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Research Article

Acute toxicity studies of *Catharanthus roseus* aqueous extract in male Wistar rats

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Background: The knowledge of the healing virtues of *Catharanthus roseus* and a host of other medicinal plants has been in existence since ancient times. *Catharanthus roseus* is traditionally used more commonly as anti-diabetic and anti-malaria remedy. Lack of adequate pharmacological and toxicological data of natural products to support their use is a major setback.

Objective: To establish the acute toxicity effect of *Catharanthus roseus* crude aqueous extract on some hematological and biochemical parameters.

Methodology: *Catharanthus roseus* aqueous extract was orally administered once to two groups of male rats at 1000 and 5000 mg/kg body weight respectively alongside a control group which received 2ml distilled water. Hematological and biochemical assays were done at 48 hours and the 14th day. The data was analyzed using SPSS 17.0.

Results: White blood cells (WBC), creatinine, urea, alanine aminotransferase and aspartate aminotransferase showed significant increase while mean cell volume reduced significantly at 48 hours in the high dose group. The body weight change was also significantly reduced.

Discussion: The alterations of the body weight gain, various biochemical and hematological parameters reflect the effect of toxicity after exposure of the tested extract doses. Total proteins concentration was not altered suggesting that the renal and liver functions were not adversely affected.

Key words: *Catharanthus roseus*, acute toxicity, rats

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1. Introduction

Natural products remedies are believed to be safer and less damaging to the human body than the synthetic drugs (Alam et al, 2011). However, their safety has continually been questioned due to reported illness and fatality of the test animals (Park et al, 2010). Little pharmacological and toxicological data is available for most common such remedies (Fragoso et al, 2008). It is therefore important to determine the safety of these remedies in order to support their use.

Acute toxicity is an initial screening step required in the toxic assessment and evaluation characteristics of all biological compounds (Akhila et al, 2007) and it establishes the median lethal dose (LD_{50}) of a substance (Robinson et al, 2007). Acute toxicity is usually defined as the adverse changes occurring immediately or a short time following a single exposure to a substance within 24 hours (OECD, 2000). An adverse effect results in functional impairment and/or biochemical lesions and affects the performance of the whole organism (Rhodes, 1993). Furthermore, the information on acute systemic toxicity generated by the test is used in hazard identification and risk management in the context of production, handling, and use of pharmacological products (Leahy, 1997). Regulatory safety assessment for natural products relies on both the assessment of

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cases of adverse reactions and the review of published toxicity information (De Smet, 1995).

Catharanthus roseus is a renowned medicinal plant, and is a rich source of alkaloids, which are distributed in all parts of the plant (Sing et al, 1997). It has traditionally been used to treat diverse ailments such as eye inflammations, rheumatism and diabetes. Among the Luo community in Kenya, *C. roseus* (rosy periwinkle) is used as an antimalarial and antidiabetic remedy (Kokwaro, 1976). Two of the dimeric alkaloids vinblastine and vincristine mainly present in the aerial parts, have found extensive application in the treatment of human neoplasm. Among the monomeric alkaloids ajmalicine (raubacine) found in the roots has been confirmed to have a broad application in the treatment of circulatory diseases, especially in the relief of obstruction of normal cerebral blood flow (Aslam et al, 2010). *C. roseus* exhibits high in vitro antiplasmodial activity, which may be due to the presence of compounds such as alkaloids, terpenoids flavonoids and sesquiterpenes that were previously isolated from the plant (Jaleel and Panneerselvam, 2007; Collu et al, 2001; Vimala , 2001; Hirose and Ashihara, 2004)

Reported indications of *C. roseu*s extract include cytotoxic effect on cell division and malfunctioning of the nerves which control digestion, cardiac and sexual functions (Lobert et al, 1997; Alexandrova et al, 2000). Therefore the impairment of vital organs by the crude extract of this plant when consumed cannot be ruled out. *C. roseus* alkaloids toxicity has previously been reported (Rosazza et al, 1992). This showed selective reversible inhibition of monoamine oxidase-B (MAO-B) that is important in the biotransformation of xenobiotic. Isolated alkaloids have also been indicated in neurotoxicity and bone marrow depression (Barnett et al, 1978).

2. Materials and Methods

2.1 Sample collection and authentication

Catharanthus roseus leaves were collected from the flower gardens of the University of Nairobi. Kenya. Identification and authentication was done at the herbarium, School of Biological Sciences, University of Nairobi. A voucher specimen (ZNK/2015/01) was deposited in the herbarium.

2.2 Extraction procedure

The *C. roseus* leaves were cleaned and rinsed with distilled water and air dried at room temperature (22-26°C) to a constant weight. The dried leaves were ground to a uniform powder using an electric mill. The powder (100 g) was soaked in 1L distilled water for 48 hours. The mixture was filtered through cotton wool and then with filter paper (125 mm). The filtrate was frozen at -20° C for 24 hours followed by freeze drying. The powdered extract was weighed into air tight polythene bags and stored sealed in the refrigerator at 4° C and used within five days.

2.3 Laboratory animals

Male Wistar albino rats (205 – 225 g) were obtained from the animal house of the department of Biochemistry, University of Nairobi and housed in the research room of this department. The room was well ventilated and maintained on light for 12 hours and 12 hour darkness. Temperatures were maintained at 27–30 °C. The rats were provided with the standard rat pellets and clean water *ad libitum*.

2.4 Acute toxicity study

The animals were randomly assigned into three groups of 5 rats each and kept overnight fasting prior to extract administration. Group 1 served as the control and the rats were orally administered with 2ml distilled water. Two concentrations of the *C. roseus* aqueous extract; 1000 and 5000 mg/kg body weight were constituted each in 2ml distilled water and orally administered to groups 2 and 3 respectively through a rat gavage. Food was withheld for further 3 hours.

The rats were observed after every 30 minutes post extract administration for the first 2 hours and latter once a day up to the 14th for changes in skin and fur, eyes and mucus membranes, behavior pattern, tremors, salivation, diarrhea, sleep, coma, mortality, moribund, ill health or any visible reaction to treatment. Weight recording was done before extract administration, at 48 hours, day 7 and day 14 using a sensitive balance.

2.5 Animal bleeding, hematological and biochemical assays

Blood from each rat was collected at 48 hours and the 14th day post extract administration via the tail lateral vein using a 2 ml hypodermic syringe and needle when the animal was restrained. A blood aliquot (1.3 ml) was put into EDTA tubes and thoroughly mixed for hematological analysis. The remaining 0.5 ml was put in plain tubes for biochemical assays.

Hemoglobin concentration (Hb), packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), mean corpuscular hemoglobin (MCH), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and thrombocytes (THROMB) were analyzed using hematological analyzer (Melet Schoesing MS4, France) within six hours of blood harvesting. The blood in the plain tubes was centrifuged at 3000 revolutions per minute (rvm) for 10 minutes to extract serum. The serum was kept at -20 °C awaiting analysis.

UV-Vis spectrophotometer (UVmini-1240, Shimadzu) was used for biochemical assays. Total proteins, ALAT and AST were assayed using 'Human' commercial kits coded EN-GPTU INF122120D for the GPT enzymes and SU-PROT INF 157001GB for total proteins. EMEKYN urea kit and EUROCHEM creatinine kits (Euromed, UK) were used for the assay of urea and creatinine respectively. The manufacturer's protocol was followed.

2.6 Statistical Analysis

The data was analyzed using SPSS 17.0 and the results were expressed as mean ± standard deviation of the mean (SD). One-way analysis of variance (ANOVA) was employed for between and within group comparison. 95 % level of significance ($p \leq 0.05$) was used for the statistical analysis.

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2.7 Ethical Considerations

The animal studies were in compliance with the ethical procedure for the care and use of laboratory animals approved by the Animal Care and Use Committee (ACUC) of the Faculty of Veterinary Medicine University of Nairobi.

3. Results

3.1 Cage side observations:

None the 15 rats died during the experimental time and therefore the median lethal dose (LD_{50}) of the aqueous extract in Wistar rats was estimated to be ≤ 5000mg/kg/body. The rats fed with 5000mg were found to have limited morbidity for the first 6 hours but latter normalized.

No other clinical sign was observed. The general behavior of the rats was found to be normal throughout the study period.

3.2 Weight, hematological, biochemical changes

The two treatment groups showed very significantly reduced total weight gain in comparison with the non-treated group, as shown in **Table 1**.

White blood cells (WBC) had been significantly elevated at 48 hours for the 1000 mg/kg group and at 14th for the 5000mg/kg group. Mean cell volume mean (MCV) at 48 hours was reduced significantly for the 5000mg/kg group but latter normalized at day 14 (**Table 2**). All the other tested hematological parameters were not significantly different from the control. Table 2 shows that the 5000mg/kg treatment group had significantly high ALAT, P< 0.05 at day 14. The mean AST at 48 had no significant difference with the control, but at day 14 there was significant increase in the enzyme levels in the two treatment groups. A significant increase in the mean creatinine was recorded at 48 hours testing in 5000mg/kg group while the mean urea in the same group was significantly high. Mean total proteins was not significant different from the control in all the testing.

Table 1: Weight profile of the male Wister rats in *C. roseus* acute toxicity testing

	Control	1000mg	5000mg
Initial weight	217.4±9.4	222.7±10.0	217.3±9.0
At 48 hours	219.2±8.9	220.5±9.5	214.5±10.4
7 days	226.9±8.7	228.2±9.5	220.8±7.2
14 days	239.2±8,6	235±8.2	226.7±6,5
Total gain	22±1.5	*12.3±2.9	*9.4±3.3
% weight gain	8.5	*4.6	*3.6

** Significantly different from the control*

Table 2: Effect of *C. roseus* aqueous extract on hematological and biochemical measurements in male Wister rats after 48 hours and at day 14

Parameter	Control 48 hr.	Control day 14	1000 mg 48 hr.	1000 mg day 14	5000 mg 48 hr.	5000 mg day 14
WBC (10^3/µl)	10.1 ± 2.7	9.1±.34	*29.0 ±12	14.1±1.4	12.6 ±.27	*15.7±1.8
RBC (10^6xµl)	5.6 ± .22	5.7± .57	5.25 ±.1	5.8±.43	6.7 ±.20	6.3±.32
PCV (%)	35.1 ± 1.5	35.2± 3.1	35.6 ±1.5	36.3±2.0	38 ±1.2	38.2±.76
Hb (g/dl)	14.0 ± .81	12.8± 1.5	14.4 ±.39	14.8±.83	14.7 ±.42	14.9±.46
MCV(fl)	63.9 ± .60	63± 1.4	66.1 ±2.7	64.1±2.4	*22.9±13	61.2±24.2
MHC(pg)	24.0 ± 1.2	23.8±. 82	27.3 ±.59	25.7±1.4	22.1 ±.20	24.2±.92
MCHC (g/dl)	38.5 ± .91	38± 1.1	41.6 ±2.4	40.2±.63	38.2 ±1.2	38.7±.82
Thromb. (10^3/µl)	336.2±59.8	386± 20	208.2 ±2.4	398±60	347 ±78.6	361.6±11.4
AST (U/L)	45.6± 15.1	45.6± 15.1	43.4±10.1	43.7±6.1	53±8.0	43.8±7.3
ALAT(U/L)	46.4 ±2.4	46.4±2.4	42.9±4.4	38.6±8.5	*27.4±4.6	*58.1 ±4.3
T. proteins (g/dl)	7.7± 5.2	7.7±5.2	5.9±1.6	8.0±1.1	6.2±1.1	7.9±1.0
Creatinine(mg/d)	1.7± .36	1.7±.36	1.7±.65	1.3±.50	1.5±.64	*16.6±3.7
Urea (mg/dl)	42.9± 3.7	42.9±3.7	*70.6±15	42.0±7.2	29±6.3	*56.1±10.0

** Significantly different from the control*

4. Discussion

C. roseus is a well-known medicinal plant and is widely used in the Kenya ethnomedicine (Kokwaro, 1996). The current study used water solvent for crude extraction justifying the ethnomedical practice with the traditional healers (Johns et al, 1990).The median lethal dose (LD_{50}) estimated to be more than 5000mg/kg/body weight in the Wistar rats is regarded as in safe category of drugs (OECD, 2000). Previous studies showed that 4000mg/kg body weight of aqueous *C. roseus* extract orally administered to mice did not cause adverse effects to these animals (Chattopadhyay et al, 1991). The altered weight gains implied narrow safety margin and therefore this extract should be used cautiously. It was suggested that the tested doses could have interfered with the normal metabolism consequently affecting the uptake of food (Chokshi, 2007; Kevin et al, 2012)

The WBC in 1000mg/ kg group at 48 hours recorded a significant increase and then normalized at day 14, tables 2, suggesting that the extract might have caused an interference in the production of lymphocytes The mean cell volume (MCV) is an index of the size of the RBC. The low MCV reported is an implication of pathological changes of the kidney or a micryotic anemia (Chernecky et al, 2001). In this study the toxic effect was produced only at the higher dose and at 48 hours.

Elevated ALT and AST at day 14 agrees with James et al, (2007), where these enzymes were significantly elevated in rabbits fed with aqueous extract of *C.roseus*. Alanine aminotransferase, a cytoplasmic enzyme, is found in very low concentration in the liver and is released into the plasma following hepatocellular damage. Therefore this study suggested that this extract could possesses hepatotoxic effect. Similar observation was made by Pinkerton et al *(*1988) who tested a continuous infusion of *C. roseus* alkaloid Vincristine and found a transient increase in liver enzymes.

Elevated creatinine and urea levels, table 2, suggested a possible dysfunctioning of the kidneys. These two biochemical metabolites are critical and sensitive indicators of kidney function (Obidah et al, 2009). Earlier studies found an increment tread of these compounds in mice treated with of *C. roseus* leaves extract (James et al 2007*).*

The total proteins content in the treatment groups did not show any significant difference with the control group, an implication that the liver and renal functions were not adversely impaired (Kachmar and Grant, 1982).

5. Conclusion

According to the current findings, the use of *C. roseus* extract as an infusion for disease remedy may be well tolerated since there was no mortality or sever adverse effects of the test animals hence supporting the therapeutic use of this plant. However there is a risk of renal- hepato- and hematopoeitic toxicity at the tested doses. The study recommends the use of lower concentrations than 1000mg/kg in order to increase the safety margin. Safety measures that include monitoring of the vital serum enzyme and hematological parameters are recommended when this extract is being administered. Comprehensively screening for possible toxicity on sub-acute, sub-chronic and chronic levels was also recommended. The findings of this study provide basis for the selection of doses for use in long-term toxicity studies.

Conflict of Interest declaration

The authors declare no conflict of interest.

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