ACUTE AND SUB-CHRONIC TOXICITY EVALUATION OF 6-SHOGAOL-ENRICHED DRY GINGER EXTRACT IN EXPERIMENTAL MODELS

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This study aimed to evaluate the acute and sub-chronic toxicity of the 6-shogaol-enriched dry ginger extract in experimental animals. The acute toxicity test was performed in Swiss albino mice using the Behrens method, with a single oral dose ranging from 5 to 10 g/kg body weight. The sub-chronic toxicity study was carried out following the guidelines of the Ministry of Health, in which Wistar rats received oral doses of 60 mg/kg and 180 mg/kg daily for four consecutive weeks. The results of the acute toxicity study showed that the oral LD_{50} of the 6- shogaol–enriched dry ginger extract was 7.84 g/kg (95% CI: 7.23 – 8.45 g/kg). In the four-week sub- chronic toxicity assessment, the overall clinical condition, hematological parameters, serum biochemical indices, and histological structures of the liver and kidneys in the treated groups were not significantly different from those of the control group. In the high-dose group (180 mg/kg body weight/day), AST and ALT levels decreased markedly ($p < 0.05$ and $p < 0.01$, respectively), while plasma cholesterol levels showed significant reductions at week 2 ($p < 0.05$) and week 4 ($p < 0.001$).

Keywords: 6-shogaol-enriched dry ginger extract, acute toxicity, sub-chronic toxicity.

I. INTRODUCTION

Herbal medicine plays a crucial role in public health, particularly in developing countries where it remains a key option for healthcare. Despite their long history of use, many herbal products still lack systematic safety evaluations. The global shift towards natural therapies emphasizes the urgent need to identify plant-derived compounds that are both effective and safe. One such compound is 6-shogaol, a bioactive component of *Zingiber officinale*, which has garnered increasing attention for its powerful pharmacological properties and therapeutic potential.

Zingiber officinale (ginger, family Zingiberaceae) is widely cultivated in Vietnam and traditionally used as a culinary spice and a medicinal herb. It exerts warming, antispasmodic, antiemetic, anti-inflammatory, and digestive-supporting effects, and is usually employed in treating colds, abdominal pain, nausea, and digestive disorders.[1,2] The rhizome contains phenolic constituents such as gingerols, shogaols, zingerone, and paradols. Among these, 6-shogaol-the dehydrated form of 6-gingerol-exhibits potent pharmacological activity. Extensive studies have shown anti-inflammatory, antioxidant, anticancer, cardioprotective, and neuroprotective properties for 6-shogaol and 6-gingerol.[3,4] 6-Shogaol often displays superior bioactivity compared with 6-gingerol, including inhibition of tumor proliferation and induction of apoptosis across various cancer cell lines.[5] It also enhances hepatic antioxidant defenses via

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Nrf2/HO-1 activation and NF-κB suppression, thereby mitigating oxidative and inflammatory liver injury.[6,7] In models of diclofenac sodium and acetaminophen-induced hepatotoxicity, 6-shogaol–enriched ginger extracts reduced AST, ALT, and MDA while increasing GSH, improving hepatic histology.[8,9] Additionally, 6-shogaol has been shown to lower lipid levels, improving fatty liver disease.[10] Despite these promising pharmacological effects, safety data for standardized 6-shogaol–enriched dry ginger extract remain limited. This study, therefore, evaluated acute and sub-chronic oral toxicity in experimental animals to provide preclinical safety evidence for further development.

II. MATERIALS AND METHODS

1. Subjects

Test material

A 6-shogaol–enriched dry ginger extract was prepared from fresh ginger harvested in Nghe An, Vietnam. The ginger was air-dried to reduce moisture to less than 13%, fermented to increase 6-shogaol content, and extracted with 90% ethanol under reflux. The concentrate was dried with HPMC E6 to improve solubilization. The standardized 6-shogaol–enriched dry ginger extract contained 110 mg of 6-shogaol per gram (HPLC), was uniformly dispersed in distilled water. It was stored in tightly sealed amber containers at 4 °C, protected from light and humidity to maintain stability and 6-shogaol content.

Animals

Swiss albino mice (20 ± 2g) and *Wistar* rats (150 ± 20g) of both genders were obtained from the Experimental Animal Center, Vietnam Military Medical University. Animals were acclimatized for 7 days in a controlled condition with a temperature of 25 ± 2°C, air humidity, and 12-hour light/dark cycles with free access to food and water.

2. Methods

Acute toxicity test

Performed according to the Behrens method.[11] Six groups (n = 10 per group) of mice received single oral doses of 5, 6, 7, 8, 9, or 10 g/kg. Animals were monitored for 14 days for clinical signs and mortality. The LD_{50} value was calculated based on two adjacent doses surrounding the 50% mortality level. The following equation was applied:

$$LD_{50} = D_1 + \frac{(50\text{-}a) \times d}{b - a}$$

Where D_1 and D_2 are the lower and higher doses (g/kg), $d = D_2 - D_1$, and a and b represent the mortality percentages at D_1 and D_2, respectively.

Sub-chronic toxicity test

Followed the Vietnam Ministry of Health guideline.[12] *Wistar* rats were randomly assigned into three groups (n = 10/group): Lot 1 (control: vehicle 10 mL/kg), Lot 2 (low-dose: 60 mg/kg/day), and Lot 3 (high-dose: 180 mg/kg/day). Dosing was administered orally once daily for 4 weeks. Clinical signs, body weight, hematology (RBC, Hb, HCT, WBC, leukocyte differential, platelets), and biochemistry (AST, ALT, total cholesterol, creatinine) were measured at baseline (T0), week 2 (T2), and week 4 (T4). Liver and kidney histopathology were examined at the end of the experiment**.** The study was conducted at the Institute of Biomedicine and Pharmacy, and the Department of Pathology, 103 Military Hospital, Vietnam Military Medical University.

Statistics

Data were analyzed with SPSS v27. Normality was assessed using the Shapiro–Wilk test. Normally distributed data were presented as Mean ± SD and analyzed with one-way ANOVA; non-normal data as Median

(min–max) and analyzed by Kruskal–Wallis test. Significance was set at $p < 0.05$.

3. Research ethic

Procedures complied with institutional guidelines and were approved by the Vietnam Military Medical University Ethics Committee.

III. RESULTS

1. Acute toxicity study

Table 1. Evaluation of acute oral toxicity of 6-shogaol–enriched dry ginger extract in mice

Group	Dose (g/kg body weight)	n	Deaths (24 h)	Deaths (72 h)	Deaths (14 days)	Survivors (14 days)
Lot 1	10	10	10	10	10	0
Lot 2	9	10	8	8	8	2
Lot 3	8	10	6	6	6	4
Lot 4	7	10	2	2	2	8
Lot 5	6	10	0	0	0	10
Lot 6	5	10	0	0	0	10

During the first 12 hours after administration, clinical signs of toxicity were observed only in mice receiving doses of 8 g/kg or more, including lethargy, decreased mobility, and mild convulsions before death. No abnormal sign was seen in groups receiving 6 g/kg or less. After 24 hours, no additional death occurred. The surviving animals were monitored continuously for 14 days and remained healthy, with normal appetite, activity, and body-weight gain, and no delayed toxicity was observed.

The oral LD_{50} of the extract was 7.84 g/kg (95% CI: 7.23 – 8.45 g/kg), calculated by the Behrens method, indicating very low acute toxicity (Category 5 according to GHS Rev. 10, 2023).

2. Sub-chronic toxicity study

Effect of 6-shogaol–enriched dry ginger extract on overall clinical condition and body weight changes in rats

Overall clinical condition: Throughout the 4-week subchronic study, all rats in the control and treatment groups (60 and 180 mg/kg) remained healthy, with normal fur, activity, and excretion patterns.

Table 2. Effect of 6-shogaol–enriched dry ginger extract on body weight

Group	Body weight (gram)		
	Week 0	Week 2	Week 4
Lot 1 (Control)	150.50 ± 13.95	163.40 ± 13.28	175.70 ± 13.74
Lot 2 (low-dose 60mg/kg)	166.30 ± 16.10*	164.60 ± 17.42	178.80 ± 26.24
Lot 3 (high-dose 180mg/kg)	167.20 ± 20.79*	170.20 ±22.06	185.30 ± 28.43
p_{21}	*< 0.05*	*> 0.05*	*> 0.05*
p_{31}	*< 0.05*	*> 0.05*	*> 0.05*

*All data are presented as mean ± SD (n=10). Statistical differences among groups were analyzed using one-way ANOVA. * $p \leq 0.05$ compared to the control group*

The body weight of rats in the groups receiving the 6-shogaol–enriched dry ginger extract showed no statistically significant difference compared with the control group (p > 0.05).

Effect of 6-shogaol–enriched dry ginger extract on hematological parameters

Table 3. Effect of 6-shogaol–enriched dry ginger extract on erythrocyte

Parameter	Time	Lot 1 (Control) (n = 10)	Lot 2 (low-dose 60 mg/kg) (n = 10)	Lot 3 (high-dose 180 mg/kg) (n = 10)	p21	p31
RBC (T/L)	T0	8.76 ± 1.05	8.32 ± 1.34	8.32 ± 0.61	> 0.05	> 0.05
	T2	8.33 ± 0.77	8.78 ± 0.89	9.07 ± 0.45*	> 0.05	< 0.05
	T4	8.16 ± 0.57	8.30 ± 1.00	8.22 ± 0.73	> 0.05	> 0.05
Hb (g/dL)	T0	15.37 ± 1.33	14.65 ± 2.32	14.89 ± 1.33	> 0.05	> 0.05
	T2	14.22 ± 1.44	15.05 ± 1.16	15.99 ± 0.68*	> 0.05	< 0.05
	T4	14.82 ± 0.93	14.76 ± 1.42	14.47 ± 1.51	> 0.05	> 0.05
HCT (%)	T0	44.73 ± 4.15	42.39 ± 6.68	42.79 ± 4.00	> 0.05	> 0.05
	T2	43.97 ± 3.78	45.10 ± 4.06	47.37 ± 3.34*	> 0.05	< 0.05
	T4	42.97 ± 2.03	42.47 ± 4.37	42.31 ± 4.24	> 0.05	> 0.05

*All data are presented as mean ± SD. Statistical differences among groups were analyzed using one-way ANOVA. * p ≤ 0.05 compared to the control group; (T0: baseline; T2: week 2; T4: week 4)*

At all observation time points, the red blood cell count, hemoglobin concentration, and hematocrit values of the groups receiving the 6-shogaol–enriched dry ginger extract showed no statistically significant difference compared with the control group (p > 0.05).

Table 4. Effect of 6-shogaol–enriched dry ginger extract on leukocyte and platelet

Parameter	Time	Lot 1 (Control) (n = 10)	Lot 2 (low-dose 60 mg/kg) (n = 10)	Lot 3 (high-dose 180 mg/kg) (n = 10)	p21	p31
WBC (G/L)	T0	12.66 ± 2.29	12.49 ± 5.74	12.48 ± 3.64	> 0.05	> 0.05
	T2	8.97 ± 2.18	11.36 ± 2.80*	8.66 ± 3.04	< 0.05	> 0.05
	T4	15.37 ± 4.67	16.23 ± 3.90	14.48 ± 4.69	> 0.05	> 0.05
Neutrophils (%)	T0	3.16 ± 0.37	3.48 ± 0.76	3.00 ± 0.42	> 0.05	> 0.05
	T2	3.61 ± 0.62	4.16 ± 0.89	3.43 ± 0.82	> 0.05	> 0.05
	T4	3.49 ± 0.71	3.92 ± 0.72	3.06 ± 0.43	> 0.05	> 0.05

Parameter	Time	Lot 1 (Control) (n = 10)	Lot 2 (low-dose 60 mg/kg) (n = 10)	Lot 3 (high-dose 180 mg/kg) (n = 10)	p21	p31
Lymphocytes (%)	T0	52.34 ± 8.15	50.63 ± 8.08	57.08 ± 6.46	> 0.05	> 0.05
	T2	58.13 ± 9.77	52.72 ± 6.78	55.75 ± 14.09	> 0.05	> 0.05
	T4	54.52 ± 6.48	52.69 ± 6.68	56.50 ± 6.29	> 0.05	> 0.05
Platelets (G/L)	T0	592.30 ± 99.96	571.30 ± 196.91	634.60 ± 88.45	> 0.05	> 0.05
	T2	596.10 ± 170.43	650.00 ± 245.85	552.90 ± 222.39	> 0.05	> 0.05
	T4	754.80 ± 139.19	777.50 ± 261.87	725.20 ± 176.27	> 0.05	> 0.05

*All data are presented as mean ± SD. Statistical differences between groups were analyzed using one-way ANOVA. * p ≤ 0.05 compared to the control group. (T0: baseline; T2: week 2; T4: week 4)*

No statistically significant difference was observed in total white blood cell count, leukocyte differential (neutrophil and lymphocyte percentages), or platelet count between the rats treated with the 6-shogaol–enriched dry ginger extract and those in the control group ($p > 0.05$).

Effect of 6-shogaol–enriched dry ginger extract on serum biochemical parameters

Table 5. Effect of 6-shogaol–enriched dry ginger extract on serum biochemical parameters

Parameter	Time	Lot 1 (Control) (n = 10)	Lot 2 (low-dose 60 mg/kg) (n = 10)	Lot 3 (high-dose 180 mg/kg) (n = 10)	p21	p31
AST (IU/L)	T0	132.30 ± 18.36	130.40 ± 13.15	128.50 ± 21.60	> 0.05	> 0.05
	T2	140.80 ± 28.10	124.00 ± 18.21	155.30 ± 95.84	> 0.05	> 0.05
	T4	118.50 ± 9.85	121.20 ± 20.37	103.50 ± 15.09*	> 0.05	< 0.05
ALT (IU/L)	T0	120.90 ± 36.32	98.60 ± 13.69	98.50 ± 15.76	> 0.05	> 0.05
	T2	73.30 ± 15.16	63.60 ± 12.08	69.50 ± 50.35	> 0.05	> 0.05
	T4	66.10 ± 8.12	65.80 ± 16.78	50.90 ± 11.29*	> 0.05	< 0.01
Cholesterol (mmol/L)	T0	1.26 ± 0.40	1.02 ± 0.21	1.38 ± 0.47	> 0.05	> 0.05
	T2	1.37 ± 0.23	1.32 ± 0.33	1.52 ± 0.58*	*> 0.05*	< 0.05
	T4	2.41 ± 0.29	2.04 ± 0.46	1.78 ± 0.29*	< 0.05	< 0.001
Creatinin (µmol/L)	T0	85.70 ± 6.52	86.50 ± 5.04	86.10 ± 5.24	> 0.05	> 0.05
	T2	80.10 ± 3.25	79.80 ± 4.52	71.50 ± 18.17	> 0.05	> 0.05
	T4	73.40 ± 3.27	73.20 ± 3.16	75.80 ± 3.43	> 0.05	> 0.05

*All data are presented as mean ± SD. Statistical differences among groups were analyzed using one-way ANOVA. * p ≤ 0.05 compared to the control group. (T0: baseline; T2: week 2; T4: week 4)*

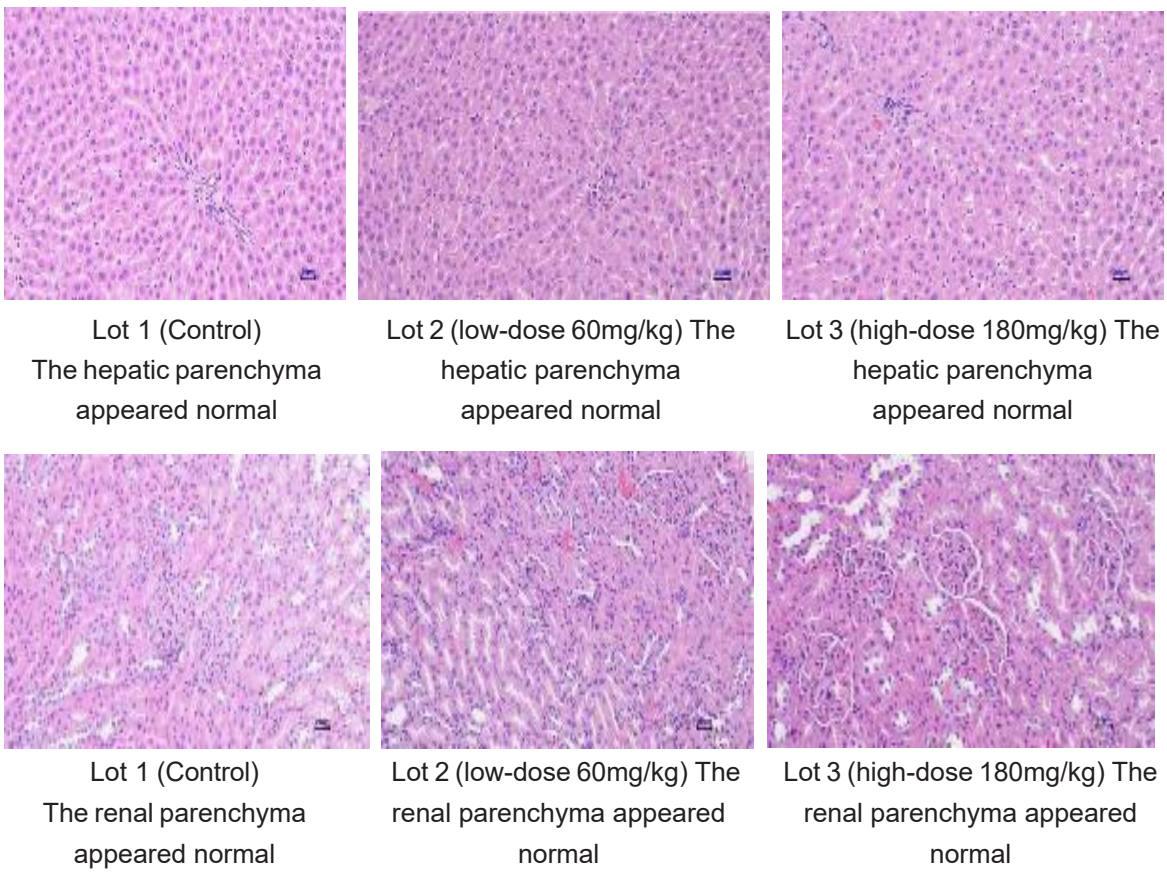
The hepatic enzyme activities (AST and ALT) and the plasma concentrations of cholesterol and creatinine in rats from both treatment groups (60 mg/kg and 180 mg/kg) remained within normal physiological ranges. Compared to the control group, the high-dose group (180 mg/kg) exhibited a statistically significant decrease in AST, ALT activity, and plasma cholesterol level at week 4 ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively), whereas plasma creatinine concentration showed no significant difference ($p > 0.05$).

Histopathology examination

Gross observation

Macroscopic observation with the naked eye and under a magnifying lens (25×) in all experimental rats, including both control and treated groups, revealed no gross pathological change in the liver or kidneys.

Histological observation



Lot 1 (Control) The hepatic parenchyma appeared normal

Lot 2 (low-dose 60mg/kg) The hepatic parenchyma appeared normal

Lot 3 (high-dose 180mg/kg) The hepatic parenchyma appeared normal

Lot 1 (Control) The renal parenchyma appeared normal

Lot 2 (low-dose 60mg/kg) The renal parenchyma appeared normal

Lot 3 (high-dose 180mg/kg) The renal parenchyma appeared normal

Figure 1. Histological structure of the liver and kidneys of rats after 4 weeks of oral administration of 6-shogaol–enriched dry ginger extract (H&E staining, ×400)

After 4 weeks of oral administration, the hepatic and renal histological structures in both treatment groups were normal and comparable to those of the control group, with no observable histopathological alteration.

IV. DISCUSSION

In natural health product development, evaluating acute and sub-chronic toxicity is a fundamental step to establish the safe dose range and identify target organs, while also

providing a scientific basis for subsequent clinical trials and optimized dosage-form development.[13] Current WHO guidelines emphasize implementing internationally standardized methodologies (OECD, GHS) to ensure the comparability and reliability of toxicological data.[14]

This study evaluated the 6-shogaol–enriched dry ginger extract for acute oral toxicity using the Behrens method with a 14-days observation period. The results showed an oral LD_{50} of 7.84 g/kg (95% CI: 7.23 – 8.45 g/kg), classifying the extract as *Category 5 – Practically non-toxic* according to the UN GHS classification (Rev. 10, 2023).[14] This finding demonstrates that the formulation possesses very low acute toxicity, consistent with the report by Ali et al. (2008), in which a ginger extract exhibited an LD_{50} > 5 g/kg body weight in rats.[15]

In sub-chronic toxicity studies, hematological and biochemical indices are key indicators reflecting the systemic effects of repeated exposure to test substances. The hematological parameters, including RBC count, hemoglobin, hematocrit, WBC count, and platelet count, were selected to evaluate potential effects on bone marrow activity, oxygen-carrying capacity, and immune status. Any alteration in these indices may indicate hemopoietic suppression, anemia, or inflammatory response following prolonged treatment.

Similarly, biochemical indices such as AST, ALT, total cholesterol, creatinine, and blood urea were monitored as sensitive markers for hepatic and renal integrity. Elevations in AST and ALT reflect hepatocellular injury, while increased creatinine and urea concentrations are indicative of impaired renal filtration or tubular damage. These markers collectively provide an integrated assessment of target-organ safety, particularly for natural products with multiple bioactive constituents like 6-shogaol.

In the four-week sub-chronic toxicity assessment, the overall clinical condition, body weight, and behavior of the experimental rats remained stable across all groups. The erythrocyte indices – red blood cell count (RBC), hemoglobin (Hb), and hematocrit (HCT) – showed no significant change compared with the control group ($p > 0.05$), indicating that the extract did not affect peripheral hematopoiesis. This result agrees with Borekar et al. (2020), who reported no remarkable hematological alteration in rats administered standardized ginger extract for 28 days.[16]

Leukocyte and platelet count also remained within physiological limits, with no statistically significant difference between groups. These findings indicate that the 6-shogaol – enriched dry ginger extract neither induced systemic inflammation nor caused immunosuppression, consistent with the known immunomodulatory and anti-inflammatory properties of 6-shogaol, mediated through NF-κB inhibition and suppression of pro-inflammatory cytokine production.[6,7]

Regarding serum biochemical parameters, the high-dose group (180 mg/kg) exhibited a statistically significant reduction in ALT activity and plasma cholesterol concentration compared with the control group ($p < 0.05$ and $p < 0.001$, respectively), whereas AST and creatinine levels remained unchanged ($p > 0.05$). This pattern confirms the absence of hepatocellular or renal damage and suggests a hepatoprotective and lipid-regulating effect of 6-shogaol. Numerous in vivo studies have shown that 6-shogaol activates the Nrf2/HO-1 pathway, enhances antioxidant enzyme activities (SOD, GSH), and inhibits NF-κB, thereby reducing oxidative stress and hepatic inflammation.[6,7]

Moreover, clinical meta-analyses have demonstrated that ginger extract can significantly reduce total cholesterol, triglycerides, and LDL-C, while increasing HDL-C.[17] These outcomes are entirely consistent with the present findings, where cholesterol levels markedly decreased in the high-dose group, further supporting the lipid-modulating and hepatoprotective potential of the 6- shogaol–enriched dry ginger extract. The observed reduction in ALT and serum cholesterol supports potential hepatoprotective and lipid-regulating benefits and the development of this extract as a safe natural health product.

V. CONCLUSION

The results of this study demonstrated that the 6-shogaol–enriched dry ginger extract exhibited very low acute toxicity, with an oral LD_{50} of 7.84 g/kg (95% CI: 7.23 – 8.45 g/kg). The 4-weeks sub-chronic toxicity study in Wistar rats orally administered the extract suspension at 60 mg/kg/day and 180 mg/kg/day revealed no hematological, biochemical, or histopathological alteration in the liver or kidneys. Moreover, the extract showed a tendency to improve hepatic enzyme activities and modulate plasma lipid profiles.

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