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Acute and Sub-acute Toxicity Evaluation in Rats of PPOJ5 and ADOJ6 Herbal Remedies Used Traditionally in the Management of HIV Infection

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Authors' contributions

This work was carried out in collaboration between all authors. Author OJ designed the study, wrote the protocol, participated in animal care and treatment, performed the statistical analysis under the mentorship of author FB and wrote the first draft of the manuscript. Author KJM performed laboratory experimentation and analysis of samples and proof reading of the manuscript. Author NR participated in animal care and treatment, proof read both draft proposal and manuscript. Author TJK proof read the draft proposal and manuscript. Author LI participated in proposal development and writing of first draft manuscript. Author FB mentored the junior researchers on statistical analysis and proof read both the proposal and manuscript. Author AAG mentored the junior researchers on proposal writing, proof read both proposals and drafted the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The use of herbal medicine in the treatment of many ailments is on the rise. It's a common practice in many rural communities where access to health care is poor but also in the developed world. There is however, no much attention paid to the potential toxicity of these herbal products. This study was conducted to determine the toxicity of two herbal remedies; PPOJ5 and ADOJ6, being used for the management of patients with HIV. Both acute and sub-acute toxicity were evaluated using a rat model. Liver, renal and haematological parameters were measured. PPOJ5 was found slightly toxic with an estimated LD50 of 1.341 g/kg body weight and it significantly elevated lymphocyte count. ADOJ6 was safe in both acute and sub-acute toxicity studies. There is a need to evaluate the extracts of both PPOJ5 and ADOJ6 on isolated human Peripheral Blood Mononuclear Cells (PBMCs) to determine their safety level and possible immunostimulatory effects of PPOJ5.

Keywords: Acute; sub-acute; toxicity; herbal; HIV.

1. INTRODUCTION

The quest for new drug leads has refocused the attention of many researchers to natural products [1,2]. In many parts of the world, the use of herbal medicine for the treatment and management of many conditions has also increased to 80% [3], 21% use of herbal medicine by hypertensive patients in South Africa [4], 72.6% use out of 372 respondents surveyed in Jamaica [5] and 33.7% use among HIV/AIDS patients on Highly Active Antiretroviral Therapy (HAART) in Uganda [6]. This increase has however, focused on the efficacy with little attention being paid to the potential toxicity that many herbal medicines may possess. Toxicological studies in the form of acute, sub-acute, sub-chronic and chronic are a requirement for many products used as medicine. Acute toxicity refers to toxic effects manifested in an animal after the administration of a single or multiple dose(s) of a substance in a period not exceeding 24 hours, up to a limit of 2000 mg/kg. The animals under test are normally observed for up to a period of 14 days for delayed toxic effects resulting from the effects of single or multiple doses administered within a 24 hour period. The observation normally involves recording of behaviours, body weight, duration and reversibility of the toxic effects [7,8]. In the determination of the toxic characteristics of a substance, an acute toxicity test is normally performed. The route of administration has to be reflective of the intended route for use in humans. For substances intended to be used as drugs, several tests are normally conducted including acute, sub-acute, sub-chronic and chronic among others. Acute toxicity testing involves the estimation of the lethal dose in 50 percent of test animals, commonly referred to as the LD50 [9,10]. For products destined for human use, results of acute toxicity studies are required by regulatory bodies for the purpose of registration. The same results have also been traditionally used to set appropriate dose levels for repeated toxicity studies in animals and to support the effects of overdose in humans. The same results can also be used to support doses for First-in-Man (FIM) studies and give early indication of target organ toxicity [9]. Sub-acute toxicity refers to effects of drugs or toxic substances that manifested over a period of 14 days. It is the shortest form of a repeated dose toxicity study in the investigation of medicinal products for their safety following exposure for that period. Effects on organ functions, haematological indices and behavioural patterns, weight and reversibility of toxic effects that may manifest following brief exposure are also investigated during sub-chronic exposure.

The use of herbal products has been reported to stand at about 25% worldwide [11]. In the less developed countries, the use of herbal products for the treatment of various conditions is even greater as it is the cheapest, easily available form of medicine to the majority of the population. It is thus imperative that in such areas, studies are conducted on the efficacy of those products and most importantly safety studies as the products are in wide use because of their perceived efficacy and thus safety is not of concern. This study was conducted primarily on two medicinal plants, PPOJ5 and ADOJ6 which are being used for the management of persons with HIV/AIDS by a traditional healer. Major areas of concern were acute toxic effects and sub-acute toxicity, focusing on the haematological and biochemical parameters.

2. MATERIALS AND METHODS

2.1 Research Design and Site

This was a short term experimental study for 16 days. The research was conducted at the

Pharmaceutical chemistry laboratory and Animal Research Facility of Mbarara University of Science and Technology from June to July 2015.

2.2 Requirements

Wistar albino Male rats (12 for acute toxicity study and 48 for the subacute toxicity study), plant extracts (PPOJ5 and ADOJ6), Blender (Bajaj, India), Funnels, Filter papers (Whatman No. 1, England), heating source (locally fabricated electric heating coil), Boiling Saucepan, Sieves, Muslin cloth, Animal cages, Glass observation Cages (Locally fabricated), laptop with web cameras (HP), syringes, oral cannulas, Insulin syringe, gloves, BD Vacutainers (heparinised and non heparinised), dissecting kits, laboratory oven (……) and laboratory record book.

2.3 Animal Procurement and Care

Male rats weighing between 140 to 180g were procured from Kampala International University Western Campus Animal Facility and used in the study. The animals were brought to the animal research facility at Mbarara University of Science and Technology and acclimatised for 2 weeks before using them in the experiment. They were treated humanely as per the National Academy of Sciences guidelines [12]. They had free access to food and water, kept in groups of 6 per cage and the cages were cleaned twice a week. Each cage was lined with soft wood shavings. At the end of the treatment period, the animals were anaesthetized using high dose ketamine in combination with diazepam before dissecting and collecting blood sample by cardiac puncture.

2.4 Collection and Processing of Plant Materials

The plant materials were collected fresh from the traditional healer, transported to the laboratory, shade dried for two weeks and ground to powder form for extraction. The plant materials could not be taxonomically identified as the traditional healer did not provide us the other required part for identification. This was because of his security purpose and had communicated to the researcher who did the collection as precondition for providing the plant materials to us. Care was however taken to ensure that the materials were not contaminated with any other plant product or a synthetic drug by the traditional healer.

2.5 Extraction and Drying

All plant materials were extracted by warm maceration as directed by the traditional healer, but in a laboratory setting. The macerate was filtered using a muslin cloth and finally using Whatman filter paper number 1 and dried in an oven (Mermmet®) at a controlled temperature of 40℃.

2.6 Phytochemical Screening

Phytochemical screening was done qualitatively following the methods described in Trease and Evans Pharmacognosy [13] in which positive colour changes as expected indicated the presence of the tested phytochemical and the reverse was true. All chemicals used to prepare the test reagents were of analytical grade.

2.7 Acute Toxicity Study

Acute toxicity studies for both extract were conducted following the Lorke's method [14]. In this method, 3 animals were tested using geometrically increasing doses of both PPOJ5 and ADOJ6. The highest dose for extract not killing any animal and the lowest dose that killed the animal were noted and used to calculate the Median lethal dose in 50 percent of the rats (LD50). Other signs of toxicity were also noted and recorded.

2.8 Sub-acute Toxicity Study

Sub-acute toxicity study was conducted for 14 days in which animals were divided into 3 groups of 6 animals each. Only animals 3-4 month old, weighing between 140 g to 180 g were selected for the study. The groups were assigned as A control group, B low dose level, C medium and D high dose level. Group A received 2 ml of distilled water each day, Group B received extract at 100 mg/kg, group C at 200 mg/kg and group D at 400 mg/kg. On the 15 day blood samples were obtained from all the animals and put in both heparinized and non-heparinized Vacutainers for analysis. Thereafter all animals were sacrificed humanely.

2.9 Data Analysis

Data from the study was analysed using graph pad prism software version 5 and was considered statistically significant at $P<0.05$. The data was analysed using one way analysis of variance (ANOVA) followed by Turkey multiple comparison test.

3. RESULTS

3.1 Phytochemical Screening

Phytochemical screening was conducted following methods described by Trease and Evean's Pharmacognosy.

Table 1. Phytochemical screening results

Phytochemical	PPOJ5	ADOJ6
Alkaloids	+	+
Flavonoids	+	-
Saponins	+	+
Tannins	+	-
Steroids	-	+
Terpenoids	+	+
Protein	+	-
Amino acids	+	-
Glycosides	+	+
Cardiac glycosides	+	+
Reducing sugars	+	+
Phenolic compounds	+	+

Key: +=Present, - = Absent

3.2 Acute Toxicity

PPOJ5 treated animals were noted to show gnawing from about 10 minutes after administration, redness at the tip of their nose, unable to eat properly and were drinking water poorly; the animals became weak and lethargic. At the dose of 3000 mg/kg, after 5 hours of observation, one animal went into coma and died in less than 6 hours post administration. The next day in about 18-19 hours, all the animals were found to have died in the night. Further reduction in dose levels produced the highest dose not killing at 1200 mg/kg and 1500 mg/kg as the lowest dose that killed all the three animals. The LD50 was then calculated and estimated at 1341 mg/kg (1.341 g/kg).

ADOJ6. Did not cause any mortality up to 5000 mg/kg and the test was terminated and LD50 considered to be above 5000 mg/kg.

3.3 Sub-acute Toxicity Study

The Table 2 indicates that aqueous extract of PPOJ5 significantly increased lymphocyte count in the experimental animals. There was no significant difference in the effects of ADOJ6.

Table 3 indicates that the extract of PPOJ5 had no significant effects on both the liver and renal function.

4. DISCUSSION

A number of phytochemicals were detected in the extract of both PPOJ5 and ADOJ6 (Table 1). Saponnins were especially found in high quantity

Table 2. Haematological test results

Parameter	Results			
	Control	100 mg/kg	200 mg/kg	400 mg/kg
WBC (10^3/µL)	7.22±0.7	6.98±1.3	10.44±0.6	4.98±0.5
NEU (%)	7.22±0.7	6.18±0.8	7.42±0.8	5.12±0.4
LYM (%)	68.28±1.6	85±1.9***	82.5±3.8**	92.7±0.6****
MO (%)	3.18±0.5	3.78±0.3	3.44±0.4	2.58±0.3
EO (%)	0.2±0.07	0.2±0.1	0.22±0.1	0.14±0.02
BA (%)	0.82±0.2	1.24±0.4	1.2±0.4	0.66±0.1
RBC(10^6/µL)	7.8±0.2	7.96±0.4	7.59±0.5	7.07±0.2
HB (g/dL)	14.68±0.5	15.36±0.5	14.4±0.8	14.04±0.3
HCT (%)	40.66±1.1	42.72±1.8	39.64±2.3	42.4±2.1
MCV (fL)	51.8±0.4	53.6±0.9	52.2±0.4	52.18±0.6
MCH (pg)	18.84±0.1	19.4±0.4	19±0.3	19.88±0.3
MCHC (g/dL)	36.58±0.07	36.06±0.3	36.28±0.3	37.04±0. 6
PLT (10^3/µL)	6.22±0.1	6.24±0.2	6.56±0.2	5.92±0.1

*Key: * = significance, number of * indicates level of significance at P=0.05, WBC=White blood cell, NEU=Neutrophils, LYM=Lymphocytes, MO= Monocytes, EO= Eosinophils, BA= Basophil, RBC = Red Blood Cell, HB = Haemoglobin, HCT = Haematocrit, MCV = Mean Cell Volume, MCH = Mean Cell Haemoglobin, MCHC = Mean Cell Haemoglobin Concentration, and PLT = Platelet*

Table 3. Liver and renal function studies

Parameter	Results			
	Control	100 mg/kg	200 mg/kg	400 mg/kg
ALT (u/l)	34.8±3.1	37±3.1	32.2±4.1	33.4±3.8
AST (u/l)	30.8±2.4	32.4±1.5	33±2	31.8±2.3
Urea	36.6±1.6	31±4.6	32±1.3	32.8±1.1
Creatinin	0.32±0.1	0.36±0.1	0.37±0.1	0.4±0.1

Key: ALT = Alanine Aminotransferase, AST = Aspartate Aminotransferase

in the extract of PPOJ5. Available reports indicate that saponnins are toxic and also that they have immunostimmulatory effects [15]. Other phytochemicals such as flavonoids, tannins and proteins were only found in the extract of PPOJ5 and not in the extract of ADOJ6. Several studies have reported the potential toxicity of flavonoids [16].

The results of the acute toxicity study have indicated the estimated LD50 of aqueous extract of PPOJ5 at about 1.341 g/kg body weight. These results put the extract of this herb at the slightly toxic and slightly irritating category [17]. It is thus important that this plant extract be used with caution. ADOJ6 on the other hand was safe as per the toxicity rating categorization [17]. It is thus not clear if the toxicity of PPOJ5 could be attributed to some of the phytochemicals found in its aqueous extract and not in the extract of ADOJ6.

Several phytochemicals have been reported to have both toxic and beneficial effects. A 14 day toxicity study in this research did not show any significant toxicity of the two herbal extracts; PPOJ5 and ADOJ6. It was however noted that, there was a significant increase in the lymphocyte count in the animals treated with the extract of PPOJ5 in comparison to those in the control group (Table 2). A possible indication that the extract could be having an immunomudulating activity. [15,18,19,20] reported that saponnins possess immunostimulatory activity and since saponnins were found in high quantity in the extract of PPOJ5, they could be responsible for the increase in the lymphocyte count in the groups treated with PPOJ5. This plant extract thus requires evaluation for their immunostimulatory or antiretroviral activity. The extract did show any significant effects on both liver and renal function (Table 3). However, there was an increase in the levels of AST which was not dose dependent. While for the case of renal function, there was an increase in creatinine levels which was dose dependent. The increase in both AST and creatinine levels independent of ALT and urea did provide enough evidence to show that the extract of PPOJ5 is toxic to the liver and the kidneys. This is an indication that the dose levels used in this experiment are safe.

5. CONCLUSION

The extract of PPOJ5 was found slightly toxic and increased lymphocyte count in the experimental animals. The extract may be helpful in the management of HIV by stimulating the immune system. Further studies are thus needed on its possible immunostimulatory activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was sought from Mbarara University Research and ethics Committee (MUST-REC) and the Uganda National Council for Science and Technology (UNCST).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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