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Research Article

Acute And Subacute Oral Toxicity Study Of Aphrodisiac Herbal Formulation In Rats

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ABSTRACT

The objective of this study is to evaluate the acute and subacute toxicity of the of Aphrodisiac Herbal Formulation in albino rats. The acute toxicity was performed where the limit dose of 2000 mg/kg body weight used. Observations were made and recorded for 24 h, and once daily further for a period of 14 days. The rats were weighed and various observations, like mortality, behaviour, injury, or any signs of illness were conducted once daily during the period. For subacute study, four groups of 10 animals (female rats) received 60mg/kg, 120mg/kg and 200mg/kg oral by dissolving in water for 28 days. of freshly-prepared Aphrodisiac Herbal Formulation, respectively, every 24 h orally for 28 days. At the end of each study, haematological analysis and biochemical parameters were evaluated. Histopathological examination of vital organs of the animals were taken for gross findings, compared to controls. There was no significant difference ($p > 0.05$) observed in the relative organs, body weights, haematological, biochemical parameters, and gross abnormalities, compared to the control. No mortality was recorded. Therefore, analysis of results may lead to the conclusion that the medium-term oral administration of the Aphrodisiac Herbal Formulation for 28 days does not cause toxicity.

Keywords: acute toxicity; biochemical analysis; haematological parameters; Aphrodisiac Herbal; subacute toxicity; histopathology

Introduction

1.1 Toxicity:

The level of harm that a chemical compound or specific chemical combination can do to an organism is known as its toxicity. The fundamental idea of toxicology is that a toxicant's effect is dose dependent. The large dose may produce the toxicity. The toxicity may damage whole animal or may damage the cells and tissues.

1.2 Toxicology:

According to the US Food and Drug Administration (FDA), screening novel compounds for pharmacological activity and hazard potential in animals is critical. The term "study of all the adverse effects of chemicals or toxic hazards interacting with living beings" is used to describe toxicology. Sometime people refer toxicology as a "Science of Safety" because of toxicology is deal with the science to investigating poisons and harmful consequences of chemical exposure. Toxicology utilizes science to make predictions about which substances may be harmful and in what manner, then distributes that information to safeguard public health.

1.3 Herbal toxicity:

Ayurveda is a traditional medicine system of India. In ayurvedic medicinal system, herbs are prescribed as a medicine. While using herb as a medicine, it may show the toxicity, it is known as herbal toxicity. The toxicity may be produced by the primary or secondary metabolites of plant, active constitute of plant, phytochemicals present in the plant or high dose of medicine.

1.4 Acute toxicity:

The acute toxicity lasts for the 14 days. The dose is given to the animal single time. The rodent and nonrodent animal are used in the acute toxicity. The experimental product is given to the animal at different dose level

after that the animal is observed for 14 days. The observational parameter that is observed includes morphological, biochemical, and histological. All the parameters are recorded and compared with the control group.

1.5Subacute toxicity:

The dosing time in subacute toxicity is 28 days. The dosing can be multiple or single time. In the repeated dosing toxicity, the rodents of either sex are employed. The fix dose of experimental product is given to the animals for 28 days. During the dosing time, the observational parameters are observed and noted including biochemical, morphological and histological.

2. Materials and methods

2.1 Introduction of Aphrodisiac herbal formulation:

This is a poly herbal formulation consisting 12 plant extract showed in to the table. These plants have been used as aphrodisiac since years ago. The toxicity study of this formulation is not done yet. The acute and subacute oral safety profile of this aphrodisiac herbal formulation in rats has to be done as per the OCED guideline 420 and 407 respectively in present study.

The aphrodisiac herbal formulation composition is as following:

Sr no.	**Extract name**	Amount of extract
1	*Tribulus terrestris* extract	84 mg
2	*Chlorophytum borivilianum* extract	62 mg
3	*Mucuna pruriens* extract	62 mg
4	*Withania somnifera* extract	62 mg
5	*Tinospora cordifolia* extract	62 mg
6	*Argyeia nervosa* extract	33 mg
7	*Asparagus racemosus* extract	33 mg
8	*Phyllanthus emblica* extract	33 mg
9	*Sida cordifolia* extract	33 mg
10	*Dioscorea bulbifera* extract	12 mg
11	*Chopachini smilax glabra* extract	12 mg
12	*Pueraria tuberose* extract	12 mg
13	Carbohydrate	0.54 g
14	Protein	0.07 g
15	Fat	0.05 g

[Table 1.1: Composition of aphrodisiac herbal formulation]

2.2 Animal Care and Husbandry

Rat Albino Wistar Male and Female Age 2 to 3 months Source Cadila healthcare Limited, Zydus research center, Gujarat. The experimental animals were housed at temperature 22°C (±3°C). The relative humidity should be at least 30% and should not exceed 70%. Artificial lighting should be used, with 12 hours of light and 12 hours of darkness. The rats were kept in autoclaved cages and has a stainless-steel top grill. Autoclaved wheat husk will utilize as bedding. The beading material change at least once per week.

2.3 Acute oral toxicity:

Group (n=5)	**Method**	**No. of animals**
Control	Water orally	5 female
Treatment	2000 mg/kg of AHF orally	5 female

2.3.1 Acute oral toxicity as per OECD guideline 420:

Single oral dose was administered to animals on 1st day On 14th day, the animals were sacrificed Blood collected and serum prepared Biochemical parameters and haematological parameters were done Histological evaluation of brain, heart, lungs, liver, kidney, and uterus Tabulation, compilation of results and statistical analysis

2.3.2 Hematological and Biochemical analysis

Observational parameters like: Skin and fur, Lethargy, Diarrhoea, Sedation, Tremor, Clonic convulsion, Tonic extention, Straub reaction, Pilo erection, Muscle spasm, Spasticity, Ptosis, Lacrimation, Salivation and Mortality.

Body weight changes

Food and Water consumption

Hematological parameter like: RBC, WBC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), platelet count, Hb. Level.

Biochemical investigation like: Glucose, Total cholesterol, Uric acid, Bilirubin, Creatinine, triglyceride, Total protein in serum. Enzymes [alanine aminotransferase (SGPT), aspartate aminotransferase (SGOT)] in serum

Histopathological evaluation: Organs: Brain, Heart, Kidney, Liver, Lungs, Testis/Uterus of control and high dose group

Blood pressure

2.4 Subacute study for 28 days:

Group (n=10)	Method	No. of animals
Control	Vehicle only	10 (5 male + 5 female)
Low dose (60 mg/kg)	60 mg/kg of herbal formulation for 28 days orally	10 (5 male + 5 female)
Medium dose (120 mg/kg)	120 mg/kg of herbal formulation for 28 days orally	10 (5 male + 5 female)
High dose (200 mg/kg)	200 mg/kg of herbal formulation for 28 days orally	10 (5 male + 5 female)

2.4.1 Subacute oral toxicity as per OECD guideline 407:

Oral dose was administered to animal once in a day for 28 days daily On 29th day, the animals were sacrificed Blood collected and serum prepared Biochemical parameters and haematological parameters were done Hispothological evaluation of brain, heart, lungs, liver, kidney, and testis/uterus Tabulation, compilation of results and statistical analysis.

2.4.2 Haematological and Biochemical analysis

Observational parameters like: Skin and fur, Lethargy, Diarrhea, Sedation, Tremor, Clonic convulsion, Tonic extension, Straub reaction, Pilo erection, Muscle spasm, Spasticity, Ptosis, Lacrimation, Salivation and Mortality.

Body weight changes (Weekly)

Food and Water consumption (Daily)

Haematological parameter like: RBC, WBC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Haematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), platelet count, Hb. Level.

Biochemical investigation like: Glucose, Total cholesterol, Uric acid, Bilirubin, Creatinine, triglyceride, Total protein in serum. Enzymes [alanine aminotransferase (SGPT), aspartate aminotransferase (SGOT)] in serum.

Histopathological evaluation: Organs: Brain, Heart, Kidney, Liver, Lungs, Testis/Uterus of control and high dose group.

Blood pressure (Weekly):

3 Results and Discussion:

3.1 Results of Acute oral toxicity:

3.1.1 Effect of AHF on observation parameters of rats:

Observation	Control														Treatment (2000 mg/kg AHF)												
Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Skin and fur	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lethargy	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sedation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tremor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clonic convulsion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tonic extention	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub reaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Muscle spasm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spasticity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(Table no. 1.2 Effect of AHF on observation parameters)

3.1.2 Effect of AHF on body weight of rats:

Effect of single oral administration of Aphrodisiac Herbal Formulation on body weight of rats All values are represented as MEAN ± SEM, n=5. There was no statistically significant difference was seen in body weight as compared to the control group.

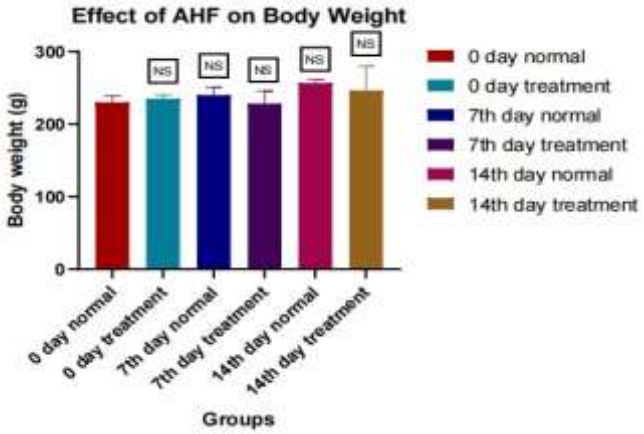

Figure 1.1. Effect of single oral administration of AHF on Body weight of rats.

3.1.3 Effect of AHF on food consumption of rats:

Effect of Aphrodisiac Herbal Formulation on food consumption of rat during 14 days experimental period All values are expressed as Mean ± SEM. There was no statistically significant difference was seen in food consumption as compared to the control group.

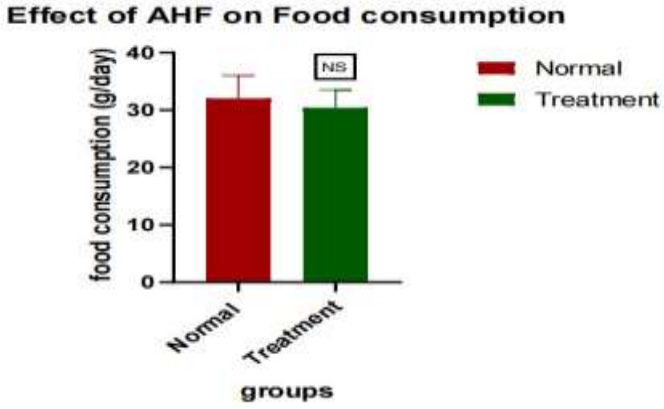

Figure 1.2. Effect of single oral administration of AHF on food consumption of rats.

3.1.4 Effect of AHF on Water consumption of rats:

Effect of Aphrodisiac Herbal Formulation on Water consumption of rat during 14 days experimental period All values are expressed as Mean ± SEM.There was no statistically significant difference was seen in water consumption as compared to the control group.

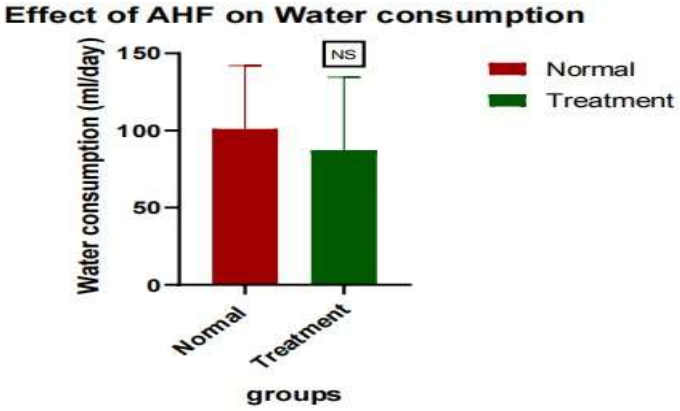

Figure 1.3 Effect of single oral administration of AHF on water consumption of rats

3.1.5 Effect of AHF on haematological parameters of rats:

Table no 1.3 Effect of Aphrodisiac Herbal Formulation on haematological parameters of rats. All values are expressed as Mean ± SEM. There was no statistically significant difference was seen in haematological parameters as compared to the control group.

Parameter (n=5)	Normal	Treatment
Hemoglobin (g/dL)	14.40 ± 0.35	14.60 ± 0.10
RBC (10^6/mm^3)	7.06 ± 0.34	7.49 ± 0.06
WBC (/mm^3)	9454.00 ± 1305.90	9288.40 ± 889.00
Platelets (/mm^3)	544800.00 ± 10622.62	531000.00 ± 37636.42
HCT (%)	40.54 ± 1.48	41.28 ± 0.10
MCV (Fl)	153.91 ± 95.76	151.28 ± 96.30
MCH (pg)	20.50 ± 0.67	19.27 ± 0.11
MCHC (g/dL)	35.60 ± 0.53	35.12 ± 0.26
RDW-CV (%)	14.16 ± 0.04	14.28 ± 0.04
Neutrophils (%)	20.40 ± 4.30	19.00 ± 1.67
Lymphocytes (%)	72.60 ± 4.66	76.20 ± 2.22
Eosinophils (%)	1.20 ± 0.20	1.20 ± 0.20
Monocytes (%)	5.20 ± 0.48	3.40 ± 0.74

Table no 1.3 Effect of Aphrodisiac Herbal Formulation on haematological parameters of rats.

3.1.6 Effect of AHF on biochemical parameters of rats:

Table no.1.4 Effect of Aphrodisiac Herbal Formulation on biological parameters of rats. All values are expressed as Mean ± SEM. There was no statistically significant difference was seen in biochemical parameters as compared to control group.

Parameter (n=5)	Normal	Treatment
Glucose (mg/dL)	126.89 ± 12.51	126.27 ± 11.88
Cholesterol (mg/dL)	137.40 ± 13.51	146.95 ± 19.14
TG (mg/dL)	85.05 ± 17.61	80.78 ± 16.10
Bilirubin (mg/dL)	1.03 ± 0.07	0.92 ± 0.11
SGOT (U/L)	233.33 ± 19.35	219.80 ± 30.04
SGPT (U/L)	61.72 ± 13.02	71.08 ± 1.68
Total protein (g/dL)	7.84 ± 0.17	7.92 ± 0.23
Creatinine (mg/dL)	1.00 ± 0.06	0.93 ± 0.09
BUN (mg/dL)	150.66 ± 9.80	168.20 ± 17.70
Uric acid (mg/dL)	2.75 ± 0.29	2.40 ± 0.47

Table no.1.4 Effect of Aphrodisiac Herbal Formulation on biological parameters of rats.

3.1.7 Effect of AHF on blood pressure of rats.

Table no.1.5 Effect of Aphrodisiac Herbal Formulation on blood pressure of rats All values are expressed as Mean ± SEM. There was no statistically significant difference was seen in Blood pressure as compared with control.

Time	BP of rats (mm/Hg)
0 min	120.3 ± 0.8
30 min	118.0 ± 1.7
1 hr.	122.0 ± 1.1
2 hr.	117.6 ± 0.6
3 hr.	115.3 ± 0.6
4 hr.	117.3 ± 0.8
8 hr.	114.0 ± 1.5
12 hr.	117.0 ± 0.5
24 hr.	118.6 ± 0.3

Table no.1.5 Effect of Aphrodisiac Herbal Formulation on blood pressure of rats

3.1.8 Effect of Aphrodisiac Herbal Formulation on histopathology: 1. Brain

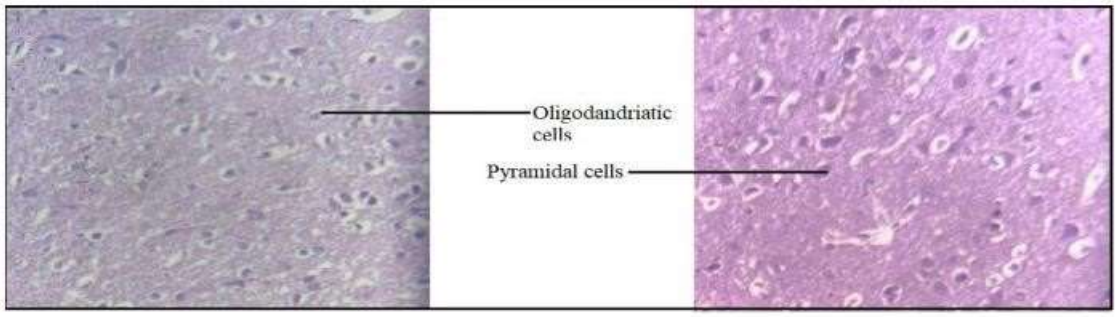

Figure 3.1.8.1 Brain of female normal rat

Figure3.1.8.2 Brain of female treatment rat

2. Heart

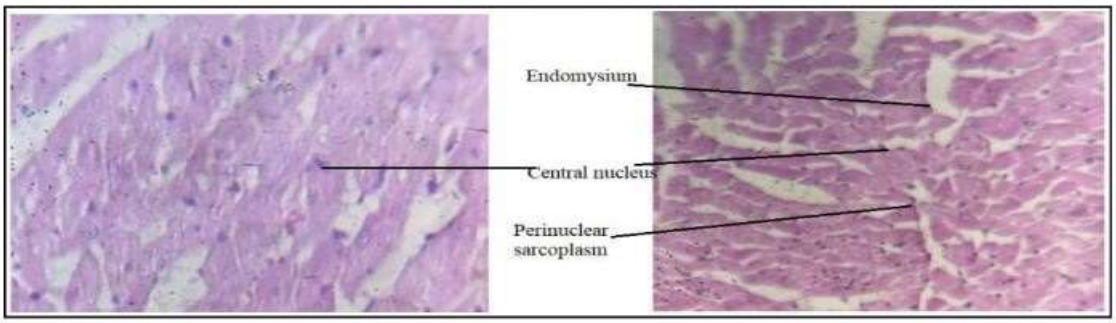

Figure3.1.8.3 Heart of female normal rat

Figure 3.1.8.4 Heart of female treatment rat

3. Kidney

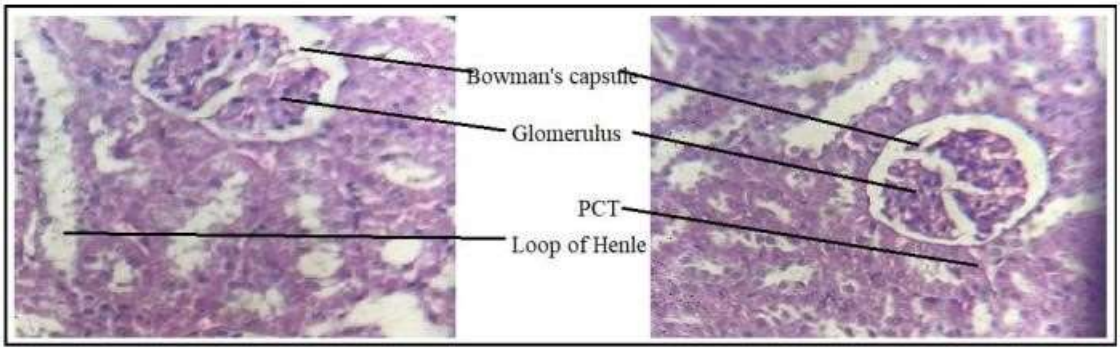

Figure 3.1.8.5 Kidney of female normal rat

Figure 3.1.8.6 Kidney of female treatment rat

4. Liver

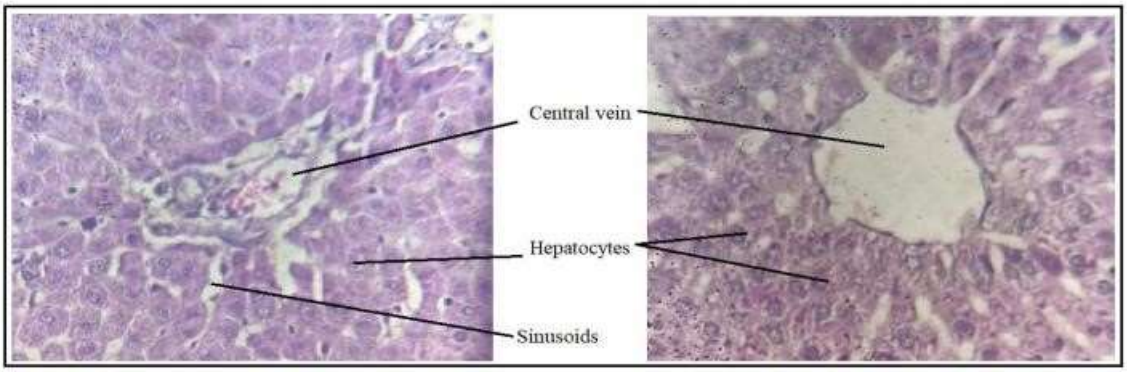

Figure 3.1.8.7 Liver of female normal rat Figure 3.1.8.8 Liver of female treatment rat

5. Lung

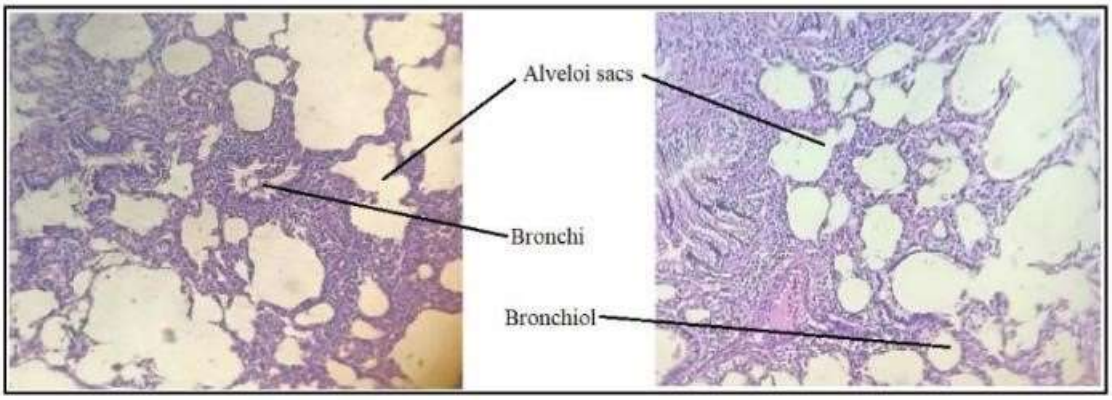

Figure 3.1.8.9 Lung of female normal rat Figure3.1.8.10 Lung of female treatment rat

6. Uterus

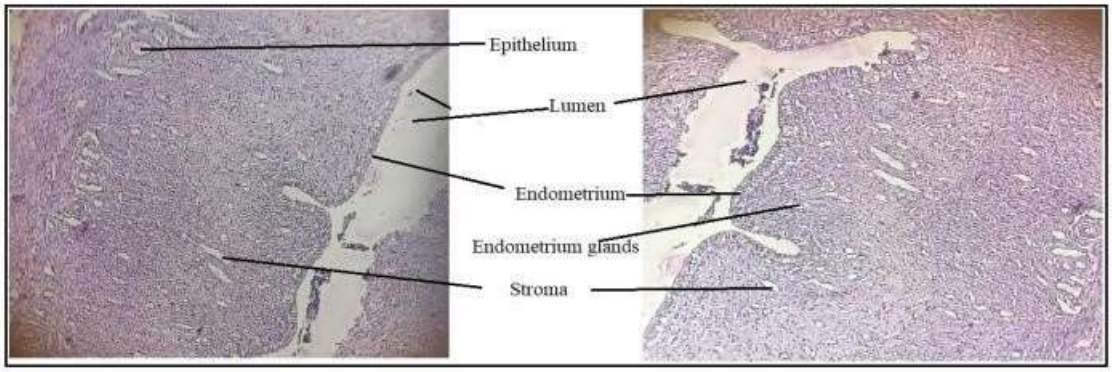

Figure 3.1.8.11 Uterus of female normal rat Figure 3.1.8.12 Uterus of female treatment rat

3.2 Discussion of Acute oral toxicity:

There were no treatment related death or signs of toxicity developed in both the control and treated animals throughout the study. No significant difference in body weight was also observed. Further, there were no gross pathological abnormalities found in both control and treated groups. Thus, the LD50 value was found to be greater than 2000 mg/kg in rats.

3.3 Results of Subacute oral toxicity:

3.3.1 Effect of AHF on observation parameters of rats:

Table no 1.6 Effect of Aphrodisiac Herbal Formulation on observation parameters of rats

Observation parameter (n=10)	Week 1				Week 2				Week 3				Week 4			
	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4
Skin & fur	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lethargy	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	✓	-	-	✓	-	-	-	-	-
Sedation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tremor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clonic convulsion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tonic extension	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub's reaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pilo erection	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Muscle spasm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spasticity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

3.3.2 Effect of AHF on body weight change in rats:

Table no 1.7 Effect of Aphrodisiac Herbal Formulation on body weight changes in rats All values are expressed as Mean ± SEM. There was no statistically significant difference was seen in bodyweight of 60 mg/kg AHF, 120 mg/kg AHF and 200 mg/kg AHF treated group during 28 days as compared with control group.

Groups(n=10)	Weeks			
	Week 1	Week 2	Week 3	Week 4
Control (M)	240 ± 10	226.66 ± 6.66	240 ± 5.77	243.33 ± 6.66
Control (F)	190 ± 15.2	206.6 ± 21.8	216.6 ± 21.8	216.6 ± 3.3
60AHF(M)	223.3 ± 10	230 ± 8.81	233.5 ± 8.81	230 ± 11.54
60 AHF (F)	206.6 ± 13.3	250 ± 8.8	213.3 ± 18.5	250 ± 15.2
120 AHF(M)	213.33 ± 6.66	226.66 ± 8.81	230 ± 10	226.66 ± 14.59
120 AHF (F)	216.6 ± 17.6	226.6 ± 28.4	216.6 ± 24.3	226.6 ± 28.4
200 AHF (M)	220 ± 11.54	233.33 ± 13.33	220 ± 10	233.33 ± 6.66
200 AHF (F)	206.6 ± 13.3	230 ± 6.6	216.6 ± 14.5	230 ± 11.5

3.3.3 Effect of AHF on food consumption in rats:

Table no 1.8 Effect of Aphrodisiac Herbal Formulation on food consumption in rats All values are expressed as Mean ± SEM. There was no statistically significant difference was seen in food consumption of 60 mg/kg AHF, 120 mg/kg AHF and 200 mg/kg AHF treated group during 28 days as compared with control group

Group (n=10)	Food consumption (gm/day)
Control	57.28 ± 2.65
60 mg/kg	56.94 ± 1.69
120 mg/kg	58 ± 1.93
200 mg/kg	54.55 ± 1.99

3.3.4 Effect of AHF on water consumption in rats:

Table no 1.9 Effect of Aphrodisiac Herbal Formulation on water consumption in rats All values are expressed as Mean ± SEM. There was no statistically significant difference was seen in water consumption of 60 mg/kg AHF, 120 mg/kg AHF and 200 mg/kg AHF treated group during 28 days as compared with control group.

Group (n=10)	Water consumption (ml/day)
Control	82.23 ± 3.60
60 mg/kg	82.41 ± 2.21
120 mg/kg	86.60 ± 3.75
200 mg/kg	80.80 ± 2.07

3.3.5 Effect of AHF on hematological parameters of rats:

Table no 1.10 Effect of Aphrodisiac Herbal Formulation on hematological parameters of rats All values are expressed as Mean ± SEM. There was no statistically significant difference was seen in water consumption of 60 mg/kg AHF, 120 mg/kg AHF and 200 mg/kg AHF treated group during 28 days as compared with control group

Parameters (n=10)	Groups			
	Control	60 mg/kg	120 mg/kg	200 mg/kg
Hemoglobin (g/dL)	15.53 ± 0.41	15.75 ± 0.19	15.66 ± 0.32	15.31 ± 0.25
RBC (10^6/mm^3)	7.12 ± 0.55	6.90 ± 0.57	7.638 ± 0.23	7.67 ± 0.35
WBC (/mm^3)	15846 ± 61473	16050 ± 1961	12889 ± 870	12983 ± 1746
Platelets (/mm^3)	526667 ± 61473	504500 ± 41447	441000 ± 26932	431500 ± 42166
HCT (%)	43.05 ± 1.37	41.85 ± 3.56	43.98 ± 1.20	43.21 ± 0.72
MCV (FL)	61.75 ± 3.42	60.73 ± 3.00	57.33 ± 1.23	56.65 ± 1.94
MCH (pg)	22.36 ± 1.40	23.68 ± 2.07	20.43 ± 0.35	20.08 ± 0.67
MCHC (g/dL)	36.11 ± 0.48	39.55 ± 4.51	35.68 ± 0.54	35.46 ± 0.54
RDW-CV (%)	14.06 ± 0.08	14.25 ± 0.03	13.86 ± 0.14	14 ± 0.11
Neutrophiles (%)	28 ± 5.41	17.66 ± 3.66	18.33 ± 3.05	21 ± 7.57
Lymphocytes (%)	65 ± 6.03	76.16 ± 3.12	75.5 ± 3.71	73 ± 7.96
Eosinophiles (%)	1.16 ± 0.16	1.16 ± 0.16	1.16 ± 0.16	1 ± 0
Monocytes (%)	5.83 ± 0.54	5 ± 0.68	5 ± 0.85	5 ± 0.44

3.3.6 Effect of AHF on biochemical parameters of rats:

Table no 1.11 Effect of Aphrodisiac Herbal Formulation on biochemical parameters of rats All values are expressed as Mean ± SEM. There was no statistically significant difference was seen in biochemical parameters of 60 mg/kg AHF, 120 mg/kg AHF and 200 mg/kg AHF treated group as compared to control group.

Parameters (n=10)	Groups			
	Control	60 mg/kg	120 mg/kg	200 mg/kg
Glucose (mg/dL)	113.13 ± 16.71	94.85 ± 9.80	111.64 ± 11.91	122.47 ± 10.23
Cholesterol (mg/dL)	92.27 ± 9.4	70.65 ± 2.64	102.64 ± 19.29	105.75 ± 16.83
TG (mg/dL)	122.45 ± 13.89	115.5 ± 18.42	119.78 ± 10.53	119.39 ± 12.92
Bilirubin (mg/dL)	1.25 ± 0.07	1.25 ± 0.10	1.19 ± 0.09	1.21 ± 0.08
SGOT (U/L)	148.96 ± 10.65	153.01 ± 5.05	127.67 ± 6.37	141.96 ± 5.37
SGPT (U/L)	66.18 ± 4.49	67.62 ± 0.08	79.39 ± 8.89	68.77 ± 1.94
Total protein (g/dL)	7.51 ± 0.18	7.79 ± 0.19	8.11 ± 0.28	7.95 ± 0.28
Creatinine (mg/dL)	0.83 ± 0.03	0.89 ± 0.07	0.84 ± 0.04	0.85 ± 0.07
BUN (mg/dL)	35.83 ± 4.01	44.7 ± 2.93	48.11 ± 4.92	47.78 ± 5.55
Uric acid (mg/dL)	3.24 ± 0.29	3.53 ± 0.09	3.28 ± 0.24	3.27 ± 0.44

3.3.7 Effect of AHF on blood pressure of rats:

Table no 1.12 Effect of Aphrodisiac Herbal Formulation on blood pressure of rats All values are expressed as Mean ± SEM. There was no statistically significant difference was seen in blood pressure of 60 mg/kg AHF, 120 mg/kg AHF and 200 mg/kg AHF treated group as compared to control group.

Time (hr.)	Blood pressure (mm/Hg)			
	Control	60 mg/kg	120 mg/kg	200 mg/kg
Week 1	125.5 ± 1.05	126.5 ± 1.05	124.3 ± 1.30	123.8 ±0.94
Week 2	124 ± 1.12	126 ± 0.51	124.1 ± 1.62	123.5 ± 0.88
Week 3	125.6 ± 1.40	124.8 ± 1.40	123 ± 0.96	125.1 ± 0.98
Week 4	123 ± 0.57	125.3 ± 0.91	120.1 ± 0.83	123.5 ± 1.43

3.3.8 Effect of AHF on histopathology of rats:

Male Histopathology

Brain Histopathology

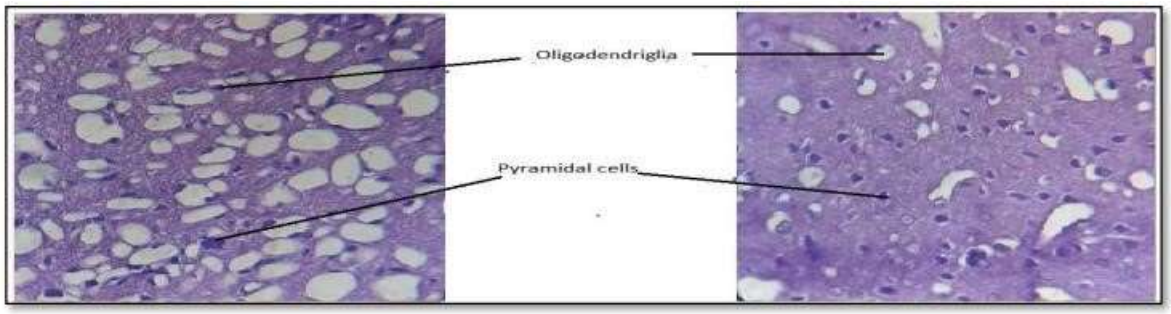

Fig.3.3.8.1 Brain of Control Male

Fig.3.3.8.2 Brain of 60 mg/kg Male

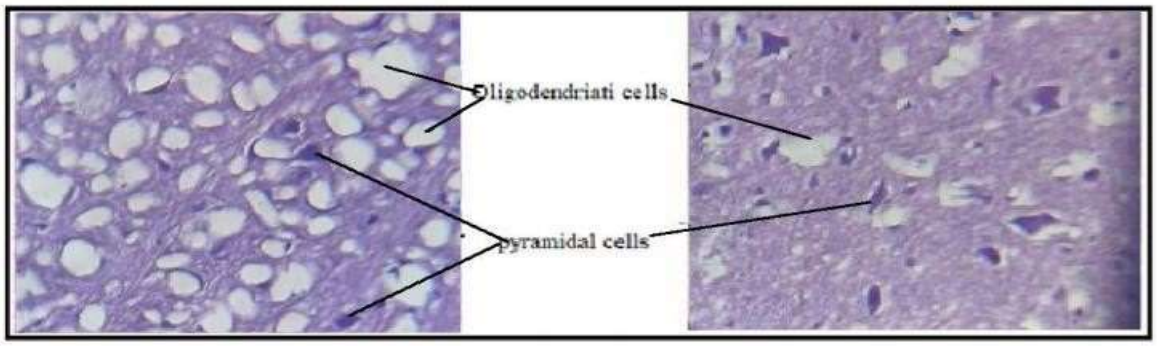

Fig.3.3.8.3 Brain of 120 mg/kg Male

Fig3.3.8.4 Brain of 200 mg/kg Male

In brain there was no change observed. Oligodendroglia and pyramidal cells were seen in figure and no change was seen in 60, 120 and 200 mg/kg AHF rat brain as compared to control rat Brain.

Heart Histopathology

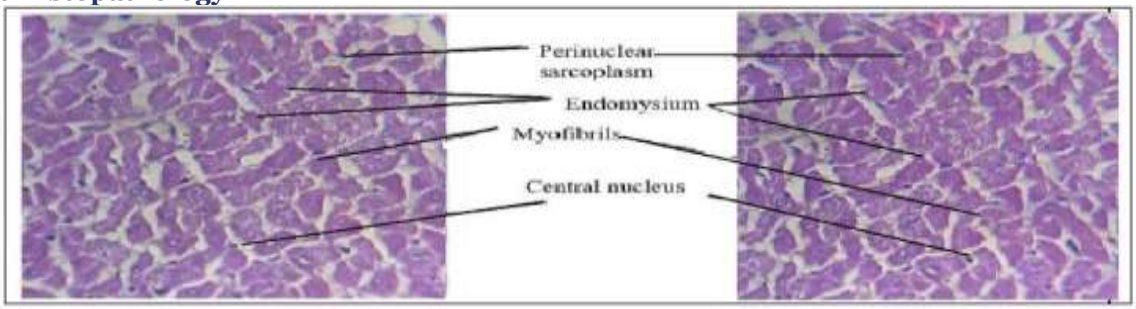

Fig.3.3.8.5 Heart of Control Male

Fig.3.3.8.6 Heart of 60 mg/kg Male

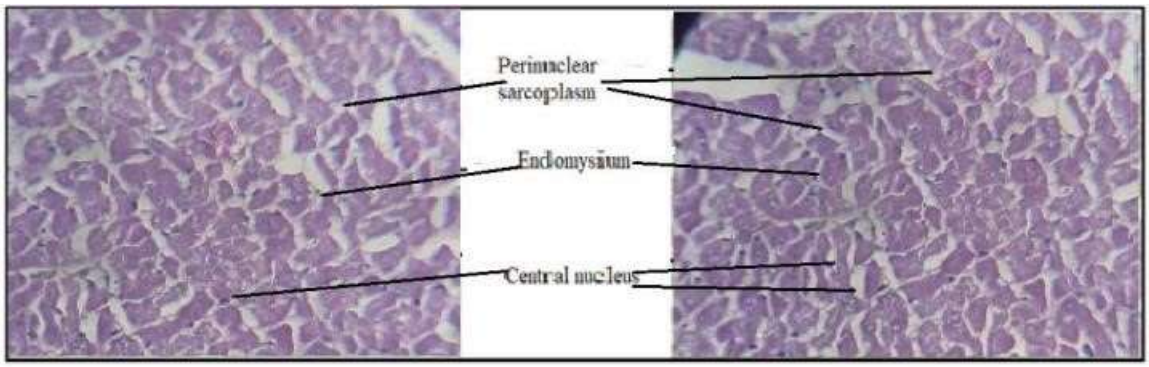

Fig.3.3.8.7 Heart of 120 mg/kg Male Fig.3.3.8.8 Heart of 200 mg/kg Male In heart there was no change observed. Perinuclear cells, Endomysium and central nucleus were seen in figure and no change was observed in 60, 120 and 200 mg/kg AHF rat heart as compared to control rat heart

Lung histopathology

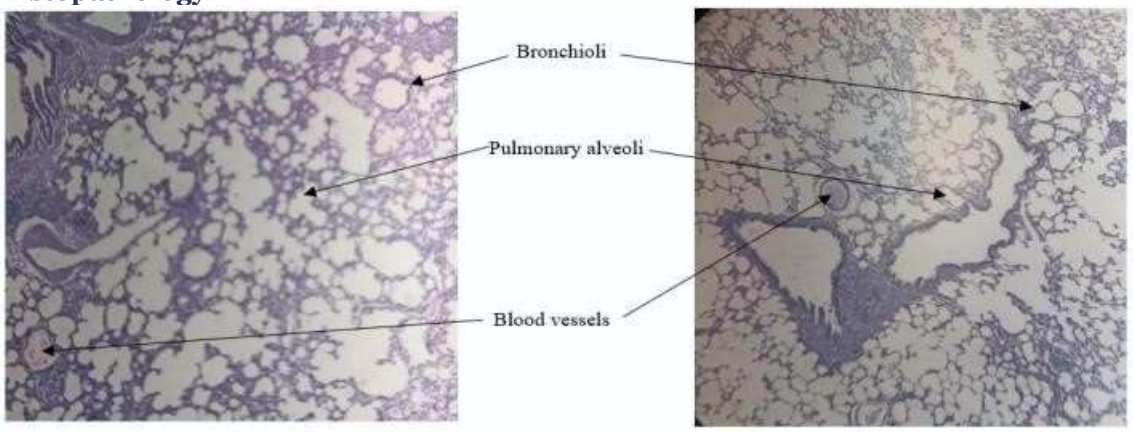

Fig.3.3.8.9 Lungs of Control Male Fig.3.3.8.10 Lungs of 60 mg/kg AHF Male

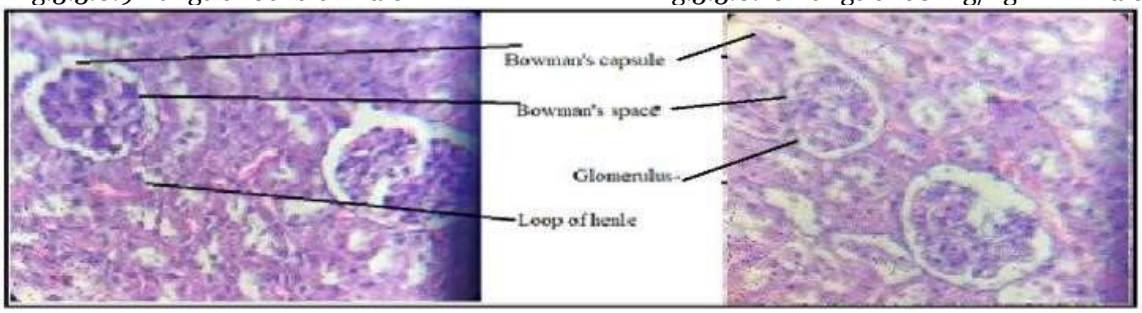

Fig.3.3.8.11 Lungs of 120 mg/kg Male Fig.3.3.8.12 Lungs of 200 mg/kg Male In lungs there was no change observed. Pulmonary alveoli, blood vessels and Bronchiole were seen in figure and no change was observed in 60, 120 and 200 mg/kg AHF rat lungs as compared to control rat lungs.

Kidney Histopathology

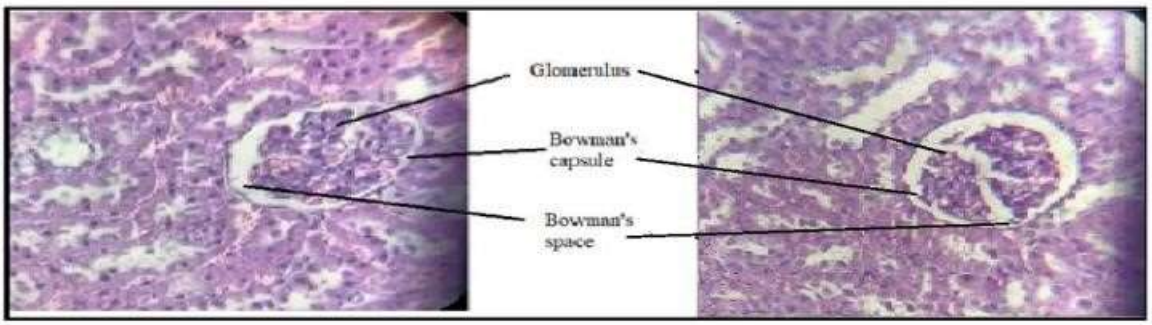

Fig.3.3.8.13 Kidney of Control Male Fig.3.3.8.14 Kidney of 60 mg/kg Male

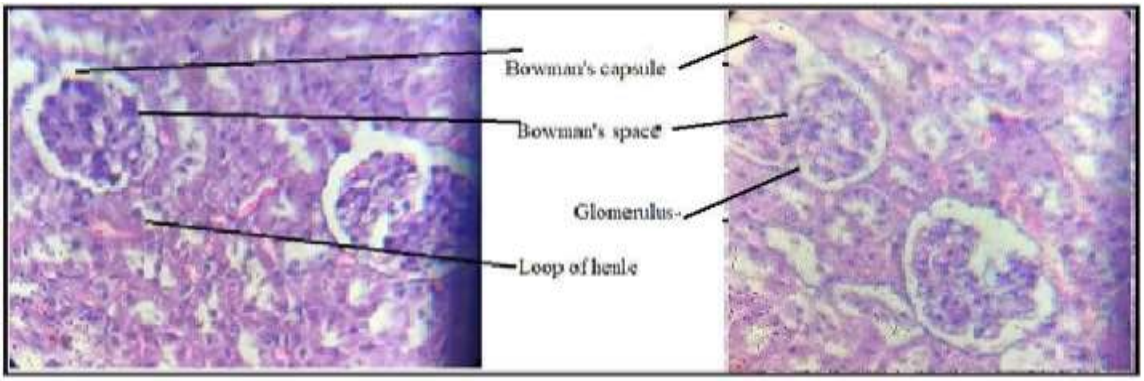

Fig.3.3.8.15 Kidney of 120 mg/kg Male Fig.3.3.8.16 Kidney of 200 mg/kg Male

In kidney there was no change observed. Bowman's capsule with glomeruli and Bowman's space were seen in figure and no change was observed in 60, 120 and 200 mg/kg AHF rat kidney as compared to control rat kidney.

Liver Histopathology

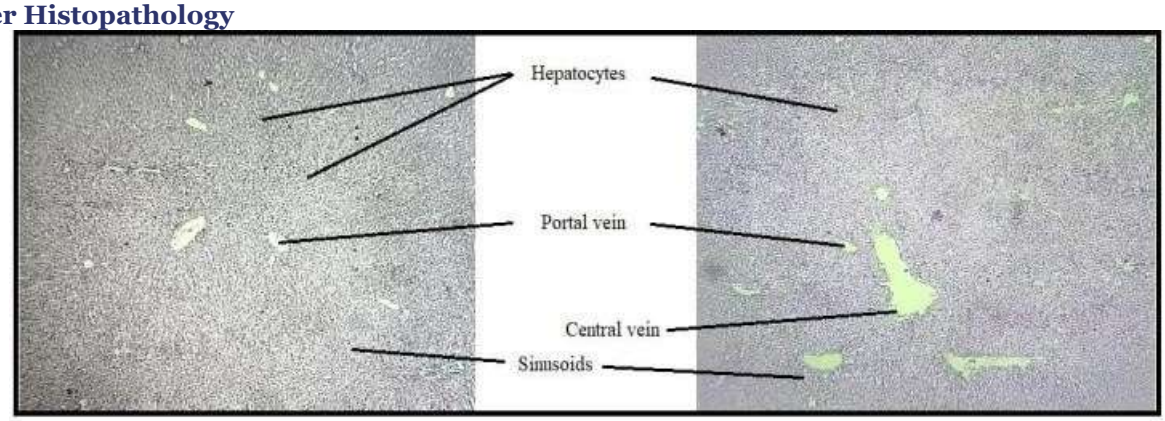

Fig.3.3.8.17 Liver of Control Male Fig.3.3.8.18 Liver of 60 mg/kg Male

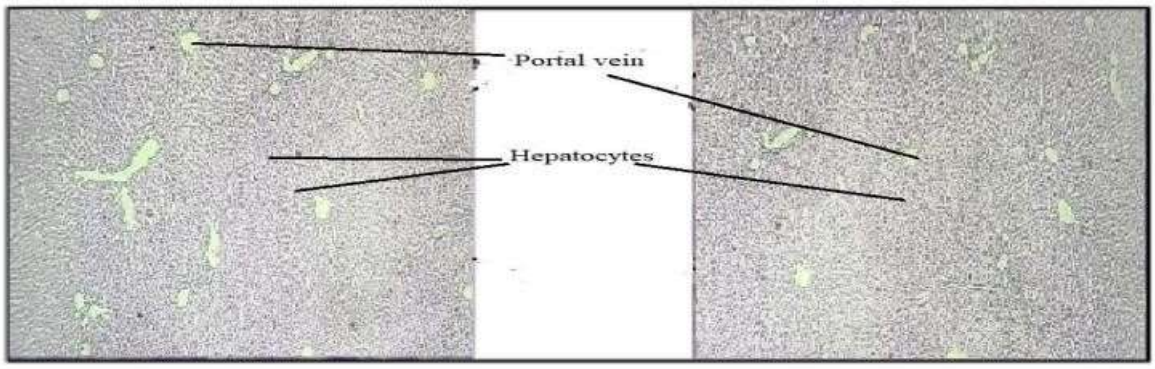

Fig.3.3.8.19 Liver of 120 mg/kg Male Fig.3.3.8.20 Liver of 200 mg/kg Male In liver there was no change observed. Hepatocytes were seen in figure and no change was observed in liver of 60, 120 and 200 mg/kg AHF rat liver as compared to control rat liver.

Testis Histopathology

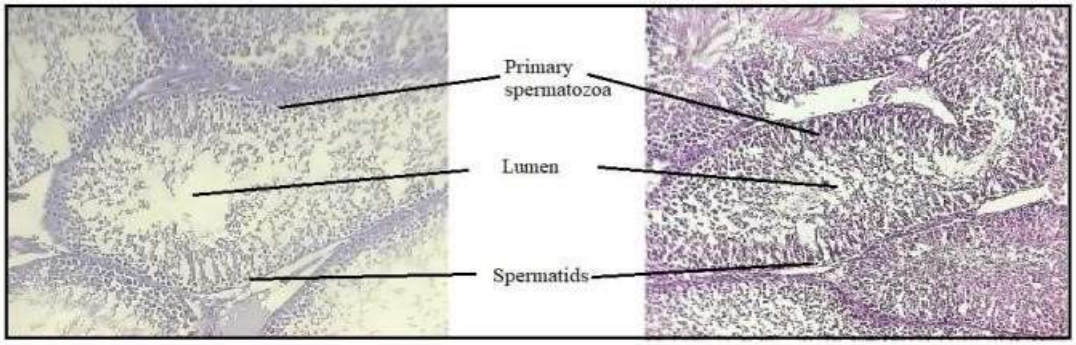

Fig.3.3.8.21 Testis of Control Male Fig.3.3.8.22 Testis of 60 mg/kg Male

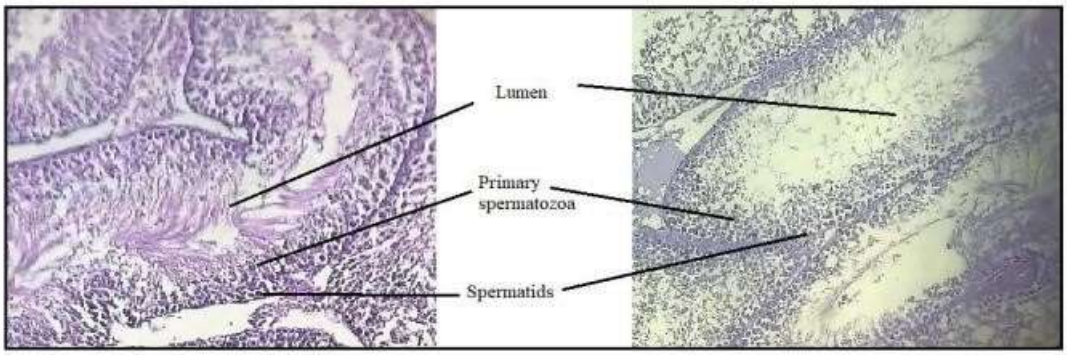

Fig.3.3.8.23 Testis of 120 mg/kg Male Fig.3.3.8.24 Testis of 200 mg/kg Male In Testis there was no change observed. Lumen, primary spermatozoa were seen in figure and no change was observed in Testis of 60, 120 and 200 mg/kg AHF rat testis as compared to control rat testis

Female Histopathology

Brain Histopathology

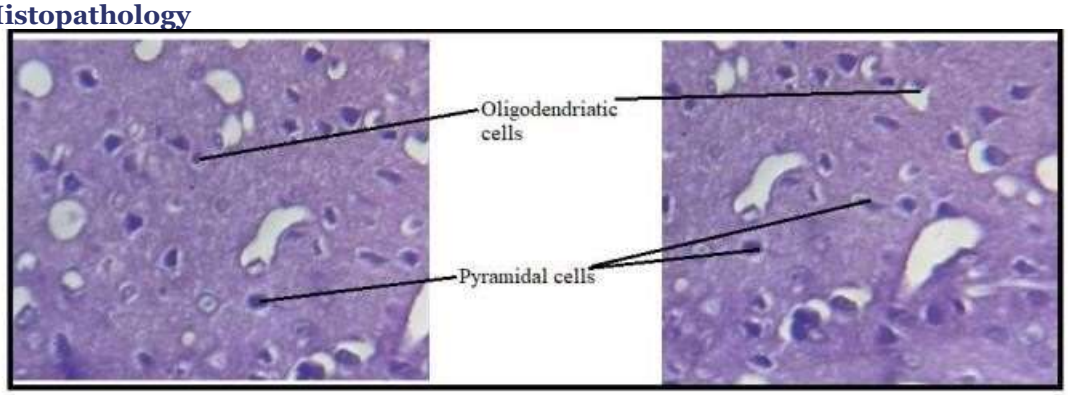

Fig.3.3.8.25 Brain of Control Female Fig.3.3.8.26 Brain of 60 mg/kg Female

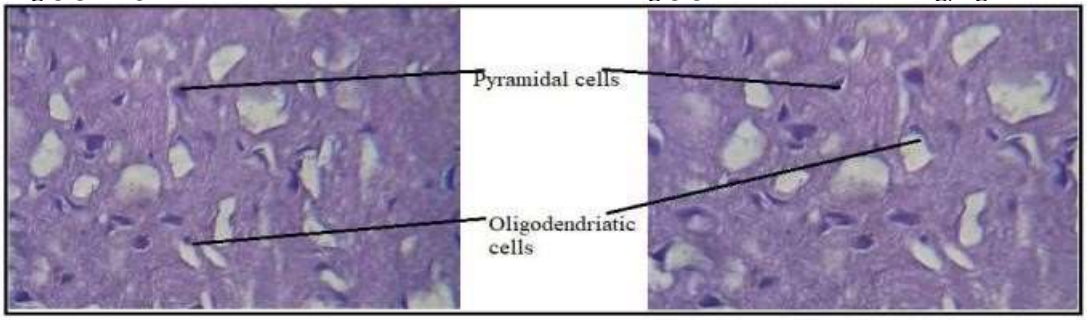

Fig.3.3.8.27 Brain of 120 mg/kg Female Fig.3.3.8.28 Brain of 200 mg/kg Female In brain there was no change observed. Oligodendroglia and pyramidal cells were seen in figure and no change was seen in 60, 120 and 200 mg/kg AHF rat brain as compared to control rat brain

Heart Histopathology

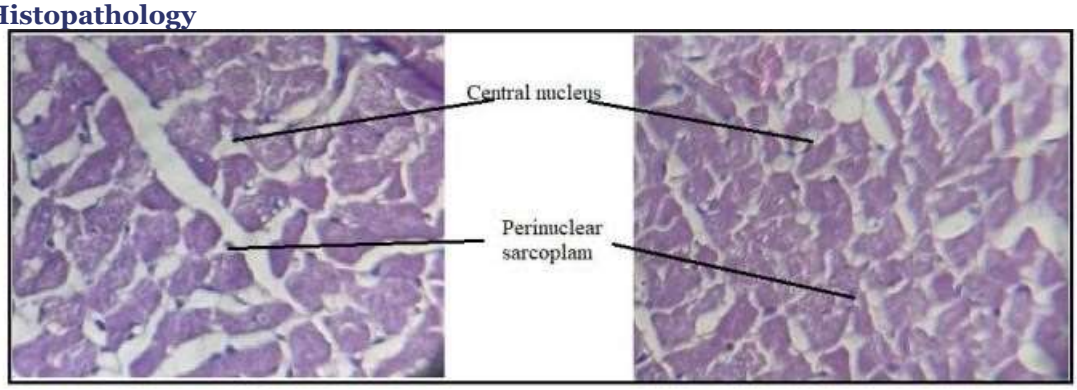

Fig.3.3.8.29 Heart of Control Female Fig.3.3.8.30 Heart of 60 mg/kg Female

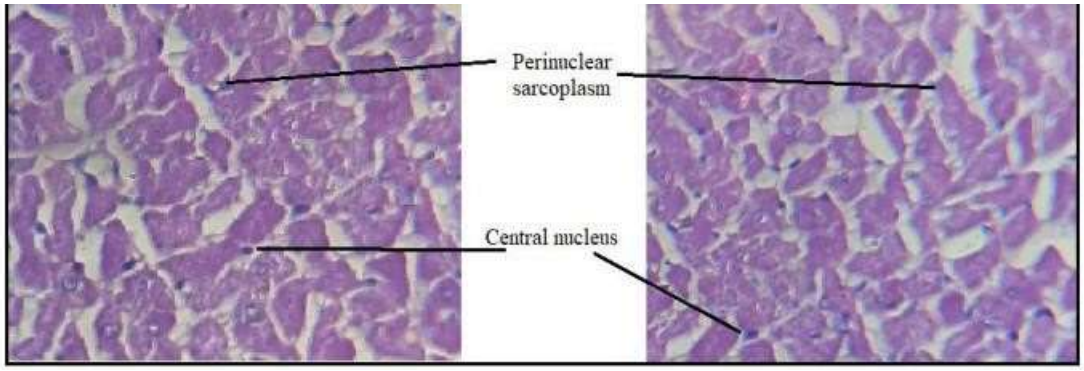

Fig.3.3.8.31 Heart of 120 mg/kg Female Fig.3.3.8.32 Heart of 200 mg/kg Female In heart there was no change observed. Perinuclear cells, Endomysium and central nucleus were seen in figure and no change was observed in 60, 120 and 200 mg/kg AHF rat heart as compared to control rat heart.

Lung histopathology

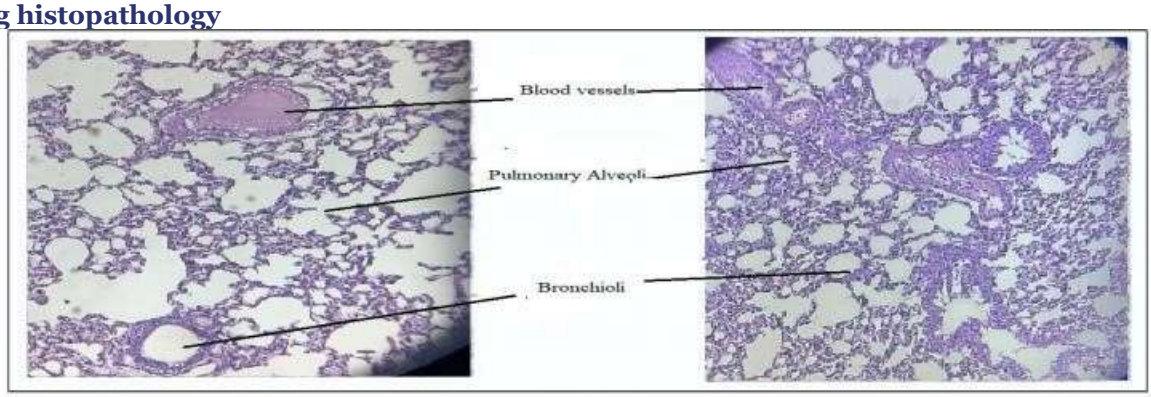

Fig.3.3.8.33 Lungs of Control Female Fig3.3.8.34 Lungs of 60 mg/kg Female

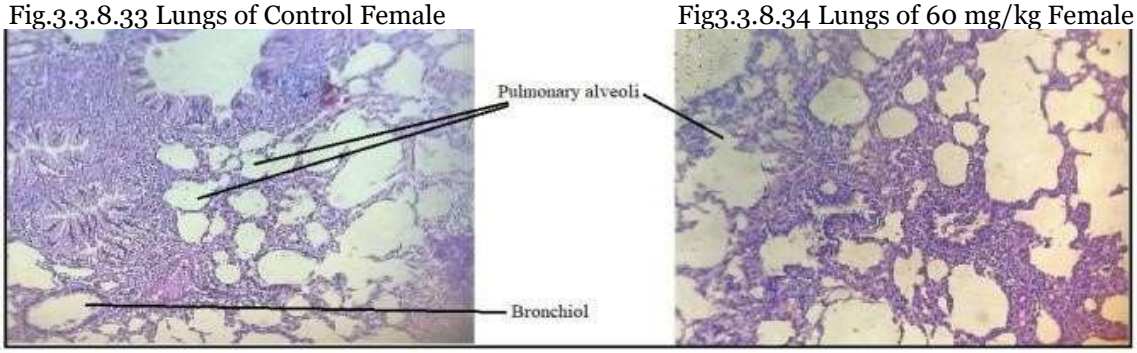

Fig.3.3.8. 35 Lungs of 120 mg/kg Female Fig.3.3.8.36 Lungs of 200 mg/kg Female In lungs there was no change observed. Pulmonary alveoli, blood vessels and Bronchiole were seen in figure and no change was observed in 60, 120 and 200 mg/kg AHF rat lungs as compared to control rat lung

Kidney Histopathology

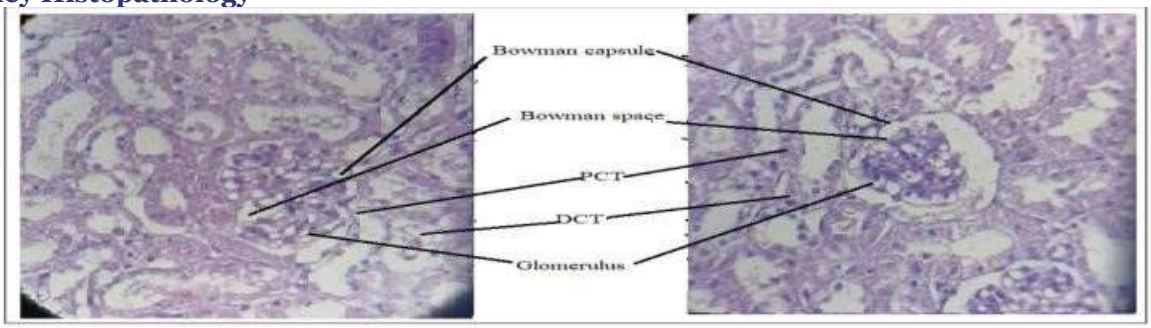

Fig.3.3.8.37 Kidney of Control Female Fig.3.3.8.38 Kidney of 60 mg/kg Female

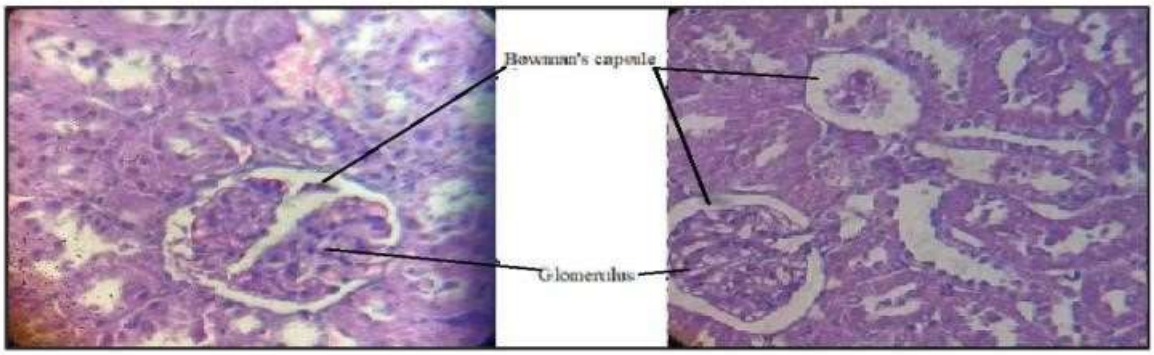

Fig.3.3.8.39 Kidney of 120 mg/kg Female Fig.3.3.8.40 Kidney of 200 mg/kg Female

In kidney there was no change observed. Bowman's capsule with glomeruli and Bowman's space were seen in figure and no change was observed in 60, 120 and 200 mg/kg AHF rat kidney as compared to control rat kidney.

Liver Histopathology

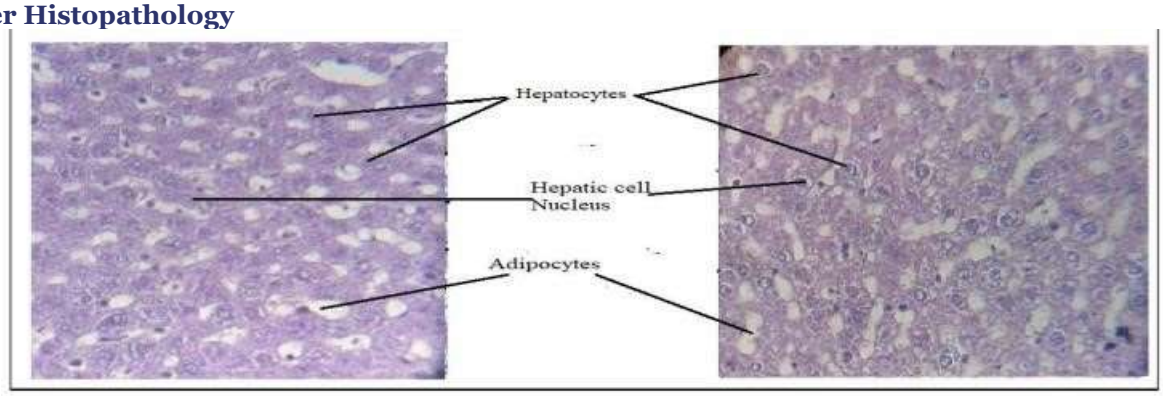

Fig.3.3.8.41 Liver of Control Female Fig.3.3.8.42 Liver of 60 mg/kg Female

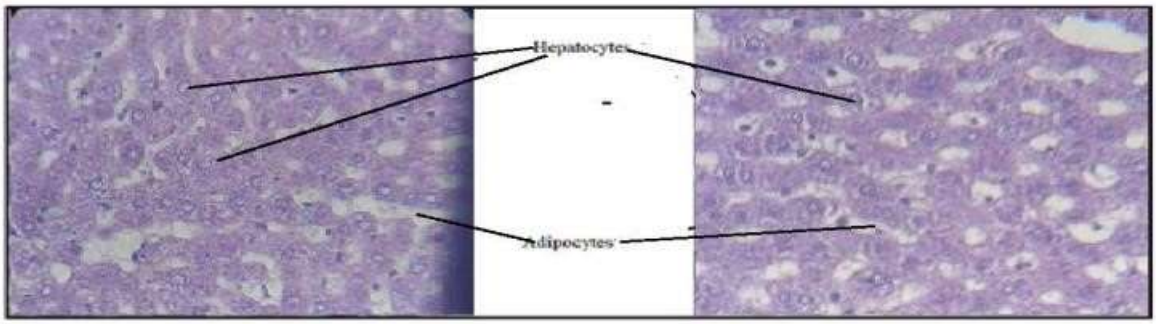

Fig.3.3.8.43 Liver of 120 mg/kg Female Fig.3.3.8.44 Liver of 200 mg/kg Female In liver there was no change observed. Hepatocytes were seen in figure and no change was observed in liver of 60, 120 and 200 mg/kg AHF rat liver as compared to control rat liver.

Uterus Histopathology

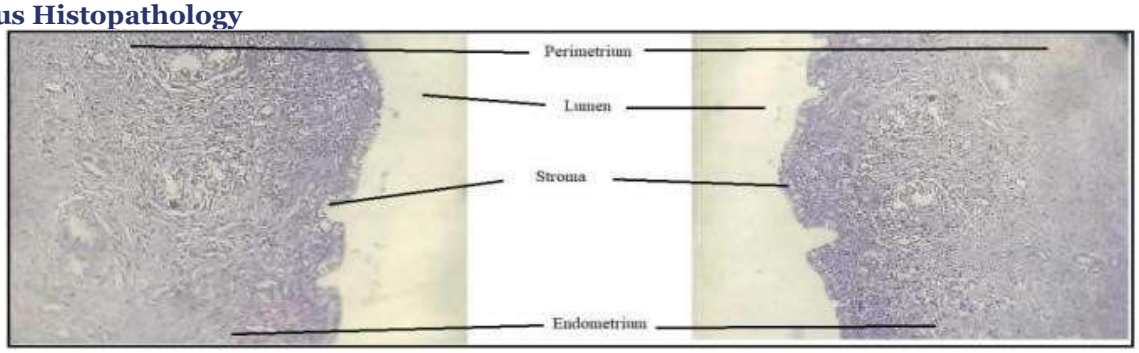

Fig.3.3.8.45 Uterus of Control female Fig.3.3.8.46 Uterus of 60 mg/kg female

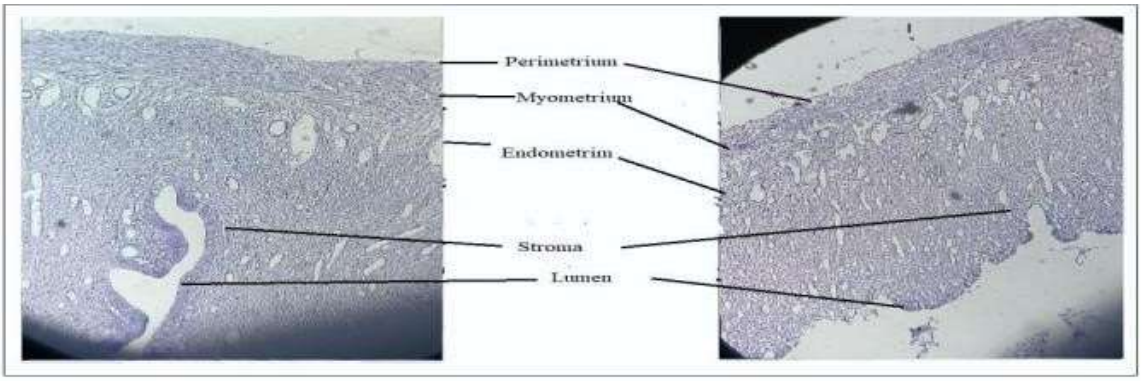

Fig.3.3.8.47 Uterus of 120 mg/kg female Fig.3.3.8.48 Uterus of 200 mg/kg female In Uterus there was no change observed. Lumen, Endometrium, Stroma were seen in figure and no change was observed in Testis of 60, 120 and 200 mg/kg AHF rat uterus as compared to control rat uterus.

3.3.9 Discussion of Sub acute oral toxicity:

There were no treatment-related toxic sign and mortality observed in both sex of rats treated at 60, 120 and 200 mg/kg orally for a period of 28 days. No significant difference in body weight gain was observed between control and treated groups during the study. There was no significant change found in food and water intake in treatment group compared to control group. All the haematological parameters and biochemical parameters were found to be within the clinical range. Gross necropsy saw that there was no significant difference in the organ structure. Present study reveals no histological change in organ like brain, heart, kidney, liver, lungs, testis and uterus of treated groups as compared to control group.

3.4 conclusion:

Based on the outcome of studies on the acute and subacute oral toxicity of herbal aphrodisiac formulation, the following inference could be established: The acute oral toxicity study reveals that the LD50 of AHF is greater than the 2000 mg/kg in both sex rats because there was no any mortality found at single dose of 2000mg/kg. This AHF produced no change in behaviour, body weight, food and water consumption, haematological parameters, histopathology of rats after treated with AHF for 28 days at dose 60mg/kg, 120 mg/kg and 200 mg/kg. The subacute oral toxicity demonstrated that No Observed Adverse Effect Level of AHF is greater than the 200 mg/kg/day oral in both sex rats. After performing the study, conclude that the AHF was found to be non-toxic at tested dose but further study is required for establishment of sufficient safety evidence for human use.

3.5 Acknowledgement:

We extend our sincere gratitude to sponsor U-Liva Nutrition LLP, for providing invaluable support and resources throughout the course of this research. Special thanks to the faculty and staff whose guidance and expertise significantly contributed to the success of our study. We also appreciate the institution's commitment to fostering a conducive research environment.
This work is a testament to the collaborative spirit fostered by B.K. Mody Government Pharmacy College, Rajkot.

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