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# Evaluation of red ginger (*Zingiber officinale* var. rubrum) and paracetamol combination on inflammatory and stress markers in a rat model of NGF-induced low back pain during pregnancy

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**ABSTRACT**

Low back pain (LBP) during pregnancy is associated with heightened maternal stress and systemic inflammation, contributing to adverse outcomes such as preterm birth and low birth weight. While paracetamol is commonly used for pain relief in pregnant women, its effects on inflammatory pathways and pregnancy outcomes remain inconclusive. Red ginger (*Zingiber officinale* Roscoe) has demonstrated anti-inflammatory potential through downregulation of Interleukin 6 (IL-6) and tumor necrosis factor-alpha and upregulation of interleukin 10 (IL-10) in various inflammatory models. However, studies evaluating the combined use of red ginger and paracetamol in animal models have not yet been conducted. This study aimed to evaluate the effects of red ginger extract, alone and in combination with paracetamol, on inflammatory biomarkers, physiological stress, and pregnancy outcomes in Wistar rats with nerve growth factor (NGF)-induced LBP. Thirty pregnant rats were divided into five groups: normal control, negative control (NGF induction), paracetamol only, red ginger only, and combination therapy. Treatments were given orally from gestational day 7 to 20. Measured parameters included systemic and placental IL-6, IL-10, plasma cortisol, uterine Heat Shock Protein 70 (HSP70), and NGF-induced LBP, which increased IL-6, cortisol, and uterine HSP70 but decreased IL-10**,** accompanied by reduced fetal growth and shorter gestation. Red ginger, alone or combined with paracetamol, shifted these biomarkers toward normal and improved fetal growth and gestational length (with the combination showing the largest median changes among treatment groups). In summary, red ginger extract demonstrates therapeutic potential as a complementary co-therapy to mitigate inflammation and stress during pregnancy with LBP, with enhanced effects when combined with paracetamol.

## 1. INTRODUCTION

Preterm birth and low birth weight (LBW) remain major global causes of neonatal morbidity, long-term developmental impairment, and perinatal mortality. In Indonesia, the prevalence of preterm birth exceeds 15%, with approximately 12.4% of infants in Indonesia born with LBW, contributing significantly to neonatal morbidity and long-term developmental risks [1–3]. Concurrently, low back pain (LBP), a prevalent musculoskeletal complaint during pregnancy, affects between 40% and 54% of pregnant women worldwide [4]. Beyond compromising maternal quality of life, LBP may induce physiological stress, reduce mobility, and promote systemic inflammation, all of

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which contribute to adverse pregnancy outcomes, including LBW and preterm delivery [5].

The clinical burden of LBP during pregnancy is compounded by the limited pharmacologic options deemed safe for maternal and fetal health. Paracetamol remains the most commonly used analgesic in pregnancy due to its general safety profile. However, concerns regarding its limited anti-inflammatory effects and potential long-term neurodevelopmental consequences have been increasingly raised [6]. Evidence suggests that prolonged paracetamol exposure may be associated with altered fetal brain development and behavior [7], prompting a search for safer alternatives capable of addressing both pain and inflammation. Despite its widespread use, the molecular pathways through which paracetamol influences inflammatory cascades are still incompletely understood, especially within the complex physiological environment of progressive LBP during pregnancy.

Activation of the hypothalamic–pituitary–adrenal (HPA) axis and elevated cortisol levels, as observed in chronic stress models [8–10], may also occur in chronic pain conditions such as LBP, potentially impairing uteroplacental function and fetal development. This neuroendocrine stress response is accompanied by inflammatory dysregulation, characterized by increased expression of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), and decreased levels of interleukin-10 (IL-10), further disrupting intrauterine homeostasis [9,11]. These molecular alterations are closely linked with premature labor, fetal growth restriction, and poor neonatal outcomes [12].

In this context, interest has grown in natural alternatives with safer profiles. Red ginger (*Zingiber officinale Roscoe*), a phenotypic variant rich in gingerol, shogaol, and terpenoids, has been reported to affect NF-κB/MAPK signaling, cytokines, and oxidative stress [13–15], although pathway markers were not assessed in the present study. Previous studies have demonstrated its immunomodulatory and antidepressant-like properties in non-pregnant models [16,17]; however, evidence is currently lacking to support its use in pregnancy, particularly in stress-compromised conditions such as LBP. While previous research has mainly examined paracetamol as a monotherapy for pregnancy-related pain, with limited evaluation of its effects on inflammatory and stress pathways, studies on ginger have largely focused on nausea, vomiting, or general anti-inflammatory effects. To the best of our knowledge, no previous studies have specifically investigated red ginger (*Zingiber officinale Roscoe var. rubrum*) in pregnant models of LBP, nor explored its combination with paracetamol. Our study is therefore novel in proposing and testing this combined approach as a potential complementary therapy to measure the risk for maternal and fetal outcomes.

To address this gap, the present study evaluates the therapeutic potential of red ginger extract, alone and in combination with paracetamol, in a Wistar rat model of nerve growth factor (NGF)-induced LBP during pregnancy. We assessed systemic and placental inflammation (IL-6 and IL-10), physiological stress markers (cortisol and HSP70), and fetal outcomes (fetal weight, crown–rump length, and gestational age). This study examines the effects of red ginger—alone and combined with paracetamol—on inflammatory and stress markers in a pregnant rat model of NGF-induced LBP.

## 2. MATERIALS AND METHODS

### 2.1. Study design and animal model

All animal procedures in this study were reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret, Indonesia, under ethical clearance number 244/UN27.06.11/KEP/EC/2024. The research was conducted in accordance with institutional guidelines and international standards for the ethical treatment of laboratory animals, including the ARRIVE 2.0 guidelines, and the corresponding checklist is provided as supplementary material [18].

This study was a laboratory-based experimental research study employing a post-test-only control group design. A total of 30 pregnant female Wistar rats (*Rattus norvegicus*), aged 4 months and weighing between 200 to 300 g, were used as the animal model due to their physiological stability and responsiveness to pharmacological interventions, particularly in inflammation-related pregnancy research. Female rats were mated overnight with proven fertile males at a 2:1 ratio. The presence of a vaginal plug the following morning was considered gestational day 0 (GD0), indicating successful mating and presumptive pregnancy [19]. Only plug-positive females were included for subsequent NGF induction and treatment.

### 2.2. Preparation, extraction, and characterization of red ginger extract

Red ginger (*Zingiber officinale* Roscoe var. rubrum) rhizomes were sourced from a certified supplier in Lampung, Indonesia, and taxonomically authenticated by the Department of Pharmacognosy. Cleaned and sliced rhizomes were dried at 40°C and extracted with 70% ethanol by maceration for 72 hours at a 1:10 (w/v) ratio of dried material to solvent. The extraction procedure was performed at the Botany Laboratory, Universitas Lampung (UNILA). Filtrates were filtered, concentrated under reduced pressure at ≤45°C, and evaporated to yield a viscous extract; the yield was recorded. To ensure standardization and quality control, the extract was subjected to quantitative phytochemical assays [20], showing total phenolics of 2.24%–2.35%, total flavonoids of 0.47%, and antioxidant activity of 61.39%–61.78%. Gas Chromatography–Mass Spectrometry (GC–MS) analysis, conducted at the Faculty of Pharmacy, Universitas Gadjah Mada (UGM), confirmed the chemical profile of the extract, with major peaks corresponding to terpenoid constituents (zingiberene, β-sesquiphellandrene, ar-curcumene, and β-bisabolene) and the phenolic compound [6]-shogaol. These compositional data were used as reference values for extract standardization. The extract was stored at 4°C in airtight containers until use.

### 2.3. Animal housing and group allocation

All animals were acclimatized for seven days under controlled laboratory conditions (temperature: 22°C–25°C; humidity: 55%–70%; 12-hour light/dark cycle). Inclusion criteria comprised plug-positive, healthy, pregnant Wistar rats

aged 12 weeks with a body weight (BW) ranging from 200 to 300 g. No animals or data points were excluded during the study. To minimize baseline variability, animals were pre-matched based on BW prior to group allocation. Exclusion and potential dropout criteria were considered during the selection process. Randomization was applied to assign animals into five experimental groups ($n$ = 6 per group) as follows: Group N (Normal Control): no intervention. Group K (Negative Control): NGF 1.0 µg/200 g BW. Group P1: NGF + paracetamol 45 mg/kg body BW. Group P2: NGF + red ginger extract 90 mg/kg BW. Group P3: NGF + paracetamol 45 mg/kg BW + red ginger extract 90 mg/kg BW. Blinding procedures were not implemented; however, assay-level/procedural blinding was applied (coded specimen IDs, randomized ELISA order, duplicate wells, and calibrated measurements; see Section 2.4).

### 2.4. Treatment protocol

To induce LBP, NGF was administered intramuscularly at a dose of 1.0 µg/200 g BW into the left multifidus muscle at a depth of 3 mm from the L5 spinous process, under isoflurane anesthesia [21]. The injection protocol consisted of two administrations: the first on gestational day 8 (designated as day 0 of LBP induction) and the second on gestational day 13 (corresponding to day 5 post-induction). This dual-injection approach has been validated in previous studies to induce persistent mechanical hyperalgesia, effectively modeling chronic musculoskeletal pain associated with pregnancy [22].

Paracetamol and red ginger extract were administered orally once daily via gastric gavage from gestational day 7 to 20. Paracetamol was given at a dose of 45 mg/kg body weight, and red ginger extract at 90 mg/kg BW. The dosage selection was based on preliminary experiments conducted prior to the main study, which demonstrated that oral administration of these agents effectively reduced inflammatory markers without exhibiting any signs of toxicity in pregnant rats or their fetuses.

Animals were randomly allocated to five groups using a computer-generated sequence. Although complete blinding of treatment allocation was not possible, procedural blinding was implemented: biospecimens were assigned coded IDs and assayed by personnel blinded to group, ELISA plates were analyzed in randomized order with duplicate wells (intra-assay CV <10%), and fetal measurements were obtained on calibrated instruments using preset criteria. Euthanasia and necropsy were conducted in a randomized sequence, and husbandry/handling procedures were identical across groups. Outcomes were objective and quantitative (biomarker concentrations, fetal weight, and crown–rump length).

#### *2.4.1. The experimental protocol is illustrated in Figure 1*

Thirty pregnant Wistar rats were randomly divided into five groups: (1) normal control without intervention (N), (2) negative control (K−) with NGF induction only, (3) treatment group with paracetamol (P1) (45 mg/kgBW), (4) treatment group with red ginger extract (P2) (90 mg/kgBW),

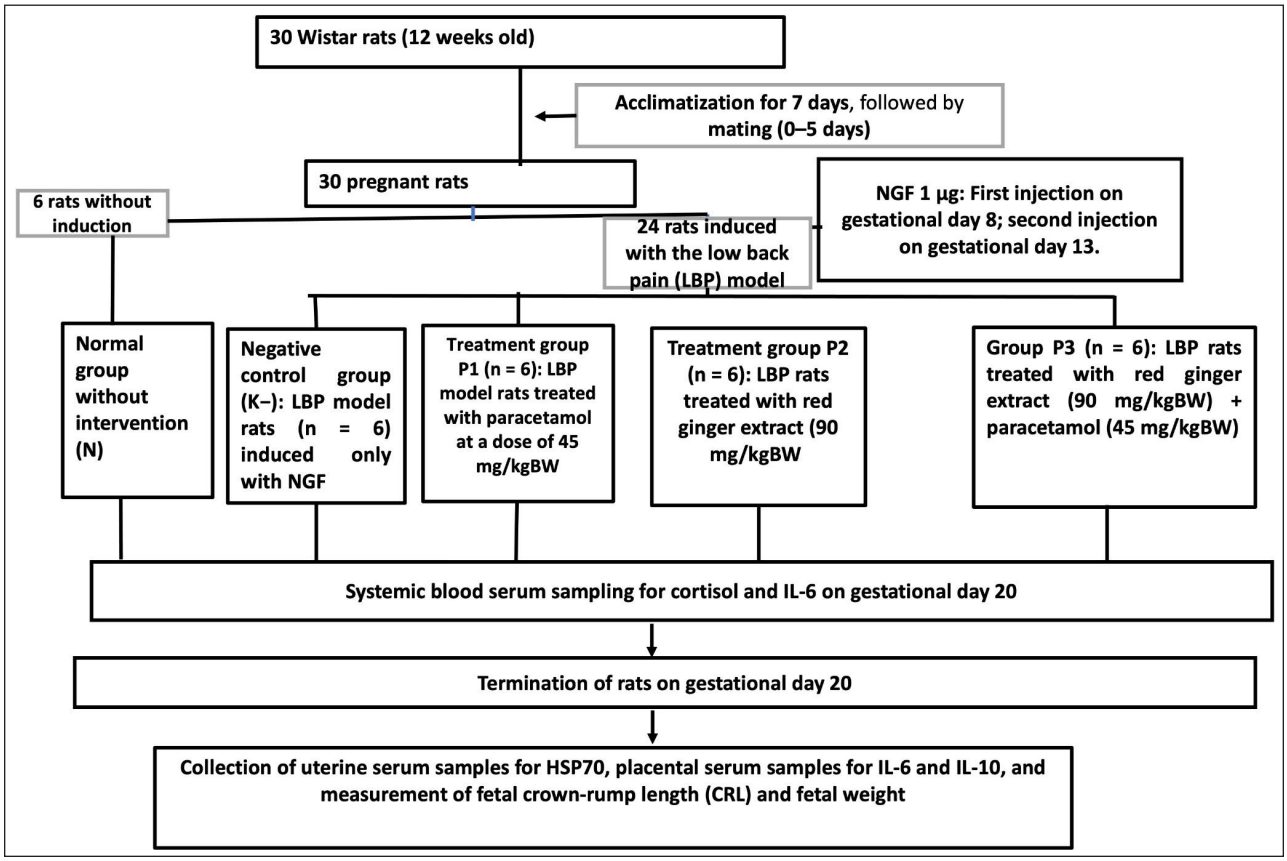


**Figure 1.** Flowchart of experimental protocol in pregnant Wistar rats: evaluation of paracetamol and red ginger extract in an NGF-induced LBP Model.

and (5) combination therapy group with red ginger extract (P3) (90 mg/kgBW) and paracetamol (45 mg/kgBW). NGF induction was performed on gestational days 8 and 13. Blood and tissue samples were collected on day 20 for biomarker analysis (cortisol, IL-6, IL-10, and HSP70) and fetal growth assessment (crown–rump length [CRL] and fetal weight).

### 2.5. Monitoring and termination

#### *2.5.1. Animal welfare and monitoring*

NGF induction and sampling were performed under inhalational anesthesia (isoflurane). To avoid confounding nociceptive/inflammatory readouts, no routine systemic analgesics were given post-NGF; dams received nesting material, moist chow, and thermal support.

#### *2.5.2. Humane endpoints*

Humane endpoints were predefined (rapid weight loss >15%, persistent anorexia/dehydration >24 hours, self-mutilation, severe unalleviated distress, or moribund state).

#### *2.5.3. Rescue plan*

Veterinary assessment and subcutaneous buprenorphine; any dam receiving rescue analgesia was excluded from analysis.

#### *2.5.4. Maternal monitoring*

Maternal monitoring was performed twice daily for 72 hours post-NGF (behavior, posture, grooming, food/water intake, and vaginal bleeding) and once daily thereafter until term. Throughout the study, dams were observed for clinical signs, physical activity, food intake, and signs of preterm labor; maternal body weight was recorded daily. Termination was conducted on gestational day 20 under ketamine (75 mg/kg BW) and xylazine (10 mg/kg BW) anesthesia. Animals delivering prior to day 20 were categorized as having experienced preterm delivery.

### 2.6. Sample collection and biomarker assessment

Blood samples were collected via intracardiac puncture immediately after termination for the measurement of systemic IL-6 and plasma cortisol levels. Placental and uterine tissues were harvested for the analysis of placental IL-6, IL-10, and uterine HSP70 concentrations using a sandwich ELISA method. Readings were obtained using a microplate ELISA reader (Zenyth 200RT, Biochrom, UK). Fetuses were counted, weighed using an analytical balance (±0.001 g accuracy), and measured for CRL using a digital caliper. Gestational age was calculated based on the detection of vaginal plugs to the day of termination or spontaneous delivery.

### 2.7. Statistical analysis

Statistical analyses were performed using SPSS software version 27.0 [23]. Data normality was assessed using the Shapiro–Wilk test, and homogeneity was assessed using Levene's test. Due to non-normal data distribution, the Kruskal–Wallis test was utilized for group comparisons, followed by Bonferroni-adjusted Mann–Whitney *U* tests for post hoc analysis. A $p$-value <0.05 was considered statistically significant. Results are presented as median (Q1–Q3).

## 3. RESULTS

A total of 30 pregnant Wistar rats were successfully included in the study and allocated into five groups. The effects of NGF-induced LBP and the subsequent therapeutic interventions were evaluated across multiple parameters, including fetal growth outcomes, inflammatory cytokines, stress biomarkers, and gestational indicators.

Throughout gestation, dams remained active with stable food intake and continuous body-weight gain, indicating normal maternal condition. Preterm delivery occurred only in the NGF control group (2/6 dams); all treated groups reached term. At necropsy, no resorptions or external malformations were detected in any group. Collectively, these observations indicate that the procedures and treatments were well tolerated and did not produce adverse maternal or fetal effects. The findings are summarized in the following sections and tables.

### 3.1. Fetal growth outcomes

#### *3.1.1. Fetal weight (Table 1)*

The induction of LBP through NGF injection resulted in a significant intrauterine growth restriction (IUGR), as evidenced by a marked reduction in fetal weight in the negative control group (K−), with a median value of 1.84 g (Q1–Q3: 1.73–1.92 g), compared to the normal control group (N), which had a median value of 4.32 g (Q1–Q3: 4.30–4.49 g) ($p < 0.001$). This finding confirms the detrimental impact of systemic inflammation and physiological stress on fetal growth.

Administration of paracetamol monotherapy (P1) and red ginger extract (P2) each demonstrated protective effects on fetal growth, with respective median weights of 4.09 g (Q1–Q3: 4.07–4.10 g) and 4.11 g (Q1–Q3: 4.04–4.22 g). The combination therapy group (P3) exhibited the most optimal improvement, achieving a median fetal weight of 4.36 g (Q1–Q3: 4.30–4.37 g), closely approximating the physiological parameters observed in the normal control group (Table 1).

#### *3.1.2. CRL (Table 2)*

Fetal CRL, a key indicator of axial longitudinal development, exhibited a pronounced reduction following LBP induction. The K− showed a median CRL of 2.50 cm (Q1–Q3: 2.50–2.82 cm), which was substantially lower than that of the normal group (N) with a median of 5.03 cm (Q1–Q3: 4.99–5.17 cm) ($p < 0.001$). An increase in CRL was observed in both the paracetamol (P1) and red ginger extract (P2) groups, with median values of 4.54 cm (Q1–Q3: 4.52–4.54 cm) and 4.56 cm (Q1–Q3: 4.48–4.67 cm), respectively. The highest restorative effect was observed in the combination therapy group (P3), which reached a median CRL of 5.03 cm (Q1–Q3: 4.96–5.04 cm), indicating nearly complete restoration of fetal development to physiological conditions (Table 2).

#### *3.1.3. Gestational age (Table 3)*

The gestational age was markedly reduced in the K−, with a median value of 18.00 days, compared to the normal group (N), which maintained a gestational duration of 20.00 days ($p < 0.001$), suggesting a potential risk of preterm birth as

**Table 1.** The effects of red ginger extract on fetal weight in a pregnant rat model of LBP.

| Group | Fetal weight (g) (Median) | Quartile (Q1–Q3) | *p*-value (Kruskal–Wallis test) |
|---|---|---|---|
| Normal (N) | 4.32 | 4.30–4.49 | |
| Negative control (K−) | 1.84 | 1.73–1.92 | |
| Paracetamol (P1) | 4.09 | 4.07–4.10 | $p < 0.001$* |
| Red ginger extract (P2) | 4.11 | 4.04–4.22 | |
| Paracetamol + Red ginger (P3) | 4.36 | 4.30–4.37 | |

Values are presented as median (Q1–Q3). Group N = normal pregnant rats without intervention; K− = rats with LBP induced by NGF; P1 = NGF-induced rats treated with paracetamol; P2 = NGF-induced rats treated with red ginger extract; P3 = NGF-induced rats treated with a combination of paracetamol and red ginger extract. Fetal weight differed significantly among groups as determined by the Kruskal–Wallis test ($p < 0.001$). *$p < 0.05$ indicates significant difference based on Kruskal–Wallis test.

**Table 2.** The effects of red ginger extract on CRL in a pregnant rat model of LBP.

| Group | Crown–rump length (cm) median | Percentile (Q1–Q3) | *p*-value (Kruskal–Wallis test) |
|---|---|---|---|
| Normal (N) | 5.03 | 4.99–5.17 | |
| Negative control (K−) | 2.50 | 2.50–2.82 | $p < 0.001$* |
| Paracetamol (P1) | 4.54 | 4.52–4.54 | |
| Red ginger extract (P2) | 4.56 | 4.48–4.67 | |
| Paracetamol + Red ginger (P3) | 5.03 | 4.96–5.04 | |

Values are presented as median (Q1–Q3). Group N = normal pregnant rats without intervention; K− = rats with LBP induced by NGF; P1 = NGF-induced rats treated with paracetamol; P2 = NGF-induced rats treated with red ginger extract; P3 = NGF-induced rats treated with a combination of paracetamol and red ginger extract. CRL differed significantly among groups as determined by the Kruskal–Wallis test ($p < 0.001$). * $p < 0.05$ indicates significant difference based on Kruskal–Wallis test.

**Table 3.** The effects of red ginger extract on gestational age in a pregnant rat model of LBP.

| Group | Gestational age (days) (Median) | *p*-value (Kruskal–Wallis test) |
|---|---|---|
| Normal (N) | 20.00 | |
| Negative control (K−) | 18.00 | |
| Paracetamol (P1) | 20.00 | $p < 0.001$* |
| Red ginger extract (P2) | 20.00 | |
| Paracetamol + Red ginger (P3) | 20.00 | |

Values are presented as median (Q1–Q3). N = normal pregnant rats without intervention; K− = NGF-induced pregnant rats with LBP and no treatment; P1 = NGF-induced rats treated with paracetamol; P2 = NGF-induced rats treated with red ginger extract; P3 = NGF-induced rats treated with both paracetamol and red ginger extract. Gestational age was significantly shorter in the K− group (18 days) compared to all other groups, which remained at 20 days ($p < 0.001$, Kruskal-Wallis test). * $p < 0.05$ indicates significant difference based on Kruskal–Wallis test.

a consequence of inflammation and chronic stress. In contrast, all treatment groups—including paracetamol (P1), red ginger extract (P2), and the combination therapy (P3)—exhibited a median gestational age of 20.00 days, identical to the normal control group. This finding underscores the efficacy of both single and combined interventions in preventing preterm labor and preserving gestational integrity (Table 3).

These findings collectively indicate that the combined intervention of paracetamol and red ginger extract confers superior efficacy in preserving fetal growth parameters and preventing premature labor compared to monotherapies.

### 3.2. Placental inflammatory biomarkers

#### *3.2.1. IL-6 expression (Table 4)*

Placental IL-6 levels were markedly elevated in the K− following NGF-induced inflammation, with a median value of 166.50 pg/ml (Q1–Q3: 166.50–166.50), in contrast to the normal group (N), which exhibited physiological concentrations of 69.83 pg/ml (Q1–Q3: 68.17–79.61) ($p < 0.001$). Administration of paracetamol (P1) and red ginger extract (P2) significantly reduced IL-6 expression to 101.50 pg/ml (Q1–Q3: 100.11–103.44) and 87.06 pg/ml (Q1–Q3: 84.56–88.17), respectively, reflecting substantial anti-inflammatory effects. Notably, the combined treatment group (P3) demonstrated the most pronounced reduction in IL-6 levels, with a median of 76.78 pg/ml (Q1–Q3: 74.83–81.50), closely approximating physiological levels. These findings highlight the therapeutic potential of combination therapy in mitigating placental inflammation triggered by chronic pain (Table 4).

#### *3.2.2. IL-10 expression (Table 5)*

Placental IL-10 expression, a critical anti-inflammatory cytokine involved in fetal immune tolerance, was significantly suppressed in the K− following NGF-induced LBP, with a median level of 34.00 pg/ml (Q1–Q3: 34.00–34.00 pg/ml), markedly lower than the physiological range observed in the normal group (N), which exhibited a

**Table 4.** Effects of red ginger extract on placental IL-6 expression in a pregnant rat model of LBP.

| Group | Placental IL-6 (Median) pg/ml | Quartile Q1–Q3 | *p*-value (Kruskal–Wallis test) |
|---|---|---|---|
| Normal (N) | 69.83 | 68.17–79.61 | |
| Negative control (K−) | 166.50 | 166.50–166.50 | |
| Paracetamol (P1) | 101.50 | 100.11–103.44 | $p < 0.001$* |
| Red ginger extract (P2) | 87.06 | 84.56–88.17 | |
| Paracetamol + Red ginger (P3) | 76.78 | 74.83–81.50 | |

Values are presented as median (Q1–Q3). N = normal pregnant rats without intervention; K− = NGF-induced pregnant rats with LBP and no treatment; P1 = NGF-induced rats treated with paracetamol; P2 = NGF-induced rats treated with red ginger extract; P3 = NGF-induced rats treated with both paracetamol and red ginger extract. Placental IL-6 expression was significantly higher in the K− group compared to all others ($p < 0.001$), whereas treatment, particularly the combination (P3), reduced IL-6 levels toward normal values. * $p < 0.05$ indicates significant difference based on Kruskal–Wallis test.

**Table 5.** The effects of red ginger extract on placental IL-10 expression in a pregnant rat model of LBP.

| Group | Placental IL-10 (Median) pg/ml | Quartile (Q1–Q3) | *p*-value (Kruskal–Wallis test) |
|---|---|---|---|
| Normal (N) | 80.36 | 79.61–83.85 | |
| Negative control (K−) | 34.00 | 34.00–34.00 | |
| Paracetamol (P1) | 51.58 | 49.45–53.55 | $p < 0.001$* |
| Red ginger extract (P2) | 64.00 | 62.94–64.00 | |
| Paracetamol + Red ginger (P3) | 70.06 | 70.06–71.12 | |

Values are presented as median (Q1–Q3). N = normal pregnant rats without intervention; K− = NGF-induced pregnant rats with LBP and no treatment; P1 = NGF-induced rats treated with paracetamol; P2 = NGF-induced rats treated with red ginger extract; P3 = NGF-induced rats treated with both paracetamol and red ginger extract. Placental IL-10 expression was lowest in the K− group, while the combination therapy (P3) resulted in the highest IL-10 levels among treated groups ($p < 0.001$, Kruskal–Wallis test). * $p < 0.05$ indicates significant difference based on Kruskal–Wallis test.

median IL-10 concentration of 80.36 pg/ml (Q1–Q3: 79.61–83.85 pg/ml) ($p < 0.001$). Monotherapy with paracetamol (P1) moderately improved IL-10 levels, reaching a median of 51.58 pg/ml (Q1–Q3: 49.45–53.55 pg/ml), whereas red ginger extract (P2) further enhanced IL-10 expression to a median of 64.00 pg/ml (Q1–Q3: 62.94–64.00 pg/ml). The combined intervention (P3) produced the most optimal restorative effect, elevating IL-10 to 70.06 pg/ml (Q1–Q3: 70.06–71.12 pg/ml), closely approaching physiological homeostasis. These findings underscored the enhanced anti-inflammatory potential of combined red ginger extract and paracetamol therapy in mitigating immune dysregulation at the maternal–fetal interface (Table 5).

These findings indicated that the combination of paracetamol and red ginger extract provides enhanced regulatory effects on placental inflammatory homeostasis, characterized by the suppression of pro-inflammatory IL-6 and the upregulation of anti-inflammatory IL-10 expression.

### 3.3. Uterine cellular stress marker

#### *3.3.1. HSP70 expression (Table 6)*

Uterine expression of heat shock protein 70 (HSP70), a molecular marker of cellular stress, showed a significant alteration following NGF-induced LBP. The K− exhibited a markedly **elevated** HSP70 level, with a median of 9.94 pg/ml (Q1–Q3: 9.90–9.96), compared to the normal group (N), which had a median of 1.64 pg/ml (Q1–Q3: 1.59–1.67; $p < 0.001$). This elevation reflected heightened cellular stress response mechanisms under chronic pain conditions.

Treatment with paracetamol (P1) and red ginger extract (P2) significantly reduced HSP70 expression relative to K−**,** to 2.83 pg/ml (Q1–Q3: 2.73–2.89) and 2.52 pg/ml (Q1–Q3: 2.44–2.58), respectively. Notably, the combination therapy group (P3) demonstrated a further restoration of HSP70 expression to a median of 2.07 pg/ml (Q1–Q3: 2.06–2.11), suggesting an effect that most closely approaches normal cellular homeostasis (Table 6).

These results indicate that both paracetamol and red ginger mitigated uteroplacental cellular stress, with the combined treatment demonstrating the greatest efficacy in normalizing tissue homeostasis.

HSP70 expression, an essential molecular chaperone involved in cellular stress response, was significantly increased in the K−, with a median value of 9.94 pg/ml (Q1–Q3: 9.90–9.96), compared to the physiological baseline observed in the normal group (N), which was 1.64 pg/ml (Q1–Q3: 1.59–1.67) ($p < 0.001$). This **elevation** indicated a **heightened** cellular stress response due to NGF-induced LBP.

Monotherapy with paracetamol (P1) significantly **reduced** HSP70 levels relative to 2.83 pg/ml (Q1–Q3: 2.73–2.89), and red ginger extract (P2) yielded a similar reduction to 2.52 pg/ml (Q1–Q3: 2.44–2.58). Remarkably, the combination therapy group (P3) demonstrated HSP70 levels of 2.07 pg/ml (Q1–Q3: 2.06–2.11), more closely approximating the normal physiological range.

The Kruskal–Wallis test confirmed a statistically significant difference across all groups ($p < 0.001$), supporting the enhanced efficacy of red ginger and paracetamol in **reducing** HSP70 expression relative to K− and mitigating cellular stress in pregnant rats with NGF-induced inflammatory LBP.

### 3.4. Systemic inflammatory and stress markers

#### *3.4.1. Systemic IL-6 levels (Table 7)*

Systemic IL-6 levels, indicative of circulating pro-inflammatory activity, were markedly elevated in the K− following NGF-induced LBP, with a median of 143.17 pg/ml (Q1–Q3: 141.92–144.83), compared to the normal group, which exhibited a median of 64.00 pg/ml (Q1–Q3: 61.92–66.09; $p < 0.001$). Treatment with paracetamol (P1) and red ginger extract (P2) significantly reduced systemic IL-6 levels to 93.17 pg/ml (Q1–Q3: 91.92–94.42) and 78.38 pg/ml (Q1–Q3: 76.83–79.42), respectively. Notably, the combination treatment group (P3) achieved a further reduction to 71.50 pg/ml (Q1–Q3: 70.25–72.75), closely approximating the physiological baseline observed in the normal group (Table 7).

#### *3.4.2. Plasma cortisol levels (Table 8)*

Plasma cortisol concentrations, an established biomarker of physiological stress, were markedly elevated in the negative control (K−) group following NGF-induced

**Table 6.** Effects of red ginger extract on uterine HSP70 expression in a pregnant rat model of LBP.

| Group | HSP70 (Median) pg/ml | Quartile (Q1–Q3) | *p*-value (Kruskal–Wallis test) |
|---|---|---|---|
| Normal (N) | 1.64 | 1.59–1.67 | |
| Negative control (K−) | 9.94 | 9.90–9.96 | |
| Paracetamol (P1) | 2.83 | 2.73–2.89 | $p < 0.001$* |
| Red ginger extract (P2) | 2.52 | 2.44–2.58 | |
| Paracetamol + Red ginger (P3) | 2.07 | 2.06–2.11 | |

Values are presented as median (Q1–Q3). N = normal pregnant rats without intervention; K− = NGF-induced pregnant rats with LBP and no treatment; P1 = NGF-induced rats treated with paracetamol; P2 = NGF-induced rats treated with red ginger extract; P3 = NGF-induced rats treated with both paracetamol and red ginger extract. Uterine HSP70 expression was highest in the K− group (9.94 pg/mL), indicating cellular stress due to LBP. All treatment groups (P1, P2, P3) showed significantly lower HSP70 levels ($p < 0.001$), with P3 closest to normal. *$p < 0.05$ indicates significant difference based on Kruskal–Wallis test.

**Table 7.** Effects of red ginger extract on systemic IL-6 levels in a pregnant rat model of LBP.

| Group | Systemic IL-6 (Median) pg/ml | Quartile (Q1–Q3) | *p*-value (Kruskal–Wallis test) |
|---|---|---|---|
| Normal (N) | 64.00 | 61.92–66.09 | |
| negative control (K−) | 143.17 | 141.92–144.83 | $p < 0.001$* |
| Paracetamol (P1) | 93.17 | 91.92–94.42 | |
| Red ginger extract (P2) | 78.38 | 76.83–79.42 | |
| Paracetamol + red ginger (P3) | 71.50 | 70.25–72.75 | |

Values are presented as median (Q1–Q3). N = normal pregnant rats without intervention; K− = NGF-induced pregnant rats with LBP and no treatment; P1 = NGF-induced rats treated with paracetamol; P2 = NGF-induced rats treated with red ginger extract; P3 = NGF-induced rats treated with both paracetamol and red ginger extract. Systemic IL-6 levels were significantly elevated in the K− group ($p < 0.001$), while the combination (P3) achieved the greatest reduction, approaching normal values. * $p < 0.05$ indicates significant difference based on Kruskal–Wallis test.

**Table 8.** The effects of red ginger extract on plasma cortisol levels in a pregnant rat model of LBP.

| Group | Plasma cortisol (Median) ng/ml | Quartile (Q1–Q3) | *p*-value (Kruskal–Wallis test) |
|---|---|---|---|
| Normal (N) | 17.16 | 17.09–17.28 | |
| Negative control (K−) | 21.71 | 21.66–21.85 | |
| Paracetamol (P1) | 18.46 | 18.34–18.52 | $p < 0.001$* |
| Red ginger extract (P2) | 17.82 | 17.75–17.92 | |
| Paracetamol + Red ginger (P3) | 17.28 | 17.16–17.45 | |

Values are presented as median (Q1–Q3). N = normal pregnant rats without intervention; K− = NGF-induced pregnant rats with LBP and no treatment; P1 = NGF-induced rats treated with paracetamol; P2 = NGF-induced rats treated with red ginger extract; P3 = NGF-induced rats treated with both paracetamol and red ginger extract. Plasma cortisol levels were highest in the K− group ($p < 0.001$), whereas the combination therapy (P3) reduced cortisol levels close to those of the normal group. *$p < 0.05$ indicates significant difference based on Kruskal–Wallis test.

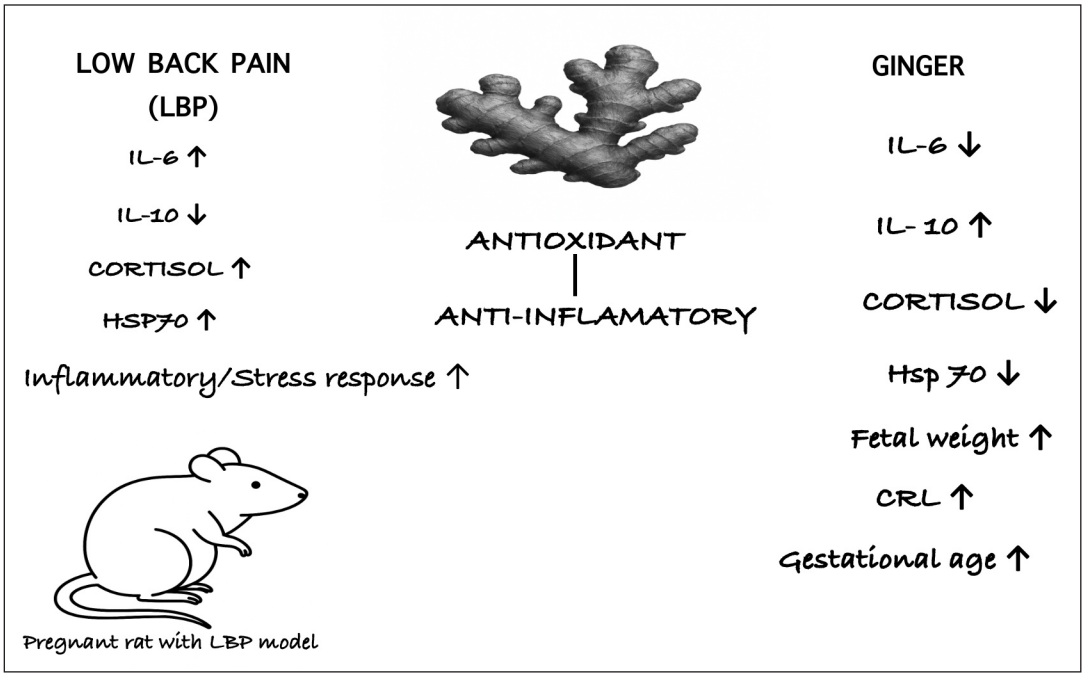


**Figure 2.** Schematic illustration of red ginger's modulatory effects on inflammation and stress biomarkers in a rat model of pregnancy-associated LBP.

LBP (21.71 ng/ml, Q1–Q3: 21.66–21.85), compared to the normal group (17.16 ng/ml, Q1–Q3: 17.09–17.28; $p < 0.001$). Administration of paracetamol (P1) and red ginger extract (P2) independently attenuated plasma cortisol levels to 18.46 ng/ml (Q1–Q3: 18.34–18.52) and 17.82 ng/ml (Q1–Q3: 17.75–17.92), respectively. The combination treatment (P3) further normalized plasma cortisol to 17.28 ng/ml (Q1–Q3: 17.16–17.45), closely aligning with physiological values. These findings underscore the enhanced potential of paracetamol and red ginger extract in mitigating stress responses in pregnant rats experiencing inflammatory LBP (Table 8).

Together, these findings highlighted the ability of both agents, particularly when combined, to suppress systemic inflammation and restore neuroendocrine balance in pregnant rats exposed to chronic pain-induced stress.

Collectively, these findings provided compelling evidence of the therapeutic efficacy of red ginger extract, particularly when combined with paracetamol, in modulating systemic inflammation and physiological stress in pregnancies complicated by LBP. The broader implications of these outcomes are elaborated in the subsequent discussion.

## 4. DISCUSSION

### 4.1. Fetal weight

The present study demonstrated a significant reduction in fetal weight among pregnant rats exposed to NGF-induced LBP, indicating IUGR is likely mediated by sustained maternal physiological stress and systemic inflammation. This finding aligns with existing literature that implicates chronic stress and persistent nociceptive stimuli in impairing uteroplacental function by disrupting nutrient and oxygen transport to the fetus [24,25]. The resulting overactivation of the HPA axis leads to excessive cortisol secretion, which directly limits fetal cell proliferation and growth [26–28].

Paracetamol (P1) administration significantly improved fetal weight, suggesting its role in mitigating nociceptive stress and possibly attenuating neuroendocrine dysregulation [27]. However, concerns regarding its long-term safety on fetal neurodevelopmental trajectories require cautious interpretation [29]. Red ginger extract (P2), rich in active constituents such as gingerol, shogaol, and flavonoids, yielded comparable improvements. These compounds possess potent anti-inflammatory and antioxidant effects, which may suppress cytokine-mediated placental inflammation, regulate maternal cortisol levels, and enhance placental angiogenesis [30–32].

The combined intervention (P3) produced fetal weights statistically indistinguishable from those of the normal group, suggesting a greater overall improvements without inferring interaction that effectively mitigates inflammatory damage and restores intrauterine homeostasis [31]. These results underscore the potential of integrated pharmacological–phytotherapeutic strategies in addressing pregnancy complications associated with musculoskeletal pain.

### 4.2. CRL

CRL is widely recognized as a reliable morphological parameter for assessing axial fetal development. In this study, CRL was significantly diminished in the K−. This finding parallels the known inhibitory effects of maternal stress and inflammation on embryonic cell differentiation and skeletal elongation [26,32]. Elevated maternal levels of cortisol and pro-inflammatory cytokines such as IL-6 and TNF-α have been

shown to impair osteogenesis and delay fetal tissue maturation, which supports these findings [32].

Paracetamol (P1) and red ginger extract (P2) individually demonstrated the capacity to counteract these effects. Paracetamol is understood to function by modulating nociceptive perception and indirectly reducing central HPA axis overactivation [33]. Conversely, red ginger suppresses oxidative stress and NF-κB pathway activation, thereby supporting cellular proliferation and matrix development [25,34]. The most pronounced restoration was seen in the P3 group, which exhibited CRL values approaching those of the normal group, highlighting the combined benefit of dual intervention in enhancing linear fetal growth.

These results are further supported by previous studies by Yusof *et al*. [24] and Hassan *et al*., [35], which demonstrated that ginger supplementation increases placental vascularization and fetal somatic growth, largely due to the upregulation of vascular endothelial growth factor (VEGF) and improved uterine perfusion.

### 4.3. Gestational age

Preterm birth is frequently linked to chronic maternal stress, and in this study, rats in the K− group experienced significantly reduced gestational age. The early onset of labor can be attributed to the biochemical cascade triggered by nociceptive stress, including the upregulation of placental corticotropin-releasing hormone (CRH), prostaglandins, and oxytocin—all of which contribute to myometrial contractility and cervical softening [36]. Concurrent elevations in IL-6 and cortisol serve as both upstream and downstream amplifiers in this parturition cascade, further accelerating labor onset [34,37].

Paracetamol and red ginger, when administered individually (P1 and P2), were effective in preserving the gestational period until term. Their protective effect is believed to arise from suppression of neuroinflammatory mediators and stabilization of the maternal HPA axis [34,38,39]. Remarkably, the combination therapy (P3) entirely normalized gestational length, suggesting enhanced efficacy in preventing premature labor through the simultaneous targeting of nociceptive, inflammatory, and neuroendocrine pathways. These findings align with recent evidence highlighting the role of maternal stress in preterm birth risk [40,41] and the benefits of reducing inflammatory and endocrine disruptions during gestation [42].

### 4.4. Placental IL-6

Placental expression of IL-6 was significantly elevated in the K−, indicating a robust local inflammatory response driven by persistent nociceptive stress during pregnancy. IL-6, as a central pro-inflammatory cytokine, not only disrupts trophoblast differentiation but also accelerates placental aging and compromises nutrient transport capacity [19,42]. This local inflammatory environment is detrimental to fetal growth and is often linked with adverse obstetric outcomes.

Both paracetamol (P1) and red ginger extract (P2) reduced placental IL-6 levels significantly. Paracetamol's effect is primarily attributed to its inhibition of prostaglandin synthesis via the cyclooxygenase (COX) pathway [43,44]. In contrast, red ginger appears to exert broader immunomodulatory actions, including MAPK and NF-κB inhibition, as well as activation of endogenous antioxidant defenses through the Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway [45,46]. However, neither monotherapy restored IL-6 to baseline values. Interestingly, the combination group (P3) achieved near-complete normalization of placental IL-6, suggesting complementary pharmacological actions that target both the oxidative and inflammatory axes simultaneously.

Supporting studies by Mashhadi *et al*. [32] and others have highlighted that phytotherapeutic agents, especially those with antioxidant and anti-inflammatory properties, are particularly effective in reversing placental inflammation when used alongside conventional medications [42,47].

### 4.5. Placental IL-10

In contrast to IL-6, IL-10 is a pivotal anti-inflammatory cytokine that is critical for maintaining maternal–fetal immune tolerance. A dramatic reduction in placental IL-10 observed in group K− underscores the extent of immunological imbalance induced by LBP-related stress. Low IL-10 levels have been associated with trophoblast dysfunction, IUGR, and increased risk of spontaneous preterm labor [48].

Paracetamol (P1) treatment resulted in a moderate increase in IL-10, likely through indirect pathways involving a reduction in maternal cortisol and central stress mediators [49]. Red ginger extract (P2) demonstrated a more pronounced effect, consistent with its known ability to activate Signal Transducer and Activator of Transcription 3 (STAT3) and other transcription factors that directly promote IL-10 synthesis [50]. The combined treatment (P3) restored IL-10 levels to nearly physiological values, indicating effective immunological rebalancing.

These findings are in agreement with Saito *et al*. [48], who emphasized the importance of maintaining IL-10 levels in pregnancy to prevent premature uterine contractions and promote placental tolerance mechanisms. The combined effect observed in this study suggests that dual therapy not only reduces pro-inflammatory signals but also actively enhances anti-inflammatory immune adaptation during gestation [51].

### 4.6. Uterine HSP70

HSP70 is a critical molecular chaperone that safeguards cells against proteotoxic stress and maintains placental homeostasis. In this study, uterine expression of HSP70 was significantly upregulated in the K− compared to the normal group, reflecting a heightened cellular stress response to sustained nociceptive load. This elevation indicates increased protein-folding demand, augmented chaperone activity, and heightened vulnerability to placental dysfunction, which may predispose to fetal growth restriction and adverse pregnancy outcomes [52–54].

Treatment with paracetamol (P1) reduced HSP70 levels toward normal, likely through its ability to attenuate prostaglandin synthesis and metabolic demand. Red ginger extract (P2) exhibited a greater reduction, consistent with its potent antioxidant activity and ability to suppress oxidative damage by modulating MAPK and related stress pathways [55]. Notably, the combination therapy (P3) achieved the largest

decrease, with HSP70 expression approaching that of the normal control, suggesting a combined effect between analgesic and phytochemical antioxidant mechanisms.

These findings align with recent evidence that emphasizes the importance of maintaining placental oxidative balance and cytoprotective responses in pregnancies at risk of fetal growth restriction [56]. Furthermore, bioactive compounds of red ginger, particularly 6-gingerol, have been shown to protect trophoblasts against oxidative injury through mitophagy regulation [57], reinforcing the translational potential of red-ginger–based interventions in mitigating maternal–fetal stress and preserving healthy gestation.

### 4.7. Systemic IL-6

The systemic profile of IL-6 followed a similar pattern to the placental expression. The K− group showed marked elevation of circulating IL-6, indicating widespread inflammatory activation. Its implications were not only for uterine contractility but also for maternal vascular tone, immune surveillance, and fetal neurodevelopment [34,47]. Elevated IL-6 in the maternal bloodstream can exacerbate the inflammatory loop by further stimulating the HPA axis.

Paracetamol (P1) reduced IL-6 levels through inhibition of the COX pathway, although its effect remains modest compared to more potent anti-inflammatories [38]. Red ginger (P2), on the other hand, achieved greater reductions via NF-κB inhibition and enhancement of superoxide dismutase and glutathione peroxidase activity [32,38]. The P3 group displayed systemic IL-6 approaching the normal control, reflecting a highly effective combined anti-inflammatory response.

These results are strongly supported by the findings of Zhao *et al*. [57] and Mashhadi *et al*. [32], which demonstrate that phytochemical compounds, when co-administered with traditional analgesics, can produce amplified systemic anti-inflammatory effects.

### 4.8. Plasma cortisol

As a primary biomarker of physiological stress, plasma cortisol was significantly elevated in the K− group, consistent with chronic HPA axis activation in response to nociceptive load. Sustained high cortisol levels are known to impair placental 11β-HSD2 activity, thus increasing fetal exposure to biologically active glucocorticoids and potentially disrupting neurodevelopmental outcomes [57–60].

Both paracetamol (P1) and red ginger extract (P2) lowered plasma cortisol significantly. Paracetamol's effect is believed to occur indirectly via attenuation of central stress signaling [38,49]. In contrast, red ginger's adaptogenic and anxiolytic effects operate through suppression of CRH and ACTH synthesis and modulation of excitability within the limbic system [24,61,62]. The most favorable result was observed in the P3 group, where cortisol levels were restored to near-baseline.

This observation supports the findings of Gholamrezaei *et al*. [61] and O'Connor *et al*., [62], who have shown that reducing maternal stress via targeted interventions can prevent fetal HPA axis programming abnormalities [63].

The cumulative findings of this study highlight the therapeutic advantage of combining paracetamol and red ginger extract in mitigating the adverse sequelae of pregnancy-associated LBP. The dual intervention effectively restored key inflammatory (IL-6, IL-10), stress (cortisol, HSP70), and fetal outcome (birth weight, CRL, gestational age) indicators to near-normal levels. In contrast to monotherapies, the combination therapy exhibited a combined effect, offering more robust normalization of maternal immunological and neuroendocrine function.

In pregnant rats with LBP, inflammatory and stress biomarkers (IL-6, cortisol, HSP70) were elevated, while the anti-inflammatory cytokine IL-10 was suppressed. Administration of red ginger extract reversed these alterations—decreasing IL-6, cortisol, and HSP70 levels while increasing IL-10 expression—resulting in improved fetal weight, CRL, and gestational age. This illustration highlights red ginger's antioxidant, anti-inflammatory, and anti-stress mechanisms in mitigating adverse pregnancy outcomes.

Physiologically, maternal inflammation and stress represent tightly linked systems that, when dysregulated, perpetuate a cycle of placental dysfunction and fetal compromise. IL-6 and cortisol serve as central mediators within this bidirectional axis [26,42,58,64], contributing to increased HSP70 expression and suppression of IL-10, thereby disrupting fetal tolerance mechanisms and amplifying the risk of preterm labor [52]. The observed improvements across multiple biomarkers in the combination group suggest that co-targeting nociceptive, oxidative, and inflammatory pathways can substantially improve pregnancy outcomes.

### 4.9. Mechanistic insights: complementary actions of paracetamol and red ginger

Mechanistically, paracetamol exerts central effects by dampening prostaglandin synthesis and modulating pain–stress pathways [32,37]. At the same time, red ginger offers peripheral anti-inflammatory, antioxidant, and immunomodulatory properties through NF-κB, Nrf2, and MAPK axis inhibition [24,38,47,65]. Their complementary pharmacological profiles present a compelling rationale for integrated interventions, especially in cases where chronic pain and systemic inflammation coexist during gestation. Although the present findings suggest that red ginger and paracetamol exert their protective effects through modulation of inflammatory and stress pathways, no direct assessment of downstream signaling cascades such as NF-κB, MAPK, or Nrf2 was performed in this model. The mechanistic interpretation is therefore based on changes in IL-6, IL-10, HSP70, and cortisol levels, in conjunction with existing molecular evidence on the bioactive compounds of red ginger. These outcomes are in line with prior findings indicating that dual-modality therapies not only alleviate maternal symptoms but also address the underlying pathophysiology of pregnancy complications [42].

### 4.10. Translational implications for prenatal care

Furthermore, the use of a validated animal model and inclusion of both systemic and tissue-specific biomarkers enhance the study's relevance and reproducibility of the present

findings [38,53]. This preclinical study provides insight into the management of pregnancy-related LBP and stress-linked inflammation. The observed improvements with red ginger extract alone and in combination with paracetamol are consistent with complementary actions and generate hypotheses for translation, rather than constituting clinical recommendations. Given the widespread use of paracetamol and growing interest in evidence-informed phytochemicals, this approach warrants evaluation as a complementary co-therapy in prenatal care, particularly in resource-limited or high-risk settings, pending formal studies of safety and efficacy.

Future research should prioritize dose-finding, pharmacokinetics, **maternal–fetal safety**, and long-term offspring outcomes in pregnancy models, followed by rigorously designed clinical trials. Biomarker-guided strategies targeting the cortisol–cytokine–oxidative stress axis may improve risk stratification and early prevention of obstetric complications. The integration of nutraceuticals such as red ginger into conventional perinatal care should remain investigational until supported by adequate clinical evidence.

## 5. LIMITATIONS

This study used a rat model, which, although informative, has limited translational value for human pregnancy. Moreover, our five-group, non-factorial design with non-parametric analyses precludes formal interaction (synergy) testing; combination findings are interpreted without inferring interaction**.** Key differences must be considered: (i) metabolism, as drug absorption and enzyme activity (e.g., cytochrome P450) differ between rats and humans, affecting pharmacokinetics; (ii) immune response, since rodent placental structure and cytokine regulation do not fully reflect human maternal–fetal tolerance; and (iii) safety, because doses considered safe in rats may not predict teratogenicity or long-term developmental risks in humans. Randomization and laboratory blinding were implemented as described**,** but full blinding of gavage administrators was not feasible, which may introduce performance bias. Only short-term outcomes until gestational day 20 were assessed, without evaluation of postnatal development, maternal lactational physiology, or offspring neurobehavioral outcomes. The cytokine panel was limited, and genetic/epigenetic markers were not analyzed. Additionally, the study focused solely on female subjects without considering paternal or intergenerational influences that may be relevant for stress-adaptation mechanisms. These limitations highlight the need for rigorously designed, blinded, and longitudinal clinical studies to confirm the efficacy, safety, and developmental impact of this combined intervention.

## 6. CONCLUSION

This study demonstrates that the combined administration of red ginger extract and paracetamol exerts enhanced therapeutic effects in a pregnant rat model of LBP. The intervention significantly enhanced fetal growth, normalized key inflammatory and stress biomarkers (IL-6, IL-10, HSP70, and cortisol), and preserved gestational duration. Compared to monotherapy, the combination more effectively restored systemic and intrauterine homeostasis. These findings support the integration of phytotherapy and pharmacotherapy in managing complex pregnancy-related conditions. Further clinical validation is needed to assess its safety and effectiveness in real-world obstetric care.

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## 8. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

## 9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## 10. ETHICAL APPROVALS

Ethical approval details are given in the ‘Materials and Methods’ section.

## 11. DATA AVAILABILITY

All data generated and analyzed are included in this research article.

## 12. PUBLISHER’S NOTE

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## 13. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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## SUPPLEMENTARY MATERIAL

The supplementary material can be accessed at the link here: https://japsonline.com/admin/php/uploadss/4739_pdf.pdf