**ORIGINAL ARTICLE**

# Transdermal Drug Delivery System of Glipizide in Streptozotocin Induced Diabetic Mice

AJAZ FATIMA[1*], SHAZIA ASIM[2*], HAMMAD HUSSAIN [3*], ASIA FIRDAUS[4*], WARDAH SIDDIQUE[5*], ALIYA SHABBIR[6*]
*Professor, Department of Pharmacology, Lahore Medical & Dental College, Lahore*
*Associate Professor, Department of Pharmacology, Lahore Medical & Dental College, Lahore*
*Deputy registrar University of Health Sciences, Lahore*
*Assistant Professor, Department of Pharmacology, Lahore Medical & Dental College, Lahore*
*Assistant Professor, Department of Pharmacology, Lahore Medical & Dental College, Lahore*
*Assistant professor Shalamar Medical and Dental College, Lahore*
*Correspondence to Dr. Shazia Asim: Email: shaziasim33@hotmail.com, Cell: 00923349911022*

## ABSTRACT

**Background:** Glipizide, is the most commonly approved oral hypoglycemic drug in type 2 diabetes mellitus, but it is known to cause fluctuations in bioavailability and poor patient compliance due to frequent dosage and side effects.
**Aim:** In this study comparison of the transdermal and oral administration of glipizide was made, in streptozotocin induced diabetic (SID) albino mice, in terms of hypoglycemic effect and duration of action.
**Methods:** Amongst four groups of albino mice, one group served as normal control while diabetes mellitus was induced in the rest of the three groups by a single intra-peritoneal injection of streptozotocin (STZ). One groups of diabetic mice served as diabetic control while the remaining two were designated as STZ-oral glipizide and STZ-transdermal glipizide (TG) groups on basis of mode of intervention in each of these groups.
**Results:** Changes in blood glucose (BG) levels were recorded in two diabetic groups receiving oral and TG as well as in normal control and diabetic control groups at specific intervals of time for 48 hours. TG group showed significant hypoglycemic activity when compared with orally administered set of mice. Oral glipizide showed sharp fall in glucose levels in blood in the first hours, whereas the decline with the transdermal glipizide at 6 hours was also significant but less as compared to oral glipizide. Increase in bioavailability of glipizide with transdermal application indicated its possibility for use in diabetes mellitus in humans to improve glycemic control and compliance.
**Conclusion:** in this study, matrix design of transdermal delivery system of glipizide, exhibited optimum and sustained decrease in blood glucose levels for 24 hours in STZ induced diabetic mice, in comparison to oral glipizide, which had peak hypoglycemic effect at 6 hours.
**Keyword**: Diabetes Mellitus, Glipizide, Blood Glucose (BG), Transdermal Patch (TP), Transdermal Glipizide (TG)

## INTRODUCTION

Diabetes consist of group of disorders of abnormal carbohydrate metabolism that causes hyperglycemia due to a range of conditions that include impaired insulin secretion or insulin resistance, or both. Worldwide more than 400 million are diabetics. Correct figures for the prevalence of diabetes in Pakistan are not available because only few small scale studies have been carried out in some parts of the country. According to JPMA the current prevalence of type 2 diabetes mellitus in Pakistan is 11.77%, in females 9.19% and among males it is 11.20% (Sultan Ayoub 2016).

The oral hypoglycemic that are currently in use are Sulfonylureas (Insulin secretagogues), Meglitinides (repaglinide), Biguanides (metformin), Alpha-glucosidase inhibitors and Thiazolidinediones. Sulfonylureas is amongst most commonly prescribed oral hypoglycaemic group but have certain limitations due to repeated dosing alterations, side effects like hypoglycaemia, weight gain, gastrointestinal disturbances, liver toxicity etc, and cost of the treatment, which results in poor compliance (Kennedy, 2012). Type 2 diabetic patients, although not dependent on insulin for their survival, but almost one third of them would require insulin for lowering their glucose levels in blood and controlling long-term complications (WHO, 2002).

Amongst many options available, Glipizide is an oral hypoglycaemic agent of sulfonylurea group, which is most commonly used. In experimental animals mechanism of action of glipizide is to increase secretion of insulin from pancreatic beta cells causing in lowering of BG (Pfizer, 2000). UK Prospective Diabetes Study has recognized that, amongst type 2 diabetes patients, an intensive control of blood-glucose by either insulin or sulphonylureas, significantly lowers the risk of micro-vascular complications, such as retinopathy, neuropathy and nephropathy. Therefore, in diabetes, iatrogenic hypoglycemia has been considered as the main complication to long term glycemic control and better quality of life. In type 2 diabetes, risk of hypoglycaemia, frequent dosage and gastrointestinal upsets are the factors resulting in poor compliance to oral hypoglycaemic drugs, especially sulphonylureas.

Due to above mentioned factors the researchers are motivated to find alternate possibilities to find new therapeutic agents e.g. herbs and other bioactive substances. In addition, drugs are also being tried through different routes other than conventional routes (especially through transdermal route), as the skin is a readily

Received on 17-11-2020
Accepted on 27-02-2021

accessible surface for drug delivery. The advantage of TP over conventional routes is that this route is more feasible in achieving consistent serum levels of the drug thus lowering hazards of side effects (Tiwary et al., 2007). Hence, the controlled-release system of such kind delivers a drug at a slower rate for longer period of hours, days or even months rather than just minutes to hours. The dosage formulation contains extra drug than a conventional form of tablet, and needs less frequent dosing and enhanced patient compliance.

Routes of traditional transdermal drug delivery include intercellular, transcellular and follicular routes. Drugs have to penetrate through the stratum corneum, epidermis and dermis of skin to go into the circulation (Wilkosz and Bogner, 2003). For augmented permeation of drugs through stratum corneum, several techniques like micro-poration, chemical penetration enhancers, needle-less jet injections, thermal patches, medicated tattoos, iontophoresis, phonophoresis and liposomal delivery are presently center of promising research (Nanda et al., 2006). Several researchers have estimated pharmacokinetic potential of glipizide for transdermal use mainly through in-vitro permeation studies by full thickness abdominal skin of mouse or Wister rats as membrane (Mutalik et al., 2003, Shankar et al., 2011; Tan et al., 2009; Ghosal et al., 2009). This study shows a comparison of the oral versus transdermal administration of glipizide in SID mice.

## METHODS

Experimental study (Randomized Control Trial Design) was carried out in Experimental Research Laboratory and Department of Pharmacology, University of Health Sciences Lahore, Lahore (UHS).

**Sample size and grouping:** Study was conducted on 40 male albino mice, age between 8-12 weeks and weighing between 30-45 grams, from Experimental Research Laboratory of UHS.

After acclimatization, the study animals were randomly allocated to four groups by balloting method (A, B, C and D), each containing 10 animals. Groups A (normal control group) and B (diabetic control group) served as control and Groups C (STZ-Oral Glipizide Group) and D (STZ- TG Group) were the experimental groups.

**Materials:**

**Chemicals and equipment:** The drugs and chemicals were procured from the different sources. Glipizide, Streptozotocin, Ethyl cellulose and Dibutyl phthalate were bought from Sigma-Aldrich. Polymethyl methacrylate (low molecular weight), Gum acacia2 % suspension and ACCU-CHEK® Performa Glucometer, was from Roche Diagnostics.

**Experimental Procedure:** Group A mice were separated and kept as normal control. Mice in remaining three groups (B, C and D) were starved for 16 hours and diabetes was induced by a single intraperitoneal injection of freshly dissolved streptozotocin (150 mg/kg body weight) in 0.1 M citrate buffer, pH 4.5 (Song et al., 2005).

Following streptozotocin injection, mice were given food and drinking water with 5% glucose solution for 48 hours to control early mortality due to release of the stored insulin from impaired pancreatic islets. One week after streptozotocin injection, BG levels were estimated in mice and those with fasting BG over 250 mg/dl were considered diabetic and were recruited in the research protocol (Takamura et al., 1999).

**Blood sampling and estimation of glucose level in the blood:** BG estimation was done at 0, 6, 12, 24 and 48 hours intervals (Hoff, 2000), by Glucometer (ACCU-CHEK® Performa, Roche Diagnostics) as this required only a tiny drop of blood i.e., < 1 µl of blood (Roche Diagnostics, 2009). Blood was collected from tail vein of mice at intervals of 0, 6, 12, 24 and 48 hours after giving oral glipizide and TG in Groups C and D, and BG levels were estimated. For the untreated Groups A and B, BG levels were measured at comparable intervals.

**Dose translation for glipizide:** Mouse dose of oral glipizide for Group C was calculated using the normalization of body surface area formula (Reagan-Shaw et al., 2007):

Human Equivalent Dose HED (mg/kg) = Animal dose (mg/kg) x Animal $K_m$/ Human $K_m$

$K_m$ value for mice is 3 and for human it is 37 (Food and Drug Administration, 2005).

For example maximum human recommended dose of glipizide is 30 mg/day, calculated around 0.43 mg/kg. From the formula it translated to the mice dose as follows:

Animal dose (mg/kg) =0.43 x 37/3= 5.30 mg/kg

In SID mice within group C, glipizide was given orally at a dose of 5.30 mg/kg/d in 2 % acacia suspension with the help of gavage needle for 2 consecutive days with concurrent recording of BG levels at the intervals stated above.

**Preparation and application of Transdermal Patch:** TP were made according to the guidelines of mercury substrate method, by dissolving Polymethyl methacrylate and Ethyl Cellulose (drug and the polymers) in a ratio of 9.8: 0.2 with dibutyl phthalate (30 % weight/weight of polymer), in chloroform. This solution was transferred into a leveled mercury surface and the organic solvent was evaporated for 24 hours. After this, the excised individual films (4 $cm^2$ and 5 mg of glipizide) were prepared with an aluminum foil support and an adhesive tape (Sridevi et al., 2000). In SID mice in group D, hair in the abdomen of these animals were cleared and TP were applied.

Changes in BG levels were recorded in the two diabetic groups of mice receiving oral and TG as well as in normal control and diabetic control groups at specific intervals of time for 48 hours.

**Statistical analysis:** ANOVA and Tukey HSD (Honestly Significant Difference) test were applied and the results showed that the mean BG values were statistically significant $p< 0.001$, when BG levels of Group-D (STZ induced diabetes+ Transdermal glipizide) were compared with Group-B (Diabetic control group). Group-D was also compared with groups A and C. The effect of TG on glucose level in blood was less as compared to the effect of oral glipizide.

## RESULTS AND DISCUSSION

This study was meant to observe ant hyperglycemic effects of TG in SID mice for duration of 48 hours. As expected,

increase in levels of BG after administration of STZ was detected in Group C (STZ induced diabetes + Oral glipizide). Oral glipizide administration in Group C, lead to a significant decline in BG levels. The mean BG level was ± SEM. 154.00 ± 0.257 mg/dl, 177.2mg/dl, 236.6mg/dl and 263mg/dl at 6 hours, 12 hours, 24 hours, and 48 hours, respectively in Group C. Mean BG values were statistically significant p< 0.001, when comparison of BG levels was made between Group C and Group-B, as shown in figs 1, 2, 3, 4, and 5, respectively.

Similarly, an increase in BG level was observed after administration of STZ into Group D (STZ induced diabetes + TG). Glucose levels in blood significantly decreased after application of TP in this group. The mean BG level was ± SEM. 170.20± 0.257 mg/dl, 175mg/dl, 190.3mg/dl and 237.7mg/dl at 6 hours, 12 hours, 24 hours, and 48 hours, respectively in Group D mice. Mean BG values were statistically significant (p< 0.001), when levels were compared between Group D and Group-B, as shown in figs 1, 2, 3, 4, and 5.

The study shows that maximum hypoglycemic effect in Group C was detected at 6 hours after oral glipizide, followed by gradual and steady rise of glucose (in blood) up to 48 hours as shown in Figures. 1, 2, 3, 4 and 5. In comparison hypoglycemic activity of TG patches in Group D was sustained up to 24 hours followed by rise in BG level as observed at 48 hours, shown in Figures. 1, 2, 3, 4, and 5.

When comparison was made between groups D and C, the hypoglycemic activity of TG in Group D was significant. A maximum hypoglycemic effect in group D was detected at 6 hours and stayed persistent for up to 24 hours. This sustained and gradual hypoglycemic effect was probably due to steady and slow permeation rate of the drug from the device. In Group C, the hypoglycemic effect of oral glipizide reduced slowly after 6 hours. This phenomena was may be due to short half-life of the drug. There was a steep fall in sugar level in blood in the initial hours after oral administration of glipizide, whereas the decline in glusoe with the TG at 6 hours was significant but less as compared to oral glipizide.

The justified rationale behind above mentioned results is probably rapid absorption of glipizide after oral administration as compared to its transdermal application. It may also be supposed that the absorption of glipizide from the transdermal patch was slow but constant. It is hence deducted that glipizide concentration in blood released from transdermal drug delivery system (TDDS) is low as compared to when given through oral route, but on the other hand plasma concentration of glipizide stayed high for prolonged time period with TDDS, which resulted in a sustained reduction in BG level as shown in figs 3 and 4.

A comparison can be made between above results with those of Udupa and Mutalik. Their study showed hypoglycemic activity of membrane moderated TG streptozotacin induced diabetic mice, on comparing with oral glipizide. Udupa and Mutalik told that hypoglycemic effect of oral and TG was significant, P < 0.05, upto 10 and 24 hours, when compared with the control group. Oral glipizide produced a maximum hypoglycemic response at 2 hours, while the same effect was observed after 6 hours in TG treated group. Hypoglycemic effect in transdermal group remained stable for entire duration of their experiment, i.e., 24 hours (Mutalik et al., 2006).

Our study is in confirmation with above mentioned study. Contrastingly, as compared to membrane moderated system with Ethyl vinyl acetate, as rate controlling membrane in afore mentioned study, our experiment intricate matrix type of TDDS.

Irrespective of design of patch and other procedural differences, a surge in bioavailability of glipizide with transdermal application shows its possibility for potential use in diabetes mellitus in humans to improve glycemic control and compliance. However, keeping in view the species differences in structure of mammalian skin and permeation across skin, it would necessitate pharmacokinetic studies in human volunteers to analyze target permeation rate and optimum application area of TG to acquire minimum effective drug concentration,.

Figure 1. Mean BG at 0 hour.

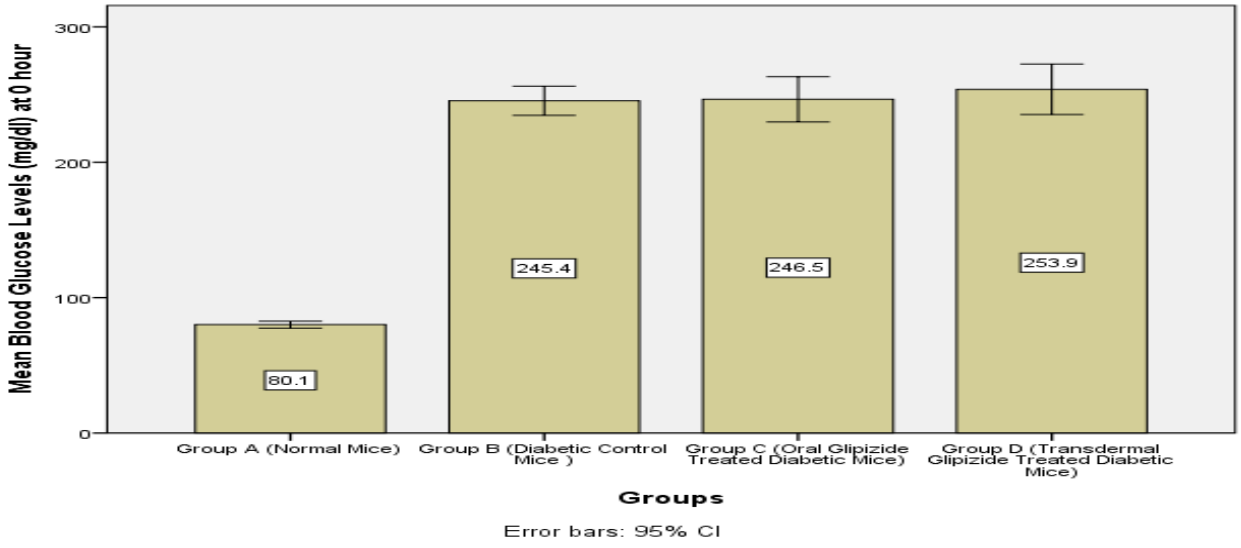


Figure 2. Mean BG at 6 hours.

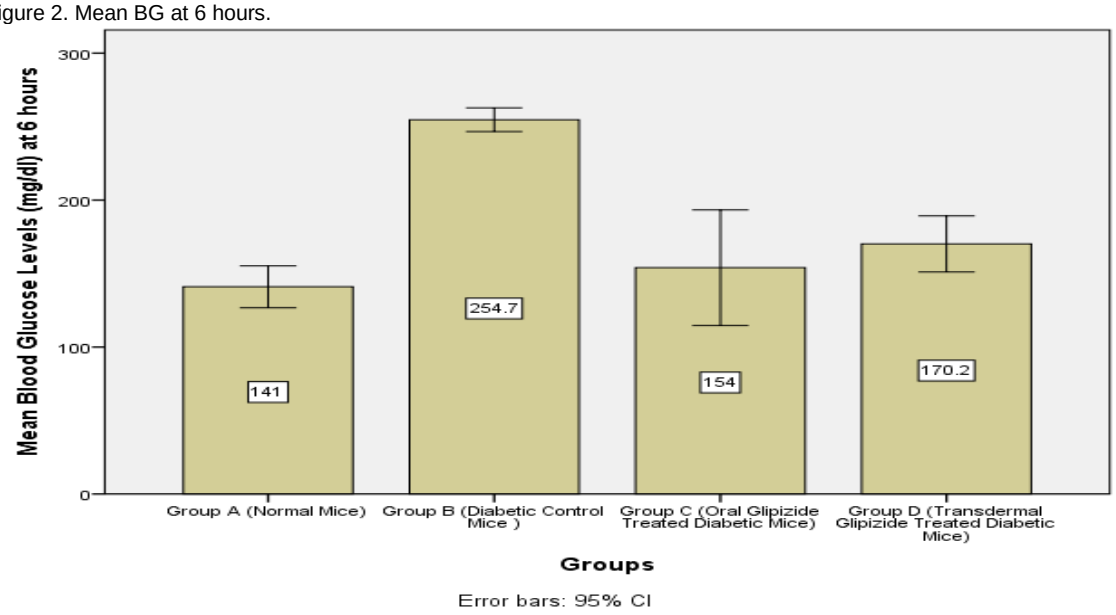



Figure 3.Mean BG at 12 hours

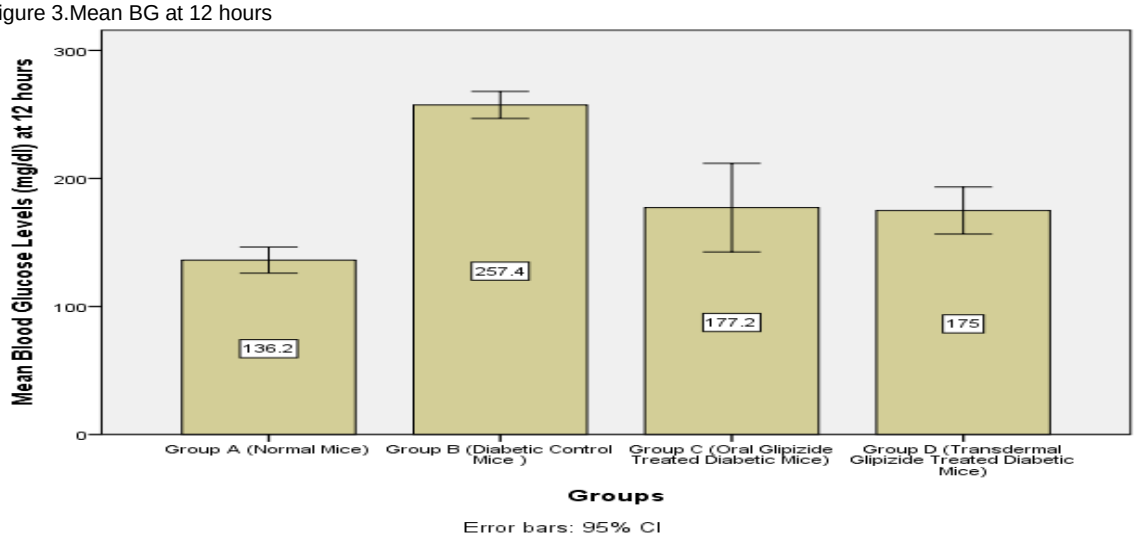



Figure 4. Mean BG at 24 hours.

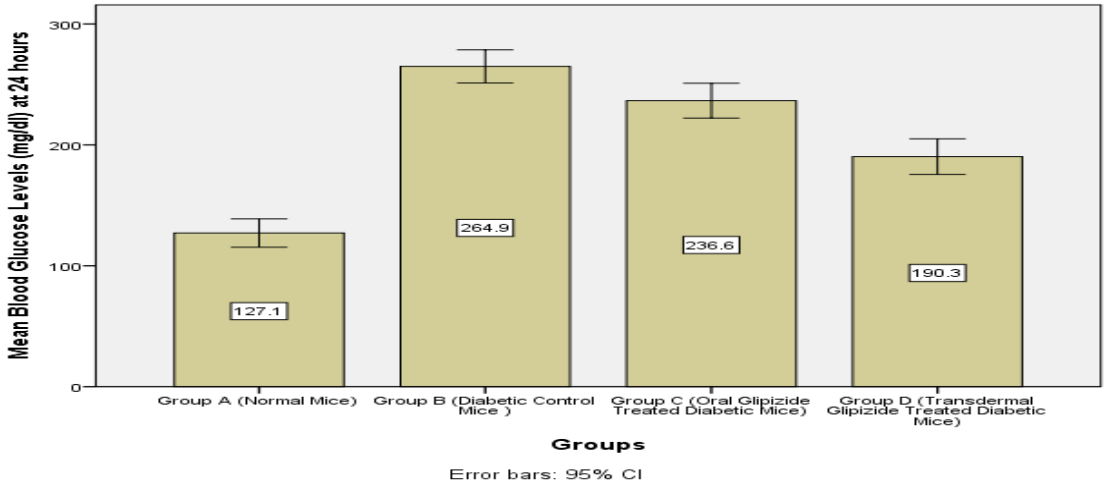
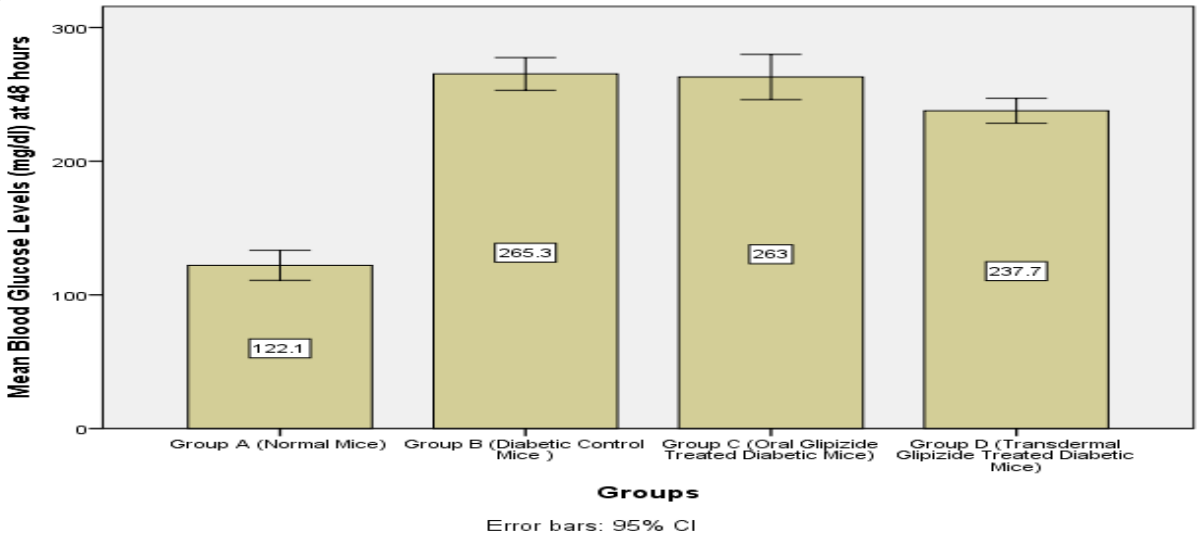


Figure 5. Mean BG at 48 hours.




## CONCLUSION

In conclusion, in this study, matrix design of transdermal delivery system of glipizide, exhibited optimum and sustained decrease in blood glucose levels for 24 hours in STZ induced diabetic mice, in comparison to oral glipizide, which had peak hypoglycemic effect at 6 hours.

## REFERENCES


1. Ammar, H. O., Salama, H. A., Ghorab, M., El-Nahhas, S. A., and Elmotasem, H., 2006. A transdermal delivery system for glipizide, Current Drug Delivery. **3**(3)**:** 333-341.
2. Transdermal drug delivery systems: A review. Girish Chopda. 12 January 2006.
3. Ghosal, K., Rajan, R. and Nanda, A., 2009.Effects of Chemical Enhancers on the Release of Glipizide through matrix Patch. International Journal of ChemTech Research, **1**, (4): 1128-1130.
4. Kennedy, M.S.N., 2012. Pancreatic Hormones & Antidiabetic drugs. In: Katzung, B.G. ed. 2012. Basic & Clinical Pharmacology. New York: McGraw Hill Lange, **41**:753-760.
5. Mutalik, S. and Udupa, N., 2003. Effect of some penetration enhancers on the permeation of glibenclamide and glipizide through mouse skin.Pharmazie, **58**(12):891-894.
6. Mutalik, S. and Udupa, N., 2006. Pharmacological evaluation of membrane-moderated transdermal system of glipizide. Clin. Exp.Pharmacol. Physiol., **33**: 17–26.
7. Mutalik, S., Udupa, N., Kumar, S., Agarwal, S., Subramanian, G. and Ranjith, A.K., 2006. Glipizide matrix transdermal systems for diabetes mellitus: Preparation, in vitro and preclinical studies. Life Sciences, **79**: 1568–1577.
8. Nanda, A., Nanda, S. and Ghilzai, N.M.K., 2006. Current developments using emerging transdermal technologies in physical enhancement methods, Curr. Drug Deliv., **3**(3): 232-242.
9. Pfizer Inc. Glucotrol, Full U.S. Prescribing Information [online].2000[cited 2013 May 21]; Available from: http://www.pfizer.com/pfizer/download/uspi_glucotrol.pdf
10. Reagan-Shaw, S., Nihal, M. and Ahmad, N., 2007. Dose translation from animal to human studies revisited. FASEB J., **22**: 659–661.
11. Roche Diagnostics, 2009. ACCU-CHEK PERFORMA, Package insert.
12. Shankar, S.J., Varalakshmi, C., Raken, P. and Manoj, K., 2011. Development of a membrane-controlled transdermal therapeutic system for glipizide. International Journal of Pharmaceutical Research and Development, **3** (4): 131-138.
13. Song, Y., Wang, J., Li, Y., Du, Y., Arteel, G.E., Saari, J.T., Kang, Y.J. and Cai, L., 2005. Cardiac Metallothionein Synthesis in Streptozotocin-Induced Diabetic Mice, and Its Protection against Diabetes-Induced Cardiac Injury. Am. J. Pathol., **167**(1):17–26.
14. Sridevi, S., Chary, M.G., Krishna, D.R. and Diwan, P.V., 2000. Pharmacodynamic evaluation of transdermal drug delivery system of glibenclamide in rats. Indian J. Pharmacol., **32**: 309-12.
15. Sultan Ayoub M, Inam Zia, Ishfaq A Bukhari, Shoukat Ali Arain 2016. Type 2 diabetes mellitus in Pakistan: Current prevalence and future forecast. JPMA., V 66, 12
16. Takamura, T., Ando, H., Nagai, Y., Yamashita, H., Nohara, E. and Kobayashi, K., 1999. Pioglitazone prevents mice from multiple low-dose streptozotocin-induced insulitis and diabetes. Diab. Res. and Clin. Prac., **44**: 107–114.
17. Tiwary, A.K., Sapra, B. and Jane, S., 2007. Innovations in Transdermal Drug Delivery: Formulations and Techniques, Recent Pat. Drug Deliv.Formul., **1**(1): 23-36.
18. UK Prospective Diabetes Study (UKPDS) Group, 1998. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33), The Lancet, **352**: 837-853.
19. UK Prospective Diabetes Study (UKPDS) Group, 1983. UK prospective study of therapies of maturity onset diabetes: I. Effect of diet, sulphonylurea, insulin or biguanide therapy on fasting glucose and bodyweight over one year. Diabetologia, **24**: 404–411.
20. WHO, Diabetes Mellitus, Fact sheet N°138, Revised April 2002
21. Wilkosz, M.F. and Bogner, R.H., Transdermal drug delivery, Part 1: current status. U.S. Pharmacist [online]. 2003[cited 2013 May 29];