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IN-VIVO ANTI-PLASMODIAL ACTIVITY OF *HAGENIA ABYSSINICA* [FAMILY: ROSACEAE] IN *PLASMODIUM BERGHEI* INFECTED IN MICE

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ABSTRACT: Malaria is a significant public health problem in the world, and the rising problem of drug resistance and the availability of a limited number of effective anti-malarial drugs underline how essential it is to find new anti-malarial agents. This study was undertaken to evaluate the *in-vivo* antimalarial activities of an extract of *Hagenia abyssinica* that is used traditionally to treat malaria in Ethiopia. The rodent malaria *Plasmodium berghei* was used to infect healthy 7-week-old Swiss albino mice weighing 24-28 gm. Each of the extracts (100 mg/kg, 200 mg/kg, and 400 mg/kg) was administered to different groups of mice. The parameters of parasitemia, survival time, body weight, and packed cell volume were measured using repository test and Peter's test. The extract significantly inhibited parasitemia and increased survival time in infected mice in a dose-dependent manner. The extract prevented loss of weight, packed cell volume. This study concluded that the extract has a promising anti-malarial activity which supports the *in-vitro* anti-plasmodial activity and the traditional use of the plant for treatment of malarial infection.

INTRODUCTION: Malaria is the most prevalent and lethal protozoan disease in the world infecting 216 million and killing 445,000 persons in 2017, mostly pregnant women and children under five. Most of these cases (88%) and deaths (90%) occurred in Sub-Saharan African countries. Most deaths and drug-resistant infections occur due to infection with *Plasmodium falciparum*, which is the most virulent *Plasmodium species* [1].

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Even though malaria disease is preventable and curable, it is still one of the global public health issues especially Africa. Greater than 75% of the country is malarious with approximately 68% of the population living in areas at risk of malaria, making malaria the major public health issues in Ethiopia [1, 2].

Resistance to anti-malarial drugs has been developed for all anti-malarials agents, and it is a major threat to the global effort for the control of malaria. The inappropriate, ineffective, and inefficient use of anti-malarials drugs increases drugs resistance. There is broad consensus on the urgent need for new drugs developed to treat malaria, with broad therapeutic potential, good

pharmacokinetic character, acceptable safety and novel mode of action, to widen the range of treatment and to prevent the development of anti-malarial drug resistance [2, 3]. One approach to this is the investigation of medicinal plants and natural products. The identification of bioactive compounds from medicinal plants is a highly promising potential approach for isolating new, safe and effective lead compounds [3]. In History, the most of the anti-malaria drugs have been isolated from plants or structures modification on plant lead compounds. More than 50% of all drugs in clinical use today contain certain substances of natural origin; most of these products are from plant sources [4]. Previous findings of anti-malarial agents such as quinine isolated from cinchona bark extract and artemisinin found from *Artemisia annua* encouraged the possibility of finding new anti-malarial drugs from plant sources [4, 5].

Ethiopia, which has different kinds of medicinal plants has accumulated over generations a great number of well-known remedies *Hagenia abyssinica* (Family: Rosaceae), is one of them. *H. abyssinica* (Bruceae) J. F. Gmel is a deciduous tree with distinct male and female trees, both of which are endowed with colorful flowers. Ethnobotanical studies revealed that *H. abyssinica* has a wide range of therapeutic use since ancient times against malaria, fever, intestinal worms (tapeworm), stomachache, diarrhea, healing of the wound, typhoid and livestock disease [6]. Several bioactive metabolites characterize the chemical composition of the *Hagenia abyssinica*. As active principles in this species, the phloroglucinol derivatives Including kosotoxin, protokosin, koisdine and kosin isolated. It also has volatile oil, acrid resin, tannic acid, quercetin 3-O-β-glucoside, rutin and quercetin glucuronide [7, 8]. The phenolic compound, Kosin which is isolated from the flowers have comparable taenicides potency as the marketed drugs, niclosamide.

Hagenia abyssinica has a spasmolytic activity which relieves nonspecific abdominal complaints which could arise from the high secretion of acetylcholine and histamine in the gastrointestinal tract. *Hagenia abyssinica* has cytotoxic activity against colonic adenocarcinomas with varying growth characteristics and morphology. The hexane extracts of leaves of *H. abyssinica* were the most active extracts with the highest zones of inhibition against *S. aureus*, MRSa and *P. aeruginosa* [8, 9]. From 1817 to1954, *Hagenia abyssinica* was listed in the pharmacopeias of 29 countries. To our knowledge, the stem bark extract of *H. abyssinica* also found to have significant *in-vitro* anti-malaria activity against *Plasmodium falciparum* [10]. In the endemic area including Ethiopia where malaria prevails, traditional herbal medicines are often used for antipyretic and anti-malarial therapy. But, there is little scientific information is available to the safety and efficacy of these herbal remedies. Thus, based on ethnobotanical and *in-vitro* studies mentioned above the present study evaluated the *in-vivo* anti-malarial activities of *H. abyssinica* stem bark extracts of against *Plasmodium berghei* in mice [11, 12].

MATERIALS AND METHODS:

Collection and Preparation of the Plant Materials: The plant material (stem bark) was collected during dry season from Wegera district 780 km North-west of Addis Ababa, Central Ethiopia and identified by in the National Herbarium, Department of Biology, Addis Ababa University, where a specimen with voucher number (HA 01) was deposited. The stem was cleaned peeled and air dried under open shade at room temperature. Then the dried materials were ground to a coarse powder using mortar and pestle.

Extraction of Plant Materials: The crude extracts were prepared by cold maceration technique. The coarse powder (50 g) was macerated in one liter 80% methanol for 72 h on an orbital shaker (VWR, USA) at 120 rotations per min to facilitate the extraction process. The filtrate was separated from the mark using Whatman filter paper 18 cm diameter and 0.1 µm pore diameter, and the mark was re-macerated two times. The filtrates were dried in an oven at the temperature not exceeding 40 ℃ then further drying process was carried out in desiccators for removal of water. Finally, the dried extract was stored with the vial in a refrigerator at 4 °C until the beginning of the actual experiment [13, 14].

Experimental Animals: Swiss albino mice are weighing 24-30 gm and aged 7 weeks of both sexes were inbred at the Animal House of from University of Gondar animal house, transported

within a cage and acclimatized in the laboratory for one week before the study conducted. The mice were kept at room temperature and exposed to a 12h light/ dark cycle, allowed free access to food (pellets) and water in plastic cages with a metal cover for free passage of air. This study was undertaken in line with the internationally accepted laboratory animal use and care guideline [15].

Rodent Parasites (*Plasmodium berghei*): Chloroquine (CQ) sensitive *P. berghei*, ANKA strain was used in this study. The parasites were obtained from Aklilu Lemma Institute of Pathobiology, by inoculation of uninfected mice with blood from *P. berghei* infected mice. The parasites were maintained by successive inoculations of mice every five days with 0.2 ml of the infected blood.

Drugs and Reagents: Chloroquine (Addis Pharmaceuticals Factory, Ethiopia), trisodium citrate (Deluxe Scientific Surgico, India), ketamine (Deluxe Scientific Surgico, India), methanol (Okhla Industrial, India), Giemsa (Science lab, USA) and normal saline (Addis Pharmaceuticals Factory, Ethiopia) were used. All reagents were analytically graded and procured from legal pharmaceutical suppliers.

Acute Toxicity Testing: A preliminary toxicity study was performed to determine the safe dose range that could be used for the studies rather than to give full toxicity data on the plant. Acute toxicity testing was done using OECD-425 guidelines. A single limit dose of 2000 mg/kg body weight of the extract was administered to the first mouse with the oral route by gavage, and then other four mice (24-28 gm each) were sequentially treated based on the result of the first mouse. Before administration of the extract, the mice were inhibited from free access to food for 3 h. Similarly, food was withheld for 1 h after the extract administration. Following administration of the extract, the mice were observed for toxicities like diarrhea, hair erection, tremors, convulsions, salivation, weight loss, lethargy, and paralysis periodically for the first four hours during the 24 h period and later followed for 14 days to assess any death [16].

Phytochemical Screening of the Crude Extract: Phytochemical screening consists of performing simple chemical tests used to analyze the presence of particular phytochemical using standard procedures according to [17, 18].

Detection of Alkaloids (Mayer's Test): 1.36 gm of mercuric chloride and 5 gm of potassium iodide were dissolved in 60 ml and 10 ml of distilled water respectively. These two solvents were mixed and diluted to 100 ml using distilled water. To 1 ml of acidic aqueous solution of samples, a few drops of reagent were added. Formation of white or pale precipitate indicates the presence of alkaloids.

Detection of Saponins (Foam Test): A drop of sodium bicarbonate was added in a test tube containing 50 ml of an aqueous extract of the plant. The mixture was shaken and kept for 3 min. A honeycomb like froth formation shows the presence of saponins.

Detection of Tannins (Lead Acetate Test): 5 gm of the extract was stirred in 10 ml of distilled water, filtered and a few drops of 1% solution of lead acetate were added to the filtrate. Yellow or red precipitate formation indicates the presence of tannins.

Detection of Cardiac Glycosides (Keller Killian's Test): 0.5 gm crude extract was stirred in 1 ml of glacial acetic acid that contain one drop of ferric chloride solution. This was then under layer with 1 ml of concentrated sulphuric acid. Brown ring formation at the interface shows the presence of a de-oxy sugar characteristic of cardenolides.

Detection of Resins (Precipitation Test): 30 gm extracts were further extracted with 5 ml 96% ethanol. The resultant alcoholic extract was added into 20 ml of distilled water in a beaker. Precipitate shows the presence of resins.

Detection of Phenols (Ferric Chloride Test): To 1 ml of an alcoholic solution of the crude extract, 2 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green color shows the presence of phenols.

Detection of Flavonoids (Shinoda's Test): 2 mg of the extract was dissolved in 5 ml of ethanol, and to this 10 drops of diluted HCl and the small amount of magnesium were added and the solution

was boiled for few minutes. The appearance of reddish pink or brown colour showed the presence of flavonoids.

Detection of Anthraquinone (Ninhydrin Test): 1 gm extract was boiled for two minutes with 5 ml of 0.5N KOH and 0.5 ml of 5% H_2O_2 mixture. After cooling, the suspension is filtered through the glass wool. The filtrate was treated with six drops of acetic acid, and the resulting solution was mixed with 5 ml of toluene. The upper layer is separated with a pipette and transferred to a test tube and 2 ml of 0.5N KOH was added. Infra-red color appearance in the aqueous layer indicates the presence of anthraquinone.

Detection of Terpenoid (Copper Acetate Test): 1 ml of conc. H_2SO_4 and 2 ml of chloroform were added to 1 mg of extract, and the appearance of reddish brown color indicated the presence of terpenoid.

***In-vivo* Anti-plasmodial Activity Testing:**

Infecting of Mice: Donor mouse blood which is infected with the *P. berghei* used for inoculums preparation. After anesthetizing with ketamine (120 mg/kg) by intraperitoneal route, blood was collected from a donor mouse having parasitemia of 30 to 37% by heart puncture into a test tube having anticoagulant (0.5% trisodium citrate). Then, the infected blood was diluted with normal saline (0.9% NaCl) so that the final suspension contained about 1×10^7 infected erythrocytes in every 0.2 ml of suspension. This 0.2 ml suspension was inoculated intraperitoneally to initiate infection.

Grouping and Dosing of Mice: Twenty-five infected mice of both sexes were grouped randomly into five groups of five mice each. Group, I mice were treated with distilled water (used to dissolve the crude extract, 10 ml/kg, served as negative control), group II, III and IV mice were treated with 100, 200, and 400 mg/kg of crude extract, respectively and group (V) mice were treated with the standard drug (chloroquine,10 mg/kg, served as positive control). The safety data of the plant was obtained from acute toxicity testing as the crude extract was safe at 2 g/kg body weight in mice and this was a base for selecting the doses (100, 200 and 400 mg/kg) of the extract for the current anti-malarial effect study.

Test on Early Malaria Infection (4-Day Suppressive Test): Parasite suppressive effect of the crude extract was assessed using the method mentioned by Peters [19]. Twenty-five mice were first infected intraperitoneally with the inoculum (1×10^7 *P. berghei* erythrocyte, 0.2 ml) on the first day (D0). After 3 h post-infection, the test groups were treated with 100, 200 and 400 mg/kg/day doses of the extract. The reference drug group was treated with chloroquine (10 mg/kg) and the control group received distilled water 0.2 ml/kg.

All the treatments were repeated for the next three days (D1 to D3). On the fifth day (D4) thin blood films were fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 min which is taken from the tail of each mouse. Average percent parasitemia and suppression was determined by using the following formula [20].

Percentage of parasitaemia = (Number of parasitized RBC) / (Total number of RBC count) × 100%

Average % suppression= (A-B) / A × 100%

Where, A = Mean parasitaemia of negative control group, B = Mean parsitaemia of extract treated group.

Prophylactic Anti-malarial Test (Repository Test): When the extract found effective in four-day suppressive test further evaluation the extract for its prophylactic effect undertaken. The prophylactic effect of the extract was assessed by applying the method described by Fidock *et al.* [21] Twenty-five mice of both sexes were weighed and randomly assigned into two control and three test groups Group I mice was given distilled water 0.2 ml/kg body weight. Groups II, III and IV, were given 100, 200 and 400 mg extract/kg body weight orally respectively while group V mice received 10 mg chloroquine/kg body weight orally daily for four consecutive days (D0 to D3). On the fifth day (D4), a standard inoculum (1×10^7 *P. berghei* erythrocytes, 0.2 ml) was administered intraperitoneally for each mouse. After 72 h (D7), blood smears were prepared from the tail of each mouse on a microscopic slide to determine parasitemia level.

Monitoring of Body Weight and PCV Changes: Body weight loss is one feature of rodent malaria

infections. Body weight change is a crucial indicator used to measure the efficacy of the crude extracts [21]. The body weight of each mouse was recorded before infection (day 0) and on (day 4) after treatment using sensitive digital balance. PCV measurement is another essential indicator to know the effectiveness of the crude extracts in preventing red blood cell hemolysis due to the parasite infection. The PCV of each mouse was recorded before infection (day 0) and on (day 4) after treatment. Blood was collected from the tail of each mouse with heparinized microhaematocrit capillary tubes. The tubes were sealed by crystal seal and placed in a microhematocrit centrifuge with the sealed ends outwards. The blood was centrifuged at 12,000 rpm.

PCV = (Volume of erythrocytes in a given volume of blood) / (Total blood volume) × 100%

Monitoring of Mean Survival Time (MST): MST is a crucial indicator that is used to evaluate the efficacy of antiplasmodial activity of the crude extracts. A crude extract that results in survival time greater than that of infected non-treated mice were considered as active [22]. Mortality of the study mice was recorded daily and the number of days from the time of infection with *P. berghei* until the incidence of death was recorded for each mouse in both the treatment and control groups throughout the follow up period. MST for each group was calculated using the following formula:

MST = Sum of survival time of all mice in a group daily / Total number of mice in the group

Data Analysis: Data were entered, cleaned, coded, checked and analyzed by using SPSS version 20. The one-way analysis of variance (ANOVA) followed by Tukey‟s HSD posthoc test, was used to determine the significance in the comparisons of parasitemia suppression, weight change, PCV change, and survival time among the groups. Paired t-test was also used to compare some parameter between initial and final results. The results were considered significant when P<0.05.

RESULTS:

Phyto-chemical Analysis: Preliminary phyto-chemical screening stem bark crude extract of *H. abyssinica* showed the presence of alkaloids, anthraquinones, terpenoids, phenolic compounds, tannins and flavonoids

TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF METHANOL EXTRACT OF THE STEM BARK OF *H. ABYSSINICA*

Secondary metabolite	Reagent	Test Result
Alkaloids	Mayer's test	+
Anthraquinone	Ninhydrin Test	+
Resins	Precipitation test	+
Cardiac glycosides	Legal's Test	-
Terpenoid	Copper acetate Test	+
Flavonoids	Shinoda's Test	+
Phenols	Ferric Chloride test	+
Phytosterol	Salkowski's Test	+
Saponins	Foam Test	-
Tannins	Lead acetate test	+

(+) present (-) absence

Toxicity Test: The acute toxicity study showed the crude extract did not cause mortality of mice within 24 h up to 2 g/kg. Gross behavioral and physical observation of mice also did not display signs of acute toxicity like salivation, lacrimation, hair erection, tremors, convulsions and reduction in feeding and motor activities. Based on these evidence the median lethal dose (LD_{50}) of the extract after single oral administration in five female mice was found to be greater than 2 g/kg body weight.

Effect of the 4-Day Suppressive Test: The result of the 4-day suppressive test showed that the crude extract has significant (P<0.001) average percentage of chemosuppression against *P. berghei* at all dose levels (100-400 mg/kg) when compared to negative control (distil water treated) group. The dose of the crude extract at 400 mg/kg showed the highest percentage parasite suppression activity (83.33%, P<0.001), followed by 200 (79.6%, P<0.001) and 100 (65.3%, P<0.05). Significant parasitemia reduction (P<0.05) was also observed in all test groups treated with the crude extract.

But the standard drug, chloroquine (10 mg/kg), has a parasitemia suppression of 100%. All the mean survival time of the extract treated group was significant (P<0.05) when compared to distill water treated group of mice. The mean survival time increases as the dose increases which is dose-dependent effect. The standard drug, chloroquine (10 mg/kg), showed a significant effect on percentage parasite suppression and mean survival time.

TABLE 2: EFFECT OF EXTRACT ON PARASITAEMIA IN FOUR-DAY SUPPRESSIVE TEST

Test Dose	Average % Parasitaemia	Average % Suppression	Mean Survival time ± SEM (days)
NC	24.0 ± 1.87	0.0	6.8 ± 0.37
HA100 mg/kg	8.33 ± 1.67^{a3}	65.29 ± 1.7^{a3}	10.0 ± 0.58^{a1}
HA 200 mg/kg	5.0 ± 1.87^{a3}	79.6 ± 2.2^{a3}	12.4 ± 1.17^{a3}
HA 400 mg/kg	4.0 ± 1.87^{a3}	83.33 ± 4.3^{a3}	15.2 ± 0.97^{a3}
CQ 10 mg/kg	0.0^{a3b1c1}	100 ± 0^{a3b1c1}	28 ± 0.00^{a3b1c1}

Values are expressed as percentage and mean ± SEM (n=5). a as compared to NC; b as compared to HA100 mg/kg; c as compared to HA 200 mg/kg; d as compared to HA 400 mg/kg; 1 P<0.05; 2 P<0.01; 3 P<0.001. HA, *Hagenia abysinica*; CQ, Chloroquine; NC, Negative control; SEM, Standard error of mean.

TABLE 3: EFFECT OF EXTRACT ON BODY WEIGHT AND PACKED CELL VOLUME OF *P. BERGHEI*-INFECTED MICE IN 4-DAY SUPPRESSIVE TEST

Test Dose	BW D0 ± SEM	BW D4 ± SEM	% Change	PCV D0 ± SEM	PCV D4 ± SEM
Dit. H_2O 0.2 ml	24.4 ± 0.9	23.8 ± 0.9	-2.46^{b3c3d3e3}	61.43 ± 0.75	51.33 ± 1.39
HA100 mg/kg	24.3 ± 0.7	25.5 ± 0.6	4.94	60.24 ± 1.14	51.67 ± 1.23
HA 200 mg/kg	25.2 ± 0.6	26.8 ± 1.1	6.34	52.13 ± 1.48	49.87 ± 1.51
HA 400 mg/kg	23.7 ± 0.7	25.8 ± 1.8	8.86	56.32 ± 2.03	55.75 ± 1.5
CQ 10 mg/kg	24.3 ± 0.6	26.5 ± 0.92	9.05	54.26 ± 3.43	56.01 ± 3.45
Dit. H_2O 0.2 ml	24.4 ± 0.9	23.8 ± 0.9	-2.46^{b3c3d3e3}	61.43 ± 0.75	51.33 ± 1.39

Data expressed as a percentage and meant ± SEM (n=5). A as compared to 0.2 ml distal water; b as compared to 100 mg/kg; c as compared to 200 mg/kg; and d as compared to 400 mg/kg; e as compared to CQ 1 P<0.001; 2 P<0.01. HA, *Hagenia abyssinica*; CQ, Chloroquine; PCV, Packed cell volume; D0, day 0; D4, day 4; SEM, Standard error of the mean.

As presented in **Table 3** the body weight of the mice was measured before and after the treatment day four. The comparison analysis showed that the crude extract treated groups prevented weight loss significantly (P<0.05) at all doses level when compared to distilled water treated control group. The crude extract treatment, in a dose-dependent manner, prevented from a significant decrease in PCV. The decrease in PCV was highest at 100 (P<0.001) followed by 200 (P>0.05) and 400 (P>0.05) as compared to chloroquine (10 mg/kg). But, there was no difference shown a change in PCV between the distilled water treated group and HA100 treated group.

Effect on Repository Test: The crude extract treatment, significantly reduced parasitemia level by 58.3%, 66.6%, and 78.3% for HA100, HA200, and HA400, respectively, as compared to the distilled water treated control group (P<0.001 in all treated groups). The inhibition by chloroquine (10 mg/kg) was significantly higher than the inhibition by all crude extract doses and the distilled water treated control group (P<0.001).

TABLE 4: PROPHYLACTIC EFFECT THE EXTRACT ON MICE INFECTED BY *P. BERGHEI*

Test Dose	Average % Parasitaemia	Average % suppression	Mean Survival time ± SEM(days)
NC	60.0 ± 04.0	0.0	7.67 ± 0.33
HA100 mg/kg	25.0 ± 22.0	58.3^{a1}	8.16 ± 0.17^{a1}
HA200 mg/kg	20.0 ± 5.3^{a1}	66.6^{a1}	8.67 ± 0.42^{a1}
HA400 mg/kg	13.0 ± 4.5^{a1}	78.3^{a1}	14.00 ± 0.58^{a1b1c1}
CQ 10 mg/kg	0	100^{a1b2}	20.00 ± 0.0^{a1b1c1d1}
NC	60.0 ± 04.0	0.0	7.67 ± 0.33

Data expressed as a percentage and meant ± SEM (n=5). a as compared to 0.2 ml distil water; b as compared to 100 mg/kg; c as compared to 200 mg/kg; and d as compared to 400 mg/kg; 1 P<0.001; 2 P<0.01. HA, *Hagenia abyssinica*; CQ, Chloroquine; PCV, Packed cell volume; D0 day 0; D4, day 4; SEM, Standard error of the mean.

DISCUSSION: The emergence of multi-drug resistant malaria-causing parasite and the absence of safe and effective malaria vaccine attracted scientists' attention to search a new anti-malarial agent. It is interesting that candidate agents from medicinal plants utilized in traditional medicine for the management of malaria to be evaluated. The therapeutic effects of plants occur due to the presence of bioactive agents [21]. The *in-vivo* anti-plasmodial effect of the extract displayed that the stem bark of *H. abyssinica* has a bioactive agent that have anti-malaria effect [5, 6, 27, 28].

Phytochemical screening of the extract showed the presence of triterpenes, tannins, alkaloids, terpenoids, flavonoids, anthraquinones, glycosides

and phenols **Table 1**. Alkaloids which are found in this extract are known bioactive agent for their anti-malarial effect in other plants including cinchona bark (*Cinchona officinalis*) from which the most effective anti-malarial agent quinine was discovered [21, 22]. The crude extract also contains phenols which are well known for its anti-oxidant and free radical scavenging activity that can prevent oxidative damage induced by the parasite. Anthraquinones & flavonoids were identified as active anti-malarial agent and the later classes of agents supposed to act by blocking fatty acid synthesis of the parasite [23]. Also, to these activities, the crude extract may also have another crucial effect on the hosts, such as acting as anti-inflammatory, analgesics, anti-oxidant, and antipyretics or as immune stimulatory activity. The crude extract may act either of the above singly or synergistically by different mechanisms suggested or via another unknown mechanism [18, 23].

Hydroalcoholic (80% methanol) solvent was used in the extraction process because it extracts polar and moderately polar bioactive constitutes (s) [24]. The stem bark was prepared as a decoction to in a similar way of traditional preparation by traditional practitioners. Young mice were also used to prevent the effect of anemia in old mice and some physiological changes due to aging which may affect the treatment outcome. The *in-vivo* model was used since it is suitable to know the pro-drug effect and the effect of the immune system in the removal of the parasite. A rodent malaria model was used in predicting the effect due to their similar disease features to those of plasmodial infection in a human, when infected with *P. berghei* and also several anti-malarial drugs such as quinine mefloquine, halofantrine, and artemisinin derivative were found using this model [25]. The 4-day suppressive method is a standard test which commonly utilized to evaluate the anti-malarial effect of the candidate agent on early infections, and repository test which is frequently used to evaluate the prophylaxis effect of the candidate agent. In both evaluations method determination of percentage parasitemia suppression is the most reliable parameter in the anti-malarial drug discovery [22, 26].

The results showed that percentage of parasite suppression in the repository test and 4-day suppressive test significantly reduced in extract-treated groups, when compared to mice in the negative control group with 83%, and 78.3% respectively. This shows that the candidate agent has the anti-plasmodial effect. An *in-vivo* anti-malarial impact of a crude extract categorized as moderate, good, and very good, if the crude extract displayed percentage parasitemia suppression equal to or greater than 50% at a dose of 500, 250, and 100 mg/kg b.w per day, respectively [27]. Based on this category, the extract has an excellent anti-malarial effect.

PCV was measured to assess the effectiveness of the candidate agent in preventing hemolysis because *P. berghei* infected mice suffer from anemia due to the destruction of infected erythrocytes, dyserythropoiesis, and erythropoietic suppression. In this study, the extracts prevented reduction of PCV in extract administered as compared with that in the negative control mice. This shows the extract prevented anemic conditions that were manifested negative control mice due to severe infection. This may be due to decreased parasitemia level in the in mice treated with the extracts in a dose-dependent manner. In the untreated mice, the parasitemia level increased and resulting in more red blood cells destruction and marked decrease of hematocrit PCV [28].

The life-prolonging potential of the extract in malaria-infected mice could be seen from its prolongation of the mean survival time. According to Peters the mean survival time of mice is considered as evidence for the anti-malarial activity of the plant extract. A plant extract that results in survival time greater than that of infected non-treated mice were considered as bioactive. In this study, there was lower parasitemia level in the extract administered group and longer survival time of the mice at the highest doses of the extract indicating strongly associated with a dose-dependent pharmacological activity in other words the extract suppressed the growth of *P. berghei*, and this was in agreement with another *in-vivo* anti-malarial test [29].

These findings were further supported by measuring Body weight change in mice which is an important parameter that measures the antiplasmodial activity of plant extracts. Because

loss of body weight is one of the manifestations of *P. berghei*-infected mice, possibly due to the appetite suppressant and the disordered metabolic activity effect of the parasite [30]. All Mice treated with the extract showed an increased in body weight gain when compared to the negative control group, hence this might indicate that the anti-malarial effect of the extract. The present study on body weight is in agreement with that of Dicasso [31].

CONCLUSION: This study has shown the efficacy of the stem bark extract of *H. abyssinica* supporting its traditional usage to malaria. The results also indicated that the extract has wide safety of margin making it a potential source for isolation of safer and cost-effective alternative drug with a new mode of action in the prophylaxis and treatment of malaria. Therefore, further studies on the extract could be carried out to isolate and characterize the active principle from this plant.

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COMPETING INTEREST: The authors declare that they have no competing interests

ETHICS APPROVAL: The Ethical clearance to experiment on mice was obtained from the Institutional Ethics Review Board of College of health science, the University of Gondar with registration number UOG/18/363/07. The mice were handled by national guidelines for handling laboratory animals.

REFERENCES:

1. WHO: World malaria fact sheet. Geneva: World Health Organization 2016.
2. Thiengsusuk A, Chaijaroenkul W and Na-Bangchang K: Anti-malarial activities of medicinal plants and herbal formulations used in Thai traditional medicine. Parasitology Research 2013; 112(4): 1475-81.
3. Silva JR, Ramos AD, Machado M, de Moura DF, Neto Z, Canto-Cavalheiro MM, Figueiredo P, do Rosário VE, Amaral AC and Lopes D: A review of antimalarial plants used in traditional medicine in communities in Portuguese-speaking countries: Brazil, Mozambique, Cape Verde, Guinea-Bissau, São Tomé and Príncipe and Angola. Memorias do Instituto Oswaldo Cruz 2011; 106: 142-58.
4. Biamonte MA, Wanner J, and Le Roch KG: Recent advances in malaria drug discovery. Bioorganic and Medicinal Chemistry Letters 2013; 23(10): 2829-43.
5. Balunas MJ and Kinghorn AD: Drug discovery from medicinal plants. Life sciences 2005; 78(5): 431-41.
6. Karumi EW, Maitai CK and Okalebo FA: Anthelmintic and antibacterial activity of *Hagenia abyssinica* (Bruce) JF Gmel (Rosaceae). East and Central African Journal of Pharmaceutical Sciences 2013; 16(3): 75-80.
7. Nibret E and Wink M: Trypanocidal and antileukemic effects of the essential oils of *Hagenia abyssinica*, *Leonotis ocymifolia*, *Moringa stenopetala*, and their main individual constituents. Phytomed 2010; 17(12): 911-20.
8. Singh I and Bharate S: Phloroglucinol compounds of natural origin. Natural Product Reports 2006; 23(4): 558-591.
9. Assefa B, Glatzel G and Buchmann C: Ethnomedicinal uses of *Hagenia abyssinica* (Bruce) JF Gmel. among rural communities of Ethiopia. Journal of Ethnobiology and Ethnomedicine 2010; 6(1):20.
10. Pankhurst R: Europe's discovery of the Ethiopian taenicide--kosso. Medical History 1979; 23(3): 297.
11. Vial H, Taramelli D, Boulton IC, Ward SA, Doerig C and Chibale K: CRIMALDDI: Platform technologies and novel anti-malarial drug targets. Malaria Journal 2013; 12(1): 396.
12. Anthony MP, Burrows JN, Duparc S, JMoehrle J and Wells TN: The global pipeline of new medicines for the control and elimination of malaria. Malaria Journal 2012; 11(1): 316.
13. Zohra SF, Meriem B and Samira S: Phytochemical screening and identification of some compounds from mallow. J Nat Prod Plant Resour 2012; 2(4): 512-6.
14. Prohp TP and Onoagbe IO: Determination of phyto-chemical composition of the stem bark of *Triplochiton scleroxylon* K. Schum. (sterculiaceae). International Journal of Applied Biology and Pharmaceutical Technology 2012; 3(2): 68-76
15. National Research Council. Guide for the care and use of laboratory animals. National Academies Press 2010.
16. OECD, Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. Paris: OECD Publishing; 2008. doi: http://dx.doi.org/10.1787/9789264071049-en.
17. Harborne JB: Phytochemical methods. Chapman and Hall, Ltd., London, 1973: 49-188.
18. Evans WC. Trease and Evans' Pharmacognosy. Elsevier Health Sciences 2009.
19. Peters W: Rational methods in the search for anti-malarial drugs. Transactions of the Royal Society of Tropical Medicine and Hygiene 1967; 61(3): 400-10.
20. Petros Z: The need of standardized herbal remedies as alternate sources of anti-malarial products in Ethiopia-updated review. Pharmacologyonline 2011; 3: 1440-7.
21. Kalra BS, Chawla S, Gupta P and Valecha N: Screening of antimalarial drugs: An overview. Indian J Pharmacol 2006; 38: 5-12.
22. Oliveira AB, Dolabela MF, Braga FC, Jácome RLRP, Varotti FP and Póvoa MM: Plant-derived anti-malarial agents: new leads and efficient phytomedicines. Part I. Alkaloids. An Acad Bras Cienc 2009; 81: 715-40.
23. Onguene PA, Ntie-Kang F, Lifongo LL, Ndom JC, Sippl W and Mbaze LM: The potential of anti-malarial compounds derived from African medicinal plants. Part I: a pharmacological evaluation of alkaloids and terpenoids. Malar J 2013; 12: 449.
24. Otsuka H: Purification by solvent extraction using partition coefficient. Methods in Biotechnology Natural Products Isolation. Totowa, NJ: Human Press 2006: 269-73.
25. Kalra B, Chawla S, Gupta P and Valecha N: Screening of anti-malarial drugs: an overview. Indian J Pharm 2006; 38: 5-12.

26. Fidock DA, Rosenthal PJ, Croft SL, Brun R and Nwaka S: Anti-malarial drug discovery efficacy models for compound screening. Nat Rev Drug Disco 2004; 3: 512-20
27. Okokon JE, Ofodum KC, Ajibesin KK, Danladi B and Gamanil KS: Pharmacological screening and evaluation of anti-plasmodial activity of *C. zambesicus* against *P. berghei* in mice. Indian J Pharmacol 2005; 37: 243-246.
28. Ayodele T: Studies on *Azadricha indica* in malaria. Presented in: 4th OAU inter African symposium. Abijan, Ivory Cost 1979.
29. Peters W: Drug resistance in *Plasmodium berghei* vincke and lips, 1948; I. Chloroquine resistance. Exp Parasitol 1965; 17: 80-9.
30. Basir R, Fazalul Rahiman SS, Hasballah K, Chong WC, Talib H and Yam MF: *Plasmodium berghei* ANKA Infection in ICR Mice as a Model of Cerebral Malaria. Iranian J Parasitol 2012; 7: 62-74.
31. Dikasso D, Makonnen E, Debella A, Abebe D, Urga K, Makonnen W, Melaku D, Assefa A and Makonnen Y: *In-vivo* anti-malarial activity of hydroalcoholic extracts from *Asparagus africanus* Lam. In mice infected with *Plasmodium berghei*. Ethiopian Journal of Health Development 2006; 20(2): 112-8.

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