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Immunoanalysis of Oleandrin; Study the Cross-Reactivity Oleandrin/Digoxin for the Technique KIMS

[1]Said Nadji, [2]Abderahmen Abdaoui, [1]Hanane Ouerdane, [3]Mohamed Azzouz, [1]Rania Abtroun, [3]Mohamed Reggabi and [4]Bachra Alamir

[1]Laboratory of Toxicology, University Hospital of Bab El Oued, 53 Boulevard Said Touati, Algiers, Algeria
[2]Laboratory of Toxicology, Military Central Hospital, Ain Naadja, Algiers, Algeria
[3]Laboratory of Biology and Toxicology, Hospital Ait Idir, Boulevard Abderrezak Hadad, Algiers, Algeria
[4]National Center of Toxicology, Route Petit Staoueli NIPA, Dely Brahim, Algiers, Algeria

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Corresponding Author:
Said Nadji,
Laboratory of Toxicology,
University Hospital of Bab El Oued,
53 Boulevards Said Touati,
Algiers, Algeria

ABSTRACT

The chemical structure of oleandrin is similar to that of digoxin, this similarity is the cause of cross-reactivity in immunoanalysis. The objectives of this study was to evaluate the performance of the technique KIMS (Kinetic interaction of microparticules in solution) for the determination of oleandrin using a cassette designed for the assay of digoxin, calculation of cross-reactivity between oleandrin and digoxin, as well as between components of the leaves of the extractum of Nerium *oleander* and digoxin by KIMS. Concentration levels were prepared by spiking increasing amounts of oleandrin in free serum and into a pool of serums from patients treated by digoxin to study the correlation oleandrin/apparent concentration of digoxin and the cross-reactivity between oleandrin and digoxin respectively. A pool of plasma from patients treated by digoxin was loaded with increasing concentrations of a Nerium *oleander* leaves extractum for the study of cross-reactivity between Nerium *oleander* leaves extractum components and digoxin. The cross-reactivity between oleandrin and digoxin was higher for the KIMS technique. The average cross-reactivity between oleandrin and digoxin was 11.445%, while that between the leaves of the extractum of Nerium oleander and digoxin was 12.842%. The cross-reactivity between oleandrin, digoxin for the digoxin assay was huger for the technique KIMS, it can be exploited to diagnose oleander poisoning cases, the cross-reactivity between the molecules of Nerium *oleander* and digoxin for the KIMS technique was mainly due to oleandrin.

Key words: Oleandrin, digoxin, immunoassay, KIMS, cross-reactivity, Nerium *oleander*

INTRODUCTION

The poisoning with Nerium *oleander* may be serious and occurs after the ingestion of the plant material especially the leaves[1], the clinical picture of intoxication was similar to that of digitalis and can be a source of confusion for diagnosis[2]. Analysis of biological samples remains the only effective way to identify and quantify the incriminated molecules, immunoassay techniques were widely used because of their low cost and ease compared to chromatographic techniques, however they remain less specific[3]. Cross-reactivity between oleandrin and digoxin was common to immunochemical techniques for the determination of digoxin; however, it differs from one technique to another[4]. The technique KIMS is widely used in laboratories for the determination of

digoxin, as an homogeneous phase immunoassay based on the measurement of variations in the scattered light or the resulting absorbance during the aggregation of activated microparticles.

The microparticles are coated with digoxin, they aggregate rapidly in the presence of an anti-digoxin antibody solution. When introducing a sample containing digoxin, the aggregation reaction is partially inhibited. The antibodies bind to the drug of the sample, which makes them then no longer free to cause the aggregation of the particles and the formation of the agglomerate of particles is therefore hindered[5]. The objectives of this study were:

- Evaluate the cross-reactivity between oleandrin and digoxin for digoxin assay by KIMS
- Evaluate the cross-reactivity between the components of the leaves extract of Nerium *oleander* and the digoxin
- Evaluate the performance of the technique KIMS for the determination of oleandrin using a cassette made for the assay of digoxin

MATERIAL AND METHOD

Instrumentation:

- COBAS INTEGRA 400 plus. (Measurement mode: Absorbance, wavelength: 652 nm)
- Vortex
- Centrifuge: Nüve Dikkat
- Balance: maximum Kern BLT100 100 g, d = 0.01 mg

Reagents:

- Oleandrin standard; CAS number: 465-16-7, laboratory: Phytolab GmbH and Co. KG, Vestenbergsgreuth, Germany, quantity: 10 mg, batch: 6922, chromatographic purity: 97.48%
- HPLC quality methanol from Scharlau, batch number: 13087014
- Plasma pool of 10 patients taking digoxin treatment provided by the National Center of toxicology
- Fresh frozen plasma provided by the blood transfusion center of the university-hospital of Bab El Oued
- Cassette for the assay of digoxin
- Paradigm calibrators Preciset TDM calibrators
- Quality control type "Roche TDM OnLine controls"

Development: To study the correlation oleandrin/apparent concentration in digoxin, the chosen concentrations points were prepared by loading plasma with oleandrin at concentrations 10, 25, 50 and 100 ng mL^{-1}, while that the concentration 0 ng mL^{-1} corresponds to the oleandrin-free plasma.

The cross-reactivity between oleandrin and digoxin was studied by loading the plasma pool of patients treated by digoxin with increasing amounts of oleandrin to obtain the concentrations: 10, 25, 50 and 100 ng mL^{-1}, point 0 corresponds to the value obtained with the plasma pool of patients treated with digoxin (oleandrin-free). For the study of the cross-reactivity digoxin/leaves extractum of Nerium *oleander*, the extract of leaves of a shrub from the Hamdania region (Algeria), whose oleandrin concentration was previously determined by a validated chromatographic method (SFSTP protocol) and which gave a content of 1015.04 ppm[6], was diluted to one thousandth (1/1000) with methanol. The plasma pool of patients on digoxin was loaded with Nerium *oleander* extractum solution supposed to contains 1 ppm of oleandrin to prepare solutions whose concentrations: 10, 25, 50 and 100 ng mL^{-1}, point 0 corresponds to the value obtained of the plasma pool of patients under digoxin treatment (free of Nerium *oleander* extractum). The results were compared with similar published studies.

RESULTS AND DISCUSSION

Correlation oleandrin/apparent concentration in digoxin:

The apparent concentrations obtained with the assay of 3 replicates from each concentration point of the increasing concentrations of oleandrin with digoxin cassette are obtained in the (Table 1), the results are expressed as mean±standard deviation.

In order to evaluate the performance of the technique, the obtained results were compared with those published in the literature. In a comparative study[7], three immunological techniques were compared for oleandrin/digoxin cross-reactivity: FPIA (Abbott Laboratories) and two recent techniques Digoxin II and Digoxin III AxSYM analyzer (Abbott Laboratory).

Table 1: Apparent concentrations corresponding to oleandrin

Oleandrin concentration (ng mL^{-1})	Apparent concentration in digoxin (ng mL^{-1}) (n = 3)
0	0.252±0.040
10	1.057±0.06
25	2.320±0.07
50	4.483±0.09
100	8.608±0.107

Mean±Standard Deviation

Indeed, the results obtained show that the cross-reactivity between oleandrin and digoxin was more marked with the technique KIMS. For a concentration of 10 ng mL^{-1} of oleandrin, the apparent concentration obtained was 1.057 ng mL^{-1}. A similar result was obtained with the study of Amitava *et al.*[7], but with an oleandrin concentration of 1000 ng mL^{-1} for the techniques FPIA and Digoxin III, which represents a difference of 1/100, this difference becomes approximately 1/200 while increasing the concentration of oleandrin. In fact, For the KIMS technique 50 ng mL^{-1} of oleandrin gives an apparent digoxin concentration of 4.483 ng mL^{-1}, close values were obtained with a concentration of 10000 ng mL^{-1} for the FPIA and Digoxin III techniques (4.94 and 3.31 ng mL^{-1}, respectively)[7].

Another similar study which interested on the apparent digoxin concentration obtained with concentrations of oleandrin reveals that, for a drug-free serum supplemented with 250 ng mL^{-1} of oleandrin, the observed apparent digoxin concentrations obtained by Dimension Vista, FPIA, EMIT and Tina Quant digoxin assays, were 1.2, 1.3, 0.9 ng mL^{-1} and none detected, respectively[8]. Moreover, a further one found that spiked serum with an oleandrin concentration of 1000 ng mL^{-1}, gave apparent concentration of digoxin around 2.2 ng mL^{-1} with Dimension Vista assay, 2 ng mL^{-1} using the FPIA and 0.7 ng mL^{-1} with Tina Quant assay[9]. Beside it was shown that, the magnitude of the cross-reactivity between oleandrin and digoxin was approximately 65% less for the Beckman Coulter technique comparing to FPIA[9].

A recent study, shows a marginal cross-reactivity between oleandrin and digoxin for the assay of digoxin related to the techniques Siemens Immulite, Roche 501, Abbot AxSym, Abbott Architect and Roche 601, which gave approximately: 6, 3.5, 4, 1.5, 1.5 ng mL^{-1}, respectively for a concentration of 100000 ng mL^{-1} of oleandrin, these results show a very low sensitivity of these assays for the detection of oleandrin[10]. By comparing all these assays to KIMS, it may be noted that, KIMS has the highest sensitivity to determine oleandrin with a digoxin assay due to its highest cross-reactivity.

Other studies reported the feasibility to use digoxin assays in order to detect or quantify cardiac glycosids as convallatoxin, that gave an apparent concentration of digoxin of 0.50, 0.88, 1.70, 2.55 ng mL^{-1} for concentrations of convallatoxin of: 50, 100, 250, 500 ng mL^{-1}, respectively for the luminescent oxygen channeling technology-based Digoxin assay (Siemens Diagnostics)[11]. A similar study with oleandrin gave apparent concentrations of digoxin of: 0.11, 0.15, 0.19 and 0.46 ng mL^{-1} for the same range of concentrations (from 50 to 500 ng mL^{-1})[12], that revealed the highest sensitivity to convallatoxin comparing to oleandrin, which shows less signal, nevertheless, the technique ADVIA Centaur Digoxin showed no sensitivity to convallatoxin in the range from 10 to 100 ng mL^{-1}, where it was none detected[13].

The diagnosis of poisoning with oleander generally uses HPLC methods coupled with mass spectrometer to quantify the concentration of oleandrin in biological samples[14,15], however, these methods remain complex and have a high cost. The sensitivity offered by the KIMS technique for the assay of oleandrin allows an alternative, calibration curve could be drawn and the result of the oleandrin concentration may be given as apparent concentration in digoxin.

Cross reactivity oleandrine/digoxin: The results obtained with KIMS technique show an additive type cross-reaction because apparent concentrations of digoxin corresponds approximately to the addition of the apparent concentrations obtained with oleandrin alone to the fixed value which was in fact the concentration of digoxin in the plasma pool (1.181 ng mL^{-1}, Table 2).

The formula to calculate the cross-reactivity is given as:

Cross-reactivity (%) = 100 × (Apparent concentration obtained−Concentration of the analyte)/Concentration of the interferent[16] (1)

The results of the calculations are shown in Table 3.

The average cross-reactivity expressed as a percentage between oleandrin and digoxin for the technical KIMS turns around 11.445%. By comparison to the technique luminescent oxygen channeling technology-based Digoxin assay, where the cross-reactivity as a bias was negative (-3.1 and -12.2% for

Table 2: The values of apparent concentrations in digoxin as a function of the concentrations oleandrin (pool of plasma from patients taking digoxin)

Oleandrin concentration (ng mL^{-1})	Apparent concentration in digoxin (ng mL^{-1}) (n = 3)
0	1.181±0.07
10	2.447±0.06
25	4.042±0.113
50	6.960±0.134
100	11.270±0.122

Mean±Standard Deviation

Table 3: Calculation of the oleandrin/digoxin cross-reactivity expressed as a percentage

Oleandrin concentration (interferent) (ng mL^{-1})	Digoxin concentration (analyte) (ng mL^{-1})	Cross-reactivity (%)
10	1.181	12.660
25		11.444
50		11.588
100		10.089
Average of the cross-reactivity		11.445

Mean±Standard Deviation

Table 4: Apparent concentrations in digoxin according to concentrations of oleandrin in the extractum of leaves

Oleandrin concentration in Nerium *oleander* leaves extractum (ng mL^{-1})	Apparent concentration in digoxin (ng mL^{-1}) (n=3)
0	1.181±0.05
10	2.784±0.09
25	4.591±0.15
50	7.020±0.12
100	11.204±0.07

Mean±Standard Deviation

Table 5: Calculation of cross-reactivity between the molecules of leaves extractum/digoxin expressed as a percentage

Oleandrin Concentration in Nerium *oleander* leaves extractum (ng mL^{-1})	Cross-reactivity (%)
10	16.03
25	13.64
50	11.678
100	10.023
Average of the cross-reactivity	12.841

Mean±Standard Deviation

the concentration 50 and 100 ng mL^{-1} of oleandrin, while that digoxin concentration was 0.98 ng mL^{-1})[12], the cross-reactivity obtained for the techniques FPIA and Vista (Flex cartridge) was around 1% for concentration of oleandrin 50 and 100 ng mL^{-1}, whereas, it was around 0.5% for EMIT and 0% for Tina Quant and with negative values (-3.1, -12.2%) for the luminescent oxygen channeling technology-based Digoxin assay[8,12].

Cross-reactivity between leaves extractum from Nerium *oleander* / digoxin: Comparison of the results obtained with the oleander extract loaded into the plasma pool of patients taking digoxin and those obtained with oleandrin solely, show similar values of apparent concentrations in digoxin (Table 2, 4). The difference between the apparent digoxin concentrations obtained with the extractum of Nerium *oleander* and those obtained with oleandrin alone was more marked with lower concentrations of oleandrin (10 and 25 ng mL^{-1}) with 0.337 and 0.549 ng mL^{-1}, respectively, becomes insignificant while increasing concentrations of oleandrin (0.06 and 0.066 ng mL^{-1}) for 50 and 100 ng mL^{-1} of oleandrin, respectively. The average of the differences between apparent concentration in digoxin for leaves extractum of Nerium *oleander* and oleandrin are 0.22 ng mL^{-1}. The cross-reactivity between the leaves extractum of Nerium *oleander* and digoxin (12.842%) for the KIMS technique is close to that noted between oleandrin and digoxin (11.445%) (Table 3, 5). Therefore the cross-reactivity in the assay of leaves extractum is mainly due to oleandrin, which represents the major heteroside of the leaves extractum of Nerium *oleander*. On the other hand, The technique luminescent oxygen channeling technology- based Digoxin assay, gave a bias of (-6.1, +21.1 and +74.6%) for concentration of 0.1, 0.5 and 1 µL mL^{-1} of oleander extract[12].

CONCLUSION

The KIMS technique has a higher cross-reactivity between digoxin and oleandrin (11.445%) that may lead to overestimation of the concentration of digoxin in blood. Besides, it is more sensitive compared to more recent techniques (FPIA, Digoxin II, Digoxin III) for the detection of oleandrin, this sensitivity can be exploited for the detection of Nerium *oleander* intoxication and provides speed, ease and lower cost compared to chromatographic techniques. The cross-reactivity between Nerium *oleander* extractum and digoxin for the KIMS technique is mainly due to oleandrin, this is explained by the fact that oleandrin is the major constituent.

REFERENCES

1. Huang, K.C., 1998. The Pharmacology of Chinese Herbs. 2nd Edn., CRC Taylor and Francis, Boca Raton, pp: 544.
2. David, W.N., 1997. Poisonous plants and animals of Florida and the Caribbean. Pineapple Press Inc., Florida, pp: 315.
3. Amitava, D. and D. Pradip, 2004. Rapid detection of oleander poisoning by using digoxin immunoassays: Comparison of five assays. Ther. Drug. Monit., 26: 658-663.
4. Amitava, D., 2009. Advances in Chromatographic Techniques for Therapeutic Drug Monitoring. CRC Taylor and Francis, Boca raton, pp: 475.
5. Amitava, D., 2011. Health Educator's Guide to Understanding Drugs of Abuse Testing. Jones and Bartlett Learning, Boston pp: 208-210.
6. Said, N., A. Abderahman and O. Hanane, 2017. Determination of Oleandrin levels by HPLC-DAD invegetalmaterial collected throughout Algeria and the study of some Influencing factors. Res. J. Phytoch., 11: 74-84.
7. Amitava, D., S.A. Risin, M. Reyes and J.K. Actor, 2008. Rapid detection of oleander poisoning by Digoxin III, a new digoxin assay. Am. J. Clin. Pathol., 129: 548-553.
8. Amitava, D. , K. Kimberley, A.R. Semyon and K.A. Jeffrey, 2011. Rapid detection of oleander poisoning by dimension vista digoxin assay (Flex Reagent Cartridge). J. Clin. Lab. Anal., 25: 105-109.
9. Amitava, D., 2011. Effects of Herbal Supplements on Clinical Laboratory Test Results. Walter de Gruyter, Houston, pp: 449-459.
10. Jamie, M.E., A.K. Yumi, D. Barbara, W. Amer and D. Amitava, 2015. The iDigoxin assay is more sensitive than LOCI digoxin assay for rapid detection of convallatoxin, the active cardiac glycoside of lily of the valley. Ann. Clin. Lab. Sci., 45: 323-326.

11. Amitava, D., J.W. Kerry, A.H. Shen, J. Myrtle and K.A. Jeffrey, 2014. Bidirectional (Negative/Positive) Interference of Oleandrin and Oleander Extract on a Relatively New LOCI Digoxin Assay Using Vista 1500 Analyzer. J. Clin. Lab. Anal., 28: 16-20.
12. Kerry, J.W., S.P.H. Richard, K.A. Jeffrey and D. Amitava, 2014. Rapid detection of the active cardiac glycoside convallatoxin of lily of the valley using LOCI Digoxin assay. Am. J. Clin. Pathol., 142:307-312.
13. Amitava, D. and B. Laverne, 2018. Convallatoxin, the active cardiac glycoside of lily of the valley, minimally affects the ADVIA Centaur digoxin assay. J. Clin. Lab. Anal., 32: e22583.
14. Antoine, T., K. Pascal, F. Branche and L. Bertrand, 1998. Confirmation of oleander poisoning by HPLC/MS. Int. J. Legal Med., 111: 32-40.
15. Ibrahim, A.W., Z. Omar, A.A. Nawal and A.A. Anwar, 2008. A fatal case of oleandrin poisoning. Forensic Sci. Int., 179: e31-36.
16. Nccls, N.C., 1986. Interference Testing in Clinical Chemistry; Proposed Guideline, Villanova, pp: 6.