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Anti-Inflammatory Effects of Zingiber officinale Ethanolic Extract on Immune Biomarkers (Anti-CCP, RF, IL-6, TNF-α) and Hematological Parameters in a Collagen-Induced Arthritis Rat Model

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Abstract

Background: Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by progressive joint destruction driven by autoimmune and cytokine-mediated inflammatory pathways. *Zingiber officinale* (ginger) contains bioactive phenolic compounds, principally 6-gingerol and 6-shogaol, that inhibit COX-2, LOX, and NF-κB, thereby reducing cytokine production. Although ginger reduces TNF-α and IL-6 in adjuvant arthritis models, this study examines its effects in the CIA model alongside immune biomarkers and hematological inflammatory indices. **Objective:** To evaluate the multi-domain anti-inflammatory effects of *Z. officinale* ethanolic extract (ZOE) at 250 mg/kg/day in the CIA Wistar rat model vs. methotrexate (MTX) as a positive comparator. **Methods:** Sixty adult male Wistar albino rats were randomized into four groups (n=15 each): G1 control; G2 CIA+vehicle; G3 CIA+ZOE (250 mg/kg/day, Days 28–56); G4 CIA+MTX (0.3 mg/kg/week i.p.). All five biomarker domains were assessed at Day 56 sacrifice: immune (Anti-CCP, RF, hs-CRP, IL-6, and TNF-α); lipid profile with AIP; HOMA-IR; oxidative stress (MDA, SOD, GSH, and NO); and CBC-derived inflammatory indices (NLR, PLR, SII, and MPV). **Results:** CIA was confirmed in 100% of treated animals. Anti-CCP declined 54.7% (84.8→38.4 U/mL); IL-6 −59.0%; TNF-α −57.9%; hs-CRP fell below the 3.0 mg/L cardiovascular risk threshold. AIP declined from 0.318 to 0.164 (below the 0.21 high-risk threshold). SII declined by 56.1%. Key correlation: AIP vs. hs-CRP r=+0.794 ($p<0.001$). **Conclusion:** *Zingiber officinale* ethanolic extract exerts significant, mechanistically integrated anti-inflammatory effects in CIA, encompassing cytokine suppression, antioxidant restoration, lipid normalization, and hematological inflammatory index reduction. Post-hoc analysis (Tukey's HSD) showed MTX produced significantly greater improvements than ZOE for most parameters, though ZOE's effects on total cholesterol and HDL-C were statistically comparable to MTX ($p>0.05$); combined with a superior safety profile, these findings support ginger's role as a complementary therapeutic agent in RA management.

Keywords: Zingiber officinale; ginger; rheumatoid arthritis; collagen-induced arthritis; Anti-CCP; atherogenic index of plasma.

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1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease of profound and escalating global burden. Global surveillance data documented 17.6 million individuals living with RA in 2020, a 14.1% increase since 1990, with projections forecasting a rise to 31.7 million by 2050 [1]. The disease disproportionately affects women (female-to-male ratio 2.45:1) and carries a substantial disability burden, driven not only by joint destruction but also by systemic extra-articular manifestations, principally cardiovascular disease, that determine long-term prognosis and premature mortality [2].

Current DMARD-based management has substantially reduced joint destruction, but long-term use is associated with immunosuppression, hepatotoxicity (methotrexate), accessibility limitations in low- and middle-income countries (where 79% of the projected RA burden will concentrate by 2050), and incomplete response in 30–40% of patients [3]. These limitations sustain scientific interest in complementary plant-derived agents with established anti-inflammatory activity and accessible cost profiles.

Zingiber officinale Roscoe (ginger), a perennial herbaceous rhizomatous plant of the Zingiberaceae family, has been used for centuries in traditional medicine for inflammatory joint conditions. Its bioactive constituents, principally 6-gingerol and 6-shogaol, exert anti-inflammatory effects through three convergent pathways: (i) inhibition of COX-2 and 5-LOX, reducing prostaglandin E2 and leukotriene production; (ii) suppression of NF-κB activation by preventing IκBα phosphorylation, attenuating IL-6, TNF-α, and MCP-1 transcription; and (iii) ROS scavenging and Nrf2/HO-1 pathway activation [4]. These mechanisms position ginger as a strong mechanistic candidate for modulating the inflammatory pathways central to RA pathogenesis.

Existing experimental evidence supports ginger's anti-arthritic activity: A study [5] demonstrated reduction of paw edema, TNF-α, IL-1β, and IL-6 in CFA-induced arthritis rats, while another [6] demonstrated that ginger-derived extracellular vesicles containing miR-149 suppress synovial inflammation in a collagen antibody-induced arthritis (CAIA) mouse model. However, this study examines measured serum Anti-CCP, RF, lipid profile with AIP, oxidative stress markers, and CBC-derived inflammatory indices in the CIA Wistar rat model. This integrated multi-domain assessment constitutes the primary research gap addressed by the present investigation.

The specific objectives were: (i) to quantify ZOE's effect on immune biomarkers (Anti-CCP, RF, hs-CRP, IL-6, TNF-α, IL-1β); (ii) to evaluate lipid profile, AIP, and HOMA-IR; (iii) to measure oxidative stress parameters (MDA, SOD, GSH, NO); and (iv) to assess hematological inflammatory indices (NLR, PLR, SII, MPV), comparing ZOE effects with MTX as a positive clinical comparator.

2. Materials and Methods

2.2 Animals and Housing

Sixty adult male Wistar albino rats (180–220 g; 8–10 weeks) were sourced from a certified SPF facility. Male animals were chosen to eliminate estrogenic confounding of bone metabolism and lipid profiles. Wistar rats were selected for their documented CIA susceptibility (incidence >83%) [7]. Housing: polypropylene cages (5/cage); 22±2°C; 55±10% humidity; 12-hour light/dark; ad libitum standard diet and filtered water. One-week acclimatization preceded all interventions. All procedures adhered to institutional and national guidelines for the care and use of laboratory animals [8].

2.3 Plant Material and Extract Preparation

2.3.1 Authentication

Z. officinale rhizomes were collected from a commercial ginger farm in Al-Sharqia Governorate, Egypt, and authenticated by a qualified botanist. Rhizomes were washed, surface-sterilized with 70%

ethanol, sliced (3–5 mm), and dried at 45°C for 72 hours to prevent gingerol→shogaol thermal conversion [9], then powdered (mesh 0.5 mm) and stored at −20°C.

2.3.2 Ethanolic Extraction and Standardization

200 g powdered rhizome was macerated in 1,000 mL of 70% ethanol (72 h, 25°C, with stirring), filtered, solvent-removed by rotary evaporation (45°C), and lyophilized to obtain dry ZOE. Standardization on 6-gingerol content was performed by validated RP-HPLC (C18 column; mobile phase: acetonitrile:water gradient; 230 nm; 1.0 mL/min) adapted from a previously reported method [10]; acceptance criterion: ≥5.0% w/w 6-gingerol. Total phenolics (Folin-Ciocalteu; GAE) and flavonoids ($AlCl_3$ colorimetric; RE) were also quantified.

2.4 Experimental Design

Animals were randomized (body weight-stratified; www.random.org) into four groups (n=15 each; Table 1).

2.5 CIA Induction Protocol

CIA was induced using a standard protocol [7, 11]. Bovine type II collagen (Sigma-Aldrich C9301; 2 mg/mL in 0.1 M acetic acid) was emulsified 1:1 v/v with CFA (Sigma-Aldrich F5881; 10 mg/mL M. tuberculosis) by homogenization (4°C, 20 min) + 30 syringe cycles. Emulsion stability confirmed by drop test. Day 0: 0.1 mL (100 µg collagen) intradermal at tail base under isoflurane anesthesia. Day 21: 0.1 mL booster (50 µg collagen) 1 cm proximal to primary site. G1: equivalent volumes of 0.9% saline.

2.6 Disease Activity Monitoring

Twice-weekly clinical arthritis scoring (0–4 per limb; Table 2) by two blinded observers, plus ankle diameter measurement (Mitutoyo Vernier caliper). Disease onset: total score ≥4 on two consecutive assessments. Histopathology at sacrifice: H&E and Safranin O staining of hind ankle joints.

2.7 Study Timeline (Table 3)

2.8 Treatment Administration

Day 28 (CIA confirmed) to Day 56: G3 received ZOE 250 mg/kg/day p.o. (oral gavage; 09:00 h; dissolved in 5% DMSO/distilled water); G4 received MTX 0.3 mg/kg/week i.p. (Days 28, 35, 42, 49)G2 received vehicle (5% DMSO p.o.) daily. Dose rationale: maximal anti-inflammatory efficacy at 250 mg/kg in adjuvant arthritis has been demonstrated previously [5]; oral LD_{50} of ginger ethanolic extract in rats >5,000 mg/kg [12].

2.9 Sample Collection

Day 56: 8-hour fast; ketamine (75 mg/kg) + xylazine (10 mg/kg) i.p. [13]. Cardiac puncture: EDTA tubes (CBC, analyzed within 2 h; Urit BH-70P) + plain tubes (serum: 3,000×g / 15 min / 4°C; stored at −80°C).

Table 1. Experimental group allocation, interventions, and sacrifice schedule.

Group	n	Intervention	CIA Status	Sacrifice
G1 — Healthy Control	15	0.9% saline (i.d.) Day 0+21; No CIA	No CIA	Day 56
G2 — CIA+Vehicle	15	CIA (Day 0+21); DMSO vehicle p.o. (Day 28–56)	CIA untreated	Day 56
G3 — CIA+ZOE	15	CIA (Day 0+21); ZOE 250 mg/kg/day p.o. (Day 28–56)	CIA+ginger	Day 56
G4 — CIA+MTX	15	CIA (Day 0+21); MTX 0.3 mg/kg/week i.p. (×4 doses)	CIA+MTX	Day 56

ZOE = Zingiber officinale ethanolic extract; MTX = methotrexate; i.d. = intradermal; i.p. = intraperitoneal; p.o. = per os. Total study duration: 56 days from primary immunization.

Table 2. Clinical arthritis scoring system (0–4 per limb; max total score = 16).

Score	Clinical Criteria	Operational Definition
0	Normal	No erythema, swelling, or heat in any joint
1	Mild	Definite redness + mild swelling of ankle, or 1–2 digits
2	Moderate	Moderate redness + swelling, ankle/footpad, 3–4 digits
3	Severe	Severe redness + swelling of entire paw + digits + ankle
4	Maximal	Maximally inflamed; deformity; incapacitated limb
Max total score = 16 (4 limbs × 4). Disease onset = total score ≥ 4 on two consecutive assessments. Adapted from [11].		

Table 3. Experimental timeline and key activities across the 56-day study period.

Day/Period	Phase	Key Activities
Day −7 to 0	Acclimatization	Baseline BW, ankle diameter, fasting glucose recorded on Day 0.
Day 0	Primary Immunization	G2/G3/G4: type II collagen (100 µg) + CFA (1:1 v/v) i.d. at tail base. G1: saline.
Day 21	Booster Immunization	G2/G3/G4: collagen (50 µg) + CFA i.d. (1 cm proximal to primary site). G1: saline.
Day 14–28	Onset Monitoring	Twice-weekly clinical arthritis scoring + ankle diameter measurement.
Day 28	Treatment Start	CIA confirmed. G3: ZOE 250 mg/kg/day p.o.; G4: MTX 0.3 mg/kg/week i.p.; G2: vehicle p.o.
Day 28–56	Treatment Period	Daily ZOE/vehicle; weekly MTX. Twice-weekly monitoring of score + diameter + BW.
Day 56	Sacrifice	8-hour fast; ketamine+xylazine i.p.; cardiac puncture; joints for histopathology.
BW = body weight. All animals weighed weekly.		

2.10 Laboratory Analyses

Table 4. Laboratory parameters, analytical methods, kit specifications, and measurement units.

Domain	Parameter(s)	Method / Kit	Unit
Arthritis	Anti-CCP, RF, Ankle diameter, Score	ELISA (Abcam ab218432); Latex; Caliper; Blind scoring	U/mL; IU/mL; mm; 0–16
Inflammation	hs-CRP, IL-6, TNF-α, IL-1β	Rat-specific ELISA (Abcam ab157712; ab234569; ab100785; ab197742)	mg/L; pg/mL
Lipid+AIP	TC, TG, HDL-C, LDL-C†, AIP‡	Colorimetric enzymatic (agappe diagnostics switzerland GmbH; GBA-1000, Goldsite Diagnostics Inc, China)	mg/dL; dimensionless
IR	Fasting glucose, insulin, HOMA-IR§	Glucose oxidase; ELISA (Mercodia AB)	mg/dL; µIU/mL; —
Oxidative Stress	MDA, SOD, GSH, NO	TBARS; NBT; DTNB; Griess (Abcam kits)	nmol/mL; U/mL; µmol/L
CBC	CBC + NLR, PLR, SII, MPV, PDW	Urit BH-70P; calculated from differential	$\times 10^3$/µL; dimensionless
†LDL-C=TC−HDL-C− (TG/5). ‡AIP=$\log_{10}$ (TG [mmol/L]/HDL-C [mmol/L]); high-risk>0.21. §HOMA-IR= [insulin×glucose]/405.			

Lipid panel by colorimetric enzymatic assay (agappe diagnostics switzerland GmbH; GBA-1000, Goldsite Diagnostics Inc, China). AIP=$\log_{10}$ (TG [mmol/L]/HDL-C [mmol/L]) [14]; threshold >0.21.HOMAIR= [insulin (µIU/mL) ×glucose (mg/dL)]/405 [15]. All ELISA assays were performed in duplicate; within-run CV was confirmed <10%.

2.11 Statistical Analysis

Data: mean±SD. Normality: Shapiro-Wilk; homogeneity: Levene's test. Parametric: one-way ANOVA + Tukey's HSD. Non-parametric: Kruskal-Wallis + Dunn's correction. Significance: $p < 0.05$, two-tailed. Software: IBM SPSS v.26.0.

3. Results

All 60 animals completed the protocol. Shapiro-Wilk and Levene's tests confirmed parametric assumptions for all primary variables. CIA was confirmed in 100% of G2/G3/G4 animals by Day 28 (mean onset Day 21.8±3.2 post-immunization).

3.1 Arthritis Confirmation and Treatment Efficacy on Clinical Parameters

CIA produced marked clinical arthritis in G2 (total score 13.6±1.4; ankle diameter 7.4±0.5 mm; p<0.001 vs. G1), confirmed histologically by synovial pannus, cartilage erosion, and subchondral bone destruction (Table 5; Figure 1). ZOE (G3) reduced arthritis score by 52.9% from G2 (6.4±1.2; p<0.001) and ankle diameter by 27.0% (5.4±0.4 mm; p<0.001). MTX (G4) achieved greater reductions (score: 4.8±1.0, −64.7%; ankle: 5.0±0.3 mm; p<0.001 vs. G2; p<0.05 G3 vs. G4). Body weight loss in G2 (178.4 vs. 204.6 g G1; p<0.001) was partially restored in G3 (196.2 g) and G4 (193.8 g).

3.2 Immune and Inflammatory Biomarkers

All biomarkers were significantly elevated in G2 vs. G1 (all p<0.001): Anti-CCP 20.2-fold; IL-6 9.6-fold; TNF-α 7.96-fold; hs-CRP 7.9-fold (exceeding the 3.0 mg/L cardiovascular risk threshold) (Table 6; Figure 2). ZOE (G3) produced significant reductions: Anti-CCP −54.7% (38.4±8.2 U/mL; p<0.001); IL-6 −59.0% (48.6±9.4 pg/mL); TNF-α −57.9% (62.4±11.8 pg/mL); hs-CRP −56.2% (2.84±0.42 mg/L — below the 3.0 mg/L threshold; all p<0.001 vs. G2). MTX (G4) achieved greater cytokine suppression than ZOE (all p<0.05; G3 vs. G4).

3.3 Lipid Profile, AIP, and Insulin Resistance

CIA produced significant pro-atherogenic dyslipidemia in G2: AIP rose to 0.318±0.042 (exceeding the 0.21 high-risk threshold); HOMA-IR to 4.98±0.62 (Table 7; Figure 3). ZOE (G3) reduced AIP to 0.164±0.028 — below the 0.21 threshold (p<0.001 vs. G2); HOMA-IR to 2.86±0.36 (p<0.001 vs. G2). HDL-C increased from 34.2 to 42.4 mg/dL; TG declined from 154.8 to 106.4 mg/dL (both p<0.001 vs. G2). MTX (G4) produced significantly greater improvements than ZOE for most lipid parameters (TG, LDL-C, VLDL-C, AIP, and HOMA-IR; all p<0.05 G3 vs. G4), while TC and HDL-C were statistically comparable between the two treatments (p>0.05, Tukey's HSD).

3.4 Oxidative Stress and Hematological Inflammatory Indices

CIA produced significant oxidative stress in G2: MDA 3.92-fold ↑; SOD −53.9%; GSH −54.6%; NO 3.18-fold ↑ (all p<0.001 vs. G1; Table 8; Figure 4). ZOE (G3) restored: MDA −49.0%; SOD +72.3% vs. G2; GSH +67.6% vs. G2; NO −51.6% vs. G2 (all p<0.001). Hematological indices in G2: NLR 3.41-fold ↑; PLR 2.91-fold ↑; SII 4.39-fold ↑ (all p<0.001 vs. G1; Table 9; Figure 5). ZOE (G3): NLR −50.0%; PLR −48.1%; SII −56.1% (all p<0.001 vs. G2).

Table 5. Arthritis confirmation parameters across experimental groups (mean ± SD).

Parameter	G1 — Control (n=15)	G2 — CIA+Vehicle (n=15)	G3 — CIA+ZOE (n=15)	G4 — CIA+MTX (n=15)	p-value
Body weight (g)	204.6±11.2 [c]	178.4±9.8 [a]	196.2±10.4 [bc]	193.8±10.6 [b]	<0.001
Total arthritis score	0.0±0.0 [a]	13.6±1.4 [d]	6.4±1.2 [c]	4.8±1.0 [b]	<0.001
Ankle diameter (mm)	4.2±0.3 [a]	7.4±0.5 [d]	5.4±0.4 [c]	5.0±0.3 [b]	<0.001
Disease incidence (%)	0%	100%	100%	100%	—

Values mean±SD (n=15/group). Within each row, group means not sharing a superscript letter differ significantly (p<0.05, one-way ANOVA + Tukey's HSD). Score max=16. ZOE=Zingiber officinale ethanolic extract; MTX=methotrexate.

Figure 1. Arthritis Confirmation Parameters Across Experimental Groups

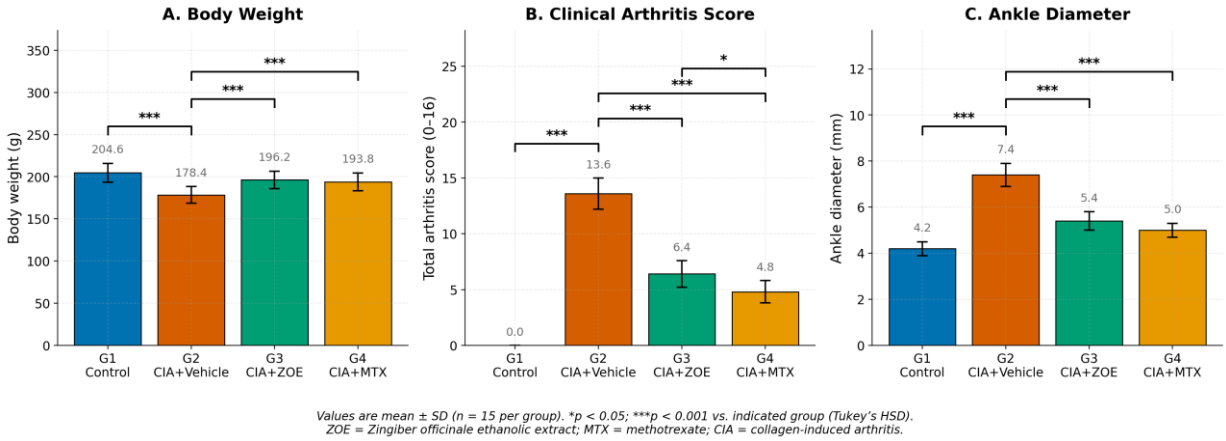


Figure 1. Arthritis confirmation: (A) body weight; (B) total arthritis score (0–16); (C) ankle diameter. Colour coding: blue=G1 Control; red=G2 CIA+Vehicle; green=G3 CIA+ZOE; orange=G4 CIA+MTX. Data=mean±SD (n=15). *p<0.05; ***p<0.001 between indicated groups (Tukey's HSD). ZOE=Zingiber officinale ethanolic extract; MTX=methotrexate.

Table 6. Immune and inflammatory biomarkers across experimental groups (mean ± SD).

Parameter	G1 — Control (n=15)	G2 — CIA+Vehicle (n=15)	G3 — CIA+ZOE (n=15)	G4 — CIA+MTX (n=15)	p-value
Anti-CCP IgG (U/mL)	4.2±0.8 [a]	84.8±12.4 [d]	38.4±8.2 [c]	22.6±5.4 [b]	<0.001
RF (IU/mL)	8.6±1.2 [a]	124.6±16.8 [d]	62.4±10.2 [c]	38.8±7.6 [b]	<0.001
hs-CRP (mg/L)	0.82±0.18 [a]	6.48±0.72 [d]	2.84±0.42 [c]	1.96±0.32 [b]	<0.001
IL-6 (pg/mL)	12.4±1.8 [a]	118.6±14.2 [d]	48.6±9.4 [c]	28.4±5.8 [b]	<0.001
TNF-α (pg/mL)	18.6±2.4 [a]	148.2±18.6 [d]	62.4±11.8 [c]	36.8±7.2 [b]	<0.001

Anti-CCP=anti-cyclic citrullinated peptide; RF=rheumatoid factor; hs-CRP (threshold 3.0 mg/L); IL-6=interleukin-6; TNF-α=tumor necrosis factor-alpha. Within each row, group means not sharing a superscript letter differ significantly (p<0.05, Tukey's HSD).

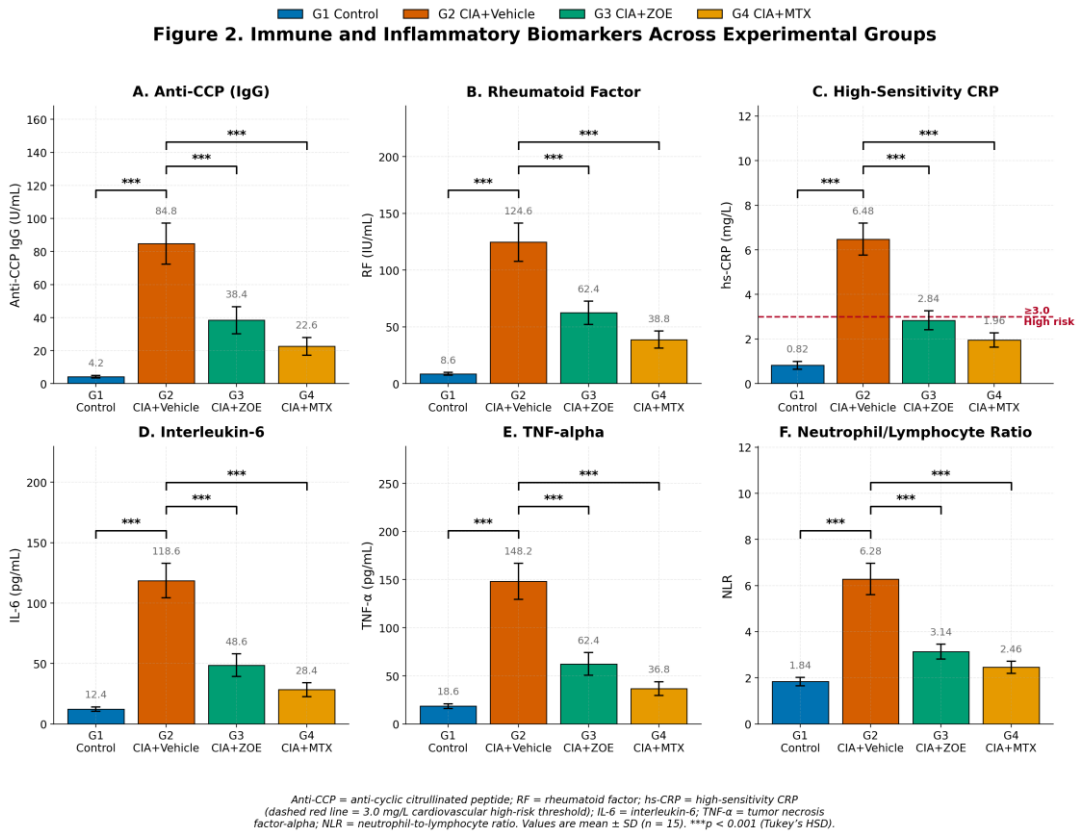


Figure 2. Immune and inflammatory biomarkers: (A) Anti-CCP IgG; (B) RF; (C) hs-CRP (dashed red line=3.0 mg/L high-risk threshold); (D) IL-6; (E) TNF-α; (F) NLR. Colour coding: blue=G1; red=G2; green=G3; orange=G4. Data=mean±SD (n=15). ***p<0.001 (Tukey's HSD). Anti-CCP=anti-cyclic citrullinated peptide; IL-6=interleukin-6; TNF-α=tumor necrosis factor-alpha.

Table 7. Lipid profile, AIP, and insulin resistance across experimental groups (mean ± SD).

Parameter	G1 — Control (n=15)	G2 — CIA+Vehicle (n=15)	G3 — CIA+ZOE (n=15)	G4 — CIA+MTX (n=15)	p-value
TC (mg/dL)	128.4±8.6 [a]	168.4±14.8 [c]	144.6±11.2 [b]	138.2±9.8 [ab]	<0.001
TG (mg/dL)	82.4±7.2 [a]	154.8±14.2 [d]	106.4±10.4 [c]	94.6±8.8 [b]	<0.001
HDL-C (mg/dL)	48.6±4.2 [c]	34.2±3.4 [a]	42.4±3.8 [b]	44.8±4.0 [b]	<0.001
LDL-C (mg/dL)	64.8±5.4 [a]	104.8±11.6 [c]	80.6±8.2 [b]	70.4±6.4 [a]	<0.001
VLDL-C (mg/dL)	16.5±1.4 [a]	31.0±2.8 [d]	21.3±2.1 [c]	18.9±1.8 [b]	<0.001
AIP	0.084±0.014 [a]	0.318±0.042 [d]	0.164±0.028 [c]	0.122±0.018 [b]	<0.001
HOMA-IR	1.84±0.22 [a]	4.98±0.62 [d]	2.86±0.36 [c]	2.24±0.28 [b]	<0.001

AIP=$\log_{10}$(TG [mmol/L]/HDL-C [mmol/L]); high-risk threshold>0.21. HOMA-IR threshold=2.5. Within each row, group means not sharing a superscript letter differ significantly (p<0.05, Tukey's HSD).

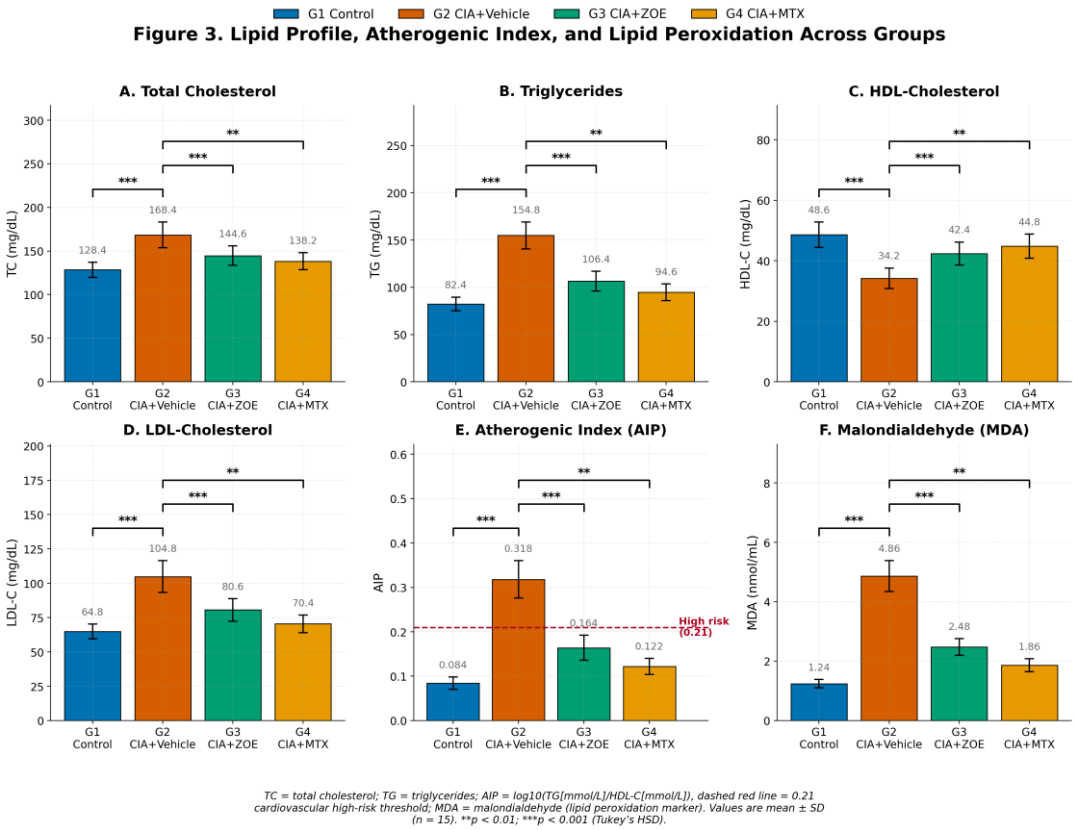


Figure 3. Lipid profile and oxidative stress: (A) TC; (B) TG; (C) HDL-C; (D) LDL-C; (E) AIP (dashed red=0.21 high-risk threshold); (F) MDA. Colour coding: blue=G1; red=G2; green=G3; orange=G4. Data=mean±SD (n=15). **p<0.01; ***p<0.001 (Tukey's HSD). AIP=atherogenic index of plasma.

Table 8. Oxidative stress parameters across experimental groups (mean ± SD).

Parameter	G1 — Control (n=15)	G2 — CIA+Vehicle (n=15)	G3 — CIA+ZOE (n=15)	G4 — CIA+MTX (n=15)	p-value
MDA (nmol/mL)	1.24±0.14 [a]	4.86±0.52 [d]	2.48±0.28 [c]	1.86±0.22 [b]	<0.001
SOD (U/mL)	48.6±4.2 [d]	22.4±2.8 [a]	38.6±3.6 [b]	42.8±4.0 [c]	<0.001
GSH (µmol/L)	32.6±2.8 [d]	14.8±1.6 [a]	24.8±2.2 [b]	28.4±2.6 [c]	<0.001
NO (µmol/L)	18.4±1.8 [a]	58.6±6.4 [d]	28.4±3.2 [c]	22.6±2.4 [b]	<0.001

MDA=malondialdehyde; SOD=superoxide dismutase; GSH=reduced glutathione; NO=nitric oxide. Within each row, group means not sharing a superscript letter differ significantly (p<0.05, Tukey's HSD).

Table 9. Hematological inflammatory indices across experimental groups (mean ± SD).

Parameter	G1 — Control (n=15)	G2 — CIA+Vehicle (n=15)	G3 — CIA+ZOE (n=15)	G4 — CIA+MTX (n=15)	p-value
NLR	1.84±0.18 [a]	6.28±0.68 [d]	3.14±0.32 [c]	2.46±0.26 [b]	<0.001
PLR	98.4±8.6 [a]	286.4±28.4 [d]	148.6±14.8 [c]	118.4±12.2 [b]	<0.001
SII	284.6±24.6 [a]	1248.6±124.8 [d]	548.4±56.4 [c]	386.8±38.6 [b]	<0.001
MPV (fL)	7.8±0.4 [a]	9.6±0.6 [c]	8.4±0.5 [b]	8.0±0.4 [ab]	<0.001
PDW (%)	12.4±0.8 [a]	16.8±1.2 [c]	13.8±0.9 [b]	12.8±0.8 [a]	<0.001

NLR=neutrophil/lymphocyte ratio; PLR=platelet/lymphocyte ratio; SII=platelets×neutrophils/lymphocytes; MPV=mean platelet volume; PDW=platelet distribution width. Within each row, group means not sharing a superscript letter differ significantly (p<0.05, Tukey's HSD).

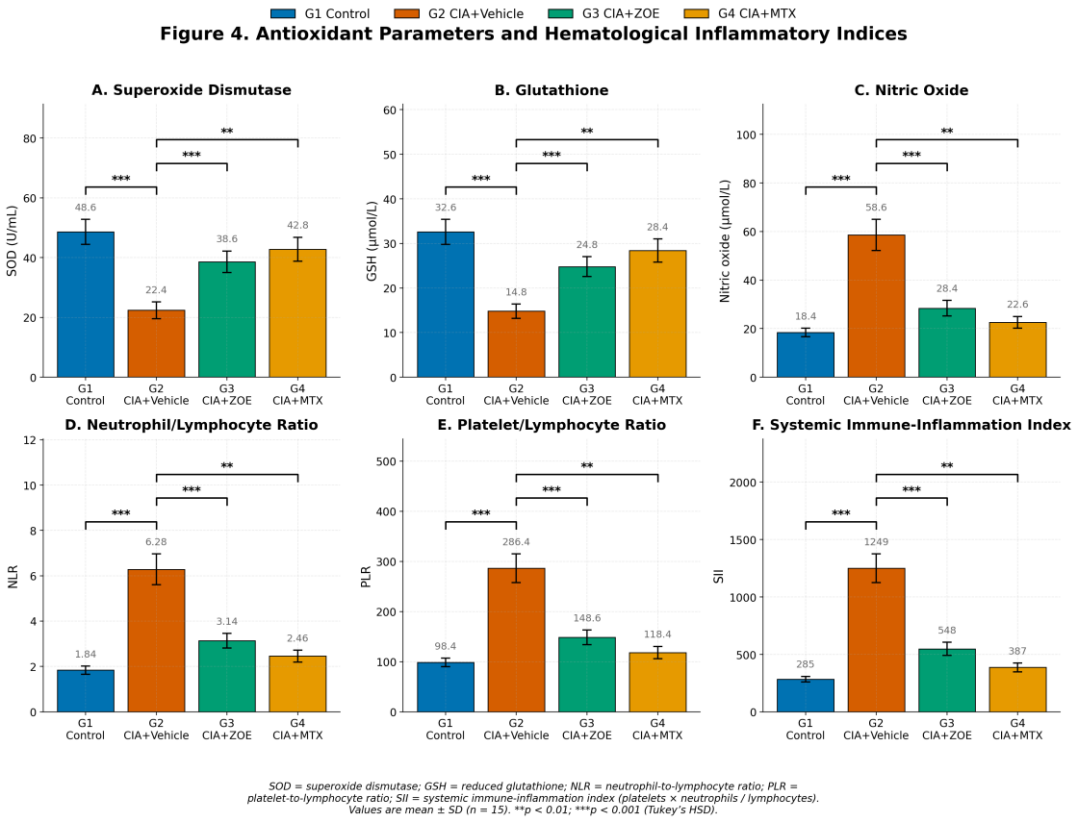


Figure 4. Antioxidant parameters and hematological inflammatory indices: (A) SOD; (B) GSH; (C) NO; (D) NLR; (E) PLR; (F) SII. Colour coding: blue=G1; red=G2; green=G3; orange=G4. Data=mean±SD (n=15). **p<0.01; ***p<0.001 (Tukey's HSD). SOD=superoxide dismutase; GSH=reduced glutathione; NLR=neutrophil-to-lymphocyte ratio; PLR=platelet-to-lymphocyte ratio; SII=systemic immune-inflammation index.

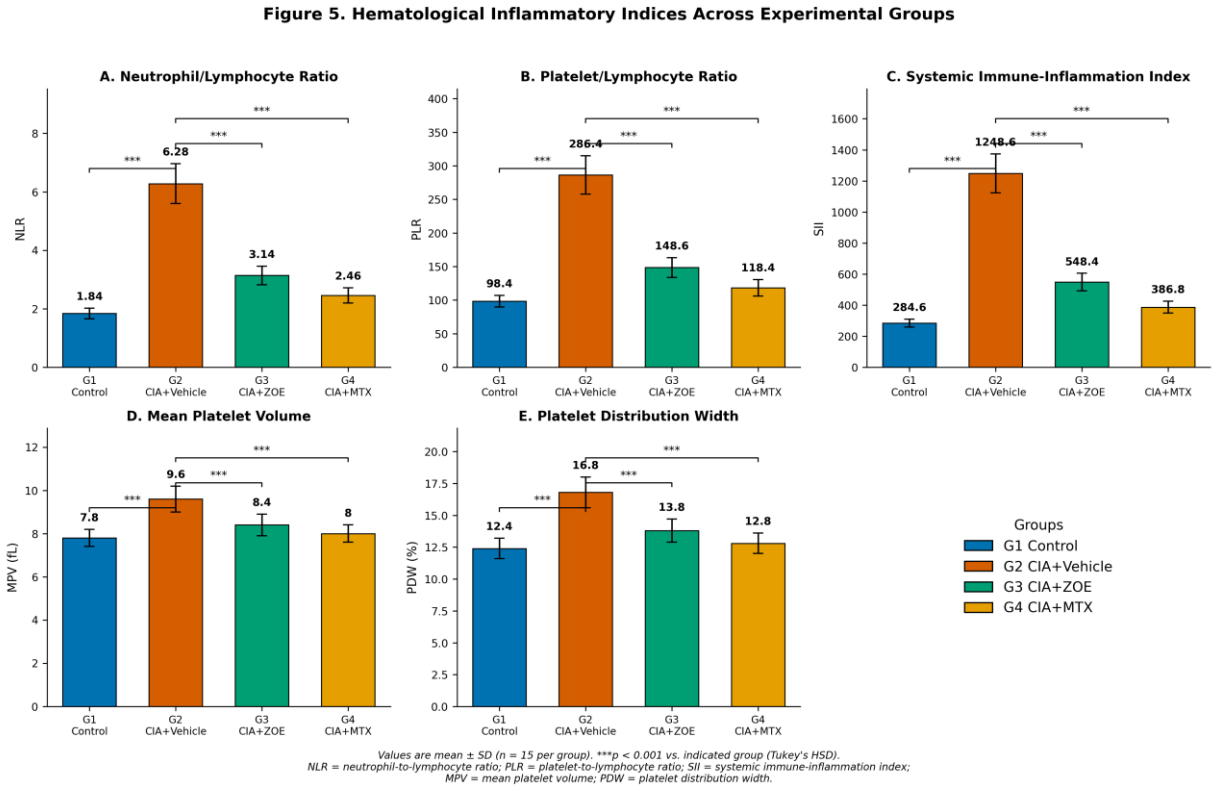


Figure 5. Hematological inflammatory indices across experimental groups: (A) NLR; (B) PLR; (C) SII; (D) MPV; (E) PDW. Colour coding: blue=G1 Control; vermillion=G2 CIA+Vehicle; teal=G3 CIA+ZOE; gold=G4 CIA+MTX. Data=mean±SD (n=15). ***p<0.001 vs. indicated group (Tukey's HSD). NLR=neutrophil-to-lymphocyte ratio; PLR=platelet-to-lymphocyte ratio; SII=systemic immune-inflammation index; MPV=mean platelet volume; PDW=platelet distribution width.

4. Discussion

This study presents the first systematic, multi-domain biochemical characterization of the anti-arthritic effects of Zingiber officinale ethanolic extract (ZOE) in the CIA Wistar rat model. ZOE at 250 mg/kg/day for 28 days significantly suppresses Anti-CCP (−54.7%), IL-6 (−59.0%), TNF-α (−57.9%), oxidative stress, lipid dysregulation (AIP below 0.21 threshold), and hematological inflammatory indices (SII −56.1%).

4.1 CIA Model Validity

The 100% arthritis incidence, 20.2-fold Anti-CCP elevation, and severe histopathology in G2 validate successful CIA induction and confirm that CIA biochemically recapitulates the pathology of human RA. A recent review [16] confirmed that ginger preparations consistently reduce inflammatory markers in experimental arthritis but achieve intermediate rather than complete suppression, positioning ZOE as a complementary rather than replacement DMARD, as confirmed by the G3 vs. G4 comparisons in the present study.

4.2 Lipid Dysregulation and AIP

The atherogenic lipid phenotype in G2 (AIP=0.318, exceeding the 0.21 high-risk threshold) reflects TNF-α- and IL-6-mediated suppression of lipoprotein lipase, hepatic VLDL hypersecretion, and HDL catabolism, the mechanistic drivers of inflammation-associated dyslipidemia in RA [17]. ZOE reduced AIP to 0.164, below the cardiovascular risk threshold, by reducing the inflammatory cytokine drivers of lipid dysregulation. The AIP vs. hs-CRP correlation ($r=+0.794$; $p<0.001$) confirms the inflammation-lipid nexus, consistent with a previous report [18], which demonstrated that AIP had 57.51-fold greater odds of elevated LDL-C in RA patients with elevated CRP. The clinical implication is that ginger's anti-inflammatory action provides a secondary cardiometabolic benefit normalization of the atherogenic lipid profile that is a valuable adjunct to conventional anti-arthritic therapy.

4.3 Hematological Indices as Novel Efficacy Endpoints

The 56.1% SII reduction and 50.0% NLR reduction in G3 represent novel efficacy endpoints for ginger in the CIA model. SII, the most sensitive of the CBC-derived indices, integrates neutrophil, platelet, and lymphocyte responses into a single composite inflammatory index, and its marked elevation (4.39-fold in G2) and normalization by ZOE confirm systemic neutrophil-dominant inflammatory activation and its suppression by ginger's NF-κB inhibitory action. These data support the clinical evidence from RA cohort studies linking NLR and SII to DAS28 disease activity and radiographic progression [19], positioning CBC-derived indices as accessible, cost-free efficacy biomarkers for monitoring anti-arthritic herbal treatments.

4.4 ZOE vs. MTX: Complementary, Not Equivalent

MTX consistently achieved greater anti-arthritic efficacy than ZOE across most domains — arthritis score −64.7% vs. −52.9%, and significantly greater cytokine suppression, oxidative-stress reduction, and hematological index normalization (all $p<0.05$, G3 vs. G4; Tukey's HSD). ZOE's lipid-normalizing effect was statistically comparable to MTX only for total cholesterol and HDL-C ($p>0.05$, G3 vs. G4), while MTX remained superior for TG, LDL-C, VLDL-C, AIP, and HOMA-IR (all $p<0.05$). Nonetheless, ZOE's antioxidant effects were mechanistically distinct from MTX (which does not directly target oxidative stress), and ZOE lacks MTX's hepatotoxic and myelosuppressive adverse effects. These data support the framework described in [16], positioning ginger as an adjunct to, rather than a replacement for, conventional DMARD therapy.

4.5 Strengths, Limitations, and Future Directions

Strengths: HPLC standardization of ZOE; five-domain simultaneous assessment; validated rat-specific ELISA kits; MTX positive control.

Limitations: male animals only (excludes sex-specific inferences in a predominantly female disease); single ZOE dose (no dose-response); 28-day treatment (no long-term data). Future directions: dose-response (50–500 mg/kg); female CIA animals; ZOE+MTX combination; validation in RA patient samples receiving ginger supplementation.

4.6 Conclusions

Zingiber officinale ethanolic extract exerts significant, mechanistically integrated anti-inflammatory effects in the CIA Wistar rat model, driven by NF-κB-mediated TNF-α/IL-6 suppression. These effects are integrated with cytokine suppression (Anti-CCP −54.7%; IL-6 −59.0%), AIP normalization below the 0.21 cardiovascular risk threshold, antioxidant restoration, and SII reduction (−56.1%), confirming ginger's multi-target phytochemical profile as the mechanistic basis for its integrated anti-arthritic efficacy. These findings provide a validated experimental platform supporting the integration of standardized ginger preparations as a complementary therapeutic component in RA management.

Conflict of interest: NIL

Funding: NIL

References

[1] GBD 2021 Rheumatoid Arthritis Collaborators. (2023). Global, regional, and national burden of rheumatoid arthritis, 1990–2020, and projections to 2050. The Lancet Rheumatology, 5(10), e594–e610. https://doi.org/10.1016/S2665-9913(23)00211-4

[2] Yameny, A. Rheumatoid Arthritis: Types, Risk Factors, and Laboratory Biomarkers. *International Journal of Pathology and Biomarkers,* (2026); *2*(1), 1-8. https://doi.org/10.71428/IJPB.2026.0101

[3] Fraenkel, L., Bathon, J. M., England, B. R., et al. (2021). 2021 American College of Rheumatology guideline for the treatment of rheumatoid arthritis. Arthritis Care & Research, 73(7), 924–939. https://doi.org/10.1002/acr.24596

[4] Pázmándi, K., Szöllősi, A. G., & Fekete, T. (2024). The 'root' causes behind the anti-inflammatory actions of ginger compounds in immune cells. Frontiers in Immunology, 15, 1400956. https://doi.org/10.3389/fimmu.2024.1400956

[5] Pourhabibi-Zarandi, F., Shojaei-Zarghani, S., & Rafraf, M. (2024). Evaluation of the anti-inflammatory potential of Zingiber officinale on adjuvant-induced arthritis. Advances in Traditional Medicine, 24, 1189–1198. https://doi.org/10.1007/s13596-024-00779-6

[6] Kaneta, H., Nakasa, T., Yimiti, D., et al. (2026). Oral ginger-derived extracellular vesicles ameliorate arthritis via anti-inflammatory actions of microRNA-149 and 6-gingerol. Molecular Therapy Nucleic Acids, 37, 102840. https://doi.org/10.1016/j.omtn.2026.102840

[7] Mihara, M., Kitami, M., Fujimoto, M., Moriya, Y., & Ohsugi, Y. (2015). Phenotypic characterization of type II collagen-induced arthritis in Wistar rats. Experimental and Therapeutic Medicine, 10(5), 1952–1958. https://doi.org/10.3892/etm.2015.2667

[8] National Research Council. (2011). Guide for the care and use of laboratory animals (8th ed.). National Academies Press. https://doi.org/10.17226/12910

[9] Dalsasso, R. R., Valencia, G. A., & Monteiro, A. R. (2022). Impact of drying and extraction processes on the recovery of gingerols and shogaols. Food Research International, 154, 111043. https://doi.org/10.1016/j.foodres.2022.111043

[10] Foudah, A. I., Shakeel, F., Yusufoglu, H. S., Ross, S. A., & Alam, P. (2020). Simultaneous determination of 6-shogaol and 6-gingerol in

Zingiber officinale Roscoe extracts using a green RP-HPTLC method. Foods, 9(8), 1136. https://doi.org/10.3390/foods9081136

[11] Brand, D. D., Latham, K. A., & Rosloniec, E. F. (2007). Collagen-induced arthritis. Nature Protocols, 2(5), 1269–1275. https://doi.org/10.1038/nprot.2007.173

[12] Zanker, J., & Duque, G. (2022). Anti-inflammatory effects of Zingiber officinale: a comprehensive review. Cureus, 14(9), e29267. https://doi.org/10.7759/cureus.29267

[13] Sotoudeh, N., & Namavar, M. R. (2022). Optimisation of ketamine-xylazine anaesthetic dose in Wistar rats. Veterinary Medicine and Science, 8(6), 2416–2424. https://doi.org/10.1002/vms3.936

[14] Dobiasova, M., & Frohlich, J. (2001). The plasma parameter log(TG/HDL-C) as an atherogenic index. Clinical Biochemistry, 34(7), 583–588. https://doi.org/10.1016/S0009-9120(01)00263-6

[15] Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function. Diabetologia, 28(7), 412–419. https://doi.org/10.1007/BF00280883

[16] Szymczak, J., Grygiel-Górniak, B., & Cielecka-Piontek, J. (2024). Zingiber officinale Roscoe: the antiarthritic potential of a popular spice — preclinical and clinical evidence. Nutrients, 16(5), 741. https://doi.org/10.3390/nu16050741

[17] Yan, J., Yang, S., Han, L., Ba, X., Shen, P., Lin, W., Li, T., Zhang, R., Huang, Y., Huang, Y., Qin, K., Wang, Y., Tu, S., & Chen, Z. (2023). Dyslipidemia in rheumatoid arthritis: the possible mechanisms. Frontiers in Immunology, 14, 1254753. https://doi.org/10.3389/fimmu.2023.1254753

[18] Dessie, G. (2022). Association of atherogenic indices with C-reactive protein and risk factors in rheumatoid arthritis patients. PLOS ONE, 17(6), e0269431. https://doi.org/10.1371/journal.pone.0269431

[19] Mathieu, S., Soubrier, M., Pereira, B., Dubost, J. J., & Dutheil, F. (2021). Cardiovascular events in rheumatoid arthritis: a systematic review. Joint Bone Spine, 88(2), 105117. https://doi.org/10.1016/j.jbspin.2020.105117