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TOXICITY EVALUATION OF DHADHU VIRTHI KULIGAI IN WISTAR RATS

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ABSTRACT

Evaluation of toxicity is important before starting clinical trial of any drug. Need for traditional medicines have been increasing in the recent past, so it is necessary to ensure that the medicine prescribed are safe. Aim of the study is to evaluate the safety profile of Dhadhu Virthi Kuligai (DVK) through Acute Oral Toxicity study, 28-Day Repeated Dose Oral Toxicity Study and 90-Day Repeated dose oral toxicity as per OECD guidelines. In Acute study, Animals were divided into groups. Three Female animals are used for each group. The dose level of 5, 50, 300 and 2000 mg/kg body weight was administered stepwise. Animals were observed for toxic signs for 14 days and gross pathology was performed at the end of the study. In repeated dose toxicity study, the animals were divided into four groups. The first group was treated as control (milk) and second, third, fourth groups were treated with Low dose 11.7mg/kg/ b. wt, Mid dose 58.5mg/kg/b. wt, High dose 117mg/ kg/ b. wt of DVK. 80 Wistar Albino Rats (40M + 40F) were selected and divided into 4 groups. First group treated as a control (milk) and other three group were treated with test drug (Low dose 11.7 mg/kg/ b. wt, Mid dose 58.5 mg/kg/ b.wt and High dose 117mg/kg/ b. wt) for 90 days. In acute oral toxicity study, no treatment related death or toxic signs were observed. 28-day repeated dose study and 90 day repeated dose oral toxicity,

did not show evidence of any significant treatment related changes in all observations from low dose to the high dose level, when compared with the control. Histopathological examination also revealed that no abnormalities. This study ensures that the drug is safe.

KEYWORDS: Dhadhu Virthi Kuligai, Toxicity, OECD guidelines.

INTRODUCTION

Siddha system of medicine dates back to many centuries. It has been practiced by Tamil vaithiyars (native healers of Tamil Nadu) who taught people an impeccable lifestyle that trails in societies of Tamil Nadu. The current accepted modern medicine has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies.[1] Since the beginning of the human race plants were the preferable source of medicines. Medicinal plants are often used without satisfactory demonstration of their pharmacological activities. Moreover, many people believe that traditional medicines have no adverse effects. During the past few years it is observed that, the adverse effects of phytomedicines, as well as its adulteration, toxicity, and drug interaction are common problems related to public health.[2] *Dhadhu virthi kuligai* is a poly herbal formulation referred from *Noikalukku siddha parigaram*[3], The ingredients of this kuligai are used for male infertility in Siddha system. Many poly herbal formulations are mentioned in Siddha literatures for male infertility, *Dhadhu virthi kuligai* is one among them and indicated for male infertility, loss of libido, poor sperm quality and burning micturation. For justifying safety of the drug, there is no evidence of toxicological data. So the present study aimed to evaluate the toxicological effect of DVK through acute and 28 days repeated dose oral toxicity studies.

Procurement and Authentication of Raw Drugs

The drugs were purchased from authorized country raw drug store in Chennai. All the plant materials, *Mucuna pruriens, curculiogo orchiodes, Hygrohila auriculate, Asparagas racemosus* and *Acacia nilotica* were identified and authenticated by Botanist, National Institute of Siddha, Tambaram Sanatorium, Chennai.

Preparation of Dadhu virthi kuligai

All these ingredients were powdered and soaked in lime juice for 24 hours and ground well with the same juice for 3 days and allowed to dry. Again, ground with tender coconut water

for 3 days, until it attains a waxy consistency, then it was made into small size pills (5 grains-325mg).

Animal Care and Husbandry

Sexually mature male and female Wistar albino rats (150-200gm) were obtained from TANUVAS, Madhavaram, Chennai. All the animals were kept under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore). Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the, ***Dhadhu virthi kuligai***. The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design. IAEC approved number: 1248/AC/09/CPCSEA-9/DEC-2013/12.

ACUTE ORAL TOXICITY STUDY

Dhadhu virthi kuligai suspended in milk and was administered to the groups of wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals are used for each group. The dose level of 5, 50, 300 and 2000mg/kg body weight was administered stepwise. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously as per the guideline after substance administration. The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. Finally, the number of survivors was noted after 24 h and these animals were then monitered for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.[4]

28 DAY REPEATED DOSE ORAL TOXICITY

Randomization, Numbering and Grouping of Animals

48 Wistar Albino Rats (24M + 24F) were selected and divided into 4 groups. Each group consist of 12 animals (Male -6, and Female-6). First group treated as a control and other three group were treated with test drug (low, mid, high) for 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

Dose Selection

As per OECD guideline three dose levels were selected for the study. They are low dose (X), mid dose dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose (650mg) and the body surface area of the rat (0.018). i.e X dose is 11.7mg/animal, 5X dose is 58.5mg/animal,10X dose is 117 mg/animal.

Preparation and Administration of Dose

Dhadhu virthi kuligai was suspended in with milk to obtain concentrations of 200mg/ml. It was administered to animals at the dose levels of X, 5X, 10X. The test substance suspensions were freshly prepared every two days once for 28 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 28 consecutive days.[5]

REPEATED DOSE 90-DAY ORAL TOXICITY

Randomization, Numbering and Grouping of Animals

80 Wistar Albino Rats (40M + 40F) were selected and divided into 4 groups. Each group consist of 20 animals (Male -10 and Female-10). First group treated as a control and other three group were treated with test drug (low, mid, high) for 90 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

Justification for Dose Selection

As per OECD guideline three dose levels were selected for the study. They are low dose (X), mid dose dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose (650 mg) and the body surface area of the rat (0.018). i.e X dose is 11.7mg/animal, 5X dose is 58.5 mg/animal, 10X dose is 117 mg/animal.

Preparation and Administration of Dose

Dhadhu virthi kuligai was suspended in milk with distilled water to obtain concentrations of 200mg/ml. It was administered to animals at the dose levels of X, 5X, 10X. The test substance suspensions were freshly prepared every two days once for 90 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 90 consecutive days.[6]

Collection and analyses of blood

At the end of treatment period, animals were fasted overnight, then anesthetized to collect blood samples from the abdominal aorta in two tubes: one with EDTA for haematological parameters, another one without anticoagulant and was centrifuged at 4000 rpm at 4°C for 10 minutes to obtain the serum for biochemical parameters. Blood samples of control and drug treated rats were analyzed for haemoglobin (Hb), total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Platelet, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), were calculated by auto analyzer. Serum samples of control and experimental animals were analyzed for, Bilirubin, BUN, Creatinine, Triglyceride, Total Cholesterol, HDL, LDL, VLDL, using standard methods. Activities of glutamate oxaloacetate transaminase/Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine aminotransferase (GPT/ALT) were estimated as per the colorimetric procedure.

Histopathology

The organs included liver, kidneys, spleen, brain, heart, lungs, stomach, testis and uterus of the animals were preserved, and they were subjected to Histopathological examination. Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of all the animals (low, mid, high) w e r e preserved and fixed in 10% formalin for 24 hrs. Samples were dehydrated in an auto technic and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50^{o}C and then in a cubical block of paraffin made by the "L" molds. It was followed by microtome and the slides were Prepared then stained with Haematoxylin-eosin.

Statistical analysis

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by dunnet test using a computer software programme – Graphpad Instat-3 version.

RESULTS AND DISCUSSION

The treated rats with 2000mg/kg in the Acute toxicity study did not show any mortality, any untoward clinical sign, any behavioural signs, alterations in body weight and necropsy findings at the end of the study. This indicates that the dosages administered were below toxic level and proves the safety of the drug. [Table 1, Table 5].

The Repeated dose 28 day oral toxicity and Repeated dose 90-day oral toxicity study of *Dhadhu virthi kuligai* in Wistar albino rats were studied. The treated animals survived throughout the study period of 28 days and 90 days did not reveal any treatment related major abnormal clinical signs at the test dose levels. The overall percentage of body weight gain in rats treated with the drug was found to be normal indicating that the test animals were in a healthy condition during the 90days of observation period.[Table1, Table The P values of haematological parameters [Table] and biochemical parameters [Table] of the tested rats were not significant indicating that the drug exerted nil impact on the parameters and they were within the reference range. In histopathological study on DVK high dose treated rats no significant abnormalities were seen (Fig: 5 to 8). The necropsy studies showed no remarkable changes.

This strongly stress the fact of the drug having no toxic effect on the body metabolism.The necropsy studies showed no remarkable changes. So the trial drug Dhadhu virthi kuligai hope fully use for human trails.

Table 1: Body weight changes of test animals in 28 days repeated oral toxicity study of Dhadhu virthi kuligai.

DOSE	DAYS				
	1	7	14	21	28
CONTROL	163.6±33.673	169.4±39.315	173.7±37.771	173.7±39.73	174.7±37.311
LOW DOSE	151.1±31.115	155.9 ± 31.77	161.5±31.716	161 ± 31.77	166.7 ± 14.77
MID DOSE	157.6± 13.57	174.3 ± 31.71	175.7 ± 39.11	179.1±33.37	176.7 ± 33.11
HIGH DOSE	177.5± 37.75	177.7 ± 33.77	193.7 ± 33.47	199 ± 31.93	207 ± 37.94

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by dunnett's (N=10); $^{ns}p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, calculated by comparing treated groups with control group.

Table 2: Water intake changes in grams of test animals in 28 days repeated oral toxicity study of Dhadhu virthi kuligai.

DOSE	DAYS				
	2	7	23	22	28
CONTROL	37±5.37	38.5±3.22	39.5±3.37	38.5±3.37	37±3.12
LOW DOSE	33.7±2.98	35.3±2.23	35.2±2.28	35.2±3.32	35.9±2.52
MID DOSE	37.3±3.75	37.2±3.70	37.2±3.25	39±3.98	39.2±3.53
HIGH DOSE	30.2±3.35	30.3±3.88	39.9±3.78	40.3±3.30	40.2±3.83

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N=10); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table 3: Effect of 28 days repeated dose of Dhadhu virthi Kuligai on Haematological parameters.

Category	Control	Low dose	Mid dose	High dose
Haemoglobin(g/dl)	13.7±0.88	13.60±0.86	14.15±0.88	15.28±0.96
Total WBC (×10^3 l)	10.91±0.59	11.35±0.73	11.48±0.91	10.20±1.17
Neutrophils (%)	21.85±0.65	25.73±0.73	26.41±1.36	25.0±2.20
Lymphocyte (%)	73.84±1.48	76.18±3.12	72.60±2.66	74.30±2.76
Monocyte (%)	0.76±0.07	0.74±0.09	0.82±0.03	0.79±0.06
Eosinohil(%)	0.34±0.09	0.43±0.02	0.26±0.06	0.17±0.04
Platelets cells/ul	347.17±8.76	398.71±8.16	383.18±9.0	387.16±9.74
Total RBC (cells/cu.mm)	5.99±0.12	6.99±0.57	8.82±0.59	8.05±0.72
PCV%	39.79±0.6	43.35±1.13	42±1.78	45.83±2.56
MCHC g/dl	31.6±1.23	34.09±1.09	35.98±1.12	33.03±1.54
MCV fl	48.07±3.67	55.20±1.51	55.40±1.35	56.28±1.45

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N=10); p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table 4: Effect of 28 days repeated dose of Dhadhu Virthi Kuligai on Biochemical parameters.

BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
GLUCOSE (R) (mg/dl)	118.45±13.4	113.16±8.44	117.26±11.20	122.42±11.6
T. CHOLESTEROL (mg/dl)	82.26±1.83	83.45±1.83	85.42±1.78	90.22±1.73
TRIGLYZERIDES (mg/dl)	44.35±1.48	45.32±1.48	44.58±1.30	46.62.66±1.33*
UREA (mg/dl)	12.35±0.99	15.31±0.76	16.07±1.38	16.48±1.42
CREATININE (mg/dl)	0.48±0.007	0.66±0.06	0.92±0.07	0.86±0.05
URIC ACID (mg/dl)	4.37±0.35	5.11±0.43	5.9±1.25*	4.48±0.43
T BILIRUBIN (mg/dl).	0.60±0.07	0.85±0.06	0.89±0.08	0.86±0.05
SGOT(U/dl)	14.95±1.39	16.35±0.51	17.01±1.53	16.55±1.03
SGPT(U/dl)	51.23±1.28	55.91±1.59	58.34±1.48	55.32±0.68
ALP(U/dl)	196.25±8.77	151±16.17	148.16±24.07*	154.33±14.65*
T PROTEIN (mg/dl)	6.32±0.38	7.48±0.34	7.016±0.23	6.53±0.46

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N=10); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table No 5: Body weight changes of test animals in 90 day repeated oral toxicity study of Dhadhu virthi kuligai.

DAYS	GROUPS			
	Control	Low dose	Mid dose	High dose
1	163.6±33.683	150.1 ± 21.105	158.6± 13.57	168.5± 28.75
15	172.8 ± 28.870	161.5 ± 21.706	175.7 ± 29.01	182.8 ± 32.48
30	185.8 ± 28.310	186.7 ± 14.88	186.8 ± 32.11	190 ± 28.94
45	204.5± 27.73	205.5± 29.76	206.7± 19.75	207± 22.75
60	227.6±33.683	233.6±23.683	237.6±33.683	224.6±23.783
75	242.8 ± 26.85	247.8 ± 28.870	242.8 ± 28.870	243.8 ±26.870
90	265± 27.320	267± 27.320	268± 27.320	266± 36.320*

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N=10); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table 6: Effect of 90 days repeated dose of Dhadhu virthi Kuligai on Haematological parameters.

Category	Control	Low dose	Mid dose	High dose	P value (p)*
Haemoglobin (g/dl)	13.95±0.94	13.18±0.98	14.8±1.30	13.15±1.39	N.S
Total WBC (cells/cu.mm)	11.65±1.22	11.23±0.91	11.48±0.65	11.35±0.35	N.S
Neutrophils(%)	24.2±2.41	25.98±1.29	26.43±1.32	24.63±2.35	N.S
lymphocyte (%)	73.35±2.62	76.70±1.41	74.25±1.95	76.54±2.85	N.S
Monocyte (%)	0.74±0.09	0.79±0.07	0.79±0.08	0.73±0.08	N.S
Eosinohil(%)	0.48±0.12	0.42±0.07	0.53±0.07	0.28±0.05	N.S
Basophil (%)	0.22±0.07	0.19±0.07	0.23±0.06	0.21±0.05	N.S
Platelets cells/ul	379.05±9.48	395.91±16.9	379.86±17.90	378.23±15.9	N.S
Total RBC (cells/cu.mm)	8.15±1.09	7.52±0.84	8.80±0.80	7.78±0.97	N.S
PCV%	45.56±1.39	44.92±0.78	48.37±0.76	44.36±0.98	N.S
MCHC g/dl	35.85±1.46	35.74±1.21	36.91±4.31	34.83±2.03	N.S
MCV Fl	54.7±1.98	57.92±8.13	56.3±8.17	59.55±6.16	N.S

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N=10); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table 7: Effect of 90 day repeated dose of Dhadhu virthi Kuligai on Biochemical parameters.

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
UREA (mg/dl)	15.15±1.76	14.93±3.23	16.14±1.79	14.55±1.57	N.S
CREATININE(mg/dl)	0.80±0.91	0.71±0.21	0.81±0.28	0.64±0.08	N.S
T.BILIRUBIN(mg/dl)	0.75±0.1	0.79±0.1	0.89±0.25	0.80±0.13	N.S
SGOT(U/dl)	16.7±0.98	16.25±0.56	16.7±0.98	16.15±0.87	N.S
SGPT(U/dl)	58.5±3.53	54.25±1.45	63.58±1.28	54.7±0.86	N.S
ALP(U/dl)	119.15±8.77	120±16.17	101.16±24.07*	126.33±14.55*	N.S
T.PROTEIN(mg/dl)	7.41±0.38	7.46±0.54	7.106±0.23	6.53±0.46	N.S
GLUCOSE (R) (mg/dl)	118.15±13.4	120.15±3.17	119.91±3.15	122.41±2.15	N.S
T.CHOLOSTEROL (mg/dl)	87.15±2.62	86.53±1.45	92.45±1.36	91.76±1.46	N.S
TRIGLY(mg/dl)	46.05±2.18	46.31±1.48	47.31±3.15	46.21.±2.19*	N.S

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N=10); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Histopathology of Brain

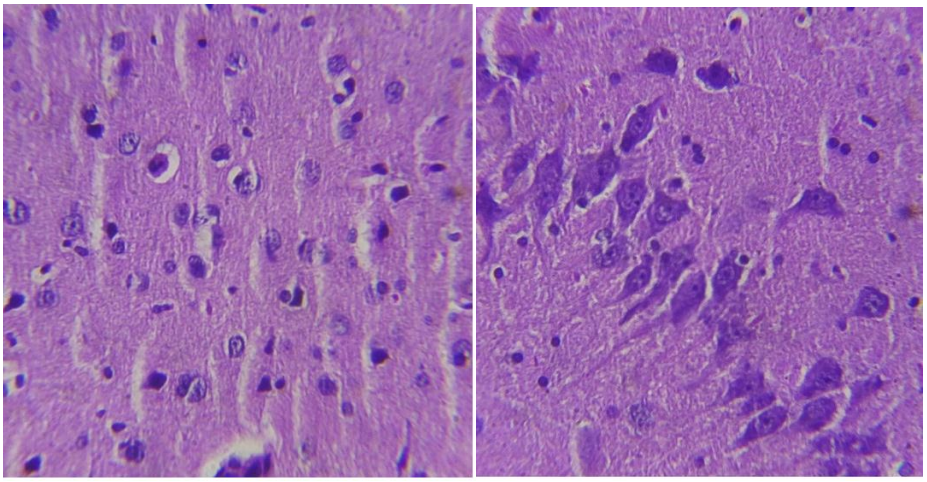

Plate A: Control Male. **Plate B: Control Female.**

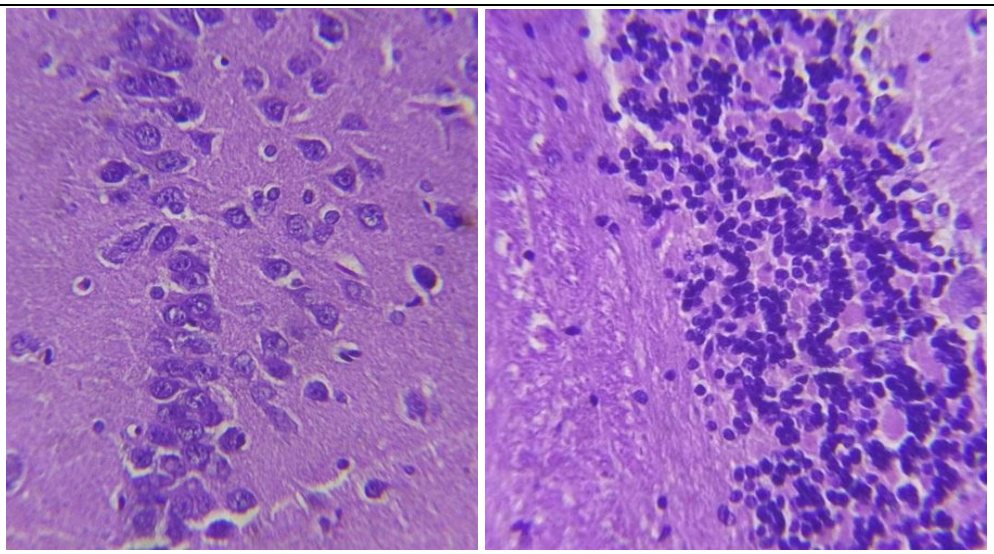

Plate C: High Dose Male. **Plate D: High Dose Female.**

Figure.1:

Plate A: Regular marginal alignment on the neurons with promising histology was observed.

Plate B: The CA zones of brain hippocampi are filled with densely packed Pyramidal cells.

Plate C: Arrangement of neurons on cerebral cortex appears normal and dense.

Plate D: Three layers of cerebellar cortex, the molecular, purkinje and granular layers, appeared clear and distinct without any changes in their cells.

Histopathology of Heart

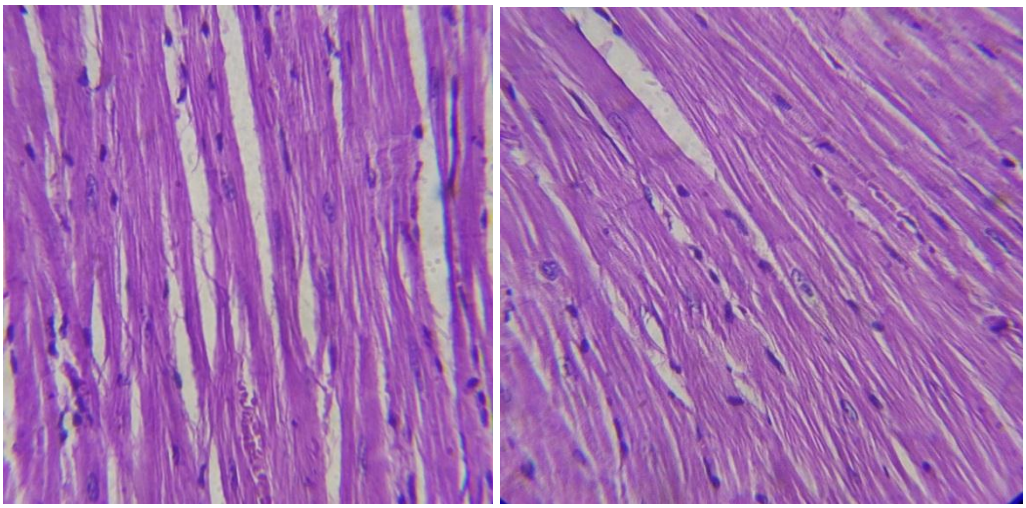

Plate A: Control Male. **Plate B: Control Female.**

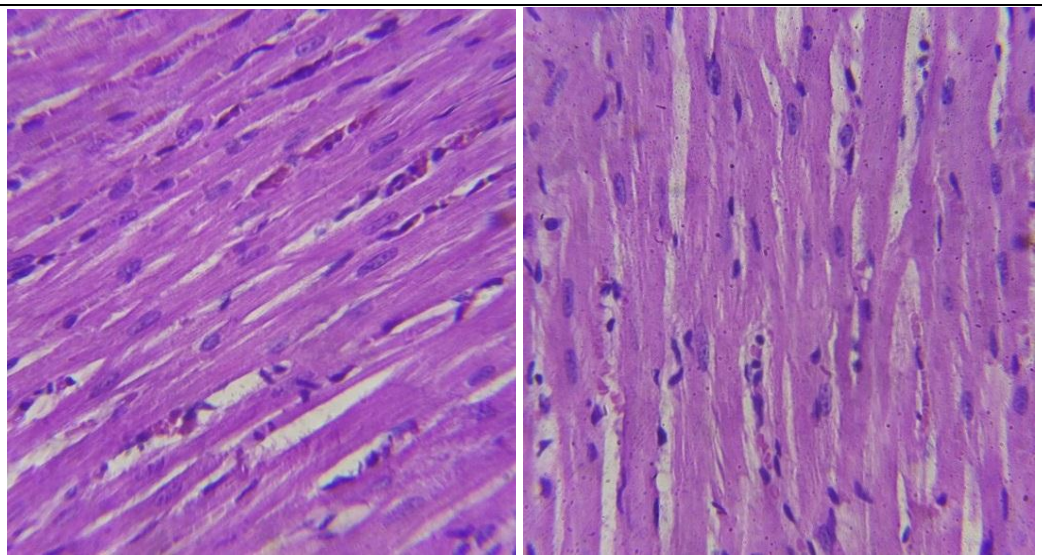

Plate C: High Dose Male. **Plate D: High Dose Female.**

Figure 2:

Plate A: Nucleus appears prominent with regular arrangement of fibres. No evidence of pyknotic nucleus.

Plate B: Myocardial cells appears normal with well-defined mycoplasma and prominent nucleus and nucleolus.

Plate C: Appearance of fibrils and cross striations are normal and equidistant.

Plate D: Appearance of cardiac myocyte was normal.

Histopathology of Lung

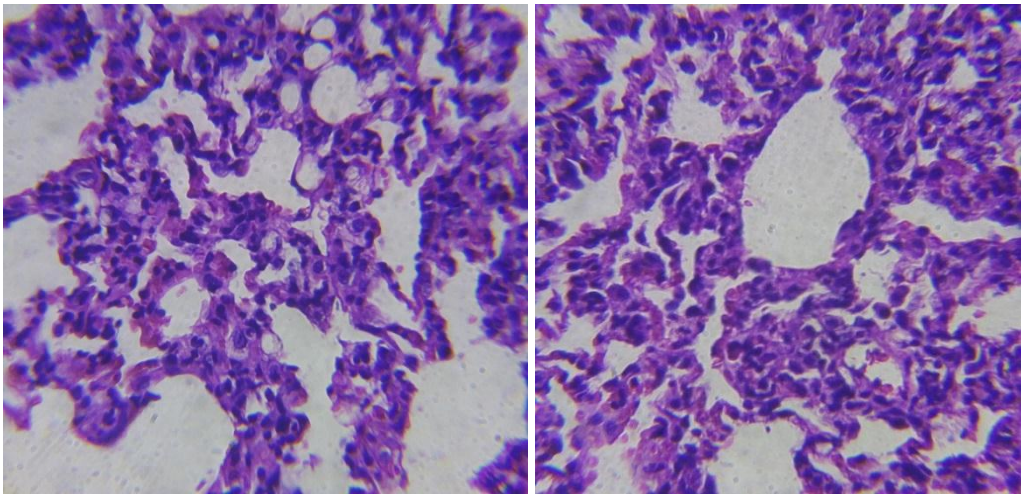

Plate A: Control Male. **Plate B: Control Female.**

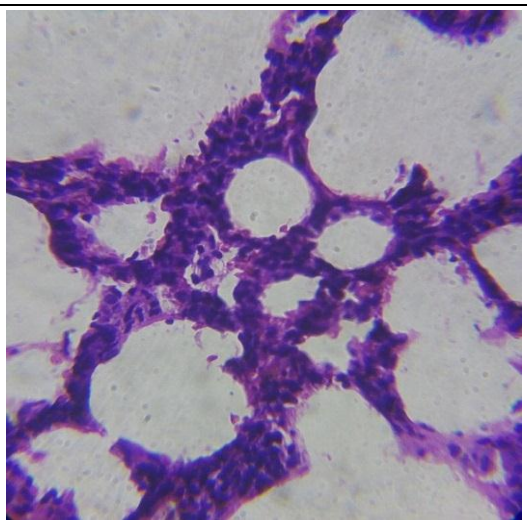
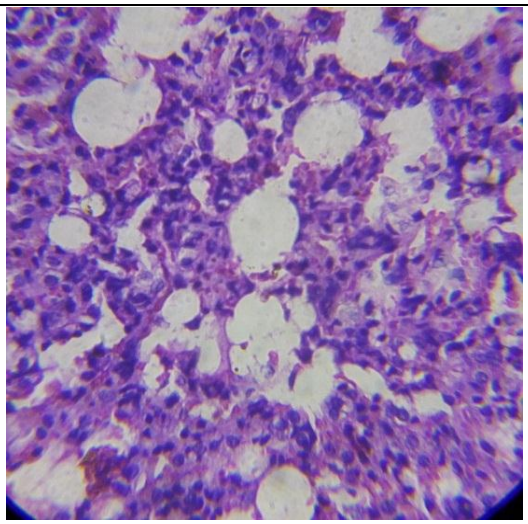

Plate C: High Dose Male. **Plate D: High Dose Female.**

Figure 3:

Plate A: Arrangement of epithelial and muscular appears normal.

Plate B: Lung parenchyma appears normal with regular arrangement of alveoli and alveolar sac with no signs of lymphocyte infiltration and pulmonary fibrosis.

Plate C: Perfect network of simple squamous epithelium were observed. Inter alveolar septum and alveolar capillary appears normal.

Plate D: Pneuomocyte and capillary appears normal.

Histopathology of Stomach

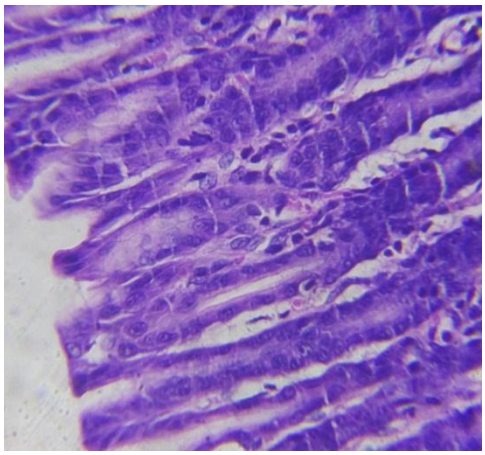
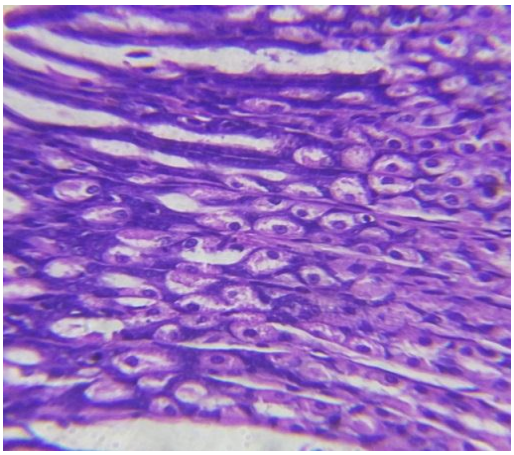

Plate A: Control Male. **Plate B: Control Female.**

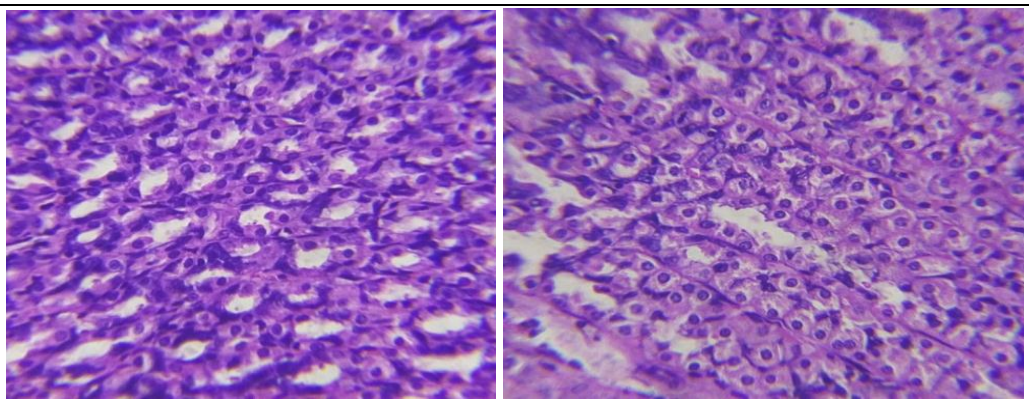

Plate C: High Dose Male. **Plate D: High Dose Female.**

Figure 4:

Plate A: Lumina of blood vessels appears normal. Appearance of glandular lumen was normal.

Plate B: Regular arrangement of muscularis externa and outer longitudinal muscle were observed.

Plate C: Gastric glands including secretary sheath appears normal.

Plate D: Normal gastric mucosa containing intact gastric gland cells, parietal cells which are spherical cell with deeply stained dark nucleus.

Histopathology of Liver

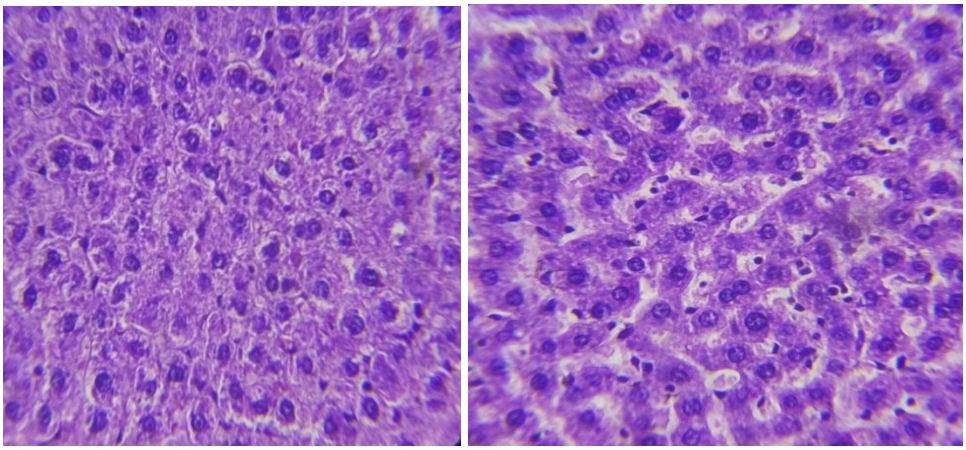

Plate A: Control Male. **Plate B: Control Female.**

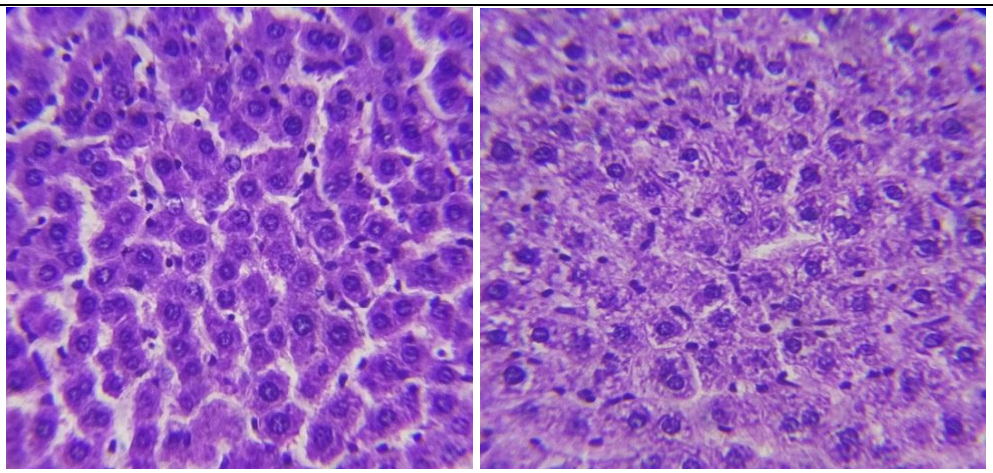

Plate C: High Dose Male. **Plate D: High Dose Female.**

Figure 5:

Plate A: Cytoplasm appears normal with widen portal tract.No signs of nodular degeneration and cirrhosis.

Plate B: The walls of the lumen appears normal with no evidence of ischemic changes.

Plate C: Liver parenchyma appears normal with no evidence of necrosis. Rare appearance of Kupffer cells with no evidence of phagocytosis in intracytoplasmic region.

Plate D: The centrilobular hepatocytes appears normal with stained cytoplasm.

Histopathology of Kidney

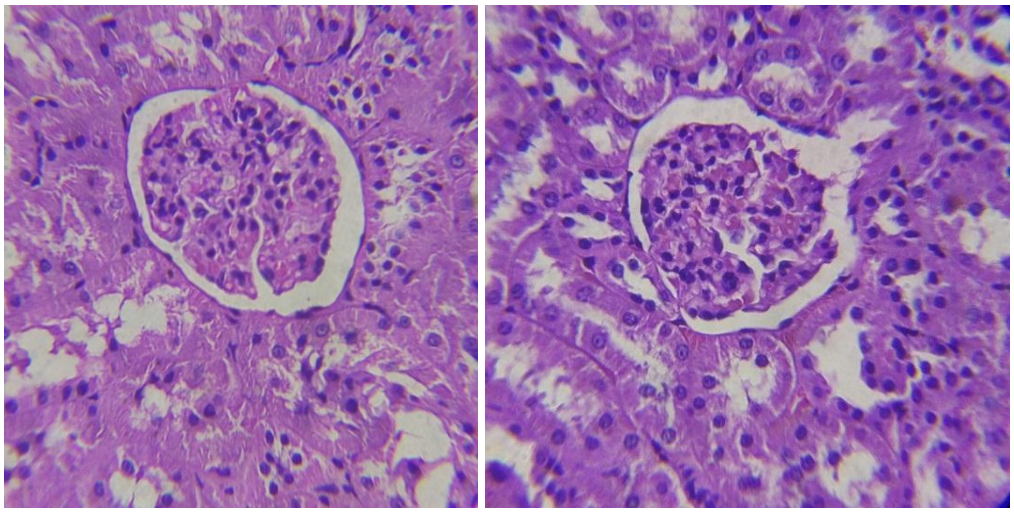

Plate A: Control Male. **Plate B: Control Female.**

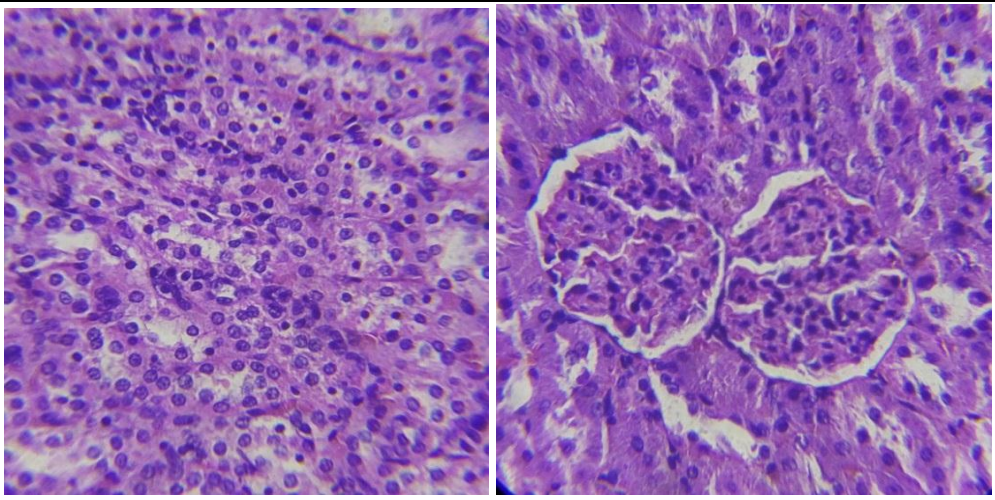

Plate C: High Dose Male. **Plate D: High Dose Female.**

Figure 6:

Plate A: Appearance of central artery and marginal sinus are normal. No abnormalities found in lymph node.

Plate B: Appearance of glomerular basement membrane was normal.

Plate C: Foot processes of podocytes are separated from one another by a regular narrow Filtration Slit.

Plate D: Bowman's capsule appears normal and surrounded with Proximal Convoluted Tubule, Distal Convoluted Tubule and Collecting Duct.

Histopathology of Spleen

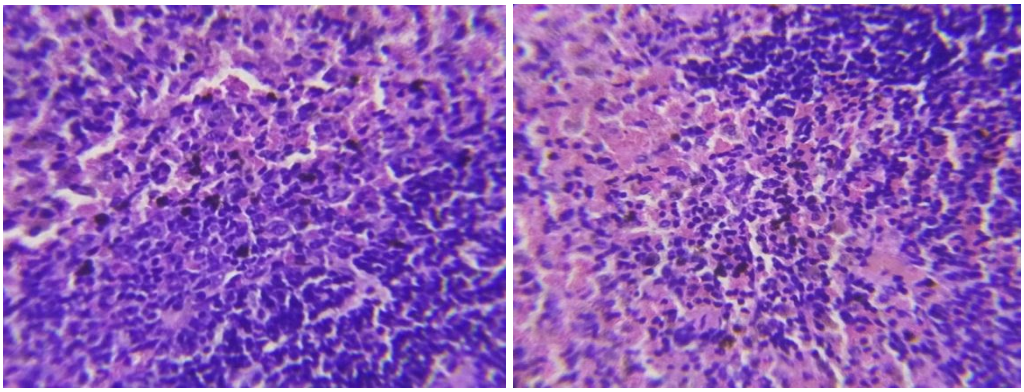

Plate A: Control Male. **Plate B: Control Female.**

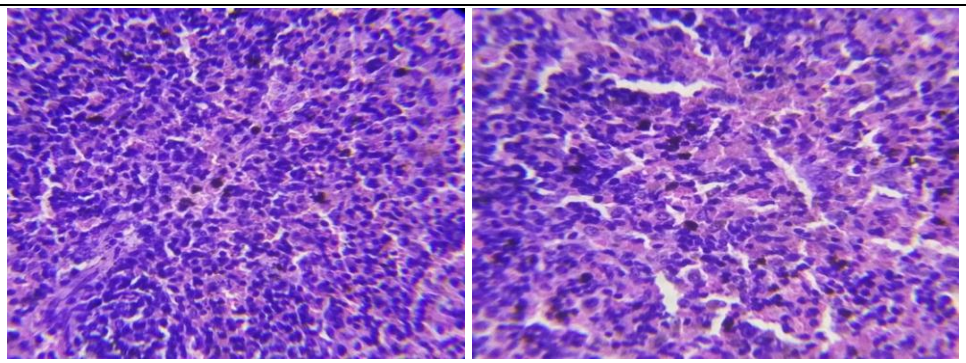

Plate C: High Dose Male. **Plate D: High Dose Female.**

Figure 7:

Plate A: Normal renal structure with rounded renal corpuscles formed of the Glomerulus. Increased bowman space around glomeruli.

Plate B: Marginal vascular zone radiated in between red and white pulp. Appearance of splenic red pulp was normal.

Plate C: Lymphoid follicles appears normal.

Plate D: Erythropoietic cells (EP) are scattered throughout the red pulp with increased number of megakaryocytes.

Histopathology of Testes

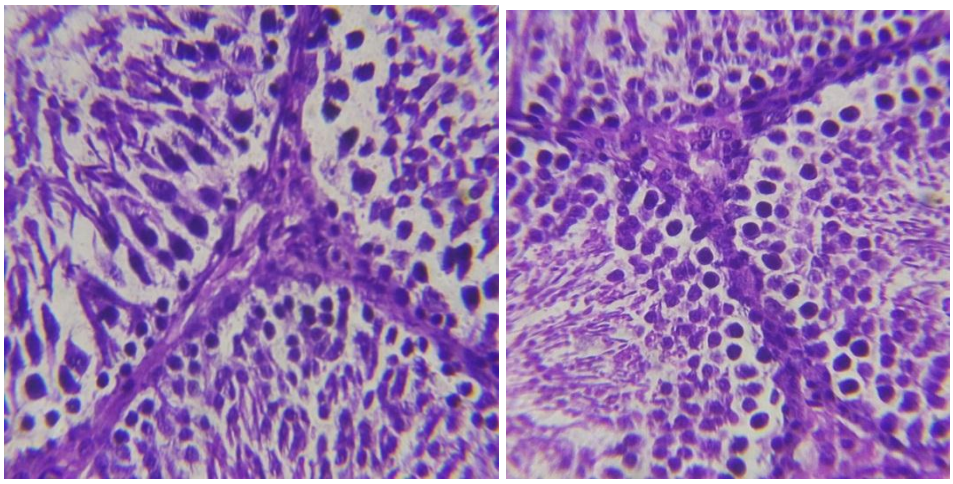

Plate A: Control Male. **Plate B: High Dose Male.**

Figure 8:

Plate A: Histo cytology of testicular tissue shows well differentiated germ cells with respect of spermatogonia includes spermatid and sperm were observed.

Plate B: Normal sertoli cell aligned properly on the basement membrane with oval dome shaped nucleus.

Histopathology of Uterus

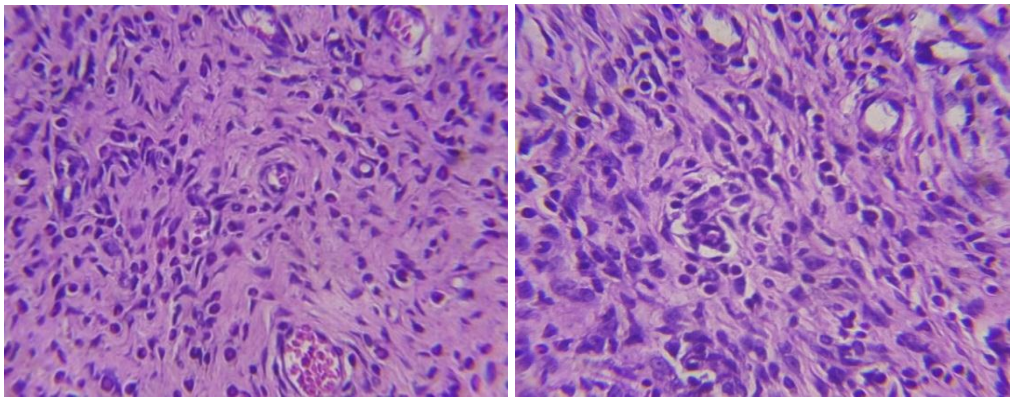

Plate A: Control Female. **Plate B: High Dose Female.**

Figure 9:

Plate A: Appearance of endometrium, myometrium and uterine glands was normal.

Plate B: Endometrial stroma; G, gland; M, myometrium; P, perimetrium; L, lumen exhibits normal histological aspect of endometrium and myometrium.

Histopathology of Ovary

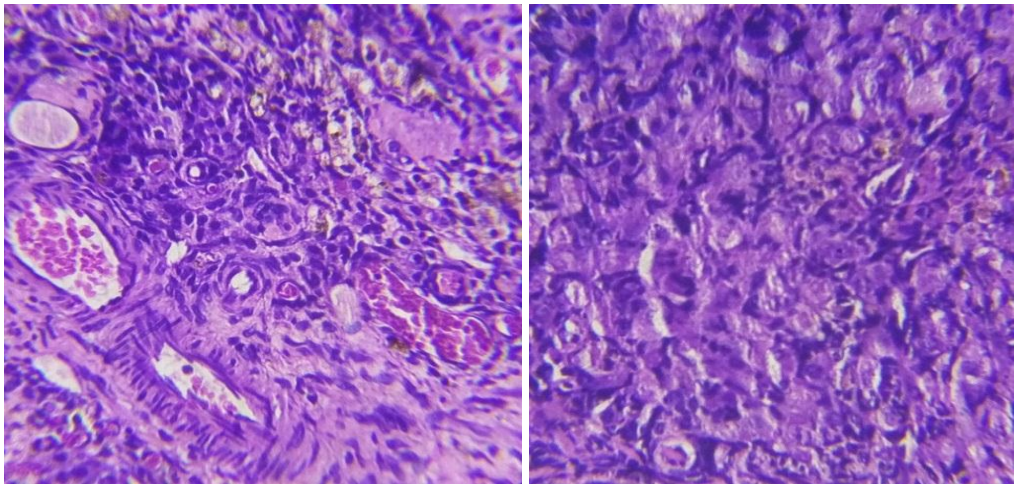

Plate A: Control Female. **Plate B: High Dose Female.**

Figure 10:

Plate A: Follicular cells, cytoplasm and nucleus appear normal.

Plate B: Corpora lutea, atretic follicles and interstitial tissue appears normal.

CONCLUSION

From the results of analytical evaluation of the test drug *Dhadhu Virthi Kuligai*, it is inferred that quality and stability was good when prepared under the standard protocol mentioned in this study. In vivo toxicity study reveals the drug DVK shows no mortality and signs of toxicity upto 2000mg/Kg bodyweight in acute oral administration. In 28 days repeated oral

toxicity study and 90 Day repeated dose oral toxicity study there was no significantly changes in haematological, biochemical parameter in control and treatment group. The histopathology report also ensures that there were no remarkable cellular changes at all the dose level. Based on the results, it can be concluded that, the dose level of *Dhadhu Virthi Kuligai*, is a safe for human consumption.

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Conflict of interest

The authors declare that there is no conflict of interest.

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