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The New LOCI Digoxin Assay on the Vista 1500 Analyzer Is Virtually Free From Interferences of Herbal Supplements Hawthorn and Ashwagandha (Indian Ginseng)

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Herbal supplements hawthorn and ashwagandha (Indian ginseng) are indicated for cardiac illnesses and may be taken by patients receiving digoxin therapy. Because both hawthorn and ashwagandha are known to interfere with serum digoxin measurements using certain digoxin immunoassays, we investigated potential interference of these two herbal supplements with the new homogenous sequential chemiluminescent assay for digoxin based on the luminescent oxygen channeling technology (LOCI digoxin) for application on the Dimension and Vista platform. When aliquots of a drug-free serum pool were supplemented with various amounts of hawthorn (three different commercial preparations) or ashwagandha (two different commercial preparations) and apparent digoxin values were measured using LOCI digoxin assay on Dimension Vista 1500 analyzer we observed none-detected values except when aliquots were supplemented with very high amounts of the herbal extracts. When aliquots of a serum digoxin pool (prepared by pooling specimens from patients receiving digoxin) where further supplemented with various amounts of these supplements and digoxin concentrations were remeasured, statistically significant falsely higher digoxin values were observed only in specimens containing very high amounts of these supplements. Such interference may not be clinically significant. We conclude that new LOCI digoxin assay is virtually free from interferences of herbal supplements, hawthorn, and ashwagandha. J. Clin. Lab. Anal. 26:227–231, 2012.

Key words: hawthorne; ashwagandha; LOCI digoxin assay

INTRODUCTION

Herbal remedies were the only medicines available to treat various illnesses in the early years of human population development (1). Hawthorn fruit has historically been used to treat heart diseases. Currently, extracts of hawthorn leaves and flowers are used by herbalists to treat patients with heart failure and coronary artery disease. Hawthorn may play a role in the prevention and treatment of cardiovascular disease such as hypertension, hyperlipidemia, and particularly congestive heart failure (2). Clinical studies have indicated that symptoms such as dyspnea and fatigue improved significantly after hawthorn treatment as compared with placebo, while reported adverse effects of hawthorn are usually mild and transient and include nausea, dizziness, cardiac, and gastrointestinal complaints (3). Exercise tolerance was shown to be significantly increased with hawthorn extract consumption and symptoms such as shortness of breath and fatigue improved (4).

Ashwagandha (*Withania somnifera*), also known as winter cherry and Indian ginseng, grows in India, Africa, parts of Europe as well as North America. Ashwagandha has been used in Ayurvedic medicine for more than 3,000 years for treating various conditions including cardiac

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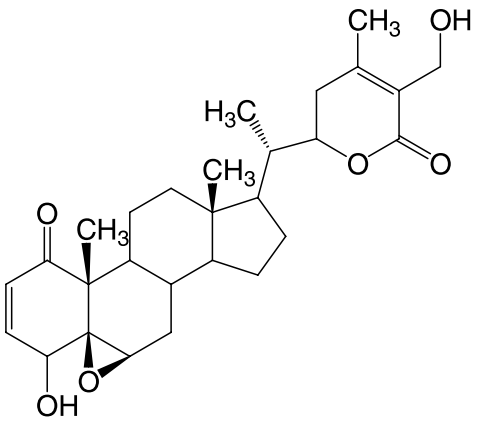

Fig. 1. Chemical structure of Withaferin A, an active component of ashwagandha.

dysfunction. Ashwagandha is effective in limiting myocardial injury after ischemia and reperfusion in Wistar rats (5).

Digoxin, a cardiac glycoside, is found in foxglove plants (*Digitalis lanata*). The main pharmacologic effects of digoxin include a dose-dependent increase in myocardial contractility and negative chronotropic action. Digitalis also increases the refractory period and decreases impulse velocity in certain myocardial tissue (such as the AV node). Digoxin has a narrow therapeutic index. Therefore, therapeutic drug monitoring is essential for achieving optimal efficacy as well as for avoiding toxicity. The therapeutic range of digoxin is usually considered as 0.8–1.8 ng/ml, but there is a substantial overlap between therapeutic and toxic concentrations. Digoxin toxicity may occur with a lower digoxin level, if hypokalemia, hypomagnesemia, or hypothyroidism coexists. Likewise, the concomitant use of drugs such as quinidine, verapamil, spironolactone, flecainide, and amiodarone can increase serum digoxin levels thereby increasing the risk of digoxin toxicity. One clinical trial indicated that the beneficial effect of digoxin was observed at serum concentrations of 0.5–0.9 ng/ml, whereas serum concentrations at or more than 1.2 ng/ml appeared harmful (6).

Because both hawthorn and ashwagandha are readily available without prescription and are indicated for treating congestive heart failure, a person receiving digoxin for heart failure may also be taking hawthorn or ashwagandha. In addition, epicatechin, chlorogenic acid, isoquercitin, and hyperoside, the major active flavonoids of hawthorn have a polycyclic ring structure similar to digoxin (7) Withaferin A, a major biochemical constituent of ashwagandha has structural similarity to digoxin (Fig. 1). Interference of hawthorn on Digoxin III immunoassay marketed by the Abbott Laboratories for application on the AxSYM analyzer has been reported (8). Similarly, ashwagandha also interferes with serum digoxin measurement using immunoassays including the Digoxin III immunoassay (9,10). Recently, Siemens Diagnostics has marketed a new digoxin immunoassay based on the LOCI technology (luminescent oxygen channeling technology) for application on the Dimension Vista analyzer but effect of hawthorn and ashwagandha on serum digoxin measurement using this immunoassay has not been reported before. Here, we report our findings on the effect of these herbal supplements on serum digoxin measurement using LOCI digoxin immunoassay.

MATERIALS AND METHODS

Three hawthorn (*Crataegus* spp.) liquid extracts were purchased locally: Brand 1 (Herb Pharm, William, OR), Brand 2 (Health and Herbs, Albany, OR), and Brand 3 (Gaia Herbs, Brevard, NC). Both extracts from Brand 1 and 3 used for this study contain 12% ethanol in water but the extract of Brand 2 was alcohol free. Two ashwagandha products from two different manufacturers were used in this study. Both products were liquid extract of ashwagandha in ethanol/water (60:40 by vol). The first product (Ashwagandha Brand 1) was manufactured by Herb Pharm (Williams, OR) and the second product (Ashwagandha Brand 2) was available from Herbs etc (Santa Fe, New Mexico).

New LOCI digoxin immunoassay for application on the Dimension Vista analyzer was obtained from Siemens Diagnostics (Deerfield, IL) and all assays were run on the Dimension Vista 1500 analyzer following manufacturer's recommended protocol. The LOCI Digoxin assay is a homogenous sequential chemiluminescent assay based on the luminescent oxygen channeling technology and utilizes a specific mouse monoclonal antibody against digoxin and requires no sample pretreatment prior to the assay. The analytical measurement range of this assay is from 0.06 to 5.0 ng/ml while the calibration range is 0–5.0 ng/ml of serum digoxin concentration. The limit of detection is 0.06 ng/ml. Therefore any apparent digoxin value less than 0.06 ng/ml was considered as a "none detected" digoxin value.

We used one drug-free serum pool for this study. No digoxin was detected in the drug-free serum pool. In order to ensure that the serum pool was indeed drug and DLIS (digoxin like immunoreactive substances) free, we further treated this serum pool with activated charcoal (50 mg per milliliter of serum) for 20 min. Activated charcoal was purchased from Aldrich Chemical Company (Milwaukee, WI). After treatment with activated charcoal, the pool was centrifuged at a high speed in order to separate activated charcoal from the serum. The resulting supernatant was used for further experiments. We also prepared a digoxin pool from surplus serum specimens that were submitted to our clinical laboratory for therapeutic drug monitoring of

digoxin and that would have been discarded after testing. These specimens are stored in the laboratory for a week after performing and reporting results to the ordering clinicians and then are discarded.

We used hawthorn and ashwagandha extracts directly for this study. In the first set of experiments, microliter quantities of liquid hawthorn or ashwagandha extracts were added to drug-free serum to achieve final volumes of 5, 10, or 20 μl of extract per milliliter of serum. These volumes reflect *in vivo* concentrations after usage of recommended doses while higher concentrations represent those for suspected severe overdose as described earlier (8–10). After supplementation, apparent digoxin concentrations were measured in triplicate using LOCI digoxin assay. In order to ensure that the small amount of ethanol present in the extract did not affect immunoassays, appropriate extracts were placed into a glass test tube and the organic phase was evaporated under nitrogen at room temperature almost to dryness. The resulting, moist residue was reconstituted with drug-free serum. Each measurement was performed in triplicate and values were expressed as the mean and one standard deviation.

Because cross-reactivity of a compound should be tested in the presence of the primary analyte (11), one digoxin serum pool was prepared by combining serum specimens from patients receiving digoxin. Aliquots of the digoxin pool were further supplemented with various amounts of hawthorn (Brand 1–3) or ashwagandha (Brand 1 and 2) extract and digoxin concentrations were again measured using the LOCI digoxin assay. Values were compared with the initial digoxin concentration measured in each sample of the individual serum pools, each measurement being performed in triplicate, with the values expressed as mean and standard deviation.

Statistical analyses were performed using independent *t*-test two tailed. A difference was considered statistically significant only at a 95% confidence interval or higher ($P < 0.05$) using two-tailed analysis.

RESULTS

Measurable apparent digoxin concentrations were observed with the LOCI digoxin assay only with Brand 3 of hawthorn extract at higher concentrations (10 and 20 μl/ml extract per milliliter of drug-free serum) when the aliquots of the drug-free serum pool were supplemented with various amounts of hawthorn extracts (Brand 1–3). However, with ashwagandha extracts, both Brand 1 (at 20 μl/ml extract per milliliter of drug-free serum) and Brand 2 extract (10 and 20 μl/ml extract per milliliter of drug-free serum) showed measurable apparent digoxin concentrations. Highest apparent digoxin concentration of 0.21 ng/ml (mean of three replicates) was observed when an aliquot of drug-free serum pool was supplemented with 20 μl/ml of Brand 2 ashwagandha extract (Table 1). However, all observed apparent digoxin concentrations were relatively low.

TABLE 1. Apparent Digoxin Concentrations in Aliquots of Drug-Free Serum Pool Supplemented with Various Amounts of Hawthorn or Ashwagandha Extract and Digoxin Concentration Measured by the LOCI Digoxin Assay

Specimen	Apparent digoxin, ng/ml, mean (SD), $n = 3$ LOCI digoxin assay
Drug-free serum pool	None detected
+ 5 μl/ml Hawthorn Brand 1	None detected
+ 10 μl/ml Hawthorn Brand 1	None detected
+ 20 μl/ml Hawthorn Brand 1	None detected
+ 5 μl/ml Hawthorn Brand 2	None detected
+ 10 μl/ml Hawthorn Brand 2	None detected
+ 20 μl/ml Hawthorn Brand 3	None detected
+ 5 μl/ml Hawthorn Brand 3	None detected
+ 10 μl/ml Hawthorn Brand 3	0.08 (0.01)
+ 20 μl/ml Hawthorn Brand 3	0.16 (0.04)
+ 5 μl/ml Ashwagandha Brand 1	None detected
+ 10 μl/ml Ashwagandha Brand 1	None detected
+ 20 μl/ml Ashwagandha Brand 1	0.09 ((0.02)
+ 5 μl/ml Ashwagandha Brand 2	None detected
+ 10 μl/ml Ashwagandha Brand 2	0.14 (0.01)
+ 20 μl/ml Ashwagandha Brand 2	0.21 (0.02)

When aliquots of the digoxin pool (prepared from patients receiving digoxin) were supplements with various amounts of hawthorn or ashwagandha extract, statistically significant increases in serum digoxin concentrations were observed with only Brand 3 of hawthorn extract as well Brand 2 of ashwagandha extract. For example, the mean digoxin concentration in the pool was 0.98 ng/ml. When an aliquot of this digoxin pool was supplemented with 20 μl/ml of hawthorn Brand 3 extract, the mean digoxin concentration was increased to 1.07 ng/ml. The highest increase in the digoxin concentration was observed when another aliquot of the digoxin pool was supplemented with 20 μl/ml ashwagandha Brand 2 extract. The observed digoxin concentration was 1.13 ng/ml (Table 2). Therefore, the digoxin concentration increased by 15.3%.

DISCUSSION

There is no published study stating concentrations of active ingredients in blood after ingestion of hawthorn or ashwagandha. Therefore, we calculated expected in vitro concentrations of extract after recommended dosage or ingesting excessive dosage representing overdose with both hawthorn and ashwagandha. These calculations were discussed in our previous publications (8–10). Moreover, there is no published report regarding presence of metabolites of active ingredients of hawthorn and ashwagandha. Therefore, we assumed that because

TABLE 2. Effect of Supplementing Aliquots of Digoxin Pools with Various Amounts of Hawthorn or Ashwagandha Extract on Serum Digoxin Measurements by LOCI Digoxin Assay

Specimen	Digoxin, ng/ml, mean (SD), $n = 3$ LOCI digoxin assay
Digoxin serum pool	0.98 (0.01)
+ 5 μl/ml Hawthorn Brand 1	0.96 (0.01)
+ 10 μl/ml Hawthorn Brand 1	0.97 (0.03)
+ 20 μl/ml Hawthorn Brand 1	0.94 (0.03)
+ 5 μl/ml Hawthorn Brand 2	0.98 (0.01)
+ 10 μl/ml Hawthorn Brand 2	0.98 (0.02)
+ 20 μl/ml Hawthorn Brand 3	0.96 (0.01)
+ 5 μl/ml Hawthorn Brand 3	1.00 (0.03)
+ 10 μl/ml Hawthorn Brand 3	1.05 (0.01)[a]
+ 20 μl/ml Hawthorn Brand 3	1.07 (0.02)[a]
+ 5 μl/ml Ashwagandha Brand 1	0.99 (0.01)
+ 10 μl/ml Ashwagandha Brand 1	1.02 (0.03)
+ 20 μl/ml Ashwagandha Brand 1	1.00 (0.01)
+ 5 μl/ml Ashwagandha Brand 2	1.02 (0.03)
+ 10 μl/ml Ashwagandha Brand 2	1.07 (0.02)[a]
+ 20 μl/ml Ashwagandha Brand 2	1.13 (0.03)[a]

[a]Significantly greater than the corresponding value of the digoxin pool (control) by independent *t*-test, two tailed ($P < 0.05$).

metabolites should be similar in structure to active ingredients, if no significant interference was observed with the extract, most likely metabolites should not cause significant interference. However, this hypothesis requires further validation from in vivo studies.

Because of the perception that both hawthorn and ashwagandha can improve cardiac health, a patient taking digoxin may also take hawthorn or ashwagandha. Therefore, for therapeutic drug monitoring of digoxin, it is advisable to use a specific digoxin assay that has minimal cross-reactivity with these herbal supplements. Our study clearly indicates that Brand 1 and Brand 2 of hawthorn extract demonstrated no interference with the new LOCI digoxin assay even when a high amount of extract was used for supplementing aliquots of digoxin pool. The Brand 3 of hawthorn extract showed statistically significant increases in digoxin values in the presence of 10 and 20 μl/ml of extract but again no interference when an aliquot was supplemented with 5 μl/ml of the extract, an in vivo concentration expected after recommended highest dosage of hawthorn. Moreover, the discordance between this observed value and the value of digoxin in the original pool was less than 10%, a clinically insignificant change. Similarly with ashwagandha extract, highest discrepancy observed was a 15.3% increase in serum digoxin value in the presence of 20 μl/ml of ashwagandha extract Brand 2. Landt et al. commented that a difference of 17% or higher in serum digoxin measurement is clinically significant (12). However, as reported earlier, with the Digoxin III assay, such high concentrations of hawthorn produced not only statistically significant but also clinically significant difference because the values were increased up to 40% (8). The digoxin values were increased by more than 25% in the Digoxin III assay in the presence of ashwagandha (10).

A relatively high interference of ashwagandha has been reported with the FPIA (fluorescence polarization immunoassay) for digoxin for application on the TDx analyzer (Abbott Laboratories, Abbott Park, IL) (9). This assay used a polyclonal antibody against digoxin. However, this assay is no longer available from the Abbott Laboratories. The new LOCI digoxin assay utilizes a specific monoclonal antibody against digoxin, which makes this assay to be virtually free from interferences of both hawthorn and ashwagandha. Another interesting observation from this work is that the magnitude of interference of hawthorn varied with different brands of hawthorn products. Brand 1 and 2 showed no statistically significant change in serum digoxin measurement even in the presence of very high amounts of extract. As herbal supplements are not prepared following the same rigorous protocols adopted by pharmaceutical industries, both active ingredients as well as inactive ingredients are present in the herbal supplement and amounts of such ingredients may vary significantly between different brands of products. Bergonzi et al. observed that the concentration of hypericin, the active component of herbal antidepressant St. John's wort varied from 0.03 to 0.2% in commercially available extracts of St. John's wort prepared by various manufacturers (13). Unlike single pharmaceutical products, active ingredients of botanical preparations are highly variable because their chemistry and morphology depend on the genotypic, phenotypic variations as well as geographic variations, weather exposure, harvesting variations, and processing condition (14).

We conclude that the new LOCI digoxin assay is virtually free from interferences of both hawthorn and ashwagandha. However, our initial report requires further validation from in vivo studies.

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