

## Interaction of Insulin with Prokinetic Drugs in STZ-induced Diabetic Mice

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### ABSTRACT

**AIM:** Prokinetic drugs have been used for gastroparesis in diabetic patients for a relatively long time already and some data about the interactions with insulin in the clinic should be available. To study the possible interactions of metoclopramide, domperidone or erythromycin in streptozotocin (STZ)-induced diabetic mice treated with insulin in different parameters.

**METHODS:** Effects of the individual as well as combined drugs were studied in diabetic mice via estimation of the blood glucose and serum insulin levels, small intestinal transit, gastric emptying, xylose absorption and glucose tolerance tests. The groups included were normal control, diabetic control, insulin 2 IU/kg (s.c.), metoclopramide 20 mg/kg (p.o.), domperidone 20 mg/kg (p.o.) and erythromycin 6 mg/kg (p.o.) individually and in combination. The first set of experiments was carried out to investigate the subchronic effect of one week of daily dose of the tested drugs individually as well as the combination of insulin with each prokinetic drug in diabetic mice on blood glucose and serum insulin levels. The other five sets of experiments were carried out to investigate the acute effect of a single dose of each drug individually and in combination on blood glucose and serum insulin levels, small intestinal transit, gastric emptying, oral xylose absorption and glucose tolerance tests.

**RESULTS:** The study included test prokinetic drugs i.e., metoclopramide (20 mg/kg), domperidone (20 mg/kg) and erythromycin (6 mg/kg), as well as insulin (2 IU/kg), which was individually effective in decreasing SIT, enhancing GE and increasing xylose absorption significantly ( $P<0.05$ ) in diabetic mice.

Erythromycin tended to decrease blood glucose level and increase serum insulin level after one week of daily dose administration in diabetic mice. Erythromycin potentiated the effect of insulin given on blood glucose level and serum insulin level where other prokinetic agents failed to do so after repeated dose administration in diabetic mice. Metoclopramide or erythromycin in combination with insulin significantly ( $P<0.05$ ) decreased small intestinal transit in diabetic mice, which was less than that of insulin alone. Administration of test prokinetic drugs along with insulin antagonized the action of insulin on xylose absorption. These combinations also increased the rate of glucose absorbed from the gut.

**CONCLUSIONS:** The present study suggests that prokinetic drugs could potentially improve glycemic control in diabetic gastroparesis by allowing a more predictable absorption of nutrients, matched to the action of exogenous insulin. The use of prokinetics i.e. erythromycin may be interesting in the clinic in order to decrease the need for insulin in diabetic patients. The dose of insulin may be safely decreased with erythromycin in chronic treatments.

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**Key words:** Streptozotocin; Gastrointestinal motility; Insulin; Prokinetic drugs; Intestinal absorption

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### INTRODUCTION

Diabetes mellitus is the most common cause of gastroparesis and disturbed gastric and small intestine motility. Gastroparesis is a syndrome characterized by delayed gastric emptying in absence of mechanical obstruction of stomach. The cardinal symptoms include postprandial fullness (early satiety), nausea, vomiting, bloating, or epigastric pain. Symptoms attributable to gastroparesis are reported by 5 to 12% of patients with diabetes<sup>[1,2]</sup>. There is an association between self-reported glycemic control and psychological distress and development of gastrointestinal symptoms in diabetics<sup>[3]</sup>. Impaired function of the gastrointestinal tract related to diabetes mellitus results from diabetic autonomic neuropathy, impaired sensory innervation and a direct effect of persistent hyperglycemia<sup>[4]</sup>. Once established, diabetic gastroparesis tends to persist, despite amelioration of glycemic

control. Thus, gastric emptying and symptoms are stable during  $\geq 12$  years follow-up, despite improved glycemic control<sup>[5]</sup>. Gastroparesis affects nutritional state and, in diabetics, it also has deleterious effects on glycemic control and secondary effects on organs that lead to increased mortality<sup>[6]</sup>. First-line treatment includes restoration of nutrition and medications using prokinetic drugs. Therefore, searching for therapeutic interventions of prokinetic drugs that will improve the specific alterations associated with diabetic gastroparesis represents the most important aim of the present study. Prokinetic drugs have been used for gastroparesis in diabetic patients for a relatively long time already and some data about the interactions with insulin in the clinic should be available. It deemed of interest to investigate the possible drug-drug interactions, which may develop from co-administration of insulin and certain prokinetic drugs i.e. metoclopramide, domperidone and erythromycin. Also, the study aims to warn about the possibility that prokinetics might increase the hypoglycemic effect of insulin.

Prokinetic drugs, commonly used to treat delayed gastric emptying, have variable effects on small intestinal motility, and little is known about their effects on glucose absorption. The prokinetic drugs act primarily through neurons since peristalsis is based on neural reflexes. Dopamine antagonists such as metoclopramide and domperidone<sup>[7]</sup> are used in this study. Motilides such as erythromycin enhances peristalsis by acting on motilin receptors<sup>[8]</sup>.

In the present study, streptozotocin (STZ)-induced diabetic mice were treated with insulin, prokinetic agents i.e. metoclopramide, domperidone or erythromycin individually and in combination. Acute and subchronic study were carried out to determine whether the prokinetic drugs could improve the blood glucose level and neuropathy changes in diabetic conditions treated with insulin. This was achieved by measuring some of the biochemical parameters affected by persistent hyperglycemia via estimation of blood glucose and serum insulin levels. Acute study were carried out to determine the effect of the test drugs on the gastrointestinal tract motility represented in small intestinal transit and gastric emptying, knowing that all of the prokinetic drugs used produce acute actions on the gut. The rate of gastric emptying is an important determinant of carbohydrate absorption and thus of the blood glucose profile<sup>[9]</sup>. Oral xylose absorption and glucose tolerance tests were used as representative indices of carbohydrates absorption changes.

## MATERIALS AND METHODS

### Animals

Healthy adult male albino mice weighting between 20-30 g were used in the present study. They were obtained from the animal house of the research department of Kahira pharmaceutical company, Cairo, Egypt. All animals were fed a standard pellet chow and had free access to water. They were maintained under controlled laboratory conditions (temperature, humidity) throughout the study. New groups of mice were recommended for each test done. Animals were sacrificed under mild ether anesthesia. Experiments were conducted in accordance with the guidelines set by the animal's health research ethics training initiative, Egypt.

### Drugs and Reagents

Insulin (Regular insulin, Novonordisk, Denmark), Metoclopramide (Memphis Pharmaceutical Co., Cairo, Egypt), Domperidone (El Kahira Pharmaceutical Co., Cairo, Egypt) and Erythromycin ethylsuccinate (Abbott Laboratories, Cairo, Egypt) were obtained. Glucose Reagent Kit (Biomérieux, France), Insulin IRMA Kit IM3210 (Immunotech Beckman coulter, Czech Republic), Streptozotocin (Sigma Aldrich Chemie, Germany), Phloroglucinol (Sigma chemical Co., USA) and D-xylose (Acros Organics, USA)

were used. Insulin injection was diluted with normal saline solution for obtaining a suitable strength. Hydroxypropylmethylcellulose (1%) was used as vehicle to administer prokinetic drugs. The other reagents were the highest grade of commercially available products.

### Drug treatments

Control groups received the equal volumes of vehicle through corresponding routes. The groups included were normal control, diabetic control, insulin 2 IU/kg (s.c.), metoclopramide 20 mg/kg (p.o.), domperidone 20 mg/kg (p.o.) and erythromycin 6 mg/kg (p.o.) individually and in combination. The doses were selected based on the earlier reports and recommended clinical doses and prior pilot experiments<sup>[10,11,12]</sup>. Metoclopramide, domperidone or erythromycin in the dose mentioned above were given alone 15 minutes before the administration of insulin/vehicle. Insulin was given 50 minutes before determination of blood glucose and serum insulin levels. Six main sets of experiments were carried out. The first set of experiments carried out to investigate the subchronic effect of one week of daily dose of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin in STZ-induced diabetic male mice on blood glucose and serum insulin level. The other five sets of experiments carried out to investigate the acute effect of a single dose of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin on blood glucose and serum insulin level, small intestinal transit, gastric emptying, oral xylose absorption and glucose tolerance tests.

### Experimental induction of diabetes

Diabetes mellitus was induced in overnight fasted mice by a single intraperitoneal injection of freshly prepared solution of streptozotocin 100 mg/kg body weight in 0.1 M cold citrate PH 4.5<sup>[13,14]</sup>. The animals were allowed to drink 5% glucose solution to overcome STZ-induced hypoglycemia<sup>[15]</sup>. The control mice were injected with citrate buffer alone. Two week following STZ injection, blood samples were collected from the tail venopuncture of the mice and used for the estimation of blood glucose levels using advanced Glucometer ACCU-CHEK (Roche, Germany)<sup>[16,17]</sup>. Overnight fasted mice with blood glucose level above 200mg/dL were selected and used in the present study.

### Measurement of blood glucose and serum insulin levels

Blood was collected from retro-orbital venous plexus according to the method of Cocchetto<sup>[18]</sup>. Blood was collected using heparinized microhematocrit capillaries into Wassermann tubes. Blood glucose level was measured using advanced Glucometer ACCU-CHEK. Serum was separated by centrifugation at 11000 rpm for 2 min. and serum glucose level was determined immediately using glucose kit<sup>[19]</sup>. There is no significant difference in glucose levels between the two methods. The remaining amount of serum was kept frozen at -20°C for insulin determination. Serum insulin was estimated by immunoradiometric assay (IRMA) technique<sup>[20]</sup> using insulin IRMA Kit. This estimation was done 2 min before drug/vehicle administration and 50 min after insulin/vehicle administration.

### Small intestinal transit (SIT)

The passage of a charcoal meal through the gastrointestinal transit in mice was used as parameter for intestinal motility<sup>[21,10]</sup>. Overnight fasted mice were treated with test prokinetic drug orally 45 min and/or insulin subcutaneously 30 min before administration of charcoal

meal (0.3 mL of a 5% suspension of charcoal in 2% hydroxypropylmethylcellulose solution). After 20 min, animals were killed by cervical dislocation just after mild ether anesthesia. Abdomen opened, the charcoal marker was identified in the small intestine and tied immediately to avoid movement of marker. The entire intestine was removed by cutting at pyloric and ileocaecal ends and then washed in water. The distance the meal was traveled through the intestine as indicated by the charcoal is measured and expressed as percent of the total distance from the pylorus to the caecum.

Small intestinal transit (SIT) = (Distance travelled by charcoal / Total length of the small intestine) × 100.

### Gastric emptying

Gastric emptying was determined by the phenol red method<sup>[22,12]</sup>. The test prokinetic drug was given alone 45 min before administration of phenol red meal. Insulin (s.c.) was injected 30 min before the administration of the meal. A solution of 1.5% Hydroxypropylmethylcellulose containing 0.05% phenol red as a marker was given intragastrically (0.5 mL/mouse) to overnight fasted mice. 15 min later, Animals were sacrificed by cervical dislocation just after mild ether anesthesia. The abdominal cavity was opened, and the cardiac and pyloric ends of the stomach were clamped, then the stomach was removed and washed with normal saline. The stomach was cut into pieces and homogenized with 25 mL of 0.1 N NaOH. The suspension was allowed to settle for 1 h, and then 5 mL of the supernatant was added to 0.5 mL of 20% trichloroacetic acid (w/v) and centrifuged at 3000 rpm for 20 min. To one ml of supernatant 4 mL of 0.5 N NaOH was added. The absorbance of this pink colour liquid was measured using spectrophotometer at 560nm (Model: Shimadzu 150-20). Phenol red recovered from animals that were sacrificed immediately after administration of the test meal was used as a standard (0% emptying). Gastric emptying (%) in the 15 min period was calculated according to the following equation: Gastric emptying (GE) % = 100 - (X × Y - 1 × 100); X = Absorbance of phenol red recovered from the stomach of animals sacrificed 15 min after test meal; Y = mean (n=5) absorbance of phenol red recovered from the stomachs of control animals (killed at 0 min following test meal).

### Oral D-xylose Loading Test

This test measures intestinal carbohydrates absorption by calculating the plasma concentration of D-xylose after ingestion of a known amount of d-xylose<sup>[23,24]</sup>. The test prokinetic drug was given alone 45 min before administration of xylose. Insulin (s.c.) was injected 30 min before the administration of xylose. A 30% solution containing D-xylose (0.8 g/kg body weight) was administered by oral gavage to overnight fasted mice. After 60 min of xylose administration, blood samples were drawn from retro-orbital venous plexus and blood samples were centrifuged at 11 000 rpm for 2 min, plasma xylose concentrations were measured using a colorimetric assay. The assay involved incubation of 20 µL of plasma with 1 mL of colour reagent containing 1 g phloroglucinol in 200 mL glacial acetic acid and 20 mL concentrated HCL and heated for 4 min at 100°C, followed by reading of the absorbance at 554 nm using spectrophotometer (Model: Shimadzu 150-20).

### Oral Glucose Tolerance Test

The OGTT was used to evaluate intestinal absorption. The test was carried out according to the method of Stümpel *et al* and Sachin *et al*<sup>[25,26]</sup>. After the mice were fasted for 12 h, the test compound was administered half an hour before the glucose loading. A 50%

glucose solution (2.5 g/kg of body weight) was orally administered, and blood was taken from the tail vein at 0, 30, 60 and 120 min afterward. Blood glucose concentrations were determined immediately using an Accu-chek (Roche Diagnostics, Germany). The difference between the value of the diabetic control group and the diabetic treated groups represent the amount of glucose absorption from the intestine affected by the different drugs used in this study in addition to other factors. The extent of absorption of the glucose was estimated using the total area under the curve, which represents blood glucose level from t0 to t120. AUC<sub>total</sub> is calculated using the trapezoidal rule from t0 to t120.

### Statistical analysis

All data were expressed as the mean ± standard error with 6 to 10 mice per group. Statistical analysis was performed using two ways analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. For all the statistical tests, the level of significance was fixed at  $P < 0.05$ .

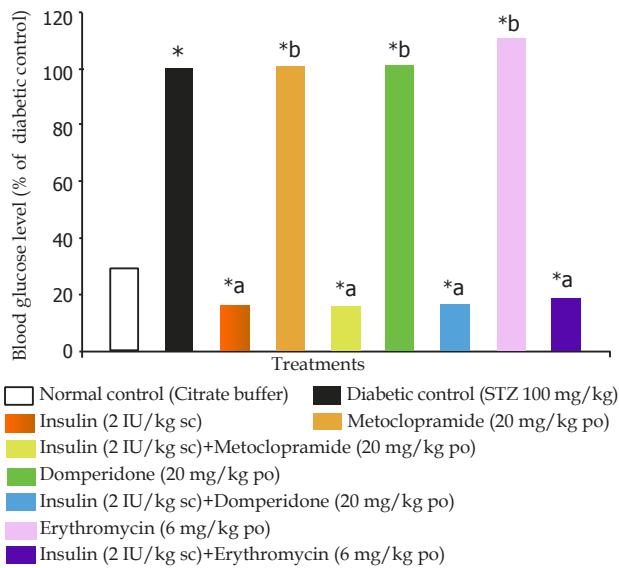
## RESULTS

### *Effects of insulin and certain prokinetic drug individually or combined on blood glucose and serum insulin levels in STZ-induced diabetic mice.*

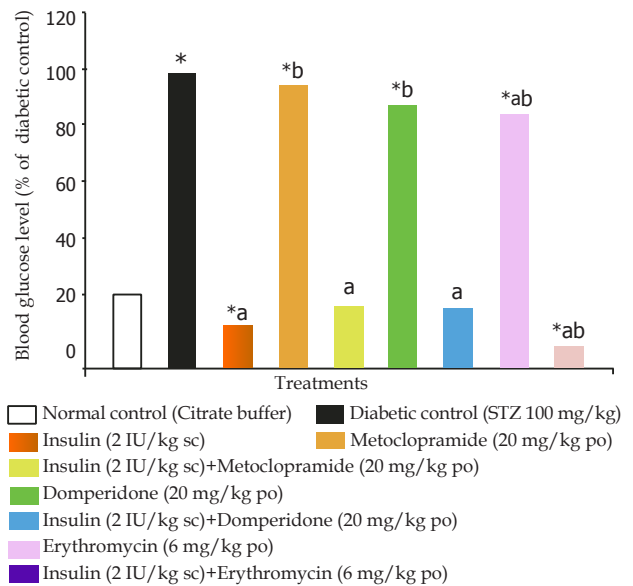
Figure 1 and Figure 3 showed antihyperglycemic effect of insulin against STZ-induced diabetic mice. Acute administration of insulin (2 IU/kg) significantly ( $p < 0.05$ ) decreased blood glucose level to 45.37 ± 4.57 mg/dL and increased serum insulin level to 1.96 ± 0.1 uIU/kg in diabetic mice close to hypoglycemic value. Acute effect of single dose of Metoclopramide (20 mg/kg), Domperidone (20 mg/kg) or erythromycin (6 mg/kg) individually did not affect blood glucose level and serum insulin level in diabetic mice. Acute effect of metoclopramide, domperidone or erythromycin did not affect the action of insulin on blood glucose and serum insulin level (Figure 1, Figure 2). Erythromycin tended to decrease blood glucose level and increase serum insulin level after one week of daily dose administration in diabetic mice. Daily dose administration of insulin (2 IU/kg) for one week significantly ( $p < 0.05$ ) decreased blood glucose level to 45.94 ± 2.6 mg/dL and increased serum insulin level to 2.01 ± 0.02 uIU/kg in diabetic mice close to hypoglycemic value. There is no interaction between insulin and the test prokinetic namely metoclopramide or domperidone on blood glucose level and serum insulin level after one week of daily dose administration in diabetic mice. On the other hand, Combination of insulin and erythromycin significantly ( $p < 0.05$ ) decreased blood glucose level to 22.9 ± 1.91 mg/dL and increased serum insulin level to 2.18 ± 0.12 uIU/kg in diabetic mice (Figure 3, Figure 4).

### Small intestinal transit

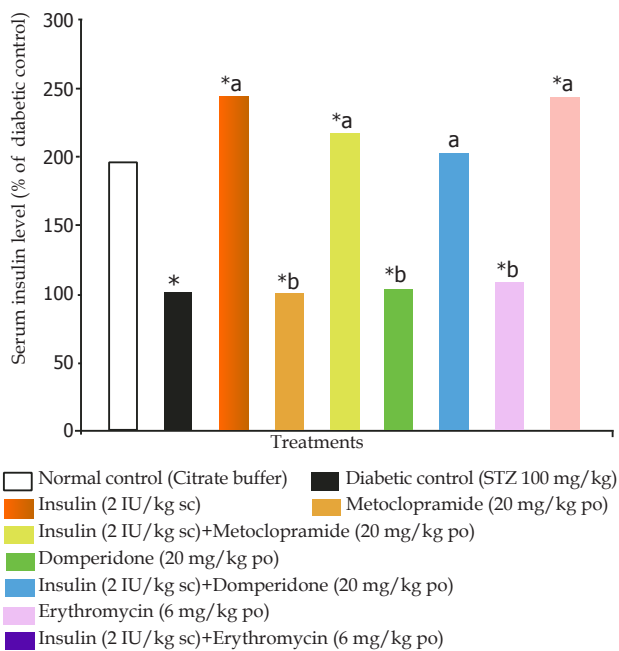
The normal control value of the small intestinal transit was 56.61 ± 2.58 % of the total length of the small intestine. Induction of diabetes in mice significantly ( $p < 0.05$ ) increased SIT to 76.9 ± 6.12%. Insulin at dose (2 IU/kg) significantly ( $p < 0.05$ ) decreased SIT in diabetic mice to 61.05 ± 3.85% as compared to diabetic control group. The test prokinetic drugs namely metoclopramide (20 mg/kg), domperidone (20 mg/kg) and erythromycin (6 mg/kg) significantly ( $p < 0.05$ ) decreased SIT in the diabetic mice to 50.04 ± 2.42%, 48.7 ± 4.53%, and 43.05 ± 3.5% respectively. Either of metoclopramide or erythromycin in combination with insulin significantly ( $p < 0.05$ ) decreased SIT in diabetic mice and this effect was less than that of insulin alone. Domperidone did not affect the action of insulin on SIT in diabetic mice (Table 1).



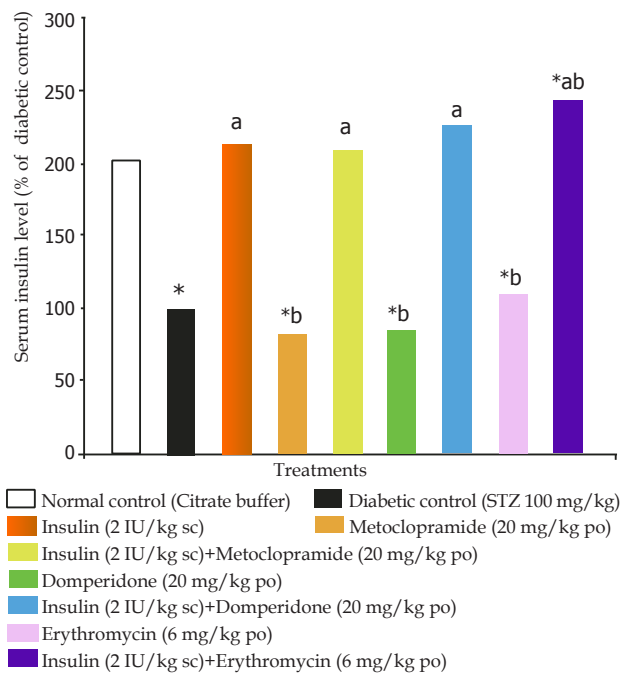
**Figure 1** Acute effect of a single dose of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin on blood glucose level in STZ-induced diabetic mice. Values represent the mean±s.e.m. of eight mice per group. \* Significantly different from the normal control value at  $P<0.05$ . <sup>a</sup> Significantly different from the diabetic control value at  $P<0.05$ . <sup>b</sup> Significantly different from insulin value at  $P<0.05$ .



**Figure 3** Subchronic effect of one week daily dose administration of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin on blood glucose level in STZ-induced diabetic mice. Values represent the mean±s.e.m. of eight mice per group. \* Significantly different from the normal control value at  $P<0.05$ . <sup>a</sup> Significantly different from the diabetic control value at  $P<0.05$ . <sup>b</sup> Significantly different from insulin value at  $P<0.05$ .



**Figure 2** Acute effect of a single dose of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin on serum insulin level in STZ-induced diabetic mice. Values represent the mean±s.e.m. of eight mice per group. \* Significantly different from the normal control value at  $P<0.05$ . <sup>a</sup> Significantly different from the diabetic control value at  $P<0.05$ . <sup>b</sup> Significantly different from insulin value at  $P<0.05$ .



**Figure 4** Subchronic effect of one week daily dose administration of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin on serum insulin level in STZ-induced diabetic mice. Values represent the mean±s.e.m. of eight mice per group. \* Significantly different from the normal control value at  $P<0.05$ . <sup>a</sup> Significantly different from the diabetic control value at  $P<0.05$ . <sup>b</sup> Significantly different from insulin value at  $P<0.05$ .

### Gastric emptying

The normal control value of the gastric emptying was  $72.50 \pm 1.68$  % of the total amount of the phenol red meal given. High blood glucose level in diabetic control mice delayed GE significantly ( $p<0.05$ ) to  $55.23 \pm 9.30$ %. Insulin (2 IU/kg), metoclopramide (20 mg/kg), domperidone (20 mg/kg) or erythromycin (6 mg/kg) in the doses employed increased gastric emptying significantly ( $p<0.05$ )

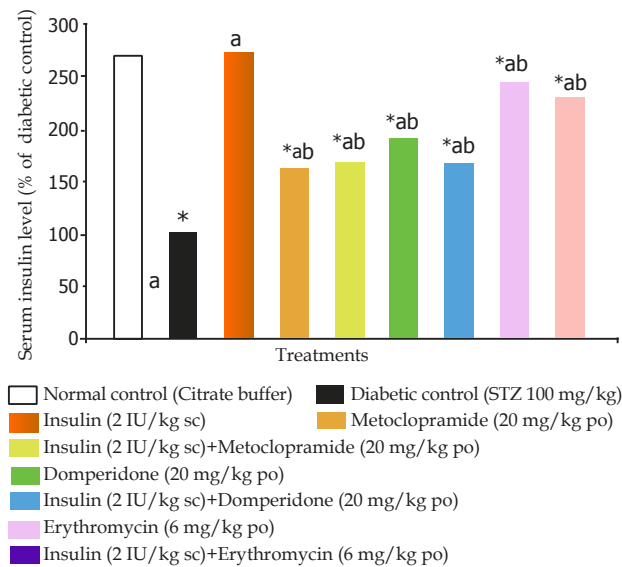
to  $95.87 \pm 2.41$ %,  $76.38 \pm 7.67$ %,  $90.92 \pm 4.92$ % and  $84.77 \pm 2.11$ % respectively compared with diabetic control mice. Administration of prokinetic drugs namely metoclopramide, domperidone or erythromycin along with insulin (2 IU/kg) did not affect the action of insulin on GE (Table 1).



**Table 1** Effects of insulin and prokinetic drugs alone and in combination on small intestinal transit and gastric emptying in diabetic mice.

Treatments	SIT (%)	GE (%)
Normal control(Citrate buffer)	56.61±2.58	72.50±1.68
Diabetic control (STZ 100 mg/kg)	76.90±6.12 *	55.23±9.30 *
Insulin (2 IU/kg s.c.)	61.05±3.85 <sup>a</sup>	95.87±2.41 <sup>ab</sup>
Metoclopramide (20 mg/kg p.o.)	50.04±2.42 <sup>ab</sup>	76.38±7.67 <sup>ab</sup>
Insulin (2 IU/kg s.c.)+Metoclopramide (20 mg/kg p.o)	54.30±3.46 <sup>a</sup>	94.91±1.01 <sup>ab</sup>
Domperidone (20 mg/kg p.o.)	48.70±4.53 <sup>ab</sup>	90.92±4.92 <sup>ab</sup>
Insulin (2 IU/kg s.c.)+Domperidone (20 mg/kg p.o)	62.60±3.07 <sup>a</sup>	92.87±1.14 <sup>ab</sup>
Erythromycin (6 mg/kg p.o.)	43.05±3.50 <sup>ab</sup>	84.77±2.11 <sup>a</sup>
Insulin (2 IU/kg s.c.)+Erythromycin (6 mg/kg p.o)	49.14±4.57 <sup>ab</sup>	90.86±3.20 <sup>ab</sup>

Values represent the mean±SE. of eight mice per group. \* Significantly different from the normal control value at  $P<0.05$ ; <sup>a</sup> Significantly different from the diabetic control value at  $P<0.05$ . <sup>b</sup> Significantly different from insulin value at  $P<0.05$ .



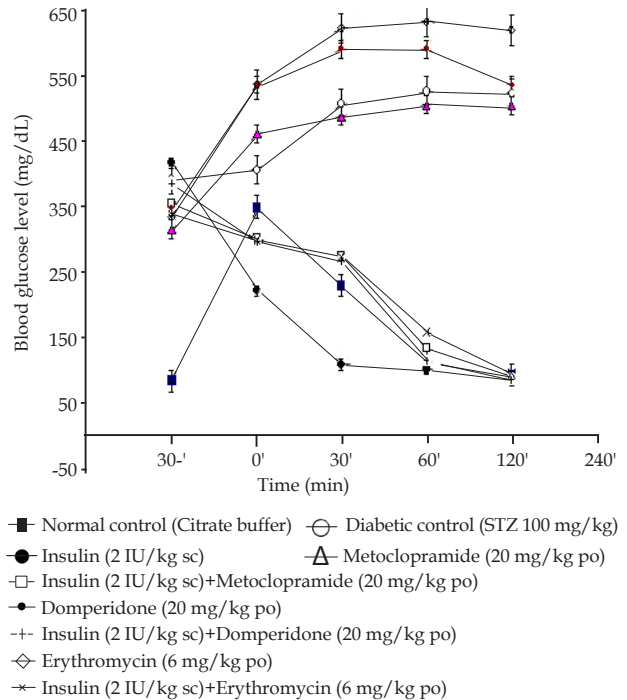
**Figure 5** Effect of a single dose of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin on oral d-xylose absorption test in STZ-induced diabetic mice. Values represent the mean ± s.e.m. of eight mice per group. \* Significantly different from the normal control value at  $P<0.05$ . <sup>a</sup> Significantly different from the diabetic control value at  $P<0.05$ . <sup>b</sup> Significantly different from insulin value at  $P<0.05$ .

**Oral d-xylose absorption test**

The normal control value of serum d-xylose concentration was 1.63 ±0.10 mg/mL after 60 min of d-xylose administration (0.8 g/kg). The amount of xylose absorbed from the GIT significantly ( $p<0.05$ ) decreased in the diabetic mice to 0.606±0.03 mg/mL as compared to normal control group. Insulin (2 IU/kg), metoclopramide (20 mg/kg), domperidone (20 mg/kg) and erythromycin (6 mg/kg) in the doses employed individually increased xylose absorption to 1.64±0.16 mg/ml, 0.989±0.03 mg/mL, 1.162±0.03 mg/mL and 1.469±0.03 mg/mL respectively. Administration of prokinetic drugs i.e. metoclopramide, domperidone or erythromycin along with insulin antagonized the action of insulin (2 IU/kg) on xylose absorption (Figure 5). There is antagonism between insulin and the test prokinetic drugs on xylose absorption in diabetic mice.

**Oral d-glucose tolerance test**

The oral glucose tolerance test (OGTT) can be used to evaluate blood glucose homeostasis and also indirectly evaluate glucose absorption. As shown in figure 6, glucose load (2.5 gm/kg) in normal mice produced rapid increase in blood glucose levels at 30min and returned to baseline values within 120 min. In contrast, STZ-induced

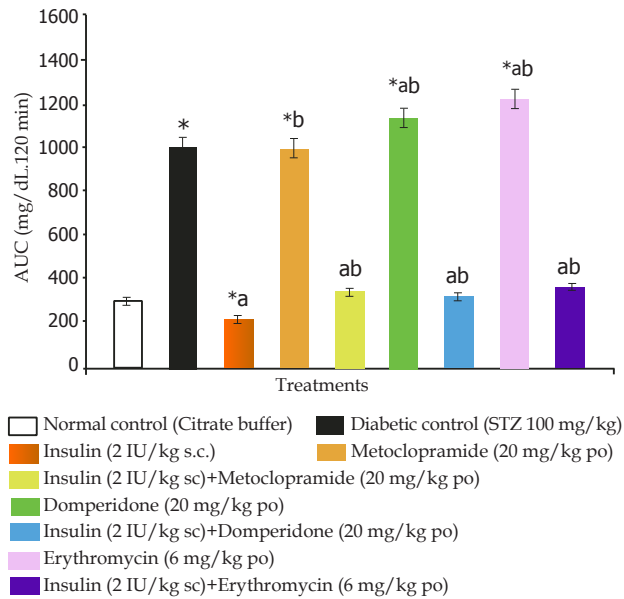


**Figure 6** Effect of a single dose of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin on oral d-glucose tolerance test in STZ-induced diabetic mice. Values represent the mean±s.e.m. of eight mice per group. \* Significantly different from the normal control value at  $P<0.05$ . <sup>a</sup> Significantly different from the diabetic control value at  $P<0.05$ . <sup>b</sup> Significantly different from insulin value at  $P<0.05$ .

diabetic mice demonstrated basal hyperglycemia (399±14 mg/dL) which remained above 400 mg/dL during all time points determined. The peak increase in serum glucose concentrations in diabetic mice was observed after 60 min of glucose treatment, while that of normal mice observed after glucose loading, indicating delayed glucose homeostasis in diabetic mice. STZ significantly ( $p<0.05$ ) increased the area under the curve (Figure 7). Insulin at dose (2 IU/kg) significantly ( $p<0.05$ ) decrease blood glucose level to 107.16±8.51 mg/dl and 100 mg/dL after 30 min and 60 min of glucose loading resp. and the effects persisted till 120 min (Figure 6). The area under the curve was significantly reduced to 226.53±12.28 mg/dL. 120 min (Figure 7). Metoclopramide at dose (20mg/kg) did not affect blood glucose level where BGL was 487.5±13.6 mg/dL and 505.5±14.55 mg/dL after 30 min and 60 min of glucose loading respectively. While domperidone at dose 20 mg/kg and erythromycin at dose 6 mg/kg produced significant ( $p<0.05$ ) increase in blood glucose level reach to 590±13 mg/dL, 590.8±17.4 mg/dL and 622±23.11 mg/dL, 631.5±21.48 mg/dL after 30 min and 60 min of glucose loading respectively (Figure 6). In addition, domperidone and erythromycin significantly ( $p<0.05$ ) increased the area under the curve (Figure 7). Administration of metoclopramide, domperidone or erythromycin along with insulin significantly ( $p<0.05$ ) increases blood glucose level as compared to insulin treated value (Figure 6). Combination of insulin with metoclopramide, domperidone or erythromycin significantly ( $p<0.05$ ) increased the area under the curve as compared to insulin treated value (Figure 7).

**DISCUSSION**

Findings of the present investigation revealed that STZ-induced diabetes resulted in a significant increase in small intestinal transit



**Figure 7** Effect of a single dose of insulin, metoclopramide, domperidone and erythromycin individually as well as the combination of insulin with metoclopramide, domperidone or erythromycin on the area under the curve of blood glucose concentrations during oral glucose absorption test in STZ-induced diabetic mice. Values represent the mean  $\pm$  s.e.m. of eight mice per group. \* Significantly different from the normal control value at  $P < 0.05$ . <sup>a</sup> Significantly different from the diabetic control value at  $P < 0.05$ . <sup>b</sup> Significantly different from insulin value at  $P < 0.05$ .

and significant decrease in gastric emptying. Abnormalities in gastric emptying and small intestinal motor functions were also reported in diabetic mice<sup>[27,28]</sup>. The mechanism of action of increased intestinal transit may be, partially, due to increased cholinergic and decreased beta-adrenergic receptor activities in diabetic animals<sup>[29]</sup>. The delay in gastric emptying could be partially attributed to the decrease in the number of myenteric neurons in stomach as a result of diabetes<sup>[30,31,32]</sup>. Similarly, the increased in intestinal transit could be mediated through the same mechanism. All of the stomach's smooth muscle cells have the ability to produce electric depolarizations "slow waves" from resting potential. These rhythmic contractions are thought to originate in the non-smooth muscle pacer cells in the interstitial cells of Cajal<sup>[33]</sup>. Gastric emptying is delayed because the number of gastric interstitial cells (ICCs) is markedly diminished in diabetes<sup>[34]</sup>. Data of the current study showed that insulin induce hypoglycemia significantly attenuated small intestinal transit and accelerated gastric emptying in diabetic mice. These results are partly in agreement with earlier reports<sup>[35,36,37,38]</sup>. The mechanism of action of the obtained results might be due to direct effect of insulin and not only due to antidiabetic effect of insulin that decreased blood glucose level leading to decrease SIT. This effect could be due to counter-regulation of hypoglycemia. The mechanisms of insulin on stomach could be mediated via insulin stimulant effect on the vagus nerve as reported by Quigley *et al.*<sup>[39]</sup>.

Data of the present investigation showed that STZ-induced diabetes resulted in a significant decrease in xylose absorption. This result is in agreement with Fuessl<sup>[40]</sup>. The decrease in xylose absorption could be mediated via decreasing the rate of gastric emptying which resulted from elevation in BGL as reported by the present study. This explanation coincides with that given by Rayner *et al.*<sup>[9]</sup> and Marianne *et al.*<sup>[41]</sup>.

Recent studies have shown that modifications of systemic glycemia in OGTT reflect the activity of the intestinal glucose

transporter SGLT1<sup>[42]</sup>. In the present study, STZ-induced diabetic mice demonstrated basal hyperglycemia which remained above 400 mg/dL during all time points determined. The capacity of the small intestine to absorb glucose increases in experimentally induced diabetic animals as a consequence of the enhanced activity and abundance of SGLT1 as shown by Fedorak *et al.*<sup>[43]</sup> suggesting SGLT1 as a potential target for glycemic control in diabetic animals. STZ-induced diabetic mice exhibited severe hyperglycemia with increased  $\text{Na}^+$  dependent glucose uptake activity compared with normal mice<sup>[44]</sup>. Acute effect of insulin induced hypoglycemia increased xylose absorption and glucose absorption from the GIT in the diabetic mice. In the present study, the effects of insulin on the intestinal absorption of sugar did not differentiate between an effect of insulin on the absorption capacity of the mucosa and other factors that may affect total sugar absorption. Some studies have shown that the effect of insulin leads to increase  $\text{Na}^+$  dependent glucose carrier activity in the small intestine, which leads to increase glucose absorption<sup>[45]</sup>. Some studies reported that insulin induced hypoglycemia accelerates gastric emptying in type 1 diabetes<sup>[37]</sup>, which decrease time of movement of sugar from stomach to the small intestine, in addition to its therapeutic effect by decreases the rate of glucose production and increases the rate of glucose utilization by cells<sup>[46]</sup>.

By studying the prokinetic drugs in the current study individually, domperidone (20 mg/kg po) was found to be the most effective prokinetic agent in the diabetic mice when compared to diabetic control group as well as the other prokinetic drugs. Domperidone, metoclopramide and erythromycin significantly ( $p < 0.05$ ) decreased small intestine transit and accelerated gastric emptying in STZ-induced diabetic mice. Similar results have been reported by recent studies<sup>[47,48,49,50,51]</sup>. The inhibitory effect of domperidone on small intestinal transit is probably mediated via its action on dopamine since it is dopamine antagonist<sup>[52]</sup>. Dopamine has an indirect inhibitory effect via inhibition of cholinergic transmission in the myenteric plexus, which regulates the gastrointestinal tract<sup>[53]</sup>. It could be suggested that metoclopramide produced its action through inhibition of presynaptic and postsynaptic  $\text{D}_2$  receptors, stimulation of presynaptic excitatory 5-HT<sub>4</sub> receptors and/or antagonism of presynaptic inhibition of muscarinic receptors. The aforementioned are in accordance with that given by Valenzuela *et al.*<sup>[54]</sup>. The action of erythromycin is probably mediated via its agonistic activity to motilin receptors, which accelerates gastric emptying<sup>[8,33]</sup>.

Metoclopramide significantly increased xylose absorption but did not affect glucose absorption in STZ-induced diabetes. These finding is in harmony with that given by Paul *et al.*<sup>[55]</sup>. The action of metoclopramide is mediated via increased plasma concentrations of glucagon like peptide-1 and glucose dependant insulinotropic polypeptide, which responsible for delay in glucose absorption, this action did not affect rate of xylose absorption. Domperidone (20 mg/kg) and erythromycin (6 mg/kg p.o.) significantly increased xylose absorption and glucose absorption in the diabetic mice as compared to diabetic control group. The effect of erythromycin could be mediated through the action of erythromycin on motilin receptors in the GIT. The action of erythromycin is probably mediated via its agonistic activity to motilin receptors, which accelerates gastric emptying and increases the rate of sugars absorption.

Combination of domperidone (20 mg/kg), metoclopramide (20 mg/kg) or erythromycin (6 mg/kg) with insulin (2 IU/kg) decreased the amount of xylose absorbed from the GIT as compared to insulin given alone in the diabetic mice which indicates antagonistic interaction between each two drugs on xylose absorption. It is difficult to satisfactory explain this action on the bases of the few

lectures available on the two drugs in this respect. Combination of insulin with metoclopramide, domperidone or erythromycin increased glucose absorption from the intestine as compared to the effect of insulin alone in the diabetic mice. These results suggest that combination of prokinetics with insulin may lead to increase Na<sup>+</sup> dependent glucose carrier activity in the small intestine. In addition to treating symptoms, prokinetic drugs could potentially improve glycemic control in diabetic gastroparesis by allowing a more predictable absorption of nutrients, matched to the action of exogenous insulin.

The present study warned about the possibility that prokinetics may increase the hypoglycemic effect of insulin. Erythromycin tended to decrease blood glucose level and increase serum insulin level after one week of daily dose administration in STZ-induced diabetic mice. Erythromycin in the dose of 6 mg/kg p.o. potentiated the effect of insulin on blood glucose and serum insulin levels after one week of daily dose administration in diabetic mice where other prokinetic agents failed to do so after repeated dose administration in diabetic mice. Similar results have been reported by Ueno *et al.*<sup>[56]</sup>. The action of erythromycin on insulin could be mediated via its action as a motilin agonist. It is to be noted that motilin controls cyclic release of insulin through vagal cholinergic muscarinic pathways as reported by Suzuki *et al.*<sup>[57]</sup>. Itoh *et al.*<sup>[58]</sup> found that there are no motilin receptors in the pancreas. Therefore, the action of erythromycin on insulin secretion is probably mediated via vagal-cholinergic muscarinic pathways stimulation linking to serotonergic receptors, a common mechanism in the stimulatory effect of motilin on muscle contraction in the stomach and on pancreatic polypeptide secretion from the endocrine pancreas<sup>[59,60]</sup>.

The action of erythromycin on gastrointestinal motility in the present study is on discrepancy to some studies which state that motilin receptors do not exist in rodents. They only exist as pseudogenes<sup>[61,62]</sup>. However, the present results are in agreement with other studies<sup>[63,64,65]</sup>. It is suggested that the action of erythromycin is mediated by binding to central motilin receptors which might be involved in regulation of gastric motility in diabetic rats.

This study dealt with an important issue concerning the use of prokinetics in insulin-treated diabetic individuals. Not all diabetic patients develop gastrointestinal motility disorders or gastroparesis, but the use of prokinetics might be also interesting in these patients. Erythromycin potentiates the effect of insulin given on blood glucose level and serum insulin level after repeated dose administration in diabetic mice. It seems to suggest that the use of prokinetics i.e. erythromycin might be interesting in the clinic in order to decrease the need of insulin.

In conclusion, combination of insulin with metoclopramide, domperidone or erythromycin increases glucose absorption. And this leads to suggestion that this prokinetic may guard against the risk of severe hypoglycemia associated with diabetic mice treated with insulin. The present study suggests that the use of prokinetics i.e. erythromycin may be interesting in the clinic in order to decrease the need for insulin in diabetic patients. The dose of insulin may be safely decreased with erythromycin in chronic treatments.

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