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An experimental study to evaluate the potentiating effect of Selenium and Vitamin-E as supplement to Sulfonylureas on Alloxan induced diabetic rats

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KEYWORDS

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Hypoglycemic activity

A B S T R A C T

Selenium has a significant role in the defense against oxidative stress and is essential for carbohydrate and lipid metabolism. Vitamin E found to have hypoglycemic property when used in type 2 diabetes. Suflonylureas are very commonly used oral hypoglycemic agents. Effect of these drugs and their potentiating effect on the blood glucose levels when used in combination was compared and studied. 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 was used to induce diabetes in albino rats. Diabetic rats which were divided into four groups of six animals each and one group containing six normal rats were control. This study was carried out over a period of 21 days with daily oral administration of drugs and at the end of the study blood glucose levels were tested using glucometer. A comparison between group IV and group V showed that the decrease in blood glucose levels in group V was equal to that of group IV.

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 (Ramaiya, 2005). The aim of therapy for diabetes mellitus is to manage complications of diabetes like retinopathy, cardiovascular disease, nephropathy, neuropathy and other complications (Gonec *et al.*, 2005; Erdmann, 2005). 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 (Ozeren *et al.,* 2003; 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). Also some epidemiological studies have shown an inverse relationship between coronary heart disease and selenium intake by possible anti-atherogenic activity due to its antioxidant activity by protecting LDL from oxidation leading to decrease of body fat and triglyceride levels (Kardinaal *et al.,* 1997). Vitamin E a fat soluble vitamin, is useful in diabetes because of its antioxidant and hypoglycemic properties, which are well documented. Some studies suggest that, vitamin E reduces the risk of cardiovascular diseases and microvascular complications in people with diabetes (Lonn *et al.,* 2002).

A combined administration of vitamin E with selenium will act synergistically, moreover selenium and vitamin E supplementation will modulate fatty acid composition in heart, kidney and aorta. Both selenium and vitamin E have been associated with insulin like properties.

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 and vitamin E 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 with selenium and vitamin E 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

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. Vitamin E obtained from Microlabs, Queens Road, Bangalore .

Induction of diabetes mellitus

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°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 (Mueller and Pallauf, 2006; Jelodar *et al.,* 2005). In a well restrained rat, the tail

was embedded in 45^0C 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, Vitamin E, 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
Groups - V (6 Rats) : Diabetic: were given Glibenclamide, Selenium and Vitamin E
Dose – Glibenclamide 5 mg / kg /day, oral route
Selenium - 0.54 µg/100gm/day, oral route

Vitamin E -135µg/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

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 (Table 1). The second group of animals with diabetes acted as diabetic control (Table 2). 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 when they are used in combination were studied. Animals treated with Glibenclamide alone (Group – III) (Table 3) and combination of Glibenclamide & Selenium (Group IV) (Table 4) led to a decrease in blood glucose levels which was statistically highly significant ($p < 0.001$) and a combination of Glibenclamide, Selenium & Vitamin E (Group V) (Table 5) 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.

This present study was aimed to study a potentiation of hypoglycemic effect when Glibenclamide, Selenium and Vitamin E are used in combination as compared with Glibenclamide alone in Alloxan induced diabetic rats.

Table.1 This table represents the blood glucose levels in normal control rats without diabetes Group – I, Normal Control – No Diabetes

Fasting Blood Glucose (mg/dl)		
Rats	**Initial Reading**	**Final Reading (After 21 days)**
1	97	99
2	94	98
3	100	97
4	101	96
5	99	97
6	98	96

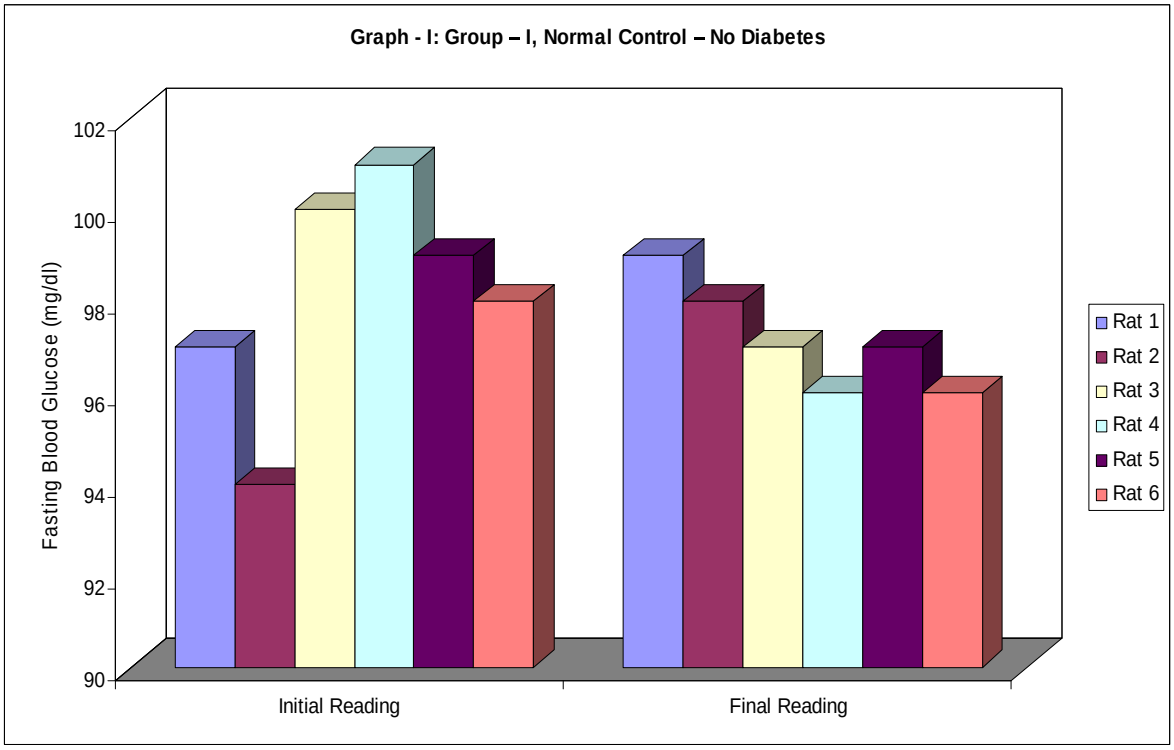


Table.2 The difference between the initial readings and the final readings in group II is statistically insignificant.
Group – II, Diabetic Control, No Drug (Given Normal Saline)

Fasting Blood Glucose (mg/dl)		
Rats	**Initial Reading**	**Final Reading (After 21 days)**
1	261	278
2	266	280
3	299	304
4	291	304
5	280	293
6	299	301

(n = 6) t = 1.84 p > 0.05

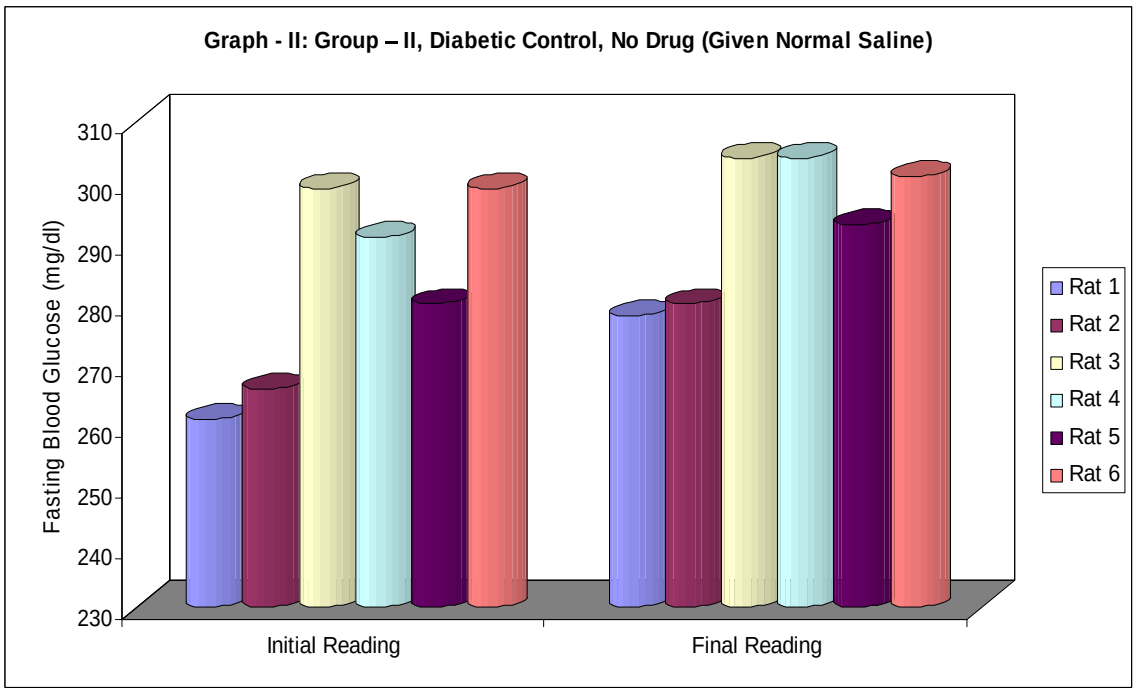


Table.3 The difference between the initial readings and the final readings in the group III is statistically highly significant

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

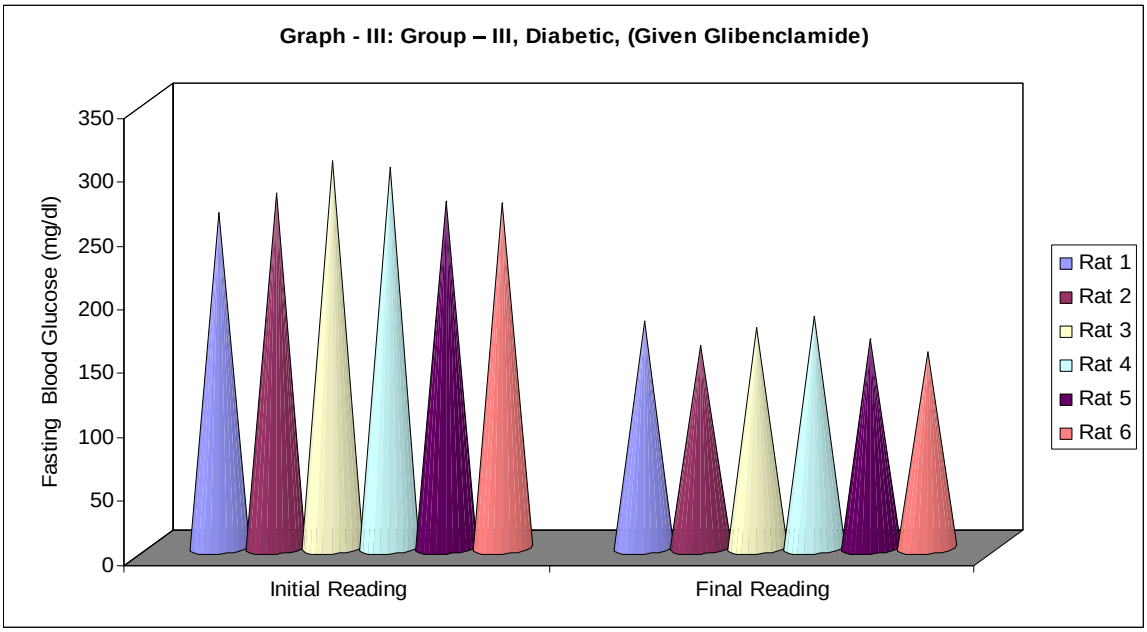


Table.4 The difference between the initial readings and the final readings in the group IV is statistically highly significant

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

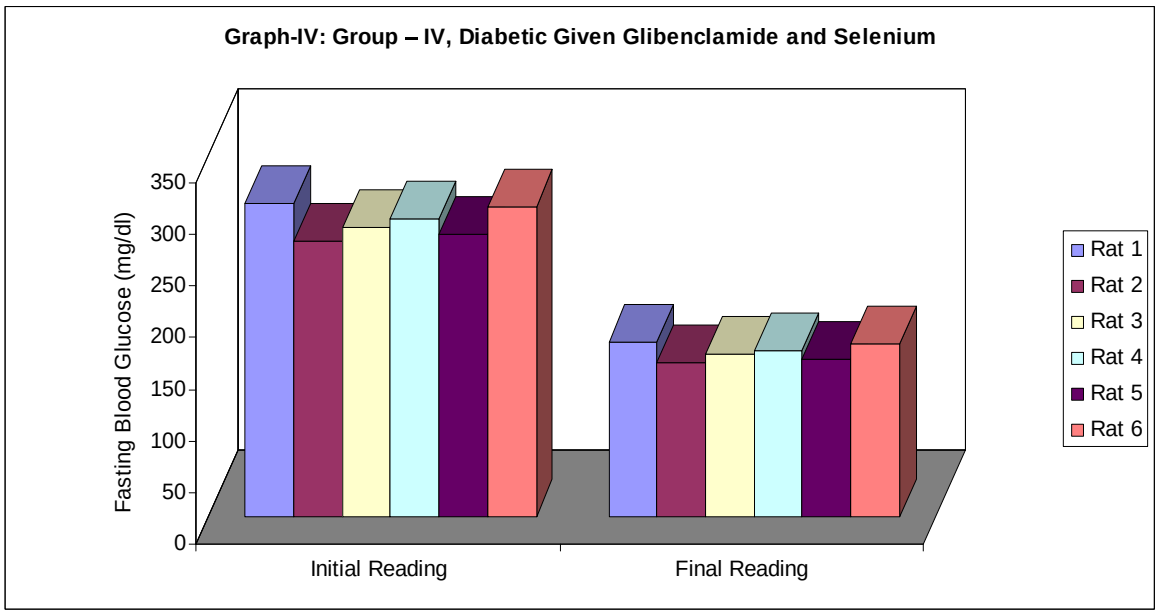


Table.5 The difference between the initial readings and the final readings and the reading in the group V is statistically highly significant
Group – V, Diabetic Rats (Given Glibenclamide, Selenium and Vitamin E)

Fasting Blood Glucose (mg/dl)		
Rats	**Initial Reading**	**Final Reading (After 21 days)**
1	266	150
2	288	146
3	301	155
4	297	142
5	263	154
6	282	157

(n = 6) t = 17.77 p < 0.001

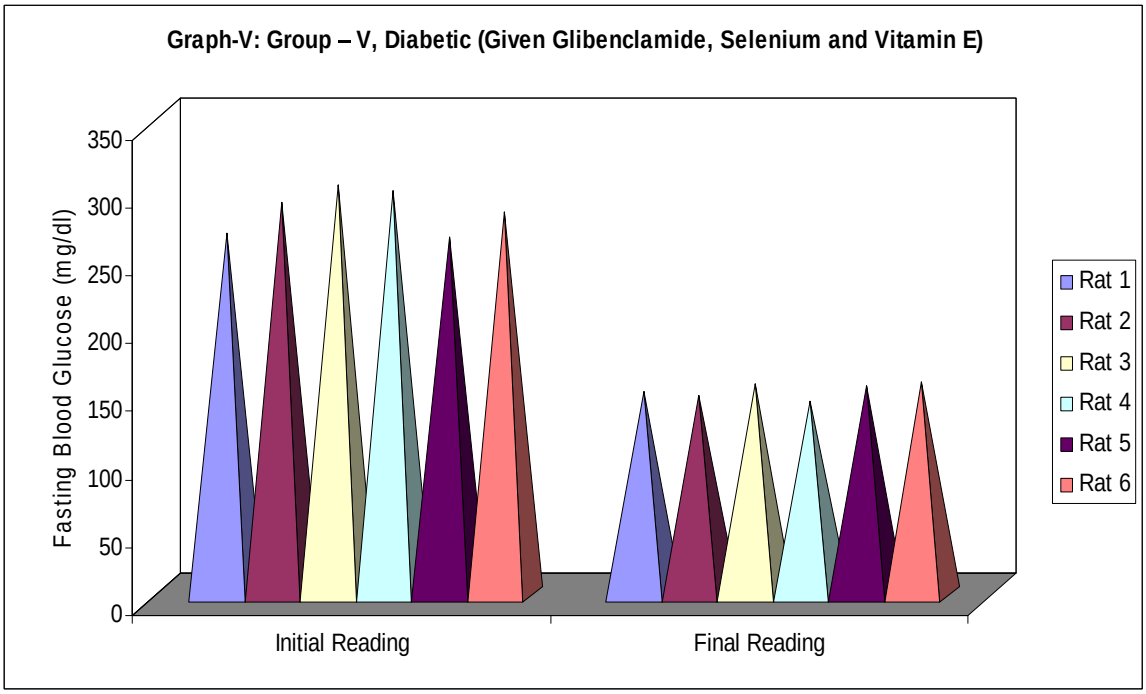
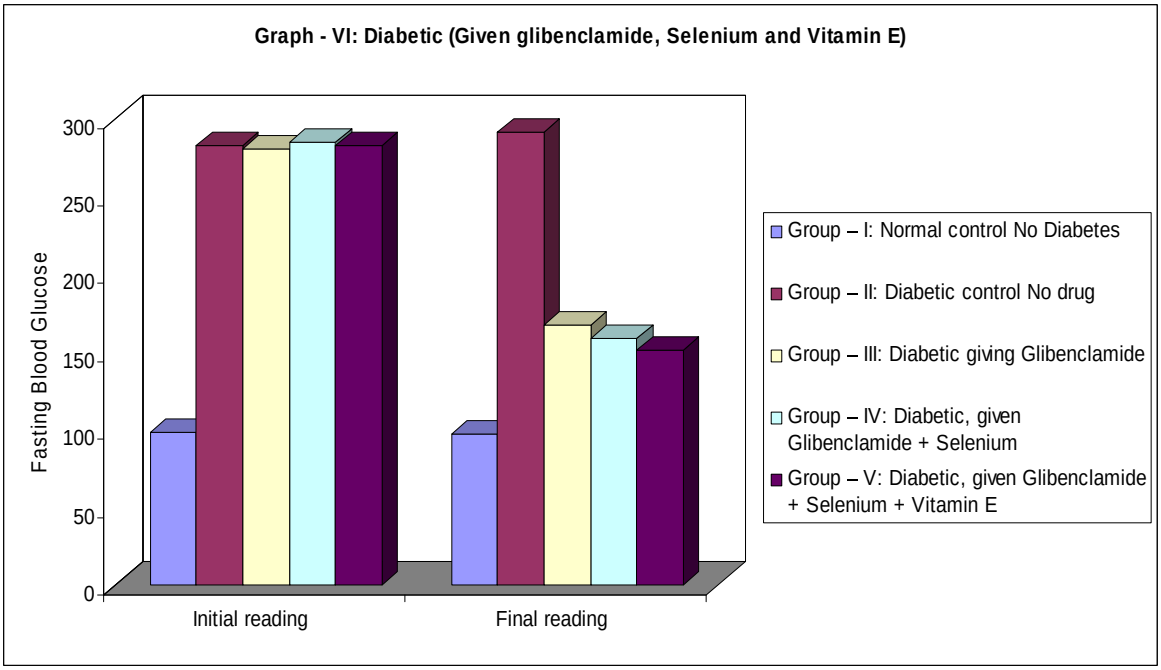


Table.6 Comparison of the effect of Glibenclamide, Glibenclamide and Selenium, and Glibenclamide, Selenium and Vitamin E on the blood glucose levels of alloxan induced diabetic rats

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
Group – V: Diabetic, given Glibenclamide + Selenium + Vitamin E	282.83 ±15.72	150.67 ± 5.79
F – Ratio	0.06	320.93
P Value	p > 0.05 Not significant	p < 0.05 Significant



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
Group III Vs Group V	t = 3.43, p < 0.01 - Significant
Group IV Vs Group V	t = 1.93, p < 0.01 – Significant

Glibenclamide inhibits hepatic glucose production and promotes utilization of glucose in the peripheral tissues, muscles and adipose tissue by significantly influencing 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. It was observed that rats of Group-III which were given Glibenclamide 5 mg/kg/day, oral route, showed a statistically highly significant ($p < 0.001$) decrease in blood glucose levels.

The inorganic form of Selenium, Selenate mimics insulin like activity in experimental models by phosphorylation of the thyrosyl residues present on cellular and ribosomal proteins which are involved in insulin post-receptor effects like translocation of glucose transporter to the plasma membrane. Hypoglycemic effect of selenium is also due to the acceleration of glucose metabolism and inhibition of glucose synthesis in liver. Muller and Pallauf (2006) 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. On comparison between Group III and Group IV we found that the blood glucose levels decreased to a much lower level when a combination of Glibenclamide 5 mg/kg/day and Selenium - 0.54 µg/100gm/day, oral route was used rather when Glibenclamide was used alone.

Excessive oxidative stress in diabetes leads to increase in Liver thiobarbituric acid reactive substance (TBARS) and generation of malondialdehyde (MDA) leading to cytotoxic actions and DNA/protein alterations. Combination of selenium and vitamin E preserves oxidative status and polyunsaturated content of tissues by accelerating detoxification of malondialdehyde (MDA).

Diabetic rats belonging to the Group V were given a combination of glibenclamide (5mg/kg/day), Selenium (0.54 µg/100gm/day) and vitamin E (135µg/100gm/day). The decrease in the blood glucose levels of all animals were statistically significant ($p < 0.001$) (Table 6).

Apart from the synergistic effect, both selenium and vitamin E have been associated with insulin like properties thus forming a promising combination (Gallaher *et al.,* 1993). On comparison between Groups III, IV and V we found that there is maximum decrease in the blood glucose levels when the combination of all the three drugs was used (glibenclamide, selenium & vitamin E) and is statistically highly significant. Therefore the combination of these drugs could be synergistic in leading to a potentiation of action in the decrease of blood glucose levels in diabetic rats.

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