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The effect of halofuginone administration on IL-1β levels in obese rat models with osteoarthritis

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ABSTRACT: Osteoarthritis (OA) is a degenerative joint disease frequently associated with obesity, which elevates proinflammatory cytokines such as interleukin-1β (IL-1β) and exacerbates joint degeneration. Obesity, defined clinically as a body mass index above 30 kg/m², continues to rise globally due to high-calorie diets, sedentary lifestyles, and low public awareness of healthy habits. In Southeast Asia, including Indonesia, Malaysia, Brunei, and Singapore, the prevalence of obesity reaches an average of 23.4%, with Indonesia reporting 35.5% of adults, 20% of children aged 5–12 years, and 14.8% of adolescents aged 13–18 years as overweight or obese. Halofuginone, a potential anti-inflammatory agent, has been suggested to modulate IL-1β levels and influence OA progression. This study aimed to analyze and determine the effect of Halofuginone administration on IL-1β levels in obese rat models with OA. An experimental study was conducted using obese rats induced with OA, divided into control and treatment groups, with the latter receiving Halofuginone. IL-1β levels were quantified using enzyme-linked immunosorbent assay (ELISA), and statistical analyses were performed to compare the groups. Results indicated that Halofuginone administration significantly reduced IL-1β levels in the treatment group compared to the control group, demonstrating its modulatory effect on inflammatory responses in obese OA models. These findings provide novel evidence of Halofuginone's role in obesity-related OA, highlighting its potential translational application as a targeted anti-inflammatory therapy. The study contributes to future research directions on cytokine modulation and OA management, offering insights into therapeutic strategies to mitigate inflammation-driven joint degeneration in obese populations.

1. INTRODUCTION

Obesity is not only linked to metabolic syndromes such as type 2 diabetes mellitus, hypertension, and dyslipidemia but also plays a crucial role in the development of musculoskeletal disorders, particularly osteoarthritis (OA). Increased body weight imposes excessive mechanical stress on weight-bearing joints, particularly the knees and hips, accelerating articular cartilage degradation (Koonce and Bravman, 2013). However, as highlighted by Koonce and Bravman (2013) in their article

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"Obesity and Osteoarthritis: More than Just Wear and Tear", the association between obesity and OA extends beyond mechanical overload. Traditionally, OA has been viewed as a degenerative "wear-and-tear" disease of the joints, but emerging evidence shows that obesity contributes to OA through complex systemic and metabolic mechanisms. Adipose tissue in obese individuals acts as a biologically active organ that secretes proinflammatory cytokines and adipokines, inducing chronic low-grade inflammation and accelerating joint degeneration (Thijssen et al., 2015).

Supporting this perspective, a later study published in Rheumatology titled "Obesity and Osteoarthritis, More Than Just Wear and Tear: Pivotal Roles for Inflamed Adipose Tissue and Dyslipidaemia in Obesity-Induced Osteoarthritis" further elucidates the biological pathways linking obesity to OA (Thijssen et al., 2015). The study found that inflamed adipose tissue and dyslipidemia play central roles in mediating cartilage destruction through metabolic and inflammatory cascades. Specifically, elevated levels of inflammatory mediators such as leptin, adiponectin, and resistin derived from hypertrophic adipose tissue were shown to disrupt cartilage homeostasis and promote synovial inflammation. Additionally, dyslipidemia was identified as a contributing factor that exacerbates oxidative stress and lipid accumulation in joint tissues, further amplifying cartilage degradation (van der Heijden et al., 2015).

Collectively, these findings reinforce the notion that obesity serves as both a mechanical and a metabolic risk factor for OA. Therefore, weight reduction is not only beneficial in relieving joint stress but also essential in modulating inflammatory and metabolic pathways that underlie OA progression. Effective management of OA in obese patients should thus adopt a comprehensive approach addressing both biomechanical loading and systemic metabolic dysregulation to achieve optimal therapeutic outcomes.

Moreover, adipose tissue functions as an active endocrine organ that secretes proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and leptin. These cytokines contribute to chronic, low-grade inflammation in joint tissues, triggering cartilage catabolism, upregulating matrix metalloproteinases (MMPs), and suppressing chondrocyte regeneration (Wang and He, 2018). Hence, obesity contributes to OA pathogenesis through both biomechanical overload and systemic inflammatory mechanisms (Fuggle et al., 2020; Wang and He, 2018).

OA is a chronic degenerative joint disease characterized by progressive damage to articular cartilage and subchondral bone, driven by mechanical stress and complex biological interactions (Nedunchezhiyan et al., 2022). It remains one of the leading causes of musculoskeletal disability globally, with the incidence of knee OA estimated at 240 per 100,000 people per year (Fransen et al., 2011). In Indonesia, its prevalence increases with age, 5% in individuals under 40 years, 30% between 40 and 60 years, and up to 65% in those over 61 years. Moreover, knee OA accounts for approximately 74.9% of the global disability burden from OA (Butarbutar et al., 2024; Fransen et al., 2011).

Among the various risk factors, obesity is one of the most significant contributors to OA development, particularly in the lower limbs. Even mild overweight can increase the risk of joint damage by altering gait, posture, and mechanical loading distribution (Nedunchezhiyan et al., 2022). The coexistence of obesity and OA results in persistent activation of inflammatory pathways, notably the NF-κB pathway, which amplifies cytokine production, including TNF-α, IL-1β, and MMPs. This continuous inflammatory cycle leads to tissue degeneration and perpetuates the chronicity of OA (Wang and He, 2018).

Inflammatory cytokines play a central role in the pathophysiology of OA. Elevated IL-1β is one of the key mediators that stimulate chondrocyte apoptosis, increase MMP expression, and inhibit extracellular matrix synthesis. The chronic upregulation of IL-1β thus directly contributes to the progressive destruction of cartilage and worsening of joint function (Estee et al., 2023). Therefore, controlling IL-1β expression represents a critical therapeutic target in obesity-related OA (Choi et al., 2019).

Conventional management of OA involves both non-pharmacological and pharmacological approaches. Weight control, low-impact exercise, and physiotherapy help reduce mechanical stress, while nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are often prescribed to alleviate inflammation (Katz et al., 2021; Tong et al., 2023; Wang and He, 2018). However, prolonged NSAID or corticosteroid use may cause adverse effects such as gastrointestinal ulcers, hepatic and renal impairment, and cardiovascular complications (Juma et al., 2024). Hence, alternative and complementary treatments are being increasingly explored to provide safer anti-inflammatory effects and prevent further cartilage damage (Fuggle et al., 2020; Juma et al., 2024).

One promising compound is Halofuginone, a synthetic derivative of febrifuginone, an alkaloid extracted from Dichroa febrifuga (commonly known as Gigil). This traditional medicinal plant, widely found in Indonesia, particularly in West and Central Java, has long been used to treat fever, malaria, and inflammation (Katz et al., 2021; Wenbo et al., 2017). Modern pharmacological research has demonstrated Halofuginone's potential as an antifibrotic, immunomodulatory, and anti-inflammatory agent. By suppressing collagen type I synthesis and inhibiting TGF-β and NF-κβ

signaling pathways, Halofuginone is believed to reduce cytokine-mediated inflammation and oxidative stress (Cui et al., 2016).

Given its pharmacological profile, Halofuginone may offer therapeutic benefits in obesity-related OA by attenuating the inflammatory cascade and lowering IL-1β levels. Nevertheless, scientific evidence on its effectiveness in this context remains limited (Demir et al., 2024).

Based on the aforementioned background, OA remains a major degenerative joint disorder associated with obesity, driven by chronic inflammation mediated by IL-1β and related cytokines. Conventional pharmacological therapies carry notable side effects, highlighting the urgent need for alternative anti-inflammatory agents.

Therefore, this research seeks to analyze the effect of Halofuginone administration on IL-1β levels in obese rat models of OA.

2. MATERIAL AND METHOD

2.1. Research design

This study employed a quantitative, *in vivo* experimental design with a *Post-Test Control Group*. The purpose of the experiment was to analyze the effect of Halofuginone administration on interleukin-1 beta (IL-1β) levels in obese rat models of OA. The study was conducted under controlled laboratory conditions, allowing the researcher to minimize the influence of extraneous variables (Sugiyono, 2015).

Thirty-six male *Rattus norvegicus* (Wistar strain) rats were randomly divided into six groups, each consisting of six rats:

1. K (Control Group): Healthy rats without any intervention.
2. KN1 (Negative Control 1): Rats induced with obesity using a High-Fat Diet (HFD).
3. KN2 (Negative Control 2): Rats induced with obesity and knee OA through Anterior Cruciate Ligament Transection (ACLT) and Destabilization of the Medial Meniscus (DMM).
4. KP (Positive Control): Obese OA rats treated with paracetamol (150 mg/kgBW/day).
5. P1 (Halofuginone Group): Obese OA rats treated with Halofuginone (1.5 mg/kgBW/day).
6. P2 (Combination Group): Obese OA rats treated with Halofuginone (1.5 mg/kgBW/day) and paracetamol (75 mg/kgBW/day).

To prevent postoperative infection after ACLT and DMM, ceftriaxone (100 mg/kg BW, subcutaneously, twice daily) was administered for three consecutive days before the pharmacological intervention (Aydogdu et al., 2018).

2.2. Research location and duration

All experimental procedures, including HFD induction, surgical induction of OA, and biochemical examinations (ELISA for IL-1β), were carried out at the Central Laboratory, Faculty of Medicine, Universitas Islam Bandung (UNISBA). Histopathological analyses were conducted at the Department of Anatomical Pathology, Al-Ihsan Hospital, Bandung.

The research was conducted from June to September 2025, spanning animal model preparation, intervention, data collection, and analysis.

2.3. Experimental subjects

The research subjects were male *Rattus norvegicus* (Wistar strain), aged 8–10 weeks, weighing 250–300g. The sample size was determined using Federer's formula (1963):

$$(k - 1)(n - 1) \geq 15$$

With six groups (k = 6), the minimum sample size per group was five rats. To compensate for potential dropouts, each group contained six rats, for a total of 36 animals.

2.4. Sampling technique and criteria

Purposive sampling was applied based on the following criteria:

Inclusion criteria

Male *Rattus norvegicus* (Wistar strain), 8–10 weeks old, 250–300 g body weight.

Exclusion criteria

Rats showing signs of illness or physiological abnormalities (e.g., piloerection, dull fur, dehydration, or inactivity).

2.5. Experimental procedures

2.5.1. Induction of obesity

Rats in the obesity groups (KN1, KN2, KP, P1, and P2) were fed a High-Fat Diet (HFD; 60% kcal fat; Research Diets D12492) for four weeks. Body weight was recorded periodically, and obesity status was confirmed using the Lee Index (Aydogdu et al., 2018).

2.5.2. Induction of osteoarthritis

OA was induced in groups KN2, KP, P1, and P2 by surgical transection of the anterior cruciate ligament and

destabilization of the medial meniscus (ACLT + DMM) under general anesthesia (ketamine and xylazine). Post-surgical care included subcutaneous ceftriaxone to prevent infection.

2.5.3. Halofuginone and paracetamol administration

After confirming obesity and OA conditions (day 63), treatments were administered orally by gavage for 28 days: (a) **Halofuginone:** 1.5 mg/kgBW/day (P1, P2); (b) **Paracetamol:** 150 mg/kgBW/day (KP) or 75 mg/kgBW/day (P2).

2.5.4. Blood collection and ELISA analysis

On day 91, all animals were anesthetized, and blood samples were collected via cardiac puncture. Serum IL-1β concentrations were quantified using a Rat IL-1β ELISA kit (ELISA method) following standard procedures:

a. Samples and standards were added to antibody-coated wells.
b. After incubation and washing, enzyme-linked detection antibodies were applied.
c. Substrate (TMB) was added, and the reaction was stopped after color development.
d. Optical density was measured at 450 nm using an ELISA reader.
e. IL-1β concentrations were calculated from a standard curve and expressed in pg/mL.

2.6. Data analysis

Data were analyzed using SPSS version 25. Normality was tested using the Shapiro–Wilk test, and homogeneity was tested using Levene's test. If the data were normally distributed and homogeneous, a One-Way ANOVA followed by post hoc Tukey's test was performed. For non-parametric data, the Kruskal–Wallis test followed by Dunn's test was applied. A p-value < 0.05 was considered statistically significant.

2.7. Ethical considerations

All experimental protocols were reviewed and approved by the Animal Ethics Committee of the Faculty of Medicine, Universitas Islam Bandung (UNISBA), ensuring adherence to ethical standards for animal research.

3. RESULT

3.1. IL-1β level data results

The results of IL-1β concentration measurements obtained through ELISA examination are expressed in pg/mL. Table 4.1 presents the mean IL-1β levels in each experimental group. The mean IL-1β level in the control group (K) was 14.67 ± 0.58, in the negative control group 1 (KN-1) was 32.85 ± 4.76, and in the negative control group 2 (KN-2) was 74.51 ± 8.59. Meanwhile, for the positive control group (paracetamol) (KP), the intervention group (halofuginone administration) (P1), and the combination group (Halofuginone and paracetamol) (P2), the mean IL-1β levels were 25.91 ± 1.53, 24.92 ± 0.79, and 20.18 ± 0.90, respectively.

IL-1β concentrations measured by ELISA are reported in pg/mL. As shown in Table 1, the mean IL-1β levels varied across all experimental groups. The control group (K) showed the lowest IL-1β level (14.67 ± 0.58 pg/mL), representing normal conditions without obesity or OA induction.

The negative control-1 group (KN-1), consisting of obese rats without intervention, showed increased IL-1β levels (32.85 ± 4.76 pg/mL), indicating systemic inflammation associated with obesity. The negative control-2 group (KN-2), representing rats with both obesity and OA, demonstrated the highest IL-1β concentration (74.51 ± 8.59 pg/mL). This significant increase suggests that the coexistence of obesity and OA synergistically elevates the inflammatory response.

In contrast, the positive control group (KP) that received paracetamol showed a reduction in IL-1β levels (25.91 ± 1.53 pg/mL) compared with the negative control groups, indicating paracetamol's anti-inflammatory effect. The intervention group (P1) that received Halofuginone also exhibited a decreased IL-1β level (24.92 ± 0.79 pg/mL), suggesting that Halofuginone effectively suppresses inflammation. The combination group (P2), treated with both Halofuginone and paracetamol, had the lowest IL-1β level among the treatment groups (20.18 ± 0.90 pg/mL), suggesting a synergistic anti-inflammatory effect between the two agents.

Overall, the data indicate that halofuginone administration, either alone or in combination with paracetamol, can significantly reduce IL-1β levels in obese rat models with OA,

Table 1
The mean result of the IL-1β examination.

Kelompok	n	Mean IL-1β	Median IL-1β	SD IL-1β
K	6	14.67	14.82	0.58
KN-1	6	32.85	32.36	4.76
KN-2	6	74.51	78.16	8.59
KP	6	25.91	25.42	1.53
P1	6	24.92	24.95	0.79
P2	6	20.18	20.16	0.90

Note: Group K (control), KN-1 (negative control-1/obesity without intervention), KN-2 (negative control-2/knee OA with obesity), KP (positive control/administration of paracetamol), P1 (administration of Halofuginone), P2 (administration of halofuginone combined with paracetamol).

supporting its potential role in modulating inflammatory responses associated with obesity-induced OA (Eitner et al., 2017).

Table 1 presents the mean, median, and standard deviation (SD) of IL-1β levels measured by ELISA across six experimental groups, expressed in pg/mL.

The control group (K), which consisted of normal rats without obesity or OA induction, showed the lowest mean IL-1β concentration (14.67 ± 0.58 pg/mL). This finding indicates a normal physiological condition with minimal inflammatory response.

The negative control-1 group (KN-1), representing obese rats without intervention, exhibited a noticeable increase in IL-1β levels (32.85 ± 4.76 pg/mL). This elevation reflects the onset of chronic low-grade inflammation commonly associated with obesity.

A further increase was observed in the negative control-2 group (KN-2) (74.51 ± 8.59 pg/mL), which consisted of obese rats with OA. The markedly high IL-1β levels in this group confirm that the coexistence of obesity and OA intensifies systemic inflammation, as IL-1β plays a central role in cartilage degradation and joint inflammation.

The positive control group (KP), which received paracetamol treatment, showed a substantial decrease in IL-1β concentration (25.91 ± 1.53 pg/mL) compared to the negative controls. It suggests that paracetamol exerts a mild anti-inflammatory effect, contributing to the reduction of inflammatory cytokines.

Similarly, the intervention group (P1), treated with Halofuginone alone, demonstrated a comparable reduction in IL-1β levels (24.92 ± 0.79 pg/mL), indicating that halofuginone effectively suppresses inflammatory activity in obese rats with OA.

The combination treatment group (P2), which received both halofuginone and paracetamol, had the lowest mean IL-1β value (20.18 ± 0.90 pg/mL). This result suggests a synergistic anti-inflammatory effect between Halofuginone and paracetamol, leading to a more pronounced reduction in IL-1β production.

It might be said that the data in Table 4.1 demonstrate that IL-1β levels were highest in the obese OA group (KN-2) and decreased progressively following pharmacological interventions. Halofuginone, either alone or in combination with paracetamol, effectively reduced IL-1β levels, supporting its potential as a therapeutic agent for controlling inflammation in obesity-related OA models.

Figure 1 illustrates the distribution of IL-1β levels (pg/mL) across the six experimental groups through a boxplot representation. The figure visually supports the numerical findings presented in Table 1, showing distinct variations among groups.

The control group (K) shows the lowest IL-1β levels, with a narrow interquartile range and minimal variability, indicating a stable, normal inflammatory condition.

In the negative control-1 group (KN-1), representing obese rats without intervention, the boxplot shows a clear elevation in IL-1β concentration with moderate variation among samples. This increase reflects the inflammatory response associated with obesity.

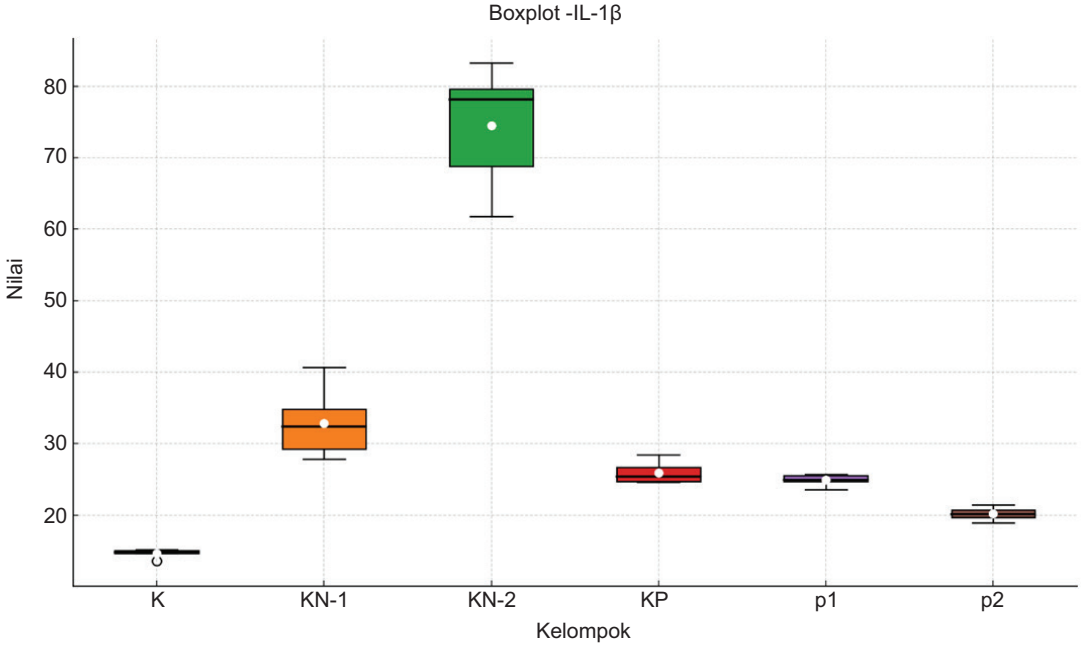

Figure 1. Boxplot of IL-1β levels.

The negative control-2 group (KN-2) exhibits the highest IL-1β levels with a wide box range and a higher upper whisker, indicating pronounced inflammatory activity and biological variability among obese rats with OA. The elevated median and mean values confirm that the coexistence of obesity and OA significantly exacerbates inflammation.

Conversely, the positive control group (KP) treated with paracetamol shows a marked reduction in IL-1β levels compared to the negative control groups, suggesting an anti-inflammatory effect of paracetamol. The data distribution is relatively compact, reflecting a consistent response to treatment.

Similarly, the intervention group (P1), which received halofuginone alone, showed a further reduction in IL-1β levels, comparable to that in the paracetamol-treated group. The narrow interquartile range indicates uniform suppression of inflammatory cytokines among samples.

The combination treatment group (P2), receiving both halofuginone and paracetamol, exhibits the lowest IL-1β values among all treated groups, with minimal variability. This finding implies a synergistic effect between halofuginone and paracetamol in reducing IL-1β production and controlling inflammation.

The boxplot clearly visualizes the trend observed in the descriptive data: IL-1β levels increase sharply in the obese OA model (KN-2) and progressively decrease following pharmacological interventions. The pattern reinforces the hypothesis that Halofuginone has a significant anti-inflammatory effect, particularly when combined with paracetamol, in obese rat models with OA.

Table 1 and Figure 1 present quantitative and visual descriptions of IL-1β levels (pg/mL) across six experimental groups, as determined by ELISA. Both the numerical data and the boxplot illustrate a clear difference in IL-1β concentrations between the control, negative control, and treatment groups.

As indicated in Table 1, the control group (K) showed the lowest mean IL-1β value (14.67 ± 0.58 pg/mL), representing normal physiological conditions with minimal inflammation. This result is visually supported in Figure 1, where the boxplot for group K is at the lowest position and has a narrow range, indicating stable, homogeneous data.

The negative control-1 group (KN-1), representing obese rats without intervention, exhibited a notable increase in IL-1β levels (32.85 ± 4.76 pg/mL). The corresponding boxplot shows a higher median and a moderate interquartile range, reflecting the inflammatory effects of obesity-induced cytokine activation.

A significant escalation is seen in the negative control-2 group (KN-2), with the highest IL-1β concentration (74.51 ± 8.59 pg/mL). The boxplot visually reinforces the finding that KN-2 is at the top of the distribution, indicating severe inflammation and high biological variability. The elevation in both mean and spread values confirms that the coexistence of obesity and OA amplifies IL-1β production as part of a chronic inflammatory process.

In contrast, the positive control group (KP) receiving paracetamol demonstrated a substantial decline in IL-1β (25.91 ± 1.53 pg/mL). The boxplot supports this finding, with a visibly lower median compared to both negative control groups, indicating paracetamol's anti-inflammatory effect on cytokine expression.

The intervention group (P1) treated with Halofuginone showed a comparable reduction (24.92 ± 0.79 pg/mL), as shown in the table and boxplot, which depict a stable, consistent downward trend. It suggests that Halofuginone effectively suppresses IL-1β expression, likely by inhibiting proinflammatory signaling pathways, such as TGF-β and NF-κB.

The combination group (P2), receiving both Halofuginone and paracetamol, exhibited the lowest IL-1β level among the treated groups (20.18 ± 0.90 pg/mL). The boxplot clearly shows this result, with the lowest median and the smallest variability range, indicating a synergistic anti-inflammatory effect between Halofuginone and paracetamol.

In summary, the pattern from both Table 1 and Figure 1 demonstrates a consistent relationship:

a. IL-1β levels were markedly elevated in obese rats, especially those with OA (KN-2).
b. Pharmacological treatments (paracetamol, Halofuginone, and their combination) effectively reduced IL-1β concentrations.
c. The combination treatment (P2) produced the most excellent anti-inflammatory effect, indicating a potential synergistic mechanism.

These findings collectively support the hypothesis that Halofuginone significantly reduces IL-1β levels in obese rat models of OA, either alone or in combination with paracetamol, highlighting its therapeutic potential for controlling inflammation associated with obesity-induced OA.

The normality test was conducted to determine whether the IL-1β data in each treatment group followed a normal distribution. The results show that all groups, including the control (K), negative controls (KN1 and KN2), positive control (KP), and treatment groups (P1 and P2), have p-values greater than 0.05, specifically 0.054, 0.652, 0.192, 0.196, 0.419, and 0.922, respectively. These values indicate that the data in all groups are normally distributed ($p > 0.05$). Thus, it can be concluded that the IL-1β data meet the assumption of normality, allowing for further analysis using parametric statistical tests.

The K group ($p = 0.054$) is close to the significance threshold. However, it remains within the normal range,

Table 2
Normality test of data.

Pemeriksaan	K (p)	Dist	KN1 (p)	Dist	KN2 (p)	Dist	KP (p)	Dist	P1 (p)	Dist	P2 (p)	Dist
IL-1β	0.054	N	0.652	N	0.192	N	0.196	N	0.419	N	0.922	N

*Shapiro–Wilk Test, N: Normal, TN: Not Normal, K: Control group, KN1: Negative control-1, KN2: Negative control-2, KP: Positive control, P1: Intervention group, P2: Combination intervention group.

Table 3
ANOVA result.

Examination	Group	K	KN1	KN2	KP	P1	P2	p-value
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
IL-1β		14.67 ± 0.57	32.85 ± 4.76	74.51 ± 8.58	25.90 ± 1.52	24.92 ± 0.78	20.18 ± 0.90	0.000

suggesting that the data in the primary negative control group are relatively homogeneous, with no significant deviations from normality.

Groups KN1 and KN2 exhibit relatively high p-values (0.652 and 0.192, respectively), indicating a symmetrical, stable distribution of data among individuals in the obesity control groups.

Similarly, groups KP, P1, and P2 display p-values well above 0.05 (0.196, 0.419, and 0.922, respectively), suggesting that the administered treatments, including Halofuginone, did not cause extreme variations among individuals that could affect data homogeneity.

Therefore, the assumption of normality is met, allowing the use of parametric statistical analyses, such as ANOVA, to compare group differences.

ANOVA results for IL-1β levels: **The table presents the mean and standard deviation (SD) of IL-1β levels in each group:**

a. K (Control): **14.67 ± 0.57**
b. KN1 (Negative Control-1/obesity without intervention): **32.85 ± 4.76**
c. KN2 (Negative Control-2/knee osteoarthritis with obesity): **74.51 ± 8.58**
d. KP (Positive Control/paracetamol): **25.90 ± 1.52**
e. P1 (Halofuginone): **24.92 ± 0.78**
f. P2 (Halofuginone + Paracetamol): **20.18 ± 0.90**

The p-value = 0.000 (<0.05) indicates a statistically significant difference between at least two groups. The KN2 group showed the highest IL-1β level, indicating that obesity combined with knee OA leads to a marked increase in inflammation. The intervention groups (P1 and P2) and the positive control (KP) demonstrated reduced IL-1β levels compared to the negative controls, suggesting anti-inflammatory effects of Halofuginone and/or paracetamol administration. The combination treatment (P2) resulted in the lowest IL-1β level among the intervention groups, approaching that of the normal control group (K), indicating a potential synergistic effect in reducing inflammation.

The data for IL-1β, MMP-13, TNF-α, TGF-β1, PGE2, MDA, and clinical assessments through knee range of motion (ROM) measurements in each treatment group were normally distributed ($p > 0.05$). Therefore, parametric statistical tests were applied, specifically ANOVA, followed by post hoc tests to identify the most effective group.

The effect of Halofuginone on IL-1β expression, clinical assessment via knee ROM, and histopathological evaluation using the OARSI score in an obese rat model with knee OA is presented in Table 4.10.

Based on Table 4.10, halofuginone administration significantly reduced IL-1β levels in obese rats with knee OA ($p = 0.000$, $p \leq 0.05$).

To further examine group comparisons, a post hoc analysis was performed, with the results presented in Table 4.

To analyze and determine the effect of halofuginone administration on IL-1β levels in obese rat models with OA, the post hoc results presented in Table 4.11 indicate the following:

a. Halofuginone administration significantly reduced IL-1β levels in the KP, P1, and P2 groups compared to the Control group (K) ($p = 0.000$, $p \leq 0.05$).
b. IL-1β levels were significantly lower in the K, KN2, P1, and P2 groups compared to KN1 ($p = 0.000$, $p \leq 0.05$), except between KP and KN1, which was not statistically significant ($p > 0.05$).
c. Significant reductions in IL-1β were observed in the K, KN1, KP, P1, and P2 groups compared to KN2 ($p = 0.000$, $p \leq 0.05$).
d. Compared to KP, IL-1β levels were significantly lower in the K, KN1, and KN2 groups ($p = 0.000$, $p \leq 0.05$), while no significant differences were found between P1 or P2 and KP ($p > 0.05$).

Table 4
Post hoc table for IL-1β.

Treatment (I)	Treatment (J)	p-value
K	KN1	0.000
K	KN2	0.000
K	KP	0.001
K	P1	0.002
K	P2	0.001
KN1	K	0.000
KN1	KN2	0.000
KN1	KP	0.063
KN1	P1	0.024
KN1	P2	0.000
KN2	K	0.000
KN2	KN1	0.000
KN2	KP	0.000
KN2	P1	0.000
KN2	P2	0.000
KP	K	0.001
KP	KN1	0.063
KP	KN2	0.000
KP	P1	0.998
KP	P2	0.181
P1	K	0.002
P1	KN1	0.024
P1	KN2	0.000
P1	KP	0.998
P1	P2	0.363
P2	K	0.213
P2	KN1	0.000
P2	KN2	0.000
P2	KP	0.181
P2		

*Post hoc test (significant at $p \leq 0.05$).
Note: K = Control; KN1 = Negative Control-1; KN2 = Negative Control-2; KP = Positive Control; P1 = Intervention Group; P2 = Combination Intervention Group.

e. The P1 group showed significantly lower IL-1β levels compared to K, KN1, and KN2 ($p = 0.000$, $p \leq 0.05$), except for KP and P2, which did not differ significantly ($p > 0.05$).
f. For the P2 group, IL-1β levels were significantly reduced compared to KN1 and KN2 ($p = 0.000$, $p \leq 0.05$), whereas no significant differences were observed when compared to K, KP, or P1 ($p > 0.05$).

These findings indicate that Halofuginone, alone or in combination with paracetamol, effectively lowers IL-1β levels in obese rats with knee OA, with the combination therapy (P2) showing a strong effect in several comparisons (Chen et al., 2020; Deshpande et al., 2025).

4. DISUCSSION

These results show that Halofuginone administration (P1 group) and the combination of Halofuginone + Paracetamol (P2 group) significantly reduced IL-1β levels compared to the Control group ($p = 0.000$) and the negative controls (KN1, KN2). These studies support the findings that Halofuginone exerts a strong anti-inflammatory effect.

For example, the mechanisms described by Batarfi et al. (2025)—inhibition of TGF-β signaling, reduction of subchondral bone remodeling, and decreased angiogenesis—demonstrate that Halofuginone acts not only on joint tissue but also on inflammatory pathways, affecting cytokines such as IL-1β. It further strengthens the argument that Halofuginone directly contributes to the reduction of IL-1β in obese OA models.

The findings presented in Table 1 align with established knowledge of IL-1β's role in obesity-related OA. IL-1β is a key proinflammatory cytokine involved in cartilage degradation, synovial inflammation, and the progression of OA (Baechle et al., 2023). The low IL-1β levels observed in the control group (K) reflect the physiological baseline of inflammatory activity. In contrast, the significant elevation in the KN-1 and KN-2 groups demonstrates how obesity and the coexistence of OA amplify systemic inflammation, consistent with prior studies linking metabolic dysregulation and inflammatory cytokines to joint pathology (Nedunchezhiyan et al., 2022).

The observed reduction in IL-1β levels in the halofuginone-treated group (P1) and the combination group (P2) can be mechanistically explained by Halofuginone's modulation of the TGF-β signaling pathway and inhibition of proinflammatory processes. (Sobieh et al., 2023) reported that Halofuginone attenuates OA progression by suppressing TGF-β activity and reducing subchondral angiogenesis, which indirectly decreases the production of proinflammatory cytokines, including IL-1β. Additionally, Halofuginone has been shown to modulate Th17/Treg cell balance, reducing systemic inflammatory signaling (Cui et al., 2016), which may further contribute to the observed suppression of IL-1β in obese OA rats.

The combination treatment (P2) yielded the lowest IL-1β concentrations, suggesting a potential synergistic effect between Halofuginone and paracetamol. Paracetamol is known to exert mild anti-inflammatory and analgesic effects, primarily by inhibiting prostaglandin synthesis (Juma et al., 2024). When combined with Halofuginone, the dual mechanism likely targets both cytokine production and inflammatory mediator pathways, resulting in a more pronounced reduction in IL-1β levels than either agent alone.

Overall, these results corroborate previous research indicating that Halofuginone effectively reduces inflammatory cytokines and protects joint tissue in OA models. The findings

also support the therapeutic potential of Halofuginone, alone or in combination with conventional analgesics such as paracetamol, for controlling IL-1β-mediated inflammation in obesity-associated OA.

Table 1 and Figure 1 provide both quantitative and visual representations of IL-1β levels (pg/mL) across six experimental groups, as determined by ELISA. The data consistently show significant differences in IL-1β concentrations among the control, negative control, and treatment groups, reflecting the influence of obesity, OA, and pharmacological interventions on systemic inflammation.

The control group (K) exhibited the lowest mean IL-1β value (14.67 ± 0.58 pg/mL), representing normal physiological conditions with minimal inflammatory activity. It is consistent with the homeostatic regulation of proinflammatory cytokines under non-pathological conditions (Goldring & Otero, 2011). The corresponding boxplot in Figure 4.1 further confirms this, showing a narrow range and low variability, indicative of stable basal IL-1β expression.

The negative control-1 group (KN-1), representing obese rats without intervention, showed a notable increase in IL-1β levels (32.85 ± 4.76 pg/mL), reflecting chronic low-grade inflammation associated with obesity. It aligns with prior studies demonstrating that obesity induces systemic inflammation through adipose tissue-mediated cytokine production, including IL-1β (De Bari et al., 2015). The boxplot illustrates a moderate spread, highlighting biological variability in response to obesity-induced inflammatory stress.

A further significant increase was observed in the negative control-2 group (KN-2) (74.51 ± 8.59 pg/mL), consisting of obese rats with OA. This marked elevation confirms that the coexistence of obesity and OA exacerbates systemic inflammation. IL-1β plays a central role in cartilage degradation and synovial inflammation, and its elevated levels in KN-2 support the established pathophysiology of obesity-aggravated OA (Kapoor et al., 2011). The boxplot's high median and wide interquartile range indicate both severe inflammation and increased biological variability.

Treatment with paracetamol (KP) reduced IL-1β levels substantially (25.91 ± 1.53 pg/mL), demonstrating its mild anti-inflammatory effects by inhibiting prostaglandin synthesis (Juma et al., 2024). The boxplot confirms this reduction, showing a lower median and decreased variability compared to negative controls.

Halofuginone treatment alone (P1) similarly reduced IL-1β levels (24.92 ± 0.79 pg/mL). It aligns with findings from studies showing that Halofuginone attenuates OA by inhibiting TGF-β signaling and reducing subchondral angiogenesis. These mechanisms indirectly suppress proinflammatory cytokine production, including IL-1β, suggesting that Halofuginone modulates inflammatory signaling pathways such as NF-κB and TGF-β to exert anti-inflammatory effects. The boxplot shows a stable, consistent downward trend, reinforcing the reliability of this effect.

The combination treatment group (P2), receiving both Halofuginone and paracetamol, exhibited the lowest IL-1β level among all treated groups (20.18 ± 0.90 pg/mL). The boxplot shows the lowest median and the least variability, indicating a synergistic anti-inflammatory effect between Halofuginone and paracetamol. This synergism may result from simultaneous targeting of cytokine production (Halofuginone) and prostaglandin-mediated inflammatory pathways (paracetamol), enhancing overall suppression of IL-1β.

The marked elevation of IL-1β levels in the KN-2 group reflects the combined impact of obesity and OA, which synergistically activate inflammatory pathways, particularly through NF-κB and MAPK signaling cascades. This activation enhances transcription of proinflammatory mediators, including IL-1β, TNF-α, and MMPs, leading to cartilage matrix degradation and joint inflammation (Nedunchezhiyan et al., 2022; Wang and He, 2018). These results are consistent with prior findings demonstrating that obesity exacerbates osteoarthritic pathology by amplifying systemic and local inflammatory responses.

Halofuginone administration (P1) significantly reduced IL-1β expression, indicating potent anti-inflammatory activity. Mechanistically, Halofuginone inhibits TGF-β/Smad3 signaling and suppresses Th17 differentiation, thereby decreasing IL-1β and TNF-α synthesis (Aydogdu et al., 2018). The combination treatment (P2) produced the most significant reduction in IL-1β levels, suggesting a synergistic interaction between Halofuginone's molecular inhibition of cytokine synthesis and paracetamol's peripheral anti-inflammatory mechanism via cyclooxygenase inhibition (Katz et al., 2021).

These findings are consistent with previous studies indicating that IL-1β suppression correlates with slower cartilage degradation and improved joint structure in osteoarthritic models (De Roover et al., 2023). The normal distribution of the data, confirmed by the Shapiro-Wilk test ($p > 0.05$), reinforces the reliability of the findings and validates the use of parametric tests to interpret the effects of Halofuginone.

The results presented in Table 4.1 reveal apparent variations in IL-1β levels across the six experimental groups: control (K), negative control 1 (KN1), negative control 2 (KN2), positive control (KP), treatment 1 (P1), and treatment 2 (P2). The control group (K) exhibited the lowest mean IL-1β concentration (14.67 ± 0.58 pg/mL), reflecting baseline physiological conditions with minimal inflammatory activity. In contrast, the KN1 (32.85 ± 4.76 pg/mL) and KN2 (74.51 ± 8.59 pg/mL) groups showed marked elevations, representing

the inflammatory impact of obesity and obesity-associated OA, respectively. These increases align with established knowledge that obesity and OA synergistically enhance systemic and local inflammatory responses, primarily through activation of NF-κB and MAPK pathways, which stimulate the transcription of proinflammatory cytokines such as IL-1β, TNF-α, and MMPs (Koonce & Bravman, 2013).

The positive control group (KP) demonstrated a reduction in IL-1β (25.91 ± 1.53 pg/mL) following paracetamol administration, consistent with its known peripheral anti-inflammatory action through cyclooxygenase inhibition (Graham et al., 2013). Treatment with Halofuginone alone (P1, 24.92 ± 0.79 pg/mL) or in combination with paracetamol (P2, 20.18 ± 0.90 pg/mL) further decreased IL-1β levels, highlighting halofuginone's potent anti-inflammatory effects. Mechanistically, halofuginone suppresses TGF-β/Smad3 signaling and inhibits Th17 differentiation, leading to downregulation of proinflammatory cytokine synthesis, including IL-1β and TNF-α (Jain et al., 2021). The combination treatment (P2) displayed the most pronounced reduction, suggesting a synergistic effect between halofuginone's molecular inhibition of cytokine production and paracetamol's prostaglandin-mediated anti-inflammatory mechanism (Katz et al., 2021).

The boxplot visualization corroborates these findings, showing elevated IL-1β in KN1 and KN2, followed by a progressive decline in KP, P1, and P2 groups. The narrow interquartile ranges observed in the treatment groups indicate more uniform anti-inflammatory responses among subjects. Normality testing confirmed that all data were suitable for parametric analysis ($p > 0.05$), validating the use of ANOVA to assess statistical differences. ANOVA results (Table 4.10) showed a significant effect of treatment on IL-1β levels ($p = 0.000$), confirming that halofuginone administration alone or in combination with paracetamol significantly reduces IL-1β levels compared to the control and negative control groups.

These findings are consistent with prior research demonstrating that IL-1β suppression correlates with slower cartilage degradation and reduced joint inflammation in OA models (Fransen et al., 2011). By attenuating IL-1β production, Halofuginone may mitigate cytokine-mediated.

The epistemology of this study emphasizes that the validity of scientific knowledge arises not from a single type of data but from the convergence of evidence across molecular, functional, and structural levels. The generated knowledge is then compared with prior literature to assess consistency, expand understanding, and strengthen causal inferences about the effects of Halofuginone on cartilage damage and joint integrity in obesity-associated OA.

In the context of OA, IL-1β occupies a central role as the initial trigger of the inflammatory cascade that leads to joint degradation. Under physiological conditions, synovium and chondrocytes maintain homeostasis, tightly balancing anabolic and catabolic signals. However, in obesity, adipose tissue releases adipokines such as leptin, resistin, and visfatin, which induce low-grade systemic inflammation. Activation of tissue macrophages and synoviocytes elevates IL-1β production even prior to mechanical damage. Upon mechanical injury or OA induction, IL-1β becomes the dominant inflammatory mediator, acting through IL-1R1 receptors on chondrocytes and synoviocytes and activating the MyD88–IRAK–TRAF6 signaling pathway, ultimately triggering NF-κB and p38 MAPK pathways. NF-κB activation promotes transcription of inflammatory genes, including COX-2, mPGES-1, TNF-α, and MMPs, particularly MMP-13. These pathways not only amplify inflammation but also directly accelerate cartilage matrix degradation.

In this study, IL-1β levels in the control group (K) remained within the physiological range. In contrast, obese rats (KN-1) showed a nearly twofold increase in IL-1β, consistent with obesity-induced systemic inflammatory tone via TLR4 and NF-κB activation. When OA was induced (KN-2), IL-1β levels dramatically rose to approximately 74.5 pg/mL, indicating strong joint inflammation. This data demonstrates that obesity establishes an inflammatory background that exacerbates the inflammatory response in OA, illustrating the interaction between metabolic and mechanical factors in OA pathogenesis.

Administration of Halofuginone alone (P1) reduced IL-1β levels to around 24.9 pg/mL, while the combination of Halofuginone and paracetamol (P2) further decreased IL-1β to 20.2 pg/mL. This reduction was more significant than in the paracetamol-only group (KP), indicating that Halofuginone acts directly on upstream inflammatory mechanisms rather than merely providing peripheral analgesia. This effect is likely due to halofuginone's inhibition of NF-κB p65 and p38 MAPK activation, reducing IL-1β transcription at both mRNA and protein levels. Several studies support these mechanisms: Mu et al. (2018) demonstrated selective inhibition of Smad and NF-κB by Halofuginone in inflammatory models; Baechle et al. (2023) reported its strong immunomodulatory effects via regulation of proinflammatory gene transcription; and Xu et al. (2025) observed reduced IL-1β expression and cartilage-protective effects in MIA-induced OA rat models.

Epistemologically, the measurement of IL-1β in this study provides strong evidence for halofuginone's ability to intervene at the molecular level in the upstream inflammatory axis of OA. The significant reduction of IL-1β in OA with obesity indicates that Halofuginone is effective even under conditions of elevated baseline inflammation. It extends previous findings, which typically utilized OA models without obesity. From an epistemic perspective, IL-1β serves as a key pillar

for causal inference, demonstrating that halofuginone functions as an anti-inflammatory modulator rather than merely a symptomatic analgesic.

This research provides the first scientific evidence in an obesity-related OA model that Halofuginone effectively reduces the expression of inflammatory biomarkers, particularly IL-1β.

5. CONCLUSION

This study demonstrated that halofuginone administration significantly reduced IL-1β levels in obese rat models with OA, confirming its anti-inflammatory potential. The findings revealed that obesity and OA synergistically elevated IL-1β expression, indicating a heightened inflammatory response in comorbid conditions. Quantitative analysis showed the highest IL-1β levels in the obese OA group (KN2: 74.51 ± 8.59 pg/mL), while treatment with Halofuginone alone (P1: 24.92 ± 0.79 pg/mL) and in combination with paracetamol (P2: 20.18 ± 0.90 pg/mL) markedly reduced cytokine expression.

Statistical analysis (ANOVA, $p = 0.000$) confirmed significant differences among groups, and post-hoc testing revealed that Halofuginone, either alone or combined with paracetamol, produces an anti-inflammatory effect comparable to or superior to that of paracetamol alone (van der Heijden et al., 2015). The combination therapy demonstrated the most significant suppression of IL-1β, suggesting a synergistic mechanism for modulating inflammatory pathways.

Overall, the results indicate that Halofuginone effectively attenuates systemic inflammation in obesity-induced OA by downregulating IL-1β. These findings support the potential application of Halofuginone as an adjunctive or alternative therapeutic agent for managing inflammation and joint degeneration in obesity-related OA. Further studies are recommended to elucidate its molecular mechanisms, optimal dosage, and long-term safety profile in clinical settings.

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