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**Original Research Article**

# Anti-diabetic Effects of Aqueous plant *(Citrullus colocynthis)* Extract in Streptozotocin Induced Diabetic Mice

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## Abstract

The aim of this study was to find out anti-diabetic effects of *Citrullus colocynthis* commonly known as bitter apple. Random blood glucose levels were measured before and after plant extract administration. Powdered form of plant extract was used as an oral treatment. Diabetes was induced in mice using artificial diabetes inducer named-Streptozotocin. Maximum anti-hyperglycemic effect and reduction in glucose level was observed with individual treatment of extract of *C. colocynthis* @400mg/kg, which was 75%±1.3. This treatment was more effective than amaryl @3mg/kg, which shows effectiveness of 52%±2.4 and Glucophage @500mg/kg, which shows effectiveness of 29%±2.1. Results showed that bitter apple have insulin tropic activity which helps in regeneration of beta cells of pancreas which turn normalize blood glucose levels without any significant side effects such as hypoglycemia. Further research is still required before implementation of this plant to treat diabetic patients.

**Keywords:** *Citrullus colocynthis*, anti-hyperglycemic, diabetes, regeneration, pancreas, Insulin, glucose levels.



## INTRODUCTION

Holistic medicine has been used as a treatment by various cultures for thousands of years. Drugs are important to prevent and treat disease. Traditional medicines are isolated from natural sources like plants, marine sources, microorganisms, etc with plants being the most effective source among them for years (Sikand, & Laken, 1998). The main reason of worldwide usage of herbal medicine is that they have less health risk than pharmaceuticals (Etkin, 2008). Herbal extracts exert synergistic pharmacological effects by down regulating different proteins of the same signaling networks (Ulrich-Merzenich, 2014). Herbal medicines can have toxic as well as non-toxic effects both (Zhang *et al*., 2014). Misidentified herbal drugs cause a latent health risk. Medicinal plants have been mainly used by cultures of underdeveloped countries (Zhao *et al*., 2012). Plants belonging to the Cucurbitaceae family contain biologically active substances that upgrade the scale of medical significance. Doshi & Kanade, (2019) has reported that *Cucurbita pepo* containing novel compounds such as carotene, carbohydrate, mineral, fatty acids and linoleic acid. Whereas, the chemical constituents present in *Trichosanthes cucumerina* include flavonoids, carotenoids, phenolic acids and, insoluble dietary fibers (Devi N.D 2017, Liyanage R2016). Singh *et al*., 2016 has reported the subsequent compounds in *Trichosanthes cucumerina*: tannins, terpenoids, alkaloids, saponins, glycosides and, phenols. *Citrullus colocynthis* schard commonly referred to as bitter apple, bitter gourd, etc is a member of family Cucurbitaceae and grows in the Egyptian desert, alluvial plains and along the east coasts as well as

Rabian, Sahara desert, Sudan, Saudi Arabia, Pakistan and, India.

Several active constituents of *Citrullus colocynthis* are reported such as alkaloids, flavonoids, saponins, tannins, carbohydrates. Cucurbitacins and flavones are reported as the main constituent of this plant. It has cytotoxic, hepatoprotective, cardiovascular, and anti-diabetic anti-lipedimic , anti-cancer as well as hepato-protective and anti-inflammatory effects (Abdel-Hassan *et al*., 2000; Sebbagh *et al*., 2009; Jeyanthi & Christy, 2011). The seeds of this plant are major source of various fatty acids such as myristic acid, palmitic acid, oleic acid, linoleic acid as well as linoleic acid. Almost more than 400 plant species have shown anti-diabetic activity (Abbas *et al*., 2006). The plant has about 8.25% protein content comprising of novel amino acids named lycine, leucine, and methionine (Shaheen *et al*., 2003) and phytochemicals including flavonoids, phenols, alkaloids tannins, glycosides and saponins (Benariba, *et al.*, 2009). It is used to treat various ailments such as diabetes, cancer, leukemia, rheumatism, constipation, menorrhagia (Arivoli, & Samuel, 2011).

Diabetes is a chronic metabolic disorder in which body metabolism is disturbed. Diabetes is one of the leading causes of morbidity and mortality in the world. There are two types of diabetes mellitus; Type I and type II. Type I diabetes mellitus is caused by oxidative stress due to environmental as well as nutritional factors which lead to increased production of reactive nitrogen and reactive oxygen species which cause damage of beta cells (Rahimi *et al*., 2005). Loss of beta cells is the main indicator of diabetes mellitus (Amin *et al*., 2017). Although there are many synthetic drugs available for diabetic patients but these drugs are expensive and have unavoidable side effects. Therefore, we are in dire need to test plants to find their anti-diabetic effects, so that they can be given to help diabetic patients. Type II diabetes mellitus is a common disease that imposes serious health problems to general population, caused either by reduced production of insulin or resistance to action of insulin (Arulrayan *et al*., 2007). This disorder leads to destruction or dysfunction of beta cells of pancreas, which leads to insulin resistance which in turn leads to alteration of carbohydrate, proteins and fat metabolism (Djrolo *et al*., 1998). Anti-diabetic effects of this plant may be due to its insulin tropic activity (Jayaraman *et al*., 2009). The aim of this study is to find out anti-diabetic effects of aqueous and alcoholic extracts of *Citrullus colocynthis* in diabetic induced mice.

## MATERIALS AND METHODOLOGY

Animals were procured from animal house of university. Ethical approval was taken from the institutional review board.

### Preparation of *Citrullus colocynthis* extract

Dried Fruits of *Citrullus colocynthis* were purchased from the market (Figure). Mortar and pestle were used to prepare the powder form of dried fruits and their seeds. After that 500 ml distilled water and 500 ml of 70% ethanol were added to 100 g powder of *Citrullus colocynthis* to make aqueous and ethanol extract of plant respectively. The mixture was allowed to boil under reflux for 3 hours at 60℃ in the case of aqueous extract and 15 min in the case of ethanolic extract. The mixture was then filtered using Whatman filter paper No.1. The filtrate obtained was poured into Petri-dishes and placed in an electrical oven for 2 days at 50℃ to obtain the dried extract. These dried extracts of yellow color were then shifted in 50 ml falcon tubes separately, labeled, and covered with aluminum foil to protect from contamination. Then these tubes were placed at 4℃ and diluted with distilled and/or normal saline at the time of usage.


**Fig-1: *Citrullus colocynthis* fruits (a), preparation of powdered form (b), dehydration of extract in the electrical oven after pouring in petri-dishes (c), dried form of ethanolic and aqueous extract.**

**Experimental animals**

Male albino adult mice were used for experimental purposes. They were 3-4 months in age and weight of about 37g each. 40 mice were opted and arranged into four groups. Each group was comprised of 10 mice. Mice were kept in polypropylene cages for one week and acclimatized for the experiment. Animals were kept under observation to notice their behavioral changes and were kept at a controlled room temperature of about 35-37℃. Twelve hour day and twelve-hour night exposures were maintained. The animals were properly fed with commercially available diet and water for mice available from the local market.


**Fig-2: Male albino adult mice were used for Experiment.**

**Diabetes induction in mice**

**0.05M Sodium Citrate buffer preparation (pH 4.5)**

0.1M stock solution of sodium citrate buffer was prepared. 1.2044 g sodium citrate dihydrate and1.1341g citric acid was added in 100 ml distilled water. This is 0.1 M concentration of stock solution. 0.05 ml citrate buffer was obtained by mixing 50 ml stock solution into 50 ml distilled water. Its pH was adjusted to 4.5. It was stored at 4℃ in the freezer. The buffer should be freshly prepared and was kept in ice before inducing diabetes.

**Streptozotocin-sodium citrate buffer mixture preparation**

Streptozotocin was purchased from Riphah International University, Lahore. Three doses of STZ – sodium citrate buffer were prepared by mixing 50 mg/kg STZ in 0.3 ml sodium-citrate buffer and were administered intraperitoneally for 3 days in each mouse. Mice were kept fasted before and after administration of STZ for 12 hours. Disposable restrainers were used to inject mice. Mice can also be handled by using scruffing. The buffer should be ice cold before mixing with streptozotocin. Streptozotocin- sodium citrate buffer mixture was prepared immediately before injecting into mice because the buffer could degrade streptozotocin within 15 minutes after mixing. Glucose was measured 72 hours after streptozotocin-sodium citrate buffer mixture administration by pricking the tail of mice.


**Fig-3**: **Preparation of sodium citrate and citric acid for buffer (a), maintenance of buffer pH at 4.5 (b), buffer kept in ice during performance (c) and Streptozotocin (d).**

**Calculation of STZ dose per mouse**

1000g mice required STZ dose= 0.04 g/mouse
40 g mouse required STZ dose=X
1000X=0.04x40

**X=0.04x40/1000=1.6/1000=0.0016g/mouse**

**STZ per mouse**

STZ (40 mg/kg): 20/1000 x 40= (Table 1)
STZ (50 mg/kg): 20/1000 x 50= (Table 1).

**Table-1: STZ –Na citrate dose.**

| Streptozotocin Dose (mg/kg) | Mouse weight (g) | STZ-Na citrate dose |
|---|---|---|
| 40 mg/kg | 20g | 0.0008g STZ+0.3 ml buffer |
| 50 mg/kg | 20g | 0.001g STZ +0.3 ml buffer |

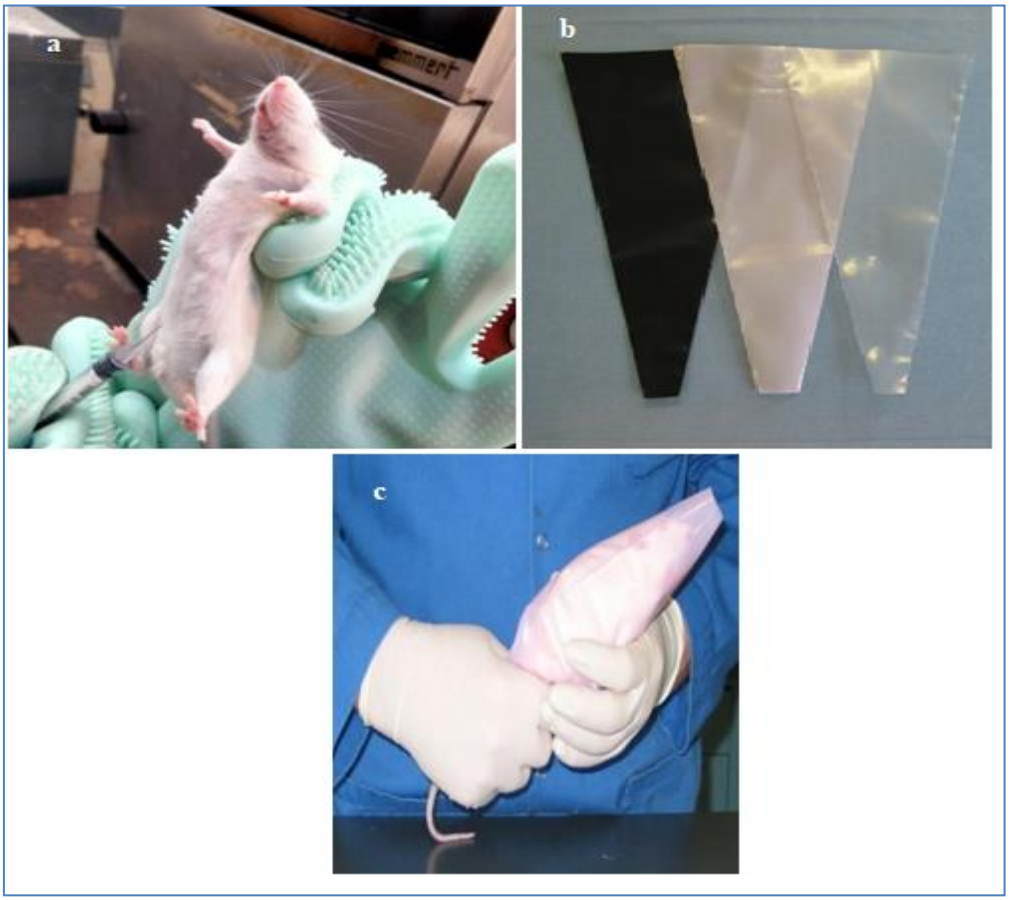


**Fig-4**: **Intraperitoneally injection of STZ (a), disposable restrainer (b), and mouse retrained with restrainer (c).**

**Administration of various treatments**

**Table-2: Experiment design for administration of various treatments.**

| Groups | Treatment |
|---|---|
| **Group 1(Negative control)G1** | Non -diabetic mice without any treatment, only on a regular diet |
| **Group 2 (Positive control)G2** | Diabetic and untreated mice without treatment, on a regular diet |
| **Group 3 (G3)** | Treatment with Aqueous extract of *Citrullus colocynthis* in diabetic mice |
| **Group 4 (G4)** | Treatment with a standard drug named Glucophage in diabetic mice |

**Administration of *Citrullus colocynthis* extract**

Plant extract was prepared by adding 500 ml distilled water and 500 ml of 70% ethanol to 100 g *Citrullus colocynthis* powder (400mg/kg) to make aqueous and ethanol extract of plant respectively. Vortex mixer was used to make homogenous mixture. This prepared solution was administered orally for once a week. Mice can be handled by scruffing or restrainer technique. Blood glucose levels were measured before and after treatment (Table 3). Dose calculation formula= weight (g)/1000 x 400mg/1000.

**Table-3: Effect of *Citrullus colocynthis* extract (400 mg/kg) in diabetic mice. (Group 1) Glucose level (mg/dl)**

| Sr no. of mouse | Negative control* | Positive control^ | After STZ | After treatment with C.C | % Reduction in BGLs | Treatment effect remain for days |
|---|---|---|---|---|---|---|
| 1 | 97 | 290 | 300 | 150 | 74% | 18 |
| 2 | **90** | **328** | **353** | **120** | **89%** | **21** |
| 3 | 72 | 300 | 312 | 160 | 63% | 15 |
| 4 | 89 | 250 | 306 | 122 | 85% | 11 |
| 5 | 91 | 300 | 340 | 180 | 64% | 20 |

Standard mean percentage decrease in BGLs ±SEM came out to be 75 % ±1.3
*Non diabetic and untreated, ^Diabetic and untreated, *> 0.05 considered significant.*

**Administration of Amaryl**

Amaryl is a synthetic drug used by diabetic patients. Medicinal extract was prepared by adding amaryl powder (3mg/kg dose) in 0.1 ml of distilled water. Homogenous mixing was done using vortex mixer. This prepared solution was administered once a week. Scruffing technique was used to handle mice. Blood glucose levels were measured before and after treatment (Table 4). Dose calculation formula=weight (g)/1000 x 3mg/1000

**Table-4: Effect of Amaryl (3mg/kg) in diabetic mice. Group 2 Glucose level (mg/dl)**

| Mice No. | -Ve control | +Ve control | After STZ | After Amaryl treatment | % reduction in BGLs | Treatment effect remain for days |
|---|---|---|---|---|---|---|
| 1 | 92 | 315 | 325 | 209 | 50% | 03 |
| 2 | 71 | 361 | 341 | 230 | 41% | 05 |
| 3 | 102 | 322 | 300 | 200 | 51% | 02 |
| 4 | 97 | 291 | 324 | 190 | 59% | 04 |
| 5 | **90** | **300** | **320** | **182** | **60%** | **07** |

Standard mean percentage decrease in BGLs ±SEM came out to be 52 % ±2.4
STZ: Streptozotocin, BGLs: Blood glucose levels)

Standard mean percentage decrease in BGLs ±SEM came out to be 29%±2.1%; *Non diabetic and untreated, ^Diabetic and untreated, *> 0.05 considered significant.*

**Administration of glucophage**

Amaryl is a synthetic drug used by diabetic patients. Medicinal extract was prepared by adding glucophage powder (500mg/kg dose) in 0.2 ml of distilled water. Homogenous mixing was done using vortex mixer. This prepared solution was administered once a week. Scruffing technique was used to handle mice. Blood glucose levels were measured before and after treatment (Table 5). Dose calculation formula=weight (g)/1000 x 500 mg/1000.

**Table-5: Effect of Glucophage (500mg/kg) in diabetic mice**

| sr. no of mouse | Negative control* | Positive control^ | After STZ | after treatment | %reduction in BGLs | Treartment remain for days |
|---|---|---|---|---|---|---|
| 1 | 84 | 349 | 362 | 300 | 22% | 07 |
| 2 | **90** | **300** | **320** | **230** | **39%** | **07** |
| 3 | 93 | 371 | 328 | 248 | 34% | 03 |
| 4 | 106 | 291 | 315 | 270 | 22% | 05 |
| 5 | 73 | 322 | 309 | 240 | 29% | 02 |

**Estimation of Glucose levels**

Testing glucometer was used for estimation of blood glucose levels of each mouse mouse before and after treatments. Glucose level more than 150mg/dl was considered diabetic.

## STATISTICAL ANALYSIS

SPSS was used to perform the statistical analysis of different conditions. Paired t-test was conducted to calculate the significance of each result obtained. In addition, ANOVA was also applied to find significance of results. P-value obtained was significant showing the quality of results obtained. This P-value is denoted by alpha (α). If the value of α is less than 0.05, then the results are considered significant.

Reduced BGLs= (BGLs after STZ-Control) –BGLs after treatment-Control)
% reduction in BGLs= Reduced BGLs/BGLs after STZ-Control x 100

## RESULTS

**Effect of aqueous extract of *Citrullus colocynthis* in diabetic mice**

After induction of diabetes, the anti-diabetic effect of *Citrullus colocynthis* aqueous extract was

evaluated. Mice in treatment group 3 (G3) were treated orally with aqueous extract of *Citrullus colocynthis* with increasing doses from 300-400 mg/kg for two weeks. No death was noted. *Citrullus colocynthis* not only controlled diabetes but also controlled the body weight of all mice in a dose and time-dependent manner. The maximum percentage of reduction of blood glucose level was about 75±1.2%. No treatment was given to positive control. The negative control group was treated with normal 0.9 % saline of about 1ml/100g body weight. No difference was seen in the weight and blood glucose levels of mice in a negative group. Animals were fasted for 12 hours before the induction of diabetes and before the measurement of blood glucose levels in mice after 72 hours of STZ induction. The standard group includes diabetic mice that were treated with a standard anti-diabetic drug named Glucophage for two weeks to compare our results with the standard drug. Our results were comparable. The average percentage of reduction of blood glucose level with extract was 70% and that with the standard was 52 ±2.4%.

## DISCUSSION

Diabetes mellitus is a chronic metabolic disorder leading to disturbed metabolism of carbohydrates, proteins and lipids. Liver and other body tissues are involved in body metabolism (Ulrich-Merzenich, 2014). Plants contain specific components that help to treat various diseases. About 400 species of plants have anti-diabetic effects. It has cytotoxic, hepatoprotective, cardiovascular, and anti-diabetic anti-lipidimic, anti-cancer as well as hepato-protective and anti-inflammatory effects (Abdel-Hassan *et al*., 2000; Sebbagh *et al*., 2009; Benariba *et al*., 2009; Jeyanthi & Christy, 2011). Anti-diabetic effects of this plant may be due to its insulin tropic activity (Nmila *et al*., 2000). Plant extract can partially reverse hyperglycemic condition in STZ induced diabetic mice. It is helpful in improving the level of glucose by partial regeneration of beta cells and stimulation of insulin secretion. The activity of this fraction could be attributed for more extent to the presence of saponin and glycosidic components.

Some side effects including diarrhea,nephrosis, vomiting and hematochezia ,seizures etc and death by circulatory collapse has made it necessary for herbalists to use this plant with care . A clinical trial was found for this plant that confirmed antidiabetic properties of 200 mg /kg fruit given for 14 days (Alia *et al*., 2017). Abdel-Hassan *et al*., 2000 has studied the hypoglycemic effect of aqueous 300 mg/kg, glycosides 50 mg/kg and saponins 50 mg/kg extracts of *Citrullus Colocynthis* epicarp. They have reported that aqueous and glycosides extracts produce significant decrease in blood glucose levels 6 hours after oral administration of extracts in normal rabbits. Saponins showed a hypoglycemic effect in normal and alloxan diabetic rabbits after oral administration of numerous doses (Abdel-Hassan *et al*., 2000). Another study conducted by Jayaraman *et al*., (2009) has studied anti-diabetic effects of petroleum extract of *C. colocynthis* in Streptozotocin induced diabetic mice. Two different doses (300 and 500mg/kg) of fruit extract were administered which significantly reduced blood glucose levels. Glibenclamide (0.5 mg/kg) was used as reference drugs. Another study done by has shown the anti-diabetic effect of aqueous extract of *C. colocynthis* by administrating 2.5g/kg. Glucose reduction was about 57%. In our study maximum anti-hyperglycemic effect and reduction in glucose level was observed with individual treatment of about 400mg/kg *C. colocynthis* extract which was 75% ±1.3. This treatment was more effective than amaryl (3mg/kg) which shows effectiveness of 52% ±2.4 and Glucophage (500mg/kg) which shows effectiveness of 29±2.1 %.

## CONCLUSION

Results indicate that *C. colocynthis* have insulin tropic activity and stimulate secretion of insulin by partial regeneration of beta cells of liver but due to side effects care has to be taken while administration in order to avoid side effects, Still more research, experiments are required to be performed in order to implement it on humans for treatment purpose.

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