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Selenium-enriched crude polysaccharide from *Rosa roxburghii Tratt* ameliorates cadmium-induced acute kidney injury in mice by modulating intestinal microorganisms

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ABSTRACT

Cadmium is a toxic heavy metal that can cause serious damage to the body. It can trigger the oxidative stress response and damage various organs of the body (kidney, liver, brain, lung, testis, etc.). Selenium polysaccharides are considered to possess better antioxidant, immune regulation, and heavy metal removal activities than other polysaccharides, But few reports focused on Selenium Polysaccharides in *Rosa roxburghii* Tratt. The purpose of this study was to isolate crude polysaccharides (RRP), and crude Selenium polysaccharides (SeRRP) from *Rosa roxburghii Tratt* fruit and determine their structure, antioxidant activity, and protective effects on cadmium-exposed mice (PONY-2020-FL-62). Results showed that SeRRP had lower half-maximal inhibitory concentration (IC50) and higher superoxide dismutase (SOD) activity. The intake of food and body weight decreased, while the kidney index and liver index increased significantly after acute cadmium exposure. Most significantly, SeRRP ameliorates kidney injury by improving the kidney index. Furthermore, changes in the gut microbiota may be related to SeRRP or RRP. SeRRP and RRP decreased the Firmicutes/Bacteroidetes ratio, and increased the abundance of beneficial bacteria (*Lachnospiraceae*, *Muribaculaceae*, and *Ruminococcaceae*, etc.). These findings indicate that SeRRP and RRP have the potential to be functional food against oxidant and heavy metal exposure.

1. Introduction

With the development of industrialisation, the species, quantity, and opportunities of human exposure and use of heavy metals have increased sharply, so heavy metals pose an increasing threat to us [1]. Cadmium is a highly toxic heavy metal found in food, water, and air [2]. In the human body, cadmium exposure is mainly due to high cadmium content in food, inhalation of cigarette smoke, and living, working in areas with severe cadmium pollution. Cadmium can accumulate in the body, trigger oxidative stress and inflammation, and damage various organs of the body (Kidney, liver, brain, lungs, testes, etc.), and it is very toxic to bacteria, causing intestinal microbial imbalance, which in turn causes various diseases [1,3–5]. Gut microbiota is susceptible to Cd, and Cd exposure via digestive tract can reduce the abundance of certain gut microbes such as *Lachnospiraceae* and *Streptococcaceae* while promoting the colonization of *Coriobacteriaceae* and *Lactobacillaceae* [6,7]. In the gut microbiota, Cadmium exposure may lead to increased

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lipopolysaccharide (LPS) production and affect the metabolic activities of the gut microbiome With increased LPS production, impaired barrier function leads to endotoxemia and systemic inflammation [2]. In the intestinal wall, Cd exposure induces an inflammatory response and cellular damage, leading to increased intestinal permeability to macromolecules, which further may lead to increased bacterial translocation into tissues [8,9]. To reduce the adverse effects of Cadmium on human beings. On the one hand, health risk assessment of Cadmium exposure is needed to identify major sources of cadmium and minimize Cadmium exposure to humans in daily life; On the other hand, Cadmium readily accumulates in crops and enters the food chain, adversely affecting the environment and human health, so the development of safe and effective strategies for Cadmium toxicity is necessary. Effective dietary strategies (essential micronutrients antagonizing Cadmium, probiotics and edible plants and dietary phytochemical supplements, etc.) play an important role in reducing or preventing Cadmium toxicity [10]. The intestinal ecosystem is an important line of defense to limit the absorption and diffusion of heavy metals and other pollutants [11]. Plant polysaccharides cannot be completely digested or absorbed in the upper digestive tract but can be fermented by the microbiota in the large intestine. It has the function of a probiotic element, which can promote the proliferation of beneficial bacteria, produces material benefits to the human body, and participates in the metabolism of the organism [12].

Rosa roxburghii Tratt, also known as mountain king fruit and thorn berry fruit, belongs to Rosaceae [13–15]. As one of the main bioactive components, *Rosa roxburghii Tratt* polysaccharide can not only resist oxidation, delay aging, and duce blood sugar but also alleviate heavy metal toxicity and regulate gut microbes [16,17]. In vivo antioxidant experiments, the concentrations of *Rosa roxburghii* polysaccharide were 200 mg/kg and 400 mg/kg. It could significantly increase the activities of antioxidant enzymes in the serum of aging mice induced by D-galactose [14]. *Rosa roxburghii Tratt* polysaccharide RTFP-3 can safely enter the large intestine and is fermented by the intestinal microbiota as a prebiotic to produce short-chain fatty acids, change the composition of the intestinal microbiota, and promote the growth of certain beneficial bacteria, such as *Bifidobacteriaceae, Lactobacilliaceae*, and so on [17]. In a cell experiment, *Rosa roxburghii Tratt* polysaccharide had neurotrophic activity and had an obvious protective effect on neural stem cell injury induced by sodium thiosulfate and glutamate [18]. In addition, *Rosa roxburghii Tratt* polysaccharide can significantly reduce the wound healing rate of ovarian cancer A2780 cells, inhibit their migration and invasion, and inhibit the expression of MMP-9 [19].

Selenium is one of the essential trace elements for human beings, which affects the important physiological functions of the human body by changing the expression of at least 30 selenoproteins. These selenoproteins can be used as antioxidants, regulate thyroid hormone metabolism and immune system function, improve sperm production and quality, and prevent cancer, etc [20,21]. Selenium can not only balance the redox level in the body, protect cells from oxidative damage and maintain the normal function of cells but also resist all kinds of damage caused by a variety of heavy metals [22]). Studies have shown that selenium can reduce the index of liver organs, improve the activity of antioxidant enzymes and increase the ability to scavenge free radicals in cadmium-exposed mice by antagonizing the heavy metal cadmium [23]. However, the content of selenium polysaccharides in natural resources is extremely low. Therefore, the best way to obtain organic selenium is the selenization of polysaccharides [24]. The combination of selenium and polysaccharide is better than selenium or polysaccharide alone. Selenium-rich polysaccharides can further enhance their antioxidant capacity, which has great therapeutic potential [25]. It has been reported that dietary fiber and selenized lentinan can change the structure, increase the diversity of the intestinal flora of mice, and slow down chronic pancreatitis caused by oxidative stress [26–28]. *Rosa roxburghii Tratt* polysaccharide can increase the abundance of beneficial bacteria and reduce the abundance of harmful bacteria in diabetes mellitus [16].

It is speculated that selenium-enriched *Rosa roxburghii Tratt* polysaccharides and *Rosa roxburghii Tratt* polysaccharides play a positive role in alleviating the body damage and intestinal homeostasis imbalance caused by cadmium exposure, which needs to be carefully verified by research. To characterize the structure and antioxidant activity of polysaccharides and Selenium polysaccharides in *Rosa roxburghii* Tratt, we explore their effects on the gut microbiota of cadmium-exposed mice. Firstly, common *Rosa roxburghii Tratt* was used as raw material, and response surface methodology was used to optimize the extraction of *Rosa roxburghii Tratt* polysaccharides. Secondly, selenium-enriched *Rosa roxburghii Tratt* was obtained by foliar spraying selenium fertilizer, and selenium polysaccharide was extracted from *Rosa roxburghii Tratt*. Compared the difference in composition, chemical structure, and antioxidant capacity of two polysaccharides. Finally, through animal experiments, taking cadmium-exposed mice as the research object, two kinds of polysaccharide intervention were used to study its effect on the intestinal flora of cadmium-exposed mice, and its protective effect on cadmium-exposed mice was studied from the perspective of intestinal flora.

2. Materials and methods

"Guinong No.5" *Rosa roxburghii Tratt* and selenium-enriched *Rosa roxburghii Tratt* (foliar spray with 50 mg/L selenium fertilizer) were harvested from the *Rosa roxburghii Tratt* cultivation base in Panzhou City, Guizhou Province. The fresh *Rosa roxburghii Tratt* fruits were transported to the laboratory at low temperatures. The dried *Rosa roxburghii Tartt's* fruits were crushed into powders and screened through 60 mesh, stored in a dryer for standby. RRP and SeRRP in *Rosa roxburghii Tratt* were optimally extracted by response surface methodology, analyzed for chemical composition and monosaccharide composition, determined molecular weight, and structurally characterized by infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) , and the specific methods and results are shown in **Supplementary Information (SI)**.

2.1. Antioxidant activities of SeRRP and RRP

2.1.1. Assay of DPPH radical scavenging

The scavenging activity of DPPH free radical (DPPH•) was measured by the reported method [13]. In brief, 1.0 mL of DPPH solution

(0.4 mM, in ethanol) was mixed with 3.0 ml of the RRP or SeRRP at various concentrations. After mixing vigorously for 10 s, the mixture was incubated at 25 °C in the dark for 30 min. Then, the absorbance of the resulting solution was measured at 517 nm. The DPPH radical scavenging effect was calculated as follows **formula 1**:

$$\text{DPPH Scavenging effect } (\%) = [(A_0 - A_1) / A_0] \times 100 \quad (1)$$

where A_0 was the DPPH of the mixture without sample and A_1 was the sample DPPH. Ascorbic acid was carried out at a positive control.

2.1.2. Assay of ABTS radical scavenging

The ABTS assay was performed following a previously described method [29], with some modifications. ABTS was dissolved in 0.01 M PBS (pH 7.4) at a 7 mM concentration. The prepared ABTS + solution was diluted to an absorbance of 0.70 ± 0.02 at 734 nm. An aliquot of 0.1 mL of RRP or SeRRP water solution (5–50 μg/mL) was mixed with 2.9 mL of ABTS + solution. After reacting for 6 min at room temperature, the absorbance was immediately measured at 734 nm. Each sample was measured in triplicate and averaged. ABTS radicals scavenging effect was calculated according to the following equation **formula 2**:

$$\text{ABTS scavenging effect } (\%) = A_0 - (A_1 - A_2)\ A_0 \times 100 \quad (2)$$

where $\mathbf{A_0}$, $\mathbf{A_{734}}$ of ABTS without sample; $\mathbf{A_1}$, $\mathbf{A_{734}}$ of sample and ABTS; and $\mathbf{A_2}$, $\mathbf{A_{734}}$ of the sample without ABTS. Ascorbic acid was carried out as a positive control.

2.1.3. Determination of SOD activity

Then the activity of SOD was detected. Test kits for the determination of SOD were obtained from Solarbio Technology (Beijing Solarbio Technology Co., Ltd, Beijing China).

2.2. Animal experiment

2.2.1. Diets and experimental groups

56 SPF grade male BAL b/c mice aged 7 weeks were reared in an environment of alternating light and shade for 12 h, temperature 21–25 °C, and air humidity 45–65% for one week. The experiment was approved by the animal ethics guidelines of the China Agricultural University Experimental Animal Ethics Committee. After one week of acclimation, they were randomly divided into 7 groups with 8 mice in each group. The grouping information and drug administration were shown in Table 1. The corncob bedding of mice was replaced every three days and supplemented with purified water, growth, and breeding feed. Normal saline was prepared and stored in a 4 °C refrigerator after sterilization at a high temperature. $CdCl_2$ solution with a concentration of 0.6 mg mL^{-1} and SeRRP, RRP ascorbic acid solution with a concentration of 10 mg mL^{-1} with sterilized normal saline were prepared. The weight, food intake, and water consumption of the mice were recorded at 9:00 a.m. every day, and 3 mg/kg BW $CdCl_2$ was injected intraperitoneally into the mice in the Cd, PCG, SRPC, and RPC groups. For the CG, SRP, and RP groups mice were intraperitoneally injected with the same amount of normal saline. At about 8:00 p.m., all groups were fed by force-feeding, Cd and CG groups were given sterilized saline, the PCG group was given 100 mg/kg BW ascorbic acid, SRP and SRPC groups were given 100 mg/kg BW SeRRP, and mice in RP and RPC groups were given 100 mg/kg BW RRP. The experiment lasted for 14 days, and fasting 12 h before dissection [30,31].

2.2.2. Sample collection

After 14 days of feeding, the mice in each experimental group fasted 12 h before dissection. Their blood was collected in the blood collection vessel and centrifuged at 3000 r/min at 4 °C for 15 min. The upper serum was extracted into a sterile, enzyme-free centrifuge tube (1.5 ml) and stored at −80 °C. The cecum of mice in each group was frozen in liquid nitrogen and placed in a refrigerator at −80 °C. The microbial structure in the cecum was detected by 16S RNA amplicon sequencing as soon as possible. The cecum was token in a labeled sterile cryopreservation tube and placed in a −80 °C refrigerator. 16S RNA amplicon sequencing was used to detect the microbial structure. The kidney and liver were weighed and recorded immediately after anatomy. The left kidney and left liver were placed in a formaldehyde fixation solution, while the right kidney and right liver were placed in a cryopreservation tube. All samples were stored at −80 °C for use [32,33].

Table 1
Animal experiment grouping and dosage.

group/administration method	Method and dose of administration	
	intraperitoneal injection	Force-feeding
model set (Cd)	3 mg/kg BW $CdCl_2$	physiological saline
blank control (CG)	physiological saline	physiological saline
positive control (PCG)	3 mg/kg BW $CdCl_2$	100 mg/kg BW ascorbic acid
***Rosa roxburghii* Selenium polysaccharide (SRP)**	physiological saline	100 mg/kg BW SeRRP
***Rosa roxburghii* Selenium polysaccharide + Cd (SRPC)**	3 mg/kg BW $CdCl_2$	100 mg/kg BW SeRRP
***Rosa roxburghii* polysaccharide (RP)**	physiological saline	100 mg/kg BW RRP
***Rosa roxburghii* polysaccharide + Cd (RPC)**	3 mg/kg BW $CdCl_2$	100 mg/kg BW RRP

2.2.3. Viscera index

The kidney index and liver index of mice were calculated by **formulas 3 and 4**, respectively [34].

$$\text{kidney index } (\%) = \frac{\text{kidney weight(g)}}{\text{Weight of mice(g)}} \times 100\% \tag{3}$$

$$\text{liver index } (\%) = \frac{\text{liver weight(g)}}{\text{Weight of mice(g)}} \times 100\% \tag{4}$$

2.2.4. Gut microbial gene sequencing and information analysis

Gut microbial composition was detected according to the process shown in Fig. 1a, and bio-information analysis was conducted according to the detected data and the process shown in Fig. 1b. For specific experimental steps, refer to the method of Wang Lei et al. [35].

2.3. Statistical analysis

SPSS 20 was used for significant difference analysis and correlation analysis ($P \leq 0.05$). Origin 95 was used for mapping. Uparse v7.0.1001 was used for clustering. Qiime software (version 1.9.1) was used to calculate the alpha index and UniFrac distance, and R language (version 2.15.3) was used to draw the dilution curve and rank endurance Lefse software was used for lefse analysis.

3. Results and discussion

3.1. Antioxidant activity analysis in vitro

3.1.1. Assay of DPPH radical scavenging

The scavenging rate of vitamin C, SeRRP and RRP on DPPH free radicals at different concentrations were shown in Fig. 2a Within the experimental concentration range, the scavenging rate of vitamin C on DPPH free radicals was the highest, which was significantly higher than that of SeRRP and RRP ($P < 0.05$). The IC50 of SeRRP and RRP were 40.49 μg/mL and 59.96 μg/mL, respectively. When the polysaccharide concentration was 20–80 μg/mL, the clearance rate of SeRRP was significantly higher than that of RRP ($P < 0.05$), but when the concentration was higher than 160 μg/mL (including 160 μg/mL), the clearance rate of SeRRP was lower than that of RRP.

3.1.2. Assay of ABTS radical scavenging

The scavenging rate of each sample to ABTS free radicals was shown in Fig. 2b Vitamin C, SeRRP, and RRP had a dose-dependent

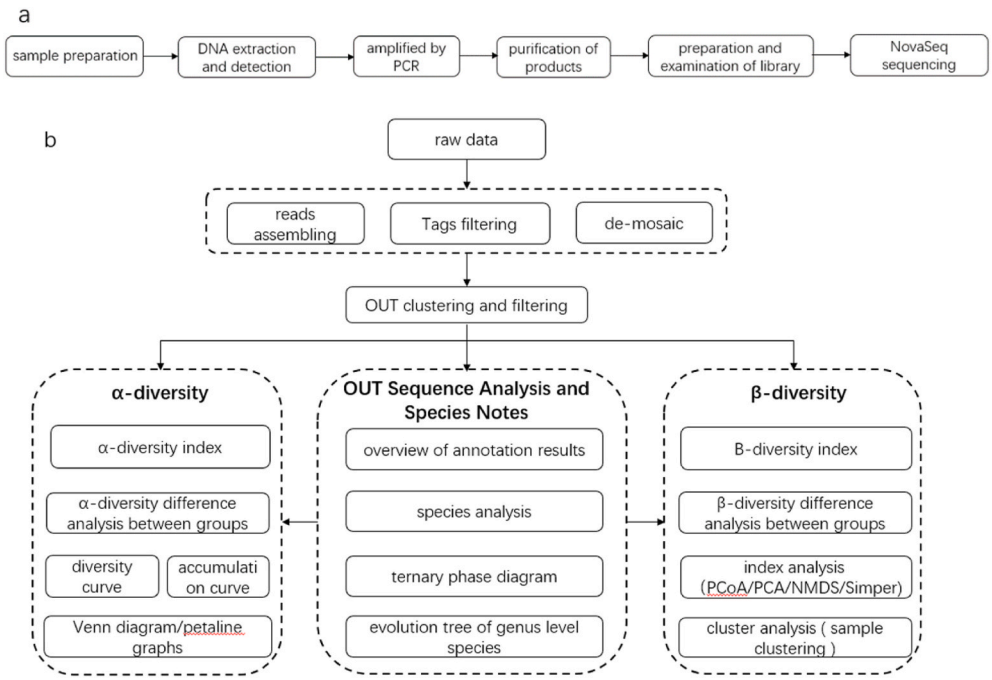

Fig. 1. Flow chart, a: experimental computer, b: information analysis.

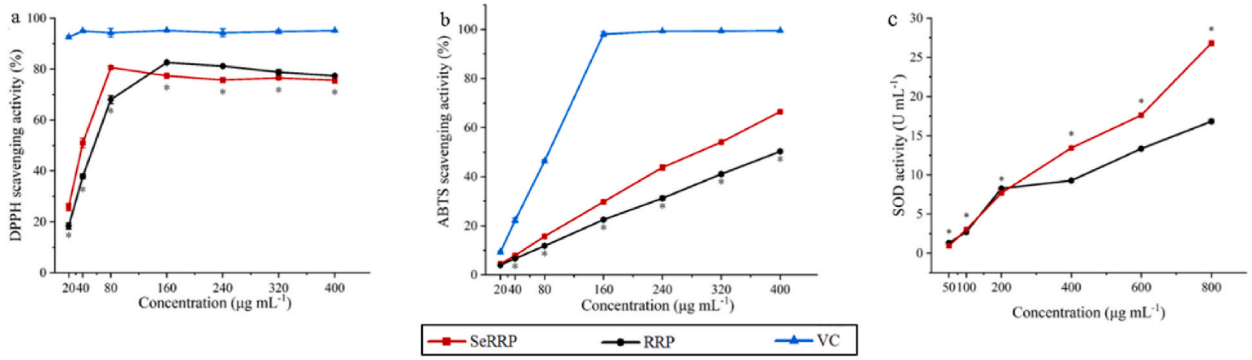

Fig. 2. DPPH radical scavenging activity (a), ABTS radical scavenging activity (b), SOD activity (c).

inhibitory effect on ABTS free radicals, and they all increased with the increase of concentration within the experimental concentration range. The scavenging rate of vitamin C on ABTS free radicals was much higher than that of SeRRP and RRP. The inhibition rate of SeRRP was significantly higher than that of RRP ($P < 0.05$), and the IC50 of the two polysaccharides was 275.88 μg/mL and 443.63 μg/mL. When the concentration was 400 μg/mL, the inhibition rates of SeRRP and RRP were the highest, which were 66.67 ± 0.30% and 50.31 ± 0.51%. It is suggested that the antioxidant capacity of SeRRP was higher than that of RRP, which might be due to the higher selenium content in SeRRP, or the existence of selenium might change the composition and structure of *Rosa roxburghii* polysaccharide, resulting in the difference in antioxidant activity.

3.1.3. Determination of SOD activity

The SOD activity of SeRRP and RRP were shown in Fig. 2c The SOD activity of the two polysaccharides had a dose relationship with the concentration, and the SOD activity increased with the increase of the concentration. When the concentration was 100 μg/mL or higher than 200 μg/mL, the SOD activity of SeRRP was significantly higher than that of RRP ($P < 0.05$); when the polysaccharide

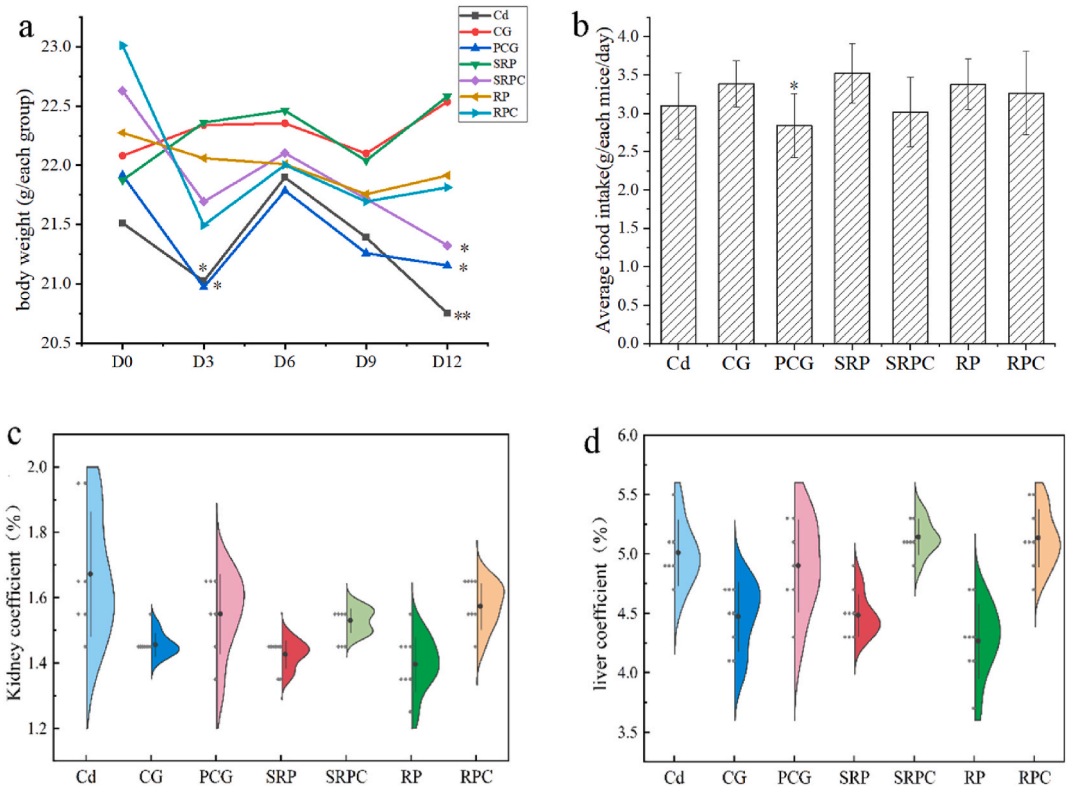

Fig. 3. Changes in body weight (a), food intake (b), Kidney coefficient (c), and liver coefficient (d) of mice in each treatment group. * Indicates that there is a significant difference at the level of $P < 0.05$ compared to CG (control group); ** Indicates a significant difference at the level of $P < 0.01$ compared to CG, Same as below 3.3. Effects of SeRRP and RRP on gut microbial composition.

concentration was 800 μg/mL, the SOD activity of the two polysaccharides were the highest, 26.82 ± 0.32 U/mL and 16.82 ± 0.28 U/mL. The difference in SOD activity between the two kinds of polysaccharides could lead to differences in their antioxidant capacity and other physiological activities.

According to the antioxidant activity determinations, the antioxidant activities of RRP and SeRRP were different. Low molecular weight polysaccharides may have better antioxidant activity, because they expose more of the reducing ends in accepting and eliminating free radicals compared to high molecular weight polysaccharides [36]. Lower molecular weight increases the antioxidant capacity of bitter gourd polysaccharides and *Laminaria japonica* polysaccharides [37,38]. The antioxidant activity of SeRRP is relatively higher than RRP due to its lower molecular weight (Table S5).

These results indicated that SeRRP and RRP might serve as a potential antioxidant with notable DPPH, ABTS radical scavenging activity and SOD activity. However, Wu et al. showed that the DPPH radical scavenging activity of purified RRP was not significantly different from that of ascorbic acid [36]. It may be due to the fact that RRP and SeRRP contain impurities such as inorganic salts, lipids, proteins and low molecular nonpolar substances (Fig S3). In addition, considering the presence of co-extracted low molecular weight compounds may also have an impact on the results, but it is uncertain whether it is positive or negative. Further research is also needed to explore the next. It is possible to purify and extract SeRRP, RRP and other possible active compounds and study their antioxidant activity, etc.

3.2. Changes in food intake, body weight, and organ coefficient of mice

During the experiment, all the mice could take in food and water normally, but compared with the blank group, the mice injected with $CdCl_2$ reduced food intake, and decreased fur gloss and depression. On the 9th day, one mouse in the Cd group died, while no death occurred in other groups. As shown in Fig. 3a, in 12 days, the weight of mice injected with $CdCl_2$ decreased, and without injection increased, indicating that acute cadmium exposure could significantly reduce the weight of mice, and ascorbic acid had no slowing effect. Especially on the third day. Mice in the Cd and PCG groups significantly reduced their body weight compared with the control ($P < 0.05$), but the SRPC and RPC groups were no significant difference. Indicating that in the short term, SeRRP and RRP could reduce the effect of $CdCl_2$ on the body weight of mice, and had a certain protective effect on mice exposed to cadmium, but had little effect on mice exposed to cadmium for a long time. As shown in Fig. 3b, compared with the mice without $CdCl_2$ injection, the food intake of mice injected with $CdCl_2$ is generally lower, and the PCG group was significantly lower ($P < 0.05$). It indicated that cadmium exposure affected the food intake of mice, and there was no significant difference in the effect of SeRRP and RRP on the food intake of mice exposed to cadmium ($P < 0.05$).

The organ coefficient was an important physiological index of animals. The increase in organ coefficient indicated edema or hyperplasia in the organs, while the decrease in organ coefficient indicated atrophy of the corresponding organs [39,40]. The calculated organ coefficients were shown in Fig. 3c and d Compared with the CG group, the kidney coefficient and liver coefficient of the Cd group increased significantly ($P < 0.01$), indicating that cadmium exposure could significantly affect the kidney and liver of mice. However, there was no significant difference between the SRPC group injected with $CaCl_2$ and the control group, which indicated that SeRRP had a better protective effect on the kidney of cadmium-exposed mice and could reduce the toxic effect of $CdCl_2$ on the kidney, while ascorbic acid and RRP had a little protective effect on the kidney of cadmium exposed mice. The liver coefficient of the Cd group, PCG group, SRPC group, and RPC group were significantly different from the CG group at the level of $P < 0.01$, indicating that ascorbic acid, SeRRP, and RRP could not reduce the toxic effect of cadmium on the liver of mice.

Moreover, considering the presence of co-extracted low molecular weight compounds in SeRRP and RRP, there could be a potential impact on the above results. Due to the uncertainty of the specific composition of these compounds, their synergistic effect needs to be further investigated.

To analyze the altered gut microbial composition in mice induced by Cadmium and the effect of the RRP and SeRRP, a metagenomic DNA sequencing was performed. Alpha diversity and beta diversity were used to evaluate the diversity and richness of intestinal microorganisms in each group. Alpha diversity analyzed the complexity of samples. OTU number, Shannon index, Simpson index, Chao index, ACE index, and coverage rate were selected to characterize the diversity and evenness of species distribution in samples and visually displayed the depth and data volume of sequencing sequences [41–43]. As shown in Table 2, compared with the control group, there was no significant difference in the diversity of the gut microbiota in each group of mice. In addition, the Shannon index and Simpson index of the RP group and RPC group were significantly lower than the CG group, indicating that the RRP could significantly affect the diversity and uniformity of gut microbes in normal and cadmium-exposed mice. At the phylum level, as shown in Fig. 4a. *Firmicutes* and *Bacteroides* were the dominant flora in each group, accounting for more than 95% of the total flora, which was

Table 2
Alpha diversity index of intestinal microbes in mice of different experimental groups.

group	OUT number	Shannon	Simpson	Chao	ACE	coverage
Cd	423.25 ± 4.35	6.68 ± 0.18	0.98 ± 0.00	459.79 ± 26.57	458.51 ± 16.65	0.9983 ± 0.0006
CG	416.50 ± 13.89	6.70 ± 0.06	0.98 ± 0.01	463.45 ± 21.21	457.9 ± 11.19	0.9980 ± 0.0005
PCG	402.00 ± 13.37	6.57 ± 0.21	0.98 ± 0.00	432.67 ± 18.72	435.59 ± 14.67	0.9980 ± 0.0000
SRP	423.25 ± 33.09	6.53 ± 0.32	0.97 ± 0.01	475.80 ± 91.94	465.08 ± 61.59	0.9980 ± 0.0014
SRPC	445.50 ± 45.05	6.60 ± 0.43	0.97 ± 0.02	481.52 ± 52.04	482.55 ± 52.93	0.9983 ± 0.0005
RP	391.25 ± 7.14	6.31 ± 0.17*	0.96 ± 0.01*	419.53 ± 23.96	427.05 ± 15.29	0.9983 ± 0.0005

consistent with the research results in other kinds of literature [28,35]. Notably, a small amount of *Cyanobacteria* was detected in the SRPC group, which was not detected in other groups. The ratio of *Firmicutes* to *Bacteroides* (F/B) was used to measure the structural changes of intestinal microorganisms. Cd exposure could increase the F/B value of intestinal microorganisms, but ascorbic acid, SeRRP, and RRP could regulate the intestinal flora of cadmium-exposed mice and decrease the F/B value. This phenomenon was consistent with previous findings that demonstrated that polysaccharides from *Rosa roxburghii* could reduce the F/B ratio [16,17]. The composition of species' relative abundance at the order classification level was shown in Fig. 4b. *Clostridium* and *Bacteroides* account for the highest proportion. Compared with the control group, the clostridial orders of the Cd group increased, while the PCG group, SRPC group, and RPC group decreased; the *Bacteroides* orders decreased in the Cd group, and the PCG group, SRPC group, and RPC group all increased. It showed that ascorbic acid, RRP, and SeRRP could all change the ratio of *Clostridium* and *Bacteroides* in the intestinal microbes of mice exposed to cadmium. In addition, 1.26% of *Bifidobacteriales* were detected in the SRPC group, which were not detected in the other experimental groups. The relative abundance composition of species at the family taxonomic level was shown in Fig. 4c. *Trichospiraceae*, *Muribaculaceae*, and *Rumenomycetes* account for a relatively high proportion, which was the common dominant bacteria families of the experimental group of mice; In addition, in the SRPC group, 1.26% of *Bifidobacteriaceae* were detected, but not in the other groups. *Muribaculaceae* was reduced by 33.04% in the Cd group. Compared with the Cd group, the proportions of *Trichospiraceae*, *Muribculaceae*, and *Rumenomycetes* in the PCG, SRPC, and RPC groups were adjusted to be similar to the normal group, indicating that ascorbic acid, RRP, and SeRRP could adjust the dominant flora of cadmium-exposed mice at the taxonomic level. The taxonomic level of the genus, as shown in Fig. 4d, compared with the control group, the proportion of *Alipipes*, *Rumenella*, and *Rossella* in the Cd group increased. Mice exposed to cadmium were orally taken with selenium SeRRP and RRP could adjust the intestinal flora of mice by changing the proportions of *Alipipes*, *Trichospira*, *Rossella*, *Gallophilus*, and *Akkermansia*.To find the differences between the different groups and species of the influence of intestinal flora. IEfSe analysis was carried out with IEfSe software, and the LDA values between the Cd group, CG group, RP group, RPC group, SRP group, and SRPC group were plotted. The

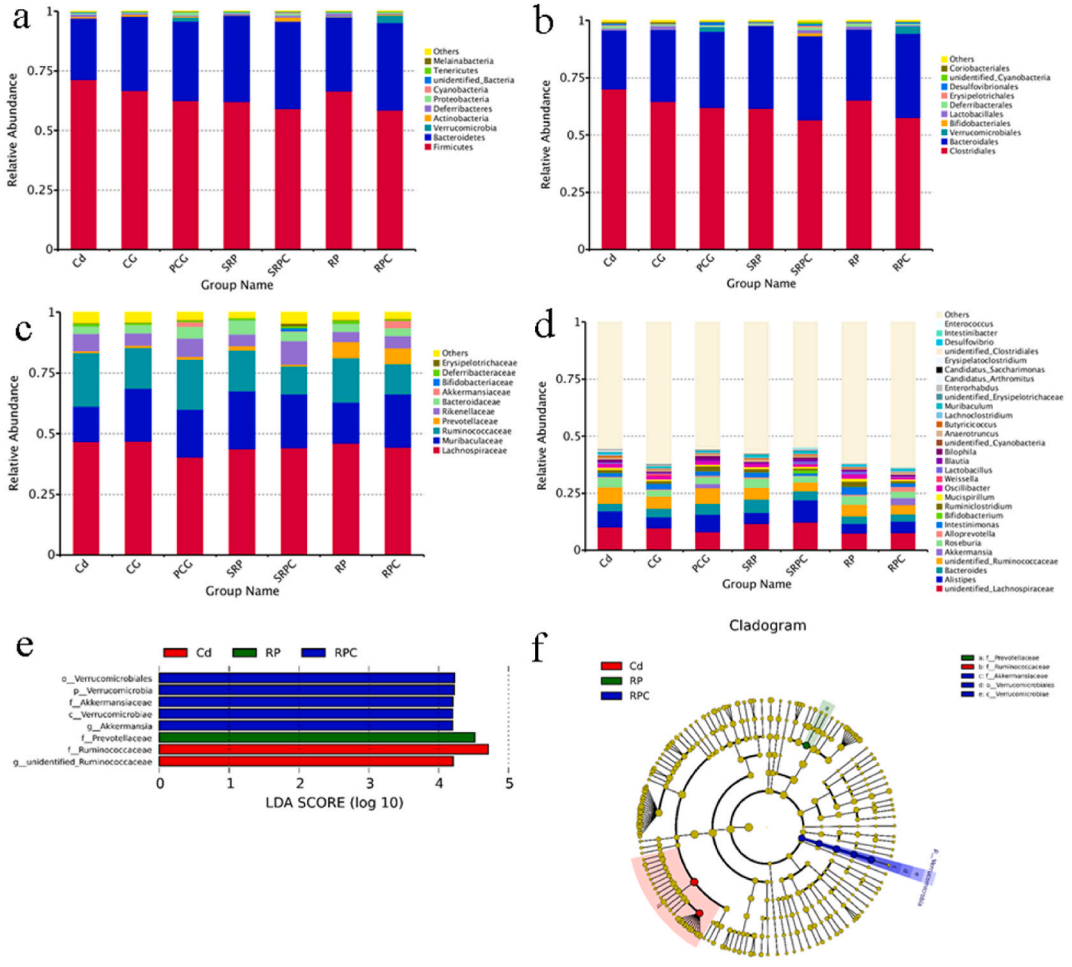

Fig. 4. Gut microbial composition of different samples at the phylum level (a), order level (b), family level (c), genus level (d); LDA distribution histogram (e); evolutionary branch graph (f). Note: The bar chart of the distribution of LDA values shows species with LDA Score greater than a set value (set to 4 by default), i.e., Biomarker with statistically significant differences between groups . .

distribution histogram and evolutionary clade diagram were shown in Fig. 4e and f. LDA value represented the influence of different species, the LDA value greater than 2 was the credible difference. The greater the value, the greater the impact of the different species [44]. In this experiment, the LDA value was set to 4 to screen the different species more strictly. As can be seen from the figure, a total of 8 mice gut microbes had been found to have a greater impact on the marker microorganisms. Among them, there were two marker microorganisms in the Cd group, which belonged to the rumen bacteria, and one kind of marker microorganism in the RP group was *prevotellaceae*. RPC group included 5 species of *Verrucomicrobia*, *Akkermaniaceaea*, and so on, all belonging to *Verrucomicrobia*.

Gut microbiota is considered to be an important target mediating toxic effects of cadmium [45]. Gut microbiota is susceptible to Cd, and Cd exposure via digestive tract can reduce the abundance of certain gut microbes, destroyed intestinal microecology [2,7,46]. Growing evidence suggests that Higher F/B ratio strongly associated with obesity-related inflammation and type 2 diabetes mellitus [47,48]. Previous studies have reported that polysaccharides from *Rosa roxburghii* reversed the ratio of F/B, thereby reducing obesity [49,50]. In this study, the Cd group exhibited a higher F/B ratio compared to RRP and SeRRP that reduced this ratio, suggesting that RRP and SeRRP have a positive effect on the gut microbiota. *Muribaculaceae* has anti-inflammatory properties and could inhibit the CD8 T cell activation to tolerate the immunity stimulation [51]. Our results showed a 33.04% reduction of *Muribaculaceae* in the Cd group. SeRRP and RRP enhanced *Muribaculaceae* abundance in Cd-induced mice. Notably, *Bifidobacterium* are of great significance to inhibit obesity, diabetes, and other metabolic diseases [52,53] In this study, 1.26% of Bifidobacterium bifidum was detected in the SRPC group. This indicates that the effects of SeRRP and RRP may be caused by the increased populations of these beneficial species. Several studies have shown that *Alistipes* are pro-inflammatory bacteria that are pathogenic in colorectal cancer and are associated with psychosomatic signs of depression [54,55]. The results showed that the Cd group exhibited a higher abundance of *Alistipes*. However, *Alistipes* were modulated by oral administration of SeRRP and RRP in Cd-exposed mice. *Rumen* bacteria were Gram-positive bacteria belonging to Firmicutes. Studies had found that *Rumen* Bacteria was related to the metabolism of carbohydrates. It could decompose polysaccharides to produce short-chain fatty acids. Butyrate could regulate cell apoptosis, differentiation, and gene expression, and relieve intestinal inflammation [56,57]4. These results indicated that after intracorneal injection of $CdCl_2$, the body would produce a stress response and increase the intestinal microorganism content related to intestinal detoxification and energy metabolism to reduce the toxic effect of cadmium exposure on mice. Studies have reported that the content of *Ackermanniaceae* in *Microflora verrucosa* was negatively correlated with obesity, and some other intestinal disorders such as enteritis and appendicitis, which could maintain the integrity of intestinal mucosa and improve immunity and was a kind of beneficial microorganism [58–60]. In this experiment, the amount of *Ackermanniae* in the RPC group was significantly increased, indicating that RRP could regulate the intestinal microbial structure of mice exposed to cadmium by upregulating the proportion of beneficial bacteria in mice exposed to cadmium and played a positive role in alleviating the damage of cadmium to the intestinal tract of mice. In addition, no marker microorganism was detected in the CG group, SRP group, and SRPC group, indicating that the structure of intestinal microorganisms did not change significantly after oral administration of SeRRP in both normal and cadmium-exposed mice. SeRRP would not unbalance the intestinal microorganism of normal mice but could regulate the intestinal flora of cadmium-exposed mice to reach the normal level.

In conclusion, the results showed that SeRRP and RRP could decrease the ratio of Firmicutes/Bacteroidetes and increase the abundance of some beneficial bacteria. Suggests that SeRRP and RRP may lead to changes in the gut microbiome. The reduction of cadmium-induced damage in mice may be related to the modulation of the intestinal microbiome by SeRRP and RRP.

However, there are shortcomings in our study. The antioxidant indices of mice were not examined in the animal experiments, thus preventing a more comprehensive assessment of the beneficial effects of RRP and SeRRP. Our previous data indicate that SeRRP and RRP have high antioxidant capacity. Animal studies from Chen and Kan revealed that the polysaccharide extracted from *Rosa roxburghii Tratt* fruit can significantly increase the antioxidant capacity to some extent, as well as decrease the level of malondialdehyde (MDA) in both serum and liver of d-Gal aging-induced mice [14]. Adequate antioxidant intake appears to be essential for effective immune system functioning. Some studies have shown that *Rosa roxburghii Tratt* polysaccharides have certain immunomodulatory effects [61,62]. Therefore, it is hypothesized that SeRRP and RRP may reduce the hazardous effects of cadmium exposure in mice through antioxidant and immunomodulatory effects, and further studies will be needed in the future to confirm this result and the possible mechanisms.

Additionally, the beneficial effects of RRP and SeRRP on the gut microbiome may also be related to the co-extraction of low molecular weight compounds. These co-extracted compounds may contain active ingredients that contribute positively to the results. Further purification and extraction experiments and *in vitro* and in vivo studies are needed to explore the future.

4. Conclusion

In summary, SeRRP was extracted for the first time in this study. Revealing the modulatory effects of RRP and SeRRP on the intestinal microbiota of cadmium-exposed mice. Firstly, RRP and SeRRP were detected to have strong antioxidant capacity; And then, the mice from RRP and SeRRP group contained lower levels of potentially harmful bacteria (e.g., *Alistipes*) and higher levels of potentially beneficial bacteria (e.g., *Muribaculaceae* and *Bacteroidaceae*). These results suggested that RRP and SeRRP might potentially be explored as an effective functional ingredient for the management of Cadmium exposure.

In this study, RRP and SeRRP were extracted by water extraction and alcohol precipitation, other extraction methods such as microwave-assisted extraction, ultrasonic-assisted extraction, enzyme extraction, and supercritical CO_2 can be further studied to explore more efficient extraction conditions. The purity of RRP and SeRRP extracted in this experiment is not high. It can further remove impurities and purify the crude polysaccharide, and deeply study the primary, secondary, tertiary, and quaternary structures of pure polysaccharides. In addition, it can tap other potential functions of polysaccharides and develop corresponding functional health

products or selenium-enriched *Rosa roxburghii* related products.

It can be concluded from animal experiments that changes in the gut microbiota may be related to SeRRP or RRP. 8 kinds of marker microorganisms that have a great impact on the intestinal microbial structure were found in different groups, belonging to *rumen bacilli*, *prevotellaceae,* and *verruca (verruca, verruca, and ackermaniidae)*. On the one hand, the functional role of each kind of bacteria can be deeply studied, On the other hand, we can explore the effect of long-term low-dose Cadmium exposure on mouse intestinal flora, and study the methods to reduce the damage of Cadmium to the body from the perspective of regulating intestinal flora. Moreover, after feeding RRP and SeRRP, the mice were in a good mental state, fed and drank water normally, and there was no mental depression or death. Compared with normal mice, there was no significant difference in body weight, food intake, organ index, and intestinal flora. It can be preliminarily judged that the safety of RRP and SeRRP is high, but a more rigorous and systematic method is needed to study whether SeRRP has potential side effects and its safe dose.

In conclusion, our study was the first to show the beneficial effects of SeRRP and RRP on mice exposed to heavy metal cadmium. Because of the efficacy of selenium, selenium-rich foods are widely popular. Due to the lack of standards, there is abuse in the application of selenium fertilizer. Good operating practices are needed to accurately apply fertilizer to make the selenium content meet the standard and not exceed the amount. A series of studies on *Rosa roxburghii Tratt* can be carried out in the future. In addition, the beneficial effects of the two polysaccharides have been observed in animal experiments, and the maximum dose of their beneficial effects can be further explored.

Ethical approval

This study and included experimental procedures were approved by the institutional animal care and use committee of Pony Testing International Group Co., Ltd., with the approval number: PONY-2020-FL-62. All animal housing and experiments were conducted in strict accordance with the institutional guidelines for the care and use of laboratory animals.

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Author contribution statement

Xingmiao Lu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the da …

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

All authors disclosed no relevant relationships.

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