ISSN 2347-6893

Toxicological evaluation of chronic administration of Catharanthus roseus extract in the adult albino rats

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ABSTRACT

Herbal drugs are common use in many developing countries because of its effectiveness and low cost. World Health Organization approved its use as treatment for many diseases. So it should study efficacy and safety the natural source of these drugs. Catharanthus roseus is a popular ornamental plant. It contains about seventy alkaloids and used for multiple therapeutic purposes**.** Available literature on Cathranthus indicated to controversial issue about its safety. This study evaluates toxicological effect the leaf extract of Catharantus roseus in the rats by assessment biochemical and histopathological changes of liver and kidney, morphological changes of pancreas and heart with study some hematological parameters. Sixty albino rats were divided into three groups, each group consists of twenty rats. Control group received water, while second and third group received 300 and 400mg/kg of leaf extract of Catharanthus roseus, respectively and daily by orogastric tube for three months. Prolonged use of Catharanthus roseus led to significant abnormalities of liver and renal function tests, and some hematological parameters associated with hepatic, renal, cardiac and pancreatic histopathological changes which increased based on the dose. Repeated use of Catharanthus roseus caused hepatic, renal, hematological, cardiac and pancreatic toxicity depending on the dose.

Indexing terms/Keywords

Catharanthus; Chronic use; Toxicity

Academic Discipline And Sub-Disciplines

Education; biology; pathology;

SUBJECT CLASSIFICATION

Library of Congress Classification

TYPE (METHOD/APPROACH)

Sixty albino rats were divided into three groups, each group consists of twenty rats. Control group received water, while second and third group received 300 and 400mg/kg of leaf extract of Catharanthus roseus, respectively and daily by orogastric tube for three months

Council for Innovative Research

Peer Review Research Publishing System

Journal: Journal of Advances in Biology

Vol. 6, No. 1

editorsjab@gmail.com , editor@cirjab.com

ISSN 2347-6893

Introduction

Plant and phytochemical products play an important role in the modern medicine. In recent years, Many drugs are botanical because it has been isolated from natural source. It is used as treatment for many diseases although some of these drugs have harmful side effects (1). Herbal drugs or its extracts are wide use because of its effectiveness and low cost although its unknown active principles. In many developing countries, herbal medicine is an important source of health care. After approval the World Health Organization "WHO" for its use as treatment for different diseases, further studies should be needed to assure the efficacy and safety of plant extracts carefully (2).

Catharanthus roseus is a popular ornamental plant because it is found in the gardens and homes across the world. It has many therapeutic effects and used as anti-inflammatory, antimalarial, antitumor, antiarrhythmic, antihypertensive, antimitotic, antifertility, antispasmodic, antifungal, antihypercholesterolemic, antimutagenic, antiviral, cardio tonic, CNS depressant, diuretic, antispermatogenic, expectorant and disinfectant agents (3).

Catharanthus roseus (Vinca rosea) is known as Madagascar periwinkle. It is a perennial herb of the Apocynaceae family (4). It contains about seventy alkaloids such as cartharathine, lochnenine, vindoline vindolinenine, vincristine, vinblastine, tetrahydroalstronine, reserpinne, serpentine (5). Available literature on Cathranthus only indicated toxic effects of the synthetic alkaloids and cytotoxic effects of the crude extract on cell division and the nerves which control digestion, bowels, cardiac and sexual function (6). Researchers discovered the presence of highly toxic alkaloids in tissues of Catharanthus roseus. These alkaloids are vincristine and vinblastine which are approved by the US Food and Drug Administration (FDA) for use in the cancer chemotherapy. It is now used in treatment the different types of cancer such as Hodgkin's disease, leukemia, Kaposi's sarcoma and malignant lymphomas. It raised the survival rate in the childhood leukemia from less than 10 % in 1960 to over 90 % today (7, 8).

Liver and kidney are essential organs for metabolism and excretion of drugs, and toxic agents. Drugs and other foreign substances are metabolized and inactivated in the liver. Therefore, it is susceptible to toxicity of these agents. Certain medical agents may injure the liver, when it is taken at high doses or even within therapeutic doses administration (9). Kidneys eliminate the end toxic metabolites of any agent because about 20% of total cardiac output flows through the kidneys (10).

So, the present study aims to evaluate the toxicological effect of chronic administration the aqueous leaf extract of Catharantus roseus by assessment hepatic and renal functions, some hematological parameters and morphological changes of liver, kidney, pancreas and heart.

Material and methods

Sixty healthy adult albino rats weighing 200-300 g were obtained from the animal house of king Abdel Aziz University-Jeddah. Rats were exposed to 12 hr day-night cycles. It had free access to water and food during the experimental period. Animals divided into three groups, each group consists of twenty rats. The first group (control) received distilled water only while the second received 300 mg/kg of leaf extract of Catharanthus roseus**.** The third group received 400 mg/kg of leaf extract of Catharanthus roseus (11). Administration the leaf extract of Catharanthus roseus and distilled water were done daily by orogastric tube for three months.

Preparation the Catharanthus roseus leaf extract

Catharanthus roseus was collected from medicinal garden (Cairo- Egypt). It was identified by botanical expert. Fresh leaves of Catharanthus roseus were washed with distilled water and then dry at room temperature for 15 days. The dried material was grinded to become powder. 100 gram of powder was ready for mixing with 500 ml of distilled water. It was stirred in the container for one day at room temperature**.** Liquid extract was evaporated by vacuum distillation to become dry. The residue was filtered and then dried to remove wetness. The dried residue was weighed and then dissolved in 5ml sterile water and stored at 4°C (12).

Blood Sample Collection

At the end of experiment, the rats were anesthetized with diethyl ether. Blood samples were obtained from the orbital sinus using heparinized capillary tubes and collected into EDTA tubes for blood cells and platelets counts. Prothrombin time was measured with a coagulometer (Sysmex® CA-1500-Siemens-Healthcare Diagnostics) (13). The samples were centrifuged at 3000 rounds at 4°C for 10 min to separate the serum for hepatic and renal biomarkers assessment. Assay of AST was performed by mixing the serum to buffered solution of L- aspartic acid and 2- ketoglutarate and then incubated for one hour at 37 °C. After incubation, 1 mm of DNPH and 0.4m of NaOH was added (14). Assay of ALT was performed by mixing the serum to buffered solution of DL- alanine and 2- ketoglutarate, and then incubated for thirty minutes at 37 °C. After incubation, 1 mm of DNPH and 0.4m of NaOH was added. Assay of ALk.Ph was performed by using p- nitrophenol phosphate as substrate, in alkaline buffer with fresh unhemolysed serum for 45 min at 12°C (15). Assay of albumin was performed by using the dye binding method. Bromocresol green reacted with albumin in acid solution to get blue-green colour complex**.** Assay of bilirubin was performed by spectophotometric method. It involved measurement the maximum absorbance of bilirubin at 437 nm (16). Urea and creatinine were determined by routine colorimetric methods using the commercial kits and quantify on clinical biochemistry autoanalyser (17).

Histopathological studies

All animals were sacrificed under excess anesthesia after 24 hours from the last administration of leaf extract of Catharanthus roseus. Incision was performed in the chest and abdomen for kidney, liver, pancreas and heart excision. Tissue specimens (liver, kidney pancreas and heart) were collected from three groups and then fixed in 10% neutral buffered formalin. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned at 4-6 µm thickness and stained by haematoxylen and eosin, Periodic Acid Schiff (PAS) and Mallory stain (18).

Statistical analysis

Statistical analysis was performed using SPSS version 17. Variability of results was expressed as mean ± SD. Results were analyzed by using one-way ANOVA and post-hoc multiple comparison test (TUKEY) to investigate the difference among groups. *P* value of 0.05 was considered statistically significant.

Ethical considerations

The most appropriate animal species was chosen for this research. Promotion of a high standard of care and animal well-being at all times was done. Appropriate sample size was calculated by using the fewest number of animals to obtain statistically valid results.

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Painful procedures were performed under anesthesia to avoid distress and pain. Our standards of animal care and administration met those required by applicable international laws and regulations.

Results

Table (1) represents mean ±SD values of liver function tests in the rats. Mean ±SD values of **AST** in control group which received distilled water, second group which received 300 mg/kg of Catharanthus roseus leaf extract and third group which received 400 mg/kg of Catharanthus roseus leaf extract, were 33.5±2.2, 149.9±4.2 and 189.4±5.4 respectively. Value of F was 7472.16 and statistical significant whereas P > 0.001. The Mean ±SD values of **ALT** in control group, second group, and third group were 33.9±2.6, 108.1±2.6 and 206.8±7.4 respectively. Value of F was 6553.94 and statistical significant whereas p > 0.001. The Mean ±SD values of **ALK.Ph** in control group, second group and third group were 74.7±2.9, 239.4±7.4 and 277.4±9.2 respectively. Value of F was 4639.17 and statistical significant whereas p > 0.001. The Mean ±SD values of **albumin** in control group, second group, and third group were 4.5±0.22, 7.3±0.23 and 8.4±0.31 respectively. Value of F was 1176.54 and statistical significant whereas p > 0.001. The Mean ±SD values of **bilirubin** in control group, second group, and third group were 0.5±0.06, 1.8±0.15 and 2.7±0.14 respectively. Value of F was 1600.02 and statistical significant whereas p > 0.001. Mean ±SD values of **prothrombin time** in control group, second group and third group were 12.2±0.1, 20.6±0.39 and 22.8±0.43 respectively. Value of F was 5371.74 and statistical significant whereas p > 0.001.

Table (1). Effect of chronic administration the Catharanthus roseus leaf extract on Mean + SD of rats liver function tests.

Group / Parameter	First M±S.D	Second M±S.D	Third M±S.D	F
AST	33.5±2.2	149.9±4.2*	189.4±5.4**	7472.16
ALT	33.9±2.6	108.1±2.6*	206.8±7.4**	6553.94
Alk. Ph.	74.7±2.9	239.4±7.4*	277.4±9.2**	4639.17
Albumin	4.5±0.22	7.3±0.23*	8.4±0.31**	1176.54
Bilirubin	0.5±0.06	1.8±0.15*	2.7±0.14**	1600.02
PT	12.2±0.1	20.6±0.39*	22.8±0.43**	5371.74

Number per group = 20 SD = standard deviation
AST = Aspartate aminotransferase.
ALT = Alanine aminotransferase.
ALk.Ph = Alkaline phosphatase.
PT = Prothrombin Time
First group (control) received distilled water.
Second group received 300 mg/kg of Catharanthus roseus leaf extract.
Third group received 400 mg/kg of Catharanthus roseus leaf extract
* = p < 0.001 (significant difference in comparison with control group)
** = p < 0.001 (significant difference in comparison with second group)

Table (2) represents mean ±SD values of renal function tests in the rats. Mean ±SD values of **urea** in control group, second group, and third group were 27.9±4.06, 82.5±1.4 and 98.0±3.1 respectively. Value of F, was 2868.83 and statistical significant whereas p > 0.001. Mean ±SD values of **creatinine** in control group, second group and third group and were 0.71±0.11, 1.8±0.11 and 2.8±0.11 respectively. Value of F 1708.67 and statistical significant whereas p > 0.001.

Table (2). Effect of chronic administration the Catharanthus roseus leaf extract on Mean +SD of rats renal function tests.

Group / Parameter	First M±S.D	Second M±S.D	Third M±S.D	F
WBC_S	5.7±0.18	7.6±0.20*	9.0±0.58**	402.12
RBC_S	5.8±0.05	3.8±0.09*	3.1±0.02*	8884.69
PLAT	463.2±1.8	442.6±1.5*	413.1±7.3**	638.862

Number per group = 20 SD = standard deviation
First group (control) received distilled water.
Second group received 300 mg/kg of Catharanthus roseus leaf extract.
Third group received 400 mg/kg of Catharanthus roseus leaf extract
* = p < 0.001 (significant difference in comparison with control group)
** = p < 0.001 (significant difference in comparison with second group)

Table (3) represents mean ±SD values of some hematological parameters in the rats. The Mean ±SD values of **white blood cells count** in control group, second group and third group were 5.7±0.18, 7.6±0.20 and 9.0±0.58 respectively. Value of F was 402.12 and statistical significant whereas p > 0.001. The Mean ±SD values of **red blood cells count** in control group, second group and third group were 5.8±0.05, 3.8±0.09 and 3.1±0.02 respectively. Value of F was 8884.69 and statistical significant whereas p > 0.001. The Mean ±SD values of **platelets count** in control group, second group and third group were 463.2±1.8, 442.6±1.5 and 413.1±7.3 respectively. Value of F 638.862 and statistical significant whereas p > 0.001.

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Table (3). Effect of chronic administration the Catharanthus roseus leaf extract on Mean +SD of some hematological parameters in the rats.

Group / Parameter	First M±S.D	Second M±S.D	Third M±S.D	F
Urea	27.9±4.06	82.5±1.4*	98.0±3.1**	2868.83
Creatinine	0.71±0.11	1.8±0.11*	2.8±0.11**	1708.67

Number per group = 20 SD = standard deviation
WBC_S= White blood cells
RBC_S= Red blood cells
PLAT= Platelets
First group (control) received distilled water.
Second group received 300 mg/kg of Catharanthus roseus leaf extract.
Third group received 400 mg/kg of Catharanthus roseus leaf extract
* = $p < 0.001$ (significant difference in comparison with control group)
** = $p < 0.001$ (significant difference in comparison with second group)

Histopathological findings

A. Hepatic histopathological findings by light microscope.-

Histopathological assessment of the control liver section group, showed normal hepatic architecture **(Fig.1$_a$)** with normal distribution of collagen and small amount of wavy fibrils **(Fig.2$_a$)** and normal positive reaction of PAS based on mucopolysaccharide granules in the cytoplasm of hepatocytes **(Fig.3$_a$).** However, hepatic tissues rats of second group which received 300mg/kg of leaf extract of Catharanthus roseus, showed marked hepatic disorganization, coarse, pink vacuoles in the cytoplasm, inflammatory cellular infiltration with small contracted and abundant fragmented pycknotic nuclei around the central vein, necrosis of hepatocytes, degenerated kupffer cells and fibrosis of central vein (**Fig.1$_b$**) with thick bundles of collagen fibers which consists of wavy sporadic or fused fibrils **(Fig.2$_b$)** and mild reduction the positive reaction of PAS in comparison with control group.**(Fig.3$_b$)**. And transverse section of hepatic tissues in the rats of third group which received 400mg/kg of leaf extract of Catharanthus roseus, revealed severe damage of hepatic architecture, increase the number of vacuoles and inflammatory cellular infiltration, small abundant fragmented pycknotic nuclei around the central vein with hypertrophy of some hepatocytes **(Fig.1$_c$)** with more deposition of collagen fibers **(Fig.2$_c$)** and severe reduction the positive reaction of PAS **(Fig.3$_c$).**

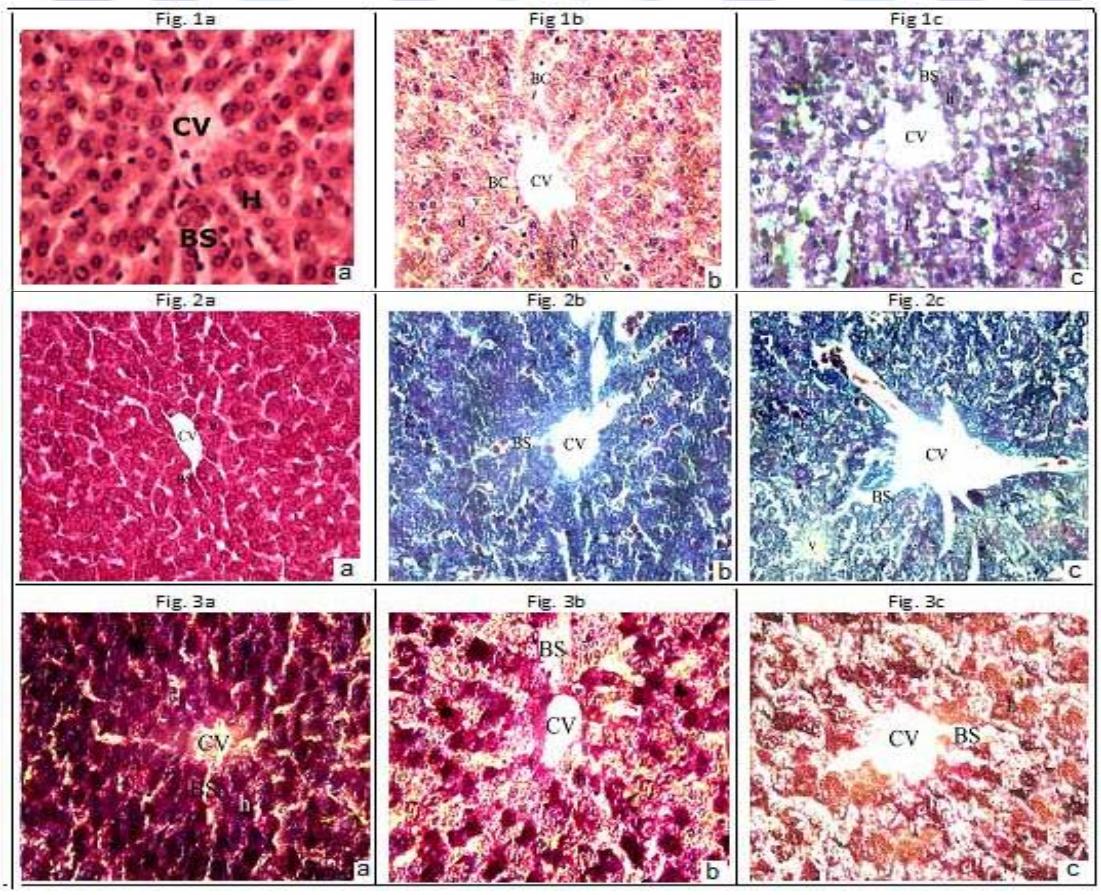

Fig (1a) a photomicrograph of transverse section in the control rat liver shows normal hexagonal hepatic lobules with portal triads at the vertices and a central vein (CV) in the middle. Hepatocytes (H) are arranged into hepatic cords and separated by adjacent blood sinusoids (BS). (1b) second group rat liver shows widening of central vein (CV), foamy hepatocyte cytoplasm filled with vacuoles (v), necrosis of some hepatocytes (h) and contracted, pycknotic nuclei with

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condensed chromatin (P). Widening of blood sinusoids (BS) with degenerated area (d). (1c) a third group rat liver shows more widening of central vein (CV), more foamy hepatocyte cytoplasm with increase the size of vacuoles (v), necrosis of some hepatocytes (h) with contracted, pycknotic nuclei and condensed chromatin (P), widening of blood sinusoids (BS) and degenerated area (d). (H&E X400). Fig (2a) a photomicrograph of transverse section in the control rat liver shows normal distribution of collagen and small amount of wavy fibrils, normal hepatocytes (h), central vein (CV) and blood sinusoids (BS). (2b) a section of second group rat liver shows thick bundles of wavy collagen fibrils around the central vein (CV),enlarged hepatocytes (h) and widening of blood sinusoids (BS). (2c) a section of third group rat liver shows more deposition of collagen fibers around the central vein (CV), enlarged hepatocytes (h) and widening of blood sinusoids (BS). (Mallory X400). Fig (3a) a photomicrograph of transverse section in the control rat liver shows normal positive reaction of PAS, central vein (CV), hepatocytes (h) and blood sinusoids (BS). (3b) a section of second group rat liver shows mild decrease in PAS reaction, hepatocytes (h) with increase the number of vacuoles (v), widening of central vein (CV) and blood sinusoids (BS). (3c) a section of third group rat liver shows marked decrease in PAS reaction, hepatocytes (h) with increase the number of vacuoles (v), widening of central vein (CV) and blood sinusoids (BS). (Periodic acid-Schiff's X400).

B. Renal histopathological findings by light microscope.-

Histopathological assessment of the control kidney section group showed normal renal structure **(Fig.4$_a$)** with normal distribution of collagen **(Fig.5$_a$)** and normal positive reaction of PAS **(Fig.6$_a$).** But renal tissues in rats of second group which received 300mg/kg of leaf extract of Catharanthus roseus, showed enlarged of vascular glomeruli, tightness of the glomerular capsular space, atrophied of some vascular glomeruli and degeneration of epithelial lining of most renal tubules (**Fig.4$_b$**) with fibrous tissue deposition in the vascular glomeruli, degenerated epithelial lining Bowman's capsule with oedema and fibrosis of tubular epithelium cells **(Fig.5$_b$)** and mild reduction the positive reaction of PAS in comparison with control group **(Fig.6$_b$)**. And transverse section of renal tissues in the rats of third group which received 400mg/kg of leaf extract of Catharanthus roseus, showed marked distortion of glomeruli and epithelial lining of renal tubules with tightness in the glomerular capsular space **(Fig.4$_c$)** with marked fibrous tissue deposition in vascular glomeruli, and more collagen and connective tissues deposition **(Fig.5$_c$)** with marked reduction the positive reaction of PAS **(Fig.6$_c$).**

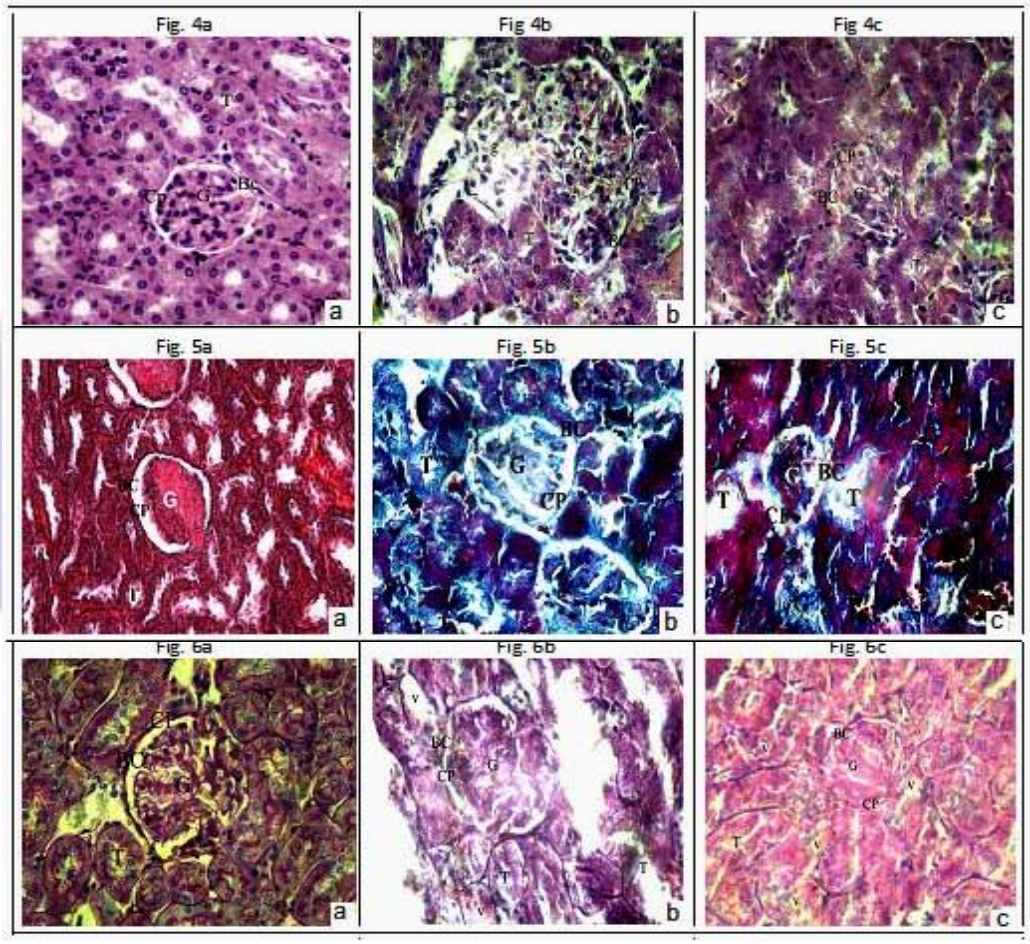

Fig (4a) a photomicrograph of transverse section of control rat kidney shows normal glomeruli (G), normal glomerular capsular space (GS), with flat epithelium lining the Bowman's capsule (BC) and normal cells in the lining epithelium of the tubules (T). (4b) a section of second group rat kidney shows enlarged vascular glomeruli (G), decrease of glomerular capsular space (GS), with flat epithelium lining of Bowman's capsule (BC), degeneration of some epithelial lining of tubule (T) and complete destruction of epithelial cells of other tubules (t). (4c) a section of third group rat kidney shows small and shrink vascular glomeruli (G), decrease of glomerular capsular space (GS), with destruction the epithelial lining of Bowman's capsule (BC) and renal tubule (T), and complete destruction of epithelial cells of other tubules (t). (H&E X400). Fig (5a) a photomicrograph of transverse section of control rat kidney shows normal glomeruli (G), flat epithelium lining

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glomerular capsule (BC) with distinct capsular space (CP) and normal tubules (T). (5b) a photomicrograph of transverse section in the second group rat kidney shows fibrous deposition in the vascular glomeruli (G), tightness of glomerular capsular space (C), with degenerated epithelial lining Bowman's capsule (BC), oedema and fibrosis of tubular epithelium cells (T). (5c) a photomicrograph of transverse section in the third group rat kidney shows marked fibrous deposition in the vascular glomeruli (G), tightness of glomerular capsular space (CP), with marked degeneration of epithelial lining Bowman's capsule (BC), oedema and fibrosis of tubular epithelium cells (T). (Mallory X400)
Fig (6a) a photomicrograph of transverse section of control rat kidney shows normal positive reaction of PAS, normal vascular glomeruli (G), glomerular capsular space (CP), with normal lining Bowman's capsule (BC) and tubular epithelium cells (T). (6b) a section of second group rat kidney shows moderate decrease in PAS reaction with degenerate vascular glomeruli (G), tightness in the glomerular capsular space (CP), irregular lining Bowman's capsule (BC) and tubular epithelium cells(T), and increase the number of vacuoles(v). (6c) a section of third group rat kidney shows marked decrease in PAS reaction with degenerate vascular glomeruli (G), tightness in the glomerular capsular space (CP), irregular lining Bowman's capsule (BC) and tubular epithelium cells (T), and increase the number of vacuoles (v). (Periodic acid-Schiff's X400)

C. Pancreatic histopathological findings by light microscope.-

Histopathological assessment of the control pancreas section group showed normal acini and cells the islets of langerhans **(Fig.7$_a$).** But pancreatic tissues in rats of second group which received 300mg/kg of leaf extract of Catharanthus roseus, showed a relative reduction the number of islets of langerhans with decrease the dimensions of acini in comparison with the control group **(Fig.7b).** Pancreatic tissues in the rats of third group which received 400mg/kg of leaf extract of Catharanthus roseus showed extensive damage the islets of langerhans with marked decrease the dimensions of acini (**Fig.7$_c$**).

D. Cardiac histopathological findings by light microscope.-

Histopathological assessment of the control cardiac section group showed normal appearance of myocardium branching and anastomosis cardiac muscle fibers, acidophilic sarcoplasm, and central elongated vesicular nuclei with capillaries in between **(Fig.8$_a$).** But cardiac tissues in rats of second group which received 300mg/kg of leaf extract of Catharanthus roseus, showed loss the normal cardiac architecture with fragmentation and degeneration of the myocardial fibers in comparison with the control group **(Fig.8b).** Cardiac tissues in the rats of third group which received 400mg/kg of leaf extract of Catharanthus roseus showed severe and marked widespread fragmentation and degeneration of myocardial fibers

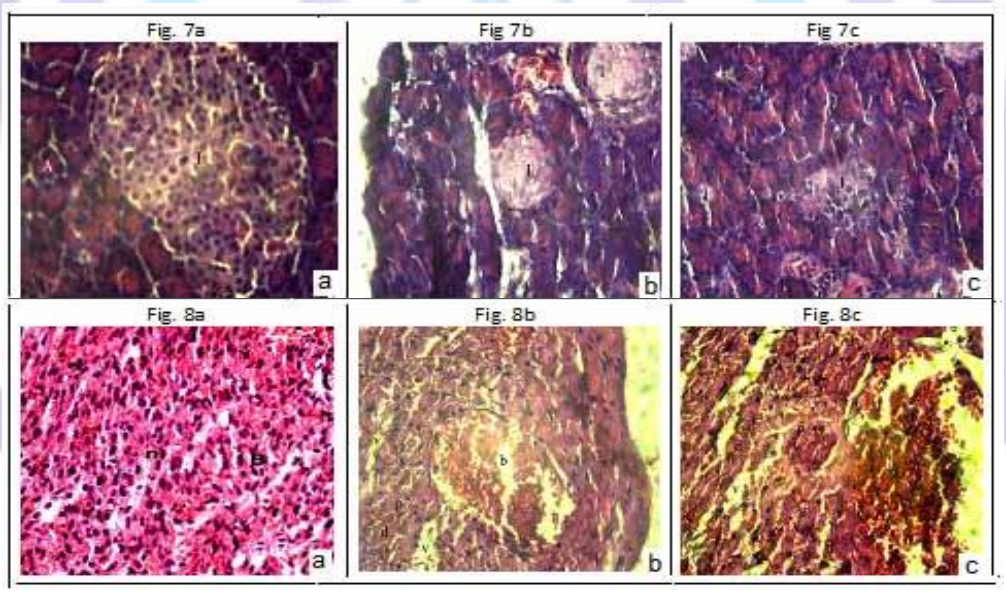

Fig (7a) a photomicrograph of section of control rat pancreas shows normal acini (A) and cells the islets of langerhans "I". (7b) a section of second group rat pancreas shows reduction the number of islets of langerhans "I" and decrease the dimensions of acini (A). (7c) a photomicrograph of section in third group rat pancreas shows extensive damage the islets of langerhans "I" and marked decrease the dimensions of acini (A). Fig (8a) a photomicrograph of section of control rat heart shows normal branching and anastomosis cardiac muscle fibers (B), acidophilic sarcoplasm and central elongated vesicular nuclei (n). (8b) a section of second group rat heart shows fragmentation and degeneration of the myocardial fibers (p), large size of vacuoles (v) acidophilic sarcoplasm, degenerated area (d) with degenerated nuclei (n) and congested blood vessels (b). (8c) a photomicrograph of section in third group rat heart shows marked widespread fragmentation and degeneration of the myocardial fibers, huge number and size of vacuoles (v), acidophilic sarcoplasm, degenerated nuclei (n) and pycknotic nuclei (p), increase the number and size of degenerated areas (d), and increase the size of congested blood vessels(b). (H&E X400).

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Discussion

Most the medicinal plants or its different parts such as roots and leaves, has therapeutic effects. According to WHO, 70-80% of people in the worldwide depend on herbal medicine as therapeutic methods for many diseases. Every day, the global demand for herbal medicine is growing although the information regarding the safety of these herbal plants is still inadequate (19). Although Catharanthus roseus is widespread use in various herbal remedies, there are controversial published articles about its toxicity. Thus, the present study aims to investigate the toxicological effect of Catharanthus roseus on vital organs of the body in the rats.

Our results showed statistical significant increase of liver function, hepatic disorganization, necrosis of hepatocytes, degenerated kupffer cells and fibrosis of central vein in second group which received 300mg/kg leaf extract of Catharanthus roseus in comparison with control group. These findings become more severe in the third group which received 400mg/kg leaf extract of Catharanthus roseus in comparison with second group. According to Sherlock (20), ALT is localized to hepatocytes alone with high specificity. It is increased after hepatic cellular injury. AST is increased in the serum after hepatic tissue necrosis because most of the circulating AST activity is derived from the hepatocytes isoenzyme which presents in the liver cell (21). Elevation of serum alkaline phosphatase is resulted from any disease which affects hepatocyte secretion because it is represented near the canalicular membrane of the hepatocyte (22). According to Aragon and Younossi (23), any injury to liver cells induced by test substances leads to leak the hepatic enzymes into the circulation and then its raised serum levels.

James et al., (5) are consistent with our results for AST, ALT, ALK.Ph and morphological changes of liver which referred to hepatotoxicity of Catharanthus roseus but they are not agree with significant elevations of other hepatic parameters (albumin, bilirubin and prothrombin time) which confirmed Catharanthus roseus toxicity on the liver. Results of present study are consistent with Kevin et al., (24) who referred that hepatotoxicity of Catharanthus roseus is enhanced based on its dose and duration of use. Upmanyu et al., (25) showed that toxic effect of Catharanthus roseus on the liver due to hepatotoxic effect of vincristine which is one of its main alkaloids. On the contrary the results of current study, Adekomi (11) confirmed that Catharanthus roseus has not any toxic effect on the liver by using the similar doses but in shorter period.

The present study demonstrated that there is a statistical significant increase of renal biomarkers (urea and creatinine) and renal histopathological changes (fibrosis of glomeruli with degeneration of renal tubules epithelial lining and Bowman's capsule) in the second group which received 300mg/kg leaf extract of Catharanthus roseus in comparison to control group. These findings become more severe in the third group which received 400mg/kg leaf extract of Catharanthus roseus in comparison with second group. This is in contrast with Adekomi, (11) and Kevin et al., (24) who confirmed that Catharanthus roseus has not any toxic effect on the kidney by using different doses and periods.

The current study refereed that some hematological parameters (red blood cells and platelets) in the second group which received 300mg/kg leaf extract of Catharanthus roseus, had statistical significant decrease in comparison to control group. On contrary with white blood cells count which had statistical significant increase in its value. These findings were statistically significant exaggerated in the third group which received 400mg/kg leaf extract of Catharanthus roseus in the same direction of reduction the red cells and platelets count, and increase the white cells count. These results are consistent with James et al., (5) who suggested that leaf extract of Catharanthus roseus has two alkaloids (vinblastine and vincristine) which produce severe prolonged inflammatory reaction induced the increase of white cells count and also lead to bone marrow depression resulting thrombocytopenia and red blood cells count reduction. Ahmad et al., (6) explained the increase of white cells count because of its proliferation and this could be due to the effect of Catharanthus roseus extract.

The present investigation indicated that Catharanthus roseus leads to reduction the number of islets of langerhans with decrease the dimensions of acini in the second group in comparison with the control group. Histopathological changes of pancreas were more marked in the third group which received 400mg/kg of leaf extract of Catharanthus roseus. These histopatholgical results are in contrast with Ahmed et al., (6) who confirmed that leaf extract of Catharanthus roseus helps regeneration the damaged pancreatic tissues and then its healing exerting antidiabetic action.

This study refered that rats cardiac tissues of the second group which received 300mg/kg of leaf extract of Catharanthus roseus showed fragmentation and degeneration of the myocardial fibers in comparison with the control group. These changes become marked widespread in the rats of third group which received 400mg/kg of leaf extract of Catharanthus roseus. Conversely, James et al., (5) who confirmed that Catharanthus roseus has not any toxic effect on the heart. Furthermore, Rasineni et al., (26) referred to the beneficial effect of Catharanthus roseus for decrease the risk of cardiovascular diseases.

Our explanation for toxic affection of heart and pancreas could be due to slow metabolic rate and excretion of vinca alkaloids and then its accumulation in the body for longer period, and toxic effect of leaf extract which is based on its concentration, duration of treatment and route of administration and this explanation was in agreement with Kevin et al., (24) who use high doses of leaf extract for prolonged period.

Conclusion

Chronic administration of Catharanthus roseus leads to toxic effect on liver and kidney which are manifested as histopathological changes and biochemical abnormalities. It also causes abnormalities of some hematological parameters, cardiac and pancreatic histiopathological changes. Severity of toxicity depends on dose of administration.

Recommendations

According to the results of this study, the use of Catharanthus roseus as medication for long period and by high doses should be restricted as much as possible because of its toxicity on the vital organs. It is recommended that further researches in human should be performed to verify our results. We suggest further studies with other different doses and periods to complete this work.

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