Antioxidant Properties and Effect of *Abrus precatorius* Leaves Extract on Haematological and Biochemical Parameters in Rats

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A B S T R A C T

Background

Abrus precatorius (*A. precatorius*) is a herbal remedy commonly used in traditional medicine. We investigated the phytochemical, antioxidant, toxic properties, and safety of *A. precatorius* leaves in rats.

Methods

Phytochemical studies were conducted using standard procedures. The antioxidant properties were studied using the reducing power and 2, 2′- diphenyl-1-picrylhydrazyl (DPPH) scavenging assays. The effect of the extract on biochemical and haematological parameters were evaluated after the oral administration of the extract at daily doses of 200, 400 and 600 mg/kg of the rats' body weight for 28 days.

Results

Saponin was the most abundant (202.25±9.25 mg/100g) while alkaloids (9.69±0.34 mg/100g) were the least phytochemical content of *A. precatorius*. The extract had an LD_{50} value of >5000 mg/kg, demonstrating significant reducing power and DPPH scavenging activities (IC50 = 106.22 µg/mL).

The extract significantly decreased the serum bilirubin and AST concentrations but increased the total proteins concentration compared with the controls ($p<0.05$). Urea, creatinine, ALP, ALT, chloride and albumin concentrations in the treated rats were not significantly different from those of the controls ($p>0.05$). The 600 mg/kg dose significantly increased the Hb, PCV, RBC and MCHC of the treated rats, compared to those of the controls ($p<0.05$). All doses of the extract tested significantly increased the platelet count but did not alter the counts of MCV, MCH, TWBC, RDW, lymphocyte and neutrophils ($p<0.05$).

Conclusions

This study demonstrated that the *A. precatorious* leaves possess antioxidant and erythropoietic properties. It was also found to be relatively safe with regards to the liver and kidney integrity at concentrations up to 600 mg/kg body weight of the rats.

Keywords

Abrus precatorius; Antioxidant; Biochemical; Haematology; Phytochemicals.

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INTRODUCTION

Natural products are the free gifts of nature bestowed upon humanity with numerous therapeutic properties (1). Medicinal plants are rich sources of secondary metabolite with antioxidant properties that serve to prevent such diseases as cancer, diabetes, atherosclerosis, Alzheimer, diabetes, infections and more (2). According to the recent reports from the World Health Organisation (WHO), about 25% of current drugs are plant-based and more than 75% of the world's population rely on medicinal plants for primary health care needs (3). Also, WHO has recommended application of herbal medicine, especially in areas with limited access to modern healthcare services. However, WHO emphasizes that the safety of all herbal medicine should be the overriding criterion in their selection and usage.

The plant, *A. precatorius* (Fabacea family) is locally known as "Idon Zakara" in Hausa dialect of Nigeria. It

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grows widely in tropical areas, such as southern India and Africa. In Nigeria, *A. precatorius* leaves have been used for the treatment of numerous diseases, including malaria, typhoid, hepatitis and respiratory tract infections (4). The leaves have also been reported for their cytotoxicity, anti-diabetic and antimicrobial activities (5). The root and leaves are used in traditional medicine to treat cancer (6), chronic nephritis (7), bronchitis, fever, asthma, stomatitis, and diabetes (8). The stem, root and leaves of this plant are also used to treat snake bites, tuberculosis, protozoal infections and insecticide poisoning (9,10). The seeds have been reported for the toxic effect on kidneys, liver, heart, spleen, intestine and lungs (4). However, the leaves have been known to contain low concentrations of a deadly poison known as "*abrin*" (11,12). The aim of the present study was to evaluate the phytochemical, antioxidant, toxic properties, and the safety profile of *A. precatorius* leaves in rats.

MATERIALS AND METHODS

Sample Collection and Preparation: Fresh leaves of *A. precatorius* were collected from Minna Niger State in Nigeria and were identified by an expert botanist at the Department of Biological Science, Federal University of Technology, Minna, Nigeria (FUTMINNA).

Experimental Animals: Healthy albino rats were procured from the animal holding unit at FUTMINNA. They were allowed unrestricted access to rat pellet food and water.

Ethical Approval: The ethical principles governing the use of laboratory animals as set by the Federal University of Technology, Minna; Committee on Ethics for Medical and Scientific Research. Also, the existing internationally accepted principles of laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review were duly observed.

Reagent and Chemicals: Ascorbic acid was obtained from Merck Co. (Darmstadt, Germany) and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) from Sigma-Aldrich Co. (St. Louis, USA). All biochemical assay kits were obtained from either Randox Laboratories (Ardmore, UK) or Agappe Diagnostics (Kerala, India). All other chemicals used in this study were of analytical grades and obtained locally.

Sample Preparation and Extraction: The collected leaves were washed and dried out for 2 weeks at 37°C and powdered, using a grinding mill. A 50g-sample of the material was extracted with 200 ml of methanol, using soxhlet apparatus (Fujian, China) and the resulting extract was concentrated, using a water bath at low temperature.

Phytochemical Analysis: Quantitative phytochemical analysis of the crude extract of *A. precatorius* was carried out, using standard procedures as described previously (13,14).

Antioxidant Study: The antioxidant activity of the extracts was measured at varying concentrations (20-100 µg/ml), using ascorbic acid as the reference and DPPH assay (15). The reducing power of the extract was determined by a previously described method (16).

Toxicological Study: The acute toxicity of the extract was evaluated as described by Lorke's (17). The subacute toxicity was determined based on the method described by Yusuf *et al*. (18).

Animals & Grouping: Twenty albino rats were randomly divided into 4 groups of 5 rats each. Group 1 rats were given normal saline orally (10 ml/kg) and served as the controls. Groups 2, 3 and 4 received 200, 400 and 600 mg/kg the methanol extract of *A. precatorius,* respectively, for 28 days. Blood samples were collected and centrifuged to separate the sera for biochemical analyses as described previously (19).

Biochemical Parameters: The serum levels of AST, ALT, ALP, total proteins, albumin, bilirubin, urea, creatinine and chloride were determined spectrophotometrically, using standard methods (20-24).

Haematological Parameters: The hematological constituents, such as haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and the concentration (MCHC), white blood cells (WBC), platelet count (PLT) and differential counts: granulocytes, lymphocytes, eosinophils, monocytes and neutrophils were determined, using an automated haematologic analyzer (SYSMEX KX21, Japan), employing the methods described by Dacie and Lewis (25).

Data Analysis: The data were analyzed using statistical package for social science (SPSS, v18). The differences among the groups were compared using analysis of variance (ANOVA) followed by Duncan's multiple range test at $P<0.05$ confidence level.

RESULTS

Phytochemical & Acute Toxicity Study: The quantitative analysis revealed saponin to be the most abundant phytochemical component (202.25±9.25 mg/100g), followed by tannins (129.12±9.30 mg/100g) while alkaloids were the least abundant phytochemical content (9.69±0.34 mg/100g) of the *A. precatorius* extract (Table 1). The acute toxicity study did not cause mortality, with the LD_{50} concentration being greater than 5000 mg/kg in the rats (Table 2).

Table 1. Quantitative phytochemical contents of *A. precatorius* extract.

Phytochemical	Concentration (mg/100g)
Flavonoids	60.97±3.20
Phenols	121.95±11.36
Tannins	129.12±9.30
Saponins	202.25±9.25
Alkaloids	9.69±0.34

Table 2. Acute toxicity profile of *A. precatorius* extract.

Group	Dose (mg/kg)	No of animals	Mortality
Group 1	10	3	0/3
Group 2	100	3	0/3
Group 3	1000	3	0/3
Group 4	1600	3	0/3
Group 5	2900	3	0/3
Group 6	5000	3	0/3

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In-vitro Antioxidant Activities: The extract of *A. precatorius* inhibited DPPH radicals with an IC_{50} value of 106.22 µg/ml while ascorbic acid had an IC_{50} value of 33.970 µg/ml (Figure 1). The reducing power analysis of the extract also showed some antioxidant activity, resulting in an increase in absorbance with the increasing concentrations of the extract (Figure 2).

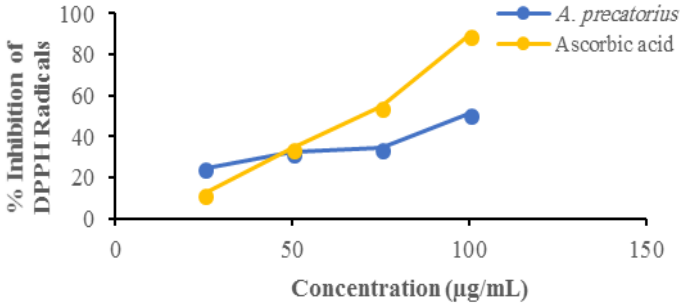

Figure 1. DPPH Radical Scavenging Activity of *A. precatorius* extract.

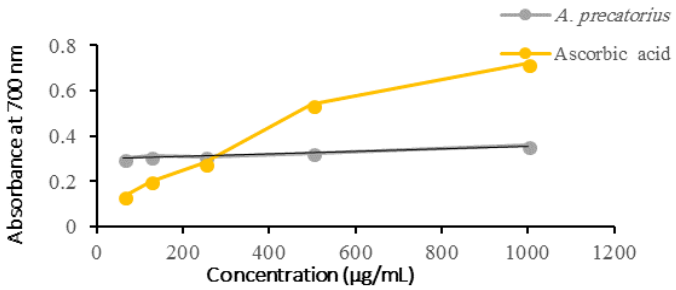

Figure 2. The reducing activity of *A. precatorius* extract.

Biochemical Parameters: The extract of *A. precatorius* at 200, 400 and 600 mg/kg concentrations increased total serum proteins from 97.85±3.50 mg/dl (controls) to 119.70±0.00, 125.40±32.30 and 113.05±25.65 mg/dl, respectively (Table 3). At the same concentrations, the extract decreased the bilirubin concentrations from 0.70±0.01 mg/dl (controls) to 0.64±0.03, 0.38±0.00 and 0.25±0.00 mg/dl, respectively. Similarly, the AST activities declined from 77.40±1.80 U/l (controls) to 45.00±1.80, 37.80±9.00, and 43.21±10.81 U/l in rats treated with 200, 400 and 600 mg/kg of the extract, respectively. At the same extract concentrations, urea, creatinine(Table 4), ALP, ALT, chloride and albumin concentrations were not significantly different in experimental rats from those in the control group.

Hematological Parameters: At a dose of 600 mg/kg, the extract significantly increased the haemoglobin, packed cell volume, red blood cells and MCHC of the treated rats compared with those of the controls. All doses of the extract tested significantly increased the platelet count ($p<0.05$) compared to that of the controls. However, MCV, MCH, TWBC, red blood cells distribution width (RDW), lymphocyte and neutrophil counts were not significantly different among rats treated with any concentrations of the extract compared to those for the controls (Table 5).

Table 3. Effect of *A. precatorius* extract on liver function.

Group	Total Proteins	Bilirubin	ALP	Albumin	AST	ALT
Control	97.85±3.50 [a]	0.70±0.01 [d]	96.25±3.75 [a]	5.69±0.06 [a]	77.40±1.80 [b]	57.35±3.67 [a]
200 mg/kg	119.70±0.00 [b]	0.64±0.03 [c]	94.22±4.66 [a]	4.46±0.71 [a]	45.00±1.80 [a]	55.68±3.67 [a]
400 mg/kg	125.40±32.30 [b]	0.38±0.00 [b]	91.28±3.74 [a]	5.63±0.52 [a]	37.80±9.00 [a]	61.90±4.32 [a]
600 mg/kg	113.05±25.65 [b]	0.25±0.00 [a]	96.25±3,.75 [a]	5.63±0.29 [a]	43.21±10.81[a]	58.35±4.35 [a]

Values are mean ± SEM of 3 determinations. The values along the same row with different superscripts were significantly different (p<0.05). The superscript alphabet "**d**" indicates the highest significance level followed by "**c**" and "**b**", while "**a**" indicates the least significance level at p< 0.05.

Table 4. Effect of *A. precatorius* extract on urea, creatinine and electrolytes.

Group	Urea	Creatinine	Chloride
Control	19.72±1.69 [a]	2.51±0.50 [a]	190.00±0.00 [a]
200 mg/kg	17.00±3.28 [a]	2.00±1.01 [a]	149.99±8.57 [a]
400 mg/kg	25.92±0.00 [a]	1.99±0.00 [a]	156.42±2.14 [a]
600 mg/kg	20.56±2.53 [a]	1.99±1.00 [a]	163.68±26.54 [a]

Values are mean ± SEM of 3 determinations. The values along the same row with different superscripts were significantly different (p<0.05). The superscript alphabet "**d**" indicates the highest significance level followed by "**c**" and "**b**", while "**a**" indicates the least significance level at p< 0.05.

Table 5. Effect of *A. precatorius* extract on hematological parameters.

Component	Controls	200 mg/kg	400 mg/kg	600 mg/kg
Hb	14.56±0.78 [a]	15.43±0.24 [a]	14.53±1.90 [a]	16.35±1.67 [b]
PCV	42.34±0.45 [a]	43.45±1.82 [a]	44.32±1.98 [a]	49.75±1.32 [b]
MCV	45.42±2.67 [a]	46.43±2.56 [a]	45.42±2.67 [a]	43.45±2.34 [a]
MCH	14.32±0.56 [a]	15.45±0.78 [a]	15.21±0.89 [a]	16.56±0.88 [a]
MCHC	40.89±1.99 [a]	48.32.±2.34 [a, b]	42.89±0.89 [a]	52.34±1.90 [b]
RBC	8.03±0.23 [a]	8.17±0.79 [a]	8.91±0.67 [a, b]	9.4.62±0.46 [b]
PLC	198.23±4.56 [a]	219.67±3.78 [b]	235.56±3.45 [b]	226.76±4.56 [b]
TWBC	5.91±0.46 [a]	5.32±0.46 [a]	5.06±0.45 [a]	5.39±0.29 [a]
Neutrophils	33.57±0.68 [a]	33.46±0.56 [a]	30.67±0.56 [a]	31.24±0.98 [a]
Lymphocyte	43.46±0.89 [a]	45.435±0.79 [a]	42.35±1.35 [a]	41.57±1.68 [a]
RDW+	34.56±0.45 [a]	33.56±0.57 [a]	35.67±0.45 [a]	33.56±0.7 [a]

Values are mean ± SEM of 3 determinations. The values along the same row with different superscripts were significantly different (p<0.05). The superscript alphabet "**d**" indicates the highest significance level followed by "**c**" and "**b**", while "**a**" indicates the least significance level at p< 0.05.

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DISCUSSION

The significant amount of secondary metabolites, including flavonoids, alkaloids and saponins in *A. precatorius* leaves is an indication that they contain phytochemicals that could confer considerable health benefits to individuals who consume the plant appropriately. Flavonoids occur in foods either as free monomers (quercetin, catechin) or oligomers (procyanidins), bound to saccharides as glycosides. The significant amount of phytochemicals in the extact, as reported in this study, is in close agreement with the amounts reported by Ikechukwu *et al*. (26) for the contents of saponins (8.22 % w/v), alkaloids (6.00 % w/v) and flavonoids (30.05% w/v) in *A. precatorius* leaves (Table 1).

The consumption of flavonoid-rich foods is associated with low incidence of coronary heart disease, myocardial infarction, cancer, neurodegenerative diseases, chronic or infectious disorders (4-8). In addition to their antioxidant properties, flavonoids, alkaloids and saponins have been reported to exhibit multiple biological benefits against viral, bacterial, inflammatory, vasodilatory, cancer and ischemic conditions (27). In the pathogenicity of these diseases, oxidative stress has been assumed to play a major role, suggesting that flavonoids exert their health benefits through antioxidant mechanism (2). The activities of the extract against DPPH radicals could, therefore, be attributed to the presence of these phytochemicals.

The dose-dependent increase in the antioxidant activities of *A. Precatorious, as* observed in this study, is in line with that reported by another study (28). This indicates that the antioxidant potentials of *Newbouldia laevis* and *Crateva adansonii* extracts increase as the concentrations rise. The IC_{50} values of *A. precaterious* was also far better than the scavenging activities reported for some other medicinal plants, such as *Padina pavonica* (IC_{50} = 5.59 mg/ml), *Laurenica majuscule* (IC_{50} =14.3 mg/ml), and *Laurencia catarinensis* (IC_{50} = 53.8 mg/ml) (29). The high antioxidant activities could be translated to higher medicinal value of the plant extract. The high flavonoid contents of *A. precatorius* leaves may have contributed significantly to the findings in this study, which were in line with those reported by Kostic *et al*. (30).

The estimated LD_{50} of the extracts being greater than 5000 mg/kg suggests that the extract is fairly safe with a very low toxicity. This finding is in agreement with a previous study (26), reporting that the extract of *A. precatorius* leaves are safe for consumption as no signs of toxicity or death was ever recorded even at a concentration of 500 mg/kg of the extract (26). This is contrary to the findings of Tion *et al*. (1) who reported that the estimated LD_{50} of the *A. precatorius* seed extracts to be at doses of 175-187.5 mg/kg. This finding also contradicts the report of Saganuwan *et al*. (31) who indicated that the LD_{50} of the aqueous extract in mice was 2558.9 mg/kg.

The toxicity of natural products particularly plant extracts has been implicated in the alterations of biochemical parameters (32). Transaminases are liver enzymes at low concentrations in the serum. The marked decreases of aspartate aminotransferase concentration may not necessarily indicate a compromised liver function in the appropriate clinical context (33). This is because it has been established that AST activities are elevated when there is injury to the liver or other organs such as heart, muscle, brain and kidneys (33). However, aminotransferases play a major role in amino acid metabolism through intracellular biochemical regulations (19). The marked decrease in AST could; however, affect amino acid metabolism and consequently impact ATP generation in animals. However, the preserved activities of aspartate transaminase, alkaline phosphatase and serum albumin concentration suggest that the functional integrity of liver had not been compromised by the administration of *A. precatorius* extract in rats.

The concentrations of total serum proteins, albumin, bilirubin, urea, creatinine and electrolytes are useful markers of secretory, synthetic and excretory functions of the liver and kidneys (34). The observed increases in the total serum proteins and a decrease in bilirubin suggest a compromised synthetic ability of the liver due to the administration of the extract. The extract might have increased the functional activity of the liver by interfering with the equilibrium in the synthesis and degradation, removal or clearance of total proteins and bilirubin in the animals. A similar finding has previously been reported in rats that were given snail haemolymph (34). The increase in the total serum proteins could; however, lead to dehydration which is detrimental to cellular homeostasis. This will negatively affect the metabolic activities of the liver and consequently the health of the animals. Urea and creatinine are known to accumulate in the urine, when the normal glomerular function is compromised. Therefore, the preserved concentration of urea and creatinine in rats treated with *A. Precatorious* extract reflects a preserved glomerular function (19).

The erythrocyte parameters including RBC, PCV, Hb, MCH, MCHC, and RDW are useful indicators of the levels of circulatory erythrocytes and serve as useful indices of the bone marrow capacity to produce RBC in response to the administration of drugs, toxins or plant extracts (35). The significant increase in haemoglobin, packed cell volume, red blood cells and MCHC following treatment with 600 mg/kg of *A. Precatorious* is an indication that erythropoiesis can be stimulated by the extract. The extract must have increased the rate of erythropoietin synthesis and its release in the kidneys, which is the humoral regulator of RBC production (32). Therefore, the extract could be used in the management of anaemia. Similar observation has been made in rats treated with the extract of *T. occidentalis* (35). The observation with the white blood cell and lymphocyte counts indicated that the extract exerted leucopoetic and possibly immunomodulatory effects on the treated animals (34).

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CONCLUSIONS

The findings demonstrated that the methanol extract of *A. precatorious* has antioxidant and erythropoietic properties. The extract was also found to be relatively safe at concentrations of up to 600 mg/kg in rats with respect to the function and integrity of the liver and kidneys after four weeks of daily administration.

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CONFLICT OF INTEREST

The authors declared no conflict of interests in conducting this study with any internal or external entity.

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