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Assessment of Long-term Toxicity of Siddha Polyherbal formulation Thiripalathi Kashayam in Wistar Rats

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KEYWORDS

Siddha, Thiripalathi kashayam, wistar rats, long-term toxicity study, noeal

ABSTRACT:

Introduction: Medicines derived from natural sources have long served as a foundation for the development of pharmaceutical drugs. Traditional herbal medicines consist of naturally occurring plant-based substances that have been widely used for the prevention, treatment and management of various diseases as well as for nutritional support. Toxicological evaluation plays a crucial role in ensuring safety by assessing the potential risks and benefits of both synthetic and natural substances. Several investigations have reported the toxicity assessment of different medicinal plants and herbal formulations. Thiripalathi kashayam is an antidiabetic medicine mentioned in the Siddha literature; its toxicological evaluation is essential, particularly in the present context of the increasing demand for safe herbal antidiabetic drugs.

Objectives: Objective of this study to evaluate the adverse effect of Thiripalathi Kashayam after long term administration for 90 days in Wistar albino rats

.**Methods**: Animals were divided into five groups, each treated with 4, 8, 16 ml/kg body weight of Thiripalathi Kashayam corresponding to half, one and double times the proposed human therapeutic dose, for 90 consecutive days. The fifth group (satellite) received 16 ml/body weight of Thiripalathi Kashayam for 120 days. Body weight and food intake were measured weekly. At the end of the study, all animals were euthanised & serum, blood, and organs were collected and analysed using serum biochemistry, haematology and histopathology. The satellite group was kept for 4 weeks after treatment.

Journal of Chemical Health Risks

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Results: No significant treatment-related toxicological findings were noted in the clinical features, bodyweight, laboratory investigations and pathological findings of the high-dose treated groups, when compared to those of the control group.

Conclusions: No observed adverse effect level for Thiripalathi kashayam at the dose of 16 ml/kg bodyweight.

1. Introduction

The Siddha system of medicine is firmly rooted in strong philosophical principles and experimental knowledge. It is a life science that focuses on individualized therapy and a holistic approach to healthcare. Siddha is also a comprehensive medical system that promotes overall well-being by addressing mental, emotional, ethical and spiritual aspects of health. Indian Materia medica contains nearly 2000 naturally derived drugs in various traditional medical systems and practices. Toxicology explains how chemical substances interact with living systems and helps determine safe exposure limits. Since any substance can become harmful at higher doses, toxicity depends mainly on the level of exposure- small doses may be safe or even beneficial. Pre-clinical evaluation of herbal drugs also offers scientific evidence for their traditional use and confirms their safety and effectiveness.[1]

Thiripalathi Kashayam is one among the classical Siddha polyherbal formulations mentioned in the text "Mega Nivarana Pothini Ennum Neerizhivu Maruthuvam" 1/157,106. Thiripalathi Kashayam contains the following ingredients: Stem bark of Aavarai (*Cassia auriculata (Linn.),* Kadalazhinjil (*Salacia reticulata Wight*), dry unripe fruit of Nellimulli(Emblica officinalis *Linn),* epicarp of unripe fruit of Thanrikkai (*Terminalia bellerica Roxb*) and Manjal Kadukkai (*Terminalia chebula Retz),* each 4gms indicated for the treatment of Type II Diabetes mellitus (Madhumegam).[2]

This study was designed to evaluate the combined toxicological effects of the multiple ingredients present in the Thiripalathi kashayam. All experiments were designed in accordance with the WHO Research guidelines for evaluating the safety and effectiveness of herbal medicines.[3]

2. Objectives

Our study combines traditional knowledge with modern scientific methods to better understand the safety profile of Thiripalathi Kashayam. Through this integrated approach, the research aims to explore the complex safety aspects associated with this formulation. By doing so, it adds valuable information to the existing knowledge on herbal formulations and their long-term therapeutic potential. Furthermore, this investigation not only highlights the beneficial characteristics of Thiripalathi kashayam but also provides a comprehensive model for systematically assessing the safety of polyherbal medicines in a holistic manner.

3. Methods

1. Animals

The study protocol was reviewed and approved by the Institutional Ethical Committee (IAEC protocol approval number- NCP/IAEC/2025-15). The study was performed at the lab animal house facility, Nandha College of Pharmacy, Erode, India (CPCSEA Reg No:688/PO/Re/S/02/CPCSEA). A total of 100 in-house-bred Wistar rats (6-8 weeks old) were used in this study. The animals were acclimatized for 7 days and maintained under controlled conditions in sterile polypropylene cages, with a 12-12-hour light-dark cycle, temperature of 23±1℃, and relative humidity 55±5%. All rats were provided with standard pelleted feed (M/s. Hindustan Lever Ltd, Mumbai) and purified drinking water (Kent RO system) *ad libitum.*

2. Experimental design

Table.1. Experimental outline

Group No.	Groups	Dose of Thiripalathi Kashayam ml/Kg B. W	No.of males	No.of Females

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 992-999 | ISSN:2251-6727

I	Control group (Vehicle alone)	10 ml/kg of distilled water	10	10
II	Low dose group ((½ TD))	4	10	10
III	Mid dose group (TD)	8	10	10
IV	High dose group (2 TD)	16	10	10
V	Satellite group (2 TD)	16	10	10

The administered doses were determined based on the approved human dose, using a conversion factor of 0.018 [4]. The therapeutic dose of Thiripalathi kashayam in humans is 45 ml twice a day. The maximum dose was selected based on an acute oral toxicity study of Thiripalathi kashayam.(unpublished data)

3. Dose preparation

Thiripalathi kashayam was freshly prepared and then administered orally to animals with oral gavage daily for 90 days. Control group received distilled water for 90 days. The dosing volume for each animal was determined using a standard volume of 10ml/kg body weight.

4. Long term toxicity study

All experiments were designed accordance with the WHO Research guidelines for evaluating the safety and effectiveness of herbal medicines [3]. All groups were treated for 90 days. All dosages are given in Table 1. The satellite group was maintained for an additional 4 weeks following the completion of the 90-day study period.

5. Observations

The following parameters were observed (before, after dosing and during): general appearance, abnormal behavior, aggression, lacrimation, salivation, gait pattern, body position, & posture, motor coordination, autonomic nervous system function, neurological signs (tremor, convulsion), reaction to physical handling & environmental stimulation.

6. Mortality & clinical signs

During the entire study period, animals were monitored twice daily for morbidity & mortality. Any indication of ill health, behavioral abnormalities, or adverse effects were noted daily. In addition, thorough physical examinations were carried out once every week.

7. Body weights

Body weights were measured before the start of dosing & then recorded weekly throughout the study until completion.

8. Clinical pathology

At the end of the treatment period, blood was collected from overnight-fasted animals by cardiac puncture & transferred into two separate tubes for haematological and biochemical assessments. For the satellite group, blood samples were obtained on day 120 after treatment initiation to evaluate the reversibility of any treatment related changes. Before blood collection, all animals were anesthetized using Ketamine (75mg/kg) and Xylazine (10mg/kg).

1. Hematological parameters

Hematological parameters such as hemoglobin concentration (Hb), white blood cell count (WBC), red blood cell count (RBC), Differential count (DC), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Packed cell volume (PCV) & platelet count were measured.

2. Serum Biochemistry

After incubating the whole blood at 4-8℃, serum was separated and stored at -20℃ for subsequent analysis. Standardized diagnostic kits were used for estimating aspartate transaminase (AST/SGOT) (U/L), alanine transaminase (ALT/SGPT) (U/L), total protein (mg/dl), total bilirubin (mg/dl), Alkaline phosphatase (ALP)(IU/L), serum albumin (g/dL), Blood urea nitrogen (mg/dL), creatinine (mg/dL), uric acid (mg/dL), Total cholesterol (mg/dL), Triglycerides (mg/dL), High density lipoprotein (HDL) (mg/dL), Low density lipoprotein (LDL) (mg/dL), Very low-density lipoprotein (VLDL) (mg/dL).

3. Gross pathology and histopathology

All surviving animals from each group were euthanized by excess anesthesia. A complete necropsy was

Journal of Chemical Health Risks
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JCHR (2026) 16(2), 992-999 | ISSN:2251-6727

performed, and major organ including Brain, Heart, Liver, Kidney & pancrease were collected. These organs were weighed & prepared for histopathological evaluation. Animals in the satellite group were sacrificed on day 120, and the same procedure was followed. The collected tissues were fixed, routinely processed & stained with hematoxylin & eosin (H&E). All organs & tissues were first examined microscopically, and any gross lesions along with the specified tissues were sectioned and stained. Microscopical evaluation were carried out to identify histopathological alterations. When notable changes were detected in organs from the high-dose group, the corresponding organs from the remaining group were examined further. If no significant findings were noted, histopathological evaluation was not extended to the other groups.

9. **Statistical Analysis**

Results were represented as mean ± SEM. The data were analysed by using one way analysis of variance (ANOVA) followed by Dunnett's '*t*' test using GraphPad version 3. P values < 0.05 were considered as significant.

4. Results

5. General symptoms

In both sexes there were no changes in General appearance including skin colour, fur, mucosa, gait pattern, sensorimotor responses to visual, acoustic, tactile and painful stimuli. Clinical symptoms such as posture, motor coordination, , neurological signs (tremor, convulsion), reaction to physical handling & environmental stimulation were similar in all groups.

6. Mortality

No treatment-related mortality or abnormal clinical signs were observed in any of the treated animals. Gross necropsy & histopathological examination did not show any toxicologically significant lesions.

7. Body weight

No statistically significant changes in body weight were observed across all treated groups when compared to the control group.(Table 2)

8. Feed & water consumption

No statistically significant changes in food & water intake observed across all treated groups when compared to the control group.

1. Clinical Pathology

1. Haematological parameters

No significant changes in haematological parameters observed across all treated groups when compared to the control group (Table 3, 4)

2.

epatic and Renal Biomarkers

Hepatic & renal parameters in all treated groups are comparable to the normal control and remains within the range. (Table 5).

3. Lipid profile

Lipid parameters in all treated groups are comparable to the normal control and remains within the range (Table 6).

2. Histopathology

1. Macroscopic Findings

Gross pathological examination of animals from both control & treated groups did not show any lesions of toxicological relevance.

External

External examination of animals from all groups did not reveal any significant lesion.

Internal

Visceral examination of animals from all groups did not reveal any significant lesion.

2. Microscopic Findings

No treatment related microscopic lesions were detected in different tissues from the high dose group**.**

9. Discussion

Toxicity studies of Thiripalathi kashyam in experimental animals are carried out to determine the maximum tolerated dose and to evaluate any treatment related changes in hematological parameters, serum biochemical markers & histopathological findings.Body weight changes are key indicator for assessing an animal's overall health during studies. A reduction in body weight is often the earliest sign of an emerging adverse effect. Any dose producing a body weight loss of 10% or more is generally regarded as toxic.[5] Body weight variation (Table.2) in the treated groups showed no significant difference compared to the normal control group, indicating that long-term administration of Thiripalathi kashayam did not produce any toxic effect in rats.No significant alterations in the hematological parameters in

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 992-999 | ISSN:2251-6727

the treated groups. All hematological values within normal physiological ranges and were not considered toxicologically relevant.Clinical biochemistry was performed to assess any potential changes in Liver & Kidney functions that may be affected by the test drug. Biochemical evaluations showed no significant differences in the measured parameters compared with the respective control groups.Gross pathological examination was performed, and histopathological evaluation of the vital organs showed normal tissue architecture, comparable to that of the control group.

10. Conclusion

Wistar rats successfully tolerated long-term dosing of Thiripalthi kashaym at 16ml/kg bodyweight (equivalent to double the therapeutic dose) for 90 days. No treatment-related adverse effects were observed in clinical signs, feed & water intake, biochemical, hematological parameters and histopathological findings. Therefore, the no-observed -adverse-effect level (NOAEL) for Thiripalathi kashayam can be considered as double the therapeutic dose.

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12. Conflict of Interest

The authors declare no Conflict of Interest

13. Funding

Nil

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Table: 2. Effect of *Thiripalathi Kashayam* on body weight in Wistar rats

Drug Treatment	Body Weight (gms)				
	Control group	Low dose group	Mid dose group	High dose group	Satellite group
Initial	80.22±5.23	82.37±4.97	79.63±6.34	80.71±4.32	81.28±3.90
7th Day (1st week)	88.37±4.78	91.25±5.04	89.06±5.26	92.66±4.87	91.27±4.06
14th Day (2nd Week)	97.14±6.66	101.84±5.33	98.67±5.67	100.15±5.85	99.50±5.67
21st Day (3rd Week)	108.23±6.91	109.58±5.71	109.37±5.70	112.87±6.30	111.22±6.85
28th Day (4th Week)	118.33±7.20	121.21±7.21	122.88±5.31	126.30±7.45	124.32±6.57
35th Day (5th Week)	126.61±6.35	129.66±4.64	129.46±6.24	134.88±6.67	135.60±6.37
42nd Day (6th Week)	134.92±7.54	135.80±7.20	136.20±6.30	140.74±6.80	142.98±7.04
49th Day (7th Week)	156.17±8.21	157.55±8.35	159.55±7.22	164.90±6.04	163.23±6.82
56th Day (8th Week)	173.68±8.24	175.21±7.66	178.64±6.22	180.11±6.27	179.40±6.66
63rd Day (9th Week)	195.82±6.33	195.93±6.47	201.07±7.58	207.24±7.22	206.52±6.30

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 992-999 | ISSN:2251-6727

70th Day (10th Week)	215.37±8.87	219.20±8.93	221.84±8.57	227.38±8.07	228.07±7.49
77th Day (11th Week)	235.27±8.62	238.99±7.64	240.70±9.67	243.33±7.15	243.80±7.29
84th Day (12th Week)	260.22±9.75	265.31±8.09	270.45±8.05	275.14±7.68	276.20±7.12
91st Day (13th Week)	293.85±7.07	305.04±7.64	310.64±9.64	312.49±8.93	313.07±7.95

The Values are expressed as mean ± SEM (n=20), The data were analysed by using one-way ANNOVA followed by Dunnett's '*t*' test for comparison. Level of significance P < 0.01, P<0.05

Table 3. Effect of *Thiripalathi Kashayam* on haematological parameters (WBC, RBC, Hb, Platelet and DC) in Wistar rats

The Values are expressed as mean ± SEM (n=20), The data were analysed by using one-way ANNOVA followed by

Groups	Drug Treatment	WBC ($X10^3$/cmm)	RBC (x10/cmm)	Hb (gms %)	Platelet ($x10^3$/cmm)	Differential Count (%)				
						Neutrophils	*Lymphocytes*	*Eosinophil*	*Monocytes*	*Basophils*
I	**Control group**	8.16± 0.62	6.14± 0.41	13.19 ± 1.12	862.31± 1.27	27.62± 1.22	70.24± 5.36	0.20± 0.01	1.22± 0.10	1.02± 0.10
II	**Low dose group**	7.93± 0.51	6.02± 0.59	12.90 ± 1.06	860.27± 2.87	26.90± 2.08	71.36± 6.60	0.18± 0.01	1.20± 0.12	0.96± 0.05
III	**Mid dose group**	8.15± 0.77	6.15± 0.54	13.20 ± 1.30	861.05± 1.84	26.45± 2.04	72.66± 6.98	0.21± 0.02	1.19± 0.10	1.02± 0.11
IV	**High dose group**	8.16± 0.80	6.17± 0.55	13.18 ± 1.22	860.06± 1.45	26.42± 2.17	72.03± 5.89	0.22± 0.01	1.2± 0.11	1.0± 0.06
V	**Satellite group**	8.16± 0.72	6.18± 0.60	13.20 ± 1.20	861.24± 1.50	27.20± 2.27	72.21± 3.57	0.22± 0.01	1.3± 0.10	1.01± 0.07

Dunnett's '*t*' test for comparison. Level of significance P < 0.01, P<0.05

Table 4. Effect of *Thiripalathi Kashayam* on haematological parameters (PCV, MCV, MCH and MCHC) in Wistar rats

Group	Drug Treatment	PCV	MCV (fl)	MCH (pg)	MCHC (g/dl)
I	**Control group**	39.33± 3.27	60.10± 4.21	21.45± 1.83	33.80± 2.93

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 992-999 | ISSN:2251-6727

II	**Low dose group**	38.49± 3.52	58.88± 3.93	21.40± 2.04	33.60± 3.05
III	**Mid dose group**	40.62± 3.66	59.37± 3.27	21.10± 1.60	33.04± 2.85
IV	**High dose group**	39.97± 3.09	60.82± 4.55	21.55± 1.63	34.17± 3.22
V	**Satellite group**	38.38± 2.93	60.07± 3.67	21.87± 1.41	33.97± 3.27

The Values are expressed as mean ± SEM (n=20), The data were analysed by using one-way ANNOVA followed by Dunnett's '*t*' test for comparison. Level of significance P < 0.01, P<0.05

Table: 5. Effect of *Thiripalathi Kashayam* on hepatic & renal biomarkers in Wistar rats

Groups	Drug Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Bilirubin (mg/dl)	Total Protein (g/dl)	Serum Albumin (g/dl)	Blood Urea Nitrogen (mg/dl)	Creatinine (mg/dl)	Uric Acid (mg/dl)
I	**Control group**	58.67± 4.23	36.24± 3.06	87.24± 7.52	0.54± 0.03	6.89± 0.54	3.19± 0.21	20.27± 2.04	0.54± 0.03	1.22± 0.10
II	**Low dose group**	58.32± 4.06	37.59± 2.86	86.55± 7.20	0.53± 0.04	6.86± 0.44	3.18± 0.25	20.64± 1.14	0.53± 0.02	1.21± 0.12
III	**Mid dose group**	59.55± 4.36	36.27± 3.21	87.67± 8.05	0.535± 0.03	6.87± 0.60	3.20± 0.26	20.89± 1.27	0.53± 0.03	1.22± 0.09
IV	**High dose group**	60.70± 4.27	36.52± 3.18	87.37± 7.42	0.54± 0.04	6.88± 0.62	3.19± 0.24	20.97± 2.27	0.52± 0.04	1.22± 0.08
V	**Satellite group**	59.92± 5.22	36.82± 3.11	88.53± 8.21	0.54± 0.05	6.89± 0.60	3.20± 0.30	20.07± 2.07	0.53± 0.05	1.22± 0.11

The Values are expressed as mean ± SEM (n=20), The data were analysed by using one-way ANNOVA followed by Dunnett's '*t*' test for comparison. Level of significance P < 0.01, P<0.05

Table: 6. Effect of *Thiripalathi Kashayam* on lipid profile in Wistar rats

Groups	Drug Treatment	Lipid Profiles (mg/dl)				
		Total Cholesterol	Triglyceride	HDL	LDL	VLDL
I	**Control group**	118.62± 1022	67.25± 4.38	42.24± 2.94	62.94± 4.75	13.43± 1.27
II	**Low dose group**	114.85± 9.11	65.80± 4.14	43.80± 3.67	61.56± 4.21	12.40± 1.14
III	**Mid dose group**	105.87. ± 9.33	66.13± 5.52	45.60± 4.06	61.50± 4.06*	13.02± 1.07

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 992-999 | ISSN:2251-6727

IV	**High dose group**	117.27± 8.04**	67.37± 4.18	46.77± 3.44	62.80± 3.87**	13.58± 0.95
V	**Satellite group**	118.83± 7.20**	67.43± 4.87	46.80± 4.01	62.58± 3.08**	13.47± 0.11

The Values are expressed as mean ± SEM (n=20), The data were analysed by using one-way ANNOVA followed by Dunnett's '*t*' test for comparison. Level of significance P < 0.01, P<0.05

Table: 7. Histopathology of organs

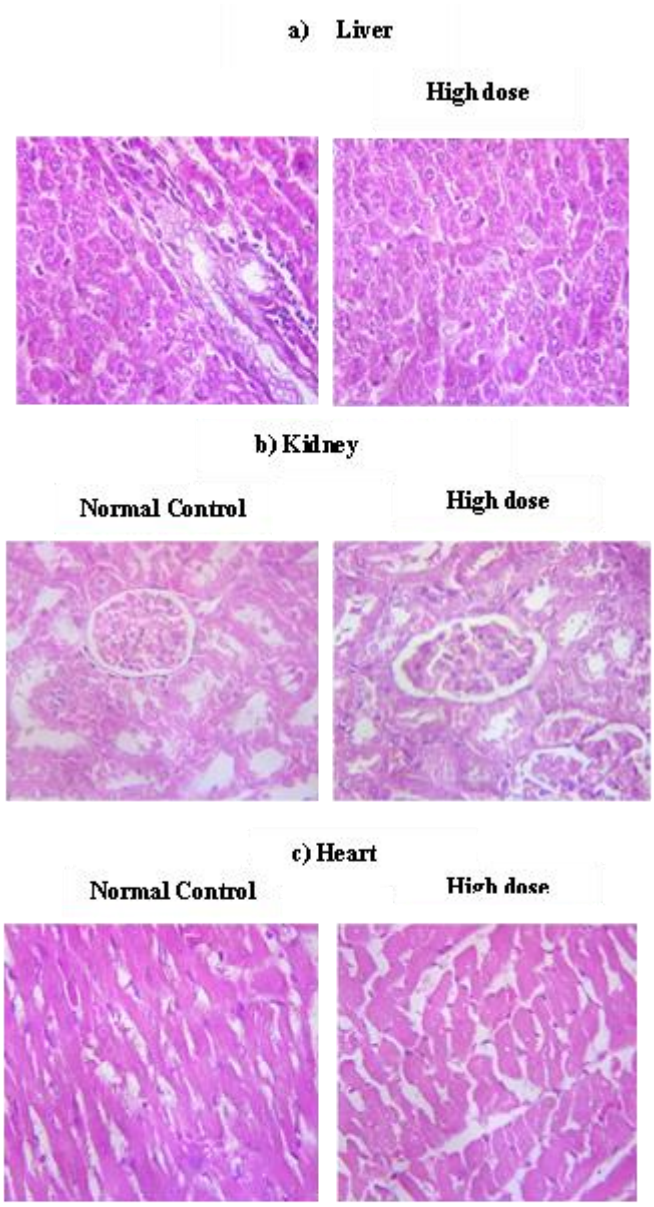

Figure 1 Histopathology images of normal control & High dose group a) showing normal histology of Liver, b) showing normal histology of Kidney, c) showing normal histology of Heart

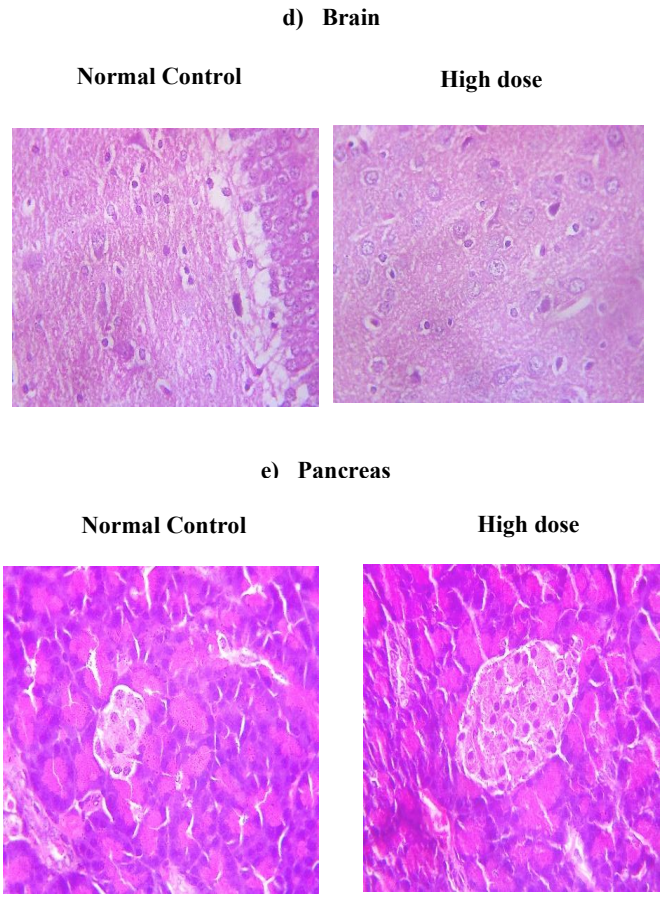

Figure 2. Histopathology images of normal control & High dose group d) showing normal histology of Brain, e) showing normal histology of Pancreas