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Determination Effects of *Rheum ribes* L. against High Calorie Diet-induced Obesity: Investigation of Changes in Immunologic and Neurologic Enzymes Activities

Bedia BATİ

ABSTRACT: This study was carried out to investigate the immunotoxic and neurotoxic effects of *Rheum ribes* L. (Rr) plant extract on liver, brain and heart tissues in rats with an experimental obesity model. Wistar albino male rats were used in the study. In this study, 4 groups were formed, one of which was the control group, and each group consisted of 6 rats. The groups were the "control (CG)", "high calorie diet (HCD) control (HCDG)", 'HCD + Rr (200 mg/kg) (HCDRHE1)" and "HCD + Rr (400 mg/kg) (HCDRHE2)". At the end of the study, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), adenosineaminase (ADA) and myeloperoxidase (MPO) biomarkers were evaluated in tissues. According to the study findings, the ADA and MPO activity levels of the obese group increased significantly compared to the control group, and these enzyme levels in the groups administered Rr plant extract approached the control group levels. On the other hand, the AChE and BChE activity levels of the obese group decreased compared to the control group, and there was a statistically significant increase in the groups administered plant extracts compared to the control group. In conclusion, Rr plant root extract suppresses neurotoxicity and immunotoxicity in rats with obesity induced by a high-calorie diet (HCD).

Keywords: Acetylcholinesterase, adenosineaminase, butyrylcholinesterase, myeloperoxidase, obesity, *Rheum ribes*

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Ethics Committee Approval: The study was carried out with the approval of Van Yuzuncu Yil University Experimental Animals Unit Ethics Committee dated 23.12.2021 and numbered 05.

INTRODUCTION

Today, obesity is not only a disease, but also among the causes of many diseases such as insulin resistance, oxidative stress, inflammation, hypertension and cardiovascular mortality (Petrie et al., 2018; Lasker et al., 2019). It is known that obesity is caused by an imbalance between energy intake and consumption. In addition, this disease is thought to increase the incidence of chronic diseases that affect quality of life such as cardiovascular diseases, type 2 diabetes and cancer, as well as causing abnormal fat accumulation in the body (WHO, 2020; Tung et al., 2020). Especially in modern societies, lifestyles involving high calorie food intake, inactivity and lack of physical exercise are among the causes of weight gain (Golombek et al., 2013; Spiegel et al., 2009; Tung et al., 2020).

Studies about obesity reported that butyrylcholinesterase (BChE) and its variants are associated with obesity (Vaisi-Raygani, 2008; Lima et al., 2013; Gok, 2021). In addition, recent studies show that butyrylcholinesterase and acetylcholinesterase (AChE) are considered as diagnostic markers of low-grade systemic inflammation (Das., 2007; Kurdoglu et al., 2012; Bitzinger et al., 2019) and are one of the important forms of cholinesterase in mammals (Caglayan et al., 2019). Cholinesterase is a family of enzymes that hydrolyze acetylcholine (ACh) to choline and acetic acid, including BChE and AChE (Kurdoglu et al., 2012). Cholinesterase activity is one of the biomarkers of neurotoxicity (Olson, 2018). It has been reported that there are two types of cholinesterase in the body, AChE and BChE (Gulcin et al., 2016; Turkan 2021).Similarly, adenosineaminase and myeloperoxidase are biomarkers of the immune system in vertebrates. While ADA is an enzyme that catabolizes adenosine or deoxyadenosine, MPO is an enzyme that catalyzes the formation of hypochlorous acid or hypothiocyanide (Tamura et al., 2016; Lu et al., 2018; Dogan et al., 2020).

It is thought that determining the factors causing obesity in medical nutrition can increase success of obesity treatment and in this way, some active substances in foods may be used to support obesity treatment (Macit and Köksal., 2020). From the past to the present, medicinal plants have been used as an alternative treatment for various diseases due to their effectiveness (Almalki et al., 2019; de Lima et al., 2018). In addition, plant extracts such as alkaloids, polyphenols, terpenoids have highly beneficial effects on anti-obesity through various mechanisms (Yang et al. 2020; Wang et al., 2022). One of these plants is *Rheum ribes* L.. *Rheum ribes* (Rr) is widely used in Turkey and around the world as a food source, medicinal and auxiliary drug since ancient times (Ozturk et al., 2007; Lajter et al., 2013; Bati et al., 2020). Rr is one of the medicinal plants and is a perennial plant belonging to the Polygonaceae family (Amiri et al., 2015; Takcı et al., 2021). The root of Rr has strong antioxidant activity and contains phenolic compounds, tannins and anthracene derivatives. Anthraquinone and stilbene are considered its main components (Hussaini et al., 2021; Amiri et al., 2015). Rr roots are known to be used for the treatment of hypertension, diabetes, obesity and kidney diseases (Keser et al., 2020).

In recent years, research trends about new neuroprotective drugs from natural sources have emerged that awaken new therapeutic hopes. *Rheum ribes* L. is one of the most promising medicinal plants known for its antioxidant properties and pharmaceutical effects. There is no experimental study investigating the metabolic activity of this plant on neurotoxicity and immunotoxicity in rat tissues. Therefore, in this study, the effects of metabolic enzyme activities of Rr plant root extract on neurotoxicity and immunotoxicity in rat tissues with obesity-induced toxicity linked to a high calorie diet (HCD) were investigated.

MATERIALS AND METHODS

Chemicals

Acetylthiocholine iodide (AChI), butyrylthiocholine iodide (BChI), 5-5' - dithiobis -(2-nitrobenzoic acid) (DTNB), adenosine, phenol, sodium hydroxide, sodium nitroprusside, ammonium sulfate, sodium hypochlorite, ethylene diamine tetra acetic acid (EDTA), potassium phosphate (KH_2PO_4), hexadecyl thrimethylammonium bromide and o-dianisidine dihyrochloride of technical grade used in this study were supplied by Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of plant extract

The lyophilized purified water extract of plant roots was prepared according to the modified method of Dalar and Konczak (2013). For this, 50 g of the ground root sample was weighed, 1000 ml of distilled water was added to it, and it was crushed in a blender for 3 minutes. The mixture was left in the shaker and homogenized for 5 hours at +4 °C and then placed in the centrifuge and centrifuged for 20 minutes. The supernatants obtained had solvent removed at +37 °C with the help of an evaporator. After this process, the sample was frozen (-50 °C) and kept in the lyophilized device at 50 millitor pressure conditions for one week. The resulting lyophilized distilled water fraction was stored in the freezer (-20 °C) until the study day.

Animals and experimental design

Wistar albino male rats were used in the study. The study was carried out with the approval of Van Yuzuncu Yil University Experimental Animals Unit Ethics Committee dated 23.12.2021 and numbered 05. Rats (25 ±1 °C) were fed *ad libitum* at room temperature in a 12 hour light/ 12 hour dark light period. In this study, which lasted for 12 weeks, 4 groups were formed, one of which was the control group, and each group consisted of 6 rats. The feeds used in the study were obtained from Research Diet. The groups in the study were formed as follows;

- Normal Control group (CG); normal rat food
- HCD Control group (HCDG); high calorie rat food
- HCD+ *Rr* group (HCDRHE1); High calorie rat food + Rr plant extract (200 mg/kg)
- HCD+ *Rr* group (HCDRHE2); High calorie rat food + Rr plant extract (400 mg/kg)

In the 56th day of the study, body mass index (BMI) was calculated for the subjects in both groups (Altunkaynak et al., 2008; Bati et al., 2021). As a result of the calculation, the groups consuming HCD feed were obese, and at the end of the 56th day, in addition to the HCD feed, the plant extract was given to the HCDRHE1 and HCDRHE2 groups by oral gavage (OECD, 2008). The obtained extract was weighed in the amount determined according to the weight of the rat, and after it was dissolved in some pure water, gavage was performed (Dogan, 2015). The experimental study was terminated by sacrificing the rats. Liver, heart and brain tissue samples were taken from rats.

Biochemical analysis

AChE and BChE activities were measured with a AE-S90-MD UV/ VIS spectrophotometer using acetylthiocholine iodide and butyrylthiocholine iodide as substrates, respectively, according to the method of Ellman et al. (1961). ADA was assayed according to Giusti and Bergmeyer (1974). MPO was assayed using the method described by Bradley et al. (1982).

Statistical analysis

Data obtained from the study groups had statistical analysis performed with the Kruskal Wallis test and the R program (R Core Team, 2022). Additionally, significance in statistical tests was taken as $p<0.05$.

RESULTS AND DISCUSSION

In this study AChE, BChE, ADA, and MPO activities were evaluated for neurotoxicity and immunotoxicity activities in liver, heart and brain tissues. Figure 1 gives the weekly weight values for rats during the experiment.

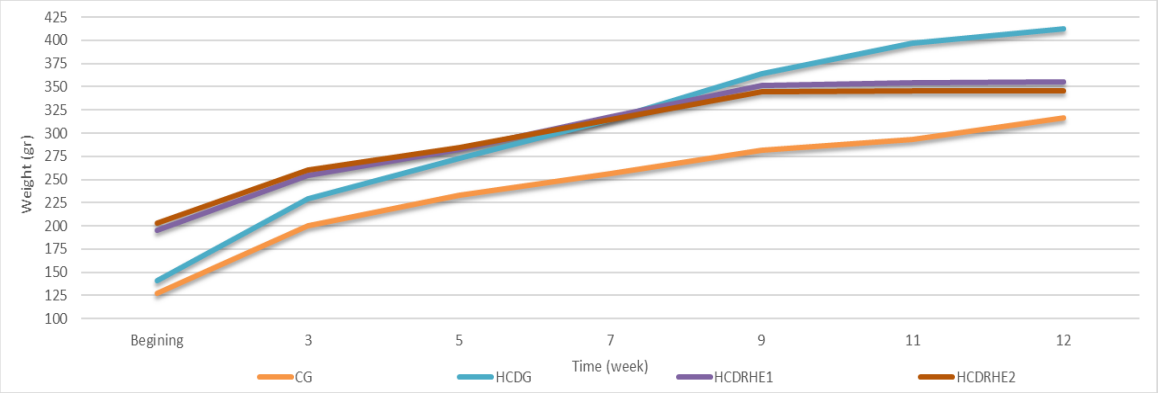


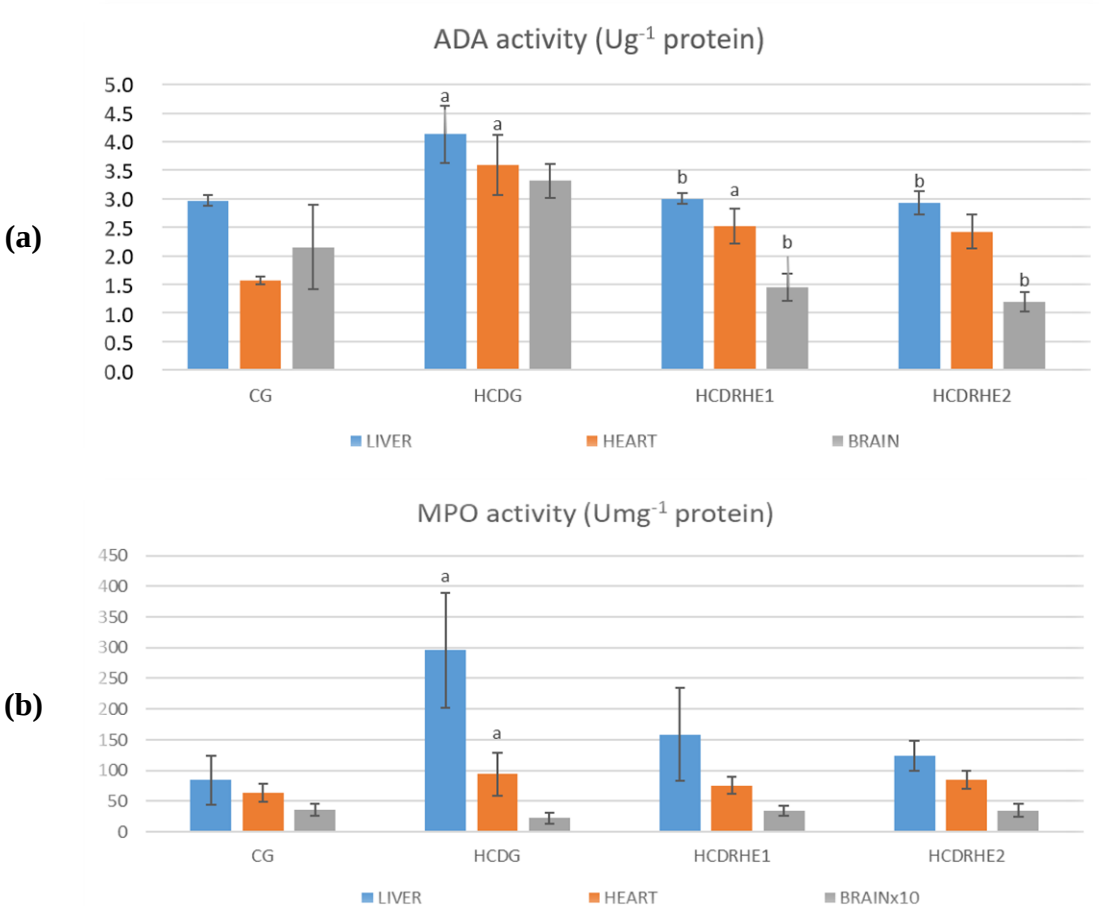
Figure 1. Time-linked variation in body weight in experimental groups

The effect of Rr plant extract on neurotoxicity and immunotoxicity activities in rats with an obesity model induced with HCD was investigated and the results are presented in Table 1, Figure 2 and Figure 3.

Table 1. Immunologic and Neurologic enzymes activity values in tissues (mean ± standard deviation)

		CG (mean±SD)	HCDG (mean±SD)	HCDRHE1 (mean±SD)	HCDRHE2 (mean±SD)
AChE (Ug^{-1} protein)	Liver	0.045±0.00	0.019±0.01[a]	0.041±0.00	0.042±0.00[b]
	Heart	0.041±0.01	0.035±0.02	0.050±0.01	0.048±0.02
	Brain	0.049±0.01	0.051±0.01	0.044±0.00	0.044±0.00
BChE (Ug^{-1} protein)	Liver	0.020±0.01	0.010±0.01	0.025±0.01	0.022±0.02
	Heart	0034±0.01	0.016±0.01	0.025±0.01	0.028±0.00
	Brain	0.057±0.01	0.006±0.00	0.031±0.01	0.042±0.01
ADA (Ug^{-1} protein)	Liver	2.971±0.10	4.131±0.50[a]	3.005±0.09[b]	2.927±0.20[b]
	Heart	1.570±0.07	3.590±0.53[a]	2.521±0.30[a]	2.425±0.30
	Brain	2.153±0.73	3.312±0.30	1.450±0.23[b]	1.196±0.17[b]
MPO (Umg^{-1} protein)	Liver	84.454±40.17	295.939±93.62[a]	158.672±75.15	124.249±24.00
	Heart	63.981±14.52	93.993±35.30[a]	75.974±14.42	85.056±15.00
	Brainx10	36.603±10.00	22.559±9.00	34.685±8.00	35.050±11.00

a: The difference compared to the CG group is statistically significant ($p<0.05$)
b: The difference compared to the HCDG group is statistically significant ($p<0.05$)



a: The difference compared to the CG group is statistically significant ($p<0.05$)
b: The difference compared to the HCDG group is statistically significant ($p<0.05$)

Figure 2. (a) Effects of *Rheum ribes* extracts on ADA enzyme. (b) Effects of *Rheum ribes* extraction on MPO enzyme

According to the findings of the study in Figure 2 (a), there was a statistically significant increase in the liver tissue of the HCDG group compared to the CG group, and there was a significant decrease in the HCDRHE1 and HCDRHE2 groups compared to the HCDG group. However, a significant increase in heart tissue was observed in the HCDG and HCDRHE1 groups compared to the CG group. In addition, there was a significant increase in brain tissue in the HCDRHE1 and HCDRHE2 groups compared to the HCDG group. According to the results in Figure 2 (b), there was a statistically significant increase in the liver tissue of the HCDG group compared to the CG group, while a significant increase was found in the heart tissue in the HCDG group compared to the CG group.

When the studies on this subject are examined, Ozok and Celik (2019) stated that ADA is related to immune system functions and that its activity increases in cases of increased immunity and decreases in cases of decreased immunity. It was also stated that increased liver MPO level is closely related to liver damage. In a study by Nussbaum et al. (2013), they stated that the inhibitory effect of neutrophils, MPO and ADA is associated with the inhibition of activated cells, since MPO is abundantly expressed in neutrophils and is directly related to the phagocytic activity of these cells. In addition, ADA activity is important in the stimulation of receptors that play a role in regulating extracellular adenosine concentrations and, consequently, modulating the inflammatory response (Antonioli et al., 2012). However, MPO is mostly associated with inflammation and neutrophils. It is used as an indicator of filtration (Ozkol et al., 2012). During neutrophil and macrophage stimulation, MPO and other tissue-disrupting substances are released from cells (i.e. ROS and cytotoxic proteins) into the extracellular

space. Ozkol et al. (2017) conducted a study to determine the protective effect of N-acetylcysteine (NAC) and vitamin E (Vit E) against ethanol (EtOH) intoxication (Se) in rats. In this study, they stated that the activity of MPO in the hepatic and cerebral tissues of the EtOH group increased significantly, and that neutrophils and macrophage activation played critical roles in the damaging effect of EtOH. According to Kalaz et al. (2016) EtOH-induced rats had similar high MPO activity. Our study results showed that there was an increase in ADA and MPO levels in groups consuming a HCD diet, similar to the literature. This situation is thought to be caused by obesity complications that develop due to high calorie diet consumption.

Medicinal plants and their bioactive compounds are used against immunotoxic and neurotoxic damage caused by chemicals, pesticides and heavy metals (Dogan et al., 2020). Ozok and Celik (2019), in their study to determine the immunotoxic effects of *Tiliaplatyphyllos Scop* plant infusion of ethanol in rats, stated that ADA activity in liver tissue decreased significantly, and the plant extract had a positive effect on enzyme activity with its antioxidant effect. In addition, there was increased MPO activity and EtOH in liver, spleen, heart, kidney and brain tissues. It was stated that it is associated with damage due to the increase in ROS production as a result of the effect of its toxicity. However, in a study on rats by Rahman et al. (2021), MPO activities increased in liver tissue after CCl_4 administration. An increase occurred in the group given polystachya leaf (PT) extract; therefore, treatment with PT leaf extract had the ability to restore MPO activities to a near-normal level in CCl4-treated rats. The results of our study show parallelism with the literature studies. In the study, it was observed that ADA and MPO levels increased in the HCDG group, but approached the levels close to the CG group in the groups given plant extract. As a functional component, the strong binding of free radicals and antioxidant properties of Rr with high phenolic content is considered to be the reason for this situation (Meral, 2011).

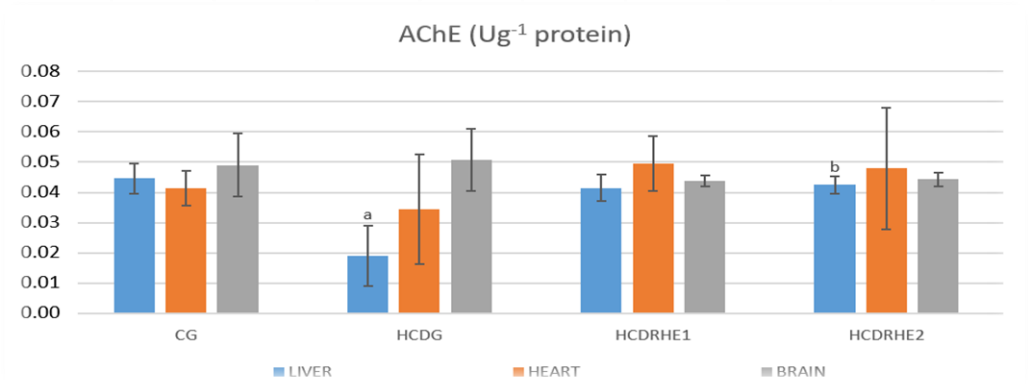
Ugbaja et al. (2021) found that evaluated the neuro-corrective effects of lycopene on neurological disorders caused by obesity. In the study, an increase was observed in the high activities of the neurosignaling-related enzymes AChE, ADA, MAO, NTPdase and 5ND in the brains of obese rats. In similar studies, ADA activity increased in obese subjects (Kurtul et al., 2006; Jadhav and Jain 2012). According to the brain tissue results from our study, it was observed that ADA levels increased in obese rats, similar to the literature, and ADA levels in the plant extract groups showed a significant decrease compared to the HCDG group and approached the levels of the CG group (Figure 2 (a), Table 1).

According to the results in Figure 3 (a), there was a significant decrease in liver tissue in the HCDG group compared to the CG group, but a significant increase was detected in the HCDRHE2 group compared to the HCDG group. According to the results in Figure 3 (b), the BChE levels in the HCDRHE1 and HCDRHE2 groups for all tissues approached the CG group levels.

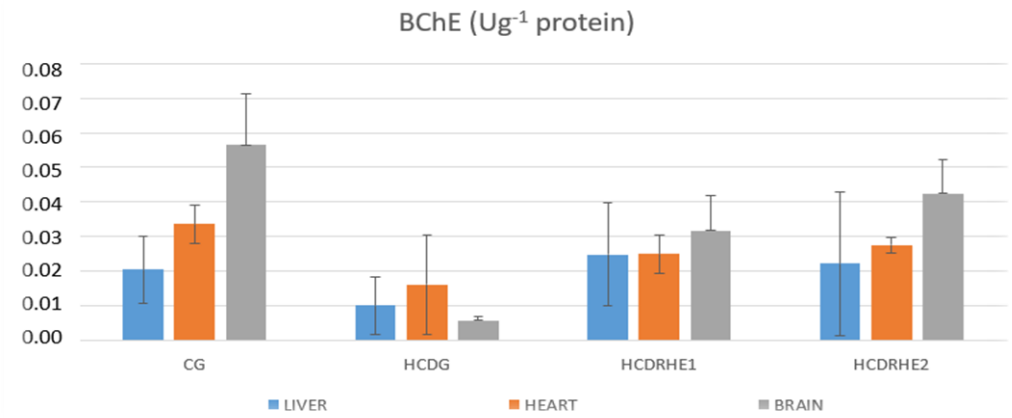
Manzoni et al., (2019) showed an increase in ROS levels, MPO and ADA activities in hyperlipidemic rats in their study. Curcimine reduced ADA and MPO activities in rats. They stated that pretreatment with curcumin prevented inflammation caused by hyperlipidemia. Our study is similar to the previous study, and we can state that Rr administration can correct the negative changes that may occur in ADA and MPO activities in rats fed a hypercaloric diet.

Bitzinger et al. (2019) examined polymorphonuclear cholinesterase inhibitors in the early stage of sepsis neutrophil (PMN) functions and their potential therapeutic effects on cecal ligation and in sepsis caused by puncture (CLP). In their study to investigate the roles of AChE and BChE as inflammatory markers, they investigated sepsis caused by CLP. They stated that it caused a significant time-dependent decrease in AChE activity. In addition, BChE activity was observed in hepatic septic rats at 24 hours. There was a decrease that may be associated with dysfunction.

(a)



(b)



a: The difference compared to the CG group is statistically significant ($p<0.05$)
b: The difference compared to the HCDG group is statistically significant ($p<0.05$)

Figure 3. (a) Effects of *Rheum ribes* extracts on AChE enzyme. (b) Effects of *Rheum ribes* extracts on BChE enzyme

According to the findings of our study, the decrease in AChE and BChE levels in HCD-consuming groups is compatible with the study by Bitzinger et al. (2019). Thus, the decrease in the available BChE activity in liver tissue is also associated with hepatic dysfunction. In addition, decreased AChE activity may be associated with increased proinflammatory effects caused by obesity in HCD-induced rats. Al-Kassab and Vijayakumar (1995) in their study of hepatic values in septic syndrome stated the importance of BChE as an indicator of dysfunction. In another study, AChE activity was evaluated as an early indicator of acute systemic inflammation (Müller et al., 2019; Bitzinger et al., 2019). Many studies showed that AChE and BChE serve as diagnostic markers of low-grade systemic inflammation (Das, 2007; Kassab and Vijayakumar, 1995; Chiarla et al., 2011; Bitzinger et al., 2019).

CONCLUSION

The results of this study showed that Rr reduced oxidative stress in the tissues of HCD-fed rats. Therefore, it is possible to say that Rr can regulate abnormal increases or decreases in metabolic enzyme activities. Considering the therapeutic effects of different parts of the Rr plant, more clinical studies are needed in this area.

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