Immuno Modulatory Potentials and Histopathological Effects of Aqueous Extract of Abrus Precatorius Leaf in Mus Musculus

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Abstract: Aqueous extract of Abrus precatorius leaf is being used by Nupe community of Nigeria to treat infectious diseases including malaria, pneumonia, typhoid, diarrhoea, wound and candidiasis. The aim of the research was to study immunomodulatory potentials and histopathological effects of the extract. Forty-two mice of either sex weighing 28.25+5.92 g used for the study were divided into 6 groups of seven mice each administered 0, 12.5, 25, 50, 100 and 200 mg/kg bodyweight for a period of 3 weeks. The body weight and blood samples of the mice were obtained prior to the administration of the extract and subsequently weekly. One-third milliliter (0.33 ml) of blood was obtained from the tail vein of each mice and placed in ethylene diamine tetra acetate (EDTA) sample bottles for haematology. Liver, lung, spleen and heart were harvested for histopathology. Observed are significantly increased ($p<0.05$) packed cell volume (PCV), erythrocytes, lymphocytes, decreased neutrophils, monocytes, basophils and eosinophils. Histopathology revealed interstial haemorrhage and mononuclear cells infiltration of hepatocytes, thinning of alveolar septae and widening of alveolar spaces, myocarditis with mononuclear cells infiltration and moderate hyperplasia of white pulp of spleen. Hence, the plant may be used in the treatment of anaemia, asthma and immune-compromised diseased conditions. Abrus agglutinin and abrin have been responsible for immunomodulatory and cytotoxic activities of the plant respectively.

Keywords: Abrus precatorius leaf, anaemia, asthma, immunity, hepatitis, myocardiatis.

INTRODUCTION

Abrus precatorius L. belongs to Papillionaceae family [1] that twines around trees, shrubs and hedges with glabrous internodes and leaves [2]. Each leaflet is about 1.2 to 1.8 cm long [3]. It has fruit, which is a small pod with flat truncate shape and deflexed beak. The fruit contains 3 to 6 ovoid red seeds of about 0.6 cm in diameter with a black mark at the base. It grows in tropical and subtropical regions of the world [4]. The leaves are used to treat diabetes, asthma [5], microbial infections caused by Streptococcus pyogenes, Streptococcus pneumonia [6], Klebsiella pneumonia, Salmonella typhimurius, Escherichia coli [7], Staphylococcus aureus and Candida albicans [8] and malaria [9]. Abrus precatorius leaf extract have hematonic, plasma expander, antiplasmodial effects [10], cytotoxic to A-549 cancer cell lines [11] kidney, liver, pancreatic, cardiac and gastrointestinal cells of mice [4]. Other uses of the plant are in stomatitis, bronchitis [12] antitubercular, antiviral, cytotoxic [13]. Suppeuration, acne, boils, abscesses, tetanus, rabies [14], cough and sore throat [1]. Ligha *et al.* reported that the seed extract of A. precatorius could protect rat's kidney against alcohol-induced parenchymal injury [15]. The LD_{50} of intraperitoneal aqueous extract of Abrus precatorius leaf was estimated at 638 mg/kg body whereas the oral LD_{50} of aqueous leaf extract of the plant was between 2559.5 to 3,123.3 mg/kg body weight [6, 17, 18]. In view of the reported uses of Abrus precatorius leaf for the treatment of infectious diseases such as malaria, typhoid, pneumonia, diarrhoe and wound. There is need to investigate immune-modulatory potentials of the plant.

MATERIALS AND METHODS

Experimental Animals

A total of forty-two (42) mice of either sex weighing between 28.25 $\pm$ 5.92 g were obtained from Nigeria Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. All the mice were fed mice feed (finisher®) formulated by Grand Cereals and Oils Mills Limited (GCOML) and clean water was provided *ad libitum*. Laboratory animal care was provided according to CIOMS [19] guidelines, and the recommended procedures of the Department of Veterinary Physiology and Pharmacology ethical committee as approved by the authority of University of Agriculture Makurdi, Benue State, Nigeria.

Collection of the Plant Materials

The plant used for the study was identified by a botanist in the Herbarium of the Department of

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Biological Science, Usmanu Danfodiyo University Sokoto, where a voucher specimen was deposited.

Extract Preparation

The plant materials (leaves) collected were air dried to a constant weight under an open shade and pulverized with the help of a mortar and pestle to fine powder. Fifty (50) gramme of *Abrus precatorius* leaf powder was dissolved in 950 ml of distilled water in a conical flask. The mixture was shaken intermittently throughout a whole day using glass rod stirrer and allowed to stand overnight. The mixture was filtered with whatman filter paper No. 1 into measuring cylinder and concentrated at 60 ^{o}C to powder form in an incubator and stored in a refrigerator at 4 ^{o}C until required for use [6, 7].

Preparation of Stock Solution

Ten (10) gramme of the extract of *Abrus precatorius* leaf obtained from cold water maceration was measured and placed in a beaker and 90ml of distilled water added to prepare 10% stock solution that was stored in a refrigerator at 4 ^{o}C until required [4].

Administration of Aqueous Leaf Extract of Abrus Precatorius

The method of Yamba *et al.* [20] was used for the selection of doses used in haematological and pathological studies. The selected doses were within the range between tenth and one hundredth of the estimated median lethal dose (2,559.5 to 3,123.3mg/kg body weight) [21]. Forty-two (42) mice of either sex weighing 28.25 + 5.92 g used for the study were divided into 6 groups of seven per group. Five groups were orally administered aqueous extract of *Abrus precatorius* leaf at dose levels of 12.5, 25.0, 50.0, 100 and 200 mg/kg body weight daily for a period of 21 days. The sixth group of the animals was administered 1 ml of distilled water only serving as a control. The body weight and blood samples of the mice were obtained prior to the administration of the extract and water, and subsequently weekly during the period of extract treatment. One-third milliliter (0.33 ml) of blood was obtained from the heart of each mice with the help of needle and syringe and placed in a tube containing potassium ethylene diammine tetra-acetate (EDTA). The anticoagulated blood was used for the determination of hematological parameters. Toxicity signs in the treated mice were recorded, and mice that either died or survived during the study were subjected to necropsy [4].

Haematological Parameters

Haematological parameters were determined according to the method of Cheesbrough [22]. The parameters include erythrocytes (RBCs) count, packed cell volume (PCV), haemoglobin concentration (HB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBCs) count and differential white blood cells count (DWBC).

Pathological Examinations

Mice that died or survived during the period of the study were subjected to post mortem examinations. Samples of the organs and tissues (liver, heart, spleen and lung) were taken and fixed in 10% formalin solution. The tissues were thereafter embedded in paraffin wax and cut into 5m thick and stained with haematoxylin and eosin (H & E) stain. Histopatho-logical examination of the tissues using light microscope was thereafter carried out for presence of lesions [23].

Statistical Analysis

The data on haematological parameters were expressed as mean $\pm$ SEM. Repeated measure analysis of variance was used to analyze the data on haematological parameters at 5% level of significance [24].

RESULTS

The effects of prolonged administration of aqueous leaf extract of *A. precatorius* on the mean red blood cells count is presented in Table **1**. Treatment with the extract did not show significant increase in the red blood cell values (P>0.05). Before treatment, the red blood cells counts were 6.55$\pm$0.14, 6.66$\pm$0.31, 6.55$\pm$0.41, 6.73$\pm$0.35 and 6.03$\pm$0.42 x 10^{12}/L for groups of mice administered 12.5, 25, 50, 100 and 200 mg/kg body weight of the extract respectively. However, at 21 days of treatment with the extract the values were increased to 7.50$\pm$0.40, 7.00$\pm$0.23, 8.70$\pm$0.50, 9.00$\pm$1.00 and 7.43$\pm$0.45 x 10^{12}/L respectively. The RBC counts of the control group was not altered during the study period. The effects of various doses of aqueous extract of *A. precatorius* leaf on mean packed cell volume in mice is presented in Table **1**. Treatment with the extract resulted in significantly increased (P<0.05) values of packed cell volume when compared to the control. Mice treated with 12.5, 25, 50, 100 and 200 mg/kg of the extract had

Table 1: Effects of Prolonged Administration of Varying Doses of Aqueous Extract of Abrus Precatorius Leaf on Erythrocytes, Packed Cell Volume, Haemoglobin Concentration and Mean Corpuscular Volume

Parameters	Treatment dose (mg/kg)	Week of treatment			
		Before treatment	7	14	21
Erythrocytes (x 10^{12}/L)	0.0	6.79±0.55	6.96±0.30	6.70±0.30	6.25±0.73
	12.5	6.55±0.24	6.94±0.37	7.30±0.28	7.50±0.40
	25	6.66±0.31	7.28±0.28	7.72±0.21	7.00±0.23
	50	6.55±0.41	7.01±0.36	7.02±0.31	8.70±0.50
	100	6.73±0.35	6.75±0.37	7.95±0.86	9.00±1.00
	200	6.03±0.42	6.84±0.30	7.30±0.41	7.43±0.45
Packed cell volume (%)	0.0	34.94±2.96	32.68±2.46	36.04±2.57	36.45±3.00
	12.5	32.17±0.93	40.37±1.05	39.46±1.42	39.10±1.60[b]
	25	29.13±1.76	36.44±1.49	33.83±1.44	39.93±1.48[b]
	50	32.63±2.15	39.71±2.16	37.52±1.04	39.70±0.70[b]
	100	31.75±1.37	35.33±1.56	39.43±1.97	43.25±2.41[b]
	200	32.38±1.99	36.36±2.61	35.78±1.97	38.62±0.1.09[b]
Haemoglobin concentration (g/dL)	0.0	42.90±1.20	43.05±2.19	43.68±1.02	43.80±2.04
	12.5	42.60±0.63	44.97±1.20[b]	45.54±1.44[b]	44.31±2.70[b]
	25	42.30±2.13	43.38±1.53	44.31±1.08[b]	44.79±1.44[b]
	50	42.27±1.59	41.10±1.86	42.66±1.86	44.40±0.00[b]
	100	42.27±1.59	39.12±2.91[b]	42.75±3.06	44.40±1.48[b]
	200	40.80±1.32	30.85±3.27	44.04±3.06[b]	43.35±2.28[b]
Mean corpuscular volume (fL)	0.0	49.74±5.04	43.86±3.48	47.36±4.27	45.83±6.92
	12.5	49.45±1.81	59.43±4.14	54.16±1.33	52.63±4.63[b]
	25	43.98±2.22	51.43±3.51	54.11±2.60	50.70±2.32[b]
	50	50.83±4.11	57.89±4.84	53.96±3.31	55.60±0.00[b]
	100	47.73±2.32	52.93±2.76	51.18±5.03	58.10±3.80[b]
	200	54.99±3.91	53.82±5.34	58.50±4.17	59.43±2.56[b]

Mean + SEM of seven observations
[a] = significantly lower (P<0.05) in comparison with the pretreatment values
[b] = significantly higher (P<0.05) in comparison with the pretreatment values

pre-treatment PCV values of 32.17±1.93, 29.13±1.76, 32.63±2.15, 31.75±1.37 and 32.38±1.99% respectively and at 21 days of treatment, the values were 39.10±1.60, 39.93±1.48, 39.70±0.70, 43.25±2.41 and 38.62±1.09% respectively. The haemoglobin values were not significantly altered in the groups treated with varying doses of the extract compared to the control (Table **1**).

The mean corpuscular volumes (Table **1**) of the mice treated with the various doses of the aqueous extract of *Abrus precatorius* leaf were significantly (P<0.05) increased when compared to the pre-treated values and that of the control group. Before treatment, the values were 49.74±5.04, 49.45±1.81, 43.98±2.22, 50.83±4.11, 47.73±2.32 and 54.99±3.91 fL in the control, 12.5, 25, 50, 100 and 200 mg/kg treated groups respectively, and at 21 days of treatment, the values were 45.83±6.92, 52.63±4.63, 50.70±2.32, 55.60±0.00, 58.10±3.80 and 59.43±2.56 fL respectively. The mean corpuscular haemoglobin (Table **2**) of mice treated with varying extract doses, decreased significantly (P<0.05) when compared to the pre-treatment values and the value of the control group. The mean corpuscular haemoglobin concentration was also decreased (P<0.05) in the groups treated with varying doses of the extract in comparison with the control mice (Table **2**).

The white blood cells counts of mice treated with various doses of aqueous extract of *Abrus precatorius* leaf is presented in Table **2**. The white blood cells counts in animals treated with 12.5, 25, 50 and 100 mg/kg were significantly (P<0.05) increased from day 7 of treatment. However, the animals treated with 200 mg/kg extract dose had significantly decreased WBC values from day 14 of treatment. The mean WBC counts of the control were statistically the same throughout the experimentation period. The differential leucocytes counts (DLC) of mice treated with various doses of the aqueous extract of *A. precatorius* leaf are presented in Table **3**. Following treatments with the

Table 2: Effects of Prolonged Administration of Varying Doses of Aqueous Extract of Abrus Precatorius Leaf on Mean Corpuscular Haemoglobin, Corpuscular Hemoglobin Concentration and White Blood Cells

Parameters	Treatment dose (mg/kg)	Week of treatment			
		Before treatment	7	14	21
Corpuscular mean haemoglobin (pg)	0.0	22.10±1.96	21.08±0.43	23.14±1.03	22.03±1.66
	12.5	22.18±0.34	20.89±1.13	20.92±1.05	19.70±0.69[a]
	25	21.26±1.80	20.37±0.87	18.98±0.70	18.98±0.94[a]
	50	21.95±1.28	19.86±3.00	20.34±0.99	17.0±0.00[a]
	100	22.24±1.32	19.34±1.11	18.48±1.91	16.40±1.60[a]
	200	23.34±1.69	19.08±1.75	20.22±1.43	19.70±1.74[a]
Corpuscular haemoglobin concentration (g/L)	0.0	454.75±31.57	444.68±34.05	410.58±21.22	407.03±32.94
	12.5	459.36±11.87	370.92±12.19	386.10±14.66	379.10±27.97[a]
	25	498.43±39.06	401.01±21.66	423.32±11.59	403.00±10.22[a]
	50	441.25±27.76	349.09±15.82	380.46±20.81	372.80±00.00[a]
	100	471.31±30.03	374.58±18.58	363.93±31.11	346.30±85.70[a]
	200	431.05±28.40	353.50±48.90	414.05±31.47	376.50±30.30[a]
White blood cells count ($x10^9$)	0.0	5.28±0.46	5.68±0.34	5.88±0.71	5.18±0.90
	12.5	5.13±0.22	6.28±0.23[b]	5.82±0.20	5.80±0.32
	25	4.50±0.37	6.20±0.27[b]	5.54±0.65[b]	5.23±0.39[b]
	50	5.61±0.55	5.47±0.78	5.45±0.85	5.60±0.00
	100	5.54±1.55	5.70±0.45[a]	6.78±0.49[b]	6.50±1.30[b]
	200	6.80±0.26	6.80±0.26	5.30±0.29[a]	4.70±0.59[b]

Mean ± SEM of seven observations
[a] = Significantly lower (P<0.05) in comparison with the pretreatment values
[b] = Significantly higher (P<0.05) in comparison with the pretreatment values.

extract, the neutrophil values of the various treatment groups were statistically similar to that of the control except in the group treated with 200 mg/kg with a decreased neutrophil value at day 21 of treatment. The percentage lymphocytes were also similar to those of the control animals, except on day 21 of treatment when the value was significantly (P<0.05) higher than that of the control, group. The percentage eosinophils count was only altered (P<0.05) by treatment with 25 mg/kg of the extract (Table **3**).

The mean monocyte values were decreased (P<0.05) by treatments with 12.5, 25, and 200 mg/kg of *A. precatorius* leaf extract, while extract doses of 50 and 100 mg/kg induced an initial decrease in monocyte count followed and by an increase (Table **3**). There was significant decrease (P<0.05) in basophils value among the treatment groups as well as between the control and the treatment groups (Table **3**).

Histopathology of the liver treated with 12.5mg/kg of the extract revealed interstial haemorrage (Figure **1**) whereas there was thinning of the alveolar septae and widening of alveolar spaces (Figure **2**), mild myocarditis with mononuclear cells infiltration (Figure **3**) and moderate white pulp hyperplasia of spleen (Figure **4**).

Table 3: Effect of Aqueous Leaf Extract of *Abrus precatorius* on the Mean Differential Leucocyte Counts (DLC) of Mice

Parameters	Treatment Dose (mg/kg)	Weeks of treatment			
		0	1	2	3
Neutrophils (%)	Control	43.50±4.91	37.60±16.82	40.00±2.72	38.75±4.23
	12.5	43.50±4.91	39.63±4.03	40.00±2.07	39.00±8.54
	25.0	41.75±4.13	30.86±3.49	46.00±3.68	41.50±3.69
	50.0	38.38±3.13	36.71±3.62	36.20±3.77	43.00±0.00
	100.0	40.13±4.68	35.33±6.36	43.28±3.25	41.00±0.00
	200.0	42.88±5.48	43.00±4.47	34.75±7.43	30.25±5.66[a]

Table 3 Continue …..

Parameters	Treatment Dose (mg/kg)	Weeks of treatment			
		0	1	2	3
Lymphocytes (%)	Control	52.50±4.28	59.00±4.17	56.40±2.50	57.50±4.13
	12.5	52.50±4.28	55.50±4.19	56.00±2.43	56.00±4.36
	25.0	51.38±4.20	64.57±3.33	51.83±3.61	55.00±3.71
	50.0	56.38±3.38	58.71±3.10	60.80±4.03	50.00±0.00
	100.0	54.63±4.79	61.33±6.17	53.25±3.28	53.50±0.50
	200.0	52.00±5.16	51.80±4.50	62.50±7.44	65.25±5.02[b]
Eosinophils (%)	Control	0.62±0.32	1.20±0.37	1.20±0.37	1.00±0.40
	12.5	0.62±0.32	2.00±0.19	1.00±0.45	1.33±0.33
	25.0	2.38±0.38	1.71±0.42	0.33±0.21	0.50±0.22[a]
	50.0	0.88±0.35	1.43±0.57	1.00±0.55	1.00±0.00
	100.0	1.38±0.50	1.33±0.54	0.75±0.25	1.50±0.50
	200.0	1.00±0.50	2.40±0.68	1.25±0.48	1.00±0.71
Monocytes (%)	Control	3.38±0.65	2.20±0.58	2.40±0.51	2.75±0.63
	12.5	3.93±0.17	2.38±0.46	3.00±0.45	3.67±0.67[a]
	25.0	4.13±0.44	3.00±0.49	2.00±0.37	3.00±0.58[a]
	50.0	4.38±0.57	3.14±0.91	2.00±0.32	6.00±0.00[b]
	100.0	3.75±0.77	1.67±0.21	2.75±0.48	4.00±0.00[b]
	200.0	4.00±0.94	2.40±1.25	2.00±0.41	3.50±0.65[a]
Basophils (%)	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	12.5	0.13±0.07	0.00±0.00	0.00±0.00	0.00±0.00[a]
	25.0	0.38±0.18	0.00±0.00	0.00±0.00	0.00±0.00[a]
	50.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	100.0	0.13±0.13	0.00±0.00	0.00±0.00	0.00±0.00[a]
	200.0	0.13±0.13	0.00±0.00	0.00±0.00	0.00±0.00[a]

Mean ± SEM of seven observations
[a] = Significantly lower (P<0.05) in comparison with the pretreatment values
[b] = Significantly higher (P<0.05) in comparison with the pretreatment values.

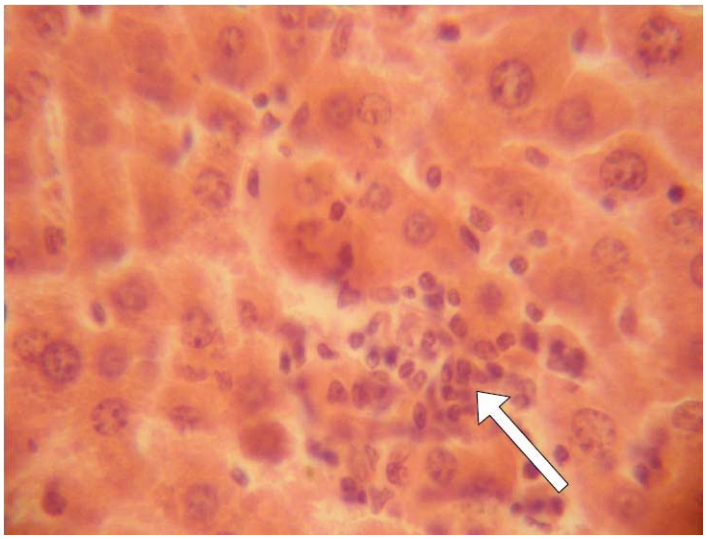

Figure 1: Photomicrograph of mouse liver treated with 12.5 mg/kg body weight of *Abrus precatorius* aqueous leaf extract for 21 days showing interstitial haemorrhage and mononuclear cells infiltration (arrows) H&E x400.

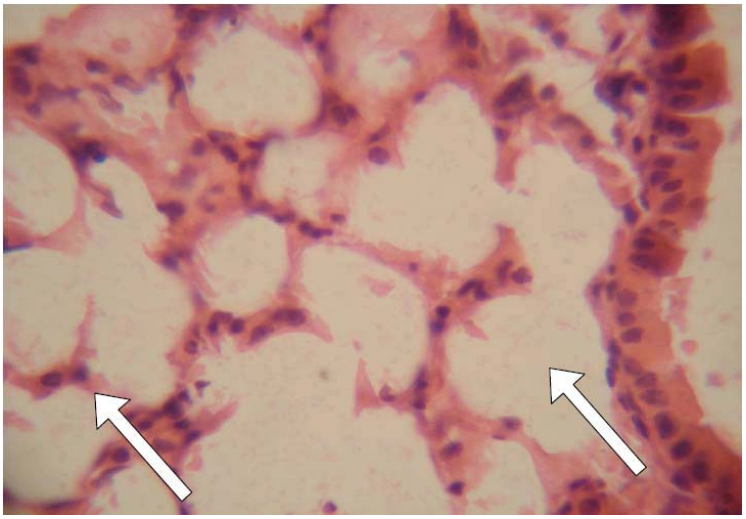

Figure 2: Photomicrograph of mouse lung treated with 12.5 mg/kg body weight of Abrus precatorius aqueous leaf extract for 21 days showing thinning of the alveolar septae and widening of alveolar spaces H&E x400.

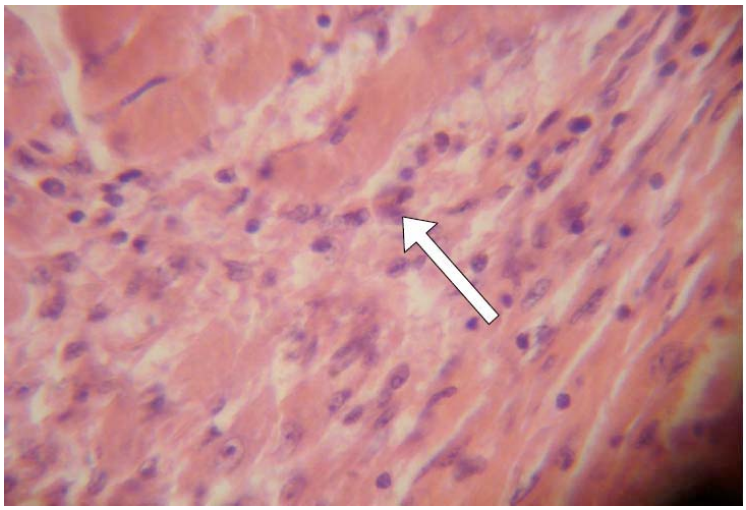

Figure 3: The heart of mice treated with 50 mg/kg of the extract leaf for 21 days showing mild myocarditis with mononuclear cells infiltration, (Arrow). (H & E: x400).

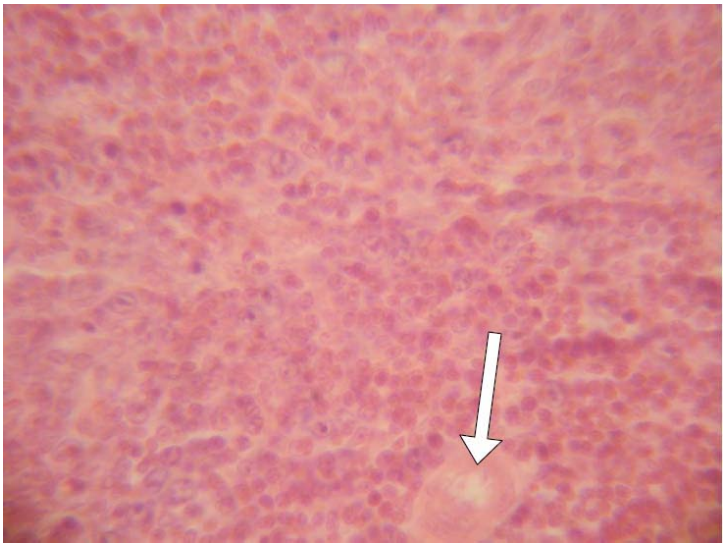

Figure 4: Photomicrograph of mouse spleen treated with 50 mg/kg body weight of Abrus precatorius aqueous leaf extract for 21 days showing moderate white pulp hyperplasia H&E x400.

DISCUSSION

The improvement of RBC and PCV values of treated animals in the present study is an indication of antianaemic effect of the extract. Substances with anti-anemic properties are known to stimulate increased production of RBC and improve the values of PCV and Hb [25]. The increase in mean corpuscular volume of the mice treated with varying doses of the extract and decreases in the values of the mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration of the treated animals suggest the presence of macrocytic red blood cells which are hypochromic. This could occur as a result of deficiency of some essential nutrients such as folic acid, vitamin C and iron. Macrocytic hypochromic condition is regenerative with increased number of reticulocytes that are more than the mature RBCs with uncompleted haemoglobin synthesis [26]. The improvement of some haematological parameters (e.g. PCV and erythrocytes) agrees with the report of Saganuwan and Onyeyili [10] indicating that aqueous leaf extract of A. precatorius has hematonic effect. However, Adedapo *et al.* [27] reported that aqueous extract of Abrus precatorius leaf caused decreased PCV and RBCs of rat at 400-1,600 mg/kg body weight indicating anti-anaemic effect at higher dose levels.

The administration of the extract at the dose level of 12.5 to 100 mg/kg stimulated increased production of absolute WBC. This could be a result of possible stimulation of immune defense system [28]. Reports have shown that persistent antigen load in the body results in increased lymphocytes. Lymphocytosis may be primarily responsible for the increase in WBC counts in the present study. However, the group treated with 200 mg/kg dose had decreased WBC counts. Our finding agrees with the report of Adedapo *et al.* [27] indicating that aqueous abrus leaf can cause lymphocytopenia. The increases in the level of lymphocytes could be due to insult caused by the active components of the extract on the body defense mechanism. This significant increase in the WBC counts of the treated mice may be due to increased WBC production by the bone marrow and lymphoid tissues [25]. The active principles present in the extract may be responsible for bone marrow and lymphoid tissue stimulation. Selective enhancement of the immune response is a primary goal in prevention of diseases. Drugs and chemicals have been known to induce lymphocytosis in man and animals [29]. Aggregation of mononuclear cells in hepatocytes, myocytes and moderate white pulp hyperplasia further suggest that the plant has immune stimulatory potentials. Lymphocytopoeisis takes place in the spleen, the cranial lymphomyeloid tissues, the thymus, and possible in other less well defined lymphomyeloid structures [30]. White pulp comprise of white cells that are manufactured similar to those manufactured in the lymphnodes. They are part of body immune system [29]. The neutropenia, lymphocytosis, eosinopenia, monocytopenia and basopenia caused by varying doses of aqueous extract of Abrus leaf is a clear evidence that the plant has immune-modulatory potentials. The decreased WBC counts observed in mice treated with 200 mg/kg may be due to immunosuppression indicating that as from 200 mg/kg dose and above, the immune-stimulatory potential of the Abrus leaf extract may be lost. The factor responsible for that is not known. Saganuwan and Onyeyili [10] had earlier reported that the plant has immune-modulatory property. Abru agglutinin, a component of abrin is the immune-modulatory agent present in the plant [31]. The mild pathological lesions seen in the liver, lung, heart and spleen of the experimental mice agrees with the report of Adedapo *et al.* [27] and Saganuwan and Onyeyili [10] indicating that Abrus precatorius leaf is toxic when administered for 3 weeks, at dose range between 12.5-200 mg/kg and 400-1,600 mg/kg body weight respectively. But thinning of alveolar septae and widening of alveolar spaces may suggest hyper-ventilatory activity of the extract and so may be used to treat asthma. Our finding agrees with the report of Saganuwan [5] who had earlier reported that local people used the plant extract to treat asthma. Taur and Patil [32] also reported that the ethanolic extract of Abrus precatorius leaves may be used in the management of asthma.

The plant contains toxalbumin (phytoprotein) abrin, which may be responsible for the observed toxic effects [33]. Abrin consists of abrus agglutinin, and toxic lectins [a] to [d], the five toxic glycoproteins found in the plant. Abrus agglutinin is non-toxic to animal cells but a potent haemagglutinator. The toxic portion of abrin is heat-stable to incubation at 60 ^{o}C for 30 minutes. But our extract was dried at 60 ^{o}C for a period of 7 days. At 80 ^{o}C most of the toxicity is lost in 30 minutes [34]. Hence the incubation of the Abrus leaf extract at 60 ^{o}C for 1 week must have reduced the toxicity of the leaf used. However, abrin is very stable in the gastrointestinal tract, from where it is slowly absorbed and thereby making it less toxic. Abrin's toxic effect is due to its direct action on the parenchymal cells (e.g. liver, kidney and red blood cells) [34]. Both subunits from which abrins [a] through [d] are made up are required for its toxic effects. Although, the plant

particularly the seed is known to be highly poisonous [3, 33], the leaf in the present study was observed to be slightly toxic.

The lesions seen in the organs such as heart, lung, spleen and liver in the 3 week extract administration are suggestive of toxic effects of Abrus leaf on muscular tissues. These findings are in disagreement with the report of Yamba *et al.* [20], indicating that the effective doses should be between 10^{th} and 100^{th} of the estimated LD_{50} in mice.

Aqueous extract of Abrus leaf is toxic and may be used as blood tonic and as an immune-stimulant in anaemic and immune-compromised diseased conditions. But it cause myocarditis, hepatitis and thinning of alveolar septae and widening of alveolar spaces, hence the plant may be also used to treat asthma.

REFERENCES

[1] Mann, A., Gbate, M., Nda, Umar, A. Eds. Medicinal and Economic plants of Nupeland. Bida, Jube Evans Books and publications, 2003; p. 225.

[2] Budavari, S. Jecquirity Bean. The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, 10th edition, New Jersey, Merck & Co., Inc, 1989; p. 560.

[3] Davis, J.H. *Abrus Precatorius* (rosary pea). The most common lethal plant poison. Journal of Florida Medical Association 1978; 65: 189-191.

[4] Saganuwan, S.A., Onyeyili, P.A. Biochemical effects of aqueous leaf extract of Abrus precatorius (Jecquirity bean) in Swiss albino mice. Herba polonica 2010; 56 (3): 63-80.

[5] Saganuwan, S.A. Some tropical plants with antihypertensive, antiasthmatic and antidiabetic value. Journal of Herbs, Spices and Medicinal Plants 2009; 15 (1): 24-44. http://dx.doi.org/10.1080/10496470902787477

[6] Saganuwan, S.A., Gulumbe, M.L. *In vitro* antimicrobial activities testing of Abrus precatorius cold water leaf extract on Streptococcus pyogenes and Streptococcus pneumonia. Proceeding of 2nd Annual Conference Nigerian Society Indigene Knowledge Develop, CRUTECH, Obabra, Cross River 2005; pp. 93-97.

[7] Saganuwan, S.A., Gulumbe, M.L. *In vitro* antimicrobial activities testing of *Abrus precatorius* cold water leaf extract on *Salmonella typhimurium, Escherichia coli* and *klebsiella pneumoniae.* Science Technology Research 2005; 4(3): 70-73.

[8] Adelowotan, O., Albinu, I., Adenipekun, E., Odugbemi, T. The *in vitro* antimicrobial activity of Abrus precatorius (L) Fabaceae extract on some clinical pathogens. Nigerian Postgraduate Medical Journal 2008; 15(1): 32-37.

[9] Saganuwan, S.A., Onyeyili P.A., Ameh, I.G. *In vivo* antiplasmodial activity by aqueous extract of Abrus precatorius in mice. Revista Latinoamericana de Quinica 2011; 39(1-2): 32-44.

[10] Saganuwan, S.A., Onyeyili, P.A. Haematonic and plasma expander effects of aqueous leaf extract of Abrus precatorius in Mus musculus. Comparative Clinical Pathology 2012; 21(6): 1249-1255. http://dx.doi.org/10.1007/s00580-011-1274-8

[11] Sivakumar, R., Alagesaboopathi C. Studies on cytotoxicity and antitumor screening of red and white forms of Abrus precatorius. African Journal of Biotechnology 2008; 7(22): 3988.

[12] Kirtikar, K.R., Basu, B.D. Indian Medicinal Plants, Vol. 1, International Book Distributors, Dehra Dun, India, 1980.

[13] Limmatvapirat, C., Sirisopanapom, Kittakoop, P. Antitubercular andtiplasmodial constitutents of Abrus prectorius. Planta Medica 2004; 70(3): 276-278. http://dx.doi.org/10.1055/s-2004-818924

[14] Watt, J.M., Breyer-Brandiwijk, A. The Medicinal Poisoning Plants of Southern and Eastern Africa, 2nd edition, Livingstone Publishers, London, UK, 1962.

[15] Ligha, A., Jaja, B., Numbere, N. Protective effect of Abrus precatorius seed extract following alcohol induced renal damage. Eurasian Journal of Scientific Research 2009; 25(3): 428-436.

[16] Saganuwan, S.A., Onyeyili, P.A., Suleiman, A.O. Comparative toxicological effects of orally and intraperitoneally administered aqueous extract of Abrus precatorius leaf in Swiss albino mice. Herba Polonica 2011; 57(3): 32-44.

[17] Saganuwan, S.A. Toxicological and antimalarial effects of aqueous leaf extract of Abrus precatorius (Jecquirity bean) in Swiss albino mice. PhD Thesis, Usmanu Danfodiyo University, Sokoto, Nigeria, 2012.

[18] Saganuwan, S.A. Principles of Pharmacological calculations, 1st edition, Ahmadu Bello University Printing Press, Zaria 2012; p. 527.

[19] CIOMS. International Guiding Principles for Biomedical Research Involving Animals, c/o WHO 121, Geneva 1985; p. 9.

[20] Yamba, O., Innocent, P.G., Odile, G.N. Biological and toxicological study of aqueous root-extract from *Mitragyna inermis* (Willd oktze) Rubiaceae. International Journal of Pharmacology 2007; 3(1): 80-85. http://dx.doi.org/10.3923/ijp.2007.80.85

[21] Saganuwan, S.A. A modified arithmetical method of Reed and Muench for determination of a relatively ideal median lethal dose (LD_{50}). African Journal of Pharmacy Pharmacology 2011; 5(12): 1543-1546. http://dx.doi.org/10.5897/AJPP11.393

[22] Cheesbrough, M. District Laboratory Practice in Tropical countries. Part 1, low price edition, Cambridge, Cambridge University Press, 2005; p. 434.

[23] Carlton, H.M. *Carlton's Histological techniques*, 5th edition. Churchill Livingstone, Edinburgh, 1980.

[24] Zar, J.H. Biostatistical Analysis. New Delhi, Dorling Kindersley pvt. Ltd. 2008; p. 663.

[25] Brown, B.A. Hematology principles and procedures. 2nd edition. Philadelphia, Lea and Febiger, 1976; p. 200.

[26] Tvedten H., Weiss, D. Clinical Diagnosis by Laboratory Methods. 2nd edition. Philadelphia London Toronto Montreal Sydney Tokyo, W.B. Saunders Company, A Division of Harcourt Brace & Company, 1999; p. 395.

[27] Adedapo, A.A., Omoloye, O.A., Ohore, O.G. Studies on the toxicity of an aqueous extract of the leaves of Abrus precatorius in rats. Onderstepport Journal of Veterinary Research 2007; 74: 31-36. http://dx.doi.org/10.4102/ojvr.v74i1.137

[28] Kastinath, R.T. Hypolipidemic effect of disulphide in rats fed high lipids diet and/or ethanol. PhD Thesis University of Bangalore, India, 1990; pp. 221-225.

[29] Guyton, A.C., Hall, J.E. *Textbook of Medical Physiology*, 11th edition, Saunders, An imprint of Elsevier, Philadelphia, Pennsylvania, 2007; p. 1116.

[30] Fange, R., Sundell, G. Lymphomyeloid tissue, blood cells and plasma proteins in chimaera monstrosa (Piscea holocephali). Acta Zoologica 1969; 50: 155. http://dx.doi.org/10.1111/j.1463-6395.1969.tb00537.x

[31] Tripathi, S., Maith, T.K. Immuno-modulatory role of native and heat denatured agglutinin from *Abrus precatorius.* International Journal of Biochemistry Cell Biology 2005; (3713): 451-462. http://dx.doi.org/10.1016/j.biocel.2004.07.015

[32] Taur, D.J., Patil, R.Y. Effect of Abrus prectorius leaves on milk induced leukocytosis and eosinophilia in the management of asthma. Asian Pacific Tropical Journal 2013; 1(1): 40-42. http://dx.doi.org/10.1016/S2221-1691(11)60119-6

[33] Garg, S.K. Abrus precatorius. In: Garg, S.K., Ed. *Veterinary Toxicology*, New Delhi, CBS publishers & Distributor, Darya Ganji 2006; p. 321.

[34] Hart, M. Jecquirity bean poisoning. New England Journal of Medicine 1963; 268: 885-886. http://dx.doi.org/10.1056/NEJM196304182681608

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