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EFFECT OF MINOXIDIL ON BLOOD GLUCOSE LEVELS IN EUGLYCEMIC AND TYPE2 DIABETIC MODELS AND ITS PHARMACODYNAMIC INTERACTION WITH GLIBENCLAMIDE : AN EXPERIMENTAL STUDY

Pharmacology

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ABSTRACT

OBJECTIVES: Minoxidil, is proven to be effective in the treatment of alopecia. As Minoxidil and diazoxide, which are potassium channel openers have been used in severe hypertension. The commonest side effect of diazoxide is hyperglycemia, due to opening of potassium channel in beta cells producing hyperpolarisation that inhibits insulin release. So when concomitantly K^+ channel activators are used, they may oppose the actions of commonly used sulfonylureas in type2 Diabetics with hypertension. There is paucity of literature in pre-clinical studies regarding the consequences of pharmacodynamic drug interaction between the two drugs and non-diabetic and diabetic persons on blood glucose levels. To evaluate the effect of Minoxidil on euglycemic and type2 diabetic and its interaction with glibencalamide, in type2 diabetic animal models.

METHODS: Type2 diabetes was induced by High fat diet-fed and low dose streptozotocin 35mg/kg i.p. Rats with blood glucose level between 250-350mg% were considered and divided into various groups according to protocol.

RESULTS: In 28 days treatment with Minoxidil (500µ/Kg,oral) produced euglycemia in non-diabetic rats, hyperglycemia in diabetic rats and antagonised the effect of glibenclamide in diabetic rats.

CONCLUSION: These results indicate that Minoxidil has hyperglycemic effect in diabetic rats, euglycemic effect in non-diabetic rats and it reduced the hypoglycemic effect of glibenclamide when it is used in type2 diabetic models of rats.

KEYWORDS

Minoxidil, Glibenclamide, Euglycemic, Hyperglycemia, High fat diet-fed, Streptozotocin.

INTRODUCTION

Diabetes mellitus is described since ancient times. For the past 200 years, it has featured in, the history of modern medicine. It is one of the commonest non- communicable disease of modern world. It is a chronic metabolic disorder characterized by hyperglycaemia, glycosuria, hyperlipidaemia and negative nitrogen balance.[1] Type 2 diabetes is caused by two primary metabolic defects, progressive pancreatic beta cell dysfunction and insulin resistance.[2] Typically, at the time of diagnosis, nearly 50% of beta cell function has been lost and less than 60% of normal insulin sensitivity is present.[3]

It is a chronic illness, which in most cases is treated for life; the results of prevalence studies of diabetes mellitus in India were systematically reviewed with emphasis on those utilizing the standard WHO criteria for diabetes diagnosis. According to International Diabetes Federation Atlas update 2014, more than 425 million people in the world suffer from Diabetes.[4] There were million cases of diabetes in India in 2014.[4] The prevalence of diabetes in adults was found to be 2.4% in rural and 4-11.6% in urban dwellers.[5]

The lifestyle modification, diet and exercise of moderate intensity are used to improve insulin sensitivity and are recommended as an integral part of treatment of Type 2 diabetes mellitus (T2Dm). [6] When the lifestyle modification, diet and exercise fail to maintain the adequate glycaemic control, oral hypoglycaemic agents (OHA) are introduced as a treatment approach. [2,3] OHAs can be used either alone or in combination with other OHAs or insulin. The Canadian Diabetes Association 2003 Clinical Practice Guidelines for the Prevention and management of Diabetes recommends a target HbA1c concentration of 7.0% or less for all patients with diabetes. [7]

Diabetes and hypertension are the commonest clinical problems of the present day in past middle age group individuals and they may co-exist. Further diabetes mellitus can increase the risk of macrovascular and microvascular diseases. Macrovascular diseases like hypertension, angina, myocardial infarction, cardiomegaly, stroke and peripheral vascular disease. Microvascular diseases like retinopathy, nephropathy and neuropathy. The management of individual disease is much easier than when they co- exist. As diabetes and hypertension/angina may coexist in some patients, a combination of two or more drugs may be required.

Second generation sulfonylureas (like glibenclamide) are the commonly used oral hypoglycaemic drugs in the management of type 2 diabetes mellitus which act by closing the ATP sensitive potassium channels. Concomitantly administered drugs to treat associated conditions or to manage complications may influence the hypoglycaemic action of oral antidiabetic drugs through pharmacokinetic and pharmacodynamics interactions.

Wide varieties of drugs are used in the management of hypertension and angina. Potassium channel openers (like nicorandil) are one of the group of drugs that are useful in hypertension and angina, which act by opening the ATP sensitive potassium channels bring about hyperpolarization and produce vasodilatation of vascular smooth muscles. The vasodilator effect of nicorandil is partly antagonised by glibenclamide.[8] The Potassium channel openers like Minoxidil and diazoxide, were using since long in severe hypertension and hypertensive emergencies, they also act by opening ATP sensitive potassium channels bringing hyperpolarisation and vasodilatation. One of the commonest side effect of diazoxide is hyperglycemia. This is due to opening of potassium channels in beta cells producing hyperpolarisation that inhibits insulin release. Thus hyperglycaemia is the side effect with use of diazoxide in type 2 diabetic patients who are on sulfonylureas. [9] Minoxidil is proven to be effective in the treatment of alopecia and severe hypertension as it activates K+ channels and causes vasodilatation. It also releases NO. [10]

Hence when concomitantly K+ channel activators are used, they may oppose the actions of sulfonylureas in type II Diabetics with hypertension. There is paucity of literature in pre-clinical studies regarding the consequences of pharmacodynamic drug interaction between the two drugs on blood glucose levels. The effect of K+ channel openers on blood glucose levels is not clear on diabetic and non-diabetic persons.

Hence, an attempt is being made to study the effect of Minoxidil on blood glucose levels in normal and diabetic rats and to evaluate the pharmacodynamic drug interactions between Minoxidil and glibenclamide in diabetic rats.

This may suggest for adjustment of dose of glibenclamide in such a situation in treatment of diabetics.

OBJECTIVES

In view of inconclusive reports regarding the hyperglycaemic activity of minoxidil and paucity of literature regarding its interaction with commonly used sulfonylureas like glibenclamide. The present study was planned with the following objectives:

1) To study the effect of Minoxidil on blood glucose levels in euglycemic rats.
2) To study the effect of Minoxidil on blood glucose levels in type 2 diabetic rats.

3) To study the pharmacodynamics interaction of glibenclamide and minoxidil on blood glucose levels of type 2 diabetic rats.

Methodology

The present study was conducted in the Postgraduate Research Laboratory Department of Pharmacology, S Nijalingappa Medical College, Navanagar, Bagalkot, Karnataka after obtaining the clearance from the Institutional Animal Ethics Committee of S.N.M.C. (Reg No: 829/AC/04/CPCSEA), (Ref: SNMC//IAEC/2014- 15/3879). It was a prospective, randomized, interventional, controlled experimental study.

Materials:

The materials used in this study are:-

Chemicals and drugs:

a. Streptozotocin: (obtained from Sigma – aldrich, St. Louis, MO, USA).
b. Citrate buffer at PH-4.4
c. Pure minoxidil sulphate: Test drug (obtained from Maruti futuristic pharma Private Limited)
d. Pure glibenclamide: Standard OHA (obtained from Triveni chemicals)
e. High Fat Diet (Mixture of coconut oil (from Marico Industries Ltd,Mumbai) and vanaspathi ghee (Gemini Edibles and Fats India private limited,AP) procured from local market.
f. Vehicle (Normal saline, distilled water)

Experimental Animals:

A total of 60 adult Wistar albino rats weighing 150-200g of either gender were procured from the Central Animal house RegNo: 829/AC/04/CPCSEA, Department of Pharmacology, S N Medical College, Bagalkot. Pregnant rats, animals with infection, animals with injuries, deformities were excluded from the study. Prior to and during study, all the animals were maintained under standard animal house conditions at 12: 12 hour dark: light cycle, 25±2°C, and 35%-60% humidity and other micro and macro environment conditions as suggested by CPCSEA (Committee for the purpose of control and supervision of experiment on animals). All animals were housed in a polypropylene cage covered with a stainless steel wire mesh and a paddy husk bed, with adequate provision for feed and water. All the animals were maintained on standard laboratory diet (VRK nutritionals, Pune) and water was provided ad libitum. All the animals were taken care of under ethical consideration.

Equipments:

1. *Mouth gag:* It is used to facilitate the introduction of oral feeding tube into the stomach of the animals.
2. *Oral feeding tube*: It is used to administer the test and standard drugs, normal saline into the stomach of the animals.
3. *Tuberculin syringe*: It is used to administer the test and standard drug, normal saline with oral feeding tube.
4. *Rat holder:* It is used to restrain the rat for getting the blood sample.
5. *Glucometer and strips*: It is used to know the blood glucose levels of rats. It was obtained from Accu-check Active Glucometer, Roche Diagnostics, Germany.

Other equipment's - Beaker, glass jar, glass rod, marking pen.

EXPERIMENTAL DESIGN

For the study 60 Adult Wistar albino rats of age 2months old weighing approximately 150-200g were used. The study was carried out from the month of November to February.

They were divided into 6 groups with 10 animals [11] in each group.
Group 1: Normal rats treated with Normal saline
Group 2: Normal rats treated with Minoxidil(500µg/Kg), oral [12]
Group 3: Diabetic rats treated with Normal saline
Group 4: Diabetic rats treated with Glibenclamide (5mg/kg), oral [13]
Group 5: Diabetic rats treated with Minoxidil(500µg/Kg), oral
Group 6: Diabetic rats treated with Minoxidil(500µg/Kg), oral and Glibenclamide (5mg/kg), oral

Induction of Diabetes Mellitus

[High fat diet- fed and low dose streptozotocin – treated Type 2 diabetic model of rats.] [14] The experiment was carried out for a period of 10 weeks. For this purpose, sixty healthy wistar albino rats of both gender and weighing approximately 150-200 gm were selected. Before starting the experiment, the animals were allowed to acclimatize to the laboratory environment for one week.

I) ***Method of Preparation of High Fat Diet*** [15]**:** Edible coconut oil and vanaspathi ghee mixed together in the ratio of 2:3 respectively v/v as per method of Shyamala MP [15] et al.

II) ***Method of Inducing Diabetes:***

i) A high fat diet, consisting of coconut oil and vanaspathi ghee, in a ratio of 2:3 v/v at a dose of 10 ml / kg body weight, was fed to the animals, per orally, daily in addition to normal diet for 6 weeks.

ii) **Injection of streptozotocin:** After 6 week's low dose of streptozocin (35mg/kg) was dissolved in a citrate buffer (PH- 4.4) with a concentration of 15mg/ml was given intraperitonially [16]

iii) **Confirmation of diabetes** [17] **:** one week after streptozocin injection, blood glucose level was measured by using portable glucometer from the tip of rat tail. Animals with random blood glucose level 250 - 300mg/dl were considered as diabetic. The diabetic animals were weighed, recorded, numbered and randomly divided into four groups of 10 animals each (n=10) [11]

III) ***Grouping of animals:***

Group I: Normal Control
Received normal diet+ normal saline (10ml/kg body weight)

Group II: Standard drug group
Received normal diet +Minoxidil500µ/Kg, oral [12]

Group III: Diabetic control group
Received normal diet + normal saline 10ml/kg body weight

Group IV**:** Diabetic Standard group
Received normal diet + 5mg/kg, oral of Glibenclamide [13]

Group V: Diabetic test group
Received normal diet + 500µ/Kg, oral of Minoxidil [12]

Group VI**:** Diabetic test group + Standard group
Received normal diet + 500µ/Kg, oral of Minoxidil [12] + 5mg/kg, oral of Glibenclamide [13]

Treatment with drugs was started on the 7th day of Streptozotocin treatment (i.e., day1) and was continued for 28 days. All the drugs were administered as a single dose in the morning to the rats, according to the group to which they belonged. Blood glucose was measured before starting the treatment (day1) and weekly thereafter up to end of treatment period (i.e., at the end of 28 days).

METHOD OF ORAL FEEDING

The test drug Minoxidil, glibenclamide and normal saline were administered Orally to all the diabetic and non-diabetic rats by using polythene tubing sleeved on An 18-20 gauge blunted hypodermic needle (oral Eustachean catheter), according to The group to which they belonged.

All the animals used for the experiment were kept under observation for daily food intake. The drugs were administered to the animals in the doses mentioned before for 6 weeks, by means of an oral feeding tube. At the end of the 6th week, all the animals were taken group wise and blood collected from each of them for assessing the blood glucose levels.

IV*) METHOD OF COLLECTION OF BLOOD*

Blood sample for glucose estimation was collected from the tip of rat's tail [15].

Procedure: In a well restrained rat, the tail was embedded in 45▫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.

METHOD OF BLOOD GLUCOSE ESTIMATION [16]

Blood glucose was estimated by glucose oxidase method using Accu-check Active glucometer in both euglycemic and diabetic rats.

It uses glucose oxidase specific strips and works on principle called as Reflectance Photometry. It is easy to use, quick to perform and reliable. Blood sample was directly placed on the test strip inserted in the glucometer, and the results appeared as mg% on the screen within 5 seconds.

There is a reasonable co-relation between laboratory results and those obtained with glucometer. [16]

Statistical analysis: All results are expressed as the mean ± SEM. The results were analyzed for statistical significance. Statistical differences between the means of the various groups were evaluated using one way analysis of variance followed by post hoc dunnet's multiple test.

Statistical evaluation was done using student's't' test value of p less than 5% ($p<0.05$) was considered statistically significant. Statistical analysis was done by using SPSS version 19 software

RESULTS

The drugs were administered orally to animals once daily for 28 days. Euglycemic as well as HFD-STZ induced diabetic animals were subjected for various treatments as mentioned earlier.

The blood glucose was estimated with the help of glucometer and the mean values expressed as mg% were calculated and compared with that of control and the standard.

Euglycemic animals

***Group 1*:** Control group (vehicle treated): had no change in blood glucose levels. [Table 1 & Fig 1]

***Group 2*:** Minoxidil treated group: had no change in blood glucose levels. [Table 1 & Fig 1]

Diabetic animals

***Group 3*:** Control group (vehicle treated): had an increase in blood glucose levels

***Group 4*:** Glibenclamide treated group: had significant decrease ($p<0.001$) in blood glucose levels. [Table 2 & Fig 2]

***Group 5*:** Minoxidil treated group: had significant increase ($p<0.001$) in blood glucose levels. [Table 2 & Fig 2]

***Group 6*:** Glibenclamide + Minoxidil treated group: had significant increase ($p<0.001$) in blood glucose levels. [Table 2 & Fig 2]

DISCUSSION

Minoxidil is a potassium channel opener. It acts on KATP channels. Potassium channels are also present in beta islet cells of pancreas where they regulate glucose metabolism by resulting in release of insulin. In presence of glucose, it enters into the beta cells through GLUT2 transporter, these ATP sensitive potassium channels close with subsequent opening of calcium channels resulting in insulin secretion.

Our study was based on the hypothesis that the test drug Minoxidil is a potassium channel opener which results in hyperglycaemic state by inhibiting insulin secretion either by keeping potassium channels patent or open, the closed potassium channel in beta cells of pancreas.

High fat diet-fed and low dose streptozotocin, type II diabetic animal model represents a good experimental diabetic state with insulin resistance, remnant insulin production by pancreatic beta cells. Fat-fed/STZ rats provide a novel animal model for type 2 diabetes, simulates the human syndrome. Therefore this animal model was selected for the study.

Our data revealed that one week after streptozotocin injection; rats exhibited an increase in blood glucose levels. Blood glucose level was measured by using portable glucometer from the tip of rat tail. Animals with random blood glucose level 250 - 300mg/dl were considered as diabetic.

In this study the standard drug Glibenclamide was given in the dose of 0.5mg/kg b.w and the test drug Minoxidil 500µg/kg, orally for 28days both individually and in combination in different groups of diabetic animals. Data from present work demonstrated that, there was no significant change in blood glucose levels between normal control rats treated with normal saline and normal rats treated with minoxidil throughout the study period.

Administration of HFD + STZ caused a significant increase in blood glucose levels throughout the study period compared with the control group ($p<0.05$).

Diabetic animals treated with glibenclamide showed a significant reduction in blood glucose levels compared with diabetic control group throughout the study period. It acts by releasing insulin from beta cells by binding with specific receptors in ATP dependent potassium channels and closing the channels which results in opening of voltage dependent calcium channel and calcium enters the cell and insulin is released by degranulation and exocytosis. Thus decrease in the blood glucose levels.

Minoxidil treated diabetic animals showed significant increase in blood glucose levels compared with control diabetic rats. As Minoxidil is a potassium channel opener and thus it causes decreased insulin release and rise in blood glucose levels.

Diabetic animals treated with both glibenclamide and minoxidil showed significant increase in blood glucose levels when compared with diabetic control rats. As Glibencalimde acts by closing only on ATP sensitive potassium channels to release insulin, these channels are opened by minoxidil co-administration and hence decreased release of insulin and rise in blood glucose levels.

KCOs like minoxidil, diazoxide, nicorandil, pinacidil, cromakalim and levcromakalim act by enhancing the ATPase activity of SUR1 subunit and the resultant channel opening causes hyperpolarisation [18] It has been found that hyperglycemia is one of the commonest side effects of potassium channel opener like diazoxide due to inhibition of insulin release. [9] In a study Kulkarni J S et al of chronic treatment with cromakalim and glibenclamide in alloxan induced diabetic rats produced significant hyperglycemia, hypoinsulinemia. [19] In a study by Suresha R. N. et al Nicorandil has shown significant rise in basal blood glucose levels in albino wistar rats [20] and in a study by Gupta anupam, Nicorandil has shown increase in blood glucose level in alloxan induced diabetic rats. [21]

Minoxidil also has cardioprotective action mediated through mitochondrial KATP channel by improving coronary blood flow. [17] Nicorandil has been approved for angina, hypertension and cardiac ischemia. [22]

In an in vitro study, glibenclamide was shown to antagonise the relaxant effect of levocromakalim on longitudinal smooth muscle of rat ileum. [23] Also glibenclamide has been found to increase small intestinal transit in mice. [24] Similarly in an invivo study of mice, glibenclamide significantly decreased small intestinal transit when administered along with morphine and mosapride. [25] This shows that concurrent administration of glibenclamide with mosapride and morphine counteracts the effect of mosapride. In the same study minoxidil was found to enhance the effect of morphine on gastrointestinal delay in the presence of mosapride. [25]

A study of comparison of the effects of the K^+ channel openers cromakalim and minoxidil sulphate on vascular smooth muscle reported antagonism of cromakalim by glibenclamide was apparently competitive, the antagonism of minoxidil sulphate by glibenclamide apparently non-competitive. [26] Our study results shows minoxidil sulphate antagonise glibenclamide competitively as both the drugs act on the same SUR subunits (SUR1, SUR2A,SUR2B). [18]

In the present study, significant hyperglycaemia is evident with diabetic rats treated with minoxidil and coadministration of glibenclamide and minoxidil. In normal rats treated with Minoxidil there is no significant change in blood glucose levels when compared to normal control rats.

The diabetic rats when treated with Minoxidil alone shown 123.82% increase in blood glucose levels when compared to diabetic control. The diabetic rats treated with both the drugs glibenclamide and minoxidil the rise in blood glucose levels was 68.13% when compared to blood glucose levels of diabetic control.

The results of our study are in accordance to action of other potassium channel openers of previous studies, increase in the blood glucose levels but minoxidil and glibenclamide competitively antagonised which is controversial to previous study.

So the present study proves hyperglycaemic effect of minoxidil in diabetic rats and its interaction with glibenclamide.

The interaction is probably pharmacodynamics in nature.

CONCLUSION

In accordance with the hypothesis it is proved that, minoxidil has shown significant rise in blood glucose level in diabetic rat models and also reduced hypoglycemic efficacy of glibenclamide when it is co-administered with Minoxidil in type 2 diabetic rat model. There is no significant change in blood glucose levels in normal rats where Minoxidil is administered.

If the findings of animal experiments are extrapolated on human beings, it is expected that the patients treated with minoxidil may develop hyperglycemia and latent or potentially diabetic individuals may develop clinical diabetes mellitus. It may also interfere with the maintenance of euglycemia in patients of cardiovascular disorders simultaneously suffering from diabetes mellitus receiving oral hypoglycemic drugs and may cause an increase in dose requirement of the later.

But extensive studies on diabetic model of animals and subsequently in human beings are required to substantiate the present work.

Table 01: Blood glucose levels (mean + standard) from day 1to day 28 of in normal groups

Groups	Day 1 M + S	Day 3 M + S	Day 7 M + S	Day 14 M + S	Day 21 M + S	Day 28 M + S
Group1 Normal saline	81.16 + 2.00	79.60 + 2.87	81.66 + 7.77	81.66 + 2.10	82 + 2.09	83 + 10.21
Group2 Minoxidil	72.8 + 10.14	73.50 + 7.79	71.83 + 10.68	75.33 + 7.60	80.16 + 10.60	88 + 9.93
T	0.828	0.551	1.074	0.691	.173	.556
P	0.427	0.594	0.308	0.505	0.866	0.584

Blood glucose levels were analysed between group 1 and group 2 according to student's't' test, value of p less than 5% *($p<0.05$) was considered stastically significant

Table 02: Blood glucose levels (mean + standard) from day 1 to day 28 of in Diabetic groups

Groups	Day 1 M ± S	Day 3 M ± S	Day 7 M ± S	Day 14 M ± S	Day 21 M ± S	Day 28 M ± S
Group3 Diabetic + Normal saline	258.33 ± 10.73	262.5 ± 10.86	264.5 ± 10.03	268 ± 8.48	265.66 ± 9.24	269.33 ± 8.91
Group4 Diabetic + Glibenclamide	257 ± 6.00	216.66 ± 7.44	191.33 ± 7.31*	144.66 ± 7.86**	127.5 ±4.88***	106 ± 9.71***
Group5 Diabetic + Minoxidil	292.16 ±9.46	440 ± 8.12***	474.5 ± 8.62***	552 ± 9.49***	558.66 ± 9.25***	602.83 ± 9.80***
Group 6 Diabetic + Glibenclamide + Minoxidil	299.33 ± 7.17	302.33 ± 7.50	305.66 ± 7.27	362.66 ± 9.56**	408 ± 8.95***	452.83 ± 9.11***

Blood glucose levels were analysed according to ANOVA followed by post hoc dunnet's multiple test and shown as mean ± SEM (Standard error mean). Based on post hoc analysis when compared with diabetic control * $p<0.05$, ** $p<0.01$, *** $p<0.001$

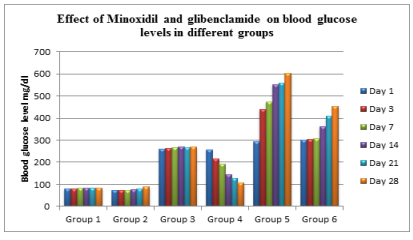

Fig 01: Bar diagram shows mean blood glucose levels in normal rats and diabetic rats.

Group1- normal saline, group2- Minoxidil, group3- diabetic+ normal saline, group4 – diabetic + glibenclamide, group5- diabetic + minoxidil, group6- diabetic + glibenclamide+minoxidil

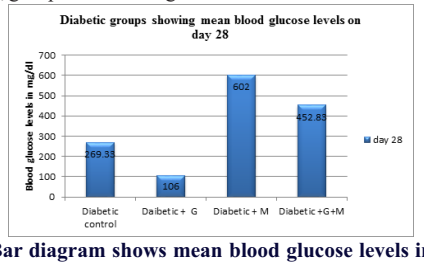

Fig 02: Bar diagram shows mean blood glucose levels in normal rats.

Blood glucose levels were calculated according to ANOVA followed by post hoc dunnet's multiple test and shown as mean ± SEM. Based on post hoc analysis when compared with control. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. (G= Glibenclamide, M=Minoxidil)

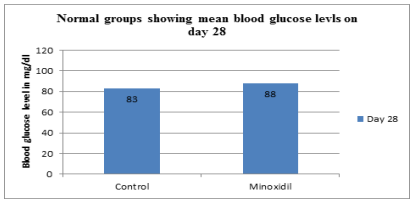

Fig 03: Bar diagram shows mean blood glucose levels in normal rats with normal saline and minoxidil.

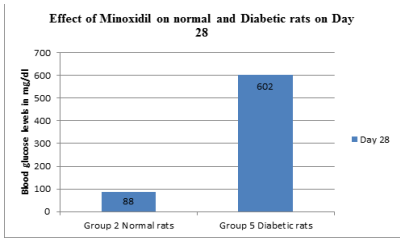

Fig 04: Bar diagram shows mean blood glucose levels of Minoxidil in normal rats and Diabetic rats.

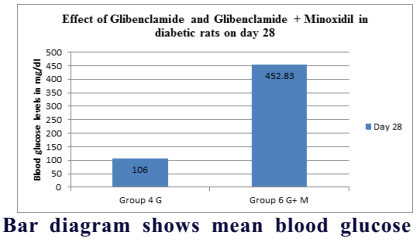

Fig 05: Bar diagram shows mean blood glucose levels of Glibenclamide and combination of Glibenclamide and Minoxidil in diabetic rats. (G= Glibenclamide, M=Minoxidil)

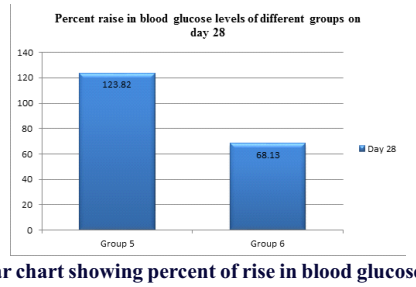

Fig 06: Bar chart showing percent of rise in blood glucose levels in group 5 (diabetic rats treated with only Minoxidil) and group 6 (diabetic rats treated with both the drugs Glibenclamide and Minoxidil) on day 28 in comparison with diabetic control (group3)

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