

Sub-lethal assessment of aqueous dried leaf extract of *catharanthus roseus* (linn.) g. don in male albino rats

Abstract

Catharanthus roseus (Linn.) G. Don is one of the famous plants used in folk medicine for the treatment and management of many forms of diseases and infections. The ubiquitous nature of this plant sprang up the interest to ascertain the sub-lethal toxicity of aqueous dried leaf extract of the plant in male albino rats. Thirty two (32) male albino rats were used in the study and were broadly divided into two: twelve (12) rats were used for acute toxicity study while the remaining twenty (20) rats for sub-acute toxicity study. Median lethal dose (LD₅₀), body weight, relative organ weight, haematological indexes and liver function enzymes were all determined after fourteen (14) days oral administration of the plant extract using standard analytical methods. This research disclosed that there was no mortality throughout the period of both studies even after the administration of the highest dose (10,000mg/kg body weight) of the extract. Similarly, it was observed that most of the examined indexes revealed dose dependent variations and there were no significant differences when compared to the control animals at (p<0.05) level of significance. Furthermore, the effects of the extract in the activities of hepatocellular enzymes (ALT, AST and ALP) and haematological indexes (MCV, WBC and Platelets) were found to be concentration dependent. This suggests that the administration of the aqueous extract of *C. roseus* could trigger off hepatocellular damage and haematological disorder if taken in large doses.

Keywords: *catharanthus roseus*, aqueous leaf extract, lethal dose, haematologic indexes, hepatocellular enzymes

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Abbreviations: RBC, red blood cells; PCV, packed cell volume; MCV, mean corpuscular volume; MCHC, mean cell hemoglobin concentration; MCH, mean corpuscular haemoglobin; HB, hemoglobin; WBC, white blood cells

Introduction

The complete dependence on orthodox medicine by mankind to balance the biological doshas is significantly losing firm grip by the day, and this has been observed over the years to be as a result of population size, inadequate supply of orthodox drugs, prohibitive treatment and toxic effects of several synthetic drugs.¹ The introduction of herbal medicine to bridge this established therapeutic gap has renewed man's hope for survival and longevity. Medicinal plant; an essential ingredient of herbal medicine, is any plant or group of plants which confers good health benefits to man when religiously used. These plants have remained man's friend; thus providing food, medicine and shelter.² The essentiality of plants and its products as panacea to ailments is not unconnected with the following benchmarks: bioaccumulation of phytoconstituents otherwise known as Phytochemicals, safety margin, less toxic, affordability, more compatible with the human body, reduced side effects and ability to target biochemical pathways.³⁻⁶ This is in consonance with the observation of the World Health Organization that about 80% of the world's population depends on medicinal plants to treat and manage infections and diseases; and in Africa, this rate is much higher.⁷

One of such plants used in the world today for relieving ailments is *Catharanthus roseus* (Linn.) G. Don. 1838 *Catharanthus roseus*, an

ornamental plant of the family Apocynaceae and order Gentianales, was previously referred to as *Vinca rosea* and commonly called Madagascar periwinkle.⁸ The leaves are oval to oblong in shape, 2.5-9.0cm long and 1.0-3.5cm broad, glossy green, hairless, with a pale midrib and a short petiole of about 1.0-1.8cm long and they are arranged in opposite pairs. The flowers look white to dark pink, with a darker red centre and a basal tube of 2.5-3.0cm long with a corolla of about 2.0-5.0cm diameter with five petals like lobes. The fruits are found to be a pair of follicles of about 2.0-4.0cm long and 3mm broad.⁹ *C. roseus* extracts have been used as a herbal medicine in India, Malaysia and China for the treatment of malaria, diabetes, insomnia, leukemia, Hodgkin's lymphoma and cancer due to the presence of active compounds in the plants.¹⁰ A pile of information on the ethno pharmaceutical relevance of the plant has been recently added in the pharmacopoeia, attributing the following biological activities to the leaf extracts of *C. roseus*: antihypertensive; hepatoprotective; antioxidant, antifungal, hypolipidemic, antibacterial, antidiabetic anticancer.¹¹⁻¹³ Unfortunately, extreme toxicity was observed when humans consumed extracts of the plants orally and therefore, its cultivation, possession or sale was outlawed in the state of Louisiana. However the use of the plant was strictly for aesthetic, landscaping or decorating purposes but never for medicinal purposes.¹⁴ It is against this background that the present study was developed. Therefore, the thrust of this study was to evaluate the sub-lethal assessment of aqueous dried leaf extract of *Catharanthus roseus* (Linn.) G. Don in male albino rats by quantifying their hematological and hepatic enzyme indexes.

Materials and methods

Collection and identification of the plant

The leaves of *Catharanthus roseus* (Figure 1) were harvested fresh from the courtyard of the Faculty of Biological and Physical Sciences and identified at the Department of Plant Science and Biotechnology, Abia State University, Uturu, Nigeria.



Figure 1 Pictorial representation of *Catharanthus roseus* (Linn.) G. Don.

Preparation and processing of plant extract

The leaves of *Catharanthus roseus* were properly sorted to remove dust and decayed materials, washed with distilled water and air-dried for seven (7) days. After drying, the leaves were ground into fine powder using a mechanical homogenizer. The fine powder was poured into a clean dry container and was stored at room temperature before used for the analyses. Exactly 80g of *Catharanthus roseus* dried leaves was soaked in aqueous medium of 750ml and allowed to stand for 24hours. It was filtered using a clean sieve. The filtrate was concentrated using rotary evaporator, and the extract stored in the refrigerator prior to use.

Laboratory animals and experimental design

Exactly Thirty two (32) weaned male albino rats, weighing 85-105g were obtained from the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, and were allowed to acclimatize for two (2) weeks in the Animal House of the Department of Biochemistry, Abia State University, Uturu. These animals were fed on grower's mash. All the animals used had free access to clean water. They were kept in well ventilated rooms with 12/12h light/dark condition and ambient room temperature. The experimental procedures used in this study conform to the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research¹⁵ and are grouped as shown below Table 1.

Table 1 Experimental design for acute and sub-lethal studies

A. Acute Toxicity	
Groups (n=3)	Extract Administration (mg/kg Body Weight)
A (Control)	0.25ml of Distilled Water
B	2000
C	5000
D	10000
B. Sub-Acute Toxicity	
Groups (n=5)	Extract Administration (mg/kg Body Weight)
I (Control)	0.25ml of Distilled Water
II	250
III	500
IV	1000

Lethal dose (LD50) study

A standard method was adopted for the determination of median lethal dose (LD₅₀) of extract.¹⁶ The aqueous dried leaf extract of *C. roseus* was given to various groups (Table 1A). The animals were then observed for behavioral changes and mortality for 24 hours.

Sub-lethal study

Animals set for sub-acute toxicity study were orally administered with the respective concentrations of the aqueous dried leaf extract of *C. roseus* (Table 1B) for fourteen (14) days. They were fasted overnight, anaesthetized with chloroform and sacrificed on the fifteenth day.

Blood collection

Blood was collected by cardiac puncture using syringe and needle into plain and Ethylene diamine tetra-acetic acid (EDTA) bottles for liver function and haematological analyses respectively. The bottles were allowed to stand for 15 minutes, spun at 12,000rpm for 5 minutes using the centrifuge and serum and plasma were decanted respectively using a Pasteur pipette into another set of plain test tubes, covered and stored in a refrigerator prior to further analysis.

Procedure for haematological parameters

Standard operating procedures as described by Afia and Momoh¹⁷ using the BC-3200 Auto-Haematology Analyzer was used for estimation of the haematological parameters. Namely: white blood cells (WBC), neutrophils, eosinophilic, basophil, lymphocyte, monocyte, red blood cells (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean cell hemoglobin concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), hemoglobin (Hb) and platelet were then calculated.

Procedures for serum chemistry analysis

The liver function enzymes; Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were spectrophotometrically determined using standard for the kits of Randox Laboratory Ltd, Co. Antrim, United Kingdom.

Statistical analysis

Results were expressed as mean±SD (standard deviation). Statistical analysis was performed by One-way analysis of variance (ANOVA) with the Graph Path Prism® Statistic software package, version 7.01. One-way ANOVA with a Tukey's multiple comparisons test was used to identify statistical differences among groups. A *p*-value of ≤ 0.05 was considered statistically significant.

Results

Table 2 showed the acute (oral) toxicity studies of aqueous dried leaf extract of *Catharanthus roseus* on male albino rats post 24 hours administration. From the result obtained, none of the twelve (12) rats died during the experimental time and therefore the median lethal (LD₅₀) of the aqueous dried leaf extract of *Catharanthus roseus* in albino rats was estimated to be greater than 10,000mg/kg per body weight. The rats fed with 5000mg/kg body weight of the extract were found to be dull and calm for the first four (4) hours but later normalized. Table 3 showed the effect of aqueous dried leaf extract of *Catharanthus roseus* on the body weight of male albino rats post 14days of administration. The result revealed that there was improvement in the percentage weight of the experimental animals when compared prior and post administration of the aqueous dried leaf extract. The percentage weight gain for control and the highest dose of the extract are 26.33 and 6.82% respectively. Table 4 showed the effects of aqueous dried leaf extract of *Catharanthus roseus* on the relative organ weight of male albino rats. The result revealed that there is no significant difference (*p*<0.05) in relative organ weight of the animals between the control and the test groups. The organ weight was not altered in all groups treated with various concentrations, ranging from 250, 500, and 1000mg/kg body weight when compared with the control group. Table 5 showed the effect of aqueous dried leaf extract of *Catharanthus roseus* on serum concentrations of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) of male albino rats. The result revealed that there was an increase in the serum concentration of Alanine aminotransferase (ALT) however, this increase was not statistically significant at (*p*<0.05). The result further showed that the concentration of Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) increased significantly (*p*<0.05) between the control and test groups (250, 500 and 1000mg/kg body weight doses of the extract).

Table 2 Acute (oral) toxicity studies of albino rats after 24hrs of administration of aqueous dried leaf extract of *Catharanthus roseus*

Group	Dose (mg/kg)	D/T	Sign of toxicity
A	0.25ml (H ₂ O)	0/3	No toxic effects observed
A	2000	0/3	No toxic effect observed
B	5000	0/3	Dullness was observed within 5 minutes
C	10000	0/3	Dullness and calmness

Table 6 showed the effect of aqueous dried leaf extract of *Catharanthus roseus* on haematological indexes (Packed Cell Volume, Haemoglobin; Red Blood Cells, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin; Mean Corpuscular Haemoglobin Concentration; White Blood Cell [total and differential] and platelets) of male albino rats. There was a significant decrease (*p*>0.05) in concentrations of platelets between the control and the test groups.

All other parameters assayed (Packed Cell Volume, Haemoglobin; Red Blood Cells, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin; Mean Corpuscular Haemoglobin Concentration; White Blood Cell, Neutrophil, Eosinophilic, Basophil, Lymphocyte and Monocytes) showed dose dependent variation, but the change was not statistically significant at *p*<0.05.

Discussion

The world today depends largely on products derived from plants to manage diseases and infections. *Catharanthus roseus* (Linn.) G. Don is one of such plants that have been exploited over the years to remedy ailments. It is against this background that the present study was developed to determine the sub-lethal assessment of aqueous dried leaf extract of *Catharanthus roseus* (Linn.) G. Don in male albino rats. The result of the acute (LD₅₀) toxicity studies of the aqueous dried leaf extract of *Catharanthus roseus* showed no mortality at the maximum dose of 10,000mg/kg body weight (Table 2). Therefore, the median lethal dose estimated to be more than 10,000mg/kg body weight in the albino rats is regarded as in safe category of drugs.¹⁸ Previous studies showed that 5000mg/kg body weight of aqueous leaf extract of *C. roseus* orally administered to male albino rats did not cause adverse effects to the experimental animals.¹⁹ The result revealed that there was significant decrease in the percentage weight gain of the experimental animals between the control and the test groups. The degree in percentage weight gain is in the order 1000mg/kg<500mg/kg<250mg/kg<Control (Table 3). The alteration in weight gain implies narrow safety margin and hence, the aqueous dried leaf extract of the plant should be used cautiously. It was suggested that the tested doses could have interfered with normal metabolism consequently affecting the uptake of food.^{19,20} The relative weight of organs (liver, spleen, kidneys, lungs, and heart) was not altered in all the groups treated with aqueous dried leaf extract of *C. roseus* when compared to the control group (Table 4). This implies that the plant extract at 14 days of exposure did not impact negatively on the relative organ weights. It has been reported that some herbal mixtures have hepatotoxic effects.^{21,22} The loss of integrity and functionality of this homeostatic organ (liver) would certainly lead to the increase in hepatocellular indexes: Alanine transaminase (ALP), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP). ALT and AST are cytoplasmic enzymes found in very low concentrations in the liver and are released into the plasma following hepatocellular injury while ALP is a membrane bound enzyme that is released in the serum following pathological phenomena.²³ The increase in the serum concentrations of these enzymes as observed in this study (Table 5) suggests the hepatotoxic effects of the aqueous dried leaf extract of *C. roseus*. These findings are in line with previous works of James et al.,²³ and Kabubii et al.,¹⁹ who reported that the ingestion of the aqueous leaf extracts of the plant resulted in the elevation of ALT, AST and ALP at different doses of 1.0g/kg and 5.0g/kg body weight in New Zealand breed of rabbits, Sprague Dawley female rats and albino rats respectively.^{19,20,23} Similar observation was made by Upmanya et al.,²⁴ who tested the intravenous administration of 1.0mg/kg of *C. roseus* alkaloid Vincristine and found a significant increase in liver enzymes.²⁴ Therefore, this study suggested that the aqueous dried leaf extract of the plant could only be hepato-friendly if taken at lower doses.

The results of this study also demonstrated a marked level in haematological parameters within the references range. It has been shown that certain conventional drugs or chemicals adversely affect

the various blood components. Haematotoxicity sets in when there is elevation of these blood components beyond their reference ranges.²⁵ The effect of *C. roseus* on some haematological index is indicated in MCV, WBC and platelets. MCV is the measure of the size of the red blood cells. Smaller MCV indicates that RBCs will be smaller than normal and this is described as microcystic; elevated MCV indicates that RBCs will be larger than normal and is termed macrocystic; whereas RBCs of normal size are termed normocystic.²⁶ These size categories are relied upon in the classification of anaemia types. In this study, the toxic effect was significant only at the highest dose

of 1000mg/kg. This observation was in agreement with the findings of Kabubii et al.,¹⁹ who reported a significant decrease in blood concentration of platelets.¹⁹ The significant decrease in the blood level of platelets implied that long term exposure to aqueous dried leaf extract of *C. roseus* could be dangerous to health. However, the findings suggested that the aqueous dried leaf extract of the plant may have no adverse effect on the bone marrow (the chief organ for haematopoietic processes and susceptible targets of toxic compounds) and the liver (the central hub of metabolism) if cautiously used (that is at low doses).

Table 3 Effects of aqueous dried leaf extract of *Catharanthus roseus* on the body weight of albino rats after 14 days of administration

Number of days	Control	Group I (250mg/kg)	Group II (500mg/kg)	Group III (1000mg/kg)
0	83.00±5.57	102.20±12.76	89.20±11.30	98.40±12.20
14	112.67±12.50	124.80±9.76	103.40±12.64	105.60±9.15
Weight gain (g)	29.67	22.6	14.2	7.2
Weight gain (%)	26.33	18.1	13.73	6.82

Values represent the mean±SD for N= 5.

Table 4 Effects of aqueous dried leaf extracts of *Catharanthus roseus* on the relative organ weight of albino rats

Organ	Control	Group I (250mg/kg)	Group II (500mg/kg)	Group III (1000mg/kg)
Liver	4.93±0.25 ^a	4.62±1.20 ^a	5.62±0.65 ^a	4.62±0.69 ^a
Spleen	0.72±0.04 ^a	0.75±0.29 ^a	0.99±0.11 ^a	0.73±0.20 ^a
Kidneys	0.92±0.05 ^a	0.88±0.11 ^a	0.99±0.17 ^a	0.84±0.05 ^a
Lungs	0.97±0.29 ^a	1.01±0.31 ^a	0.99±0.25 ^a	0.82±0.18 ^a
Heart	0.41±0.03 ^a	0.44±0.05 ^a	0.49±0.12 ^a	0.43±0.05 ^a

Table 5 Effect of aqueous dried leaf extract of *Catharanthus roseus* on hepatic enzymes of albino rats

Parameter	Control	Group I (250mg/kg)	Group II (500mg/kg)	Group III (1000mg/kg)
ALT (U/L)	32.33±6.03 ^a	36.67±4.16 ^a	35.67±3.51 ^a	37.67±0.58 ^a
AST (U/L)	30.33±1.53 ^a	33.33±1.53 ^a	38.00±1.00 ^b	39.67±3.21 ^c
ALP (U/L)	85.59±2.92 ^a	87.97±2.56 ^a	90.76±4.37 ^b	92.18±9.85 ^c

Values represent the mean±SD for N=5. Values in the same rows bearing the same letter of the alphabet are not significantly different from each other (p>0.05). Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP).

Table 6 Effect of aqueous dried leaf extract of *Catharanthus roseus* on haematological indexes of albino rats

Indexes	Control	Group I (250mg/kg)	Group II (500mg/kg)	Group III (1000mg/kg)
PCV(%)	51.70±6.84 ^a	50.93±1.78 ^a	50.43±4.05 ^a	49.80±3.90 ^a
Hb (g/dl)	12.67±0.76 ^a	12.43±1.25 ^a	12.93±0.61 ^a	12.91±0.79 ^a
RBC (×10 ¹² /L)	6.26±0.95 ^a	6.76±1.21 ^a	7.47±0.52 ^a	7.56±1.10 ^a
MCV (fl)	82.94±5.83 ^a	76.89±12.49 ^a	67.50±1.86 ^a	66.66±1.70 ^a
MCH (pg)	20.39±2.11 ^a	18.55±1.60 ^a	17.29±0.56 ^a	16.50±0.61 ^a
MCHC (g/L)	244.67±21.13 ^a	243.33±16.74 ^a	256.33±8.02 ^a	249.33±12.01 ^a
WBC (×10 ⁹ /L)	7.40±0.78 ^a	8.49±3.67 ^a	6.87±3.18 ^a	6.95±0.36 ^a

Table continued...

Indexes	Control	Group I (250mg/kg)	Group II (500mg/kg)	Group III(1000mg/kg)
Neutrophil (%)	51.67±1.53 ^a	51.67±2.52 ^a	51.33±1.15 ^a	54.67±1.53 ^b
Lymphocyte (%)	43.00±1.00 ^a	41.33±2.52 ^a	45.00±1.00 ^a	41.00±1.00 ^a
Eosinophil (%)	1.00±0.00 ^a	1.67±0.58 ^a	0.67±0.58 ^a	1.00±0.00 ^a
Basophil (%)	0.33±0.58 ^a	1.00±0.00 ^a	0.67±0.58 ^a	0.67±0.58 ^a
Monocytes (%)	4.00±1.00 ^a	4.33±0.58 ^a	2.33±0.58 ^a	2.67±0.58 ^a
Platelets (x10 ⁹ /L)	680.00±110.24 ^a	624.00±168.01 ^b	638.00±158.71 ^b	536.33±72.97 ^c

Conclusion

The results of the present study showed that the oral administration of aqueous dried leaf extract of *Catharanthus roseus* may lead to hepatocellular and haematological disorders when consumed in large doses, hence, rebutting its widespread applications in ethno medicine for the treatment and management of diseases. Consequently safety measures, have to be taken in the administration of the aqueous dried leaf extract of the plant.

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Conflict of interest

The author declares no conflict of interest.

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