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# COMPARATIVE ANTIDIABETIC STUDY OF *TRIGONELLA FOENUM GRAECUM* AND *GLYCYRRHIZA GLABRA* IN ALLOXAN-INDUCED DIABETIC RATS

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## Abstract

*This study investigated the comparative antidiabetic activity of the plant Trigonella foenum graecum and Glycyrrhiza glabra, both of which belong to the family Fabaceae. An ethanolic extract of T. foenum graecum (fenugreek) and Glycyrrhiza glabra was prepared. The phytochemical analysis and in vitro antioxidant action of both plant extracts were performed. Diabetes was established in Wistar rats by administering alloxan monohydrate intraperitoneally at a dosage rate of 120mg/kg body weight. Animals were split into six groups, with six rats per each. Different doses of both plant extracts were administered by oral route and continued for 21 days. The standard group received Metformin (500mg/kg body weight). The body weight, fasting blood glucose, and physical parameters of rats were measured on alternate days. Both plants showed considerable reduction in blood glucose levels ($p<0.05$) and improve body weight of diabetic rats, so that the 500mg/kg of T. foenum graecum and of G. glabra were the efficacious dose strengths. Hence, the experimental study establishes the fact that Trigonella foenum graecum and Glycyrrhiza glabra possess antidiabetic potential when given separately and markedly improve fasting blood glucose and body weight of rats when given in combination of both extracts. So, this study may be considered for the isolation of novel chemically active elements having antidiabetic activity for new drug development.*

## INTRODUCTION

Diabetes mellitus is characterized by persistent hyperglycemia linked to anomalies in the metabolism of carbohydrates, lipids and protein (Kamboj et al., 2024). According to reports of the International Diabetes Federation, more than 783 million people will develop diabetes by 2045 (Kashif et al., 2023). Like other developing countries, Pakistan is also facing a sharp growth in the prevalence of diabetes mellitus (Akhtar et al., 2019). Diabetes occurs due to an elevation of reactive oxygen species (ROS) level is due to an increase in catalase enzyme and a decrease in glutathione peroxidase and superoxide dismutase (SOD) antioxidants (Harding et al., 2019). The symptoms of diabetes mellitus include polydipsia,

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polyuria, and polyphagia, along with an abnormal increase in body weight and a glucose level≥200mg/dL (11.1 mmol/L) or a fasting glucose concentration ≥126mg/dL (7.8 mmol/L). Non-insulin-dependent diabetes is the most common form of diabetes mellitus, characterized by high sugar levels in the blood, resistance to insulin, in which cells do not respond to insulin and relative insulin deficiency instead of absolute deficiency. An important causative factor for the development of type 2 diabetes is obesity. Uncontrolled eating habits and a motionless lifestyle of people cause a concurrent increase in obesity and diabetes, which is termed a disability epidemic (Kumar et al., 2020).

Several anti-diabetic herbs have been used since long time as part of traditional medicine. These traditional medicines consist of several herbal and non-herbal constituents and these constituents are necessary for the development of a new compound with possible low side effects for treating diabetes (Eijaz et al., 2024). Nowadays, it is estimated that a proportion of the world population (~80%) depends on herbal products to cure several diseases. Herbal plants have different phytochemical constituents in treating oxidative stress mediated pathological conditions in diabetes and its related complications (Alzahrani et al., 2024).

One of the indigenous plants used for medicinal purposes is *Trigonella foenum-graecum*, commonly known as fenugreek. Maithi is a common name of fenugreek in Pakistan. It has numerous therapeutic properties, including antibacterial, anti-diabetic, anti-inflammatory, galactagogue, and rejuvenating effects. According to the literature, it possesses numerous pharmacological effects, including anti-oxidant, antibacterial, antidiabetic, antiulcer, antifungal, anticancer, and hepatoprotective qualities (Alzahrani et al., 2024). The presence of flavonoids in the ethanolic extract of Glycyrrhiza glabra roots has been shown to reduce blood glucose levels through metabolism via their peroxisome proliferator-mediated receptor ligand binding activity. So, flavonoids in licorice roots play an important role in reducing blood sugar levels in diabetic rats (Prabhu et al., 2021). The purpose of this study is to compare the antidiabetic potential of both plants because they belong to the same family.

## Materials and Methods

### Plant extraction

Ethanol extract of *Trigonella foenum graecum* seed and *Glycyrrhiza glabra* roots were prepared by infrequently shaking, stirring and trembling. The plants were identified by a taxonomist from the Botany department, University of Agriculture (UAF).

### Filtration and storage of plant extracts

After collection and identification, seeds and roots were washed with distilled water and then subjected to crushing and grinding to obtain coarse powder. The powder of both plants was soaked in 90% ethanol for 72 hours with infrequent shaking. Solvents were filtered through Whatman filter paper no.2. The filtrate was then subjected to a rotary evaporator to get a concentrated extract. The plant extracts were stored in amber-colored bottles below 10°C in a refrigerator separately (Abbas et al., 2025). Different qualitative phytochemical analysis carried out using the plants extract.

### *In-vitro* antioxidant study

### DPPH or α-diphenyl-β picrylhydrazyl radical scavenging activity

The DPPH assay of ethanol extracts of both plants was measured by the modified method. 900µl of DPPH was added under dark light with 0.1ml of both extracts at various concentrations separately. The reaction mixtures were kept at 37°C for 15 minutes. Absorbance of both samples was recorded at 517nm by a spectrophotometer. The ascorbic acid is used as a reference or standard dissolved in distilled water.

The scavenging activity of *T. foenum graecum* and *G. glabra was determined by following the equation:*

Inhibition%=1-(*A standard-A blank* ÷ *A control*) × 100

### Reducing power assay

2ml of plant extract, 5ml of potassium ferricyanide and dimethyl sulfoxide were added to a flask to incubate for 30 minutes in a water bath at 50°C.

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Finally, 3ml of TCA 10% used to cease the reaction. Then 5ml of the supernatant portion of the reaction mixture is mixed with 5ml of distilled water and 1ml of $FeCl_3$ solution 0.01%. Then absorbance was taken spectrophotometrically at 593nm and allowed to cool down at room temperature for ten minutes using a blank solution.

**Study Protocol**

Thirty-six Albino rats weighing 180g to 200g were divided into six groups and each group having six healthy rats. Different doses of plant extracts were given orally through gastric intubation. All rats except the normal control group were made diabetic by injecting 120mg/kg body weight alloxan monohydrate intraperitoneally. The standard group was treated with the oral hypoglycemic agent metformin 500mg/kg/OD. The treatment 1 and treatment 2 groups received ethanolic extract of *Trigonella foenum graecum* seed and *Glycyrrhiza glabra* root (500mg/kg/day), respectively. The Treatment 3 group received a combination of both plant extracts. Blood samples were collected at day 0 (after diabetes induction), 11, and 21 (after treatment) for different biochemical tests.

**Evaluation parameters**

Qualitative phytochemical screening, *in vitro* antioxidant study, different physical and biochemical parameters were carried out.

**Statistical analysis**

Data was evaluated through using the two-way ANOVA and Dunnett's Multiple Range test (DMR). Data was showed as (mean ± SD).

## RESULTS

**Phytochemical analysis**

Different chemical tests were carried out to reveal the presence of alkaloids, phenols, tannins, saponins, flavonoids and carbohydrates in both extracts, as shown in Table 1. Results of DPPH and reducing power are shown in Table 2.

**Table-1: Qualitative phytochemical analysis**

| Phytochemicals | *T. foenum graecum* | *G. glabra* |
|---|---|---|
| Alkaloids | + | + |
| Phenols | + | + |
| Tannins | + | + |
| Saponins | + | + |
| Flavonoids | + | + |
| Carbohydrates | + | + |

**Table-2:** Quantitative antioxidant activity

| Oxidative test | *T. foenum graecum* | *Glycyrrhiza glabra* |
|---|---|---|
| %Inhibition of DPPH | 69.70% | 41.935% |
| Reducing power % | 19.76% | 21.52% |

**Body weight of rats**

Results showed that the body weight of the diabetic control group animals had decreased significantly ($p<0.01$) as the experiment proceeded relative to week 0. This observation indicates that animals treated with the fenugreek seed extract (500mg/kg) and licorice root extract (500mg/kg) and a combination of both extracts have a substantial effect on the physical parameter (weight) of the body and improve the body weight of experimental rats to normal weight after treatment for 21 days. Animal groups treated with plant extracts significant increase ($p<0.05$) in body

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weight throughout the whole experimental period, as shown in Figure 1.

**Feed intake**

The results showed that feed intake in the diabetic group was slightly decreased as compared to the normal group. Treatment of rats with *Trigonella foenum graecum* extract 500mg/kg and *Glycyrrhiza glabra* extract 500mg/kg gradually improved the feed intake towards the normal as compared to the diabetic group, as shown in Figure 1.

**Fasting Blood Glucose (mg/dL)**

In diabetic rats, an average fasting blood glucose concentration increased significantly (P<0.05) in the first week after induction of diabetes, as seen in Figure 1. Average fasting blood glucose level was significantly (P<0.05) decreased after treatment with *T. foenum graecum* 500mg/kg, *G. glabra* 760mg/kg and a combination of both plant extracts in all weeks.

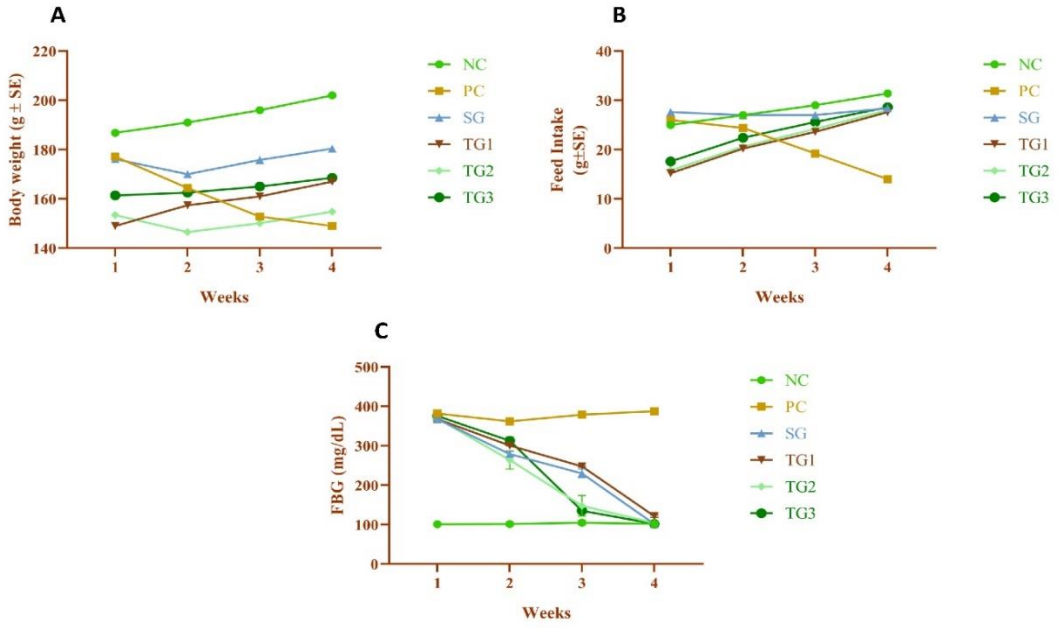


**Figure 1. Effect of treatment on physical parameters where A= Body Weight, B= Feed Intake and C= Fasting Blood Glucose level in diabetic rats**

**Serum Blood Glucose (mg/dL)**

Experimental results showed increased random blood glucose levels in group 2 as compared to the control group after three weeks. After three weeks of testing, treatment groups exhibited a substantial drop in randomized blood glucose compared to disease group (Figure 2).

**Serum insulin level (mg/dL)**

Serum insulin the level was very low (P<0.05) in the diabetic group, but serum insulin levels in the standard and treated groups were significantly(P<0.05) raised close to normal level according to time-dependent manner on the 21st day of study as shown in Figure 2.

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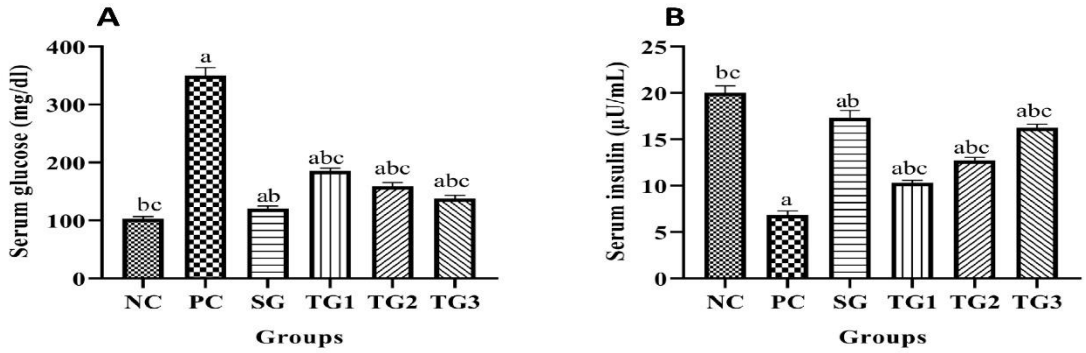


**Figure 2. Effect of plant extracts on A= Serum Glucose and B= Serum insulin level. a shows significance of normal control with others, b shows significance of disease control with other groups and c shows significant differences of standard drug treated group with other groups.**

**Health Markers**

The results of statistical analysis showed that the average values of aspartate aminotransferase AST (U/L), alanine aminotransferase ALT (U/L), creatinine, and Urea levels were significantly ($P<0.05$) high in diabetic rats. Results described that treated groups showed changes, the treatment doses considerably decreased ALT, AST, Creatinine, and Urea levels, as shown in Figure 3.

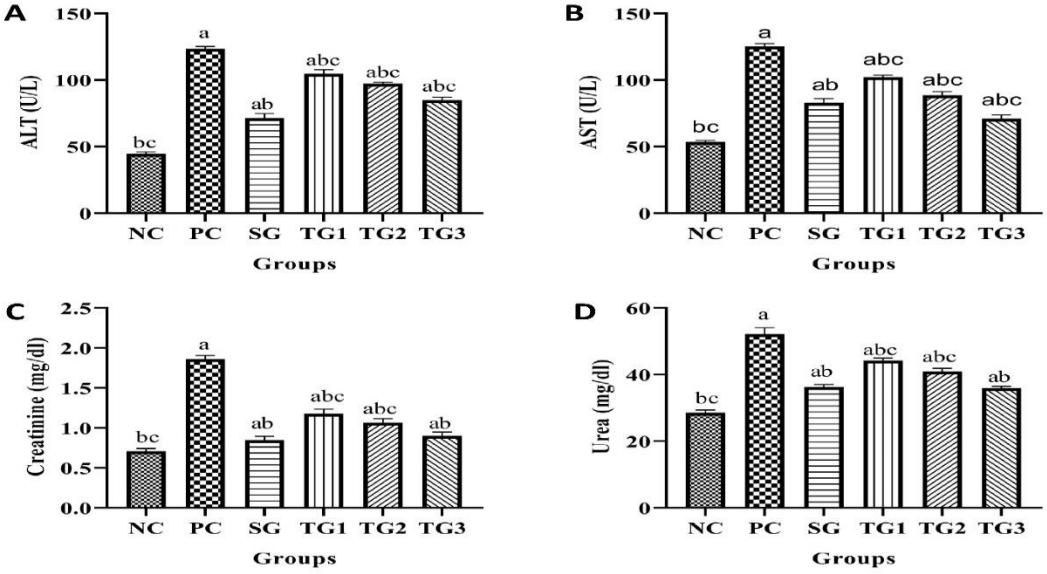


**Figure 3. Effect of plant extract on health markers where A= ALT, B= AST, C= Creatinine level and D= Urea level in serum**

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**Anti-Inflammatory and Anti-oxidants**

The level of TNF-α and IL-6 was significantly higher, while CAT and SOD level were lower in diabetic rats. Treatment with plant extracts significantly improved the anti-inflammatory and antioxidant levels, as shown in Fig. 4.

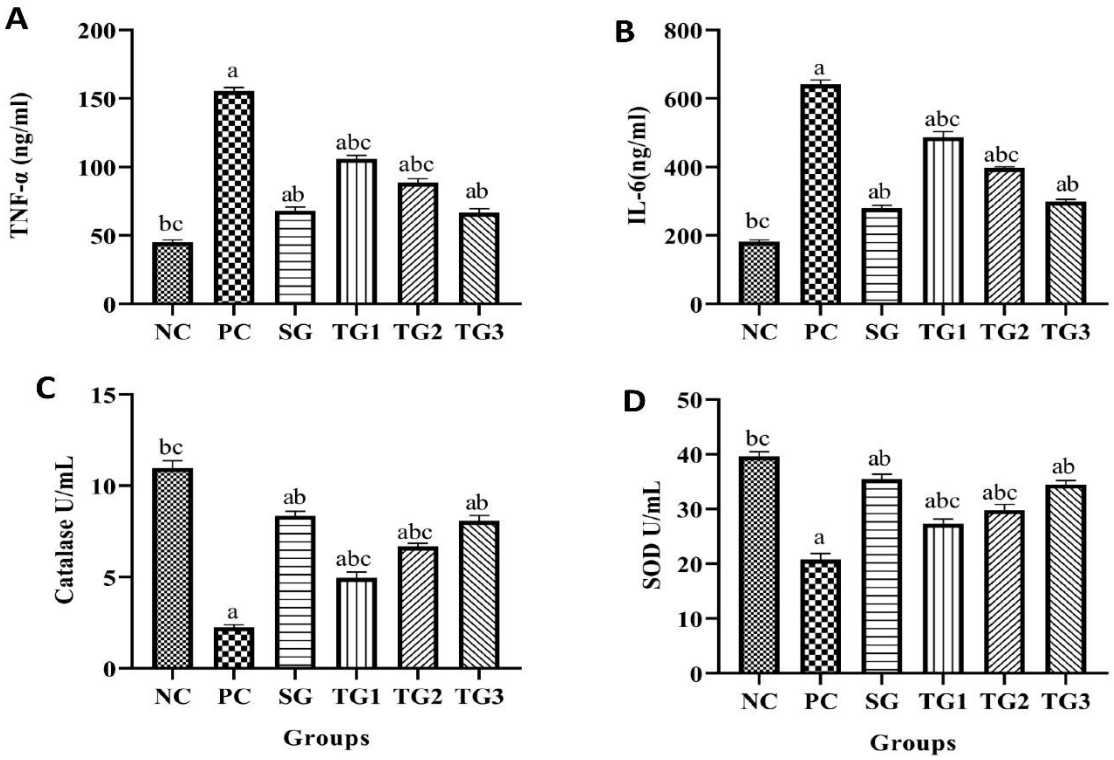


**Figure 4. Effect of plant extract on inflammatory and oxidative stress markers where A= TNF-α, B= IL-6, C= Catalase and D= SOD level in serum**

## DISCUSSION

Diabetes mellitus is a metabolic disorder of carbohydrate, fat, or protein metabolism that causes an abnormally high blood glucose level (Shaw et al., 2010). Currently present pharmacological therapies include exogenous insulin administration and oral hypoglycemic agents. It added a substantial economic burden and has a harsh impact on the individual's finances and on their families, especially in developing countries (Sharma et al., 2010). This makes it difficult to provide proper healthcare medications, especially in poor and developing countries. Furthermore, the available therapies have quite a few adverse effects (Bandawane et al., 2011).

Plants include a variety of phytochemical and bioactive components, including flavonoids, alkaloids, tannins, saponins, polyphenols, glycosides, terpenoids, and protein-based chemicals, all of which contribute to insulin synthesis and glucose uptake. Nowadays, many synthetic drugs and insulin, including human insulin, are used for the treatment of diabetes, but have many side effects on prolonged use. Therefore, scientists all over the world are working on medicinal plants useful for the treatment of diabetes mellitus (Tran Kien et al., 2021). Medicinal plants used for human health care are part of the traditional medicinal system (Karunamoorthi et al., 2013).

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Fenugreek is the common name of *Trigonella foenum-graecum* and it is widely used in many Ayurveda preparations. Due to the nutritive and curative properties of fenugreek, it has been used in folk medicine for treating a wide range of diseases, including diabetes, fever, abscesses and boils. Various reports revealed that fenugreek seeds have a hypoglycemic effect when used by diabetic patients (Shaw et al., 2010). In the present study, the ethanol extract of *Trigonella foenum graecum* seeds shows a hypoglycemic effect in diabetic rats. After administration, the seed extract shows noticeably reduced blood glucose levels in diabetic rats.

*Glycyrrhiza glabra* is usually known as liquorice or licorice. The rhizome and roots of this plant are useful because they have a sweet taste and can be utilized as spices and flavoring. According to the literature, it has numerous pharmacological effects, including antiulcer, antioxidant, anti-inflammatory, antibacterial, antifungal, antidiabetic, anticancer, and hepatoprotective activity. The extract of ethanol of *Glycyrrhiza glabra* roots includes polyphenolic chemicals that inhibit amylase as well as glucosidase in vitro. The results showed that *Glycyrrhiza glabra* plant roots possess antidiabetic and antioxidant properties (Karkanis et al., 2018).

The presence of flavonoids in the ethanol-based extract of *Glycyrrhiza glabra* roots has been shown to reduce blood glucose levels through metabolism via peroxisome proliferator-induced receptor ligand binding activity. *Glycyrrhiza glabra* enhance insulin mediated glucose removal in muscles and also enhances GLUT-1 transport from the intracellular site to the plasma membrane.

**Conclusion**

In the present scenario, the usage of alternative remedies provides unique opportunities for plant-based product development. Developed methods in the past are significantly contributing to the isolation of phytochemical compounds possessing therapeutic nature. The lifestyle changes are related to the increased prevalence of diabetes and its complications. This study showed the antioxidant capacity of *T. foenum graecum* and *G. glabra* due to the presence of phytochemical constituents, which have antioxidant and anti-diabetic properties. Moreover, the present study provided evidence that both plants extract responsible for maintaining body weight and exhibiting antioxidant activity. Thus, these plants may be considered as a source for the separation of a novel antidiabetic agent.

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