Archives of Clinical Toxicology

Yi G, et al., Arch Clin Toxicol. 2025;7(1):77-87.

Original Research

A 90-day oral toxicity study of Grains of Paradise Extract

Gloria Yi[1], George Gao[1], Wayne Yi[1], Long Jiang[1,*]

[1]Nanjing Nutrabuilding Bio-tech Co., Ltd, Nanjing, China

*Author for correspondence: Email: ljjiang@nnbnutrition.com

Received date: July 25, 2025
Accepted date: August 14, 2025

Abstract

Grains of Paradise (*Aframomum melegueta* [Roscoe] K. Schum.), a spice native to West Africa, has a rich ethnopharmacological history. Studies suggest Grains of Paradise possesses anti-inflammatory and analgesic properties. However, despite these potential therapeutic uses, comprehensive toxicological data on the extract of Grains of Paradise in animals remains limited. This study aimed to evaluate the subchronic toxicity of Grains of Paradise Extract in Sprague Dawley rats following 90 days of oral administration by gavage at doses of 0 (vehicle control), 135, 270, and 340 mg/kg bw/day, followed by a 28-day recovery period for the high-dose and control groups. No mortality, adverse clinical signs, or treatment-related differences in body weight, organ weights, and feed consumption were observed. Hematology, clinical chemistry, and histopathology did not reveal toxicologically significant changes. While some statistically significant, non-dose-dependent alterations occurred, such as minor T3/T4 elevations at 340 mg/kg, these lacked toxicological relevance without thyroid dysfunction. Based on the overall data, the No Observed Adverse Effect Level (NOAEL) for Grains of Paradise Extract was determined to be 270 mg/kg bw/day. These results support the long-term safety of this botanical, facilitating its potential application as a functional food or therapeutic.

Keywords: Grains of Paradise Extract, Subchronic toxicity, Sprague Dawley rats, NOAEL, 90-day toxicity study, Oral administration, OECD

Abbreviations: bw: Body weight; kg: Kilogram; mg: Milligram; NOAEL: No Observed Adverse Effect Level; OECD: Organization of Economic Co-operation and Development

Introduction

Grains of Paradise (*Aframomum melegueta* [Roscoe] K. Schum.), also known as Guinea pepper or Alligator pepper, is a member of the Zingiberaceae family indigenous to the West African coast, including countries such as Ghana, Liberia, Ivory Coast, Togo, and Nigeria. It produces purple flowers that develop into long pods containing small, reddish-brown, aromatic, and pungent seeds. The seeds have a long history of use in West Africa, serving both as a spice to flavor food and as a remedy in ethnobotanical practices [1].

The therapeutic potential of Grains of Paradise has been investigated in both animal and human research. Animal studies have demonstrated its anti-inflammatory and analgesic properties. The active compounds in Grains of Paradise, such as gingerols, paradols and shogaols, are considered to be the main sources of its pharmacological activities, partially achieved by inhibiting the activity of COX-2 enzyme and the expression of pro-inflammatory genes. The presence of these compounds is also consistent with its application in traditional medicine, such as for the treatment of pain and discomfort related to inflammation [2–7].

Moreover, it has been found to have hypoglycemic effects, positioning it as a potential candidate for the treatment of diabetes [8]. In human studies, the ingestion of Grains of Paradise has been shown

to augment whole-body energy expenditure and decrease visceral fat, suggesting its potential as a dietary supplement for weight regulation [9]. Despite these promising findings, the toxicological profile of Grains of Paradise is not fully elucidated. A 28-day toxicity study in rats showed dose-related increases in liver weight and alkaline phosphatase levels, indicating possible hepatotoxic effects [10]. However, the long-term effects and the mechanisms underlying these toxicological outcomes remain unclear.

The purpose of this 90-day OECD 408-compliant toxicology study is to provide a comprehensive evaluation of the safety of Grains of Paradise Extract following extended administration. By examining the chronic effects on various physiological systems, this study aims to determine the No Observed Adverse Effect Level (NOAEL) and to identify any potential long-term toxicological risks associated with the consumption of Grains of Paradise Extract. This research is crucial for developing safe usage guidelines for Grains of Paradise Extract, both as a culinary spice and a potential therapeutic agent, and for deepening our understanding of its toxicological profile to ensure its safe and efficacious application.

Materials and Methods

Test article

The test article, Grains of Paradise Extract (CaloriBurn GP®, Nanjing Nutrabuilding Bio-tech Co., Ltd), is an ethanol-extracted preparation derived from the seeds of *Aframomum melegueta*. The extract is stored in tightly sealed containers, protected from moisture and sunlight, with a shelf life of 24 months when kept at room temperature.

Ninety-day repeated dose oral toxicity study on Grains of Paradise Extract

A 90-day repeated dose oral toxicity study was specifically designed and conducted in accordance with OECD guideline 408 to assess the toxicological potential of Grains of Paradise Extract.

Male and female Sprague Dawley rats, aged 7-8 weeks, were randomly assigned based on body weight to be administered via gavage 0 (G1, control group, 0.5% Carboxyl Methyl Cellulose Sodium vehicle), 135 (G2), 270 (G3), or 340 mg/kg bw/day (G4) of Grains of Paradise Extract. The animals in the main study group (n=10 rats/sex/dose group) were administered Grains of Paradise Extract for 90 consecutive days and were sacrificed on day 91. In addition, two satellite groups of animals were either untreated (control recovery, G1R) or administered 340 mg/kg bw/day (G4R) of Grains of Paradise Extract for an additional 28 consecutive days.

Detailed clinical examinations were conducted prior to the first administrations during acclimatization and following randomization and a minimum of once weekly afterward. These include changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, and behavior patterns were assessed once before the start of administration and a minimum of once weekly (on the day of body weight measurement) thereafter. Cage-side observations were conducted for mortality and morbidity twice daily. The rats were assessed for tremors, convulsions, salivation, diarrhea, lethargy, and coma. Body weights were measured when they were received by the facility, on the day of randomization, once per week during the administration period, and on the final day of dosing. The amount of feed consumed was recorded weekly on the day the rats were weighed and feed input was also measured weekly. Ophthalmological examinations were performed on the last day of treatment for the main study groups and at the end of the recovery period for the recovery group.

At the end of the 90-day administration period for the main study and on day 119 for the recovery groups the rats were fasted overnight but were given access to water, and blood samples were collected for hematology and biochemistry analyses via the retro-orbital sinus. Hematology analyses included white blood cell (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV), neutrophil (NEUT), lymphocyte (LYMP), monocyte (MONO), eosinophil (EOS), large unstained cell (LUC), basophil (BASO), and reticulocyte (Retic). Clinical chemistry and hormone analyses included albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium (Ca), total cholesterol (T.Chol), creatinine kinase (CK), creatinine (Creat), gamma - glutamyltransferase (GGT), glucose (Glu), high density lipoprotein - cholesterol (HDL - C), inorganic phosphorous (Pi), lactate dehydrogenase (LDH), low density lipoprotein - cholesterol (LDL - C), total bilirubin (T. Bil), total protein (T.Pro), triglyceride (Trig), blood urea nitrogen (BUN), albumin/globulin (A/G), sodium (Na), potassium (K), chloride (Cl), urea, globulin, thyroxine (T4), triiodothyronine (T3) and thyroid-stimulating hormone (TSH). Urine samples were collected after the overnight fast and the volume, pH, specific gravity, color, urine albumin, and appearance were determined. Urine concentration of glucose, urobilinogen, ketone bodies, and blood in urine were measured.

On the day of necropsy, the rats were sacrificed using carbon dioxide, and the adrenals, brain, testes, epididymides, liver, kidneys, heart, spleen, thymus, lungs, thyroid with para thyroid gland, pituitary gland (in males), ovaries, uterus with cervix (in females) were collected and preserved. Blood smears were performed. All gross lesions were examined. Histopathological analyses were performed on all animals in the control and Grains of Paradise Extract treatment groups on all preserved organs. The following were weighed with paired organs being weighed together: adrenal glands, brain, thyroid with parathyroids, thymus, epididymides, heart, kidneys, liver, ovaries, spleen, testes, and uterus with cervix.

Statistical analysis

For the 90-day toxicity assay, GraphPad Prism software version 9.5.0 was used for statistical analysis of body weight, feed consumption, clinical chemistry, hematology, electrolytes, and organ weights. Means ± SD were calculated and one-way ANOVA with Dunnett's test was used to determine the significant difference between control and treatment groups. P-values of less than 0.05 were considered significant.

Results

No unscheduled deaths of animals occurred during the study and no clinical signs were observed. No significant reduction in body weight was observed for animals treated with Grains of Paradise Extract compared with animals treated with control for the main and recovery study groups (**Table 1**-Body Weight Males, **Table 2**-Body Weight Females).

Table 1. Grains of Paradise Extract Body Weight Males.

Week	Groups					
	G1	G2	G3	G4	G1R	G4R
1	153.31±11.18	141.89±12.76	155.42±14.09	157.50±10.10	153.18±14.35	148.64±18.35
2	163.75±11.32	151.56±13.12	166.11±13.68	167.77±10.37	163.94±13.71	158.42±18.75
3	174.99±11.36	164.21±12.66	177.82±13.53	179.21±9.90	175.02±13.80	170.02±18.83
4	186.61±10.78	176.84±14.81	188.57±14.58	190.03±7.85	188.49±16.66	180.94±19.74
5	197.11±10.76	187.50±14.48	198.88±15.26	200.48±7.95	200.14±17.23	191.61±20.16
6	207.05±11.04	196.63±14.42	208.57±14.88	210.01±8.11	210.09±17.14	200.77±19.69
7	217.96±10.29	209.83±14.33	220.39±17.00	221.22±7.65	221.50±18.73	211.61±21.16
8	227.77±9.70	220.22±14.71	230.65±15.80	231.21±8.00	232.19±17.07	222.32±22.53
9	237.13±10.19	228.84±13.88	238.76±16.63	239.35±8.97	241.13±16.71	231.16±21.96
10	245.48±10.33	237.97±13.87	247.18±16.02	247.49±9.06	250.40±17.46	239.87±22.03
11	253.08±10.12	244.93±13.42	254.28±15.97	255.12±9.45	260.70±17.05	249.35±20.56
12	260.98±9.84	251.34±13.24	261.02±16.23	261.81±9.73	266.93±17.21	256.06±20.25
13	269.07±10.68	259.11±12.75	269.91±16.76	270.64±10.36	276.14±16.47	264.12±19.96
14	273.08±10.81	263.65±12.86	274.56±16.79	275.41±10.69	285.01±16.21	273.02±19.90
15	-	-	-	-	293.85±16.00	280.96±19.25
16	-	-	-	-	303.15±16.73	288.73±19.09
17	-	-	-	-	312.62±16.44	298.58±19.93
18	-	-	-	-	321.41±16.09	309.03±19.75

Values were expressed as mean±SD (n=10), Statistical significance was compared between Control (G1) versus other treatment groups (P≥0.05).

G1: Control Main Study; G2: Low Dose (135 mg/kg) Main Study; G3: Middle Dose (270 mg/kg) Main Study; G4: High Dose (340 mg/kg) Main Study; G1R: Control Recovery Study; G4R: High Dose (340 mg/kg) Recovery Study.

Table 2. Grains of Paradise Extract Body Weight Females.

Week	Groups					
	G1	G2	G3	G4	G1R	G4R
1	144.71±14.12	155.20±5.79	147.53±9.36	153.52±7.38	150.91±9.74	150.24±12.72
2	151.27±12.84	160.56±5.49	153.99±9.99	159.00±7.59	156.86±10.05	156.62±12.74
3	160.37±12.71	170.58±5.31	161.79±9.97	167.18±7.72	164.22±9.87	164.50±13.56
4	168.48±12.15	179.23±6.49	170.97±11.48	174.83±8.12	172.75±10.39	173.40±14.09
5	175.94±11.96	185.58±6.35	179.31±11.58	181.88±8.08	180.85±11.15	181.56±13.62
6	184.62±11.92	193.79±6.32	186.96±12.03	190.12±7.62	188.87±12.31	189.65±13.10
7	192.80±12.32	202.28±6.96	195.13±13.05	198.72±8.28	196.72±13.86	197.62±12.95
8	200.97±12.84	210.76±7.97	203.30±14.29	207.31±9.24	204.57±15.55	205.59±13.05
9	206.16±13.15	215.70±7.97	208.31±14.32	212.46±9.18	209.28±15.87	210.52±12.68
10	210.74±13.48	220.52±7.78	213.37±14.34	217.22±9.31	214.27±15.58	215.52±12.28
11	216.27±12.62	225.41±7.23	218.01±13.92	221.59±9.05	218.65±14.98	220.48±12.37
12	222.48±12.47	231.13±7.44	224.03±14.24	227.04±9.06	224.62±14.85	226.60±12.82
13	227.73±12.89	236.74±7.65	228.90±14.44	233.91±10.03	229.58±15.36	232.27±12.47
14	234.51±12.50	243.63±7.82	234.71±14.77	240.70±9.70	235.61±15.96	238.48±12.66
15	-	-	-	-	243.39±16.02	244.80±12.69
16	-	-	-	-	247.74±15.75	249.46±12.50
17	-	-	-	-	253.68±15.65	255.08±12.78
18	-	-	-	-	259.75±16.01	260.41±12.60

Values were expressed as mean±SD (n = 10), Statistical significance was compared between Control (G1) versus other treatment groups (P≥0.05).

G1: Control Main Study; G2: Low Dose (135 mg/kg) Main Study; G3: Middle Dose (270 mg/kg) Main Study; G4: High Dose (340 mg/kg) Main Study; G1R: Control Recovery Study; G4R: High Dose (340 mg/kg) Recovery Study.

There was no significant difference in feed consumption for males and females in the Grains of Paradise Extract groups compared with the control group for the main or recovery study group. Ophthalmological examinations and grip strength tests also showed no differences. Gross pathological examination revealed no abnormalities at necropsy. Histopathological assessment revealed minimal lesions in both the control (G1) and high-dose (G4) groups of males and females, including minimal mononuclear cell infiltration (occasionally with early alveolar fibrosis) in the lungs, minimal mixed cell infiltration in the liver, minimal inflammation of the urothelium and minimal mononuclear cell infiltration in the kidneys, and minimal mononuclear cell infiltration in the heart. Histopathological examination of the thyroid gland (including parathyroid) showed no treatment-related microscopic alterations, with all animals exhibiting normal histology within normal limits. Importantly, all observed lesions in the lungs, liver, kidneys, and heart were sporadic, with frequencies not significantly different from the control group, and other lesions were isolated and spontaneous.

Several statistically significant alterations in hematological parameters were discovered. A statistically significant decrease in Eosinophils (EOS) was observed in males in the G2, G3, and G4 groups when compared to the control group (G1). In contrast, statistically significant increases were recorded. Males in the G4R group showed a statistically significant increase in EOS and White Blood Cells (WBC). Females in the G4R group exhibited a statistically significant increase in Reticulocytes count. The study indicates notable changes in hematological parameters, including decreases in EOS in certain male groups and increases in EOS, WBC, and Reticulocytes in other groups (**Table 3**-Hematology Males, **Table 4**-Hematology Females). However, these discrepancies were not attributed to the treatment because no distinct dose-response relationship was found.

There were no significant difference in most clinical biochemistry parameters including ALT, AST, ALP, calcium, creatinine, GGT, HDL-C, LDL-C, total bilirubin, and triglycerides when compared to the control groups in both male and female rats. However, statistically significant alterations were observed in specific parameters. Males in the G4 group exhibited a decrease in total protein levels. Males in the G2 group showed decreased blood urea nitrogen (BUN) and urea concentrations. Sodium levels were elevated in males of the G3 and G4 groups. Both male and female rats in the G4R group had increased glucose levels. Furthermore, males in the G4R group presented decreased globulin and increased potassium levels (**Table 5** - Clinical Biochemistry Males, **Table 6** - Clinical Biochemistry Females). These statistically significant changes are considered incidental and unrelated to treatment, as they lacked a dose-dependent relationship.

Table 3. Grains of Paradise Extract Hematology Males.

Parameter	Groups					
	G1	G2	G3	G4	G1R	G4R
WBC (10^9/L)	10.90±1.17	10.96±3.09	12.41±2.66	11.02±3.34	10.38±3.17	15.01*±3.83
RBC (10^{12}/L)	8.99±0.34	9.28±0.27	9.11±0.33	8.58±1.12	8.63±0.51	8.59±0.52
HGB (g/L)	149.00±7.32	155.10±7.06	151.00±6.73	145.00±15.53	144.83±9.87	144.50±4.59
HCT (L/L)	0.47±0.02	0.49±0.03	0.47±0.01	0.46±0.06	0.45±0.03	0.44±0.02
MCV (fL)	52.72±1.74	52.84±1.59	52.12±1.56	53.73±2.54	51.75±2.08	51.42±2.93
MCH (pg)	16.58±0.71	16.71±0.71	16.57±0.46	16.97±0.88	16.77±0.83	16.87±0.96
MCHC (g/L)	314.60±6.85	316.90±18.51	318.00±5.98	316.00±8.31	323.83±4.07	328.17±5.27
RDW (%)	13.54±0.38	13.79±0.74	13.54±0.62	13.20±0.84	13.98±1.11	13.42±0.53
PLT (10^9/L)	957.20±115.84	905.60±115.29	945.60±67.72	952.90±76.02	1008.00±76.38	1040.33±137.43
MPV (fL)	7.26±0.22	7.28±0.27	7.19±0.28	7.39±0.20	7.57±0.16	7.38±0.26
NEUT (%)	24.04±7.07	24.37±9.24	27.75±8.60	23.98±10.97	31.40±8.54	27.97±8.77
LYMP (%)	66.17±8.31	68.13±8.74	65.05±7.95	68.83±11.54	61.22±7.51	61.02±9.74
MONO (%)	2.87±1.32	2.90±0.73	2.47±0.99	2.53±0.89	2.75±0.45	2.15±0.87
EOS (%)	5.60±2.43	3.48*±0.87	3.48*±2.04	2.74*±1.29	3.55±1.68	7.75*±3.09
LUC (%)	1.26±0.57	1.09±0.25	1.18±0.29	1.89±2.17	1.07±0.19	1.12±0.35
BASO (%)	0.04±0.05	0.03±0.05	0.03±0.05	0.01±0.03	0.02±0.04	0.02±0.04
Retic (10^{12}/L)	0.18±0.02	0.18±0.05	0.18±0.06	0.15±0.05	0.18±0.04	0.16±0.04
Retic (%)	1.98±0.21	1.89±0.58	2.02±0.67	1.76±0.56	2.11±0.38	1.86±0.58

WBC: White Blood Cell; RBC: Red Blood Cell Count; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: Red Blood Cell Distribution Width; PLT: Platelet; MPV: Mean Platelet Volume; NEUT: Neutrophil; LYMP: Lymphocyte; MONO: Monocyte; EOS: Eosinophil; LUC: Large Unstained Cell; BASO: Basophil; Retic: Reticulocyte Count.

Values were expressed as mean±SD (n=10), Statistical significance was compared between Control (G1) versus other treatment groups (* P Value <0.05;** P Value <0.001; *** P Value <0.0001).

G1: Control Main Study; G2: Low Dose (135 mg/kg) Main Study; G3: Middle Dose (270 mg/kg) Main Study; G4: High Dose (340 mg/kg) Main Study; G1R: Control Recovery Study; G4R: High Dose (340 mg/kg) Recovery Study.

Table 4. Grains of Paradise Extract Hematology Females.

Parameter	Groups					
	G1	G2	G3	G4	G1R	G4R
WBC (10^9/L)	8.60±2.11	10.32±2.38	9.20±3.00	9.25±3.49	7.89±1.16	10.86±4.06
RBC (10^{12}/L)	8.53±0.39	8.57±0.45	8.35±0.41	8.47±0.37	8.38±0.29	8.11±0.66
HGB (g/L)	149.30±6.67	148.60±3.44	149.60±4.20	149.30±5.85	146.00±3.46	138.83±11.44
HCT (L/L)	0.47±0.02	0.47±0.01	0.45±0.02	0.47±0.02	0.45±0.01	0.43±0.03
MCV (fL)	54.71±1.98	54.61±1.88	54.53±1.64	55.62±1.22	53.95±1.12	52.75±1.73
MCH (pg)	17.49±0.63	17.34±0.82	17.93±0.74	17.64±0.33	17.42±0.49	17.17±0.68
MCHC (g/L)	319.80±1.62	318.00±7.72	329.30±15.53	317.30±4.74	322.83±3.06	325.17±5.85
RDW (%)	12.15±0.57	12.16±0.51	11.73±0.48	12.54±1.62	11.83±0.64	12.22±0.55
PLT (10^9/L)	925.20±142.92	999.50±137.73	1023.60±131.83	951.80±137.65	944.83±81.17	1009.50±234.74
MPV (fL)	7.46±0.22	7.33±0.17	7.24±0.23	7.39±0.23	7.47±0.20	7.47±0.05
NEUT (%)	23.15±4.61	19.34±6.83	26.28±5.00	20.57±5.89	28.30±7.87	29.33±11.66
LYMPH (%)	69.13±6.58	72.67±9.48	65.57±5.10	71.87±8.12	62.92±8.91	61.93±12.46
MONO (%)	2.56±0.95	2.24±0.83	2.75±0.94	2.80±0.64	2.58±0.58	2.48±0.71
EOS (%)	3.81±2.29	4.28±3.37	4.03±1.28	3.25±2.36	4.75±2.63	4.35±1.28
LUC (%)	1.32±0.34	1.42±0.25	1.31±0.28	1.51±0.60	1.43±0.41	1.88±1.12
BASO (%)	0.02±0.04	0.02±0.04	0.05±0.05	0.01±0.03	0.00±0.00	0.02±0.04
Retic (10^{12}/L)	0.14±0.03	0.13±0.04	0.14±0.05	0.14±0.03	0.10±0.01	0.18±0.08
Retic (%)	1.63±0.45	1.54±0.54	1.70±0.53	1.71±0.39	1.23±0.16	2.24*±1.09

WBC: White Blood Cell; RBC: Red Blood Cell Count; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: Red Blood Cell Distribution Width; PLT: Platelet; MPV: Mean Platelet Volume; NEUT: Neutrophil; LYMP: Lymphocyte; MONO: Monocyte; EOS: Eosinophil; LUC: Large Unstained Cell; BASO: Basophil; Retic: Reticulocyte Count.

Values were expressed as mean±SD (n=10), Statistical significance was compared between Control (G1) versus other treatment groups (* P Value <0.05;** P Value <0.001; *** P Value <0.0001).

G1: Control Main Study; G2: Low Dose (135 mg/kg) Main Study; G3: Middle Dose (270 mg/kg) Main Study; G4: High Dose (340 mg/kg) Main Study; G1R: Control Recovery Study; G4R: High Dose (340 mg/kg) Recovery Study.

Table 5. Grains of Paradise Extract Clinical Biochemistry Male.

Parameter	Groups					
	G1	G2	G3	G4	G1R	G4R
ALB (g/L)	30.81±0.91	30.70±1.88	30.37±2.28	30.92±2.84	28.92±1.80	30.17±1.87
ALP (U/L)	178.40±75.80	223.10±75.53	197.10±46.25	191.80±81.59	203.50±98.51	156.67±19.94
ALT (U/L)	50.40±8.49	55.90±13.28	55.30±11.12	54.40±9.18	53.83±7.99	50.67±10.91
AST (U/L)	116.60±14.55	132.50±20.61	130.20±30.26	132.50±47.73	133.67±11.74	140.67±31.24
Ca (mmol/L)	2.47±0.05	2.47±0.06	2.48±0.07	2.48±0.06	2.38±0.03	2.40±0.07
T.Chol (mmol/L)	1.42±0.19	1.49±0.17	1.57±0.21	1.65±0.36	1.39±0.27	1.36±0.09
CK (U/L)	200.10±92.74	253.80±360.23	215.60±204.04	139.10±89.25	483.00±600.28	253.67±91.62
Creat (μmol/L)	27.70±3.16	27.70±2.98	26.20±3.01	25.00±6.11	32.50±5.47	32.50±4.04
GGT (U/L)	0.80±0.63	0.80±0.42	0.90±0.57	1.10±0.32	1.83±0.75	1.00±0.63
Glu (mmol/L)	7.04±0.75	6.44±0.82	6.29±0.76	6.55±0.51	4.80±0.80	6.00*±0.73
HDL - C (mmol/L)	0.81±0.12	0.83±0.11	0.86±0.11	0.89±0.16	0.78±0.12	0.77±0.06
Pi (mmol/L)	1.55±0.16	1.59±0.19	1.58±0.22	1.62±0.18	1.63±0.22	1.50±0.24
LDH (U/L)	98.60±20.22	120.20±60.68	125.40±57.45	104.00±29.34	199.00±49.21	164.67±34.98
LDL - C (mmol/L)	0.36±0.05	0.40±0.07	0.41±0.06	0.42±0.07	0.35±0.09	0.30±0.03
T. Bil (μmol/L)	2.63±0.18	2.56±0.33	2.70±0.30	2.76±0.34	3.19±0.35	2.96±0.11
T.Pro (g/L)	76.62±2.50	75.02±3.80	74.47±3.35	72.65*±3.96	76.93±4.55	72.43±2.61
Trig (mmol/L)	0.77±0.32	0.73±0.16	0.82±0.34	0.86±0.37	0.54±0.29	0.63±0.16

BUN (mmol/L)	6.61±0.44	5.87*±0.82	6.12±0.63	6.06±0.65	5.57±0.52	5.51±0.54
Urea (mmol/L)	6.61±0.44	5.87*±0.82	6.12±0.63	6.06±0.65	5.57±0.52	5.51±0.54
Globulin (g/L)	45.81±2.94	44.32±4.68	44.10±4.71	41.73±4.51	48.02±6.16	42.27*±1.22
A/G (Ratio)	0.68±0.06	0.70±0.10	0.70±0.10	0.75±0.14	0.62±0.11	0.71±0.04
Na (mmol/L)	140.80±1.22	141.98±1.23	142.29*±1.63	143.06*±1.03	142.03±3.87	141.63±3.79
K (mmol/L)	3.76±0.41	3.65±0.27	3.46±0.15	3.70±0.21	3.09±0.08	3.54*±0.28
Cl (mmol/L)	114.99±0.88	115.88±1.13	114.05±1.05	114.34±1.02	109.93±2.44	109.43±0.91

ALB: Albumin; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; Ca: Calcium; T.Chol: Total Cholesterol; CK: Creatinine Kinase; Creat: Creatinine; GGT: Gamma - Glutamyltransferase; Glu: Glucose; HDL - C: High Density Lipoprotein - Cholesterol; Pi: Inorganic Phosphorous; LDH: Lactate Dehydrogenase; LDL - C: Low Density Lipoprotein - Cholesterol; T. Bil: Total Bilirubin; T.Pro: Total Protein; Trig: Triglyceride; BUN: Blood Urea Nitrogen; A/G: Albumin/Globulin; Na: Sodium; K: Potassium; CL: Chloride.

Values were expressed as mean±SD (n=10), Statistical significance was compared between Control (G1) versus other treatment groups (* P Value <0.05;** P Value <0.001; *** P Value <0.0001).

G1: Control Main Study; G2: Low Dose (135 mg/kg) Main Study; G3: Middle Dose (270 mg/kg) Main Study; G4: High Dose (340 mg/kg) Main Study; G1R: Control Recovery Study; G4R: High Dose (340 mg/kg) Recovery Study.

Table 6. Grains of Paradise Extract Clinical Biochemistry Female.

Parameter	Groups					
	G1	**G2**	**G3**	**G4**	**G1R**	**G4R**
ALB (g/L)	33.69±3.48	32.09±2.02	33.90±3.38	34.43±2.63	32.37±3.77	31.08±3.41
ALP (U/L)	166.40±45.49	177.90±82.16	208.10±100.55	181.70±137.16	132.00±43.86	151.67±53.08
ALT (U/L)	51.10±12.90	54.30±12.96	49.50±12.26	55.40±15.07	53.83±10.61	42.83±13.24
AST (U/L)	123.50±26.32	146.00±29.97	119.70±33.34	124.30±29.17	129.33±17.15	119.50±26.76
Ca (mmol/L)	2.48±0.06	2.47±0.06	2.51±0.08	2.55±0.08	2.45±0.11	2.47±0.12
T.Chol (mmol/L)	1.84±0.33	1.82±0.40	1.80±0.30	1.79±0.34	1.84±0.40	1.56±0.30
CK (U/L)	120.50±49.31	130.10±85.51	91.70±32.60	77.10±26.98	126.50±36.59	147.17±69.16
Creat (µmol/L)	33.50±2.88	34.00±3.46	31.90±3.31	32.80±2.90	39.00±2.00	39.83±6.68
GGT (U/L)	0.60±0.52	0.90±0.32	0.70±0.67	0.90±0.57	1.33±0.52	1.50±1.05
Glu (mmol/L)	5.94±0.77	6.31±0.90	6.30±0.73	6.76±1.31	5.18±0.62	5.94*±0.54
HDL - C (mmol/L)	1.08±0.17	1.08±0.19	1.08±0.18	1.09±0.18	1.13±0.23	0.94±0.18
Pi (mmol/L)	1.31±0.14	1.28±0.18	1.37±0.27	1.38±0.19	1.16±0.15	1.20±0.18
LDH (U/L)	106.50±39.10	140.80±66.19	100.70±39.43	91.50±22.57	159.83±31.75	119.17±37.51
LDL - C (mmol/L)	0.40±0.10	0.37±0.10	0.36±0.07	0.36±0.13	0.36±0.10	0.30±0.05
T. Bil (µmol/L)	2.99±0.18	2.85±0.20	2.99±0.25	3.05±0.36	3.52±0.25	3.59±0.49
T.Pro (g/L)	77.37±2.86	77.56±2.39	77.86±3.57	79.28±4.65	80.52±4.70	79.07±3.74
Trig (mmol/L)	0.57±0.23	0.51±0.24	0.58±0.32	0.57±0.22	0.38±0.16	0.43±0.15
BUN (mmol/L)	6.46±0.86	6.73±0.74	6.92±0.77	6.73±0.78	6.82±0.74	6.75±0.91
Urea (mmol/L)	6.46±0.86	6.73±0.74	6.92±0.77	6.73±0.78	6.82±0.74	6.75±0.91
Globulin (g/L)	43.68±4.23	45.47±2.54	43.96±4.08	44.85±4.39	48.15±3.27	47.98±2.38
A/G (Ratio)	0.78±0.13	0.71±0.07	0.78±0.12	0.78±0.11	0.68±0.09	0.65±0.08
Na (mmol/L)	141.50±3.20	138.76±3.51	139.83±3.15	139.36±3.37	142.40±4.48	140.95±3.98
K (mmol/L)	3.53±0.11	3.44±0.23	3.35±0.22	3.37±0.21	3.30±0.30	3.13±0.32
Cl (mmol/L)	114.27±0.87	113.73±1.08	114.23±1.15	115.40±1.48	111.42±1.77	110.30±0.91

ALB: Albumin; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; Ca: Calcium; T.Chol: Total Cholesterol; CK: Creatinine Kinase; Creat: Creatinine; GGT: Gamma - Glutamyltransferase; Glu: Glucose; HDL - C: High Density Lipoprotein - Cholesterol; Pi: Inorganic Phosphorous; LDH: Lactate Dehydrogenase; LDL - C: Low Density Lipoprotein - Cholesterol; T. Bil: Total Bilirubin; T.Pro: Total Protein; Trig: Triglyceride; BUN: Blood Urea Nitrogen; A/G: Albumin/Globulin; Na: Sodium; K: Potassium; CL: Chloride.

Values were expressed as mean±SD (n=10), Statistical significance was compared between Control (G1) versus other treatment groups (* P Value <0.05;** P Value <0.001; *** P Value <0.0001).

G1: Control Main Study; G2: Low Dose (135 mg/kg) Main Study; G3: Middle Dose (270 mg/kg) Main Study; G4: High Dose (340 mg/kg) Main Study; G1R: Control Recovery Study; G4R: High Dose (340 mg/kg) Recovery Study.

No significant alterations in thyroid-stimulating hormone (TSH) were observed in either male or female rats across all dose levels when compared to the respective control groups. In males, T3 and T4 levels remained comparable to controls at low and mid doses, while a significant increase in both T3 and T4 was noted in the high-dose (G4) group. A similar trend was observed in females, where T3 and T4 levels were unchanged at low and mid doses but were significantly elevated in the high-dose (G4) group. After the recovery period, T3 remained significantly elevated in high-dose females compared to controls, whereas T4 levels no longer differed in either sex (**Table 7** - Extract Thyroid Hormone Profile Males, **Table 8** - Extract Thyroid Hormone Profile Females). These statistically significant elevations in thyroid hormones were confined to the high-dose groups and were not accompanied by changes in TSH or any thyroid organ abnormalities; therefore, they are considered incidental and not indicative of a treatment-related effect, as no consistent dose-dependent relationship was evident.

Urinalysis showed no change related to the administration of Grains of Paradise Extract compared with the control group. Evaluation of organ weights revealed that for the majority of organs, neither absolute nor relative weights (organ-to-body weight ratios) showed statistically significant differences between treatment groups and their respective controls in both sexes and for both the main and recovery phases. However, in female rats of the high-dose recovery (G4R) group, statistically significant increases were observed in both the absolute and relative weights of the adrenal glands compared to the control recovery group (**Table 9** – Absolute Organ Weights Males; **Table 10** – Absolute Organ Weights Females; **Table 11** – Relative Organ Weights Males; **Table 12** – Relative Organ Weights Females). However, these differences were confined to the G4R group and did not exhibit a dose-dependent pattern, as no corresponding changes were observed in the high-dose (G4) group or any other dosage groups. Given the absence of a dose-response relationship and the lack of corroborating histopathological findings

Table 7. Grains of Paradise Extract Thyroid Hormone Profile Male.

Parameter	Groups					
	G1	G2	G3	G4	G1R	G4R
T3 (ng/mL)	10.95±0.53	11.15±1.03	10.64±1.02	12.11*±1.16	8.09±0.79	9.19±1.47
T4 (ng/mL)	505.42±75.70	578.05±60.22	628.95*±152.25	647.61*±98.09	365.84±29.49	387.96±50.57
TSH (ng/mL)	0.045±0.003	0.046±0.005	0.046±0.003	0.046±0.005	0.033±0.004	0.034±0.004

T3: Triiodothyronine; T4: Thyroxine; TSH: Thyroid Stimulating Hormone.

Values were expressed as mean±SD (n=10), Statistical significance was compared between Control (G1) versus other treatment groups (* P Value <0.05; ** P Value <0.001; *** P Value <0.0001).

G1: Control Main Study; G2: Low Dose (135 mg/kg) Main Study; G3: Middle Dose (270 mg/kg) Main Study; G4: High Dose (340 mg/kg) Main Study; G1R: Control Recovery Study; G4R: High Dose (340 mg/kg) Recovery Study.

Table 8. Grains of Paradise Extract Thyroid Hormone Profile Female.

Parameter	Groups					
	G1	G2	G3	G4	G1R	G4R
T3 (ng/mL)	10.71±0.84	11.55±0.96	11.70±1.57	13.45*±2.30	7.77±1.70	10.41*±0.84
T4 (ng/mL)	523.07±29.32	582.12±79.36	604.86±73.58	709.38*±125.33	380.77±35.42	412.30±18.35
TSH (ng/mL)	0.045±0.003	0.045±0.004	0.045±0.003	0.045±0.004	0.032±0.005	0.033±0.003

T3: Triiodothyronine; T4: Thyroxine; TSH: Thyroid Stimulating Hormone.

Values were expressed as mean±SD (n=10), Statistical significance was compared between Control (G1) versus other treatment groups (* P Value <0.05; ** P Value <0.001; *** P Value <0.0001).

G1: Control Main Study; G2: Low Dose (135 mg/kg) Main Study; G3: Middle Dose (270 mg/kg) Main Study; G4: High Dose (340 mg/kg) Main Study; G1R: Control Recovery Study; G4R: High Dose (340 mg/kg) Recovery Study.

Table 9. Grains of Paradise Extract Absolute Organ Weights Male.

Parameter	Groups					
	G1	G2	G3	G4	G1R	G4R
Adrenals (g)	0.052±0.013	0.050±0.007	0.055±0.009	0.058±0.013	0.049±0.006	0.052±0.009
Testes (g)	3.115±0.216	3.005±0.342	3.228±0.261	3.109±0.381	3.251±0.430	3.468±0.074
Epididymides (g)	1.388±0.211	1.242±0.234	1.299±0.171	1.356±0.252	1.268±0.156	1.382±0.134
Liver (g)	9.389±0.970	8.416±1.122	9.404±1.560	8.861±1.706	9.329±2.003	9.942±0.665
Kidneys (g)	2.240±0.400	1.949±0.268	2.214±0.331	2.047±0.299	2.386±0.445	2.508±0.241
Heart (g)	1.021±0.134	0.954±0.089	1.102±0.172	0.999±0.159	1.048±0.249	1.175±0.101
Brain (g)	1.946±0.114	1.932±0.126	2.001±0.099	1.951±0.102	1.868±0.327	2.069±0.046
Spleen (g)	0.814±0.169	0.814±0.091	1.016±0.227	0.828±0.244	0.831±0.172	0.908±0.153

Thymus (g)	0.236±0.073	0.237±0.078	0.278±0.133	0.271±0.086	0.228±0.084	0.234±0.090
Lungs (g)	2.478±0.493	2.495±1.134	2.681±0.597	2.237±0.299	2.918±0.594	2.488±0.163
Thyroid with para thyroid gland (g)	0.021±0.003	0.023±0.004	0.021±0.003	0.023±0.003	0.024±0.004	0.022±0.002
Pituitary gland (g)	0.011±0.003	0.011±0.002	0.011±0.002	0.011±0.002	0.012±0.003	0.010±0.001

Values were expressed as mean±SD (n=10), Statistical significance was compared between Control (G1) versus other treatment groups (* P Value <0.05; ** P Value <0.001; *** P Value <0.0001).

G1: Control Main Study; G2: Low Dose (135 mg/kg) Main Study; G3: Middle Dose (270 mg/kg) Main Study; G4: High Dose (340 mg/kg) Main Study; G1R: Control Recovery Study; G4R: High Dose (340 mg/kg) Recovery Study.

Table 10. Grains of Paradise Extract Absolute Organ Weights Female.

Parameter	**Groups**					
	G1	**G2**	**G3**	**G4**	**G1R**	**G4R**
Adrenals (g)	0.065±0.018	0.067±0.013	0.073±0.017	0.076±0.019	0.047±0.003	0.054*±0.006
Ovaries (g)	0.113±0.027	0.128±0.034	0.121±0.029	0.111±0.034	0.097±0.021	0.110±0.015
Uterus with Cervix (g)	0.785±0.438	0.745±0.287	0.751±0.262	0.714±0.305	0.557±0.159	0.676±0.145
Liver (g)	6.580±1.294	6.439±0.780	6.721±1.045	7.394±1.228	5.580±1.114	6.653±1.513
Kidneys (g)	1.572±0.227	1.501±0.111	1.556±0.199	1.683±0.158	1.387±0.206	1.601±0.306
Heart (g)	0.792±0.089	0.797±0.030	0.785±0.122	0.831±0.064	0.729±0.116	0.831±0.116
Brain (g)	1.867±0.106	1.905±0.088	1.881±0.095	1.934±0.152	1.797±0.060	1.914*±0.091
Spleen (g)	0.592±0.147	0.617±0.153	0.581±0.076	0.663±0.004	0.524±0.122	0.709±0.210
Thymus (g)	0.221±0.054	0.185±0.066	0.234±0.067	0.207±0.002	0.218±0.071	0.206±0.069
Lungs (g)	1.909±0.383	2.143±0.452	2.042±0.185	2.298±0.425	2.182±0.475	1.992±0.387
Thyroid with para thyroid gland (g)	0.020±0.004	0.023±0.004	0.023±0.004	0.024±0.008	0.022±0.004	0.021±0.002
Pituitary gland (g)	0.012±0.002	0.010±0.002	0.011±0.002	0.011±0.002	0.011±0.001	0.011±0.001

Values were expressed as mean±SD (n=10), Statistical significance was compared between Control (G1) versus other treatment groups (* P Value <0.05; ** P Value <0.001; *** P Value <0.0001).

G1: Control Main Study; G2: Low Dose (135 mg/kg) Main Study; G3: Middle Dose (270 mg/kg) Main Study; G4: High Dose (340 mg/kg) Main Study; G1R: Control Recovery Study; G4R: High Dose (340 mg/kg) Recovery Study.

Table 11. Grains of Paradise Extract Relative Organ Weights Male.

Parameter	**Groups**					
	G1	**G2**	**G3**	**G4**	**G1R**	**G4R**
Terminal B.W (g)	262.81±11.68	254.27±13.38	264.92±17.12	265.37±11.53	312.71±13.96	300.35±18.72
Adrenals (%)	0.020±0.005	0.020±0.003	0.021±0.003	0.022±0.005	0.016±0.002	0.018±0.003
Testes (%)	1.186±0.067	1.184±0.144	1.224±0.146	1.173±0.143	1.044±0.165	1.157±0.059
Epididymides (%)	0.530±0.091	0.490±0.098	0.494±0.085	0.511±0.092	0.406±0.047	0.462±0.053
Liver (%)	3.575±0.371	3.314±0.450	3.579±0.737	3.353±0.732	2.988±0.664	3.323±0.330
Kidneys (%)	0.852±0.144	0.768±0.110	0.841±0.157	0.774±0.131	0.765±0.156	0.836±0.073
Heart (%)	0.389±0.048	0.376±0.038	0.418±0.078	0.378±0.063	0.337±0.085	0.394±0.051
Brain (%)	0.741±0.042	0.763±0.081	0.759±0.071	0.736±0.043	0.601±0.118	0.691±0.053
Spleen (%)	0.310±0.063	0.321±0.038	0.387±0.102	0.310±0.080	0.266±0.056	0.305±0.068
Thymus (%)	0.090±0.028	0.094±0.033	0.108±0.056	0.102±0.032	0.073±0.026	0.079±0.032
Lungs (%)	0.942±0.175	0.991±0.488	1.022±0.263	0.846±0.127	0.932±0.173	0.830±0.052
Seminal Vesicle with Coagulation Gland and Prostate (%)	0.669±0.082	0.640±0.174	0.631±0.112	0.591±0.110	0.004±0.001	0.003±0.001
Thyroid with para thyroid (%)	0.008±0.001	0.009±0.002	0.008±0.001	0.009±0.001	0.008±0.001	0.008±0.001
Pituitary gland (%)	0.004±0.001	0.004±0.001	0.004±0.001	0.004±0.001	0.004±0.001	0.003±0.001

Values were expressed as mean±SD (n=10), Statistical significance was compared between Control (G1) versus other treatment groups (* P Value <0.05; ** P Value <0.001; *** P Value <0.0001).

G1: Control Main Study; G2: Low Dose (135 mg/kg) Main Study; G3: Middle Dose (270 mg/kg) Main Study; G4: High Dose (340 mg/kg) Main Study; G1R: Control Recovery Study; G4R: High Dose (340 mg/kg) Recovery Study.

Table 12. Grains of Paradise Extract Relative Organ Weights Female.

Parameter	Groups					
	G1	G2	G3	G4	G1R	G4R
Terminal B.W (g)	229.93±11.31	238.59±8.37	230.49±15.23	233.86±10.34	252.63±15.21	253.08±11.08
Adrenals (%)	0.028±0.008	0.028±0.006	0.032±0.007	0.033±0.008	0.019±0.002	0.021±0.002*
Ovaries (%)	0.049±0.013	0.054±0.015	0.052±0.013	0.048±0.015	0.039±0.009	0.043±0.004
Uterus with Cervix (%)	0.347±0.208	0.314±0.130	0.324±0.101	0.303±0.124	0.223±0.067	0.266±0.051
Liver (%)	2.871±0.594	2.703±0.355	2.917±0.416	3.168±0.555	2.226±0.515	2.619±0.521
Kidneys (%)	0.685±0.105	0.630±0.050	0.677±0.085	0.721±0.069	0.553±0.101	0.631±0.105
Heart (%)	0.346±0.045	0.334±0.017	0.340±0.040	0.356±0.026	0.291±0.057	0.328±0.038
Brain (%)	0.815±0.070	0.799±0.052	0.818±0.047	0.829±0.081	0.713±0.049	0.757±0.035
Spleen (%)	0.259±0.067	0.260±0.067	0.253±0.039	0.284±0.048	0.209±0.055	0.279±0.074
Thymus (%)	0.096±0.024	0.078±0.027	0.102±0.028	0.089±0.026	0.088±0.032	0.081±0.025
Lungs (%)	0.832±0.168	0.899±0.184	0.890±0.103	0.988±0.203	0.871±0.217	0.784±0.129
Thyroid with para thyroid (%)	0.009±0.002	0.010±0.002	0.010±0.002	0.010±0.002	0.009±0.002	0.008±0.001
Pituitary gland (%)	0.005±0.001	0.004±0.001	0.005±0.001	0.005±0.001	0.004±0.001	0.005±0.001

Values were expressed as mean±SD (n=10), Statistical significance was compared between Control (G1) versus other treatment groups (* P Value <0.05; ** P Value <0.001; *** P Value <0.0001).

G1: Control Main Study; G2: Low Dose (135 mg/kg) Main Study; G3: Middle Dose (270 mg/kg) Main Study; G4: High Dose (340 mg/kg) Main Study; G1R: Control Recovery Study; G4R: High Dose (340 mg/kg) Recovery Study.

in the adrenals or other organs, these isolated changes in adrenal weights are considered incidental and not attributable to treatment with Grains of Paradise Extract.

In summary, the 90-day subchronic repeated dose oral toxicity study of Grains of Paradise Extract with a 28-day recovery period reported no mortality, morbidity, or clinical signs at doses of 135, 270, or 340 mg/kg. Body weight, feed consumption, observations, organ weights, hematology, gross pathology, histopathology, and the majority of clinical chemistry parameters remained comparable across all treated groups. While some statistically significant, but non-dose-dependent changes were observed, such as minor elevations in T3 and T4 levels in the high-dose (340 mg/kg) group, these findings lacked toxicological relevance due to the lack of accompanying alterations in thyroid-stimulating hormone (TSH) or histopathological changes. Based on the overall absence of adverse effects at the 270 mg/kg dose and the marginal T3/T4 increases observed at 340 mg/kg without pathological correlates, the NOAEL for Grains of Paradise Extract was determined to be 270 mg/kg bw/day, pending further evaluation of the high-dose hormonal findings.

Discussion and Conclusion

Grains of Paradise is rich in bioactive compounds, primarily including gingerols, paradols, and shogaols [11]. These constituents have been implicated in various health benefits and pharmacological activities.

The potential of Grains of Paradise in enhancing metabolic and glycemic health, particularly in the management of obesity and diabetes, has drawn substantial interest. Studies have shown that Grains of Paradise Extract can increase energy expenditure and reduce visceral fat accumulation [12]. This thermogenic effect is linked to the activation of brown adipose tissue (BAT), which plays a crucial role in dissipating energy as heat. 6-paradol has been shown to promote BAT activation and browning of white adipose tissue, offering a promising approach to combat obesity [13–15]. These findings suggest that Grains of Paradise could be a valuable adjunct to lifestyle interventions for weight management. In terms of its potential in glycemic regulation, the components of Grains of Paradise display potential hypoglycemic effects *in vitro* by inhibiting the activities of α-amylase and α-glucosidase. Notably, its mechanism of action differs from that of antidiabetic drugs such as Acarbose, indicating that it may represent an alternative candidate for antidiabetic therapies [16–18].

Beyond metabolic and glycemic benefits, Grains of Paradise has also been shown to modulate inflammatory pathways. Inhibition of cyclooxygenase-2 (COX-2) enzyme activity and suppression of pro-inflammatory gene expression have been observed [2–7]. This inhibition of COX-2, a pivotal mechanism shared by numerous non-steroidal anti-inflammatory drugs (NSAIDs), plays a crucial role in alleviating inflammation and preventing the onset of chronic inflammatory diseases [19–21].

Despite its well-documented health benefits, the long-term safety profile of Grains of Paradise Extract has remained inadequately characterized due to limited toxicological data. To address this knowledge gap, the present study conducted a 90-day subchronic repeated dose oral toxicity experiment, followed by a 28-day recovery period, using Sprague Dawley rats. The results provide crucial insights into the safety of Grains of Paradise Extract.

The findings demonstrate that Grains of Paradise Extract was well tolerated up to the highest dose of 340 mg/kg bw/day, with no treatment-related mortality, morbidity, or adverse clinical signs observed. Body weight, food consumption, hematology, clinical chemistry, urinalysis, organ weights, and histopathological examinations did not reveal any significant differences between the Grains of Paradise Extract-treated groups and the control group.

A comprehensive assessment of hepatic and renal toxicity was conducted using established biomarkers. Regarding liver function, key enzymes ALT, AST, and ALP showed no statistically significant

alterations at any dose level. Complementary parameters including total bilirubin, total protein, and albumin remained within normal physiological ranges without dose-dependent trends, despite isolated fluctuations. Renal function markers creatinine, BUN, and urea similarly exhibited no biologically relevant changes. These biochemical findings, corroborated by normal urinalysis and absence of histopathological lesions in liver/kidney tissues, confirm the absence of treatment-related hepatorenal toxicity.

While an earlier 28-day study by Nebojsa *et al.* (2010) [10] reported dose-dependent increases in liver weight and serum ALP in rats administered Aframomum melegueta extract, our 90-day OECD 408-compliant study observed no such hepatotoxicity at doses up to 340 mg/kg. Possible explanations include: (1) differences in extract composition—variations in botanical source, preparation process, or standardization can alter the bioactive profile and thereby affect toxicological outcomes; (2) dose design, as the effects in Nebojsa *et al.*'s study occurred at 450 and 1,500 mg/kg, higher than the maximum dose in the present study; and (3) study duration, as short-term effects may not persist with longer exposure due to adaptive responses. Thus, this divergence likely reflects differences in extract composition, dosing, and study duration. Our test article showed no evidence of hepatotoxicity under the intended dosing regimen.

Some statistically significant changes were noted in specific hematological parameters, clinical chemistry values, and absolute organ weights, these alterations were not dose-dependent and were considered to be within normal biological variation and unrelated to Grains of Paradise Extract administration. The minimal lesions observed in the lungs, liver, kidneys, and heart during histopathological examination were sporadic, with frequencies not significantly different from the control group, further supporting the safety of Grains of Paradise Extract.

Of note, a statistically significant increase in serum T3 and T4 levels was observed in the high-dose (340 mg/kg) group compared to the control. However, these hormonal changes were not accompanied by alterations in thyroid-stimulating hormone (TSH) or histopathological changes in the thyroid gland. The lack of a dose-response relationship and the absence of thyroid dysfunction or pathology suggest these hormonal changes were likely incidental fluctuations rather than compound-related adverse effects. Further investigation may be warranted to elucidate the potential mechanisms underlying these mild thyroid hormone alterations at the highest dose.

Taken together, the results of this 90-day subchronic toxicity study demonstrate that Grains of Paradise Extract has a favorable safety profile, with a NOAEL of 270 mg/kg bw/day. These findings support the long-term safe use of Grains of Paradise Extract and provides a scientific basis for understanding its traditional medicinal value and modern health applications.

Conflicts of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgments

The study was conducted by Vedic Lifesciences, Pvt. Ltd, Mumbai, India. The study was sponsored by Nanjing Nutrabuilding Bio-tech Co., Ltd., Nanjing, China.

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