Scutellaria Polysaccharide Mediates the Immunity and Antioxidant Capacity of the Giant Freshwater Prawn (Macrobrachium Rosenbergii)

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Research

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Scutellaria polysaccharide mediates the immunity and antioxidant capacity of the giant freshwater prawn (*Macrobrachium rosenbergii*)

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Abstract

Background: The giant freshwater prawn (*Macrobrachium rosenbergii*) is a commercially valuable freshwater crustacean species that is cultivated throughout the world. *M. rosenbergii* is frequently affected by a variety of diseases. Therefore, feed additive research is necessary to improve the immunity and survival rate of *M. rosenbergii*. Scutellaria polysaccharide (SPS) extracted from *Scutellaria baicalensis* (a Chinese medicinal herb) can enhance the antioxidant ability of organisms.

Methods: In this study, *M. rosenbergii* were fed with 50 mg/kg, 100 mg/kg, and 150 mg/kg of SPS. Following a four-week feeding trial, SPS had no positive effect on the growth of *M. rosenbergii* compared with the control group. Then, the immunity and antioxidant capacity of *M. rosenbergii* were tested by qRT-PCR and enzyme activities.

Results: The results showed that the expressions of prophenoloxidase (proPO) and toll receptor (Toll-R) mRNA showed no changes in hemocytes of *M. rosenbergii*. However, the expressions of heat shock protein 70 (HSP70) and nuclear factor κB (NF-κB) mRNA were up-regulated during the first two weeks of culture and were down-regulated in weeks 3 and 4. The mRNA expressions of HSP70, NF-κB, and Toll-R (participating in the immune response) in heart, muscle, and hepatopancreas were decreased after four weeks of SPS feeding. This indicated that long-term feeding of SPS could regulate the immune responses of *M. rosenbergii.* The activity levels of antioxidant biomarkers, alkaline phosphatase (ACP), acid phosphatase (AKP), polyphenol oxidase (PPO), catalase (CAT), and superoxide dismutase (SOD), in

hemocytes, heart, muscle, and hepatopancreas increased during the feeding time, indicating that the antioxidant capacity of *M. rosenbergii* was improved by SPS supplementation in the feed.

Conclusions: In summary, SPS was conducive to enhancing the antioxidant capacity of *M. rosenbergii*. These results provide a theoretical basis in supporting of SPS addition to the feed of *M. rosenbergii.*

Keywords: *Macrobrachium rosenbergii*; Scutellaria polysaccharide; immunity capacity; antioxidant capacity

Background

The giant freshwater prawn (*Macrobrachium rosenbergii*) is a commercially valuable freshwater crustacean species, which is cultured all over the world. Numerous pathogens, such as the white spot syndrome virus (WSSV), *Vibrio harveyi*, and *V. alginolyticus* negatively impact the health of *M. rosenbergii*, thus threatening the *M. rosenbergii* aquaculture industry. The resistance of *M. rosenbergii* to external pathogens relies on humoral and cellular immunity [1]. Prophenoloxidase (proPO), a key enzyme in the melanization cascade, is the zymogen form of phenoloxidase (PO), which is involved in invertebrate immune reactions [2-4] . The antioxidant system can eliminate reactive oxygen species (ROS) and protect organisms from oxidative damage [5]. Therefore, enhancing the immunity and antioxidant capacity of *M. rosenbergii* is helpful to combat the damage caused by pathogens on the shrimp.

Recently, Chinese herbal medicines have been extensively investigated for their antiviral, antimicrobial, and anti-inflammatory effects [6, 7]. Chinese herbal medicines are widely used in aquaculture because of their excellent efficacy (no drug resistance and residue, and few side-effects) [8]. In previous studies, various Chinese herbs synergistically improved the nonspecific immunity of numerous fish species, such as the common carp (*Cyprinus carpio*) [9], the tilapia (*Oreochromis niloticus*) [10], the Chinese prawn (*Fenneropenaeus chinensis*) [11], and the flounder (*Paralichthys olivaceus*) [12, 13].

Scutellaria polysaccharide (SPS) is extracted from the root of *Scutellaria*

baicalensis, which is a traditional Chinese herb and widely prescribed to treat bacterial infections in humans [14]. A recent study showed that the stem and leaves (aerial parts) of *S. baicalensis* had extensive antibacterial effects on *Aeromonas hydrophila*, *Edwardsiella tarda*, *V. alginolyticus*, and *V. harveyi* [6]. The root of *S. baicalensis* exerted the strongest antioxidant activity compared with leaves, stems, and flowers [15]. *S. baicalensis* roots work as a strong free radical scavenger with high antioxidant capacity [16-20]. Therefore, it can protect cells from oxidative stress [21], immune hepatitis [22], and allergic dermatitis [23, 24]. SPS has excellent antioxidant effects on organisms [25-28]. SPS has been widely added to animal feed to prevent cardiovascular diseases in pigs [29] and improve the immune capacity of chickens [30]. However, the antioxidant and immunomodulatory effects of SPS on *M. rosenbergii* still remain unclear.

In this study, different concentrations of SPS were used as feed supplement for *M. rosenbergii.* Furthermore, the levels of immune and antioxidant indexes were traced. The results provide insight into the immunoregulatory and antioxidant functions of SPS in *M. rosenbergii*.

Materials and methods

Experimental materials

M. *rosenbergii* was collected from the Zhejiang Freshwater Fisheries Research Institute (Huzhou, Zhejiang, China). SPS was obtained from Xi'an XIAOCAO

Botanical Development Co., Ltd., China. 50 mg/kg, 100 mg/kg, and 150 mg/kg of SPS (85%) were added to the basic feed (Guangdong Haid Group Co., Ltd., China), containing high-quality fish meal, soybean meal, peanut bran, shrimp shell powder, flour, yeast powder, multimineral and multi-dimensional food inducers. It was composed of 40% crude protein, 4% crude fat, 15% crude ash, 12% moisture, 5% crude fiber, 4% calcium, and 1% phosphorus.

Experimental design

A total of 720 prawns (body weight of 15.84 ± 2.87 g) were randomly divided into four groups, namely the control, 50 mg/kg, 100 mg/kg, and 150 mg/kg groups. Each group used three replicates with 60 tails per replicate. The experiment was continued for one month, and samples were drawn every other week. The growth performance, as well as the antioxidant and immunity capacity of *M. rosenbergii* were determined.

Sample collection

The prawns were anesthetized with tricaine methanesulfonate (MS-222, A5040, Sigma, USA) and sampled every other week. From each group, the prawns of 10 trails were weighed and counted. The prawns were anesthetized, and their blood was drawn into sterile centrifuge tubes. The heart, muscle, and hepatopancreas were dissected and placed in sterile centrifuge tubes. All samples were snap-frozen in liquid nitrogen and temporarily stored at -80 °C for further analysis.

Expression analysis of immune factors by quantitative real-time polymerase chain reaction (qRT-PCR)

The mRNA levels of proPO, heat shock protein 70 (HSP70), toll receptor (Toll-

R), and nuclear factor κB (NF-κB) were determined by qRT-PCR. Total RNA was extracted from the samples by Trizol (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. The RNA was reverse-transcribed into complementary DNA (cDNA) (TaKaRa, Japan) and stored at -20 °C for qRT-PCR. The primer sequences of proPO, HSP70, Toll-R, and NF-κB genes were designed using online Primer 3 (NCBI/Primer-BLAST) (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) (Table 1). β-actin was used as internal control. Each sample was measured in triplicate according to the following procedure: incubation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 55 °C for 20 s, 72 °C for 20 s, and 4 °C for 5 min. A melting-curve analysis was performed to determine the target specificity. The relative expression ratio of the target genes versus the β-actin gene was calculated using the $2^{-\Delta\Delta CT}$ method, and all data were given in terms of relative mRNA expression.

Determination of antioxidant enzyme activities

The activity levels of alkaline phosphatase (ACP), acid phosphatase (AKP), polyphenol oxidase (PPO), catalase (CAT), and superoxide dismutase (SOD) were determined with the respective enzyme activity assay kits (Nanjing Jiancheng Bioengineering Institute, China). The protein concentration in each sample was determined by protein assay kit (A045-4, Nanjing Jiancheng Bioengineering Institute, China). The article numbers of the kits are A060 (ACP), a059-2 (AKP), a136-1-1 (PPO), A007-1 (CAT), and a001-3 (SOD). The tissues were collected, and sample diluent was added as substrate. After incubation for 30 min, the enzyme activities were measured using a microplate reader at absorbances of 520 nm (ACP), 520 nm (AKP), 420 nm

(PPO), 450 nm (SOD), and 405 nm (CAT). To compare the parameters of enzyme activity, the control group was homogenized over the four weeks. The enzyme activity of 50 mg/kg, 100 mg/kg, and 150 mg/kg groups was compared with those of the control group. The activity of the control group was one every week. The relative value was used to evaluate the antioxidant indicators and identify changes.

Results

Effect of SPS on the growth of M. rosenbergii

The body weights of control and experimental groups is shown in Fig. 1. The body weights of control and experimental groups increased from 16 g/10 prawn in the first week to 55 g/10 prawn in the fourth week. The body weight of the experimental groups significantly decreased compared with the control group, indicating that SPS had no growth-promoting effect. The survival rates of prawns in control, 50 mg/kg, 100 mg/kg, and 150 mg/kg SPS groups exceeded 93%. However, there was no significant difference between these groups, indicating that SPS did not affect the survival rates of prawns.

Effect of SPS on the immunity capacity of M. rosenbergii hemocytes

In invertebrates, the inactive proPO is converted into the active prophenoloxidase (PO) to participate in the immune response. HSP70 is a basic indicator for stress responses in organisms. NF-κB regulates the immune and inflammatory responses in

prawn tissues. Toll-R participates in nonspecific immunity and bridges nonspecific and specific immunity. After culturing prawns for four weeks, the mRNA levels of proPO and Toll-R showed no change in the experimental groups, while Toll-R was up-regulated in the second week of culture. HSP70 and NF-κB mRNA expressions were up-regulated in the second week of culture and down-regulated in the third and fourth weeks of culture (Fig. 2).

Effect of SPS on the immunity capacity of M. rosenbergii tissues

During the breeding period, the HSP70 level followed a decreasing trend (Fig. 3A). After one week of culturing prawns the expression of HSP70 was slightly decreased in the 50 mg/kg and 100 mg/kg groups. Furthermore, decreases of HSP70 were observed in the 100 mg/kg and 150 mg/kg groups after culturing for two weeks, especially in the muscle tissue and the hepatopancreas.

As shown in Fig. 3B, the expression level of NF-κB was increased in the first week and significantly decreased in the fourth week. In the 50 mg/kg and 100 mg/kg groups in the third feeding week, the mRNA expression levels had increased in muscle tissue but decreased in the liver and hepatopancreas.

As shown in Fig. 3C, the mRNA expression of Toll-R decreased in the three tissues (heart, muscle, and hepatopancreas) of *M. rosenbergii* over the four weeks of culture. After four weeks of culture, the mRNA expression of Toll-R was significantly decreased in the hepatopancreas.

Effect of SPS on antioxidant capacity of M. rosenbergii hemocytes

ACP and AKP are the most important indexes of antioxidant enzymes in crustaceans. PPO is another important index of antioxidant enzyme in crustaceans. Both SOD and CAT are members of the antioxidant enzyme defense system, and scavenge free oxygen radicals in the body. SOD can protect cells from oxidative damage, and CAT activity reflects the anti-lipid peroxidation ability of the body. The activities of ACP, AKP, and PPO increased in hemocytes over the entire feeding time. The CAT activity increased in 50 and 100 mg/kg groups, but decreased in the 150 mg/kg group (Fig. 4).

Effect of SPS on antioxidant capacity of M. rosenbergii tissues

The ACP levels exhibited a complex trend in heart, muscle, and hepatopancreas (Fig. 5A). The level of ACP expression in the heart increased in the second and third weeks of culture. The enzyme activity significantly decreased in the hepatopancreas in the third week of culture and remained stable in the fourth week.

After one week of culture, the activity of AKP increased in both heart and muscle tissues (Fig. 5B). After two weeks of culture, the activity of the AKP enzyme increased significantly in muscle tissue. After four weeks of culture, enzyme activity decreased in heart and muscle tissue and increased in the hepatopancreas. These results suggest that muscle AKP might be sensitive to the feeding duration of SPS.

After two weeks of culture, the heart and muscle PPO levels decreased, and the

hepatopancreas PPO level increased (Fig. 5C). Over three weeks of culture, PPO levels increased in the heart, muscle, and hepatopancreas. After four weeks of culture, PPO decreased, which indicates that polysaccharides can increase the activity of PPO at the appropriate time.

The SOD levels increased in the heart, muscle, and hepatopancreas over the four weeks of cultivation (Fig. 5D). However, a significant increase in the SOD level was found in the hepatopancreas after two weeks of culture, indicating that the hepatopancreas is the most sensitive organ to SPS.

As shown in Fig. 5E, the CAT levels increased significantly in heart, muscle, and hepatopancreas tissues after three weeks of culture. In the fourth week, the CAT activity level was higher than levels in the third week of culture. The results showed that long-term feeding of SPS enhanced CAT activity and overall antioxidant capacity.

Discussion

Nutrition and immunity are two crucial factors that affect the health of organisms. The different nutrient levels affect the immune function of the body, whereas the immune system influences the nutrient requirements of the organism [31]. Chinese herbal medicine can enhance the immunity of the body. Chinese herbal medicine enhanced the humoral immunity and cellular immune function of immunosuppressed mice [32]. Addition of a Chinese traditional herbal complex to diets had beneficial effects on the immunity of pigs [33]. Traditional Chinese medicine could successfully

232 stimulate the immunity of aquatic animals and improve the nonspecific immune
233 function [9]. *S. baicalensis* was reported to stimulate the growth and antimicrobial
234 activities of flounder (*P. olivaceus*) [34]. SPS increases the antioxidant activity of the
235 liver [35], offers resistance to viruses [36], and has immune functions [37] in both
236 humans and animals. SPS significantly inhibited the infectivity of Newcastle disease
237 virus in chicken embryo fibroblasts and showed antiviral activity [38]. SPS could
238 inhibit virus replication at the cellular level as indicated by Trypan blue exclusive assay,
239 immunofluorescence assay, and PCR methods [36]. SPS could inhibit NF-κB signaling
240 and NLRP3 inflammasome activation, thus decreasing the disease activity index and
241 myeloperoxidase activity [28]. SPS could improve the nonspecific and specific immune
242 abilities of mice by increasing the IgG, IgM, and IgA levels in serum [39]. However,
243 the influence of SPS on the immune and antioxidant abilities of M. rosenbergii has not
244 been investigated to date. In this study, the effects of SPS on the immunity and
245 antioxidant capacity of M. rosenbergii were analyzed.

246 The immune response of prawns is relatively primitive. The prawn mainly relies
247 on innate immunity to resist pathogenic microorganisms. Therefore, the research on the
248 immune mechanism of prawn mainly focuses on the antioxidant enzyme system, Toll
249 receptor, HSP70, and other immune-related factors [40]. In crustaceans, hemocytes play
250 a vital role in both cellular and humoral immunity. Immune indicators were tested in
251 hemocytes. The proPO activation system activates PO activity, which is involved in the
252 immune response of invertebrates against pathogens [41]. In this study, the mRNA
253 expressions of proPO showed no change in hemocytes. The results demonstrated that

254 the effects of proPO on the immunocompetence of *M. rosenbergii* were not significant
255 with prolonged feeding time. The PO activity of the Chinese prawn was high 4 h after
256 injection with peptidoglycan [42] and 48 h after dietary administration of fungal
257 polysaccharides [43]. Moreover, long-term feeding of immune polysaccharides can
258 affect the immune function both positively and negatively [44, 45]. HSP70 is a member
259 of the heat shock protein family, which is induced in stressed cells of organisms. Heat
260 shock proteins are well-known agents that protect organisms and cells, and the relative
261 expression of these proteins determines the anti-stress ability of organisms [46]. In this
262 study, the expression of HSP70 significantly increased in hemocytes after culturing for
263 two weeks and decreased after culturing for three weeks in all groups. In tissues, the
264 expression of HSP70 significantly decreased in the 100 mg/kg and 150 mg/kg groups
265 after culturing for two weeks. This indicates that the appropriate concentration and
266 feeding time of SPS could improve the health of *M. rosenbergii*. Chinese herbal
267 medicine is a bidirectional immunomodulator [47], and can promote low immune
268 function and inhibit hyperfunction. NF-κB and the Toll-R induce a range of immune
269 responses in crustaceans [48, 49]. NF-κB is involved in the development of various
270 inflammation-related diseases, and is key in modulating the immune and inflammatory
271 responses by controlling the expression of inflammatory cytokines [28, 50]. In this
272 study, the relative expression level of NF-κB significantly decreased in the fourth week
273 of culture in hemocytes, heart, muscle, and hepatopancreas, suggesting that long-term
274 SPS feeding could regulate the immunity of *M. rosenbergii*. *S. baicalensis* has been
275 reported to reduce inflammatory responses by inhibiting NF-κB and MAPK pathways

276 [51], suggesting that the roots of *S. baicalensis* can inhibit NF-κB and enhance
277 immunity. Furthermore, Toll-R expression level decreased in all tissues and feeding
278 stages, suggesting that SPS can indirectly regulate the expression of the Toll-R gene
279 through the NF-κB pathway. This hinders the production of inflammatory factors by
280 the innate immune system, thus improving the phagocytic ability and enhancing the
281 killing ability of natural immune cells. The immune capacity of shrimp can be improved
282 by immune stimulation and can be maintained for a certain period (several days to
283 several months); however, the mechanism of SPS on *M. rosenbergii* should be further
284 studied.

285 The antioxidant capacity can also reflect the immune capacity and health status.
286 ACP, AKP, and PPO play important roles in the immune system of shrimp or prawn.
287 Phosphatase can catalyze the hydrolysis of various phosphorous compounds.
288 According to their optimal pH characteristics, phosphorous compounds can be divided
289 into AKP and ACP. In this study, after feeding SPS at different concentrations, ACP and
290 AKP enzyme activities increased in hemocytes over the whole feeding time. They also
291 increased in heart and muscle tissues after the first feeding week, but decreased in the
292 fourth feeding week. These results indicate that long-term feeding of SPS did not
293 significantly enhance the activity of ACP and AKP. It has been demonstrated that long-
294 term feeding of polysaccharides may fail to stimulate immune and antioxidant functions,
295 or even decrease these [44]. Short term administration of SPS can promote ACP and
296 AKP activities, which may stimulate the transfer and metabolism of ACP phosphate
297 groups, and may provide more inorganic phosphoric acid for ADP phosphorylation to

298 improve immunity and antioxidant capacity. PPO can recognize the invasion of foreign
299 bodies and regulate the immune and antioxidant functions. Therefore, the activities of
300 ACP and PPO enzymes reflect the immune function of prawn. Activated PPO can
301 stimulate the synthesis of quinones in organisms, which spontaneously produce melanin.
302 Melanin helps to protect from the invasion of foreign pathogens [52, 53]. It has been
303 reported that *in vivo* and *in vitro* stimulation of the giant tiger prawn (*Penaeus monodon*)
304 with yeast glucan significantly enhanced the PPO activity of hemolymph tissue [54].
305 The results of the present study showed that the PPO level increased in hemocytes,
306 heart, muscle, and hepatopancreas in the third week of culture, indicating that
307 polysaccharides can increase the PPO activity.

308 SOD and CAT are representative indexes of the antioxidant function [55, 56]. SOD
309 and CAT are important immune-related factors in the immune system of the body,
310 which can reflect the body's nonspecific immune function [57]. In this study, SPS
311 significantly increased the SOD and CAT activities in 50 mg/kg and 100 mg/kg groups
312 in hemocytes, heart, muscle, and hepatopancreas. Another study found that SOD was
313 strongly expressed in the heart of white-leg shrimp (*Penaeus vannamei*), stimulated by
314 ROS after one week of culture [58]. S. baicalensis decreased the ROS level and
315 maintained the equilibrium of ROS through biosynthesis [59]. S. baicalensis Georgi
316 flower extract (SFE) could significantly reduce oxidative damage of aging rats by
317 increasing their SOD level in the serum [60]. These results indicate that both short- and
318 long-term administration of SPS benefits the antioxidant capacity of *M. rosenbergii*.

319

Conclusion

The possible mechanism through which SPS regulates the immunity and antioxidant capacity of *M. rosenbergii* has been identified (Fig. 6). In summary, the SPS supplementation in feed regulated the mRNA of proPO, NF-κB, HSP70, and Toll-R in *M. rosenbergii*. The activities of antioxidant-related enzymes (ACP, AKP, PPO, SOD, and CAT) increased. However, different tissues and their immune indexes had different sensitivities to SPS. The experimental data showed that long-term feeding of SPS could improve the antioxidant capacity of *M. rosenbergii*, which provides data for the preparation of compound Chinese herbal medicine.

Ethics approval and consent to participate

All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no.85-23 revised 1996).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the authors on reasonable request.

Competing interests

The authors have no conflicts of interest to declare.

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Authors' contributions

Lindan Sun, Feng Lin, Keping Chen and Li Lin designed the study. Lindan Sun and Feng Lin conducted the experiment, performed and collected the data. Lindan Sun analyzed the data and wrote the manuscript. Zhendong Qin, Fei Shi, Youlu Su, Chun Liu, Lijuan Zhao, Jun Li, Keping Chen and Li Lin read, revised and approved the final manuscript.

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Table 1

Primers used in qRT-PCR on mRNA of immune parameters.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Gene ID
proPO	TACATGCACCAGCAAATTATCG	AGTTTGGGGAAGTAGCCGTC	HF570111.1
HSP70	CTCTGCCCAAGCAAGTAT	GAATCTGTGCCTTATCCA	HG001455.1
NF-κB	GTGGCTCACTTACGACTC	AAGGTCCATACTCTTTGC	KR827675.1
Toll-R	TCTACGACCGCAACGAGC	CGGAGTGGGAGTGAACAG	JF895474.1
β-actin	GTGCGTGACATCAAGGAA	GTGCGTGACATCAAGGAA	AF221096.1

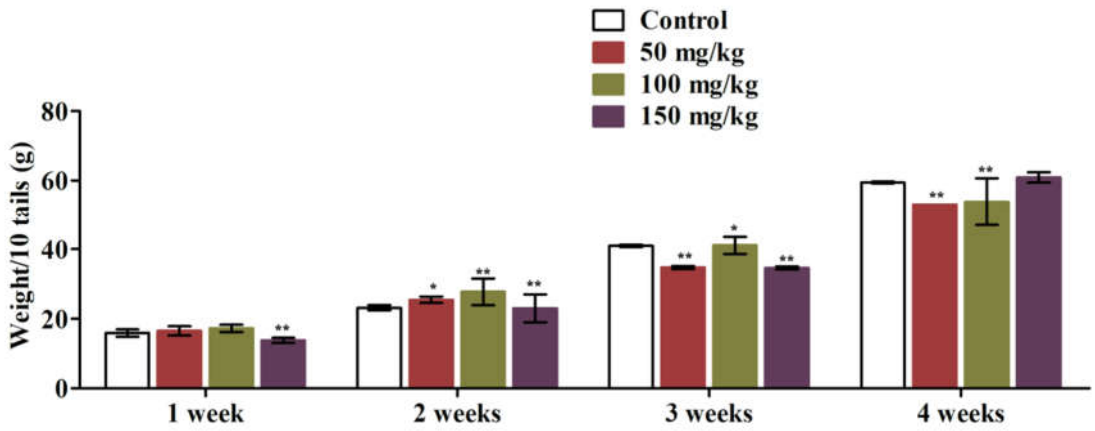

Fig.1 Effects of SPS on the body weight of *M. rosenbergii* (10 tails) for four weeks in control, 50 mg/kg, 100 mg/kg and 150 mg/kg groups. Data presented are from three independent experiments, and values represent means and standard deviations (SD). * indicates $P < 0.05$, ** indicates $P < 0.01$. Error bars indicate mean ± SD, n = 3.

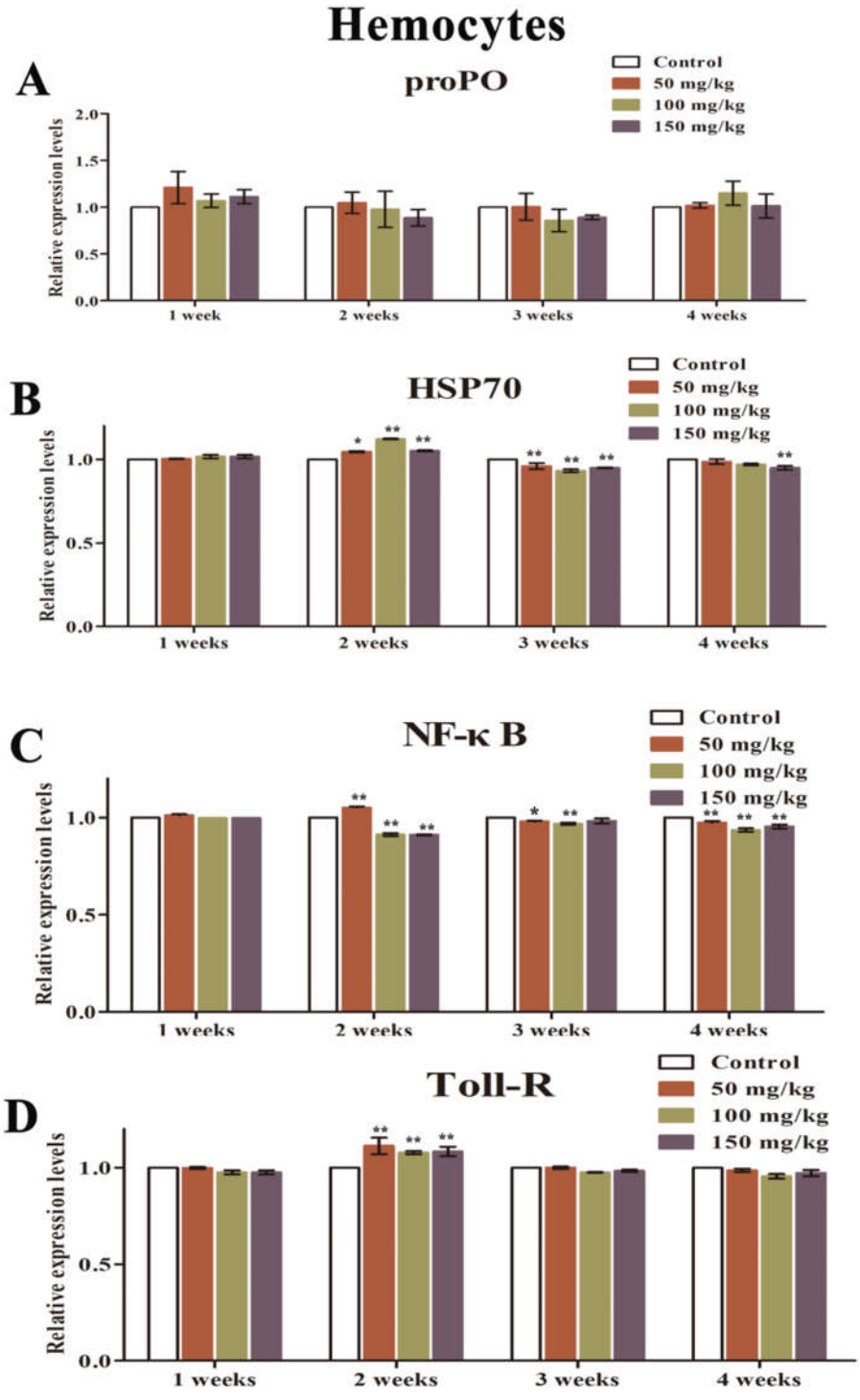

Fig.2 Effects of SPS on mRNA levels of immune factors in hemocytes. The mRNA level of proPO (A), HSP70 (B) , NF-κB (C) and Toll-R (D) from hemocytes for four weeks. Data presented are from three independent experiments, and values represent means and standard deviations (SD). * indicates $P < 0.05$, ** indicates $P < 0.01$. Error bars indicate mean ± SD, n = 3.

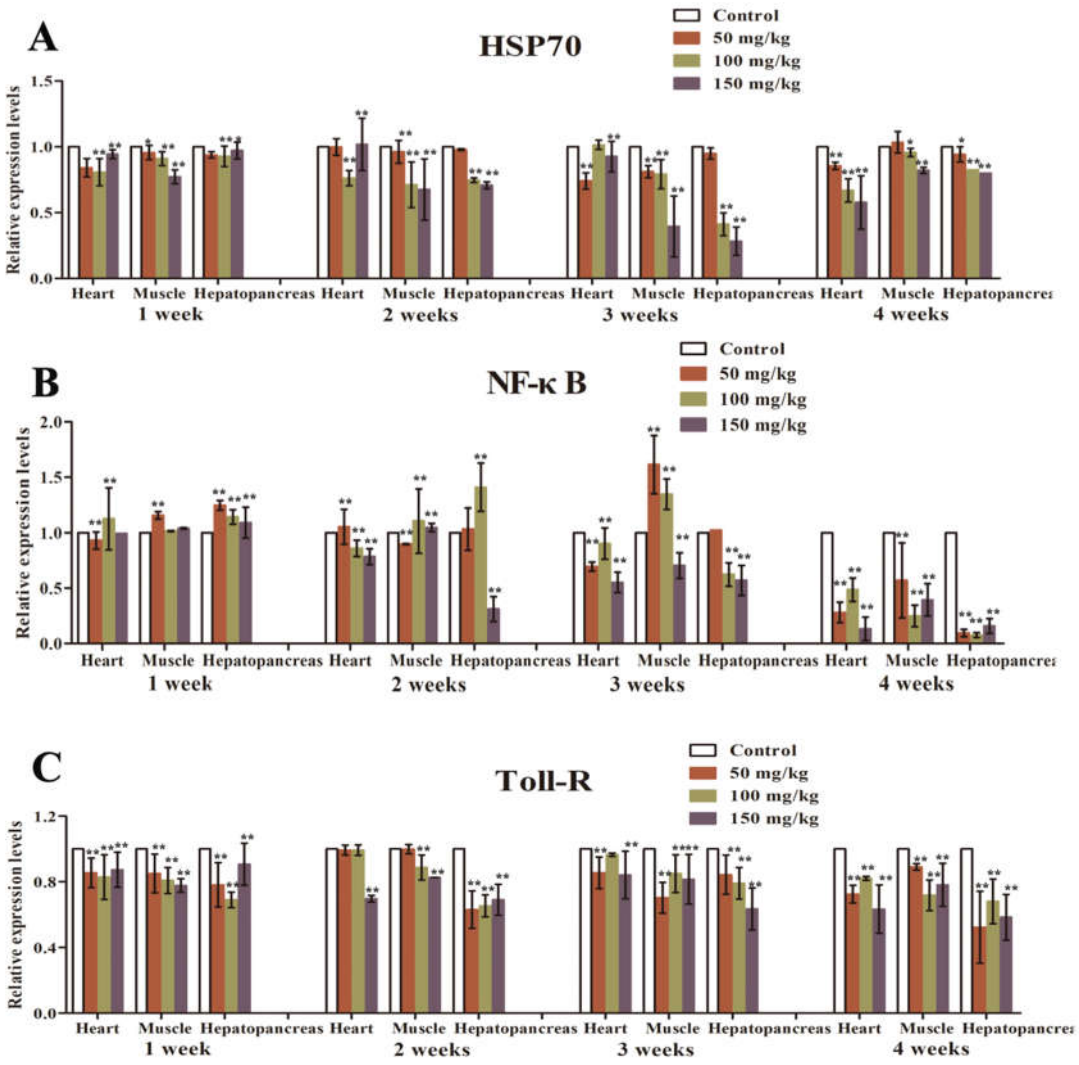

Fig. 3 Effects of SPS on mRNA levels of immune factors in tissues. The mRNA level of HSP70 (A) , NF-κB (B) and Toll-R (C) from heart, muscle and hepatopancreas for four weeks. Data presented are from three independent experiments, and values represent means and standard deviations (SD). * indicates $P < 0.05$, ** indicates $P < 0.01$. Error bars indicate mean ± SD, n = 3.

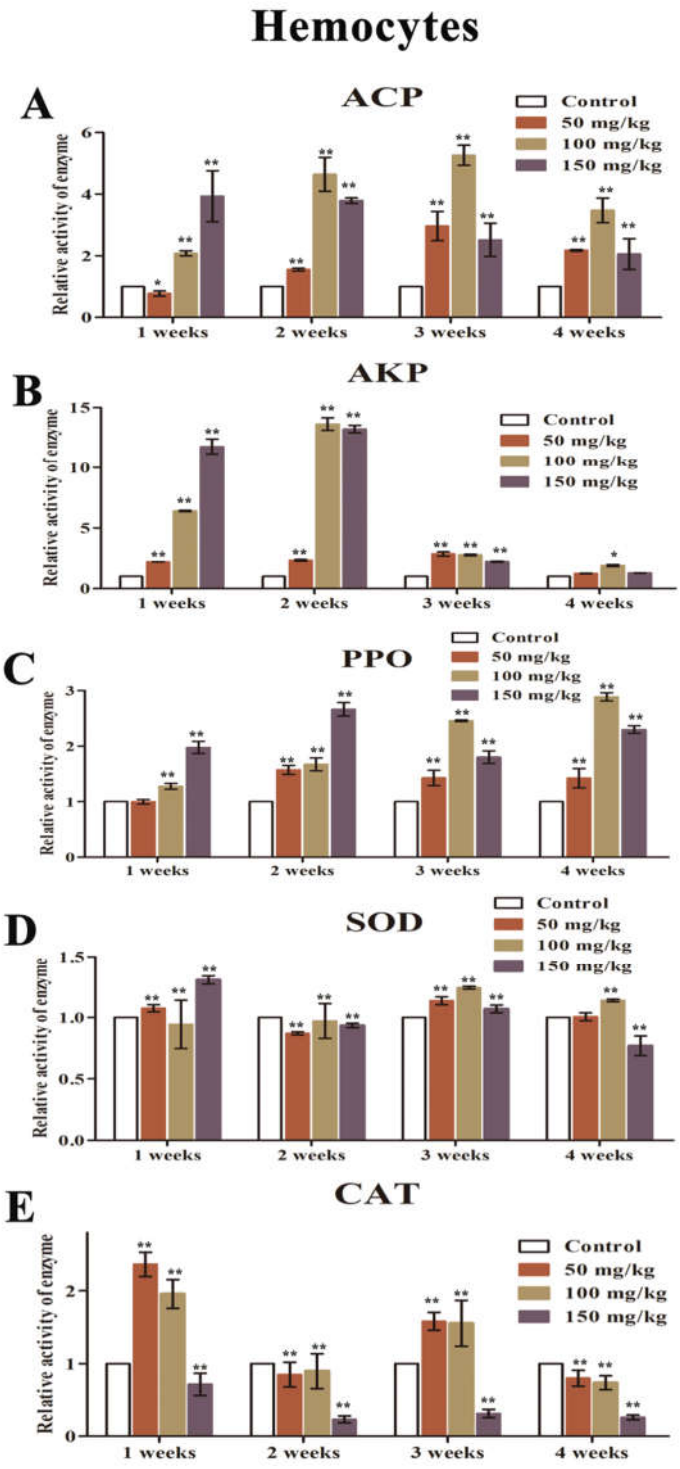

Fig. 4 Effect of SPS on the activities of antioxidant enzymes in *M. rosenbergii*. The activities of ACP (A), AKP (B), PPO (C), SOD (D) and CAT (E) from hemocytes for four weeks. Data presented are from three independent experiments, and values represent means and standard deviations (SD). * indicates $P < 0.05$, ** indicates $P < 0.01$. Error bars indicate mean ± SD, n = 3.

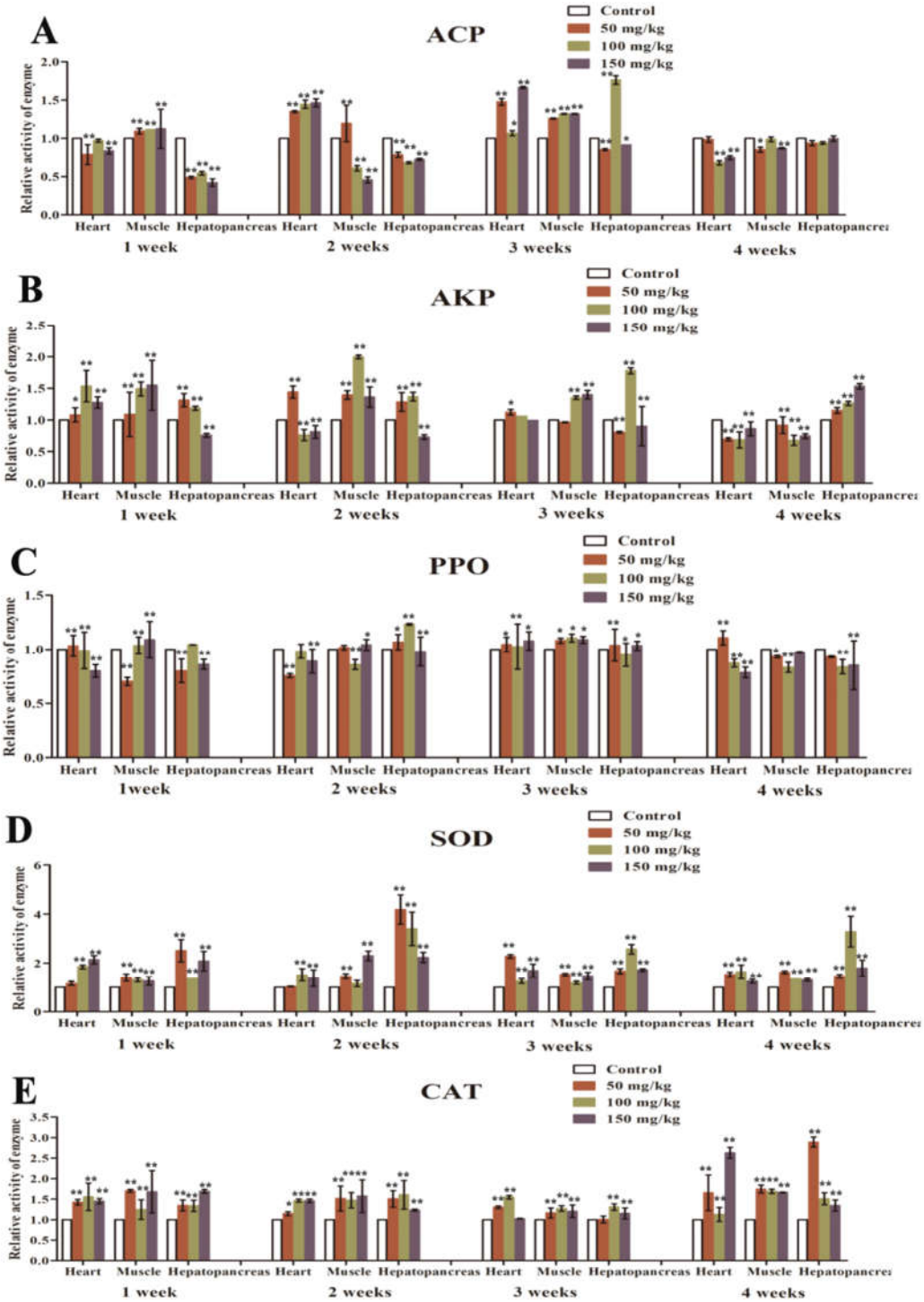

Fig. 5 Effect of SPS on the activities of antioxidant enzymes in *M. rosenbergii*. The activities of ACP (A), AKP (B), PPO (C), SOD (D) and CAT (E) from heart, muscle and hepatopancreas for four weeks. Data presented are from three independent experiments, and values represent means and standard deviations (SD). * indicates $P < 0.05$, ** indicates $P < 0.01$. Error bars indicate mean ± SD, n = 3.

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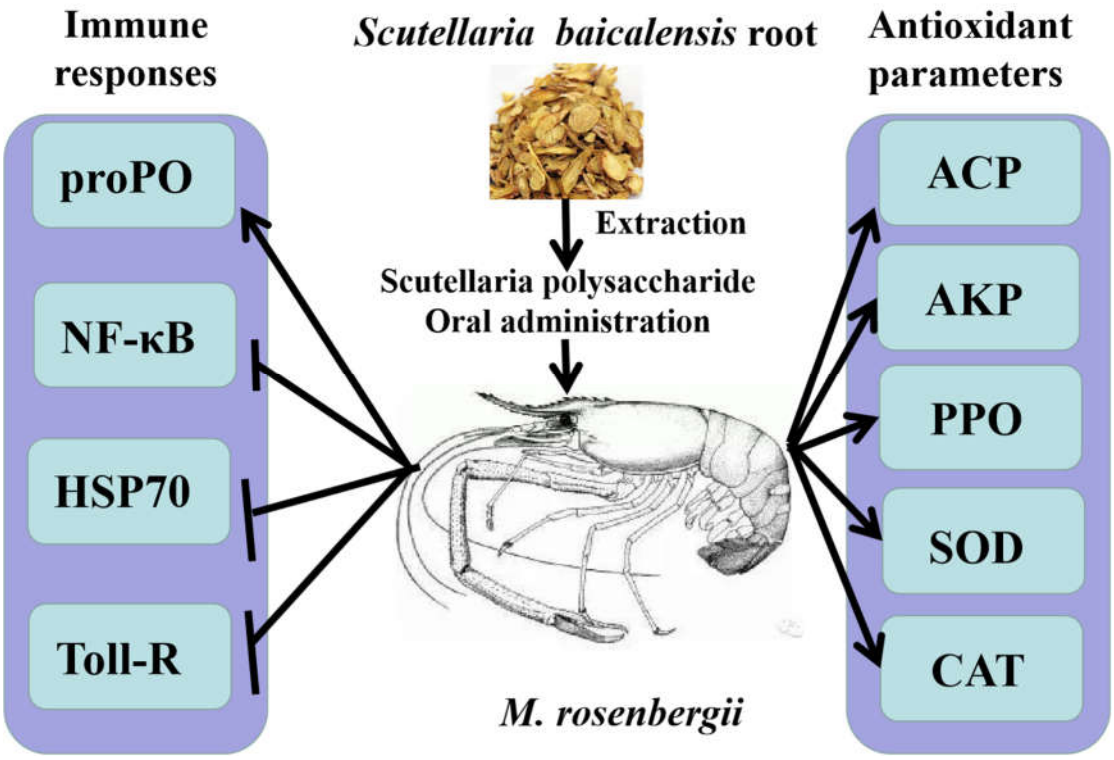

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587 **Fig. 6** Effect of SPS on immunity and antioxidant capacity of *M. rosenbergii*. The

588 arrows show facilitation and the horizontal lines show inhibition.

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Figures

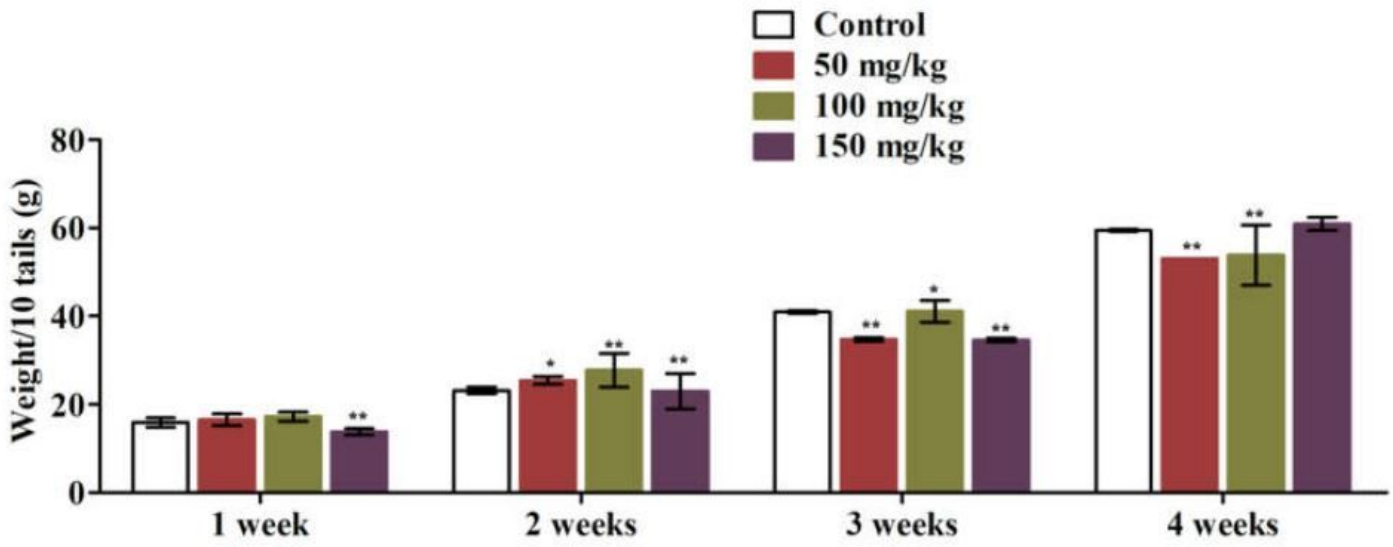

Figure 1

Effects of SPS on the body weight of M. rosenbergii (10 tails) for four weeks in control, 50 mg/kg, 100 mg/kg and 150 mg/kg groups. Data presented are from three independent experiments, and values represent means and standard deviations (SD). * indicates $P < 0.05$, ** indicates $P < 0.01$. Error bars indicate mean ± SD, n = 3.

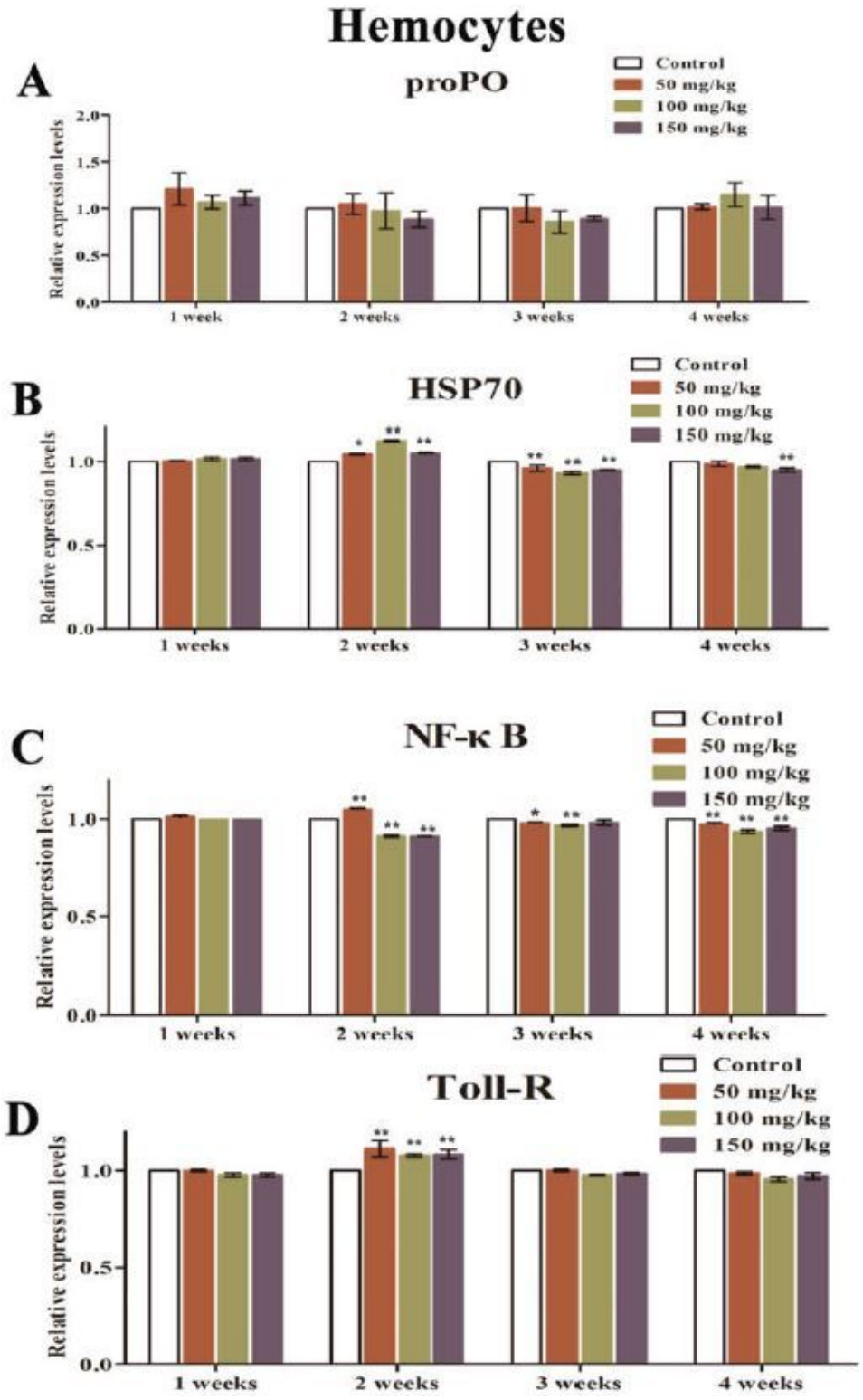

Figure 2

Effects of SPS on mRNA levels of immune factors in hemocytes. The mRNA level of proPO (A), HSP70 (B) , NF-κB (C) and Toll-R (D) from hemocytes for four weeks. Data presented are from three independent experiments, and values represent means and standard deviations (SD). * indicates $P < 0.05$, ** indicates $P < 0.01$. Error bars indicate mean ± SD, n = 3.

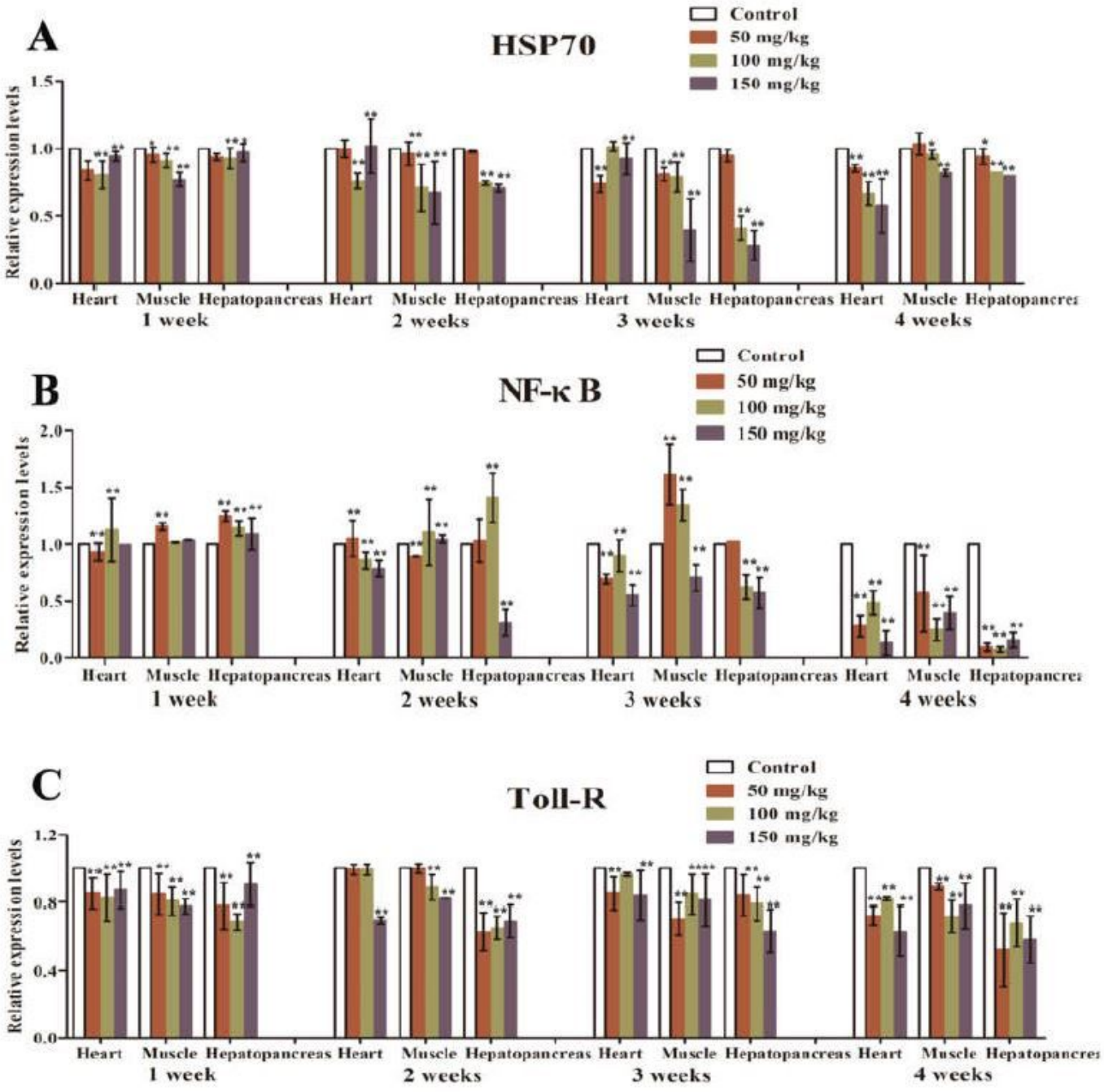

Figure 3

Effects of SPS on mRNA levels of immune factors in tissues. The mRNA level of HSP70 (A) , NF-κB (B) and Toll-R (C) from heart, muscle and hepatopancreas for four weeks. Data presented are from three independent experiments, and values represent means and standard deviations (SD). * indicates $P < 0.05$, ** indicates $P < 0.01$. Error bars indicate mean ± SD, n = 3.

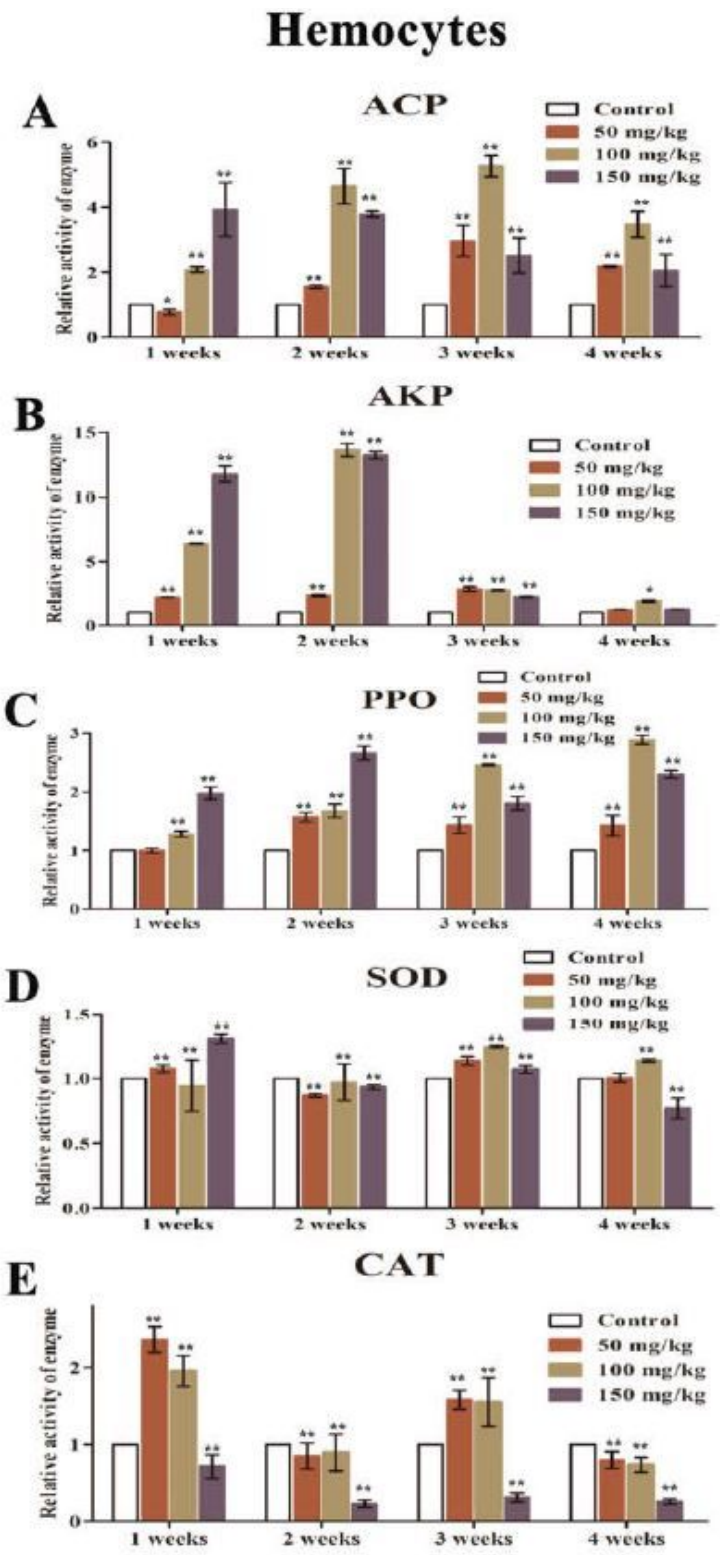

Figure 4

Effect of SPS on the activities of antioxidant enzymes in M. rosenbergii. The activities of ACP (A), AKP (B), PPO (C), SOD (D) and CAT (E) from hemocytes for four weeks. Data presented are from three independent experiments, and values represent means and standard deviations (SD). * indicates $P < 0.05$, ** indicates $P < 0.01$. Error bars indicate mean ± SD, n = 3.

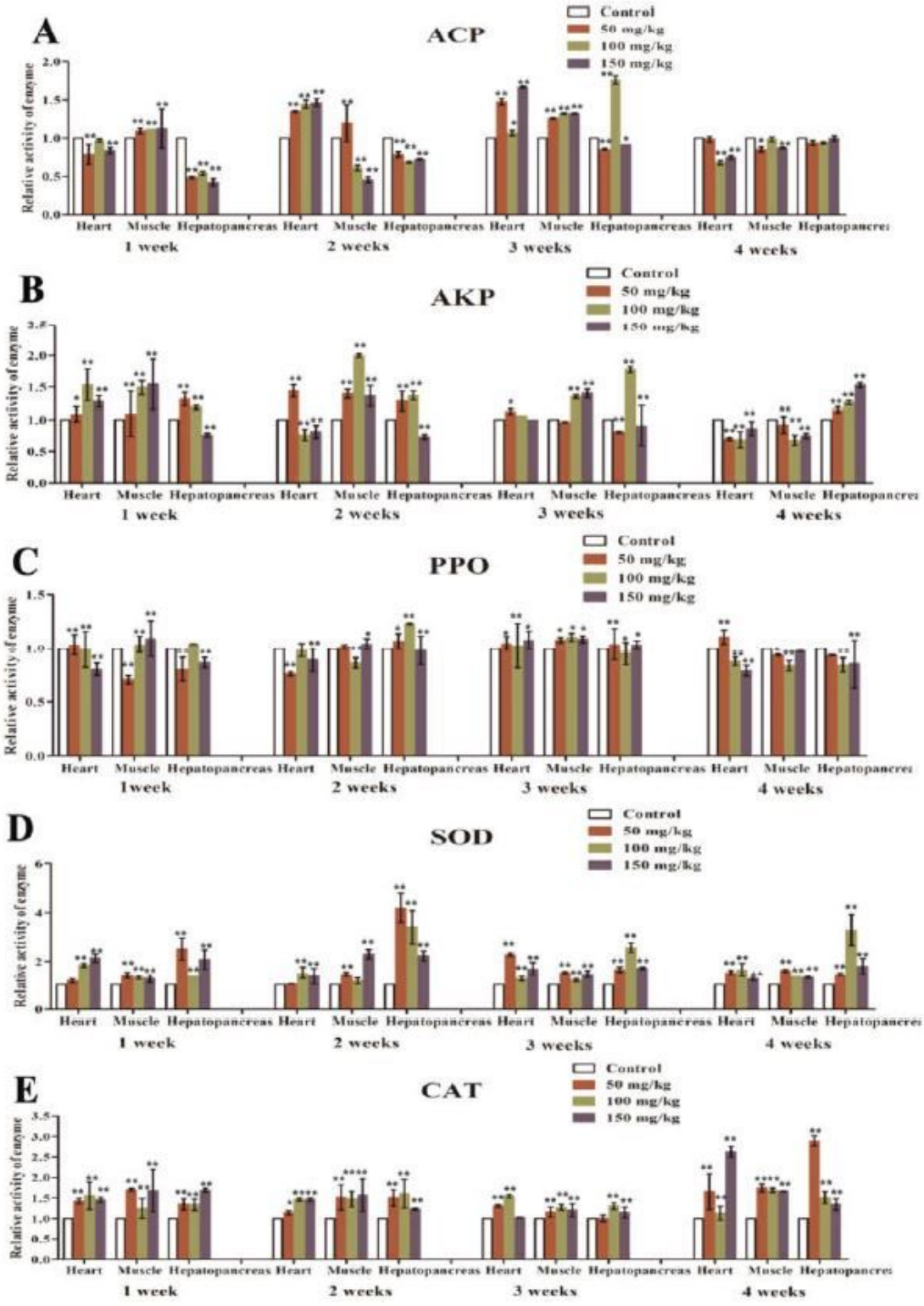

Figure 5

Effect of SPS on the activities of antioxidant enzymes in M. rosenbergii. The activities of ACP (A), AKP (B), PPO (C), SOD (D) and CAT (E) from heart, muscle and hepatopancreas for four weeks. Data presented are from three independent experiments, and values represent means and standard deviations (SD). * indicates $P < 0.05$, ** indicates $P < 0.01$. Error bars indicate mean ± SD, n = 3.

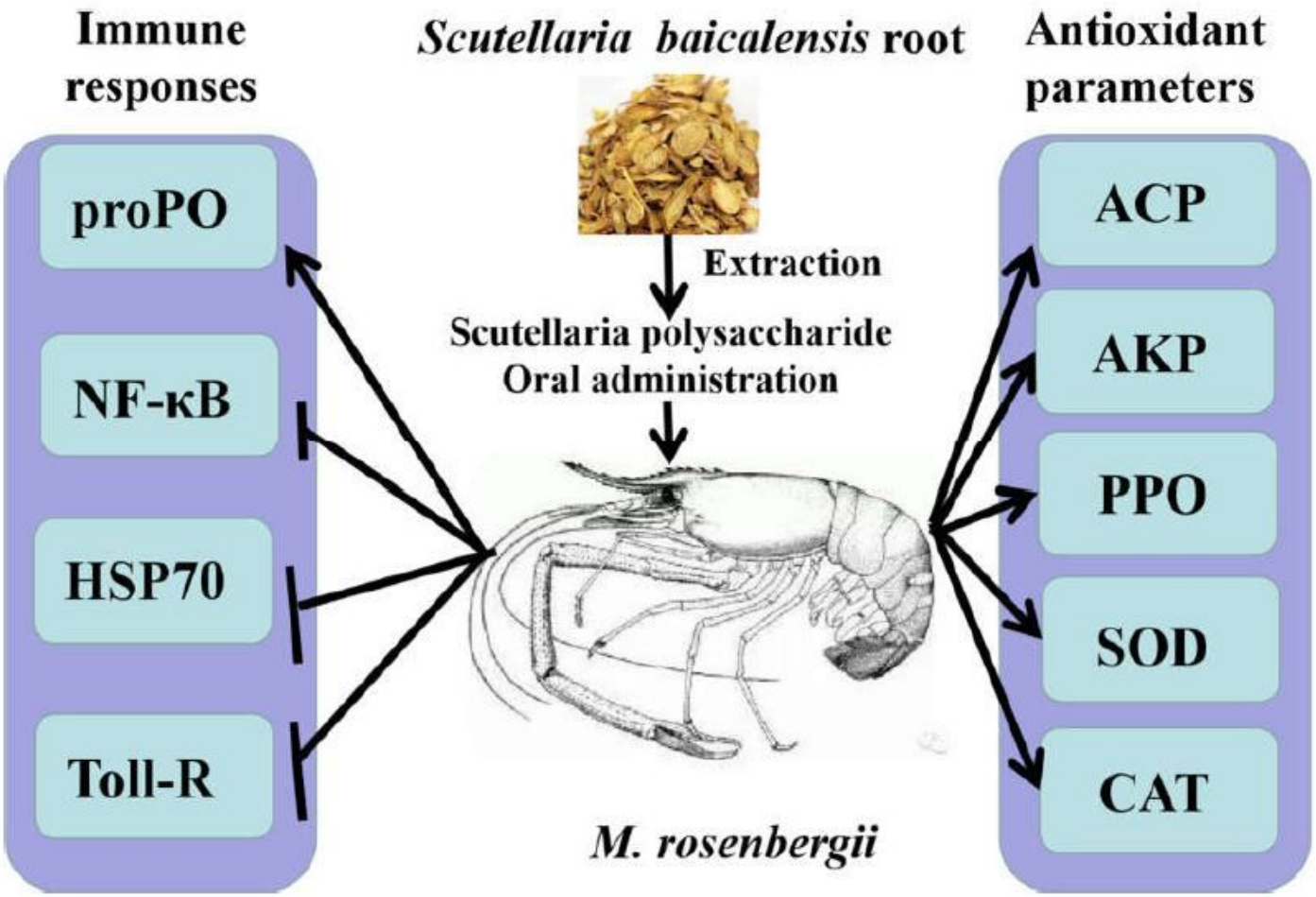

Figure 6

Effect of SPS on immunity and antioxidant capacity of M. rosenbergii. The arrows show facilitation and the horizontal lines show inhibition.