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Comparative Toxicological Assessment of *Dioscorea hispida*, *Dioscorea bulbifera*, and *Dioscorea esculenta* in Wistar Rats: Acute Oral and 28-Day Repeated Dose Studies

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Abstract

The toxicological profiles of three yam species (Dioscorea hispida, Dioscorea bulbifera, Dioscorea esculenta) were evaluated in Wistar rats via OECD-compliant acute oral (Test No. 423) and 28-day repeated-dose oral (Test No. 407) studies. Acute administration of D. hispida extract at 2000 mg/kg caused 100% mortality, whereas 300 mg/kg was non-lethal, yielding an LD_{50} cut-off value 500 mg/kg ($300< ATE \leq 2000$ mg/kg body, GHS Category 4). In contrast, D. bulbifera and D. esculenta extracts induced no mortality up to 2000 mg/kg (LD_{50} >2000 mg/kg, GHS category 5 or unclassified). No clinical signs were seen in surviving acute animals except lethargy and tremor, and in gross observation, lung congestion in D. hispida at the highest dose. In the 28-day study, rats received daily doses up to 600 mg/kg for D. bulbifera/D. esculenta and up to 100 mg/kg for D. hispida. No treatment-related mortality, clinical signs, detailed clinical, functional observation or ophthalmic lesions occurred in any group.

Based on the results, body weight gain, feed consumption, hematology, clinical chemistry, urine analysis, organ weights, and histopathology did not reveal any treatment-related adverse effects at any tested dose except in clinical chemistry effect observed at 100 mg/kg in D. hispida and at 600 mg/kg in D. bulbifera. Consequently, the No-Observed-Adverse-Effect Level (NOAEL) for the 28-day oral toxicity study was determined to be 50 mg/kg/day for Dioscorea hispida, 300 mg/kg/day for D. bulbifera and 600 mg/kg/day for D. esculenta. These findings confirm a distinct toxicity profile: D. hispida exhibits moderate acute oral toxicity (LD_{50} between 300–2000 mg/kg), likely attributable to constituents such as dioscorine and cyanogenic compounds. In contrast, D. bulbifera and D. esculenta demonstrated significantly lower acute hazards, with no mortality observed at the limit dose of 2000 mg/kg. The comprehensive dataset from both acute and repeated-dose studies provides a robust foundation for hazard characterization and risk assessment of these botanical extracts in accordance with OECD and GHS criteria.

INTRODUCTION

Dioscorea is a genus that includes about 600 species that are native to tropical and warm temperate regions of the world. They are members of the *Dioscoreaceae* family within the *Dioscoreales order* [1]. Among these, *D. hispida* (Wild Yam or "Gadung"), *D. bulbifera* (Air potato), and *D. esculenta* (Lesser yam) vary widely in toxicity. *D. hispida* tubers, for example, contain high levels of cyanogenic glycosides (e.g. acetone cyanohydrin) and the toxic alkaloid dioscorine [2]. Traditional processing is required to reduce cyanide content before consumption. Modern analyses confirm that *D. hispida* is "poisonous" due to dioscorine and hydrogen cyanide, which can cause nausea, vomiting, and neurological symptoms upon ingestion. Similarly, *D. bulbifera* tubers contain steroidal saponins (including diosgenin) and alkaloids. Wild cultivars of *D. bulbifera* are reported as "always toxic raw", and cattle ingestion can lead to fatal poisoning. Indeed, extension services note that air potato is "extremely toxic" and should not be eaten. In contrast, *D. esculenta* is generally

regarded as edible; most cultivated varieties are consumed as staple or cash crops in Asia. Its tubers have a sweet flavour and nutritional value (high starch, vitamins) and are cooked as vegetables. Although "some varieties can be poisonous," most *D. esculenta* strains are considered safe to eat.

Given these divergent profiles, a controlled toxicology study is needed to characterize and compare their hazards. Regulatory guidelines emphasize the use of standard rodent models: Wistar rats are commonly employed due to well-known physiology and historical database (OECD, 2001). The acute oral toxicity protocol (OECD TG 423) uses a stepwise dosing of three female rats per step [3]. The 28-day repeated-dose study (OECD TG 407) assesses potential subacute effects, target organs, and NOAEL (organ weight, clinical pathology, histopathology) [4]. Accordingly, we conducted both an acute toxicity test and a 28-day repeated-dose test of ethanolic extracts of *D. hispida*, *D. bulbifera*, and *D. esculenta* in Wistar rats. Our objectives were to determine the LD_{50} (or cut-off) and GHS hazard category for each species, and to identify any systemic toxicity after 28-day exposure. Comparative analysis across species and doses was done via well-structured tables, integrating outcomes from provided experimental data with literature benchmarks. This comprehensive assessment will inform safety evaluations and guide the usage of these yam extracts in traditional or nutraceutical applications.

METHODOLOGY

Test Substances and Preparation

Ethanolic extracts of *D. hispida*, *D. bulbifera*, and *D. esculenta* tubers were obtained from authenticated plant material. Each extract was standardized for consistency as per the Indonesian Herbal Pharmacopoeia II. Test articles were formulated daily at the required concentrations by suspending in 1% w/v carboxymethylcellulose (CMC) in water (vehicle), which was selected based on solubility tests. Formulations were prepared fresh each day with continuous stirring to ensure homogeneity. Dosing volume was 1 mL/100 g body weight for all groups.

Experimental Animals and Husbandry

Wistar rats (*Rattus norvegicus*) females were used in an acute study, and both sexes were used in a subacute (28 days) study, sourced from a reputable breeder (Global Bioresearch Solutions Pvt., Ltd., India). In an acute study, 8-11 weeks old animals were used, while in a subacute (28 days) study 7–8 weeks old animals were used at start, with body weights within ±20% of the mean for each sex. Standard laboratory conditions were maintained: temperature 22±3°C, humidity 30–70%, 12 h light/dark cycle, and at least 12 air changes per hour. Rats were housed in groups of 3 per polypropylene cage with sterile bedding, and allowed *ad libitum* access to pelleted rodent feed (Nutrivet Life Sciences, batch #010325) and reverse-osmosis water. A 7-day acclimatization period preceded dosing, during which all animals were confirmed to be healthy by a veterinarian. Animals were identified by cage and tail markings, and randomly assigned to treatment groups. Animal use and care complied with guidelines of the Institutional Animal Ethics Committee and were in accordance with national (CCSEA) regulations.

Study Design Overview

Both the acute and 28-day studies were conducted under GLP conditions [5]. Tables 1–2 summarize the experimental groups and dosing for each study.

Table 1. Acute Oral Toxicity Study Design (OECD 423)

Species	Step	Dose (mg/kg)	No. of Animals (Female)	Outcome
D. hispida	Step I	2000 mg/kg	3	All 3 animals died
	Step II	300 mg/kg	3	No Mortality or Morbidity
	Step III	300 mg/kg	3	No Mortality or Morbidity
D. bulbifera	Step I	2000 mg/kg	3	No Mortality or Morbidity
	Step II	2000 mg/kg	3	No Mortality or Morbidity
D. esculenta	Step I	2000 mg/kg	3	No Mortality or Morbidity

	Step II	2000 mg/kg	3	No Mortality or Morbidity

For acute testing, each extract was evaluated as per OECD 423 using three female rats per step. For *D. hispida*, initial dosing at 2000 mg/kg resulted in 100% mortality (Step I). A lower dose (300 mg/kg) was then tested with 0/3 mortality (Steps II and III). For *D. bulbifera* and *D. esculenta*, both steps at 2000 mg/kg produced no deaths.

Table 2. 28-Day Repeated Dose Study Design (OECD 407)

Species	Group	Dose (mg/kg/day)	No. of Animals (M/F)	Recovery Group (M/F)
D. hispida	G1	0 (vehicle)	5/5	Yes (G5) 5/5
	G2	25	5/5	No
	G3	50	5/5	No
	G4	100	5/5	Yes (G6) 5/5
D. bulbifera	G1	0 (vehicle)	5/5	Yes (G5) 5/5
	G2	150	5/5	No
	G3	300	5/5	No
	G4	600	5/5	Yes (G6) 5/5
D. esculenta	G1	0 (vehicle)	5/5	Yes (G5) 5/5
	G2	150	5/5	No
	G3	300	5/5	No
	G4	600	5/5	Yes (G6) 5/5

Each species underwent a 28-day oral toxicity study following OECD 407. For *D. hispida*, doses of 25, 50, 100 mg/kg/day were chosen based on acute toxicity; for *D. bulbifera* and *D. esculenta*, higher doses (150, 300 and 600 mg/kg/day) were used. Control groups (G1) received a vehicle. An additional recovery group (G5) for each species received control (vehicle) and high dose recovery G6 (100 mg/kg for *D. hispida* or 600 mg/kg for *D. bulbifera* and *D. esculenta*) for the 28-day period, plus a 14-day recovery. No mortality or clinical effects were predicted at these doses, but this design allows comprehensive observation of possible target organ effects.

Table 3. Study Schedules

Day	Acute Study Activities
-1	Fasting (Overnight)
0	Body weight, Dosing of 3 rats per step (see Table 1); mark as Day 0
1-14	Observe acute rats for mortality/signs; weigh on Day 7, 14; euthanize on Day 14 for necropsy and gross pathology
Day	**28-Day Study Activities**
-1	Randomization, baseline measurements
1-28	Body weight, First dose administration (all groups), Daily dosing (G1–G4) Days 1–28; weekly body weight and feed consumption; daily clinical observations; detailed clinical examination, ophthalmic exams (pre and week 4); Functional Observation, Urine Analysis (week 4)
29	Terminal sacrifice of main groups; blood/organ collection, gross pathology
29-42	Recovery phase (no dosing, observations continue) weekly body weight and feed consumption; daily clinical observations; detailed clinical examination, ophthalmic exams (pre and week 6); Functional Observation, Urine Analysis (week 6)
43	Sacrifice of recovery groups; Blood and organ collection, Gross pathology

Observations and Measurements

In an acute study, all animals were observed for mortality and morbidity twice daily. Animals were observed for clinical signs during acclimatization period and treatment period once daily. On the day of dosing, animals were observed during the first 30 minutes and at 1 hour, 2 hours, 3 hours and 4 hours after dosing. The observations included conditions of skin and fur, eyes and mucus membrane, respiratory, circulatory, and autonomic and central nervous system, somato-motor activity and behavioural pattern. Specific observations were made for tremors, convulsions, salivation, diarrhoea, lethargy, sleep, coma and other. The body weight of the individual animal was observed and recorded on day 0 prior to dosing and in weekly intervals thereafter (day 7 and 14). At the end of the 14-day observation period, all the surviving rats were euthanized by overdose of CO_2. All the animals were observed for external and internal gross pathology.

In a subacute (28 days) study, all experimental animals were observed at least twice daily for morbidity and mortality throughout the study period. General clinical signs observations of rats from all the groups were made once a day, preferably at the same time each day, considering the peak period of anticipated effects after dosing. Body weight and feed consumption were recorded at a weekly interval. All animals were subjected to detailed clinical observation prior to test item administration and at weekly intervals thereafter during the treatment period and recovery period. During detailed clinical examination, all rats were observed for changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g. lacrimation, piloerection, pupil size, unusual respiratory patterns), changes in gait, posture and response to handling as well as the presence clonic or tonic movements, stereotypies (e.g., excessive grooming, repetitive circling) and bizarre behaviour (e.g. self-mutilation, walking backwards). Ophthalmological examination of all animals was performed with an ophthalmoscope prior to the start of treatment, at the end of the treatment period and reversal period. Before examination, Mydriasis was induced using a 1% solution of Tropicamide. Functional observations such as Sensory Observation (Approach Response, Touch Response, Sound Response, Tail Pinch Response, Pupillary Reflex, Proprioception Response and Surface or Air Righting Reflex), Foot splay Measurement, Activity Measurement and Grip Strength Measurement (Fore limb and Hind limb) were carried out in surviving animals in the last week of the dosing phase and reversal period.

At scheduled sacrifices, animals were fasted overnight, and blood samples were collected (via retro-orbital sinus under light anaesthesia) for hematology and clinical chemistry. Hematology included RBC count, hemoglobin, hematocrit, WBC count with differential, platelet count, MCV, MCH, MCHC, reticulocyte count, and Clotting time. Serum biochemistry included albumin (ALB), Alkaline phosphatase (ALP), bilirubin total (BT), Calcium (CAL), Cholesterol (CHOL), Cholinesterase, Creatinine (CREAT), Glucose (GLU), Phosphorus (PHO), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), total protein (TP), Triglyceride, Urea, Sodium (Na), Potassium (K), Chloride (Cl). Urine was collected overnight (pre-terminal) for routine analysis (glucose, protein, blood, pH, specific gravity color, volume, Bilirubin, Urobilinogen, etc.). All animals underwent gross necropsy. Absolute and relative organ weights were measured (Adrenals, Testes/Ovaries, Brain, Kidneys, Liver, Heart, Spleen, Epididymides/Uterus, Thyroid). Tissues were fixed, processed, and examined microscopically for pathology.

Data Analysis and Ethics

Data were summarized as group means ± standard deviation. Body weights, feed consumption, clinical pathology, foot splay, grip strength, motor activity, and organ weights were analyzed by one-way ANOVA (parametric) or Kruskal–Wallis (nonparametric) with Dunnett's or Dunn's post-hoc tests, respectively, to compare treated groups vs. control. A p-value <0.05 was considered statistically significant. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC Approval: TXI/09/02 and TXI/09/13) and complied with OECD Good Laboratory Practice principles. In particular, the study design followed OECD TG 407 for repeated dose toxicity and OECD TG 423 for acute toxicity (stepwise dosing).

Explanation of Methodology Tables

Table 1 (Acute Toxicity Design) outlines the stepwise dosing regimen per OECD 423. Three female rats per step were given escalating doses of each extract. Female Wistar rats were fasted overnight prior to dosing. The feed was withheld approximately 3.0 hours to 3.5 hours post dosing, but drinking water was provided

ad libitum. The dose was calculated based on the fasted body weight of each rat and administered in a single dose by gavage using a stainless steel 18G cannula. The time interval between dosing was determined by the onset, duration and severity of toxic signs. The sequence of doses (2000→300→300 mg/kg for *D. hispida*; 2000→2000 for *D. bulbifera* and *D. esculenta*) and observed outcomes (mortality) are summarized. This table clarifies how the LD_{50} ranges were derived: all three *D. hispida* rats died at 2000 mg/kg, so a lower limit of toxicity was established at 300 mg/kg (where no deaths occurred). In contrast, *D. bulbifera* and *D. esculenta* had no deaths at 2000 mg/kg, indicating much higher LD_{50} values.

Table 2 (28-Day Toxicity Design) details the group structure for the subacute study. For each species, six groups were used: four dose levels in the main study (control, low, mid, high) and a two recovery group (control recovery and high dose recovery). Each dose group had 10 rats (5 male, 5 female). The recovery group was split into control and high-dose subsets to allow evaluation of reversibility. This table conveys the experimental scheme and justifies dose selection: *D. hispida* doses (25–100 mg/kg) are lower than those for the other species (150–600 mg/kg), reflecting its higher acute toxicity. The "Recovery Group" column indicates which high dose and control rats were observed an extra 14 days without treatment. The doses were calculated based on the recent body weight of each rat and administered daily in a single dose for 28 consecutive days by gavage using a suitable gavage needle. The dose volume for administration was 1 mL/100 g of body weight. Dose volume was constant at all the dose levels.

Table 3 (Study Schedules) presents the timeline of major procedures. The acute study had dosing on Day 0 with 14 days of observation, whereas the 28-day study had daily dosing for 28 days, plus 14 days for recovery animals. Key activities (e.g. fasting, dosing, observations, sampling) are aligned with study days. This schedule ensures compliance with OECD testing protocols: the acute study covers a 14-day observation period, and the 28-day study covers 28-day dosing plus a recovery period as recommended for detecting delayed toxicity. Each table here aids comprehension of the protocol by summarizing complex procedures in a structured format.

RESULTS AND DISCUSSION

Acute Oral Toxicity Results

All observations from the acute study are summarized in Table 4. For *D. hispida*, all the animals were found dead at Step I treated with 2000 mg/kg body weight and no mortality was observed at 300 mg/kg body weight (Step II and Step III) throughout the 14 days observation period. At 2000 mg/kg body weight (Step I), all animals were found normal at 30 minutes, 1 hour and 2 hours after dosing, whereas lethargy and tremors were observed in all animals at 3 and 4 hours after dosing, and all the animals were found dead on day 1. Animals of Step II and Step III at 300 mg/kg body weight were found to be normal throughout the 14 day observation period. The mean body weight of all the surviving animals was observed with a gain on day 7 and 14, as compared to day 0. No external and internal gross pathological changes were seen in any of the animals of Steps II and III treated with 300 mg/kg body weight. Lung congestion was observed in all the animals found dead in Step I treated with 2000 mg/kg body weight.

In contrast, *D. bulbifera* and *D. esculenta* at 2000 mg/kg produced no mortality or signs of toxicity in any rat (Step I and II). The mean body weight of all the animals was observed with a gain on day 7 and 14, as compared to day 0. No external and internal gross pathological changes were seen in any of the animals of Steps I and II treated with 2000 mg/kg body weight. Under GHS, substances with LD_{50} >2000 mg/kg fall into Category 5 ("may be harmful") or remain unclassified [6]. These results agree qualitatively with the literature: for example, high LD_{50} values for *D. bulbifera* bulbs [7].

Table 4. Acute Oral Toxicity Outcomes

Species	Dose (mg/kg)	No. Dead/No. Dosed	Clinical Sign and Mortality	Gross Pathology
D. hispida	2000	3/3 (Day 1)	Lethargy and Tremors	Lung congestion

D. hispida	300	0/3 (Day 0–14)	No mortality or clinical signs	No abnormality detected
D. hispida	300	0/3 (Day 0–14)	No mortality or clinical signs	No abnormality detected
D. bulbifera	2000	0/3 (Day 0–14)	No mortality or clinical signs	No abnormality detected
D. bulbifera	2000	0/3 (Day 0–14)	No mortality or clinical signs	No abnormality detected
D. esculenta	2000	0/3 (Day 0–14)	No mortality or clinical signs	No abnormality detected
D. esculenta	2000	0/3 (Day 0–14)	No mortality or clinical signs	No abnormality detected

DISCUSSION: The data confirm that *D. hispida* is significantly more acutely toxic than the other two species. The LD_{50} range (300–2000) is consistent with its known toxic constituents (dioscorine and cyanide) [8]. In literature, *D. hispida* tuber extracts have been reported to cause similar lethality around 300–1000 mg/kg in rats [9]. The absence of any mortality for *D. bulbifera* and *D. esculenta* up to 2000 mg/kg is notable; these species confirming their low acute toxicity profile reported in literature [10], [11]. Their GHS Category 5 rating indicates "low acute toxicity," consistent with *D. bulbifera* being edible after cooking and *D.* esculenta being a traditional food crop.

28-Day Repeated Dose Toxicity Results

Survival and Clinical Signs: All animals in the 28-day study survived through scheduled sacrifice or recovery sacrifice. No treatment-related deaths occurred in any group of any species. By study end, all high-dose animals appeared clinically normal. Thus, there was **no mortality or persistent clinical toxicity** associated with any extract at any dose.

Body Weight and Food Consumption: Mean body weight show normal growth in all groups. By Day 28, treated rats had gained weight comparable to controls (no statistically significant differences) (Table 5). Similarly, weekly body weight change percentages did not differ significantly between control and treated groups. Food consumption (g/animal/day) was also equivalent across groups (data summarized in Table 6). In summary, **no adverse effect on general health or growth** was evident in any treated group, indicating that none of the extracts impaired appetite or metabolism at the tested doses. These findings correspond with earlier subacute toxicity assessments on Dioscorea species, where no major alterations were noted at similar dose ranges.

Table 5. Mean Body Weight (g)

Species: *Dioscorea bulbifera*

Group (N)	**G1 (05)**		**G2 (05)**		**G3 (05)**		**G4 (05)**		**G5 (05)**		**G6 (05)**	
Dose (mg/kg b.wt.)	**0**		**150**		**300**		**600**		**0**		**600**	
Day	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Sex	**Male**											
Day 1	189.40	12.90	184.60	12.30	185.00	17.01	189.80	11.82	187.00	15.70	193.00	15.67
Day 8	217.20	12.97	212.40	10.95	212.80	18.57	217.60	12.99	214.80	14.55	220.80	16.90
Day 15	246.40	13.76	241.60	10.97	242.00	18.52	246.80	12.48	244.00	14.40	250.00	16.25
Day 22	277.20	14.70	272.40	10.50	272.80	15.12	277.60	9.45	274.80	14.96	280.80	13.83
Day 28	303.00	15.65	298.20	11.10	298.60	14.89	303.40	8.71	-	-	-	-
Day 29	-	-	-	-	-	-	-	-	300.60	15.85	306.60	13.79
Day 36	-	-	-	-	-	-	-	-	331.40	17.17	337.40	12.03
Day 42	-	-	-	-	-	-	-	-	357.20	18.05	363.20	12.13
Sex	**Female**											

Day 1	181.60	9.02	176.20	8.17	181.00	8.94	184.00	10.12	184.40	8.56	179.60	6.31
Day 8	194.40	9.13	189.00	7.97	193.80	8.76	196.80	10.06	197.20	8.67	192.40	6.31
Day 15	209.40	9.42	204.00	8.00	208.80	9.26	211.80	9.34	212.20	8.07	207.40	6.66
Day 22	224.60	9.26	219.20	8.14	224.00	9.38	227.00	9.35	227.40	7.89	222.60	6.58
Day 28	242.00	9.43	236.60	8.23	241.40	9.76	244.40	8.88	-	-	-	-
Day 29	-	-	-	-	-	-	-	-	244.80	7.43	240.00	6.82
Day 36	-	-	-	-	-	-	-	-	257.60	7.64	252.80	6.91
Day 42	-	-	-	-	-	-	-	-	272.60	7.16	267.80	7.43

Species: *Dioscorea esculenta*

Group (N)	**G1 (05)**		**G2 (05)**		**G3 (05)**		**G4 (05)**		**G5 (05)**		**G6 (05)**	
Dose (mg/kg b.wt.)	**0**		**150**		**300**		**600**		**0**		**600**	
Day	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Sex	**Male**											
Day 1	190.00	12.21	193.00	10.86	190.80	12.15	185.20	12.99	191.80	12.74	190.60	11.70
Day 8	217.80	12.68	220.80	11.82	218.60	10.88	213.00	11.98	219.60	13.58	218.40	13.72
Day 15	247.00	11.90	250.00	11.09	247.80	11.37	242.20	11.41	248.80	13.26	247.60	13.28
Day 22	277.80	11.54	280.80	11.23	278.60	14.14	273.00	13.51	279.60	10.11	278.40	10.99
Day 28	303.60	10.11	306.60	9.69	304.40	14.08	298.80	13.94	-	-	-	-
Day 29	-	-	-	-	-	-	-	-	305.40	9.50	304.20	10.33
Day 36	-	-	-	-	-	-	-	-	336.20	6.65	335.00	8.86
Day 42	-	-	-	-	-	-	-	-	362.00	5.96	360.80	8.23
Sex	**Female**											
Day 1	185.00	6.24	186.00	6.04	184.60	4.93	182.40	8.05	175.00	4.30	179.40	10.90
Day 8	197.80	6.91	198.80	5.85	197.40	4.34	195.20	7.92	187.80	4.38	192.20	11.23
Day 15	212.80	7.82	213.80	5.89	212.40	3.71	210.20	7.63	202.80	3.90	207.20	11.08
Day 22	228.00	7.18	229.00	6.00	227.60	4.28	225.40	7.70	218.00	3.67	222.40	10.69
Day 28	245.40	7.60	246.40	6.11	245.00	4.12	242.80	7.56	-	-	-	-
Day 29	-	-	-	-	-	-	-	-	235.40	3.29	239.80	10.47
Day 36	-	-	-	-	-	-	-	-	248.20	3.56	252.60	10.88
Day 42	-	-	-	-	-	-	-	-	263.20	3.42	267.60	10.85

Species: *Dioscorea hispida*

Group (N)	**G1 (05)**		**G2 (05)**		**G3 (05)**		**G4 (05)**		**G5 (05)**		**G6 (05)**	
Dose (mg/kg b.wt.)	**0**		**25**		**50**		**100**		**0**		**100**	
Day	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Sex	**Male**											
Day 1	187.40	15.04	193.60	16.04	185.20	11.03	183.00	10.34	193.20	15.22	185.40	6.88
Day 8	215.20	14.48	221.40	17.78	213.00	12.10	210.80	9.58	221.00	16.19	213.20	5.85

Day 15	244.40	15.16	250.60	17.34	242.20	12.79	240.00	8.92	250.20	16.83	242.40	6.02
Day 22	275.20	17.71	281.40	16.07	273.00	10.56	270.80	11.37	281.00	16.72	273.20	6.72
Day 28	301.00	18.26	307.20	16.15	298.80	10.76	296.60	11.72	-	-	-	-
Day 29	-	-	-	-	-	-	-	-	306.80	17.28	299.00	6.40
Day 36	-	-	-	-	-	-	-	-	337.60	17.95	329.80	8.79
Day 42	-	-	-	-	-	-	-	-	363.40	18.57	355.60	8.73
Sex	**Female**											
Day 1	177.80	2.95	180.20	10.08	184.20	6.65	179.60	10.55	179.00	9.59	181.80	10.50
Day 8	190.60	2.61	193.00	10.79	197.00	6.40	192.40	10.06	191.80	8.96	194.60	11.06
Day 15	205.60	2.19	208.00	11.68	212.00	5.70	207.40	10.04	206.80	8.58	209.60	11.74
Day 22	220.80	2.39	223.20	10.99	227.20	5.89	222.60	10.48	222.00	9.19	224.80	11.17
Day 28	238.20	2.28	240.60	11.37	244.60	5.50	240.00	10.63	-	-	-	-
Day 29	-	-	-	-	-	-	-	-	239.40	9.18	242.20	11.45
Day 36	-	-	-	-	-	-	-	-	252.20	8.58	255.00	12.02
Day 42	-	-	-	-	-	-	-	-	267.20	8.38	270.00	12.77

Body weight gains over 28 days did not differ between control and treated groups for any species. No dose-related trends and no statically significant difference were observed (e.g. *D. bulbifera*, mean body weight on day 28 of male in high dose 600 mg/kg was 303.40 vs 303.00 in control).

Table 6. Feed Consumption (g/animal/day)
Species: *Dioscorea bulbifera*

Group (N)	**G1 (05)**		**G2 (05)**		**G3 (05)**		**G4 (05)**		**G5 (05)**		**G6 (05)**	
Dose (mg/kg b.wt.)	**0**		**150**		**300**		**600**		**0**		**600**	
Day	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Sex	**Male**											
Day 1-8	18.32	2.62	17.78	2.01	18.43	2.07	18.05	2.41	18.36	2.08	17.60	2.06
Day 8-15	18.58	2.22	18.90	1.46	19.05	2.04	17.90	2.17	18.76	2.02	18.74	1.67
Day 15-22	19.54	2.00	20.32	2.20	19.92	2.18	19.91	1.74	19.57	2.19	19.77	1.93
Day 22-28	21.04	1.64	21.39	2.04	20.91	1.90	22.05	2.04	-	-	-	-
Day 22-29	-	-	-	-	-	-	-	-	20.78	1.77	21.21	2.09
Day 29-36	-	-	-	-	-	-	-	-	21.80	2.30	21.86	2.21
Day 36-42	-	-	-	-	-	-	-	-	23.78	2.16	23.73	2.10
Sex	**Female**											
Day 1-8	15.94	3.33	15.80	2.41	16.04	2.33	15.91	2.44	15.69	2.49	15.83	2.41
Day 8-15	17.26	3.21	16.99	3.29	17.35	2.31	17.22	3.01	17.01	3.17	17.13	2.09
Day 15-22	18.02	3.31	18.00	2.31	18.49	3.41	17.79	2.44	17.58	2.49	18.30	3.49
Day 22-28	19.33	3.24	18.89	2.32	19.05	2.36	18.79	2.28	-	-	-	-
Day 22-29	-	-	-	-	-	-	-	-	18.57	2.39	18.76	2.41
Day 29-36	-	-	-	-	-	-	-	-	19.41	3.39	19.36	3.36
Day 36-42	-	-	-	-	-	-	-	-	20.01	2.27	19.99	2.30

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Species: *Dioscorea esculenta*

Group (N)	**G1 (05)**		**G2 (05)**		**G3 (05)**		**G4 (05)**		**G5 (05)**		**G6 (05)**	
Dose (mg/kg b.wt.)	**0**		**150**		**300**		**600**		**0**		**600**	
Day	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Sex	**Male**											
Day 1-8	17.90	2.52	17.36	1.91	18.01	1.97	17.63	2.31	18.69	2.15	17.93	2.13
Day 8-15	18.16	2.12	18.48	1.36	18.63	1.94	17.48	2.07	19.09	2.09	19.07	1.74
Day 15-22	19.12	1.90	19.90	2.10	19.50	2.08	19.49	1.64	19.90	2.26	20.10	2.00
Day 22-28	20.62	1.54	20.97	1.94	20.49	1.80	21.63	1.94	-	-	-	-
Day 22-29	-	-	-	-	-	-	-	-	21.11	1.84	21.54	2.16
Day 29-36	-	-	-	-	-	-	-	-	22.13	2.37	22.19	2.28
Day 36-42	-	-	-	-	-	-	-	-	24.11	2.23	24.06	2.17
Sex	**Female**											
Day 1-8	15.52	3.23	15.38	2.31	15.62	2.23	15.49	2.34	16.02	2.56	16.16	2.48
Day 8-15	16.84	3.11	16.57	3.19	16.93	2.21	16.80	2.91	17.34	3.24	17.46	2.16
Day 15-22	17.60	3.21	17.58	2.21	18.07	3.31	17.37	2.34	17.91	2.56	18.63	3.56
Day 22-28	18.91	3.14	18.47	2.22	18.63	2.26	18.37	2.18	-	-	-	-
Day 22-29	-	-	-	-	-	-	-	-	18.90	2.46	19.09	2.48
Day 29-36	-	-	-	-	-	-	-	-	19.74	3.46	19.69	3.43
Day 36-42	-	-	-	-	-	-	-	-	20.34	2.34	20.32	2.37

Species: *Dioscorea hispida*

Group (N)	**G1 (05)**		**G2 (05)**		**G3 (05)**		**G4 (05)**		**G5 (05)**		**G6 (05)**	
Dose (mg/kg b.wt.)	**0**		**25**		**50**		**100**		**0**		**100**	
Day	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Sex	**Male**											
Day 1-8	18.97	2.85	18.43	2.24	19.08	2.30	18.70	2.64	19.12	2.31	18.36	2.29
Day 8-15	19.23	2.45	19.55	1.69	19.70	2.27	18.55	2.40	19.52	2.25	19.50	1.90
Day 15-22	20.19	2.23	20.97	2.43	20.57	2.41	20.56	1.97	20.33	2.42	20.53	2.16
Day 22-28	21.69	1.87	22.04	2.27	21.56	2.13	22.70	2.27	-	-	-	-
Day 22-29	-	-	-	-	-	-	-	-	21.54	2.00	21.97	2.32
Day 29-36	-	-	-	-	-	-	-	-	22.56	2.53	22.62	2.44
Day 36-42	-	-	-	-	-	-	-	-	24.54	2.39	24.49	2.33
Sex	**Female**											
Day 1-8	16.59	3.56	16.45	2.64	16.69	2.56	16.56	2.67	16.45	2.72	16.59	2.64
Day 8-15	17.91	3.44	17.64	3.52	18.00	2.54	17.87	3.24	17.77	3.40	17.89	2.32
Day 15-22	18.67	3.54	18.65	2.54	19.14	3.64	18.44	2.67	18.34	2.72	19.06	3.72
Day 22-28	19.98	3.47	19.54	2.55	19.70	2.59	19.44	2.51	-	-	-	-

Day 22-29	-	-	-	-	-	-	-	-	19.33	2.62	19.52	2.64
Day 29-36	-	-	-	-	-	-	-	-	20.17	3.62	20.12	3.59
Day 36-42	-	-	-	-	-	-	-	-	20.77	2.50	20.75	2.53

Feed intakes were stable across dose levels. For each weekly interval and sex, treated groups consumed as much feed as controls (e.g., in last week, *D. bulbifera* male rats on 600 mg/kg ate ~21.63 g/day vs. 20.62 g in controls). No dose-related reduction in appetite was found. This suggests the extracts did not adversely affect nutrition or metabolism. Collectively, normal weight gain and feed consumption indicate **lack of overt toxicity** at doses up to 100 mg/kg for *D. hispida* and 600 mg/kg for the others.

Clinical Pathology (Hematology and Biochemistry)

Hematological and serum biochemistry results are presented in Tables 7 and 8. Analysis revealed distinct patterns between species. For *D. bulbifera*, hematological parameters across all dose groups remained within normal physiological ranges. However, in clinical chemistry, statistically significant elevations were noted. In high-dose males (G4, 600 mg/kg), Alkaline Phosphatase (ALP) increased to 360.15 ± 36.13 U/l (vs. 237.88 ± 8.90 in control), Alanine Aminotransferase (ALT) to 93.36 ± 5.07 U/l (vs. 63.02 ± 10.06), and Aspartate Aminotransferase (AST) to 155.18 ± 17.16 U/l (vs. 133.78 ± 26.81). A similar pattern was observed in high-dose females (G4), with ALP elevated to 387.19 ± 23.13 U/l (vs. 231.76 ± 14.38), ALT to 98.06 ± 10.20 U/l (vs. 59.36 ± 5.08) and AST to 152.94 ± 5.43 U/l (vs. 119.74 ± 21.77). These elevations were absent in the corresponding recovery groups (G6), indicating reversibility. Similar hepatotoxic tendencies due to steroidal saponins and related phytoconstituents have been reported previously [10].

For *D. hispida*, hematology also showed no significant treatment-related changes. In clinical chemistry, high-dose males (G4, 100 mg/kg) exhibited marked increases in ALT (137.00 ± 6.03 U/l vs. 64.52 ± 10.06 in control) and AST (206.64 ± 9.18 U/l vs. 134.98 ± 26.81). High-dose females (G4) showed similar significant elevations in ALT (133.92 ± 9.95 U/l vs. 60.86 ± 5.08) and AST (211.46 ± 8.73 U/l vs. 128.16 ± 33.84). For *D. hispida*, significant elevations in ALT and AST at 100 mg/kg also indicate hepatic involvement, in agreement with known toxicity of dioscorine and hydrogen cyanide derivatives found in this species [9]. These changes returned to control levels in recovery groups (G6). In contrast, for *D. esculenta*, all hematological and clinical chemistry parameters across all dose groups, including the high dose (600 mg/kg), remained within normal limits. No statistically significant or biologically relevant changes were observed, and values were comparable to controls in both main and recovery phases. This aligns with the species widely documented safe consumption profile and low inherent toxicity.

Other parameters, including kidney function markers (urea, creatinine), electrolytes, total protein, albumin, and glucose, showed no consistent, dose-related changes across all three species.

Table 7. Mean Hematology Results (Mean ± SD)
Species: *Dioscorea bulbifera* (Sex: Male)

Group	Parameters	Hb (g/dl)	PCV (%)	Total RBC ($x10^6$/ cmm)	RBC Indices			Total WBC ($x10^3$/ cmm)	Differential WBC (%)				Platelets ($x10^3$/ cmm)	Clotting time (Sec.)	Reticulocyte (%)
					MCH (pg)	MCV (fl)	MCHC (g/dl)		N	L	E	M			
G1	**Mean**	14.03	41.95	8.71	16.21	57.17	28.40	11.53	27.00	69.80	2.20	1.00	875.20	105.60	2.38
	SD	0.81	2.97	0.91	1.40	4.69	2.03	1.25	3.08	4.97	1.30	1.00	81.89	10.31	0.19
G2	**Mean**	12.21	41.45	9.05	13.67	54.05	25.32	11.81	27.20	69.40	1.80	1.60	846.60	112.00	2.56
	SD	0.65	3.16	1.03	2.01	7.23	2.07	1.22	3.11	4.51	0.84	1.14	85.68	13.32	0.11
G3	**Mean**	14.95	41.73	8.91	17.09	51.32	33.46	11.43	26.40	71.00	2.00	1.00	854.20	111.80	2.44
	SD	1.23	3.05	1.13	3.25	10.19	3.19	1.74	3.21	2.24	1.22	0.71	46.77	13.99	0.21
G4	**Mean**	13.33	43.81	9.43	14.30	54.47	26.33	12.51	26.40	69.20	2.80	1.60	841.80	95.80	2.76
	SD	1.17	4.42	1.28	1.96	7.44	2.16	2.19	2.51	2.59	0.84	0.89	58.33	9.36	0.45
G5	**Mean**	13.95	42.64	9.84	14.35	50.50	28.66	11.67	23.20	73.80	2.20	0.80	855.20	104.60	2.62
	SD	0.63	2.35	1.28	1.81	8.76	2.01	1.70	3.42	4.21	1.10	0.84	62.50	3.65	0.19

G6	**Mean**	13.85	41.33	9.89	14.34	49.32	29.32	11.91	29.00	67.20	2.40	1.40	854.80	106.80	2.42
	SD	0.29	1.37	1.74	2.33	10.25	1.64	2.35	3.81	3.77	1.67	1.14	57.68	8.53	0.15

Species: *Dioscorea bulbifera* (Sex: Female)

Group	Parameters	Hb (g/dl)	PCV (%)	Total RBC ($x10^6$/ cmm)	RBC Indices			Total WBC ($x10^3$/ cmm)	Differential WBC (%)				Platelets ($x10^3$/ cmm)	Clotting time (Sec.)	Reticulocyte (%)
					MCH (pg)	MCV (fl)	MCHC (g/dl)		N	L	E	M			
G1	**Mean**	14.03	42.13	9.33	15.16	52.38	28.93	10.93	26.60	70.00	2.00	1.40	863.40	110.00	2.48
	SD	0.75	4.15	0.73	1.94	4.69	2.49	0.59	3.21	4.64	0.71	1.14	53.57	15.17	0.19
G2	**Mean**	13.81	43.49	9.31	14.87	54.43	27.45	10.11	25.80	70.20	2.40	1.60	830.00	110.40	2.50
	SD	0.73	3.70	0.55	1.00	6.01	1.89	0.47	3.49	4.09	0.55	1.14	78.73	12.28	0.32
G3	**Mean**	13.91	40.53	9.21	15.14	51.09	29.74	10.97	27.60	69.40	1.60	1.40	844.20	105.40	2.60
	SD	0.72	2.46	0.42	1.20	4.40	2.53	0.82	2.70	3.78	0.89	1.14	25.41	3.36	0.16
G4	**Mean**	13.43	43.91	8.77	15.39	59.56	26.12	10.89	28.20	67.60	2.40	1.40	869.20	108.80	2.40
	SD	0.63	4.42	0.85	0.93	8.15	2.89	1.04	2.59	1.82	1.14	1.14	38.69	12.68	0.22
G5	**Mean**	13.03	41.90	9.73	13.62	49.83	27.18	11.57	26.80	70.20	2.00	1.00	881.40	98.20	2.54
	SD	1.13	2.04	1.20	2.57	5.70	2.33	1.58	2.17	1.92	0.71	1.00	43.40	6.94	0.11
G6	**Mean**	14.17	41.99	10.39	13.64	45.76	30.09	11.53	25.60	71.80	1.80	0.80	825.60	110.60	2.52
	SD	0.62	2.52	0.47	0.24	5.30	2.90	2.08	2.30	1.92	1.30	1.10	87.76	8.50	0.19

Species: *Dioscorea esculenta* (Sex: Male)

Group	Parameters	Hb (g/dl)	PCV (%)	Total RBC ($x10^6$/ cmm)	RBC Indices			Total WBC ($x10^3$/ cmm)	Differential WBC (%)				Platelets ($x10^3$/ cmm)	Clotting time (Sec.)	Reticulocyte (%)
					MCH (pg)	MCV (fl)	MCHC (g/dl)		N	L	E	M			
G1	**Mean**	14.15	42.68	8.88	15.96	56.49	28.40	12.33	24.40	72.00	2.00	1.60	842.80	114.60	2.50
	SD	0.98	1.73	0.44	1.30	5.08	2.96	1.12	2.07	2.35	1.22	0.89	48.13	8.44	0.10
G2	**Mean**	13.56	43.20	9.39	14.49	53.31	27.26	11.48	22.80	72.20	3.00	2.00	842.20	107.60	2.74
	SD	0.36	3.38	0.56	1.05	4.88	1.59	1.37	3.83	1.92	2.00	0.71	51.67	9.04	0.11
G3	**Mean**	14.31	43.27	9.44	15.18	53.24	28.71	11.98	25.60	71.80	1.60	1.00	860.00	102.20	2.54
	SD	1.40	1.88	0.89	0.98	6.02	2.59	1.58	2.07	2.77	1.14	1.00	48.33	11.05	0.22
G4	**Mean**	13.41	42.89	8.95	15.14	56.36	26.79	12.09	23.80	72.20	2.40	1.60	856.60	110.20	2.56
	SD	1.38	2.75	0.62	2.74	6.67	2.59	1.73	4.60	6.22	1.14	1.52	61.32	12.44	0.24
G5	**Mean**	13.98	42.78	9.14	15.35	55.02	28.14	11.16	28.20	67.80	2.20	1.80	811.00	118.80	2.60
	SD	0.91	2.64	0.75	0.98	6.81	3.05	0.90	2.59	2.95	0.84	0.45	46.50	9.58	0.20
G6	**Mean**	13.39	44.77	9.45	14.35	55.91	25.91	12.29	29.80	67.00	1.80	1.40	856.80	109.40	2.48
	SD	0.76	2.81	1.19	1.91	9.91	2.91	1.19	1.92	2.12	0.84	0.89	79.36	9.79	0.08

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Species: *Dioscorea esculenta* (Sex: Female)

Group	Parameters	Hb (g/dl)	PCV (%)	Total RBC ($x10^6$/ cmm)	RBC Indices			Total WBC ($x10^3$/ cmm)	Differential WBC (%)				Platelets ($x10^3$/ cmm)	Clotting time (Sec.)	Reticulocyte (%)
					MCH (pg)	MCV (fl)	MCHC (g/dl)		N	L	E	M			
G1	Mean	13.15	43.12	9.76	13.64	51.23	26.69	11.57	23.40	71.80	3.20	1.60	883.80	106.00	2.54
	SD	1.04	3.25	1.02	2.25	8.03	2.71	0.54	2.88	1.92	0.84	0.89	38.40	9.03	0.17
G2	Mean	13.40	43.36	9.34	14.44	54.50	26.69	11.32	24.60	71.40	2.20	1.80	836.60	106.00	2.80
	SD	0.76	2.54	0.88	1.46	7.79	2.19	0.80	3.36	4.39	0.84	0.84	77.71	9.67	0.22
G3	Mean	13.31	43.01	9.01	14.81	56.24	26.58	11.29	26.80	68.00	2.60	2.60	825.60	116.40	2.46
	SD	0.94	1.75	0.65	1.12	7.23	2.96	0.70	3.63	3.24	1.14	0.55	33.83	9.84	0.11
G4	Mean	14.20	40.34	9.67	14.75	48.05	31.02	11.02	26.60	68.80	2.20	2.40	831.40	108.20	2.52
	SD	0.96	3.63	0.72	1.42	7.60	3.17	0.86	3.78	3.70	0.84	0.55	82.63	13.74	0.29
G5	Mean	13.25	42.03	9.18	14.59	53.42	27.27	11.14	28.20	68.00	2.20	1.60	828.60	107.80	2.60
	SD	1.28	3.15	0.93	2.34	5.90	2.87	0.78	2.59	2.65	0.84	0.55	78.39	12.54	0.16
G6	Mean	13.83	41.97	9.19	15.11	53.36	28.59	11.97	29.20	68.00	1.80	1.00	834.40	101.20	2.48
	SD	0.82	2.43	0.85	0.97	6.54	3.39	1.79	2.39	4.12	0.84	1.00	82.56	7.19	0.18

Species: *Dioscorea hispida* (Sex: Male)

Group	Parameters	Hb (g/dl)	PCV (%)	Total RBC ($x10^6$/ cmm)	RBC Indices			Total WBC ($x10^3$/ cmm)	Differential WBC (%)				Platelets ($x10^3$/ cmm)	Clotting time (Sec.)	Reticulocyte (%)
					MCH (pg)	MCV (fl)	MCHC (g/dl)		N	L	E	M			
G1	Mean	14.00	42.53	8.73	15.81	56.34	28.25	12.18	22.40	74.00	2.00	1.60	830.80	111.60	2.30
	SD	0.98	1.73	0.44	1.30	5.08	2.96	1.12	2.07	2.35	1.22	0.89	48.13	8.44	0.10
G2	Mean	13.41	43.05	9.24	14.34	53.16	27.11	11.33	20.80	74.20	3.00	2.00	830.20	104.60	2.54
	SD	0.36	3.38	0.56	1.05	4.88	1.59	1.37	3.83	1.92	2.00	0.71	51.67	9.04	0.11
G3	Mean	14.16	43.12	9.29	15.03	53.09	28.56	11.83	23.60	73.80	1.60	1.00	848.00	99.20	2.34
	SD	1.40	1.88	0.89	0.98	6.02	2.59	1.58	2.07	2.77	1.14	1.00	48.33	11.05	0.22
G4	Mean	13.26	42.74	8.80	14.99	56.21	26.64	11.94	21.80	74.20	2.40	1.60	844.60	107.20	2.36
	SD	1.38	2.75	0.62	2.74	6.67	2.59	1.73	4.60	6.22	1.14	1.52	61.32	12.44	0.24
G5	Mean	13.83	42.63	8.99	15.20	54.87	27.99	11.01	26.20	69.80	2.20	1.80	799.00	115.80	2.40
	SD	0.91	2.64	0.75	0.98	6.81	3.05	0.90	2.59	2.95	0.84	0.45	46.50	9.58	0.20
G6	Mean	13.24	44.62	9.30	14.20	55.76	25.76	12.14	27.80	69.00	1.80	1.40	844.80	106.40	2.28
	SD	0.76	2.81	1.19	1.91	9.91	2.91	1.19	1.92	2.12	0.84	0.89	79.36	9.79	0.08

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Species: *Dioscorea hispida* (Sex: Female)

Group	Parameters	Hb (g/dl)	PCV (%)	Total RBC ($x10^6$/ cmm)	RBC Indices			Total WBC ($x10^3$/ cmm)	Differential WBC (%)				Platelets ($x10^3$/ cmm)	Clotting time (Sec.)	Reticulocyte (%)
					MCH (pg)	MCV (fl)	MCHC (g/dl)		N	L	E	M			
G1	Mean	13.00	42.97	9.61	13.49	51.08	26.54	11.42	21.40	73.80	3.20	1.60	871.80	103.00	2.34
	SD	1.04	3.25	1.02	2.25	8.03	2.71	0.54	2.88	1.92	0.84	0.89	38.40	9.03	0.17
G2	Mean	13.25	43.21	9.19	14.29	54.35	26.54	11.17	22.60	73.40	2.20	1.80	824.60	103.00	2.60
	SD	0.76	2.54	0.88	1.46	7.79	2.19	0.80	3.36	4.39	0.84	0.84	77.71	9.67	0.22
G3	Mean	13.16	42.86	8.86	14.66	56.09	26.43	11.14	24.80	70.00	2.60	2.60	813.60	113.40	2.26
	SD	0.94	1.75	0.65	1.12	7.23	2.96	0.70	3.63	3.24	1.14	0.55	33.83	9.84	0.11
G4	Mean	14.05	40.19	9.52	14.60	47.90	30.87	10.87	24.60	70.80	2.20	2.40	819.40	105.20	2.32
	SD	0.96	3.63	0.72	1.42	7.60	3.17	0.86	3.78	3.70	0.84	0.55	82.63	13.74	0.29
G5	Mean	13.10	41.88	9.03	14.44	53.27	27.12	10.99	26.20	70.00	2.20	1.60	816.60	104.80	2.40
	SD	1.28	3.15	0.93	2.34	5.90	2.87	0.78	2.59	2.65	0.84	0.55	78.39	12.54	0.16
G6	Mean	13.68	41.82	9.04	14.96	53.21	28.44	11.82	27.20	70.00	1.80	1.00	822.40	98.20	2.28
	SD	0.82	2.43	0.85	0.97	6.54	3.39	1.79	2.39	4.12	0.84	1.00	82.56	7.19	0.18

Table 8. Biochemistry Results (Mean ± SD)

Species: *Dioscorea bulbifera* (Sex: Male)

Group (N)	G1 (5)		G2 (5)		G3 (5)		G4 (5)		G5 (5)		G6 (5)	
Dose (mg/kg b.wt.)	0		150		300		600		0		600	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Albumin (g/dl)	4.35	0.38	4.30	0.30	4.23	0.46	4.19	0.03	4.41	0.37	4.36	0.29
Alkaline Phosphatase (U/l)	237.88	8.90	230.20	18.75	232.78	15.74	360.15	36.13	231.54	12.65	234.70	13.80
Bilirubin Total (mg/dl)	0.40	0.03	0.40	0.04	0.40	0.05	0.38	0.05	0.39	0.02	0.38	0.04
Calcium (mg/dl)	10.81	0.18	10.62	0.35	10.73	1.01	10.87	0.40	10.86	0.20	10.67	0.35
Cholesterol (mg/dl)	54.92	7.27	59.38	5.36	52.80	12.40	51.86	1.32	55.14	7.14	59.60	5.48
Cholinesterease (U/l)	1033.54	94.18	1034.38	39.24	1055.80	53.25	1028.72	75.88	1033.56	94.40	1034.40	39.53
Creatinine (mg/dl)	0.78	0.11	0.86	0.18	0.84	0.10	0.84	0.15	0.60	0.00	0.68	0.11
Glucose (mg/dl)	106.66	11.35	112.19	9.95	86.02	10.15	96.22	6.87	106.52	11.27	112.05	10.01
Phosphorus (mg/dl)	5.75	0.67	6.49	0.50	6.38	1.64	6.46	0.94	5.79	0.68	6.54	0.50
Asparate aminotransferase (U/l)	133.78	26.81	126.36	10.60	138.04	9.18	155.18	17.16	133.60	26.66	126.18	10.63
Alaninine Aminotransferase (U/l)	63.02	10.06	66.42	8.04	71.16	3.63	93.36	5.07	62.96	9.99	66.36	8.02
Total Protein (mg/dl)	7.85	0.19	8.40	0.35	8.10	0.62	8.00	0.33	7.90	0.19	8.45	0.36
Triglycerides (mg/dl)	78.28	34.44	95.50	7.57	97.78	26.58	97.32	20.79	78.56	34.40	95.78	7.54

Urea (mg/dl)	42.46	5.34	43.04	7.10	46.22	9.49	34.34	8.13	42.44	5.24	43.02	7.03
Sodium (mmol/l)	145.46	2.85	146.40	2.53	146.80	1.87	147.54	1.35	145.28	2.80	146.22	2.53
Potassium (mmol/l)	4.64	0.28	4.80	0.31	4.72	0.14	4.93	0.59	5.12	0.23	5.28	0.41
Chloride (mmol/l)	100.14	2.85	98.34	1.51	98.20	1.30	96.34	3.11	100.36	2.91	98.56	1.46

Species: *Dioscorea bulbifera* (Sex: Female)

Group (N)	**G1 (5)**		**G2 (5)**		**G3 (5)**		**G4 (5)**		**G5 (5)**		**G6 (5)**	
Dose (mg/kg b.wt.)	**0**		**150**		**300**		**600**		**0**		**600**	
Parameter	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Albumin (g/dl)	4.31	0.16	4.22	0.26	4.45	0.19	4.28	0.16	4.33	0.17	4.24	0.25
Alkaline Phosphatase (U/l)	231.76	14.38	238.52	6.05	241.38	3.99	387.19	23.13	239.90	13.25	234.82	11.28
Bilirubin Total (mg/dl)	0.50	0.02	0.56	0.07	0.60	0.09	0.46	0.03	0.52	0.02	0.57	0.08
Calcium (mg/dl)	10.84	0.36	10.48	0.48	10.86	0.37	10.59	0.52	10.88	0.34	10.52	0.50
Cholesterol (mg/dl)	56.00	2.50	55.44	11.66	52.88	7.51	50.48	3.27	56.22	2.64	55.66	11.71
Cholinesterease (U/l)	1567.30	216.70	1583.08	293.60	1561.32	214.30	1411.98	268.64	1567.72	216.82	1583.50	293.55
Creatinine (mg/dl)	0.89	0.12	0.91	0.23	0.90	0.15	0.91	0.13	0.71	0.03	0.73	0.13
Glucose (mg/dl)	102.12	25.81	108.90	10.88	99.36	11.31	108.28	6.58	101.98	25.81	108.76	10.93
Phosphorus (mg/dl)	4.78	0.49	4.76	0.39	4.73	0.49	5.16	0.88	4.77	0.49	4.74	0.41
Asparate aminotransferase (U/l)	119.74	21.77	129.58	11.91	137.10	11.09	152.94	5.43	126.78	33.98	136.58	23.58
Alaninine Aminotransferase (U/l)	59.36	5.08	66.72	2.54	65.32	10.05	98.06	10.20	59.20	4.98	66.56	2.56
Total Protein (mg/dl)	7.97	0.42	8.05	0.43	8.12	0.64	7.86	0.33	7.94	0.40	8.02	0.43
Triglycerides (mg/dl)	58.48	14.09	54.36	14.33	54.90	5.12	58.04	6.92	58.26	14.11	54.14	14.41
Urea (mg/dl)	37.04	5.80	34.42	2.96	34.78	3.65	38.16	4.92	36.92	5.77	34.30	2.97
Sodium (mmol/l)	147.33	2.14	147.44	1.48	151.52	5.62	148.84	3.53	146.95	2.09	147.06	1.44
Potassium (mmol/l)	4.99	0.22	5.22	0.16	5.18	0.50	4.92	0.44	4.87	0.24	5.10	0.23
Chloride (mmol/l)	101.69	1.64	99.42	0.44	99.54	2.76	99.94	2.06	102.01	1.69	99.74	0.42

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Species: *Dioscorea esculenta* (Sex: Male)

Group (N)	**G1 (5)**		**G2 (5)**		**G3 (5)**		**G4 (5)**		**G5 (5)**		**G6 (5)**	
Dose (mg/kg b.wt.)	**0**		**150**		**300**		**600**		**0**		**600**	
Parameter	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Albumin (g/dl)	4.43	0.38	4.38	0.30	4.31	0.46	4.27	0.03	4.49	0.37	4.44	0.29
Alkaline Phosphatase (U/l)	237.44	6.45	239.48	7.65	237.40	9.13	236.80	10.64	233.94	12.52	232.88	10.90
Bilirubin Total (mg/dl)	0.43	0.03	0.43	0.04	0.43	0.05	0.41	0.05	0.42	0.02	0.41	0.04
Calcium (mg/dl)	10.84	0.18	10.65	0.35	10.76	1.01	10.90	0.40	10.89	0.20	10.70	0.35
Cholesterol (mg/dl)	55.01	7.27	59.47	5.36	52.89	12.40	51.95	1.32	55.23	7.14	59.69	5.48
Cholinesterease (U/l)	1034.24	94.18	1035.08	39.24	1056.50	53.25	1029.42	75.88	1034.26	94.40	1035.10	39.53
Creatinine (mg/dl)	0.58	0.11	0.66	0.17	0.62	0.08	0.66	0.13	0.40	0.00	0.48	0.11
Glucose (mg/dl)	107.86	11.35	113.44	9.95	87.22	10.15	97.42	6.87	107.72	11.27	113.30	10.01
Phosphorus (mg/dl)	5.83	0.67	6.57	0.50	6.46	1.64	6.54	0.94	5.87	0.68	6.62	0.50
Asparate aminotransferase (U/l)	134.58	26.81	127.16	10.60	138.84	9.18	139.64	9.18	134.40	26.66	126.98	10.63
Alaninine Aminotransferase (U/l)	63.82	10.06	67.22	8.04	71.96	3.63	70.40	6.03	63.76	9.99	67.16	8.02
Total Protein (mg/dl)	7.92	0.19	8.47	0.35	8.17	0.62	8.07	0.33	7.97	0.19	8.52	0.36
Triglycerides (mg/dl)	79.08	34.44	96.30	7.57	98.58	26.58	98.12	20.79	79.36	34.40	96.58	7.54
Urea (mg/dl)	43.16	5.34	43.74	7.10	46.92	9.49	35.04	8.13	43.14	5.24	43.72	7.03
Sodium (mmol/l)	145.26	2.85	146.20	2.53	146.60	1.87	147.34	1.35	145.08	2.80	146.02	2.53
Potassium (mmol/l)	4.84	0.28	5.00	0.29	4.92	0.15	5.14	0.61	5.32	0.23	5.48	0.40
Chloride (mmol/l)	100.54	2.85	98.74	1.51	98.60	1.30	96.74	3.11	100.76	2.91	98.96	1.46

Species: *Dioscorea esculenta* (Sex: Female)

Group (N)	**G1 (5)**		**G2 (5)**		**G3 (5)**		**G4 (5)**		**G5 (5)**		**G6 (5)**	
Dose (mg/kg b.wt.)	**0**		**150**		**300**		**600**		**0**		**600**	
Parameter	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Albumin (g/dl)	4.39	0.16	4.30	0.26	4.53	0.19	4.36	0.16	4.41	0.17	4.32	0.25
Alkaline Phosphatase (U/l)	235.95	10.04	238.90	8.96	236.22	8.48	232.66	8.62	239.08	7.46	240.76	9.51
Bilirubin Total (mg/dl)	0.53	0.02	0.59	0.07	0.63	0.09	0.49	0.03	0.55	0.02	0.60	0.08
Calcium (mg/dl)	10.87	0.36	10.51	0.48	10.89	0.37	10.62	0.52	10.91	0.34	10.55	0.50

Cholesterol (mg/dl)	56.09	2.50	55.53	11.66	52.97	7.51	50.57	3.27	56.31	2.64	55.75	11.71
Cholinesterease (U/l)	1568.00	216.70	1583.78	293.60	1562.02	214.30	1412.68	268.64	1568.42	216.82	1584.20	293.55
Creatinine (mg/dl)	0.70	0.14	0.74	0.24	0.70	0.14	0.72	0.13	0.52	0.04	0.56	0.13
Glucose (mg/dl)	103.32	25.81	110.10	10.88	100.56	11.31	109.48	6.58	103.18	25.81	109.96	10.93
Phosphorus (mg/dl)	4.86	0.49	4.84	0.39	4.81	0.49	5.24	0.88	4.85	0.49	4.82	0.41
Asparate aminotransferase (U/l)	127.76	33.84	137.56	23.66	137.90	11.09	133.70	14.99	127.58	33.98	137.38	23.58
Alaninine Aminotransferase (U/l)	60.16	5.08	67.52	2.54	66.12	10.05	67.32	9.95	60.00	4.98	67.36	2.56
Total Protein (mg/dl)	8.04	0.42	8.12	0.43	8.19	0.64	7.93	0.33	8.01	0.40	8.09	0.43
Triglycerides (mg/dl)	59.28	14.09	55.16	14.33	55.70	5.12	58.84	6.92	59.06	14.11	54.94	14.41
Urea (mg/dl)	37.74	5.80	35.12	2.96	35.48	3.65	38.86	4.92	37.62	5.77	35.00	2.97
Sodium (mmol/l)	147.10	2.14	147.24	1.48	151.32	5.62	148.64	3.53	146.72	2.09	146.86	1.44
Potassium (mmol/l)	5.18	0.20	5.42	0.16	5.38	0.50	5.14	0.46	5.06	0.24	5.30	0.23
Chloride (mmol/l)	102.06	1.64	99.82	0.44	99.94	2.76	100.34	2.06	102.38	1.69	100.14	0.42

Species: *Dioscorea hispida* (Sex: Male)

Group (N)	**G1 (5)**		**G2 (5)**		**G3 (5)**		**G4 (5)**		**G5 (5)**		**G6 (5)**	
Dose (mg/kg b.wt.)	**0**		**25**		**50**		**100**		**0**		**100**	
Parameter	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Albumin (g/dl)	4.50	0.38	4.45	0.30	4.38	0.46	4.34	0.03	4.56	0.37	4.51	0.29
Alkaline Phosphatase (U/l)	236.28	8.95	227.00	18.76	231.18	15.75	229.58	15.77	231.48	12.52	231.68	10.90
Bilirubin Total (mg/dl)	0.46	0.03	0.46	0.04	0.46	0.05	0.44	0.05	0.45	0.02	0.44	0.04
Calcium (mg/dl)	10.95	0.18	10.80	0.33	10.87	1.01	11.01	0.40	11.00	0.20	10.81	0.35
Cholesterol (mg/dl)	55.72	7.27	61.92	6.41	53.60	12.40	52.66	1.32	55.94	7.14	60.40	5.48
Cholinesterease (U/l)	1035.64	94.18	1033.75	35.73	1057.90	53.25	1030.82	75.88	1035.66	94.40	1036.50	39.53
Creatinine (mg/dl)	0.68	0.11	0.77	0.15	0.72	0.08	0.76	0.13	0.50	0.00	0.58	0.11
Glucose (mg/dl)	108.16	11.35	112.70	9.26	87.52	10.15	97.72	6.87	108.02	11.27	113.60	10.01
Phosphorus (mg/dl)	5.87	0.67	6.61	0.50	5.80	0.58	6.01	0.78	5.91	0.68	6.66	0.50
Asparate aminotransferase (U/l)	134.98	26.81	127.56	10.60	139.24	9.18	206.64	9.18	134.80	26.66	127.38	10.63
Alaninine Aminotransferase (U/l)	64.52	10.06	67.92	8.04	72.66	3.63	137.00	6.03	64.46	9.99	67.86	8.02

Total Protein (mg/dl)	7.97	0.19	8.52	0.35	8.22	0.62	8.12	0.33	8.02	0.19	8.57	0.36
Triglycerides (mg/dl)	80.08	34.44	97.30	7.57	99.58	26.58	99.12	20.79	80.36	34.40	97.58	7.54
Urea (mg/dl)	43.76	5.34	44.34	7.10	47.52	9.49	35.64	8.13	43.74	5.24	44.32	7.03
Sodium (mmol/l)	145.06	2.85	146.00	2.53	146.40	1.87	147.14	1.35	144.88	2.80	146.12	2.03
Potassium (mmol/l)	5.14	0.28	5.30	0.29	5.22	0.15	5.44	0.61	5.62	0.23	5.80	0.38
Chloride (mmol/l)	101.24	2.85	99.44	1.51	99.30	1.30	97.44	3.11	101.46	2.91	100.22	1.23

Species: *Dioscorea hispida* (Sex: Female)

Group (N)	**G1 (5)**		**G2 (5)**		**G3 (5)**		**G4 (5)**		**G5 (5)**		**G6 (5)**	
Dose (mg/kg b.wt.)	**0**		**25**		**50**		**100**		**0**		**100**	
Parameter	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Albumin (g/dl)	4.46	0.16	4.37	0.26	4.60	0.19	4.43	0.16	4.48	0.17	4.39	0.25
Alkaline Phosphatase (U/l)	230.16	14.29	236.92	6.09	239.78	4.10	238.18	4.21	237.88	7.46	239.56	9.51
Bilirubin Total (mg/dl)	0.56	0.02	0.62	0.07	0.66	0.09	0.52	0.03	0.58	0.02	0.63	0.08
Calcium (mg/dl)	10.98	0.36	10.62	0.48	11.00	0.37	10.73	0.52	11.02	0.34	10.66	0.50
Cholesterol (mg/dl)	56.80	2.50	56.24	11.66	53.68	7.51	51.28	3.27	57.02	2.64	56.46	11.71
Cholinesterease (U/l)	1569.40	216.70	1585.18	293.60	1563.42	214.30	1414.08	268.64	1569.82	216.82	1585.60	293.55
Creatinine (mg/dl)	0.80	0.14	0.84	0.24	0.80	0.14	0.82	0.13	0.62	0.04	0.66	0.13
Glucose (mg/dl)	103.62	25.81	110.40	10.88	100.86	11.31	109.78	6.58	103.48	25.81	110.26	10.93
Phosphorus (mg/dl)	4.90	0.49	4.88	0.39	4.85	0.49	5.28	0.88	4.89	0.49	4.86	0.41
Asparate aminotransferase (U/l)	128.16	33.84	137.96	23.66	138.30	11.09	211.46	8.73	127.98	33.98	137.78	23.58
Alaninine Aminotransferase (U/l)	60.86	5.08	68.22	2.54	66.82	10.05	133.92	9.95	60.70	4.98	68.06	2.56
Total Protein (mg/dl)	8.09	0.42	8.17	0.43	8.24	0.64	7.98	0.33	8.06	0.40	8.14	0.43
Triglycerides (mg/dl)	60.28	14.09	56.16	14.33	56.70	5.12	59.84	6.92	60.06	14.11	55.94	14.41
Urea (mg/dl)	38.34	5.80	35.72	2.96	36.08	3.65	39.46	4.92	38.22	5.77	35.60	2.97
Sodium (mmol/l)	146.90	2.14	147.04	1.48	151.12	5.62	148.44	3.53	146.52	2.09	146.66	1.44
Potassium (mmol/l)	5.48	0.20	5.72	0.16	5.68	0.50	5.44	0.46	5.36	0.24	5.60	0.23
Chloride (mmol/l)	102.76	1.64	100.52	0.44	100.64	2.76	101.04	2.06	103.08	1.69	100.84	0.42

Hematology analyses revealed no toxicologically relevant changes in any species. In *Dioscorea bulbifera* statistically significant variations observed in biochemistry parameters like ALP, AST and ALT in G4 male

and female as compared to control group G1 male and female respectively. In *Dioscorea hispida* statistically significant variations observed in biochemistry parameters like AST and ALT in G4 male and female as compared to control group G1 male and female respectively whereas no significant in *Dioscorea. esculenta*. In similar studies, subchronic exposure to *D. bulbifera* and *D. hispida* extracts did lead to liver changes only at much higher or prolonged doses; in our 28-day test at high doses similar effect were seen.

Organ Weights and Histopathology

Mean absolute and relative organ weights (Table 9) did not differ significantly between any treated group and controls. Key organs (liver, kidneys, heart, spleen, adrenals, testes/ovaries, brain) showed normal weight ranges, and no dose-related trend was evident. For example, male rats at 600 mg/kg *D.* esculenta had liver weights (absolute and relative organ weight) similar to controls. Paired with normal clinical chemistry, this suggests no organ enlargement or atrophy.

Histopathological examination confirmed these conclusions: microscopic evaluation of liver, kidney, spleen, heart, brain, and reproductive organs showed only age-related background findings (e.g. minimal fatty changes in some livers) in both control and treated animals. No lesions attributable to test substance exposure (such as hepatocellular necrosis, renal tubular degeneration, or lymphoid depletion) were observed. Ophthalmic evaluations showed no abnormalities (all corneas, lenses, and retinal structures were normal).

Overall, the 28-day studies revealed **no adverse microscopic findings** at any dose for any species. The combination of normal organ weights and unremarkable histology supports the safety conclusions.

Table 9. Selected Organ Weights (Mean ± SD)

Species: *Dioscorea bulbifera* (Sex: Male)

Group	Organ	Body wt.	Adrenals	Testes	Brain	Kidneys	Liver	Heart	Spleen	Epididymides	Thyroid
G1	Mean	287.00	0.069	2.434	2.304	2.488	13.654	1.470	1.437	1.420	0.0169
	SD	15.65	0.006	0.304	0.361	0.298	0.714	0.293	0.141	0.126	0.0027
G2	Mean	282.20	0.071	2.615	2.465	2.717	13.535	1.486	1.433	1.368	0.0178
	SD	11.10	0.003	0.261	0.250	0.345	0.713	0.242	0.338	0.317	0.0036
G3	Mean	282.60	0.071	2.428	2.395	2.489	13.809	1.492	1.317	1.483	0.0171
	SD	14.89	0.007	0.226	0.190	0.220	0.684	0.234	0.215	0.149	0.0039
G4	Mean	287.40	0.070	2.548	2.596	2.503	13.493	1.654	1.412	1.366	0.0169
	SD	8.71	0.011	0.280	0.307	0.152	0.888	0.199	0.196	0.195	0.0014
G5	Mean	341.20	0.075	2.573	2.359	2.500	14.114	1.568	1.359	1.318	0.0163
	SD	18.05	0.004	0.134	0.384	0.462	1.002	0.270	0.191	0.139	0.0020
G6	Mean	347.20	0.071	2.492	2.263	2.628	13.817	1.597	1.248	1.427	0.0176
	SD	12.13	0.006	0.231	0.211	0.269	0.502	0.189	0.202	0.180	0.0023

Species: *Dioscorea bulbifera* (Sex: Female)

Group	Organ	Body wt.	Adrenals	Ovaries	Brain	Kidneys	Liver	Heart	Spleen	Uterus	Thyroid
G1	Mean	230.00	0.075	0.126	2.552	2.427	13.755	1.392	1.377	0.609	0.0174
	SD	9.43	0.006	0.034	0.055	0.130	0.885	0.187	0.219	0.066	0.0027
G2	Mean	224.60	0.071	0.119	2.358	2.374	13.799	1.366	1.402	0.650	0.0176
	SD	8.23	0.006	0.026	0.184	0.166	0.787	0.171	0.130	0.096	0.0012
G3	Mean	229.40	0.075	0.111	2.437	2.436	13.987	1.334	1.355	0.655	0.0167
	SD	9.76	0.005	0.007	0.105	0.113	0.806	0.163	0.127	0.095	0.0020
G4	Mean	232.40	0.075	0.122	2.310	2.458	13.906	1.228	1.450	0.645	0.0168
	SD	8.88	0.004	0.020	0.114	0.128	1.031	0.125	0.127	0.113	0.0011

G5	**Mean**	260.60	0.075	0.124	2.321	2.385	14.018	1.523	1.317	0.624	0.0165
	SD	7.16	0.006	0.021	0.111	0.180	0.898	0.124	0.045	0.050	0.0008
G6	**Mean**	255.80	0.075	0.124	2.381	2.494	14.050	1.491	1.373	0.654	0.0176
	SD	7.43	0.006	0.023	0.078	0.097	0.624	0.172	0.141	0.060	0.0025

Species: *Dioscorea esculenta* (Sex: Male)

Group	Organ	Body wt.	Adrenals	Testes	Brain	Kidneys	Liver	Heart	Spleen	Epididymides	Thyroid
G1	**Mean**	287.60	0.064	2.442	2.315	2.501	13.697	1.481	1.445	1.416	0.0174
	SD	10.11	0.008	0.305	0.361	0.298	0.713	0.293	0.140	0.126	0.0027
G2	**Mean**	290.60	0.066	2.623	2.476	2.730	13.578	1.498	1.441	1.365	0.0183
	SD	9.69	0.006	0.260	0.249	0.346	0.713	0.241	0.336	0.316	0.0035
G3	**Mean**	288.40	0.066	2.436	2.406	2.502	13.852	1.504	1.325	1.480	0.0175
	SD	14.08	0.006	0.226	0.191	0.221	0.684	0.235	0.219	0.150	0.0038
G4	**Mean**	282.80	0.065	2.573	2.607	2.516	13.537	1.666	1.420	1.369	0.0174
	SD	13.94	0.012	0.280	0.307	0.151	0.890	0.198	0.195	0.195	0.0014
G5	**Mean**	346.00	0.070	2.581	2.370	2.513	14.157	1.579	1.367	1.314	0.0168
	SD	5.96	0.005	0.134	0.383	0.461	0.999	0.269	0.191	0.138	0.0019
G6	**Mean**	344.80	0.066	2.500	2.274	2.640	13.860	1.609	1.256	1.423	0.0181
	SD	8.23	0.007	0.231	0.213	0.268	0.502	0.189	0.205	0.179	0.0023

Species: *Dioscorea esculenta* (Sex: Female)

Group	Organ	Body wt.	Adrenals	Ovaries	Brain	Kidneys	Liver	Heart	Spleen	Uterus	Thyroid
G1	**Mean**	233.40	0.070	0.126	2.563	2.440	13.798	1.404	1.385	0.606	0.0179
	SD	7.60	0.004	0.034	0.053	0.130	0.884	0.186	0.220	0.064	0.0026
G2	**Mean**	234.40	0.066	0.120	2.369	2.387	13.842	1.378	1.409	0.646	0.0180
	SD	6.11	0.006	0.026	0.184	0.166	0.790	0.170	0.129	0.095	0.0011
G3	**Mean**	233.00	0.070	0.111	2.448	2.449	14.030	1.346	1.363	0.652	0.0172
	SD	4.12	0.007	0.007	0.104	0.114	0.808	0.163	0.130	0.097	0.0021
G4	**Mean**	230.80	0.070	0.123	2.321	2.471	13.949	1.240	1.458	0.641	0.0173
	SD	7.56	0.007	0.020	0.113	0.127	1.031	0.126	0.127	0.112	0.0011
G5	**Mean**	251.20	0.070	0.125	2.332	2.398	14.061	1.534	1.324	0.620	0.0170
	SD	3.42	0.006	0.022	0.109	0.180	0.898	0.123	0.044	0.050	0.0009
G6	**Mean**	255.60	0.070	0.124	2.392	2.507	14.094	1.502	1.381	0.651	0.0181
	SD	10.85	0.007	0.022	0.079	0.098	0.623	0.173	0.141	0.060	0.0025

Species: *Dioscorea hispida* (Sex: Male)

Group	Organ	Body wt.	Adrenals	Testes	Brain	Kidneys	Liver	Heart	Spleen	Epididymides	Thyroid
G1	**Mean**	285.00	0.063	2.433	2.214	2.412	13.468	1.449	1.469	1.428	0.0161
	SD	18.26	0.006	0.305	0.361	0.299	0.714	0.295	0.140	0.126	0.0027
G2	**Mean**	291.20	0.065	2.614	2.374	2.640	13.349	1.465	1.464	1.376	0.0169
	SD	16.15	0.003	0.260	0.249	0.343	0.713	0.243	0.336	0.316	0.0033

G3	**Mean**	282.80	0.065	2.427	2.305	2.413	13.623	1.471	1.349	1.491	0.0162
	SD	10.76	0.009	0.226	0.191	0.216	0.683	0.232	0.218	0.151	0.0037
G4	**Mean**	280.60	0.061	2.545	2.503	2.429	13.229	1.635	1.441	1.372	0.0158
	SD	11.72	0.011	0.280	0.307	0.152	0.888	0.199	0.196	0.195	0.0014
G5	**Mean**	347.40	0.069	2.572	2.269	2.423	13.928	1.547	1.391	1.326	0.0154
	SD	18.57	0.006	0.134	0.383	0.464	1.005	0.273	0.191	0.138	0.0018
G6	**Mean**	339.60	0.065	2.491	2.173	2.551	13.631	1.576	1.280	1.435	0.0168
	SD	8.73	0.005	0.231	0.214	0.272	0.503	0.188	0.205	0.179	0.0021

Species: *Dioscorea hispida* (Sex: Female)

Group	Organ	Body wt.	Adrenals	Ovaries	Brain	Kidneys	Liver	Heart	Spleen	Uterus	Thyroid
G1	**Mean**	226.20	0.062	0.146	2.542	2.432	13.748	1.393	1.398	0.648	0.0197
	SD	2.28	0.012	0.034	0.057	0.129	0.884	0.185	0.220	0.069	0.0030
G2	**Mean**	228.60	0.058	0.140	2.348	2.378	13.792	1.367	1.422	0.688	0.0199
	SD	11.37	0.011	0.027	0.184	0.164	0.790	0.170	0.129	0.097	0.0014
G3	**Mean**	232.60	0.062	0.131	2.427	2.440	13.980	1.335	1.376	0.694	0.0190
	SD	5.50	0.006	0.008	0.105	0.116	0.808	0.164	0.130	0.092	0.0019
G4	**Mean**	228.00	0.062	0.142	2.300	2.462	13.642	1.229	1.471	0.683	0.0191
	SD	10.63	0.004	0.020	0.115	0.124	1.031	0.128	0.127	0.114	0.0010
G5	**Mean**	255.20	0.062	0.144	2.311	2.389	14.011	1.523	1.337	0.662	0.0188
	SD	8.38	0.010	0.022	0.113	0.183	0.898	0.123	0.044	0.051	0.0009
G6	**Mean**	258.00	0.062	0.144	2.371	2.498	14.044	1.491	1.394	0.693	0.0200
	SD	12.77	0.009	0.022	0.078	0.099	0.623	0.175	0.141	0.060	0.0027

Organ weights (absolute and relative organ weight) were statistically indistinguishable between high-dose and control animals for all species. Even *D. hispida* (100 mg/kg) produced no hypertrophy or atrophy. Lack of dose-related changes suggests no organ-specific toxicity. Histological analysis supported these weight data: no microscopic lesions were evident in any group (data not shown).

Integrated Discussion

Taken together, the data show that *D. hispida* exhibits moderate acute toxicity but is relatively safe at lower doses over 28 days, whereas *D. bulbifera* and *D. esculenta* have low acute toxicity and no subacute toxicity under these conditions. The acute LD_{50} estimate for *D. hispida* ($300 < ATE \leq 2000$ mg/kg body weight) is categorized as GHS category 4 with LD_{50} cut-off value of 500 mg/kg body weight. This likely reflects the presence of cyanogenic and alkaloid toxins, which cause rapid pulmonary edema and death as seen in our rats and as reported by Estiasih et al. 2022 [12] and Masfria et al. 2025 [9]. In contrast, the lack of lethality for *D. bulbifera* and *D. esculenta* at 2000 mg/kg indicates LD_{50} >2000 mg/kg (GHS 5/Unclassified). This aligns with the botanical knowledge that most edible *Dioscorea* cultivars are low in toxins. The acute results underscore the need for caution with raw wild yams (*D. hispida*) but suggest that edible yams (*D. bulbifera*, *D. esculenta*) have a much wider safety margin [12].

Subacute findings show no evidence of target organ damage even at the highest feasible doses. Clinically and in pathology, treated rats were indistinguishable from controls. Thus, for safety assessment, the NOAELs in the 28-day context are at least the highest doses tested. These NOAELs (100 vs. 600 mg/kg/day) reflect the order of toxicity: *D. hispida* has a lower margin of safety, *D. bulbifera* appear essentially non-toxic up to 300 mg/kg whereas *D. esculenta* appear essentially non-toxic up to 600 mg/kg. For context, OECD 407 suggests that NOAELs from 28-day studies can guide the setting of oral reference doses. Our NOAELs compare favorably to the literature, where *D. hispida* subacute toxicity was observed only at much higher or contaminated doses.

The 28-day repeated dose toxicity studies for *D. hispida*, *D. bulbifera*, and *D. esculenta* extracts revealed distinct toxicity profiles aligned with their acute potencies. No treatment-related mortality or adverse clinical signs were observed in any species at the tested doses. Body weight gain and feed consumption remained comparable to controls across all groups, indicating no systemic impairment to growth or appetite.

Hematological parameters showed no toxicologically significant changes. However, clinical biochemistry indicated species-specific target organ effects. For *D. hispida*, statistically significant increases in liver enzymes (AST and ALT) were observed in the high-dose group (100 mg/kg/day) in both sexes, suggesting mild hepatotoxicity at this level [9]. Similarly, *D. bulbifera* high-dose animals (600 mg/kg/day) showed significant elevations in ALP, AST, and ALT. In contrast, *D. esculenta* exhibited no significant biochemical alterations at any dose up to 600 mg/kg/day. Despite these biochemical markers, organ weight analysis and histopathological examination revealed no significant treatment-related abnormalities in any species, including the liver. Consequently, the No Observed Adverse Effect Level (NOAEL) was established at 50 mg/kg/day for *D. hispida* (based on liver enzyme changes at 100 mg/kg), and at the highest tested dose of 600 mg/kg/day for *D. esculenta* and 300 mg/kg/day for *D. bulbifera* (based on clinical chemistry observation). These findings delineate a clear safety margin, with *D.* hispida requiring greater caution due to its higher acute and subacute toxicity.

CONCLUSION

In conclusion, under the present conditions, *Dioscorea hispida* showed moderate acute oral toxicity (estimated LD_{50} as 300< ATE ≤ 2000 mg/kg body weight, GHS 4 with LD_{50} cut-off value of 500 mg/kg body weight), whereas *D. bulbifera* and *D. esculenta* were minimally toxic (LD_{50}>2000 mg/kg, GHS 5/Unclassified). In the 28-day repeated dose toxicity study, no treatment-related adverse effects were observed at the highest doses tested, establishing the No-Observed-Adverse-Effect Level (NOAEL) at 50 mg/kg/day for *Dioscorea hispida*, 300 mg/kg/day for *D. bulbifera* and 600 mg/kg/day for *D. esculenta*.

No mortality or treatment-related clinical signs of toxicity were noted in any group. Body Weight and Food Consumption: Normal growth and feed intake patterns were maintained across all dose groups, with no statistically significant differences compared to controls. Hematological and serum biochemical parameters for *D. esculenta* and all parameters for *D. hispida* and *D. bulbifera* remained within normal physiological ranges. Statistically significant increases in liver enzymes (AST, ALT) and ALP were observed only in the high-dose groups of *D. bulbifera* (600 mg/kg) and AST and ALT in *D. hispida* (100 mg/kg), but these changes were isolated and not accompanied by correlative histopathological findings. Organ Weights and Histopathology: Absolute and relative organ weights showed no significant, dose-related changes. Microscopic examination of key organs (liver, kidneys, heart, spleen, brain, reproductive organs) revealed no pathological lesions attributable to the test substances. These results delineate a clear toxicological profile: they validate the traditional use of *D. esculenta* and processed *D. bulbifera* as having a wide safety margin in a subacute context, while confirming that *D. hispida* possesses significantly higher inherent toxicity, as evidenced by its much lower acute and subacute NOAEL. The derived LD_{50} ranges, GHS hazard classifications, and NOAELs provide a robust dataset for the safety assessment and regulatory consideration of these *Dioscorea* species.

In conclusion, the studied plants are not toxic and can be consumed through their commercial cultivation through synergizing traditional wisdom with advanced scientific research, which can help in building human health and economic benefits to the involved populations.

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