Invivo Antimalarial Activity of *Dodonaea Angustifolia* Seed *Extracts Against Plasmodium Berghei* in Mice Model

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ABSTRACT

Dodonaea angustifolia has a wide range of therapeutic applications against various diseases including malaria. This plant is traditionally used for treatment of malaria in different countries including Ethiopia. However, the antimalarial effect and safety of *D. angustifolia* are not studied. The aim of this study was to evaluate antimalarial activity of *Dodonaea angustifolia* in *Plasmodium berghei* infected mice. In the present study, aqueous and hydroalcoholic extracts as well as solvent fractions of the aqueous extract of *D. angustifolia* seeds were investigated for their antimalarial activity using Peters' 4-day suppressive test method. Different concentrations of the crude extracts and the fractions of the water extract, most active extract, were orally administered to screen for their antimalarial activities. The extracts significantly inhibited parasitemia and prevented packed cell volume reduction ($p < 0.05$) dose-dependently. Crude extracts and fractions of the aqueous extract of *D. angustifolia* seeds increased the survival time of infected mice. None of the extracts, however, prevented body weight loss. The aqueous extract of *D. angustifolia* was found to produce 35.79% parasite suppression. From this extract, three fractions were produced by solvent fractionation technique using butanol, chloroform and water and tested *in vivo* against *P. berghei* in mice. The butanol fraction was found to be the most active producing inhibition of 48.6% at 100 mg/kg. The test substance observed to be safe with no toxicity on the mice even at 4500 mg/kg. The results of the present work supported the traditional use of the plant against malaria and confirmed the antimalarial activity of the plant. Moreover, antimalarial compounds can be isolated from this plant and tested against human malaria parasite in the future.

Key words: Malaria; *Dodonaea angustifolia*; Extracts; *Plasmodium berghei*

1. INTRODUCTION

Malaria is one of the serious health problems worldwide. At present, around 3.2 billion people are at risk of malaria each year globally (WHO, 2005), with 2-3 million deaths occurring each year (Snow et al., 2005). In Africa, Malaria accounts for 10% of the total disease burden. Over 90% of deaths occur in sub-Sahara Africa (WHO, 2005). Each year an estimated 300 to 500 million clinical cases of malaria occur, making it one of the most common infectious diseases worldwide. Malaria can be, in certain epidemiological circumstances, a devastating disease with high morbidity and mortality demanding a rapid and comprehensive effort (Bloland, 2001). In Ethiopia, malaria is one of the leading causes of high morbidity and mortality. The annual

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number of clinical cases is 4 to 5 million, with approximately 70,000 deaths (Betemariam and Yayeh, 2002).

Four Plasmodium species are responsible for human malaria: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. *P. falciparum* is the most virulent parasite, and is responsible for the majority of malaria related morbidity and mortality (WHO, 2005). Antimalarial drug resistance has become one of the greatest challenges against malaria control. Resistance to antimalarial drugs has been described for two of the four species of malaria parasites that naturally infect humans, *P. falciparum* and *P. vivax. P. falciparum* has developed resistance to nearly all antimalarial drug in current use (Bloland, 2001). Morbidity and mortality due to malaria in Africa has risen, primarily due to increasing resistance to chloroquine and sulfadoxine-pyrimethamine (SP) in *Plasmodium faciparum* (Snow et al., 2001). *P. falciparum* in Ethiopia is resistant to chlroquine and resistance to SP is also highly prevalent in Ethiopia (WHO, 2001). Resistance to SP has been reported with *in vivo* failure rates of 68 - 78% in different localities of Ethiopia (Checchi et al., 2006).

With the problems of increasing levels of drug resistance, traditional medicines could be an important and sustainable source of treatment (Wilcox and Bodeker, 2004). In Africa, the use of indigenous plants still plays an important role in malaria treatment and these plants might be interesting sources for the detection of novel antiplasmodial compounds (Hilou et al., 2006).

Dodona angustifolia L. is a shrub belonging to family Sapindaceae. The centre of origin of *D.angustifolia* is believed to be Australia, but it is also widely distributed throughout the tropics and subtropics (Little and Skolmen, 1989). This plant has a wide range of therapeutic applications since ancient times against pneumonia and tuberculosis (Watt and Breyer-Brand Wijk, 1981), as a botanical pesticide (Ghosh and Ulaganathan, 2004), as a medicine to treat infections, musculo-skeletal and digestive system disorders and injuries (Cook, 1995). *D. angustifolia* is also reported to have analgesics and antipyretic (Amabeoku et al., 2001), antiretroviral (Asres et al., 2001a) and antimalarial activities (Lemordant, 1971; Ali et al., 2004; Simonsen et al., 2001; Clarkson et al., 2004). In addition, by personal communication with traditional healers and informal survey, the plant is traditionally claimed to have antimalarial activity in Ethiopia. However, the antimalarial effect and toxicity of *D. angustifolia* are not studied. The aim of the present study was, therefore, to determine the *in vivo* antimalarial effect of the plant.

2. MATERIALS AND METHODS

2.1. Collection of Plant Materials

Whole plant was collected from North Shoa near Debresina which is 190 kilo meters North of Addis Ababa between February and March 2007. Species identification was done at the National Herbarium of the Department of Biology, Addis Ababa University with voucher number HM02/2006.

2.2. Preparation of Extracts

The seeds of *Dodona angustifolia* L. were collected, cleaned, and air dried under shade at room temperature and powdered by using a grinding mill (Hamburg 76 West Germany). The powdered plant material was weighed by sensitive balance and soaked separately with distilled water and hydroalcohol, 80:20 methanol: water mix and kept on orbital shaker at 130 rotations per minute (rpm). After 72 hours, the extract was filtered by Whatman filter paper number 1 with pore size 0.7µm. This step was repeated three times. Methanol was removed from the hydroalcolic extract under reduced pressure by rotary evaporator (Buchi Rota vapor, Germany) in distillation flask at 45 rpm and 40 $^{o}_{C}$ to obtain the crude extracts. The extract was further concentrated to dryness in a water bath. For aqueous extract, the filtrate was frozen in deep freezer overnight and then freeze dried with a lyophilizer at -40^{o}C and vacuum pressure (200 mBar). The crude extracts were stored in a refrigerator at 4^{0}C in air tight plastic containers until used.

Table 1. Experimental Design of the Study.

Treatment group	***Number of Mice***	***Extract/drug/vehicle dose mg/kg***
I	6	100 mg/kg extract
II	6	200 mg/kg extract
III	6	400 mg/kg extract
IV	6	1ml distilled water
V	6	10 chloroquine

2.3. Experimental Design

This study is animal based experiment where a total of 30 mice for each experiment were randomly assigned in to five groups for each plant extract with six mice per group (Table 1). Three groups of mice received the extracts at 100, 200, and 400 mg/kg respectively. The other

two groups of mice were treated by 1 ml distilled water and chloroquine at dose of 10 mg/kg respectively.

2. 4. Phytochemical Screening

Preliminary phytochemical analysis of the powdered plant material was carried out to screen chemical constituents to detect the major secondary metabolites present in the plant by using the standard methods described by Sofowora (1993).

2.5. In Vivo Antimalarial Screening of Crude Extracts

The study was conducted on male albino mice 8 weeks old; weighing 30.77 ± 2.98 grams. They were obtained from the laboratory animal unit of Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa, Ethiopia. The mice were randomly assigned to treatment and control groups and maintained on a 12-h light/dark cycle. They were provided with adequate amount of food and water. Six mice were used in each group, and each mouse was employed only for one experiment.

A method described by Peters and Robinson (1992) was used for this test. In this study a total of 30 mice were randomly assigned into three treatment groups and two controls (negative and positive control) with six mice per group for each extract.

Plasmodium berghei was obtained from Drug Research Department of EHNRI. Albino mice previously infected with *P. berghei* having variable parasitaemia were used as donors. The parasitemia of the donors was first determined. These mice were then sacrified by head blow, and blood was collected in a Petri dish with an anticoagulant (0.5% trisodium citrate) by severing the jugular vein. The blood was then diluted with physiological saline (0.9%) based on the parasitemia of the donor mice and the RBC count of normal mice in such a way that 1ml blood contains $5x10^7$ infected erythrocytes. Each mouse received 0.2 ml of diluted blood containing $1x10^7$ *P. berghei* infected erythrocytes by intraperitoneal route. To avoid variability in parasitemia, the blood collected from all donor mice was pooled together, and the parasite was maintained by weekly passage to other mice.

Three hours after inoculation of the parasite (Trager and Jensen, 1976), the mice in the three treatment groups received the extracts in doses of 100, 200, 400 mg/kg for four consecutive days in a volume of 1 ml. Two control groups, the negative control receiving distilled water daily for

four consecutive days and the positive control receiving 10mg/kg/day chloriquine phosphate daily for four consecutive days (BNo.6215C1RJB, Ipca laboratory) were used in each experiment. Test concentrations of the extracts, the vehicle and the standard drug used in this study were administered through oral route with the aid of an oral needle.

2.6. Determination of Body Weight and Temperature

The body weight of each mouse in all the groups was taken before infection (day 0) and on day 4. The rectal temperature of the mice was measured with a digital thermometer before infection, three hours after infection and then daily up to day 4 to see the effect of the extracts on body temperature.

2.7. Determination of Parasitemia

Peters' 4-day suppressive test against *P. berghei* infection in mice was employed (Trager and Jensen, 1976). On day 4 of the experiment, thin smears were prepared from tail blood on microscopic slides, dried and fixed with methanol. The blood films were stained with Giemsa and examined under the microscope. The parasitemia was determined by counting minimum of three fields per slide with 100 RBC per field (Zucker and Campbell, 1993). The percentage suppression of parasitaemia was calculated for each test concentration by comparing the parasitaemia in infected controls with those received different concentrations of the test extract. Percent parasitemia and percent parasitemia inhibition (% PI) were calculated using the modified Peters and Robinson formula (1992):

$$Parasitemia = \frac{Number\ of\ parasited\ RBC}{Total\ Number\ of\ RBC\ examined} \times 100$$

and

$$\%PI = \frac{Parasitemia\ in\ negative\ control - parasitemia\ in\ treatment}{Parasitemia\ in\ negative\ control} \times 100$$

2.8. Determination of PCV

Blood was collected from tail of each mouse in heparinized microhaematocrit capillary tubes. The tubes were sealed by crystal seal and placed in a microhematocrit centrifuge (Hettich haematokrit, Germany) with the sealed ends out wards. The blood was centrifuged at 12,000 rpm

for 15 minutes. The packed cell volume (PCV) of each mouse was then measured before infection and on day 4 after infection using the formula:

$$PCV = \frac{Volume\ of\ erythrocytes\ in\ a\ given\ volume\ of\ blood}{Total\ blood\ volume\ examined} \times 100$$

2.9. Determination of Mean Survival Time

Mortality was monitored daily and the number of days from the time of incoculation of the parasite up to death was recorded for each mouse in the treatment and control groups throughout the follow up period. The mean survival time (MST) for each group was calculated as:

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

2.10. Fractionation of the more Active Extract

Aqueous crude extract of *Dodonaea angustifolia* had better antiplasmodial activity and was further fractionated by solvent fractionation technique. 14 g crude aqueous extract of *D. angustifolia was* dissolved in 350 ml distilled water. This was subjected to fractionation using two additional solvents of increasing polarity (chloroform and butanol). The chloroform and butanol fractions were concentrated in a Rota vapor to obtain the respective fractions. The aqueous residue was freeze dried to get the aqueous fraction.

2.11. Antimalarial Activity Test with Fractions

The chloroform, butanol and aqueous solvent fractions were evaluated for their antimalarial effects following similar methods as for the crude extracts. The three fractions were tested for their antiplasmodial activity at 50 mg/kg body weight.

2.12. Acute Toxicity Test

The study mice were fasted over night. The mice were then randomly assigned into 5 groups of 10 mice each (5 males and 5 females), caged separately and the weight of each mouse was determined. According to the method of Weil, (1952), the aqueous extract of *D. angustifolia*

which showed better anti-malarial activity was orally administered at doses of 2000, 3000, 4500 and 6750 mg/kg. A fifth group of 10 mice (5 males and 5 females) was orally administered with distilled water used as negative control. Signs of acute toxicity and mortality in each group within 24 hours were recorded.

2.13. Data Analysis

The data were calculated by using Microsoft-excel 2003, and expressed as mean ± SD for each dose level. Data were analyzed using SPSS version 13 for windows software. Statistical analysis was undertaken by one-way analysis of variance (ANOVA) tests coupled to Least Significant Difference (LSD) to compare result between doses and among treatment and control groups. Mean PCV and body weight before and after infection and treatment were compared by two-tailed paired *t*-test. The result was considered statistically significant at 95% confidence level and P-value<0.05. The LD_{50} was calculated with the aid of SPSS software employing probit regression analysis of the dose and the respective mortalities of the mice.

Table 2. Effect of crude extracts of *D. angustifolia* seed on body weight of *P. berghei* infected mice.

Treatments	***Dose (mg/kg)***	***Mean Body weight (g) ±SD***		
		D0	***D4***	***% change***
Hydroalcoholic extract	100	29.83±1.25	25.08±1.56	-15.92
	200	32.08±1.17	26.16±1.43	-18.45
Distilled wáter**	400	31.68±2.25	24.22±1.02	-23.55
Chloroquine*	1ml	30.23±1.13	26.08±1.26	-13.73
	10	29±2.6	27.55±2.47	-5.00
Aqueous extract	100	34±0.87	31.42±1.64	-7.59
	200	32.9±1.44	28.74±2.44	-12.64
	400	32.62±1.49	27.56±1.66	-15.51
Distilled wáter**	1ml	33.08±3.01	29.91±2.8	-9.58
Chloroquine*	10	28.12±2.85	28±2.06	-0.43

Note: Body weight expressed as mean ± SD, n=6; * positive control; ** negative control.

3. RESULTS

The result of general phytochemical screening of powdered plant materials of *Dodonaea angustifolia* showed the presence of many secondary metabolites, such as tannins, alkaloids, phytosteriods, saponnins and polyphenols. There was a significant ($p<0.05$) loss of body weight between days 0 and 4 in both negative control and extract treated groups (Table 2). Treatment

with crude extracts did not prevent body weight loss due to parasitemia. The extracts reduced body temperature as compared with the negative and positive controls (Fig. 1).

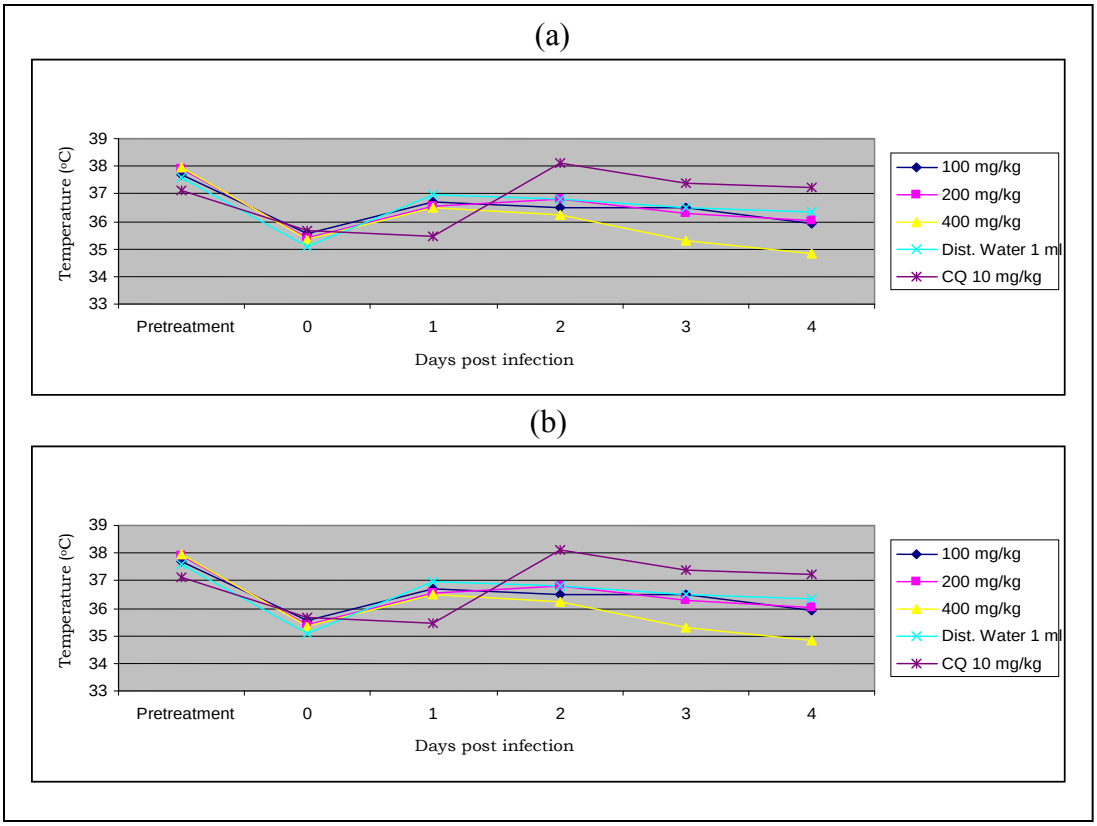

Figure 1. Effect of *D. angustifolia* seed (a) hydroalcoholic crude extract and (b) aqueous crude extracts on rectal temperature of *P. berghei* infected mice. (***Note***: Pretreatment: time of parasite inoculation; 0: three hours after parasite inoculation).

Table 3. Effect of crude extracts of *D. angustifolia* seed on parasitemia and mean survival time of *P. berghei* infected mice.

Treatments	Dose (mg/kg)	Mean Parasitemia±SD	Average chemo-suppression (%)	Mean Survival time (day) ±SD
Hydroalcoholic extract	100	37.25±3.98	3.45	9±0.89
	200	28.88±3.9	25.14	8.67±0.82
	400	26.45±3.51	31.44	8.17±0.41
Distilled Water**	1ml	38.58±2.33	0.00	7.67±0.52
Chloroquine*	10	0.00	100	14.83±2.23
Aqueous extract	100	31.58±4.39	17.07	9.67±2.25
	200	27.93±6.44	26.65	9.83±2.04
	400	24.45±4.61	35.79	10.5±2.07
Distilled Water*	1ml	38.08±0.66	0.00	8.5±0.55
Chloroquine*	10	0.00	100	14.33±1.51

Note: * positive control; ** negative control

The extracts prevented packed cell volume reduction (Fig. 2), and the mean PCV on days 0 and 4 did not show statistically significant difference ($p>0.05$). The aqueous extract of *D. angustifolia* was found to be more active than the hydroalcoholic extract on day 4 ($P < 0.05$) (Table 3) in reducing parasitemia. Parasitemia of the mice in the extract treated groups on day 4 is significantly lower ($p<0.05$) than that of the mice in the negative control group (Table 3).

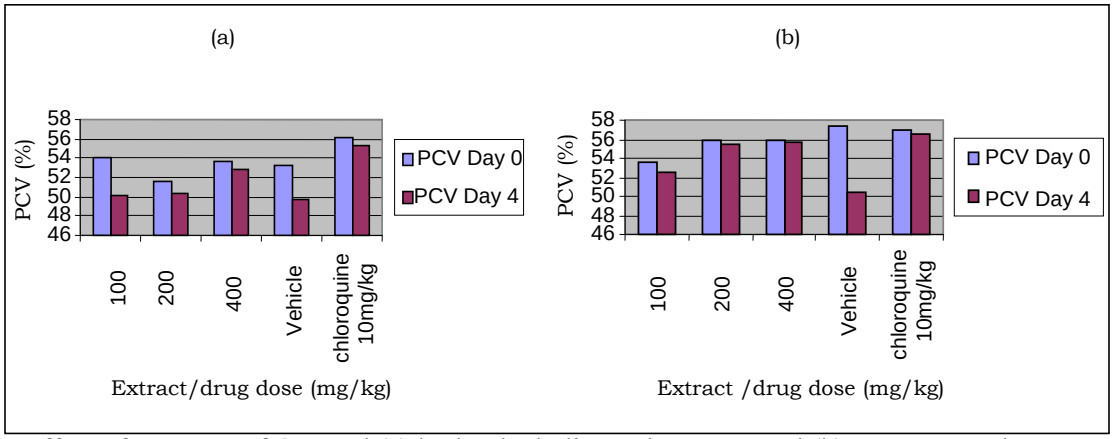

Figure 2. Effect of *D. angustifolia* seed (a) hydroalcoholic crude extract and (b) aqueous crude extracts on PCV of *P. berghei* infected mice on days 0 and 4.

Table 4. Effect of solvent fractions of *D. angustifolia* on body weight of *P. berghei* infected mice.

Fractions	***Dose (mg/kg)***	***Mean body weight (g) ±SD***		***% change***
		D0	***D4***	
Chloroform	50	29.18±1.73	28±1.23	-4.04
Butanol	50	30.58±1.94	29.26±2.87	-4.32
Aqueous residue	50	27.95±2.33	26.03±1.68	-6.87
Distilled water**	1ml	30.83±2.85	26.63±2.53	-13.62
Chloroquine*	10	27.55±3.54	27.08±2.65	-1.71

Chloroform and butanol fractions of *D. angustifolia* prevented body weight reduction significantly ($p< 0.05$) (Table 4). The prevention of body weight reduction by butanol fraction was dose dependent. The mice treated with the butanol fraction had significantly higher body weight ($p<0.05$) than the mice treated with aqueous fraction and the negative control. The mean rectal temperature of the study mice on day 4 in the extract treated and negative control groups was not different; however the mice treated with chloroform and butanol fractions had higher temperature than that of the aqueous fraction treated group (Fig. 3).

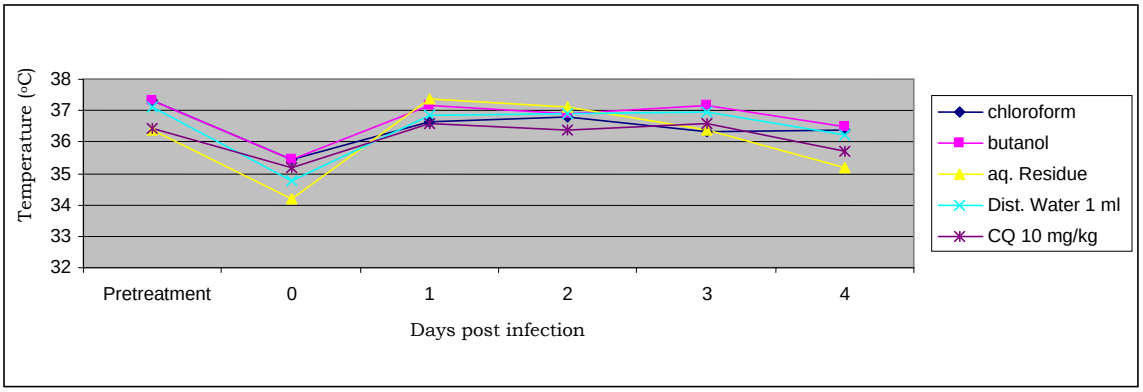

Figure 3. Effect of *D. angustifolia* seed aqueous extract fractions on rectal temperature of *P. berghei* infected mice (Note: Pretreatment: time of parasite inoculation; 0: three hours after parasite inoculation).

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The butanol fraction of *D. angustifolia* aqueous extract was the fraction with the highest parasite inhibition (Table 5). The butanol fraction of *D. angustifolia* aqueous extract prolonged survival time of the study mice (10.17 ± 2.93 days) as compared to that of the mice in the negative control group (8.33 ± 1.03 days). The % inhibition with butanol fraction was in a dose dependent manner. All the fractions prolonged the mean survival time of the treated mice as compared to the negative controls. However, none of the mice cured from the infection.

Table 5. Effect of *D. angustifolia* seed aqueous extract fractions on parasitemia and mean survival time of *P. berghei* infected mice.

Fraction	***Dose (mg/kg)***	***MeanParasitemia±SD***	***Average chemo suppression %***	***Mean Survival time (day) ±SD***
Chloroform	50	46.17±1.72	22.18	9±2.28
Butanol	50	33±4.65	44.38	10.17±2.93
Aqueous	50	51.5±9.91	13.2	8.17±0.75
water**	1ml	59.33±6.31	0.00	8.33±1.03
Chloroquine**	10	0.00	100	16±4.2

The chloroform and butanol fractions prevented PCV reduction. The aqueous residue did not prevent PCV reduction. The mice treated with butanol and chloroform fractions had higher PCV than the mice in the negative control and aqueous residue groups (Fig. 4).

The aqueous extract of *Dodonaea angustifolia* was tolerated by the study mice when administered orally. No death was observed in the animals receiving *D. angustifolia* aqueous extract up to a dose of 4500 mg/kg body weight which is about 11 times the maximum effective

dose tested (400 mg/kg). This shows that the LD_{50} for *D. angustifolia* is greater than 4500 mg/kg body weight.

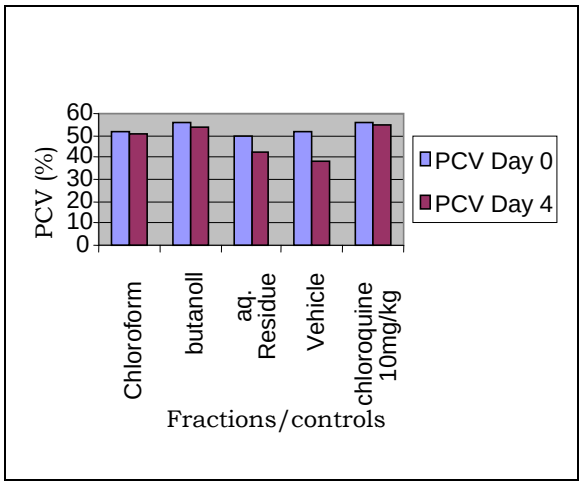

Figure 4. Effect of *D. angustifolia* seed aqueous extract fractions on PCV of *P. berghei* infected mice on days 0 and 4.

4. DISCUSSION

The extracts of *Dodonaea angustifolia* contain different secondary metabolites that have antiplasmodial activity in other plants (Abdulelah and Zainal-Abidin, 2007). Tannins (Asres et al., 2001b), alkaloids (Saxena et al., 2003), and phenols (Hilou et al., 2006) which have been suggested to be responsible for antiplasmodial activity of other plants were also detected in the seed of *D. angustifolia*. As the antiplasmodial activity observed in many plants (Okokon et al., 2005) was considered to result from single or combined action of these metabolites, same could be said for the present study.

The phenols present in this plant which have antioxidant effect (Alexandru et al., 2007) may also contribute to the antimalarial activity due to inhibition of haem polymerization (Taramelli et al., 1999). The phytosteroids and flavonoids detected in this plant could as well be responsible for the antimalarial effect as these metabolites have been proved to possess potential immunomodulatory effects in other plants (Aherne et al., 2007).

The loss of body weight in the extract treated mice was possibly due to appetite suppressant effect of the crude extracts. This result is in agreement with that of a previous study on other plants (Chinchilla et al., 1998). The result of the present study on body weight, however, is not in agreement with that of of Dikasso et al. (2006a).

A decrease in the metabolic rate of infected mice occurred before death and was accompanied by a corresponding decrease in internal body temperature (Hansen and Pappas, 1977). In this study, however, the extracts reduced body temperature as compared with the negative control and standard antimalarial drug. This might suggest that the extracts have hypothermic effect on the study mice.

Extracts of *D. angustifolia* seed showed anti-malarial activity against *P. berghei* infection in mice as evidenced by the percentage of parasite inhibition. As shown from the results of the *in vivo* antiplasmodial studies, aqueous extract of *D .angustifolia* exhibited higher suppressive activity on *P. berghei* than its hydroalcoholic extract. The plant extracts are less effective when compared to that of the standard antimalarial drug, chloroquine phosphate. The effects on parasitemia in this study are similar to the ones reported by previous studies such as on *Asparagus africanus* (Dikasso et al., 2006a), *Amarantus spinosus* (Hilou et al., 2006), *Withania somnifera* (Dikasso et al., 2006b) and *Clerodendrum myricoides* (Muregi et al., 2007). However, relatively higher antiplasmodial activities than the present result have been reported on *Nigella sativa* (Abdulelah and Zainal-Abidin, 2007), *Croton zambesicus* (Okokon et al., 2005), *Anonna senegalensis* (Ajaiyeoba et al., 2006), *Boscia angustifolia* (Muthaura et al., 2007), and *Azadirachta indica* (Valecha et al., 2001).

According to Taylor and Hurd, (2001), the effect of rodent malaria on PCV as measured by haematocrit was parasite-induced fall down to 43–44%, which occurred approximately 48 hours post-infection. *P. berghei* infected mice suffer from anaemia because of RBC destruction, either by parasite multiplication or by spleen reticuloendotelial cell action as the presence of many abnormal RBC stimulates the spleen to produce many phagocytes (Chinchilla et al., 1998). In this study, the extracts of *D. angustifolia* prevented significant PCV reduction in a dose dependent manner.

The extracts prolonged the mean survival time of the study mice indicating that the extracts suppressed *P. berghei* and reduced the overall pathologic effect of the parasite on the study mice. However, neither the extracts nor the standard drug cured the infection. This could be due to recrudescence of *P. berghei* parasites after apparent cure. Our result on mean survival time is in agreement with similar studies done on extracts of *Boscia angustifolia* (Muthaura et al., 2007), *Momordica foetida* (Waako et al., 2005), and *Nigella sativa* (Abdulelah and Zainal-Abidin, 2007).

Fractions of *Dodonaea angustifolia* aqueous extract showed varying degrees of malaria parasite suppression. The butanol fraction was found to be most active. This fraction also prevented body weight loss, temperature reduction, and PCV fall significantly. The mean survival time of mice treated with this fraction was longer than the mice treated with the other fractions. This might lead to the hypothesis that the active components responsible for the antiplasmodial activity of this plant might be more concentrated in this fraction. This may also suggest the potential for isolating pure compounds with much higher antimalarial activity (Bhat and Surolia, 2001). However, this is not always true as the antiplasmodial activity of plants could be due to synergistic activity of the components. Although medicinal plants are assumed to be safe, many of them are potentially toxic (Ajaiyeoba et al., 2006). The absence of mortality up to a dose equal to 11 times the maximum effective dose, and no serious signs of toxicity in aqueous extract of *D .angustifolia* may imply the fact that this plant could safely be used to treat malaria.

5. CONCLUSION

The results obtained from the present work support the traditional use of *Dodonaea angustifolia* against malaria and confirmed the antimalarial activity of the plant. *D. angustifolia* can be a potential source of lead molecule(s) for the development of a new antimalarial drug and alternative antimalarial drugs. Novel antimalarial compounds can also be isolated from the most active fraction of *D. angustifolia* and tested against human malaria parasites in the future. Prior to recommendation for its application, however, further studies have to be conducted on its antiplasmodial activity as well as safety profile.

6. ACKNOWLEDGEMENT

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