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ASSESMENT OF ACUTE AND SUB-ACUTE TOXICITY OF HYDRO-ETHANOLIC EXTRACT OF *Catharanthus roseus* LEAVES EXTRACT

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Abstract

Objective: This study investigated the acute and subacute toxicity of hydro-ethanolic extract of *Catharanthus roseus* leaves in rats for evaluating its safety profile.

Methods: *Catharanthus roseus* leaves extract (CRLE) for the acute (2000mg/kg) and subacute (100, 200 and 400 mg/kg) toxicity studies were administered orally to rats according the guidelines of Organization for Economic Cooperation and Development (OECD), respectively. Food and water intake as well as body and organ weight of animals were recorded. Signs of toxicity were assessed, and hematological, biochemical and histopathological analysis were performed.

Results: In the acute toxicity study, a single dose of the hydro-ethanolic extract of *Catharanthus roseus* leaves at 2000 mg/kg caused no mortality in the animals, suggesting that the median lethal dose is greater than 2000 mg/kg. In the subacute toxicity study, administration of the extract for 28 days, at all doses, caused no significant changes in the body weights or organ weights of rats in the treated groups when compared with the control group. In addition, hematological and biochemical parameters also revealed no toxic effects of the extract on rats. Histological sections of the liver and kidney from test animals showed no signs of degeneration. On the basis of acute and sub-acute toxicity studies, the minimal effective dose of 200mg/kg is taken for efficacy studies.

Keywords: *Catharanthus roseus* leaves; Acute and subacute toxicity; Biochemical analysis; Hematological parameters; Histopathological studies.

INTRODUCTION

Herbal medicine is an alternative natural remedy in primary health care in developing countries. Medicinal plants are commonly used as therapeutic agents because they represent an efficient, inexpensive alternative to traditional medicine, that are generally considered safe. However, this may be a misconception, since their action may cause side effects (Bello *et al.,* 2016; Rokaya *et al.*, 2014). Plants synthesize a variety of metabolites, some of which may be beneficial or potentially toxic to mankind (Kale *et al.,* 2019). Also, it has been true that pharmaceutical drugs may be therapeutic at one dose and toxic at another (Choubey *et al.,* 2010; Sharif *et al.,* 2015). The use of plant based products in both traditional and modern

societies as herbal remedies or crude drugs, or as purified compounds have a long history. Currently, herbal medicine is increasingly becoming popular throughout the world, especially in developing countries, where medicinal plants are available, accessible, and are at the reach of the poor people. Even though the use of these plants has shown promising potential phytotherapeutic effects with high global demand, but there are still concerns not only about their use but also about their safety (Suntar, 2020; Kharchoufa *et al.,* 2020).

Nowadays in all over the world people give preference to plant origin drugs as a source for medication, because of undesirable effects of synthetic drugs, which are believed to be suitable for chronic treatment. Traditional plants might provide new compounds, which can counter the high cost and toxic effects of the current medicines for many rural populations in developing countries. In recent years, The medicinal plants used *in vitro* and *in vivo* studies have received special attention (Vivekanandhan *et al.,* 2018). In this sense, toxicity tests are important for evaluating possible effects that phytocompounds may develop before their use in clinical practice (Lekshmi *et al.,* 2019). Considering several reasons justify the need to evaluate the biological activity of natural products, it is necessary to study acute toxicity *in vivo*, evaluating its possible effects on hematological, biochemical, and organ histology parameters. The objective of the present study is to evaluate the acute and subacute toxicity of the hydro-ethanolic extract of *Catharanthus roseus* leaves.

MATERIALS AND METHODS

Animals

Acute toxicity study carried out accordance with The Organization for Economic Cooperation and Development (OECD) guidelines for the Testing of Chemicals. Male albino rats of Wistar strain approximately weighing 180-200gms were used in this study. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2ºC and 12 hrs light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water *ad libitum*. They were acclimatized to the environment for 1 week prior to experimental use. All the animal experimental protocols were approved (Approval number: XXV/VELS/PCO/CPCSEA/IAEC/09.10.2021) by the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Preparation of extract

10grams of *Catharanthus roseus* leaves powder were used for extraction. Extraction was performed with cold extraction using the maceration method into hydro-ethanol (70%) solvent for 24 hours using the "intermittent shaking" method to obtain an extract. The extracts were filtered using Whatman filter No 1 paper and filtrate was used for toxicity studies. Toxicity studies were followed by the method of OECD (1995 and 2001).

Acute toxicity studies

Albino rats were randomly assigned into two groups of each six rats. Group 1 is control, fed daily with only normal laboratory diet and water. Group 2 treated with ethanol extract of *Catharanthus roseus*. All the groups were treated with respective extract at a dose of 2000 mg/kg body weight for 14 days through an oral needle following a period of 10-h fasting. All animals were maintained on standard laboratory diets with water *ad libitum*. After administration of the extract, animals were monitored continuously for every two hours for a day to detect acute changes in behavioral responses, spontaneous activity, irritability, corneal reflex, tremors, convulsion, salivation, diarrhea, lethargy if any, and also monitored for any mortality during the course of toxicity study.

Sub-acute toxicity studies

Albino rats were randomly assigned into four groups of each six rats. Group 1 is control, fed daily with only a normal laboratory diet and water. Group 2, Group 3 and Group 4 were treated with hydro-ethanol extract of 100, 200 and 400mg/kg of Plant extract respectively. The rats were administrated *Catharanthus roseus* leaves extract orally daily for 28 days.

Group I : Normal saline (0.5ml)
Group II : *Catharanthus roseus* leaves extract 100mg/kg of body weight.
Group III : *Catharanthus roseus* leaves extract 200mg/kg of body weight.
Group IV : *Catharanthus roseus* leaves extract 400mg/kg of body weight.

Collection of samples

At the end of 28 days, overnight-fasted rats were sacrificed by cervical dislocation and blood was collected with and without EDTA to obtain plasma and serum for analysis of various biochemical parameters, blood samples were used for the analysis of haematological parameters. At the same time, kidney and liver tissues were carefully excised for histopathological examination.

Biochemical analysis

Haemoglobin was estimated by Cyanmethaemoglobin method (Dacie and Lewis, 1968) (Beacon Diagnostic Kit). RBC, WBC counted and PCV by the method of Ochei and Kolhatkar, (2000). Protein was estimated by the method of Lowry *et al.* (1951). Albumin was estimated by the method of Rodkey (1965). The serum total bilirubin was estimated by the method of Malloy and Evenlyn (1937). The serum SGOT and SGPT were estimated by the method of Reitman and Frankel (1957). The serum alkaline phosphatase activity was estimated by the method of Kind and King's (1954). Urea was estimated by the method of Natelson (1957). Serum creatinine was carried out by alkaline picrate method of Boneses and Taussy (1954). Serum sodium was estimated by colorimetric method of Maruna and Trinders (1958). Serum potassium was estimated by method of Maruna (1957). Cholesterol and HDL were assayed by Allain *et al* (1974). Triglyceride was assaed by Werner *et al* (1981) method. HDL cholesterol was determined by the method of Allain *et al.* (1974). LDL cholesterol was calculated as per Friedewald's (1972) equation. Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Superoxide dismutase activity was assayed by the procedure of Kakkar *et al* (1984). The activity of catalase was determined by the method of Beers and Sizer (1952). Reduced glutathione

was determined by method of Moron *et al* (1979). The activity of glutathione peroxidase was estimated by the method of Rotruck *et al* (1973). The level of ascorbic acid was assayed by the method of Omaye *et al* (1979). α-tocopherol was estimated by the method of Baker *et al* (1980).

Histopathological studies

The organs, namely liver and kidney were carefully excised and weighed. These organs were preserved in a fixation medium of 10% buffered formalin for histopathological study. Slides were viewed on a photographic microscope to find out the histological changes in liver and kidney. Histological studies carried out by the method of Ochei and Kolhatkar, (2000).

Statistical analysis

Values are expressed as Mean ± SD for 6 rats (each group). Data were analyzed by one-way ANOVA followed by post-hoc DMRT test using SPSS ver. 22. $P< 0.05$ was considered significant.

RESULTS AND DISCUSSION

Medicinal plants are used a lot because all that is natural is free of toxic effects. However, research has identified the toxic nature of some plants. The scientific knowledge of the toxicity study is very necessary to unveil the clinical potential of (Eran *et al.,* 2016). The scientific knowledge of the toxicity study is very necessary to unveil the clinical potential of *Catharanthus roseus*. Hence, the current study was undertaken to evaluate and focus on the acute and subacute toxicity of *Catharanthus roseus* leaves in an animal model.

Acute toxicity study

General appearance and behavioral observations

Plant-based medicines have been utilized to prevent, diagnose, and treat various diseases. This therapy has been carried out for the past 20 years because it is more comfortable to access multiple regions. However, the use of doses in traditional medicine has not been scientifically studied with certainty. Therefore, toxicity testing must be done to ensure the safety of the plant extracts. Toxicity testing is essential to estimate the level of damage caused by compounds to biological and non-biological materials. The clinical signs and symptoms exerted by drugs on vital body organs are considered as principal observations among toxicity indicators. The acute toxic effect of hydro-ethanolic extract of *Cathranthus roses* leaves extract was determined as per the OECD guideline. On the 14 days treatment of hydro-ethanolic extract of *Cathranthus roses* leaves extract, the rats were survived throughout the entire study period. No treatment-related toxic symptoms or mortality were observed after oral administration of tested extract. None of these rats had shown any abnormal behavioral responses in any dose range. There was no change in behavioral responses, spontaneous activity, irritability, corneal refex, tremors, convulsion, salivation, diarrhea and lethargy if any when compared to control group (Table 1).

Table 1: Acute toxicity study of extracts in wellness parameters of rats

Observations	Animal group	
	Control rat	Extract (2,000 mg/kg body wt)
Consciousness	+	+
Grooming	-	-
Touch response	+	+
Sleeping duration	+	+
Movement	+	+
Gripping strength	+	+
Righting re flex	+	+
Food intake	+	+
Water consumption	+	+
Tremors	-	-
Diarrhea	-	-
Hyper activity	-	-
Pinna reflex	+	+
Corneal reflex	+	+
Salivation	+	+
Skin color	+	+
Lethargy	-	-
Convulsion	-	-
Morbidity	-	-
Sound response	+	+

Note: + indicate normal - indicate absent

There were generally no significant differences observed in the relative body weights in this study (Table 2). From the present study it was seen that there was no significant change in the haematological and biochemical parameters in the *Cathranthus roses* leaves extract treated group compared to the normal control group (Tables 2 to 7). Gross examination at autopsy and histopathological evaluations of liver and kidney organs stained with haematoxylin and eosin revealed no significant differences (Figure 1). Acute oral toxicity effects of hydro-ethanolic extract of *Cathranthus roses* leaves extract on rats were studied and no animal deaths in rats receiving 2000 mg/kg of extract. No sign of toxicity was observed in the wellness parameters during the 14-days observation period. Therefore, the approximate acute lethal dose (LD_{50}) of *Cathranthus roses* extract in rat was estimated to be higher than 2000 mg/kg.

Table 2: Effect of *Catharanthus roseus* on animal and organ weight of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	*P* Value
Initial day (gm)	185.50±3.62	186.68±4.18	$^{NS}P>0.05$
Final day (gm)	193.93±5.00	191.56±4.50	$^{NS}P>0.05$
Liver weight (gm)	5.50±0.25	5.50±0.22	$^{NS}P>0.05$
Kidney weight (gm)	1.52±0.13	1.43±0.14	$^{NS}P>0.05$
Acute Oral Toxicity Effects (N = 6)			
Animal live (Nos.)	6±0	6±0	$^{NS}P>0.05$
Animal dead (Nos.)	Nil	Nil	
% of Mortality	Nil	Nil	

Values are expressed as Mean ± SD for six rats. Data was calculated by student *t*-Test (Independent sample, *P* value two tail) using SPSS ver. 20. Statistically significant level 0.05. **P*<0.05 significant and NS non-significant ($^{NS}P>0.05$).

Biochemical Analysis

The effects of acute administration of *Catharanthus roseus* leaves extract (CRLE) on biochemical parameters are presented in Table 3. The CRLE had no effect on serum electrolytes (Na and K). The kidney function parameters, like urea, and creatinine, did not reveal any significant changes. No statistically significant differences in the liver function parameters like alanine aminotranferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were observed. Additionally, no relevant changes were found in total protein, albumin, and globulin content (Table 3 and 4).

Table 3: Effect of *Catharanthus roseus* on liver profile of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	*P* Value
Protein (mg/dl)	7.33±0.12	7.39±0.14	$^{NS}P>0.05$
Albumin (mg/dl)	4.35±0.13	4.27±0.12	$^{NS}P>0.05$
Bilirubin (mg/dl)	0.71±0.02	0.73±0.06	$^{NS}P>0.05$
ALT (IU/L)	26.79±1.73	26.67±2.16	$^{NS}P>0.05$
AST (IU/L)	46.92±1.62	46.36±2.24	$^{NS}P>0.05$

ALP (IU/L)	51.80±0.80	52.26±1.34	^{NS}P>0.05

Values are expressed as Mean ± SD for six rats. Data was calculated bystudent *t*-Test (Independent sample, *P* value two tail) using SPSS ver. 20. Statistically significant level 0.05. *P<0.05 significant and NS non-significant (^{NS}P>0.05).

Table 4: Effect of *Catharanthus roseus* on kidney profile of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	*P* Value
Creatinine (mg/dl)	0.91±0.04	0.92±0.03	^{NS}P>0.05
Urea (mg/dl)	23.35±2.60	23.59±1.44	^{NS}P>0.05
Sodium (Meq/L)	151.77±4.24	151.72±5.44	^{NS}P>0.05
Potassium (Meq/L)	4.41±0.32	4.44±0.24	^{NS}P>0.05

Values are expressed as Mean ± SD for six rats. Data was calculated by student *t*-Test (Independent sample, *P* value two tail) using SPSS ver. 20. Statistically significant level 0.05. *P<0.05 significant and NS non-significant (^{NS}P>0.05).

Hematological Analysis

The effects of acute administration of CRLE on haematological parameters (Hb, RBC, WBC, PCV, MCV, MCH and MCHC) are shown in Table 5. Administration of CRLE (2000mg/kg) did not cause any significant difference in most of the hematological parameters when compared with the control group.

Table 5: Effect of *Catharanthus roseus* on hematology profile of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	*P* Value
Hb (gm/dl)	13.13±1.17	13.41±0.27	^{NS}P>0.05
RBC ($\times 10^6$ /mm^3)	4.52±0.32	4.49±0.16	^{NS}P>0.05
WBC ($\times 10^3$ /mm^3)	7.45±0.21	7.42±0.18	^{NS}P>0.05
PCV (%)	22.60±1.97	24.15±1.85	^{NS}P>0.05
MCV (famato litre)	50.02±4.34	53.84±5.59	^{NS}P>0.05
MCH (pico gram)	29.18±3.70	29.84±0.81	^{NS}P>0.05
MCHC (%)	58.59±8.33	55.81±4.44	^{NS}P>0.05

Values are expressed as Mean ± SD for six rats. Data was calculated by student *t*-Test (Independent sample, *P* value two tail) using SPSS ver. 20. Statistically significant level 0.05. **P*<0.05 significant and NS non-significant (^{NS}P>0.05).

Oxidative stress markers

The effect of *Catharanthus roseus* on oxidative stress profile of control and experimental rats (acute toxicity) is shown in table 6. The administration of CRLE (2000mg/kg) **has no effect on** the oxidative stress parameters when compared with the control group.

Table 6: Effect of *Catharanthus roseus* on oxidative stress profile of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	*P* Value
MDA (nmol of MDA formed/L)	7.43±0.28	7.44±0.25	^{NS}P>0.05
SOD (U/ml)	4.40±0.19	4.53±0.26	^{NS}P>0.05
CAT (U/ml)	6.50±0.29	6.41±0.22	^{NS}P>0.05
GPx (U/ml)	8.40±0.19	8.56±0.15	^{NS}P>0.05
GSH (mg/dl)	4.49±0.23	4.36±0.15	^{NS}P>0.05
Vit-C (µg/dl)	3.49±0.27	3.34±0.14	^{NS}P>0.05
Vit-E (µg/dl)	2.49±0.20	2.54±0.29	^{NS}P>0.05

Values are expressed as Mean ± SD for six rats. Data was calculated by student *t*-Test (Independent sample, *P* value two tail) using SPSS ver. 20. Statistically significant level 0.05. **P*<0.05 significant and NS non-significant (^{NS}P>0.05).

Table 7 showed the effect of *Catharanthus roseus* leaves extract on lipids profile of control and experimental rats (Acute toxicity). There is no significant (*P*>0.05) changes were observed lipid profile as cholesterol, triglyceride, HDL and LDL on acute administration of CRLE.

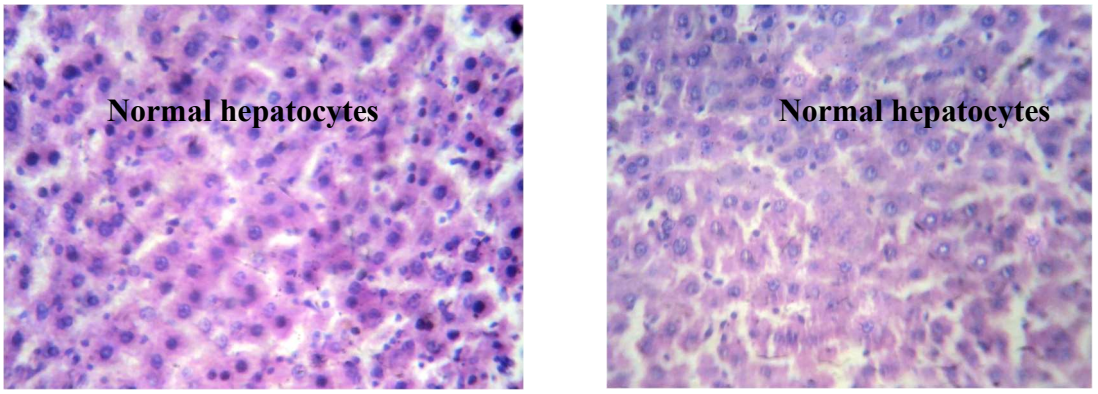
Table 7: Effect of *Catharanthus roseus* on lipids profile of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	*P* Value
Cholesterol (mg/dl)	92.05±3.62	89.88±2.58	^{NS}P>0.05
Triglyceride (mg/dl)	114.22±4.28	114.36±2.75	^{NS}P>0.05
HDL (mg/dl)	33.31±1.94	34.14±2.25	^{NS}P>0.05
LDL (mg/dl)	35.89±3.82	32.86±4.24	^{NS}P>0.05

Values are expressed as Mean ± SD for six rats. Data was calculated by student *t*-Test (Independent sample, *P* value two tail) using SPSS ver. 20. Statistically significant level 0.05. **P*<0.05 significant and NS non-significant (^{NS}P>0.05).

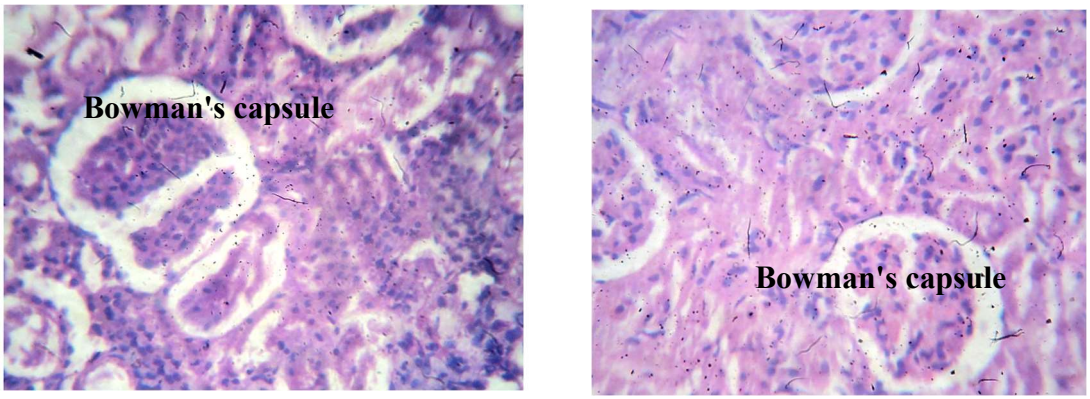
Histological observation

Histopathological studies were conducted on kidney and liver of all the rats. The microscopic examination viewed under the light microscope using magnification (10x40x) powers. Gross examination of the organs did not show any signs of necropsy and abnormal morphological changes. The microscopic examination of the hematoxylin eosin stained tissue sections also recorded insignificant changes in *Catharanthus roseus* leaves extract treated rats as compared with the control rats' tissues (Plate 1).



Group I (Normal) **Group II (2000mg/kg)**

Liver histopathology (10 × 40X)



Group I (Normal) **Group II (2000mg/kg)**

Kidney histopathology (10 × 40X)

Plate 1: Histology of liver and kidney in control and *Catharanthus roseus* leaves extract treated animal shows normal architecture

Acute toxicity

The clinical use of herbal drugs without any standard dosage criteria together with lack of adequate scientific evidence has raised concerns regarding their toxicity status. Thus, it becomes essential to assess the safety and toxicity of herbal medicines before their human consumption. Various experimental animal models have been used to evaluate the toxicity of herbal drugs to select a safe dose for future human use. The acute toxicity study may provide initial information on the mode of toxic action of an agent, acts as the basis for classification and labelling, and helps in deciding the dose of novel compounds in animal studies. Moreover, if a high dose (e.g., 2000 mg/kg) is found to be survivable, no further acute testing will be conducted (NRC, 2006). In this study, *Catharanthus roseus* **leaves at a dose of 2000 mg/kg had no adverse effect on the treated rats in up to 14 days of observation.**

According to Raina *et al.,* (2010) and Cajuday and Pocsidio (2010), the weights of the organs are markers of pathological and physiological wellness status of animals. Changes in organ weights are hall-marks of toxicity in experimental animals, which are determined by toxicity tests (El Hilaly *et al*., 2004). The toxic effect of ingested herbal remedies in the body is most likely to be felt by important organs such as the spleen, heart, liver and kidneys because of the vital roles that they play in the body (Ezeja *et al*., 2014). The liver and kidneys are major targets of xenobiotic action, with the liver being the main organ for xenobiotic biotransformation, while the kidney serves as excretory organ of xenobiotics (Hoff-Brand and Pettit, 2000). Our findings on organ weight revealed that there was no significant increase in organ weight, suggesting that the plant extract was not toxic to the animals at the tested doses.

The wellness status of animals is hinged on changes in bodyweight (Muhammad *et al*., 2015). After 28 days of treatment, all the animals exhibited a steady increase in body weight. It indicates that the daily intake of the extract did not alter food intake. In addition, it possibly shows that weight gain and appetite stability were not impeded by the extract during the exposure period. This validates the oral route folkloric usage of the plant. **The bone marrow is a major location for novel blood cell manufacture and a vulnerable tissue targeted by toxic compounds in the hematopoietic system (Kifayatullah *et al*., 2015). The hematological parameters between control and treated groups showed the extract was non-toxic to the haemopoietic system. The liver biomarkers are specific tools in examining liver toxicity during drug biotransformation (Mukinda and Syce, 2007).**

Additionally, most of the biochemical parameters were not altered. No relevant changes were found in levels of ALT, AST, ALP, creatinine, which are good indicators of liver and kidney functions. No gross lesions were found in histopathology examinations. Kidney disease can be detected by measurements of kidney indices like creatinine, uric acid, urea, potassium, sodium and chlorides and their normal levels reflect a reduced likelihood of renal problems (Dalle *et al.*, 2006). No statistically significant differences in the liver function parameters like ALT, AST, ALP was observed. In the present study, no significant alterations in ALT, AST, ALP, creatinine, urea, potassium, sodium. Additionally, no relevant changes were found in total protein, albumin and globulin levels in *Catharanthus roseus* **extract fed rats when compared to the control was observed. This indicates that the**

functional integrity of the liver and kidney were not compromised after treatment with graded doses of the extract. Similarly, *Catharanthus roseus leaves* extract oral administration non-significant changes ($p>0.05$) in total cholesterol (TC), serum triglyceride (TG), HDL and LDL levels were observed.

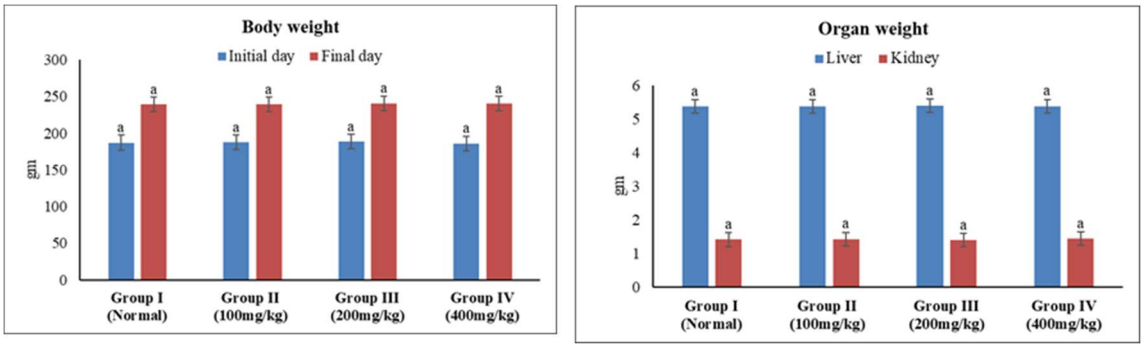
Histological studies are used as benchmarks for determining pathological changes in tissues and organs. Histological analysis of liver and kidney revealed no abnormalities in cellular architecture of these vital organs in the extract treated rats when compared with the control rats; *Catharanthus roseus leaves* extract did not adversely affect the morphology of the vital organs. This also supports our results that liver and kidney injury biomarkers were elevated in groups treated with *Catharanthus roseus leaves* extract.

Effects of *Catharanthus roseus* extract on lipid peroxidation were evaluated by measuring MDA, SOD, Catalase, GPx, GSH, Vitamin C and E enzymatic and non-enzymatic activities. Elevation in oxidative stress in biological entities thereby interfering with the system's antioxidant defence mechanisms (Pajero *et al.,* 2002). However, in this study, *Catharanthus roseus* administration at 2000 mg/kg bw did not cause any significant difference in all of the oxidative stress parameters when compared with the control group. **Since no toxic stress were found during the acute toxicity study, further study was conducted to evaluate the subacute toxicity of** *Catharanthus roseus* **leaves extract up to 28 days to prepare inclusive toxicological records on this plant.**

Sub-acute toxicity studies

A body weight was determined on initial (0) day and 28th days and the organs liver and kidney weight of four groups. The first one is the control, Group I is CRLE of 100 mg/kg, II CRLE of 200 mg/kg, and the last group, named as Group III, is CRLE of 400 mg/kg. No significant ($p > 0.05$) changes in the body weight were observed (Figure 1)

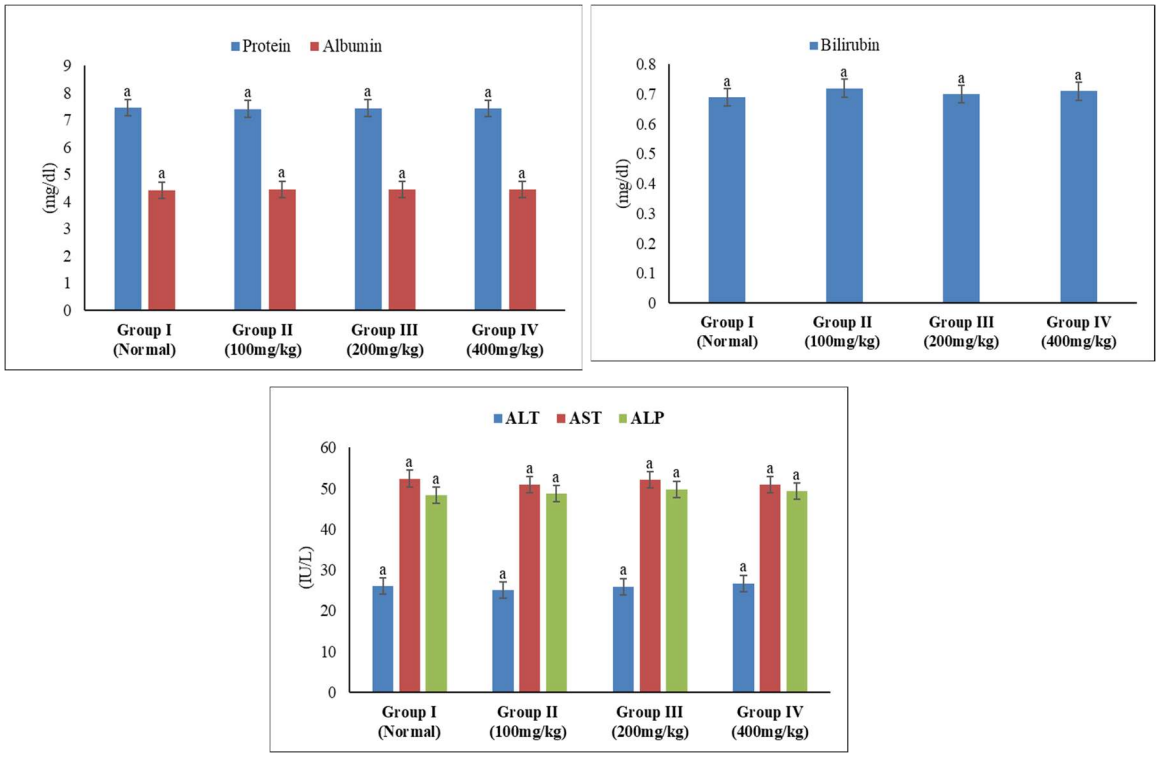
Figure 1: Effect of *Catharanthus roseus* leaves extract on animal and organ weight of control and experimental rats (Sub-acute toxicity)



Values are expressed as Mean ± SD for six rats. Mean values within the Row followed by different letters (Superscript) are statistically significant ($P<0.05$) from each other group and same letter was statistically non-significant ($P>0.05$) were comparison by ANOVA, Duncan's multiple range test (DMRT), significant level alpha 0.05.

The effect of sub-acute administration of CRLE on liver indices is presented in figure 2. A non-significant ($p > 0.05$) changes observed protein, albumin, bilirubin content and enzymes AST, ALP and ALP activities were observed in 100, 200 and 400mg/kg treated groups as compared with control rats.

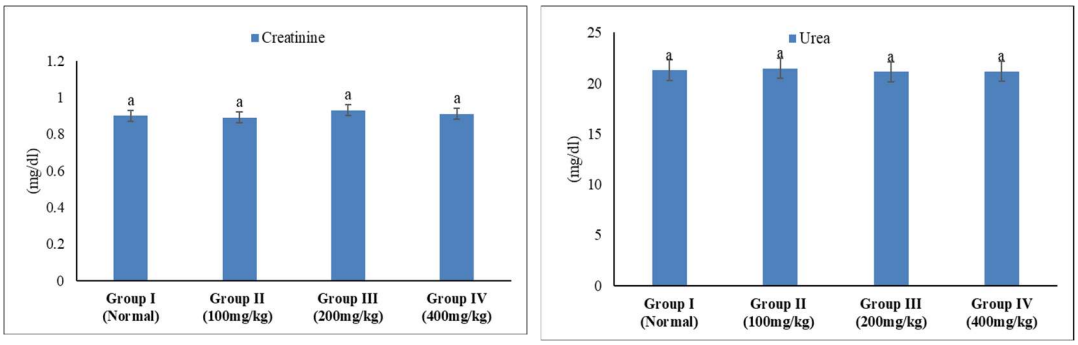
Figure 2: Effect of *Catharanthus roseus* leaves extract on liver profile of control and experimental rats (Sub-acute toxicity)



Values are expressed as Mean ± SD for six rats. Mean values within the Row followed by different letters (Superscript) are statistically significant ($P<0.05$) from each other group and same letter was statistically non-significant ($P>0.05$) were comparison by ANOVA, Duncan's multiple range test (DMRT), significant level alpha 0.05.

Sub-acute administration of CRLE in the treated rats caused no significant difference ($p > 0.05$) in the kidney parameters (creatinine, sodium, potassium, and urea levels) investigated (Figure 3). The effects of sub-acute administration of CRLE on haematological parameters (Hb, RBC, WBC, PCV, MCH, MCHC and MCV) are shown in Figure 4. Daily administration of CRLE for 28 days did not cause any significant difference in most of the hematological parameters when compared with the control group.

Figure 3: Effect of *Catharanthus roseus* leaves extract on kidney profile of control and experimental rats (Sub-acute toxicity)



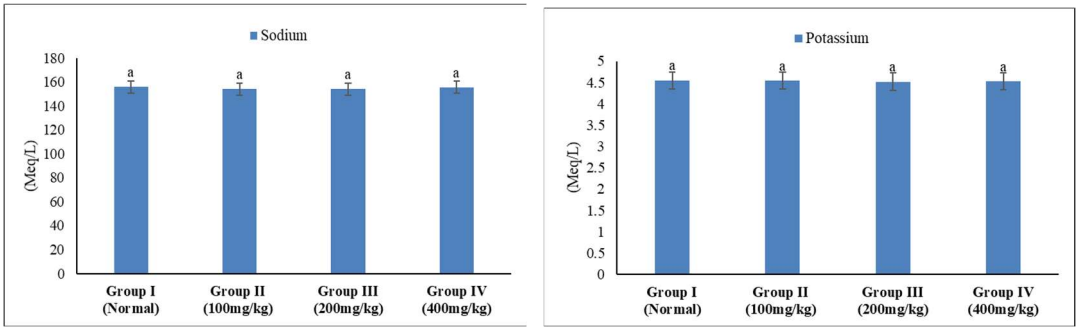
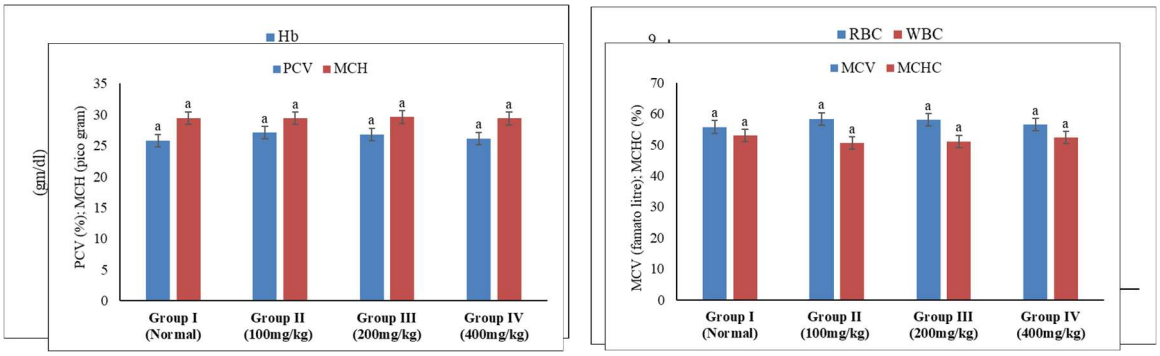


Figure 4: Effect of *Catharanthus roseus* leaves extract on hematology profile of control and experimental rats (Sub-acute toxicity)

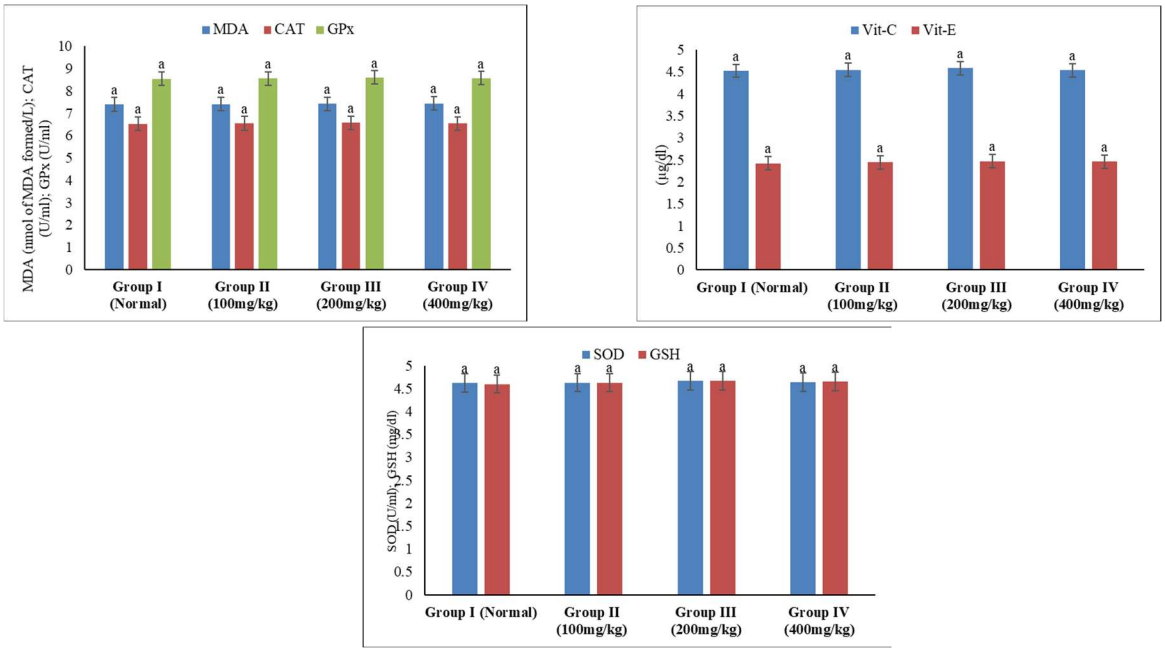


Values are expressed as Mean ± SD for six rats. Mean values within the Row followed by different letters (Superscript) are statistically significant (P<0.05) from each other group and same letter was statistically non-significant (P>0.05) were comparison by ANOVA, Duncan's multiple range test (DMRT), significant level alpha 0.05.

The effects of sub-acute administration of CRLE on oxidative stress parameters (MDA, superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione (GSH), Vitamin C and E) are shown in Figure 5. Daily administration of CRLE for 28 days did not cause any significant difference in MDA parameters when compared with the control group. The enzymatic antioxidants superoxide dismutase, catalase and glutathione peroxidase activities of both control and CRLE-fed rats are indicated in Figure 5. The results indicated no significant difference in these enzymes

activity after sub-acute treatment with different doses of CRLE for 28 days when compared to control set. The non-enzymatic antioxidants reduced glutathione (GSH), Vitamin C and E content of both control and CRLE-fed rats are indicated in Figure 5. The results indicated no significant difference in non-enzymatic antioxidants after sub-acute treatment with different doses of CRLE for 28 days when compared to control set.

Figure 5: Effect of *Catharanthus roseus* leaves extract on oxidative stress profile of control and experimental rats (Sub-acute toxicity)



Values are expressed as Mean ± SD for six rats. Mean values within the Row followed by different letters (Superscript) are statistically significant (P<0.05) from each other group and same letter was statistically non-significant (P>0.05) were comparison by ANOVA, Duncan's multiple range test (DMRT), significant level alpha 0.05.

Effects of sub-acute administration of CRLE on the lipid profile of experimental rats are shown in Figure 6. CRLE treatment resulted in non-significant changes (p>0.05) in TC and TG concentrations as compared to control rats. CRLE treatment at 100, 200 and 400 mg/kg both resulted in non-significant changes (p>0.05) in HDL and LDL levels when compared to the control.

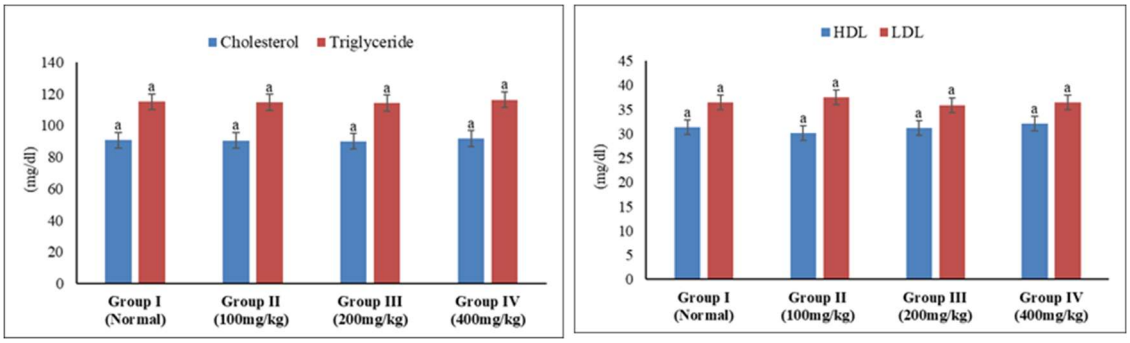


Figure 6: Effect of *Catharanthus roseus* leaves extract on lipids profile of control and experimental rats (Sub-acute toxicity)

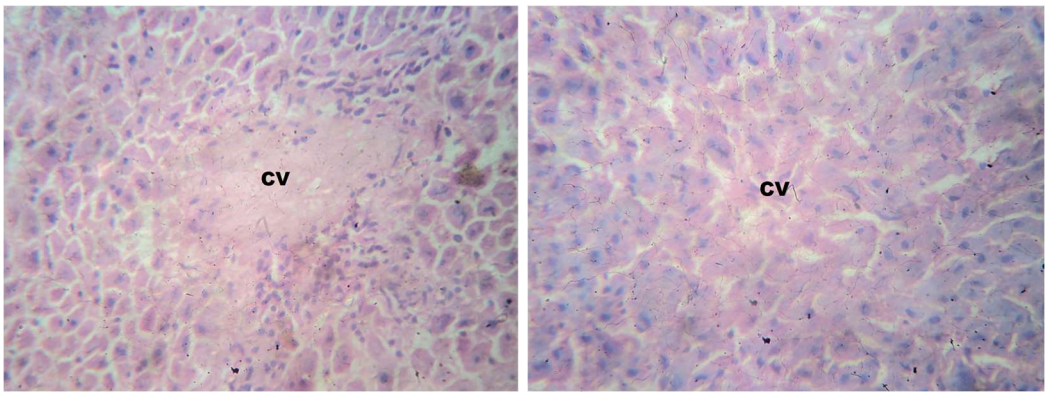
Values are expressed as Mean ± SD for six rats. Mean values within the Row followed by different letters (Superscript) are statistically significant (P<0.05) from each other group and same letter was statistically non-significant (P>0.05) were comparison by ANOVA, Duncan's multiple range test (DMRT), significant level alpha 0.05.

Histopathological studies

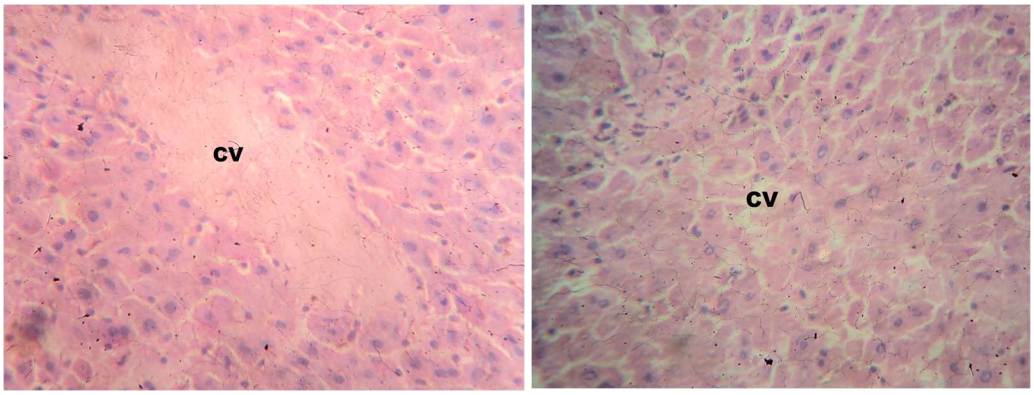
Histological studies revealed no abnormalities in liver and kidney tissues in extract-treated rats. Thus, the histopathological evaluation indicated that the extract did not have any adverse effacts on the morphology of the tissues and these observations supported the biochemical results mentioned. Therefore, it is concluded that the extract did not produce any toxic effects in male albino rats.

Liver

Histological studies of subacute toxicities of the liver in the control and experimental group of rats observed that the liver cells are arranged into lobules in both control and experimental groups (Plate 2). Liver cells of hepatocytes are arranged flat. A discontinuous layer of cells lines the sinusoids. The central vein is lined by epithelial cells' predominant nucleus. There are no abnormalities in the histology of the liver were observed in all the dose treated groups.



Group I (Normal) **Group II (100mg/kg)**



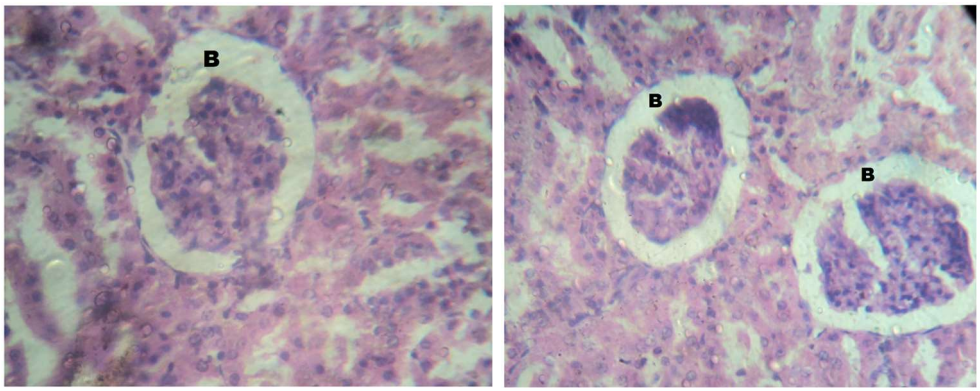
Group III (200mg/kg) **Group IV (400mg/kg)**

CV: Central veins of liver

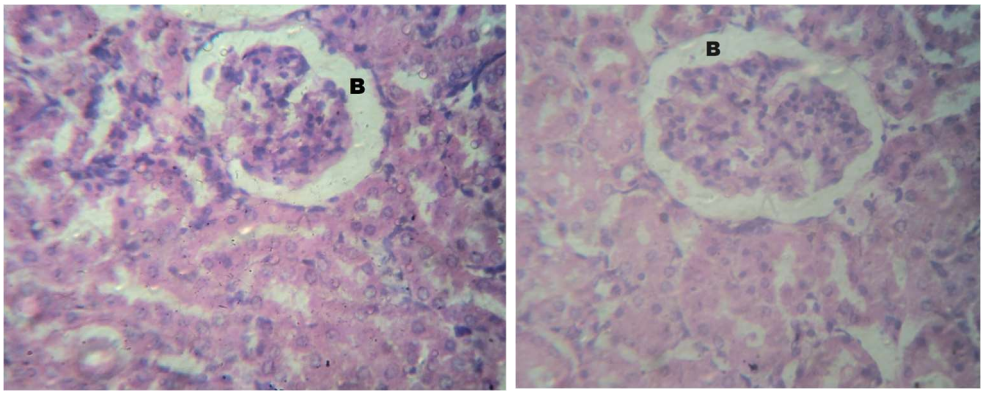
Plate 2: Histopathology of liver (10 × 40X) in control and experimental rats

Kidney

The normal architecture of kidneys was observed in both the control and experimental group of rats (Plate 3). The Renal corpuscles in the centre display a slight shrinkage artifact and thus clearly demonstrate Bowman's space. The renal corpuscles are surrounded by cross-sections of proximal convoluted tubules, distal convoluted tubules and macula densa. There are no abnormalities in the histology of the kidneys were observed in all the dose treated groups.



Group I (Normal) **Group II (100mg/kg)**



Group III (200mg/kg) Group IV (400mg/kg)
B: Bowman's capsule of kidney
Plate 3: Histopathology of kidneys (10 × 40X) in control and experimental rats

Subacute toxicity

The subacute toxicity as the advance effects occurring as result of the repeated daily (oral) dosing of a chemical to experimental annual for part of the life span. Although opinions different on the length of exposure. Exposure in a subacute study is generally from 1-3 months. The National Academy of Science (NAS) defines subacute exposure from a few days to 6 months (**Maclachlan and Dixon**, 1976). Consequently, in this study, the leaves of ***Catharanthus roseus*** were evaluated in rats at doses of 100, 200 and 400 mg/kg for 28 days. The body weight changes serve as a sensitive indication of general health status of animals (Hilaly *et al.*, 2004). After 28 days of treatment of the extract, all the animals exhibited a normal increment in body weight. It can be stated that leaves of ***Catharanthus roseus*** did not interfere with the normal metabolism of animals. The significant increment in food and water intake is considered as being responsible for augmentation in body weight gain. Similarly, no significant changes in the weight of the liver and kidney were observed, suggesting that administration of ***Catharanthus roseus*** leaves at subacute oral doses produces no effect on the normal growth. The protocol of weighing relative organs in toxicity studies includes their sensitivity to predict toxicity and it correlates well with histopathological changes (Kluwe, 1981). The results of this study revealed no significant changes in the relative organ weight of control and treated groups which showed that none of the organs were adversely affected, nor showed any signs of toxicity throughout the study.

In toxicity studies, haematology analyses also play a major role in evaluating the possible toxic effects induced by the oral treatment of the test material (**Yakubu *et al*., 2007)**. The haematological parameters can be used to determine the blood relating functions of plant extract. The haemopoietic system is one of the most sensitive targets of toxic compounds and an important index of physiological and pathological status in both humans and animals. The extract indicated a significant difference on the RBC indices which suggested that the ***Catharanthus roseus*** leaves does not affect erythropoiesis, morphology, or osmotic fragility of red blood cells (Odeyemi *et al.,* 2009). WBC's are the first line of cellular defines that respond to infectious agents, tissue injury,

or any inflammation. Furthermore, significant changes were observed in **PCV, MCH, MCHC and MCV** in the leaves of ***Catharanthus roseus*** suggesting that the extract might not have exerted challenge on the immune system of the animals.

In toxicity studies, serum biochemistry analyses play a major role in evaluating the possible toxic effects induced by the oral treatment of the test material (**Yakubu *et al*., 2007)**. Evaluation of biochemistry was done to monitor the any alterations in renal and hepatic functions on treatment with extract. The total protein, albumin, globulin, and total bilirubin did not affecting the hepatocelluar and secretory functions of the liver. The non-significant in the levels of ALT, AST, ALP, **creatinine, sodium, potassium and urea** which are good indicators of liver and kidney functions (Olorunnisola, *et al.,* 2012), suggests that sub-chronic administration of extract did not alter hepatocytes and kidneys of normal metabolism of the animals. These observations were further confirmed by the histological assessment of the liver and kidney organs. Based on the results found in our study, we concluded that leaves of ***Catharanthus roseus*** hydro-ethanol extract was safer and non-toxic and could be well used for pharmacological and therapeutic purposes. The results indicated no significant difference in enzymatic and non-enzymatic antioxidants after sub-acute treatment with different doses of hydro-ethanol **extract of** *Catharanthus roseus leaves* for 28 days when compared to control set.

In toxicity studies, **oxidative stress markers play a major role in evaluating the stress effects induced by the oral treatment of the test material.** *Catharanthus roseus leaves* supplemented to rats were assessed the oxidative stress markers as lipid peroxidation by measuring malondialdehyde (MDA) levels, enzymatic antioxidants SOD, catalase GPx and non-enzymatic antioxidants GSH, Vitamin C and E. Reduction of enzymatic and non-enzymatic antioxidants and increases in MDA levels connotes an elevation in oxidative stress in biological entities thereby interfering with the system's antioxidant defence mechanisms (Pajero *et al*., 2002). However, in this study, CRLE administration at 100, 200 and 400 mg/kg bw non-significantly increased ($p>0.05$) the MDA and antioxidant levels in comparison to the control. This suggests that hydro-ethanol **extract of** *Catharanthus roseus leaves* possesses beneficial properties due to its content of phytochemicals, in boosting the body's defense. *Catharanthus roseus leaves* extract oral administration non-significant changes ($p>0.05$) in total cholesterol (TC), serum triglyceride (TG), HDL and LDL levels were observed. This study recommended that CRLE administration may prove effective in the management of cardiovascular ailments, diabetes as well as deregulated blood pressure. Many researchers supported the biochemical parameters, haematological, oxidative stress parameters, lipid profile and histological studies of present study (Osafanme *et al.,* 2020; Ashutosh *et al.*, 2022). Considering non-signiant changes in all the parameters of treated rats compared to the control group rats in both toxicity studies, it is possible to suggest that the oral treatment of CRLE is non-toxic to rats.

Conclusion

This study showed that the administration of the *Catharanthus roseus* leaf extract to Wistar rats was not toxic in any of the tested doses. The extract did not have a direct impact

on the liver and kidney functions as corroborated by results from hematological and blood chemistry analysis. Also, the extract did not bring about any change in food intake, water consumption or body weight. Histopathological examinations of internal organs such as liver and kidney of rats in all test groups in both toxicity studies showed a normal cellular architecture and were similar to those of the control group rats. Furthermore, the results obtained from both acute and subacute toxicity studies of *Catharanthus roseus* leaf extract could thus give insight into its safety in humans. These findings indicate that the no observed adverse effect level of *Catharanthus roseus* leaf extract was greater than 2000 mg/kg/day. On the basis of toxicity studies, the minimal effective dose of 200mg/kg is taken for efficacy studies.

References

Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W. and Fu, P.C. (1974) Enzymatic determination of total serum cholesterol, Clin. Chem., 20, 470-475.

Ashutosh KumarBrijesh KumarRajesh KumarAjay KumarManish SinghVinod TiwariAnshuman TrigunayatParamita PaulPratistha Singh Acute and subacute toxicity study of ethanolic extract of *Calotropis procera* (Aiton) Dryand flower in Swiss albino mice. Phytomedicine Plus 2 (2022) 10022, 1-6

Baker H, Frank O, De Angeles B and Feinglod S. (1980) Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. Nutrition Reports International, 21: 531.

Beers R and Sizer I. (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. Journal of Biological Chemistry, 195: p133.

Bello, I., Bakkouri, A. S., Tabana, Y. M., Al-Hindi, B., Al-Mansoub, M. A., Mahmud, R., & Asmawi, M. Z. (2016). Acute and sub-acute toxicity evaluation of the methanolic extract of Alstonia scholaris stem bark. *Medical Sciences*, *4*(1), 4.

Beuge JA and Aust SD. (1978) The thiobarbituric acid assay. Methods in enzymology 52: pp 306-307.

Boneses RN and Taussk HA (1945). On the colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem. 158: pp 581-591.

Cajuday LA. Pocsidio GL. Effects of Moringa oleifera Lam. (Moringaceae) on the reproduction of male mice (Mus musculus). J Med Plants Res 2010;4 (12):1115–21.

Choubey, A., Parasar, A., Choubey, A., Iyer, D., Pawar, R. S., & Patil, U. K. (2010). Potential of medicinal plants in kidney, gall and urinary stones. *International Journal of Drug Development and Research*, *2*(2), 431-447.

Dacie JV and Lewis SM. (1968) Practical Hematology, 4th edition J and A, Churchill, UK, 37.

Dalle, D. I., Rossi, R., Colombo, R., Giustarini, D., & Milzani, A. (2006). Biomarkers of oxidative damage in human disease. *Clinical Chemistry*, *52*(4), 601–623.

El Hilaly J, Israili ZH, Lyoussi B. Acute and chronic toxicological studies of Ajuga iva in experimental animals. J Ethnopharmacol 2004;91(1):43–50.

Eran, B.-A.; Noah, S.; Lee, H.G.; Kamer, M.; Suha, O.; Elad, S. Potential risks associated with traditional herbal medicine use in cancer care: A study of middle eastern oncology health care professionals. Cancer ***2016*****,** 122**, 598–610** .

Ezeja MI, Anaga AO, Asuzu IU. Acute and sub-chronic toxicity profile of methanol leaf extract of Gouania longipetala in rats. J Ethnopharmacol 2014;151(3):1155–64.

Friedewald's WT., Levy RT and Fredrickson DS. (1972) Estimation of low-density lipoprotein cholesterol in plasma, without use of the preparative centrifuge. Clin. Chem., 23: 499.

Hilaly, J.; Israili, H.; Lyoussi, B. Acute and chronic toxicological studies of Ajuga iva **in experimental animals.** J. Ethnopharmacol. ***2004*****,** 91**, 43–50.**

Hoff-Brand AV, Pettit JE. Hematological parameters in essentials of haematology. 4th ed. New Jersey: Blackwell Science; 2000.

Kakkar P, Das B and Viswanathan PN. (1984) A modified spectrophotometric assay of SOD. *Ind J Biochem Biophy,* 21: 130-132.

Kale, O. E., Awodele, O., & Akindele, A. J. (2019). Subacute and subchronic oral toxicity assessments of Acridocarpus smeathmannii (DC.) Guill. & Perr. root in Wistar rats. *Toxicology reports*, *6*, 161-175.

Kharchoufa, L., Bouhrim, M., Bencheikh, N., El Assri, S., Amirou, A., Yamani, A., & Elachouri, M. (2020). Acute and subacute toxicity studies of the aqueous extract from Haloxylon scoparium Pomel (Hammada scoparia (Pomel)) by oral administration in rodents. *BioMed Research International*, *2020*.

Kifayatullah, M., Mustafa, M. S., Senguptha, P., Sarker, M. M. R., Das, A., & Das, S. K. (2015). Evaluation of the acute and sub-acute toxicity of the ethanolic extract of Pericampylus glaucus (Lam.)Merr inBALB/c mice. *Journal of Acute Disease*, *4*(4), 309–315.

Kind, E.J., King, R.P.N. (1954). Determination of alkaline phosphatase activity by colorimetric method.*J. Clin. Path*. 7, 322.

Kluwe, W.M. Reanl functions tests as indicators of kidney injury in subacute toxicity studies. Toxicol. Appl. Pharmacol. ***1981*****,** 57**, 414–424.**

Lekshmi, V.S.; Rauf, A.A.; Kurup, G.M. (2019) Toxicological Evaluation of Bioactive Sulfated Polysaccharides from the Edible Marine Algae Padina tetrastromatica and Ulva fasciata in Sprague-Dawley Rats. J. Biol. Act. Prod. Nat. , 9, 79-96,

Lowry OH, Rosenbrough N, Fair AC, Randall RJ (1951). Protein measurements with folin phenol reagent. J.Biol.Chem. 193:265-275.

Maclachlan J. A. and R. L. Dixon, Advances in modern toxicology: new concepts in safety evaluation, Vol. Part 1 (Hemisphere publishing, Washington D.C., 1976. pp. 423–448.

Malloy, H.T., Evenlyn, K.A., 1937. J. Biol. Chem. 119, 481.

Maruna and Trinders SR (1958) Determination of serum sodium by colorimetric method. Clin.Chem Act 2.1.581.

Maruna RFL. (1957) Determination of serum potassium by colorimetric method. Clinica chemica acta, 2(2): pp131-133.

Moron MS, DsePierre JW and Manerwik KB.(1979) Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. Biochimicaet Biophysica Acta, 582: pp67-68.

Muhammad S, Hassan LG, Dangoggo SM, Hassan SW, Umar KJ, Aliyu RU. Acute and subchronic toxicity studies of kernel extract of Sclerocarya birrea in rats. Sci World J 2011;6(3):11–4.

Mukinda, J., & Syce, J. A. (2007). Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. *Journal of Ethnopharmacology*, *112*(1), 138–144.

Natelson S. (1957) Micro-techniques of clinical chemistry for the routine laboratory. C.C.Thomas, Spring-Field, Illinois, p: 381.

National Research Council (NRC). Toxicity Testing for Assessing Environmental Agents**; Interim Report; National Academics Press: Washington, DC, USA, 2006.**

Ochei J and Kolhatkar A (2000). Medical Laboratory Science, Theory and Practice, Tata McGraw-Hill Publishing Company Limited, New Delhi. 276-287.

Odeyemi, O.O.; Yakubu, M.T.; Masika, P.J.; Afolayan, A.J. Toxicological evaluation of the essential oil from Menthalongifolia **L. subsp. capensis leaves in rats.** J. Med. Food. ***2009*****,** 12**, 669–674.**

Olorunnisola, O.S.; Bradley, G.; Afolayan, A.J. Acute and subchronic toxicity studies of methanolic extract of Tulbaghiaviolacea **rhizomes in Wistar rats.** Afr. J. Biotechnol. ***2012*****,** 11**, 14934–14940.**

Omaye ST, Tumball JD and Sauberlich HE. (1979) Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods in Enzymology, 62: 1-11.

OECD (Organization for Economic Co-operation and Development), "Test no. 423: acute oral toxicity-acute toxic class method," The OECD Guideline for Testing of Chemicals , OECD, Rome, 2001.

OECD (Organization for Economic Co-Operation and Development), "Test no. 407: repeated dose 28-day oral toxicity study in rodents," The OECD Guidelines for Testing of Chemicals, OECD, Paris, France, 1995.

Osafanme Lucky Iserhienrhien & Paulinus Ngozi Okolie, Cogent Acute and sub-acute toxicity profile of methanol leaf extract of *Geophila obvallata* on renal and hepatic indices in Wistar rats. Food & Agriculture (2020), 6: 1794240.1-12.

Pajero, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Fieriage, N., Burillo, J., & Codina, C. (2002). Between the free radical scavenging activity and anti- oxidant activity of six distilled and non distilled Mediterranean herbs and aromatic plants. Journal of Agricultural and Food Chemistry, 50(23), 6882–6890

Raina P, Chandrasekaran CV, Deepak M, Aggarwal A. Ruchika KG. Evaluation of subacute toxicity of methanolic/aqueous preparation of aerial parts of O. sanctum in Wistar rats: clinical, hematological, biochemical and histopathological studies. J Ethnopharmacol 2015;175:509–17.

Reitman and Frankel S. (1957) A colorimetric method for the determination of serum glutamate oxaloacetic and glutamate pyruvate transaminases. Am J Clin Pathol, 28: 56-63.

Rodkey FL. (1965) Direct spectrophotometric determination of albumin in human serum. Clinical Chemistry 11: pp478-9.

Rokaya, M. B., Uprety, Y., Poudel, R. C., Timsina, B., Münzbergová, Z., Asselin, H., ... & Sigdel, S. R. (2014). Traditional uses of medicinal plants in gastrointestinal disorders in Nepal. *Journal of ethnopharmacology*, *158*, 221-229.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG and Hoekstra WG. (1973) Selenium: biochemical roles as component of glutathione peroxidase. Sci, 179: 588-590.

Sharif, H. B., Mukhtar, M. D., Mustapha, Y., Baba, G., & Lawal, A. O. (2015). Acute and subchronic toxicity profile of Euphorbia pulcherrima methanol extract on Wistar albino rats. *Advances in Pharmaceutics*, *2015*.

Suntar, I. (2020). Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. *Phytochemistry Reviews*, *19*(5), 1199-1209.

Vivekanandhan, P., Senthil-Nathan, S., & Shivakumar, M. S. (2018). Larvicidal, pupicidal and adult smoke toxic effects of Acanthospermum hispidum (DC) leaf crude extracts against mosquito vectors. *Physiological and Molecular Plant Pathology*, *101*, 156-162.

Werner M. Gabrielson D.G and Eastman G (1981). Ultramicro determination of serum triglycerides by bioluminescent assay. Clinical Chemistry. 27: pp268-271.

Yakubu M. T., M. A. Akanji, and A.T. Oladiji, "Haematological evaluation in male albino rats following chronic administration of aqueous extract of Fadogiaagrestis stem," Pharmacognosy Magazine, vol. 3, no. 9, pp. 34–38, 2007.