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Improvement of the extracts of *Rosa roxburghii* Tratt on prostatic fibrosis induced by high-fat diet

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ABSTRACT: A high-fat diet (HFD) has been implicated in the development of prostatic fibrosis, which, either independently or in combination with benign prostatic hyperplasia (BPH), can result in lower urinary tract symptoms (LUTS) in elderly men. *Rosa roxburghii* Tratt (RRT), a food with both edible and medicinal properties, is rich in a diverse range of bioactive compounds, including terpenoids, organic acids, polyphenols, coumarins, glycosides, amino acids, flavonoids, and phthalides. In this study, we employed an HFD-induced prostatic fibrosis mouse model to investigate the anti-fibrotic effects of RRT extracts and the underlying mechanisms. Treatment with RRT extracts mitigated the progression of prostatic fibrosis by addressing lipid metabolic dysregulation, inflammation, oxidative stress, and imbalances in sex hormone levels. Furthermore, RRT extracts reduced the incidence of bladder urine retention in HFD-fed mice and inhibited the TGF- β /Smad signaling pathway, a key mediator of fibrosis. Collectively, these findings suggest that RRT extracts may ameliorate HFD-induced prostatic fibrosis through a multi-target, multi-component approach. This study provides valuable experimental insights into the pathogenesis of HFD-induced prostatic fibrosis and supports the potential therapeutic application of RRT in health, food, and medicinal contexts.

Keywords: High-fat diet, Obesity, Prostatic fibrosis, LUTS, RRT

1. Introduction

Lower urinary tract symptoms (LUTS) represent a common health concern in elderly men, encompassing a range of bothersome voiding symptoms, including increased urinary frequency, urgency, nocturia, a slow stream, and postmicturition dribble^[1]. BPH is widely regarded as the primary cause of LUTS in this population^[2]. Recent studies have highlighted that prostatic fibrosis is closely associated with the development of LUTS, acting either as an independent pathological factor or in synergy with BPH to exacerbate symptoms^[3]. Prostatic fibrosis disrupts the elasticity of the prostatic urethra, increases resistance at the bladder outlet, and contributes to male LUTS. Notably,

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research has shown a strong correlation between HFD-induced obesity, particularly central obesity, and the occurrence of clinically significant LUTS^[4]. Conversely, weight loss has been associated with a reduction in LUTS symptoms^[5, 6]. Gharaee-Kermani et al. reported that HFD-induced obesity may promote lower urinary tract fibrosis in murine models^[7]. Additionally, our previous study observed that patients with metabolic syndrome, particularly those with hyperlipidemia, exhibit more advanced prostatic fibrosis in histopathological samples^[8].

As awareness of essential nutrients vital for human growth continues to expand, there is a growing focus on the health benefits of food and its potential medical applications. The use of functional foods has emerged as an important strategy for the prevention and treatment of various diseases. *Rosa roxburghii* Tratt (RRT), also known as ShanWang Guo or the cranberry fruit, is a perennial deciduous shrub in the Rosaceae family. It is widely distributed across altitudes ranging from 500 to 2500 meters in the mountainous regions of southwest and south-central China, with a particular high prevalence in Guizhou Province^[9]. RRT fruit is an underutilized functional food that has attracted increasing attention due to its rich composition of bioactive compounds^[10]. Studies have demonstrated that RRT fruit possesses anti-inflammatory, antioxidant, hypoglycemic, hypolipidemic, radioprotective, immunomodulatory, anticancer, and anti-aging properties^[11, 12]. Recently, it was reported that RRT juice could inhibit TGF- β ₁-induced epithelial-mesenchymal transition (EMT) and fibroblast differentiation by targeting the Smad2/3 signaling pathway, thereby exerting anti-pulmonary fibrosis effects^[13]. However, no studies have yet explored whether RRT extracts possesses an anti-prostatic fibrosis effect. Our previous studies have demonstrated that prostatic fibrosis may be linked to prostatic inflammation, altered sex hormone levels, and upregulation of the TGF- β /Smad signaling pathway^[14, 15]. Thus, it is of interest to investigate whether RRT extracts could ameliorate obesity-related prostatic fibrosis through mechanisms such as anti-inflammation, modulation of sex hormone levels, or down-regulation of the TGF- β /Smad pathway.

In this study, we constructed a mouse model of HFD-induced prostatic fibrosis and investigated the effects of RRT extracts intervention. Our findings confirm that RRT extracts alleviated HFD-induced prostatic fibrosis in mice by modulating lipid metabolism, exerting anti-inflammatory and antioxidant effects, and correcting sex hormone imbalances. These results provide valuable insights into the prevention and treatment of prostatic fibrosis and contribute experimental data to support the development and application of RRT-based products. Our study offers novel insights into the potential therapeutic role of RRT extracts and underscores the importance of further exploration in this area.

2. Materials and methods

2.1 Animals

Thirty C57BL/6J male mice (8weeks old, weighing 18-20g) were purchased from Chongqing Tengxin Biotechnology Co., Ltd. (Chongqing, China). The mice were placed in a specific

pathogen-free (SPF) room maintained at an air-conditioned temperature (23-25)°C and a relative humidity of 50%-60% on a 12-hour light/dark cycle. All animal care procedures and experiments were approved by the Experimental Animal Ethics Committee of Guizhou University (Approval Number: EAE-GZU-2024-E048).

2.2 RRT extracts sample preparation

RRT juice was sourced from Guizhou Peihua Agricultural Science and Technology Development Co., Ltd (Guizhou, China). The RRT juice was evaporated and concentrated through rotating evaporation and vacuum freeze-drying. Finally, the RRT freeze-dried powder was stored at 4°C in sterilized glass bottles.

2.3 Analysis of common genes associated with prostatic fibrosis

Genes linked to prostatic fibrosis were searched in the GeneCards (<https://www.genecards.org/>, accessed on 5 May 2025) and NCBI (National Center for Biotechnology Information) (<https://www.ncbi.nlm.nih.gov/>, accessed on 5 May 2025) databases. Duplicate genes were removed from each database individually. A comparison between the two databases was then conducted to screen for common genes associated with prostatic fibrosis. These common genes were then annotated using the Sangerbox (<http://www.sangerbox.com/tool>, accessed on 5 May 2025) platform for conducting Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis ($P < 0.05$).

2.4 Extracts of RRT ingredients analysis and target genes prediction

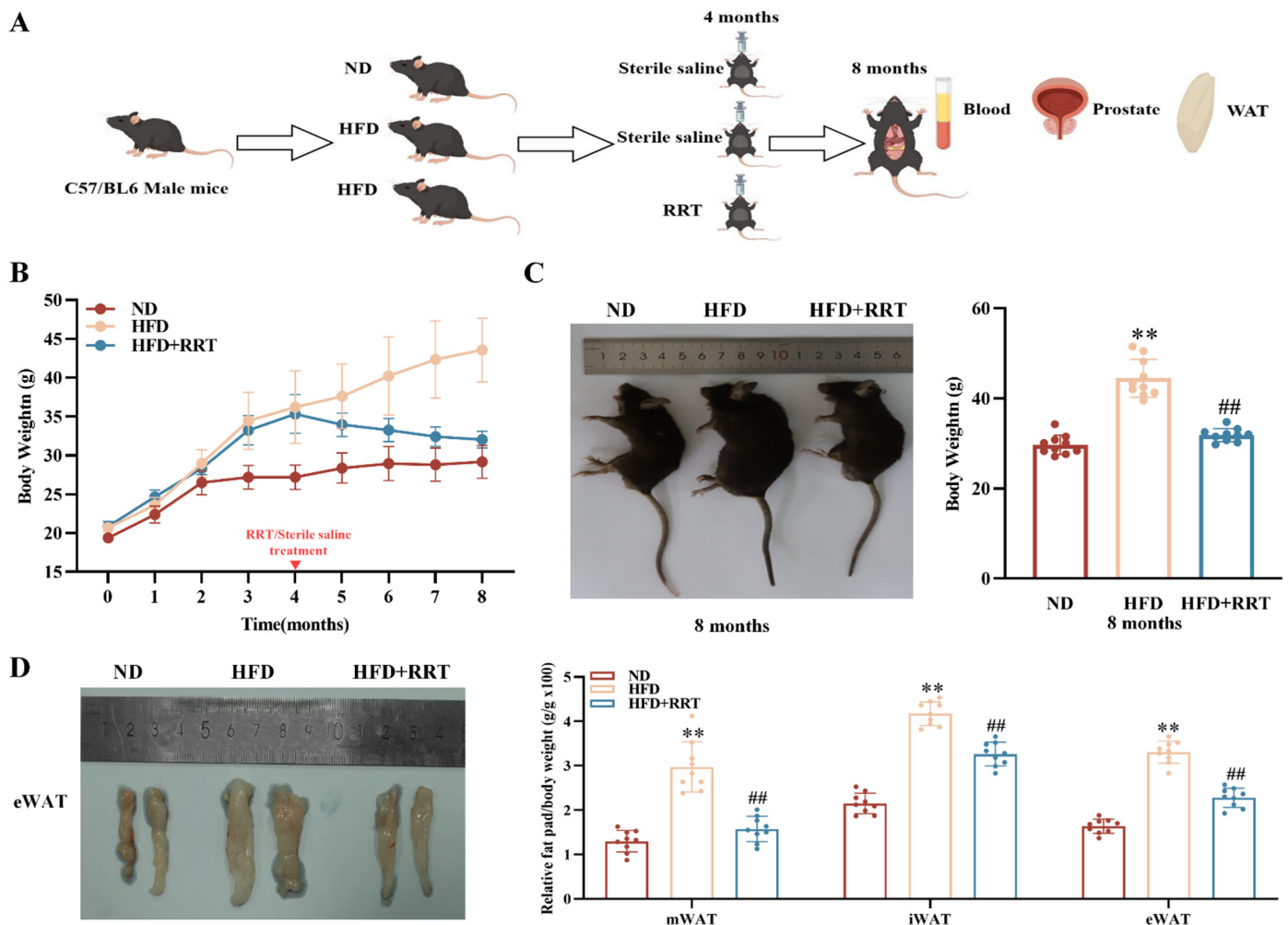
The ingredients of RRT extracts were analyzed using liquid chromatography-mass spectrometry (LC-MS). Utilizing the Traditional Chinese Medicine Systems Pharmacology database and analysis platform (TCMSP) (<https://tcmssp.com/tcmssp.p-hp>, accessed on 5 May 2025), active ingredients within RRT extracts exhibiting oral bioavailability (OB) $\geq 20\%$ and drug-like properties (DL) ≥ 0.18 were screened. The identified ingredients predicted targets were further obtained from both TCMSP and Swiss Target Prediction (www.swisstargetprediction.ch/, accessed on 5 May 2025). These predicted targets of RRT extracts were then cross-referenced with commonly identified genes associated with prostatic fibrosis to pinpoint the core targets pertinent to both the disease and RRT extracts.

The protein-protein interaction (PPI) relationships among these core targets, the STRING database (<https://string-db.org/>, accessed on 5 May 2025) was queried online. The retrieved PPI data were imported into Cytoscape 3.10.2 software for visualization and analysis, ultimately constructing a PPI network diagram. Subsequent to this, the Sangerbox platform was employed to annotate the core targets and conduct KEGG enrichment analysis ($P < 0.05$).

2.5 Establishment of the HFD-induced obesity mouse model and treatment with RRT extracts

Mice were randomly assigned to three groups of ten each: the Normal diet group (ND group), the HFD group, and the HFD+RRT extracts-treated group (HFD+RRT group). The ND group and HFD

group were fed either a normal diet (12.95% of calories from fat, 24.02% protein, and 63.03% carbohydrates) or a high-fat diet (60.93% of calories from fat, 19.79% protein, and 19.27% carbohydrates), respectively, for 8 months. Starting from the fifth month, mice in the HFD+RRT group were administered RRT extracts daily via gavage at a dose of 200 mg/kg, which was determined based on preliminary experimental results from our previous studies (this dosage did not affect the mice's food intake or behavioral state). Water was provided ad libitum. At the conclusion of the experiment (spanning 8 months), the mice were weighed, and orbital blood was collected before they were euthanized. After measuring the diameter of the isolated bladder using a vernier caliper, prostate specimens were collected and weighed. In addition, the mesenteric white adipose tissue (mWAT), inguinal white adipose tissue (iWAT), and epididymal white adipose tissue (eWAT) were harvested and weighed individually. The relative organ weights were determined by dividing the organ weight by the body weight and multiplying by 100. The experimental treatment procedure is clearly depicted in Fig. 1A drawn by Figdraw.



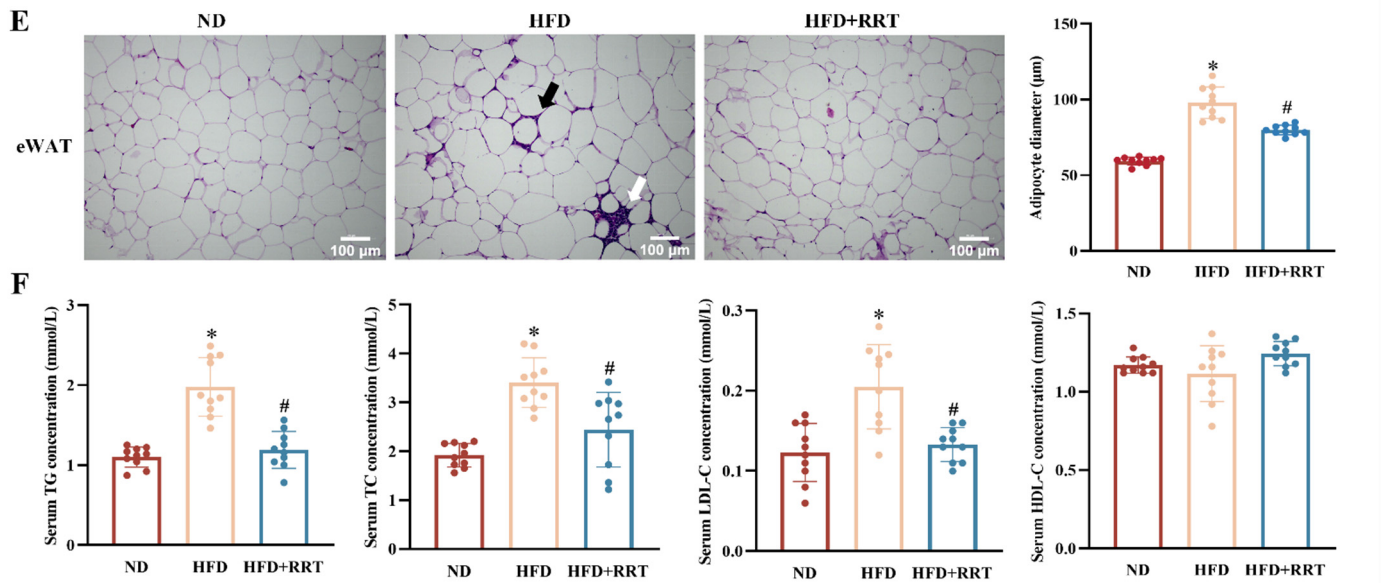


Fig. 1 RRT extracts relieve HFD-induced obesity. (A) The experimental treatment procedure of mice model; (B) Body weight change of mice in each group within 8 months; (C) Macroscopic views of body size and body weight of mice in each group at 8 months; (D) Macroscopic views of eWAT and the relative weight of mWAT, iWAT, and eWAT of mice in each group at 8 months; (E) Representative images of HE-stained eWAT and adipocyte diameter quantification of mice in each group at 8 months (HE×100; Scale bar=100 μm); with black arrows pointing to CLS and white arrows pointing to foci of inflammatory cells; (F) Serum lipid concentrations of the mice in each group. Data are presented as the mean ± SD. * $P < 0.05$, ** $P < 0.01$ compared to the ND group; # $P < 0.05$, ## $P < 0.01$ compared to the HFD group.

2.6 Serum lipid measurements

Serum lipids, including total triglycerides (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), and high density lipoprotein-cholesterol (HDL-C) were assessed using kits obtained from Nanjing Jiancheng Institute of Biological Engineering (Nanjing, China).

2.7 Serum hormone measurements

Serum oestradiol (E₂) and testosterone (T) levels were determined by using E₂ enzyme-linked immunosorbent assay (ELISA) kits (Uscn Life Science, Wuhan, China) and T enzyme-linked immunosorbent assay (ELISA) kits (Ruixin, Wuhan, China) according to the manufacturer's instructions.

2.8 Antioxidant indexes measurements

The total superoxide dismutase (T-SOD) and malondialdehyde (MDA) of mice prostatic tissues were determined using kits from the Nan Jing Jian Cheng Bioengineering Institute (Nanjing, China) according to the manufacturer's instructions.

2.9 Histopathological examination

The urethra-prostate complexes and eWAT were fixed in 10% neutral formalin solution for 24 hours at room temperature (RT), dehydrated in ethanol, cleared in xylene and embedded in paraffin. Paraffin-embedded urethra-prostate complexes (covering the whole prostate) or eWAT were cut into serial cross-sections (4μm thickness). Subsequently, the sections were stained with haematoxylin and

eosin (HE), and histopathologic changes in the prostate and eWAT were observed. The quantification methods for the degree of inflammation (in the prostate and eWAT), the adipocyte diameter and CLS (in eWAT) as follows. Under a 400× magnification field of view, the number of inflammatory cells in 5 randomly selected non-overlapping fields of view in the prostate of each mouse is counted; under a 40× magnification field of view, 10 non-overlapping fields of view were randomly selected in the eWAT of each mouse to measure adipocyte diameter, the number of CLS, and number of inflammatory cell foci. The average values are taken as the quantitative indicators for adipocyte diameter, CLS, and inflammatory infiltration in prostate or eWAT.

2.10 Masson's trichrome staining

Masson's trichrome staining was performed according to the manufacturer's instructions (Solarbio, Beijing, CHN). Briefly, the sections were placed in Weigert's working haematoxylin for 10 minutes (min). After washing in distilled water, the sections were stained for 10 min in Masson's trichrome solution. Sections were rinsed in 95% alcohol, dehydrated, cleared, and coverslipped. Collagen fiber and smooth muscle were stained blue and red, respectively. The quantitative analysis of the relative area of collagen fibers was performed as follows. Under a 100× magnification field of view, 4 non-overlapping fields of view were randomly selected in the prostate of each mouse. The ImageJ software was employed to measure the area of the blue-stained regions between prostate acini and around the glands (excluding the glands themselves and the blue regions within the glands) to calculate the relative area of collagen fibers. The average values are taken as the quantitative indicators for collagen fibers in the prostate of each mouse.

2.11 Immunohistochemical staining

Immunohistochemical (IHC) staining was performed to detect the expression of collagen I, TGF- β_1 , estrogen receptor α (ER α), androgen receptor (AR), CD68, Lysozyme (Lyso), CD20 and CD45RO, respectively. Briefly, the sections were treated with blocking buffer (Dako Denmark A/S, Glostrup, Denmark) for 30 min at RT and thereafter incubated with anti-ER α antibody (1:300 dilution), anti-AR antibody (1:100 dilution), anti-CD68 antibody (1:300 dilution), anti-lysozyme antibody (1:200 dilution), anti-CD20 antibody (1:200 dilution), or anti-CD45RO antibody (1:200 dilution) overnight at 4°C, respectively. After thorough rinsing with Tris-NaCl buffer, the sections were incubated with biotinylated goat anti-rabbit IgG (1:200 dilution in Tris-NaCl buffer) for 60 min at RT. The sections were subsequently incubated with an avidin-biotinylated enzyme complex and 3,3'-diaminobenzidine (DAB). All the antibodies mentioned above were obtained from Abcam. Collagen I, TGF- β_1 , ER α and AR staining was scored based on the percentage of positive cells and staining intensity. The percentage of positive cells was denoted by four scores as follows: <5% = 0, 6% to 25% = 1, 26% to 50% = 2, 51% to 75% = 3, and >75% = 4. The staining intensity was scored as follows: no staining = 0, weak staining = 1, moderate staining = 2, or intense staining = 3. The histologic score (H-score) for each sample was determined by the following formula: H-score =

percentage score $\times$ intensity score. The expression of collagen I, TGF- β_1 , ER α and AR was semiquantified by the H-score.

2.12 Real-time quantitative PCR

The mRNA levels of *Il-1 β* , *Il-6*, *Nf-kb*, *Cxcl1*, *Ccl2*, *Ptgs2*, *Col1a1* (encoding collagen I), *Acta2* (encoding α -SMA), *Tgfb1*, *Esr1* (encoding ER α), *Ar* and *Gapdh* gene were detected by Real-time quantitative PCR (RT-qPCR). Total RNA was extracted from mice prostates with TRIzol reagent (Invitrogen, Carlsbad, CA). The RNA was reverse transcribed to cDNA and then subjected to RT-qPCR. RT-qPCR was performed by using SYBR Green Premix Pro Taq HS qPCR Kit (AG). Primer sequences are shown in Supplementary Table 1. The relative mRNA levels were calculated by $2^{-\Delta\Delta C_t}$.

2.13 Western blotting

Total protein was extracted, and proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto polyvinylidene difluoride (PVDF) membranes. After blocking with 5% dry milk, the PVDF membranes were incubated with anti- α -SMA antibody, anti-Collagen I antibody, anti-TGF- β_1 antibody, anti-Smad2 antibody, anti-p-Smad2 antibody, anti-Smad3 antibody, anti-p-Smad3 antibody and anti-GAPDH antibody for 120 min at RT, respectively. After washing, membranes were incubated with secondary antibodies for 2 hours at RT. Protein signals were detected using an ECL chemiluminescent substrate. The intensity of the signal on the film was quantitated using a computer-assisted imaging system and normalized to the corresponding signal for GAPDH. The primary antibody information can be found in Supplementary Table 2.

2.14 Statistical analysis

Data from the groups are expressed as the means $\pm$ standard deviations (SD). Means were compared by a one-way analysis of variance (ANOVA) and t-test using SPSS software version 20.0 (IBM, Armonk, NY, USA) to determine if there were statistically significant differences. A P -value < 0.05 indicated a statistically significant difference.

3. Results

3.1 RRT extracts relieve HFD-induced obesity

At 4 months, the body weight of mice fed with HFD was significantly higher than that of mice fed with ND. From this point onward, the body weights of mice in the HFD+RRT group, which received daily RRT extracts-treated via gavage, gradually declined in comparison to those in the HFD group (Fig. 1B). At the conclusion of the experiment (at 8 months), the body weights and relative weights of WAT (including mWAT, iWAT, and eWAT) were significantly increased in the HFD mice compared to the ND mice. In contrast, a decrease in both body weights and relative WAT weights (mWAT, iWAT and eWAT) was observed after 4 months of treatment with RRT extracts ($P < 0.01$) (Fig. 1C and D). The HE staining results of eWAT revealed that mice in the HFD group had significantly larger adipocyte diameters compared to those in the ND group (P

< 0.01), and macrophage rings (crown-like structures, CLS) surrounding the white adipocytes, along with foci of inflammatory cell infiltration were observed in the HFD group. However, in the HFD+RRT group, the adipocyte diameters of eWAT were smaller than those in the HFD group ($P < 0.05$) (Fig. 1E), and no significant CLS or inflammatory cell infiltration were observed. Additionally, serum lipid levels were assessed across the three groups of mice. The findings indicated that TG, TC, and LDL-C levels were elevated in the HFD mice compared to the ND mice. In contrast, these levels were significantly reduced in the HFD+RRT group compared to the HFD group ($P < 0.05$). No significant changes in high-density lipoprotein cholesterol (HDL-C) levels were detected among the three groups (Fig. 1F).

3.2 RRT extracts improve HFD-induced prostatic fibrosis

There were no significant differences in the relative prostate weight among the three groups. However, we observed that many mice in HFD group exhibited increased bladder diameter and urinary retention in the bladder compared to the ND group. After treatment with RRT extracts, only a few mice in HFD+RRT group showed mild urinary retention in the bladder, and their bladder diameter was significantly reduced (Fig. 2A). Histological analysis revealed more pronounced morphological alterations in the HFD group. The HFD group showed hyperplastic prostatic stroma, with a thickened fibromuscular layer around the acini and focal or diffuse infiltration of inflammatory cells, compared to ND group. Interestingly, the group treated with RRT extracts exhibited a thinner fibromuscular layer and fewer inflammatory cells compared to the HFD group (Fig. 2B). Masson's staining results indicated that the relative collagen fiber area was significantly greater in the HFD group compared to the ND group. In contrast, RRT extracts treatment significantly improved this collagen fiber area in the prostate compared to the HFD group ($P < 0.01$) (Fig. 2B and C), suggesting that RRT extracts may ameliorate HFD-induced prostatic fibrosis. Furthermore, we used RT-qPCR and IHC to examine fibrosis-related markers in the prostate of mice among three groups. RT-qPCR results showed that the mRNA expression levels of *Colla1* and *Tgfb1* were elevated in the prostate of HFD mice compared to ND mice. However, treatment with RRT extracts led to a significant reduction in the expression levels of *Colla1* and *Tgfb1* mRNA in the prostate of HFD+RRT mice ($P < 0.05$, $P < 0.01$) (Fig. 2D). The IHC results demonstrated that collagen I and TGF- β_1 -positive cells are mainly expressed in the fibromuscular layer surrounding the acini and in the stromal cells between the prostatic acini, with higher protein levels of collagen I and TGF- β_1 in HFD mice than ND mice. Notably, these protein levels were significantly lower in HFD+RRT mice compared to HFD mice. These findings were corroborated by WB results, which aligned with the IHC observations. However, no significant differences in the expression of mRNA (*Acta2* gene) or α -SMA protein among the three groups (Fig. 2D and F). Furthermore, we measured the expression of proteins related to the TGF- β /Smad pathway. The results showed that the expression ratios of p-Smad2 to Smad2 and p-Smad3 to Smad3 were significantly higher in the HFD group compared to the ND group. Treatment with RRT extracts significantly down-regulated the expression ratios of p-Smad2/Smad2 and p-Smad3/Smad3 ($P < 0.05$) (Fig. 2G).

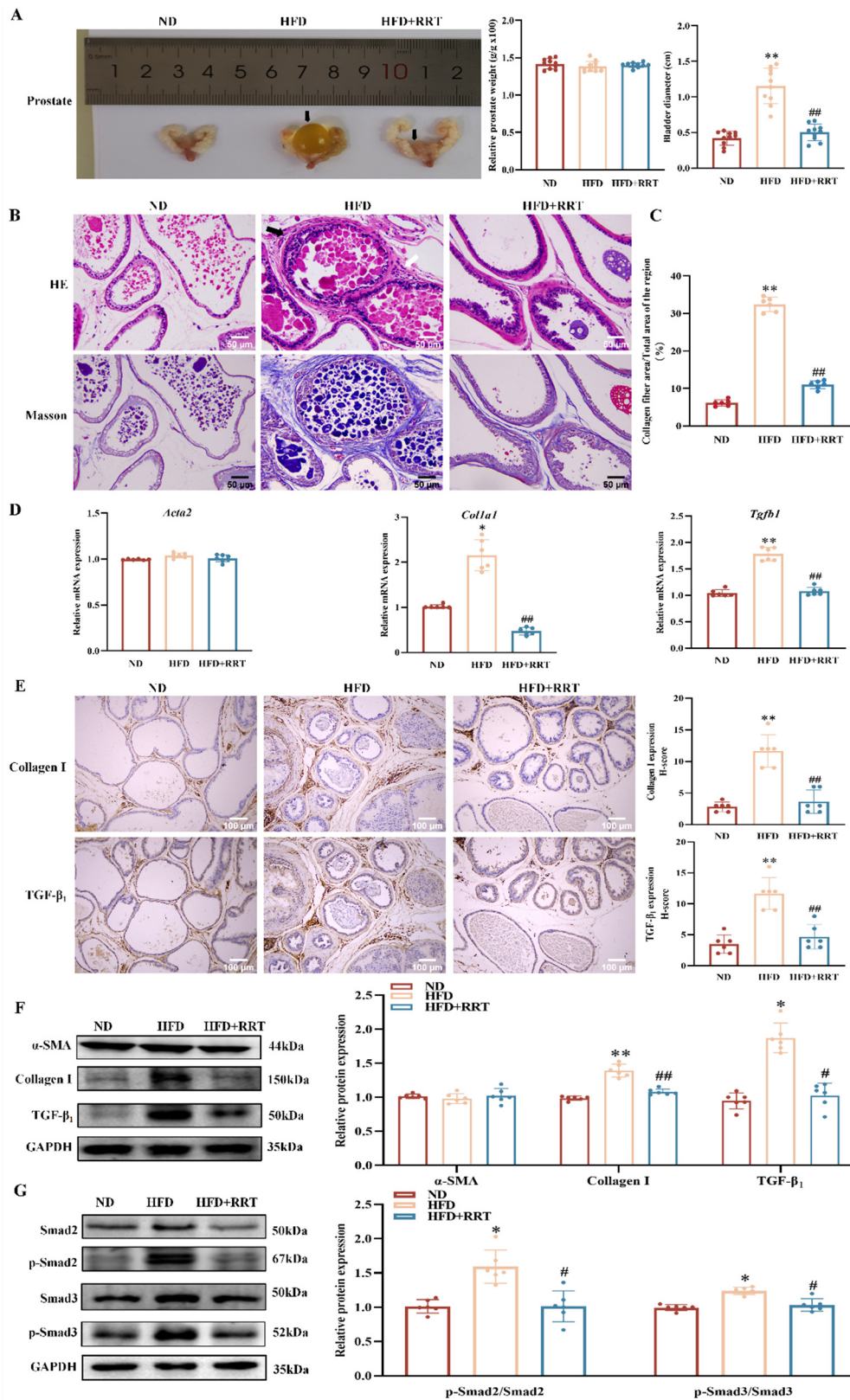


Fig. 2 RRT extracts improve HFD-induced prostatic fibrosis. (A) Macroscopic views of prostate and urinary retention in the bladder, along with the relative weights of mice prostates and the diameters of the bladders in each group, with black arrows pointing to urinary retention in the bladder; (B) HE and Masson's staining of mice prostate of each group, with black arrows pointing to fibromuscular layer around the acini and white arrows pointing to focal inflammatory cells (HE×200; Masson×200; Scale bar=50 μm); (C) Relative collagen fiber area of mice prostate in each group; (D) The mRNA expression level of *Acta2*, *Colla1* and *Tgfb1* of mice prostate in each group; (E) IHC staining and quantitative analysis of collagen I and TGF-β₁ in mice prostate of each group (IHC×100; Scale bar=100 μm); (F) The protein expression level of α-SMA, collagen I and TGF-β₁ of mice prostate in each group. (G) The protein expression level of TGF-β/Smad pathway-related proteins of mice prostate in each group. Data are presented as the mean ± SD. **P* < 0.05, ***P* < 0.01 compared to the ND group; #*P* < 0.05, ##*P* < 0.01 compared to the HFD group.

3.3 RRT extracts may improve prostatic fibrosis through its multi-ingredient and multi-target properties

To identify key genes involved in the progression of prostatic fibrosis, we conducted a comprehensive analysis of genomic data from the publicly available databases GeneCards and NCBI. Initially, we analyzed genes with differential expression patterns associated with prostatic fibrosis in each database. After performing a joint analysis, we identified 283 genes with consistent patterns of change across both databases (Fig. 3A). Interestingly, KEGG enrichment analysis highlighted several pathways of interest, including those related to lipid metabolism. Specifically, insulin resistance, adipocytokine signaling pathway, and sphingolipid signaling pathway were enriched ($P < 0.05$). This suggests that lipid metabolism and genes associated with HFD may be key players in the development and progression of prostatic fibrosis. Our analysis also revealed that the KEGG pathway enrichment included inflammation, oxidative stress, and estrogen-related pathways ($P < 0.05$), suggesting a connection between prostatic fibrosis and not only lipid metabolism but also inflammation, oxidative stress and alterations in sex hormones (Fig. 3B).

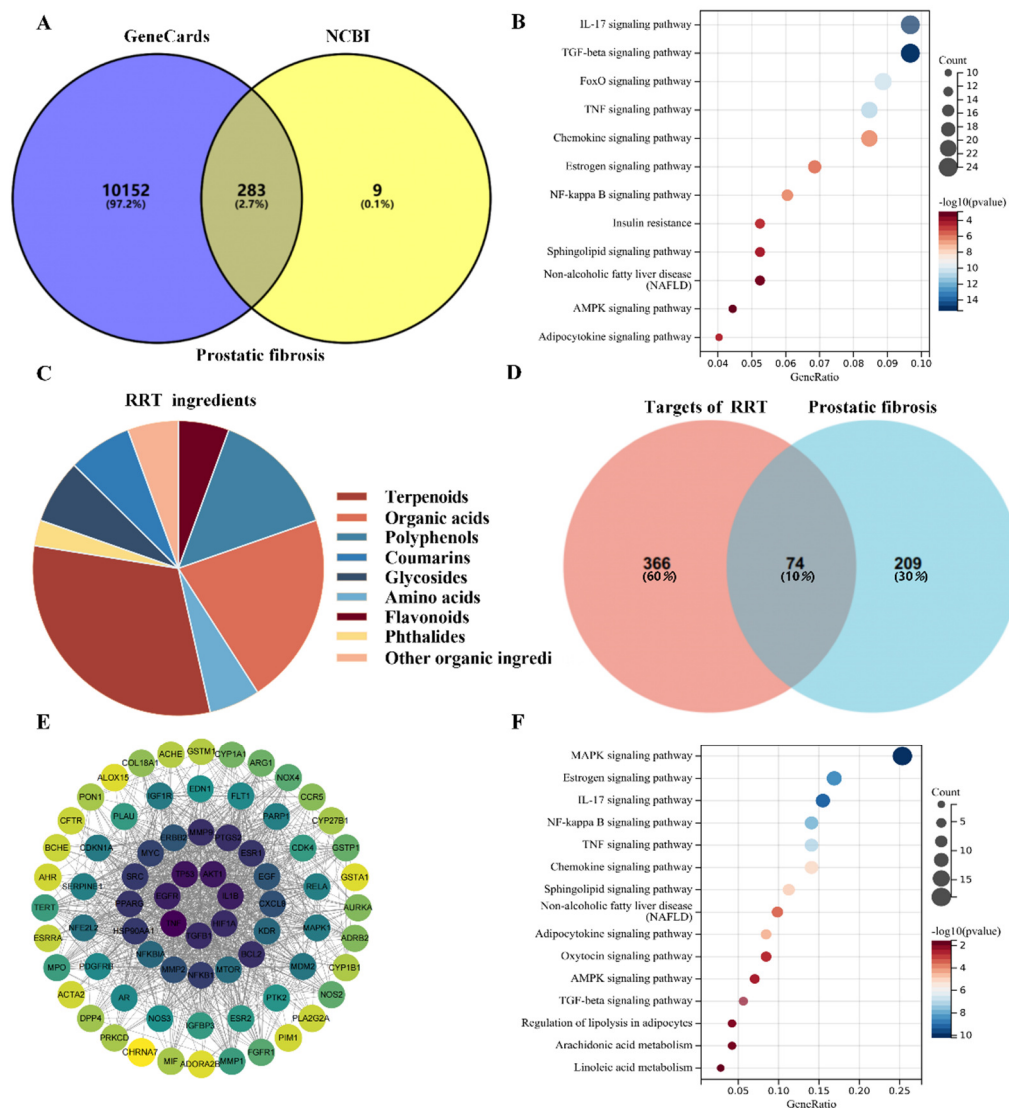


Fig. 3 RRT extracts may improve prostatic fibrosis through its multi-ingredient and multi-target properties. (A) Venn diagram of prostatic fibrosis-related genes between GeneCards and NCBI; (B) KEGG enrichment analysis of 283 key genes related to the progression of prostatic fibrosis; (C) Classification of RRT ingredients; (D) Venn diagram of target genes of 11 active ingredients of RRT extracts and 283 key genes related to the progression of prostatic fibrosis; (E) Association map (PPI network map) of 74 potential genes for RRT in the treatment of prostatic fibrosis; (F) KEGG enrichment analysis of 74 potential genes for RRT in the treatment of prostatic fibrosis.

Furthermore, we analyzed the primary ingredients of RRT extracts, revealing that there are 71 major ingredients in RRT extracts (refer to Supplementary Table 3), including terpenoids, organic acids, polyphenols, coumarins, glycosides, amino acids, flavonoids, phthalides, and other ingredients (Fig. 3C). Subsequently, using the TCMSP database, we screen 71 ingredients with OB of at least 20% and a DL score of 0.18 or higher were screened. From this selection, 11 active ingredients, including Bilobalide, Cianidanol, Ellagic acid, Emodin, Eriodictyol, Ginkgolide B, Limonin, Lindenenol, Morin, Nodakenin, and Procyanidin B1 were identified (Table 1). These ingredients were then cross-referenced with the Swiss Target Prediction Database, revealing 440 potential targets associated with the 11 active ingredients. These 440 genes were subsequently integrated with the 283 key genes previously identified for the development of prostatic fibrosis. This analysis led to the identification of 74 potential targets for the treatment of prostatic fibrosis by RRT (Fig. 3D). STRING and KEGG enrichment analyses indicated that RRT might mediate prostatic fibrosis by modulating lipid metabolism, inflammation, oxidative stress, and sex hormones, among other factors (Fig. 3E and F).

Table 1 11 active ingredients of RRT extracts

NO	Chemical ingredient	OB%	DL
1	Bilobalide	84.42	0.36
2	Cianidanol	54.83	0.24
3	Ellagic acid	43.06	0.43
4	Emodin	24.4	0.24
5	Eriodictyol	71.79	0.24
6	Ginkgolide B	46.14	0.73
7	Limonin	21.3	0.57
8	Lindenenol	52.05	0.18
9	Morin	46.23	0.27
10	Nodakenin	57.12	0.69
11	Procyanidin B1	67.87	0.66

3.4 RRT extracts reduce the inflammatory and oxidative stress of prostate in HFD-fed mice

The histopathological examination of the prostate revealed that ND mice did not exhibit significant inflammatory cells infiltration. In contrast, HFD mice displayed diffuse or focal infiltration of inflammatory cells between their prostate acini, a condition that was notably ameliorated in HFD mice treated with RRT extracts ($P < 0.01$) (Fig. 4A). We also examined alterations in inflammatory mediators within the three mouse prostate tissues. The relative mRNA expression levels of *Il-1 β* , *Il-6*, *Ptgs2*, *Cxcl1*, *Ccl2* and *Nf- κ b* were significantly higher in the prostate of HFD mice compared to ND mice, and RRT extracts treatment reduced the expression of those inflammatory mediators in the prostate of HFD mice ($P < 0.05$, $P < 0.01$) (Fig. 4B). Furthermore, we assessed the levels of oxidative stress in mice prostates of three groups, and found that the T-SOD content was reduced, and the MDA content was elevated in the prostates of HFD mice compared to ND mice ($P < 0.05$, $P < 0.01$). Conversely, in the prostates of HFD mice supplemented with RRT extracts, T-SOD content was elevated, and MDA content was reduced compared to HFD mice ($P < 0.05$, $P < 0.01$) (Fig. 4C). The HE staining results of eWAT revealed that mice in the HFD group had CLS surrounding the white

adipocytes, along with foci of inflammatory cell infiltration compared to the ND group. However, after treatment with RRT extracts, the number of CLS and inflammatory cell foci significantly decreased ($P < 0.01$) (Fig. 4D). Additionally, IHC analysis of eWAT further confirmed the eWAT of HFD mice exhibited CLS composed of macrophages (CD68⁺ and Lyso⁺) and foci inflammatory cells infiltrated composed of B lymphocytes (CD20⁺) and T lymphocytes (CD45RO⁺) (Fig. 4E). These results suggest that treatment with RRT extracts effectively mitigated CLS and inflammatory cell infiltration in eWAT, which may lead to the amelioration of adipose tissue inflammation.

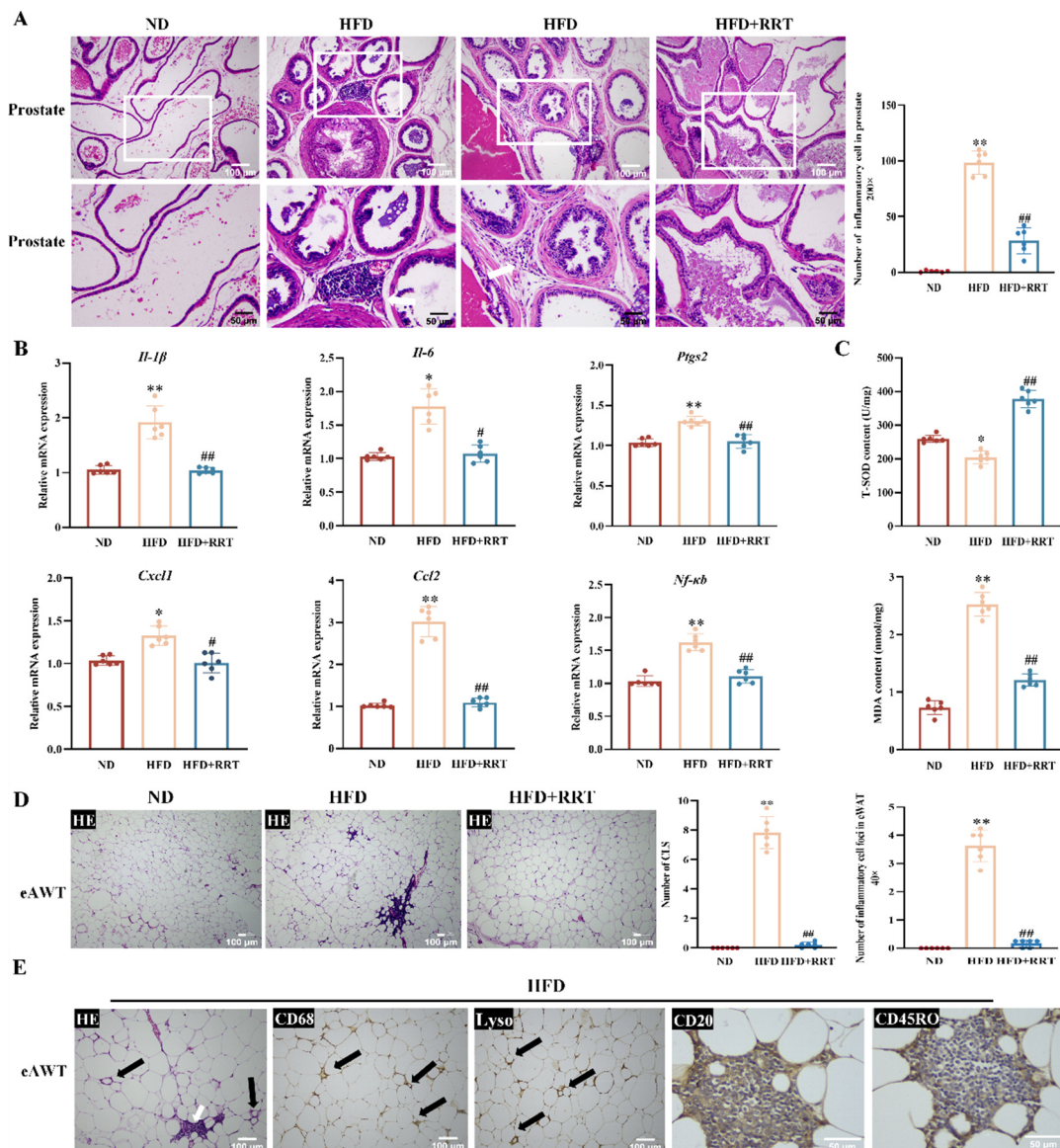


Fig. 4 RRT extracts reduce inflammatory and oxidative stress of prostate in HFD-fed mice. (A) Representative images of HE-stained prostate in each group (HE×100; Scale bar=100 μm), the image below shows a higher magnification view of the white box area in the above image (HE×200; Scale bar=50 μm), with white arrows pointing to diffuse or focal inflammatory cells infiltration; quantitative analysis of inflammatory cells in prostate; (B) The relative mRNA expression levels of some inflammatory mediators in mice prostate of each group; (C) T-SOD and MDA contents in mice prostate of each group; (D) HE staining of eWAT in three groups of mice; quantitative analysis of CLS and inflammatory cells foci in mice WAT; with black arrows pointing to CLS and white arrows pointing to focal inflammatory cells infiltration (HE×100; Scale bar=100 μm); (E) HE and IHC staining of CD68, Lyso, CD20 and CD45RO of mice eWAT in HDF group; with black arrows pointing to CLS and white arrows pointing to focal inflammatory cells infiltration (HE×100; IHC×100; IHC×400; Scale bar=100 μm; Scale bar=50 μm). Data are presented as the mean ± SD. * $P < 0.05$, ** $P < 0.01$ compared to the ND group; # $P < 0.05$, ## $P < 0.01$ compared to the HFD group.

3.5 RRT extracts improve serum sex hormones, ER α and AR expression of prostate in HFD-fed mice

Our results indicated that serum E₂ levels were elevated, whereas T levels were reduced in HFD group of mice compared to ND mice. However, HFD mice treated with RRT extracts demonstrated lower serum E₂ levels and higher T levels compared to the HFD group ($P < 0.05$, $P < 0.01$) (Fig. 5A). We examined the mRNA and protein expression of ER α and AR in the prostate of mice from each group using RT-qPCR and IHC, respectively. The RT-qPCR results indicated that the mRNA level of *Esr1* (encoding ER α) was elevated in the HFD group compared to that in the ND group, and RRT extracts treatment notably decreased *Esr1* mRNA expression relative to HFD mice ($P < 0.01$). However, no significant alterations in *Ar* mRNA were observed among the three groups (Fig. 5B). The IHC image revealed that ER α -positive cells were located in the fibromuscular layer surrounding the acini and in the stromal cells between the prostatic acini, while AR-positive cells were found within the acinar lumina and ductal epithelium (Fig. 5C). The HFD group showed a higher number of ER α -positive interacinar stroma cells compared to the ND group, and the expression of ER α was lower in the HFD+RRT group than in the HFD group, although the difference was not statistically significant. In line with the mRNA levels of *Ar*, the expression of AR protein did not significantly differ among the three groups (Fig. 5D).

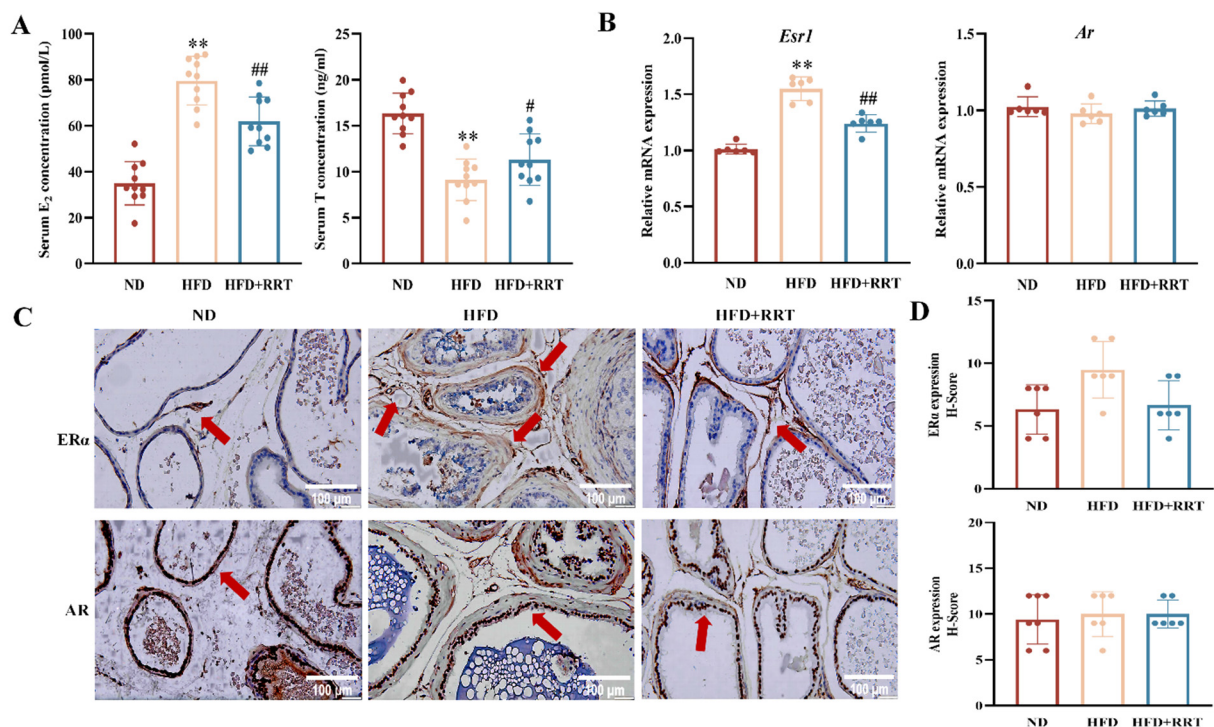


Fig. 5 RRT extracts improve serum sex hormones, ER α and AR expression of prostate in HFD-fed mice. (A) Mice serum E₂ and T concentrations in each group; (B) The mRNA expression of *Esr1* (encoding ER α) and *Ar* in mice prostate of each group; (C) IHC staining of ER α and AR in mice prostate of each group, with red arrows pointing to ER α -positive stromal cells and AR-positive acinar and ductal epithelium cells (IHC $\times 200$; Scale bar=100 μ m); (D) The protein expression of ER α and AR in mice prostate of each group. Data are presented as the mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$ compared to the ND group; # $P < 0.05$, ## $P < 0.01$ compared to the HFD group.

4. Discussion

It has been recognized that HFD-induced obesity is associated with the development of prostatic fibrosis. However, the precise mechanism remains to be elucidated. RRT has garnered attention for its therapeutic

effects on cardiovascular diseases, diabetes, and cancer, attributed to its various active substances^[12, 16]. In this study, we found that RRT extracts are rich in a variety of natural products and nutrients, including organic acids, phenolic compounds, flavonoids, and terpenes, and possesses strong free radical scavenging ability and anti-inflammatory effects. Excitingly, we demonstrated that RRT extracts significantly alleviated HFD-induced prostatic damage and prostatic fibrosis. These protective effects may be mediated through the regulation of lipid metabolism, anti-inflammatory actions, antioxidant stress, modulation of sex hormone levels, and inhibition of the Smad2/3 signaling pathway downstream of TGF- β ₁.

After eight months of high-fat feeding, we successfully established a mouse model of HFD-induced obesity, characterized by increased body weight and WAT, as well as elevated serum levels of TG, TC, and LDL-C. These findings firmly establish that a HFD induces obesity over time. Following four months of RRT extract intervention, we observed a marked reduction in body weight, relative WAT weight, and serum levels of TG, TC, and LDL-C. Zhang et al. reported that RRT can effectively lower serum TG, TC, and LDL-C levels in patients with cerebral infarction, thereby ameliorating symptoms of atherosclerosis and reducing the recurrence rate of the disease^[17]. Numerous studies have confirmed that RRT is rich in a variety of bioactive compounds, including flavonoids, terpenoids, phenolic acids, and polysaccharides, all of which contribute to its lipid-lowering properties^[18]. Therefore, our study indicated that treatment with RRT extracts may alleviate obesity caused by the HFD, potentially reducing the risk of associated diseases, including prostatic fibrosis.

The phenomenon of fibrosis induced by a HFD has been widely reported in various tissues, including the lungs, liver, and heart. In the present experiment, we observed that mice develop HFD-induced obesity concurrent with prostatic fibrosis, characterized by a thickened fibromuscular layer surrounding the prostate acini, an increase in collagen I expression and upregulation of molecules associated with the TGF- β /Smad signaling pathway. We were pleased to observe that treatment with RRT extracts effectively alleviated HFD-induced prostatic fibrosis, as evidenced by a thinner fibromuscular layer around the prostate acini and a reduction in Collagen I expression and the TGF- β /Smad signaling pathway proteins. However, we did not observe any changes in α -SMA expression in the prostate at either the protein or mRNA level. D'Arcy and colleagues reported that activation of the IL-4/IL-13 induces prostate fibroblasts to express ECM but not to differentiate completely to myofibroblasts. They refer to this fibroblast cell types that secrete ECM but lack α -SMA expression or contractile activity as activated fibroblasts^[3]. In this study, whether the treatment with RRT extracts affects these stromal cells to improve prostatic fibrosis remains to be further explored in future research. Although no significant difference in relative prostate weight was observed among the three groups, we found that many mice in the HFD group exhibited a notable increase in bladder diameter and urinary retention in the bladder. In contrast, only a few mice in the HFD+RRT group showed mild urinary retention, with a significant reduction in bladder diameter. Although we did not design a void spot assay to directly assess urinary dysfunction in mice, these results indirectly suggest that HFD mice may experience urinary dysfunction, which can be alleviated by RRT extracts treatment. We hypothesize that the significant urinary

retention observed in HFD-fed mice may be related to prostatic fibrosis, as HFD feeding promotes the accumulation of periurethral collagen contributes to the development of prostatic fibrosis, which in turn leads to lower urinary tract obstruction and urinary retention. It has been reported that RRT polysaccharides exhibit anti-renal fibrosis properties and can improve kidney function in cases of chronic kidney disease^[11]. Additionally, a previous study indicated that RRT freeze-dried powder effectively prevented renal fibrosis and injury resulting from unilateral ureteral obstruction in rats, which was associated with the inhibition of oxidative stress and the TGF- β /Smad signaling pathway^[9]. Based on these findings, we conclude that HFD-induced prostatic fibrosis can lead to urinary obstruction and retention in mice, and that RRT extracts may alleviate these issues by reducing prostatic fibrosis induced by the HFD.

Inflammation is closely associated with the development of tissue fibrosis in various organs, including the prostate. Inflammatory cell infiltration is commonly observed in tissue samples from patients with BPH/LUTS. BPH patients with prostatitis often show significant proliferation of prostate stromal cells, accompanied by marked fibrotic changes^[19-21]. In our study, we found that, compared with ND mice, HFD mice exhibited significant inflammatory cell infiltration in the prostate and the mRNA expression levels of inflammatory mediators (*Il-1 β* , *Il-6*, *Ptgs2*, *Cxcl1*, *Ccl2* and *Nf- κ b*) showed a significant increase. Our previous studies have confirmed that in mice with prostatic fibrosis induced by sex hormone imbalance, the above-mentioned inflammatory mediators are all significantly increased in prostate (data not published). *Il-1 β* and *Il-6* are important cytokines in the interleukin family that play crucial roles in information transmission, immune cell activation, and inflammatory responses. *Ptgs2* is an inflammatory marker that can be induced by various inflammatory signals^[22]. *Cxcl1* and *Ccl2* are classified as CC- and CXC- chemokines, respectively, and they can recruit inflammatory cells to tissues, thereby further enhancing the inflammatory response. *Nf- κ b* is a powerful transcription factor that mediates the expression of multiple genes encoding inflammatory mediators, including several interleukins, CC- and CXC-type chemokines, and TGF- β ^[23, 24]. Furthermore, we observed a significant reduction in the above-mentioned inflammatory mediators and inflammatory cell infiltration in the prostates of mice in the HFD+RRT group. This suggests that HFD induces an inflammatory response in the prostate, while RRT extracts significantly improve HFD-induced prostate inflammation. In fact, HFD affects not only the prostate; we found that HFD mice showed a significant increase in the relative weight of WAT, larger adipocyte diameter, increased inflammatory cell infiltration, and the emergence of CLS. WAT is a highly secretory organ that significantly contributes to inflammatory changes in adjacent tissues. In obesity induced by HFD, increased macrophage infiltration in eWAT leads to the release of pro-inflammatory mediators, which may result in the prostate being situated in an inflammatory microenvironment^[25-27]. Furthermore, existing data indicate that obese men exhibit an elevated risk of prostatitis, which may also be linked to the pro-inflammatory effects of adipokines^[8, 28]. After treatment with RRT extracts, the inflammatory cell infiltration in eWAT of HFD mice was significantly reduced. Previous studies have shown that a polysaccharide from RRT can enhance the expression of tight junction proteins (ZO-1, claudin-1, and occludin) and decrease the levels of inflammatory cytokines^[29]. Our results show that

RRT extracts mainly contains 11 active ingredients, such as ellagic acid and emodin. Ahad et al. identified the antioxidant and anti-inflammatory properties of ellagic acid, which significantly improved diabetes-induced nephropathy, inhibited NF- κ B activity, stimulated anti-inflammatory factor synthesis, and reduced oxidative stress in kidney tissues^[30]. Emodin reduces the secretion of IL-1 β , IL-6, and tumor necrosis factor- α (TNF- α) by macrophages and also inhibits the NF- κ B signaling pathway to diminish the expression of inflammatory factors^[31]. Moreover, in the present study, mice fed with HFD exhibited reduced expression of T-SOD and increased expression of MDA. However, this phenomenon was reversed by RRT extracts treatment, suggesting that HFD induces oxidative stress and promotes the production of ROS, which in turn stimulate the production of ECM. Treatment with RRT extracts can suppress oxidative stress levels in HFD-fed mice, thereby hindering the progression of prostatic fibrosis. A study by Chen et al. showed that a novel polysaccharide isolated from RRT exhibited significant scavenging activities against DPPH, hydroxyl, and superoxide radicals^[32]. Collectively, these findings suggest that RRT extracts may exert anti-fibrotic effects on the prostate through its anti-inflammatory and antioxidant actions.

The prostate is a sex hormone-dependent organ, with its function and structure significantly influenced by the levels of sex hormones. Our previous study has also shown that changes in sex hormone levels and their ratios can lead to prostatic fibrosis^[15]. Based on this, we further explored the effects of HFD and RRT extracts on sex hormones. Compared to circulating hormones levels, the levels of sex hormones within prostate tissue can play a more critical role, as circulating hormones do not fully reflect local tissue hormone levels. Notably, although we did not measure the sex hormone levels in the prostate of each group, we found that mice fed HFD exhibited elevated E₂ levels and reduced T levels. Additionally, We observed that elevated ER α expression occurred in mice fed a HFD at both the mRNA and protein levels, although the difference in ER α protein levels was not statistically significant. Treatment with RRT extracts reversed the effects of HFD on serum sex hormone levels and prostate ER α expression. In men, most estrogens are produced through the aromatization of androgens in extraglandular tissues, particularly in WAT^[33, 34]. This conversion process is particularly significant in obese individuals, as the content of aromatase in fat tissue is higher and its activity may be enhanced, thereby promoting the conversion of more androgens to estrogens^[35, 36]. Flavonoids have been proven to modulate the activity of aromatase^[37]. Therefore, we speculate that the effects of RRT extracts on HFD-induced sex hormone changes may be due to two potential mechanisms: First, flavonoids in RRT extracts may influence aromatase activity. Second, RRT extracts may reduce systemic fat content, inhibit estrogen conversion, thereby affecting the estrogen signaling pathway.

5. Conclusion

In this study, RRT extracts were found to mitigate HFD-induced prostatic fibrosis by inhibiting obesity, exerting anti-inflammatory effects, combating oxidative stress, modulating sex hormone levels, or down-regulating the TGF- β /Smad pathway. Although this is a preliminary investigation, the findings provide valuable experimental data and insights for further research and practical applications of RRT products, highlighting the critical role of a healthy diet in the prevention of prostatic diseases. Research has confirmed

that RRT contains various bioactive ingredients; however, the specific ingredients that inhibit HFD-induced prostatic fibrosis and their underlying mechanisms remain unclear at present. In future research, it is necessary to investigate the bioavailability of some crucial compounds identified in RRT extracts, as well as whether they reach the prostate and act on it directly.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Data availability

Data will be made available on request.

Acknowledgements

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