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HEPATOPROTECTIVE EFFECT OF ETHANOLIC LEAF EXTRACTS OF ABRUS PRECATORIUS IN PLASMODIUM BERGHEI INFECTED IMPRINTING CONTROL REGION (ICR) MICE; A HISTOPATHOLOGICAL PERSPECTIVE

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ABSTRACT

The aim of this study is to investigate the histological perspective of hepatoprotective activity of ethanolic leaf extract of *A. precatorius* in *P. berghei* infected Imprinting Control Region (ICR) mice. Thirty-six (36) ICR mice were separated into six groups of six mice each. Five groups were intravenously inoculated with *P. berghei* and changes in parasitaemia was then monitored and recorded daily to confirm infection. The sixth group was neither infected nor treated (the normal control group). Seven days after infection, four groups were administered orally with 30, 50 and 100mg/kg of the extract and 4mg/kg of Artemeter Lumefanthrine for five consecutive days, whereas the fifth group was not treated (negative control). Changes in *P.berghei* parasitaemia on days 0,1,2,3 and 4 during treatment were then ascertained. Haematological analysis of blood samples and histopathological evaluation of liver and spleen sections were then carried out to determine the tissue regenerative effect of the extract. Mice treated with 30, 50, 100mg/kg of extract and 4mg/kg of Artemether Lumefanthrine, had significantly ($p<0.05$) lower malaria parasitaemia and high parasite clearance accompanied with regeneration of hepatocytes in liver sections. Also, there was significant closure of parasitophorous vacuoles in treated groups, as compared with the negative control group that showed severe hepatocyte derangement and conspicuous parasitophorous vacuoles around parasitized hepatocytes. The ethanolic leaf extract of *A. precatorius* showed significant histological evidence of hepatoprotective activity ($p<0.05$) against *P. berghei* infected ICR mice.

Key Words: *Abrus precatorius*, hepatoprotective activity, *Plasmodium berghei*, ICR mice.

INTRODUCTION

The plant, *Abrus precatorius* is known commonly as jequirity, crab's eye, rosary pea, precatory pea or bean, John Crow bead, Indian licorice, Akar Saga, gideegidee or Jumbie bead in Trinidad and Tobago (Wag staff, 2008). In Ghana, it is known among the Ashantes as damma - bo, obrekuo aniwa, and the Fantes as dabraboma or anyan-enyiwwa. It is a slender, perennial climber that twines around trees, shrubs, and hedges. It is a legume with long, pinnate-leafleted leaves and the flowers are small and pale violet in color with fruits scarlet in color with glossy texture (Kare, 2007) .The plant is best known for its seeds, which are used as beads and in percussion instruments, and it is also known for its toxic nature due to the presence of abrinn in seeds (Bisby *et al*., 1994). The seed is found in a variety of colors such as black, orange, and most

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commonly, red with a glossy appearance with the black band at the end that attaches to the plant (Fernando, 2001).

Laboratory study of the plant has revealed that an ethanolic extract of *A.precatorius* has antioxidant, anti-inflammatory and analgesic potential in rodents (Arora *et al*., 2011). The methanolic extract of the leaves of *A.precatorius* has been evaluated for possible bronchodilator activity by using various in vivo and in vitro models in guinea pigs and the results revealed that the methanolic extract produced dose-dependent bronchodilator activity, thus justifying to some extent the traditional use of the plant *A.precatorius* in asthma (Asaase *et al*., 2011). In another research, neuromuscular effects of the crude extracts of the leaves of *A.precatorius* was investigated using isolated toad rectus abdominals and rat phrenic nerve-diaphragm muscle preparations as well as young chicks. Also Studies on aqueous, methanolic and chloroform extracts of *A.precatorius* showed greater inhibitory activity against a number of disease causing bacteria such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli* (Kekuda *et al*., 2010 ; Sudipta *et al*., 2012).

Malaria is the world's worst tropical disease, which claims 2.7 – 3 million lives annually. About 1.5 billion people live in malarious regions such as Nigeria and Ghana and is responsible for 1 in 5 deaths of children in Africa (Akubue, 2006). It is a life-threatening disease caused by parasites transmitted through the bites of sporozoite infected female *anopheles* mosquitoes. In 2013, malaria caused an estimated 584 000 deaths mostly among African children (WHO, 2014).There are four main parasite species that cause malaria in humans: *Plasmodium falciparum, P. vivax, P. malariae and P. ovale,* though *P. knowlesi* is also known to cause zoonotic malaria in man occasionally with other known species of Plasmodium infecting rodents, example *P. yoelli, P. vinckei, P.chabaudi* and *P. berghei* (Gillespie and Pearson, 2001). *P. berghei* is one of the species of the plasmodium that have been described in African murine rodents and is also transmitted through the bite of female *Anopheles* mosquito (Jambou *et al*., 2011). Over the years, there have been several attempts to develop potent and efficacious antimalaria agents to combat the degree of resistance. However, most of the drugs such as artesunate have potential liver damaging effect (Ranjita *et al*., 2014). Research has shown that antimalarial agents produce carbon-centered radical to influence the biochemical environment of certain vital organs such as liver and kidney which manifests histologically (Olayemi *et al*., 2012) in tissue sections.

Hepatocyte disturbances are mostly known to be mediated by xenobiotics (Nasreddin & Beydoun, 2007; Santo *et al*., 2008) and malarial infection. Hepatocytes of zone 1 of the hepatic acinus are subject to cellular damage usually when xenobiotics are the major stimuli. Jaundice and liver dysfunction are common in severe malaria caused by *P. falciparum* (Autino *et al*., 2012) in malarious regions. Most of the hepatic pathologies occur during the pre-erythrocytic cycle. During this stage, the sporozoite enters the hepatocyte and creates parasitophorous vacuole within which it develop into merozoites. Unlike red blood cells which rupture to release the trophozoite, the exit from hepatocyte is by attachment of the merosomes to the inner surface of the plasma membrane of the hepatocyte and releasing the trophozoite out by exocytosis leaving a hollow parasitophorous vacuole behind. Inability of the empty parasitophorous vacuole to close under normal physiological conditions may result in the death of hepatocyte by necrosis or apoptosis. Though *A. precatorius* is known to have antioxidant activity and hepatoprotective effect, the histological mechanism through which this is achieved is not established.

METHODS

The plant materials (leaves) used for the study was obtained from the University of Cape Coast Botanical Garden and authenticated by the botanist at the School of Biological Science, University of Cape Coast.

Ethanolic extract of the plant materials

The method for the preparation of the plant extract was as follows. The leaves were shade dried and pulverized to fine powder using warring blender.49.4 grams of *A.precatorius* leaf powder was macerated in 500ml solution(70% v/v ethanol) in a conical flask. The mixture was thoroughly shaken intermittently throughout the period of extraction and was allowed to stand for seventy two hours (72hrs). The filtrate of the extract was reduced to a dark green syrupy liquid using a water bath and then dried into its solid form (Saganuwan and Gulumbe, 2005a).

Phytochemical Constituents of the Extract

Qualitative phytochemical screening was performed on the extract to determine the presence of Saponins, Tannins, Triterpenes, Alkaloids, Flavonoids and Glycosides using the procedure described by (Kapoor *et al*., 1969; Odebiyi *et al*., 1978).

Antioxidant activity of A. precatorius

The nitric oxide radical scavenging activity was determined according to the method described by (Hallowell and Gutteridge, 1999) with slight modifications. A volume of 2 ml of 10 mM sodium nitroprusside prepared in 0.025 M of phosphate buffer (pH 7.4) was mixed with 0.5 ml of plant extracts and standards, (gallic acid and ascorbic acid) individually at concentrations of (20-200µg/ml). The mixture was incubated at 25°C for 150 min. A volume of 0.5 mL of the incubated solution was mixed with 0.5 ml of Griess reagent (prepared by mixing in a ratio 1:1, 1% w/v sulphanilamide reagent prepared in distilled water and heated on a water bath at 100 OC and

0.1% w/v naphthylenediamine dichloride dissolved in 5% phosphoric acid). The mixture was further incubated at room temperature for 30 min after which the absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthylenediamine dichloride was measured at 540 nm against a phosphate buffer blank. Control experiment without the test sample but with equivalent amount of the solvent used to dissolve the sample was conducted in an identical manner. All experiments were conducted in triplicates.

Parasite inoculation and animal treatment

A total of thirty-six (36) ICR mice were put into 6 groups; 6 mice per group (thus 4 test groups and two control group). All the mice in the test groups and the positive control group were inoculated intraperitoneally with *P. berghei* and mice were examined daily for the presence of the parasites.

Seven days after inoculation, mice in group 1, 2 and 3 were treated with 30, 50 and 100mg/kg of ethanolic extract of *A. precatorius* leaf respectively. Mice in group 4 were treated with Artemether and Lumefanthrine (a standard antimalaria drug). The fifth group, the positive control group was left untreated. However, group 6 mice were neither inoculated nor treated. The mice were treated for seven consecutive days and were monitored until the parasites were cleared off except in group 5.

Blood samples were obtained from the nicked tail vein of the infected mice each day of treatment and examined using thick and thin blood films for parasite density and parasitized erythrocytes count respectively (Cheesbrough, 2005). This was done by microscopy using oil immersion. At the end of the treatment, the mice in all the groups were sacrificed for hematological and histopathological analysis.

Hematological analysis

Some selected hematological parameters were determined. The parameters include red blood cell (RBC) count, packed cell volume (PCV), Haemoglobin concentration (HB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and white blood cells (WBCs) count.

Histopathological procedure

The liver and spleen were removed and examined macroscopically and then fixed with 10% formalin for about 48 hours. The tissues were then processed routinely, stained with routine Haematoxylin and Eosin (H & E) stain as described elsewhere (Bancroft and Gamble, 2008). The slides were observed under the light microscope at magnifications of X10 and X40 for histological analysis.

RESULTS

Mean percentage parasitaemia

The mean percent parasitaemia of mice infected with *P. berghei*and subsequently treated with varying doses of the ethanolic extract of *A. precatorius* leaves. The pre-infection mean percent parasitaemia were similar in all the groups. Following infection, the mean percent parasitaemia decreased in all the treatment groups but increased in the infected untreated group. The observed increase in mean percent parasitaemia in the infected untreated group was significant ($P<0.05$) compared to the groups treated with either the extract or halofantrine. The mean percent parasitaemia for the infected untreated group increased from 52.1 ±3.513 on day 0 to 71.8±3.513 on day 4.The treated groups decreased in mean percent parasitaemia from day 0 up to day 4. The normal group remained unchanged throughout the period of experimentation.

Mean percent reduction parasitaemia

Table 4.2 shows the mean percent reduction of mice infected with *P.berghei* and subsequently treated with varying doses of the ethanolic extract of *A. precatorius* leaves. The mean percent reduction was highest in the positive control group with percent reduction of 10.5±0.587% followed by the treatment groups with percent reduction of 9.83±0.587%, 8.60±0.587%, and 7.90±0.587%, in the 30,100 and 50 mg/kg extracts.

Hematological parameters for the various groups

The hematological parameter of mice infected with *P. berghei* and subsequently treated with varying doses of the ethanolic extract of *A. precatorius* leaves showed significant variations.

The hemoglobin concentration on the day 4 was highest in the positive control group (15.5±12.67), followed by 30,100 and 50 mg/kg extract with recorded values of 13.0±7.14, 10.5±8.86 and 8.5±8.13 respectively. The RBCs count was lowest in the infected untreated control and highest in the positive control on the day 4. However, the WBC recorded was highest in the infected untreated group with a value of 8.7±1.78 and was lowest in the positive control group with a value of 3.6±12.67. The highest MCHC value was 58.7±12.67 and was recorded for the positive control group on day four while the least value was 8.3±1.78 and was recorded for the infected untreated group. The differences in the hematological parameters were very significant in the various treatment groups ($p<0.05$).

The wave length reading for Nitric oxide (NO) radical scavenging activity of A. precatorius

The absorbance (UV) light absorption reading decreased from 0.0903 to 0.0544 with concentrations 1.0 and 0.2 respectively for the plant extract. The gallic acid, the ascorbic acid and the blank also decreased from 0.0791, 0.0471 and 0.1086 with 1.0 concentration to 0.0565,0.0430 and 0.1071 respectively.

Figure 1 shows the mean percent parasitaemia of mice infected with *P. berghei* and subsequently treated with varying doses of the ethanolic extract of *A. precatorius* leaves. The pre-infection mean percent parasitaemia were similar in all the groups. Following infection, the mean percent parasitaemia decreased in all the treatment groups but increased in the infected untreated group.The mean percent parasitaemia for the infected untreated group recorded the highest value whereasthe treated groups decreased in mean percent parasitaemia from day 0 up to day 4. The normal group remained unchanged throughout the period of experimentation.

Plates A and B are liver tissues obtained from negative control mice (x40 & x 10 magnifications respective). In this section (x40), there are several infected hepatocytes per field with highly basophilic nuclei. Cells are found to contain merosomes and larger parasitophorous vacuoles with tissue sections which appear to be edematous. Hepatic sinusoids are found to be severely enlarged and contain hemosiderin deposits. Central vein has moderately fragmented endothelium and contain many basophilic neutrophils (x10). Plates C and D are liver tissues obtained from positive control mice (x40 & x 10 magnifications respective). Tissue shows moderate number of infected hepatocytes per field with evidence of hepatic necrosis due to presence of basophilic nuclei and merosomes. Parasitophorous vacuoles are of similar size compared with those of negative control. Hepatic sinusoids are found to be moderately enlarged and contain hemosiderin deposits due to hemolysis. Central vein has subnormal endothelium and contain few basophilic neutrophils (x10). Plates E and F are liver tissues obtained from mice treated with 30mg/Kg of ethanolic extract *Abrus precatorius* leaf. Tissue shows very few infected hepatocytes per field with no evidence of hepatic necrosis. Parasitophorous vacuoles are found to be highly closed with normal hepatic sinusoids and normal central vein. Plate G is liver tissue obtained from a normal control mouse showing normal histological architecture.

Plate I is photomicrograph of a spleen obtained from negative control, showing white and red pulp, with the presence of haemosiderin deposits.

Table 1. Results showing the phytochemical screening of *A. precatorius*

TEST	RESULTS
Saponine	+++
triterpin	_ _ _
alkaloid	+++
tanin	+++
Flavonoid	+++

+++ indicates positive results. --- indicates negative result.

Table 2. Mean percentage parasitaemia

DOSE/DAY	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
30mg	52.3±7.13	40.5±7.13*[a]	30.3±7.13*	18.4±7.13*	13.4±7.13*
50mg	46.8±5.68	41.8±5.68*[b]	38±5.68*[bc]	25.3±5.68*[bc]	15.2±5.68*
100mg	50.2±6.13	35.9±6.13*	29.7±6.13*	20.2±6.13*	15.8±6.13*
PoC	55.6±7.83	47.9±7.83*	37.3±7.83*	21.6±7.83*	13.8±7.83*
NeC	52.1 ±3.51	54.7±3.51	59.5±3.51	65.8±3.51	71.8±3.51
NC	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00

PoC= Positive control, NeC= negative control, NC= Normal control. (*) indicate significant difference between negative control and treated groups. ([a]) is significant difference between 100mg/Kg and 30mgKg, ([b]) is significance difference between 100mg/Kg and 50mg/Kg, and ([c]) significance difference between 50mg/Kg and 30mgKg.

Table 3. Mean percent reduction parasitaemia

Dosage(mg/Kg)	30	50	100	PoC	NeC.	NC
% reduction.	9.83±0.59	7.90±0.59	8.60±0.59	10.5±0.59	-10.8±0.58	0±0.00

Values are expressed as mean± standard error of mean (Mean± SEM).PoC= Positive control, NeC= Negative control, NC= Normal control

Table 4. Hematological parameters for the various groups

Dosage(mg/kg)	WBC (K/µL)	RBC (M/µL)	HGB (g/dL)	MCHC(g/dL)
30	3.70±7.14*	7.11±7.14*	13.00±7.14*	35.50±7.14*[a]
50	5.60±8.13*	4.98±8.13*	8.50±8.13*	38.30±8.13*
100	4.40±8.86*	4.67±8.86*	10.50±8.86*[c]	41.90±8.86*
PoC	3.60±12.67*	7.79±12.67*[a]	13.50±12.67*[a]	58.70±12.67*
NeC	8.70±1.78	1.08±1.78	3.10±1.78	8.30±1.78
NC	4.28±0.72	7.92±0.73	14.20±6.10	34.60±0.13

Values are expressed as mean± standard error of mean (Mean± SEM). PoC= Positive control, NeC= negative control, NC= Normal control. (*) indicate significant difference between negative control and treated groups. ([a]) is significant difference between 100mg/Kg and 30mgKg, ([b]) is significance difference between 100mg/Kg and 50mg/Kg, and ([c]) significant difference between 50mg/Kg and 30mgKg.

Table 5. Wave length readings for Nitric oxide (NO) radical scavenging activity of *A. precatorius*

Conc./Abs	1.0	0.8	0.6	0.4	0.2
Plant extract	0.0903±0.001	0.0796±0.001	0.0689±0.001	0.0665±0.001	0.0544±0.001
Gallic acid	0.0791±0.012	0.0763±0.012	0.0700±0.012	0.0690±0.012	0.0565±0.012
Ascorbic acid	0.0471±0.010	0.0466±0.010	0.0454±0.010	0.0437±0.010	0.0430±0.010
Blank	0.1086±0.023	0.1085±0.023	0.1081±0.023	0.1079±0.023	0.1071±0.023

Values are expressed as mean± standard error of mean (Mean± SEM).

Fig 1. Daily antimalarial activity of ethanolic leaf extract of *A. precatorius* and Artemeter Lumefanthrine on *P. berghei* infected ICR mice

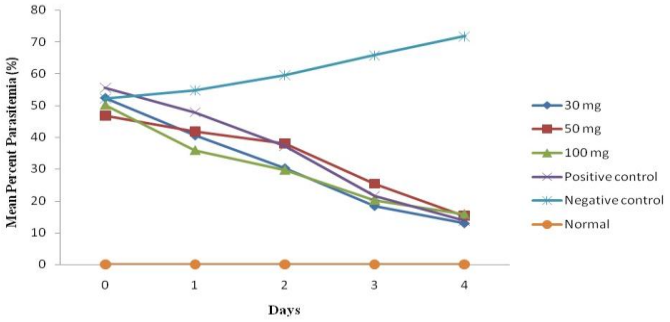


Fig 2. Photomicrograph of liver obtained from various mice

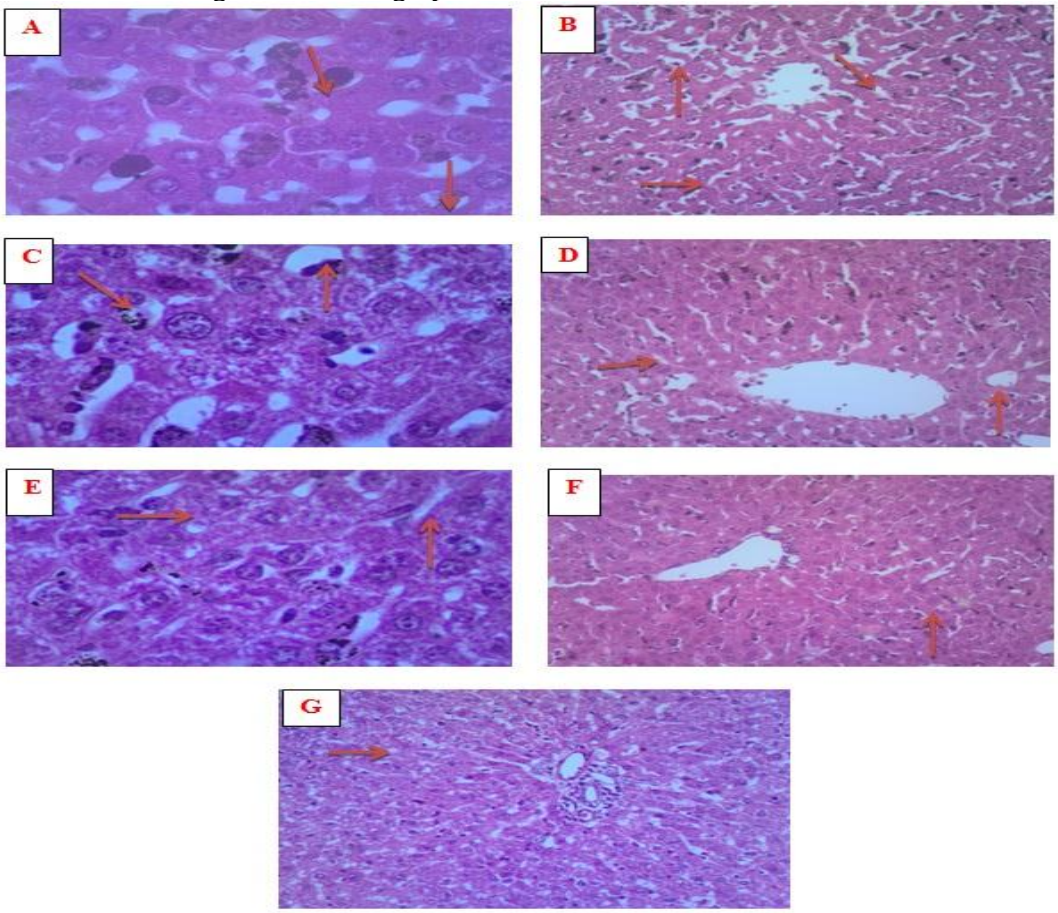
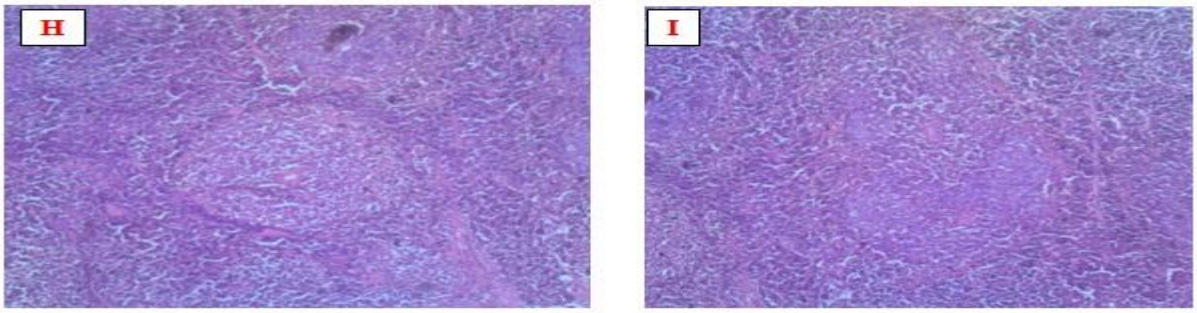


Fig 2. Plate H is a photomicrograph of spleen taken from a mouse treated with ethanolic extract of *A. precatorius* showing white and red pulp, with no distinct marginal zone and I shows photomicrograph of a spleen from negative control rats, showing white and red pulp, with the presence of haemosiderin



DISCUSSION

From the present study, *P. berghei* produced a significant parasitaemia, weakness, anaemia and loss of body weight within seven days of infection. The signs observed in this study were similar to those reported in a research conducted elsewhere (Saganuwan *et al*., 2010). The signs observed also conform to the clinical signs observed in acute malaria infection in mice (Ajagbonna *et al*., 2002). The weight of the *P. berghei* infected untreated mice decreased significantly compared with the weights of the infected treated mice. This might be due to glucose depletion, loss of appetite and anaemia in the untreated mice. This shows that malarial infection can induce significant decrease in body weight. This observation agrees with the report of Health Exchange (2001) indicating that malaria could cause decreased weight gain.

The parasitological findings show that when the mice were treated with the various doses of *A. precatorius* (30, 50 and 100 mg/kg) and Artemether Lumefanthrine respectively, there was a significant decreased in mean percent parasitaemia. The mean percent parasitaemia for the 30mg, 50mg and 100mg decreased from 52.3±7.125%, 46.8±5.683%, and 50.2±6.127% on day 0 to 13±7.125%, 15.2±5.683% and15.8±6.127% respectively on day seven. The decrease in mean percent parasitaemia might be due to the fact that the extract was able to clear the *P. berghei* parasites from the blood. However, there was a significant increase in mean percent parasitaemia in the negative control (infected untreated). The increase in mean percent parasitaemia might be due to tremendous multiplication of the *P. berghei* in the mice in the absence of treatment. This observation agrees with the report of Saganuwan and Onyeyili (2010), where the antimalarial effect of aqueous extract of *A. precatorius* was studied in *P. berghei* infected mice (Saganuwan and Onyeyili, 2010). The mean percent parasitaemia for the normal group, however, remained the same throughout the experimental process. From the results, it can be inferred that the standard antimalarial drug (Artemeter Lumefanthrine) has the highest mean reduction percentage parasitaemia (10.5±0.587%), followed by the 30mg extract with a mean reduction percentage parasitaemia of 9.38±0.587%.

The hematological findings showed that the red blood cells (RBC) and hemoglobin (Hb) levels decreased after infection with *P. berghei*. The decrease in RBC and Hb levels is an indication of hemolytic anaemia. This finding confirms the results obtained by Ajagbonna and colleagues who studied the hematological effects of *A. precatorius* in *P. berghei* infected mice. They also observed a progressive fall in RBC, and Hb values in plasmodium infections (Ajagbonna*et al*., 2002).

In the present study, the RBC, and Hb values increased in the treated on day 4 of treatment. The increased level of the hematological parameters noticed in this study may be due to the antimalarial effect of the extract and Artemether Lumefanthrine on the *Plasmodium* parasites, since the increase in the blood parameters corresponded to the decreased parasite load. The infected mice showed leukocytosis which decreased to normal with the progressive disappearance of the parasites following treatment. This phenomenon is in agreement with the findings of Guyton and Hall (2007), and might be due to lymphocytosis, eosinophilia and monocytosis. The WBC counts returned to almost pre-infection levels following treatment with the extract and Artemeter Lumefanthrine and may suggest that *A. precatorius* leaf extract has antimalaria effects similar to Artemeter Lumefanthrine.

The results from the histopathological study also revealed severe pathological changes in the liver of the infected untreated (negative control) mice. There was evidence of severe haemosiderosis in the hepatic sinusoid and bile canaliculi. Critical examination of the plasma membrane of hepatocytes in zone 1 of the hepatic sinus indicated moderate signs of coagulative hepatic necrosis accompanied by density of conspicuous parasitophorous vacuole around infected hepatocyte per field of view. There was also evidence of endothelia cell derangement in the central vein (CV) and portal triad in the same mice. The severe histological damages observed in the liver of the negative control mice were as a result of parasite life cycle during hepatic phase of *Plasmodium* development. The infection of host cell by plasmodium sporozoites

require sporozoite motility (Prudencio *et al*., 2006) and presumably involve invagination of the plasma membrane with the formation of parasitophorous vacuole around the parasite. The sporozoites migrate from the hepatic sinusoids, reach and infect hepatocytes and form parasitophorous vacuole within which they multiply. This is where the sporozoites develop into several merozoites and surrounded by a membrane called merosomes. Upon maturation of merozoites, they exit the hepatocyte by exocytosis. This mechanism is known to cause disruption in the plasma membrane of the hepatocyte thereby inducing spontaneous necrosis of the affected liver cells. The *Plasmodium* parasite may also breakdown glucose to form lactic acid and release free radicals into the extracellular compartment of the liver to cause bleb of the plasma membrane of the hepatocytes. This is the most probable cause of ischemia of cells underlying the blockade, and resulting coagulative necrosis. The ischemia, lactic acid production and free radical present could be a major contributing factor of endothelia cell derangement in the bile canaliculi, hepatic sinusoids, central vein and parts of the portal triads.

The number of hepatocyte infected determines the pathological state of the liver. In the absence of treatment, more hepatocytes are invaded and high level of hepatic necrosis is observed. In response to the damage caused, Kupffer cells actively proliferate to break down ruptured cellular debris by phagocytic action leading to Kupffer cell hypertrophy and hyperplasia. The iron in the haemoglobin molecule accumulates in the liver resulting in haemosiderosis which progresses to haemochromatosis in the absence of treatment. This iron accumulation also resulted in micronodular cirrhosis which appeared microscopically as Prussian blue stain. Despite all these cellular damages observe, periportal mononuclear cell infiltration was absent.

Histological section of liver from the mice treated with Artemeter Lumefanthrine indicated a mild haemosiderosis in the hepatic sinusoid and bile canaliculi, and little evidence of hepatic necrosis. Presence of hemosider in the hepatic sinusoid and bile canaliculi indicates that Artemeter Lumefanthrine has hemolytic effect. Cytoplasm of few hepatocytes was found to be bleb in the hepatic acini. However, there was no difference in parasitophorous vacuoles in hepatocytes of liver of mice treated with Artemeter Lumefanthrine and the negative control group.

The ethanolic leaf extract of *A. precatorius* at a dose of 30mg/Kg showed greatest antimalarial activity close to that of Artemeter Lumefanthrinein *P. berghei* infected ICR. However, the histopathological changes in both cases were different. In the mice treated with 30mgKg of the *A. precatorius*, there was no evidence of haemosiderosis or Kupffer cell hyperplasia. There were very mild hepatic necrosis with very little parasitophorous vacuole and merosomes in few hepatocytes compared with the group treated with Artemeter Lumefanthrine. The large parasitophorous vacuole observed in the negative control group was found to be significantly closed in this case. This evidence could be traced to the antioxidant activity of *A. precatorius* extract (Arora *et al*., 2011) which has potentiated the wound healing effect of the liver thereby closing the parasitophorous vacuoles. It is wealth admitting the fact that, the antioxidant activities of the extract mopped up most free radicals introduced by the presence of the *P. berghei* and helped stabilize the plasma membrane of the hepatocyte thereby reducing any possible necrotic effect caused by the plasmodium.

Gross anatomical observation showed hypertrophy of the spleen in the extract treated mice but not in the positive control. This is in agreement with research work carried out elsewhere (Alebachew *et al*., 2014). Histological study reveals hyperplasia of the spleen with white and red pulp, with no distinct marginal zone. In the negative control, there was white and red pulp, with the presence of haemosiderosis.

CONCLUSION

The ethanolic leaf extract of *A. precatorius* showed significant antimalarial activity in *P. berghei* infected ICR mice and was effective at the dose of 30 and 100mg/Kg. Histological evidence also showed that the extract was effective at a dose of 30mg/Kg, which had a strong hepatoprotective effect in *P. berghei* infected ICR mice.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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