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Study on the mechanisms of *Suaeda rigida* polysaccharides on the heart inhibition and skeletal muscle promotion in the frog

[*Estudo sobre os mecanismos dos polissacarídeos de* Suaeda rigida *na inibição do coração e promoção da musculatura esquelética no sapo*]

A.L. *Sha*[1], H.Y. *Hao*[2*]

[1]School of Teacher Education, Chongqing Three Gorges University, 404120, Wanzhou, Chongqing, P.R. China
[2]School of Environmental and Chemical Engineering, Chongqing Key Laboratory of Water Environment Evolution and Pollution Control in Three Gorges Reservoir Area, Chongqing Three Gorges University, 404120, Wanzhou, Chongqing, P.R. China

ABSTRACT

The objectives of this study were to investigate the effects of different concentrations of *Suaeda rigida* polysaccharides (SRPs) on the physiological characteristics of the frog heart and gastrocnemius muscle, compare their similarities and differences, and analyze the mechanisms. $CaCl_2$ and acetylcholine (Ach) were selected respectively to be co-incubated with the high concentration SRPs to observe the effects on the heart contraction of frog. The effects of different concentrations of the SRPs on the activities of acetylcholinesterase (A-CHE), Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase in the isolated frog heart were detected by UV-Vis spectrophotometry. The gastrocnemius muscle was immersed in the high concentration of SRPs for 10 min, and the systolic indexes were recorded. The effects of SRPs on the Ach content and A-CHE activity at the sciatic nerve-gastrocnemius junction were determined by UV-Vis spectrophotometry and enzyme-linked immunosorbent assay (ELISA). The results showed that the SRPs had significant inhibitory effects on the contractile amplitude of isolated heart and the contractile amplitude induced by $CaCl_2$ and Ach, respectively (P<0.01). The activity of Ca^{2+}-Mg^{2+}-ATPase was significantly promoted, and the activity of A-CHE was significantly inhibited (P<0.01). The contraction amplitude, contraction rate, relaxation rate of gastrocnemius muscle and the Ach content at the junction of sciatic nerve-gastrocnemius muscle were significantly increased (P<0.01), and the activity of A-CHE at the junction was significantly inhibited (P<0.01) by the SRPs. All the results suggested that the SRPs could inhibit the contraction of heart and promote the contraction and relaxation of skeletal muscle. The mechanism was related to blocking the fast I_{Na} channel, inhibiting the $I_{Ca\text{-}L}$ and activating the M receptors of myocardial membrane and then inhibiting external Ca^{2+} influx, increasing Ca^{2+}-Mg^{2+}-ATPase activity, decreasing a-che activity.

Keywords: *Suaeda rigida* polysaccharides, heart, sciatic nerve-gastrocnemius muscle, contraction, Ca^{2+}, Ach

RESUMO

Os objetivos deste estudo foram investigar os efeitos de diferentes concentrações de Suaeda rigida *polissacarídeos (SRPs) sobre as características fisiológicas do coração de rã e do músculo gastrocnêmio, comparar suas semelhanças e diferenças, e analisar os mecanismos. CaCl2 e acetilcolina (Ach) foram selecionados respectivamente para serem co-incubados com os SRPs de alta concentração para observar os efeitos sobre a contração do coração de rã. Os efeitos das diferentes concentrações dos SRPs sobre as atividades da acetilcolinesterase (A-CHE), Na+-K+-ATPase e Ca2+-Mg2+-ATPase no coração isolado de rã foram detectados pela espectrofotometria UV-Vis. O músculo gastrocnêmio foi imerso na alta concentração de SRP por 10 min, e os índices sistólicos foram registrados. Os efeitos das SRPs no conteúdo de Ach e na atividade de A-CHE na junção nervo-gastrocnêmio ciático foram determinados*

*Corresponding author: saldky@126.com
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pela espectrofotometria UV-Vis e pelo ensaio de imunoabsorção enzimática (ELISA). Os resultados mostraram que os SRPs tiveram efeitos inibidores significativos sobre a amplitude contrátil do coração isolado e a amplitude contrátil induzida por CaCl2 e Ach, respectivamente (P<0,01). A atividade do Ca2+-Mg2+-ATPase foi significativamente promovida e a atividade do A-CHE foi significativamente inibida (P<0,01). A amplitude de contração, a taxa de contração, a taxa de relaxamento do músculo gastrocnêmio e o conteúdo de Ach na junção do músculo nervo ciático-gastrocnêmio foram significativamente aumentados (P<0,01), e a atividade do A-CHE na junção foi significativamente inibida (P<0,01) pelas SRPs. Todos os resultados sugeriram que os SRPs poderiam inibir a contração do coração e promover a contração e o relaxamento do músculo esquelético. O mecanismo estava relacionado ao bloqueio do canal INa rápido, inibindo a ICa-L e ativando os receptores M da membrana miocárdica e depois inibindo o influxo externo de Ca2+, aumentando a atividade de Ca2+-Mg2+-ATPase, diminuindo a atividade a-che.

Palavras-chave: Suaeda rigida *polissacarídeos, coração, músculo nervo-gastrocnêmio ciático, contração, Ca2+, Ach*

INTRODUCTION

Suaeda rigida Kung et G.L.Chu is an endemic species in Tarim Basin, which belongs to Caryophyllales, Chenopodiaceae, Suaeda (Feng *et al*., 2003). With high nutrient content, it is an excellent forage resource in Tarim Basin. Sheep, rabbits, and camels all like to eat it, and locals often collect its tender leaves for wild vegetables (Sha *et al*., 2013a). It is expected to provide new ideas for the development of its medicinal and feeding value by studying the effective nutrients of its pharmacological effects. Our previous research on the chemical components, feeding and medicinal value of *Suaeda rigida* have shown that *Suaeda rigida* contains polysaccharides, alkaloids, flavonoids, volatile oil, saponins and other chemical components (Hao and Sha, 2013), and dietary supplementation of *Suaeda rigida* can improve the antioxidant capacity (Hao *et al*., 2017), growth performance and disease resistance of Karakul sheep (Sha *et al*., 2013a). These results indicated that *Suaeda rigida* contains a variety of active components, which has good feeding and medicinal value. Our previous studies in mice have shown that *Suaeda rigida* polysaccharides (SRPs) can play a protective role in the normal tissue cells by significantly inhibiting nitric oxide synthase (NOS) and ultimately inhibiting excessive nitric oxide (NO) production (Sha *et al*., 2013b). Therefore, it has been confirmed that the polysaccharide is one of the important active ingredients of *Suaeda rigida*, but there are no reports on other medicinal and feeding values of the SRPs. Previous studies have shown that plant polysaccharides can protect myocardium and delay skeletal muscle fatigue (Zhu *et al*., 2018; Go *et al*., 2018). The aims of this study are to investigate the effects of the SRPs on the physiological characteristics of the frog heart and gastrocnemius muscle, compare their similarities and differences, and analyze the mechanisms. It is expected to provide theoretical foundation and scientific basis for the development and utilization of other medicinal and feeding values of the SRPs. The heart of a frog is composed of cardiac muscle, while its gastrocnemius is composed of skeletal muscle. The physiological characteristics and contraction mechanism of cardiac muscle and skeletal muscle are different in cell structure, contractility, excitability, conductivity and so on. Therefore, when the SRPs act on the heart and gastrocnemius of frog, both the degree of influence and the changes on the physiological characteristics of the two are likely to be different.

MATERIALS AND METHODS

Healthy adult *Rana nigromaculata* (110±10 g, n=56 for both sex), were purchased from the Experimental Animal Center attached to Tarim University. They were kept for 3 days in large tanks with aerated circulating water and standard food. The care and use of frogs were approved by the Ethic-Scientific Committee for Experiments on Animals of Tarim University (Number of the project approval certificate 2019-022815).

Suaeda rigida was collected in the 12th Regiment of the First Division of Xinjiang Production and Construction Corps, which was identified by Dr. Zhaoping Yang, Department of Botany, Tarim University, then dried in the

shade and crushed. Acetylcholine chloride (Ach), verapamil hydrochloride (VPLh) and pilocarpine nitrate (PCPn) were purchased from Sinopharm Chemical Reagent Co., Ltd., China. Commercial kits used for the determination of acetylcholine esterase (A-CHE), adenosine triphosphatase (ATPase, including Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase), coomassie brilliant blue protein were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Acetylcholine (Ach) ELISA kit was from cloud-clone Co., USA. Ringer's solution (NaCl 0.111 mol/L, $NaHCO_3$ 0.2×10^{-2} mol/L, KCl 0.2×10^{-2} mol/L, NaH_2PO_4 0.8×10^{-4} mol/L, $CaCl_2$ 0.1×10^{-2} mol/L, glucose 0.011 mol/L in distilled water; all reagents used were analytical pure, purchased from Sinopharm Chemical Reagent Co., Ltd., China).

The preparation program of SRPs was improved according to our previous research methods (Sha *et al*., 2013b). 500 g of *Suaeda rigida* powders were weighed (sieved through a 40-mesh sieve), 85% ethanol was added to reflux together in a water bath for 1h. They were defatted 3 times and then filtered, the filtrate was discarded, and the residue was placed in a distillation flask (500 ml), sealed for 3 h (80°C). The filtrate was filtered and collected, and the residue was repeatedly extracted for 3 times. The filtrates were combined, and the protein was removed by Sevage method. After decolorization with 30% H_2O_2 (40°C, 4h), the concentrated solution was precipitated with anhydrous ethanol, centrifuged at 3000 rpm for 30 min, the precipitates were collected, dried in vacuum (60°C) and weighed to obtain 38.10 g SRPs. The gray-white solid flocculent FOPs (2.00 g) were dissolved in distilled water through a 100 mL volumetric flask to obtain the high concentration of SRPs solution (20mg/mL). Then, the solution was diluted twice, and 10, 5 mg/mL medium and low concentrations of SRPs were obtained, respectively.

The brain and spinal cord of the frog were destroyed to expose the whole heart. Intubation was carried out using the conventional method of frog's heart intubation, after the frog's heart cannula was fixed, the blood of isolated heart was replaced with fresh Ringer's solution in time (Ai, 2014). The frog's heart apex was picked out by frog heart clips and connected with the tension transducer through a thin wire. Then, the Medlab biological signal acquisition and processing system (Nanjing Medease Science and Technology Co., Ltd., China) was connected through the guide electrodes, and the system parameters were set according to the conventional method (Ai, 2014). The isolated hearts were perfused with low, medium and high concentrations of the SRPs solution respectively. After each perfusion, the hearts were washed with the Ringer's solution for 3-5 times, and the next step was performed after the cardiac contraction curve was restored. The myocardial contraction amplitude and heart rate observed before and after administration were recorded respectively. It was observed that the myocardial contraction amplitude and heart rate returned to before the addition of the SRPs solution after about 20-30 seconds of rinse with the Ringer's solution. The effect of the SRPs solution is reversible in myocardium.

When the systolic curve of frog was stabilized, 2 drops of $CaCl_2$ (0.1 mol/L) or Ach (0.1μmol/L) were added into the Ringer's solution in the cannula, and after 6 minutes of treatment, the high concentration of the SRPs were added to observe and record the changes of the frog's systolic amplitude. The VPLh (0.1μmol/L) or PCPn (0.1μmol/L) was given as a positive control.

The frogs were randomly divided into 4 groups, i.e., the Ringer's solution group (control group), SRPs-treated groups of the low, medium, and high concentration (n=9 for each group). The isolated frog hearts of each group were prepared, soaked with drugs of each group for 5min, then cut into pieces, and the ice bath homogenate was prepared into 10% tissue homogenate. After centrifugation at 4000 rpm for 8 min, the supernatant was taken, and the activities of A-CHE, Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase and the protein content of each group were determined according to the respective detection kits by UV-Vis (Shanghai Lengguang Technology Co., Ltd., China).

According to our previous research methods (Sha *et al*., 2017), the sciatic nerve-gastrocnemius muscle specimens of frog were prepared in vivo, and the Ringer's solution was dripped into the gastrocnemius muscle for 10-20min to infiltrate the gastrocnemius muscle. A thin wire was used to connect the gastrocnemius muscle with the

tension transducer, and then a guide electrode was used to connect the MedLab biological signal acquisition and processing system. The system parameters were set according to the conventional method and the contraction indexes of the gastrocnemius muscle were recorded (Ai, 2014). The gastrocnemius muscle was completely immersed into a culture dish containing the high concentration SRPs (20mg/mL). After 10min of immersion, the indexes of gastrocnemius muscle contraction were recorded immediately. The threshold stimulation, maximum stimulation of the sciatic nerve-gastrocnemius specimen and the contraction latency, contraction amplitude, contraction time and diastolic time of the gastrocnemius muscle were recorded before and after administration. After repeated rinse, the gastrocnemius muscle was immersed in the Ringer's solution for 10 min. After re-measurement and observation, the contraction indexes of gastrocnemius muscle could be restored to before soaking the high concentration SRPs. The effect of the SRPs solution is reversible in gastrocnemius muscle.

A sharp blade was used to take the specimens from the sciatic nerve-gastrocnemius junction. Part of the specimens was immediately placed in a plastic tube, sealed and frozen with liquid nitrogen to inactivate the A-CHE. Then the content of Ach was quickly detected by a powerwave XS full wavelength Microplate Reader (Bio-Tek, USA), according to the ELISA kit instructions provided by cloud-clone Co., USA. In addition, 0.1g of tissue samples were taken, and 1 mL of extract was added to ice bath homogenate. After centrifugation at 4℃ at 8000 rpm for 10min, the supernatant was taken, and the activity of A-CHE was determined according to the kit instructions provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

The contraction rate and diastolic rate of the gastrocnemius were calculated by using the formulas: contraction rate (g/ms) = contraction amplitude / contraction time, and diastolic rate (g/ms) = contraction amplitude / diastolic time. The formulas were used: inhibition rate (%) = (mean value before administration - mean value after administration) / mean value before administration×100%; promotion rate (%) = (mean value after administration - mean value before administration) / mean value before administration×100%, in which the rates of change were divided into inhibition rate and promotion rate, and the inhibitory rate was indicated by adding "-" before the number. All the experimental data were expressed as the mean plus or minus standard deviation (SD). SPSS23.0 statistical software (SPSS Inc.) was used to conduct t-test or one-way ANOVA on the data. One-way ANOVA was used to compare the differences between the control groups and the experimental groups of the SRPs, and Dunnett's t-test for further comparison among groups. Paired t-test was used to compare the differences between the two groups before and after treatment of the SRPs. Differences were regarded as significant at $P<0.05$, extremely significant at $P<0.01$.

RESULTS

As shown in Table 1 and Figure 1, compared with those before administration, the three SRPs-treated groups (5, 10, 20mg/mL) had extremely significant inhibitory effect on the myocardial contraction amplitude of the frog ($P<0.01$). The inhibition rates from low to high concentrations were 52.34%, 36.00% and 70.31%, respectively.

Table 1 Effects of the SRPs on the contractile amplitude of the isolated frog heart（$\bar{x}$±SD, n=9）

Groups	Concentrations / mg. ml^{-1}	Contractile amplitude（g）		Inhibition rate (%)
		Before administration	After administration	
Low	5.00	3.96±0.38	1.89±0.35**	52.34%
Intermediate	10.00	4.07±0.47	2.61±0.75**	36.00%
High	20.00	4.06±0.27	1.20±0.51**	70.31%

Note: compared with before administration, $^{**}P<0.01$.

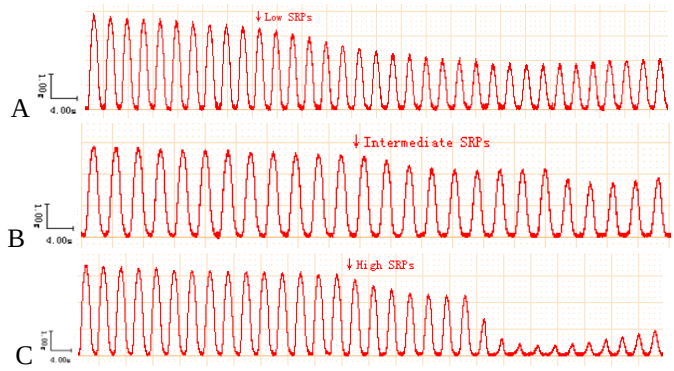

Figure 1 Effects of the SRPs on the contraction of the isolated frog heart

Compared with those before administration, the low concentration of SRPs (5mg/mL) had inhibitory effect on the heart rate of frog (inhibition rate 9.78%), while the intermediate and high concentration of SRPs (10, 20mg/mL) had promoting effect on the heart rate of frog (promotion rate was 6.25% and 5.28%, respectively). Although different concentrations of the SRPs had slight effects on the heart rate of frogs, the effects were not significant (P>0.05) (Table 2, Figure 1).

Table 2 Effects of the SRPs on the heart rate of the frog ($\bar{x}$ ±SD, n=9)

Groups	Concentrations / mg. ml^{-1}	Heart rate（times·min^{-1}）		Rate of change (%)
		Before administration	After administration	
Low	5.00	30.67±2.88	27.67±4.23	-9.78%
Intermediate	10.00	32.00±3.58	34.00±3.58	6.25%
High	20.00	34.33±5.68	36.33±7.28	5.28%

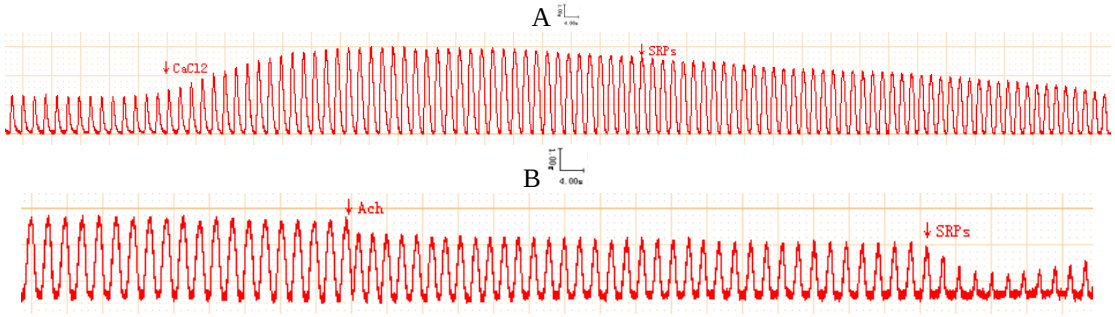

Figure 2 Effects of the SRPs on the contraction of the isolated frog heart induced by $CaCl_2$ and Ach, respectively

The application of $CaCl_2$ (0.1mol/L) alone significantly increased the systolic amplitude (7.58±0.82g) of the isolated frog heart compared with the one before administration (P<0.01). The systolic amplitude was significantly decreased (5.46±0.47g) after addition of the high concentration SRPs (20mg/mL) (P<0.01), and the effect was like that of VPLh positive control (5.21±0.43g). The cardiac systolic amplitude (2.85±0.23g) of the isolated frog was significantly reduced by single application of Ach (P<0.01), and the systolic amplitude was further decreased (2.04±0.18g) by addition of the high concentration SRPs (P<0.01), and the effect was equivalent to that of PCPn positive control (1.90±0.14) (Table 3, Figure 2).

Table 3 Effects of the SRPs on the systolic amplitude of the isolated frog heart induced by $CaCl_2$ and Ach, respectively （$\bar{x}$ ±SD, n=9）

	Before administration	Induced drugs	High SRPs	positive control
Systolic amplitude（g）	4.05±0.31	7.58±0.82**（$CaCl_2$）	5.46±0.47$^{\#\#}$	5.21±0.43$^{\#\#}$(VPLh)
Systolic amplitude（g）	4.04±0.29	2.85±0.23**（Ach）	2.04±0.18$^{\#\#}$	1.90±0.14$^{\#\#}$(PCPn)

Ach: Acetylcholine chloride; PCPn: Pilocarpine nitrate; VPLh: Verapamil hydrochloride.
Note: **indicates comparison before and after administration, P<0.01; $^{\#\#}$ Compared with the intervention of $CaCl_2$ or Ach, $^{\#\#}P$<0.01.

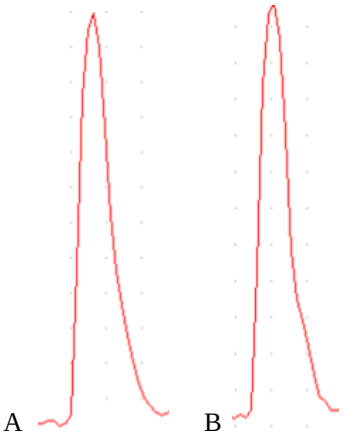

Figure 3 Effect of the SRPs on the single contraction of gastrocnemius muscle of the frog in vivo
Note: A. before infiltration; B. after infiltration of the SRPs

As shown in Table 4, the A-CHE activity in the isolated frog heart tissues of the three SRPs-treated groups (5, 10, 20mg/mL) was lower than that of the Ringer's solution group, while the Ca^{2+}-Mg^{2+}-ATPase activity was higher than that of the control group, and all showed a certain dose-dependent effect. Among which the differences in the intermediate and high concentration groups (10, 20mg/mL) were extremely significant (P<0.01), the difference of A-CHE activity in the low concentration group (5mg/mL) was extremely significant (P<0.01), and the difference of Ca^{2+}-Mg^{2+}-ATPase activity in the low concentration group was significant (P<0.05). The Na^{+}-K^{+}-ATPase activity of the three SRPs-treated groups was higher than that of the control group, but the differences were not significant (P>0.05).

Table 4 Effects of the SRPs on the activities of A-CHE, Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase in the isolated frog heart tissues（$\bar{x}$ ±SD，n=9）

Groups	Concentrations / mg．ml^{-1}	A-CHE (U/mg)	Na^{+}-K^{+}-ATPase (U/mg)	Ca^{2+}-Mg^{2+}-ATPase (U/mg)
Control group	—	0.29±0.03	0.39±0.07	1.46±0.15
Low SRPs	5.00	0.22±0.03**	0.44±0.08	1.82±0.30*
Intermediate SRPs	10.00	0.18±0.02**	0.41±0.10	2.13±0.34**
High SRPs	20.00	0.13±0.04**	0.43±0.07	2.40±0.28**

A-CHE: acetylcholinesterase.
Note: Compared with the control groups. $^{**}P$<0.01, * P<0.05.

In this study, the effects of the SRPs on the physiological characteristics of the frog gastrocnemius muscle were expressed by the experimental data of sciatic nerve-gastrocnemius muscle specimens in vivo.

Compared with those before infiltration, the threshold and maximum stimulation of sciatic nerve-gastrocnemius muscle of the frog in vivo were increased after infiltration of the high concentration SRPs (promotion rate was 11.76% and 32.14%, respectively). Among them, the threshold stimulation was not significantly increased (P>0.05), but the maximum stimulation was very significantly increased (P<0.01) (Table 5).

As shown in Table 6 and Figure 3, the high concentration SRPs (20mg/mL) increased the contraction latency, contractile amplitude, contractile rate, relaxation rate of gastrocnemius muscle and the Ach content at the junction of sciatic nerve-gastrocnemius muscle of the frog compared with the ones before infiltration. Among them, there was no significant effect on the contraction latency (P>0.05), but the effects on the contractile amplitude, contractile rate, relaxation rate and ACh content were very significant (P<0.01), especially the relaxation rate. The relaxation rate of gastrocnemius muscle was greatly increased after infiltration of the high concentration SRPs, and the promotion rate was as high as 136.36%. However, the A-CHE activity at the junction of sciatic nerve-gastrocnemius muscle was significantly decreased (inhibition rate 53.81%) after infiltration of the SRPs (P<0.01). The results showed that the SRPs had significant effects on the contraction amplitude, contraction rate and relaxation rate of the gastrocnemius muscle in vivo, as well as the content of Ach and the activity of A-CHE at the junction of sciatic nerve-gastrocnemius muscle.

Table 5 Effects of the SRPs on the threshold and maximum stimulation of sciatic nerve-gastrocnemius muscle of the frog in vivo

Parameter	Before infiltration	After infiltration of the SRPs	Promotion rate (%)
Threshold stimulus (mV)	0.17±0.04	0.19±0.06	11.76%
Maximum stimulus (mV)	0.28±0.03	0.37±0.04**	32.14%

Note: compared with that before infiltration, **P<0.01. The same as table 6.

Table 6 Effects of the SRPs on the contraction latency, contractile amplitude, contractile rate, relaxation rate of gastrocnemius muscle and the Ach content, A-CHE activity in the joint of sciatic nerve-gastrocnemius muscle of the frog in vivo

Parameter	Before infiltration	After infiltration of the SRPs	Promotion rate (%)
Contraction latency (ms)	31.09±5.73	34.28±7.16	10.26%
Contractile amplitude (g)	7.52±0.93	13.40±1.05**	78.19%
Contractile rate (g/ms)	0.16±0.02	0.29±0.04**	81.25%
Relaxation rate (g/ms)	0.11±0.02	0.26±0.03**	136.36%
Ach（umol/g）	1.76±0.22	2.95±0.51**	67.61%
A-CHE（U/L）	94.08±9.73	43.46±6.25**	-53.81%

Ach: Acetylcholine chloride; A-CHE: acetylcholinesterase.

DISCUSSION

The continuous orderly and coordinated contraction and relaxation of the heart is a necessary condition for the realization of blood pumping function, and this function of the heart depends on the physiological characteristics of myocardial cells, including automatic rhythm, contractility, conductivity, excitability, etc. The rhythm of the frog's ventricle is controlled by the automatic rhythm of the venous sinus, which is the normal pacing point of the frog. The mechanisms of its automatic rhythmicity mainly depend on the delayed rectifier potassium current (I_K), inward ion current (I_f current) and T-type calcium current (I_{Ca-T}) (Wang, 2018). The action

potential from the venous sinus is transmitted to the ventricle through a special conduction system, which induces the action potential of ventricular myocytes. The mechanisms of action potential generation mainly depend on voltage-gated fast I_{Na} channel, I_{Ca-T} during the depolarization and repolarized transient outward current (I_{to}), L-type calcium current (I_{Ca-L}), I_K and inward rectifier potassium current (I_{K1}) during the repolarization (Wang, 2018), especially the depolarization process. Ventricular muscle contraction is triggered by the excitation-contraction coupling of the sarcolemma action potential, which leads to myofilament sliding. Because the sarcoplasmic reticulum of cardiomyocytes is not as developed as that of skeletal muscle, the amount of Ca^{2+} stored is very small, its excitation-contraction coupling process is highly dependent on exogenous Ca^{2+} influx and is very sensitive to changes in extracellular Ca^{2+} and drug effects (Hussein *et al*., 2001; Wang, 2018). The results showed that the SRPs could significantly inhibit the contraction amplitude of the frog's isolated heart (P<0.01), but had no significant effect on the heart rate (P>0.05), which indicated that the SRPs had no effect on the I_K, I_f current and I_{Ca-T}, but could block the fast I_{Na} channel by binding to target 1; the SRPs could inhibit exogenous Ca^{2+} influx by inhibiting the I_{Ca-L} of myocardial membrane. This is consistent with the results of our previous research on *Paris polyphylla* polysaccharides (Wu *et al*., 2021). The SRPs had significant inhibitory effects on the systolic amplitude of the isolated frog heart induced by $CaCl_2$ and Ach, respectively (P<0.01). The effect was equivalent to that of VPLh and PCPn, which have been the commonly used I_{Ca-L} blocker and muscarinic receptor agonist, respectively. Therefore, it showed that the SRPs might affect the extracellular Ca^{2+} influx by affecting I_{Ca-L}. By activating the M receptor on the myocardial cell membrane, the intracellular cAMP concentration was reduced, and finally the extracellular Ca^{2+} influx might be inhibited.

Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase are sodium-potassium pumps and calcium pumps existing in the myocardial membrane, respectively. Na^+-K^+-ATPase can transport Na^+ in the myocardium to the outside of the myocardial membrane and K^+ outside membrane to the myocardium, Ca^{2+}-Mg^{2+}-ATPase can transport Ca^{2+} in the myocardium to the outside of the myocardial membrane (Yang *et al*., 2019). A-CHE is a decomposing enzyme in the process of Ach metabolism, and its activity can reflect the degree of Ach decomposition. The results showed that the activity of Ca^{2+}-Mg^{2+}-ATPase was significantly promoted, and the activity of A-CHE was significantly inhibited (P<0.01), but the activity of Na^+-K^+-ATPase in the isolated heart was increased not significant (P＞0.05) by the SRPs. The results and degree of the effects on the activities of three enzymes were consistent with those of Yang *et al*. (2019). Therefore, it suggested that the SRPs might not only promote Ca^{2+} efflux from the myocardial membrane by increasing the activity of Ca^{2+}-Mg^{2+}-ATPase, but also activate the cholinergic M receptor by decreasing the activity of A-CHE and further increasing the content of Ach, and finally inhibit the contraction of isolated frog's heart.

There are many motor units with different excitability in the in vivo sciatic nerve-gastrocnemius specimen. When the stimulation intensity reaches the threshold stimulation, a small number of motor units with high excitability will be excited, showing that a small number of muscle fibers contract at the same time to produce small muscle tension. When the stimulation intensity reaches the maximum stimulation, the motor units with low excitability will also be excited, thus the gastrocnemius muscle contract to produce the maximum tension. The results showed that the high concentration SRPs had no significant promotional effects on the threshold stimulation of the frog's sciatic nerve-gastrocnemius muscle (P＞0.05), but the maximum stimulation was significantly increased (P<0.01), which indicated that the SRPs could enhance the excitability of many motor units with low excitability but had little effects on those with high excitability. The results of this study were consistent with the results of Tibetan medicine *Lycium ruthenicum* on the sciatic nerve-gastrocnemius muscle of toads (Cao *et al*., 2019).

The excitatory transmission at the junction of sciatic nerve-gastrocnemius muscle requires electrical signals to trigger Ca^{2+} channel in the anterior membrane of the junction, and the synaptic vesicles quantum release Ach, which combines with the cation channel of N_2 type Ach

receptor in the endplate membrane to cause endplate potential, thus the excitatory transmission is completed, and then Ach is hydrolyzed by A-CHE in the endplate membrane (Zhao *et al*., 2013). The skeletal muscle is composed of myotube system, sarcomere, and myofibril. Due to the action of longitudinal calcium pump on sarcoplasmic reticulum in the myotube system, the concentration of Ca^{2+} in terminal cistern is very high. $I_{Ca\text{-}L}$ in the T-tube membrane and sarcomere can release Ca^{2+} in the terminal cistern into the cytoplasm along difference of concentrations, thus triggering the myofilament sliding (Wang, 2018). In this experiment, the contraction amplitude, contraction rate, relaxation rate of gastrocnemius muscle and the Ach content at the junction of sciatic nerve-gastrocnemius muscle were significantly increased (P<0.01), and the activity of A-CHE at the junction was significantly inhibited (P<0.01) by the SRPs. The results suggested that the SRPs could not only promote gastrocnemius contraction by decreasing the activity of A-CHE and further increasing the content of Ach, but also enhance the activity of the calcium pump on the longitudinal sarcoplasmic reticulum, stimulate the $I_{Ca\text{-}L}$ in the T-tube membrane and sarcolemma to increase the release of Ca^{2+} in the terminal cistern, and finally the combination of Ca^{2+} with troponin leads to the enhancement of gastrocnemius contraction. This is consistent with the results of our previous research on *Paris polyphylla* polysaccharides (Wu *et al*., 2021). Sun and Zhao (2012) studied the betacyanins in *Suaeda salsa*, which belonging to Suaeda, and found that it could promote the contraction of gastrocnemius muscle in toad, which was consistent with the results of this study.

There are differences between myocardial contraction and skeletal muscle contraction. Due to the different excitation time of skeletal muscle cells and cardiac muscle cells, the skeletal muscle cells produce action potential quickly, so it can produce tetanic contraction. And the duration of action potential of the cardiac muscle cell is long, especially long effective refractory period in the excitation process, so the cardiac muscle will not produce tetanic contraction, its contraction and diastole can be carried out alternately from beginning to end. In addition, there are also differences in cell structure, conductivity and contraction mechanism between the two. Many differences lead to the different degrees of influence and changes of the SRPs on the physiological characteristics of the myocardium and skeletal muscle. Studies have shown that Polygonatum polysaccharide can protect the myocardium by inhibiting cardiomyocyte apoptosis and anti-oxidative stress (Zhu *et al*., 2018), Astragalus polysaccharide can delay the fatigue of skeletal muscle by improving the activity of antioxidant system and inhibiting oxidative stress induced by exhaustive exercise (Go *et al*., 2018), which have the same effects as the SRPs in this study.

Based on the results of this study, the SRPs had different effects on the physiological characteristics of frog heart and gastrocnemius muscle. It could inhibit the contraction of isolated frog heart, cooperate with the inhibition of Ach on the contraction amplitude of isolated frog heart, and inhibit the promotion of $CaCl_2$ on it. The mechanism of action is that the SRPs could not only promote Ca^{2+} efflux from the myocardial membrane by increasing the activity of Ca^{2+}-Mg^{2+}-ATPase, but also activate the cholinergic M receptor by decreasing the activity of A-CHE and further increasing the content of Ach. By blocking the $I_{Ca\text{-}L}$ and activating the M receptor on the myocardial cell membrane, the intracellular cAMP concentrations were reduced, and finally the extracellular Ca^{2+} influxes were inhibited. In addition, it may be related to block the fast I_{Na} channel by binding to target 1. The SRPs could promote the contraction and relaxation of skeletal muscle, and the mechanism of action is that the SRPs could not only promote gastrocnemius contraction by decreasing the activity of A-CHE and further increasing the content of Ach, but also enhance the activity of the calcium pump on the longitudinal sarcoplasmic reticulum, stimulate the $I_{Ca\text{-}L}$ in the T-tube membrane and sarcolemma to increase the release of Ca^{2+} in the terminal cistern. In addition, it may be related to enhance the excitability of many motor units with low excitability.

The main reasons why the frogs were selected for this experiment are as follows: the anatomical structure and physiological characteristics of the ventricle and sciatic nerve-gastrocnemius of the frog are like those of human and mammalian, such as the bioelectrical activities of skeletal muscle cells and cardiomyocytes, the contraction

and relaxation of myocardium and skeletal muscle, etc (Sha *et al*., 2017). The physiological theories such as the myofilament sliding can also be applied to explain the physiological characteristics of frogs. The frog is an amphibian, belonging to a typical kind of poikilothermic animal, after the heart is isolated, it does not need to ensure a constant temperature environment. The heart has no coronary artery, and the cardiomyocytes directly obtain nutrients and oxygen from the blood in the heart cavity without additional oxygen. Although the brain and spinal cord of the frog have been destroyed because it is a poikilothermic animal and an in vivo specimen, there is no need to worry about drying in the air. Therefore, the sciatic nerve-gastrocnemius specimen can maintain good physiological activity at room temperature for a period of time. In conclusion, the survival conditions of isolated heart and in vivo sciatic nerve-gastrocnemius muscle of frogs are relatively simple, easy to master and control, and can maintain the physiological activity at room temperature for a period of time (Sha *et al*., 2017). Therefore, the frog was selected as the experimental material to study the effects of the SRPs on the physiological characteristics of the heart and skeletal muscle of human and mammalian. The results showed that the SRPs could inhibit cardiac contraction and promote skeletal muscle contraction and relaxation. Therefore, it can be inferred that the SRPs are expected to be used in the research and development of L-type calcium channel blockers, antihypertensive drugs, antifatigue drugs (Cao *et al*., 2019), and the treatment of skeletal muscle diseases (such as muscular dystrophy, myotubular myopathy, infectious myopathy, metabolic myopathy).

ETHICAL STATEMENT

All applicable international, national, and institutional guidelines for the care and use of animals were followed. All the protocols on living animals used in this paper came from the Experimental Animal Center attached to Tarim University. Moreover, all efforts were made to minimize the suffering of the frogs.

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