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EVALUATION OF SUBACUTE ORAL TOXICITY INDUCED BY ETHANOLIC EXTRACT OF *DALBERGIA SISSOO* LEAVES IN EXPERIMENTAL RATS

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ABSTRACT

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 subacute oral toxicity of ethanolic extract *Dalbergia sissoo* (*D. sissoo*) in male and female Wistar rats. The 28-day subacute toxicity study was conducted to detect the no-observed adverse effect level (NOAEL). 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 1 until day 28. At the end of the study, the animals were humanely sacrificed and assessed for the effect extract of *Dalbergia sissoo* leaves 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: subacute oral toxicity, *Dalbergia sissoo*, 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 modes of herbal treatment are a part of the culture and the dominant method of healing therapy. 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.[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. 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 an alternative medicine is vary important before it is further developed into a new medicinal herbal therapy.[3]

Dalbergia sissoo, popularly known as shisham in India, is an erect deciduous tree. *D. sissoo* is widely available throughout the Indian subcontinent. Various pharmacological properties of *D. sissoo*, including stimulation of new cell growth and tissue regeneration, have been reported. In Indian medicinal practice, the leaf juice of *D. sissoo* is prescribed for eye ailments. In our phytopharmacological evaluation program aimed at finding an effective alternative therapy for postmenopausal osteoporosis, we recently reported that several phytoestrogens, particularly methoxy isoflavones, were present in the crude extract made from the leaves of *D. sissoo* and exhibited in vitro bone-forming activity.[4] Comprehensive investigation of *D. sissoo* reported to contain estrogenic flavonoids and some sterols with estrogenic activity. The reported results of phytochemical analysis indicated to the presence of flavonoids in Dalbergia sissoo.[5]

However, no studies on the toxicity of *D. sissoo* leaves have been described in the literature. Therefore, in the present investigation, we aimed to investigate the

toxicity (oral subacute) of *D. sissoo* leaves in order to increase the confidence in their safety to humans to treat various ailments.

MATERIALS AND METHODS

Collection and Identification of Plant Material

D. sissoo leaves were collected from the surrounding area of rural Pune during September 2018. The plant was identified and authenticated by M/s. Shamantak Enterprises, Dr. Gautam, Botanist, Pune, India.

Preparation of Plant Extract

A weighed quantity (50g) of the air-dried powdered leaves of D. sissoo was drawn and then it was extracted with 90% ethanol in a Soxhlet extractor. 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 D. sissoo was given according to body weight of animals.[6]

Experimental Animal

Male and female Wistar rats 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 rats were randomized into experimental and control groups and housed individually in sanitized cages 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/PC-08/01-2K18).

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.[7,8]

Wistar 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 *D. sissoo* 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 CO_2 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.[2]

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%.[9]

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 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.

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.

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

Subacute toxicity study

The subacute toxicity study of the D. sissoo extract was determined as per OECD guideline 407. All study animals were given D. sissoo 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 D. sissoo extract on food and water intakes

Table 1 depicts the effect of the D. sissoo 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 D. sissoo extract on food and water intake per animal.

Treatment	Sex	Average food intake (g/d)	Average water intake (mL/d)
Control	Female	11.67±1.54	18.74±2.45
	Male	17.38±1.87	21.78±4.21
200 mg/kg D. sissoo	Female	12.69±1.94	16.92±1.61
	Male	18.68±0.86	24.22±3.47
500 mg/kg D. sissoo	Female	12.57±2.21	18.64±5.43
	Male	19.34±1.76	24.68±2.29
1000 mg/kg D. sissoo	Female	12.09±0.98	16.37±0.73
	Male	18.90±1.06	23.51±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 D. sissoo extract on the relative organ weights

Daily administration of D. sissoo 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 D. sissoo extract on relative organ weight.

Group	Heart	Lungs	Liver	Kidneys
Male				
Control	0.55±0.04	0.32±0.09	3.12±1.73	0.79±0.12
200 mg/kg D. sissoo	0.47±0.08	0.28±0.06	2.97±1.23	0.81±0.06
500 mg/kg D. sissoo	0.49±0.03	0.31±0.12	2.67±1.14	0.78±0.09
1000 mg/kg D. sissoo	0.51±0.09	0.29±0.07	2.93±1.67	0.75±0.11
Female				
Control	0.49±0.08	0.33±0.10	3.14±1.05	0.752±0.17
200 mg/kg D. sissoo	0.46±0.09	0.31±0.12	2.67±1.64	0.69±0.12
500 mg/kg D. sissoo	0.51±0.11	0.34±0.08	2.74±1.87	0.76±0.09
1000 mg/kg D. sissoo	0.46±0.14	0.29±0.06	2.88±0.94	0.71±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 D. sissoo extract on body weight

Table 3 shows the body weight of rats before, during and after the treatment. The daily oral administration of D. sissoo extract at all doses 200, 500 and 1000 mg/kg for 28 days did.

Table 3: Effect of D. sissoo extract on the body weights (g).

Group	Day 0	Day 14	Day 28
Male			
Control	163.08±13.78	164.70±13.77	165.83±14.03
200 mg/kg D. sissoo	199.66±11.43	204.16±11.76	208.5±13.25
500 mg/kg D. sissoo	262.66±11.15	277.33±9.92*	281±9.48*
1000 mg/kg D. sissoo	277±10.87	258.66±24.29*	272.66±11.87*
Female			
Control	162.87±10.60	162.87±10.60	163.5±10.72
200 mg/kg D. sissoo	190±8.52	198.66±7.03*	204.83±4.41*
500 mg/kg D. sissoo	193.33±3.51	198.16±3.28*	200.66±3.57*
1000 mg/kg D. sissoo	239.5±7.01	242±8.06*	242.33±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 D. sissoo extract on hematological parameters

The effects of the subacute oral administration of the D. sissoo extract on hematological parameters are represented in Table 4.

Table 4: Effect of D. sissoo extract on the hematological parameters.

Parameters	Control	200 mg/kg	500 mg/kg	1000 mg/kg
Male				
Hb (gm %)	16.85±1.58	19.025±1.29	19.52±0.39	22.85±07*
RBC (x $10^3/cm^2$)	7.61±0.17	7.20±0.04	6.84±0.132*	6.47±0.19**
WBC (x $10^3/cm^2$)	14.6±0.34	13.7±0.17	12.95±0.59*	13.35±0.15
PLT (x $10^5/cm^2$)	43.52±0.28	41.85±0.37*	41.77±0.20*	41.17±0.43**
PCV (%)	58.27±0.67	58.15±0.11	58.55±0.37	59.02±0.48
MCV (fl)	54.71±0.30	33.67±0.80*	34.17±0.99*	34.52±0.96*
MCH (pg)	18.33±0.41	18.05±0.32	18.77±0.16	18.54±0.36
MCHC (gm/dl)	673.75±14.07	645.5±14.25	720.75±4.88*	687.5±8.12
Neutrophils (N %)	17.52±0.36	21.97±0.24*	19.55±0.68*	23.55±0.51*
Eosinophils (E %)	7.48±0.17	7.02±0.17	6.87±0.05*	6.62±0.11*
Lymphocytes (L %)	12.9±0.14	13.7±0.09*	13.7±0.18*	13.07±0.23
Monocytes (M %)	42.97±0.33	42.15±0.23	41.3±0.40*	41.47±0.47
Female				
Hb (gm %)	18.25±2.49	21.27±1.78	19.2±1.12	21.92±0.41
RBC (x $10^3/cm^2$)	6.93±0.09	6.76±0.08	6.32±0.11*	6.85±0.04
WBC (x $10^3/cm^2$)	11.12±1.26	13.1±0.38	13.15±0.35	13.25±0.26
PLT (x $10^5/cm^2$)	40.42±0.35	37.87±0.67	37.07±0.53*	37.7±0.84*
PCV (%)	58.8±0.18	58.5±0.38	57.02±0.28*	56.92±0.20*
MCV (fl)	49.8±0.52	35.6±0.29*	35.12±1.00*	35.8±0.24*
MCH (pg)	19.55±0.39	20.3±0.28	19.55±0.21	20.02±0.08
MCHC (gm/dl)	675.75±24.28	678.5±21.74*	676.75±32.95	688.5±11.89
Neutrophils (N %)	15.3±0.27	15.9±0.55	15.45±1.24*	15.5±0.65*
Eosinophils (E %)	7.56±0.23	6.47±0.08*	6.35±0.18*	6.65±0.09*
Lymphocytes (L %)	12.12±0.34	12.76±0.30*	13.1±0.31	12.67±0.11*
Monocytes (M %)	39.35±0.45	38±0.73	37.75±0.37*	38.52±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 D. sissoo extract on biochemical parameters

The effects of the subacute oral administration of the D. sissoo extract on biochemical parameters are represented in Table 5.

Table 5: Effect of D. sissoo extract on the hematological parameters.

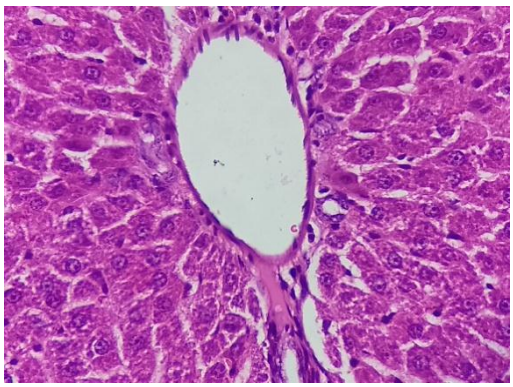
Parameters	Control	200 mg/kg	500 mg/kg	1000 mg/kg
Male				
Urea (mmol/L)	6.22±0.20	5.31±0.27	5.91±0.46	5.223±0.18*
Urea Nitrogen (mmol/L)	7.61±0.23	5.44±0.22*	5.04±0.50*	5.06±0.28*
Creatinine (µmol/L)	44.67±1.17	41.11±1.44	43.37±2.13	48.45±0.59
Total protein (g/L)	66.81±1.01	73.20±1.59	90.345±4.54*	81.52±2.38*
Albumin (g/L)	35.6±1.19	28.66±0.99*	30.95±0.91*	32.94±0.09
ALP (U/L)	286.46±6.37	281.14±0.29	310.56±5.11	298.89±10.98
AST (U/L)	114.22±5.86	119.97±6.02	125.65±4.52	127.58±9.26
ALT (U/L)	50.20±1.22	52.39±0.87	49.32±1.20	54.84±0.73
Bilirubin (Total) (mg/dl)	0.54±0.04	0.44±0.38	0.44±0.03	0.54±0.05
Female				

Urea (mmol/L)	6.22±0.20	5.78±0.17	6.04±0.46	6.22±0.31
Urea Nitrogen (mmol/L)	7.61±0.23	5.79±0.30*	5.87±0.35*	5.43±0.21*
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
ALP (U/L)	286.46±6.37	358.89±13.70*	290.30±18.39	238.82±9.82
AST (U/L)	114.22±5.86	136.15±7.39	119.97±4.19	130.94±5.99
ALT (U/L)	50.20±1.22	51.00±1.43	53.29±0.73	54.60±0.72
Bilirubin (Total) (mg/dl)	0.54±0.04	0.43±0.03	0.46±0.03	0.62±0.04

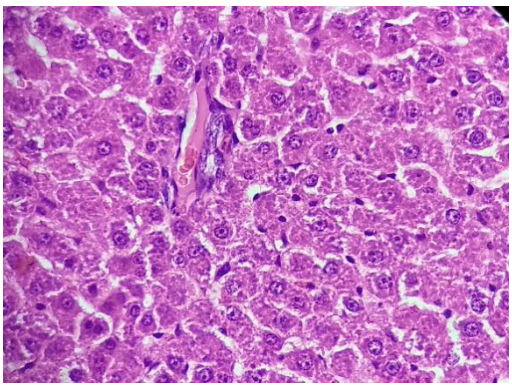
*Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. *p>0.05.*

Histopathological Examination

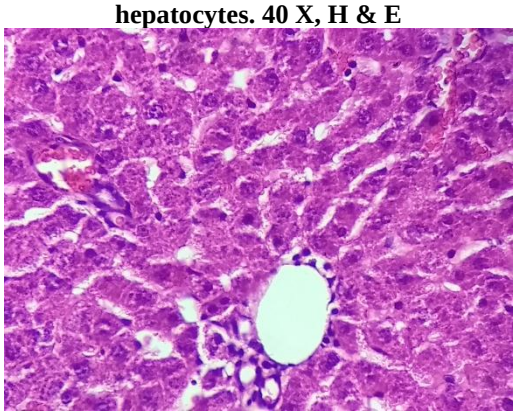
Liver: Microscopic examination of liver showed minimal to mild focal hepatocellular infiltration of inflammatory cells in both male and female animals treated with extract at 500 and 1000 mg./kg body weight when compared with control group (Figure 1).



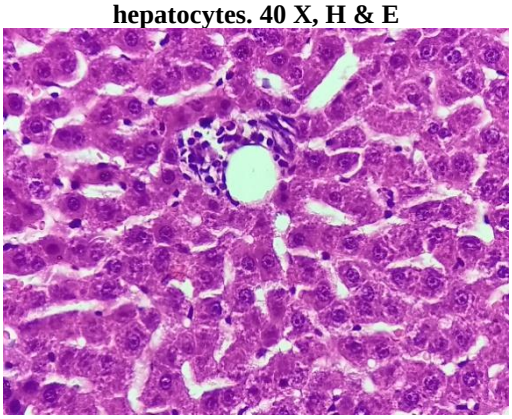
Control; Male; Liver; Showing normal periportal hepatocytes. 40 X, H & E



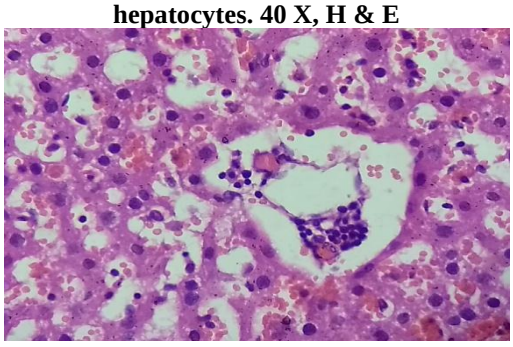
Control; Female; Liver; Showing normal Centrilobular hepatocytes. 40 X, H & E



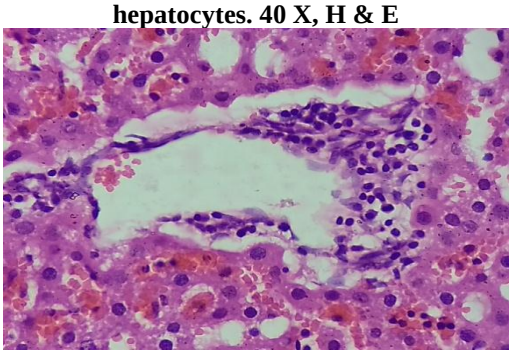
Low Dose; Male; Liver; Showing normal Centrilobular hepatocytes. 40 X, H & E



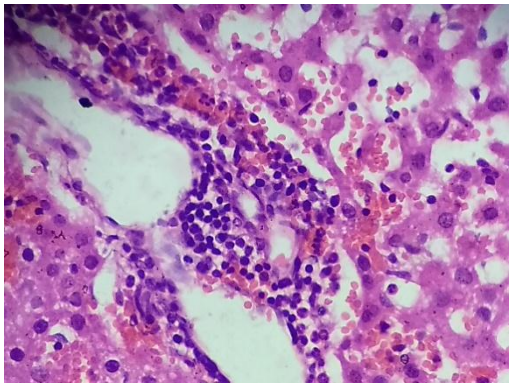
Low Dose; Female; Liver; Showing normal Centrilobular hepatocytes. 40 X, H & E



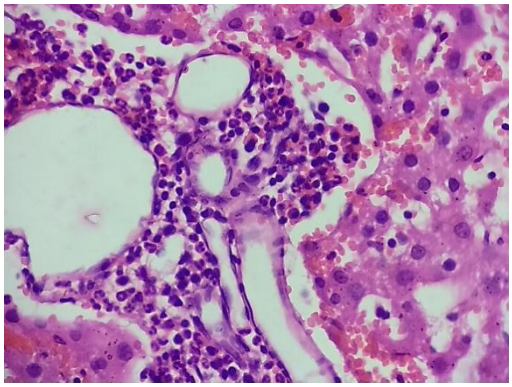
Medium Dose; Male; Liver; Showing mild sinusoidal dilation with infiltration of inflammatory cell (Minimal). 40 X, H & E



Medium Dose; Female; Liver; Showing mild sinusoidal dilation with infiltration of inflammatory cell (Minimal). 40 X, H & E



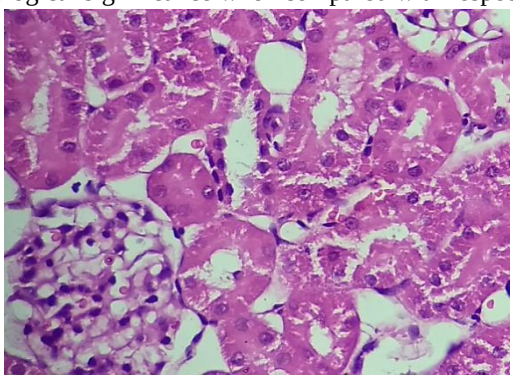
High Dose; Male; Liver; Showing mild sinusoidal dilation with infiltration of inflammatory cell (Mild). 40 X, H & E



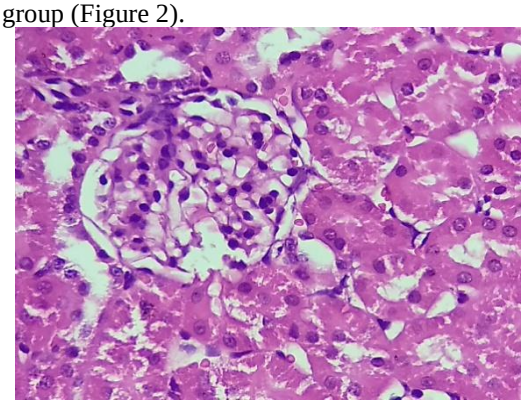
High Dose; Female; Liver; Showing mild sinusoidal dilation with infiltration of inflammatory cell (Mild). 40 X, H & E

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

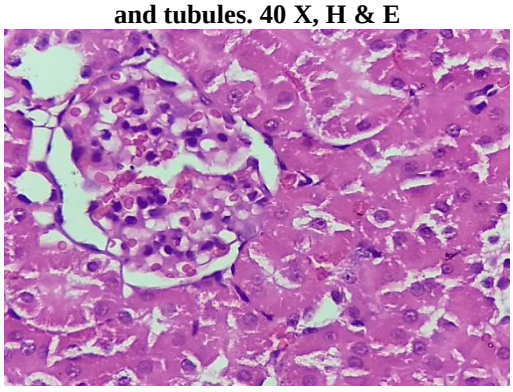
Kidney: Microscopic examination of Kidneys from both male and female of all groups did not show any lesion of pathological significance when compared with respective control group (Figure 2).



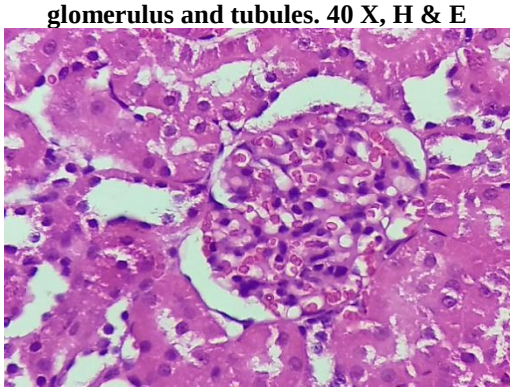
Control; Male; Kidneys; Showing normal glomerulus and tubules. 40 X, H & E



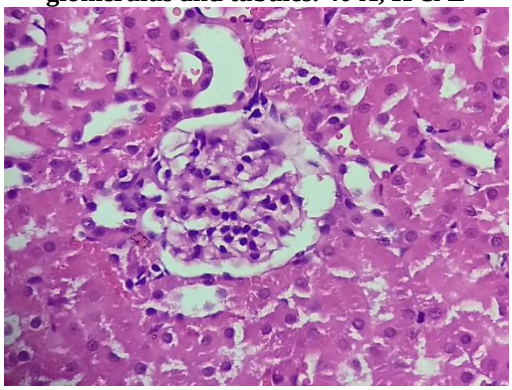
Control; Female; Kidneys; Showing normal glomerulus and tubules. 40 X, H & E



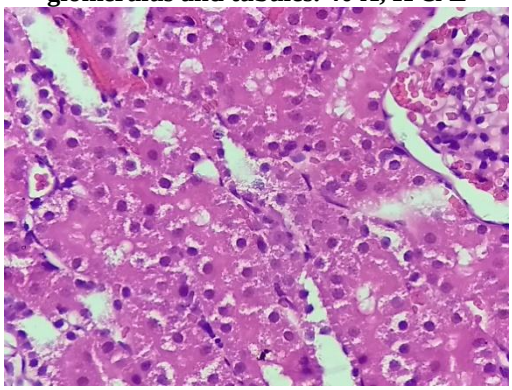
Low Dose; Male; Control; Kidneys; Showing normal glomerulus and tubules. 40 X, H & E



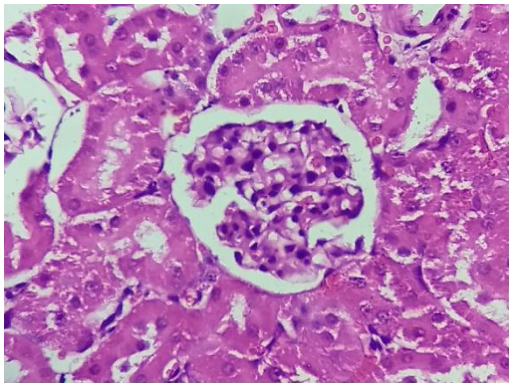
Low Dose; Female; Control; Kidneys; Showing normal glomerulus and tubules. 40 X, H & E



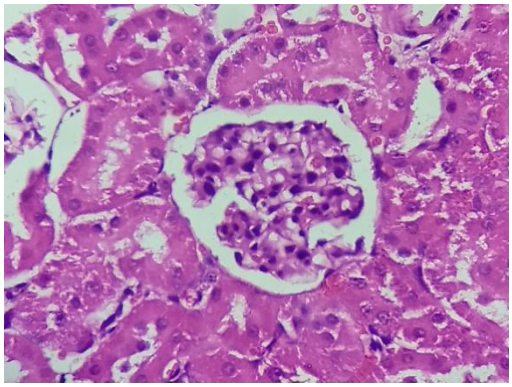
Medium Dose; Male; Kidneys; Showing normal glomerulus and tubules. 40 X, H & E



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



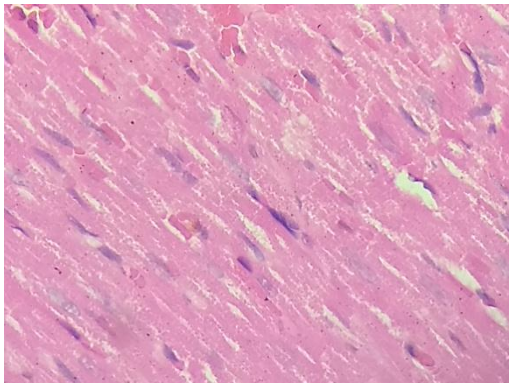
High Dose; Male; Kidneys; Showing tubular degeneration. 40 X, H & E



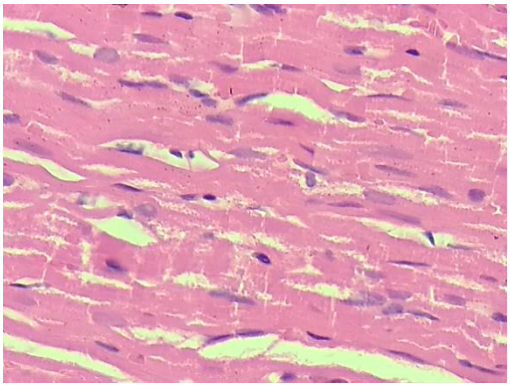
High Dose; Female; Kidneys; Showing tubular degeneration. 40 X, H & E

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

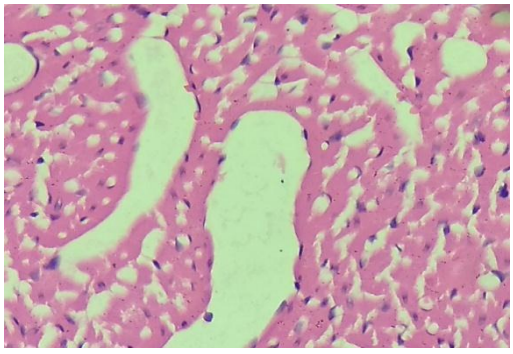
Heart: Microscopic examination of heart from both male and female of all groups did not show any lesion of pathological significance when compared with respective control group (Figure 3).



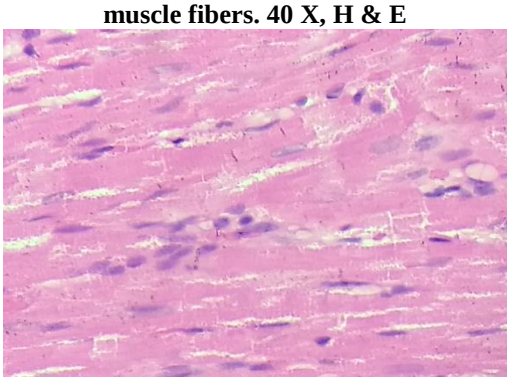
Control; Male; Heart; Showing normal myocardial muscle fibers. 40 X, H & E



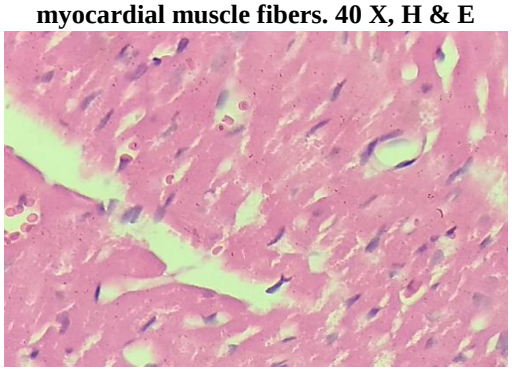
Control; Female; Heart; Showing normal myocardial muscle fibers. 40 X, H & E



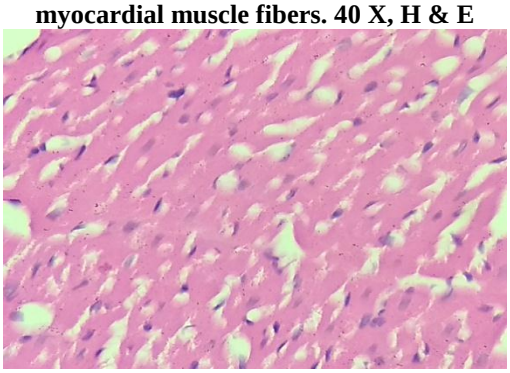
Low Dose; Male; Control; Heart; Showing normal myocardial muscle fibers. 40 X, H & E



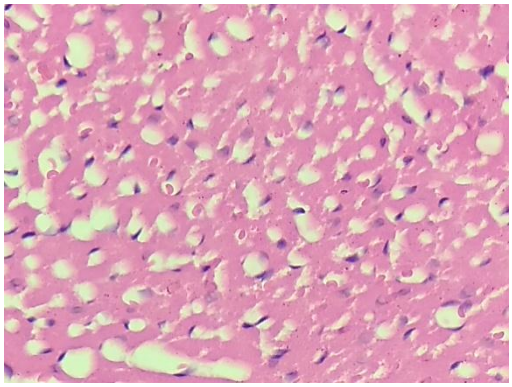
Low Dose; Female; Control; Heart; Showing normal myocardial muscle fibers. 40 X, H & E



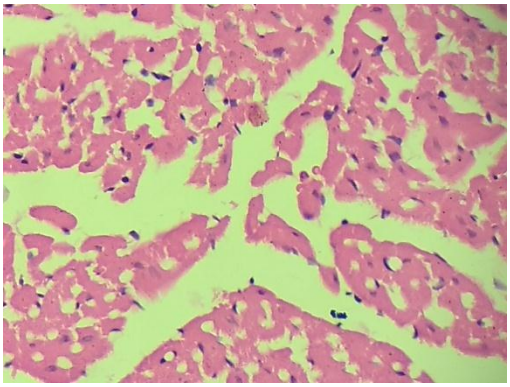
Medium Dose; Male; Heart; Showing normal myocardial muscle fibers. 40 X, H & E



Medium Dose; Female; Heart; Showing normal myocardial muscle fibers. 40 X, H & E



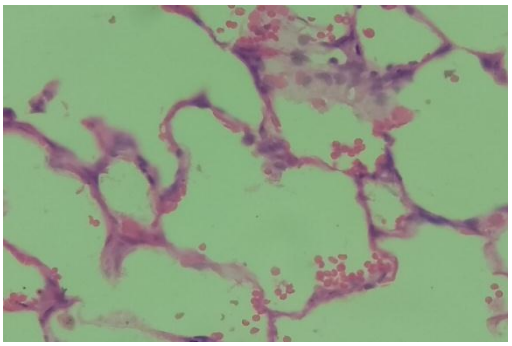
High Dose; Male; Heart; Showing normal myocardial muscle fibers. 40 X, H & E



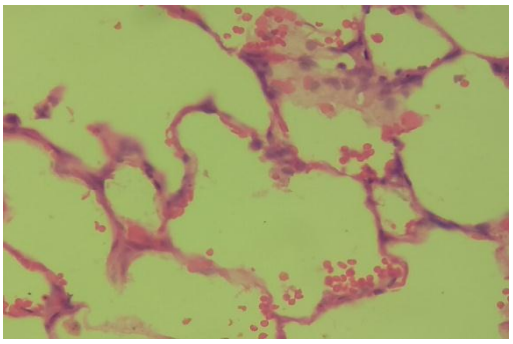
High Dose; Female; Heart; Showing normal myocardial muscle fibers. 40 X, H & E

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

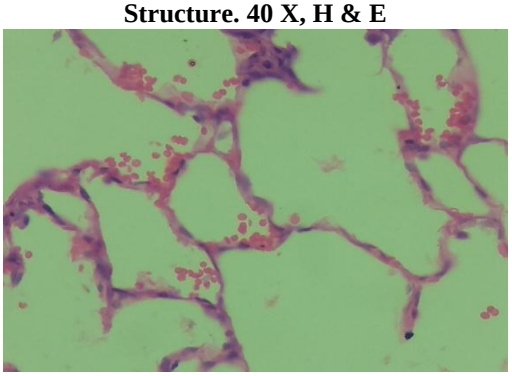
Lungs: Microscopic examination of Lungs from both male and female of all groups did not show any lesion of pathological significance when compared with respective control group (Figure 4).



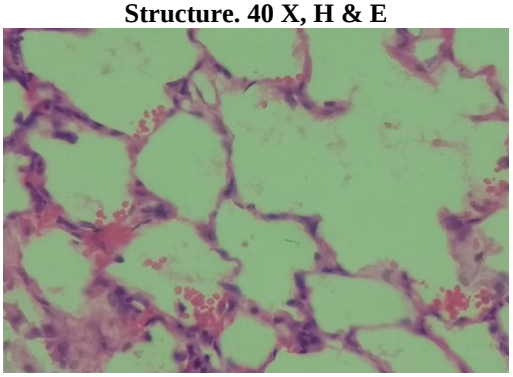
Control; Male; Lungs; Showing normal alveolar Structure. 40 X, H & E



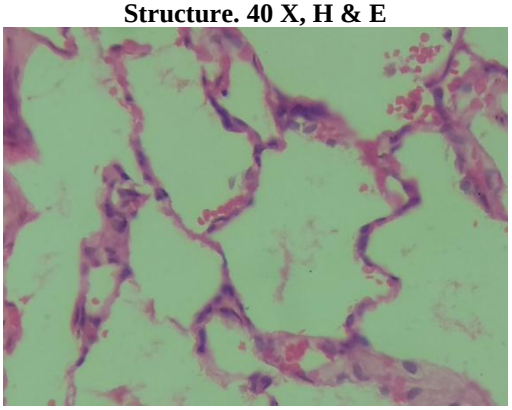
Control; Female; Lungs; Showing normal alveolar Structure. 40 X, H & E



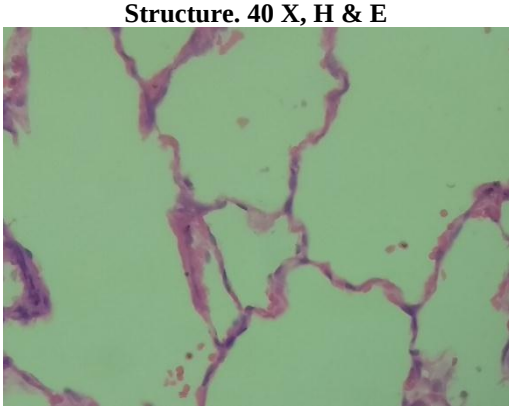
Low Dose; Male; Lungs; Showing normal alveolar Structure. 40 X, H & E



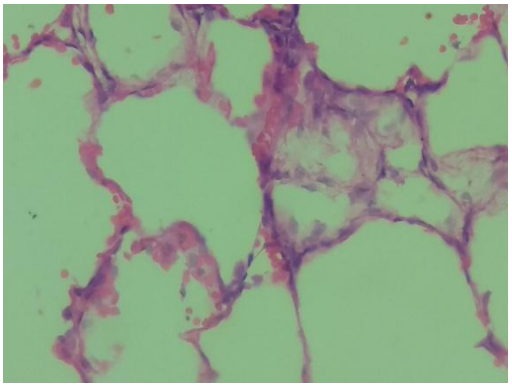
Low Dose; Male; Lungs; Showing normal alveolar Structure. 40 X, H & E



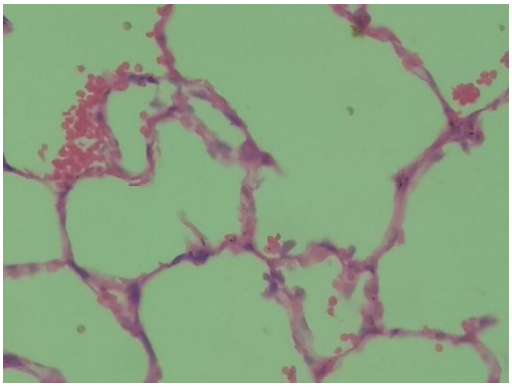
Medium Dose; Male; Lungs; Showing normal alveolar Structure. 40 X, H & E



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

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

Therefore, on the basis of histopathology findings, it can be concluded that animals treated with extract at 1000 mg/kg body weight showed hepatocellular infiltration of inflammatory cells in liver 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.

DISCUSSION

The use of herbal medicines as alternative treatments has been increasing worldwide and gaining popularity in developing countries. Although medical plants may have biological activities that are beneficial to humans, the potential toxicity of these bioactive substances has not been well established[10]. Thus, the safety and efficacy of these plants must be studied thoroughly to maximize their benefits for mankind. 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 hallmarks of pathological and physiological wellness status of animals.[11,12] Changes in organ weights are hallmarks of toxicity in experimental animals, which are determined by toxicity tests.[13] The toxic effect of ingested herbal remedies in the body is most likely to be felt by important organs such as the heart, liver, lungs and kidneys because of the vital roles that they play in the body.[14] The liver and kidneys are major targets of xenobiotic action, with the liver being the major organ for xenobiotic biotransformation, while the kidney serves as excretory organ of xenobiotics.[15] Our findings on organ weight revealed that there was no significant increase in organ weight (Table 2), suggesting that the D. sissoo extract was not toxic to the animals at the tested doses.

The wellness status of animals is hinged on changes in body weight.[16] After 28 days of treatment, all the animals exhibited a steady increase in body weight. It indicated that the daily intake of D. sissoo extract did not alter food intake. Furthermore, it possibly shows that weight gain and appetite stability were not impeded by the extract during the exposure period. This validates the oral route folkloric usage of the D. sissoo extract.

The evaluation of hematological parameters is of great importance in determining the health status of an individual.[17] 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 increased release of WBC's is a notable biomarker of stress and also aids in defending the body against some inflammatory conditions, such as bacterial infections, leukemia and hemorrhage. The result obtained from this study revealed that D. sissoo extract did not cause any significant changes in the levels of WBC count, or in their subtypes, including neutrophils, lymphocytes, monocytes and eosinophils, at any of the doses, relative to control (Table 4). This suggests that the D. sissoo extract is nontoxic. The retention of creatinine, electrolytes, urea and uric acid in the body is indicators of kidney damage[16, 17, 18]. Alteration in the levels of some electrolytes such as Na^+, K^+, Cl^- and Mg^{2+} can also be a sign of renal injury kidney disease.[19] Our findings revealed that there was no significant difference in the level of creatinine, urea or uric acid in the three dose groups, when compared with the control group, in both sexes of the rats. This provides further support for the safety of D. sissoo extract at these doses, as there was no alteration in kidney function. Elevated levels of serum transaminase enzymes (ALT, AST and ALT) are clear indications of hepatic impairment in animals. The insignificant changes in plasma levels of AST, ALT and ALP at the doses of 200, 500 and 1000 mg/kg in both sexes of the animals are clear indications that the extract caused no damage to the liver. Inflammatory responses are mostly triggered by a decrease in plasma albumin levels[20]. There was no significant difference in the levels of total protein, albumin, total bilirubin as shown in Table 5. This indicates that D. sissoo extract did not obstruct kidney's ability to excrete the above-mentioned metabolites.

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 D. sissoo extract.

D. sissoo 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 D. sissoo 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 chemistry analysis on both sexes. Also, the extract did not bring about any changes in food intake, water consumption or body weight and produced no evident histopathological damage in the rat organs, regardless of gender. Furthermore, the results obtained from subacute toxicity studies of D. sissoo could thus give insight to its safety in humans. There is need for further subacute toxicity studies of the extract on pregnant rats and fetus to further explore the safety of this plant.

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