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Stochastic modelling of the oral cancer proliferation and death in the presence of Thymoquinone as anticancer therapeutics

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Abstract. Oral cancer is one of the most commonly known cancer worldwide. Thymoquinone (TQ) an extract from *Nigella sativa*, has clinically been proven as an anticancer therapeutic agent for oral cancer due to its intrinsic pharmacological characteristics. Understanding the mechanisms of oral cancer proliferation and death in the presence of TQ is crucial so that the insight of the interaction of cancer cells and TQ can be discovered. Cancer cells in the presence of TQ is subjected to the uncontrolled factors of the environmental noise. Deterministic model is inadequate to explain this behaviour. Herein, a stochastic model is proposed to illustrate the dynamics of HSC-3 oral cancer cell lines in the presence of TQ. The deterministic model is perturbed with the noisy behaviour which then leads to the stochastic model. The model is simulated by using a four-stage stochastic Runge-Kutta (SRK4) method and the kinetic parameters are estimated by using the maximum likelihood estimation (MLE) method. The prediction quality of the model is measured by using root mean square error (RMSE). The low values of RMSE show the best-fit of the stochastic model.

1. Introduction

Cancer is a general term for a group of diseases that arise when abnormal cells replicate out of control in almost every organ and tissue of the body. Oral cancer is a subtype of head and neck cancer that affects the tongue, jawbone, palate, salivary glands, teeth, mouth floor, and oral mucosa. Oral cancers were mostly diagnosed late in their progression, leaving patients with a poor prognosis. Early diagnostic and treatment may possibly enhance the patient survival up to 80% for head and neck cancer [1, 2]. According to the report [1], almost 19.3 million new cancer cases with 10 million death from cancer have been reported. World Health Organization (WHO) has reported in the year 2019, cancer was the leading cause of mortality under the age of 70 in 112 of the 183 countries surveyed [3]. Oral cancer is one of the most common and deadliest disease all over the world with a five-year survival rate of only 50 % [4]. While the organisation of Global Burden Disease has estimated 3.5 billion people have been affected by this disease in 2017 globally. Generally, in oral cancer, the oral malignancy appears in the form of an oral cavity either on the lips or inside the mouth. Oral squamous cell carcinoma associated



with the development of multiple other carcinomas also which includes melanoma and type of neoplasms. Along with other treatment methods, 50% of oral squamous cell carcinoma patients have been treated with adjuvant chemotherapy and radiotherapy. These treatments unfortunately damage the normal cell. To prevent the harmful effects of chemotherapy and radiotherapy effects, natural remedies medications are a good choice for preventing cancer. For natural treatment, thymoquinone (TQ) is the main active constituent of *Nigella sativa* seeds that has numerous pharmacological activities including anticancer, antioxidant, and antimicrobial [5]. Many studies have investigated the role of TQ as an antitumor and anti-metastatic agent in cancer regulation and prevention [6]. Inhibition of human oral carcinoma proliferation is occurred through apoptosis process [7]. The apoptosis is a programme cell death in cancer cells associated with up and down-regulation caspases proteins [8]. A control regulation of apoptosis can target the oral cancer cells, hence able to treat the oral cancer [9]. TQ-based induction of p53 gene-dependent apoptosis has been reported via activation of caspase-3 [10]. However, the dynamic behaviour of the interaction between TQ and apoptosis is still in question. Therefore, it is important to model the dynamics of the oral cancer cells in the presence of TQ as anticancer therapeutics.

Deterministic modelling has been widely used to study the interaction of cancer cells and its treatment [11, 12]. The interaction of TQ and cancer cells is subjected to the uncontrolled factors of noisy behaviour such as stress effect, deamination, changes in blood pressure, cellular metabolism and so on [13]. However, due to presence of stochasticity particularly in oral cancer when it reacts with TQ, deterministic model required to be extending into its stochastic counterpart. Therefore, present research is at aim to model the interaction of HSC-3 cell line (oral cancer cell) with TQ as anticancer therapeutics. As TQ induces the apoptosis (TQ-cancer cell death) pathway, the interaction of TQ and oral cancer cells in the apoptosis pathway is converted into chemical reaction. Further, this reaction is transformed into mathematical model of ordinary differential equation (ODE). The perturbation of the noisy behaviour (White noise) is done through growth rate parameter to form a mathematical model of stochastic differential equation (SDE). Model parameters are estimated using maximum likelihood estimator (MLE) and the model is simulated using four stage stochastic Runge-Kutta (SRK4). The performance of ODE and SDE models are measured using RMSE.

2. Material and Methods

Originally, HSC-3 cells are oral squamous carcinomas from the human tongue in different patients. HSC-3 cells started as oral squamous carcinomas from various patients' tongues. Those enzymes first were identified in 1989 at Tokyo Medical and Dental University, Faculty of Dentistry's Department of Oral and Maxillofacial Surgery [14]. The pro-apoptotic BAX, Noxa, and p53 AIP1 proteins are required for p53 activation mutated, which results in cell-programmed death through induction. Even then, as the majority of p53 is muted, more stem cells pathways develop, resulting in cell proliferation to original phases [15]. The details about the materials and methods of the experimentation for HSC-3 cell lines interact with TQ are summarised in Table 1.

Table 1. Materials and Methods of the experimentation of TQ react with HSC-3.

Materials	
Reagents	TQ and Cisplatin (CDDP, has proven to be one of the more efficient anticancer chemotherapeutic agents because it targets multiple intracellular sites to induce death in tumour cells [13]) were purchased from Sigma-Aldrich, USA. TQ and CDDP stock solutions were freshly formulated in 100 mg/mL concentrations before each experiment by dissolving with molecular grade Dimethyl Sulfoxide (DMSO), Gibco, USA.
Cell Culture	The HSC-3, HSC-4, and HACAT cell lines used for the present study were gifted from Prof. Masaaki Ikeda, Tokyo Medical and Dental University, Japan. The Integrated Centre for Research Animal Care and Use (ICRACU), International Islamic University Malaysia, generously supported the human gingival fibroblast (HOF) cells (IIUM). The cells were cultured in Dulbecco's Modified Eagle Medium ((DMEM)), Gibco, USA in a humidified incubator with 5 % CO ₂ at 37° C, combined with 10 % fetal bovine serum (FBS) (Gibco, USA) and 1 % Penicillin-Streptomycin.

Equipment The used biosafety cabinets class 2 were from ESCO (laboratory) (USA) and Jouan (French). CO₂ incubator (Thermo Fisher™, Germany), waterbath (Mettler GmbH+ Co., Germany), centrifuge (Hettich® MIKRO 120, Germany), and vortex (VELP Scientifica Srl, Italy) were applied to perform the experimentation. Deep freezer (Sanyo, Japan) was used to preserve the sample at -80° C. The inverted focal microscope (Meiji Techno, Japan) and conventional microscope CX31 (Olympus Microscope, Japan) were used to observe characteristics of cell lines. Water purification and distilled water systems were from the PURELAB option by ELGA, UK. The microplate reader (Infinite® 200 PRO Nano quant, Tecan, Männedorf, Switzerland) was used for measuring the absorbance of the sample in all assays.

Methods

In Vitro Cytotoxic Study (MTT Assay)	For 24 hours, the cells were seeded in 96-well plates until they reached a density of 70-80 per cent confluency per well. TQ and CDDP samples were presented to cells for 24 and 48 hours at specified concentrations (0 - 0.01 mg/mL for TQ and 0 - 0.008 mg/mL for CDDP). In each well, 20 mL MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich, US) was applied at a concentration of 5 mg/mL (in Phosphate-buffered saline PBS). In a humidified climate, the cells were then incubated for 4 hours at 37° C. The supernatants were discarded, and the formazan crystals were dissolved in 100 litres of DMSO for 1 hour before being read in a microplate reader. At a wavelength of 570 nm, the absorbance was determined in a spectrophotometer (reference: 630 nm). At least two times, each experiment was carried out. The standard error mean (SEM) of three replicates is recorded, and it is expressed as a percentage of the control values.
Caspase 3/7 Activity Assay	The activation of caspase 3/7 was determined by using the caspase-Glo 3/7 assay Kit (Promega, US). The cells were grown overnight in 96-well flat clear bottom white plates at a density of 3 X 10 ⁴ /well. The cells were treated in the same manner as those described in the flow cytometry experiment. The assay was conducted according to the manufacture protocol following sample treatment in 24 hours by adding the caspase-Glo 3/7 assay reagent directly to the well. The absorbance was read in a luminometer 30 min after the addition of the reagent into the wells. Each sample was performed in triplicate.

Herein, the data used is for in vitro toxicity effect of TQ concentration, TQ's effect on oral cancer is being studied. The HSC-3 cells were exposed with graded concentrations of TQ (5µL, 10µL, 20 µL, 40 µL, 60 µL) for 24 and 48 hours. For 24 and 48 hours, HSC-3 cells were exposed to graded concentrations of TQ (5L, 10L, 20L, 40L, 60L). To reflect normal cells, HOF and HaCaT cells were used. Due to its ease of propagation and near-normal phenotype. The keratinocyte model HaCaT has long been used. The TQ has been shown in several experiments to reduce the viability of oral cancer cells while having marginal effects on normal cells. TQ caused cell-type-dependent cytotoxicity in HSC-3 cells [16]. TQ decreases the viability of HSC-3 cells in a concentration and time-based way, with IC₅₀ values of 3 g/mL and 8 g/mL for 24 and 48 hours, respectively. The TQ was estimated by using the normal cell line such as human gingival fibroblast HOF and HACAT cells. The cells exposed to an IC₅₀ dose of TQ with HSC-3 cells [17, 18]. The data used for this study was obtained from [19].

3. Mathematical Model

We have adopted the stochastic modelling approach to describe the interaction of the HSC-3 and TQ in the present study. TQ induced apoptosis signalling pathway is presented in Figure 1. In human oral cancer cells, TQ helps in regulating apoptosis proliferation, which leads to apoptotic cell death and apoptosis triggered by caspase-3 activation.

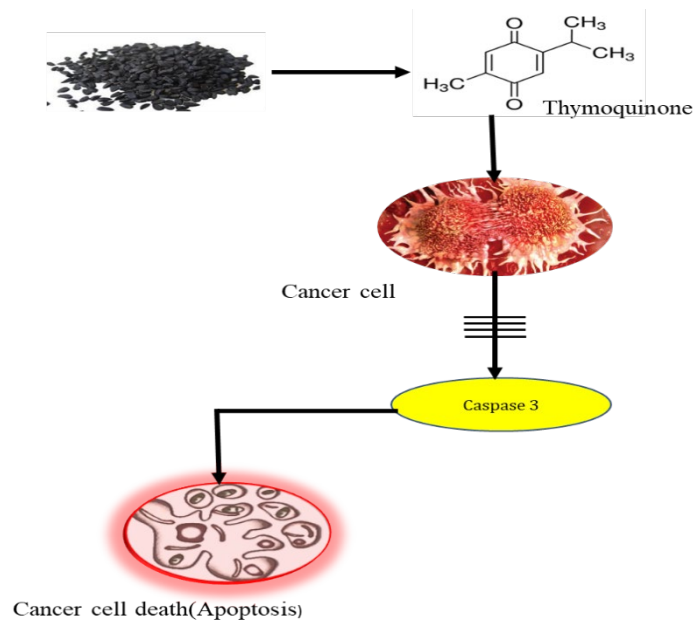


Figure 1. Model diagram of TQ induces apoptotic pathway.

The interaction rate of TQ with the cancer cells is denoted as k_1 . The activated caspase 3 (Figure 1) promotes the cancer cell death or specifically known as the apoptosis mechanism at the rate of k_2 . This pathway can be converted into the chemical reaction in mathematical form as



Herein $\lambda = 48.7199$ is the observed IC_{50} value in the experimentation. The IC_{50} is referred to the inhibition concentration of TQ that capable to reduce the cell viability up to 50%. X is the cancer cell, Y is the activation of caspase 3, k_1 and k_2 are the kinetic parameters. Equation (1) can be transformed into a system of ordinary differential equation (ODE) form as

$$\begin{aligned} dX &= -k_1 \lambda X dt \\ dY &= (k_1 \lambda X - k_2 Y) dt \end{aligned} \tag{2}$$

Equation (2), which is the form of ODEs can be converted into stochastic differential equation (SDEs) by perturbing the parameters k_1 and k_2 such that

$$k_1 \rightarrow k_1 + \sigma_1 \frac{dW}{dt} \tag{3}$$

$$k_2 \rightarrow k_2 + \sigma_2 \frac{dW}{dt} \tag{4}$$

This yield,

$$\begin{aligned} dX &= -k_1 \lambda X dt + \sigma_1 X dW(t) \\ dY &= (k_1 \lambda X - k_2 Y) dt + \sigma_2 Y dW(t) \end{aligned} \tag{5}$$

Here, $\sigma_1, \sigma_2 > 0$ are diffusion coefficients and the wiener process for $t \geq 0$ is a Gaussian white noise process with mean zero and variance, Δt .

4. Results and Discussion

The simulated trajectories of the model (5) in the time interval $[t_0, T]$ are obtained SRK4 over the time increment $h_n = t_n - t_{n-1} = \frac{T}{N}$. The kinetic parameters are estimated using the MLE method and the results are depicted in Table 2.

Table 2. Maximum Likelihood Estimates of Stochastic Model Parameter

Parameters	k_1	k_2	σ_1	σ_2
Deterministic Model	- 0.00013	- 0.00013	-	-
Stochastic Model	- 0.00013	- 0.00013	- 0.007	0.5

The initial conditions of HSC-3 cell lines, $X(0)=1$. Figure 2 shows the simulated results of the deterministic model (2) and the corresponding experimental data.

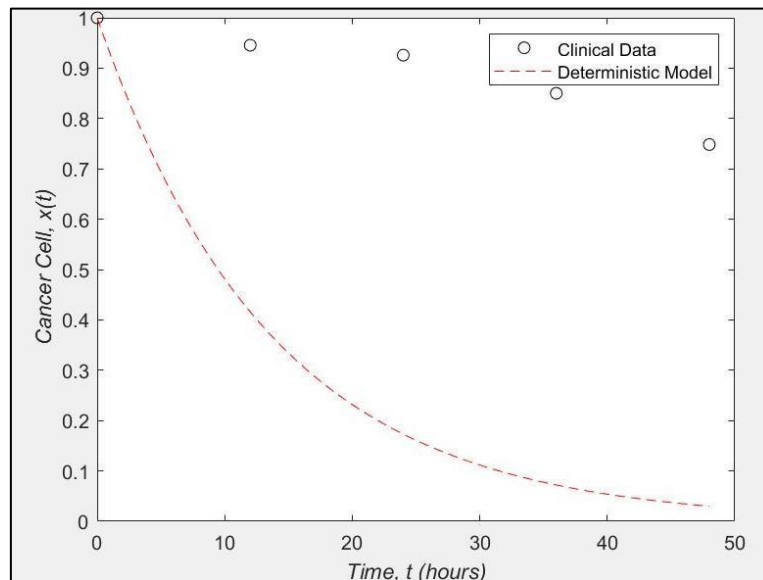


Figure 2. Graphical simulation results of deterministic model for ODE with experimental data of cancerous growth with treatment.

TQ with a dosage of $3\mu\text{g}/\text{mL}$ is promising in HSC-3 cancer treatment under in vitro experimentation. Graphical visualisation of results shows the medicinal effect of TQ ($3\mu\text{g} / \text{mL}$) on cancer cell growth in 0, 12, 24, 36 and 48 hours. HSC-3 cancer cell lines show the decreasing trend of growth under the presence of TQ, hence indicate TQ is a promising treatment in the fight against HSC-3 cancer cell lines (in vitro experiment). However, the simulated result of the deterministic model is inconsistent with the experimental data. Figure 3 illustrates the simulated result of SDE (5).

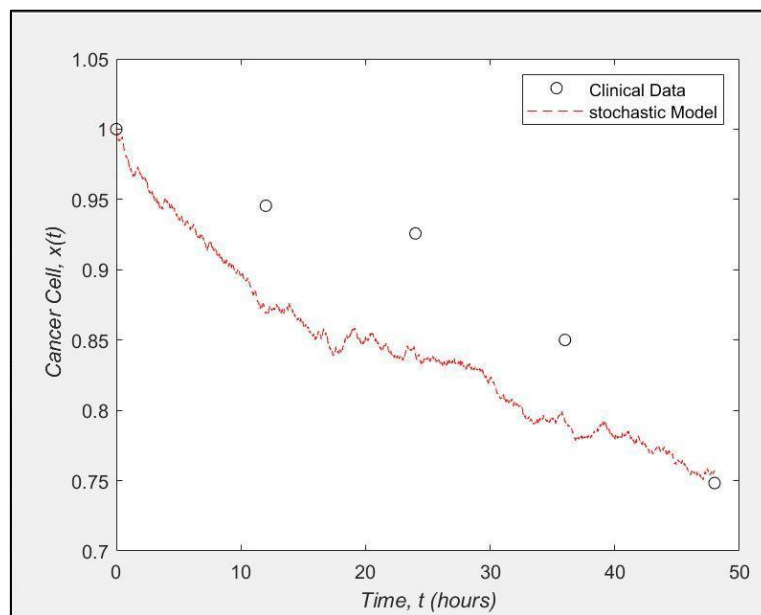


Figure 3. The Stochastic model’s simulations for SDE with experimental data of cancerous growth with treatment.

Figure 3 shows the consistency of the simulated results with the experimental data as depicted by the decreasing trend of the sample path that follows the experimental data. Graphical visualization shows the medicinal effect of TQ ($3\mu\text{g/mL}$) on cancer cell growth in 0, 12, 24, 36 and 48 hours. Cancer interacting with TQ is subjected to the noisy behaviour as reflected by the up and down of the simulated results. RMSE is computed to measure the error of both models as indicated in Table 3. The SDE model shows a low value of RMSE, hence indicating a good fit of the model.

Table 3. RMSE (Root Mean Square) Value for ODE and SDE Model

Deterministic Model (ODE)	0.6114
Stochastic Model (SDE)	0.0573

5. Conclusion

The numerical simulations for deterministic and stochastic models were presented for cancer cell growth in response to the TQ for the HSC-3 cancer cells line. Herein, the cancer cell growth is subjected to noisy behaviour as it is influenced by various uncontrolled factors. The developed model can well explain the effect of TQ on oral cancer for HSC-3 cell lines. TQ is the promising targeted treatment of HSC-3 cancer cells line as depicted by the decreasing curve of the cancer cells in the presence of TQ. The lowest RMSE observed in this study concluded that the stochastic model is more adequate to oral cancer for HSC-3, which is fitted good to the experimental data. To prevent tumour growth, drug-targeted therapy should be started as soon as a cancer cell is identified and examined regularly.

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