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ORIGINAL ARTICLE

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Pharmacokinetics of Oleandrin Following Administration of a Nerium oleander Extract in Mice

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ABSTRACT

Background. *Nerium oleander* Linn is native of Mediterranean regions of Africa and Europe, where it is used in folk medical practices. The whole plant is known to be toxic. Various studies have shown its antimicrobial and anticancer properties. Oleandrin, the main toxic component in *Nerium oleander* is an inhibitor of P-glycoprotein. Cardiac glycosides, including oleandrin, are substrates of P-glycoprotein. The presence of other inhibitors in the alcoholic extract may potentially modify the pharmacokinetics of oleandrin. Given the therapeutic potential and the associated toxicity of the alcoholic extract, it is important to investigate the kinetics of oleandrin within this extract. **Purpose**. The objective of this study was to determine the pharmacokinetic parameters in mice following oral and I.V. administrations of a hydroalcoholic extract of *Nerium oleander*. **Methods**. Pharmacokinetic investigations of oleandrin, a cardiotonic glycoside and main active compound in *Nerium oleander*, were conducted in mice following intravenous administration (30 mg/kg) and oral administration (150 mg/kg) of the hydrolcoholic extract *of Nerium oleander*. For the oral route, seven times were chosen for the mice sampling. Four times were chosen for the intravenous route. Three mice per group were used at each time point. In the excretion study, seven mice were housed in a mouse urine collection device and a dose of 150 mg/g was administered. A urine sample was collected after 48 hours. Oleandrin was measured by LC-MS/MS validated method. MS data were acquired in the positive ion electrospray ionization (ESI) mode. Two deuterated internal standards, cocaine-d3 and digoxin-d3, were used. The retention times were 8.47 min for oleandrin, 4.78 min for deuterated internal standard cocaine d3 and 5,37 min for deuterated internal standard digoxin d3. **Results.** The equivalent doses of oleandrin administred to mice were 1710 ug/kg for the oral route and 342 ug/kg for the intravenous route. Oleandrin was rapidly absorbed after oral administration (Cmax at 10 min). The AUC0-inf (ug/L*min) values obtained after intravenous and oral dosing were 34797.7 and 107222 respectively, resulting in an oral bioavailability of 61.6 %. The apparent volume of distribution (Vss) was 0.55 L/kg and Clearance ClT was 0.01 kg* L/min.

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1. INTRODUCTION

Nerium oleander Linn (Figure 1) is a member of the Apocynaceae family. It originates from Mediterranean regions in Africa and Europe (1). N. oleander is an attractive shrub typically reaching heights of 2 to 5 meters, distinguished by smooth stems containing a thick, whitish latex. Its leaves are persistent, leathery, elongated, lanceolate, smooth, and have very short petioles (2). The flower buds, resembling torches, open into fragrant blooms (2). The flowers range in color from white to pink to deep red, each featuring five spreading petals (1).

Figure 1. Nerium oleander

N. oleander is documented in numerous traditional pharmacopeias in its native regions, traditionally employed for treating ailments such as leprosy, malaria, venereal diseases, scabies, skin conditions, diabetes, and as an abortifacient (1–4). The entire plant is toxic, whether fresh or dried, and remains poisonous even after boiling (2). Every part of the plant contains cardiac glycosides, including the stems, leaves, young shoots, flowers, nectar, sap, and residues from combustion (1,5).

The presence of cardiotonic glycosides, particularly oleandrin (Figure 2), renders *N. oleander* similarly toxic to digitalis (6). Ingestion can lead to systemic toxicity, predominantly affecting the heart, digestive system, and nervous system in both humans and animals (1,2,5,6). However, alongside its toxicity, therapeutic potential has been identified. Oleandrin has exhibited neuroprotective and antiglioma effects(7). Extracts from the leaves have demonstrated antimicrobial and anti-cancer properties (2,8–16).

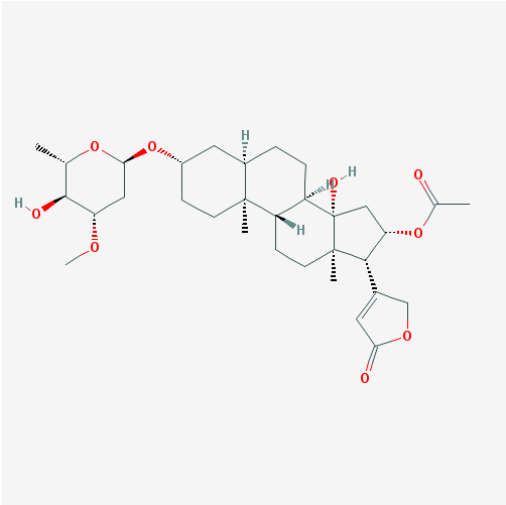

Figure 2. Oleandrin (24)

Some compounds of a methanolic extract of *Nerium oleander* are expected to be lead compounds in development of anti-multidrug resistance (MDR) cancer agents (17). One mechanism of MDR involves P-glycoprotein overexpression (17). P-glycoprotein inhibitors reverse P-glycoprotein mediated efflux enhancing drug transport across the epithelial membrane. These inhibitors influence metabolism, absorption, distribution, and elimination of P-glycoprotein substrates thereby modulating pharmacokinetics(18,19). This action is one of the mechanisms of action for bioenhancers of herbal origin(18). Oleandrin is an inhibitor of P-glycoprotein(7) while cardiac glycosides like digoxine are substrates for P-glycoprotein(20–22). If oleandrin is a substrate of P-glycoprotein, the presence of other inhibitors in the alcoholic extract could modify the pharmacokinetics of oleandrin. Due to the therapeutic interest(17) and the toxicity (23) of the alcoholic extract studying the kinetics of oleandrin within this extract holds significant importance.

The aim of this study is to determine pharmacokinetic parameters in mice following oral and I.V. administrations of hydroalcoholic extract of *Nerium oleander*.

2. MATERIAL AND METHODS

Oleander extract

Following macroscopic and microscopic identification, 270 grams

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of dried *N. oleander* leaves powder was macerated in 70 % ethanol at a ratio of 1 volume of powder to 4 volumes of solvent for 48 hours at room temperature. The resulting hydroalcoholic extract was obtained through filtration. Subsequently, a second filtration was performed on the oleander extract after treatment with 10% lead acetate to remove impurities. Ethanol was removed from the filtrate through evaporation under reduced pressure using a rotavap (Büchi Rotavapor R-200). The resulting extract underwent freeze-drying for 30 hours (Christ®LCG) and was stored in a freezer.

Animals

The subjects were 12-week-old male Swiss mice, obtained from the Department of Pharmacy, Faculty of Medicine, Constantine 3 University, Algeria. Their average weight was 21,8 g ± 20% for the plasma study and 23g ± 20% for the excretion study. Food and water were available ad libitum. The animals were housed in a standard controlled environment with a temperature range of 22° C and relative humidity of 55 %. Experimentally naive mice were used in each experiment, with three mice per group at each time point.

Pharmacological studies in mice

Oral studies: mice were fasted during eight hours and a dose of 150 mg/g was administered by gastric feeding tube. Seven times were chosen for the mice sampling : 10, 20, 30, 40, 60, 120, 180 min. Retro-orbital sampling were performed at defined times. Blood samples were collected in tubes with heparin and centrifuged at 3500 tr/min for 10 min to obtain plasma. The samples obtained in each group were processed together. Plasma (0.2±0.5 ml) was frozen at -60° C until analysis.

I.V. studies : mice were fasted during one hour and a dose of 30 mg/g was administered by intravenous injection into the tail vein after dilatation. Four times were chosen for the mice sampling : 10, 30, 60, 120 min. Retro-orbital sampling were performed at defined times. Blood samples were collected in tubes with heparin and centrifuged at 3500 tr/min for 10 min to obtain plasma. Samples obtained in each group were processed together. Plasma (0.2±0.5 ml) was stored at -60°C until analysis.

Excretion : seven mice were fasted one hour and a dose of 150 mg/g was administered by gastric feeding tube and housed in a mouse urine collection device(25). A urine sample was collected at 48 h. Urine was centrifuged at 3500 tr/min for 10 min and supernatant was stored at -60°C until analysis.

Sample preparation

One mL of sample (oleander extract, plasma, urine) was mixed with 10 µL of the deuterated standard solution (cocaine-d3 [0.5 µg / mL] and digoxin-d3 [2 µg / mL]) and 2 mL of acetonitrile (standards were obtained from Sigma-Aldrich for Oleandrin, LGC Standards for Digoxin-d3 and Lipomed for Cocaine-d3). The mixture was centrifuged at 3000 g for 10 minutes using Fisher Bioblock Scientific®Sigma 2-6E. The ACN fraction of the supernatant was evaporated using Techne®Sample Concentrator 2475-1100500 set at 45°C. Oasis HLB cartridges (3 mL, 60 mg) from Waters® were conditioned with 1 volume of MeOH and of 1 volume deionized water. The supernatant of the pretreated sample was passed through the cartridges. The cartridges were eluted with 3 x 0.5 mL of MeOH, the solvent was then evaporated under 45 ° C and the extract was redissolved in 50 µl of mobile phase buffer (5 mM acetate buffer pH = 4.5 / ACN, 90/10), vortexed and centrifuged at 14000 g during 4 min. The supernatant was analysed.

LC-MS/MS analysis

An UHPLC-HRMS / MS on Ultimate 3000 (Dionex®) coupled with a Q-Exactive (Thermo®) was used in all analysis. The analytical column was a C18 2,1 x 150 mm (1,8 um) (Waters® UPLC Acquity HSS). The injection volume was 10 μL. The mobile phase consisted of 2 mM formate buffer pH = 3.0 / ACN 0.1% formic acid at a flow rate of 0,4 mL/min under a gradient of 5% to 95% of ACN 0.1% formic acid over 17 min. All the reagents were of LC-MS grade. The retention times were 8.47 min for oleandrin, 4.78 min for deuterated internal standard cocaine d3 and 5,37 min for deuterated internal standard digoxin d3. MS data were acquired in the positive ion electrospray ionization (ESI) mode, using the following conditions: sheath gaz flow rate : 60 ; sweep gaz flow rate : 5 ; spray voltage (|kV|) : 2 ; capillary temp (°C) = 300 ; gaz heater temp (°C) : 250 ; scan type : full MS ; duration : 0.5 – 11 min ; scan range : 120 – 1050 m/z.

The mass transitions were 577,3381 and 578,3410 m/z for oleandrin, 801,4801 and 802,4833 m/z for digoxin-d3, 307,1737 m/z for cocain-d3.

Pharmacokinetic analysis

The computer software PK Solver 2.0 was used for calculation of pharmacokinetic parameters ($t_{1/2}$, T_{max}, C_{max}, $AUC_{0±1}$, Cl_T, and V_{ss}) through standard non compartmental analysis of plasma concentration-time data.

3. RESULTS

Oleander extract

The amount of oleandrin in the extract was 11,4 g of oleandrin by kg of extract. The equivalent doses of oleandrin administred in mice are then 1710 ug/kg for P.O. and 342 ug/kg for I.V.

Plasma oleandrin in mice after I.V. and P.O. administration of extract

Oleandrin concentrations in plasma after I.V. and P.O. administration of the hydrolacoholic extract are presented in Figure 3 and calculated from the eq dose of oleandrin. After P.O. administration, oleandrin was rapidly absorbed with a maximum concentration (Cmax) of 1471,3 ug/L achieved the first time sampling 10 min (Tmax). The oral bioavailability (F) was 61,6 %

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and was calculated according to the following formula

$F\ \% = (AUC_{0\text{-inf P.O.}} / AUC_{0\text{-inf I.V.}} \times Dose_{I.V.} / Dose_{P.O.}) \times 100$

Pharmacokinetic parameters derived according to I.V. and P.O. administration of oleandrin extract are summarized in Table 1.

Cumulative excretion

Forty-eight hours after mice received a P.O. dose of extract 150 mg/kg (1710 ug/kg eq. dose of oleandrin), 437,7 ng/mL of oleandrin were excreted in 6,2 mL of urine. This represents 0,986% of the initial dose administred.

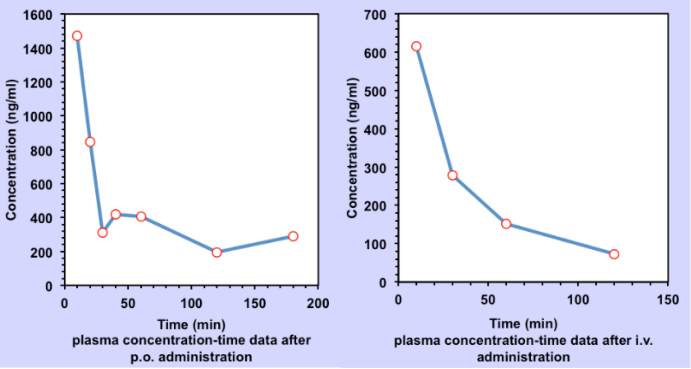

Figure 3. plasma concentrations of oleandrin after P.O. and I.V. administration.

4. DISCUSSION

LC-MS/MS which provides rapid and unequivocal determination of oleandrin (26), was used to determine the amount of oleandrin in biological fluids and the hydroalcoholic extract. The latter, made it possible to estimate the equivalent dose of oleandrin administered in mice.

Oleandrin in the hydroalcoholic extract has been absorbed faster (Tmax 10 min in the present study) than oleandrin alone (20 min in Ni et al.'s study) (27). The bioavailability is also twice greater (61% in this study versus 30 % in Ni et al.'s study)(27). The presence of other compounds in the extract may increase the rate of absorption and the amount absorbed. As previously documented, mice administered oleander extract exhibited elevated levels of oleandrin in their brain and plasma compared to mice given an equal amount of oleandrin alone. This indicates that certain additional constituents within the oleander extract might augment oleandrin's capacity to penetrate the blood-brain barrier (27), as could potentially affect gastrointestinal absorption.

The initial elimination of oleandrin from plasma was swift. The half-life (t1/2) was notably prolonged in mice following oral (P.O.) administration compared to intravenous (I.V.) administration. Corresponding findings have been documented (27).

Table 1. Pharmacokinetic parameters of oleandrin in mice.

Route of administration	Per os	intravenous
Eq-Dose (ug/kg)	1710	342
T_{max} (min)	10	/
C_{max} (ug/L)	1471,3	613,9
$T_{1/2}$ (min)	91,6	48
$AUC_{0\text{-inf}}$ (ug/L*min)	107222	34797,7
Cl_T (kg*L/min)	0,015	0,01
V_{ss} (L/kg)	/	0,55
Bioavailability (%)	61,6 %	/

eq dose : equivalent dose; Tmax : maxium time ; Cmax : maximum concentration ; t1/2 : Half-life ; AUC : area under curve ; ClT : total clearance ; Vss : apparent volume of distribution at steady state

Given that the average blood volume in male mice is approximately 85 mL/kg, with a hematocrit of 47% (28), the volume of distribution was calculated to be 12 times greater than the plasma volume. This suggests significant diffusion of oleandrin into tissues.

Twenty-four hours after mice were administered a P.O. dose of oleandrin extract, approximately 1% of the equivalent oleandrin dose was detected in urine. This aligns with previous research indicating that 60% of administered oleandrin is recovered in feces (27).

Although blood and urine samples were gathered in this study, these measurements may not capture potentially significant interindividual differences.

Additional studies are necessary to establish the pharmacokinetic characteristics of oleandrin across diverse routes of administration, various animal species, different dosage levels, and varied formulations.

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Competing interests: The authors declare that they have no competing interest.

List of Abbreviations

N. oleander : Nerium oleander Linn

I.V. : IntraVenous

ACN : Acetonitrile

MeOH : Methanol

LC-MS/MS : Liquid chromatography tandem–mass spectrometry

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LC-MS : Liquid chromatography–mass spectrometry

MS : mass spectrometry

$t_{1/2}$: Half-life

T_{max} : Time of maximum concentration observed

C_{max} : maximum concentration

AUC : area under curve

Cl_T : total clearance

V_{ss} : apparent volume of distribution at steady state

P.O. : per os

eq dose : equivalent dose

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