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Phytochemical-Mediated Modulation of Oxidative Stress and Inflammatory Pathways: A Preclinical Evaluation of Turmeric (*Curcuma longa*) Rhizome Methanolic Extract in Albino Rats

Kabiru Usman[1], Sambo L. Sambo[1], Mansur Usman[2], A. B. Hamza[1], N.I. Danmaliki[1], A. A. Naibi[1], Umar Adamu[1], Nafiu Bello[1], Aliyu Garba[1], Abubakar Yusuf[1]

[1]Department of Biochemistry, Federal University Gusau, Zamfara State, Nigeria.

[2]Department of Microbiology, Federal University Gusau, Zamfara State, Nigeria.

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Corresponding Author:
Kabiru Usman

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ABSTRACT

Turmeric is an important plant with many health benefits classified under the family of ginger. This study was conducted to evaluate the phytochemical composition, antioxidant capacity, and anti-inflammatory effects of turmeric extract using qualitative, quantitative, *in vitro*, and in *vivo* approaches. Methods such as DPPH, FRAP, and lipoxygenase inhibition assays were employed. Result of the qualitative phytochemical screening revealed the presence of several secondary metabolites, including alkaloids, flavonoids, phenols, tannins, terpenoids, steroids, glycosides, and volatile oils, with flavonoids, phenols, and tannins being particularly abundant. Quantitative analysis confirmed high concentrations of phenols (112.5 mg GAE/g), flavonoids (84.6 mg QE/g), tannins (42.8 mg TAE/g), and terpenoids (27.5 mg/g), indicating strong antioxidant potential. *In vitro* antioxidant assays demonstrated concentration-dependent activity. The DPPH assay showed up to 81.5% radical scavenging at 400 µg/mL (IC_{50} = 122.4 µg/mL), while the FRAP assay confirmed considerable ferric-reducing capacity, though slightly lower than that of ascorbic acid. *In vivo* studies revealed that toxin exposure significantly decreased antioxidant enzymes (SOD, CAT, and GSH) and increased lipid peroxidation (MDA). Treatment with turmeric extract (200 and 400 mg/kg) markedly improved antioxidant enzyme levels and reduced MDA in a dose-dependent manner. The 400 mg/kg dose nearly restored normal values and showed effects comparable to Vitamin E. Furthermore, the extract significantly reduced pro-inflammatory cytokines (TNF-α and IL-6), with the higher dose demonstrating anti-inflammatory activity similar to diclofenac. Findings from this study demonstrate that turmeric extract is a potent source of bioactive compounds with robust antioxidant and anti-inflammatory activities. Its ability to enhance endogenous antioxidant defenses and suppress pro-inflammatory cytokines may support its therapeutic potential in conditions involving oxidative stress and inflammation.

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INTRODUCTION

Turmeric scientifically called *Curcuma longa*, is a rhizomatous herbaceous plant that belongs to the family of ginger and has been extensively studied due to its medicinal properties. These medicinal properties are primarily attributed to the presence of important active components that turmeric harbors, including curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Sharifi-Rad *et al.,* 2020). However, curcumin, a polyphenolic is the most studied and constitutes approximately 3-8% of turmeric powder (Brown, 2024).Curcumin and other phenolic compounds present in the rhizome are responsible for the antioxidant properties of turmeric extracts where they scavenge free radicals and inhibit lipid peroxidation, thereby protecting cells from oxidative damage (Zhou *et al.,* 2022). In addition to its antioxidant activity, curcumin present in turmeric has been reported to activate the Nrf2 pathway, which regulates the expression of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase (Jafar *et al.,* 2023). It is this activation that leads to a decline in oxidative stress markers in different types of cells of the body such as human epithelial and immune cells (Brown, 2024). Studies using in vitro assays showed that extracts of turmeric has the potential to reduce damage caused by oxidative stress in human cells. For example, treatment of curcumin has been reported to be associated with reduced levels of reactive oxygen species (ROS) and enhanced cellular antioxidant capacity (Anggraeni *et al.,* 2022). Curcumin is central in turmeric's anti-inflammatory effects where it modulates inflammatory responses. It exerts this effect by inhibiting the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β by downregulating NF-kB signaling pathways (Zhou *et al.,* 2022).Curcumin has also been reported to suppress the activation of different mediators of inflammation in human cells exposed to lipopolysaccharides (LPS), a common inflammatory stimulus (Sharifi-Rad *et al.,* 2020).This study was therefore conducted to investigate the therapeutic potential of turmeric in combating oxidative stress and inflammation which are important key contributors to various chronic diseases in Gusau, Zamfara State, Northwestern Nigeria using albino rats.

MATERIALS AND METHODS

Samples Collection and Preparation

Turmeric rhizomes were obtained from Gusau Central Market, Zamfara State. The samples were washed and dried and the dried rhizomes were grounded into a fine powder and subjected to solvent extraction using methanol. The extract was filtered and concentrated under reduced pressure. The plant was authenticated at botany unit, department of biological sciences, Federal University Gusau, with a voucher number FUG/BIO/HEB/2025/249.

Experimental Animals

A total of thirty-five apparently healthy Wistar albino rats of both sexes, weighing between 180-200g, were obtained from animalfacility of the Department of Biological sciences at Bayaro University kano, Nigeria. The animals were housed under standard laboratory conditions, maintained at approximately 22^0C with a 12-hour light/dark cycle, and provided with ad libitium access to water and standard pellet feed. Prior to the experiment, the rats were acclimatized to the laboratory environment for one week. The study protocols received approval from the Ethics committee of the Federal University Gusau.

Experimental Design and Treatment (Antioxidant)

A total of 35 Wister albino rats weighing between 180-200g were randomly assigned into five groups each consisting of 7 rats.

Group 1 (Normal Control): Rats received olive oil (vehicle only) orally by gavage daily for 14 days and were not exposed to CCl_4.

Group 2 (CCl_4 Control): Rats were administered CCl_4 (dissolved in olive oil, 1:1) intraperitoneally 2–3 times per week during the first 7 days and received only the vehicle thereafter.

Group 3 (Standard Drug – Vitamin E): Rats were induced with CCl_4 intraperitoneally during the first 7 days and treated with Vitamin E, orally for 14 days as the standard protective group.

Group 4 (Turmeric Extract 200 mg/kg): Rats were induced with CCl_4 during the first 7 days and treated with turmeric extract at 200 mg/kg orally, for 14 days to evaluate its protective effect.

Group 5 (Turmeric Extract 400 mg/kg): Rats were induced with CCl_4 during the first 7 days and treated with turmeric extract at 400 mg/kg orally, for 14 days to assess dose-dependent protective activity (Sriram *et al.,* 2018).

Experimental Design and Treatment (Anti Inflammatory)

A total of 35 Wister albino rats weighing between 180-200 g were randomly assigned in to five groups each consisting of 7 rats.

Group 1 (Normal Control): Rats received distilled water orally, for 14 days without lipopolysaccharides (LPS) induction to serve as the normal control group.

Group 2 (LPS Control): Rats were induced with lipopolysaccharide (i.p.) during the first 7 days and received only the vehicle, serving as the inflammation control group.

Group 3 (Standard Drug – Diclofenac): Rats were induced with LPS and treated with diclofenac (100 mg/kg) orally, for 14 days as the standard anti-inflammatory group.

Group 4 (Turmeric 200 mg/kg): Rats were induced with LPS and treated with turmeric extract at 200 mg/kg orally, for 14 days to evaluate its anti-inflammatory effect.

Group 5 (Turmeric 400 mg/kg): Rats were induced with LPS and treated with turmeric extract at 400 mg/kg orally for 14 days to assess the dose-dependent anti-inflammatory activity (Sriram *et al.,* 2018).

Qualitative Phytochemical Analysis

Saponin, glycoside, cardiac glycosides, alkaloids, steroids, flavonoids, tannins, and saponin were screened according to the standard method of Sofowora (1993).

Assessment of Antioxidant Activity Using DPPH Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was employed to evaluate the antioxidant activity of turmeric extract. This method is based on the reduction of DPPH radicals in the presence of antioxidants (Boroumand *et al.,* 2018).

DPPH Preparation

A DPPH solution (0.1 mM) was prepared in methanol. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer.

Sample Test.

Different concentrations of turmeric extract (e.g., 50, 100, 150 µg/mL) were mixed with the DPPH solution and incubated in the dark for 30 minutes. The decrease in absorbance was measured, and the percentage inhibition was calculated using the formula:

Percentage Inhibition $(AO - A1/A0 \times 100)$

Where:

A_O = Absorbance of the control

A_1= Absorbance of the sample (Healthline, 2024).

Anti-inflammatory activity of Turmeric Extract on TNF-α and IL-6 Cell

Extraction of TNF-α and IL-6 cell

To extract TNF-α and IL-6 from whole blood samples, 5mL of whole blood was collected using sterile techniques, ensuring it was drawn into EDTA tubes to prevent coagulation. The samples were centrifuged at 600 rpm for 10 minutes at room temperature to separate the plasma from the cellular components. The supernatant (plasma) was carefully transferred into serum separator tubes without disturbing the pellet, as this plasma contained the soluble cytokines TNF-α and IL-6. Finally, the separated plasma was aliquoted into cryovials and stored at -80°C until further analysis to prevent degradation of the cytokines (Zhang and An, 2015).

Exposure of TNF-α and IL-6 to Inflammatory Stimuli

To evaluate the anti-inflammatory effects of turmeric extract, human cells were exposed to inflammatory stimuli such as lipopolysaccharides (LPS), and the levels of TNF-α and IL-6 were measured.

Cell Culture

TNF-α and IL-6 cells were maintained in RPMI-1640 medium supplemented with fetal bovine serum (FBS). Cells were differentiated into macrophages using phorbolmyristate acetate (PMA).

Treatment Protocol

After differentiation, cells were treated with various concentrations of turmeric extract (50, 100, 200 µg/mL) for 24 hours prior to LPS stimulation (100 ng/mL).

Quantification of TNF-α and IL-6 levels

To quantify TNF-α and IL-6 levels, an ELISA was performed according to the manufacturer's instructions. This typically involved coating a 96-well plate with captured antibodies specific to TNF-α and IL-6, followed by incubation overnight at 4°C. The wells were then washed with PBS containing 0.05% Tween-20 before adding diluted plasma samples (100 µL) to each well and incubated for 2 hours at room temperature. Afterward, biotinylated detection antibodies were added, followed by avidin-horseradish peroxidase (HRP). A substrate solution was added, and the reaction was stopped with sulfuric acid after a specified time. Finally, absorbance was measured at 450 nm using an ELISA reader (Zhang and An, 2015; NCBI, 2013).

RESULT

Table 1: Qualitative Phytochemical Screening of Turmeric Extract

Table below shows the result of qualitative analysis of turmeric extract. The result reveals that turmeric extract contains a broad spectrum of secondary metabolites, including alkaloids, flavonoids, phenols, tannins, terpenoids, steroids, glycosides, and volatile oils. The abundance (++/+++) of flavonoids, phenols, and tannins is particularly noteworthy since these compounds are strongly linked with antioxidant and anti-inflammatory properties as shown below.

Table 1: Phytochemical Constituents of Turmeric Extract (Qualitative)

Phytochemical	Presence (+) / Absence (–)
Alkaloids	++
Flavonoids	+++

Phenols	++
Tannins	++
Saponins	+
Terpenoids	++
Steroids	++
Glycosides	+
Volatile oil	++

Note: *+ = partially present; ++ = moderately present; +++ = strongly present; – = absent.*

Table 2: Quantitative Phytochemical Constituents of Turmeric Extract

Quantitative Analysis confirmed high levels of phenols (112.5 mg GAE/g) and flavonoids (84.6 mg QE/g), followed by tannins (42.8 mg TAE/g) and terpenoids (27.5 mg/g) suggesting that turmeric is a rich source of natural antioxidants.

Table 2: Phytochemical Constituents of Turmeric Extract (Quantitative)

Compounds	**Concentration (mg/g) (Mean ± SD)**
Total Phenols (GAE)	112.5 ± 3.2
Flavonoids (QE)	84.6 ± 2.8
Tannins (TAE)	42.8 ± 1.9
Saponins	21.4 ± 1.5
Alkaloids	18.2 ± 1.2
Terpenoids	27.5 ± 1.8

(GAE = Gallic Acid Equivalent; QE = Quercetin Equivalent; TAE = Tannic Acid Equivalent)

Table 3: DPPH Radical Scavenging Activity of Turmeric Extract

The following table shows the result of DPPH Assay of turmeric extract. The extract demonstrated concentration-dependent radical scavenging, with 81.5% inhibition at 400 µg/mL. The IC_{50} value (122.4 µg/mL) is higher (less potent) than that of the standard ascorbic acid (92.5 µg/mL), but still indicative of strong antioxidant activity as shown below.

Table 3: DPPH Radical Scavenging Activity of Turmeric Extract

DPPH **Concentration (µg/mL)**	**% Inhibition (Mean ± SEM)**	**FRAP Concentration (µg/mL)**	**FRAP (Mean ± SEM)**
50	28.6 ± 1.5	50	0.214 ± 0.01
100	46.2 ± 2.0	100	0.382 ± 0.02
200	63.8 ± 2.4	200	0.655 ± 0.03
400	81.5 ± 2.8	400	0.912 ± 0.04
IC_{50} (µg/mL)	122.4	Standard(Ascorbic Acid, 400 µg/mL)	1.052 ± 0.05

Standard (Ascorbic Acid): IC_{50} = 92.5 µg/mL

Table 4: Ferric Reducing Power of Turmeric Extract

The table below shows the result of FRAP Assay of the extract. The extract showed a dose-dependent ferric-reducing capacity, although slightly lower than the standard at equivalent concentrations (0.912 vs 1.052 at 400 µg/mL).

Table 4: Ferric Reducing Power of Turmeric Extract

Concentration (µg/mL)	**(Mean ± SEM)**
50	0.214 ± 0.01
100	0.382 ± 0.02
200	0.655 ± 0.03
400	0.912 ± 0.04
Standard (Ascorbic Acid, 400 µg/mL)	1.052 ± 0.05

Note: *Values are Mean ± SEM.*

Table 5: Effect of Turmeric Extract on Antioxidant Enzyme Activities in Experimental Rats

The toxin/disease-induced group showed drastic decreases in SOD, CAT, and GSH, alongside a sharp rise in MDA (marker of lipid peroxidation) an indicative of oxidative stress and tissue damage. Turmeric-treated groups (200 & 400 mg/kg) significantly

restored antioxidant enzyme activities and reduced MDA levels, nearly matching the protective effect of Vitamin E. The 400 mg/kg dose performed best, showing near-normal enzyme activities (SOD = 7.80 U/mg, CAT = 63.8 U/mg, GSH=7.55 µmol/mg, MDA=2.32 nmol/mg).

Table 5: Effect of Turmeric Extract on Antioxidant Enzyme Activities in Experimental Rats

Group	SOD (U/mg)	CAT (U/mg)	GSH (µmol/mg)	MDA (nmol/mg)
Grp 1: (Control)	8.25 ± 0.45	65.2 ± 3.1	7.85 ± 0.42	2.10 ± 0.15
Grp 2: (Toxin/Disease-induced)	4.12 ± 0.32*	34.8 ± 2.7*	3.22 ± 0.28*	5.92 ± 0.28*
Grp 3: (Stand drug,Vit. E)	7.45 ± 0.38#	60.1 ± 2.8#	7.15 ± 0.31#	2.45 ± 0.20#
Grp 4 (Turmeric Extract (200 mg/kg)	6.92 ± 0.40#	58.6 ± 2.5#	6.85 ± 0.35#	2.68 ± 0.22#
Grp 5 (Turmeric Extract (400 mg/kg)	7.80 ± 0.36#	63.8 ± 3.2#	7.55 ± 0.30#	2.32 ± 0.18#

Notes: Data are expressed as Mean ± SEM (n = 5 rats/group). There was a statistically significant difference between the disease group and the control group ($p < 0.05$). Both Vitamin E and turmeric-treated groups showed significant improvement compared to the disease group ($p < 0.05$), with no significant difference found between the treatment groups and the control group.

Figure 1:Anti-inflammatory Activity of Turmeric Extract on Cytokines in Experimental Rats.

In the figure below, lipopolysaccharide injections group had significantly elevated TNF-α and IL-6 levels, confirming an inflammatory response. Turmeric extract (200 & 400 mg/kg) reduced pro-inflammatory cytokines in a dose-dependent manner. The higher dose (400 mg/kg) produced values close to the standard drug diclofenac, indicating strong anti-inflammatory potential.

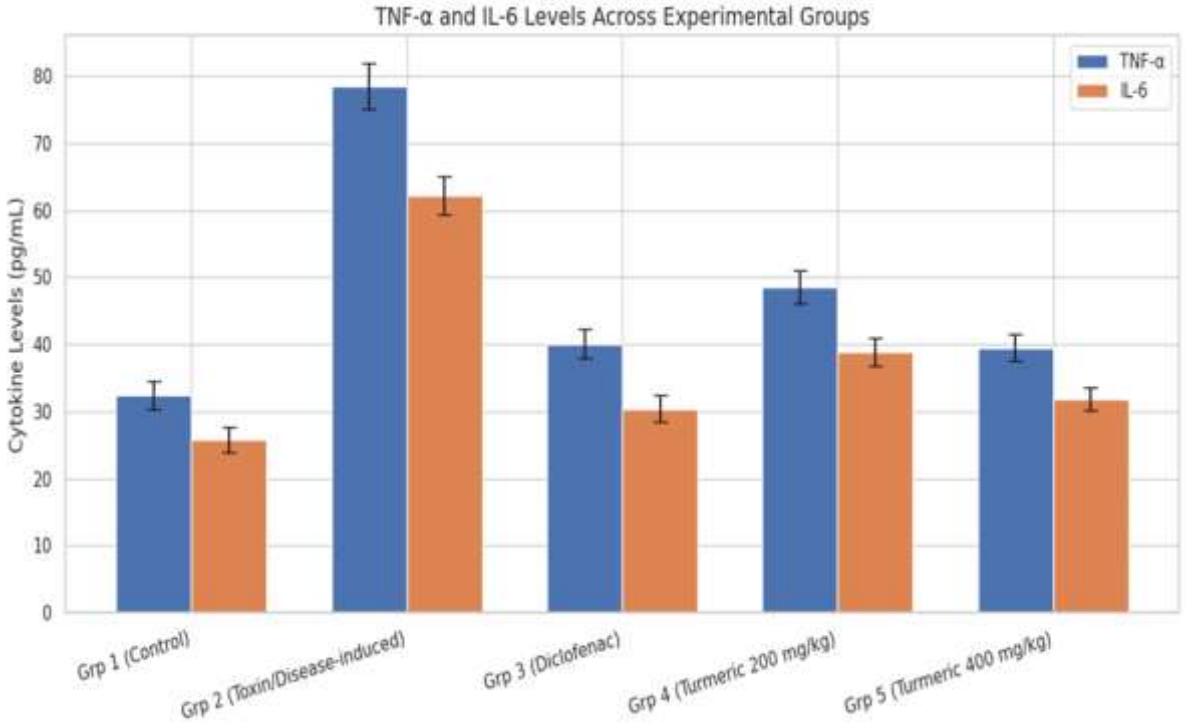

Figure 1: Anti-inflammatory Activity of Turmeric Extract on Cytokines in Experimental Rats

DISCUSSION

Qualitative screening performed on the extract of turmeric revealed the presence of alkaloids, flavonoids, phenols, tannins, terpenoids, steroids, glycosides and volatile oils. The bioactive compounds, Curcuminoids (chiefly curcumin, demethoxycurcumin and bis-demethoxycurcumin) alongside abundant terpenoids and phenolic/flavonoid compounds constitute the principal bioactive pools in turmeric and are repeatedly implicated in antioxidant and anti-inflammatory potentials of turmeric. The observed high abundance (++/+++) of flavonoids, phenols and tannins (Table 1), therefore provides a plausible chemical basis for the antioxidant and anti-inflammatory actions of turmeric as these compounds are well known radical scavengers and redox modulators (Fuloria *et al.,* 2022).The quantitative profile showing elevated concentrations of phenols (112.5 mg GAE/g) and flavonoids (84.6 mg QE/g) indicates that turmeric possesses a strong antioxidant matrix (table 2). Phenolic compounds in

particular, are known antioxidants capable of donating hydrogen atoms to neutralize free radicals. Through this mechanism, they interrupt lipid peroxidation and stabilize reactive oxygen species (ROS) (Shahidi and Ambigaipalan, 2015). The high phenolics observed in this study align with previous studies that reported accumulated substantial phenolic metabolites in turmeric which are suspected to contribute to its redox-modulating capacity (Naz *et al.,* 2022). Flavonoids, present at similarly elevated levels in this study, further strengthen turmeric's antioxidant potential. Flavonoids are known metabolites that act through several mechanisms including ROS scavenging, metal-ion chelation, and modulation of cellular antioxidant enzymes such as superoxide dismutase and catalase (Panche *et al.,* 2016). Their abundance supports evidence that turmeric-derived flavonoids synergize with curcuminoids to enhance overall antioxidant efficacy (Prasad *et al.,* 2021).The moderate concentrations of tannins (42.8 mg TAE/g) and terpenoids (27.5 mg/g) likewise, contributes to the broad-spectrum bioactivity of turmeric Agbor*et al.,* 2014),Tannins exhibit strong reducing power and lipid-peroxidation-inhibiting effects due to their polymeric phenolic structures (Agbor *et al.,* 2014), while terpenoids participate in oxidative-stress modulation by quenching singlet oxygen and regulating cellular signaling pathways involved in inflammation and oxidative balance (Nunes *et al.,* 2020). The presence of these compounds supports the conclusion that turmeric contains a diversified antioxidant network rather than relying solely on curcumin or phenolics (Shahidi and Ambigaipalan, 2015).

The DPPH radical scavenging results demonstrate that the turmeric extract exhibits strong free-radical quenching activity, achieving 81.5% inhibition at 400 µg/mL (table 3). Although the IC_{50} value (122.4 µg/mL) is higher than that of ascorbic acid (92.5 µg/mL), this still reflects substantial antioxidant potency, consistent with earlier reports that turmeric extracts typically show moderate-to-high DPPH scavenging ability depending on solvent polarity, curcuminoid content, and phenolic concentration (Zhang *et al.,* 2019; Amalraj *et al.,* 2017). The slightly reduced potency relative to ascorbic acid is not unexpected, as pure ascorbic acid is a single, highly reactive reductant, whereas turmeric extracts contain a spectrum of metabolites that contribute collectively but may have lower individual reaction kinetics with DPPH radicals (Rahman *et al.,* 2022).

The FRAP data further corroborates the extract's redox activity, with a dose-dependent ferric-reducing ability reaching a value of 0.912 at 400 µg/mL, compared to 1.052 obtained for the standard (table 4). This pattern aligns with published findings that turmeric's ferric-reducing efficacy is substantial but typically lower than isolated reference antioxidants (Suresh *et al.,* 2020). The reducing power observed is attributable to the abundant phenolics, flavonoids, tannins, and terpenoids quantified earlier, all of which possess electron-donating capacity that facilitates the reduction of Fe^{3+} to Fe^{2+} (Shahidi and Ambigaipalan, 2015).Importantly, while the extract demonstrates slightly lower potency than ascorbic acid in both assays, the broad diversity of phytochemicals present in turmeric may support synergistic antioxidant activity, enhance bioavailability and provide more sustained effects in biological systems. Previous studies have highlighted that turmeric's complex mixture of curcuminoids, flavonoids, and other secondary metabolites may act through complementary mechanisms including free-radical scavenging, metal-ion chelation, modulation of endogenous antioxidant enzymes, and suppression of oxidative stress-related signaling pathways (Prasad *et al.,* 2021; Hewlings and Kalman, 2017).

The marked reduction in superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) in the toxin/disease-induced group, accompanied by a significant elevation in malondialdehyde (MDA), clearly indicates the establishment of oxidative stress and lipid peroxidation (Table 5). Declines in endogenous antioxidant enzymes are a typical biochemical response to toxic insults, reflecting excessive reactive oxygen species (ROS) generation and depletion of cellular redox reserves (Li *et al.,* 2022). Concurrently, the rise in MDA a major by-product of polyunsaturated lipid degradation, confirms membrane oxidative injury and impaired cellular integrity (Ayala *et al.,* 2014). Administration of turmeric extract at 200 and 400mg/kg produced a significant restorative effect on the antioxidant defense system (Table 5). The extract effectively elevated SOD, CAT, and GSH levels while reducing MDA concentrations, demonstrating a reversal of oxidative damage. These findings align with previous reports showing that turmeric phytochemicals, particularly curcuminoids and flavonoids, enhance endogenous antioxidant enzyme activities and protect tissues from free-radical–induced degeneration (Rahmani *et al.,* 2018; Kunnumakkara *et al.,* 2017).The 400 mg/kg dose produced the most pronounced biochemical improvements, with SOD (7.80 U/mg), CAT (63.8 U/mg), GSH (7.55 µmol/mg), and MDA (2.32 nmol/mg) approaching the protective profile observed with Vitamin E. This dose-dependent enhancement mirrors the established pharmacological trend that higher concentrations of turmeric extracts often yield stronger antioxidant responses due to greater bioavailability of active secondary metabolites (Amalraj *et al.,* 2017).

The findings from this study demonstrate that administration of lipopolysaccharide (LPS) in experimental rats significantly elevated the levels of pro-inflammatory cytokines TNF-α and IL-6, confirming the induction of a robust inflammatory response (Figure 1). LPS is a well-established endotoxin known to activate immune cells and trigger the production of cytokines, serving as a standard model for studying systemic inflammation in vivo (Aggarwal and Harikumar, 2009). Treatment with turmeric extract at doses of 200 and 400 mg/kg resulted in a dose-dependent reduction of TNF-α and IL-6 levels (figure 1). Notably, the higher dose (400 mg/kg) produced cytokine levels approaching those observed in animals treated with the standard anti-inflammatory drug diclofenac, highlighting the potent anti-inflammatory potential of turmeric extract. Results of this study are in total agreement with previous studies that reported that,curcuminwhich is the most important bioactive compound in turmeric has the potential of suppressing cytokine production and inhibiting inflammatory signaling pathways such as NF-κB and COX-2 (Hoseini *et al.,* 2021;

Panahi *et al.,* 2008). The observed immunomodulatory effects of turmeric may likely arise from its ability to regulate immune responses through suppression of pro-inflammatory mediators. This aligns with its traditional use in managing inflammatory conditions such as arthritis and reinforces the ethnopharmacological relevance of turmeric (Rahimi *et al.,* 2023). Moreover, the extract's rich composition of bioactive phytochemicals contributes to its many pharmacological effects.

CONCLUSION

To the best of our knowledge, this is the first time a work of this nature was conducted. From our findings, it was shown that turmeric extract possessed a vast array of bioactive phytochemicals that confer strong antioxidant and anti-inflammatory properties. Its *in vitro* radical-scavenging activity and *in vivo* restoration of antioxidant defenses, coupled with suppression of pro-inflammatory cytokines, demonstrate a dual protective effect against oxidative stress and inflammation. The dose-dependent efficacy, particularly at 400 mg/kg, approaches standard pharmacological agents such as diclofenac and vitamin E, validating turmeric's therapeutic potential. These findings may support the traditional use of turmeric in managing inflammatory disorders and suggest its potential as a natural adjunct or complementary agent for oxidative stress and inflammation-related conditions, especially, in developing countries, where access to health care is a challenge.

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