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Antidiabetic Activity of Polyherbal Formulation Containing *Trigonella foenum, Cuminum cyminum, Trachyspermum ammi, Cicer arietinum* (TCTC) in Alloxan Induced Diabetic Rats

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ABSTRACT

The WHO has recommended and encouraged the use of alternative therapy especially in countries where access to the conventional treatment of diabetes is not adequate. The ingredients present in polyherbal formulation i.e Fenugreek, Cumin, ajwain, cheak pea are widely used in various systems of medicine for a wide range of properties. The polyherbal TCTC is one of such herbal remedies prepared from the seeds of Fenugreek, Cumin, Ajwain, Cheak pea used to evaluate antidiabetic activity. The extraction value of Fenugreek seed was 12.7% w/w, Cumin seed was 7.2%w/w, Ajwain 10%w/w and Cheak pea seed was 8.32%w/w. we concluded that TCTC, a combination of four herbal plants exerts a significant antidiabetic effect. This could be due to different types of active principles from various plants, which may have different mechanism of action. Therefore, combination may be beneficial; the main aim of present study was an effort to study of novel polyherbal formulation for antidiabetic activity containing Fenugreek, Cumin, Ajwan, Cheak Pea in alloxan induced Diabetic rats. The polyherbal formulation is one of such herbal remedies prepared from the seeds of *Trigonella foenum, Cuminum cyminum, Trachyspermum ammi, Cicer arietinum* used to evaluate antidiabetic activity.

INTRODUCTION

Diabetes mellitus is a disease in which the body doesn't produce or properly use insulin. Insulin is a hormone produced in the pancreas, an organ near the stomach. Insulin is needed to turn sugar and other food into energy. When diabetes occurs, body can't make enough insulin or can't use its own insulin as it should, or both. This causes sugars to build up too high in the blood. (International Diabetes Federation) The body has to maintain the blood glucose levels at a very narrow range, which is done with insulin and glucagons. Insulin is a hormone produced by special cells called beta cells in the pancreas. Herbal medicines for the treatment of diabetes mellitus have gained importance throughout the world. The available literature shows that there are more than 400 plant species showing hypoglycaemic activity. Though some of these plants have great reputation in the indigenous system of medicine for their antidiabetic activities, many remain to be scientifically established.

MATERIAL AND METHOD

Drugs and Chemicals

The Alloxan monohydrate (Ozone International Mumbai),

Ethanol, Normal saline solution 5% glucose. Tween 80 (5%)

Authentication of plant

All plants seeds were collected from local market of Chikhli Town and Authenticated from Shri Shivaji Senior College Botany Department Chikhli Dist-Buldana Maharashtra India.

Extraction of the Plants:-

The air dried powder was subjected to hot continuous extraction with ethanol in a Soxhlet extractor and filtered. The filtrate was evaporated at room temperature to concentrate extract. The yield of ethanolic extract of Fenugreek, seeds was 12.7 % Cumin 7.2 % Ajwain 10% and cheak pea- 8.32 w/w. Weighed quantity was dissolved in distilled water using 1.5% Tween 80 to prepare drug solution of concentration of 100 mg/ml and used for pharmacological studies. The individual extracts of *Trigonella Foenum Cuminum cyminum Trachyspermum ammi*, *Cicer arietinum* L which were prepared above were mixed in equal proportions using 5% Tween 80 solution for pharmacological experiments.

Trigonella foenum graecum

The seeds were collected, air-dried and powdered. 1500 grams of the powdered seeds were extracted in Erlenmeyer flask with 90% ethanol for 48 hours by maceration process with occasional shaking and stirring.

Cuminum cyminum

The seeds were collected, air-dried and powdered. 1500 grams of the powdered seeds were extracted in Erlenmeyer flask with 90% ethanol for 48 hours by maceration process with occasional shaking and stirring.

Trachyspermum ammi

The seeds were collected, air-dried and powdered. 1500 grams of the powdered seeds were extracted in Erlenmeyer flask with 90% ethanol for 48 hours by maceration process with occasional shaking and stirring.

***Cicer arietinum* L**

The seeds were collected, air-dried and powdered. 1500 grams of the powdered seeds were extracted in Erlenmeyer flask with 90% ethanol for 48 hours by maceration process with occasional shaking and stirring.

Preparation of Polyherbal formulation

The individual extracts of *Trigonella foenum-graecum, Cuminum cyminum, Trachyspermum ammi, Cicer arietinum* L which were prepared above were mixed in equal proportions using 5% Tween 80 solution for pharmacological experiments.

Detection of Phytochemical constituents

The FCAC was evaluated for presence of various phytochemical constituents as per the methods described by Khandelwal.

Animals

Male albino rats were purchased from wockhardt pharmaceutical Aurangabad. These were housed under standard condition of temperature 25 ± 1ºC and relative humidity of 45% to

55% under 12-h light: 12-h dark cycle. The animals had free access to food pellets and water. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) approved 751/PO/Re/S/03/CPCSEA 03.03.2003 of Anuradha College of Pharmacy Chikhli Dist-Buldana. The experimental design was approved by institutional Animal Ethical committee and the study was performed according to the committee for the purpose of control and supervision of experiments on Animals (CPCSEA) guidelines for the use and care of animals.

Toxicity studies

Non-diabetic adult Male albino rats of male sex were subjected to acute toxicity studies as per guidelines (AOT no. 425) suggested by the Organization for Economic Cooperation and Development. The rats were observed continuously for 2 h for behavioral, neurological and autonomic profiles and for any lethality or death for the next 48 h.

Induction of diabetes

The rats were injected alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body weight, intraperitoneally (IP). Since alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (15–20 ml) intraperitoneally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycaemia.

Chronic treatment model

Rats were divided into six groups of five rats (n = 5) each. Groups 1 & 2 served as control and diabetic untreated control respectively. Group 3, 4 & 5 was treated with the ethanolic extract of *Trigonella Foenum, Cuminum Cyminum, Trachyspermum Ammi Cicer arietinum* L at of and 100 mg/kg, 200 mg/kg and 500 mg/kg per oral/day. Group 6 served as standard and was treated with 5 mg/kg/day glibenclamide for 21 days. Blood glucose levels and body weight were measured on day 1, 7, 14 and 21 of the study.

The diabetic rats were divided into six groups (n =5),

- Group I- Normal control rats received 5% Tween 80 in distilled water p.o. at 5 ml/kg b.w.
- Group II - Diabetic control rats received 5% Tween 80 in distilled water p.o.

- Group III - Diabetic rats received polyherbal formulation (TCTC) 100mg/kg b.w., p.o.
- Group IV- Diabetic rats received polyherbal formulation (TCTC) 200mg/kg b.w., p.o.
- Group V - Diabetic rats received polyherbal formulation (TCTC) 500mg/kg b.w., p.o.
- Group VI - Diabetic rats received glibenclamide at the dose of 5mg/kg b.w.p.o.

The administrations of extracts were continued for 21 days, once daily. Blood samples were collected through the lateral tail vein on days 1, 7, 14 and 21 after drug administration and the blood glucose levels were estimated using Accu-check glucometer.

All drugs were given orally by oral feeding needle.

Acute study

Acute toxicity study of polyherbal formulation was carried out in rats. It was observed that there was no gross evidence of any abnormalities up to 4 hrs and no mortality was observed in animals up to the end of 48 hours at the maximum tested dose level of 2000mg/kg b.w. in rats. This was considered as Maximum Tolerated Dose (MTD) and thus, 1/10th of MTD i.e., 100 mg/kg b.w. was taken as test dose and the test dose i.e., 500 mg/kg b.w. was also selected for the experimental studies.

Subacute study

All the animals were administered the respective drugs doses at prefixed time for 28 days. GLs were estimated on 1, 7, 14 and 21 days. At the end of 21 days the drug administration was stopped and a rest period of 7 days was given. The GLs were estimated on 28th day. The data were represented as mean GL ± standard error of mean (SEM).

Body weight

All the rats were weighed daily during study period of 21 days. The body weights were noted and presented as mean change in body weights.

Oral Glucose Tolerance Test (OGTT)

Animals were fasted for 24 hours before experiment but were allowed free access to water. Fasted rats were divided into three groups of 6 animals each (WHO, 1999).

- Group I - Control animals received 5% Tween 80 in distilled water at 5ml/kg b.w.p.o.
- Group II - 100 mg/kg b.w. of polyherbal formulation (TCTC) p.o.
- Group III - 200 mg/kg b.w. of polyherbal formulation (TCTC) p.o.
- Group IV- 500 mg/kg b.w. of polyherbal formulation (TCTC) p.o.

After 30 minutes of the treatment to the Groups I, II and III, 2gm/kg body weight glucose was given orally to the animals. Blood samples were collected from tail just prior to glucose administration and at 60, 120 and 180 minutes after glucose loading. The glucose levels were estimated for all the three groups by lateral tail vein puncture method using Accu-check glucometer.

Statistical analysis

Results were expressed as Mean ± SEM. Statistical analysis were performed with Graph pad prism 5 software using one-way analysis of variance (ANOVA) followed by Dunnett's t test. P values less than *p<0.05, p**<0.01 was considered to be statistically significant, when compared with control and standard group as applicable (Diabetes and Metabolism 1989).

Acute Toxicity Study (OECD Guideline 423)

Animals were fasted prior to dosing, food but not water was withheld overnight. Following the period of fasting, the animals were weighed and test substance was administered. After the substance had been administered, food was withheld for further 3-4 hours. As a dose was administered in fractions over a period, it was necessary to provide the animals with food and water depending on the length of the period. (Ghosh MN, 1984; Turner R, 1965)

Three animals were used for each step. The dose level of the extract to be used as the starting dose was selected from one of the four fixed doses levels 500, 1000, 1500 and 2000mg/kg body weight (Lorke D, 1983). The starting dose levels such that which was most likely to produce mortality in some of the dosed animals. After administration of the test sample, the animals were observed continuously for first four hours for behavioral changes and at the end of 48 hour for mortality, if any.

RESULTS

Experimental Observation

The dried powdered parts of the respective plants were extracted using soxhlet method and percentage yield of the extract are tabulated in table 1.

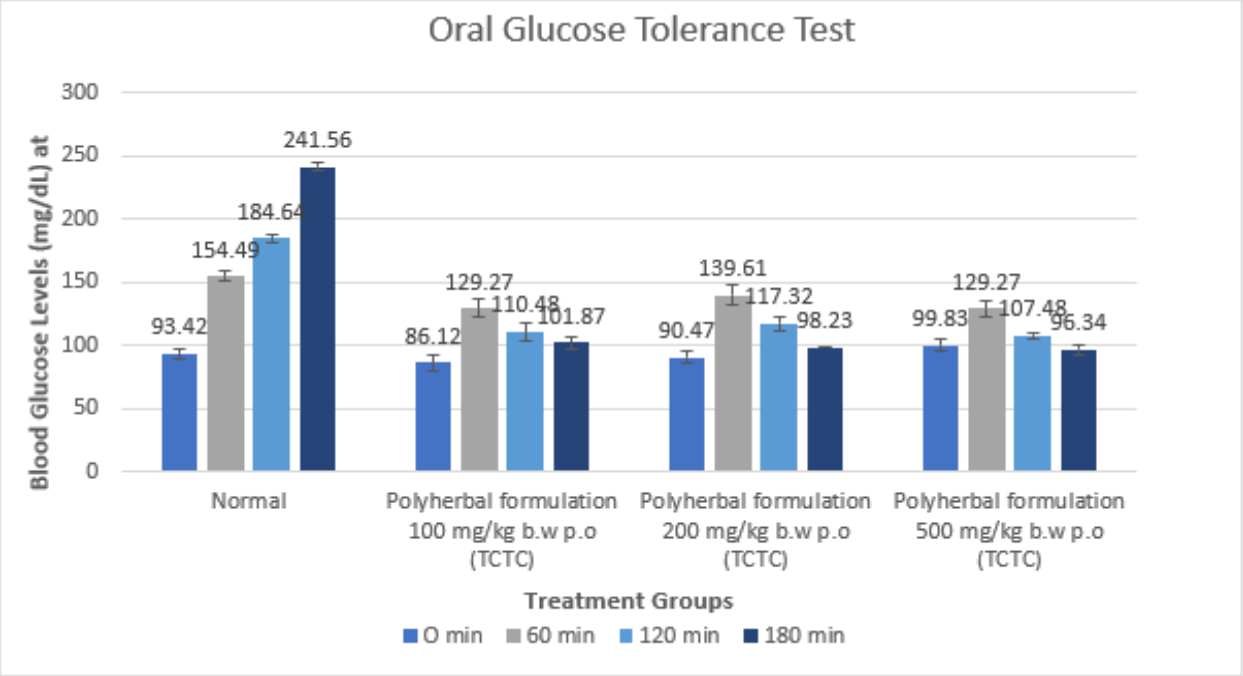
Table No. 1:- Percentage Yield of Extracts

Sr. No.	Plant Extract	Part used	Extraction Method	Solvent used	% yield of extract(w/w)
1.	Fenugreek	Seed	Soxhlation	Water+alcohol	12.7%
2.	Cumin	Seed	Soxhlation	Water+alcohol	7.2%
3.	Ajwain	Seed	Soxhlation	Water+alcohol	10%
4.	Cheak Pea	Seed	Soxhlation	Water+alcohol	8.32%

Table No. 2:-Oral Glucose Tolerance Test

Treatment Groups	Blood Glucose Levels (mg/dL) at			
	O min	60 min	120 min	180 min
Normal Control	93.42±3.87	154.49±3.78	184.64±2.94	241.56±2.44*
Polyherbal formulation 100 mg/kg b.wp.o	86.12±5.92	129.27±6.87**	110.48±7.81**	101.87±4.85**
Polyherbal formulation 200 mg/kg b.wp.o	90.47±4.63	139.61±7.62**	117.32±5.83**	98.23±9.78**
Polyherbal formulation 500 mg/kg b.wp.o	99.83±4.39	129.27±6.36**	107.48±2.93**	96.34±3.91**

Values are expressed as Mean±SEM (n=5) *P<0.05 *P<0.01 was considered significant with respect to control group using ANOVA followed by Dunneet's t-test.

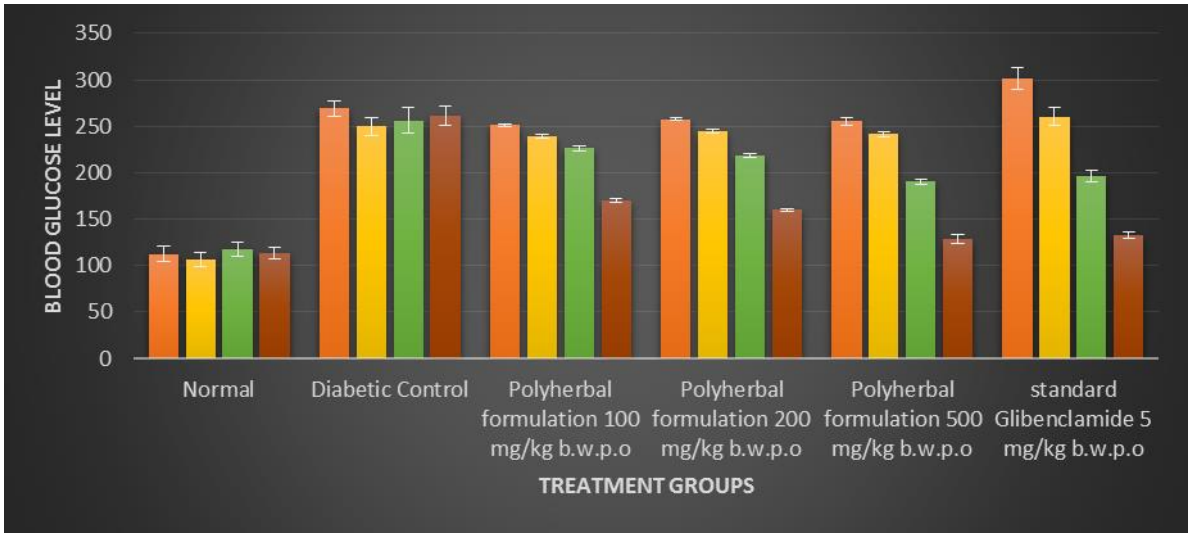


Graph 1-Oral Glucose Tolerance Test

Table No. 3:- Effect of Polyherbal Formulation on Blood Glucose Level

Treatment Groups	**Blood Glucose Levels (mg/dL) on**			
	Day 1	**Day 7**	**Day 14**	**Day 21**
Normal	112.59±8.63	106.53±8.25	117.32±7.39	113.55±6.39
Diabetic Control	269.06±8.24	249.99±9.84	256.51±13.81	261.49±9.84
Polyherbal formulation 100 mg/kg b.wp.o (TCTC)	251.25±1.69	239.65±2.05*	226.16±2.54**	170.72±2.05**
Polyherbal formulation 200 mg/kg b.wp.o (TCTC)	257.60±1.24	244.52±2.44*	218.44±2.05**	165.05±1.63**
Polyherbal formulation 500 mg/kg b.wp.o (TCTC)	255.45±4.21	241.56±2.38*	190.64±2.94**	128.49±4.78**
Standard Glibenclamide 5 mg/kg b.w.p.o	301.70±11.62	260.57±9.75*	196.34±5.85**	132.61±3.92**

Values are expressed as Mean±SEM (n=5) *P<0.05 *P<0.01 was considered significant with respect to control group using ANOVA followed by Dunneet's t-test.



Graph 2- Effect of Polyherbal formulation on Blood Glucose Level

DISCUSSION

One of the most commonly used chemical agent predominantly in laboratories to induce diabetes in animal is alloxan which is an oxidized product of uric acid that tends to destroy the islet cells of the pancreas by oxidation mechanism and producing Type 1 diabetes known as '*alloxan diabetes'*. Glibenclamide is the standard drug is used to stimulate insulin from β cells of islets of langerhans for many years. Hence Glibenclamide (5 mg/Kg) was selected as standard drug. Alloxan, a β-cell cytotoxin induces chemical diabetes in a wide variety of animal's species including rats by damaging the insulin-secreting β-cells of the pancreas. Alloxan causes time and concentration dependent degradation lesions of the pancreatic β-cells leading to hyperglycemia. present study was evaluated that the polyherbal *TCTC* is one of such herbal remedies prepared from the seeds of Fenugreek, Cumin, ajwain, Cheak pea used to evaluate antidiabetic activity. The extraction value of Fenugreek seed was 12.7%w/w, Cumin seed was 7.2%w/w, Ajwain 10%w/w and Cheak pea seed was 8.32%w/w. The acute toxicity studies and behavioral pattern records indicated that *TCTC* was quite safe at the dosage employed, as no visible signs of toxicity or adverse effects were observed in treated animals and no change was observed in the normal behavioral pattern of study animals.

The antihyperglycemic activity of one of the polyherbal formulation was screened using glucose tolerance test. The formulation tested for this activity exhibited significant antihyperglycemic activity at a dose level 500 mg/kg b.w. (113.55±6.39) as compared to control (247.72±10.22) at 28 day. The polyherbal formulation of drug was effective in

decreasing the blood glucose levels in diabetic rat at both the low and high dose significantly. FCAC, a combination of seeds of fenugreek, Cumin, Ajwain, Cheak pea in this present investigation showed significant antihyperglycemic. So, it can be used as an agent for treatment of diabetic effect of *TCTC* may be due to increase in insulin secretion or decrease in insulin resistance or increased glucose absorption.

CONCLUSION

The polyherbal formulation also reduced elevated levels of selected biochemical parameters and prevented other complication of hyperglycemia. Search for an effective drug, alone or in combination, for treatment of diabetes still remain elusive. Herbal formulations used extensively in traditional systems of medicine may provide a suitable alternative for this. Therefore, present study was designed to evaluate the effect of a four weeks treatment of polyherbal formulation consisting of (*TCTC*), at doses of 100, 200 and 500 mg/kg on blood glucose level and other biochemical parameters like cholesterol, urea, creatinine, billirubin and SGPT in alloxan (150 mg/kg, IP) induced diabetic rats. Oral administration of polyherbal formulation to diabetic animals up to four weeks dos dependently reduced the blood glucose level, which was comparable to that of glibenclamide (5 mg/kg). Significant decrease in body weight also was observed with diabetic control, which was partially restored upon administration of polyherbal formulation.

These findings provide scientific evidences to anti-diabetic use of a traditional formulation and suggest that administration of polyherbal formulation to rats, in a dosage used safely by humans, reduces the production of various diabetes causing biochemical parameters and concomitantly prevents the development of Type 2 (NIDDM) diabetes in established animal models.

TCTC combination of four herbs and it is more effective in the treatment of diabetes mellitus as determined by extreme statistically significant p-value < 0.01 in alloxan induced diabetic rats. The mechanism of the hypoglycemic activity of poly herbal formulation *TCTC* may be due to enhance the effect of insulin and stimulates the insulin secretion from beta cells of pancreas. So this study suggests that the *TCTC* had potent antidiabetic effect which could be used in the management of diabetes mellitus effectively.

In traditional systems of medicine, many plants have been documented to be useful for the treatment of various systemic disorders. Many of the traditional/indigenous systems of

medicine are effective than the modern system of medicine, but they suffer from lack of complete standardization which is one of the important challenges faced by the traditional system of medicine. The concept of polyhedral formulation is well documented in the ancient literature. Compared to the single herb, the polyherbal formulation has better and extended therapeutic potential. Hence, the present study was planned to formulate and standardize a polyherbal formulation using a plant having known antidiabetic activity and evaluate its therapeutic effects in rats.

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