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# THE EXPERIMENTAL STUDY OF INDRAVARUNI MOOL (CITRULLUS COLOCYNTHIS SCHRAD.) ON HYPERGLYCEMIA

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**ABSRACT**
The aim of this study was to examine the effect of root of *C. colocynthis* on blood glucose levels of normal and alloxan-induced diabetic rats. Diabetes mellitus was induced by intraperitoneal (150 mg/kg b.w.) injection of alloxan monohydrate for three days into all animals group and the animals showing blood glucose level in the range of 180 -280 mg/dL were selected for study. The blood glucose concentrations of the animals were measured before inducing hyperglycemia, after inducing and at end of 30 days drug trial. Aqueous extract of roots of *Citrullus colocynthis* showed significant reduction in blood sugar level when given in double dose of standard dose when compared with normal saline treated group(232 mg/dl) and standard dose of aq extract of citrullus root treated group (158 mg/dl). The Plant root and metformin treated rats significantly reduce the elevated levels of glucose. So these results indicate that Root of Citrullus colocynthis showed Hypoglycemic effect.

**KEYWORDS:** *C. colocynthis*, Hypoglycemic effect.

## INTRODUCTION

Diabetes mellitus is an endocrine disorder characterized by persistent hyperglycemia that result from defects in insulin secretion, or its action, or both1. The global increase in the prevalence of diabetes is due to population growth, aging, urbanization and an increase of obesity and physical inactivity The use of plants for medicinal purposes is as old as our civilization. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones**.** The World Health Organization has also recommended the evaluation of the effectiveness of plants in conditions where safe modern drugs are beyond the reach of general people.[1] The plant *Citrullus colocynthi*s claims to possess hypoglycemic property as reviewed from various literatures.[2,3] The plant subjected for the current research work had been used traditionally as antidiabetic, the fruit is scientifically proved as antidiabetic; therefore, it was thought interesting to evaluate the antidiabetic profile of the selected plant part (root), which has not yet been scientifically undertaken.

## MATERIAL AND METHODS

The present study was carried out in the Bilwal Medchem and Research Laboratory Pvt. Ltd. Jaipur. **A**ll experiments were performed according to institution animal ethical committee(IACE) (Approval No. BMRL/AD/CPCSEA/IAEC/2018/3/iv).

### A. Efficacy study: Alloxan induced diabetic in wister albino rats

✓ **Test drug dose Calculation**

Dose fixation of test drug for rats will be calculated on the base of body surface area ratio by referring to table of **Paget & Barnes (0.018 x Adult Dose x 5).**

✓ **Housing and feeding conditions**

The temperature in the experimental animal room had been 22ºC (+ 3ºC). Although the relative humidity had been at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

✓ **Marking of albino wistar rat for identification**

The albino rat was marked with Picric acid in each group as H, B, T, HB, BT, and HT where:-

- H stand for head of albino rat
- B stand for Back of albino rat,
- T stand for Tail of albino rat
- HB stand for head back of albino rat
- BT stand for Back Tail of albino rat
- HT stand for head tail of albino rat.

✓ **Procedure**

The animals were fasted overnight and diabetes was induced by a single intra-peritoneal injection of a freshly prepared solution of Alloxan (150mg/kg body weight) in 0.9% NaCl saline solution into all the animals in group 1, 2, 3 and 4.

✓ **Group Design**

**Group-1:** Six Hyperglycemia induced wistar rats had received normal saline 5ml/kg/day/P.O. for 30 days.
**Group-2:** Six Hyperglycemia induced wistar rats had received 10% aqueous solution of Indravaruni in 270 Dose mg/ kg/day/P.O. for 30 days.
**Group-3:** Six Hyperglycemia induced wistar rats had received 10% aqueous solution of Indravaruni in 540 Dose mg/kg/day/P.O. for 30 days.
**Group-4:** Six Hyperglycemia induced wistar rats had received 5% aqueous solution of Metformin150 mg/kg/day/P.O. for 30 days.

✓ **Evaluation**

- Blood glucose levels.
- Histopathology study of Pancrease.

✓ **Blood Collection**

Blood glucose levels were assessed by Glucometer in all the animal groups by using blood from Retro-orbital sinus of the rats.

✓ **Sample collection for Histopathological analysis**

At the end of the stipulated 30 days feeds were withdrawn, the rats were subjected to a 12 hours fast but had access to water. Sacrificed using $Co_2$ chamber. The harvested Pancrease was carefully dissected out, trimmed of all fat and connective tissue blotted dry to remove any blood. The tissues were fixed in 10% formal saline, and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at onehour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicularly to the long axis of the pancreas. The sections were designated "vertical sections". Serial sections of 5 µm thick were obtained from a solid block of tissue, fixed on clean albuminized slides to prevent sections coming off the slides and later stained with Haematoxylin and Eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mountant, allowed to dry at room temperature and observed Histopathologically under digital light microscope.

✓ **Statistical analysis**

All the values were expressed as mean ± standard error of the mean (S.E.M) of six animals each across the groups. Statistical analysis of data was carried out using one-way analysis of variance (ANOVA) with help of Graph pad Prism software. Dunnett's multiple comparisons test. P value < 0.05 was considered to be statistically significant.

## OBSERVATIONS AND RESULTS

✓ **Efficacy Study**

- **Observation and Results of Blood Glucose levels in Alloxan induced Diabetic Wister Albino Rats before and after 30 days of Drug administration**

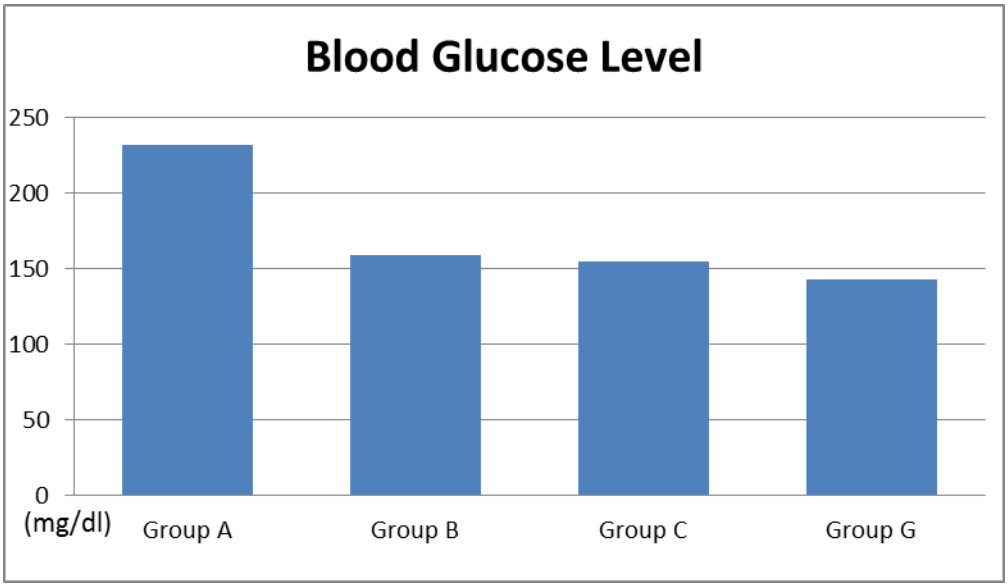
| Group A | | | |
|---|---|---|---|
| **Marking on rats** | **Before inducing Hyperglycemia, blood glucose (mg/dl)** | **After inducing Hyperglycemia, blood glucose(mg/dl)** | **End of Trial blood glucose (mg/dl)** |
| **H** | 109 | 194 | 210 |
| **B** | 98 | 189 | 198 |
| **T** | 110 | 204 | 215 |
| **HB** | 113 | 224 | 241 |
| **BT** | 104 | 201 | 249 |
| **HT** | 124 | 249 | 279 |
| **Mean** | **109.67** | **210.17** | **232.00** |
| **SD** | **8.78** | **22.50** | **30.03** |
| **SEM** | **3.58** | **9.18** | **12.26** |

| Group B | | | |
|---|---|---|---|
| **Marking on rats** | **Before inducing Hyperglycemia, blood glucose (mg/dl)** | **After inducing Hyperglycemia, blood glucose(mg/dl)** | **End of Trial blood glucose (mg/dl)** |
| **H** | 104 | 189 | 162 |
| **B** | 106 | 251 | 296 |
| **T** | 110 | 241 | 126 |
| **HB** | 92 | 214 | 120 |
| **BT** | 98 | 189 | 124 |
| **HT** | 109 | 241 | 123 |
| **Mean** | **103.1666667** | **220.8333333** | **158.5** |
| **SD** | **6.940220938** | **27.5566084** | **69.1483912** |
| **SEM** | **2.833333333** | **11.24993827** | **28.2297125** |

| Group C | | | |
|---|---|---|---|
| **Marking on rats** | **Before inducing Hyperglycemia, blood glucose (mg/dl)** | **After inducing Hyperglycemia, blood glucose(mg/dl)** | **End of Trial blood glucose (mg/dl)** |
| **H** | 96 | 219 | 165 |
| **B** | 97 | 241 | 177 |
| **T** | 93 | 198 | 143 |
| **HB** | 110 | 237 | 173 |
| **BT** | 104 | 185 | 146 |
| **HT** | 107 | 231 | 125 |
| **Mean** | **101.1666667** | **218.5** | **154.833333** |
| **SD** | **6.794605704** | **22.57210668** | **20.1634984** |
| **SEM** | **2.773886163** | **9.215023965** | **8.23171374** |

| Group D | | | |
|---|---|---|---|
| **Marking on rats** | **Before inducing Hyperglycemia, blood glucose (mg/dl)** | **After inducing Hyperglycemia, blood glucose(mg/dl)** | **End of Trial blood glucose (mg/dl)** |
| **H** | 105 | 214 | 148 |
| **B** | 110 | 243 | 179 |
| **T** | 99 | 243 | 127 |
| **HB** | 106 | 186 | 149 |
| **BT** | 95 | 205 | 130 |
| **HT** | 96 | 211 | 125 |
| **Mean** | **101.8333333** | **217** | **143** |
| **SD** | **6.047037842** | **22.36962226** | **20.5231577** |
| **SEM** | **2.468692862** | **9.132360045** | **8.37854403** |

| **Blood Glucose** | **Group A MEAN ± SEM** | **Group B MEAN ± SEM** | **Group C MEAN ± SEM** | **Group G MEAN ± SEM** |
|---|---|---|---|---|
| | 232 ±12.258 | 158.5 ± 28.230 | 154.83 ± 8.232 | 143 ± 8.379 |




- **Statistically analysis of Blood Glucose (One Way Anova)**

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant? | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| **Group A vs. Group B** | 73.5 | 14.32 to 132.7 | Yes | * | 0.0134 |
| **Group A vs. Group C** | 77.17 | 17.99 to 136.3 | Yes | ** | 0.0094 |
| **Group A vs. Group D** | 89 | 29.82 to 148.2 | Yes | ** | 0.0030 |

Statistically significant results were observed in Group C when rats administerd with dose 540 mg/kg/day/p.o. Though the results are less significant than Group D when standard drug were administerd.

✓ **Histopathology Study**

- In Group A Diabetic Rat's Pancreas showing Degenerative and Necrotic changes and reduced dimension of Islets of Langerhans.
- In Group B Diabetic Rats treated with Indravaruni in 270 mg/kg showing slightly improvement of Islets of Langerhans.
- In Group C Diabetic Rats treated with Indravaruni in 540 mg/kg showing marked improvement of Islets of Langerhans. In Group D Diabetic Rats treated with metformin showing marked improvement of Islet of Langerhans.

## DISCUSSION

- **Efficacy Study**
- **Alloxan induced diabetic in wister albino rats**

Alloxan is a well-known Diabetogenic agent that is used to induce Diabetes in experimental animals. The underlying mechanism starts with the selective uptake of the compound due to its structural similarity to glucose as well as highly efficient uptake mechanism by Glut -2 transporter in the pancreatic beta-cells. In this regard, Alloxan, an analogue of urea selectively destroys the beta-cells. This toxic action involves oxidation of essential sulphydryl (-SH groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis. After administration of single Intraperitoneal injection of Alloxan 150 mg/kg Diabetes was induced and normal saline, test sample and standard drug (Metformin) was administered orally for 30 days.

- **In Group A**- when 5ml normal saline given to diabetic rats for 30 days trial , before treatment blood glucose mean was 210 (mg/dl), and after 30 days blood glucose mean was 232 (mg/dl).There is no decrese in blood glucose levels instead increase in glucose level were shown.
- **In Group B**- when aqueus extract of root of indravaruni at dose 270mg/kg was given to diabetic rats for 30 days, before test drug given, Blood glucose level mean was 220(mg/dl) and after 30 days treatment mean was 158(mg/dl). There is decrese in blood glucose levels.
- **In Group C**- when aqueus extract of root of indravaruni at dose 540mg/kg was given to diabetic rats for 30 days, before test drug given, Blood glucose level mean was 218(mg/dl) and after 30 days treatment mean was 154(mg/dl). There is decrese in blood glucose levels.
- **In Group D**- when standard drug metformin at dose 150mg/kg was given to diabetic rats for 30 days, before standard drug given, Blood glucose level.

mean was 217(mg/dl) and after 30 days treatment mean was 143(mg/dl). There is decrese in blood glucose levels.

- **Intergroup comparision of parameters was done using Anova test**

✓ When Group A(negative control) compared to Group B, Study revealed that mean value of glucose in Group A was 232 mg/dl and mean value of Group B was 158 mg/dl. It showed that Group B is more significant than Group A.

- ✓ When Group A(negative control) compared to Group C, Study revealed that mean value of glucose in Group A was 232 mg/dl and mean value of Group C was 154 mg/dl. It showed that Group C is more significant than Group A.
- ✓ When Group A(negative control) compared to Group D, Study revealed that mean value of glucose in Group A was 232 mg/dl and mean value of Group D was 143 mg/dl. It showed that Group D is more significant than Group A.
- ✓ Both Grp B & Grp C drugs show effect on hyperglycemia and both decrease blood glucose level but Grp D have shown more affinity than Grp C to decrease blood glucose levels.
- ✓ After 30 day Blood glucose levels, Histopathology study of pancreas was observed and found Blood glucose levels at both dose 270 mg/kg and 540 mg/kg found statically significant in comparison of normal saline (p value: 0.0134, 0.0094) but group C (extract Root of Citrullus colocynthis at dose 540 mg/kg) had found more potent than lower dose.
- ✓ Histopathology study of pancreas shows that degeneration of Islet of Langerhans was rejuvenated after administration of test sample at both dose. But histological changes in group C (at dose 540 mg/kg) had found more significant response.
- ✓ After observation test sample found dose dependent therapeutic response higher dose show more therapeutic response than lower dose.

## CONCLUSION

When Group D, Standard Drug (Metformin) at dose of 150 mg/kg compare with Group C, at test sample dose of 540 mg/kg found more statistically significant.

- We can say that, test drug is less significant than standard drug and test drug is lies between negative control and positive control.
- So we can say that Diabetes is not self limiting disease as we seen that in Group A, no therapeutically changes found after oral administration of normal saline. Although found increase in blood glucose levels.
- So Diabetic patient if untreated than blood glucose levels will increase regularly.
- Histopathology study of pancreas shows that degeneration of Islet of Langerhans was rejuvenated after administration of test sample at both dose. But histological changes in group C (at dose 540 mg/kg) had found more significant response.
- ➢ After all observations, Test sample found dose dependent therapeutic response. Higher dose show more therapeutic response than Lower dose.

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