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Effect of Asian Ginseng, Siberian Ginseng, and Indian Ayurvedic Medicine Ashwagandha on Serum Digoxin Measurement by Digoxin III, a New Digoxin Immunoassay

Amitava Dasgupta,[1]* Gertie Tso,[1] and Alice Wells[2]

[1]*Department of Pathology and Laboratory Medicine, University of Texas Health Sciences Center at Houston, Houston, Texas*

[2]*Memorial-Hermann Hospital Laboratory Services, Houston, Texas*

Asian ginseng, Siberian ginseng, and Indian Ayurvedic medicine Ashwagandha demonstrated modest interference with serum digoxin measurements by the fluorescent polarization immunoassay (FPIA). Recently, Abbott Laboratories marketed a new digoxin immunoassay, Digoxin III for application on the AxSYM analyzer. We studied potential interference of these herbal supplements on serum digoxin measurement by Digoxin III assay in vitro and compared our results with the values obtained by Tina-quant assay. Aliquots of drug-free serum pool were supplemented with various amounts of Asian ginseng, Siberian ginseng, or Ashwagandha approximating expected concentrations after recommended doses and overdoses of these herbal supplements in serum. Then digoxin concentrations were measured by the Digoxin III and Tina-quant (Roche Diagnostics) assay. We also supplemented aliquots of a digoxin pool prepared from patients receiving digoxin with various amounts of these herbal supplements and then measured digoxin concentrations again using both digoxin immunoassays. We observed modest apparent digoxin concentrations when aliquots of drug-free serum pool were supplemented with all three herbal supplements using Digoxin III assay (apparent digoxin in the range of 0.31–0.57 ng/ml), but no apparent digoxin concentration (except with the highest concentration of Ashwagandha supplement for both brands) was observed using the Tina-quant assay. When aliquots of digoxin pool were further supplemented with these herbal supplements, digoxin concentrations were falsely elevated when measured by the new Digoxin III assay. For example, we observed 48.2% (1.63 ng/ml digoxin) increase in digoxin concentration when an aliquot of Digoxin pool 1 (1.10 ng/ml digoxin) was supplemented with 50 μl of Asian ginseng extract (Brand 2). Measuring free digoxin does not eliminate the modest interferences of these herbal supplements in serum digoxin measurement by the Digoxin III assay. J. Clin. Lab. Anal. 22:295–301, 2008.

Key words: Asian ginseng; Siberian ginseng; Ashwagandha; Digoxin III; Tina-quant digoxin assay; interference; serum digoxin

INTRODUCTION

Herbal medicines are readily available worldwide from stores without prescriptions and the use of herbal medicines among the general population is on the rise. A recent report indicates that 18.6% (over 38 million) US adults are using herbal remedies. Factors associated with highest rates of use of herbal remedies include ages 40–64, female gender, non-black/non-Hispanic race, and annual income of $65,000 and higher (1). The ginseng that grows in Manchuria is *Panax ginseng*, which is commonly marketed as "Asian Ginseng." It is used as an antioxidant, anti-inflammatory agent, anticancer remedy as well as a cardioprotective agent in traditional

*Correspondence to: Amitava Dasgupta, Department of Pathology and Laboratory Medicine, University of Texas Medical School at Houston, 6431 Fannin, MSB 2.292 Houston, TX 77030.
E-mail: Amitava.Dasgupta@uth.tmc.edu

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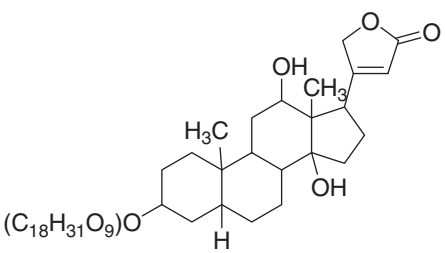
Chinese medicines, but its pharmacological properties have not been established by rigorous research (2). For thousands of years, ginseng has been considered as a heart tonic in China. Although Asian ginseng (*P. ginseng*) is commonly used, there are other types of ginseng such as Siberian ginseng, which is derived from the roots of *Eleutherococcus senticosus*. Siberian ginseng is different from Asian ginseng in that Panax-type ginsenosides are not found in Siberian ginseng. According to one published report, in the United States, ginseng is the most common herbal remedy taken by the general population (20%) followed by Echinacea (19%), *Ginkgo biloba* (15%), and St. John's wort (14%) (3).

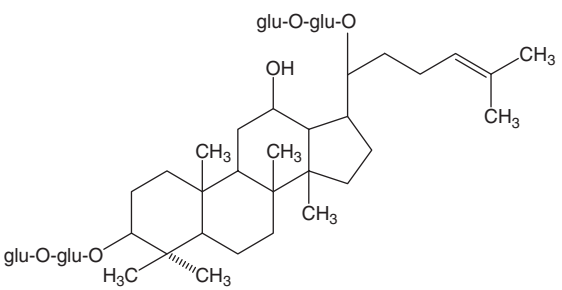
P. ginseng contains active components termed as ginsenosides (4). *P. ginseng* may be protective against adriamycin-induced heart failure in rats (5). Ginsenoside Rb 1, one of the active components of *P. ginseng*, has been reported to release nitric oxide and decrease intracellular-free calcium in cardiac myocytes, both of which play important roles in anti-hypertrophic effect. Treatment with ginsenoside Rb 1 can inhibit right ventricular hypertrophy in rats induced by monocrotaline (6).

Ashwagandha (*Withania somnifera*), also known as winter cherry, grows in India, Africa, and some parts of Europe as well as North America. Ashwagandha is considered as a wonder shrub of India and has been used in Ayurvedic medicine for over 3,000 years for treating various conditions including cardiac dysfunction. Ashwagandha preparations are readily available in the United States from herbal stores. Interestingly, several brands of St. John's wort, a popular herbal antidepressant, also contain Ashwagandha as a constituent. Ashwagandha is effective to limit myocardial injury after ischemia and reperfusion in Wister rats (7). Widodo et al. reported that leaf extract of Ashwagandha selectively kills tumor cells. Ashwagandha extract selectively activates p53 function in tumor cells thus causing either the arrest of the growth of tumor cells or causing apoptosis (8). Owais et al. demonstrated antibacterial efficacy of Ashwagandha against salmonella infection. Oral administration of aqueous extract of Ashwagandha successfully obliterated salmonella infection in Balb/c mice as revealed by increased survival rate as well as less bacterial load in various vital organs of the treated mice (9). Therapeutic efficacy of Ashwagandha against experimental aspergillosis in Balb/c mice has also been reported (10). Chorpa et al. reported potential efficacy of RA-11, an Ayurvedic medicine containing Ashwagandha as a major component, in treatment of symptomatic osteoarthritis of the knee over 32 weeks of therapy (11).

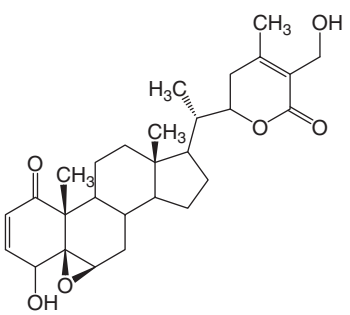
Because of the structural similarity between ginsenosides (several compounds that are structurally related) and digoxin, Asian ginseng interferes with serum digoxin measurement by the fluorescence polarization immunoassay (FPIA) (12). Structures of digoxin and ginsenoside Rb 1 are given in Figure 1. McRae reported a case where Siberian ginseng interfered with serum digoxin measurement. In one patient a serum digoxin level of 5.2 ng/ml was reported, but the patient had no symptoms of digoxin toxicity. The patient was taking Siberian ginseng. On discontinuation of Siberian ginseng, his digoxin level returned to therapeutic range (13). We were unable to confirm such high interference of Siberian ginseng with the FPIA of digoxin in our previous study, but we observed that Siberian ginseng interferes moderately with digoxin measurement using the FPIA assay, which uses a polyclonal antibody



Digoxin



Ginsenoside Rb-1



Withaferin A

Fig. 1. Chemical structure of digoxin, ginsenoside Rb 1, and withaferin A.

against digoxin. More specific monoclonal antibody-based digoxin immunoassays are virtually free from interference of Asian and Siberian ginseng (12). Withaferin A, a major biochemical constituent of Ashwagandha, has structural similarity with digoxin (Fig. 1). Other novel withanolides have been isolated and characterized from Ashwagandha (14,15). We reported earlier that Ashwagandha interferes with serum digoxin measurements by the FPIA on the TDx analyzer, but the Beckman digoxin assay that uses a monoclonal antibody against digoxin demonstrated minimal interference (16). Recently the Abbott Laboratories (Abbott Park, IL) released a new digoxin assay (Digoxin III) for application on the AxSYM analyzer (Abbott Park, IL). We studied the potential interference of Asian ginseng, Siberian ginseng, and Ashwagandha with serum digoxin measurement by the new Digoxin III assay, which uses a polyclonal antibody against digoxin and compared the results obtained by Tina-quant Digoxin assay (Roche Diagnostics, Indianapolis, IN), which utilizes a monoclonal antibody against digoxin in the assay design. Here, we report our findings.

MATERIALS AND METHODS

Two different brands of Asian ginseng were used in this study. The first brand of Asian ginseng was Song Shiu Pan Panax Ginseng manufactured in Shanghai, China. The second brand of Asian ginseng was also manufactured in China (location unknown) and distributed by Peace of Heaven Inc. (Hayward, CA). Only one brand of Siberian ginseng (Z-T brand liquid extract manufactured in China and distributed by Z-T Universal Inc. Glen Head, NY) is available in the local Houston market as revealed by surveying several Chinese herbal stores. Two Ashwagandha products 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). The Digoxin III immunoassays were purchased from the Abbott Laboratories and the assays were run on the AxSYM analyzer also available from the Abbott Laboratories. The Tina-quant digoxin assays (Roche Diagnostics) were run on the Integrated Modular System.

Neither Digoxin III nor Tina-quant digoxin assay requires any specimen pre-treatment. The new Digoxin III assay is linear up to a serum digoxin concentration of 4.0 ng/ml and the sensitivity of the assays is 0.30 ng/ml. The Tina-quant assay is linear up to a serum digoxin concentration of 5.0 ng/ml and the sensitivity of the assay is 0.15 ng/ml. Therefore, any value less than the sensitivity of the assay was considered as "none-detected."

In the first set of experiments microliter quantities of liquid extract of these herbal supplements were added to milliliter amounts of aliquots of drug-free serum. These concentrations mimic in vivo concentration after recommended doses of use; the higher concentrations represent those for suspected overdose as described earlier (12,16). After supplementation, apparent digoxin concentrations were measured in triplicate using Digoxin III and Tina-quant assays. In order to ensure that the small amount of alcohol present in the extract did not affect the individual immunoassay, appropriate extracts were placed into a glass test tube and the organic phase was evaporated using nitrogen at room temperature. The almost dry 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, we prepared four digoxin serum pools by combining serum specimens from patients receiving digoxin after removing the patient identities. These specimens are routinely submitted to our laboratory for therapeutic drug monitoring. Left over specimens were used after performing and reporting all results to the ordering clinicians and after holding these specimens for one week as required by our laboratory protocol. Aliquots of the digoxin pool were further supplemented with Asian ginseng, Siberian ginseng, or Ashwagandha and digoxin concentrations were measured again by Digoxin III and Tina-quant assays and the values were compared with the original digoxin concentration observed in the respective digoxin pools. Each measurement was performed in triplicate and the values expressed as the mean and one standard deviation.

In another set of experiments we studied the possibility of overcoming interference of Asian ginseng, Siberian ginseng, or Ashwagandha in serum digoxin measurement by measuring free digoxin concentrations. For this purpose, aliquots of another serum digoxin pool (Digoxin Pool 4) were supplemented with 10, 25, or 50 µl of each herbal supplement extract per milliliter of the digoxin pool. Then total and free digoxin concentrations were measured using the Digoxin III assay. Protein-free ultrafiltrate was prepared by centrifuging each specimen using a Centrifree Micropartition System filter (Amicon, Danvers, MA) for 30 min at $1{,}500 \times g$ and free digoxin concentration was measured in the protein-free ultrafiltrate.

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

RESULTS

When aliquots of drug-free serum were supplemented with Asian ginseng, Siberian ginseng, or Ashwagandha, significant apparent digoxin concentrations were observed when Digoxin III assay was used for serum digoxin measurement, but no apparent digoxin concentration was observed when serum digoxin concentrations were measured by the Tina-quant assay, except for highest concentration of Ashwagandha supplemented aliquots of the drug-free serum pool. For example, when aliquots of drug-free serum pool were supplemented with 25 or 50 µl of Asian ginseng (Brand 1) extract per milliliter of serum, the observed apparent digoxin concentrations were 0.35 and 0.47 ng/ml, respectively. When other aliquots of drug-free serum pool were supplemented with 25 or 50 µl of Siberian ginseng extract per milliliter of serum, the observed apparent digoxin concentrations were 0.31 and 0.41 ng/ml, respectively. Similarly, when other aliquots of drug-free serum pool were supplemented with 50 µl Ashwagandha extract per milliliter of the serum, the observed apparent digoxin concentration was 0.59 ng/ml by the Digoxin III assay (Brand 1) and 0.47 ng/ml (Brand 2), respectively. Modest apparent digoxin concentrations (0.37 ng/ml with the Brand 1 product and 0.33 ng/ml with the Brand 2 product) using the Tina-quant assay were also observed when aliquots of drug-free serum pool were supplemented with 50 µl of Ashwagandha per milliliter of serum (high Ashwagandha concentration expected only in case of severe overdose) (Table 1).

When aliquots of the digoxin pool prepared from patients receiving digoxin were further supplemented with Asian ginseng, Siberian ginseng, or Ashwagandha, we observed falsely elevated digoxin concentrations when measured by the Digoxin III assay, but no significant difference was observed between the digoxin value in the original pool and digoxin values observed when Tina-quant assay was used for serum digoxin measurements except for with Ashwagandha. In this case, with the highest amount of Ashwagandha concentration (expected only with severe overdose), the concentration of digoxin increased significantly from the digoxin concentration of the original pool. For example, the digoxin concentration in the Digoxin Pool 3 increased from 1.63 to 1.90 ng/ml in the presence of 50 µl of Ashwagandha per milliliter of the digoxin pool. With lower concentrations of Ashwagandha as expected in individuals taking this supplementation, no statistically significant increases in the digoxin concentration

TABLE 1. Cross-Reactivity of Asian Ginseng, Siberian Ginseng, and Ashwagandha With the New Digoxin III Assay and Tina-Quant Assay

Specimen	Digoxin III Digoxin (ng/ml) Mean (SD), $n = 3$	% Increase[a]	Tina-quant Digoxin (ng/ml) Mean (SD), $n = 3$	% Increase[b]
Drug-free serum	None detected		None detected	
+10 µl/ml Asian ginseng, Brand 1	None detected	0.0	None detected	0.0
+25 µl/ml Asian ginseng, Brand 1	0.35 (0.02)	16.6	None detected	0.0
+50 µl/ml Asian ginseng, Brand 1	0.47 (0.03)	56.6	None detected	0.0
+10 µl/ml Asian ginseng, Brand 2	0.31 (0.01)	3.3	None detected	0.0
+25 µl/ml Asian ginseng, Brand 2	0.40 (0.05)	33.3	None detected	0.0
+50 µl/ml Asian ginseng, Brand 2	0.57 (0.02)	90.0	None detected	0.0
+10 µl/ml Siberian ginseng	None detected	0.0	None detected	0.0
+25 µl/ml Siberian ginseng	0.31 (0.01)	3.3	None detected	0.0
+50 µl/ml Siberian ginseng	0.41 (0.03)	96.7	None detected	0.0
+10 µl/ml Ashwagandha, Brand 1	None detected	0.0	None detected	0.0
+25 µl/ml Ashwagandha, Brand 1	0.39 (0.01)	30.0	None detected	0.0
+50 µl/ml Ashwagandha, Brand 1	0.59 (0.04)	96.7	0.37 (0.06)	146.6
+10 µl/ml Ashwagandha, Brand 2	None detected	0.0	None detected	0.0
+25 µl/ml Ashwagandha, Brand 2	0.31 (0.01)	3.3	None detected	0.0
+50 µl/ml Ashwagandha, Brand 2	0.47 (0.05)	56.6	0.33 (0.06)	120.0

[a] Percentage increase calculated by subtracting 0.30 (detection limit of Digoxin III assay) from the observed value then divide the difference by 0.30 in order to calculate the percentage increase.

[b] Percentage increase calculated by subtracting 0.15 (detection limit of Tina-quant assay) from the observed value then divide the difference by 0.15 in order to calculate the percentage increase.

TABLE 2. Effect of Supplementing Aliquots of Digoxin Pools With Various Ginsengs on Serum Digoxin Measurements by Digoxin III and Tina-Quant Digoxin Assays

Specimen	Digoxin III Digoxin (ng/ml) Mean (SD), n = 3	% Increase[b]	Tina-quant Digoxin (ng/ml) Mean (SD), n = 3
Digoxin Pool 1	1.10 (0.05)		1.03 (0.06)
+10 µl/ml Asian ginseng, Brand 1	1.26 (0.03)[a]	14.5	1.00 (0.00)
+25 µl/ml Asian ginseng, Brand 1	1.37 (0.02)[a]	24.5	1.07 (0.06)
+50 µl/ml Asian ginseng, Brand 1	1.51 (0.01)[a]	37.2	1.03 (0.06)
+10 µl/ml Asian ginseng, Brand 2	1.39 (0.01)[a]	26.4	1.03 (0.06)
+25 µl/ml Asian Ginseng, Brand 2	1.49 (0.06)[a]	35.5	1.07 (0.06)
+50 µl/ml Asian ginseng, Brand 2	1.63 (0.09)[a]	48.2	0.97 (0.06)
+10 µl/ml Siberian ginseng	1.20 (0.05)[a]	9.0	1.00 (0.00)
+25 µl/ml Siberian ginseng	1.33 (0.03)[a]	20.9	0.97 (0.06)
+50 µl/ml Siberian ginseng	1.43 (0.01)[a]	30.0	0.93 (0.06)
Digoxin Pool 2	1.08 (0.08)		0.97 (0.06)
+10 µl/ml Ashwagandha, Brand 1	1.33 (0.07)[a]	23.1	1.03 (0.06)
+25 µl/ml Ashwagandha, Brand 1	1.46 (0.06)[a]	35.2	1.10 (0.10)
+50 µl/ml Ashwagandha, Brand 1	1.63 (0.03)[a]	50.9	1.30 (0.10)[a]
+10 µl/ml Ashwagandha, Brand 2	1.21 (0.04)[a]	12.0	0.97 (0.06)
+25 µl/ml Ashwagandha, Brand 2	1.39 (0.08)[a]	28.7	0.97 (0.06)
+50 µl/ml Ashwagandha, Brand 2	1.45 (0.06)[a]	34.3	1.00 (0.00)
Digoxin Pool 3	1.74 (0.01)		1.63 (0.06)
+10 µl/ml Ashwagandha, Brand 1	1.97 (0.01)[a]	13.2	1.67 (0.06)
+25 µl/ml Ashwagandha, Brand 1	2.08 (0.07)[a]	19.5	1.73 (0.06)
+50 µl/ml Ashwagandha, Brand 1	2.21 (0.05)[a]	27.0	1.90 (0.10)[a]
+10 µl/ml Ashwagandha, Brand 2	1.84 (0.02)[a]	5.7	1.60 (0.10)
+25 µl/ml Ashwagandha, Brand 2	1.93 (0.06)[a]	10.9	1.63 (0.06)
+50 µl/ml Ashwagandha, Brand 2	2.08 (0.09)[a]	19.5	1.70 (0.06)

[a]Significantly greater than the corresponding value of the digoxin pool by independent *t*-test two tailed ($P < 0.05$).
[b]Percentage increase in digoxin value as measured by Digoxin III assay in the presence of various ginsengs compared with the digoxin value of the serum pool. Percentage increase in digoxin values as measured by Tina-quant assay is negligible and not shown.

was observed, indicating that the Tina-quant assay is virtually free from interference of Ashwagandha (Table 2).

In a separate experiment we studied the potential of eliminating the interference of Asian ginseng, Siberian ginseng, and Ashwagandha in serum digoxin measurement by measuring free digoxin concentrations using the new Digoxin III assay. Because the interferences of these herbal supplements in serum digoxin measurements by the Tina-quant assay were minimal, we did not perform any additional experiments to investigate the effect of these herbal supplements on free digoxin measurements using the Tina-quant assay. Our results indicate that interference of Asian ginseng, Siberian ginseng, and Ashwagandha on serum digoxin measurement by the Digoxin III assay cannot be eliminated by measuring free digoxin concentrations. For example, the concentrations of total and free digoxin in the Digoxin Pool 4 were 1.24 and 0.83 ng/ml, respectively. In the presence of 10 µl of Ashwagandha Brand 1 extract, the total and free digoxin concentrations were 1.61 and 0.93 ng/ml, respectively. In the presence of 25 µl of Ashwagandha Brand 1 extract, the corresponding total and free digoxin concentrations were 1.78 and 1.04 ng/ml, respectively (Fig. 2).

DISCUSSION

The US Food and Drug Administration (FDA) regulates drugs and requires that drugs should be both safe and effective. Most complementary and alternative medicines are classified as dietary supplements or foods

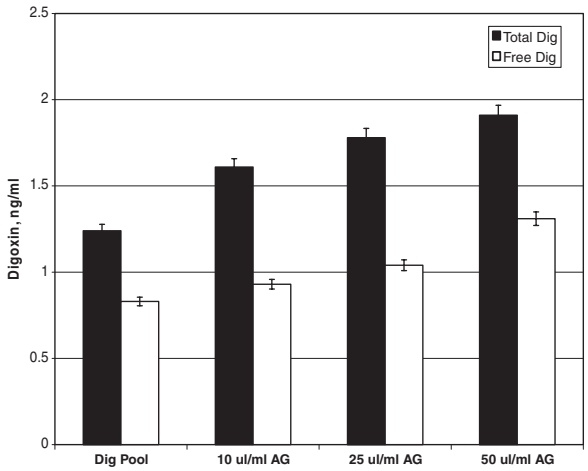


Fig. 2. Effect of supplementing aliquots of a digoxin pool (Digoxin Pool 3) with various amounts of Ashwagandha Brand 1 on total and free digoxin concentrations as measured by the Digoxin III assay. AG: Ashwagandha Brand 1.

and are marketed pursuant to the Dietary Supplement Health and Education act of 1994. FDA does not require documentation of efficacy of herbal supplements as long as these products do not claim any treatment benefit. Complementary and alternative medicines including Ayurvedic medicines are becoming increasingly popular in the United States, Europe, and other parts of the world. These products are freely available to the general population from health food stores and herbal drug stores without a prescription. In developing countries, as much as 80% of the indigenous populations use traditional systems of medicines. Within the European market, herbal medicines represent an important pharmaceutical market with annual sales of seven billion US dollars. In the United States, the sale of herbal medicine increased from 200 million dollars in 1988 to over 3.3 billion dollars in 1997 (17). Most people consider herbal medicines safe and do not report their physicians regarding use of herbal supplements. Abnormal test results can be observed in individuals taking herbal supplements including unexpected test results in therapeutic drug monitoring in patients who demonstrated therapeutic concentrations of a drug before (18).

Interferences in digoxin immunoassays are common from variety of factors including digoxin-like immunoreactive factors, spironolactone, potassium canrenoate, and related steroids as well as complementary and alternative medicines (19–21). Therefore, an ideal digoxin immunoassay should be free from potential cross-reactants. Our data indicate that Asian ginseng, Siberian ginseng, and Ashwagandha cross react modestly with the new Digoxin III assay. This may be because of the fact that digoxin assay utilizes a polyclonal antibody in the assay design. In contrast, the Tina-quant digoxin assay, which uses a specific monoclonal antibody against digoxin, is not affected by Asian or Siberian ginseng and virtually free from interference of Ashwagandha.

Although interferences of all three herbal supplements to the new Digoxin III assay are modest, the magnitude of interferences varied between two brands of Asian ginseng and two brands of Ashwagandha. Because herbal remedies are not prepared using the rigorous standard of pharmaceutical products, significant variations of active ingredients are common with herbal products. In one study authors reported that the hypericin content, the active component of St. John's wort, varied from 0.03 to 0.29% among eight brands of commercially available St. John's wort (22).

One limitation of this study is that our results are preliminary in vitro data. Owing to potential toxicity of these herbal supplements we did not peruse any experiments with human volunteers. We conclude that components of Asian ginseng, Siberian ginseng, and Ashwagandha modestly interfere with serum digoxin measurements by the new Digoxin III assay in vitro. On the other hand, presence of apparent digoxin activity in serum of a patient with suspected ginseng or Ashwagandha overdose but not taking digoxin (as measured by the Digoxin III) may indirectly further validate the suspected overdose. However, for medical legal cases, this initial finding should be confirmed by a more sophisticated analytic technique such as high-performance liquid chromatography combined with tandem mass spectrometry. Recently, Yang et al. described a high-performance liquid chromatography-electrospray ionization-tandem mass spectrometric method for determination of ginsenoside in human plasma (23). Khajuria et al. described a method for quantification of selected withanolides including withaferin A from Ashwagandha using high-performance liquid chromatography and UV (DAD) as well as positive ion electrospray ionization mass spectrometry (24).

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