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In vivo Trials of Potential Antimalarial from *Beta vulgaris* Extracts in Jeddah, Saudi Arabia

[1]Haleema H. Albohiri, [1]Najia A. Al-Zanbagi and [2]Saad Hasan Albohairi

[1]Biology Department, Science College, King Abdulaziz University, Jeddah, Saudi Arabia
[2]Medical laboratory Technologist in King Abdulaziz University Hospital, Saudi Arabia

Abstract: Malaria is caused by *Plasmodium sp.*, it is responsible for around half million people dying yearly. Malaria control is imaginable only with coordinated efforts of governmental sectors with health care workers. This study was aimed to investigate the *in vivo* antiplasmodial activity of *Beta vulgaris* aqueous and methanol extracts in mice infected with chloroquine sensitive *Plasmodium berghei berghei*. The effectiveness of extracts was evaluated against early infection, the EDs were estimated for the best concentration of tested antimalarial extracts and its activity in curative and prophylactic tests. Methanol extract 30 mg/kg did not show high inhibition activity with 12.3% while 30 mg/kg aqueous extract gave 79.4% inhibition level in early infection (suppressive test). The aqueous extract at all the doses 5 - 400 mg/kg did not showed dose-dependent activity against the parasite in the suppressive test. Aqueous extract 50 mg/kg gave the best inhibitory level with 90.2%, 41.4% and 64% in early infection, curative and in the 4th day prophylactic test respectively. This effort provides a stimulus to conduct further investigation on *B. vulgaris* aqueous extract in *in vitro* study for *P. falciparum* and to proceed for more advanced extraction to examine *B. vulgaris* active components as malaria inhibitor compounds.

Key words: *Plasmodium berghei* · Beet Root · Antiplasmodium · Curative Test · Prophylactic Test

INTRODUCTION

Malaria is responsible for the death of over one million people annually, approximately three billion people are at risk of infection by both *P. falciparum* and *P. vivax* parasites. About 90% of disease occurs in the most poverty-stricken sub-Saharan African countries. However, countries of Asia, Latin America, Middle East and Europe are also affected [1].

Control of malaria involves three living beings: Firstly Man, as a moving target host that can take the disease too far and wide. Second *Plasmodium*, the agent which is highly adaptable, hides in humans and mosquitoes and has also developed resistance to drugs. Finally Anopheles mosquito, the vector is moving and highly adaptable and has shown resistance to insecticides [2].

In various parts of the world and for thousands of years the traditional herbal medicines have been used for malaria treatment. Many people have been used plants as medicament against malaria fever or other disease symptoms, some of these discovered plants have the antimalarial efficacies which were scientifically confirmed and their active compounds were isolated as well as their possible action mechanisms were considered [3].

In the past, compounds containing new structure from natural origin represent main source for discovery novel drugs for many diseases such as plant and marine extracts, Alkaloids, Terpenes, Quassinoids, Flavonoids, Limonoids, Chalcones, Peptides, Xanthones, Quinones and Coumarines and different antimalarials from nature [4]. These natural products have showed encouraging treatment activities *in vitro* and *in vivo*, which can be improved into safe medicines by using synthetic strategies to obtain antimalarials active components against not only drug sensitive, but also for multidrug resistant strains of *Plasmodium* [5].

Numerous parts of *Beta vulgaris* are used in the traditional medication for several curative properties. The plant roots are expectorant, diuretic and used as medicine for cerebral disturbance, liver and kidney diseases, energizing the immune and hematopoietic systems and as particular diet in cancer treatment [6].

MATERIALS AND METHODS

Experimental Mice: Swiss albino male mice, weighing 19-25 gram age 6–8 weeks were used. They were housed in protective plastic cages covered by minute holes net to

Corresponding Author: Haleema H. Albohiri, Biology Department, Science College, King Abdulaziz University, Jeddah, Saudi Arabia. E-mail: halbohiri@kau.edu.sa.

prevent small insect's entry. Mice were maintained under humidity (65%), room temperature (20±2) and artificial illuminated of 12 hours dark/light cycle, they were fed on standard diet *ad libitum* with free access to water.

Malaria Parasites Strain: Chloroquine-sensitive *Plasmodium berghei berghei* "*ANKA*" was obtained from Institute of Immunology and Infection Research in European Malaria Reagent Repository, Edinburgh. Strain sample was received safely and saved directly in liquid nitrogen at Bio Bank, King Fahad Medical Research Center, Jeddah, Saudi Arabia. Parasites were maintained by continuous re-infestation in mice [7].

Parasites Inoculation: Parasitized erythrocytes *P. berghei ANKA* were obtained from orbital plexus in EDTA tubes of donor infected mouse by determining percentage parasitemia and erythrocytes count and diluting them Phosphate Buffered Saline (PBS) in proportions indicated by both determinations [8]. Experimental mice were inoculated with 0.2 ml suspension of $1{\times}10^6$ parasitized erythrocytes *P. berghei ANKA strain* using the intraperitoneal injection route [9].

Plant under Study: *Beta vulgaris* was tested as beetroot aqueous extract and beetroot methanol extract. Aqueous beetroot extract was obtained from Beijing Herbal Health Biotech Limited Liability Company, Beijing, China. Fresh beetroots were purchased from local market then were washed with water, the methanol extract was extracted according to standard procedure of extraction [10].

Antimalarial Activity: Standardized laboratory screening was passed through primary, secondary and tertiary screening as described in David *et al*. [11] protocol.

Primary Screening: Four-day suppressive test was conducted using mice injected intraperitonealy with standard inoculation of *P. berghei* containing 10^6 infected erythrocytes in Phosphate Buffered Saline (PBS) with 47% parasitemia level and CBC was 2.9 (10^6/µl). After 3 hours of inoculation, infected mice were randomly divided into 4 groups, each group of 9 mice in 3 cages. They were treated orally by 0.2 ml dose from tested materials for 4 consecutive days (Day 0 to Day 3).

Mice were classified in groups as: group 1 (Negative control mice that were treated by distilled water), group 2 (Mice were treated by dose 4 mg 100% *B. vulgarius* aqueous extract with 1 ml distilled water (4:1 equal to 30 mg/kg), group 3 (Mice were treated by dose 4 mg 100% *B. vulgarius* methanol extract with 1 ml distilled water (4:1 equal to 30 mg/kg), group 4 (positive control mice that were treated by 5 mg/kg Chloroquine). On Day 3, thin blood films were made from all mice tails in every group. Means of parasitemia were estimated to calculate suppression% of malaria infection for each extract tested using formula mentioned in Sumsakul *et al.* [12] as follow:

$$\text{Suppression\%} = \frac{\text{Parasitemia of negative control mice} - \text{Parasitemia of treated mice}}{\text{Parasitemia of negative control mice}} \times 100$$

Secondary Screening: *Beta vulgarius* aqueous extract was tested in different doses following standard 4-day suppressive test to estimate Effective Doses (EDs). Mice were inoculated intraperitonealy with standard inoculation of *P. berghei* containing 10^6 infected erythrocytes in PBS with 71% parasitemia level and CBC was 1.5 (10^6/µl). Infected mice were administered orally by 0.2 ml from different doses of extracts, then were randomly divided into 3 groups as: group 1 (Negative control mice were only received distilled water), group 2 (Infected mice were administered separately concentrations of 5 - 400 mg/kg 100% *B. vulgarius* aqueous extract for each concentration 5 mice), group 3 (Positive control mice were administered 5 mg/kg Chloroquine).

Tertiary Screening: Best inhibitory concentration of *B. vulgarius* aqueous extract was studied in two tests, Curative Test and Prophylactic Test.

On Day 0 of Curative Test, mice were weighed and injected intraperitoneally with standard inoculum of 10^6 *P. berghei* infected erythrocytes in PBS with 56% parasitemia level and CBC was 1.78 (10^6/µl). After 72 hours, on Day 3 and according to parasitemia level confirmation, mice were divided into 3 groups, in each group 9 mice separated in 3 cages as: group 1 (Negative control mice were orally administered only distilled water), group 2 (Positive control mice were orally received 5 mg/kg Chloroquine), group 3 (Mice were orally treated by the best suppressive concentration of *B. vulgarius* aqueous extract). Malaria treatment procedure lasted for 5 consecutive days as single dose per day. At the end of experimental period, blood smears for every mouse from each group were collected to be examined microscopically for monitoring parasitemia level.

In Prophylactic Test, modification of David *et al*. [11] protocol was done concerning estimation of mice survival duration. Mice were divided into 3 groups of 6 mice per group in 2 cages. They received daily different doses from Day 0 to Day 3 of experiment as: group 1 (Normal mice

were administered dose 50 mg/kg/day *B. vulgarius* aqueous extract for 4 consecutive days), group 2 (Positive control mice were received dose 5 mg/kg/day Chloroquine), group 3 (Negative control mice were administered only distilled water). On Day 4, all mice were inoculated with standard inoculums of 10^6 *P. berghei* infected erythrocytes in PBS with 55% parasitemia level and CBC was 1.71 (10^6/µl). Increase or decrease in parasitemia level was estimated in all mice by doing blood smears every 48 hours and monitoring the mice survival time in days was determined.

Statistical Analysis: SPSS for Windows (Version 19.0) was used to analyze data and Microsoft Excel 2007 for figures designing was also used. Values were expressed as mean ± standard deviation and the t-test, One-way ANOVA with Post Hoc test Two-way ANOVA were analyzed statistically the results obtained. Significant differences between experimental mice groups were considered at P-values less than 0.05 levels. Effective doses evaluation was based on Leitchfield and Wilcoxon [13] to approximate median effective dose and slope of dose-percent effect curves as well as to specify Confidence limits of these parameters for 5% probability. Graphs were drawn using Sigmaplot program.

RESULTS

Primary Screening: Inhibition levels of parasitemia in *P. berghei* infected mice were estimated (Figure 1) by using 5 mg/kg Chloroquine, *B. vulgaris* aqueous extract and methanol extract in doses of 4:1 (30 mg/kg) for 4 successive days.

Mean of parasitemia level in all infected mice was calculated given high value in those untreated mice (Distilled water) as 8.1 and the percentage of inhibition was none. In positive control group, the parasitemia mean was 0.39 giving the highest inhibition level as 95.19% with parasites survival rate as 4.81% (Table 1, Figures 2 & 3), followed by *B. vulgaris* aqueous extract in 30 mg/kg, malaria survival rate was 20.59% with mean parasitemia level as 1.67 and the parasites inhibition level was 79.4%. There was no clear inhibition effect by using 30 mg/kg *B. vulgaris* methanol extract and *P. berghei* survival rate was high (87.67%), the parasitemia level was 7.1 and it gave low inhibition activity as 12.3%.

S.D: Standard Deviation: There were significant differences in the parasitemia level in *P. berghei* infected mice (*P*= 0.0) between negative control group and mice treated by *B. vulgaris* aqueous extract and Chloroquine. No significant difference in parasitemia level between malaria infected mice treated by *B. vulgaris* methanol extract and those taken only distilled water (*P*= 0.31), also no significant difference in parasitemia level between infected mice treated by *B. vulgaris* aqueous extract or those medicated by Chloroquine (*P* = 0.2).

Secondary Screening: The effective doses (Eds) of *B. vulgaris* aqueous extract were estimated following the standard 4th day suppressive test.

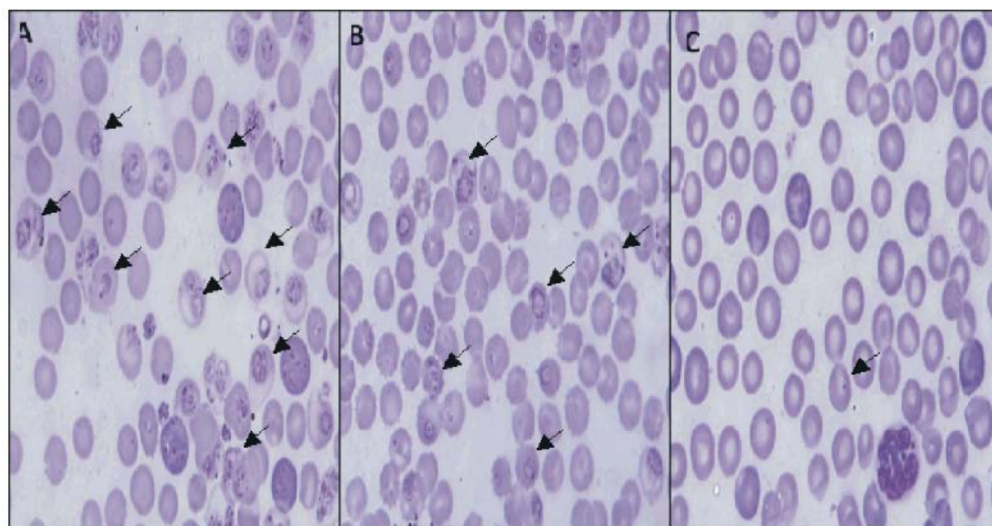


Fig. 1: Stained blood smears of *Plasmodium berghei* infected mice in primary screening test. A- Mice were treated by using Distilled water (-ve control), B- Mice were treated by 30 mg/kg from *Beta vulgaris* aqueous extract, C- Mice were treated by 5 mg/kg Chloroquine (+ve control) showing decreasing in the infected RBCs in both of that treated by *B. vulgaris* aqueous extract and Chloroquine. Infected RBC (Arrow).

Table 1: Inhibition levels of parasitemia in *Plasmodium berghei* infected mice. Mice treated by using doses of 30 mg/kg from *Beta vulgaris* aqueous extract, *B. vulgaris* methanol extract and 5 mg/kg Chloroquine (+ve control) and distilled water (-ve control)

	Parasitemia level (%)*			
Plasmodium berghei		---------- *Beta vulgaris* (4:1 doses) ------------		
infected mice	Distilled water (-ve control)	Aqueous extract	Methanol extract	Chloroquine 5mg/kg(+ve control)
1	10	2.5	5	1
2	9	1.5	6	1
3	6	2.5	5	0
4	11	1	15	0
5	13	2	5	0
6	7	1.5	7	1
7	4	2	6	0.5
8	9	1	7	0
9	4	1	8	0
Total of parasited RBC	73	15	64	3.5
Mean of parasitemia ±S.D	8.11 ± 3.10	1.67 ± 0.61	7.11 ± 3.14	0.39 ± 0.49
Inhibition level (%)**	0	79.41	12.33	95.19
Survival rate (%)***	100	20.59	87.67	4.81

* Parasitemia level %: (Number of parasited RBC / Total number of examined RBC) X 100

** Inhibition level %: Mean of (Parasitemia in negative control mice - Parasitemia in treated mice) / Mean of Parasitemia in negative control mice X 100

*** Survival rate %: (100 - inhibition level) X 100

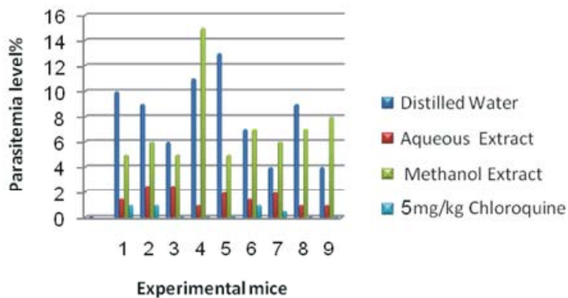


Fig. 2: Parasitemia levels in *Plasmodium berghei* infected mice. Mice were treated by using doses of 30 mg/kg from *Beta vulgaris aqueous* extract, *B. vulgaris* methanol extract and the Chloroquine (+ve control) and distilled water (-ve control)

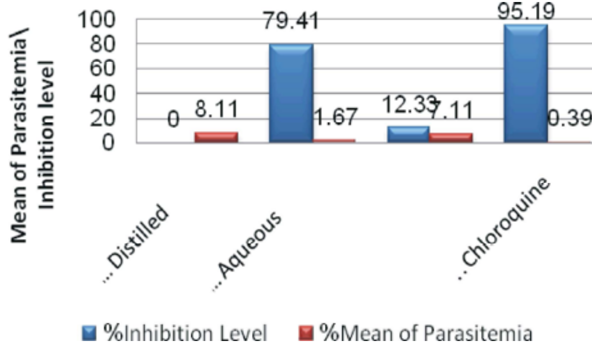


Fig. 3: Inhibition levels of parasitemia in *Plasmodium berghei* infected mice. Mice treated by using doses of 30 mg/kg from *Beta vulgaris* aqueous extract, *B. vulgaris* methanol extract and Chloroquine (+ve control) and distilled water (-ve control)

The inhibition ratio of parasitemia increased gradually in low concentrations to reach the peak of inhibition as 90.2% in 50 mg/kg, the mean of parasitemia was 0.86 and the percentage of survival rate was 9.7%. In higher concentrations 100 mg/kg to 400 mg/kg, gradual decline in the inhibition level was noticed, it reached 17.05% in 400 mg/kg with parasitemia level as 7.3 and the parasites survival rate was 82.9% (Table 2, Figure 4). Lower concentration of beetroot aqueous extract as 5 mg/kg showed no activity (-2.27%) in malaria infected mice, there was increasing in the parasitemia level and in malaria survival rate, as well as that recorded for distilled water. Chloroquine gave the highest percentage of parasitemia suppression as 96.59% that the mean of parasitemia was 0.3 with low percentage of parasite survival rate (3.4%).

There were no significant differences in parasitemia level between malaria infected mice treated by 50 mg/kg *B. vulgaris* aqueous extract and 30 mg/kg *B. vulgaris* aqueous extract (P= 0.6) and 5 mg/kg Chloroquine (P= 0.8). No significant differences in parasitemia level between negative control group and that treated by 5 mg/kg *B. vulgaris* aqueous extract (P=0.9) and 400 mg/kg (P= 0.5). There was significant difference in parasitemia level between mice treated by 200 mg/kg and 50 mg/kg *B. vulgaris* aqueous extract (P= 0.02) or 5 mg/kg Chloroquine (P= 0.01), but there were no significant differences between doses of 400 mg/kg and 10 mg/kg (P= 0.8).

Table 2: Inhibition levels of parasitemia in *Plasmodium berghei* infected mice by using ascending concentrations. By using *Beta vulgaris* aqueous extract as well as the 5 mg/kg Chloroquine (+ve control) and distilled water (-ve control)

	Parasitemia level (%)*								
Plasmodium berghei infected mice	Distilled Water (-ve control)	*Beta vulgaris* aqueous extract 5 mg/kg	10mg/kg	30mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	Chloroquine 5mg/kg (+ve control)
1	12	2	12	2.5	0.5	4	5	6.5	0
2	10	20	4	1.5	1	3	4	6	0.5
3	7	2	11	2.5	0.8	1	9	11	0
4	6	5	1	1	1	5	6.5	8	0
5	9	16	7	2	1	6	6	5	1
Total of parasited RBC	44	45	35	9.5	3.9	19	30.5	36.5	1.5
Mean of parasitemia± S.D	8.8 ± 2.39	9 ± 8.42	7±4.64	1.9± 0.65	0.86± 0.22	3.8± 1.92	6.1± 1.88	7.3± 2.33	0.3± 0.45
Inhibition level (%)**	0	-2.27	20.45	78.41	90.23	56.82	30.68	17.05	96.59
Survival rate (%)***	100	102.27	79.55	21.59	9.77	43.18	69.32	82.95	3.41

* Parasitemia level %: (Number of parasited RBC / Total number of examined RBC) X 100

** Inhibition level %: Mean of (Parasitemia in negative control mice - Parasitemia in treated mice) / Mean of Parasitemia in negative control mice X 100

*** Survival rate %: (100 - inhibition level) X 100

S.D: Standard Deviation

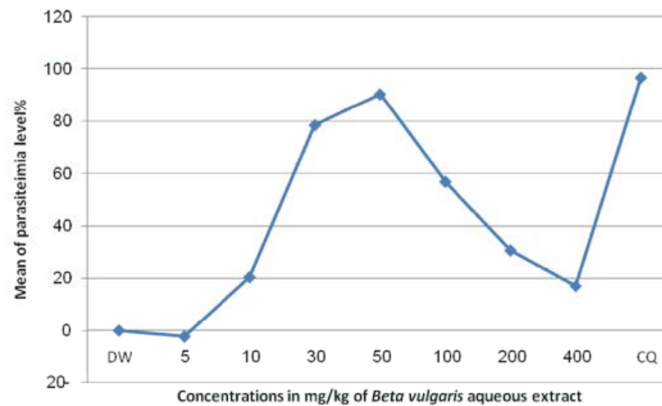
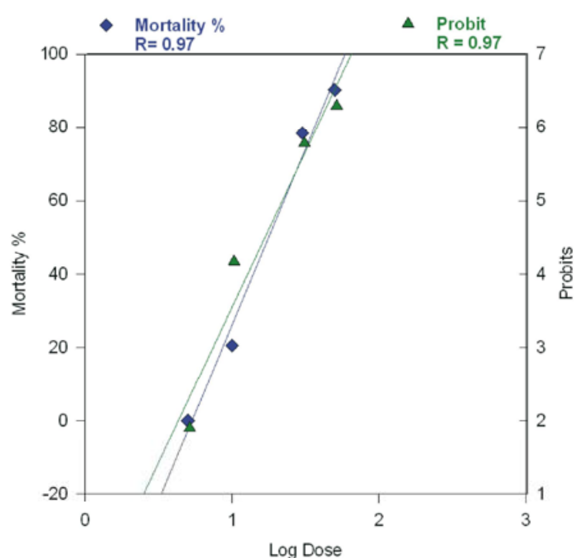


Fig. 4: Inhibition levels of parasitemia in *Plasmodium berghei* infected mice by using ascending concentrations. Using doses of *Beta vulgaris* aqueous extract as well as the 5 mg/kg Chloroquine (+ve control) and distilled water (-ve control) (DW: Distilled Water, CQ: Chloroquine)

Estimation of EDs: ED_{50} and ED_{90} of *B. vulgaris* aqueous extract were estimated as 20.9 (13–33) mg/kg and 42.6 (21–85) mg/kg respectively at 5% confidence limits (Table 3, Fig 5).

Table 3: ED50 and ED90 of *Beta vulgaris* aqueous extract against the parasitemia in *Plasmodium berghei* infected mice.

Materials used	concentrations used (mg/kg)	Mean of parasitemia level	Inhibition level (%)	Inhibition concentrations with 5% confidence limits* ED_{50}	ED_{90}
Beta vulgaris aqueous extract	5	9	0	20.9 (13 - 33)	42.6 (21 - 85)
	10	7	20.45		
	30	1.9	78.41		
	50	0.86	90.23		
Distilled water (-ve control)	0	8.8	0	-	-



ED_{50}= 20.9 (5% confidence limits: 13 - 33)

ED_{90}= 42.6 (5% confidence limits: 21 - 85)

Fig. 5: ED_{50} and ED_{90} (after 4 days in suppressive test) of *Beta vulgaris* aqueous extract for inhibiting the parasitemia in *Plasmodium berghei* infected mice

Curative Test: Means of parasitemia levels of *P. berghei* on 3rd day of infection showed 34.1% in negative control group, it reached 50.4% after 5 days with increasing in malaria survival rate (100%). On 3rd day of infection, treated mice groups recorded 31.1, 33.56 and 15.1 in the parasitemia level by using 50 mg/kg *B. vulgaris* aqueous extract and 5 mg/kg Chloroquine. After 5 days of treatment, beetroot aqueous extract showed decreasing in parasitemia level as 18.2 with inhibition level as 41.4%. Chloroquine (+ve control) showed minimum parasitemia level as 2.2 in the day 7 of infection and the inhibition level was 85.3% (Table 4 & Figures 6 & 7).

In all mice groups, there was significant differences between pre and post parasitemia level (*P*= 0.0), also there was significant differences between mice group untreated (Distilled water) and other mice treated groups (*P*= 0.0).

Prophylactic Test: Monitoring the malaria infection through 20 days of prophylactic test showed 100% mice were still alive until 6th day of infection in negative control group and mean of parasitemia was 31.67, while it was

Table 4: Parasitemia levels in *Plasmodium berghei* infected mice following the curative test procedure. By using 50 mg/kg of *B. vulgaris* aqueous extract, 5 mg/kg Chloroquine (+ve control) and distilled water (-ve control)

	Parasitemia level (%)*					
	Distilled Water (-ve control)		Aqueous Extract 50%		Chloroquine 5mg\kg (+ve control)	
Plasmodium berghei infected mice	Pre	Post	Pre	Post	Pre	Post
1	32	47	32	16	10	4
2	34	50	39	28	15	0
3	30	45	31	17	7	0
4	33	43	42	26	8	0
5	35	52	45	34	17	5
6	45	61	42	29	18	3
7	33	52	12	2	16	0
8	28	49	8	0	19	1
9	37	55	29	12	26	7
Total of parasited RBC	307	454	280	164	136	20
Mean of parasitemia± S.D	34.11± 4.86	50.44± 5.43	31.11± 13.21	18.22± 12.04	15.11± 6.01	2.22± 2.63
Inhibition level (%)**	-47.87	41.43	85.31			
Survival rate (%)***	100	58.57	72.5			

* Parasitemia level %: (Number of parasited RBC / Total number of examined RBC) X 100

** Inhibition level %: Mean of (Parasitemia in negative control mice - Parasitemia in treated mice) / Mean of Parasitemia in negative control mice X 100

*** Survival rate %: (100 - inhibition level) X 100

S.D: Standard Deviation

Pre: Parasitemia level in day 3 of infection before treatment

Post: Parasitemia level in day 7 of infection, after 5 days of treatment

S.D: Standard deviation

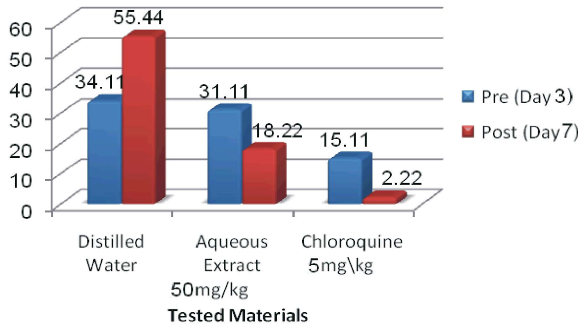


Fig. 6: Parasitemia levels in *Plasmodium berghei* infected mice following the curative test procedure. By using 50 mg/kg of *B. vulgaris* aqueous extract, 5 mg/kg Chloroquine (+ve control) and distilled water (-ve control)

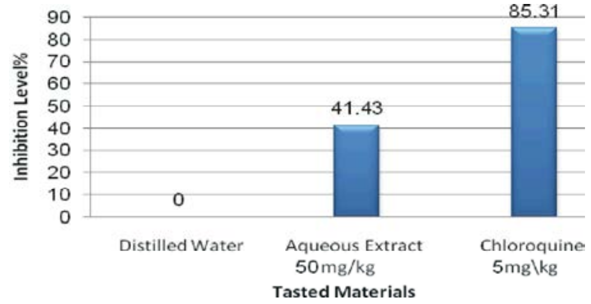


Fig. 7: Inhibition levels of parasitemia in *Plasmodium berghei* infected mice following the curative test procedure.By using 50 mg/kg of *Beta vulgaris* aqueous extract, 5 mg/kg Chloroquine (+ve control) and distilled water (-ve control)

Table 5: Inhibition levels of parasitemia in *Plasmodium berghei* infected mice following the prophylactic test procedure. By using 50 mg/kg of *Beta vulgaris* aqueous extract, 5 mg/kg Chloroquine (+ve control) and distilled water (-ve control)

Plasmodium berghei infected mice		Parasitemia level (%)* / day by using *Beta vulgaris* aqueous extract (50mg/kg)																			
		D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8	D_9	D_{10}	D_{11}	D_{12}	D_{13}	D_{14}	D_{15}	D_{16}	D_{17}	D_{18}	D_{19}	D_{20}
Distilled water(-ve control)	1		0.5		12		27		46	X											
	2		0.5		11		21		42		65X										
	3		0		22		35		49		59 X										
	4		0.8		20		31		48		X										
	5		0		19		45	X													
	6		0		9		31		X												
Total of parasited RBC			1.8		93		190		185		124										
Mean of parasitemia			0.3		15.5		31.67		46.25		62										
No. of dead mice in each day		0	0	0	0	0	0	1	1	1	3										
Aqueous Extract (50mg/kg)	1		0		8		12		20		32		46		56		X				
	2		0		10		16		25		36		52		X						
	3		0		4		13		23		42		55X								
	4		0		0.5		0.8		5		18		29		40		57		73		X
	5		2		8		14		22		36		50		64		X				
	6		0		3		10		21		38		48		58		73		81	X	
Total of parasited RBC			2		33.5		65.8		116		202		280		218		130		154	-	-
Mean of parasitemia			0.3		5.58		10.97		19.33		33.67		46.67		54.5		65		77	-	-
Inhibition level (%)**			0		64		65.2		58.2		45.7		-		-		-		-	-	-
No. of dead mice in each day		0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	2	0	0	1	1
Chloroquine (5mg/kg)(+ve control)	1		0		7		16		26		39		48		53X						
	2		0		4		14		23		38		51X								
	3		0		10		22		39		45		55		X						
	4		0.8		6		17		24		43		52		65		X				
	5		0		9		17		28		32		40		49		58		75X		
	6		0		5		13		25		39		46		55		X				
Total of parasited RBC			0.8		41		99		165		236		292		222		58		75		
Mean of parasitemia			0.13		6.83		16.5		27.5		39.33		48.67		55.5		58		75		
Inhibition level (%)**			56.7		55.9		47.9		40.5		36.6		-		-		-		-		
No. of dead mice in each day		0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	2	0	1		

* Parasitemia level %: (Number of parasited RBC / Total number of examined RBC) X 100

** Inhibition level %: Mean of (Parasitemia in negative control mice - Parasitemia in treatedmice) / Mean of Parasitemia in negative control mice X 100

☐ : No examination was done, X: Death mice were observed

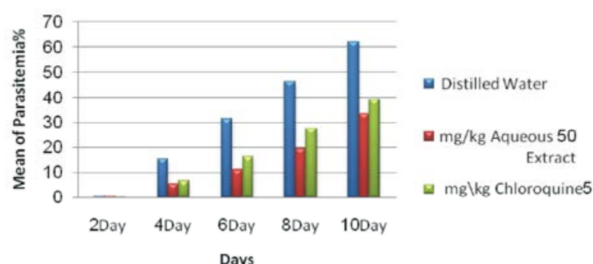


Fig. 8: Mean of parasitemia in *Plasmodium berghei* infected mice following the prophylactic test procedure. By using 50mg/kg of *Beta vulgaris* aqueous extract, 5 mg/kg Chloroquine (+ve control) and distilled water (-ve control) after 10 days of infection

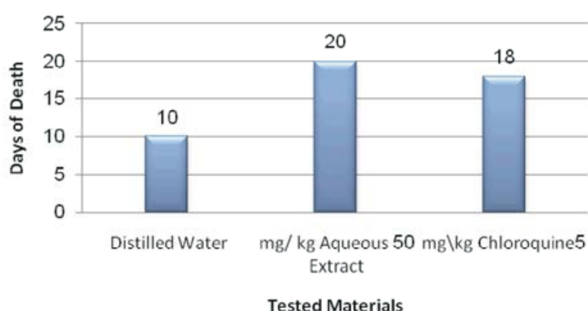


Fig. 9: Total days of death in *Plasmodium berghei* infected mice following the prophylactic test procedure. By using 50mg/kg of *Beta vulgaris* aqueous extract, 5 mg/kg Chloroquine (+ve control) and distilled water (-ve control) after 10 days of infection

10.97 by using 50 mg/kg beetroot aqueous extract and 16.5 by using 5 mg/kg Chloroquine. Deaths started in untreated mice group in day 7 of experiment and all mice died by 10th day. All mice still alive until 10th day of experiment using beetroot aqueous extract and Chloroquine, with inhibition level as 45.7% and 36.6% respectively (Table 5, Figures 8 & 9). One mouse in aqueous beetroot extract group survived until day 20 but in Chloroquine group the last mouse died in day 18 with 75 parasitemia.

There were significant differences in parasitemia level (P= 0.0) between 50 mg/kg of *B. vulgaris* aqueous extract, 5 mg/kg Chloroquine (+ve control) and distilled water (-ve control). No significant difference in parasitemia level between malaria infected mice treated by *B. vulgaris* aqueous extract and Chloroquine (P= 0.05). Based on days, there were significant difference in parasitemia level between the survival days for all treated mice groups (P= 0.000) and (P= 0.001).

DISCUSSION

The *Dodonaea angustifolia* and *Bombax buonopozense* queous extract were less effectiveness than resulted from *B. vulgaris* aqueous extract in the present study [14&15].

The antimalarial activity of leaf, stem bark and root bark methanol extracts of *Trichilia megalantha* at dose 100 mg/kg, leaves extract didn't show any malaria suppression [16]. Methanol leaves extract of *Berlina grandiflora* and *Maerua crassifolia* at 100 mg/kg exhibited malaria suppression as 83% and 71% respectively [8,17] gave better suppression than 30 mg/kg *B. vulgaris* methanol extract (12%). Also, 200 mg/kg methanol leaf extract of *Bombax buonopozense,* 50 mg/kg methanol bark extract of *Aphloia theiformis* which was 65% as malaria chemosuppression levels [18].

Chloroquine as 5 mg/kg gave 95.2% parasitemia inhibition in the suppressive test, the same doses showed same suppression with 95.4% malaria inhibition [19] while it recorded higher inhibitory activity as 96.7% [20].

The gradient concentrations of 5 to 400 mg/kg *B. vulgaris* aqueous extract did not show dose dependent chemosuppressive effect with different levels of parasitemia inhibition similar with that proved for the effect of *Paullinia pinnata* ethanol leave extract [21].

Comparing the best inhibitory oral daily doses of 50 mg/kg *B. vulgaris* aqueous extract that caused 90% malaria suppression effect, Ramli *et al.* [22] found greatly lower suppressive effectiveness as 15% with the same dose from ethanolic extract of *Plectranthus amboinicus*.

There are some studies investigated *in vitro* the IC_{50} of antiplasmodial components against different strains of *Plasmodium falciparum* [23] who recorded the IC_{50} values of methanolic extract of *Aphloia theiformis* bark and leaves of *Terminalia bentzoe*, *Psiadia arguta* and *Nuxia verticillata* against 3D7 and W2 *Plasmodium* strain were as 13.3, 24.8, 22.4 and 32.7 µg/ml respectively. Results were completely differed from that of *B. vulgaris* aqueous extract in two points which related to the experimental assay type and to the malaria parasite species.

The result of *B. vulgaris* aqueous extract in curative test as 41.4% was better than that gained by using *Plectranthus amboinicus* leaves extract at dose 50 mg/kg [22] who found no significant reduction of parasitemia on the curative test, but it was lower than the result of 100 mg/kg *Morinda lucida* aqueous extract.

Chloroquine at 5 mg/kg showed lower chemosuppression with 85.3% in the curative test compared with 97.84% malaria inhibition at the same daily doses of Chloroquine [19].

The tested blood smears in the prophylactic test showed 64% malaria suppression on day four after infection and the mice life span was extended to 20 survival days by using *B. vulgaris* aqueous extract. There are no previous studies using the survival days as a factor to determine the antimalarial activity in the prophylactic test. The parasitemia inhibition by using 50 mg/kg *B. vulgaris* aqueous extract recorded in day four after infection was better than 59.4% that resulted from using Xylopic acid on petroleum ether in 100 mg/kg after 72 hours of infection [9] 59% gained from *Gongronema Latifolium* ethanolic extract in 200 mg/kg [24] 60% resulted from *Verbena hastate* ethanol leaves extract in 200 mg/kg [25] and 33.15% came from *Morinda lucida* aqueous extract in 100 mg/kg [19] but it is lower than that 50 mg/kg *Plectranthus amboinicus* leaves extract which showed 83.33% in decreasing parasitemia on the third day of rodent malaria infection [22].

The result of 5 mg/kg Chloroquine was 56.7% after 48 hours of infection in the prophylactic test and it was lower than the result of using the same dose of Chloroquine for its inhibitory effect as 97.84% after 72 hours of infection [19].

Red beetroot is rich in vitamin B folate that is considered essential for different cellular functions starting from carbon donation to the growth of normal tissues and cognitive processes [26]. Folate or Folic acid shows an antioxidant activity [27]. The beetroot extract contains sugars (34% sucrose), protein (13%) and betaine (1.0%), all based on percentage dry weight [28]. The red beet pigments content are decreased when the beet diameter increased, that a large beets had lower pigments content than the small ones. The beetroot pigments are completely extracted by using water, methanol or ethanol solutions in dilutions of 20–50% [29]. Extraction beetroot with water increases the stability of its pigments and with slight acidification by using ascorbic acid, the beetroot color stability and its oxidation resistance will increased [30,31].

Red beetroot pigments are communally nominated as Betalains which are divided into two major classes, Betacyanins and Betaxanthins, the first has the red violet color but the second has yellow color [32]. The antioxidant betacyanins characters are due to their free phenolic groups. The cyclic amine group of betalamic acid moiety is considered to act as a hydrogen donor both in betacyanins and betaxanthins [33]. The exclusive health benefits of betalain pigments are due to its nitrate content which includes nitrogen and amino acid [34].

The Red beets have a number of endogenous enzymes as b-glucosidases and peroxidases [30] these enzymes in the beetroot peel indicates their obvious contribution in the defense functions by the peroxides scavenging or by oxidizing other molecules that formed in the surface [35]. Also they showed antioxidant and radical scavenging activities [36,37,38]. They interfere from human alimentary canal to its blood stream make the Betalains to be bioavailable for the human organisms [39].

CONCLUSION

Beta vulgaris is considered as effective inhibitory malaria substance for *Plasmodium berghei* that is adequately effective to give protection at low doses in early and stable infections as well as considerable prevention of malaria future infection. These results are encouraging to study the histopathological effect of *B. vulgaris* aqueous extract in the brain of *Plasmodium berghei* infected mice, make an *in vitro* experiment of *B. vulgaris* effect on human strains, *Plasmodium falciparum*, examine the extract active constituent efficacy as chemotherapeutic and chemopreventive agents, study the combination treatment with the Chloroquine to attain the interactive effect and reduction of the drug toxicity with possible for lowering the effectual dosage, finally evaluating *B. vulgaris* genotoxicity, biochemical and histological studies evaluation.

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