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The effect of calcium in water hardness on digoxin plasma levels in an experimental rat model

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Original Article

Abstract

BACKGROUND: Digoxin is a drug for ventricular rate control in atrial fibrillation (AF). The major challenge in digoxin therapy is to adjust the appropriate concentration range for this drug due to its narrow therapeutic index. Unique physiochemical properties of drinking water affect the pharmacological actions and delivery of drugs to the body whether they are administered orally, topically, or by injection. The aim of this study was to evaluate water hardness effect on digoxin therapy in an experimental rat model.

METHODS: 48 rats weighing 200-220 g were randomly assigned to three groups that received drinking water with 50, 400, and 800 mg/l hardness degrees for 28 days. Then each group was assigned into two groups. One received digoxin 0.2 mg/kg a day orally for four days. The other group received normal saline (as control group). Continuous recording of electrocardiogram (ECG) was performed by PowerLab system (AD Instruments Company) before and day 4 of digoxin treatment. Then serum samples were collected and assessed for digoxin, sodium, potassium, calcium, magnesium, blood urea nitrogen (BUN), and creatinine levels.

RESULTS: Water hardness in the range of 50-800 mg/l had no effect on serum digoxin levels ($P > 0.050$), but consuming hard drinking water (400 and 800 mg/l) could increase serum calcium levels and then cause mortality (37.5% in both groups), following changes in ECG due to digoxin consumption.

CONCLUSION: Consuming hard drinking water probably interferes with digoxin pharmacodynamics in the way of toxicity induction.

Keywords: Digoxin; Atrial Fibrillation; Electrocardiogram; Calcium

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Introduction

Digoxin and other cardiac glycosides (digitals) have been used for years in the treatment of congestive heart failure (CHF) and atrial fibrillation (AF).[1] AF is the most common supraventricular arrhythmia associated with an increased risk of stroke and cardiovascular death.[2] Heart failure (HF) is a common cardiac disorder associated with AF and may contribute to worsening prognosis of patients presenting with both conditions.[3] Digoxin is a purified glycoside extracted from the foxglove plant that increases the force and velocity of myocardial systolic contraction (positive inotropy) and

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decreases conduction velocity through the atrioventricular (AV) node by prolonging its refractory period. The most common dose-dependent adverse effects that happen when digoxin serum levels exceed 2 ng/ml include nausea, vomiting, anorexia, diarrhea, heart block, vision disturbances (yellow/green halo effect), headache, weakness, dizziness, and confusion.[4] Digoxin reversibly binds to the extracellular side of Na^+/K^+ -ATPase in cardiac cells and inhibits this enzyme, thereby increases intracellular sodium, which, in turn, inhibits the Na^+/Ca^{2+} exchanger leading to intracellular calcium level increase; and through this mechanism, digoxin exerts its positive inotropic effect.[5] However, digitals are responsible for many cases of iatrogenic morbidity and mortality due to high risk of toxicity; many cardiologists prefer to use digoxin in patients who have both AF and CHF.[6] It is reported that in patients with AF on good anticoagulation control with vitamin K antagonists such as warfarin or acenocumarol, digoxin use was associated with a higher rate of total and cardiovascular mortality.[7] In fact, digoxin like most inotropes, may shorten life and cause life-threatening arrhythmias.[8] Although in a case-control study consisting of 6116 subjects, it was demonstrated that only persons who at least received 1 prescription for digoxin within 7 days before the date of diagnosing acute pancreatitis may have the high relative odds of this outcome.[9] Furthermore, in a recent study, it is demonstrated that digitoxin is a well-tolerated and therapeutic alternative to angiotensin-converting enzyme inhibitors (ACEIs) and beta blockers in patients with HF due to cardiac involvement in myotonic dystrophy type 1 (DM1).[10] There is an increased risk of death from any cause associated with digoxin therapy among women that have HF and depressed left ventricular (LV) systolic function.[11] It is documented that patients with hypercalcaemia should discontinue digoxin therapy and be evaluated for the presence of rhythm disorders while receiving appropriate treatment for hypercalcaemia.[12] Levine et al. found no support for this historical belief of calcium contraindication in patients with digoxin intoxication.[13] However, Gupta et al. believe that several methodological concerns limit the validity of that conclusion.[14]

Our country, Iran, is located in an area with dry climate and as we preliminarily examined, in the most cities in center, east, and south of Iran, the hardness degrees of tap water are in the range of 500 to 600 parts per million (ppm) or even more. Water hardness is a measure of the quantity of divalent ions in water. Calcium and magnesium are the most common sources of water hardness. The hardness of water is reported in milligrams per liter or ppm as calcium carbonate (mg/l $CaCO_3$). It is shown that under similar conditions, the absorbability of the calcium in the drinking water is comparable to the absorbability of calcium in milk. As a result, high-calcium water consumption can make a considerable contribution to total calcium intake. Milk-alkali syndrome is characterized by high blood calcium caused by taking in too much calcium and absorbable alkali that if not be treated may lead to kidney failure or death. Safe and tolerable upper level of calcium intake is set at 2500 mg/day in adults, but this amount is inadequate for pregnant and lactating women. Consuming calcium above this level may produce adverse health effects.[15] On the other side, it is suggested that consumption of high mineral (calcium) drinking water could be beneficial for osteoporosis treatment or in maintaining of bone density.[16] By two different mechanisms, orally-consumed calcium is absorbed from the intestinal lumen: 1) active, transcellular absorption that involves in calcium import into the enterocyte through voltage-insensitive transient receptor potential (TRP) channels, transporting across the cell and exporting into extracellular space via a calcium-ATPase fluid and then blood and 2) passive, paracellular absorption that involves in calcium transport into the blood and it occurs when its dietary level is moderate or high. There are some reports about the effect of water hardness on different aspects of diseases and pharmacologic properties of drugs.

A study in 2003 showed that water hardness reduces bioavailability of enrofloxacin in broilers. Data showed that when water hardness increased, a linear decay of concentration in steady state (Cs) maximum has occurred.[17] In another study, water hardness enhancement under alkaline conditions increased calcium complexation with clofibrate anions; therefore, it increased the removal of clofibric acid by activated carbon filters.[18] In contrast, water hardness decreases caffeine absorption onto activated carbon filters because of caffeine competition between inorganic ions (such as calcium and magnesium).[19] Hair that contains more anionic moieties as a result of chemical treatments such as bleaching and chemical relaxing, has a higher cationic binding capacity and is, thus, more susceptible to water hardness metal uptake than virgin hair.[20]

Dilution of florfenicol, a broad-spectrum antibiotic

as a veterinary medication, in drinking water with different degrees of hardness showed that this drug was stable under a range of water hardness (low to high).[21] Although, in 2015, Wasana et al. found that high prevalence of chronic kidney disease of unknown etiology (CKDU) existed in areas that hardness of drinking water was in the range of 121-180 mg/l $CaCO_3$, aluminium, cadmium, and arsenic concentrations were almost comparable or below World Health Organization (WHO) recommendations. Fluoride concentration in drinking water in the most parts of this area was higher than the WHO recommendations.[22] Conversely, another study in an area in Pakistan reported that hypertension (HTN) was no more common in people taking hard water than those using fresh water.[23]

Obviously, there are conflicting documents about effect of water hardness on biological subjects. In so doing and because of narrow therapeutic index of digoxin, we performed this study to examine if drinking water hardness could influence digoxin plasma levels or induce its toxicity. Furthermore, we determined different electrolytes in serum blood urea nitrogen (BUN) and creatinine as functional renal bio-monitoring indexes.

Materials and Methods

Digoxin as a white, bitter, odorless powder with molecular weight of 780.95 g/mole, anhydrous $CaCO_3$, disodium ethylenediaminetetraacetic acid (EDTA) dehydrate, sodium salt of 1-(1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid (No. 203 in the Color Index), 2,2',2"-nitrilotriethanol [also called triethanolamine (TEA)], and 2-methoxyethanol (also called ethylene glycol monomethyl ether) were purchased from Sigma Chemical Company (Tehran, Iran).

Preparing standard calcium solution (hard water):[24] To clarify the role of calcium in water hardness in our experiment, hardness was caused only by the presence of calcium; a $CaCO_3$ value of 100 mg/l represents a free calcium concentration of 40 mg/l (dividing $CaCO_3$ value by 2.5). After weighing 1000 g anhydrous $CaCO_3$ powder into a 500-ml dry erlenmeyer flask, we placed a funnel in the flask neck and added a little at a time, drop by drop, hydrochloric acid (HCl) 1N until all $CaCO_3$ was dissolved. Then we added 200 ml distilled water and boiled it for a few minutes to expel carbon dioxide (CO_2). After cooling and adding a few drops of methyl red indicator and adjusting to the intermediate orange color by adding 3N ammonium hydroxide (NH_4OH) or drop by drop HCl 1N, as required, we transferred it quantitatively and diluted it to 1000 ml with distilled water (1 ml = 1.00 mg $CaCO_3$). Hardness of this sample is 1000 mg/l. However, to determine the distinct hardness, it was measured by chemical titration. After resolving the absolute hardness, we used it as the stock solution and prepared the water hardness of 50, 400, and 800 mg/l by this equilibrium formula:

$C_1 \times V_1 = C_2 \times V_2$; C_1: 1000 mg/l; V_1: The volume from stock solution needed to prepare solutions with required hardness (50, 400, or 800 mg/l); C_2: required hardness (50, 400, or 800 mg/l); V_2: Final volume of each solution with required hardness (50, 400, or 800 mg/l)

Preparing standard EDTA titrant (0.01 M): 3.723 g of analytical reagent grade disodium EDTA was dissolved in distilled water and diluted to 1000 ml.

Preparing indicator

Eriochrome Black T: We dissolved 0.5 g dye [sodium salt of 1-(1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid (No. 203 in the Color Index)] in 100 g 2,2',2"-nitrilotriethanol (TEA) or 2-methoxyethanol.

Titration of sample: We diluted 25.0 ml sample to about 50 ml with distilled water in a porcelain casserole or other suitable vessel then added 1 buffer solution to give a pH of 10.0 to 10.1. Then we added 2 drops of indicator solution. For titration, we added standard EDTA titrant slowly into the sample, with continuous stirring, until the last reddish tinge disappeared. The last few drops were added at 3- to 5-second intervals. At the end point, the solution normally was blue [hardness (EDTA) as mg $CaCO_3$/l = A × B × 1000/ml sample; A = ml titration for sample and B = mg $CaCO_3$ equivalent to 1.00 ml EDTA titrant].

Animal experiment: Male Sprague-Dawley rats (200-220 g) from the Babol University of Medical Sciences Breeding Colony, Babol, Iran, were fed ad libitum for pelleted chow as food but restricted to special water access and housed at 22 ± 2 °C under a 12:12-h light-dark cycle (lights on at 7 AM). Our study complies with the Declaration of Helsinki and all experimental procedures involving animals were approved by the Animal Care Committee of Mashhad University of Medical Sciences, Mashhad, Iran.

Animals (n = 48) were randomly assigned into six treatment groups: 1) Free access to drinking water with hardness of 50 mg/l for 32 days (n = 8), 2) Free access to drinking water with hardness of 400 mg/l for 32 days (n = 8), 3) Free access to drinking water with hardness of 800 mg/l for 32 days (n = 8) (these

three groups were examined to confirm about the effect of water hardness on mortality rate alone), 4) Free access to drinking water with hardness of 50 mg/l for 32 days (n = 8) and 0.2 mg/kg digoxin orally by gavages in the last five days, 5) Free access to drinking water with hardness of 400 mg/l for 32 days (n = 8) and 0.2 mg/kg digoxin orally by gavages in the last five days, and 6) Free access to drinking water with hardness of 800 mg/l for 32 days and 0.2 mg/kg digoxin orally by gavages in the last five days (n = 8). The oral lethal dose, 50% (LD_{50}) of digoxin in rat is 28.27 mg/kg.[25]

At the first day of experiment and day 32, 4 hours after the last digoxin administration, rats were anesthetized with ketamine plus xylazine [80 and 8 mg/kg, respectively, intraperitoneal (i.p.)], then continuous recording of electrocardiogram (ECG) was performed by PowerLab (AD Instruments Company) system. At the first day, rats with unmoral ECGs were excluded from the experiment. At the first day of study and after the last ECG recording, blood samples were taken and processed to serum by centrifuging at 3000 revolutions per minute (rpm) for 20 minutes. The serum samples were stored at -20 °C until analysis.

ECG recording: Continuous recording of ECG was performed by PowerLab (AD Instruments Company) system. ECG was recorded in lead I by silver/silver chloride (Ag-AgCl) electrode. The output of PowerLab system was inserted in a computer by USB network communication software interface (Chart for Windows Software, Version 7) used to display and save the signal in the computer. For processing the signal, MATLAB software (version 7.6) was used to extract the feature from the ECG. In this paper, QRS complex, ST, and RR interval, T and P waves were extracted from ECG signals by MATLAB. The waves in ECG were obtained in each cardiac cycle by the MATLAB software. Figure 1 shows the comparison of ECGs.

Electrolytes, BUN, creatinine, and digoxin concentration evaluation: To evaluate the calcium, magnesium, potassium, and sodium levels, atomic absorption spectrophotometry was used. The BUN and creatinine levels were measured by using High Performance Liquid Chromatography (HPLC) mass spectrometry. Also, the digoxin concentration was measured by enzyme-linked immunosorbent assay (ELISA).

Continuous variables were reported as mean ± standard deviation (SD). In analytical statistics, the normality of quantitative variables was firstly assessed using Kolmogorov-Smirnov test (K-S test). To assess and compare the two groups, Mann-Whitney U test was used. For evaluating the features in 3 levels, analysis of variance (ANOVA) test and Games-Howell test as post-hoc were used. The extracted features from ECG, after and before measurement, were compared using Wilcoxon signed-rank test. A significance level of $P < 0.05$ was considered to be significant. All statistical analyses were performed using SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA).

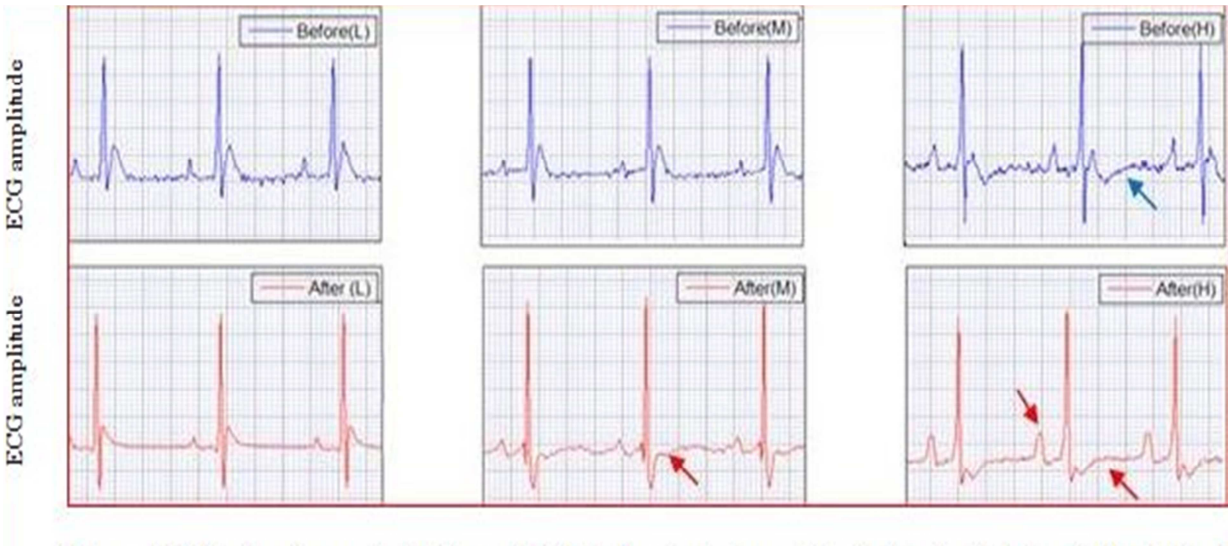

Figure 1: Electrocardiogram (ECG) signals recorded before and 4 hours after digoxin treatment (day 4 after first digoxin administration)
L, M, and H represent low, medium, and high hardness degrees of drinking water

Results

Effect of water hardness on survival rate: Values showed 37.5% reduction in survival rate as water hardness increased. From 8 rats that existed in each of 6 groups, in groups with free access to drinking water with hardness degrees of 400 and 800 mg/l for 32 days (n = 8) and 0.2 mg/kg digoxin orally by gavage in the last five days, there were 3 dead ones at the last day of experiment. There was no other mortality in the other groups in the whole study.

Effect of water hardness on digoxin serum levels: At the first day of study and after the last ECG recording, blood samples were collected. Analysis of the samples did not show any significant difference in digoxin levels in groups with different degrees of water hardness ($P < 0.050$).

Effect of water hardness on serum electrolytes, BUN, and creatinine: As we showed in table 1, free access to drinking water with different hardness did not cause any differences in serum magnesium, sodium, BUN, and creatinine levels. However, calcium levels in both groups with water hardness of medium and hard degrees were significantly increased compared to low water hardness access group ($P < 0.010$ in both comparisons). Moreover, these differences happened with or without digoxin treatment ($P < 0.010$). In all digoxin-treated groups, there were significant differences ($P < 0.001$) in potassium levels before and after digoxin treatments. Water hardness had no influence on these decreases and they happened in all different degrees of water hardness.

Effect of water hardness on ECG recording: Evaluation of the extracted features in 3 levels showed differences in ST, T, and QT intervals (ms) and ST and P amplitudes (v) as shown in table 2. ECG recording views are shown in figure 1.

ST intervals: ST intervals significantly decreased in medium and high water hardness consuming groups compared to low water hardness consuming group (4.01 ± 0.83, $P < 0.010$ and 4.84 ± 0.72, $P < 0.050$, compared to 5.88 ± 0.64, respectively). It also happened in digoxin-treated groups (4.25 ± 0.65, $P < 0.050$ and 3.88 ± 0.35, $P < 0.010$, compared to 5.36 ± 0.34, respectively).

T intervals: T intervals significantly increased in high water hardness consuming group compared to low water hardness consuming group (82.66 ± 16.32 compared to 44.25 ± 15.45, $P < 0.001$, respectively), but it did not happen in digoxin-treated groups. As the result of digoxin treatment, T intervals significantly decreased in high water hardness access groups (82.66 ± 16.32 compared to 39.25 ± 6.19, $P < 0.001$, respectively).

QT intervals: QT intervals significantly increased in high water hardness consuming group compared to low water hardness consuming group (106.32 ± 10.39 compared to 65.07 ± 9.32, $P < 0.001$, respectively), but it did not happen in digoxin-treated groups. As the result of digoxin treatment, QT interval significantly decreased in medium and high water hardness access groups (65.90 ± 6.57 vs. 49.07 ± 6.38, $P < 0.010$ and 106.32 ± 10.39 vs. 58.08 ± 3.87, $P < 0.001$, respectively).

ST amplitudes: ST amplitudes significantly increased in medium and high water hardness consuming group compared to low water hardness consuming group (0.096 ± 0.016, $P < 0.010$ and 0.107 ± 0.011, $P < 0.001$ compared to 0.064 ± 0.008, respectively). As the result of digoxin treatment, ST amplitudes significantly decreased in high water hardness access group (0.107 ± 0.011 vs. 0.047 ± 0.028, $P < 0.001$, respectively).

P amplitudes: P amplitudes significantly increased in high water hardness consuming group compared to low water hardness consuming group (0.083 ± 0.016 compared to 0.047 ± 0.008, $P < 0.010$, respectively). As the result of digoxin treatment, P amplitudes significantly increased in medium and high water hardness access group (0.055 ± 0.011 vs. 0.085 ± 0.014, $P < 0.010$ and 0.083 ± 0.016 vs. 0.121 ± 0.025, $P < 0.001$, respectively).

Discussion

According to our findings, consuming water with different degrees of hardness for 32 days did not affect serum digoxin levels as a drug as well as magnesium, sodium, BUN, and creatinine levels. However, calcium levels in both groups with water hardness of medium and high degrees significantly increased compared to low water hardness access group ($P < 0.010$ in both comparisons). Moreover, these differences happened with or without digoxin treatment ($P < 0.010$). Although serum calcium levels increased in both groups with medium and high hardness degrees of consuming water, they were still in the normal range (9-10.5 mg/dl). Analyzing ECGs showed that as hardness degree of consumed water increased, ST intervals significantly decreased and in contrast, T and QT intervals and ST and P amplitudes significantly increased, but these changes did not cause any mortality. On the other side, digoxin treatment caused ST, T, and QT intervals and ST amplitude reduction and P amplitude elevation. In a study, the first important finding after digoxin toxicity in the P-QRS-T complex included wide and tall P wave.[26]

Table 1. Serum levels of magnesium, calcium, sodium, potassium, creatinine, and blood urea nitrogen (BUN) before and after experiment in low, medium, and high degrees of drinking water hardness groups

Digoxin	Water hardness (mg/l)	Magnesium (mg/dl)		Calcium (mg/dl)		Potassium (mEq/l)		Calcium/potassium		Sodium (mEq/l)		BUN (mg/dl)		Creatinine (mg/dl)	
		Day1	Day32	Day1	Day32	Day1	Day32	Day1	Day32	Day1	Day32	Day1	Day32	Day1	Day32
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
+	50 (low)	2.66 ±0.27	2.32 ±0.31	9.55 ±0.39	9.52 ±0.48	6.22 ±0.95	7.05±0.63	1.53 ±0.29	1.35 ±0.15	138.10±1.02	136.83±0.84	23.84 ±1.56	24.33±2.34	0.45±0.05	0.50±0.04
	400 (medium)	2.42 ±0.21	2.35 ±0.15	9.41 ±0.43	10.00±0.30#	6.81 ±0.39	7.12±0.47	1.32 ±0.21	1.40 ±0.39	137.12±1.81	136.20±0.84	23.10 ±1.01	23.00 ±1.73	0.50±0.05	0.48±0.04
	800 (high)	2.61 ±0.20	2.76 ±1.32	9.53±0.29	10.81 ±0.88#	6.69 ±0.98	7.76 ±1.33	1.42 ±0.15	1.39 ±0.25	135.72±0.88	137.16 ±2.23	22.27 ±2.01	23.66±1.50	0.50± 0.05	0.48 ±0.04
-	50 (low)	2.22 ±0.31	2.25 ±0.30	9.85±0.32	8.83 ±0.31	6.52 ±1.11	5.45±0.67**	1.51 ±1.94	1.62 ±0.29	136.52±0.89	135.83± 4.26	22.34±1.87	21.83±1.94	0.47±0.04	0.50±0.02
	400 (medium)	2.31 ±0.12	2.49 ±0.17	9.51 ±0.41	9.60 ±0.26#	6.44 ±1.02	4.55 ±0.33**	1.48 ±0.25	2.10 ±1.94*	136.32 ±2.78	137.25 ±1.71	22.92 ±1.43	22.25 ±1.50	0.50±0.04	0.50 ±0.04
	800 (high)	2.56 ±0.25	2.52 ±0.14	9.63 ±0.21	9.57 ±0.25#	6.47 ±0.44	4.52 ±0.27**	1.48 ±0.29	2.17 ±0.39*	138.00 ±2.05	139.50 ±1.00	24.00 ±2.74	22.25 ±1.71	0.45±0.05	0.50±0.03

Data were determined on day 1 and 4 hours after last digoxin administration (day 4 after first digoxin administration).

Data are shown as mean ± standard deviation (SD) (n = 8); * Statistical differences between before and after digoxin administration shown as * $P < 0.050$, ** $P < 0.001$; # Statistical differences compared to low degree of hardness shown as # $P < 0.010$; BUN: Blood urea nitrogen

Table 2. The mean and standard deviation (SD) of extracted features from electrocardiogram (ECG) signals

Water hardness (mg/l)	Effect of water hardness after 32 days			Effect of water hardness after 32 days + digoxin 0.2 mg/kg in the last 4 days		
	50 (low)	400 (medium)	800 (high)	50 (low)	400 (medium)	800 (high)
QRS (ms)	14.940 ± 2.780	15.470 ± 1.620	18.820 ± 3.880	15.540 ± 4.240	18.710 ± 4.780	14.950 ± 4.120
ST (ms)	5.880 ± 0.640	4.010 ± 0.830$^{\#\#}$	4.840 ± 0.720	5.360 ± 0.340	4.250 ± 0.650$^{\#}$	3.880 ± 0.350$^{\#\#}$
ST amplitude (v)	0.064 ± 0.008	0.096 ± 0.016$^{\#\#}$	0.107 ± 0.011$^{\#\#\#}$	0.058 ± 0.014	0.084 ± 0.016$^{\#}$	0.047 ± 0.028***
RR (ms)	231.060 ± 27.570	219.590 ± 25.160	227.180 ± 29.360	244.110 ± 18.220	257.740 ± 27.940	212.770 ± 25.910
T (ms)	44.250 ± 15.450	46.420 ± 9.750	82.660 ± 16.320$^{\#\#\#}$	33.350 ± 7.070	27.110 ± 5.230	39.250 ± 6.190***
QT (ms)	65.070 ± 9.320	65.900 ± 6.570	106.320 ± 10.390$^{\#\#\#}$	54.250 ± 4.350	49.070 ± 6.380**	58.080 ± 3.870***
P (ms)	26.510 ± 4.070	20.330 ± 6.140	30.660 ± 3.740	28.330 ± 5.110	21.450 ± 5.570	22.590 ± 4.160
P amplitude (v)	0.047 ± 0.008	0.055 ± 0.011	0.083 ± 0.016$^{\#\#\#}$	0.066 ± 0.012	0.085 ± 0.014**	0.121 ± 0.025$^{\#\#\#***}$
PR (ms)	43.280 ± 11.780	44.240 ± 7.470	42.440 ± 8.120	42.480 ± 10.150	45.370 ± 8.710	40.710 ± 8.390

Extracted features from electrocardiograms (ECGs) are shown as mean ± standard deviation (SD) (n = 8)

* Statistical differences between before and after digoxin administration shown as ** P < 0.010, and *** P < 0.001; $^{\#}$Statistical differences compared to low degree of hardness shown as $^{\#}$ P < 0.050; $^{\#\#}$ P < 0.010; and $^{\#\#\#}$ P < 0.001

In another clinical study of digoxin-poisoned cases, ECG changes included sinus bradycardia, extrasystole, ST segment depression (a sagging appearance), lowering of the T wave, obvious or apparent shortening of the Q-T duration, first-degree heart block, and finally complete heart block.[27] These findings support our result that in high water hardness consuming group, some degrees of digoxin toxicity have occurred. Digoxin treatment in both medium and high water hardness groups caused 37.5% reduction in survival rate. In all digoxin-treated groups, there were significant differences ($P < 0.001$) in potassium levels before and after digoxin treatments. These changes happened in all different degrees of water hardness. It is reported that along therapeutic serum concentrations of digoxin with automaticity, higher calcium to potassium ratios may exist.[28]

In this study, calcium to potassium ratios after digoxin administration increased significantly in all water hardness degrees ($P < 0.050$). An important part of calcium balance control in the body is parathyroid hormone (PTH) secretion that is stimulated by hypocalcemia and inhibited by hypercalcaemia. PTH works to increase Ca^{++} levels by stimulating bone resorption, decreasing urinary loss of Ca^{++}, and indirectly stimulating Ca^{++} absorption in the small intestine by stimulating synthesis of $1,25(OH)_2D$ in the kidney. As a result, consuming high-calcium containing water did not increase blood calcium levels beyond the normal values, but it could cause higher calcium levels (in comparison to low-calcium containing water). These elevations did not cause any mortality by themselves but could induce digoxin toxicity and its consequence, mortality. It is a concern in digoxin therapy when the patient consumes hard drinking water. Altogether, all these documents support this fact that it is not suitable to consume hard water.

Conclusion

Our data showed that consuming water with high degrees of hardness could induce digoxin toxicity even in therapeutic serum levels. Patients and physicians must be sensitive and alert of this important concern.

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Conflict of Interests

Authors have no conflict of interests.

References

1. Yang EH, Shah S, Criley JM. Digitalis Toxicity: A fading but crucial complication to recognize. Am J Med 2012; 125(4): 337-43.
2. Fuster V. Atrial fibrillation: An epidemiologic, scientific and clinical challenge. Nat Rev Cardiol 2005; 2: 225.
3. Heist EK, Ruskin JN. Atrial fibrillation and congestive heart failure: Risk factors, mechanisms, and treatment. Prog Cardiovasc Dis 2006; 48(4): 256-69.
4. McEvoy GK. AHFS drug information: Essentials 2006-2007. Bethesda, MD: American Society of Health-System Pharmacists; 2002. p. 1552.
5. Katzung BG, Masters S, Trevor A. Basic and clinical pharmacology. New York, NY: McGraw Hill Professional, 2012.
6. Bavishi C, Khan AR, Ather S. Digoxin in patients with atrial fibrillation and heart failure: A meta-analysis. Int J Cardiol 2015; 188: 99-101.
7. Pastori D, Farcomeni A, Bucci T, Cangemi R, Ciacci P, Vicario T, et al. Digoxin treatment is associated with increased total and cardiovascular mortality in anticoagulated patients with atrial fibrillation. Int J Cardiol 2015; 180: 1-5.
8. Eade E, Cooper R, Mitchell AR. Digoxin-time to take the gloves off? Int J Cardiol 2013; 164(3): 365-7.
9. Lai SW, Lin CL, Liao KF. Digoxin use may increase the relative risk of acute pancreatitis: A population-based case-control study in Taiwan. Int J Cardiol 2015; 181: 235-8.
10. Finsterer J, Stollberger C. Beneficial effect of digitoxin for heart failure from noncompaction in myotonic dystrophy 1. Int J Cardiol 2020; 160(3): e50-1.
11. Rathore SS, Wang Y, Krumholz HM. Sex-based differences in the effect of digoxin for the treatment of heart failure. N Engl J Med 2002; 347(18): 1403-11.
12. Vella A, Gerber TC, Hayes DL, Reeder GS. Digoxin, hypercalcaemia, and cardiac conduction. Postgrad Med J 1999; 75(887): 554-6.
13. Levine M, Nikkanen H, Pallin DJ. The effects of intravenous calcium in patients with digoxin toxicity. J Emerg Med 2011; 40(1): 41-6.
14. Gupta A, Su M, Greller H, Majlesi N, Hoffman RS. Digoxin and calcium: The verdict is still out. J Emerg Med 2010; 39(1): 102-3.
15. Cotruvo JA, Bartram J. Calcium and magnesium in drinking-water: Public health significance. Geneva, Switzerland: World Health Organization; 2009.

16. Ogata F, Nagai N, Ito Y, Kawasaki N. Fundamental study on effect of high-mineral drinking water for osteogenesis in calciprivia ovariectomized rats. Yakugaku Zasshi 2014; 134(5): 679-85.
17. Sumano LH, Gutierrez OL, Aguilera R, Rosiles MR, Bernard BM, Gracia MJ. Influence of hard water on the bioavailability of enrofloxacin in broilers. Poult Sci 2004; 83(5): 726-31.
18. Mestre AS, Nabico A, Figueiredo PL, Pinto MsL, Santos MSC, Fonseca IM. Enhanced clofibric acid removal by activated carbons: Water hardness as a key parameter. Chem Eng J 2016; 286: 538-48.
19. Couto Jr OM, Matos I, da Fonseca IM, Arroyo PA, da Silva EA, de Barros MA. Effect of solution pH and influence of water hardness on caffeine adsorption onto activated carbons. Can J Chem Eng 2015; 93(1): 68-77.
20. Evans AO, Marsh JM, Wickett RR. The uptake of water hardness metals by human hair. J Cosmet Sci 2011; 62(4): 383-91.
21. Hayes JM, Eichman J, Katz T, Gilewicz R. Stability of florfenicol in drinking water. J AOAC Int 2003; 86(1): 22-9.
22. Wasana HM, Aluthpatabendi D, Kularatne WM, Wijekoon P, Weerasooriya R, Bandara J. Drinking water quality and chronic kidney disease of unknown etiology (CKDu): Synergic effects of fluoride, cadmium and hardness of water. Environ Geochem Health 2016; 38(1): 157-68.
23. Razzaque S, Aidrus F, Kumar A, Kumar A, Ghauri I, Ullah S. Hypertension in people consuming hard water in Haji Samoa Goth Keti-Bundar Sindh. Annals of Abbasi Shaheed Hospital & Karachi Medical & Dental College 2015; 20(1): 17.
24. American Public Health Association. Standard methods for the examination of water and wastewater. Denver, CO: American Water Works Association; 1999.
25. Yoshida A, Fujita M, Owada E, Ito K. Effects of diltiazem on plasma and tissue digoxin levels in mice. J Pharmacobiodyn 1984; 7(8): 511-6.
26. Malmasi AA, Selk Ghafari M, Sorouri S. Electrocardiographic manifestation of digoxin toxicity in a Pomeranian dog. Iranian Journal of Veterinary Medicine 2009; 3(1): 55-8. [In Persian].
27. Hammad SAE-H, Amin SA, El-Seidy AM, Habib NM. Clinical study of poisoned cases by some drugs admitted to the Menoufia poisoning control center over 1 year (2011), with a focus on ECG changes. Menoufia Medical Journal 2014; 27(2): 260.
28. Sonnenblick M, Abraham AS, Meshulam Z, Eylath U. Correlation between manifestations of digoxin toxicity and serum digoxin, calcium, potassium, and magnesium concentrations and arterial pH. Br Med J (Clin Res Ed) 1983; 286(6371): 1089-91.