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Original Article

# Evaluation of Antihyperglycemic Activity of *Citrullus Colocynthis* Fruit Pulp in Streptozotocin Induced Diabetic Rats

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ABSTRACT

In the present study the selected plant extract have been screened for its anti-hyperglycemic potential and thereby for its possible role in inhibition of the pathways leading to diabetic complications in STZ induced diabetic rats. The herb namely fruit pulp of *Citrulluscolocynthis (CC)*. Initially hypoglycemic studies were conducted without inducing of diabetes mellitus. The extract which is showing % reduction of glucose between 30 -50% were selected in order to avoid hypoglycemic shock. Treatment with the selected plant extract significantly increased the body weight and decreased food and water intake in a dose dependent manner. The selected plant extract was found to decrease glucose levels at 3rd and 8th hours and standard drug was found to decrease glucose levels at 1st & 6th hours significantly after every week (biphasic reduction). The selected plant extract was found to increase insulin levels at same time intervals where there is peak reduction of glucose.

**Keywords:** Diabetes mellitus (DM), Diabetic complications, Citrullus colocynthis (CC), Streptozocin(STZ).

## 1. INTRODUCTION

Diabetes mellitus is group of syndrome characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins with an increased risk of complications such as retinopathy and nephropathy vascular disease etc., [1]it is of mainly two types. Type1Diabetes mellitus: It is also called as "Insulin Dependent Diabetes Mellitus" and Type2 Diabetes Mellitus:It is said to be Non –Insulin Diabetes Mellitus.

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Diabetes mellitus is a most common endocrine disorder, affecting more than 300million people worldwide. For this, therapies developed along the principles of western medicine (allopathic) are often limited in efficacy, carry the risk of adverse effects, and are often too costly, especially for the developing world[2]. Therefore, treating diabetes mellitus with plant derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive. The field of pharmacology and therapeutics to develop evidence-based alternative medicine to cure different kinds of diabetes in man and animals. Isolation & identification of active constituents from these plants, preparation of standardized dose & dosage regimen can play a significant role in improving the hypoglycemic action[3, 4].

**Citrullus colocynthis:**

Citrullus colocynthis (L.) Schrad is a valuable cucurbit plant, widely distributed in the desert areas of the world. Citrullus colocynthis fruits are usually recognized for its wide range of medicinal uses as well as pharmaceutical and nutraceutical potential. It is also known as bitter apple. The plant has been reported to possess a wide range of traditional medicinal uses including in diabetes, leprosy, common cold, cough, asthma, bronchitis, jaundice, joint pain, cancer, toothache, wound, mastitis, and in gastrointestinal disorders such as indigestion, constipation, dysentery, gastroenteritis, colic pain and different microbial infections. Several bioactive chemical constituents from fruits were recorded, such as, glycosides, flavonoids, alkaloids, fatty acids and essential oils. The main constituents of fruit pulp are colocynthin (the bitter principle upto 14 %), colocynthein (resin), colocynthetin, pectin gum. Seed contain a fixed oil (17 %) and albuminiods (6 %).[5,6,7]

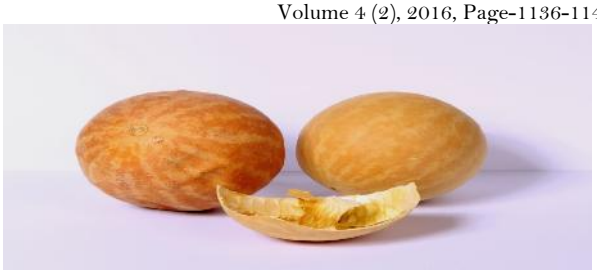

**Fig 1: Fruit pulp of Citrullus**

The selected plant extract have been evaluated for their Antihyperglycemic activity in the present study by estimation of blood glucose, serum insulin levels. Streptozotocin (STZ; *N*-nitro derivative of glucosamine) is a cytotoxic chemical that is particularly toxic to the pancreatic, insulin producing beta cells in mammals[3] used for inducing diabetes. Normalization of blood glucose by intensive insulin therapy reduces the risk of development of diabetic complications. The selected plant extract was administered once daily orally to STZ induced diabetic rats. The study period was maintained for 4 weeks to get the induction of diabetes.[8, 9] At the end of each week of 4 week study period change in body weight, food and water intake, serum glucose estimation and insulin levels were measured to evaluate antihyperglycemic activity.

## 2. MATERIALS AND METHODS

***Induction of diabetes mellitus***

The animals fasted overnight and diabetes was induced by a single intra peritoneal injection of freshly prepared STZ (60 mg/kg body weight of rats) in 0.1 M citrate buffer (pH 4.5). The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. The animal housing and handling were in accordance with CPSCEA guidelines and was approved by CPSCEA for conducting animal experiments with the registration No. 516/01/A/CPCSEA. The prior permission for the study was obtained from our institutional Animal Ethical Committee (IAEC). Control rats were injected with citrate buffer alone. The animals were considered as

diabetic, if their blood glucose values were above 250 mg/dL on the third day after the STZ injection. The treatment started on the fourth day after the STZ injection and this day was considered the first day of treatment. The treatment was continued for 30 days by using above extracts through oral route. Since the extract is aqueous, water is used as vehicle for administration of extract. After 30 days of treatment [10].

*a). Acute toxicity studies*: Acute toxicity studies were performed according to OECD guidelines 423 [11].

*b). Estimation of Glucose*:

Glucose estimation was done in all the groups at the end of the day of each week of study i.e. 8th ,15th ,22nd , and 29th days at 0, 1,2,3,4,6,8,10 and 12 hour intervals.

*Reagents*

- Enzymatic (GOD / POD ) Merck kit
- Distilled water
- Anhydrous glucose (500 gm) Merck
- Washing solution

***Steps of measuring glucose oxidase method*:**

- Draw 2-3 ml of blood from retro orbital vein in a clean dry test tube.
- After 10 minutes centrifuge it at 3000 RPM for 3 minutes.
- Separate serum and within one hour perform glucose estimation.

| | Test | Standard | Blank |
|---|---|---|---|
| **Working Solution** | 3 ml | 3 ml | 3 ml |
| **Serum** | 0.02ml | - | - |
| **Standard** | - | 0.02ml | - |
| **Distilled water** | - | - | 0.02ml |

Mix and incubate at 37°C for 15 minutes or at room temperature for 30 minutes. Read the absorbance of the test and standard against blank at 505 nm.

***Estimation of serum Insulin*:**

Serum Insulin estimation was done at the time intervals where there is a peak % reduction in blood glucose levels and also at final hour. The estimations were carried out at the end of every week of treatment. For standard, control and disease control groups insulin was estimated at zero, 1st, 6th, and final hour intervals. All the treatment groups shown the peak % reduction in glucose levels at 3rdand 8th levels. So, insulin levels were estimated at zero, 3rd, 8th, and final hours. Insulin estimation was done by ELISA method using steps of the manual given in Mercoida rat insulin assay kit [12-14].

## 3. RESULTS

**Table 1: Effect of selected plant extracts on percentage increase of body weight in rats**

| S.NO | Group | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|
| 1. | Normal control | 0.11± 0.03 | 0.71± 0.94 | 0.28± 0.21 | 0.51± 0.08 |
| 2. | Diabetic control | 15.27± 0.73@ | 18.81± 0.84@ | 26.73± 1.25@ | 32.52± 1.18@ |
| 3. | Diabetic standard | 23.76± 0.14 | 18.38± 0.93x | 10.82± 0.94y | 0.97± 0.08cz |
| 4. | CC (100 mg/Kg) | 21.14± 1.12 | 15.18± 1.19x | 11.28± 1.0 y | 2.74± 0.25by |
| 5. | CC (200 mg/Kg) | 21.02± 0.94 | 13.66± 0.94x | 9.36± 0.94z | 0.69± 0.08cz |
| 6. | CC (400 mg/Kg) | 18.39± 1.23 | 12.18±0.95y | 7.92± 0.87z | 0.34± 0.04cz |

Data were expressed as Mean± SEM (gm) and analyzed by performing two ways ANOVA with significant value at P > 0.05. 1st week values are compared with 2nd, 3rd and 4th weeks in every group. For each week, % of change in body weight of every group is compared with control group, and a non-parametric test (Dunnte's multiple comparison test) was applied for multiple comparisons.

- @ = Normal control group is compared with disease control
- a = *, b = ** and c = ***, within each group change in body weight after 2nd, 3rdand 4th weeks was compared with change in body weight.
- x = #, y = ## and z = ###, whereas change in body weight all groups werecompared with control group after every week of treatment.

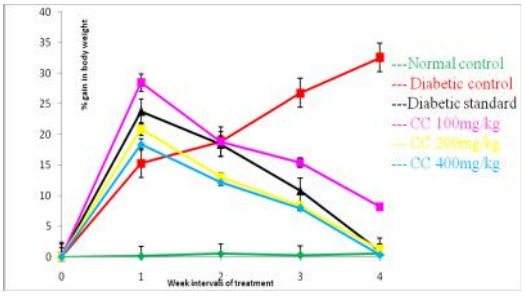


**Fig 2: Effect of selected plant extracts on percentage increase of body weight**

**Table2: Effect of extracts on percentage reduction in food intake**

| S. NO | Group | 0 week | 1st week (Average) | 2nd week (Average) | 3rd week (Average) | 4th week (Average) |
|---|---|---|---|---|---|---|
| 1. | Normal control | 00.00 | 0.64 | 0.63 | 0.13 | 3.40 |
| 2. | Diabetic control | 00.00 | 10.12@ | 8.32@ | 6.38@ | 4.06@ |
| 3. | Diabetic standard | 00.00 | 8.13 | 11.92x | 24.29y | 31.38cz |
| 4. | CC(100 mg/Kg) | 00.00 | 00.00 | 7.45x | 9.09x | 27.87y |
| 5. | CC(200 mg/Kg) | 00.00 | 00.00 | 12.45x | 14.75x | 29.55z |
| 6. | CC (400 mg/Kg) | 00.00 | 00.00 | 15.35y | 16.23y | 33.59z |

Data were expressed as Mean and analyzed by performing two way ANOVA with significant value at P > 0.05. 1st week values are compared with 2nd, 3rd and 4th weeks in every group. For each week, % reduction in food intake of every group is compared with control group, and a non-parametric test (Dunntte's multiple comparison test) was applied for multiple comparisons.

- @ = Normal control group is compared with disease control
- a = *, b = ** and c = ***, within each group change in intake of food and water after 2nd, 3rdand 4th weeks was compared with change intake of food and water after 1st week.
- x = #, y = ## and z = ###, whereas change in intake of food and water of all groups were compared with control group after every week of treatment

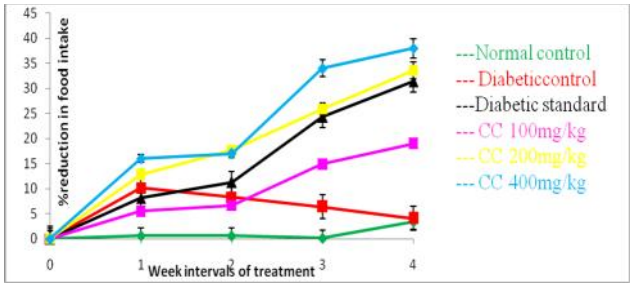


**Fig 3: Effect of selected plant extracts on percentage reduction of food intake**

**Table3: Effect of extracts on percentage reduction in water intake**

| S.No | Group | 0 week | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|---|
| 1. | Normal control | 00.00 | 1.21 | 1.28 | 0.78 | 4.05 |
| 2. | Diabetic control | 00.00 | 8.04@ | 7.97@ | 6.03@ | 5.71@ |
| 3. | Diabetic standard | 00.00 | 11.94x | 12.57x | 24.94y | 32.03cz |
| 4. | CC (100 mg/Kg) | 00.00 | 8.43x | 9.74x | 28.52y | 32.34by |
| 5. | CC (200 mg/Kg) | 00.00 | 13.43x | 15.40x | 30.20z | 33.61cz |
| 6. | CC (400 mg/Kg) | 00.00 | 14.93y | 16.88y | 34.24z | 38.33cz |

Data were expressed as Meanandanalyzed by performing two way ANOVA with significant value at P > 0.05. 1st week values are compared with 2nd, 3rd and 4th weeks in every group. For each week, % reduction in water intake of every group is compared with control group, and a non-parametric test (Dunntte's multiple comparison test) was applied for multiple comparisons.

- @ = Normal control group is compared with disease control
- a = *, b = ** and c = ***, within each group change in intake of food and water after 2nd, 3rdand 4th weeks was compared with change intake of food and water after 1st week.
- x = #, y = ## and z = ###, whereas change in intake of food and water of all groups were compared with control group after every week of treatment..

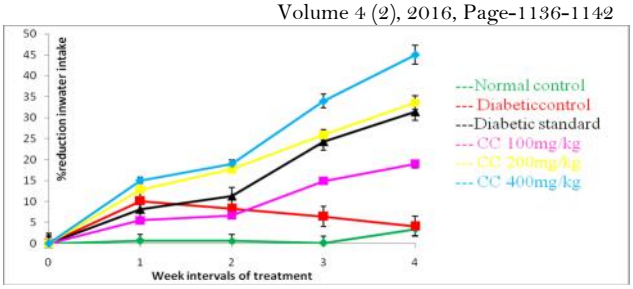


**Fig 4: Effect of selected plant extracts on percentage reduction of water intake**

**Table 4: Effect of selected plant extracts on percentage reduction values of glucose after 1st week**

| Treatment Group | Time intervals 1st Hr | 2nd Hr | 3rd Hr | 4th Hr | 6th Hr | 8th Hr |
|---|---|---|---|---|---|---|
| Normal Control | -1.89± 0.46 | 8.17±0.47 | 6.13± 0.64 | 9.22± 0.59 | 15.76± 0.43 | 3.63± 0.46 |
| Diabetic control | 1.11±0.20 | 2.12±0.52 | 3.50±0.53 | 5.03±0.65 | 9.85±0.88@ | 10.72±1.0@ |
| Diabetic standard | 31.65±0.7 | 19.78±1.26 | 12.13± 1.84 | 6.66± 1.19 | 30.08± 0.47 | 16.13± 0.95 |
| CC(100 mg/Kg) | 6.01± 0.47 | 9.95± 0.82 | 18.54± 0.51 | 10.98±1.27 | 5.25± 0.53 | 17.99± 0.71 |
| CC(200 mg/Kg) | 6.01± 0.86 | 11.10±0.70 | 25.53± 0.59 | 12.00±1.04 | 6.08± 1.10 | 24.61± 0.96 |
| CC(400 mg/Kg) | 6.43± 0.63 | 13.20±1.08 | 30.05± 0.45 | 16.04±1.67 | 8.85± 0.56 | 29.46± 0.79 |

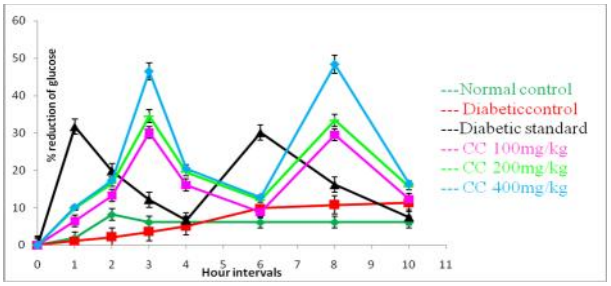


**Fig 5: Effect of selected plant extracts on percentage reduction of glucose levels after 1st week**

**Table 5: Effect of selected plant extracts on percentage reduction values of glucose after 2nd week**

| Treatment Group | Time intervals 1st Hr | 2nd Hr | 3rd Hr | 4th Hr | 6th Hr | 8th Hr | 10th Hr |
|---|---|---|---|---|---|---|---|
| Normal Control | 3.76± 2.09 | 4.26± 0.73 | 4.03± 1.63 | 5.30± 1.79 | 6.13± 2.68 | 7.29± 2.61 | 3.34± 4.48 |
| Diabetic control | 1.02±0.20 | 2.04±0.49 | 2.89±0.44 | 3.69±0.51 | 7.23±0.80 | 7.95±0.71@ | 8.91±0.61 |
| Diabetic standard | 32.81±0.66 | 21.26± 1.53 | 14.98± 1.56 | 10.14±1.52 | 32.06±1.64 | 16.78± 1.11 | 10.49± 1.60 |
| CC (100 mg/Kg) | 9.95± 0.94 | 24.06± 0.57 | 46.04±0.40* | 30.83±0.71 | 20.78±0.85 | 45.34± 0.13* | 24.10± 0.99 |
| CC (200 mg/Kg) | 11.85±0.94 | 26.87± 0.57 | 48.04±0.40* | 33.65±0.71 | 22.65±0.85 | 48.34± 0.13* | 27.09± 0.99 |
| CC (400 mg/Kg) | 12.95±0.94 | 27.31± 0.57 | 52.05±0.40* | 34.92±0.71 | 23.94±0.85 | 53.34± 0.13* | 29.56± 0.99 |

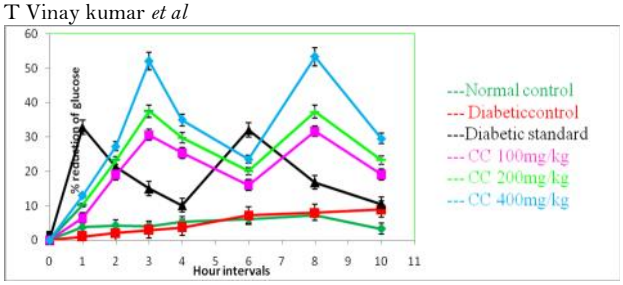


**Fig 6: Effect of selected plant extracts on percentage reduction of glucose levels after 2nd week**

**Table 6: Effect of selected plant extracts on percentage reduction values of glucose after 3rd week**

| Treatment Group | Time intervals | | | | | |
|---|---|---|---|---|---|---|
| | 1st Hr | 2nd Hr | 3rd Hr | 4th Hr | 6th Hr | 8th Hr |
| Normal Control | 1.16± 3.09 | 1.79± 2.19 | -3.02± 2.03 | 2.45± 3.25 | -5.27±3.23 | 2.94± 2.14 |
| Diabetic control | 0.98± 0.17 | 1.93± 0.18 | 2.51± 0.24 | 2.96± 0.28 | 4.33± 0.28 | 5.19±0.28@ |
| Diabetic standard | 44.94± 0.15d | 21.43±0.92 | 12.83± 1.27 | 6.53± 1.03 | 45.34± 0.20 d | 20.71±0.86 |
| CC (100 mg/Kg) | 15.73± 0.47 | 28.55±1.17 | 52.94± 0.31d | 20.06± 0.76 | 22.04±1.15 | 51.94±0.30d |
| CC (200 mg/Kg) | 17.49± 0.47 | 32.45±1.17 | 55.94±0.31d | 24.40±0.76 | 24.05±1.03 | 56.94±0.30d |
| CC (400 mg/Kg) | 18.06± 0.47 | 34.94±1.17 | 61.94±0.31d | 27.12±0.76 | 25.09±1.10 | 60.94±0.30d |

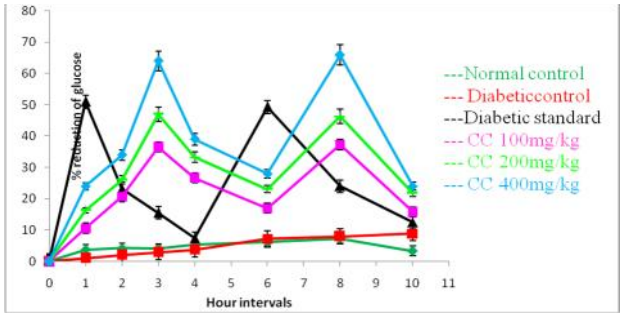


**Fig 7: Effect of selected plant extracts on percentage reduction of glucose levels after 3rd week**

**Table 7: Effect of selected plant extracts on percentage reduction values of glucose after 4th week**

| Treatment Group | Time intervals | | | | | |
|---|---|---|---|---|---|---|
| | 1st Hr | 2nd Hr | 3rd Hr | 4th Hr | 6th Hr | 8th Hr |
| Normal Control | -1.80± 2.55 | -0.51±2.82 | 3.41±3.36 | -2.26± 3.43 | -2.09± 2.63 | -9.17± 3.20 |
| Diabetic control | 0.79 ± 0.16 | 1.59± 0.22 | 2.35± 0.21 | 2.72± 0.23b | 3.52±0.26d | 4.36± 0.32d |
| Diabetic standard | 50.96± 0.98d# | 23.19±0.5 | 15.51± 0.86 | 7.39± 1.10 | 49.29± 0.84d# | 23.99± 0.89 |
| CC(100 mg/Kg) | 17.20± 0.91 | 28.41±0.6 | 57.73±1.31d | 35.06±0.80 | 24.26± 1.28 | 54.94±1.03d |
| CC(200 mg/Kg) | 19.34± 0.91 | 31.03±0.6 | 61.73±1.31d | 36.94±0.80 | 26.52± 1.28 | 60.94±1.03d |
| CC(400 mg/Kg) | 23.43± 0.91 | 33.46± 0.96 | 63.73± 1.31d# | 38.73± 0.80 | 27.93± 1.28 | 65.94± 1.03d# |

Data were expressed as Mean± SEM (mg/dL) and analyzed by performing two way ANOVA with significant value at P > 0.05. 1st week values are compared with 2nd, 3rd and 4th weeks and a non-parametric test (Dunntte's multiple comparison test) was applied for multiple comparisons. a= *, b= **, c=***and d= ****. Values compared in between weeks and hour intervals of each group individually. # = Values compared of high doses of all extracts compared with control group at 4th week of the study.Where, # = x, ## = y and ### = z. @ = % reduction of glucose levels of Normal control group were compared with Disease control.

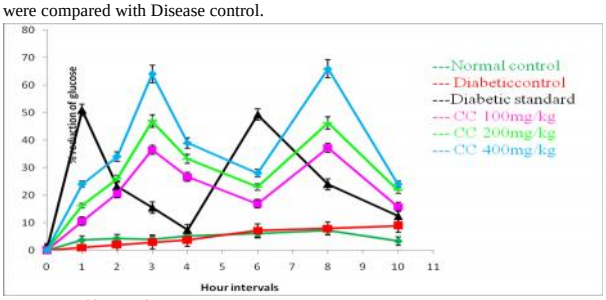


**Fig 8: Effect of selected plant extracts on percentage reduction of glucose levels after 4th week**

**Table 8: Percentage increase values of insulin levels after 1st week**

| S.No. | Treatment groups | Time intervals | | |
|---|---|---|---|---|
| | | First peak | Second peak | Final hour |
| 1. | Normal Control | 0.15± 0.11 | 0± 0.00 | 0.60± 0.11 |
| 2. | Diabetic control | 7.28± 0.04 | 10.53± 0.09@ | 2.19± 0.15 |
| 3. | Diabetic standard | 37.14± 0.54 | 57.63± 0.77 | 1.70± 0.14 |
| 4. | CC (100 mg/Kg) | 41.95± 0.53 | 60.74± 0.21x | 15.43± 0.14 |
| 5. | CC (200 mg/Kg) | 44.41± 0.35 | 63.93± 0.24x | 18.95± 0.23 |
| 6. | CC (400 mg/Kg) | 48.83± 0.37 | 66.02± 0.73y | 20.83± 0.32 |

**Table 9: Percentage increase values of insulin levels after 2nd week**

| S.No. | Treatment groups | Time intervals | | |
|---|---|---|---|---|
| | | First peak | Second peak | Final hour |
| 1. | Normal Control | 0.13± 0.02 | 0.24± 0.03 | 0.51± 0.04 |
| 2. | Diabetic control | 5.21± 0.11@ | 4.32± 0.10 | 2.54± 0.17 |
| 3. | Diabetic standard | 41.84± 0.06 | 61.42± 0.31a | 2.84± 0.12 |
| 4. | CC (100 mg/Kg) | 45.26± 0.41 | 66.24± 0.32ay | 17.92± 0.78 |
| 5. | CC (200 mg/Kg) | 48.84± 0.09 | 67.46± 0.31ay | 19.54± 0.54 |
| 6. | CC (400 mg/Kg) | 51.52± 0.18 | 71.42± 0.21by | 22.40± 1.03 |

**Table 10: Percentage increase values of insulin levels after 3rd week**

| S.No. | Treatment groups | Time intervals | | |
|---|---|---|---|---|
| | | First peak | Second peak | Final hour |
| 1. | Normal Control | 0.34± 0.2 | 0.45± 0.94 | 0.60± 0.30 |
| 2. | Diabetic control | 5.02± 0.05 | 7.53± 0.04@ | 1.19± 0.43 |
| 3. | Diabetic standard | 41.84± 0.06 | 61.42± 0.31 | 3.57± 0.10 |
| 4. | CC (100 mg/Kg) | 50.36± 0.14 | 70.40± 0.58by | 18.63± 0.74 |
| 5. | CC (200 mg/Kg) | 53.94± 0.84 | 71.62± 0.48by | 20.23± 0.72 |
| 6. | CC (400 mg/Kg) | 56.62± 0.93 | 75.58± 0.14cz | 23.13± 0.72 |

**Table 11: Percentage increase values of insulin levels after 4th week**

| S.No. | Treatment groups | Time intervals | | |
|---|---|---|---|---|
| | | First peak | Second peak | Final hour |
| 1. | Normal Control | 0.14± 0.04 | 0.64± 0.08 | 0.81± 0.15 |
| 2. | Diabetic control | 4.24± 0.14@ | 2.35± 0.14 | 1.53± 0.23 |
| 3. | Diabetic standard | 50.99± 0.73 | 68.70± 0.81a | 3.86± 0.03 |

| | | | |
|---|---|---|---|
| 4. CC (100 mg/Kg) | 54.41± 0.94 | 73.52± 0.94$^{bz}$ | 18.92± 0.06 |
| 5. CC (200 mg/Kg) | 57.99± 0.85 | 74.74± 0.92$^{bz}$ | 20.52± 0.14 |
| 6. CC (400 mg/Kg) | 60.67± 0.79 | 78.70± 0.93$^{cz}$ | 23.42± 0.18 |

Data were expressed as Mean± SEM (% of insulin increase) and analyzed by performing two way ANOVA with significant value at P > 0.05. 1st week values are compared with 2nd, 3rd and 4th weeks and a non-parametric test (Dunntte's multiple comparison test) was applied for multiple comparisons. Where,

- a= *, b= **, c=***and d= ****. Values compared in between weeks of each group individually.
- # = Values compared of high doses of all extracts compared with control group at end of every week of the study. Where, # = x, ## = y and ### = z.
- @ = % of increase of insulin levels of Normal control group were compared with Disease control.
- For diabetic standard group first peak, second peak were observed at 1st and 6th hours respectively.
- All treatment groups shown peaks at 3rd and 8th hours respectively.

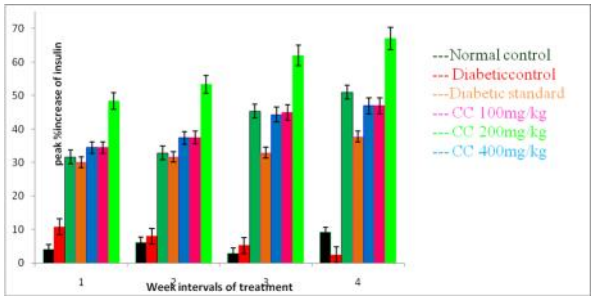


**Fig 9: Effect of selected plant extracts on peak percentage increase of insulin levels**

## 4. DISCUSSION

In STZ induced diabetic rats body weight was found to be decreased and food and water intake were found to be increased. Treatment with the selected plant extract significantly increased the body weight and decreased food and water intake in a dose dependent manner. The increase in food and water intake in diabetic rats might be due to excessive thirst and hunger (Polyphagia and Polydypsia). [15-16] Excessive food and water intake in diabetes might be due to glucose sensors that are present on hypothalamus are directly linked to the nerves which regulates energy equilibrium. The glucose sensors on hypothalamus are connected to nerves of the portal vein and carotid body, which can increase eating and drinking habits in diabetes Weight loss in diabetic rats might be due to increase in muscle wasting as described by, catabolism of fats and proteins, due to insulin deficiency and decreased protein content in muscular tissue by proteolysis The decrease in food intake, water intake and increase in body weight with the treatment of selected plant extract.

## 5. CONCLUSION

In this study the aqueous extract of the herb namely fruit pulp of *Citrullus colocynthis (CC)*, is evaluated for their antihyperglycemic activity and further for their role in the inhibition of development of diabetic complications. The literature review revealed that the active constituents (polyphenols) were more in their respective selected parts. Hence in the present study, respective parts of selected plant is evaluated for their beneficial role in diabetes and diabetes induced complications.

The doses of the selected plant extract is fixed by performing acute toxicity studies according to OECD 425 guidelines and same doses were fixed as low dose (100mg/Kg), mid dose (200mg/Kg) and high dose (400mg/Kg) for the selected plant extract and also evaluated for their antihyperglycemic activities and for their role in preventing diabetic complications in STZ induced diabetic rats.

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