Ashdin Publishing
Journal of Drug and Alcohol Research
Vol. 12 (2023), Article ID 236227, 14 pages
**DOI:** 10.4303/JDAR/236227

*Research Article*

# *In Vivo* Schizonticidal Activity of Tribal Folklore Herbs of Western Ghats, Coimbatore, Tamil Nadu

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**Received:** 01 March 2023; Manuscript No: JDAR-23-97267; **Editor assigned:** 03 March 2023; PreQC No: JDAR-23-97267 (PQ); **Reviewed:** 17 March 2023; QC No: JDAR-23-97267; **Revised:** 22 March 2023; Manuscript No: JDAR-23-97267 (R); **Published:** 29 March 2023; **DOI:** 10.4303/JDAR/236227



**Abstract**

**Background:** Folklore medicinal plants *Solanum trilobatum, Spathodea campanulata, Syzygium jambos and Tylophora indica* fractions were examined for their antiplasmodial property in rodent malaria parasite *Plasmodium berghei* infected Swiss albino mice model.

**Method:** Three *in vivo* assays (Peter's four-day test, Rane's curative and Prophylactic assay) were used in the study to determine the efficacy of plant extracts against *Plasmodium berghei* infection in the rodent model.

**Results:** Peter's four-day test showed significant parasite suppression of 97.72% ($p<0.001$) in Chloroquine (CQ) control group prolonging the mean survival time of animals for 32 days. Furthermore, effective parasite control ($p<0.01$) of 72.72% and 63.63% has been noticed in TICLE and SJCLE respectively at 400 mg/kg b.wt, whereas the SJMLE unveiled 61.36% chemosuppression accomplishing a statistical significance of $p<0.05$. The leaf extracts TICLE, SJCLE and SJMLE prolonged the mean survival time of mice groups up to 24.3 ± 2.84, 21 ± 0.57 and 20.6 ± 0.8 days respectively. Among the optimistic test extracts TICLE, SJCLE and SJMLE screened from the Peter's four day test, the TICLE exceptionally disclosed dose dependent parasite suppression of 46%, 57%, and 63% at 100 mg/kg, 200 mg/kg, and 400 mg/kg b.wt respectively in Rane's Curative assay but not up to the level of CQ control.

**Conclusion:** The present investigation establishes; the selected plant species were effective in assorted range of antiplasmodial activity and could be a potential source in antimalarial drug discovery.

**Keywords:** Antiplasmodial activity; Apicomplexan; Chemosuppression; *Plasmodium berghei*; Schizonticidal

## Introduction

Among vector born parasitic infections, malaria stands as a major health concern in the list of top 3 diseases all over the world. The impact of malaria in the developing countries is contemplated with an utmost implication in effective treatment regimen and eradication. A fact sheet of world malaria report 2022 appraised 6,19,000 malaria deaths globally in 2021 compared to 6,25,000 deaths in the first year of pandemic and 5,68,000 in 2019 before the pandemic struck. The report also projected that the malaria cases raised between 2020 and 2021, but slower rate than in the period 2019 to 2020 [1].

According to the WHO report global tally of malaria cases are 247 million in 2021, where India accounted for 79% of cases in the region with 83% of all malaria deaths in the WHO South-East Asia Region. Globally, 53% of the *P. vivax* burden is in the WHO South-East Asia Region, with the majority being in India (47%). In 2021, India accounted for about 79% of all malaria cases and about 40% of all cases in the region were due to *P. vivax*. Of 67 *P. falciparum* Therapeutic Efficacy Studies (TES) conducted in the WHO South-East Asia Region enrolling at least 20 patients, no TES reported a greater than 10% treatment failure rate. However, the presence of mutations associated with resistance to Sulfadoxine-Pyrimethamine (SP) in central India could be an early warning sign before failure of Artesunate(AS)+SP [2,3].

The above fact sheet reports are much concern that malaria apicomplexan parasite has emerged with resistance for conventional antimalarial agents and its incessant epitope change limited the success of malarial vaccines. Since from the discovery of Quinine from Cinchona bark in 1820,

search of antimalarials from plants has been focused with utmost importance till date. The emergence of drug resistance by the malarial parasite made a great attention among the researchers to come out with a novel drug discovery. Plants are veritable sources for their valuable phytometabolites that being considered in the therapy and remedy for several ailments. For the past few decades in the field of medication an upsurge in phototherapy has been recognized for safe treatment with nil side effects. Among different plant species the folklore *Solanum trilobatum*, *Spathodea campanulata*, *Syzygium jambos* and *Tylophora indica* herbs flagged for ailments between the tribal communities of Western Ghats, Tamilnadu. Several references reported the therapeutic applications of *S. trilobatum*, *S. campanulata*, *S. jambos* and *T. indica* and no report exists on antimalarial activity of these plants with an exception of *S. campanulata* [4-7]. The present investigation deals with an *in vivo* antiplasmodial activity of above cited plant extracts in *Plasmodium berghei* infected Swiss albino mice model.

## Materials and Methods

### Collection of plant material

The plant samples were collected based on the tribal knowledge residing around Western Ghats, Coimbatore, Tamil Nadu. Authenticated at Botanical Survey of India, Southern Regional Centre, Coimbatore. The voucher specimens *Solanum trilobatum* L. (No.1269), *Spathodea campanulata* P. Beauv. (No.1371), *Syzygium jambos* L. Alston (No.1408) and *Tylophora indica* (Burm.f.) Merr (No.1194) were deposited in the Department of Microbiology, Rathnavel Subramaniam (RVS) College of Arts and Science, Sulur for future references.

### Maintenance of *Plasmodium berghei*

The Chloroquine (CQ) sensitive rodent malarial parasite *Plasmodium berghei* NK-65 strain used in the present study was provided as a kind gift by Dr. R. Usha, Associate Professor, Department of Microbiology, Karpagam Academy of Higher Education,Coimbatore. The blood-stage parasite was maintained in an adult donor mouse by serial blood passage and the mice with 20%-30% rising parasitaemia was anaesthetized, bled by cardiac puncture for blood parasites (schizonts). The blood collected in a heparinized tube containing 0.5% trisodium citrate buffer was adjusted with 0.9% physiological saline, so that each 0.2 mL aliquots contains 1 x $10^7$ infect-ed RBC/mL of blood.

### Animals

Swiss albino mice of both sexes weighing about 25 grams-32 grams used in the study were maintained accord-ing to the international guidelines in the animal house, fed with standard mice pellet and water *ad libitum* [8].

### Plant leaf extracts

The disease-free shade dried powdered leaves were defatted with petroleum ether and extracted with organic solvents *viz.* methanol and chloroform by cold maceration method. After a week of socking, filtration was conducted with whatmann filter paper no.1, and concentrated *via.* rotary vacuum evaporator. The concentrated crude extracts were stored at 4°C for further analysis.

### Acute toxicity testing

The acute toxicity of 8 extracts from 4 plant species was performed according to the method described [8,9]. About 48 Swiss albino mice were divided randomly into 16 groups of 3 mice per group. After 2 h of fasting, Group I and II were orally administered with 2 g/kg b.wt and 5 g/kg b.wt of crude STMLE, STCLE, SCMLE, SCCLE, SJMLE, SJCLE, TIMLE and TICLE respectively. Each group of mice were observed closely during the first 30 minutes after dosing, periodically during the first 24 h with special attention and once daily thereafter for a total of 7 days in the case of Gp. I (administered with 2 g/kg b.wt of crude extracts) and 14 days in the case of Gp. II (administered with 5 g/kg b.wt of crude extracts). The animals were monitored for any toxicity signs to assess the safety of plant extracts.

### Phytoconstituent analysis

The crude leaf extracts of *Solanum trilobatum* and *Spathodea campanulata* were subjected to qualitative phytochemical tests, whereas the phytochemical screening of *Syzygium jambos* and *Tylophora indica* leaf extracts were already determined in our previous study [6,7,10].

### *In vivo* antiplasmodial test

**Suppressive assay:** The *in vivo* antiplasmodial activity was carried out adapting classical Peter's 4-day suppressive test [11]. The experimental protocol was approved by the Institutional Animals Ethical Committee (IAEC) (Reg. IAEC1012/C/06/CPSEA-Resolution No.7B). In brief, the animals were randomly allotted into 5 groups (Gp.I-Gp.V) of 6 mice in each group following intraperitonial inoculation with 0.2 mL aliquots of blood containing $1 \times 10^7$ *P. berghei* infected erythrocytes. To evaluate the efficacy, the plant extracts were first dissolved in 5 drops of 10% solvent (solvent used in the extract preparation) in 10 mL double distilled water. Three hours after infection Gp. I-III orally received diluted plant extracts of 100 mg/kg b.wt/day, 200 mg/kg b.wt/day and 400 mg/kg b.wt/day respectively for 4 consecutive days (D0-D3). Simultaneously, the negative and positive control groups

received 0.2 mL of vehicle (5 drops of 10% solvent in double distilled water) and Chloroquine 10 mg/kg b.wt/day respectively. On D4 (day 5) the blood parasitaemia in all the groups were determined from the tail vein blood smears fixed with methanol and staining with Giemsa stain.

**Rane's curative assay:** In the established infection the most active crude extracts exhibiting ≥ 50% parasite suppression (schizonticidal) screened from the suppressive test were used to evaluate the restorative potential according to the method described [12]. On day 0 (D0) the mice groups were intraperitoneally infected with 1 x $10^7$ *P. berghei* infected erythrocytes followed by a random division of 5 groups with 6 mice in each group (individual experimental groups for each plant extract). After 72 h observation, the Gp.I-III orally received the plant drug of 100 mg/kg b.wt/day, 200 mg/kg b.wt/day and 400 mg/kg b.wt/day respectively. The Gp. IV (negative control) and Gp. V (positive control) received 0.2 mL of vehicle and chloroquine phosphate 10 mg/kg b.wt/day respectively. The animals were dosed accordingly once daily for 5 consecutive days (D3-D7). On D8 the blood parasitaemia in all the groups were determined by Giemsa staining. The mean survival of the treatment groups were arithmetically determined by calculating the average survival time in days starting from the day of infection to 30 days (D0-D29).

**Prophylactic assay:** The *in vivo* antiplasmodial repository activity was carried out adopting classical Peter's 4-day suppressive test [11]. To evaluate the efficacy of the plant drug in repository activity, the plant extracts were first dissolved in a solution containing 5 drops of 10% solvent (used in the extract preparation) in 10 mL double distilled water. The Gp. I-III orally received diluted plant extracts of 100 mg/kg body weight/day, 200 mg/kg body weight/day and 400 mg/kg body weight/day respectively for 4 consecutive days (D0-D3). Simultaneously, the negative and positive control groups received 0.2 mL of vehicle (5 drops of 10% solvent in double distilled water) and Chloroquine 10 mg/kg body weight/day respectively. On D4 after 96 h (day 5) the animals in all the groups were intraperitoneally infected with 0.2 mL aliquots of blood containing 1 × $10^7$ *P.berghei* infected erythrocytes. After 72 h from the day ofinfection (D7) blood parasitaemia in all the groups were determined from the tail vein blood smears fixed with methanol and Geimsa stained. The rectal temperature, body weight and mean survival in days of all the groups from the day of experiment was recorded to determine the significance in the prophylactic assay.

**Blood smears**

The blood parasitemia (schizont stage) of all the infected mice groups were recorded on D4 in Peter's 4-day, D8 in Rane's and Prophylactic test. Thin blood smears fixed with absolute methanol and Geimsa stained were microscopically examined under 100-x magnification for parasitaemia count. The number of parasitized erythrocytes examined under 3 different fields on each slide was averaged to obtain the parasitaemia of individual animal. The percentage parasitaemia and suppression was calculated for all the doses of plant extracts as follows:

$$\% \text{ Parasitaemia} = \frac{\text{Number of infected red blood cells}}{\text{Total number of RBCs examined}} \times 100$$

$$\% \text{ Suppression} = \frac{\text{Parasitaemia in negative control} - \text{Parasitaemia in drug treated group}}{\text{Parasitaemia in negative control}} \times 100$$

**Determination of body weight and temperature**

With an aid of digital weighing balance and thermometer, the body weight and temperature in each group of mice was recorded. In the Peter's test the body weight was recorded 1 h before parasite infection on D0 and at the end of D3 after treatment completion. Similarly, the rectal temperature was measured 1 h before and 3 h after parasite infection and then consecutively for 4 days to monitor the consequence of plant extracts on mice body temperature. In Rane's test, the body weight and temperature were measured 3 h before infection on day 0 (D0) and consecutively from D3-D8 during the treatment period to establish the effect of plant drug in malarial mice. The temperature and body weight observation was made before and after infection in Prophylactic assay up to 8 days.

**Determination of MST**

Mortality in each group was monitored daily from the time of parasite inoculation in the treatment as well as in the control groups throughout the study. In the suppressive test, the survival determination was monitored from day 0–day 4. In the curative (Rane's test) and prophylactic assay the survival was observed for 30 days (D0-D29). Further, the mean survival time (MST) of each group was calculated as follows:

$$\% \text{ MST} = \frac{\text{Average of survival days of all mice in a group}}{\text{Total number of mice in a group}}$$

**Statistical data analysis**

Each experimental was done in duplicate, data's were expressed as mean ± Standard Error of Mean (SEM). Statistical significance determined by One-Way Analysis Of Variance (ANOVA) by Dunnett's multiple comparison test. The P-value less than or equal to 0.05 in percentage parasitaemia suppression, rectal temperature, body weight and mean survival time among the experimental groups were considered as statistically significant by comparing with

control groups.

## Results

### Phytochemical screening

Previous reports on phytochemical analysis of *S. jambos* and *T. indica* revealed the presence of phytoconstituents in the plant extracts [6,7]. The present investigation on phytochemical screening of *S. trilobatum* and *S. campanulata* confirmed the presence of alkaloids, flavonoids, terpenoids, saponins, tannins and steroids in the plant extracts.

### Determination of acute toxicity

The extracts from 4 plant species *S. trilobatum, S. campanulata, S. jambo* and *T. indica* showed varying degrees of toxicity and their results with $LD_{50}$ were depicted in the Table 1. All the extracts were found to be safer up to 5000 mg/kg b.wt with exception of STCLE and SJCLE, which displayed 2000 mg/kg b.wt was safer but the higher doses 5000 mg/kg b.wt killed the animals.

**Table 1:** Determination of acute toxicity of plant extracts with $LD_{50}$

| Test extract | Drug dose/Behavioral changes | | $LD_{50}$ |
|---|---|---|---|
| | Group-I (2000 mg/kg b.wt) | Group II (5000 mg/kg b.wt) | |
| **STMLE** | No behavioral changes or mortality | Salivation and trebling was observed from 3 to 6 h after drug administration, but recovered within 4 h | ≥ 5000 |
| **STCLE** | Salivation observed after 5 h and recovered in 24 h. | Seizures, hair erection, and respiratory disturbances were observed on the 2nd day. The animals died on 4th day | >2000 |
| **SCMLE** | No behavioral changes or mortality | Except hair erection no other symptoms were observed | ≥ 5000 |
| **SCCLE** | Salivation and restless behavior was observed from the 5th h-18th h and recovered within 24 h. | The animals found restless for 48 h and became normal from the 3rd day. | ≥ 5000 |
| **SJMLE** | No behavioral changes or mortality | No behavioral changes or mortality | ≥ 5000 |
| **SJCLE** | No behavioral changes or mortality | Diarrhoea and salivation was observed after 12 h, the animals died on 5th day | >2000 |
| **TIMLE** | No behavioral changes or mortality | No behavioral changes or mortality | ≥ 5000 |
| **TICLE** | No behavioral changes or mortality | No behavioral changes or mortality | ≥ 5000 |

**STMLE:** S.Trilobatum Methanolic Leaf Extract, **STCLE:** S.Trilobatum Chloroform Leaf Extract, **SMLE:** S.Campanulata Methanolic Leaf Extract, **SCCLE:** S.Campanulata Chloroform Leaf Extract, **SJMLE:** S.Jambos Methanolic Leaf Extract, **SJCLE:** S.Jambos Chloroform Leaf Extract, **TIMLE:** T.Indica Methanolic Leaf Extract, **TICLE:** T.Indica Chloroform Leaf Extract, **$LD_{50}$:** Lethal Dose 50%; **h:** Observation in hours.

### Peter's four-day suppressive test

In the Peter's four day test at the termination of treatment with CQ and plant extracts, the blood smears from the tail vein was used to determine the parasite suppression in the *P. berghei* infected mice. Eight extracts (STMLE, STCLE, SCMLE, SCCLE, SJMLE, SJCLE, TIMLE, and TICLE) from 4 plant species exhibited varying degrees of antiplasmodial activity in malaria mice model. The assay results of mean parasitaemia, percent parasite suppression, variation in rectal temperature and body weight, as well as mean survival time of treatment groups compared with negative control was depicted in Table 2. Most significant parasite suppression of 97.72% ($p<0.001$) was observed in Chloroquine (CQ) control indicating the potential effect of synthetic drug. It is also obvious that still a day treatment may be required for the achievement of 100% chemo-suppression in the positive control group. Blood smears from the untreated control on D4 (day 5) disclosed nil parasite reduction with a mean parasitaemia of 29.3 ± 0.81 parasites in the blood compared to the CQ control with 0.67 ± 0.67 parasites indicating the drug was effective and further treatment obligatory to clear the parasites.

**Table 2:** Peter's 4 Day test depicting mean parasitaemia, percent parasite suppression, rectal temperature, body weight and mean survival time of *Plasmodium berghei* infected mice groups treated with plant extracts and control drug

| Plant extract/ Drug | Dose mg/kg b.wt. | Mean Parasitaemia Mean ± SEM | % Parasitaemia Suppression | Rectal Temperature (°C) | | | | | | | Mean Body weight (g) ± SEM | | | Mean survival days |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | D0 BT. | D0 AT | D1 | D2 | D3 | D4 | % Change | D0 | D4 | % Change | |
| Negative control | 0.2 | 29.3 ± 0.81 | 0 | 37.2 ± 1.32 | 37.6 ± 1.32 | 37.9 ± 0.85 | 36.4 ± 1.85 | 35.2 ± 0.0 | 35 ± 1.0 | -6.28 | 30.3 ± 0.31 | 26.4 ± 1.23 | -15 | 7.3 ± 0.33 |
| Positive Control (CQ) | 10 | 0.67 ± 0.62 | 97.72 ± 0.88*** | 37.4 ± 0.23 | 37.5 ± 0.25 | 36.8 ± 0.32 | 37 ± 1.77 | 37 ± 0.0 | 37.5 ± 1.0 | 0.26*** | 29.4 ± 0.23 | 30.2 ± 1.24 | 2.64** | 32.6 ± 0.33*** |
| STMLE | 100 | 26 ± 1.0 | 11.36 ± 0.33 | 37.6 ± 0.23 | 37.8 ± 1.33 | 36.52 ± 1.52 | 36.8 ± 0.22 | 36.4 ± 1.2 | 36.5 ± 1.71 | -2.7 | 28.6 ± 1.2 | 27.4 ± 1.2 | -4.4 | 7.6 ± 0.3 |
| | 200 | 20 ± 1.0 | 31.81 ± 0.88 | 36.7 ± 1.85 | 37 ± 0 | 36.8 ± 1.12 | 36.7 ± 0.52 | 37 ± 0.63 | 36.2 ± 0.25 | -2 | 27.5 ± 0.41 | 27.3 ± 0.23 | -0.7 | 11 ± 0.57 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 400 | 14 ± 1.5 | 52.27 ± 0.33 | 37.54 ± 0.52 | 37.8 ± 1.52 | 36 ± 0.65 | 36 ± 0.6 | 36 ± 0.26 | 36.54 ± 0.31 | -0.5** | 25.5 ± 0.54 | 25.8 ± 0.7 | 1.16 | 14.3 ± 0.3 |
| STCLE | 100 | 28.6 ± 0.3 | 2.27 ± 0.33 | 37.450.22 | 37 ± 0.32 | 36.54 ± 0.75 | 35.4 ± 0 | 35.1 ± 1.62 | 35.2 ± 0.54 | -6.4 | 30.2 ± 1.25 | 28.6 ± 0.58 | -5.6 | 7 ± 0.57 |
| | 200 | 27.6 ± 1.76 | 5.68 ± 0.57 | 37.2 ± 1.52 | 37.2 ± 1.66 | 36 ± 0 | 35.1 ± 0.31 | 35.2 ± 0 | 35 ± 0.58 | -6.3 | 29.65 ± 0.62 | 28.2 ± 0.42 | -5.1 | 13.6 ± 6.6 |
| | 400 | 23.3 ± 1.2 | 20.45 ± 0.33 | 37.6 ± 0.36 | 37.5 ± 1.54 | 36 ± 0.87 | 37 ± 0.15 | 36 ± 0.53 | 36.2 ± 0.25 | -3.9 | 27.56 ± 1.52 | 27.12 ± 1.3 | -1.6 | 13 ± 4 |
| SCMLE | 100 | 28 ± 1 | 4.54 ± 0.57 | 37.5 ± 0.63 | 37.7 ± 0 | 36.4 ± 0.99 | 36.9 ± 1.9 | 36.5 ± 1.4 | 36.5 ± 1.50 | -0.7 | 30.14 ± 0.35 | 28.45 ± 1.24 | -5.9 | 6 ± 0.5 |
| | 200 | 24.6 ± 1.45 | 15.9 ± 0.88 | 37.22 ± 0.32 | 37.1 ± 0.22 | 36 ± 0.05 | 36 ± 1.52 | 36.4 ± 0.52 | 36.5 ± 1.7 | -0.5 | 28.14 ± 1.21 | 27 ± 1.5 | -4.2 | 8.6 ± 0.8 |
| | 400 | 16.6 ± 0.8 | 43.18 ± 0.33 | 36 ± 1.52 | 37.2 ± 1.52 | 36.4 ± 0 | 36.8 ± 0.95 | 36.2 ± 1.65 | 35.8 ± 2.1 | -0.4** | 29 ± 0.55 | 28.2 ± 2.1 | -2.8 | 10.3 ± 0.33 |
| SCCLE | 100 | 28.6 ± 1.2 | 2.27 ± 0.33 | 36.5 ± 1.35 | 37.6 ± 0.5 | 36.4 ± 053 | 35.6 ± 1.22 | 35 ± 0.12 | 35.2 ± 1.21 | -3.7 | 28.23 ± 0.25 | 27.3 ± 0.57 | -3.4 | 8.6 ± 0.33 |
| | 200 | 25.7 ± 1.2 | 12.49 ± 1.33 | 37.3 ± 0.25 | 37.5 ± 0 | 37 ± 0.25 | 36 ± 145 | 36 ± 1.85 | 36 ± 1.33 | -3.6 | 29 ± 0.9 | 28.4 ± 0.69 | -2.1 | 13.6 ± 1.33 |
| | 400 | 22 ± 2.08 | 24.99 ± 0.57 | 36.48 ± 1.52 | 36.5 ± 0 | 36 ± 0.85 | 35 ± 1.59 | 36 ± 0.55 | 36 ± 1.14 | -1.3 | 26.25 ± 0.62 | 25.2 ± 1.7 | -4.2 | 18 ± 0.57 |
| SJMLE | 100 | 19.7 ± 0.8 | 32.95 ± 0.00 | 36.9 ± 0.52 | 37.8 ± 1.52 | 37.52 ± 0.36 | 37 ± 2.13 | 37 ± 1.86 | 36.65 ± 0.7 | -3.4 | 28 ± 1.25 | 27.4 ± 1.77 | -2.2 | 15 ± 1.0 |
| | 200 | 16.3 ± 0.6 | 44.31 ± 1.20 | 37.2 ± 1.33 | 38 ± 0.23 | 37 ± 1.56 | 37 ± 1.96 | 37.5 ± 1.63 | 37 ± 0.7 | -2.8 | 28.25 ± 0.32 | 28.54 ± 0.92 | 1.01 | 16.6 ± 0.33 |
| | 400 | 11.3 ± 0.8 | 61.36 ± 0.33** | 37.4 ± 0.71 | 38 ± 0.75 | 38 ± 0.65 | 37.4 ± 0.99 | 38 ± 0.33 | 37.24 ± 1.4 | -0.8*** | 27 ± 0.36 | 28 ± 1.23 | 3.57* | 20.6 ± 0.8* |
| SJCLE | 100 | 21 ± 0.57 | 28.4 ± 0.33 | 37 ± 0.25 | 37 ± 1.32 | 36.7 ± 0 | 36 ± 1.56 | 35.8 ± 1.45 | 36 ± 0.56 | -2.8 | 31.22 ± 0 | 31.8 ± 1.84 | 1.82 | 14.3 ± 0.3 |
| | 200 | 15 ± 0.5 | 48.86 ± 0.33 | 37.5 ± 1.9 | 37.2 ± 1.52 | 36.3 ± 1.32 | 36.6 ± 0.13 | 36 ± 0.54 | 36.8 ± 1.21 | -1.9 | 29.2 ± 0.25 | 29 ± 1.69 | -0.7 | 17.7 ± 0.31 |
| | 400 | 10.6 ± 1.2 | 63.63 ± 0.57* | 36.62 ± 1.25 | 37 ± 0.52 | 36.5 ± 0.21 | 36 ± 0.03 | 36.9 ± 0.65 | 36.8 ± 0.41 | 0.48** | 27 ± 0.38 | 27 ± 1.4 | 0** | 21 ± 0.57** |
| TIMLE | 100 | 26.6 ± 0.8 | 9.09 ± 0.57 | 36.9 ± 0.52 | 37.2 ± 0 | 36 ± 1.33 | 36.5 ± 1.95 | 36.2 ± 0.95 | 35.7 ± 0.87 | -3 | 27.6 ± 1.53 | 28.33 ± 1.52 | -2.4 | 8 ± 0.5 |
| | 200 | 22.6 ± 0.8 | 22.72 ± 0.57 | 37.28 ± 0.21 | 38.2 ± 1.66 | 37 ± 1.45 | 37.2 ± 0 | 37.5 ± 0.54 | 37 ± 0.22 | -2.7 | 25.6 ± 1.46 | 25.5 ± 0.84 | -0.4 | 12 ± 0.5 |
| | 400 | 14.3 ± 1.8 | 51.13 ± 0.33 | 37.2 ± 0.23 | 37.2 ± 1.26 | 37.5 ± 1.85 | 36.2 ± 0.77 | 36.5 ± 0.64 | 36.2 ± 0.45 | -1.4** | 27.9 ± 0.23 | 28.6 ± 1.5 | 3.49 | 14.3 ± 0.32 |
| TICLE | 100 | 17.3 ± 0.3 | 40.9 ± 0.88 | 36.9 ± 0 | 37.4 ± 0.9 | 36.3 ± 0.45 | 36.9 ± 1.85 | 36.7 ± 1.4 | 36.5 ± 0.54 | -1.1 | 28.6 ± 1.52 | 29.4 ± 1.22 | 2.72 | 13.6 ± 0.82 |
| | 200 | 11 ± 0.5 | 62.49 ± 0.00 | 37 ± 1.03 | 37.5 ± 0.85 | 36.53 ± 0.63 | 36 ± 1.75 | 36 ± 0 | 37.2 ± 0.21 | 0.53 | 30.8 ± 1.52 | 30.7 ± 1.42 | -0.3 | 15 ± 0.1 |
| | 400 | 8 ± 1.73 | 72.72 ± 2.84** | 37.4 ± 0.23 | 36.9 ± 1.52 | 35.8 ± 0.85 | 35.65 ± 0.23 | 35.5 ± 0.85 | 37.3 ± 0.35 | -0.3** | 29.4 ± 0.32 | 31 ± 1.12 | 5.16** | 24.3 ± 2.84** |

Data's expressed as mean ± SEM; SEM: Standard Error Mean; CQ-Chloroquine; BT-temperature recorded one hour before infection and treament; AT-temperature recorded 3 hours after infection before treatment; D0-D3-treatment day 0to day 3; D4-day 4 observation; ***, **, and * represent the statistical significance of treatment groups at the levelof p<0.001, p<0.01 and p<0.05 compared with the negative control by Dunnet's multiple comparison test.

At higher doses of 400 mg/kg b.wt effective parasite suppression ($p<0.01$) was observed in TICLE and SJCLE exhibiting 72.72% and 63.63% respectively, whereas the SJMLE exhibited 61.36% chemo-suppression accomplishing a statistical significance of $p<0.05$. Besides, the other extracts STMLE and TIMLE at 400 mg/kg b.wt exhibited 52.27% and 51.13% parasite reduction respectively but not up to the suppressive effect of CQ, TICLE, SJCLE and SJMLE. A moderate to minimum parasite reduction was also observed in SCMLE, SCCLE and STCLE with 43.18%, 24.9% and 20.45% effectiveness respectively.

Body temperature at all circumstances of energy necessity play a crucial role in mammalian metabolism. In the post infection the analysis of rectal temperature initially revealed a slow escalation in negative control for 24 h-48 h followed by a gradual decline on day 3 (36.4°C) and day 4 (35.2°C). The initial temperature prior (1 h before) to infection in the control group was recorded as 37.2°C, which in turn after parasite infection has raised to 37.9°C on day 2 and declined on day 5 as 35°C indicating the struggle in the internal metabolism has subsided the rectal temperature. The CQ and plant extract treated groups displayed trivial temperature fluctuation compared to the negative control. The effect of TICLE and SJMLE was highly significant ($p<0.001$) in maintaining the body temperature at higher doses of 400 mg/kg b.wt comparable to CQ treated group. A statistical significance ($p<0.01$) of temperature conservation was also observed in SCMLE, SJCLE, TIMLE and STMLE compared to the negative control.

Analysis of mean body weight revealed the plant extracts has a positive influence in averting body weight reduction compared to the negative control with apparent weight loss, which may be due to the worsen metabolism under parasitaemia. The CQ, TICLE and SJCLE treated groups displayed a significant ($p<0.01$) retentive body weight compared to SJMLE ($p<0.05$) and the other extracts *viz.*

STMLE, STCLE, SCMLE, SCCLE and TIMLE.

An assorted survival time was perceived at all doses in treated groups compared to the untreated control. The observation of mean survival time in day's represents the CQ significantly ($p<0.001$) protected the animals up to 32 days compared to the negative control survived only for 7 days. The extracts TICLE, SJCLE and SJMLE at 400 mg/kg b.wt prolonged the mean survival time of the infected mice up to 24.3 ± 2.84 ($p<0.01$), 21 ± 0.57 ($p<0.01$) and 20.6 ± 0.8 ($p<0.05$) days respectively revealing that the plant drug was defensive against plasmodial infection in the animal model compared to the negative control but significantly inferior to the CQ control.

## Curative assay

The Dunnet's multiple comparison test was employed in curative assay to determine the statistical significant of plant extracts on parasitaemia reduction, rectal temperature, body weight, and mean survival in days comparing with the control groups (Tables 3 and 4). In the established infection an extensive parasite reduction 82.55% ($p<0.001$) was found in CQ group determining that an exploit of 100% parasite suppression can be auspicious only on further CQ treatment. The active anti-plasmodial extracts TICLE, SJCLE and SJMLE screened from the suppressive test were subjected in the curative assay. A notably significant ($p<0.001$) parasite suppression was observed in all the 3 doses (100 mg/kg b.wt, 200 mg/kg b.wt, 400 mg/kg b.wt) effective after day 4 from drug initiation at 72 h. Exclusively, the TICLE at 100 mg/kg b.wt, 200 mg/kg b.wt, and 400 mg/kg b.wt disclosed dose dependent suppressive effect of 46.3%, 57%, and 63.75% respectively compared to the SJCLE and SJMLE. Furthermore, the SJCLE and SJMLE influenced a parasite suppression of 53% and 59% respectively at the higher dose of 400 mg/kg b.wt.

**Table 3:** Rane's curative assay depicting mean parasitaemia, percent parasite suppression, and rectal temperature of *Plasmodium berghei* infected mice groups treated with plant extracts and control drug

| Dose mg/kg b.wt. | Mean ± SEM | % Parasitaemia Suppression | Rectal temperature (°C)/Day | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | D0 | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | % Change |
| 0.2 | 37.25 ± 3.62 | 0 | 36.9 ± 0.12 | 37.4 ± 0.81 | 37.4 ± 0.61 | 36.8 ± 0.05 | 36.6 ± 0.95 | 36.1 ± 1.23 | 35.1 ± 1.05 | 34.7 ± 1.23 | 34.2 ± 1.23 | -7.89 |
| 10 | 6.5 ± 4.94 | 82.55*** | 37 ± 0.30 | 37.24 ± 0.55 | 36.2 ± 0.41 | 35.5 ± 0.13 | 36.5 ± 0.15 | 37.2 ± 0.56 | 37.5 ± 1.4 | 37 ± 0.21 | 36.9 ± 0.91 | -0.27** |
| 100 | 20.75 ± 2.54 | 44.29 | 37.6 ± 0.21 | 36.2 ± 0.93 | 36.1 ± 0.62 | 35.2 ± 0.66 | 36.4 ± 0.15 | 37.2 ± 0.78 | 37 ± 0.37 | 37 ± 0.64 | 37.1 ± 0.97 | -1.34 |
| 200 | 20 ± 2.85 | 46.3 | 36.5 ± 0.47 | 36 ± 0.27 | 36 ± 0.12 | 35 ± 0.51 | 36.2 ± 1.22 | 37.2 ± 0.35 | 36.8 ± 0.14 | 36.2 ± 0.23 | 36.4 ± 0.67 | -0.27 |
| 400 | 17.5 ± 2.67 | 53.02*** | 37.4 ± 0.72 | 37.2 ± 1.43 | 36 ± 0.63 | 36 ± 0.52 | 36.1 ± 1.23 | 36.7 ± 0.33 | 36.9 ± 0.69 | 37.1 ± 0.12 | 37.3 ± 0.51 | -0.26* |
| 100 | 23.5 ± 1.86 | 36.91 | 37.4 ± 0.54 | 37 ± 0.35 | 37 ± 0.54 | 36 ± 0.62 | 36.9 ± 0.44 | 36.2 ± 0.32 | 36.2 ± 1.12 | 36.9 ± 1.51 | 37 ± 0.38 | -1.08 |
| 200 | 21 ± 2.43 | 43.62 | 37.3 ± 1.1 | 36.5 ± 1.21 | 36.5 ± 0.82 | 35.8 ± 0.01 | 36 ± 0.59 | 36 ± 0.15 | 36.8 ± 1.20 | 37 ± 1.71 | 37 ± 0.25 | -0.81 |
| 400 | 15.25 ± 1.69 | 59.06*** | 37.3 ± 0.84 | 36 ± 0.12 | 36.2 ± 0.67 | 35 ± 1.02 | 36.5 ± 0.58 | 37 ± 1.91 | 37.2 ± 0.22 | 37.2 ± 0.91 | 37.1 ± 0.16 | -0.53* |
| 100 | 20 ± 2.08 | 46.3 | 37.7 ± 0.0 | 37 ± 0.91 | 36.58 ± 0.46 | 34 ± 0.41 | 34.8 ± 0.45 | 36 ± 1.71 | 37.5 ± 0.81 | 37.2 ± 0.35 | 37.5 ± 0.19 | -0.53 |
| 200 | 16 ± 2.54 | 57.04 | 37.4 ± 0.65 | 37.9 ± 0.22 | 36.58 ± 0.45 | 35 ± 0.78 | 35.2 ± 0.23 | 36.1 ± 1.03 | 36.6 ± 0.77 | 37 ± 0.0 | 37.3 ± 1.24 | -0.26 |
| 400 | 13.5 ± 3.10 | 63.75*** | 37.56 ± 1.23 | 36.3 ± 1.01 | 36.2 ± 1.32 | 36 ± 0.85 | 36.8 ± 1.13 | 37.2 ± 1.02 | 37.2 ± 1.41 | 37.5 ± 0.0 | 37.5 ± 1.31 | -0.16** |

Data's expressed as mean ± SEM; SEM: Standard Error Mean; CQ-Chloroquine; D0-D8-day 0 to day 8; **, and * represent the statistical significance of treatment groups at the level of $p<0.001$, $p<0.01$ and $p<0.05$ compared with the control by Dunnet's multiple comparison test.

**Table 4:** Rane's curative assay depicting mean body weight and mean survival in days of *Plasmodium berghei* infected mice groups treated with plant extracts and control drug

| Plant extract/ Drug | Dose mg/kg b.wt. | Mean Body weight (gms) ± SEM/Day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D0 | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | % Change | Mean survival days |
| Negative control | 0.2 | 28.9 ± 0.14 | 28.3 ± 0.37 | 28.6 ± 1.40 | 28.6 ± 1.77 | 26.4 ± 0.91 | 25.1 ± 0.81 | 24.6 ± 1.22 | 23.6 ± 1.11 | 23.2 ± 1.02 | -24.6 | 8.7 ± 0.33 |
| Positive control (CQ) | 10 | 28.6 ± 0.21 | 28.3 ± 015 | 27.4 ± 1.03 | 26 ± 1.23 | 26.7 ± 0.33 | 27.2 ± 1.51 | 27.2 ± 1.03 | 27.5 ± 0.92 | 28.5 ± 1.02 | -0.35*** | 33 ± 1.15*** |
| SJMLE | 100 | 31.7 ± 0.35 | 31 ± 1.23 | 29.58 ± 0.74 | 28.2 ± 0.21 | 28 ± 0.55 | 28 ± 1.35 | 28.5 ± 0.44 | 28.4 ± 0.12 | 29.9 ± 0.33 | -6.02 | 15.7 ± 0.6 |
| | 200 | 29.4 ± 1.80 | 28.9 ± 0.43 | 28.58 ± 1.47 | 27.9 ± 0.33 | 27.4 ± 0.21 | 28.2 ± 0.53 | 28.42 ± 1.20 | 28.1 ± 0.00 | 28.7 ± 0.56 | -2.43 | 17.7 ± 0.7 |
| | 400 | 29.4 ± 1.62 | 28.1 ± 0.61 | 28 ± 1.11 | 27.7 ± 0.52 | 27.2 ± 1.04 | 27.3 ± 0.81 | 28.5 ± 0.00 | 28.5 ± 0.03 | 28.7 ± 0.66 | -2.43* | 19 ± 0** |
| SJCLE | 100 | 27.5 ± 1.21 | 27.2 ± 0.24 | 26.6 ± 1.35 | 24.8 ± 1.90 | 25.7 ± 0.91 | 25.9 ± 0.21 | 26.3 ± 0.00 | 26.8 ± 0.33 | 27 ± 1.08 | -1.85 | 11.6 ± 0.33 |
| | 200 | 30.4 ± 0.35 | 30 ± 0.01 | 30 ± 1.05 | 29 ± 1.03 | 29.2 ± 0.07 | 29.14 ± 1.23 | 29.8 ± 0.35 | 29.9 ± 1.54 | 29.9 ± 0.87 | -1.67 | 16 ± 0.57 |
| | 400 | 29.2 ± 0.66 | 28.2 ± 0.00 | 27.6 ± 0.66 | 26.67 ± 1.07 | 27.4 ± 0.61 | 27.5 ± 0.56 | 28.9 ± 0.91 | 28.6 ± 0.55 | 28.9 ± 1.07 | -1.03* | 19.3 ± 0.33* |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TICLE | 100 | 29.8 ± 0.47 | 28.6 ± 1.61 | 27.8 ± 0.28 | 26.7 ± 0.22 | 28.42 ± 0.77 | 29 ± 0.22 | 29.2 ± 1.27 | 29.4 ± 0.43 | 29.4 ± 1.11 | -1.36 | 15.3 ± 0.8 |
| | 200 | 31.1 ± 0.28 | 31.2 ± 1.02 | 30.5 ± 0.56 | 29.4 ± 1.43 | 29.44 ± 0.25 | 29.7 ± 0.83 | 29.8 ± 1.02 | 29.9 ± 0.59 | 30.7 ± 0.00 | -1.3 | 20 ± 0.57 |
| | 400 | 30.2 ± 0.11 | 30.2 ± 0.07 | 29.6 ± 0.47 | 28.4 ± 0.09 | 28.6 ± 0.77 | 28.9 ± 1.22 | 29.7 ± 0.00 | 29.7 ± 1.54 | 29.9 ± 1.35 | -1** | 21.7 ± 0.33** |

Data's expressed as mean ± SEM; SEM: Standard Error Mean; CQ-Chloroquine; D0-D7-treatment day 0 to day 7; D8-day 8 observation; ***, **, and * represent the statistical significance of treatment groups at the level of p<0.001, p<0.01 and p<0.05 compared with the control by Dunnet's multiple comparison test.

In post inoculation test the negative control exposed a trifling raise in rectal temperature initially for the first 2 days followed by a gradual decline from day D3 to D8 in the observation recounting the fact increase in blood parasitaemia and disturbance in normal body metabolism, hence there was attenuation in rectal temperature. The CQ group displayed a statistical significance ($p<0.01$) in recovering the rectal temperature from the instant next day (D4) of treatment initiation. Among the 3 extracts (SJMLE, SJCLE, TICLE) the TICLE was comparable to CQ revealing a significant ($p<0.01$) temperature retentive effect compared to the SJCLE and SJMLE ($p<0.05$ respectively) at all doses in the treatment assay.

In the curative assay a key drop in body weight was observed in the untreated control (Table 4). The mean body weight in negative control prior to infection on D0 was weighed as 28.9 g ± 1.03 g, which has reduced in course on D8 as 23.2 g ± 1.02 g. The loss of body weight in the negative control group denotes, parasitaemia in blood has effectively reduced the body metabolism, which in turn has abridged the body weight and rectal temperature with mortality of 1 animal on day 8 in the observation. Furthermore, the CQ group displayed a weight reduction for the first 3 days prior to drug treatment and a steady raise in body weight was detected from D4 after the initiation of drug followed by a normal recovery of weight on D8 in the surveillance. To rule out the effectiveness of plant extracts in established infection the similar dose levels (100 mg/kg b.wt, 200 mg/kg b.wt, 400 mg/kg b.wt) used in the suppressive assay was followed after 3 days in the study. Among the 3 extracts screened from the suppressive test, the TICLE displayed statistically significant ($p<0.01$) body weight recovery compared to the SJCLE and SJMLE ($p<0.05$ respectively). In the entire analysis the CQ group displayed a leading effect compared to the extract treatment groups and no mortality was noticed in all the groups till the treatment termination signifying the drug was safer and active in the prescribed doses.

The observation of mean survival time in days revealed that the drug prolonged the survival time of mice compared to the untreated group (Table 4). The CQ group animals showed 34 ± 1.15 days ($p<0.001$) of survival compared to the negative control, which displayed only 8.7 ± 0.33 days of survival. Dose dependent mean survival in days were observed in all mice groups treated with plant extracts compared to the negative control. At higher doses of 400 mg/kg b.wt TICLE and SJMLE protected the mice for about 19 ± 0 days and 21.7 ± 0.33 days respectively with the significance of $p<0.01$ confidence. The SJCLE protracted the survival of mice for 19.33 ± 0.33 days at the higher doses of 400 mg/kg b.wt with a statistical significance of $p<0.05$ confidence.

**Prophylactic assay for residual activity**

The increasing failure of antimalarials including chloroquine and sulphadoxine-pyrimethamine in many endemic sites of Africa and Asian countries has stimulated terrific public health concern. The failure of malarial drugs has imposed more investigation of plants for newer antimalarials and optimization with prevailing antimalarial activity, when used individually as compound remedies or in amalgamation with orthodox medicine [13].

The present study has not only validated the antiplasmodial activity of 4 medicinal plants and their extracts but has also demonstrated the outstanding effect of individual herb among the 4 selected through a series of *in vivo* methods such as suppressive, curative and prophylactic assays against chloroquine sensitive *P. berghei* infection in mice. Among 8 extracts from 4 plant species the suppressive assay has presented 3 extracts SJMLE, SJCLE and TICLE with an outstanding parasite control posting the herbs to curative assay. The curative assay on its part has concluded the effectiveness of 3 extracts with >50% antiplasmodial activity dispatching the extracts to the next level of screening in prophylactic assay.

In the prophylactic test, 3 extracts SJMLE, SJCLE and TICLE subjected in the curative assay were used to identify the schizonticidal activity against *Plasmodium berghei* infected mice model. In the assay prior to parasite infection to verdict the efficacy of plant extracts, an analogous dose levels 100 mg/kg, 200 mg/kg, 400 mg/kg body weight used in the curative assay was followed for the first 4 days (D0-D3) in the study. The blood parasitaemia of animals 3 days after the infection showed enhancing results with decrease in parasites. Dunnet's multiple comparison test was employed to compare the efficacy of plant drug in the repository to determine the statistical significance in blood parasite reduction, rectal temperature, body weight, and mean survival in days comparing with the control groups (Table 5). All the 3 extracts showed parasite reduction >50% achieving a significance in a dose dependent manner of 100 mg/kg

body weight, 200 mg/kg body weight and 400 mg/kg body weight (Table 6). The negative control exposed 100% parasitaemia conferring the parasite exponential state in the vehicle group. Besides, the positive control animals (CQ) displayed 96.7% significant ($p<0.001$) parasite reduction on observation after 72 h from the day of parasite infection, forecasting 100% parasite conquest can be acquired only on further CQ treatment.

**Table 5:** Prophylactic assay depicting mean parasitaemia, percent parasite suppression, rectal temperature and percent change in rectal temperature of *Plasmodium berghei* infected mice treated with plant extracts along with control groups

| Plant extract/ Drug | Dose mg/kg b.wt. | Mean Parasitaemia Mean ± SEM | % Parasitaemia Suppression | Rectal temperature (°C) ± SEM/Day | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Plant extract/drug treatment days | | | | Day infected | Days observed | | | |
| | | | | D0 (0 h) | D1 (24 h) | D2 (48 h) | D3 (72 h) | D4 (96 h) | D5 (120 h) | D6 (144 h) | D7 (168 h) | % Change |
| Negative control | 0.2 | 30.4 ± 0.67 | 0 | 37.1 ± 0.21 | 37.1 ± 1.25 | 37.3 ± 0.87 | 37.2 ± 0.01 | 37.1 ± 2.14 | 37.6 ± 1.35 | 36.2 ± 0.21 | 36 ± 0.55 | -3.1 |
| Positive Control (CQ) | 10 | 1 ± 0.6 | 96.71*** | 36.9 ± 1.56 | 36.8 ± 0.54 | 38.8 ± 0.01 | 37.1 ± 1.87 | 37.2 ± 0.36 | 36.9 ± 0.33 | 36.8 ± 0.48 | 36.9 ± 0.33 | 0* |
| SJMLE | 100 | 26.25 ± 1.03 | 13.65 | 37.2 ± 2.3 | 37.1 ± 1.35 | 37.1 ± 0.55 | 37.3 ± 1.84 | 37.2 ± 0.98 | 37.4 ± 0.25 | 37 ± 1.22 | 37 ± 1.22 | -0.5 |
| | 200 | 20.5 ± 0.37 | 32.56 | 37.2 ± 0.99 | 37.8 ± 0.01 | 37 ± 0.00 | 36.9 ± 1.23 | 36.8 ± 0.66 | 36.6 ± 0.56 | 36.8 ± 0.33 | 37.2 ± 0.33 | 0 |
| | 400 | 15 ± 0.32 | 50.65 | 37.1 ± 1.26 | 36.9 ± 0.85 | 37.2 ± 2.14 | 36.9 ± 2.01 | 36.8 ± 1.32 | 37.2 ± 0.54 | 37.4 ± 0.11 | 37.2 ± 1.22 | 0.27* |
| SJCLE | 100 | 22.75 ± 0.6 | 25.16 | 37.2 ± 0.33 | 37 ± 0.21 | 37.1 ± 1.55 | 36.9 ± 1.34 | 37.2 ± 0.99 | 36.9 ± 0.57 | 37 ± 0.52 | 37.2 ± 1.11 | 0 |
| | 200 | 14 ± 0.86 | 53.94 | 37.2 ± 2.1 | 36.9 ± 0.99 | 37.1 ± 0.85 | 37.2 ± 0.65 | 37 ± 1.25 | 37.5 ± 1.25 | 37.1 ± 0.36 | 37.2 ± 0.66 | 0 |
| | 400 | 8.75 ± 1.11 | 71.21* | 36.8 ± 0.52 | 37 ± 1.25 | 37.1 ± 1.8 | 37.1 ± 0.74 | 37 ± 1.12 | 37.4 ± 0.02 | 37.8 ± 0.58 | 37.2 ± 2.22 | 1.08* |
| TICLE | 100 | 25.75 ± 0.7 | 15.29 | 37.2 ± 0.88 | 37.1 ± 2.15 | 37.1 ± 0.01 | 37.3 ± 0.08 | 37.2 ± 1.77 | 37.4 ± 0.56 | 36.9 ± 0.32 | 37 ± 1.25 | -0.5 |
| | 200 | 19.25 ± 0.95 | 36.67 | 36.9 ± 0.23 | 36.9 ± 0.36 | 37.1 ± 0.35 | 37 ± 0.25 | 36.9 ± 0.27 | 37.2 ± 0.58 | 37 ± 1.35 | 37 ± 1.24 | 0.27 |
| | 400 | 13.5 ± 0.4 | 55.59 | 37 ± 1.66 | 37 ± 0.01 | 36.9 ± 1.33 | 37 ± 0.88 | 37.1 ± 1.62 | 37.4 ± 1.25 | 37.8 ± 0.58 | 37.2 ± 0.58 | 0.53* |

Data's expressed as mean ± SEM; SEM: Standard Error Mean; CQ-Chloroquine; D0-D7-treatment day 0 to day 7; D7-day 7 observation; ***, and * represent the statistical significance of treatment groups at the level of $p<0.001$ and $p<0.05$ compared with the control by Dunnet's multiple comparison test.

**Table 6:** Prophylactic assay depicting mean body weight and mean survival in days of *Plasmodium berghei* infected mice groups treated with plant extracts and control drug

| Plant extract/ Drug | Dose mg/kg b.wt. | Mean Body weight (gms) ± SEM/Day | | | | | | | | Mean survival days |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Plant extract/drug treatment days | | | | Day infected | Days observed | | | |
| | | D0 | D1 | D2 | D3 | D4 | D5 | D6 | D7 | % Change |
| Negative control | 0.2 | 29.8 ± 1.41 | 29.6 ± 0.23 | 29.4 ± 0.51 | 29.6 ± 0.22 | 29.4 ± 1.22 | 26.9 ± 0.56 | 25.4 ± 0.33 | 22.8 ± 1.54 | -30.7 | 8.3 |
| Positive control (CQ) | 10 | 30.3 ± 0.01 | 30.2 ± 0.21 | 30.4 ± 0.11 | 30.4 ± 0.64 | 30.2 ± 0.48 | 29.8 ± 0.99 | 30.7 ± 1.27 | 31 ± 0.75 | 2.25*** | 39.6*** |
| SJMLE | 100 | 29.4 ± 1.6 | 29.2 ± 0.58 | 29.8 ± 0.35 | 29.8 ± 0.33 | 28.6 ± 2.12 | 27.3 ± 0.25 | 28.5 ± 0.01 | 28.9 ± 0.72 | -1.73 | 15.3 |
| | 200 | 31 ± 0.01 | 31.2 ± 1.25 | 31 ± 0.55 | 31 ± 0.54 | 30.9 ± 1.55 | 30.1 ± 0.56 | 30.2 ± 1.11 | 30.8 ± 0.99 | -0.64 | 18.3 |
| | 400 | 30 ± 0.58 | 30.2 ± 0.21 | 30.2 ± 1.39 | 30.4 ± 1.26 | 30.4 ± 0.85 | 29.8 ± 0.64 | 30 ± 1.24 | 30 ± 0.93 | 0* | 23* |
| SJCLE | 100 | 31.2 ± 0.56 | 30.9 ± 0.01 | 30.7 ± 1.20 | 31 ± 0.25 | 31.1 ± 1.36 | 31 ± 0.99 | 31 ± 0.23 | 31 ± 0.01 | -0.64 | 19 |
| | 200 | 29.1 ± 1.22 | 29.2 ± 0.33 | 29.1 ± 1.91 | 29.3 ± 1.23 | 29.2 ± 0.26 | 29 ± 1.63 | 29.2 ± 0.55 | 29.3 ± 0.25 | 0.68 | 25.3 |
| | 400 | 30.2 ± 0.81 | 30.1 ± 1.33 | 30.2 ± 0.71 | 30.4 ± 0.69 | 30.4 ± 0.66 | 30 ± 1.52 | 30 ± 1.29 | 30.2 ± 0.14 | 0* | 30.7** |
| TICLE | 100 | 31.3 ± 0.02 | 31.2 ± 1.02 | 31.4 ± 0.25 | 31.3 ± 0.58 | 31.3 ± 2.01 | 30.8 ± 0.58 | 30.8 ± 1.05 | 30.4 ± 1.05 | -2.96 | 13 |
| | 200 | 30.2 ± 1.02 | 30.1 ± 1.33 | 30.1 ± 1.02 | 30.3 ± 0.84 | 30.2 ± 0.84 | 29.4 ± 0.55 | 29.6 ± 0.88 | 29.8 ± 0.61 | -1.34 | 17 |
| | 400 | 29 ± 1.36 | 29 ± 0.95 | 29.1 ± 0.66 | 29.2 ± 1.55 | 29 ± 0.14 | 28.2 ± 0.66 | 28.8 ± 0.71 | 28.8 ± 0.42 | -0.69** | 25.6* |

Data's expressed as mean ± SEM; SEM: Standard Error Mean; CQ-Chloroquine; D0-D7-treatment day 0 to day 7; D8-day 8 observation; ***, **, and * represent the statistical significance of treatment groups at the level of $p<0.001$, $p<0.01$ and $p<0.05$ compared with the control by Dunnet's multiple comparison test

A specific subside in parasite proliferation was perceived dose dependently in SJCLE group revealing 25.16%, 53.94% and 71.21% at 100 mg/kg body weight, 200 mg/kg body weight, and 400 mg/kg body weight, yet unparalleled to CQ but displayed a significance of $p<0.05$. The other 2 extracts TICLE and SJMLE however not significant, unveiled 55.5% and 50.6% parasite drop respectively at the highest concentration of 400 mg/kg body weight and however was not analogous to the activity of CQ and SJCLE in the assay.

The negative control exposed appropriate raise in rectal temperature from the subsequent day (D4) of infection to D5 in the observation. Due to the parasite multiplication and aberration in the internal body metabolism, there was a hasty decline in body temperature from D6 onwards demonstrating the negative consequence of parasitaemia on body temperature. The CQ group along with the 3 extracts

(SJMLE, SJCLE, TICLE) displayed a statistical significance ($p<0.05$) in convalescing the rectal temperature from the instant next day (D5) after infection with parasites and all the extracts were comparable to CQ control revealing a temperature retaining efficacy at all doses in the treatment.

A crucial drop in body weight was observed in the untreated control (Table 6), where the mean body weight prior to infection on D4 was weighed as 29.4 g ± 1.22 g, which has reduced in course on D7 as 22.8 g ± 1.54 g. The reduction in body weight in the negative control signifies the blood parasitaemia has efficiently reduced the internal metabolism, which in turn has curtailed the body weight and rectal temperature with mortality of all animals on day 8 and day 9 (from the day of infection) in the observation. Furthermore, the CQ group displayed a diminutive weight reduction for the first day (D5) after infection and a steady raise in body weight was detected from D6 in the investigation. The TICLE ($p<0.01$) displayed a statistically significant body weight recovery compared to SJMLE and SJCLE which showed the statistical significance of $p<0.05$, where the CQ control showed a pronounceable significance of $p<0.001$.

In the prophylactic analysis the CQ group displayed a significant ($p<0.001$) survival ≥ 39.6 days compared to the extract treatment groups. Dose dependent mean survival in days were observed in all mice groups treated with plant extracts compared to the negative control (8.3 days). The mean survival of SJMLE and TICLE treated groups at 400 mg/kg body weight were found to be 23 and 25.6 days respectively demonstrating the significance of $p<0.05$, whereas the mean survival of animals in SJCLE treated group was up to 30.7 days with a notable significance of $p<0.01$. The results were hopeful that all the dose levels of plant extracts were operative in sustaining the survival of animals imperiled to parasite infection.

**Discussion**

The rodent malaria parasite *P. berghei* has proved to be analogous to human malarial parasites in most essential aspects of structure, physiology, life cycle and makes as a valuable model for the investigation of biology of malaria parasites, parasite-host interactions, vaccine development and drug testing [14,15]. In the present study, the chloroquine sensitive *P.berghei* NK 65 strain was used to check the efficacy of plant extracts in antiplasmodial activity. Along with the conventional antimalarials such as chloroquine, mefloquine, halofantrine and artemisinin an upsurge in herbal therapy derivatives has been recognized using rodent malaria model [16]. In the present study the most reliable *in vivo* antiplasmodial efficacy of plant drug was appraised by Peter's four-day test, Rane's curative and prophylactic assay using mice model. The present methods were convenient in the determination of percent parasitaemia suppression and a mean parasitaemia level ≤ 90% to that of vehicle treated group indicating that the compound in test can be considered in standard screening studies [17].

The chemosuppression measured in the Peter's four-day test indicates that the candidate plant extracts SJCLE, SJMLE, STMLE, TIMLE and TICLE has an active suppressive sequel on parasitaemia ascertaining that the plant was endowed with antimalarial activity. From the suppressive trial 3 extracts TICLE (72.72%), SJCLE (63.63%) and SJMLE (61.36%) displaying parasitaemia suppression at 400 mg/kg b.wt were screened for curative assay. The screened extracts TICLE, SJCLE and SJMLE were also effective in curative assay exhibiting above 50% antiplasmodial activity. The *in vivo* antiplasmodial activity can be classified as very good, good, and moderate if extracts display percentage parasitemia suppression equal to or greater than 50% at a dose of 100 mg/kg, 250 mg/kg and 500 mg/kg body weight per day, respectively [18,19]. This remains the fact that both chloroform and methanol fractions were active in blood schizontocidal activity demonstrating the individual and synergistic effect [20,21].

Crude extracts of all the 4 plants displayed alkaloid presence in the phytochemical screening proving that the alkaloids may be the major phytoconstituents for antiplasmodial activity. Active phytoconstituents such as alkaloids, phenolic compounds and terpenoids present in plant extracts could be responsible for the antimalarial activity. The chosen plants were also effective against fungal strains resolving the synergistic effect of plant metabolites [22]. Among alkaloids quinine was recognized and reported experimentally for its schizonticidal activity and hence the presence of alkaloids in the plant extracts set downs the compounds responsible for antiparasitic activity of the plants that might be concentrated in semi-polar and non-polar fractions.

Compared to the untreated control the treatment groups showed positive results in parasite control in Peters four-day test. In the suppressive test no much difference in the body temperature or weight loss was noticed as the course of study was only for 4 days and the treatment was initiated within an hour after infection with the *P. berghei*. But in the curative assay a great difference in mean parasitaemia, parasite suppression, rectal temperature, and body weight was observed before and after treatment. The untreated control group was prone to temperature fall, body weight loss and animal death on the day 9 due to the upshot of parasitaemia. Our results concur with the reports stating acute anemia, loss of body weight and temperature are the general features of malaria-infected mice [23].

Studies have reported the detrimental effects of malarial parasites on host organs involving phagocytosis of infected

and uninfected erythrocytes, tissue anoxia, and degeneration of liver, spleen, lymphoid and adrenal tissue [24]. The negative consequence on host carbohydrate, lipid and protein metabolism under malarial parasitaemia has also been reported [25,26]. Reviews relate the metabolism and body temperature are interrelated; any disturbance in the metabolism may lead to the fluctuation in the host internal body temperature and body weight. Such temperature changes may accompany by an alteration of the metabolic rate in the host.

Reports confirms that a change in metabolic rate after *P. berghei* post infection in Swiss albino mice increased rectal temperature for 5 days and temperature decline after day 5 due to parasitaemia demonstrating the reduced metabolism dampens the body temperature [27]. Hence, in antimalarial screening the drug should prevent loss of body weight under parasitemia. The crude extract of all the 4 selected plants suggestively prevented weight loss associated with increase in parasitemia level; despite the fact the increase in body weight was not persistent with increase in extract doses.

In the present assay much higher doses (>400 mg/kg b. wt) of plant drug were not tested in antiplasmodial activity to identify the weight loss in the mice but our acute toxicity test ruled the symptoms and outcomes associated with higher doses of plant extracts in the mice model. The $LD_{50}$ of 2 extracts *viz* STCLE and SJCLE were found to be toxic between 2000 mg/kg b.wt and 5000 mg/kg b.wt with a fatal consequence in animals. Whereas the other extracts were safer in exploit upon administration in mice reflecting minor distresses. Schizonticidal effect was noticed at the maximum of 400 mg/kg b.wt of all the extracts in both suppressive and curative assay without weight loss but no gain in body weight at any point. The study reports that the chance of appetite suppressive activity may occur under the circumstances of higher plant drug doses. Recent studies on animal model have proved that under higher dose administration appetite loss may be attributed to phenolic compounds, flavonoids, saponins, and glycosides found in the crude extract [28-30].

Malaria infections are complicated syndromes involving many inflammatory responses which may enhance cell-to-cell interaction (cytoadherence), cell stimulation involving malaria-derived antigens/toxins and host-derived factors such as cytokines. Moderate amounts of cytokines are though good for the host causes fever [31]. Clinically, it is crucial to reverse the effects of both toxins and cytokines to prevent further complications of malaria. In the present work the extracts of selected 4 plants acted as consummate agent in antiplasmodial activity as they exerted suppressive, curative and prophylactic properties in mutable levels in *P. berghei* induced malaria mice. Most of the inflammatory condition of malaria is characterized by free radical generation, activation of phospholipase activity with subsequent generation of prostaglandins and other cytokines (TNF, IFN-$\gamma$, and IL-1$\beta$). The cytokines upregulate the expression of adhesion molecules such as ICAM-1 binding the parasitized red blood cells to the vascular endothelium, where these inflammatory mediators as well as parasite sequestration are responsible for the consequence in malaria [32]. The antiplasmodial property of the selected plant extracts may be due to the inhibition of the production and/or release of these inflammatory mediators associated with mice malaria. Indeed, plant extracts has analgesic and potent anti-inflammatory properties and hence the prophylactic effect of the extracts may be attributed to its direct cytotoxic effect on the parasites in a mechanism similar to the CQ.

Reports suggests that the rectal temperature preferably declines as parasite level worsens with attenuation in the metabolic rate of infected mice prior to death and escorted by a corresponding diminution in internal body temperature [33]. In the present assay suppressive test did not show much attention in body temperature except chloroquine but there was a notable effect in retentive body temperature in the curative assay concurring medicinally active phytometabolites averts the rapid dropping of rectal temperature.

It is worth noting that plant extracts drove least to significant extermination of parasites from the blood of the mice depending on the shoot-up in dose level of the extract and is compared to the standard CQ for the activity. Chloroquine enters the red blood cell, inhabiting parasite cell, and digestive vacuole by simple diffusion, where it becomes protonated (CQ2+), as the digestive vacuole is known to be acidic (pH 4.7); chloroquine then cannot leave by diffusion. The CQ caps hemozoin molecules to prevent further biocrystallization of heme, thus leading to heme buildup and at the same time the CQ binds to heme (FP) to form the FP-CQ complex, which is highly toxic to the cell and disrupts membrane function. Action of the toxic FP-CQ and FP results in cell lysis and ultimately parasite cell undergoes autodigestion [34]. In essence, the parasite cell drowns in its own metabolic products and in such a way the activity of plant extracts may also be analogous in its activity.

Our results concur with the exercise reported that at the highest dose of antimalarials, a greater extent destroyed the parasites circulating in the lumen of the liver blood vessels [35]. The lower percentage of parasite suppression may be due to the insufficiency of drug in the blood circulation or its existence in the liver. Our results corresponds with the

report [36] describing that the inflammatory processes of liver in the *P. berghei* infected mice might be induced by the circulating parasite and due to low doses of drug treatment and the highest dose of drug could attribute to the complete elimination of the parasites from circulation. In the present study both CQ and plant drug prolonged the survival times of the mice at different dose levels, which could be attributed to the high parasitaemia clearance (reduced parasite burden) observed for the extracts subjected.

The SJCLE with 71.21% parasite suppression showed comparable efficacy to CQ (96.7%) in the prophylaxis assay indicating the indiscrimination of SJCLE on the stages of malaria parasite in the infected animals. It is not clear how the selected plant extracts exert prophylactic activity on *P. berghei* infection but it may be inhibiting the multiplication of the parasites as well as direct cytotoxic effect on the parasites [37]. It may modulate the membrane properties of the erythrocytes preventing parasite invasion [38].

The Sulfadoxine/Pyrimethamine (SP) used in one of the studies exerted prophylactic activity *via* the inhibition of dihydropteroate synthetase and dihydrofolate reductase enzymes of the parasites [39]. Generally, prophylactic antimalarial drugs work by disrupting the initial development of malaria parasites in the liver (causal activity). They may act by suppressing the emergent asexual blood stages of the parasite (suppressive activity) or by preventing the relapses induced by the latent liver forms (hypnozoites) [40]. The present study plants can therefore be used for malaria prophylaxis as well as a curative agent. Although the rodent model presents with some limitations, it has successfully been validated through the identification of several conventional antimalarials including the currently used antimalarials, halofantrine artemisinin derivatives, and the atovaquone/proguanil (Malarone), a drug approved in USA for malaria treatment and prophylaxis [41].

Day wise blood smears in untreated and treated groups showed a range of parasite increase and reduction respectively. Choloroquine treatment was found to be effective in chemosuppression, similarly TICLE, SJMLE and SJCLE were also effective but not up to the level of synthetic drug chloroquine.

Study results indicate that the chloroquine was active from day of treatment, whereas the plant extracts exhibited protective effect on successive doses ensuing parasite reduction, preventing body weight and temperature loss. The delayed activity in the above mentioned parameters may be due to the insufficiency in plant dose levels indicative of the need or the extracts might have a delayed onset of defensive action. Dose dependent parasite suppression and related parameters indicates, the increase in plant extract doses leads to the increase in activity in suppressive, curative and prophylactic assay. Similar to our report, previous reports on plant study determines the dose-dependent parasite suppressive effect at higher doses [42,43]. The SJMLE, SJCLE and TICLE prolonged the mean survival time in suppressive and established infection relevant to the antiplasmodial study of *Melanthra scandens* and *Croton macrostachyus* [42,44]. Our previous phytochemical study reports on *S. trilobatum*, *S. campanulata*, *S. jambos*, and *T. indica* demonstrated the presence of active metabolites such as alkaloids, flavonoids, polyphenols and terpenoids. Literature reviews on these metabolites in single or in synergism attribute to have different extent of antimalarial activity [31-33]. From least to significant level all the selected plants possessed antiplasmodial activity in the present assay. Extracts of *T. indica* and *S. jambos* screened from suppressive test was found to be potentially active in established antiplasmodial activity. The analogous doses of screened plant extracts from the Ranes curative assay also displayed an effective prophylactic activity in the final component of the assay.

The methanolic and chloroform extracts of *S. jambos* and chloroform extract of *T. indica* exerted suppression against *P. berghei* infection in the mice curatively and prophylactically. This suggests that the candidate plants leaf extracts screened from the suppressive assay can overwhelm parasite growth if administered orally for curative and prophylactic purposes in higher doses. The suppressive, curative and prophylactic effect of *S. jambos* and *T. indica* to some extent be ascribed to the presence of phytochemicals such alkaloids, flavonoids, polyphenols, anthraquinones, glycosides, saponins, steroids, tannins and triterpenoids, where these phytochemicals have indeed demonstrated antimalarial properties [45,46].

In the present study, the prophylactic activity of the extracts may be attributed to alkaloids, as well as may be due to the presence of saponins and glycosides. Interestingly, some glycosides and terpernoids have been identified to attack the broad stage of malaria infection thereby acting as both curative and prophylactic agents [47]. It is possible that the alcoholic and chloroform extract contained this broad stage acting glycosides thereby acting as both curative and prophylactic agent. The terpenoids in the extracts may be devoid of broad stage anti-plasmodial effect and studies are underway to investigate this claim. It is however possible that other phytochemicals not detected by the screening method employed may also contribute to the observed anti-plasmodial effect in the present investigation.

Malaria infection is a complicated syndrome involving inflammatory responses and pyrexia due to host derived factors [48]. It is therefore likely that the antimalarial action

of the extracts may be accompanied by anti-inflammatory and anti-pyretic activities. Because the *S. jambos* and *T. indica* leaf extracts appeared to be more promising than the other extracts used in treating and preventing schizont multiplication in the experimental mice, it could be effective for further future studies in the inflammatory and pyrexia models. Since the study was a pilot performance participating in the screening of active anti-plasmodial extracts, further research focus of compound identification is yet to be carried out with a fundamental hint from the 3 method of approaches such as suppressive, curative and prophylactic assay.

## Conclusion

The present study indicates the methanolic and chloroform leaf extracts of *S. trilobatum*, *S. campanulata*, *S. jambos*, and *T. indica* were effective in antiplasmodial activity at varying degree with differential effects on all parameters measured. The acute toxicity of plant extracts evidenced the toxicity profile of all the extracts determining the safer dose levels in experimental model. The crude TICLE, SJMLE and SJCLE screened from the suppressive test was found to be potentially active in the curative assay witnessed with the reduction of *P. berghei* burden in the blood stream of mice groups comparable to the chloroquine control. The present study submits the antimalarial phyto-metabolites present in the crude extracts may be synergistic or discrete in action.

## Funding

The infrastructure for this research was funded by DST-"Fund for Improvement of S&T Infrastructure (FIST)" Department of Biotechnology-Star College Scheme, New Delhi, Science and Technology Project Scheme under TN-SCST, Chennai.

## Acknowledgement

The authors, therefore, gratefully acknowledge DST-FIST, DBT Star College Scheme and TNSCST.

## Conflict of Interest

We declare that, we all authors have no conflict of interest.

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