

ANTIMALARIAL EFFICACY OF *Bergenia ciliata* (SAXIFRAGACEAE) LEAF  
EXTRACT *IN VITRO* AGAINST *PlasmodiumP. falciparum* AND *IN VIVO* AGAINST  
*PlasmodiumP. berghei*

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## ABSTRACT

**Aim:** ~~The traditional medicine plant *Bergenia ciliata* was used traditionally for its medicinal properties is being to~~ evaluated for its antiparasmodial activity against *Plasmodium P. falciparum* *in vitro* and preventive and curative activity against *Plasmodium P. berghei* *in vivo*. The ~~study also tests the~~ safety of the ethanolic leaf extract of *Bergenia ciliata* (ELEBC) to the liver and kidney functions of the rodent host ~~was also tested~~.

**Place and Duration of the study:** Parasitology Laboratory, Department of Zoology, Panjab University, Chandigarh, India, between October 2014 to November 2015.

**Methodology:** The *in vitro* antiparasmodial activity of the ELEBC against both chloroquine-resistant (RKL-9) and sensitive (MRC-2) strains of *P. falciparum* was assessed by ~~using the~~ WHO method. The cytotoxicity of the extract against human cancer and normal cell lines was tested by MTT assay. The *in vivo* repository and curative efficacy of the extract against *P. berghei* were tested using ~~the~~ Peter's method and modified method of Ryley and Peters respectively. The biochemical assays were performed as per standard methods.

**Results:** ELEBC exhibited considerable inhibitory activity against both RKL-9 and MRC-2 strains of *P. falciparum* with  $IC_{50}$  ~~=of~~ 6.4  $\mu$ g/ml and  $<5$   $\mu$ g/ml respectively. The extract exhibited no toxicity against both cancer and normal cell lines with  $CC_{50}$   $>1000$   $\mu$ g/ml and selectivity index (SI)  $>10$ . Maximum chemosuppression of 74.45% and 91.96% was observed on day 7 at a concentration of 1000  $\mu$ g/kg (repository activity) and 250  $\mu$ g/kg (curative activity), respectively. 83.33% survival of mice was observed in G6 (750  $\mu$ g/kg) while in all other ELEBC treated groups 50% survival was recorded on day 28 of study in the curative test. ~~–~~Hepatic function (SGOT, SGPT, ALP and bilirubin) and renal function biomarkers (creatinine and urea) in serum were observed to be significantly ( $P < 0.0005$ ) lower as compared to the infected control (G2).

**Conclusions:** ELEBC possesses considerable antimalarial activity against both sensitive and resistant strains of *P. falciparum*. It also exhibits significant efficacy as a preventive and curative remedy against the disease without any side effects on hepatic and renal functions of the rodent host.

**Key words:** Malaria, *Bergenia ciliata*, *Plasmodium*, traditional medicine, Mean survival Time, Chemosuppression, cytotoxicity

## 1. INTRODUCTION

Malaria is a major cause of morbidity and mortality in the tropical and subtropical areas. The World Malaria Report 2015 shows that the mortality rates due to the disease have fallen by 47% globally and by 54% in the African region since 2000 [1] [due to the application of antimalarial drugs, like ????](#). However, the increased spread of drug resistant strains of *Plasmodium* has necessitated the need for development of new antimalarial drugs for the containment of the disease. The use of traditional herbal remedies in the treatment of various ailments is universal and is believed to be safe and efficacious, [which](#) have led to the development of plant based medications [2]. The high chemical diversity, complexity, biochemical specificity and different molecular properties have established natural product medicines as lead structures for the development of new drugs [3, 4]. Even the most important antimalarial drugs (quinine and artemisinin) are plant derived.

The Indian subcontinent is blessed with wide varieties of aromatic and medicinal plants in which the Western Ghats, North Western Himalayas and North Eastern states constitute a rich repository of medicinal wealth. *Bergenia ciliata* (Saxifragaceae) has been used traditionally as an antipyretic or fever reducing agent [5] in the Himalayan region. It is a rhizomatous, evergreen, clump forming, perennial herb. The rhizome juice is used for [treating](#) cough [6], asthma, cold and urinary problems. The plant is also known to possess anti-inflammatory, antibacterial [7], antiviral [8], antidiabetic [9] and antioxidant [10, 11] properties.

The ethanolic leaf extract of *Bergenia ciliata* (ELEBC) has been reported to possess considerable suppressive activity with  $IC_{50} < 5 \mu\text{g/ml}$  and  $ED_{50} < 250 \text{ mg/kg}$  against rodent malaria parasite *P. berghei* [12]. Secondary metabolites viz. flavonoids, phenols, steroids and diterpenes were detected in the qualitative phytochemical screening tests of ELEBC. The present study explores the *in vitro* antimalarial efficacy of the extract against the human malaria parasite *Plasmodium falciparum* and its *in vivo* potential as a preventive and curative

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remedy against the disease. The study also highlights the safety of ELEBC treatment to the liver and kidney functions of rodent host.

## 2. METHODOLOGY

### 2.1 Plant specimen

Leaves of *Bergenia ciliata* were collected from Shimla district of Himachal Pradesh, India. Taxonomic identification of the plant (voucher specimen no. 2138) was carried out at Botany Department, Panjab University, Chandigarh, India. Leaves were washed thoroughly with water, shade-dried and powdered. The permission for collection of leaves of *Bergenia ciliata* was obtained from Himachal Pradesh State Biodiversity Board, State Council for Science Technology and Environment, Kasumpti, Shimla (letter no. SCSTE/SBB-959).

### 2.2 Preparation of plant extract

Soxhlet extraction method was used to prepare the ethanolic leaf extract of *Bergenia ciliata* (ELEBC). The ethanolic extract was filtered, concentrated under vacuum in Rotary evaporator followed by lyophilization and stored at -4°C.

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### 2.3 Experimental animals

White Swiss albino female mice, *Mus musculus*, 4-6 weeks old, weighing about 22-25 g, were used in the present study. These were obtained from L.L.R University of Animal and Veterinary Sciences, Hisar, Haryana, India and kept in Central Animal House, Panjab University, Chandigarh. The animals were fed on commercially available standard pellet diet (Ashirwad Industries, Chandigarh, India) and water *ad libitum*. The treatment of mice was done according to the guidelines of committee for the purpose of control and supervision on experiments on animals (Animal ethical clearance registration no. 45/1999/CPCSEA), Panjab University, Chandigarh, India.

### 2.4 Parasite

***P. falciparum* culture:** The chloroquine-resistant (RKL-9) and chloroquine-sensitive (MRC-2) strains of *P. falciparum* were obtained from NIMR (National Institute of Malaria Research), New Delhi, India. Modified method of Trager and Jenson (1976) was used

to maintain *P. falciparum* culture *in vitro* in human red blood cells (blood group A+) using RPMI-1640 as culture medium supplemented with 10% human AB+ serum [13].

***P. berghei* (NK-65) strain:** NK- 65 (chloroquine-sensitive) strain of rodent malaria parasite *P. berghei* was maintained by intraperitoneal inoculation of  $1 \times 10^6$  infected erythrocytes to normal mice in citrate saline. The percent parasitaemia was monitored by preparation of Giemsa stained thin blood smears on glass slides through tail vein incision of infected mice.

### 2.5 *In vitro* antiplasmodial efficacy of ELEBC against *P. falciparum*

The *in vitro* antiplasmodial activity of ELEBC was evaluated by WHO protocol based on the inhibition of schizont maturation [14]. The stock solution (10 mg/ml) of the extract was made in 1% (v/v) DMSO (Dimethyl Sulphoxide) and further diluted with incomplete medium, to make different concentrations (5-100 µg) of ELEBC. Schizont maturation inhibition as compared to the negative control wells was determined by the method of Walter and Bagai (2014) [15], using the following formula and IC<sub>50</sub> was calculated using Sigmaplot software:

$$100 - \frac{\text{Average schizont maturation in extract/drug treated well}}{\text{Average schizont maturation in control well}} \times 100$$

### 2.6 Cytotoxicity assay

*In vitro* cytotoxicity of the ELEBC was assessed on *HeLa* cell lines and primary culture of normal human dermal fibroblasts employing the MTT assay [16]. *HeLa* cells were purchased from National Center for Cell Science (NCCS), Pune, India. Primary culture of dermal fibroblasts was obtained from Department of Dermatology, P.G.I.M.E.R, Chandigarh, India. Both cell lines were maintained at 37°C in atmosphere with 5% CO<sub>2</sub> in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) foetal calf serum, glutamine 2 mM, and antibiotics streptomycin (100 µg/ml) and penicillin (100 µl/ml).

The cells in DMEM were seeded into 96-well culture plate in 100 µl of complete medium containing 5000-10000 cells per well. Cells were then incubated with different concentrations (10-1000 µg) of ELEBC in duplicate after a period of 24 h. This was followed by the addition of 10 µl of tetrazolium reagent (1 mg/ml) in each well after 48 h incubation

and further incubated for 1 h. The supernatant was removed, and ~~dimethyl sulphoxide~~ [DSMO](#) (100  $\mu$ l/well) was added to allow [the](#) Formosan solubilization. The optical density (OD) of each well was read at 450 nm and [proportion \(%\) of](#) cell viability was calculated to determine the CC<sub>50</sub> value.

## 2.7 Selectivity index

The selectivity index (SI) i.e. ratio of CC<sub>50</sub> determined on cell lines to the IC<sub>50</sub> determined on the parasite was also calculated.

## 2.8 *In vivo* antiplasmodial efficacy of ELEBC against *P. berghei*

*In vivo* preventive and curative efficacy of ~~*B. ergenia*~~ *ciliata* was determined by [the](#) following methods:

### 2.8.1 Assessment of preventive activity of ELEBC

The preventive (repository) activity of ELEBC was evaluated by Peter's method [17]. Mice were divided into eight groups consisting of [six](#) mice ~~six~~ each. GP1, GP2 and GP3 were designated as normal, infected and vehicle control respectively. Four groups (GP4-GP7) were administered with different concentrations of ELEBC (250-1000 mg/kg, [at a interval of 250 mg/kg](#)) respectively once daily for four consecutive days (D0-D3) orally (Table 1). The extract was dissolved in standard suspension vehicle (SSV) before administration. Pyrimethamine (1.2 mg/kg) was used as positive control (GP8). On day 5 (D4), the mice were injected i.p. with ~~1 $\times$~~ 10<sup>6</sup> *P. berghei* infected erythrocytes. The parasitaemia level was checked by preparation of Giemsa stained thin blood smears on day 7 to determine the average [% of](#) chemosuppression for each group.

### 2.8.2 Assessment of curative activity of ELEBC

A modified method similar to [that](#) described by Ryley and Peters [\[18\]](#) was used to assess curative (schizontocidal) activity of ELEBC in established infection of *P. berghei* ~~[18]~~. Mice were divided into eight groups consisting of [six](#) mice ~~six~~ each. On D0, all experimental mice (except G1) were inoculated i.p. with 1 $\times$ 10<sup>6</sup> ~~*P. lasmodium*~~ *berghei* infected red blood cells. On D4 (seventy-two hours later), G4-G7 mice were administered different concentrations of the ELEBC (250-1000 mg/kg). Chloroquine (5 mg/kg) was given to the positive control (G8) (Table 1). The drug/extract was given once daily for 4

consecutive days orally dissolved in SSV. Giemsa stained thin blood films were prepared on day 7, 14, 21 and 28 to monitor the parasitaemia level in each group. The mean survival time (MST) of mice in each group was calculated during the follow up period of 28 days (D0–D29).

## 2.9 Biochemical assays for liver and kidney function tests

The biochemical assays were performed in the serum obtained from blood of mice by tail vein drainage on D7 and survivors of experimental groups on D28. All biochemical assays were performed using commercially available diagnostic kits (Reckon Diagnostic Pvt. Ltd. and Transasia Bio Medicals Ltd., Baddi, Distt. Solan, Himachal Pradesh, India).

### 2.9.1 Liver function test

The activity of [alkaline phosphatase \(ALP\)](#) [19], [xxxx \(SGOT\)](#) and [xxxxx \(SGPT\)](#) [20] and concentration of bilirubin [21] were checked in serum of mice for assessment of hepatic function.

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### 2.9.2 Kidney function test

Serum levels of urea [22] and creatinine [23] were measured for assessment for renal function.

## 2.10 Statistical analysis

Statistical analysis was carried out employing Microsoft Excel. Experimental data [has been were](#) expressed as Mean±SD for each group. For antiplasmodial and cytotoxicity activities the 50% inhibitory concentration (IC<sub>50</sub>/CC<sub>50</sub>) was calculated by probit regression analysis using Sigmaplot software. The statistical significance of inter group difference of various *in vivo* parameters [has been was](#) determined by unpaired Student's *t* test using Graph pad software. Comparison was made between the test groups and control group of mice and *P*-value <0.05 was termed as statistically significant value.

## 3. RESULTS AND DISCUSSION

Considering the importance of plant derived drugs in the management of malaria, testing of different medicinal plants for their potential as antimalarials can provide newer, safe

and effective drugs against malaria. *B. ciliata* is well known for its antioxidant, anti-inflammatory, antipyretic, antiviral and antibacterial properties and has been used traditionally for the treatment of various ailments.

Ethanollic leaf extract of *B. ciliata* (ELEBC) exhibited considerable inhibitory activity against both chloroquine-resistant and sensitive strains of the parasite with  $IC_{50} = 6.4 \mu\text{g/ml}$  and  $<5 \mu\text{g/ml}$  respectively (Fig. 1A, B). In case of chloroquine-resistant (RKL-9) strain, maximum inhibition of  $92.57 \pm 0.38\%$  was observed at a high concentration (100  $\mu\text{g/ml}$ ) of the extract, while  $77.47 \pm 11.65\%$  inhibition of schizont maturation was evident at 10  $\mu\text{g/ml}$  of ELEBC (Fig. 2).

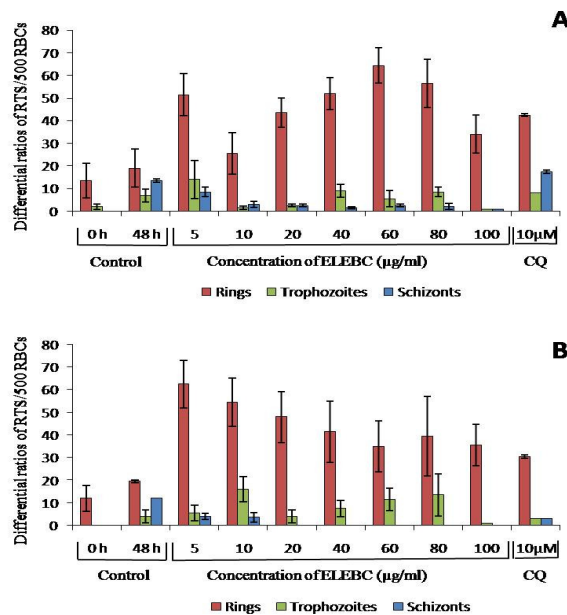


**Fig. 1: Effect of ELEBC (5-100  $\mu\text{g/ml}$ ) on schizont maturation of chloroquine-resistant [A] and chloroquine-sensitive [B] strains of *P. falciparum* in vitro.**

The extract exhibited better efficacy against the chloroquine-sensitive (MRC-2) strain of the parasite. Inhibition of schizont maturation was observed to be in a dose dependant manner with  $62.5 \pm 17.67\%$  chemosuppression at the lowest tested concentration (5  $\mu\text{g/ml}$ ) and complete inhibition of schizont maturation (100%) above 20  $\mu\text{g/ml}$  concentration of extract (Fig. 2). The standard drug CQ exhibited  $75 \pm 0\%$  chemosuppression. Based upon WHO

recommendations and other studies, extracts exhibiting  $IC_{50} < 5 \mu\text{g/ml}$  are classified as highly active,  $IC_{50}$  between  $5\text{--}15 \mu\text{g/ml}$  as promising antimalarials, moderate activity at  $IC_{50}$   $15\text{--}50 \mu\text{g/ml}$  and inactive  $IC_{50} > 50 \mu\text{g/ml}$  [24]. ELEBC can thus be categorised as promising antimalarial against RKL-9 strain and highly active against the MRC-2 strain.

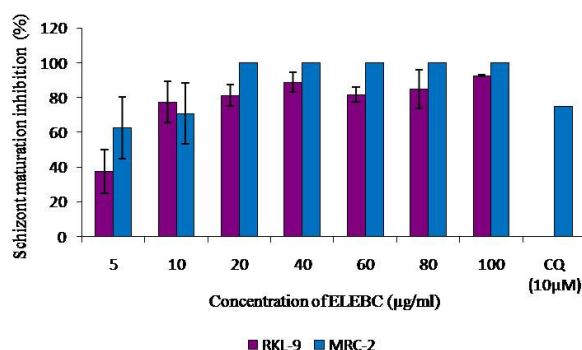


**Fig. 2: Percent inhibition of schizont maturation of both RKL-9 and MRC-2 strains *P. falciparum* at different concentrations (5-100  $\mu\text{g/ml}$ ) of ELEBC after 48 h of incubation.**

The safety of any compound/drug exhibiting good antiplasmodial activity is a major concern during antimalarial drug development. The candidate drug/extract should not only exhibit high activity against the parasite, but ~~it should~~ also be non-toxic to the host. *B. ~~ergenia~~ ciliata* leaf extract (ELEBC) exhibited no signs of toxicity against both cancer (*HeLa*) and normal (dermal fibroblasts) cell lines. The cell viability (%) was ~~observed to be~~  $67.1 \pm 7.37\%$  at  $1000 \mu\text{g/ml}$  of the extract in case of *HeLa* cells (Fig. 3). In case of normal human dermal fibroblasts  $>70\%$  viability was observed at all the tested concentrations ( $10\text{--}1000 \mu\text{g/ml}$ ) of ELEBC. The  $CC_{50}$  was ~~calculated to be~~  $>1000 \mu\text{g/ml}$  against both cell lines.

The selectivity index (SI) was ~~determined to be~~  $156.25$  and  $>200$  for ~~the both~~ resistant and sensitive strains of *P. falciparum*, respectively. According to Valdes *et al.* [25] any extract/drug exhibiting high selectivity index ( $SI > 10$ ) offers a potential for safe therapy and is considered to be an active antimalarial [25]. Thus, ELEBC can be classified as active antimalarial ~~against both the strains of the parasite~~. The high selectivity index ( $SI > 10$ ) also

signifies that the pharmacological activity of the extract is due of their specific antiplasmodial efficacy and not due to general toxicity [26].

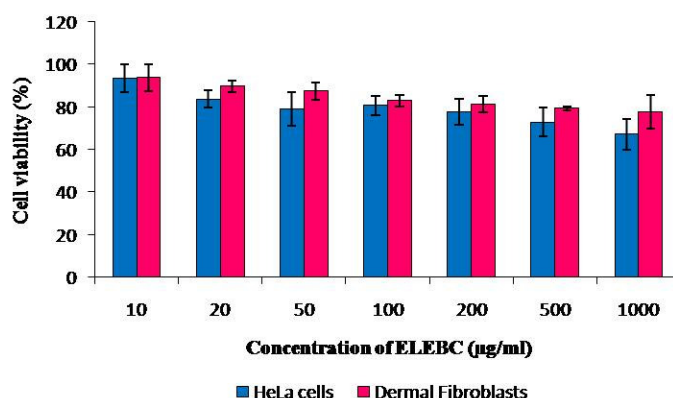


**Fig. 3: Percent viability of cancer (*HeLa*) and normal (dermal fibroblasts) cell lines upon treatment with ELEBC (10-1000 µg/ml).**

Any compound or drug exhibiting promising *in vitro* activity can be further evaluated for their potential *in vivo* antiplasmodial activity. The positive control (pyrimethamine, 1.2 mg/kg) used for the evaluation of preventive activity of extract in the present study is an inhibitor of dihydrofolate reductase (pDHFR) enzyme essential for DNA synthesis and multiplication of the parasite [27]. It was widely used as a prophylactic drug for malaria before the development of resistance. Dose dependent inhibition of parasite development was evident after administration of ~~ethanolic leaf extract of *Bergenia ciliata*~~ (ELEBC) on day 7. Chemosuppression of 13.91%, 38.37%, 61.08% and 74.45% was observed on day 7 at ~~various the ELEBC concentrations (of 250-1000 mg/kg) of ELEB\_C~~ in comparison to pyrimethamine (78.78%).

Initially mice treated with 1000 mg/kg of the extract exhibited low parasitaemia ( $1.89 \pm 0.47\%$ ) on day 7. However, during the follow up period ~~mortality of mice was evidently decreased~~ at higher doses (750 mg/kg and 1000 mg/kg) of the extract. At lower doses (250 mg/kg and 500 mg/kg) 50% survival was recorded with low grade infection of  $5.3 \pm 1.69\%$  and  $4.85 \pm 1.43\%$  respectively. Only one mouse administered with pyrimethamine

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survived till day 21 post inoculation with  $1.59 \pm 0\%$  infection (Fig. 4). These findings illustrate the preventive potential of the extract against the parasite as well its safety to the rodent host leading to enhancement of the Mean Survival Time of mice. The observed activity of ELEBC can be attributed to the presence of phytochemicals such as phenols, flavonoids, steroids and diterpenes [12] ~~were~~ detected in the ELEBC, which have been implicated in providing protection against the malaria parasite [28, 29].

**Table 1: Evaluation of preventive and curative activity of *Bergenia ciliata* against *P. berghei* infection.**

| Groups<br>n= 6                                          | Preventive activity<br>Dose (D0-D3)<br>(0.2 ml/mouse/OD/oral) |                 | Groups<br>n= 6                     | Curative activity<br>Dose (D4-D7)<br>(0.2 ml/mouse/OD/oral) |                 |                           |
|---------------------------------------------------------|---------------------------------------------------------------|-----------------|------------------------------------|-------------------------------------------------------------|-----------------|---------------------------|
|                                                         | P (%)<br>Day 7                                                | CS (%)<br>Day 7 |                                    | P (%)<br>Day 7                                              | CS (%)<br>Day 7 | MST (days)                |
| GP1 (DW)<br>Normal control                              | -                                                             | -               | G1 (DW)<br>Normal control          | -                                                           | -               | 28±0                      |
| GP2 (DW)<br>Infected control                            | 7.4±3.13                                                      | -               | G2 (DW)<br>Infected control        | 33.84±0.38                                                  | -               | 8.33±1.15                 |
| GP3 (SSV)<br>Vehicle control                            | 5.56±0.83                                                     | -               | G3 (SSV)<br>Vehicle control        | 36.86±1.89                                                  | -               | 9.5±2.08                  |
| GP4 (250 mg/kg)                                         | 6.37±1.52 <sup>#</sup>                                        | 13.91           | G4 (250 mg/kg)                     | 2.72±0.92 <sup>***</sup>                                    | 91.96           | 25.16±6.01 <sup>***</sup> |
| GP5 (500 mg/kg)                                         | 4.56±1.68 <sup>#</sup>                                        | 38.37           | G5 (500 mg/kg)                     | 6.57±3.29 <sup>***</sup>                                    | 80.58           | 19.66±9.15 <sup>***</sup> |
| GP6 (750 mg/kg)                                         | 2.88±0.51 <sup>**</sup>                                       | 61.08           | G6 (750 mg/kg)                     | 3.31±3.20 <sup>***</sup>                                    | 90.21           | 25±7.34 <sup>***</sup>    |
| GP7 (1000 mg/kg)                                        | 1.89±0.47 <sup>***</sup>                                      | 74.45           | G7 (1000 mg/kg)                    | 3.58±2.25 <sup>***</sup>                                    | 90.11           | 22.66±8.35 <sup>***</sup> |
| GP8<br>(Pyrimethamine<br>1.2 mg/kg)<br>Positive control | 1.57±0.25 <sup>***</sup>                                      | 78.78           | G8 (CQ 5mg/kg)<br>Positive control | 4.48±2.72 <sup>***</sup>                                    | 86.76           | 17.75±6.94 <sup>*</sup>   |

Data is expressed as Mean± SD for six mice per group as compared to infected control. . n= total no of mice in each group.

\*\*\* $P < .0005$  extremely statistically significant, \*\* $P < .005$  very statistically significant, # $P > .05$  not statistically significant.

P (%) percent parasitaemia; CS (%) percent chemosuppression; SSV (standard suspension vehicle) and DW (distilled water).

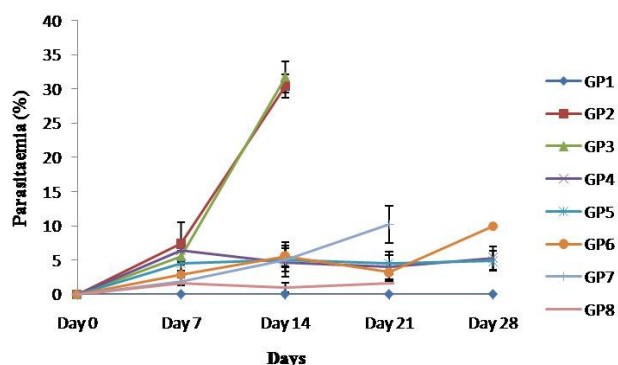


**Fig. 4:** Course of infection (Mean±SD) in various mice groups on administration of ELEBC during the follow up period in the preventive test.

ELEBC *Bergenia ciliata* leaf extract also exhibited considerable curative activity *in vivo*. Non-dose dependent chemosuppression of 91.96%, 80.58%, 90.21% and 90.11% was observed at various the concentrations (between 250- and 1000 mg/kg), respectively on day 7, which was greater than that in the treatment of chloroquine (86.76%). However, the suppressive effect became dose dependent by day 14 post inoculation (Table 1). A low parasitaemia of  $3.34 \pm 1.34\%$ ,  $2.36 \pm 1.27\%$ ,  $3.1 \pm 1.36\%$  and  $4.41 \pm 1.78\%$ , respectively, was observed at various the concentrations (of 250- to 1000 mg/kg) of ELEBC on day 28 (Fig. 5).

Maximum MST ( $25 \pm 7.34$  days) was observed at 750 mg/kg of the extract. It was extremely statistically significant ( $P < .0005$ ) as compared to untreated control (G2), which exhibited a MST of  $8.33 \pm 1.15$  days (Table 1). MST of  $17.75 \pm 6.94$  days was evident in the chloroquine (5 mg/kg) treated group. Maximum survival (83.33%) was observed in G6 (750 mg/kg), whereas, in all the other ELEBC treated groups 50% survival was recorded on day 28 of study.

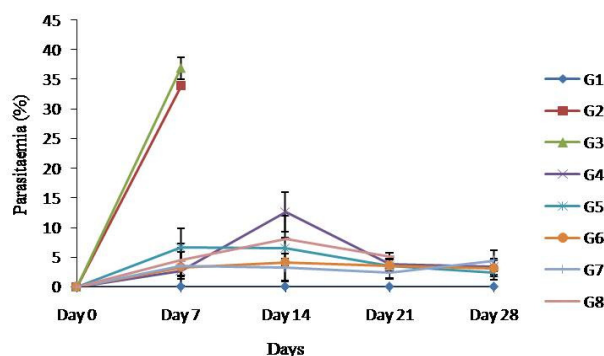


**Fig. 5: Course of infection (Mean±SD) in various groups on administration with ELEBC during the follow up period in the curative test.**

The functioning of the liver and kidney upon ELEBC administration in suppressive test was also assessed by measuring serum levels of alkaline phosphatase, SGOT, SGPT, bilirubin, creatinine, and urea. Alkaline phosphatase (ALP) is located on the hepatocyte membrane in the liver as well in the placenta, intestine, kidney, bone and platelets. Elevated level of serum ALP has been reported in malaria infection [30]. Similar observation has been made-In the present study, where extremely statistically significant ( $P<.0005$ ) increase in the activity of serum ALP was observed in infected control (G2) and vehicle control as compared to normal G1 ( $210.18\pm6.75$  IU/L). The increased serum ALP activity indicates perturbation of the host hepatocytes membrane due to infection leading to leakage of this enzyme out of liver cells [31]. Among the ELEBC treated groups ALP activity was observed to be significantly ( $P<.0005$ ) low as compared to infected control (G2) (Table 2), which indicates the safety of the extract to the liver function of the rodent host.

Bilirubin is a breakdown product of haem present in haemoglobin, which is released by the breakdown of senescent erythrocytes. Rise in bilirubin level in malaria is due to liver damage and haemolysis of parasitized and non-parasitized cells [32]. The total bilirubin is an indicative of jaundice. In the present study too, the concentration of bilirubin was observed to increase significantly ( $P<.0005$ ) in the infected control ( $3.60\pm0.41$  mg/dl) on day 7 (Table 2). This increase in the bilirubin levels are attributed to intravascular haemolysis and associated renal failure resulting in decreased excretion of bilirubin [33]. In the ELEBC treated groups slight elevation in bilirubin concentration was evident at higher concentrations (750-1000

mg/kg) of the extract (Table 2). However, the level was significantly ( $P<.0005$ ) lower as compared to the infected control mice. These findings point to the safety of the extract to host's liver at lower doses. Increase in serum bilirubin concentration was recorded in chloroquine treated mice on the 28 day of study (Table 2).

**Table 2: Serum SGOT, SGPT, ALP, bilirubin, urea and creatinine concentration in mice treated with various concentrations of ELEBC along with normal and infected control on day 7 and survivors of experimental groups on day 28.**

| GROUPS      | ALP<br>(IU/L)  | Bilirubin<br>(mg/dl) | SGOT<br>Activity<br>(IU/L) | SGPT<br>Activity<br>(IU/L) | Creatinine<br>(mg/dl) | Urea<br>(mg/dl) |
|-------------|----------------|----------------------|----------------------------|----------------------------|-----------------------|-----------------|
| G1<br>Day 7 | 210.18±6.75    | 0.58±0.39            | 25.80±4.83                 | 24.51±4.56                 | 0.59±0.18             | 33.58±7.44      |
| G2<br>Day 7 | 454.86±7.71*** | 3.60±0.41***         | 82.65±5.93***              | 79.70±2.69***              | 1.99±0.47***          | 67.83±7.36***   |
| G3<br>Day 7 | 406.93±4.50*** | 2.39±0.26***         | 94.29±5.09***              | 91.34±4.41***              | 1.49±0.17***          | 55.39±1.99***   |
| G4<br>Day 7 | 378.72±9.79*** | 0.7±0.18***          | 22.1±6.25***               | 17.68±0***                 | 0.82±0.23***          | 42.61±4.54***   |
| G5<br>Day 7 | 169.55±2.47*** | 0.5±0.09***          | 22.1±6.25***               | 39.78±6.25***              | 1.55±0.78#            | 53.65±1.90**    |
| G6<br>Day 7 | 313.75±4.45*** | 1.05±0.45***         | 17.68±0***                 | 22.1±3.42***               | 1.25±0.21***          | 23.01±3.09***   |
| G7<br>Day 7 | 247.70±5.09*** | 1.18±0.11***         | 15.47±3.12***              | 13.26±6.25***              | 1.77±0.78#            | 34.51±1.67***   |
| G8<br>Day 7 | 228.15±7.70*** | 0.78±0.26***         | 55.98±4.14***              | 68.15±6.46***              | 0.79±0.45***          | 38.70±8.46***   |
| Day 28      | 224.43±6.06    | 1.27±0.70            | 61.43±4.51                 | 56.11±9.31                 | 0.44±0.14             | 51.66±5.18      |

Data is expressed as mean± SD for six mice per group as compared to infected control.

\*\*\* $P<.0005$  extremely statistically significant, \*\* $P<.005$  very statistically significant, # $P>.05$  not significant.

Liver enzymes (both SGOT and SGPT) have been reported to be considerably elevated in malaria hepatopathy patients [34, 35]. Rise in plasma level of SGOT has been observed to be accompanied by increase in SGPT level in liver diseases. In the present study, also a similar observation has been made where the level of serum SGOT and SGPT was observed to be significantly ( $P<.0005$ ) elevated in the infected control (G2) and vehicle control (G3) as compared to the normal control. The SGOT and SGPT activity recorded in the all the ELEBC treated groups was significantly ( $P<.0005$ ) lower in comparison to the infected

control ( $82.65 \pm 5.93$  IU/L) on day 7 (Table 2), which points to the safety of *B. ergenia ciliata* leaf extract to the host's hepatic function.

Serum urea and creatinine levels are used for the assessment of kidney function [36]. Increased serum levels of creatinine and urea are indications of deficiency in renal function [37, 38]. The results of the present study are in accordance with these observations where significantly ( $p < 0.0005$ ) elevated creatinine and urea levels were observed in *P. berghei* infected mice (G2) and the vehicle control (Table 2) on day 7. Among ELEBC treated groups creatinine concentration was ~~observed to be~~ significantly ( $P < 0.0005$ ) lower than the infected control at a low concentration of 250 mg/kg (G4). However, considerable elevation in the creatinine concentration was recorded at higher concentrations of the extract (G5-G7). On the other hand, serum urea concentration was ~~observed to be~~ significantly ( $P < 0.0005$ ) lower than the infected control (G2) in mice treated with ELEBC except G5 ( $53.65 \pm 1.90$  mg/dl) (Table 2). These findings illustrate the safety of extract administration to the host's kidney function at lower doses.

#### 4. CONCLUSIONS

The present study indicates that ELEBC possesses considerable *in vitro* antiplasmodial activity with good selectivity against the deadliest species of malaria parasite, causing cerebral malaria. It also exhibited good repository and excellent curative activity against *P. berghei* *in vivo*. Treatment with ELEBC is able to cause parasite inhibition as well as enhance the mean survival time of host. The biochemical parameters also points towards the safety of ELEBC to the liver and kidney function of the rodent host at lower doses. Further studies can be carried out to isolate active principles responsible for its antiplasmodial efficacy as new lead compounds for the treatment of the dreaded disease.

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### **CONFLICT OF INTEREST**

Authors declare that they have no conflict of interest.

### **AUTHOR'S CONTRIBUTIONS**

This work has been done in collaboration between both the authors. Dr. Neha S. Walter contributed to collection of plant specimen; identification and herbarium, performed the laboratory work, wrote the protocol, performed analysis of data and wrote the first draft of the manuscript. Dr. Upma Bagai designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. Both the authors read and approved the final manuscript.

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