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Stability and antioxidant activity of flavonoids from *Lycium barbarum* L. leaves during digestion *in vivo*

Yanli FAN[1*], Maimai MA[1], Jinghua CHEN[1], Yufang PEI[1], Xiazhi SUN[1]

Abstract

It has been determined that *Lycium barbarum* L. leaves are a vital source of bioactive chemicals and have good potential for creating nutritious formulations. In this work, gavage injections of *Lycium barbarum* L. leaves flavonoids (LBLF) at various doses – 50, 150, and 300 mg/kg BW – were administered to Sprague-Dawley (SD) rats. Four main LBLFs (rutin, chlorogenic acid, kaempferol, and quercetin) were assessed for stability and antioxidant activity *in vivo*. The findings demonstrated that within 4 h of the LBLF intervention, the plasma levels of rutin, chlorogenic acid, kaempferol, and quercetin were significantly dose-dependent. The rutin content peaked at 762.16 ± 36.63 µg/g at 1 h after the intervention, while the other three peaked at 383.07 ± 3.19 µg/g, 29.44 ± 1.95 µg/g and 18.511 ± 1.99 µg/g, at 3 h after the intervention respectively. Additionally, the levels of malondialdehyde (MDA) were significantly lower, and the activities of glutathione peroxidase (GSH-P_x), catalase (CAT), and total superoxide dismutase (T-SOD) were all considerably higher than those of the control group. This will establish a basis for elucidating the LBLF metabolic pathway *in vivo*, additional research into techniques to increase its stability, and investigation into its therapeutic activity.

Keywords: *Lycium barbarum* L. leaves flavonoids; *in vivo* metabolism; antioxidant activity; stability.

Practical Application: The leaves of *Lycium barbarum* L. are traditionally utilised for tea and are even discarded as byproducts. The findings of this study indicate that the flavonoids isolated from *Lycium barbarum* L. leaves may be used by experts in the fields of health and nutrition, as well as to increase the nutritional content of LBLF.

1 Introduction

The leaves of *Lycium barbarum* L. are abundant in protein, amino acids, vitamins, and minerals, which strengthen the stomach, preserve the liver and kidneys, eliminate constipation, and alleviate sleeplessness. The study revealed that the crude protein, calcium, iron, thiamine, and niacin content of *Lycium barbarum* L. leaves was greater than that of *Lycium barbarum* L. (Salazar et al., 2006). In the past, systematic and in-depth research has been conducted on the pharmacologically active components and product development of *Lycium barbarum* L. leaves. However, there are few studies on the in vivo metabolism of LBLF and how to improve their stability.

The primary bioactive compounds in the leaves of *Lycium barbarum* L. are flavonoids. According to research, the most prominent monomer compounds in the leaves of *Lycium barbarum* L. from Ningxia include rutin, kaempferol, quercetin, chlorogenic acid, caffeic acid, P-coumaric acid, and ferulic acid, also known collectively as LBLF (Zhao et al., 2019). It was found that rutin, quercetin and chlorogenic acid all had a wide range of physiological activities, such as anti-oxidant (Şenocak et al., 2020; Agunloye et al., 2019), anti-inflammatory (Zheng et al., 2017), anti-tumor (Alonso-Castro et al., 2013; Kim et al., 2019) and anti-bacterial (Deepika et al., 2019; Li et al., 2018). According to Nassef (2019), the flavonoid aglycone quercetin prevents human platelet aggregation. Chlorogenic acid has also boosted fluoroxidal action (Miao & Xiang, 2020). The leaves of *Lycium barbarum* L. show intriguing possibilities for resource development and application.

Current free radical medicine holds that the pathogenic process of various diseases involves an imbalance between free radical metabolism and lipid peroxidation. Excess oxygen free radicals are responsible for many diseases and premature ageing in the human body, and excessive accumulation of free radicals destroys proteins and DNA through oxidative processes, causing cell death and tissue damage in animals (Habib & Ibrahim, 2011). There are enzyme systems in living organisms that eliminate free radicals, such as GSH-P_x, CAT, T-SOD, etc. This enzyme family can utilise redox processes to oxidise free radicals and protect cells from harm. The accumulation of free radicals attacks the polyunsaturated fatty acids on the biofilm when free radical generation is excessive, leading to lipid peroxidation and the production of MDA. However, MDA can cause crosslinking of essential macromolecular polymers, such as proteins and nucleic acids, and affect the fluidity and permeability of cell membranes, limiting their normal function (Qiu et al., 2021).

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[1] *School of Food & Wine, Ningxia University, Yinchuan, Ningxia, China*
*Corresponding author: fanyanli.nxu@qq.com

Antioxidants, both endogenous and exogenous, are used by organisms to stop cellular damage (Liu et al., 2017). LBLF can have anti-oxidant and liver-protective effects as an exogenous antioxidant (Dong et al., 2009). According to research, all food and supplements must be digested in the gastrointestinal tract before being absorbed and used. This process may need transformation. The bioavailability and absorption of bioactive compounds in blood circulation directly influence their physiological efficiency (Wood & Tamura, 2001). LBLF are a type of naturally occurring antioxidant, so it is imperative to research the stability of its digestion *in vivo* and its absorption, metabolism, and antioxidant activity. The changes in major monomer content and antioxidant activity in the plasma and liver of rats given various dosages of LBLF at multiple periods after gavage were examined in this research using the SD rat model and UPLC-MS technology. To provide a scientific foundation and valuable reference for the innovation of *Lycium barbarum* L. leaves resource value, the improvement of resource utilisation efficiency, the development of the health industry, the changes of the medlar and the use of microscope full-scanning technology to observe its liver lesions.

2 Materials and methods

2.1 Materials and chemicals

Lycium barbarum L. leaves were purchased from Yuxin *Lycium barbarum* Seed Industry Co. LTD (Ningxia, China). The LBLF were self-made in the laboratory.

The antioxidant assay kits were procured from Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). Methanol and methanoic acid (HPLC grade) were from Fuyu Fine Chemical Co., LTD (Tianjin, China). Rutin, chlorogenic acid, Kaempferol and quercetin (HPLC grade) were from Meilun Biotechnology Co., LTD (Dalian, China).

2.2 Laboratory animals

All experimental animals were purchased and raised in the barrier of Ningxia Medical University Experimental Animal Center, certificate number: 20190016009561. All animal experiments were permitted by the Animal Welfare and Ethics Committee of Ningxia Medical University (No. IACUC-NYLAC-2020-176).

2.3 Experimental instruments and equipment

ME203E electronic balance was procured by Mettler Co., LTD (Shanghai, China). HWS12 thermostat water bath cauldron was from Wuxiang Instrument (Shanghai, China). Xiangyi Centrifuge Instrument Co., LTD procured TGL-16M high-speed desktop refrigerated centrifuge. LTQ Orbitrap XL Ultra Performance Liquid Chromatography-Electrostatic Field Orbital Mass Spectrometer was from Thermo Fisher, USA LTD.

2.4 Experimental methods

Extraction and purification of samples

Based on the previous research in the laboratory, LBLF were obtained (Niu et al., 2021).

Animal experiment

The SD rats were from Ningxia Medical University's Experimental Animal Center. They were divided into four groups, with the LBLF high, medium, and low dose groups and the blank control group, after becoming accustomed to rearing for three to five days. Three animals in each group were used in the blank group, which received a subcutaneous injection of physiological saline (0.25 mL/10 g Normal Saline). Throughout the experiment, all rats consumed freely. All rats were given 2.5 mL of LBLF solution following the high, medium, and low dose groups (300, 150, and 50 mg/kg BW, respectively), after fasting without water for 12 h. Blood samples were obtained by the capillary after intervals of 0, 0.5, 1, 2, 3, and 4 h at each time point while being sedated with hydrated acetaldehyde. The blood was drawn under aseptic conditions, mixed well, and centrifuged at 3000 revolutions per minute for 10 min before the plasma was absorbed into the frozen storage tube. After the final blood sample was taken, the rats were killed by CO inhalation. The liver tissues were dried using filter paper, rinsed with pre-chilled normal saline, and then put in a corning tube. To obtain an initial sample with a concentration of 10% and place it in a Freezing Tube, homogenise the tissue at 4 °C with ice physiological saline. The plasma and liver samples were frozen in tanks of liquid nitrogen.

Stability determination of monomers in vivo

Sample handling

Thaw the plasma at room temperature and take 200 μL plasma, put it into a small test tube, and quickly add 100 μL of methanol and 100 μL of 10 mol/L hydrochloric acids to participate in the hydrolysis, vortex for 30 s, and hydrolyze the mixture in 37 °C water bath for 120 min. After hydrolysis, immediately keep it in cold water to cool, add 50 μL of ammonia (7.5 mol/L) to terminate the hydrolysis, add 1 mL of methanol to a small test tube, ultrasonic for 5 min, centrifuge at high speed at 10000 r/min for 5 min at room temperature. And take the supernatant of 1.5 mL of the solution, blow-dry under dark conditions, and store the dry sample at -80 °C for later use. During the determination, a dry sample was taken, redissolved with 200 μL methanol, vortexed for 2 min, centrifuged at high speed of 12000 r/min for 5 min, and 20 μL supernatant was taken for analysis. Thaw the liver tissue sample at room temperature, add 2 mL of methanol to the tissue homogenate, vortexed for 60 s, centrifuged at 12000 r/min for 5 min, absorb about 2 mL of supernatant and blow-dry under dark conditions. The residue was dissolved with 100 μL methanol, vortexed for 2 min, centrifuged at 10000 r/min for 5 min, and underwent 20 μL supernatant injection analysis.

Test conditions

Chromatographic conditions: Chromatographic column: C18 column (150 nm × 4.6 mm, 5 μm) was eluted at 25 °C. Flow rate: 1.0 mL/min; Injection volume: 10 μL; Column temperature: 30 °C. Mobile phase A: 0.1% FA (Formic Acid) + H_2O. B: MeOH (Methanol). Gradient elution condition: 0 → 5 min, 30% A, 5 → 30 min, 60% A, 30 → 35 min, 30% A.

Mass spectrometric conditions: scan from m/z 100 to 1000 using an electrospray ion mass spectrometer (ESI-MS) in positive ion mode. The conditions are as follows: the needle voltage is 3.5 kV, electrospray ionization, enter the mass spectrometer mobile phase split to 0.5 mL/min, capillary voltage 3.5 kV, the capillary temperature is 350 °C, the temperature of the dryer is 350 °C, atomizing gas (N_2) pressure 40 psi, dry gas (N_2) flow rate 12 L/min^{-1}.

Establishment of the standard curve: take the phenolic acid monomer standard (rutin, chlorogenic acid, kaempferol, and quercetin) from the purified flavonoids of *Lycium barbarum* L. leaves and prepare it with ultrapure water with the concentration of 0.3, 1, 3, 5, 10 and 20 μg/mL standard solution, liquid quality test. A large number of pre-experimental results showed that the four monomers existed in the plasma and liver of SD rats.

Stability of antioxidant activity in vivo

T-SOD, CAT, GSH-P_x, and MDA contents in plasma and liver were measured at 0, 0.5, 1, 2, 3, and 4 h after gavage. The determination methods are carried out according to the instructions of the kit.

Histology observation

The liver tissue was soaked in 4% paraformaldehyde solution and fixed for 24 h. The liver tissue was removed, rinsed with alcohol gradient solutions, and dehydrated. It was placed in xylene solution for transparent treatment, embedded in a 5 um thick piece of paraffin. After HE staining, the pathological tissue was observed under the microscope for image acquisition and analysis. The final staining result was that the nucleus was blue, and the cytoplasm was red.

2.5 Analysis of data

All experiments were performed three times with three parallel samples. Standard deviation (SD) was used to express the data as mean (mean). SPSS 17.0 was utilised to examine the data's correlation and variance. The significance of the mean value was tested using the LSD test or one-way ANOVA; a difference of $p < 0.05$ was considered to be significant, and a difference of $p < 0.01$ was considered to be highly significant. The peak of the mass spectrometry data was located using the Xcalibur 2.2 software, and the origin 2017, DPS 2019, and Excel programmes were used to visualise the results.

3 Results and discussion

3.1 Changes in flavonoids monomer content in Lycium barbarum L. leaves during in vivo digestion

Changes of main monomer contents of LBLF in plasma of SD rats

UPLC-MS showed peaks in the plasma chromatogram (high, medium, and low dose groups) following LBLF administration. They were deemed to be absorbed LBLF monomers (rutin, chlorogenic acid, quercetin, and kaempferol prototype), which were not detected in the blank group's chromatogram. Based on a significant number of prior studies conducted by the study team, it was determined that the purified LBLF monomer content consisted primarily of rutin, chlorogenic acid, quercetin, and kaempferol. When doing in vivo trials, the analysis was based on these four main flavonoid monomers (Chen et al., 2020).

The changes in rutin, chlorogenic acid, kaempferol and quercetin contents in plasma after the intervention of LBLF in rats are shown in Figure 1. As can be seen from A in Figure 1, there is an apparent dose-dependent relationship in the high, medium and low dose groups. As shown in B of Figure 1, rutin reached its maximum concentration in the three dose groups (168.80 ± 15.25 μg/g, 331.99 ± 30.24 μg/g, 762.16 ± 36.63 μg/g) after 1 h, but the other three substances reached their maximum concentrations at 3 h.

The fact that rutin occupies more than 80% of the flavonoid monomers in *Lycium barbarum* L. leaves may explain why rutin differs from it. After entering the blood, rutin exhibited a rapid metabolism rate and a short half-life, reducing the peak time. In conclusion, rutin reached its most significant value 1 h after the intervention. In contrast, the other three compounds reached their maximum value 3 h after the intervention, and the maximal absorption peak arrived. The difference between rutin and the other three flavonoids may be because rutin makes up more than 80% of flavonoids in *Lycium barbarum* L. leaves and has a rapid metabolic rate and a short half-life after entering the circulation, hence decreasing the peak time. The concentration of each monomer component diminishes with digestion time, and in rats was exceedingly unstable. This condition likely resulted from these flavonoids' constant metabolism and excretion (Wood & Tamura, 2001). After administering quercetin to rats, it was discovered that the compound was promptly taken into the blood in the intestine and detected as prototype compounds, showing that rats can absorb and metabolise quercetin. According to research, when quercetin was administered to rats it was promptly taken into the blood in the intestine and discovered as a prototype compound, demonstrating that rats can absorb and metabolise quercetin (Yang et al., 2016). Rats were administered various doses of quercetin and kaempferol via gavage to examine their stability during digestion in the body (Xiao et al., 2019). Hydrogen bonding has shown that kaempferol can bind to sialyltransferase to increase its stability. Using van der Waals force and electrostatic attraction, quercetin can bind with sialyltransferase to increase its stability within the body. The detection and identification of these plasma samples demonstrated that flavonoids isolated from *Lycium barbarum* L. leaves might be absorbed into rat plasma via the gastrointestinal system, consequently exerting various antioxidant properties.

Changes of main monomer contents of LBLF in SD rat liver

The blood inflow components of LBLF can also be detected in the liver in the prototype. As depicted in Figure 2, the low, medium, and high dose groups are strongly dose-dependent, and the overall content of rutin > chlorogenic acid > quercetin > kaempferol; the content of the high dose group was 327.25 ± 2.01 μg/g > 195.54 ± 20.39 μg/g > 11.54 ± 1.12 μg/g > 10.07 ± 2.50 μg/g. The content of four monomers is far lower than that in plasma. The reason for the extremely low rutin content in the liver

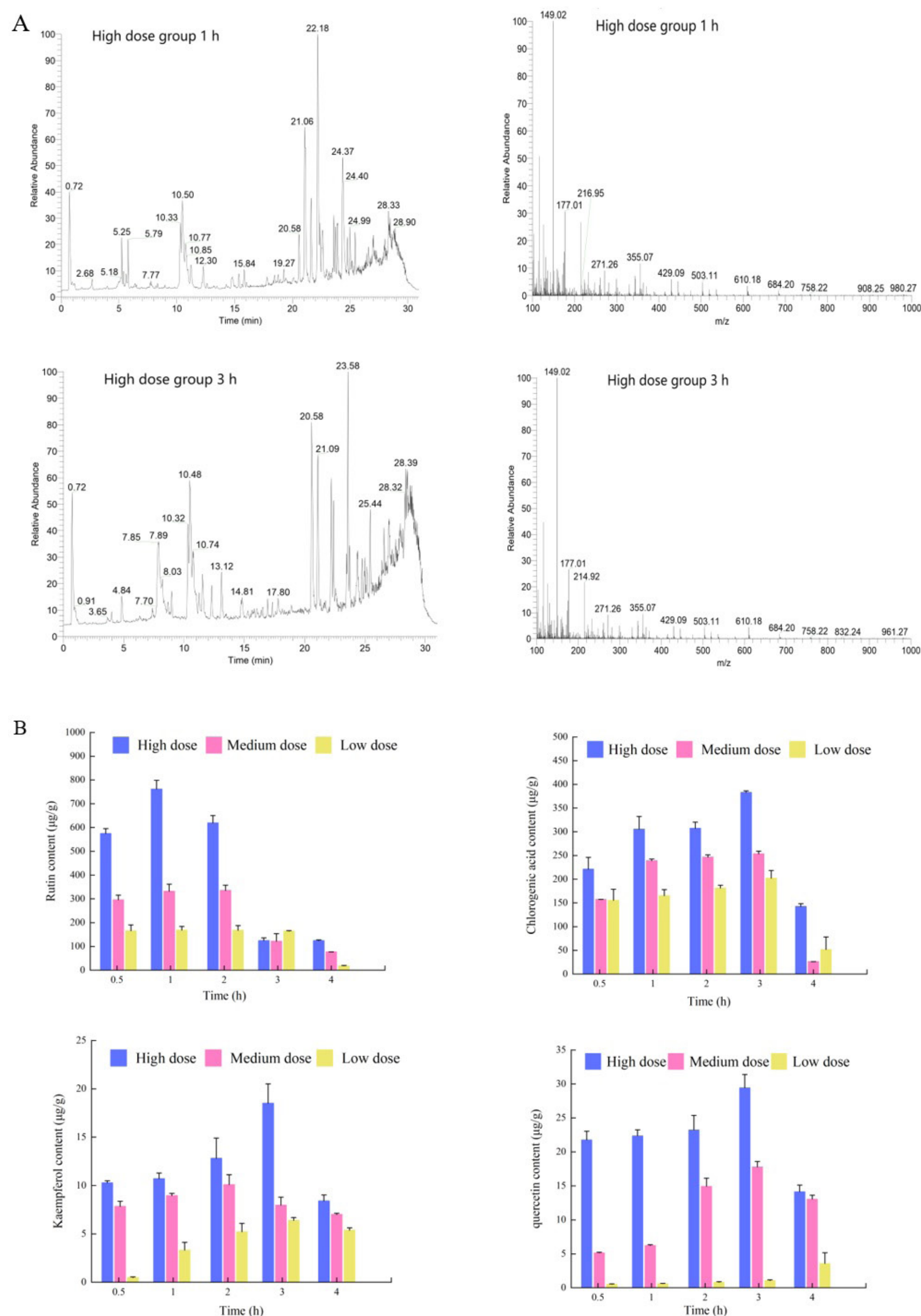

Figure 1. A. For directional and quantitative investigation, chromatograms and mass spectra of the monomer concentrations in rat plasma at 1 h and 3 h following intervention with high dosage flavonoids were utilised. B. The correlation between the plasma concentrations of rutin, chlorogenic acid, kaempferol, and quercetin and the digestion time of rats following administration of flavonoids.

may be that rutin monomer components after the intervention are hydrolyzed and metabolized into other components in the intestine to play an active role (Xie et al., 2017).

3.2 Changes in antioxidant activity of flavonoids in Lycium barbarum L. leaves during digestion in vivo

$GSH\text{-}P_x$ is an important peroxidase in the body and plays a vital role in scavenging free radicals (Sharapov et al., 2021). $GSH\text{-}P_x$ can catalyze the conversion of glutathione from reduced to oxidized form, reducing toxic peroxides to non-toxic hydroxyl compounds. At the same time, it can promote the decomposition of H_2O_2 into H_2O and O_2 and protect cell membrane function from damage by peroxides (Fan et al., 2016). Studies have shown that decreased $GSH\text{-}P_x$ activity will lead to the loss of lipid peroxidation and cell touch function (Cavalaro et al., 2019). This work evaluated the effects of LBLF on $GSH\text{-}P_x$ activity in rat plasma and liver. The outcomes were

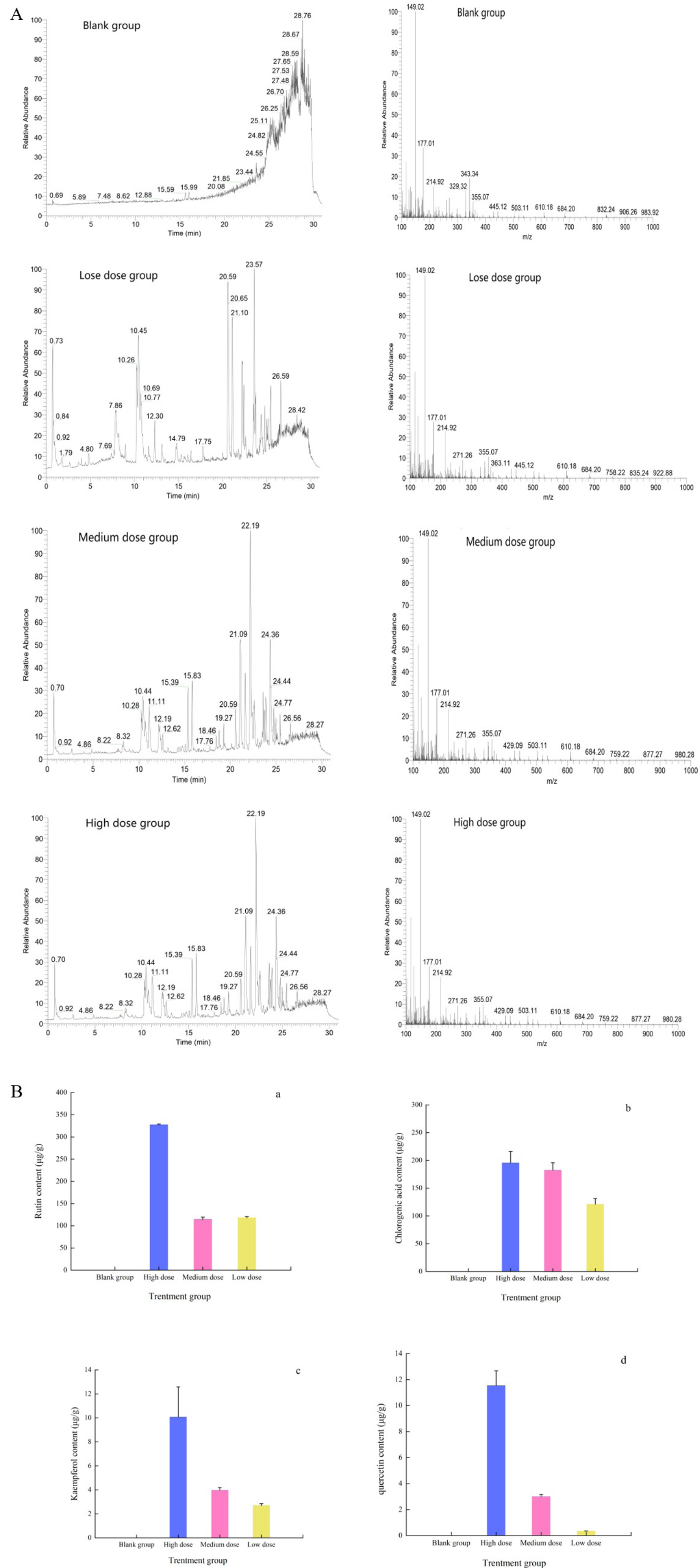

Figure 2. A. Chromatograms and mass spectra of the key monomers in the liver of rats treated with different dosages of flavonoids (blank group, low-dose group, medium-dose group, and high-dose group) were studied, as were the concentrations of the main monomers. B. Variations in the major monomer content and digesting time in rat liver following administration of (a) rutin, (b) chlorogenic acid, (c) kaempferol, and (d) quercetin.

presented in Tables 1 and 2. The change in GSH-P_x activity in plasma at different times after gavage increased significantly at 3 h ($p < 0.05$), which was caused by the maximum content of flavonoids (rutin, chlorogenic acid, kaempferol, and quercetin) in *Lycium barbarum* L. leaves at that time. Within the intragastric dosage range, this action exhibited a specific dose dependence. It is demonstrated that LBLF increased GSH-P_x activity in SD rats and negatively impacted endogenous peroxides.

CAT is present in numerous tissues of the body, particularly liver tissue. CAT might break down lipid peroxidation and hydroxyl free radicals to safeguard the intracellular environment and the regular function of cell membranes (Wang et al., 2015). This investigation examined the effect of LBLF on CAT activity in SD rat plasma and liver. The results are presented in Tables 1 and 3. The medium and high doses of LBLF significantly raised the CAT activity in the plasma and liver of SD rats compared to the control group ($p < 0.05$). At different times of gastric perfusion, plasma CAT activity increased significantly ($p < 0.05$), although liver antioxidant activity was lower than plasma levels. The plasma high-dose group boosted CAT activity considerably at 3 h, demonstrating that high-dose LBLF is an essential source of dietary antioxidants that promote the enhancement of CAT activity in the body (Pavan et al., 2014).

SOD can protect the body's inner membrane structure, maintain the body's metabolic balance, and maintain the body's antioxidant balance (Shang et al., 2018). SOD activity in the body can indirectly reflect the strength of the free radical scavenging ability of the body (Xiaoming et al., 2012). The effects of LBLF on SOD activity in the plasma and liver of rats are shown in Tables 1 and 4. Compared with the blank control group, LBLF high-dose group significantly increased SOD activity in plasma and liver of SD rats ($p < 0.05$), and there was no significant change in low and medium dose groups. This shows that LBLF can increase SOD activity in serum and liver of SD rats at high doses and eliminate oxygen-free radicals produced in the body. The results showed that the flavonoids of *Lycium barbarum* L. leaves in the high-dose group significantly promoted SOD activity in the plasma and liver of SD rats.

MDA is a physiological ketone aldehyde that develops when polyunsaturated fatty acids peroxide on the membrane of a living cell. The variation in MDA concentration will affect the function of the cell membrane. MDA concentration might indirectly indicate the extent of cell membrane damage (Pavan et al., 2014). Previous research has demonstrated that MDA is typically employed as a lipid peroxidation evaluation index *in vivo* (Jimenez-Escobar et al., 2020). This study examined the effects of LBLF on the MDA content of rat plasma and liver. The MDA content in the liver and plasma of SD rats in the experimental group with LBLF intervention fell directly to the intervention dose, as demonstrated in Tables 1 and 5. It indicates that LBLF can suppress the body's lipid peroxidation response, enhancing anti-oxidant defence. Significantly decreasing oxidative stress will lessen the oxidative damage to rat tissues and organs (Jimenez-Escobar et al., 2020). This study yielded identical results to a previous investigation, indicating that pumpkin seed sterol had the same effect on SD rats (Özbek & Ergönül, 2020).

3.3 Study on in vivo toxicity of flavonoids from Lycium barbarum L. leaves

As can be seen in Figure 3, the liver tissue of the SD rats in the blank control group had a normal liver cell structure with distinct borders, intact cytoplasmic preservation, a prominent nucleus, a visible central vein, and liver cells arranged in strips (Figure 3A). The absence of phenomena like steatosis, necrosis, and inflammatory cell infiltration in the cytoplasm, as well as the lack of a significant difference in the liver section diagram between different LBLF dose groups, can be seen in the low, medium, and high dose groups of LBLF (Figure 3B-3D) close to the blank control group. This suggests that LBLF is safe and

Table 1. Effects of different doses of flavonoids from *Lycium barbarum* L. leaves on liver indexes of SD rats.

Treatment group	Index			
	GSH-P_x (U/mg)	CAT vitality	MDA content	Total SOD activity
Blank group	3.22 ± 0.67 ^{c}	0.19 ± 0.02 ^{d}	8.36 ± 0.52 ^{a}	8.72 ± 0.95 ^{d}
Low dose group	4.83 ± 2.46 ^{a}	0.93 ± 0.05 ^{c}	7.22 ± 0.20 ^{b}	15.02 ± 0.18 ^{c}
Medium dose group	32.21 ± 3.11 ^{b}	1.11 ± 0.06 ^{b}	7.13 ± 0.03 ^{bc}	22.00 ± 0.32 ^{b}
High dose group	113.28 ± 2.78 ^{c}	1.49 ± 0.11 ^{a}	6.64 ± 0.16 ^{c}	46.45 ± 0.03 ^{a}

Different small letters in the same column indicate significant differences at different times ($p < 0.05$); different capital letters in the same line have a significant difference compared with the blank control group ($p < 0.05$), the same below.

Table 2. Effects of different doses of flavonoids from *Lycium barbarum L.* leaves on GSH-P_x activity in plasma of SD rats.

Time (h)	Treatment group			
	Blank group	Low dose group	Medium dose group	High dose group
0	22.53 ± 2.30 ^{bX}	21.48 ± 14.52 ^{cX}	23.14 ± 2.06 ^{eX}	22.81 ± 3.45^{eX}
0.5	21.66 ± 1.67 ^{bZ}	216.37 ± 6.71 ^{aY}	258.25 ± 43.11 ^{cX}	728.41 ± 3.45 ^{dW}
1	24.92 ± 2.56 ^{aZ}	126.17 ± 54.89 ^{bY}	159.46 ± 22.37 ^{dX}	1737.62 ± 3.45 ^{aW}
2	23.72 ± 6.73 ^{bZ}	253.42 ± 29.80 ^{aY}	627.11 ± 27.06 ^{bX}	1722.05 ± 35.82 ^{aW}
3	23.89 ± 4.15 ^{bZ}	101.47 ± 7.02 ^{bY}	689.39 ± 18.57 ^{aX}	1568.32 ± 6.70 ^{bW}
4	23.11 ± 3.69 ^{bZ}	28.45 ± 6.51 ^{cY}	649.66 ± 48.88 ^{abX}	1380.76 ± 7.52 ^{cW}

Different small letters in the same column indicate significant differences at different times ($p < 0.05$); different capital letters in the same line have a significant difference compared with the blank control group ($p < 0.05$), the same below.

Table 3. Effects of different doses of flavonoids from *Lycium barbarum* L. leaves on CAT activity in the plasma of SD rats.

Time (h)	Treatment group			
	Blank group	Low dose group	Medium dose group	High dose group
0	4.62 ± 0.67 ^{aW}	4.54 ± 1.20 ^{cW}	4.44 ± 1.11 ^{eW}	5.22 ± 0.09 ^{dW}
0.5	4.70 ± 0.29 ^{aZ}	7.34 ± 0.16 ^{bX}	6.43 ± 0.30 ^{dY}	10.72 ± 1.77 ^{cW}
1	4.72 ± 0.36 ^{aY}	7.66 ± 0.27 ^{bX}	7.78 ± 0.13 ^{cX}	12.10 ± 1.64 ^{bcW}
2	4.69 ± 0.72 ^{aZ}	8.02 ± 0.94 ^{bY}	10.26 ± 0.15 ^{bX}	13.53 ± 1.22 ^{bW}
3	4.75 ± 0.67 ^{aZ}	9.75 ± 0.78 ^{aY}	12.12 ± 0.32 ^{aX}	16.56 ± 0.72 ^{aW}
4	4.69 ± 0.54 ^{aZ}	7.78 ± 0.92 ^{bY}	10.46 ± 0.48 ^{bX}	13.46 ± 1.40 ^{bW}

Different small letters in the same column indicate significant differences at different times ($p < 0.05$); different capital letters in the same line have a significant difference compared with the blank control group ($p < 0.05$), the same below.

Table 4. Effects of different doses of flavonoids from *Lycium barbarum L.* leaves on SOD activity in plasma of SD rats.

Time (h)	Treatment group			
	Blank group	Low dose group	Medium dose group	High dose group
0	32.53 ± 1.67 ^{aW}	31.98 ± 2.12 ^{cW}	30.46 ± 3.96 ^{abW}	33.48 ± 3.81 ^{cW}
0.5	33.64 ± 1.97 ^{aX}	33.35 ± 0.72 ^{bcX}	33.94 ± 2.50 ^{abX}	44.47 ± 3.95 ^{bW}
1	33.13 ± 3.92 ^{aX}	34.83 ± 1.14 ^{bcX}	36.50 ± 2.53 ^{aX}	47.31 ± 3.35 ^{bW}
2	33.25 ± 3.65 ^{aX}	33.33 ± 1.10 ^{aX}	33.50 ± 4.39 ^{abX}	52.71 ± 2.09 ^{aW}
3	32.09 ± 1.44 ^{aX}	32.54 ± 3.97 ^{aX}	34.05 ± 6.59 ^{abX}	55.23 ± 0.47 ^{aW}
4	33.20 ± 3.67 ^{aY}	36.46 ± 1.69 ^{bX}	38.08 ± 1.25 ^{bX}	43.23 ± 2.79 ^{bW}

Different small letters in the same column indicate significant differences at different times ($p < 0.05$); different capital letters in the same line have a significant difference compared with the blank control group ($p < 0.05$), the same below.

Table 5. Effects of different doses of flavonoids from *Lycium barbarum* L. leaves on MDA content in plasma of SD rats.

Time (h)	Treatment group			
	Blank group	Low dose group	Medium dose group	High dose group
0	8.23 ± 0.32 ^{aW}	8.33 ± 0.54 ^{aW}	8.06 ± 0.19 ^{aW}	8.19 ± 0.33 ^{aW}
0.5	8.27 ± 0.42 ^{aW}	7.89 ± 0.56 ^{abW}	7.99 ± 0.03 ^{aW}	7.18 ± 0.11 ^{bW}
1	8.26 ± 1.67 ^{aW}	7.02 ± 0.19 ^{cW}	7.01 ± 0.07 ^{bW}	7.13 ± 0.10 ^{bW}
2	8.30 ± 1.44 ^{aW}	6.04 ± 0.50 ^{dX}	6.80 ± 0.26 ^{bX}	6.28 ± 0.01 ^{dX}
3	8.26 ± 2.06 ^{aW}	7.17 ± 0.31 ^{bcX}	6.08 ± 0.05 ^{cX}	6.81 ± 0.07 ^{cX}
4	8.15 ± 0.90 ^{aW}	7.04 ± 0.02 ^{cW}	7.02 ± 0.19 ^{bW}	7.05 ± 0.08 ^{bcW}

Different small letters in the same column indicate significant differences at different times ($p < 0.05$); different capital letters in the same line have a significant difference compared with the blank control group ($p < 0.05$), the same below.

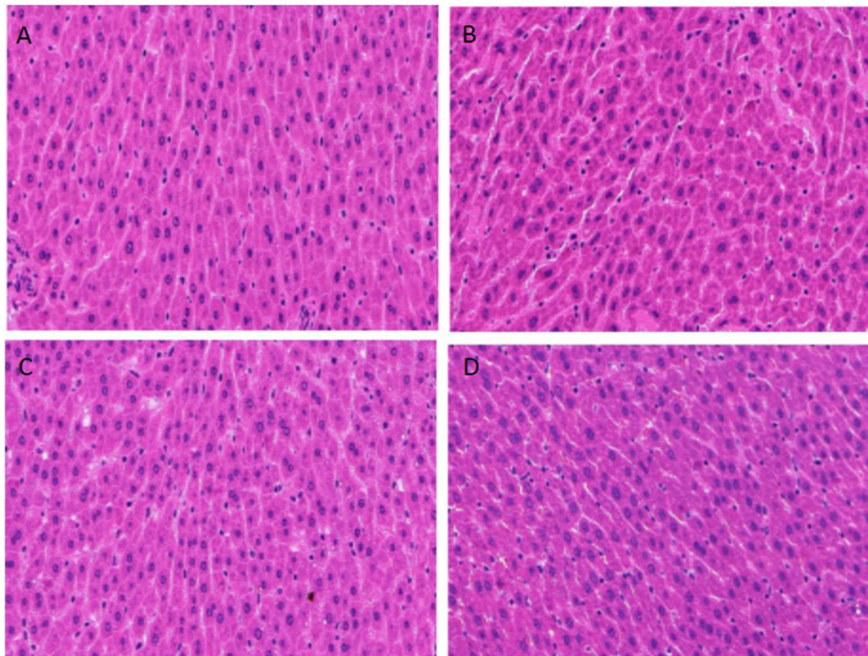

Figure 3. Effects of *Lycium barbarum* L. leaves flavonoids on histopathological changes in SD rats in (A) blank group, (B) low-dose group, (C) medium-dose group, and (D) high-dose group.

non-toxic within the intragastric dose range. However, research has revealed testing the long-term toxicity of quercetin by administering it to rats in a specific dose. Male rats fed at a dosage of 1900 mg $kg^{-1}d^{-1}$ for two years showed focal epithelial hyperplasia of the renal tubules. According to Yang et al. (2018), there was a small but significant rise in the incidence of chronic renal disease as quercetin consumption rose. This finding suggests that the occurrence of safety issues may be influenced by the dose of LBLF, exposure duration, and cellular redox status.

4 Conclusion

The findings demonstrated that after LBLF intervention in SD rats, the monomer chemicals rutin, chlorogenic acid, kaempferol, and quercetin appeared in the form of prototype compounds in plasma and the liver. High-dose LBLF therapy can significantly increase GSH-P_x, T-SOD, CAT, and liver tissue activity in SD rats after the intervention ($p < 0.05$), decrease MDA content ($p < 0.05$), and have good antioxidant activity *in vivo*. There was no discernible change in the liver slices of SD rats exposed to various doses of LBLF, indicating that the flavonoids extract derived from the separation and purification of *Lycium barbarum* L. leaves was safe and non-toxic within the intervention dose range.

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