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EFFECT OF MICROCAPSULES SOLID DISPERSION OF METFORMIN HCl ORAL ADMINISTERED FORMULATION ON HYPERGLYCEMIA IN RATS

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ABSTRACT

The present study is to examine In-*vivo pharmacodynamics* antidiabetic effect following oral administration of the selected optimized microcapsules in comparison with oral metformin solution. Solid dispersions microcapsules were prepared using solvent evaporation method which enclosed preparation of a uniform dispersion of Metformin HCl in (Hydroxy propyl methylcellulose k100, Ethyl cellulose, Eudragit RL PO, Eudragit RS PO and Compritol 888 ATO). A two-factor, General factorial statistical design was used to quantitate the effect of polymer type(X1) and drug: polymer ratio(X2) on the release profile. Where polymer type and drug: polymer ratio were selected as independent variables, while Y1 (cumulative drug release after 1 h) and Y2 (cumulative drug release in 3 h), Y3 (cumulative drug release in10 h),Y4 (angle of repose) and Y5(Hausner ratio) were selected as dependent variables. A convenient statistical model was made and a significantly controlled release rate was exhibited. The solid dispersions were characterized for their in vitro- release rate. The oral administration of formulae (T20) which consists of metformin HCl and compritol 888 ATO) in drug/polymer ratio (1:4) was chosen as optimum formula resulted in a clear long lasting Statistically significant anti-hyperglycemic effect up to 12 h as compared to diabetic control Group and metformin HCl solution treated group. Factorial design suggested only one optimized combination of the polymer by which maximum desirability obtained. The oral administration of formulae (T20) resulted in a clear long lasting statistically significant anti-hyperglycemic effect.

KEYWORD: Controlled release, Factorial design, In-vivo study, Metformin HCl, Solid dispersion.

INTRODUCTION

Blood glucose (BG), the major energy source for cells, should be maintained stable at all times despite intermittent food intake and variable demands. In patients with DM, an increased blood glucose concentration (hyperglycemia) caused undesirable symptoms. For many patients, diabetes is only diagnosed and aggressively treated when one of the characteristic diabetic complications develops.[1]

Diabetes Mellitus can be classified into two types: type 1 (Insulin-Dependent DM) and type 2 (Non-Insulin-dependent DM), with the vast majority of diabetic patients suffering from type 2.[2] Metformin hydrochloride is an oral anti-hyperglycemic agent, an orally administered biguanide, which is widely used in the management of type II diabetes, shows incomplete absorption from the gastrointestinal tract and the absolute bioavailability is (50 –60 %) with a short plasma half-life of (1.5 -4.5 h). An obstacle to the more successful use of Metformin therapy is the high incidence of concomitant gastrointestinal symptoms, such as abdominal discomfort, nausea, and diarrhea.[3]

Controlled drug delivery is one which delivers the drug at a predetermined rate and controls release rate that specified period of time. Controlled release drug delivery maintain uniform blood level for utilizes drug-encapsulating devices from which therapeutic agents may be released at controlled rates for long periods of time. Such systems are preferred over traditional methods of drug delivery due to numerous advantages including modifying of drug release rates, protection of fragile drugs and improve patient comfort and compliance.[4]

Solid dispersion methods have been used widely in a various formulation to enhance dissolution rate and bioavailability of poorly water soluble drugs.[5,6] Preparation of matrices with water insoluble and water swell able polymers using solid dispersion method is valuable in the production of controlled release products.

Many studies have been reported in the literature for the preparation of controlled release system, using solid dispersion technique.[7,8]

The target of this research is in-vivo study of formulated sustained release (SR) microcapsules of Metformin HCl. Oral glucose loading animal model, this method is often referred to as physiological induction of DM where the BG level of the animal transiently increased with no damage to the pancreas. In the clinical setting, it is known as glucose tolerance testing (GTT) and it is taken as standard procedure often used for the diagnosis of borderline diabetic patients. The animals, usually rabbits or rats, are fasted overnight prior to oral glucose load (1-5 g/kg body weight) administration and BG level is monitored over a period of time.[9,10]

MATERIALS AND METHODS

Materials

For Microcapsules formulation

Metformin HCl was brought as a gift from Ferchem Srl Co., Milano (Italy), Eudragit RLPO, Eudragit RSPO were brought as a gift from Rohm pharma, GMBH (Germany).Hydroxy propyl methylcellulose. HPMC K100 and compritol ATO 888 were obtained from colorcon limited, Luna supplier. All of the other chemicals and solvents were of reagent grade.

For In-vivo study

Metformin HCl powder was in the form of white crystalline powder, dissolved in distilled water forming solution and was given orally by gavage in a dose of 150 mg /kg /day.[11]

Streptozotocin (STZ) was purchased from sigma chemical Company inc., USA). It was in the form of white powder. Freshly made solutions of STZ dissolved in citrate buffer (pH 4.5) injected oncely intraperitoneally in a dose of 58 mg/kg.[12]

Metformin HCl prepared microcapsules, it was in the form of capsules, the capsules were opened and the content was suspended in a mixture from tween and distilled water (10:90 v/v) forming a suspension and was given orally by gavage in a dose of 75 mg/kg and 150 mg /kg. Tween 80, it was purchased from EL Nasr Pharmaceutical Company.

METHODS

Preparation of solid dispersions

Microcapsules were produced by solvent evaporation method11.By dispersing accurately weight quantities of Metformin HCl and polymers individually(ethyl cellulose300 cps, Eudragit RLPO, Eudragit RSPO, compritol ATO888 and HPMC K 100) dissolved at different ratios (1:1 to1:4) in a solvent(combination of mixture dichloromethane: chloroform(1:1)with continuous stirring and evaporation in open air, subsequently the solid mass was pulverized and passed through sieve No 18 ,the sieved granules were stored at 25 °C in a well-closed container until use.

Formulation Design

General factorial design has 2 to 15 factors, each factor must have at least 2 levels and at most 100 levels, but the number of levels can be different for each factor. The number of runs is limited to 10000, thus there is no catalog of available designs. In this study general factorial design containing 2 independent variables evaluated. One of them X1(a type of polymer) at 5 levels (HPMC, EC, Eudragit RLPO, Eudragit RS PO and Compritol) and the other X2(drug -polymer ratio) at 4 levels (1:1, 1:2, 1:3 and1:4).The experimental trials were performed at 20 combinations.

Evaluation of Solid Dispersions

In-vitro Dissolution studies

Fig.1 showing the dissolution rate of Metformin HCl was estimated for all formulations using USP type II with six rotating basket, speed 100 rpm (Mumbai, India) and the dissolution media utilized was 900 ml of HCl pH 1.2 for 2 h then converted to phosphate buffer pH 6.8 at 37ºC by adding30 gm of Tri- sodium orthophosphate.12 H2O13. At suitable intervals, 5 ml of each sample was taken and assayed at 232 nm by UV-visible spectrophotometer and replaced by fresh dissolution media. Fig. 1 showing dissolution profile of Metformin HCL compritol microcapsules at different ratios (1:1,1:2,1:3 and 1:4).The data were analyzed by MINI TAB SOFTWARE(version 17). Metformin HCl microcapsules were optimized based on the percentage drug release criteria test to confirm acceptance criteria of USP as follow:

Release at 1h: 20 to 40%
Release at 3 h: 45 to 65%
Release at 10 h: Not less than 85%

Drug content and percent yield

Metformin HCl content of microcapsules was determined by an extraction method. [6] Microcapsules equivalent to (50mg) were weighed accurately, crushed and added to Methanol (20 ml) in volumetric flask and make up the volume with 100 ml 0.1 N HCl, And The solution is shacked well and filtered through whatman filter paper no 44 and 10ml of filtrate was taken out and diluted up to100ml with 0.1 N HCl, again 2 ml was taken out and diluted up to 10 ml with 0.1 N HCl and absorbance was assayed spectrophotometrically at 232 nm against 0.1 N HCl as a blank, the percent yield of each formulation was also calculated.

Characterization the physical properties of the produced Metformin HCl microcapsules

Determination of Densities before and after tapping and true density also Hausner Ratio and Compressibility Percent of the produced Metformin HCl Microcapsules were achieved.

Data Analysis
The response values for this study are Y1 (Cumulative drug release in 1 h), Y2 (Cumulative drug release in 3 h), Y3 (Cumulative drug release in 10 hr) Y4 (Angle of repose) and Y5 (Hausner ratio).The multiple regression analysis was made using MINI TAB 17 software. Interaction plots and Contour plots are obtained from designed experiments by Analysis of data of full factorial design using ANOVA.The response values are subjected to multiple regression analysis to show the relationship between the factors and the responses values.

Formulations Optimization
The optimized formulation was estimated by using software MINI TAB 17 and applying constraints on dependent variables. One optimized formulation is obtained. The optimized batch(s) was further investigated by DSC, FTIIR, XRD, and SEM.

In-vivo pharmacodynamics study
The pharmacodynamics study of metformin HCL from selected drug loaded microcapsules was applied on male albino Sprague Dawly rats. The results were compared to those obtained with immediate-release dosage (pure solution of metformin HCI in distilled water). The protocol of the studies (REC-FPSPI- 11/71) was approved by the Research Ethics Committee for experimental and clinical studies at the Faculty of Pharmaceutical Sciences and Pharmaceutical Industries, Future University in Egypt.

Animal handling and drug administration
Male albino rats, Sprague Dawley, weighing between (160 and 180) g. were used in this study. The animals were fed a normal diet and water at libitum and in a constant temperature of 25°C with a period 7 days for animals' acclimatization. Thirty rats randomly divided into 5 groups, each consisting of 6 rats. Induction of diabetes was performed in rats of groups (2 to 5).

Induction of diabetes using streptozotocin
Streptozotocin (STZ) dissolved in cold 0.1M citrate buffer, pH 4.5 and was administered intraperitoneally oncely, which was freshly prepared (within 10 min of preparation), in a dose of (58 mg/kg). [13, 14, 15] Blood samples were checked 72 h post administration using Bionime 300 glucometer. Rats blood glucose values (250-300 mg /Dl) were considered diabetic and were included in the study.[16]

Experimental design
Metformin HCl was administered 150 mg /kg as shown in Table 1. The rats received the assigned treatment according to the following scheme.

The rats were divided into 5 groups comprising 6 animals in each group as follows.

Group I (Negative Control):
(Normal non -diabetic, control rats) were treated daily with saline and served as the negative control.

Group II (Positive control):
Animals were injected with a single dose of Streptozotocin (58 mg/kg) by the intraperitoneal route to induce diabetes and served as a positive control.

Group III (Pure metformin HCL):
Diabetic rats were treated with 150 mg/kg body weight of pure metformin HCl

Group IV (Formulation SR metformin, low dose):
Diabetic rats were treated with 75 mg/kg body weight of metformin HCL containing microcapsules.

Group V (Formulation SR metformin, high dose):
Diabetic rats were treated with 150 mg/kg body weight of metformin HCL containing microcapsules.

Blood sampling
Blood samples, obtained from rats via the tail vein, and immediately placed on the glucose strips and blood glucose levels (BG) levels were determined using the Bionime Glucometer. Pharmacodynamics analysis**.**

All quantitative data were collected from experiments performed and expressed as mean ± S.E. Statistical analyses were performed using one-way ANOVA Tukey's post-tests, using Graph Pad Prism 5.2 (Graph Pad software). Differences were considered significant for $p < 0.05$.The anti-hyperglycemic activity of different metformin HCL formulations in terms of percentage change from baseline BG (obtained at zero time) and percentage reduction of BG level at different time points time post treatment were calculated.

Table 1: Protocol for treatment administration in different animals groups.

Treatment	group No*	Administration form	Metformin HCL dose(mg/kg)
Control	1 (Normal)	Normal saline	0
Control	2 (Diabetic)	Normal saline	0
Reference	3 (Diabetic)	Metformin solution	150
Formulation (low dose)	4 (Diabetic)	Metformin microapsules	75
Formulation (high dose)	5 (Diabetic)	Metformin microapsules	150

RESULTS
The responses were recorded and data was analyzed using ANOVA. The individual parameter was evaluated using F-test and a polynomial equation for each response was generated using Multiple Regression analysis In ANOVA, values of "Prob > F" less than 0.05 indicate model terms are significant. In this case X1, X2, X1*X2 are significant terms. The interaction plot of X1*X2 on Y1 showed that HPMC at level (1:1), and Eudragit RL at level(1:3) and compritol (1:4) showed the optimum

release rate at 1 h (20-40%). Contour plot of X1*X2 on Y1 shows that HPMC allows different cumulative percent of drug released after 1 h using different drug polymer ratio at level (1:1) drug to polymer ratio above 80 % of drug released. Increasing ratio at (1:2, 1:3) percent of drug released decreased to be between (60-80%).At (1:4) level decreased the percent of drug released to be (20-40%). The interaction plot of X1*X2 on Y2 showing levels (compritol,EUD RL, and EUD RS) at a drug : polymer ratio(1:4) showing the optimized release at 3 hrs (45-65%).The contour plot of X1*X2 on Y2 showed that Eudragit RS allows percent of drug release less than 20% after 3h in all drug polymer ratio. Ethylcellulose at drug- polymer ratio (1:1, 1:2) allows percent of drug release (20-40%) after 3hrs. when increasing the ratio to (1:3, 1:4) percent of drug released decreased (less than20 %). Compritol at (1:1, 1:2, 1:3) drug-polymer ratio released percent of drug range (40-60%) when increasing the ratio to (1:4) drug release percent decreased. The interaction plot in Fig. 2 of X1*X2 on Y3 indicated that using HPMC at levels (1:2,1:3,1:4) and Eudragit RL at levels (1:2,1:2,1:3) and compritol (1:1,1:2,1:3,1:4) optimized the release after 10 h.

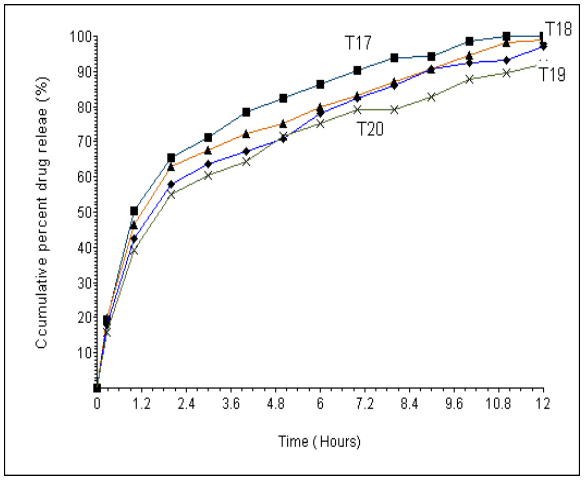

Fig. 1: In-vitro release of Metformin HCL capsules containing drug Compritol ATO 888.

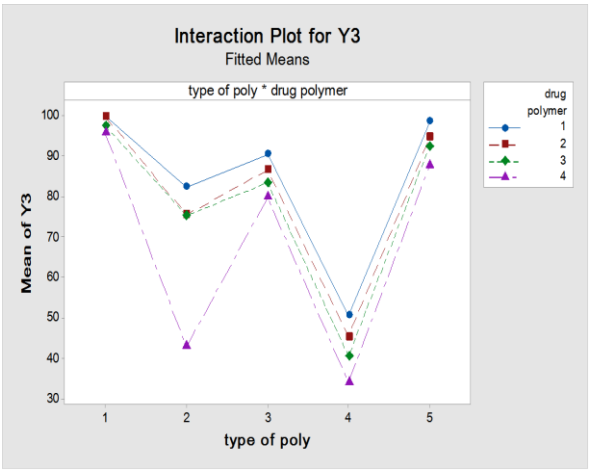

Fig. 2: Interaction plot of Y3 versus x1, x 2.

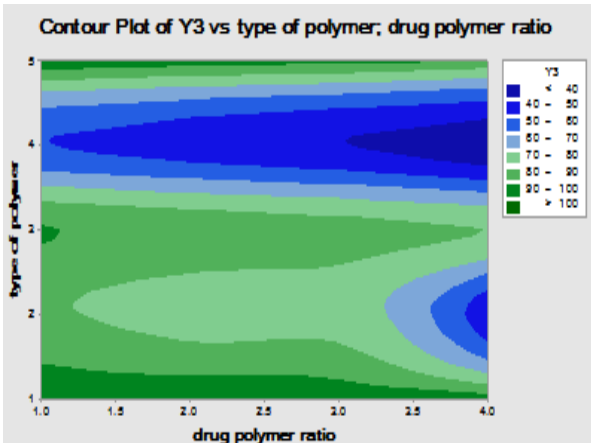

Fig. 3: Contour plot of Y3 versus x1, x2.

The contour plot in Fig. 3 showed that HPMC released percent of drug (90-100%) in all drug- polymer ratios after 10 hrs. Ethyl cellulose at level 1(1:1) released (80-100 %) after 10 hrs which decreased by increasing drug polymer ratio (1:2,1:3) to be (70-80%) ,(20-40%)cumulative amount of drug release ratio. the interaction plot of X1*X2 on Y4 and contour plot of both X1*X2 on angle of repose indicated that using HPMC (1:3), EC(1:2), Eudragit RL (1:1), Eudragit RS (1:3) and compritol (1:2) gives the minimum value for angle of repose which means good flow character The interaction plot and the contour plot of both X1*X2 effect on Y5 (Hausner ratio) indicated that using HPMC(1:3), EC(1:3), Eudragit RL (1:4),Eudragit RS (1:1) and compritol (1:1) gives the minimum value for Hausner ratio, less than 1.1 which means good flow character. On the basis of dissolution studies of formulations and constraints applied,the results of factorial design suggested only one optimized combination of polymers by which maximum desirability has been achieved. The check-out batch was selected for further studies.

Differential Scanning Calorimetric

The thermogram of Metformin HCl Fig. 4 (a) did not show any endothermic transition between 30°C – 450°C because of its melting point at 230.051°C and an associated enthalpy 358.895 J/G indicating its anhydrous crystalline state. In order to study Metformin HCl polymer interactions the analysis will depend on polymer characteristic thermal properties as follow: Compritol ATO 888 polymer alone Fig. 4(c) showed a sharp endothermic peak, with a melting point at 69.635 ^{o}C because of its crystalline nature and associated fusion enthalpy 118.124 J/G. The DSC thermogram of Metformin-compritol microcapsules Fig. 4(b) showed a sharp endothermic peak at 232.295oC of Metformin an endothermic peak at 78.851 of compritol. This slight change of melting points of both of the components may be related to the dilution factor.

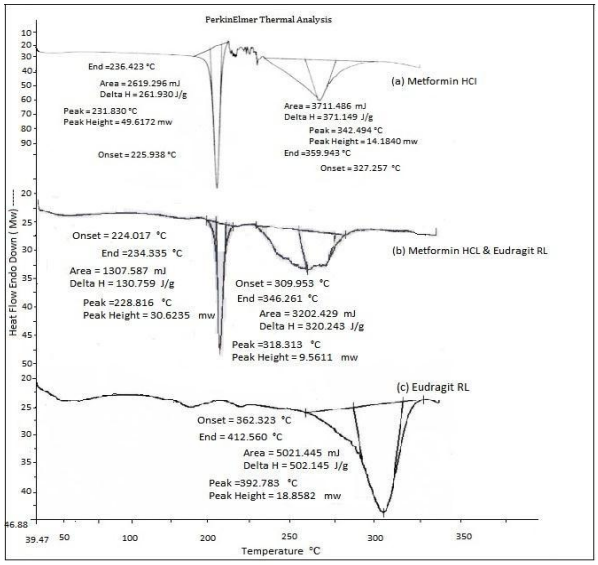

Fig. 4: The thermogram of Metformin HCL-compritol888 ATO Microcapsules (a) Metformin HCL (b) Metformin HCL-compritol microcapsules (c) compritol ATO 888.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectrums of Metformin HCl and Metformin HClcompritol microcapsules were applied from which we found Pure Metformin HCl Fig. 5(a) shows characteristic bands from different functional group at:(3373.9-3299.5) cm–1 due to NH3 group primary stretching vibrations,(3173.2) cm–1 due to secondary stretching NH3 group, (1631.1-1573.4) cm–1 due to -C=N group stretching vibrations, and (1061,933.4) cm–1 due to C-N stretching vibrations and -NH out of plane bending. These results are in good agreement with the finding of other authors on Metformin HCl. Fig 5(b) of Metformin HCl- compritol microcapsules shows the presence of all characteristic peaks of Metformin HCl and decrease in the intensity of the characteristic Metformin HCL bands at (3372.3 cm–1, 3174.7 cm–1) and also at (1570 cm–1), which may be due to the weak hydrogen bonding between the drug and the polymer.

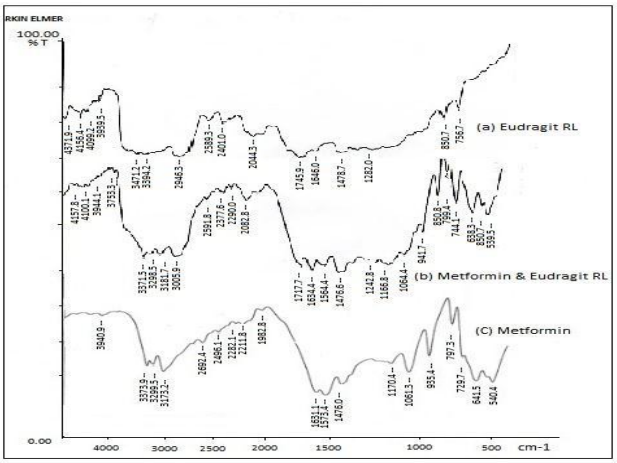

Fig. 5: The FTIIR spectra of (a) Metformin HCL (b) Metformin HCL-Compritol microcapsules (c) compritol888 ATO

Powder X-ray diffraction studies

X-ray diffraction studies were applied on Metformin HCl alone, polymer alone and drug loaded polymer. 1- X-ray diffraction pattern of pure Metformin HCl showed sharp numerous distinct peaks notably at 2θ angles, were 12.2°, 23.7°, 24.5°, 37.1° and 50°. This series of sharp and intense diffraction peaks were emphasized the crystalline state of pure Metformin HCl. 2- X-ray diffraction pattern of compritol ATO 888shows two peaks at 21° and 23 ° due to lipid Polymorphism. 3- The X-ray diffraction pattern of drug loaded microcapsules prepared by solid dispersion using compritol ATO 888 showed all the intense peaks of both the drug and the polymer. The crystallinity of drug in the solid dispersion was less than that observed before preparation. But the pattern still showing the typical signals of Metformin HCl but the intensity is weakened as shown in Fig. 6.

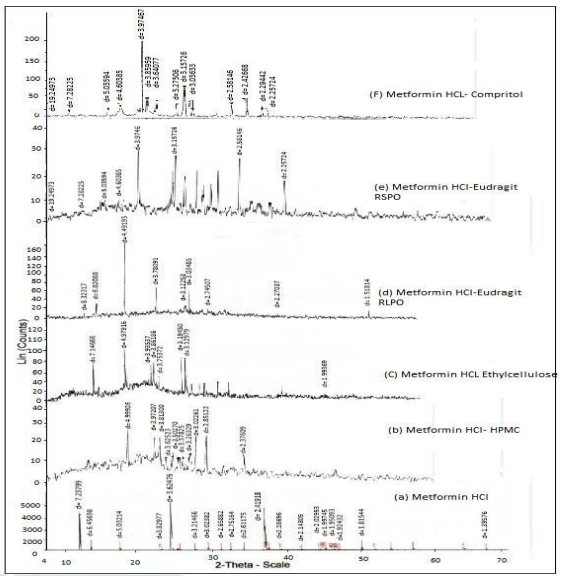

Fig. 6: X-ray pattern of (a) pure Metformin HCl (b) Metformin HCl– HPMC (c) Metformin HCl-Ethylcellulose (d)Metformin HCL- Eudragit RL (e) Metformin HCl-Eudragit RSPO (F)Metformin HCl-Compritol solid dispersions.

Scanning Electron Microscopy

Electron-microscopic technique was used to give us a clear picture about the shape and the surface of the pure crystalline powder of Metformin HCL and the prepared Metformin HCl microcapsules. It was found that the majority of Metformin HCl microcapsules were irregular in the shape and the sizes of the tested formulae were different in diameters as shown in (Fig. 7) this was clear because the sample from each formula was taken before doing the sieve analysis.

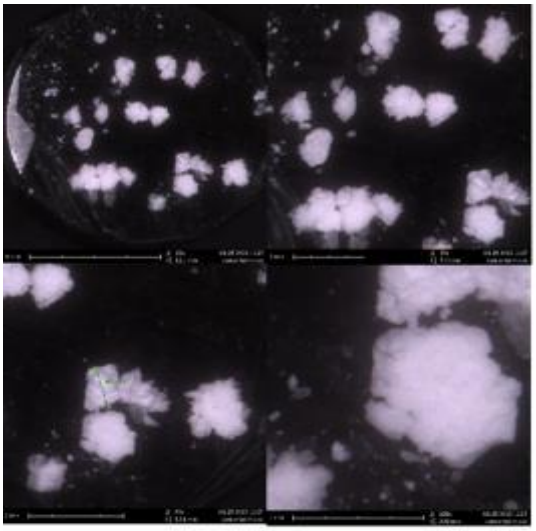

Fig. 7: SEM of Metformin HCL-compritol micro capsules.

In-vivo pharmacodynamics study

Blood glucose levels

Blood glucose levels of five groups through 4 weeks treatment were studied at 4 time points every week for all rats and the pharmacodynamics effect of the developed formulation T20 was studied and compared with the pure drug, Based on previous study, a 25 % reduction in the BG level was considered significant anti-hyperglycemic effect.[17]

The BG levels expressed as a percentage from basal levels and the percentage reduction of BG after administration of the different treatments versus time curves are investigated.

Body weight change

Another parameter which is changed during the study and related to the efficiency of the treatment is shown in Fig. 8.

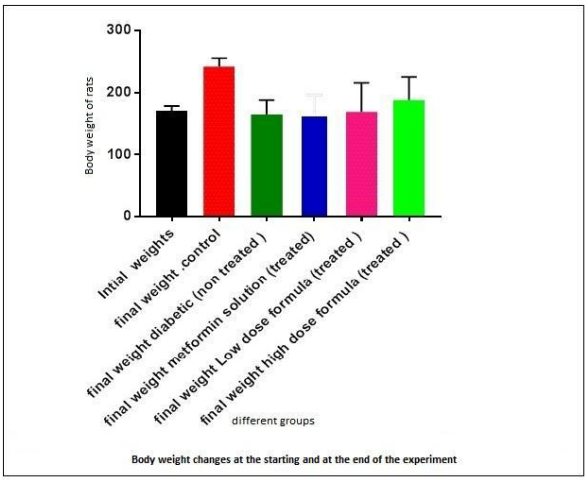

Fig. 8: Body weight changes of different groups at the end of the biological experiment.

Blood glucose levels

Group 1 rats showed normal BG levels throughout the study indicating that animals were not stressed by the experimental procedures. On the other hand, extended high BG levels followed oral administration of Group 2 rats with normal saline solution, the BG levels of animals did not increase after administration of microcapsules either low or high dose of formulae (T20) which showed statistically significant BG lowering as compared to that of diabetic control up to 12 hrs. Thus, the non-diabetogenic nature of the selected polymer was evidenced, confirming its good choice as metformin carrier. BG levels and % reduction in BG levels obtained. The effect of different formulations on blood glucose lowering activity can be clearly seen by close examination of Table 2, blood glucose levels gradually decreased reaching significant reduction within 3h.

Following oral administration of metformin solution the blood glucose level gradually declined, showing a significant reduction in blood glucose at 3 h and Lasting 6 h. before decreasing below the significant level 12 h post drug administration. Fig. 9, 10, 11 are shown BG levels and % reduction in BG levels obtained after administration of selected formulae.

Table 2: Effect of different formulations of metformin HCl on blood glucose lowering activity to diabetic rats.

Low dose Formula (treated) group		High dose Formula (treated) group		Metformin solution (treated)		Diabetic(Non t-treated)		Control Group		
% Reductionin BG level	BGlevel	% ReductioninBG level	BGlevel	% Reduction in BG level	BG level	% Reductionin BG level	BG level	% Reductionin BGlevel	BG level	Time(h.)
0	294.8±22.4	0	284±23.8	0	302.1±17	0	272.8±24.4	0	66.8±2.3	0
- 37.8±7.8	184.3±7.8	-40.9±20.5	167.8±20.5	- 34.8±15.2	196.8±15.2	36.2±23	371.8±23	7.7±4.8	72±4.8	3 h
-41.6±10.6	172±10.6	- 58.5±8.6	117.8±8.6	- 10.3±14.4	270.8±14.4	37.2±23.5	374.5±23.5	- 12.9±3.9	58.1±3.9	6 h
- 26.5±10	216.5±10	-38.5±9	174.6±9	32.8±21.1	401.3±21.1	45.2±24	396.3±24	4±4.5	69.5±4.5	12 h

All results are expressed as mean of 6 determinations ± SE

Percentage change in blood glucose level after 3 h

Fig. 9 showed that (control) normal group showed non-significant increase in blood glucose level, Diabetic group (non-treated) showed significant increase in % of blood glucose level of 36.29 compared to zero time recorded after 3h while diabetic treated groups showed significant anti-hyperglycemic effect with % BG level reduction (34.86, 37.82, 40.90) for (metformin solution, low dose formula, high dose formula), respectively.

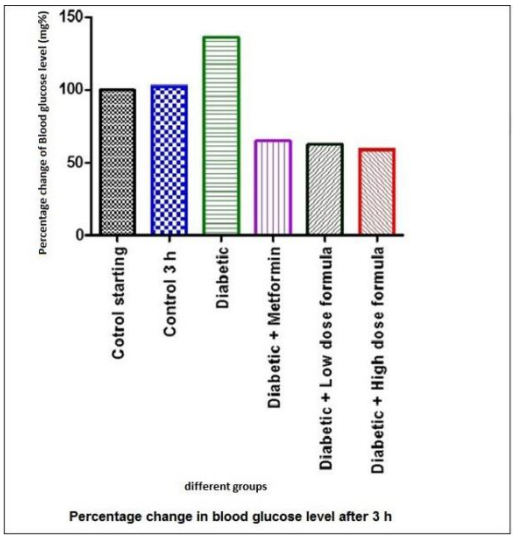

Fig. 9: Percentage change in blood glucose level after 3 h.

Percentage change in blood glucose level after 6 h

Fig. 10 showed that there is a non-significant increase in blood glucose in (control) normal group. Diabetic group (non-treated) showed significant increase in % of blood glucose level of 37.26 compared to zero time recorded after 6h while diabetic treated groups showed significant anti-hyperglycemic effect with % BG level reduction (10.37,41.66, 58.50) for (metformin solution, low dose formulae, high dose formulae), respectively.

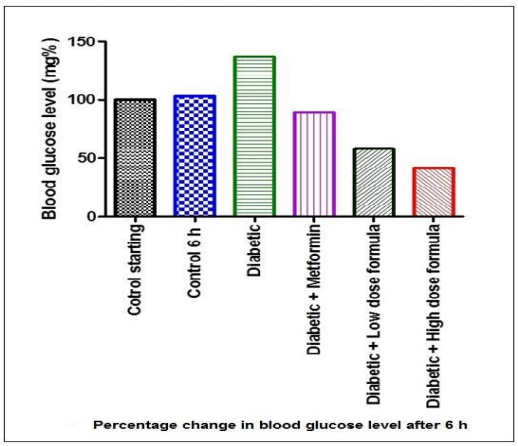

Fig. 10: Percentage change in blood glucose level after 6 h.

Percentage change in blood glucose level after 12 h

Fig. 11 showed that the blood glucose level of normal (control) group showed non-significant change, Diabetic group (non-treated) showed significant increase in % of blood glucose level of 45.27% compared to zero time recorded after 12 h. Metformin solution treated group showed significant increase in blood glucose level by about 32.82% compared to zero time, moreover (low and high dose formula) showed a significant decline in blood glucose level by about (26.7 % and 38.52 %) respectively. Regarding blood glucose level at different time points among control groups (non-treated groups) and diabetic(non-treated) there are non-significant change in control (non- diabetic, non-treated group) while diabetic (non-treated) showed significant % BG increase in all time points 36.29%,37.26 and 45.27 at 3, 6 and 12 h, respectively.

Metformin (solution) treated group showed significant decrease by 34.86% after 3 h followed by decrease 10.37 % after 6h but reversed significant increase by 32.82 % expressed as percentage from basal levels. The oral administration of Metformin (low dose formulation and, high dose formulation) treated groups showed a significant reduction in all points. The high dose formulation showed the maximum reduction in blood glucose 59.5% and continues its efficacy in lowering blood glucose level by 38.5 % which is higher percent reduction than low dose formulation after 12 h.

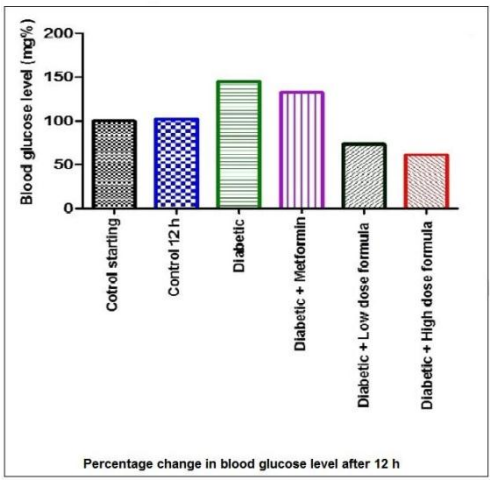

Fig. 11: Percentage change in blood glucose level after 12h.

Body weight changes

Fig. 8 showed that the body weight of normal control group was significantly increased by 53.2% when compared with starting time of the study, while diabetic (non-treated group) showed mild decrease in body weight by about (2.7 %), furthermore diabetic treated group were changed from starting point and decreased in weight by (9.7 % and 1.43 %) for (metformin solution and low dose formulae) respectively. Group treated with high dose formulae showed mild increase in weight by about 4.15%.

DISCUSSION

Compatibility studies were performed using DSC, FTIR and X ray diffraction. The DSC thermogram of metformin HCl-ethyl cellulose solid dispersions showed sharp endothermic peak of metformin HCl which decreased from 230^{0}C to 226.461 ^{0}C due to dilution so no significant change of melting point. The DSC thermogram of metformin HCl-HPMC solid dispersions showed no significant change of T_m of metformin HCl and no significant interaction. The DSC thermogram of metformin-Compritol solid dispersions showed sharp endothermic peak at 232.295 ^{0}C of metformin and endothermic peak of Compritol at78.851 (Fig. 4). This change of melting points of both of the components may be attributed to due to dilution factor. The DSC thermograms of metformin HCl-Eudragit polymers showed sharp endothermic peak of metformin HCl at 229.219 ^{0}C indicating crystalline nature of metformin HCl and polomorphic nature of Eudragit RLPO and Eudragit RSPO in Fig. 4. For FTIR analysis, metformin HCl–ethyl cellulose solid dispersion showed the presence of all characteristic peaks of metformin HCl. This result indicates chemical stability of metformin HCl in polymer film matrix and the absence of interactions between ethyl cellulose and metformin HCl. chemical stability of metformin HCl in polymer film matrix and the absence of interaction between metformin HCl and HPMC. Metformin HCl-Compritol solid dispersions in Fig. 5 showed the presence of all characteristic peaks of metformin HCl and reduction in the intensity of the characteristic metformin bands at (3372.3 cm^{-1}, 3174.7 cm^{-1} and also at 1570 cm^{-1} which may be due to the weak hydrogen bonding between the drug and the polymer. Similar trends of results were obtained with Compritol 888 ATO and metformin HCl. no significant shift or reduction in the intensity of the FTIR bands of metformin HCl which observed, confirming the absence of interaction between Eudragit RLP0 and Eudragit RSPO, also with metformin HCl. Series of sharp and intense diffraction peaks indicated the crystalline state of pure metformin HCl. Ech of ethyl cellulose, HPMC K100, Eudragit RSPO and Eudragit RLPO polymers are amorphous in nature due to the absence of complete stereo regularity and presence of bulky side groups. The lipid polymorphism Nature of Compritol 888 ATO.

General factorial design was used for formulating metformin HCl solid dispersions. It deals with optimization of formulation variables to improve *in-vitro* release of metformin HCl. A two-factors, X1 (type of polymer), X2 (drug polymer ratio), X1 has 5 levels and X2 has 4 levels). The production yield is a measure of the accuracy of the solid dispersions technique, since it measures the actual weight of the prepared solid dispersions (drug + polymer + any other additives). The range of the production yield of the prepared solid dispersions was found to be between 79.18% and 99.5% The best value appeared in formula T2 (99.5%) while the worst value appeared in formula T13 (79.19%). Formula T20 gave the best drug content of the prepared metformin HCl solid dispersion dispersions (97.6%).

Micrometric properties proved that the arithmetic mean diameter of the good distribution formulae lied between (450 – 500) µm. Metformin HCl solid dispersions which represent a low distribution observed a lower value for bulk and tap densities ranged from 0.3100 to 0.3844 gm/cm^3 while metformin HCl solid dispersions which represent a high distribution gave a higher value of bulk densities ranged from 0.3848 to 0.6353 gm/cm^3. The studied metformin HCl solid dispersions can be arranged in descending order regarding the Hausner ratio as follow: T1, T20, T7, T9, T13, T19, T18, T11, T15, T16, T4, T2, T6,T10, T12,T8,T3,T14,T5 and T17.

The studied metformin HCl solid dispersions can be arranged in a descending order regarding the compressibility percent as follow: T1, T20, T7, T9, T13, T19, T18, T11, T15, T16, T4, T2, T6, T10, T12, T8, T3, T14, T5, and T17.

The studied metformin HCl solid dispersions can be arranged in a descending order regarding the angle of repose as follow: T7,T17, T19, T10,T20, T2, T13, T11, T1, T12,T16, T18,T14,T4, T8, ,T5,T15,T6, T9 and T3.

The results concluded that the majority of metformin HCl solid dispersions were irregular in shape. The best formulae were observed to be T20, T19, T4 and T3 for the *in-vitro* release of metformin HCl solid dispersions after the whole dissolution period. The *in-vitro* release of metformin HCl solid dispersions from the different formula studied followed different kinetic models and no one kinetic model can express the drug release from a specific formula of metformin HCl. Factorial design analysis for 20 formulae (T1-T20) and Response optimization for selection of the best batch by composite desirability determination, the result of the response optimization showed the best combination of polymer and drug: polymer ratio was the formula T20 (which consists of metformin HCl and compritol 888 ATO) in D/P ratio (1:4). This formula gave the best drug content, *in-vitro* release and flow character.

Oral glucose loading animal model method is often referred to as physiological induction of DM where the BG level of the animal transiently increased with no damage to the pancreas. In the clinical setting, it is known as glucose tolerance testing (GTT) and it is taken as standard procedure often used for the diagnosis of borderline diabetic patients.

Metformin HCl prepared solid dispersions proved successful treatment of hyperglycemia in rats and sustained effect compared to those obtained with immediate- release dosage (pure solution of metformin HCl in distilled water).

CONCLUSION

It was clear that the use of solid dispersions can control the drug release, which was achieved by dispersion in polymeric carriers, HPMC k100, Ethyl cellulose, Eudragit RLPO, Eudragit RSPO and Compritol ATO 888. On the basis of dissolution studies of formulations the ratio of polymers and Metformin HCl required to control release of Metformin HCl was obtained and optimized using 2 factor general full factorial designs. The results of factorial design suggested only one optimized combination of the polymer by which maximum desirability obtained. The oral administration of formulae (T20) which consists of metformin HCl and compritol 888 ATO) in drug/polymer ratio (1:4) resulted in a clear long lasting Statistically significant anti-hyperglycemic effect up to 12 h as compared to diabetic control Group and metformin HCl solution treated group . Regarding body weight, there is obvious significant increase in body weight in control group at the end of the experiment, while non-significant increase or decrease in body weight among the treated group, confirming the effect of diabetes on Rats' body weight.

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