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Experimental and Mathematical Model for the Antimalarial Activity of the Ethanolic Stem Extract of *Azadirachta indica A. Juss* in Swiss Mice Infected with *Plasmodium berghei berghei NK65*

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOR helped in literature searches, designed the study, performed the statistical analysis and designed the model. Author MJO designed the experimental protocols and wrote the first draft of the manuscript. Author AMO assisted in the statistical analysis and the design of the model. All authors read and approved the final manuscript.

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Abstract

In spite of the control measures, malaria remains one of most health challenges of our time and will remain a problem until the transmission agent seize to exist which is impossible. This paper presents a five-dimensional ordinary differential equation modelling the transmission of *Plasmodium* between Swiss male albino mice that were induced with *Plasmodium berghei berghei NK65* and did not recover from the infection, but was suppressed with time to a reasonable level. The study consists of five groups of five Swiss mice each. Group A, B, C and D were healthy mice, infected mice without treatment, infected mice that received chloroquine (5 mg/kg) and infected mice that received mixture of chloroquine (5 mg/kg)

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and folic acid (5 mg/mL) respectively. Group E mice were infected and administered with 400 mg/kg body weight of *Azadirachta indica A. Juss* stem extract. The prophylactic activities of the study were performed by administring Swiss mice with chloroquine, folic acid and *A. indica* stem extract for 3 days. On the 4th day, the mice were inoculated with *Plasmodium berghei berghei NK65*. The parasite density was estimated for each mouse 72 hours post-parasite inoculation. The standard drug and the extract significantly reduced the parasite. The standard drug and the extract of *A. indica* ameliorate the effect of the parasite on the liver architecture. In the mathematical model, we perform the analysis of the basic reproductive number R_0 using the next generation matrix, determine the endemic state equilibrium of the model as well the determination of the local and global stability of the model using comparison theorem. Our model results show that the disease-free equilibrium is asymptotically stable at threshold parameter less than unity and unstable at threshold parameter greater than unity. Numerical simulations were carried out to confirm the analytic results and explore the possible behavior of the formulated model which was in agreement with the experimental analysis of this work.

Keywords: Antimalaria; Azadirachta indica A. Juss stemextract; histomorphology study; mathematical model; Plasmodium berghei berghei NK65.

1 Introduction

Malaria is one of the most deadly infectious diseases that have claimed million of lives around the world globally. 3.3 billion people or half of the world's population in 104 countries are at the risk of getting infected by malaria disease [1-3]. It has been estimated that between 300 and 500 million individuals of all ages are infected annually and between 1.5 and 2.7 million people die of malaria every year [4]. Malaria is widely spread in tropical and subtropical regions, including Africa, Asia, Latin America, the Middle East and some parts of Europe. The most cases and deaths occur in sub-Saharan Africa. In particular, thirty countries in sub-Saharan Africa account for 90% of global malaria deaths [1]. Shockingly, the disease kills an African child every 30 seconds and over 2,000 young lives are lost daily across the globe [5-6]. For example, malaria accounts for 60% of outpatient visits and 30% of hospitalizations among children under five years of age in Nigeria [7]. The disease, malaria, which remains one of the most prevalent and lethal human infection worldwide, is caused by infection with single-celled (protozoan) parasites of genus *Plasmodium* and is characterized by paroxysms of chills, fever, headache, pain and vomiting. The parasites are transmitted to humans through the bites of infected female *Anopheles* mosquitoes (vectors). Of the five parasite species (*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium knowlesi*) that cause malaria in humans, *Plasmodium falciparum* is the most deadly form and it predominates in Africa [3]. The parasite is responsible for the greatest number of deaths and clinical cases in the tropics. Its infection can lead to serious complications affecting brain, lungs, kidneys and other organs [5]. Traditionally used medicinal plants have played important role in malaria treatment across the globe [8].

Azadirachta indica A. Juss (*A. indica*) commonly known as Neem is one of the most useful traditional medicinal plant. Every part of the tree has been used as traditional medicine for various human ailments. The taxonomic classification of *Azadirachta indica* is as follows: Kingdom: *Plantae*, Order: *Rutales*, Suborder: *Rutinae*, Family: *Meliaceae*, Subfamily : *Melioideae*, Genus: *Azadirachta*, Species: *indica* [9].

Mathematical models for transmission dynamics of malaria are useful in providing better insights into the behavior of the disease. The models have played great roles in influencing the decision making processes regarding intervention strategies for preventing and controlling the insurgence of malaria. The study on malaria using mathematical modelling began in 1911 with Ronald Ross [10]. He introduced the first deterministic two-dimensional model with one variable representing human and the other representing mosquitoes where it was shown that reduction of mosquito population below a certain threshold was sufficient to eradicate malaria. Macdonald modifies the Ross's model by considering the latency period of the parasites in mosquitoes and their survival during that period [11]. However, in this case, it was shown

that reducing the number of mosquitoes is an inefficient control strategy that would have little effect on the epidemiology of malaria in areas of intense transmission. This paper presents a five compartmental model which comprises group of some Swiss male albino rats, divided into susceptible, infected and infected treated respectively. The infected mice are treated with standard drug (chloroquine), chloroqine with folic acid and *A. indica* (Dongoyaro) extract respectively and expected to suppress the plasmodium with time (t). Thus, our model is based on the susceptible-infective-infected treatment (SIT) in mice' population after inducing with *Plasmodium berghei berghei NK65.* The suppressive rate corresponds to how quickly parasites are reduced in the mice host due to treatment.

2 Materials and Methods

2.1 Collection and identification of plant extract

The *Azadirachta indica A. Juss* plant was obtained from Ikorodu in Lagos State, Nigeria The plant was authenticated from the department of Botany, University of Lagos, Lagos-Nigeria. Authentication number for the *A. indica* was given (6967).

2.2 Preparation of ethanolic stem extract of *Azadirachta indica*

The stem of *Azadirachta indica* was washed, air dried under shade in the Biochemistry Laboratory, pulverised into coarse power using industrial machine. Extraction was carried out by dispersing 100 g of the grounded *Azadirachta indica* plant material into 1L of 70% ethanol and shaking was done with GFL shaker for 72 hours. This was followed by vacuum filtration and concentrated by rotary evaporator at a temperature not exceeding 40°C. The concentrated extract was dried to complete dryness in an aerated oven at 40°C for 48 hours. The extract was later stored in a refrigerator at 4°C.

2.3 Malaria parasites

The chloroquine-sensitive *Plasmodium berghei berghei NK65* was used to assess the antimalarial activity of *A. indica.* It was obtained from NationalInstitute for Medical Research (NIMR) Lagos, from Dr Aina, O.O. The parasites were maintained by continuous re-infestation in mice.

2.4 Inocula

Parasitized erythrocytes were obtained from a donor infected mouse by occular puncturing. This was prepared by determining percentage parasitaemia and the erythrocytes count of the donor mouse and diluting them with normal saline in proportions indicated by both determinations [12]. Each mouse was inoculated intraperitoneally with infected blood suspension (0.2 mL) containing 1×10^7 *P. berghei NK65* parasitized red blood cells.

2.5 Grouping of animals

Group A. Healthy uninfected Swiss mice
Group B. Mice infected with *P. berghei berghei NK65* received no treatment (negative control)
Group C. (Positive control I) = 5 mg/kg B.WT of chloroquine (CQ) + *P. berghei berghei NK65*
Group D. (Positive control II) = 5 mg/kg B.WT of chloroquine (CQ) + folic acid (FA) +*P. berghei berghei NK65*
Group E = 400 mg/kg B.WT of *A. indica* stem extract + *P. berghei berghei NK65*

2.6 Repository test

This test was carried out by using the method described by [13]. Tweenty-five Swiss albino mice weighing between 18-30 g were randomly divided into 5 groups of 5 mice per cage. The mice were orally administered with 400 mg/kg B.WT of the extract, 5 mg/kg B.WT of chloroquine and 5 mg/kg B.WT folic acid and 0.2 mL normal saline (negative control) for three consecutive days. On the fourth day, the mice were passaged intraperitoneally with standard inocula of *P. berghei berghei NK65* containing $1x10^7$ infected erythrocytes. Seventy-two hours later (day 7), thin and thick films were made from the tail blood of each mouse. The films were fixed with methanol (thin), stained with 10% Giemsa and parasitaemia level assessed by microscopically counting the parasitized red blood cells on at least 2000 red blood cells in 10 different fields [12].

1. % parasitaemia = $\frac{\text{No. of parasitized RBC}}{\text{Total no. of RBC counted}}$ X 100
2. Percent suppression of parasitemia was calculated:

   Parasitemia of negative control-parasitemia of test/ Parasitemia of control x100.

2.7 Histopathological study and observations

The histopathological analyses were assayed in the Department of Anatomy, college of Medicine, University of Lagos, Idi-Araba, Surulere, Lagos, Nigeria. The Swiss albino mice were sacrificed and their abdomens were cut open to remove their liver. Histopathologies of the liver of the mice were determined.

2.7.1 Mathematical model formulation

The mice population is subdivided into susceptible $S(t)$, infected mice $I(t)$, and infected treated mice $T(t)$. The infected mice were further divided into four groups as shown above.

The total population of the mice is denoted by

$$N(t) = S(t) + I(t) + T_{SD}(t) + T_{SDF}(t) + T_E(t) \tag{1}$$

$\alpha_1, \alpha_2, \alpha_3$ are the proportion of infected rats to be treated with Standard Drug (CQ), Standard Drug plus Folic Acid (CQ+FA) and Dongoyaro extract (*A. indica*) respectively. The mice in group B may die as a result of the disease at the rate σ since they are not treated ,the natural death rate for each compartments is μ ,the suppressive rate for rats in group C, group D and group E are $\varepsilon_1, \varepsilon_2$ and ε_3 respectively.

Thus, the model is presented by the following ODE:

$$\left.\begin{aligned} S' &= N - (1-\alpha_1-\alpha_2-\alpha_3)\frac{\beta SI}{N} - \mu S \\ I' &= \frac{\beta SI}{N} - (\sigma+\mu)I \\ T'_{SD} &= \frac{\beta SI}{N}\alpha_1 - (1-\varepsilon_1)I_B - \mu I_B \\ T'_{SDF} &= \frac{\beta SI}{N}\alpha_2 - (1-\varepsilon_2)I_C - \mu I_C \\ T'_E &= \frac{\beta SI}{N}\alpha_3 - (1-\varepsilon_3)I_D - \mu I_D \end{aligned}\right\} \tag{2}$$

The assumptions for the model (2) are:

(i) It is a closed population (ii) No reproduction since all mice are male. (iii) No mouse recovered completely from the disease, due to the numbers of day the drug and extracts were administered.

2.7.2 Model analysis

By normalizing equation (2) by setting

$$X_1 = \frac{S}{N}, X_2 = \frac{I}{N}, X_3 = \frac{T'_{SD}}{N}, X_4 = \frac{T'_{SDF}}{N}, X_5 = \frac{T'_E}{N}., \tag{3}$$

System (2) transform to

$$\left.\begin{aligned} X'_1 &= 1-(1-\alpha_1-\alpha_2-\alpha_3)\beta X_1 X_2 - \mu X_1 \\ X'_2 &= \beta X_1 X_2 - (\sigma+\mu)X_2 \\ X'_3 &= \alpha_1 \beta X_1 X_2 - (1-\varepsilon_1)X_3 - \mu X_3 \\ X'_4 &= \alpha_2 \beta X_1 X_2 - (1-\varepsilon_2)X_4 - \mu X_4 \\ X'_5 &= \alpha_3 \beta X_1 X_2 - (1-\varepsilon_3)X_5 - \mu X_5 \end{aligned}\right\} \tag{4}$$

2.8 Existence and stability analysis of disease-free equilibrium, P_0

The model has a disease-free equilibrium (DEF), which is obtained by setting the right-hand side of equation (4) to zero and solving for $X_1, X_2, X_3, X_4,$ and X_5 to get

$$P_0: \ (X'_1, X'_2, X'_3, X'_4, X'_5) = \left(\frac{1}{\mu}, 0,0,0,0\right) \tag{5}$$

2.8.1 Local stability of the disease free equilibrium (DFE) P_0

The Jacobian matrix of equations (4) at the disease-free equilibrium point P_0 is

$$J(P_0) = \begin{pmatrix} -\mu & \frac{-\beta(1-\alpha_1-\alpha_2-\alpha_3)}{\mu} & 0 & 0 & 0 \\ 0 & \frac{\beta}{\mu}-(\sigma+\mu) & 0 & 0 & 0 \\ 0 & \frac{\alpha_1\beta}{\mu} & -((1-\varepsilon_1)\gamma_1+\mu) & 0 & 0 \\ 0 & \frac{\alpha_2\beta}{\mu} & 0 & -((1-\varepsilon_1)\gamma_1+\mu) & 0 \\ 0 & \frac{\alpha_3\beta}{\mu} & 0 & 0 & -((1-\varepsilon_1)\gamma_1+\mu) \end{pmatrix} \tag{6}$$

Clearly the eigenvalues of (6) are

$$\lambda_1 = -\mu,\ \lambda_2 = -(1-\varepsilon_1)+\mu\ ,\lambda_3 = -((1-\varepsilon_2)+\mu), \lambda_4 = -(1-\varepsilon_3)+\mu)$$

$$\lambda_5 = (\sigma+\mu)\left(\frac{\beta}{\mu(\sigma+\mu)}-1\right) = (\sigma+\mu)(R_0-1) < 0,\ \text{provided if } R_0 < 1.$$

Now, introduce $R_0 = \dfrac{\beta}{\mu(\sigma+\mu)}$ which is defined as the average number of secondary infection caused by a single mouse in a population of susceptible throughout its period of infectiousness.

The reproduction number was calculated using the next generation matrix approach [1] by considering the dominant Eigen-value of the matrix, FV^{-1} where F and V denoted the transmission matrix and transition matrix, hence

$$F = \begin{pmatrix} \dfrac{\beta}{\mu} & 0 & 0 & 0 \\ \dfrac{\alpha_1\beta}{\mu} & 0 & 0 & 0 \\ \dfrac{\alpha_2\beta}{\mu} & 0 & 0 & 0 \\ \dfrac{\alpha_3\beta}{\mu} & 0 & 0 & 0 \end{pmatrix}$$

$$V = \begin{pmatrix} (\sigma+\mu) & 0 & 0 & 0 \\ 0 & ((1-\varepsilon_1)\gamma_1+\mu) & 0 & 0 \\ 0 & 0 & ((1-\varepsilon_2)\gamma_2+\mu) & 0 \\ 0 & 0 & 0 & ((1-\varepsilon_3)\gamma_3+\mu) \end{pmatrix}$$

Thus, the disease free equilibrium P_0 of system (4) is locally asymptotically stable (LAS) if $R_0 < 1$

The result is written as a theorem as follows:

Theorem 1: The disease free equilibrium (DFE) P_0 of the system (4) is locally asymptotically stable if $R_0 < 1$ and unstable if $R_0 > 1$.

2.9 Global stability of the disease free equilibrium (P_0)

The comparison approach method is used here to prove the global stability of the DFE P_0.

The rate of change of the infected and recovered classes of system (4) can be written as

$$\begin{bmatrix} X_2' \\ X_3' \\ X_4' \\ X_5' \\ \end{bmatrix} \leq (F-V) \begin{pmatrix} X_2 \\ X_3 \\ X_4 \\ X_5 \\ \end{pmatrix} \tag{7}$$

Where F and V retain their original meaning, and according to Castillo- Chavez and song (2004), all Eigen-values of matrix $(F-V)$ are real negative eigenvalues that is

$$\lambda_1 = (-(1-\varepsilon_3)+\mu), \lambda_2 = -((1-\varepsilon_2)+\mu), \lambda_3 = (-(1-\varepsilon_1)+\mu), \lambda_4 = (\sigma+\mu)(R_0-1)$$

Obviously, λ_4 is negative if $R_0 < 1$,hence the linearized differential inequality (6) is stable whenever $R_0 < 1$ .consequently , $(X_2, X_3, X_4, X_5) \to (0,0,0,0)$ as $t \to \infty$.

Evaluating system (4) at $X_2 = X_3 = X_4 = X_5 = 0$ make $X_1 \to \frac{1}{\mu}$ for $R_0 < 1$ .Thus, the disease free equilibrium P_0 is globally asymptotically stable whenever $R_0 < 1$.

The following theorem summarizes the above result:

Theorem 2: Assuming that system (4) describes a male mouse population, then the disease free equilibrium P_0 of system (4) is globally asymptotically stable (GAS) if $R_0 < 1$, otherwise unstable.

2.9.1 Local asymptotic stability of endemic equilibrium P^*

Observe that system (4) have the endemic equilibrium point

$P^* = (X^*_1, X^*_2, X^*_3, X^*_4, X^*_5)$, such that

$$X_1^* = \frac{\sigma+\mu}{\beta} = \frac{1}{\mu R_0}$$

$$X_2^* = \frac{\mu(R_0-1)}{\beta(1-\alpha_1-\alpha_2-\alpha_3)}$$

$$X_3^* = \frac{\alpha_1(\sigma+\mu)(R_0-1)}{\beta((1-\varepsilon_1)\gamma_1+\mu)(1-\alpha_1-\alpha_2-\alpha_3)}$$

$$X_4^* = \frac{\alpha_2(\sigma+\mu)(R_0-1)}{\beta((1-\varepsilon_2)\gamma_2+\mu)(1-\alpha_1-\alpha_2-\alpha_3)}$$

$$X_5^* = \frac{\alpha_2(\sigma+\mu)(R_0-1)}{\beta((1-\varepsilon_3)\gamma_3+\mu)(1-\alpha_1-\alpha_2-\alpha_3)}.$$

The Jacobian matrix of (4) at P^* is

$$\begin{pmatrix} -\mu R_0 & \frac{-(1-\alpha_1-\alpha_2-\alpha_3)\beta}{\mu R_0} & 0 & 0 & 0 \\ A & 0 & 0 & 0 & 0 \\ B & \frac{\alpha_1\beta}{\mu R_0} & -((1-\varepsilon_1)+\mu) & 0 & 0 \\ C & \frac{\alpha_2\beta}{\mu R_0} & 0 & -((1-\varepsilon_2)+\mu) & 0 \\ D & \frac{\alpha_3\beta}{\mu R_0} & 0 & & -((1-\varepsilon_3)+\mu) \end{pmatrix} \quad (8)$$

Where

$$A=\frac{\mu(R_0-1)}{(1-\alpha_1-\alpha_2-\alpha_3)}, B=\frac{\alpha_1\mu(R_0-1)}{(1-\alpha_1-\alpha_2-\alpha_3)}, C=\frac{\alpha_2\mu(R_0-1)}{(1-\alpha_1-\alpha_2-\alpha_3)}, D=\frac{\alpha_3\mu(R_0-1)}{(1-\alpha_1-\alpha_2-\alpha_3)}$$

And the Eigen-values of (8) is obtained as

$$\lambda_1=-(1-\varepsilon_3)+\mu), \lambda_2=-((1-\varepsilon_2)+\mu), \lambda_3=-((1-\varepsilon_1)+\mu) \text{ , and}$$

$$f(\lambda)=R_0\lambda^2+\mu R^2{}_0\lambda+\beta(R_0-1)=0 \quad (9)$$

It follows that (9) will have two real negative roots if $R_0>1$, hence all eigenvalues of (7) are real and negative if $R_0>1$ implying that endemic equilibrium point is locally asymptotically stable. The foregoing discussion is summarized as follows:

Theorem 3: The endemic equilibrium point (EE) *P** of system (4) is locally asymptotically stable if $R_0>1$ otherwise unstable.

2.10 Statistical analysis

Data was expressed as mean ± SD and parasitemia of the different groups were statistically assessed by unpaired t-test using Graphpad Instat Demo version 5.00. A *P*-value < 0.05 was considered significant.

3 Results

3.1 Numerical simulations

In this section, we numerically study the model. For the graphs presented under this section, the number of Swiss mice in each group are equal, so that $\alpha_1=\alpha_2=\alpha_3$, the transmission rate β was assumed to be small since the transmission was self-induced through injecting the mice, the natural death rate was assumed to be equal and small for all the groups. Considering the period of the experiment, the chances for disease induced death rate σ in the infected compartment without treatment is assumed small (if there was any).

Table 1. Repository effects of the ethanolic stem extract of *Azadirachta indica* against *Plasmodium berghei berghei NK65*

Group	Dose (mg/Kg)	Mean Parasitemia density (D7)	% Supprression
A	0.2 mL	NIL	NIL
B	0.2 mL	19.07±1.95	NIL
C	5	2.15± 0.19	88.73*
D	10	2.21±0.23	88.41*
E	450	5.43±0.25	71.52*

Values are expressed in mean ± SD for five mice in each group. D7= Day seven
**significant different from negative control at P<0.05 (n=5)*

4 Discussion

The GC-MS analysis of the stem extract of *A. indica* has been presented in our previos result [14]. We show that it contains six compounds: Hexadecanoic acid, methyl ester (5.64%), 11-Octadecenoic acid, methyl ester (78.39%), (E)-9-Octadecenoic acid ethyl ester (2.81%), Methyl stearate (6.29%), Methyl 18-methylnonadecanoate (2.62%) and Docosanoic acid, methyl ester (4.24%). We also show in our previous result that the stem contains the following minerals: iron, copper, potassium, calcium, magnesium and sodium. These minerals are responsible for the important uses of the plants [14]. We also show that the methanolic stem extract of *A. indica* contains some secondary metabolites like alkaloids, saponins, glycoside, tannin, anthraquinone, reducing sugar and flavonoids. Studies have shown that secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolic compounds are responsible for the antimalarial activities of the plants [15-17]. In this study, chloroquine was used as the standard antimalarial drug. Chloroquine has been used for prophylactic, curative and suppressive antiplasmodial activities. A mean parasitaemia level that is ≤90% of that of the vehicle treated animals usually indicates that the test compound is active [18]. Group C, D and E animals significantly ($P<0.05$) reduce the percentage parasitemia of all the tested groups compared to the negative control group (group B). The repository test revealed that *A. indica* stem extract significantly reduces parasitaemia in animal models in a dose-dependent manner comparable to that of the standard drugs tested. Different studies have shown the antiplasmodial activities of *Azadirachta indica* [19-20]. Multiplication of the parasite in the blood causes anemia and damage of essential organs of the host such as the lungs, spleen and liver. *P. berghei* infections may also affect the brain causing cerebral complications in laboratory mice with symptoms comparable to those of patients infected with *P. falciparum* [21,22].

The photomicrographs of this study revealed that the histoarchitecture of the liver in group A mouse has hepatocytes that were normal with normal appearance of liver architecture (Fig. 1). The liver architecture of parasitized mice without treatment (group B) showed severe degeneration of cells, severe hepatocyte damage cells and severe inflammatory cell infiltration (Fig. 2). Dharmeshkumer et al. [23] reported severe hepatic dysfunction during maximum parasitemia where the liver was enlarged with more sinusoidal dilatation and hyperplasia of Kupffer cells. Fig. 3 shows histologic section of liver tissue stained with hematoxylin and eosin for infected mouse treated with 5 mg/kg body weight of chloroquine. The photomicrograph shows radial plates of hepatocytes with mild infiltration of parenchyma by diffuse infiltrates of inflammatory cells. Fig. 4 shows histologic section of liver tissue stained with hematoxylin and eosin for infected mouse treated with 5 mg/kg body weight of chloroquine and 5 mg/kg body weight of folic acid. Histologic sections of liver tissue show radial plates of hepatocytes. There is infiltration of parenchyma by diffuse infiltrates of inflammatory cells forming aggregates mild inflammation. The infected mice treated with 400 mg/kg body weight of *A. indica* stem extract showed moderate hepatocytes, mild inflammatory cells and the liver architecture structure is not severely damaged (Fig. 5). This is an indication that the administration of the extract reduces the effect of liver damaged caused by *P. berghei berghei NK65* induction.

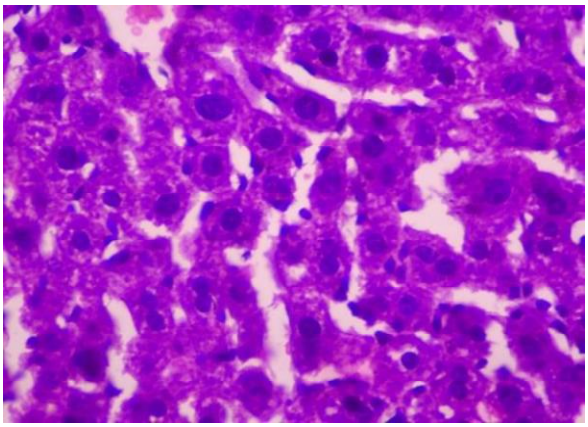

Fig. 1. Photomicrographs of liver sections stained with hematoxylin and eosin. Hepatic tissue of control uninfected mouse (group A). Examined at a magnification of × 400 under a microscope

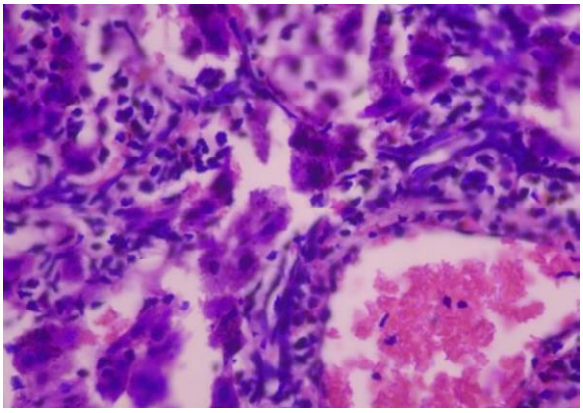

Fig. 2. Histologic sections of liver tissue stained with hematoxylin and eosin for infected mouse without treatment (group B). Examined at a magnification of × 400 under a microscope

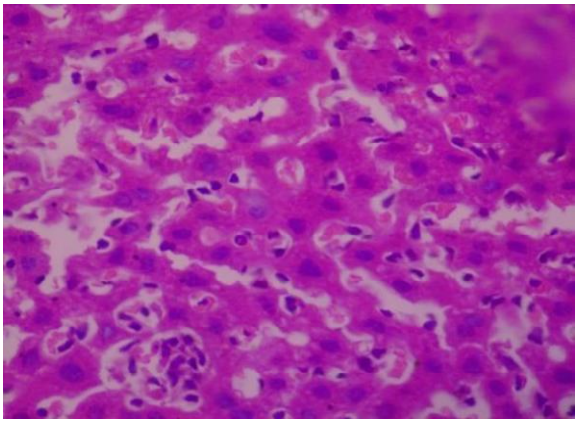

Fig. 3. Histologic section of liver tissue stained with hematoxylin and eosin for infected mouse administered with 5 mg/kg body weight of chloroquine. Examined at a magnification of × 400 under a microscope

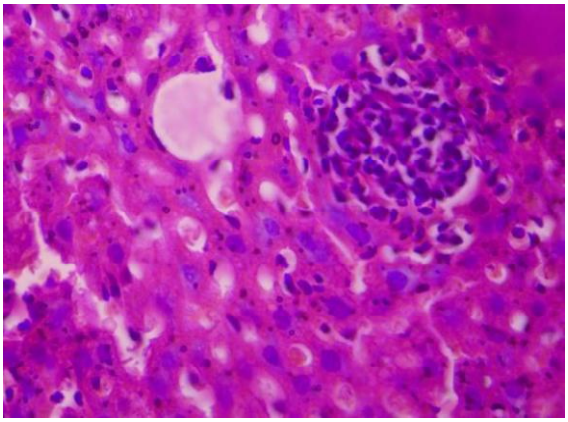

Fig. 4. Histologic section of liver tissue stained with hematoxylin and eosin for infected mouse administered with 5 mg/kg body weight of chloroquine and 5 mg/kg body weight of folic acid. Examined at a magnification of × 400 under a microscope

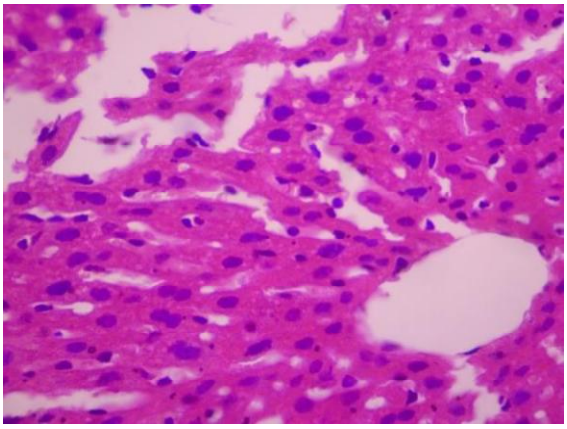

Fig. 5. Photomicrograph of liver tissue stained with hematoxylin and eosin for infected mouse administered with 400 mg/kg B.W of *A. indica* stemextract. Examined at a magnification of × 400 under a microscope

The mathematical model shows that Fig. 6 affirms the claim of our analytical result that if $R_o < 1$ the total population is stable. The administration of the standard drug (CQ), Standard drug (CQ) with folic acid and Dongoyaro extract as shown in Fig. 6 revealed that the recovered mice increases while the infected and treated mice decreases in the level of parasitemia. However, if $R_o > 1$, Fig. 7 support our analytical result shows that the infection persist in the mice population, thus, the observed increase in the infected mice population with the normal mice (susceptible) declining and little mice recovered from the infections. The efficacy of the chloroquine, chloroquine with folic acid and *A. indica* extract to suppress the *P. berghei berghei NK65* were considered in Fig. 8, Fig. 9 and Fig. 10 respectively. The analytical result is in perfect agreement with our experimental result that the chloroquine, chloroquine with folic acid and *A. indica* extract are able to suppress the plasmodium to an acceptable level which accounts for the increase observed in the recovered mice as shown in the figures. An experimental and mathematical model that considered the efficacy of chloroquine, chloroquine with folic acid and *A. indica* stem extract were considered and studied in this work. It was shown that the infection in the mice population were cleared out if the associated basic reproduction $R_0 < 1$ while the infection persist in the population if $R_0 > 1$. The study revealed that the *A. indica* ethanolic stem extract showed good suppressive property in suppressing the plasmodium parasites compared to chloroquine and chloroquine with folic acid.

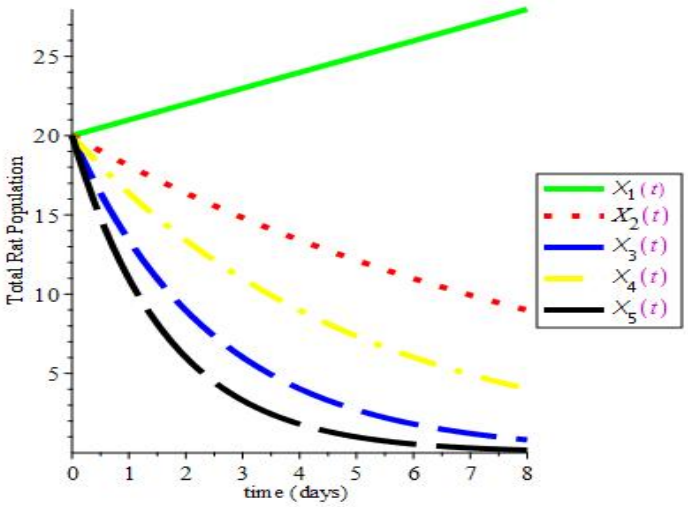

Fig. 6. The graph of the total mice population against time for parameters value at $\alpha_1 = 0.2, \alpha_2 = 0.2, \alpha_3 = 0.2, \beta = 0.00001, \mu = 0.0001, \sigma = 0.00001, \varepsilon_1 = 0.6, \varepsilon_2 = 0.8, \varepsilon_3 = 0.4$

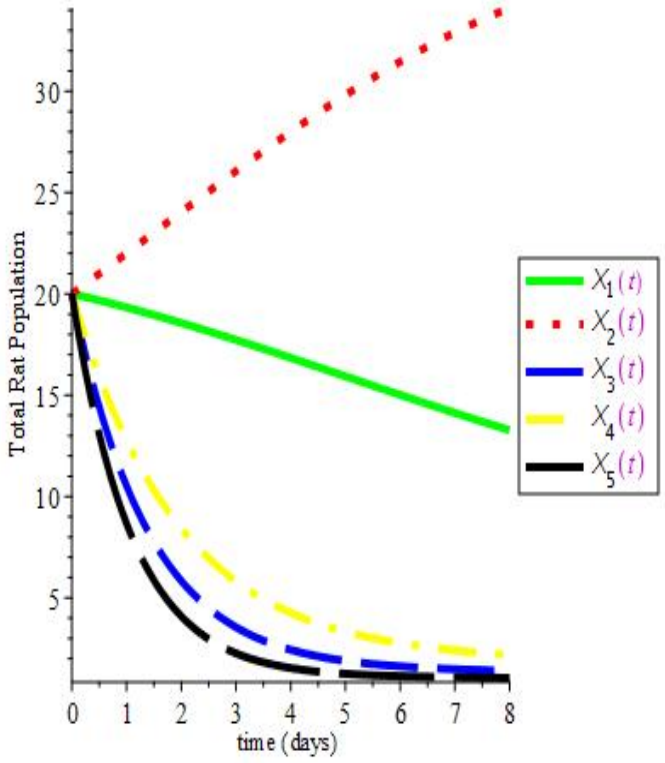

Fig. 7. The graph of the total mice population against time for parameters value at$\alpha_1 = 0.2, \alpha_2 = 0.2, \alpha_3 = 0.2, \beta = 0.01, \mu = 0.001, \sigma = 0.1, \varepsilon_1 = 0.3, \varepsilon_2 = 0.5, \varepsilon_3 = 0.1$

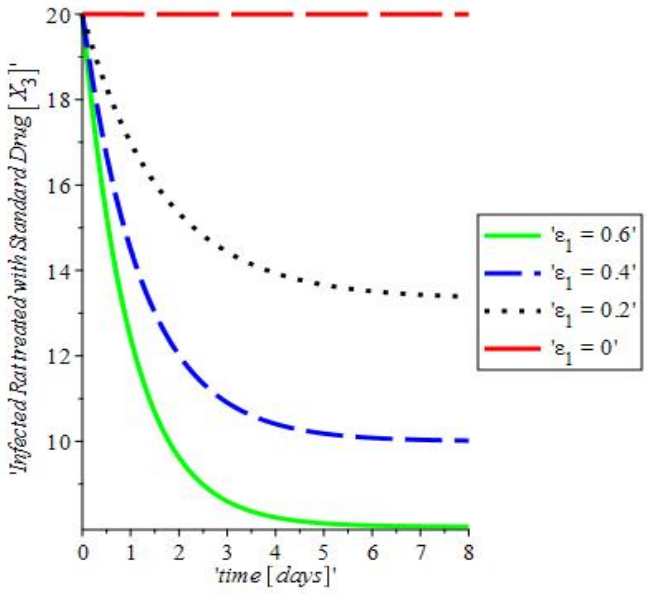

Fig. 8. The variation of proportion of Infected treated mice with Standard drug (CQ) against time at different values of the efficacy with other parameters fixed at $\alpha_1 = 0.2, \beta = 0.1, \mu = 0.0001$

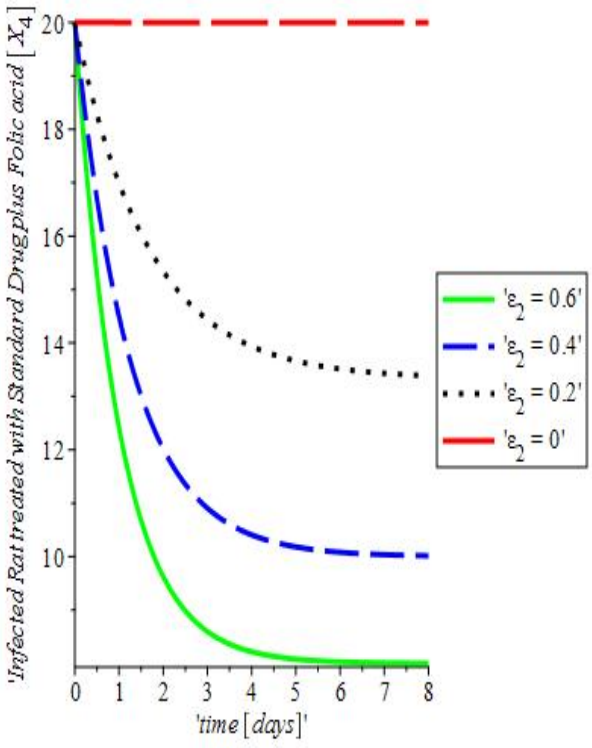

Fig. 9. The variation of proportion of Infected Treated mice with Standard drug and Folic acid against time at different values of the efficacy with other parameters fixed at $\alpha_2 = 0.2, \beta = 0.1, \mu = 0.0001$

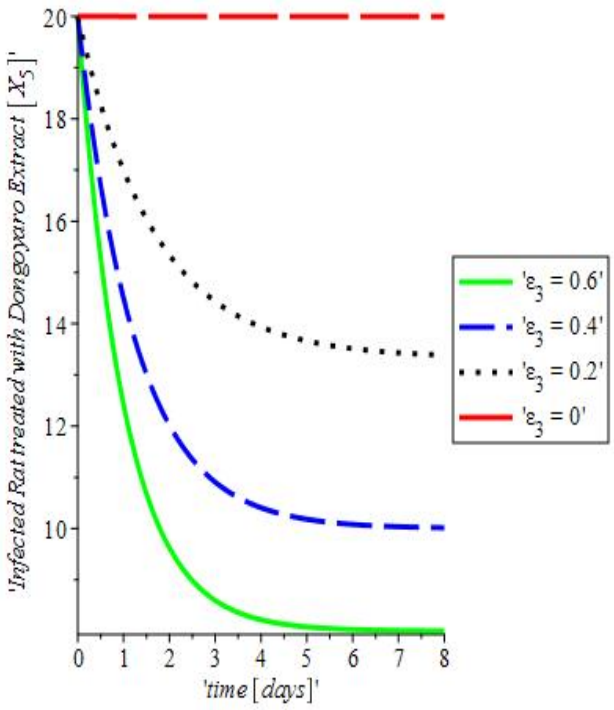

Fig. 10. The variation of proportion of Infected Treated mice with *A. indica* extract against time at different values of the efficacy with other parameters fixed at $\alpha_3 = 0.2, \beta = 0.1, \mu = 0.0001$

5 Conclusion

In conclusion, the stem extract of *A. indica* has significantly reduced parasitemia in Swiss albino mice induced with *P. berghei berghei NK65* and the experimental work has been supported by the mathematical model.

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Competing Interests

Authors have declared that no competing interests exist.

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