve has not been thoroughly studied in the past, but ***Marx et al., (2006)*** did note morphological changes associated with apoptosis in chick embryos. These effects are brought on by cardiac glycosides found in *Urginea maritima*, which elevate intracellular Ca^{++} by inhibiting Na^{+}/K^{+}-ATPase function. The excessive intracellular calcium may harm astrocytes and axonal neuroglia.

In treated rats that received 1/20 of the LD_{50} of the alcoholic extract of *Urginea maritima*, histopathological examination of the brain indicated pathologic alterations, like vacuolar degeneration of neurons, significant cell body shrinkage, dilated congested arteries, moderate edema, and loss of striations. Chromatin condensed and shrank in the pyknotic nuclei Fig. (11).

As a result of receiving 1/20 of the LD_{50} dose of *Urginea maritima* alcoholic extract, treated rats' sciatic nerves underwent pathologic changes. The nerve trunk displayed deteriorated axons with incomplete myelin sheath, scattered congested capillaries, and disrupted fascicles due to interstitial edema that was clearly visible, Schwann

cells showed degeneration with foci of shrinkage and foci of swelling which nuclei are deeply stained Fig. (13).

Conclusion:

The findings of this study led us to the conclusion that oral administration of an alcoholic extract of *Urginea maritima* at a dose of (1/20 LD_{50}) once daily for 45 days caused pathological and biochemical changes in the heart, brain, and sciatic nerve.

As a result, *Urginea maritima* should be used with caution in the pharmaceutical industry; further investigation is needed to ascertain the precise mechanisms underlying the biochemical and clinical alterations caused by *Urginea maritima*, particularly in the heart and neurological system.

Each locality's and district's common and possibly deadly poisonous flora and animals should be known to all healthcare professionals.

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الملخص العربى

دراسات سمية على نبات بصل العنصل السيناوى فى الجرذان البيضاء

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الملخص العربي

تنتشر بمصر عامة وفى سيناء خاصة النباتات الطبية التى تنمو طبيعيا والتى تدخل فى نطاق واسع فى الاستخدامات الطبية سواء فى العلاجات البدائية أو فى صناعة الأدوية عن طريق استخلاص

المواد الفعالة من هذه النباتات ,بعض هذه النباتات لها تأثير سام كما أن بعضها يستخدم فى مكافحة القوارض والحشرات.
استهدفت هذه الدراسة البحث عن الآثار السمية لنبات بصل العنصل الذى ينمو بشمال سيناء والذى له العديد من الاستخدامات الطبية كما أنه يستخدم فى مكافحة القوارض وقد استخدم لهذا الغرض عدد48 جرذ زنة 100±20جم عدد 24 جرذ ذكر وعدد 24 جرذ أنثى استخدمت منها 24 جرذ لحساب الجرعة النصف مميتة .
تم استخدام عدد 24 للدراسات السمية للنبات وقد قسمت الى اربع مجموعات كالتالى :-
* المجموعة الاولى تكونت من 6 جرذان ذكور تم تجريعها داى ميثيل سلفوكسيد وذلك لمدة 45 يوم واستخدمت كمجموعة ضابطة .
المجموعة الثانية تكونت من 6 جرذان إناث تم تجريعها داى ميثيل سلفوكسيد وذلك لمدة 45 يوم واستخدمت كمجموعة ضابطة.
- استخدمت المجموعة الثالثة والتى تكونت من 6 جرذان ذكور لدراسة التأثير السمى للإعطاء المتكرر للمستخلص الكحولى لنبات بصل العنصل بجرعة 36 مجم/كجم من وزن الجسم وذلك لمدة خمسة واربعون يوما.
- استخدمت المجموعة الرابعة والتى تكونت من 6 جرذان إناث لدراسة التأثير السمى للإعطاء المتكرر للمستخلص الكحولى لنبات بصل العنصل بجرعة 26 مجم/كجم من وزن الفأر وذلك لمدة خمسة واربعون يوما.
وقد تم ذبح الجرذان عند نهاية التجربة وتجميع العينات التالية:
- عينات دم لدراسة الأثر على القياسات البيوكيميائية
- عينات من الاعضاء الداخلية (القلب والمخ بالاضافة الى العصب الوركى) لدراسة الاثر الباثولوجى
ولقد اسفرت نتائج هذه الدراسة عن الاتى :
- تم حساب الجرعة النصف مميتة للمستخلص الكحولى لنبات بصل العنصل وقد وجدت 716.14 مجم / كجم فى ذكور الجرذان و517.61 مجم / كجم فى الاناث.
- تم دراسة اثر التجريع اليومى لتخفيف 20/1 من الجرعة النصف مميتة من المستخلص الكحولى لنبات بصل العنصل للجرذان لمدة 45 يوم على نشاط انزيمات كرياتين كاينار و لاكتيك ديهيدروجيناز وعلى مستويات الكالسيوم ، البوتاسيوم والصوديوم فى الدم .
- القلب والمخ والعصب الوركي تم فحصهم ميكروسكوبيا لدراسة اى تغير فى هذه الانسجة.
- وبدراسة التحليل الإحصائي للنتائج وجد ان التجريع اليومى لتخفيف 20/1 من الجرعة النصف مميتة من المستخلص الكحولى لنبات بصل العنصل لذكور واناث الجرذان لمدة 45 يوم أدى الى ارتفاع نشاط انزيمات كرياتين كاينار و لاكتيك ديهيدروجيناز وكذلك مستوى البوتاسيوم فى الدم ونقص مستوى الكالسيوم والصوديوم فى ذكور واناث الجرذان التى تم تجريعها وذلك مقارنة بالمجموعات الضابطة .
- تحاليل الانسجة:
- أظهرت النتائج ان التجريع الفمى للمستخلص الكحولى لنبات بصل العنصل بجرعة ستة وثلاثون مجم/كجم للذكور وستة وعشرون مجم /كجم للاناث لمدة خمسة واربعون يوما الى بعض التغيرات الباثولوجية فى القلب والمخ والعصب الوركى وذلك مقارنة بالمجموعة الضابطة.
ومن تلك الدراسات نستخلص أن نبات بصل العنصل المتواجد بشمال سيناء يمكن استخدامه كمبيد للقوارض بينما ننصح بعدم استخدامه لفترات طويلة أو يسخدم بحذر فى المجالات الطبية.