Research Article

Phytopharmacological evaluation of combined extract of *Trigonella foenum-graceum* and *Holarrhena pubescens* for the management of alloxan induced type-2 diabetes in rats

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Abstract

Objectives: To evaluate the antioxidant, antidiabetic effect of the polyherbal formulation of methanolic extract of *Trigonella foenum-graceum* (seed) and *Holarrhena pubescens* (stem) on alloxan induced type-2 diabetes in rats. **Materials and Method:** Rats were divided into five groups (n=6). Group-I (Normal control) received normal saline. Group-II (Negative control) received 120 mg/kg of Alloxan, treated subsequently with normal saline.Group-III and IV (Test group Test-1&2) received 250, 500 mg/kg bw of TFG+HP methanolic extract. Group-V (Standard) received Glibenclamide (3mg/kg p.o). The qualitative analysis of various phytochemical constituents and quantitative analysis were determined. Theantidiabetic effects studied using in vivo glucose uptake in an isolated alloxan-induced diabetic rat model. The *in vitro* anti-oxidant activity of extracts of *Trigonella foenum-graecum* and *Holarrhena pubescens* was evaluated by DPPH radical scavenging activity. **Results:** Their phytochemical analysis revealed variable phytoconstituents in the methanolic extracts. In vitro antioxidant activity was measured using the one, DPPH assay, with methanolic extracts from *Trigonella foenum-graecum* and *Holarrhena pubescens* showing IC_{50} values of 37.05 and 32.32, respectively, close to the standard (Ascorbic acid). The test samples showed a significant ($P<0.05$) reduction in blood glucose levels compared to the alloxan-treated group. The body weights for the normal control, alloxan-treated, and polyherbal formulation-treated groups (at doses of 250 mg and 500 mg) were recorded. **Conclusion:** The results obtained in present study clearly demonstrate that the methanolic extracts from *Trigonella foenum-graecum* seeds and *H. pubescens* stems exhibited significant antioxidant activity and moderate antidiabetic activity, suggesting the need for further exploration in animal models and the isolation of active constituents.

Keywords: *Trigonella foenum-graecum, Holarrhena pubescens*, alloxan-induced, phytochemical, antidiabetic, polyherbal

Introduction

Diabetes mellitus (DM), characterized by persistent hyperglycemia, is a prevalent condition affecting over 124 million individuals globally (Laakso et al., 2001; Quinn et al., 2001). DM is linked to an elevated risk of atherosclerosis, renal, nervous system, and ocular complications. The primary biochemical abnormality underlying the increased oxidative load in DM appears to be uncontrolled hyperglycemia. This heightened oxidative stress is implicated in the pathogenesis of diabetic complications and premature age-related changes associated with DM (Hasanain et al., 2002). Numerous studies have consistently demonstrated that type 2 diabetes is accompanied by increased oxidative damage to various biomolecules, particularly lipids. Both animal models and human studies have shown that diabetes is correlated with oxidative stress, as indicated by elevated blood levels of lipid peroxidation products, especially incases of poor blood glucose control (Oranje et al.,1999; Laight et al., 2000; Sundaram,1996; Nourooz-Zadeh et al., 1997; Bonnefont-rousselot et al., 2000; Jennings et al.,1987; Mooradian et al., 1996;Velazquez et.al 1991; Salah et al., 1995; Feillet-Coudray et al., 1999). Given the etiopathogenesis of DM, concerns about the adverse effects of synthetic drugs, limitations of current therapies in addressing all aspects of diabetes, the high costs associated with modern medications,

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and limited access to advanced treatments in rural areas of developing nations, there is a pressing need for alternative approaches to pharmacotherapy for diabetes mellitus. The utilization of medicinal plants is gaining recognition in primary healthcare for diabetes, offering a potential breakthrough in treatment. Recent experiences highlight the advantages of natural remedies, emphasizing their relatively low toxicity, safety, and minimal occurrence of severe side effects (Dangi et al., 2004)

Trigonella foenum-graecum, a beneficial medicinal plant from the Fabaceae family, is an annual herb cultivated globally, including in Ethiopia. Widely recognized, the seeds serve as spices worldwide, while the leaves are incorporated into diets as green leafy vegetables. Notably, *Trigonella foenum-graecum* seeds, known for their bitter taste, have been esteemed for their medicinal qualities over an extended period (Sauvaire et al., 1998; Gupta et al., 2001; Geberemeskel et al., 2019). Throughout ancient literature, religious scriptures, travel records, and anecdotes spanning various continents and historical periods, a diverse range of medicinal properties linked to *Trigonella foenum-graecum* has been documented. These medicinal uses encompass a broad spectrum, ranging from wound healing to potential benefits in bust enhancement. This not hardly holds promise for the incident of new drug leads but further focuses their potential as edible supplements to existing therapeutic approaches (Zahara et al., 2020). Numerous pharmacological effects have been attributed to this herb, including antipyretic, antidysentery, antidiarrheal, anthelmintic, antibacterial, and anti-amoebic properties (Kokate et al., 2014).

Considering the factors mentioned earlier, this current research conducted an experiment involving varying dosages of plant extracts derived from *Trigonella foenum-graecum* and *Holarrhena pubescens* on rats with induced diabetes from alloxan. The objective is comparing hypoglycemic impacts of these diverse doses with effects of a recognized antidiabetic medication (Glibenclamide) on the induced diabetic rats. Additionally, the study aimed to perform initial qualitative and quantitative phytochemical analyses on the raw extract and assess the potential antioxidant effects and toxicity levels associated with these crude extracts.

Materials and methods

Collection of Plant Material

The medicinal plant *Trigonella foenum-graecum* (Seed) and *Holarrhena pubescens* (Stem) (300 gm) was collected locally from Bhopal, M.P. After cleaning, plant (Seed and Stem) were dried under shade at room temperature for 3 days and Then in oven at 45°C till complete dryness. Dried plant *Trigonella foenum-graecum* (Seed) and *Holarrhena pubescens* (Stem) were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration.

Preparation of plant extract

Trigonella foenum-graecum and *Holarrhena pubescens* plant portions (300 gm) were coarsely grounded and then extracted using soxhlet assembly& sequential extraction with Pet ether & methanol organic solvents for 36 hours. Each extract was then dried by evaporation at low pressure using a rotary evaporator and the resulting residue was kept in an airtight container (Kokate et al., 1994).

Preliminary Phytochemical investigation

The Preliminary Phytochemical screening was performed by using pet. Ether and methanolic extract of both the plant to determine the presence of phytochemicals such as carbohydrates, alkaloids, flavonoids, glycosides, proteins & amino acids, saponins, triterpenoids and steroids, tannins and other phenolic compounds (Table 3). To identify the constituents in plant extracts, specific qualitative phytochemical tests were performed. The colour intensity or the precipitate formation was used as medical responses to tests (Tongco et al., 2015).

Quantitative Phytochemical Estimation

Total phenolic content (TPC)

The total phenolic content of *Trigonella foenum-graecum* and *Holarrhena pubescens* extract was determined using the Folin-Ciocalteu Assay. The *Trigonella foenum-graecum* and *Holarrhena pubescens* extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent mg/gm. Concentration of 20, 40, 60, 80, and 100 μg/mL of Gallic aid was prepared. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically (Parthasarathy et al., 2009).

Total flavonoid content (TFC)

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of *Trigonella foenum-graecum* and *Holarrhena pubescens* extract solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allowed to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and

mixed thoroughly. Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Rutin was prepared. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight (Adisa et al., 2011).

In-vitro Antioxidant Activity using DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

For the preparation of DPPH reagent, 0.1mM solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol was prepared. Then sample/standard was prepared by taking freshly 1 mg/ml methanol extract of *Trigonella foenum-graecum* and *Holarrhena pubescens* extracts/standard. Different volume of extracts/standard (20 – 100µl) was taken from stock solution in a set of test tubes and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly and absorbance was recorded at 517 nm after 30 minutes incubation in dark at room temperature. For control, Take 3 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm (Sikarwar et al., 2010).

Experimental Animals

Healthy Male Wistar rats (200±50 gm) with no prior drug treatment were selected to carry out all the present studies. The animals were used after an acclimatization period of 10 days to the laboratory environment .They were housed in standard metal cages and provided with food and water ad libitum. All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) and the approval reference number was granted is PBRI/IAEC/02-05-23/019. Animals were housed in a group of six in separate cages under controlled conditions of temperature (22 ± 2°C).

Acute oral toxicity

Acute oral toxicity of the polyherbal formulation was carried out as per the guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423. The principle involves a stepwise procedure with the use of a minimum number of animals per step to obtain sufficient information on the acute toxicity of the test substance to enable its classification. Healthy wistar rats (3 animals/dose) of either sex were used for the experiment. Overnight fasted rats were orally fed with the plant extracts in increasing dose levels of 50, 300, 500 and 2000 mg/kg body weight, respectively. The animals were observed for their behavioral (alertness, restlessness, irritability, and fearfulness), profiles continuously for 24 h. After a period of 24 h, the animals were observed for 14 days for mortality (Mahajan and Baviskar, 2021).

Preparation of Polyherbal formulation

They were taken in the ratio of 1:1 (500:500 mg). Suspension was prepared by using plant extracts of selected plant materials by triturating method in mortar and pestle using the suitable suspending agent of Tween 80 (0.01% v/v) and Sodium carboxy methyl cellulose (CMC 0.7%) along with Methyl paraban (0.2% w/v) and lemon oil (0.01ml) excipient. The dried extracts were mix in water and additives Tween-80, Sodium CMC were added (Table 1). The suspending agent, sodium CMC in the aqueous medium containing preservatives was added in mortar and pestle along with extracts of selected plant material with continuous triturating (Print et al., 2015; Shah et al., 2014).

Evaluation parameter of Suspension

Sedimentation volume

The sedimentation volume is the ratio of the ultimate height of the sediment to the initial height of the total suspension as the suspension settles in a cylinder under appropriate standard conditions. It was evaluated by keeping a measured volume of suspension in a graduated cylinder in an undisturbed state for a certain period and note that the volume of the sediment is expressed as ultimate height (Print et al., 2015).

Table 1. Composition of Polyherbal formulation

S. No	Ingredients	Quantities
1.	*Trigonellafoenum-graecum* and *HolarrhenapubescensExtract*	1:1
2.	Tween 80	0.01 % v/v
3.	Sodium carboxy methyl cellulose	0.7 %
4.	Methyl paraban	0.2 % w/v
5.	Propyl paraben	0.02 % w/v
6.	lemon oil	0.01ml
7.	Distilled water	q.s

Redispersibility

The suspension was allowed to settle in a measuring cylinder. The mouth of the cylinder was closed and was inverted through 180º and the number of inversions necessary to restore a homogeneous suspension was determined (Print et al., 2015).

pH determination

A pH meter (EI) was used to analyze the suspension's pH level (Print et al., 2015).

Particles size analysis

Particle size distribution has a crucial role in stability of a suspension. Optical microscopy was used to determine particle size distribution in highly diluted solutions (Print et al., 2015).

Induction of diabetes in rats

In order to induce diabetes in the rat, an overnight fasting was imposed to standardize conditions. Diabetes was induced through the intraperitoneal injection of Alloxan monohydrate, dissolved in sterile normal saline. The injection was administered at a dosage of 120 mg/kg, with the specific doses determined based on the individual body weights of the animals. Prior to the administration of Alloxan monohydrate, all the rats were subjected to blood glucose level evaluations. Subsequently, after a 72-hour period, blood glucose levels were once again assessed. Rats with blood glucose levels surpassing 250 mg/dl were classified as diabetic, rendering them eligible for inclusion in the study (Strate et al., 2005).

Experimental design

Diabetes was induced in groups aside from the normal control via a singular intraperitoneal injection of Alloxan at a dose of 120 mg/kg. Throughout the experimental period, Glibenclamide was orally administered, suspended in 0.9% NaCl warm water. Treatment was initiated on the fourth day following diabetic induction, marking the first day of treatment, and sustained for a duration of 21 days. Observations were made at regular intervals of 0, 3, 7, 14, and 21 days post-treatment, encompassing measurements of body weight and blood glucose levels.

Animal Grouping

For the purpose of proper experimentation, the animals were methodically divided into five separate groups, each of which consisted of six animals.

The animal grouping structure was as follows:

Group-I encompassed the normal control and was treated with a solution of normal saline.

Group-II served as the negative control, comprising alloxan-induced diabetic rats that received an injection of 120 mg/kg of Alloxan, treated subsequently with normal saline.

Group-III represented Test-1, wherein alloxan-induced diabetic rats were treated with a combination of *Trigonella foenum-graecum* and *Holarrhena pubescens* extracts at a dose of 250 mg/kg bw (TFG+HP).

Group-IV, or Test-2, entailed alloxan-induced diabetic rats being treated with 500 mg/kg bw of the same combination (TFG+HP). Finally,

Group-V, designated as the standard, involved alloxan-induced diabetic rats that were administered Glibenclamide orally at a dose of 3 mg/kg.

Once again, diabetes was induced via a single intraperitoneal injection of Alloxan, with the exception of Group I. Glibenclamide administration followed the same procedure as previously outlined.

Blood sample collection & blood glucose determination

Blood samples for glucose analysis were collected from the tail tips of the experimental rats. The measurements occurred on the days indicated in the study-specifically, days 0, 3, 7, 14. The blood glucose levels were quantified using the milligrams per deciliter (mg/dl) unit. This methodology boasted sufficient sensitivity, with the added benefit that minimal blood volumes (1-2 µL) were requisite for the analysis. The blood sample was procured by delicately cutting the tail tips using a sharp blade. The glucose test strips embedded within the glucometer were employed to facilitate the analysis process.

Histopathology of pancreas

The whole pancreas from each animal was removed after sacrificing the animal and washed on ice cold saline immediately. A portion of pancreatic tissue was fixed in 10% neutral formalin fixative solution for histological studies. After fixation tissues were embedded in paraffin, solid sections were cut at 5 µm and the sections were stained with haematoxylin and eosin (Tan et al., 2019).

Results

Formulation of Polyherbal suspension

In Polyherbal formulation it was observed that, sedimentation volume (0.28), PH slightly alkaline pH (6.2), viscosity (51.3), particle size (19.03), Density (1.21gm/ml) and good redispersibility observed .

Percentage yield

The yield of extracts received from the *Trigonella foenum-graecum* and *Holarrhena pubescens* is summarized in Table 2. The % yield of petroleum ether extracts of *Trigonella foenum-graecum* and *Holarrhena pubescens* were 0.52% and 0.43% respectively, and the of the methanolic extracts were1.86% and 1.62%. Methanolic extracts of both the plant provide highest % yield compared to petroleum ether extracts (Table 2).

Table 2. Results of percentage yield of crude extracts of *Trigonella foenum-graecum and Holarrhena pubescens* extract

S. N.	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Trigonellafoenum-graecum*	Pet ether	300	1.56	0.52%
		Methanol	350	5.58	1.86%
2	*Holarrhena pubescens*	Pet ether	300	1.30	0.43%
		Methanol	367	5.95	1.62%

Table 3. Results of phytochemical analysis of both the plant extracts

Chemical Constituents	*Trigonellafoenum-graecum*		*Holarrhena pubescens*	
	Pet. Ether Extract	Methanolic Extract	Pet. Ether Extract	Methanolic Extract
Carbohydrates	-	+	-	-
Glycosides	-	+	-	+
Alkaloids	-	+	-	+
Terpenoids and Steroids	-	+	-	+
Flavonoids	-	++	-	+
Tannins and Phenolic Compounds	-	++	-	+
Saponins	-	+	-	+
Protein and Amino acids	-	+	-	-

Soxhlet extraction method, + and – designates presence & absence of respective SMs in test extract,

Preliminary Phytochemical study

Qualitative Analysis

The preliminary phytochemical investigation of the *Trigonella foenum-graecum* methanolic extract revealed the presence of Carbohydrates, saponins, protein and amino acid, terpenoids, alkaloids, carbohydrate and phenolic compounds such as tannins, and flavonoid and *Holarrhena pubescens* methanolic extract revealed the presence of saponins, glycosides, alkaloids, and phenolic compounds such as tannins, and flavonoid, while the petroleum ether extract of both the plants does not shows the presence of any phytoconstituents. Hence methanolic extract of both the plants were analysed for the further studies. These compounds are likely to be responsible for the observed antioxidant and antidiabetic activity of this extract either single or in synergy with one another (Table 3).

Quantitative Analysis

Preliminary phytochemical testing of crude extracts confirmed the presence of phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed and the results are summarized in table 4. TPC of *Trigonella foenum-graecum* and *Holarrhena pubescens* was 76 mg/gm and 93 mg/gm respectively and TFC of *Trigonella foenum-graecum* and *Holarrhena pubescens* was 25 mg/gm and 39 mg/gm respectively. However, the extract of *Trigonella foenum-graecum* shows the higher TPC and TFC compared to extract of Holarrhena pubescens (Table 4).

In vitro Antioxidant Assays

In the present investigation, the in vitro anti-oxidant activity of extracts of *Trigonella foenum-graecum* and *Holarrhena pubescens* was evaluated by DPPH radical scavenging activity. The results are summarized in Tables 5. *Trigonella foenum-graecum* and *Holarrhena pubescens* methanolic extract showed IC50 value 37.05 % and 32.32 % for DPPH method, which was comparable to that of ascorbic acid (IC50 =22.65%). DPPH is a free radical, stable at room temperature, which produces a purple colour solution in methanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured methanol extract (Table 5).

***In vivo* anti -diabetic study**

All groups prior to extract administration (0 day) showed no apparent difference in body weight compared to normal control group. Significant body weight gain was recorded for *Trigonella foenum-graecum* and *Holarrhena pubescens* at

Table 4: Results of Total Phenolic and Total Flavanoids contents

***Trigonella foenum-graecum* extract**		***Holarrhena pubescens* extract**	
Total Phenolic Content(mg/gm equivalent to gallic acid)	Total Flavanoids contents(mg/gm equivalent to rutin	Total Phenolic Content(mg/gm equivalent to gallic acid)	Total Flavanoids contents(mg/gm equivalent to rutin
76	25	93	39

Table 5: DPPH radical scavenging activity of methanol extract of both the plants and standard

Extracts	**DPPH IC_{50} (% inhibition)**
Trigonella foenum-graecum	37.05±1.54
Holarrhena pubescens	32.32±1.62
Standard (Ascorbic acid)	22.65±1.08

All the values were expressed as Mean±SEM. Statistical difference were determined by one way ANOVA followed by Dunnett"s test. P<0.05 consider as statistical significant.

Table 6: Effect of *Trigonella foenum-graecum* and *Holarrhena pubescens* extract on Body weight of the rats

Groups	**Treatment**	**Body weight(gms)**			
		0 day	**3 day**	**7 day**	**14 day**
Group 1	Normal control	204.94±0.26	207.11±0.23	211.38±0.22	214.12± 0.21
Group II	Alloxan treated Dose (120 mg/kg)	215.93±0.21	208.18±0.138	206.10±0.18	197.10±0.25
Group III	250mg/kg bw (PHF)	211.03±0.22	209.14±0.26	208.37±0.39	213.99±0.15
Group IV	500 mg/kg bw (PHF)	213.95±0.25	210.29±0.20	210.53±0.13	216.13±0.12
Group V	Glibenclamide (3 mg/kg)	217.95±0.24	217.06±0.27	221.18±0.18	221.02±0.20

the 7th day of treatment compared to diabetic control group. All doses of the extracts and standard showed a significant improvement in body weight at the 14th day when compared to diabetic control. By contrast, the body weight of the diabetic control group was significantly decreased at the 14th day compared to a normal control group (Table 6).

Blood glucose determination

Glucose is also the main fuel in the body tissues and has the function to generate energy. Blood glucose level is closely related to diabetes mellitus. Diabetes mellitus is a disease that arises in a person which is indicated by the presence of blood glucose levels exceeding the normal level (hyperglycemia) due to the deficiency of the insulin hormone in the body. If the disease is uncontrolled or the patient is unaware of the disease, there will be various fatal chronic complications

Histopathology of pancreas

Histopathological studies (Figure 1) shows normal acini and normal cellular population in the islets of langerhans in pancreas of normal control and lesions in diabetic rats which maintained significantly after treatment by standard drug and PHF up to normal.

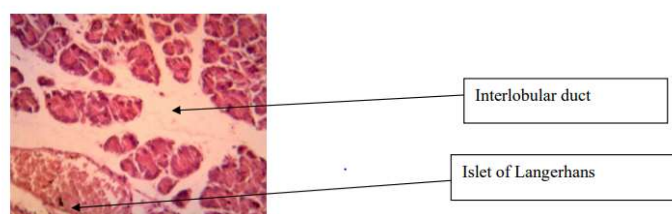
Discussion

The prevalence of Type 2 diabetes has been increasing exponentially, and a high prevalence rate has been observed in developing countries and in populations undergoing "westernization" or modernization. The failure of the current therapies, and financial costs for the treatment of this disease make it necessary to develop new efficient therapy strategies and appropriate prevention measures for the control of Type 2 diabetes. Herein, we summaries our current understanding about the epidemiology of Type 2 diabetes, the roles of genes, lifestyle and other factors contributing to rapid increase in the incidence of Type 2 diabetes. The core aims are to bring forward the new therapy strategies and cost – effective intervention trials of

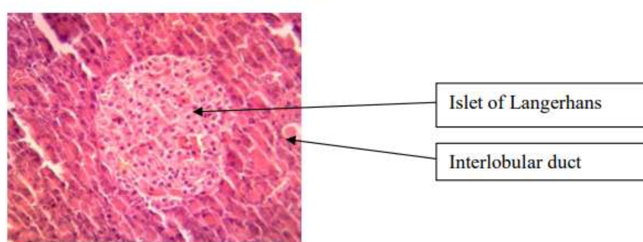
Table 7: Impact of test samples of extract upon Blood Glucose Level in experimental rats

Groups	Treatment	Blood Glucose Level (gms)			
		0 day	3 day	7 day	14 day
Group 1	Normal control	85.07±0.19	87.06±0.20	84.38±0.33	85.16±0.19
Group II	Alloxan treated Dose (120 mg/kg)	84.19±0.17	274.85±0.26	288.12±0.23	295.21±0.30
Group III	250 mg/kg bw (PHF)	85.09±0.22	254.36±0.104	243.87±0.49	219.22±0.28
Group IV	500 mg/kg bw (PHF)	78.13±0.09	241.09±0.14	229.99±0.20	182.07±0.23
Group V	Glibenclamide (3 mg/kg)	76.52±0.35	239.83±0.26	226.02±0.12	173.14±0.27

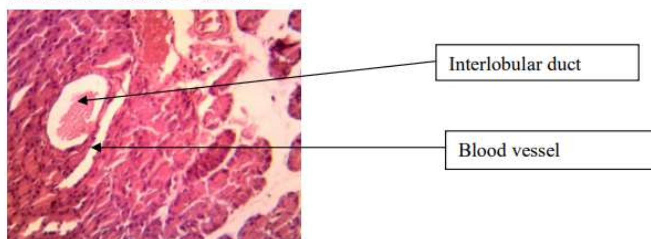
Normal Group 1



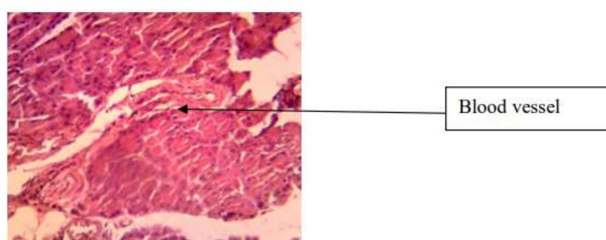
Group II Alloxan treated Dose (120 mg/kg)



Group III 250mg/kg bw (PHF)



Group IV500 mg/kg bw (PHF)



Group V Glibenclamide (3 mg/kg)

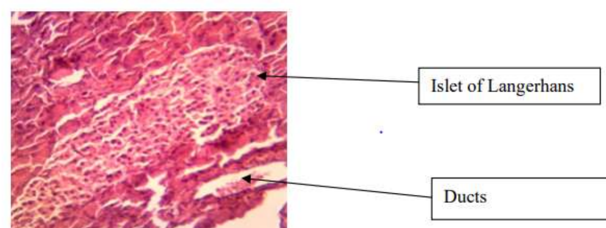


Figure 1: Histopathology of Pancreas

Type 2 diabetes acute toxicity study of the Methanolic extracts demonstrated that *Trigonella foenum-graecum* and *Holarrhena pubescens* were non-toxic throughout the experiment. The lethality was found to be zero in the groups of *Trigonella foenum-graecum* and *Holarrhena pubescens* extracts.

The low dose of Alloxan (120 mg/kg body wt. i.e.) was chosen for this study there might have been many surviving beta cells, capable of undergoing regeneration. In conclusion, the present study shows that the Methanolic extracts of *Trigonella foenum-graecum* and *Holarrhena pubescens* has potential anti diabeticaction in Alloxan induced diabetic rats and the effect was found to be more similar to the reference drug glibenclamide. Further study needs investigation to pinpoint the mechanism of activity. The present investigation discusses the anti diabetic potential of the *Trigonella foenum-graecum* and *Holarrhena pubescens* in Alloxan- induced diabetic rats. In present work, administration of the *Trigonella foenum-graecum* and *Holarrhena pubescens* herbal formulation dose (250 and 500 mg/kg) resulted in a significant hypoglycemic effect in normoglycemic rat. The hypoglycemic effect of glibenclamide was evident due to the stimulation of insulin release from pancreatic β-cells and inhibition of glucagon secretion (Table 7).

Conclusions

The results obtained in the present study clearly demonstrate that the methanolic extract of *Trigonella foenum-graecum* and *Holarrhena pubescens*, which can effectively scavenge reactive oxygen species/free radicals under in vitro conditions and antidiabetic activity. Phytochemical analysis indicated the presence of key bioactive compounds. The extracts demonstrated in vitro antioxidant activity, providing insight into their potential mechanisms of action. This antioxidant activity may be due to the number of stable oxidized products that it can form after oxidation or radical scavenging. The formulated polyherbal suspension

exhibited desirable characteristics, such as sedimentation volume, pH, viscosity, redispersibility, density, and particle size. In the in vivo study, the extracts exhibited favorable effects on body weight and blood glucose levels in treated rats. These findings suggest the potential of *Trigonella foenum-graecum* and *Holarrhena pubescens* extracts as adjuncts in diabetes management. Further investigations are warranted to elucidate their precise mechanisms and therapeutic potential.

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