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Original Article

CURCUMIN-ZN-ARTEMETHER COMBINATION THERAPY FOR *PLASMODIUM BERGHEI* INFECTED MICE

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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-artemether. Oral administration of curcumin-Zn-artemether 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. Administration of curcumin-Zn artemether combination reduced the parasitemia in mice more effectively compared to that in mice treated with a single drug.

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.

INTRODUCTION

Resistance 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 1979 and has since spreading 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), artesunate, 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 the known ACTs. Both drugs have short half-lives and no resistance is known to curcumin. Curcumin, in addition to having a direct killing effect as an antimalarial, is also able to activate the immune system against *P. berghei* [4, 5].

An important issue with curcumin is its poor bioavailability, less stability, rapid metabolism and short half-life [6]. Several attempts are under way to improve bioavailability through the use of preparations such as liposomes, phospholipid complexes, nanoparticles or microparticles [7, 8]. Complexing curcumin with transition metals is one of the useful ways to overcome the problem related to solubility, bioavailability and stability [9]. In present study curcumin-Zn complex was prepared and evaluated for antimalarial activity as single drug therapy and in combination with artemether.

MATERIALS AND METHODS

Curcumin was purchased from Phytopharma actives Pvt. Ltd., Mumbai. Giemsa stain (Himedia), tween 60, zinc sulfate and glycerol (Research lab) were purchased from local markets.

Animals

Inbred albino rats were obtained from the animal house of Pravara Medical College, Pravaranagar. The research was conducted in accordance with standard institutional guidance given by the Institutional Animal Ethics Committee (IAEC). The Labs used for the purpose was approved by Committee for the purpose of control and supervision of experiments on animals, Ministry of social justice and empowerment, Govt. Of India (Registration No.-448/01/c/CPCSEA).

Preparation of curcumin-Zn complex

Zinc sulfate ($ZnSO4{\cdot}7H2O$) was mechanically mixed in a mortar with curcumin ($Zn2+$: Curcumin 1/1 mole) until homogenous powder mixture was obtained. Then, glycerol/water (1: 1 v/v) solution was added to mixture, followed by mechanical shaking at 25 °C until pasty combination was obtained. Pasty product was dried at 50 °C and free glycerol was eliminated by washing with distilled water. A dark-colored powder complex of curcumin-Zn was obtained [10].

Characterization of curcumin and curcumin-Zn complex [11, 12]

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR spectroscopy of curcumin and curcumin-Zn complex was performed on FTIR (Jasco FT/IR-4100) spectrophotometer. About 5 mg of sample was mixed with 100 mg of Potassium bromide (KBr)

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 spectrophotometric 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 10^7 *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 incoculation 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:

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

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.

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

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

$$\%\ Parasitemia\ Suppression = \frac{A-B}{A} \times 100$$

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.

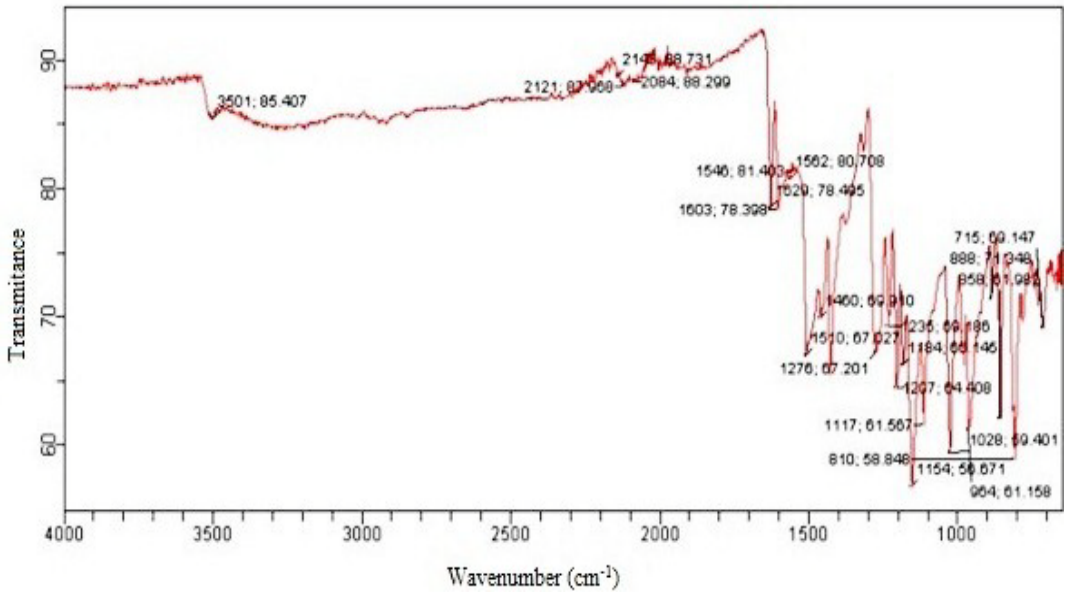

Fig. 1: FTIR spectrum of curcumin

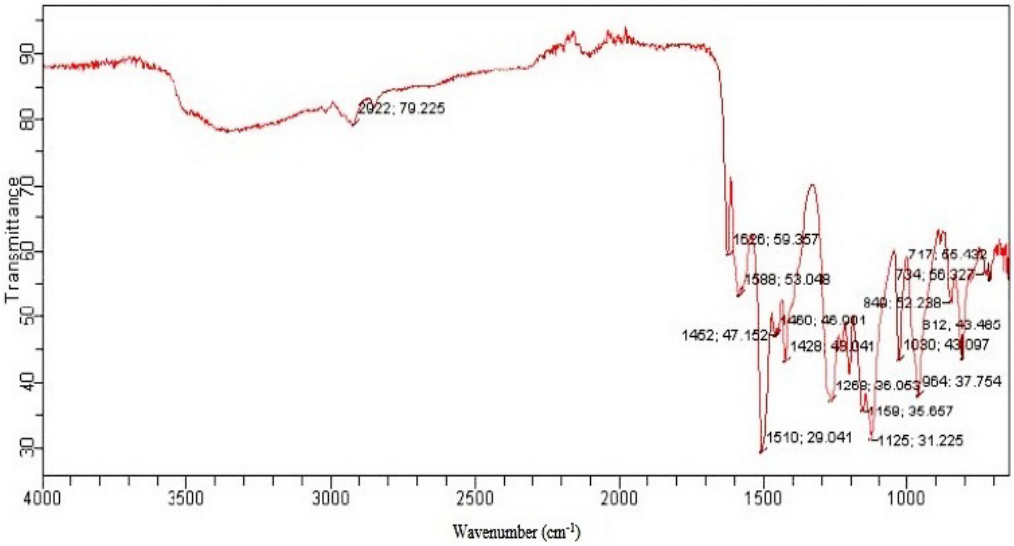

Fig. 2: FTIR spectra of curcumin-Zn complex

Table 1: Interpretation of the FTIR spectrum

Reported (Cm-1)	Observed for curcumin (cm-1)	Observed for curcumin-Zn(cm-1)	Functional group Determination
3510.2	3502	-	Phenolic OH (Streching)
1627.8	1629	1626	Ketone C=O (Streching)
1596	1603	1588	Aromatic C=C
1276	1276	1269	C-O

The 1629 and 1603 cm-1 bands correspond to the mixtures of stretching vibrations of (C=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.

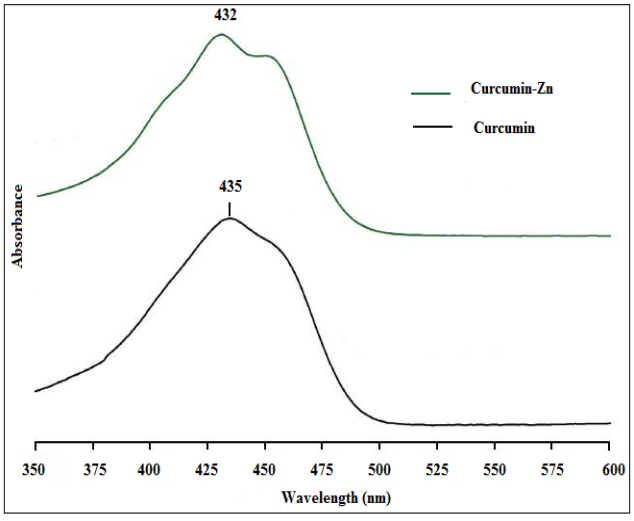

Fig. 3: UV spectral data of curcumin and curcumin-Zn complex

Their spectra was deconvoluted with an absorption band at 435 nm for curcumin, at 432 nm for curcumin-Zn complex (fig. 3). From FTIR and UV analysis of curcumin and curcumin-Zn, it was confirmed that curcumin-Zn complex was successfully prepared.

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 mean 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

Gr No.	Treatment	Dose	Mean survival time (days)
1	Control		6.1
2	Curcumin Zn complex	3 D 5 mg/d	10.2
3	Curcumin	3 d 5 mg/d	7.6
4	Curcumin-Zn+ artemether combination	3 D 5 mg/d +1.5 mg i. p.	40**
5		3 D 5 mg/d +1.0 mg i. p.	40**
6		3 D 5 mg/d +0.5 mg i. p.	22**
7	Artemther	1.5 mg i. p.	33.4*
8		1.0 mg i. p.	24.6*
9		0.5 mg i. p.	17.2*

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

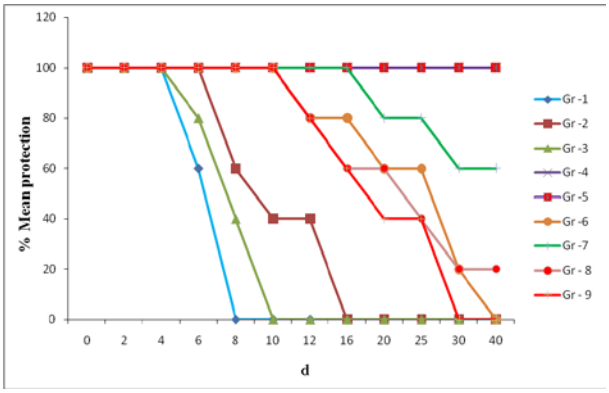

Fig. 4: Percent mean protection in mice receiving different treatments over time (40 d post treatment), * (n=5)

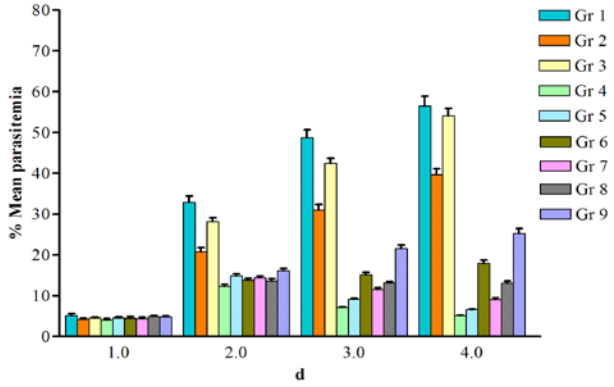

Fig. 5: Percent mean parasitemia in mice receiving different treatments

Table 3: Percent suppresion of parasitemia and % mean parasitemia of mice

Group no.	Treatment	Dose	% Suppression of parasitemia on the following d after treatment				% mean parasitemia on 4th day
			1	2	3	4	
1	Control		-	-	-	-	56.51±5.2
2	Curcumin Zn complex	3Days,5 mg/d	17.44	36.68	35.17	30.9	39.65±3.81*
3	Curcumin	3Days,5 mg/d	11.43	14.28	11.03	4.22	54.12±0.9*
4	Curcumin-Zn(II)+artemether combination	3Days,5 mg/d +1.5 mg i. p	21.31	62.55	85.73	90.86	5.16±0.9**
5		3Days,5 mg/d +1.0 mg i. p	11.62	54.83	80.9	88.35	6.58±0.8**
6		3Days,5 mg/d +0.5 mg i. p	12.59	58.02	68.23	68.21	17.96±2.8**
7	Artemther	1.5 mg i. p	14.53	56.23	75.79	83.98	9.05±1.5**
8		1.0 mg i. p	5.03	58.35	72.48	76.78	13.12±2.2**
9		0.5 mg i. p	6.97	51.06	54.98	55.29	25.26±2.2

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

1. M Geyer. editor. Antimalarial drug combination therapy-Report of WHO technical consultation. Geneva: WHO; 2001.
2. N Valecha, KD Tripathi. Artemisinin: current status in malaria. Indian J Pharmcol 1997;29:71-5.
3. A Shahiduzzaman, H Daugschies. Curcumin: a natural herb extract with antiparasitic properties. In: Heinz Mehlhorn. editor. Nature Helps. Berlin, Heidelberg: Springer; 2011. p. 141-52.
4. G Padmanaban, VA Nagaraj, PN Rangarajan. Artemisinin-based combination with curcumin adds a new dimension to malaria therapy. Curr Sci 2012;102:704-11.
5. NM Patrice, D Taramelli, L Vivas. The plant-based immunomodulator curcumin as a potential candidate for the development of an adjunctive therapy for cerebral malaria. Malar J 2011;10(1):1-9.
6. YJ Wang, MH Pan, AL Cheng. Stability of curcumin in buffer solutions and characterization of its degradation products. J Pharm Biomed Anal 1997;15:1867–76.
7. RB Laware, BS Kuchekar. Formulation, optimization and evaluation of microparticles of curcumin-Zn complex. Int J Pharm Biol Sci 2015:6:567-78.
8. BM Patrick, C Régis, P Véronique. An oral malaria therapy: Curcumin-loaded lipid-based drug delivery systems combined with β-arteether. J Controlled Release 2013;172:904–13.
9. Rahaman, MA Rahman, MR Awal. Synthesis and characterization of metal complexes containing curcumin (C21H20O6) and study of their anti-microbial activities and DNA binding properties. J Sci Res 2014;6:97-109.
10. B Zebib, Z Mouloungui, V Noirot. Stabilization of curcumin by complexation with divalent cations in glycerol/water system. Bioinorg Chem Appl 2010;1:1-8.
11. VA Joshi, MG Ahmed, S Suresh, R Kowti. Comparative study: Solution stability and dissolution behavior of solid dispersions curcumin. Indian J Novel Drug Delivery 2010;2(3):88-95.
12. PS Devendra, C Jayanthi, K Joshi, GB Hanumanthachar. Enhancement of aqueous solubility of curcumin by solid dispersion technology. World J Pharm Pharm Sci 2013;2:4109-18.
13. PG Vathsala, C Dende, VA Nagaraj, D Bhattacharya, G Das, PN Rangarajan, *et al*. Curcumin-arteether combination therapy of *Plasmodium berghei*-infected mice prevents recrudescence through immunomodulation. PLoS One 2012;7:1-10.
14. DN Nandakumar, VA Nagaraj, PG Vathsala, PN Rangarajan, G Padmanaban. Curcumin-artemisinin combination therapy for malaria. Antimicrob Agents Chemother 2006;50(5):1859-60.
15. http://www.cdc.gov [Last accessed on 25 Mar 2015].