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Original Article

Phytochemical screening, proximate analysis, median lethal dose (LD_{50}), hematological and biochemical effects of various extracts of *Abrus precatorius* seeds in *Mus musculus*

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ABSTRACT

Objective: *Abrus precatorius* is a universal panacea in herbal medicine. In view of this, phytochemical screening, proximate analysis, median lethal dose (LD_{50}), hematological and biochemical effects of extracts of *A. precatorius* seed was studied in *Mus musculus*.

Materials and methods: Nineteen (19) mice were used for the study. Four (4) mice were used for determination of median lethal dose of the aqueous and ethyl acetate extracts respectively. The LD_{50} of aquous and ethyl acetate extracts was estimated at 187.5±62.5 mg/Kg and 175±75 mg/Kg respectively. The remaining fifteen (15) mice divided into 3 groups of 5 each were used for hematological and biochemical studies. Group 1 was administered 1 mL of distilled water while groups 2 and 3 were administered 1/10th (18.75 mg/Kg) of LD_{50} (187.5 mg/Kg) of methanolic and ethanolic seed extracts, for a period of 4 weeks.

Results: Proximate analysis showed the presence of moisture, ash, crude protein and crude fiber. Carbohydrate and organic matter were calculated. Phytochemical screening showed alkaloids, flavanoids, tannins, saponins, and reducing sugars in both ethanolic and aqueous extracts. Cardiac glycosides were present in aqueous extract. Hematology revealed increased packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) whereas red blood cell (RBC) and white blood cell (WBC) were significantly *($P<0.05$)* decreased. Biochemistry revealed significantly decreased *($P<0.05$)* total protein, albumin, cholesterol, globulin and albumin/globulin ratio whereas creatinine and alkaline phosphatase were significantly increased.

Conclusion: *A. precatorius* seed extracts are very toxic and can be used as blood tonic, immunosupressant, hypocholesterolemic and renotoxic.

KEYWORDS

Abrus precatorius seed; Blood tonic; Hypocholesterolemic; Immunosuppressant; Renotoxicity

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INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and one-quarter of modern drugs have been isolated from natural sources (Saganuwan, 2013). Large quantities of drugs are produced synthetically nowadays, and about 25% of the drugs produced are extracted from plants. According to World Health Organization WHO, 80% of the world's population relies on drugs derived from plants for treatment of ailments (Haghiroalsadat et al., 2011).

Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. Phytochemicals are bioactive, non-nutrient, naturally occurring plant compounds or chemicals which are used for medicinal purposes (Okarter et al., 2009). These chemicals often referred to as "secondary metabolities" are classified into four major categories. These four categories include terpenoids (such as carotenoids, sterols, cardiac glycosides and plant volatiles), phenolics (such as lignans, phenolic acid, tannins, coumarins, lignins, stilbenes and flavonoids), nitrogen containing compounds (such as non-protein amino acids, cyanogenic glucosides and alkaloids) and sulphur containing compounds (such as GSH, GSL, phytoalexins, thionins, defensins and lectins) (Mazid et al., 2011). Phytochemicals are present in a variety of plants utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables (Okwu, 2005).

Proximate and nutrient analyses of edible plant and vegetables play a crucial role in assessing their nutritional significance (Pandey et al., 2006). As various medicinal plant species are also used as food along with their medicinal benefits, evaluating their nutritional significance can help to understand the worth of these plant species (Pandey et al., 2006). WHO had emphasized on the need and importance of determining proximate and micronutrients composition of medicinal plants. Such herbal formulations must pass through standardization processes (Niranjan and Kanaki, 2008).

A. precatorius L. is a member of the Fabaceae family and known in various communities with different names. The name Abrus, meaning beautiful or graceful, is used to describe the appearance of the seed (Prabha et al., 2015). The names include cat's eye, bead tree, rosary pea and jequirity bean (Pandey et al., 2006). *A. precatorius* leaf extract is cytotoxic, has antimicrobial and anti-diabetic activities (Alayande et al., 2017). The leaves and roots are sweetish and traditionally used to cure fever, stomatitis, bronchitis, asthma and diabetes (Saganuwan, 2009), chronic nephritis (Ligha et al., 2009), cancer (Sivakumar and Alagesaboopathi, 2008), sores, scratches, wounds, leucoderma, tetanus, boils, abscesses, for prevention of rabies (Saganuwan and Onyeyili, 2010), with hematonic, plasma expander known effect (Saganuwan and Onyeyili, 2011). A. precatorius leaf, stem and root are also used as antimicrobial (including *Mycobacteria tuberculosis*), antiprotozoal, insecticidal and anti-snake venom remedies (Attal et al., 2010; Teklay et al., 2013; Sunday et al., 2016). The plant has reported toxic effect on kidney, liver, heart, spleen, intestine and lungs (Saganuwan and Onyeyili, 2010). The reported phytochemical components of the plant are abricin, abrin, abrisin, abrine, abraline, abrasine, abrus- sic acid, anthocyanins, ash, campestrol, cycloartenol, picatorine, precasine among others (Windholz, 1983). Abrin A and abrin B agglutinins are also reported type IV ribosome inactivating proteins that inhibit protein synthesis in eukaryotes and induce apoptosis (Bagaria et al., 2006).

The seeds are taken for tuberculosis and painful swellings (Attal et al., 2010). The leaves have also been used in Nigeria for the treatment of many diseases including malaria, typhoid, cough, respiratory tract infections and hepatitis (Saganuwan et al., 2011a). Since immuno-modulatory agents are useful in the control, prevention and treatment of diseases, hematological and biochemical effects of the seed extract was studied in mice and results extrapolated to dogs with a view to identifying its immunomodulatory potentials in dogs.

MATERIALS AND METHODS

Experimental animals and ethical approval: A total of nineteen (19) Swiss albino mice of both genders weighing 25.3±1.7 gm were used for the study. They were obtained from Nigerian veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. All the mice were fed mice feed (Finisher) formulated by Grand Cereals and Oil Mills Limited (GCOML) Jos, Plateau State, Nigeria. Clean water was provided *ad libitum*. The mice were kept under hours of light and dark (12:12) respectively. The animals were housed in polypropylene cages containing sterile paddy husk (procured locally) as bedding. All animals were handled according to the International guiding principles for biomedical research involving animals (CIOMS, 1985). This study was approved by the Federal University of Agriculture, Makurdi, Nigeria and certified by the Animal Ethic Committee of the College of Veterinary Medicine with a certificate on experiment reference No 011.

Collection of plant material: The plant material (seeds) used for the study were collected from Kwande and Makurdi Local Government Areas of Benue State, Nigeria between the months of September and November and identified by a botanist in the Department of Biological Sciences, University of Agriculture, Makurdi where a voucher specimen was deposited.

Preparation of extract: The plant parts (seeds) were thoroughly washed with tap water, dried on filter papers and air dried under open shade for more than a month. The seeds were pulverized with the help of a mortar and pestle to coarse powder and finally ground into fine powder using a grinding machine. Fifty grams (50 gm) of *A. precatorius* seed powder was dissolved in four hundred and fifty milliliter (450 mL) of each aqueous, methanolic and ethyl acetate in separate conical flasks. The mixtures were shaken intermittently throughout a whole day using glass rod stirrer and allowed to stand overnight. The mixtures were separately filtered with Whatman filter paper No. 1 into measuring cylinder and concentrated at 60^{o}C in an incubator and stored in a refrigerator at 4^{o}C until required for use (Saganuwan and Gulumbe, 2005a, b).

Administration of *A. precatorius* seed extracts: The method of Yamba et al. (2007) was used for the selection of doses used in hematological and serum biochemistry studies. The selected doses were within the range between tenth and one hundredth of the estimated median lethal dose of aqueous (187.5 mg/Kg) and ethyl acetate (175 mg/Kg) extract. Fifteen (15) mice of either sex weighing 25.3±5.92 gm used for the study were divided into 3 groups of 5 each. The first group was administered 1 mL of distilled water serving as control. Groups 2 and 3 were orally administered one-tenth of the LD_{50} (187.5 mg/Kg body weight) of ethanolic and methanolic extract of *A. precatorius* seed daily for a period of 21 days. 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 mouse 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 while the plasma was used for determination of serum biochemical parameters. Toxicity signs of the treated mice were recorded, and mice that died or survived during the study were subjected to necropsy (Saganuwan and Onyeyili, 2010).

Hematological parameters: Hematological parameters were determined according to the method of Cheesbrough (2005). The parameters include red blood cells (RBCs) count, packed cell volume (PCV), hemoglobin concentration (HB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBCs) count and differential white blood cells count (DWBC).

Serum biochemical parameters: Serum biochemical parameters were determined. The parameters include Total protein, albumin, globulin, albumin/globulin ration, cholesterol, creatinine, alkaline phosphatase (ALP) and alanine aminotransferases (ALT).

Proximate analysis: Determination of proximate composition was carried out in accordance with AOAC (1990). Proximate contents of carbohydrates, protein, fat, crude fibre, ash and moisture as well as caloric value were determined. Moisture content, ash, crude protein and crude fiber were determined (AOAC, 1990). Carbohydrate content was estimated by subtracting the obtained fat and protein from organic matter. The percentage of organic matter was calculated by subtracting the percentage of ash from one hundred (100) (Onwuka, 2005).

Statistical analysis: The data on hematological and biochemical parameters were expressed as mean±standard error of mean (SEM). Repeated measure analysis of variance was used to analyze the data on hematological and biochemical parameters at 5% level of significance (Zar, 2008).

RESULTS

Phytochemical analysis of ethanol and water extracts of *A. precatorius* show presence of alkaloids, flavanoids, tannins and reducing sugars. However, cardiac glycoside was present in water extract (**Table 1**). Proximate analysis of the *A. precatorius* seed revealed moisture (10.54%), ash (2.98%), crude lipid (15.47%), crude fiber (8.05%), crude protein (18.70%), carbohydrate (44.26%) and dry matter (89.46%) respectively (**Table 2**). The estimated median lethal dose (LD_{50}) of aqueous seed extract of *A. precatorius* was 187.5±62.5 mg/Kg (**Table 3**) whereas the LD_{50} of ethyl acetate was estimated at dose level of 175±75.0 mg/Kg bwt, respectively (**Table 3**).

Table 1: Phytochemical constituents of ethanolic and water extracts

Metabolites	Ethanol	Water
Alkaloids	+ve	+ve
Anthraquinones	-ve	-ve
Cardiac glycosides	-ve	+ve
Flavanoids	+ve	+ve
Tannins	+ve	+ve
Saponins	+ve	+ve
Steroids	-ve	-ve
Reducung sugars	+ve	+ve
Phlobatanin	-ve	-ve
Terpenoids	-ve	-ve
Volatile oil	-ve	-ve

+ve = Present, -ve = absent

Table 2: Proximate contents of Abrus precatorius

Parameters	Composition
Moisture content (%)	10.54
Ash (%)	2.98
Ether Extract (Crude lipid) (%)	15.47
Crude Fiber (%)	8.05
Crude Protein (%)	18.70
Carbohydrate (%)	44.26
Dry Matter (%)	89.46

Table 3: The median lethal dose (LD_{50}) of aqueous extract of *Abrus precatorius* seed extract in mice (*Mus musculus*)

Sex	Weight (gm)	Dose (mg/Kg)	Survival status
Female	27.5	250	Died
Male	22.1	125	Survived
	24.8±2.7	187.5±62.5	-

Table 4: The median lethal dose (LD_{50}) of ethyl acetate extract of *Abrus precatorius* seed extract in mice (*Mus musculus*)

Sex	Weight (gm)	Dose (mg/Kg)	Survival status
Male	26.6	250	Survived
Male	25.0	100	Died
	25.8±0.8	175±75.0	-

Methanolic and ethanolic extracts caused significantly increased (P<0.05) hemoglobin and MCH respectively of mice and dogs. But ethanolic extract caused significantly increased in PCV of mice and dogs whereas RBC was significantly decreased (P<0.05) in mice and dogs administered methanolic and ethanolic extract, respectively. WBC in both mice and dogs was significantly decreased by methanol and ethanol extract respectively (**Table 5** and **7**). The effects of methanol and ethanol extracts caused significant (P<0.05) decrease in total protein, albumin, albumin-globulin ratio and cholesterol of mice and dogs respectively. Methanol and ethanol extracts significantly increased (P<0.05) creatinine and alkaline phosphatase of mice and dogs respectively. Alanine aminotransferase and globulin did not increase significantly (P<0.05) in mice and dogs administered methanolic and ethanolic extract (**Table 6** and **8**).

DISCUSSION

Phytochemical principles are responsible for biological activity of plants. The presence of alkaloids, flavanoids, tannins and saponins in the water and ethanol extracts of *A. precatorius* seed shows that the seed may have an array of biological activities. The findings agree with the report of Saganuwan et al. (2011b) indicating that the aqueous extracts of *A. precatorius* have phytochemical principles that may have biological activities. Alkaloids, flavonoids and saponins have antiviral, anti-bacterial, anti-inflammatory, vasodilatory, anti-cancer and anti-ischemic effects (Prochazkova et al., 2011). Alkaloids are used as analgesics, antimalarials, antiseptics and antibacterial agents, saponins exhibit natural antibiotics effect by attacking bacteria and fungi (Okwu and Emenike, 2006). Alkaloids help to defend the plant against herbivores and pathogens (Khan, 2016b; Pervez et al., 2016). The bioactivities of tannins include cardioprotective activity, histamine release inhibition, cytotoxic activity (Beretta et al., 2009; Karthikeyan et al., 2007), antidiabetic and antiobesity bioactivities (Serrano et al., 2009). The estimated LD_{50} of the extracts at dose level of 187.5±62.5 and 175±75.0 mg/Kg bwt show that the seed extract is very toxic. This finding disagrees with the report of Saganuwan (2001), Saganuwan et al. (2016) indicating that the LD_{50} of aqueous leaf extract in mice was 2558.9 mg/Kg bodyweight.

The increased PCV caused by ethanolic extract agrees with the report of Saganuwan et al. (2011a) indicating that extract of *A. precatorius* have hemopoeitic activity. However, hyperhemoglobinemia and anemia show that the plant could cause hemolysis. Saganuwan et al. (2016) and Adedapo et al. (2007) had earlier reported that higher dose of *A. precatorius* extract can cause hemolysis in mice and rats respectively. The leucopenia observed in the present study may indicate immunomoinhibitory potential of the plant. Hypoproteinemia, hypoalbuminemia and low albumin-globulin ratio may suggest immunomodulatory activity of *A. precatorius* seed extracts. Our findings disagree with the report of Saganuwan and Onyeyili (2010), indicating that aqueous extract of *A. precatorius* could cause hyperproteinemia, hyperalbuminemia and high albumin-globulin ratio. Hypocholesterolemia observed in the present study agrees with the report of Saganuwan (2009) and Reddy et al. (2014) who

Table 5. The effects of methanolic and ethanolic extracts on hematological parameters of mice (*Mus musculus*)

Parameters	Treatment Groups		
	Control	Methanolic extract	Ethanolic Extract
PCV (%)	36.00±2.58	37.33±4.84	44.00±3.06[a]
Hb (gm/dL)	11.99±0.86	12.45±1.61[a]	14.67±1.02[a]
RBC ($X10^{12}$)	9.60±1.02	7.13±0.34[b]	6.37±0.60[b]
MCV (fL)	39.00±5.07	52.80±8.02[a]	70.97±10.87[a]
MCH (pg)	12.99±1.68	17.60±2.67[a]	23.66±3.62[a]
MCHC (gm/dL)	33.31±0.23	33.34±0.00	33.33±0.00
WBC ($X10^{9}$)	10.65±0.92	4.87±1.62[b]	8.93±5.26[b]

a= Data are significantly higher in comparison with the control
b= Data are higher significantly lower in comparison with the control P<0.05

Table 6. The effects of methanolic and ethanolic extracts on biochemical parameters of mice (*Mus musculus*)

Parameters	Treatment Groups		
	Control	Methanolic extract	Ethanolic Extract
Total protein(gm/dL)	8.95±0.75	4.63±2.68[b]	5.27±2.12[b]
Albumin (mg/dL)	4.70±2.15	0.73±0.22[b]	0.67±0.43[b]
Globulin (mg/dL)	4.25±1.40	3.90±2.46	4.60±1.69
Albumin: globulin	1.11	0.19[b]	0.15[b]
Cholesterol (mg/dL)	113.38±26.77	36.87±19.35[b]	15.87±6.95[b]
Creatinine (mg/dL)	0.40±0.15	0.90±0.17[a]	3.20±0.81[a]
ALT (u/L)	37.10±21.54	17.70±7.13	110.17±38.53
ALP (u/L)	98.03±30.86	123.67±57.09[a]	220.90±24.63[a]

a= Data are significantly higher in comparison with the control
b= Data are higher significantly lower in comparison with the control P<0.05

Table 7. The extrapolated effects of methanolic and ethanolic extracts on hematological parameters of Nigerian native dogs

Parameters	Treatment Groups		
	Control	Methanolic extract	Ethanolic Extract
PCV (%)	5.22±0.37	5.41±0.02	6.38±0.44[a]
Hb (gm/dL)	1.74±0.13	1.81±0.23[a]	2.13±0.15[a]
RBC ($X10^{12}$)	1.39±0.15	1.04±0.05[b]	0.92±0.09[b]
MCV (fL)	5.66±0.74	7.66±1.63[a]	10.29±1.58[a]
MCH (pg)	1.88±0.24	2.55±0.39[a]	3.43±0.53[a]
MCHC (gm/dL)	4.83±0.00	4.83±0.00	4.83±0.00
WBC ($X10^{9}$)	1.54±0.13	0.71±0.24[b]	1.29±0.76[b]

a= Data are significantly higher in comparison with the control
b= Data are higher significantly lower in comparison with the control P<0.05

Table 8. The extrapolated effects of methanolic and ethanolic extracts on biochemical parameters of Nigerian native dogs

Parameters	Treatment Groups		
	Control	Methanolic extract	Ethanolic Extract
Total protein(gm/dL)	1.30±0.11	0.67±0.39[b]	0.76±0.31[b]
Albumin (mg/dL)	0.68±0.31	0.11±0.03[b]	0.10±0.06[b]
Globulin (mg/dL)	0.62±0.20	0.57±0.36	0.67± 0.25
Albumin: globulin	0.16	0.03[b]	0.02[b]
Cholesterol (mg/dL)	16.44±3.88	5.35±2.81[b]	2.30±1.01[b]
Creatinine (mg/dL)	0.06±0.02	0.13±0.03[a]	0.46±0.12[a]
ALT (u/L)	5.38±3.12	2.57±1.03	15.98±5.59
ALP (u/L)	14.21±4.48	17.93±8.28[a]	32.03±3.57[a]

a= Data are significantly higher in comparison with the control
b= Data are higher significantly lower in comparison with the control P<0.05

reported that *A. precatorius* play a role in hypocholesterolemia by inhibiting the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, a key enzyme in cholesterol biosynthesis. This indicates that *A. precatorius* seed could be used in the management of diabetes and obesity. Hyperalkalemia may not be a sign of serum pathological effect. But hypercreatinemia observed in the present study may show toxic potential of the seed extract on kidney as earlier reported by Saganuwan and Onyeyili (2010) and Saganuwan et al. (2011b). However,

immunomodulatory potential of *A. precatorius* is dose-dependent (Saganuwan and Onyeyili, 2010).

Therefore, the seed extract of the plant could be used in the management of diabetes, obesity and hyperimmuno-stimulatory diseases such as systemic lupus erythematosus, psoriasis and inflammatory bowel disease by causing decreasing immune response.

CONCLUSION

Water and ethanol extracts of *A. precatorius* seeds contain alkaloids, flavanoids, saponins and tannins. The extracts are very toxic and may have immuno-inhibitory effects on hyper-immuned diseased conditions.

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CONFLICT OF INTEREST

The authors have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS' CONTRIBUTION

MTT carried out the study ,whereas HF and SAS designed the study and SAS analyzed the data. All the authors proof read the manuscript.

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