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GLIPIZIDE AND VALDECOXIB COMBINATION MUCOADHESIVE TABLETS: FORMULATION, *IN VITRO* AND *IN VIVO* EVALUATION

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ABSTRACT

Aim: The main purpose of present study was to develop mucoadhesive tablets of Glipizide and Valdecoxib to achieve controlled plasma level of the drug especially in diabetes mellitus patients, who has additional pain therapy.

Methods: The drug-excipient compatibility studies were performed by Differential Scanning Calorimetric (DSC) and Fourier Transform Infrared spectroscopic (FTIR) studies. Physicochemical characteristics and *in vitro* drug dissolution and *in vivo* drug release properties were characterized. The *in vitro* drug release pattern and the dissolution data was treated with mathematical modeling. Accelerated stability studies were also carried out to the optimized formulation (F-5). The DSC and FTIR studies revealed that drugs were compatible with the excipients used. The optimized formulation (F-5) was found to have good physicochemical, *in vitro* release pattern and *in vivo* release characteristics. The *in vitro* dissolution data was perfectly fitting to zero order and the release of drug from the formulation followed Higuchi's release. The accelerated stability studies revealed that the tablets retain their characteristics even after stressed storage conditions.

Conclusion: From this study it was concluded that Glipizide and Valdecoxib combination mucoadhesive tablets is a good combination for diabetics who associated with pain and inflammation therapy.

INTRODUCTION

Glipizide is an oral hypoglycemic agent, commonly prescribed drug for the treatment of patients with type II diabetes mellitus [1]. Glipizide is a weak acid with a pKa of 5.9 [1]. It is practically insoluble in water and acidic solutions but as per the Biopharmaceutical Classification System (BCS) it is highly permeable (class 2) and it has a biological half-life of 3.4±0.7h. The normal dose is 2.5 to 10 mg twice or thrice daily [2].

Valdecoxib is a non-steroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, analgesic and antipyretic properties was chosen as a model drug due to its high first pass metabolism [3]. It undergoes both P450 and non-P450 dependent (Glucuronidation) metabolism [3]. The mechanism of action is believed to be due to inhibition of Prostaglandin synthesis primarily through inhibition of cyclooxygenase-2 (COX-2).

Glipizide and Valdecoxib combination mucoadhesive tablets were prepared by using Sodium Carboxy methyl cellulose, Hydroxy Propyl Methyl Cellulose, Carbopol-934P and Poly Vinyl Pyrrolidone. Glipizide and Valdecoxib combination mucoadhesive tablets are not available commercially. So an attempt has been made to develop and evaluate a combination sustained release mucoadhessive formulation containing an anti-diabetic drug with NSAID.

MATERIALS AND METHODS

Materials

Glipizide and Valdecoxib were obtained from Dr. Reddy's laboratories (Hyderabad, India). Hydroxy propyl methyl cellulose (HPMC) K4M, Carbopol-934P, Sodium Carboxy Methyl Cellulose-H, Poly Vinyl Pyrrolidone-K30, Ethanol (50%) and magnesium stearate were procured from SD fine chemicals,Mumbai,all other ingredients used were of analytical grade.

Drug-excipient compatibility studies

Differential Scanning Calorimetric (DSC) analysis

Differential Scanning Calorimetry (DSC) thermo grams were obtained by a differential scanning calorimeter (Schimadzu DSC-50, Tokyo, Japan) at a heating rate of 10°C/min from 30-300°C in nitrogen atmosphere (20 ml/min) with a sample weight of 3mg.

Fourier Transform Infrared Spectroscopic (FTIR) analysis

The FTIR spectrums of Glipizide, Valdecoxib and Formulation (F-5) blend were studied by using Fourier Transform Infrared (FTIR) spectrophotometer (Perkin Elmer, spectrum-100, Japan) using the KBr disk method (5.0015 mg sample in 300.0021 mg KBr). The scanning range was 500 to 4000 cm^{-1} and the resolution was 1 cm^{-1}. This spectral analysis was employed to check the compatibility of drugs with the polymers used.

Preparation of mucoadhesive Tablets

Mucoadhesive tablets were prepared in 3 steps [4-6].

a) Preparation of Core Layer's Mixture

Glipizide, Valdecoxib, HPMC, Carbopol-934P, Sodium Carboxy Methyl Cellulose-H, Poly vinyl Pyrrolidone-K30 and Magnesium stearate were mixed well by using glass mortar and pestle. This mixture was used for the preparation of core layer of the tablet. The composition of core layer was represented in Table No1.

Table No 1: Composition of mucoadhesive tablets core layer

INGREDIENTS (MG)	FORMULATION				
	F-1	F-2	F-3	F-4	F-5
Glipizide	10	10	10	10	10
Valdecoxib	20	20	20	20	20
Hydroxy Propyl Methyl Cellulose	5	10	15	20	25
Carbopol-934P	10	20	30	40	50
Sodium Carboxy Methyl Cellulose-H	5	10	15	20	25
Poly Vinyl Pyrrolidone-K30	2	4	6	8	10
Spray dried Lactose	94	72	50	28	6
Magnesium stearate	4	4	4	4	4

Total Weight = 150 mg

b) Preparation of Backing Layer's Granules

Carbopol-934P, Poly Vinyl Pyrrolidone and Magnesium stearate were mixed well in glass mortar and pestle. Gradually ethanol was added to the dry mixture to get a wet mass/lump. Then this lump was passed through the sieve # 40. Then wet granules were dried in a Hot Air Oven at a temperature 50^{o}C for 20 min. To this dried granules, magnesium stearate lubricant was added. These granules were used for the preparation of backing layer of the tablet. The composition of backing layer was represented in Table No 2.

Table No 2: Composition of mucoadhesive tablet backing layer

Ingredients	Quantity (mg)
Magnesium stearate	15
Carbopol-934P	35
Poly Vinyl Pyrrolidone-K30	25
Ethanol (50%)	q.s

c) Compression

Single punch rotary tablet compression machine with die punch set having diameter of 10mm was used. Firstly, the mixture of drug and polymers (weighed quantity-150mg) was compressed, then upper punch was removed and granules of backing layer (weighed quantity-75mg) were added over the first layer and compressed and the bilayer tablet was prepared.

Physical evaluation of tablets

Thickness

The thickness of the tablets was determined using a screw gauge (ISC Technologies, Kochi, India). 5 tablets from each batch were used and the mean values were calculated.

Uniformity of Weight Test

20 tablets of each formulation were weighed using an electronic balance (YPX202N, China) and the test was performed as per the official procedures.

Hardness test

The hardness of the tablets was measured using Monsanto tablet hardness tester (MHT 51, China). It is expressed in kg/cm^2. Three tablets were randomly picked and analyzed for hardness. The average and standard deviation values were also calculated.

Friability test

The friability of tablets was determined using Roche Friabilator (Campbell Electronics, Mumbai, India). The friabilator was operated at 25 rpm for 4 min. The % friability was then calculated by the following equation [7].

$$F = W_{initial} - W_{final} / W_{initial} \times 100$$

Where, F= friability (%), $W_{initial}$ = initial weight and W_{final} = Final weight

Uniformity in drug content

The formulated tablets were tested for uniformity in Glipizide and Valdecoxib contents by using UV/ Visible double beam spectrophotometer (Elico SL 210, India) at 223 nm and 252 nm for Glipizide and Valdecoxib respectively.

Surface pH

The surface pH of the mucoadhesive tablets was determined to find out the possibility of any side effects when swallowed. An acidic or alkaline pH may cause irritation to the mucosa. The tablet was allowed to swell by keeping it in contact with 1 ml of distilled water (pH 6.5 ± 0.02) for 2h at room temperature [8]. The pH was measured by using digital pH meter (PHS-25, China).

Moisture absorption studies of mucoadhesive tablet

A 5% w/v solution of Agar prepared in hot water and transferred into petri dishes and allowed to solidify. Five pre weighed tablets from each formulation were placed in vacuum oven overnight to remove moisture and laminated on one side with a water impermeable backing membrane. The tablets were placed on the surface of the agar and incubated at 37^{o}C for 1h. Then the tablets were removed and weighed and the percentage of moisture absorption was calculated by using the following equation [9].

% Moisture absorption = {(final weight – initial weight)/initial weight} x100

Mucoadhesive Force Measurement

Mucoadhesive force measurement of tablets was done by modifying balance method. The right pan was replaced with a glass beaker container and on the left side beaker with a copper wire. Teflon block of 1.5 cm diameter and 3 cm height was adhered strongly with the glass beaker. The two sides were then adjusted, so that the left hand side was exactly 5 g heavier than the right. Stick the stomach on the teflon block with help of the cynoacrylate glue and fill the beaker with acidic buffer till the tissue remains in a moist condition. Stick the tablet to beaker and put on the tissue for 15 min, later add water slowly into right beaker until the tablet detaches. Weigh the water required for the tablet detachment. Calculate Actual weight for detachment and force of adhesion in dynes by following equation [10].

Actual weight for detachment (W) = weight for detachment (g)

Matrix Erosion

Each tablet weighed (W_1) were immersed in a phosphate buffer pH 7.4 for predetermined time (1, 2, 4, 8 and 12h). After immersion, tablets were wiped off by the excess of surface water by the use of filter paper. The swollen tablets were dried at 60^{o}C for 24h in an oven and kept in a desiccator for 48h prior to be reweighed (W_2). The matrix erosion was calculated using the following equation [10].

$$\text{Matrix Erosion} = (W_1 - W_2)/ W_1 \times 100$$

Swelling behavior of matrix tablets

The swelling behavior of formulation F-1, F-2, F-3, F-4 and F-5 were studied. One tablet from each formulation was kept in a Petri dish containing phosphate pH 7.4. At the end of 2h, the tablet was withdrawn, kept on tissue paper and weighed, repeated for every 2h till the end of 12h. The % weight gain by the tablet was calculated by following equation [11].

$S.I = \{(M_t - M_0) / M_0\} \times 100$ Where, S.I = Swelling Index; M_t = Weight of tablet at time 't' and M_0 = Weight of tablet at time 0.

***In vitro* Dissolution Studies**

The dissolution of the mucoadhesive tablets were performed using USP XXIII dissolution apparatus (paddle method) using 500 ml of 0.1M HCl solution for first 2 h followed by phosphate buffer solution (pH 7.4) for next 10h as the dissolution medium, which was maintained at 37±0.5^{o}C and stirred at 50 rpm. Tablet was glued with Cyanoacrylate adhesive (Evobond) from backing layer side to the glass slide and it was placed at the bottom of jar of dissolution apparatus to avoid movement of tablet. Aliquots of 5ml of samples were withdrawn with a bulb pipette at different time intervals of 1h till 12h and replaced with equal volume of dissolution medium at each withdrawal, filtered it through Whatmann Filter Paper No.1. The samples were then analyzed using double beam UV visible spectrophotometer (Elico SL 210, India) at 223 nm and 252 nm for Glipizide and Valdecoxib respectively. The cumulative amount of drug released at various time intervals was calculated [12-15].

***In Vivo* Pharmacodynamic Studies**

Selection and maintenance of animals

Wistar albino rats of either sex weighing 200-250 g were employed for the study (procured from National Institute of Nutrition, Hyderabad, India). The rats were maintained under standard laboratory conditions (temperature of 25±2^{o}C and relative humidity of 50±15%) and normal photo period was used for the experiment [16-18]. Commercial pellet diet (Ratan Brothers, India) and water were provided when desired. The experimental protocol has been approved by the Institutional Animal Ethics committee and by the Regulatory body of the government (Regd no.1563/PO/a/11/CPCSEA).

Hypoglycemic study

Two groups of rats each of 5 animals, that were fasted (water) at least 12 h before the experiments were used for the study. Before drug administration, a blood sample as a control was taken from each rat from behind the eyeball through the angle of ocular cavity using small capillary tubes. The blood glucose level for the control and test samples was determined using the glucose-measuring instrument Medisence (Abbott Laboratories, USA). The instrument was self–calibrated, and the samples were allowed to dry before the results were read to avoid contamination of the lens. Pure Glipizide and mucoadhesive tablet (F-5) were administered orally to each group using stomach intubations. A dose of 800 µg/kg of Glipizide was administrated in a suspension form (freshly prepared) for each rat. Blood samples were collected at 1h intervals up to 12h and the blood glucose level was performed as per method described earlier. The % reduction in blood glucose level was measured [19].

Anti-inflammatory study

The anti-inflammatory actions were measured by comparing the maximal oedema response during 6 h [20, 21] A 0.1 ml of 1% carrageenan suspension was subcutaneously given into the sub plantar tissue of the hind paw of each rat. Group I normal rats treated with placebo suspension (1% Sodium CMC), which served as normal control, Group II rats were treated with Valdecoxib pure drug (2 mg/kg body weight), which serves as standard and Group III rats were treated with formulation suspension at a dose of 2mg/kg body weight respectively. All the doses were administered orally according to their body weight.

Accelerated Stability Studies

To assess the drug and formulation stability, stability studies were done according to ICH and WHO guidelines. Optimized formulation (F-5) was sealed in aluminum packaging coated inside with polyethylene, and then kept in stability chamber maintained at 45^{o}C and 75% RH for 3 months [22]. At the end of studies, samples were analyzed for the drug content, *in vitro* dissolution, floating behavior and other physicochemical parameters

RESULTS AND DISCUSSION

Compatibility studies

The FTIR of Glipizide showed characteristic peaks at 3373.70 (3300-3500) (N-H); 2934.57 and 2855.22 (2850 – 3000) (C-H); 2789.04 and 2706.85(3300- 2500 (O-H); 1529.59, 1462.94 and 1346.73 (1350–1550) (N=O); 1025.67 (1220 -1020) (C-N); 1157.94 and 1123.29 (1000 – 1300) (C-O). The FTIR spectrum was shown in Fig. 1.

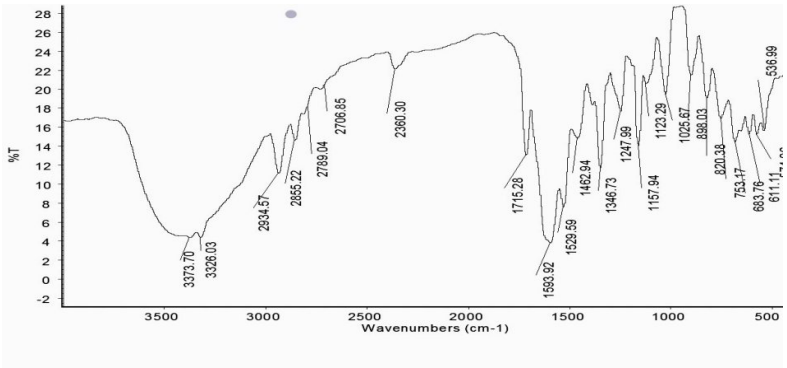

Fig.1: FTIR spectrum of Glipizide

Whereas the FTIR spectra of Valdecoxib showed characteristic peaks at 3441.05 (3300-3500) (N-H); 2909.68 and 2805.61 (2850–3000) (C-H); 2805.61 (3300-2500 (O-H); 1521.66, 1447.00 and 1405.77 (1350–1550) (N=O); 1217.98, 1153.91, 1127.73 and 1080.53 (1220 - 1020) (C-N); 1283.52 and 12487(1000–1300) (C-O). The FTIR spectrum was shown in Fig.2.

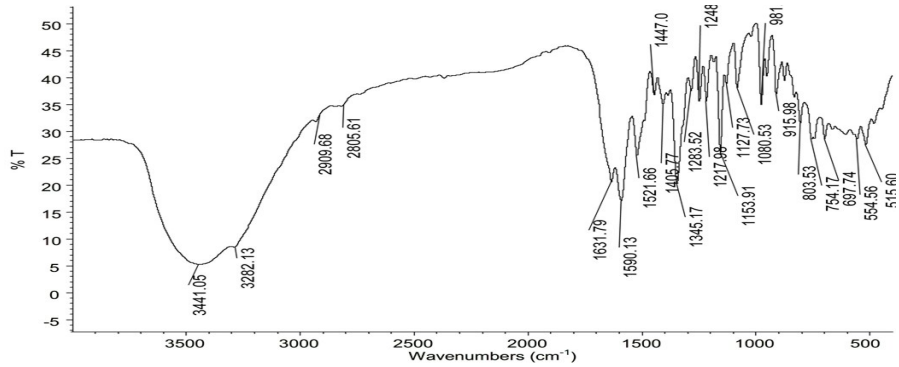

Fig.2: FTIR spectrum of Valdecoxib

The formulation showed characteristic peaks at 3439.40 (3300-3500) (N-H); 2942.54 (2850–3000) (C-H); 2739.88 (3300-2500 (O-H); 1485.61, 1446.91 and 1387.02 (1350–1550) (N=O); 1035.13 (1220-1020) (C-N) and 1156.96 (1000 –1300) (C-O). The FTIR spectrum was shown in Fig. 3.

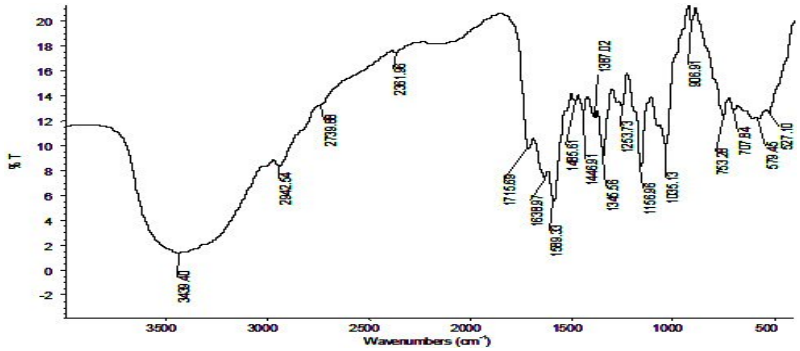

Fig.3: FTIR spectrum of formulation blend (F-5)

Evaluation of tablets

Average weights of the formulated tablets were uniform and ranged from 225±6.55 to 227±2.11mg. The thickness of the formulated tablets was ranged from 10.0±0.19 to 10.2±0.15mm, indicates uniformity in weight and thickness. The hardness of the formulated tablets were ranged from 6.8±0.06 to 8.9±0.08 kg/cm^2, which was more than 4kg/cm^2 and the weight loss on friability was ranged from 0.29±0.02 to 0.84±0.05%. The hardness and friability results revealed that the formulated tablets have good mechanical strength, which helps in maintaining the rigidness of tablet during handling and transport. All these physical and mechanical properties of formulated tablets showed in Table No 3. The swelling index increases by increasing the contact time as the polymers gradually absorbs the water due to hydrophilic nature with resultant swelling. The formulated tablets showed good Swelling behavior and showed in Fig. 4.

Table No 3: Evaluation of physical parameters of different mucoadhesive tablets

Formulation	Average Weight (mg)	Thickness (mm)	Friability (%)	Hardness (kg/cm^2)
F-1	226±1.41	10.2±0.15	0.54±0.05	7.7±0.05
F-2	225±7.58	10.1±0.58	0.84±0.05	6.9±0.04
F-3	225±6.55	10.0±0.19	0.77±0.03	5.8±0.16
F-4	226±4.51	10.0±0.59	0.66±0.01	6.5±0.19
F-5	227±2.11	10.1±0.046	0.29±0.02	8.5±0.29

All values mentioned in mean ± SD; Number of experiments (n) =6

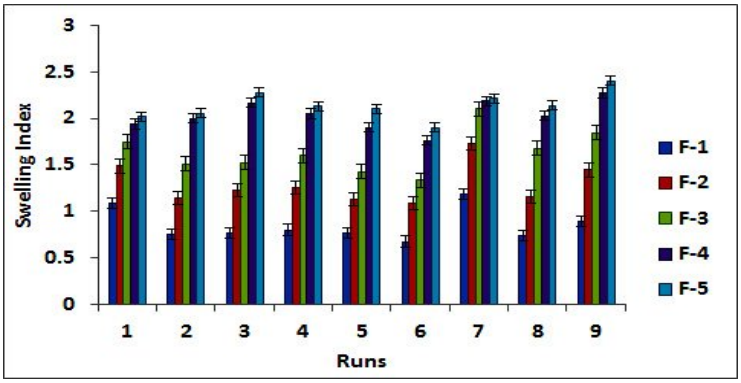

Fig.4: Swelling Index of formulated tablets

The surface pH was ranged from 6.67±0.88 to 7.07±0.11. The percentage water absorption was ranged from 38.48±2.01 to 39.87±0.55%. The formulated tablets showed good mucoadhesive strength which was ranged from 18.21±4.52 to 24.44±2.59. The mucoadhesion strength increases as the concentration of polymers increased. The percentage Glipizide in formulated tablets was ranged from 97.89±5.74 to 99.99±5.44% and Valdecoxib was ranged from 93.02±3.52 to 99.54±2.52% indicating the uniformity of drug content in formulation All these values were shown in Table No 4.

Table No 4: Evaluation parameters of different mucoadhesive tablets

Formulation	Surface pH	Water absorption (%)	Mucoadhesion strength (g)	Drug content (%)	
				Glipizide	Valdecoxib
F-1	6.91±0.24	38.54±0.11	18.21±4.52	99.99±5.44	95.01±5.84
F-2	6.99±0.61	39.87±0.55	20.67±2.16	96.98±6.59	93.02±3.52
F-3	7.07±0.11	38.48±2.01	21.79±1.54	97.89±5.74	99.54±2.52
F-4	7.05±0.46	39.18±1.03	22.44±2.57	98.44±9.56	96.44±5.84
F-5	6.67±0.88	39.12±1.26	24.44±2.59	99.04±1.25	98.11±5.26

All values mentioned in mean ± SD; Number of experiments (n) =6

Table No 5: Matrix Erosion of formulated tablets

Formulation	Percent matrix erosion after time (%)				
	2h	4h	6h	8h	12h
F-1	4.11±0.44	4.55±0.19	4.62±0.45	5.62±0.29	6.52±0.08
F-2	4.12±0.18	5.44±0.19	6.35±0.32	6.85±0.05	7.16±0.09
F-3	5.04±0.11	5.99±0.48	6.15±0.14	7.12±0.16	8.05±0.05
F-4	4.48±0.09	5.11±0.16	6.05±0.08	7.04±0.25	8.15±0.03
F-5	4.54±0.08	5.84±0.19	6.94±0.08	7.69±0.32	9.13±0.11

All values mentioned in mean ± SD; Number of experiments (n) =6

The plots result from *in vitro* dissolution study was shown in Fig. 5 and 6. The optimized formulation (F-5) was tested for drug content, Surface pH, mucoadhesion strength and Swelling Index before and after accelerated stability studies. The study proved that the formulations retain their characteristic parameters before and after accelerated stability studies. The values were shown in Table No 6.

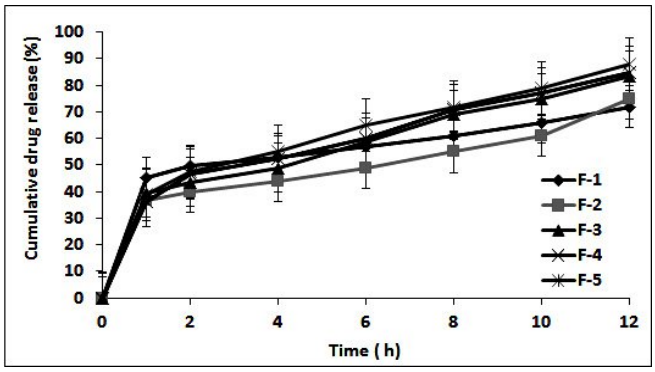

Fig.5: *In vitro* drug release from formulated tablets (Glipizide)

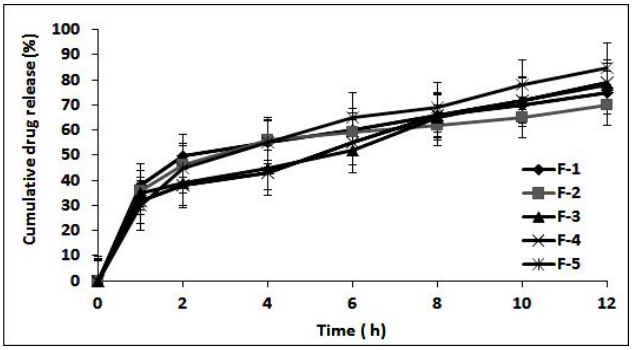

Fig.6: *In vitro* drug release from formulated tablets (Valdecoxib)

***In Vivo* Pharmacodynamic Studies**

Hypoglycemic study

The % reduction in blood glucose levels in Group-I was found to maximum at 3rd h and declined to base level by 8th h. On the other hand the formulation (F-5) showed % reduction in blood glucose level was maintained even after 12th h. The results were shown in Fig. 7.

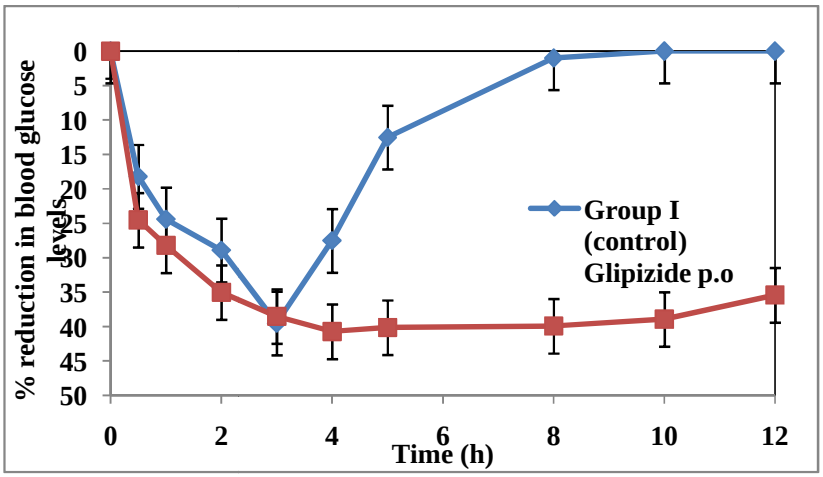

Fig.7: Reduced blood glucose levels (%) of optimized mucoadhesive tablets (F-5) vs. Glipizide oral control

Anti-inflammatory study

The % inhibition of paw edema with formulation (F-5) was better than pure drug. The results were shown in Fig. 8.

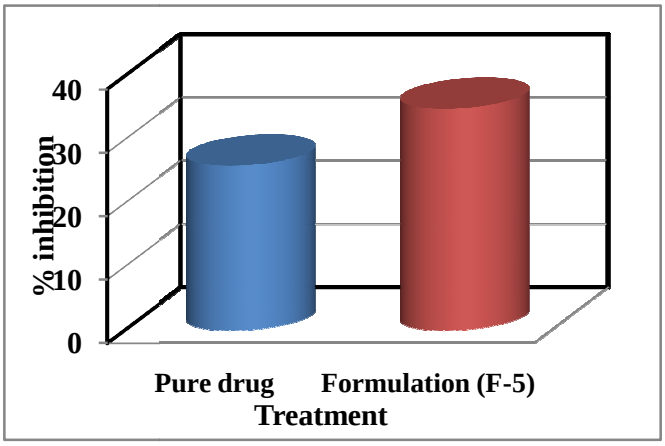

Fig.8: % inhibition of paw edema with formulation (F-5) vs. Valdecoxib pure drug

Table No 6: Parameters before and after stability studies of formulation F-5

Parameter	Initial	After stability studies
Drug content (%)	99.94±5.64 (Glipizide)	99.94±5.58
	99.97±8.54 (Valdecoxib)	99.97±8.12
Surface pH	6.68±0.15	6.68±0.46
Mucoadhesive strength (g)	23.68±2.59	23.67±2.86
Swelling Index (%)	85.65±5.68	85.64±5.24

All values mentioned in mean ± SD; Number of experiments (n) =3

CONCLUSION

This study revealed that Glipizide and Valdecoxib combination mucoadhesive tablets are a good combination for treatment of diabetic patients who are with additional treatment of pain and inflammation.

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