

Madagascar periwinkle (*Catharanthus roseus*) enhances kidney and liver functions in Wistar rats

Damilare A. Adekomi

Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria.

SUMMARY

Botanical drugs are complementary therapies in the management and treatment of clinical conditions. This study was aimed at investigating the possible changes in the structural and functional entities of two vital organs, the liver and kidney, following oral administration of ethanolic leaf extracts of *Catharanthus roseus*. Thirty-two wistar rats were used for this study and were randomly assigned into three treatment (n=24) and control (n=8) groups. The animals in the treatment groups - A, B, and C respectively- received 400 mg, 300 mg and 200 mg per kg body weight of Madagascar periwinkle extract for twenty-one days, while the animals in the control group (group D) received an equal amount of phosphate buffered saline (PBS). The administration was performed orally using an orogastric tube over twenty-one days (21d). Twenty-four hours after the last administration, all the animals were sacrificed by cervical dislocation. Laparotomy was performed and the liver and kidney excised, trimmed free of fat, and rinsed in cold phosphate buffered saline solution. The liver was quickly fixed in 10% formol-saline, while the kidney was fixed in Bouin's

fluid for histological processing. Blood samples collected from the abdominal aorta and portions of the liver stored at -20°C in the refrigerator were used for the biochemical analysis of kidney metabolites and liver enzymes respectively. It was observed that the activities of the kidney metabolites and liver enzymes following the administration of the ethanolic extract of *C. roseus* were statistically and significantly reduced in a dose-dependent pattern in all the experimental groups when compared with the control group. The results obtained from this study suggest that the oral administration of ethanolic extracts of *C. roseus* has no compromising effect on the kidney and liver and may enhance the functional features of the organs of Wistar rats to a greater extent.

Key words: Phosphate buffered saline – Kidney enzyme – Liver enzyme – Laparotomy – Cervical dislocation

INTRODUCTION

Catharanthus roseus (*Vinca rosea*) is known as the common or Madagascar periwinkle. It is a perennial herb of the Apocynaceae family

originally native to Madagascar (Don, 1999; Morton, 1991). It measures about two feet in height and has dark green glossy leaves and pale pink or white flowers. The organic extracts of *C. roseus* are used in the ethnobotanical treatment of diabetes, malaria, leukemia, wasp stings, sore throat, eye irritation, and infections (Stolle and Greoger, 1967). It is also used as an astringent, a diuretic and an expectorant. The plant contains about seventy alkaloids, some of which include cartharathine, lochnenine, vindoline, vindolinine, vincristine, vinblastine, tetrahydroalstronine, reserpinne, serpentine, etc. (Gordon et al., 1964).

About 20% of total cardiac output flows through the mammalian kidneys. The nephron is the functional and structural unit of the kidney. The kidneys of mammalian species show variations in the ratio of cortical to juxtamedullary nephrons but have in common a unique structural characteristic; i.e., the presence of two capillary networks: the glomerular and the peritubular. Endothelial cells of the peritubular capillaries show endocrine activity, represented in the synthesis and release of the erythropoietic hormone. The kidneys are the main source- around 85% of the circulating amount- of this hormone (Junqueira et al., 1998; Ganong, 1999).

The liver is the largest organ in the mammalian body and is the centre of all metabolic activities in the body. Drugs and other foreign substances are metabolized and inactivated in the liver and the organ is therefore susceptible to the toxicity of these agents. Certain medicinal agents when taken at excessively high doses, and sometimes even when administered within therapeutic ranges, may injure the liver. It has a peculiar macro- and micro- structure. It receives both venous and arterial blood from the gastrointestinal tract via the hepatic portal system and through the hepatic artery respectively. The micro-morphological features of the liver is highlighted by the lobules and their sinusoids, which are lined with highly active Kupffer cells, central veins and hepatocytes plates that are closely associated with the terminal lymphatics and bile canaliculi (Webster and Webster, 1974; Lesson et al., 1988; Berne and Levy, 1998; Constanzo, 1998). The morphological features distinguished to date allow the liver to carry out a vast array of functions. The hepatic venous circulation qualifies the liver as a blood reservoir, with a significant lymph outflow. The

macrophage population is efficient in its blood-cleansing activities. The hepatocytes have metabolic functions that deal with very essential processes such as detoxification, deamination, transamination, the removal of ammonia in the form of urea, biosynthesis and the release of the non-essential amino acids and plasma proteins, with the exception of immunoglobulins, gluconeogenesis, the storage of glycogen, the conversion of carbohydrates and proteins into lipids, the synthesis of lipoproteins, phospholipids and cholesterol, the oxidation of fatty acids, the storage of iron in the form of ferritin as well as the storage of vitamins A, D and B₁₂. The hepatocytes are efficient at uptaking blood bilirubin to conjugate it mainly with glucuronic acid and then excrete it into the bile. Potassium and sodium salts of the conjugated bile acids are also excreted into the bile. Based on specific biochemical reactions, several functional tests have been formulated to explore hepatic status (Johnson, 1995; Stryer, 1995; Guyton and Hall, 1996; Ganong, 1999; Nelson and Cox, 2000).

These vital organs have the ability to carry out several essential functions. The maintenance of body fluid volumes and their electrolytes within normal limits participates in regulation of acid-base balance and blood pressure, the excretion of non-protein nitrogenous compounds, such as urea and uric acid, and the elimination of endogenous toxic waste agents. The kidneys help get rid of hazardous compounds such as endogenously produced creatinine and many metabolites of hormones, as well as ingested and administered chemicals and drugs (Griffin and Ojeda, 1996; Guyton and Hall, 1996; Nelson and Cox, 2000; Feraille and Doucet, 2001).

When a herbal product is ingested, the body interacts with it in an attempt to get rid of any harmful toxins, especially if the body cannot convert the foreign substance into cellular components. Common manifestations due to such insults are changes in enzyme levels and those of other cellular components. The enzymes commonly involved include aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), and amylase. Products such as urea and uric acid are also vital diagnostic tools for toxicity. In a previous study, Wannang et al. (2005) found that altered serum levels of these products in rats are an indication of the potential toxicity of the plant. Toxicity could also result

in tissue or organ damage. The organs most commonly affected are the liver, pancreas, and kidney among others. The aim of this study is therefore to investigate the effect of the plant extract on some kidney and liver parameters.

MATERIAL AND METHODS

Collection of plant and preparation of plant extracts

Fresh leaves of *C. roseus* were collected from the premises of the University of Ilorin Teaching Hospital, Ilorin, Nigeria. The leaves of *Catharanthus roseus* plant were air-dried and the dried plant material was weighed using a Gallenkamp (FA2104A, England) electronic weighing balance and ground with a Blender/Miller III, (model MS - 223, China).

Five hundred and fifty two grams (552 g) of the dried powdered sample was soaked in five liters of 70% ethanol for 24 hours at room temperature with constant shaking (Stuart Scientific Orbital Shaker, UK), and then filtered through silk cloth. The filtrate was concentrated using a rotary evaporator (Rotavapor® R-210) at 42–47°C to obtain 56.50 g alcohol-free residual extract of *Catharanthus roseus*.

Toxicity evaluation

The method of Lorke (1983) was adopted to determine the dose of the extract that would be lethal to 50% of the population of animals. Three dose points (450, 500 and 550 mg/kg) were chosen for the pilot experiment, in which doses of 400, 300 and 200 mg/kg were administered to the animal in the extract-treated groups respectively.

Animal care and experimental design

Thirty-two wistar rats of the first filial generation were randomly assigned into three extract treatment (n=24) and one control (n=8) groups. The animals in the extract treatment groups, designated A, B, and C, received 400 mg, 300 mg and 200 mg per kg body weight of the ethanolic Madagascar periwinkle extract for twenty-one days respectively, while the animals in the control group (group D) were administered with equal amount of phosphate buffered saline (PBS). All animals were housed in clean cages with dimensions of 33.0×20.5×19.0 cm contained in well ventilated standard housing conditions (temperature: 28–31°C; humidity: 50–55%). Their cages were cleaned every day.

All experimental procedures followed the recommendations provided in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institute of Health (1985). The rats were fed with standard rat chow at a recommended dose of 100 g/kg as advised by the International Centre of Diarrheal Disease Research, Bangladesh (ICDDR, B) daily. Drinking water was supplied *ad libitum*.

Twenty-four hours after the last administration, all animals were sacrificed by cervical dislocation. A laparotomy was performed and the kidney and liver were excised, trimmed free of fat and rinsed in cold phosphate buffered saline solution. The liver was fixed in 10% formal saline, while the kidneys were fixed in Bouin’s fluid for routine histological processing. Blood samples collected from the abdominal aorta and portions of the liver stored at -20°C (for 5 hrs) in the refrigerator were used for the biochemical analysis of kidney metabolites and liver enzymes respectively.

Histological procedure

After fixing the kidney and liver of both the extract-treated and control animals, the tissues were processed for Hematoxylin and Eosin (Carleton, 1967) staining techniques. After fixation, the tissues were embedded in paraffin wax; serial sections of 5 μ m thick were obtained using a Leitz Rotary microtome (Leitz 1512 Microtome). The sections were mounted in DPX and examined with the aid of an Olympus (XSZ-107BN, No. 071771) binocular light microscope. The photomicrograph of each slide was taken with a DXM1200F Nikon Digital Camera (Nikon, Japan) for subsequent histological analyses.

Estimation of liver enzymes

The levels of alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were estimated in liver homogenates. A 10% homogenate of the tissues in chilled 5% sucrose solution was immediately prepared with a Potter homogenizer (GPE, Bedfordshire, England). The homogenate was centrifuged at 3000 rpm for 10 minutes at 4°C. The supernatant was used for the assay of liver enzymes.

Alanine aminotransferase (ALT): An ultra-violet light source was used to measure the activity of the enzyme ALT in homogenate samples spectrophotometrically. The optimal

wavelength was 340 nm. Enzyme assays were performed using specific kits manufactured and marketed by Randox Laboratories Ltd, UK.

Aspartate aminotransferase (AST): The activity of the aspartate aminotransferase enzyme in serum samples was measured using a photometric method. A spectrophotometer (Beckman, USA) was used. The assay kit of Randox Laboratories Ltd, UK was used.

Estimation of kidney metabolites

Blood samples were collected (using a sterilized needle and syringe) from the abdominal aorta of each rat (both the treatment and control groups) in marked conical test tubes, and the blood in the test tubes was allowed to clot. The test tubes were centrifuged to harvest serum. These harvested serum samples were employed to run routine biochemical tests for the kidney function indexes. In order to investigate kidney function in the experimental animals, serum samples were analyzed for their contents of urea (mg/dl) and creatine (mg/dl).

Serum creatinine: Creatinine Analyzer- 2 (Beckman Coulter Inc., USA) in combination with a specific reagent kit (Hichem Creatine Pak, Elan Diagnostics, USA) were employed to evaluate the creatinine content of the serum samples.

Serum urea: The urea content in the serum samples was estimated by means of an automated Blood Urea Analyzer (Beckman Coulter Inc., USA). The kit used for the analysis was the Hichem kit for blood urea nitrogen analyzers. The kit was supplied by Elan Diagnostics, USA.

Statistical analyses

Data were evaluated statistically using Student's t-test with SPSS/14.0 software (SPSS Inc, Chicago, USA) and Excel 2007 (Microsoft Corporation, USA) and were expressed as means \pm standard error of mean (SEM). A value of $p < 0.05$ was considered to indicate a significant difference between groups.

RESULTS

Toxicity evaluation

The method of Lorke (1983) was used to determine the dose of the extract that would be lethal to 50% of the population of animals. Three dose points (450, 500 and 550 mg/kg) were chosen for the pilot experiment, from

which doses of 200, 300 and 400 mg/kg respectively were administered to one animal per group in the second phase. The geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where death occurred) was calculated and taken as the LD 50 value.

All the animals administered 500 and 550 mg/kg body weight of the plant extract died at 48hrs after administration. Although prior to their death it was observed that they abstained completely from their feed, there was loss of motor activities, since the animals rarely moved. At the 45th hour after administration, the animals began to convulse and were completely immobile.

However, the animals administered 450mg/kg body weight of the ethanolic plant extract were moderately active but later became docile. They also abstained from their feed, to return to normal activity at about 7hrs after administration of that particular dose. According to Chattopadhyay (1999), a 70% ethanol extract of leaves of *Catharanthus roseus* in an oral dose of 400 mg/kg is 20% as effective as tolbutamide in diabetic rats, although safer.

Body weight

The effects of different doses of ethanolic leaf extracts of *Catharanthus roseus* on the body weight of the animals in the treatment groups as compared with that in the control group is shown in Fig. 3. After 21 days of treatment, the body weights of the treated animals were observed to increase significantly ($p < 0.05$) in comparison with the controls. Among the treated groups, higher body weight was recorded in group (A), which received 400 mg/kg bw of the extract followed by group (B) receiving 300 mg/kg bw, and then group (C) receiving 200 mg/kg bw. Thus the administration of the ethanolic leaf extract of *Catharanthus roseus* increased the body weight of the treated animals in a dose-dependent pattern. The results of the present study are in agreement with the reports of Mostofa et al. (2007) and Iweala and Okeke (2005).

Gross observations

No gross alterations were observed in the cytoarchitecture and morphology of the kidney and liver of the animals in the treatment and control groups after termination of the experimental procedure. The kidney and liver (with all their component parts) of the ani-

mals in both the treatment and control groups appeared morphologically normal.

Microscopic observations of the kidney

The histological preparations of kidney from the treated and control rats showed that the various segments of kidney tubules were well preserved. Abundant glomeruli, nephrons with interspersed blood capillaries

were also clearly seen. Several regions of kidney tubules appeared to be normal without any change in mesangial thickening or hyaline deposition. The renal parenchyma showed no evidence of distortion of any kind. It is evident at this magnification (x1950) that the tubules constituted the bulk of the parenchyma with different shapes, diameters and staining intensities (Fig. 1).

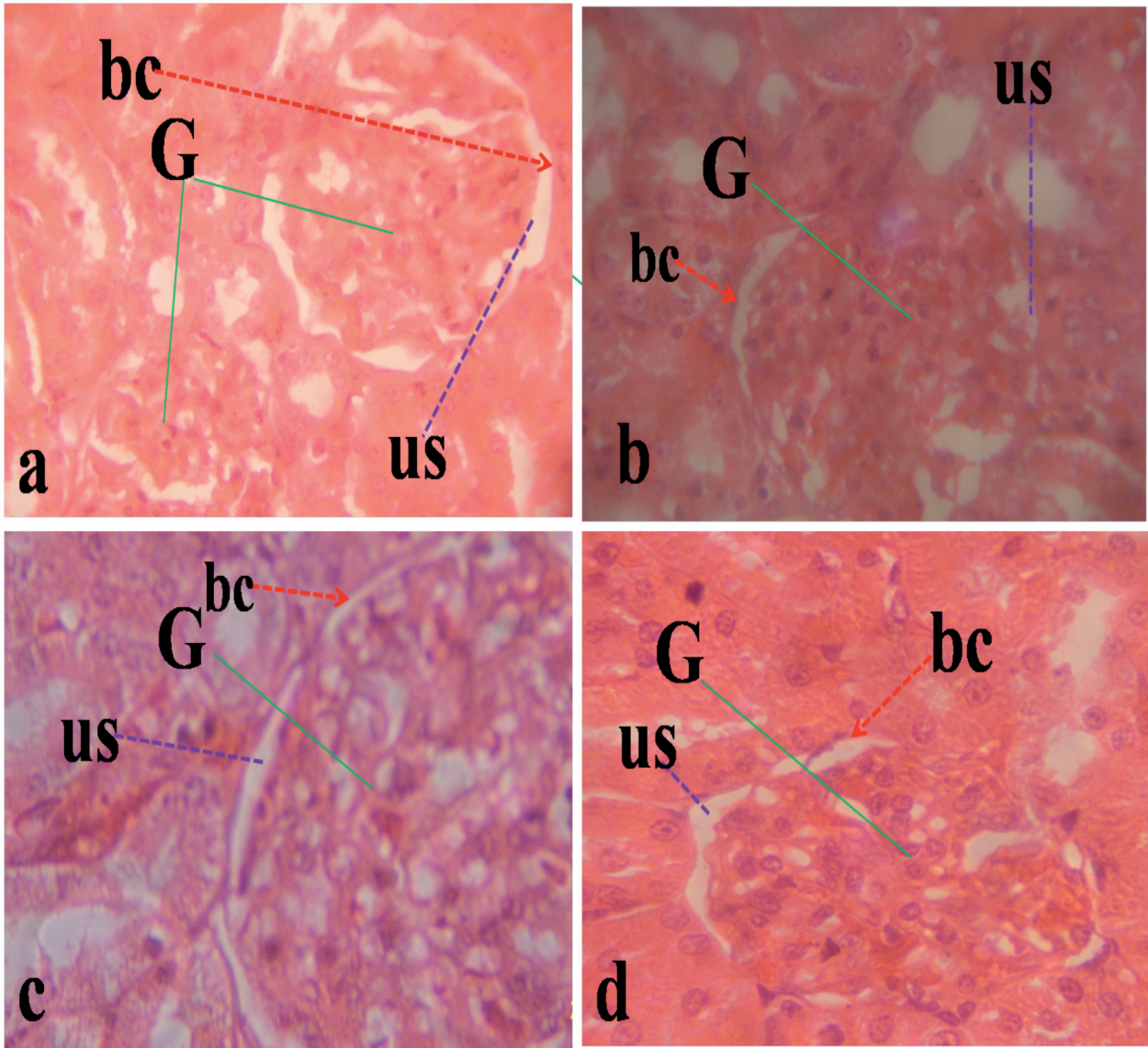


Fig. 1. Cortical portion of the kidney in the treatment groups (a, b, and c) and the control group (d); glomerulus (G), Bowman's capsule (bc), urinary space (us). (H&E, x 1920).

Microscopic observations of the liver

When the sections obtained from the histological processing of the liver were viewed under the microscope it was observed that they conformed to normal histological fea-

tures. The sinusoids in the sections of the treated animals were devoid of occlusions and were not distorted. The extent of conformity to the normal histological outline was observed to be higher in the treated groups as compared with the control group (Fig. 2).

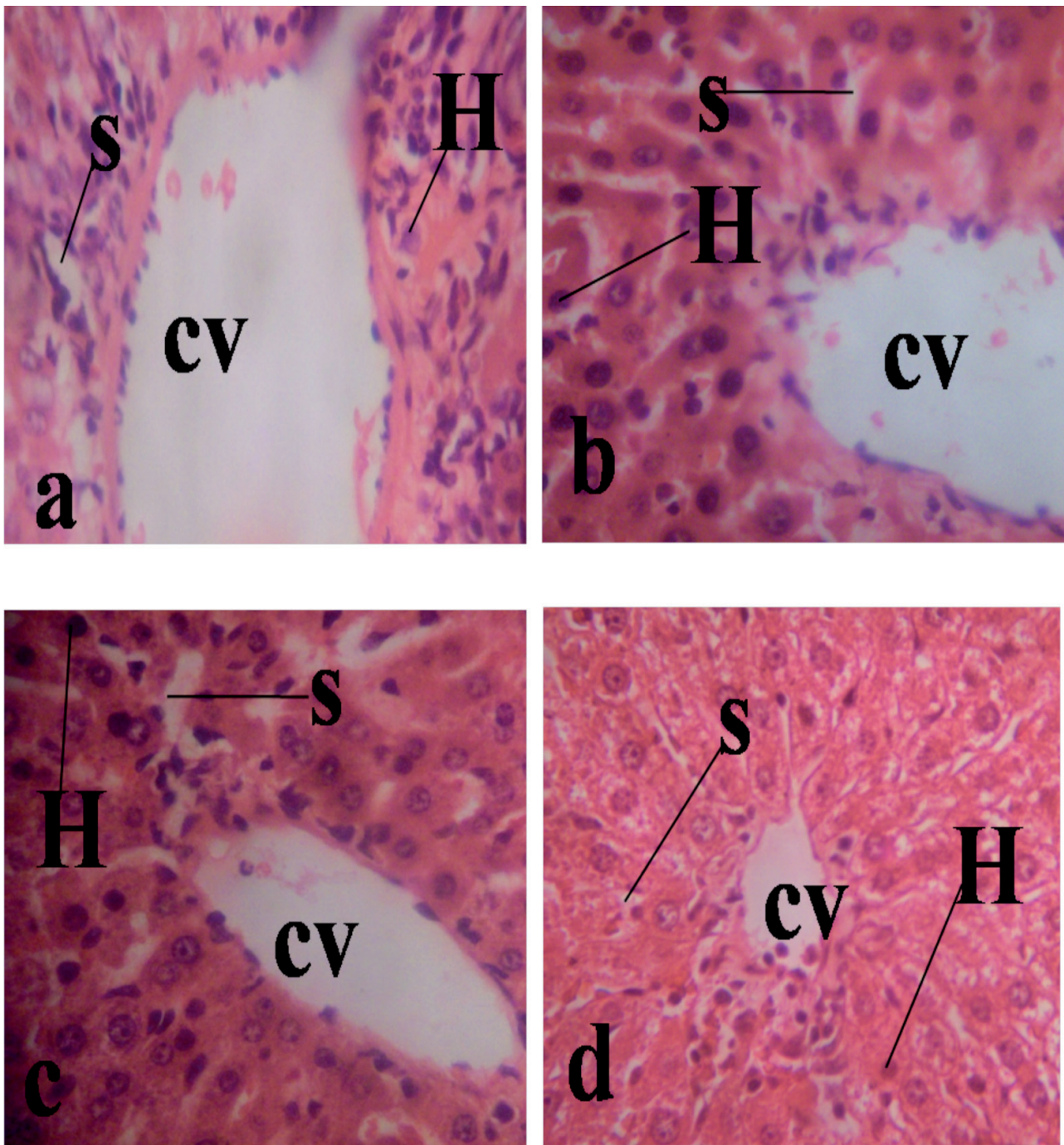


Fig. 2. Liver sections of the treatment groups (a, b, and c) and the control group (d); hepatocyte (H), sinusoid (s), and central (centrolobular) vein (cv). (H&E, x 1920).

Table 2. Liver enzyme indices of control and extract-treated rats.

Group	No of Rats	ALT (Mean±SEM) (U/L)	AST (Mean±SEM) (U/L)
A (1.0 ml)	8	21.87±1.45	94.50±2.50
B (0.5 ml)	8	24.35±1.20	95.50±6.50
C (0.1 ml)	8	28.66±1.20	98.56±8.00
D (control)	8	30.84±3.21	103.10±1.00

A *p* value of less than 0.05 indicates a significant difference between the compared means.

Table 3. Kidney metabolite indices of control and extract-treated rats.

Group	No of Rats	Serum Urea (mg/dl)	Serum Creatinine (mg/dl)
A (1.0 ml)	8	24.57±1.21	0.50±0.04
B (0.5 ml)	8	26.97±1.74	0.53±0.09
C (0.1 ml)	8	27.21±1.37	0.59±0.11
D (control)	8	30.57±1.21	0.80±0.07

A *p* value of less than 0.05 indicates a significant difference between the compared means.

Alanine aminotransferase (ALT)

Table 2 shows the changes in liver ALT levels in the treatment groups as compared with the control. ALT levels were significantly low ($p < 0.05$) in all the treatment groups at the expiration of the study.

Aspartate aminotransferase (AST)

The changes observed in the levels of AST activities in the livers of the treatment groups versus the control are also shown in (Table 2). The changes in AST levels were comparative to the trend obtained for ALT. At the end of the study, the levels of AST activities in the liver of the treatment groups was significantly low ($p < 0.05$).

Activities of urea and creatinine

As observed in Table 3, the activities of serum urea and creatinine in the treatment groups was significantly reduced in comparison with the values obtained in the control group. The difference between means was significant ($p < 0.05$). Blood serum samples from the rats that received the ethanolic extract of *Catharanthus* contained less urea nitrogen ($p = 0.05$) as compared with the mean urea nitrogen values of the control, hence the low level of the activities of urea.

DISCUSSION

A search of the available literature revealed no published report on the toxicity of *Catharanthus roseus* in humans despite the widespread use of the plant in various herbal remedies. In this study, oral administration of the ethanolic extract of *Catharanthus roseus* to rats produced no observable toxicity in the liver of the rats at 21d of administration. Estimations from use of the adopted method of Lorke (1983) provided a tolerable dose of the ethanolic extract of *Catharanthus roseus* by the animals. Dapar et al. (2007) stated that the estimation of tolerable doses of plant extract is of immense importance in view of the large-scale human consumption of these plants in managing or combating certain ailments and that they should be a matter of concern.

However, toxicity evaluation of plant extracts or drugs is not a very reliable procedure in the determination of toxicity since there is considerable variation in results among different species and even in the same specie under different experimental conditions. Moreover, toxicity tests do not provide comprehensive information on which system failure led to the death of the animals. Some deaths may have been due to the quantity of the test substance causing gastric rupture or other morbidity unrelated to the actual toxicity of the extract. Notwithstanding, toxicity tests in conjunction with photomicrographs of stained and processed histological tissue sections provide a clue as to the toxic characteristics of the drug or plant (Dapar et al., 2007).

It was observed during the course of this study that the plant extract had a modulating effect on the body weight of rats exposed to the extract. There was a decrease in the body weight of the animals in groups A and B administered 400 mg/kg and 300 mg/kg body weight of the extract on the 7th and 14th day respectively after administration as compared with other animals in the other treatment and the control groups (Fig. 3). Histological examination of the kidneys and livers of these animals showed intact normal histological features, and this suggests that the decrease in body weight observed in the animals in these groups (i.e. A and B) on day seven (7) and fourteen (14) of the study was not an result of nutrient absorption (Constanzo, 1998). However, for the time being what was actually responsible for the decrease in body weight

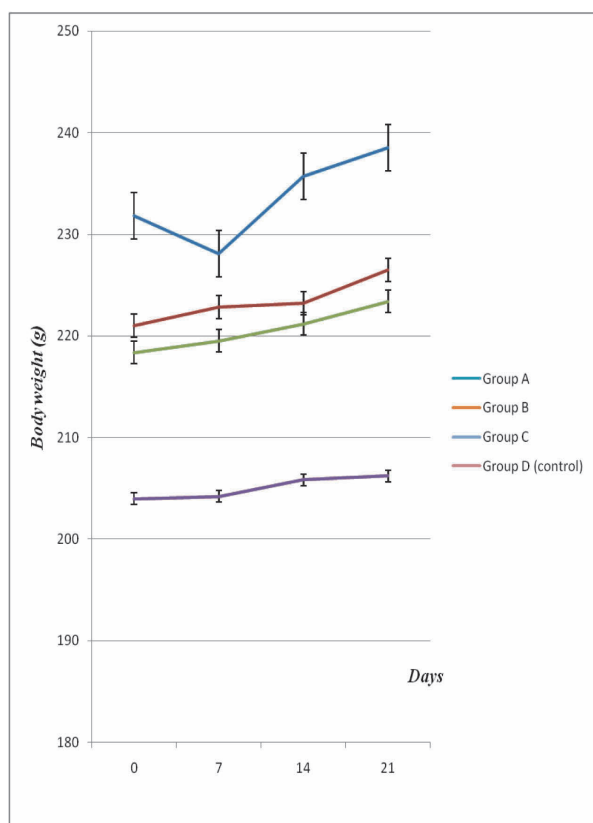


Fig. 3. Graph showing body weight changes in grams.

cannot be ascertained. Thereafter, the weight of the animals in all the treatment groups began to increase and this was observed along the remaining days of study. A similar study by Prasad et al. (2009) reported a statistically significant increase in the body weight of rats treated with leaf extracts of *C. roseus*.

From the histological sections of the livers of treated rats, there were no features of cellular degeneration, since the sinusoids in the sections of the treated animals were devoid of congestion, occlusions and were not waterlogged. There was no necrosis or oedema of the hepatocytes. There was no evidence of Mallory bodies in the liver parenchyma. The portal tracts were free of inflammation.

It is known that when certain types of cells are damaged, the enzymes they contain may become compromised. Alanine aminotransferase is one such enzyme. It is markedly elevated in acute liver damage. The enzyme aspartate aminotransferase has a similar role, but is found in various body tissues such as the heart, kidney, brain, lungs, muscles, and liver. This enzyme is compromised when these organs are damaged and is often used as a marker in determining the extent of damage. In viral hepatitis and other forms of liver disease associated with liver necrosis, the levels of AST and ALT are elevated before the clinical signs and symptoms of the diseases appear (Dufour et al., 2000).

Determination of ALT activity is a relatively sensitive indicator of hepatic damage in certain animal species and can help to determine whether further diagnostic tests (e.g., determination of creatine kinase activity, bile acid concentrations, or a liver biopsy) are necessary (Bain, 2003). The mechanisms of increased ALT activity include enzyme release from damaged cells and the induction of enzyme activity due to drug administration. The release of ALT from the cytosol may occur secondary to cellular necrosis or as a result of cellular injury, with membrane damage and bleb formation (Stockham and Scott, 2002).

In dogs, cats, rats, rabbits, and primates, ALT activity is highest in hepatocytes. Therefore, elevations in serum ALT activity are considered to be relatively specific for liver disease. However, measurement of serum ALT activity does not test hepatic integrity alone because increased serum ALT activity also may occur with striated muscle necrosis or injury (Stockham and Scott, 2002; Valentine et al., 1990). Ruminants, pigs, horses, and birds

have a much lower level of hepatocellular ALT activity. In these species, increased serum ALT activity is usually a reflection of skeletal muscle necrosis (Stockham and Scott, 2002).

Increased ALT activity can be caused by reversible or irreversible damage to hepatocytes including necrosis, ischaemia, enzyme induction (e.g., *anticonvulsants*, *glucocorticoids*, and *thiacetarsamide*), drug-induced hepatotoxicity (e.g., tetracycline in cats, carprofen in dogs), cholestasis, and trauma (Stockham and Scott, 2002; Hadley et al., 1990; Kaufman and Greene, 1993; MacPhail et al., 1998; Muller et al., 2000). These forms of hepatic damage may be acute or chronic. Acute hepatocellular injury tends to result in more marked elevations of ALT activity. In chronic hepatic disease, ALT activity may be within the reference interval or only mildly elevated (Stockham and Scott, 2002).

The reduction in the values of serum urea and creatinine following the administration of the ethanolic leaf extract of *Catharanthus roseus* suggests that the kidney may not be damaged by the administration of the aqueous extract. The physiology of the kidneys' functional units with regard to getting rid of urea and creatinine was not biochemically impaired.

Conclusions

In this study, oral administration of the ethanolic extract of *Catharanthus roseus* significantly reduced the level of AST and ALT in the liver, and the values of urea and creatinine were also significantly reduced. The histological outline of the plant extract on the kidney and liver as seen in the histological sections obtained from the treated rats supports this claim. This is indicative of the absence of a toxic effect of the ethanolic extract of the leaf of *C. roseus* on the kidney and liver at the doses administered to the animals here. In conclusion, the data obtained from this study show that oral administration of ethanolic leaf extract of *C. roseus* has no adverse effects on kidney and liver morphology. Our observations suggest that *C. roseus* is not hepatotoxic and has no adverse effect on the liver and kidney enzymes of the treated rats. Further studies should be directed towards isolating the specific component(s) of the plant responsible for the positive enhancing effects in order to standardize plant preparations for maximum therapeutic benefit.

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