International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 656-663
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Antiplasmodial Activity of Alstonia Scholaris

M. Swapna Reddy [1,2] and B. Ramya Kuber* [1]
[1]Sri Padmavathi Mahila Visvavidyalayam, Tirupathi.
[2]Vaagdevi Pharmacy College, Bollikunta, Warangal.

Received: 16 Jan 2019 / Accepted: 18 Mar 2019 / Published online: 1 Apr 2019
Corresponding Author Email: rkuberpharma@yahoo.com

Abstract

As there is an increasing resistance to available antimalarial agents, there is a broad consensus on the need to develop new antimalarial drugs. 4-day suppressive test and Ranes tests were used to evaluate the Antiplasmodial activity of the Ethanolic Bark extract of *Alstonia scholaris* against *P. falcipuram* infection in mice by determining parasitemia and percentage inhibition. In addition, each pre-treated mouse was used daily and monitored for the determination of survival time, rectal temperature, packed cell volume (PCV) and also body weight was recorded regularly. Out of all the doses of the bark extracts B600 exhibited more significant parasite suppression, prevented the reduction of temperature, loss of weight and was also capable of significantly increasing the survival time in comparison with control. The bark extract in a dose-dependent manner reduced parasitemia by 59%, 66%, and 95% for doses of B200, B400, and B600, respectively, compared to the controls. The inhibition by CQ15 was significantly higher than the inhibition by B200 and B400 but it was not statistically significant with B600. Further studies are required to determine the chemical constituents responsible for the antimalarial activity of Alstonia scholaris.

Keywords
Antiplasmodial activity, Alstonia scholaris, parasitemia, survival time, rectal temperature and packed cell volume.

*****

1. INTRODUCTION

Malaria is one of the most important tropical diseases and the greatest cause of hospitalization and death. It causes an estimated 0.7–1 million deaths per year. Approximately one-half of the world's population is at risk of contracting malaria. Most cases (78%) occur in the African region, followed by Southeast Asia (15%) and Eastern Mediterranean regions (5%). Because of increasing resistance to available antimalarial agents, there is broad consensus on the need to develop new antimalarial drugs. The development of two important drugs Quinine and derivatives of artemesinin from natural sources and the utilization of many plants traditionally in various parts of the world trigger the conduction of *in vitro* and *in vivo* studies because natural products can be a source of new antimalarial drugs.[1]

Alstonia scholaris is a traditionally important medicinal plant. This evergreen tree is native to the Indian subcontinent and Southeast Asian countries. The plant is used in traditional, Ayurvedic, Unani, Homoeopathy and Sidha alternative medicinal systems against different ailments such as asthma, fever, dysentery, diarrhoea, epilepsy, skin diseases, snakebite etc. Among the phytochemicals, alkaloids are mostly reported[2].

2. MATERIALS AND METHODS

2.1. Plant materials

The Bark of *Alstonia scholaris* was collected from local areas of Warangal.

2.2. Animals and the parasite

Male Swiss albino mice (age, 8 weeks; weight, 23–27 grams (g)) were used; they were bred and maintained. They were housed in the animal house at a temperature of 22 ± 3 °C, relative humidity of 40–50% and 12-hour light/12-hour dark cycle. The animals were acclimatized for 2 weeks to the experimental environment and provided with commercial food and water *ad libitum*. A chloroquine sensitive strain of *P. falcipuram (Plasmodium falcipuram)* (ANKA) was obtained from Kakatiya medical college, Warangal. The parasites were maintained by serial passage of blood from infected mice to uninfected mice on a weekly basis[3].

2.3. Extraction

The plant material was air dried at room temperature under shade and reduced to appropriate size by grinding with an electric mill. A total of 500 g of the dried bark was extracted by soxhalation for 6 hours. The filtered extract was then dried by a rotary evaporator (Buchi Rota Vapor) at a temperature of 40 °C. After drying, 54 g (10.8%) of the dry extract of bark was obtained. The dried extract was maintained at –20 °C (centigrade) until use[3].

2.4. Phytochemical screening

Following standard procedures, preliminary screening tests were performed on the crude extracts to detect different secondary metabolites. Phytochemical screening of the crude bark extract was carried out by employing standard procedures and tests (Trease & Evans), to reveal the presence of chemical constituents such as Alkaloids, Flavonoids, Tannins, Terpenes, Saponins, Anthraquinones, reducing sugars, cardiac Glycosides. Mayer and Dragendorff reagents for alkaloids; Reaction of Liebermann for Steroids/triterpene; Reaction with ferric chloride and Sodium hydroxide for tannins and Polyphenols; Cyanidine test for flavonoïds[4]

2.5. Acute toxicity testing

Nine Swiss albino mice were randomly divided into three groups of three mice per cage. 10,100 and 1000 mg/kg of the bark extract dissolved in distilled water, and these doses were administered to all the groups. Then the mice groups were observed for any gross changes such as loss of appetite, hair erection, lacrimation, tremors, convulsions, salivation, diarrhoea, mortality, and other signs of toxicity[4].

2.6. *In-vivo* antimalarial tests

2.6.1. Parasite inoculation

The donors were four albino mice which were previously infected with *P. falcipuram* and had parasitemia levels of 20%, 24%, 25%, and 25%. The parasitemia of the donor mice was first determined. The donor mice were then sacrificed by decapitation and their blood was collected into a Petri dish treated with 0.5% trisodium citrate. The blood was then diluted with physiological saline (0.9%). The dilution was based on the parasitemia of the donor mice and the red blood cell count of the normal mice so that 1 ml blood contained 5×10^7 infected erythrocytes. Each mouse was administered intraperitoneally with 0.2 ml of this diluted blood, which contained 1×10^7 *P. falcipuram* infected erythrocytes[3,4].

2.6.2. Grouping and dosing of animals

Thirty mice were infected and were administered with methnolic bark extracts of varying doses. Then these mice were randomly divided into five groups of six mice per group. The mice in groups were used to evaluate the antimalarial effects of the bark using Peter's test and Rane's test. Three bark extract doses were used for treatment i.e. 200 mg/kg, 400 mg/kg, & 600 mg/kg and were administered to 2nd, 3rd and 4th groups respectively. The negative (1st Group) and positive control (5th group) were orally administered with 2% Tween 80 (1 ml/100 g of mouse body weight) and chloroquine15 mg/kg (CQ15), respectively. The doses were selected based on the results of the acute toxicity study. A standard Gavage was used for the oral administration of the extracts, the vehicles, and the standard drug. The duration of the administration depended on the type of test[3,4,5].

2.6.3. The 4-day suppressive test

This test was used to evaluate the antiplasmodial activity of the extracts against *P. falcipuram* infection in mice. The method used was described by Peter et al. The infected mice were randomly divided into 5 respective groups. Treatment with bark extracts and standard was started 3 hours after the mice had been inoculated with the parasite on Day 0. Treatment was then continued daily for 4 days from Day 0 to Day 3. After giving the treatment for 4 days, a thin blood film was obtained on Day 4 to determine parasitemia and percentage inhibition. In addition, each mouse was attended daily for the determination of rectal temperature, Packed Cell Volume (PCV), body weight and survival time after treatment was also recorded[5].

2.6.4. Rane's test

The curative potential of the crude extract was evaluated using the method described by Ryley and Peters. On Day 0, the standard inocula of 1×10^7 infected erythrocytes were inoculated in mice Intraperitoneally. Seventy-two hours later, the mice

were randomly divided into their respective groups and treated with varying doses of B200, B400, B600 and CQ15 mg/kg bodyweight once daily for 4 days. A Geimsa-stained thin blood film was prepared from the tail blood of each mouse daily for 5 days to monitor the parasitemia level. The survival time for all the groups was determined by finding the average survival time (days) of mice, starting from the day of infection to 30 days (D0–D29). Rectal temperature, PCV, and body weight values were also recorded[6, 7]

2.6.5. PCV measurement

The PCV was measured to predict the effectiveness of the test extracts in preventing haemolysis resulting from increased parasitemia associated with malaria. Heparinized capillary tubes were used to collect blood from the tail of the mouse. The capillary tubes were filled with blood to three-quarters of their height and sealed at the dry end with sealing clay. The tubes were then placed in a microhematocrit centrifuge with the sealed end outwards, and centrifuged for 5 minutes at 11,000 rpm. The tubes were then removed from the centrifuge and the PCV was determined using the standard Micro-Hematocrit Reader. The PCV is a measure of the proportion of red blood cells (RBC) to plasma and is measured before inoculating the parasite and after treatment by the following relationship:

PCV = volume of erythrocytes in a given volume of blood / total blood volume[8]

2.6.6. Parasitemia measurement

Thin smears of blood were prepared from the blood obtained from the tail of each mouse on Day 4 for Peter's test and Days 3–7 for Rane's test. The smears were applied on microscope slides, fixed with absolute methanol for 15 minutes and stained with 10% Geimsa stain at pH 7.2 for 15 minutes. The stained slides were then washed gently using distilled water and then air dried at room temperature. Two stained slides for each mouse were examined under an Olympus microscope with an oil immersion nose piece at 100× magnification power. Three different fields on each slide were examined to count the infected RBC. The average of the results was used to calculate the average parasitemia level. The percent parasitemia suppression of the extracts was compared with respect to the controls[9].

2.6.7. Monitoring of body weight and temperature changes

The body weight of each mouse in all groups was measured using a sensitive digital weighing balance before infection and after the completion of the treatment for Peter's test and Rane's test. The rectal temperature of the mice in all groups was measured by a digital rectal thermometer before injection and after the completion of the treatment for both tests[8,9].

2.7. Ethical consideration

The animals were maintained and cared for in accordance with guidelines for the use and maintenance of experimental animals. The study protocol was approved by institutional animal ethical committee. (------)

2.8. Data analysis

Data are expressed as the mean ± standard error of the mean. The data were analyzed using Windows SPSS Version 16.0. For comparisons of parasitemia suppression, weight, PCV, rectal temperature, and survival time among groups, One-way Analysis of Variance (ANOVA) followed by *post-hoc* test was used to determine statistical significance. Values of $p < 0.05$ were considered statistically significant[10].

3. RESULTS

3.1. Phytochemical screening

Phytochemical screening of the alcoholic crude bark extracts of *Alstonia scholaris* revealed the presence of alkaloids, phenolic compounds, tannins, terpenoids, saponins, flavonoids anthraquinones and cardiac glycosides.

Table1. Phytochemical constituents of bark extract of *Alstonia scholaris*.

Secondary metabolites	Bark extract
Alkaloids	+
Saponins	+
Phenolic compounds	+
Cardiac glycosides	–
Tannins	+
Flavonoids	+
Terpenoids	+
Anthraquinones	–
Phlobatannins	–

Note: '-' indicates absent. '+' indicates present.

3.2. Toxicity studies

The acute toxicity study indicated that extract caused no mortality at various doses used within the first 24 hours and for the next 14 days. Physical and behavioural observations of the experimental mice also revealed no visible signs of acute toxicity such as lacrimation, loss of appetite, tremors, hair erection, salivation and diarrhoea.

3.3. Effect of the 4-day suppressive test

The results of the study showed that the bark extract displayed a very good suppressive activity against *P. falcipuram.* Percentage inhibition analysis indicated that the extracts produced a dose-dependent decrease in all animals on the 5th day of parasitemia ($p < 0.001$), compared to the negative controls. The bark-extract B600 exhibited, significant parasite suppression, compared to the other doses shown in figures 1, 2 and 3. The extract at all other doses were also capable of significantly increasing the survival time.

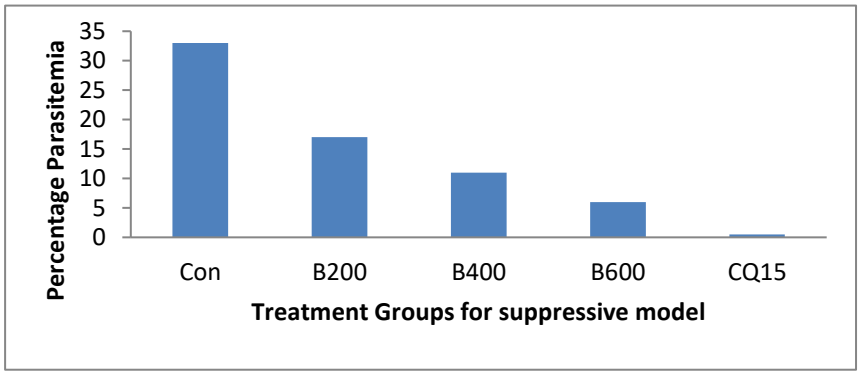

Figure 1: Parasitemia measurements in the 4 day suppressive test of bark extract of *Alstonia scholaris.*

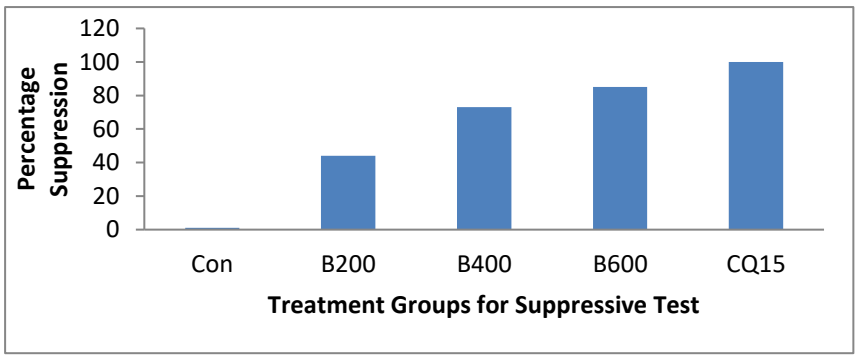

Figure 2: Percentage suppression measurements in the 4 day suppressive test of bark extract of *Alstonia scholaris*.

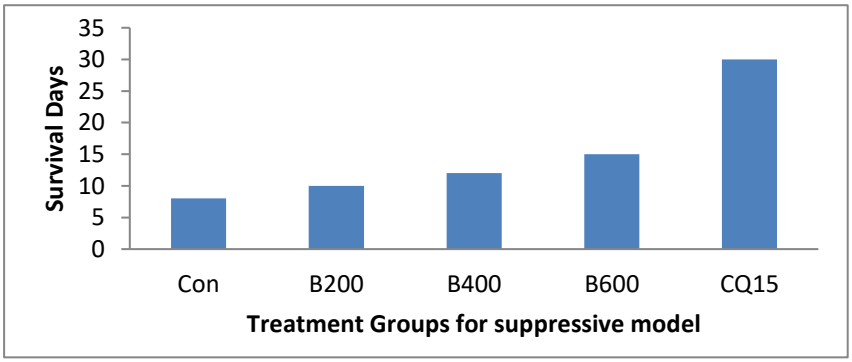

Figure 3: Survival time measurements in the 4-day suppressive test of bark extract *of Alstonia scholaris.*

Analysis of the rectal temperature revealed that 80% methanolic bark extract of *Alstonia scholaris* significantly prevented the reduction of temperature in a dose-dependent manner ($p < 0.01$ for 200, 400

and $p < 0.001$ for 600). The test extract prevented the loss of weight associated with the increase in parasitemia at all dose levels, compared to the negative controls. However, the increase in body weight was dose-dependent; the highest reduction was caused by 600 ($p < 0.001$), followed (in decreasing order) by 400 ($p < 0.01$) and 200 ($p < 0.05$). There were no detectable differences between the extracts and standard in preventing weight loss associated with increasing parasitemia shown in figures 4, 5 and 6.

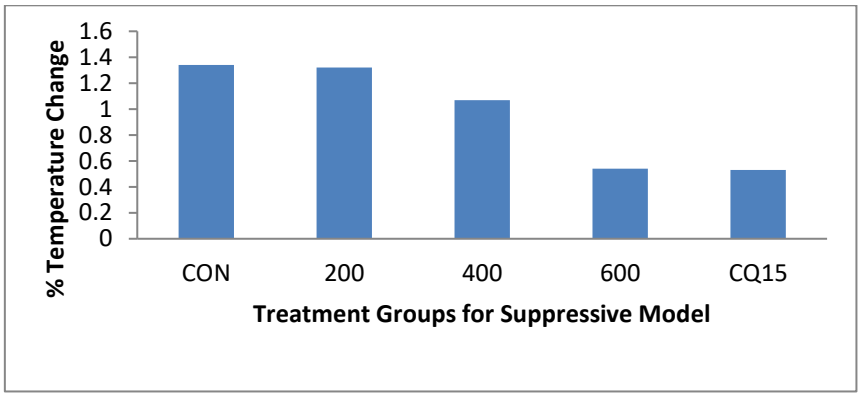

Figure 4: Temperature measurements in the 4 day suppressive test of the Bark extracts of Alstonia scholaris.

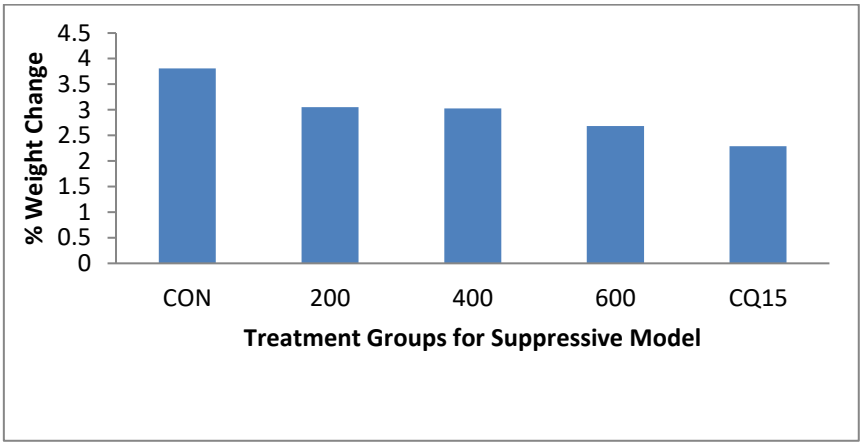

Figure 5: Weight measurements in the 4 day suppressive test of the Bark extracts of Alstonia scholaris.

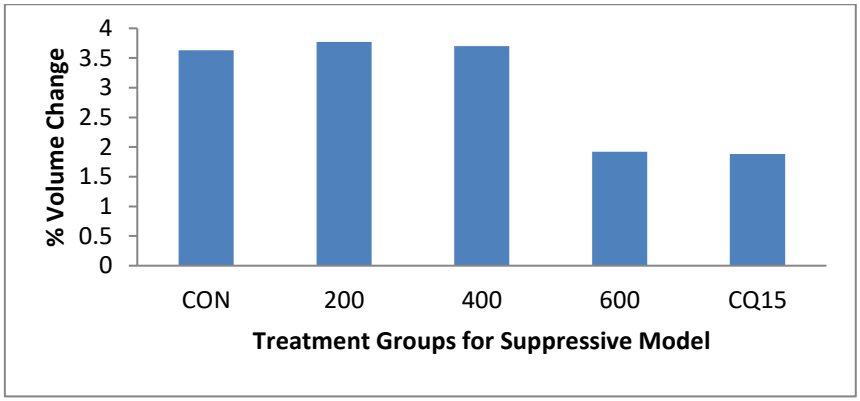

Figure 6: PCV measurements in the 4 day suppressive test of the Bark extracts of Alstonia scholaris.

3.4. Effect on curative test

The bark extract in a dose-dependent manner reduced parasitemia by 59%, 66%, and 95% for 200, 400, and 600, respectively ($p < 0.001$ in all animals), compared to the controls. The inhibition by CQ15 was significantly higher than the inhibition by B200 ($p < 0.001$) and B400 ($p < 0.05$), but it was not statistically significant with B600. Survival time was significantly increased by B200, B400 ($p < 0.001$) and B600 ($p < 0.001$). However, this increase could not match that obtained with chloroquine shown in figures 7, 8.

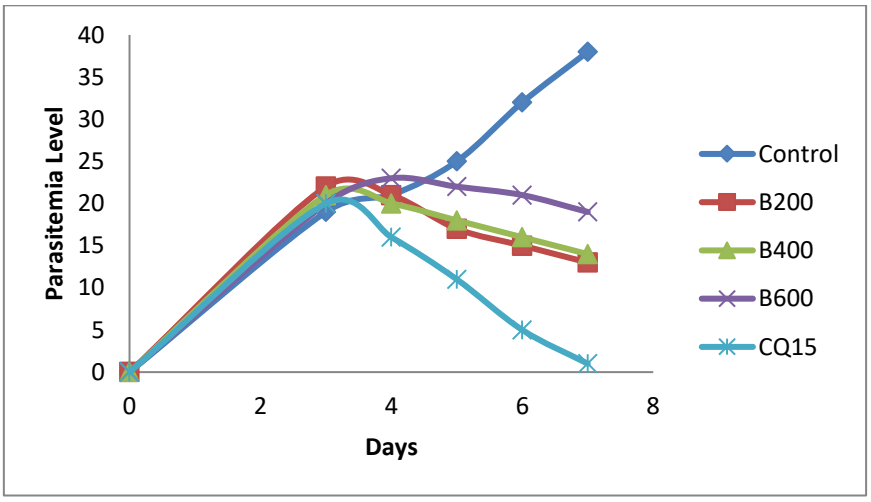

Figure 7: Parasitemia measurements for Rane's test of Bark extracts of Alstonia scholaris.

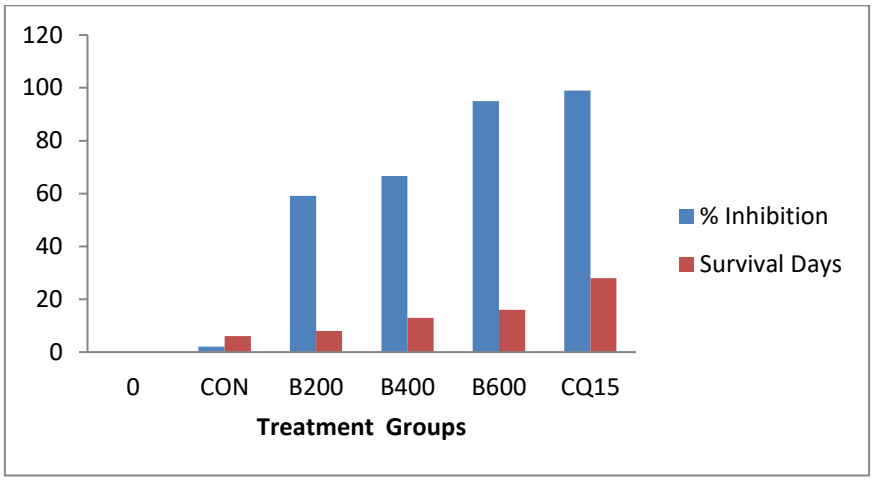

Figure 8: Survival time measurements for Rane's test of Bark extracts of Alstonia scholaris.

Rectal temperature analysis indicated that both extracts significantly prevented the reduction of rectal temperature in comparison to the control i.e. B200 ($p < 0.01$) < B400 ($p < 0.001$) < B600 ($p < 0.001$). A comparison of the doses did not reveal any apparent change. No detectable changes were observed between the extract doses and CQ15. Similar to the 4-day suppressive test, extracts failed to show any protective activity against the reduction in the PCV as shown in figure 9, 10 and 11. The extracts exhibited a preventive effect in the weight reduction of *P. falciparum* infected mice at all dose levels, compared to the controls. There was no significant difference among the extract doses and CQ15.

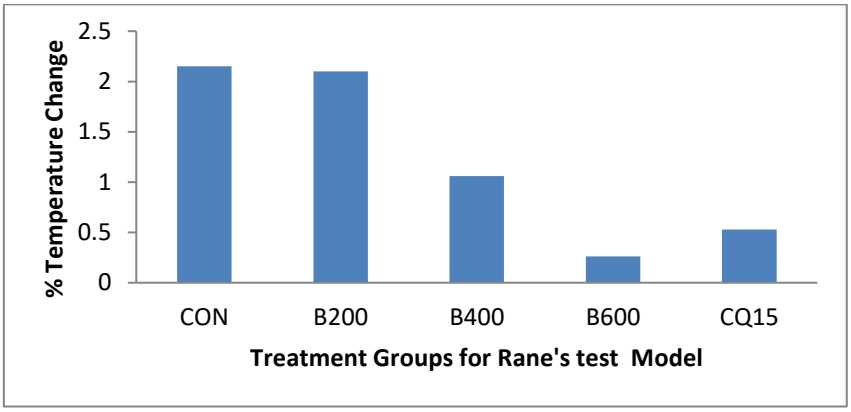

Figure 9: Rectal temperature measurements in Rane's test of bark extract of Alstonia scholaris.

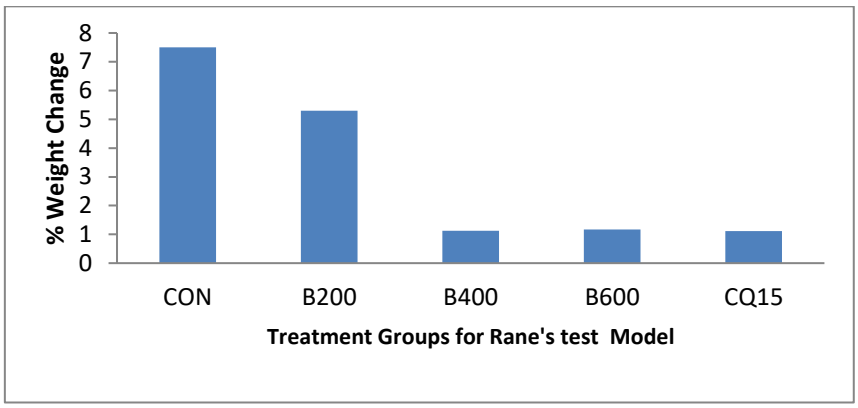

Figure 10: Weight measurements in Rane's test of bark extract of Alstonia scholaris.

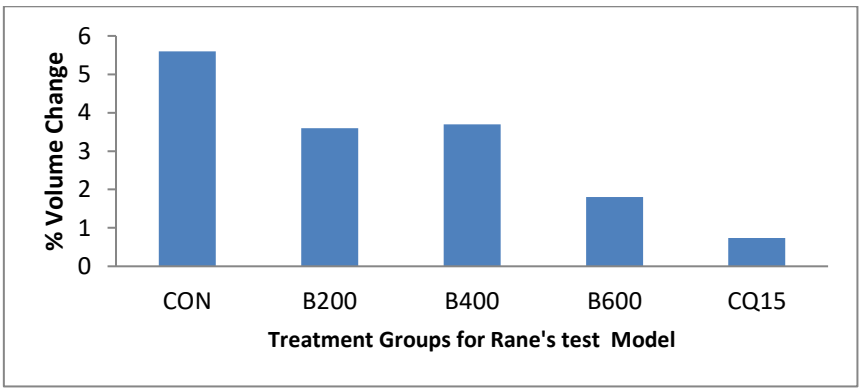

Figure 11: PCV measurements in Rane's test of bark extract of Alstonia scholaris.

Discussion

An In-vivo model was employed for this study, because it takes into account a possible prodrug effect and possible involvement of the immune system in the eradication of an infection.

The 4-day suppressive test, which primarily evaluates the antimalarial activity of candidate agents on early infections, and Rane's test, which evaluates the curative capability of candidate extracts on established infections. The above two tests are commonly used for antimalarial drug screening. In both the methods, the determination of the percent inhibition of parasitemia is the most reliable parameter. A mean group parasitemia level that is ≤ 90% of that the level in control animals usually indicates that the test compound is active in standard screening studies.

The results from the study indicated that, in *P. falciparum* infected mice, the percentage parasitemia measured in the 4-day test was significantly reduced in extract-treated groups, compared to mice in the negative control group. This indicates that the plant is endowed with antimalarial activity. In fact, the Bark extract with 89% and 88% parasitemia suppression for Peter's test and Rane's test, respectively, have comparable antimalarial activity. Alkaloids, phenolic compounds, and terpenoids in these extracts could be responsible for their antimalarial activity.

Anemia, body weight loss, and body temperature reduction are the general features of malaria-infected mice. Therefore, the ideal antimalarial agents from plants are expected to prevent body weight loss in parasite-infected mice. Despite the fact that an increase in weight was not consistent with an increase in the dose, the Bark extract of *Alstonia scholaris* significantly prevented weight loss associated with an increase in the parasitemia level, a decrease in the metabolic rate in infected mice occurs before death and is accompanied by a corresponding decrease in the internal body temperature. The rectal temperature ideally decreases as the parasite level escalates. Active compounds should prevent the rapid dropping of the rectal temperature. Extracts had a protective effect against temperature reduction in a dose-dependent manner.

The PCV was measured to evaluate the effectiveness of the crude extract in preventing haemolysis due to a rising parasitemia level. The underlying cause of anaemia includes the following mechanisms: the clearance and/or destruction of infected RBC, the clearance of uninfected RBC, and erythropoietic suppression and dyserythropoiesis. Each of these mechanisms has been implicated in human and in mouse malarial anemia. Extracts prevented PCV reduction.

The active compound has yet to be identified, although the antimalarial activity of *Alstonia scholaris* could be attributed to a single metabolite or a combination of its secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolic compounds. These metabolites have been reported in the literature as having different extents of antimalarial activity. Many species of the genus *Alstonia* also reportedly have promising antimalarial activity in different in vitro and *in vivo* studies.

CONCLUSION

The results obtained from the present study revealed that bark extracts prevented a reduction in body weight, PCV and in rectal temperature, which are associated with increasing parasitemia; Survival time of the extract-treated mice was also significantly prolonged. In this study, bark extracts produced significant parasitemia suppression in both models. Further studies are required to determine the chemical constituents responsible for the antimalarial activity of *Alstonia scholaris*.

CONFLICT OF INTEREST

None.

REFERENCES

1. Batista, B, Júnior, S.J.A, Oliveira B.A, Plant-derived antimalarial agents, new leads and efficient phytomedicines, part II. Non-alkaloidal natural productsMolecules, 14, pp. 3037-3072, (2009).
2. *Alstonia scholaris*:phytochemistry and pharmacology :A concise review, Journal of applied pharmaceutical science. 1(6):51-57. August (2001).
3. Irungu, B.N, Mbabu, M.J, Kiboi, E D.M, Moindi, J. Kinyua, M. Romano *In vivo* antimalarial and acute toxicity properties of hexane and chloroform extracts from Clausena anisata (Willd.) Benth AJPT, 1 pp. 24-29, (2012).
4. Tekalign, D, Yalemtsehay, M, Abebe, A, *In vivo* antimalarial activity of Clerodendrum myricoids, Dodonea augustifolia and Aloe debrana against Plasmodium berghei Ethiop J Health Dev, 24, pp. 25-29, (2010).
5. Hilou, A, Nacoulma, G, Guiguemde, T.R, In vivo antimalarial activities of extracts from Amaranthus spinosus and Boerhaavia erecta in mice J Ethnopharmacol, 103 pp. 236-240, (2006).
6. Peter, W, Portus, H, Robinson L, The four-day suppressive *in vivo* antimalarial test Ann Trop Med Parasitol, 69, pp. 155-171, (1995).
7. Okokon, J.E, Effiong, I, Ettebong, E, *In vivo* antimalarial activities of ethanolic crude extracts and fractions of leaf and root of *Carpolobia lutea* Pak J Pharm Sci, 24, pp. 57-61, (2011).
8. Rosenthal, J.P, Antimalarial drug discovery, old and new approaches J Exp Biol, 206, pp. 3735-3744, (2003).
9. Wright W.C, Plant derived antimalarial agents, new leads and challenges Phytochem Rev, 4, pp. 55-61, (2005).
10. Kalra, B.S, Chawla, S, Gupta, P, Valecha N, Screening of antimalarial drugs Ind J Pharmacol, 38, pp. 5-12, (2006)