



28 DAYS REPEATED DOSE ORAL TOXICITY STUDY OF HYDROALCOHOLIC EXTACT OF SESBANIA SESBAN LEAVES IN EXPERIMENTAL RATS

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ABSTRACT

Natural medication is the hotspot for the inquiry of numerous novel helpful mixes in agricultural nations. Prior to utilized as medication, drugs from plant cause must be guaranteed safe. The study is aimed at evaluating the possible toxicity in 28-day subacute oral toxicity of hydroalcoholic extract *Sesbania sesban* (*S.sesban*) in male and female Wistar rats. The 28-day subacute poisonousness study was directed to recognize the no-watched unfriendly impact level (NOAEL). In this investigation, a sum of 48 rodents were isolated into the control, low dose (200 mg/kg), medium dose (500 mg/kg) and high dose (1000 mg/kg) gatherings. The HAESS was given daily from day 1 until day 28. At the last day of the study, the animals were humanely sacrificed and assessed for the effect extract of *Sesbania sesban* leaves on body weight and relative organ weights and hematological, biochemical and histopathological parameters.

KEYWORDS: subacute oral toxicity *Sesbania sesban*, biochemical analysis, hematological parameters, histopathology.

INTRODUCTION

Herbal medicine is the source for the search of many novel therapeutic compounds in developing countries. Before used as medicine, drugs from plant origin must be ensured safe.^[1] Plant-derived medicines are used in all civilizations and cultures and, hence, plants have always played a key role in health care systems worldwide. In most developing countries, the indigenous methods of home grown treatment are an aspect of the way of life and the prevailing strategy for recuperating treatment. These remedies, with impressive degree of adequacy, are socially acknowledged, financially reasonable and, generally, are the main accessible source. This raises concerns about the potential toxic effect resulting from chronic use of such medicinal plants. Consequently, assessing the toxicological impacts of any therapeutic plant extricate expected to be utilized clinically or preclinically, is a significant aspect of its appraisal of possible poisonous impacts.^[2] Recently, increasing interest in herbal medicines is the belief that because these medicines are natural and have been traditionally used, they are safe and harmless. *Sesbania sesban* Linn. consist of dried bark of the plant *Sesbania Sesban* Linn. (Fabaceae) is found all through the fields of India and generally called as Jayanti (SANS) and Shevri (MAR).^[3] The plant is inreached with full of medicinal uses. According to ethno medicinal claims the poultice of leaves of *Sesban* Linn. Advances festering of bubbles

and abscesses and retention of provocative rheumatic swellings. Juice of new leaves is credited with Anthelmintic properties.^[4] However, no studies on the toxicity of *S. sesban* leaves have been described in the literature. In this way, in the current examination, we intended to research the toxicity (oral subacute) of *S. sesban* leaves so as to expand the trust in their security to people to treat different illnesses.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The part of plant *S sesban* is leaves were collected from georai, district of Beed, Maharashtra and authenticated by M/s. shamantak enterprises, pune. Certificate of Aunthentication number of *Sesbania sesban* is SE/AC/ 2019/05.

Preparation of Plant Extract

The leaves of *S. sesban* were collected from the mature plants, shade dried and powdered (80mesh). The powdered leaves (2000g) was defatted with petroleum ether and later extracted (soxhlet) using 90% ethanol and water. The hydroalcoholic extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50° C to get a solid residue. Different concentration (200mg/kg and 400 mg/kg p.o.) of hydroalcoholic extract of leaves of *S. sesban* was given according to body weight of animals.

Experimental Animal

Wister rats of either sex weighing 200–250 g was used for the subacute toxicology studies. The rats were maintained at a room temperature of 22–24 °C, with a 12 h light/dark cycle and humidity around (50 ± 5) %. During acclimatization, the rodents were randomized into test and control gatherings and housed independently in sterilized confines housed with sterile paddy husk as bedding. The animals had free access to food and water ad libitum throughout study. All experimental procedures were in compliance with the Institutional Animal Ethical Committee approved protocol (CPCSEA/IAEC/PC08/01-2K19).

Subacute Oral Toxicity

Study Subacute oral toxicity study was performed according to the Organization of Economic Co-Operation and Development (OECD) guideline 407 for testing of chemicals and World Health Organization guideline.^[5,6] Wistar rats of male and females was divided randomly into 4 groups (n=12; six males and six females for every gathering), and their loads were recorded. The standardized hydroalcoholic extract of *Sesbania sesban* prepared in distilled water was administered orally and daily for 28 days in single doses of 200 mg/kg (group 1), 500 mg/kg (group 2) and 1000 mg/kg (group 3) body weight. The control rats (group 4) received only vehicle (distilled water). Harmful indications and mortality were watched every day for 28 days. At the end of each week, the body weights of the apparent multitude of rodents were recorded. At the end of the 28 days of administration, all of the rats were given anesthesia under CO₂ inhalation, and blood tests were gathered via cardiac puncture into both non-heparinized and EDTA-containing tubes for biochemical and hematological analyses, respectively. The rats were sacrificed by clavicle dislocation. The vital organs like liver, kidney, heart and lungs were then fixed in 10% formalin for histopathological study.^[12]

Relative organ weight

The previously mentioned organs were immediately eliminated and weighed independently. Every organ to body weight proportion (relative organ weight) was determined as (weight of organ/body weight of rat upon the arrival of penance)*100%.^[7]

Blood Sampling

Blood samples were collected using retro orbital route of blood withdrawal. Blood was divided into two parts; one part was collected in plain bulbs (non EDTA), while second part was collected in EDTA bulbs. The blood samples were subsequently centrifuged at 3000 rpm for 20 min using bench centrifuge (Remi Laboratory Instruments, India) to obtain serum and plasma. The serum and plasma were separated, with the serum transferred into fresh plain sample Eppendorf tubes. Hematological and biochemical analyses were performed.

Hematological Analyses

Complete blood cell counts were evaluated measuring red blood cells (RBCs) count, hemoglobin concentration (Hb), hematocrit or packed cells volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBCs) count, platelets (PLT), Neutrophils (N %), Eosinophils (E %), Lymphocytes (L %) and monocytes (M %). Following hematological determinations were carried out using Nihon Cohden Celltac alpha.

Biochemical Analyses

The biochemical analyses carried out included measurement of liver functions such as aspartate aminotransferase (AST) alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, renal function markers (urea, nitrogen urea and creatinine) and protein profile (albumin and total protein). Following biochemical parameters were performed using Chariot Prince Biochemistry Analyzer.

Histopathological Examination

Liver, heart, Liver, kidneys, heart and lungs extracted from every treatment bunch were exposed to histopathological assessments. In the wake of fixing the tissues in 10 % formalin, they were dried out and mounted in paraffin blocks and lungs extracted from every treatment bunch were exposed to histopathological assessments. In the wake of fixing the tissues in 10 % formalin, they were dried out and mounted in paraffin blocks. The sections of 3-5 µ thickness were cut and stained with hematoxylin-eosin stain.

Statistical Analysis

Statistical analysis was carried out using the GraphPad Instat 3. All of the data are shown as the mean ± standard error of the mean (S.E.M) and were analyzed using oneway analysis of variance (ANOVA). Significant differences between the control and experimental groups were determined using Tukey – Kramer's all comparison test, P <0.05 was considered significant.

RESULTS

Subacute toxicity study

The subacute toxicity study of the *S. sesban* extract was determined as per OECD guideline 407. All study animals were given *S. sesban* extract daily at doses 200, 500 and 1000 mg/kg po. All the animals survived the entire 28 – day period. No signs of toxicity were observed in the extract treated group compared to control group.

Effects of *S. sesban* extract on food and water intakes

Table 1 depicts the effect of the *S. sesban* on the food and water intake in subacute treatment. The single daily administration of the extract at doses 200, 500 and 1000 mg/kg for 28 days have no significant changes (p>0.05) in food and water intakes per animal when compared with control group.

Table 1: Effect of S. sesban extract on food and water intake per animal.

Treatmene	Sex	Average food intake (g/d)	Average water intake(ml/d)
Control	Female	11.57±1.53	17.64±2.45
	Male	16.38±1.77	20.75±4.11
200 mg/kg S.sesban	Female	11.65±1.94	16.82±1.61
	Male	17.68±0.86	21.21±3.47
500 mg/kg S.sesban	Female	11.55±2.21	16.54±5.43
	Male	17.32±1.76	21.58±2.29
1000 mg/kg S.sesban	Female	12.09±0.88	17.37±0.73
	Male	17.80±1.06	20.52±6.24

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. *p<0.05.

Effects of S. sesban extract on the relative organ weights

Daily administration of S. sesban for 28 days did not cause any significant alteration (p<0.05) in organ

weights in the experimental groups relative to control (Table 2). The results revealed that the vital organs such as liver, kidney, heart and lungs were not adversely affected throughout the treatment period.

Table 2: Effect of S.sesban extract on relative organ weight.

Group	Heart	Liver	Lungs	Kidneys
Male				
Control	0.52±0.04	3.10±1.72	0.31±0.08	0.75±0.10
200mg/kg S. sesban	0.48±0.08	2.97±1.23	0.23±0.05	0.81±0.05
500Mg/kg S.sesban	0.48±0.03	2.66±1.14	0.30±0.12	0.77±0.09
1000Mg/kg S. Sesban	0.53±0.09	2.95±1.67	0.25±0.07	0.76±0.11
Female				
Control	0.48±0.08	3.16±1.05	0.34±0.10	0.762±0.17
200Mg/kg S.sesban	0.43±0.09	2.63±1.64	0.34±0.12	0.68±0.12
500Mg/kg S.sesban	0.53±0.11	2.76±1.87	0.31±0.08	0.75±0.09
1000Mg/kg S.sesban	0.48±0.14	2.85±0.94	0.28±0.06	0.72±0.08

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. *p>0.05.

Effect of S. sesban extract on body weight

Table 3 shows the body weight of rats before, during and after the treatment. The daily oral administration of S.

sesban extract at all doses 200, 500 and 1000 mg/kg for 28 days did.

Table 3: Effect of S. sesban extract on the body weights (g).

Group	Day 0	Day14	Day 28
Male			
Control	162.08±13.78	162.70±13.77	163.83±14.03
200 mg/kg S sesban	195.66±11.43	202.16±11.76	206.5±13.25
500 mg/kg S.sesban	263.66±11.15	274.33±9.92*	280±9.48*
1000mg/kg S. sesban	276±10.87	255.66±24.29*	273.66±11.87*
Female			
Control	164.87±10.60	164.87±10.60	166.5±10.72
200 mg/kg S.sesban	193±8.52	195.66±7.03*	205.83±4.41*
500 mg/kg S.sesban	190.33±3.51	194.16±3.28*	202.66±3.57*
1000 mg/kg S.sesban	238.5±7.01	243±8.06*	244.33±8.46*

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. *p>0.05.

Effect of S. sesban extract on hematological parameters
The effects of the subacute oral administration of the S.

sesban extract on hematological parameters are represented in Table 4.

Table 4: Effect of *S. sesban* extract on the hematological parameters.

Parameter	Control	200 mg/kg	500 mg/kg	1000mg/kg
Male				
Hb (gm%)	15.85±1.58	18.025±1.29	18.52±0.39	26.85±07*
RBC(x10 ³ /cm ²)	8.61±0.17	7.20±0.04	6.84±0.132*	6.47±0.19**
WBC(x10 ³ /cm ²)	15.6±0.34	15.7±0.17	14.95±0.59*	17.35±0.15
PLT (x 105/cm2)	46.52±0.28	44.85±0.37*	44.77±0.20*	45.17±0.43**
PCV (%)	57.27±0.67	57.15±0.11	57.55±0.37	55.02±0.48
MCV (fl)	55.71±0.30	34.67±0.80*	35.17±0.99*	35.52±0.96*
MCH (pg)	17.33±0.41	19.05±0.32	17.77±0.16	15.54±0.36
MCHC (gm/dl)	623.75±14.07	655.5±14.25	730.75±4.88*	677.5±8.12
Neutrophils (N%)	15.52±0.36	22.97±0.24*	18.55±0.68*	24.55±0.51*
Eosinophils (E %)	9.48±0.17	7.02±0.17	6.87±0.05*	6.62±0.11*
Lymphocytes (L%)	16.9±0.14	12.7±0.09*	42.3±0.40*	16.07±0.23
Monocytes(M%)	41.97±0.33	43.15±0.23	43.3±0.40*	43.47±0.47
Female				
Hb (gm%)	19.25±2.49	23.27±1.78	17.2±1.12	22.92±0.41
RBC(x10 ³ /cm ²)	7.93±0.09	7.76±0.08	6.32±0.11*	6.85±0.04
WBC(x10 ³ /cm ²)	10.12±1.26	14.1±0.38	14.15±0.35	14.25±0.26
PLT (x 105/cm2)	42.42±0.35	38.87±0.67	36.07±0.53*	36.7±0.84*
PCV (%)	57.8±0.18	57.5±0.38	58.02±0.28*	54.92±0.20*
MCV (fl)	48.8±0.52	36.6±0.29*	36.12±1.00*	35.8±0.24*
MCH (pg)	18.55±0.39	21.3±0.28	18.55±0.21	21.02±0.08
MCHC (gm/dl)	665.75±24.28	668.5±21.74*	666.75±32.95	678.5±11.89
Neutrophils (N%)	16.3±0.27	14.9±0.55	15.45±1.24*	16.5±0.65*
Eosinophils (E %)	7.56±0.23	6.47±0.08*	6.35±0.18*	6.65±0.09*
Lymphocytes (L%)	11.12±0.34	13.76±0.30*	16.1±0.31	13.67±0.11*
Monocytes(M%)	38.35±0.45	39±0.73	36.75±0.37*	39.52±0.38

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. *p>0.05.

Effect of *S. sesban* extract on biochemical parameters *S. sesban* extract on biochemical parameters are
The effects of the subacute oral administration of the represented in Table 5.

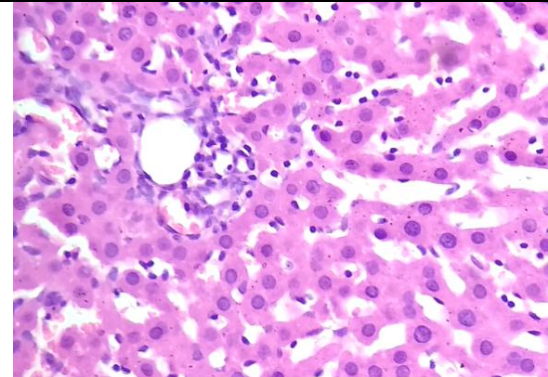
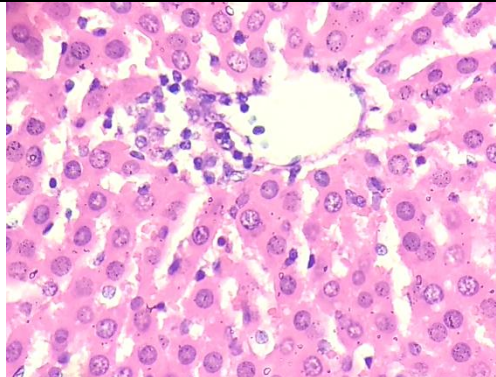
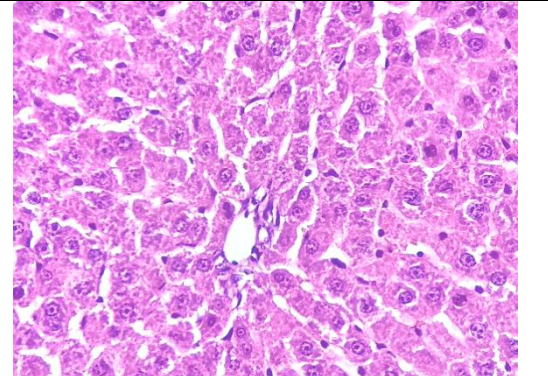
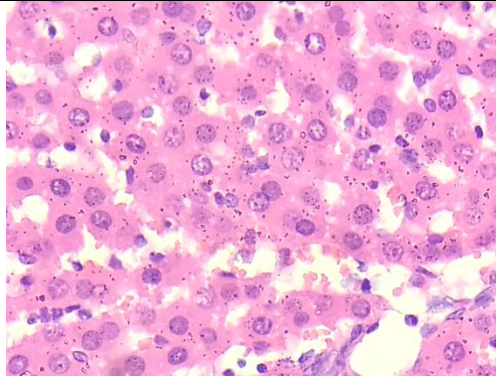
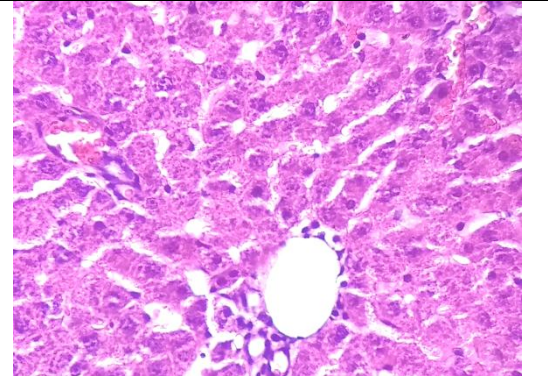
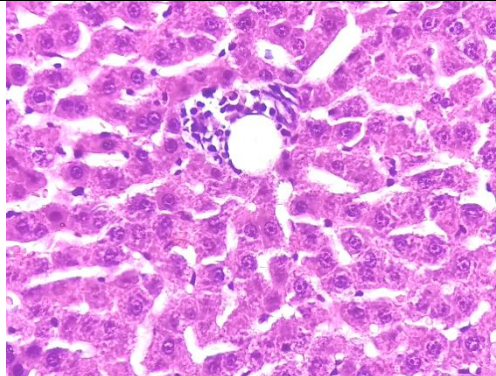
Table 5: Effect of *S. sesban* extract on the biochemical parameters.

Parameter	Control	200 Mg/kg	500 mg/kg	1000mg/kg
Male				
Urea (mmol/L)	7.22±0.20	5.31±0.27	6.91±0.46	5.123±0.18*
Urea Nitrogen (mmol/L)	8.61±0.23	5.44±0.22*	5.04±0.50*	5.06±0.28*
Creatinine (μmol/L)	43.67±1.17	42.11±1.44	44.37±2.13	44.45±0.59
Total protein (g/L)	65.81±1.01	74.20±1.59	91.345±4.54*	85.52±2.38*
Albumin (g/L)	36.6±1.19	27.66±0.99*	32.95±0.91*	32.94±0.09
ALP (U/L)	256.46±6.37	285.14±0.29	311.56±5.11	295.89±10.98
AST (U/L)	124.22±5.86	118.97±6.02	126.65±4.52	123.58±9.26
ALT (U/L)	52.20±1.22	53.39±0.87	48.32±1.20	55.84±0.73
Bilirubin (Total) (mg/dl)	0.52±0.04	0.41±0.38	0.43±0.03	0.44±0.05
Female				
Urea (mmol/L)	7.22±0.20	6.78±0.17	6.05±0.46	6.32±0.31
Urea Nitrogen (mmol/L)	8.61±0.23	6.79±0.30*	5.88±0.35*	5.53±0.21*
Creatinine (μmol/L)	45.67±1.17	42.44±1.77	43.33±2.40	48.77±1.14
Total protein (g/L)	64.81±1.01	77.30±3.38	82.48±3.96*	87.00±3.23*
Albumin (g/L)	31.6±1.19	33.84±1.31	32.85±1.14	35.45±0.72
ALP (U/L)	266.46±6.37	328.89±13.70*	291.30±18.39	237.82±9.82
AST (U/L)	124.22±5.86	126.15±7.39	116.97±4.19	131.94±5.99
ALT (U/L)	51.20±1.22	52.00±1.43	55.29±0.73	55.60±0.72
Bilirubin (Total) (mg/dl)	0.51±0.04	0.42±0.03	0.46±0.03	0.63±0.04

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. *p>0.05

Histopathological Examination

Liver: Microscopic examination of liver showed minimal to mild focal hepatocellular infiltration of inflammatory cells in both male and female animals treated with extract at 500 and 1000 mg./kg body weight when compared with control group (Figure 1).

	
Control; Male; Liver; Showing normal periportal hepatocytes. 40 X, H & E	Control; Female; Liver; Showing normal Centralobular hepatocytes. 40 X, H & E
	
Low Dose; Male; Liver; Showing normal Centralobular hepatocytes. 40 X, H & E	Low Dose; Female; Liver; Showing normal Centralobular hepatocytes. 40 X, H & E
	
Medium Dose; Male; Liver; Showing mild sinusoidal dilation with infiltration of inflammatory cell (Minimal). 40 X, H & E	Medium Dose; Female; Liver; Showing mild sinusoidal dilation with infiltration of inflammatory cell (Minimal). 40 X, H & E

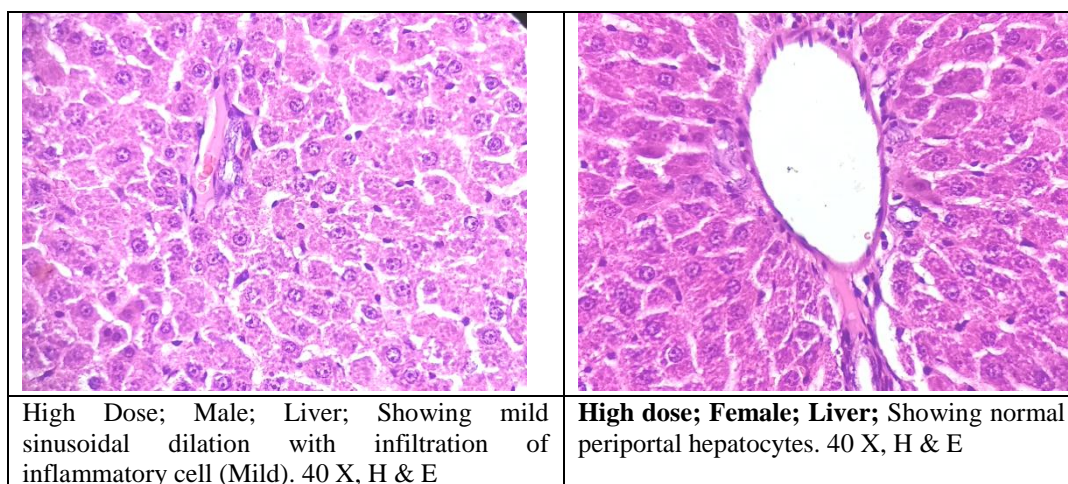
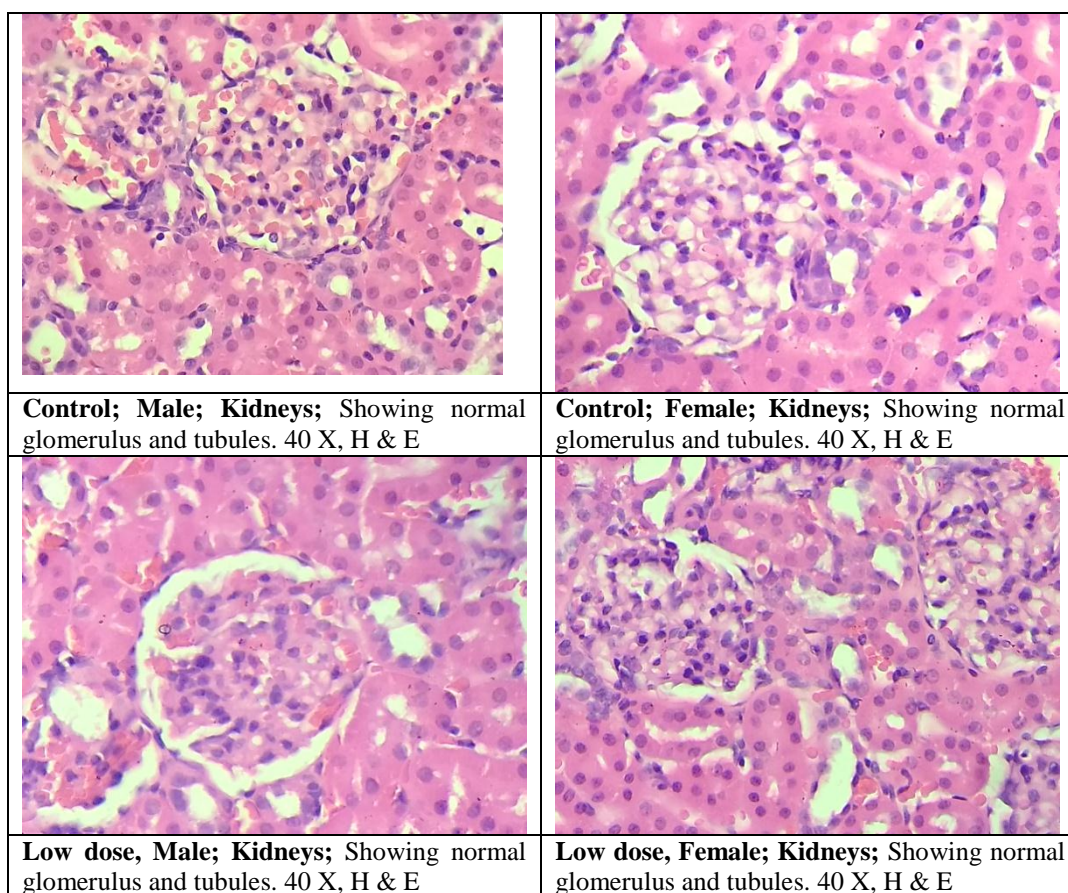


Fig. 1: Histopathological examination of Liver of rats in subacute oral toxicity study.

Kidney: Microscopic examination of Kidneys from both male and female of all groups did not show any lesion of

pathological significance when compared with respective control group (Figure 2).



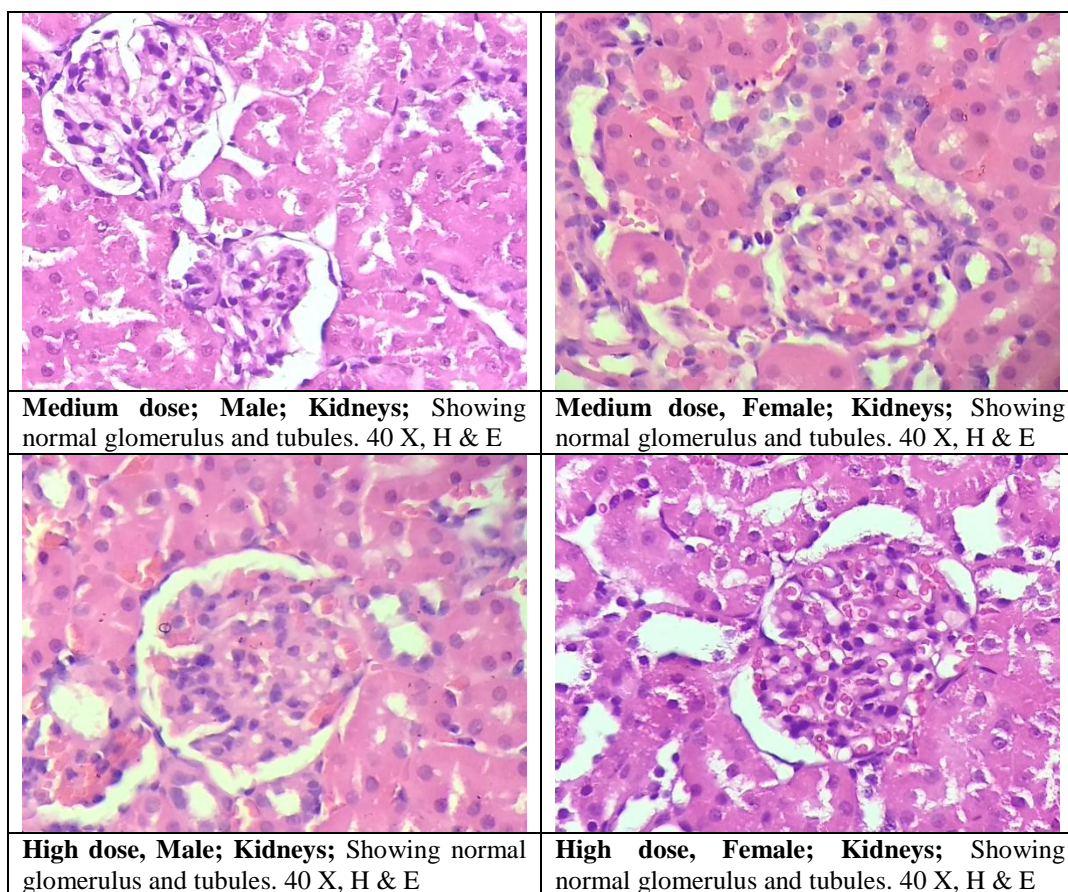
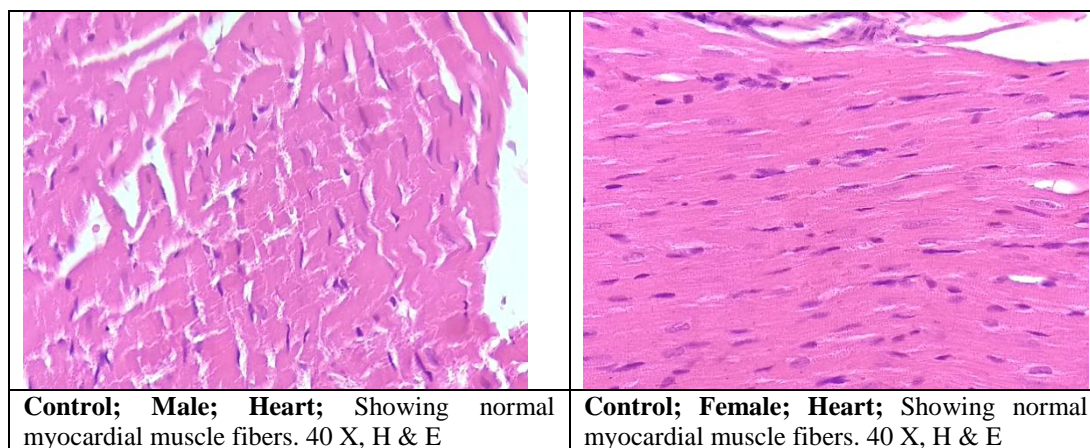


Fig. 2 Histopathological examination of Kidney of rats in subacute oral toxicity study.

Heart: Microscopic examination of heart from both male and female of all groups did not show any lesion of

pathological significance when compared with respective control group (Figure 3).



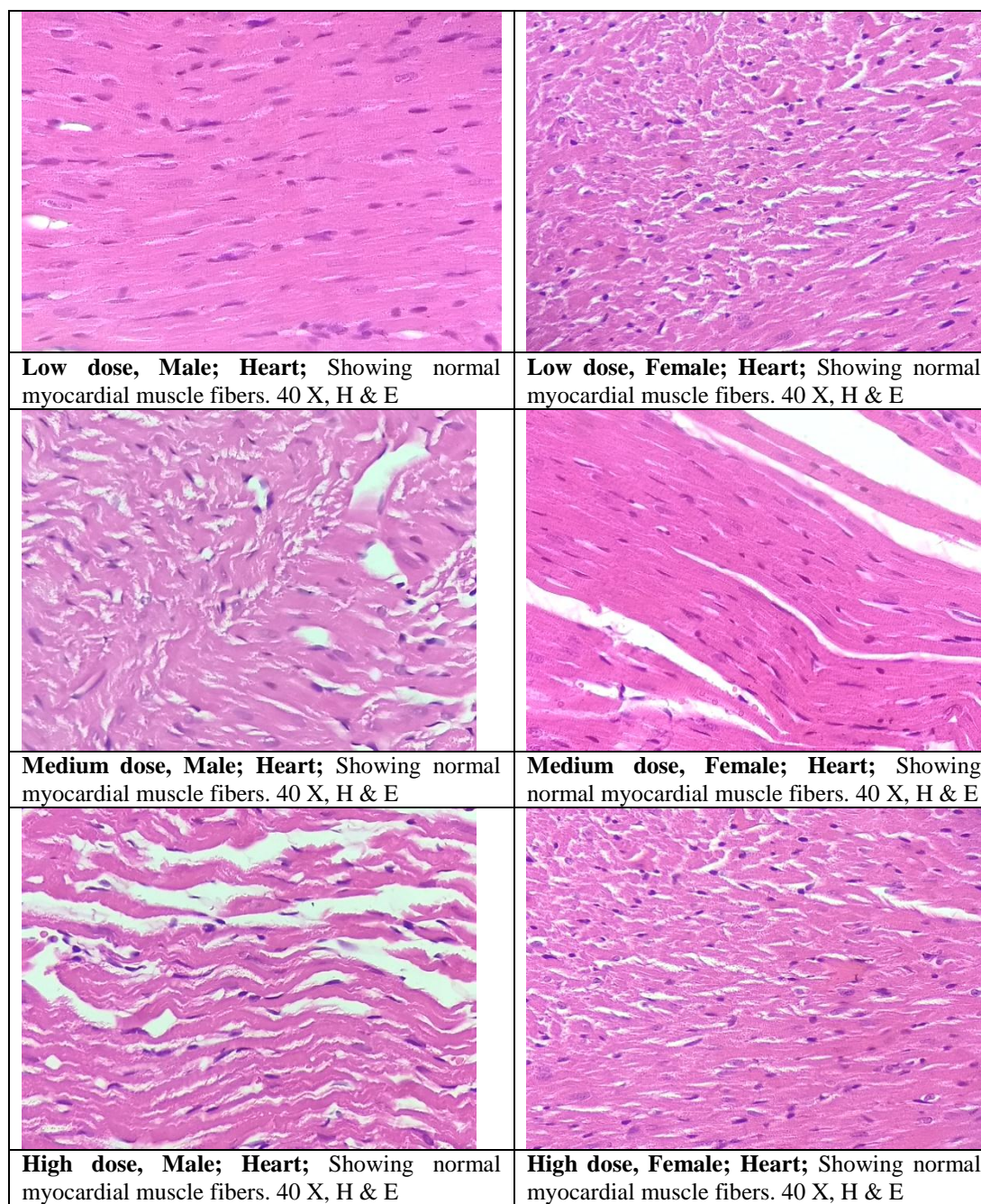


Fig. 3 Histopathological examination of Heart of rats in subacute oral toxicity study.

Lungs: Microscopic examination of Lungs from both male and female of all groups did not show any lesion of

pathological significance when compared with respective control group (Figure 4).

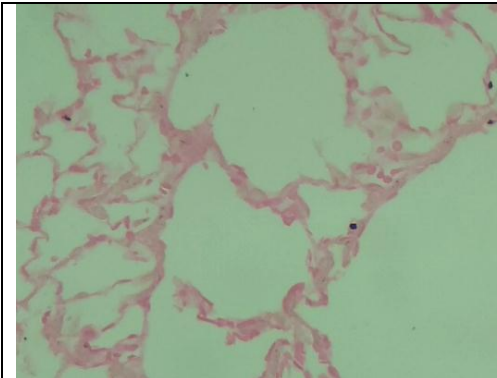
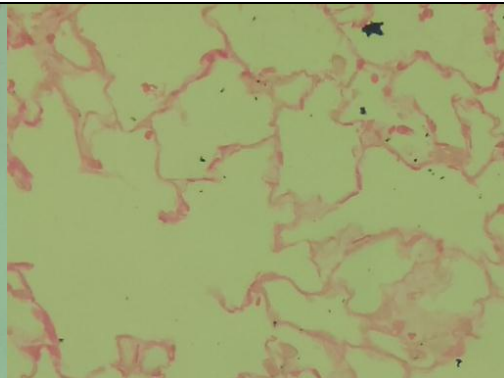
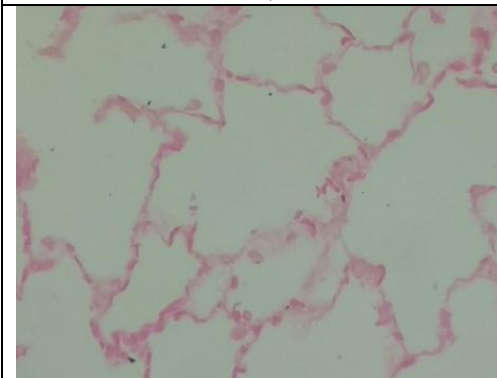
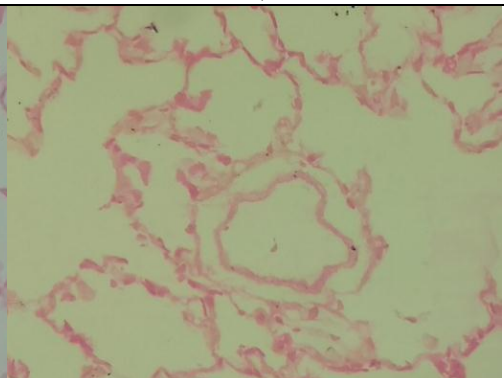
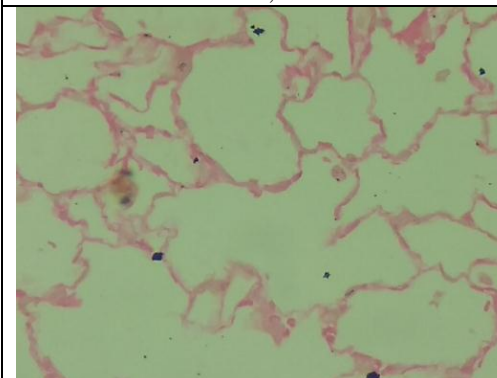
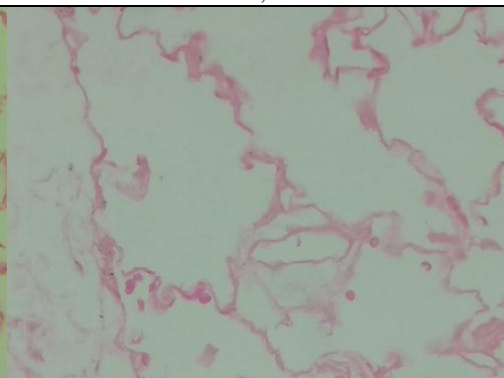
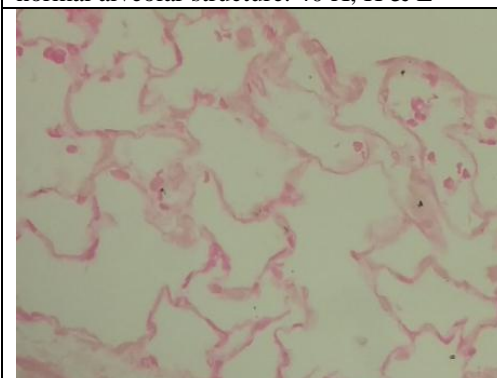
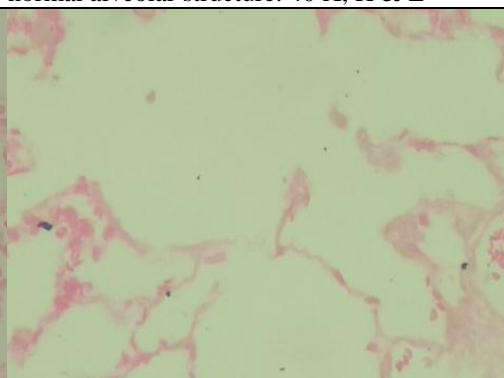
	
Control; Male; Lungs; Showing normal alveolar structure. 40 X, H & E	Control; Female; Lungs; Showing normal alveolar structure. 40 X, H & E
	
Low dose, Male; Lungs; Showing normal alveolar structure. 40 X, H & E	Low dose Female; Lungs; Showing normal alveolar structure. 40 X, H & E
	
Medium dose, Male; Lungs; Showing normal alveolar structure. 40 X, H & E	Medium dose, Male; Lungs; Showing normal alveolar structure. 40 X, H & E
	
High dose, Male; Lungs; Showing normal alveolar structure. 40 X, H & E	High dose, Female; Lungs; Showing normal alveolar structure. 40 X, H & E

Fig. 2 Histopathological examination of Lung of rats in subacute oral toxicity study.

DISCUSSION

The use of herbal medicines as alternative treatments has been increasing worldwide and gaining popularity in developing countries. Although clinical plants may have organic exercises that are gainful to people, the likely poisonousness of these bioactive substances has not been well established.^[8] Thus, the wellbeing and adequacy of these plants must be concentrated completely to amplify their advantages for humankind. To achieve this objective, a toxicological evaluation is performed using various experimental animals to provide guidelines for selecting a “safe” dose for human uses. The weights of the organs are signs of obsessive and physiological wellbeing status of creatures.^[9,10] Changes in organ loads are signs of harmfulness in test creatures, which are dictated by poisonousness tests.^[11] The poisonous impact of ingested home grown cures in the body is well on the way to be felt by significant organs, for example, the heart, liver, lungs and kidneys due to the imperative jobs that they play in the body. The liver and kidneys are significant focuses of xenobiotic activity, with the liver being the significant organ for xenobiotic biotransformation, while the kidney fills in as excretory organ of xenobiotics.^[12] Our discoveries on organ weight uncovered that there was no huge increment in organ weight (Table 2), suggesting that the *S. Sesban* extract was not harmful to the animals at the tried dosages. The wellness status of animals is hinged on changes in body weight.^[13] After 28 days of treatment, all the animals exhibited a steady increase in body weight. It indicated that the daily intake of *S. sesban* extract did not alter food intake. Besides, it conceivably shows that weight increase and hunger soundness were not blocked by the concentrate during the introduction time frame. This approves the oral course folkloric utilization of the *S. sesban* extract. The evaluation of hematological parameters is of great importance in determination the health status of an individual.^[14] These parameters do not only depict the harmful effects of herbal remedies, but also reveal the blood-relating potential. We observed that there were no noticeable hemolytic changes on WBC, RBC, Hb, PCV, MCH, MCV, MCHC, granulocytes and leukocytes. The expanded arrival of WBC's is a prominent biomarker of stress and furthermore helps in guarding the body against some incendiary conditions, for example, bacterial diseases, leukemia and discharge. The outcome got from this investigation uncovered that *S. sesban* remove didn't cause any critical changes in the degrees of WBC tally, or in their subtypes, including neutrophils, lymphocytes, monocytes and eosinophils, at any of the portions, comparative with control (Table 4). This proposes that the *S. sesban* extract is nontoxic. The retention of creatinine, electrolytes, urea and uric acid in the body is indicators of kidney damage.^[13,14,15] Our discoveries uncovered that there was no noteworthy distinction in the degree of creatinine, urea or uric corrosive in the three portion gatherings, when contrasted and the benchmark group, in both genders of the rodents. This offers further help for the security of *S. sesban* separate at these portions, as there was no

adjustment in kidney work. Raised degrees of serum transaminase chemicals (ALT, AST and ALT) are away from of hepatic disability in creatures. Incendiary reactions are generally set off by a diminishing in plasma egg whites levels.^[16] There was no noteworthy contrast in the degrees of complete protein, egg whites, all out bilirubin as appeared in Table 5. This demonstrates that *S. sesban* remove didn't block kidney's capacity to discharge the previously mentioned metabolites. Histological investigations are utilized as benchmarks for deciding neurotic changes in tissues and organs. Histological examination of heart, liver and kidney (Fig. 1) revealed no abnormalities in cellular architecture of these vital organs in the morphology of vital organs.

S. sesban leaves have been widely used for the treatment of many ailments. Many studies have demonstrated their utility, including their biological activities, in vitro and their therapeutic benefits in rodents.

CONCLUSION

This study showed that the administration of the *S. sesban* extract to Wistar rodents was not poisonous in any of the tried dosages. The concentrate didn't directly affect the liver and kidney works as substantiated by results from hematological and blood science examination on both genders. Likewise, the concentrate didn't achieve any adjustments in food admission, water utilization or body weight and delivered no obvious histopathological harm in the rodent organs, paying little mind to sex. Moreover, the outcomes acquired from subacute harmfulness investigations of *S. sesban* could subsequently offer knowledge to its wellbeing in people.

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