Camellia oleifera seed extract attenuated abdominal and hepatic fat accumulation in rats fed a high-fat diet

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Title

Camellia oleifera seed extract attenuated abdominal and hepatic fat accumulation in rats fed a high-fat diet

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Abstract

The objective of this study was to evaluate the effects of the hot-water extract of defatted *Camellia oleifera* seeds (CSE) on body and liver fat accumulation in rats. Forty rats were divided into five groups and each group was fed either an isocaloric control diet (C) or a high-fat liquid diet with 0% (H), 0.12% (H1), 0.24% (H2), or 0.48% CSE (H3) for 8 weeks. Ingestion of the high-fat liquid diet increased abdominal and liver fat accumulation, although no difference was found in body weights compared to rats fed the control diet. We found that rats fed the H2 and H3 diets had lower plasma alanine aminotransferase activities than the H group in the 4th and 8th weeks. At the end of the study, the H2 and H3 groups also had lower epididymal and retroperitoneal fat masses, and all CSE groups had lower circulatory leptin levels than the H group. CSE consumption decreased hepatic fat accumulation in terms of liver triglycerides and a histopathology analysis, and ameliorated high-fat diet-induced elevation of hepatic tumor necrosis factor- α levels. We also found that CSE groups had lower malondialdehyde and hydroxyproline levels in the liver. Our results suggested that CSE may exert beneficial effects through decreasing body fat accumulation and hepatic steatosis and regulating adipokine levels in diet-induced non-alcoholic fatty liver disease.

Keywords: camellia; abdominal fat; adipokine; fatty liver; inflammation

1. Introduction

Obesity is a major health problem currently, and excess intake of energy and fat leads to increases in adipose tissue and also ectopic fat adiposity, especially in the liver. Non-alcoholic fatty liver disease (NAFLD) is now a common cause of chronic liver disease, and the prevalence of NAFLD continues to increase worldwide and is related to insulin resistance, obesity, and metabolic syndrome (Pappachan et al., 2017; Seidell, 2005). Body fat accumulation, fatty liver, and insulin resistance play important roles in the progression of NAFLD from fatty liver to hepatosteatosis, and even fibrosis. Overconsumption of dietary lipids leads to abnormal lipid metabolism and increased hepatic fat accumulation, which activates the expression of cytochrome P450 2E1 (CYP2E1) and increases oxidative stress and inflammatory responses *in vivo* (Jiang et al., 2016; Johann et al., 2006). In addition to the increase in hepatic cytokine levels, adipose tissue can also release adipokines which have certain physiological effects. A high-fat diet and increased adipose tissues, especially abdominal adipose tissue, may lead to an imbalance of adipokine excretion (Falcao-Pires et al., 2012). Elevation of the circulating leptin concentration and a reduction in the adiponectin concentration are related to insulin resistance, secretion of proinflammatory cytokines, and further cardiovascular complications (Ntaios et al., 2013).

The tea tree is one of the most important crops in Taiwan. *Camellia oleifera* Abel. is traditionally used to produce tea seed oil, and the pomace is used as a detergent and traditional remedies (Ye et al., 2013). Recent studies reported that tea leaves, flower buds, and seeds exhibit antioxidative, hypoglycemic, hypolipidemic, and antiobesity activities due to their phenolic, saponin, and polysaccharide contents (Kuo et al., 2010;

Wang et al., 2013; Zhang et al., 2012). However, the physiological activities of seed extract still remain very few and unclarified. Therefore, we used a rat model with high-fat-liquid-diet-induced NAFLD (with fat as approximately 70% of the total energy) to investigate the effect of the hot-water extract of defatted *C. oleifera* seeds (CSE) on abdominal and hepatic fat accumulation, and related cytokine concentrations.

2. Materials and Methods

2.1. Preparation of *Camellia oleifera* seed extract (CSE)

The defatted seed pomace of *Camellia oleifera* was collected from oil factories in Nantou County, Taiwan. Seed pomace of *C. oleifera* was defatted with n-hexane and grounded into powder. Ten grams of the powder was extracted with 100 mL of hot water (90~100°C) 3 times for 1 h each. The extracted solution was then filtered, and the filtrate was lyophilized and ground into powder as our CSE sample. Total phenolic contents of the CSE was detected with Folin-ciocalteu reagent and the blue complex was detected spectrophotometrically at 735 nm using gallic acid as the standard (Dicko et al., 2002). Total saponin contents was detected at 550 nm spectrophotometrically with vanillin and sulfuric acid and using diosgenin as the standard (Hiai et al., 1976). Total polysaccharide content was determined using an UV spectrometry method as previously reported (Albalasmeh et al., 2013). We found that CSE contains 81.3 ± 0.6 mg/g phenolic compounds, 206.3 ± 8.7 mg/g total saponins and $6.3 \pm 0.4\%$ total polysaccharides.

2.2. Experiment design

Forty male Wistar rats (200~250 g) were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Investigators at the Taipei

Medical University Laboratory Animal Center followed protocols described in the “Guide for the Care and Use of Laboratory Animals” (Approval ID: LAC-2014-0294). Animals were fed a standard rat chow diet (Rodent Laboratory Chow 5001, Purina Mills, St. Louis, MO, USA) for 1 week, and then fed a control liquid diet for 1 week for adaptation. Then, 32 rats were assigned to the experimental groups with a high-fat diet (carbohydrate as 11%, protein as 17%, and fat as 72% of the total energy) (Lieber et al., 2004) with different amounts of CSE (H group, high-fat diet, n=8; H1 group, high-fat diet + 0.12% CSE, n=8; H2 group, high-fat diet + 0.24% CSE, n=8; H3 group, high-fat diet + 0.48% CSE, n=8), and the remaining 8 rats were assigned to a control group (C group, n=8) and fed an isocaloric control diet for 8 weeks. The doses of CSE used were according to effectiveness of tea seed saponins reported previously (Ye et al., 2013). Food and water were provided with free access. Food intake was recorded every day, and the body weight (BW) was recorded every week. After an 8-wk experimental period, rats were sacrificed to collect blood, liver, and adipose tissues. To evaluate the effects of the CSE on abdominal fat accumulation, we collected the retroperitoneal and epididymal adipose tissues and weighed them.

2.3. Blood collection and analysis

Blood samples were collected from the tail vein every 4 weeks to determine alanine aminotransferase (Looi and Walterfang, 2013) and aspartate aminotransferase (Falcao-Pires et al., 2012) levels. After 8 weeks, rats were sacrificed after being anesthetized with sodium pentobarbital. Blood samples were collected from the inferior vena cava into tubes containing an anticoagulant. Blood samples were immediately centrifuged, and the plasma was stored at -80°C until being analyzed. Plasma total

cholesterol (TC), triglyceride (TG), free fatty acids (FFAs), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined using a Hitachi 7170 autoanalyzer (Tokyo, Japan). Plasma glucose (Randox Laboratories, Antrim, UK) and insulin (Mercodia Rat Insulin ELISA, 10-1250-01, Uppsala, Sweden) levels were determined using commercial kits. To determine malondialdehyde (MDA) levels, plasma samples were measured by the thiobarbituric acid-reactive substance (TBARS) method (Urias-Silvas et al., 2008). Plasma leptin (Mouse and Rat Leptin ELISA, RD291001200R, Biovendor, Brno, Czech Republic) and adiponectin (AssayMax Rat Adiponectin ELISA, ERA2500-1, Assaypro, MO, USA) levels were also determined using commercial kits.

2.4. Liver analysis

After being perfused with saline, livers were collected and stored at -80°C. TC, TG, and FFA concentrations in the liver were determined using diagnostic kits (Randox Laboratories). To determine MDA levels, the liver was homogenized in buffer containing 0.25 M sucrose, 1 mM EDTA, and 10 mM Tris-HCl, and determined as previously described [15]. To determine tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, and IL-10 concentrations in the liver, samples were homogenized in buffer containing 50 mM Tris-base, 150 mM NaCl and 1% Triton-X 100. After centrifugation, the supernatants were measured with an enzyme-linked immunosorbent assay kit (ELISA; rat TNF- α /TNFSF1A, Rat IL-1 β , and Rat IL-6, DuoSet® ELISA Development System; R&D Systems, MN, USA). To measure the hydroxyproline concentration, an early indicator of fibrosis, the liver was homogenized in distilled water. After centrifugation,

the supernatant was hydrolyzed with 4 N NaOH at 120°C, reacted with chloramine T and Ehrlich's reagent, and determined at 550 nm.

2.5. Fecal analysis

Fecal samples were collected for 1 day in week 8, dried for 48 h, and stored at -80°C until being analyzed. Fecal lipids were extracted with chloroform and methanol. Fecal bile acid was extracted with methanol. Fecal cholesterol and bile acid concentrations were analyzed with commercial kits (Randox Laboratories). Total lipids were determined using commercial kits (Fortress BXC0263, Northern Ireland, UK).

2.6. Histopathological analysis

The dissected livers of rats were fixed in formaldehyde. Samples were stained with hematoxylin and eosin (H&E). Biopsies were examined by a pathologist on a blinded basis. To evaluate the fatty change, the liver was observed at $\times 200$ magnification with a scale. Degrees of fatty liver were assessed by a pathologist (Brunt, 2007).

2.7. Statistical analysis

Data are reported as the mean $\pm$ standard error of the mean (SEM). Statistical analyses were performed using the SAS software (version 8.2; SAS Institute, Cary, NC, US). Data were analyzed by a one-way analysis of variance (ANOVA) and Fisher's least significant difference test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. BW and food intake

Rats were fed isocaloric control or high-fat diets for 8 weeks. No difference was found in the food intake, final BW, or BW change among all groups (Table 1). The

results indicated that no dose of CSE consumed in this study affected the growth or appetite of rats.

3.2. Plasma biochemical analysis

The liver function of rats was determined by plasma AST and ALT activities (Fig. 1). No differences in AST or ALT levels were found at the baseline. Group H had higher AST and ALT activities than the C group from the 4th week to the end of the study. Groups H2 and H3 both had lower AST and ALT activities compared to the H group from the 4th week to the end of the study. We found no differences in fasting plasma glucose, insulin, or MDA concentrations among all groups. Rats fed the high-fat diet had a significant higher plasma leptin level and lower plasma adiponectin level compared to the control group, while rats consuming the diets containing 0.24% and 0.48% CSE showed no differences in either leptin or adiponectin levels compared to the control group (Table 2). The H group also showed a higher plasma FFA level than the C group when consuming the CSE.

3.3. Liver steatosis and inflammation

We found a greater liver weight (Table 1) and hepatic cholesterol level (Table 3) in the H group than in the C group, and no differences were found among the H, H1, H2, and H3 groups. Hepatic TG, MDA, TNF- α , IL-1 β , and hydroxyproline levels were higher in the H group than in the control group. All CSE groups had lower hepatic hydroxyproline levels than the H group. Rats consuming 0.48% CSE had significant lower TG and MDA levels compared to the H group and had no difference in MDA, TNF- α , or hydroxyproline concentrations compared to the C group.

3.4. Histopathology analysis

In the histopathological analysis, the C group shows normal histology while the H group reveals a picture of steatohepatitis with macrovesicular and microvesicular steatosis, and CSE consumption ameliorated high-fat-diet-induced steatosis (Fig. 2). In the fatty change score analysis, the H group was significantly higher than the C group, and all CSE consuming groups were significantly lower than the C group.

3.5. Abdominal fat accumulation

We collected epididymal and retroperitoneal fat tissues of rats to evaluate the abdominal fat accumulation. We found that the H group had higher epididymal and retroperitoneal fat masses than the control group. Both the H2 and H3 groups had lower epididymal and retroperitoneal fat masses than the H group, and there were no differences in abdominal fat compared to the C group (Table 1).

3.6. Fecal lipid excretion

At the end of the study, we collected feces for 24 h and measured the fecal lipid excretion to understand the mechanisms of CSE's effects. We found an increase in total lipid excretion only in the H3 group compared to the H group. No differences were found in bile acid or cholesterol excretion in any CSE intervention group compared to the H group.

4. Discussion

C. oleifera seeds are traditionally used as a source of dietary oil, and the defatted pomace is used as a detergent or is discarded. However, studies on the physiological effects of CSE are very few and limited. Recent studies have reported that *C. oleifera* seed extract may show antioxidative and anti-inflammatory effects *in vivo* due to its saponin constituents (Wei et al., 2014; Ye et al., 2013). Although plant-derived saponins

were reported to have potentials in regulating lipid metabolism and modulating adipokine balance (Elekofehinti et al., 2017; Marrelli et al., 2016), to our knowledge, this is the first study to investigate the effects of CSE on diet-induced fat accumulation *in vivo*. During the 8-week experimental period, none of the 3 dosages of dietary CSE affected normal growth or food intake of rats, but did affect the rats' body composition. We found that the high-fat liquid diet increased epididymal and retroperitoneal fat masses, and CSE consumption decreased the abdominal fat mass while no difference in BWs was found among all groups.

Abdominal fat accumulation is related to systemic inflammation and cardiovascular risks. Recent reports indicated that abdominal adiposity rather than the body-mass index is a better predictor of an increased risk of cardiovascular mortality (Czernichow et al., 2011) and many metabolic disorders (Piya et al., 2013). Adipose tissues have endocrine functions and secrete various kinds of bioactive factors called adipokines. In obese subjects, the release of proinflammatory cytokines such as leptin, TNF- α , and IL-6 increases, and that of anti-inflammatory cytokines such as adiponectin and IL-10 decreases (Ouchi et al., 2011). Leptin production is reported to be positively correlated with the body fat mass (El-Shazly et al., 2015). Consistent with previous studies, we found that a high-fat diet caused elevation of the abdominal fat mass and circulating leptin levels, and we also found that CSE groups had less abdominal fat and lower plasma leptin concentrations. In addition, CSE also ameliorated the decrease in circulating adiponectin induced by the high-fat diet. Although the roles of leptin and adiponectin in NAFLD are still controversial, many studies showed that leptin is related to stimulation of proinflammatory actions and may have profibrogenic effects, while on the other hand,

adiponectin is related to suppression of hepatic lipogenesis and inhibition of proinflammatory cytokine release (Chen and Liu, 2017; Marra and Bertolani, 2009). In our study, we found that CSE ameliorated diet-induced fatty liver and elevations of hepatic TNF- α and hydroxyproline (an early-stage discriminator of fibrosis (Toyoki et al., 1998)) concentrations. Polysaccharides derived from the *C. oleifera* exhibited an antioxidative capacity *in vitro* (Jin and Ning, 2012), and we also found lower hepatic MDA concentrations in the CSE groups. These results suggested that CSE can reduce abdominal fat accumulation and protect against liver injury via regulating inflammatory responses and oxidative stress.

FFAs play important roles in the progression of NAFLD. The excess intake of energy and fat lead to imbalanced lipid metabolism, while excess circulating FFAs lead to lipid accumulation in the liver and insulin resistance (Liu et al., 2010). In the present study, rats fed the high-fat diets had higher circulatory FFA levels. Although we did not find differences in plasma lipid profiles among the high-fat control and CSE groups, we found that CSE consumption significantly lowered circulating FFA levels. Excess FFAs also stimulate the excretion of inflammatory cytokines such as TNF- α , IL-1 β , and IL-6 through activation of the nuclear factor (NF)- κ B pathway (Dowman et al., 2010), which may ultimately lead to insulin resistance and cause liver injury (Piya et al., 2013). The results of our liver tissue analysis and histopathological analysis both showed that CSE can ameliorate hepatic steatosis, and both the H2 and H3 groups had lower plasma AST and ALT activities than the H group from the 4th week on. These results suggest that the CSE may be hepatoprotective via regulation of FFAs. However, we found no difference in food intake and only a significant elevation of fecal lipid excretion in the H3 group.

Therefore, the effects of CSE on abdominal and hepatic adiposity cannot completely be explained by the increase in lipid excretion.

TNF- α is an important factor in obesity-related proinflammatory responses and insulin resistance (Meriga et al., 2017; Stanley et al., 2011). In the present study, we found that CSE consumption reduced lipid peroxidation in a dose-dependent manner and ameliorated high-fat diet-induced elevation of hepatic TNF- α and hydroxyproline levels. However, no difference was found in fasting plasma glucose or insulin concentrations among all groups, and this may have been because the duration of the experiment was not long enough for rats to show diet-induced insulin resistance. These results showed that CSE can decrease the inflammatory response and hepatic injury in the early stage of diet-induced obesity. Future studies are needed to clarify the effects of long-term consumption of the CSE on obesity-related metabolic disorders.

5. Conclusion

In this study, we first investigated the effects of the CSE on high-fat diet-induced fat accumulation *in vivo*. We concluded that the CSE exerts beneficial effects through decreasing body fat accumulation and hepatic steatosis and regulating adipokine levels in diet-induced NAFLD. Future studies can evaluate the effects of CSE over longer experimental periods and clarify the possible underlying mechanisms, and the results of this study may be useful in the development of functional food products.

6. Conflict of interest

The present study was funded by the National Science Council (NSC 102-2320-B-214-005, Taipei, Taiwan). Neither author has any other financial conflicts of interest, and all authors adhered to the Committee on Publication Ethics guidelines on research and publication ethics.

7. Abbreviation

CSE	<i>Camellia oleifera</i> seeds
NAFLD	non-alcoholic fatty liver disease
CYP2E1	cytochrome P450 2E1
TG	triglycerides
FFA	free fatty acids
HDL-C	high-density lipoprotein-cholesterol
LDL-C	low-density lipoprotein-cholesterol
AST	aspartate aminotransferase
ALT	alanine aminotransferase
MDA	malondialdehyde
TBARS	thiobarbituric acid-reactive substance
TNF	tumor necrosis factor
IL	interleukin

H&E	hematoxylin and eosin
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ANOVA	analysis of variance
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Tables

Table 1. Body weight, food intake, liver weight, and abdominal fat mass

	C	H	H1	H2	H3
Body weight (0 wk, g)	252.6±7.6	256.0±8.8	253.7±8.1	252.2±9.1	247.5±7.0
Body weight (8 wk, g)	539.1±13.3	558.3±15.4	596.6±16.3	545.1±17.0	549.7±13.5
Weight change (g)	286.5±14.9	302.4±13.6	315.9±15.2	292.9±15.7	302.1±9.7
Food intake(g)	53.9±0.7	52.3±1.1	52.5±0.8	52.0±0.8	52.9±0.6
Liver weight (g)	12.7±0.6 ^a	18.2±1.5 ^b	18.6±0.8 ^b	16.4±0.9 ^b	16.8±0.7 ^b
Epididymal fat mass (g)	10.2±0.8 ^c	17.5±2.0 ^a	16.2±1.1 ^{ab}	12.2±1.0 ^{bc}	12.2±1.0 ^c
Retroperitoneal fat mass (g)	16.6±1.7 ^b	24.7±2.1 ^a	22.7±1.9 ^a	18.7±1.6 ^b	15.4±1.0 ^b

Values are presented as the mean ± SEM. N group, control diet ($n=8$); H group, high-fat diet ($n=8$); H1 group, high-fat diet + 0.12% hot-water extract of defatted *Camellia oleifera* seeds (CSE) ($n=8$); H2 group, high-fat diet + 0.24% CSE ($n=8$); H3 group, high-fat diet + 0.48% CSE ($n=8$). ^{abc}Values in a row with different superscript letters significantly differ ($p < 0.05$).

Table 2. Plasma biochemical analytical results at the end of the study

	C	H	H1	H2	H3
Total cholesterol (mmol/L)	1.8±0.1	2.0±0.1	1.9±0.1	1.8±0.1	1.9±0.1
Triglycerides (mmol/L)	1.2±0.1 ^a	0.7±0.1 ^b	0.7±0.1 ^b	0.7±0.1 ^b	0.8±0.1 ^b
High-density lipoprotein-cholesterol (mmol/L)	1.6±0.1	1.7±0.05	1.6±0.05	1.6±0.1	1.6±0.07
Low-density lipoprotein-cholesterol (mmol/L)	0.2±0.0 ^b	0.4±0.04 ^a	0.3±0.0 ^a	0.3±0.0 ^a	0.4±0.0 ^a
Free fatty acids (mmol/L)	0.4±0.2 ^b	0.6±0.3 ^a	0.3±0.1 ^b	0.4±0.2 ^b	0.3±0.1 ^b
Thiobarbituric acid-reactive substances (μmol/L MDA)	7.0±0.4	7.2±0.4	7.6±0.3	7.8±0.4	7.8±0.2
Glucose (mmol/L)	5.8±0.3	5.6±0.2	5.6±0.3	5.6±0.2	5.8±0.2
Insulin (μg/L)	0.6±0.1	0.6±0.1	0.6±0.1	0.5±0.0	0.5±0.0
Leptin (ng/mL)	7.0±0.8 ^b	10.8±1.6 ^a	6.0±0.6 ^b	5.7±0.9 ^b	4.5±0.8 ^b
Adiponectin (μg/mL)	2.3±0.2 ^a	1.6±0.1 ^b	1.8±0.2 ^b	1.9±0.2 ^{ab}	2.0±0.1 ^{ab}

Values are presented as the mean ± SEM. N group, control diet ($n=8$); H group, high-fat diet ($n=8$); H1 group, high-fat diet + 0.12% hot-water extract of defatted *Camellia oleifera* seeds (CSE) ($n=8$); H2 group, high-fat diet + 0.24% CSE ($n=8$); H3 group, high-fat diet + 0.48% CSE ($n=8$). ^{ab}Values in a row with different superscript letters significantly differ ($p < 0.05$).

Table 3. Liver lipids, lipid peroxides, cytokines, and hydroxyproline concentrations at the end of the study

	C	H	H1	H2	H3
Total cholesterol ($\mu\text{mol/liver}$)	27.5 $\pm$ 1.1 ^b	47.9 $\pm$ 4.6 ^a	44.6 $\pm$ 4.8 ^a	45.4 $\pm$ 2.0 ^a	47.1 $\pm$ 1.7 ^a
Triglycerides ($\mu\text{mol/liver}$)	48.7 $\pm$ 3.7 ^c	215.5 $\pm$ 24.8 ^a	212.3 $\pm$ 14.6 ^a	191.8 $\pm$ 11.4 ^{ab}	170.8 $\pm$ 7.9 ^b
Thiobarbituric acid-reactive substances ($\mu\text{mol/g protein}$)	0.25 $\pm$ 0.01 ^c	0.31 $\pm$ 0.01 ^a	0.30 $\pm$ 0.01 ^b	0.29 $\pm$ 0.01 ^b	0.26 $\pm$ 0.02 ^{bc}
Tumor necrosis factor- α (ng/mg protein)	8.6 $\pm$ 0.4 ^b	10.9 $\pm$ 1.0 ^a	10.5 $\pm$ 0.6 ^a	9.6 $\pm$ 0.5 ^{ab}	9.2 $\pm$ 0.6 ^b
Interleukin (IL)-1 β ($\mu\text{g/mg protein}$)	33.6 $\pm$ 1.3 ^b	50.8 $\pm$ 3.2 ^a	51.3 $\pm$ 1.4 ^a	50.5 $\pm$ 2.6 ^a	51.1 $\pm$ 2.3 ^a
IL-6 (ng/mg protein)	29.5 $\pm$ 1.1	32.8 $\pm$ 3.1	30.5 $\pm$ 1.4	28.8 $\pm$ 1.4	28.8 $\pm$ 0.7
IL-10 ($\mu\text{g/mg protein}$)	9.7 $\pm$ 1.0	10.2 $\pm$ 0.5	11.9 $\pm$ 0.8	11.7 $\pm$ 1.2	12.0 $\pm$ 0.7
Hydroxyproline ($\mu\text{g/mg protein}$)	196.9 $\pm$ 10.0 ^c	313.2 $\pm$ 14.7 ^a	255.0 $\pm$ 10.8 ^b	242.7 $\pm$ 4.4 ^b	199.8 $\pm$ 16.0 ^c

Values are presented as the mean $\pm$ SEM. N group, control diet ($n=8$); H group, high-fat diet ($n=8$); H1 group, high-fat diet + 0.12% the hot-water extract of defatted *Camellia oleifera* seeds (CSE) ($n=8$); H2 group, high-fat diet + 0.24% CSE ($n=8$); H3 group, high-fat diet + 0.48% CSE ($n=8$). ^{abc}Values in a row with different superscript letters significantly differ ($p < 0.05$).

Figures

Figure 1. Plasma aspartate aminotransferase and alanine aminotransferase activities of rats fed different diets for 0, 4, and 8 weeks.

Values are presented as the mean $\pm$ SEM. C group: control diet (n=8); H group: high-fat diet (n=8); H1 group: high-fat diet + 0.12% hot-water extract of defatted *Camellia oleifera* seeds (CSE) (n=8); H2 group: high-fat diet + 0.24% CSE (n=8); H3 group: high-fat diet + 0.48% CSE (n=8). ^{ab}Values with different superscript letters at the same time point significantly differ ($p < 0.05$).

Figure 2. Histopathology of hepatic fatty change (a) (H&E stain $\times 200$) and score (b) at the end of the study.

Values are presented as the mean $\pm$ SEM. N group, control diet (n=8); H group, high-fat diet (n=8); H1 group: high-fat diet + 0.12% hot-water extract of defatted *Camellia oleifera* seeds (CSE) (n=8); H2 group, high-fat diet + 0.24% CSE (n=8); H3 group, high-fat diet + 0.48% CSE (n=8). ^{abc}Values with different superscript letters at the same time point significantly differ ($p < 0.05$).