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The Effect of *Otostegia integrefolia* Leaf Extracts on the Packed Cell Volume, Body Weight and Survival Time of *Plasmodium berghei* Infected Mice

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Abstract: The antimalarial suppressive test of *Otostegia integrefolia* leaf extracts previously demonstrated and showed promising results in mice. The present study is aimed at evaluating the protective capability of the crude extracts of the leaves of the plant against loss of body weight, reduction in Packed Cell Volume (PCV) and shortening survival time caused by the malaria parasite using *Plasmodium berghei* infected mice. A standard 4 days Peter's antimalarial test was carried out. PCV and body weight of mice were measured before parasite inoculation and after 4 days successive treatment. Starting from parasite inoculation up to death, survival time of each mouse in each group was also recorded. Aqueous, methanol and chloroform crude extracts of the plant material at tested doses of 200, 400 and 800 mg kg^{-1} were administered orally for the treatment and vehicles for the control groups. The result showed that, extracts of the plant material were able to prevent significant ($p<0.05$) loss of body weight and reduction in the PCV of mice. Similarly, aqueous and methanol extract treated mice lived longer survival time than the respective negative controls. Thus, polar solvent extracts of the plant material are able to reduce the overall pathogenic effects of the *Plasmodium berghei* parasitaemia in mice.

Key words: Body weight, *in vivo*, *Otostegia integrefolia*, packed cell volume, sub-acute, survival time

INTRODUCTION

Malaria is a complex blood borne parasitic infection places a huge burden on the most vulnerable population living in Sub-Sahara African countries. More than 90% of the global malaria related mortality is reported in this region (AMREF, 2005). Due to the spread of multidrug resistant strains of malaria parasites, currently available antimalarial drugs are unable to cure the disease and relief disease related pathologies (White, 1998; Noedl *et al*., 2008; Dondorp *et al*., 2009; WHO, 2010a). Thus, development of new antimalarial medicines is mandatory. An alternative searching for new antimalarial drugs is from traditional herbal medicines in which more than 80% of the world population is still depends on (WHO, 2010b; Ginsburg and Deharo, 2011).

For thousands of years, herbal medicines have been used to manage the malaria disease. In malaria endemic areas particularly Africa where there are problems of availability and affordability of standard antimalarial drugs, herbal medicines have been the main stay treatments against malaria related pathologies (Willcox, 2004; Muthaura *et al*., 2007; Adebayo and Krettli, 2011; Koudouvo *et al*., 2011). It is recorded that over 1,277 species of plant have been used for the treatment of malaria worldwide (Willcox and Bodeker, 2004).

In Ethiopia, traditional herbal medicine has also played a critical role in the country's history of malaria treatment (Bekele, 2007). *Otostegia integrefolia* commonly called as "Tenjut'' in the native language "Amharic'' is among the well-known herbal medicine in the country. The medicinal value of the different plant parts were previously reported by several researchers (Wilson and Mariam, 1979; Giday *et al*., 2007; Andemariam, 2010; Parvaz and Yadav, 2010).

Similarly, Endale *et al*. (2013) and Yeshanew and Mekonnen (2013) demonstrated the antimalarial suppressive effect of the leaves of the plant extracts *in vivo* in mice and showed promising results. This study therefore, aimed at evaluating the efficacy of extracts of *O. integrefolia* leaves against loss of body weight, reduction in PCV and shortening survival time of *Plasmodium berghei* infected mice.

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MATERIALS AND METHODS

Plant material collection and authentication: During the months of September and October (2011) fresh leaves of *Otostegia integrefolia* were collected from "Dembecha" which is located 350 km Northwest of Addis Ababa, Ethiopia. Identification and authentication of the plant was done at the National Herbarium of Addis Ababa University. Voucher specimen was then deposited under the collection number of sy02/2011.

Crude extracts preparation: Fresh leaves of the plant were cleaned, cut into pieces and air dried under shade. The dried leaves then ground in to coarse powder and crude extracts were prepared by cold maceration technique (soaking the plant powder in 1:8 w/v ratio with distilled water, methanol and chloroform in separate Erlenmeyer flasks).

Experimental animals and pathogen: Swiss albino mice of 6-8 weeks old weighing 23-32 g were obtained from the Animal house of the College of Natural Sciences, Addis Ababa University. They were used in accordance with NIH Guide for the care and use of laboratory animals (NRC, 1996). The antimalarial test of extracts were performed using CQ-sensitive strain of the rodent malaria parasite, *Plasmodium berghei.*

Sub-acute toxicity tests: To determine sub-acute toxicity level of the plant extracts, twenty healthy mice were used. They were randomly categorized into four groups of 5 mice per group. They were given oral dose of 500, 1000 and 2000 mg kg^{-1} body weight of the extracts by dissolving each dose extract with 0.4 mL of the vehicles. The fourth group (control) was given orally 0.4 mL of the, respective vehicle. Adminstration of extracts and respective vehicles was continued for 4 consecutive days in a 24 h schedule. Body weight and PCV were measured before and after the treatment. Then, comparision of both paramaters were made over time within each group.

Pathogen inoculation: A standard 4 days Peter's antimalarial test was employed (Peters, 1967). Twenty mice were randomly clustered into four groups of 5 mice per group. At day zero (D_0) each mouse in all groups was infected with *Plasmodium berghei* parasitaemia with standard inoculums (1×10^6 *P. berghei* infected RBCs) intraperitoneally (Krettli *et al.*, 2009). About 3 h after parasite inoculation, the three groups were administered orally with 200, 400 and 800 mg kg^{-1} of extract by dissolving the extract with 0.2 mL of the respective vehicle for each mouse using an oral needle for 4 consecutive days in a 24 h schedule. The fourth group (negative controls) was given with same volume of the respective vehicles.

Determination of body weight and PCV: To determine the effectiveness of the extract in preventing loss of body weight and PCV reduction by the parasite, both parameters were measured before parasite inoculation and after treatment in all the extract treated and negative control groups using digital sensitive balance and heparinized microhaematocrit tube, respectively. The mean PCV and body weight were calculated according to the following mathematical equation described by Bull *et al.* (2000):

$$\text{Mean PCV} = \frac{\text{Volume of erythrocyte in a given blood} \times 100}{\text{Total blood volume}}$$

$$\text{Mean body weight} = \frac{\text{Total weight of mice in a group}}{\text{Total number of mice in that group}}$$

Determination of mean survival time: Mortality was supervised daily and the number of days from the time of parasite inoculation up to death was recorded for each mouse in the treatment and control groups throughout the follow up observation period. The Mean Survival Time (MST) for each group was then calculated as indicated by Samanta *et al.* (2011):

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

Statistical analysis: Student's paired t-test was conducted to determine the significance level of PCV and Body weight of *P. berghei* infected mice between D_0 and D_4. One way Analysis of Variance (ANOVA) also carried out to compare the survival times of the *P. berghei* infected mice between the control and extract treated groups at a fixed time. All the results were presented as the Mean±SEM (Standard Error Mean) and statistical significance were considered at a 95% confidence interval ($p<0.05$).

RESULTS AND DISCUSSION

Sub-acute toxicity tests: Acute toxicity test of extracts of the plant material was demonstrated and it was found safe in mice that received up to a single dose of 2000 mg kg^{-1} (Yeshanew and Mekonnen, 2013). For sub-acute toxicity determination of these extracts, healthy mice were

administered with all the toxicity test doses for 4 consecutive days. No mortality of mice was observed during a week follow up observation period. Similarly, the effect of extracts on the PCV and body weight of healthy mice were also assessed. Extracts of the plant part did not causes serious side effects in both parameters. However, extract treated mice were found to have slight decrement in their body weights and PCV values while the controls resulted in slight increments at the fifth day of post treatments. Even though there were changes when the value of both parameter were compared over time with in a group, it was no stastically significant ($p>0.05$) change (Table 1).

Antimalarial tests: Although there was not significant ($p>0.05$) change, reduction in PCV values were observed in both the negative controls and extract treated *P. berghei* infected mice. Similarly, at all tested doses, extracts of the plant material was also unable to prevent a minor loss of body weight of the same mice. However, mice administered with the vehicles showed statistically significant ($p<0.05$) weight loss when compared overtime within a group. On the other hand, mice feed with 200 mg kg^{-1} of the methanol and 800 mg kg^{-1} chloroform extracts, loss of body weight was found to be statistically significant ($p<0.05$). But when extract treated groups were in comparison with the respective negative controls, no significant ($p>0.05$) difference was observed (Table 2).

Extract administered mice lived longer time than the corresponding negative controls. In all extracts, the highest survival time was recorded in mice that received 800 mg kg^{-1}. Mice that administered with 200, 400 and 800 mg kg^{-1} of aqueous extract lived 6.75±0.48, 7.25±0.48 and 7.75±0.63 days respectively while the respective negative control lived 6.75±0.25 days. At the same tested doses, the methanol and chloroform extract treated mice lived 7.5, 8.25±0.25 and 13.50±0.87 days and 8.50±0.64, 9.25±0.25 and 10.50±0.87 days when their respective negative control lived 7.00±0.41 and 9.50±1.20 days respectively. About 400 and 800 mg kg^{-1} methanol extract treated mice lived statistically significant ($p<0.05$) longer survival time than the corresponding negative controls. On the other hand, mice that were given 200 and 400 mg kg^{-1} chloroform extract lived shorter time than the respective negative controls (Fig. 1).

After 4 days consecutive treatments of the crude extracts, a slight decrement in the PCV and body weight were observed in mice that were subjected for sub-acute toxicity tests. However, it was not statically significant ($p>0.05$). Whereas, the negative controls that received the

Table 1: Sub-acute toxicity test results of extracts of the leaves of *Otostegia integrefolia* in mice

		PCV (%)			Body weight (g)		
Treatments	Dose (mg kg^{-1})	D_0	D_4	p-value	D_0	D_4	p-value
dH_2O extract	dH_2O (NC)	48.50±0.78	48.50±0.78	0.368	26.07±0.27	27.67±0.22	0.639
	500	51.40±0.80	50.50±1.46	0.124	27.27±0.26	26.87±0.46	0.435
	1000	51.42±1.26	49.96±1.33	0.906	29.73±0.90	30.80±0.32	0.098
	2000	51.15±0.70	51.39±1.06	0.538	31.23±0.55	29.60±0.57	0.906
MeOH extract	20% DMSO (NC)	48.50±0.78	48.94±0.54	0.368	26.07±0.27	27.67±0.22	0.639
	500	50.93±0.80	50.55±1.64	0.043	25.80±0.42	23.96±0.69	0.278
	1000	50.98±0.77	50.73±0.78	0.972	29.07±0.70	29.03±0.54	0.758
	2000	50.96±1.01	48.75±1.10	0.627	30.57±0.27	28.60±1.31	0.134
$CHCl_3$ extract	20% DMSO (NC)	48.50±0.78	48.94±0.54	0.368	26.07±0.27	27.67±0.22	0.639
	500	50.90±0.92	49.38±0.78	0.932	26.30±0.70	26.30±0.72	0.801
	1000	50.43±0.80	50.10±0.70	0.405	33.80±1.08	33.63±1.24	0.763
	2000	50.45±0.97	50.03±0.60	0.114	32.40±0.55	32.36±0.60	0.770

Table 2: Effect of *Otostegia integrefolia* leaf extracts on the PCV and body weight of *Plasmodium berghei* infected mice *$p<0.05$

		PCV (%)			Body weight (g)		
Treatments	Dose (mg kg^{-1})	D_0	D_4	p-value	D_0	D_4	p-value
dH_2O extract	dH_2O (NC)	50.80±0.58	47.26±0.72	0.170	23.30±0.75	22.62±0.47	0.034*
	200	50.18±0.79	49.54±0.74	0.634	27.46±0.71	24.82±1.06	0.129
	400	49.22±0.57	50.66±0.39	0.334	27.50±0.48	27.20±0.32	0.280
	800	51.00±0.45	51.28±0.40	1.000	29.98±1.09	28.40±1.41	0.677
MeOH extract	20% DMSO (NC)	49.68±1.51	49.96±0.98	0.664	29.28±0.79	27.98±0.90	0.028*
	200	51.04±0.73	48.00±1.08	0.134	27.40±0.95	26.00±0.79	0.017*
	400	50.46±0.62	48.68±1.78	0.683	29.18±0.55	27.68±0.60	0.286
	800	50.66±0.40	49.24±0.97	0.397	30.10±1.20	28.98±1.13	0.667
$CHCL_3$ extract	20% DMSO (NC)	51.34±0.66	49.40±0.79	0.858	26.08±0.59	23.70±0.49	0.024*
	200	51.06±0.33	49.82±0.73	0.962	29.65±0.81	28.50±0.89	0.310
	400	50.80±1.03	50.80±1.03	0.503	32.32±1.95	28.45±1.71	0.724
	800	50.40±0.53	52.50±1.16	0.438	31.80±1.11	28.45±0.37	0.044*

NC = Negative Control; dH_2O = Distilled water; MeOH = Methanol; $CHCl_3$= Chloroform; PCV = Packed Cell Volume; DMSO = Dimethylsulfoxide
D_0 = Treatment at day zero; D_4 = Treatment at day four

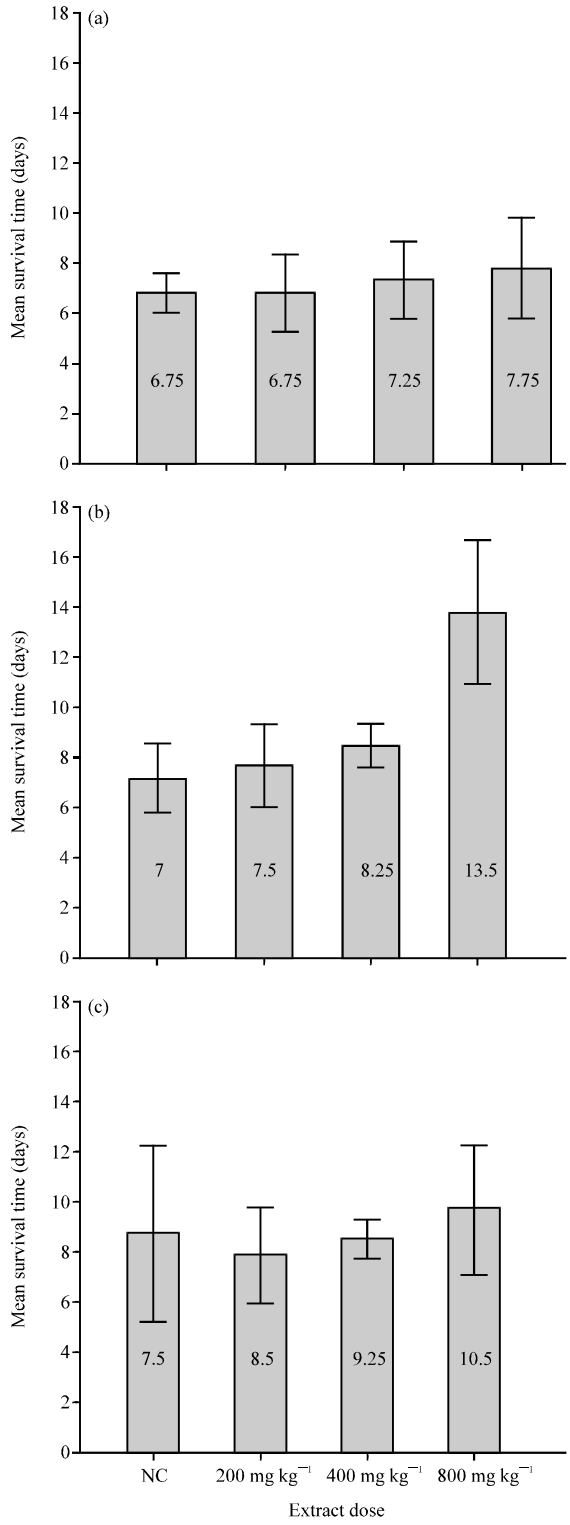

Fig. 1: Effect of a) aqueous; b) methanol and c) chloroform extracts of the leaves of *Otostegia integrefolia* on the survival time (day) of *Plasmodium berghei* infected mice

vehicles were in contrary showed a slight increment in both parameters after the treatment. The depressing effect of crude extracts of the plant material on the appetite and feeding activities of mice could possibly is the cause of decrements in PCV and body weight of mice that received the extracts at day 4 of post treatment. But, other serious toxic symptoms were not observed during 1 week follow up observation time. This possibly provides a scientific basis for the ethno-medicinal use of the plant in traditional healers of Ethiopia.

P. berghei infected experimental mice that were treated with the antimalarial tested doses of extracts, resulted in the reduction of PCV value on the 5th day of post-infection. However, the reduction was found not statistically significant ($p>0.05$). This result might be implicated with the depressive effect of the crude extracts on the food intake of mice. Similar effects were also observed in healthy mice that received higher doses of same extracts.

Body weight loss is one feature of rodent malaria infections (Perlmann and Troye-Blomberg, 2007). The crude extracts in this study did not prevent minor body weight loss of *P. berghei* infected mice even though the loss was not statically significant ($p>0.05$). But mice given with the vehicles were found statically significant ($p<0.05$) losses at day 4 of post infection. This result therefore, indicates that the extracts were able to prevent a significant ($p<0.05$) loss of body weight of mice. Similarly, minor reduction in body weight was also recorded in healthy mice that received higher doses of extracts of the plant material. Thus, the loss possibly is due to the depressing effect of the crude extracts on appetite and food intake of mice and might not by the parasite.

Mice treated with extracts of the plant material lived relatively longer time than the corresponding negative controls. The result confirmed that the crude extracts of the plant material suppressed *P. berghei* parasitaemia and probably reduces the overall pathogenic effect of the parasite. Longer survival time was recorded in mice that administered with the water and methanol extracts justifying the traditional usage of water and ethanol (local alcohols) as common solvents. Thus, chewing or taking the water or ethanol extracts of the plant material may serve to eases malaria related sickness until the patient gets modern medical treatments.

About 400 and 800 mg kg^{-1} methanol extract treated mice lived significantly ($p<0.05$) longer time than the respective negative control. The result may be due to the potent antimalarial suppressive effect of the plant material that was demonstrated in the methanol extract (Endale *et al*., 2013; Yeshanew and Mekonnen, 2013).

However, mice treated with 200 and 400 mg kg^{-1} of chloroform extract treated groups lived shorter time than the respective negative controls. It could possibly be due to the lowest antimalarial suppressive effects of the chloroform extract (Yeshanew and Mekonnen, 2013) and may be the depressing effect of the chloroform extracts on appetite and food intake in mice.

CONCLUSION

Crude extracts of the plant material are safe and able to prevent loss of body weight and reduction in the PCV of *P. berghei* infected mice. Similarly, it lengthens the survival time of the same mice. Therefore, it is recommended that bioactivity guided fractionation of the water and methanol crude extracts of the plant material could result in identifying active fractions and further more in active compounds that can help in controlling parasitaemia load better.

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