EVALUATION OF SUBACUTE ORAL TOXICITY STUDY INDUCED BY ETHANOLIC EXTRACT OF SESBANIA GRANDIFLORA FLOWER EXTRACT IN EXPERIMENTAL RATS.

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Abstract:-

The objective of this study to evaluate the sub-acute toxicity of the ethanolic extract of sesbania grandiflora flower [family- Fabaceae] in wistar rat. 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. The study is aimed at evaluating the possible toxicity in 28-day sub-acute oral toxicity of ethanolic extract *Sesbania grandi*flora flower extract) in male and female Wistar rats. The 28-day sub-acute toxicity study was conducted to detect the no-observed adverse effect level . In this study, a total of 48 rats were divided into the control, low dose (200 mg/kg), medium dose (500 mg/kg) and high dose (1000 mg/kg) groups. The extract was administered daily from day 28. At the end of the study, the animals were sacrificed and assessed for the effect extract of *Sesbania Grandiflora* flower extract on body weight and relative organ weights and hematological, biochemical and histopathological parameters. The hematological and serum biochemical parameters for the assessment of kidney and liver injuries were carried out. Results of hematological and serum biochemistry results showed no changes in the control and treated groups. In the histopathology, evaluation of kidney tissues in all treated groups showed no significant ($p > 0.05$) lesions.

KEYWORDS: sub-acute oral toxicity, Sesbania grandiflora, biochemical analysis, hematological parameters, histopathology.

INTRODUCTION

Advanced in medical technology have encouraged studies examining the development and novel use of biological resources. A number of natural substances are widely used as raw materials of medicine, health function foods and home remedies. . These remedies, with considerable extent of effectiveness, are socially accepted, economically viable and, mostly, are the only available source. This raises concerns about the potential toxic effect resulting from chronic use of such medicinal plants. Therefore, evaluating the toxicological effects of any medicinal plant extract intended to be used clinically or preclinically, is a crucial part of its assessment of potential toxic effects.[1] 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. Nevertheless, their natural origin is not a guarantee of safety, as concerning the risks associated with the use of herbal products have noted. Hence, scientific information regarding the safety of this plant for use as

alternative medicine is very important[2] . Natural products are believed to be safer than chemical products. The toxicity studies drug extract provide preliminary information of drug extract and same time used to provide LD 50.Differnt sign and symptoms are observed during the gross observation studies during the drug given .On the basis of toxicities , the therapeutic dose and route of administration of drug can also be known. Photochemical [3] with biological activity have great utility as pharmaceutical and pharmacological action. These type of activities' of herbal drugs are due to the presence of various active principals or Phytoconstituent like alkaloid, saponins , tannin, resin , phytosterol ,flavonoids, organic acids, essential oil, fixed oil, Although in recent times, synthetic drugs are used extensively in modern medicine systems. However many modern medicines are developed through the clues obtained from phytochemicals. More over the phytochemicals are even today are important resources for medicinal uses.[4,5] The plant products are becoming more popular than the synthetic drugs due to their low toxicity and long standing experience of exposure of these drugs in ethnic medicine system like Ayurveda.

Sesbania grandiflora [Linn] belong to the plant family Fabaceae is found in topical area of India. It is cultivated in **October** month , plant grows wild in hedges and shady forest. A short lived, quick, growing, soft wooded tree .[6,7]

The present study is done to identify the phytochemical constituent of the flower and to evaluate the toxicity of extract in both male and female Wister rat.

MATERIAL AND METHOD :-

Collection and identification of plant material

Fresh flowers of sesbania grandiflora were collected from the local area of pune and the collected in the month of October and November and authenticated by M/S Shamantak Enterprises, Dr Gautam, Botanist , pune, India .The sample was identified at the national . Herbarium where some specimen was already available with the number B/II/293/0206776

Preparation of Plant extracts :

The flowers of Sesbania grandiflora (Linn.) were collected and shade dried. The dried flowers were coarse powdered and the powder was packed in to soxhelt column and extracted successively with petroleum ether (50 – 80^{0}C), methanol (60^{0}C) and distilled water. The extracts were concentrated by using rotary flash evaporator under reduced pressure. The dried extracts were stored in airtight container in refrigerator below 10^{0}C.

Experimental Animals

Young male and female Wister rats [200- 250g] body weight were bread at the department of pharmacology In the animal house of AISSMS COLLEGE OF PHARMACY PUNE under standard environmental condition of the temperature 22–24 °C, with a 12 h light/dark cycle and humidity around (50 ± 5) %. During acclimatization, the rats were randomized into experimental and control groups and housed individually in sanitized cages housed with sterile 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/PC-06/01-2K18

Acute toxicity :

[8]The bioassay was conducted according to the world health organization guideline for the evaluation of safety and efficacy of herbal medicine . For the study were divided into six group of 10 animal each group contain 5 male and 5 female animal . The acute toxicity study for methanolic and aqueous extract of flower was determined in albino rat maintained under standard condition The animal were fasted overnight and prior to the experiment fixed dose method was adopted as per OECD guideline no 423 of CPCSEA

Sub- acute toxicity

Sub-acute 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. [9, 10]

Wister rats of either sex was divided randomly into 4 groups (n=12; six males and six females per group), and their weights were recorded. The standardized 90% ethanolic extract of S .Grandiflora 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). Toxic manifestations and mortality were observed daily for 28 days. At the end of each week, the body weights of all the rats were recorded. At the end of the 28 days of administration, all of the rats were given anesthesia under CO2 inhalation, and blood samples were collected 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.

Relative organ weight

The above-mentioned organs were quickly removed and weighed individually. Each organ to body weight ratio (relative organ weight) was calculated as (weight of organ/body weight of rat on the day of sacrifice) *100%.

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.

Biochemical Analyses

The biochemical analyses carried out included measurement of liver functions such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), 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.

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 volume (MCV), mean corpuscular hemoglobin (MCH), 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.

Histopathological Examination

Liver, kidneys, heart and lungs excised from each treatment group were subjected to histopathological examinations. After fixing the tissues in 10 % formalin, they were dehydrated 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 one-way 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:

Sub-acute toxicity study

The sub-acute toxicity study of the *S. Grandiflora* extract was determined as per OECD guideline 407. All study animals were given *S. Grandiflora* 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. Grandiflora flower extract on food and water intakes

Table 1 depicts the effect of the S. Grandiflora on the food and water intake in sub-acute 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 when compared with control group.

Table 1:- Effect of s. Grandiflora flower extract on food and water intakes in sub- acute toxicity study

Treatment	Sex	Average food intake (g/d)	Average water intake (mL/d)
Control	Female	13.67±1.54	26.74±2.45
	Male	17.38±1.87	21.78±4.21
200 mg/kg S. Grandiflora	Female	14.69±1.94	19.92±1.61
	Male	19.68±0.86	27.22±3.47
500mg/kg S. Grandiflora	Female	14.57±2.21	18.64±5.43
	Male	19.34±1.76	24.68±2.29
1000mg/kg S. GRANDIFLORA	Female	14.09±0.98	17.37±0.73
	Male	19.90±1.07	25.51±6.23

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. Grandiflora extract on body weight

Table 2 shows the body weight of rat before and after drug treatement. The daily oral administration of S. Grandiflora extract at all doses 200,500,1000,mg/kg body weight for 28 days.

Group	Day 0	Day 14	Day 28
Male			
Control	216±8.93	229.06±10.72	218.4±14.66
200mg/kg S.Grandiflora	246.4±2.80	230.8±8.97	218.5±13.25
500 mg/kg S. Grandiflora	262.66±11.15*	277.33±9.92*	281±9.48*
1000mg/kg S.Grandiflora	246.2±3.35	216.6±4.63*	210.6±11.69*

Female			
Control	188.4±12.16	206.6±10.91	218.8±13.23
200mg/kg S.Grandiflora	228.2±10.16	181.4±10.09**	204.83±4.41*
400mg/kg S.Grandiflora	243.±4.66	213.4±4.27*	200.66±3.57*
1000mg/kg S.Grandiflora	245.8±6.37	232.9±9.28	207.4±8.70*

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.

Table no 3 -Effect of S. Grandiflora extract on hematological parameters

The effects of the sub-acute oral administration of the S .Grandiflora extract on hematological parameters are represented in Table 3

TEST	CONTROL GROUP		LOW DOSE		MEDIUM DOSE		HIGH DOSE	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
RBC COUNT 9X 106/µL	6.8±0.34	6.25±0.26	6.66±0.18	6.15±0.11	6.065±0.29	5.43±0.32	7.22±0.31**	5.78±0.28*
HEMATOCRIET [%]	41.78±0.64	37±0.56	41.02±0.51	31.89±0.85	39.21±0.49	32.22±0.33	39.69±1.06	35.92±0.46
MCV[fl]	50.53±0.67	51.20±1.14	56.91±1.68*	48.91±1.14*	55.3±0.46**	55.36±0.29**	52.11±0.87**	52.45±1.07**
MCH [pg]	15.43±0.27	17.93±0.27	16.65±1.32	17.24±0.90	16.32±1.39	18.05±0.30	14.64±0.77	15.47±0.59
MCHC [g/dl]	29.13±0.57	30.29±1.050	32.90±0.51	31.17±0.42	30.98±1.19	29.14±0.69	29.80±0.54	33±0.54

HEMOGLOBIN [HB][g/dl]	12.81± 0.40	18.25±2. 49	14.44± 0.45	31.57±1 5.8	19.56± 0.39	26.3±1.1 2	22.86± 07**	23.92±0 .42*
Platelet count [X 10^3/µL]	746±3 3	724.33±2 7.51	672.66 ±9.52	824.5±1 3.69	725.34 ±7.30	727.66±8 .38*	851.5± 15.51	748.83± 13.43*
TOTAL LEUKOCYTE COUNT[X10^3/µ L	9.43±0 .28	9.92±0.4 3	11.07± 0..34	9.075±0. 34	9.54±0 .39	11.44±0. 29	11.7±0 .34*	10.56±0 .20*
BASOPHIL[%]	15.42± 0.58	14.1±0.3 8	17.93± 0.58	14.70±0. 35	14.35± 0.51	14.43±0. 58	14.61± 0.38	14.27±0 .27
LYM PHOCYTE[%]	12.9±0 .14	12.12±0. 34	13.7±0 .09*	13.76±0. 30**	13.7±0 .18**	13.1±0.3 1	13.07± 0.23	13.67±0 .11*
MONOCYTE [%]	42.9±0 .33	39.35±0. 41	43.15± 0.23	38.65±0. 73	41.3±0 .40*	39.75±0. 37*	41.47± 0.47	42.52±0 .38
NEUTROPHIL [%]	17.52± 0.36	15.7± 0.26	22.97± 0.25**	16± 0.56	20.55± 0.69*	25.45±1. 28**	23.55± 0.51	25±0.76 **
EOSINOPHIL[%]	9±0.17	8.48±0.2 3	7.48±0 .17	7.56±0.2 3*	7.02±0 .19	6.35±0.1 8**	6.48±0 .05	6.69±0. 09*

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. Grandiflora extract on biochemical parameters

The effects of the sub-acute oral administration of the S.Grandiflora extract on biochemical parameters are represented in Table 4

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

Parameters	Control	200 mg/kg	500 mg/kg	1000 mg/kg
Male				
Creatinine (µmol/L)	44.67±1.17	41.11±1.44	43.37±2.13	48.45±0.59
Total protein (g/L)	66.81±1.01	73.20±1.59	90.345±4.54**	81.52±2.38*
Albumin (g/L)	35.6±1.19	28.66±0.99**	30.95±0.91*	32.94±0.09
ALT (U/L)	0.655±0.018	0.653±0.028	0.791±0.023*	0.798.89±10.98

AST (U/L)	114.22±5.86	119.97±6.02	129±0.76	123.78±
Bilurubim mg/dl	0.151±0.12	0.325±0.20*	0.365±0.0182***	0.530±0.018*

Parameters	**Control**	**200 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Female				
Creatinine (µmol/L)	44.67±1.17	41.44±1.77	42.33±2.40	47.77±1.14
Total protein (g/L)	66.81±1.01	78.30±3.38	85.48±3.96*	88.00±3.23*
Albumin (g/L)	35.6±1.19	32.84±1.31	31.85±1.14	34.45±0.72
AST (U/L)	0.645±0.013	0.638±0.023	0.781±0.096**	119.94±5.99
ALT (U/L)	0.513±0.036	0.651±0.027	0.788±0.079***	0.795±0.026**
Bilirubin (Total) (mg/dl)	0.153±0.01	0.33±0.016**	0.46±0.03	0.53±0.18**

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. Grandiflora extract on the relative organ weights

Daily administration of S. Grandiflora 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. Grandiflora extract on relative organ weight

Group	**Heart**	**Liver**	**Lungs**	**Kidneys**
Male				
Control	0.55±0.04	3.7±1.73	1.43±0.23	1.03±0.12
200 mg/kg S.Grandiflora	0.51±0.08	3.4±1.23	1.5±0.23	0.56±0.20
500 mg/kg S. Grandiflora	0.45±0.03	3.9±1.14	2.2±0.12	1.16±0.012
1000 mg/kg S. Grandiflora	0.40±0.09	3.5±1.67	2.9±0.17**	1.06±0.20
Female				
Control	0.47±0.08	2.71±1.05	1.56±0.26	0.76±0.17

200 mg/kg S.Grandiflora	0.48±0.09	2.18±1.64	1.23±0.23	0.67±0.12
500 mg/kg S.Grandiflora	0.47±0.11	2.98±1.87	1.61±0.08	0.83±0.09
1000 mg/kg S. Grandiflora	0.44±0.14	3.10±0.94	1.46±0.26	0.69±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.

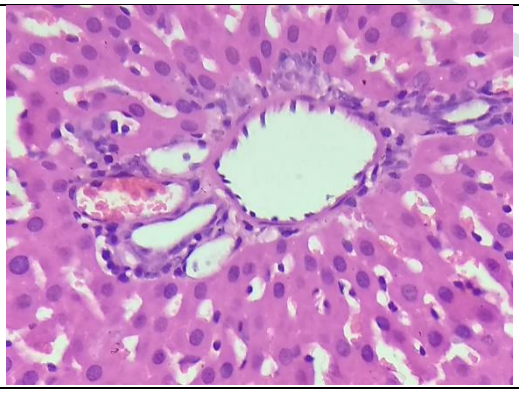
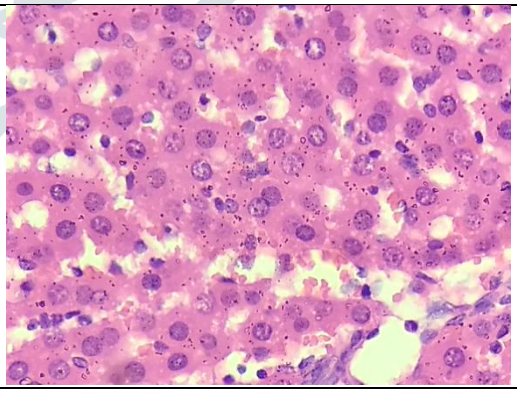
1.1 **Histopathology**

All the preserved organs/tissues samples such as Liver, Heart, Lungs and Kidneys from both male and female animals from G1 to G4 groups were processed routinely and embedded in paraffin. The sections of 3-5 µ thickness were cut and stained with hematoxylin-eosin stain. Histopathology examination of all the organs were carried out by board certified Toxic pathologist.

Microscopic Examination of liver :-

Microscopic examination of liver showed minimal to mild; focal to multifocal hepatocellular vacuolation (microvesicular and macrovesicular) in both male and female animals treated with Sesbania Grandiflora extract at 500 and 1000 mg/kg body weight however low dose group (G2) animals did not show any change when compared with control group.

Table 1:- Images **of Liver**

	
Control; Male; Liver; Showing normal periportal hepatocytes. 40 X, H & E	**Control; Female; Liver;** Showing normal Centralobular hepatocytes. 40 X, H & E

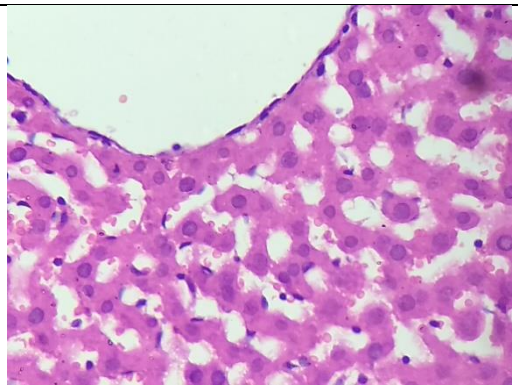
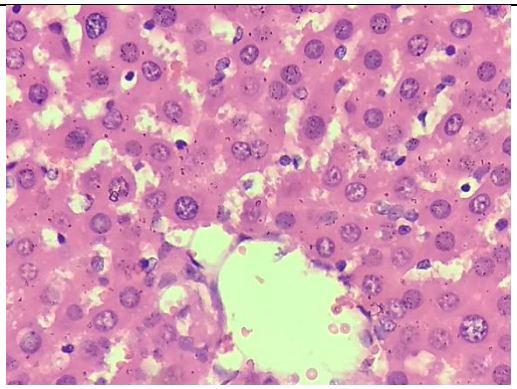
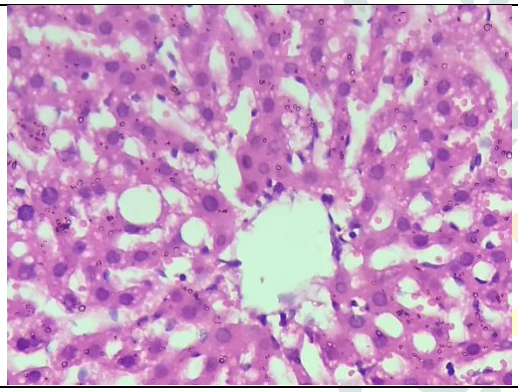
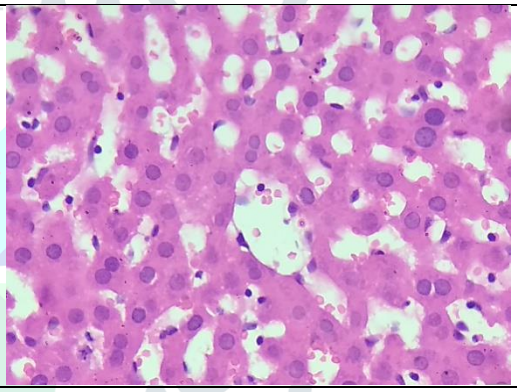
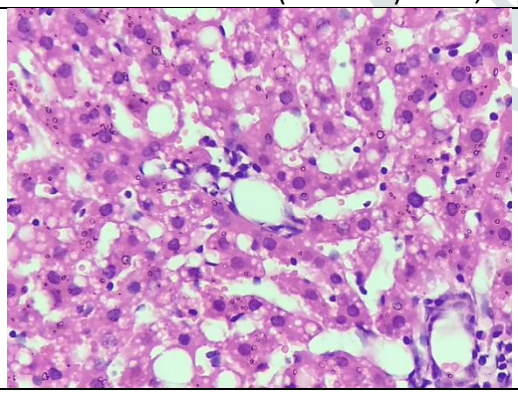
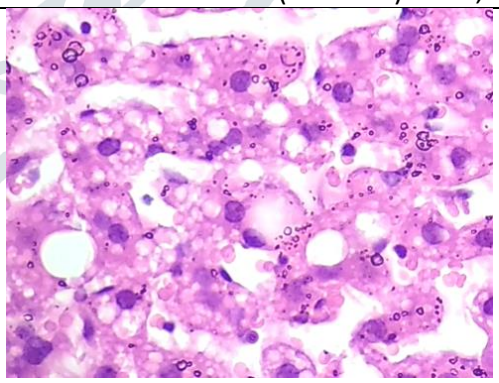
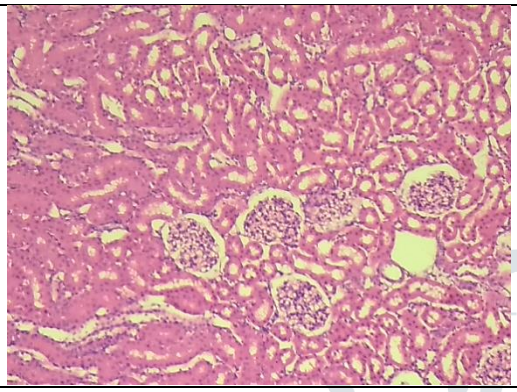
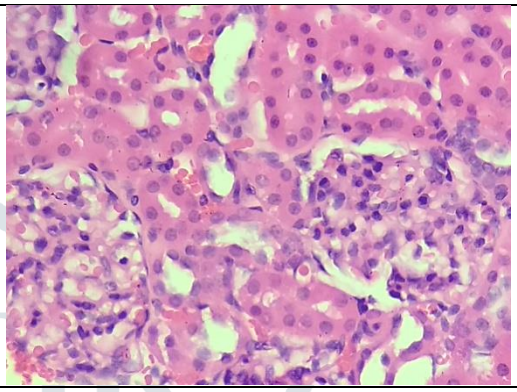
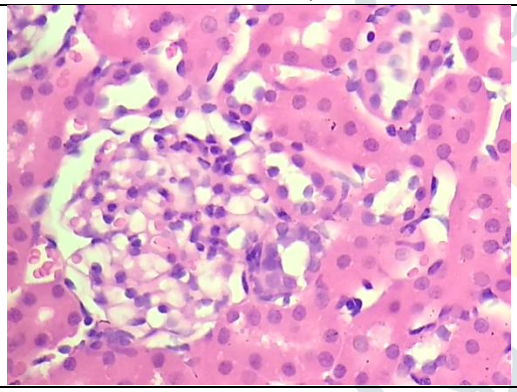
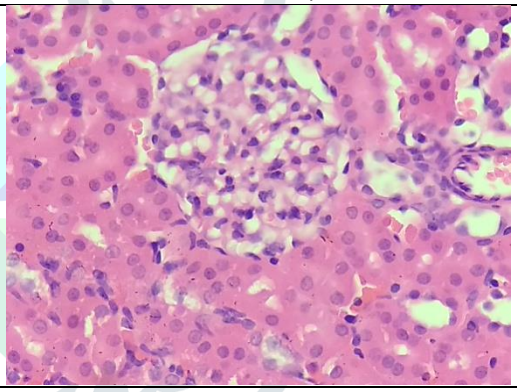
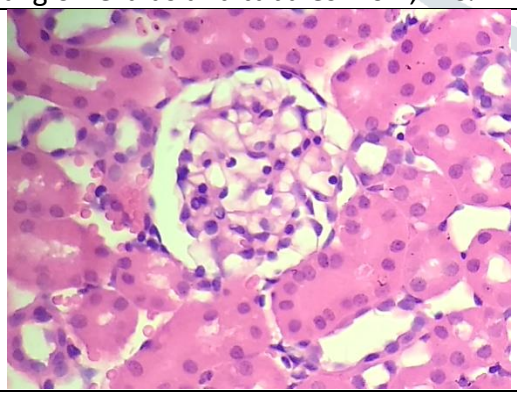
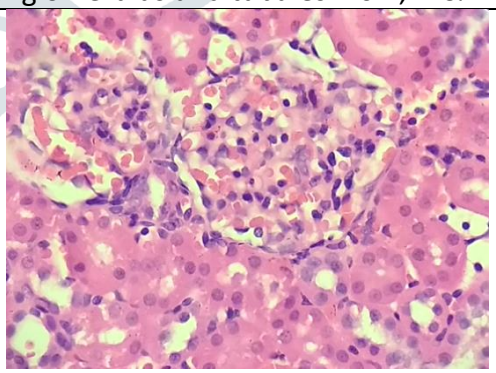
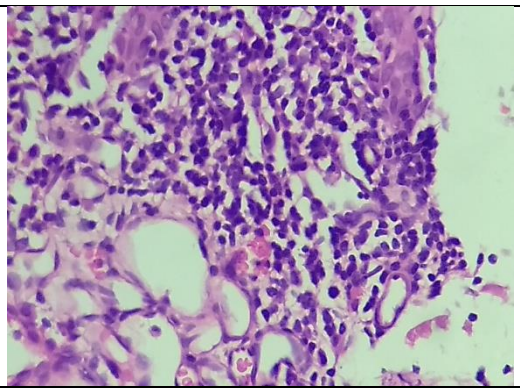
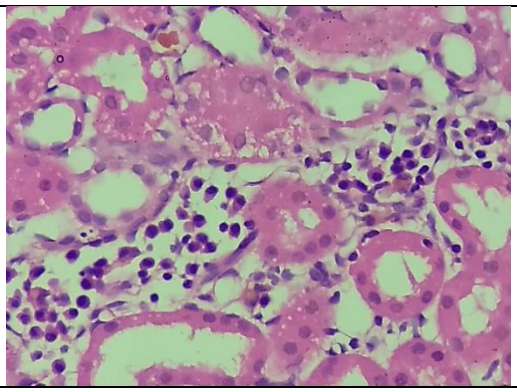
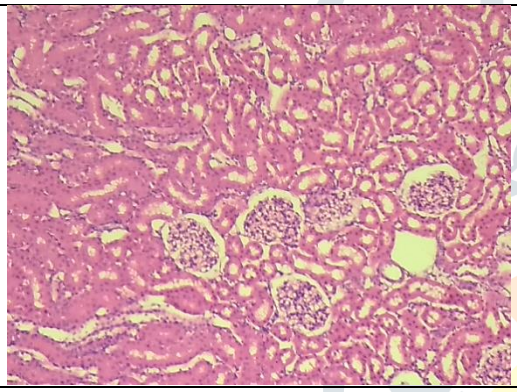
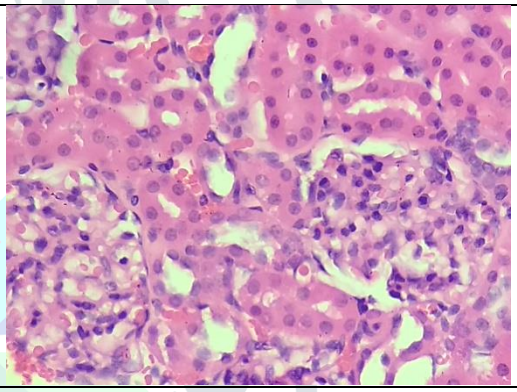
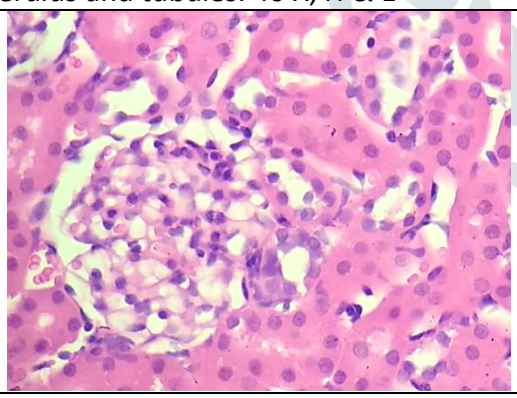
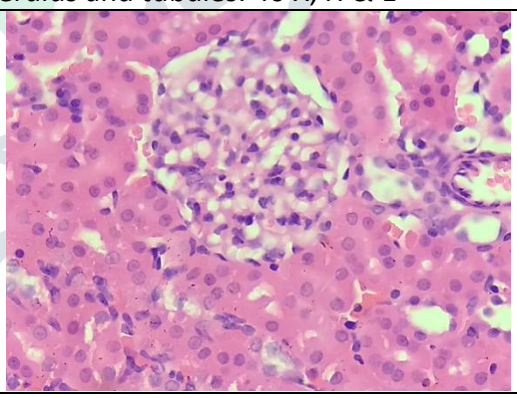
	
Low Dose; Male; Control; Liver; Showing normal hepatocellullar parenchyma. 40 X, H & E	**Low Dose; Female; Control; Liver;** Showing normal hepatocellullar parenchyma 40 X, H & E
	
Medium Dose; Male; Liver; Showing mild hepatocellular vacuolation (Minimal). 40 X, H & E	**Medium Dose; Female; Liver;** Showing mild hepatocellular vacuolation (Minimal). 40 X, H & E
	
High Dose; Male; Liver; Showing Mild hepatocellular vacuolation. 40 X, H & E	**High Dose; Female; Liver;** Showing Mild hepatocellular vacuolation 40 X, H & E

Table 2:- Images Kidneys

Microscopic examination of Kidneys showed minimal multifocal infiltration of inflammatory cells in both male and female animals treated with Sesbania Grandiflora extract at 1000 mg/kg body weight however low (G2) and medium dose (G3) groups animals did not show any change when compared with control group.

	
Control; Male; Kidneys; Showing normal glomerulus and tubules. 40 X, H & E	**Control; Female; Kidneys;** Showing normal glomerulus and tubules. 40 X, H & E
	
Low Dose; Male; Control; Kidneys; Showing normal glomerulus and tubules. 40 X, H & E	**Low Dose; Female; Control; Kidneys;** Showing normal glomerulus and tubules. 40 X, H & E
	
Medium Dose; Male; Kidneys; Showing normal glomerulus and tubules. 40 X, H & E	**Medium Dose; Female; Kidneys;** Showing normal glomerulus and tubules. 40 X, H & E

	
High Dose; Male; Kidneys; Showing Infiltration of inflammatory Cells. 40 X, H & E	**High Dose; Female; Kidneys;** Showing Infiltration of inflammatory Cells. 40 X, H & E
	
Control; Male; Kidneys; Showing normal glomerulus and tubules. 40 X, H & E	**Control; Female; Kidneys;** Showing normal glomerulus and tubules. 40 X, H & E
	
Low Dose; Male; Control; Kidneys; Showing normal glomerulus and tubules. 40 X, H & E	**Low Dose; Female; Control; Kidneys;** Showing normal glomerulus and tubules. 40 X, H & E

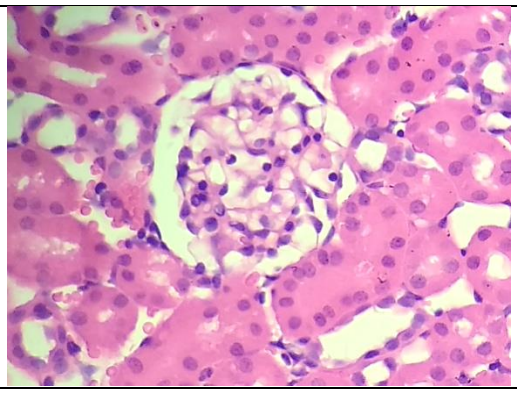
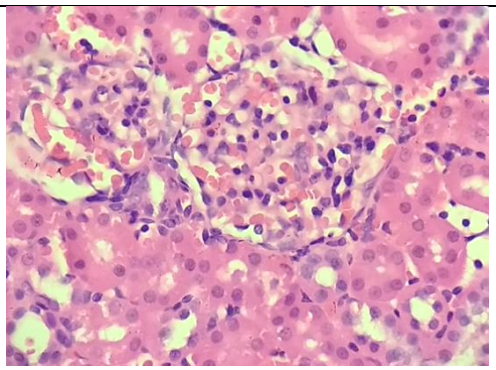
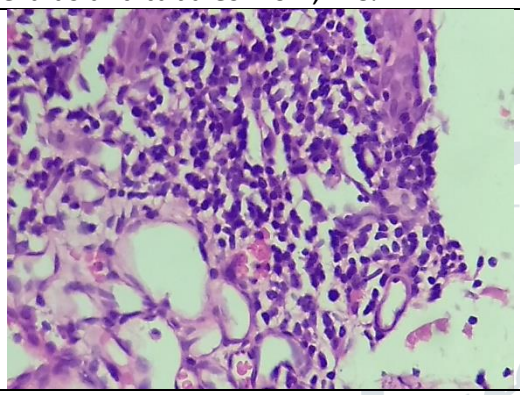
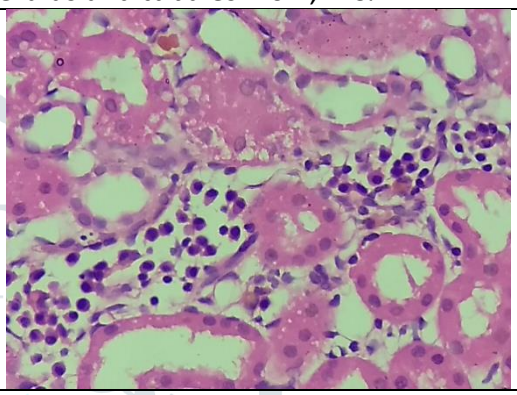
	
Medium Dose; Male; Kidneys; Showing normal glomerulus and tubules. 40 X, H & E	**Medium Dose; Female; Kidneys;** Showing normal glomerulus and tubules. 40 X, H & E
	
High Dose; Male; Kidneys; Showing Infiltration of inflammatory Cells. 40 X, H & E	**High Dose; Female; Kidneys;** Showing Infiltration of inflammatory Cells. 40 X, H & E

Table 3 images of heart.

Microscopic examination of heart from both male and female of all groups (G1 to G4) did not show any lesion of pathological significance when compared with respective control group.

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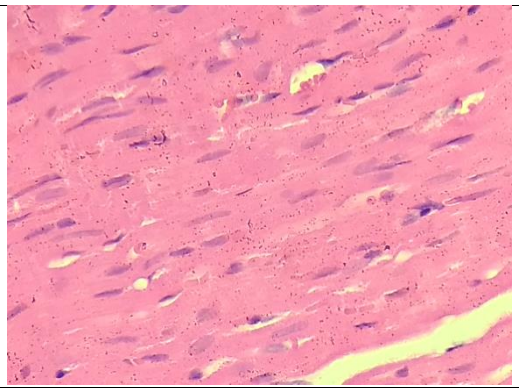
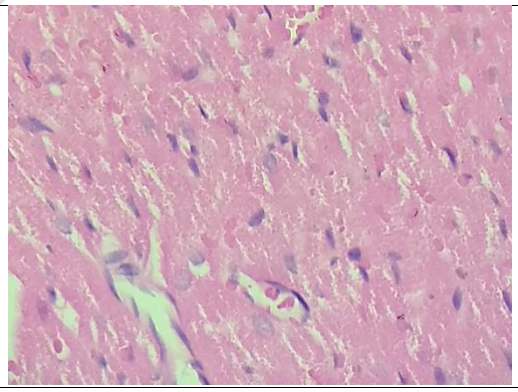
	
Control; Male; Heart; Showing normal myocardial muscle fibers. 40 X, H & E	**Control; Female; Heart;** Showing normal myocardial muscle fibers. 40 X, H & E

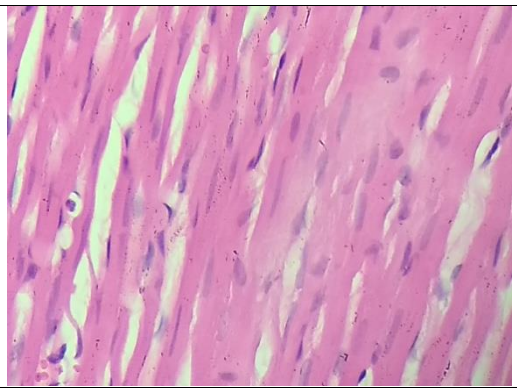
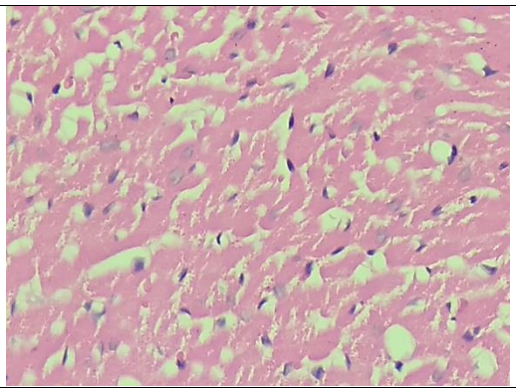
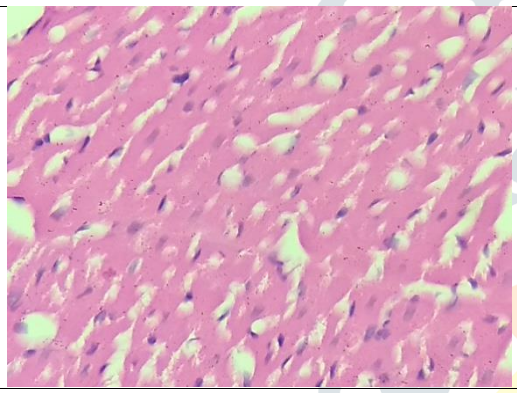
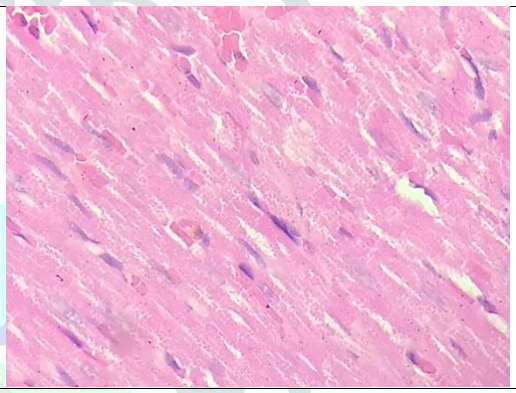
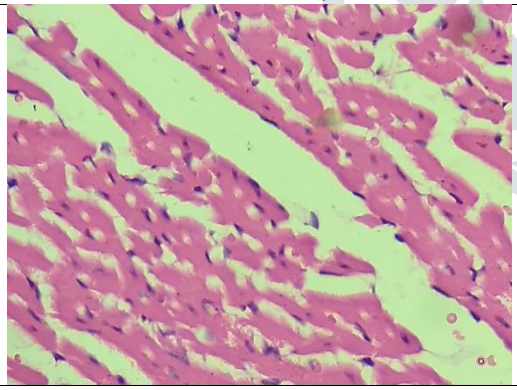
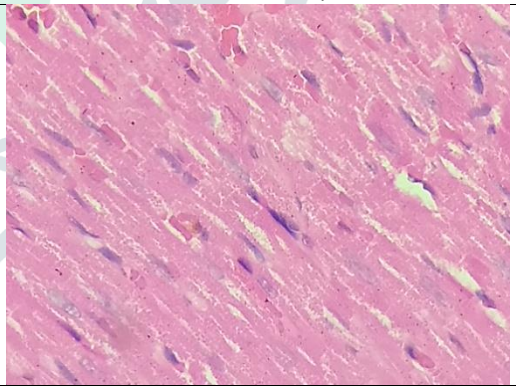
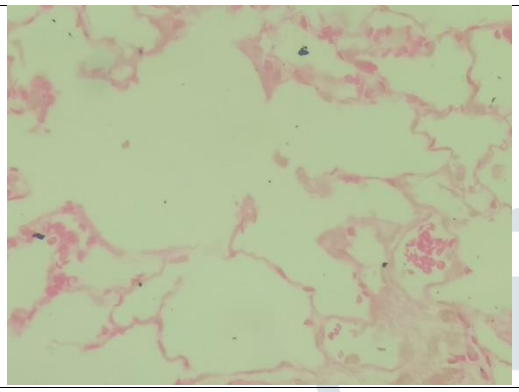
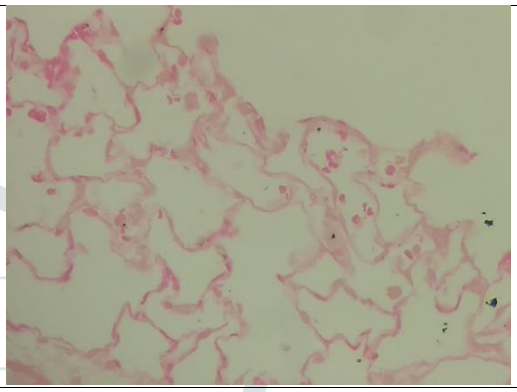
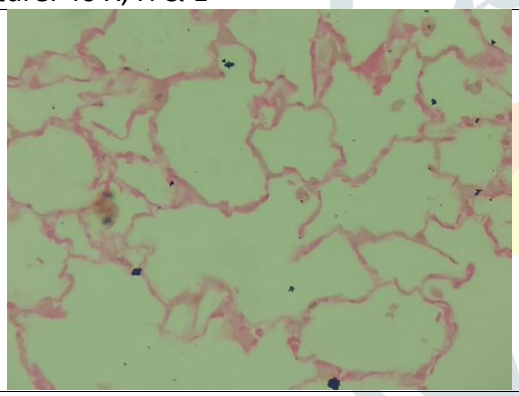
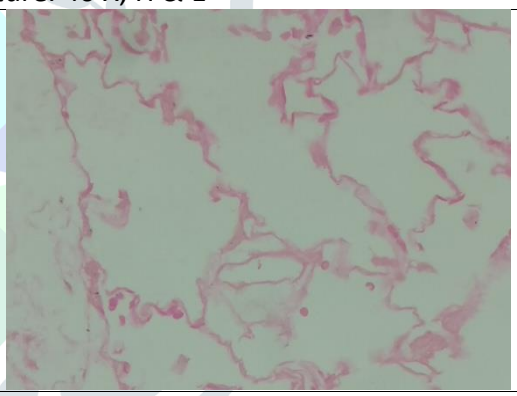
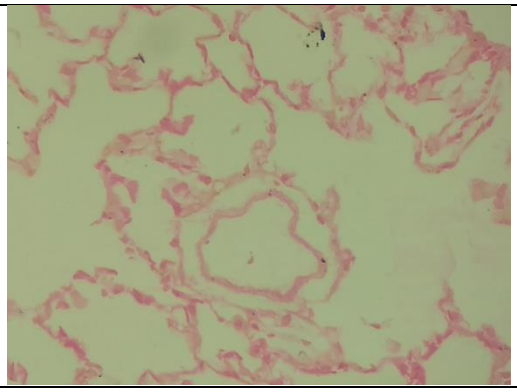
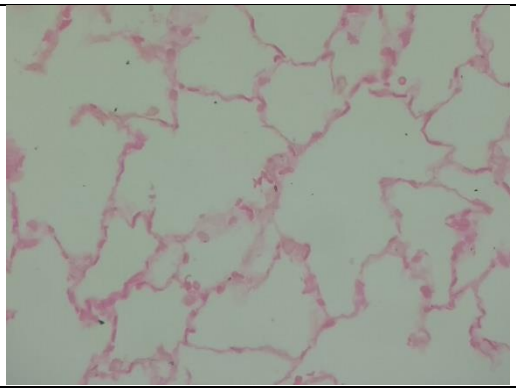
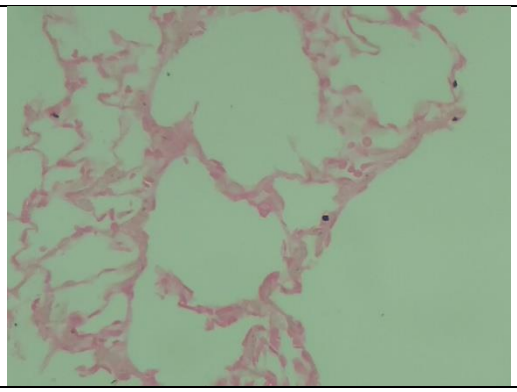
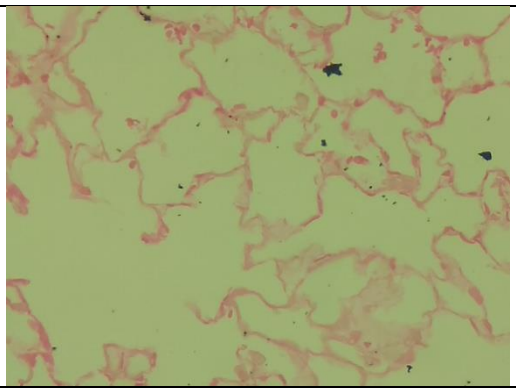
	
Low Dose; Male; Control; Heart; Showing normal myocardial muscle fibers. 40 X, H & E	**Low Dose; Female; Control; Heart;** Showing normal myocardial muscle fibers. 40 X, H & E
	
Medium Dose; Male; Heart; Showing normal myocardial muscle fibers. 40 X, H & E	**Medium Dose; Female; Heart;** Showing normal myocardial muscle fibers. 40 X, H & E
	
High Dose; Male; Heart; Showing normal myocardial muscle fibers. 40 X, H & E	**High Dose; Female; Heart;** Showing normal myocardial muscle fibers. 40 X, H & E

Table no 4 Images of lung

Microscopic examination Microscopic examination of Lungs from both male and female of all groups (G1 to G4) did not show any lesion of pathological significance when compared with respective control group.

	
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; Male; Lungs;** Showing normal alveolar Structure. 40 X, H & E

	
Medium Dose; Male; Lungs; Showing normal alveolar Structure. 40 X, H & E	**Medium Dose; Female; 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

On the basis of histopathology findings, it can be concluded that animals treated with extract Sesbania grandiflora flower at1000 mg/kg body weight showed hepatocellular infiltration of inflammatory cells in liver and kidney changes at 500mg/kg body weight however kidneys, heart and lungs did not show any changes when compared with control group. Hematological analysis of extract treated animals were comparable with control group animals, Showed the liver toxicity.

DISCUSION

The herbal medicines and their formulations have been considered to be safe and effective due to their negligible side effects. This assumption may have influenced the indiscriminate use of these formulations to a large extent amongst the rural populace. These formulations are usually administered over a long period of time without proper dosage monitoring by the experts and lack of awareness of the toxic effects that might result from such prolonged usage [11]. Hence, the current study was undertaken to evaluate and focus on the sub-acute toxicity of Sesbania Grandiflora flower extract in an animal model.

In screening natural products for pharmacological activity, the evaluation of the toxic characteristics of medicinal products (extract, isolated compounds, and formulation) is usually a preliminary step. And During such evaluation, the determination of LD50 is usually an initial step to be conducte[12]. The acute toxicity study may provide initial information on the mode of toxic action of an agent, acts as the basis for classification and labeling, and helps in deciding the dose of novel compounds in animal studies. In this study S .Grandiflora flower at 1000mg/kg had no adverse effect on the treated rats in up to 14 days of observation. There were increase significant changes in the weight and the organs of the rats. The hematological parameters between control and treated groups showed the extract was non-toxic to the haemopoietic system. Additionally, most of the biochemical parameters were not altered. No relevant changes were found in levels of ALT, AST, or creatinine, which are good indicators of liver and kidney functions. No gross lesions were found in histopathology examinations. Therefore further study was conducted to evaluate the sub-acute toxicity of S .Grandiflora up to 28 days to prepare inclusive toxicological records on this plant. Sub-acute studies provide information on dosage regimens, target organ toxicity, and identify observable adverse effect that may affect the average life span of experimental animals. Consequently, in this study, the leaves of S.Grandiflora were evaluated in rats at doses of 200, 500, and 1000 mg/kg for 28 days. The body weight changes serve as a sensitive indication of general health status of

animals [13]. After 28 days of treatment of the extract, all the animals exhibited a normal increment in body weight. It can be stated that flowers of S .Grandiflora did not interfere with the normal metabolism of animals. The significant increment in food and water intake is considered as being responsible for augmentation in body weight gain.

Similarly, no significant changes in the weight of the heart, liver, lung, spleen and kidney were observed, suggesting that administration of MTE leaves at subacute oral doses produces no effect on the normal growth. The protocol of weighing relative organs in toxicity studies includes their sensitivity to predict toxicity and it correlates well with histopathological changes [14]. The results of this study revealed no significant changes in the relative organ weight of control and treated groups which showed that none of the organs were adversely affected, nor showed any signs of toxicity throughout the study.

The haematological parameters can be used to determine the blood relating functions of plant extract. The haemopoietic system is one of the most sensitive targets of toxic compounds and an important index of physiological and pathological status in both humans and animals. The extract indicated a non-significant difference on the RBC indices which suggested that the MTE does not affect erythropoiesis, morphology, or osmotic fragility of red blood cells [15]. WBC's are the first line of cellular defines that respond to infectious agents, tissue injury, or any inflammation. Furthermore, no significant changes were observed in neutrophils, lymphocytes, and monocytes in the leaves of MTE suggesting that the extract might not have exerted challenge on the immune system of the animals.

Evaluation of serum biochemistry was done to identify the possible alterations in renal and hepatic functions affected by extract. Total protein, albumin, globulin, and total bilirubin also affecting the hepatocelluar and secretory functions of the liver. The lack of significant alterations in the levels of ALT, AST, ALP, creatinine , which are good indicators of liver and kidney functions [16], suggests that sub-chronic administration of extract neither altered hepatocytes and kidneys of rats, nor the normal metabolism of the animals. These observations were further confirmed by the histological assessment of the organs showed in Figure 2. Based on the results found in our study, we concluded that the flowers of S.Grandiflora ethanolic extract was safer and non-toxic and could be well used for pharmacological and therapeutic purposes. Moreover, no work has been reported on its flowers (i.e., isolation of chemical constituents and their characterization). Very few species of S.Grandiflora possess neurological toxicity

Histological studies are used as benchmarks for determining pathological changes in tissues and organs. Histological analysis of heart, liver and kidney (Fig. 1) revealed no abnormalities in cellular architecture of these vital organs in the morphology of vital organs. This also supports our results that liver and kidney injury biomarkers were not elevated in groups treated with S .Grandiflora extract.

S .Grandiflora 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

Conclusions

This study showed that the administration of the S.Grandiflora flower extract to Wistar rats was not toxic in any of the tested doses. The extract did not have a direct impact on the liver and kidney functions as corroborated by results from hematological and blood sample of both sexes. Also the extract did not change in food and water intake. The histology examination revealed no remarkable changes in the

internal organs, like kidney, liver, spleen, lung, and heart of the rats, in both control and treated groups. Furthermore, the data of acute and sub-acute toxicity studies on this plant were obtained in order to increase the confidence in its safety to humans for the use in the development of pharmaceuticals. The studies also need further experimental activities, like sub-chronic toxicity study, the effect of the extract on pregnant rats, foetuses, and their reproductive capacity to complete the safety profile of this plant.

Acknowledgments

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