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Evaluation of anti-diabetic activity of aqueous and alcoholic extract citrullus colocynthis and trigonella foenum graceum in STZ induced diabetes in rats

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***Abstract*---**The aim of this study was to assess the effects of Aqueous and Alcoholic Extract Citrullus Colocynthis and Trigonella Foenum Graceum in different ratio through biochemical factors and histopathological changes in streptozotocin-induced diabetic rats. The current experimental study was performed on 48 male rats, which became diabetic with 60 mg/kg body weight intraperitoneal injection of streptozotocin (STZ). The animals were divided into eight groups: untreated diabetic and diabetic treated with alcoholic and aqueous extract, respectively. The animals were treated with different ratio (i.e. 25:75, 50:50, 75:25) of both alcoholic and aqueous extract of Citrullus Colocynthis and Trigonella Foenum Graceum orally for 14 days. The results indicated that blood sugar, OGTT level shows significantly decrease in alcoholic extract of Citrullus Colocynthis and Trigonella Foenum Graceum (50:50) in diabetic rats treated with the extract compared to the other groups. Histopathological findings showed STZ-induced diabetic complications in the pancreas were improved following treatment with aqueous and alcoholic extract of C. colocynthis and Trigonella Foenum Graceum.The administration of both aqueous and alcoholic extract of C. colocynthis and Trigonella Foenum Graceum had a significant anti-hyperglycemic, compared to all, alcoholic extract of both (50:50) shows a prominent effect in decrease in blood sugar level. In addition, C. colocynthis and Trigonella Foenum extract may have a protective effect on the pancreas.

Keywords---diabetes mellitus, streptozotocin, citrullus colocynthis, trigonella foenum graceum, hypoglycemic effect.

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from the defects in insulin secretion, insulin resistance or both. [1] It is considered to be one of the five foremost causes of death in the world. [2] There are reports that incidence of diabetes mellitus was 2.8% in 2000 and is expected to be increase to 4.4% in 2030. [3] For a long time, diabetes mellitus has been treated with a number of medicinal plants or their extracts based on folklore medicine. [4] The oral hypoglycemic agents (sulfonylurea, biguanide, thiazolidinedione, α-glycosidase inhibitor and DPP-IV inhibitor) can produce several undesirable side effects and in addition, they are not suitable for use in pregnancy. [5] Thus, the management of diabetes without any side effects is still a challenge. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of active research. Since, the oral hypoglycemic agents cause several side effects it has become a need to search for a new herbal drug with little side effects. [6]. In present study, we try to investigate antidiabetic effect of Aqueous and alcoholic extract of both plants in different ratio in streptozotocin induced diabetic albino wistar rat. Various researches gave evidences regarding antidiabetic activity of these plants. Based on the available literature we planned to develop a novel polyherbal formulation more potent than currently available medicines. [7-12]

Materials and Methods

Drugs and chemicals

Citrullus colocynthis and *Trigonella foenum graceum* were collected from the herbarium of Bilwal medchem and research laboratory pvt. ltd., Jaipur in the month of august 2020. Both plants was taxonomically identified and authenticated by botany dept. of Bilwal medchem and research laboratory pvt ltd, reference number BMRL/AD/PA-124/2020 as *Citrullus colocynthis* (Family- Cucurbitaceae) and *Trigonella foenum graceum* (Family- Fabaceae). Roots of *Citrullus colocynthis* and seeds of *Trigonella foenum graceum* were used to carry out the experimental work. Streptozotocin (STZ) and other chemicals and reagents used in the study were of analytical grade chemicals.

Experimental animals

Albino Wistar rats (200-250 g) of either sex were obtained. All animals were maintained under standardized condition (12-h light/dark cycle, 24 ± 2°C & humidity 35-60 %) and they were provided with standard pellet diet and water ad libitum. The rats were left for 48 h for adaptation prior to the beginning of the experiment. The study was carried out in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animal) guidelines.

Toxicity study

At the basis OECD guideline, the acute oral toxicity was carried out in albino Wistar rats of either sex weighing 200-250g. Animal were divided into two groups. Sign and symptoms were observed till 14 days and haematology analysis were performed.

- Group A: Aqueous extract of *Citrullus colocynthis* and *Trigonella foenum graceum* (50:50), 2000 mg/kg
- Group B: Ethanolic extract of *Citrullus colocynthis* and *Trigonella foenum graceum* (50:50), 2000 mg/kg

Induction of type 2 diabetes

Sreptozotocin (60 mg/kg, i.p.) were administered to overnight fasting albino Wistar rats (200-250 g) to induce Type 2 diabetes. Streptozotocin (dissolved in citrate buffer, pH 4.5) was administered. [13-15] Hyperglycemia was confirmed by elevated blood glucose levels at 72 h and then on day 6 after injection and only animals with fasting blood glucose level greater than 200 mg/dl were selected for antidiabetic study.

Experimental design

Forty eight adult albino wistar rats were divided into eight groups having six rats in each. These groups received different treatment in following manner:

- Group 1: Diabetic induced+ (50:50) aqueous extract of *Citrullus colocynthis* and *Trigonella foenum graceum*
- Group 2: Diabetic induced+ (50:50) ethanolic extract of *Citrullus colocynthis* and *Trigonella foenum graceum*
- Group 3: Diabetic induced+ (25:75) aqueous extract of *Citrullus colocynthis* and *Trigonella foenum graceum*
- Group 4: Diabetic induced+ (25:75) ethanolic extract of *Citrullus colocynthis* and *Trigonella foenum graceum*
- Group 5: Diabetic induced+ (75:25) aqueous extract of *Citrullus colocynthis* and *Trigonella foenum graceum*
- Group 6: Diabetic induced+ (75:25) ethanolic extract of *Citrullus colocynthis* and *Trigonella foenum graceum*
- Group 7: Diabetic induced+ Standard Drug (Glibenclamide 10 mg/kg)
- Group 8: Diabetic induced+ Normal Control (Receive normal saline 5ml/kg)

All the above mentioned treatments were given orally, started one week (7 days) after induction of diabetes and treatments continued for 14 days. Parameters observed under anti diabetic activity are Blood Glucose and Oral Glucose tolerance test.

Histopathology

After sacrifice, pancreas tissues of each group were rapidly dissected out and washed immediately with saline and fixed in 10% phosphate buffered formalin. Paraffin-embedded specimens were cut into 5 µm-thick sections and stained with hematoxylin and eosin (H&E). The sections were examined under the light microscope.

Statistical analysis

All the data are expressed as mean. The statistical significance between groups was tested using one-way ANOVA.

Results

Toxicity study analysis

Toxicity Study of *Citrullus colocynthis* and *Trigonella foenum graceum* in group A and group B were observed on various paramaters as shown in Table 1 and Table 2. No significant changed has been observed.

Table 1
Aqueous extract of *Citrullus colocynthis* and *Trigonella foenum graceum* (50:50), 2000 mg/kg

Group A						
Observation	30min.	4hr.	24hr.	48hr.	1w	2w
Skin and Fur	N	N	N	N	N	N
Eyes	N	N	N	N	N	N
Mucous Membrane	N	N	N	N	N	N
Salivation	Ab	Ab	Ab	Ab	Ab	Ab
Lethargy	Ab	Ab	Ab	Ab	Ab	Ab
Sleep	N	N	N	N	N	N
Coma	Ab	Ab	Ab	Ab	Ab	Ab
Convulsions	Ab	Ab	Ab	Ab	Ab	Ab
Tremors	Ab	Ab	Ab	Ab	Ab	Ab
Diarrhea	Ab	Ab	Ab	Ab	Ab	Ab
Morbidity	Ab	Ab	Ab	Ab	Ab	Ab
Mortality	Ab	Ab	Ab	Ab	Ab	Ab

Table 2
Ethanolic extract of *Citrullus colocynthis* and *Trigonella foenum graceum* (50:50), 2000 mg/kg

Group B						
Observation	30min.	4hr.	24hr.	48hr.	1w	2w
Skin and Fur	N	N	N	N	N	N
Eyes	N	N	N	N	N	N

Mucous Membrane	N	N	N	N	N	N
Salivation	Ab	Ab	Ab	Ab	Ab	Ab
Lethargy	Ab	Ab	Ab	Ab	Ab	Ab
Sleep	N	N	N	N	N	N
Coma	Ab	Ab	Ab	Ab	Ab	Ab
Convulsions	Ab	Ab	Ab	Ab	Ab	Ab
Tremors	Ab	Ab	Ab	Ab	Ab	Ab
Diarrhea	Ab	Ab	Ab	Ab	Ab	Ab
Morbidity	Ab	Ab	Ab	Ab	Ab	Ab
Mortality	Ab	Ab	Ab	Ab	Ab	Ab

Table 3
Hematology Analysis of Aqueous extract and Ethanolic extract of *Citrullus colocynthis* and *Trigonella foenum graceum* (50:50), 2000 mg/kg

S. No.	Parameters	Value (Mean±S.E.M)		Normal Range
		Group A	Group B	
1.	Haemoglobin	14.35	14.32	11.5-16.1 grams per decilitre
2.	WBC	7.4	8.2	6.6-12.6 x 10^3/mm^3
3.	RBC	8.14	7.96	6.76-9.75 x 10^6/ mm^3
4.	Neutrophils	2.12	3.10	1.77-3.38 x10^3/ mm^3
5.	Lymphocytes	6.24	7.15	4.78-9.12 x 10^3/ mm^3
6.	Eosinophils	0.02	0.02	0.03-0.08 x 10^3/ mm^3
7.	Monocytes	0.03	0.02	0.01-0.04 x 10^3/ mm^3
8.	Basophils	0.01	0.01	0.00-0.03 x 10^3/ mm^3

Both Group A and Group B shows no significant change In Hematology parameters and found under normal ranges.

Effect of blood glucose level

Effect of extract Aqueous and Ethanolic extract of *Citrullus colocynthis* and *Trigonella foenum graceum* on blood glucose level in rats are shown in Table 4.

Table 4
Effect of Extract on Blood Glucose Level

Blood Glucose	H	B	T	HB	BT	HT	Blood Glucose (Mean± STD)
Group 1	142	156	138	161	154	149	150 ± 8.74
Group 2	112	126	130	124	114	112	119.66 ± 7.94
Group 3	153	160	158	145	166	162	157.33 ± 7.42
Group 4	140	148	126	139	152	145	141.66 ± 9.09
Group 5	161	158	166	170	168	176	166.5 ± 6.44
Group 6	130	124	118	134	142	132	130 ± 8.29
Group 7	104	100	106	98	97	102	101.16 ± 3.48
Group 8	212	198	204	207	190	198	201.5 ± 7.79

The mean results shows that alcoholic extract of Group 2 significantly decrease in blood glucose level as compare to aqueous extract of Group 1.

OGGT analysis

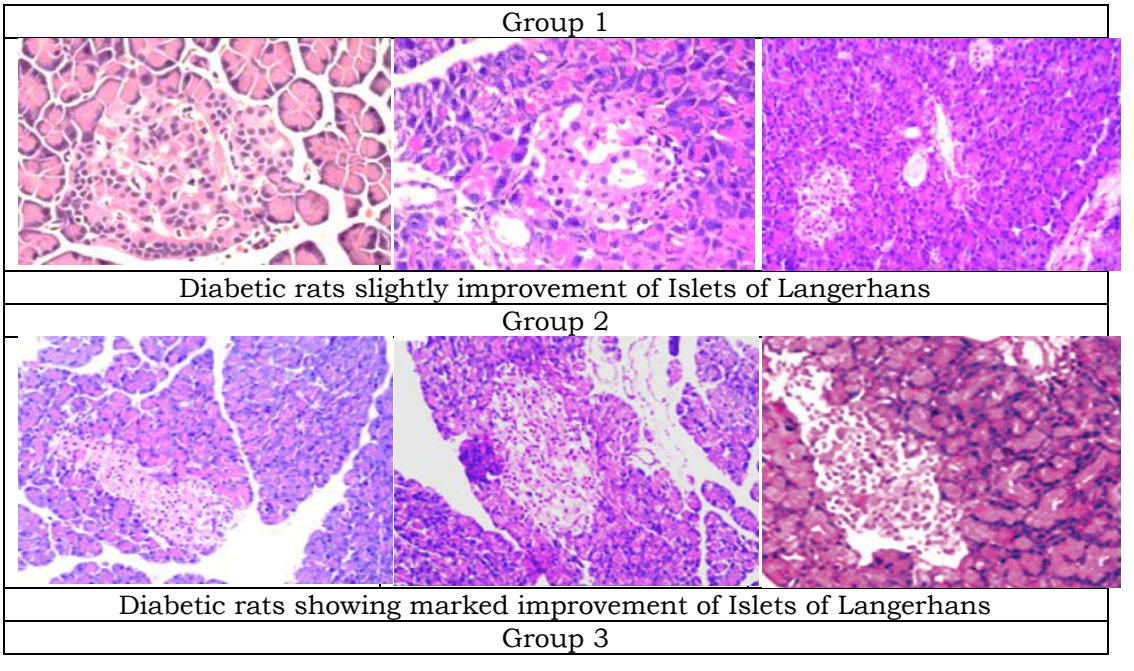
Oral Glucose Tolerance test are observed at 0 min, 60 min and 120 min in each group as shown in Table 5.

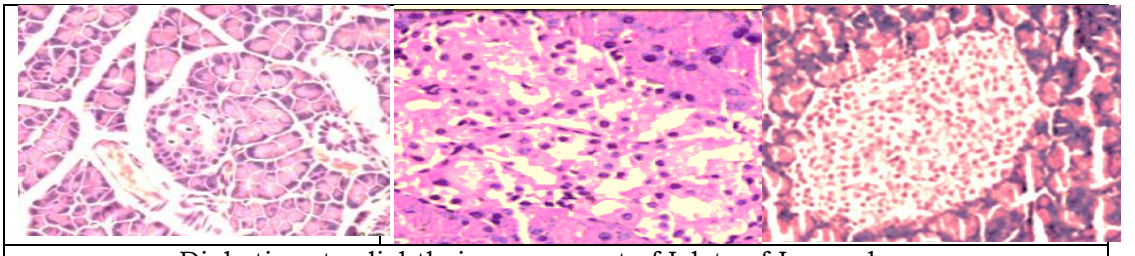
Table 5
OGTT Analysis

Oral glucose tolerance test			
Groups	0 hr MEAN ± STD	60 min MEAN ± STD	120 min MEAN ± STD
Group 1	150.66 ± 7.20	151.33 ± 6.91	154.33 ± 6.65
Group 2	120.66 ± 6.05	121.83 ± 4.53	122.5 ± 7.34
Group 3	154.5 ± 7.47	158.66 ± 7.06	159.66 ± 8.01
Group 4	140.33 ± 7.22	142.33 ± 6.37	144.5 ± 3.39
Group 5	160 ± 8.46	162.83 ± 10	165.5 ± 7.84
Group 6	125 ± 8.24	126.33 ± 10.40	128.66 ± 10.26
Group 7	108.66 ± 8.28	109.83 ± 5.38	110.83 ± 7.67
Group 8	209.5 ± 14.19	214.83 ± 12.90	230.33 ± 13

Histopathological study

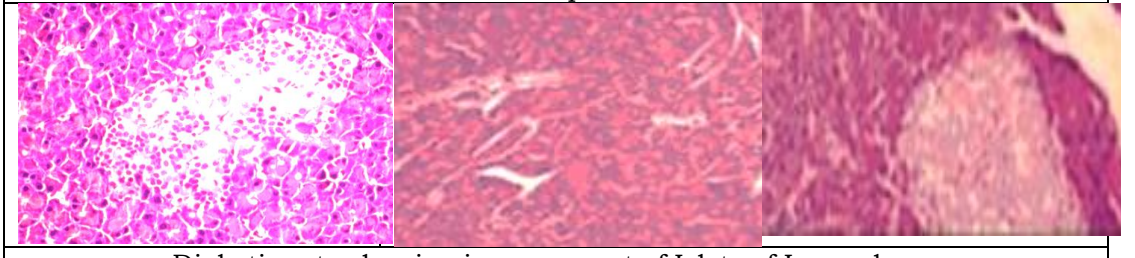
In normal control rats, appearance of pancreas was shown normal. Pancreas of diabetic control rats showing reduced islet cells. However, the treatment with extract showed a recovery of islet cells to near normal appearance (Figure 1).





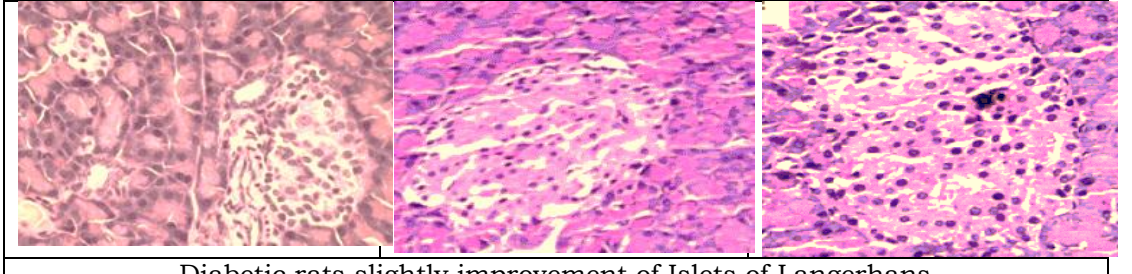
Diabetic rats slightly improvement of Islets of Langerhans

Group 4



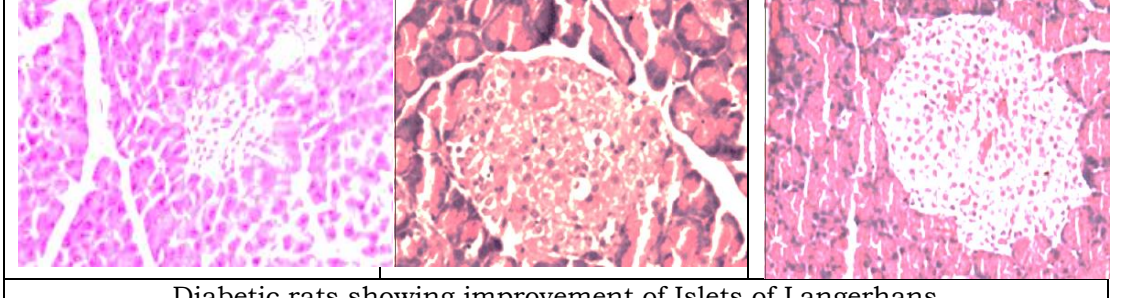
Diabetic rats showing improvement of Islets of Langerhans

Group 5



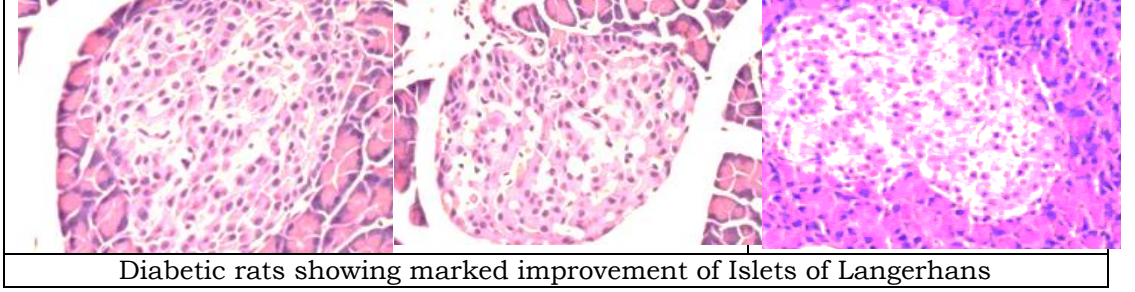
Diabetic rats slightly improvement of Islets of Langerhans

Group 6



Diabetic rats showing improvement of Islets of Langerhans

Group 7



Diabetic rats showing marked improvement of Islets of Langerhans

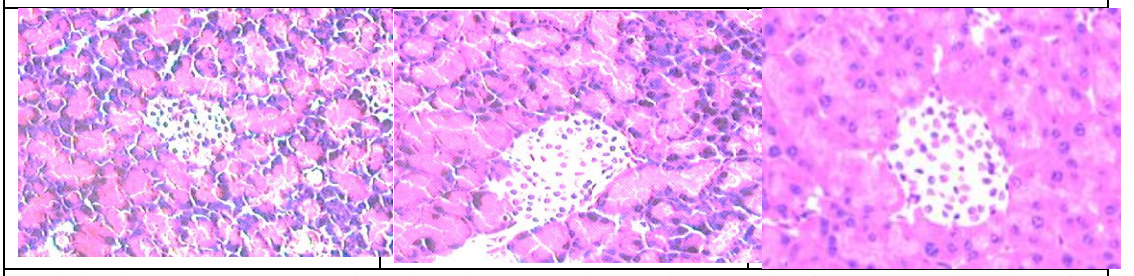
Group 8

Diabetic rats pancreas showing degenerative and necrotic changes and reduced dimension of Islets of Langerhans.

Figure 1. Histopathalogical analysis of rats

Discussion

Administration of streptozotocin caused diabetes, which may be because of destruction of beta cells of the islet of Langerhans of the pancreas. [16] Excessive production and decreased utilization of glucose by the tissue are the fundamental basis of hyperglycemia in diabetes mellitus. [17]. In addition, a daily oral intervention of alcoholic extract of Citrullus Colocynthis and Trigonella Foenum Graceum (50:50) indicated that blood sugar, OGTT level shows significantly decrease in in STZ induced diabetic rats treated compared to the other groups of aqueous and alcoholic extract of different concentration. This result confirmed a finding of other studies, which investigated the effect of extract on the blood glucose in rats and extracts of C. colocynthis and Trigonella Foenum Graceum in humans.

Both doses aqueous and alcoholic extract of C. colocynthis and Trigonella Foenum Graceum significantly decreased the glucose level as compared to the diabetic control rats. This effect may be due the decrease in glucose absorption from the intestines or induction of glycogenic process along with decrease in glyconeogenesis and glycogenolysis. [18]. Fenugreek seed extract administration reduced blood glucose levels, possibly due to the high content of alkaloid trigonelline and steroidal saponins in fenugreek, especially the 4-hydroxy-isoleucine compound that is said to be insulinotropic. [19, 20]. When aqueous and alcoholic extract of C. colocynthis and Trigonella Foenum Graceum was administered to glucose loaded overnight fasted normal rats, hypoglycemia was observed after 60 min.

Conclusion

From this study, it was concluded that the administration of alcoholic leaf extract of C. colocynthis and Trigonella Foenum Graceum shows a significant anti-hyperglycemic effect as compared to aqueous extract, with accompanied by a protective and beneficial effect on the histopathological changes on the pancreas. These findings confirmed the traditional usage of the C. colocynthis and Trigonella Foenum Graceum extract for the treatment of diabetes.

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