"The study of drug interaction of Glipizide with Nicorendil on blood glucose level in diabetic mice"

Dr. Devendra singh Doneriya	Assist. Professor, Pharmacology, G. R. Medical College, Gwalior, India.
Dr. Vijayendra Gautam	Assist. Professor, Pharmacology, G. R. Medical College, Gwalior, India.
Dr. Savita Vyas	Associate Prof. Pharmacology, MGM Medical College, Indore, India.

ABSTRACT A patient who suffers from type 2 diabetes has a 2–4 times greater risk of death from cardiovascular causes than the patient without diabetes. Thus, chances of concomitant administration of CVS drugs such as anti-anginal, anti-hypertensive and others along with the anti-diabetic drugs is a common therapeutic strategy for the treatment of patients suffering from diabetes with CVS diseases.

So, this study was planned to evaluate the interaction of Glipizide with Nicorandil on blood glucose level in alloxan induced diabetic mice. This study on drug interaction of nicorandil (3 mg/kg p.o.), glipizide (2.5 mg/kg p.o.) and combination of glipizide with nicorandil (2.5 + 3 mg/kg, p.o.) showed that glipizide reduced blood glucose levels at 2, 3 and 5 hrs period and the difference were statistically significant at 3 and 5 hrs in comparison to control group.

The study also showed insignificant lowering of blood glucose by nicorandil as compared to control group (p>0.05). However, statistically significant difference was observed in comparison to glipizide group at 3 and 5 hrs. (p<0.05). Nicorandil per se has not reduced blood glucose level as it is reduced by glipizide group. Similarly, the combination group (G+N) did not produce any significant change in blood glucose levels as compared to control group at 2,3 and 5 hours. Yet, at 5 hr the effect of combination was significant as compared to glipizide group (p<0.05). It also increases blood glucose level in combination group even in presence of glipizide. Thus, Combination group found to reduce efficacy of glipizide when nicorandil was administered in combination,

So, on the basis of this study we conclude that Glipizide per se lower blood glucose level in diabetic mice and Nicorendil per se hinder reduction in blood glucose level. It may reduce efficacy of glipizide when administered in combination, suggesting nicorendil should be avoided as anti-anginal or anti-hypertensive drug in patients suffering from diabetes with CVS diseases or proper glucose monitoring should be done.

INTRODUCTION –

Drug interaction (DI) can be defined as a modification of the effect of a drug when administered with another drug. The effect may be an increase or a decrease in the action/s of either substance, or it may be an adverse effect that is not normally associated with either drug [1]. Drug Interactions (DIs) are an important cause of drug related problems and this includes significant morbidity and mortality.

Epidemiological studies on drug interaction supports that incidence of adverse drug interactions has been estimated to be between 2.2 and 30% in hospitalized patients and between 9.2 and 70.3% in ambulatory patients [2-5]. Drug interactions are important in clinical practice and have been estimated to account for 6-30% of all adverse drug reactions (ADRs) [6]. Review of studies of the epidemiology of DDIs in hospital admissions found that the reported incidence ranged from 0-2.8% [7]. In the Harvard Medical Practice Study of adverse events, 20% of events in an acute hospital in-patient setting were drug related. Of these, 8% were considered to be due to DDIs [8]. The Boston Collaborative Drug Surveillance Program examined 83,200 drug exposures in 9,900 hospitalized patients and identified 3,600 ADRs. A total of 234 (6.5%) adverse drug reactions caused were attributed to DIs [9]. A patient who suffers from type 2 diabetes has a 2–4 times greater risk of death from cardiovascular causes than the patient without diabetes. [10]

Thus, chances of concomitant administration of CVS drugs such as anti-anginal, anti-hypertensive and others along with the anti-diabetic drugs is a common therapeutic strategy for the treatment of patients suffering from diabetes with CVS diseases.

So, this study was planned to evaluate the interaction of Glipizide with Nicorandil on blood glucose level in diabetic mice.

Glipizide is an orally effective standard antidiabetic drug. It is a second generation sulfonylurea. Nicorandil, a novel anti-anginal agent, has been characterized as having potent coronary vasodilator properties. It belongs to the group of potassium channel-opening vasodilators.

AIMS & OBJECTIVES

1. To evaluate, whether oral anti-anginal drug (nicorandil) affect blood glucose level in diabetic mice i.e. per se effect of nicorandil on blood glucose level.

2. To asses any acute change in blood glucose level has been observed on administration of oral anti- anginal drug (nicorandil) in combination with glipizide in diabetic mice.

Material & Methods – This study was conducted on swiss albino mice in the department of pharmacology at MGM Medical college, Indore, MP.

Animals-
Swiss albino mice of either sex were used for the experiments

Drugs-
Alloxan (Power Alloxan Monohydrate, Suvidhinath, India)
Nicorandil (Tab. Korandil, Sun Pharma, Sikkim, India)
Glipizide (Tab.Glynase, USV limited, India)
Gum Acacia (Himedia laboratories)

Equipments / Instruments-
• Glucometer – Accu Check Active: Made in Ireland .
• Singal Pan Electronic Analytical balance A&D.JAPAN
• Electronic weighing machine
• Mice holder
• Tuberculin syringes (1 ml)
• Needles (22, 23, 24 G)
• Feeding needle (16 G)
• Oral gavages

- Test tubes, beakers, flasks
- Glass mortar pestle
- Surgical hand gloves and Spirit.

Ethical approval –

The study project was submitted for approval to the Institutional Animal Ethics committee (IAEC) of our institution – M.G.M. Medical College, Indore (Reg. NO. 709).

Methodology

1. Method for oral administration of drug.

A 16 or 18 gauge needle was suitably covered with flexible polythene tubing, where the edge was made blunt, the needle was fixed to 1ml tuberculin syringe. The mice was held firmly in left hand ,the needle was moistened with glycerin and inserted right in to the esophagus and gently pressing plunger for drug administration, and this was followed by 0.2ml of distilled water to ensure administration of correct dose of drug.

2. Induction of diabetes in mice: Using alloxane[11,12,13]

Procedure: Swiss albino mice (20-30 g) were procured from our central animal house. They were kept under standard environmental conditions of temperature, relative humidity and were fed with standardized diet and water ad libitum during an acclimatization period. The mice were fasted for 18 hours before experimentation but were allowed free access to water. Diabetes was induced by the injection of 150 mg/kg (i.p.) of fresh prepare alloxan monohydrate soluble in water for injection immediately before use.

Seventy-two hours later, the fasting blood glucose level in the mice was determined [14].The blood glucose levels these animals were measured through tail clipping method using a one touch Glucometer device with strips [15]. In this method the mouse was held in a mild restraining device and the distal 1 to 2 mm of the tail was clipped using a sterilized razor [16] and the droplet of blood collected directly on the glucometer strip. Only one droplet was sufficient for blood glucose determination on each occasion.

Diabetes was further confirmed after 8 days and animals with fasting blood glucose of 250-350 mg % were considered appropriate and were used in the study [17,18]

3 Preparation of drugs for animal experimentation:

 The suspension of Glipizide , Nicorendil and solutions of both to be given orally to the experimental animals as standard or in combination, were prepared in 2% gum acacia. Gum acacia here acted as a vehicle. Control group was given a 2% gum acacia suspension (in the standard dose of 10 ml/kg) orally. For all the studies 6 animals were kept in each group (n=6).

Drug Interaction study

To estimate and demonstrate change in blood glucose level on administration of Glipizide with Nicorendil using oral route in diabetic mice:

A single dose study employing serial sampling of blood was used and blood glucose level was estimated using glucometer.

Animals: Albino mice; Swiss strain (20-30 gm)

Groups-

CON. - Control Group I; (2% gam acasia)

GLP. - Glipizide Group II; (2.5mg/kg BW Glipizide)

NIC.- Nicorendil Group III; (3mg/kg BW Nicorendil)

G+N. - Glipizide +Nicorendil combination Group IV ; (2.5mg/kg+3mg/kg BW respectively)

Procedure:

Alloxan induced diabetic albino mice were selected for the study by following the procedure mentioned above. Each animal was weighed and individual doses (volume of drug solution to be given) were calculated. After keeping the animals overnight fasting in the laboratory for acclimatization, random (pre dose) blood glucose levels (0 hr) were measured.

Then drugs were administered orally to groups I, II, III, IV the stipulated doses and the time of dosing was noted for all the animals in each group.

Blood glucose levels were again measured in all the animals at 0, 2, 3, 5 hrs respectively after the drugs were administered.

OBSERVATIONS & RESULTS –

Table N-1: Statistical analysis of effect of drugs on Blood Glucose Level in alloxane- induced diabetic mice

Treatment (Dose (p.o.) BW)	Blood Glucose Levels mg% ± SEM			
	0 hr	2 hr	3hr	5hr
CON (2 % Gum acacia)	291.2 ± 4.686	285.0 ± 5.502	273.0 ± 4.091	263.7 ± 4.088
GLP (2.5 mg/kg)	288.2 ± 6.215	269.7 ± 5.469	245.0* ± 4.789	223.5* ± 5.880
NIC (3 mg/kg)	288.5 ± 5.071	281.3 ± 6.998	271.2# ± 5.782	267.5# ± 7.715
G + N (2.5 + 3 mg/kg)	294.5 ± 6.334	280.2 ± 6.595	264.3 ± 5.897	253.5# ± 5.227
One-way ANOVA	F	0.2727	1.135	6.08	11.88
	Df	3,20	3,20	3,20	3,20
	p	>0.05	>0.05	<0.05	<0.001

CON- Control, GLP- Glipizide, NIC- Nicorandil, G+– Glypizide-Nicorandil combination, Values are mean ± SEM, n=6 in each group, *p<0.05 compared with control, # p<0.05 Compared with GLP (Tukey's test)

Inference –

- Glipizide showed depletion in blood glucose levels at 2, 3 and 5 hrs. Period and the difference were statistically significant at 3 and 5 hrs in comparison to control group (p< 0.05).
- The nicorandil group, though showed lowering of blood glucose levels at 2, 3 and 5 hrs., the decrease was not statistically significant as compared to control group (p>0.05). However, it showed statistically significant difference in comparison to glipizide group at 3 and 5 hrs. (p<0.05), since blood glucose reduction was significantly less in comparison to lowering of blood glucose by glipizide group.
- The combination group (G+N) did not produce any significant change in blood glucose levels as compared to control group at 2,3 and 5 hours. Yet, at 5 hr the effect of combination was significant as compared to glipizide group (p<0.05).
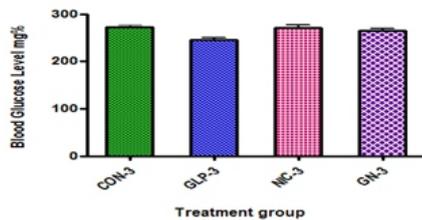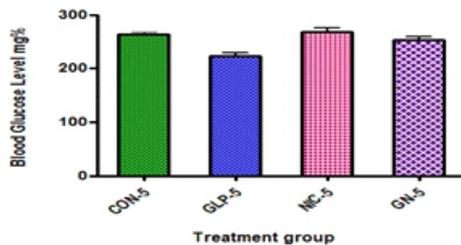
Graphs N-1Change in Blood Glucose Level at 0 hrs. in diabetic mice
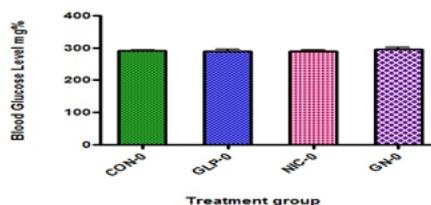
Graphs N-1.1: Change in Blood Glucose Level at 2 hrs. in diabetic mice
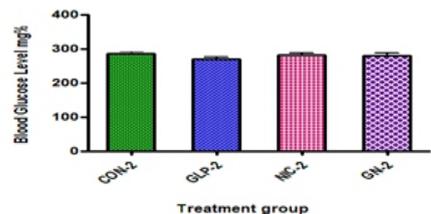
CON- Control, GLP- Glipizide, NIC- Nicorandil, G+– Glypizide-Nicorandil combination

Graphs N-1.2: Change in Blood Glucose Level at 3 hrs. in diabetic mice

Graphs N-1.3: Change in Blood Glucose Level at 5 hrs. in diabetic mice

CON- Control, GLP- Glipizide, NIC- Nicorandil, G+– Glypizide-Nicorandil combination

Table N-2 : Percentage change in Blood Glucose Levels from fasting (0hr) in diabetic mice.

Treatment (Dose (p.o.) BW)	percentage change in Blood Glucose Level (% ± SEM)			
	0hr	0-2hr	0-3hr	0-5hr
CON (2 % Gum acacia)	0	-2.13 ± 0.771	-6.22 ± 0.560	-9.42 ± 0.858
GLP (2.5 mg/kg)	0	-6.36 ± 1.400	-14.90 ± 1.447	-22.37 ± 1.740
NIC (3 mg/kg)	0	-2.53 ± 1.060	-6.02 ± 1.010	-7.81 ± 1.320
G + N (2.5 + 3 mg/kg)	0	-4.83 ± 1.482	-10.10 ± 2.312	-13.70 ± 2.700

CON- Control, GLP- Glipizide, NIC- Nicorandil, G+– Glypizide-Nicorandil combination

Inference-

All the groups showed varying percentage of decreased in blood glucose levels. Glipizide showed sharp decrease in blood glucose level as compared to other groups. Where, combination G+ N did not reduce blood glucose to that extent to which glipizide reduced it when given alone. Thus, indicating reduced efficacy of glipizide when given in combination with nicorandil.

Graph N-2 : Effect of drugs on percent change in blood glucose levels in 5 hours
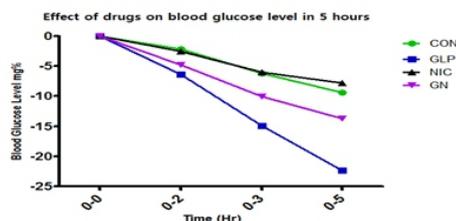
CON- Control, GLP- Glipizide, NIC- Nicorandil, G+– Glypizide-Nicorandil combination

DISSCUSSION & CONCLUSION –

Day-by-day new medications are continually being introduced to the market and knowledge of drug interactions to clinicians is essential for dealing with the challenging drug interactions; as therapeutic outcome is significantly affected by negative DIs. A drug interaction is considered clinically significant when it occurs between two or more co-administered agents and results in the need for a dosage adjustment of one of the agents or need to use of other alternative medical intervention [19].

This study on drug interaction of nicorandil (3 mg/kg p.o.), glipizide (2.5 mg/kg p.o.) and combination of glipizide with nicorandil (2.5 + 3 mg/kg, p.o.) showed that glipizide reduced blood glucose levels at 2, 3 and 5 hrs period and the difference were statistically significant at 3 and 5 hrs in comparison to control group (p< 0.05),(table N-1 and graphs N-1 to N-1.3). Glipizide is an oral rapid- and short-acting anti-diabetic drug from the sulfonylurea class. The action is produced by blocking potassium channels in the beta cells of the islets of Langerhans and the cell depolarization of cells, which results in signaling leading to calcium influx. The increase in calcium will initiate more insulin release from each beta cell. [20]

The study also showed insignificant lowering of blood glucose by nicorandil as compared to control group (p>0.05). However, statistically significant difference was observed in comparison to glipizide group at 3 and 5 hrs. (p<0.05). Nicorandil per se has not reduced blood glucose level as it is reduced by glipizide group. Similarly, the combination group (G+N) did not produce any significant change in blood glucose levels as compared to control group at 2,3 and 5 hours. Yet, at 5 hr the effect of combination was significant as compared to glipizide group (p<0.05). It also increases blood glucose level in combination group even in presence of glipizide. Thus, Combination group found to reduce efficacy of glipizide when nicorandil was administered in combination, (table N-2, graph N-2). This effect of nicorandil might be due to its pharmacodynamics. Nicorandil is a unique anti-anginal agent, reported to act as both an ATP-sensitive K (+) channel opener (PCO) and a nitric oxide donor. A study conducted to prove directly the effect of ATP-sensitive potassium (KATP) channels activity on glucose transport into cultured human skeletal muscle cells; where nicorandil, dose-dependently inhibited insulin-stimulated glucose uptake.

So, on the basis of this study we conclude that Glipizide per se lower blood glucose level in diabetic mice and Nicorendil per se hinder reduction in blood glucose level. It may reduce efficacy of glipizide when administered in combination, suggesting nicorendil should be avoided as anti-anginal or anti-hypertensive drug in patients suffering from diabetes with CVS diseases or proper glucose monitoring should be done.

Though, because of pharmacokinetic and pharmacodynamic variation between animals and human species further studies are required to confirm these results in human diabetic subjects.

REFERENCES –
1. http://medical-dictionary.thefreedictionary.com/drug-drug+interaction
2. Gosney M, Tallis R. Prescription of contraindicated and interacting drugs in elderly patients admitted to hospital, Lancet, 1984;1: Pp 564-7.
3. Kinney E. Expert system detection of drug interactions: results in consecutive patients, Comput Biomed Res, 1986; 19: Pp 462-7.
4. Dambro MR, Kallgren MA. Drug interaction in a clinic using COSTER, Comput Biol Med, 1988; 18: Pp 31-8.
5. Shinn AF, Shrewsbury RP, Anderson KW. Development of a computerized drug database (MEDICOM) for use in a patient specific environment, Drug Inf J, 1983; 17: Pp 205-10.
6. Classen DC, Pestotnick SL, Evans RS, Burke JP. Computer surveillance of adverse drug events in hospital patients, JAMA, 1991; 266: Pp 2847-51.
7. Jankel C A, Fitterman L K. Epidemiology of drug-drug interactions as a cause of hospital admissions, Drug safety, 1993; 9 (1): Pp 55-9.
8. Leape L, Brennam TA, Laired N et al. The nature of adverse events in hospitalized patients: results of the Harvard Medical Practice Study II, N Engl J Med, 1992; 324: Pp 377-84.
9. Boston Collaborative Drug Surveillance Program: Adverse drug interactions, JAMA, 1972; 220: Pp1238-9.
10. Ehud Grossman a Franz H. Messerli, Fisman EZ, Tenenbaum A (eds): Cardiovascular Diabetology: Clinical, Metabolic and Inflammatory Facets. Adv Cardiol. Basel, Karger, 2008, vol 45, pp 82–106].
11. Mohammed Fazil Ahmed, Syed Mohammed Kazim, Syed Safiullah Ghori, et al., "Antidiabetic Activity of Vinca rosea Extracts in Alloxan-Induced Diabetic Rats," International Journal of Endocrinology, vol. 2010; 6.
12. Etuk et al., Evidence based analysis of chemical method of induction of diabetes

mellitus in experimental animals. Asian J. Exp. biol. sci., vol 1 (2)2010: 331-336.

13. Lenzen, S: The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 51, 216-226, 2008.

14. Oladiende FO et al., Effect of Cnidoscolus aconitifolius leaf extract on the blood glucose and insulin levels of inbred type 2 diabetic mice. Cell Mol Biol (Noisy-le-grand). 2007 May 15; 53(3):34.

15. Dewanjee S, Bose SK, Sahu R, Mandal SC. Antidiabetic effect of matured fruits of Diospyros peregrina in alloxan-induced diabetic rats. Int J Green Pharm 2008; 2:95-9.

16. Tuli JS, Smith JA, Morton DB. 1995. Corticosterone, adrenal and spleen weight in mice after tail bleeding, and its effect on nearby animals. Lab Anim 29:90–95.

17. Ezeigbo II. Antidiabetic potential of methanolic leaf extracts of Icacina trichantha in alloxan-induced diabetic mice. Int J Diab Dev Ctries 2010; 30:150-2.

18. Etuk, E.U., Animal models for studying diabetes mellitus. Agriculture And Biology Journal Of North America. ISSN Print: 2151-7517, ISSN Online: 2151-7525.

19. Williams D, Feely J. Pharmacokinetic–pharmacodynamic drug interactions with HMG-CoA reductase inhibitors. Clin Pharmacokinet 2002: 41: 343–370.

20. http://en.wikipedia.org/wiki/Glipizide.