Evaluation of antidiabetic activity of miglitol nanoparticles in streptozotocin induced diabetic rats

Karuppusamy C*[1], Venkatesan P[1], Kalaiselvan R[2]

[1]Department of Pharmacy, Annamalai University, Annamalainagar 608002, Tamil Nadu, India
[2]Research & Development Centre, Sun Pharmaceutical Industries Ltd., Sarhaul, Sector-18, Gurgaon 122015, Haryana, India

ABSTRACT

Diabetes mellitus is a group of metabolic disorder characterized by a complete lack of insulin, a relative lack of insulin, or insulin resistance. In response to the need for better control of diabetes, several new classes antidiabetic drugs were introduced. Among these are Alpha Glucosidase Inhibitors which are used to treat the Type II Diabetes mellitus by inhibiting the Alpha Glucosidase enzymes in the small intestine. The biological half-life of Miglitol is 2 Hrs and by conventional dosage form it requires oral administration of three times daily. Hence to overcome this problem, we developed controlled release formulation of Miglitol loaded nanoparticles will help to release the drug in continuously for 12 Hrs. The effect of newly formulated Miglitol loaded nanoparticles was studied in diabetic rates. Diabetes was induced by streptozotocin in Wistar rats. At first, Maltose was administered orally to all the groups. Along with maltose, the newly formulated Miglitol loaded nanoparticles and marketed conventional release tablets was administered in diabetic rates and Blood glucose was measured at predefined interval. After the initial Maltose load, in the interval of 6 Hrs, Maltose load to the animal was repeated for two more times. After administration of the first maltose load, there was steady attenuation in the plasma glucose values after the same time point in the groups treated with Miglitol formulations. After initial administration of drug, the pharmacological actions are similar for marketed conventional release dosage form and Nanoparticle. However, the conventional release dosage form could not control the peak postprandial plasma glucose after second & third loading of maltose. Whereas, the Nanoparticle formulation significantly (P<0.001) controlled the peak postprandial plasma glucose after second & third loading of maltose.

Keywords: Eudragit; Maltose load; Postprandial plasma glucose; Type II Diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that affects approximately 25% of population in the world and afflicts 150 million people and is set to rise to 300 million by 2025 (Vats *et al.,* 2005). In response to the need for better control of diabetes, several new classes antidiabetic drugs were introduced in 1990s. Among these are Alpha Glucosidase Inhibitors such as Acarabose, Voglibose and Miglitol. These drugs having high affinity for the enzyme Alpha Glucosidase. A carbohydrate comprises of starch and sucrose are metabolised by this Alpha Glucosidase enzymes into monosaccharides in the small intestine before they are absorbed. This leads to delay in carbohydrate metabolism, prolongation of digestion time, and reduction in the rate of glucose absorption, finally resulting in inhibition of the rise of postprandial glucose levels. (Jean – Pierre JE sels *et al.,* 1999). The biological half-life of Miglitol is 2hrs and by conventional dosage form it requires oral administration of three times daily (TID). Hence to overcome these problems associated with the use of conventional dosage forms of Miglitol for the treatment of Type II Diabetes Mellitus, we developed controlled release formulation of Miglitol -loaded nanoparticles will help to release the drug in continuously for 12 Hrs. The objective of the present study is to evaluate the effect of Miglitol nanoparticles in Streptozotocin (STZ) induced diabetes and to compare the effect of Miglitol nanoparticles with marketed conventional dosage form by using rat as an animal model.

MATERIALS AND METHODS

Male Wistar Rats having 180 to 200 g weight obtained from P Rami Reddy Memorial College of pharmacy Animal Breeding House. The animals were housed in a room at 25°C ±2°C with lighting from 6:00 to 18:00hrs (12hrs light/dark phase). The animals were fed on standard pellet diet (Hindustan Lever, Mumbai, India) and water *ad libitum*. Animals were acclimatized to

their environment for one week before experimentation.

Before the study, Institutional Animal ethical committee approval from P Rami Reddy Memorial College of pharmacy, Kadapa, AP was obtained. (Approval No. PRRMCP / CPCSEA / COP / 10/2015, Date: 04-Nov-2015).

MISOBIT® Tablets (Lupin Labs, India) was used as the marketed conventional release formulation in the study.

Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of a freshly prepared streptozotocin solution (Sigma, No. 242-646-8) (50 mg/kg Body Weight) to overnight fasted rats. (Zecharia Madar, 1989; Burcelin, R., *et al.,* 1993; Venkatesan .N *et al.,* 2013).

In vivo study

After 48 Hrs, all animals were treated with STZ, fasting plasma glucose levels was measured. Only rats with fasting blood glucose levels of 150 to 200mg/dl have been included in the study.

These diabetic rats were sub-divided into 3 groups (Group II, III & IV) and each group consisting of 6 animals. All four groups of Wistar Rats received the following treatment schedule, (M. A. Tormo *et al.,* 1998) Treatment schedule is shown in Figure 1.

Group I: Normal control. (Citrate buffer)

Group II: Diabetic Control

Group III: Miglitol Marketed 10 mg/kg., p.o,

Group IV: Miglitol Nanoparticles contains Miglitol 30 mg/kg, p.o

Following 12 Hrs fast, blood glucose was measured in the early morning (Time 0).

Then For all four groups, without anesthesia, with a gastric probe attached to a syringe, Maltose was administered orally (2g/kg) dissolved in 0.9% NaCl.

For Group III rats, along with Maltose, crushed marketed Miglitol Conventional release dosage form contains Miglitol 10 mg/kg. Dispersed in water was administered orally. (M. A. Tormo *et al.,* 1998)

For Group IV rats, along with Maltose, Optimized Miglitol Nanoparticles, Miglitol 30 mg/kg. Dispersed in water was administered orally.

Blood sample was collected from Lateral Tail vein of the animal by using temporary Tail cannulation method. Blood glucose was determined by using One Touch Select Simple Glucometer® (Blood Glucose Testing meter). This measuring device contains Test strip and meter. Once the blood sample has made it on to the glucose strip, a device called a glucose meter is used to measure the glucose in the blood. In each test strip,

there is a chemical called glucose oxidase. This glucose oxidase reacts with the glucose in the blood sample and is created into an acid called gluconic acid. This current is then able to read and determine how much glucose is in the sample of blood on the testing strip. The number is then relayed on the screen of the glucose testing meter. (M. A. Tormo *et al.,* 1998; Parasuraman S *et al.,* 2010; Sunil K. Panchal *et al.,* 2011; Karl-Heinz Diehl *et al.,* 2001)

To perform a test, a test strip was inserted in the meter with clean, dry hands as far as it will go. The each test strip has been immediately used after removing it from the vial. After switch ON the meter, drop of blood was placed in the Test strips. Line up the test strip with the blood drop so that the narrow channel on the edge of the test strip is almost touching the edge of the blood drop. The blood drop was drawn into the narrow channel and the confirmation window filled completely. When the confirmation window was full, blood glucose level appears on the display, along with the unit of measure. The test strips are one time measurement only, so each time fresh test strips has been used.

Blood Sampling Interval

Blood samples were collected prior to Maltose loading (0) and thereafter at periodic intervals (0.5, 1, 1.5, 2, 3, 6.5, 7, 7.5, 8, 9, 12.5, 13, 13.5, 14 and 15 Hrs of initial Maltose load).

After the initial Maltose load, in the interval of every 6 Hrs, Maltose load to the animal was repeated for two more times.

RESULTS AND DISCUSSION

Miglitol Nanoparticle was evaluated with diabetic rats along with the marketed conventional release Miglitol tablets. Diabetes was induced by streptozotocin (STZ) Injection. Postprandial glucose levels after administration of different formulations (Marketed conventional release and Miglitol Nanoparticles) are displayed in Table 1 and Figure 2 (Blood Plasma Glucose Level at Different Time Points).

After initial maltose load, the postprandial plasma glucose level has been slightly increased for the normal control group animals (Group I). After one hour of maltose loads, the blood glucose levels are increased from 88.67 mg/dL to 111.33 mg/dL. However blood glucose level has been reached its normal level in 3rd hour. (94.67 mg/dL). Again after second maltose load, the postprandial plasma glucose level has been slightly increased. The postprandial plasma glucose level reached at 119.67 mg/dL (sampling interval 7 Hrs). Again on 9th hour of sampling interval, the blood glucose returned to normal level. (89.83 mg/dL). The same pattern continued for 3rd maltose load as well.

The diabetic control group (Group II) is not treated with any of the drug. After first maltose load, the postprandial plasma glucose level has been significantly
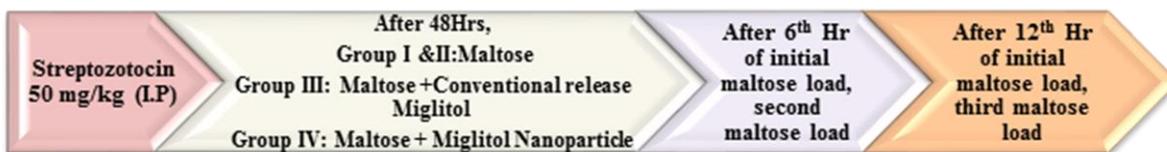
Figure 1: Treatment schedule

Table 1: Blood Plasma Glucose Level at Different Time Points

Treatment Group	Sampling interval in Hrs			
	I	II	III	IV
0	88.67±2.63	176.17±4.61	169.33±3.67	171.50±3.67
0.5	102.67±4.54	189.17±3.42	174.00±3.62*	181.33±3.07
1	111.33±2.49	264.00±1.88	176.17±3.21***	185.67±3.84***
1.5	112.50±2.67	263.17±4.88	173.00±3.54***	183.33±6.21***
2	100.83±4.17	216.83±4.83	172.67±2.65***	176.50±4.53***
3	94.67±3.60	181.67±2.56	170.83±4.43	172.17±4.64
6.5	105.00±2.31	183.67±3.79	179.17±2.66	181.83±3.43
7	119.67±1.38	282.17±3.07	289.83±4.79	185.67±4.06***
7.5	110.33±2.95	263.83±5.97	276.50±3.56	185.83±3.36***
8	102.00±2.19	227.50±2.99	245.50±2.36**	176.33±4.88***
9	89.83±2.23	182.17±3.10	199.17±2.93**	171.83±4.89
12.5	103.67±2.69	187.50±2.95	184.67±4.02	180.00±5.12
13	116.5.00±2.63	290.17±2.82	292.67±6.09	190.00±3.76***
13.5	108.67±2.67	282.83±4.15	284.17±3.47	184.67±3.67***
14	96.00±2.16	231.33±2.95	244.17±3.92*	179.50±4.70***
15	91.17±4.02	186.33±2.56	189.83±3.82***	174.67±3.81*

The values are expressed as Mean ± SEM, n=6. The statistical analysis was carried out using One Way ANOVA followed by post-hoc Dunnett's test was performed.

*denotes $p < 0.5$, ** denotes $p < 0.05$ and *** denotes $p < 0.001$ compared to Diabetic Control group (Group II). Graphpad prism 4 software was used for the calculation
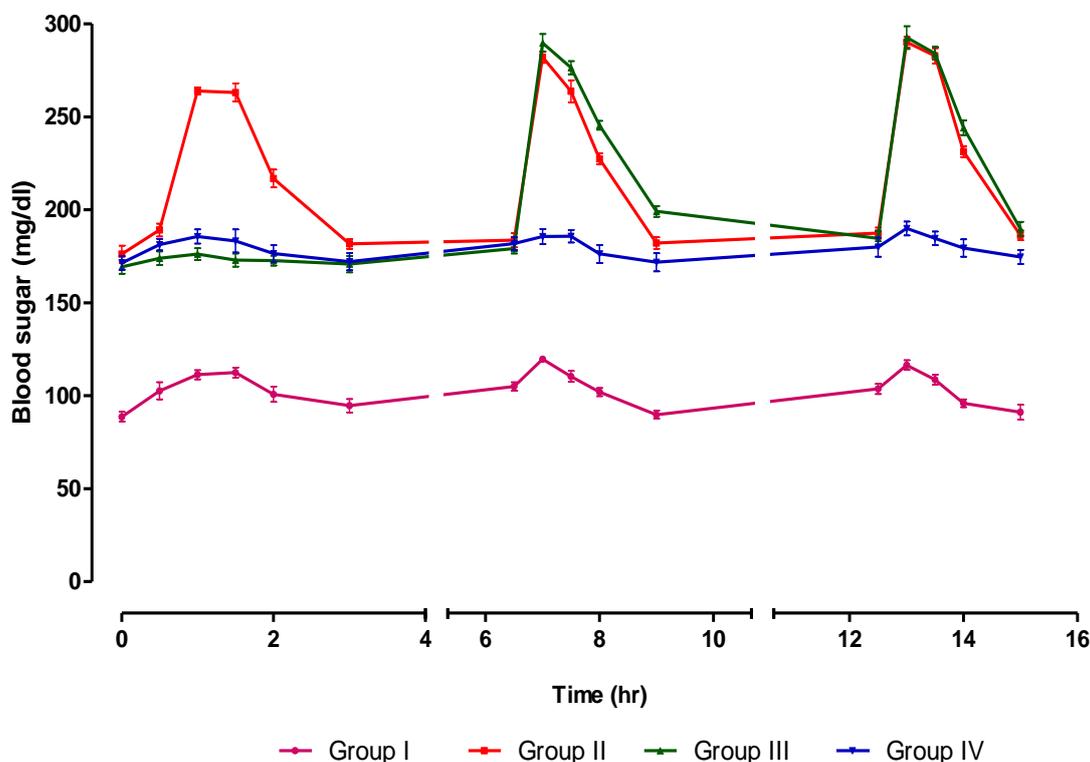

Figure 2: Blood Plasma Glucose Level at Different Time Points

increased and reached 264.00 mg/dL. After 2 Hrs of maltose load, the plasma glucose level has been gradually decreased and reached 181.67 mg/dL at 3rd hour. The same pattern continues for 2nd and 3rd maltose load as well. After the maltose load the postprandial plasma glucose level has been significantly increased for the diabetic control group (Group II).

Group III animals were treated with Marketed Miglitol conventional release dosage form. After initial maltose load, there is no significant increase in postprandial plasma glucose level observed in Group III. The blunt postprandial plasma glucose peak has been seen. One hour after maltose load, the plasma glucose has not been increased as compared with 0 Hr sampling. Before maltose load (0 Hr) the observed plasma glucose level was 169.33 mg/dL, after one hour of maltose load, the postprandial plasma glucose increased up to 176.17 mg/dL. This indicated that the Marketed Miglitol conventional release dosage form significantly (P<0.001) controlled the postprandial plasma glucose level after initial maltose load. The second maltose load has been given 6th hour of the study. After second maltose load, the postprandial plasma glucose level has been significantly increased and reached 289.83 mg/dL. The same pattern observed after 3rd maltose load also. The 3rd maltose load has been given at 12th Hour of the study. After 3rd maltose load the postprandial plasma glucose level has been significantly increased and reached 292.67 mg/dL. This result indicates that the conventional release dosage form could not control the elevation of postprandial plasma glucose level after 2nd maltose load.

Group IV animals were treated with Miglitol nanoparticles. After initial maltose load, there is no significant increase in postprandial plasma glucose level observed in Group IV animals. The blunt postprandial plasma glucose peak has been seen. One hour after maltose load, the plasma glucose has not been increased as compared with 0 Hr sampling. After one hour of initial maltose load, the postprandial plasma glucose increased only up to 185.67 mg/dL. This indicated that the newly formulated Miglitol nanoparticles significantly (P<0.001) controlled the postprandial plasma glucose level after initial maltose load when compared with controlled group. Again second maltose load has been given at 6th hour of the study. The postprandial plasma glucose level was measured at 7th, 7.5 and 8th hour of the study. The test result indicates that after second maltose load, there has been no significant increase in postprandial plasma glucose level observed in Group IV animals. The observed plasma glucose level at 7th, 7.5 and 8th hour are 185.67 mg/dL, 185.83 mg/dL and 176.33 mg/dL respectively. After second maltose load, the blunt postprandial plasma glucose peak has been seen. This indicated that the newly formulated Miglitol nanoparticles significantly (P<0.001) reduce the postprandial plasma glucose level after second maltose load when compared with marketed formulation.

Again third maltose load has been given at 12th hour of the study. The postprandial plasma glucose level was measured at 13th, 13.5 and 14th hour of the study.

The test result indicates that after third maltose load, there is no significant increase in postprandial plasma glucose level observed in Group IV animals. The observed plasma glucose level at 13th, 13.5 and 14th hour are 190.00 mg/dL, 184.67 mg/dL and 179.50 mg/dL respectively. After third maltose load, the blunt postprandial plasma glucose peak has been seen. This indicated that the newly formulated Miglitol nanoparticles significantly (P<0.001) reduce the postprandial plasma glucose level after third maltose load as well when compared with marketed formulation.

In diabetic rats there was a clear reduction in blood glucose after the initial administration of drug. After initial administration of drug, the pharmacological actions are similar for marketed conventional release dosage form and Nanoparticle. However, the immediate release dosage form could not control the peak postprandial plasma glucose after second & third loading of maltose (after 6 Hrs & 12 Hrs respectively). Whereas, the Nanoparticle formulation significantly (P<0.001) reduced the peak postprandial plasma glucose after second & third loading of maltose (after 6 Hrs & 12 Hrs respectively). Miglitol nanoparticles pharmacological action has been sustained significantly and reduced peak postprandial plasma glucose up to about 12 Hrs. The marketed conventional release dosage form pharmacological action could not sustained more than 3 Hrs.

Hence this study clearly indicates that the newly prepared nanoparticle formulation can significantly reduce peak postprandial plasma glucose up to 12 Hrs.

DISCUSSION

Miglitol is a reversible inhibitor of a-Glucosidase enzymes (e.g., sucrase, trehalase, glucoamylase, isomaltase) in the brush border of the small intestine. These enzymes are responsible for breaking down complex carbohydrates, such as sucrose and starch, into glucose and other monosaccharide that can be absorbed in the small intestine. Miglitol does not produce any action on glucose and other monosaccharide it's only prevents the conversion of complex carbohydrates to monosaccharide. Hence instead of glucose, maltose has been selected for this study. (Bischoff, 1994; .M. A. Tormo *et al.,* 1998).

For Group III and group IV animals, after first maltose load, there is no significant increase in postprandial plasma glucose level observed. The postprandial plasma glucose level for Group III and group IV animals are respectively 176.17 mg/dL and 185.67 mg/dL, where as in the same time period, postprandial plasma glucose level for Group II (Untreated diabetic control) is 264.00 mg/dL.

When an oral maltose overload was administered in the presence of Miglitol, We observed a reduction in the appearance of glucose in the blood. These results are similar to those found by other authors in humans and laboratory animals. (M.A Tormo *et al.,* 1998; Lembcke B *et al.,* 1991). It is clear that, in accordance with data in the literature, the observed reduction in blood glucose after the oral maltose overload is due to inhibition of the intestinal hydrolysis of maltose and the resulting effect of inhibiting glucose absorption (M.A.Tormo *et al.,* 1998).

After second and third maltose load, there is no significant reduction in postprandial plasma glucose level observed in Group III animals which are treated with marketed conventional release dosage form. The observed postprandial plasma glucose level is 289.83 mg/dL which is similar to untreated Diabetic control animals (Group III). This because of unavailability of Miglitol at site of action (small intestine) to produce required pharmacological action. The elimination half-life of Miglitol is two to three hours (Ahr HJ *et al.,* 1997). Whereas, group IV animals, which are treated with Miglitol nanoparticles, we could not observe any steep postprandial glucose peak concentrations after second and third maltose load as well. The Nanoparticle formulation significantly ($P<0.001$) controlled the peak postprandial plasma glucose after second & third loading of maltose (after 6 Hrs & 12 Hrs respectively). This is due to availability of Miglitol at site of action (small intestine) to produce required pharmacological action. Miglitol nanoparticle was coated with Eudragit and Eudragit contains quaternary ammonium group which may provide positive surface charge and effective adhesion of nanoparticles to the negatively charged mucous membrane in the GI track. (Pankaj Ranjan Karn *et al.,* 2011). Hence Eudragit ensured the availability of drug for 12 Hrs at the site of action. Further the Miglitol was loaded with PLGA nanoparticle which facilitate the control release of drug for the prolonged period of time (i.e., up to 12 Hrs). Hence the Miglitol nanoparticles pharmacological action has been sustained and releases the drug significantly and reduced peak postprandial plasma glucose up to about 12 Hrs.

CONCLUSION

Miglitol Nanoparticle was evaluated with diabetic rats along with the marketed conventional release Miglitol tablets. The present study results demonstrated that Miglitol Nanoparticle significantly reduced the peak postprandial plasma glucose. Miglitol nanoparticles pharmacological action has been significantly sustained by prolonged release of drug and reduces the peak postprandial plasma glucose up to about 12 Hrs. The marketed conventional release Miglitol dosage form pharmacological action could not sustained more than 3 Hrs. Hence this study clearly indicates that the newly prepared Miglitol nanoparticle formulation can produce the required pharmacological action up to 12 Hrs.

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