Resistant to first line drugs to treat malaria is the prime problem in controlling it. Parasitic resistance to chloroquine and then to others was first noticed in the 1970s and has since spread all over the world. Chloroquine resistance is associated with reduced sensitivity to other drugs such as quinine and amodiaquine. Immediate measures are needed to replace antimalarial drugs which are rapidly becoming ineffective with newer, cheaper and effective antimalarial [1].

Combination therapies preferably using “novel” antimalarial drugs are the way forward for improving therapeutic efficacy and delaying development of resistance in antimalarial treatment. Artemisinin (qinghaosu), artemunate, artemether and dihydroartemisinin have all been used in combination with other antimalarial drugs for the treatment of malaria. Artemisinin derivatives are eliminated rapidly and have a short half-life. When given in combination with a longer half-life “partner” antimalarial drug allows a reduction in the duration of treatment, while at the same time enhancing efficacy and reducing the likelihood of resistance development [2].

The natural product curcumin is a polyphenolic compound extracted from the rhizome of Curcuma longa L. In India, it is commonly used as a spice to add color and flavor to the food. In Ayurveda, use of curcumin is well documented for the treatment of various ailments [3]. Studies have shown that a new combination therapy with artemether (ARM) and curcumin is unique, with potential advantages over known Artemisinin Combination Therapy (ACT). The problems of poor solubility, stability and bioavailability of curcumin can be overcome by preparing curcumin metal complex. In present study curcumin-Zn complex was prepared and evaluated for antimalarial activity in combination with artemether.”

**ABSTRACT**

**Objective:** Studies have shown that a new combination therapy with artemisinin derivatives and curcumin is unique, with potential advantages over known Artemisinin Combination Therapy (ACT). The problems of poor solubility, stability and bioavailability of curcumin can be overcome by preparing curcumin metal complex. In present study curcumin-Zn complex was prepared and evaluated for antimalarial activity in combination with artemether.

**Methods:** Curcumin Zn complex was prepared using zinc sulfate. The mice survival and % parasitemia were studied in Plasmodium berghei (P. berghei) infected albino mice treated with curcumin, curcumin-Zn complex and combination of curcumin-Zn with artemether.

**Results:** The mean survival time in mice infected with P. berghei was compared after treatment with curcumin, curcumin-Zn, artemether and combination of curcumin-Zn-artermether. Oral administration of curcumin-Zn-artermether prolonged the survival of P. berghei infected mice. All the mice were treated with Curcumin-Zn (5 mg/day) artemether (1000 µg) survived for more than 40 d and recovered with no detectable parasitemia.

**Conclusion:** In vivo antimalarial activity of curcumin-Zn complex was found superior to curcumin. A single dose of 1000 µg of artemether in combination with curcumin-Zn gives complete protection in P. berghei infected mice. Such suppressive action was superior to that of administration of the single drug at the same dose. This may reduce the chances of drug resistance.

**Keywords:** Curcumin-Zn complex, Artemether, Mice survival, % parasitemia.
and compressed to form pellets. The spectra of sample was scanned from a wave number range of 650 to 4000 cm⁻¹.

**UV spectroscopy**

Solution of curcumin and its metal complex were prepared in Dimethyl Sulphoxide (DMSO) and scanned on UV spectrophotometer in the range 350 to 600 nm to find λ max. UV spectroscopic analysis was done to confirm the formation of curcumin-Zn complex.

**Antimalarial activity [13, 14]**

**Mice survival study**

Male albino mice were used for the study. Tap water and mouse feed was provided *ad libitum*. *P. berghei* (ANKA strain) erythrocytic stages were maintained by serial passing of infected blood in male albino mice. Animals were divided into nine groups based on the treatment. Mice were injected intraperitonally (i. p.) with 10⁷ *P. berghei* infected mouse erythrocytes. Control group (group no 1) was only given 5% tween 60. Mice in group 2 and 3 received an oral suspension of curcumin-Zn and curcumin respectively in corn oil at a dose equivalent to 5 mg of curcumin on d 1, 2 and 3. Animals in group 4, 5 and 6 received by oral route a combination of curcumin-Zn (equivalent to 5 mg curcumin on d 1, 2 and 3) and i. p. injection of artemether at single dose of 0.5, 1 or 1.5 mg, respectively (on d 1). While artemether was injected intraperitonally as a suspension in 5% tween 60 to animals in group 7, 8 and 9 with a dose of 0.5, 1 or 1.5 mg (on d 1) respectively. The survival time (over 40 d post treatment) of mice infected with the erythrocytic stages of *P. berghei* was compared in different groups.

**Determination of mean survival time**

Mortality was monitored daily and the number of d from the time of inoculation of the parasite up to death was recorded for each mouse in the treatment and control groups throughout the follow up period. The mean survival time (MST) for each group was calculated as:

\[ \text{MST} = \frac{\text{Sum of survival time in all mice in group (days)}}{\text{Total number of mice in that group}} \]

**Percent mean parasitemia in mice [15]**

Parasitemia was monitored by light microscopy (oil immersion, 1000× magnification) by examining thin smears of blood from the tail veins of the mice.

Blood films are made by applying 4.5 microlitres of blood to microscope slides as soon as the specimen is received. Thin blood films were fixed in methanol and stained with Giemsa stain immediately after slide production. The parasitemia level was determined by counting, in random fields of the microscope, the number of parasitized RBCs. The percent of infected RBCs were determined by enumerating the number of infected RBCs in relation to the number of uninfected RBCs. A minimum of 500 RBCs was counted per sample.

\[ \% \text{Parasitemia} = \frac{\text{Number of infected RBC}}{\text{Total number of RBC counted}} \times 100 \]

Inoculation of *P. berghei* to mice was done on d 0, while percent mean parasitemia was measured on d 1, 2, 3 and 4. The blood samples were collected after 4 hours of receiving treatment as per specified above and at the same time on the next days.

**% Suppression of parasitemia**

The percentage suppression of parasitemia was calculated for each test concentration by comparing the parasitaemia in infected controls with those received different concentrations of the treatments. Percent parasitemia suppression were calculated as

\[ \% \text{Parasitemia Suppression} = \frac{A - B}{A} \times 100 \]

Where, A is mean % parasitemia in the control group and B is % parasitemia in the treatment group.

**Statistical analysis**

All data were expressed as mean±SD. The statistical analysis of all the observations was carried out using one-way ANOVA followed by a multiple comparison test of Tukey, where necessary. P<0.05 was considered as significant compared with the control group and all data were analysed at a 95% confidence interval.

**RESULTS AND DISCUSSION**

Curcumin and curcumin-Zn complex in powdered form were scanned between wave number 4000 to 650 cm⁻¹. The resultant spectrum obtained has shown in fig. 1 and 2. The major peaks of the spectrum were then interpreted so as to determine the respective functional groups present. The results are shown in table 1.
The 1629 and 1603 cm\(^{-1}\) bands correspond to the mixtures of stretching vibrations of (C=\(\equiv\)C) and (C=O) in curcumin were red shifted to 1625 and 1588 cm\(^{-1}\) in the curcumin-Zn complex respectively.

**UV Spectrophotometric determination**

UV spectra of curcumin and its metal complex were developed by dissolving it in DMSO.

**Antimalarial study**

**Mice survival test**

The mean survival time of mice infected with the erythrocytic stages of *P. berghei* was compared in different groups as shown in table 2. All mice in the control group died in 7 d post exposure to infection. Animals in group 2 and 3 have shown 100% mortality by the 16th and 10th d respectively. It was observed that animals in group 2 treated with curcumin-Zn have survived for longer time than animals treated with curcumin (group 3) suggesting the superior antimalarial action of curcumin-Zn than curcumin. But it failed to give complete protection against *P. berghei* infection in mice.

All animals in group 4 and 5 have survived by the 40th d with 100% protection against *P. berghei* infection (fig. 4). Comparing mean survival time and % protection between same dose (artemether) groups viz. 6 and 9, 5 and 8, 4 and 7, it was observed that the combination of artemether and curcumin-Zn has offered more protection than artemether (single drug) administration. The combination of curcumin-Zn artemether prolonged the survival time as compared to administration of single drug at the same dose as all the animals in group 4 and 5 have survived by the d 40.

**Percent mean parasitemia in mice**

There was marked variation in percent mean parasitemia in mice receiving control, curcumin, curcumin-Zn, combination of curcumin Zn-artemether and artemether for the first 4 d post infection as shown in fig 5. The percent mean parasitemia in control mice was drastically increased showing that more RBCs are parasitized with time.

The values for percent mean parasitemia on 4th d and % suppression of parasitemia in mice receiving various treatments are reported in table 3. Animals in group 2 and 3 showed an increase in percent parasitemia to 39.65 and 54.12 respectively on d 4. Increase in percent mean parasitemia in animals of group 2 was less than animals of group 3 thus supporting the claim of superior antimalarial action of curcumin-Zn than curcumin.
Animals in group 4 and 5 showed maximum % parasitemia suppression, respectively 90.86 and 88.35 on d 4. It was observed that combination drug treatment has led to suppression of parasitemia more effectively than single drug administration. The animals in group 4 and 5 recovered with no detectable parasitemia after 40 d in the mice survival study.

### Table 2: Mean survival time in *P. berghei* infected mice

<table>
<thead>
<tr>
<th>Gr No.</th>
<th>Treatment</th>
<th>Dose</th>
<th>Mean survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Curcumin Zn complex</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Curcumin</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Curcumin-Zn+artemether combination</td>
<td>40**</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>40**</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>22**</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Artemether</td>
<td>33.4**</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>24.6**</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>17.2**</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.001 compared to control

### Table 3: Percent suppression of parasitemia and % mean parasitemia of mice

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Dose</th>
<th>% Suppression of parasitemia on the following d after treatment</th>
<th>% mean parasitemia on 4th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>56.5±5.2</td>
</tr>
<tr>
<td>2</td>
<td>Curcumin Zn complex</td>
<td>3 Days, 5 mg/d</td>
<td>17.44</td>
<td>39.65±3.81</td>
</tr>
<tr>
<td>3</td>
<td>Curcumin</td>
<td>3 Days, 5 mg/d</td>
<td>11.43</td>
<td>54.12±0.9**</td>
</tr>
<tr>
<td>4</td>
<td>Curcumin-Zn(II)+artemether combination</td>
<td>3 Days, 5 mg/d +1.5 mg i. p</td>
<td>21.31</td>
<td>5.16±0.9**</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>3 Days, 5 mg/d +1.0 mg i. p</td>
<td>11.62</td>
<td>6.58±0.8**</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>3 Days, 5 mg/d +0.5 mg i. p</td>
<td>12.59</td>
<td>17.96±2.8**</td>
</tr>
<tr>
<td>7</td>
<td>Artemether</td>
<td>1.5 mg i. p</td>
<td>14.53</td>
<td>9.05±1.5**</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>1.0 mg i. p</td>
<td>5.03</td>
<td>13.12±2.2**</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>0.5 mg i. p</td>
<td>6.97</td>
<td>25.26±2.2</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD (n = 5); *P<0.05, **P<0.001 compared to control

### CONCLUSION

Curcumin a natural polyphenolic compound possess antimalarial activity, but its use is restricted because of poor solubility, stability and bioavailability. In both mice survival and % parasitemia inhibition study, it was found that curcumin-Zn complex has superior antimalarial activity than curcumin. Administration of the combination of curcumin-Zn and artemether has increased survival rates to 100% and reduced the parasitemia more effectively in mice. Such suppressive action was superior to that of administration of the single drug at the same dose. The antimalarial potential of curcumin can be re-explored by using curcumin metallo complex with improved solubility, stability and bioavailability.
CONFLICT OF INTERESTS
Declared None

REFERENCES