

# Effect of a Herbal Formula Song Zhi Wan on Non-alcoholic Fatty Liver Disease in Obese Mice

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**Abstract:** *Background:* Effective treatment against non-alcoholic fatty liver disease (NAFLD) is lacking. Song Zhi Wan (SZW), a Chinese formulation medicine comprising eight herbal ingredients, has been demonstrated to confer a liver protective effect in chronic hepatitis C patients. We aimed to investigate the effect of SZW on NAFLD using a high-fat diet (HFD; 60% fat) induced obese mouse model. *Methods:* C57BL/6J mice were fed with HFD for 10 weeks, followed by daily oral administration of various dosages of SZW (low [n=6], normal [n=10], high [n=10]) or water (n=10) for 8 weeks. Another formulation of SZW (modified SZW), in which two ingredients were replaced by radish seed and barley, was tested. Serum total cholesterol and triglyceride levels, liver transaminases, and histologic steatosis were assessed. *Results:* At the end of experiment, the HFD-fed placebo mice had a mean increase in serum total cholesterol and triglyceride by 57.7% and 35.0%, respectively. HFD-fed mice receiving either SZW formulation had a smaller increase in serum total cholesterol (mean increase 7.9% – 39.4%) and a significant reduction in triglyceride (mean reduction 4.2% – 27.4%;  $P < 0.05$ ). A dosage dependent effect on serum total cholesterol and triglyceride was observed with modified SZW ( $=0.043$  and  $0.006$ , respectively). 90% of placebo mice and 59% of SZW-treated mice had severe steatosis ( $P=0.079$ ). With an escalating dosage of original SZW, there was a decreasing proportion of mice with severe steatosis ( $P=0.013$ ). *Conclusion:* SZW administration can attenuate hypercholesterolemia, reduce dyslipidemia and alleviate steatosis in HFD-induced obese mice.

**Keywords:** Dyslipidemia, Herbal Medicine, Liver Steatosis, Non-alcoholic Fatty Liver Disease, Obese Mice

## 1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is defined by excessive fat accumulation in the liver that is not a result of alcohol and other definite hepatotoxins. NAFLD is a common liver disease with increasing global health concern, with an estimated global prevalence of 25.2% [1]. It consists of a broad spectrum of diseases, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH),

which may lead to advanced fibrosis, cirrhosis or its related complications, and hepatocellular carcinoma. NAFLD has been mostly observed and studied in the Western world, with a prevalence of 24 – 46% [2]. Recently, with the improvement of living standards and changes in lifestyle and diet, the prevalence of NAFLD is also growing rapidly in Asia. A meta-analysis conducted by Li et al, has found that the pooled prevalence of NAFLD in Mainland China is 20.1% [3]. In urban cities in China like Beijing, Shanghai,

and Hong Kong, as well as in other Asian cities, the prevalence of NAFLD has been reported to be over 40% [2]. These data suggested the importance of NAFLD as a major cause of chronic liver disease (besides chronic hepatitis B) in Asia. However, the mechanism of the pathogenesis of NAFLD has not been completely elucidated, and effective treatment for NAFLD is lacking.

Song Zhi Wan (SZW), a formulation based on a Chinese herb originally developed in Hunan Province in China, is composed of 8 Chinese herbal ingredients, namely *Gardenia jasminoides* Ellis root (16% by weight), *Cudrania cochinchinensis* (14% by weight), *Poria cocos* (16% by weight), *Ilex asprella* (11% by weight), *Salvia miltiorrhiza* (12% by weight), *Cortex Lycii* (13% by weight), *Pseudostellaria heterophylla* (11% by weight), and *Amomum villosum* (8% by weight). Preclinical pharmacological data have shown that SZW can induce the production of interferon- $\gamma$  in mice and rats [4]. In phase II-III clinical trials with chronic hepatitis C patients, SZW has demonstrated a protective effect on liver, with significant normalization of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and reduction of hepatitis C virus RNA levels, as well as a good safety and tolerance profile [5, 6]. SZW has been patented in China for the treatment of chronic hepatitis C [7], and is an approved Chinese herbal medicine by the National Medical Products Administration (NMPA; formerly China Food and Drug Administration) [8]. However, data on the effect of SZW on liver function in NAFLD are lacking. As SZW possesses liver protective function in chronic hepatitis C, we hypothesized that SZW may improve NAFLD-induced liver injury. To test this hypothesis, we performed experiments with C57BL/6J mice, which have been demonstrated to be a suitable animal model to mimic human metabolic abnormalities [9]. This study aimed to evaluate the efficacy of SZW in reducing steatosis, alleviating liver damage, and regulating dyslipidemia in this high-fat diet (HFD) induced NAFLD mouse model.

## 2. Methods

### 2.1. Testing Reagents

In addition to testing the original formula of SZW (original SZW), we tested a modified formula of SZW (modified SZW), in which *Ilex asprella* and *Cudrania cochinchinensis* in the original ingredient list were replaced by two health food ingredients, radish seed and barley. Both original and modified SZW were kindly provided in powder form by NT Pharma (Hong Kong) Limited.

Dosage calculation in this animal study was based on the SZW dosage used in chronic hepatitis C patients [6]. The normal dosage of SZW in adult patients is 10g/day. Dosage estimation was performed using allometric scaling parameters, with an exponent of 0.75. Assuming an average man weighs 65 kg, we determined the normal dosage of SZW in a 50g mouse was 46.2 mg.

### 2.2. Animal Model and Study Design

Six to eight week old male C57BL/6J mice, house-bred and maintained at the Laboratory Animal Unit at The University of Hong Kong, were used as an animal model to mimic human metabolic abnormalities [9]. At the start of the experiment, mice (mean body weight: 22 g; range 19 – 24 g) were randomly group-housed (3-5 mice per cage) in a pathogen-free environment with well-controlled temperature ( $22\pm 2^{\circ}\text{C}$ ) and humidity (40 – 60%), along with a 12:12 hour light/dark cycle in the Laboratory Animal Unit, The University of Hong Kong. All mice were fed *ad libitum*. Following a period of 7 days for environmental adaptation with standard chow, the mice were randomly assigned to either be fed with HFD (Research Diets, New Brunswick, NJ; catalog number D12492), which consist of 60% fat, or its corresponding low-fat control diet (LFD; Research Diets; catalog number D12450J), with matched 7% sucrose content with HFD but with only 10% fat content. The mice were randomly divided into different groups according to the diet and intervention (Table 1). To avoid excessive use of mice, we limited the number of mice used to the minimum. Assuming a 50% difference between animal groups, a sample size of 10 mice per group would yield a statistical power of 80% with a 90% confidence level. For all groups, the mice were first fed with either LFD or HFD for 10 weeks, followed by an intervention period (with SZW or water as placebo) for 8 weeks. Each group consisted of 10 mice, except for the 2 low dose SZW groups, which consisted of 6 mice. Group 1 consisted of LFD-fed mice ( $n=10$ ), which served as the low fat normal control. Group 2 was the model control with HFD-fed mice ( $n=10$ ). Groups 3, 4 and 5 were HFD-fed mice administered with various concentrations of original SZW at low, normal or high dose respectively, and groups 6, 7 and 8 were HFD-fed mice receiving modified SZW at low, normal or high dose respectively. The normal dosage was determined at 46.2 mg SZW /50g mouse (see above). SZW was prepared as a suspension form in water and administered daily in the afternoon via oral gavage. The concentration of high dose SZW was set as twice as the concentration of the normal dosage (92.4 mg SZW/ 50g mouse) and low dose was set as half the concentration of the normal dosage (23.1 mg/ 50g mouse). As placebo, the intervention-free control mice were administered with water at a volume equivalent to that of SZW. Mice were weighed weekly, and the dosages of SZW (or water) for each mouse were weight-based. SZW was administered for a period of 8 weeks.

At baseline, (before SZW treatment/placebo), 50  $\mu\text{L}$  blood was collected from the tail vein. Mice were euthanized by intraperitoneal injection of pentobarbital at overdose (150 mg/kg) at the end of the experiment (after 8 weeks of intervention), and blood and liver tissues were collected for biochemical and histological analyses. Licenses to conduct animal experiments were obtained from the Department of Health, Hong Kong SAR Government and were subject to the provisions of the Cap. 340 Animals (Control of Experiments) Ordinance and Regulations. All animal experiments were performed in accordance with the guidelines for the use of experimental

animals by the Committee on the Use of Live Animals in the Teaching and Research, the University of Hong Kong.

**Table 1.** Diets and treatment regimens of mice in different groups.

Group number	Diet	Intervention	Intervention duration	Dosage*	Number of mice
1	Low-fat diet	Water	8 weeks	N/A	10
2	High-fat diet	Water	8 weeks	N/A	10
3	High-fat diet	Original SZW	8 weeks	Low	6
4	High-fat diet	Original SZW	8 weeks	Normal	10
5	High-fat diet	Original SZW	8 weeks	High	10
6	High-fat diet	Modified SZW	8 weeks	Low	6
7	High-fat diet	Modified SZW	8 weeks	Normal	10
8	High-fat diet	Modified SZW	8 weeks	High	10

SZW: Song Zhi Wan

\* Dosage definition: normal=46.2 mg in a 50g mouse (equivalent to a normal dosage of 10g SZW in a 65 kg man); low=half of normal dose (23.1 mg in a 50g mouse); high=double the normal dose (92.4 mg in a 50g mouse).

### 2.3. Biochemical Analysis

Serum ALT and AST were assessed using the ALT/GPT Liqui-UV and AST/GOT Liqui-UV assays according to the manufacturer's instructions respectively (both from Stanbio Laboratory, Boerne, TX, USA). Serum triglycerides and total cholesterol were analyzed using the LiquiColor Triglycerides and Cholesterol LiquiColor assays according to the manufacturer's instructions, respectively (both from Stanbio Laboratory).

### 2.4. Histologic Analysis

Liver tissue specimens were fixed in 10% buffered formalin, embedded in paraffin, cut at 5- $\mu$ m thickness and stained with hematoxylin and eosin (both from Sigma-Aldrich Inc, St. Louis, MO, USA). The degree of steatosis was assessed by a pathologist who was blinded to the experimental design and in accordance with the system described previously [10]. Fatty change was expressed as percentage of cells with steatosis and categorized as previously described: no steatosis (<5% fatty change); minimal steatosis (5 – 33% fatty change); moderate steatosis (>33 – 66% fatty change); and severe steatosis (>66% fatty change) [10].

### 2.5. Statistical Analysis

Statistical analysis was performed using SPSS 25.0 (SPSS, Chicago, IL, USA). Continuous variables were presented as the mean $\pm$ standard error of mean (SEM) and analyzed using the student t-test or the one-way ANOVA test. Categorical

variables were analyzed using the Chi-square test or the Fisher's Exact test. A *P* value < 0.05 was considered statistically significant.

## 3. Results

### 3.1. Induction of Hypercholesterolemia and Hypertriglyceridemia with High-fat Diet

Prior to the feeding with different diets, the mice assigned to have LFD and HFD had comparable body weight (22.5 $\pm$ 0.3 g vs. 22.1 $\pm$ 0.1 g, respectively; *P*=0.331). At week 0 (baseline) of intervention (i.e. after 10 weeks of differential diet and before SZW administration), mice fed with HFD (mean body weight: 44.1 $\pm$ 0.5 g) were significantly heavier than those fed with LFD (mean body weight: 29.9 $\pm$ 0.6 g; *P* < 0.001). The mean weight change after 10 weeks of differential diet in the LFD-fed mice was 7.4 $\pm$ 0.6 g, while the mean weight change in the HFD-fed mice was 22.0 $\pm$ 0.5 g (*P* < 0.001). HFD-fed mice had a significantly higher level of serum total cholesterol (129.6 $\pm$ 3.5 mg/dL) than the LFD-fed mice (79.1 $\pm$ 8.4 mg/dL; *P* < 0.001), while there was a trend of higher serum triglyceride levels in the HFD-fed mice (76.0 $\pm$ 6.1 mg/dL) than in the LFD-fed mice (41.4 $\pm$ 3.8 mg/dL; *P*=0.052).

The HFD-fed mice were then assigned to receive different forms and dosages of SZW (or water as a placebo control). At baseline (starting of SZW treatment), the body weight, serum total cholesterol and triglyceride across the different HFD-fed mouse groups receiving different treatment were well balanced and comparable (Table 2).

**Table 2.** Baseline characteristics of the mice in different groups.

Groups	1 (n=10)	2 (n=10)	3 (n=6)	4 (n=10)	5 (n=9*)	6 (n=6)	7 (n=10)	8 (n=10)
Diet	LFD	HFD	HFD	HFD	HFD	HFD	HFD	HFD
Treatment	Water	Water	Original SZW	Original SZW	Original SZW	Modified SZW	Modified SZW	Modified SZW
Dosage	N/A	N/A	Low	Normal	High	Low	Normal	High
Body weight, g	29.9 $\pm$ 0.6	43.2 $\pm$ 1.3	45.1 $\pm$ 0.9	43.3 $\pm$ 1.5	42.8 $\pm$ 1.0	46.6 $\pm$ 1.5	43.5 $\pm$ 1.9	45.6 $\pm$ 1.2
Total cholesterol (mg/dL)	79.1 $\pm$ 8.4	112.5 $\pm$ 4.2	114.5 $\pm$ 8.6	136.9 $\pm$ 5.0	127.4 $\pm$ 4.8	114.0 $\pm$ 3.8	142.6 $\pm$ 10.6	157.8 $\pm$ 14.5
Triglyceride (mg/dL)	41.4 $\pm$ 3.8	76.2 $\pm$ 9.7	75.4 $\pm$ 9.0	89.0 $\pm$ 18.7	89.1 $\pm$ 16.6	53.9 $\pm$ 4.6	73.5 $\pm$ 21.3	53.7 $\pm$ 4.3

All variables were expressed as mean $\pm$ SEM

\* 1 mouse died during the experiment (at week 5 of SZW treatment)

LFD: low-fat diet; HFD: high-fat diet; SZW: Song Zhi Wan; N/A: not applicable

### 3.2. Effect of SZW on Mice with High-fat Diet Induced Fatty Liver

At the end of the experiment, the mean body weight of the HFD-fed placebo control mice (group 2) was  $49.2 \pm 0.68$  g, which represented a 14.8% increase in weight comparing with that at the time before the treatment period (baseline). The increase of body weight in the HFD-fed control mice at the end of the experiment was significantly greater than that in the LFD-fed control mice (group 1), which had a mean increase of body weight of 3.3% ( $P=0.041$ ). There was also an increase in body weight in the HFD-fed mice receiving original SZW (mean increase: 11.3 – 11.9%) and modified SZW (mean increase: 5.1 – 12.0%). The increases in body weight in the individual treatment groups were slightly less than that in the HFD placebo group, but the differences in body weight change were not statistically significant. A dosage effect on body weight change was not observed in both the original and modified SZW groups (Figure 1). Throughout the study, we did not find any signs of adverse events and animal discomforts such as prolonged inability to eat or drink, persistent anorexia or dehydration, consistent or rapid body weight loss of >20% and lack of vitality.

In the LFD-fed control mice, the total cholesterol levels further increased by 16.9% at the end of treatment (compared with baseline), whereas the HFD-fed placebo mice had a 57.7% increase of total cholesterol at the end of treatment ( $P=0.073$ ). An increase of total cholesterol was also observed in the HFD-fed mice receiving low, normal and high dosage of

original SZW (mean increase from baseline: 25.0%, 11.5% and 21.7%, respectively). However, there were no significant differences in the mean increase in serum cholesterol between the placebo and original SZW treatment groups ( $P$  values for low, normal and high dosage were 0.143, 0.054, and 0.109, respectively; Figure 2A). For the mice treated with modified SZW (Figure 2B), the mean increase in total cholesterol levels by the end of low, normal, and high dose SZW treatment were 39.4%, 14.6% and 7.9%, respectively; with  $P$  values (compared with placebo)=0.388, 0.075, and 0.049, respectively. Overall, by comparing the placebo group and the three dosage groups of modified SZW, a dosage effect on serum total cholesterol was observed ( $P=0.043$ ).

Compared with baseline, the serum triglyceride levels of the LFD-fed mice further increased by 6.5% by the end of the experiment. The mean increase in serum triglyceride in the LFD-fed mice was significantly smaller than that in the HFD-fed placebo control mice (35.0% increase compared with baseline;  $P=0.022$ ). In all mice treated with either original or modified SZW, there was a decrease in serum triglyceride at the end of treatment. The mean reduction in serum triglyceride in the mice treated with low, normal and high dose original SZW were 27.4%, 10.1% and 11.1% respectively, while the mean triglyceride reduction in the low, normal and high dose modified SZW were 4.2%, 11.2% and 18.6% respectively (all  $P < 0.05$  compared with the placebo groups; Figure 3). A dosage dependent reduction in serum triglyceride levels was observed for the modified SZW treated mice ( $P=0.006$ ).

**Table 3.** Liver enzyme levels of mice in different groups at the end of treatment.

Groups	Treatment assignment	ALT, U/L	AST, U/L
1	N/A (Low-fat control)	$17.6 \pm 7.8$	$69.3 \pm 5.9$
2	N/A (High fat placebo group)	$55.8 \pm 11.0$	$128.3 \pm 23.1$
3	Low dose original SZW	$31.0 \pm 10.4$	$63.7 \pm 6.8^*$
4	Normal dose original SZW	$36.7 \pm 12.7$	$94.0 \pm 9.6$
5	High dose original SZW	$24.1 \pm 9.7$	$64.6 \pm 5.5^*$
6	Low dose modified SZW	$26.4 \pm 9.5$	$71.9 \pm 8.3^*$
7	Normal dose modified SZW	$33.4 \pm 10.2$	$78.0 \pm 11.6$
8	High dose modified SZW	$33.7 \pm 8.7$	$83.8 \pm 6.9$

All variables were expressed as mean  $\pm$  SEM

\* $P < 0.05$  compared with placebo group (group 2)

SZW: Song Zhi Wan; ALT: alanine aminotransferase; AST: aspartate aminotransferase

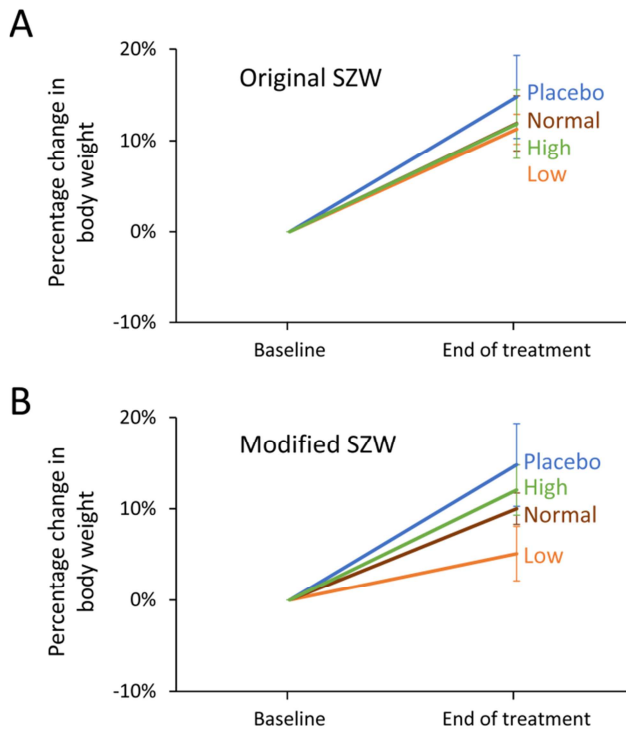
**Table 4.** Degree of steatosis in different groups of mice at the end of treatment.

Group number	Treatment	Proportion (%) of mice with			
		No steatosis	Minimal steatosis	Moderate steatosis	Severe steatosis
1	N/A (Low-fat diet control)	10/10 (100%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
2	N/A (High fat placebo group)	0/10 (0%)	0/10 (0%)	1/10 (10%)	9/10 (90%)
3	Original SZW (low dose)	0/6 (0%)	0/6 (0%)	2/6 (33%)	4/6 (67%)
4	Original SZW (normal dose)	2/10 (20%)	0/10 (0%)	2/10 (20%)	6/10 (60%)
5	Original SZW (high dose)*	1/9 (11%)	1/9 (11%)	4/9 (45%)	3/9 (33%)
6	Modified SZW (low dose)	0/6 (0%)	1/6 (16.5%)	1/6 (16.5%)	4/6 (67%)
7	Modified SZW (normal dose)	3/10 (30%)	0/10 (0%)	1/10 (10%)	6/10 (60%)
8	Modified SZW (high dose)	0/10 (0%)	0/10 (0%)	3/10 (30%)	7/10 (70%)

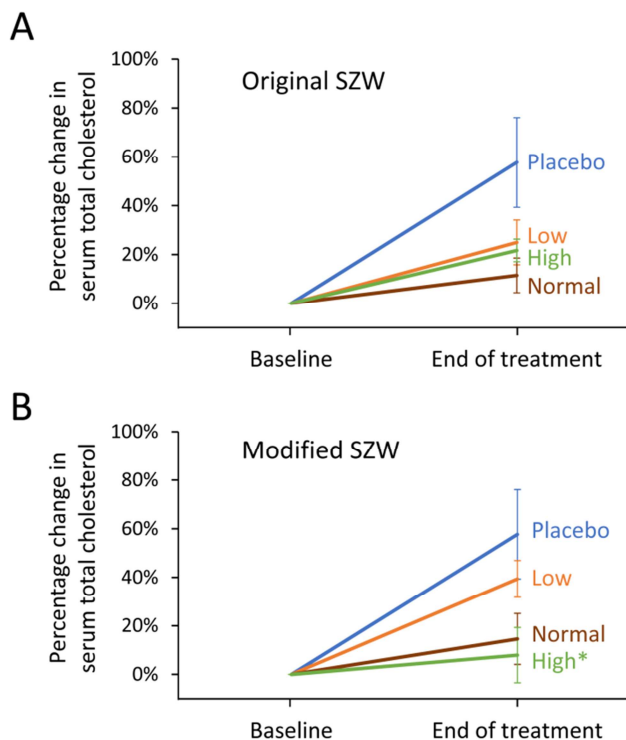
Steatosis grading was categorized according to previously published criteria [10]. No steatosis (<5% cells with fatty change); minimal steatosis (5 – 33% cells with fatty change); moderate steatosis (>33 – 66% cells with fatty change); severe steatosis (>66% cells with fatty change)

\* 1 mouse died during the experiment (at week 5 of SZW treatment)

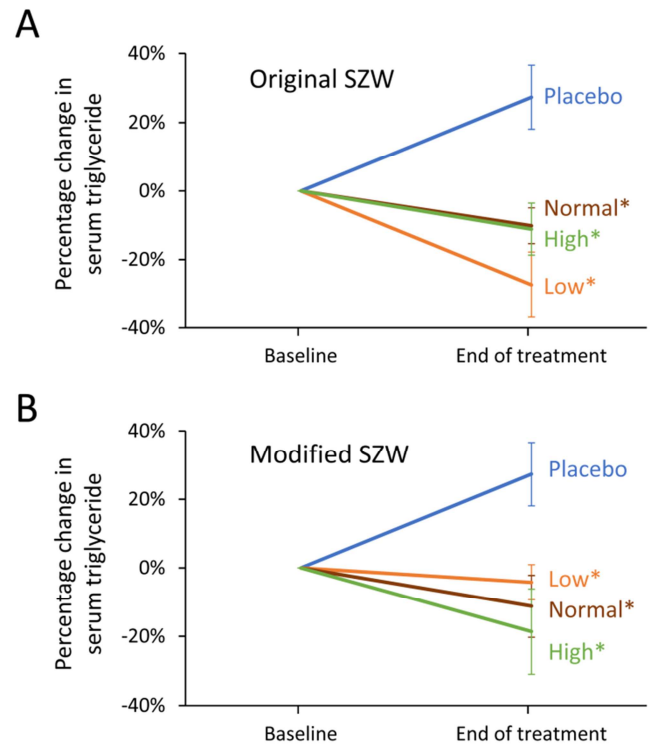
SZW: Song Zhi Wan; N/A: not applicable



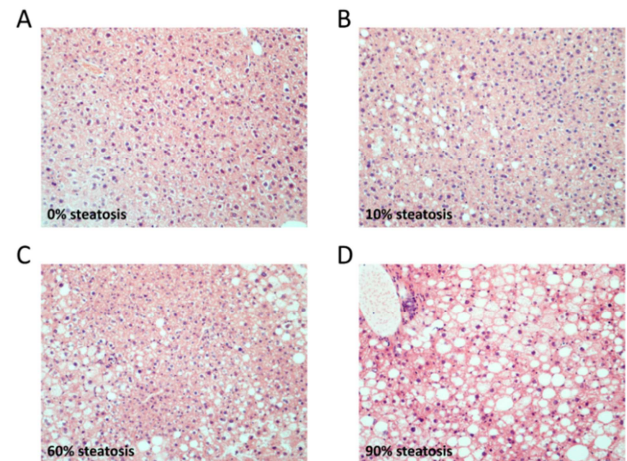
**Figure 1.** Change in body weight in the high-fat diet-fed mice treated with A. Original Song Zhi Wan (SZW) and B. Modified SZW. Data were expressed as percentage change compared with baseline, with mice treated with water (placebo, in blue), low dose SZW (orange), normal dose SZW (brown), and high dose SZW (green).



**Figure 2.** Change in serum total cholesterol levels in the high-fat diet-fed mice treated with A. Original Song Zhi Wan (SZW) and B. Modified SZW. Data were expressed as percentage change compared with baseline, with mice treated with water (placebo, in blue), low dose SZW (orange), normal dose SZW (brown), and high dose SZW (green). Statistical significance (compared with placebo group) was denoted by an asterisk (\*).



**Figure 3.** Change in serum triglyceride levels in the high-fat diet-fed mice treated with A. Original Song Zhi Wan (SZW) and B. Modified SZW. Data were expressed as percentage change compared with baseline, with mice treated with water (placebo, in blue), low dose SZW (orange), normal dose SZW (brown), and high dose SZW (green). Statistical significance (compared with placebo group) was denoted by an asterisk (\*).



**Figure 4.** Representative histological images of mouse liver tissues showing varying degrees of steatosis (H&E, 200× magnification). A. Control mouse fed with low-fat diet and H<sub>2</sub>O (Group 1; 0% steatosis); B. Mouse receiving high dose original SZW (Group 5; 5-10% steatosis); C. Mouse receiving normal dose original SZW (Group 4; 60% steatosis); D. Mouse fed with high-fat diet and H<sub>2</sub>O (Group 2; 90% steatosis).

At the end of the treatment period, mean serum ALT and AST levels of the LFD-fed control mice were  $17.6 \pm 7.8$  U/L and  $69.3 \pm 5.9$  U/L, respectively, which were lower than those of the HFD-fed placebo mice (ALT:  $55.8 \pm 11.0$  U/L;  $P=0.013$  and AST:  $128.3 \pm 23.1$  U/L;  $P=0.035$ ). Compared with the placebo group, mice treated with SZW (either in the original

or modified formula) had lower ALT and AST levels, albeit that the differences were mostly statistically insignificant, and a dosage effect was not observed (Table 3).

The degree of liver steatosis in the liver at the end of the experiment is shown in Table 4. All of the LFD-fed control mice had <5% fatty change (no steatosis), while 9 out of 10 (90%) HFD-fed placebo mice had severe steatosis (the remaining mouse had moderate steatosis). As a whole, of the 51 mice treated with either original or modified SZW, 30/51 (59%) had severe steatosis, and 8/51 (16%) mice had no or minimal steatosis. The proportion of SZW-treated mice with severe steatosis tended to be lower than that in the untreated HFD-fed placebo mice ( $P=0.079$ ). For the mice treated with original SZW, with escalating dosage, there was a decreasing trend of the proportion of mice with severe steatosis ( $P=0.013$ ), but this trend was not observed with an escalating dosage of modified SZW. Representative histologic results are shown in Figure 4.

## 4. Discussion

SZW has been shown to improve liver functions in patients with chronic hepatitis C [5, 6]. However, preclinical data on its effect on liver function in NAFLD are lacking. In this study, we used a HFD-induced mouse model to study whether SZW administration can improve liver function, reduce metabolic disorder and alleviate the degree of steatosis induced by HFD.

Compared with the LFD-fed mice, the HFD-fed mice were significantly heavier, had higher levels and serum total cholesterol and triglyceride, and showed signs of liver steatosis, indicating that the HFD did induce dyslipidemia and NAFLD.

During the intervention period, the HFD mice receiving water as placebo continued to gain weight and had increased levels of serum cholesterol and triglyceride. Mice receiving SZW also had an increased body weight and serum cholesterol during the treatment period, but the increase was smaller in the SZW treatment group. Notably, compared with the placebo group, mice receiving a high dose of modified SZW had a significantly smaller increase in serum cholesterol levels. Both forms of SZW caused a decrease in triglyceride levels, and the change was statistically significant when comparing with the placebo group. However, a dosage effect on the change in serum cholesterol and triglyceride levels was not observed, except in the mice treated with modified SZW, in which its effect on serum cholesterol level was dose dependent.

The effect of SZW on improvement of NAFLD was directly assessed by histologic assessment of fatty change. In general, mice treated with SZW, in either the original or modified forms, had less degree of steatosis than the placebo mice. A statistically significant dosage dependent effect was observed with the original SZW. These data suggested that SZW, in either the original or modified form, were able to reduce the steatotic changes induced by HFD. Whether a longer duration of SZW treatment will further reduce the degree of steatosis deserves further studies.

SZW is made up of extracts from eight herbal components,

some of which have been shown to possess hepatoprotective effects in NAFLD. For example, *gardenia jasminoides* Ellis, the major component of SZW, has been shown to possess hepatoprotective and hypolipidaemic effects in mice and attenuate fibrosis and hepatocellular injury in rats [11, 12]. *Gardenia jasminoides* Ellis is also an ingredient of two traditional Chinese formulae, Yinchenhao Decoction and Qushi Huayu Decoction, both of which have been shown to reduce hepatic fat accumulation in rats [13]. Likewise, many *in vivo* studies have demonstrated the hepatoprotective and hypolipidaemic effects brought about by other SZW ingredients such as *Cudrania cochinchinensis* [14], *Poria cocos* [15, 16], *Ilex asprella* [17], *Salvia miltiorrhiza* [18–20], *Cortex Lycii* [21], and *Amomum villosum* [22]. Although the exact mechanisms of the combination of these ingredients in SZW on the alleviation of NAFLD, as well as the possible synergy involved, remain to be determined, taken together with its effect on body weight, serum total cholesterol and triglyceride, SZW was demonstrated to show an improvement of liver function in this NAFLD mouse model.

This study has certain limitations. First, baseline liver biopsies were not available to monitor the change in steatosis before and after SZW treatment. Second, due to the limited availability of blood samples collected from tail vein, other metabolism related parameters such as glucose or insulin levels were not measured. In addition, this study only investigated the effect of SZW as a whole on liver steatosis; the mechanisms of the effect of SZW, as well as its individual components, on the alleviation of NAFLD, deserves further studies.

## 5. Conclusion

In summary, both the original and modified formulas of SZW demonstrated some effects in the amelioration of dyslipidemia and the alleviation of HFD-induced steatosis. In particular, HFD-fed mice receiving SZW showed a significant reduction in triglyceride and a better cholesterol profile than mice receiving water as placebo. There were also fewer SZW-treated mice with severe steatosis than placebo mice. Further animal and human studies are warranted to confirm the safety and efficacy of SZW for the treatment of NAFLD.

## Author Contributions

Conceptualization: DKHW, FL. Methodology: DKHW, FL. Formal analysis: DKHW, SZ, GW. Investigation: DKHW, SZ, GW, SYSC, RCLL. Resources: SZ, GW, FL. Data curation: DKHW, GW. Writing – Original Draft: DKHW. Writing – Review & Editing: GW, SZ, LM, PX, WKS, MFY. Supervision: MFY. Project administration: DKHW. Funding acquisition: MFY.

## Conflicts of Interest Statement

The authors declare that they have no competing interests.



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