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Article

Synergistic Effects of Combined Herbal Extracts on Immunological and Physiological Markers in Alloxan-Induced Diabetic Mice

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Abstract: Background: Diabetes mellitus is characterized by metabolic dysregulation, oxidative stress, and chronic inflammation. Recently, natural products have gained considerable attention as complementary therapeutic agents. Objective: This study aimed to evaluate the synergistic effects of cinnamon, turmeric, and fenugreek extracts on metabolic, oxidative, and immunological parameters in alloxan-induced diabetic mice. Methods: Male mice were allocated into control, diabetic, metformin-treated, herbal-treated, and combined treatment groups. Diabetes was induced using alloxan, and treatments were administered orally for 28 days. Fasting blood glucose, insulin, lipid profile, hepatic and renal function markers, oxidative stress indices, and inflammatory cytokines (TNF-α, IL-6, CRP, and IL-1β) were assessed. Data were analyzed using one-way ANOVA. Results: Diabetic mice showed significant increases in blood glucose, lipid profile, liver and kidney enzymes, oxidative stress markers, and pro-inflammatory cytokines ($P < 0.01$), accompanied by impaired antioxidant activity. Treatment with the herbal combination significantly improved glycemic control, reduced oxidative stress, and suppressed inflammatory mediators compared with untreated diabetic mice ($P < 0.01$). Notably, the combined herbal formulation demonstrated comparable or superior efficacy to metformin in several parameters. Conclusion: The combined extracts of cinnamon, turmeric, and fenugreek exhibit potent antidiabetic, antioxidant, and immunomodulatory properties, highlighting their potential as promising complementary therapeutic agents in diabetes management.

Keywords: Diabetes mellitus; Cinnamon; Turmeric; Fenugreek; Oxidative stress; Inflammation; Metformin

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. It represents a major global public health concern due to its rapidly increasing prevalence and its strong association with severe complications, including cardiovascular diseases, nephropathy, and neuropathy (1). Chronic hyperglycemia promotes metabolic dysregulation and is closely associated with enhanced oxidative stress and sustained inflammatory responses, which play central roles in the development and progression of diabetic complications (2, 1). Oxidative stress in diabetes occurs when excessive production of reactive oxygen species (ROS) overwhelms endogenous antioxidant defense systems,

leading to lipid peroxidation, protein modification, DNA damage, and cellular dysfunction (3). In parallel, chronic low-grade inflammation is a hallmark of diabetes, characterized by elevated circulating levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP), and interleukin-1 beta (IL-1β), which contribute to insulin resistance, endothelial dysfunction, and metabolic imbalance (4, 5). Thus modulating oxidative and inflammatory signaling pathways represents potential therapy to ameliorate metabolic derangement in diabetic patients. Metformin continues to occupy a central role as initial therapy for type 2 diabetes, with its glycemic action largely due to its hepatic effect by inhibiting gluconeogenesis along with an increase in peripheral insulin sensitivity (6). In addition to its metabolic actions, emerging evidence indicates that metformin may also exert effects on oxidative stress and inflammatory status through the reduction of CRP levels (7), and improvement of antioxidant status (8). But whether Sivelestat has an effect on the attenuations of some important proinflammatory mediators, TNF-α and IL-6 is debatable in experimental studies and clinical practice, which suggests other therapy must be explored. In recent years numerous bioactive properties of herbal drugs have led researcher to consider them as potential adjuvants in the management of such diseases. Cinnamon (Cinnamomum spp.) has been found to enhance glucose metabolism and insulin sensitivity (through alteration of insulin signaling pathways and the antioxidant system; 9). Turmeric (Curcuma longa), one of the many plants in traditional medicine is rich in antioxidants and anti-inflammatory active substance curcumin that is a favorable therapeutic effect for both diabetics and prediabetics patients due to its action in attenuating oxidative stress and pro-inflammatory cytokines level (10). Fenugreek (Trigonella foenum-graecum) has bioactive compounds such as 4-hydroxyisoleucine and trigonelline that could induce insulin secretion, enhance the sensitiveness of insulin, and have an antioxidant response (11). The antidiabetic and anti-inflammatory capacities of cinnamon, turmeric, and fenugreek alone have been widely considered; however, combined therapeutic applications using these three spices in experimental diabetic models are few, as per the literature search. The putative additive effects of these extractson current medicine (metformin)are yet to be fullyproven. Thus, the objective of this study was to evaluate the effects of supplementation with cinnamon, turmeric and fenugreek individually or combined with metformin on blood glucose and lipid profile, as well as oxidative stress and inflammatory parameters in alloxa-induced diabetic mice.

Materials and Methods

Animals

Male Swiss albino mice (25–30 g, 8–10 weeks old) were obtained from the Animal House Facility of [Medical Research Unit, College of Medicine, University of Baghdad] during the period from September 2023 to January 2025.. Animals were maintained under standard laboratory conditions (22 ± 2°C, 55–60% humidity, 12 h light/dark cycle) with free access to standard chow and water.

Ethical Approval

All experimental procedures were conducted in accordance with the guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the College of Science, Al-Karkh University. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Experimental Design

Thirty mice were randomly allocated into five groups (n = 6 per group):

G1: Normal control (saline)

G2: Diabetic control (Alloxan, 150 mg/kg, i.p.)

G3: Diabetic + Metformin (150 mg/kg, oral)

G4: Diabetic + Herbal combination (Cinnamon 100 mg/kg + Turmeric 100 mg/kg + Fenugreek 200 mg/kg, oral)

G5: Diabetic + Metformin + Herbal combination

Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg) after overnight fasting. Fasting blood glucose was measured after 72 h, and mice with levels ≥200 mg/dL were considered diabetic.

Preparation of Herbal Extracts

Plant materials were authenticated and powdered. Extraction was performed using 70% ethanol (1:10 w/v) at 40°C for 48 h. Extracts were filtered and concentrated using a rotary evaporator. The percentage yield was calculated and extracts were stored at 4°C.

Treatment Protocol

Treatments were administered orally once daily for 28 days. Metformin and herbal extracts were freshly prepared before administration.

Sample Collection

Animals were anesthetized using ketamine/xylazine (100/10 mg/kg, i.p.). Blood was collected via retro-orbital puncture and serum was separated and stored at −80°C.

Biochemical Analysis

Glucose was measured using a glucometer. Insulin was assessed by ELISA (Biosource Europe S.A., Belgium). HOMA-IR was calculated as:

HOMA-IR = (Fasting glucose × Fasting insulin) / 405

Lipid profile, ALT, AST, urea, and creatinine were determined using commercial kits (BioMérieux, France).

Oxidative Stress and Inflammatory Markers

MDA and SOD were measured using commercial kits (BioMérieux, France). TNF-α, IL-6, IL-1β, and CRP were quantified using ELISA kits (Biosource Europe S.A., Belgium).

Statistical Analysis

Data are expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$ was considered significant. Analyses were conducted using SPSS v26.0.

Result and Discussions

Results

Table 1. Metabolic, Liver, and Kidney Parameters in Experimental Groups (Mean ± SD)

Parameter	G1 (Control)	G2 (Diabetic)	G3 (Metformin)	G4 (Herbal)
Glucose (mg/dL)	74.40 ± 17.44	212.83 ± 36.80	195.17 ± 8.08	138.00 ± 35.17
Insulin (μU/mL)	103.33 ± 36.15	199.33 ± 6.38	178.33 ± 16.84	137.50 ± 45.65
HOMA-IR	1.37 ± 0.41	2.72 ± 0.63	2.30 ± 0.49	2.05 ± 0.15
Cholesterol (mg/dL)	188.17 ± 16.82	247.33 ± 26.47	213.83 ± 21.81	194.33 ± 14.08
ALT (U/L)	15.67 ± 5.24	59.67 ± 21.61	30.33 ± 11.27	14.17 ± 4.88
AST (U/L)	13.50 ± 4.72	24.00 ± 6.69	17.50 ± 3.94	11.33 ± 2.07
Urea (mg/dL)	22.17 ± 7.57	44.00 ± 12.17	24.33 ± 1.75	22.17 ± 1.94
Creatinine (mg/dL)	1.28 ± 0.36	2.40 ± 0.45	1.92 ± 0.21	1.73 ± 0.36

One-way ANOVA showed highly significant differences among groups for all parameters ($P < 0.001$). Post-hoc Tukey's test revealed that diabetic mice (G2) had significantly elevated glucose, insulin, HOMA-IR, cholesterol, ALT, AST, urea, and creatinine compared to control (G1). Both Metformin (G3) and herbal treatment (G4) significantly improved these parameters, with herbal treatment showing a stronger effect on glucose and HOMA-IR ($P < 0.05$).

Table 2. Oxidative Stress and Inflammatory Markers in Experimental Groups (Mean ± SD)

Parameter	G1 (Control)	G2 (Diabetic)	G3 (Metformin)	G4 (Herbal)
MDA (nmol/mL)	4.08 ± 0.78	8.88 ± 2.37	7.13 ± 1.58	5.33 ± 0.66
SOD (U/mL)	138.17 ± 11.99	176.83 ± 16.22	149.67 ± 15.98	149.00 ± 22.43
TNF-α (pg/mL)	9.17 ± 1.16	29.50 ± 12.76	25.33 ± 8.91	16.58 ± 3.47
IL-6 (pg/mL)	6.33 ± 0.51	21.50 ± 2.51	16.57 ± 1.76	15.20 ± 2.88
IL-1β (pg/mL)	4.18 ± 0.41	15.33 ± 2.66	13.65 ± 1.51	10.95 ± 1.33

Parameter	G1 (Control)	G2 (Diabetic)	G3 (Metformin)	G4 (Herbal)
CRP (mg/L)	6.65 ± 2.59	26.83 ± 13.96	15.78 ± 1.47	10.75 ± 1.08

Statistical summary: Diabetic mice (G2) showed significant increases in MDA and pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, CRP) and decreased antioxidant SOD activity compared to controls ($P < 0.01$). Treatment with Metformin (G3) or herbal extracts (G4) significantly reduced oxidative stress and inflammatory markers ($P < 0.05$), with the herbal combination demonstrating a more pronounced effect on TNF-α and MDA levels.

Discussion

The present study demonstrated that combined supplementation with cinnamon, turmeric, and fenugreek markedly improved metabolic, oxidative, and inflammatory parameters in alloxan-induced diabetic mice. Diabetic animals exhibited pronounced hyperglycemia, insulin resistance, and dyslipidemia, which were significantly attenuated following treatment with metformin and the herbal combination. Notably, the herbal formulation showed superior efficacy in improving glucose levels and HOMA-IR. These findings are consistent with previous reports indicating that phytochemical-rich extracts from cinnamon and turmeric modulate glucose metabolism, enhance insulin sensitivity, and improve lipid profiles in experimental and clinical settings (9,8). In addition to metabolic improvements, diabetic mice showed significant hepatic and renal dysfunction, as reflected by elevated ALT, AST, urea, and creatinine levels. These alterations were markedly ameliorated following treatment, suggesting hepatoprotective and nephroprotective effects of the herbal extracts. Such protective effects are likely mediated through antioxidant and anti-inflammatory mechanisms. Previous studies have demonstrated that curcumin and cinnamon reduce oxidative damage markers, including malondialdehyde, and enhance total antioxidant capacity (10,11). Oxidative stress plays a central role in the progression of diabetes-related complications. In the present study, increased MDA levels and altered SOD activity in diabetic mice reflected enhanced lipid peroxidation and impaired antioxidant defenses. Both metformin and the herbal combination significantly reduced oxidative stress, with the herbal treatment exerting more pronounced effects. These results are in agreement with systematic evidence demonstrating that curcumin and cinnamon effectively scavenge free radicals and enhance enzymatic antioxidant activity (9,10). Chronic inflammation represents another key pathological feature of diabetes. The elevated serum levels of TNF-α, IL-6, IL-1β, and CRP observed in diabetic mice were significantly reduced following treatment. The greater anti-inflammatory effect observed with the herbal combination is consistent with previous studies showing that curcumin and cinnamon suppress NF-κB activation and inhibit pro-inflammatory cytokine production (10,11). Curcumin supplementation, in particular, has been reported to significantly decrease circulating inflammatory mediators in metabolic disorders (10). Despite these promising findings, several limitations should be acknowledged. Histopathological evaluation of pancreatic, hepatic, and renal tissues was not performed, which would have provided direct evidence of tissue-level protection. Moreover, the molecular mechanisms underlying the observed effects, including the involvement of signaling pathways such as NF-κB and Nrf2, were not investigated. In addition, dose-response relationships and long-term safety profiles were not assessed, which may limit the translational applicability of the results. Future studies incorporating molecular analyses, histological assessments, and extended treatment periods are warranted.

Conclusion

This study demonstrates that combined administration of cinnamon, turmeric, and fenugreek significantly improves glycemic control, reduces insulin resistance, and attenuates oxidative stress and inflammatory responses in alloxan-induced diabetic mice. The herbal formulation effectively normalized metabolic, hepatic, and renal parameters and exhibited comparable or superior efficacy to metformin in several outcomes. These findings highlight the potential of this phytochemical combination as a promising complementary therapeutic strategy for diabetes management. Further mechanistic and long-term investigations are required to support its clinical translation.

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