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An Investigation into the Anti-Plasmodial Effect of the Ethanol Extract of the Leaves of *Helianthus annus* in Swiss Albino Mice

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Abstract: The increasing problem of malaria parasite resistance to several of the available pharmacological agents has necessitated the search for newer effective therapies. We investigated the anti-plasmodial effect of the ethanol extract of the leaves of *Helianthus annus* in Swiss albino mice infected with chloroquine sensitive *Plasmodium berghei berghei*. Twenty (20) mice were divided into 4 treatment groups with 5 mice per group. Two treatment groups received two different doses of the ethanol extract (EE) of *H. annus* leaves (2g/kg/day and 4g/kg/day) for 3 days while the other 2 groups served as controls; the positive control received chloroquine (25mg/kg in three divided doses) while the negative control group had 0.5ml/kg distilled water. Pretreatment and post treatment blood collected by tail laceration were used to prepare thick blood films and the parasite count, percentage change in parasitaemia and percentage parasite chemosuppression were determined afor each group. The mean percentage chemo-suppression was observed to be as high as 98.1% and 98.3% in mice which received 2g/kg /day and 4g/kg/day respectively. The chloroquine treated mice showed a 98.8% chemosuppression and, there was an increment in parasitaemia of about 97.93% in the negative control. These results suggested a significant ($P<0.01$) anti-plasmodial effect that is comparable if not equivalent to chloroquine.

Key words: Anti-Plasmodial Effect · Ethanol Extract · Leaves · Helianthus annus · Mice

INTRODUCTION

Malaria is an infectious disease believed to have started before human history [1]. Major impact on human survival reportedly started about a thousand years ago coinciding with the neolithic revolution [2]. aThe causative organism is the protozoa of the family Plasmodium which is transmitted to humans by an infected female anopheles mosquito during a blood meal. Of the over forty recognized species of the plasmodium parasites, four species namely; *P. vivax, P. malariae, P. ovale; and P. falciparum* have been reported to infect humans [3]. aA few other species such as *P. berghei* and *P yeolli* have also been reported to be infective in other mammals like rodents and monkeys [1, 4]. aCulex fatigan transmitted malaria has also been reported in Sparrows [5]. aMalaria today is a pandemic acute tropical /sub-tropical infectious disease most prevalent in South East Asia, Middle East, Haiti, Dominican Republic, India, Papua New Guinea, Central America and Africa [6]. Unlike developed countries, infectious diseases have been reported to constitute the major cause of morbidity and mortality in poor developing regions of the world [7]. Of these, malaria for a long time has been recognized as among the world's most devastating human infection with 300 to 500 million clinical cases and nearly 3 million deaths each year [8, 9].

Most of the mortalities from this disease have been attributed to infections with *Plasmodium falciparum* which have been reported to pose the greatest risk to non immune individuals and children less than 5 years of age [3].

In many parts of the world malaria like other infectious diseases is still being treated by both orthodox and traditional medicines [10]. The commonly reported tools of traditional medical practice include herbs, animal

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products, minerals, rituals and incantations; of these herbs are the most popular [11].

Plants have been the source of many important compounds with pharmacologic effects including useful anti-malaria drugs like quinine and Artemisinine [12]. a*Helianthus annus* has been used for medicinal purposes. A tea made from the leaves is astringent, diuretic and expectorant and used in the treatment of fevers [13]. The tincture of *Helianthus annus* has been used in Russia and in the Caucasus, the inhabitants are known to employ leaves of this sunflower in the treatment of malaria. aA tincture prepared from the seed with rectified spirit of wine is useful for intermittent fevers and ague instead of quinine. aIt has been employed in Turkey and Persia, whereas quinine and arsenic have failed, being free from any of the inconveniences which often arise from giving large quantities of the other drugs [1]. The median lethal dose of the ethanol extract of the leaf of H. annus had been previously reported as 14g/Kg in Wistar rats asuggesting that it is very safe [14].

The present scenario of increasing prevalence of multi-drug resistant strains of the parasite with an attendant rise in treatment failure by several available drugs that used to be effective. This has necessitated the need to keep looking for new sources of safer and effective drugs.

The objective of this study was to investigate the anti-plasmodia effect of *Helianthus annus* in mice infected with *P. berghei* and to compare any effect with those of chloroquine and distilled water treatment on chloroquine sensitive *P. berghei*.

MATERIALS AND METHODS

Preparation of Plant Extract: The wild variety of the plant was identified by taxonomists at the Botany Department of the Delta State University, Abraka, Nigeria.

The fresh leaves (2kg) of the plant were harvested, washed and air dried to a constant weight before ground pulverized. a100g of it was measured and soaked in 500ml of absolute ethanol (BDL 95%) for 24 hours. The mixture was then filtered using Watman 2.0 filter paper. The filtrate was evaporated to dryness in a Rotary evaporator at a temperature of 40°C.

Preparation of Stock Solution: The stock solution was prepared by dissolving 10g of the extract in 100ml of distilled water to give a stock concentration of 0.10g/ml.

Preparation of Blood Film: Blood was collected from the animals by snipping of the distal end of the tail with a pair of scissors after cleaning with methylated spirit swab. Three drops of blood were allowed to drop on a slide and a thick film was made. This was air dried and the blood smear was stained with 10% Giemsa solution for 5 minutes,

Parasite Density: WBCs and *Plasmodium* trophozoites were counted in 50 high power fields in relation to the predetermined average total number of white blood cells in rats (8000/µL). Level of parasitaemia was determined by the formulae below [15]:

No. of parasites/No. of WBC X 8000 = No. of parasites/µl

Animals: Twenty male Swiss albino mice (25-32g) were procured from the breeding colony of the College of Health Science of the Delta State University, Abraka.

The animals were housed in mosquito screened cages and acclimatized for a period of 10 days. They were maintained on a standard rat diet (Pfizer) and water *ad libitum* with 12hours light/dark exposure cycles.

Parasite Inoculation: Chloroquine sensitive *Plasmodium berghei* strains were obtained from the Institute of Medical Research in Advance Technology (IMRAT), Ibadan, Oyo state, Nigeria and were maintained in mice. Each experimental and control mouse was inoculated intraperitoneally with 0.2ml of infected blood containing about 1.0×10^7 *P. berghei* parasitized red blood cells obtained from a donor mouse having about 65% parasitaemia [16].

Drug Administration: The drug chloroquine, used in this study was administered intramuscularly while the extract and distilled water were administered orally with the aid of an intravenous medicut/mediflon cannula used as an improvised oral cannula [17].

Evaluation of Blood Schizonticidal Activity in Established Infection: A modified method similar to those by Ryley and Peters [18] was used. a Seventy-two hours post parasite inoculation, blood obtained from mice in each group through tail laceration was used to prepare pre-treatment thick blood films.

Sequel to which two different doses (2 and 4g/kg/day for 3days) of the Helianthus annus leaf extract were respectively administered orally to the extract treated

groups while chloroquine (25mg/kg in three divided daily doses of 10, 10 and 5mg/kg/day) was given to the positive control group as a standard anti-malaria drug. The other control group had distilled water (0.5ml/kg/day for 3 days). After which the mice tails were lacerated again to obtain blood used to prepare their post-treatment thick blood films.

The average percentage suppression of parasite was calculated in comparison to control using the method described by Knight and Peters [19].

$$\frac{\text{Av. parasiteamia in control - Av. Parasiteamia in treated groups}}{\text{Av. Parasiteamia in control}}$$

Statistical Analysis: The results were expressed as Mean ± SEM alevel off parasitaemia and percentage parasite chemosuppression. The data were statistically analyzed using students t-test and single ANOVA values of $P<0.05$ were considered significant.

RESULTS

Pre-treatment mean level of parasitaemia (/ul) decreased from 761.75± 123, 1,014.5± 376.55 and 1,624.75 ± 284.00 to 633.0 ± 126.65, 574.75 ± 168.70, 413.75 ± 126 in the 2g/kg EE, 4g/kg EE and Chloroquine treated mice, respectively. aPost-treatment parasitaemia in control mice was 33,484 ± 4004.06 which was higher compared to the pre-treatment level of 691.5± 129.89 (Table 1).

The percentage change in parasite levels were 16.9, 43.35 and 74.5 reduction in the 2g/kg EE, 4g/kg EE and chloroquine treated groups, respectively but 97.93 increase in t he control treated with distilled water (Figure 1).

The percentage chemo-suppression was 98.1, 98.3 and 98.8 for the 2g/kg EE, 4g/kg EE and chloroquine treated groups, respectively (Figure 1).

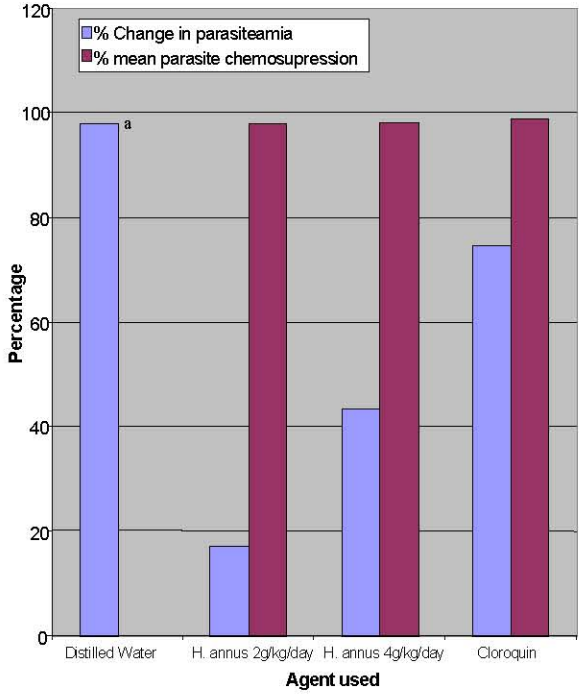
Table 1: Mean Levels of Plasmodial Parasitaemia in Ethanol extract of H. annus Leaves, Chloroquine Treated and Control Mice

Parasite Level		
Treatment Received	Pre-Treatment	Post-Treatment
Control (5ml/kg distilledH_2O)	691.5±129.89	[a]33484.50±4004.06
H.annus Leaf Extract (2g/kg)	761.75±123.64	[b]633.0±126.65
H. annus Leaf Extract (4g/kg)	1014.50±376.55	[b]574.75±168.70
Chloroquine (25mg/kg)	1624.75± 284	[b]413.75± 126.

Mean ± SEM (n = 4).

Pre-treatment of extract of vs Post-treatment (t-Test) [a]$P < 0.01$ significant

Post-treatment vs Control Post-treatment (t-Test) [b]$P < 0.05$ significant



a - Percentage change in parasitaemia increased in control as against decreasing in EE and Chloroquine treated

DISCUSSION

This study revealed the presence of anti-plasmodia activity in the crude ethanol extract of *Helianthus annus* leaf extract in mice infected with *Plasmodium berghei*. aThe mean percentage chemo-suppression was about 98.1, 98.3 and 98.8 for 2g/kg/day, 4g/kg/day extract and chloroquine treated groups, respectively (Figure 1). aHowever, with respect to change in parasitaemia between the pre-treatment and post-treatment of each group, it was observed that while the 2g/kg/day, 4g/kg/day extract and chloroquine treatment resulted in 16.90%, 43.35% and 74.5% decrease, respectively, the control group that received distilled water showed 97.93% increase in parasitaemia. The result obtained in table 1 showed that the post treatment parasitaemia reduced drastically in the extract treated groups (2 g/kg/day and 4g/kg/day), when compared to the negative control ($P<0.01$). The effect showed no significant difference from the chloroquine treated group. aThe extract and chloroquine treated group also showed no significant change between their pre and post-treatment count. Meanwhile for the control group a high significant change in parasitaemia in post treatment count when compared with the pre-treatment count of the same population ($P<0.01$) was observed, suggesting a high level of parasite

chemo-suppression by the extract and chloroquine treatment. It is also important to note that there were no significant difference between pre-treatment count of all the groups, indicating that whatever difference was observed between the post - treatment mean parasite counts of extracts, chloroquine treated and control mice were not chance findings ($P<0.05$). aThe botanical family of *H annus*; *Asterecea* have been previously reported to be endowed with many photochemical constituents with potentials to serve as sources of lead compounds for new drugs.

The presence of saponins, tannins, glycosides and carbohydrate in the extract had been previously reported [14]. Some of these components have also been reported to have anti-plasmodia activities suggesting that the activity observed in this study could ahave been due to aa single, additive or synergistic action of these compounds [20]. The continued need to discover newer and effective anti-malaria drugs or lead compounds in the face of increasing prevalence of multi-drug resistance of the parasite would justify an investigation of any herbal medicine useful in treatment of malaria as such may reveal an active compound that can be exploited to improve treatment and prophylaxis against the present malaria pandemic.

In conclusion, this study showed that the ethanol extract of the leaves of *Helianthus annus* have anti-plasmodia effect that is comparable to that of chloroquine against chloroquine sensitive *Plasmodium berghei* strains. Although this study was somehow limited in scope in that the extract was not tested on resistant strains of the parasite which is at the frontline of current anti - malaria researches it however lends validity to the use of the leaves of *H. annus* in the treatment of malaria as long as it is ain chloroquine sensitive areas.

Recommendation: This plant has shown a promising anti - malaria activity and to further enhance its worth both in orthodox medicine and otherwise it will be necessary to look into the following areas.

- Isolation, identification and characterization of the active anti-malaria principle in this plant.
- Effort should be made to demonstrate the effect of this plant on Chloroquine resistant strains of plasmodium parasite.
- Characterization of the plants pharmacological parameters (pharmacokinetic parameters) to further enhance its use.

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