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Effects of *Citrullus Colocynthis* and *Cucumis Callosus* Extract on Blood Glucose Levels in Alloxan-Induced Diabetic Rats

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Abstract

Polyherbal mixture is better option for treatment of diabetes mellitus having the advantage of producing maximum therapeutic efficacy at low dose. Effects of the ethanolic extracts of the fruits of *Citrullus colocynthis* and *Cucumis callosus* on the blood glucose levels were investigated in alloxan induced diabetic rat. In diabetic wistar rats, oral administration of ethanolic extract of *C. colocynthis* (300 mg/kg) and *C. callosus* (500 mg/Kg) produced significant reduction in plasma glucose after 7 days and highly significant after 15 days. But the combination of both plant extract at the half dose *C. colocynthis* (150 mg/Kg) and *C. callosus* (250 mg/Kg) produced more significant effect in the compare of diabetic control group. The effect was more pronounced with the combination of both plant extract, significantly lowered the fasting glucose levels after 7 days and highly significant (P<0.01) after 15 days, when given orally to alloxan diabetic rats. Phytochemical screening revealed that the fruits of *C. colocynthis* and *C. callosus* contains tertiary and quaternary alkaloids, glycoside and saponin components. These results suggest that the ethanolic extract of fruits of *C. colocynthis* and C. callosus possesses antihyperglycemic effect and its combination (*C. colocynthis* and *C. callosus*) produced synergistic action at lower dose of ethanolic plant extract.

***Keywords*:** Polyherbal mixture, *Citrullus colocynthis*, *Cucumis callosus*, Phytochemical screening, Antihyperglycemic.

Introduction

Diabetes mellitus is not a single disease. Instead, it is a heterogeneous group of syndromes all characterized by increasing blood glucose caused by a relative or absolute deficiency of insulin, insulin is a hormone manufactured by the beta cells of the pancreas, which is required to utilize glucose from digested food as an energy source [1]. Virtually all forms of diabetes are due to either in decrease in the circulating level of insulin (insulin deficiency) or a decrease in response of target tissues to insulin (insulin resistance). In diabetes mellitus carbohydrate metabolism is reduced while that of protein and lipids are increased. Insulin deficiency causes hyperglycemia, as hyperglycemia increases, there is loss of glucose through urine (glycosuria). Chronic hyperglycemia is associated with microvascular and macrovascular complications that can lead to visual impairment, blindness, kidney disease, nerve damage, amputations, heart disease, and stroke, including sexual dysfunction [2].

Diabetes mellitus has two major forms:

- Type- 1 (Insulin Dependent Diabetes Mellitus or IDDM)
- Type-2 (Non Insuline Dependent Diabetes Mellitus or NIDDM)

Recently two more diabetes disease states have also been added. These are:

- Type-3 (Drug Induced Diabetes)
- Type-4 (gestational Diabetes)

TYPE-1(Insulin Dependent Diabetes Mellitus or IDDM):

Insulin dependent diabetes mellitus most commonly afflicts juveniles but IDDM can also occur among adults. The disease is characterized by an absolute deficiency of insulin caused by massive beta cells lesions or necrosis. In case of Type 1 diabetes loss of beta cell function may be due to invasion by viruses, the action of chemical toxins, or usually, through the action of autoimmune antibodies directed against the beta cells. As a result of destruction of beta cells, the pancreases fail to respond to ingestion of glucose. Type-1 diabetes shows classic symptoms of insulin deficiency (polydipsia, polyphagia and polyuria) [3].

TYPE-2 (Non-Insulin Dependent Diabetes Mellites Or NIDDM) :

Most diabetics are in this category. Genetic factors rather than viruses or autoimmune antibodies are apparently causal. The metabolic alteration observed are milder than those described for IDDM, but the long term clinical consequences can be just as devastating(e.g.: vascular complications and subsequent infection can lead to amputation or the lower limbs). In INDDM pancreas retain some beta cells function, resulting in variable insulin levels that are insufficient to maintain glucose homeostasis. Patient with Type-1 diabetes are often obese. Type-2 diabetes is frequently accompanied by target organ insulin resistance that limits responsiveness to both endogenous and exogenous insulin. In some cases, insulin resistance is due to a decreased no. of mutation of insulin receptors [4].

TYPE-3 Diabetes (Drug Induced Diabetes):

Many pharmacological agents have side effects which include the raising of blood glucose level and cause reversible diabetes. A number of drugs, corticosteroids, thiazids diuretics, beta blockers, antipsychotics, statin and protease inhibitors have been linked with increased risk development of diabetes [5].

TYPE-4 Diabetes (Gestational Diabetes):

It is also called “Gestational Diabetes Mellitus” (GDM). It is observed in approximately 4-5% of all pregnancies. Elevated blood sugar levels are usually observed in second or last trimester of pregnancy and usually resolved during the postpartum period. There is no genetic predisposition. The most plausible cause is that during pregnancy, the placental hormones promote insulin resistance [6].

EPIDEMIOLOGY

Diabetes is a leading cause of morbidity and mortality for a growing proportion of the world’s population. The World Health Organization predicts a worldwide increase of 50% in diabetes related deaths in the next 10 years, with a majority of deaths occurring in relatively wealthier nations.

In 2000, according to the World Health Organization (WHO), at least 171 million people worldwide suffer from diabetes, or 2.8% of the population. Its incidence is increasing rapidly, and it is estimated that by 2030, this number will almost double. The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet. This has suggested an environmental (i.e., dietary) effect, but there is little understanding of the mechanism at present, though there is much speculation, some of it most compellingly presented [7].

PLANT UNDER STUDY

1. *Citrullus colocynthis:*

Citrullus colocynthis (family: Cucurbitaceae) is a desert viny plant that grows in sandy arid soils. It is native to the Mediterranean Basin and Asia and is distributed among the west coast of northern Africa, eastward through the Sahara, Egypt until India. It grows also in southern European countries as in Spain and on the islands of the Grecian archipelago. It is an annual or a perennial plant (in wild) in Indian arid zone and has a great survival rate under intense xeric conditions. It can tolerate annual precipitation of 250 to 1500 mm and an annual temperature of 14.8 to 27.8 °C.

The main chemical contain of fruit pulp is colocynthin (the bitter principle up to 14 %), colocynthein (resin), colocynthetin, cucurbitane type triterpen glycoside viz colocynthoside A & B, cucurbitacin E 2-O-beta-D-glcoside, aglycone Cucurbitacin E, pectin gum and glucopyranosylcucurbitacin [8, 9].

2. *Cucumis callosus*

It is most probably a variety of the melon that is *Cucumis melo* var *agrestis* (Cucurbitaceae). Native to dry areas of India being common throughout the south America, areas of Thailand, Egypt and Africa, eastward through Iran to India and other parts of tropical Asia. Has been known since Biblical times and cultivated in the Mediterranean region, especially in Cyprus and in India for many centuries.

The major component is α- and β-amyrins, taraxerol, lupeol, cycloartenol, dehydro-cycloartanol, euphol, tirucallol, amino acids,

ascorbic acid, sugars fructose, glucose, sucrose, raffinose, and stachyose [10, 11].

MATERIALS AND METHOD

Collection of Plants:

The fruit of *C. colocynthis* and *C. callosus* were purchased from local herb store of Jaipur in August 2013. The fruits were cleaned, washed, chopped to small pieces and then dried at room temperature. The dried fruit was powdered and passed from 40-50 mesh size sieves.

Extraction of Plants:

100 gm of plant powder were taken in soxhlet apparatus and extracted into 800 mL ethanol (90%) for 48 hours. It was shaken frequently during the first 6 hours and allowed to stand for 18 hours. There after it was filtered rapidly and then filtrate was evaporated with the help of vacuum evaporator at the temperature of 45^0c.

Chemicals Used

Alloxan hydrate and glibenclamide were obtained from Central Drug House (CDH), New Delhi and all chemicals were obtained commercially and were of analytical grade.

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the crude extract of was *Citrullus colocynthis* and *Cucumis callosus* carried out in order to ascertain the presence of its constituents utilizing standard conventional protocols.

Test Animals

Experimental rats were processed in accordance to *Committee for the Purpose of Control and Supervision of Experiments on Animals* (*CPCSEA*). Albino wistar male rats weighing 150-200 g was used for the study. They were maintained in the animal house for experimental purpose. The animals were maintained under controlled conditions of temperature (22 ± 3°C), humidity (30 to 70 %) and 12-h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water ad libitum. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress.

Induction of Diabetes Mellitus

Healthy Wistar strain albino rats weighing about 150- 200 grams were taken in each group. Animals are divided into six groups and each group containing 8 animals. Animals were deprived to food for 16 hour but allowed free access to water**.** Diabetes mellitus was induced by intraperitoneal injection of freshly prepared solution of alloxan monohydrate (150 mg/Kg) dissolved in physiological saline solution in overnight fasted rats. Since alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic release of insulin. After a period of three days the rats with a blood glucose levels greater than 200 mg/dL were considered diabetic and used for this research work.

Experimental Design

The alloxan induced diabetic Wistar rats were randomly assigned into six groups (1-6) of eight rats (n=8) each as follows, namely

Group 1- Received normal saline i.p

Group 2- Received alloxan (Diabetic control, 120 mg/Kg, i.p)

Group 3-Received *Citrullus colocynthis* Group (300 mg/Kg, orally)

Group 4- Received *Cucumis callosus* Group (500 mg/Kg, orally)

Group 5- Received *Citrullus colocynthis* and *Cucumis callosus* Group (150 mg/Kg & 250 mg/Kg, orally)

Group 6- Received Glibenclamide Group (5 mg/Kg, i.p)

Determination of Blood Glucose Levels

Blood samples were collected retro orbital plexus of the rats, for blood glucose determination at intervals of 0, 3, 7 and 15 days. Determination of the blood glucose level was done by the Achu – Check glucometer and results were reported as mg/dl.

Statistical Analysis

Blood glucose levels were expressed in mg/dl as mean ± SD. The data were statistically analyzed using ANOVA followed by Dunnet's test. The values of $p<0.05$ were considered as statically significant.

RESULT

Preliminary Phytochemical Analysis

The preliminary phytochemical screening of extract of *Citrullus colocynthis* gave positive tests for carbohydrates, resins, saponin, anthraquinone, steroids and alkaloids and extract of *Cucumis callosus* gave positive tests for carbohydrate, alkaloid, protein, saponin, flavonoids, tannin and glycosides.

Table 1: Phytochemical screening of *Citrullus colocynthis* and *Cucumis callosus* fruits etract

Tested For	Tests	C. colocynthis extract	C. callosus extract
Carbohydrate	Molisch	(+)	(+)
Alkaloid	Wagner's	(-)	(+)
Protein	Biuret	(-)	(+)
Steroids	Salkowski	(+)	(-)
Glycosides	Sodium hydroxide	(+)	(+)
Saponins	Foam	(+)	(+)
Tannins	Ferric chloride	(-)	(+)
Flavonoids	Ferric chloride	(-)	(+)
Resin	Ferric chloride	(+)	(-)

+ = Present, - = Absent

Anti diabetic Study

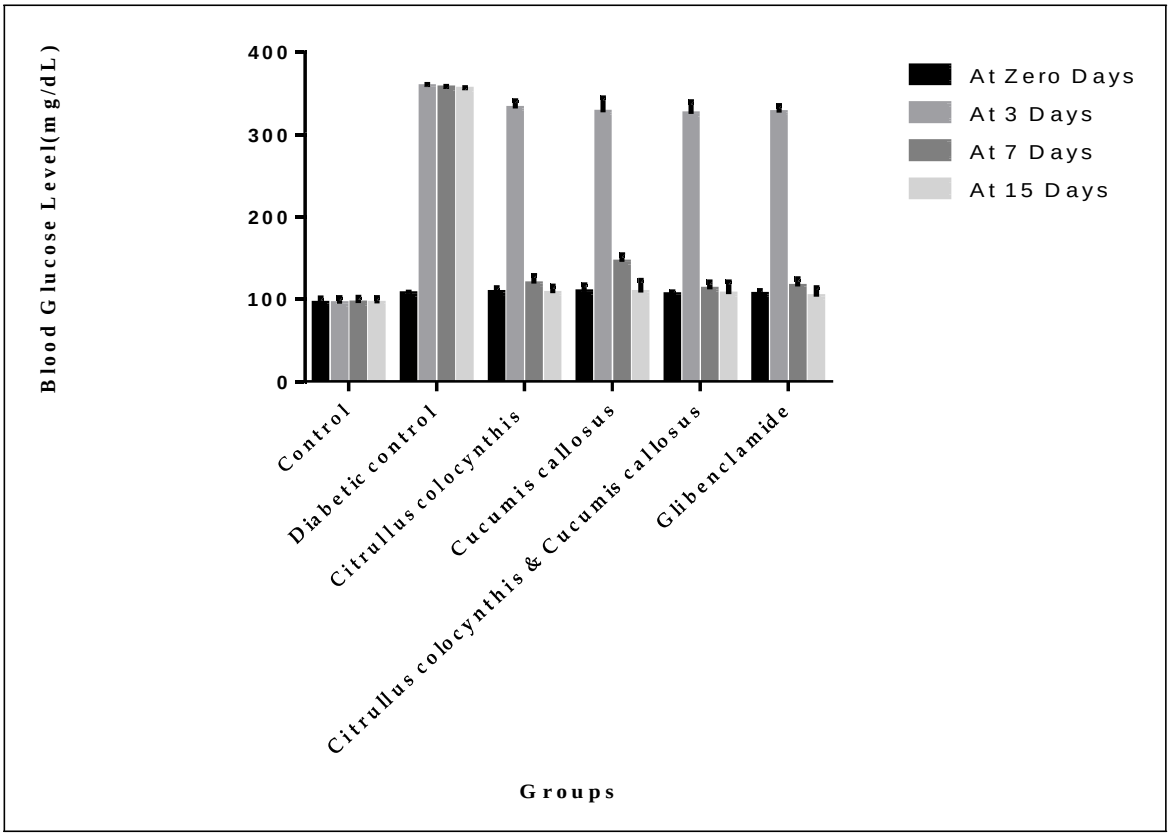
The effect of combination therapy on blood glucose level in normal and alloxan induced diabetic rats is shown in Table 2. At 3 days of alloxan administration the peak blood glucose level increased rapidly from initial value at 0 days and subsequently decreased after 7 and 15 days of test drug treatment. The combination therapy of low doses of *C. colocynthis and C. callosus* showed more significant ($P<0.001$) reduction in blood glucose level compared to the individual treatment of *C. colocynthis*, *C. callosus* at high doses and similar level of significance of standard drug, glibenclamide.

Table 2: Effect of fruit of ethanolic *Citrullus colocynthis and Cucumis callosus* and their combination on blood glucose level of alloxan-induced diabetic Wistar rats

Treatment	Blood Glucose Level (mg/dL)			
	0 Days	After 3 Days of Alloxan Administration	After 7 Days of Treatment	After 15 Days of Treatment
Group 1 Control, (N/Saline)	95.12±6.578	94.88±7.120	95.75±7.086	95.25±6.861
Group 2 Diabetic Control	106.80±2.0	358.2±2.7	356.4±2.2	355.0±1.9
Group 3 *Citrullus colocynthis* (300 mg/Kg), Orally	108.50±5.806 *	331.88±9.141 a*	119.12±10.063 *	107.62±8.484**
Group 4 *Cucumis callosus* (500mg/Kg), Orally	109.25±8.565 *	327.12±17.716 *	145.62±8.651 *	108.50±15.024**
Group 5 *Citrullus colocynthis*(150 mg/Kg) & *Cucumis callosus* (250 mg/Kg), orally	105.75±3.845 *	325.12±14.555 *	112.50±8.536 *	106.50±15.024**
Group 6 Glibenclamide (5mg/Kg), i.p	106.25±4.652 *	326.88±8.271 *	115.75±9.407 *	103.62±10.954**

Vales are given as mean ± SD for 8 rats in each group; experimental groups are compared with diabetic control group. Values are statistically significant at *=P<0.05 ns=not significant and **=P<0.01.

Figure 1: Showing decrease blood glucose level in all extracts.



DISCUSSION

Treatment employing two or more herbs in combination known as, "polyherbal therapy" has the advantage of producing maximum therapeutic efficacy than the single herb treatment at lower dose. Polyherbal therapy may provide synergistic, potentiative pharmacological properties within themselves because of presence of vast range of phytobioactive constituents [12].

The present work was focused to establish the therapeutic efficacy and probable benefit associated with the combination therapy at low doses of ethanolic extracts of fruits of *C. colocynthis* and *C. callosus* in comparison to their individual treatments and standard antidiabetic drug, glibenclamide.

Alloxan-induced hyperglycaemia has been described as a useful experimental model to study the activity of hypoglycemic agents because alloxan, β-cytotoxin cause a massive destruction of β-cells of islets of Langerhans, resulting in reduced synthesis and release of insulin. Alloxan-induced diabetes is characterized by loss in body weight and increased intake of food. Body weight loss might be the result of protein wasting due to deficiency in carbohydrate metabolism and unnecessary breakdown of tissue protein [13].

Oral administration of *C. colocynthis* and *C. callosus* and the combination therapy of low doses of *C. colocynthis* and *C. callosus* showed significant (P<0.01) reduction in blood glucose level after 7 and 15 days of treatment. The reduction in blood glucose level was more significant (P<0.001) with the combination therapy than the single treatment. This suggests that the administration of combination of low doses of *C. colocynthis*

and *C. callosus* can more significantly reduce the hyperglycemia.

C. colocynthis and *C. callosus* attributed to the vital phytoconstituents contained in both the plants, viz. – carbohydrate, glycosides and Saponins. The anti-oxidant and free radical scavenging properties of flavonoids and other polyphenolic compounds of the two plants might be responsible for the antidiabetic activity of the combination therapy.

CONCLUSION

In conclusion, the experiment evidence obtained in the present laboratory animal study indicate that ethanolic extract *Citrullus colocynthis* and *Cucumis callosus* fruits possess anti-diabetic properties and combination of these plant extract at half dose shows synergistic action, which suggest the presence of biologically active components which may be worth further investigation and elucidation.

The results observed suggest the enhanced synergistic antihyperglycemic effect of the combination therapy of low doses of *Citrullus colocynthis* and *Cucumis callosus*. This also gives an opportunity to reduce the dose of herbs in order to avoid the burden of herbal over dosing and at the same time proper precaution and care should be exercised as the combination herbal therapy may pose the condition of severe hypoglycaemia.

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