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IN VITRO AND *IN VIVO* CHARACTERIZATION OF GLIMEPIRIDE MICROPARTICLES

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ABSTRACT: In the present investigation, controlled release microparticles of Glimepiride were prepared by emulsion solvent evaporation technique using cellulose acetate as release retardant polymer. The microparticles were evaluated for *In-Vitro* release study and *In-Vivo* evaluation. The microparticles were subjected to *In-vitro* release studies by employing 7.8 pH phosphate buffer as dissolution medium. The rate of drug release from these microparticles followed zero order kinetics and the mechanism of drug release was governed by peppas mechanism. The exponential coefficient (n) value indicating that drug release followed non fickian mechanism. The *in vivo* performance of pure drug and optimized Glimepiride microparticles were evaluated in rabbits in a randomized cross-over design. The estimation of glimepiride in serum was carried out by HPLC method. The parameters such as maximum plasma concentration (Cmax), time for peak plasma concentration (tmax), mean residence time (MRT) and area under curve ($AUC_{0-\alpha}$) were significantly ($P< 0.001$) differed following glimepiride microparticles compared to pure drug administration. The relative bioavailability of glimepiride was increased about five fold after microparticles administration as compared to pure drug. This may be due to the slow controlled release of Glimepiride. In the case of optimized Glimepiride microparticles administration, percentage blood glucose reduction was observed after 4.0 hrs and was prolonged over a period of 16 hrs. It was concluded that the Glimepiride microparticles administration, resulted in increased relative bioavailability and reduced frequency of administration.

INTRODUCTION: Microparticles are a type of drug delivery systems where the particle size ranges from one micron to few mm. These microparticles allow protection of drug from the environment, stabilization of sensitive drug substances, elimination of incompatibilities, or masking of unpleasant taste.

Hence, they play an important role as drug delivery systems aiming at improved bioavailability of conventional drugs and minimizing side effects[1].

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Glimepiride is an oral blood sugar-lowering drug as a third generation Sulfonylurea for the management of type-II diabetes mellitus. It is absorbed at the gastrointestinal tract. Due to its low biological half lives (5 hrs), it requires frequent administration. To reduce the dosing frequency and to improve patient compliance prolonged release dosage forms are required [2]. Hence, there is a scope for continued interest and need for developing controlled release formulations.

In the present investigation solvent evaporation method was employed with an objective of developing microparticles for obtaining controlled release of Glimepiride. The microparticles were evaluated for In-Vitro release study and *In-Vivo* evaluation. The *in vivo* performances of pure drug and Glimepiride microparticles were evaluated in rabbits in a randomized cross-over design. The

pharmacokinetic and pharmacodynamic parameters of both pure drug and Glimepiride microparticles were compared statistically to reveal the enhanced therapeutic efficacy of Glimepiride microparticles in comparison with pure drug.

MATERIALS AND METHODS:

Glimepiride (gift sample from Natco Pharm. Pvt. Ltd. Hyderabad), cellulose acetate (S.D Fines chemicals Ltd. Mumbai), Acetone (Qualigens), Span 80 (S.D Fines chemicals Ltd. Mumbai), Liquid paraffin (S.D Fines chemicals Ltd. Mumbai) were of pharmaceutical grade and obtained commercially.

Animals: New Zealand male rabbits (1.2 - 1.8 kg) maintained at 25 ± 1°C was used for the study after getting the approval from Institutional Animal Ethical Committee. The animals were housed in stainless steel metabolic cages and provided standard diet and water ad libitum.

Preparation of Microparticles:

All the microparticles formulations were prepared by emulsion solvent evaporation technique[3]. 1gm of Glimepiride and polymer (cellulose acetate) in 1:3 ratio were dissolved in 10ml of acetone. The organic solution was then slowly added to 100ml of liquid paraffin containing 1% of span 80 as surfactant with constant stirring for 1hr. The resulting microparticles were separated by filtration and washed with petroleum ether. The microparticles finally air dried over a period of 12 hrs and stored in a desiccator.

In Vivo Evaluation:

Pharmacokinetic evaluation of Glimepiride microparticles:

The pharmacokinetic performance of Glimepiride microparticles was studied in a randomized crossover study design in rabbits. Twelve healthy rabbits with a mean age of 10±2 weeks and with a mean body weight of 3±0.2 kg were used. Two groups of rabbits with 6 in each were fasted for 12 hrs prior to study. The animal dose of pure Glimepiride and its microparticles was calculated relevant to human dose. A dose of 180 µg/kg of pure Glimepiride and 115 µg/kg Glimepiride equivalent microparticles were administered orally in the form of suspension for two groups of rabbits. The rabbits were restrained in a wooden rabbit holder. The ears of the rabbits were cleaned and the hair was removed with the help of depilatory. Before withdrawal, the ear veins were dilated by swabbing with cotton or by application of warm water. The marginal ear vein of the left ear was punctured with a help of a 24 gauge needle. About 1 ml of blood samples were drawn at 0 (before drug administration), 0.5, 1.0, 2.0, 3.0, 4.0 and 6.0 hrs after pure drug administration and at 0, 1, 2, 4, 6, 8, 12, 16, 20, and 24 hrs after administration of Glimepiride microparticles[4].

Blood sample volume was replaced by administration of isotonic saline. The blood samples were collected in a micro centrifuge tube and centrifuged at 3500 rpm for 10 min. Later the supernatant layer (serum layer) was collected and utilized for estimation of Glimepiride concentration. The estimation of Glimepiride in serum was carried out by HPLC method[5]. The pharmacokinetic parameters of Glimepiride such as K_e (hr^{-1}), $t_{1/2}$ (hr), K_a (hr^{-1}), AUC $_{(0-\alpha)}$ (µg-hr/ml), MRT (hr), C_{max} (µg/ml), T_{max} (hr) were estimated by non-compartmental methods [6].

Pharmacodynamic evaluation of Glimepiride microparticles:

Hypoglycemic effect of Glimepiride microparticles were performed on normal healthy wistar rats weighing 250 to 300 g each. The rats were maintained at normal photo period (12 hour dark/12 hour light) with an ambient temperature of 23-26ºC under adequate light and ventilation. Commercial pellet diet and water were provided to the Rats. The approval of the Institutional Animal Ethics Committee was obtained before starting of the study. Two groups of Wistar rats (6 in each group) were fasted with water for 12 hours. Before drug administration, a blood sample as a control was taken for each rat from behind the eyeball through the angle of ocular cavity using small capillary tubes.

Pure Glimepiride and Glimepiride microparticles were administered orally to each group using stomach intubations. A dose of 180 µg/kg of Glimepiride was administered in suspension form for each rat. The blood glucose level for the control and test sample was determined using the glucose

measuring instrument[7]. Blood samples were collected using the retro orbital puncture method at predetermined time at 1 hour interval up to 12 hour collected. The percentage reduction in blood glucose level was measured. The hypoglycemic activity of Glimepiride at anytime (t) in rats was calculated as the percent blood glucose change at that time with respect to initial blood glucose according to the formula given below [8].

Percentage blood Glucose reduction at time

$$'t' = \frac{a - b}{a} X\,100$$

Where 'a' is Initial blood glucose level and 'b' is Blood glucose level at time't'.

RESULTS AND DISCUSSIONS:

The microparticles were prepared by emulsion solvent evaporation technique by using cellulose acetate as polymer and span 80 as surfactant. The method employed gave discrete, spherical, non-sticky and free flowing microparticles. As aggregates these microparticles were also non-sticky and free flowing. The optimal concentration of span 80 was found to be 1.0%. Microscopic examination of the formulations revealed that the microparticles were spherical and appeared as aggregates or discrete particles.

The microparticles were subjected to *In-vitro* release studies by employing 7.8 pH phosphate buffer as dissolution medium. The rate of drug release from these microparticles followed zero order kinetics and the mechanism of drug release was governed by peppas mechanism. The in vivo performance of pure drug and optimized Glimepiride microparticles were evaluated in rabbits in a randomized cross-over design. High-performance liquid chromatographic (RP-HPLC) method was developed for determination of Glimepiride in rabbit serum. Typical chromatogram of Glimepiride in serum was given in **Fig.1**.

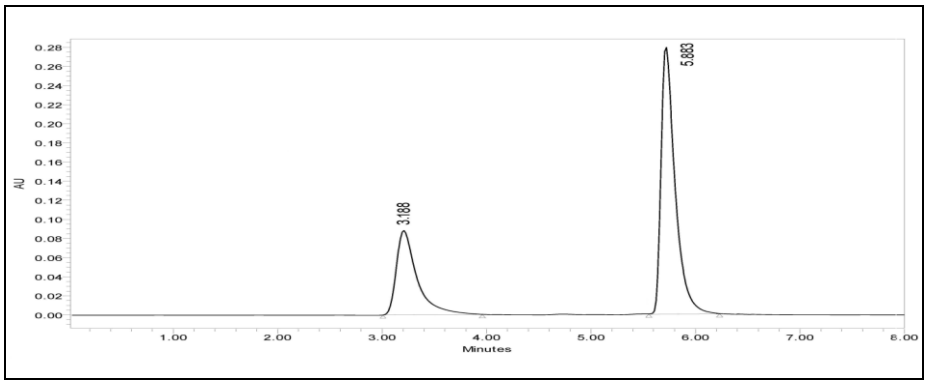

FIG.1: HPLC CHROMATOGRAM SHOWING GLIMEPIRIDE AND INTERNAL STANDARD PEAKS

Serum Glimepiride concentrations at different times after pure drug administration and optimized microparticles administration were calculated and are shown in **Fig.2**.

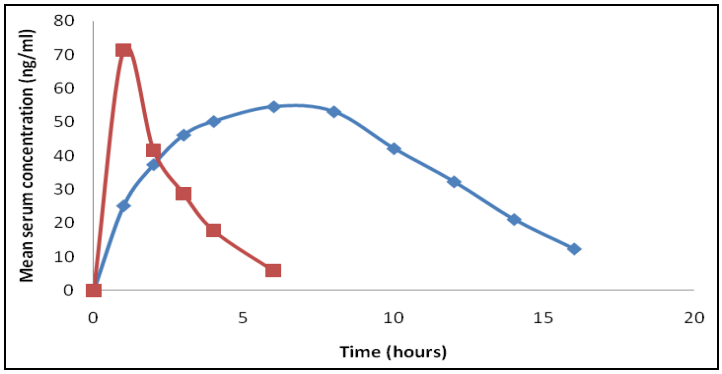

FIG.2: COMPARATIVE SERUM CONCENTRATION -TIME CURVE OF GLIMEPIRIDE FOLLOWING PURE DRUG AND OPTIMIZED MICROPARTICLES ADMINISTRATION

(-■-) Serum Concentration -Time Curve of Glimepiride following pure drug administration

(-♦-) Serum Concentration -Time Curve of Glimepiride following optimized microparticles administration

Statistical Treatment of Pharmacokinetic Parameters of Glimepiride obtained with pure drug and optimized microparticles were calculated and are shown in **Table 1**.

TABLE 1: STATISTICAL TREATMENT OF PHARMACOKINETIC PARAMETERS (Mean ± S.d.) OF GLIMEPIRIDE OBTAINED WITH PURE DRUG AND OPTIMIZED MICROPARTICLES

Pharmacokinetic parameter	Pure Drug	Optimized Microparticles	Calculated value of 't'
C_{max} (ng/ml)	71.36 ± 0.31	54.6 ± 0.42	36.70***
MRT (h)	2.84 ± 0.01	12.42 ± 0.18	75.50***
$t_{1/2}$ (h)	1.53 ± 0.011	5.09 ± 0.072	40. 75***
K_{el} (h^{-1})	0.58 ± 0.012	0.31 ± 0.014	8.87***
K_a (h^{-1})	1.68 ± 0.01	0.53 ± 0.02	19.67***
$AUC_{0-\square}$ (ng h/ml)	191 ± 1.43	1018.1.±2.07	256.60***

Null hypothesis (H_o): There is no significant difference between the pharmacokinetic parameters of Glimepiride obtained with pure drug and optimized microparticles. Table value of 't' with 10 DF at the 0.001 level is 4.587.

Result: H_o is not accepted as the calculated 't' value more than the table Value of 't' with 10 DF at 0.001 levels of significance. It was therefore concluded that there was significant difference between the pharmacokinetic parameters of obtained with pure drug and optimized microparticles.

The results indicated that the parameters significantly differed following optimized microparticles administration, compared to pure drug administration. The concentration of selected drugs in serum was found to be stabilized and maintained in a narrow range over the study period up to 16 hrs for optimized microparticles formulation where as the concentration was decreased rapidly up on pure drug administration. The maximum plasma concentration (C_{max}) was attained at 1 hrs after pure drug administration and it was observed after 6 hrs upon application of optimized microparticles formulation of same dose. The mean residence time (MRT) was found to be increased significantly ($p<0.001$) for optimized microparticles formulation on comparison with pure drug administration. Though both the formulations containing an equivalent amount of Glimepiride (180 µg), the $AUC_{0-\infty}$ values observed with optimized microparticles formulations ($p<0.001$) was found to be fivefold than that of pure drug administration.

The low t_{max} and high C_{max} values following pure drug administration was due to rapid absorption from the gastro intestinal tract, in contrast the low C_{max} and prolonged t_{max} after optimized microparticles administration was due to slow release of drug from microparticles.

The *in vivo* pharmacokinetic studies revealed that the optimized microparticles exhibited controlled release and absorption kinetics over longer periods of time which in turn maintained the desired serum concentrations over longer periods of time of 12 hours. The hypoglycemic effect was studied in healthy rats and compared the hypoglycemic effect of pure drug with the hypoglycemic effect of optimized microparticles formulation. The plot of percentage reduction of blood glucose Vs time was shown in figure 3. In the study of hypoglycemic effect of pure drug , the maximum percentage of reduction of blood glucose was found to be 37.67% at 2nd hour and in case of optimized microparticles formulation the maximum percent reduction of blood glucose was 36.40% at 4th hour. The maximum hypoglycemic effect increased from 2nd hour to 4th hour and maintained constantly up to 8th hour. The constant effect was due to slow release and slow absorption of Glimepiride over a longer period of time.

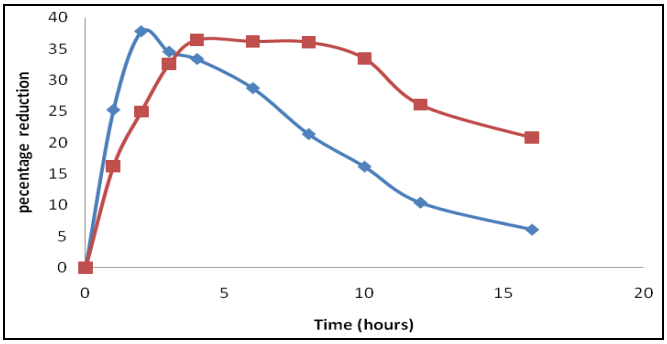

FIG.3: PERCENTAGE REDUCTION OF BLOOD GLUCOSE LEVELS OF GLIMEPIRIDE FOLLOWING PURE DRUG AND OPTIMIZED MICROPARTICLES ADMINISTRATION

(-♦-) Percentage reduction of blood glucose levels of Glimepiride following pure drug administration

(-■-) Percentage reduction of blood glucose levels of Glimepiride following optimized microparticles administration

CONCLUSION: The Glimepiride microparticles were successfully prepared by Emulsion Solvent Evaporation method by using Cellulose Acetate as a release rate retardant. Drug release from the microparticles followed zero order kinetics and controlled by peppas mechanism. The *in vivo* pharmacokinetic studies revealed that the optimized microparticles exhibited controlled release and absorption kinetics over longer periods of time which in turn maintained the desired serum concentrations over longer period of 12 hours.

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