

## Original Article

# Effect of Shenmaisanjie capsules on hyperthyroidism-induced liver damage in rat

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**Abstract:** Shenmaisanjie capsule (SMSJC) is a preparation of traditional Chinese medicine and has been administered to out-patients with hyperthyroidism. This study was designed to examine the effect and mechanism of SMSJC on hyperthyroidism-induced liver damage in rats. Fifty Sprague-Dawley rats were randomly divided into euthyroid group, hyperthyroid group and three SMSJC-treated hyperthyroid groups (0.24, 0.48 and 0.96 g/kg SMSJC were administered, respectively). The hyperthyroidism-induced liver damage rat model was established by administration of L-thyroxine (800 µg/kg) daily for 6 weeks. After 6 weeks, serum levels of triiodothyronine ( $T_3$ ), tetraiodothyronine ( $T_4$ ), free  $T_3$  (FT<sub>3</sub>), free  $T_4$  (FT<sub>4</sub>), thyroid-stimulating hormone (TSH), thyrotropin receptor antibodies (TRAbs), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin (TBIL), tumor necrosis factor (TNF-α), factor associated suicide (Fas) and Fas ligand (FasL) were determined. Livers were extracted for histological analysis and examination of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and malondialdehyde (MDA) level. SMSJC (0.48 and 0.96 g/kg) significantly reduced the elevated levels of thyroid hormones, ALT, AST, ALP, TBIL, TNF-α, Fas and FasL. In addition, SMSJC markedly inhibited MDA, enhanced hepatic activity of SOD, GSH-Px and CAT, and improved liver morphologic changes. The results indicate that SMSJC could be used to prevent experimental hyperthyroidism-induced liver damage through regulation of the antioxidant system, lipid peroxidation and death receptor apoptosis pathway.

**Keywords:** Shenmaisanjie capsule, hyperthyroidism, liver damage, rat, oxidative stress, apoptosis

## Introduction

Thyroid hormones influence almost all the tissue and organ system in the body, and play an important role in metabolism, thermoregulation, development and growth [1, 2]. Hyperthyroidism is a disease caused by overproduction and secretion of thyroid hormones, combined with the complications of untreated thyrotoxicosis including loss of weight, osteoporosis, oxidative damage of liver, atrial fibrillation and even cardiovascular collapse and death [3-5]. Cardiopathy is most typical complication in hyperthyroidism and has been studied more, in recent years, liver dysfunction is often observed in patients with hyperthyroidism and the occurrence reported varies from 37% to 77.9% among different studies [6-8]. The hepatic injury in hyperthyroidism varies from mild liver dysfunction associated with nonspecific histologic changes to severe central hepat-

ic ischemia. In most cases, the changes in the liver are characterized by cholestasis, some degree of fatty infiltration, cytoplasmic vacuolization, nuclear irregularity and hyperchromatism in hepatocytes [9, 10]. Until recently, the pathogenesis of hepatic dysfunction in severe hyperthyroidism is not fully understood.

The results of previous researches provided some explanations for liver dysfunction associated with hyperthyroidism. It was well established that oxidative stress and mitochondrial oxygen consumption increased in hyperthyroid rat liver [11, 12]. Hyperthyroidism-induced apoptosis in rat liver involved the activation of mitochondria-dependent pathway (intrinsic way) and death receptor-mediated pathways (extrinsic way) [13, 14]. They were two classic activation mechanisms of apoptosis. The intrinsic pathway involved the release of cytochrome c from mitochondria, generation of reactive oxy-

gen species (ROS) and loss of mitochondrial trans-membrane potential. Extrinsic or death receptor-mediated pathway was mediated through cell-surface death receptors, such as TNF- $\alpha$  and Fas. Binding of TNF- $\alpha$  and FasL to their respective receptors induced apoptosis through the apical caspase-8 pathway. In addition, a study showed that higher FT<sub>4</sub> concentration and higher TRAbs value were independent risk factors predicting abnormal liver function [15]. However, the association between thyroid function indexes and liver injury remains controversial. Though the number of severe hyperthyroidism liver damage case has been decreased significantly since the clinical use of anti-thyroid drugs (ATD), but long-term use of propylthiouracil (PTU) and methimazole (MMI) may result in hepatic injury. It's very urgent to study the mechanism and treatment drug of hyperthyroidism-induced hepatic damage. Previous research suggested that both vitamin E and curcumin, low molecular mass antioxidants, had differential regulation on complexes I and II mediated mitochondrial respiration and a protective role against L-thyroxine induced hepatic dysfunction and oxidative stress [12]. To date, clinical treatment of hyperthyroidism-induced hepatic damage is mainly to control clinical symptoms of hyperthyroidism, nevertheless drugs that can protect liver function and reduce transacylase and prevent hepatic damage are rare. Therefore, it is deserved to discover a drug or preparation with remarkable hepatoprotective and antithyroid efficacy, and slight adverse drug reactions.

Traditional Chinese medicine (TCM) has been used over thousands of years and are based on experiences and practices, which has the characteristics of multiple targets and less adverse reaction. SMSJC, a traditional Chinese medicine preparation, is prepared by the Affiliated Hospital of Southwest Medical University based on Yiqiyangyin and Ruanjiansanjie theory. The preparation is extracted from nine medicinal herbs, such as codonopsis radix, ophiopogonis radix, schisandrae chinensis fructus, ziziphi spinosae semen, poria, carthami flos, persicae semen, fructus tritici levis and fritillariae thunbergii bulbus. The preparation, acting as a protective agent against hyperthyroidism and hyperthyroidism-induced hepatic damage, has been used in clinical for more than ten years. Nevertheless, the mechanism of SMSJC for the

treatment of hyperthyroidism-induced hepatic damage remains unclear. Now, this study was designed to evaluate the therapeutic effect of SMSJC on hyperthyroid-induced liver damage in the rat and the possible mechanism of its action. Some ingredients of SMSJC have been reported to possess a variety of biological and pharmacological activities of enhancing immunity, anti-inflammatory, anti-tumor, improving microcirculation and anti-oxidant and so on [16-20]. Here, we would investigate the role of oxidative stress and death receptor-mediated pathway including TNF- $\alpha$ , Fas and FasL in hyperthyroidism-induced apoptosis in rat liver.

### Materials and methods

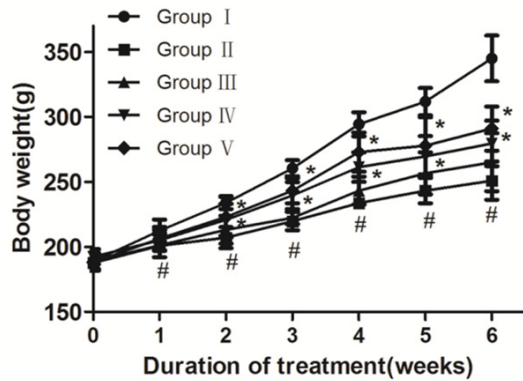
#### *Animals and experimental design*

Adult Sprague-Dawley rats of both genders weighing 180-200 g were obtained from the Medical Experimental Animal Center of Southwest Medical University. The animals were maintained under standard laboratory conditions (12 h light/12 h dark cycle, room temperature 25 $\pm$ 2 $^{\circ}$ C) with free access to tap water and food. The animals were allowed 1 week to adapt to the laboratory conditions, then they were randomly divided into weight-matched five groups (10 rats in each group): Group I, euthyroid; Group II, hyperthyroid; Group III, hyperthyroid with low-dose SMSJC; Group IV, hyperthyroid with medium-dose SMSJC; Group V, hyperthyroid with high-dose SMSJC.

Except for Group I, all the other rats were perfused intragastrically with L-thyroxine sodium tablet (800  $\mu$ g/kg) every morning for 6 weeks [13, 14, 21]. Meanwhile, rats in Groups III-V were intragastrically given SMSJC at a dose of 0.24, 0.48, 0.96 g/kg respectively every afternoon for 6 weeks. Rats in Group I were treated with distilled water instead. The intake of water and food, appearance and behavior were observed daily, and body weight and body temperature were recorded weekly. All animal procedures were undertaken in accordance with the Guidelines on the Care and Use of Laboratory Animals issued by the Chinese Council on Animal Research and the Guidelines of the Animal Care.

At the end of the 6 weeks period, about 24 hours after the last dose, except the dead, all

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**Figure 1.** Effect of SMSJC on bodyweight at different time point. Data are expressed as mean  $\pm$  SD, n=10. #P<0.01 versus Group I; \*P<0.01 versus Group II.

rats were anaesthetized with an intraperitoneal injection of sodium pentobarbital (17 mg/kg) [22]. The livers and blood samples were collected for further assessments. Blood samples were collected from the abdominal aorta, kept in an ice bath for 2 hours and then centrifuged at 1000 rpm for 15 min (4°C). The serum was aliquoted and stored at -20°C until being assayed. The livers were rapidly removed, right lobes were fixed in 0.4 g/L formaldehyde solution and embedded in paraffin prior to processing for histopathology under light microscopy, the other livers were weighed, snap-frozen in liquid nitrogen and stored at -70°C until being assayed for malondialdehyde (MDA) content and superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) activity.

## Biochemical assays

The serum contents of triiodothyronine ( $T_3$ ),  $T_4$ , free  $T_3$  ( $FT_3$ ),  $FT_4$  and TSH were assayed by radioimmunoassay (RIA). Serum levels of transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and total bilirubin (TBIL) were measured using an automatic biochemical analytic instrument (Hitachi 7060). The expression of TNF- $\alpha$ , Fas and FasL in serum were detected by ELISA.

The samples of liver were rapidly thawed and cut into slices, which were then manually homogenized, using a Potter homogenizer with a glass pestle, in 19 volumes of ice-cold 100 mM Tris-HCl buffer containing 0.1 mM ethylenediaminetetraacetic acid and 0.1% (v/v) Triton X-100, pH 7.8. All procedures were performed on ice. Homogenates were centrifuged at 1,500 rpm at 4°C for 15 min and the resul-

tant supernatants were aliquoted and stored at -70°C until analysis. The concentration of MDA and the activities of SOD, GSH-Px and CAT in liver tissue were detected by spectrophotometric method with commercially available test kits. The presence of protein in the supernatant was detected by the Coomassie brilliant blue method.

## Histological analysis of liver

Following sacrifice, liver tissue was immediately fixed for histological studies in freshly prepared formol sublimate, dehydrated in graded ethanol series, cleared in xylene and embedded in paraffin wax. Tissues were sectioned (5  $\mu$ m) and stained with hematoxylin and eosin for general histopathological examination.

## Drugs and reagents

L-thyroxine sodium tablets were purchased from Merck KGaA (Darmstadt, Germany). Shenmaisanjie capsules were provided by preparation room of the Affiliated Hospital of Southwest Medical University (Luzhou, China). Radioimmunoassay kits for  $T_3$ ,  $FT_3$ ,  $T_4$ ,  $FT_4$  and TSH and ELISA kits for TNF- $\alpha$ , Fas and FasL were obtained from Beijing sino-uk institute of biological technology (Beijing, China). Assay kits for ALT, AST, ALP, TBIL, SOD, GSH-Px, CAT, MDA and TRAb were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) and R&D Systems China Co. Ltd. (Shanghai, China). All other reagents and solvents used in experiments were of analytical grade.

## Statistical analysis

Calculations and statistical analyses were performed using the GraphPad Prism 6.0 software. The data are presented as mean  $\pm$  standard deviation (SD). The level of significance was determined by one-way analysis of variance (ANOVA) followed by post hoc multiple comparisons using SPSS 22.0. Least-significant Difference (LSD) or Dunnett's  $T_3$  was used for the data with equal variances or not. The values of P<0.05 and P<0.01 were used as the criterion for statistical significance.

## Results

### Effect of SMSJC on bodyweight and rectal temperature

All the rats were alive at the end of the 6th week. **Figure 1** shows the change of body

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**Table 1.** Effect of SMSJC on rectal temperature at different time point (°C,  $\bar{x} \pm s$ , n=10)

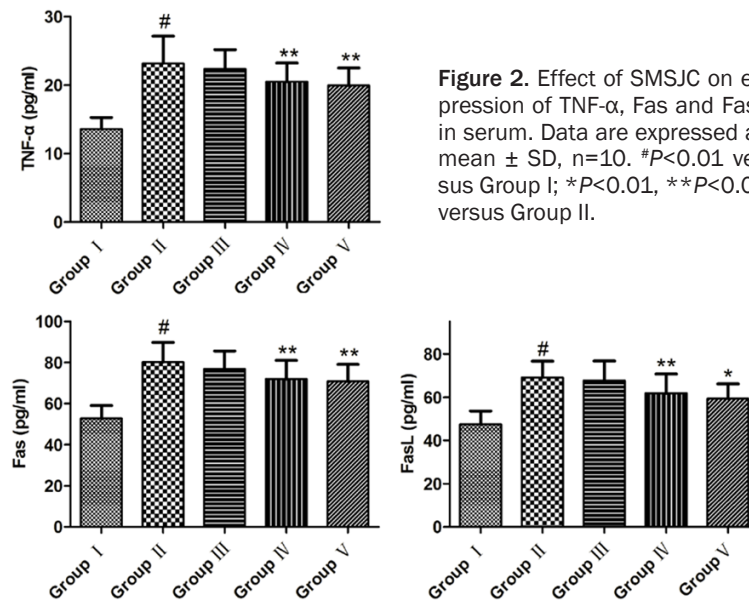
Groups	0 w	1 w	2 w	3 w	4 w	5 w	6 w
I	37.73±0.33	37.75±0.41	37.82±0.58	37.71±0.34	37.73±0.42	37.96±0.30	38.00±0.49
II	37.87±0.38	38.23±0.51 <sup>##</sup>	38.84±0.37 <sup>#</sup>	39.01±0.38 <sup>#</sup>	39.13±0.51 <sup>#</sup>	39.43±0.35 <sup>#</sup>	39.68±0.40 <sup>#</sup>
III	37.88±0.31	38.02±0.23	38.74±0.26	38.78±0.37	38.89±0.43	38.88±0.51 <sup>*</sup>	39.34±0.55 <sup>**</sup>
IV	37.93±0.45	38.00±0.46	38.67±0.35	38.63±0.30 <sup>**</sup>	38.45±0.55 <sup>*</sup>	38.56±0.63 <sup>*</sup>	38.84±0.36 <sup>*</sup>
V	37.75±0.35	38.02±0.55	38.41±0.43 <sup>**</sup>	38.35±0.39 <sup>*</sup>	38.11±0.47 <sup>*</sup>	38.43±0.46 <sup>*</sup>	38.46±0.29 <sup>*</sup>

<sup>#</sup>P<0.01, <sup>##</sup>P<0.05 versus Group I; <sup>\*</sup>P<0.01, <sup>\*\*</sup>P<0.05 versus Group II.

**Table 2.** Effect of SMSJC on thyroid hormones and TSH ( $\bar{x} \pm s$ , n=10)

Groups	T <sub>3</sub> (nmol/L)	T <sub>4</sub> (nmol/L)	FT <sub>3</sub> (pmol/L)	FT <sub>4</sub> (pmol/L)	TSH (mIU/L)
I	1.74±0.24	40.76±4.52	4.65±0.68	33.03±2.37	2.91±0.22
II	12.42±1.02 <sup>#</sup>	188.27±20.90 <sup>#</sup>	11.72±1.02 <sup>#</sup>	77.22±3.62 <sup>#</sup>	0.92±0.11 <sup>#</sup>
III	12.08±0.93	182.65±16.45	11.13±0.70	72.99±6.85	0.93±0.13
IV	10.93±0.77 <sup>**</sup>	153.93±13.81 <sup>*</sup>	11.08±0.97	70.38±6.42	0.96±0.15
V	10.97±0.79 <sup>**</sup>	140.10±14.27 <sup>*</sup>	10.50±1.05 <sup>*</sup>	61.55±8.56 <sup>*</sup>	0.97±0.13

<sup>#</sup>P<0.01 versus Group I; <sup>\*</sup>P<0.01, <sup>\*\*</sup>P<0.05 versus Group II.



**Figure 2.** Effect of SMSJC on expression of TNF- $\alpha$ , Fas and FasL in serum. Data are expressed as mean  $\pm$  SD, n=10. <sup>#</sup>P<0.01 versus Group I; <sup>\*</sup>P<0.01, <sup>\*\*</sup>P<0.05 versus Group II.

cant rectal temperature decrease from the second week ( $P<0.01$  or  $P<0.05$ ) (Figure 1 and Table 1). However, the improvement of symptoms in Group III was mild ( $P>0.05$ ).

### Effect of SMSJC on serum levels of T<sub>3</sub>, T<sub>4</sub>, FT<sub>3</sub>, FT<sub>4</sub>, TSH and TRAb

Compared with rats in Group I, serum T<sub>3</sub>, T<sub>4</sub>, FT<sub>3</sub> and FT<sub>4</sub> levels increased significantly while serum TSH decreased markedly in Group II. Serum T<sub>3</sub> and T<sub>4</sub> levels decreased significantly in Groups IV and V ( $P<0.05$ ) and serum FT<sub>3</sub> and FT<sub>4</sub> levels decreased significantly in Group V as compared to Group II ( $P<0.01$  or  $P<0.05$ ), whereas no significant difference of serum TSH level was noticed in Groups III-V ( $P>0.05$ ) as compared to hyperthyroid rats. However, serum TRAb level was not significantly different in five groups of rats (data not shown).

Effect of SMSJC on serum levels of ALT, AST, ALP, TBIL, TNF- $\alpha$ , Fas and FasL

### Effect of SMSJC on serum levels of ALT, AST, ALP, TBIL, TNF- $\alpha$ , Fas and FasL

L-thyroxine treatment resulted in significant increase in serum levels of ALT, AST, ALP, TBIL,

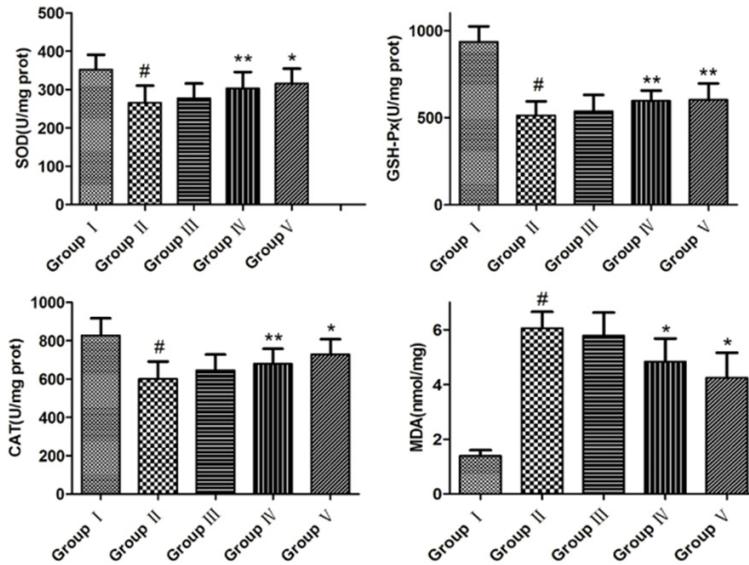
weight in the five groups of rats. The rats in the control group grew normally and moved freely. Significant loss in bodyweight was observed in Group II-V compared to rats in Group I. The rats in Group II experienced hyperthyroidism with symptoms like active, irritability, aggressiveness, erected hair, water intake increase and body temperature enhancement compared to rats in Group I ( $P<0.01$ ). Compared with Group II, the rats in Group IV, V showed relief in irritability and aggressive behavior, water intake decrease, alleviation in bodyweight loss and signifi-



**Table 3.** Comparison of hepatic function among five groups of rats ( $\bar{x} \pm s$ , n=10)

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TBIL
I	52.84±3.83	148.53±18.08	134.28±27.43	1.82±0.29
II	70.27±6.51 <sup>#</sup>	231.39±22.11 <sup>#</sup>	168.49±18.41 <sup>#</sup>	2.23±0.26 <sup>#</sup>
III	67.47±5.06	222.78±19.65	151.91±28.98	2.15±0.18
IV	63.80±6.95 <sup>**</sup>	212.60±23.14 <sup>**</sup>	146.43±27.06	1.96±0.15 <sup>**</sup>
V	57.81±5.96 <sup>*</sup>	198.42±18.85 <sup>*</sup>	137.35±36.17 <sup>**</sup>	1.91±0.30 <sup>*</sup>

<sup>#</sup>P<0.01 versus Group I; <sup>\*</sup>P<0.01, <sup>\*\*</sup>P<0.05 versus Group II.



**Figure 3.** Effect of SMSJC on activities of SOD, GSH-Px, CAT and MDA level in liver homogenate. Data are expressed as mean  $\pm$  SD, n=10. <sup>#</sup>P<0.01 versus Group I; <sup>\*</sup>P<0.01, <sup>\*\*</sup>P<0.05 versus Group II.

TNF- $\alpha$ , Fas and FasL in Group II compared to euthyroid rats. Whereas, serum levels of ALT, AST, ALP, TBIL, TNF- $\alpha$ , Fas and FasL declined significantly in Groups IV and V rats in comparison to hyperthyroid rats ( $P<0.01$  or  $P<0.05$ ). Furthermore, among the 3 SMSJC treatment groups, the highest dose group showed the best effect (**Figure 2** and **Table 3**).

*Effect of SMSJC on hepatic SOD, GSH-Px, CAT activities and MDA level*

Significant decrease in activities of SOD, GSH-Px and CAT and significant augment in MDA level in liver homogenate were observed in L-thyroxine treatment group in comparison to euthyroid rats ( $P<0.01$ ). Administration of SMSJC (medium and high dose) to hyperthyroid rats resulted in marked increase in the activities of SOD, GSH-Px and CAT and decrease in

MDA level in liver homogenate compared to those in hyperthyroid rats ( $P<0.01$  or  $P<0.05$ ) (**Figure 3**).

*Effect of SMSJC on liver histopathology*

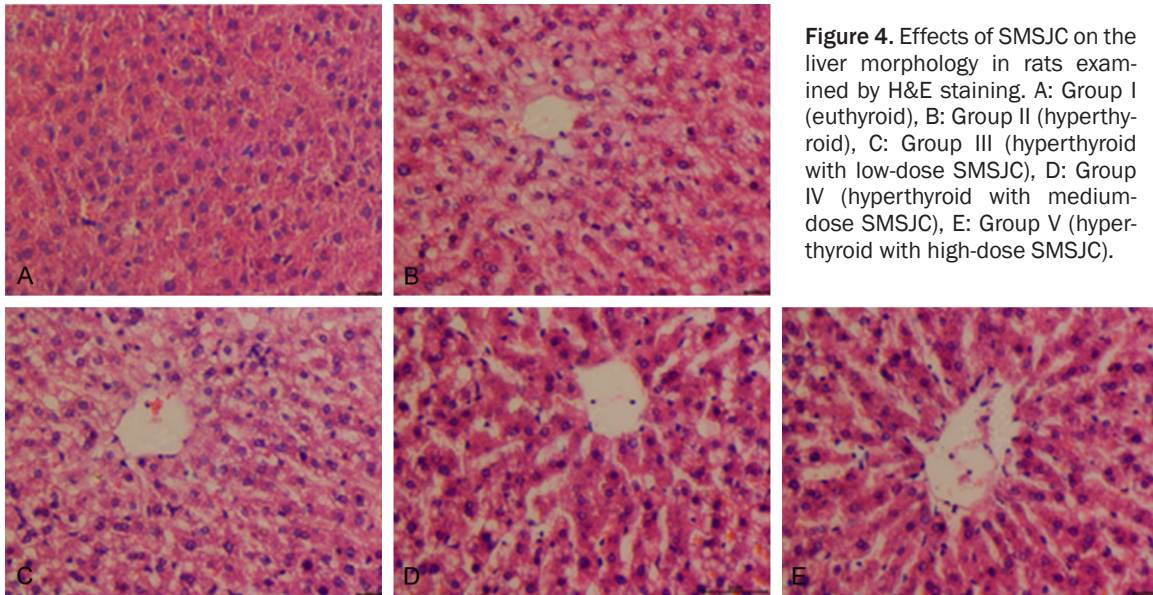
HE staining was performed to evaluate pathological changes. Histopathological section staining showed that the structure of the hepatic lobules was normal, hepatic cells were orderly arranged, cytoplasmic staining was red and no vacuoles, hepatic turbidity, or fatty degeneration was seen in the cytoplasm in the control rats (**Figure 4A**). In L-thyroxine treatment group, hepatic lobules could be observed but hepatic cords were irregular, the number of hepatocytes was increased, and congestion in sinusoidal spaces, fatty degeneration in hepatic cytoplasm and cytoplasmic vacuolization were more obvious compared with euthyroid group (**Figure 4B**). Liver sections of SMSJC treatment rats showed a reduction in hepatocyte number along with increase in sinusoidal spaces

in comparison to hyperthyroid rats, conditions in Group V were better than those in Group III and IV.

**Discussion**

Thyroid hormones affect all tissues and modulate the rate of metabolic activity, and liver is a major target organ with important biological and medical implications. Overt thyrotoxicosis, whether endogenous or exogenous, is characterized by excess thyroid hormones and suppressed TSH in serum. The severity of thyrotoxic symptoms is proportional to the elevation in the serum levels of FT<sub>4</sub> and FT<sub>3</sub>. Nevertheless, serum TSH levels are considerably more sensitive than direct thyroid hormone measurements for assessing thyroid hormone excess [23]. In the present study, loss in bodyweight, higher rectal temperature, decrease in serum TSH and

## Shenmaisanjie capsules prevent hyperthyroidism-induced liver damage



**Figure 4.** Effects of SMSJC on the liver morphology in rats examined by H&E staining. A: Group I (euthyroid), B: Group II (hyperthyroid), C: Group III (hyperthyroid with low-dose SMSJC), D: Group IV (hyperthyroid with medium-dose SMSJC), E: Group V (hyperthyroid with high-dose SMSJC).

elevation in serum  $T_3$ ,  $T_4$ ,  $FT_3$  and  $FT_4$  in L-thyroxine treated rats confirmed the hyperthyroid state. Clinical diagnosis of liver damage is commonly assessed by monitoring status of serum ALT, AST, ALP,  $\gamma$ -GGT activities and TBIL, albumin levels [24]. Our results showed that significant increases in serum ALT, AST, ALP and TBIL were present in model group. These findings are compatible with the results of other studies [25-27]. Investigators have demonstrated that more than 70% cases of newly diagnosed and untreated hyperthyroidism patients had at least one liver function test abnormality in which the most commonly elevated parameters were ALT and ALP, and patients in hepatic dysfunction had significantly higher  $FT_3$ ,  $FT_4$  and TRAb concentration. Moreover, there was a positive correlation between degree of liver damage and the serum level of  $T_3$  and  $T_4$ . However, we found that TRAb level measured was not significantly different in five groups. Graves' disease (GD) is an autoimmune disorder with elevation of TRAbs. TRAbs stimulate the TSH receptor and then increase thyroid hormone production, which may contribute to hepatic dysfunction in patients with GD [28]. The difference indicated that GD model is not replicated in the present study, and the reasons remain to be further investigated.

In the present study, hyperthyroidism was established by intragastrical administration of L-thyroxine (800  $\mu\text{g}/\text{kg}$ ) for 42 days on the

basis of previous studies [11, 29, 30]. The reason of extending the time was that: (i) hyperthyroidism-induced liver injury is chronic and is commonly found in patient with longer course, (ii) the plasma half-life of  $T_4$  is 7 days, the release of thyroid hormone stored inside thyroid takes about 2 weeks, therefore ATD exerts the therapeutic action after 4 weeks, (iii) SMSJC and L-thyroxine were perfused at the same time, and (iiii) SMSJC is a traditional Chinese medicine preparation, which may take longer time to play a role of treatment compared with other ATDs. In addition, SMSJC has complex ingredient, in order to avoid drug interactions, L-thyroxine and SMSJC was perfused in the morning and afternoon, respectively.

For the first time, the data suggest that oral supplementation of SMSJC to hyperthyroid rats can markedly alleviate the serum levels of ALT, AST, TBIL and thyroid hormones, slightly change serum ALP and TSH. An assessment of serum  $FT_4$  and TSH are required before treatment and at intervals after starting the treatment. Serum TSH may remain suppressed for several months after starting therapy and is therefore not a good parameter to monitor therapy early in the course. Hyperthyroidism patients often have a high bone metabolic rate, increase of osteoblast and osteoclast activity. Moreover, thyroid hormone has the effect of direct stimulation of bone re-absorption in vitro, cause osteoporosis. Hence, the augment of ALP is derived from not only liver but also bone, and the ALP level

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can't reflect the severity of hepatobiliary disease in hyperthyroid. Meanwhile, after SMSJC treatment, hyperthyroidism signs and symptoms were improved significantly. But the symptom of hyperorexia was not obvious, which may be due to the decreased appetite after liver dysfunction. These findings indicated that exogenous SMSJC could protect liver and thyroid function. In addition, liver histology found that L-thyroxine resulted in increase of hepatic cell number, fatty degeneration in hepatic cytoplasm and cytoplasmic vacuolization. Thyroid hormone is known to play an essential role in hepatocyte proliferation of rat liver [31]. Supplementation of SMSJC to rats resulted in restoration of normal number and histoarchitecture. Though severe damage is not evident in the liver histology, it points towards the immense therapeutic potential of the exogenous antioxidants, SMSJC, in liver disease.

It is well established that thyroid hormone modulates the metabolism of ROS, which include superoxide radicals, hydrogen peroxide and hydroxyl radicals. To detoxify ROS, mammalian cells have evolved a complex antioxidant system. Hyperthyroid state is accompanied with an increase in prooxidant to antioxidants ratio, which leads to oxidative stress [32]. In the present study, liver MDA level increased significantly, SOD, CAT and GSH-Px declined significantly in hyperthyroid rats. The results indicated that excess thyroid hormone stimulated massive oxygen free radical and lipid peroxide, consumed a lot of SOD, GSH-Px and CAT, which resulted in antioxidant defense system damaged and oxygen free radical-induced hepatic injury in hyperthyroidism rats. Administration of SMSJC to hyperthyroid rats resulted in marked increase in activities of SOD, GSH-Px and CAT and decrease in MDA level in liver homogenate. The findings indicated that SMSJC suppressed lipid peroxidation, enhanced antioxidant enzyme activity and the ability of scavenging free radicals, corrected imbalances of oxidation and anti-oxidation system, thus which indicated that SMSJC could protect mitochondria and reverse liver dysfunction. A variety of ingredients in SMSJC, i.e. codonopsis radix, ophiopogonis radix, schisandrae chinensis fructus, ziziphi spinosae semen, have antioxidation and scavenging free radicals actions. The antioxidant effect of SMSJC may be the result of synergistic action of components. Previously the

study found that hyperthyroidism-induced apoptosis in rat liver involved the activation of death receptor and mitochondria-mediated pathways. In this study, we found that SMSJC alleviated expression of TNF- $\alpha$ , Fas and FasL in hyperthyroid rats. The finding indicated that SMSJC inhibited death receptors apoptosis pathway in hyperthyroidism-induced liver injury. In mitochondria-mediated apoptotic pathway, a large number of pro-apoptotic proteins are released from mitochondria into cytoplasm through mitochondrial permeability transition pore, trigger the activation of caspase-9 and the subsequent caspase cascade, which lead to apoptosis. Whether these mechanisms are involved in the protective effects of SMSJC deserved to be studied in the future.

In our study, the changes of almost all the parameters were not significant in low-dose SMSJC group, whereas the changes were significant in medium- and high-dose groups in comparison to hyperthyroid rats. But there was no difference between medium-dose group and high-dose group, which indicated that medium dose was most appropriate. The medium dose (0.48 g/kg) used in our study was transferred from the human clinical dosage (5.4 g/70 kg BW) by the index of Body Surface Area (200 g BW rat to 70 kg BW adult human is 0.018). In addition, these compounds in SMSJC are remarkably free of toxicity, as shown by the fact that they have been approved for human consumption and are widely used as safe traditional Chinese medicine.

The present study clearly demonstrate that excess thyroid hormones increase production of splanchnic oxygen free radicals, decrease antioxidant enzymes, enhance expression of several death receptors and their ligands, including TNF- $\alpha$ , Fas and FasL. Oxidative stress and death receptor apoptosis pathway play essential role in hyperthyroidism hepatic damage. Administration of SMSJC is proved to be beneficial in reversing hepatotoxicity and thyrotoxicity. It is proposed that the effect is concerned with decreasing the levels of thyroid hormone, transaminase, TNF- $\alpha$ , Fas and FasL in serum and suppressing oxidative stress. The results indicate that SMSJC could be used to prevent experimental hyperthyroidism-induced liver damage through regulation of the antioxidant system, lipid peroxidation and death



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receptor apoptosis pathway. Moreover, future interventional studies are needed to confirm these provocative results and discover other protective mechanism of SMSJC.

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## Disclosure of conflict of interest

None.

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