Preclinical Safety Pharmacology Studies of *Taiwanofungus camphoratus* Extract

Lin JY and Chiu E*

Level Biotechnology Inc. CRO-Preclinical Testing Center, Taiwan

*Corresponding author: Emerson Chiu, Level Biotechnology Inc. CRO-Preclinical Testing Center, No. 80, Lane 169, Kangning Street, Hsi-Chih District, New Taipei City 221, Taiwan ROC, Tel: 886-02-2695-9935; Fax: 886-02-2692-1444; Email: emerson@mail.level.com.tw

Abstract

Taiwanofungus camphoratus is a unique mushroom that only grows in Taiwan. It has been used as fold medicine for a long history. Recent studies have demonstrated *T. camphoratus* possessed multiple pharmacological effects including anti-cancer, hepatoprotective and immunomodulatory effects. *T. camphoratus* extract was composed of extracts from cut-log cultivated fruiting body and solid-state culture of *T. camphoratus*. This article presents the testing results of *T. camphoratus* extract in *in vitro* hERG assay and *in vivo* safety pharmacology studies on central nervous, respiratory and cardiovascular systems.

Results

1) The hERG transfected HEK293 cells were treated with *T. camphoratus* extract at concentrations of 5, 10 and 25 µg/mL showed no significant effect on hERG current. 2) *T. camphoratus* extract was found to have no significant effects on central nervous and respiratory systems of male rats and female rats at oral doses up to 3400 mg/kg and 1700 mg/kg, respectively. 3) Beagle dogs received *T. camphoratus* extract orally up to the dosage of 1000 mg/kg did not cause physiological abnormalities on cardiovascular system. Accordingly, these results provided the safety information of *T. camphoratus* extract for human consumption.

Keywords: *Taiwanofungus camphoratus*; hERG; Central Nervous System; Respiratory System; Cardiovascular System

Introduction

Taiwanofungus camphoratus (syn. *Antrodia cinnamomea*, *Antrodia camphorata*) is an edible and medicinal mushroom originating in Taiwan. Taiwan aborigines have commonly used the fruiting body of *T. camphoratus* as folk medicine for health promotion and treating liver disease, drug and food intoxication, hypertension and cancer [1,2]. Many pharmacologic studies have noted that *T. camphoratus* possessed a variety of biological activities including anti-oxidant, anti-cancer, liver protection, anti-inflammation, and immunomodulatory effects [3-11]. Additionally, many bioactive components of *T. camphoratus* have been identified, including terpenoids, polysaccharides, benzenoids, lignans, nucleic acid, benzoquinone

derivatives, steroids, and maleic/succinic acid derivatives [1,12]. For the demand of market, it has been developed many kinds of cultivation methods to produce *T. camphoratus* including liquid fermentation, solid-state culture, cut wood culture, and dish culture. The components of *T. camphoratus* will depend on the culture techniques.

With the wild applications of *T. camphoratus* used in health food supplements in Taiwan, the safety issue of *T. camphoratus* has become increasingly important to consumers. Several toxicological studies have been done to support the safety of *T. camphoratus*. In 2013, Chang et al [13], reported that no abnormal findings were observed in male and female mice up to 1666.67 mg/kg. Huang et al [14], demonstrated that under the dosage of 6 g/kg of *T. camphoratus* showed no sub-chronic toxicity and teratogenicity in SD rats. Our previous studies also found the health food product "Leader Deluxe *Antrodia cinnamomea*" and "Leader Antrodia cinnamomea capsule" have no obvious toxic evidences in rats at dose of 2800 and 2500 mg/kg, respectively [15,16]. The maximum tolerated dose (MTD) of the solid-state cultivated mycelial powder of *Antrodia cinnamomea* (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 [17].

T. camphoratus extract was composed of extract from cut-log cultivated fruiting body and solid-state culture of *T. camphoratus*. The previous studies from our laboratory revealed *T. camphoratus* extract showed no toxicity evidences at dose of 1700 mg/kg in 90 and 180 days repeated oral dose toxicity studies in rats (data not published). In this article, we evaluated the effects of *T. camphoratus* extract in *in vitro* primary cardiovascular test (hERG test) and *in vivo* core batteries of safety pharmacology studies on central nervous, respiratory and cardiovascular systems. All studies were designed according to the suggestions of "ICH (2001) S7A Safety Pharmacology Studies for Human Pharmaceuticals". This is the first study to explore the safety pharmacology effects of *T. camphoratus* and the results would provide more safety evidences for *T. camphoratus*.

Material and Methods

Test Substance

T. camphoratus extract, LEAC-102, was composed of extracts from cut-log cultivated fruiting body and solid-state culture of *T. camphoratus* that provided by Taiwan Leader Biotech Corp. (Taipei, Taiwan).

Evaluation on hERG Channel Current

Cell culture: Human ether-a-go-go-related gene transfected human embryonic kidney 293 cells (hERG transfected HEK293 Cells) were obtained from the University of Wisconsin (Madison, WI). The cells were maintained and passaged in Minimum Essential Medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10 % heat-inactivated fetal bovine serum, 100 units/mL penicillin-streptomycin, 1 mmol/L sodium pyruvate, MEM non-essential amino acids (all supplements were from Life Technologies Corporation, Carlsbad, CA) and 400 µg/mL G 418 (Geneticin; Sigma-Aldrich, St. Louis, MO). The cells were cultured in a 5% CO_2 incubator at 37°C.

Electrophysiological experiments: The tests were conducted with the whole-cell patch-clamp method at physiological temperature (37±1.0°C). *T. camphoratus* extract was dissolved in dimethylfloxide (DMSO) and subsequently prepared by diluting the DMSO solutions with the superfusing solution (137 mmol/L NaCl, 4 mmol/L KCl, 1.8 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 10 mmol/L HEPES, and 10 mmol/L D(+)-glucose, pH adjusted to 7.4 with NaOH) to obtain the test solutions of 5, 10, and 25 µg/mL. The test solutions were filtered through a membrane filter (0.5 µm; PTFE Hydrophilic membrane; Advantec Tokyo Kaisha, Ltd.) to remove fibril-like forms undissolved in the superfusing solution. The resultant solutions were used for the hERG-current measurement. The peak amplitude of the hERG tail currents was measured from 4 individual cells, which were assigned to each experimental group including test solutions at the respective concentrations (5, 10, and 25 µg/mL), 0.5 vol% DMSO (vehicle control) or 0.1 µmol/L E-4031 (positive control). The pipette solution was composed of 130 mmol/L KCl, 1 mmol/L $MgCl_2$, 5 mmol/L EGTA, 10 mmol/L HEPES, and 5 mmol/L MgATP (pH adjusted to 7.2 with KOH).

The hERG currents passing through the cell membrane were measured under voltage clamp mode by the whole-cell patch-clamp technique. A schematic diagram of the voltage protocol to elicit the hERG tail current is shown below (Figure 1); the membrane potential of the cell was held at −80 mV, and depolarizing step pulses were given every 15 seconds to elicit the hERG tail current. The effects of the vehicle control, test substance, or positive-control substance on the hERG current were determined by changes in the peak amplitude of the tail current elicited by a partially repolarizing step pulse from +20 mV to −50 mV for 500 milliseconds following a depolarizing step pulse from the holding potential of −80 mV to +20 mV for 500 milliseconds. The peak value of the tail

Lin JY and Chiu E. Preclinical Safety Pharmacology Studies of Taiwanofungus camphoratus Camphoratus Extract. Adv Clin Toxicol 2019, 4(1): 000146.

Copyright© Lin JY and Chiu E.

current was computed based on the holding current. After confirming a stable baseline for the peak tail currents, the test solution was applied to the cell for 11 mins at a flow rate of 5 mL/min with a peristaltic pump (WM-120S/DV; Watson-Marlow Limited, Falmouth, UK).

Figure 1: hERG Tail Current.
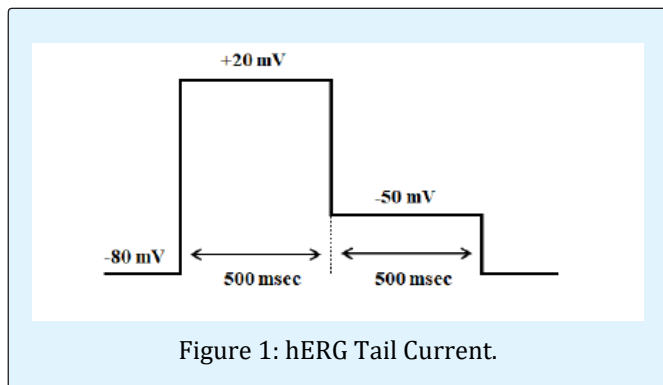
Data acquisition and analysis: The hERG currents were measured with an amplifier (Axopatch 200B; Molecular Devices, LLC., Sunnyvale, CA). Electric signals were recorded onto computer hard drive by software (pCLAMP 10; Molecular Devices, LLC., Sunnyvale, CA). The peak tail currents obtained before and 11 mins after beginning the application were compared, and the change rate (suppression rate) was calculated. The suppression rate in each cell was compensated for by the mean suppression rate in the vehicle-control group with the formula described below. Effects of the test substance and positive-control substance were evaluated with the compensated suppression rates.

• X: Suppression rate (%)

$X = [(A - B) / A] \times 100$

A: Peak tail current in each cell immediately before application

B: Peak tail current in each cell at completion of application

• Xc: Compensated suppression rate (%)

$Xc = [(A - B) / (100 - B)] \times 100$

A: Suppression rate in each cell (%)

B: Mean suppression rate in vehicle-control group (%)

The data are represented as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (one-way ANOVA) or student's t-test (SAS®, Ver. 9.3; SAS Institute Japan Ltd. and EXSUS, Ver. 8.0; CAC Croit Corporation). The significance levels were defined at $p<0.05$.

Evaluation of Central Nervous System (CNS) in Rats

The 8 weeks old Sprague Dawley (SD) (BioLASCO, Taiwan Co. Ltd.) rats were randomly divided into four groups with 10 males and 10 females in each group. The animals were housed in the AAALAC International accredited facility of Level Biotech. Inc. under 12 h light/12h dark cycle and the temperature of animal room was at 19.7-20.0℃ with relative humidity 45.9-63.0 %. The test article, *T. camphoratus* extract, was suspended in water for injection (WFI) to obtain dosing solutions. The dosing solutions were orally administered to animals at dose of 0, 170, 1700 and 3400 mg/kg for male rats and 0, 170, 850 and 1700 mg/kg for female rats. The control group was administrated with WFI only. The motor activity was conducted on all animals at pre-dose, 30 ± 5 mins post-dose and 24 ± 2 h post-dose and the functional observation battery (FOB) was performed at pre-dose, 1.5 ± 0.5 h post-dose and 24 ± 2 h post-dose. The FOB was performed of all animals including cage-side observation and handling observation (the testing parameters were including lethality, convulsion, tremor, straub tail, sedation, excitation, abnormal gait, jumps, loss of balance, motor incoordination, fore-paw treading, abnormal writhes, piloerection, stereotypies, head twitches, scratching, respiration, fear, touch response, sedation intensity, excitation intensity, aggressiveness, right reflex, ptosis, exophthalmia, grip strength, akinesia, catalepsy, corneal reflex, analgesia, defecation, salivation, lacrimation, pupillary light reflex and body temperature). All data were calculated and expressed as mean ±SD or percentage. Comparisons of parametric data collected from treated and control groups were performed by one-way ANOVA, followed by Dunnett's method (SPSS, Ver. 12.0). Non-parametric data was analyzed by Kruskal-Wallis nonparametric ANOVA method. The significance level was defined at $p<0.05$. The study was approved by the Institutional Animal Care and Use Committee (IACUC number: 170206-02).

Evaluation of Respiratory System in Rats

The 8-9 weeks old SD (BioLASCO, Taiwan Co. Ltd.) rats were randomly divided into four groups with 10 males and 10 females in each group. The animals were also housed in Level Biotech. Inc. under 12 h light/12h dark cycle and the temperature of animal room was at 19.7-20.0℃ with relative humidity 45.7-61.9 %. The test article, *T. camphoratus* extract, was suspended in WFI to obtain dosing solutions. The dosing solutions were orally administered to animals at dose of 0, 170, 1700 and 3400 mg/kg for male rats and 0, 170, 850 and 1700 mg/kg for

Lin JY and Chiu E. Preclinical Safety Pharmacology Studies of Taiwanofungus camphoratus Camphoratus Extract. Adv Clin Toxicol 2019, 4(1): 000146.

Copyright© Lin JY and Chiu E.

female rats. The control group was administrated with WFI only. The respiratory parameters were detected on all animals before study for 1 h recording as baseline, 4 h recording continuously after dosing and the 24 to 25 h recording after dosing. The whole-body plethysmography (emka TECHNOLOGIES, Paris, France) was used for detecting the respiratory parameters in freely moving animals. The parameters included inspiratory time, expiration time, peak inspiratory flow, peak expiratory flow, tidal volume, expired volume, relaxation time, minute volume, frequency of breathing, end-inspiratory pause, end-expiratory pause, enhanced pause and mid-expiratory flow. All study data acquisition and analysis were operated under iox software system (emka TECHNOLOGIES, Paris, France) and an average value of each selected parameter was calculated from detectable peaks at 1 min interval. The data for each respiratory parameter was calculated and presented as baseline value (one hour recording data prior to dosing), four hours continuous recording data at one hour interval after dosing and 24 to 25 h recording after dosing (denoted as the 24th h time point). All data were expressed as mean ± SD and analyzed by one-way ANOVA, followed by Dunnett's method (SPSS, Ver. 12.0). Besides, an additional paired t-test was used if the ANOVA results were statistically significant. The significance level was defined at $p < 0.05$. The study was approved by the Institutional Animal Care and Use Committee (IACUC number: 180101).
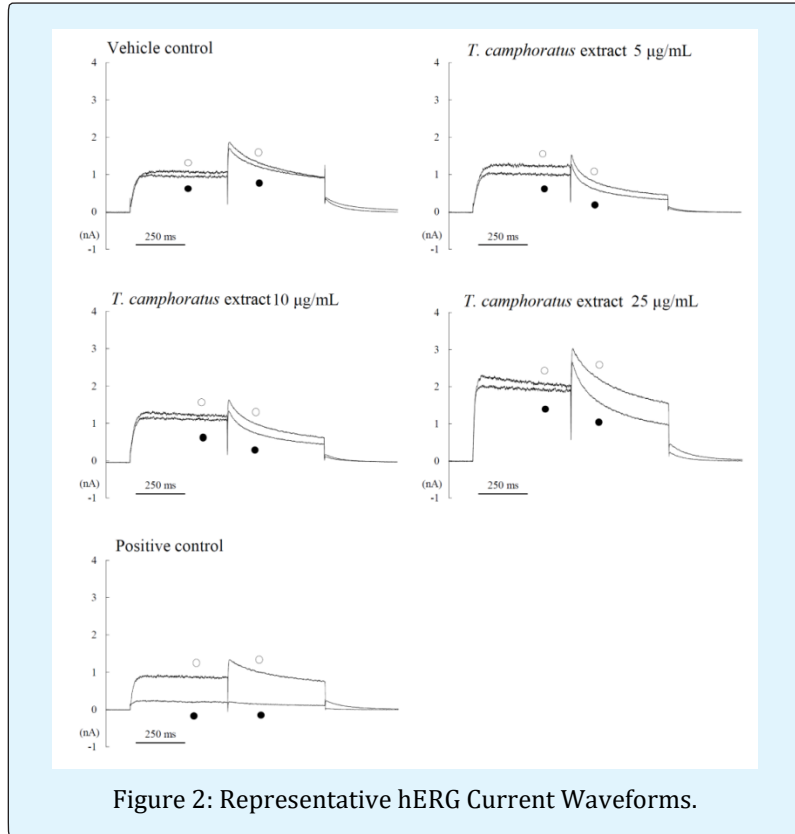
Evaluation of Cardiovascular System in Beagle dogs

The 6 months old beagle dogs (Covance Inc., Cumberland, VA) were housed in the AAALAC International accredited facility of Level Biotech. Inc. under 12 h light/12h dark cycle and the temperature of animal room was at 19.2-22.1℃ with relative humidity 43.7-67.2%. Total six beagle dogs (3 male and 3 female) were used and treated with vehicle control (Empty Porcine Hard Gelatin Capsules) first and following treated with each dosage of $T.$ $camphoratus$ extract (54, 540, 1000 mg/kg) in gelatin capsules with at least 1-week washout period between treatments. The emkaPACK$4G$ noninvasive telemetry system (emka TECHNOLOGIES, Paris, France) was used for detecting the cardiovascular parameters in freely moving animals. The parameters included RR interval, PR interval, P wave duration, QRS wave interval, QT interval, QTcB (Bazett's method), QTcF (Fridericia's method), heart rate, diastolic arterial pressure, systolic arterial pressure and mean arterial pressure. The electrocardiograms parameters were detected on all animals during pre-dose period for 1 h recording as baseline, and 24 h recording continuously after each dosage. The tail artery blood pressure was measured (ecgAUTO software NIBP) during the pre-dose period, 0-4th and 23th-24th h post-dosing periods. All study data acquisition was operated under iox software system (emka TECHNOLOGIES, Ver. 2.9.4.25). Study data analysis was operated under ecgAUTO software system (emka TECHNOLOGIES, Ver. 3.3.0.21). All ECG parameters were extracted from the lead II configuration. All data were expressed as mean ± SD and analyzed by one-way ANOVA, followed by Dunnett's method (SPSS, Ver. 12.0). Besides, an additional paired t-test was used if the ANOVA results were statistically significant. The significance level was defined at $p < 0.05$. The study was approved by the Institutional Animal Care and Use Committee (IACUC number: 171106).

Results and Discussion

Effects of $T.$ $camphoratus$ Extract on hERG Current in HEK293 Cells

The effects of the soluble fraction of $T.$ $camphoratus$ extract on hERG current were shown in Figures 2 & 3. The hERG-current-suppression rates, compensated for by the vehicle-control group rate (11.9%), were 2.6%, 4.7%, and 5.2% at dose of 5, 10, and 25 μg/mL, respectively. There was no statistically significant difference between $T.$ $camphoratus$ extract groups and vehicle-control group. A hERG channel inhibitor, E-4031, was used at 0.1 μmol/L as a positive-control substance, and its hERG-current-suppression rate, compensated for by the mean suppression rate in the vehicle-control group, was 86.3%. This rate was statistically significant when compared to the vehicle-control group, thereby confirming the validity of this experimental system to evaluate the suppressive effects of the test substance on the hERG current. However, $T.$ $camphoratus$ extract was a kind of herb material and couldn't completely dissolve in DMSO solution. The test solutions were filtered through a 0.5μm PTFE Hydrophilic membrane to remove a few fibril-like forms for prevention of interference the experiments. Overall, $T.$ $camphoratus$ extract had no significant effect on the hERG current at nominal concentrations of up to 25μg/mL, which were based on the actual weight of the test substance in preparation of the DMSO solution, under the conditions of this study.

Lin JY and Chiu E. Preclinical Safety Pharmacology Studies of Taiwanofungus camphoratus Camphoratus Extract. Adv Clin Toxicol 2019, 4(1): 000146.

Copyright© Lin JY and Chiu E.

Figure 2: Representative hERG Current Waveforms.

hERG-current waveforms recorded from a single HEK293 cell before (open circle) and 11 minutes after initiating the application (closed circle) are superimposed.

Vehicle control: 0.5-vol% DMSO; Positive control: 0.1 µmol/L E-4031.

Figure 3: Effects of *T. Camphoratus* Extract on Tail Current from hERG Channels Expressed in HEK293 Cells.
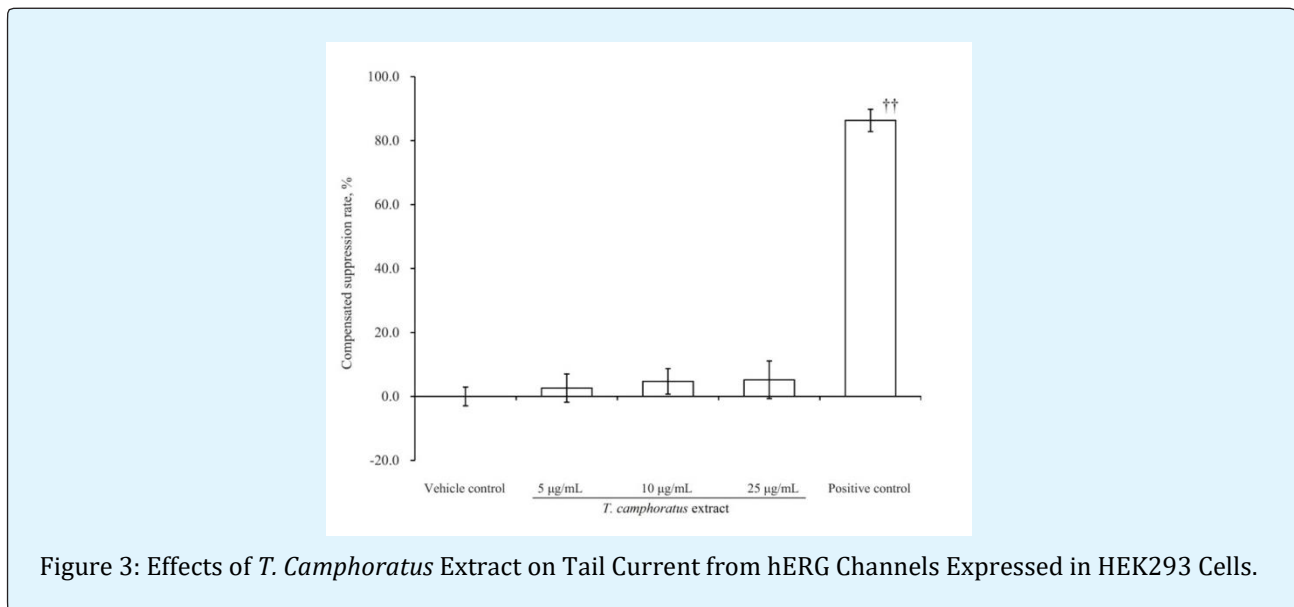
No significant suppressive effect of *T. camphoratus* extract on the hERG current. The mean suppression rate compensated for by 11.9%, the mean suppression rate in the vehicle-control group (0.5-vol% DMSO), 11 minutes after application was as follows: 2.6% ± 4.4% at 5 µg/mL, 4.7% ± 4.0% at 10 µg/mL, and 5.2% ± 5.9% at 25 µg/mL. The mean compensated suppression rate in the positive-control group (0.1 µmol/L E-4031) was 86.3% ± 3.5%. Each column represents the mean ± SD (n = 4). No statistically significant difference was noted among the test substance and vehicle-control groups, one-way ANOVA, ††p<0.01, Student's *t*-test.

Effects of *T. camphoratus* Extract on Central Nervous System (CNS) in Rats

The male and female rats received *T. camphoratus* extract at doses up to 3400 mg/kg and 1700 mg/kg, respectively, showed no significant effects on CNS. Results of motor activity and functional observational battery (FOB) responses were shown in Tables 1 & 2. No treatment related changes were observed in motor activity at pre-dose period, 30 mins and 24 h post-dosing periods. In FOB measurements, the value of grip strength in high-dose (3400 mg/kg) males was statistically significantly higher than vehicle control group at 24 h post-dosing period. However, this change was considered incidental and unrelated to treatment because the change was not correlated with other parameters of motor activity and FOB. Based on the results, *T. camphoratus* extract showed no adverse effects on CNS in rats that could provide safety information for human exposure.

Parameters	Gender	Dose (mg/kg)	Pre-dose	30 min	24 h
Average speed (cm/sec.)					
	Male	0	5.424±0.966	4.183±1.034	4.203±1.355
		170	5.315±0.597	4.271±0.816	4.099±1.161
		1700	5.502±0.898	4.305±0.644	4.797±0.741
		3400	5.338±1.012	4.973±0.682	4.230±0.772
	Female	0	5.430±0.624	4.113±0.952	4.504±0.895
		170	5.382±1.720	4.056±1.256	4.824±1.265
		850	5.478±1.328	4.231±1.104	4.615±1.346
		1700	5.288±1.641	4.198±0.850	5.256±1.402
Max speed (cm/sec.)					
	Male	0	41.996±10.904	34.101±11.971	27.244±12.993
		170	40.515±7.521	31.513±5.760	28.439±12.583
		1700	42.572±15.210	33.105±7.395	37.382±8.473
		3400	46.482±8.687	41.703±10.611	38.373±11.421
	Female	0	51.748±8.954	40.166±17.737	43.893±18.941
		170	45.023±16.185	35.371±22.634	40.370±17.980
		850	46.491±8.777	37.963±15.302	45.788±15.866
		1700	59.697±34.940	48.554±19.337	55.333±8.855
Total distance (cm)					
	Male	0	3270.646±1149.900	2458.525±1023.302	2642.129±1344.129
		170	3346.333±722.597	2657.786±712.840	2840.399±1169.978
		1700	3967.793±1340.863	2854.312±1062.888	3574.599±877.609
		3400	3862.154±1466.586	2974.510±1011.123	2900.317±1037.412
	Female	0	2994.735±712.093	2152.536±658.927	2767.476±1208.073
		170	3689.441±1805.136	2503.654±740.367	3344.445±1093.070
		850	3734.458±2002.942	2376.107±1022.169	3343.206±1081.230
		1700	3521.432±1479.436	2502.781±645.590	3649.590±1210.729

Table 1: Effect of *T. Camphoratus* Extract on Motor Activity in Rats.
All data presented as mean ± SD.

Lin JY and Chiu E. Preclinical Safety Pharmacology Studies of Taiwanofungus camphoratus Camphoratus Extract. Adv Clin Toxicol 2019, 4(1): 000146.

Copyright© Lin JY and Chiu E.

Parameters	Gender	Dose (mg/kg)	Pre-dose	1.5 h	24 h
Respiration (breath per min.)					
	Male	0	126.6±14.0	106.8±7.4	111.6±9.9
		170	125.4±10.8	115.8±8.0	117.6±9.5
		1700	120.6±7.7	110.4±11.7	117.0±12.1
		3400	122.4±13.6	112.8±6.8	112.2±13.6
	Female	0	114.6±11.1	96.0±16.2	94.2±9.8
		170	119.4±16.1	96.6±10.0	88.2±13.9
		850	105.6±12.1	99.6±13.0	93.0±11.7
		1700	108.0±12.0	97.8±12.7	92.4±11.7
Grip strength (kg)					
	Male	0	0.63±0.07	0.69±0.06	0.66±0.07
		170	0.69±0.07	0.66±0.11	0.68±0.04
		1700	0.63±0.08	0.69±0.06	0.69±0.06
		3400	0.66±0.07	0.68±0.06	0.74±0.05 *
	Female	0	0.60±0.07	0.65±0.08	0.65±0.07
		170	0.59±0.06	0.62±0.06	0.69±0.06
		850	0.62±0.06	0.66±0.05	0.68±0.04
		1700	0.58±0.06	0.64±0.08	0.68±0.08
Defecation					
	Male	0	0.5±0.5	1.0±0.0	0.7±0.5
		170	0.8±0.4	0.7±0.5	0.4±0.5
		1700	0.5±0.5	1.0±0.0	0.4±0.5
		3400	0.5±0.5	0.6±0.5	0.5±0.5
	Female	0	0.7±0.5	0.8±0.4	1.0±0.0
		170	0.9±0.3	0.9±0.3	0.8±0.4
		850	0.9±0.3	0.7±0.5	1.0±0.0
		1700	0.9±0.3	0.9±0.3	0.9±0.3
Body temperature Scores					
	Male	0	-0.1±0.3	0.0±0.0	0.0±0.0
		170	0.0±0.0	0.0±0.0	0.0±0.0
		1700	0.0±0.0	0.0±0.5	0.0±0.0
		3400	-0.1±0.3	0.0±0.0	0.0±0.0
	Female	0	0.0±0.0	0.0±0.0	-0.2±0.4
		170	0.0±0.0	0.0±0.0	-0.2±0.4
		850	0.0±0.0	0.0±0.0	0.0±0.0
		1700	0.0±0.0	0.0±0.0	-0.1±0.3

Table 2: Effect of *T. Camphoratus* Extract on Functional Observation Battery in Rats.
All data presented as mean ± SD.
* *p* < 0.05 compared to vehicle control group.

Effects of *T. camphoratus* Extract on Respiratory System in Rats

The male and female rats received *T. camphoratus* extract at doses up to 3400 mg/kg and 1700 mg/kg, respectively, showed no significant effects on respiratory system. There was no statistical difference noted in all respiratory parameters among all treated groups during each time period (Table 3). Based on the test results, *T. camphoratus* extract administered to rats via oral gavage up to the dosage of 3400 mg/kg for males and 1700 mg/kg for females did not cause significant abnormal respiratory effects in this study. All results generated from this study will provide safety information for human exposure.

Lin JY and Chiu E. Preclinical Safety Pharmacology Studies of Taiwanofungus camphoratus Camphoratus Extract. Adv Clin Toxicol 2019, 4(1): 000146.

Copyright© Lin JY and Chiu E.

Parameters	Gender	Dose (mg/kg)	Pre-dose	1 h	2 h	3 h	4 h	24 h
Inspiratory Time (msec)								
	Male	0	200.34 ± 25.95	169.74 ± 27.42	218.75 ± 24.24	216.94 ± 14.52	219.42 ± 22.57	201.12 ± 26.08
		170	216.34 ± 22.56	169.43 ± 40.69	209.00 ± 33.81	214.32 ± 20.86	211.94 ± 36.31	217.51 ± 19.92
		1700	206.80 ± 17.05	179.65 ± 27.40	209.31 ± 27.36	212.86 ± 27.88	210.93 ± 34.55	206.79 ± 18.14
		3400	209.92 ± 26.24	161.14 ± 28.06	210.60 ± 24.84	225.75 ± 20.21	220.75 ± 26.18	215.81 ± 27.27
	Female	0	187.38 ± 58.44	178.32 ± 27.67	260.15 ± 28.83	248.82 ± 22.91	254.34 ± 36.85	240.97 ± 36.48
		170	161.36 ± 44.39	165.94 ± 36.96	232.59 ± 32.92	259.87 ± 37.36	262.10 ± 24.54	230.52 ± 51.11
		850	188.80 ± 40.22	175.45 ± 34.88	241.26 ± 45.74	250.23 ± 30.97	255.69 ± 22.14	238.89 ± 23.05
		1700	189.44 ± 42.26	207.44 ± 40.58	248.24 ± 25.60	235.01 ± 59.60	244.48 ± 47.57	249.50 ± 29.30
Expiration Time (msec)								
	Male	0	284.01 ± 37.39	264.67 ± 36.04	313.36 ± 37.68	314.75 ± 33.81	324.63 ± 42.57	295.30 ± 34.41
		170	290.18 ± 31.09	248.84 ± 46.29	298.65 ± 33.30	305.01 ± 36.65	303.86 ± 50.67	305.75 ± 29.16
		1700	274.96 ± 25.10	262.90 ± 40.88	294.78 ± 33.96	295.36 ± 35.08	299.09 ± 33.90	289.19 ± 26.32
		3400	286.84 ± 54.55	252.51 ± 46.63	294.48 ± 43.32	306.74 ± 46.06	306.30 ± 45.20	299.14 ± 50.31
	Female	0	329.39 ± 97.33	323.53 ± 68.06	456.26 ± 78.57	421.07 ± 44.61	436.07 ± 81.17	413.01 ± 73.20
		170	274.69 ± 78.53	291.07 ± 51.51	410.96 ± 80.30	437.24 ± 72.00	449.93 ± 77.51	386.97 ± 93.80
		850	296.45 ± 61.10	300.88 ± 52.91	394.30 ± 52.12	397.16 ± 44.19	407.98 ± 44.11	392.59 ± 31.93
		1700	301.30 ± 65.78	383.85 ± 134.95	431.83 ± 123.98	379.18 ± 86.56	386.96 ± 68.95	398.67 ± 46.46
Peak Inspiratory Flow (mL/s)								
	Male	0	10.93 ± 2.90	13.53 ± 2.36	8.22 ± 1.98	8.66 ± 1.41	8.51 ± 1.63	11.07 ± 3.01
		170	8.93 ± 2.29	13.86 ± 4.77	9.35 ± 3.57	8.42 ± 2.28	9.56 ± 4.31	9.35 ± 2.61
		1700	9.68 ± 1.41	12.67 ± 1.88	9.83 ± 2.18	9.28 ± 2.89	9.55 ± 2.36	9.93 ± 1.01
		3400	9.26 ± 1.79	13.99 ± 2.21	9.04 ± 2.05	7.66 ± 1.39	7.79 ± 1.63	10.11 ± 4.83
	Female	0	13.21 ± 3.92	13.17 ± 2.24	7.07 ± 1.25	7.84 ± 1.52	7.37 ± 2.02	8.00 ± 2.44
		170	15.38 ± 6.75	14.03 ± 6.09	9.34 ± 2.72	7.24 ± 2.01	7.74 ± 4.71	9.03 ± 3.54
		850	13.13 ± 3.56	12.74 ± 3.34	8.35 ± 3.51	7.87 ± 2.34	7.29 ± 1.58	8.19 ± 1.50
		1700	12.73 ± 3.39	10.71 ± 3.23	7.36 ± 2.02	9.30 ± 3.64	8.28 ± 2.66	7.04 ± 1.65
Peak Expiratory Flow (mL/s)								
	Male	0	11.59 ± 2.12	12.39 ± 1.00	9.61 ± 1.66	9.83 ± 1.59	9.97 ± 1.49	11.93 ± 2.78
		170	10.42 ± 2.20	12.77 ± 3.37	10.39 ± 2.39	9.73 ± 2.10	10.68 ± 2.87	11.07 ± 3.05
		1700	10.70 ± 1.43	11.64 ± 1.63	10.45 ± 1.60	10.18 ± 1.72	10.31 ± 1.35	10.99 ± 1.51
		3400	10.56 ± 1.33	12.35 ± 1.52	9.83 ± 1.14	9.22 ± 0.89	9.35 ± 1.08	11.41 ± 3.89
	Female	0	11.81 ± 2.60	11.28 ± 1.86	7.77 ± 0.64	8.12 ± 1.22	7.83 ± 1.47	8.61 ± 1.33
		170	14.30 ± 6.11	12.50 ± 5.96	9.69 ± 2.74	8.03 ± 2.19	8.32 ± 4.01	9.26 ± 2.03
		850	12.81 ± 3.14	11.05 ± 2.28	8.77 ± 1.81	8.35 ± 1.77	8.10 ± 1.31	8.85 ± 1.09
		1700	12.31 ± 2.81	9.81 ± 1.89	7.84 ± 1.92	9.28 ± 2.46	8.40 ± 1.33	7.81 ± 1.40
Tidal Volume (mL)								
	Male	0	1.12 ± 0.20	1.17 ± 0.18	1.02 ± 0.17	1.04 ± 0.14	1.03 ± 0.13	1.20 ± 0.23
		170	1.03 ± 0.23	1.14 ± 0.21	1.05 ± 0.18	1.01 ± 0.21	1.04 ± 0.21	1.12 ± 0.22
		1700	1.11 ± 0.09	1.22 ± 0.15	1.08 ± 0.14	1.05 ± 0.11	1.09 ± 0.12	1.16 ± 0.18
		3400	1.10 ± 0.08	1.16 ± 0.12	1.01 ± 0.11	1.00 ± 0.09	1.00 ± 0.09	1.17 ± 0.28
	Female	0	1.00 ± 0.05	0.99 ± 0.06	0.87 ± 0.07	0.89 ± 0.10	0.87 ± 0.07	0.92 ± 0.10
		170	1.02 ± 0.31	0.98 ± 0.30	0.98 ± 0.29	0.88 ± 0.24	0.90 ± 0.28	0.92 ± 0.11
		850	1.04 ± 0.10	1.05 ± 0.12	0.91 ± 0.12	0.92 ± 0.09	0.91 ± 0.07	0.93 ± 0.08
		1700	0.98 ± 0.12	0.95 ± 0.14	0.87 ± 0.16	0.93 ± 0.12	0.91 ± 0.10	0.87 ± 0.11
Expired Volume (mL)								
	Male	0	1.12 ± 0.20	1.16 ± 0.17	1.02 ± 0.17	1.05 ± 0.14	1.02 ± 0.14	1.19 ± 0.23
		170	1.04 ± 0.25	1.14 ± 0.21	1.05 ± 0.18	1.02 ± 0.21	1.03 ± 0.20	1.14 ± 0.24
		1700	1.13 ± 0.09	1.22 ± 0.12	1.10 ± 0.12	1.05 ± 0.11	1.09 ± 0.13	1.17 ± 0.21

Lin JY and Chiu E. Preclinical Safety Pharmacology Studies of Taiwanofungus camphoratus Camphoratus Extract. Adv Clin Toxicol 2019, 4(1): 000146.

Copyright© Lin JY and Chiu E.

	3400	1.10 ± 0.08	1.18 ± 0.09	1.01 ± 0.11	1.01 ± 0.09	0.99 ± 0.09	1.17 ± 0.28
Female	0	1.00 ± 0.05	1.00 ± 0.07	0.87 ± 0.07	0.88 ± 0.11	0.89 ± 0.06	0.91 ± 0.11
	170	1.03 ± 0.31	0.98 ± 0.30	0.96 ± 0.27	0.89 ± 0.25	0.91 ± 0.28	0.92 ± 0.11
	850	1.05 ± 0.08	1.05 ± 0.12	0.90 ± 0.12	0.92 ± 0.09	0.92 ± 0.06	0.93 ± 0.08
	1700	0.99 ± 0.13	0.96 ± 0.13	0.86 ± 0.18	0.96 ± 0.10	0.95 ± 0.10	0.88 ± 0.11

Relaxation Time (msec)

Male	0	122.26 ± 11.22	119.61 ± 10.02	129.47 ± 13.59	131.90 ± 14.28	132.31 ± 14.27	127.24 ± 12.34
	170	123.81 ± 11.61	116.87 ± 16.00	127.44 ± 11.17	128.19 ± 10.80	125.30 ± 15.99	128.15 ± 11.72
	1700	123.53 ± 7.60	124.82 ± 18.40	130.09 ± 11.75	129.98 ± 13.69	131.30 ± 13.03	126.67 ± 8.35
	3400	123.74 ± 11.39	118.98 ± 12.42	126.23 ± 9.39	131.61 ± 9.46	130.01 ± 11.80	126.89 ± 12.30
Female	0	132.33 ± 27.18	138.18 ± 28.97	177.75 ± 40.53	170.53 ± 31.60	173.17 ± 35.63	155.82 ± 23.36
	170	113.63 ± 25.11	127.74 ± 27.73	165.54 ± 55.50	179.34 ± 62.47	184.04 ± 60.26	142.38 ± 18.61
	850	123.56 ± 19.09	133.79 ± 17.38	152.94 ± 18.85	158.39 ± 17.18	160.81 ± 15.11	148.80 ± 8.23
	1700	122.02 ± 18.37	156.85 ± 52.22	189.01 ± 90.36	167.60 ± 56.79	161.13 ± 26.39	152.71 ± 16.32

Minute Volume (mL)

Male	0	168.58 ± 44.00	205.95 ± 31.33	125.63 ± 29.63	129.28 ± 19.95	128.45 ± 27.36	168.64 ± 44.76
	170	139.58 ± 36.75	220.04 ± 81.29	143.37 ± 50.06	131.99 ± 35.91	145.40 ± 62.42	144.06 ± 40.28
	1700	152.41 ± 20.74	196.85 ± 30.44	147.68 ± 32.41	142.00 ± 43.18	144.42 ± 33.26	154.43 ± 16.99
	3400	149.82 ± 31.87	219.80 ± 36.34	139.27 ± 31.80	120.96 ± 21.60	122.39 ± 23.45	157.52 ± 67.16
Female	0	199.42 ± 70.03	196.41 ± 42.45	92.52 ± 22.78	108.16 ± 22.97	100.79 ± 33.96	111.87 ± 37.45
	170	251.26 ± 119.10	220.78 ± 110.35	133.84 ± 46.14	99.42 ± 29.84	104.54 ± 65.93	126.00 ± 56.07
	850	207.71 ± 65.02	192.09 ± 51.63	114.62 ± 49.39	111.03 ± 34.81	101.54 ± 24.52	113.47 ± 20.54
	1700	200.40 ± 66.01	154.98 ± 55.67	100.97 ± 34.90	134.63 ± 61.49	118.07 ± 40.20	100.22 ± 25.15

Frequency of breathing (bpm)

Male	0	151.26 ± 28.28	181.64 ± 39.36	124.50 ± 21.65	126.81 ± 13.06	125.47 ± 25.24	140.68 ± 27.35
	170	132.55 ± 16.62	191.63 ± 52.49	136.67 ± 32.41	131.53 ± 25.98	139.22 ± 37.54	127.31 ± 16.17
	1700	136.98 ± 14.91	170.14 ± 30.94	134.91 ± 20.48	134.08 ± 32.16	132.20 ± 27.17	133.55 ± 16.26
	3400	137.29 ± 26.62	197.79 ± 40.82	138.86 ± 26.02	121.07 ± 17.21	123.97 ± 21.86	133.03 ± 27.51
Female	0	201.60 ± 71.84	198.35 ± 41.89	103.29 ± 23.84	121.14 ± 24.40	114.68 ± 35.22	122.23 ± 38.53
	170	244.77 ± 84.82	221.74 ± 50.13	139.50 ± 41.87	112.48 ± 22.49	108.41 ± 35.16	138.24 ± 63.74
	850	202.11 ± 58.91	190.68 ± 48.26	125.20 ± 48.49	121.21 ± 35.33	109.03 ± 20.78	121.24 ± 20.93
	1700	202.13 ± 61.13	161.13 ± 47.62	115.01 ± 31.25	143.62 ± 67.68	130.99 ± 47.60	115.54 ± 24.13

End-Inspiratory Pause (msec)

Male	0	4.29 ± 0.95	3.58 ± 0.65	4.31 ± 0.69	4.18 ± 0.40	4.19 ± 0.40	3.83 ± 0.57
	170	4.33 ± 0.74	3.61 ± 0.72	3.84 ± 0.73	4.27 ± 1.01	4.34 ± 1.15	4.06 ± 0.55
	1700	4.36 ± 0.54	4.43 ± 2.74	4.93 ± 1.99	5.08 ± 1.67	4.50 ± 1.20	3.95 ± 0.44
	3400	3.98 ± 1.13	3.21 ± 0.50	4.36 ± 1.25	4.44 ± 0.89	4.29 ± 1.14	4.36 ± 1.24
Female	0	4.71 ± 1.64	5.55 ± 3.71	9.83 ± 7.44	9.21 ± 6.66	9.33 ± 6.58	6.21 ± 1.66
	170	3.76 ± 1.21	4.92 ± 3.37	6.02 ± 1.71	7.94 ± 3.46	7.62 ± 3.24	5.18 ± 2.02
	850	4.57 ± 1.90	4.56 ± 1.85	7.68 ± 3.53	8.09 ± 3.50	8.40 ± 4.31	5.28 ± 1.05
	1700	5.21 ± 2.55	7.42 ± 6.04	10.30 ± 9.15	9.80 ± 8.62	9.60 ± 7.42	6.06 ± 1.96

End-Expiratory Pause (msec)

Male	0	54.13 ± 11.92	45.13 ± 10.78	61.79 ± 12.81	60.39 ± 11.77	62.97 ± 13.78	55.86 ± 11.24
	170	57.44 ± 9.55	42.32 ± 11.69	57.14 ± 14.39	59.25 ± 13.88	58.90 ± 12.71	61.17 ± 8.57
	1700	52.54 ± 8.60	44.07 ± 8.05	54.77 ± 9.97	54.04 ± 9.64	55.46 ± 9.48	55.81 ± 9.62
	3400	55.48 ± 17.28	42.71 ± 13.68	56.03 ± 11.79	58.43 ± 12.83	58.23 ± 12.87	59.72 ± 14.38
Female	0	56.52 ± 20.23	51.48 ± 11.57	78.98 ± 16.36	73.63 ± 9.27	75.95 ± 13.91	78.04 ± 16.29
	170	49.55 ± 19.30	48.79 ± 12.18	77.44 ± 28.06	82.18 ± 21.79	83.97 ± 16.90	76.85 ± 33.19
	850	55.74 ± 15.72	47.54 ± 10.46	70.23 ± 13.09	70.93 ± 10.14	75.51 ± 9.17	75.08 ± 7.90
	1700	56.87 ± 16.84	58.59 ± 15.98	70.95 ± 20.08	63.04 ± 22.65	66.12 ± 15.79	76.93 ± 12.53

Enhanced Pause								
	Male	0	1.73 ± 0.43	1.43 ± 0.47	2.00 ± 0.54	1.91 ± 0.38	2.10 ± 0.45	1.72 ± 0.39
		170	1.89 ± 0.22	1.35 ± 0.39	1.89 ± 0.54	1.94 ± 0.37	2.08 ± 0.45	1.98 ± 0.32
		1700	1.57 ± 0.22	1.28 ± 0.23	1.65 ± 0.35	1.72 ± 0.36	1.70 ± 0.24	1.67 ± 0.23
		3400	1.79 ± 0.53	1.24 ± 0.47	1.85 ± 0.41	1.91 ± 0.45	1.96 ± 0.53	1.87 ± 0.40
	Female	0	1.72 ± 0.64	1.55 ± 0.49	2.49 ± 0.86	2.18 ± 0.56	2.27 ± 0.58	2.47 ± 0.70
		170	1.62 ± 0.62	1.50 ± 0.36	2.58 ± 0.96	2.51 ± 0.77	2.39 ± 0.56	2.59 ± 1.28
		850	1.64 ± 0.49	1.39 ± 0.37	2.32 ± 0.68	2.21 ± 0.51	2.26 ± 0.45	2.37 ± 0.45
		1700	1.77 ± 0.58	1.98 ± 0.68	2.17 ± 0.71	1.85 ± 0.77	2.01 ± 0.72	2.41 ± 0.42
Mid-expiratory flow (mL/s)								
	Male	0	8.35 ± 1.75	9.14 ± 0.81	6.47 ± 1.22	6.71 ± 1.23	6.67 ± 1.26	8.30 ± 2.06
		170	7.49 ± 1.53	9.91 ± 3.19	7.33 ± 1.88	6.74 ± 1.53	7.46 ± 2.40	7.71 ± 2.44
		1700	7.83 ± 1.22	8.61 ± 1.51	7.48 ± 1.25	7.23 ± 1.59	7.26 ± 1.14	7.71 ± 1.30
		3400	7.60 ± 1.23	9.26 ± 1.46	7.07 ± 1.04	6.38 ± 0.72	6.44 ± 0.82	8.18 ± 3.09
	Female	0	9.47 ± 2.68	8.94 ± 1.99	5.43 ± 0.63	5.68 ± 1.03	5.49 ± 1.35	6.09 ± 1.30
		170	12.05 ± 5.78	10.29 ± 5.52	7.12 ± 2.21	5.73 ± 1.69	5.89 ± 3.49	6.66 ± 1.90
		850	10.36 ± 3.30	8.48 ± 2.36	6.25 ± 1.73	5.90 ± 1.54	5.61 ± 1.05	6.27 ± 1.04
		1700	10.06 ± 2.95	7.34 ± 1.84	5.54 ± 1.56	6.90 ± 2.55	5.99 ± 1.41	5.38 ± 1.14

Table 3: Effect of *T. camphoratus* extract on respiratory parameters in rats.
All data presented as mean ± SD.

Effects of *T. camphoratus* Extract on Cardiovascular System in Beagle Dogs

The beagle dogs were given gelatin capsules containing *T. camphoratus* extract at doses of 0 (empty capsule), 54, 540, 1000 mg/kg orally and the ECG parameters and blood pressures were measured at indicated time periods. In both sexes, no statistical difference was noted in baseline data of all cardiovascular data. In male dogs, a significant increase in QTcB and QTcF were observed at 4 h at 54 mg/kg, at 3 h and 4 h at 540 mg/kg, at 3 h and 4 h (QTcB only) at 1000 mg/kg as compared to vehicle control. These values, however, were compared to baseline data by paired *t*-test and no significant difference was noted except the QTcF value of 1000 mg/kg at 3 h. These findings described above were not considered to be of toxicological significance because the changes were not dose related and within normal physiological range. Next, no statistical difference was noted in all cardiovascular data of female dogs (heart rate, RR interval, PR interval, P duration, QRS duration, QT interval, and corrected QT (QTc)) at each indicated time period. Furthermore, the blood pressure parameters in *T. camphoratus* extract treated male and female dogs showed no statistical difference at each indicated time period as compared to vehicle control (Tables 4 & 5).

Parameters	Gender	Dose (mg/kg)	Pre-dose	1 h	2 h	3 h	4 h	24 h
RR Interval (ms)								
	Male	0	798.413 ± 59.868	748.537 ± 28.625	764.893 ± 80.637	800.077 ± 101.283	827.943 ± 115.681	716.803 ± 91.396
		54	578.047 ± 108.284	582.813 ± 127.615	664.927 ± 163.928	617.427 ± 95.058	620.623 ± 82.525	771.383 ± 130.829
		540	703.587 ± 186.107	683.137 ± 119.233	650.053 ± 127.592	663.040 ± 119.734	601.870 ± 111.554	801.173 ± 158.028
		1000	720.617 ± 169.553	569.373 ± 114.235	624.447 ± 124.155	639.270 ± 69.644	598.487 ± 69.776	703.330 ± 115.312
	Female	0	706.220 ± 88.543	706.773 ± 44.127	702.807 ± 114.886	703.727 ± 70.382	724.317 ± 96.812	691.520 ± 123.706
		54	589.973 ± 102.244	549.167 ± 67.720	617.430 ± 86.254	596.647 ± 66.039	642.023 ± 90.679	658.123 ± 85.785
		540	757.533 ± 192.680	681.860 ± 154.167	739.573 ± 111.680	668.697 ± 104.978	645.270 ± 108.574	757.487 ± 132.135
		1000	683.033 ± 238.674	634.803 ± 174.501	648.720 ± 182.701	694.740 ± 191.545	672.997 ± 189.251	661.173 ± 45.326
PR Interval (ms)								
	Male	0	78.547 ± 7.793	77.820 ± 8.338	77.073 ± 6.758	76.920 ± 7.228	83.430 ± 8.877	79.360 ± 10.650
		54	78.453 ± 5.905	78.503 ± 6.079	79.927 ± 6.030	79.787 ± 6.900	83.517 ± 9.692	80.597 ± 5.993
		540	81.067 ± 5.261	84.453 ± 8.486	79.307 ± 7.151	82.943 ± 7.964	81.617 ± 9.115	79.240 ± 6.221
		1000	81.887 ± 7.768	82.583 ± 8.166	82.740 ± 6.859	80.860 ± 4.779	79.970 ± 4.493	85.890 ± 7.518

Lin JY and Chiu E. Preclinical Safety Pharmacology Studies of Taiwanofungus camphoratus Camphoratus Extract. Adv Clin Toxicol 2019, 4(1): 000146.

Copyright© Lin JY and Chiu E.

Advances in Clinical Toxicology

	Female	0	93.587 ± 6.351	85.920 ± 2.882	88.937 ± 8.371	87.247 ± 5.864	87.880 ± 8.315	89.810 ± 3.775
		54	91.630 ± 6.395	89.163 ± 3.995	88.340 ± 2.409	85.183 ± 3.445	91.230 ± 7.962	91.010 ± 5.254
		540	90.647 ± 7.097	89.200 ± 3.810	90.050 ± 8.087	85.137 ± 0.701	88.273 ± 4.077	90.087 ± 4.650
		1000	99.893 ± 12.184	95.203 ± 8.709	94.670 ± 11.690	92.050 ± 6.231	90.963 ± 8.570	92.100 ± 4.723
P wave Duration (ms)								
	Male	0	35.770 ± 5.350	34.847 ± 4.064	35.080 ± 4.052	36.190 ± 3.428	42.330 ± 4.476	35.680 ± 7.207
		54	35.087 ± 3.264	35.843 ± 3.360	37.133 ± 3.928	37.340 ± 4.688	40.470 ± 3.785	36.537 ± 4.505
		540	39.120 ± 4.793	43.760 ± 3.050	37.997 ± 5.213	44.170 ± 5.116	40.577 ± 3.131	37.117 ± 4.885
		1000	36.080 ± 4.402	37.693 ± 3.592	37.277 ± 3.406	37.680 ± 3.964	36.847 ± 4.052	38.497 ± 3.124
	Female	0	37.870 ± 2.102	36.037 ± 1.409	37.877 ± 2.410	36.637 ± 2.249	36.790 ± 2.102	38.213 ± 2.027
		54	37.043 ± 6.300	35.997 ± 2.884	35.347 ± 4.119	34.120 ± 3.108	38.560 ± 8.982	36.377 ± 3.851
		540	38.673 ± 2.791	36.793 ± 2.010	38.900 ± 4.948	35.290 ± 2.266	35.320 ± 4.157	35.713 ± 3.203
		1000	41.470 ± 4.936	39.493 ± 4.003	40.280 ± 5.974	39.307 ± 4.157	37.907 ± 5.972	37.160 ± 2.833
QRS wave Duration (ms)								
	Male	0	40.737 ± 3.340	39.530 ± 1.882	39.440 ± 1.384	39.010 ± 2.653	38.933 ± 3.622	39.983 ± 2.174
		54	39.333 ± 1.444	39.197 ± 2.113	39.933 ± 1.826	39.103 ± 1.985	40.913 ± 4.180	39.880 ± 1.228
		540	39.610 ± 3.879	38.920 ± 3.916	39.927 ± 3.435	39.080 ± 3.981	39.963 ± 3.591	40.413 ± 4.524
		1000	38.860 ± 2.223	39.050 ± 1.254	39.387 ± 1.030	39.650 ± 2.001	39.280 ± 1.508	39.867 ± 2.140
	Female	0	40.853 ± 1.955	38.577 ± 2.916	39.417 ± 4.525	38.740 ± 3.248	38.620 ± 2.025	42.017 ± 6.946
		54	40.373 ± 4.674	37.230 ± 2.089	38.600 ± 3.212	40.270 ± 5.963	37.597 ± 2.752	38.023 ± 3.410
		540	40.600 ± 3.182	38.343 ± 2.361	39.367 ± 0.731	38.863 ± 2.486	39.053 ± 2.754	39.670 ± 1.573
		1000	37.693 ± 2.546	37.073 ± 1.848	37.720 ± 1.340	37.073 ± 1.409	36.610 ± 2.201	37.757 ± 2.214
QT Interval (ms)								
	Male	0	206.057 ± 1.141	201.877 ± 1.916	206.367 ± 5.905	203.893 ± 2.259	202.930 ± 3.292	210.413 ± 6.634
		54	191.583 ± 14.820	186.527 ± 11.450	196.833 ± 12.281	195.250 ± 9.689	201.210 ± 9.257	202.630 ± 5.552
		540	204.273 ± 20.341	202.880 ± 7.816	200.253 ± 12.000	206.530 ± 9.097	197.860 ± 10.753	205.930 ± 9.756
		1000	201.030 ± 12.067	188.980 ± 10.458	195.600 ± 8.354	202.487 ± 9.031	196.073 ± 4.124	210.107 ± 4.766
	Female	0	204.360 ± 9.544	190.747 ± 6.205	199.027 ± 18.670	197.817 ± 10.616	198.280 ± 12.799	203.600 ± 19.481
		54	195.887 ± 12.476	183.640 ± 12.736	191.120 ± 8.444	187.437 ± 6.908	198.163 ± 12.725	199.873 ± 11.167
		540	213.013 ± 27.911	198.193 ± 20.540	207.780 ± 31.052	196.570 ± 8.557	199.530 ± 16.356	209.890 ± 20.110
		1000	202.463 ± 30.136	197.110 ± 26.206	195.013 ± 19.255	201.963 ± 29.364	200.973 ± 30.712	200.737 ± 20.575
QTcB (ms)								
	Male	0	242.907 ± 6.692	243.660 ± 6.197	248.547 ± 5.228	241.397 ± 9.210	236.070 ± 9.328	258.943 ± 15.129
		54	258.383 ± 8.989	251.897 ± 13.068	252.303 ± 12.607	256.753 ± 5.337	263.070 ± 7.214 #	242.643 ± 6.930
		540	254.887 ± 1.825	255.627 ± 8.920	257.947 ± 7.875	263.823 ± 10.519 #	263.713 ± 6.470 #	240.467 ± 10.800
		1000	250.220 ± 8.885	256.427 ± 12.486	255.067 ± 15.687	260.670 ± 4.626 #	260.097 ± 8.056 #	259.977 ± 16.944
	Female	0	256.907 ± 19.104	236.567 ± 10.190	249.990 ± 20.813	247.377 ± 15.665	245.403 ± 14.907	256.907 ± 4.757
		54	261.820 ± 14.408	254.100 ± 10.237	252.130 ± 9.935	249.173 ± 10.426	257.200 ± 20.161	256.577 ± 12.554
		540	259.277 ± 32.079	250.763 ± 19.598	253.230 ± 27.836	251.100 ± 16.082	258.127 ± 8.173	252.877 ± 9.634
		1000	258.943 ± 9.687	257.490 ± 5.664	252.857 ± 8.804	254.310 ± 16.623	255.803 ± 14.263	253.970 ± 17.136
QTcF (ms)								
	Male	0	228.973 ± 4.524	228.053 ± 4.147	232.630 ± 4.046	227.137 ± 5.765	223.437 ± 5.206	240.823 ± 9.602
		54	233.347 ± 7.490	227.303 ± 6.329	231.440 ± 5.177	233.730 ± 1.535	239.993 ± 3.820 #	227.633 ± 3.101
		540	235.830 ± 6.452	235.900 ± 2.805	236.320 ± 2.015	242.367 ± 4.452 #	238.987 ± 0.655 #	227.510 ± 3.836
		1000	231.650 ± 4.715	231.150 ± 4.893	232.903 ± 7.997	239.080 ± 4.492 *	236.260 ± 3.948 #	241.460 ± 9.898
	Female	0	237.030 ± 14.782	219.433 ± 8.118	230.733 ± 18.505	228.717 ± 13.100	227.553 ± 12.429	236.720 ± 10.037
		54	237.153 ± 10.328	227.547 ± 10.176	229.223 ± 6.562	226.117 ± 6.983	235.077 ± 16.229	235.297 ± 10.258
		540	241.640 ± 26.874	230.963 ± 16.278	236.070 ± 28.170	230.620 ± 11.319	236.103 ± 9.233	236.650 ± 11.816
		1000	237.367 ± 12.446	234.683 ± 12.643	231.050 ± 6.433	234.493 ± 18.764	235.080 ± 18.502	234.250 ± 18.580
Heart Rate (bpm)								
	Male	0	75.430 ± 5.615	80.237 ± 3.134	79.000 ± 7.967	75.750 ± 8.963	73.417 ± 10.213	84.697 ± 11.660
		54	106.190 ± 19.256	106.157 ± 22.123	93.740 ± 21.483	98.720 ± 15.092	97.927 ± 14.099	79.180 ± 12.379
		540	88.873 ± 20.390	89.490 ± 14.260	94.827 ± 19.413	92.583 ± 17.472	102.090 ± 19.533	76.803 ± 14.618

Lin JY and Chiu E. Preclinical Safety Pharmacology Studies of Taiwanofungus camphoratus Camphoratus Extract. Adv Clin Toxicol 2019, 4(1): 000146.

Copyright© Lin JY and Chiu E.

		1000	86.330 ± 19.731	108.670 ± 24.613	98.937 ± 21.587	94.643 ± 10.827	101.200 ± 12.232	86.973 ± 15.244
	Female	0	85.930 ± 11.608	85.123 ± 5.496	87.067 ± 15.555	85.860 ± 9.018	83.920 ± 12.151	88.493 ± 14.495
		54	103.667 ± 17.091	110.410 ± 14.069	98.553 ± 14.795	101.367 ± 10.892	94.830 ± 14.584	92.300 ± 12.997
		540	83.207 ± 23.738	91.260 ± 21.825	82.417 ± 12.844	91.350 ± 15.524	94.813 ± 16.372	80.723 ± 13.057
		1000	96.020 ± 35.797	98.977 ± 24.479	97.157 ± 25.056	90.943 ± 25.247	93.757 ± 24.830	91.040 ± 6.416

Table 4: Effect of *T. camphoratus* Extract on ECG Parameters in Beagle Dogs.

All data presented as mean ± SD.

*$p < 0.05$ [Statistical difference from the control group by Dunnett's method and from baseline by paired *t*-test.]

#$p < 0.05$ [Statistical difference from the control group by Dunnett's method, but with no statistical difference from the baseline by paired *t*-test.].

Parameters	Gender	Dose (mg/kg)	Pre-dose	1 h	2 h	3 h	4 h	24 h
Diastolic Arterial Pressure (mmHg)								
	Male	0	71.723 ± 10.717	74.730 ± 5.697	72.787 ± 17.095	85.827 ± 22.943	71.780 ± 9.820	72.033 ± 8.915
		54	75.653 ± 13.462	84.387 ± 31.292	64.973 ± 13.569	72.243 ± 8.954	72.173 ± 16.368	73.593 ± 6.770
		540	70.243 ± 6.934	67.113 ± 5.577	65.300 ± 10.888	67.040 ± 2.746	75.883 ± 10.167	72.167 ± 4.491
		1000	64.037 ± 8.878	82.787 ± 33.932	72.613 ± 27.037	62.340 ± 13.232	54.913 ± 8.358	64.083 ± 7.796
	Female	0	67.503 ± 12.804	82.767 ± 10.919	74.023 ± 4.972	68.727 ± 5.416	67.130 ± 9.918	70.747 ± 14.513
		54	61.410 ± 5.577	70.997 ± 9.352	61.027 ± 9.095	68.193 ± 5.443	81.777 ± 26.263	64.497 ± 6.579
		540	64.857 ± 4.945	66.803 ± 1.589	65.270 ± 7.665 [a]	76.647 ± 15.126	61.960 ± 8.825	63.763 ± 4.128
		1000	64.527 ± 8.057	71.050 ± 8.440	65.943 ± 6.659	56.537 ± 10.009	60.953 ± 7.543	59.430 ± 15.599 [a]
Systolic Arterial Pressure (mmHg)								
	Male	0	119.173 ± 19.173	129.633 ± 15.506	123.827 ± 28.079	132.487 ± 21.398	116.603 ± 7.987	119.427 ± 18.234
		54	122.290 ± 15.031	140.467 ± 30.572	126.777 ± 21.013	123.560 ± 10.139	123.687 ± 15.826	124.293 ± 11.099
		540	126.227 ± 16.320	121.060 ± 5.983	120.343 ± 17.277	121.477 ± 15.623	125.267 ± 12.978	127.173 ± 19.665
		1000	108.950 ± 6.190	123.440 ± 24.995	118.163 ± 18.314	104.717 ± 7.843	109.430 ± 10.546	115.063 ± 16.114
	Female	0	106.443 ± 10.599	124.920 ± 11.499	121.900 ± 16.123	116.410 ± 13.126	120.020 ± 22.471	116.490 ± 10.157
		54	105.440 ± 5.856	116.863 ± 12.171	105.197 ± 6.617	110.187 ± 4.226	128.307 ± 32.081	109.897 ± 11.591
		540	104.160 ± 18.689	115.327 ± 9.046	111.595 ± 9.595 [a]	115.917 ± 11.057	102.357 ± 5.889	101.993 ± 9.488
		1000	103.693 ± 5.809	111.997 ± 11.882	110.540 ± 12.109	103.937 ± 8.532	103.387 ± 6.739	106.865 ± 9.991 [a]
Mean Arterial Pressure (mmHg)								
	Male	0	93.233 ± 14.943	99.557 ± 11.079	97.340 ± 25.772	106.733 ± 23.823	91.627 ± 9.782	93.693 ± 14.800
		54	96.060 ± 11.596	111.737 ± 31.787	90.910 ± 9.945	93.963 ± 10.440	94.610 ± 16.973	97.237 ± 7.714
		540	97.303 ± 11.184	94.377 ± 6.963	88.163 ± 14.131	93.830 ± 12.647	98.510 ± 11.621	99.267 ± 17.554
		1000	86.113 ± 5.078	100.857 ± 30.118	92.763 ± 23.177	81.473 ± 10.426	73.910 ± 8.958	84.627 ± 10.630
	Female	0	84.833 ± 12.458	100.740 ± 9.972	95.927 ± 9.899	89.720 ± 7.922	88.547 ± 13.674	90.990 ± 10.507
		54	81.087 ± 6.153	91.720 ± 10.414	81.060 ± 7.453	88.200 ± 5.587	101.930 ± 27.703	85.550 ± 9.611
		540	82.320 ± 10.849	88.760 ± 3.631	87.025 ± 8.224 [a]	95.253 ± 14.125	79.360 ± 5.323	80.507 ± 8.033
		1000	82.310 ± 5.916	88.903 ± 10.707	85.977 ± 9.414	77.630 ± 9.768	80.873 ± 6.403	76.955 ± 17.458 [a]

Table 5: Effect of *T. camphoratus* Extract on Blood Pressure in Beagle Dogs.

All data presented as mean ± SD.

a : N = 2 (The peak blood pressure of one female at 540 mg/kg was unrecognizable during 1 to 2 h post-dosing period and cannot be calculated reliably by iox system. In addition, no blood pressure was recorded in one female at 1000 mg/kg during 23 to 24 h post-dosing period, because the tail cuff was loose. Therefore, the data were not included in statistical calculation.)

The ECG morphology at each indicated time period was evaluated by a veterinary cardiologist that the sinus arrest noted in all six dogs and large negative T wave noted in two dogs before and after the *T. camphoratus* extract treatment (data not shown). In general, T wave changes are very non-specific. Tall T wave could be as a

normal variant. This could occur with hyperventilation, anxiety, and even positional changes. However, tall T wave could also be a warming sign of myocardial hypoxia or electrolytes disturbance (hyperkalemia). Sinus arrest is frequently caused by high parasympathetic tone due to one or many factors. It is commonly in brachycephalic breed dogs with strenuous respiratory efforts that irritate the pharynx and cause reflex vagal stimulation. Other factors may cause sinus arrest include surgical stimulation, impingement upon the vagus nerve (neoplasia), drug toxicity (digitalis or β-blockers). No treatment is needed for this conducting disturbance, unless syncope is developed [18,19]. In this study, large (negative) T wave was found in one male and one female dog and sinus arrest was seen in all six dogs in both the predose and post stages. The link between these abnormal findings and *T. camphoratus* extract was not indicated. Based on the results, the dogs received *T. camphoratus* extract via oral administration up to the dosage of 1000 mg/kg did not cause physiological abnormalities on cardiovascular system in this study.

Conclusion

Results from *in vitro* hERG test and *in vivo* core batteries of safety pharmacology studies revealed that *T. camphoratus* extract had no significant effect on the hERG current at nominal concentrations of up to 25 μg/mL and had no obvious toxicity evidences on central nervous, respiratory and cardiovascular system (up to doses at 1700 mg/kg in female rats, 3400 mg/kg in male rats and 1000 mg/kg in both sexes of dogs). The results would provide the evidences to support the safety of *T. camphoratus* extract as a food supplement and for clinical usage.

Conflict of Interests

The authors declare that there are no conflicts of interests.

References

1. Geethangili M, Tzeng YM (2011) Review of Pharmacological Effects of Antrodia camphorata and Its Bioactive Compounds. Evid Based Complement Alternat Med 2011: 17.

2. Lu MC, El-Shazly M, Wu TY, Du YC, Chang TT, et al. (2013) Recent research and development of Antrodia cinnamomea. Pharmacol Ther 139(2): 124-156.

3. Wang JJ, Wu CC, Lee CL, Hsieh SL, Chen JB, et al. (2017) Antimelanogenic, Antioxidant and Antiproliferative Effects of Antrodia camphorata Fruiting Bodies on B16-F0 Melanoma Cells. PLoS One 12(1): e0170924.

4. Wu MD, Cheng MJ, Wang WY, Huang HC, Yuan GF, et al. (2011) Antioxidant activities of extracts and metabolites isolated from the fungus Antrodia cinnamomea. Nat Prod Res 25(16): 1488-1496.

5. Lu MC, Du YC, Chuu JJ, Hwang SL, Hsieh PC, et al. (2009) Active extracts of wild fruiting bodies of Antrodia camphorata (EEAC) induce leukemia HL 60 cells apoptosis partially through histone hypoacetylation and synergistically promote anticancer effect of trichostatin A. Arch Toxicol 83(2): 121-129.

6. Yen IC, Lee SY, Lin KT, Lai FY, Kuo MT, et al. (2017) *In Vitro* Anticancer Activity and Structural Characterization of Ubiquinones from Antrodia cinnamomea Mycelium. Molecules 22(5).

7. Chiu HW, Hua KF (2016) Hepatoprotective Effect of Wheat-Based Solid-State Fermented Antrodia cinnamomea in Carbon Tetrachloride-Induced Liver Injury in Rat. PLoS One 11(4): e0153087.

8. Li ZW, Kuang Y, Tang SN, Li K, Huang Y, et al. (2017) Hepatoprotective activities of Antrodia camphorata and its triterpenoid compounds against CCl4-induced liver injury in mice. J Ethnopharmacol 206: 31-39.

9. Huang TT, Wu SP, Chong KY, Ojcius DM, Ko YF, et al. (2014) The medicinal fungus Antrodia cinnamomea suppresses inflammation by inhibiting the NLRP3 inflammasome. J Ethnopharmacol 155(1): 154-164.

10. Chen YY, Lo CP, Lin CC, Hsieh YH (2018) Effects of *Taiwanofungus camphoratus* on non-specific and specific immune activities in mice. Mycology 9(2): 129-135.

11. Lin YH, Kuo JT, Chen YY, Kumar KJS, Lo CP, et al. (2018) Immunomodulatory Effects of the Stout Camphor Medicinal Mushroom, *Taiwanofungus camphoratus* (Agaricomycetes)-Based Health Food Product in Mice. Int J Med Mushrooms 20(9): 849-858.

12. Yue PY, Wong YY, Chan TY, Law CK, Tsoi YK, et al. (2012) Review of biological and pharmacological activities of the endemic Taiwanese bitter medicinal

Lin JY and Chiu E. Preclinical Safety Pharmacology Studies of Taiwanofungus camphoratus Camphoratus Extract. Adv Clin Toxicol 2019, 4(1): 000146.

Copyright© Lin JY and Chiu E.

mushroom, Antrodia camphorata (M Zang et CH Su) Sh. H. Wu et al. (higher Basidiomycetes). Int J Med Mushrooms 14(3): 241-256.

13. Chang JB, Wu MF, Lu HF, Chou J, Au MK, et al. (2013) Toxicological evaluation of Antrodia cinnamomea in BALB/c mice. In Vivo 27(6): 739-745.

14. Huang CC, Nam MK, Tsai YT, Lan A (2014) Evaluation of the Sub-Chronic Toxicity and Teratogenicity of Antrodia cinnamomea mycelia. Life Sci J 11(11): 1090-1098.

15. Lin YH, Kumar KJS, Vani MG, Liao MG, Lin CC, et al. (2016) In vitro and in vivo toxicological assessments of *Antrodia cinnamomea* health food product (Leader *Antrodia cinnamomea* Capsule). Fundamental Toxicological Sciences 3(5): 205-216.

16. Lin CC, Kumar KJS, Liao JW, Kuo YH, Wang SY, et al. (2015) Genotoxic, teratotoxic and oral toxic assessments of *Antrodia cinnamomea* health food product (Leader Deluxe *Antrodia cinnamomea* (R)). Toxicol Rep 2: 1409-1417.

17. Lo CP, Chen YY, Lin CC, Kumar KJS (2016) Genotoxicity, Acute and Sub-Chronic Toxicity Studies of Solid-State Cultivated Mycelial Powder of *Antrodia cinnamomea*. Advances in Clinical Toxicology 1(1): 1-10.

18. Euler DE, Guo H, Olshansky B (1996) Sympathetic influences on electrical and mechanical alternans in the canine heart. Cardiovasc Res 32(5): 854-860.

19. Fox PR, Sisson D, Moïse NS (1999) Textbook of canine and feline cardiology: principles and clinical practice. 2nd (Edn.), Philadelphia; London: Saunders. 10(6): 955.