Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 11 (2)

Prophylactic Study of Ethanol Leaf Extract of *Vernonia amygdalina* (ELVA) In Cerebral Cortex of Young Mice Inoculated with *Plasmodium berghei.*

***Abdulazeez MA, Adebisi SS and Musa SA**
Department of Human Anatomy, Faculty of Basic Medical Science, College of Health Science, Ahmadu Bello University, Zaria, Nigeria.

Corresponding Author: Abdulazeez MA
E-mail: mradvocacy@gmail.com; +234-70-34806649

ABSTRACT
In developing countries traditional medicine still plays an important role in local health care systems. Estimation has shown that up to 80% of some Asian and African countries depend on traditional medicine for their health care needs. *Vernonia amygdalina* has been reported to show a significant inhibitory activity *in-vitro.* This study was conducted to investigate the histological changes of ethanol leaf extract of *Vernonia amygdalina* (ELVA) in cerebral cortex of young mice inoculated with *Plasmodium berghei (Pb).* A total of twenty-five (25) young mice, mixed sex, aged 2-3 weeks, weighing between 5-8g were divided into five groups with five mice per group. Group 1 (distilled water), Group 2 (*Pb)*, Group 3 (*Pb* + ELVA 250mg/kg), Group 4 (*Pb* + ELVA 125mg/kg) and Group 5 was treated with (*Pb* + 1.2mg/kg Pyrimethamine). The mice were treated with ELVA for 72 hours prior to inoculation with *P. berghei,* while parasite quantification was recorded up till the 8th day before sacrifice. Brain tissues of sacrificed mice were removed and fixed in Bouin's fluid, processed for histopathology using Haematoxyline and Eosin (H and E), and Cresyl Echt Violet stain. ELVA at doses 2500mg/kg, 1250mg/kg and Pyrimethamine at 1.2mg/kg exhibited parasitemia level of no statistical significance and percentage suppression of group 3 (72.7%), group 4 (76.2%) and group 5 (61%) ($p<0.057$) respectively as compared to control group. In this prophylactic design there was no significant changes between the ELVA treated groups and the untreated. The study shows that ELVA has a minimal dose dependent effect on the parasite quantification and the histopathological observations.

Keywords: *Vernonia amygdalina,* ELVA, *P. berghei,* Pyrimethamine.

INTRODUCTION
Malaria infection are caused by the novel *Plasmodium falciparum* which are characterized by defective immune response and complicated by poor efficacy which may be associated immunopathology.[1] This endemic can be traced back to time immemorial and over a space of human existence it has coevolved as the parasite finds human host most suitable for its life cycle. *Plasmodium falciparum* infection continues to cause high rates of morbidity and mortality with 216 million cases and an estimated 445, 0000 deaths in 2016, largely in African children[2]. Pregnant women, unborn children and children under age of 5years are more vulnerable to malaria which remain a major cause of complications like maternal anemia, prenatal mortality and low birth weight. It account for 40% of public health expenditure, 30-50% of in-patient admission and up to 50% of out-patient. This endemic sequel has a strong hold on the socio-economy development of a nation.

In the etiology and treatment of malaria infection various response are influenced by strong interaction (s) between the immune system of the host and the parasite. In 1-2% of the world cases on malaria infections with *Plasmodium falciparum* cerebral malaria (CM) the most severe complication of falciparum develops, resulting in about 80% of all the malaria deaths, mainly of children, pregnant women and non-immune adults[3].In many cases survivors present neurocognitive sequels, such as behavioral changes, learning deficits, cognitive impairments in the short or long term after the episodes.

MATERIALS AND METHODS
Materials: The following are some of the materials: Digital Microscope (Celestron and Olympus), Distilled water, Laptop Computer, Bouin's fluid, Beakers, Specimen bottles, Digital weighing balance, 1ml and 5ml syringes and injecting needles, Dissecting tray and Dissecting kit.

Plant Collection and Extraction: The fresh leaves of *Vernonia amygdalina* plant were collected based on Ethnobotanical description and with the help of local traditional healers around Mada Area Development Council in Gusau local government area of Zamfara State, Nigeria, in May 2017. The plant was identified and authenticated at Botany Department, Faculty of Life Science, Ahmadu Bello University Zaria, and given a

voucher number (12063). The Fresh leaves of *Vernonia amygdalina* were cleaned from extraneous materials, air-dried under shade at room temperature then pounded into powder in Biochemistry Laboratory of Umaru Musa Yaradua University Katsina state, Nigeria. The powdered plant material was weighed (500g) using SCIENTECH Mode No SL 3100D Rev-c accuracy class (II)[4]. Powdered *Vernonia amygdalina* (500g) was macerated with 5000ml, 80 % of ethanol for 72 hours with intermittent agitation by Orbital shaker at 120 revolutions per minute. The supernatant part of agitated material filtered with 15 cm Whatman grade1 filter paper two times. The filtrate of *Vernonia amygdalina* was then concentrated using Rotary evaporator (BUCHI R250,Switzerland) at 40°C to remove methanol and further dried using in a lyophilizer (CHRIST, 3660 Osterode/harz/, France) to remove water and the extract were kept at - 20°C until used[4]. A total of 235g (53%) yield of the extract was obtained from the 500g of the powder.

Experimental Animals Preparation: The animals employed for this study were premature young male and female 2-3 weeks old Albino Swiss mice (5-8g). Thirty mice were obtained from the Animal Facility, Faculty of Pharmaceutical Sciences of Ahmadu Bello University Zaria, Nigeria. The mice were put to full experimental condition because the experiment took place in the same venue of purchase by first screening the mice for any possible parasitic infection. Blood was collected from the tail with anticoagulant (EDTA) of each mouse. A thin blood smear was made from each blood sample and stained. These were used to screen the mice for haemoparasites. The packed cell volume (PCV) of each mouse was also determined. Twenty five Mice with normal PCV range of 39.0 – 47.0 and free of haemoparasites were used for the experiment[5]. They were then housed in cages and given standard pellet diet and water *ad libitum*. The animals were maintained under the natural light-dark cycle throughout the duration of the study. For in-vivo anti-malarial assays of plant extracts, the mouse-infective chloroquine sensitive strain of *P. berghei* NK65 obtained from National Institute of Medical Research Lagos State was kept alive by continuous intraperitoneal (i.p.) serial blood passage from mice to mice on research demand by the laboratory staff of the animal facility of Faculty of Pharmaceutical science, Ahmadu Bello University Zaria.

Prophylactic test (Repository test): Evaluation of prophylactic potential of the extract was done using Peters methods with slight modification[6]. Twenty five Swiss albino young mice were divided into five groups with five mice per group (Table 1). 72 Hrs. after treatment is inoculation, then follow by thin blood films on day 5 to monitor the parasitaemia level. Rectal temperature was also measured by a digital thermometer[7].

Table 1: Grouping and Dosage

Groups	Treatment
Group 1	Distilled water
Group 2	*Pb*
Group 3	*Pb* + ELVA 250mg/kg
Group 4	*Pb* + ELVA 125mg/kg
Group 5	*Pb* + 1.2mg/kg Pyrimethamine

Pb- Plasmodium berghei, ELVA- Ethanol leaf extract of *Vernonia amygdalina.* All administration via Oral route. Pre inoculation treatment is 3 days, inoculation on the 4th day and sacrifice on the 8th day.

Parasite and its quantification: Blood sample were collected from tail snip of each mouse according to methods of[8]. The smears were made on clean microscope slides, fixed with absolute methanol for 15 min and stained with 15% Geimsa stain at pH 7.2 for 15 min. The stained slides were then washed gently using distilled water and air dried at room temperature. Each stained slide was examined under Olympus microscope (CHK2-F-GS, Taiwan) with an oil immersion objective of 100x magnification power to evaluate the percent suppression of each extract with respect to the control groups. The microscope eye piece shows about 100 red blood cells per field[9].

The parasitemia level was determined by counting minimum of five fields per slide with about 100 RBC in random field of the microscope.

Percent parasitemia and percentage of suppression was calculated using formula described in all model[7].

$$\% \text{ Parasitaemia} = \frac{\text{Number of Parasitized RBC}}{\text{Total RBC counted}} \times 100\ \%$$

$$\% \text{ Suppression} = \frac{(\text{Parasitaemia in normal control}) - (\text{parasitaemia in treated group})}{\text{Parasitaemia in normal control}} \times 100\ \%$$

Animal Sacrifice and Tissue Samples: At the end of the administration, mice were sacrificed and brain tissue dissected out, fixed immediately in Bouin's fluid[10].

Histology process: Brain tissue were extracted and preserved in Bouin's fluid for routine histological procedure. In the histology lab it was then dehydrated in

graded ethanol, cleared in xylene and embedded in paraffin wax. Afterwards coronal sections were then taken for the various individual staining procedure which includes the H & E and Cresyl violet.

Statistical Analyses: Data collected were presented as Mean ± SEM and were statistically analysed using Statistical Package for Social Science (SPSS), Version 20 (IBM, Incorp, NY). A *p*-value < 0.05 was considered significant. ANOVA was used to compare the levels of parasitaemia of the *P. berghei* infected mice between the controls and extract treated groups at a fixed time. The results were presented as the Mean ± SEM (Standard Error of the Mean) and statistical significance was considered at a 95% confidence interval (P<0.05).

Ethical Consideration: Ethical approval was collected from Ahmadu Bello University, research and ethics committee on the use of animals with an approval code of ABUCAUC/2017/029.

RESULTS

Ld_{50} of ELVA: The acute exposure of mice to ethanol extract of *Vernonia amygdalina* (ELVA) at the doses of 2900 and 5000mg/kg body given during *in vivo* study showed no abnormalities. There was no death or any sign of toxicity in any of the mice.

Basic indicators: The mice inoculated with *P. berghei* had features of fever and these includes decrease in food intake with consequent loss of weight. The rectal temperatures were significantly higher in the infected mice. Hair coats in these mice were rough. Signs of the infection subsided on the treatment groups.

Mean Parasitaemia quantification: The highest mean percentage parasitaemia was observed in group 2 (17.82 ± 0.53%) during the prophylactic study (Table 2). The decrease in parasitaemia appeared to be dose dependent, being 11.38 ± 2.62% (Group 3) and 12.28 ± 0.85% (Group 4), while group 5 (treated with 1.2 mg/kg of pyrimethamine) had 16.26 ± 0.38%. The level of suppression was least (61.7%) among mice treated with pyrimethamine group 5 and highest (76.2%) in group 3. However, these variations in mean percentage parasitemia and level of suppression were not statistically significant (p < 0.05) among the groups.

Table 2: Percentage Parasitaemia Suppression

Groups	Treatment	Dose	% Parasitaemia	% Suppression	P-Value
1	Distilled water	P/bw	---------	----------	
2	*Pb*	0.3ml	17.82±0.53	----------	
3	*Pb* + ELVA	250mg/kg	11.38±2.62	76.2	0.057
4	*Pb* + ELVA	125mg/kg	12.28±0.85	72.7	
5	*Pb* + Py	1.2mg/kg	16.26±0.38	61.7	

Pb- Plasmodium berghei, ELVA- Ethanol leaf extract of *Vernonia amygdalina,* Py- Pyrimitamine Where N < Numbers of animal (5), P/bw- Per body weight

Body Weight Determination.

Ethanol extract treated mice shows statistically significant (p<0.05) increments of body weight across all groups on day 5 i.e. before induction of malaria parasite. (Table 3). The result indicates that at Day 5 the mice in group 3 (12.54 ±0.33) was significantly higher compare to Group 2 (10.76±0.56), group 4 (11.50± 0.16), group 5 (11.02±0.28) and group 1 (11.36±0.29), while there is no significant difference at Day 8 across the groups.

Table 3: Pre and Post Treatment Body Weight

Groups	Treatment	Dose	Body Weight		P-value	P-value
			Pre D5 (g)	Post D8 (g)	D5	D8
1	Distilled H_20	P/bw	11.36±0.29	11.06±0.28		
2	*Pb*	0.3ml	10.76±0.56	10.94±0.59	0.019	0.203
3	ELVA	250mg/kg	12.54±0.33	12.56±0.72		
4	ELVA	125mg/kg	11.50±0.16	11.52±0.36		
5	Py	1.2mg/kg	11.02±0.28	11.74±0.45		

Pb- Plasmodium berghei, ELVA- Ethanol leave extract of *Vernonia amygdalina, Py*- Pyrimethamine Where N < Numbers of animal (N<5), P/bw- Per body weight, Pre D5- before (Day 5), Post D8- After (Day 8).

PCV Determination on Day 5 and Day 8 of the Prophylactic Test

The Packed cell volume result indicates that at Day 8 the mice in group 5 (29.60±0.51) was significantly lower ($p < 0.001$) compare to Group 1 (39.80±0.49), group 2 (38.40±0.68), Group 3 (38.80±0.37) and group 4 (39.40±0.40) while at Day 5 there is no significant different observed across the groups. This could possibly be as a result of the circumstance of the drug administration and probably the age of mice is also a factor responsible for the difference.

Table 4: Mean value of PCV

Groups	Treatment	Dose	PCV		P-Value	
			D 5	D 8	D 5	D 8
1	Distilled	P/bw	39.60±1.29	39.80±0.49[a]		
2	*Pb*	0.2ml	38.80±0.66	38.40±0.68[b]	0.029	□ 0.001
3	ELVA	250mg/kg	40.80±0.66[b]	38.80±0.37[c]		
4	ELVA	125mg/kg	34.40±1.08[a]	39.40±0.40[d]		
5	Py	1.2mg/kg	37.00±2.03	29.60±0.51[abcd]		

P.b- induced with *Plasmodium berghei*, V.A- *Vernonia amygdalina* treated, Py- Pyrimithamine Where N <Numbers of animal (N<4), P/bw- Per body weight, D5- (Day 5), D8- (Day 8).

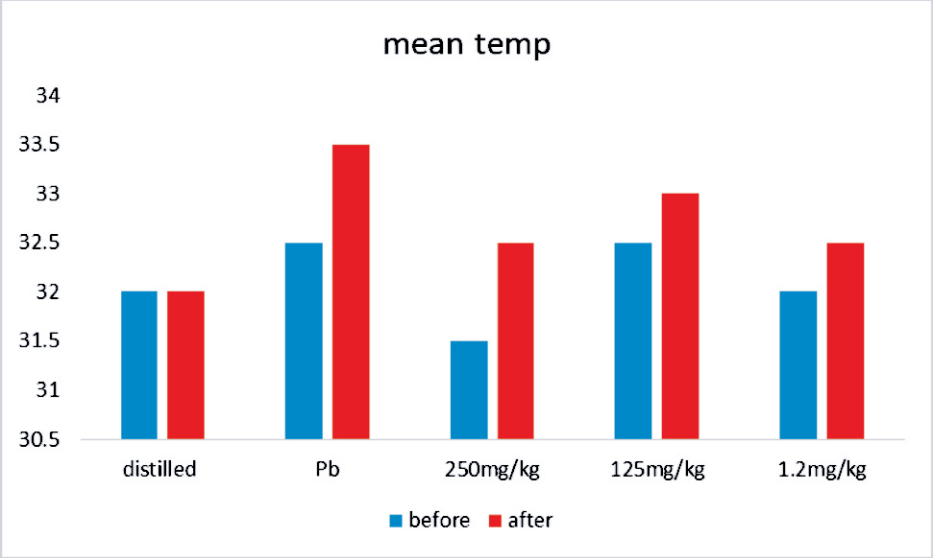

Figure 1. Representation of the mean temperature

Histopathology of cerebral cortex of mice during prophylactic study. There were normal pyramidal and stellate cells in the cerebral cortex of mice in group 1 during the prophylactic study. The layer I and II of the cerebral cortex of the mice in group 2 showed
scattered areas of necrosis. The cerebral cortex of layer I and II of the group 3 and group 4 revealed.

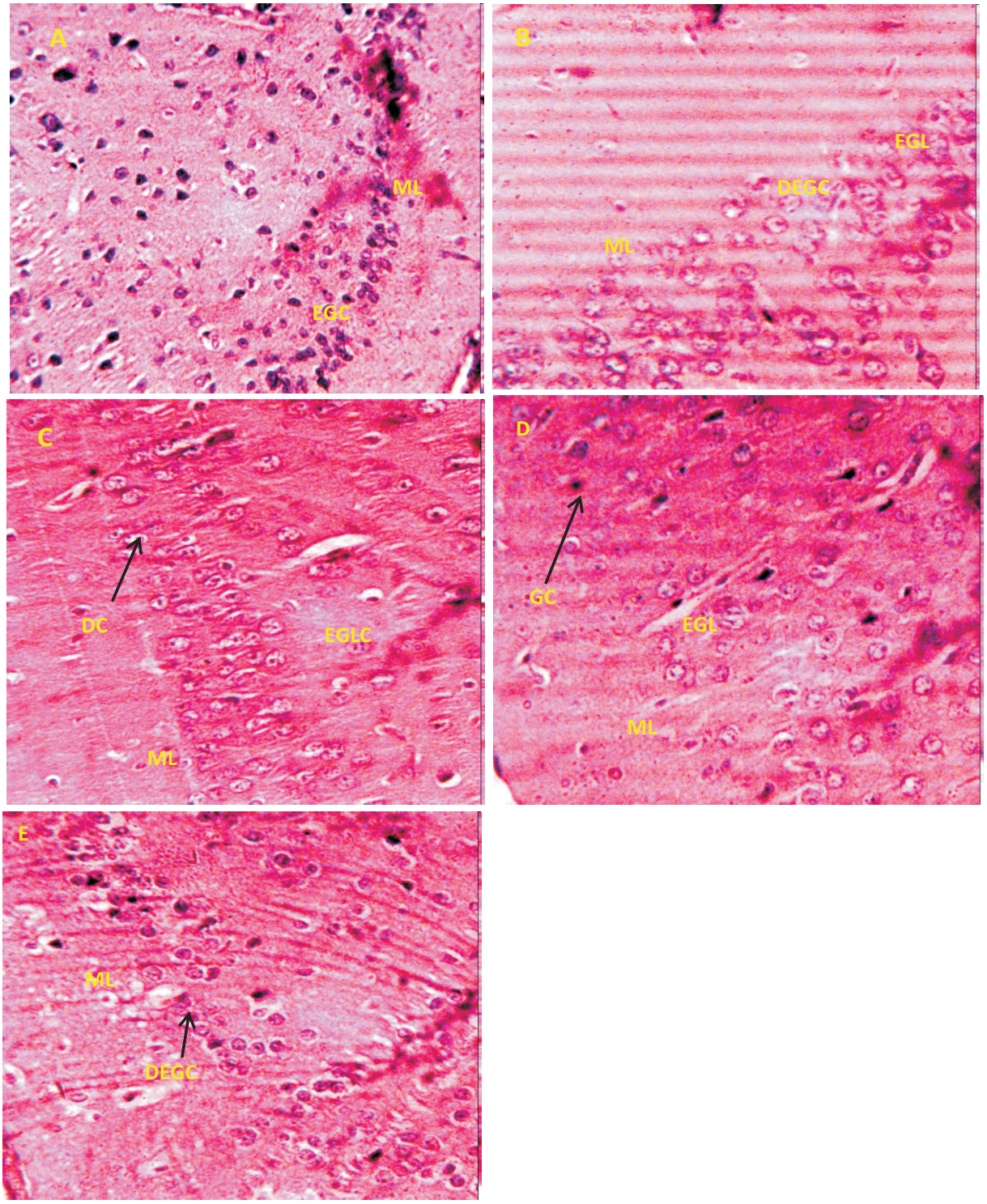

Plate I: A- Treated with Distilled Water, B- Infected and Not Treated, C-treated with 250mg/Kg Of Extract, D-treated With 125mg/Kg Of Extract, E- Treated With Pyrimethamine 1.2mg/Kg. EGC- External Granular Cells, EGL-external Granular Layer, ML-Molecular Layer, DEGC-Degenerated External Granular Cells. (H and E X 250

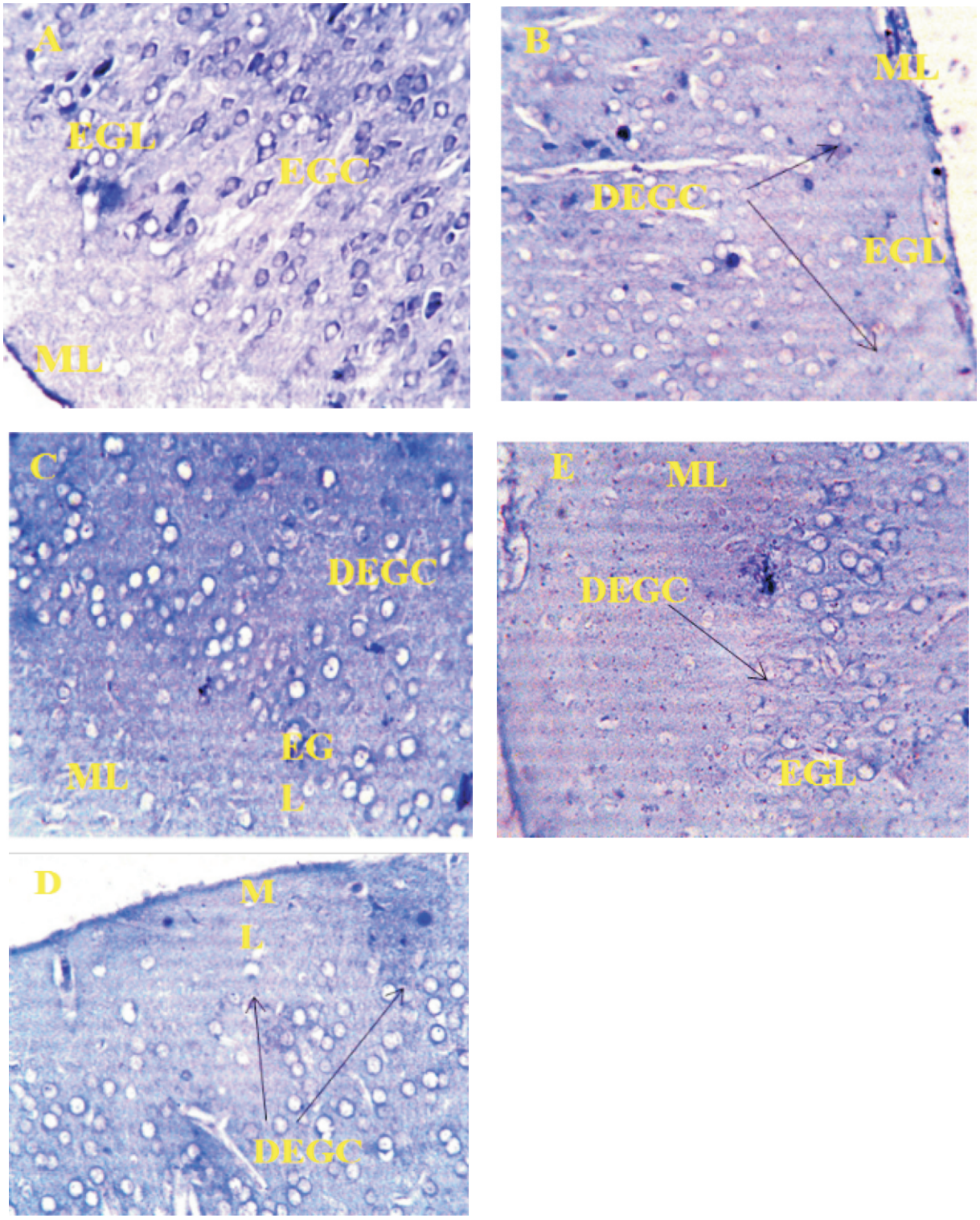

Plate II: A- Distilled Water, B- Infected C- 250mg/Kg ELVA, D- 125mg/Kg , E- Treated With Pyrimethamine 1.2mg/Kg. EGC- External Granular Cells, EGL-External Granular Layer, ML-molecular Layer, DEGC-degenerated External Granular Cells. (Cresyl Echt Violet X 250)

DISCUSSION

In order to deal with the expanding problem of drug resistance which continues to challenge malaria control efforts, new antimalarial drugs are needed. The indigenous people are exploiting a range of herbals for effective treatment of various diseases involving malaria. Modern drugs have been deducted from folklore and traditional medicine. There are about 1200 plant species from 160 families used to treat malaria[11]. According to WHO, about 60 % of world's people use herbal medicine for treating their sickness[12].The extracts were considered active when parasitaemia was reduced by > 30%[13] The present study showed the prophylactic effect of ethanol extract of *V. amygdalina* with significant (P<0.057) parasitaemia suppression effect at the dose of 125 mg/kg with 72.7% respectively as compared to normal control group. While the standard drug, pyrimethamine, showed the highest effect (76.2%, P<0.057) at the dose of 1.2 mg/kg as compared to normal control group.

Even if much data has not been obtained on the repository effect on *V. amygdalina* other report during a repository study with the use of medicinal plant extract by[14], in the treatment of mice infected with *P. berghei* with aqueous leaf extract of *Morinda lucida,* with 70.18% at the dose of 800mg/kg activity were recorded. Study done by[15] who also checked the prophylactic effect of other traditional medicinal plant extract against *P. berghei* also exhibited a significance (75.6%, P < 0.05) effect at the dose of 600mg/kg. In this study it was also observed that ethanol leaf extract of *Vernonia amygdalina* exerted a dose dependent percentage suppression with the extract treated group which is in accordance with reports of [16] where the percentage suppression is dose dependent for the aqueous treated group and it is significant different across the group

CONCLUSIONS

The spread of resistant malaria parasites to the available antimalarial drugs call for a new chemotherapeutic agent to control the disease. Medicinal plants are constantly screened for bioactivity in our quest for the discovery of new and effective therapeutic agents. The results of this study could help encourage more identification and validation of natural products which has shown drug properties thus facilitating the development of a new generation of antimalarial.

In conclusion, the results obtained from current study indicated that;

- The present study indicated that the extract did not exhibit any signs of acute toxicity up to the dose of 5000mg/kg.
- Based on the results of the studies it was concluded that ethanol extract of *V. amygdalina* shows less prophylactic properties.

ACKNOWLEDGEMENTS

Our immense gratitude goes to the entire staffs of the animal facility of Faculty of Pharmaceutical science, Staffs of Histology unit of Department of Human Anatomy, and Friends from hematological laboratory of Veterinary Medicine for providing the bench space.

COMPETING INTREST

No competing interest.

REFERENCES

1. WHO, "Malaria spread sheet, World Health Organization, Geneva, 2019
2. WHO, "Malaria: retreat of a century old-scourge" World Health Organization, Geneva, 2017.
3. WHO, World Malaria Report 2016. (World Health Organization, Geneva).
4. Debella A., "Manual for phytochemical screening of medicinal plants". Ethiopian Health and Nutrition Research institute, Addis Ababa, Ethiopia. 2002; pp 35-47.
5. Delwatta, Shehani & Gunatilake, Mangala & Baumans, Vera & Seneviratne, Melanie & Dissanayaka, Manjula & Batagoda, Siyani & Udagedara, Asanga & Walpola, Prasad, "Reference values for selected hematological, biochemical and physiological parameters of Sprague-Dawley rats at the Animal House, Faculty of Medicine, University of Colombo, Sri Lanka". *Animal Models and Experimental Medicine*. 2018; 1. 10.1002.
6. Peters, W., "Rational methods in the search for antimalarial drugs". *Transnational Royal Society Tropical Medicine and Hygiene*, 1967; 61: 400-410.
7. Al-Adhroey A.H., Nor, Z.M., Al-Mekhlafi A.A., Amran, R., Mahmud, "Evaluation of the use of Cocos nucifera as antimalaria remedy in Malaysian folk medicine" *Journal of Ethnopharmacology* 2011; 134 (3): 988-991.
8. Kalra, B.S., "Screening for antimalaria drugs: an overview", *Indian Journal of Pharmacology*. 2006; 38: 5-12.
9. Dikasso, D., Mekonnen, E. Debella, A., Abebe, D., Urga, K., Mekonnen, W., Melaku, D., Assefa, A. and Mekonnen, Y., "In vivo anti-malarial activity of hydro alcoholic extracts from *Asparagus africanusLam* in mice infected with *Plasmodium berghei". Ethiopia Journal of Health Development.* 2006; 20: 112-118.
10. Bancroft, J.D. and Gamble, M, "Theory and Practice of Histological Techniques". Text book, 6th Edition, Churchill Livingstone, Elsevier, China, 2008.
11. Willeox ML, Bodeker G., "Traditional herbal medicines for malaria". *British Medical Journal*. 2004; 329:1156-59.
12. WHO, "General guidelines for methodologies on research & evaluation of traditional medicine". WHO/EDM/TRM/2000.1. World Health Organization, Geneva, 2000.
13. Carvalho, L.H., Brandao, M. G., Santos-Filho, D., Lopes, J. L. and Krettli, A.U., "Antimalarial activity of crude extracts from Brazilian plants studied in vivo in Plasmodium berghei-infected mice and in

vitro against Plasmodium falciparum in culture". *Brazilian journal of medical and biological research,* 1990; 24(11): 1113-1123.

14. Ebiloma GU, Igoli JO, Katsoulis E, Donachie AM, Eze A, Gray AI, de Koning HP, "Bioassay-guided isolation of active principles from Nigerian medicinal plants identities new trypanocides with low toxicity and no cross-resistance to diamidines and arsenicals". *Journal of Ethnopharmacology.* 2012; 202: 256-264.
15. Petros, Z., Melaku, D., "*In vivo* anti-plasmodial activity of *Adhatoda Schimperiana* leaf extract in mice" 2012. http://pharmacologyonline.silae.it ISSN: 1827-8620.
16. Ebiloma, Godwin, "Suppressive, Curative and Prophylactic Potentials of Morinda lucida (Benth) against Erythrocytic Stage of Mice Infective Chloroquine Sensitive *Plasmodium berghei* NK-65". *British Journal of Applied Science & Technology*. 2011; 131-140.