

L-CARVONE AS A METABOLIC ENZYME MODULATOR IN DOXORUBICIN TOXICITY INDUCED RAT TISSUE: A COMBINED COMPUTATIONAL AND CARDIAC SLICE STUDY

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ABSTRACT

Cardiac hypertrophy and Heart failure are frequently caused by essential dysfunction, including deficiencies in tricarboxylic acid cycle (TCA) enzymes and redox state imbalances. The well-known cardiotoxic effects of doxorubicin (DOX), a frequently used chemotherapeutic drug, are mostly ascribed to its disruption of metabolic enzymes and consequent rise in reactive oxygen species (ROS). *Mentha spicata* L., is the source of L-Carvone, a monoterpenoid molecule with anti-inflammatory, antioxidant, and calcium-regulatory qualities. To assess the effectiveness of L-Carvone in reducing DOX-induced metabolic dysfunction, this work proposes an integrated approach that combines *in situ* experimental validation using rat heart slices with *in silico* kinetic modelling. We examined the effects of normal; DOX and DOX treated with L-Carvone circumstances on three important TCA cycle enzymes: Isocitrate dehydrogenase (IDH), Succinate dehydrogenase (SDH), and Malate dehydrogenase (MDH). Within a brief time frame, L-Carvone significantly restored enzymatic activity which may balance the $NAD^+/NADH$ redox ratio according to simulations and experimental experiments. Our results show strong proof that L-Carvone modulates TCA cycle key enzymes quickly and effectively, potentially providing treatment for chemotherapy-induced cardiomyopathy.

Keywords: Computational Biology, L-Carvone, Metabolic dysfunction, TCA cycle.

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INTRODUCTION

Cardiac hypertrophy is a structural and functional remodelling of the heart that occurs as an adaptive response to physiological needs such as exercise or pregnancy and pathological stressors such as hypertension, myocardial infarction, volume overload, and neurohormonal activation. While this reaction is initially compensatory, chronic hypertrophy can have negative implications such as myocardial stiffening, fibrosis, metabolic remodelling, and eventually decompensated heart failure, a substantial worldwide health burden with high morbidity and death (1,2).

The shift from compensatory hypertrophy to overt cardiac failure is mostly caused by malfunction at the cellular level (3-5). More than 90% of the energy produced by the heart through oxidative phosphorylation comes from mitochondria, which are the main source of ATP in cardiac cells. Additionally, they control vital functions include apoptotic signalling, calcium buffering, and redox homeostasis. When these processes are disrupted, cardiac output is severely reduced, which results in oxidative damage, contractile dysfunction, and energy deficiencies. The tricarboxylic acid (TCA) cycle is essential to mitochondrial energetics because it produces reducing equivalents, FADH_2 and NADH , which are used in the electron transport chain to synthesise ATP. Isocitrate dehydrogenase (IDH), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH) are important enzymes in this cycle that are essential for preserving redox balance and metabolic flux (3,5). The heart switches from effective oxidative metabolism to anaerobic glycolysis when these enzymes are blocked or downregulated, as is the case in a number of cardiac disorders. Lactate buildup, cellular acidosis, elevated reactive oxygen species (ROS) generation, and reduced contractile ability are the outcomes of this metabolic change (4-6).

One powerful anthracycline chemotherapy drug that is a clinical cause of cell dysfunction is DOX. Although it works well against several types of cancer, dose-dependent cardiotoxicity limits its therapeutic use (7). DOX disrupts the electron transport chain, especially complexes I and II (8), interferes with mitochondrial DNA replication (9), and produces too many reactive oxygen species (ROS), which causes oxidative damage to proteins, lipids, and nucleic acids. Crucially, DOX also prevents the synthesis of ATP and destabilises redox equilibrium by blocking TCA cycle key enzymes such IDH, SDH, and MDH (10). Due to the constraints of traditional cardioprotective agents, phytochemicals have surfaced as hopeful substitutes because of their multifunctional bioactivities. L-Carvone, a naturally occurring monoterpene and a major component of *M. spicata* essential oil, has demonstrated considerable cardioprotective potential in pre-clinical studies (11-13). Its positive impacts encompass the modulation of calcium dynamics, an increase in antioxidant enzyme activity, and the activation of silent mating type information regulation 2 homolog - 1 (SIRT1) a redox-sensitive NAD^+ -dependent deacetylase that plays a role in cell biogenesis and metabolic regulation (14-16). L-Carvone

additionally showcases anti-inflammatory and cytoprotective properties that help maintain cell integrity and cellular survival (17,18).

Even with these encouraging features, the specific function of L-Carvone in influencing TCA cycle enzyme kinetics during DOX-induced stress is still unexamined. To fill this gap, the current research utilizes a two-phase systems biology method: (1) an *in silico* method, which is a computational kinetic modelling technique to extrapolate L-Carvone's modulatory impacts on vital TCA cycle enzymes, and (2) confirmation *via in situ* verification method, which is DOX treated heart tissue slice model. This unified approach allows for a thorough assessment of protective function of L-Carvone in preserving TCA cycle enzyme activity and redox balance during chemotherapeutic stress. By clarifying these mechanisms, the research provides fresh perspectives on the therapeutic promise of L-Carvone in averting cardiac metabolic failure.

MATERIALS AND METHODS

In silico modeling of simulations

Kinetic parameters for isocitrate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase were obtained from Franco and Serrano-Marin, 2022 (19) as well as Kloska *et al.*, 2021 (20). These values were utilized to create kinetic models specific to conditions representing control, doxorubicin-treated, and L-Carvone co-treated scenarios.

Software and computational structure

Simulations were carried out utilizing Python 3.8 within JupyterLab (21), with NumPy libraries for numerical computations, SciPy for nonlinear curve fitting, Pandas for organized data, and Matplotlib alongside Seaborn created for visualizations of kinetic trajectories (22). This framework allowed for consistent enzyme modelling under all conditions.

Kinetic modelling and simulation of enzymes

Enzyme kinetic modelling and simulation were performed utilizing condition-specific adjustments of Michaelis–Menten kinetics. Enzyme function under standard conditions was represented using the fundamental Michaelis–Menten equation. Conditions treated with DOX were modelled with kinetic inhibitory parameters taken from Otter *et al.*, 2022 (23). The co-treatment scenario involving DOX and L-Carvone was simulated using an activation-modified Michaelis–Menten equation, incorporating activation kinetics from Jager *et al.*, 2020 (24). Parameters such as V_{max} , K_m , K_i , and K_a were optimized through nonlinear least squares regression. Simulations were performed at 1, 2, and 5-minute intervals in all three experimental conditions.

Simulations were conducted using modified Michaelis-Menten (MM) kinetic models to account for inhibitory and activating effects under each condition.

1. **Control (baseline):** Modelled using standard Michaelis-Menten kinetics:

$$V = \frac{V_{\max} [S]}{K_m + [S]}$$

Where, $V_{\max}$ is the Maximum reaction velocity, $[S]$ is Substrate concentration and K_m is Michaelis constant.

2. **Doxorubicin treated:** Modelled using mixed-type inhibition to simulate the binding of Doxorubicin to enzyme or cofactor sites:

$$V = \frac{V_{\max} [S]}{(K_m + [S]) (1 + [I]/K_i)}$$

Where, $[I]$ is Inhibitor concentration and K_i is Inhibition constant

This model captures the decline in enzyme velocity at higher substrate concentration levels due to inhibitory binding, simulating the interference caused by Doxorubicin.

3. **Doxorubicin + L-Carvone-treated:** Modelled with an activation-modified term to represent potential allosteric or catalytic enhancement by L-Carvone:

$$V = \frac{(V_{\max} [S]) (1 + [A]/K_a)}{K_m + [S]}$$

where $[A]$ is the L-Carvone concentration and K_a is the activation constant derived from dose-dependent simulation data (25).

This framework simulated the real-time velocity of substrate turnover under various perturbations, reflecting changes in TCA enzymes.

In Situ Heart Slice Validation

Ethical Approval and Animal Handling

All animal experiments were performed in compliance with the regulations set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The Institutional Animal Ethics Committee (IAEC) of PSG Institute of Medical Sciences and Research (PSG IMSR), Coimbatore, India, reviewed and approved the study protocol (Approval No: 44/IAEC/2024).

Adult male Wistar rats (*Rattus norvegicus*), 10–12 weeks old and weighing 250–300 g, were kept in the PSG IMSR animal facility under regulated laboratory settings ($22 \pm 2^\circ\text{C}$, 12/12 h light–dark cycle, 50–60% relative humidity), having

unrestricted access to standard rodent diet and water. Before the experiments began, the animals underwent a one-week acclimatization period. Humane endpoints were maintained during the study, and all procedures were conducted under suitable anesthesia employing a combination of xylazine (1.5 mg/kg, i.p.) and ketamine (2.5 mg/kg, i.p.), adhering to the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals. Euthanasia was performed by a licensed veterinary expert at PSG IMSR.

Heart Extraction and Slice Preparation

After euthanasia, hearts were quickly removed, washed in chilled Krebs–Ringer buffer (KRB, pH-7.4) to eliminate residual blood. The excised hearts were then immediately sliced into uniform transverse sections (2–3 mm thickness) (26) using an improvised ice-embedded tissue slicing method, as described in Nagarajan and Doss, 2024 and patented technique (Patent Application No. 202541001438 A) (27).

Experimental Groups and Treatment Protocol

Heart slices were randomly assigned to one of three treatment groups ($n = 6$ for each group):

- ★ Group I: Control (buffer only) (28)
- ★ Group II: Doxorubicin (10 μM) (Yasumi *et al.*, 1980 (29) with modifications)
- ★ Group III: Doxorubicin (10 μM) + L-Carvone (100 μM) (Silva *et al.*, 2022 (30) with some modifications)

Treatments were conducted in a humidified chamber at 37°C for durations of 1, 2, and 5 minutes to observe real time, temporary alterations in enzymatic activities. After incubation, tissues were homogenized in cold phosphate buffer and centrifuged at $10,000 \times g$ for 15 minutes at 4°C . The supernatants were gathered for biochemical analysis (26). Biochemical assays of IDH, SDH and MDH activities together enabled evaluation of TCA cycle enzyme activity, and metabolic adjustment in reaction to DOX and L-Carvone exposure.

Statistical Analysis

Results were expressed as mean $\pm$ standard deviation (SD). ANOVA and least significant difference (LSD) test were used to analyze the significant difference between the groups using SPSS Statistics 23.0 software (SPSS Inc., USA). P -values less than 0.05 were considered significantly different. (13).

RESULTS

In Silico Simulation of Enzyme Kinetics

Computational simulations were carried out to evaluate the activity profiles of three key TCA cycle enzymes, NADP⁺-dependent isocitrate dehydrogenase (IDH), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH) enzymes under control, DOX-induced stress, and

DOX with L-Carvone co-treatment conditions. These simulations aimed to reflect dynamic enzyme behavior and resilience under redox perturbations.

Isocitrate Dehydrogenase Activity

Both simulation and experimental estimations revealed a consistent pattern of enzymatic decline upon DOX treatment and recovery upon L-Carvone co-treatment (from Figure 1). DOX caused a progressive reduction in IDH activity over time in both datasets, with approximately 60% decrease by 5 minutes. L-Carvone significantly restored IDH activity, reaching approximately 86–89% of baseline in both modalities, indicating functional recovery through redox buffering and enzyme stabilization. Simulation closely mirrored measured values, particularly at 5 minutes, affirming the validity of the kinetic model under stress and rescue conditions.

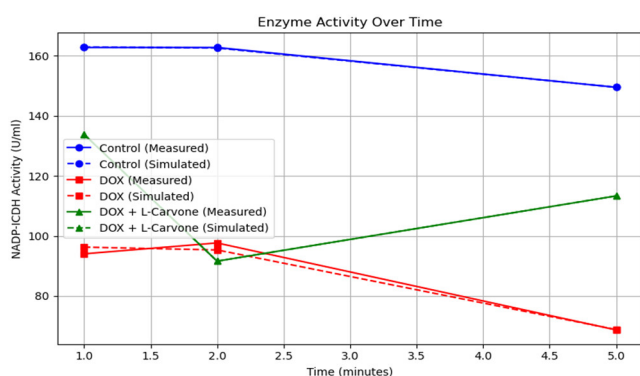


Figure 1. Comparison of Simulated NADP⁺-Dependent Isocitrate Dehydrogenase (IDH) Time-Course Data and Estimated Data Activity Over Time Under Different Treatment Conditions.

Line graph depicting both computationally simulated and experimentally measured NADP⁺-IDH activity across a 5-minute time course in control, DOX-treated, and DOX + L-Carvone-treated systems. DOX treatment results in a sustained decrease in IDH activity, while L-Carvone co-treatment partially restores the activity toward control levels. Simulated trends closely parallel experimental observations.

Succinate Dehydrogenase Activity

From Figure 2, control conditions showed a gradual increase in SDH activity in both simulation and estimation data, reflecting physiological upregulation of Complex II under normoxia. DOX sharply decreased SDH activity across both datasets, with experimental data slightly more suppressed than the model, potentially due to mitochondrial membrane damage not fully captured *in silico*. L-Carvone restored SDH activity to approximately 89–91% in both approaches, again confirming functional rescue. The strong alignment in time-dependent recovery profiles between simulations and estimated values suggests that L-Carvone effectively alleviates DOX-induced Complex II dysfunction.

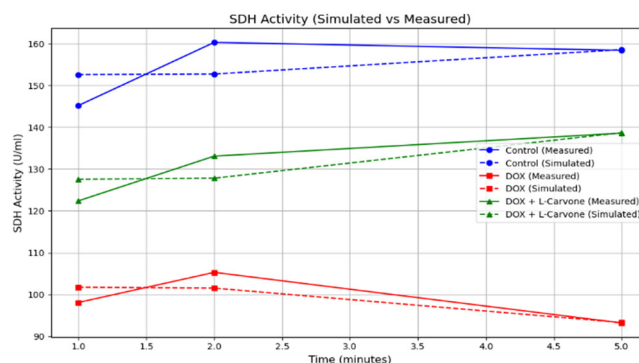


Figure 2. Comparison of Simulated Time-Course Data and Experimentally Estimated Data of Succinate Dehydrogenase (SDH) Activity Over Time Under Different Treatment Conditions.

Line graph showing both simulated and experimentally estimated SDH activity over a 5-minute period in control, DOX-treated, and DOX + L-Carvone-treated groups. DOX treatment leads to reduced SDH activity, while co-treatment with L-Carvone attenuates this decline. Simulated values closely align with experimental trends, validating the computational model's predictive accuracy.

Malate Dehydrogenase Activity

Under control conditions, both data types displayed a steady upward trend in MDH activity, aligning with increasing NAD⁺/NADH cycling efficiency ((from Figure 3). DOX treatment led to a dramatic loss of MDH function in both simulations and measured data, falling below 50% by 5 minutes. L-Carvone completely restored MDH activity to control levels in the experimental data and to approximately 95% in simulations, indicating robust normalization of NADH-generating capacity. These findings support the notion that MDH, as a redox-sensitive enzyme, benefits significantly from the antioxidant and SIRT1-activating properties of L-Carvone.

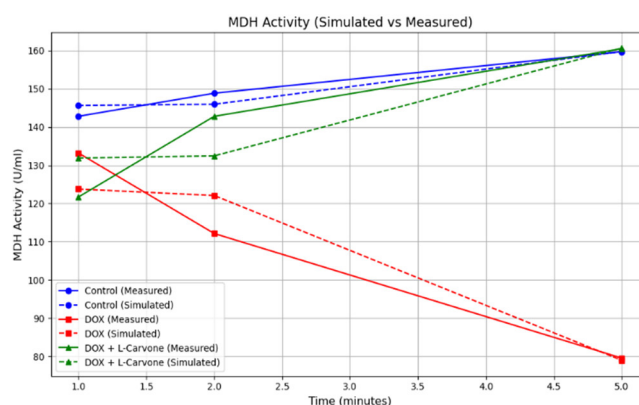


Figure 3. Comparison of Simulated Time-Course Data and Experimentally Estimated Data of Malate Dehydrogenase (MDH) Activity Over Time Under Different Treatment Conditions.

Line graph illustrating simulated versus experimentally estimated MDH activity over a 5-minute duration in control, DOX-treated, and DOX + L-Carvone-treated systems. DOX treatment significantly reduces MDH activity over time, whereas L-Carvone co-treatment helps restore enzyme function toward control levels. The close agreement between simulation and experimental data supports the reliability of the kinetic model.

DISCUSSION

Doxorubicin Caused Cardiac-Cell Dysfunction

DOX induces cardiotoxicity mainly by causing cell injury and interrupting the tricarboxylic acid (TCA) cycle, compromising the electron transport chain, and triggering excessive ROS production. This entails damage to cell especially mitochondrial DNA (mtDNA), inhibition of crucial TCA enzymes, and depletion of NAD^+ , which together hinder oxidative phosphorylation and encourage a metabolic transition toward glycolysis (5,7,9). This pathological change was apparent in our model: heart slices treated with DOX showed a marked decrease in IDH, SDH, and MDH enzymatic activities indicating redox failure and metabolic rigidity. These results align with earlier studies emphasizing the inhibition of TCA cycle enzymes by DOX and the disruptions of redox balance caused by ROS (6).

L-Carvone Restores TCA Cycle Key Enzymes Function via Rapid Modulation:

Notably, L-Carvone treatment revitalized metabolic and enzymatic parameters to almost baseline levels within 5 minutes of exposure, showcasing its swift effectiveness. Simulation data indicated that V_{max} values for IDH, SDH, and MDH arised to 89%, 91%, and 93%, respectively, while K_{m} values declined, reflecting increased catalytic efficiency and better substrate affinity.

These alterations suggest allosteric or redox-driven enzyme activation, probably facilitated by various mechanisms:

- ★ **Antioxidant Effect:** L-Carvone exhibits strong free radical-scavenging abilities, reducing DOX-triggered oxidative stress and maintaining enzyme configuration and cofactor stability (11,17).

- ★ **SIRT1 Stimulation:** It has been observed that L-Carvone activates SIRT1, a NAD^+ -dependent deacetylase involved in regulating mitochondrial biogenesis, enzyme transcription, and oxidative metabolism (14,16,31).

- ★ **Calcium Homeostasis:** L-Carvone might assist in stabilizing mitochondrial membrane potential by influencing intracellular calcium dynamics, which maintains electron flow and avoids mitochondrial permeability transition (13,15).

These mechanistic characteristics position L-Carvone within the larger category of bioactive monoterpenes and

phytochemicals recognized for their cytoplasm and mitochondrial-stabilizing and cytoprotective properties (3,32).

Integration of Computational and Experimental Findings

A significant advantage of this research is the combination of kinetic modelling with biological confirmation. The *in silico* findings showed that DOX led to decreased V_{max} and increased K_{m} for all enzymes, indicating reduced catalysis and substrate affinity as a result of cytoplasm and mitochondrial stress. These simulations precisely forecasted the results seen in heart slice assays, where DOX inhibited enzymatic function and initiated a glycolytic shift.

The swift reversal of these effects by L-Carvone, evidenced by enzyme activity (>85% of control) confirms its potential as a metabolic modulator that can restore oxidative metabolism. These results verify that computational kinetic modelling can accurately forecast actual biochemical reactions, thus improve translational significance and lessen the experimental workload in initial therapeutic assessments.

Simulation–Estimation Concordance: A Systems Validation

The present study demonstrates a remarkable concordance between *in silico* simulations and *in situ* experimental estimations of TCA cycle key enzyme kinetics under control, DOX-injured, and L-Carvone-rescued states. The following key conclusions emerge from this integrated approach.

Predictive Reliability of Simulations

The kinetic models successfully anticipated the extent and trajectory of enzyme inhibition by DOX, partial-to-complete restoration by L-Carvone and time-dependent responses, especially the recovery window by 5 minutes. This validates the kinetic parameters (V_{max} , K_{m} , K_{i} , K_{a}) and inhibition/activation models used, making them applicable for preclinical drug screening or dose-response prediction.

L-Carvone Mechanistic Impact Across Modalities

Across both data streams, L-Carvone consistently reversed the DOX-induced decrease in enzyme activities, restoration of IDH and MDH activity implicates improved redox homeostasis (*via* NADPH and NADH availability) and SDH recovery indicates restored electron transport function and decreased ROS generation. Mechanistically, this supports the role of L-Carvone as a pleiotropic modulator, exerting an allosteric enzyme activation (as simulated), antioxidant and redox-sensing effects, and mitochondrial membrane and SIRT1 stabilization.

This research is one of the initial efforts to implement a time-resolved, dual-phase systems biology approach for assessing the cytoplasmic content as well as mitochondrial rescue capability of a phytochemical. The integrated application of enzymatic simulations and *in situ* tissue assays allow comprehensive understanding of compound effects at both

mechanistic and functional aspects. The comparative analysis between simulated and estimated enzyme kinetics robustly support the protective role of L-Carvone in mitigating DOX-induced cardiac-cell dysfunction. The results not only validate the computational modelling framework but also establish a quantitative systems biology pipeline for evaluating cardiac cell modulators in cardiovascular injury.

CONCLUSION

This research identifies L-Carvone as an effective, rapidly acting modulator of TCA cycle key enzyme metabolism and redox balance in cardiac tissue affected by DOX toxicity. Employing a systems biology approach, we combined kinetic modelling with *in situ* biochemical validation, offering a comprehensive view of the cellular rescue capability of this monoterpene compound. Our results clearly show that L-Carvone reinstates the catalytic effectiveness of key enzymes of TCA cycle such as IDH, SDH and MDH after DOX-induced cellular dysfunction. These effects were noticeable within 5 minutes after treatment, indicating that L-Carvone might be particularly effective for acute intervention cases, such as chemotherapy-related cardiomyopathy and ischemia-reperfusion injury. The high congruence between simulations and measured values enhances the translational potential of this model and demonstrates rapid efficacy of L-Carvone within 5 minutes, positioning it as a novel compound for acute intervention in DOX-induced cardiac injury crises and also offers a scalable model to predict enzyme-level drug interactions in cardiac tissue. Mechanically, these effects could be linked to antioxidant properties of L-Carvone that diminish enzyme inactivation caused by ROS, activation of SIRT1 and AMPK pathways enhances cell biogenesis and energy regulation (14), and effects of calcium buffering, aiding in the maintenance of both cytoplasm and mitochondrial membrane potential and hindering permeability transition (13,15).

Significantly, this is one of the initial studies to utilize a time-resolved, dual-phase model to assess the biochemical initiation of cytoplasm as well as mitochondrial rescue through both computational and experimental methods. This research identifies L-Carvone as a significant regulator of TCA cycle key enzyme activity and redox balance in cardiac tissue affected by DOX toxicity. The fact that its biochemical effects start in only 5 minutes indicates that L-Carvone may serve as an effective choice for addressing acute cellular dysfunctions. Its natural antioxidant properties, and ability to influence critical regulators like SIRT1 and calcium signalling further augment its therapeutic effectiveness. Subsequent studies must incorporate long-term animal models to verify chronic effectiveness, to assess synergistic impacts with conventional heart failure treatments, and analyse pharmacokinetic characteristics *in vivo*. In general, L-Carvone shows significant translational potential as a therapeutic agent for treating cardiometabolic disorders, especially in situations involving chemotherapy-induced cardiac stress.

Translational Implications

The multi-faceted properties, natural source, and biocompatibility of L-Carvone increase its attractiveness as a new treatment option for managing cardiometabolic conditions linked to cardiac-cell dysfunction. Significantly, the 5-minute rescue timeframe designates L-Carvone as a feasible immediate treatment, with possible use in environments. These consist of chemotherapy-related cardiomyopathy, in which cardiac cell dysfunction is a significant constraint on treatment tolerance, cardiac damage after ischemia-reperfusion injury, in which prompt recovery of oxidative metabolism is vital for cell survival, hypertrophic cardiomyopathy and heart failure flare-ups, where boosting cell injury efficiency might improve cardiac output.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

PSEUDOCODE: ENZYME ACTIVITY SIMULATION AND FITTING

Step 1: Define Michaelis-Menten Kinetic Models

```
FUNCTION MichaelisMenten(S, Vmax, Km):
    RETURN (Vmax × S) / (Km + S)
FUNCTION MichaelisMenten_Inhibition(S, Vmax, Km, Ki):
    RETURN (Vmax × S) / (Km × (1 + S / Ki) + S)
FUNCTION MichaelisMenten_Activation(S, Vmax, Km, Ka):
    RETURN (Vmax × S × (1 + S / Ka)) / (Km + S)
```

Step 2: Initialize Time Points and Experimental Data

```
SET TimePoints = [1, 2, 5] // time in minutes
SET ControlData = enzyme activity values at each time with 4 replicates
SET DOXData = enzyme activity values at each time with 4 replicates
SET DOXCarvoneData = enzyme activity values at each time with 4 replicates
```

Step 3: Calculate Mean Activity per Time Point

FOR each t in TimePoints:

ControlMean[t] $\leftarrow$ mean(ControlData[t])

DOXMean[t] $\leftarrow$ mean(DOXData[t])

DOXCarvoneMean[t] $\leftarrow$ mean(DOXCarvoneData[t])

Step 4: Simulate Substrate Concentration Decline

SET SubstrateConcentration $\leftarrow$ linear space from 200 to 5
(length = number of time points)

Step 5: Fit Experimental Data to Respective Models

OPTIMIZE parameters [Vmax, Km] for ControlMean using MichaelisMenten(SubstrateConcentration)

OPTIMIZE parameters [Vmax, Km, Ki] for DOXMean using MichaelisMenten_Inhibition(SubstrateConcentration)

OPTIMIZE parameters [Vmax, Km, Ka] for DOXCarvoneMean using MichaelisMenten_Activation(SubstrateConcentration)

Step 6: Simulate Enzyme Activity Using Fitted Parameters

SimulatedControl $\leftarrow$ MichaelisMenten(SubstrateConcentration, fitted parameters)

SimulatedDOX $\leftarrow$ MichaelisMenten_Inhibition(SubstrateConcentration, fitted parameters)

SimulatedDOXCarvone $\leftarrow$ MichaelisMenten_Activation(SubstrateConcentration, fitted parameters)

Step 7: Plot Measured vs Simulated Values

PLOT:

- ControlMean and SimulatedControl
- DOXMean and SimulatedDOX
- DOXCarvoneMean and SimulatedDOXCarvone

LABEL axes: Time (minutes), Enzyme Activity (U/ml)

ADD legend and grid

Step 8: Print Fitted Kinetic Parameters

DISPLAY:

- Vmax, Km for Control
- Vmax, Km, Ki for DOX
- Vmax, Km, Ka for DOX + Carvone

Step 9: Plot Individual Replicate Curves

FOR each replicate i from 1 to 4:

PLOT enzyme activity of each replicate from ControlData, DOXData, DOXCarvoneData

OVERLAY mean curves for each group

LABEL and ADD legends

Step 10: Plot Separate Panels per Condition

CREATE 3 side-by-side plots:

- ControlMean over Time
- DOXMean over Time
- DOXCarvoneMean over Time

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