Research article

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The Chronic Toxicity Studies of Camellia Seed Oil Containing Tea Saponins on Mice Blood and Organs

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ABSTRACT

Tea saponins are normal non-ionic surfactants with surface-dynamic (surfactant) properties, isolated from the *Camellia oleifera* seed. These saponins are more prominent than 10% of the camellia seed and are used as a natural tenside that is thoughtful to the environment. Camellia seed oil containing tea saponins was used as the main material. Three experimental groups were used: Low, Medium, and High groups with different doses. 30 days feeding in mice experiment. Mice blood and organs were used to analyze the chronic toxicity among the experimental rats in 30 d and olive oil used as a control group. The study showed that, there were significant differences in some index and there were no vital changes in the other indicators. The analysis of the results indicates that, camellia oil containing tea saponins have some toxic effects in functions of viscera, peripheral blood in mice. The results provides a theoretical basis for the utilization and safety of camellia oil. The chronic toxicity of camellia oil containing tea saponin was not strong, feeding on mice in a high dose (255 mg/kg·bw low tea saponin/oil) for 30 days each viscera and other features and growth have no significant impacts.

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Introduction

Camellia oil coming from Camellia oleifera, it's a natural, edible oil in China; it has been considered as a very old edible vegetable oil in southern China, contains high unsaturated fatty acids, polyphenols, vitamin E and carotene. FAO recommended it as a healthcare plant oil [1, 2], with similar properties as olive oil, can also be used as cooking oil [3]. Tea saponin was frequently disposed of with the oil cake or used as manure in the conventional oil preparation techniques. Tea saponin is viewed as a solid frothing, emulsifying,

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scattering and wetting agent, with various therapeutic uses. It has also been broadly utilized in various fields [4] such as in food, chemical, industrial, agricultural and construction materials, metallurgy and metal processing [5, 6.] Generally, saponins are amphipathic glycosides amassed phenomenologically by the cleanser like foam they give when shaken in watery arrangements, and fundamentally by having, in any event, one hydrophilic glycoside moieties got together with a lipophilic triterpene or steroid derivative [7]. It has a very high bioactive components. Previous research showed that tea saponinfoaming ability is better than fine soap [8]. The structure of tea saponin is shown in Figure1, with a molecular weight of 1222.54 [9]. This study aims to investigate the impact of camellia seed oil containing tea saponins on mice blood and organs.

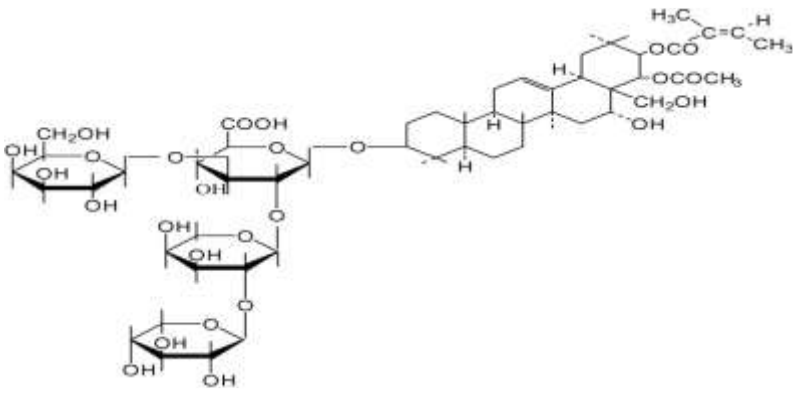

Fig 1 Structures of saponins isolated from leaves of the tea plant (*Camellia sinensis var. sinensis*) [10].

Materials and Methods

The experimental drug

96% of tea saponin of purple reagent was obtained from a factory in Shanghai, China; Qiagen integral protein, albumin kit, cereal third transaminase kits, aspirate aminotransferase kits, triglyceride kits, and total cholesterol kits all were obtained from Nanjing Science and Technology Co., China.

The experimental animal

60 healthy male Kunming mice of five weeks old were obtained, weighing 25 to 30 g specific pathogen free (SPF) were obtained from the Center for Disease Control (CDC), Hubei province, China, were used. Mice were fed with tea oil containing tea saponin for 30 days; were housed in a room with temperature (18 to 24ºC), and relative humidity (40% to 60%); all the experiments were done at College of Food Science and Technology; Huazhong University; Wuhan City, Hubei Provence.

Experimental instrument

Optical microscope (EX20) Ningbosyunny Instruments Co., Ltd., Bench top high speed refrigerated centrifuge (H1850R) Hunan Xiangyi Laboratory Instrument Development Co., Ltd., Microplate Reader (51119200), America Thermo Scientific Company and Electronic balance BS224S Sartorius analytical instrument (Beijing) Co., Ltd.

Test's methods

Animal and dose

Mice were separated into six cages, every cage containing 10. They were stamped, weighed, and fed with essential feed, with versatile feeding and observed for seven days. The mice were then arbitrarily partitioned into six groups of 10 mice each. Standard saline (control A), olive oil (control B) as control groups, tea saponin/water, 25 mg/kg b.w. (C), low, 255 mg/kg b.w. (D), medium, 382.5 mg/kg b. w. (E) and high, 510 mg/kg b.w. (F) dose group. All animal treatments were performed according to the standards and methodology illustrated in the National Institutes of Health Guide on the Care and Use of Laboratory Animals [11, 12]. The study was endorsed and inspected by Institutional Animal Welfare and Research, Ethics Committee guidelines of Huazhong Agricultural University, Wuhan, China (Approval number: 31273519).

All mice were fed *ad libitum*; they were given an intra-gastric administration every morning at 8:00–9:00 once a day for 30 days. Control A was given standard saline; control B was given olive oil (0.01 mL each). The other groups were given the test samples (tea oil containing tea saponin) 28, 255, 382.5, and 510 mg/kg.bw, respectively. The mice were weighed every week and the activity of the mice, feeding, presence of poisoning and death were observed every day, and drug dose was balanced by body weight.

Blood measurement

Method of Abliz et al. [13] was adopted with some modifications. Mice treated for 30 days, and fasted one day before the end of the trial. On the last day, the mice weighed and fixed. The blood was collected from their eyes. The blood was allowed to remain at room temperature for 0.5 hr, later was centrifuged at 3000 rpm for 10 min. Serum was pipetted for serum lipid concentration estimation, the serum blood was determined using the automatic blood cell analyzer [14-19].

Mice organs measurement

After blood collection, the liver, spleen, and kidney immediately taken, and every each organ, washed with super cold saline; blotted on filter paper and weighed, the organ's indexes were indicated by the accompanying formula:

$$\text{Organ index\%} = \left(\frac{\text{organ weight(g)}}{\text{animal weight(g)}}\right) * 100$$

The organs washed and dried, and three examples of every one taken. These were rapidly placed into glass vials loaded up with formaldehyde 4%, cooled to 4°C, for histopathological examination [12]. Serum was utilized in biochemical assays. Triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) were determined using TG kit, TC kit, HDL kit and LDL kit (Beijing wantai DRD CO., LTD., Beijing, PR China) [20].

Statistical analysis

The analysis of variance (ANOVA) was used to compare the means. Analyses were carried out using IBM® SPSS® Statistics V25 statistical software to compare treatment groups, and observations at $P<0.05$ were considered statistically significant.

Results

The general status and body weight of mice

Daily changes in mice were observed before and after lavage signs; drinking water, and feeding, activities. Fine lavage tea saponin/camellia oil for each group of mice, and olive oil, movement is not active, to eat and drink is compared with the normal group and tea

saponin/water are generally low, and lavage mice before and after the change is not obvious, the entire process of experimental mice were no poisoning or death situation.

All the mice were given same diet throughout the study, and there was difference between the different treatment groups in dose. In the process of experiment, tea saponin dose/camellia oil, olive oil group and normal group, tea saponin/aqueous solution are in the trend of steady growth. The body weight of mice and rats weight growth stage before the experiment, the normal growth conform to animals, each dose group mice weight there was no significant difference in comparison with other groups.

Hemogram and histological results

From Table 1 it can be observed that, during the period of feeding, according to the records of each food intake in mice, it was observed that the tea saponin/camellia oil food utilization rate of each dose group compared with normal group found that, there was no significant difference.

Table 2 and 3, showed the experimental results of tea saponin in each dose group compared with the control. The LYM, MONO, NEU, and EO figures of the treated samples were statistically ($P > 0.05$) not different from the control (Table 2), the result of each index was within the normal range. In the same manner WBC, and HGB treated samples were statistically ($P > 0.05$) not different from the control, while RBC, and PLT figures of the treated samples were statistically ($P > 0.05$) different from the control (Table 3). The effects of tea saponins of camellia oil on blood lipids in mice are shown in Table 4. In comparison with control group A, TC and TG were the important index reflecting the body lipid metabolism. Table 5 shows the effect of tea saponins from camellia oil on Alanine transaminase (ALT), Aspartate transaminase AST, and Total protein (TP) in serum of mice for 30 days to assay the toxicity. Table 6 shows the weight/body of the liver, spleen, and kidney ascertained; and compared according to the experimental results. Figure 1 -3, shows no adverse effects of camellia oil containing tea saponin of all mice organ's kidney, liver and spleen physiology compared with control groups.

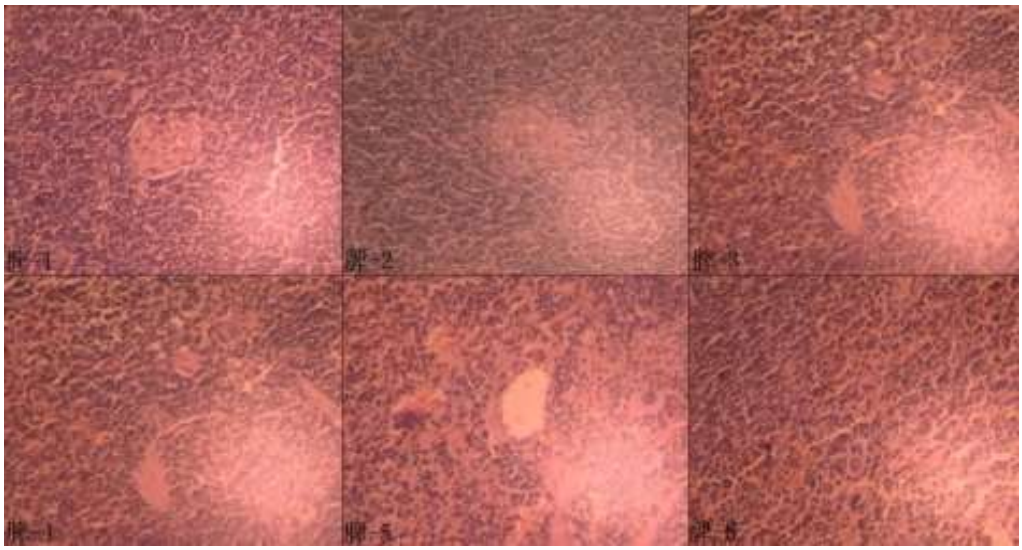

Fig 2. Histological observation of kidney from mice feed tea saponins of camellia oil. *Where are: 1: Normal control; 2: TS/ aqueous solution; 3: Low dosage of TS/ camellia oily solution ; 4: Medium dosage of TS/ camellia oily solution ; 5: High dosage of TS/ camellia oily solution ; 6:Olive oil* **.**

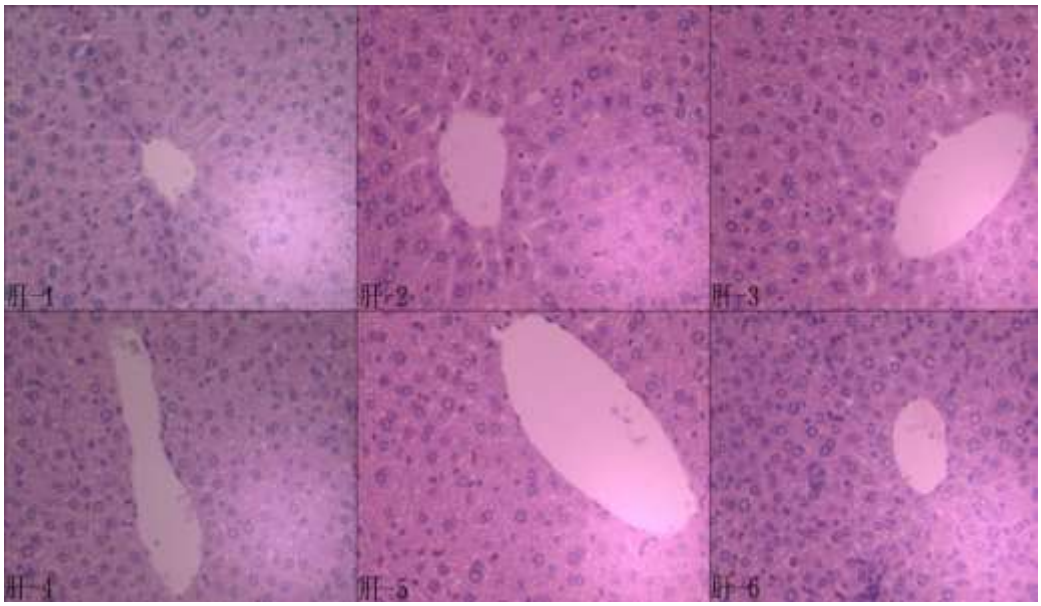

Fig 3. Histological observation of Liver from mice feed tea saponins of camellia oil. Where are: 1: Normal control; 2: TS/ aqueous solution; 3: Low dosage of TS/ camellia oily solution; 4: Medium dosage of TS/ camellia oily solution; 5: High dosage of TS/ camellia oily solution; 6:Olive oil .

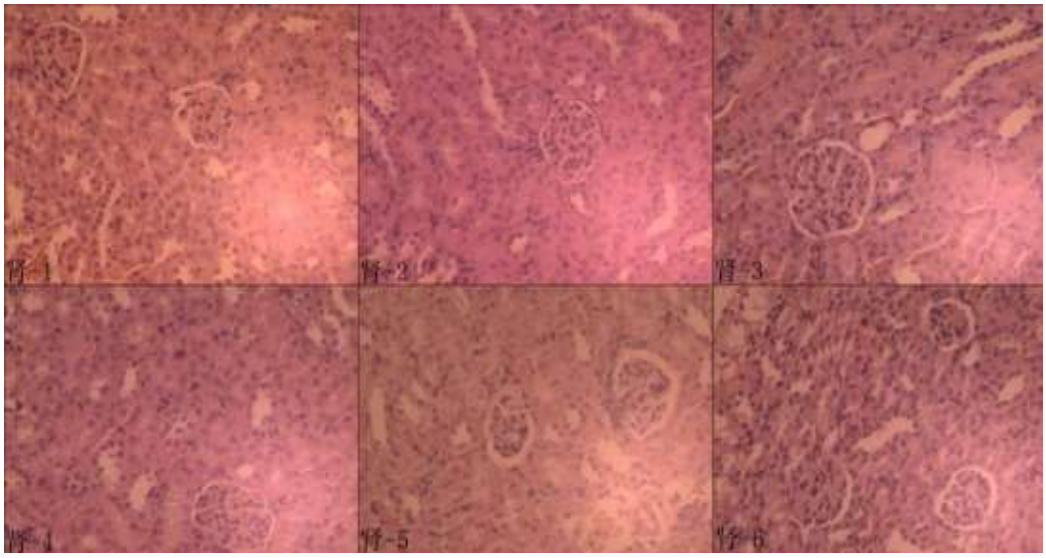

Fig 3. Histological observation of Spleen from mice feed tea saponins of camellia oil. Where are: 1: Normal control; 2: TS/ aqueous solution; 3: Low dosage of TS/ camellia oily solution ; 4: Medium dosage of TS/ camellia oily solution ; 5: High dosage of TS/ camellia oily solution.

Discussion

Body weight and food utilization

From Table 1, the weight of mice was increased each day after tea oil saponin treatment. No changes in eating, drinking and exploratory behavior have seen in the mice treated with tea oil saponin. The body weight plot demonstrated that, the weights of the mice were decreased and the tea oil saponin-treated groups were differ significantly when compared with control groups ($p>0.05$). This proposed the tea oil saponin did not make any unfriendly impact to the body weight of the mice. Nevertheless, the body weight of tea oil saponin-treated groups did not differ significantly when compared with each other ($p > 0.05$). The observed body weight loss in experimental groups may be due to appetite affected by the tea oil saponin, leading to decreased food consumption. The feed utilization ratio in groups treated with tea oil saponin (255, 382.5 and 510 mg/kg) decreased significantly when compared to the control group ($p<0.05$). Tea oil saponin had no effect on organ tissue histopathology compared to control groups

Table 1 Effects of tea saponins in camellia oil on food utilization ratio in mice

Group and dosage	Weight changes (g)	Food utilization ratio (%)
Control A	4.34[a]	14.42[a]
Olive oil control B	0.42[b]	7.74[b]
28 mg/kg·bw tea saponin/water	3.76[c]	11.48[c]
255 mg/kg·bw low tea saponin/oil	3.11[d]	8.86[d]
382.5 mg/kg·bw med. tea saponin/oil	3.01[d]	8.04[d]
510 mg/kg·bw high tea saponin/oil	3.22[d]	8.19[d]

Data are expressed as mean ± SD and analyzed by one-way ANOVA followed by Dunnett's test for each parameter separately.The numbers with different letters in columns represent the data has significant difference from control group

Peripheral hemogram

In Tables 2 ad 3, each dose group inside the treated groups, there were no significant differences, which can be concluded that, camellia oil containing tea saponin has no effect on lymphocytes in mice. However, all the haematological values in all treated groups were still within the normal range and the alteration had no clinical significance. These results are comparable with our recent results [11], where tea oil saponin fed to mice for 90 days showed no significant difference on histopathological values when compared with control groups.

Table 2 Hematological parameters (peripheral hemogram) of mice treated orally with different doses of tea saponins in camellia oil for 30 days

Group and dosage	LYM (109/L)	MONO (109/L）	NEU（109/L）	EO(109/L）
Control A	1.76±0.26 [a]	0.01±0.01 [a]	0.11±0.01[a]	0.02±0.01[a]
Olive oil control B	2.14±1.51 [a]	0.37±0.17 [b]	0.22±0.17[b]	0.02±0.02[a]
28 mg/kg·bw tea saponin/water	2.19±0.07 [a]	0.01±0.01[a]	0.13±0.03[c]	0.01±0.01[b]
255 mg/kg·bw low tea saponin/oil	2.19±0.62 [a]	0.03±0.02[a]	0.15±0.06[c]	0.01±0.01 [b]
382.5 mg/kg·bw med. tea saponin/oil	2.34±0.68[b]	0.02±0.02[a]	0.13±0.11[c]	0.02±0.01[a]
510 mg/kg·bw high tea saponin/oil	2.25±0.21[a]	0.01±0.00[a]	0.14±0.02[c]	0.02±0.01[a]

Data are expressed as mean ± SD and analyzed by one-way ANOVA followed by Dunnett's test for each parameter separately, LYM: lymphocytes, EO: eosinophils, NEU: neutrophils, BA: basophiles, MONO: monocytes. The numbers with different letters in columns represent the data has significant difference from control group

Table 3 Effects of tea saponins in camellia oil on peripheral hemogram in mice

Group and dosage	WBC (109/L)	RBC(1012/L)	PLT(109/L)	HGB (g/L)
Control A	2.13±0.29[a]	8.51±0.36[a]	809.00±59.65[a]	149.75±1.89 [a]
Olive oil control B	3.90±0.07[b]	9.21±0.20[b]	534.50±338.18[b]	146.00±0.00[a]
28 mg/kg·bw tea saponin/water	2.33±0.06 [a]	8.60±0.27 [a]	820.33±15.89[c]	149.60±2.51[a]
255 mg/kg·bw low tea saponin/oil	2.20±0.14 [a]	9.18±0.51[b]	793.00±69.46[d]	155.50±9.47[b]
382.5 mg/kg·bw med. tea saponin/oil	2.37±0.15 [a]	8.97±0.38[c]	749.67±35.70[e]	151.40±5.55[a]
510 mg/kg·bw high tea saponin/oil	2.25±0.07 [a]	9.39±0.12[d]	698.50±16.26[f]	150.50±3.54[a]

Data are expressed as mean ± SD and analyzed by one-way ANOVA followed by Dunnett's test for each parameter separately. WBC: white blood corpuscles RBC: red blood corpuscles, HGB: hemoglobin, PLT: blood platelet count. The numbers with different letters in columns represent the data has significant difference from control group

Serum lipids

Table 4. in comparison with control group A, TC and TG were the important index reflecting the body lipid metabolism. All measured parameters were dose dependent and has not significant impact, which can explain the effect of camellia oil containing tea saponin on mice lipid metabolism. These results are in good agreement with Ahmed et al. [11] who extracted tea saponin using different techniques and studied its effect on mice lipid metabolism, they reported the same findings. Results showed lipid profile total cholesterol had decreased in various degrees, and significant differences were recorded between dosed and control groups. However, TG did not reveal any significant alteration.

Table 4 Effects of tea saponin in camellia oil on blood lipids of mice

Group and dosage	TC（mmol/L）	TG (mmol/L)
Control A	1.92±0.05[a]	0.25±0.10
Olive oil control B	1.96±0.03[b]	0.25±0.05
28 mg/kg·bw tea saponin/water	1.88±0.05[c]	0.23±0.20
255 mg/kg·bw low tea saponin/oil	1.90±0.04[d]	0.25±0.10
382.5 mg/kg·bw med. tea saponin/oil	1.89±0.05[e]	0.24±0.08
510 mg/kg·bw high tea saponin/oil	1.92±0.05[a]	0.25±0.02

Data are expressed as mean ± SD and analyzed by one-way ANOVA followed by Dunnett's test for each parameter separately TC: total cholesterol, TG: total triglyceride. The numbers with different letters in columns represent the data has significant difference from control group.

Serum AST, ALT and TP

From Table 5 it was clear that, the doses of 28, 255, 382.5 and 510 mg/kg/bw increased the ALT, and AST, levels in serum compared with the two control groups; while there these doses decreased the TP levels in serum compared with the two control groups; olive oil group and normal control group of extremely significant differences ($P < 0.01$). This result is in good agreement with our previous results [11] which, demonstrated that tea oil saponin increased ALT, AST activities. But olive oil group is not the main research object of this experiment; the experiment set olive oil group is just making a reference of grease, but its special circumstances, worthy of our attention and discussion. Mice in the serum total protein determination of TP, according to the results of olive oil group the low, middle and high dose group was significantly lower than control water ($P < 0.01$), due to the special control of total protein of olive oil. Generally, it was a little higher in the treated groups.

Table 5 Effects of tea saponins in camellia oil on ALT, AST and TP in mice

Group and dosage	ALT(mmol/L)	AST (mmol/L)	TP (mmol/L)
Control A	14.36±1.09^{f}	64.39±0.99^{f}	2.07±0.07^{e}
Olive oil control B	8.16±0.40^{a}	152.44±0.34^{a}	1.48±0.04^{a}
28 mg/kg·bw tea saponin/water	14.81±0.75^{d}	65.12±0.04^{d}	1.65±0.03c^{a}
255 mg/kg·bw low tea saponin/oil	14.43±0.48^{e}	64.55±0.10^{ef}	1.76±0.02^{cd}
382.5 mg/kg·bw med. tea saponin/oil	14.78±0.31^{c}	66.19±0.14^{bc}	1.60±0.03^{bc}
510 mg/kg·bw high tea saponin/oil	14.40±0.5^{ab}	67.19±0.09^{ab}	1.36±0.04b^{a}

Data are expressed as mean ± SD and analyzed by one-way ANOVA followed by Dunnett's test for each parameter separately,ALT: Alanine transaminase, AST: Aspartate transaminase, TP: Total protein. The numbers with different letters in columns represent the data has significant difference from control group.

Organ indexes

Following 30 days of administrations, the mice sacrificed and the significant organs were gathered. The organ indexes (organ weight/body weight) of the liver, spleen, and kidney ascertained; and compared according to the experimental results (Table 5). The outcome demonstrated that the tea oil saponin had undeniable impact on the organ indexes. No significant difference was detected between the control and treated groups on kidney, and spleen but on liver significant differences were detected between two control groups and treated groups. Livers were bigger in all groups compared with other organs. These findings disagreed with the results of Yoshikawa et al. [21] who studied the inhibitory activity of camellia saponins extracted, from the seeds of *Camellia japonica* on mice, they reported no obvious changes in the body weight, organ index and biochemical parameters.

Table 6 Effects of tea saponins in camellia oil on visceral index in mice

Group and dosage	Liver-index (mg/g)	Renal index (mg/g)	Spleen-index (mg/g)
Control A	44.03±0.19[a]	12.23±0.09[a]	3.78±0.03[a]
Olive oil control B	38.35±0.22[b]	9.86±0.02[a]	4.48±0.02[a]
28 mg/kg·bw tea saponin/water	40.84±0.21[c]	11.28±0.04[a]	3.50±0.02[a]
255 mg/kg·bw low tea saponin/oil	43.29±0.14[d]	10.37±0.03[ad]	3.43±0.02[b]
382.5 mg/kg·bw med. tea saponin/oil	40.00±0.20[ce]	10.46±0.03[ac]	3.16±0.04[cd]
510 mg/kg·bw high tea saponin/oil	40.00±0.16[ce]	10.92±0.04[ab]	3.19±0.01[c]

Data are expressed as mean ± SD and analyzed by one-way ANOVA followed by Dunnett's test for each parameter separately. The numbers with different letters in columns represent the data has significant difference from control group

Mice organs histopathological results (kidney, liver, spleen)

Anatomy of mice organs (liver, kidney, spleen and heart) was observed by the naked-eye; it was found that, no differences between the control groups and doses groups concerning edema, hyperplasia, and atrophy lesions, with bright-color appearance, as the normal organs and without any hitopathological changes. Finally, based on the corresponding viscera, mainly by the formalin preserved and dyeing embedding tissue section using the optical microscope in each slice observation, above for the observations.

Figure1 shows kidney histopathological tissue in Kunming mice, the performance of kidney tissues in the doses groups was roughly same as control groups with no significant difference, where there was clear renal tubular structure, complete epithelium; no crystals within the lumen, also no any inflammatory cells. Figure 2 shows liver pathological tissue in Kunming mice, the figure showed an ordinary liver tissue, no changes in the appearance of the controls and dose groups. There were big nucleus, neat veins closely surrounding cells tissue, and regular lobular, beside no cavitations, no abnormal accumulation or precipitation. Generally, the dose groups showed a normal liver tissue, fatty degeneration also not found in the liver [16] and no toxic effects. Figure 3 shows spleen histopathological tissue in Kunming mice, the figure showed tissue morphology; all groups were characterized by clear organizational structure, some big volume and irregular polygonal cells, scattered distribution in the edge area of the spleen cells, red pulp spleen sonne and lymph follicle germinal center can be clearly seen. All treatment groups compared with control groups showed as normal, and there was no significant difference.

So as the general results, camellia oil containing tea saponin of all mice organ's kidney, liver and spleen physiology without adverse effects.

The study indicates that, the appearance of the mouse performance is normal, but in food, intake and body weight changes exist certain differences. The weight of mice showed normal speed growth, prove that camellia oil containing tea saponin have little impact on weight and food utilization in mice. AST and ALT in each group were no significant differences between groups; so that tea saponin in camellia oil didn't damage liver cells, so its non-toxic . TC and TG is one of the important indices for detecting small body lipid metabolism. The results prove that camellia oil containing tea saponin in mice don't disruptive effects of lipid metabolism. Sung et al [22] investigated the effects of saponin extracted from *Asparagus cochinchinensis* on the weights of eight organs and seven urine factors. Their results indicated no significant differences in the weights of any of the organs, while significant alterations were observed in ALT, AST, and LDH.

In conclusion, the results of the present study suggest that feeding on mice in high dose 30 days each viscera and other features and growth are not a significant impact. This study confirmed no toxicity for tea saponin when using tea saponin less than 255mg/kg.bw and it was dose-dependent. Furthermore, these findings provide vital information regarding the histopathological studies which, revealed that, all treatment groups compared with control groups showed no significant difference.

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