

Acute Toxicity Effect of Artemisia Afra Plant Extracts on the Liver, Kidney, Spleen and in Vivo Antimalarial Assay on Swiss Albino Mice

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Abstract: *Artemisia afra* (Jacq. Ex. Wild), or "African Wormwood" belonging to the family of Astereaceae and is widely used traditionally for health care in the eastern part of Africa with few research evidence substantiating its safety. The aim of this study was to investigate the safety of the ethanolic, dichloromethane, and hexanolic extracts of *Artemisia afra* by determining its pharmacotoxicological effects after an acute oral administration in mice and to test also their *in vivo* antimalarial effects. Oral acute doses of *Artemisia afra* extracts were given to thirty mice at the doses of 1000, 2000 and 2500 mg/kg of body weight. The mice were then observed for fourteen days, toxicity signs, body weight, organs weight and biochemical parameters were checked. Four days petter's test was run on mice to determine the *in vivo* antimalarial activity of the plant extracts and the IC₅₀ for each extract was determined. The results show few toxicity signs from the first two days after oral administration. There were no differences in organs weight and body weight for the experimental mice when compared to the control group. The level of alanine transaminase (ALT) and aspartate transaminase (AST) were found do not be statistically different from the control. The LD₅₀ of the extracts was found to be greater than 2500 mg/kg of body weight. The results also showed a high antimalarial effect of the extracts when tested *in vivo* using *Plasmodium Berghei Anka*. In Conclusion *Artemisia afra* is a strong drug candidate for malaria with no toxic effects in high dosage.

Keywords: Oral Acute Toxicity, Medicinal Plant, Antimalarial Assay, *Plasmodium Berghei Anka*, *Artemisia Afra*, Biochemical Test

1. Introduction

A large percentage of the African population depends on medicinal plants for health care [1, 2]. The World Health Organization (WHO) has recognized that 80% of the African

population makes use of traditional medicine [3]. *Artemisia afra* is one of the most widely used herbs in traditional medicine. In recent years, it has gained significant attention from the scientific community. Studies have been conducted either to verify or to substantiate the traditional use of this

herb. Furthermore, its use is also being investigated in the treatment of modern diseases like diabetes, cardiovascular diseases, cancer, and respiratory diseases [4]. In Africa, more than 2,000 plants have been identified and used as herbal medicines to treat several ailments, but very few of these plants have been screened for their safety and efficacy [5].

Artemisia afra grows naturally in a land with high altitude and it is distributed widely from South Africa to the Cap. The aqueous extract of *A. afra* has shown bronchodilator activity [6], as well as anti-histaminic and analgesic properties [7]. The ethanolic and dichloromethane extracts of the plant have been shown to have *in vitro* hypotensive and anti-tuberculosis effects, respectively [8]. *Artemisia afra* is also traditionally used in the treatment of malaria [9]. The aqueous, ethanolic and methanolic extracts have also shown high *in vitro* antimalarial effect [9, 10]. Despite its multiple uses, very little is known about the toxicity of this plant [4] mostly, the toxicity when this plant is extracted using another solvent different from Water. Toxicity data are required to predict the safety associated before the use of medical products, it helps to decide whether a new drug should be adopted for clinical use or not [11]. This study aimed to investigate the toxicity of *Artemisia afra* plant when extracted with Ethanol, Dichloromethane, and Hexane at the same time to test their *in vivo* antimalarial effect using mice.

2. Material and Methods

2.1. Plant Materials

Aerial part of *Artemisia afra* was collected from Rumonge in the Southern part of Burundi. The leaves were dried under shadow, grounded and sent to Nairobi under plastic bags. The phytosanitary certificate was provided to facilitate the transfer of the powdered leaves from Burundi to Kenya where the study was carried out.

2.2. Extraction Process

Powdered leaves of *Artemisia afra* was extracted with Dichloromethane; Ethanol and Hexane. The powder leaves were extracted with these three solvents in a flat bottom flask and sorely mixed on an orbital shaker. After gentle maceration for 48 hours, the extracts were filtered through a Whatman filter paper chart. The filtrate was concentrated under reduced pressure using rotary evaporator at 20 rpm and 40°C bath temperature. Finally, concentrated extracts were collected in vials and placed on a water bath at 40°C to evaporate the remaining solvents and stored at room temperature for complete dryness.

2.3. Ethical Approval

This study was approved by the Animal Care and Use Committee (ACUC) of Kenya Medical Research Institute (KEMRI) to carry out that work. The mice were handled following the standard operating procedures guidelines for the care and use of laboratory animals.

2.4. Experimentations on Animals

The animals used in this study were provided by KEMRI animal laboratory facility. The animals were kept for 24 hours in the acclimatization room before starting the experiment to avoid any stress. Thirty healthy adult male and female mice weighing between 20- 22g were used for the acute toxicity test. Mice were randomly assigned into 10 groups and were housed 3 animal per cages. Male and female were separated to avoid them to mate. The animals were kept in separate aluminium cages and provided with bedding of clean paddy husk. All animals had free access to water and food. The three extracts of *Artemisia afra* dissolved in 5% DMSO and in PBS, were prepared for the acute toxicity test, three concentrations were made for each extract 2500 mg/kg; 2000mg/kg; 1000mg/kg. Selection of doses was made based on the OECD guideline [12]. Mice were separated in 10 cages each with three individual per cage, 1 cage for control where the mice were inoculated with a mixture of phosphate buffer saline 1X with 5% DMSO and 9 others cages where every mice was inoculated with the extracts with only one single dose for each. The body weight of each mice was recorded before dosing. The first set, groups 2 to 4 received DCM extract with respectively three designated doses (1000mg/kg, 2000mg/kg 2500mg/kg of the formulation per body weight), the second set, groups 5 to 7 received ethanolic extract with respectively three designated doses (1000mg/kg, 2000mg/kg 2500mg/kg of the formulation per body weight), and the last set, group 8 to 10 received hexanolic extract with respectively three designated doses (1000mg/kg, 2000mg/kg 2500mg/kg of the formulation per body weight) to see a range of toxic effects and mortality rates in 48 hours observation.

After the single dose inoculation, mice were observed for 14 days toxicity signs and weight of animals were recorded every day. At the end of the experiment, mice were sacrificed using a CO₂ chamber and put on dissecting board where the organs were collected and the blood was drawn by cardiac puncture and kept in a vacuum heparin tube.

The blood samples in the tubes were centrifuged at 2500 rpm for 15 minutes and the serum was drawn and transferred into other clean vials and kept at -20°C until analysis for clinical chemistry measurements. The organs (Liver, Kidneys; and spleen) were collected and kept in phosphate buffered formalin solution for tissue processing, and the gross pathological observation of these organs was performed to check for any lesions.

2.5. *In Vivo* Antimalarial Assay

Fifty-six Swiss albinos' mice were used for *in vivo* antimalarial assays. The mice were infected with *P. berghei* *anka* and the assay was based on 4 days Peter test [13]. The parasite was provided by KEMRI and passage several times using donor's mice by intraperitoneal injection (IP) way. Briefly, an aliquot of 0.2 ml of infected blood from donor's mice was used to infect the experimental mice through intraperitoneal injection. The mice were infected with the

same amount of parasitized erythrocytes and divided randomly in a group of 4 individuals. Fourteen groups were made among which negative and positive group.

Two to 4 hours after infection (Day 0) mice were treated daily during 4 days with a single dose of test sample at a volume of 0.2ml by oral route. The positive group was treated with artemether 10mg/kg of body weight dissolved in tween 80 and the negative group was given of saline buffer constitute with PBS buffer, DMSO (5%) and 10% of tween 80. The remaining experimental groups were treated with *Artemisia afra* extracts. All the extracts were dissolved in DMSO and in Tween 80 and 4 dosages were made for DCM; hexanolic extracts (400 mg/kg, 200 mg/kg, 100 mg/kg, 50 mg/kg) and for ethanolic extracts (200 mg/kg, 100 mg/kg, 50 mg/kg, 25mg/kg).

Parasitaemia was determined daily (24 hours interval) with a thin blood smear sampled from the tail and stain with 10% of Giemsa solution. At the end of the 4 days petter's test thin blood smear was similarly made (96 h post-infection) for all the animals and stained as described above. Parasitaemia was calculated under a microscope by counting four fields of approximately 200 erythrocytes per field. The difference between the mean value of the control group (taken as 100%) and those of the experimental groups was calculated and expressed as the percentage of Chemo-Suppression, according to the following formula [10]:

$$\% \text{Chemo-Sup.} = [(A-B)/A] \times 100$$

A= Mean parasitaemia in the negative control group on day 4.

B= Corresponding parasitaemia in the test group.

2.6. Statistical Analysis

All data were organized and analyzed using SPSS version 25 statistical software. The values of body and organ weight and the biochemical parameters were analyzed by SPSS and the results were expressed as MEAN \pm SE(x) (standard error of the mean). Differences between the experimental and control groups were compared using one-way analysis of variance (ANOVA), followed by Dunnett's T-test to determine their level of significance. $P < 0.05$ were considered as statistically significant.

3. Results

3.1. Effect of Acute Toxicity of *Artemisia Afra* Extracts on the Weight and Behaviour of the Mice

After oral administration of the extracts, from the 30 mice used for acute toxicity test, only one died 24 hours after oral administration. That mouse belongs to the group treated with 2500mg/kg of dichloromethane extract (DCM group1). Changes like loss of appetite, hypoactivity, lethargic, dizziness were observed at the dose of 2500mg/kg and disappeared after the washout period after 2 days of observation.

Both the treated and control groups of mice had stable initial body weight during the first week and during the second week of the observation period, there is a proportional gain in their body weight (Figure 1, Figure 2, Figure 3).

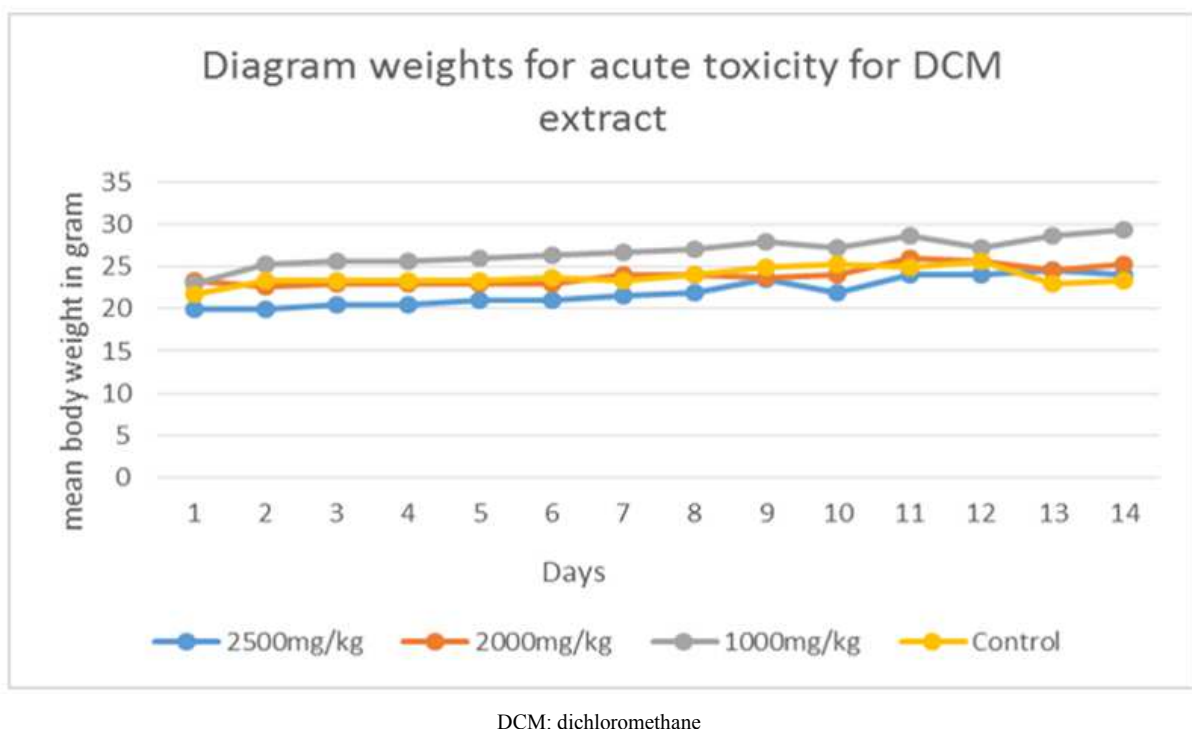


Figure 1. Mean body weight change in mice treated with 2500mg/kg; 2000mg/kg, 1000mg/kg DCM extracts one single dose.

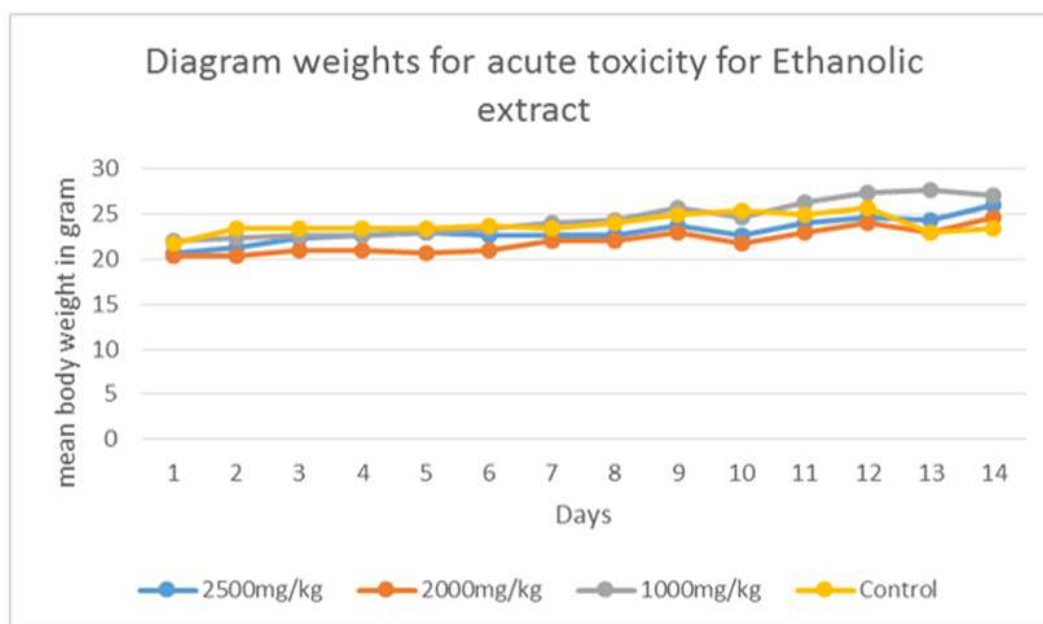


Figure 2. Mean body weight change in mice treated with 2500mg/kg; 2000mg/kg, 1000mg/kg Ethanolic extracts one single dose.

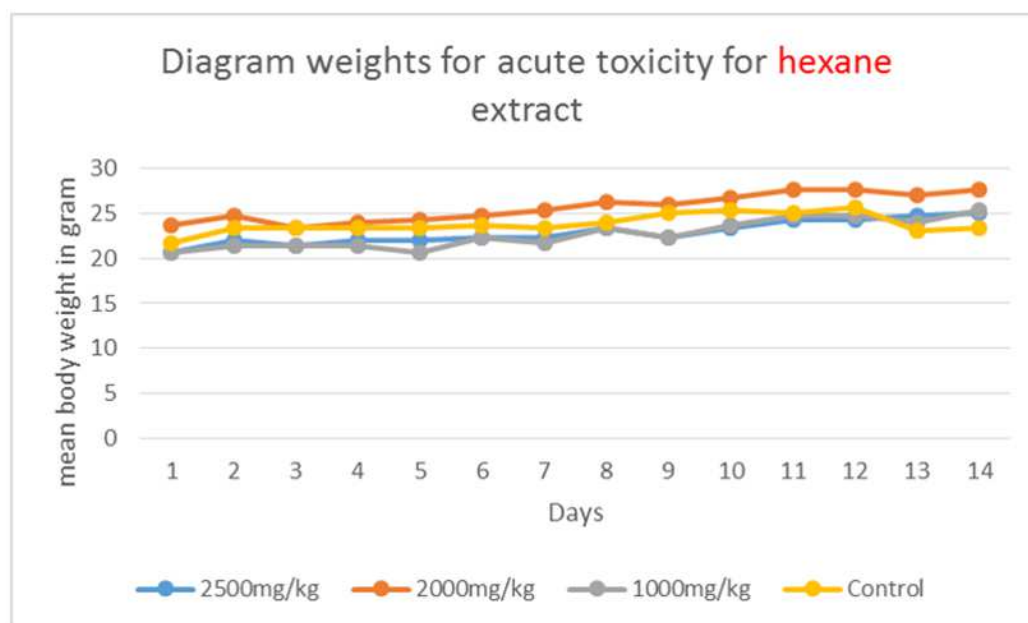


Figure 3. Mean body weight change in mice treated with 2500mg/kg; 2000mg/kg, 1000mg/kg Hexane extracts one single dose.

During the period of 14 days of acute toxicity test weight of animal was recorded daily and the statistical analysis of the weight between animal treated with extracts and the control showed some slight differences (Table 1).

Table 1. Mean body weight of mice treated with 3 extracts as compared to the control during 14days. Results presented as the mean of the body weight plus standard Error of the mean (P-value are in bracket).

Groups	Doses mg/kg	DCM (Mean body weight+SE)	Ethanol (Mean body weight +SE)	Hexane (Mean body weight +SE)
Group0	Control	23,8095±,28966	23,8095±,28966	23,8095±,28966
Group1	2500	22,0357±,43998** (.008)	23,095238±(.584)	22,8571±,35414 (.287)
Group2	2000	23,9524±,28996(.993)	21,976190±** (.010)	25,6429±,41561** (.006)
Group3	1000	26,7857±,44145** (.000)	24,500000±(.610)	22,6667±,42510 (.150)

SE: standard error of the mean;

DCM: dichloromethane;

P-value 0.05 is considered as significant

Mice treated with DCM extract the difference in body weight was significant when compared to the control in group 1 and group 3. In group 1 their weight was slightly small compared to the control but for group 3 it was in contrast since showed progressive body weight gain (figure 1) in the same time the group3 had received the smallest doses and the group1 had received the highest doses.

For mice inoculated with ethanolic extract, there was a significant difference in body weight compared to the control. The mice in group 2, had a slight loss of their body weight and a similar observation was reported in those treated with hexanolic extract although there was an increase of their body weight for the mice in group2 treated with 2000mg/kg.

For the last group of mice inoculate with hexanolic extract, when we compared each group to the control a gain of weight was observed in group 2.

3.2. Effect of Acute Toxicity of *Artemisia afra* Extracts on the Organs Weight of the Mice

After the 14th day, all the mice were sacrificed. Observation on the gross appearance of internal organs including liver, kidney, spleen and stomach of treated mice did not show any abnormal changes in texture, shape, size or colour in comparison to that of the control group. No lesion was noted in the internal organs in all groups.

Liver, kidney and spleen of each experimental group of mice were weight and compared to the control. *A. afra* extract did not produce any significant effect on the weight of the organs. The statistical analysis revealed that there were no significant differences between the organs of the mice inoculated with extracts (DCM, ETOH, and Hexanolic extracts) and the organs of the mice from control (Tables 2-4).

Table 2. Mean weight organs of mice treated with DCM extract as compared to the control (P-value in bracket).

Doses	Mean weight±SE for DCM extract		
	Liver	Kidney	Spleen
Control	1,5400±,07572	,3500±01528	,1000±,00000
1000 mg/kg	1,6267±,13383 (1,000)	,4933±,05487(,037)	,1033±,01333(1,000)
2000 mg/kg	1,3067±,08647(,829)	,3000±,02000(,978)	,0933±,02404(1,000)
2500 mg/kg	1,2533±,03756(,586)	,3433±,00882(1,000)	,1033±,00882(1,000)

SE: standard error

DCM: dichloromethane

Table 3. Mean weight organs of mice treated with HEX extract as compared to the control (P-value in bracket).

Doses	Mean weight±SE for hexane extract		
	Liver	Kidney	Spleen
Control	1,5400±,07572	,3500±,01528	,1000±,00000
1000 mg/kg	1,2867±,06438(,746)	,2800±,01202(,993)	,0800±,01000(1,000)
2000 mg/kg	1,2800±,01732(,715)	,2800±,01764(,442)	,0967±,00333(1,000)
2500 mg/kg	1,1600±,06429(,202)	,2800±,02082(,813)	,1000±,00000(1,000)

SE: standard error

Table 4. Mean weight organs of mice treated with EtOH extract as compared to the control (P-value in bracket).

Doses	Mean weight±SE for Ethanolic extract		
	Liver	Kidney	Spleen
Control	1,5400±,07572	,3500±,01528	,1000±,00000
1000 mg/kg	1,4300±,12166(1,000)	,4300±,02517(,661)	,1867±,06692(,419)
2000 mg/kg	1,1233±,12252(,118)	,3500±,03215(1,000)	,0767±,02186(1,000)
2500 mg/kg	1,3900±,09018(,992)	,3300±,01528 (1,000)	,1267±,03180(1,000)

EtOH: Ethanol

SE: standard error

3.3. Effect of Acute Toxicity of *Artemisia Afra* Extracts in the Biochemical Parameters Compared to the Control Group

AST and ALT level was tested in plasma of the mice treated with extracts and compared to the control to detect

any sign of toxicity on the liver. When we compared the biochemical parameters between the experimental mice and the control, there were no significant changes for the ALT and AST level in all doses of *Artemisia afra* extracts (Dichloromethane, hexane, and ethanolic) (Table 5).

Table 5. Effect of DCM, EtOH, and Hexane Extracts on AST and ALT biochemical parameters, mean of experimental groups compared to the control (Expressed in Mean \pm SDE, P-value in bracket).

Liver Function Test (AST in μ l)				
Groups	Doses (mg/kg)	DCM	Ethanol	Hexane
Control	-	196,3333 \pm 25,89938	196,3333 \pm 25,89938	196,3333 \pm 25,89938
Group1	1000	259,0000 \pm 19,07(0,852)	189,0000 \pm 65,96(1,000)	422,3333 \pm 63,83(,440)
Group2	2000	206,6667 \pm 42,58(1,000)	305,0000 \pm 57,422(,906)	508,0000 \pm 122,40 (,667)
Group3	25000	304,0000 \pm 66,39(,951)	264,6667 \pm 27,5095(,901)	370,3333 \pm 45,03(,397)

Liver Function Test (ALT in μ l)				
Groups	Doses (mg/kg)	DCM	Ethanol	Hexane
Control	-	33,2333 \pm 1,6835	33,2333 \pm 1,6835	33,2333 \pm 1,6835
Group1	1000	41,8000 \pm 6,23(0,975)	45,2333 \pm 13,57 (0,999)	63,4000 \pm 3,43(,055)
Group2	2000	34,6667 \pm 3,10(1,000)	51,2000 \pm 13,55 (,974)	119,0667 \pm 36,72 (,0715)
Group3	2500	37,0500 \pm 2,62(0,994)	48,0667 \pm 9,71(0,944)	79,2333 \pm 18,48 (,673)

3.4. In Vivo Antimalarial Assay of Artemisia Afra Extracted with DCM, EtOH, and Hexane

Antimalarial effects of extracts of *Artemisia afra* on *Plasmodium berghei* in mice are summarized in tables 6 and 7. The suppression of the Parasitaemia (chemo-suppression) and the ED₅₀ were used as measured of efficacy. Four Dosages were used to do the *in vivo* antimalarial assay. Samples were categorized as highly active when chemo-suppression was above 60% or moderately active between 30 and 60%, but lowly active below 30%. At the dosage of 200mg/kg of body weight for all the different extracts, we got around the same percentage of suppression (ETOH

61.64%; DCM 63.82%; and Hexane 66.89%) all results showed a high suppression of the parasites. When dosage was decreased the parasitaemia changed as follows, at the dosage of 100 mg/kg EtOH extract were the most active and showed moderate suppression compared to the others with a percentage of chemo-suppression which is still high at 57.07% for ETOH extract; following by DCM extract 42.17% and come in last Hexane extract 32.19%. The same observation was observed at the dosage of 50mg/kg of body weight the activity is still moderate for the *A. afra* ethanolic and DCM extracts but low for the hexane extract.

Table 6. Effect of DCM, EtOH and Hexanolic extracts of Artemisia afra on P. Berghei infected mice.

Dosage / %Chemosup.	Dosage 1 200mg/ml	Dosage 2 100mg/ml	Dosage 3 50mg/ml	Dosage 4 25mg/ml
DCM	63.82% \pm 6.54%	42.17% \pm 9.66	32.26 \pm 9.25%	5.84% \pm 2.20
ETOH	61.64% \pm 6.04	57.07% \pm 11.97	50.52% \pm 7,93	35.42% \pm 4.3

Dosage	Dosage 1 400mg/ml	Dosage 2 200mg/ml	Dosage 3 100mg/ml	Dosage 4 50mg/ml
Hexane	73.23% \pm 13.52	66.89% \pm 5.07	32.19% \pm 23.34	22.86% \pm 18.88
Positive control	88.33%			

The ethanolic extract of *A. afra* has the highest in vivo antimalarial activity with the highest ED₅₀ = 6.43mg/ml following by the DCM extract ED₅₀=11.99mg/ml then came is last the hexanolic extract ED₅₀= 14.79mg/ml.

Table 7. ED₅₀ from in vivo antimalarial assay for DCM, ETOH and Hexanolic extracts of Artemisia afra collected from Burundi.

Afra extracts	Ethanolic	DCM	Hexane
ED ₅₀ (mg/ml)	6.43	11.99	14.79

4. Discussions

Artemisia afra is widely used as a traditional medicinal plant in many countries e.g. South Africa [5] for its wild medicinal properties. Acute (single dose) toxicity was conducted in mice swiss albinos for a period of 14 days to determine the toxicity of the plant. Most studies have tested toxicity only on the aqueous extract. The toxicity of *A. afra* extracted with other solvents (DCM; ethanol, hexane...) needs to be investigated because having high antimalarial activities due to interest in the medicinal value of the plant. In this present study, the ethanolic, hexane, and DCM extract

of *A. afra* were tested for their toxicity and also *in vivo* antimalarial activity. The result of the acute toxicity did not report mortality with single oral dose up to 2500mg/kg body weight. Only one single mortality was recorded for the DCM extract at the dosage of 2500mg/kg body weight. The present result, therefore, suggests that the oral LD₅₀ of all the extracts are greater than 2500mg/kg. Others studies after an acute toxicity test have reported an LD₅₀ more than 5000mg/kg of body weight but for Aqueous extract of *Artemisia afra* [14] that showed that the aqueous extract is much safer. During the 14 days test, the body weight was recorded daily. Body weight changes are indicators of adverse side effects of drugs and chemicals [15, 16], as the animals that survive cannot lose more than 10% of the initial body weight [17]. An Increase of the body weight for all groups is observed during week 2. The increment in body weight determines the positive health status of the animals [18]. However, differences between the experimental and control group were noted. A decrease of body weight was observed for the group of mice treated with DCM extract at the dosage of 2500mg/kg and also for ETOH extract at 2000mg/kg compared to the control, but for hexane extract

of *A. afra* at the dosage of 2000mg/kg of body weight there is a gain of weight compare to the control. For the other groups, the weight gain is not significantly different from that of the control group. Therefore, the overall weight gain in both treated and control mice might indicate a good health status of the experimental animals. Research done by Eshetu *et al* in 2016 [19], the same observation was done during an acute oral administration test of aqueous extract of *Artemisia afra*. For the weight of the organs, there are no significant differences between the experimental groups and the control. Organs weight is an index of swelling, atrophy or hypertrophy [20]. Comparison of organ weights between control and treated groups have conventionally been used to predict the toxic effects of test material [21-23] and help to know if the organs were exposed to injury or not. However, in the present study, there was no significant change in organ (liver, spleen, and kidney) weights and visual gross examination of the organs of both treated mice and the controls. These showed normal architecture, no color change and no morphological disturbances, indicating that the acute toxicity oral doses of *A. afra* extracts administered had no effect on the organs of the mice and was well tolerated.

ALT and AST biochemical test were evaluated using the plasma from the blood of the mice to obtain further toxicity related information, not detected by direct examination of organs and body weight analysis. No statistical differences were noted when we compare the experimental mice with the control, which suggested that the extracts have no effect on the liver function. Generally, liver cell damage is characterized by a rise in serum enzymes like AST, ALT, ALP, etc. [24, 25]. In our results AST is more expressed compare to ALT this is due to that AST is expressed in higher concentration in a number of tissues (liver, kidney, heart and pancreas) and it is released slowly compare to ALT [26]. *Artemisia afra* is a promising medicinal plant with a high antimalarial effect. Mostly the acute toxicity for *A. afra* plant is done for the aqueous extract it is also very important to check the toxicity of the plant extracts when others solvents are used for the extraction. The antimalarial effect is more important when the plant is extracted with others solvent for example with methanol or ethanol compare to when it is extracted with water because this solvents (methanol, ethanol) extract more bioactive compounds that may have medicinal importance. The problem using those solvents is they may have toxic effect why toxicity test is important to run for them.

The *in vivo* antimalarial assay of *A. afra* extracts (EtOH, DCM and Hexanolic extracts) was found to have high activities. *A. afra* extracted with ethanolic had the highest activity, following the DCM extract then by the *A. afra* hexanolic extract. The results are in line with the study done by Gathirwa *et al.*, who found a high chemo-suppression activity of *A. afra* plant extracted with MeOH during *in vivo* antimalarial activity with *P. berghei anka* [10]. This plant not only had a big antimalarial activity but also was found to inhibit rapid growing of *Mycobacterium aurum* and virulent *Mycobacterium tuberculosis* replication [27], to reduce also blood glucose and may have beneficial effects on

complications of diabetes [28], and also to have potential anticancer properties [1].

5. Conclusion

Artemisia afra plant has a remarkable *in vivo* antimalarial activity. The LD₅₀ after acute oral toxicity was greater than 2500mg/kg of body weights. No significant changes in general behaviour and biochemical parameters, except a slight increase in AST activity. No significant changes were observed also in organ weights. In conclusion, findings suggest that *A. afra* extracts have a significant *in vivo* activity and are no toxic when given orally up to 2500mg/kg. This plant constitutes a great source to find new antimalarial compounds. Further studies, however are required to study the safety of that plant during a sub-chronic test.

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Conflict of Interest

The author declare no conflict of interest.

Ethical Considerations

This study was approved by the Animal Care and Use Committee (ACUC) of Kenya Medical Research Institute (KEMRI) to carry out that work.

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