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Research Article

Sub-chronic Toxicity Study Of T-AYU-HM™ Premium: A Herbo-mineral Formulation

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ABSTRACT

To perform a sub-chronic toxicity study and to generate scientific data regarding the safety profile of T-AYU-HM™ Premium, a herbo-mineral formulation used for sickle cell disease. Experimental animals (Mice) were divided into six groups and were acclimatized and treated with 125 mg T-AYU-HM™ Premium/kg body weight (T1 LD), 625 mg T-AYU-HM™ Premium/kg body weight (T2 MD), and 1250 mg T-AYU-HM™ Premium/kg body weight (T3 HD) and two group of satellite daily for 90 days. 0.5% CMC was administered to the control group as a vehicle. The satellite groups were treated with 125 mg T-AYU-HM™ Premium/kg body weight (S1 LD) & 1250 mg T-AYU-HM™ Premium/kg body weight (S2 HD, 1250 mg/kg) receiving low & high dose respectively. The mice were closely observed for a clinical sign of toxicity, stereotypical behavior and alteration in autonomic activity during the entire study period. Hematological and blood biochemical parameters were observed on days 0, 60, 90. Motor coordination activity and sensory stimuli assessment were performed after the 11th week. At the termination of the study, all animals were sacrificed, and organs such as the heart, brain, kidney, liver, etc., were collected & observed for histopathology. There was no change in the normal gross behavior of animals in the sensory and motor assessment activity in the treatment group compared to the control group. Evaluation of hematological parameters shows a significant increase in red blood corpuscles. Histopathological examination of various organs shows a normal architecture in all the treated groups. T-AYU-HM™ Premium was found to be safe on repeat dose oral administration in NOAEL, dose up to 1250 mg/kg body weight when administered orally for 90 days in both the sexes of Swiss Albino mice.

INTRODUCTION

Sickle cell anemia is an autosomal recessive hemoglobin disorder that affects millions of children of underprivileged areas across the globe. The factors like acidosis, infection and hypoxia generate pressure on red blood corpuscles and induce lysis of red blood corpuscles known as sickling. This sickling phenomenon of red blood corpuscles induces various complications that are becoming a major concern in managing sickle cell anemia. The clinical complications in sickle cell patients are heterogeneous. This hemoglobin disorder requires a drug that can be tolerated well throughout life. The drug has to be safe, effective, and affordable as well. Many options are available for symptomatic management, but preventing mortality and providing a pain-free quality of life is most challenging.[1-5]

T-AYU-HM™ is a herbo-mineral formulation prepared by incorporating various time-tested herbal ingredients. The information regarding the profile of formulation is mentioned in Table 1. The formulation has documented acute oral toxicity and in vitro efficacy studies.[6-8] As per the OECD guideline, the oral sub-chronic toxicity study was performed to detect toxic effects after oral administration of testing formulation in animals for a portion of the animal's lifetime, but not more than 10% of the animal's

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Table 1: Profile information about T-AYU-HM™ Premium.

Product	T-AYU-HM™ Premium
Batch no.	AHP-069
Mfg. Date	June-2019
Exp. Date	May-2022
Appearance	Brown color tablet
Manufacture by	ATBU Harita Pharmaceuticals Pvt. Ltd, Gujarat
Product Ingredients	Each 300 mg tablet is composed of Calyx of Mica (25 mg) Calyx of iron (12.5 mg), *Terminalia chebula* (25 mg), *Zingiber officinale* (25 mg), *Asparagus racemosus* (25 mg), Punica granatum (12.5 mg), *Myristica fragrans* (25 mg), *Piper longum* (37.5 mg), *Tinospora cordifolia* (37.5 mg), *Leptadiniareticlata* (37.5 mg).
Storages condition	Store in a dry and cool place, Keep away from direct sunlight. Do not refrigerate.

lifetime.[9] The primary objective of performing a sub-chronic toxicity study is to acquire information on the toxic effects of formulation that are undetectable in acute oral toxicity study, detailed information on possible toxic effects after repeated exposure of the testing compound for an observational period, and information on the dose that does not cause toxic effects. The secondary objective of performing a sub-chronic toxicity study is to investigate the compound's cumulative and reversibility effects.

Methodology

Objective and Animal Ethic Approval

The sub-chronic toxicity test of T-AYU-HM™ Premium was conducted according to OECD guideline 408. The animal ethics committee approved the study protocol ROFEL/IAEC/2019/04 of ROFEL Shri G. M. Bilakhia College of Pharmacy, Vapi.

Chemicals

Distilled Water, Ethylene diamine tetraacetic acid (EDTA), Sodium Chloride, Carboxy Methyl Cellulose (CMC), Glucose, Ketamine, Xylazine, etc. were provided by the Institute.

Experimental Animals and Conditions

Species: Swiss Albino mice and Sex: Male and Female. Weight of animals: 28gm to 46 gm for females and 26 to 48gm for males at the initiation of dosing. Acclimatization: 7 days and Temperature: 22 ± 3°°C. Relative Humidity: 30-70%. Light cycle: 12-hour light-dark cycle was maintained throughout the study period.Water& Conventional diet: ad libitum was given to mice.Housing: In polypropylene cages with not more than five animals per cage.

Treatment Groups and Dose Administration

Experimental animals (Mice) were divided into six groups and acclimatized. Three dose levels were selected: low 125 mg/kg, mid 625 mg/kg, and high 1250 mg/kg. The selection was based on a ratio of 1:5:10 based on the human therapeutic dose (2 tablets of 300 gm per day) and converted allometrically to mice dose applying standard conversion factor considering human body weight (60 kg). There were 20 animals (10 males and 10 females) in each group (Control, T1 LD, T2 MD, and T3 HD). The satellite groups (S1 LD and S2 HD) consists of 10 animals each (5 males and 5 female). The experimental animals were treated with 125 mg T-AYU-HM™ Premium/kg body weight (T1 LD), 625 mg T-AYU-HM™ Premium/kg body weight (T2 MD), and 1250 mg T-AYU-HM™ Premium/kg body weight (T3 HD) and two groups of satellite for 90 days. The vehicle was administered to the control group as a vehicle. The other two groups were satellite groups treated with 125 mg T-AYU-HM™ Premium/kg body weight (S1 LD) and 1250 mg T-AYU-HM™ Premium/kg body weight (S2 HD, 1250 mg/kg) receiving low and high doses, respectively. T-AYU-HM™ Premium was administered orally to animals using disposable syringes (1, 2, and 5 mL) tipped with an oral gavage needle (18 gauge).

Observations Parameters

Clinical Signs and Mortality

All experimental animals were noticed once regularly for clinical signs of toxicity and twice regularly for morbidity and mortality over the study period. Clinical signs such as home cage observations (Body position, sedation, excitation, writhing, tremor, convulsion), handling observations (body tone, abdominal tone, aggressiveness to the handler, tail suspension, touch escape, position struggle, corneal reflex, lacrimation, salivation, grasp irritability, urination, defecation), open field observations (transfer arousal, tail elevation, tail pinch, finger approach, finger withdrawal, ataxia) were observed and scored using Modified Irwin test.[10,11] In the 12th week before termination of the study, reactivity to the sensory stimuli was checked using a photoactometer, and motor coordination assessment was carried out using the rota-rod model.[12-14] The elevated plus-maze model was also performed to discover the anxiety and depression-like behavior in all the treated, satellite, and control groups.[15]

Bodyweight

The body weight of all the experimental mice was recorded before the first dose of the study, followed by once a week throughout the study duration. Bodyweight was documented before the scheduled euthanasia at the termination of the study. A change in body weight was observed.

Laboratory Parameters

On days 0, 60, and 90, the blood from the individual animals (of all the groups) was collected in the vial containing EDTA anti-coagulant solution via Retro-orbital route under the anesthetized condition to determine hematological parameters and biochemical estimation.

Hematological Parameters

The blood sample was analyzed for parameters such as hemoglobin, red blood corpuscles, platelets, white blood cells, differential white blood cells.

Blood Biochemistry

This includes parameters such as glucose, proteins, albumin, globulin, creatinine, cholesterol, bilirubin, urea nitrogen.

Gross Pathology

All the animals were euthanized at the end of the study and subjected to gross necropsy. Organ weight and histopathological study of brain, heart, liver, kidney, spleen, uterus (for female) of the animals were recorded.

Statistical Analysis

Data of detailed clinical observation, body weight, hematological parameters, blood biochemical Analysis, and individual organ weight were analyzed for differences among treated and control groups by using in-housed validation software. As required, data were analyzed for normal distribution by one-way ANOVA followed by Dunnett's test and Tukey's Multiple Comparison Test. Data were obtained as non-parametric – the data were analyzed using the Kruskal Wallis test. Data's statistical significance was reported at the 5% significance level ($p < 0.05$).

Results and Discussion

Clinical Signs

There were no treatment-related clinical signs. No mortality was observed in treated animals of any group when compared to control throughout the entire study duration.

Bodyweight

The mean body weight of mice of both the sexes in all the treated and satellite groups showed a progressive increase during the first week of the study. Weekly mean values of body weight of all the treated animals were found statistically non-significant in both the sexes when compared with the control group. There was an increase in body weight, but not significant when observed with control. The normal gain and loss of mean body weight were observed in all the groups when compared with control throughout the study period. But this was considered being normal concerning these species. No treatment-related loss in body weight was observed in Figs. 1A and B.

The Reaction Towards Sensory Stimuli and Motor-Activity Assessment

Reactivity towards sensory stimuli and motor coordination assessment was recorded before the first dose and during the last week of exposure. It did not exhibit any statistically significant changes or treatment-related changes compared with the control. The results are mentioned in Tables 2 and 3.

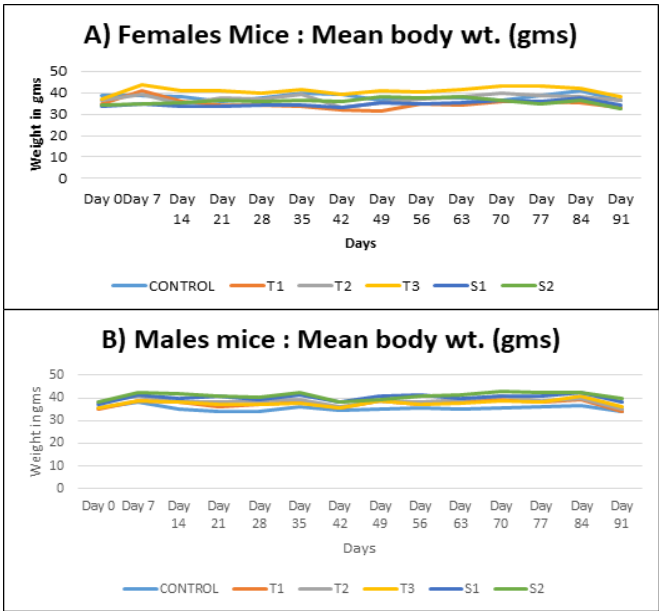

Fig. 1: (A) Female mice and (B) males mice. n = 10 (control – T3HD) and n = 5 (S1 & S2). All values are expressed as Mean ± SEM. *$p < 0.05$, the significant difference compared to control group using one-way ANOVA continued by Turkey's Multiple Comparison Test.)

Table 2: Reactivity of mice toward sensory stimuli-Photoactometer (No. of Cut-off Beams)

	Females		*Males*	
Groups	*Pre-treatment (PT)*	*After11thweek (DT)*	*Pre-treatment (PT)*	*After11thweek (DT)*
Control	168.6 ± 3.23	169.2 ± 3.48	163.4 ± 3.29	164.8 ± 3.57
T1LD	165.3 ± 3.19	165.4 ± 3.033	164.2 ± 3.22	166.2 ± 3.17
T2MD	163 ± 3.01	165.25 ± 3.36	165 ± 3.30	164 ± 3.02
T3HD	167.4 ± 3.15	165.88 ± 3.13	166.7 ± 3.08	165.9 ± 3.02
S1LD	165 ± 4.09	163.33 ± 3.75	165.6 ± 4.05	169.4 ± 4.57
S2HD	168.8 ± 4.32	168.75 ± 2.68	163 ± 4.604	162.8 ± 3.51

n = 10(control–T3HD) & n=5(S1&S2).All values are expressed as Mean±SEM.
*$p < 0.05$, the significant difference compared to control group using one way ANOVA continued by Dunnett test

Table 3: Motor-activity assessment through Rotarod Model (Fall of Time (in a sec))

	Females		*Males*	
Groups	*Pre-treatment(PT)*	*After11thweek(DT)*	*Pre-treatment(PT)*	*After11thweek(DT)*
Control	282.6 ± 5.01	281.9 ± 3.45	286 ± 4.516	285.3 ± 4.90
T1LD	280 ± 5.31	280.1 ± 3.94	279.5 ± 4.93	278.7 ± 3.91
T2MD	285.5 ± 3.84	283.75 ± 3.4	278.1 ± 5.22	278 ± 5.62
T3HD	284.4 ± 4.64	281.5 ± 5.98	281.8 ± 4.84	280.4 ± 4.48
S1LD	288.2 ± 3.12	288 ± 3.51	285 ± 5.63	285 ± 4.76
S2HD	280 ± 2.62	280 ± 5.30	283.8 ± 5.96	283.8 ± 5.14

n = 10 (control–T3HD) & n = 5(S1&S2).All values are expressed as Mean ± SEM.
*p < 0.05, the significant difference compared to control group using one-way ANOVA continued by Dunnett test

Table 4: Time spent in the open arm of Elevated plus maze (Time spent in open arms (in a sec))

	Females		*Males*	
Groups	*Pre-treatment (PT)*	*After 11thweek (DT)*	*Pre-treatment (PT)*	*After11thweek (DT)*
Control	133.4 ± 3.19	134.5 ± 3.36	131.3 ± 3.07	132 ± 2.94
T1LD	132.8 ± 3.55	133.5 ± 3.63	133.3 ± 2.86	133.5 ± 2.75
T2MD	135.3 ± 2.42	136.5 ± 3.41	133.5 ± 2.37	134.1 ± 2.91
T3HD	135.3 ± 2.53	136.6 ± 3.80	134.8 ± 2.83	135.2 ± 3.33
S1LD	130.8 ± 5.75	136 ± 4.93	130.4 ± 4.20	131.6 ± 4.19
S2HD	133.6 ± 4.15	132.75 ± 4.95	129.2 ± 4.34	130.6 ± 3.66

n = 10 (control–T3HD) and n = 5(S1&S2). All values are expressed as Mean ± SEM.
*p < 0.05, the significant difference compared to control group using one-way ANOVA continued by Dunnett test.

Table 5: Results of hematological Analysis.

FEMALE	*Control*	*T1 LD*	*T2 MD*	*T3 HD*	*S1HD*	*S2 HD*
Hb (g/dl)	8.56 ± 0.37	8.35 ± 0.32	8.38 ± 0.35	8.55 ± 0.39	8.71 ± 0.51	8.57 ± 0.55
RBC (10^6/mm^3)	7.73 ± 0.22	8.06 ± 0.36*	8.05 ± 0.34	7.8 ± 0.3	8.03 ± 0.44	8.1 ± 0.37*
Platelets (10^3/mm^3)	284.55 ± 4.08	283.1 ± 5.14	283 ± 3.01	284 ± 3.2	284 ± 5.85	280 ± 3.89
WBC (10^3/mm$^{3)}$	10.2 ± 0.44	9.4 ± 0.64	9.85 ± 0.63	9.8 ± 0.6	10 ± 0.57	10.3 ± 0.4
Monocytes (%)	1.11 ± 0.21	1.05 ± 0.19	1.3 ± 0.16	1.4 ± 0.2	1.03 ± 0.06	1.1 ± 0.22
Neutrophils (%)	26 ± 2.94	25 ± 2.49	24 ± 2.85	26.37 ± 2.89	25 ± 1.15	26 ± 3.55
Lymphocytes (%)	56 ± 1.83	61 ± 1.93	58 ± 2.44	57.5 ± 1.91	59 ± 2.33	59 ± 3.67
Eosinophils (%)	2.27 ± 0.3	1.74 ± 0.27	1.65 ± 0.26	1.98 ± 0.26	2.3 ± 0.36	2.32 ± 0.37
Basophils (%)	0.13 ± 0.02	0.1 ± 0.01	0.11 ± 0.01	0.15 ± 0.017	0.14 ± 0.01	0.13 ± 0.00
PCV (%)	44.22 ± 1.22	43 ± 1.19	41.76 ± 0.91	42.91 ± 0.65	41.5 ± 1.25	42 ± 1.22
Male						
Hb (g/dl)	8.3 ± 0.42	8.25 ± 0.28	8.3 ± 0.29	8.3 ± 0.28	8.32 ± 0.48	8.2 ± 0.48
RBC(10^6/mm^3)	8 ± 0.31	8.2 ± 0.32	8.3 ± 0.33	8.3 ± 0.32*	8.16 ± 0.45	7.96 ± 0.34
Platelets (10^3/mm^3)	272.5 ± 3.03	271 ± 5.15	270 ± 4.95	272 ± 4.19	272 ± 4.63	270 ± 5.79
WBC (10^3/mm$^{3)}$	10.1 ± 0.49	10.36 ± 0.57	9.7 ± 0.62	10 ± 0.52	10 ± 0.52	9.86 ± 0.53
Monocytes (%)	1.8 ± 0.15	1.91 ± 0.27	1.9 ± 0.23	1.8 ± 0.23	2.1 ± 0.29	2 ± 0.31
Neutrophils (%)	24 ± 2.1	25.1 ± 2.45	26.2 ± 2.14	25 ± 2.21	25.8 ± 3.18	25.2 ± 3.33
Lymphocytes (%)	64 ± 2.69	65.7 ± 2.7	62 ± 2.12	66.3 ± 3.25	62 ± 2.21	59.6 ± 2.9
Eosinophils (%)	1.3 ± 0.2	1.4 ± 0.25	1.47 ± 0.23	1.2 ± 0.24	1.12 ± 0.34	1.46 ± 0.32
Basophils (%)	0.15 ± 0.01	0.14 ± 0.01	0.11 ± 0.02	0.10 ± 0.04	0.12 ± 0.04	0.14 ± 0.02
PCV (%)	42.56 ± 0.76	42 ± 0.9	40.64 ± 0.96	42 ± 1.22	44.22 ± 1.35	42 ± 0.8

(All values are expressed as Mean ±SEM. *p < 0.05,the significant difference compared to control group using one-way ANOVA continued by Tukey's Multiple comparison test)

Assessment of Anxiety or Depression-like Behavior

The assessment for anxiety or depression-like behavior did not reveal any significant changes in treatment groups compared with the control mentioned in Table 4.

Hematological Parameters

Evaluation of hematological parameters on the 90th day revealed no significant changes. There was a significant increase in RBCs in female mice (T1 LD and S2 HD) & male mice (T3 HD) compared to control. The results are presented in Table 5

Biochemical Analysis of Blood

The biochemical analyses of blood are presented below. There was no significant difference observed in carbohydrate, cholesterol, and protein utilization throughout the study period. There was no significant variation between the treatment and control groups

Table 6: Reports of biochemical Analysis of blood.

Female	*Control*	*T1 LD*	*T2 MD*	*T3 HD*	*S1*	*S2 HD*
Protein	5.92 ± 0.33	6.15 ± 0.27	6.31 ± 0.26	6.15 ± 0.31	5.9 ± 0.55	5.8 ± 0.57
Albumin	3.75 ± 0.21	3.65 ± 0.19	3.81 ± 0.34	3.73 ± 0.19	3.53 ± 0.6	3.3 ± 0.3
Globulin	0.53 ± 0.02	0.53 ± 0.02	0.5 ± 0.03	0.53 ± 0.03	0.51 ± 0.02	0.52 ± 0.03
Glucose	116.8 ± 4.79	121 ± 5.21	120.5 ± 5.02	120.2 ± 4.62	118 ± 5.77	112.5 ± 5.54
BUN	17.01 ± 0.93	18.57 ± 1.07	14.22 ± 0.37	18.01 ± 0.93	15.86 ± 1.04	17 ± 0.7
Creatinine	0.6 ± 0.04	0.58 ± 0.04	0.55 ± 0.04	0.54 ± 0.04	0.57 ± 0.07	0.62 ± 0.05
Bilirubin	0.48 ± 0.05	0.49 ± 0.04	0.49 ± 0.04	0.5 ± 0.03	0.48 ± 0.06	0.52 ± 0.05
Cholesterol	59.33 ± 4.04	62 ± 4.29	56 ± 4.65	59 ± 4.08	53.66 ± 4.7	57.75 ± 5.17
Male						
Protein	5.63 ± 0.35	6.09 ± 0.29	6.4 ± 0.21	6.6 ± 0.25	6.1 ± 0.50	5.84 ± 0.37
Albumin	3.2 ± 0.2	3.15 ± 0.2	3.3 ± 0.2	3.31 ± 0.2	3.14 ± 0.22	3.06 ± 0.12
Globulin	0.5 ± 0.03	0.4 ± 0.02	0.5 ± 0.02	0.51 ± 0.03	0.48 ± 0.04	0.47 ± 0.03
Glucose	122 ± 4.96	120.4 ± 4.84	124.5 ± 4.12	124 ± 4.92	118 ± 5.54	125 ± 5.92
BUN	19.96 ± 1.66	21.24 ± 1.2	22.02 ± 1.14	21.18 ± 1.44	19.18 ± 1.65	19.4 ± 1.24
Creatinine	0.52 ± 0.05	0.49 ± 0.05	0.54 ± 0.03	0.49 ± 0.05	0.49 ± 0.06	0.51 ± 0.06
Bilirubin	0.49 ± 0.07	0.53 ± 0.04	0.53 ± 0.03	0.53 ± 0.03	0.5 ± 0.04	0.53 ± 0.05
Cholesterol	52.2 ± 5.11	53.2 ± 3.12	54.1 ± 2.92	53 ± 4.56	50 ± 5.55	52 ± 5.83

All values are expressed as Mean ±SEM. *p < 0.05, the significant difference compared to control group using one-way ANOVA continued by Tukey's Multiple comparison test.

Table 7: Summary of Individual organ weight (in gms).

		Groups					
Organs	*Gender*	*Control*	*T1 LD*	*T2 MD*	*T3 HD*	*S1 LD*	*S2 HD*
Heart	Female	0.28 ± 0.02	0.27 ± 0.02	0.29 ± 0.01	0.26 ± 0.01	0.28 ± 0.03	0.26 ± 0.02
	Male	0.28 ± 0.02	0.29 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.29 ± 0.02	0.28 ± 0.03
Brain	Female	0.67 ± 0.01	0.66 ± 0.01	0.68 ± 0.01	0.69 ± 0.01	0.68 ± 0.01	0.66 ± 0.03
	Male	0.66 ± 0.01	0.67 ± 0.01	0.66 ± 0.02	0.65 ± 0.02	0.65 ± 0.02	0.68 ± 0.02
Liver	Female	2.4 ± 0.17	2.3 ± 0.17	2.29 ± 0.15	2.51 ± 0.12	2.39 ± 0.11	2.19 ± 0.17
	Male	2.31 ± 0.16	2.43 ± 0.14	2.27 ± 0.11	2.5 ± 0.14	2.29 ± 0.14	2.47 ± 0.18
Kidney	Female	0.38 ± 0.04	0.37 ± 0.03	0.35 ± 0.02	0.36 ± 0.02	0.38 ± 0.05	0.39 ± 0.05
	Male	0.38 ± 0.02	0.39 ± 0.03	0.36 ± 0.03	0.35 ± 0.0	0.37 ± 0.05	0.38 ± 0.04
Spleen	Female	0.26 ± 0.03	0.27 ± 0.01	0.29 ± 0.02	0.28 ± 0.01	0.26 ± 0.02	0.25 ± 0.03
	Male	0.28 ± 0.02	0.27 ± 0.01	0.26 ± 0.02	0.25 ± 0.02	0.29 ± 0.03	0.28 ± 0.03
Uterus	Female	0.36 ± 0.02	0.35 ± 0.02	0.34 ± 0.01	0.38 ± 0.02	0.37 ± 0.03	0.36 ± 0.03

n = 10 (Control, T1LD, T2MD, T3HD) & n = 5 (S1 & S2). All values are expressed as mean ± SEM.
*p < 0.05, the significant difference compared to the control group using one way ANOVA continued by Dunnett Test.

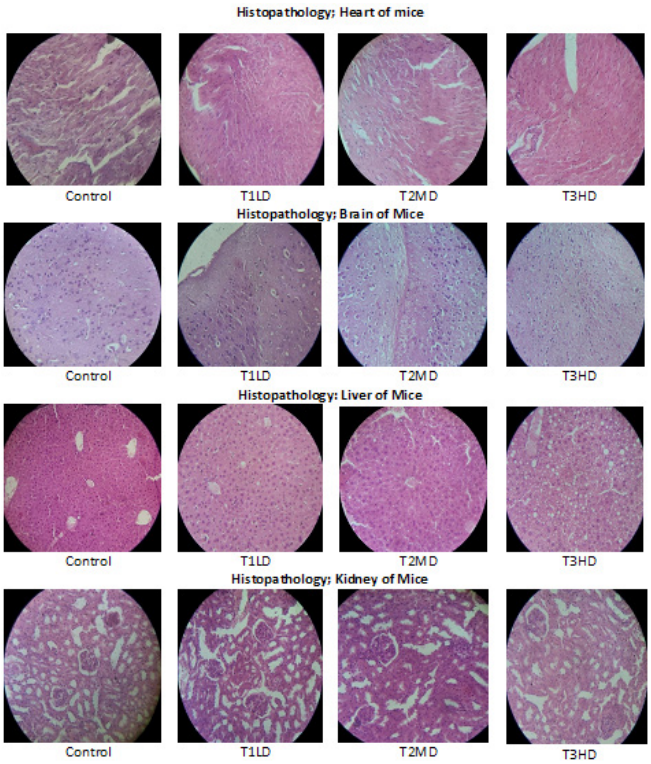

Fig. 2: Histopathology of some organs of mice

in terms of liver and kidney function can also observe through various biochemical parameters in Table 6.

Individual Organ Weight

No significant differences were observed in organ weights of both sexes in treatment groups when compared with the control group mentioned in Table 7. The absence of significant changes in the relative organ weight of primary organs suggests that long-term use of the herbs-mineral formulation did not cause major changes in metabolic processes. The observed changes were incidental findings and, hence, not considered as treatment-related.

Gross Pathology

After completion of the study, all surviving animals were terminally sacrificed and necropsied. External gross examination of all the animals did not reveal any abnormalities. Internal examination of the terminally sacrificed animals of all the groups did not unveil any abnormalities of pathological alteration.

Histopathological Examination

The gold standard for detecting toxicity-related alterations in herbal medications is histopathology examination mentioned in Fig. 2. Microscopic evaluation of the collected organs such as heart, brain, liver, kidney, spleen, and uterus does not reveal any cell structure alteration and possesses normal architecture with no significant pathology compared to control. In comparison to healthy control mice, there were no significant changes in hepatocyte organization or inflammatory or necrotic changes in formulation-treated mice. Furthermore, no granuloma or malignancy was seen in the liver tissues following a sub-chronic oral administration of the different doses of sample formulation.

Conclusion

Administration of the drug at a dose up to 1250 mg T-AYU-HM^{TM} Premium /kg body weight in mice for 90 consecutive days did not reveal any signs of toxicity, either on clinical parameters, laboratory parameters and macroscopic or microscopic observations of internal organs. During the study period, the level of hemoglobin nearly appears maintained. This observation may require attention, especially when the formulation attempt to treat hemoglobin disorder. Therefore, based on the results of this study T-AYU-HM^{TM} Premium was found to be safe on repeat dose administration in NOAEL (No Observed Adverse Effect Level) dose up to 1250 mg T-AYU-HM^{TM} / kg body weight when administered orally for 90 days in both the sexes of Swiss Albino mice.

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