

**AN EXPERIMENTAL STUDY TO EVALUATE THE EFFECT OF SINGLE DOSE OF CLARITHROMYCIN AND GLIBENCLAMIDE COMBINATION IN COMPARISON WITH MONOTHERAPY OF CLARITHROMYCIN AND GLIBENCLAMIDE ON BLOOD GLUCOSE LEVEL IN NEW ZEALAND ALBINO RABBIT.****Rohini Gambre, Akash Khobragade\*, Sadiq Patel, Sarita Jaiswar, Kiran Paripelli and Mansi Brid**

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**ABSTRACT**

**Introduction:** Aim of the study is to evaluate the effect of a combination therapy of Clarithromycin and Glibenclamide given orally as single dose on Blood Glucose level (BGL) at different time intervals 1 hour, 2 hour and 4 hour in albino rabbits. **Methods:** 20 hrs fasting animal's blood was collected for baseline investigations. After feeding, the rabbits were given a single oral dose of 2% gum acacia, Clarithromycin, Glibenclamide and combination of Clarithromycin and Glibenclamide to their respective group. Blood collections were done at 1 hour, 2 hour, 4 hour after drug administration. **Results:** After 20 hrs fasting mean value of BGL in control, Clarithromycin, Glibenclamide and combination of Clarithromycin and Glibenclamide group were  $98.62 \pm 0.24$ ,  $98.40 \pm 0.15$ ,  $97.73 \pm 0.24$  and  $98.39 \pm 0.13$  respectively. After 1 hour the mean values of BGL in Control group, Clarithromycin, Glibenclamide and Clarithromycin + Glibenclamide group were  $99.20 \pm 0.34$ ,  $98.22 \pm 0.14$ ,  $81.72 \pm 0.65$  and  $79.41 \pm 0.39$  respectively. At 2 hour the mean value of BGL in Control group, Clarithromycin, Glibenclamide and Clarithromycin + Glibenclamide group were  $99.59 \pm 0.32$ ,  $97.64 \pm 0.19$ ,  $81.58 \pm 0.33$ , and  $77.69 \pm 0.25$  respectively. By the end of 4 hour the mean value of BGL in Control group, Clarithromycin, Glibenclamide and Clarithromycin + Glibenclamide group were  $99.77 \pm 0.24$ ,  $97.39 \pm 0.18$ ,  $80.66 \pm 0.18$  and  $78.68 \pm 0.44$  respectively. **Conclusion:** Combination of Clarithromycin & Glibenclamide shows significant decrease in BGL as compared to Clarithromycin and Glibenclamide alone in non-diabetic albino rabbits at different time intervals for single dose for a single day.

**KEYWORDS:** Diabetes Mellitus, Clarithromycin, Glibenclamide, BGL.**INTRODUCTION**

Diabetes mellitus<sup>[1]</sup> is a condition characterized by chronic hyperglycemia and disturbance of carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion and /or insulin action. The cardinal symptoms are polyuria, polydipsia, asthenia and weight loss. There is also increased in the susceptibility to bacterial and fungal infections.

The two broad categories of DM are designated as type 1 and type.<sup>[1]</sup> Type I diabetes is treated with *insulin*,<sup>[1,2]</sup> while type II is treated by dietary modification, exercise, *oral hypoglycaemic drugs* (sulphonylureas, biguanides, glitazones etc.) and one third may ultimately require insulin.<sup>[1,2]</sup>

Being a chronic condition, diabetes mellitus requires lifelong therapy. Besides, an increased susceptibility to infections, makes the simultaneous use of antibiotics imperative in these patients. Several antibiotics are being used for infection occurring in diabetic patients such Fluoroquinolones, macrolides and beta

lactamase inhibitors etc.<sup>[3]</sup> Clarithromycin is macrolide with 14 membered lactone rings to which are attached one or more deoxy sugars. Macrolide antibiotics are bacteriostatic agents that inhibit protein synthesis by binding reversibly to the 50S ribosomal subunits of sensitive organisms. Alternatively, macrolides may bind and cause a conformational change that terminates protein synthesis by indirectly interfering with transpeptidation and translocation.<sup>[4]</sup>

The antimicrobial spectrum of Clarithromycin include Mycobacterium avium complex (MAC), other Atypical mycobacterial, Mycobacterium leprae, most active against sensitive strains of gram positive cocci, Moraxella, legionella, Mycoplasma. Pneumoniae, H.Pylori. Clarithromycin is indicated for the treatment of infections such as bronchitis, pneumonia, pharyngitis, sinusitis, folliculitis, cellulitis, erysipelas,<sup>[5]</sup> eradication of H. Pylori, Disseminated or localised mycobacterial infections and also disseminated Mycobacterium avium complex infection in HIV - infected patients with CD4 lymphocyte counts less than or equal to 100/mm<sup>4</sup>

Clarithromycin is potent mechanism based inhibitors of CYP3A4 reducing the clearance of a number of well characterized CYP3A4 substrate such as midazolam, cyclosporine, carbamazepine and terfenadine.<sup>[4,6,7]</sup>

Rhabdomyolysis, co-incident with the co-administration of Clarithromycin, and HMG-CoA reductase inhibitors, such as lovastatin and simvastatin has been reported.<sup>[8]</sup> A number of studies and case reports have described clinically important drug interactions with oral hypoglycemics.<sup>[9]</sup>

Several antibiotics can reduce the metabolism of oral hypoglycemics. It is common to see blood sugar concentrations change during an infectious episode. The infection itself may cause elevations of blood glucose, whereas reduced food intake during an acute illness would tend to lower glucose concentrations. These disease induced alterations in blood glucose will occur in addition to any changes caused by the presence of an interacting drug.

The presence of several blood glucose modifiers mandates careful monitoring of the patient's blood sugar, especially during concurrent therapy with drugs known to inhibit the metabolism of oral hypoglycemics.<sup>[10]</sup> Multiple drug-drug interactions have been reported to potentiate the effect of sulfonylureas. These include anti-inflammatory agents, sulfa antibiotics, bishydroxycoumarin, and antidepressants. All the sulfonylurea agents stimulate insulin release from pancreatic islet cells. The sulfonylurea agents with the longest half-lives cause the most problems and risk of hypoglycaemia. Of the newer second generation agents, Glibenclamide has been reported to cause hypoglycaemia more often than glimepiride.<sup>[11]</sup>

Sulfonylurea-induced hypoglycaemia can be particularly problematic in patients with impaired renal or hepatic function.<sup>[12]</sup>

There are multiple case records studies indicating the occurrence of hypoglycaemic events with combined use of Clarithromycin with Glibenclamide in type II DM elderly patients with mild to moderate renal impairment. These patients were prescribed in identical dosages for respiratory infections. They developed hypoglycaemia within 48 hours of starting Clarithromycin.<sup>[13]</sup> Also, two case control and two crossover studies using US medicamaid data showed that few antibiotics examined were associated with elevated risk of severe hypoglycaemia in Glibenclamide users. In this, statically significant association was found with Clarithromycin [Odd ratio – 5.02; 95% CI – 3.35 – 7.54] using cefalexin as reference.<sup>[14]</sup>

As Clarithromycin is potent CYP3A4 inhibitor whereas Glibenclamide is metabolised extensively by Cytochrome P450 enzyme in liver mainly – CYP2C9. So

role of CYP3A4 enzyme in causation of severe hypoglycaemia may not be implicated.<sup>[14]</sup> Also there are studies which shows that Clarithromycin does not appear to alter in vivo catalytic activity of CYP 1A2, CYP2C9 and CYP2D6 in healthy individuals.<sup>[15]</sup> Owing to lack of definitive reports, the suspected mechanism for this potentially severe hypoglycaemia which is of concern in the treatment of elderly patients with type 2 diabetes is unclear which remain as shortcomings in these reports. Therefore, this study is being planned to evaluate whether Clarithromycin is main reason for hypoglycaemia or simultaneous administration of Clarithromycin and Glibenclamide has led to such reactions due to drug interaction.

## MATERIALS AND METHODS

**A. Study design:** Single centric, prospective, randomized control, parallel group study.

### B. Study centre & approval

Study was conducted in central animal house of Department of pharmacology, Grant Medical College & Sir J.J. Group of Hospitals, Byculia, Mumbai, after approval from Institutional Animals Ethics Committee. This study was done in collaboration with biochemistry lab for measuring the blood sugar levels.

**C. Study model:** New Zealand albino rabbits.

### D. Animals

All animals were procured from animal house of Department of Pharmacology, Grant Medical College & Sir J.J Group of Hospitals, Mumbai.

- New Zealand albino rabbits – total sample size of was 24.

- Body Weight between 1-2 kg

- Age above 24 wks

- Animals of either sex.

- Housing: These animals was housed under standard laboratory condition in a well- ventilated room and fed on Leucine grass and standard pellet diet (Amrut Laboratory animal feed, Nav Maharashtra Chakan oil Mills ltd. Crude protein 22.2%, Crude fibre 71%, ash 7.2%, Sand silica 1.1%). The animals had free access to diet and water and will be placed in clean, neatly labeled cages containing single albino rabbits in each cage.

- Those animals who are non-diabetic & not involved in any other study.

### E. Instruments used

- Rabbit restrainer – to hold the rabbit in restrained position.

- Mouth gag – for introduction of feeding tube

- Feeding catheter – for feeding test and standard drugs to experimental animals.

- Syringes – 5 ml & 3 ml – to collect the blood

- Sterile hypodermic needles – 20G

- Cotton swab – to withhold the bleeding

- Spirit – for antiseptic purpose

- Scissors – to cut the aural hairs
- Ear clamp – to make the veins prominent.
- Fluoride bulbs & plain tubes for blood collection.
- Labels – for labelling bulbs collected from each groups with timing mentioned.
- Cage tag – for identification of different groups
- Weighing balance machine – to weigh the weight of the animals & to measure the weights of the drugs.
- Gloves

#### F. Drugs & duration<sup>[4]</sup>

- Study drug: Clarithromycin
  - Standard drug: Glibenclamide
  - Control drug: Gum acacia (Vehicle /control)<sup>[16]</sup>
  - Tab. Clarithromycin: The human dose of Tablet Clarithromycin is 500 mg twice a day (i.e.1000/day). The dose for rabbit extrapolated from the human dose was 50 mg/kg/day using body surface chart.<sup>[17]</sup>
  - Tab. Glibenclamide: Clinical dose of Glibenclamide is 5 to 20 mg daily.
- In the present study Glibenclamide was administered in the dose of 0.5 mg/kg/day.

#### Table1: Group Division

##### For Single dose study (Abbreviation A)

Groups	Group name	Drugs	Doses	Duration	Animals
IA	Control	Gum acacia 2 %	(2 ml/kg)	Single dose	6
IIA	Study drug	Clarithromycin	50mg/kg	Single dose	6
IIIA	Standard drug	Glibenclamide	0.5mg/kg	Single dose	6
IVA	Combination	Clarithromycin + Glibenclamide	50 mg/kg + 0.5 mg/kg	Single dose	6

#### Procedure for acute/ Single dose study

Animals were fasted for 20 hours. After 20 hours fasting 5 ml blood was collected from lateral marginal vein of the ear by using 20G needle with syringe for carrying out baseline investigations like BGL, SGPT, SGOT, BUN and Sr. Creatinine. This reading was considered as baseline (0 hour) reading. While withdrawing blood all aseptic precautions were kept in mind. Following the blood withdrawal for baseline investigations all animals were fed with same quantity of standard diet in mg/kg. After feeding, the rabbits were given a single oral dose of 2 % gum acacia, Clarithromycin, Glibenclamide and combination of Clarithromycin and Glibenclamide to their respective group in the above mentioned doses. 6 rabbits of group IA were fed 2% gum acacia (single) as an oral suspension.

6 rabbits of group IIA were fed then 50 mg/kg of Clarithromycin (single oral dose) as an oral suspension in 2% gum acacia.

6 animals of group IIIA were given 0.5mg/kg Glibenclamide (single oral dose) as an oral suspension in 2% gum acacia.

6 animals of group IVA were given combination of 50 mg/kg of Clarithromycin (single oral dose) and

- Combination therapy: Glibenclamide (0.5mg/kg) plus Clarithromycin (50 mg/kg) oral once.

Study medication was administered to the fasting animals after drawing blood samples for baseline values. Blood collection mainly done from right marginal ear vein with 24 G needle after cleaning the skin with spirit. For all groups oral suspension of the drug was made in 2% gum acacia and dose was adjusted to a volume of 2-5 ml for albino rabbits, which was administered with the help of a feeding catheter. Study medication was administered as a suspension in 2% gum acacia, as single dose in acute study.

**G. Study groups and schedule:** This study was planned in to evaluate the effects of Clarithromycin alone and in combination with Glibenclamide – (single oral dose study) taking into consideration safety and tolerability profile of animals.<sup>[17]</sup>

For Single dose study, animals were divided in 4 groups each group consisting of 6 albino rabbits.

0.5mg/kg Glibenclamide (single oral dose) as an oral suspension in 2% gum acacia.

Blood collections were done at 1 hour, 2 hour, 4 hour after drug administration.

Blood collection was done by keeping in mind the time taken to post prandial blood sugar level and onset of action of Clarithromycin and Glibenclamide which was same for all the conditions mentioned above.

#### H. Assessment parameter

- Acute study: Drug Blood sugar level estimation – Baseline, 1, 2, 4 hour.
- Tolerability and safety parameters: Gross Body weight, SGPT, SGOT, BUN, S. Creatinine.

**I. Methodology for blood collection:** Collection of blood sample:<sup>[17]</sup> Blood was collected from the lateral marginal ear vein, for that purpose lateral margin of the ear was shaved & swabbed with spirit. Ear was grasped between thumb & index finger in a well restrained rabbit using 20 gauge needle with bevel up. Venepuncture was made at site immediately proximal to the thumb along the marginal vein. Blood was placed in neatly labelled fluoride & plain tubes.

Blood samples were kept at room temperature for coagulation to be completed, subsequently samples were subjected to centrifugation and supernatant plasma and serum was separated. After the drug doses were given, serial blood collections were done to assess the parameters. Blood was sent for investigations to biochemistry laboratory.

## J) Methodology<sup>[18]</sup>

**1. Blood glucose level:** Blood glucose was estimated by GOD (Glucose – peroxidase) method.

**Principle:** This is an enzymatic calorimetric method for the quantitative determination of glucose in serum / plasma. In this single reagent system, glucose oxidase converts glucose to gluconic acid and hydrogen peroxide. Hydrogen peroxide is broken down to water and oxygen by peroxidase. The oxygen reacts with 4-aminophenazone in presence of phenol to form pink colour compound hydroxybenzoate. The intensity of the colour formed is proportional to the glucose present in the sample.

**Table 2: Mean Baseline values of BGL of control and different groups**

	Group IA (Control)	Group IIA (Cla)	Group IIIA (Gli)	Group IVA (Cla + Gli)	P value
<b>BGL (mg/dl)</b>	98.62 ± 0.24	98.40 ± 0.15	97.89 ± 0.15	98.11 ± 0.19	0.06 (NS)

All values are expressed in mean + SEM

ANOVA test with post hoc tukey's test is applied

P < 0.05 is considered to be significant

All groups are comparable with respect to Blood glucose level (BGL) at 0 hour baseline value.

**Table 3: Mean values of blood glucose level in control and different groups at different time intervals.**

Groups	Time factor				P value
	Baseline	1 hour	2 hour	4 hour	
<b>Group IA (Control)</b>	98.62 ± 0.24	99.20 ± 0.34	99.59 ± 0.32	99.77 ± 0.24	0.055(NS)
<b>Group IIA (Cla)</b>	98.40 ± 0.15	98.30 ± 0.14	98.47 ± 0.17	98.82 ± 0.21	0.21 (NS)
<b>Group IIIA (Gli)</b>	97.89 ± 0.15	81.72 ± 0.26***	81.56 ± 0.33***	80.66 ± 0.18***	<0.0001 (S)
<b>Group IVA (Cla + Gli)</b>	98.11 ± 0.19	79.26 ± 0.45***	77.69 ± 0.26***	78.68 ± 0.44***	<0.0001(S)

All values mentioned in the table are Mean + SEM values.

\*\*\* P<0.001, \*\*P<0.01, \*P<0.05 when compared to Group I A(Control).

**At Baseline:** At baseline i.e. after animals were fasted for 20 hour & before giving the different drugs mean value of BGL(blood glucose level) in control group was 98.62 ± 0.24 & the mean values of BGL in Clarithromycin, Glibenclamide and combination of Clarithromycin and Glibenclamide were 98.40 ± 0.15, 97.73 ± 0.24 and 98.39 ± 0.13 respectively, which were nearly similar in all groups.

### AT 1 HOUR

After 1 hour of administration of the control & different drugs, the mean values of BGL in Control group was 99.20 ± 0.34. The mean values noted at 1 hour in Clarithromycin, Glibenclamide and Clarithromycin + Glibenclamide group were 98.22 ± 0.14, 81.72 ± 0.65 and 79.41 ± 0.39 respectively, which were decreased when compared with 1 hour value in control group. The decrease in blood sugar level

## J. Statistical analysis

All quantitative data was presented as mean ± standard error of mean (SEM). For calculation and presentation of data, MS – Excel 2007 was used and for statistical analysis the software which was used – “Graphpad Instat 3”, San Diego California USA. The treatment groups were analyzed with control for baseline comparability by ANOVA and comparisons of parameters between groups for statistical significance at any given time were also done by ANOVA. For individual comparison of data, post hoc Tukey's test was applied for within group comparison. Within group data with different time variables was compared by Paired 't' test. For all tests a 'p' value of <0.05 was considered as statistically significant.

## RESULTS

We tried to find out the effect of Clarithromycin given orally in single dose in acute study and combined with Glibenclamide.

in Group IIA (Clarithromycin) is not significant (p value - > 0.05) but statistically significant for Glibenclamide and Clarithromycin + Glibenclamide (P<0.001) when compared to Control group.

Also when Group IV A (Combination of Clarithromycin and Glibenclamide) is compared with Group IIIA, statistically significant difference was found between two groups. (p value - <0.001)

### At 2 HOUR

At 2 hour of administration of the control & different drugs, the mean value of BGL in Control group was 99.59 ± 0.32. The mean values noted at 2 hour in Clarithromycin, Glibenclamide and Clarithromycin + Glibenclamide group were 97.64 ± 0.19, 81.58 ± 0.33, and 77.69 ± 0.25 respectively, which were decreased when compared with 2 hour value in control group.

The decrease in blood sugar level in Group IIA (Clarithromycin) is not significant ( $p$  value -  $> 0.05$ ) but statistically significant for Glibenclamide and Clarithromycin + Glibenclamide ( $P < 0.001$ ) when compared to Control group.

Also when Group IVA (Combination of Clarithromycin and Glibenclamide) is compared with Group IIIA, statistically significant difference was found between two groups. ( $p$  value -  $< 0.001$ ).

#### At 4 HOUR.

By the end of 4 hour the mean value of BGL in Control group was  $99.77 \pm 0.24$ . The mean values noted at the end of 4 hour in Clarithromycin, Glibenclamide and

Clarithromycin + Glibenclamide group were  $97.39 \pm 0.18$ ,  $80.66 \pm 0.18$  and  $78.68 \pm 0.44$  respectively, which were decreased when compared with 4 hour value in control group. The decrease in blood sugar level in Group IIA (Clarithromycin) is not significant ( $p$  value -  $> 0.05$ ) but statistically significant for Glibenclamide and Clarithromycin + Glibenclamide ( $P < 0.001$ ) when compared to 4 hour value of Control group.

Also when Group IVA (Combination of Clarithromycin and Glibenclamide) is compared with Group IIIA, statistically significant difference was found between two groups. ( $p$  value -  $< 0.001$ )

#### Comparison of mean value of BGL in Group IA (Control) at different time Interval

**Table 4: Mean values of Blood Glucose level (BGL) in Group IA (Control) at different time intervals in acute study.**

Parameter	Time factor				P value
	Baseline	1 hour	2 hour	4 hour	
<b>BGL</b>	$98.62 \pm 0.24$	$99.20 \pm 0.34$	$99.59 \pm 0.32$	$99.77 \pm 0.24$	0.055

Footnote – all values are expressed in mean + SEM

ANOVA test with post hoc tukey's test is applied

$P < 0.05$  is considered to be significant

#### BGL at different time intervals in Group IA (Control)

- The mean value of BGL at 0 hour was  $98.62 \pm 0.24$ . At 1 hour the BGL level was  $99.20 \pm 0.34$  which was increased and statistically insignificant ( $p > 0.05$ ) as compared to 0 hour BGL level.

- At 2 hour the mean BGL level was  $99.59 \pm 0.32$  which was increased and statistically insignificant ( $p > 0.05$ ) as compared to mean BGL values at 0 hour.
- By the end of 4 hour mean BGL value was  $99.77 \pm 0.24$ , which was increased and statistically insignificant ( $p > 0.05$ ) as compared to 0 hour.

**Table 5: Mean values of blood glucose level in Group II A (Clarithromycin) at different time intervals**

Parameter	Time factor				P value
	Baseline	1 hour	2 hour	4 hour	
<b>BGL</b>	$98.40 \pm 0.15$	$98.3 \pm 0.14$	$98.47 \pm 0.17$	$98.82 \pm 0.21$	0.21

All values mentioned in the table are Mean + SEM values.

ANOVA test with posthoc tukey's test is applied

\*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$  when compared to Baseline.

#### Mean value of BGL at different time intervals in Group II A

- The mean value of BGL at 0 hour was  $98.40 \pm 0.15$ . After 1 hour the BGL level dropped to  $98.32 \pm 0.14$ .
- At 2 hour the BGL mean level was  $98.47 \pm 0.17$ , it was decreased when compared to mean BGL values at 0 and 1 hour.

- By the 4 hour mean BGL value was  $98.82 \pm 0.21$ , which was decreased when compared to mean BGL values at 0, 1 and 2 hour. This decrease was statistically insignificant ( $P > 0.05$ ) when compared to mean BGL value at 0 hour.

**Table 6: Mean values of BGL in Group IIIA (Glibenclamide) at different time Intervals**

Parameter	Time factor				P value
	Baseline	1 hour	2 hour	4 hour	
<b>BGL</b>	$97.89 \pm 0.15$	$81.72 \pm 0.26^{***}$	$81.58 \pm 0.33^{***}$	$80.66 \pm 0.18^{***}$	$< 0.0001$

All values mentioned in the table are Mean+ SEM values.

ANOVA test with post hoc tukey's test is applied

\*\*\*  $P < 0.001$  as compared to baseline value in Group IIIA (Glibenclamide).



### BGL values at different time intervals in Group IIIA (Glibenclamide)

- The fasting mean value of BGL at 0 hour was  $97.89 \pm 0.15$ .

After 1 hour, the BGL level decreased to reading of  $81.72 \pm 0.26$  this decrease in

- BGL value is statistically significant when compared with BGL at 0 hour in Group IIIA. (p value - < 0.001)

- At 2 hour, the BGL mean level further decreased to  $81.56 \pm 0.33$  indicating significant difference in BGL value between 2 hour and 0 hour. (p value - < 0.001)

- At 4 hour the BGL value further decreased to  $80.66 \pm 0.18$ , which was less when compared to mean BGL values at 0 and 1 hour. This decrease was statistically significant (P<0.001) when compared to mean value of BGL in group IIIA at 0 hour.

**Table 7: Mean values of BGL in Group IVA (Clarithromycin + Glibenclamide) at different time intervals.**

Parameter	Time factor				P value
	Baseline	1 hour	2 hour	4 hour	
BGL	$98.11 \pm 0.19$	$79.26 \pm 0.45^{***}$	$77.69 \pm 0.26^{***}$	$78.68 \pm 0.44^{***}$	<0.0001

All values are expressed in mean + SEM

ANOVA test with post hoc tukey's test is applied

\*\*\* P<0.001 as compared to baseline value in Group IVA (Clarithromycin + Glibenclamide).

### BGL value at different time intervals in Group IV A (Clarithromycin + Glibenclamide)

- The mean value of BGL at 0 hour was  $98.11 \pm 0.19$ . After 1 hour the BGL level dropped to  $79.26 \pm 0.45$ .

- At 2 hour the BGL mean level decreased further to  $77.69 \pm 0.26$  which statically significant. (p < 0.001)

- At 4 hour the BGL value becomes  $78.68 \pm 0.44$ , which was less when compared to mean BGL values at 0 and 1 hour. This decrease was statistically significant (P<0.001) when compared to mean value of BGL in group IIIA at 0 hour.

## RESULTS AND DISCUSSION

In this study, 4 groups were made, Group IA control 2% Gum acacia, Group IIA Clarithromycin, Group IIIA Glibenclamide, Group IVA & Clarithromycin + Glibenclamide combination group. In all the 4 groups of acute phase study division, we had evaluated effect of control and other drugs on blood glucose levels at 1, 2 and 4 hours in non diabetic rabbits. The rabbits were fasted for 20 hours and baseline collection was done for investigations. The rabbits were fed with equal quantity of diet, immediately after which the drugs were given. All the values of Clarithromycin, Glibenclamide & combination group were compared with control group at different time intervals.

**Effect of test and standard drugs on BGL at different time intervals:** After giving Clarithromycin, Glibenclamide and combination of Clarithromycin + Glibenclamide, Clarithromycin group did not show any significant decrease in Blood glucose level whereas Glibenclamide and combination of Clarithromycin and Glibenclamide groups showed statistically significant (p<0.001) reduction in blood glucose levels when compared to control at 1, 2 and 4 hours respectively. The maximum reduction in blood

glucose level was shown by Clarithromycin + Glibenclamide combination group at 2 hour followed by Glibenclamide group when compared to control at 1, 2 & 4 hours. Also this reduction shown by combination group was statistically significant when compared to 2 hour blood glucose value in individual Clarithromycin and Glibenclamide groups respectively.

**Effect of control on blood glucose level at different time intervals:** In control group the blood glucose levels reached to maximum levels at 1 hour after intake of the food which was physiological. It shows that BGL increase steadily over this time period. This was probably due to the compensatory mechanisms which come into play during starvation i.e. ↑ Glucagon levels, ↑ adrenergic response mediated by α2 pathway, ↑ hepatic gluconeogenesis etc.<sup>[22]</sup>

**Effect of Clarithromycin (group IIA) on blood Glucose level at different time intervals:** In Clarithromycin treated group, the blood Glucose level changed from baseline value of 98.40 mg/dl to 98.30, 98.47 and 98.82 mg/dl at 1 hour, 2 hour and 4 hour respectively. The changes shown in blood Glucose level by Clarithromycin were not statistically significant when compared to group I A (control). Effect of Glibenclamide (group IIIA) on blood Glucose level at different time intervals: In Glibenclamide treated group, the Blood Glucose level are decreased at 1,2 and 4 hour when compared to baseline value and these differences are statistically significant (p value - <0.001).

**Effect of Clarithromycin and Glibenclamide combination in group IV on different parameters at different time intervals:** In Clarithromycin + Glibenclamide group, the maximum reduction is seen at 2 hour BGL values. The BGL are decreased at 1 hour, 2 hour and 4 hour when compared to baseline value. These decreases in BGL at different time interval are found to be statistically significant. (p value - <0.001).

From the above mentioned finding, it is seen that Clarithromycin on its own doesn't have any effects on blood Glucose level but when combined with Glibenclamide show statically significant decrease in blood glucose even on single dose therapy as seen in acute study.

## CONCLUSIONS

1. Clarithromycin, a macrolide antibiotic does not show any significant decrease in blood glucose level in non-diabetic albino rabbits at different time intervals if given as single dose for one day only.
2. Combination of Clarithromycin & Glibenclamide shows significant decrease in blood Glucose levels as compared to Glibenclamide alone in non-diabetic albino rabbits at different time intervals when checked for single dose for a single day .

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