ORIGINAL ARTICLE

Zingiber Officinale Roscoe and Echinops Kebericho Mesfin Showed Antiplasmodial Activities against Plasmodium Berghei in a Dose-dependent Manner in Ethiopia

Abdissa Biruksew1*, Ahmed Zeyunud1, Yonas Alemu1, Lemu Golassa5, Moti Yohannes2, Asfaw Debella3, Geme Urge6, Bart De Spiegeleer7, Sultan Suleman4

OPEN ACCESS

Citation: Abdissa Biruksew, Ahmed Zeyunud, Yonas Alemu, et al. Zingiber Officinale Roscoe and Echinops Kebericho Mesfin Showed Antiplasmodial Activities against Plasmodium Berghei in a Dose-dependent Manner in Ethiopia. Ethiop J Health Sci. 2018;28(5):655. doi:http://dx.doi.org/10.4314/ejhs.v28i5.17

ABSTRACT

BACKGROUND: The emergence and spread of Plasmodium falciparum resistance to antimalarial drugs necessitated the search for new drugs from natural products. Zingiber officinal Roscoe and Echinops Kebericho Mesfin are traditional herbal medicines widely used for the treatment of malaria in Ethiopia. The aim of the study was to assess the toxicity profile and in vivo antiplasmodial activities of 70% methanol crude extracts of both plant materials against Plasmodium berghei.

METHODS: Healthy male Swiss Albino mice of age 4-5 weeks and weight 25-36 g were infected by P. berghei. The extracts were administered orally at doses 5000, 2500 and 1250 mg/kg for acute toxicity of E. kebericho Mesfin. Graded doses at 1000, 500 and 250 mg/kg used for four days suppressive studies. Parasitemia, body weight, packed cell volume (PCV) and survival times. Statistical significance was determined by one-way ANOVA. Independent t-test was used to compare results. Results were presented as a mean ± standard error of the mean (M ± SEM). All data were analyzed at a 95% confidence interval (α= 0.05).

RESULTS: At the dose of 5000 mg/kg, E. kebericho Mesfin showed no toxic effects. The LD50 of extract could go beyond the dose used. In vivo antiplasmodial activity of extracts showed excellent chemo suppression at 500 and 1000 mg/kg in a dose dependent manner compared with the negative control. The chemo suppressions of the 1000 mg/kg of both plant extracts were 49.53 ± 1.90% and 32.83 ± 1.03%, respectively. The survival times of P. berghei infected mice were also a dose dependent manner while failed to prevent weight loss.

CONCLUSION: The extracts of both medicinal plants showed antiplasmodial activities against P. berghei. It confirmed the literature findings and their traditional uses.

KEYWORDS: Echinops kebericho Mesfin, Zingibir officinale Roscoe, Plasmodium berghei, oral acute toxicity, antiplasmodial
INTRODUCTION

Malaria is a global public health threat infecting an estimated 212 million and causing 429,000 deaths in 2015. However, the current prevention and control measures have led to a 29% reduction in malaria mortality rates globally. Sub-Saharan Africa still carries a huge share of malaria burden. Thus, the continent has been home to 90% of malaria morbidity and 92% of mortality rates (1). In response to the emergence and spread of Plasmodium falciparum resistance to chloroquine (CQ) and sulfadoxine-pyrimethamine (SP), the World Health Organization (WHO) has recommended the use of artemisinin-based combination therapy (ACT). ACTs have been used in reducing malaria burden and play an important role in maintaining the current success of control programs (2). Nowadays, the increase in global travel and population movement augments resistant parasite spread to any part of the world (3-5) leading to treatment failures and decreased efficacies. Emergence and spread of P. falciparum resistance to ACTs in South-eastern Asia threaten global malaria efforts. The declining efficacy of the classical antimalarial medicines, resistance of vectors to insecticides and failure of developing effective vaccines have led to an urgent need for searching new and efficient antimalarial candidates from natural products (6,7).

Studies uncovered that modern pharmacopeia still contains at least 25% drugs derived from plants and many others which are synthetic analogs built on prototype compounds isolated from plants (8,9). About 90% of the Ethiopian population rely on traditional medicines including herbal products for their primary health care needs (10). Moreover, medicinal plants have played a profound role for the treatment of human malaria due to the presence of antimalarial compounds in several plant species (11). Quinine, for instance, was extracted from Cinchona bark, while CQ and primaquine were synthesized as new antimalarial agents, artemisinin, a potent partner drug in ACTs, was discovered from the Chinese medicinal plant by the Noble prize winner Chinese Professor, Tu Youyou (12).

Artemisia annua, the only available effective antimalarial drug against resistant strains of Plasmodium parasites (13). It attracted the attention of many researchers and stakeholders to look for new antimalarial remedies from natural products.

In Ethiopia, there are a number of medicinal plants traditionally used for the treatment of malaria and/or fever. Among which Zingiber officinale Roscoe and the indigenous plant, Echinops kebericho Mesfin are the two most common ones (14, 15).

Echinops is one of the genera classified under the family Asteraceae. E. kebericho Mesfin (Qabarichoo in Afan Oromoo) is endemic to Ethiopia confined only to the highlands (14). Kebericho is so far known from a few localities in Ethiopia (Shawa and Gojam Provinces) at altitudes of 2300-2600 m. It is an erect perennial herb or shrub up to 1.2 m high, commonly from a massive root stock with leafy stems (16). It has long been traditionally used specifically by the local practitioners (healers) against a wide range of ailments. These include malaria, migraine, mental illness, heart pain, lung Tuberculosis, leprosy, kidney disease, bilharzia, syphilis and amoebic dysentery (14).

Z. officinale Roscoe is commonly called Ginger, African ginger or Black ginger. It belongs to Zingiberaceae family. Ginger is a 2-4 foot tall perennial with grass like leaves up to a foot in length. Z. officinale Roscoe is mostly distributed in Asia, Africa, India, Jamaica, Mexico, and Hawaii (17) and it has been widely used in West African countries as an antimalarial agent (18-20). In general, the two medicinal plants have wider medicinal applications; therefore, this study was designed to investigate the traditional claims of antimalarial efficacy and safety of their use within the community using in vivo antimalarial activity and acute toxicity profiles.

METHODS

Extraction of the plant material was conducted at Jimma University Laboratory of Drug Quality
The acute toxicity and antiplasmodial activity studies were conducted at Jimma University College of Agriculture and Veterinary Medicine, Experimental Research Laboratory from January to May 2012.

**Collection and preparation of plant material:** Fresh rhizomes of *Echinops kebericho* Mesfin and roots of *Z. officinale* Roscoe were collected from Gindabarat, Gonfi Kedida which is located at an attitude of 9 33' 00" and longitude 37 53' 00" (21), West Oromia 200 km from Addis Ababa, Ethiopia. The plant materials were wrapped with plastic sheets during transportation. Collection of the plants was guided by the traditional healers, and species identification of the plants was made by a botanist at Jimma University herbarium. Plant specimens were deposited with voucher numbers 2348 and 2347 for *E. kebericho* Mesfin and *Z. officinale* Roscoe, respectively, at Jimma University Herbarium.

**Extraction of plant materials:** The plant materials were air dried at room temperature under shade and grounded to a powder using mortar and pestle. A total of 100 g of each plant material was extracted using Soxhlet apparatus with 400 ml of 70% methanol. The mixtures were filtered using Whatman filter paper No.2 (Whatman®, England). Methanol was evaporated from the extracts by a rotary evaporator (Buchi Rota vapor, Switzerland) under low pressure, and the final filtrate was stored in a tightly closed bottle container at 4°C until used (22). Each fresh experimental solute was prepared by 3% Tween 80 (Sigma - Aldrich).

**The parasites:** Chloroquine sensitive P. berghei strain ANKA was obtained from Ethiopian Public Health Institute (EPHI) and maintained at the Animal House of Jimma University College of Agriculture and Veterinary Medicine (JUCAVM). The viability of parasites was maintained by serial passage at a standard inoculum of 1×10^7 of parasitized erythrocytes from donor mice to healthy ones in the experimental laboratory as described elsewhere (23, 24).

**The experimental animal:** Male Swiss albino NMRI mice 4-5 weeks of age and 25-36 g were procured from the breeding colony of School of JUCAVM, and donor mice were obtained from EPHI. They were then maintained on commercial pellet and water ad libitum and kept in 12 h dark and 12 h light in clean and fly-proof house stable one week prior to the commencement of experiment for acclimatization purpose. The health of the mice was followed by a veterinarian. The care of experimental animals was followed according to the standard set by the international guideline for animal care and use. The animals were then randomly assigned to different treatment groups of five, each in a cage.

**Oral acute toxicity test:** Twenty albino mice aged 4-5 weeks and weighing 25-36 g were randomly grouped into four groups of 5 mice; three treatment groups and one control group. The mice in the control group received 3% tween 80 (vehicle), whereas the crude extracts of *E. kebericho* Mesfin were orally administered in a vehicle using oral gavages at doses 5000, 2500 and 1250 mg/kg body weight.

Then, the mice were observed continuously for 4 h after administering the crude extract and followed for 24 h for any manifestations of toxicity. The median lethal dose (LD50) of the crude extracts was determined according to the method described earlier (25). Oral acute toxicity parameters such as body weight (measured on day 1, day 7 and day 14), hematological assay (26) and histopathological indices were determined on day 14 (27). It was reported that the toxicity of ginger had already been conducted and considered negligible with oral LD50 values in various animals exceeding 5 g/Kg(28). Besides this fact, the plant has been recognized by U.S. Food and Drug Administration generally recognized as safe food supplement (29). Because of this report, we did not conduct the toxicity profiles of *Z. officinale* Roscoe.

**In vivo antiplasmodial activity studies:** Antiplasmodial activity of each of the test extracts was performed in a 4-day suppressive standard test as described earlier (30). Forty male Swiss Albino mice 4-5 weeks and weight 25-36 g were used to evaluate the antiplasmodial activity of both plant extracts. Donor mice infected with
rodent malaria parasite, *P. berghei* (with a raised parasitemia of 20-30%) were sacrificed, and blood was collected via cardiac puncture with a sterile and disposable needle and syringe. The blood was diluted with normal saline in such a way that 0.2 ml of blood contained approximately 1-10^7 infected red blood cells (31). Each mouse was inoculated intraperitoneally with 0.2 ml of infected blood on the first day (D 0). The mice were then divided randomly into eight groups of five mice in each group. Three groups of mice were assigned to different treatment groups, and the other two groups were used as common controls (positive and negative). Three hours post infection, the treatment groups were orally administered with 1000, 500 and 250 mg/kg/day doses of each plant extract. Chloroquine tablet, (Batch Number 0123C3RJA, Addis Pharmaceuticals Factory PLC Adigrat, Ethiopia) at the dose of 25 mg/kg/day and vehicle (3% Tween 80) were administered orally to the positive and negative control groups, respectively, for four consecutive days (D 0 to D 3).

On the fifth day (D 4), drops of blood were taken from the tail of each mouse and thick, and thin smears were made on the microscopic slide. The smears were then fixed with methanol and stained with 10% Giemsa. Four fields were examined on each slide. The number of infected and uninfected red blood cells were counted and the mean was taken according to previous works (31):

\[
\text{Parasitaemia} = \frac{\text{Total iRBCs}}{\text{Total RBCs counted}} \times 100\%
\]

The percentage parasitemia and chemo-suppressions were calculated according to the standardized test methods (24,32). Therefore, the difference between the mean parasitemia value of the control group (taken as 100%), and those of the experimental groups was calculated and expressed as percent reduction (activity) using either of the following Equations 1 and 2 (31):

\[
\text{Activity} = 100 - \frac{\text{mean parasitaemia in treated}}{\text{mean parasitaemia in control}}
\]

Or

\[
\%\text{chemosuppression} = \frac{\text{Parasitaemia in Negative control} - \text{Parasitaemia in study group}}{\text{Parasitaemia in Negative control}}
\]

Thus, a plant extract is considered to have antiplasmodial activity if it shows a reduction in parasitemia ≥ 30% (33).

**Determination of packed cell volume and body weight of the mice:** The PCV was measured to predict the effectiveness of the test extracts (34) on D 4. Blood from the tail of each animal was drawn in duplicate, and measurements were done as follows:

\[
\text{Packed Cell Volume} = \frac{\text{Volume of erythrocytes in a given volume of blood}}{\text{Total blood Volume}}
\]

On D 0 and D 4, the body weight of each mouse was determined to observe whether the test extracts prevented the weight loss. The body weights of mice are commonly reduced with increasing parasitemia in infected mice (26).

**Monitoring mean survival time:** Mortality was monitored daily, and the number of days 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 using Equation 4 (35):

\[
\text{MST} = \frac{\text{Sum of survival times of all mice in a group}}{\text{Total numbers of mice in that group}} \text{days}
\]

**Data analysis:** Results were presented as a mean ± standard error of the mean (M ± SEM). SPSS Version 20 was used for the analysis of data of parasitemia, body weight, PCV, and survival times. Statistical significance was determined by one-way ANOVA. Independent t-test was used to compare results. All data were analyzed at a 95% confidence interval (α= 0.05) (26).

**RESULTS**

**Oral acute toxicity assessment of Echinops Kebericho Mesfin:** Oral acute toxicity studies revealed that the graded doses of 70% methanol extract of *E. Kebericho Mesfin* (maximum 5,000...
mg/kg) did not show any signs of toxicity in mice. The experimental maximum dose did not show any lethal effects; revealing that the LD50 could be beyond the dose used. In this in vivo acute toxicity study, there was no gross physical and behavioral changes such as rigidity, sleep, diarrhea, depression, abnormal secretion and hair erection. Moreover, there were no significant differences between the control groups and the experimental arms with regard to body weight, hematological and histopathological parameters (Tables 1 and 2) at the aforementioned doses.

Table 1: Effects of 70% methanol rhizome extract of *Echinops kebericho* Mesfin on body weight of mice in oral acute toxicity studies

| Treatment group (does in mg/kg) | Body weight(g) on three different days |  |  |  |
|-------------------------------|-----------------|---|---|---|
|                               | Day 0           | Day 7          | Day 14         | p-value |
| 5000 mg/kg                   | 33.00±1.650     | 32.67±1.43     | 35.0±1.26      | 0.490   |
| 2500 mg/kg                   | 32.00±1.00      | 35.33±1.28     | 38.33±0.61     | 0.002   |
| 1250 mg/kg                   | 28.50±0.99      | 32.83±0.98     | 37.0±0.816     | 0.000   |
| Control                      | 26.17±2.50      | 33.00±2.113    | 36.67±1.23     | 0.007   |

Key: Values are expressed as MEAN±SEM, n=6, day 0= initial day pretreatment weight: D 7=weight on day 7; D 14= weight on day 14

Table 2: The effect of 70% methanol extracts of the rhizome of *Echinops kebericho* Mesfin on Haematological parameters in oral acute toxicity studies

| Treatment group (does in mg/kg) | WBC(10^3/mm³) | RBC(10^6/mm³) | Hgb (mg/dl) | HCT % |
|-------------------------------|---------------|---------------|-------------|-------|
| 5000                          | 3.53±0.79     | 7.93±1.64     | 10.78±2.22  | 40.18±8.31 |
| 2500                          | 3.17±0.52     | 9.78±0.53     | 13.32±0.63  | 50.21±2.72 |
| 1250                          | 4.14±0.59     | 10.25±0.62    | 13.97±0.71  | 47.82±1.03 |
| Control                       | 3.17±0.15     | 8.78±0.35     | 12.88±0.45  | 49.70±0.96 |

Key: Values are expressed as Mean±SEM: n=6: TWBC=Total White Blood Cells: RBC= Red Blood Cells: Hgb=Haemoglobin: HCT=Haematocrit

**In vivo antiplasmodial Studies**: In a four day suppressive tests, the in vivo antiplasmodial studies of the methanol extracts of both plants showed significant chemo-suppressions in a dose-dependent manner as compared to the negative control (Table 4). Accordingly, the highest parasitemia suppressive effects of the rhizome extract of *E. Kebericho* Mesfin were 49.53 ± 1.90, 34.66 ± 0.76, and 22.13 ± 0.87 for 1000, 500, and 250 mg/kg/body weight; respectively (Table 4). The antiplasmodial activities of *Z. officinale* Roscoe were also 32.83 ± 1.03, 23.49 ± 1.47, 19.87 ± 0.84 for 1000, 500, and 250 mg/kg/body weight; respectively. It can easily be inferred that the first and the second highest doses of both plant extracts showed profound chemo-suppression (P <0.001) in *P. berghei* infected animals. On the other hand, 1000 mg/kg of *E. Kebericho* Mesfin rhizome extract was found to be promising antiplasmodial activity than other graded doses.
Table 4: Effects of 70% methanol rhizome extracts of *Echinops kebericho* Mesfin and *Z. officinale* Roscoe on % parasitemia, chemo-suppression and survival times of *P. berghei* infected mice

| Extracts          | Doses (mg/kg) | % Parasitemia | % Inhibition (activity) | Survival time (days) |
|-------------------|---------------|---------------|-------------------------|----------------------|
| *E. kebericho*    | 1000          | 50.18±1.90    | 49.53±1.90              | 10.31±0.67           |
|                   | 500           | 65.28±0.764   | 34.66±0.76              | 9.91±0.63            |
|                   | 250           | 77.780±0.87   | 22.13±0.87              | 6.78±0.20            |
| *Z. officinale*   | 1000          | 67.06±1.03    | 32.83±1.03              | 7.33±0.51            |
|                   | 500           | 76.31±1.49    | 23.49±1.47              | 6.38±0.24            |
|                   | 250           | 80.03±0.84    | 19.87±0.84              | 6.28±0.20            |
| 25 mg/kg CQ       |               | 5.14±0.37     | 94.79±0.37417           | 13.00±0.00           |
| Negative control  |               | 100.00±0.00   | 0.00                    | 6.18±0.20            |

Key: Values are expressed as Mean±SEM, n=5: TWN80=Tween80

Extracts of both plants failed to protect weight loss of mice infected with rodent malaria at all doses levels (p > 0.05)) and no significant difference was observed between the experimental arm and the untreated groups (Table 3).

Table 3: Effects of 70% methanol extracts of both plant materials on body weights of *P. berghei* infected mice in the antiplasmodial activity studies

| Extracts          | Doses (mg/kg) | Body weight (gm) |
|-------------------|---------------|------------------|
|                   |               | D 0     | D 14     | P- value |
| *E. kebericho*    | 1000          | 33.20±2.200  | 31.60±1.60 | 0.001   |
|                   | 500           | 31.20±0.97   | 28.40±1.50 | 0.009   |
|                   | 250           | 31.20±2.50   | 28.60±2.70 | 0.007   |
| *Z. officinale*   | 1000          | 32.20±0.970  | 26.50±1.17 | 0.00    |
|                   | 500           | 30.00±1.00   | 26.00±0.81 | 0.00    |
|                   | 250           | 27.40±2.31   | 24.60±2.23 | 0.002   |
| 25 mg/kg CQ       |               | 24.40±1.88   | 22.80±1.65 | 0.016   |
| Negative control  |               | 26.60±4.00   | 31.0±3.51   | 0.054   |

Key: Values are significant at 95% CI and p-value <0.05: TWN80=Tween 80, n=5

Assessment of the mean survival times of mice in all test groups was compared to the negative control groups (Table 4). It was also observed that mice treated with all doses of extracts lived longer than the negative control in a dose dependent manner. The study showed that 70% methanol crude extracts were able to protect reduction in PCV in a dose depended manner when compared to the control groups (Table 5).
Table 5: The effects of 70 % methanol rhizome extracts of *E. kebericho* Mesfin and *Z. officinale Roscoe* on PCV of *P. berghei* infected treated mice

| Extract doses | PCV (%) on day 4 |
|---------------|------------------|
|               | *E. kebericho* Mesfin | *Z. officinale Roscoe* |
| 1000mg/kg     | 45.96±1.08        | 33.46±1.08            |
| 500mg/kg      | 39.70±0.44        | 20.04±0.32            |
| 250mg/kg      | 33.00±0.81        | 22.10±1.18            |
| 25 mg/kg CQ   | 49.62±0.34        | 49.62±0.34            |
| Negative control | 20.98±0.70      | 20.98±0.70            |

**DISCUSSION**

Using plants as traditional remedies started from the antiquity to the present day for every day health care needs of human being including malaria (36). Accordingly, about 90% of Ethiopians, and 80% of the world population rely primarily on plant products for their healthcare for more than a century (10). Societies always believe that plant product is safe, non-toxic or have negligible health hazards. Accordingly, *E. Kebericho* Mesfin and *Z. officinale Roscoe* are among traditionally used medicinal plants against wide ranges of diseases including malaria in Ethiopia (37). *P. berghei* causes acute malaria in rodents and sensitive to CQ and non-infectious to human. It is widely used elsewhere in the world for in vivo evaluation of antimalarial agents. The oral acute toxicity evaluation of *E. Kebericho* Mesfin was conducted prior to antimalarial studies to rule out the LD50. Hence, the current finding showed that 70% methanolic rhizome extract of the plant was non-toxic at 5 g/ kg of body weight in all study parameters.

The antimalarial activities of both plant materials in a four day suppressive test showed remarkable chemo - suppression of 49.53 ± 1.90% and 32.83 ± 1.03 respectively .The finding further reflected that the corresponding longer mean survival times compared to all other doses and the negative controls in a dose dependent manner. This suggests that 1000 mg/kg of an extract of *E. Kebericho* Mesfin might be the desirable therapeutic dose in the experimental animals. Increasing the dose of *Z. officinale Roscoe* beyond 1000 mg/kg body weight could result in best chemo - suppressive effect than the currently explained findings.

The antimalarial activities of these plants suggest that inhibitory effects on parasite replication. These could be due to the presence of active compounds in the test extracts (38-40) that are evenly distributed among plant species of the family. Studies elsewhere revealed that high degree of parasitemia suppression might be explained by mechanisms of biomarker actions having an indirect effect on the immune system or by other pathways that are not yet fully understood (41) in mice.

Previously isolated chemical compounds from Echinops species include alkaloids, saponins, phytosterols, polyphenols, carotenoids, Sesquiterpenes (alcohols/ lactones), lignans, acetylenic & thiophene compounds and essential oil. Specially, Sesquiterpenes was claimed to be antimalarial agents (42) which strongly supports the current finding. In vitro studies from the family Asteraceae like *Dicoma tomentosa* (43), *Microglossa pyrifolia* (44), *Oncosiphon piluliferum*, *Artemisia gorgonum Webb*, *Anthemis auriculata Boiss* (45) reported to show antimalarial activities against *P. falciparum*. Thus, this evidence coupled with the traditional claims suggested the antimalarial biomarkers are evenly distributed among the species of the same family. The antimalarial effect of *Z. officinale Roscoe* is also in support with previous in vitro studies such as *Aframomum zambesiacum K. Schum* and *Kaempferia marginata Carey*.
reported to have antiplasmodial activities from the family Zingiberaceae (45). The current findings are strongly supported by two evidences. First, the traditional claims of both plants as antimalarial remedies. Secondly, the presence of Sesquiterpenes as an antiplasmodial agent in the crude extract of E. kebericho Mesfin and in vitro studies of both plant species of the same families.

In conclusion, at the maximum experimental dose level of 5g/kg, E. Kebericho Mesfin showed no acute toxicity; and therefore, the LD50 of the plant was found to be beyond the concentration we used. The 70% methanolic extracts of both plants showed a dose dependent suppression of parasitemia. Hence, this study indicated that both plants have promising antiplasmodial activities against P. berghei whichsupports their traditional uses. Finally, the authors recommend that further in vitro studies on P. falciparum should be conducted to validate the antiplasmodial activities of both plants for routine health care use.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Jimma University for the financial assistance. We would also like to thank the Ethiopian Public Health Institute for a donation of experimental animals, provision of standard parasites and pertinent training for the principal investigator. We would also like forward our gratitude to Professor Bart De Spiegeleer and Dr. Game Urge for commenting and editing the whole manuscript.

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