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Original Article

Effect of Uzarin Isolated from *Calotropis procera* on Blood Electrolytes and Cardiac Related Enzymes

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ABSTRACT

Background: Cardiac glycosides are known to be one of the most important drugs that extracted from medicinal plants. Cardiac glycosides have been used as a medicine for a long time with congestive heart failure. **Objectives:** In this study, we aim to investigate the biochemical changes happened in male rats by Uzarin, the cardiac glycoside isolated from *Calotropis procera*, to monitor the biochemical changes caused by cardiac glycosides in the long-term therapy or the overdose intake as well. **Materials and Methods:** Uzarin is isolated and purified from *Calotropis procera* by chromatographic methods. Uzarin structure was determined by 2 D NMR techniques. Determination of Serum Electrolytes Concentrations and cardiac related enzymes were done using biochemical methods. **Results:** This study may reflect the changes in serum electrolytes and cardiac related enzymes that happen with cardiac glycosides-dependent patients. **Conclusion:** Uzarin could significantly decrease K, Na, Ca, Mg and Cl levels 24 hr after injection. In serum, Creatine Kinase (CK) and Lactate Dehydrogenase (LDH) as well as γ-Glutamyl Transferase (GGT) activities were highly increased.

Keywords: Uzarin, Cardiac glycosides, *Calotropis procera*, Serum electrolytes, Cardiac related enzymes.

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INTRODUCTION

Cardiac glycosides are a large class of medicinal compounds isolated from many plants (cardenolides) and animals (bufadienolides). The most abundant part of this class is cardenolides which is known as digitalis glycosides referring to genus digitalis. It consists of a common skeleton which consists of a steroidal part with a five-membered α, β-unsaturated γ-lactone ring (butenolide) at C17. The sugar moiety is attached at C3.[1]

Cardiac glycosides are used in treatment of congestive heart failure, heart cannot sufficiently pump enough blood to maintain body needs.[2] On the other hand, cardiac glycosides are an important cause of drug poisoning occur during long-term treatment as well as after an overdose. One of the most cardio tonic medications still in use around the world is Digoxin.[3] The clinical use of cardiac glycosides requires attention and full comprehension of all the pitfalls and dangers by both the physician and the patient. Because of the narrow therapeutic range of cardiac glycosides, their prescription requires more care, and the ideal dosage has to be determined for each individual carfully.[4] The concentration of free cardiac glycosides in the blood is between 10^{-9} and 5 x 10^{-9} M. The toxic concentration of cardiac glycosides is considered at a two-fold or three-fold increase. In such concentration, the characteristic toxicity symptoms appear.[5-8] The uptake of ^{42}K was inhibited by concentrations equivalent to those found in the blood of intoxicated subjects as shown from the studies on human heart slices.[7,9]

The binding sites for cardiac glycosides in the cell membrane are specific receptors for these potent drugs. These receptors are part of the Na^+, K^+-ATPase molecule. As such as possible, the drug-receptor interaction shows its effects on this enzyme system and the inhibition of its activity accompanied with the binding process appears. Some other effects such as conformational change of the tertiary structure of cardiac plasmalemmal proteins, inhibition of specific ^{22}Na binding to the cell membranes, inhibition of ^{42}K binding and inhibition of ^{45}Ca efflux have been suggested.[10-16] It was reported that cardiac glycosides-ATPase interaction is the backbone for the medical effect of these drugs. There are two explanations for such an effect. The first one is a direct influence of cardiac glycosides on the ATPase activity. The second one is a carrier mediated cardiac glycoside-transport into a distinct compartment of the myocardial cell.[17]

There are three methods used to manifest the clinical interactions between potassium and cardiac glycosides. First, hypokalemia

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enhances digitalis-induced automaticity. Second, hyperkalemia prevents digitalis-induced automaticity. Third, Potassium may affect digitalis-induced changes in atrioventricular conduction.[18] Hypomagnesemia decreases the amount of cardiac glycoside required to induce ectopic rhythms in both experimental animals and humans.[19,20] Exchange system of Na^+ and Ca^{2+} operating across the sarcolemmal membrane of cardiac muscle cells has been implicated in the regulation of myocardial contractility and ionic exchange.[21-24] It has been reported that high doses of cardiac glycosides resulted in an increased sodium excretion may be due to inhibition of tubular sodium reabsorption. The main anion to accompany the cardiac glycoside is chloride which induces an increase in urinary sodium extraction.[25-28]

It was found that cardiac glycosides resulted in alterations in physiological performance of the heart. This was represented in high Lactate Dehydrogenase (LDH) and Creatine Kinase (CK) activities.[29]

The principal goal of the present study is to investigate and evaluate the influence of high doses of uzarin on serum electrolyte levels, cardiac related enzymes. The high doses used in this investigation were due to low toxicity of cardiac glycosides in rats and mice as compared with guinea pigs, cats and dogs.[30-34] This low toxicity resulted from the fact that the Na^+, K^+- ATPase of the myocardium is less sensitive and the cardiac glycosides are more rapidly removed than in other species.[35,36]

MATERIALS AND METHODS

Plant material

Calotropis procera stems were collected from Jeddah and identified by Dr. Emad A. Al Sherif at the Plant Taxonomy Department, University of Jeddah, Saudi Arabia. Voucher specimens are deposited at the herbarium of the University of Jeddah.

Extraction and isolation

The freshly ground stems of *Calotropis procera* (30 kg) were extracted exhaustively at room temperature with 70% ethanol. The solvent was removed under vacuum at 49°C then the residue was fractionated by column chromatography using silica gel on flash chromatography (30-60 mm). The main fraction containing most of cardiac glycosides was eluted with benzene and chloroform, followed by an increasing the amount of methanol in chloroform during the course of elution. Elution with chloroform-methanol (9:2) gave rise to of uzarin crude as a semi solid material which was purified on medium-pressure column chromatography using silica gel 60 (15-40 mm) and eluted with chloroform/methanol by gradient elusion to obtain to 112 mg of uzarin with m.p. 285-288°C; UV: λ_{max} 216 nm.

Structure elucidation

NMR spectra were recorded in pyridine-d5 at room temperature using a Bruker Avance DRX-500 spectrometer. Melting point was measured using Stuart SMP10 digital Melting Point Apparatus. IR spectrum was examined by standard analytical methods with KBr plates was utilized to measure the IR spectra on a Perkin-Elmer-1430 infrared spectrophotometer over a range of 400-4000 cm^{-1}, National Research Centre, Egypt. UV measurement was achieved using DMSO over a range of 200-1100 nm on a SHMADZU 2600 spectrophotometer, University of Jeddah, Saudi Arabia. Mass measurements were measured on JEUL JMS-AX-500 mass spectrometer.

Animal Experiments

The study was carried out according to the animal experimental ethical principles adopted by American Psychological Association (APA) after the approval of Animal experiments committee in National Research Center (NRC).

Thirty adult Sprague Dawley male rats, weighing 140-160 g, obtained from the animal house of the National Research Centre, were used throughout this study. The rats were fed and libitum and supplied with normal tap water in air-conditioned cages. The animals are subjected to ether anesthesia.

Effect of uzarin on serum electrolytes and cardiac enzymes

The first group of ten normal healthy adult male rats served as control group. They were left to live normally without any type of treatment and were taken as a control group. The second group of ten animals was injected intravenously with a single dose of 10 mg/kg body weight of uzarin. The third group of ten animals was injected intravenously with a single dose of 20 mg/kg body weight of Uzarin. Blood samples were collected by ocular vein puncture, under ether anesthesia, for both the treated fasting rats after 24 hr of the intravenous injection of uzarin and control rats after being fasted for 16 hr.

Determination of serum electrolytes concentrations

Serum potassium level was detected by the specific enzyme activation method according to the method of Kimura *et al.*[37] Sodium was determined by kinetic spectrophotometric method at 420 nm according to Berry *et al.*[38] Total calcium content of serum was determined spectrophotometrically out wavelength 580 nm.[39] Serum magnesium concentration was detected by the method of Abernethy and Fowler.[40] Serum chloride concentration was determined by ferric perchlorate method described by Law and Ertingshausen.[41]

Determination of cardiac related enzyme

Determination of Creatine Kinase (CK) activity

Creatine kinase activity was determined using kit that was purchased from pointe scientific, INC, company, according to the Rosalki method.[42]

Determination of Lactate Dehydrogenase (LDH) activity

Lactate dehydrogenase activity was determined using kit, which was purchased from pointe scientific, INC. Company according to the method described by Tietz.[43]

Determination of Gamma -Glutamyl Transferase (GGT activity)

Using kit that was purchased from pointe scientific, INC. Company according to the method of Szasz.[44]

RESULTS AND DISCUSSION

Isolation and structure elucidation of uzarin

The freshly ground stems of *Calotropis procera* (30 kg) were extracted at room temperature to give Uzarin crude material. After successive purification steps, it was obtained 112 mg of Uzarin. The purity was checked by wedged tip and two-dimensional TLC techniques with various ethyl acetate - methanol elusion systems. They showed high purity of isolated Uzarin. Figure 1 shows the isolation scheme.

The structure of uzarin was elucidated by 1 and 2 D NMR techniques to be as in Figure 2. UV-measurement revealed the absorption maximum for butenolide ring at 216 nm.[45] Its mass spectrum shows M^++1 ion peak at m/z 699 which corresponds to the molecular formula C_{35} $H_{54}O_{14}$. FTIR showed the existence of the characteristic functional groups such as hydroxyl at 3200 cm^{-1}, double bond at 1650 cm^{-1} and carbonyl group at 1710 cm^{-1} All NMR signals were assigned with the same method of the previous work.[45] All protons and carbons assignments are listed in Table 1.

Effect of uzarin on serum electrolytes and cardiac related enzymes

Cardiac glycosides poisoning is a world-wide problem. It reflects the long-standing and widespread medical use of cardiac glycosides.[46] The therapeutic applications of cardiac glycosides may be limited by the occurrence of severe side effects, e.g., rhythm and conduction disturbances.[47] Therefore, it is important to understand the basic mechanisms responsible for these detrimental actions. At drug amount over the therapeutic dose, cardiac glycosides can induce a wide range of electrical and mechanical effects, leading to rhythm or conduction disturbances, or even cardiac arrest.

Effect of uzarin on serum electrolytes

Table 2 and Figure 3 show serum potassium, sodium, calcium, magnesium, and chloride concentrations of control and treated male rats with different doses of uzarin. They reveal therapeutic interest since a more observed cardiac glycoside effect is shown when the extracellular potassium concentration is decreased. It showed a highly significant decrease in serum sodium concentration after intravenous administration of different doses of uzarin. The results of serum magnesium concentration after intravenous administration of different doses of Uzarin revealed a highly significant decrease.

The initiating step of the mechanism of action of cardiac glycosides is the blockade of Na^+, K^+-pump by cardiac glycoside. Large doses produce an inhibition of the Na^+, K^+-pump. The mechanism supposes that cardiac glycosides exert their effects by depolarizing the resting membrane potential gradually because of the reduction of the K^+ equilibrium potential, E_K. This led to a high significant decrease in serum potassium concentration with administration of high doses of uzarin. Table 2 and Figure 3 represent a therapeutic interest since a more observed cardiac glycoside effect is shown when the extracellular potassium concentration is reduced, therefore, this finding is a good

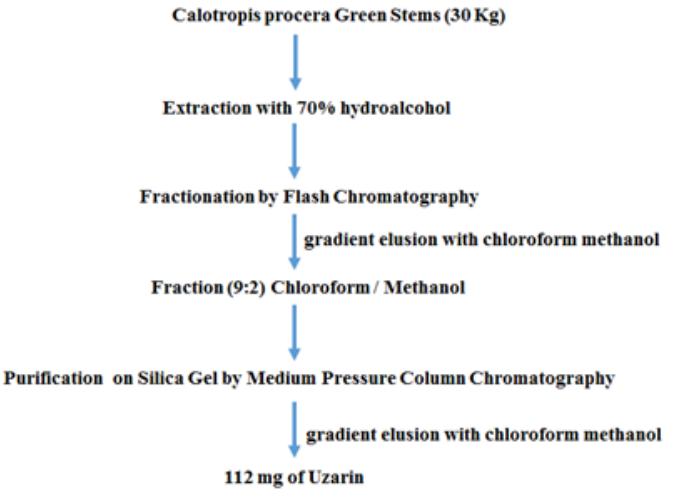

Figure 1: Isolation and purification scheme of uzarin.

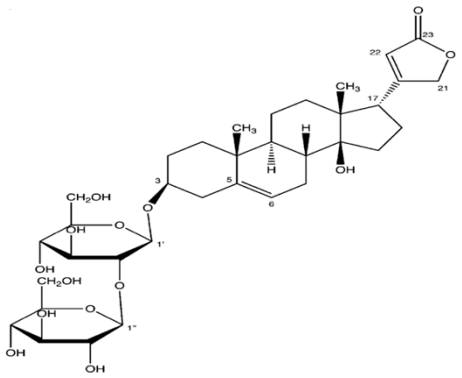

Figure 2: Structure of uzarin.

Table 1: ^{1}H and ^{13}C chemical shifts and characteristic HMBC and ROESY responses of uzarin in pyridine-*d*5.

Sl. No		^{1}H	^{13}C	HMBC	ROESY
1	α	0.76	37.2	----	1β, 3α
	β	1.53	----	----	1α, 11α, 19
2	α	2.06	29.7	----	2β, 3α, 4α
	β	1.66	----	----	2α,19
3	α	3.88	78.1	----	1α, 2α,
4	α	1.91	34.5	----	2α, 4β, 6α
	β	1.51	---	----	4α, 5α, 6β
5	α	.079	44.2	----	4β, 6α
6	α	1.04	28.8	----	4α, 5α, 6β
	β	1.18	----	----	4β, 6α, 7β
7	α	1.04	27.8	----	7β, 9α, 5α, 15β
	β	2.21	----	----	6β, 7α, 8β
8	β	1.59	41.4	----	7β, 11β, 18, 19
9	α	0.75	49.7	----	7α, 11α
10		----	35.8	----	1β, 9α, 11β
11	α	1.30	21.3	----	8β, 11α, 18, 19
	β	1.09	----	----	17α
12	α	1.19	39.5	----	17α, 18
	β	1.30	----	----	7α, 15β, 17α
13		----	49.9	----	7α, 15α
14		----	84.2	----	17α
15	α	1.79	32.9	----	17α, 21b, 22
	β	1.94	----	----	12α, 12β, 16α, 16β, 18, 22
16	α	2.02	27.1	C-12, C-13, C-14, C-17	8β, 11β, 12β, 17α, 21a
	β	1.90	51.2	C-1, C-5, C-9, C-10	1β, 2β,8β, 11β
17	α	2.71	51.2	C-22	21b
18		0.93	16.0	C-22	21a
19		0.66	12.1	C-21	17α
20		----	176.1	C-3	3`, 5`
21	a	4.97	73.7	C-1`, C-3`, C-1``	1``
	b	5.25	----	C-2`, C-4`	
22		6.07	117.4	C-3`, C-6`	
23		----	174.6	----	5`, 6`b, 6`a
1`		5.02	101.0	----	5`, 6`b
2`		4.10	84.4	----	5`, 6`a
3`		4.33	77.8	C-2`	2`, 3``, 5``
4`		----	----	C-3``, C-1``	
5`		3.90*	78	C-2``, C-4``, C2``	
6`	a	4.50*	62.4	C-3``, C-6``	
	b	4.30*	----	----	6``a, 6``b
1``		5.20	106.2	----	5``, 6``b

Sl. No		^{1}H	^{13}C	HMBC	ROESY
2``			76.7	----	5``,6``a
3``		4.19	77.8	----	1β, 3α
4``		----	----	----	1α, 11α, 19
5``		3.90*	78	----	2β, 3α, 4α
6``	a	4.46*	62.4	----	2α, 19
	b	4.36*	----	----	1α, 2α,

*Exchangeable protons.

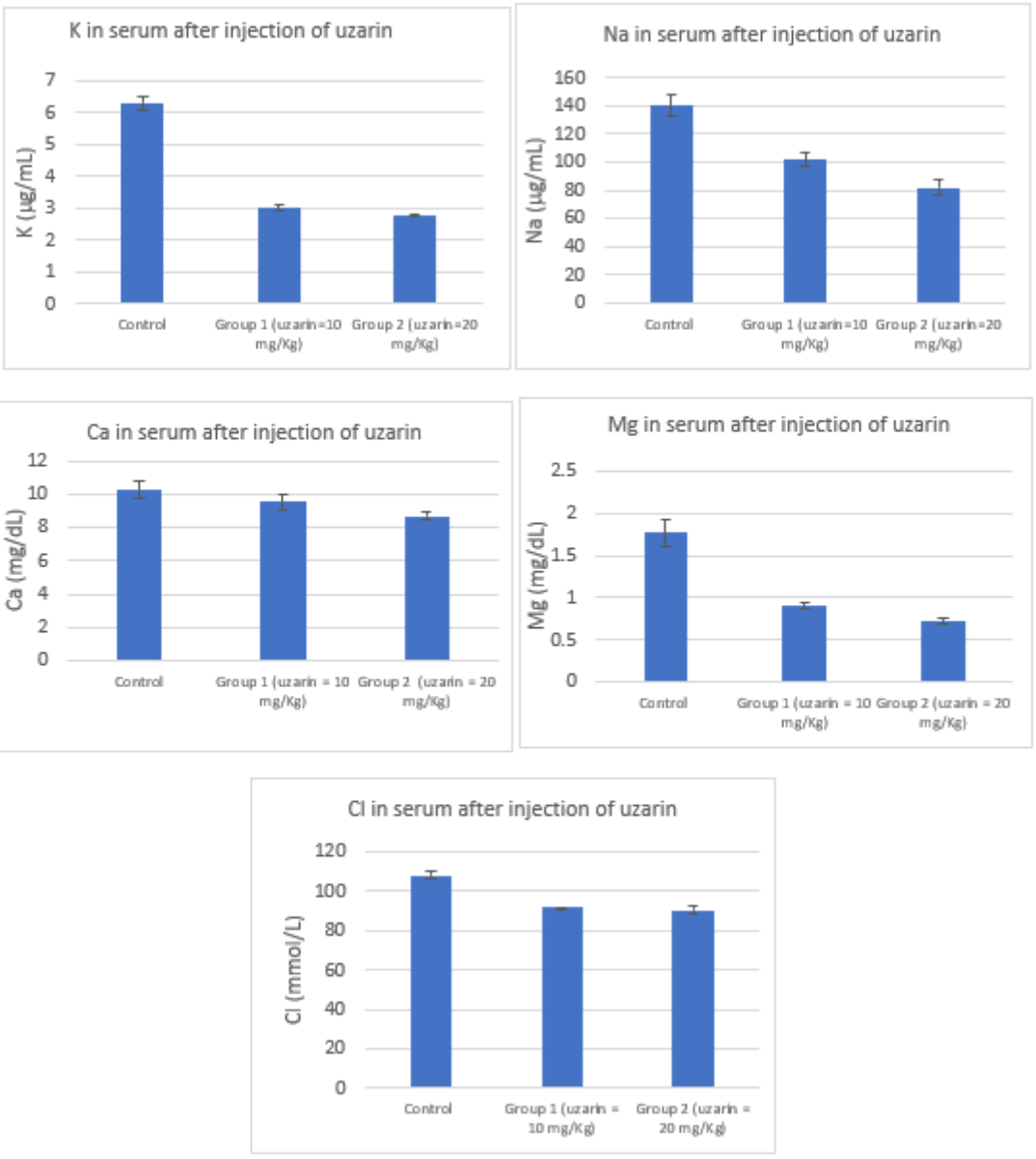

Figure 3: Serum potassium, sodium, calcium, magnesium, and chloride concentrations of control and treated male rats with different doses of uzarin.

agreement with the observation that glycoside binding to its receptor at the cellular membrane is enhanced when potassium concentration is reduced.[48] A highly significant decrease in serum sodium concentration is observed after intravenous administration of different doses of uzarin. This result can be explained by the development of the positive inotropic action of the cardiac glycosides[49-51] and an enhanced Na^{+} influx which dependent in turn upon the inhibition of Na^{+}, K^{+}-ATPase.[52] A

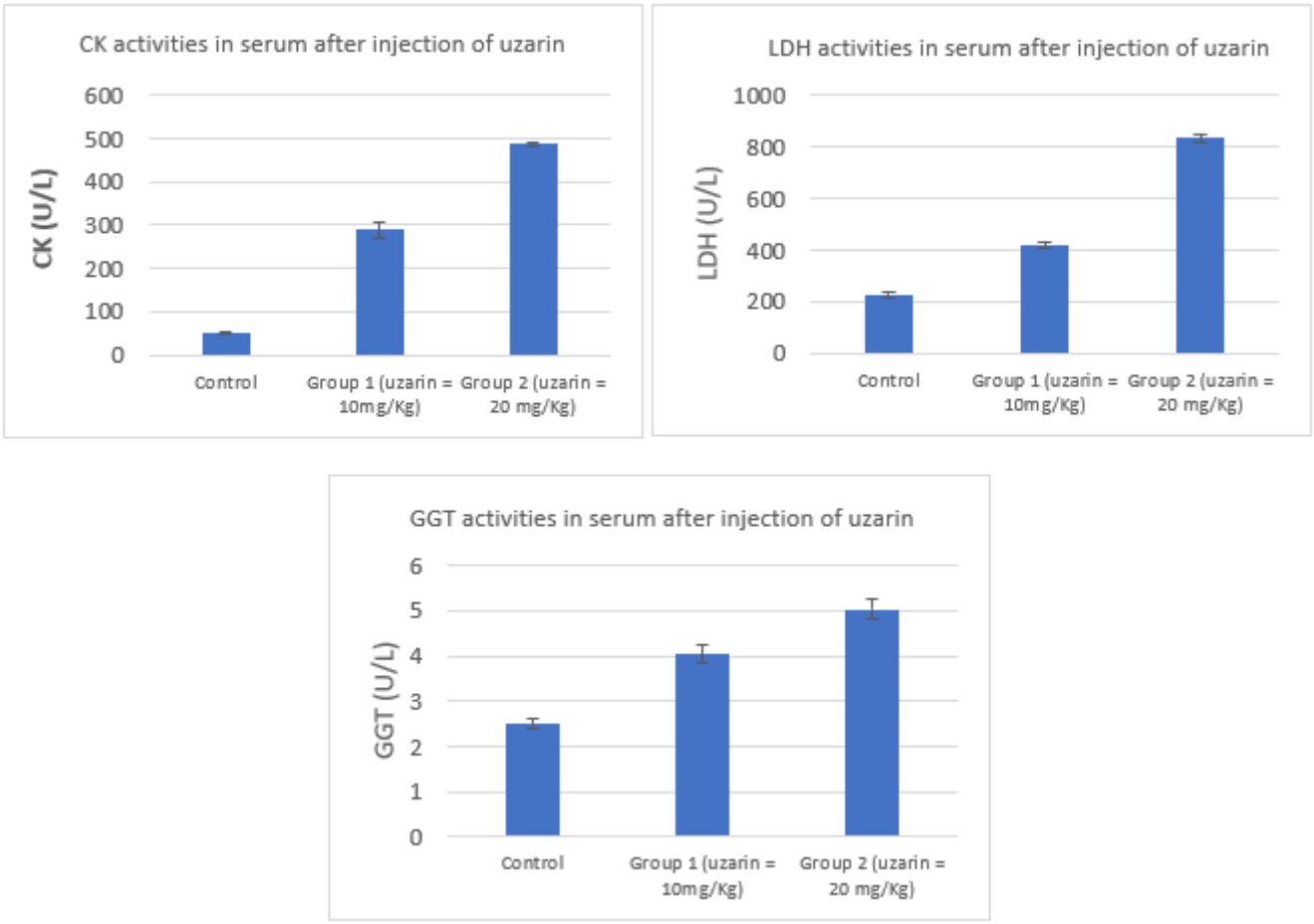

Figure 4: Creatine kinase, lactate dehydrogenase and glutamyl transferase activities in serum of control and treated male rats with different doses of uzarin.

Table 2: Serum Potassium, Sodium, Calcium, Magnesium and Chloride concentrations of control and treated male rats with different doses of uzarin.

	Change in serum electrolytes after uzarin treatment				
	K (g/mL)	Na (g/mL)	Ca (mg/dL)	Mg (mg/dL)	Cl (mmol/L)
Control	6.3±0.2	141±6.5	10.3±0.3	1.5±0.04	108 ±2.0
Group 1 (uzarin=10mg/Kg)	3.0±0.01*	102±4.5*	9.6 ±0.2 (N.S)	0.9±0.03*	90.6±1.8*
Group 2 (uzarin=20 mg/Kg)	2.8±0.03*	81±5.5*	8.8±0.2*	0.7±0.03*	88.4±2.2*

* Significant; N.S: Non-significant.

slight, but significant decrease (within normal range) of serum Ca concentration after intravenous administration of different doses of uzarin is presented in Table 2. Evidence has come from experiments implicate an increased intracellular Ca^{2+} exchange in association with the positive inotropism of the cardiac glycosides and an increased intracellular availability of Ca^{2+} occurs concurrently with the increase in developed tension.[53] The results of serum magnesium concentration after intravenous administration of different doses of Uzarin (Table 2 and Figure 3) revealed a highly significant decrease. Hypomagnesemia has been reported in subjects receiving cardiac glycosides. Hypomagnesemia occurred more frequently in subjects with clinical cardiac glycoside intoxication and was not associated with hypokalemia.[19] The causative factors for low magnesium levels in digitalized subjects are not apparent. Hypomagnesemia decreases the dose of cardiac glycosides required to induce ectopic rhythms in animals and humans[19,54] Highly significant decrease in serum chloride concentration was detected in animals treated intravenously with different doses of uzarin (Table 2 and Figure 3). This finding can be explained by the direct effect of cardiac glycosides on renal tubules that can be shown even in healthy volunteers. The main anion accompanied to cardiac glycosides is

Table 3: Creatine kinase, lactate dehydrogenase and glutamyl transferase activities in serum of control and treated male rats with different doses of uzarin.

Subject	Cardiac Related Enzymes		
	CK (U/L)	LDH (U/L)	GGT (U/L)
Control	50±2.4	227±9.8	2.5±0.1
Group 1 (Uzarin=10mg/Kg)	296±8.9*	416±10.9*	4.0±0.2*
Group 2 (Uzarin=20mg/Kg)	485±6.1*	832±16.6*	5.0±0.2*

* Significant.

chloride which induces an increase in urinary sodium excretion. In addition, therapeutic doses of cardiac glycosides decrease tubular sodium re-absorption.[20]

Effect of uzarin on cardiac related enzymes

Table 3 and Figure 4 show Creatine kinase, lactate dehydrogenase and glutamyl transferase activities in serum of control and treated male rats with different doses of uzarin. Serum Creatine Kinase (CK) and Lactate Dehydrogenase (LDH) were significantly higher in uzarin treated rats compared to the control rats.

These findings agree with Haustein,[55] who reported that in subtoxic concentrations of cardiac glycosides, several enzymes' activities of the citrate cycle are changed. cAMP transiently rises with decrease in myocardial ATP, and intracellular Lactate Dehydrogenase (LDH) and Creatine Kinase (CK) are lost from the cells. Moreover, Beller *et al.*,[56] detected an increase in serum LDH associated with cardiac glycosides in toxification. The significant increase, but within normal limits, in serum Gamma-Glutamyl Transferase (GGT) of treated animals with high doses of uzarin as compared to control reflects the toxic effect of uzarin on microsomal structures in liver cells which implies liver damage as a secondary effect to cardiac insufficiency. The enzyme level found correlates well with the duration of the drug action.[57]

CONCLUSION

Intervenors injection of isolated cardiac glycoside (uzarin) with a single dose of 10 mg/kg or 20 mg/kg in to two different groups of adult male rats induced some changes in various biochemical parameter. Uzarin, a cardiac glycoside isolated from *Calotropis procera*, could significantly decrease K, Na, Ca, Mg and Cl levels 24 hr after injection. Serum Creatine Kinase (CK) and Lactate Dehydrogenase (LDH) as well as γ-Glutamyl Transferase (GGT) activities were highly increased by uzarin intake. These results encourage us to complete this work by measuring the effect of cardiac glycosides on more biochemical indices in future.

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CONFLICT OF INTEREST

The author declared no conflicts of interest.

ABBREVIATIONS

ATP: Adenine triphosphate; **CK:** Serum creatine kinase; **GGT:** γ-glutamyl transferase; **LDH:** Lactate dehydrogenase; **cAMP:** Cyclic adenosine monophosphate.

SUMMARY

The Effect of uzarin, a cardiac glycoside isolated from *Calotropis procera*, on some biochemical indices of adult male rats is investigated. It could significantly decrease K, Na, Ca, Mg and Cl levels. In addition, Serum Creatine Kinase (CK) and Lactate Dehydrogenase (LDH) as well as γ-Glutamyl Transferase (GGT) activities were highly increased by Uzarin intake.

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