

# Genotoxicity, Acute and Sub-Chronic Toxicity Studies of Solid-State Cultivated Mycelial Powder of *Antrodia cinnamomea*

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## Research Article

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## Abstract

*Antrodia cinnamomea* is a precious medicinal mushroom endemic to Taiwan that has been used as folk medicine for health promotion and treating various diseases. In this study, the toxicological assessment of solid-state cultivated mycelial powder of *A. cinnamomea* (LE-SC) was performed using *in vitro* and *in vivo* assays. The maximum tolerated dose (MTD) of LE-SC was determined to be greater than 13.3 g/kg bw. In addition, LE-SC showed no mutagenicity in Ames test and no genotoxic activity up to the dose of 10 g/kg bw in mammalian erythrocyte micronucleus and mice sperm abnormality tests. Moreover, result from 90 days repeated dose toxicity test showed that administration of LE-SC (1.9, 3.8 and 7.6 g/kg bw) in the diet had no observable adverse effects in both male and female rats. Accordingly, the results ensured the safety of LE-SC as food supplement and for human consumption.

**Keywords:** *Antrodia cinnamomea*; Toxicological assessment; Mutagenicity; Genotoxicity; 90 days toxicity test

## Introduction

*Antrodia cinnamomea* is a rare and precious medicinal mushroom endemic to Taiwan. This fungal parasite grown inner cavity of age old *Cinnamomum kanehirai* Hayata (Lauraceae), an endemic tree species in Taiwan. The aborigines of Taiwan used *A. cinnamomea* as a health promoting herb and also used for increase liver function [1,2]. Additionally, it has been used as a folk remedy to treat liver disease, drug and food intoxication, diarrhea, abdominal pain, hypertension, skin itches and tumorigenic diseases [2-4]. Recently, the biologic activities of *A. cinnamomea* have been widely studied

based on the traditional knowledge and its therapeutic effects. Increasing evidences support the benefits of *A. cinnamomea* possessed various pharmacological effects, including anti-cancer [5-7], immunomodulatory [8-10], anti-inflammation [11,12], hepatoprotective [13-15], antioxidant [16,17] and neuroprotective effects [18, 19]. The major components of *A. cinnamomea* were identified including terpenoids, polysaccharides, benzenoids, lignans, nucleic acid, benzoquinone derivatives, steroids, and maleic/succinic acid derivatives [2,4]. A recent revealed that the stage of development, culture and storage conditions affect the components of mushroom, such as triterpenoids are predominantly found in fruiting body of *A. cinnamomea* [2].

Currently, the mycelia powders of *A. cinnamomea* is marketed in Taiwan as dietary supplements and for the demand of market, solid-state cultivation and liquid submerged cultivation are commonly used to produce the mycelia of *A. cinnamomea*. Previous toxicological studies have confirmed that the healthy food products of *A. cinnamomea* are low toxic in animals. Huang et al, demonstrated that under the dosage of 6 g/kg of *A. cinnamomea* do not induce sub-chronic toxicity and teratogenicity in SD rats [20]. The no-observed-adverse-effect-level (NOAEL) of *A. cinnamomea* has been identified to be greater than 3g/kg/day in SD rats was confirmed by 90-day repeated dose toxic study [21]. The health food product "Leader Deluxe *A. cinnamomea*" has been reported no obvious toxic evidences at dose of 2.8 g/kg in rats. However, different culture conditions or production process would lead to different concentrations of bioactive components of *A. cinnamomea* and thus affect the results of toxicity tests. Therefore, the present study was aimed to investigate the safety of solid-state cultivated mycelial powder of *A. cinnamomea* (LE-SC) by examining genotoxicity, acute and subchronic toxicity effects.

In the present study, LE-SC showed no mutagenic potential in bacterial reverse mutation study (Ames test) and no genotoxicity effects in mammalian erythrocyte micronuclei test and sperm abnormality test. The maximum tolerated dose (MTD) of LE-SC was greater than 13.3 g/kg bw and 90 days repeated dose oral toxicity studies also showed no significant toxicity signs in both male and female rats up to the dose of 7.6 g/kg bw.

## Materials and Methods

### Test substance

The solid-state cultivated mycelial powder of *Antrrodia cinnamomea* (LE-SC) was produced by Taiwan Leader Biotech Corp (Taipei, Taiwan).

### Animals

Kunming mice were purchased from Beijing HFK Bioscience Co., Ltd. (Beijing, China). Sprague Dawley (SD) rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Animals were housed in the National Institute of Nutrition and Food Safety of Chinese Center for Disease Control and Prevention (China CDC). The temperature was set at 21±2°C, relative humidity 55±20%, and lighting was 12 h per day. Autoclaved reverse osmosis (RO) treated water

was supplied *ad libitum* and laboratory rodent diet (Beijing HFK Bioscience Co., Ltd) supplied for all animals.

### Acute 14-days oral toxicity test

Test of maximum tolerated dose (MTD) was performed to investigate the acute toxic effect of LE-SC. The Sprague Dawley (SD) rats, 10 rats per sex, were administered with LE-SC at a single dose of 13.3 g/kg b.w (dosing volume: 20 mL/kg bw) via oral gavage twice a day. This dose was 100 times the human recommended daily intake based on body weight conversion basis. All animals were monitored for clinical signs of toxicity and mortality for 14 consecutive days.

### Bacterial reverse mutation test

Five doses of LE-SC (62, 185, 556, 1667 and 5000 µg/plate) were used in the bacterial reverse mutation test (Ames test) based on dose range finding study. To evaluate mutagenicity, the histidine-dependent *Salmonella typhimurium* strains TA97, TA98, TA100 and TA102 were tested with five doses of LE-SC. Positive controls, negative control, vehicle control and test solutions with or without metabolic activation were included in the evaluation. The assay with metabolic activation was performed by using rat liver S9 fraction. 0.1 mL of test solutions, 0.1 mL of fresh bacterial broth and 0.5 mL S9 mixture were mixed with the molten top agar and poured onto the surface of a minimal agar plate. The plates were incubated at 37°C for 48 h then the number of revertant colonies per plate was counted. The mutagenic activity of test articles were considered as positive, when the number of revertants in the test solutions were more than 2-fold compared with those in vehicle control and with a dose-dependent effect.

### Mammalian erythrocyte micronucleus test

The mammalian micronucleus test was performed by using Kunming mice (25-29 g) and the test article LE-SC was prepared in distilled water at concentrations of 83, 170 and 330 mg/mL (dosing volume: 10 mL/kg bw). Mice were orally administered LE-SC at doses of 2.5, 5.0 and 10.0 g/kg bw. The positive control group mice were given 40 mg/kg bw cyclophosphamide and negative control group were received distilled water. The dosing was performed twice, at 24 h intervals, and bone marrow was sampled at 30 h. The bone marrow smears were fixed in methanol and followed by Giemsa staining. The proportion of polychromatic erythrocytes (PCE) to erythrocytes was determined by counting 200

erythrocytes. 1000 PCE per animal were scored for frequency of micronucleated cells (MN%<sub>00PCE</sub>).

### Mice sperm abnormality test

The sexually mature male Kunming mice (25-28 g) were randomly assigned into five groups, 7 mice in each group. Cyclophosphamide (40 mg/kg bw) was used as positive control, distilled water was served as negative control and three LE-SC treatment groups (2.5, 5 and 10 g/kg bw). The mice were given test articles via oral *gavage* once a day for five consecutive days. 30 days after the last dose, randomly selected 5 mice from each group were sacrificed. The bilateral epididymides were collected and cut into pieces in normal saline following removal of adipose tissue. The tissue mixtures were centrifuged at 1000 rpm for 7mins and the supernatant was discarded. The sperm suspension was applied on slide, air-dried, fixed with methanol and stained with 1.5% eosin. The percentage of sperm abnormality was calculated based on evaluation of 1000 sperms of each animal.

### 90-days repeated dose oral toxicity test

The Sprague Dawley (SD) rats were randomly divided into four groups, 20 rats in each group of 10 male and 10 females. LE-SC was administered to rats on diet at dose of 1.9, 3.8 and 7.6 g/kg bw. The animals had free access to diet and water *ad libitum*. The clinical signs were observed during the experiment period. The body weight and food consumption were measured weekly for 90 consecutive days. On day 45 and the end of experiment, blood samples were collected from all surviving animals for hematological and serum biochemistry analysis. A gross necropsy was conducted on all surviving animals at end of the study. Body organs such as liver, kidney, spleen, heart, thymus and testes were collected and weighted. Organ-to-body weight ratios were calculated. Histopathological examination was performed with heart, liver, spleen, kidney, adrenals, thymus, stomach, duodenum, testis and ovaries of all animals in each dosing group.

### Statistical analysis

All data was expressed in mean  $\pm$  standard deviation (SD). If the variance was homogeneous, the data was analyzed with one-way ANOVA using PEMS software. Otherwise, non-parametric statistical analysis was performed. A value of  $p < 0.05$  was considered as statistical significance.

## Results and Discussion

### Acute (14 days) oral toxicity study

The male and female SD rats were treated with single dose of LE-SC (13.3 g/kg bw) and the clinical signs were observed for 14 consecutive days. Result shows that no obvious clinical features of toxicity signs or animal mortality related to LE-SC treatment were founded. In addition, no body weight loss was observed in both genders. The mean body weights in both male and female rats were summarized in (Table 1). The results of acute toxicity test suggested the MTD of LE-SC in rats was greater than 13.3 g/kg bw and provide the information of further studies to set the dose regimen.

Animals	Initial body weight (g)	Final body weight (g)	MTD (g/kg b.w)
Male	195.1 $\pm$ 7.1	226.9 $\pm$ 6.0	>13.3
Female	192.2 $\pm$ 4.6	289.2 $\pm$ 13.0	>13.3

Table 1: Results of acute toxicity test in rats. All data presented as mean  $\pm$  SD.

### Bacterial reverse mutation test

Bacterial reverse mutation test was performed to evaluate the mutagenicity of LE-SC. The four *his*-*S. typhimurium* strains, TA97, TA98, TA100 and TA102 have been validated to be used in this study. According to the results of dose range finding test (data not shown), 5 doses of LE-SC (62, 185, 556, 1667 and 5000  $\mu$ g/plate) were used to perform the Ames test. As shown in (Table 2), less than 2-fold increases in the number of revertants with the four *his*-*S. typhimurium* strains treated with LE-SC at 5 doses compared to vehicle control. The positive controls showed large fold increases in the number of revertants of that in untreated and vehicle control, confirming the validation of this test. Therefore, the results indicated LE-SC would not induce the *S. typhimurium* mutation in the presence or absence of S9 metabolic activation mixture.

Treatment groups ( $\mu\text{g}/\text{plate}$ )		Strains (without S9)				Strains (with S9)			
		TA97	TA98	TA100	TA102	TA97	TA98	TA100	TA102
LE-SC	65	135 $\pm$ 22.5	45.7 $\pm$ 8.1	146.3 $\pm$ 7.5	287.3 $\pm$ 2.5	139.7 $\pm$ 17.8	40.7 $\pm$ 7.6	120.0 $\pm$ 7.9	295.7 $\pm$ 16.4
	185	142.3 $\pm$ 15.7	39.3 $\pm$ 2.5	137.3 $\pm$ 22.1	276.0 $\pm$ 2.0	146.3 $\pm$ 11.0	51.7 $\pm$ 5.0	118.7 $\pm$ 9.5	287.0 $\pm$ 17.3
	556	150.7 $\pm$ 16.2	47.0 $\pm$ 6.0	144.0 $\pm$ 8.2	297.0 $\pm$ 3.5	144.3 $\pm$ 23.1	54.0 $\pm$ 2.6	121.0 $\pm$ 15.5	302.0 $\pm$ 17.8
	1667	140.3 $\pm$ 10.0	42.7 $\pm$ 5.1	137.0 $\pm$ 7.0	276.3 $\pm$ 10.4	136.0 $\pm$ 2.6	47.7 $\pm$ 1.5	142.3 $\pm$ 19.1	278.3 $\pm$ 7.1
	5000	157.7 $\pm$ 10.0	45.0 $\pm$ 9.6	125.7 $\pm$ 19.4	283.7 $\pm$ 23.4	148.7 $\pm$ 1.2	39.3 $\pm$ 3.2	160.7 $\pm$ 23.9	295.3 $\pm$ 6.8
Untreated control		120.3 $\pm$ 8.5	47.3 $\pm$ 7.6	132.3 $\pm$ 10.4	296.3 $\pm$ 7.0	144.3 $\pm$ 19.1	42.7 $\pm$ 3.8	125.0 $\pm$ 9.2	275.0 $\pm$ 13.2
Vehicle control <sup>a</sup>		137.0 $\pm$ 23.3	41.7 $\pm$ 6.0	123.3 $\pm$ 6.8	279.0 $\pm$ 8.5	164.0 $\pm$ 8.9	43.3 $\pm$ 3.2	117.0 $\pm$ 4.6	280.7 $\pm$ 20.3
Positive control <sup>b</sup>									
NaN <sub>3</sub>	1.5	-	-	1380.7 $\pm$ 224.0	-	-	-	-	-
2-AF	10	-	-	-	-	1187.3 $\pm$ 140.0	2955.3 $\pm$ 83.7	1669.3 $\pm$ 209.8	-
4-NOP	20	1308.0 $\pm$ 256.8	1871.3 $\pm$ 193.4	-	-	-	-	-	-
MMC	2.5	-	-	-	1845.3 $\pm$ 144.3	-	-	-	-
1,8-DHAQ	50	-	-	-	-	-	-	-	673.3 $\pm$ 56.8

Table 2: Results of bacterial reverse mutation test.

All data presented as mean  $\pm$  SD.

<sup>a</sup>vehicle control was sterile water.

<sup>b</sup>NaN<sub>3</sub>: sodium azide; 2-AF: 2-Aminofluorene; 4-NOP: 4-Nitro-o-phenylenediamine; MMC: Mitomycin; 1,8-DHAQ: 1,8-Dihydroxyanthraquinone

### ***In vivo* mammalian erythrocyte micronucleus test**

*In vivo* micronucleus test was conducted to investigate whether LE-SC would cause cytogenetic damage in mice. Mice were treated with LE-SC (2.5, 5 and 10 g/kg bw) twice, at 24 h interval, and the percentage and micronucleus frequency were analyzed at 30 h. The percentage of PCE in all LE-SC treated groups showed no statistically significant decreases compare to negative control (Table 3), thus suggesting LE-SC did not inhibit erythropoiesis. The micronucleus frequency of positive control (cyclophosphamide) was 13.4‰ and 15.0‰ in female and male mice, respectively; negative control was 2.0‰ and 1.6‰ in female and male mice, respectively.

The micronucleus frequency of positive control was significantly higher than negative control in both genders, thus confirming this study was valid. The micronucleus frequency of LE-SC were 1.4‰, 1.6‰, 1.4‰ in female rats; 1.8‰, 2.0‰, 1.6‰ in male rats at dose of 2.5, 5, 10 g/kg bw, respectively. The results showed no statistical significant difference ( $p > 0.05$ ) compare to negative control (Table 3). Accordingly, LE-SC did not induce the formation of micronucleated PCE at dose up to 10 g/kg bw.

Group	Dose (g/kg b.w)	PCE/RBC (%)		MN‰PCE	
		Male	Female	Male	Female
Negative control		54.7	54.4	1.6 <sup>b</sup>	2.0 <sup>b</sup>
LE-SC	2.5	54	54.6	1.8	1.4
	5	54.4	54.1	2	1.6
	10	54.8	54.4	1.6	1.4
Cyclophosphamide <sup>a</sup>	0.04	46.3	47.5	15.0 <sup>c</sup>	13.4 <sup>c</sup>

Table 3: Results of in vivo micronucleus test in mice treated with LE-SC. PCE: Polychromatic erythrocytes; RBC: Red blood cell count; MN: micronucleus.

All data presented as mean  $\pm$  SD. <sup>a</sup>positive control.

<sup>b</sup> $p > 0.05$  compared to all treatment groups by Poisson distribution.

<sup>c</sup> $p < 0.05$  compared to control group by Poisson distribution.

### Mice sperm abnormality test

To assess the possible genetic hazard effects of LE-SC, mice sperm abnormality test was performed. Various types of abnormal sperm morphology and sperm abnormality ratio were analyzed and results were summarized in (Table 4). Quantification of number of various abnormal sperms exhibit that there was no obvious difference between LE-SC treated groups and negative control group.

The frequency of sperm abnormality of positive control, cyclophosphamide was 5.38% and that showed significant difference compare to negative control (2.24%). The frequency of sperm abnormality of LE-SC treated groups (2.5, 5 and 10 g/kg bw) showed no statistically significant ( $p > 0.05$ ) compare to negative control and obviously lower than positive control group. Therefore, the results suggested LE-SC would not induce sperm abnormalities in mice.

Group	Dose (g/kg b.w)	Number of Animal	Number of Sperm Counted (per mice)	Number of Abnormal Sperm						Frequency of Abnormality (%)
				Amorphous	Hookless	Large head	Banana-shaped	others*	Total	
Negative control		5	1000	56	35	17	3	1	112	2.24 <sup>b</sup>
LE-SC	2.5	5	1000	56	39	24	2	0	121	2.42
	5	5	1000	54	38	19	2	0	113	2.26
	10	5	1000	54	34	19	1	1	109	2.18
Cyclophosphamide <sup>a</sup>	0.04	5	1000	117	72	48	24	8	269	5.38 <sup>c</sup>

Table 4: Effect of LE-SC on percentage of abnormal sperm in mice.

\*others contain double head, double tail, bent tail, and so on. <sup>a</sup>positive control.

<sup>b</sup> $p > 0.05$  compared to all treatment groups by Chi-square test. <sup>c</sup> $p < 0.01$  compared to control group by Chi-square test.

### Repeated dose 90 days oral toxicity test

No animal deaths and LE-SC administration related clinical signs of toxicity were founded during 90 days repeated dose toxicity study. The animals treated with LE-SC on diet at dose of 1.9, 3.8 and 7.6 g/kg bw showed normal activities and thick shiny hairs. There were no significant differences in mean body weights, mean weight gains and food consumption between all treated groups and vehicle control group (data not shown). In addition, no obvious change in hematology parameters were founded between all treated groups and vehicle control in both genders. Except RBC (day 45) and

neutrophil (day 90) counts of 1.9 g/kg bw female mice group and lymphocyte counts (day 90) of 3.8g/kg bw male mice group were founded statistical higher than that of vehicle control (Table 5). Moreover, serum biochemical analysis is shows there were no significant differences in both male and female rats, whereas albumin (35.0±2.1 g/L) in 3.8g/kg bw male mice group on 45 day was statistical lower than that of vehicle control (38.0±4.5 g/L) (Table 6). However, these data were within the normal historical range of the testing laboratory and without dose-response relationships.

Parameters	Male				Female			
	LE-SC (g/kg b.w)							
	Control	1.9	3.8	7.6	Control	1.9	3.8	7.6
<b>Day 45</b>								
WBC (10 <sup>9</sup> /L)	10.15±3.01	11.00±3.29	11.36±1.97	12.57±2.88	8.18±2.54	8.84±2.63	6.96±2.43	8.06±2.56
RBC (10 <sup>12</sup> /L)	8.59±0.52	8.96±0.53	8.94±0.44	8.46±0.63	7.94±0.42	8.38±0.31*	7.96±0.37	8.14±0.32
Hb (g/L)	159.1±9.4	163.9±7.8	161.6±7.0	154.3±7.7	156.4±8.2	159.5±6.1	151.0±12.3	157.0±6.5
Platelet (10 <sup>12</sup> /L)	856.7±115.1	1021.8±165.1	1123.4±279.3	988.4±261.0	903.0±103.8	858.2±124.0	834.6±137.8	933.8±140.6
Lymphocyte (%)	78.5±4.0	80.5±5.5	80.2±2.9	82.6 ± 2.9	78.8±5.6	79.0±7.9	79.3±6.8	77.2±5.2
Neutrophile (%)	14.1±3.3	11.4±5.2	11.5±2.1	10.0±2.9	12.8±4.1	13.6±5.5	13.6±5.6	15.3±4.3
Other types of WBC (%)	7.4±3.1	8.1±2.6	8.3±1.8	7.4±1.7	8.4±2.6	7.4±3.0	7.1±2.2	7.5±1.9
<b>Day 90</b>								
WBC (10 <sup>9</sup> /L)	9.88±1.67	9.28±3.20	9.85±1.27	10.42±2.74	6.08±0.63	6.74±2.64	6.76±1.02	7.07±1.83
RBC (10 <sup>12</sup> /L)	9.49±0.40	9.84±0.52	10.01±0.53	9.68±0.34	8.58±0.37	8.81±0.86	8.86±0.69	8.75±0.36
Hb (g/L)	163.8±8.9	171.3±10.5	172.3±5.5	170.8±5.6	158.6±4.4	161.3±12.6	162.8±10.0	156.3±4.6
Platelet (10 <sup>12</sup> /L)	980.5±130.8	959.9±136.9	1048.1±209.4	945.2±131.8	791.7±136.9	770.3±80.0	832.5±158.8	897.2±159.4
Lymphocyte (%)	77.3±3.3	80.1±5.5	82.6±3.3*	81.4±4.4	81.6±3.0	75.7±4.4	79.9±5.6	77.7±5.7
Neutrophile (%)	15.1±2.8	13.8±5.5	11.2±2.8	12.4±3.9	12.0±2.8	17.8±4.2*	14.1±4.9	14.9±4.8
Other types of WBC (%)	7.6±1.9	6.1±2.0	6.2±1.0	6.2±0.8	6.4±0.7	6.5±1.3	6.0±1.4	7.4±1.6

Table 5: Effect of 90 day repeated dose of LE-SC on rats-results of hematologic analysis on day 90

All data presented as mean ± SD; \**p*<0.05 compared to control.

Parameters	Male				Female			
	LE-SC (g/kg b.w)							
	Control	1.9	3.8	7.6	Control	1.9	3.8	7.6
<b>Day 45</b>								
ALT (U/L)	40.7±7.3	40.4±8.4	39.4±7.3	41.7±11.2	35.8±7.7	37.9±6.8	33.6±8.3	34.5±8.0
AST (U/L)	190.4±22.3	187.1±28.6	184.9±23.6	194.0±22.5	161.7±25.4	170.1±26.4	180.3±37.1	178.5±22.7
ALP (U/L)	209.3±41.2	186.9±33.9	184.1±28.3	167.7±32.5	98.1±21.0	84.1±15.4	97.7±17.4	99.6±20.1
BUN (mmol/L)	6.54±1.02	7.23±0.35	7.02±0.54	6.73±0.57	6.74±0.82	6.95±0.86	6.30±1.20	6.46±1.31
Creatinine (µmol/L)	62.2±12.4	60.4±8.1	63.6±7.9	62.5±5.2	69.6±9.0	65.3±4.0	61.4 ±6.3	63.1±10.2
Cholesterol (mmol/L)	1.49±0.17	1.56±0.41	1.74±0.38	1.70±0.39	2.06±0.39	2.16±0.45	2.48±0.38	2.44±0.61
Triglyceride (mmol/L)	0.67±0.08	0.77±0.13	0.80±0.20	0.68±0.09	0.69±0.08	0.85±0.15	0.78±0.17	0.74±0.15
Glucose (mmol/L)	3.04±0.55	2.82±0.23	2.94±0.33	0.75±0.28	3.30±0.53	3.50±0.27	3.07±0.60	3.13±0.40
Total protein (g/L)	61.0±4.7	61.5±6.1	62.6±3.8	62.8±5.4	68.5±3.9	69.4±2.8	64.9±3.6	66.7±4.2
Albumin (g/L)	31.6±2.3	32.0±2.4	32.8±2.9	32.7±3.2	38.0±4.5	37.9±2.1	35.0±2.1*	36.2±2.5
<b>Day 90</b>								
ALT (U/L)	37.9±5.9	40.6±7.6	37.8±7.0	40.6±7.2	37.3±9.5	34.0±11.2	38.4±7.0	38.2±7.1
AST (U/L)	212.0±16.4	206.0±21.7	190.4±27.8	193.4±25.3	146.7±35.5	154.0±21.2	168.7±23.1	176.7±30.3
ALP (U/L)	106.0±41.4	96.2±22.2	111.3±23.9	95.8±12.5	63.7±15.9	52.9±13.2	52.8±6.6	58.0±13.4
BUN (mmol/L)	6.75±0.76	6.93±0.59	6.68±0.40	6.47±0.92	7.05±0.53	6.61±0.89	6.93±0.89	7.00±0.43
Creatinine (µmol/L)	72.6±5.8	67.8±7.2	70.8±7.2	72.7±4.8	69.7±6.0	70.9±6.7	67.8±10.1	73.9±4.9
Cholesterol (mmol/L)	2.00±0.30	1.65±0.28	2.05±0.49	1.90±0.31	2.19±0.37	2.25±0.63	2.33±0.46	2.35±0.40
Triglyceride (mmol/L)	0.76±0.09	0.77±0.10	0.77±0.15	0.77±0.10	0.82±0.26	0.79±0.13	0.83±0.12	0.84±0.21
Glucose (mmol/L)	4.57±0.54	4.61±0.80	4.32±0.29	4.74±0.67	5.16±0.64	5.17±0.52	5.39±0.94	4.80±1.07
Total protein (g/L)	75.7±3.7	73.0±2.8	75.4 ± 3.0	75.4±4.8	81.6± 4.6	80.5±3.5	80.5±4.6	79.4±3.3
Albumin (g/L)	37.9±2.4	35.3±2.2	36.6±1.6	37.0±2.4	44.1± 3.0	43.2±2.7	42.3±3.4	42.3±1.9

Table 6: Effect of 90 days repeated dose of LE-SC on rats-results of serum biochemical analysis.

ALT: Alanine aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase; BUN: Blood urea nitrogen. All data presented as mean ± SD; \* $p < 0.05$  compared to control.

The results of organ weight and organ-to-body weight ratio were summarized in (Table 7). The organ weight and organ-to-body weight ratio of liver, kidney, spleen, heart, thymus and testes in LE-SC treated groups of both genders were not statistically significant differences to that of vehicle control. Additionally, no obvious abnormalities in all organs were founded by macroscopic

examination at gross necropsy. No test article related lesions were founded in histological examination of heart, liver, spleen, kidney, adrenals, thymus, stomach, duodenum, testes and ovaries. However, slightly hydropic degeneration and fatty degeneration in liver cells were noted. An animal of vehicle control group was observed focal necrosis of hepatocytes and two of high dose (7.6

g/kg bw) group with spotty necrosis of hepatocytes. The structures of kidneys were normal and clearly identified. Few animals were found convoluted tubules with few of them were founded cardiomyocytes necrosis (5/20 in vehicle control, 2/20 in high dose group). According to the severity of these histopathological lesions and without dose response effects, these changes were considered as spontaneous lesions and not related to LE-SC treated. Previously, the mycelia of *A. cinnamomea* were orally given in SD rats to study 90-day repeated dose toxic

hydropic degeneration and one animal of vehicle control were observed interstitial nephritis. Some animals showed hydropic degeneration in cardiomyocytes and a effects and indicated the NOAEL was greater than 3 g/kg bw [21]. They also found some spontaneous histopathological lesions in heart and kidneys. Our findings were comparable to their results and higher NOAEL value we found than this study. Thus, these results supported LE-SC was a low toxic material and ensured their safe use in food supplements.

	Male				Female			
	LE-SC (g/kg b.w)							
	Control	1.9	3.8	7.6	Control	1.9	3.8	7.6
<b>Body weight (g)</b>	476.9±51.5	460.9±37.7	485.6±66.4	468.2±51.2	278.6±10.1	291.5±17.9	279.1±22.8	275.6±26.7
<b>Organ weight (g)</b>								
<b>Liver</b>	12.49±1.77	12.06±1.11	12.37±1.80	12.33±1.91	7.74±0.46	7.88±0.61	7.69±0.76	7.42±1.06
<b>Kidneys</b>	3.19±0.59	2.99±0.24	3.11±0.33	3.15±0.36	2.15±0.14	2.17±0.18	2.02±0.18	2.01±0.25
<b>Spleen</b>	0.74±0.13	0.65±0.10	0.65±0.12	0.69±0.10	0.52±0.07	0.52±0.08	0.54±0.13	0.53±0.07
<b>Thymus</b>	0.48±0.14	0.48±0.21	0.47±0.11	0.37±0.13	0.40±0.11	0.42±0.10	0.37±0.13	0.42±0.13
<b>Heart</b>	1.41±0.10	1.40±0.12	1.40±0.13	1.42±0.16	1.02±0.12	1.06±0.09	1.03±0.10	0.96±0.08
<b>Testes</b>	3.25±0.33	3.10±0.44	3.41±0.36	3.48±0.27	-	-	-	-
<b>Organ to body weight ratio (%)</b>								
<b>Liver</b>	2.62±0.24	2.62±0.16	2.55±0.21	2.65±0.48	2.78±0.17	2.71±0.22	2.75±0.12	2.71±0.48
<b>Kidneys</b>	0.67±0.09	0.65±0.04	0.65±0.07	0.68±0.08	0.77±0.06	0.75±0.08	0.72±0.04	0.73±0.12
<b>Spleen</b>	0.15±0.02	0.14±0.01	0.14±0.02	0.15±0.01	0.18±0.02	0.18±0.03	0.20±0.06	0.19±0.03
<b>Thymus</b>	0.10±0.03	0.10±0.04	0.10±0.01	0.08±0.03	0.15±0.04	0.14±0.04	0.13±0.04	0.15±0.04
<b>Heart</b>	0.30±0.03	0.30±0.03	0.29±0.03	0.31±0.05	0.37±0.04	0.36±0.04	0.37±0.04	0.35±0.04
<b>Testes</b>	0.69±0.09	0.68±0.12	0.71±0.08	0.75±0.08	-	-	-	-

Table 7: Effect of 90 day repeated dose of LE-SC on rats-organ weight.

All data presented as mean ± SD.

## Conclusion

The MTD of LE-SC was greater than 13.3 g/kg bw. LE-SC showed no mutagenic activity in Ames test and did not induce chromosome damage in mammalian erythrocytes and sperm abnormalities in mice. The results of 90 days repeated oral toxicity study showed no toxic evidences related to LE-SC at highest dose of 7.6 g/kg bw. The present study concluded that LE-SC does not cause genotoxicity and mutagenicity, and the NOAEL was 7.6

g/kg bw. Therefore, this study provided a positive feedback on the safety of LE-SC for human consumption.

## Competing Interests and Funding

The author declares that he has no competing interests.



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