

An experimental study of drug interactions of Diclofenac with commonly used drugs for Diabetes Mellitus in Rabbits

Yadav S¹, Singh S², Sharma MK³, Puri JN⁴, Ansari NA⁵, Singh SP⁶

¹Dr Shailesh Yadav

Professor, Pharmacology
Maharishi Markendeshwar Institute
of Medical Sciences & Research
Mullana, Haryana, India

²Dr Sheela Singh

Professor, Pharmacology

³Dr Mithlesh Kumari Sharma

Professor, Pharmacology

⁴Dr Jagdish Nath Puri

Professor, Pharmacology

⁵Dr Niaz Ahmed Ansari

Professor, Pharmacology

⁶Dr Sheo Pratap Singh

Professor, Pharmacology

^{2,3,4,5,6}Department of Pharmacology,
Ganesh Shankar Vidyarthi
Memorial Medical College
Kanpur, Uttar Pradesh, India

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Correspondence to:

Dr Shailesh Yadav
09050365854
yshailesh41@gmail.com

ABSTRACT

Insulin, Glibenclamide and Tolbutamide are some of the frequently used drugs for the most common metabolic disorders, diabetes mellitus. Along with hypoglycaemic drugs diabetic patient is exposed to variety of drugs. NSAIDs are one of the commonly used drugs in this metabolic syndrome. When a patient on hypoglycaemic drugs receives NSAIDs there are chances of drug interactions.

This study was undertaken in the department of Pharmacology, GSVM medical college, Kanpur (UP) after approval by the institutional animal ethics committee to find out the interaction if any between Diclofenac and three hypoglycaemic drugs i.e. insulin, glibenclamide and tolbutamide.

Young healthy rabbits were divided into six groups: five rabbits in each group. First group received Insulin 1 u/kg subcutaneously; second Glibenclamide 0.05 mg/kg orally; third Tolbutamide 40 mg/kg orally; fourth Diclofenac 1mg/kg orally and insulin 1mg/kg s.c. simultaneously; fifth glibenclamide 0.05mg/kg orally at 0 hour and diclofenac 1mg/kg orally at 2 hours and sixth tolbutamide 40mg/kg orally at 0 hour and diclofenac 1mg/kg orally at 2 hours. Blood samples were taken at 0, 1, 2, 4 and 6 hours for blood sugar estimation. Mean blood sugar levels reduced significantly from their fasting levels when insulin, glibenclamide and tolbutamide were administered. However blood sugar levels did not showed any significant changes when diclofenac was administered along with insulin, glibenclamide and tolbutamide in comparison to when insulin, glibenclamide and tolbutamide were given alone. The results showed that diclofenac does not interact with insulin, glibenclamide and tolbutamide on blood sugar levels in rabbits.

Key Words: Diclofenac, Diabetes mellitus, Glibenclamide, Insulin, Tolbutamide

Introduction

Diabetes mellitus (DM) affects approximately 5 to 8% of the human population. DM has been a mass killer on globe for quite a long time. ^[1] In recent years, developed nations have witnessed an explosive increase in the prevalence of DM predominantly related to lifestyle changes and the resulting increase in prevalence of obesity. The metabolic consequences of prolonged hyperglycemia and dyslipidemia, including accelerated atherosclerosis, chronic kidney disease, and blindness, pose an enormous burden on patients with DM and on the public health system. DM affects multiple organ systems and makes the

patient prone for infections. Because of above mentioned reasons a significant number of patients of DM are on polypharmacy. However polypharmacy carries risks of drug interactions and adverse drug reactions. ^[2] DM consists of a group of syndromes characterized by altered metabolism of carbohydrate, proteins and lipids: and an increased risk of complications from vascular disease. ^[3] It can be prevented upto some extent by lifestyle modifications especially in the persons where genetic predisposition is absent. ^[4] Hyperglycemia is a common end point for all types of DM and is the parameter that is measured to evaluate and

manage the efficacy of pharmacotherapy. Insulin, Glibenclamide and Tolbutamide are few of the commonly used drugs for diabetes mellitus. [5] Inflammation is the dynamic process by which living tissues react to injury. Inflammation characterized by pain and swelling is a protective reaction of the vascularized tissue to injury, intended to eliminate the cause of cell injury. [6] Acute inflammation is characterized by 5 cardinal signs: redness, heat, swelling, pain and loss of function. [7] The process is related to vascular and connective tissues.

Drugs to treat inflammation and immune reactions are very extensively used in medical practice. [8] The nonsteroidal anti-inflammatory drugs (NSAIDs) have a variety of clinical uses as antipyretics, analgesics, and anti-inflammatory agents. As diabetic patients mostly have to be treated for their whole life; they receive various drugs including NSAIDs. A significant number of drug interactions are common with number of NSAIDs. It has been observed that NSAIDs in therapeutic and toxic doses have effects on blood sugar levels. [9] Ibuprofen drops may affect blood sugar levels. [10] Since sulfonylureas are highly bound to plasma proteins and are extensively metabolized by microsomal enzymes, coadministration of drugs capable of displacing them from their protein binding sites or inhibiting their metabolism (e.g., sulfonamide antibacterials, propranolol, salicylates, phenylbutazone, chloramphenicol, probenecid, and alcohol) also may potentiate hypoglycemia. [11] The aim of this study was to assess the effect of interaction of diclofenac with insulin, glibenclamide and tolbutamide on blood sugar levels in rabbits.

Material and Methods

This study was undertaken in the Department of Pharmacology, G.S.V.M. Medical College, Kanpur, U.P. after obtaining the approval from Institutional animal's ethics committee. This study was conducted on young healthy albino rabbits of both sexes weighing 1-1.6 Kg. All the animals were maintained under recommended animal house conditions. Rabbits were divided into six groups. Five rabbits were placed in each group. Rabbits were fasted overnight. In the morning the following drugs were given.

Group 1: Rabbits of this group received insulin 1 unit/kg subcutaneously.

Group 2: Rabbits of this group received glibenclamide 0.05 mg/kg orally.

Group 3: Rabbits of this group received tolbutamide 40 mg/kg orally.

Group 4: Rabbits of this group received insulin 1 unit/kg subcutaneously and diclofenac 1 mg/kg orally simultaneously.

Group 5: Rabbits of this group received glibenclamide 0.05 mg/kg orally at 0 hour and diclofenac 1 mg/kg orally at 2 hours.

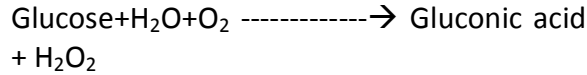
Group 6: Rabbits of this group received tolbutamide 40 mg/kg orally at 0 hour and diclofenac 1mg/kg orally at 2hours.

Blood samples were taken at 0, 1, 2, 4 and 6 hours. All Blood samples were collected from marginal vein of pinna under aseptic conditions. Immediately after taking the last blood sample rabbits received standard animal diet. Water was available ad-libidum to rabbits during the study. Estimation of blood sugar was done by using glucose oxidase-peroxidase method. Monozyme diagnostic reagent kit (CODE NO. k2020) was used. Due to specificity of enzymes, the enzymatic method is the most accurate. Enzymes used in this method are glucose oxidase and peroxide.

Principle:

Glucose is oxidized by glucose oxidase to gluconic acid and Hydrogen peroxide. [12]

Glucose oxidase



Hydrogen peroxide thus formed is broken down to water and nascent oxygen by glucose peroxidase. Oxygen acceptor accepts oxygen and forms quinolamine compound which gives a red colour. The red colour so developed is measured at 510-590 nm and is directly proportional to glucose concentration. Glucose oxidase / peroxidase method used currently is relatively free from interference. There is no interference from urates, creatinine, glutathione and heamoglobin.

Equipment:

For the estimation of blood glucose levels, "photoelectric colorimeter" having 510 nm-590 nm filters was used.

Calculation:

$$\text{Glucose(mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

Statistical analysis:

The results obtained were analyzed by using Student's *t* test and "p" value less than 0.05 and 0.01 were considered significant and highly significant respectively.

Results

The blood sugar levels at zero hour served as control in all the six groups. (Table 1) The insulin treated group (1st group) showed highly significant fall in blood sugar levels till 2 hours; this effect persisted 4 hours although it became significant from highly significant. At the end of 6 hours the fall in blood sugar levels became insignificant. (Table 1) The glibenclamide treated group (2nd group) showed highly significant fall in blood sugar levels at 2 hours. This effect persisted till 6 hours. (Table 1) The tolbutamide treated group (3rd group) did not show significant fall in blood sugar levels till 1 hour; however after 2 hours this group showed highly significant fall in blood sugar levels. This effect persisted till 4 hours; than fall became significant. (Table 1)

Table 1: Means blood sugar levels following administration of insulin (1 unit/kg s.c.), glibenclamide (0.05 mg/kg p.o.) and tolbutamide (40 mg/kg p.o.) in overnight fasted rabbits. (n=5)

Drugs	0hour (Mean±SD)	1hour (Mean±SD)	2hours (Mean±SD)	4hours (Mean±SD)	6hours (Mean±SD)
Insulin	108.72±6.35	65.64±3.81**	61.26±4.12**	99.38±2.56*	104.34±2.67
Glibenclamide	111.26±5.06	94.36±3.02**	90.02±8.05**	69.40±7.06**	71.88±4.90**
Tolbutamide	111.24±6.46	104.36±6.73	86.88±2.58**	63.76±4.17**	71.26±4.61*

*Significant (p<0.05) **Highly Significant (p<0.01)

The insulin + diclofenac treated group (4th group) showed insignificant fall in blood sugar levels from 1 hour till 6 hours in comparison to insulin treated group. (Table 2) The glibenclamide + diclofenac treated group (5th group) showed insignificant fall in

blood sugar levels from 1 hour till 6 hours in comparison to glibenclamide treated group. (Table 3) The tolbutamide + diclofenac treated (6th group) showed insignificant fall in blood sugar levels from 1 hour till 6 hours in comparison to tolbutamide treated group. (Table 4)

Table 2: Modification of insulin induced changes in blood sugar levels by Diclofenac (n=5)

Drug	1 hour (Mean±SD)	2 hours (Mean±SD)	4hours (Mean±SD)	6 hours (Mean±SD)
Insulin	65.64±3.82	61.26±4.13	99.38±2.59	104.34±2.70
Insulin+ Diclofenac	65.00±3.42	60.02±3.39	100.00±3.08	103.74±5.12
“p” value	>0.05	>0.05	>0.05	>0.05

TABLE 3: Modification of glibenclamide induced changes in blood sugar levels by Diclofenac (n=5)

Drug	1 hour (Mean±SD)	2 hours (Mean±SD)	4hours (Mean±SD)	6 hours (Mean±SD)
Glibenclamide	94.36±6.75	90.02±8.07	69.40±7.08	71.88±4.91
Glibenclamide+ Diclofenac	94.36±6.75	88.76±7.17	71.86±6.23	75.64±8.67
“p” value	>0.05	>0.05	>0.05	>0.05

TABLE 4: Modification of tolbutamide induced changes in blood sugar levels by Diclofenac (n=5)

Drug	1hour (Mean±SD)	2hours (Mean±SD)	4hours (Mean±SD)	6hours (Mean±SD)
Tolbutamide	104.36±9.25	86.88±2.59	63.76±4.18	71.26±4.62
Tolbutamide+ Diclofenac	104.36±7.80	86.24±4.74	63.14±6.73	73.12±5.20
“p” value	>0.05	>0.05	>0.05	>0.05

Discussion

Patients of DM specifically non-insulin dependent diabetes mellitus are tried to be managed by diet and exercise initially¹³. Some patients can be managed by this approach; however significant number of patients requires hypoglycaemic drugs. Once pharmacotherapy is started generally

it has to be continued for a very long duration including life time of the patient¹⁴.

DM is an autoimmune syndrome. The treatment should not only consider lowering the blood glucose level but also should focus on the correction of any associated coronary vascular disease risk factors such as smoking, hyperlipidemias,

and obesity as well as monitoring of blood pressure. Besides the drugs required for hyperlipidemias and hypertension the diabetic patients are exposed to many other drugs i.e. drugs for erectile dysfunction, antibiotics and NSAIDs.

It means many DM patients are on polypharmacy. This creates increased chances of drug interactions. Drug interactions affects by two ways i.e. increasing/decreasing the pharmacological effects of hypoglycaemic or interacting drug and increased/decreased levels of blood sugar. Significant drug interactions and patient harm that results from them are common concerns in clinical practice. One study showed that although drug interactions account for only 3.8% of emergency room visits, the patients affected by them usually have to be admitted to the hospital¹⁵. However it is not only the drug-drug interactions; the drug-disease interactions are also very common in diabetics¹⁶.

NSAIDs are known to have interactions with a number of drugs; Sulfonylureas are one of those drugs. Most of these drug interactions are because of displacement of the drugs from plasma protein binding sites. Based on the above facts we carried out this study. We tried to find out whether diclofenac has any influence on the hypoglycaemic effects produced by insulin, glibenclamide and tolbutamide. Insulin, glibenclamide and tolbutamide when given alone produced significant hypoglycaemic effect as expected. When Diclofenac was given with insulin, glibenclamide and tolbutamide there was significant fall in blood sugar levels. This means that hypoglycaemic effect of insulin, glibenclamide and tolbutamide persisted with Diclofenac. On comparison of hypoglycaemic effects

produced by insulin and insulin + diclofenac (Table 2); glibenclamide and glibenclamide + diclofenac (Table 3) and tolbutamide and tolbutamide + diclofenac (Table 4) it was found that diclofenac did not have any influence on the hypoglycaemic effects of insulin, glibenclamide and tolbutamide. The non-interaction of diclofenac with insulin, glibenclamide and tolbutamide will occur or not in human beings is the topic of further research.

Diclofenac does not interact with Insulin, Glibenclamide and Tolbutamide on blood sugar levels in rabbits. Whether these findings are true to human beings or not is the subject of further research.

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