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Original Article

Haemato-Biochemical and Oxidative Responses of Adult Wistar Rats to Methanolic Extracts of *Abrus precatorius* Linn Leaves

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Abstract

In the present study, we explored the hematological, biochemical and oxidative chanes caused by the administration of 70% methanolic leaf extracts of *A. precatorius* to adult wistar rats in sub-acute and sub chronic study of 28 and 90 days respectively. Treatment with varying doses of the extract caused a significant increase in red blood cell counts and packed cell volume when compared to the control values. The biochemical parameters studied at the end of the 28-day treatment varied within the control range, however, following the 90-day treatment the parameters showed significant upward derangement, especially serum alanine aminotransferase, aspartate aminotransferase, mean plasma total bilirubin concentrations and mean plasma conjugated bilirubin concentrations, indicative of organ injury. The extract at all doses provided significant protection against hepatic lipid peroxidation and increased antioxidant enzyme activities such as superoxide dismutase, catalase, and glutathione peroxidase. In conclusion, the extract had positive haematopoietic property but should not be taken for a long time due to its effect on the liver enzymes. Also the extract has shown potential as an antioxidant and thus may be useful in the prevention of diseases caused by oxidative stress.

Keywords: *A. precatorius,* antioxidant, biochemical, free radicals, hematopoietic, oxidative stress.

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Introduction

Indigenous medicinal plants have always been a major source of modern medicine (Gupta *et al.,* 1994). Interest in medicinal plant's pharmacognosy has increased due to the new trend of phytotherapy as alternative medicine (Das *et al.,* 1999). However, plants produce bioactive compounds that act as defense mechanism against predators but which may also cause deleterious effect to humans when consumed as food or drug (Ames *et al.,* 1990; Devappa *et al.,* 2010). Consequently, it is expedient that thorough scientific investigations should be conducted on these medicinal plants for efficacy and potential toxicity. The effects of administration of a given plant extract on the biochemical and hematological parameters in a living system provides clue to the toxicity level of the extract.

The heightened curiosity on the pharmacological importance of herbs has also been due to the search for natural replacements for synthetic antioxidants (Chatterjee *et al.,* 2013). A large number of plants have been shown to contain phytochemicals which are viable sources of natural antioxidants e.g. tocopherol, vitamin C, carotenoids, and phenolic compounds. These antioxidants act by upsetting the molecular events involved in the initiation, promotion or progression of degenerative conditions and carcinogenesis (Gul *et al.,* 2011).

Abrus precatorius is a slender, perennial climber that twines around trees, shrubs, and hedges. It is a legume with long, pinnately compound leaves of family name – Fabaceae. Its flowers are arranged in violet or pink clusters. The seed pod curls back when it opens and reveals the seeds (Sudipta *et al.,* 2013). The seeds are truncate shaped, 1.5-2cm long, with attractive scarlet - black color. The plant is native to India and Indonesia, however it now grows in tropical and subtropical areas of the world where it has been introduced (Armstrong, 2000). *Abrus precatorius* is derived from the Greek word *Abrus* which means delicate and refers to the leaflets; *precatorius* refers to 'petitioning' and was chosen because of the use of the seed in Rosaries. *A. precatorius* leaves are sweet tasting, and according to a human taste panel, the sweetness was 30-100 times greater than sucrose (Choi *et al.,* 1989). *A. precatorius* is one of the plants that have proven to be of great medicinal importance; hence this study is to determine the haematogical, biochemical and oxidative responses of adult wistar rats to methanolic extracts of *A. precatorius* leaves.

Materials and Methods

Plant Material

The leaves of A. precatorius were collected, identification and authenticated in Forestry and Wildlife Management, Faculty of Agriculture of University of Port Harcourt. The Voucher specimen was deposited at the Department of Plant Science and Biotechnology Herbarium and was assigned specimen number: UPH/NO-P-052 for the leaves.

Equipment

Photo Microscope (Olympus, Japan), Rotary Evaporator (Heildolph Instruments, Germany), Handheld tissue homogenizer (Omni International), Mettler – toledo GmbH digital weighing balance (Type BD202, SNR 06653), Photoelectric colorimeter (Gallenkamp and Sons Ltd; England) and Uniscope Laboratory Centrifuge (Model M800B, Surgifriend Medicals and Essex, England), Spectrophotometer (PerkinElmer's LAMBDA) were the equipment used. Syringes (1 mL, 5 mL), oral cannula, cotton wool, heparinised and non-heparinized sample bottles, capillary tubes, excision kit, EDTA bottles, Microscopic slides (Olympus, China) and Hand gloves were instruments also employed.

Chemicals

Albumin reagent (Sigma Diagnostic®, UK), Sodium hydroxide (NaOH), Hydrated copper(II) sulfate, Potassium sodium tartrate, Bovine serum albumin (BSA), Tris – HCl buffer, Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Phenazonium methosulphate (PMS), Nitroblue tetrazolium chloride (NBT), Sucrose buffer, Glutathione (GSH), DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical, Sodium azide (NaN3), Hydrogen peroxide (H2O2) and all solvents; n-hexane, Acetic acid glacial and Methanol used were of analytical grade and purchased from Sigma

Aldrich, USA. All reagents used were prepared according to specifications using appropriate solvents and distilled water.

Plant Extraction

Freshly harvested leaves of *A. precatorius* were washed and shade dried to constant weight. The dried samples were milled into coarse powder by a mechanical grinder and soaked in 2 litres of Methanol (70%) for 72 hrs, also with frequent agitation. Thereafter, the mixture was filtered and the solvent recovered at 60oC, leaving behind a semi solid extract designated as APL. The extract was poured hot into a beaker of known weight, and allowed to cool in a fume cupboard. The weight of dried extract was thus deduced from the weight of the beaker and its contents.

Experimental Animals

Adult wistar rats of both sexes, weighing between 200 - 250g were housed in standard well ventilated cages in the rat control room. They were allowed free access to laboratory chow and distilled water ad libitum. They were left to adapt to normal laboratory conditions for 2 weeks before initiation of the experiment and were re-weighed. For the sub-acute administration study, the animals were randomly divided into six groups of five animals each. Group 1 receiving no treatment, but an equal volume of PBS, served as the control group. Groups 2 - 6 were orally administered 15, 30, 60, 120 and 240mg/kg doses of APL respectively. The duration of treatment was for 28 days. For the sub-chronic administration study, the animals were also randomly divided into six groups of five animals each. Group 1 receiving no treatment, but an equal volume of PBS, served as the control group. Groups 2 - 6 were orally administered 15, 30, 60, 120 and 240mg/kg doses of APL respectively. The duration of treatment was for 90 days.

Sample Collection

At the end of 28th day for the sub-acute study and 90th day for the sub-chronic study, blood samples were collected by cardiac puncture from rats into non-heparinised bottles and centrifuged. Serum was then collected for hematological and biochemical studies.

Determination of Serum Biochemical and Hematological Parameters

Total protein was measured using the Biuret reaction method while albumin was measured by colorimetric estimation using the Sigma Diagnostics albumin reagent (Sigma Diagnostic®, UK), which contained bromocresol green (BCG). Globulin was estimated as the difference between total protein and albumin. Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were determined on a photoelectric colorimeter (Gallenkamp and Sons Ltd; England) as described by Toro *et al.,* 1975 and Duncan *et al.,* 1994. Serum urea and creatinine levels were also determined on a photoelectric colorimeter (Gallenkamp and Sons Ltd, England) as described by Toro *et al.,* 1975 and Coles, 1986.

Determination of haemoglobin concentration was as described by Schalm *et al.,* 1975 using the cyanomethaemoglobin method. Packed cell volume (PCV) was determined by conventional method of filling the capillary tubes with blood as described by Schalm *et al.,* (1975). Erythrocyte count was determined by the haemocytometer method as described by Coles, 1986. Total leucocytes and differential leucocyte count were also determined.

Determination of Non - Enzymatic Anti Oxidant Effect

At the end of 28th day for the sub-acute study and 90th day for the sub-chronic study, animals were sacrificed and liver was collected. The organ was then washed in running tap water to remove the blood clots and weighed amount of the organ was kept in chilled normal saline for estimation of lipid peroxides and reduced glutathione. For the estimation of lipid peroxides, 0.5 g of freshly excised liver tissue was homogenized with 5 ml Tris – HCl buffer (pH 7.5). For the estimation of reduced glutathione, homogenate of liver was prepared in the ratio of 0.5 g of wet tissue to 4 ml of phosphate buffer and then centrifuged at 5000 rpm, and the supernatant was used. Levels of reduced glutathione and lipid peroxides of liver homogenate were estimated by standard procedures (Moron *et al.,* 1978 and Fraga *et al.,* 1988). Malondialdehyde in liver homogenate was determined by reaction with thiobarbituric acid (TBA). Liver organ were

homogenized in10% (w/v) 0.1 M Tris–HCl buffer (pH 7.5). One milliliter of the homogenate was combined with 2 ml of TCA–TBA–HCl reagent (15% trichloroacetic acid (TCA) and 0.375% thiobarbituric acid (TBA) in 0.25 N HCl) boiled for 15 min. Precipitate was removed after centrifugation at 1000g for 10 min and absorbance of the sample was read at 535 nm against a blank.

Determination of Enzymatic Anti Oxidant Effect

Assay of Superoxide Dismutase (SOD)

The assay mixture contained 1.2 ml sodium pyrophosphate buffer (0.052 M, pH 8.3), 0.1 ml of 186 µM Phenazonium methosulphate (PMS), 0.3 ml of 300 µM NBT, 0.2 ml of 780 µM NADH, 1.0 ml liver homogenate (the tissue was homogenized in10% (w/v) 0.25 M sucrose buffer) and distilled water to a final volume of 3.0 ml. Reaction was started by the addition of NADH and incubated at 30ºC for 1 min. The reaction was stopped by the addition of 1.0 ml glacial acetic acid and the mixture stirred vigorously. 4.0 ml of n-butanol was added to the mixture and shaken well. The mixture was allowed to stand for 10 min, centrifuged, the butanol layer taken out and the absorbance was measured at 560 nm against a butanol blank.

Assay of Catalase (CAT)

The reaction mixture contained 2.0 ml of liver homogenate (tissue was homogenized in 10% (w/v) 50 mM phosphate buffer, pH 7.0) and 1.0 ml of 30 mM hydrogen peroxide (in 50 mM phosphate buffer, pH 7.0). A system devoid of the substrate (hydrogen peroxide) served as the control. Reaction was started by the addition of the substrate and decrease in absorbance monitored at 240 nm for 30 s at 25ºC. The difference in absorbance per unit time was expressed as the enzyme activity. One unit is defined as the amount of enzyme required to decompose 1.0 M of hydrogen peroxide per minute at pH 7.0 and 25ºC.

Estimation of Glutathione Reductase (GSR)

The activity of the enzyme, glutathione reductase also known as glutathione disulphide reductase was determined spectrophotometrically by the decrease in absorbance of NADPH at 340 nm. The reaction mixture contained 2.1 ml of 0.25 mM, potassium phosphate buffer pH 7.6, 0.1 ml of 0.001 M NADPH, 0.2 ml of 0.0165 M oxidised glutathione and 0.1 ml (10 mg/ml) of bovine serum albumin (BSA). The reaction was started by the addition of 0.02 ml of liver homogenate with mixing and the decrease in absorbance at 340 nm was measured for 3 min against a blank. Glutathione activity was expressed as nmoles NADPH oxidized/min/ mg liver protein at 30ºC.

Estimation of Glutathione Peroxidase (GPx)

The reaction mixture consists of 0.2 ml of 0.4 M Tris buffer, 0.1 ml of sodium azide, 0.1 ml of hydrogen peroxide, 0.2 ml of glutathione and 0.2ml of liver homogenate supernatant incubated at 37ºC for 10 min. The reaction was inhibited by the addition of 10% TCA and the absorbance was taken at 340 nm. Activity was expressed as nmoles/min/mg liver protein.

Statistical Analysis

Statistical analysis was carried out by using one-way analysis of variance (ANOVA) followed by Dunnett's test. Results are expressed as mean ± SEM, P values < 0.05 were considered significant.

Results

Serum Hematological Parameters

The hematological changes produced in experimental rats given varying doses of APL after 28 days and 90 days are presented in Table 1. After the 28 day study period, rats that received 120 mg/kg and 240 mg/kg doses of the extract showed a significant increase ($p < 0.05$) in the mean PCV and RBC level relative to the control. All the dose levels caused a significant ($p < 0.05$) increase in platelet count as well as the MCV when compared to the control values. The result also showed a significant increase ($p < 0.05$) in the white blood cell count in rats given 240 mg/kg relative to the controls, no significant increase ($p > 0.05$) in the lymphocyte counts and a significant decrease ($p < 0.05$) in the neutrophil counts in rats dosed with 60, 120 and 240 mg/kg of A. precatorius extract. After 90 days of administering APL extracts, there were no significant ($p>0.05$) increase in hematological

parameters beyond the values observed in the 28 day study. However this was with the exception of the platelet count which showed significantly higher values ($p < 0.05$) in all treated groups relative to the control.

Table 1: Hematological parameters in experimental animals after 28 days and 90 days of 70% Methanolic *A. precatorius* leaf (APL) extract administration.

APL extract						
	Sub-acute toxicity study (28 days)					
Parameters	**Control**	**15mg/kg**	**30mg/kg**	**60mg/kg**	**120mg/kg**	**240mg/kg**
RBC (10^6/µl)	4.2±0.2	4.9±0.5	5.2±0.4	5.8±0.7	8.3±0.9*	9.7±0.5**
PCV (%)	37.2±1.4	38.6±1.0	39.1±0.7	41.0±0.8	57.2±0.8*	76.9±1.6***
HB (g/dl)	13.0±0.2	15.0±0.7	15.6±0.4	15.6±0.2	16.0±0.5	16.2±0.1
MCV (fl)	58.5±0.6	62.1±0.3	65.5±0.1*	67.3±0.2*	74.0±0.3*	79.5±0.6*
MCH (pg)	25.8±1.2	27.2±1.2	28.8±1.6	32.3±0.9	35.3±1.4	35.3±0.8
MCHC (g/dl)	40.5±0.4	37.3±0.2	35.5±0.7	36.8±0.1	40.1±0.3	39.4±0.9
HCT (%)	30.0±0.2	33.0±0.5	33.0±0.3	36.0±0.1	37.0±0.3	41.0±0.5
WBC (10^3/µl)	12.1±0.1	13.1±0.4	14.1±0.1	14.6±0.2	16.1±0.6	32.5±0.1**
LYM (%)	62.8±0.4	63.1±0.5	63.8±0.7	64.3±0.4	64.8±0.3	64.8±0.6
PLT (%)	32.2±2.2	37.2±1.8	43.2±1.7*	49.2±2.3*	53.2±2.0*	54.2±2.8*
NEU (%)	35.6±2.1	35.2±1.9	33.2±1.3	23.1±1.5*	19.3±2.0*	15.2±1.7*
	Sub-chronic toxicity study (90 days)					
Parameters	**Control**	**15mg/kg**	**30mg/kg**	**60mg/kg**	**120mg/kg**	**240mg/kg**
RBC (10^6/µl)	4.8±0.2	5.3±0.1	5.6±0.1	6.2±0.4	6.2±0.9	6.6±0.1
PCV (%)	38.3±1.4	39.2±1.1	39.0±0.6	41.2±0.7	45.3±0.6	47.2±1.0
HB (g/dl)	14.5±0.2	15.0±0.7	15.6±0.3	15.7±0.4	16.6±0.4	18.0±0.1
MCV (fl)	63.3±0.6	67.5±0.3	68.5±0.2	68.0±0.5	69.7±0.8	68.9±0.7
MCH (pg)	28.4±1.6	28.7±1.4	29.8±1.2	30.2±1.1	32.0±1.1	33.4±1.5
MCHC (g/dl)	43.7±0.4	44.5±0.8	44.9±0.1	45.5±0.3	45.9±0.8	47.0±0.1
HCT (%)	37.1±0.2	35.0±0.9	37.6±0.3	39.1±0.6	41.0±0.2	43.7±0.4
LYM (%)	62.9±1.0	63.8±1.4	65.2±1.3	66.0±1.2	66.8±1.0	67.1±1.3
PLT (%)	37.0±2.1	44.1±1.7	47.7±2.0	54.2±1.5	55.0±1.6	58.9±1.8*
NEU (%)	37.9±0.1	35.6±0.1	34.2±0.4	34.0±0.5	33.2±0.3	34.1±0.2
WBC (10^3/µl)	13.2±1.4	13.6±1.1	13.5±1.3	14.1±2.0	14.5±1.0	15.6±0.5

Hematological Parameters: RBC: (Red Blood Cell Count), PCV: (Packed Cell Volume), HB: (Hemoglobin Count), MCV: (Mean Corpuscular Volume), MCH: (Mean Cell Hemoglobin), MCHC: (Mean Corpuscular Hemoglobin Concentration), HCT: (Hematocrit), WBC: (White Blood Cell Count), LYM: (Lymphocyte number), PLT: (Platelet Count), NEU: (Neutrophil count). Data represents the Mean ± S.E.M for each group of rats, n = 5.
*$p<0.05$ = significant difference

Serum Biochemical Parameters

Liver enzyme assay of rats treated with APL for 28 days showed that the extract caused mild increase ($p > 0.05$) in the level of ALP and AST especially with the 240 mg/kg dose. The values of creatinine in groups treated with 120mg/kg and 240mg/kg increased significantly ($p<0.05$) in comparison with the control group. Also there were slight dose dependent increase in the glucose levels of treated rats and significant decrease ($p<0.05$) in their cholesterol levels. All other studied parameters did not change markedly (table 2).

However, after the 90-day study, APL extracts at all doses produced a significant ($p<0.05$) increase in serum ALT, ALP and AST and a significant decrease in albumin when compared to that of the control (table 2).

Determination of Non-Enzymatic and Enzymatic Antioxidant Activity

The results showing the concentration of different non-enzymatic antioxidants in the liver protein are represented in table 3. Reduced glutathione were found to be maximum with higher

doses of the extract after 28 day administration. Prolonged administration did not produce a more significant effect; rather there was a slight decline in the values obtained after the 90 day study as shown in table 3. MDA and Lipid peroxide values were significantly lower in the extract treated groups relative to the control, showing that there was reduced lipid peroxidation with extract treatment.

The liver SOD, CAT, GSR and GPx levels of A. precatorius extract treated animals (15-240mg/kg) showed significant increase relative to the control. Of particular note is the dose dependent increase in SOD activity under the 28 day-study, which became more profound ($p<0.05$) with prolonged administration. Administration of the extract for 90 days at 60, 120 and 240 mg/kg doses showed SOD activity of 2.06±0.01, 2.14±0.02 and 2.78±0.01 (units/min/mg protein) respectively when compared to the control group (0.40±0.02 units/min/mg protein) (table 3).

Table 2: Biochemical parameters in experimental animals after 28 days and 90 days of 70% Methanolic *A. precatorius* leaf extract administration.

APL extract						
Sub-acute toxicity study (28 days)						
Parameters	**Control**	**15mg/kg**	**30mg/kg**	**60mg/kg**	**120mg/kg**	**240mg/kg**
TP (g/dl)	5.58±0.29	5.63±0.24	5.75±0.17	5.75±0.22	5.90±0.18	6.11±0.27
Glo (g/L)	3.10±0.17	3.12±0.21	3.16±0.13	3.30±0.19	3.32±0.23	3.41±0.13
Alb (g/L)	2.18±0.03	2.17±0.09	2.15±0.06	2.16±0.11	2.13±0.03	2.11±0.12
T bil (μmol)	0.38±0.12	0.41±0.11	0.42±0.09	0.50±0.03	0.59±0.14	0.67±0.06
Conj bil (μmol)	0.10±0.08	0.11±0.09	0.14±0.11	0.19±0.14	0.23±0.08	0.23±0.15
AST (U/l)	69.10±4.20	70.17±4.19	70.80±3.94	72.07±4.31	74.00±4.80	83.30±4.50
ALP (U/l)	69.12±5.03	69.13±5.23	70.63±5.09	71.84±5.20	73.69±5.07	74.54±4.91
ALT (U/l)	41.15±2.09	42.91±1.78	42.95±2.07	42.95±2.83	43.92±2.03	47.15±2.33
UREA (mg/dl)	3.33±0.11	3.41±0.11	3.48±0.18	3.49±0.09	3.55±0.11	3.87±0.10
CRE (mg/dl)	0.48±0.10	0.59±0.17	0.60±0.15	0.65±0.09	0.87±0.07*	0.97±0.03*
CHOL (mg/dl)	2.35 ±0.10	2.34 ±0.15	2.32 ±0.20	0.21 ±0.09*	0.18 ±0.10*	0.16 ±0.18*
GLU (mg/dl)	78.20±0.15	78.33±0.11	79.09±0.18	79.45±0.12	79.06±0.15	81.21±0.23
Sub-chronic toxicity study (90 days)						
Parameters	**Control**	**15mg/kg**	**30mg/kg**	**60mg/kg**	**120mg/kg**	**240mg/kg**
TP (g/dl)	5.54±0.13	5.68±0.16	5.78±0.27	5.85±0.25	6.34±0.18	6.57±0.27
Glo (g/L)	3.13±0.10	3.21±0.15	3.25±0.15	3.34±0.17	3.38±0.13	3.47±0.17
Alb (g/L)	2.18±0.12	2.15±0.08	2.13±0.12	2.09±0.17	1.64±0.13*	0.78±0.11*
T bil (μmol)	0.37±0.19	0.46±0.13	0.52±0.19	0.55±0.12	0.65±0.17	0.69±0.16*
Conj bil (μmol)	0.07±0.10	0.09±0.06	0.12±0.08	0.19±0.11	0.25±0.10	0.27±0.14
AST (U/l)	68.30±2.03	72.23±2.25	77.60±2.54	79.31±2.19*	80.06±2.70*	86.46±2.23*
ALT (U/l)	42.10±1.43	42.58±1.91	44.00±1.41	49.30±1.82*	58.36±1.75*	69.11±1.03*
UREA (mg/dl)	3.37±0.10	3.46±0.13	3.53±0.14	3.69±0.11	3.71±0.10	3.88±0.11
CRE (mg/dl)	0.48±0.10	0.52±0.14	0.61±0.10	0.67±0.11	0.89±0.07*	0.91±0.03*
CHOL (mg/dl)	2.32 ±0.08	2.14 ±0.04	2.05 ±0.03	2.04 ±0.06	2.01 ±0.07	1.89 ±0.09*
GLU (mg/dl)	78.20±0.09	78.35±0.10	79.12±0.16	80.03±0.17	81.08±0.09	83.17±0.204
ALP (U/l)	72.10±3.17	76.13±3.08	77.13±3.10	83.32±3.05*	85.11±3.08*	89.44±3.11*

Biochemical parameters; TP: (Total Proteins), GLO: (Globulin), ALB: (Albumin), T Bil: (Total bilirubin), Conj Bil: (Conjugated bilirubin), AST: (Aspartate transaminase), ALT: (Alanine transaminase), ALP: (Alkaline phosphatase), URIC: (Uric acid), CRE: (Creatinine), CHOL: (Cholesterol), GLU: (Glucose). Data represents the Mean ± S.E.M for each group of rats, n = 5.
*$p<0.05$ = significant difference.

Table 3: Effect of 70% Methanolic *A. precatorius* leaf extract administration on Liver Enzymatic and Non - Enzymatic Parameters in Experimental Animals.

APL extract						
	Sub-acute toxicity study (28 days)					
Parameters	**Control**	**15mg/kg**	**30mg/kg**	**60mg/kg**	**120mg/kg**	**240mg/kg**
LPx (nmol/mL)	5.12±0.12	5.07±0.14	4.69±0.12	4.41±0.19	2.92±0.21*	2.51±0.20**
rGSH (mol/mL)	3.12±0.18	3.19±0.11	3.26±0.17	3.35±0.25	3.79±0.21*	3.87±0.11**
MDA (µmol/L)	2.27±0.11	2.21±0.13	2.15±0.09	2.08±0.15	2.02±0.16	1.71±0.16**
SOD (units/min/mg protein)	0.41±0.16	0.43±0.08	0.48±0.07	0.55±0.02	0.74±0.04	0.83±0.03*
CAT(mmoles/mg tissue)	0.12±0.03	0.14±0.07	0.29±0.11	0.36±0.14	0.51±0.11	0.59±0.10*
GSR (nmoles NADPH oxidized/min/ mg liver protein)	67.70±2.14	72.12±2.17	75.36±2.22	79.11±2.10	84.32±2.40*	86.10±2.04*
GPx (nmoles/min/mg liver protein)	68.43±2.42	69.64±3.03	72.17±3.12	74.09±3.09	78.11±3.08	79.33±3.12*
Sub-chronic toxicity study (90 days)						
Parameters	**Control**	**15mg/kg**	**30mg/kg**	**60mg/kg**	**120mg/kg**	**240mg/kg**
LPx (nmol/mL)	5.16±0.10	5.13±0.07	5.08±0.11	4.87±0.15	4.53±0.14	4.45±0.17
rGSH (mol/mL)	3.13±0.16	3.23±0.13	3.29±0.11	3.41±0.12	3.72±0.15	3.73±0.13
MDA (µmol/L)	2.29±0.02	2.27±0.08	2.19±0.14	2.17±0.11	2.08±0.12	2.01±0.12
SOD (units/min/mg protein)	0.40±0.02	0.78±0.06	1.25±0.01	2.06±0.01**	2.14±0.02**	2.78±0.01**
CAT(mmoles/mg tissue)	0.12±0.02	0.17±0.03	0.24±0.11	0.45±0.11	0.67±0.05	0.74±0.06
GSR (nmoles NADPH oxidized/min/ mg liver protein)	67.57±2.09	71.24±2.03	76.16±2.22	79.68±2.03	85.12±2.09*	89.34±2.12*
GPx (nmoles/min/mg liver protein)	67.33±2.18	70.32±3.21	74.37±3.09	75.45±3.12	79.13±3.20	83.56±3.07*

LPx: (lipid peroxides), rGSH: (reduced glutathione), MDA: (malondialdehyde), SOD: (Superoxide Dismutase), CAT: (Conjugated bilirubin), GSR: (Glutathione Reductase), GPx: (Glutathione Peroxidase). Data represents the Mean ± S.E.M for each group of rats, n = 5.

*$p<0.05$ = significant difference. **$p<0.001$ = highly significant difference.

Discussion

Blood is an important index of the physiological and pathological status of an intact biological system (Schlam *et al.*, 1975). Thus in the present study, different serum haematological and biochemical parameters were evaluated to determine the effect of methanolic extract of A. precatorius on wistar rats. In addition, since oxidative stress has been implicated in a number of disease states, we also assessed the effect of extract treatment on enzymatic and non- enzymatic oxidative indices.

An earlier LD50 study by Ogbuehi *et al.*, revealed that A. precatorius leaves are relatively non-toxic at the doses studied. This report varied considerable from some published articles (Saganuwan *et al.*, 2009). This necessitated further consideration of the toxicity or pharmacological importance of the said plant using physiological parameters.

Repeated administration of drug or plant extract could result in toxicity which may cause adverse effect originally not presented in acute administration. Thus, the very high LD50 reported in an earlier study (Ogbuehi *et al.*, 2015) may not be a conclusive finding about the safety of the extract. To this end, the effect of sub-acute and sub chronic (28 and 90 days) oral administration of the extract at graded doses of 15, 30, 60, 120 and 240mg/kg respectively were evaluated.

Administration of the extract for 28 days at low doses of 15 and 30mg/kg did not significantly ($p>0.05$) affect the haematological values of experimental animals when compared with the control values. However, at higher doses 60, 120 and 240mg/kg, the extract caused a slightly significant ($p<0.05$) increase in the HB, PCV and RBC of the rats. Of particular note, is the significantly high ($p<0.05$) PCV and RBC of AP treated rats, (9.7±0.5%) and (76.9±1.6%) respectively at 240mg/kg in comparison with the

PCV and RBC of control rats (4.2±0.2%) and (37.2±1.4%). On the other hand, sub-chronic administration of the extract at all doses did not cause any marked change in the haematological values except a marked elevation of platelet count. The lymphocytosis and neutropenia observed in our study may be of inflammatory origin. Hence, methanolic leaf extract of A. precatorius may have a haematopoietic property. This agrees with the findings of Saganuwan *et al.,* 2011 who reported that the plant may be used to improve haematological parameters.

With 28-day APL treatment, the biochemical parameters studied varied within the control range. The effects were however, more profound after the chronic administration, causing marked serum elevation of liver enzymes which suggests liver damage. The liver is a key internal organ which plays an important role in the elimination of waste products and toxic substances. Dysfunctions of these organs lead to leaking of biochemical substances into the blood circulatory system (Gaw *et al.,* 1999; Moss and Henderson, 1996). Therefore, increasing biochemical values in blood is a sign of abnormal liver function due to decreased excretion of waste products. The results obtained in this study are indicative of hepatic involvement following the administration of A. precatorius leaf extract at dose 240 mg/kg and all doses in the 90-day study. Prolonged administration caused an increase in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) which may not be unconnected to hepatic damage.

ALT is a kind of cytoplasmic enzyme that increases in plasma which is an indication of injuries caused by toxic agents to the liver. Also increased activity of mitochondrial enzyme AST in plasma reflects severe tissue injuries (Uddin *et al.*, 2014). Urea is a biomarker of kidney function and retention of this product in the body indicates renal damage (Johnson *et al.,* 2013). In this present study, there were no significant increase ($p>0.05$) in the level of urea when compared with the control group.

Adedapo *et al.,* has in an earlier study published the toxic effects of A. precatorius leaves at dose levels of >400mg/kg in male wistar rats, as evidenced by decreased hematological and biochemical parameters. Saganuwan *et al.,* 2010 reported that A. precatorius caused increased plasma levels of urea and creatinine, which may be indicative of renal damage to mice at dose 12.5 – 200mg. The variation of these results with the result of the present study may not be unconnected to the solvent and method employed in the extraction.

A high serum cholesterol level is both an independent and synergistic risk factor for cardiovascular diseases and is often related to hypertension, obesity and diabetes mellitus (Khalil *et al.,* 2014). In this study, a moderately significant decrease was observed in the cholesterol level of APL treated rats. This is a positive result owing to the recent disease burden of cardiovascular diseases associated with elevated HDL cholesterol levels in developing countries such as Nigeria (Maiyaki *et al.,* 2014).

Free radicals are known to play a definite role in a wide variety of pathological manifestations while on the other hand, antioxidants fight against free radicals and protect us from various diseases by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms (Umamaheswari *et al.,* 2008). Several parameters have been used to estimate the in vitro antioxidant activity of the extract under study. Superoxide anion (O2-) is one of the most important representatives of free radicals. It acts as a precursor of more reactive oxidative species such as single oxygen with biological macromolecules and thereby inducing tissue damage, and plays a vital role in peroxidation of lipids (Aruoma, 1996; Pulido *et al.,* 2000). Superoxide dismutase (SOD) acts by accelerating the dismutation of the toxic superoxide radical to hydrogen peroxide and molecular oxygen (Petrulea *et al.,* 2012). This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4- nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye (Wen-Yuh *et al.,* 2013). The SOD activity is measured by the degree of inhibition of this reaction. One unit of SOD is the amount that causes a 50% inhibition of the rate of INT reduction. In the present study, high SOD activities were found in the methanolic extracts of A. precatorius leaves indicating that the extract contained compounds to inhibit the

reduction of INT. (table 3). High inhibitions were observed as the dose increased and were even more pronounced with prolonged administration under the sub chronic study.

Lipid peroxidation involves the formation and propagation of lipid radicals. This phenomenon, if not checked can lead to destruction of membrane lipids, unbridled inflammation and metabolic disorders. Production of malondialdehyde (MDA) is a hallmark of this process, thus increased MDA level is an important indicator of lipid peroxidation (Grotto *et al.,* 2009). This process is initiated by hydroxyl and superoxide radicals leading to the formation of peroxy radicals that ultimately propagates chain reaction in lipids. Thus, antioxidants which are capable of scavenging peroxy radicals could prevent lipid peroxidation. In the present study, we observed a considerable reduction in MDA values of treated rats when compared to the control. It may be that the extract contributed to the biosynthesis of the antioxidant enzymes thereby increasing free radical scavenging ability and reducing membrane lipid peroxidation.

On the other hand, glutathione (GSH) is an important intracellular antioxidant, which functions as free radical scavenger, protecting cells against free radicals, peroxides and other toxic compounds. Decline in the level of functional reduced glutathione levels is considered to be an indicator of oxidative stress. In this study, it was observed that the methanolic extract of A. precatorius has anti-oxidant property as there was a statistically significant decrease in lipid peroxides and a concurrent increase in reduced glutathione. The biochemical function of glutathione peroxidase is to reduce lipidhydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. Glutathione catalyzes the reduction of glutathione disulfide to the sulfhydryl form glutathione, which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell (Meister *et al.,* 1988). Glutathione reductase functions as dimeric disulfide oxidoreductase and utilizes an FAD prosthetic group and NADPH to reduce one mole of GSSG to two moles of GSH: It can act as a scavenger for hydroxyl radicals, singlet oxygen, and various electrophiles. Reduced glutathione reduces the oxidized form of the enzyme glutathione peroxidase, which in turn reduces hydrogen peroxide (H2O2), a dangerously reactive species within the cell. In addition, it plays a key role in the metabolism and clearance of xenobiotics and regenerates antioxidants such and Vitamins E and C to their reactive forms.

Several studies have alluded to the possible anti-oxidative effect of A. precatorius leaves and even seeds to provide protection against different disease conditions such as neuro-degenerative disorders, hepatocellular carcinoma and cataract (Premanand *et al.,* 2010; Ramaswami *et al.,* 2010 and Umamaheswari *et al.,* 2012) or to be incorporated in nutrition (Vanitha *et al.,* 2014). It has been postulated that plants possessing anti-oxidant property such as A. precatorius are able to inhibit oxidation of biological substrates, by virtue of the presence of flavonoids, saponins and polyphenols (Yoshiki *et al.,* 1995; Nunes *et al.,* 2012; Gul *et al.,* 2013; Namratha *et al.,* 2014). In lending support to this, Ogbuehi *et al.,* in a recent study reported the considerable presence of flavonoids and saponins in methanolic extract of A. precatorius (Ogbuehi *et al.,* 2015).

Conclusion

Methanolic leaf extract of *Abrus precatorius* may be used to improve haematological parameters. Also, the plant has shown promise as a potential antioxidant supplement for management of diseases resulting from oxidative stress.

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