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Toxicity Assessment of *Sesbania sesban* var. *bicolor*, a Traditionally-used Anthelmintic Medicinal Plant, in Rodent Models

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ABSTRACT

Sesbania sesban var. *bicolor*, a tropical shrub,is a medicinal plant in India. This assessment reports the toxic potentials of its leaf extract. Acute oral toxicity was executed in mice whereas sub-acute toxicity was carried out using rodent models. The study showed that the median lethal dose was over than 5000 mg/kg. Toxicity assessments displayed a mild increase in neutrophils and a mild decrease in eosinophil and monocyte counts in rats. Also, the SGOT and SGPT levels were found to be raised, indicating evidence of hepatotoxicity. The mitochondria of kidney and liver tissues studied by transmission electron microscopy revealed damage in the cristae and membrane. Together, it may be concluded that *S. sesban* var. *bicolor* extract possesses mild toxicity in experimental animals. Therefore, despite its efficacy in traditional medicine, the long-term use of this plant should be controlled.

Keywords: Acute toxicity; Anthelmintic; *Sesbania sesban var. bicolor*; Sub-acute toxicity; Traditional medicine

1. INTRODUCTION

Traditional medicines are commonly used as an easily accessible and affordable source of health care in India[1]. For nearly 75 % of the global population, traditional medicines use as the first line of treatment[2]. Despite of their widespread use, several such medicines have been reported to be toxic[3]. A study on two African medicinal plants, *Cassia occidentalis* and *Ziziphus mauritiana* revealed that their long-term use can cause adverse effects[4]. Similarly, *Cymbopogon giganteus*, used to treat hepatitis in Africa, was reported to cause mortality and lung inflammation[5]. Although some studies have also documented the usage of traditional medicines to be safe[5-6], these should undergo rigorous safety assessments before they are recommended to potential users[7].

S. sesban var. *bicolor* is a branched shrub distributed in tropical regions, including India[8-9]. It is used traditionally to treat diarrhea, splenomegaly, rheumatism, excessive menstrual flow, and skin diseases[9]. The anthelmintic[9], antioxidant[10] and anti-diabetic[11] potentials of this plant have also been reported. Earlier phytochemical studies have detected tannins, phenols, carbohydrates, proteins, flavonoids, steroids and alkaloids[12] which could be the source for its multiple healing effects. This assessment was executed to validate the toxic potentials of *S. sesban* var. *bicolor* leaves extract (SLE) utilizing rodent models.

2. METHODOLOGY

2.1 Plant Material and Experimental Animals

Fresh leaves of *S. sesban* var. *bicolor* were harvested from Kokrajhar. It was identified by a taxonomist and assigned the voucher number as NEHU-12084 in the Ethnopharmacology Laboratory, Department of Zoology, NEHU, Shillong. Leaves were shade-dried and extracted in methanol and the yield was 19.52 % (w/w). This extract was preserved at 4 °C. Female Swiss albino mice (25 to 30 g) and albino rats of Wistar strain (190 to 200 g) were used for acute toxicity and sub-acute toxicity study, respectively. The Institutional Ethics Committee (Animal Models), North-Eastern Hill University, approved the use of animal models (Approval: IEC, NEHU, 4/12/2014).

2.2 Toxicity Assessments

Acute toxicity study was executed on five female mice who were dosed with 2,000 mg/kg, one after the other, after careful observation for 48-h.The animals were continuously observed for 14 days, and then later dosed at 5000 mg/kg of extract, if three or more animals survived[13-14]. For sub-acute toxicity study, threedoses of extract were designated based on an earlier study[15]. Animals were placed in 6 groups (5 males and 5 females in each) and dosed orally, once daily for 28 days[16]. Group I (control) was given only the vehicle (phosphate-buffered saline). Groups II, III, and IV were orally given 100 mg/kg, 200 mg/kg and 400 mg/kg of plant extract, respectively. Animals in group V served as satellite control and was given orally only the vehicle. Animals in group VI served as the satellite group and they were given the highest dose for 28 days, but were observed for another 14 days, to detect any recovery or delayed effects of extract treatment. Any deviations from normal behaviour, weights and the amount of food and water consumed by animals were noted. On day 29, animals were euthanized and blood specimens were acquired through cardiac puncture to perform the haematological and biochemical study parameters.

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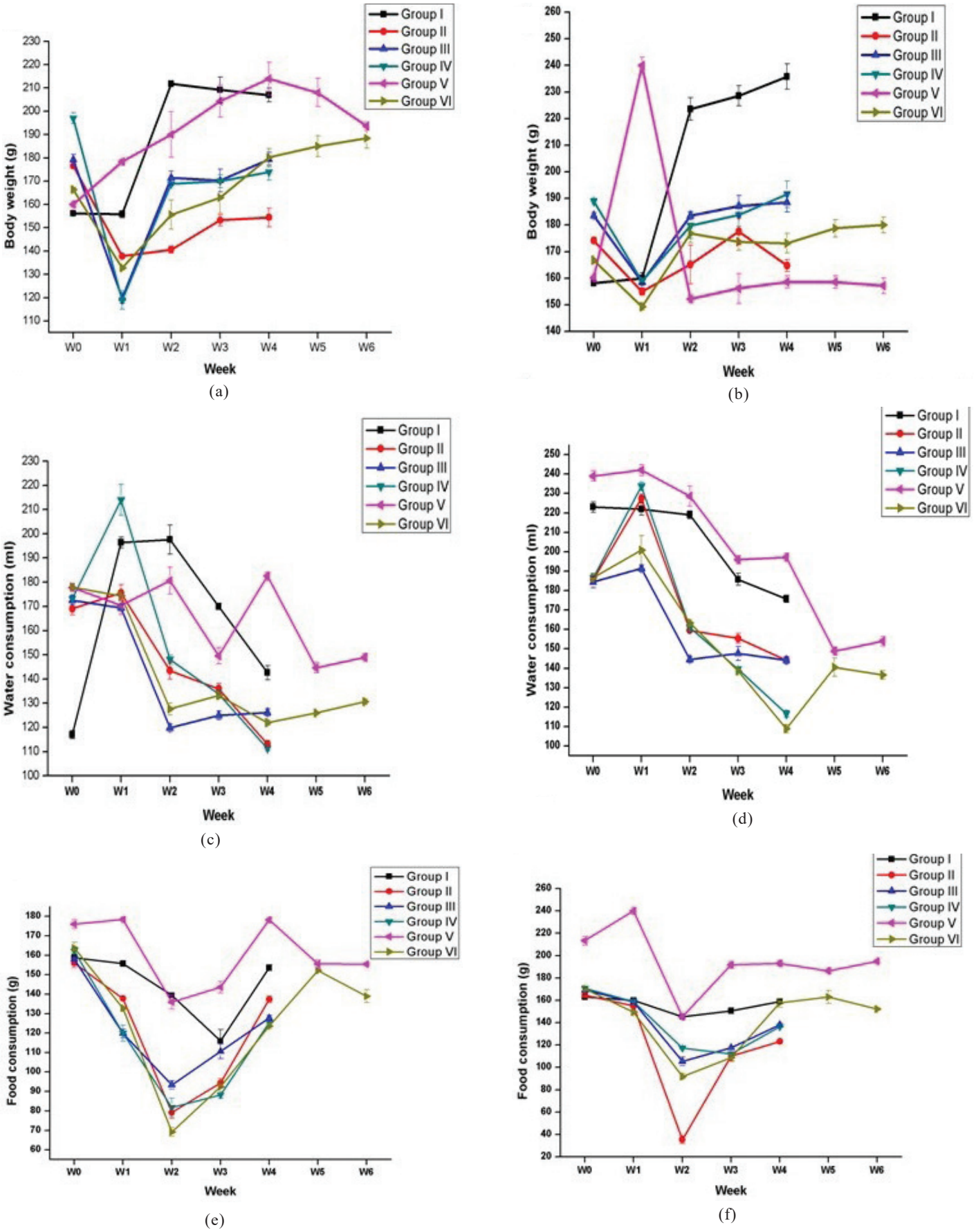

Figure 1. Effects of SLE on: A =Body weights of female rats, B = Body weights of male rats, C =Water consumption of female rats, D =Water consumption of male rats, E = Food consumption of female rats, F =Food consumption of male rats.

2.3 Transmission Electron Microscopy (TEM) Study

After necropsy of animals on day 29, liver and kidney of animals treated with the highest dose (400 mg/kg) were retrieved and processed for viewing in a JEM 100 CXII (Jeol) TEM to detect any abnormalities in the tissues resulting from plant dose administration[17].

2.4 Statistical Analysis

Analysis of the generated data were carried out by student's *t*-test and ANOVA, succeeded by Tukey's post hoc test. The data are depicted as mean ± standard error of mean (S.E.M.).

3. RESULTS AND DISCUSSION

3.1 Toxicity Assessments

Administration of 2000 and 5000 mg/kg of extract did not manifest any toxic signs nor it cause mortality of animals. All animals appeared healthy throughout the study and beyond. Hence, it was concluded that the oral LD_{50} of SLE is over 5000 mg/kg which as per the Globally Harmonized System (GHS) is considered safe[18].

Control group of animals dosed for 28-days displayed a rise in body weight. On the contrary, all treated groups showed a decline in body weights, during the first week which successively elevated during the following weeks (Fig. 1(a), (b)). The treated animals also showed a gradual decrease in water intake, whereas an increase was observed after the treatment period in the satellite groups (Fig. 1(c), (d)). The food intake decreased till week 2 and then increased gradually after that. However, the treated animals revealed a downfall in food intake all along week 2, 3 and 4 (Fig. 1(e), (f)). It is established that variations in food and water consumption indicate the toxic potentials of test materials[19]. However, no significant variations were observed in these parameters in extract treated animals.

Researchers have used both the sexes of experimental animals in their assessments to determine if sex could cause any differences in the parameters[6] as also demonstrated in this study. Haematological studies showed an elevation in neutrophil counts in all the treated groups, and a decrease in monocyte and eosinophil counts in both the sexes of animals. These parameters however were recoverable in the satellite group, indicating that the long-term usage of plant could be a prime factor for their alterations. The white blood cells (WBC's) are the first cell type to arrive at the site, indicating the presence of foreign particles[20]. The findings also revealed araised platelet count in the satellite group, depicting that the damaging effects were seen even after the dosing was discontinued. The other examined parameters did not show any deviations (Table 1). Serum examination of animals showed an elevation in SGOT and SGPT in a dose-dependent manner. These parameters were also found to be raised in the satellite group of animals (Table 2). Elevations of these enzymes are a clear indication of liver damage depicting a hepatotoxic effect[21]. A toxicity assessment by Saleem and coworkers on *Saccharum munja* projected similar findings, where these marker enzymes were noted to beraised, indicating the plant to be hepatotoxic[22]. Alterations in body weight, food and water consumption parameters were found to be insignificant in animals.

A toxicity assessment was carried out on *Salacia reticulata*, where investigators evaluated similar parameters using the same procedures as used in our study. The workers reported that the administration of plant extract did not cause any changes in the feeding habits and body weights of experimental animals. Also, organ weights and the analyzed biochemical and haematological parameters were found to be

Table 1. Effects of SLE on haematological parameters

Parameters	Unit	Group – I Control	Group – II 100 mg/kg	Group – III 200 mg/kg	Group – IV 400 mg/kg	Satellite groups	
						Group – V Control	Group – VI 400 mg/kg
Female rats							
RBC	10^6/cmm	7.40±0.06	7.26±0.06	7.36±0.07	6.88±0.21^{b}	5.96±0.10	7.35±0.15^{cd}
WBC	/cmm	5060±92.73	5760±50.99^{ab}	5820±66.33^{ab}	6940±67.82^{ab}	5880±37.41	5336±37.76^{cd}
Neutrophils	%	20.6±0.871	31.2±0.374^{ab}	30±0.316^{ab}	25.4±-0.4^{ab}	20.8±0.374	19.2±0.583^{d}
Lymphocytes	%	63.8±0.663	62.4±0.4	63.2±0.374	66.4±0.509^{b}	62.4±0.4	66.6±0.509^{cd}
Eosinophils	%	7.8±0.2	3.4±0.244^{ab}	3.6±0.244^{ab}	5.2±0.2^{ab}	8.4±0.244	6.8±0.583^{d}
Monocytes	%	7.8±0.2	3±0^{ab}	3.2±0.2^{ab}	2.8±0.2^{ab}	8.4±0.244	7.4±0.244^{d}
Haemoglobin	gm/dL	12.92±0.13	13.64±0.17^{b}	13.56±0.16^{b}	12.36±0.05	12.32±0.05	14.34±0.14^{cd}
Platelets	/cmm	651800±3624	647000±5495	662800±5580	840400±509^{ab}	718800±22762	645184±1622^{d}
Male rats							
RBC	10^6/cmm	7.63±0.04	7.38±0.04	7.59±0.09	7.50±0.08	6.60±0.11	6.88±0.04
WBC	/cmm	5940±186.01	5980±58.30	6140±60	7200±44.72^{ab}	6320±66.33	5706±41.78^{cd}
Neutrophils	%	14.2±0.374	27.6±0.509^{ab}	26.4±0.509^{ab}	25.2±0.374^{ab}	13.4±0.4	13.8±1.067
Lymphocytes	%	69.8±0.2	63.6±0.244^{ab}	64.6±0.244^{ab}	66.6±0.244^{ab}	69.4±0.244	72.8±0.374^{cd}
Eosinophils	%	8±0.316	4.8±0.374^{ab}	4.8±0.374^{ab}	5±0.316^{ab}	8.2±0.2	6.8±0.489^{d}
Monocytes	%	8±0	4±0^{ab}	4±0^{ab}	3.2±0.2^{ab}	9±0	6.6±0.4^{cd}
Haemoglobin	gm/dL	14.2±0.09	14.04±0.08	13.94±0.25	12.52±0.14	12.56±0.13	13.84±0.16
Platelets	/cmm	666800±6240	667400±5608	677600±4354	863600±9667^{ab}	881200±4188	564400±1691^{cd}

Significant at $^{a}p<0.001$, $^{b}p<0.05$ compared to control, $^{c}p<0.001$, $^{d}p<0.05$ compared to satellite control group.

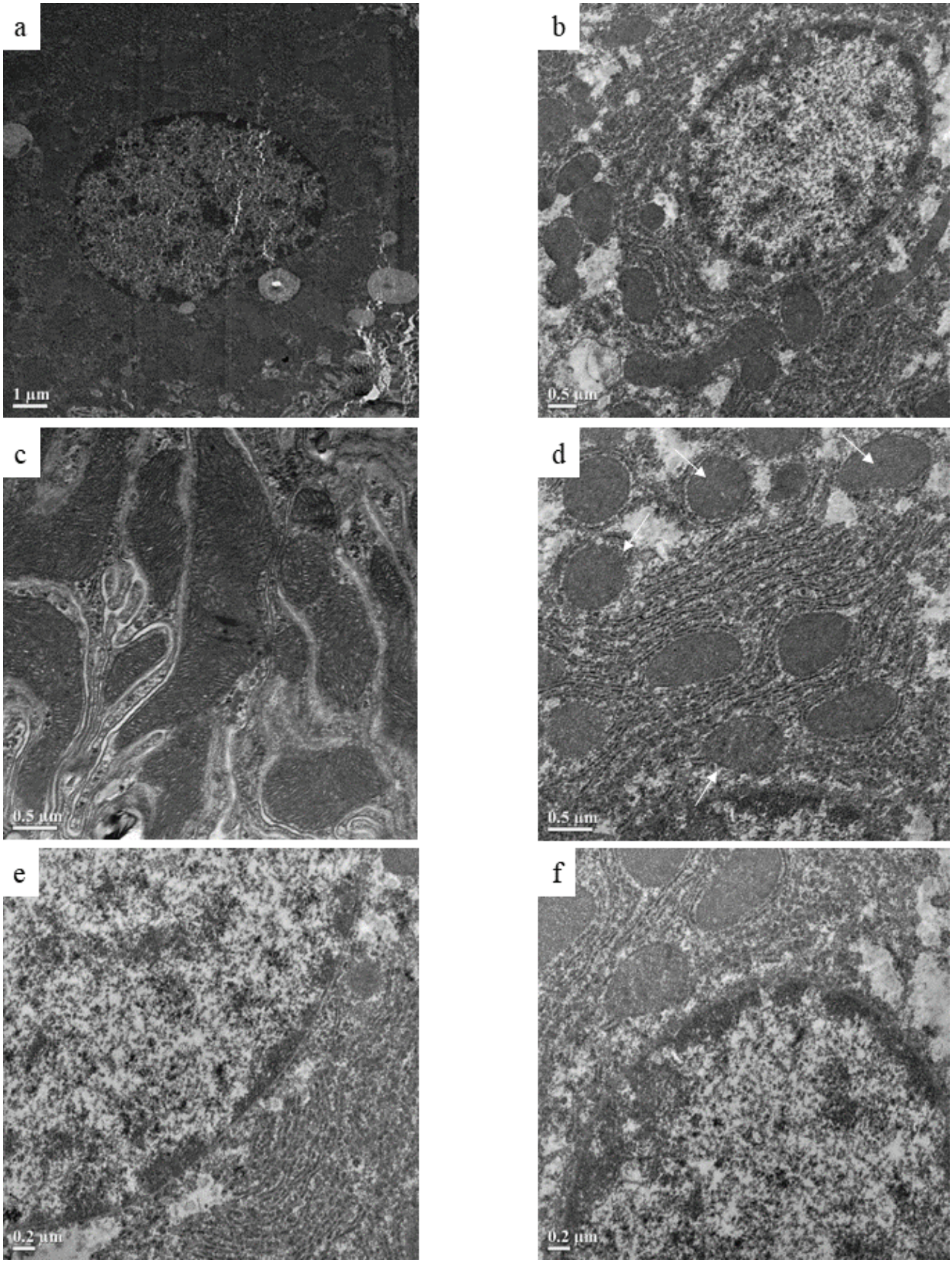

Figure 2. Transmission electron micrographs of liver of rats: A = Control animals, showing nucleus, nucleolus, and double layered nuclear membrane, B =Extract treated animals, showing regular architecture, C = Control animals, showing mitochondria, D = Extract treated, showing broken cristae and dissolute mitochondrial membrane (arrows), E = Control animals, showing ER, F = Extract treated rats, showing regular ER.

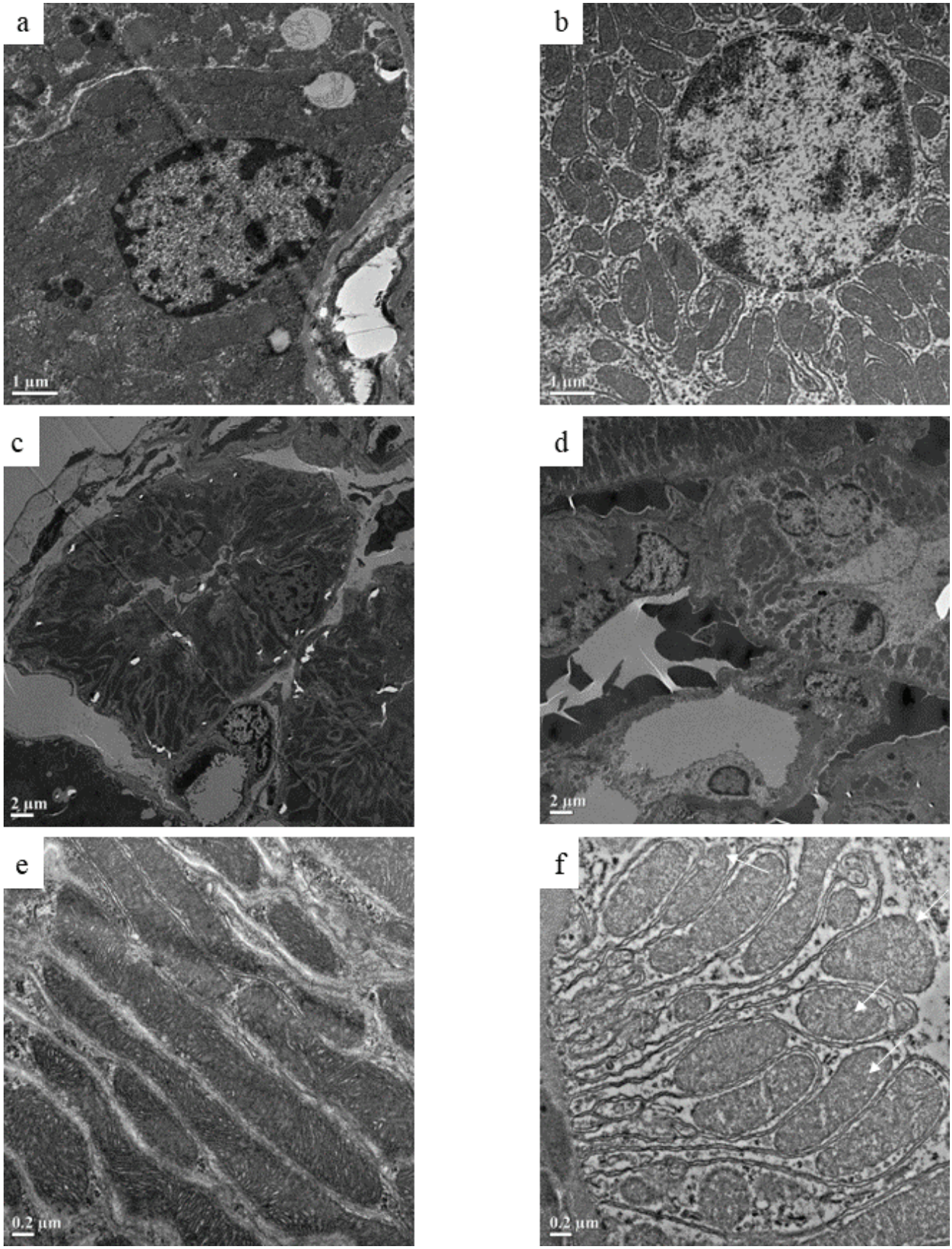

Figure 3. Transmission electron micrographs of kidney of rats: A =Control animals, showing nucleus, nucleolus, and double layered nuclear membrane, B = Extract treated animals, showing regular architecture, C = Control animals, showing normal tubule, D =Extract treated animals, showing regular tubule, E = Control animals, showing mitochondria, F =Extract treated animals, showing broken cristae and dissolute mitochondrial membrane (arrows).

Table 2. Effects of SLE on biochemical parameters

Parameters	Unit	Group – I Control	Group – II 100 mg/kg	Group – III 200 mg/kg	Group – IV 400 mg/kg	Satellite groups	
						Group – V Control	Group – VI 400 mg/kg
Female rats							
SGOT	U/L	159.01±0.78	156.32±0.42	176.46±5.83^{b}	185.72±0.44^{ab}	152.36±0.60	168.38±0.53^{cd}
SGPT	U/L	64.35±0.23	65.76± 0.37^{ab}	80.97±1.52^{ab}	82.92±0.33^{ab}	90.93±0.14	178.23±0.41^{cd}
ALP	U/L	308.52±0.56	307.39±0.31	325.45±4.14^{ab}	306.06±0.31	303.88±1.73	593.07±12.39^{cd}
Tot Bil	mg/Dl	0.86±0.003	0.80±0.003^{ab}	0.86±0.005	0.67±0.007^{ab}	0.52±0.003	0.72±0.008^{cd}
Albumin	mg/Dl	3.42±0.16	3.98±0.06	4.58±0.21^{ab}	3.63±0.13	3.40±0.14	4.74±0.18^{cd}
Creatinine	mg/dL	0.66±0.003	0.73±0.009^{b}	0.75±0.02^{b}	0.63±0.01	1.00±0.03	0.93±0.03
Urea	mg/Dl	16.07±0.25	16.21±0.26	19±0.23^{ab}	14.59±0.10^{b}	19.18±0.34	18.44±0.33
Uric acid	mg/dL	3.39±0.16	4.25± 0.08^{b}	3.59±0.23	3.20±0.02	2.49±0.15	3.92±0.04^{cd}
Male rats							
SGOT	U/L	166.79±3.26	160.59±0.24	177.37±0.05	215.92±0.39	158.75±0.26	122.03±0.32
SGPT	U/L	65.72±0.58	68.31±0.50^{ab}	83.71±0.95^{ab}	86.20±0.60^{ab}	93.21±0.46	151.10±0.53^{cd}
ALP	U/L	310.67±0.64	401.99±0.35^{ab}	400.36±0.35^{ab}	318.21±0.54^{ab}	318.35±0.36	427.40±0.45^{cd}
Tot Bil	mg/dL	0.88±0.006	0.93±0.003^{ab}	0.84±0.005^{ab}	0.71±0.008^{b}	0.56±0.008	0.72±0.02^{cd}
Albumin	mg/dL	4.04±0.03	4.64±0.11^{b}	4.49±0.25	3.98±0.06	3.98±0.06	4.36±0.14^{d}
Creatinine	mg/dL	0.69±0.007	0.82±0.006^{ab}	0.77±0.01^{ab}	0.66±0.008	1.17±0.03	1.23±0.06
Urea	mg/dL	17.26±0.83	18.09±0.29	19.70±0.09^{b}	16.92±0.03	21.24±0.33	18.08±0.25^{cd}
Uric acid	mg/dL	3.81±0.05	5.46±0.13^{ab}	3.76±0.23	3.88±0.02	3.10±0.14	4.65±0.04^{cd}

Significant at $^{a}p<0.001$, $^{b}p<0.05$ compared to control, $^{c}p<0.001$, $^{d}p<0.05$ compared to satellite control group.

regular[23]. On the basis of these findings, the authors concluded that the plant to is non-toxic in nature to its users. On the contrary, the sub-acute toxicity study of *Hibiscus rosa-sinensis* revealed that its long-term use caused elevation in SGOT, SGPT, bilirubin, urea and creatinine levels, concluding it to be toxic[24].

3.2 Transmission Electron Microscopic Study

Electron microscope studies of vital organs supplement some value in toxicity studies. Hence several investigators have performed TEM studies of liver, kidney, spleen, and other body organs[25-26]. In this study, the liver of treated animals revealed a regular nucleus (Fig. 2(b)) but in the mitochondria, the membrane was dissolute and the cristae were found to be damaged (Fig. 2(d)). The endoplasmic reticulum (ER) however, was found to be regular (Fig. 2(f)). Likewise, the kidney of the treated animals also showed the same findings where the nucleus (Fig. 3(b)) and tubule (Fig. 3(d)) portrayed regular features, but the mitochondria showed damaged cristae and membrane (Fig. 2(f)). Soren and coworkers reported similar deformities in the mitochondria in a toxicity assessment on *Cyperus compressus*[27]. Such damaged mitochondria will lose its ability to carry out the cellular functions actively. Another study on *S. sesban* revealed that, its long-term use caused damages in spleen of treated animals who showed deformed ER and mitochondria, which was also observed in this study[28]. Assessment of organs to assess organ toxicity have been carried out by several workers.

Studies on *Salacia reticulata* showed that its long-term use did not cause any alterations in the liver and kidney architecture[23]. Contrary to this, authors who evaluated the toxicity of *Hibiscus rosa-sinensis* revealed damaging effects in the kidney tissues in the form of damaged tubules and glomeruli[24].

4. CONCLUSIONS

As depicted in the results, the long-term administration of SLE is capable of causing mild signs of toxicity in experimental animals. Therefore, despite its efficacy in traditional medicine, the long-term usage of this plant should be done with caution.

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CONTRIBUTORS

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