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Turmeric extract reverses diclofenac-induced malondialdehyde elevation: comparative efficacy of two therapeutic doses

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ABSTRACT

Background: Chronic diclofenac use generates excessive reactive oxygen species, causing lipid peroxidation and elevated malondialdehyde (MDA) levels. Turmeric extract contains curcuminoids with antioxidant properties that may reverse NSAID-induced oxidative damage.

Objectives: To compare the efficacy of turmeric extract at two therapeutic doses in reversing diclofenac-induced MDA elevation in rats.

Methods: Twenty-eight male Sprague-Dawley rats were divided into four groups (n=7): normal control, negative control (diclofenac 10 mg/kg, days 8-14), treatment 1 (diclofenac + extract 100 mg/kg, days 15-28), and treatment 2 (diclofenac + extract 200 mg/kg, days 15-28). Blood MDA levels were measured spectrophotometrically and analyzed using one-way ANOVA with Dunnett's T3 post-hoc test.

Results: Diclofenac significantly elevated MDA levels by 18.0% ($p<0.001$). Both turmeric extract doses significantly reduced MDA compared to diclofenac-only treatment: 100 mg/kg achieved 14.6% reduction ($p=0.001$) while 200 mg/kg demonstrated superior efficacy with 18.3% reduction ($p<0.001$), restoring levels below baseline.

Conclusion: Turmeric extract demonstrates dose-related efficacy in reversing diclofenac-induced oxidative stress, with 200 mg/kg showing superior therapeutic effects, supporting its potential as adjunct therapy for chronic NSAID users.

Keywords: Antioxidants, Curcuma longa, diclofenac, malondialdehyde, oxidative stress

Introduction

Excessive reactive oxygen species (ROS) generation disrupts redox homeostasis, triggering oxidative damage to cellular macromolecules—including lipids, proteins, and DNA—and contributing to diverse pathological conditions such as cardiovascular disease, cancer, neurodegenerative disorders, and accelerated aging [1]. Among pharmacological ROS sources, chronic non-steroidal anti-inflammatory drug (NSAID) use represents a significant contributor to oxidative stress. Diclofenac, one of the most frequently prescribed NSAIDs globally, undergoes hepatic metabolism via cytochrome P450 enzymes, generating reactive metabolites and excessive ROS that precipitate hepatotoxic and nephrotoxic complications, particularly in elderly populations [1,2]. These adverse effects—including peptic ulcer formation, renal dysfunction with secondary hypertension, and gastrointestinal bleeding—limit long-term NSAID utility despite their therapeutic efficacy for musculoskeletal pain management.

Globally, over 30 million people use NSAIDs daily for chronic pain management, with diclofenac ranking among the most prescribed analgesics worldwide [3]. Geriatric populations, who constitute the majority of chronic NSAID users, exhibit heightened vulnerability to oxidative stress-

related adverse effects due to age-related decline in endogenous antioxidant capacity. The associated morbidity and healthcare costs underscore the urgent need for safe, cost-effective adjunct therapies to mitigate NSAID-induced oxidative complications—a significant clinical and public health priority.

Malondialdehyde (MDA), a stable end-product of lipid peroxidation, serves as a gold-standard biomarker for quantifying oxidative tissue damage, with elevated levels directly correlating with NSAID-induced hepatotoxicity and nephrotoxicity severity [4]. Unlike short-lived ROS species, MDA's relative stability in biological fluids enables accurate quantification and makes it particularly suitable for evaluating antioxidant intervention efficacy. Elevated MDA concentrations have been implicated in cardiovascular disease, hepatic dysfunction, Parkinson's disease, cancer, and metabolic syndrome, reflecting its broad utility as an oxidative stress indicator [4].

When endogenous antioxidant defenses—comprising enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (glutathione, vitamins C and E) systems—become overwhelmed by excessive ROS generation, supplementation with exogenous antioxidants becomes essential. Plant-derived antioxidants offer particular therapeutic promise due to their multi-targeted mechanisms, favorable safety profiles, and accessibility compared to synthetic alternatives.

Curcuma longa L. (turmeric), a Zingiberaceae family member widely used in traditional medicine, contains curcuminoids—principally curcumin, demethoxycurcumin, and bisdemethoxycurcumin—as major bioactive constituents with demonstrated antioxidant, anti-inflammatory, and hepatoprotective properties [5,6]. Unlike synthetic antioxidants that typically act through single mechanisms, turmeric extract offers multi-target therapeutic potential through synergistic actions of multiple phytochemicals, including flavonoids (quercetin), carotenoids (β-carotene), and phenolic acids (caffeic acid, cinnamic acid) [6]. Curcumin's antioxidant activity operates through direct ROS scavenging via hydrogen atom donation, activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway to upregulate endogenous antioxidant enzymes, and suppression of pro-inflammatory pathways (NF-κB, COX-2) that amplify oxidative damage [5,7]. Previous investigations have confirmed substantial antioxidant capacity of turmeric extracts in vitro; however, translation of these findings to clinically relevant in vivo models requires systematic evaluation.

Despite extensive documentation of turmeric's antioxidant properties, critical gaps remain in understanding its therapeutic application against NSAID-induced oxidative stress. Studies examining turmeric's efficacy in reversing diclofenac-induced lipid peroxidation are scarce, particularly regarding post-treatment interventions that better simulate clinical scenarios where oxidative damage is already present. Most existing studies employ concurrent administration protocols, which demonstrate preventive rather than therapeutic efficacy. Therefore, this study aimed to compare the efficacy of turmeric extract at two therapeutic doses (100 mg/kg vs. 200 mg/kg) in reversing diclofenac-induced oxidative stress, quantified by malondialdehyde levels in a rat model. These findings may provide foundation for evidence-based phytotherapeutic interventions and guide future comprehensive dose-response investigations.

Methods

Ethical approval

All experimental procedures were approved by the Livestock, Marine, and Fishery Ethics Committee of Universitas Nusa Cendana (Approval No.: 110/1.KT/KEPPKP/VII/2024, Date: July 18, 2024) and conducted in accordance with institutional guidelines for the care and use of laboratory animals.

Experimental animals and housing

Twenty-eight male white rats (*Rattus norvegicus*, Sprague-Dawley strain) were housed in standard polycarbonate cages with four rats per cage, provided with wood shaving bedding changed twice

weekly. Rats had ad libitum access to standard commercial pellet feed (BR-1) and tap water in sterilized bottles. All animals underwent a seven-day acclimatization period before experimental procedures to minimize stress-related variables.

Experimental design and randomization

A randomized controlled experimental design with four groups was employed. Animals were randomly assigned to groups using a computer-generated random number sequence to ensure unbiased allocation. Sample size (n = 7 per group) was determined based on previous studies and resource optimization principle for animal research [8]. The four experimental groups were: (i) normal control (NC): standard feed + distilled water (1 mL/day, oral); negative control (NEGC): standard feed + sodium diclofenac (10 mg/kg BW, oral, 7 days); (iii) treatment 1 (P1): sodium diclofenac (10 mg/kg BW, 7 days) + turmeric extract (100 mg/kg BW, 14 days); (iv) treatment 2 (P2): sodium diclofenac (10 mg/kg BW, 7 days) + turmeric extract (200 mg/kg BW, 14 days).

Plant extract preparation

Fresh turmeric rhizomes were collected from Nunsaen Village, Central Fatuleu Subdistrict, Kupang Regency, East Nusa Tenggara Province, Indonesia (9°58'34"S, 123°52'19"E) and authenticated at the Herbal NTT Laboratory, Kupang City. Fresh rhizomes (8 kg) were washed, drained, sliced, and air-dried, yielding 1,619 g. The dried material was pulverized using a mechanical blender and sieved (40-mesh) to obtain 1,500 g of fine powder (simplisia).

The powder was macerated with 95% ethanol (7.5 L, 1:5 w/v ratio) for 72 hours at room temperature with periodic agitation. After vacuum filtration through Whatman No. 1 filter paper (6,550 mL filtrate), the marc was re-macerated with fresh ethanol (3 L) for 24 hours, and filtrates were combined. The combined extract was concentrated using a rotary evaporator (Heidolph Laborota 4000) at 40°C under reduced pressure, then air-dried in a laminar flow hood to yield 139 g of crude extract (9.3% yield w/w).

Ethanol-free test

To ensure complete removal of ethanol from the extract, 1 mL of thick turmeric extract was placed in a test tube. Five drops of concentrated sulfuric acid (H_2SO_4, 98%) and 2 mL of potassium dichromate solution ($K_2Cr_2O_7$, 5% w/v) were added. A negative result (no color change to green-blue) indicates absence of ethanol, confirming the extract was suitable for animal administration without risk of ethanol-related toxicity.

Phytochemical screening

Preliminary phytochemical screening was conducted to identify major classes of secondary metabolites present in the extract using standard methods [9].

Alkaloid test: Extract (5 g) was dissolved in chloroform (10 mL) and ammonia solution (5 mL, 10%), filtered, acidified with sulfuric acid (10 drops, 2M), and tested with Mayer's, Dragendorff's, and Wagner's reagents.

Flavonoid test: Extract (0.1 g) was mixed with magnesium powder (0.1 g), amyl alcohol (0.4 mL), and ethanol (4 mL, 96%), shaken vigorously; red, yellow, or orange color indicates flavonoids.

Saponin test: Extract (0.1 g) in distilled water (10 mL) was shaken; stable foam (≥1 cm, persisting ≥10 minutes) indicates saponins.

Triterpenoid/steroid test: Extract (0.1 g) in chloroform (2 mL) with acetic anhydride (3 drops) and concentrated sulfuric acid (1 drop); green/blue indicates steroids, purple/red indicates triterpenoids.

Tannin test: Boiled extract filtrate (0.1 g in 10 mL water) with ferric chloride (5 drops, 1% $FeCl_3$); black, blue-black, or green-black color indicates tannins.

Administration of sodium diclofenac and turmeric extract

Sodium diclofenac powder was dissolved in sterile distilled water to achieve 2 mg/mL concentration. Turmeric extract stock solutions were prepared using 0.5% sodium carboxymethylcellulose (NaCMC)

as suspending agent: 1,000 mg extract in 100 mL NaCMC (10 mg/mL) for the 100 mg/kg dose, and 2,000 mg extract in 100 mL NaCMC (20 mg/mL) for the 200 mg/kg dose. All solutions were prepared fresh daily and protected from light.

All oral administrations were performed using gavage technique with a ball-tipped gavage needle. Administration volume was standardized at 1 mL per 200 g body weight, adjusted proportionally for individual variations. Diclofenac administration (days 8-14): Animals in groups NEGC, P1, and P2 received sodium diclofenac solution (10 mg/kg BW) orally; NC group received equivalent volume of distilled water. Turmeric extract administration (days 15-28): Animals in groups P1 and P2 received turmeric extract suspensions (100 mg/kg and 200 mg/kg BW, respectively) orally; NC and NEGC groups received equivalent volume of 0.5% NaCMC vehicle solution.

Sample collection

Cardiac blood samples were collected following total anesthesia via inhalation. Rats were placed in a closed container with cotton wool moistened with 0.2 mL of ether and observed until immobile. Anesthetized animals were positioned supine on a styrofoam-backed surface with limbs secured using needles. Cardiac blood was collected via thoracotomy using surgical scissors, a scalpel, and a 3 mL disposable syringe [8].

Malondialdehyde measurement

Blood samples were immediately transferred to EDTA-coated tubes, wrapped in aluminum foil, and stored at -80°C until analysis. MDA levels were determined by mixing whole blood with thiobarbituric acid (TBA) reagent, and absorbance was measured at 532 nm using a UV spectrophotometer [8].

Data analysis

MDA levels were analyzed using parametric statistical methods. Data normality was assessed using the Shapiro-Wilk test (appropriate for sample sizes <50), and variance homogeneity was evaluated using Levene's test. Group differences were analyzed using One-Way ANOVA, followed by Dunnett's T3 post-hoc test for pairwise comparisons.

Results

Phytochemical screening results

Preliminary phytochemical analysis of the turmeric extract revealed the presence of alkaloids, tannins, flavonoids, saponins, and steroids. The ethanol-free test confirmed complete removal of the extraction solvent, indicating the extract was suitable for animal administration without risk of ethanol-related toxicity.

Malondialdehyde (MDA) levels in blood

MDA levels, a biomarker of lipid peroxidation and oxidative stress, were measured in whole blood samples from all experimental groups. Data were

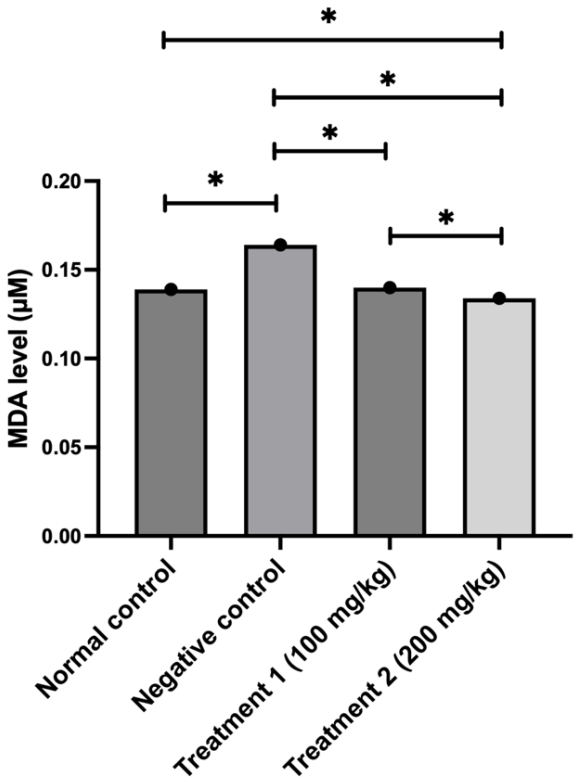

Figure 1. Turmeric extract reduces malondialdehyde levels in diclofenac-treated rats. MDA concentrations (µM) in blood samples from normal control (NC), diclofenac-treated (NEGC), and diclofenac + turmeric extract-treated groups (P1: 100 mg/kg; P2: 200 mg/kg). Data are mean (n=7). Different letters indicate significant differences between groups ($p < 0.05$, Dunnett's T3 test). *$p < 0.05$

normally distributed across all groups (Shapiro-Wilk test, $p > 0.05$) but showed heterogeneous variances (Levene's test, $p < 0.001$), necessitating the use of Dunnett's T3 post-hoc test following one-way ANOVA. Figure 1 presents the MDA levels across treatment groups, calculated using the conversion equation $y = 0.0184x + 0.1241$ ($R^2 = 0.99$).

One-way ANOVA revealed highly significant differences in MDA levels among the four groups ($p < 0.001$). Post-hoc pairwise comparisons using Dunnett's T3 test showed that the negative control group had significantly higher MDA levels than the normal control ($p < 0.001$), confirming successful induction of oxidative stress by diclofenac.

Both treatment groups demonstrated significantly lower MDA levels compared to the negative control: P1 (100 mg/kg) reduced MDA by 14.6% ($p = 0.001$), while P2 (200 mg/kg) achieved an 18.3% reduction ($p < 0.001$). Notably, the P2 group showed significantly lower MDA levels than P1 ($p < 0.001$), demonstrating dose-related efficacy with superior protection at the higher dose. The P2 group also achieved MDA levels significantly below the normal control ($p = 0.005$), suggesting robust antioxidant activity extending beyond reversal of drug-induced oxidative stress.

Discussion

This study demonstrates that turmeric extract (*Curcuma longa* L.) exerts significant dose-related antioxidant effects in reversing diclofenac-induced oxidative stress in rats, as evidenced by reduced malondialdehyde (MDA) levels. Our findings provide experimental support for the potential use of turmeric extract as an adjunct therapy in individuals requiring chronic NSAID treatment. The key findings are: (1) diclofenac administration significantly elevated MDA levels by 18.0%, confirming oxidative stress induction; (2) both turmeric extract doses (100 and 200 mg/kg) significantly reduced MDA levels compared to diclofenac-only treatment; and (3) the 200 mg/kg dose demonstrated superior efficacy, achieving an 18.3% reduction in MDA levels and restoring oxidative balance below baseline values.

The 18.0% elevation in MDA levels observed in the diclofenac-treated group (NEGC: 0.164 µM) compared to controls (NC: 0.139 µM) confirms successful induction of oxidative stress and validates our experimental model. This finding aligns with established mechanisms of NSAID-induced oxidative damage, whereby diclofenac undergoes hepatic metabolism via cytochrome P450 enzymes, generating reactive metabolites and reactive oxygen species (ROS) that overwhelm endogenous antioxidant defenses and trigger lipid peroxidation [1,3]. The magnitude of MDA elevation is consistent with previous rodent models of NSAID-induced oxidative stress [9], supporting the validity of our experimental approach.

Both doses of turmeric extract demonstrated significant therapeutic effects in reversing established oxidative stress. The 100 mg/kg dose reduced MDA levels by 14.6% compared to the negative control ($p = 0.001$), restoring values to levels statistically indistinguishable from the normal control ($p = 0.921$). The 200 mg/kg dose showed superior efficacy, reducing MDA levels by 18.3% ($p < 0.001$) and achieving levels significantly below even the normal control baseline ($p = 0.005$). This dose-related efficacy (P1 vs. P2, $p < 0.001$) aligns with pharmacological principles wherein antioxidant activity increases with higher concentrations of bioactive compounds [5,6,7].

The observed MDA reduction likely results from the synergistic action of multiple bioactive compounds in turmeric extract, particularly curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin), flavonoids, and phenolic acids confirmed by our phytochemical screening. While our study did not directly investigate molecular mechanisms, the literature suggests these compounds may act through direct ROS scavenging, activation of endogenous antioxidant pathways (such as Nrf2 signaling), and suppression of inflammatory mediators [10,11,12]. The sustained effect over 14 days of treatment suggests involvement of genomic mechanisms beyond simple radical scavenging. Future mechanistic studies measuring endogenous antioxidant enzyme activities

and pathway activation markers would provide molecular-level confirmation.

A notable aspect of this study is the post-treatment intervention design, wherein turmeric extract was administered after cessation of diclofenac rather than concurrently. This approach better simulates clinical scenarios where oxidative damage is already established when patients seek intervention, demonstrating therapeutic rather than merely preventive efficacy. The robust MDA reduction observed indicates that turmeric extract can effectively reverse established oxidative damage, which has important translational implications for chronic NSAID users who may already have accumulated oxidative injury.

Several aspects warrant further investigation. First, we measured only MDA as an oxidative stress biomarker; future studies should include additional markers (protein carbonyls, 8-OHdG, total antioxidant capacity) and assess endogenous antioxidant enzyme activities to comprehensively characterize the antioxidant response and elucidate mechanisms. Second, histopathological examination of liver and kidney tissues would provide direct evidence of tissue-level protection beyond circulating biomarkers. Third, our 14-day treatment period addressed acute reversal of oxidative stress; longer-term studies are needed to evaluate sustained efficacy and safety with chronic administration. Finally, pharmacokinetic assessment of curcumin bioavailability and plasma concentrations would establish dose-exposure relationships and guide formulation strategies to overcome curcumin's known bioavailability limitations, facilitating clinical translation.

Conclusion

Turmeric extract demonstrates significant dose-related effects in reversing diclofenac-induced oxidative stress, with the 200 mg/kg dose achieving 18.3% MDA reduction and restoring oxidative balance. These findings support its potential as an adjunct therapy for individuals requiring chronic NSAID treatment and provide foundation for future mechanistic and translational studies.

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Author contributions

Conceptualization, KAJS, ALSA, and KL; Methodology, KAJS, ALSA, RLN, and KL; Investigation, KAJS; Formal Analysis, KAJS; Data Curation, KAJS; Writing—Original Draft Preparation, KAJS; Writing—Review & Editing, KAJS, ALSA, RLN, and KL; Supervision, ALSA, RLN, and KL; Project Administration, KAJS; Visualization, KAJS; Resources, ALSA, RLN, and KL; Funding Acquisition, KL. All authors have read and agreed to the published version of the manuscript.

Declaration of interest

The authors declare that none of them has any conflict of interest with any private, public or academic party related to the information contained in this manuscript.

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