Direct RESEARCH JOURNALS
Vol.4 (3), pp. 29-34, August 2016
ISSN 2449-0814
Article Number: DRJA111712367

Direct Research Journal of Health and Pharmacology
http://directresearchpublisher.org/journal/drjhp

Research Paper

Effects of aqueous stem bark extract of *Jatropha curcas* on some haematological indices of mice infected with *Plasmodium berghei*

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Received 23 July 2016; Accepted 8 August, 2016

The effect of aqueous stem bark extract of Jatropha curcas in mice infected with Plasmodium berghei was investigated. Ninety mice divided into six groups of fifteen were used for the study. Group 1 served as normal control, group 2 was inoculated with 0.2 ml of 1.0 x 107 infected erythrocytes containing Plasmodium berghei as negative control, Groups 3, 4 and 5 were infected and treated with 250, 500 and 750 mg/kg body weight aqueous stem bark extract of Jatropha curcas respectively while Group 6 were infected and treated with arthemether (20 mg/kg body weight) for fourteen days. The infected untreated showed a significant decrease($p<0.05$) in packed cell volume(PCV), red blood cells (RBCs), haemoglobin(HB), white blood cells (WBCs), and platelet count from day 7 to day 14 compared with normal control ($p<0.05$).However, treatment with the aqueous stem bark extract of Jatropha curcas and arthemether caused a significant increase ($p<0.05$) in RBCs, PCV and platelet levels reduced by infection compared to untreated control group ($p<0.05$). Also, there was no significant ($p<0.05$) difference between the extract-treated and the arthemether-treated groups 3, 4, 5 and group 6 respectively from the normal control group ($p<0.05$). Since our findings had shown that there were improvements in all the haematological indices for the infected and treated groups, this suggests that the plant extract of Jatropha curcas possess some protective properties against malaria parasites.

Key words: Haematological, Biochemical indices, Plasmodium berghei, Jatropha curcas

INTRODUCTION

Malaria is a disease caused by a parasite that spends part of her life in humans and part in mosquitoes. The actual number of deaths caused by malaria yearly may be significantly higher, as precise statistics are unavailable in many rural areas, and many cases are undocumented. Malaria is commonly associated with poverty, and can indeed be the consequence of poverty and a major hindrance to economic development (Mandell *et al.,* 2010). The World Health Organization (WHO) estimated that in 2002 malaria caused 207 million clinical episodes, and 627,000 deaths. An estimated 91% of deaths in 2010 were in the African Region. In the African region, where malaria is the most common and serious infectious disease. In 1955 the World Health Organization (WHO) began a program to eliminate malaria worldwide. Initially there was great success as WHO employed insecticides such as DDT against the mosquito vectors. Unfortunately, strains of *Anopheles* mosquitoes began a rapid resurgence due to resistance in the mosquitoes against DDT (WHO, 2005). It is now estimated that about 400 to 600 million people are infected annually worldwide with about 4 million deaths (WHO, 2005).

According to Centre for Diseases Prevention and Control

(CDC, 2013), malaria remains one of the major killers of humans worldwide, threatening the lives of more than one-third of the world's population. It thrives in the tropical areas of Asia, Africa and Central and South America, where it strikes millions of people. Each year 350 to 500 million cases of malaria occur worldwide. Sadly, more than 1 million of its victims, mostly young children, die yearly. More than 100 different species of *Plasmodium* exist and they produce malaria in many types of animals and birds, as well as in humans.

Secondary metabolites of plants (phytochemicals) with previously unknown pharmacological activities have been extensively investigated as medicinal agents (Sofowora, 1993).

These secondary metabolites differ from plant to plant and include such examples as: anthraquinones, flavonoids, glycosides, saponins, tannins and etc. Plants also contain other compounds such as morphine, atropine, codeine, steroids, lactones and volatile oils, which possess medical values for the treatment of different diseases (Sofowora, 1993). Due to the medicinal properties of most plants, it is essential that their efficacy and toxicity risks should be evaluated. *Jatropha curcas* is a drought resistant large shrub, belonging to the genus *Euphorbiaceae* which has oil producing seeds (Jongschaap *et al*., 2007). *Jatropha curcas* L. is the commonest specie found in Nigeria. Traditionally *Jatropha curcas* is used for the treatment of fever, mouth infections, jaundice, guinea worm, sores and joint rheumatism (Van den Berg *et al.,* 2010;Villegas *et al.,* 1997).

Previous studies also reported the presence of antibacterial agents in different parts of *Jatropha curcas* L. (Mishra *et al.,* 2010).The people of Jada local government area of Adamawa State, Nigeria and its inhabitants widely use *Jatropha curcas* stem bark for the treatment of many ailments including malaria. However, there are no scientific bases for malaria treatment. Therefore, this research is designed to provide the scientific evidence for the use of the aqueous stem bark extract of *Jatropha curcas* on some haematological indices since. Our unpublished paper has earlier reported the scientific basis for the antimalarial activity.

MATERIALS AND METHODS

Collection of plant material

Fresh *Jatropha curcas* plant was collected from the Staff quarters of Government Secondary School, Jada Local Government Area of Adamawa State, Nigeria and was taxonomically identified and authenticated in the Plant Science Department of Modibbo Adama University of Technology, Yola.

Chemicals and reagents

All reagents and solvents used were of analytical grade.

Experimental animals

Ninety mice weighing between 18-22 g were obtained from Animal Facility Centre, National Veterinary Research Institute Vom, Jos, Nigeria. The mice were housed in polypropylene cages, and given standard laboratory diet and water and maintained under standard laboratory conditions.

Malaria parasite

The malaria parasite (*Plasmodium berghei* NK-65) was obtained from National Institute for Medical Research (NIMR), Lagos, Nigeria.

Extract preparation

The stem bark was carefully removed from the *Jatropha curcas* plant and washed thoroughly with tap water then rinsed twice with distilled water and allowed to dry at room temperature under shade.

The dried stem bark was crushed to powder with pestle and mortar and two hundred grams of the powder was cold macerated in 1000 ml of distilled water for 24 h with constant shaking at room temperature and filtered using Whitman's filter paper No. 1.

It was then concentrated to dryness on water bath at 40°C and the crude extract was kept in desiccator and dried.

Phytochemical screening

The powdered stem bark was screened for the qualitative presence of the following secondary metabolites such as tannins,saponins, flavonoids, anthraquinones, terpenoids, steroids, alkaloids and glycosides. The phytochemical screening was carried out following standard methods (Sofowora, 1993; Evans, 2002).

After preliminary analysis to determine the presence of these phytochemicals, the sample was further subjected to quantitative analysis to determine the percentage of the secondary metabolites that were present using the following procedures: Flavonoid was quantified using the method of Sofowora, (1993).

Glycoside, steroids, and terpenes were quantified using the method of Analytical Committee of Royal Society of Chemistry.

Alkaloids were quantified using the method of Henry, (1993) and anthraquinones was quantified using the method of Lewis and Elvin-Lewis, (1977).

Parasite inoculation

The inoculation of the parasite was carried out by determining both the percentage parasitemia and erythrocytes count of the donor mouse using a haemocytometer and appropriate dilutions of the infected blood with isotonic saline were made. Each mouse was inoculated intraperitoneally with 0.2 ml of infected blood containing about 1 x10^7 *Plasmodium berghei* parasitized red blood cells. After 72 h, parasitemia was confirmed by analyzing tail blood of the mice after which treatment commenced for twelve days.

Experimental design

Ninety Swiss albino mice were divided into six groups of fifteen (15) mice each. Groups 2-6 were inoculated with the rodent malaria parasite *Plasmodium berghei* from the same donor mouse. Groups 3, 4 and 5 were treated with 250, 500 and 750 mg/Kg body weight of the aqueous leaf stem bark extract of *Jatropha curcas* respectively per day. Group 6 was treated with 20 mg/Kg body weight of arthemether which served as positive control. Groups 1 and 2 served as normal and negative controls respectively.

After establishment of infection and the administration of the extract, the mice were sacrificed on days 0, 7, and 14; and the blood samples were collected using cardiac puncture. The blood samples were collected into sterile containers and were allowed to stand for 30 minutes to clot and centrifuged at 2500 rpm for 10 min and serum collected for hematological analysis.

Determination of Hematological Indices

Packed cell volume (coles 1980)

Blood obtained from the mice in heparinized capillary tube to about ¾ of the length. One head of the tube completely sealed using plasticine wax. The sealed capillary tube was transferred to haematocit centrifuge place so that the sealed end pointed outward. The cover of the centrifuge was tightened to prevent blood spillage and centrifuged at 1,500 rpm for 5 min. Thereafter, the capillary tubes were transferred to the graphite reader and the percentage packed cell volume was read directly.

Hemoglobin determination: Haemoglobin was determined by the Cyanmethemoglobin method of Baure, (1980).

Red cell count: Red blood cell count was carried out using the Manual method described by Baker et al. (1998).

White blood count: White blood cell count was carried out as described by the stain method of NCCLS, (1993).

Statistical analysis

Results were expressed as mean ± SEM. Student t-test was used to analyse the data between each group and control group at p values($p<0.05$) was considered significant in all cases (SPSS version 21 was used).

RESULTS AND DISCUSSION

The result of the qualitative phytochemical constituents present in the aqueous stem bark extract of *Jatropha curcas* is shown in the (Table 1). Alkaloids, terpenoids, glycosides, anthraquinones and steroids were present. The percentage concentrations of the phytochemicals determined from the aqueous stem bark extract of *Jatropha curcas* is shown in (Table 2). The extract contained high concentrations of alkaloids and anthraquinones.

Table 1: Phytochemical constituents present in the aqueous stem bark extract of *Jatropha curcas*.

Phytochemicals	Inference
Alkaloids	+
Flavonoids	–
Glycosides	+
Tannins	–
Terpenoids	+
Anthraquinones	+
Saponins	–
Steroids	+

+ = Present; – = Absent

Table 2. Phytochemicals Concentrations in the aqueous stem bark extract of *Jatropha curcas.*

Phytochemicals	Concentration (%)
Alkaloids	3.91± 0.14
Flavonoids	1.33± 0.10
Glycosides	0.90 ± 0.49
Terpenoids	0.30 ± 0.02
Anthraquinone**s**	1.58±0.88
Steroids	0.20 ± 0.00

Values are mean ± S.E.M; (n=3).

The phytochemical analysis of the aqueous stem bark extract of *Jatropha curcas* L showed that alkaloids and anthraquinones content are highest. Therefore, the protective effects of the plant on hematological Parameters may be attributed to the high content of alkaloids and anthraquinones.

Table 3. Effects of aqueous stem bark extract of *Jatropha curcas* on RBC, Hb and PCV in mice infected with *Plasmodium berghei*.

HAE. PAR.	GROUP 1 Normal control	GROUP 2 Negative control	GROUP 3 [250 mg/kg]	GROUP 4 500 mg/kg	GROUP 5 750 mg/kg	GROUP 6 Drug 20 mg/kg
RBC ($\times10^{12}$/l)						
Days						
0	4.50±0.35	2.46±0.25^{a}	2.39±0.23	2.12±0.20	3.13±0.22^{ab}	3.44±0.25^{b}
7	4.34±0.20	1.20 ±0.01^{a}	2.46 ±0.36^{b}	2.13±0.23^{b}	4.44±0.55^{b}	3.48±0.51^{b}
14	4.46±0.35	0.14±0.04^{a}	3.00±0.10^{ab}	3.16±0.29^{ab}	3.94±1.05^{b}	4.88±0.38^{b}
Hb (g/l)						
Days						
0	16.40±1.32	12.20±1.33^{a}	12.20±0.20^{a}	12.45±0.25	13.54±0.25^{a}	14.50±0.45^{a}
7	14.15±0.20	11.75±2.08^{a}	12.90±0.06^{ab}	13.11±0.13^{ab}	14.02±0.04^{b}	15.50±0.61^{b}
14	13.80±0.10	8.26±2.68^{a}	13.40±0.54^{b}	13.41±0.54^{b}	14.15±0.23^{b}	15.60±0.19^{b}
PCV (%)						
Days						
0	41.33±0.78	12.34±0.45^{a}	35.45±0.50^{ab}	42.40±0.60^{b}	42.50±0.70^{bc}	43.30±0.50^{b}
7	43.20±0.25	11.89±0.22^{a}	37.12±0.24^{b}	43.12±1.99^{bc}	45.99±1.19^{bc}	44.49±0.62^{bc}
14	49.33±0.78	11.00±0.13^{a}	41.34±2.53^{ab}	45.01±2.66^{ab}	44.76±4.35^{ab}	49.65±0.45^{b}

All values are the mean ± SEM, (n=5) at ($p<0.05$); a-Significantly lower than group 1, b- Significantly higher than group 2; c-Significantly higher than group 1, d- Significantly lower than group 2.HAE. PAR= Haematological Parameters; RBC= Red Blood Cells; Hb=Haemoglobin; PCV=Packed Cell Volume.

In this study, it was observed that at higher dose of the extract was able to exhibit a considerable effect on hematological Parameters comparable to the standard drug. This could be due to sufficient accumulation of the bioactive compounds in the plant extract. The consideration of haematological indices is due to the fact that most changes during malaria are pronounced in the blood and it's forming system. Anaemia is a common problem in malaria and in this study, was evidenced by a decrease in the levels of circulating RBC,Hb and PCV in all the infected groups which was pronounced on day 7 post infection and more significantly in the infected-untreated mice until day 14. The lysis of the RBCs may be as a result of non-immune damage of the parasitized RBCs as a result of high parasitemia or immune mediated damage of parasitized and non-parasitized RBCs because the alteration in the RBCs antigen structure caused the parasite invasion to stimulate the production of antibodies against the RBCs. The observed reduction in the hematological indices may be as result of either repeated haemolysis of the parasitized RBCs most importantly. Therefore, this triggers immune mediated RBC lysis. Also, the developing parasite destroys the intracellular protein that is haemoglobin. This accounts for further decrease in the levels of Hb. However, there was a significant increase in the RBC, Hb, and PCV after the administration of the extract. This was obviously seen from day 7 of post-infection in the extract-treated and artemether-treated groups; and more significantly in the infected-treated animals until day 14.

This suggests that the extract may have an effect on the production of RBCs, which might have contributed to the increase in Hb and PCV observed in the infected extract-treated groups on day 14 post infection. It suggest that something like an immune buster effect of the extract and the arthemether in the mice when compared with groups 1 and 2 so according to the results in the (Table 3).

The function of the white blood cells is to fight infection by phagocytosis against foreign organisms, and to produce, transport and distribute antibodies in immune responses. The increase in WBC ($\times10^{9}$/l) in the infected groups may be due to stimulation by the immune system to fight the parasites. White blood cells mainly fight infection, defend the body by phagocytosis against invasion by foreign organisms, and to produce, transport and distribute antibodies in the immune response. On day 7 post infection, there was a decrease in WBC in all the infected groups. This may imply a reduction in the ability of the mice to resist the infection (Yakubu *et al.,* 2007). However, WBC in the infected extract-treated and infected artemether-treated mice was higher than in the infected untreated mice.This is a suggestion that the extract and the standard drug were able to boost the immune system of the mice.

Furthermore, on day 14, there was an observed continued decrease in WBC in the infected untreated mice that correlates with a high parasitemia and other derangements due to the infection. The improved ability of the mice to fight infection as a result of the treatment with the extract and artemether significantly increases the WBC levels. It implies that the increase in WBC was due administration of the extract which was able to reverse the a damaging effects of the parasites.

There was also a decrease in platelets levels in the infected groups; platelets like RBCs have nucleus and are discoid, measuring about 1.5- 3.0 µm in diameter. Because the body has limited reserve of platelets, it can

Table 4. Effects of aqueous stem bark extract of *Jatropha curcas* on total WBC, LYMP and PLT in mice infected with *Plasmodium berghei.*

HAE . Par.	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6
WBC($\times 10^9$/l)						
Days						
0	9.65±1.75	7.45±0.45^{a}	6.00±0.50	8.00±0.60	8.60±0.50^{b}	9.00±0.50^{b}
7	9.50±0.50	6.24± 2.06^{a}	6.10± 0.47	8.12±0.57^{ab}	8.86±0.40^{b}	9.06±0.60^{b}
14	8.65±0.75	5.06±1.65^{a}	6.30± 0.81	8.21±0.56^{b}	9.73±1.70^{b}	9.88±0.42^{b}
LYMP (%)						
Days						
0	82.60±0.86	84.98±0.45^{c}	80.50±0.50^{ad}	79.98±0.70^{ad}	79.60±0.45^{ad}	81.40±0.51^{ad}
7	84.07±0.50	88.21±0.49^{c}	79.33±0.67^{ad}	76.41±1.72^{ad}	72.79±0.36^{ad}	77.79±0.36^{ad}
14	85.00±0.76	89.14±1.00^{c}	72.43±0.47^{ad}	71.27±2.15^{ad}	74.08±0.72^{d}	69.08±0.72^{ad}
PLT($\times 10^9$/l)						
Days						
0	421.02±2.10	345.58±2.40^{a}	359.50±0.55	368.70±0.60^{ab}	419.74±0.70^{ab}	388.77±0.80^{ab}
7	430.10±3.40	330.51±1.50^{a}	377.50±0.36^{ab}	378.51±0.23^{ab}	423.00±3.02^{ab}	398.02±2.02^{ab}
14	424.01±2.02	286.02±7.03^{a}	390.03±0.10^{ab}	396.00±0.29^{ab}	425.50±2.02^{b}	430.04±2.04^{bc}

All values are the mean ± SEM, (n=5) at ($p<0.05$); a-Significantly lower than group 1,b- Significantly higher than group 2; c- Significantly higher than group 1, d- Significantly lower than group 2.HAE Par=hematological Parameters; WBC=White Blood Cells; LYMP=Lymphocytes; PLT=Platelets.

then be rapidly depleted. Therefore, the decrease in the levels of platelets is also common in malaria and may result from increased sequestration of platelets in spleen. The observed significant increase in platelet count by the infected extract-treated and artemether-treated compared to the untreated group, suggests a stimulatory effect of the extract on platelet production. Lymphocytes are the major components or effecter molecules of the immune system, therefore the observed increase in lymphocytes may affect the immune system.

Conclusion

The phytochemical analysis of the aqueous stem bark extract of *Jatropha curcas* L showed that alkaloids and anthraquinones content are highest. Therefore, the protective effects of the plant on Haematological parameters may be attributed to the high content of alkaloids and anthraquinones. In this study, it was observed that at higher dose of the extract was able to exhibit a considerable effect on hematological parameters comparable to the standard drug. This could be due to sufficient accumulation of the bioactive compounds in the plant extract. The consideration of hematological indices is due to the fact that most changes during malaria are pronounced in the blood and it is forming system.

Recommendation

Further studies should be carried out on other parts of *Jatropha curcas* to identify, isolate and characterize the active component responsible for the antimalarial property.

Author`s declaration

We declare that this study is an original research by our research team and we agree to publish it in the journal.

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