STUDY OF EFFECT OF NICORANDIL ON INSULIN PRODUCTION IN ALLOXAN INDUCED DIABETIC RATS
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ABSTRACT: OBJECTIVE: To evaluate the effect of ATP – sensitive potassium channel opener, nicorandil on insulin production in alloxan – induced diabetic rats. METHODS: In an attempt to ascertain the involvement of ATP sensitive potassium channels in the regulation of insulin release, the effect of nicorandil on ATP sensitive potassium channel was studied. Albino rats of Wistar strain, weighing between 200–250 grams of either sex were used for the study. Diabetes was induced by injecting alloxan monohydrate 2% solution intra - peritoneally in a dose of 150 mg/kg body weight. Animals with fasting blood glucose (FBS) between 200–300 mg/dl were selected for the study. They were divided into 3 groups of six animals each. Group I serving as control received 2% gum acacia orally for 30 days, Group II as standard was given orally glibenclamide (0.5 mg/kg body weight) for 30 days & Group III was treated orally for 30 days with nicorandil (0.3 mg/kg body weight) respectively. Fasting blood sugar (FBS) was recorded in all the rats on 1st, 3rd, 7th, 14th, 21st & 28th days. RESULT: Results show that glibenclamide has significantly reduced the blood sugar levels (P<0.05), whereas nicorandil has shown a significant rise in blood sugar level (P<0.05). CONCLUSION: The study shows that nicorandil worsens existing diabetes. This may attribute to the hypothesis that the opening of ATP sensitive potassium channels on beta cells of pancreas leads to inhibition of insulin release. These findings suggest that potassium channel openers should be avoided in presence of diabetes.

KEYWORDS: Diabetes mellitus; Albino rats; Alloxan monohydrate; Fasting blood sugar; Glibenclamide; Nicorandil.

INTRODUCTION: Mankind has known diabetes for more than 2500 years. In 1869, a German medical student, Paul Langerhans, noted that pancreas contains two distinct groups of cells – the acinar cells (Which secrete digestive enzymes) and islet cells, which he suggested, served a second function. The islet cells contain beta cells which secrete insulin. In 1889, Oskar Minkowski and Joseph Von Mering showed that pancreatectomized dogs exhibit a syndrome similar to diabetes mellitus in human beings.

HISTORY OF INSULIN3: In 1921 Frederick G. Banting a young Canadian surgeon assumed that islet tissues secreted insulin but the hormone was destroyed by proteolytic digestion prior to or during extraction. Together with Charles H. Best, a 4th year medical student, he attempted to overcome the problem by tying the pancreatic ducts. The acinar tissue degenerated, leaving the islets undisturbed; the remaining tissue was then extracted with ethanol and acid. Banting and Best thus obtained a pancreatic extract that was effective in decreasing the concentration of blood glucose in diabetic dogs.
HISTORY & MECHANISM OF ACTION OF SULFONYLUREA ORAL HYPOGLYCEMIC AGENTS: In contrast to systematic studies that led to isolation of insulin, the sulfonylurea was discovered accidentally. In 1942, Janbon and colleagues noted that some sulfonamides caused hypoglycemia in experimental animals. Carbutamide became the first sulfonylurea but was withdrawn because of adverse effects on bone marrow. Later tolbutamide was widely used in type II diabetes mellitus in early 1950’s. Since then many different agents of this class have been in use worldwide. In 1997, a new class of oral insulin secretogogues called meglitinides (benzoic acid derivatives) was approved for clinical use. Repaglinide has gained acceptance as a fast acting, premeal therapy to limit postprandial hyperglycemia.

ATP sensitive potassium channels (K-ATP) determine the resting membrane potential in β-cells. Glucose stimulation of insulin secretion begins with its transport into the β-cells via a membrane transporter called GLUT-2. Glucose phosphorylation by glucokinase is the rate-limiting step that controls glucose regulated insulin secretion. Further metabolism of glucose–6 phosphate via glycolysis generates ATP which inhibits the activity of an ATP-sensitive potassium channel. This channel consists of two separate proteins: one is the receptor for certain oral hypoglycemics (example: sulfonylurea, meglitinides), the other is an inwardly rectifying K+ channel protein. Inhibition of this k+ channel induces β-cell membrane depolarization, which opens voltage-dependent calcium channels, leading to calcium influx. This calcium signal induces insulin secretion. Second generation sulfonylurea’s (like glibenclamide) are the commonly used oral hypoglycemic drugs in the management of type 2 diabetes mellitus which by closing the ATP sensitive potassium channels brings about partial depolarization and produce release of insulin from beta cells.

Diabetes and hypertension are the commonest clinical problems of the present day in past middle age group individuals and they may coexist. Further diabetes mellitus may increase the risk of macrovascular complications like hypertension, angina, myocardial infarction, cardiomyopathy, stroke and peripheral vascular disease, as well as Microvascular diseases like retinopathy, nephropathy and neuropathy. The management of individual disease is much easier than when they exist together. As diabetes is most often associated with hypertension/angina in some patients, there is a need to prescribe vasodilators. Among vasodilators nicorandil is one drug which is used in angina & hypertension.

MECHANISM OF ACTION OF NICORANDIL: Wide varieties of drugs are used in the management of hypertension and angina. Potassium channel openers (Like nicorandil) are one of the groups of drugs that are useful in angina, which by opening the ATP sensitive potassium channels brings about hyper polarization and produces vasodilatation of vascular smooth muscles. Drugs that have their primary site of action at arteriolar level (Calcium channel blockers and potassium channel openers) are not beneficial in the treatment of angina. However nicorandil also exerts a nitrate like effect, stimulating guanylyl cyclase to increase cyclic GMP, primarily in epicardial coronary arteries including stenotic segments producing vasodilatation. Hence this drug is used in the management of angina/ischemic heart disease. Further minoxidil and diazoxide, which are potassium channel openers have been used since long in severe hypertension and hypertensive emergencies. They also act by opening ATP sensitive potassium
channel bringing about hyper polarization and vasodilatation. One of the commonest side effects of diazoxide is hyperglycemia. This is due to opening of potassium channel in beta cells producing hyper polarization that inhibits insulin release. Thus hyperglycemia is the main problem with use of diazoxide in type 2 diabetic patients who are on oral hypoglycemic drugs.³

Like diazoxide the novel potassium channel opener (nicorandil) also activates ATP sensitive potassium channels, which are present both on vascular smooth muscles and membrane of beta cells of pancreas. Hence nicorandil in addition to producing vasodilatation in coronaries may also inhibit insulin secretion.

Hence in a situation where type 2 diabetes is associated with ischemic heart disease/ hypertension, there may be a chance that nicorandil may be prescribed for management of angina and this may worsen diabetic control. Thus in anticipation of the above action of nicorandil, this study has been taken up in albino rats. Diabetes was induced with intra-peritoneal injection of 2% alloxan. The results were analyzed by noting the effect of nicorandil on insulin secretion when it is administered orally.

**Note:** Increase in insulin secretion is indicated by fall in blood glucose level.
Decrease in insulin secretion is indicated by rise in blood glucose level.

**MECHANISM OF ACTION OF ALLOXAN:** The unique capability of alloxan to selectively destroy the pancreatic B-cell was first described by Dunn et al⁴ (1943). The toxic effect of alloxan in these cells seems to be closely related to increased cellular permeability⁵ and morphological abnormalities of plasma membrane of rodents exposed to alloxan has been described. Alloxan administration leads to a decrease in islet superoxide dismutase activity. Recent studies have suggested mitochondrial dysfunction in the pancreatic B-cells may also be involved in the diabetogenic action of alloxan. As a result, free calcium levels may be increased. Excess calcium concentration in the cell cytoplasm has been known to be noxious and leads to cell death.⁶,⁷ Alloxan has shown to cause IDDM in dogs, cats, sheep, rabbits, mice, monkeys, fish, turtles and birds.⁸ Only guinea pig is insensitive to the diabetogenic action of alloxan.

Administration of alloxan in to the peritoneal cavity of 2 day old rat has been reported to cause an experimental situation similar to that seen in NIDDM.⁶,⁹ Alloxan diabetic animals also possess diabetic complications such as cardiomyopathy, gastroenteropathy and autonomic neuropathy.¹⁰

**MATERIALS AND METHODS: ANIMALS:** Albino rats of Wistar strain (200-250 grams) of either sex were randomly selected from central animal facility, JSS Medical College, Mysore. Animals were housed into groups of 6 per cage at a temperature of 25 degrees +/- 1 degree and relative humidity of 45-55%. Animals had free access to food and water. The institutional animal ethical committee approved the protocol of this study.

**DRUGS AND CHEMICALS:**
1) 2% Alloxan monohydrate: For induction of Diabetes.
2) 2% Gum acacia as suspending agent.
3) Glibenclamide (0.5mg per kg body weight): 2\textsuperscript{nd} generation sulfonylurea, standard drug.
4) Nicorandil (0.3mg per kg body weight): k\textsuperscript{+} channel opener, test drug.

**EQUIPMENTS:** Mouth gag, Polythene tube, Tuberculin syringe, Glucometer.

**METHODOLOGY:** Animals were divided into three groups with six rats in each group and were fed with pellet diet and water and acclimatized to laboratory conditions before carrying out any experimental work. For measuring fasting blood glucose, blood was collected from the rat’s tail vein by tail cutting method. Following overnight fast Diabetes was induced by injecting freshly prepared 2\% Alloxan Monohydrate solution dissolved in 0.9\% Sodium Chloride intra-peritoneally in a dose of 150mg per kg body weight. Following injection, animals were carefully observed for the first 24 hours for any evidence of allergic reaction, behavioral changes and convulsions. No untoward reaction was observed in any animal. Fasting blood glucose was recorded daily morning at around 9.00 am for one week. Animals developed stable hyperglycemia after 4-5 days. Only those animals with blood glucose between 200-300 mg/ dl were selected for the study. Later they were divided into 3 groups, each group having 6 animals. The groups were named as control, standard, test group respectively.

**Administration of Drugs:** Each group of animal was orally fed with the following agents.

**CONTROL GROUP:** 6 alloxan induced diabetic rats, orally fed with 0.5 ml of 2\% gum acacia for 30 days.

**STANDARD GROUP:** 6 alloxan induced diabetic rats, orally fed with 0.5 ml of 2\% gum acacia + Glibenclamide 0.5 mg/ kg body weight for 30 days.

**TEST GROUP:** 6 alloxan induced diabetic rats, orally fed with 0.5 ml of 2\% gum acacia+ Nicorandil 0.3mg/kg body weight for 30 days.

Fasting blood glucose was recorded in all animals of each group on 0, 1\textsuperscript{st}, 3\textsuperscript{rd}, 7\textsuperscript{th}, 14\textsuperscript{th}, 21\textsuperscript{st} and 28\textsuperscript{th} day. Hyperinsulinemic action of the drug is assessed by its capacity to decrease FBS levels and the hypoinsulinemic action by its capacity to increase FBS levels.

The results will be analyzed by calculating the mean values, the standard deviation, the standard error, the ‘\textit{t}’ test, ‘\textit{p}’ value and the analysis of variance (ANOVA). \textit{P}-Value is the standard table value of ‘\textit{t}’ at (12-2)=10 degree of freedom for 0.05(5\%) level of significance. \textit{P}<0.05 = Significant

\textit{P} >0.05 = Not Significant.

The formulas are enclosed in Annexure.
RESULTS:

| Sl. No. | Groups       | 0 day | 1st day | 3rd day | 7th day | 14th day | 21st day | 28th day |
|---------|--------------|-------|---------|---------|---------|----------|----------|----------|
| A – 1   | Diabetic control | 281   | 278     | 280     | 277     | 282      | 279      | 280      |
| B – 1   | Standard     | 229   | 168     | 145     | 128     | 110      | 112      | 108      |
| C – 1   | Test Group   | 296   | 380     | 418     | 423     | 430      | 444      | 460      |

Table 1 showing the FBS values in mg/dl of 1st animal of each group from 0 to 28th day

| Sl. No. | Groups       | 0 day | 1st day | 3rd day | 7th day | 14th day | 21st day | 28th day |
|---------|--------------|-------|---------|---------|---------|----------|----------|----------|
| A – 2   | Diabetic control | 288   | 291     | 281     | 286     | 283      | 280      | 281      |
| B – 2   | Standard     | 267   | 204     | 167     | 162     | 156      | 130      | 118      |
| C – 2   | Test Group   | 277   | 374     | 391     | 402     | 440      | 456      | 480      |

Table 2 showing the FBS values in mg/dl of 2nd animal of each group from 0 to 28th day

| Sl. No. | Groups       | 0 day | 1st day | 3rd day | 7th day | 14th day | 21st day | 28th day |
|---------|--------------|-------|---------|---------|---------|----------|----------|----------|
| A – 3   | Diabetic control | 240   | 238     | 240     | 242     | 239      | 237      | 235      |
| B – 3   | Standard     | 250   | 220     | 210     | 198     | 182      | 167      | 148      |
| C – 3   | Test Group   | 247   | 350     | 380     | 402     | 418      | 430      | 456      |

Table 3 showing the FBS values in mg/dl of 3rd animal of each group from 0 to 28th day

| Sl. No. | Groups       | 0 day | 1st day | 3rd day | 7th day | 14th day | 21st day | 28th day |
|---------|--------------|-------|---------|---------|---------|----------|----------|----------|
| A – 4   | Diabetic control | 230   | 232     | 228     | 228     | 224      | 226      | 229      |
| B – 4   | Standard     | 242   | 218     | 206     | 182     | 161      | 138      | 122      |
| C – 4   | Test Group   | 236   | 258     | 289     | 302     | 348      | 376      | 401      |

Table 4 showing the FBS values in mg/dl of 4th animal of each group from 0 to 28th day.
Table 5 showing the FBS values in mg/dl of 5th animal of each group from 0 to 28th day.

| Sl. No. | Groups       | 0 day | 1st day | 3rd day | 7th day | 14th day | 21st day | 28th day |
|---------|--------------|-------|---------|---------|---------|----------|----------|----------|
| A − 5   | Diabetic control | 282   | 278     | 283     | 280     | 276      | 281      | 279      |
| B − 5   | Standard     | 276   | 242     | 236     | 212     | 186      | 152      | 146      |
| C − 5   | Test Group   | 268   | 292     | 301     | 328     | 386      | 426      | 462      |

Table 6 showing the FBS values in mg/dl of 6th animal of each group from 0 to 28th day.

| Sl. No. | Groups       | 0 day | 1st day | 3rd day | 7th day | 14th day | 21st day | 28th day |
|---------|--------------|-------|---------|---------|---------|----------|----------|----------|
| A − 6   | Diabetic control | 280   | 281     | 278     | 279     | 275      | 276      | 274      |
| B − 6   | Standard     | 282   | 260     | 242     | 202     | 176      | 138      | 120      |
| C − 6   | Test Group   | 286   | 308     | 342     | 390     | 406      | 440      | 480      |

Table 7 showing the mean values of blood glucose levels & Std. Deviations in different groups of animals.

| SL. No | Group          | DAY-0   | DAY-1     | DAY-3     | DAY-7     | DAY-14    | DAY-21    | DAY-28    |
|--------|----------------|---------|-----------|-----------|-----------|-----------|-----------|-----------|
| 1.     | Diabetic Control | 267 ± 22.8 | 266 ± 24.0 | 265 ± 24.3 | 265 ± 24.0 | 263 ± 25.1 | 263 ± 24.8 | 263 ± 24.2 |
| 2.     | Standard       | 258 ± 18.9 | 218 ± 31.7 | 201 ± 38.0 | 189 ± 31.1 | 161 ± 27.0 | 139 ± 18.7 | 127 ± 16.2 |
| 3.     | Test-A         | 268 ± 23.0 | 327 ± 48.7 | 353 ± 51.6 | 374 ± 48.0 | 404 ± 33.0 | 428 ± 27.9 | 456 ± 29.0 |

Table 8 showing a Comparison of percentage of reduction in blood glucose levels in case of Standard and percentage of increase in blood glucose levels in case of test drug.

| SL. No | GROUP | 1ST DAY | 3RD DAY | 7TH DAY | 14TH DAY | 21ST DAY | 28TH DAY | Mean   |
|--------|-------|---------|---------|---------|----------|----------|----------|--------|
| 1.     | Standard ↓ | 15.17%  | 21.78 % | 29.96 % | 37.35 %  | 45.91 %  | 50.58 %  | 33.45 %|
| 2.     | Test drug ↑ | 20 %    | 31.7 %  | 39.5 %  | 50.7%    | 59.7%    | 70.1 %   | 45.28 %|

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P < 0.05 = Significant (S) or highly significant (HS).
G = Glibenclamide.
N = Nicorandil.

ANALYSIS OF RESULTS:

A. BLOOD GLUCOSE COMPARISON WITHIN EACH GROUP:
   1. There was no significant difference in the mean values of blood glucose level from day '0' today '28' in the diabetic control group (p > 0.05).
   2. In the standard (Glibenclamide) group the difference in the mean values of blood glucose level was found to be statistically significant between 0 to 1st day, 1st to 3rd day, 3rd to 7th day, 7th to 14th day and 21st to 28th day (p < 0.05).
   3. In the Test (Nicorandil) group the difference in mean values of blood glucose level was found to be statistically significant (P < 0.05) between 0 to 1st day, 1st to 3rd day, 3rd to 7th day, 7th to 14th day, 14th to 21st day and 21st to 28th day.

B. BLOOD GLUCOSE COMPARISON BETWEEN THE GROUPS:
   Between the standard (Glibenclamide) and test (Nicorandil) group: The difference between the mean values of blood glucose levels of standard and test group was found to be highly statistically significant right from the 1st day to the 28th day. i.e., the test drug has caused significant rise in blood glucose level when compared to standard.

DISCUSSION: Diabetes is most commonly associated with hypertension and ischemic heart disease, for which a combination of drug therapy is required. The introduction of insulin secretagogues like sulfonylureas and meglitinides has revolutionized the treatment of diabetes along with biguanides and Thiazolidinediones, which reduce insulin resistance/produce insulin sparing effect.
In addition to nitrates, potassium channel openers are the other group of drugs that are useful in hypertension and ischemic coronary heart disease. 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.

In the light of this, the novel potassium channel opener nicorandil has been investigated for any hyperglycemic effect. The results obtained were compared with the hypoglycemic effect of the standard drug glibenclamide.

Albino rats of Wistar strain, weighing between 200-250gms of either sex were used for the study. Chemical diabetes model was used as an experimental method for the study. In this method, alloxan monohydrate 2% solution was injected intraperitoneally in a dose of 150mg/kg.b.wt as described by Dunn et. al. in 1943. In this study the standard drug glibenclamide was given in the dose of 0.5mg/kg.b.wt and the test drug nicorandil in the dose of 0.3mg/kg.b.wt orally for 30 days in different groups of diabetic animals.

The group in which glibenclamide was given showed a fall in blood glucose level. The mean fall in blood glucose level was 33.45% over 28 days which was found to be statistically significant.

The group in which nicorandil was given showed a rise in blood glucose level. The mean rise in blood glucose level was 45.28% over 28 days, which was found to be statistically significant.

Statistical analysis was done using paired students ‘t’ test, t-values obtained in each group were compared with P-value (standard table value of ‘t’) at 10 degree of freedom for 0.05(5%) level of significance. P<0.05 (significant) and P>0.05 (not significant).

The minimum number of animals required to conduct the study is 6 in each group and hence 6 animals were included in each group. The above results indicate that nicorandil has significant hyperglycemic effect.

Because of pharmacokinetic and Pharmacodynamic variations between animal and human species further studies are required to substantiate these results in human diabetic subjects where the minimum number of subjects required in each group is 30.

CONCLUSION: Thus from the above study it is concluded that nicorandil when administered in alloxan induced diabetic rats has shown the following effects;

1. It has worsened diabetes by increasing the blood glucose levels probably due to inhibition of insulin release by opening of ATP sensitive K+ channels on the surface of beta cells of pancreas.
2. These findings suggest that potassium channel openers should be avoided in presence of diabetes. However if coronary vasodilator effect is required for angina in presence of diabetes, then other vasodilators like nitrates can be preferred.

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ANNEXURE:
STATISTICAL FORMULAE:
If x1, x2 - - - - xn are ‘n’ Observations [say x = blood glucose level in mg/dl] then,
1. The Arithmetic mean (AM) or x is denoted by the formula.

\[ X = \text{A. M} = \left[ \frac{X_1 + X_2 + \ldots + X_n}{n} \right] = \left[ \frac{\sum X}{n} \right] \]

2. The Standard deviation (SD) is calculated by the formula.

\[ S.D = \sigma = \sqrt{\frac{\sum (X-x)^2}{n-1}} \]

3. The Standard error (SE) is calculated by the formula.

\[ S.E. = \frac{S.D}{\sqrt{n}} = \frac{s}{\sqrt{n}} \]

4. The Statistic ‘t’ is calculated by the formula.

\[ t = \frac{|m_1 - m_2|}{\text{SE} (m_1-m_2)} = \frac{|m_1 - m_2|}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}} \]
Where $m_1$ = mean of the 1st set of observations $n_1$
$m_2$ = mean of the 2nd set of observations $n_2$
$s_1$ = standard deviation of the sample of size $n_1$
$s_2$ = standard deviation of the sample of size $n_2$

5. Degree of freedom (d.f.) is the number of independent variables:
   Here d.f. = ($n_1-1$) + ($n_2-1$) = ($n_1 + n_2 -2$)

6. $P$ – value is the standard table value of 't' at (12-2 ) = 10 degree of freedom for 0.05 (5%) level of significance.
   
   $P < 0.05$ = significant or highly significant.
   $P > 0.05$ = not significant.
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