

Full length research article

**THE EFFECTS OF CATHARANTHUS ROSEUS (L) G. DON
1838 AQUEOUS LEAF EXTRACT ON SOME LIVER
ENZYMES, SERUM PROTEINS AND VITAL ORGANS**

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ABSTRACT

Plant and phytochemical products continue to play an important role in medicine. In this study, the toxicological potentials of the aqueous leaf extract of *Catharanthus roseus* G. Don 1838 in rabbits were determined. The rabbits were placed in three groups with four rabbits in each group. The control group, a second group administered with 100mg/Kg and third group administer with 360mg/Kg body weight, were tag as Group 1, 2 and 3 respectively. Increased levels of serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were found to follow the treatment of rabbits with ACR suggesting liver damage. This was confirmed by the histopathological studies that reveal centrieolobular hemorrhagic necrosis of the liver. Also, there was oxidative glomerulonephritis that set in. The effect of the extract in the activity of the hepatocellular enzymes (ALT, AST, and ALP) was found to be concentration dependent. This suggest that the infusion of the leaves could cause hepatocellular damage and kidney impairment.

Keywords: *Catharanthus roseus*, Aqueous Leaf extract, Toxicity, Alkaloids, Enzymes levels.

INTRODUCTION

The plant *Catharanthus roseus* (L) G. Don 1838 (Periwinkle or Vinca) has gained acceptance from the pharmaceutical industries as it is widely used as an infusion in different parts of world to treat diabetes (Ratsumamanga 1980; Audu 1989; Alexandrova *et al.* 2000; Heijden *et al.* 2004). Experimental evidence shows that the concentrated aqueous extract can lower blood-sugar level in rats, reduce the symptoms of diabetes and as hemostatic and tranquilizer to lower blood pressure in man and as a disinfectant (Marton 1977; Wikipedia 2006). Although attempts to confirm the active principles have met with little success, information is now available about its anti-leukemic properties (Sim 1971; Taylor and Farnsworth 1973; Alexandrova *et al.* 2000). Out of almost 6000 known alkaloids isolated from plants, more than 70 are from *C. roseus* (Sofowora 1982; Miller; 1973; Harbone 1991; Heijden *et al.* 2004).

Available literature on *Cathranthus* only indicated the toxic effects of the synthetic alkaloids and the cytotoxic effects of the crude extract on cell division, damage on the nerves which control digestion, bowels, cardiac and sexual function (Lobert *et*

al. 1997; Alexandrova *et al.* 2000; Khan 2007). As the possibility of the impairment of vital organs by the crude extract of this plant when consumed cannot be ruled out, it becomes necessary to investigate the levels of the bilirubin hepatic enzymes and other blood constituents that could be affected. This Study was carried out to monitor the effect of the aqueous leaf extracts of *C. roseus* on some liver enzymes, serum protein on heart, kidney and liver.

MATERIALS AND METHODS

Plant material: Fresh leaves of *C. roseus* were collected from flower beds. The leaves were rinsed with water, sun-dried and crushed to powder with mortar and pestle and stored in a dry clean plastic bottle. 50 g of the powdered form was soaked into 500 ml of boiling distilled water. The mixture was allowed to infuse for one hour with constant stirring. The mixture was filtered with a cloth sieve followed by cotton wool. The concentration of the aqueous extract was determined in weight per volume.

Experimental animals: 12 New Zealand breed of rabbits were randomly divided into three replicates and a control group and subjected to the following treatments:

- Group 1: Control animals were allowed access to water only
- Group 2: Animals given 100mg/kg body weight dose of the extract and water.
- Group 3: Animals given 360mg/kg body weight of the extract and water.

Feeding was done ad libitum with pelleted animal feeds. The extracts of *C. roseus* were administered orally with the aid of a plastic syringe for 9 days according to the body weight of each rabbit (Williams 1976).

Blood sample collection: Blood was collected from the experimental animals by venopuncture via the jugular vein into two sets of tubes. Into one of the tubes containing sodium citrate as anticoagulant, 10 ml of the blood was added and mixed thoroughly by inverting the tubes. The mixed blood was then centrifuged at 3000 g for 10 min, and the plasma transferred to another tube. Blood serum was prepared by allowing 10 ml of blood into each tube without anticoagulant to stand for 10 min, and centrifuged for five min at 3000g. The sera obtained was transferred into a sample vials and stored in the refrigerator.

Toxicological tests: Estimation of prothrombin time: One stage method (Quicks 1970) was used based on the activation of factors VII, V, X, II and I as excess preformed extrinsic thromboplastins, thereby passing factors XII, XI, IX and VIII. (Raphael 1983). 0.2 ml of thromboplastin calcium chloride reagent was added into a test tube and incubated with the tube containing portion of the plasma at 37 °C in water bath for 1 min. A minute later, 0.1 ml of plasma was blown into it, and a stopwatch started simultaneously. The mixture was shaken and left in the water bath for 7sec at 37°C. The tested tube was removed from the water bath and tipped gently until a clot was

formed. Immediately the clot was observed, the stopwatch was stopped, and the time noted. Same was repeated for other samples.

Determination of total serum protein: Biuret method (Dumas 1975 and the absorbance compared with a known protein concentration (Linne & Ringsurd 1979; Raphael 1983).

Determination of plasma albumin: The Dye binding method (put Ref) was employed, where Bromocresol green react with albumin in an acid solution to yield a blue-green colour complex that absorbs light at 628 nm.

Estimation of globulin: Serum globulin concentration was estimated in (g/100 ml) by subtracting the albumin concentration from the serum total protein.

Determination of albumin/globulin ratio: The albumin to globulin ratio was calculated by dividing the albumin value by that of globulin.

Estimation of aspartate amino transferase (AST): Aspartate Amino Transferase (E.C: 2.6.1.1) was estimated by monitoring the concentration of oxaloacetate hydrazine formed with 2,4 – dinitrophenyl hydrazine (DNPH) (Raphael 1983).

Estimation of alanine amino transferase (ALT): The estimation of Alanine amino transferase (E.C: 2.6.1.2) was done by monitoring the concentration of pyruvate hydrazones formed with 2,4 – dinitrophenyl hydrazine (DNPH) (Raphael 1983). The procedure of estimation of ALT is same as that of AST except for the end substrate, where alanine substrate was used.

Estimation of serum alkaline phosphates (ALP): This was determined using the method of Basssey-lowery & Brock (1946) as cited by Raphael (1983), where enzyme alkaline phosphates (ALP) hydrolyzes the P-nitrophenyl phosphate substrate, producing inorganic phosphate and p-nitrophenol. The absorbance of the colour was measured photometrically and the concentration determined.

Histopathology test: Histopathology test was conducted on the heart, liver and kidney according to Thompson (1966).

RESULTS

The aqueous leaf extract exerts a significant ($P < 0.05$) elevation on ALT, AST and alkaline phosphatase (Table 1), while studies on prothrombin time, total protein, serum albumin, globulin and Albumin/Globulin ratios shows no significant elevation ($P < 0.05$) (Table 2).

Histological studies carried out on the heart, liver and kidney to ascertain the toxic effect of *Catharanthus roseus* on these organs revealed the on set of centriobular hemorrhagic necrosis in the liver (Plate 1B). The heart shows no visible changes, while the kidney shows an oxidative glomerulonephritis that have set in within the Bowman's capsule, with few serum fibrins,

neutrophils and with extracapillary proliferative reaction (Plate 2B).

TABLE 1: EFFECTS OF AQUEOUS LEAF EXTRACT OF C. ROSEUS ON SERUM ENZYME LEVELS.

Parameters	Group 1 ^f	Group 2	Group 3
ALT (GPT) IU/L	15.64 ± 0.36	18.94 ± 4.7*	24.25 ± 4.82*
AST (GOT) IU/L	14.14 ± 0.73	17.65 ± 5.25*	17.5 ± 2.18*
Alkaline Phosphate IU/L	18.0 ± 2.16	18.0 ± 0.6	22.0 ± 4.58*

The values represent mean (± SD), n = 4

* Significant difference from the control ($P < 0.05$) (paired test).

^f Group 1: Control animals were allowed access to water only

Group 2: Given 100mg/kg body weight dose of the extract

Group 3: Given 360mg/kg body weight dose of the extract

TABLE 2: EFFECTS OF AQUEOUS LEAF EXTRACT OF C. ROSEUS ON THE PROTEIN LEVELS.

Parameters	Group 1	Group 2	Group 3
Prothrombin Time (Sec.)	9.13 ± 0.09	8.0 ± 0.00	10.62 ± 0.42
Serum Total Protein (g/100ml)	8.4 ± 90.26	8.28 ± 0.54	8.8 ± 80.84
Serum Albumin (g/100ml)	4.26 ± 0.69	3.29 ± 0.03	4.58 ± 0.35
Globulin (g/100ml)	4.23 ± 0.43	4.99 ± 0.51	4.3 ± 0.49
A/G Ratio	1.01 ± 1.60	0.66 ± 0.10	1.07 ± 0.70

NB: The value represent mean (±SD), n = 4

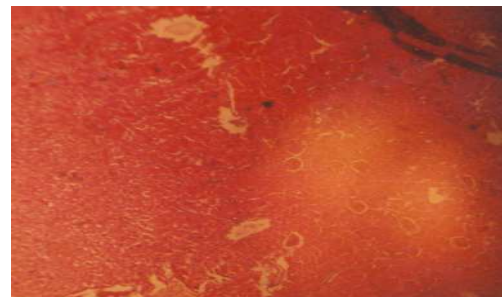


Plate 1A (Liver): Histopathological view of control group with no visible changes

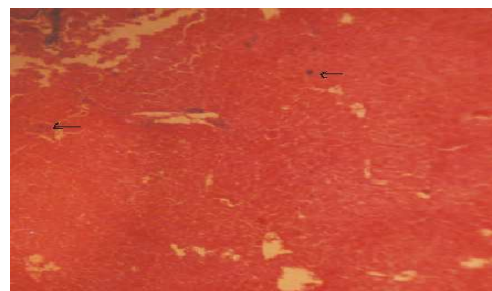


Plate 1B (Liver): Histopathological view of treated group indicating the occurrence of necrosis of sinusoidal lining with the presence of chronic passive congestion, characterized with fibrosis around the control vein.

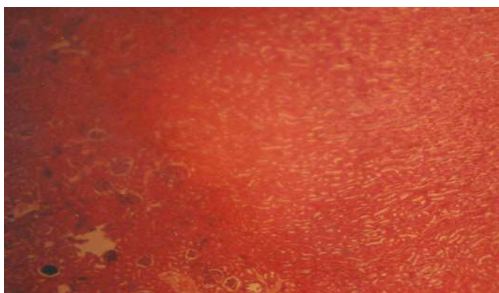


Plate 2A (Kidney): Histopathological view of control group with no visible changes

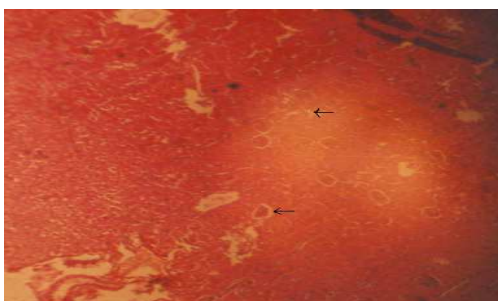


Plate 2 B (Kidney): Treated Group indicating that oxidative glomerulonephritis have set within the Bowman's capsule, with few serum fibrin, neutrophils and extracapillary proliferative reaction

DISCUSSIONS

Medicinal plants are part of Africa's cultural heritage and a very significant part of present day ailment remedies. Their medicinal benefits and toxic properties are being evaluated for the benefit of mankind (James *et al.* 2006).

Alkaline phosphatase is a membrane bound enzyme that is released in serum following a pathological phenomenon (Batt & Siest 1980; Siet *et al.* 1980). A high increase in serum alkaline phosphatase is a sensitive index of cholestasis, while a minimal increase is indicative of chronic hepatocellular disease (Wilkinson 1976). Alanine amino transferase is a cytoplasmic enzyme found in very low concentration in the liver which is released into the plasma following hepatocellular damage. The increase in concentration of this enzyme, as observed in this study suggests the hepatotoxic effect of the aqueous extract of *C. roseus*. 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 with no elevation of bilirubin. Similarly, results of this study showed no significant elevation of prothrombin, total protein, serum albumin, globulin and Albumin/Globulin ratios, agreeing with the findings of Pinkerton *et al.* (1988). *et al.* (1988).

Histological studies revealed centriobular hemorrhagic necrosis in the liver (Plate 2B) as expressed in acute toxicity of alkaloids like pyrrolizidine (Mattocks 1972), which is widely distributed in plants (Vickery & Vickery 1981). Pyrrolizidine alkaloids are hepatotoxic which could damage the liver and result in death in both man and animals (Vickery & Vickery 1981). One of the toxic effects of pyrrolizidine is veno-occlusive liver disease (Mattocks 1968). The toxicity of alkaloids is primarily due to their metabolites produced by biotransformation, and not by the alkaloids themselves (Mattocks 1972). Bull *et al.* (1968) had earlier reported that pyrrolizidine alkaloids must possess the 1-hydroxymethyl pyrrolizidine system, unsaturated in the 1, 2 – position and a hydroxyl group for esterification before the toxicity can be observed. Another possible reason for the toxic effect may be due to the very slow excretion rate and liver metabolism of *C. roseus* extract which makes the alkaloid to linger in the body (Khan 2007). The histopathological studies on the liver supports this view, as the liver tissue (Plate 1B), shows that necrotic process have set in, while the kidney reveals oxidative glomerulonephritis within the Bowman's capsule, with few serum fibrin, neutrophils and with extracapillary proliferative reaction.

Another toxic effect associated with *C. roseus* alkaloids as reported by Rosazza *et al.* (1992) include selective reversible inhibition of monoamine oxidase-B (MAO-B) that is important in the biotransformation of xenobiotic. In addition, alkaloids have been found to decrease red blood cells and the formation of granulocyte and leukocytes giving rise to bone marrow depression and neurotoxicity (Barnett *et al.* 1978; Lobert *et al.* 1998; Alexandrova *et al.* 2000; James *et al.* 2006 & Khan 2007).

The results from these studies indicate that the use of the aqueous leaf extract of *C. roseus* as infusion for disease remedy may result to hepatocellular damage when consumed in large doses. Consequently, safety measures have to be taken in the administration of these extracts that may include monitoring of the vital serum enzyme levels, administering of milk (for casein to fix alkaloid) as the alkaloids in *Catharanthus* are found to interact and be resisted by multi-drug resistance associated protein (MRP) (Khan 2007). In addition, vitamin E (an antioxidant) could be administered to further reduce the toxicity and keep cell membrane of tissues and organs intact.

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