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***Research Article***

# STUDY ON THE EFFECT OF SELENIUM AS SUPPLEMENT TO GLIBENCLAMIDE ON ALLOXAN INDUCED DIABETIC RAT

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## ABSTRACT
The study was conducted on four groups of albino rats weighing 200-250grams consisting of six animals each. To know the potentiation of the hypoglycemic effect of glibenclamide (a sulfonylurea) with selenium in Alloxan induced diabetic rats. One group acted as the normal control without any diabetes. The second group of animals with diabetes acted as diabetic control. Drugs (Glibenclamide, Selenium) were administered to the remaining groups of diabetic animals as per protocol. Effect of these drugs on the blood glucose levels, and their potentiating effect on the blood glucose levels when they are used in combination were studied.

***Keywords:*** *Alloxan Induced Diabetes, Rats, Selenium, Glibinclamide*

## INTRODUCTION
Diabetes mellitus is a group of disorders with different etiologies. It is characterized by derangements in carbohydrate, protein and fat metabolism, caused by the complete or relative insufficiency of insulin secretion, insulin action or both. Since rapid urbanization and industrialization has resulted in economic prosperity and better living standards to many, it has also resulted in considerable increase in lifestyle related diseases like diabetes (Ramachandran, 2005). The aim of therapy for diabetes mellitus is to manage complications of diabetes like retinopathy, cardiovascular disease, nephropathy, neuropathy and other complications (Ozeren *et al.,* 2003). Along with these mainline drugs, several add – ons like multivitamins, trace minerals and native herbal medicines are used in the therapy. Selenium is an essential trace element in human and animal nutrition. It is involved in the defense against the toxicity of reactive oxygen species (oxidative stress), in the regulation of thyroid hormone metabolism and regulation of the redox state of cells (Alaejos *et al.,* 2000). It has an important role to play in the carbohydrate and lipid metabolism in the body. Several studies have suggested that it potentiates the action of insulin and some studies have shown that it mimics the insulin like actions (Stapleton, 2000). An attempt has been made by this study to find out any potentiating and synergestic effect on the control of blood glucose concentration, when these three drugs i.e sulfonylurea, selenium are used in combination, than when they are used alone on alloxan induced diabetic rats. The main objectives of the study are to study the potentiation of the hypoglycemic effect of glibenclamide (a sulfonylurea) with selenium in Alloxan induced diabetic rats and to compare the potentiation of the hypoglycemic effects of above mentioned drugs with that of the standard diabetic control group.

## MATERIALS AND METHODS
### *Methodology*
The study was carried out at the Department of Pharmacology M.R. Medical College, Gulbarga on adult albino rats from central animal house of M.R. Medical College after obtaining institution ethics committee approval to undertake this study. The study was conducted according to NISA (The Indian National Science Academy) guidelines. Adult albino rats weighing 200-250grams obtained from the central animal house of M.R. Medical College were divided into 5 groups as per the protocol Alloxan monohydrate obtained from Loba – Chemie Indoaustralian Co., Mumbai, India. Glibenclamide obtained

from Dr. Reddy's Laboratories, Hyderabad.Selenium obtained from Omkar Chemicals, Badlapur 421503, Maharastra

***Induction of Diabetes Mellitus (Dhawan et al., 1996):*** A single dose (150mg/kg i.p) of freshly prepared solution of alloxan monohydrate 5% (dissolved in 5% Dextrose, pH 4.5) was administered for induction of type 2 diabetes mellitus in the rats. The animals under study were maintained at a temperature of 25 ± $1^0$C in a well ventilated animal house under natural photoperiod conditions. They were provided with standard diet and water ad libitum**.** Blood sample for glucose estimation was collected from the tip of rat's tail (Jelodar *et al.,* 2005)**.** In a well restrained rat, the tail was embedded in $45^0$C water bath and about 1mm of its end was cut and a drop of blood was collected directly on the strip placed in the glucometer (one touch). Blood glucose is estimated by using a Glucometer**.** The test drugs Selenium, Glibenclamide and Normal saline were administered orally to the diabetic and non – diabetic rats by using a polythene tubing sleeved on 18-20 gauge blunted hypodermic needle (or Eustacean Catheter), according to the group to which they belonged.

***Grouping of Animals:*** Diabetic rats with blood glucose levels in the range 250-300 mg / dl were selected for the study. They were divided in to four groups of six animals each. And one group (Group I) containing six normal animals.

Group – I (6 Rats): Normal control: were given saline
Group – II (6 Rats): Diabetic control: were given saline
Group -III (6 Rats): Diabetic: were given Glibenclamide
Dose – 5mg /kg/day, oral route
Group - IV (6 Rats): Diabetic: were given Glibenclamide and Selenium
Dose – Glibenclamide 5 mg / kg /day, oral route Selenium - 0.54 µg/100gm/day, oral route

The study was carried out over a period of 21 days with daily oral administration of drugs. The blood glucose concentrations were monitored on the first day and at the end of the study. Groups III, IV and V were compared to Group II. Handling and care of animals was according to CPSEA guidelines. Care during the animal study using diabetic animal models included food, water, shelter, prevention of infection etc.

***Statistical Methods:*** Statistical evaluation was done using student 't' test, ANOVA test ( F test) value of less than 5% ($p < 0.05$) was considered statistically significant.

## RESULTS AND DISCUSSION

### *Results*

The study was conducted on four groups of albino rats consisting of six animals each. One group acted as the normal control without any diabetes. The second group of animals with diabetes acted as diabetic control. Drugs (Glibenclamide, Selenium) were administered to the remaining groups of diabetic animals as per protocol.

Effect of these drugs on the blood glucose levels, and their potentiating effect on the blood glucose levels when they are used in combination were studied**.**

Animals treated with Glibenclamide alone (Group – III) led to a decrease in blood glucose levels which was statistically highly significant ($p < 0.001$) and A combination of Glibenclamide & Selenium (Group IV) led to a decrease in blood glucose levels which was statistically highly significant ( $p < 0.001$) as compared with animals who have not any treatment received.

***Treatment with Glibenclamide:*** Glibenclamide given to the rats of Group-III at a dose of 5 mg/kg/day caused a decrease in the levels of blood glucose in the animals, which is statistically highly significant ($p < 0.001$).

Administration of Glibenclamide alone (Group – III) led to a decrease in blood glucose levels which was statistically highly significant ($p < 0.001$).

**Table1: Effect of Glibenclamide on alloxan monohydrate induced diabetes rats**
**Group – III, Diabetic Rats (Given Glibenclamide)**

| | Fasting Blood Glucose (mg/dl) | |
|---|---|---|
| **Rats** | **Initial Reading** | **Final Reading (After 21 days)** |
| 1 | 263 | 177 |
| 2 | 278 | 158 |
| 3 | 303 | 173 |
| 4 | 298 | 181 |
| 5 | 272 | 163 |
| 6 | 270 | 154 |

*(n = 6) t = 18:59* *p < 0.001*
*Comment: The difference between the initial readings and the final readings in the group III is statistically highly significant.*

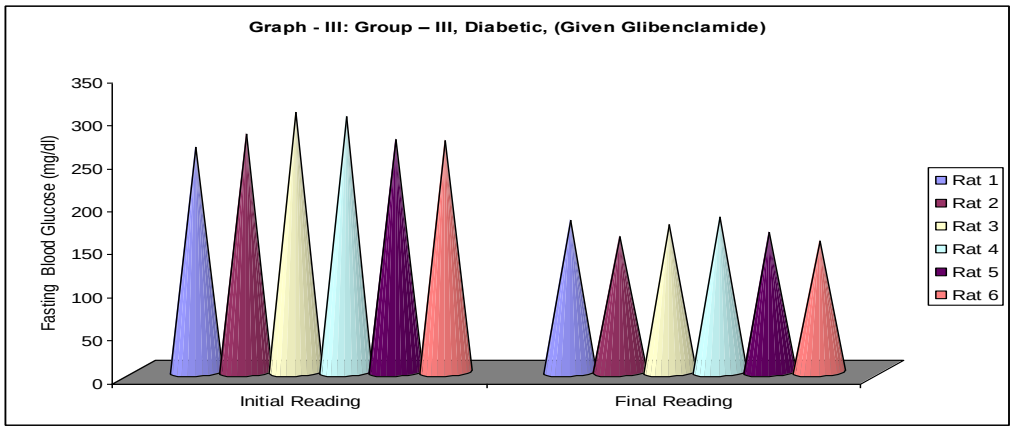



**Table 2: Effect of Glibenclamide and selenium on alloxan monohydrate induced diabetes rats**
**Group – IV, Diabetic Rats (Given Glibenclamide and Selenium)**

| | Fasting Blood Glucose (mg/dl) | |
|---|---|---|
| **Rats** | **Initial Reading** | **Final Reading (After 21 days)** |
| 1 | 302 | 168 |
| 2 | 266 | 148 |
| 3 | 280 | 156 |
| 4 | 288 | 160 |
| 5 | 273 | 152 |
| 6 | 299 | 166 |

*(n = 6) t = 47.83* *p < 0.001*
*Comment: The difference between the initial readings and the final readings in the group IV is statistically highly significant.*

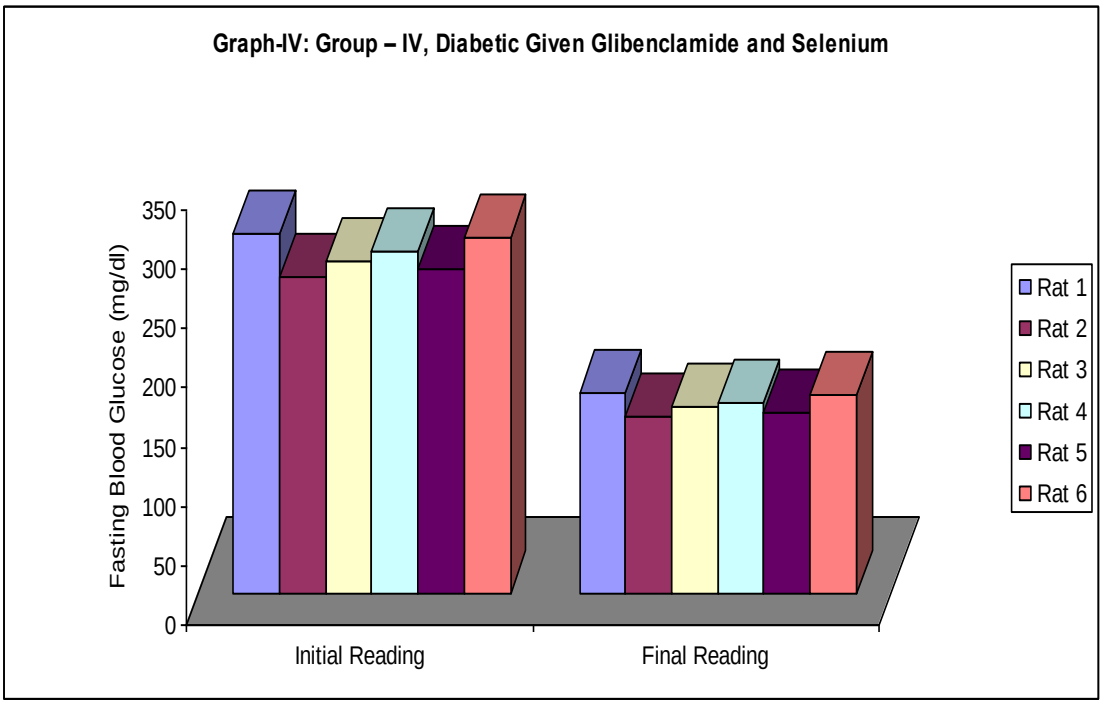
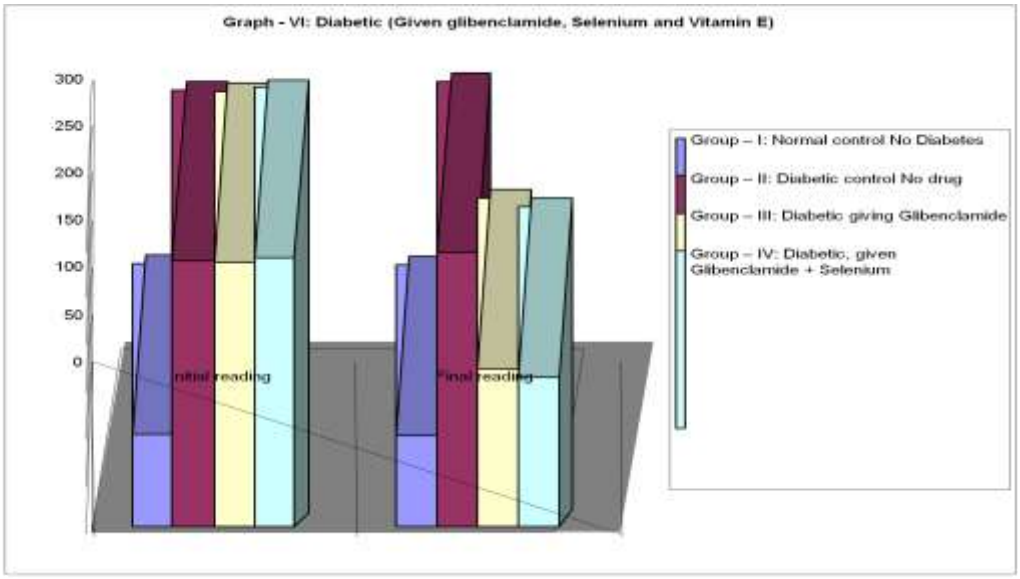



**Table 3: Comparison of the effect of Glibenclamide, Glibenclamide and Selenium on the blood glucose levels of alloxan induced diabetic rats**

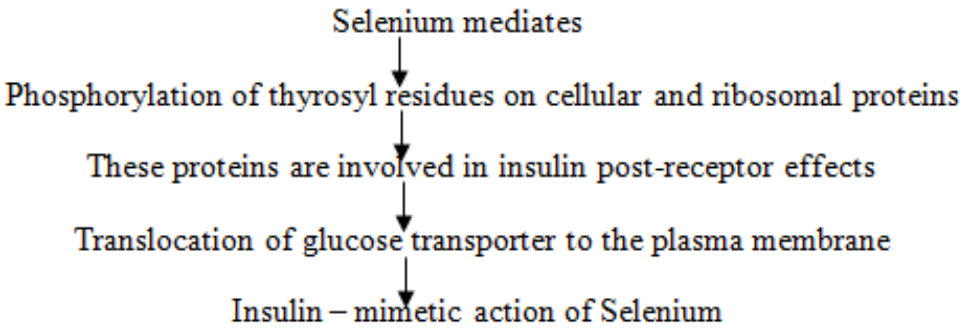
| | Fasting Blood Glucose (mg/dl) | |
|---|---|---|
| **Group** | **Initial reading (X ± SD)** | **Final reading (X ± SD)** |
| Group – I: Normal control, No Diabetes | 98.17 ± 2.48 | 97.17 ± 1.17 |
| Group – II: Diabetic control, No drug | 282.67 ± 16.48 | 290.67 ± 10.69 |
| Group – III: Diabetic, given Glibenclamide | 280.67 ± 16.17 | 167.67 ± 10.91 |
| Group – IV: Diabetic, given Glibenclamide + Selenium | 284.67 ± 14.31 | 158.33 ± 7.84 |
| F – Ratio | 0.06 | 320.93 |
| P Value | $p > 0.05$ | $p < 0.05$ |

***Final Reading***
Group II Vs Group III $t = 19.93$, $p < 0.0001$ - Highly significant
Group II Vs Group IV $t = 24.45$, $p < 0.001$ – Highly significant
Group II Vs Group V $t = 28.21$, $p < 0.001$ – Highly significant
Group III Vs Group IV $t = 1.73$, $p < 0.02$ - Significant
Administration of Glibenclamide alone (Group – III) led to a decrease in blood glucose levels which was statistically highly significant ($p < 0.001$). A combination of Glibenclamide & Selenium (Group IV) led to a decrease in blood glucose levels which was statistically highly significant ($p < 0.001$).




***Discussion***

This present study aimed to study a potentiation of hypoglycemic effect when these drugs (Glibenclamide, Selenium) are used in combination as compared with glibenclamide alone in Alloxan induced diabetic rats. Glibenclamide has got a significant influence on all these pathogenic mechanisms which characterize type 2 diabetes, namely I - Decrease in the number of insulin receptors. II- Post-receptor defects III – Diminished binding of insulin to the receptors IV – A quantitative insulin deficiency with increased hepatic glucose output. Glibenclamide inhibits hepatic glucose production and promotes utilization of glucose in the peripheral tissues, muscles and adipose tissue. Muller and Pallauf (2006)[7] had found out in their study that the dose of oral diabetic medications and insulin may need to be reduced when administered along with Selenium. In addition, an inorganic form of Selenium, Selenate mimics insulin like activity in experimental models. Basically insulin mimetic property is due to Hyperglycaemia, oxidative stress and selenium (Fridlyand and Philipson, 2006; Houstis *et al.*, 2006). Oxidative stress is caused by a relative overload of oxidants. Oxidative stress reduces insulin secretion and increases insulin resistance and may thus play a role in the pathogenesis of diabetes. Hypoglycemic effect of selenium is also due to the acceleration of glucose metabolism and inhibition of glucose synthesis in liver.




***Conclusion***

Selenium is an essential trace element involved in the complex system of defense against oxidative stress through selenium dependent glutathione peroxidase and other selenoproteins. The data obtained from this study clearly demonstrates a potentiation of hypoglycemic effect when these drugs (Glibenclamide, Selenium) are used in combination as compared with glibenclamide alone in Alloxan induced diabetic rats.

***Conflict of Interest:*** None declared.

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