International Journal of Biomedical and Health Sciences
Vol. 6, No. 4 December 31, 2010
Printed in Nigeria

0794-4748/2010 $12.00 + 0.00

http://www.asopah.org

IJBHS 2010156/6414

Madagascar periwinkle (*Catharanthus roseus*) Enhances Kidney and Liver Functions in Wistar Rats

Damilare Adedayo ADEKOMI

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

(Received September 30, 2010; Accepted November 6, 2010)

ABSTRACT: 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 the ethanolic leaf extract of *Catharanthus roseus*. Thirty-two wistar rats were used for this study and were randomly assigned into three treatments (n=24) and control (n=8) groups. The animals in the treatment groups A, B, and C respectively received 400mg, 300mg and 200mg per kilogram body weight of Madagascar periwinkle extract for twenty-one days, while the animals in the control group (group D) received equal amount of phosphate buffered saline (**PBS**). The administration was done orally using an orogastric tube for twenty-one days (21d). Twenty-four hours after the last administration, all the animals were sacrificed by cervical dislocation. Laparatomy was performed and the liver and kidney excised, trimmed free of fat, rinsed in cold phosphate buffered saline solution. The liver was quickly fixed in 10% formolsaline, 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 reduced significantly in a dose dependence pattern in all the experimental groups when compared vis-à-vis the control group. The results obtained from this study suggest that the oral administration of the ethanolic extract of *C.roseus* has no compromising effect on the kidney and liver and may enhanced to a greater extent the functional features of the organs in Wistar rats.

Key words: - Phosphate buffered saline, Kidney enzyme**,** Liver enzyme, Laparatomy, 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 [1, 2]. 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* is used in the folklore treatment of diabetes, malaria, leukemia, wasp stings, sore throat, eye irritation, infections [3]. It is also used a as an astringent, diuretic and expectorant. The plant contains about seventy alkaloids some of which include cartharathine, lochnenine, vindoline vindolinenine, vincristine, vinblastine, tetrahydroalstronine, reserpinne, serpentine, etc [4].

About 20% of the total cardiac output flows through the mammalian kidneys. The nephron is the functional and structural unit of the kidney. Kidneys of the mammalian species show variations in the ratio of the cortical and juxtamedullary nephrons but have in common a unique structural characteristic that is the presence of two capillary networks: the glomerular and the peritubular. Endothelial cells of the peritubular capillaries have endocrine activity, which is represented by synthesis and release of the erythropoietic hormone. The kidneys are the main source, around 85% of the circulating amount, of this hormone. (Junqueira, Carneiro & Kelley, 1998; Ganong, 1999) [5, 6].

Liver is the largest organ in the mammalian body. It is the centre of all metabolic activities in the body. Drugs and other foreign substances are metabolized and inactivated in the liver and is therefore susceptible to the toxicity from these agents. Certain medicinal agents when taken in overdoses 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 that are lined with highly active Kupffer cells, central veins and hepatocytes plates that are closely associated with the terminal lymphatics and bile canaliculi (Webster & Webster, 1974; Lesson *et al.*, 1988; Berne & Levy, 1998; Constanzo, 1998) [7, 8, 9,10]. The distinguished morphological features have enabled the liver to carry out a very 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, removal of ammonia in the form of urea, biosynthesis and release of the non-essential amino acids and plasma proteins with the exception of immuno gamma globulins, gluconeogenesis, storage of glycogen, conversion of carbohydrates and proteins into lipids, synthesis of lipoproteins, phospholipids and cholesterol, oxidation of fatty acids, storage of iron in the form of ferritin as well as storage of vitamins A, D and B_{12}. The hepatocytes are efficient in uptaking blood bilirubin to conjugate it mainly with glucoronic acid and then excrete it in 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, & Hall, 1996; Ganong, 1999; Nelson, & Cox, 2000) [11, 12, 13, 14, 15].

These vital organs have the ability of carrying out several essential functions. Maintenance of body fluids volumes and their electrolytes within normal limits participates in regulation of acid-base balance and blood pressure, excretion of the non-protein nitrogenous compounds, such as urea and uric acid, elimination of endogenous toxic waste agents. The kidneys help in getting rid of hazardous compounds such as the endogenously produced creatinine and many metabolites of hormones as well as ingested and administered chemicals and drugs (Griffin & Ojeda, 1996; Guyton & Hall, 1996; Nelson & Cox, 2000; Feraille & Doucet, 2001) [16, 13, 15, 17].

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. Commonly manifested by these insults are changes in enzyme levels and 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) [18] found that altered serum levels of these products in rats is an indication of the potential toxicity of the plant. The toxicity could as well result in tissue or organ damage. The organs most commonly affected are; liver, pancreas, and kidney among others. The aim of this study therefore, is to investigate the effect of the plant extract on the some kidney and liver.

Materials 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 Gallenkamp (FA2104A, England) electronic weighing balance and grinded with Blender/Miller III, (model MS - 223, China).

Five hundred and fifty two grams (552g) of the dried powdered sample was however soaked in five liters of 70% ethanol for 24 hours at room temperature with constant shaking on a shaker (Stuart Scientific Orbital Shaker, UK), and then filtered through silk cloth (Mostofa, 2007) [19]. The filtrate was concentrated using a rotary evaporator (Rotavapor® R-210) at 42- 47°C to obtain 56.50g alcohol-free residual extract of *Catharanthus roseus*.

Toxicity evaluation:

The method of Lorke (1983) [20] 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, from

which, experimental doses of 400, 300 and 200mg/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 as A, B, and C were administered 400mg, 300mg and 200mg per kilogram 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 the animals were housed in clean cages of dimensions 33.0×20.5×19.0 cm contained in well ventilated standard housing conditions (temperature: 28–31^{o}C; humidity: 50–55%). Their cages were cleaned everyday.

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 [21].

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 the animals were sacrificed using cervical dislocation [22], Laparatomy was performed and the kidney and liver were excised, trimmed free of fat and rinsed in cold phosphate buffered saline solution[23]. The liver was fixed in 10% formolsaline, 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 [24] staining techniques. After fixation, the tissues were embedded in paraffin wax; serial sections of 5µ thick were produced using Leitz Rotary microtome (Leitz 1512 Microtome). The sections were mounted in DPX and examined with the aid of Olympus (XSZ-107BN, No. 071771) binocular light microscope. The photomicrograph of each slide was taken with a Nikon Digital Camera DXM1200F (Nikon, Japan) for subsequent histological analysis**.**

Estimation Of Liver Enzymes

The levels of alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were estimated in homogenates of the liver 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 3000rpm for 10 minutes at 4°C. The supernatant was used for the assay of the liver enzymes assay.

Alanine Aminotransferase (ALT)

An ultra- violet light source was used to measure spectrophotometrically the activity of the enzyme alanine aminotransferase (ALT) in homogenate samples. The optimal wavelength is 340 nm. Enzyme assay was performed using specific kits manufactured and marketed by Randox Laboratories Ltd, UK.

Aspartate Aminotransferase (AST):

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 sample was collected (using a sterilized needle and syringe) from the abdominal aorta of each rat (both the treatment and control groups) into marked conical test tubes. 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 common routine

biochemical tests for the kidney functions 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 kit of reagents (Hichem Creatine Pak, Elan Diagnostics, USA) were employed to evaluate the creatinine content of the serum samples.

Serum Urea

Evaluation of 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 Hichem kit for blood urea nitrogen analyzer. The kit was supplied by Elan Diagnostics, USA.

Statistical Analysis

Data were statistically evaluated using the Student's t-test with SPSS/14.0 software (SPSS Inc, Chicago, USA) and Excel 2007 (Microsoft Corporation, USA) and were expressed as Mean ± 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) [20] 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, from which, doses of 200, 300 and 400mg/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 with 500 and 550 mg/kg body weight of the plant extract died after 48hrs of administration. Although, prior to their death it was observed that they abstained completely from their feed, there was loss of motor activities as the animals rarely moved. At the 45th hour of administration, the animals began to convulse and were completely immobile.

However, the animals administered with 450mg/kg body weight of the ethanolic plant extract were moderately active but later became docile and they as well abstained from their feed only to return to normal activity at about 7hrs after administration of that particular dose. According to Chattopadhyay (1999) [25], it was noted that a 70% ethanol extract of leaves of Catharanthus roseus in an oral dose of 400 mg/kg was shown to be 20% as effective as tolbutamide in diabetic rats, though 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 when compared with that in the control group is as presented 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 control. Among the treated groups, higher body weight was recorded in the group (**A**) that was administered with 400mg/kg body weight of the extract followed by group (**B**) administered with 300mg/kg body weight and then group (**C**) administered with 200mg/kg body. Thus the administration of the ethanolic leaf extract of *Catharanthus roseus* increased the body weight of the treated animals in a dose dependent pattern. Results of the present study is in agreement with the reports of Mostofa *et al*. (2007) [19] and Iweala and Okeke (2005) [26].

Gross Observation

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 the termination of experimental procedure. The kidney and liver (with all their component parts) of the animals 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. Various 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 constitute the bulk of the parenchyma with different shapes, diameters and staining intensity (Fig 1).

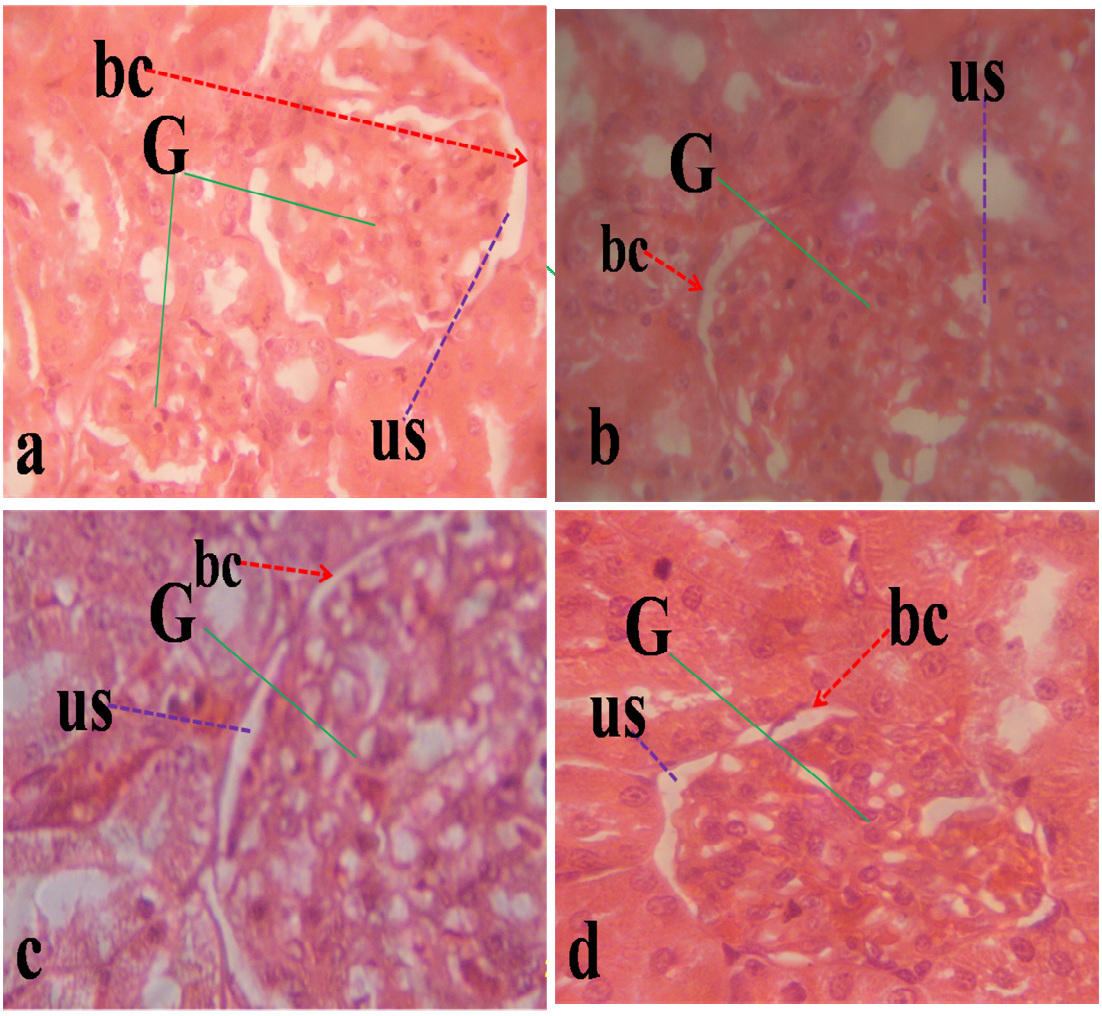

Figure 1; showing the photomicrograph of the cortical potion of the kidney in the treatment groups (**a, b,** and **c**) and the control group (**d**); the glomerulus (**G**), the Bowman's capsule (**bc**), the urinary space (**us**). (**H&E** x **1920**)

Microscopic Observations Of The Liver

When the sections obtained from the histological processing of the liver was viewed under the microscope it was observed that the sections conform to normal histological features. The sinusoids in the sections of the treated animals are devoid of occlusions and are not distorted. The extent of conformity to normal histological outline was observed to be higher in the treated groups compared with the control group (Fig 2).

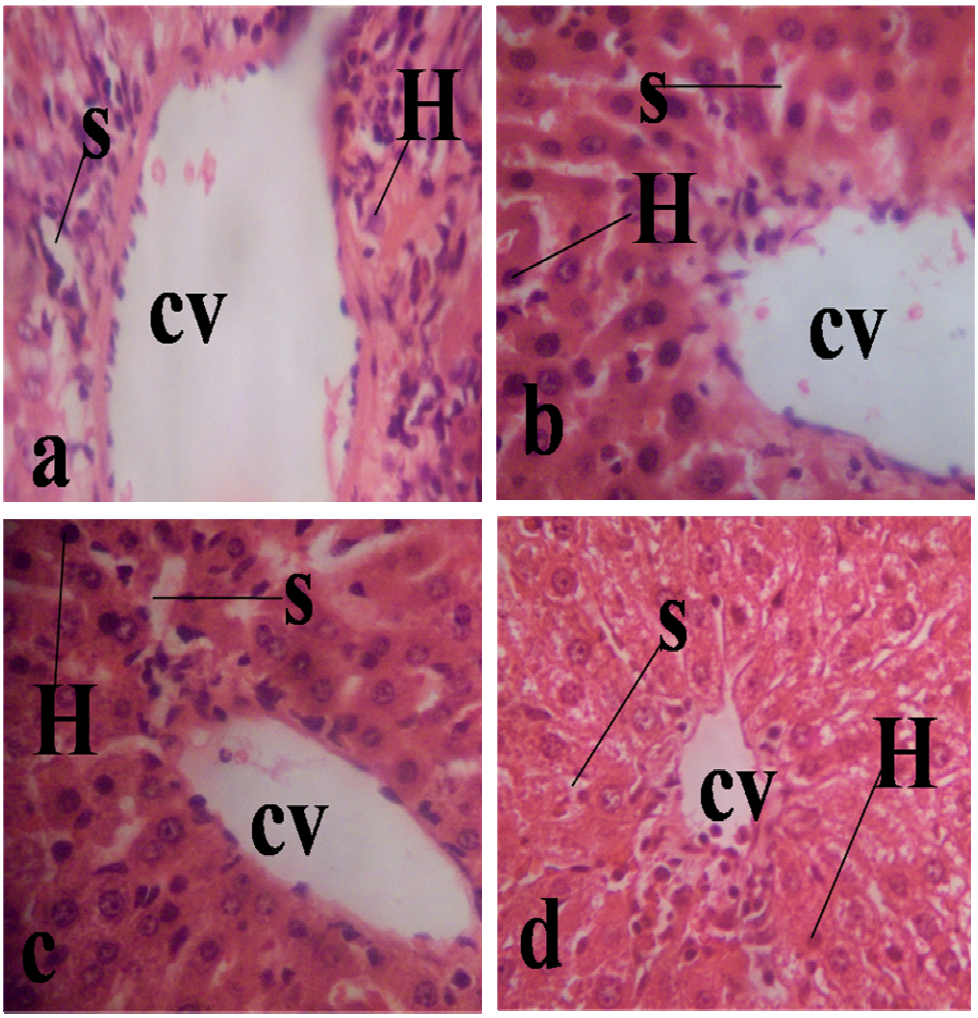

Figure 2; showing the photomicrograph of the liver in the treatment groups (**a, b,** and **c**) and the control group (**d**); the hepatocyte (**H**), the sinusoid (**s),** and the central (centrolobular) vein (**cv**)**. (H&E x 1920)**

Alanine Aminotransferase (ALT)

Table 2 shows changes in liver alanine aminotransferase (ALT) levels in various treatment groups vis-à-vis the control. Alanine aminotransferase (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 aspartate aminotransferase (AST) activities in the liver of the treatment groups versus the control are also presented in (Table 2). Changes in aspartate aminotransferase (AST) levels were comparative to the trend obtained for alanine aminotransferase (ALT). At the expiration of the study, the levels of aspartate aminotransferase (AST) activities in the liver of the treatment groups was significantly low at ($P<0.05$).

Activities Of Urea And Creatinine

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

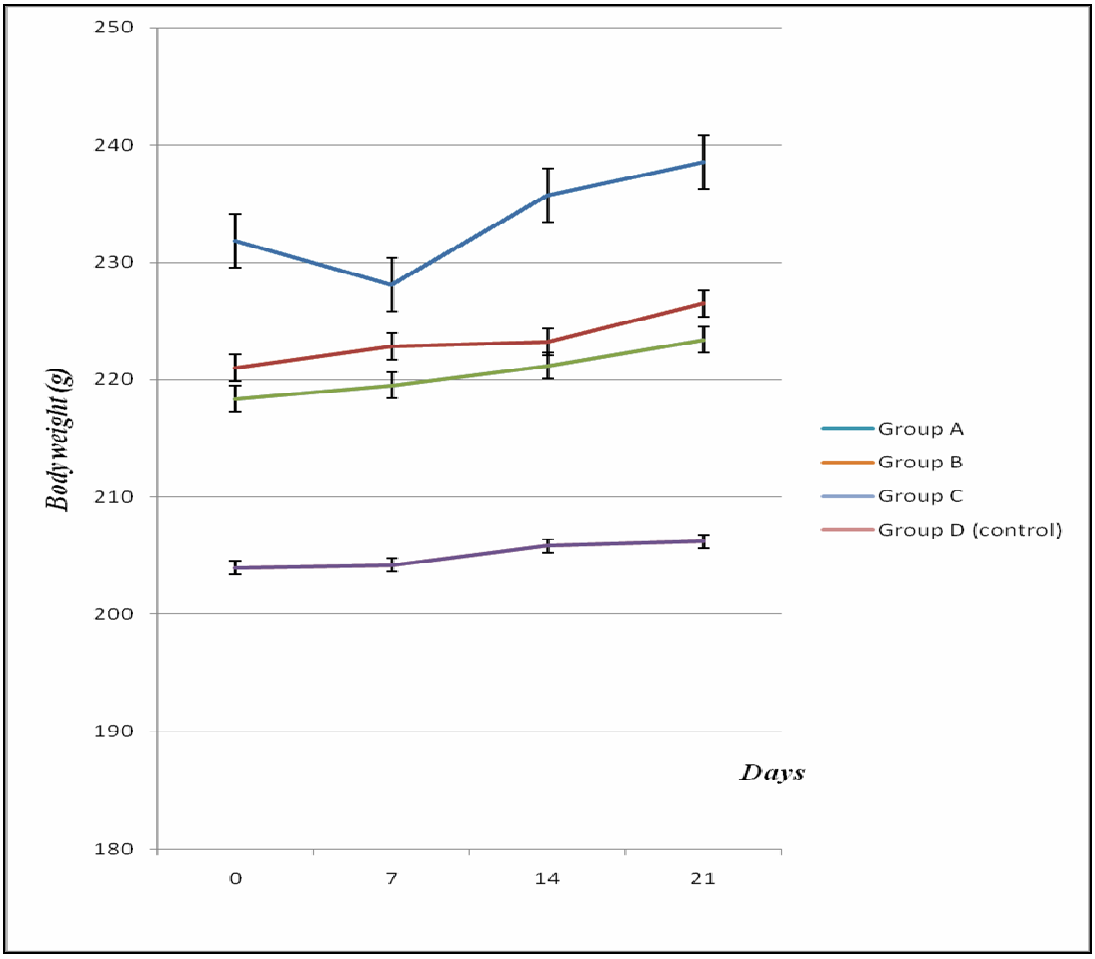

Fig 3; Graph showing the body weight changes in grams

Table 2; Liver enzymes indexes of the control and extract treated rats.

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

P value of less than 0.05 indicates a significant difference between the compared means.

Table 3; Kidney metabolites indexes of the control and extract treated rats.

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

P value of less than 0.05 indicates a significant difference between the compared means.

Discussion

The quest for the search of 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 after 21d of administration. Estimation from the use of the adopted method of Lorke (1983) [20], provided a tolerable dose of the ethanolic extract of *Catharanthus roseus* by the animals. Dapar *et. al* (2007)[27] 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 should be a matter of concern.

However, toxicity evaluation of plant extract or drug is not a very reliable procedure in the determination of toxicity as there is a wide variation in results between different species and even in the same specie under different experimental conditions. Moreover, toxicity test does not provide comprehensive information on what 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 toxicity of the extract. Notwithstanding, toxicity test in conjunction with photomicrographs of stained and processed histological tissue sections provides a clue as to the toxic characteristics of the drug or plant Dapar *et. al* (2007)[27].

It was observed during the course of this study that the plant extract has a modulating effect of 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 with 400mg/kg and 300 mg/kg body weight of the extract on the seventh (**7th**) and fourteenth (**14th**) day respectively of administration compared with other animals in the other treatment and the control groups (Fig

3). Histological examination of the kidney and liver 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 implication of nutrient absorption [10]. Although what was responsible for the decrease in body weight could not be ascertained as of now. Thereafter, the weight of the animals in all the treatment groups began to increase and this was observed through out the remaining days of study. A similar study by Prasad *et. al.,* (2009) [28] showed a statistically significant increase in the body weight of rats treated with leaf extract of *C. roseus*.

From the histological sections of the liver of treated rats, there were no features of cellular degeneration, as the sinusoids in the sections of the treated animals are devoid of congestions, occlusions and are not water lodged. 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 inflammations.

It is known that when certain types of cells are damaged, the enzymes they contained may become compromised. Alanine aminotransferase (ALT) is one of such enzyme. It is markedly elevated in acute liver damage. The enzyme, aspartate aminotransferase (AST), has 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 diseases associated with hepatic liver necrosis, the level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are elevated before clinical signs and symptoms of the diseases appear [29].

Determination of ALT activity is a relatively sensitive indicator of hepatic damage in certain animal species and can help determine whether further diagnostic tests (*i.e.,* determination of creatine kinase activity, bile acid concentration, or a liver biopsy) are necessary. [30]. Mechanisms of increased activity of ALT include enzyme release from damaged cells or induction of enzyme activity from drug administration. 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. [31]

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. [31, 32]

Ruminants, pigs, horses, and birds have a much lower level of hepatocellular ALT activity. In these species, increased serum ALT activity usually is a reflection of skeletal muscle necrosis. [31] Increased ALT activity can be caused by reversible or irreversible damage to hepatocytes including necrosis, ischemia, enzyme induction (*i.e.,* anticonvulsants, glucocorticoids, and thiacetarsemide), drug-induced hepatotoxicity (*i.e.,* tetracycline in cats, carprofen in dogs), cholestasis, and trauma. [31, 33, 34, 35, 36] These forms of hepatic damage can be acute or chronic. Acute hepatocellular injury tends to result in greater elevations of ALT activity. In chronic hepatic disease, ALT activity may be within the reference interval or mildly elevated. [31]

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

Conclusion

In this study, the oral administration of the ethanolic extract of *Catharanthus roseus* significantly reduced the level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the liver and the values of urea and creatinine was 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 support this claim. This is indicative of the non-toxic effect of the ethanolic extract of the leaf of *C. roseus* on the kidney and liver at the doses administered to the animals in this study. In conclusion, data obtained from this study showed that the oral administration of ethanolic leaf extract of *C. roseus* has no adverse effects on the kidney and liver morphology.

The observations from this study suggested 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 the plant preparation for maximum therapeutic benefit.

References

1. Don, G. (1999): *Catharanthus roseus*. In; *Medicinal plants of the World* (edited by Ross.I.A) Human press, Totowa, New Jersey, pp109-118.
2. Morton, J. F. (1991): *Major medicinal plants; botany, culture and Use* (1st Ed) Charles. C. Thomas Publishing Company, USA pp 236-241.
3. Stolle, K. and Greoger, D. (1967): *Catharanthus roseus*-A new medicinal plant. *Pharm. Zentralh. Deut*. 106:285-306.
4. Gordon, S. H., Marvin, G. and Marry, R. A. (1964): Alkaloids of *Vinca rosea*; A Preliminary report on hypoglycemic activity. *Lloydia* 27:361-363.
5. Junqueira L, Carneiro J and Kelley R (1998); *Basic histology*. Stamford: Appleton and Lange
6. Ganong W (1999); *Review of medical physiology*. Stamford: Appleton and Lange.
7. Webster D and Webster M (1974); *Comparative Vertebrate Morphology*. New York: Academic Press.
8. Lesson *et al.*, (1988); *Text / Atlas of Histology*. London: W. B. Saunders Co.
9. Berne R and Levy M (1998); *Physiology*. New York: C. V. Mosby Co.
10. Constanzo L (1998); *Physiology*. Philadelphia: W. B. Saunders Co.
11. Johnson P (1995); The assessment of hepatic function and investigation of jaundice. In W. Marshall, & S. Bangert, (Eds.), *Clinical Biochemistry: Metabolic and Clinical Aspects* (pp. 217- 236). New York: Churchill Livingstone.
12. Stryer L (1995); *Biochemistry*. New York: W. H. Freeman and Company.
13. Guyton A and Hall J (1996); *Textbook of medical physiology*. Philadelphia: W. B. Saunders Co.
14. Ganong W (1999); *Review of medical physiology*. Stamford: Appleton and Lange.
15. Nelson D and Cox M (2000); *Lehninger Principles of Biochemistry*. New York: Worth Publishers.
16. Griffin J and Ojeda S (1996); *Textbook of endocrine physiology*. Oxford: Oxford University Press Inc.
17. Feraille E and Doucet E (2001); Sodium- potassium- adenosinetriphosphatase- dependent sodium transport in the kidney: Hormonal control. *Physiological Reviews*, 81, 345- 408.
18. Wannang NN *et. al.,* (2005); Evaluation of anti-snake venom activity of the Aqueous root extract of *Securidaca longepedunculata* in rats. J. Pharm. Bio. Res. 2(2): 80-83.
19. Mostofa .M, *et. al.,* (2007); Antidiabetic Effects of *Catharanthus roseus*, Azadirachta *indica*, *Allium Sativum* and Glimepride in experimentally Diabetic induced rat, *Bangl. J. Vet. Med*. 5 (1 & 2): 99–102.
20. Lorke D (1983). A New Approach to Practical Acute Toxicity Testing, Arch. Toxicol. 54: 275-287.
21. National Institute of Health Guide for the Care and Use of Laboratory Animals: DHEW Publication (NIH), revised, Office of Science and Health Reports, DRR/NIH, Bethesda, USA, 1985.
22. Ofusori *et al*., (2008); Waterleaf (Talinum triangulare) Enhances Cerebral Functions in Swiss albino mice. Journal of Neurological Sciences [Turkish] **25: (4)** # 17; 239-246.
23. Akinola *et. al.,* (2009); Effect of Azadirachta indica on islets of Langerhans in Streptozotocin-induced diabetes mellitus.
24. Carleton H. (1967); Carleton's histological technique. 4th Ed, Oxford University Press; pp. 33-280.
25. Chattopadhyay RR (1999) "A comparative evaluation of some blood sugar lowering agents of plant origin" *J Ethnopharmacol* 67:367–72.
26. Iweala E. E. J and Okeke C. U (2005); Comparative study of the hypoglycemic and biochemical effects of C*atharanthus roseus* (Linn) g. apocynaceae (Madagascar periwinkle) and chlorpropamide (diabenese) on alloxan-induced diabetic rats. BIOKEMISTRI 17(2):149-156.
27. Dapar *et. al* (2007); The histopathologic effects of *Securidaca longepedunculata* on heart, liver, kidney and lungs of rats. African Journal of Biotechnology Vol. 6 (5), pp. 591-595.
28. Prasad *et. al.,* (2009); Antidiabetic Activity of Some Herbal Plants in Streptozotocin Induced Diabetic Albino Rats. Pakistan Journal of Nutrition, Volume: 8 | Issue: 5 | Page No.: 551-557.
29. Dufour DR, Lott JA, Nolte FS, Gretch DR, *et.al* (2000); Diagnosis and Monitoring of Hepatic Injury. II. Recommendations for laboratory test in screening, diagnosis and monitoring. Clin Chem 46; 2050-68.
30. Bain PJ (2003): Liver. *In:* Latimer KS, Mahaffey EA, Prasse KW: Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 4th ed. Ames, Iowa State Press, pp. 193-214.
31. Stockham SL and Scott MA (2002): Fundamentals of Veterinary Clinical Pathology. Ames, Iowa State University Press, pp. 434-459.
32. Valentine BA, Blue JT, Shelley SM, Cooper BJ (1990): Increased serum alanine aminotransferase activity associated with muscle necrosis in the dog. J Vet Intern Med 4:140-143.
33. Hadley SP, Hoffmann WE, Kuhlenschmidt MS, Sanecki RK, Dorner JL (1990): Effect of glucocorticoids on alkaline phosphatase, alanine aminotransferase, and gamma-glutamyltransferase in cultured dog hepatocytes. Enzyme 43:89-98.
34. Kaufman AC and Greene CE (1993): Increased alanine transaminase activity associated with tetracycline administration in a cat. J Am Vet Med Assoc 202:628-630.
35. MacPhail CM, Lappin MR, Meyer DJ, Smith SG, Webster CR, Armstrong PJ (1998): Hepatocellular toxicosis associated with administration of carprofen in 21 dogs. J Am Vet Med Assoc. 212:1895-1901.
36. Muller PB, Taboada J, Hosgood G, Partington BP, VanSteenhouse JL, Taylor HW, Wolfsheimer KJ (2000): Effects of long-term phenobarbital treatment on the liver in dogs. J Vet Intern Med 14:165-171.