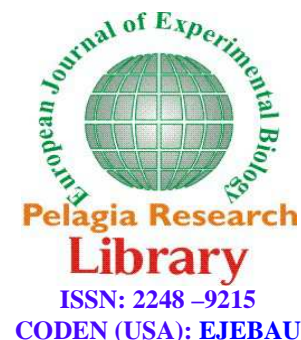




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Oral acute toxicity (LD50) study of different solvent extracts of *Abrus precatorius* Linn leaves in wistar rats

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ABSTRACT

Abrus precatorius leaves have been used in traditional medicine for the treatment of a variety of diseases including cough, malaria and infertility in women. It has also been shown to have various useful pharmacological effects. In this study, oral acute toxicity of the aqueous, 70% methanol, Petroleum ether and Acetone extracts were investigated. The graphical method of Miller and Tainter was used to estimate their LD50, using graded doses \leq 5000 mg/kg (oral limit dose). Mortality rate, body and organ weight changes were also measured in all treatment groups. Acetone extract had the lowest LD50 value (187mg/kg) indicating higher toxicity. The study also revealed that methanol was the least toxic solvent (3942mg/kg) used in the extraction. Histopathological results reveal pathological changes in the organs examined, revealing a possible hepatotoxicity, cardiotoxicity and nephrotoxicity of the extracts at the oral limit dose.

Keywords: *Abrus precatorius*, solvent, extraction, polarity, toxicity.

INTRODUCTION

Medicinal plants have increasingly become an integral part of the human society with regards to their therapeutic uses. Thus, phytomedicine research is now being promoted, as shown by the resolutions and recommendations given by the World Health Organization, which advocates the application of scientific criteria and methods for proof of safety and efficacy of medicinal plants. Particularly, in the AFR/RC49/R5 and AFR/RC50/R5 resolutions on Essential Drugs in the WHO African Region, member states were urged to encourage medicinal plant research and to promote their use in health care delivery systems [1]. Yet, safety and efficacy data are available for only a few plants. In the face of scarce information on the safety, efficacy and phytochemical characteristics of different compounds, it is difficult for drug companies to assess the potential utility or value of these compounds found in our rich indigenous plant resources.

Acute toxicity test is test in which single dose of the drug is used in each animal on one occasion only for the determination of gross behavior and LD50 (the dose which has proved to be lethal (causing death to 50% of the tested group of animals). It is usually the first step in the assessment and evaluation of the toxic characteristics of a substance. It is an initial assessment of toxic manifestations, providing information on health hazards likely to arise from short-term exposure to drugs [2]. The LD50 for a particular substance is the amount that can be expected to cause death in half (i.e. 50%) of a group of some particular animal species, usually rats or mice, when administered

by a particular route [3]. It is usually expressed as the amount of chemical administered (e.g. Milligrams) per 100 g (for small animals) or per kilogram (for bigger subjects) of the body weight of the test animal [4]. LD50 obtained at the end of a study is reported in relation to the route of administration of the test substance e.g. LD50 (oral), LD50 (dermal) etc. The most frequently performed lethal study is the oral LD50. Generally, the smaller the LD50 value, the more toxic the substance is and vice versa.

Abrus precatorius is one of the plants with a wide traditional medicinal use across different cultures, globally. It is a legume with long, pinnately compound leaves of the family name – Fabaceae. Its flowers are arranged in violet or pink clusters. The seed pod curls back when it opens and reveals the seeds [5]. The seeds are truncate shaped, 1.5-2cm long, with attractive scarlet and black color. It has slender branches and a cylindrical wrinkled stem with a smooth-textured brown bark. *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. The present study hereby investigates the oral toxicity (LD50) of different solvent extracts of *Abrus precatorius* Linn leaves in wistar rats.

MATERIALS AND METHODS

Plant Materials

The leaves of *Abrus precatorius* were collected from a farmland in Urualla, Ideato-North Local Government Area of Imo State. The botanical identification and authentication was done by Dr G.E Omokhua, of the Forestry and Wildlife Management, Faculty of Agriculture, and Dr N.L Edwin of the Department of Plant Science and Biotechnology, College of Applied and Natural Sciences, 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.

Equipment and Reagents

Photo Microscope (Olympus, Japan), Rotary Evaporator (Heildolph Instruments, Germany), Hand-held tissue homogenizer (Omni International), Mettler – toledo GmbH digital weighing balance (Type BD202, SNR 06653), Uniscope Laboratory Centrifuge (Model M800B, Surgifriend Medicals and Essex, England) are the instruments used. Syringes (1 mL, 5 mL), oral cannula, cotton wool, Heparinised and non heparinized sample bottles, capillary tubes, EDTA bottles, Microscopic slides (Olympus, China), hand gloves, Giemsa stain, Methanol, Acetone, Petroleum Ether, Silica gel (200 - 400 mesh), Picric acid, Tannic solution, Potassium mercuric iodide, Ferric chloride solution, Hydrochloric acid, Chloroform, Sodium hydroxide, Tetraoxosulphate (VI) acid and Dragendorff's reagent (Sigma Aldrich, St, Louis, MO, USA) were also used. Other chemicals such as sodium hydroxide, sodium nitrite, ferrous chloride, ammonium thiocyanate, aluminum chloride, potassium dihydrogen phosphate, dipotassium hydrogen phosphate manufactured by Merck, Germany were also used. All other reagents used were of analytical grade and were prepared according to specifications using appropriate solvents and distilled water.

Solvents

The toxic effects of 4 commonly employed solvents in the extraction process for medicinal plants were tested on wistar rats. The solvents used in the current study (in the ascending order of polarity) were petroleum ether, acetone, 70% methanol and water.

Phytochemical screening:

Qualitative phytochemical analyses of aqueous, 70% methanol, acetone and petroleum ether *A. precatorius* leaf extracts were conducted following the standard procedures [6].

Acute Toxicity Study

Rats of both sexes, in separate cages, were given a single oral limit dose of 5,000 mg/kg b.wt of the different solvent extracts while a control animal received distilled water. The treated rats were monitored for signs of toxicity and mortality at the first, second, fourth and sixth hour for immediate toxicity signs. Mortality observed in each group within 24 hrs was recorded.

Thereafter, depending on the level of tolerance of the limit dose, subsequent doses (less than the limit dose, if not well tolerated or greater than the limit dose, if well tolerated) were administered to rats of five per group. They were observed daily for an additional 7 days, for signs of delayed toxicity. The percentage mortality values were

converted to probit values by reading the corresponding probit units and plotted against log dose. Thus, the LD₅₀ was estimated graphically using the probit value that corresponds to probit 5 or 50 % [7].

The body weight of the rats was recorded before the treatment period, throughout the treatment period and after the treatment period. The group's mean body weights were also calculated. All surviving rats of the least three doses were fasted for 16-18 hours, and two rats from each group were then sacrificed for necropsy examination. The internal organs were excised and weighed. The gross pathological observations of the tissues were performed by histopathological examination.

Anesthesia was induced by administration of 1% chlorose in 25% urethane (w/v) (5ml/kg). Blood was collected from the heart for haematological and biochemical analysis. Internal organs were excised, prepared and weighed using Mettler – toledo GmbH digital weighing balance. Weight of excised organs was standardized to 100g per body weight of animals and the organs were then preserved in 10% formal saline for histo-pathological examination.

Histopathology

The liver, kidney and heart of all the animals were fixed in 10 % buffered formalin in labeled bottles, and processed routinely for histological examination. Tissues embedded in paraffin wax were sectioned 5 µm thick, stained with haematoxylin and eosin, mounted on glass slides and then examined under a standard light microscope.

Ethical Consideration

Ethical approval for the study was sought from the Research Ethics Committee of the College of Health Sciences, University of Port Harcourt. The study was conducted in accordance with the Organization for Economic Development guidelines on good laboratory practice and use of experimental animals [8].

RESULTS

Acute toxicity study for *A. precatorius* solvent extracts

The experimental rats treated with acute oral limit dose of 5000 mg/kg body weight of different solvent leaf extracts of *A. precatorius* displayed appreciable changes in physical activity and signs of apparent toxicity symptoms (fatigue, paw licking, watery stool, salivation, writhing and loss of appetite) and mortality up to 72 hours post treatment, indicating that the LD₅₀ of the crude extracts in rats is significantly less than 5000 mg/kg. Then, subsequent doses of the individual extracts were administered (240, 480, 960, 1920mg/kg).

LD₅₀ values were calculated by probit analysis within 95% confidence limits. The percentage mortality values are plotted against log-doses (Figure 1-4) and then the dose corresponding to probit 5, i.e., 50% was determined and the results are as shown in Table 3.

Table 1: Percentage Yield of Extracts

Plant Part Used	Weight of Plant Sample Used (g)	Solvent Type	Extract yield (%)
Leaves	700	Petroleum Ether	13.7g w/w
Leaves	681	Acetone	15.9 g w/w
Leaves	527	70% Methanol	18.4 g w/w
Leaves	300	Aqueous	16.1 g w/w

Table 2: Phytochemical constituents of extracts of *A. precatorius* leaves

Phytochemical constituents	Aqueous	70% Methanol	Acetone	Petroleum Ether
Alkaloids	-	+	+	+-
Glycosides	+	+++	++	+
Tannins	-	+	+++	+
Flavonoids	++	++	+	+
Saponins	+	+	+	+
Triterpenes	-	+++	-	+
Steroids	++	+	+	++
Gums and mucilage	-	-	-	-
Proteins	-	-	-	-
Starch	+	+	++	+
Fats and fixed oils	+	++	++	+++

Present = + Absent = -

Table 3: Results of LD 50 Dose Determination Following the Administration of *A. precatorius* solvent extracts

Solvents	Doses (mg/kg)	Log dose	Mortality rate	Mortality ratio	% Mortality
Aqueous	240	2.38	0	0/5	0
	480	2.68	1	1/5	20
	960	2.88	1	1/5	20
	1920	3.18	2	2/5	40
	5000	3.70	4	4/5	80
70% Methanol	240	2.38	0	0/5	0
	480	2.68	1	1/5	20
	960	2.88	1	1/5	20
	1920	3.18	2	2/5	40
	5000	3.70	3	3/5	60
Acetone	240	2.38	0	0/5	0
	480	2.68	2	2/5	40
	960	2.88	4	4/5	80
	1920	3.18	4	4/5	80
	5000	3.70	5	5/5	100
Petroleum Ether	240	2.38	1	2/5	40
	480	2.68	3	3/5	60
	960	2.88	3	4/5	80
	1920	3.18	5	5/5	100
	5000	3.70	5	5/5	100

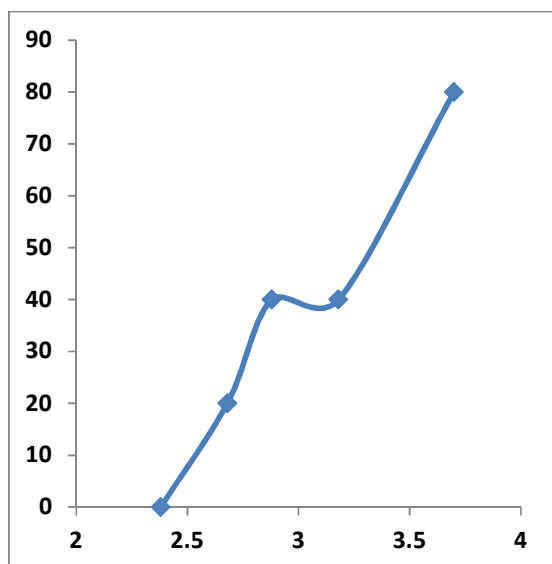


Fig 1

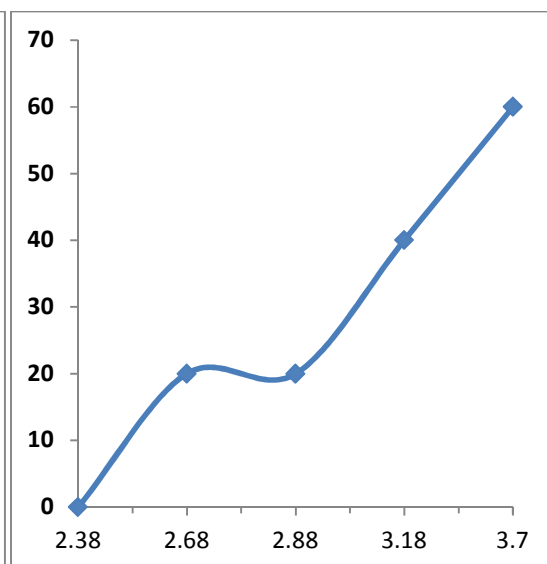


Fig 2

Table 4: Results of median lethal dose determination of *A. precatorius* solvent extracts

Solvent extracts	Log LD50	LD50 (mg/kg)
Aqueous	3.370	2,345
70% Methanol	3.596	3,942
Acetone	2.272	187
Petroleum ether	2.610	407

Effects of acute oral administration of *A. precatorius* extracts on body weights and organ weights

There were no significant differences in changes in calculated body weights of test animals compared to the control after acute administration of the aqueous extract. All animals in this group exhibited normal change in weight without a marked increase as shown in Table 5. Treatment with 70% methanol and Petroleum ether did not cause a net weight gain or loss, whereas the Acetone extract caused a drastic weight loss in test animals ($p < 0.01$).

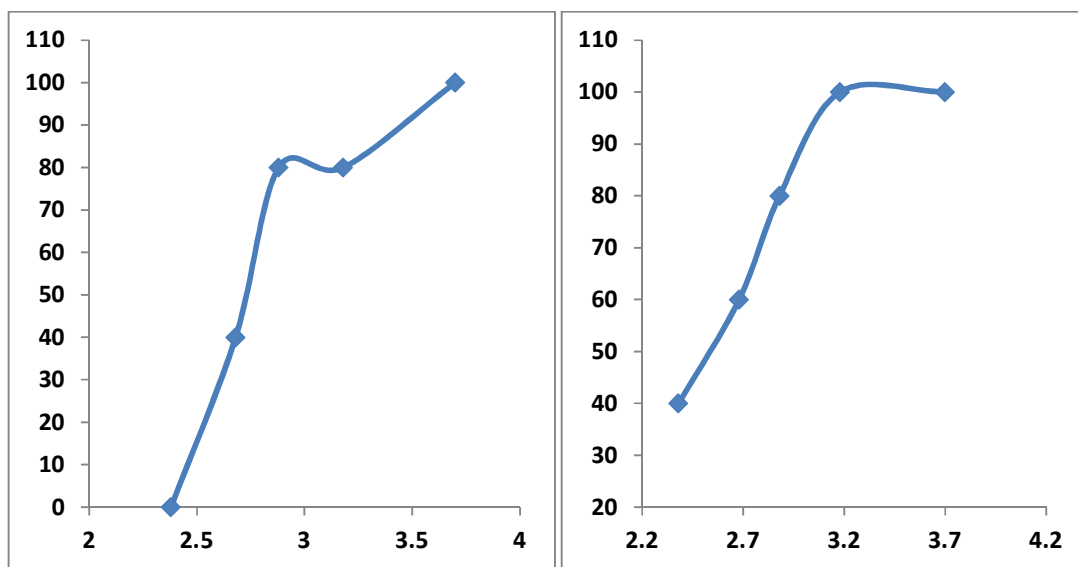


Fig 3

Fig 4

Figure 1-4: Plot of percentage mortality against log dose following oral administration of graded doses of different *A. precatorius* leaf extract. (Fig 1: Aqueous extract, Fig 2: 70% Methanol extract, Fig 3: Acetone extract and Fig 4: Petroleum ether extract)

Table 5: Body weight of experimental animals after oral acute administration of *A. precatorius* extracts

Extract	Weight (g)	Treatment groups/Doses					
		Control	240mg/kg	480mg/kg	960mg/kg	1920mg/kg	5000mg/kg
Aqueous	Initial	186 ± 4.0	185 ± 3.0	187 ± 7.0	186 ± 3.0	188 ± 8.0	187 ± 4.0
	Final	232 ± 1.0	226 ± 1.0	221 ± 5.0	216 ± 2.0	211 ± 3.0	208 ± 2.0
70% Methanol	Initial	185 ± 2.0	187 ± 2.0	188 ± 2.0	187 ± 2.0	187 ± 2.0	187 ± 2.0
	Final	234 ± 1.0	220 ± 1.0	213 ± 5.0	202 ± 2.0	198 ± 3.0*	196 ± 2.0*
Acetone	Initial	187 ± 2.0	185 ± 6.0	188 ± 3.0	187 ± 4.0	186 ± 6.0	188 ± 4.0
	Final	235 ± 4.0	190 ± 3.0*	184 ± 5.0*	178 ± 7.0*	174 ± 2.0*	166 ± 6.0*
Petroleum Ether	Initial	188 ± 3.0	187 ± 7.0	186 ± 2.0	189 ± 5.0	187 ± 3.0	189 ± 4.0
	Final	237 ± 7.0	201 ± 4.0	198 ± 6.0*	193 ± 4.0*	186 ± 8.0*	183 ± 3.0*

Data represents the Mean ± S.E.M for each group of rats, n = 5.

*p<0.05 = significant difference

Table 6: Organ weight of experimental animals after acute oral administration of *A. precatorius* leaf extracts

Extract	Weight (g)	Treatment groups/Doses					
		Control	240mg/kg	480mg/kg	960mg/kg	1920mg/kg	5000mg/kg
Aqueous	Heart	0.82±0.03	0.79±0.05	0.77±0.06	0.74±0.04	0.69±0.03	0.68±0.05
	Liver	6.67±0.14	6.55±0.13	6.47±0.11	6.39±0.14	6.37±0.14	6.21±0.11
	Left Kidney	0.59±0.03	0.57±0.04	0.57±0.02	0.56±0.01	0.57±0.07	0.56±0.03
	Right kidney	0.63±0.02	0.61±0.04	0.62±0.05	0.62±0.04	0.63±0.05	0.60±0.02
70% methanol	Heart	0.81±0.05	0.76±0.02	0.76±0.04	0.73±0.04	0.65±0.04	0.62±0.05
	Liver	6.63±0.12	6.65±0.06	6.62±0.06	6.57±0.06	6.52±0.05	6.34±0.18
	Left Kidney	0.61±0.07	0.58±0.07	0.57±0.07	0.54±0.06	0.49±0.07	0.48±0.03
	Right kidney	0.68±0.0	0.65±0.03	0.63±0.05	0.58±0.01	0.58±0.03	0.55±0.06
Acetone	Heart	0.83±0.04	0.73±0.04	0.64±0.02	0.57±0.04	0.51±0.04*	0.48±0.02*
	Liver	6.29±0.16	6.13±0.03	6.01±0.07	5.47±0.06*	5.22±0.02*	5.07±0.11**
	Left Kidney	0.66±0.0	0.56±0.02	0.55±0.03	0.49±0.06	0.49±0.02	0.48±0.04
	Right kidney	0.68±0.08	0.57±0.01	0.53±0.08	0.52±0.01	0.50±0.07	0.47±0.06
Petroleum Ether	Heart	0.84±0.05	0.72±0.05	0.63±0.05	0.62±0.04	0.56±0.08	0.55±0.04
	Liver	6.30±0.12	6.12±0.06	6.11±0.07	5.39±0.06	5.19±0.04*	5.02±0.16**
	Left Kidney	0.64±0.03	0.55±0.03	0.52±0.05	0.46±0.06	0.42±0.09	0.42±0.01
	Right kidney	0.72±0.01	0.63±0.03	0.61±0.05	0.58±0.01	0.54±0.09	0.51±0.02

Data represents the Mean ± S.E.M for each group of rats, n = 5.

*p<0.05 = significant difference; **p<0.01 = highly significant

Generally, there was weight loss in the vital organs examined ($p>0.05$) (Table 6). Marked shrinking of the liver were seen in Acetone and Petroleum Ether extract treated animals ($p<0.01$).

Histopathological examination: Histopathological examination of the liver, heart and kidney of *A. precatorius* leaf extract treated rats showed significant morphological differences, only at high doses of 5g/kg as shown in Figures 5 – 7 (H&E, $\times 300$). Treatment with acetone and petroleum ether extracts were profoundly more toxic to the rats' vital organs especially the liver as shown in the figures below. Generally, damage to organs was seen to be milder with the aqueous and 70% methanol extract treatment. On the other hand, severe vascular congestion, glomerulosclerosis, enlarged vascular glomeruli and oedema were observed in some cells of the tubular epithelium of the kidney of treated rats.

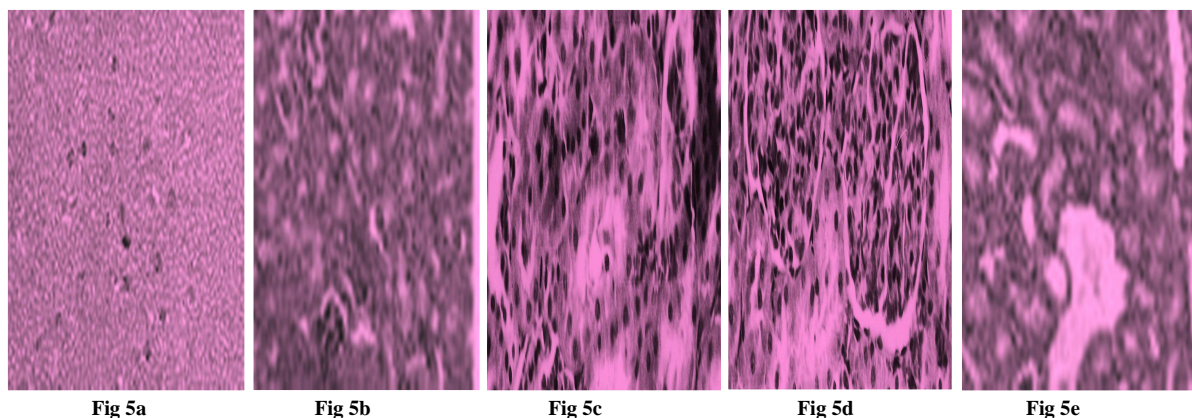


Fig 5a: Kidney of a Control Rat

Fig 5b: Kidney of an Aqueous Extract Treated Rat

Fig 5c: Kidney of a 70 % Methanol Extract Treated Rat

Fig 5d: Kidney of an Acetone Extract Treated Rat

Fig 5e: Kidney of a Petroleum Ether Treated Rat

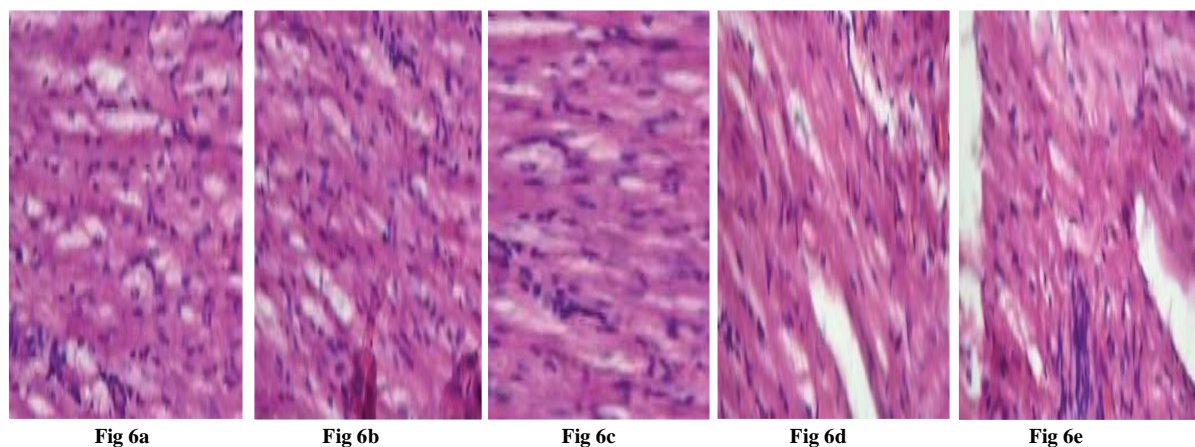


Fig 6a: Heart of a Control Rat

Fig 6b: Heart of an Aqueous Extract Treated Rat

Fig 6c: Heart of a 70 % Methanol Extract Treated Rat

Fig 6d: Heart of an Acetone Extract Treated Rat

Fig 6e: Heart of a Petroleum Ether Treated Rat

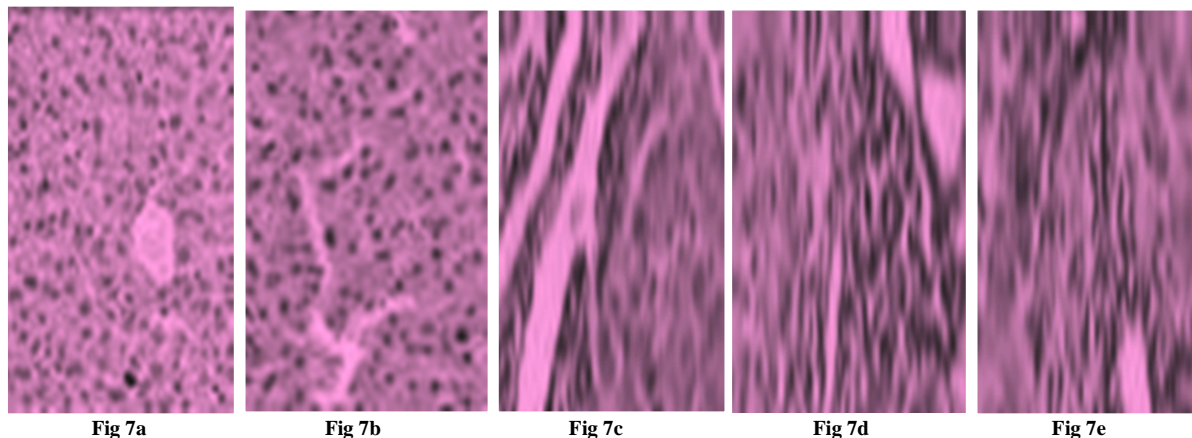


Fig 7a: Liver of a Control Rat

Fig 7b: Liver of a Aqueous Extract Treated Rat

Fig 7c: Liver of a 70 % Methanol Extract Treated Rat

Fig 7d: Liver of a Acetone Extract Treated Rat

Fig 7e: Liver of a Petroleum Ether Treated Rat

DISCUSSION AND CONCLUSION

The property of extracting solvents significantly affects the measured total phytochemical content. Solvent polarity is an important parameter that affects the yield of a plant material, thus the higher the polarity, the better the solubility of compounds such as phenols. In our investigation 70% methanol had a higher percentage yield more than the other solvents employed. Water is universal solvent, used to extract plant constituents. It is widely used by traditional medicine practitioners in extraction; however other organic solvents such as alcohols have been found to provide more abundant elements. Methanol is more polar than ethanol and was thus chosen for this study. By adding water to the absolute methanol up to 30%, the polarity of the solvent was increased [9], in addition to a possible dilution effect. Ether is commonly used selectively for the extraction of coumarins and fatty acids [10] while Acetone extraction favors more lipophilic than hypophilic substances. This is perhaps why in the present study more phyto-constituents were extracted using 70% Methanol than with Acetone and Petroleum Ether.

Saganuwan *et al* reported an estimated median lethal dose (LD₅₀) of 2559 mg/kg for aqueous extract of *Abrus precatorius* leaf [11]. This is similar to 2345 mg/kg LD₅₀ dose obtained from our toxicity studies. However, our study went further to provide estimated LD₅₀ values for 70% Methanol, Petroleum Ether and Acetone extracts as follows; 3942, 407 and 187mg/kg respectively. Thus evidently showing that 70% methanolic and aqueous extracts are safer than acetone and petroleum ether extracts.

The effect of acute oral administration of *A. precatorius* extracts on body weights and organ weights were also studied. From the present study, there was a slight increase in calculated body weights of test animals treated with the aqueous and 70% Methanol within the test period. However, Acetone and Petroleum ether treatment caused significant weight loss in the test animals. The body weight changes are indicators of adverse effects of drug or chemical and it is significant since the body weight loss was more than 10% from the initial weight in higher doses [12]. Organ weight also is an important index of physiological and pathological status in animals [13]. The relative organ weight is fundamental to diagnose whether the organ was exposed to the injury or not. Marked shrinking of the liver were seen in Acetone and Petroleum Ether extract treated animals, it may be due to the fact that the liver plays a central role in metabolizing and excretion of chemicals and is susceptible to the toxicity from these agents [14]. Little wonder then, that hepatotoxicity and drug-induced liver injury accounts for a substantial number of drug candidate failures in toxicity testing.

The photomicrograph of the heart, liver and kidney sections of the test animals (H & E x 300) showed obvious pathological changes, thus alluding to the toxic level of the extracts. The Acetone and Petroleum ether extracts were especially observed to be hepatotoxic and nephrotoxic at the oral limit dose of 5000mg/kg. However, this claim will be fully ascertained with further studies on the effect of treatment on enzymatic parameters.

In conclusion, this study has provided data on estimated LD50 of various solvent extracts of *A. precatorius* in rats; thus forming a basis for further studies on the sub-acute and chronic toxicity effect of this pharmacologically important plant. The information contained herewith also serves as a guide into choice of extracting solvents in the processing of medicinal plants.

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