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Effect of turmeric extract on glutathione levels in diclofenac-induced oxidative stress in rats

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ABSTRACT

Background: Diclofenac sodium, a commonly prescribed nonsteroidal anti-inflammatory drug (NSAID), depletes glutathione (GSH) through hepatic metabolism, causing oxidative damage. Turmeric (*Curcuma longa* L.) contains curcumin, a potent antioxidant that may restore endogenous antioxidant capacity.

Objective: To evaluate the restorative effect of turmeric extract on glutathione levels in rats following diclofenac-induced oxidative stress.

Methods: Twenty-eight male Wistar rats were divided into four groups (n=7): normal control, negative control (diclofenac sodium 10 mg/kg BW for 7 days), and two treatment groups receiving turmeric extract (100 or 200 mg/kg BW for 14 days) after diclofenac exposure. Cardiac blood glutathione levels were measured spectrophotometrically.

Results: Both turmeric extract doses significantly restored glutathione levels compared to diclofenac-only controls ($p<0.05$). The 200 mg/kg BW dose demonstrated superior efficacy, elevating GSH levels significantly above all groups ($p<0.001$) including normal controls, indicating dose-dependent antioxidant activity.

Conclusion: Turmeric extract exhibits significant dose-dependent restorative effects on glutathione following diclofenac-induced depletion, with the 200 mg/kg BW dose achieving superior GSH restoration ($p<0.001$). These findings suggest potential as an adjunct therapy for managing NSAID-induced oxidative complications, though clinical translation requires further investigation.

Keywords: curcumin, diclofenac, glutathione, oxidative stress, turmeric extract

Introduction

Free radicals are highly reactive molecules generated from both endogenous and exogenous processes, with mitochondria serving as the primary source of reactive oxygen species (ROS) formation at the cellular level [1]. Due to their unpaired electrons, free radicals can damage various cellular components including lipids, proteins, and DNA, resulting in oxidative stress. Persistent oxidative stress disrupts normal cellular and tissue function and can ultimately lead to cell death. The accumulation of oxidative damage contributes to the pathogenesis of various chronic diseases, including degenerative disorders, chronic inflammatory conditions, and neurodegenerative diseases [2].

To counteract oxidative stress, the body relies on both endogenous (internally produced) and exogenous (externally derived) antioxidants. Glutathione (GSH) is one of the principal endogenous antioxidants, playing a critical role in neutralizing ROS and protecting cells from oxidative damage [2,3].

Glutathione in mammals exists in two interconvertible forms: reduced glutathione (GSH) and oxidized glutathione (GSSG). GSH represents the active, reduced form that functions primarily in free radical neutralization [4]. Structurally, GSH is a tripeptide composed of glutamic acid, cysteine, and glycine, with the cysteine residue providing the reactive sulfhydryl (-SH) group essential for its antioxidant activity. Beyond direct ROS scavenging,

GSH participates in numerous critical biological processes including xenobiotic detoxification, amino acid transport, DNA repair, protein thiol protection, and activation of other antioxidant defense systems [5].

An imbalance between glutathione availability and free radical production leads to increased oxidative stress, which underlies the pathophysiology of many serious diseases including chronic inflammation, viral infections (such as HIV), cancer, stroke, diabetes mellitus, neurodegenerative diseases (including Alzheimer's and Parkinson's disease), and hepatic disorders [6].

Oxidative stress can arise from various endogenous and exogenous sources. Endogenous sources include normal metabolic processes such as mitochondrial respiration, during which electron leakage from the electron transport chain generates superoxide radicals, as well as enzymatic reactions catalyzed by xanthine oxidase and NADPH oxidase. Exogenous sources encompass environmental pollution, ultraviolet radiation, heavy metal exposure, and xenobiotic compounds including pharmaceutical agents. Diclofenac sodium, a widely prescribed nonsteroidal anti-inflammatory drug (NSAID), exemplifies a pharmaceutical agent that can induce oxidative stress through generation of reactive metabolites that damage cellular structures and deplete antioxidant reserves during hepatic metabolism [7].

Diclofenac sodium, though highly effective and among the most commonly prescribed NSAIDs for managing pain and inflammation, can cause serious adverse effects including hepatotoxicity and nephrotoxicity when used long-term or at high doses. These toxic effects are primarily mediated by oxidative stress resulting from diclofenac metabolism in the liver, where cytochrome P450 enzymes generate reactive quinone-imine intermediates that bind to cellular macromolecules and consume glutathione. Studies have demonstrated that diclofenac administration increases free radical production while simultaneously depleting endogenous antioxidants such as glutathione, creating a state of oxidative imbalance [6].

Turmeric (*Curcuma longa* L.), a rhizomatous herbaceous plant widely used in traditional medicine, serves as a rich source of exogenous antioxidants that can augment endogenous antioxidant defenses. Phytochemical analyses have identified numerous bioactive compounds in turmeric rhizomes, including ascorbic acid, quercetin, β-carotene, and most notably, curcumin—the primary polyphenolic constituent responsible for turmeric's characteristic yellow color and its potent antioxidant properties [8].

Curcumin exhibits multifaceted antioxidant activity through both direct free radical scavenging mechanisms and indirect pathways involving upregulation of endogenous antioxidant enzymes, including those involved in glutathione synthesis and recycling. Previous research has demonstrated that turmeric supplementation effectively increases systemic antioxidant capacity, protects cells from oxidative damage, and improves cellular redox balance [8,9].

Previous studies have demonstrated curcumin's restorative effects against drug-induced oxidative stress in rodent models. Oral curcumin administration at 100 mg/kg body weight (BW) dosage for 2 weeks in Wistar rats subjected to paracetamol-induced hepatotoxicity revealed significant increases in hepatic glutathione levels in curcumin-treated animals compared to paracetamol-only controls, indicating restoration of antioxidant capacity [10]. Similarly, dose-dependent increases in glutathione concentrations were observed following turmeric extract administration (200 mg/kg BW and 400 mg/kg BW) for 2 weeks in paracetamol-induced rats, with higher doses providing greater antioxidant protection [11]. These studies establish that curcumin/turmeric supplementation can counteract pharmaceutical-induced glutathione depletion when administered following toxic insult.

Despite evidence supporting curcumin's restorative effects against paracetamol-induced oxidative stress, limited research has examined its efficacy in ameliorating diclofenac-induced oxidative damage, which operates through distinct metabolic pathways involving quinone-imine formation rather

than the N-acetyl-p-benzoquinone imine (NAPQI) mechanism characteristic of paracetamol toxicity. Additionally, optimal dosing strategies for turmeric extract in restoring glutathione levels following NSAID-induced depletion remain incompletely characterized, particularly given the known differences in bioavailability and metabolism between paracetamol and diclofenac

Therefore, the aim of this research was to evaluate the restorative effect of turmeric extract at two dose levels (100 mg/kg BW and 200 mg/kg BW) on glutathione levels in Wistar rats following diclofenac sodium-induced oxidative stress. This study addresses an important knowledge gap by investigating whether turmeric extract can restore depleted glutathione levels after NSAID-induced oxidative damage and by characterizing the dose-response relationship for this restorative effect. Understanding the potential of turmeric extract to ameliorate NSAID-induced glutathione depletion may inform strategies for managing oxidative complications in patients requiring long-term NSAID therapy.

Methods

Study design and ethical approval

This experimental study employed a post-test-only control group design. 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) and conducted in accordance with institutional guidelines for the care and use of laboratory animals.

Experimental animals and group assignment

Twenty-eight male Wistar rats (*Rattus norvegicus*) were housed in individual cages under controlled conditions with ad libitum access to standard pellet feed and water. Rats underwent a 7-day acclimatization period before experimental procedures began. Animals were randomly divided into four groups (n=7 per group):

1. N (Normal control): Received standard feed, water, and vehicle only
2. C- (Negative control): Received diclofenac sodium only
3. P1 (Treatment 1): Received diclofenac sodium + turmeric extract 100 mg/kg BW
4. P2 (Treatment 2): Received diclofenac sodium + turmeric extract 200 mg/kg BW

Plant material collection and authentication

Fresh turmeric (*Curcuma longa* L.) rhizomes were collected at Nunsaen Village, Central Fatuleu Subdistrict, Kupang Regency, East Nusa Tenggara, Indonesia (geographic coordinates: 9°58'34"S, 123°52'19"E). Botanical identification and authentication were performed at the Herbal NTT Laboratory, Kayu Putih Village, Oebobo Sub-district, Kupang City.

Preparation of turmeric extract

Fresh turmeric rhizomes (8 kg) were thoroughly washed with clean running water, drained, and sliced into thin sections. The sliced rhizomes were air-dried at room temperature (25-30°C) until constant weight was achieved. The dried material (1,619 g) was pulverized using a mechanical blender and passed through a mesh sieve to obtain fine powder (final yield: 1,500 g) [12].

The powdered turmeric (1,500 g) was macerated in 95% ethanol (7.5 L) at room temperature for 72 hours with occasional stirring. The macerate was filtered through Whatman No. 1 filter paper, yielding 6,550 mL of liquid extract. The filtrate was concentrated using a rotary evaporator (Heidolph, Germany) at 40°C under reduced pressure to remove ethanol completely. The concentrated extract was further air-dried to obtain thick extract (final yield: 139 g, 9.3% w/w) [13].

Two stock solutions were prepared by suspending the thick turmeric extract in 0.5% sodium carboxymethylcellulose (Na-CMC) solution: 1,000 mg of thick extract was suspended in 100 mL of 0.5% Na-CMC solution for the 100 mg/kg BW dose (final concentration: 10 mg/mL), and

2,000 mg of thick extract was suspended in 100 mL of 0.5% Na-CMC solution for the 200 mg/kg BW dose (final concentration: 20 mg/mL).

Phytochemical screening

Ethanol residue test. One milliliter of turmeric extract was placed in a test tube, followed by addition of 5 drops of concentrated sulfuric acid (H_2SO_4) and 2 mL of potassium dichromate ($K_2Cr_2O_7$) solution. The absence of blue coloration indicated complete ethanol removal [14].

Alkaloid test. Five grams of extract was dissolved in chloroform, filtered, and treated with 2 M sulfuric acid. The mixture was shaken vigorously and divided into four portions: one blank and three test portions. Each test portion received one of three alkaloid reagents (Mayer's, Dragendorff's, and Wagner's reagents). Formation of white or reddish-brown precipitate indicated the presence of alkaloids.

Flavonoid test. Extract (0.1 g) was mixed with magnesium powder (0.1 g), amyl alcohol (0.4 mL), and 96% ethanol (4 mL), then shaken vigorously. Development of red, yellow, or orange coloration in the amyl alcohol layer indicated the presence of flavonoids.

Saponin test. Extract (0.1 g) was mixed with distilled water (10 mL) and shaken vigorously for 30 seconds. After addition of 1 M HCl, formation of stable foam (approximately 1 cm height) lasting for at least 10 minutes indicated the presence of saponins.

Triterpenoid and steroid test. Extract (0.1 g) was treated with 3 drops of acetic anhydride followed by 1 drop of concentrated H_2SO_4. Green coloration indicated steroids, while purple or red coloration indicated triterpenoids.

Tannin test. Extract (0.1 g) was mixed with distilled water (10 mL), allowed to stand for 5 minutes, and filtered. Five drops of 1% ferric chloride ($FeCl_3$) solution were added to the filtrate. Development of black or blue-black coloration indicated the presence of tannins.

Experimental protocol and treatment administration

The experimental protocol consisted of three phases: acclimatization (Days 1-7), induction (Days 8-14), and intervention (Days 15-28). During the induction phase (Days 8-14), animals in groups C-, P1, and P2 received diclofenac sodium (Sigma-Aldrich, USA) at a dose of 10 mg/kg BW dissolved in 0.5% Na-CMC solution, administered once daily via oral gavage for 7 consecutive days. The normal control group (N) received an equivalent volume of 0.5% Na-CMC vehicle only. This dosing regimen was selected based on established protocols for inducing oxidative stress in rodent models [6].

During the intervention phase (Days 15-28), following the diclofenac induction period, animals in treatment groups P1 and P2 received turmeric extract at doses of 100 mg/kg BW and 200 mg/kg BW, respectively, administered once daily via oral gavage for 14 consecutive days. Groups N and C- received equivalent volumes of 0.5% Na-CMC vehicle during this period. These doses were selected based on previous studies demonstrating restorative effects of curcumin against drug-induced oxidative stress [10,11].

All oral administrations were performed between 08:00 and 10:00 hours to minimize circadian variation effects. The experimental protocol consisted of three phase: acclimation (Days 1-7), induction (Days 8-14), and intervention (Days 15-28), as illustrated in Figure 1.

Blood sample collection

At the end of the experimental period (Day 29), animals were euthanized using ether inhalation. Each animal was placed in a closed induction chamber containing ether-soaked cotton until loss of consciousness and cessation of respiration were confirmed. Once deeply anesthetized, animals were positioned in dorsal recumbency on a dissection board with limbs secured using pins. The thoracic cavity was opened using surgical scissors and

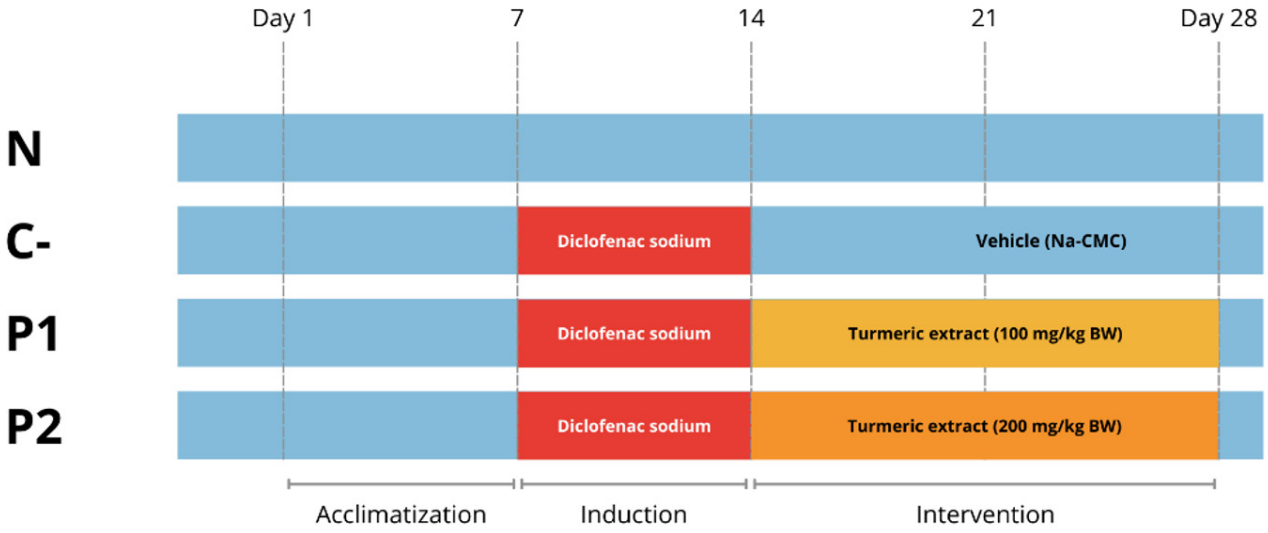

Figure 1. Intervention timeline. N: Normal control; C-: Negative control receiving diclofenac sodium (10 mg/kg BW); P1: Treatment 1 receiving diclofenac sodium (10 mg/kg BW) + turmeric extract (100 mg/kg BW); and P2: Treatment 2 receiving diclofenac sodium (10 mg/kg BW) + turmeric extract (200 mg/kg BW).

scalpel, and cardiac blood samples (approximately 3-5 mL) were collected directly from the left ventricle using a 3-mL disposable syringe.

Glutathione assay

Glutathione levels in cardiac blood samples were quantified using a colorimetric assay based on the reaction between reduced glutathione (GSH) and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), which produces the chromophore 5-thio-2-nitrobenzoic acid (TNB) [15].

A standard calibration curve was constructed by measuring absorbance at 412 nm for known GSH concentrations. The linear regression equation relating GSH concentration to absorbance was 0.413 × Absorbance + 21.16 with coefficient of determination R^2 = 0.99, indicating excellent linearity. Cardiac blood samples (250 µL) were mixed with an equal volume of DTNB reagent from the GSH Colorimetric Assay Kit and homogenized by vortexing. The mixture was then incubated at room temperature for 10 minutes to allow complete reaction between GSH and DTNB. Absorbance was measured at 412 nm using a UV-visible spectrophotometer. GSH concentrations were determined from the absorbance values using the calibration curve equation. All samples were analyzed in duplicate, and mean values were used for statistical analysis [16].

Statistical Analysis

The Shapiro-Wilk test was employed to assess normality of data distribution, selected due to the small sample size ($n < 50$ per group). Data were considered normally distributed when $p > 0.05$. Levene's test was conducted to evaluate homogeneity of variances across groups, with heterogeneous variances indicated by $p < 0.05$. One-way analysis of variance (ANOVA) was performed to compare glutathione levels among the four experimental groups, with statistical significance set at $\alpha = 0.05$. For pairwise comparisons between groups, Dunnett's T3 test was employed. This test was specifically selected because it does not assume equal variances across groups, making it appropriate for data with heterogeneous variance. Statistical significance for post hoc comparisons was set at $\alpha = 0.05$. All statistical analyses were performed using appropriate software, and data are presented as mean values with corresponding statistical test results.

Table 1. Mean glutathione concentrations by experimental group

Group	Treatment	n	Mean GSH (nmol/mg)	SD
N	Normal control	7	21.59	0.12
C-	Diclofenac only	7	21.35	0.18
P1	Diclofenac + 100 mg/kg	7	21.69	0.05
P2	Diclofenac + 200 mg/kg	7	22.10	0.18

SD: Standard deviation; GSH: Glutathione

Results

Phytochemical screening of turmeric extract

The extraction of 8 kg fresh turmeric rhizomes yielded 1,500 g of dried powder, which subsequently produced 139 g of thick extract (9.3% w/w yield from dried material). Qualitative phytochemical screening revealed the presence of multiple bioactive compounds including alkaloids, tannins, flavonoids, saponins, and steroids. The ethanol residue test confirmed complete removal of ethanol from the extract. The presence of flavonoids confirms that the extract contains curcumin, as curcumin possesses chemical and biological properties similar to flavonoid compounds [17].

Glutathione levels across experimental groups

Cardiac blood glutathione concentrations were determined spectrophotometrically at 412 nm using a standard calibration curve with excellent linearity ($R^2 = 0.99$). The mean glutathione levels differed substantially across the four experimental groups (Table 1). The negative control group (C-), which received diclofenac sodium alone, exhibited a mean GSH concentration of 21.35 ± 0.18 nmol/mg, representing a modest decrease compared to the normal control group (N: 21.59 ± 0.12 nmol/mg). This reduction of 0.24 nmol/mg (1.1%) indicates that diclofenac administration induced mild glutathione depletion.

Treatment with turmeric extract following diclofenac exposure resulted in dose-dependent increases in glutathione levels. The low-dose treatment group (P1: 100 mg/kg BW) demonstrated a mean GSH concentration of 21.69 ± 0.05 nmol/mg, which was 0.34 nmol/mg higher than the negative control and slightly elevated above normal baseline levels. The high-dose treatment group (P2: 200 mg/kg BW) exhibited the highest mean GSH concentration at 22.10 ± 0.18 nmol/mg, representing increases of 0.75 nmol/mg above the negative control and 0.51 nmol/mg above the normal control baseline.

Comparative analysis of treatment effects

Statistical analysis confirmed that glutathione levels differed significantly among the experimental groups (one-way ANOVA, $p < 0.001$). Pairwise comparisons revealed distinct patterns of treatment efficacy. The negative control group differed significantly from the normal control ($p < 0.001$), confirming that diclofenac induced measurable oxidative stress despite the modest magnitude of GSH depletion.

Both turmeric extract doses produced significant restorative effects compared to the diclofenac-only group. The low-dose treatment (P1) significantly elevated GSH levels above the negative control ($p = 0.001$), with concentrations approaching but not significantly exceeding normal baseline values ($p = 0.054$). In contrast, the high-dose treatment (P2) not only restored GSH levels but significantly elevated them above both the negative control ($p < 0.001$) and the normal baseline ($p = 0.001$), demonstrating enhanced antioxidant capacity beyond normal physiological levels.

The dose-dependent nature of the restorative effect was confirmed by direct comparison between treatment groups, with the high-dose group (P2) showing significantly higher GSH levels than the low-dose group (P1) ($p < 0.001$). This 0.41 nmol/mg difference between doses represents a 1.9%

increase in glutathione concentration, indicating that the 200 mg/kg BW dose more effectively activates endogenous antioxidant mechanisms than the 100 mg/kg BW dose.

Dose-response relationship

The relationship between turmeric extract dose and glutathione restoration demonstrated a clear dose-dependent pattern. Compared to the diclofenac-only group, the 100 mg/kg BW dose produced a 1.6% increase in GSH levels, while the 200 mg/kg BW dose produced a 3.5% increase. When compared to normal baseline, the low dose achieved near-complete restoration (0.5% above normal, not statistically significant), while the high dose produced substantial enhancement (2.4% above normal, statistically significant). This pattern indicates that while lower doses may be sufficient for restoring glutathione to baseline levels, higher doses provide additional antioxidant reserve capacity that exceeds normal physiological requirements.

Discussion

This study demonstrated that turmeric extract administration following diclofenac-induced oxidative stress resulted in dose-dependent restoration of glutathione levels in Wistar rats. The high-dose treatment (200 mg/kg BW) significantly elevated GSH concentrations above both the diclofenac-only group ($p < 0.001$) and normal baseline levels ($p = 0.001$), while the low-dose treatment (100 mg/kg BW) achieved near-complete restoration to baseline. However, the modest magnitude of diclofenac-induced GSH depletion (1.1% reduction) and the post-treatment experimental design limit conclusions about protective efficacy during concurrent NSAID exposure.

The extraction efficiency (9.3% w/w yield from dried material) aligns with previous reports [18], and the air-drying method employed preserves heat-sensitive bioactive compounds more effectively than oven-drying [19]. Phytochemical screening confirmed multiple bioactive compounds including flavonoids, indicating curcumin presence. Both curcumin and flavonoids exert antioxidant effects through electron donation, pro-oxidative enzyme inhibition, and endogenous antioxidant system upregulation [17]. However, the absence of quantitative curcumin analysis represents a significant limitation affecting reproducibility and dose-response interpretation.

The modest 1.1% GSH reduction following diclofenac administration raises questions about model adequacy. While statistically significant ($p < 0.001$), this small change may reflect the short exposure duration (7 days), moderate dose (10 mg/kg BW), or the use of cardiac blood rather than liver tissue for measurement. Diclofenac metabolism generates reactive quinone-imine intermediates that deplete glutathione through conjugation reactions [6], but hepatic GSH levels may more accurately reflect oxidative stress magnitude than circulating blood levels.

The restorative effect likely involves activation of the Nrf2 signaling pathway, though this remains speculative without molecular evidence. Curcumin disrupts Nrf2-Keap1 interaction, allowing nuclear translocation and upregulation of γ-glutamylcysteine synthetase (γ-GCS), the rate-limiting enzyme in glutathione biosynthesis [20]. This enhances de novo GSH synthesis, replenishing depleted stores. Alternative mechanisms including direct free radical scavenging and metal chelation may also contribute, though their relative importance cannot be determined from current data.

The dose-dependent response requires careful interpretation. The low dose (100 mg/kg BW) normalized GSH homeostasis without exceeding baseline, while the high dose (200 mg/kg BW) elevated concentrations 2.4% above normal. This enhanced reserve could provide greater protection against subsequent oxidative challenges, though supraphysiological GSH levels might also indicate cellular stress responses. The doses employed (100-200 mg/kg BW in rats) correspond to approximately 1,100-2,200 mg daily for a 70 kg adult when adjusted for body surface area—within therapeutic supplementation ranges but exceeding dietary intake. Curcumin's poor bioavailability due to rapid metabolism and limited absorption may limit efficacy at achievable oral doses in humans.

A critical limitation is the post-treatment design, which tests restorative rather than protective effects. Turmeric extract was administered after completing diclofenac exposure, demonstrating ability to restore depleted glutathione but not prevent depletion during concurrent NSAID exposure. Future studies employing concurrent administration would address whether turmeric extract can prevent NSAID-induced glutathione depletion during active therapy.

Additional limitations include reliance on glutathione as the sole oxidative stress biomarker. The GSH/GSSG ratio, lipid peroxidation markers (MDA, 4-HNE), protein carbonyl content, and antioxidant enzyme activities would provide more comprehensive assessment. The absence of histopathological examination prevented correlation of biochemical changes with structural evidence of hepatocellular damage. Measurement in cardiac blood rather than liver tissue may underestimate hepatic oxidative stress magnitude, and single-timepoint assessment cannot reveal temporal dynamics of depletion and restoration.

Despite these limitations, this study provides preliminary evidence that turmeric extract restores glutathione levels following diclofenac-induced depletion in a dose-dependent manner. The findings support traditional medicinal uses of turmeric and provide foundation for future research. Clinical translation faces challenges including curcumin bioavailability, potential pharmacokinetic interactions, individual metabolic variability, and unknown optimal timing and dosing schedules. Well-designed clinical trials evaluating standardized, bioavailability-enhanced formulations in patients receiving chronic NSAID therapy are needed to determine whether these preclinical findings translate to clinically meaningful benefits.

Conclusion

Turmeric extract restores glutathione levels in Wistar rats following diclofenac sodium-induced oxidative stress, with both doses (100 and 200 mg/kg BW) providing significant restorative effects and the higher dose showing superior efficacy ($p < 0.001$). The findings indicate dose-dependent antioxidant activity and suggest potential as an adjunct therapy for managing oxidative complications in patients requiring long-term NSAID treatment. However, the post-treatment experimental design limits conclusions about protective efficacy during concurrent NSAID exposure. Future research should employ concurrent treatment protocols, incorporate other oxidative stress markers including GSH/GSSG ratio.

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Declaration of interest

The authors declare that they have no conflicts of interest with any private, public, or academic party related to the information contained in this manuscript.

Author contributions

Conceptualization, CBJ and ALSA.; Methodology, CBJ and DIR.; Investigation, CBJ. and DIR; Formal Analysis, DIR; Writing—Original Draft Preparation, CBJ.; Writing—Review & Editing, ALSA. and PDP; Supervision, ALSA and PDP. All authors have read and agreed to the published version of the manuscript.

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