

EVALUATION OF FOENICULUM VULGARE AND TAGETES ERECTA FOR SYNERGIC ANTI DIABETIC ACTIVITY IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Herbal medicine sometimes referred to as Herbalism or Botanical medicine it includes use of herbs for treating pathological condition in the system. Herbal medicine have received considerable attention during last two decades as they are endowed with variety of biological activities and have wide range of therapeutic properties. Diabetes mellitus (DM) is the name given to a multiple group of disorders with different etiologies. It is characterized by derangement in protein, carbohydrate, and fat metabolism caused by the complete or relative insufficiency of insulin secretion and/or insulin action. Diabetes is a

major health problem worldwide as approximately 5% of the world's population suffers from diabetes. Diabetes is a multifaceted disease involving impaired insulin secretion and insulin resistant. The loss of glycemic control, associated with these defects results in long-term complications which are both micro vascular (e.g.; retinopathy, nephropathy, neuropathy) and macro vascular (e.g.; stroke, myocardial infraction and peripheral vascular disease). The current treatment for both of diabetes delay, but do not prevent the micro vascular disease, leading in the long run to complications, including heart disease, stroke, blindness, kidney failure and limb amputation. Traditional plant medicines are used throughout the world for a range of Diabetic presentations. Therefore, investigation on such agents from traditional medicinal plants has become more important. India has a rich history of using various potent herbs and herbal components for treating Diabetes. Many Indian plants have been investigated for their beneficial use in different types of Diabetes. *Foeniculum vulgare* belonging to family Umbelliferae is mainly used as a food condiment the chief chemical constituent of fennel is volatile oils but it also contain ketone, fenchone, phelladrine. Pharmacologically it is used as anti-inflammatory, anti-oxidant, and hepatoprotective.

Tagetes erecta of the family compositae is commonly found in parts of India, Asia, Africa and America. It is known as Marigold. The leaves are reported to be effective against piles, Kidney troubles, muscular Pain, ulcers, wound and earache. The herbs are used for the treatment of inflammatory conditions as a household remedy on experimental basis. The chief chemical constitutionsof Tagetes erecta is volatile oils, triterpinoids. The present study is mainly includes the extraction of Foeniculum vulgare and Tagetes erecta by using hydroalcohol with different concentration, and the extracts are test for its anti-diabetic activity individually and in combination for its synergistic anti diabetic activity. The test results are compared with the standard drug Glibinclamide, the Streptozotocin is used as a diabetic inducer in wister albino rats weighing 150-200 mg divided in six groups each group containing five rats in each group. The hydroalcoholic extract of Foeniculum vulgare and Tagetes erecta individually and in combination shown antidiabetic activity but it is not significant effect when the test results are compared with standard drug i.e Glibinclamide.

KEYWORDS: Diabetes mellitus, Glibinclamide, Foeniculam vulgare, Tagetes erecta, Streptozotocin.

INTRODUCTION

Diabetes mellitus (DM) is the name given to a multiple group of disorders with different etiologies. It is characterized by derangement in protein, carbohydrate, and fat metabolism caused by the complete or relative insufficiency of insulin secretion and/or insulin action.^[15]

Diabetes is a major health problem worldwide as approximately 5% of the world's population suffers from diabetes. Worldwide projections suggest that more than 300 million people will have diabetes by the year 2025 and the global cost of treating diabetes and its complications could reach US\$1trillion annually.^[41]

Diabetes is a multifaceted disease involving impaired insulin secretion and insulin resistant. The loss of glycemic control, associated with these defects results in long term complications which are both micro vascular (e.g.; retinopathy, nephropathy, neuropathy) and macro vascular (e.g.; stroke, myocardial infraction and peripheral vascular disease).^[28]

The current treatment for both of diabetes delay, but do not prevent the micro vascular disease, leading in the long run to complications, including heart disease, stroke, blindness, kidney failure and limb amputation. In type 1 diabetes the difficult of adjusting the precise

amounts of administered insulin to changing physiological condition results in episodic of hypo and hyperglycemic. Type 2 diabetes is treated in the first year following diagnosis diet, exercise and drugs that stimulate insulin secretion from a cell reduce hepatic glucose output and increase insulin sensitivity in target cells.^[24]

The effective control of blood glucose is the key in preventing or reversing diabetic complication and improving the quality of life for both type I and II diabetic patients. Although different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, none is offering complete glycemic control.^[28]

Phytochemicals play a significant role in diet based therapies to cure various maladies Consumer's trend is being widened due to awareness, spread and research interventions indicating potential health benefits associated with consumption of plants and their functional components. To date, there are hundreds of herbs and traditional herbal formulas reported to have been used for the treatment of Diabetes mellitus. (jia et al.,2008). During the latter part of the 20th century herbalism has become main stream worldwide. This is due in part to the recognition of the value of traditional and indigenous pharmacopeias the incorporation of some derived from these sources into pharmaceuticals, the need to make health care affordable for all, and the perception that natural remedies are somehow safer and more efficacious than remedies that are pharmaceutically derived.^[35]

Traditional plant medicines are used throughout the world for a range of Diabetic presentations. Therefore, investigation on such agents from traditional medicinal plants has become more important. India has a rich history of using various potent herbs and herbal compounds for treating Diabetes. Many Indian plants have been investigated for their beneficial use in different types of Diabetes.^[39]

Foeniculum vulgare belonging to family Umbelliferae is mainly used as a food condiment the chief chemical constituent of fennel is volatile oils but it also contain ketone, fenchone, phelladrine. Pharmacologically it is used as anti-inflammatory, anti-oxidant and hepatoprotective.^[67]

Tagetes erecta of the family compositae is commonly found in parts of India, Asia, Africa and America. It is known as Marigold. The leaves are reported to be effective against piles, Kidney troubles, muscular Pain, ulcers, wound and earache. The herbs are used for the

treatment of inflammatory conditions as a household remedy on experimental basis. The chief chemical constitution of *Tagetes erecta* is volatile oils, triterpenoids.^[73]

Synergistic combinations of more than one hypoglycemic were used by many physicians for proper glycemic control (Scheen *et al.*, 2005). So, it is valuable to pharmacologically screen any such combinations. Hence, the present study is an attempt to study *Foeniculum vulgare* and *Tagetes erecta* individually and also in combination against Streptozotocin induced diabetes.

AIM AND OBJECTIVE

Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. Despite the fact that it has worldwide high prevalence, morbidity, and mortality. It is regarded as a non-curable but controllable disease.^[2]

Diabetes mellitus is characterized by increased concentration of blood glucose due to derangement in carbohydrate metabolism and defective insulin production. The metabolic disturbances result in acute and long term diabetic complications.^[34]

Herbal formulations are frequently considered to be less toxic and more effective and also free from side effects than synthetic drugs.^[53]

The main aim and objective of the work is to extract the compounds from *Foeniculum vulgare* and *Tagetes erecta* with hydroalcohol with different concentration and test the extracted compound for its antidiabetic activity in streptozotocin induced diabetic rats for individual plants and in combination for its synergistic activity. The following are the parameters to be monitored for the anti-diabetic activity of extracted compounds by using standard procedures.

Parameters monitored

- Oral glucose tolerance test
- Serum glucose
- Serum cholesterol
- Serum triglyceride
- High density lipids
- Low density lipids

- Bodyweight

MATERIALS AND METHODS

Plant materials

Preparation of extracts

Each 350 g of fruits and whole plant of *Foeniculum vulgare* and *Ttagetes erecta* were collected from local market in Kosigi village Kurnool Dt A.P. and in my home garden in Kosigi vellege Kurnool Dt. A.P. its botanical identity was authenticated by Prof. K.Madavchetty, Department of Botany, Sri Venkateshwara University, Tirupathi, A.P. These were dried in sun shade and are coarsely powdered and extracted using 90% methanol for *F. vulgare* and 90% methanol for *T. erecta* by using Soxhlet extraction process. The extract was concentrated on rotatory flash evaporator to semisolid consistency. To it 1-2 drops of chloroform was added and stored at 8°C in screwed glass vials.

Experimental animals

Male wistar albino rats weighing 150-200 gm were used in the present study. They were housed in individual polypropylene cages under standard laboratory conditions of light, temperature, and relative humidity. Animals are given standard rat pellets (Pranav Argo' Ltd) and drinking water ad libitum. The experimental protocol was approved by the institutional Animal Ethical Committee of Shadan College of Pharmacy and Research Centre, Peerancheru, Hyderabad 502319.

Chemicals and Reagents

Normal Saline, Streptozotocin, Glucose Kit, Triglyceride Kit, HDL Kit, LDL Kit, Cholesterol Kit, Methanol.

Equipment

Auto analyzer (MISPEL).

Pharmacological studies

Oral glucose tolerance test (OGTT)

Rats are fasted overnight and divided into five groups with 6 animals each group. Group-1 received distilled water, to serve as control. Group-II animals were treated with Glibenclamide (0.5 mg/kg) to serve as standard. Group -III animals were treated with *F. vulgare* fruit extract (500 mg/kg B.Wt), group- IV animals treated with *T. erecta* methanolic

extract (100 mg/kg B.wt), group –V animals treated with both *F.vulgare* and *T. erecta* extracts. The control, standard and test were treated with drugs 30 min prior to the glucose load (2.5gk/kg). Blood samples were collected at 15, 30, 45, 60, 75, 90 and 120 min after glucose loading. Serum was separated and glucose levels were measured immediately.

Anti-diabetic study

In the present study, diabetes was induced by single intraperitoneal injection of streptozotocin (60 mg/kg B.wt). The streptozotocin was freshly prepared by using citrated buffer. The animals are allowed to drink 5% glucose solution over night to overcome drug induced hypoglycemia.

48 hours after injection of streptozotocin, fasting plasma blood glucose levels are estimated. Animals with plasma glucose level of > 140 mg/dl were used for the study.

The rats were divided into six groups consisting of six rats in each group; the animals were treated for 28 days.

Treatment Schedule for Antidiabetic Activity

Group No.	Treatment	Purpose
I	No treatment	To serve as normal control
II	Streptozotocin + Distilled water (60mg/kg i.p.)	To serve as disease control
III	Glibinclamide (0.5 mg/kg)	To serve as standard
IV	Foeniculum vulgare fruit extract (500 mg/kg B.wt)	To study the anti-diabetic effect of <i>F.vulgare</i>
V	Tagetes erecta whole plant extract (50 mg/ kg B.wt)	To study the anti-diabetic effect of <i>Tagetes erceta</i>
VI	Foeniculam vulgare extract (500 mg/kg) + <i>Tagetes erecta</i> (100 mg/kg)	To study the antidiabetic synergistic effect of <i>F.vulgare</i> and <i>Tagetes erecta</i>

Collection of blood sample

The blood samples were drawn on 7th, 14th, 21st and 28th day from the tail vein with the help tuberculin syringe after a fast of 12 hrs and the blood was centrifuged (2,500 rpm/10min) to get serum. The serum was used for biochemical estimation of blood glucose, triglycerides, cholesterol, HDL-cholesterol.

Biochemical Estimations

Parameters measured

Serum analytical methods

- Estimation of serum glucose.
- Estimation of triglyceride (TG).
- Estimation of total cholesterol (TC).
- Estimation of high density lipoprotein (HDL).
- Estimation of LOW density lipoprotein (LDL).
- Estimation of body weight.

1. Estimation of blood glucose

Blood glucose was estimated by using glucose kit obtained from Span Diagnostics.

METHOD

Glucose oxidase-peroxidase (GOD-POD) method

Principle Glucose oxidase (GOD) oxidizes glucose to gluconic acid and H₂O₂. In presence of enzyme peroxidase, released H₂O₂ is coupled with phenol and 4- aminoantipyrine (4-AAP) to form coloured quinoneimine dye. Absorbance of coloured dye is measured at 500 nm by using auto analyzer (Mispel Excel) and is directly proportional to glucose concentration in the sample.



Reagents used

Reagent No	Reagent	Composition	Concentration
1.	Glucose Reagent	Phosphate buffer Glucose oxidase Peroxidase 4-AAP Phenol	200 mM/L > 15 KU/L > 3 KU/L 0.3 mM/L 5 mM/L
2.	Glucose Standard	Dextrose Preservative	100 mg/dL
3.	Glucose Standard	Dextrose Preservative	400 mg/dL

Assay and Procedure: Fresh clear and unhaemolysed serum was used for the estimation.

Assay parameters

1.	Reaction type	End point
2.	Wave length	500 nm
3.	Optical path length	1 cm
4.	Temperature	37 ⁰ C
5.	Measurement	Against reagent blank
6.	Units	Mg/dL

Procedure

Pipette into tube marked	Blank	Standard	Test
Serum/Plasma	-	-	10 μ L
Glucose standard	-	10 μ L	-
Glucose reagent	1000 μ L	1000 μ L	1000 μ L

Mixed properly. Incubated at 37⁰ C for 10 minutes. The absorbance of sample and standard were measured against reagent blank at 500 nm. The absorbance was measured by using auto analyzer (Mispel Expel).

2. Estimation of triglycerides

Triglycerides were estimated by using the kit obtained from Span Diagnostics.

Method GPO-POD method

Principle: Triglycerides were hydrolysed by lipoprotein lipase (LPL) to produce glycerol and free fatty acid (FFA). In presence of glycerol kinase (GK), adenosine triphosphate (ATP) phosphorylates glycerol to produce glycerol-3-phosphate and adenosine diphosphate (ADP). Glycerol 3-phosphate is further oxidized by glycerol 3-phosphate oxidase (GPO) to produce dihydroxy acetone phosphate (DAP) and H₂O₂. In presence of peroxidase (POD), hydrogen peroxide couples with 4-aminoantipyrine (4-AAP) and 4-Chloro phenol to produce red quinoneimine dye. Absorbance o colored dye is measured at 505 nm and is proportional to triglycerides concentration in the sample.



Reagents used

Reagent No	Reagent	Composition	Concentration
1.	Triglyceride Mono reagent	Pipes buffer 4-Cholorophenol Magnesium ion ATP Lipase Peroxidase Glycerol Kinase 4-Aminoantipyrine Glycerol 3-Phosphate Oxidase	50 mM/L 5 mM/L 5 mM/L 1 mM/L > 5000 U/L > 1000 U/L > 400 U/L 0.4 mM/L > 400 U/L
2.	Triglyceride Standard	Triglyceride	200 mg/dL

Assay and Procedure: Fresh clear and unhaemolysed serum was used for the estimation.

Assay parameters

1.	Reaction type	End point
2.	Wave length	500 nm
3.	Optical path length	1 cm
4.	Temperature	37 ⁰ C
5.	Measurement	Against reagent blank
6.	Units	mg/dL

Procedure

Mixed properly. Incubated at 37⁰ C for 10 minutes. The absorbance of sample and standard were measured against reagent blank at 500 nm. The absorbance was measured by using auto analyzer (Mispel Expel).

Pipette into tube marked	Blank	Standard	Test
Serum/Plasma	-	-	10 μL
Glucose standard	-	10μL	-
Glucose reagent	1000μL	1000 μL	1000 μL

3. Estimation of Total cholesterol

(chod-Pod/Phosphotungstate Method)

Principle

Procedure

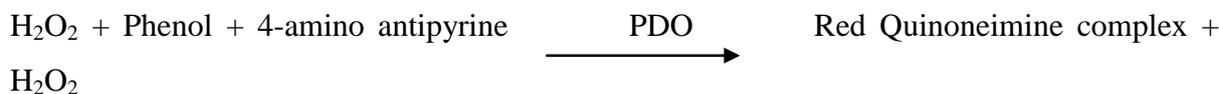
Pipette into 3 test tubes labelled blank (B), Standard (S), and Total cholesterol (T_C) as shown below.

Reagent	1.0 ml procedure			3.0 mp procedure		
	B	S	T_H	B	S	T_H
Cholesterol reagent (1)	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Cholesterol standard (2) (conc.200 mg/dl)	--	10 μ l	--	--	20 μ l	--
Specimen	--	--	10 μ l	--	--	20 μ l
Distilled water	--	--	--	2.0 ml	2.0 ml	2.0 ml

Mix well and incubate for 5 min at 37⁰ C or 10 min at room temperature Read the absorbance of standard (S), total cholesterol (T) against blank at 500 nm.

4. Estimation of HDL

Principle



On addition of precipitating reagent to the serum, followed by centrifugation, HDL fraction remains in the supernatant while the lipoproteins precipitate out.

Procedure

Step1: Pipette into the centrifuge tube.

Serum / Plasma	0.2 ml
Precipitating	0.3 ml

Mix well and allow standing at room temperature for 5 min. Centrifuge at 3000 rpm for 10 min to get a clear supernatant. If supernatant is not clear (high TGL level) dilute the sample 1:1 normal saline and multiply the result with 2.

Step2: Pipette into 3 test tubes labelled blank (B), Standard (S), HDL cholesterol (T_H) as shown below.

Reagent	1.0 ml procedure			3.0 mp procedure		
	B	S	T _H	B	S	T _H
Cholesterol reagent (1)	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Cholesterol standard (2) (conc.200 mg/dl)	--	100µl	--	--	200 µl	--
Specimen	--	--	100 µl	--	--	200 µl
Distilled water	100µl	--	--	2.2 ml	2.0 ml	2.0 ml

Mix well and incubate for 5 min at 37⁰ C or 10 min at room temperature. Read the absorbance of standard (S), HDL cholesterol (T_H) against blank at 500 nm.

5. Low density lipoprotein (LDL)^[26]

Calculation: T.C- (HDL+TG)

Statistical analysis

The results are expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) test for multiple comparison followed by Turkey- Karmer test. Statistical significance was set accordingly.

RESULTS

1. Effects of *Foeniculum vulgare* & *Tagetes erecta* on glucose tolerance in normal fasted rats.

OGTT test was studied by administration of glucose (5 mg / kg, p.o) to control (G – II) animals, a significant increase in blood glucose levels were noticed after 60 min which was followed by a reduction after 120 min.

Treatment with standard drug glibenclamide (group – III), blood glucose raised at 30 min followed by subsequent fall up to 120 min.

It was observed from present study that administration of *Foeniculum vulgare* & *Tagetes erecta* extracts increased the glucose levels were seen after 30 min and hypoglycemia effect was observed only after 120 min.

Rats treated with combination of both *Foeniculum vulgare* & *Tagetes erecta* extracts showed an increase in blood glucose levels at 30 min followed by decrease in blood glucose levels from 60 min onwards.

2. Effect of *Foeniculum vulgare* & *Tagetes erecta* on serum glucose levels

In animals treated with Streptozotocin (G – I) (60 mg / kg i.p) a significant increase in the serum glucose levels was observed on the 7th, 14th, 21st and 28th day, when compared to the normal group (G – I).

Group – III treated with standard drug (glibenclamide – 0.5 mg / kg p.o) showed a significant decrease in serum glucose levels on 7th, 14th, 21st and 28th day, when compared to the diabetic control group (G – II).

On administration of *Foeniculum vulgare* & *Tagetes erecta* extracts alone and in combination groups (G – IV, V and VI), the blood glucose levels were decreased on 7th, 14th, 21st and 28th day, when compared to the control group (G – II).

3. Effect of *Foeniculum vulgare* & *Tagetes erecta* on serum triglyceride levels

Group – II animals receiving Streptozotocin showed a significant increase in triglyceride levels on 14th, 21st and 28th day, when compared to the normal group (G – I).

Rats treated with standard drug (G – III) had significantly lowered triglyceride level on 14th, 21st and 28th day, when compared to the control group (G – II).

A significant decrease in serum triglycerides was observed in animals treated with *Foeniculum vulgare* & *Tagetes erecta* extracts alone and in combination (G – IV, V and VI), when compared to the control group (G – II).

4. Effect of *Foeniculum vulgare* & *Tagetes erecta* on serum cholesterol

The biochemical parameter, serum cholesterol has shown significant increase in Streptozotocin induced group (G – II) when compared with the normal group (G – I).

A significant decrease in the levels of serum cholesterol was observed from 14th day onwards on administration of glibenclamide (G – III), when compared with the control group (G – II).

The *Foeniculum vulgare* & *Tagetes erecta* extracts alone and in combination (G – IV, V and G – VI) caused a significant decrease in the serum cholesterol levels from the 14th onwards, when compared to the control group (G – II).

5. Effects of *Foeniculum vulgare* & *Tagetes erecta* on serum HDL level

The rats induced with Streptozotocin (G – II) a significant decrease in HDL levels was observed on 14th, 21st and 28th day, when compared to the normal group (G – I).

Group – III, receiving standard drug (glibenclamide – 0.5 mg / kg p.o) showed a significant increase in HDL levels on 14th, 21st and 28th day, when compared to the control group (G–II).

Administration of *Foeniculum vulgare* & *Tagetes erecta* extracts both alone and in combination (G – IV, V and VI) have shown a significant increase in HDL levels on 14th, 21st and 28th day, when compared to the control group (G – II).

6. Effect of *Foeniculum vulgare* & *Tagetes erecta* on serum LDL level

The rats induced with Streptozotocin(G – II) a significant increase in LDL levels was observed on 14th, 21st and 28th day, when compared to the normal group (G – I).

Group – III, receiving standard drug (glibenclamide – 0.5 mg / kg p.o) showed a significant decrease in LDL levels on 14th, 21st and 28th day, when compared to the control group (G–II).

Administration of *Foeniculum vulgare* & *Tagetes erecta* extracts both lone and in combination (G – IV, V and G – VI) have shown a significant decrease in LDL levels on 14th, 21st and 28th day, when compared to the control group (G – II).

7. Effects of *Foeniculum vulgare* & *Tagetes erecta* on body weight

The rats induced with Streptozotocin (G – II) a significant decrease in body weight was observed on 7th, 14th, 21st and 28th day, when compared to the normal group (G – I).

Group – III, receiving standard drug (glibenclamide – 0.5 mg / kg p.o) showed a significant increase in body weight on 14th, 21st and 28th day when compared to the control group (G–II).

Administration of *Foeniculum vulgare* & *Tagetes erecta* extracts both alone and in combination (G – IV, G – V and G – VI) have shown a significant increase in body weight on 14th, 21st and 28th day, when compared to the control group (G – II).

Effect of *Foeniculum vulgare* & *Tagetes erecta* on glucose tolerance in normal fasted rats

Group	Treatment	Serum glucose (mg / dl) (mean \pm SEM)				
		Time after glucose administration in minutes				
		0	30	60	90	120
I	Control	67.53 \pm 4.20	107.81 \pm 4.40	137.12 \pm 4.12	151.7 \pm 4.26	115.27 \pm 5.20
II	Standard	61.54 \pm 6.87	71.35 \pm 6.02 ^a	68.12 \pm 5.99 ^a	73.12 \pm 6.30 ^a	64.12 \pm 5.96 ^a
III	F.vulgare	70.24 \pm 4.29	82.12 \pm 4.20 ^a	94.12 \pm 5.60 ^a	97.12 \pm 4.11 ^a	67.43 \pm 5.20 ^a
IV	T. erecta	57.34 \pm 4.91	94.20 \pm 5.25 ^a	110.12 \pm 5.20 ^a	94.20 \pm 5.01 ^a	78.6 \pm 4.08 ^a
V	F.V + T.E	55.06 \pm 5.67	90.74 \pm 4.93 ^a	80.12 \pm 5.12 ^a	71.13 \pm 5.14 ^a	60.14 \pm 4.02 ^a

a = $p < 0.001$, when compared on control (G – I).

Effects of *Foeniculum vulgare* & *Tagetes erecta* on serum glucose levels in diabetic rats

Group	Treatment	Serum glucose (mg / dl) (mean \pm SEM)				
		0 th day	7 th day	14 th day	21 st day	28 th day
I	Normal	72.40 \pm 6.45	86.70 \pm 6.45	76.80 \pm 5.45	68.57 \pm 5.97	81.70 \pm 5.45
II	Control	194.70 \pm 16.56	198.00 \pm 16.46 ^a	217.80 \pm 16.26 ^a	219.60 \pm 16.85 ^a	222.30 \pm 18.44 ^a
III	Standard	180.6 \pm 16.45	93.75 \pm 6.65 ^b	92.18 \pm 10.74 ^b	91.00 \pm 7.26 ^b	85.56 \pm 9.47 ^b
IV	T.erecta	206.20 \pm 18.44	159.68 \pm 8.78 ^c	122.06 \pm 6.88 ^b	103.07 \pm 8.95 ^b	95.00 \pm 6.25 ^b
V	F.vulgare	214.00 \pm 19.50	161.87 \pm 5.49 ^c	133.43 \pm 8.35 ^b	101.22 \pm 10.49 ^b	94.23 \pm 6.38 ^b
VI	T.E+F.V	228.80 \pm 19.25	121.62 \pm 7.55 ^b	107.34 \pm 6.45 ^b	98.12 \pm 6.46 ^b	81.34 \pm 5.45 ^b

a = $p < 0.001$, when compared to normal (G – I)

b = $p < 0.001$, when compared to control (G – II)

c = $p < 0.05$, when compared to control (Group – II)

Effects of *Foeniculum vulgare* & *Tagetes erecta* on serum triglyceride levels in diabetic rats

Group	Treatment	Serum triglyceride (mg / dl) (mean \pm SEM) on				
		0 th day	7 th day	14 th day	21 st day	28 th day
I	Normal	158.30 \pm 12.44	160.20 \pm 16.45	158.00 \pm 16.44	169.21 \pm 16.83	159.80 \pm 15.48
II	Control	190.40 \pm 17.45	204.00 \pm 18.48	219.00 \pm 16.15 ^a	231.00 \pm 16.25 ^a	244.00 \pm 17.23 ^b
III	Standard	172.30 \pm 13.64	167.20 \pm 15.44	160.89 \pm 17.48 ^c	158.30 \pm 14.50 ^d	155.30 \pm 16.47 ^d
IV	T. erecta	174.00 \pm 14.32	177.20 \pm 16.42	172.30 \pm 14.45 ^c	170.20 \pm 18.99 ^c	169.40 \pm 14.42 ^d
V	F.vulgare	172.60 \pm 13.40	171.90 \pm 16.45	182.70 \pm 12.08 ^c	170.50 \pm 14.38 ^c	168.00 \pm 15.86 ^d
VI	F.V + T.E	184.00 \pm 14.55	182.10 \pm 15.11	180.80 \pm 13.09 ^c	176.91 \pm 19.07 ^c	164.10 \pm 17.03 ^d

a = $p < 0.01$, when compared to normal (Group – I)

b = $p < 0.001$, when compared to normal (Group – I)

c = $p < 0.05$, when compared to control (Group – II)

d = $p < 0.01$, when compared to control (Group – II)

Effects of *Foeniculum vulgare* & *Tagetes erecta* on serum cholesterol in diabetic rats

Group	Treatment	Serum cholesterol (mg / dl) (mean \pm SEM) on				
		0 th day	7 th day	14 th day	21 st day	28 th day
I	Normal	57.68 \pm 6.51	63.00 \pm 6.45	52.00 \pm 6.45	59.00 \pm 6.45	61.00 \pm 6.45
II	Control	146.60 \pm 12.45	154.00 \pm 13.32	168.00 \pm 16.12 ^a	161.00 \pm 16.40 ^a	159.00 \pm 16.25 ^a
III	Standard	160.00 \pm 16.46	119.00 \pm 7.20	96.00 \pm 7.14 ^b	88.00 \pm 5.22 ^b	80.00 \pm 5.05 ^b
IV	T. erecta	151.80 \pm 9.45	130.00 \pm 5.23	111.00 \pm 6.45 ^b	102.00 \pm 7.46 ^b	97.00 \pm 6.25 ^b
V	F.vulgare	143.70 \pm 11.25	119.00 \pm 6.30	108.00 \pm 7.04 ^b	96.00 \pm 6.35 ^b	92.00 \pm 7.85 ^b
VI	F.V + T.E	157.70 \pm 12.47	123.00 \pm 5.95	77.50 \pm 5.28 ^b	71.00 \pm 6.26 ^b	69.00 \pm 8.45 ^b

a = $p < 0.05$, when compared to normal (Group – I)

b = $p < 0.001$, when compared to normal (Group – II)

c = $p < 0.001$, when compared to control (Group – II)

Effects of *Foeniculum vulgare* & *Tagetes erecta* on serum HDL level in diabetic rats

Group	Treatment	Serum HDL (mg / dl) (mean \pm SEM) on				
		0 th day	7 th day	14 th day	21 st day	28 th day
I	Normal	54.40 \pm 5.54	47.50 \pm 5.57	52.73 \pm 5.00	49.70 \pm 5.56	51.30 \pm 5.54
II	Control	49.70 \pm 5.56	47.20 \pm 8.72	41.40 \pm 4.48 ^a	37.20 \pm 3.60 ^a	31.23 \pm 3.58 ^b
III	Standard	51.35 \pm 5.68	57.53 \pm 5.56	59.40 \pm 4.56 ^c	56.30 \pm 5.55 ^d	57.63 \pm 5.55 ^d
IV	T. erecta	58.93 \pm 4.74	51.53 \pm 7.69	58.30 \pm 5.05 ^c	63.63 \pm 6.69 ^d	61.30 \pm 6.64 ^d
V	F.vulgare	42.63 \pm 4.90	54.70 \pm 6.85	59.99 \pm 4.02 ^d	62.58 \pm 6.50 ^d	61.42 \pm 5.58 ^d
VI	F.V + T.E	57.40 \pm 5.59	58.60 \pm 5.57	61.30 \pm 5.00 ^c	59.32 \pm 5.47 ^c	57.70 \pm 8.51 ^c

a = $p < 0.05$, when compared to normal (Group – I)

b = $p < 0.001$, when compared to normal (Group – I)

c = $p < 0.01$, when compared to control (Group – II)

d = $p < 0.001$, when compared to control (Group – II)

Effects of *Foeniculum vulgare* & *Tagetes erecta* on serum LDL level in diabetic rats

Group	Treatment	Serum LDL (mg / dl) (mean \pm SEM) on				
		0 th day	7 th day	14 th day	21 st day	28 th day
I	Normal	43.20 \pm 6.39	52.10 \pm 6.43	48.00 \pm 6.45	54.40 \pm 6.45	53.00 \pm 6.45
II	Control	102.00 \pm 16.45	105.00 \pm 14.45	108.10 \pm 12.43 ^a	114.20 \pm 13.47 ^a	118.00 \pm 11.45 ^a
III	Standard	65.17 \pm 6.46	62.00 \pm 6.45	63.20 \pm 6.46 ^b	61.00 \pm 6.85 ^c	60.00 \pm 5.25 ^c
IV	T. erecta	78.40 \pm 6.45	82.10 \pm 7.44	70.21 \pm 4.10 ^b	72.10 \pm 5.50 ^b	71.00 \pm 6.45 ^c
V	F.vulgare	73.40 \pm 7.48	74.00 \pm 5.26	80.30 \pm 4.46 ^c	73.30 \pm 5.80 ^b	72.30 \pm 7.45 ^c
VI	F.V + T.E	62.10 \pm 6.49	68.30 \pm 6.49	62.00 \pm 6.45 ^c	66.27 \pm 5.93 ^c	61.00 \pm 6.58 ^c

a = $p < 0.001$, when compared to normal (Group – I)

b = $p < 0.01$, when compared to control (Group – II)

c = $p < 0.001$, when compared to control (Group – II)

Effects of *Foeniculum vulgare* & *Tagetes erecta* on body weight levels in diabetic rats

Group	Treatment	Serum LDL (mg / dl) (mean \pm SEM) on				
		0 th day	7 th day	14 th day	21 st day	28 th day
I	Normal	180 \pm 1.76	183.80 \pm 0.58	185.4 \pm 0.92	188.4 \pm 0.77	193.6 \pm 1.66
II	Control	176.2 \pm 0.80	159.8 \pm 0.56 ^a	147.2 \pm 1.68 ^a	142.40 \pm 1.43 ^a	138.40 \pm 1.28 ^a
III	Standard	177.4 \pm 0.67	174.6 \pm 0.50 ^c	179.2 \pm 0.37 ^b	183.2 \pm 1.06 ^b	191.4 \pm 1.40 ^b
IV	T. erecta	177.20 \pm 0.96	168.0 \pm 0.70	164.6 \pm 0.89	169 \pm 0.54 ^c	175.8 \pm 0.58 ^c
V	F.vulgare	176.6 \pm 0.67	169.6 \pm 0.50	165.8 \pm 0.94	168.8 \pm 1.49 ^c	177.70 \pm 0.89 ^c
VI	F.V + T.E	177.5 \pm 0.92	175.8 \pm 0.86 ^c	178.8 \pm 0.96 ^b	182.6 \pm 1.20 ^b	190.6 \pm 1.03 ^b

a = $p < 0.001$, when compared to normal (Group – I)

b = $p < 0.01$, when compared to control (Group – II)

c = $p < 0.001$, when compared to control (Group – II)

DISCUSSION

Diabetes mellitus is one of the leading causes of death, illness and economic loss all over the world. Insulin – dependent (Type I, IDDM) diabetes is characterized by juvenile onset and by absolute insulin deficiency. Non – insulin – dependent (Type II, NIDDM) diabetes is characterized by mature onset, by varying basal insulin levels and a frequent association with obesity. It is likely that further heterogeneity exist within these two basic types. Similarly, animal models of diabetes differ significantly from each other and none of them can be taken, without reservations, to reproduce the essentials of human diabetes.^[8]

Experimental diabetes has the advantage that it allows the analysis of the biochemical, hormonal and morphological events that take place not only during the induction of a diabetic state but also after it has taken place and during its evolution to a severe insulin deficiency or even death. This strategy has great advantages but it has to be considered that none of animal models with induced diabetes corresponds exactly to the human type – 2 diabetic mellitus, nonetheless they provide models to investigate the pathogenic mechanism that lead to hyperglycemia and its consequences.^[13]

Different chemical agents are capable of producing the alterations related to the diabetic condition (Dunn et al., 1943; Frankel et al., 1985; Ganda et al., 1976; Goldener et al., 1964; Hara et al., 1979). Streptozotocin is one of the safe diabetogenic chemical agent when Dunn and Letchie accidentally produced islet – cell necrosis in rabbits while researching the nephrotoxicity of uric acid derivatives. Streptozotocin is a specific antibiotic that destroys the β cells provoking a state of primary deficiency of insulin without affecting other islet types not more harmful compare to Alloxan (Dunn et al., 1943; Goldener et al., 1964). Hence, Streptozotocin was selected to induce diabetes in the present study.

Currently available drugs for treatment of Diabetes mellitus have a number of limitations, such as adverse effects and high rate of secondary failure.^[30] As there is a growing trend towards using natural remedies as adjuncts to conventional therapy, traditionally used plants might provide a useful source of new hypoglycemic compounds.^[13]

The extracts of *Foeniculam vulgare* have been reported to possess medicinal properties, including hypoglycemic, hypotensive and diuretic activities.^[42]

The hypoglycemic effect of *Tagetes* extract has been demonstrated using Streptozotocin induced diabetic animals.^[36] Although the importance of the hypoglycemic activity of *Foeniculum vulgare* has been recognized and antidiabetic activity of *Tagetes erecta* has not been recognized, and also its effect in combination of these herbs has not been investigated. Therefore, the present study was designed to investigate the effect of *Foeniculum vulgare* & *Tagetes erecta* in combination against Streptozotocin induced diabetes.

Recent studies have shown that modifications of systemic glycemia in OGTT reflect the activity of the intestinal glucose transporter SGLT1.^[52] We therefore further examined the effect of oral *Foeniculum vulgare* & *Tagetes erecta* in normal rats subjected to an OGTT and reduced the overall OGTT response, both individually and in combination as efficiently as the reference oral hypoglycemic drug glibenclamide.

These results therefore confirm the reduction of intestinal transport *in vivo* and may be due to increased insulin sensitivity as observed in other previous studies.^[33] Taken altogether, this consideration leads us to believe that inhibition of intestinal glucose absorption by the selected plants in the present study and their combinations may participate in their recognized antidiabetic effect.

A number of plants have been reported to possess hypoglycemic effects and the possible mechanism suggested for such hypoglycemic actions could be through an increased insulin secretion from β – cells of islets of Langerhans or its release from bound insulin or such hypoglycemic effects of plant extracts could be because of their insulin – like actions.^[49] Similar mechanisms may be considered responsible for the hypoglycemic action shown by of *Foeniculum vulgare* & *Tagetes erecta* alone and in combination in diabetic rats.

The abnormally high concentration of plasma and hepatic lipids in diabetes is mainly due to an increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits hormone sensitive lipase.^[54]

The marked hyperlipidemia that characterizes the diabetic state is regarded as a consequence of the uninhibited actions of lipolytic hormones (glucagons and catecholamines) on the fat depots (Ravi et al., 2005). On the other hand, increased LDL – Cholesterol may arise from glycosylation of the lysyl residues of apoprotein B.^[41]

The ability of LDL – cholesterol to form lipid peroxides was found to be specifically responsible for the atherogenesis in diabetic patients.^[30] It is reported that a deficiency in lipoprotein lipase activity in diabetics may contribute to significant elevation of triglycerides in blood and with insulin administration; lipoprotein lipase activity is elevated and leads to lowering of plasma triglyceride concentrations.^[33]

The *Foeniculum vulgare* & *Tagetes erecta* administration almost reversed these effects as it reduced total cholesterol and triglyceride concentrations (plasma), LDL concentration and increased HDL notably in combination. In this context, combination of *Foeniculum vulgare* & *Tagetes erecta* was found to be as effective as glibenclamide in reducing the plasma lipid profiles in diabetic rats.

The Streptozotocin treated animals, exhibited an increase in hepatic glycogen content which may be due to increase in glucose – 6 – phosphatase activity and a low level of hexokinase activity (Shiswaikar et al., 2004). The observed hypoglycemic action of *Foeniculum vulgare* & *Tagetes erecta* was reported to accompany with release of hormone insulin activity in pancreas (Rajesh et al., 2005). Fruits of *F.vulgare* acts at more than one site, namely pancreas (release of hormone insulin), muscle and intestine (uptake of glucose through specific receptor).

Tagetes erecta may be responsible for the increase in hepatic glycogen^[77] as hypoglycemia *Foeniculum vulgare* & *Tagetes erecta* administered animals suggests that the activation of glycogen synthase for which the substrate glucose – 6 – phosphate could have been readily provided by an increased hexokinase activity.^[75]

These observations clearly indicate the potential of *Foeniculum vulgare* & *Tagetes erecta* to reduce gluconeogenesis both alone and in combination. Thus, of *Foeniculum vulgare* & *Tagetes erecta* in diabetic rats reduced blood glucose levels and increased glycogenesis and glycolysis, reduced gluconeogenesis and brought the glucose metabolism towards normal levels. Moreover, the effect of combination of *Foeniculum vulgare* & *Tagetes erecta* in diabetic rats is found to be similar to that of glibenclamide.

CONCLUSION

Diabetes Mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. Despite the fact that it has a high prevalence, morbidity and mortality;

it is regarded as a non - curable but controllable disease. Herbal formulations are frequently considered to be less toxic and also free from side effects, than synthetic ones. Hence, the present study involves one such combination of herbal drugs, combination of *Foeniculum vulgare* & *Tagetes erecta* for their antidiabetic potential against alloxan induced diabetes in albino rats.

The effect of both individual and combination of *Foeniculum vulgare* & *Tagetes erecta* on blood glucose, total cholesterol, HDL, LDL, triglycerides and body weight were studied in the diabetic rats.

The results of the present study attests significant antidiabetic potential for the selected plants individually and also in combination as a prominent decrease in blood glucose, total cholesterol, LDL, triglycerides, body weight and increase in HDL, was observed in the rats treated with extracts of the selected medicinal plants. Hence, the present study provides a scientific evidence for antidiabetic potential of *Foeniculum vulgare* & *Tagetes erecta*. Further studies to isolate bioactive compounds will have pave a path to identify potential lead compounds for developing safe and efficacious antidiabetic agents.

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