
Comparative Effects of Curcumin-Gingerol Combinations and Metformin on Liver Function, Lipid Profile, and Protein Metabolism in Alloxan-Induced Diabetic Rats

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Received 19/9/2025, Accepted 3/10/2025, Published online 8/10/2025

Abstract

Diabetes mellitus is associated with profound alterations in protein metabolism, and lipid homeostasis. This study evaluated the therapeutic effects of *curcumin* and *gingerol* combinations, compared with metformin, on key biochemical parameters in alloxan-induced diabetic rats. Forty-two Wistar rats were divided into seven groups, including a normal control, negative (diabetic untreated) control, metformin (100 mg/kg), and varying doses of *curcumin* + *gingerol* (100, 200, and 400 mg/kg each). Serum biochemical assays were performed to assess total protein, albumin, globulin, bilirubin fractions, lipid profile, and liver enzymes. Results showed that metformin significantly improved protein levels, with total protein and albumin restored to near-normal values (6.00 ± 0.02 g/dL and 3.60 ± 0.09 g/dL, respectively), while the highest dose of *curcumin-gingerol* (400 mg/kg) led to a marked elevation of ALT (49.28 ± 0.18 IU/L) and AST (130.00 ± 2.31 IU/L), suggesting possible hepatotoxicity at high doses. Additionally, the 200 mg/kg dose of the plant combination significantly improved lipid parameters, reducing cholesterol (30.88 ± 1.62 mg/dL) and LDL-C (6.02 ± 1.37 mg/dL) while raising HDL-C (17.52 ± 0.25 mg/dL). These findings indicate that *curcumin-gingerol* co-administration at moderate doses may offer beneficial metabolic effects in diabetic conditions, while higher doses may compromise liver integrity.

Keywords: *Curcumin-gingerol* combination, Diabetes mellitus, Biochemical parameters, Lipid profile, Hepatotoxicity

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia due to impaired insulin secretion, action, or both. Chronic hyperglycemia in diabetes is associated with long-term damage to various organs, particularly the liver, kidneys, eyes, and cardiovascular system [1]. The liver

plays a central role in glucose and lipid metabolism and is particularly vulnerable to diabetic complications [2].

Alloxan, a potent diabetogenic compound, induces oxidative stress in pancreatic β -cells, leading to insulin deficiency and metabolic dysregulation [3]. Besides its impact on blood glucose, diabetes significantly alters protein metabolism, lipid profile, and hepatic enzyme activity, contributing to increased morbidity and mortality [4].

Whereas metformin remains the first-line treatment for type 2 diabetes, the growing interest in plant-based interventions has led to investigations into natural phytochemicals such as *curcumin*, the active compound in *Curcuma longa* (turmeric), and *gingerol*, the major pungent component of *Zingiber officinale* (ginger). *Curcumin* exhibits potent antioxidant, anti-inflammatory, and hepatoprotective effects [5-6], while gingerol has been shown to enhance insulin sensitivity and modulate lipid metabolism [7-8].

Several studies have documented the individual antidiabetic effects of *curcumin* and *gingerol*, but limited research has explored their combined therapeutic potential. Moreover, the dose-dependent effects and potential hepatotoxicity of these compounds remain unclear when administered together.

This study, therefore, aimed to evaluate the effects of combined *curcumin* and *gingerol* at varying doses on serum total protein, albumin, globulin, bilirubin levels, lipid profile, and hepatic enzyme activity in alloxan-induced diabetic rats, comparing their effects with those of standard metformin therapy. The findings are intended to clarify the safety and efficacy of these phytochemicals in managing diabetes-associated metabolic dysfunctions.

Materials and Methods

Chemicals and Reagents

Monosodium glutamate was obtained from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid was purchased from a certified Pharmaceutical supplier. All chemicals and reagents used were of analytical grade.

Collection of Plant Material

The fresh rhizomes of *Curcuma longa* and *Zingiber officinale* were harvested from the National Root Crops Research Institute, Umudike, Abia State, Nigeria, and authenticated by a botanist in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State. Voucher specimens were deposited at the university herbarium with the following voucher numbers: *Curcuma longa* – MOUAU/HB/01234 and *Zingiber officinale* – MOUAU/HB/01235. The plant materials were thoroughly washed under running tap water to remove soil and other contaminants, followed by rinsing with distilled water.

Preparation of Plant Extracts

Rhizomes of *Curcuma longa* and *Zingiber officinale* were shade-dried, powdered, and subjected to ethanol extraction using a Soxhlet apparatus. Extracts were concentrated under reduced pressure and stored at 4 °C until use.

Experimental Animals and Design

Male Wistar albino rats weighing between 150–180 g were obtained and acclimatized for two weeks under standard laboratory conditions (12 h light/dark cycle, 25 ± 2 °C, relative humidity 60–70%). The rats were provided with standard rat chow and water ad libitum. Diabetes was induced in overnight-fasted rats via a single intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight) dissolved in normal saline. After 72 hours, animals with fasting blood glucose levels ≥ 200 mg/dL were considered diabetic and selected for the study.

The diabetic rats were randomly divided into six groups (n=5 per group):

Group I: Normal Control (saline)

Group II: Diabetic Control (alloxan only)

Group III: Diabetic + Metformin (100 mg/kg)

Group IV: Diabetic + *Curcumin*, 100 mg/kg + *Gingerol* 100 mg/kg

Group V: Diabetic + *Curcumin*, 200 mg/kg + *Gingerol* 200 mg/kg

Group VI: Diabetic + *Curcumin*, 400 mg/kg + *Gingerol* 400 mg/kg

All treatments were administered orally by gavage once daily for 14 consecutive days.

Sample Collection

At the end of the treatment period, animals were fasted overnight and then euthanized via cervical dislocation under mild anesthesia. Blood samples were collected through cardiac puncture into plain tubes and allowed to clot. The clotted samples were then centrifuged at 3,000 rpm for 10 minutes to obtain serum, which was stored at -20°C for subsequent biochemical analyses.

Biochemical Assays

Serum Protein Estimation

Total protein and albumin concentrations were determined using colorimetric kits based on the Biuret and Bromocresol green methods, respectively. Globulin levels were calculated by subtracting albumin values from total protein.

Liver Function Tests

Serum bilirubin (total, direct, conjugated) and liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured using commercially available diagnostic kits (e.g., Randox Laboratories, UK), following the manufacturer's instructions.

Lipid Profile Analysis

Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were measured enzymatically using standard kits. Low-density lipoprotein (LDL-C) and very low-density lipoprotein (VLDL-C) levels were calculated using the Friedewald et al., formula [9]

Statistical Analysis

All data were expressed as mean $\pm$ standard deviation (SD). Differences between groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was accepted at $p < 0.05$. Analyses were performed using GraphPad Prism version X (or specify the version used).

Results and Discussion

Table 1: Effect of Curcumin, Gingerol, and Metformin on Serum Total Protein, Albumin, and Globulin Levels in Experimental Rats

Group	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
Normal control	6.34 $\pm$ 0.00 ^a	3.50 $\pm$ 0.00 ^c	2.85 $\pm$ 0.00 ^a
Negative control	5.71 $\pm$ 0.01 ^c	3.83 $\pm$ 0.07 ^a	1.88 $\pm$ 0.06 ^d
Metformin, 100 mg/kg	6.00 $\pm$ 0.02 ^b	3.60 $\pm$ 0.09 ^{bc}	2.40 $\pm$ 0.07 ^b
Curcumin, 100 mg/kg + Gingerol 100 mg/kg	5.39 $\pm$ 0.01 ^d	3.34 $\pm$ 0.01 ^d	2.05 $\pm$ 0.02 ^{cd}
Curcumin, 200 mg/kg + Gingerol 200 mg/kg	4.76 $\pm$ 0.05 ^f	3.09 $\pm$ 0.01 ^e	1.67 $\pm$ 0.06 ^e
Curcumin, 400 mg/kg + Gingerol 400 mg/kg	5.00 $\pm$ 0.01 ^e	2.92 $\pm$ 0.05 ^f	2.08 $\pm$ 0.07 ^c

The different superscript (^{a,b,c,d}) are statistically significant at $p < 0.05$

Table 2: Serum Liver Function Markers showing the serum levels of total bilirubin, direct bilirubin, and conjugated bilirubin

Group	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)	Conjugated bilirubin (mg/dL)
Normal control	0.65 $\pm$ 0.01 ^c	0.18 $\pm$ 0.04 ^{ab}	0.48 $\pm$ 0.03 ^b
Negative control	0.74 $\pm$ 0.03 ^a	0.15 $\pm$ 0.00 ^{bc}	0.59 $\pm$ 0.02 ^a
Metformin, 100 mg/kg	0.32 $\pm$ 0.00 ^e	0.09 $\pm$ 0.00 ^{de}	0.23 $\pm$ 0.00 ^d
Curcumin, 100 mg/kg + Gingerol 100 mg/kg	0.49 $\pm$ 0.03 ^d	0.12 $\pm$ 0.00 ^{cd}	0.37 $\pm$ 0.04 ^c
Curcumin, 200 mg/kg + Gingerol 200 mg/kg	0.62 $\pm$ 0.01 ^c	0.07 $\pm$ 0.00 ^e	0.55 $\pm$ 0.00 ^a

Curcumin, 400 mg/kg + Gingerol 400 mg/kg	0.67 ± 0.02 ^{bc}	0.19 ± 0.00 ^a	0.48 ± 0.02 ^b
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The different superscript (^{a,b,c,d}) are statistically significant at p < 0.05

Table 3: Effects of Curcumin-Gingerol Combinations and Metformin on Serum Lipid Profile in Experimental Groups.

Group	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-C (mg/dL)	VLDL-C (mg/dL)	LDL-C (mg/dL)
Normal control	61.40 ± 4.25 ^{bc}	59.17 ± 0.48 ^b	35.47 ± 0.25 ^a	11.83 ± 0.10 ^b	14.10 ± 3.91 ^b
Negative control	58.60 ± 2.63 ^c	74.17 ± 3.37 ^{ab}	28.63 ± 0.25 ^c	14.83 ± 0.67 ^{ab}	15.13 ± 1.71 ^b
Metformin, 100 mg/kg	68.42 ± 0.20 ^{ab}	62.50 ± 1.44 ^b	34.62 ± 0.25 ^{ab}	12.50 ± 0.29 ^b	21.31 ± 0.16 ^a
Curcumin, 100 mg/kg + Gingerol 100 mg/kg	62.46 ± 3.65 ^{bc}	81.67 ± 2.89 ^a	35.04 ± 0.49 ^a	16.33 ± 0.58 ^a	11.08 ± 2.58 ^{bc}
Curcumin, 200 mg/kg + Gingerol 200 mg/kg	30.88 ± 1.62 ^e	36.67 ± 0.00 ^c	17.52 ± 0.25 ^d	7.33 ± 0.00 ^c	6.02 ± 1.37 ^c
Curcumin, 400 mg/kg + Gingerol 400 mg/kg	42.46 ± 0.20 ^d	40.83 ± 0.48 ^c	13.25 ± 0.74 ^e	8.17 ± 0.10 ^c	21.04 ± 0.85 ^a

The different superscript (^{a,b,c,d}) are statistically significant at p < 0.05

Table 4: Effects of Curcumin, Gingerol, and Metformin on Liver Enzymes (ALP, AST, ALT) in Alloxan-Induced Diabetic Rats

Group	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Normal control	50.14 ± 0.98 ^c	49.20 ± 2.31 ^b	15.10 ± 0.17 ^c
Negative control	41.22 ± 0.08 ^e	45.60 ± 3.00 ^{bcd}	12.90 ± 0.06 ^d
Metformin, 100 mg/kg	53.90 ± 0.37 ^b	40.80 ± 1.62 ^d	15.60 ± 0.69 ^c
Curcumin, 100 mg/kg + Gingerol 100 mg/kg	46.25 ± 0.37 ^d	47.20 ± 2.54 ^{bc}	15.40 ± 0.23 ^c
Curcumin, 200 mg/kg + Gingerol 200 mg/kg	47.10 ± 0.94 ^d	47.60 ± 1.85 ^{bc}	17.72 ± 0.23 ^b
Curcumin, 400 mg/kg + Gingerol 400 mg/kg	62.25 ± 0.04 ^a	130.00 ± 2.31 ^a	49.28 ± 0.18 ^a

The different superscript (^{a,b,c,d}) are statistically significant at p < 0.05

This study investigated the effects of combined crude extracts of *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger) on biochemical parameters in alloxan-induced diabetic rats. Unlike studies focusing on pure compounds such as curcumin and gingerol, the use of crude extracts containing multiple bioactive substances may explain the complex and dose-dependent effects observed.

The reductions in serum total protein and globulin at higher doses (200 and 400 mg/kg) of the extracts suggest potential impairment of hepatic protein synthesis or increased protein degradation. While curcumin alone has been reported to improve protein metabolism in diabetic models [10-11], the decline observed here might result from other constituents in the crude extracts that exert hepatotoxic or pro-oxidant effects at elevated concentrations. The initial mild increase in albumin at lower doses may reflect an early protective or stimulatory effect on liver function, which diminishes at higher doses, underscoring the delicate balance of phytochemical interactions.

Bilirubin analysis further supports this dose-dependent response; moderate extract doses (100 mg/kg) reduced elevated total and conjugated bilirubin closer to the protective effects observed with metformin treatment. In contrast, higher doses increased bilirubin levels, possibly indicating hepatic dysfunction or compromised bile excretion, consistent with findings that high concentrations of phytochemicals can stress liver detoxification pathways [12-13].

The lipid profile data revealed that moderate dosing (200 mg/kg) significantly improved diabetic dyslipidemia by lowering total cholesterol, triglycerides, LDL, and VLDL levels, although HDL levels also decreased. These results align with previous reports of *curcumin* and *ginger* derivatives exerting lipid-lowering and antioxidant effects [14-15]. However, the partial reversal of these benefits at the highest dose (400 mg/kg) again highlights potential toxicity or metabolic overload associated with excessive phytochemical intake.

Elevated liver enzymes (AST, ALT, ALP) at the 400 mg/kg dose confirm possible hepatocellular injury, supporting prior studies that caution against high-dose administration of phytochemical-rich extracts due to oxidative stress and hepatotoxicity [16]. This contrasts with the well-documented hepatoprotective effects of metformin in diabetic models [17-20], which showed near-normal enzyme levels in this study.

Overall, the findings emphasize that crude extracts of *Curcuma longa* and *Zingiber officinale* can offer metabolic and hepatoprotective benefits at low to moderate doses but carry risks of hepatotoxicity and disrupted protein metabolism at higher doses [21-24]. This underscores the importance of dose optimization and the need for further research using purified active compounds to better delineate their safety and efficacy profiles.

Conclusion

Curcumin + *gingerol* combinations at lower to moderate doses ($\approx$ 100-200 mg/kg) are effective in improving disturbed protein metabolism (i.e. restoring total protein, albumin, globulin), ameliorating diabetic dyslipidemia (reducing cholesterol, triglycerides, LDL, VLDL), and maintaining liver function (ALT, AST, bilirubin) close to normal in alloxan-induced diabetic rats. The 400 mg/kg dose of the combination, though showing some improvements in lipid parameters, caused significant elevation of liver enzymes indicating potential hepatotoxicity, pointing to an upper safe limit for expression of adverse effects.

Whereas there is a substantial body of literature on *curcumin* alone and *gingerol* alone in diabetic animal models, the novelty of this work lies in exploring *curcumin* + *gingerol* administered together at multiple dose levels, which is less studied, revealing that there is a dose-dependent threshold beyond which beneficial effects reverse (i.e. potential hepatotoxicity). We simultaneously assessed protein metabolism (total protein, globulin, albumin), bilirubin fractions, lipid profile, and hepatic enzymes, giving a holistic picture of metabolic, synthetic, and detoxification liver functions, rather than focusing on one endpoint. The 200 mg/kg combination appears to be most beneficial, balancing lipid improvements and minimal liver damage; thus the work suggests an optimum dose that maximizes benefits while minimizing risk. The use of alloxan, rather than STZ or high-fat diet, and the combination therapy in this context, with these precisely measured parameters, adds to the specificity and applicability for cases of insulin deficiency effects. Therefore, *curcumin-gingerol* combinations hold therapeutic promise in diabetic metabolic disturbances but must be carefully dosed.

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