Full Length Research Paper

In vivo antimalarial activity of hydromethanolic leaf extract of *Calpurnia aurea (Fabaceae)* in Mice infected with chloroquine sensitive *Plasmodium berghei*

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Malaria is one of the most serious health problems worldwide and treatment has been compromised by drug resistance. Consequently, efforts are directed towards discovery of novel agents including from medicinal plants. The present study was aimed to evaluate the suppressive, curative and prophylactic activity of hydroalcoholic leaf extract of *Calpurnia aurea* in mice infected with chloroquine sensitive *Plasmodium berghei (*1x10^{7}parasites/mouse). The extract (15, 30 and 60 mg/kg), chloroquine (5 mg/kg) and distilled water (0.2ml/day) were administered orally for four days. Then parasitemia, packed cell volume, body weight, rectal temperature and survival time of mice were monitored. The 60 mg/kg dose in 4-day suppressive, curative and prophylactic tests had maximum parasitemia chemosuppression of 51.15, 47.77 and 36.8% (P<0.001 in all cases) respectively with significant effect on survival time (4-day suppressive test) compared to negative control. In 4-day suppressive test, the extract had prevented packed cell volume reduction at 15 and 30 mg/kg doses (P<0.05) compared to negative control. In prophylactic test, extract doses at 15mg/kg (p<0.001) and 60mg/kg (p<0.05) prevented body weight loss compared to negative control. The present work establishes antiplasmodial activity of the plant which can be a potential source of new chemotherapeutic and/or chemoprophylactic compounds.

Key words: antimalarial activity, *Calpurnia aurea*, *Plasmodium berghei*, Mice, *in vivo.*

INTRODUCTION

The number of malaria-related deaths is increasing and one key factor linked to this, is widespread drug resistance to most of the commonly available antimalarial drugs which is greatest challenge against malaria control (Trape *et al.*, 1998; Mendis *et al.*, 2001). Important attributes for the successful implementation of antimalarial drugs are good tolerability and safety, affordability, availability in endemic countries and short course regimens (Petersen *et al.*, 2011). The drug resistance of the malaria parasite is widespread, no new chemical class of antimalarials has been introduced into clinical practice since 1996 and there has recently been an increase in parasite strains with reduced sensitivity to the newest drugs. Several excellent reviews on anti-plasmodial compounds, including natural products, have been published in recent years (Claudio *et al.*, 2011). The wide spread use of traditional medicine could be attributed to cultural acceptability, physical accessibility and economic affordability and efficacy against certain types of diseases, as compared to modern medicine (Deribe *et al.*, 2006).

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 anti-plasmodial compounds (Tekalign *et al.*, 2010). Since malaria is a serious disease in Ethiopia and many developing countries, the list of traditionally used plants to control it must be backed by phytochemical studies to develop an appropriate phytomedicine (Endashaw, 2007). Studies conducted on antimalarial activity of several traditionally claimed Ethiopian medicinal plants

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indicated significant antimalarial effect (Dikasso *et al.*, 2006; Assefa *et al.*, 2007; Mengistie *et al.*, 2012).

Calpurnia aurea is used for wound healing, treatment of diarrhoea, leishmaniasis, tapeworm, trachoma, scabies, elephantiasis, swellings and antibacterial and antioxidant activity has been also reported (Adedapo *et al.*, 2008). *Calpurnia aurea* is known by several local names, chekata in Afaan Oromo and digita in Amharic. The root of *Calpurnia aurea* is claimed against amoebiasis and giardiasis, the leaf is used against malaria, leaf together with seed is used against diarrhoea, rabies and diabetes and seed is used against hypertension (Giday *et al.*, 2007). There is no any scientific/or experimental evaluation report published on the antimalarial activity of *Calpurnia*.

Therefore, the present study describes the malarial suppressive, curative and prophylactic activities of hydromethanolic leaf extract of *Calpurnia aurea* using mice infected with chloroquine sensitive *Plasmodium berghei.*

MATERIALS AND METHODS

Chemicals

The chemicals used were absolute methanol (Reagent Chemical Limited, China), geimsa stain 10% (Shenyang Xin Guang, China), chloroquine phosphate 250mg/kg (Ethiopian Pharmaceutical Manufacturing, Ethiopia), Dragendrof's reagent, Mayer reagent, concentrated sulfuric acid, glacial acetic acid, 5% ethanolic ferric chloride, 10% ethanolic ferric chloride, dilute ammonia, acetic anhydride, 1% aqueous hydrochloric acid, chloroform (BDH, Poole, England).

Plant preparation

Fresh leaves of *Calpurnia aurea* were collected from central Addis Ababa between February and March, 2012 and taxonomically identified at National Herbarium, Biology Department, Addis Ababa University. The fresh leaves were cleaned and dried at room temperature and powdered by using pestle and mortar. About 100 g of powdered leaves was macerated for 72 hrs in 1000 ml of 80% methanol in distilled water. After 72 hours, the crude extract was first filtered by using gauze then by Whatman Number 1 filter paper. Methanol was removed from the extract under reduced pressure by rotary evaporator (Buchi Rota vapor, Germany) at 40 ^{0}C to obtain the crude extract.

The filtrate was kept in an oven (Gallenkamp, England) at the temperature not exceeding 40 ^{0}C overnight and then freeze dried with a lyophilizer. A total of 22.42 g of extract was harvested from 191 g of air dried leaves of *Calpurnia aurea* giving a percentage yield of 11.74%.

Dosing of animals and parasite inoculation

The Swiss albino male mice aged 6-8 weeks weighing 25-35g were used for the whole study. The animals were acclimatized for one week to the experimental environment and provided with a commercial pellet and water ad libitum. The animals were used according to guideline for use and care of animals (National Research Council, 1996). Chloroquine sensitive *Plasmodium berghei (*ANKA strain) was used and maintained by serial blood passage in mice. The donor mice with parasitemia level of approximately 24% were used to infect mice in 4-day suppressive and prophylactic test while parasitemia level of 27% was used to infect mice in curative test. Donor mice were sacrificed and cardiac puncture was made to collect blood for mice infection. Then each mouse was inoculated intraperitoneally with 0.2ml of blood suspension containing about 1×10^7 *P. berghei* parasitized erythrocytes (Kalra *et al.*, 2006). For all experimental models, mice were randomized into five grouped (n=5). The groups I, II and III were orally administered with 15, 30 and 60 mg/kg body weight of extract respectively. Group IV was administered orally with standard (chloroquine 5mg/kg body weight) and Group V (negative control) was treated with 0.2ml distilled water.

Phytochemical screening

The phytochemical screening of leaf extract of *Calpurnia aurea* was carried out using standard procedures to identify the constituents as described by Sofowara (1993) and WHO (1978).

Acute toxicity testing

Acute oral toxicity of hydromethanolic leaf extract of *Calpurnia aurea* was evaluated in female mice aged of 6-8 weeks according to OECD guideline No 425 (OECD, 2008). The limit test dose of 2000 mg/kg was orally administered sequentially to three female mice and mortality was observed in all mice. According the OECD up and down procedure guideline, the dose was tapered to 300 mg/kg and administered and mice were observed for 24 hrs and then for 14 days. The acute toxicity study indicated that the extract did not cause mortality of mice within 24 hrs up to 300 mg/kg body weight dose. After 14 days of observation of the experimental mice, no body weight reduction was observed. Gross physical and behavioral observation also revealed no visible signs of acute toxicity.

The 4-day suppressive test

Evaluation of suppressive effect of the extract on early infection against chloroquine sensitive *P. berghei* infection

in mice was employed as described by (Peter *et al.*, 1975). The mice were randomly grouped into five groups (n=5) and inoculated with parasite as described above. Groups I, II, III were orally administered with 15, 30 and 60 mg/kg body weight of extract respectively. Group IV was administered orally with chloroquine (5 mg/kg body weight) for four consecutive days while Group V was administered orally with vehicle (0.2ml distilled water) for the same days. All treatments started 2 h post-infection. Body weight and packed cell volume were measured at day 0 and day 4 but rectal temperature was measured daily starting from day 0 till day 4. Parasitemia was measured microscopically on the 5th day from geimsa stained blood films. The mice were followed after infection till their death and survival time was recorded.

Curative test

Evaluation of the curative potential of leaf extract against established infection was carried out as described by (Ryley and Peters, 1970). Briefly, the mice were inoculated with parasites as described above and left untreated until the fourth day post-inoculation. Groups I, II, III were orally administered with 15, 30 and 60mg/kg body weight of extract respectively for four consecutive days (D_4-D_7). Positive and negative controls were treated as described above. Parasitemia level was determined from geimsa stained blood films. Parasitemia count and its mean parasitemia inhibition were recorded on day 0 and on day 4. Rectal temperature was measured daily using digital rectal thermometer while PCV and body weight were also measured at day 0 and day 4. Each mouse was followed after treatment till their death and survival time was recorded.

Prophylactic activity

The prophylactic activity of the extract was tested using the residual infection procedure described by (Peters, 1965). Groups of mice were randomized into five groups (n=5). Groups I, II, III, were orally administered daily with 15, 30 and 60 mg/kg body weight of extract respectively. Positive and negative controls were administered orally with chloroquine and vehicle. All treatments were given daily for four days and all mice were infected with the parasite on the 5th day. Thin blood films were prepared from each mouse after 72 hours of infection on day 8 and mean parasitemia was determined in each group microscopically. Body weight and packed cell volume was measured on day zero and on day four. Rectal temperature was measured daily started before inoculation till day 4. Each mouse was followed after infection till their death and survival time was recorded.

Determination of packed cell volume

Packed cell volume was measured using Wintrobe's method

(Gilmour and Sykes, 1951). Briefly, the capillary tubes were filled with blood to $3/4^{th}$ of their original height and sealed at their dry end with sealing clay. The tubes were placed in micro-hemeatocrit centrifuge with the sealed end facing the periphery and centrifuged for 5 minutes at 11,000 rpm. Finally, PCV was determined using the standard hemeatocrit reader (Hawksley Micro-Hemeatocrit Reader, England). PCV test was done from each mouse just before infection and on the first day after the end of treatment.

Parasitemia determination

Thin smears of blood were obtained from the peripheral blood on the 5th day for 4-day suppressive and curative tests and on day 8 for prophylactic test. The blood smears were applied on microscope slides fixed with absolute methanol for 10 seconds and stained with 10% geimsa stain at pH 7.2 for 15 minutes. The slides were moderately washed with running water and dried at room temperature. The number of parasitized red blood cells were counted using Olympus microscope with an oil immersion nose piece of 100x magnification power. An average of six fields counted was taken and percentage parasitemia was determined.

Data analysis

The data were expressed as mean ± standard error of mean (SEM). The differences between means of measured parameters were compared using one way analysis of variance (ANOVA) using SPSS Windows version 16.0 statistical package followed by Post-hoc test (Tukey method) multiple comparison and paired sample t-test (2-tailed). The P values <0.05 were regarded as significant.

RESULTS

Phytochemical screening

The result of preliminary phytochemical screening of powdered plant material of *Calpurnia aurea* showed the presence of several secondary metabolites including alkaloids, cardiac glycosides, flavonoids, phenols, phytosteriods, saponins, terpenoids and tannins.

Effect of extract on parasitemia

The suppressive, curative and prophylactic effects of hydromethanolic leaf extract of *C. aurea* were evaluated at three dose levels (15, 30 and 60mg/kg body weight). The result of 4-day suppressive test indicated that all dose

Table 1. Parasitemia profiles and survival time of mice infected with *P. berghei* and treated with various doses of *C. aurea* in 4-day suppressive test.

Treatment	Dose	% Parasitemia	% Suppression	Survival time (days)
Distilled water	0.2ml	67.44±5.95	0	6.40±0.55
C. aurea leaf extract	15mg/kg	36.32±2.03[a1]	46.14	7.40±1.51
	30mg/kg	38.21±2.63[a1]	43.34	8.20±0.84
	60mg/kg	32.94±1.44[a1]	51.15	9.60±0.55[a2]
Chloroquine	5mg/kg	0.00[a1,b1]	100	12.20±2.17[a2,b2]

Data are mean+SEM; n=5; a= compared to negative control; b= compared to all extract doses; 1= P<0.001; 2= p<0.05.

Table 2. The effect of hydromethanolic leaf extract of *Calpurnia aurea* on body weight of *P. berghei* infected mice in 4-day suppressive test.

Treatment	Dose	Body weight(g)		% change
		D0	D4	
Distilled water	0.2ml	30.58±0.9	29.27±1.14	- 4.51
C. aurea leaf extract	15mg/kg	26.25±0.45	25.80±0.43	-1.71
	30mg/kg	28.94±1.22	27.96±1.55	-3.31
	60mg/kg	30.6±1.20	30.26±1.17	-1.11
Chloroquine	5mg/kg	26.66±0.52	26.68±1.14[a1]	0.08

Data are mean ± *SEM*; n=5; a= compared to negative control; 1= P<0.05.

levels of the extract produced significant (p<0.001) parasitemia reduction compared to negative control (Table 1). The 15 and 60 mg/kg body weight of extract resulted in higher anti-plasmodial activity (46.14 and 51.15%, respectively), followed by the 30 mg/kg body weight (43.34%). The 60 mg/kg body weight of extract prolonged survival time of the mice (9.6 ± 0.55days)

compared to negative control (6.40 ± 0.55days). There was no significant difference in parasitemia reduction among extract doses.

In a curative test (Table 3), all the three extract doses had shown statistically significant (p<0.001) parasitemia suppression as compared to negative control. The parasitemia suppression was 37.53, 35.69 and 47.77% for

Table 3. Curative effect and survival time of hydromethanolic leaf extract of *C. aurea* in *P. berghei* infected mice.

| Treatment	Dose	% Parasitemia		% suppression	Survival time (days)
		Pre-(D3)	Post-(D7) treatment		
Distilled water	0.2ml	54.76±3.77	76.20±1.65	0	8.00±0.00
C. aurea leaf	15mg/kg	55.26±1.55	47.60±1.04[a1,c3]	37.53	8.00±0.40
extract	30mg/kg	55.06±3.34	49.00±0.80[a1,c1]	35.69	8.00±0.40
	60mg/kg	52.86±1.28	39.8±1.40[a1]	47.77	10.00±0.45
Chloroquine	5mg/kg	49.9±3.19	0.00[a1,b1]	100	16.20±1.48[a2,b2]

Data are mean±SEM; n=5; D3= Day three; D7= Day seven; a= compared to negative control; b= compared to all extract doses; c= compared to 60mg/kg; 1= p<0.001; 2= p<0.05; 3= p= 0.001.
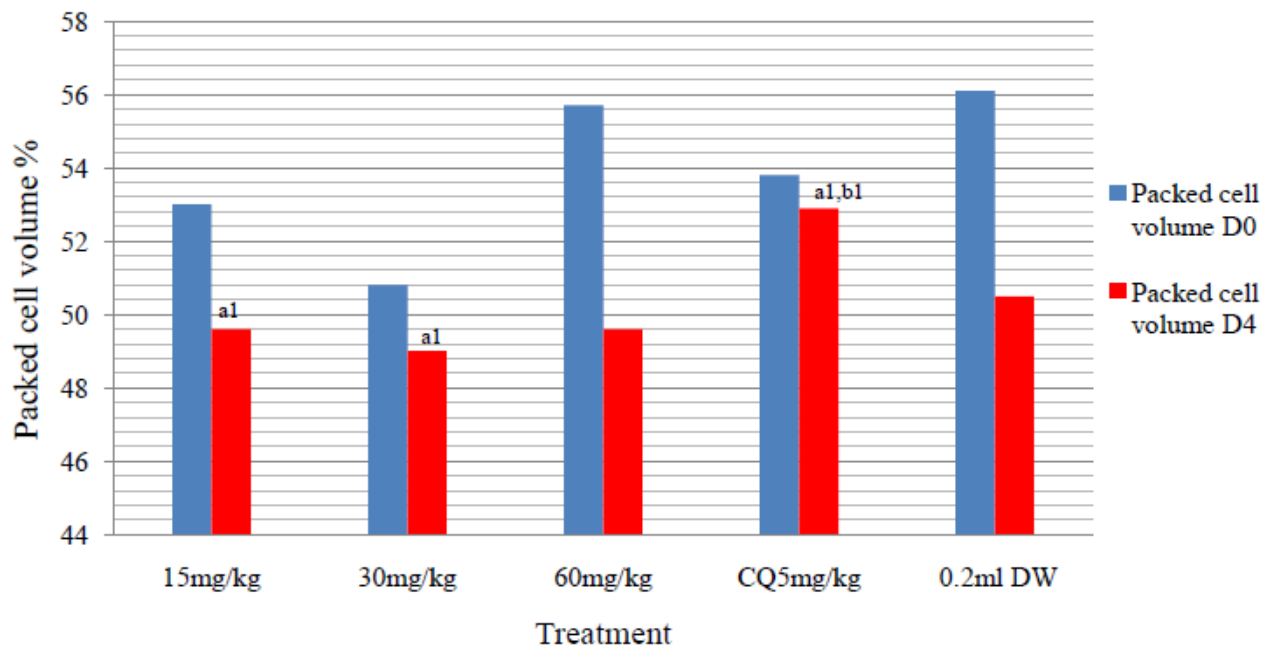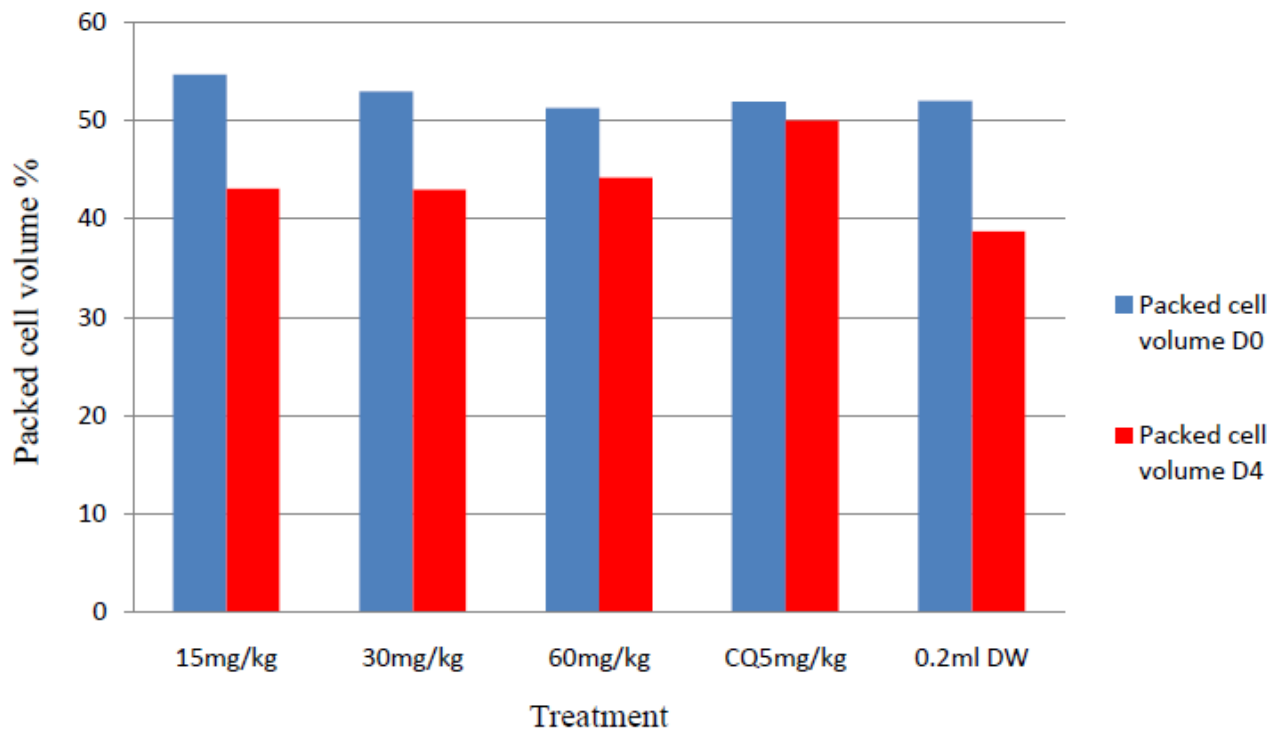

Figure 1. The effect of hydromethanolic leaf extract of *Calpurnia aurea* on packed cell volume of *P. berghei* infected mice on days 0 and 4 in 4-day suppressive test. Data are mean±SEM; n=5; DW= distilled water; D0= day 0; D4= day 4; a= compared to negative control; b= compared to all extract doses; 1= P < 0.05.

15, 30 and 60 mg/kg body weight doses respectively. Comparison among the extract dose levels indicated that the 60 mg/kg body weight dose had shown a statistically significant parasitemia inhibition compared to 15 and 30 mg/kg extract doses. However, all dose levels of the extract did not show significant prolongation of survival time when compared to negative control.

In prophylactic test, the extract of *C. aurea* displayed prophylactic activity resulting in significant (p<0.001) parasitemia inhibition at 15, 30 and 60 mg/kg body weight of extract when compared to negative control with chemosuppression rates of 32.8, 25.46 and 36.8% respectively. The survival time was prolonged only in the group treated with 60 mg/kg body weight of extract (Table 5).
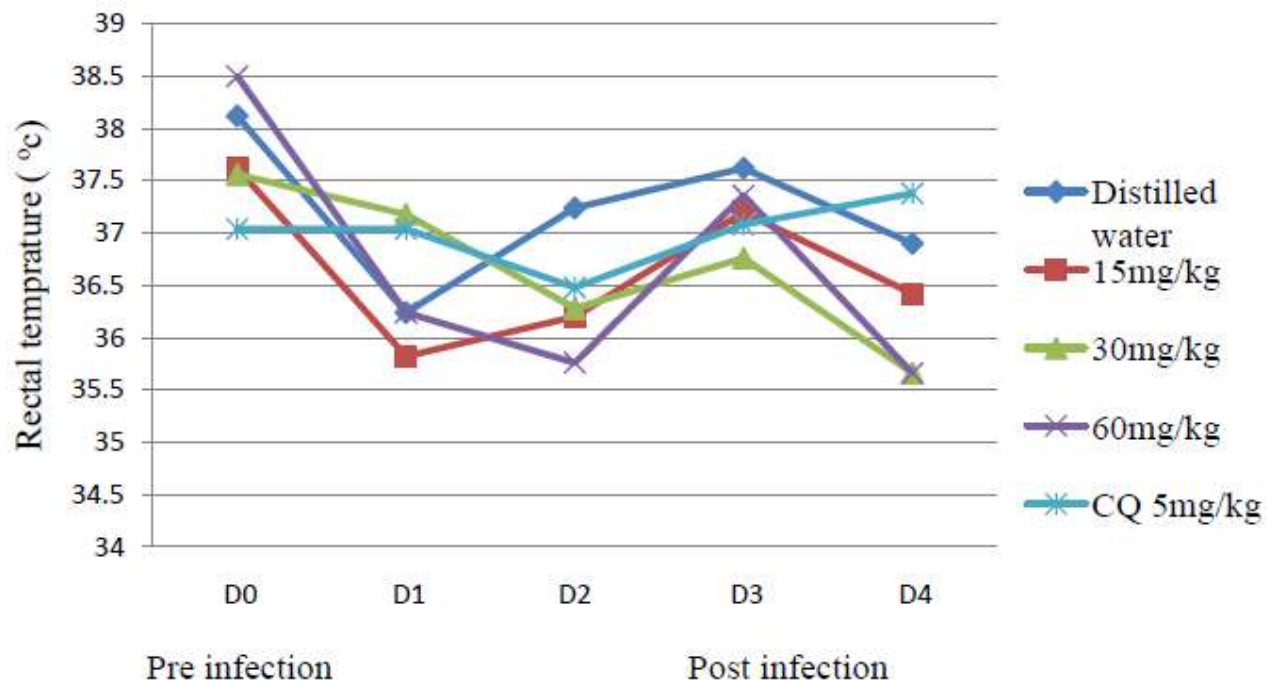
Figure 2. The effect of hydromethanolic leaf extract of *Calpurnia aurea* on rectal temperature of *P. berghei* infected mice in 4-day suppressive test. Data are mean±SEM; n=5.

Figure 3. The effect of hydromethanolic leaf extract of *Calpurnia aurea* on packed cell volume (PCV) of *P. berghei* infected mice on day 0 and 4 in curative test. Data are mean±SEM; n=5; DW= distilled water; D0= day zero; D4= day four.

Effect of crude extract on packed cell volume

In 4 day suppressive test, the effect of leaf extract of *Cal-* *purnia aurea* on PCV is indicated in Figure 1. The extract at dose levels of 15 and 30 mg/kg body weight had shown some activity on prevention against PCV reduction

Table 4. The Effect of hydromethanolic leaf extract of *Calpurnia aurea* on body weight of *P. berghei* infected mice in curative test .

Treatment	Dose	Body weight(g)		% Change
		D0	D4	
Distilled water	0.2ml	28.58±0.24	22.76±0.80	-25.57
C. aurea leaf extract	15mg/kg	28.70±0.68	24.66±0.65	-16.38
	30mg/kg	31.44±1.56	26.46±1.64	-18.01
	60mg/kg	28.14±0.66	24.63±0.37	-14.25
Chloroquine	5mg/kg	28.72±1.11	27.88±0.80[a1]	-3.01

Data are mean±SEM; n=5; a= compared to negative control; 1= p<0.05.

when compared to negative control but 60 mg/kg body weight of extract had not shown a significant (p>0.05) prevention activity against PCV reduction compared to negative control.

In curative test, the effect of extract on packed cell volume is indicated in Figure 3. There was no statistically significant difference in the mean PCV on days 0 and 4 of all dose levels when compared to negative control group indicating that the extract did not prevent significantly PCV reduction. In prophylactic test, similar to the curative test, all the extract doses did not show significant protection against the PCV reduction but chloroquine was significant (p<0.05) when compared to negative control (Figure 5).

Effect of the extract on body weight

In 4-day suppressive test and curative test, all dose levels of extract of *C. aurea* had not shown prevention against body weight loss when compared to negative control (Tables 2 and 4). Both extract treated and the negative control mice had lost some of their body weight but still, the extract treated mice had better body weight status than negative control. Chloroquine had prevented body weight reduction significantly (p<0.05) when compared to negative control.

In prophylactic test, the extract had significant prevention at 15 mg/kg (p<0.001) and 60 mg/kg (p<0.05) against body weight loss compared to negative control. When compared among the extract doses, the 15 mg/kg extract showed significant difference (p<0.05) compared to 30 mg/kg (Table 6).

Effect of extract on body temperature

In all three models, the hydromethanolic leaf extract of *C. aurea* did not cause significant prevention of rectal temperature reduction of *P. berghei* infected mice. The standard drug, chloroquine 5 mg/kg had showed significant activity in prevention against rectal temperature reduction when compared to extract treated and negative control (Figures 2, 4, 6).

DISCUSSION

Malaria is one of the most important infectious diseases in the world. Currently, antimalarial drug resistance has become one of the most important challenges to malaria control efforts (Abdulelah *et al.*, 2011; Hossein *et al.*, 2012). This has led researchers to look for other alternatives including investigation of medicinal plants. Sourcing of Artemisinin from *Artemisia annua* has further encouraged plants frequently used in the traditional management of the disease (Maje *et al.*, 2007). The present study was aimed to determine the *in vivo* anti-plasmodial activity of hydromethanolic leaf extract of *C. aurea* in *P. berghei* infected mice using 3 models; 4-day suppressive, curative and prophylactic tests.

The studies on acute toxicity revealed absence of mortality up to the dose level of 300 mg/kg body weight of extract administered orally, which is the second single high dose, recommended by OECD guidelines No 425 for testing acute toxicity-up-down procedure (UDP) when death is observed in the maximum dose 2000 mg/kg body weight within 24 hours. Therefore, the experimental

Table 5. Prophylactic effect and survival time of hydromethanolic leaf extract of *C. aurea* in *P. berghei* infected mice.

Treatment	Dose	% Parasitemia On D8	% Suppression	Survival time (days)
Distilled water	0.2ml	65.20±2.15	0	7.00±0.42
C. aurea leaf	15mg/kg	43.8±4.09[a1]	32.8	8.00±0.29
extract	30mg/kg	48.6±2.25[a2]	25.46	7.00±0.57
	60mg/kg	41.20±0.73[a1]	36.8	8.00±0.72
Chloroquine	5mg/kg	6.00±2.35[a1,b1]	91	10.00±0.56

Data are mean+SEM; n=5; D8= day eight; a= compared to negative control; b= compared to all extract doses; 1=P<0.001; 2=p=0.001.

Table 6. The effect of hydromethanolic leaf extract of *C. aurea* on body weight of *P. berghei* infected mice in prophylactic test.

| Treatment	Dose	Body weight(g)		% Change
		D0	D4	
Distilled water	0.2ml	26.60±0.81	23.20±0.49	-14.65
C. aurea leaf extract	15mg/kg	31.56±0.73	30.72±0.72[a1]	-2.73
	30mg/kg	27.08±0.77	25.00±0.45[b2]	-8.32
	60mg/kg	29.92±0.72	27.98±0.71[a2]	-6.93
Chloroquine	5mg/kg	30.14±1.83	29.37±1.99[a1]	-2.62

Data are mean±SEM; n= 5; D0= Day zero; D4= Day four; a= compared to negative control; b = compared to 15mg/kg; 1= p<0.001; 2= P<0.05.

plant was safe on treated doses. The acute toxicity result of this study suggested that the oral medial lethal dose (LD50) of the extract could be greater than 300 mg/kg body weight of extract as per the OECD guidelines. The experimental determination of lack of acute toxicity at dose of 300 mg/kg body weight of extract would justify the use of the plant extract for malaria treatment.

The phytochemical screening of hydromethanolic leaf extract of *C. aurea* indicated the presence of alkaloids, flavonoids, terpenoids, phenols, phytosteriods, saponnins, tannins and cardiac glycosides. As explained by Dharani *et al.* (2010), common antimalarial plants used to treat malaria in traditional medicine contain secondary metabolites such as alkaloids, terpenoids, coumarins, flavonoids, chalcones, quinones and xanthones. In addition to this, alkaloids and terpenoids have been implicated in anti-plasmodial activity in previous study (Okokon and Nwafor, 2009).
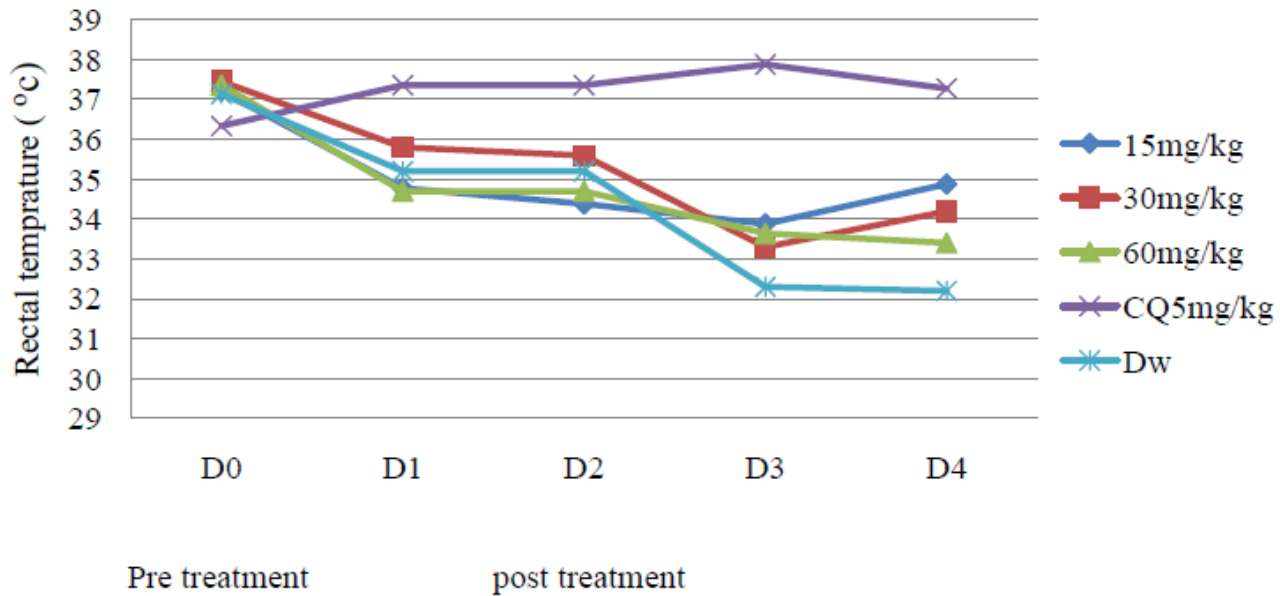
Figure 4. The effect of the hydromethanolic leaf extract of *C. aurea* on rectal temperature of *P.berghei* infected mice in curative test. Data are mean+SEM; n=5.
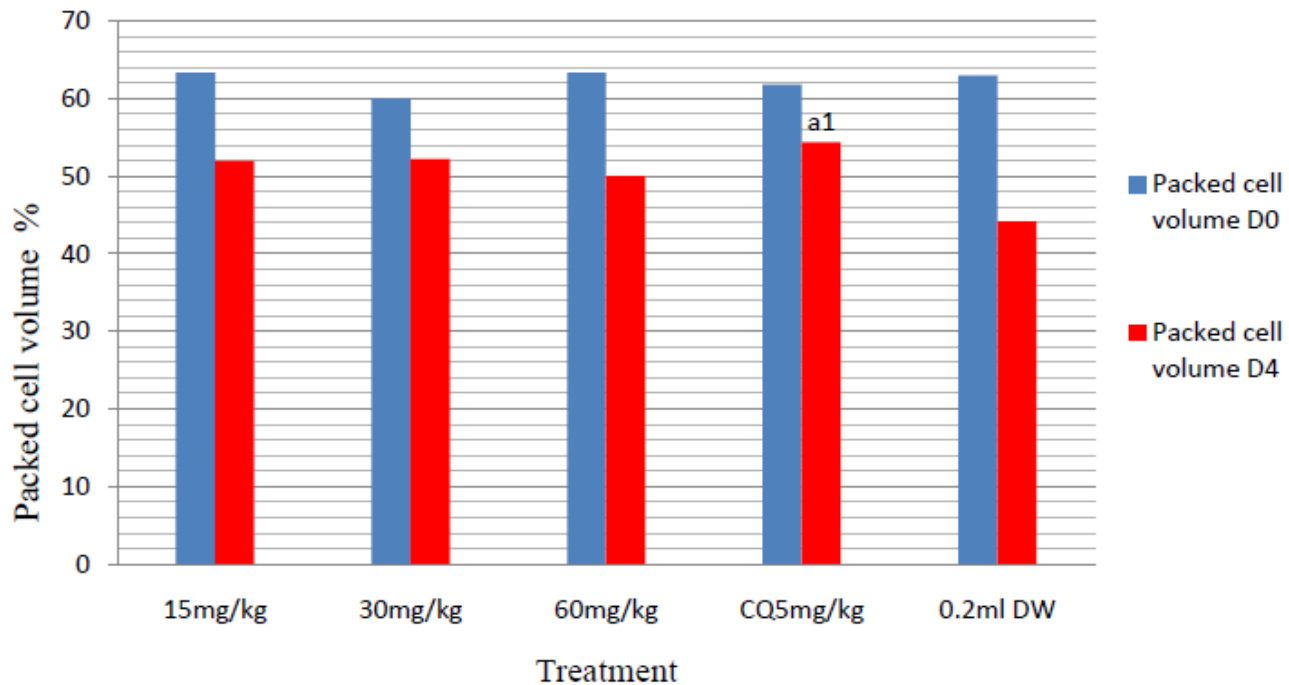
Figure 5. The effect of hydromethanolic leaf extract of *C. aurea* on packed cell volume (PCV) of *P. berghei* infected mice in prophylactic test. Data are mean±SEM; n=5; DW= distilled water; D0= day zero; D4= day four; a= compared to negative control; 1= $P<0.05$.

Flavonoids are reported to have significant anti-parasitic activities against different parasite strains of malaria, trypanosome and leishmania (Abdulelah *et al.*, 2011). The presence of tannins in the extract is likely to be responsible for the free radical scavenging effects (Ayoola *et al.*, 2008). This is in line with the *in vitro* study of methanol extracts of stem and leaf of *Calpurnia aurea* possess antioxidant properties and could serve as free radical inhibitors or scavenger or, acting possibly as primary antioxidant (Adedapo *et al.*, 2008). Phenols present
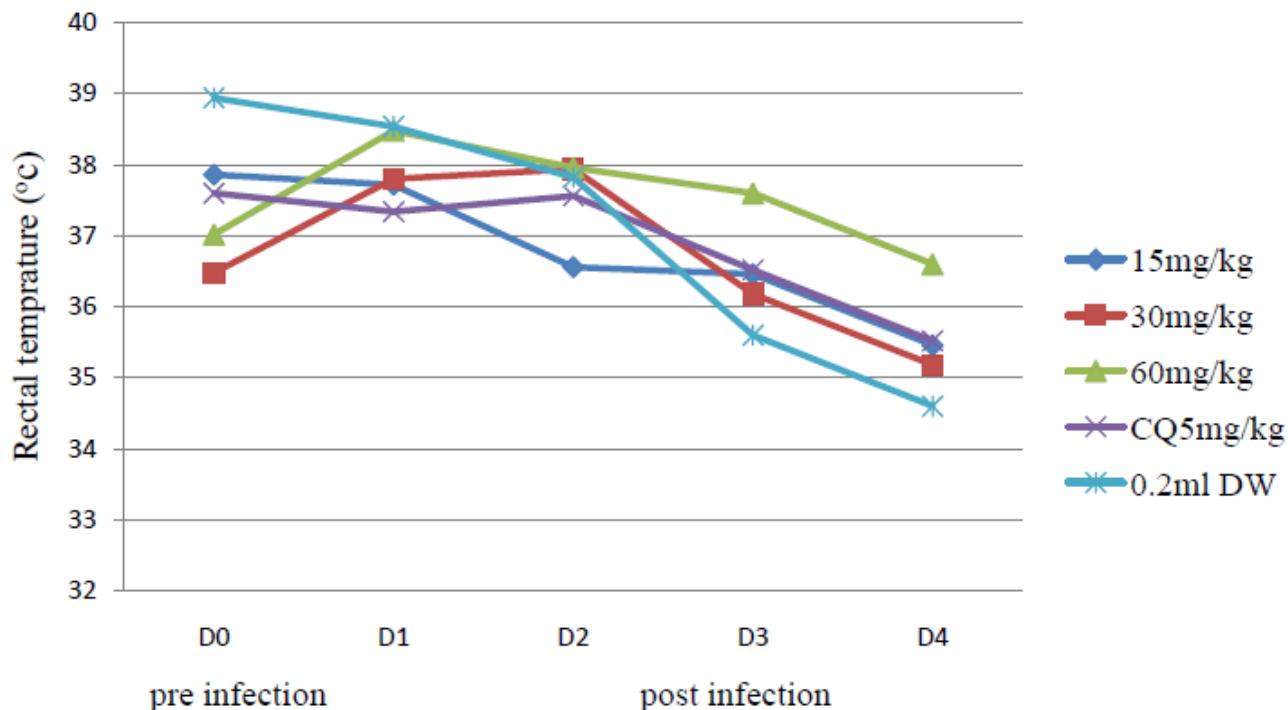
Figure 6: The effect of hydromethanolic leaf extract of *C. aurea* on rectal temperature of *P. berghei* infected mice in prophylactic test. Data are mean±SEM; n=5.

in this plant have also antioxidant effect (Alexandru *et al.*, 2007) and may also contribute to the antimalarial activity. Antioxidative activity can inhibit heme polymerization as heme has to be oxidized before polymerization, and the unpolymerised heme is very toxic for the intraerythrocytic plasmodia (Taramelli *et al.*, 1999).

Although the active compound is not identified, the results obtained from the present study showed that the hydromethanolic leaf extract of *C. aurea* possess significant suppressive effect against early *Plasmodium* infection, curative effect against established infection and prophylactic effect against residual infection in *P. berghei* infected mice, and this could probably have resulted from a single or combined effect of the secondary metabolites.

The evaluation of anti-plasmodial activity of hydromethanolic leaf extract of *C. aurea* on early malaria infection, 4-day suppressive test, had shown statistically significant parasitemia suppression of 51.15% in the highest dose, with the longest survival time compared to all the extract treated mice and negative control. Both the highest parasitemia chemosuppression and survival time suggested that 60mg/kg body weight of extract might be the optimal therapeutic dose in mice. The 60 mg/kg body weight of extract prolonged the mean survival time of the study mice indicating that the extract suppressed *P. berghei* and reduced the overall pathologic effect of the parasite on the study mice. However, neither the extract nor the standard drug cured the infection.

This could be due to recrudescence of *P. berghei* parasites after apparent cure. The 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). According to Deharo *et al.* (2001) and Munoz *et al. (*2000), an *in vivo* anti-plasmodial activity can be classified as moderate, good and very good if an extract displayed percent parasitemia suppression equal to or greater than 50% at a dose of 500 mg, 250 mg and 100 mg/kg body weight per day, respectively. Based on this classification, although the parasitemia chemosuppression was fairly below 50% at the lower doses (15 and 30 mg/kg body weight), the optimum dose has provided a drastic parasite suppression (>50%). It can thus be generally concluded that the hydromethanolic leaf extract of *C. aurea* exhibited a very good anti-plasmodial activity.

During the 4-day suppressive test, the effect of the hydromethanolic leaf extract of *C. aurea* on the packed cell volume (PCV) was tested and effect was variable depending on dose of the extract. The 15 and 30 mg/kg body weight of extract had shown prevention against PCV reduction significantly (p<0.05), this is due to the effect of the extract in preventing PCV on early infection, while 60 mg/kg body weight of extract did not prevent PCV reduction, this might be due to high level of saponins in this dose relative to the other lower doses. Saponins are known to cause hemolysis by increasing the permeability of the plasma membrane of the RBC and thereby reduce PCV (Jones and Kinghorn, 2006). Besides, the actual values of PCV are reported to be affec-

ted by nutrition status, acute and chronic blood loss, immune mediated diseases, seasonal variations, sex, age and dehydration (Ayo *et al*., 2001). Chloroquine seemed to have a good protective effect in preventing PCV reduction compared to extract treated groups and negative control.

In 4-day suppressive test, it was only chloroquine treated mice that prevented body weight loss significantly (P<0.05) but all the extract treated mice had not shown significant (P>0.05) prevention against body weight loss when compared to negative control. Body weight loss in extract treated mice might be possibly due to depressing effect of the crude extract on feed intake or appetite and this result is in agreement with that of a previous study on other medicinal plant (Mengistie *et al*., 2012). The extract doses did not prevent against rectal temperature reduction significantly compared to negative control and standard drug, chloroquine (CQ5mg/kg). This is attributed to the effect of the extract as it may have less amount of hypothermic effect on the extract treated mice. The extract treated mice had shown more prevention of rectal temperature reduction than negative control even though it was not statistically significant.

In curative test, the anti-plasmodial effect of the hydromethanolic leaf extract of *C. aurea* on the established malaria infection had shown a significant (p<0.001) parasitemia chemosuppression with maximum of 47.77% in the dose of 60 mg/kg body weight of extract (Table 3). The longest mean survival time of the mice was strongly associated with the maximum parasitemia inhibition and this was in agreement with other *in vivo* antimalarial test (Abdulelah *et al*., 2011).

Some traditional plants which showed some anti-plasmodial activity in 4-day suppressive and curative test, also showed prophylactic activity against the *P. berghei* parasite with minimum effect. Therefore, this study is also in line with other study done on other traditional plant against this rodent parasite (Godwin *et al*., 2011).

The prophylactic effect of the hydromethanolic leaf extract of *C. aurea* had shown anti-plasmodial activity against *P. berghei* infected mice with maximum parasitemia chemosuppression of 36.8% in the dose of 60 mg/kg body weight dose. Unlike 4-day suppressive and curative test, chloroquine 5 mg/kg body weight did not show 100% parasitemia eradication rather it inhibited 91% (Table 5).

The body weight gained in prophylactic test at dose of 15 and 60 mg/kg was might be due to decrease in appetite suppression effect of the crude extract on residual infection since it was given for four days prior to infection. Conversely, the 30 mg/kg dose did not show increase in body weight and this could be as a result of having the highest parasitemia level or the lowest parasitemia chemosuppression compared to the other extract doses but better than negative control.

The high parasite count in the prophylactic test in both the extract and chloroquine treated groups might be attributed to the rapid metabolism of administered extract and chloroquine (before inoculation) to inactive products where the extract and chloroquine were initially administered for four days (prophylactic test) before inoculation with *P. berghei* parasite (Dahanukar *et al*., 2000). This is in agreement with previous study done on other *in vivo* antimalarial traditional medicinal plant (Alli *et al*., 2011).

The extract doses had been observed to have lower efficacy than chloroquine, which was incomparable to extract treated groups on early, established and residual infections, may be in part due to unpurified or crude nature, inability of the parasite to develop resistance against the drug as compared to the extract, non selectivity or slow absorption and poor bioavailability of the crude extract (Othuke *et al*., 2012; Godwin *et al*. 2011; Okokon *et al*., 2007). This is in line with *in vitro* study of antibacterial properties of the methanol extracts of the leaves and stems of *Calpurnia aurea* are not as effective as the standard drugs- chloramphenicol and streptomycin, they still possess some activity against bacterial strains used (Adedapo *et al*., 2008).

The mechanism of action of the hydromethanolic leaf extract of *C. aurea* needs to be elucidated even though various mechanisms of action have been postulated for antimalarial activity of natural products. The suggested mechanisms of action for some antimalarial activity of plant sources include intercalation with the parasite DNA, inhibition of hemozoin polymerization in the parasite and inhibition of *Plasmodium falciparum* Lactate Dehydrogenase (*PF*LDH), an essential enzyme for energy generation within the parasite through glycolysis and alkylation (Adebayo and Krettli, 2011; Akuodor *et al*., 2010; Wright*,* 2005) depending on their phytochemical constituents. The extract could have elicited its action through any of the above mentioned mechanism or by some other means yet to be determined.

CONCLUSION

The results obtained from this study indicated that the hydromethanolic leaf extract of *Calpurnia aurea* has promising anti-plasmodial activity. The acute toxicity study in mice also evidenced the high toxicity profile of the plant at higher doses. The extract at the doses tested, in mice infected with rodent parasite (*Plasmodium berghei*), appreciably suppressed parasitemia and to some extent modified factors associated with the infection in all the three models used. Although it was found that *C. aurea* was not that completely efficacious in clearing the parasite burden from the blood circulation of mice infected with chloroquine sensitive *plasmodium berghei*, it definitely possesses significant antimalarial properties which can be further tapped upon for preparation of antimalarial therapeutic drugs.

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