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Ameliorative Effects Of *Curcuma Longa* And *Trigonella Foenum Graecum* On Liver Lipid Profile Of Alloxan-Induced Type-I Diabetic Rats

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Abstract

The aim of this study was to investigate the effects of *Curcuma longa* (CL), and *Trigonella foenum graecum* (TFG) in combination on protecting liver tissue from lipid peroxidation stress influenced by alloxan-induced diabetes, which is one of the most prevalent endocrine diseases globally, and exhibits a rapidly increasing growth rate among metabolic disorders. The study utilized eight groups of rats, each consisting of six individuals. Group I served as the control, followed by Groups II, III, and IV, which received treatments with CL, TFG, and CL + TFG, respectively. Type 1 diabetes was induced in rats through the intraperitoneal administration of alloxan at a single dose of

120mg/kg body weight. Group V served as the negative control (diabetic), and groups VI, VII, and VIII were diabetic rats treated with CL, TFG, and CL + TFG, respectively. The efficacy of CL and TFG extracts was determined based on lipid profiles (cholesterol, triglycerides, and phospholipids), and lipid peroxides (TBARS) in liver tissue. After 30 days treatment with CL, and TFG, a significant reduction in the levels of lipid profile, and lipid peroxidation in liver tissue was observed compared to the Group V, which served as the diabetic control. The combinational approach of CL + TFG showed greater activity when compared to the individual treatment with plant extracts. Therefore, the combination of herbal treatment significantly mitigated the adverse effects caused by alterations in lipid profile in diabetic-induced rats.

Keywords: Wistar Albino rats, Curcuma longa (CL), Trigonella foenum-graecum (TFG), antidiabetic activity, alloxan, type -1, lipid profile, liver.

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia, a pathogenesis condition often caused due to deficiencies in insulin secretion, and/or action (1). Type 2 diabetes mellitus (T2DM), comprising around 90% of DM cases, is the most prevalent form, primarily stemming from insulin resistance or inadequate insulin production (2,3). This condition significantly impacts quality of life, increasing the risk of severe complications such as stroke, amputation, kidney failure, blindness, and premature death (4). According to the International Diabetes Federation (IDF), the global prevalence of diabetes is estimated at 537 million, projected to rise to 643 million by 2030, and 783 million by 2045 (5). In India alone, approximately 77 million individuals aged 18 years or older suffer from type 2 diabetes, with nearly 25 million considered prediabetic, placing them at higher risk (6). India is recognized as the world's diabetes capital, with diabetes prevalence steadily increasing, particularly in urban areas, where it is six times more common than in rural regions (6).

The etiology of diabetes mellitus involves various factors such as reduced physical activity, increased weight, stress, dietary changes, malnutrition, alcohol consumption, and viral infections (7). Despite advancements in treatment, anti-diabetic medications can lead to severe side effects like hypoglycemic coma, liver, and kidney tissue issues (8). Consequently, there is growing interest in utilizing medicinal plants for managing metabolic diseases like DM, with approximately four billion people in developing countries relying on them (9). The World Health Organization (WHO) recommends incorporating medicinal plants into dietary practices for managing diabetes mellitus (DM) (10).

Both conventional, and complementary medicine have long used medicinal plants, and their bioactive constituents to treat, and prevent various diseases. Around 80% of the global population depends on medicinal plants for primary healthcare, particularly for managing glucose metabolism, with many plants exhibiting inhibitory effects on digestive enzymes involved in carbohydrate breakdown (11,12). Natural products, particularly those derived from medicinal plants, have garnered attention for managing diabetes, and its associated complications, which have become epidemic worldwide (13).

One such plant is *Curcuma longa* (CL), commonly known as turmeric, which has been used for millennia in Ayurvedic, and traditional Chinese medicine to treat diabetes (13). Research indicates that curcumin, a bioactive compound in turmeric, possesses hypoglycemic, antioxidant, and anti-inflammatory properties, making it a potential anti-diabetic agent (14). Similarly, *Trigonella foenum-graecum* (TFG), or fenugreek, is another plant renowned for its anti-diabetic properties, attributed to compounds like galactomannan, saponins, diosgenin, and 4-hydroxyisoleucine (15). Fenugreek has been shown to improve insulin sensitivity, and stimulate insulin secretion, making it a promising therapeutic option for diabetes management (16,17).

In light of these considerations, the present study aims to investigate the potential synergistic effects of *Curcuma longa* (CL), and *Trigonella foenum-graecum* (TFG) in alleviating liver tissue lipid profile in alloxan-induced diabetic rats.

Materials and Methods

Chemical

The chemicals used in the study was procured from the Sigma, and were of Analytical Grade. Additionally, chemicals were obtained from various scientific companies such as Merck (Mumbai, India), Qualigens (Mumbai, India), Ranbaxy (New Delhi, India), Fisher (Pittsburg, PA, USA), and Louis, MO, USA. High-quality results were ensured using specialized equipment including the Hitachi UV-2000 Spectrophotometer for measuring optical density values, the Kubota KR 200000T centrifuge for centrifuging homogenates.

Experimental Animals

A total of 48 adult male Wistar rats, weighing 180 ± 200g and three months old, were sourced from Bangalore's animal house. They were housed in groups of four in standard polycarbonate cages under laboratory conditions with a 12-hour light/dark cycle, a temperature maintained at 24±20C, and humidity ranging from 45–64 percent. The rats were provided with a standard diet obtained from Hindustan Lever Limited in Mumbai, and water was provided via plastic bottles fitted with nipples.

Experiment Groups

Rats were divided into eight groups and each group consists of six individuals.

Group -1 : Control rats
Group- 2 : Control rats treated with CL (0.25 g/Kg b.w.)
Group -3 : Control rats treated with TFG (0.25 g/Kg b.w.)
Group -4 : Control rats treated with CL (0.25 g/Kg b.w.) + TFG (0.25 g/Kg b.w.)
Group –5 : Diabetic untreated (alloxan 120mg/kg b.w)
Group -6 : Diabetic rats treated with CL (0.25 g/Kg b.w.)
Group -7 : Diabetic rats treated with TFG (0.25 g/Kg b.w.)
Group -8 : Diabetic rats treated with CL (0.25 g/Kg b.w.) + TFG (0.25 g/Kg b.w.)

After 24 hours of the 30-day treatment period, the rats were euthanized using cervical dislocation, and their liver tissue was extracted at 4°C. The tissue was rinsed with ice-cold saline, and promptly stored at a deep freeze temperature of -80°C for subsequent biochemical analysis, and enzymatic assays.

Plant Extract Preparation

The fine powder of CL rhizome, and TFG seeds powder, both bearing the AGMARK symbol, were procured from Tirupati. Cold percolation with 95% ethanol was used to extract the powder for 24 hours. The extract was then recovered, and additional 95% ethanol was added to the plant material for further extraction. This process was repeated three times, and the resulting ethanol extract was pooled, combined, and filtered. The filtrate was concentrated to dryness under reduced pressure using a rotary evaporator. The resulting ethanol extract, without further purification, was air-dried. Finally, doses equivalent to 250mg (CL + TFG) kg body weight were suspended in a 2% v/v Tween 80 solution for the experiment.

Induction of Diabetes

Diabetes was induced using alloxan to induce Insulin-dependent diabetes mellitus (IDDM) (Type-I). Rats were fasted for 18 hours prior to intraperitoneal injection of a single dose of 120mg/kg body weight of freshly dissolved alloxan (company) in saline solution (18). Following injection, rats had free access to food and water, and a 15% glucose solution was provided overnight to counter hypoglycemic shock. Diabetes onset was confirmed by observing moderate polydipsia, and marked polyuria. Fasting blood samples were collected from the second day onward via tail vein for blood glucose measurement using the Accu Chek Glucometer (Sensor Comfort). Rats with blood glucose levels exceeding 300mg/dl were administered insulin (IIU protamine Zinc insulin) to acclimatize to the diabetic condition for one week. After one week, rats with hyperglycemia (blood glucose level 250 mg/dl) were selected for the experiment.

Estimation of Total Cholesterol

Total cholesterol content was estimated using the Liebermann Burchard reaction as described by Natelson (19). Liver tissue was homogenized in isopropanol, and the resulting supernatant was treated with cholesterol reagent, and heated. After cooling, the samples were measured at 560 nm in a spectrophotometer, and the results were expressed in mg of total cholesterol per gram wet weight of tissue.

Estimation of Phospholipids

Phospholipids were assessed using the method described by Zilversmit and Davis (20). Liver tissues were homogenized with 10% trichloroacetic acid (TCA), followed by centrifugation, and addition of 60% perchloric acid (PCA). The mixture was then subjected to colorimetric analysis, and the results were expressed in mg of phospholipids per gram wet weight of tissue.

Estimation of Lipid Peroxidation (MDA Content)

Lipid peroxidation (MDA content) levels were determined according to the method outlined by Ohkawa et al. (21). Liver tissue was homogenized, and the supernatant was utilized for the assessment. The samples were subjected to various chemical treatments, followed by spectrophotometric measurement at 532 nm against a standard. The results were expressed in µ moles of malondialdehyde formed per gram wet weight of tissue.

Estimation of Triglycerides (TG - Triacylglycerol)

The Schwartz and Wolins (22) method with slight modifications, was used to determine triglyceride levels. The tissue homogenates were mixed with chloroform, and 1NH2SO4, followed by the addition of methanol, alkaline barium solution, and chronotropic corrosive reagent. The resulting supernatant was measured at 575 nm in a spectrophotometer against the reagent blank. The results were expressed in mg of triglycerides per gram wet weight of tissue.

Results

The Lipid Peroxidation values obtained from this study were depicted in table 1, and the percentage change over control in figure 1. In control rats, the level of cholesterol was measured at 62.13 mg of cholesterol per gram wet weight tissue. Treatments with CL, and TFG, both individually, and in combination (CL+TFG) in groups II, III, and IV, respectively, resulted in decreased levels of cholesterol. Conversely, in group V, the levels of cholesterol significantly increased to 99.1 mg of cholesterol per gram wet weight tissue. For diabetic rats treated with CL, TFG, and CL+TFG in groups VI, VII, and VIII, respectively, decreased levels of cholesterol were observed compared to both group I, and group V, with the most significant decrease seen in Group VI.

Table 1: Lipid Peroxidation Levels of Liver tissue of Control and Experimental Rats

Parameter / Groups	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
Cholesterol	62.13 ± 0.23	56.1 ± 0.79	57.12 ± 0.54	55.32 ± 0.61	99.1 ± 0.71	49.2 ± 0.11	50.12 ± 0.08	52.02 ± 0.67
Phospholipids	17.82 ± 0.42	16.82 ± 0.62	16.79 ± 0.52	16.62 ± 0.59	38.51 ± 0.98	14.02 ± 0.32	15.72 ± 0.46	16.1 ± 0.29
Lipid peroxidation	42.62 ± 6.39	38.8 ± 4.99	39.16 ± 5.68	37.1 ± 6.71	75.12 ± 3.72	36.14 ± 4.98	37.12 ± 5.31	35.1 ± 4.23
Triglycerides	1.315 ± 0.046	1.320 ± 0.053	1.34 ± 0.041	1.352 ± 0.031	3.542 ± 0.022	1.275 ± 0.021	1.272 ± 0.032	1.22 ± 0.019

Values are mean ± S.D. of 6 individual rats

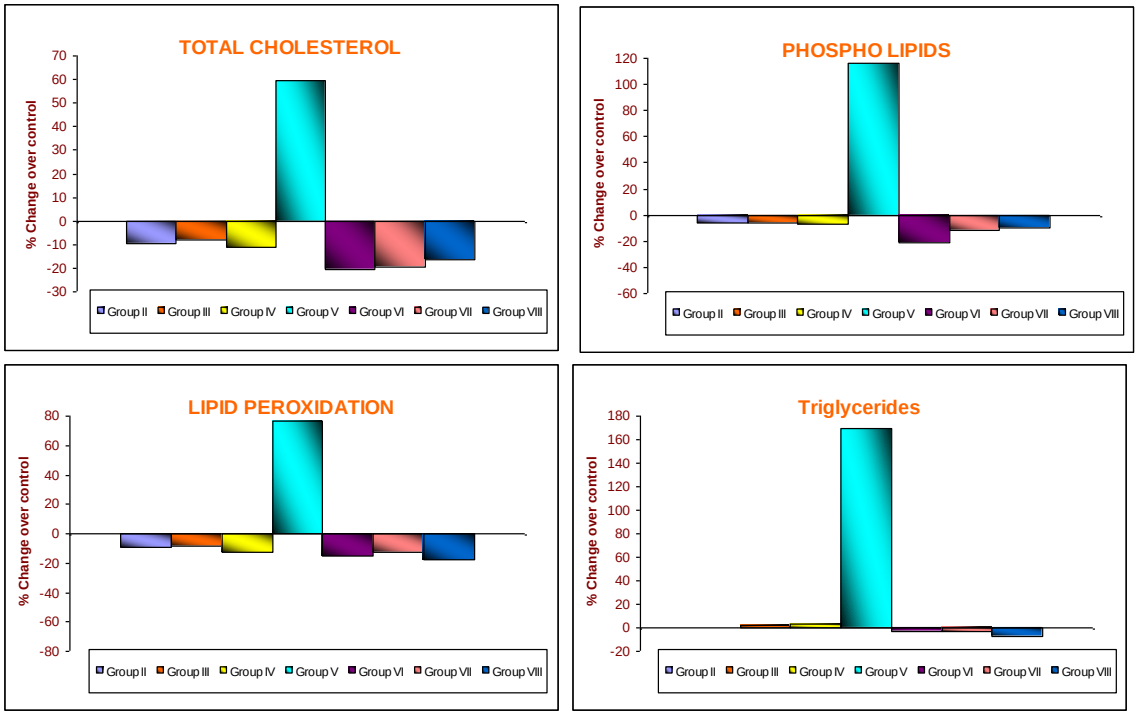
Values are significantly different from control at $P < 0.0001$

The phospholipids levels in control rats, was measured at 17.82 mg of phospholipids per gram wet weight tissue. Treatments with CL and CL+TFG in groups II and, IV, respectively, resulted in very slight decreases of phospholipids levels. However, in group III, a decrease to 16.79 mg of phospholipids per gram wet weight tissue was observed. Conversely, in group V, the levels significantly increased to 38.51 mg of phospholipids per gram wet weight tissue. For diabetic rats treated with CL, TFG, and CL+TFG in groups VI, VII, and VIII, respectively, decreased levels were observed compared to both group I, and group V, with the most significant decrease seen in Group VI.

The lipid peroxidation levels in control rats were measured at 42.62 µmoles of malondialdehyde formed per gram wet weight tissue. Treatments with CL, and TFG, both individually, and in combination (CL+TFG), resulted in decreased levels of lipid peroxidation in groups II, III, and IV, respectively. Conversely, in group V, where the rats were treated as diabetic untreated, the levels of lipid peroxidation significantly increased to 75.12 µmoles of malondialdehyde formed per gram wet weight tissue. For diabetic rats treated with CL, TFG, and CL+TFG in groups VI, VII, and VIII, respectively, decreased levels of lipid peroxidation were observed

compared to both group I (control), and group V (diabetic), with the most significant decrease seen in Group VIII.

Fig 1: Percentage Change of Lipid Peroxidation Levels of Liver tissue of Control and Experimental Rats



Values are percentage % change over Control

The triglycerides levels in control rats were measured at 1.315 mg of triglycerides per gram wet weight tissue. Treatments with CL, TFG, and CL+TFG in groups II, III, and IV, respectively, resulted in increased levels of triglycerides. However, in group V, the levels of triglycerides significantly increased to 3.542 mg of triglycerides per gram wet weight tissue. For diabetic rats treated with CL, TFG, and CL+TFG in groups VI, VII, and VIII, respectively, decreased levels of triglycerides were observed compared to both group I, and group V, with the most significant decrease seen in groups VI, VII, and VIII.

Discussion

Lipid peroxidation, the oxidative degradation of lipids, occurs when free radicals extract electrons from lipids in cell membranes, leading to cellular damage through a chain reaction mechanism (23). Disruptions in lipid organization within biological membranes can alter the activity of membrane-bound enzymes (24). Research indicates a correlation between lipid peroxidation, and reactive oxygen species, with rat liver peroxisomes exhibiting greater stability against peroxidation-induced injury compared to kidney peroxisomes (25).

Diabetic individuals often suffer from fatty liver disease and other liver disorders (26). Studies have shown that dietary supplementation with curcumin reduces albumin, urea, creatinine, and inorganic phosphorus excretion in streptozotocin-induced diabetic rats (27, 28). Additionally, curcumin supplementation decreases liver weight, and lipid peroxidation products in plasma, and urine, independent of changes in glycemia or body weight (29). Other research has also demonstrated curcumin's hypolipidemic effects (30) and its ability to reduce lipid peroxidation in liver disorder-induced rodents (31).

In our study, treatment with plant extracts resulted in decreased levels of malondialdehyde (MDA), indicating inhibition of lipid peroxidation. Spice components from *Curcuma longa* and *Abroma augusta* have shown protective effects against lipid peroxidation induced by reactive oxygen species (32). Derivatives of curcumin, and ferric acid, such as 3, 4-dihydroxy cinnamoyl methane, and caffeic acid, are potent inhibitors of lipid peroxidation (33). Furthermore, curcumin administration has been associated with reduced serum levels of cholesterol, and lipid peroxides in humans (34).

In diabetic rats treated with plant extracts, MDA levels decreased, likely due to the extracts' inhibitory effects on lipid peroxidation. Triglyceride levels were also reduced in diabetic rats treated with plant extracts, indicating anti-triglyceride activity. TFG seeds have been reported to have hypolipidemic effects on both type

1, and type 2 diabetes mellitus patients, and experimental diabetic animals (35). Oral administration of CL extract has been shown to lower blood glucose, and attenuate alloxan-induced hyperlipidemia in diabetic rabbits (36).
Phospholipids, present in cell membranes, form the majority of the surface lipoproteins, creating a lipid bilayer that interfaces with both the polar plasma environment, and the non-polar lipoprotein core (37). Increased phospholipid levels in tissues have been reported in streptozotocin-induced diabetic rats (37). The decrease in enzyme activity observed in our study may be attributed to the loss of phospholipids (38). Given the medicinal value of CL, and TFG extracts, in our study investigations shows ameliorative alterations in phospholipid content in alloxan-induced diabetic rats treated with these extracts.

Conclusion

The present study has confirmed that type II diabetic diagnosed rats treated with *Curcuma longa* (CL), and *Trigonella foenum graecum* (TFG) individually, and in combination experienced a notable reduction in cholesterol, phospholipids, lipid peroxidation, and triglycerides levels compared to control, and diabetic groups. These findings suggest that the extract of CL, and TFG represents a potent natural food source capable of managing dyslipidemia. Further research, incorporating a thorough chemical and pharmacological investigation, is warranted to isolate, and characterize specific bioactive compounds from CL, and TFG extracts, thus providing robust confirmation. Moreover, additional studies are necessary to elucidate the mechanism of action comprehensively. Furthermore, more extensive, and long-term investigations are warranted to assess the efficacy of CL rhizome, and TFG seed extract on a larger scale.

Ethics Approval

The study was approved by the Institutional Ethics Committee of Sri Venkateswara University (No.01/2011–2012/(i)/a/CPCSEA/IAEC/SVU/SSB/Dt20/06/2011), Tirupati.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation**.**

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