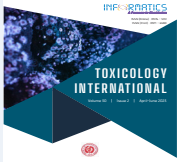
Toxicology International, *30(2) : 233-247; 2023. DOI: 10.18311/ti/2023/v30i2/32299*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.com/index.php/toxi



Safety Evaluation of Arsenicum album in Acute and Sub-Acute Toxicity Studies in Rats

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Abstract

Arsenic album is frequently prescribed in homoeopathy for many diseases. However, its safety data is not available. Thus, the study's purpose is to evaluate the oral toxicity of Arsenic album 6C, 30C, and 200C in rats. Arsenic album (6C, 30C, and 200C) was given at 2000 µl/kg for acute toxicity and observed for up to 14 days. For subacute toxicity, it was given for 28 days and observed for clinical signs, change in body weight and Mortality. Hematological, biochemical, organ weight and histopathological analyses were assessed. Results indicate no mortality of arsenic album in acute toxicity and LD50 is >2000 µl/kg. In the subacute toxicity study, arsenic album (200 µl/kg) did not show any significant changes in above parameters. It may be concluded that the arsenic album (6C, 30C, and 200C) is safe and produces no toxicity when administered orally for a prolonged duration at 200 µl/kg in rats.

Keywords: Complementary and Alternative Medicines, Homoeopathy, Safety, Toxicity

1. Introduction

In alternative medicinal systems, homoeopathy is commonly used by the general public and healthcare professionals worldwide. World Health Organization (WHO) reported that homoeopathy is practised in nearly eighty countries and has a population of about 70-80 %. Most of the countries which are in the developing phase believe in non-conventional therapy for their healthcare purposes. With the increasing use and growth in market demand of homoeopathic medicines, health authorities — and consumers — are quite concerned with their safety[1-4]. Homoeopathic medicines are typically administered at high ultra dilution and are often thought to present no major safety concerns. There is a need to assure the safety and quality of homoeopathic medicines, especially which are toxic, at the international, national, and regional levels[2,5]. There is very little literature available regarding the safety of homoeopathic medicines[6-8].

The homoeopathic remedy Arsenic album (A. album) is a trioxide of arsenic. A. album is well documented in homoeopathic literature to treat digestive disorders, respiratory disorders, disturbance in sleep, food poisoning, anxiety, allergies, depression, and obsessive-compulsive disorder[9]. A. album is also prescribed by physicians for various disorders associated with exhaustion, restless-

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Article Received on: 04.01.2023 Revised on: 13.02.2023 Accepted on: 16.02.2023

ness, anxiety, fear, fright, weakness, septic infection, and low vitality, increase thirst etc. Homoeopathic formulation of A. album has been reported to affect HT29 cells and human macrophages and it decreased NF-κB hyperactivity and decreased TNF-α release in macrophages[10]. Studies have reported that potentized A. album (30C, 200C) ameliorates arsenic-induced toxicity in mice[11,12]. In another *in-vitro* study A. album (30C, 200C) protects MT4 cells against the toxic effects of the arsenic trioxide[13]. In addition, A. album (30C) attenuates oxidative stress markers, DNA damage, and increases cell viability and antioxidant enzymes in Saccharomyces cerevisiae exposed to arsenate[14]. These studies show the anti-inflammatory, antioxidant, anti-cytotoxic, and DNA repair potential of A. album. Thus, it could prove to be an effective therapy for various human diseases. Additionally, during COVID-19 Pandemic, A. album was recommended as prophylactic medicine following the principle of *Genus epidemicus* among the population by the Ministry of AYUSH. However, there is documented evidence that potential homoeopathy drugs that have pharmacological activity have been neglected due to the lack of their safety profile[15].

Although few studies are available regarding the toxicity of A. album in patients and the effect of A. album against arsenic-induced toxicity[12]. However, there are no preclinical studies available regarding the acute and prolonged usage of A. album in healthy animals. Thus, to find out the safety profile of A. albumin (6C, 30C, and 200C) through acute and repeated dose 28-day oral toxicity studies, the present study was designed following Organization for Economic Cooperation and Development (OECD) guidelines with some modifications.

2. Materials and Methods

2.1 Drugs and Chemicals

Homoeopathic formulations, A. album in 6C, 30C, and 200C potencies were prepared following the Homoeopathic Pharmacopoeia of India (HPI) standard and provided by the in-house pharmacy of DDPR-Central Research Institute for Homoeopathy (DDPR-DRIH), CCRH, Ministry of AYUSH, Noida.

2.2 Experimental animals

Wistar albino rats of both sexes (6-8 weeks, 150-200 g) were used in the studies. The animals were purchased from the Central Animal House facility, All India Institute of Medical Sciences (AIIMS), New Delhi. Under standard conditions (25 ± 2 °C, 60–70 % humidity, 12/12 light/dark cycle), animals were housed (3 rats/cage), with free access to food and water. Animals were acclimatized to laboratory conditions for 7 days prior to the start of the experiment. The protocols of the experiment were granted by the Institutional Animal Ethical Committee (DDPR-CRIH/Pharmacology/CPCSEA/IAEC/2021/001), of Dr. DP Rastogi-Central Research Institute for Homoeopathy, Noida. According to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), all the experiments were performed.

2.3 Preparation and Dose Volume Calculation of Medicines

A homoeopathic preparation of A. album 6C, 30C and, 200C was prepared as per standard procedure (HPI) at the in-house pharmacy of DDPR-CRIH, Noida. Briefly, 1 ml of arsenic trioxide salt solution was dissolved in a glycerine and alcohol mixture. 99 ml of dispensing alcohol (90%) was added to this. To prepare potency-I, moderate, equal, and successive 10 machine-operated jerks were given. Similarly, to prepare potency-II, 1 ml of potency-1 was mixed with 99 ml of dispensing alcohol, and again 10 jerks were given. By repetition of the same above process of dilution and successive potentization, the 6th, 30th, and 200th potencies (centesimal dilution) served as Arsenic album (A. album-6C, A. album-30C, and A. album-200C) respectively. Finally, 2 ml of medicine was further diluted with 18 ml of distilled water (1:9 ratio) to prepare the final solution test medicine.

For a single oral dose acute toxicity study, the animals were dosed with A. album (6C, 30C, and 200C) at the dose of 2000 µl/kg for observing the toxic manifestations after the overdosage of the test substance within 24 hours followed by 14 days observation. For the sub-acute toxicity study (as per OECD 407), A. album (6C, 30C, and 200C) at a dose volume of 200 µl/kg body weight diluted with distilled water in a 1:9 ratio, ten times lower than the dose of the acute toxicity study, was administered orally[3].

2.4 Acute Toxicity Study

To study acute toxicity, the procedure mentioned in OECD 423 guidelines[16] was followed with slight modifications. During the procedure, after the fasting period, the female

Wistar rats (n = 3) were weighed and dosed on the same day with respective interventions in a single dose i.e. 2000 µl/kg body weight via an oral route on day 1. The animals were observed for detailed clinical signs at the 0th minute (prior dosing), 30th (±5 min) minute, 1hr (±10 min), 2hr (±10 min), 3hr (±10 min), 4hr (±10 min), and 24hr (±10 min) after the dose administration on day 1 in step 01 and step 02. Careful inspections of animals ensued twice daily during the study period for observing the mortalities if any. Other observations comprised recording body weights on days 1, 7, and 14. Following observations were recorded like general behaviour, respiratory pattern, cardiovascular signs, motor activities, reflexes, changes in skin and fur texture and changes in eyes and mucous membrane, sleep, salivation, convulsion, lethargy, diarrhoea, tremors, morbidity, and mortality. Once a week, body weight was noted down. On 14th day, animals were sacrificed and gross pathological changes were observed in all the organs (abdominal and thoracic cavities).

2.5 Repeated Dose 28-day Oral Toxicity Study

According to OECD 407, guidelines[17] with slight modifications, a repeated dose 28 days oral toxicity study was performed. Wistar albino rats of both sexes were randomly divided into five groups, n = 10/group animals (5 males, 5 females). Group I was named the normal control group and Group II was the vehicle control group. Groups III, IV and V served as the treatment groups. Group I received water, and Group II received dispensing alcohol (90%). Group III, IV, and V received A. album -6C, 30C, and 200C respectively. All the treatments were administered once daily via an oral route through an oral feeding needle attached to a graduated syringe for 28 days. The animals were observed for signs of toxicity, morbidity, and mortality daily for 28 days. After 28 days, half of the animals from all groups left without any treatment and were observed untill the recovery period, i.e., 42 days. On days 29 and 43, blood was collected from the retro-orbital puncture of the rats (overnight fasted) under isofluran for hematological and biochemical evaluations. All the surviving rats were sacrificed on Days 29 (main study animals) and Day 43 (recovery animals), using CO_2 asphyxiation followed by exsanguination. After the drug administration, the animals were monitored for signs of morbidity and mortality throughout the study period. At seven-day intervals, the body weight of the rats was measured. The mean body weight and percentage change in body weight gain were evaluated for all the animals in each group.

2.6 Hematological Analysis

Blood samples were analyzed by using a Vet hematology analyzer- (Model: Spincell-5; Make: M/s Spinreact, Spain). Parameters evaluated include; WBC, Lymphocytes, Monocytes, Neutrophils, Basophils, RBC, Hb, MCV, HCT, MCH, MCHC, and PLT.

2.7 Biochemical Parameters

Serum samples were analyzed using Biochemistry Autoanalyzer (Model number: Integra 400, Make: M/s Rosh Diagnostic India Pvt. Ltd.) for Glucose, Cholesterol, HDL, Urea, BUN, CREAT, T. Bil, D. Bil, SGOT, SGPT, ALP, TP, CA estimation.

2.8 Histopathology Examinations of Organs

The histopathology examination was carried out for the tissues collected from animals of all the groups i.e. brain, kidney, heart, lung, salivary gland, spleen, stomach, thymus, and testis/ovaries). All the collected tissues were fixed using a 10% formalin solution. All the tissues were fixed and cut into 4–5 µm thick sections. The sections were stained using hematoxylin-eosin for the microscopic assessment using a microscope (Nikon Ni-Ti-2-U upright trinocular microscope).

2.9 Statistical Analysis

Mean ± SEM was used to present the results. One-way analysis of variance (ANOVA) was used for data analysis with Dunnett's post hoc test. The results were analyzed by GraphPad Prism version 5.03, San Diego, CA, USA. Significance was measured at values of $p < 0.05$.

3. Results

3.1 Acute Oral Toxicity Study

In the acute oral toxicity study, for the development of any toxic signs or symptoms, all the animals were observed carefully at different time intervals of 0, 30 min, 1, 2, 4, 6, 8, 12 h, and then daily up to 14 days. Mild lethargy and dehydration up to 24 hours of treatment of A. album

(6C, 30C, and 200C) and alcohol (90%) were noticed. However, these symptoms disappeared after 24 hrs and all the animals recovered. Moderate abdominal breathing, vocalization, and dyspnoea were noticed from 30-40 minutes to 4 hours which worsened in 24 hours in females and lead to the death of one animal in each group of Alcohol (90%) and A. album (6C) mg/kg in step 1. In step 2 (the confirmatory step), one animal with the same sign as step 1 died in the vehicle control group. In step 2, no mortality was found in the A. album treatment group. There were no treatment-related changes noticed in feed consumption and mean body weight and mean body weight gain (per cent change in body weight with respect to Day 1) as compared to the normal control group during the observation period of 14 days. In addition, no abnormality were detected (NAD) in gross necropsy examination amongst the groups of study animals (Table 1). The results indicate that the median lethal dose (LD50) of A. album-6C upon a single oral administration as Category 5 (>2000-5000), 30C, and 200C, as Category 5 or Unclassified, as per Globally Harmonized System and greater than 2000 µl/kg bodyweight.

Table 1. Cage side observations in single dose acute toxicity study of A. album in rats

S. No.	Parameters	Step I					Step II				
		Normal control	Vehicle control	A. album 6C	A. album 30C	A. album 200C	Normal control	Vehicle control	A. album 6C	A. album 30C	A. album 200C
1.	**Lacrimation**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
2.	**Salivation**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
3.	**Piloerection**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
4.	**Resipation**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
5.	**Motor activities**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
6.	**Fur**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
7.	**Eyes**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8.	**Lethargy**	Nil	3/3 30 min -24 hrs	3/3 30 min -24 hrs	3/3 30 min -24 hrs	3/3 30 min -24 hrs	Nil	3/3 30 min -72 hrs	3/3 1 hr-2 hrs	3/3 30 min -24 hrs	3/3 30 min -24 hrs
9.	**Vocalization**	Nil	1/3 30 min -24 hrs	1/3 30 min -24 hrs	Nil	Nil	Nil	1/3 30 min -24 hrs	Nil	Nil	Nil
10.	**Tremors**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
11.	**Convulsion**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
12.	**Dehydration**	NAD	1/3 30 min -24 hrs	1/3 30 min -24 hrs	Nil	Nil	Nil	1/3 30 min -24 hrs	Nil	Nil	Nil
13.	**Nasal discharge**	NAD	1/3 30 min -24 hrs	1/3 30 min -24 hrs	Nil	Nil	Nil	1/3 30 min -24 hrs	Nil	Nil	Nil
14.	**Mortality**	0/3	1/3	1/3	0/3	0/3	0/3	1/3	0/3	0/3	0/3
15.	**Food consumption**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
16.	**Water consumption**	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

NAD: No Abnormalities Detected

Table 2. Mortality and clinical sign observations in repeated Dose 28-day oral toxicity study of A. album in rats

Sex	Male					Female				
Treatment	**Normal control**	**Vehicle control**	**A. album 6C**	**A. album 30C**	**A. album 200C**	**Normal control**	**Vehicle control**	**A. album 6C**	**A. album 30C**	**A.album 200C**
Mortality	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Clinical signs	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

NAD: No Abnormalities Detected

3.2 Sub-acute Oral Toxicity Study

The study was conducted for 43 days (28 days for the main study and 43 days for the recovery study) with A. album (6C, 30C, and 200C) 200 µl/kg/day. The results of the study revealed the survival of all the animals in each group (for 28 days in the main study and 43 days in the recovery study). In addition, no abnormal pattern in the animals was observed among the groups. In the case of feed consumption, behaviour pattern, and urinary and faecal excretion, no changes were found in the animals.

3.3 Morphological Alternation

There was no treatment-related mortality noticed in both sexes on regular administration of A. album (6C, 30C, and 200C) for 28 days. Rats of both sexes did not show any visible toxicity signs such as changes in skin, fur, eyes, and mucous membranes in each test group. Further, no behavioural changes including salivation, coma, sleep, lethargy, and diarrhoea were found in these rats during the study period. All the animals were found normal throughout the study period compared to the control group (Table 2).

3.4 Effect on Body Weight

The mean body weight and percentage change in mean body weight with respect to day 1 were recorded weekly for 43 days. During the study period, results have shown a moderate increase in mean body weight in both sexes in the control and treatment groups. Statistically, there were no significant changes in the mean body weight of the control and treatment groups throughout the study. Similarly, there was no significant percentage change in the mean body weight of the control and treatment groups recorded on the 7th, 14th, 21st, 28th, 35th and 43rd day of the study (Figure 1).

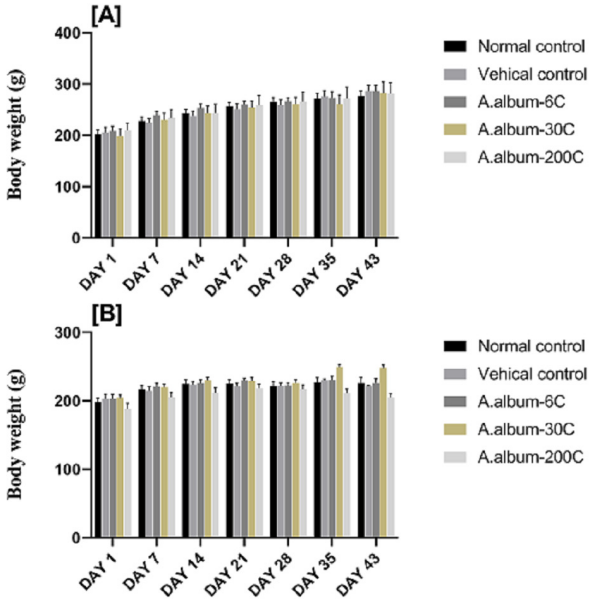


Figure 1. Body weight variation of control and treated male **(A)** and female **(B)** rats during sub-acute toxicity studies. The data is presented as mean ± SEM. One-way ANOVA was used with posthoc Dunnett's post hoc test and the weights of all the groups was compared with their relevant weight on Day 1.

3.5 Effect on Hematological Parameters

The effect of oral administration of A. album (6C, 30C, and 200C) daily for 28 days on hematological parameters was analyzed at the end of the study. The hematological parameters including WBC, RBC, Hb, HCT, MCV, MCHC, MCH, absolute reticulocyte count, reticulocyte count, and differential leucocyte count have shown no significant differences when compared with the control group (Table 3).

Table 3. Effect of A. album (6C, 30C, 200C) on hematological parameters in male and female rats

Sex	Male					Female				
T/t	Normal control	Vehicle Control	A. album-6C	A. album-30C	A. album-200C	Normal control	Vehicle Control	A. album-6C	A. album-30C	A. album-200C
WBC (10^3/µL)	3.44 ±0.32	3.77 ±0.14	3.3 ±0.28	3.78 ±0.31	4.23 ±0.46	1.92 ±0.18	2.28 ±0.18	2.19 ±0.21	2.28 ±0.24	2.03 ±0.25
Abs Lymph (10^3/µL)	2.39 ±0.26	2.41 ±0.21	2.29 .±0.2	2.49 ±0.27	2.78 ±0.31	1.23 ±0.13	1.55 ±0.13	1.22 ±0.11	1.52 ±0.21	1.33 ±0.19
Abs Mono (10^3/µL)	0.59 ±0.13	0.63 ±0.13	0.66 ±0.13	0.79 ±0.19	1.03 ±0.19	0.57 ±0.08	0.47 ±0.08	0.71 ±0.14	0.61 ±0.08	0.46 ±0.08
Abs Neut (10^3/µL)	0.4 ±0.1	0.33 ±0.09	0.32 ±0.09	0.46 ±0.14	0.39 ±0.12	0.12 ±0.03	0.24 ±0.08	0.25 ±0.13	0.14 ±0.02	0.2 ±0.09
Abs Eosin (10^3/µL)	0.05 ±0.02	0.03±0	0.02 ±0.01	0.03 ±0.01	0.03 ±0.02	0.01 ±0.01	0.03 ±0.02	0.01 ±0.01	0±0	0.02 ±0.01
Abs Baso (10^3/µL)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
RBC (10^6/µL)	8.74 ±0.22	8.04 ±0.59	8.06 ±0.27	9.85 ±0.68	9.37 ±0.32	7.91 ±0.31	7.77 ±0.26	7.62 ±0.45	8.69 ±0.4	7.97 ±0.56
HGB (g/dL)	15.26 ±0.28	14 ±0.98	14.46 ±0.42	17.73 ±1.41	17.01 ±0.85	14.85 ±0.63	14.67 ±0.52	14.78 ±0.83	16.59 ±0.66	15.32 ±0.98
HCT (%)	45.95 ±0.88	42.04 ±3.16	42.25 ±1.46	52.67 ±3.9	50.58 ±2.06	42.82 ±1.7	42.02 ±1.6	41.93 ±2.53	47.77 ±1.92	43.8 ±3.1
MCV (fL)	52.66 ±0.46	52.23 ±0.4	52.71 ±0.34	52.09 ±0.83	53.95 ±0.61	54.18 ±0.4	54.11 ±0.5	55.03 ±0.49	55.23 ±0.64	55.04 ±0.51
MCH (pg)	17.38 ±0.17	17.68 ±0.33	17.72 ±0.36	17.94 ±0.33	18.24 ±0.27	18.69 ±0.22	18.85 ±0.25	19.38 ±0.24	19.11 ±0.33	19.27 ±0.24
MCHC (g/dL)	33.09 ±0.12	33.9 ±0.57	33.91 ±0.44	33.7 ±0.28	33.79 ±0.3	34.58 ±0.2	34.91 ±0.33	35.29 ±0.25	34.67 ±0.26	35.07 ±0.26
PLT (10^3/µL)	973.9 ±49.47	877.2 ±77.13	948.7 ±79.43	755.2 ±85.05	892 ±68.62	913.6 ±85.95	795.2 ±69.74	769.4 ±76.76	782.5 ±57.18	735 ±73.37

The data are expressed as the mean and standard error of the mean. For statistical analysis One-way ANOVA with Dunnett's post hoc test was used WBC: White Blood Cell, MCH: Mean Corpuscular Hemoglobin, RBC: Red Blood Cell, MCHC: Mean Corpuscular Hemoglobin Concentration, HGB: Hemoglobin, PLT: Platelets, HCT: Hematocrit, Abs Baso: Absolute Basophils, MCV: Mean Corpuscular Volume, Abs Lymp: Absolute Lymphocyte, Abs Mono: Absolute Monocyte, Abs Eosin: Absolute Eosinophils, Abs Neut: Absolute Neutrophils.

3.6 Clinical Biochemistry

Biochemical parameters such as ALP, SGOT, SGPT, ALT, Urea, BUN, D. Bil., Creatinine, Cholesterol, HDL, LDL, Triglyceride, Serum glucose, Albumin, Total protein were analyzed at the end of the study. The statistical analysis has shown no significant changes in all biochemical parameters in the vehicle and A. album (6C, 30C, and 200C) treated groups compared with the control group (Table 4).

3.7 Effect on Organ Weight

The absolute organ weight (brain, thymus, heart, lungs, liver, stomach, kidney, spleen, testis, and ovaries) was measured during the necropsy and tabulated in Table 5. Results indicate that there was no significant difference in the absolute organ weight of A. album-treated and control group animals.

Table-4. Effect of A. album (6C, 30C, 200C) on biochemical parameters in male and female rats

Sex	Male					Female				
T/t	Normal control	Vehicle Control	A. album-6C	A. album-30C	A. album-200C	Normal control	Vehicle Control	A. album-6C	A. album-30C	A. album-200C
GLU (mg/dL)	91.40 ±2.53	91.30 ±3.83	81.70 ±7.34	89.30 ±6.74	92.30 ±5.72	77.12 ±5.33	76.39 ±5.39	82.63 ±3.25	80.10 ±4.58	81.75 ±3.50
CHOL (mg/dL)	70.20 ±4.05	70.00 ±3.76	64.30 ±5.68	65.80 ±4.69	67.20 ±5.44	63.60 ±3.80	54.30 ±5.01	57.70 ±4.10	63.50 ±7.16	60.80 ±3.35
HDL (mg/dL)	38.85 ±2.53	38.69 ±3.09	33.11 ±1.67	36.13 ±1.30	36.40 ±2.05	41.00 ±2.49	47.75 ±3.43	43.68 ±2.44	44.72 ±2.48	43.97 ±2.26
UREA (mg/dL)	32.40 ±1.80	32.40 ±2.19	26.60 ±2.88	25.30 ±3.22	29.80 ±2.98	34.68 ±1.16	34.63 ±3.76	37.17 ±2.58	34.50 ±1.27	33.81 ±1.37
BUN (mg/dL)	16.40 ±0.87	15.90 ±0.95	15.00 ±0.52	14.60 ±0.91	15.00 ±1.48	17.34 ±0.58	16.15 ±1.56	18.58 ±1.29	20.64 ±0.81	19.41 ±1.04
CREAT (mg/dL)	0.30 ±0.02	0.30 ±0.02	0.32 ±0.01	0.34 ±0.03	0.32 ±0.02	0.37 ±0.02	0.39 ±0.03	0.41 ±0.01	0.40 ±0.01	0.38 ±0.02
T.Bil (mg/dL)	0.07 ±0.01	0.05 ±0.00	0.06 ±0.00	0.06 ±0.01	0.08 ±0.01	0.03 ±0.01	0.03 ±0.00	0.04 ±0.00	0.04 ±0.01	0.05 ±0.01
D Bil (mg/dL)	0.033 ±0.00	0.04 ±0.01	0.03 ±0.01	0.03 ±0.01	0.03 ±0.01	0.03 ±0.00	0.04 ±0.01	0.03 ±0.00	0.03 ±0.01	0.03 ±0.00
SGOT (U/L)	80.43 ±3.92	85 ±4	81 ±3	73 ±5	86 ±4	90.27 ±5.96	95.14 ±6.87	100.14 ±3.95	86.58 ±6.10	101.38 ±7.79
SGPT (U/L)	41.71 ±3.48	42.53 ±2.57	47.50 ±2.81	48.90 ±3.27	51.20 ±2.50	32.74 ±1.79	40.12 ±2.41	35.36 ±3.17	37.90 ±2.52	31.88 ±1.80
ALP (U/L)	206.74 ±6.27	192.24 ±8.59	195.00 ±10.08	181.08 ±6.30	193.06 ±9.21	94.88 ±5.54	98.11 ±8.42	86.93 ±9.24	100.88 ±9.63	104.65 ±11.78
ALB (mg/dL)	4.09 ±0.22	4.03 ±0.15	3.77 ±0.10	3.84 ±0.15	3.63 ±0.12	4.26 ±0.16	4.87 ±0.40	4.65 ±0.16	4.47 ±0.20	4.54 ±0.20
TP (g/dL)	5.3 ±0.5	5.3 ±0.5	5.5 ±0.7	5.2 ±0.6	5.1 ±0.4	6.70 ±0.37	7.23 ±0.32	6.94 ±0.24	7.00 ±0.21	5.66 ±0.76
CA (mg/dL)	9.6 ±0.1	9.4 ±0.2	9.7 ±0.1	10.1 ±0.2	9.7 ±0.3	10.14 ±0.23	10.51 ±0.27	9.65 ±0.21	10.04 ±0.22	10.30 ±0.13

The data are expressed as the mean and standard error of the mean. For statistical analysis, One-way ANOVA with Dunnett's posthoc test was used. GLU: Glucose, URE: Urea, CHOL: Cholesterol, BUN: Blood Urea Nitrogen, CREAT: Creatinine, HDL: High-density lipoprotein, T.Bil: Total Bilirubin, D Bil: Direct Bilirubin, SGOT: Serum glutamic- oxaloacetic transaminase, TP: Total protein, SGPT: Serum glutamic- pyruvic transaminase, CA: Calcium, ALP: Alkaline phosphatase, ALB: Albumin.

3.8 Histopathology

The histopathological evaluation was performed in all organ tissue for each group. In addition, the macroscopic examination did not show inflammation, atrophy, edema, and hypertrophy in any organ of the treatment group compared with the control group. The histopathological examination revealed no treatment pathological changes in the sexes: male (Figures 2 and 3) and female (Figures 4 and 5). The slide image of the histological section of each organ tissue is illustrated from Figure 2 to Figure 5.

Table 5. Effect of A. album (6C, 30C, and 200C) on organ weight in male and female rats

Sex	Male					Female				
T/t	Normal control	Vehicle Control	A. album-6C	A. album-30C	A. album-200C	Normal control	Vehicle Control	A. album-6C	A. album-30C	A. album-200C
Liver	9.58 ±0.62	9.02 ±0.77	9.45 ±0.36	9.11 ±0.39	9.59 ±0.56	7.51 ±0.33	7.71 ±0.37	7.11 ±0.51	7.05 ±0.34	7.08 ±0.33
Kidneys	2.02 ±0.13	1.91 ±0.08	1.97 ±0.08	2 ±0.11	1.99 ±0.18	1.68 ±0.1	1.49 ±0.06	1.49 ±0.08	1.64 ±0.07	1.57 ±0.08
Heart	1.02 ±0.06	1.06 ±0.09	1.01 ±0.044	0.96 ±0.06	0.95 ±0.07	0.92 ±0.03	0.91 ±0.06	0.88 ±0.05	0.98 ±0.08	0.83 ±0.05
Spleen	1.15 ±0.19	2.33 ±1.24	1.05 ±0.07	1.03 ±0.09	1.11 ±0.04	0.88 ±0.07	0.81 ±0.05	0.87 ±0.13	0.85 ±0.06	0.98 ±0.08
Thymus	0.68 ±0.1	0.71 ±0.08	0.82 ±0.11	0.69 ±0.08	0.68 ±0.05	0.73 ±0.12	0.61 ±0.04	0.66 ±0.06	0.52 ±0.03	0.58 ±0.12
Stomach	1.54±0.23	1.7 ±0.12	1.34 ±0.31	1.68 ±0.06	1.88 ±0.09	1.87 ±0.06	1.56 ±0.05	1.62 ±0.09	1.67 ±0.08	1.8 ±0.26
Lungs	1.62 ±0.18	2.08 ±0.11	1.92 ±0.04	1.89 ±0.08	2.3 ±0.3	0.92 ±0.03	1.86 ±0.06	1.69 ±0.13	2.03 ±0.08	1.76 ±0.15
Pancreas	1.08 ±0.19	1.06 ±0.21	1.2 ±0.26	1.01 ±0.2	1.5 ±0.29	1.4 ±0.12	1.15 ±0.08	1.09 ±0.14	1.24 ±0.15	1.08 ±0.15
Brain	1.81 ±0.07	1.81 ±0.06	1.85 ±0.07	1.63 ±0.06	1.87 ±0.08	1.87 ±0.06	1.78 ±0.03	1.88 ±0.03	1.9 ±0.06	1.82 ±0.06
Adrenals	0.06 ±0.01	0.06 ±0.01	0.07 ±0.01	0.05 ±0	0.05 ±0	0.07 ±0.01	0.08 ±0.01	0.07 ±0.01	0.06 ±0	0.07 ±0
Testes / Ovaries	3.01 ±0.19	2.73 ±0.21	2.74 ±0.08	2.83 ±0.11	2.83 ±0.11	0.13 ±0.01	0.14 ±0.02	0.12 ±0.01	0.14 ±0.007	0.15 ±0.01

The data are expressed as the mean and standard error of the mean. For statistical analysis, One-way ANOVA with Dunnett's posthoc test was used.

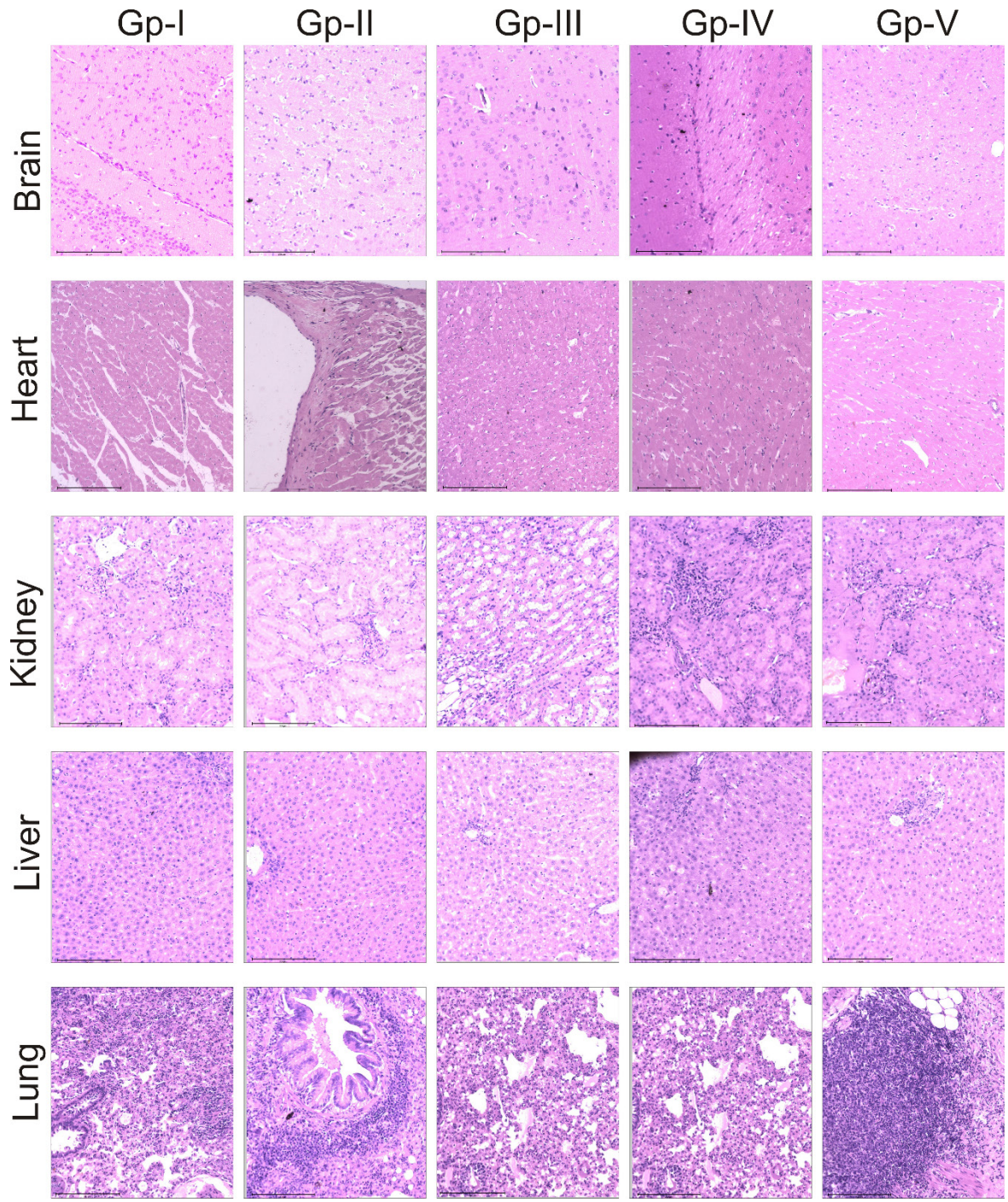


Figure 2. Histological examinations of brain, heart, kidney, liver, and lung from representative male rats of different groups with magnification 20x. Gp-I: Normal control; Gp-II: Vehicle control; Gp-III: A. album-6C; Gp-IV: A. album-30C; Gp-V: A. album-200C.

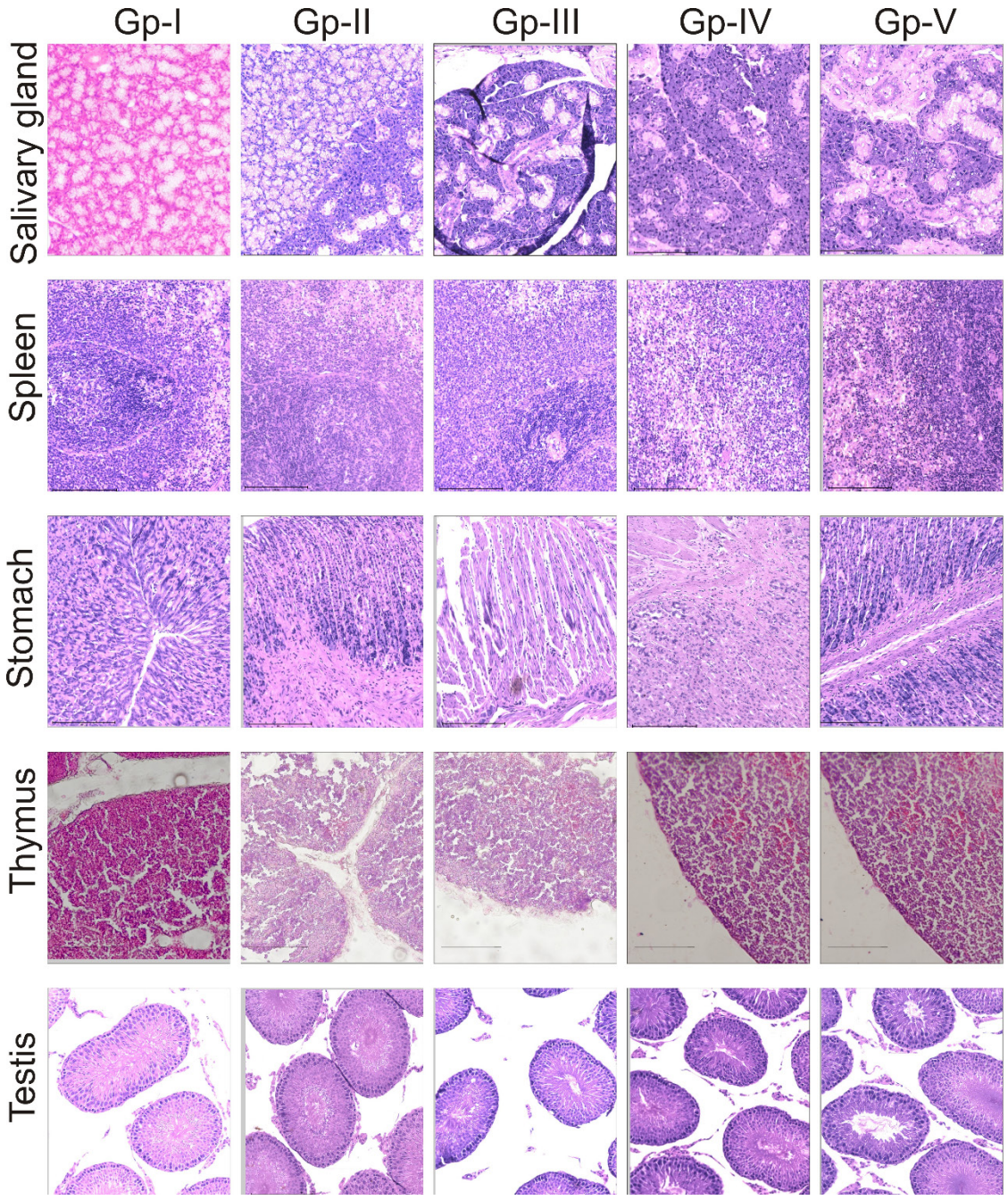


Figure 3. Histological examinations of the salivary gland, spleen, stomach, thymus, and testis from representative male rats of different groups with magnification 20x. Gp-I: Normal control; Gp-II: Vehicle control; Gp-III: A. album-6C; Gp-IV: A. album-30C; Gp-V: A. album-200C.

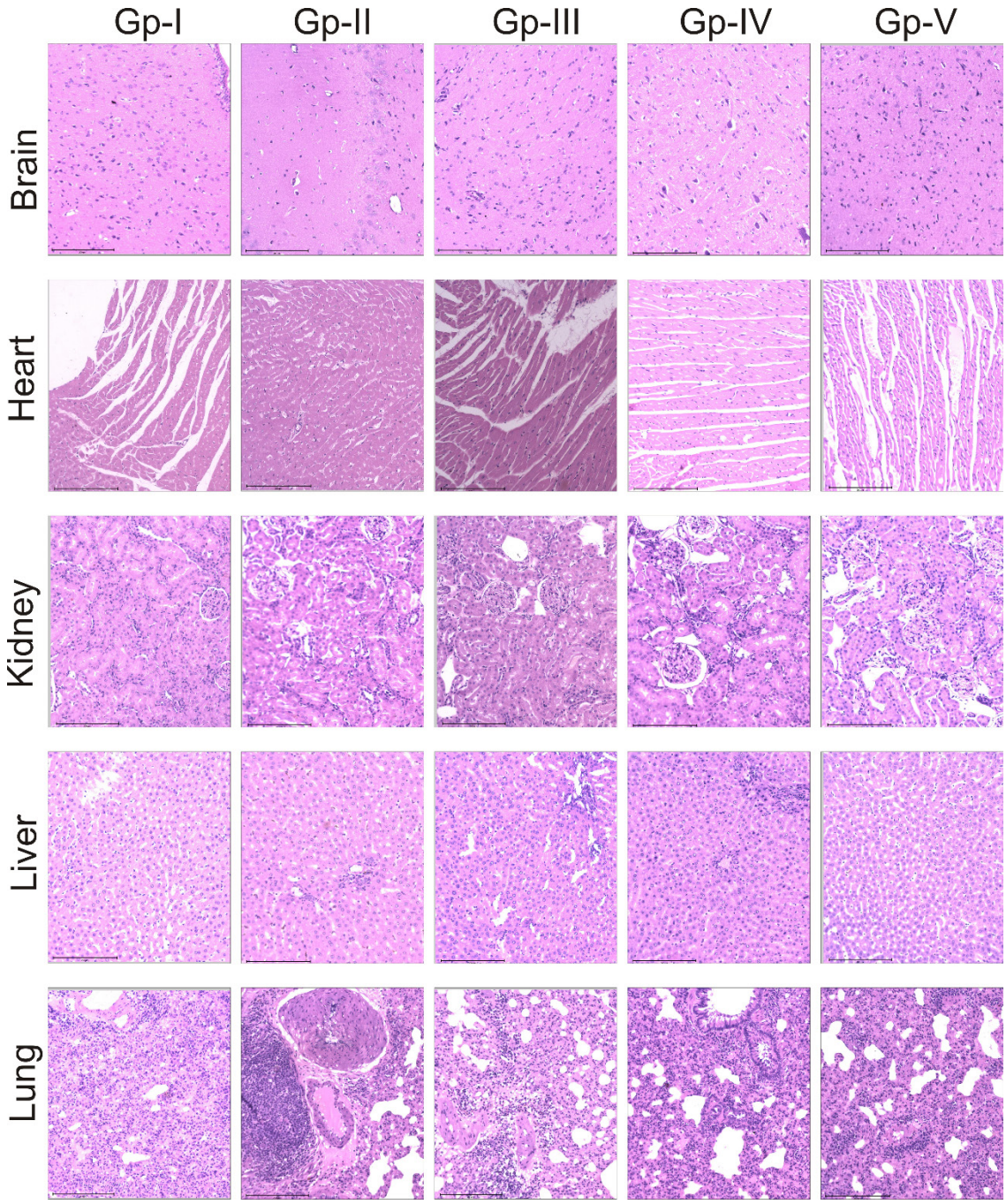


Figure 4. Histological examinations of brain, heart, kidney, liver, and lung from representative female rats of different groups with magnification 20x. Gp-I: Normal control; Gp-II: Vehicle control; Gp-III: A. album-6C; Gp-IV: A. album-30C; Gp-V: A. album-200C.

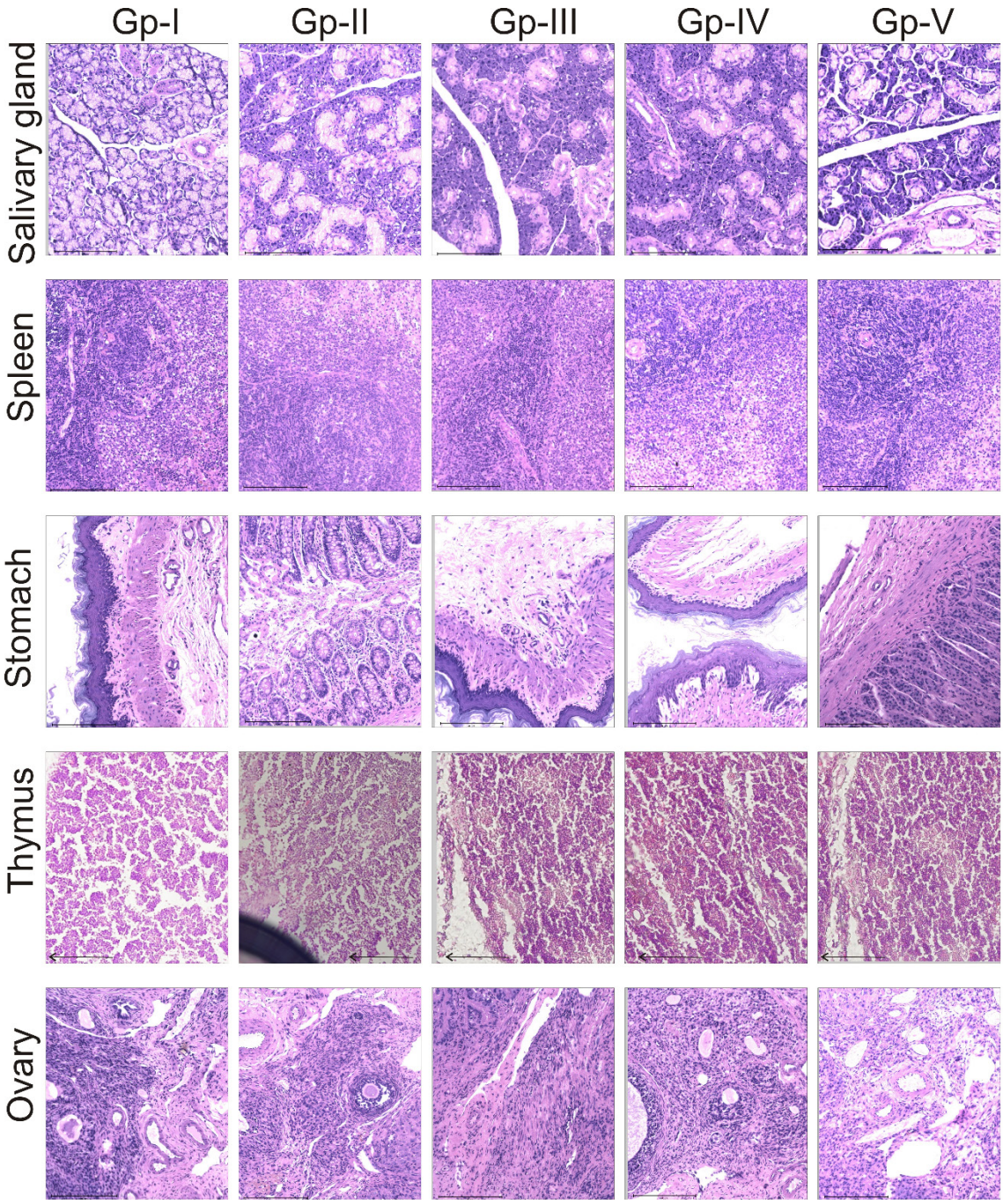


Figure 5. Histological examinations of salivary gland, spleen, stomach, thymus, and ovary from representative female rats of different groups with magnification 20x. Gp-I: Normal control; Gp-II: Vehicle control; Gp-III: A. album-6C; Gp-IV: A. album-30C; Gp-V: A.album-200C.

4. Discussion

In the present study, an acute oral toxicity study of A. album (6C, 30C, 200C) was conducted in Wistar albino rats as per the OECD 423 guideline[16] with slight modifications. It was used to estimate the LD50 range according to GHS classification. A. album (6C, 30C, 200C) was administered at a dose of 2000 µl/kg on day 1, and animals were observed for 14 days. During the experiment, mild lethargy, and dehydration was noticed up to 24 hours of treatment of A. album (6C, 30C, 200C) and alcohol (90%). However, these symptoms disappeared after 24 hrs and all the animals recovered. Moderate abdominal breathing, vocalization, and dyspnoea were noticed from 30-40 minutes to 4 hours in some animals which worsens in 24 hours in females and led to the death of animals at 2000 µl/kg of A. album (6C) and alcohol (90%). However, the clinical signs and mortality observed were not related to the drug as these symptoms and mortality were visible in the vehicle control group also suggested that the observed clinical signs and mortality were due to a high concentration of alcohol i.e. 90% dispensing alcohol. There was no drug-related mortality observed in animals after the treatment of A. albumin and its potencies in both steps.

Body weight, and percentage body weight changes (change in body weight with respect to Day 1) for all the animals in either step did not reveal any treatment-related effect throughout the experiment when evaluated statistically. The gross necropsy examination conducted on day 15 of step 01 and step 02 revealed no abnormal changes in either the external surface of the body or internal organs observed. In the present investigation, the animals were administered ten times the dose used clinically i.e. 2000 µl/kg bodyweight for investigating the toxic manifestations due to the over dosage of the test substance within 24 hours. As a result of an implicit assumption, we concluded that the clinical symptoms and mortality observed could be attributed to the vehicle (alcohol) rather than the medicine itself. It is so because the clinical signs and mortality were commonly witnessed in vehicle control (alcohol) as well as in all the treatment groups (6C, 30C, and 200C). It is reasonable to conclude that there were no toxic signs or symptoms as a result of the greater dose, which is reinforced by the fact that no mortality was observed due to treatment during the experiment.

A repeated dose 28-day oral toxicity study was performed in Wistar albino rats in accordance with OECD 407[17] with slight modifications to investigate the repeated treatment effect of A. album on potential targets of toxicity. In the present study, we evaluated the effect of A. album (6C, 30C, and 200C) 28-day repeated exposure on Body weight, feed consumption, hematology, biochemistry and organ histology. There are no treatment-related changes in behavioural and clinical signs such as gait, fur colour, eating, sleeping secretions excretions, and autonomic activity found during the experiment. The body weight change is a sensitive marker of the overall health of an animal. According to OECD and Canadian Council on Animal Care (CCAC) guidelines, more than 20 % loss of body weight was considered one of the human endpoints of the study[18,19]. The body weight change depends on feed consumption, feed palatability, feed nutritional content, digestion, and absorption[20]. The body weight, relative body weight, and feed consumption recorded on days 1st, 7th, 14th, 21st and 28th were found to be consistent throughout the study in A. album-treated groups and normal control.

Hematological parameters are important diagnostic tools in clinical settings to evaluate the toxic effect of chemical and herbal substances[21]. Hematological parameters play a pivotal role in monitoring the functioning of bone marrow and the intravascular system because bone marrow is highly sensitive to toxic substances[22]. Moreover, studies have shown that hematological alterations in rodents were found to be predictive of toxicity in humans as supported by data extrapolated from animal studies[23]. In our study, we found no treatment-related changes in the hematological parameters compared to normal control animals. The non-significant changes in blood parameters suggest that A. album (6C, 30C, and 200C) is non-toxic to hematological parameters.

In toxicological studies, clinical biochemistry plays a major role in assessing the toxic potential of the test substance. Serum biochemical parameters (Glucose, Total proteins, Creatinine, ALP, SGOT, SGPT, Urea, BUN, Cholesterol, HDL, LDL) are important in evaluating the functioning of the liver and kidney as they are essential organs for survival, metabolizing, and detoxifying chemicals and drugs[24]. The liver is the main organ involved in drug metabolism, cholesterol synthesis, and degradation. In addition, it has a role in glucose synthesis and generates glucose from hepatic glycogen stores[25]. In the present study, results indicate that A. album treatment did not cause any effect on lipid and carbohydrate metabolism. Serum ALP, SGOT, and SGPT were assessed in toxicity studies to track liver function. Increased levels of ALP,

SGPT and SGOT are an indicator of hepatic injury, cirrhosis and myocardial infarction[25,26]. The levels of ALP, SGPT and SGOT in our study found to be similar in A. album-treated and control group animals illustrating that A. album is not causing any liver toxicity. Total bilirubin an indicator of liver function and cholestasis is a byproduct of heam catabolism. It is found reduced in conditions such as aplastic anemia[27]. During the study levels of total bilirubin and BUN are not significantly different in the arsenic treatment and control group. Studies have shown that BUN is a diagnostic marker for assessing renal function in clinical settings[28]. Moreover, the level of urea and creatinine which are indicators of glomerular filtration rate for assessing renal function found to be non-significant in arsenic-treated groups compared with normal control. These results imply that A. album (6C, 30C, and 200C) does not influence renal function.

Toxic substances cause an array of metabolic reactions in vital organs (brain, liver, lung, stomach, kidney, kidney, spleen, thymus, heart, testis, and ovary) resulting in a change in colour and architecture, hypertrophy, edema considered as early signs of toxicity[29]. Macroscopic examination of animal organs during necropsy of A. album-treated animals in both studies (acute and sub-acute toxicity) did not reveal any lesions, hypertrophy, change in colour, or texture change, compared with normal control. In addition, organ weight is a significant indicator of the physiological and pathological condition of animals. Absolute organ weight and relative organ weight are fundamental markers determining any treatment-related injury in internal organs[30]. Since there was no significant change in absolute and relative organ weight of internal organs of treatment groups was observed in the study compared with control group animals. Hence, A. album treatment neither interfered with physiology nor produced a pathological change in animals.

5. Conclusions

Based on the results of this study, in an acute oral toxicity study, no mortality at a dose of 2000 µl/kg of A. album which indicates that oral LD50 of A. album (6C, 30C, and 200C) is greater than 2000 µl/kg. Repeated administration of A. album (6C, 30C, and 200C) at the dose of 200 µl/kg p.o. was found safe in Wistar albino rats. The No Observed Adverse Effect Level (NOAEL) of A. album (6C, 30C, and 200C) after 28 consecutive days of oral administration in Wistar albino rats were found to be not less than 200 µl/kg/day. No organ-related toxicity was found after the repeated oral administration of A. album. Additionally, no treatment-related histopathological changes were noticed in both the sex at 200 µl/kg/day of A. album (6C, 30C, and 200C). Therefore, it may be concluded that there is no hazardous property of homoeopathic preparation of A. album (6C, 30C, and 200C), and found safe at the dose of 200 µl/kg p.o.

6. Acknowledgements

We are thankful to ILBS for histopathology, and to the officer in charge, DDPR-CRIH, Noida for providing administrative support.

7. References

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