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ORIGINAL ARTICLE

Toxicokinetic Study and Analytical Evaluation of Atractyloside, the Main Toxic Compound of *Atractylis gummifera L*

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ABSTRACT

Atractylis gummifera L., a plant from the Asteraceae family, is traditionally recognized for its medicinal uses despite its high toxicity when ingested. Poisoning cases, often accidental, are associated with acute liver and kidney damage; however, data on the systemic behavior of its main toxin, atractyloside (ATR), remain limited. This study aimed to investigate the toxicological profile of atractyloside, the primary toxic compound of the plant, through a series of in vivo experiments. The median lethal dose (LD_{50}) was determined in mice using the Karber/Behrens and Miller-Tainter methods, yielding a value of 115 ± 22.16 mg/kg. A toxicokinetic study conducted in Wistar rats following administration of a sub-toxic dose revealed nonlinear kinetics, with a peak plasma concentration (Cmax) of 68.8 mg/L, a half-life ($t_{½}$) of approximately 90 minutes, and a free fraction of 65%. Protein binding and the role of metabolic activation were further explored using a phenobarbital induction test, which indicated a marked reduction in both Cmax and AUC, suggesting the involvement of ATR and/or its metabolites in toxicity. These findings enhance our understanding of ATR's systemic behavior and could inform clinical management of poisoning cases, particularly given the absence of a specific antidote and the current reliance on symptomatic treatment.

Keywords: gumweed, in vivo, toxicokinetics, phenobarbital.

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1. INTRODUCTION

Accidental plant poisonings remain a persistent public health concern in many Mediterranean countries, where traditional medicinal plants are still widely used. Among the most toxic species is *Atractylis gummifera L.*, a wild plant from the Asteraceae family, commonly encountered in North Africa and Southern Europe [1]. Despite its longstanding use in herbal medicine, particularly for its purported detoxifying properties, this species is responsible for numerous cases of severe poisoning, often fatal, particularly among children [2–4].

The toxic potential of *A. gummifera* is primarily due to a diterpenoid glycoside, atractyloside (ATR), and its close analogue carboxyatractyloside (CATR). These compounds exert their toxicity by targeting the mitochondria, specifically through inhibition of the adenine nucleotide translocator (ANT), a key protein responsible for ATP/ADP exchange across the inner mitochondrial membrane. This blockage leads to mitochondrial dysfunction, energy depletion, and ultimately, cell death—especially in organs with high energy demands such as the liver and kidneys.

Toxicokinetic data on ATR remain limited. There are no comprehensive studies detailing its plasma or tissue distribution, metabolic pathways, or excretion routes. Preliminary findings suggest that ATR undergoes hepatic biotransformation, with associated suppression of key detoxification enzymes including cytochrome P450 (CYP450) and cytochrome b5, possibly due to structural changes in the endoplasmic reticulum. Traces of ATR derivatives have been identified in the urine of coffee consumers, pointing to structural analogues with altered pharmacological activity [5].

Animal studies have shown dose-dependent urinary excretion of ATR, particularly after repeated intraperitoneal administration. However, no clear correlation has been established between systemic toxicity severity and plasma or urinary concentrations of ATR or its metabolites [6].

From a mechanistic standpoint, ATR competitively inhibits mitochondrial respiration by blocking the external ADP binding site on ANT. Structure-activity relationship studies indicate that multiple chemical groups (such as the isovaleryl chain, carboxyl groups, and sulfate moieties) play critical roles in modulating its inhibitory potency and cellular membrane interactions. Some analogues, like atractylogenin and epi-ATR, exhibit similar inhibitory profiles but with reduced efficacy, underlining the importance of specific substitutions [7,8]. Moreover, CATR, a non-competitive inhibitor, demonstrates even higher affinity for ANT due to an additional carboxylic group, making it a valuable kinetic model in mitochondrial research [7,9].

In this context, the current study aims to investigate the in vivo toxicity and toxicokinetic profile of ATR. We developed and validated a blood quantification method using high-performance liquid chromatography (HPLC), with a lower limit of quantification (LLOQ) of 10 mg/L and a detection limit of 2 mg/L. The study involves estimating the median lethal dose (LD50) in mice, evaluating toxicokinetics following a sub-toxic dose in rats, measuring protein-binding rates, and exploring the potential formation of toxic intermediate metabolites. These findings are intended to provide a better understanding of ATR's systemic behavior and toxicological impact.

2. MATERIALS AND METHODS

In vivo Study

The LD50 corresponds to the dose capable of killing half of the animals under specified conditions in the same animal species.

Mouse Breeding and Maintenance Protocol

For this study, male NMRI mice aged two months and weighing on average 20 ± 2 grams were used. A total of forty-two mice were included in the experiment and bred at the National Laboratory for Pharmaceutical Products Control (LNCPP).

Operating Procedure

Before the experiment, the mice were fasted for twelve hours. They were then randomly and evenly divided into seven groups, comprising one control group and six experimental groups, each receiving a specific dose of ATR administered orally. The doses, expressed in mg/kg of body weight, were 50, 75, 100, 150, 200, and 250. After administration, the animals were observed for fourteen days. The evaluation of results involved establishing the relationship between the administered dose and the intensity of observed effects, as well as calculating the LD_{50} with its confidence intervals according to the Karber and Behrens Method and the Miller and Tainter Method.

Non-compartmental Analysis

Study Plan and Population

The toxicokinetic (TK) analysis was conducted on 108 male Wistar rats, with an average weight of 180 grams. The animals were divided into three groups of thirty-six rats, with each group further subdivided into subgroups of three rats per sampling time point. Three trials were performed at different time periods, each involving thirty-six rats. Blood samples were collected from the retro-orbital sinus using a heparinized capillary tube.

Administration Schedule of ATR

A sublethal oral dose of 140 mg/kg of ATR was administered, calculated based on the LD_{50} of 210 mg/kg in Wistar rats, corresponding to LD_{50} divided by 1.5.

Blood Analysis

Blood samples for determining ATR plasma concentrations were collected at 5, 15, 30, 60, 90, 120, 180, 240, 360, 480, and 600 minutes after oral administration. Plasma concentrations were measured using a chromatographic method (HPLC/UV). The first group of three

rats served as the control group. The TK parameters of ATR were first estimated for each group using non-compartmental analysis (NCA) with Monolix® software (version 2019R1, Antony, France), and summary statistics of the parameters were subsequently calculated. Statistical data processing and TK analysis of plasma concentrations over time were performed using R® software with the ggplot2 package.

Analysis of the Free Fraction

Equilibrium dialysis (ED) is based on the principle of filtration, using a dialysis cell with two reservoirs separated by a semi-permeable cellulose membrane. This membrane is permeable to free substances but impermeable to protein-bound substances. Before analysis, it is recommended to pretreat the membrane by soaking it in a phosphate buffer at pH 7.4. The plasma sample to be analyzed and the buffer are placed in their respective reservoirs on either side of the membrane.

The dialysis system is maintained at a physiological temperature of 37 °C and continuously rotated to accelerate the attainment of binding equilibrium. At the end of dialysis, the concentration of the substance in the buffer reservoir is in equilibrium with the free concentration in the plasma reservoir. The concentration in the plasma reservoir represents the total concentration. The free fraction is calculated using the formula:

*Fu = (Buffer concentration) / (Plasma concentration) * 100*

The time required to reach equilibrium is a limiting step in ED analysis. For accurate interpretation, the free concentration in the buffer reservoir must be measured when binding equilibrium has been achieved, which is confirmed when Fu no longer changes over time.

Materials

The materials used included a Maxi-ThermoScientific rapid equilibrium dialysis (RED) device, a Rotamax 120 Heidolph orbital shaker, and a C170 BINDER® incubator to maintain a constant temperature. Reagents for preparing the buffer solution (pH 7.4) were anhydrous disodium phosphate (Na_2HPO_4) (SUPELCO N°231-913-4) and monopotassium phosphate (KH_2PO_4) (SUPELCO N°401-073-4).

Dialysis Method

The rapid equilibrium dialysis system consisted of a 96-well plate for forty-eight samples, with each sample occupying two paired chambers. In our experiments, three sets of two serum samples saturated with ATR at concentrations of 60, 80, and 100 mg/L were analyzed. For each sample, 100 µL of analyte-containing serum was placed in the sample chamber, and 600 µL of buffer was placed in the buffer chamber. The plate was incubated at 37 °C with agitation. After four hours, 250 µL from each buffer chamber was withdrawn, and analyte concentrations were measured by HPLC/UV. According to Nilsson, an ED assay lasting less than six hours does not induce sufficient pH modification to disrupt binding equilibrium [10]. Since our dialysis lasted four hours, we concluded that pH had not influenced our results.

Enzyme Induction Analysis

Plan and Study Population

Twenty-four male Wistar rats were used, divided into twelve groups of two rats each, with an average weight of 180 ± 5 g. Blood samples were collected in heparinized capillary tubes from the retro-orbital sinus. For each time point, a serum pool was prepared from the two rats, with sampling times at 5, 15, 30, 60, 90, 120, 180, 240, 360, 480, and 600 minutes.

Inducing Molecule

The enzyme inducer used was phenobarbital (GMP; BIOGALENIC LABORATORY).

Phenobarbital was selected as an enzyme inducer due to its well-established effectiveness in stimulating hepatic microsomal enzymes, particularly cytochrome P450 isoenzymes, in rodents. The dose used (20 mg/kg/day via intraperitoneal injection for seven days) corresponds to protocols that have demonstrated marked induction without evident toxicity [11]. Braga et al. (2008) [11] confirmed the effectiveness of this regimen by showing increased hepatic concentrations of cytochromes P450 and b5 in rats. Although no positive control was included in the present study, the protocol, already validated under similar conditions, and the observed variations in ATR clearance and AUC are consistent with the expected profile of enzyme induction.

Administration Schedule of ATR

The rats received an oral dose of 140 mg/kg of ATR, identical to that used for the ATR kinetic profile. These same rats had previously been treated for seven days with phenobarbital solution at 20 mg/L administered intraperitoneally at a daily dose of 20 mg/kg [11]. The control group underwent the same induction protocol without ATR administration. The TK study was performed under the same conditions as the initial phase to allow comparative analysis of the kinetic parameters.

Ethical considerations

All procedures involving animals were conducted in accordance with the ethical principles of animal experimentation and the recommendations of the International Committee for Animal Research Ethics. Animals were handled in a manner that minimized pain, stress, and suffering, and their housing complied with animal welfare standards.

3. RESULTS AND DISCUSSION

In Vivo Study

Results of the Acute Toxicity Test

The acute toxicity test of ATR in mice showed no mortality at 50 mg/kg, while 16.7 % of animals died at 75 mg/kg, 33.3 % at 100 mg/kg, and 50 % at 150 mg/kg. All animals died at doses of 200 mg/kg and above. The differences between successive doses (a) ranged from 25 to 50 mg/kg, and the mean number of deaths between doses (b) ranged from 0.5 to 6.0 animals, with calculated ab values ranging from 12.5 to 300.

Determination of the LD50 according to the KARBER and BEHRENS method

LD50=LD100-(Σab/n) =83.3 mg/kg/per os

Determination of the LD50 according to the MILLER and TAINTER method

LD50=115 ± 22.16 mg/kg/*per os* : Graphical LD50 : 115 mg/kg/per os; Standard deviation: σ=(LD84-LD16)/2=(200-67)/2=66.5; Standard deviation from the mean : $\delta = 2\sigma/\sqrt{2N'} = 2 \times 66.5 / \sqrt{(2 \times 18)} = 22.16$

In this study of acute single-dose oral toxicity in mice, we observed a sedative effect after ATR administration, which disappeared rapidly depending on the ingested concentration. However, starting from the dose of 200 mg/kg, mice exhibited abdominal writhing immediately after administration and remained motionless until death.

Indeed, *Atractylis gummifera L*. is known to have a fulminant hepatotoxic effect, which is attributed to two glycosides, ATR and CATR. Acute toxicity studies of these two molecules in rats have shown that CATR is 50 times more toxic than ATR, with LD50 values expressed in mg/kg of body weight of 2.9 and 143 mg/kg, respectively.

According to the Edward Bastarache-Hodge-Sterner classification scale, ATR and CATR are classified as extremely toxic and ultra-toxic, respectively. The LD50 values found in our experimental work are consistent with those in the literature, which are as follows:

LD50 = 83.3 mg/kg per os according to the KARBER and BEHRENS method

LD50 = 115±22.16 mg/kg per os according to the MILLER and TAINTER method.

The results of the LD50 and their impact on oxidative phosphorylation have shown specific sites of action for ATR. Researchers have identified specific ADP/ATP binding sites (pores) where nucleotides are bound and transported back and forth between the cytosol and mitochondria. Additionally, most of the in vivo effects of ATR are explained by the slowing of oxidative phosphorylation induced by inhibition of the ADP/ATP transporter (ANT) in mitochondria.

In our study, the survival of some mice and their recovery after product administration could be explained by the fact that not all adenylic nucleotide receptors (ANT: adenylate nucleotide translocase) were seemingly occupied by ATR. Consequently, the ADP/ATP exchange could still occur with partial maintenance of oxidative phosphorylation.

Since the ANT receptor has a short lifespan, the regeneration and renewal of ANT receptors will allow for the elimination of the bound toxin and subsequent recovery of the animals. This highlights the importance of dosage in intoxication by *Atractylis gummifera L.*

In Vivo studies have shown that even at high concentrations, ATR does not exhibit cytotoxicity at exposure times of 2, 4, and 6 hours; it only appears after 24 and 48 hours of incubation at the same concentrations used previously. This indicates that the mechanism by which ATR induces cell death is likely apoptotic rather than necrotic.

Non-compartmental analysis

As part of the study protocol, rats received a single oral dose of 140 mg/kg. The individual plasma toxicokinetic (TK) parameters of ATR for the three batches (ID1, ID2, and ID3), together with descriptive statistics, are summarized in Table 1.

Table1. Plasma toxicokinetic parameters of ATR in rats after a single oral administration of 140 mg/kg.

Parameter	ID1	ID2	ID3	Mean	SD	CV (%)
Tmax (min)*	90	90	90	90	-	-
Cmax (mg/L)	67.9	66.9	71.5	68.8	2.39	3.48
AUC_{0-600}min (min·mg/L)	14781	15868	17258	15969	1242	7.77
$AUC_{0-\infty}$ (min·mg/L)	15072	17483	17552	16702	1412	8.45
AUCext (%)	1.93	15.3	1.67	-	-	-
$t_{1/2}$ (min)	85.5	165	76.8	109	48.4	44.4
Vz/F (L)	0.20	0.34	0.16	0.23	0.09	40.3
Cl/F (L/min)	0.0017	0.0014	0.0014	0.0015	0.00013	8.89
MRT∞ (min)	226	281	233	247	29.9	12.1

*Cmax: maximum plasma concentration; Tmax: time to reach Cmax; AUC_{0-600}min: area under the plasma concentration–time curve from 0 to 600 min; $AUC_{0-\infty}$: area under the curve from time zero to infinity; AUCext (%): percentage contribution of the extrapolated portion to $AUC_{0-\infty}$; $t_{1/2}$: terminal half-life; Vz/F: apparent volume of distribution; Cl/F: apparent oral clearance; MRT∞: mean residence time. *Tmax expressed as median.

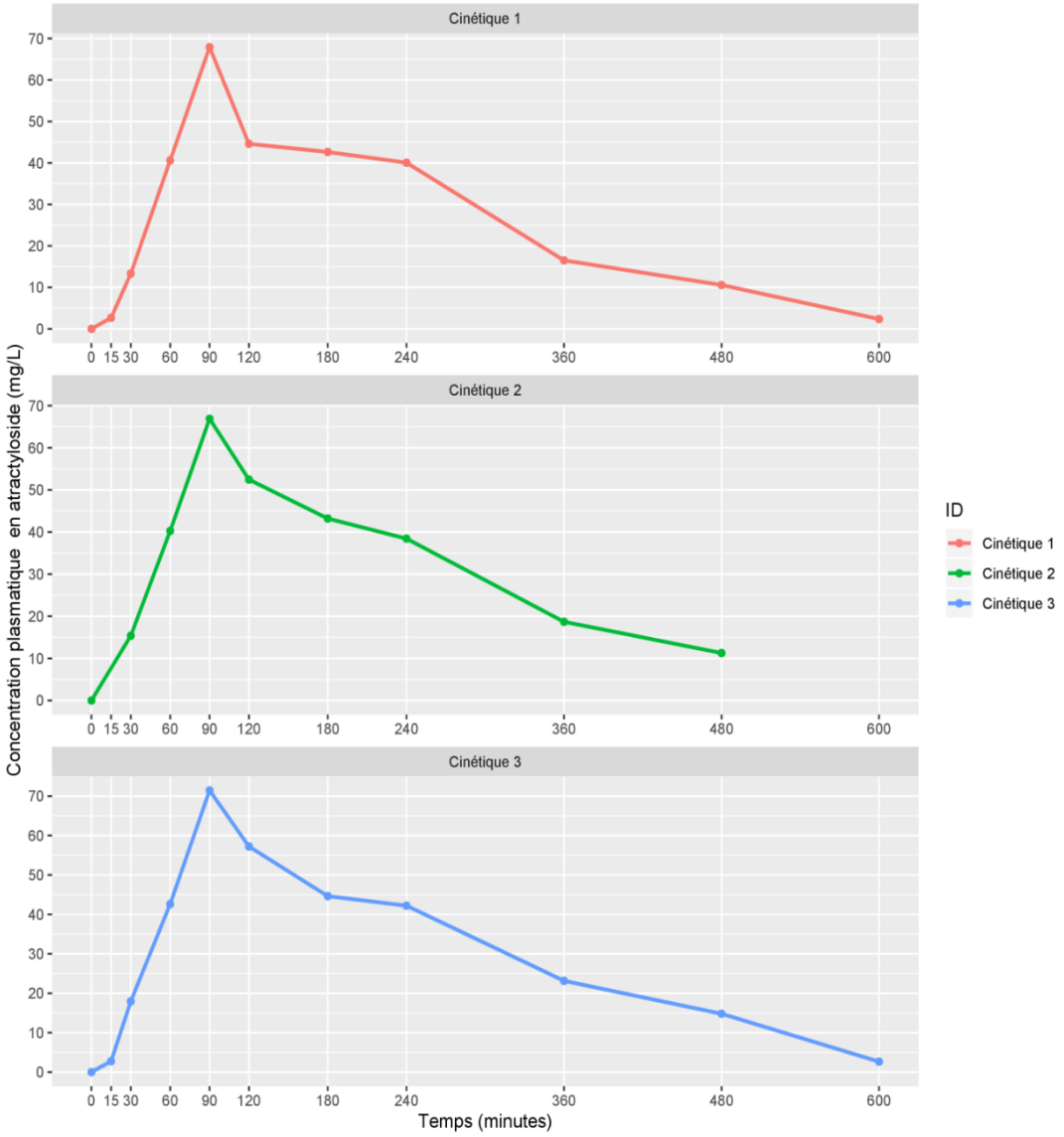

Figure 1. Individual Profiles of ATR Plasma Concentrations Over Time After a Single Oral Administration of 140 mg/kg.

The profile of individual plasma concentrations over time is shown in Figure 1. The TK profiles of ATR were comparable between batches 1 and 3, whereas the profile of batch 2 could not be fully completed as two of the three rats died before the tenth hour of exposure.

The mean plasma concentration–time curve (± standard deviation) following administration is presented in Figure 2. In the absence of bibliographical data on ATR kinetics in humans and animals, we opted for a non-compartmental analysis approach, in line with the recommendations of the 2010 Guideline on the Investigation of Bioequivalence by Gabrielsson et *al.* and Sacks et *al.* [12–14].

We notice that:

Comparing T_{max}, C_{max} and $AUC_{0-\infty}$ showed that the TK profile of ATR was comparable among the three rat lots. The average C_{max} was 68.8 mg/L, and the T_{max} was 90 minutes.

Inter-lot variability in exposure parameters (C_{max}, $AUC_{0-\infty}$, AUC_{0-600}) and clearance (Cl/F) was low, around 3 to 8%, likely due to the strong physiological homogeneity of the rats and the adjustment of the administered dose based on weight.

For lot 2, the absence of values at 5, 15, and 600 minutes led to an overestimation of $AUC_{0-\infty}$. Consequently, the values of TK parameters ($t_{1/2}$ and Vz/F) could not be accurately determined.

Regarding non-linear (zero-order) kinetics, this is typically the most common case in TK, as it is at these exposure levels that transport, metabolism, and/or excretion processes become saturated. In the case of ATR, the profile also suggests that the molecule undergoes first-pass hepatic effect, which is reinforced by the fact that the rat died within 480 minutes, likely due to enzymatic induction.

This was observed by a drastic reduction in plasma concentrations, which likely "released" (de-saturated) the extra-hepatic excretion process of the molecule. An exponential decrease (linear in a semi-logarithmic scale) in concentrations during the terminal elimination phase perfectly illustrates this phenomenon.

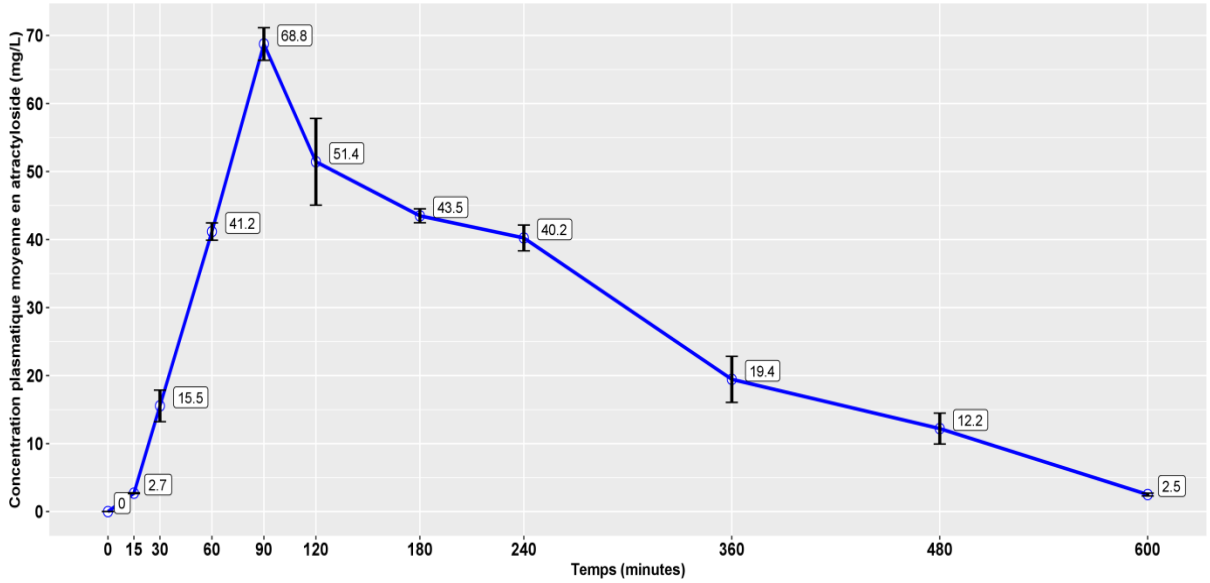

Figure 2. Profile of mean plasma concentrations (± standard deviation) of ATR over time following a single oral dose of 140 mg/kg

Free fraction analysis

After 4 hours of agitation, we proceeded to recover aliquots of buffer solutions for quantitative analysis of ATR, corresponding to the diffusible free fraction. The analysis was performed twice for the same concentration to obtain an average diffusible concentration. The concentrations in serum are the AUC and mean concentrations of ATR already performed during method validation. This concentration corresponds to the total amount of ATR (free and bound to proteins). The results are recorded in Table 2.

Table 2. *Measurement* of percentage of bound and free fraction.

Dialysis	Buffer peak area	C buffer	Serum peak area	C serum	% of bound fraction	% of free fraction
ED60	336339 340101	37.84	534654	58.83	35.68	64.32
ED80	511533 518367	56.73	731045.67	85.17	33.39	66.61
ED100	584708 601503	65.08	928996.67	100.98	35.55	64.45
Mean					34.88	65.12
Variance					1.65	1.65
Standard deviation					1.28	1.28

ED: Equilibrium dialysis

Percentage of bound fraction = $\frac{serum\ concentration - buffer\ concentration}{serum\ concentration} * 100$

Percentage of free fraction = $\frac{Buffer\ Concentration}{Serum\ Concentration} * 100$

The 95% confidence intervals for the bound fraction are [31.67 - 38.06] % and for the free fraction are [61.93 - 68.32] %. These results indicate that ATR is weakly bound to proteins.

To complete our kinetic study, we undertook to evaluate the free fraction of the molecule. This in vivo assessment was performed using the method of dialysis overload of serum samples with ATR, followed by the appropriate operating protocol. We observed that the free fraction of our molecule averages between 61 and 68 %.

Since only the free form is active, the expected effect should be rapid if the hypothesis of ATR's toxic activity was linked to the parent molecule. However, this is not the case, as the first signs in mice after administration of the DL50 only appeared after 48 hours. Similarly, the onset of deaths in rats after administration of sub-toxic doses was 480 minutes.

Enzymatic induction analysis

The implementation of the induction protocol and the evaluation of ATR toxicokinetic parameters under the same analytical conditions during the kinetic study are documented in Table 3.

Table3. Comparative study of ATR plasma TK parameters in rats, before and after phenobarbital induction.

ID	Dose (mg)	T_{max} (min)	C_{max} (mg/L)	$AUC_{0\text{-}600min}$ (min.mg/L)	R^2	$AUC_{0\text{-}\infty}$ (min.mg/L)	AUC_{ext} (%)	$t_{1/2}$ (min)	Vz/F (L)	Cl/F (L/min)	MRT (min)
1	25	90	68.8	15831	0.82	16125	1.82	81.2	0.182	0.0016	229
2	25	120	4.2	1595	0.99	2955	53.8	532	6.49	0.0085	774

ID1 : Pooled Data; ID2 : TK Data after Enzymatic Induction ; C_{max} : Maximum Plasma Concentration ; T_{max} : Time to Reach Maximum Plasma Concentration ; $AUC_{0\text{-}600min}$: Area Under the Plasma Concentration-Time Curve from 0 to 600 min ; R^2: Adjusted Coefficient of Determination ; $AUC_{0\text{-}\infty}$: Area Under the Plasma Concentration-Time Curve from time zero to infinity ; $AUC_{ext(\%)}$:Percentage of Contribution of the Extrapolated Portion to $AUC_{0\text{-}\infty}$; $t_{1/2}$: Terminal Half-life ; V_z/F : Apparent Volume of Distribution ; CL/F : Apparent Oral Clearance ; MRT : Mean Residence Time.

The kinetic plot of ATR concentrations after induction could not be finalized due to the death of rats from the last batch before the sixth hour. However, initially, a decrease in AUC following induction is observed (Figure 3).

According to the induction test conducted by *Angélica de Fátima et al*. in 2008, as well as by *Paul E. et al.* in 1981, and following the evaluation of kinetic parameters, it is noticeable that overall, the toxicokinetics of ATR was significantly reduced by the concurrent administration of an enzymatic inductive substance [11,15].

The comparison of T_{max}, C_{max} and $AUC_{0\text{-}\infty}$ showed that the clearance is increased by a factor of 5, while the C_{max} is drastically reduced by a factor of 16 and the $AUC_{0\text{-}\infty}$ by a factor of 5.

In this type of study, if the objective is to test the effect of an inhibitor or an inducer, half-life is not the ideal parameter for comparison, but rather clearance. Indeed, the $t_{1/2}$ is estimated, in non-compartmental analysis, based on the terminal elimination phase and does not consider the AUC, unlike clearance.

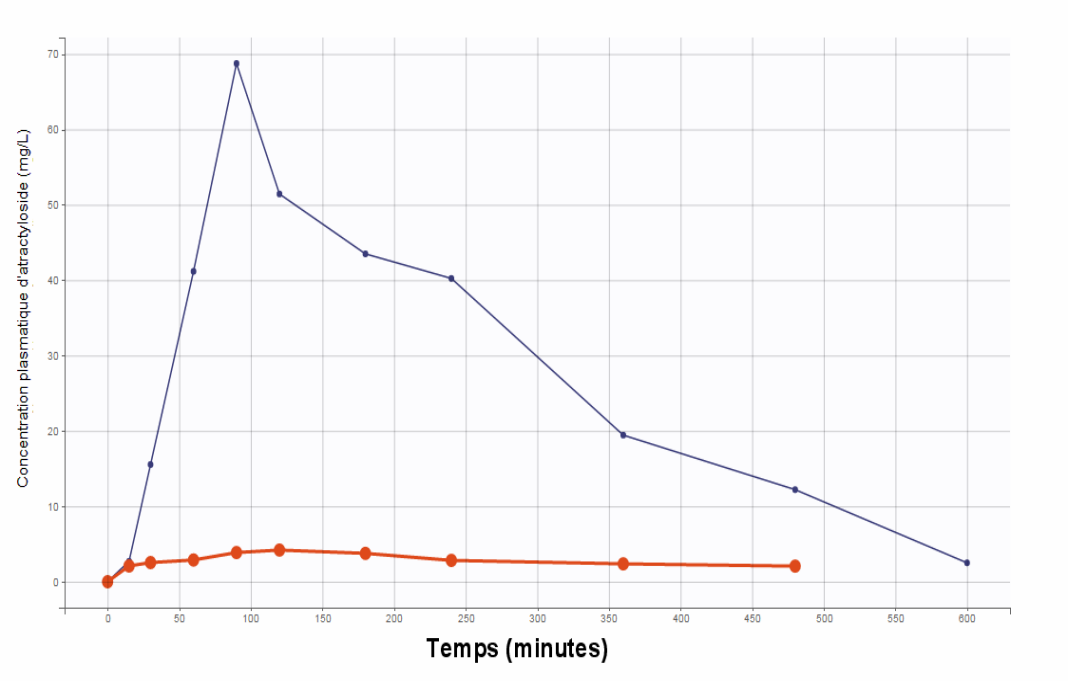

Figure 3. Profile of average plasma concentrations of ATR over time after enzymatic induction.

The high value of the half-life (532 minutes) compared to ATR administration without an inducer suggests a slower elimination rate, indicating nonlinear kinetics (saturable) at sub-toxic concentrations. The decrease in plasma concentrations induced by enzymatic induction resulted in a reduction in the elimination rate, thereby increasing the half-life.

The hypothesis, based on TK data, obtained lethal doses, and response time, suggests that toxicity results from a synergistic effect between the parent molecule and the rate of formation of toxic metabolites. These metabolites could have a higher affinity for the mitochondrial adenine nucleotide translocator (ANT) protein. This protein is responsible for transporting ADP and ATP across the inner mitochondrial membrane, leading to membrane permeabilization and the release of intra-membrane proteins, including cytochrome C, thus triggering apoptosis.

Limitations of the Study

Certain methodological limitations should be acknowledged when interpreting our results.

First, the sample sizes were relatively small (six mice per group for the LD_{50} determination and three rats per time point for the toxicokinetic analyses). This choice was based on ethical and regulatory constraints, in accordance with the 3Rs principle (Replacement, Reduction, Refinement) and following OECD guidelines No. 425 for acute toxicity and No. 417 for toxicokinetics. The objective was to minimize the number of animals used while obtaining exploitable results. However, such small sample sizes may limit statistical power and increase variability, and the pharmacokinetic parameters calculated from n=3 for a single concentration should be interpreted as descriptive rather than inferential.

Second, premature mortality of some rats before the tenth hour post-administration resulted in incomplete toxicokinetic profiles. While this may have limited the precise estimation of certain parameters (e.g., half-life, volume of distribution), it also supports the hypothesis that ATR toxicity may be partly related to the formation of a toxic metabolite or oxidative stress processes.

Finally, the absence of human pharmacokinetic data on ATR limits the contextualization and direct comparison of our results. This highlights both the novelty of our work and the need for further research, particularly clinical investigations or more advanced modeling approaches.

These factors should be considered when interpreting our results and guide future research towards larger sample sizes, extended follow-up periods, and more rigorous control groups.

4. CONCLUSION

Our study highlights the importance of understanding the toxicity of *Atractylis gummifera* and its active ingredient, ATR, in traditional medicine. The results obtained from in vivo tests, including the estimation of the LD50 and kinetic studies, shed light on the complexity of the biological response to this substance. The significant reduction in toxicity observed during induction by phenobarbital suggests the involvement of metabolites in toxic effects. Thus, a better understanding of the in vivo biology of ATR could pave the way for more effective therapeutic approaches to mitigate the severity of acute intoxications related to this plant and similar ones. These findings also underscore the urgent need for further research to develop specific treatments against the toxicity of *Atractylis gummifera*, given the current lack of targeted medical solutions and the symptomatic impact on intoxicated patients.

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

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REFERENCES

1. Dupont F, Guignard JL. Botanique: les familles de plantes. Issy-les-Moulineaux (France): Elsevier Masson; 2012. ISBN: 978-2-294-72568-5
2. Larabi IA, Azzouz M, Abtroun R, Reggabi M, Alamir B. Détermination des teneurs en atractyloside dans les racines d'Atractylis gummifera L. provenant de six régions d'Algérie. Ann Toxicol Anal. 2012;24(2):81–6.
3. Bouzidi A, Mahdeb N, Allouche L, Houcher B. Étude épidémiologique sur les plantes toxiques dans les régions de Sétif et Bordj Bou Arreridj (est de l'Algérie – Afrique du Nord). Bull Inf Toxicol. 2002;18(2):5–10.
4. Berrezoug H, Berradia A. Contribution à la prise en charge des intoxications par les végétaux: aide à la diagnose des plantes toxiques de la région de Tlemcen [thèse]. Tlemcen (Algérie): Université de Tlemcen; 2014. [cited 2023 Oct 15]. Available from: http://dspace.univ-tlemcen.dz//handle/112/7820
5. Hedili A, Warnet JM, Thevenin M, Martin C, Yacoub M, Claude JR. Biochemical investigation of Atractylis gummifera L. hepatotoxicity in the rat. Arch Toxicol Suppl. 1989;13:312–5.
6. Bye SN. The development of assays for atractyloside and its localisation in rat tissue [dissertation]. Durban (South Africa): Department of Biochemistry, University of Natal; 1991.
7. Vignais P, Vignais PV, Defaye G. Structure-activity relationship of atractyloside and diterpenoid derivatives on oxidative phosphorylation and adenine nucleotide translocation in mitochondria. Atractyloside Chem Biochem Toxicol. 1978:39–68.
8. Abtroun R. Élaboration d'un fichier analytique et toxicologique pour le Centre d'information, de contrôle et de traitement des intoxications graves [thèse de sciences médicales]. Alger (Algérie): Université d'Alger; 1986.
9. Richter H, Spiteller G. Über ein neues Atractyligenin-Glycosid aus grünen Kaffeebohnen. Chem Ber. 1978;111:3506–9.
10. Nilsson LB. The bioanalytical challenge of determining unbound concentration and protein binding for drugs. Bioanalysis. 2013;5(24):3033–50. doi:10.4155/bio.13.277
11. Braga A, Barcelos C, Braga F, Fernandes S, Oshima-Franco Y, Mantovani M, et al. Phenobarbital influence on neuromuscular block produced by rocuronium in rats. Acta Cir Bras. 2008;23(4):343–7. doi:10.1590/S0102-86502008000400005
12. European Medicines Agency. Guideline on the investigation of bioequivalence. London: EMA; 2010 Jan 20. (CPMP/EWP/QWP/1401/98 Rev.1) [cited 2024 Feb 16]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf
13. Gabrielsson J, Weiner D. Non-compartmental analysis. In: Methods Mol Biol. 2012;929:377–89. doi:10.1007/978-1-61779-109-6_24
14. Sacks LV, Shamsuddin HH, Yasinskaya YI, Bouri K, Lanthier ML, Sherman RE. Scientific and regulatory reasons for delay and denial of FDA approval of initial applications for new drugs, 2000–2012. JAMA. 2014;311(4):378–84. doi:10.1001/jama.2013.284053
15. Ward LC, Reilly PEB. Drug metabolism in rats: induction and inhibition of cytoplasmic electron transport laboratory experiments in vivo and in vitro. Biochem Educ. 1981;9(2):46–50.