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Red Ball Ginger (*Zingiber zerumbet*) Extracts-Enriched Soy Milk: Mitigation of Rheumatoid Arthritis-Related Symptoms and Lipid-Modulating Effects of Hyperlipidemia in the Experimental Animal Models

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Abstract

Zingiber zerumbet, commonly referred to as red ball ginger or pinecone ginger, is a perennial species of the family *Zingiberaceae*, native to India and Malaysia. It is primarily distributed throughout tropical Asia and the Pacific islands, where it naturally inhabits moist, shaded lowlands and hillsides. The plant is characterized by its distinctive pinecone-like inflorescences, formed by overlapping bracts that turn bright red upon maturity, enhancing its ornamental appeal. In Taiwan, *Z. zerumbet* is relatively rare, maintained through limited germplasm conservation or small-scale contract cultivation. In contrast, it is more widely recognized and utilized in regions such as India, China, and Malaysia. Beyond its horticultural value, *Z. zerumbet* is of notable ethnomedicinal interest; its rhizome has traditionally been used as a food flavoring and as a herbal remedy in folk medicine in India and Malaysia. In this study, the adult male 24 Sprague-Dawley (SD) rats (6 weeks old) with specific pathogen-free conditions were used in this study. In this experiment, all SD rats (n = 24) were divided respectively the normal control group (n = 8), the high-fat diet group (n = 8), and the *Z. zerumbet* group (n = 8). The high-fat feed (containing 0.2% cholesterol) was used to feed SD rats for 8 weeks to induce hyperlipidemia in the negative control group and the *Z. zerumbet* group; At the 5th week of the high-fat feed administration, the rheumatoid arthritis (RA) was inducted in all animals in the high-fat diet group and the Z. zerumbet group. In the normal control group, animals had ad libitum access to reverse osmosis (RO) water throughout the experimental period. In the high-fat diet group, each animal was administered soy milk daily via oral gavage at a volume of 10 mL/kg body weight (BW). In the *Z. zerumbet* group, red ball ginger (*Z. zerumbet*) powder was suspended in soy milk at a concentration of 150 mg/mL. Animals in the *Z. zerumbet* group received a daily dose of red ball ginger powder equivalent to 1,500 mg/kg BW. Hyperlipidemia and RA animal models were applied and the evaluated items in hyperlipidemia and RA were analyzed. Blood were collected before hyperlipidemia was induced (D0) and blood was collected every week after hyperlipidemia was induced. The BW of SD rats were weighed weekly. The TG (triglyceride), TCHO (total cholesterol), HDL (high-density lipoprotein), and LDL (low-density lipoprotein) contents in blood were detected and analyzed at each experimental time point. In the *Z. zerumbet* group, supplementation with *Z. zerumbet*-enriched soy milk significantly reduced serum total cholesterol and LDL cholesterol levels compared to the high-fat diet group, approaching those of the normal control group. The elevated AST/ALT ratio observed in the high-fat diet group was attenuated by *Z. zerumbet*, suggesting improved liver function. Paw swelling and arthritis clinical scores induced by RA were also significantly reduced in the *Z. zerumbet* group, indicating anti-inflammatory potential. BW gain and food intake were not significantly affected by *Z. zerumbet* supplementation after RA induction. According to the experimental results, soy milk enriched with red ball ginger (*Z. zerumbet*) extracts shows potential for lowering blood

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lipids and alleviating RA in the high-fat diet-induced rat models. Therefore, *Z. zerumbet*-enriched soy milk may be considered a promising functional food candidate for modulating blood lipids and alleviating rheumatoid arthritis symptoms.

Keywords: Blood lipid; Red ball ginger; *Zingiber zerumbet*; Soy milk; Rheumatoid arthritis; Hyperlipidemia; Experimental animal models

1. Introduction

According to global estimates by the World Health Organization (WHO), approximately 2 billion adults aged 18 years and older were overweight in 2016, of whom over 650 million were classified as obese. Overall, about 13% of the world's adult population was obese in 2016. The global prevalence of obesity has nearly tripled between 1975 and 2016. Furthermore, an estimated 38.2 million children under the age of 5 years were overweight or obese in 2019. Notably, obesity is now associated with more deaths worldwide than underweight. Obesity is characterized by excessive accumulation of body fat as a result of persistent energy intake exceeding energy expenditure [1-3]. It is defined as abnormal or excessive fat accumulation that may impair health, with the fundamental cause being an energy imbalance between calories consumed and expended. Globally, this trend has been fueled by increased consumption of energy-dense, high-fat, high-sugar foods and reduced physical activity due to increasingly sedentary work, changes in transportation modes, and urbanization. These shifts in diet and physical activity patterns are often driven by environmental and societal changes associated with development, compounded by the absence of supportive policies in sectors such as health, agriculture, transport, urban planning, environment, food production and distribution, marketing, and education [4-5].

Hyperlipidemia, a condition characterized by elevated levels of fats in the blood-primarily cholesterol and triglycerides-is a major risk factor for several serious diseases, including heart disease, hypertension, stroke, diabetes, atherosclerosis, and even kidney disease. Most individuals with hyperlipidemia are asymptomatic in the early stages, with a minority of cases attributable to genetic factors. Therefore, early detection relies on regular health examinations to monitor blood cholesterol and triglyceride levels.

In recent years, obesity, defined as abnormal or excessive fat accumulation that may impair health, has garnered growing research attention regarding its prevention and treatment. In particular, understanding the molecular mechanisms of adipocyte function has become a central focus in obesity research. Therefore, it is of considerable importance to explore and develop high-value crops with potential to lower blood lipid levels as a novel strategy to combat obesity and hyperlipidemia [1-8].

The pathogenesis of rheumatoid arthritis (RA) is attributed to chronic autoimmune dysregulation, making it a complex autoimmune disease [9-10]. Although its precise etiology remains unclear, the main pathogenic mechanism involves immune dysregulation caused by the production of autoantibodies, leading to excessive autoimmune attacks on the joints. This process triggers synovial membrane hyperplasia and infiltration of inflammatory cells-primarily lymphocytes and macrophages, along with smaller numbers of fibroblasts, plasma cells, dendritic cells, and abundant fibroblast-like synoviocytes (FLS). FLS also secrete degradative factors such as matrix metalloproteinases (MMPs). In the early stage of RA, synovitis dominates. With repeated exacerbations over time, pannus (granulation tissue with neovascularization) progressively invades the joint surface, eroding the cartilage, and ultimately destroying both cartilage and subchondral bone, resulting in joint deformity and malalignment. Subsequently, as acute inflammation subsides, the granulation tissue develops into fibrous scar tissue, leading to fibrous ankylosis, and eventually ossifies into bony ankylosis [11-16].

RA predominantly affects middle-aged women and typically involves multiple joints throughout the body, with hand joints being the most commonly affected. Patients initially experience local joint stiffness and pain, which may progress to widespread joint swelling and damage. Without timely intervention, the disease can lead to severe joint deformity and ankylosis, ultimately resulting in a complete loss of self-care ability. Currently, the mainstay of RA treatment is long-term pharmacological management. However, nonsteroidal anti-inflammatory drugs (NSAIDs) are associated with adverse effects such as gastric ulcers, and disease-modifying antirheumatic drugs (DMARDs) can cause toxicity affecting the skin and mucosa, renal and pulmonary function, hematopoiesis, and vision. The medical costs of managing these adverse drug effects may even exceed the direct costs of RA itself. Furthermore, RA significantly impairs the quality of life of patients and their families [17-18]. Therefore, improving patients' self-care abilities and developing safer and more effective therapeutic approaches are essential. Since RA is a chronic, incurable autoimmune disease in which joint damage is largely irreversible, the current clinical strategy focuses on controlling disease progression and alleviating symptoms to maintain patients' quality of life. Accordingly, it is imperative to establish appropriate RA animal models

and drug screening platforms to explore therapeutic strategies and develop novel drugs aimed at more effective treatments.

Zingiber zerumbet, a plant native to tropical and subtropical regions of Asia, has been traditionally utilized for a wide range of medicinal purposes and continues to attract scientific interest due to its diverse pharmacological properties. Nevertheless, there remains a lack of systematic evidence synthesis regarding its efficacy, highlighting the need to clarify the current research landscape on this plant. The efficacy, biological mechanisms, and safety of *Z. zerumbet* and its principal phytoconstituents in various formulations have been investigated in numerous animal studies. Reported pharmacological activities encompass a wide range of effects, including analgesic, anti-inflammatory, anti-diabetic, anti-hyperlipidemic, anti-neoplastic, immunomodulatory, antioxidant, antipyretic, hepatoprotective, nephroprotective, gastroprotective, and locomotor-suppressing activities. Among these, the ethanolic extract of *Z. zerumbet* has been shown to be well tolerated in animal models for up to 28 days of repeated administration, suggesting its potential safety for short-term use. Importantly, both *Z. zerumbet* and its major bioactive component, zerumbone, exhibit a broad spectrum of pharmacological properties, particularly notable in analgesic and anti-inflammatory models, which aligns with its traditional use as a medicinal plant in Asian cultures. Despite these promising preclinical findings, comprehensive safety evaluations remain limited, and no clinical trials in humans have been reported to date. This highlights an urgent need for further research to bridge the gap between preclinical evidence and clinical application. Future investigations should focus on standardizing the quality and composition of *Z. zerumbet* formulations, elucidating dose-response relationships, and assessing potential toxicological effects over longer durations. Well-designed clinical trials are essential to validate its efficacy and safety profile in human populations, paving the way for its potential development as a natural therapeutic agent. *Z. zerumbet* and zerumbone represent promising candidates for the development of novel treatments targeting inflammatory and metabolic disorders. However, rigorous scientific validation through clinical studies is imperative to substantiate their traditional uses and to ensure their safe and effective integration into modern healthcare [19]. Therefore, the aim of this study was to evaluate the effects of red ball ginger (*Z. zerumbet*) extract-enriched soy milk on mitigating rheumatoid arthritis–related symptoms and modulating lipid profiles associated with hyperlipidemia in experimental animal models.

2. Materials and Methods

2.1. Chemicals and Reagents

Phosphate-buffered saline (PBS; Sigma-Aldrich, Cat. No. P3813), saline (Taiwan Biotech Co., LTD, Cat. No. 100-120-1101), and Zoletil 50 (Virbac, Carros, France) were applied in this experiment.

2.2. Source of Red Ball Ginger (*Z. zerumbet*) Extracted Powders and Soy Milk

Red ball ginger (*Z. zerumbet*) extracted powders and soy milk were provided by SUNMED BIO CORP.

2.3. Experimental Animals and Experimental Design

The 24 Sprague-Dawley (SD) rats (6 weeks old) with specific pathogen-free conditions were used in this study, were purchased from BioLASCO Taiwan Co., Ltd. (Yilan, Taiwan). Before the experiment, all mice were housed in the animal room for 3-5 days. The environment was maintained room temperature (24-27°C) and 60%-70% humidity with a photoperiod of 12-hr light/12-hr dark cycle. The study will begin after a week acclimation. The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC 113053 approved by the IACUC ethics committee. In this experiment, all SD rats (n = 24) were divided respectively the normal control group (n = 8), the high-fat diet group (n = 8), and the *Z. zerumbet* group (n = 8). The high-fat feed [Diet Induced Obesity (DIO) Series diets D12451, Research Diets Inc., NJ, USA] (containing 0.2% cholesterol) was used to feed SD rats for 9 weeks to induce hyperlipidemia in the negative control group and the *Z. zerumbet* group; At the day 29 the high-fat feed administration, the rheumatoid arthritis (RA) was inducted in all animals in the high-fat diet group and the *Z. zerumbet* group. In the normal control group, the SD rats were fed with the standard laboratory diet (No. 5053, LabDiet®; PMI Nutrition International, St. Louis, MO, USA) and administrated with the reverse osmosis (RO) water ad libitum in the normal group during the experimental period. In the negative control group, each animal was administered soy milk daily via oral gavage at a volume of 10 mL/kg body weight (BW). In the *Z. zerumbet* group, red ball ginger (*Z. zerumbet*) powder was suspended in soy milk at a concentration of 150 mg/mL. Animals in the *Z. zerumbet* group received a daily dose of red ball ginger powder equivalent to 1,500 mg/kg BW. Blood were collected before hyperlipidemia was induced (D0) and blood was collected every week after hyperlipidemia was induced. The BW of SD rats were weighed weekly. The TG (triglyceride), TCHO (total cholesterol), HDL (high-density lipoprotein), and LDL (low-density lipoprotein) contents in blood were detected and analyzed at each experimental time point (Figure. 1).

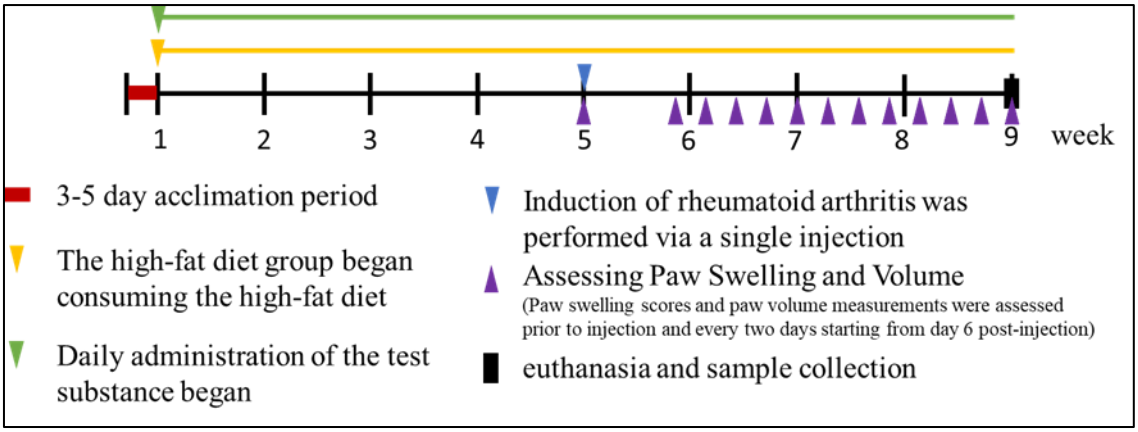

Figure 1 The experimental design comprised induction of hyperlipidemia and rheumatoid arthritis, administration of the test substance, collection of biological samples, and evaluation of paw swelling and volume

2.4. The Weighting of SD Rats' BW

To monitor the BW of SD rats every week until the end of the experiment.

2.5. Hyperlipidemia and RA Animal Models

Hyperlipidemia and RA animal models were applied and the evaluated items in hyperlipidemia and RA were analyzed [20-21]. Paw swelling was evaluated based on a standardized scoring system. Each limb was assessed separately, and a score from 0 to 4 was assigned according to the severity of swelling and redness. A score of 0 indicated a normal paw, whereas a score of 4 represented maximal swelling and redness with loss of anatomical definition. Three types of joints were observed for scoring: distal interphalangeal joints, metacarpophalangeal joints, and carpal/tarsal joints. The minimum possible score per limb was 0, and the maximum was 4.

2.6. The Analysis of Contents of TG, TCHO, HDL Cholesterol, and LDL Cholesterol in Blood of SD Rats

SD rats were collected blood and then collected sera via 4°C, 1,500 rpm for 10 minutes. The sera were analyzed the levels of TG, TCHO, HDL cholesterol, and LDL cholesterol by using Fuji DRI-CHEM NX500i (Fuji, Japan) according to the manufacturer's protocols.

2.7. Statistical Analysis

The data were expressed as mean ± SD (standard deviation). All comparisons were made by one-way ANOVA and all significant differences are reported at $^{*}p < 0.05$.

3. Results

In this experiment, the high-fat feed (containing 0.2% cholesterol) was used to feed SD rats for 8 weeks to induce hyperlipidemia in the high-fat diet group and the *Z. zerumbet* group. After the SD rats were induced with hyperlipidemia, the SD rats in the high-fat diet group were given drinking RO water every day, and the SD rats in the *Z. zerumbet* group were given a daily dose of red ball ginger powder equivalent to 1,500 mg/kg BW by gavage every day. In the experiment, blood were collected before hyperlipidemia was induced (D0) and blood was collected once per week after hyperlipidemia was induced. The BW of SD rats were weighed weekly. The TG, TCHO, HDL cholesterol, and LDL cholesterol contents in blood were detected and analyzed at each experimental time point.

3.1. BW Change of SD Rats in Each Group during the Experimental Period

At week 4, both the high-fat diet group and the *Z. zerumbet* group showed significantly higher values compared to the normal control group. In all other weeks (weeks 0, 1, 2, 3, 5, 6, 7, and 8), no significant differences were observed among the groups (Figure 2 and Table 1).

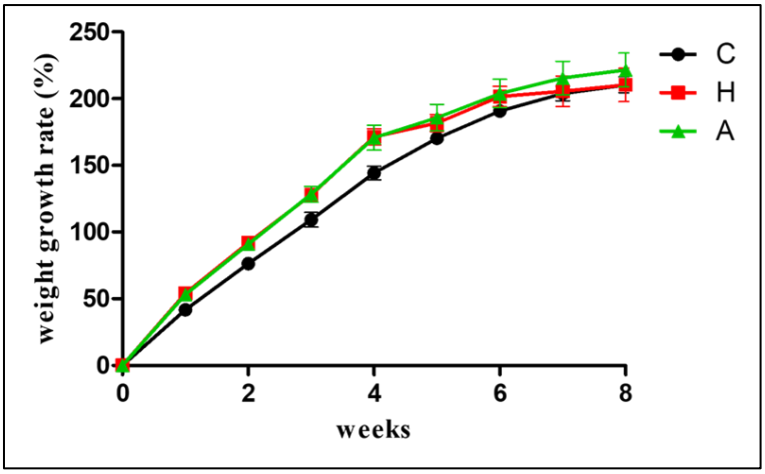

Figure 2 Body weight change rate (%) every week in each group. During the experiment, body weight (BW) was measured weekly. The weekly body weight change rate was calculated as follows: (BW at the present week-initial BW) / initial BW × 100%. Data are presented as mean ± SD (n = 8 per group). Groups: the normal control group (C), the high-fat diet group (H), and the *Zingiber zerumbet* group (A)

Table 1 BW change (g) of SD rats every week in each group

Week	Normal control group	High-fat diet group	*Zingiber zerumbet* group
W1	41.75 ± 5.38[a]	54.11 ± 5.91[a]	53.30 ± 5.25[a]
W2	76.38 ± 10.61[a]	91.99 ± 8.16[a]	90.95 ± 8.94[a]
W3	109.27 ± 12.20[a]	128.11 ± 8.26[a]	128.43 ± 14.53[a]
W4	144.28 ± 11.80[a]	171.37 ± 13.46[b]	170.76 ± 22.95[b]
W5	170.26 ± 12.48[a]	181.72 ± 18.15[a]	185.8 ± 27.86[a]
W6	190.82 ± 12.08[a]	201.63 ± 21.48[a]	203.92 ± 29.72[a]
W7	203.88 ± 15.33[a]	205.44 ± 32.17[a]	215.49 ± 35.22[a]
W8	210.37 ± 16.64[a]	210.36 ± 35.51[a]	221.55 ± 36.11[a]

BW Change of SD Rats in each group. Data are expressed as mean ± SD (n = 8 per group). Statistical comparisons among groups were performed using one-way ANOVA. Differences were considered significant at $p < 0.05$. Groups with significantly different means are denoted by different letters.

3.2. Feed Weight Change in Each Group during the Experimental Period

At week 1, the average daily food intake (g) of rats in the *Z. zerumbet* group was significantly lower than that of the normal control group ($p < 0.05$), while no significant difference was observed between the high-fat diet group and the normal control group, nor between the high-fat diet group and the *Z. zerumbet* group. From week 2 to week 8, the average daily food intake (g) in the normal control group remained significantly higher than that of the high-fat diet groups (the high-fat diet group and *Z. zerumbet* group) ($p < 0.05$), with no significant difference between the high-fat diet group and *Z. zerumbet* group. Overall, rats fed the high-fat diet (the high-fat diet group and *Z. zerumbet* group) consumed less food than those in the normal control group, but no significant difference in intake was observed between the high-fat diet group and *Z. zerumbet* group, indicating that the addition of *Z. zerumbet* did not affect the feeding behavior of rats on a high-fat diet (Figure 3 and Table 2).

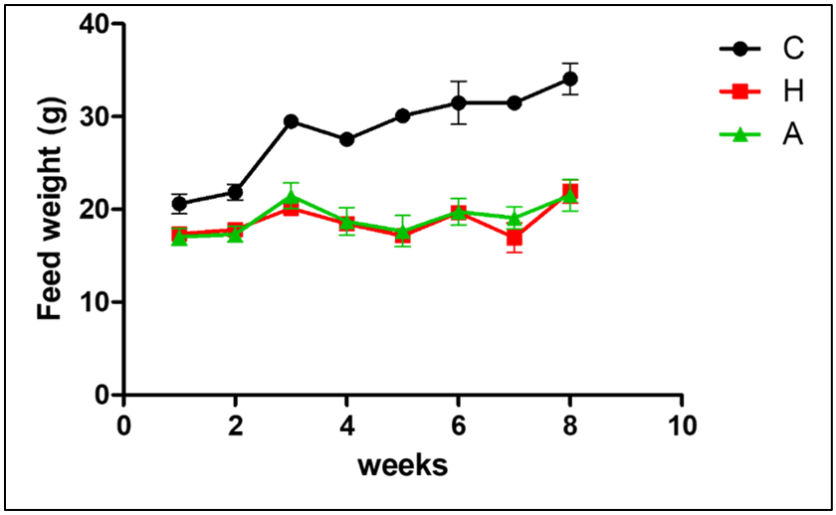

Figure 3 Feed weight (g) change every day in each group. During the experiment, feed weight was measured daily. Data are presented as mean ± SD (n = 8 per group). Groups: the normal control group (C), the high-fat diet group (H), and the *Zingiber zerumbet* group (A)

Table 2 Feed weight (g) change every day in each group. During the experiment, feed weight was measured daily

Week	Normal control group	High-fat diet group	*Zingiber zerumbet* group
W1	20.60 ± 2.10^{a}	17.37 ± 0.70^{ab}	17.05 ± 1.82^{b}
W2	21.86 ± 1.72^{a}	17.81 ± 0.88^{b}	17.32 ± 1.69^{b}
W3	29.50 ± 0.84^{a}	20.13 ± 1.19^{b}	21.44 ± 2.79^{b}
W4	27.55 ± 0.83^{a}	18.46 ± 0.39^{b}	18.70 ± 2.97^{b}
W5	30.10 ± 0.37^{a}	17.16 ± 0.97^{b}	17.66 ± 3.34^{b}
W6	31.50 ± 4.61^{a}	19.66 ± 0.85^{b}	19.74 ± 2.84^{b}
W7	31.47 ± 1.39^{a}	16.94 ± 3.20^{b}	19.09 ± 2.35^{b}
W8	34.07 ± 3.36^{a}	21.94 ± 2.50^{b}	21.51 ± 3.38^{b}
Average daily food intake in each group. Data are expressed as mean ± SD (n = 8 per group). Statistical comparisons among groups were performed using one-way ANOVA. Differences were considered significant at $p < 0.05$. Groups with significantly different means are denoted by different letters.			

3.3. Detection of the Levels of TG and TCHO in Blood of SD Rats

From week 1 to week 8, the serum total cholesterol concentration (mg/dL) in the high-fat diet group was significantly higher than that in both the normal control group and the *Z. zerumbet* group each week. In the *Z. zerumbet* group, no significant difference was observed compared to the normal control group at weeks 1, 2, 5, and 8, whereas significantly higher levels than the normal control group, but significantly lower than the high-fat diet group, were observed at weeks 3, 4, 6, and 7 (Figure 4 and Table 3).

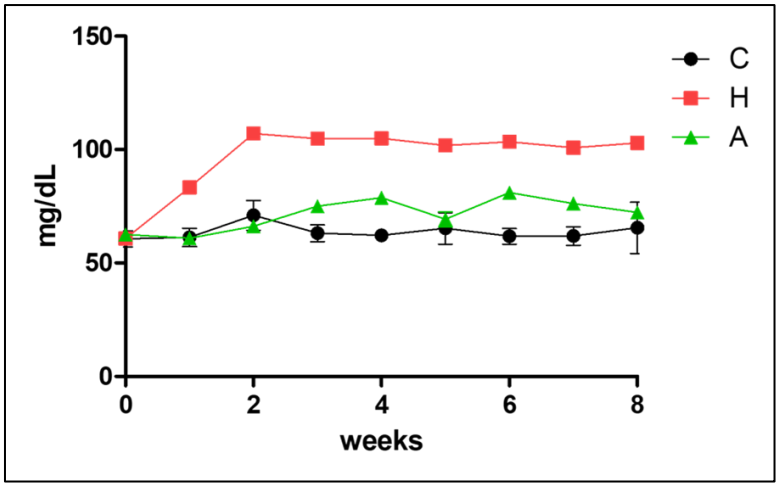

Figure 4 Detection of the level of TCHO in blood in SD rats. The level of TCHO at each experimental point. Data are presented as mean ± SD (n = 8 per group). Groups: the normal control group (C), the high-fat diet group (H), and the *Zingiber zerumbet* group (A). TCHO: total cholesterol

Table 3 The level of TCHO (mg/dL) in blood in each group

Week	Normal control group	High-fat diet group	*Zingiber zerumbet* group
W0	60.63 ± 3.50[a]	60.75 ± 3.92[a]	62.50 ± 3.78[a]
W1	61.25 ± 4.03[a]	83.25 ± 4.53[b]	60.88 ± 2.70[a]
W2	71.00 ± 6.59[a]	107.00 ± 6.63[b]	66.13 ± 6.06[a]
W3	63.13 ± 3.64[a]	104.75 ± 7.27[b]	75.00 ± 5.35[c]
W4	62.13 ± 2.17[a]	104.88 ± 7.10[b]	78.75 ± 4.03[c]
W5	65.25 ± 6.96[a]	101.75 ± 5.78[b]	69.25 ± 9.39[a]
W6	61.75 ± 3.49[a]	103.38 ± 7.44[b]	81.00 ± 3.89[c]
W7	61.88 ± 4.05[a]	100.75 ± 7.59[b]	76.13 ± 4.45[c]
W8	65.50 ± 11.28[a]	102.88 ± 4.97[b]	72.25 ± 7.4[a]
The level of TCHO in blood in SD rats in each group. Data are expressed as mean ± SD (n = 8 per group). Statistical comparisons among groups were performed using one-way ANOVA. Differences were considered significant at $p < 0.05$. Groups with significantly different means are denoted by different letters.			

3.4. Analysis of AST, ALT, AST/ALT, TG, HDL Cholesterol, LDL Cholesterol, and Total Cholesterol Contents in Blood of SD Rats

The ALT concentration (U/L) in the normal control group was significantly higher than that in both the high-fat diet group and the *Z. zerumbet* group ($p < 0.05$), with no significant difference between the high-fat diet group and the *Z. zerumbet* group (Figure 5B, Table 4). For the AST/ALT ratio, the high-fat diet group was significantly higher than the normal control group ($p < 0.05$), while the *Z. zerumbet* group did not differ significantly from either the high-fat diet group or the normal control group (Figure 5C, Table 4). No significant differences were observed among the groups in AST, triglycerides (TG), or high-density lipoprotein cholesterol (HDL-C) levels (Figures 5A, 5D, and 5F; Table 4). Regarding LDL-C and total cholesterol (TCHO), the high-fat diet group was significantly higher than both the normal control group and the *Z. zerumbet* group ($p < 0.05$), while no significant difference was observed between the normal control group and the *Z. zerumbet* group (Figures 5E, 5G; Table 4).

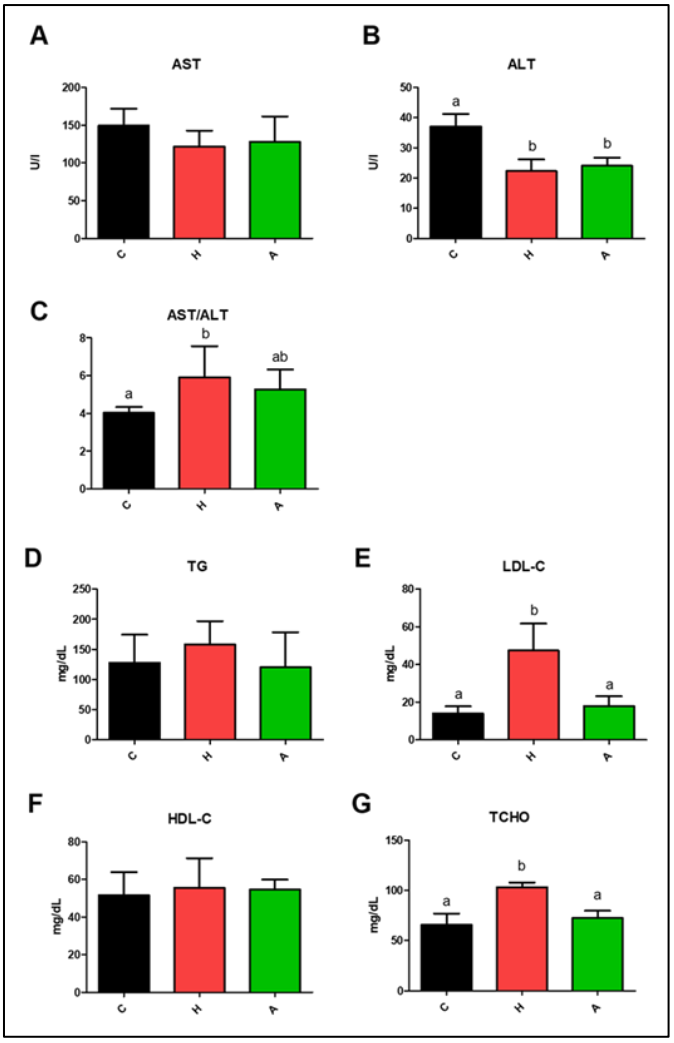

Figure 5 Analysis of AST, ALT, AST/ALT, TG, HDL, LDL, and total cholesterol contents in blood of SD rats. (A) Change in blood AST of SD rats in each group, (B) Change in blood ALT of SD rats in each group, (C) Change in blood AST/ALT of SD rats in each group, (D) Change in blood TG of SD rats in each group, (E) Changes in blood LDL cholesterol of SD rats in each group, (F) Changes in blood HDL cholesterol of SD rats in each group, (G) Changes in blood total cholesterol of SD rats in each group. Data are presented as Mean ± SD. AST: aspartate transaminase; ALT: alanine transaminase; TG: triglycerides; HDL: high density lipoprotein; LDL: low density lipoprotein; TCHO: total cholesterol

Table 4 The level of blood biochemistry values of SD rats in each group

Item	Unit	Normal control group	High-fat diet group	*Zingiber zerumbet* group
AST	U/La	149.38 ± 22.6^{a}	121.88 ± 21.03^{a}	127.88 ± 33.85^{a}
ALT	U/L	37.00 ± 4.24^{a}	22.25 ± 3.88^{b}	24.00 ± 2.73^{b}
AST/ALT		4.03 ± 0.31^{a}	5.91 ± 1.65^{b}	5.27 ± 1.05^{ab}
TG	mg/dL	127.75 ± 46.53^{a}	158.25 ± 38.8^{a}	120.13 ± 58.18^{a}
LDL-C	mg/dL	13.88 ± 3.83^{a}	47.38 ± 14.28^{b}	17.75 ± 5.37^{a}
HDL-C	mg/dL	51.63 ± 12.29^{a}	55.5 ± 15.74^{a}	54.5 ± 5.42^{a}
TCHO	mg/dL	65.50 ± 11.28^{a}	102.88 ± 4.97^{b}	72.25 ± 7.4^{a}
The level of blood biochemistry values of SD rats in each group. Data are expressed as mean ± SD (n = 8 per group). Statistical comparisons among groups were performed using one-way ANOVA. Differences were considered significant at $p < 0.05$. Groups with significantly different means are denoted by different letters.				

3.5. Changes in Paw Volume (%) of SD Rats in Each Group

During the experiment, paw volume was measured using a plethysmometer (Model: LE7500). The volume recorded on day 33 was used as the baseline, and paw volume change (%) was calculated. The high-fat diet group showed significantly higher paw volume changes compared to the normal control group on days 38, 40, 42, 44, 46, 48, and 50, and significantly higher than the *Z. zerumbet* group on days 40, 42, 44, 46, 48, and 50. No significant differences in paw volume changes were observed between the normal control group and the *Z. zerumbet* group at any time point (Figure 6 and Table 5).

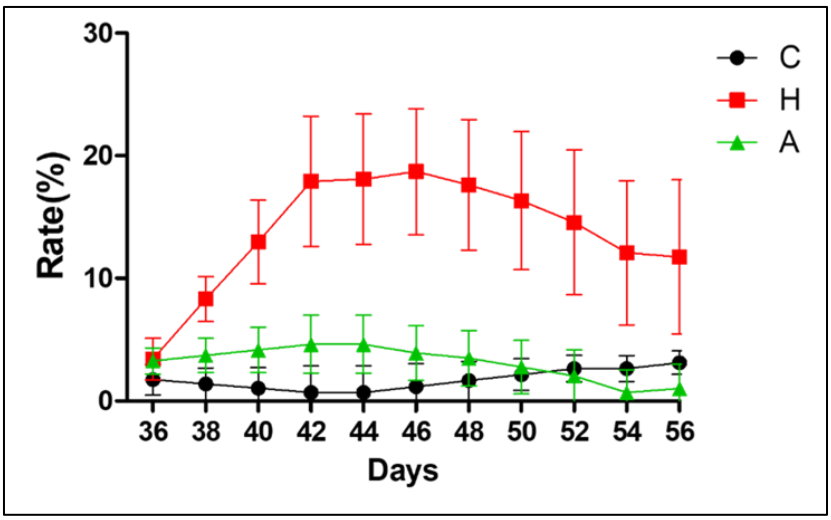

Figure 6 Changes in paw volume (%) of SD rats in each group. During the experiment, paw volume was measured once per day. Normal control group (C), High-fat diet group (H), and *Zingiber zerumbet* group (A)

Table 5 Changes in paw volume (%) of SD rats in each group

Test day	Normal control group	High-fat diet group	*Zingiber zerumbet* group
Day 36	1.74 ± 3.53[a]	3.42 ± 4.82[a]	3.26 ± 3.04[a]
Day 38	1.39 ± 3.71[a]	8.31 ± 5.17[b]	3.71 ± 3.94[ab]
Day 40	1.03 ± 4.73[a]	12.95 ± 9.62[b]	4.17 ± 5.22[a]
Day 42	0.68 ± 6.18[a]	17.91 ± 15.03[b]	4.62 ± 6.67[a]
Day 44	0.68 ± 6.18[a]	18.1 ± 15.03[b]	4.62 ± 6.67[a]
Day 46	1.17 ± 5.27[a]	18.71 ± 14.53[b]	3.90 ± 6.35[a]
Day 48	1.66 ± 4.41[a]	17.61 ± 15.08[b]	3.48 ± 6.40[a]
Day 50	2.14 ± 3.65[a]	16.34 ± 15.94[b]	2.75 ± 6.20[a]
Day 52	2.63 ± 3.05[a]	14.57 ± 16.72[a]	2.03 ± 6.05[a]
Day 54	2.64 ± 2.97[a]	12.08 ± 16.64[a]	0.69 ± 5.25[a]
Day56	3.12 ± 2.71[a]	11.75 ± 17.75[a]	1.01 ± 5.56[a]
Changes in paw volume (%) of SD rats in each group. Data are expressed as mean ± SD (n = 8 per group). Statistical comparisons among groups were performed using one-way ANOVA. Differences were considered significant at $p < 0.05$. Groups with significantly different means are denoted by different letters.			

3.6. Paw Swelling Scores in Each Group

For each rat, the three paws other than the Complete Freund's adjuvant (CFA)-injected paw were evaluated according to the paw swelling scoring criteria, and the scores were summed to obtain a total paw swelling score, with a minimum of 0 and a maximum of 12 per animal. Statistical analysis was performed using the Kruskal-Wallis test to assess differences in medians among groups. The test indicated that at least one group differed significantly from the others, and Dunn's multiple comparisons test was subsequently conducted to determine pairwise differences. The results showed that the high-fat diet group had significantly higher paw swelling scores compared to both the normal control group and the *Z. zerumbet* group, whereas no significant difference was observed between the normal control group and the *Z. zerumbet* group (Figure 7).

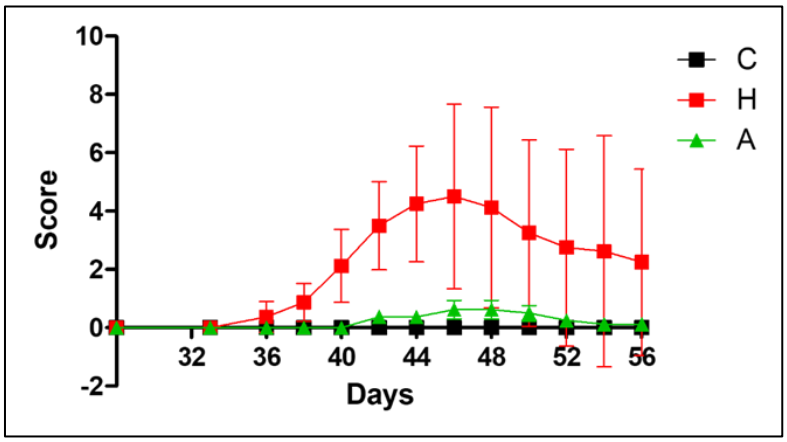

Figure 7 Paw swelling scores in each group. After induction of rheumatoid arthritis, the three non-injected paws were scored according to the paw swelling criteria. Data are presented as mean ± SD (n = 8 per group). Statistical analysis was performed using the Kruskal-Wallis test to assess differences among groups. Groups: the normal control group (C), the high-fat diet group (H), and *Zingiber zerumbet* group (A)

4. Discussion

Blood lipids refer to the content of adipose elements such as free fatty acids, phospholipids, sterols, and triglycerides in plasma. The healthy value of blood lipid content in plasma is generally below 130 mg/dL. Higher than 160 mg/dL is called excessive blood fat in medicine, that is also called hyperlipidemia. Hyperlipidemia, hypertension, and hyperglycemia are together called the "three highs", and are known as the "invisible killer" of health. They are one of the main diseases that require health management of chronic diseases [22-24].

With the improvement of living levels, there are more and more opportunities for exquisite diet. Our blood lipid value is also getting higher and higher, and there is a trend of high blood lipid value for younger people. However, many people think that this is not a disease, so they usually ignore it. If hyperlipidemia is not controlled in time, it will cause complications, and these complications are usually very serious [25-26].

Hyperlipidemia may cause many complicated disease as coronary heart disease (CHD), cerebral infarction, diabetes, fatty liver etc. The CHD is coronary atherosclerotic heart disease, and once excessive blood lipids block the coronary arteries, causing arteriosclerosis, myocardial ischemia and hypoxia. Hyperlipidemia is one of the most dangerous factors causing CHD. It was verified that an average 1% decrease of total cholesterol in serum will reduce 2% incidence of CHD. Therefore, regulating blood lipids is the most basic treatment for preventing and treating CHD. In addition, the excessive cholesterol in the blood can cause atherosclerotic plaques, resulting in narrowing and blockage of the arterial lumen. When this situation occurs in the cerebral blood vessels, it will cause cerebral infarction. A number of studies have proved that the incidence and disability rates of cerebral apoplexy are significantly related to the effect of long-term lipid-lowering treatment. Among the factors that cause stroke, such as drinking, obesity, high blood pressure, smoking, diabetes and so on. Hyperlipidemia is one of the most important influencing factors [22-28].

Hypertension, hyperlipidemia, and hyperglycemia are collectively referred to as the "three highs", which pose a serious threat to the life and health of diabetic patients. Hyperlipidemia will aggravate the patient's condition. Therefore, clinically, while treating hyperglycemia, a certain amount of blood lipid regulation will also be carried out. Moreover, hyperlipidemia is an important cause of late complications of diabetes, such as CHD, fundus necrosis, kidney disease,

neuropathy, etc. Active treatment of hyperlipidemia can effectively prevent the occurrence of these complications. Moreover, the appearance of fatty liver is mainly caused by the accumulation of fat in the liver, and patients often have hyperlipidemia. Hyperlipidemia, long-term heavy drinking, obesity, diabetes, abdominal fat accumulation, and patients with viral hepatitis are all high-risk groups for fatty liver. Therefore, regulating blood lipids in patients with hyperlipidemia is an important measure to prevent fatty liver [22-28].

RA is an autoimmune disease characterized by long-term autoimmune dysregulation involving multiple factors. It exhibits a chronic and relapsing nature [29]. Although the exact etiology of RA remains unclear, studies have highlighted a strong association with environmental factors, such as pollution and lifestyle habits [30-31], in addition to genetic predisposition and aging. Beyond joint-related symptoms, RA is also associated with an increased risk of comorbidities, including stroke and cardiovascular diseases. In patients with RA, approximately 70% develop lesions in the hands, while lesions in the feet occur in up to 90% of cases. These lesions typically appear symmetrically and are often accompanied by the formation of rheumatoid nodules. If left uncontrolled, disease progression can lead to severe joint deformity and malalignment [32]. Current RA treatment strategies primarily include pharmacological and surgical interventions. The pharmacological agents commonly used comprise nonsteroidal anti-inflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs (DMARDs), corticosteroids, and biologics such as Enbrel® (etanercept) and ILARIS® (canakinumab) [33-34]. However, these medications are often associated with significant adverse effects, particularly those containing corticosteroids. While such drugs may alleviate joint discomfort, their strong side effects impose additional health burdens. Moreover, although biologics are effective, their high treatment costs limit accessibility for many patients. Therefore, developing novel therapeutic agents for RA is of great significance.

In this study, the results were shown that

- **Body weight changes**: In the early phase of the experiment, body weight increased steadily in all groups. By week 4, the high-fat diet group and the *Z. zerumbet* group exhibited significantly higher body weights compared to the normal control group. After week 4, as complete Freund's adjuvant was administered to induce rheumatoid arthritis in the experimental groups, weight gain slowed in all groups, and no significant differences were observed thereafter.
- **Food intake**: There was no significant difference in food intake between the high-fat diet group and the *Z. zerumbet* group, indicating that the test substance did not affect feeding behavior.
- **Serum TCHO levels**: Throughout the experiment, the high-fat diet group exhibited significantly higher serum TCHO levels compared to both the normal control group and the *Z. zerumbet* group. Although the *Z. zerumbet* group showed slightly elevated TCHO levels compared to the normal control group in some weeks, they remained consistently lower than those of the high-fat diet group, demonstrating a lipid-lowering effect of *Z. zerumbet*.
- **Serum biochemical analysis**: The high-fat diet elevated the AST/ALT ratio, which is commonly regarded as an indicator of liver damage. *Z. zerumbet* administration improved liver function by reducing the AST/ALT ratio. Additionally, both LDL-C and TCHO levels were significantly higher in the high-fat diet group than in the normal control group and the *Z. zerumbet* group, indicating that *Z. zerumbet* effectively reduced both LDL cholesterol and TCHO.
- **RA-related indices**: The paw swelling volume and clinical scores demonstrated that inflammatory responses were most severe in the high-fat diet group. In contrast, the *Z. zerumbet* group significantly reduced paw swelling and clinical scores, with values approaching those of the normal control group, suggesting an anti-inflammatory potential of *Z. zerumbet*.

5. Conclusion

According to the experimental results, soy milk enriched with red ball ginger (*Z. zerumbet*) extracts shows potential for lowering blood lipids and alleviating RA in the high-fat diet-induced rat models. In terms of lipid regulation, the *Z. zerumbet* group significantly reduced TCHO and LDL cholesterol levels, approaching those of the normal control group, indicating a hypolipidemic effect. Regarding liver function, the *Z. zerumbet* group attenuated the elevated AST/ALT ratio induced by the high-fat diet, suggesting a hepatoprotective effect. In addition, the *Z. zerumbet* group significantly reduced paw swelling and arthritis clinical scores to levels comparable to the normal control group, demonstrating notable anti-inflammatory potential. Taken together, these findings indicate that soy milk enriched with red ball ginger (*Z. zerumbet*) extracts not only reduces circulating LDL-C and mitigates high-fat diet-induced liver damage, but also exerts anti-inflammatory effects in a RA model. Therefore, *Z. zerumbet*-enriched soy milk may be considered a promising functional food candidate for modulating blood lipids and alleviating RA symptoms.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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