Effects of Rosa roxburghii and Kiwifruit Composite Supplements on Improving Exercise Performance and Anti-fatigue in Mice

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Abstract

Rosa roxburghii Tratt (RR) and Actinidia chinensis Planch (AC, kiwifruit) are nutrient-dense plant foods with diverse bioactive components. This study aimed to evaluate the effect of supplementation with Rosa roxburghii and Kiwifruit (RRAC)on improving the exercise performance-enhancing and anti-fatigue effects of a RR-AC composite supplement (RRAC) in experimental murine models. Fifty male institute of Cancer Research (ICR) mice were randomly allocated to five groups (n = 10 / group): (1) blank control group (Rest, normal saline), (2) fatigue model control group (Con, normal saline), (3) RRAC-L (130 mg/kg·bw /mice/day), (4)RRAC-M (260 mg/kg·bw /mice/day), and (5) RRAC-H (520 mg/kg·bw /mice/day). Following 28 consecutive days intervention, the mice in all groups except the Rest group underwent a weigh-loaded forced swimming test. Then, mice were subsequently euthanized for serum biochemical markers and analysis and tissue collection. We found that supplementation with 28-day RRAC could significantly enhance mice's exercise endurance performance, and elevated hepatic &muscle glycogen content(all $p < 0.05$), and significantly reduced post-exercise fatigue biochemical parameters, including blood urea nitrogen (BUN), L-lactic acid (LAC), lactate dehydrogenase (LDH), creatine kinase (CK) concentration. Moreover, RRAC supplementation reduced the oxidative stress indicators malondialdehyde (MDA) activity ,and increased the superoxide dismutase (SOD) &glutathione peroxidase (GSH-Px) ($p < 0.05$). In summary, supplementation with RRAC for 28 days could significantly enhanced the exercise tolerance in mice during the weigh-loaded swimming test via glycogen accumulation and oxidative stress modulation, with no detectable adverse effects on organ structure or function.

1. Introduction

With the acceleration of the pace of life, factors such as high psychological pressure, unreasonable diet, excessive exercise, lack of rest, and a lifestyle filled with mental tension have led to an increasing number of people being in a sub-health state and experiencing "unexplained fatigue"[1].Approximately one-third of the global population suffers chronic fatigue lasting six months or longer[2].In short, fatigue is a complex physiological process that refers to the inability of the body to maintain its physiological functions at a certain level or to sustain the appropriate level of exercise intensity. This condition may lead to temporary muscle or organ dysfunction.. It is worth noting that long-term fatigue can induce the occurrence of various chronic diseases, including multiple sclerosis, Parkinson's disease, and depression, severely impairing daily living and occupational capacity[3, 4]. In recent years, the increase in public awareness of anti-fatigue has greatly promoted the development of the functional anti-fatigue product industry[5]. Functional anti-fatigue products are typically consumed as a dietary supplement, their nutritional content and composition are essentially different from those of ordinary foods[6]. The functional factors in these products are active or functional components of animals and plants, such as glucosamine, curcumin, L-carnitine, and lycopen[7]. Research reports also indicate that plant-based foods contain a wide range of bioactive compounds, including polyphenols, vitamins, phytosterols, biogenic amines, and biologically active proteins, which are beneficial to human anti-fatigue[8, 9, 10].

Rosa roxburghii Tratt (RR, CiLi), a functional fruit endemic to southwestern China (predominantly Guizhou Province),is rich in compounds, such as vitamin, superoxide dismutase (SOD), minerals, polysaccharides, phenolic compounds, triterpenoids, and organic acids, but due to its slightly sour state and astringent flavor, strong aroma and crisp texture, limiting its development and utilization as a commercial food product,[11, 12]. It is understood that juice is one of the most common RT fruit products, however, the presence of tannins in RR fruit makes the juice astringent lead to they cannot compete with other fruit beverages on the market[13]. Thus, Combining the juice with that of other fruits to form a compound juice represent a viable strategy to improve its organoleptic properties.

*Actinidia chinensis Planch (AC, kiwifruit)*is a globally distributed economic fruit crop originating in China, and known as “the king of fruits”. Due to its distinctive flavor and rich in dietary nutrients including polyphenols, vitamins, dietary fiber, and function ingredients, such as starch and protease, flavonoids, and various bioactive chemicals[14, 15], and antioxidative, antiproliferative, antiinflammatory, antimicrobial, antihypertensive, antihypercholesterolemic, neuroprotective, antiobese properties and promote gut health [16].

Conversely, there are few reports on the research of fruit based compound energy supplement products. Therefore, the purpose of this study was to explore the benefits of composite energy supplements of RR and AC in improving exercise endurance performance and delaying fatigue related blood biochemical indicators after 28 consecutive days of supplementation. In addition, we also examined organ indices and histopathology examinations to determine whether 28 consecutive days of RRAC supplementation resulted in physiological maladaptation.

2. Material and Methods

2.1 Preparation of the Rosa roxburghii and Kiwifruit composite supplements

Peel and juice mature Kiwifruit to obtain Kiwifruit pulp, wash fresh Rosa roxburghii and juice directly to obtain Rosa roxburghii pulp. Mix the above pulp in a ratio of 1:3 (Rosa roxburghiit: Kiwifrui) and mix it appropriately under heating conditions with uniform stirring, and keep it warm (70 ℃) and pack it in cans. Notably, fill the packaging bag with nitrogen to exhaust the air in the bag before bagging, sterilize the packaged product with pasteurization (80 ℃) for 10 minutes to obtain the Rosa roxburghii and Kiwifruit composite supplements. RRAC composite supplement was confirmed by the Guizhou Testing Technology Research and Application Center (Guizhou, China) and contained nutritional cntent (Table 1), vitamin (Table 2), and amino acids content (Table 3).

Table 1
Nutritional content of the RRAC composite supplement

Nutrition Facts	**/100g**
Total calories	703 kcal
Fat	2.9
Protein	1.65
Carbohydrate	33.4
Dietary fiber	1.04
SOD	1.88×10^3 (U/g)

Table 2
Vitamin and element of the RRAC composite supplement

Vitamin and Element	**mg/100g**
Vitamin E	1.27
Vitamin B1	0.245
Vitamin B2	0.245
Vitamin B6	0.375
Vitamin C	671
Na	166
Ca	351
K	3.27×10^3

Table 3
Amino acids content of the RRAC composite supplement

Amino acids	g/100g
Arginine	0.11
Lysine	0.052
Histidine	0.014
Phenylalanine	0.026
Leucine	0.041
Isoleucine	0.024
Methionine	0.016
Valine	0.033
Alanine	0.11
Glycine	0.25
Proline	0.12
Glutamic Acid	0.17
Serine	0.039
Threonine	0.035
Aspartic acid	0.097

2.2 Animal and Experimental Design

A total of Fifty healthy independent ventilation cages (IVC) grade ICR male mice, 6-8weeks old with an initial weight of 28–32 g, were purchased from the SPF Biotechnology Co.,Ltd. (Beijing, China). The animals were housed in Guizhou Foster Biotechnology Co., Ltd.(Guizhou, China), and was carried out according the standard guidelines. Standard food and water were freely provide to the animals and cared for under standard conditions with exposure to 12 light/dark cycles during the experiment period. All experimental procedures were strictly performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health, and the study protocol was approved by the Animal Research Ethics Committee of Guizhou Foster Biotechnology Co., Ltd. (IACUC No. FST/LL-03). After acclimatization for 1 week, mice were randomized into five groups(n = 10): black control group (Rest, normal saline), fatigue model control group (Con, normal saline), low-dose group (RRAC-L, 130 mg/kg·bw of RRAC), medium-dose group (RRAC-M, 260 mg/kg·bw of RRAC), and high-dose group (RRAC-H, 520 mg/kg·bw of RRAC), of which Rest group did not participate in the determination of exercise endurance. The experiment lasted 28 days, and the weights of mice were recorded every week.

2.3 Weight-Loaded Swimming Test

The weigh-loaded swimming test was carried out according to the previous studies. Briefly, 30 min after the last oral administration, a lead wire, weighing 5% of their corresponding BW, was fixed to the root of the mouse tail. Then, the mice in all group except the Rest group were subjected to swim individually in a plastic pool (50 ×50 × 40 cm) filled with water (25 ±1℃) to a depth of 30 cm. The weight-loaded swimming time was recorded when the mice sank into the water and failed to rise to the surface for breath within a period of 10 s[17]. During swimming test, a glass rod was used for stirring gently to make the water in the pool moving continuously and keep the mice swimming to exhaustion. Then, the mice were removed from the water and dried immediately. After resting for 20 min, Mice were humanely euthanized by CO_2 inhalation in strict compliance with the 2020 AVMA Guidelines for the Euthanasia of Animals. CO_2 was delivered at a flow rate of 30%–70% chamber volume per minute to minimize distress and induce rapid unconsciousness, confirmed by three sequential endpoints: marked respiratory depression, complete cessation of voluntary movement, and absence of corneal reflex. Immediately after unconsciousness verification, CO_2 delivery was terminated, and whole blood and tissue samples were promptly collected for further analyses. After dissection, the tissues were weighed to calculate the organ indexes according to the formula as follows:

$$\text{Organ indexes}(\%) = 100 \times \frac{\text{Organ weight}}{\text{Final body weight}}$$

2.4 Determination of plasma biochemical parameters on fatigue

Plasma samples were collected to determine the level of blood glucose (GLU), blood urea nitrogen (BUN), L-lactic acid (LAC), lactate dehydrogenase (LDH), creatine kinase (CK), superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) activity after 20 min of mice weight-loaded swimming. All of the above biochemical parameters were determined according to the procedures provided in the kits.

2.5 Examination of hepatic glycogen and muscle glycogen content

After dissecting the mice, part of liver and hind leg muscles were taken and weighted for glycogen content analysis. The hepatic glycogen and muscle glycogen analysis were estimated by commercial assay kits according to the manufacturer’s guide.

2.6 Histological Analysis

Liver, skeletal muscle, heart, lung, kidney, and adipose were collected from all groups. A small portion of tissue was fixed in 10% neutral buffered fromalin and covered with wax. Then 0.2μm-size sections were cut from paraffin-embedded paraffin-embedded tissue blocks, using a microtome. Slides were prepared

by deparaffinization, stained with hematoxylin and eosin (H&E), dehydrated through a series of graded alcohols (100%, 95%, and 75%), and rinsed twice in xylene. Photomicrographs were obtained by using a Motic digital pathology slide scanner.

2.7 Statistical analysis

Statistical analyses were performed using SPSS software version 24 (Statistical package for the Social Sciences software, SPSS Inc., Chicago, USA). All values are presented as the mean ±standard deviation (SD). Differences between the groups were analyzed using the one-way analysis of variance (ANOVA) test with least significant difference (LSD) methods if the data were homogeneous. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 Effect of Rosa roxburghii and Kiwifruit composite supplements (RRAC) on body weight and organ indexes in mice

As shown in Fig. 1, after 28 days of continuous gavage intervention, no significant differences were observed in the body weight values of the mice in each group ($p > 0.05$). Also, there were no significant differences in the organ indexes of the lungs, kidney, liver, spleen, and testis.

3.2 Effect of Rosa roxburghii and Kiwifruit composite supplements (RRAC) on weight-loaded swimming test

In weight-loaded swimming test, the time for the mice to resist to fatigue is normally noted to assess the anti-fatigue activity of several compounds or extracts. We observed that medium-dose group of RRAC (RRAC-M) and high dose (RRAC-H) had a significant ($p \leq 0.05$) effect on increasing the fatigue time of mice on weight-loaded swimming test, which indicated that RRAC-M and RRAC-H could markedly reinforce the exercise tolerance and ease the degree of physical fatigue of ICR mice. Meanwhile, RRAC-M had the most significant ($p \leq 0.05$) effect on elevating the fatigue time as compared to control group mice.

3.3 Effect of Rosa roxburghii and Kiwifruit composite supplements (RRAC) on the serum biochemical parameters of ICR mice.

To confirm the anti-fatigue effect exerted by Rosa roxburghii and Kiwifruit composite supplements, the blood serum levels were evaluated for BUN, CK, LAC, LDH, GLU content and MDA, SOD, GSH-Px activity after the weight-loaded forced swimming experiments.

3.4 Effect of Rosa roxburghii and Kiwifruit composite supplements (RRAC) on glycogen levels of ICR mice.

To confirm the effect of Rosa roxburghii and Kiwifruit composite supplements on liver and gastrocnemius muscle glycogen, the levels of glycogen were measured using glycogen assay kits. The glycogen levels in the liver and gastrocnemius muscle were shown in Fig. 4-A, B, when compared to black control mice, weight-loaded forced swimming augmented the liver glycogen content in other control mice. In contrast, muscle glycogen was found to decrease in control mice.

3.5 Effect of Rosa roxburghii and Kiwifruit composite supplements (RRAC) on Tissue Histology

At the end of the study, we conducted histological analysis of the heart, lungs, liver, kidney, spleen, testis, and muscle, were performed to understand the morphological damage in tissues. These results indicate that RRAC has no adverse effects on organs and tissues at the doses tested in this study.

4. Discusssion

Fatigue is an unavoidable physiological state in modern life. Preventing or reducing fatigue has been a long-standing challenge for nutrition and functional food development. Regular exercise, a balanced diet, and complementary and alternative medicine are reported to effectively relieve fatigue, including providing energy substrates[18]. Among these approaches, apart from traditional nutritional supplements like vitamins, β-Alanine and creatine, a growing body of research is now directed towards uncovering novel sources of natural anti-fatigue components as substitutes for synthetic compounds[19]. Not only traditional herbal plants, but also certain common plant foods have been discovered to exhibit significant potential, owing to their abundant bioactive compounds, including polyphenols, vitamins, phytosterols, biogenic amines, and biologically active proteins[20, 21, 22]. These diverse bioactive compounds are capable of enhancing athletic performance, postponing exercise-induced fatigue, accelerating recovery with minimal adverse effects. In this study, we have selected Rosa roxburghii and Kiwifruit as two of the most well-known plant foods abundant in nutrients.However, as mentioned previously, the presence of tannins in Rosa roxburghii makes the juice astringent, a compound of Rosa roxburghii and kiwifruit in a ratio of 1:3 was obtained to produce RRAC, and evaluate its anti-fatigue effect.

Weight-loaded forced swimming time in a forced swimming test has been widely used in animal models for evaluating the anti-fatigue efficacy of drugs or natural compounds[23]. In this study, supplementation with different dose of RRAC for 28 consecutive days significantly improved the weight-loaded forced swimming time in a time-dependent manner compared to the control.Moreover, RRAC-M has significantly longer weight-loaded forced swimming time than RRAC-L and RRAC-H, this suggests that the potential benefits of RRAC administration have a beneficial effect on weight-loaded forced swimming (Fig. 2).

Apart from swimming test, blood biochemical paraemters are also used as a marker for fatigue, studies have shown that when the organism does not acquire energy replenishment in time after intense exercise, proteins undergo deamination to produce pyruvate and a large amount of ammonia, which

must be metabolized into urea through the liver's urea cycle and excreted via the kidneys through the blood circulation system. Simultaneously, it accelerates glycolysis to generate a significant amount of lactic acid, leading to muscles fatigue. When muscles are damaged, muscle cells release creatine kinase (CK) into the blood, lactate dehydrogenase (LDH) in skeletal muscle to penetrate into the blood, and LDH can catalyze the conversion of pyruvate to lactic acid (LAC), reducing exercise tolerance. Therefore, during prolonged or high-intensity exercise disrupts the balance of energy metabolism, elevated levels of blood urea nitrogen (BUN), LAC, LDH and CK are typically observed[24, 25, 26, 27]. This seems to explain the results of study, after endurance-swimming, feeding a certain concentration of RRAC by gavage can reduce the levels of BUN, LAC, LDH and CK in mice. In addition, blood glucose is a crucial fatigue-related blood biochemical indicator, long-term or intense exercise stimulates the activity of glucose transporters on the cell membrane of muscle fibers, leading to a decrease in glucose. However, it increases the glucose uptake of cells for glycolysis, and promotes glycolysis in the liver to increase the blood sugar concentration to provide energy[28, 29], we also found that the blood glucose (GLU) levels of mice in the RRAC group were higher compared to the control group. Both the anti-fatigue and anti-oxidation effects are generally closely related in their functional metabolic pathways. The excessive oxygen free radicals generated during the prolonged and high-intensity exercises could cause oxidative damage of tissues or organs, ultimately causing fatigue in muscles[30]. As an important antioxidant enzyme, SOD and GSH-Px, important antioxidant enzymes, are commonly used as biomarkers for evaluating antioxidant capacity and oxidative damage due to their capability of scavenging oxygen free radicals in the body. The levels of SOD and GSH-Px indirectly reflect the extent of tissue damage[31, 32]. Meanwhile, MDA is a product of lipid peroxidation and serves as an indicator of oxidative stress in cells and tissues[33]. Our finding showed that RRAC significantly reduced MDA levels and restored the activities of key antioxidant enzymes (SOD, GSH-Px). The above blood biochemical paraemters indicating that long-term intake of certain concentration of RRAC can effectively relieve organism fatigue (Fig. 3).

Glycogen is an essential energy resource of organisms, including muscle glycogen and liver glycogen. After prolonged or high-intensity exercise, muscle glycogen generating ATP for energy demand and hepatic glycogen releasing glucose for other tissues maintain physiological balance, resulting in decreased muscle glycogen and liver glycogen contents in the body[34, 35]. In one study, ICR mice were randomly divided into three groups: vehicle, isocaloric, and different dose of supplementation with Santé premium silver perch essence (SPSPE) for four weeks. After swimming test, the liver glycogen and muscle glycogen amount in SPSPE was significantly higher than in other group[29]. We obtained similar results for glycogen levels. In ICR mice fed with RRAC with forced swimming, the liver glycogen and liver glycogen were elevated, with enhancement in exercise performance and anti-fatigue effect (Fig. 4).

Finally, the absence of significant changes in murine body weight and organ indices, as well as the lack of histopathological damage in key tissues and organs, following RRAC supplementation confirms the safety of the composite supplement at the tested doses. The findings demonstrate RRAC does not cause any morphological abnormalities in the heart, lung, liver, kidney, spleen, testis and muscle (Fig. 5).

5. Conclusion

In summary, our results provide evidence that supplementation with RRAC for 28 consecutive days could significantly increase exercise endurance performance by increasing glycogen storage. In addition, supplementation significantly reduced post-exercise biochemical parameters of fatigue, such as BUN, LAC, LDH concentration and CK activity. Meanwhile, the activities of SOD and GSH-Px were increased to varying degrees, and the contents of MDA were reduced significantly, while the supplementation with the proper amount of RRAC would not cause damage to various physiological indicator and organs. Therefore, RRAC can be used to mitigate fatigue during exercise and increase performance.

Declarations

Author Contributions: W.Y.Y. performed the experiments and wrote the main manuscript text; L.L.P. and X.X.L provided the idea and wrote the main manuscript text; X.X.L. andL.Y. performed the experiments and processed the data; H.H.Z. supervised the project. All authors have read and agreed to the published version of the manuscrip.

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Institutional Review Board Statement: The animal experiment was approved on 25 September 2025 by the Animal Ethics Committee of Guizhou Foster Biotechnology Co., Ltd, Guizhou, China (Approval No. FST/LL-03).

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Figures

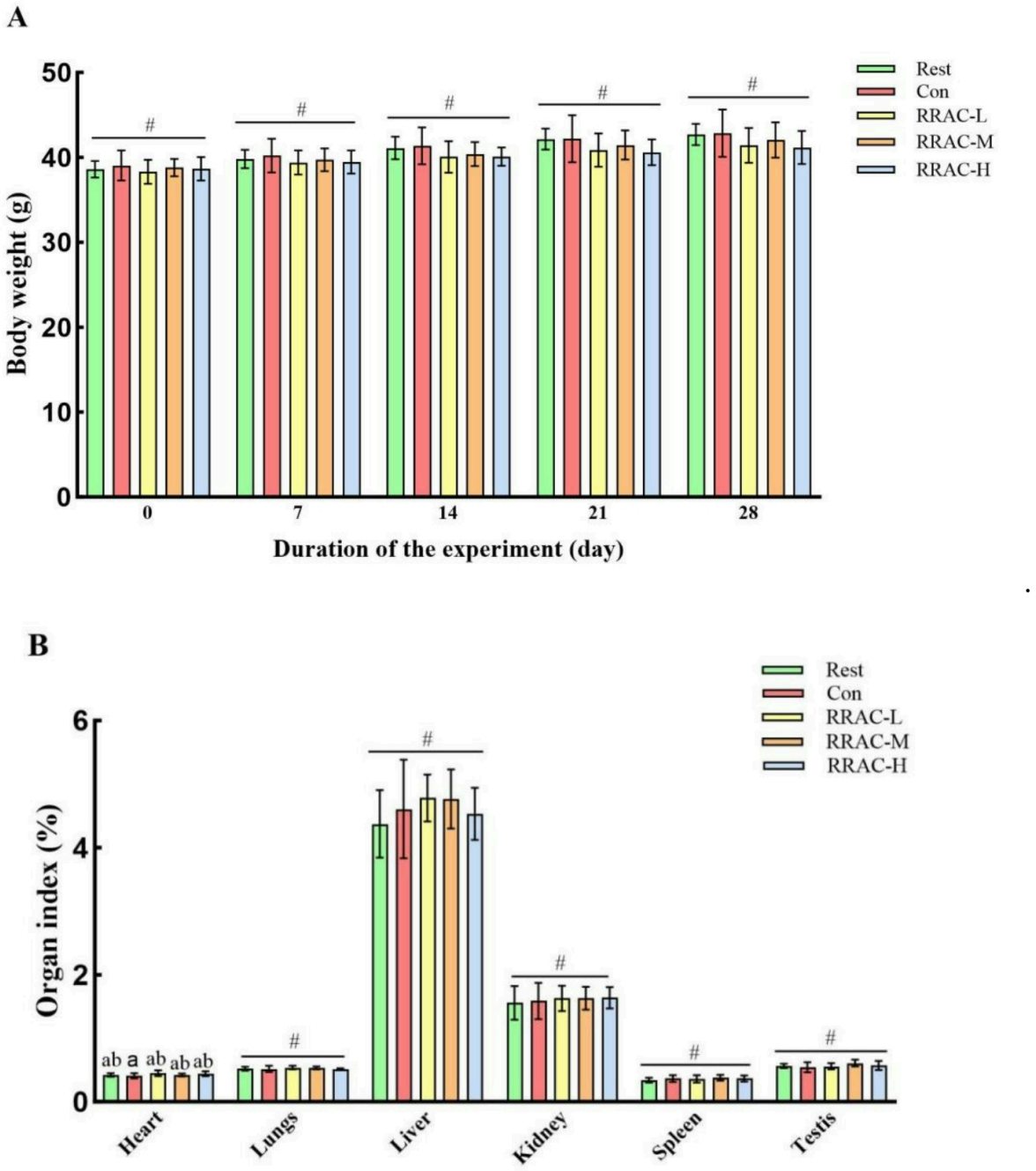

Figure 1

The effect of RRAC on mice body weight and organ index. (A) The effect RRAC intervention on body weight in ICR mice. (B) The effect of RRAC intervention on the organ index in ICR mice. The data were analyzed for significance of difference by one-way analysis of variance(ANOVA) with the LSD test($p < 0.05$); n=10 per group.[a-b] Different letters mean significant difference ($p \leq 0.05$) and # indicates that there is no significant difference between the group ($p > 0.05$). The abbreviation of group used in the figure

were: black group (Rest), fatigue model group (Con), low-dose group of RRAC (RRAC-L); medium-dose group of RRAC (RRAC-M), high-dose group of RRAC (RRAC-H).

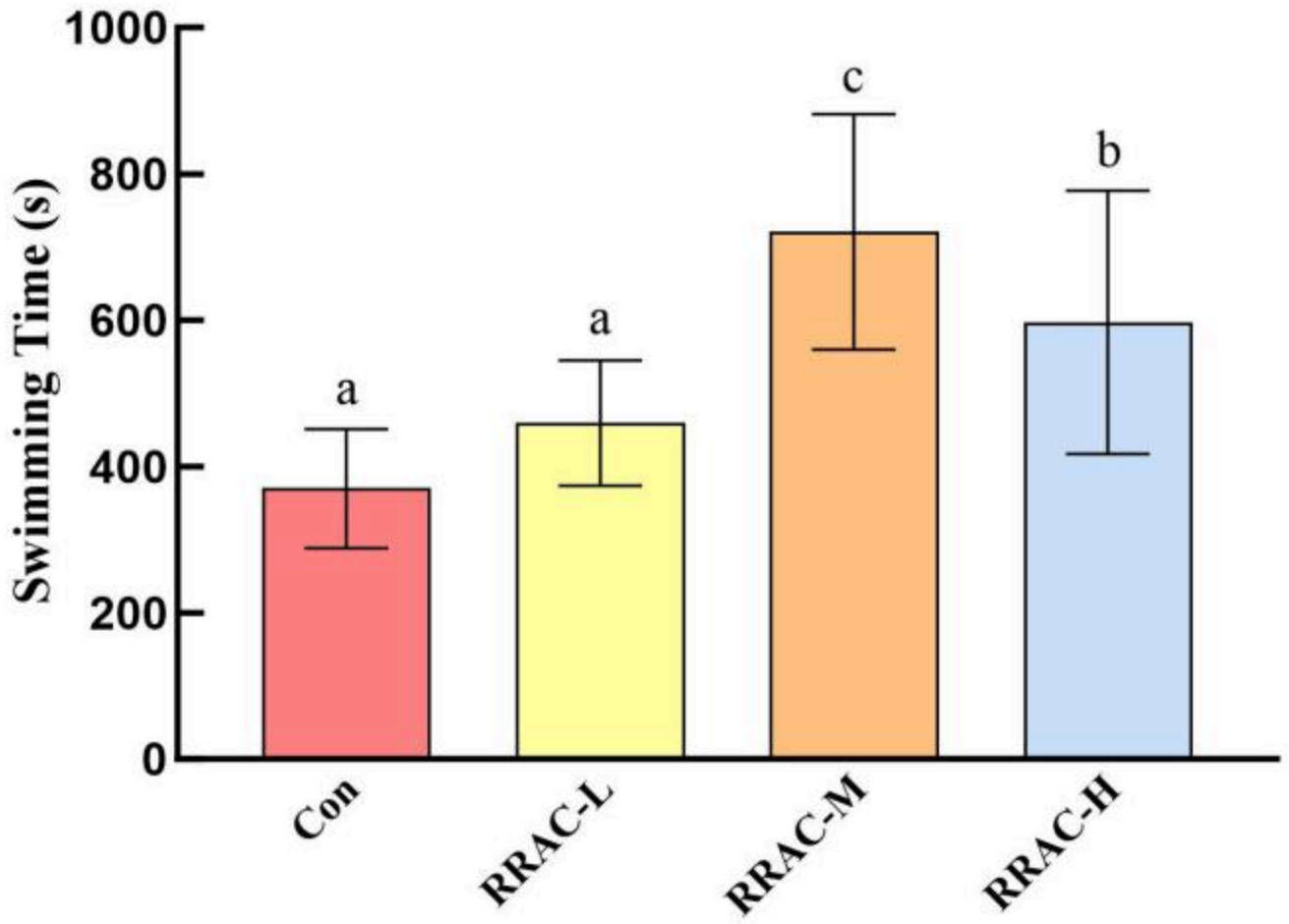

Figure 2

The effect of RRAC on swimming endurance test in ICR mice. The data were analyzed for significance of difference by one-way analysis of variance(ANOVA) with the LSD test ($p < 0.05$); n=10 per group.[a-c] Different letters mean significant difference ($p \leq 0.05$). The abbreviation of group used in the figure were: fatigue model group (Con), low-dose group of RRAC (RRAC-L); medium-dose group of RRAC (RRAC-M), high-dose group of RRAC (RRAC-H).

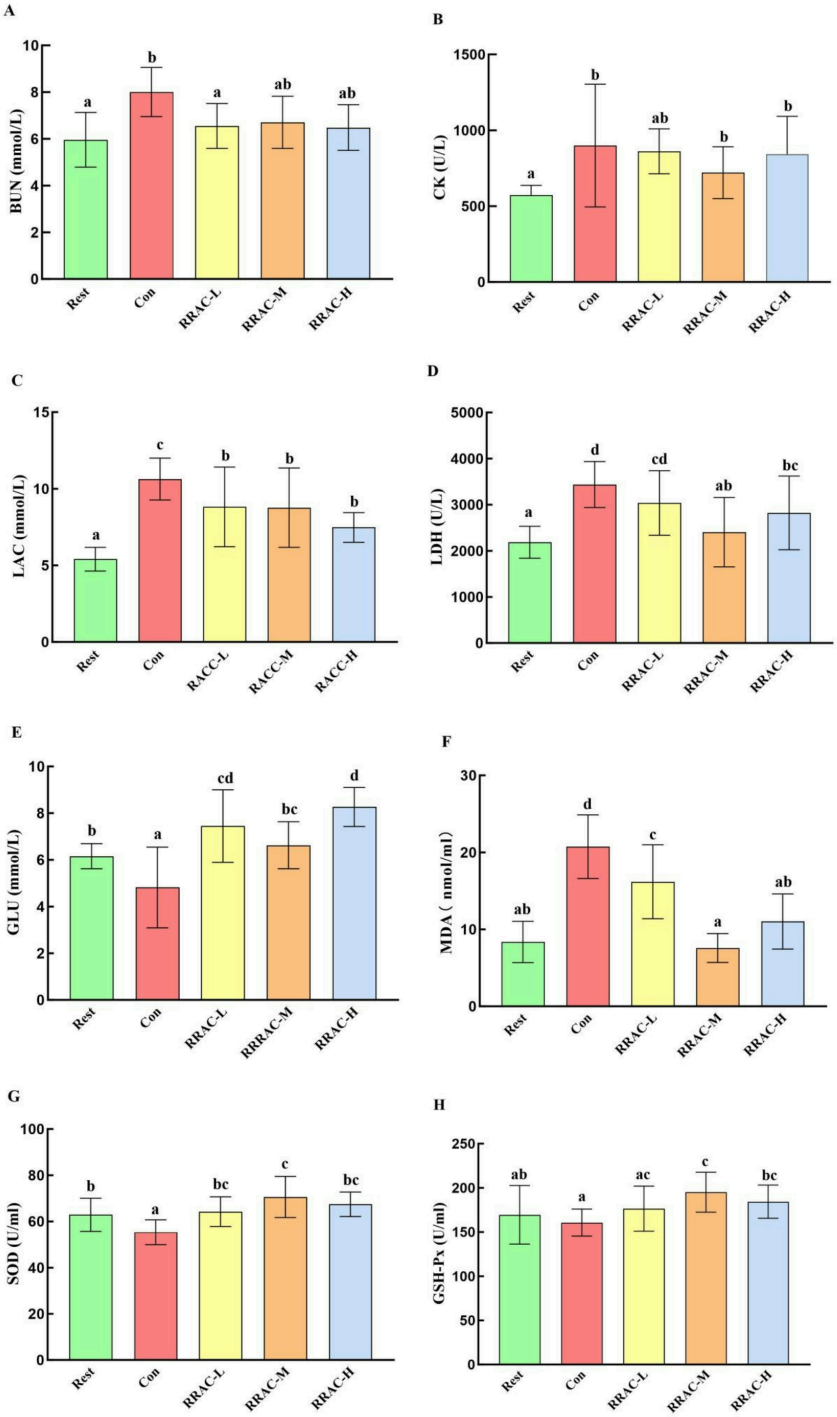

Figure 3

The effect of RRAC on biochemical parameters. (A) Blood urea nitrogen, BUN (B) Creatine kinase, CK (C) L-lactic acid, LAC (D) lactate dehydrogenase, LDH (E) glycogen, GLU, (F) malondialdehyde, MDA, (G)superoxide dismutase, SOD, (H) glutathione peroxidase, GSH-Px. The data were analyzed for significance of difference by one-way analysis of variance(ANOVA) with the LSD test ($p < 0.05$); n=10 per group.$^{a\text{-}d}$ Different letters mean significant difference ($p \leq 0.05$). The abbreviation of group used in the

figure were: fatigue model group (Con), low-dose group of RRAC (RRAC-L); medium-dose group of RRAC (RRAC-M), high-dose group of RRAC (RRAC-H).

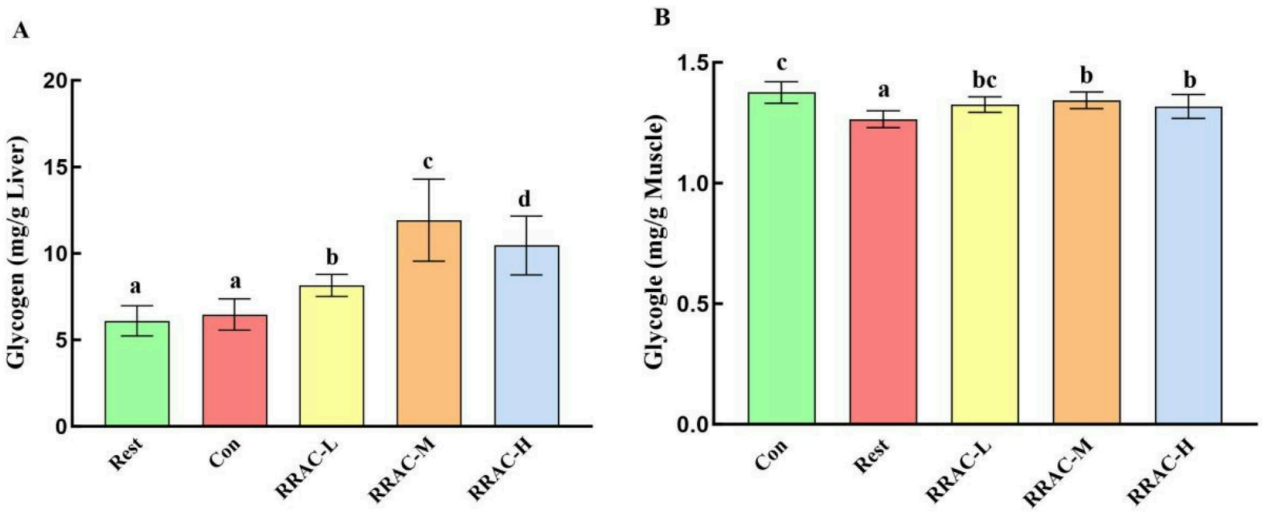

Figure 4

The effect of RRAC on liver and gastrocnemius muscular glycogen levels. The data were analyzed for significance of difference by one-way analysis of variance(ANOVA) with the LSD test ($p < 0.05$); n=10 per group.[a-d] Different letters mean significant difference ($p \leq 0.05$). The abbreviation of group used in the figure were: fatigue model group (Con), low-dose group of RRAC (RRAC-L); medium-dose group of RRAC (RRAC-M), high-dose group of RRAC (RRAC-H).

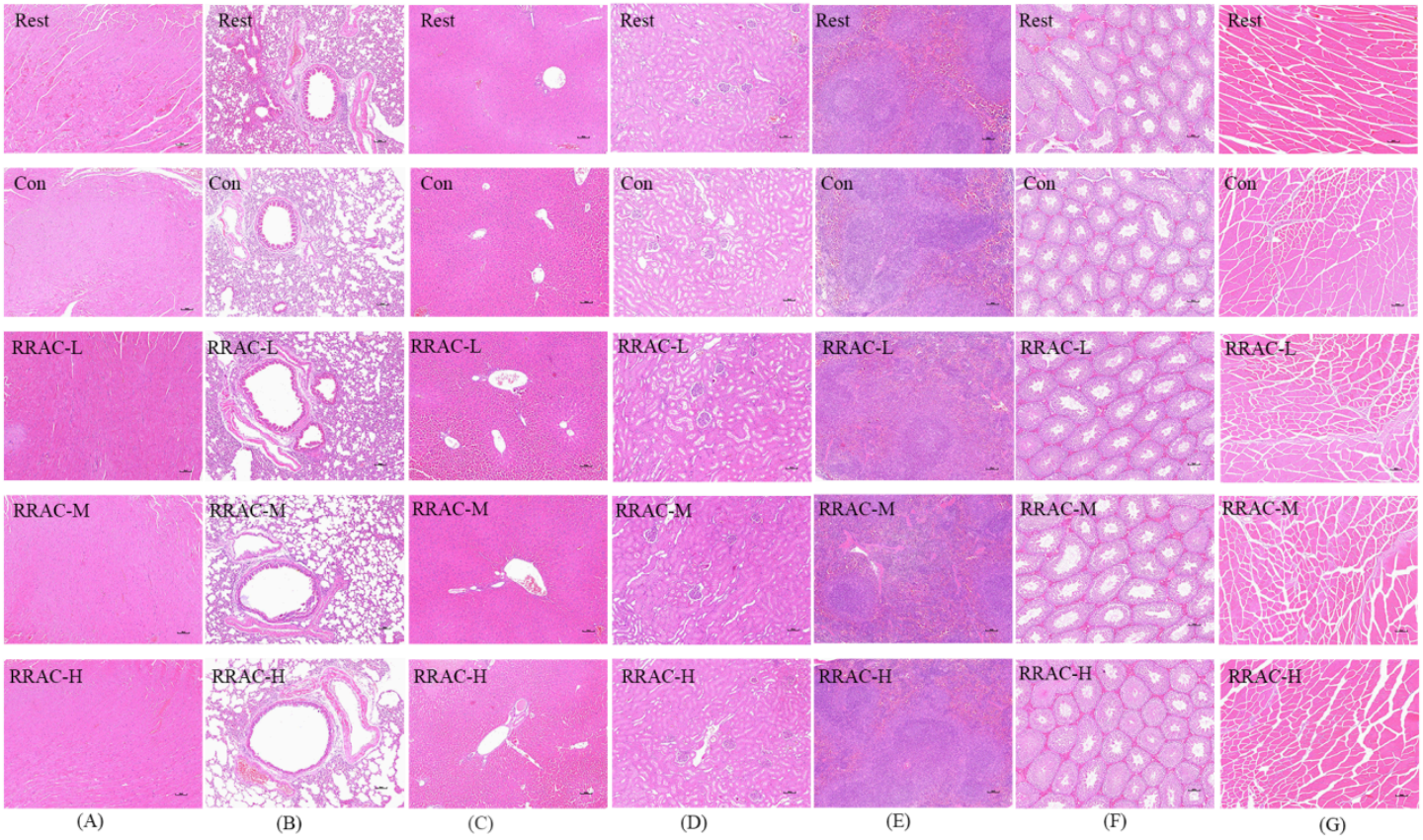

Figure 5

The effect of RRAC on histology in various organs: (A) heart, (B) lung, (C) liver, (D) kidney, (E) spleen, (F) testis, (G) muscle. H&E stain, magnification: 100×, bar, 80 μm.