Application and Development of Biochemical Transformation Kinetics for a Dengue Patient under Treatment of *Carica papaya* Leaves Extract

Gamage TV [1], Basnayake BFA[2]

[1] Department of Nursing, Faculty of Allied Health Sciences, University of Peradeniya, Sri Lanka
[2] Department of Agricultural Engineering, Faculty of Agriculture, University of Peradeniya, Sri Lanka

Abstract-. Dengue in recent years has become a worldwide public health concern. So far, there was no defined treatment for it. Therefore, secondary interpretation of a therapeutic trial study was done to evaluate the potential of *Carica papaya* leaves extract against Dengue fever by application of chemical kinetic studies.

The performed secondary analysis data set was obtained from Ahmad *et al.*, 2011. According to the study, 25 mL of aqueous extract of *C. papaya* leaves was administered to a patient infected with dengue fever twice daily for 5 consecutive days. Before and after the administration, the blood samples from the patient were analyzed for Platelets counts (PLT), White Blood Cells (WBC) and Neutrophils (NEUT). The effectiveness of *C.papaya* leaf extract was predicted by the logistic growth equation, first order and Michelis-Menten kinetics.

Michelis-Menten analysis of PLT count, WBC and NEUT showed longer time period for recovery and it can be predicted. Application of logistic growth equation to predict the rise of PLT count showed the growth coefficient of $\alpha = 0.627$, retardation coefficient $\beta = 0.0033$, the peak $\alpha/\beta = 190 \times 10^3/\mu L$ and the lowest $X_o = 50 \times 10^3/\mu L$. However, a first order equation fits better indicating virus inhibitions. A first order rate reaction allows predictions to be made even after one day's PLT count. If the slope, k is > 0.2868, between the ln values of 1^{st} and 2^{nd} day, the patient is likely to have had an attack of dengue virus.

Knowing k, can lead to prediction of hypothetical values for the next five days. Michaelis-Menten kinetics can be applied to the hypothetical values so as to make comparisons. In order to validate these findings, it is best to undertake a research involving number of patients suffering from dengue. However, this is only a preliminary study to demonstrate the soundness of the mathematical techniques used and it is recommended to undertake a large scale study to validate the findings.

Index Terms- *Carica papaya* leaves, Dengue, Michelis-Menten analysis, logistic growth equation, first order

1. INTRODUCTION

Dengue in recent years has become a worldwide public health concern. It is a Mosquito borne infection which in recent decades has become a major global public health problem. This viral disease is prevalent in tropical and sub-tropical regions around the world. Dengue Haemorrhagic Fever (DHF) affects most Asian countries and has become a leading cause of hospitalization and deaths (Hettige, 2008).There is not an exact treatment for dengue fever up to date. In 2012, dengue ranks as the most important mosquito borne viral disease in the world. The emergence and spread of all four dengue viruses ("serotypes") from Asia to the Americas, Africa and the Eastern Mediterranean regions represent a global pandemic threat. During the past five decades 50-100 million new infections are estimated to occur annually in more than 100 endemic countries. Every year hundreds to thousands of severe cases arise, including 20,000 deaths and often affecting very poor population (WHO, 2012). In Sri Lanka, dengue is currently spreading in urban to rural areas and has reached epidemic proportions. Dengue usually occurs as epidemics in Sri Lanka following monsoon seasons. The number of total cases recorded for year 2009 is 32,713 and it has become a major public health problem (Health Education Bureau, 2013).

Dengue fever (DF) is an acute febrile viral disease generally presenting with headaches, bone or joint and muscular pains, rash and leukopenia as symptoms. Dengue haemorrhagic fever (DHF) is characterized by four major clinical manifestations: high fever, haemorrhagic phenomena, hepatomegaly and in sever signs of circulatory failure. And it is a systemic and dynamic disease. Such patients may develop hypovolemic shock due to plasma leakage. This is called dengue shock syndrome (DSS). It can be fatal. Dengue viruses are transmitted to humans through the bite of infected *Aedes* mosquitoes, principally *Aedesaegypti*. Dengue virus belongs to the family *flaviviridae*. The four serotypes of dengue virus (designated DEN-1, DEN-2, etc.) can be distinguished by serological methods (WHO, 1997).

It has a wide clinical spectrum that includes both severe and non-severe clinical manifestations. After the incubation period, the disease begins abruptly and is followed by the three phases-febrile, critical and recovery phase. During febrile phase patient typically develop high-grade fever. It usually last 2-7days and is often accompanied by facial flushing, skin erythema, generalized body ache, myalgia, headache and arthralgia. Increase in capillary permeability in parallel with increasing haematocrit levels are the marks of the beginning of the critical phase and the period of clinically significant in plasma leakage usually last 24-48hours. Progressive leukopenia followed by a rapid decrease in platelet count usually precedes plasma leakage. During recovery phase haematocrit stabilizes, or may be decrease due to the dilution effect of reabsorbed fluid, leucocytes count usually starts to increase soon after defervescence but the recovery of platelet count is typically later than that of leucocyte count (WHO, 2009). Beyond those phases severe dengue is defined by one or more of the followings: plasma leakage that may lead to shock (dengue shock) and/or fluid accumulation, with or without respiratory distress and/or severe bleeding, and/or severe organ impairment. Patients with severe dengue may have coagulation abnormalities when major bleeding does occur. It is almost always associated with profound shock since this, in combination with thrombocytopenia; hypoxia and acidosis can lead to multiple organ failure and advanced disseminated intravascular coagulation (WHO, 2009). Two major pathophysiological changes occur in DHF/DSS. One is an increased vascular permeability that gives rise to loss of plasma from the vascular system. It results in haemoconcentration, low pulse pressure. Second one is disorder in haemostasis involving vascular changes, thrombocytopenia and coagulopathy. Platelet defects may be both qualitative and quantitative (WHO, 1997).

Up to date, there is no specific treatment for dengue fever. However, Dr. Ms Tharanga Kumari Wickramasinghe who is a qualified Ayurvedic doctor had already obtained the patent license for Ayurvedic treatment that can cure dengue. Patients with known as suspected dengue fever should have their thrombocyte count and haematocrit count measured daily from the third day of illness until 1-2 days after defervescene. There is no licensed DEN antiviral vaccine available for the prevention of dengue infection and immunogenic, safe tetravalent vaccines have been developed and are undergoing clinical trials. Avoid being bitten by the vector mosquito is only way to prevent dengue virus (Ahmad *et al*., 2011).

Research into phytotherapy of diseases is a current trend in the management of tropical diseases, with a view to finding cheaper, alternative medicines that the wide populace can have immediate access to. It has been reported that people consume papaya leaves extract to increase their platelet count after they contract dengue fever. Also, studies show it was an effective treatment for dengue fever. *Carica papaya* (*C. papaya*) is a member of the Caricaceae family. The papaya fruit is globally consumed as a fruit. The ripe fruit is rich source of vitamin A, C, and calcium. There are many commercial products derived from the different parts of the *C. papaya* plant, the most prominent being papain and chymopapain, which is produced from the latex of the young fruit, stem, and the leaves. *C. papaya* leaves have been used in Ayurveda medicine for centuries. Recent studies have shown its beneficial effect as an anti-inflammatory agent, anti-tumour agent as well as immune modulatory effects and as an antioxidant (Subenthiran *et al*., 2013).

Ayurveda literature reveals that *C. papaya* leaf extract has haemostatic and other medicinal properties. The studies have shown the effects of papaya leaf extract in dengue patients of elevating the total white cell counts and platelet counts (Hettige, 2008). As a folk medicine, it has been reported that people consume papaya leaves extract to increase their platelet count after they contract dengue fever. Also, studies show it was an effective treatment for dengue fever.

Clinical trials done in Malaysia, India, Sri Lanka and Pakistan have come up with evidence to prove the efficacy of *C. papaya* leaf extract in augmenting platelet count in patients with dengue fever. Their blood count was monitored and gene expression studies conducted. Toxicity studies carried out also showed that the papaya juice was safe for human consumption. Researchers from United States and Japan have discovered that enzymes found in papaya leaf have cancer fighting properties. According to the study, papaya leaves are not known to have toxic effects and their consumption does not have side effects (The Indian express, 2013).

The diagnosis of dengue fever has been a drawback in administering papaya extracts. For that reason, chemical kinetics could be a prediction tool in the absence of alternate methods of prognosis, the application of chemical kinetics is a possibility. Chemical kinetics (reaction kinetics) is the study of rates of chemical processes. Chemical kinetics includes investigations of how different experimental conditions can influence the speed of a chemical reaction and yield information about the reaction's mechanism and transition states, as well as the construction of mathematical models that can describe the characteristics of a chemical reaction. This states the speed of a chemical reaction is proportional to the quantity of the reacting substances. All of the biochemical processes considered to have been single-step reactions. But, reality is not so simple and this is particularly important for considering enzyme-catalysed reactions, because these are essentially never single-step reactions, a reaction of more than one step. A reaction that proceeds in two or more steps can reach a steady state, in which the intermediate concentrations remain essentially constant. Chemical kinetics deals with the experimental determination of reaction rates from which rate laws and rate constants are derived. The rate-determining step often can determine by using the kinetics. The main factors that influence the reaction rate include: the physical state of the reactants, the concentrations of the reactants, the temperature at which the reaction occurs, and whether or not any catalysts are present in the reaction can be determined by chemical kinetics (Wright, 2004).

The effectiveness of *C. papaya* leaf extract in augmenting platelet count can be predicted and developed by the application of enzyme kinetics. It is not reported yet. The results of kinetic studies can help to determine the administration of '*C. papaya*' leaf juice in dengue fever. It will be helpful to develop effective treatment for the management of DF in the near future. Therefore, in this study, mathematical and kinetic approaches were used to demonstrate inter and intra relationship of increasing platelet count after treatment of *C. papaya* leaf extract by using secondary data. For that, the logistic growth curve equation, first order and Michelis-Menten kinetics were used. This study provided further evidence that *C. papaya* possesses platelet increasing activity in a dose-dependent manner.

2. METHODOLOGY

Research Design

This was a secondary interpretation of a therapeutic trial study. The data were collected through secondary data analysis. The performed secondary analysis data set was obtained from journal article titled "Dengue fever treatment with *C. papaya* leaves extracts which was published by Ahmad *et al.*, 2011. Written permission from the corresponding author of this article was obtained through e-mail conversation.

Research methodology adapted by Ahmad et al., 2011

The procedure adopted by Ahmad *et al.,* 2011 for plant material preparation and the data collection by is described in the following sections.

Plant material and sample preparation

Fresh plant materials of *C. papaya* were collected from University of Peshawar. The plant material was washed with water, cut into pieces and grinded by using a blender. 25 ml of aqueous extract of *C. papaya* leaves was administered to patient infected with dengue fever. The same dose was given to the patient twice daily, morning and evening for five consecutive days (Ahmad *et al.,* 2011).

Sample preparation and administration of prepared extract

About 25ml of papaya leaves extract was administrated orally, twice daily, morning and evening for five consecutive days (Ahmad *et al*., 2011).

Study area, population and sample size

The study was conducted among a patient who diagnosed as a victim of dengue fever by basis of screening blood test examination. The study was conducted for five consecutive days before administrating *C. papaya* leaves extract and five consecutive days after administrating first *C. papaya* leaves extract (Ahmad *et al.*, 2011).

Data collection and data collection tool

Before *C. papaya* extract administration, a blood sample obtained from the patient and it was evaluated for biochemical parameters of Platelets count (PLT), White Blood Cells (WBC), Red Blood Cells (RBC), Neutrophils (NEUT). After that, blood samples were obtained from the patient in 24 hours intervals for five consecutive days and analysed for the mentioned parameters (Ahmad *et al.*, 2011).

Data collection tools

Effectiveness of the *C. papaya* extract was investigated bio chemically from blood samples. Serum was separated serum profile was used for the estimation of biochemical parameters as mentioned below (Ahmad *et al.,* 2011).
1. Platelets count (PLT)
2. White Blood Cells (WBC)
3. Red Blood Cells (RBC)
4. Neutrophils (NEUT)

Editing, Data Entry and Analysis

Initially the collected data were entered into a Microsoft Excel worksheet and then check for the correct data entry. Once the clarity of the entered data is ensured, it correctly used for kinetic analysis by application of data to Michaelis-Menten equation Line-weaver Burk model, and first order equation, logistic growth equation.

i. Mechaelis-Menten equation

$$v = \frac{v_m S}{K_m + S}$$

Where,
V = Rate of reaction
S = Substrate concentration
K_m =Michaelis-Menten Constant

ii. Line-weaver Burk model

$$\frac{1}{v} = \frac{1}{v_m} + \frac{K_m}{vS}$$

iii. Logistic growth equation

$$X_t = \frac{\alpha X_0 e^{\alpha t}}{\beta X_o e^{\alpha t} + \alpha - \beta X_0}$$

Xt = Biological transformation mass (growth) at time t
Xo = Initial value of (reactive) growth
α = Transformation or growth coefficient
β = Retardation coefficient

iv. First Order Equation

$$V = A(1 - e^{-kmt})$$ Where, $$A = \frac{km}{Vm}$$

Rate of changing values of blood cells (ds/dt) were calculated by equation as mentioned below,

$$\frac{dS}{dt} = \frac{S_n - S_{(n-1)}}{t_n - t_{(n-1)}}$$ Where, t = time in days

Cumulative value of each ds/dt was considered as v value. Then, 1/*s* and 1/***v*** values were calculated. Line-weaver Burk plot was graphed by using 1/*s* and 1/***v*** values and K_m and v_m were calculated according to the graph and then the values were predicted.

3. RESULTS AND DISCUSSION

The results of the above study have been discussed under two main topics, namely the first topic is the analysis of changes in biochemical parameters of the blood before and after administration of *C. papaya* leave extract. The second is the kinetic analysis of biochemical changes of the parameters by application of data based on Michaelis-Menten equation, Line-Weaver Burk model and Logistic growth equation.

In both topics, the following biochemical changes in blood before and after administration of *C. Papaya* leave extract were used.

1. Platelets count (PLT)
2. White Blood Cells (WBC)
3. Red Blood Cells (RBC)
4. Neutrophils (NEUT)

Table 3.1 shows biochemical changes of blood conducted for five consecutive days before administrating *C. papaya* leave extract (Ahmad *et al.*, 2011) and Table 3.2 five consecutive days after administrating first *C. papaya* leaf extract (Ahmad *et al.*, 2011).

Table 3.1: Blood test reports before the administration of *C. papaya* leaves extract (Ahmad *et al.*, 2011)

Parameter	Time (Days)				
	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}
PLT	$176\times10^3/\mu L$	$122\times10^3/\mu L$	$110\times10^3/\mu L$	$71\times10^3/\mu L$	$55\times10^3/\mu L$
WBC	$8.10\times10^3/\mu L$	$6.60\times10^3/\mu L$	$4.40\times10^3/\mu L$	$4.00\times10^3/\mu L$	$3.70\times10^3/\mu L$
RBC	$5.28\times10^6/\mu L$	$4.96\times10^6/\mu L$	4.94×10^6 /µL	5.23×10^6 /µL	$5.00\times10^6/\mu L$
NEUT	84 %	81.5 %	71.8 %	60 %	46 %

Table 3.2: Blood test reports after the administration of *C. papaya* leaves extract (Ahmad *et al.*, 2011)

Parameter	Time (Days)				
	1^{st} day	2^{nd} day	3^{rd} day	4^{th} day	5^{th} day
PLT	$73\times10^3/\mu L$	$120\times10^3/\mu L$	$137\times10^3/\mu L$	$159\times10^3/\mu L$	$168\times10^3/\mu L$
WBC	$3.80\times10^3/\mu L$	$4.40\times10^3/\mu L$	$5.30\times10^3/\mu L$	$5.90\times10^3/\mu L$	$7.70\times10^3/\mu L$
RBC	$4.71\times10^6/\mu L$	$5.33\times10^6/\mu L$	4.99×10^6 /µL	5.21×10^6 /µL	$5.26\times10^6/\mu L$
NEUT	56%	64.2%	71.1%	73%	78.3%

Study of Changes in Blood Count

1. Platelet count

There was significant continual linear decrease of platelet counts that can be observed before administration of *C. papaya* leaves extract treatment as shown in Figure 3.1. On the first day the platelet count was $176x10^3$ /µL and it reduced continuously and on 5^{th} day platelet count reduced to as low as $55x10^3$/µL. It shows that the PLT count falls beyond the normal range. The PLT count of a normal person is normally within $150x10^3$/µL - $400x10^3$/µL (Keogh, 2010).

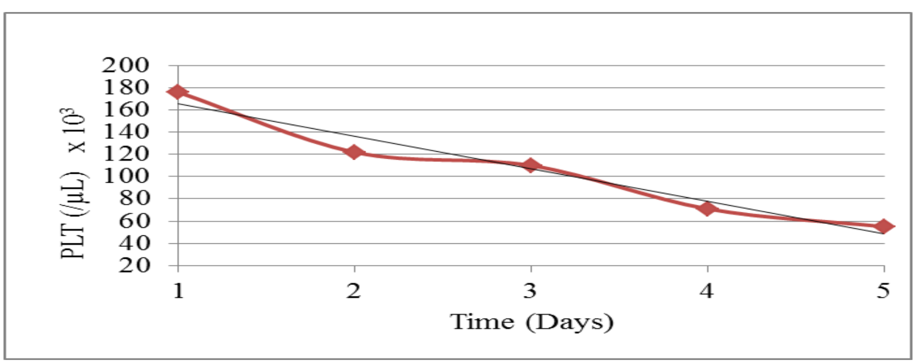

Figure 3.1: Platelet count changes with time before the administration of *C. papaya* leaves extract

After administration of *C. papaya* leaves extract, the PLT count dramatically increased as can be observed. It was almost linear increase, but reaching a plateau . On the very first day of *C. papaya* extract treatment the PLT count increased to 73×10^3/µL and lower increment towards the 4th day of *C. papaya* extract administration. The PLT count reached normal levels. Continuously, PLT count increased up to 168×10^3/µL on the 5th day.

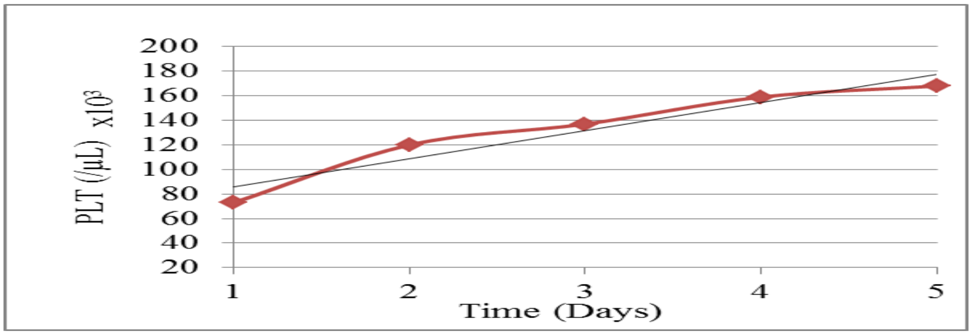

Figure 3.2: Platelet count changes after the administration of *C. papaya* leaves extract

2. White Blood Cell (WBC) Count

Similar to PLT count there was significant decrease of WBC count that can be observed before administration of *C. papaya* leaves extract treatment as shown in Figure 3.3. The comparison of the linear line show the drastic reduction up to the third day and it should be considered as the critical point. WBC count of first day was 8.10×10^3/µL and continuously it reduced and on 5th day WBC count reduced up to 3.70×10^3/µL. WBC count of a normal person ranges between 5×10^3/µL -10×10^3/µL (Keogh, 2010). Notice that after the 3rd day, the WBC count of this patient felled beyond the normal level. Low WBC count indicates viral infection (Keogh, 2010).

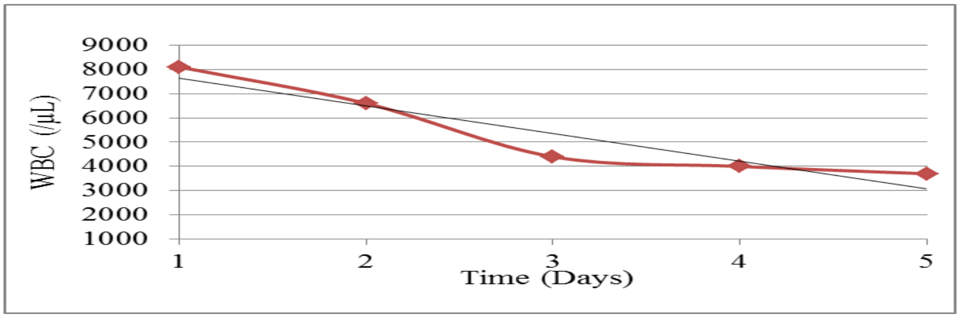

Figure 3.3: WBC count changes with time before the administration of *C. papaya* leaves extract

After administration of *C. papaya* leaves extract, the WBC count dramatically increased continuously as shown in Figure 3.4. On the very first day of *C. papaya* extract treatment, the WBC count increased up to 3.80×10^3/µL and after 3rd day of *C. papaya* extract administration, the WBC count reached to normal level. On 5th day it shows WBC count increased up to 7.70×10^3/µL.

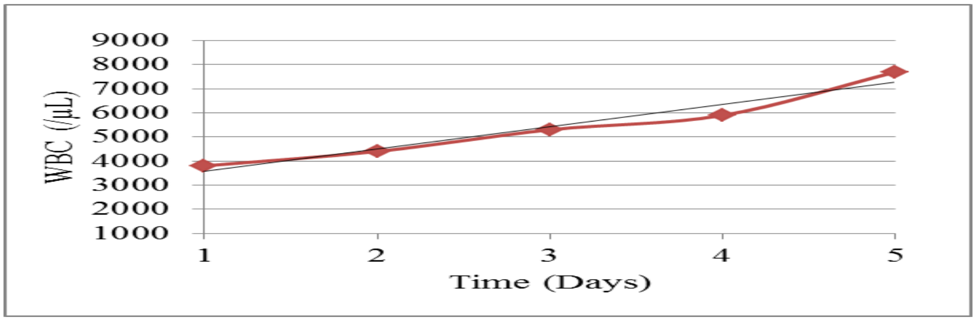

Figure 3.4: WBC count changes with after the administration of *C. papaya* leaves extract

3. Neutrophil Count

Figure 3.5 on the first day, Neutrophil count was 84% and continuously it was reducing and on 5th day Neutrophil count reduced up to 46%. Neutrophil count range of a normal person is 55% - 70% (Keogh, 2010). On 5th day, Neutrophil count of this patient ranged beyond the normal level.

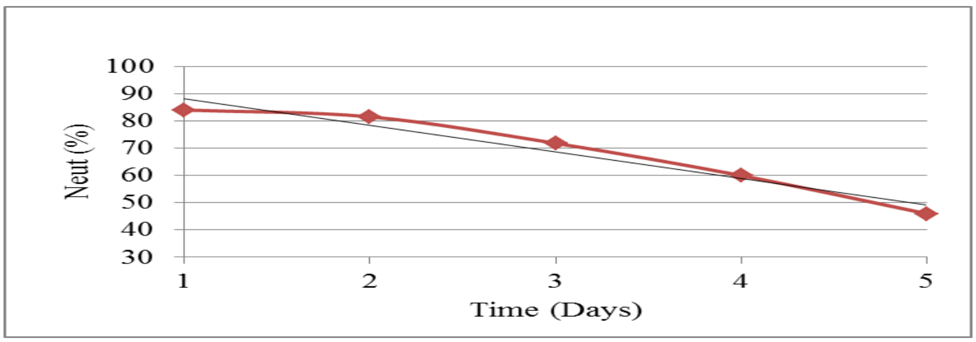

Figure 3.5: Neutrophil count changes with time before the administration of *C. papaya* leaves extract

After administration of *C. papaya* leaves extract, Neutrophil count dramatically increased as shown in Figure 3.6. And there was approximately continual linear increment up to the 5th day.

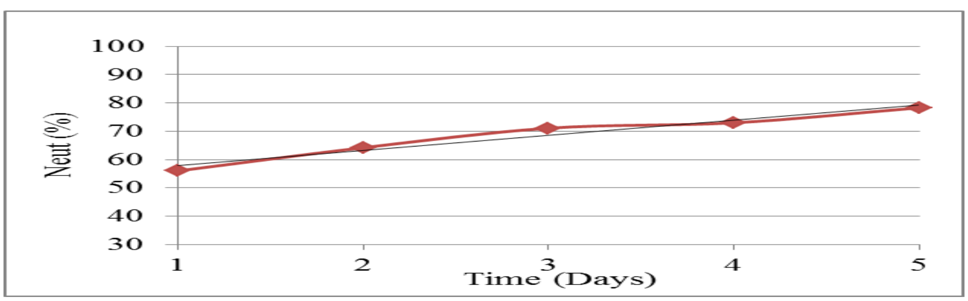

Figure 3.6: Neutrophil count changes with time after the administration of *C. papaya* leaves extract

4. Red Blood Cell (RBC) Count

During first three days, RBC count continuously reduced and on 4th day it increased on and again on 5th day the RBC count decreased up to 5.00x10^6/µL. RBC count of a normal person ranges 4.7x10^6/µL to 6.7x10^6/µL (Keogh, 2010). RBC count varies among that normal reference range. A significant continual linear decrease of RBC count could not be observed before administration of *C. papaya* leaves extract treatment.

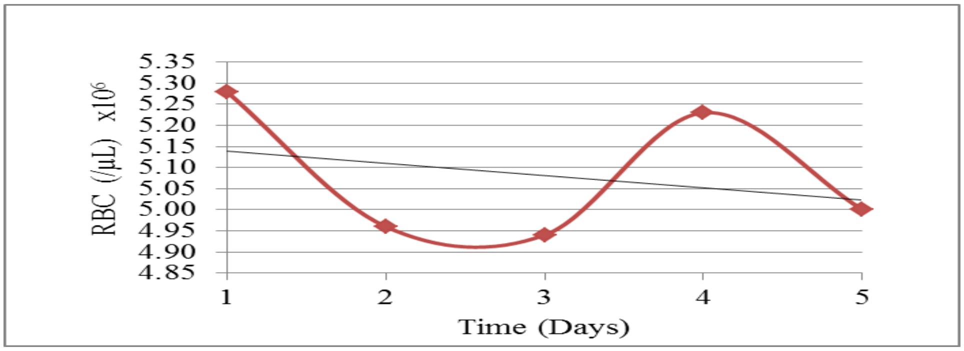

Figure 3.7: RBC count changes with time before the administration of *C. papaya* leaves extract

During first two days after administration of C. papaya leaves extract RBC count continuously increased and on 3rd day, it reduced up to 4.99x10^6/µL and again on 4th day RBC count increased up to 5.21x10^6/µL and on 5th day increased up to 5.21x10^6/µL. There was no exact significant continual linear increase of RBC count as other parameters that can be observed after administration of *C. papaya* leaves extract treatment.Therefore RBC count, perhaps cannot be used for further kinetic analysis. However, stable RBC levels were reached, rather than large variations before and after administration of C. papaya leaves extracts.

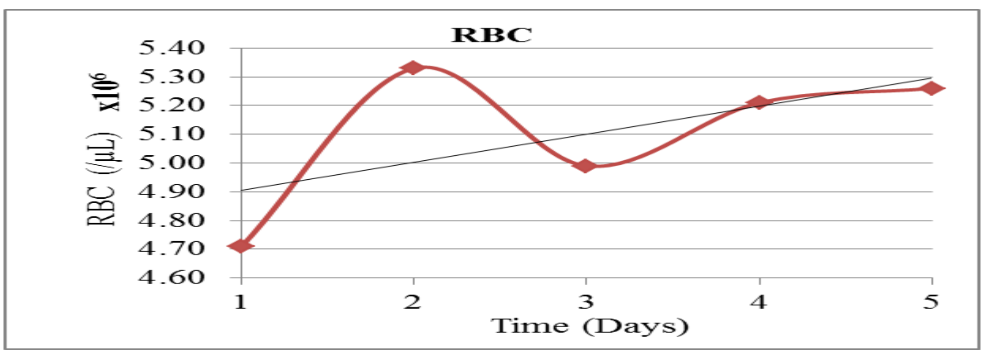

Figure 3.8: RBC count changes with time after the administration of *C. papaya* leaves extract

Kinetic Analysis

Application of Michaelis-Menten equation and Line-weaver Burk model

Dengue virus belongs to the virus family *Flaviviridae*. Leaf extracts from *C. papaya* is generally use as alternative treatment for patients with dengue fever, but there are no scientific evidences for its anti-dengue activity; hence we intended to analyze the anti-viral activity of compounds present in the leaves of *C. papaya* against dengue virus by using chemical kinetics.

According to Padmanaban *et al.*, 2013, the flavonoid quercetin from *C. papaya* has significant anti-dengue activities. The leaves of *Carica papaya* mainly consists of seven phenolic compounds namely quercetin, protocatechuic acid, p-coumaric acid, caffeic acid, chlorogenic acid, kaempferol and 5,7-dimethoxycoumarin. Table 3.3 shows K_m and v_m value for each blood type changes before and after administration of *C. papaya* extract by application of Michaelis-Menten equation.

Table 3.3: K_m and v_m value for each blood type changes before and after administration of *C. papaya*

Para.	PLTx10^3		WBC		NEUT		RBCx10^6	
	Before	After	Before	After	Before	After	Before	After
K_m	297.62	253.26	15,995	10,581.4	178.7	145.5	11.49	11.50
v_m	476.19	322.58	25,000	14,285.7	263.2	200	16.69	16.13

Interpretations

The PLT counts before and after administration of papaya show enzyme inhibition due to virus and enzyme activation from papaya extracts, see figure 3.9 and 3.10. The application of Michaelis-Menten kinetics is valid and accurate. Undoubtedly, papaya did have an influence on the recovery of the patient, since most parameters show similar pattern of recovery. Although, the suppression has a higher v_m, the patient would have reached the desired value of more than 172 x 10^3/µL. The rate of recovery is slower than under the influence of virus. However, RBC values of v_m and K_m tally for both before and after administering papaya extracts. PLT count, WBC and Neut show longer time period for recovery and it can be predicted.

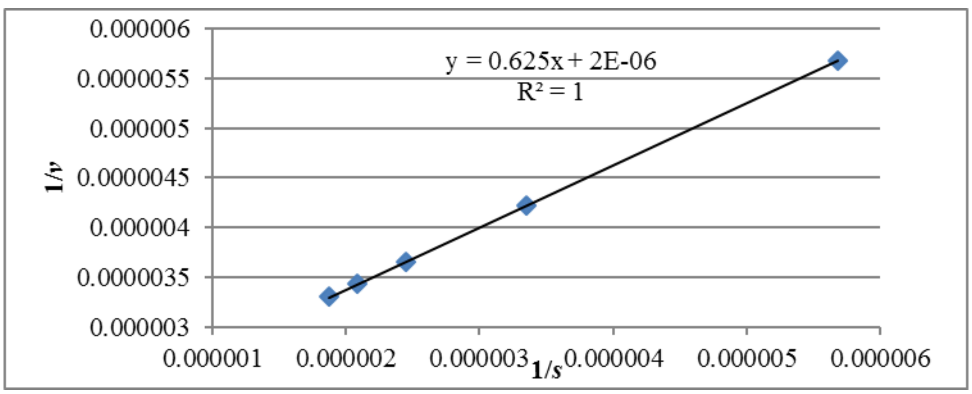

Figure 3.9: Application of Line-weaver Burk model for PLT count changes before administration of C. papaya extract

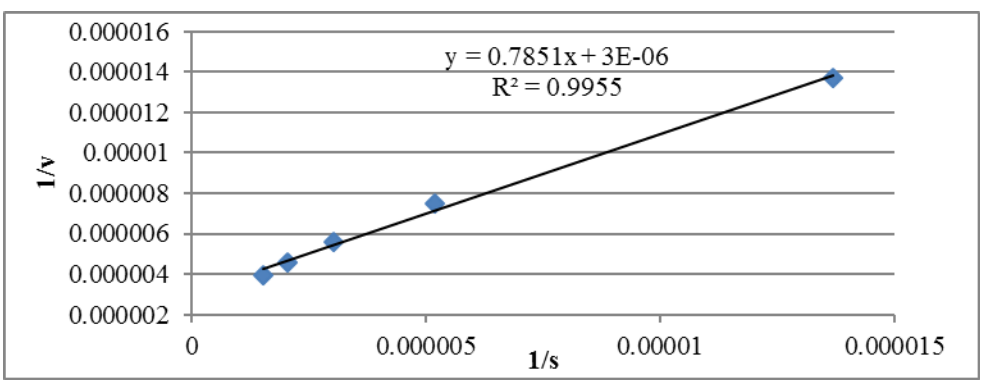

Figure 3.10: Application of Line-weaver Burk model for PLT count changes after administration of C. papaya extract

It is possible to predict the rise in PLT count using logistic growth equation. The growth coefficient of $\alpha = 0.627$, $\beta = 0.0033$ and the peak $\alpha/\beta = 190 \times 10^3/\mu L$. The Figure 3.11 illustrates the actual and prediction with logistic growth equation. The lowest value before increase in PLT count can approach a value of X_o of $50 \times 10^3/\ \mu L$. It is necessary to undertake a large sample to obtain the statistical variations of α, β and Xo. The logistic growth equation can be a useful mathematical tool to predict future changes in the PLT counts.

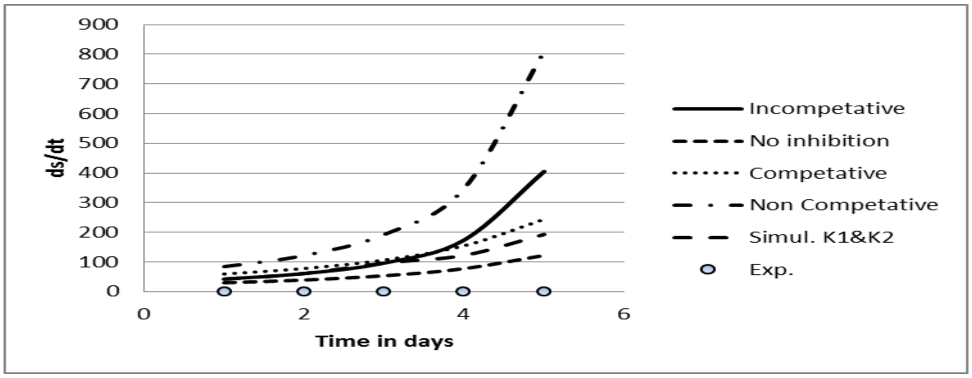

Figure 3.11: Application of logistic growth equation for PLT count changes before administration of C. papaya extract

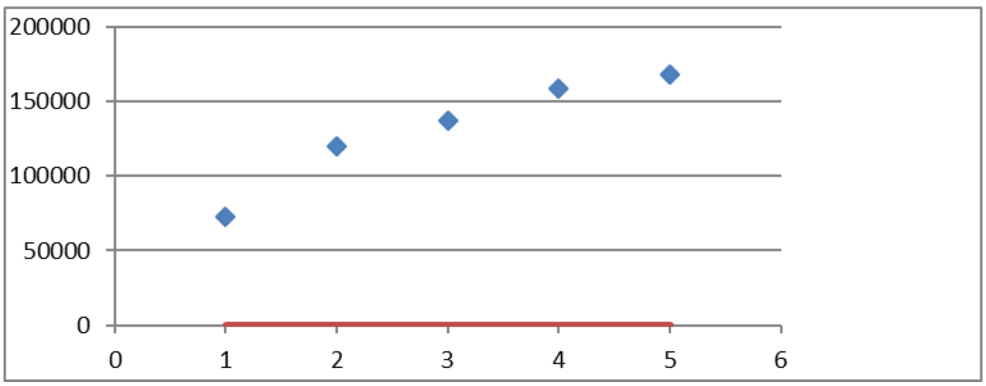

Figure 3.12: Application of first order equation for PLT count changes before administration of C. papaya extract

Interestingly, a first order equation fits better for the growth of cells after administering *papaya*. It makes more sense, since inhibitive reactions would have taken place while there were rapid growths of cells. All inhibitive reactions seem to follow first order rate reactions. The influence of the dengue virus inhibits normal enzymatic activity. Then, it is a first order rate reaction with a negative exponential function and it allows predictions to be made even after one day's PLT count. If the slope is > 0.2868, between the natural logarithmic values of first and the second day, which is the rate constant *k*, the patient is likely to have had an attack of dengue virus.

Therefore, predictions can be made for the next five days and then it is possible to generate hypothetical values for the following four days. A Michaelis -Menten kinetics can be applied to the hypothetical values so as to make comparisons of the parameters v_m and K_m of the generated and reported values of this study. In order to validate these findings, it is best to undertake a research involving all of the patients suffering from dengue

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4. CONCLUSION

The present practice of diagnosis takes long and there is no cure for dengue fever. It is possible to interpret some of the parameters to make comparisons between patients. The application of Michaelis-Menten show defined inhibitive reactions during the decline phase of PLT, WBC, NEUT and RBC. The recovery phase after administering papaya, although at a slower rate, the kinetic study validates the use of papaya as a treatment. The logistic growth equation can be applied to demonstrate the growth and retardation of PLT counts. The first order too can be applied to predict within a day the presence of dengue virus in a patient. However, this is only a preliminary study to demonstrate the soundness of the mathematical techniques used and it is recommended to undertake a large scale study to validate the findings.

ACKNOWLEDGMENT

Special gratitude to Mr. Nisar Ahamad, Department of Biotechnology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan who gave his kind permission to use secondary data from his publication on "Dengue fever treatment with *Carica papaya* leaves extracts" (Ahmad et al., 2011) for research study, thesis writing and publication purpose

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AUTHORS

First Author - T.V Gamage, Department of Nursing, Faculty of Allied Health Sciences, University of Peradeniya, Sri Lanka
vimarshanigamage@gmail.com

Second Author - Prof. B.F.A. Basnayake, Senior Professor, Department of Agricultural Engineering, Faculty of Agriculture, University of Peradeniya, Sri Lanka

Correspondence Author – T.V Gamage, vimarshanigamage@gmail.com