JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/44128
RRESEARCH ARTICLE

Evaluation of Acute and Sub-acute Toxicity Analysis of Root Powder of *Pygmaeopremna herbacea* (Roxb.) Moldenke

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Abstract

Background: Root powder of *Pygmaeopremna herbacea* (Roxb.) In traditional medicine, Moldenke, the Verbenaceae family, is widely used for managing respiratory-related disorders like bronchial asthma and pneumonia. **Aim:** This study aimed to assess the acute and subacute toxicity of the root powder of *P. herbacea*. The drug powder was prepared according to the standard traditional classical textbook. **Methods:** Sub-acute and acute toxicity were evaluated using WHO standards in Wistar albino rats and Swiss albino mice, respectively. The animals were administered 2000 mg/kg of the test drug in the acute toxicity trial, and for up to 14 days, any toxic symptoms and death were monitored. The test drug was administered to the animals for up to 28 days at dosages of 200, 100, and 50 mg/kg/BW/p.o./day in a sub-acute toxicity trial, and their morbidity and mortality were evaluated. The study was concluded with the sacrifice of all experimental animals and the completion of the evaluation of all hematological, biochemical, and histopathological assessments. **Results:** The acute toxicity investigation revealed no potentially dangerous signs of mortality. Animals in the sub-acute toxicity study did not show noteworthy variations in body weight alterations, water and food consumption, or haematological, biochemical, or histopathological features among the experiment and the control groups. **Conclusion:** According to this study, experimental animals receiving a long-term oral dosage of 200 mg/kg/BW of root powder of *P. herbacea* did not experience any harmful adverse reactions, stating that the human utilisation of the test drug is safe.

Keywords: Acute Toxicity, Bronchial Asthma, Herbal Medicine, *Pygmaeopremna herbacea*, Root Powder, Sub-acute Toxicity

1. Introduction

Reversible obstruction of the airway results from multiple manifestations of bronchospasm, mucosal oedema, and increased mucous production, which can be caused by IgE production, mast cell degranulation, eosinophil sensitization, and massive lymphocyte recruitment that define bronchial asthma,

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Article Received on: 21.05.2024 Revised on: 10.07.2024 Accepted on: 22.07.2024

a hypersensitivity disorder[1]. Due to its increasing prevalence, bronchial asthma is now included in the United Nations 2030 Agenda for Sustainable Development and the WHO Global Action Plan for the Prevention and Control of Non-Communicable Diseases[2]. Nowadays, there are a lot of bronchodilators on the market for treating bronchospasm disorders, but they frequently bring about adverse reactions that include palpitations, tremors, tachycardia, coughing, and so on[3]. Therefore, due to their extreme significance, affordability, safety, and lack of serious adverse reactions, herbal medications are extremely beneficial[4]. The development of groundbreaking herbal remedies with zero adverse side effects thus becomes needed, as described in the *Siddha* classic textbooks.

Root powder of *P. herbacea* (Roxb.) Moldenke, which is documented in the classical traditional literature *Siddha* Materia Medica, Plant division[5], belongs to the family Verbenaceae. This plant has been used as an ingredient in many of the *Siddha* preparations, which are prescribed for a variety of respiratory disorders like bronchial asthma, pneumonia and musculoskeletal disorders.

Despite the long tradition of consumption of *Siddha* herbal remedies, safety profiling as reverse pharmacology ought to be addressed for the benefit of the contemporary scientific community[6]. So, this study was therefore undertaken to assess the sub-acute and acute toxicity of the root powder of *P. herbacea*.

2. Materials and Methods

2.1 Preparation of Trial Drug

Pygmaeopremna herbacea root has been purchased, cleaned, and crushed into a fine powder by the directions provided in a typical *Siddha* classical textbook. The preparation of the trial drug is depicted in Figure 1A and 1B.

2.2 Chemicals and Reagents Used in the Study

Sigma-Aldrich supplied all the AR-grade chemicals and solvents that were used during the study. Applying commercialized test kits from Sigma-Aldrich, the biochemical parameters were analyzed.

2.3 Toxicity Profiling of Root Powder of *Pygmaeopremna herbacea* (Roxb.) Moldenke

The K.M. College of Pharmacy in Madurai, Tamil Nadu, India's Institutional Animal Ethical Committee, approved the protocol used for the experiments under the approval number IAEC/C.B.S.BHARATH CHRISTIAN/MD(S)/TNMGRMU/KMCP/84/2020 according to the Organization for Economic Co-operation and Development (OECD) guidelines at the college's animal house. This permitted the implementation of acute and sub-acute toxicity studies.

2.4 Experimental Animals

The K.M. College of Pharmacy in Madurai, Tamil Nadu, has an animal house from which we got animals for experimentation.

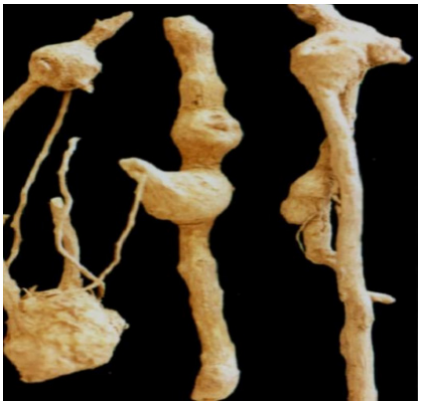

Figure 1 A. Root of *Pygmaeopremna herbacea*.

Figure 1 B. Root powder of *Pygmaeopremna herbacea*.

2.5 Selection of the Animals

Acute toxicity tests have been carried out on young adult female Swiss albino mice in good health, weighing 25±5 g, nulliparous, and not pregnant. Considering they were more sensitive to therapy, female rats were chosen. Wistar albino rats, weighing 160±20 g, male and female, were utilised to perform the sub-acute toxicity study.

2.6 Housing and Diet

The animals were housed in temperature-controlled (23 ± 2 °C) sawdust litter polypropylene cages sized 55.0 × 32.70 × 19.0 cm. For every 24 hours, the lighting had been programmed, so there was a total of twelve hours of light and twelve hours of darkness. Each of the cages has been identified with a card. This card included the test drug code, administration route, dosage level, and cage number in addition to the weight and number of animals in its interior. The animals were provided with water on demand, together with regular laboratory animal feeding pellets.

2.7 Dose Fixation

The traditional *Siddha* classical textbook recommended 2-4 g of *P. herbacea* root powder for medicinal use. The recommended dosage for mice used for acute oral toxicity was found to be 4000 mg/kg/BW based on the estimated values of Paget and Branes. The optimal dose for the low-dose, mild-dose, and high-dose groups of rats used for sub-acute oral toxicity has been established to be 400, 2000, and 4000 mg/kg/BW, respectively.

2.8 Mode of Administration of the Control Diet and Test Drug

Three hours before the administration of the medication, the animals were fasted (only food wasn't given for three hours; water was not withheld). Animals were weighed after the fasting period, and a single dosage of the test substance or control diet was administered by gavage using a specially made mouse oral needle. The mice were not given food for two hours following the administration of the test drug.

2.9 Observation Period

The animals were examined one-on-one for the first thirty minutes, then one-on-one over the following twenty-four hours. During the initial four hours and every day after that, they obtained additional medical care. This process continued for a total of fourteen days. Every rat was examined at least twice a day for any signs of illness or changes in behaviour.

2.10 Acute Oral Toxicity Study: OECD Guidelines 420 (Fixed Dose Procedure)

16 animals in all were split into 2 groups, each consisting of 8 animals (4 males and 4 females). Honey was given to the animals in group I for 14 days. The test medication, *P. herbacea* root powder (4000 mg/kg/b.w.), was given to the group II animals only once. Two hours were spent without eating after the test drug was administered. Alertness, aggression, alopecia, circling, diarrhoea, oedema, touch reaction, grip strength, grooming, lacrimation, writhing reflex, tremors, nasal sniffing, pile erection, analgesia, righting reflex, seizures, muscular spasm, hypnosis were among the criteria that were directly observed. Observations also included alterations in the respiratory, circulatory, autonomic, and central nervous systems; skin and hair; eyes and mucous membranes; somatomotor activity; and behavioural patterns. If there was one, the moment of death was noted. After a day, the number of survivors was recorded, and they were subsequently followed up every day for an additional 14 days. Weekly blood analyses were performed both during and after the course of medication. The animals were weighed and sacrificed with an injection of thiopental sodium on the fifteenth day. A thorough necropsy was performed, and the organs were examined for abnormal changes.

2.11 Sub-acute Oral Toxicity Study (28 Days): OECD Guidelines 407 (Repeated Dose Procedure)

A sub-acute toxicity test was conducted on 180 ± 10 g Wistar rats, which included both male and female animals. Four groups of five animals were assembled from the selected animals. The animal's body weight is used to determine how much drug should be administered. The weight variations are then documented by weighing the animals every seven days, starting from the beginning of the therapy. For 28 days, the animals in group I received a single oral dosage of 5 ml/kg of normal saline. For 28 days, the animals in groups II, III, and IV received oral doses of 400 mg, 2000 mg, and 4000 mg/kg/BW of *P. herbacea* root powder, respectively. Following the delivery of the test drugs, the animals were observed for any changes in behaviour. Food and water consumption

were measured every day. To document the weight differences, the animals were weighed once a week, beginning at the study's commencement. After a brief period of anaesthesia, blood samples were taken for biochemical analysis both during and after the therapy by puncturing the retro-orbital plexus. To extract plasma, the obtained blood sample was spun at 4000g for 10 minutes within 5 minutes after collection. Weekly blood analyses were performed both during and after the course of medication. The animals were weighed and sacrificed with an injection of thiopental sodium on the 29th day. The organs were kept in the formalin solution for histological examination after a gross necropsy was conducted.

2.11.1 Hematological Parameters Assessment

Blood samples collected from the control and experimental rats were analyzed for total white blood cell count, platelets count, total red blood cell count, haemoglobin, mean corpuscular haemoglobin and mean corpuscular volume.

2.11.2 Biochemical Parameters Assessment

Biochemical analysis such as renal function test (blood urea nitrogen, creatinine), liver function test (direct bilirubin, indirect bilirubin, total bilirubin, AST, ALT, albumin, globulin, total protein) and lipid profile (low-density lipoprotein, high-density lipoprotein, very low-density lipoprotein, triglycerides, total cholesterol) was done in every week and at the end of the treatment.

2.11.3 Histopathological Evaluation

The animals in the high-dose group and the control group were first examined histopathologically. The brain, heart, lungs, stomach, liver, kidney, spleen, testes, uterus, and ovary were among the organs that were taken, preserved, and sectioned into 4–6 micrometre pieces. These sections were then stained with hematoxylin and eosin stains and examined under a 40x lens to look for changes in the histopathology.

2.11.4 Statistical Analysis

The data is presented as the mean ± standard error of the mean, or SEM. A one-way analysis of variance (ANOVA) was used to statistically evaluate the haematological and biochemical markers, food and water intake, and changes in body weight of the test and control groups. Dunnett's test was used to assess all of the data using the computer program Graph Pad Instant, version 3.1. $P < 0.05$ was used to identify a statistically significant threshold.

3. Results

3.1 Acute Toxicity Study

At the 4000 mg/kg/BW dosage in the acute toxicity investigation, no Swiss Albino mice sustaining toxicity or treatment-related mortality exhibited symptoms over the study period (Table 1). As soon as the test drug was administered, and even up to fifteen minutes later, writhing reflux was seen. Every animal's morphological traits, outward appearance, and neurological systems appeared to be typical. Every animal has undergone a thorough necropsy. Neither the treatment group nor the control group's internal organs had any apparent pathological changes.

3.2 Sub-acute Oral Toxicity Study

All of the treatment group and control group animals' behavioural measures were found to be unchanged in the sub-acute toxicity investigation shown in Table 2. When compared to control rats administered normal saline, the male and female animals treated with the test drug had typical development patterns, with an approximate rise in body weight shown as in Tables 3 and 4 and an increase in food and water consumption shown as in Tables 5 to 8. The test group did not exhibit any significant change in the parameters that were found to be normal within the physiological limit when compared to the control group. Haematological parameters and calcium levels noted as in Table 9, renal function test parameters noted as in Table 10, liver function test parameters noted as in Table 11, and lipid profile parameters noted as in Table 12 were observed once a week and immediately before the necropsy. When compared to the animals in the control group, histopathological examination of several organs, including the brain, heart, lungs, stomach, liver, kidney, spleen, testes, uterus, and ovary, showed no abnormalities in the high-dose animal groups. All of the microscopic images of every organ in the male and female control and high-dose-tested animals were depicted in Figures 2 to 11.

Table 1. Effect of *Kanduparangi chooranam* on behaviour signs of Swiss Albino mice

Sl. No.	Group	Activity																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	Control	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-
2	Test 4000mg/ kg/BW	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-

1. Aggressiveness 2. Alertness 3. Alopecia 4. Circling 5. Diarrhea 6. Oedema 7. Touch Response 8. Gripping 9. Grooming 10. Lacrimation 11. Writhing reflex 12. Tremors 13. Nasal sniffing 14. Pile erection 15. Analgesia 16. Righting reflex 17. Seizures 18. Muscle spasm 19. Hypnosis 20. Mortality [(-) - Absence of activity; (+) - Presence of Activity]

Table 2. Signs of sub-acute toxicity (28 days) on exposure to root powder of *P. herbacea*

Sl. No.	Parameters Observed 2	Day													
		4	6	8	10	12	14	16	18	20	22	24	26	28	
1	Aggressiveness	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Alertness	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	Circling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	Alopecia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Oedema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Touch Response	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	Grip strength	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Writhing reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	Tremors	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	Nasal sniffing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	Pile erection	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Analgesia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	Muscle spasm	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	Hypnosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3. Effect of root powder of *P. herbacea* on body weight of male Wistar albino rats

Duration	Groups [Body weight in grams]			
	Control	Dose		
		Low	Mid	High
7th day	176.5±17.81	172.5±9.76	167.7±2.50	184.5±9.93
14th day	190.1±14.43	188.9±10.44	182.1±12.56	212.3±16.63
21st day	232.5±19.08	196.7±27.92	196.7±27.92	225.1±19.38
28th day	246.3±26.51	213.2±35.12	213.1±35.12	237.3±23.17

When n = 5, values are reported as mean± S.E.M. (Dunnett'stest)**. 0.01*P<0.05 ns P>0.05

Table 4. Effect of root powder of *P. herbacea* on body weight of female Wistar albino rats

Duration	Groups [Body weight in grams]			
	Control	Dose		
		Low	Mid	High
7th day	167.4±14.72	152.8±12.34	153.6±21.10	180.2±5.26
14th day	178±14.83	158.2±14.81	158.2±14.80	189.5±10.35
21st day	196.8±5.05	173±9.96	174.2±9.94	200.3±16.16
28th day	213.4±7.11	185.7±8.94	186.3±8.95	208.5±19.34

In the case of n=5 (Dunnett'stest)** p<0.01 *p<0.05 ns p>0.053, values are given as mean ± S.E.M.

Table 5. Effect of root powder of *P. herbacea* on water intake of male Wistar albino rats

Duration	Groups [Water intake in ml/day]			
	Control	Dose		
		Low	Mid	High
7th day	78.47±5.56	80.1±5.77	79.32±5.53	82.24±6.36
14th day	81.43±3.77	85.2±5.73	85.61±6.72	85.1±5.77
21st day	87.24±4.87	87.13±7.55	88.67±6.90	92.13±5.66
28th day	95.61±6.72	99.26±6.72	98.53±5.56	101.44±5.56

In the case of n=5 (Dunnett'stest)** p<0.01 *p<0.05 ns p>0.05, values are given as mean ± S.E.M.

Table 6. Effect of root powder of *P. herbacea* on water intake of female Wistar albino rats

Duration	Groups [Water intake in ml/day]			
	Control	Dose		
		Low	Mid	High
7th day	75.43±5.56	72.67±5.56	76.61±5.34	80.61±5.34
14th day	82.43±3.77	84.70±10.29	82.01±4.08	81.52±3.77
21st day	90.01±5.77	91.4±4.08	88.67±7.4	86.56±2.43
28th day	94.82±4.82	97.61±3.45	92.24±1.36	90.61±4.49

In the case of n=5 (Dunnett'stest)** p<0.01 *p<0.05 ns p>0.05, values are given as mean ± S.E.M.

Table 7. Effect of root powder of *P. herbacea* on food intake of male Wistar albino rats

Duration	Groups [Food intake in g/day]			
	Control	Dose		
		Low	Mid	High
7th day	43.75±0.81	41.38±1.11	42.24±1.34	45.61±0.75
14th day	47.24±1.06	44.32±1.90	45.67±0.53	47.04±0.81
21st day	50.18±1.38	45.38±0.75	47.02±0.81	50.47±1.27
28th day	55.11±2.16	49.38±1.79	49.61±0.755	54.18±0.75

In the case of n=5 (Dunnett'stest)** p<0.01 *p<0.05 ns p>0.05, values are given as mean ± S.E.M.

Table 8. Effect of root powder of *P. herbacea* on food intake of female Wistar albino rats

Duration	Groups [Food intake in g/day]			
	Control	Dose		
		Low	Mid	High
7th day	41.47±1.27	37.05±2.309	37.32±1.13	45.43±0.53
14th day	44.61±0.75	40.61±1.30	40.73±1.11	45.72±0.75
21st day	46.16±0.89	44.52±1.27	43.58±0.97	46.27±1.11
28th day	48.86±0.69	48.38±1.11	45.29±1.38	46.86±0.69

In the case of n=5 (Dunnett'stest)** p<0.01 *p<0.05 ns p>0.05, values are given as mean ± S.E.M.

Table 9. Effect of root powder of *P. herbacea* on haematological parameters and calcium

Parameters with units	Groups			
	Control	Dose		
		Low	Mid	High
Red blood cell ($\times 10^6$ µl)	6.74±1.09	6.45±0.56	6.36±1.20	6.27±1.58
Hb (%)	11.76±1.80	12.86±1.02	12.97±2.04	12.97±1.04
Leukocyte ($\times 10^3$/µl)	8.92±1.67	10.53±1.48	8.07±1.97	10.24±0.90
Platelets ($\times 10^3$/µl)	468.91±92.55	660.6±162.75	565.4±224.43	604.4±131.68
MCV (pg)	17.47±1.80	19.22±1.83	17.97±1.72	18.93±2.16
MCH (fl)	61.71±4.38	59.72±4.5	57.41±4.31	60.98±6.09
Calcium (mg/dl)	8.85 ±0.40	9.10 ±0.48	9.45 ±0.56	9.43 ±0.53

Hb - Hemoglobin; MCV - Mean Corpuscular Volume; MCH - Mean Corpuscular Hemoglobin
In the case of n=5 (Dunnett'stest)** p<0.01 *p<0.05 ns p>0.05, values are given as mean ± S.E.M.

Table 10. Effect of root powder of *P. herbacea* on kidney function test

Parameters with units	Groups			
	Control	Dose		
		Low	Mid	High
Blood Urea Nitrogen (mg/dl)	17.12±2.92	18.51±1.84	21.02±4.76	21.11±5.72
Creatinine (mg/dl)	0.57±0.17	0.72±0.24	0.74±0.22	0.67±0.25

In the case of n=5 (Dunnett'stest)** p<0.01 *p<0.05 ns p>0.05, values are given as mean ± S.E.M.

4. Discussion

Pygmaeopremna herbacea root powder has long been used by *Siddha* traditional healers and practitioners for the treatment of neuralgic disorders and bronchial asthma. But as of yet, the drug's safety hasn't been demonstrated through research. According to WHO standards, the acute and sub-acute toxicity of *P. herbacea* root powder was evaluated in this study using Swiss albino mice and Wistar albino rats as models, respectively.

During the long-term (14 days) and short-term (48 hours) observation periods, animals did not exhibit any clinical signs of acute toxicity or mortality when administered the limited dosage of 4000 mg/kg (KC) in instances of acute toxicity. This indicated that there was no acute oral toxicity resulting from *P. herbacea* root powder.

Since the extracts' mean lethal dosage (LD50) exceeded 4000 mg/kg body weight, the test drug did not cause any side effects when administered once. The data were significant at a p-value < 0.5 based on

Table 11. Effect of root powder of *P. herbacea* on function tests for liver

Parameters with units	Groups			
	Control	Dose		
		Low	Mid	High
Indirect Bilirubin (mg/dl)	0.1±00	0.1±00	0.1±00	0.1±00
Direct Bilirubin (mg/dl)	0.1±0.02	0.1±0.02	0.1±0.04	0.1±0.02
Total Bilirubin (mg/dl)	0.210±0.21	0.212±0.11	0.214±0.26	0.211±0.21
SGOT(U/L)	89±17.46	93.3±13.75	80.1±6.85	93.5±12.02
SGPT(U/L)	24.9±5.47	27±5.52	26.9±4.98	33.5±7.06
ALP (IU/L)	258.40±4.08	245.30±3.70	249.10±3.85	249.75±3.75
Total protein (g/dl)	10.20±0.87	9.48±0.47	9.22±1.21	9.48±0.45
Globulin (g/dl)	6.02±0.25	5.84±0.21	5.28±0.41	6.02±0.23
Albumin (g/dl)	3.09±0.01	3.10±0.07	3.12±0.11	3.10±0.07

In the case of n=5 (Dunnett'stest)** $p<0.01$ *$p<0.05$ ns $p>0.05$, values are given as mean ± S.E.M.

Table 12. Effect of root powder of *P. herbacea* on lipid profile

Parameters with units	Groups			
	Control	Dose		
		Low	Mid	High
HDL (mg/dl)	52.7±7.86	58.6±12.01	61.3±9.84	53.1±10.97
LDL (mg/dl)	46.5±8.71	39.3±17.02	45.9±20.42	43.1±14.14
VLDL (mg/dl)	17.1±1.73	17.71±1.42	13.46±2.41	13.68±1.92
TGL (mg/dl)	44.5±11.03	38.9±7.69	37.8±12.02	46±9.02
Total cholesterol (mg/dl)	126.15±22.46	121.62±21.71	123.4±33.02	125.09±27.82

LDL - Low-Density Lipoprotein; TGL - Triglycerides;
HDL - High-Density Lipoprotein; VLDL - Very- Low-Density Lipoprotein;
In the case of n=5 (Dunnett'stest)** $p<0.01$ *$p<0.05$ ns $p>0.05$, values are given as mean ± S.E.M.

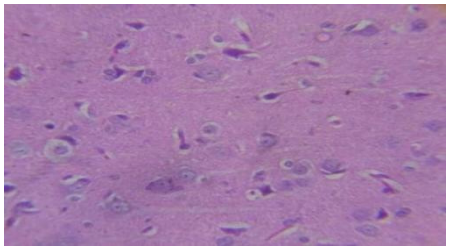

Figure 2A. Brain (Control -male) showed normal histology of striatum.

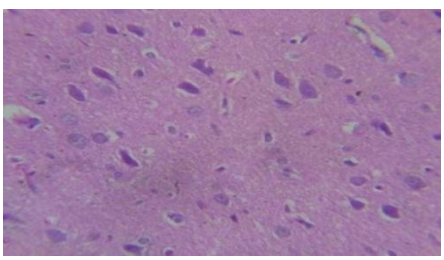

Figure 2B. Brain (High dose - male) showed a prominent nucleus with a scattered combination of medium- to large-sized neurons.

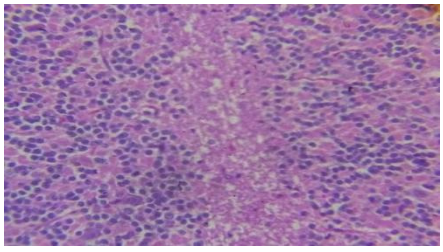

Figure 2C. Brain (Control - female) showed Purkinje cells arranged in multi-layer cells with no obvious changes.

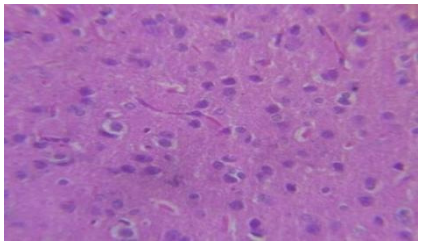

Figure 2D. Brain (High dose- female) showed cerebral region shows the neuronal populations.

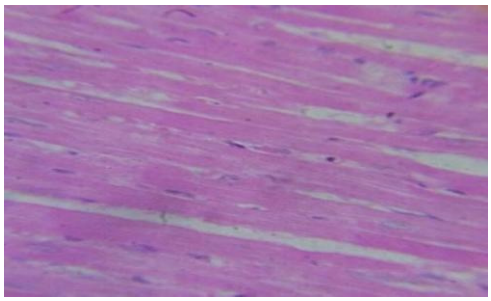

Figure 3A. Heart (Control -male) showed fibres appear normally elongated and rod-shaped.

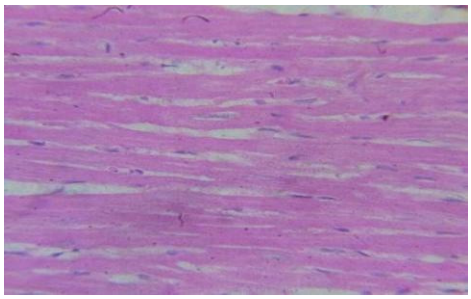

Figure 3B. Heart (High dose- male) showed normal appearance of myocyte.

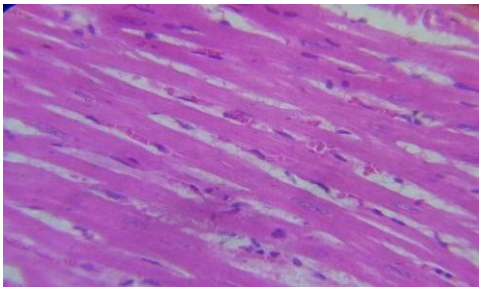

Figure 3C. Heart (Control - female) showed the normal appearance of heart fibres, there were no histological alterations.

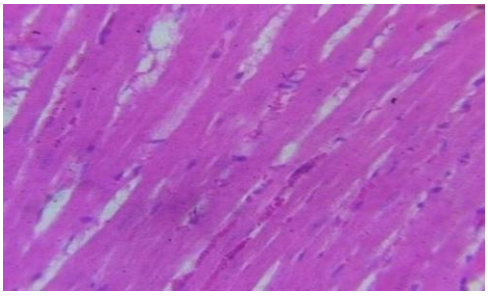

Figure 3D. Heart (High dose female) showed normal cytoarchitecture of the myocardium.

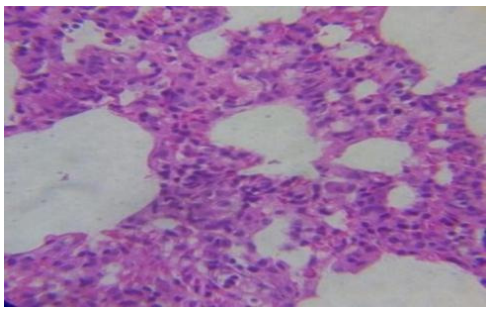

Figure 4A. Lung (Control -male) showed alveolar epithelium and capillaries appear normal.

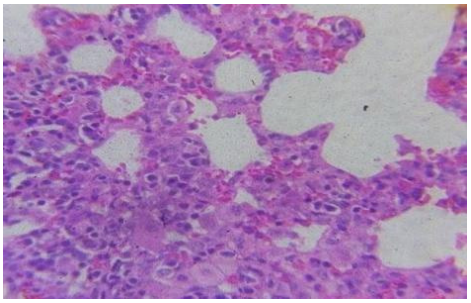

Figure 4B. Lung (High dose-male) showed the normal arrangement of epithelial and muscular cells.

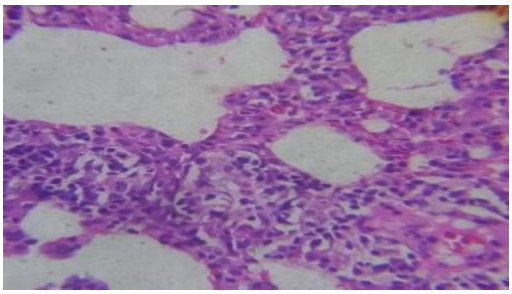

Figure 4C. Lung (Control -female) showed the appearance of the alveolar network was normal.

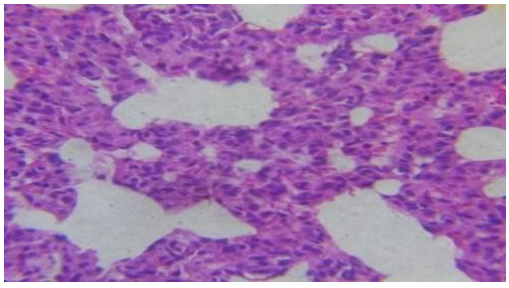

Figure 4D. Lung (High dose-female) showed the normal inter alveolar septum and alveolar capillaries.

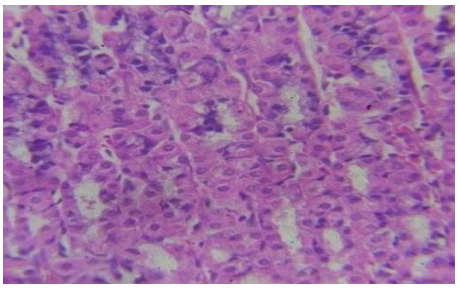

Figure 5A. Stomach (Control -male) showed the normal sub-mucosa and gastric glands.

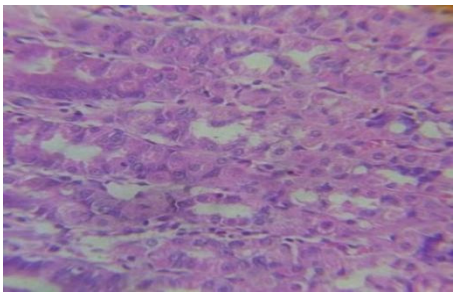

Figure 5B. Stomach (High dose-male) showed normal surface epithelium, mucosa and sub-mucosa.

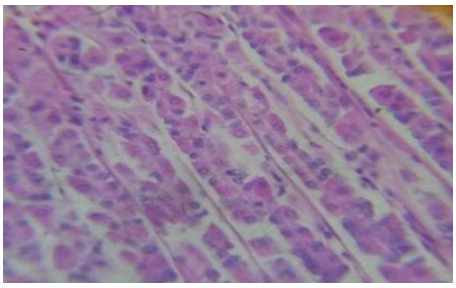

Figure 5C. Stomach (Control -female) showed the regular arrangement of outer longitudinal muscle and muscularis externa.

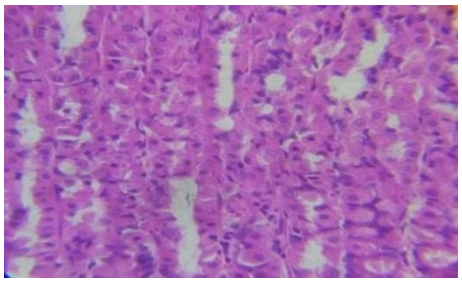

Figure 5D. Stomach (High dose- female) showed normal intra cytoplasmic zone of the mucosa.

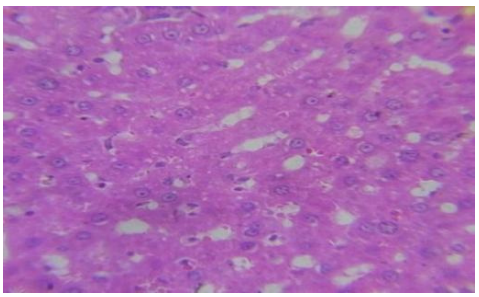

Figure 6A. Liver (Control -male) showed the presence of normal walls of the lumen without ischemia.

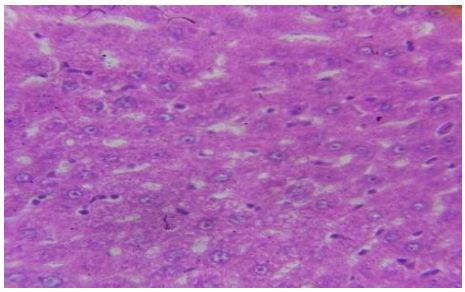

Figure 6B. Stomach (High dose - male) showed the normal liver parenchyma without any evidence of necrosis.

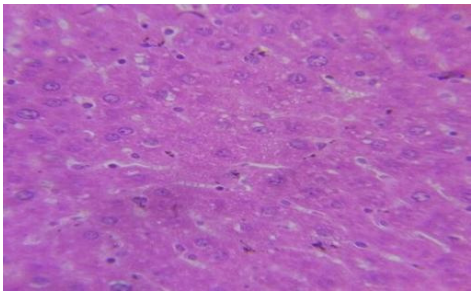

Figure 6C. Liver (Control - female) showed cytoplasm appears normal with widened portal tract.

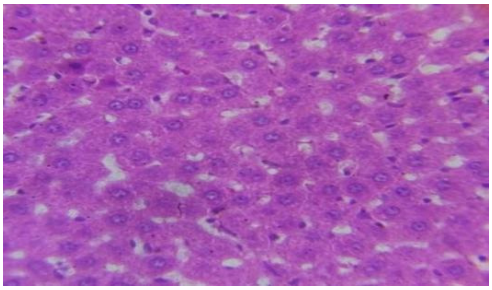

Figure 6D. Liver (High dose- female) showed normal centrilobular hepatocytes with stained cytoplasm.

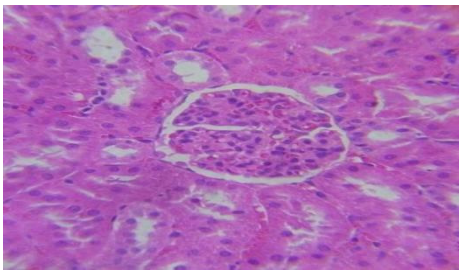

Figure 7A. Kidney (Control -male) showed the normal arrangement of the glomerular loop having regular interstitium.

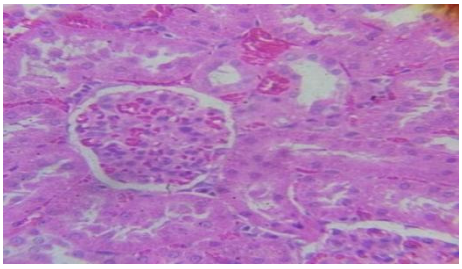

Figure 7B. Kidney (High dose-male) showed the normal lumen of vessels and bowman's space.

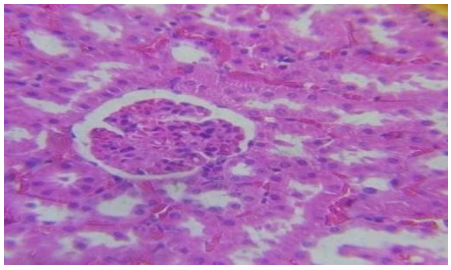

Figure 7C. Kidney (Control -female) showed normal intact renal tubules and the renal glomeruli.

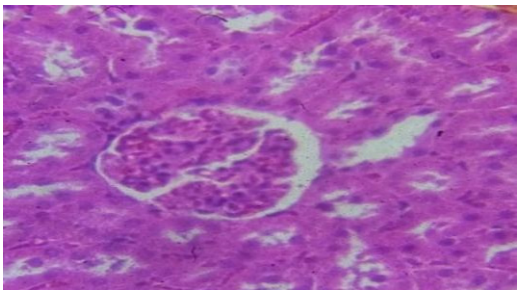

Figure 7D. Kidney (High dose- female) showed the normal appearance of proximal and distal convoluted tubules, no atrophy was noted.

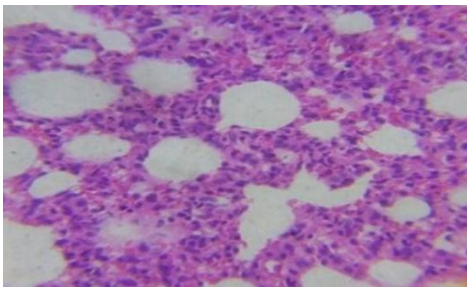

Figure 8A. Spleen (Control -male) revealed follicles and a distinct margination of the marginal zone at the red pulp's contact with the peri-arterial lymphoid sheath.

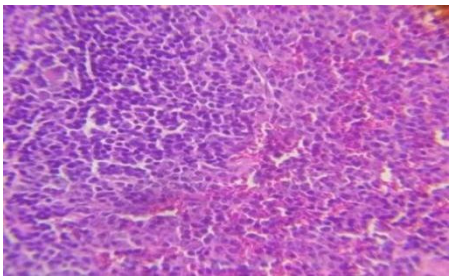

Figure 8B. Spleen (High dose - male) showed central arterioles radiating around the red pulp.

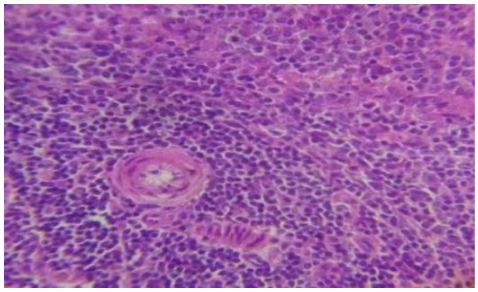

Figure 8C. Spleen (Control - female) showed the normal morphology of capsule, nodes, red and white pulp.

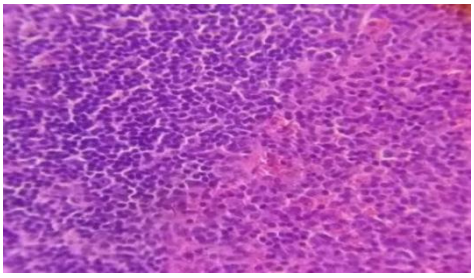

Figure 8D. Spleen (High dose - female) showed the normal Marginal Sinus (MS) of the spleen and its sinus lining cells.

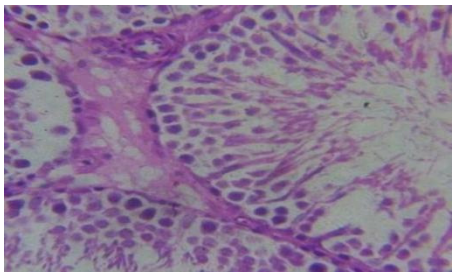

Figure 9A. Testes (Control -male) revealed the presence of primordial spermatocytes with rich chromatin and a big, centred nucleus.

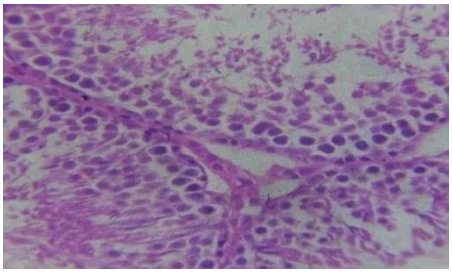

Figure 9B. Testes (High dose - male) displayed enlarged Sertoli cells and proliferating, highly divided germ cells with normal interstitial connective tissue.

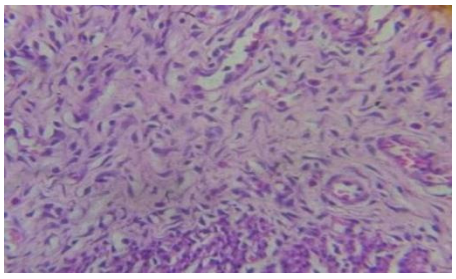

Figure 10A. Uterus (Control - female) revealed normal cytoarchitecture in layers of the uterus and glands.

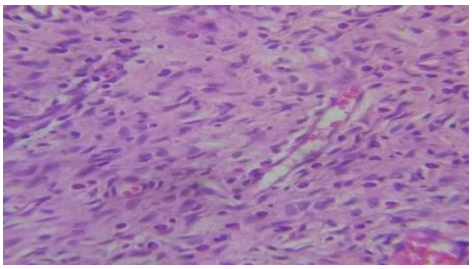

Figure 10B. Uterus (High dose - female) revealed normal epithelium and blood vessels with intact endometrial gland.

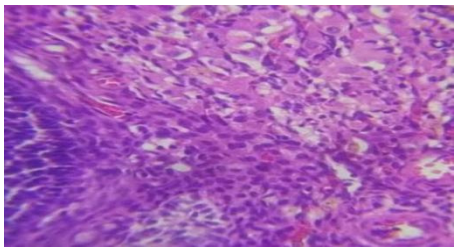

Figure 11 A. Ovary (Control - female) showed that the primary oocyte, secondary follicles, and antral follicle appearance are all good.

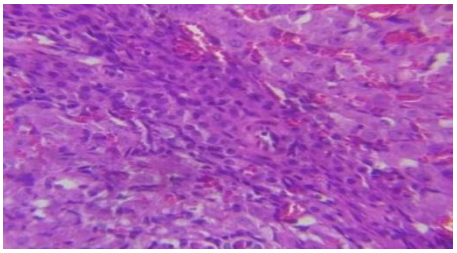

Figure 11 B. Ovary (High dose - female) Appearance of Graafian and antral follicle was normal.

the statistical analysis of the dose administered to the animals. The current investigation, which followed OECD Guidelines 420 (fixed dose procedure), showed that, even when the limit dose had been maintained at 4000 mg/kg/BW, *P. herbacea* root powder did not result in any mortality throughout the 14-day study period. All of the treatment group and control group animals' behavioural measures have been determined to be unchanged in the sub-acute toxicity study. When compared to control rats given normal saline, the male and female animals administered the test drug exhibited a typical growth pattern, increasing their consumption of food and water and slightly increasing their body weight. The test group had a rise in body weight as a result of the drug, which increased appetite.

Analyzing blood parameters is likely to be related to risk evaluation given that when data from animal research is converted to humans, changes in the haematological system have a better potential to predict human toxicity[7]. After repeated administration of the test drug over 28 days of treatment, no noticeable variations occurred between the control and test groups in every single one of the haematological parameters, which includes haemoglobin, mean corpuscular

haemoglobin, mean corpuscular volume, total red blood cell count and platelet count.

Interestingly, treatment with *P. herbacea* root powder brought about a significant increase in the amount of haemoglobin. The test drug could improve the absorption of iron, which could have been the cause. Overall, the results point to the trial drug being non-toxic to the haematological and leucopoietic systems.

Because the evaluation is based on diseases and their signs, biochemical analysis is essential when assessing the safety of medications[8]. Tests evaluating renal function, such as urea nitrogen in the blood and creatinine level, did not reveal significant changes. It indicated that there had been no kidney damage resulting from the test drugs. The test drug did not cause any nephrotoxicity, as demonstrated by the normal levels of blood urea nitrogen and creatinine.

Liver function tests, including ALT, AST, total protein, albumin, globulin, direct, indirect, and total bilirubin, in addition to lipid profile parameters like triglycerides, total cholesterol, very low-density lipoprotein, high-density lipoprotein, and low-density lipoprotein, were found to be within the physiological limits and normal. The liver predominantly synthesizes the components that makeup serum cholesterol and plasma protein levels, and changes in these constituent amounts in the serum could suggest the presence of liver damage[9]. According to the current study's findings, the test drug did not result in any hepatotoxicity.

Upon gross pathological assessment, neither the treated nor control groups' animals exhibited any signs of disease. The brain, liver, heart, kidneys, stomach, spleen, uterus, ovary, testis, and lungs were among the vital organs removed from the test groups at the end of the study and examined macroscopically to search for any apparent gross lesions when compared to the control group, which indicated no abnormal macroscopic changes. When compared to the animals in the control group, histopathological examination of several organs, including the brain, heart, lungs, stomach, liver, kidney, spleen, testes, uterus, and ovary, showed no abnormalities in the high-dose animal groups. The male and female control and high-dose tested animals' internal organs were all demonstrated to be normal under a microscope, with no significant abnormalities.

The results shown indicated that *P. herbacea* root powder has a no-observed-adverse-effect level (NOAEL). It showed the drug's safety and revealed that it could be used for a longer period without harming humans.

5. Conclusion

The results of the acute and sub-acute toxicity profiles of the root powder of *P. herbacea* (Roxb.) Moldenke showed no signs of toxicity in mice or rats, respectively, whenever the dosage was raised to 4000 mg/kg/BW. So, it is concluded that the therapeutic dose of root powder of *P. herbacea* (2 to 4g/60 kg BW) is very suitable for consumption by human beings. To demonstrate their efficacy in humans, further pharmacological properties will be investigated through experiments on animals.

6. References

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