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Anti-*Toxoplasma gondii* Properties of Ginseng polysaccharides and saponins

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Abstract: New anti-*Toxoplasma gondii* agents are in demand due to the emergence of high toxicity. Ginseng polysaccharides and saponins can be used to treat the replication of *Toxoplasma gondii* in an attempt to determine whether the medicinal uses of ginseng are supported by pharmacological effects. Anti-*Toxoplasma gondii* activities of ginseng polysaccharides and saponins were examined *in vitro* and *in vivo*. The findings are the survival time and rate of *Toxoplasma gondii* infected mice after the intake of the total polysaccharides and saponins increased compared to untreated control mice. The survival rate of mice treated with ginseng saponins was the highest at 83.3%, the phenomenon of splenomegaly of mice was decreased especially ($p < 0.05$) treated with ginseng polysaccharides. Accordingly, ginseng polysaccharides and saponins have a potential application in anti-*Toxoplasma gondii* treatments.

1. Introduction

Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan that infects humans and other warm-blooded animals, so it can be found all over the world (Dubey 1997). Although it only infects nucleated cells, its wide host range allows it to have a widespread distribution and led to severe toxoplasmosis (Holmes et al. 1977), such as encephalitis, neonatal malformations, chorioretinitis and mental retardation in the fetus especially for patients with immunosuppression, organ transplantation and AIDS (Weiss and Dubey 2009). Because of the unique lifestyle of *T. gondii*, the current clinical treatment for *T. gondii* infection is the using of sulfonamide and pyrimethamine combination (Pohl-Schickinger et al. 2012). However, the sulfonamide and pyrimethamine showed effect only in acute infection and accompany with high cytotoxicity (Walker et al. 2013). Other drugs like macrolides were also questioned by their drug resistance (Feliciano-Alfonso et al. 2019). Thus, finding medicine with high effect and low toxicity to treat for toxoplasmosis is emergency.

In these years, Chinese traditional herbs showed the anti-*T. gondii* activity, such as *Artemisia annua* L., *Zingiber officinale*, *Sophora flavescens* Aiton, *Eurycoma longifolia*, *Ginkgo biloba* sarcotesta, *Fraxinus rhychophylla*, etc. (Chen et al. 2008; Chio et al. 1996; Jiang et al. 2008; Oliveira et al. 2009). Among them, ginseng (*Panax ginseng* C. A. Meyer) has a history of over 4000 years of application, and modern mature large-scale cultivation has attracted our attention. Ginseng as a medicine to replenish “yuan qi”, an energy in human’s body to maintain individual full of vitality in Traditional Chinese Medicine theory (Lu et al. 2021). Many bioactive components in ginseng have been found and proved, such as ginsenosides, ginseng polysaccharides (Guo et al. 2021). As a non-specific immunomodulator, ginseng polysaccharides can stimulate dendritic cells, promote the proliferation of CD4+ T Lymphocytes (Park et al. 2001) and the expression of CD86 (Du et al. 2008), IL-12 (Meng et al. 2013) and TNF- α (Hwang et al. 2011), mediate the activation of Foxp3 transcription factor (Abdullahi et al. 2016), and up-regulate the immune level. Although ginseng can regulate the physiological level of the host

infected with *T. gondii*, the application of ginseng in the treatment of *T. gondii* infection is not common so far. The first record of treatment to anti-parasites for ginseng is in “Sheng Ji Zong Lu”, a medical book which was written in Song Dynasty about 1000 years ago, in addition, ginsenoside alone as an immune adjuvant combined with *T. gondii* ROP18 antigen can prolong the survival time of mice infected with *T. gondii* (Zhuo et al. 2017), while the study on the treatment of *T. gondii* infection by ginseng whole medicinal part is still blank.

In this study, we selected total polysaccharides and total saponins sourced from ginseng, red ginseng which was made from ginseng and *Panax quinquefolius* L. (American ginseng) as the research objects and explored their therapeutic effects on acute infection of *T. gondii* *in vitro* and *in vivo*.

2. Investigations and results

2.1. Effect of total polysaccharides and saponins on the selectivity index of *T. gondii* infected GES-1 cells and *T. gondii*

Anti-*T. gondii* activity was expressed as the selectivity index (SI). The IC₅₀ in GES-1 cells values are 272.6 mg/L (ginseng total polysaccharides, GP), 324.5 mg/L (red ginseng total polysaccharides, RP), 256.2 mg/L (American ginseng total polysaccharides, AP), 302.9 mg/L (ginseng total saponins, GS), 275.3 mg/L (red ginseng total saponins, RS), 295.2 mg/L (American ginseng total saponin, AS), and 225.8 mg/L (Spiramycin, SPI), respectively (Table 1). All total polysaccharides and saponins showed low cytotoxicity. The obtained SI values were 0.9 (GP), 1.1 (RP), 0.9 (AP), 1.0 (GS), 1.0 (RS), and 0.9 (AS), respectively (Table 1).

2.2. Effect of total polysaccharides and saponins on the growth inhibition of *T. gondii* *in vivo*

The numbers of tachyzoites in the peritoneal cavity were evaluated in mice for GP, RP, AP, GS, RS, and AS. The inhibition rate was also calculated. Untreated KM mice had 6.83×10^6 tachyzoites in perito-

Table 1: *In vitro* anti- *T. gondii* activity of total polysaccharides and saponins by MTT assay^a

Items	IC ₅₀ ^b in GES-1 cells (mg/L)	IC ₅₀ ^c in <i>T. gondii</i> (mg/L)	SI ^d
GP	272.6±9.61	313.8±22.07	0.9
RP	324.5±7.64	302.0±16.17	1.1
AP	256.2±9.85	282.7±8.74	0.9
GS	302.9±2.31	299.2±0.58	1.0
RS	275.3±3.79	270.9±8.14	1.0
AS	295.2±6.11	321.0±12.01	0.9
SPI	225.8±4.04	210.9±16.52	1.1

^aEach value is expressed as the mean ± SD (n = 3). ^bIC₅₀ in GES-1 cells = concentration required to reduce GES-1 cells growth by 50%. ^cIC₅₀ in *T. gondii* infected GES-1 cells = concentration required to reduce *T. gondii* infected GES-1 cells growth by 50%. ^dSI = selectivity index, a measure of efficacy, calculated by IC₅₀ in GES-1 cells / IC₅₀ in *T. gondii* infected GES-1 cells.

neal cavity (Toxo group), the tachyzoite inhibition rate in the peritoneal cavity of mice were 26.0% (GP, $p < 0.05$), 23.7% (RP, $p < 0.05$), 20.3% (AP, $p < 0.05$), 28.0% (GS, $p < 0.05$), 23.2% (RS, $p < 0.05$), 11.3% (AS), 22.0% (SPI, $p < 0.05$) after treatment, respectively, and the tachyzoite number was significantly reduced (Table 2).

Table 2: Anti- *T. gondii* activity in vivo^a

Groups	Number of tachyzoites (×10 ⁶)	Inhibition rate (%) ^b
Toxo	6.83±1.36	-
GP	5.06±1.13*	26.0
RP	5.22±1.41*	23.7
AP	5.44±0.19*	20.3
GS	4.91±0.35*	28.0
RS	5.25±1.53*	23.2
AS	6.05±0.91	11.3
SPI	5.33±0.16*	22.0

^aEach value is expressed as the mean ± SD (n=6). ^bMice peritoneal *T. gondii* inhibition rate, calculated by (untreated group – treated group)/untreated group × 100%.

2.3. Effects of total polysaccharides and saponins on the organ properties of *T. gondii* infected in vivo

To explore the organ protection effect of total polysaccharides and saponins on mice infected with *T. gondii*, the liver and spleen of mice were weight, liver and spleen index were calculated. As shown in Fig. 1, there were no significant differences in the weight increment and liver index data between each group in the liver index experiment, and they were not statistically significant. The spleen index of the normal group was significantly lower than that of the *T. gondii* infection group; it was shown that the splenomegaly of organ related to the immune system was caused by acute *T. gondii* infection ($p < 0.05$). After treatment by total polysaccharides and saponins, the phenomenon of splenomegaly of mice for *T. gondii* infection was decreased especially ($p < 0.05$) for GP.

2.4. Effects of total polysaccharides and saponins on the organ biochemical parameters in liver

To evaluate the toxicity and liver protection effect of total polysaccharides and saponins, the alanine aminotransferase (ALT), aspartate aminotransferase (AST) level of serum and glutathione (GSH), malondialdehyde (MDA) level were assessed (Fig. 2). Compared with Normal group, the serum of *T. gondii* infected mice (Toxo group) increased significantly ($p < 0.05$, $p < 0.01$), ALT increased from 22.17 ± 2.47 U/L to 74.78 ± 2.20 U/L, and AST increased from 22.52 ± 6.86 U/L rises to 70.15 ± 8.11 U/L. After the treatment of total polysaccharides and saponins, ALT and AST level are reduced to a certain extent on average. Among them, the ALT level of mice treated with GS, RS, AS and RP were significantly decreased ($p < 0.05$) and compared with SPI, it was also

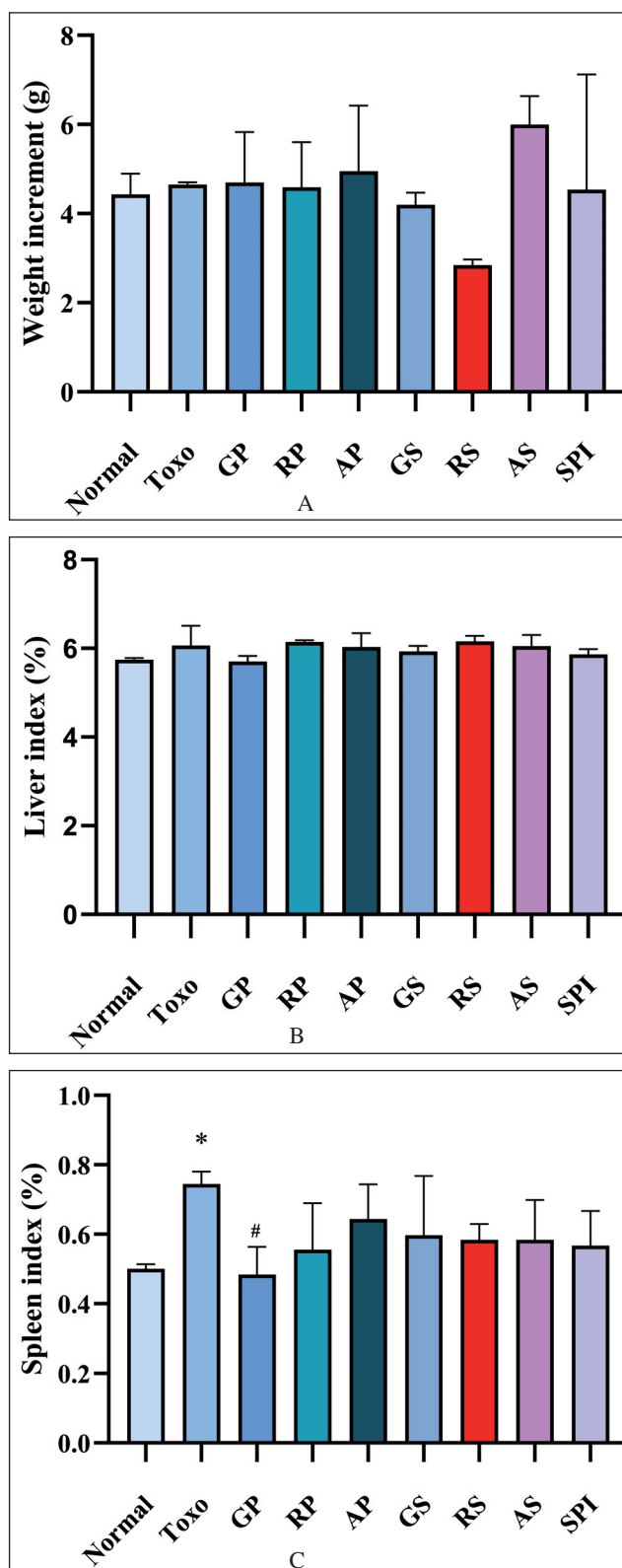


Fig. 1: Effect of total polysaccharides and saponins on weight increment (A), liver index (B), spleen index (C) in *T. gondii*-infected KM mice. Values are expressed as mean ± S.D (n=6). * $p < 0.05$ compared with Toxo group; # $p < 0.05$ compared with Normal group.

significantly decreased ($p < 0.05$) and returned to the normal level. For the change of AST level, RP, AP and GS, AS were decreased ($p < 0.05$). The infection of *T. gondii* resulted in a significant decrease in the level of GSH ($p < 0.05$), and a significant increase in MDA level, as shown in Fig. 2. The treatment of total polysaccharides and saponins, the reduction of GSH level has been alleviated, especially, for RP ($p < 0.01$), RS ($p < 0.01$) and AS

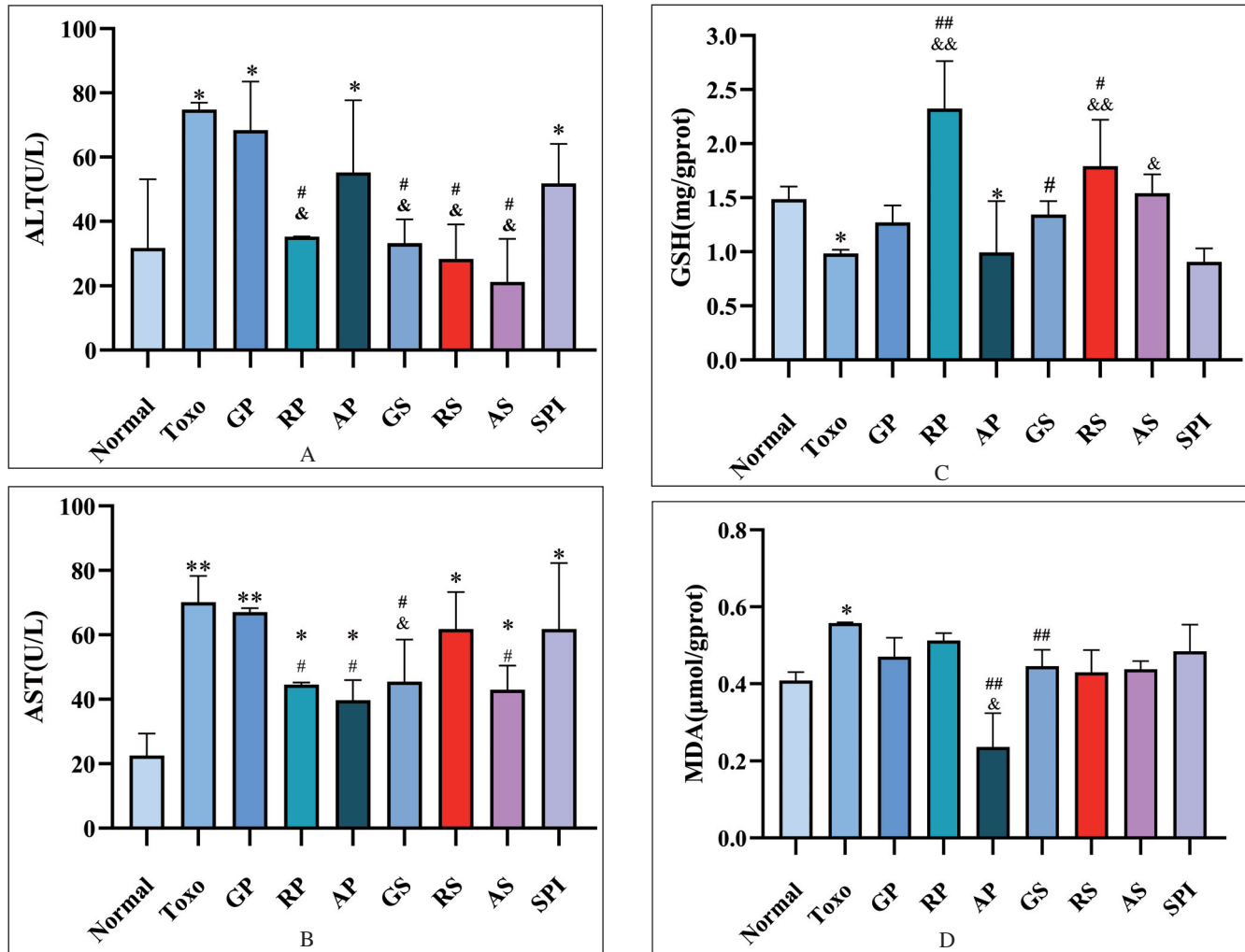


Fig. 2: Effect of total polysaccharides and saponins on ALT (A), AST (B), GSH (C), MDA (D) level in *T. gondii*-infected KM mice. Values are expressed as mean ± S.D (n=6). * $p < 0.05$, ** $p < 0.01$ compared with Normal group; # $p < 0.05$, ## $p < 0.01$ compared with Toxo group; & $p < 0.05$, && $p < 0.01$ compared with SPI group.

($p < 0.05$). The treatment of AP ($p < 0.01$), GS ($p < 0.01$) alleviated the increase of MDA which was caused by *T. gondii* infection. Although there is no significance, the other total polysaccharides and saponins can relinquish MDA level decrease to normal level.

2.5. Histology

The H&E staining was used to evaluate the liver and spleen protection effect of total polysaccharides and saponins for *T. gondii* infection, as shown in Fig. 3. The liver of the normal group was smooth and shiny, showing crimson, while the liver of other mice infected with *T. gondii* showed varying degrees of whiteness on the surface and there is white film on the liver surface, and the whole liver was darker. The treatment of total polysaccharides and saponins, the symptoms were alleviated. Toxo group has the most obvious splenomegaly, with more surface wrinkles, darker color than other groups. It may be caused by the destruction of spleen red marrow cells and melanin outflow. The treatment of total polysaccharides and saponins, this sympathy has been relieved, the folds have been reduced. In these items, GP showed significant effect. In Fig. 3A, the hepatocytes of normal group are intact, the nucleus is round, and the cytoplasm is rich. The liver of mice with acute infection of *T. gondii* had obvious lesions, hepatocyte nuclear metastasis, cytoplasm loss, the overall morphology of liver cells was missing, and inflammatory infiltration around the hepatic portal vein (as shown in Fig. 3). After treatment of total polysaccharides and saponins, the symptoms were significantly reduced. The distribution of white marrow and red marrow in the spleen cells of normal group can be seen. The structure of the spleen body is clear, the central artery is obvious, and occasionally giant nucleus cells of the spleen

are occasionally seen. *T. gondii* infected mice with a necrosis area, congestive bands around the necrosis area, the normal spleen cell structure disappears, massive loss of cytoplasm, and destruction of the complete spleen cells; after the treatment of total polysaccharides and saponins, the symptoms have improved, but spleen cell necrosis and morphology. GP is the most effective in alleviating spleen damage caused by acute infection of *T. gondii*. The overall spleen cell structure is clearly visible, the morphology is relatively intact, and the cytoplasmic deletion is reduced.

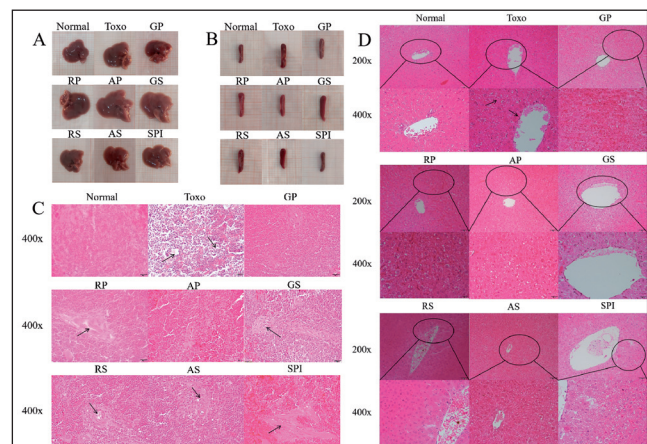


Fig. 3: Straight and histopathological photos of mice livers (A, D), spleens (B, C) with H&E staining.

2.6. Effect of total polysaccharides and saponins on the survival rate of *T. gondii*-infected mice

To verify whether total polysaccharides and saponins can prolong the survival time of mice infected with *T. gondii*, the survival rate of mice was calculated, as shown in Fig. 4. In mice acutely infected with the rapid colonization of *T. gondii*, there was a phenomenon of hair towering, body temperature drop, abdominal swelling, white contents visible in the corners of the eyes, and the appetite gradually decreased. There was no drinking water and eating phenomenon until 7th day and the death of mice was observed in Toxo group, all mice were dead in 9th day. In GP group, the death of mice was observed in 8th day, and all mice were dead in 11th day, which slightly extended the time of death of mice; the mice in RP group were dead from the 9th day until the 11th day; the mice in AP group were dead from the 8th day, and the majority of the mice were dead in the 9th day, however there are 1/6 mice survived until the end of the observation period; the survival rate of mice treated by GS is highest. Except for the death of mice in the 9th day, no mouse was dead at the rest of the observation period. Although the mice also experienced body temperature drop, hair and loss of appetite in the early stage of the observation period, this phenomenon disappeared gradually and finally returned to normal. There was no obvious abdominal swelling in mice in the GS group during the whole observation period, and the survival rate of GS group was 83.3%; the mice treated with RS began to be dead in the 8th day until the 10th day, however, the rest of the mice gradually took a turn for the better in the consecutive observation period, the survival rate of SP group is 33.3%; the mice in AS group were dead from the 8th day until the 10th day, but there was no mouse death in the 11th day, until the 13th day the remaining mice gradually took a turn for the better in the consecutive observation period; the mice treated with SPI were dead from 8th day. The mice infected *T. gondii* were dead every day until the 10th day. In summary, the survival rate of mice treated with GS was the highest at 83.3%, the survival rate in RS group was 33.3%, and the survival rate of both AP and AS group was 13.3%.

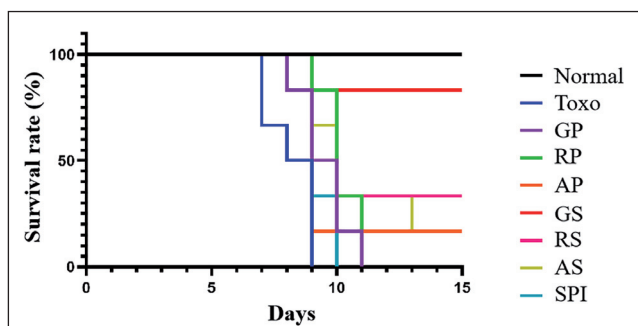


Fig. 4: Effect of total polysaccharides and saponins on the survival rate of KM mice acutely infected with 2×10^3 tachyzoites of *T. gondii* RH.

3. Discussion

Polysaccharides extracted from ginseng is one of the main active ingredients in ginseng (Wang et al. 2015), which play an important role in anti-tumor, anti-inflammatory, antioxidant, human immunity, and intestinal flora balance regulation (Jiao et al. 2014; Kim et al. 2009), also have activity to regulate hyperglycemia, hyperlipidemia, anti-fatigue, antioxidant, anti-radiation, and maintain intestinal flora (Kwak et al. 2005; Lee et al. 2006; Reyes et al. 2016; Wang et al. 2014b). Another important active ingredient in ginseng was saponins which played a variety of roles in cardiovascular diseases, diabetes, cancer, immune stimulation and inflammatory reactions (Liu et al. 2020). This situation can be linked to the Chinese medicine history of ginseng to improve individual's immunity; this promotes us to evaluate the polysaccharides and saponins of ginseng, red ginseng and American ginseng's anti-*T. gondii* activity *in vitro* and *in vivo* in this study.

In vitro anti-*T. gondii* activity was shown as SI, the stronger the activity against *T. gondii* and the lower the cytotoxicity (Jin et al. 2012). All the total polysaccharides and saponins showed low cytotoxicity to host cells (Table 1), and the SI of RP (1.1), GS (1.0), AS (1.0) was similar to SPI (1.1). It seems that RP, GS, AS showed the effect of anti-*T. gondii*, but *T. gondii* is a kind of complex and changeable parasite, the situation *in vitro* and is different from *in vivo* (Martorelli Di Genova and Knoll 2020). The effects of GP, RP, AP, GS, RS and AS *in vivo* were analyzed in acute *T. gondii* infection model in KM mice. GP, RP, AP, GS and AS can decrease the number of tachyzoites in the peritoneal cavity of infected mice significantly ($p < 0.05$) and the inhibition rate is higher than SPI except for AP (Table 2). The sequence of the inhibition rate was as follows: GS > GP > RP > RS > AP. These results indicated that GS, GP, RP, RS, AP exhibited anti-*T. gondii* activity *in vivo* and GS, GP, RP, RS were better than SPI.

Liver undertake in the metabolism and detoxification of endogenous and exogenous harmful material (Ogoke et al. 2017), acute *T. gondii* infection usually causes liver damage (Sibley and Boothroyd 1992). Spleen is responsible for coordinating innate immune responses and adaptation, thus the effect of total polysaccharides and saponins to spleen cannot be ignored. To evaluate hepatotoxicity of total polysaccharides and saponins, we measured liver index, spleen index, ALT and AST (Carobene et al. 2013). As shown in Fig. 1, the change of weight increment and liver index caused by *T. gondii* infection was not significant, but splenomegaly of organs related to the immune system was caused by acute *T. gondii* infection was obvious. Acute toxoplasmosis can attack the various tissues and organs include spleen, one of the most immune organs, would cause serious immunology response which will cause splenomegaly (Neves et al. 2009). With the treatment of GP, the splenomegaly caused by *T. gondii* infection was alleviated. GP can regulate human immunity and this activity is bidirectional immunomodulatory activity (Yang et al. 2023). Although the mechanism is not clear, GP plays an exact role on protecting spleen damage of *T. gondii* infection. This situation can be verified in histology results (Fig. 3). The liver and spleen damage caused by *T. gondii* infection were alleviated by total polysaccharides and saponins in tissue and cellular level. The levels of serum ALT and AST are extremely sensitive indicators to evaluate liver injury, and their elevation roughly reflects the degree of the damage. RP, GS, RS, AS also alleviated the ALT level and RP, GS, AS alleviated the AST level increase caused by *T. gondii* infection (Fig. 2).

T. gondii infection usually cause oxidative stress organ damage which is the unbalance of host immune system (Denkers 1999). The results (Fig. 2) of GSH and MDA showed that the total polysaccharides and saponins exhibited the ability to regulate the oxidative stress caused by *T. gondii* infection. This may be related to the polysaccharides and saponins can increase the levels of catalase and superoxide dismutase in liver to scavenge hydroxyl free radicals (Wang et al. 2014a) and alleviate organs damage.

As the above results indicated, the total polysaccharides and saponins has anti-*T. gondii* effect, thus we verified that through survival rate measurement. The Kaplan-Meier plot (Fig. 4) showed that all total polysaccharides and saponins can increase survival time of KM mice infected *T. gondii* acutely especially with the treatment of GS, RS, AS, AP, there are mice did not die and showed the increase of food intake and physical activities compared with mice with no extract treatment during the whole period. That means the total polysaccharides and saponins could be used for the chronic toxoplasmosis infection usually caused by parasite food-borne transmission. The composition of total polysaccharides and saponins was complex, kinds of biology effect were displayed (Chen et al. 2020). It's strange that there is a situation not for single compound, but the combination of several compounds will showed better therapeutic effect especially for Chinese herbs (Yang et al. 2014). Our study showed that total polysaccharides and total saponins, evaluated their anti-*T. gondii* activity. These total polysaccharides and saponins were mixtures, thus we inferred that the synergy effects of total polysaccharides and saponins led to the anti-*T. gondii* activity. In a word, AP, GS, RS and AS prolong the survival time and rate of mice infected *T. gondii* by alleviated organs damage in some biology effect.

Because ginseng, red ginseng and American ginseng contain similar chemical components, they are traditional Chinese medicinal materials and widely accepted all over the world, and ginseng, red ginseng and American ginseng have universal research value. Through the research of new anti-protozoal drugs of traditional Chinese medicine, with clear mechanism of action, safe, reliable, and completely independent intellectual property rights will improve clinical drugs and provide a new direction for the treatment of parasitic infections.

In conclusion, total polysaccharides and saponins of ginseng, red ginseng and American ginseng showed better activity on acute *T. gondii* infection with lower toxicity and hepatotoxicity. GS and RS were special in control organs damage caused by *T. gondii* infection. The oxidative stress was alleviated and more reliable protection on mice were revealed. Ginseng, red ginseng and American ginseng can be used as both of food and medicine in many countries, our study suggested total polysaccharides and total saponins of ginseng, red ginseng and American ginseng is promising medicine to be used for the treatment of toxoplasmosis, to prevent *T. gondii* infection in daily life and develop drugs for clinical treatment of toxoplasmosis.

4. Experiment

4.1. Materials

Dulbecco's Modified Eagle Medium (DMEM, HyClone®, USA), Trypsin-EDTA (Solarbio, Beijing, China), Penicillin-Streptomycin solution (Solarbio, Beijing, China) and fetal bovine serum (FBS, Gibco®, NY, USA) were used for cell culture. *Panax ginseng* C. A. Meyer (ginseng), *P. quinquefolius* L. (American ginseng) and red ginseng were purchased from Tong Ren Tang, Beijing, China. Spiramycin (SPI) was purchased from Aladdin Industrial Inc. (Shanghai, China). Extract was dissolved in dimethylsulfoxide (DMSO, Solarbio, Beijing, China) and diluted with DMEM to different concentrations, with the final DMSO concentration at less than 1% (v/v). All other chemicals were of reagent grade and purchased from Aladdin Industrial Inc. (Shanghai, China).

4.2. Total polysaccharides and saponins

Ginseng, red ginseng and American ginseng slices (50 g) dissolved in distilled water. The liquid ratio is 15:1. At 100 °C, refluxes three times in a row, 1.5 h each time, filtered, combined with three filters, decompression and concentrated, and obtain total polysaccharides and saponins of ginseng, red ginseng and American ginseng. The obtained extract is added to 95% ethanol for precipitation, centrifugation (2500 rpm, 10 min). The obtained sediment is soluble in water. Add reagent (positive alcohol: chloroform) = 1:4 Remove the protein and repeat it three times. The sugar solution is frozen in the -80 °C refrigerator to remove protein, centrifugalize, filter, and then vacuum dry the solution to get ginseng, red ginseng and American ginseng total polysaccharides. After drying the obtained supernatant, it is extracted 3 times with 75% ethanol reflux. The liquid ratio is 15:1, 1.5 hours each time. Combined with the filter, vacuum drying and purification, the total saponins are obtained. The total polysaccharides and saponins was measured by phenol-sulfuric acid (Jiang et al. 2014) and vanillin-sulfuric acid assay (Mammadov et al. 2017).

4.3. Cells, parasites and animals

GES-1 cells were cultured in RPMI 1640, supplemented with 0.01% Penicillin-Streptomycin and 10% heat-inactivated FBS and maintained at 37 °C and 5% CO₂. Cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Tachyzoites were from the virulent RH strain of *T. gondii* maintained by serial intraperitoneal passage in KM female mice, which were purchased from Experiment Center, Yanbian University. All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals in Yanbian University, China, and conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Number of license SCXK 2017-0003). All mice were kept in a central animal care facility with free access to water and rodent food during the experiment.

4.4. Anti-*T. gondii* activity and cytotoxicity in vitro

Anti-*T. gondii* activity and cytotoxicity of total polysaccharides and saponins *in vitro* were evaluated by MTT assay (Solarbio, Beijing) as a slightly modified procedure by Guo et al (Guo et al. 2019). GES-1 cells were seeded onto the 96-well plates (2 × 10³/well) for 24 h for obtaining the full monolayer, and then the host cells were infected with *T. gondii* (parasite: cell= 3:1) in complete medium. After 24 h, cells were washed to remove any extra parasites and then incubated with different concentrations (20, 200 and 500 mg/L) of total polysaccharides and saponins, SPI served as the positive control and DMSO as the negative control. After 24 h of treatment, MTT solution was added directly to the culture wells and then incubated for 4 h at 37 °C. The optical absorbance was measured at a wavelength of 548 nm using a microplate reader (Corona SH-1000Lab).

4.5. Anti-*T. gondii* activity and cytotoxicity in vivo

For the *in vivo* study, KM female mice were divided into groups consisting of 6 animals each and then intraperitoneally injected with 2 × 10³ tachyzoites of the *T. gondii*. After 4 h infection, mice were orally treated or not with different extracts: 0.2 mL normal saline (normal group and negative control group), 100 mg/kg total ginseng polysaccharides (GP group), 100 mg/kg total red ginseng polysaccharides (RP group), 100 mg/kg total American ginseng polysaccharides (AP group), 100 mg/kg total ginseng saponins (GS group), 100 mg/kg total red ginseng saponins (RS group), 100 mg/kg total American ginseng saponins (AS group) and 100 mg/kg SPI, respectively, once a day for 4 days. At the last day, heart blood samples were collected after anesthesia to separate the serum, and then mice were sacrificed by cervical dislocation. Tachyzoites were harvested from mice peritoneal cavities to determine the level of tachyzoites proliferation (Zhang et al. 2016).

4.6. Visceral weights and liver biochemical parameters

The livers and spleens were weighed. The GSH was measured according to the method (Deng et al. 2020). The liver homogenate was mixed with half volume trichloroacetic acid (20%, w/v) and centrifuged at 4000 rpm for 10 min. Then, phosphate buffer (phosphate 0.3 mol/L, pH 7.5) and 5,5-dithio-bis-(2-nitrobenzoic acid) (0.04%, w/v) were added to the separated supernatant and mixed thoroughly. After 5 min at room temperature, the absorbance was measured at 412 nm. MDA was measured by the standard method (Luan et al. 2019) with minor modifications. The liver homogenate supernatant was mixed with thiobarbituric acid (0.5%, w/v) and heated in boiling water bath for 1 h, then cooled quickly and centrifuged at 6000 rpm for 10 min, the absorbance of pink colored supernatant was measured at 532 nm. Tetraethoxypropane replaced the liver homogenate in the standard sample. Serum levels of AST and ALT were measured according to the method of (Shang et al. 2021). The substrate reaction of ALT or AST and serum was carried out under incubation at 37 °C for 30 min, then added 2,4-DNPH (2,4-Dinitrophenylhydrazine) and held for 20 min. Finally, NaOH was added and allowed to react for 5 min. The absorbance at 505 nm was measured.

4.7. Survival analysis of *T. gondii*-infected mice

Five groups of 6 mice each were intraperitoneally injected with 2 × 10³ tachyzoites of *T. gondii*. After 4 h, the negative control group was orally treated with 0.2 mL normal saline, the mice in different treated groups were orally administered with total polysaccharides and saponins. The mice were observed every day and the percentages of mice survived were calculated during 15 days.

4.8. Statistical analysis

All data was expressed as mean ± standard deviation (S.D.) triplicate. Statistical analyses and graphs were performed using SPSS 16.0 software (SPSS Inc., Chicago) and GraphPad Prism 5.0 (GraphPad Software Inc., San Diego). The method of Kaplan and Meier was used to compare the survival rates of the studied groups. A value of *p* < 0.05 was considered statistically significant.

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Conflicts of interest: None declared.

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