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Preparation and characterisation of anti-diabetic tablet containing p-GP inhibitors and herbal ingredients

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Abstract
Objective: The objective of the present research work was to develop antidiabetic formulation containing natural P-GP inhibitors in order to increase the Metformin drug permeability and a natural binder with an anti-diabetic effect.
Result: From the data it was concluded that F4 batch (Piperine 250mg) was optimized for further study as it shows complete release with higher permeation in less time than marketed and F1-F3 batches with a significant difference in percentage drug permeation. The optimized batches (F5-F8) were prepared, and their evaluation was carried out. After evaluating all parameters, it was observed that batch F8 (Linseed 15% and Dates 15%) was selected for stability study as all the parameters are found to be within their acceptable range, in compliance with IP standards.
Conclusion: The current study reveals that piperine in a suitable proportion can be highly effective in increasing the permeability of Metformin and incorporation of herbal ingredient can reduce its side effects.

Keywords: containing p-GP inhibitors, herbal ingredients

1. Introduction
P-gp, a glycosylated membrane-bound protein discovered by Juliano and Ling in Colchicine-resistant Chinese hamster ovary cells in 1976, regulated drug permeability and showed anticancer resistance [1]. P-glycoprotein (P-GP) (P-permeability) is a member of the ABC transporter superfamily that acts as a physiological barrier by eliminating pathogens and other substances from cells. In humans, P-GP is a small gene family with two isoforms MDR1/ABCB1 delivers drugs, whereas MDR2/3/ABCB4 exports phosphatidylcholine into the bile [2]. P-gp is found in the stomach, liver, kidney, brain, placenta, and pancreas, and it plays an important role in the active transport of foreign molecules out of cells [3]. P-gp can be regarded as a distinctive defense barrier network against the invasion of xenobiotics into the body due to its widespread distribution. This efflux carrier reduces the bioavailability of given drugs by limiting their adequate intracellular accumulation. As a result, drug effectiveness eventually decreases. It also influences the pharmacokinetics and pharmacodynamics of its substrates by modulating their ADME (absorption, distribution, metabolism, elimination) [4]. To overcome this problem, P-gp inhibitors were investigated as a strategy to overcome multidrug resistance and low bioavailability concerns with therapeutic P-gp substrates. Study has supported efforts to lower P-gp activity to improve therapeutic delivery and avoid resistance to antibiotics. Several synthetic and natural substances have been shown to block P-gp transport activity, leading to increased intracellular drug accumulation, MDR reversal [5], and improved pharmacokinetic and pharmacodynamic profiles of many complex molecules, which could be useful in developing possibly effective oral formulations of drugs such as tablet dosage form.
This study focused on the development of an oral anti-diabetic tablet formulation in which Metformin was utilized as the Active Pharmaceutical Ingredient (API) with absolute bioavailability ranging from 50% to 60%. The expression of an intestinal efflux transporter, P-glycoprotein (P-gp), is considered to be the cause of its bioavailability problem [6]. To overcome this limitation, effective natural P-gp inhibitors such as piperine and pectin were included in the formulation development, as well as natural binders with anti-diabetic properties such as Dates [7], Linseed, Fenugreek [8], Basil seeds, Drumstick [9] were evaluated for its binding efficacy and the optimized batch of binder were used in the final formulation development.

The ex-vivo gut sac model has been extensively researched for pharmacokinetic investigations on drug absorption, drug metabolism or prodrug conversion in gastrointestinal segments, efflux transport, multidrug resistance, and the influence of efflux transport inhibitors on drug absorption. Potential advantages of this model usually involve the presence of a mucus layer and a comparatively large surface area available for absorption [10]. The ileum gut sac from the intestine of a chicken was used in this research.

2. Materials and Methods

2.1 Materials

Metformin HCL was obtained from Aarti Drugs Limited, Sarigam, Gujarat, and Black Pepper, Orange peel powder (pectin), Dates, Linseed, Fenugreek, Basil seed, Drumstick powder were obtained in standard packs from the local market, and other materials for buffer was obtained from S.D Fine Chem Ltd., Mumbai.

2.2 Methods

2.2.1 Ex-vivo gut sac study (Non-everted)

The ex-vivo sac method was used to evaluate drug transport from the mucosal to the serosal surface by crushing the prepared tablet and preparing a solution. In this study, a small section of a non-everted chicken intestinal sac was used to illustrate the efflux mechanism of an anti-diabetic drug. The ileum was separated and dissected (10 cm each) (Figure 1 and Figure 2). After that, the segments were washed with a physiological solution (such as oxygenated tyrode's solution). After that, the ileum was clamped at 37°C and a drug solution was introduced. The opposite end of the filled intestinal segment was then clamped off. After another end was clamped, the entire intestinal sac was transferred in a beaker containing 200 ml oxygenated media at 37°C. An aeration tube was utilized to provide oxygen. The samples were collected at discrete intervals and evaluated using UV spectroscopy (Shimadzu) at 234nm.

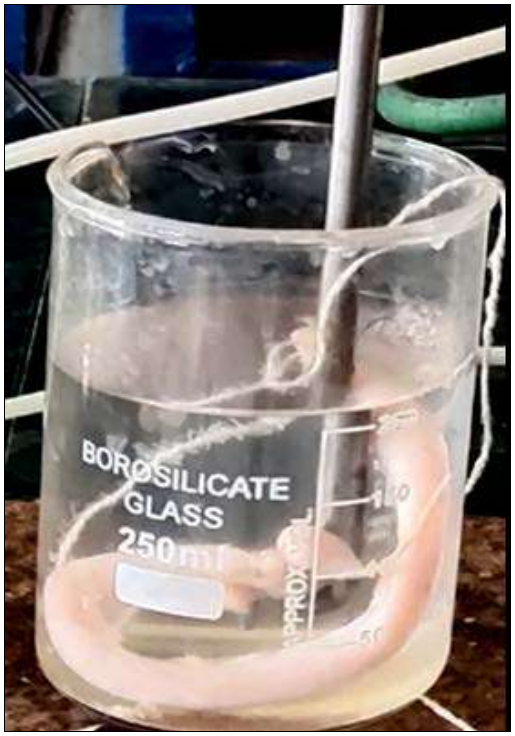

Fig 1: Assembly of non-everted gut sac study

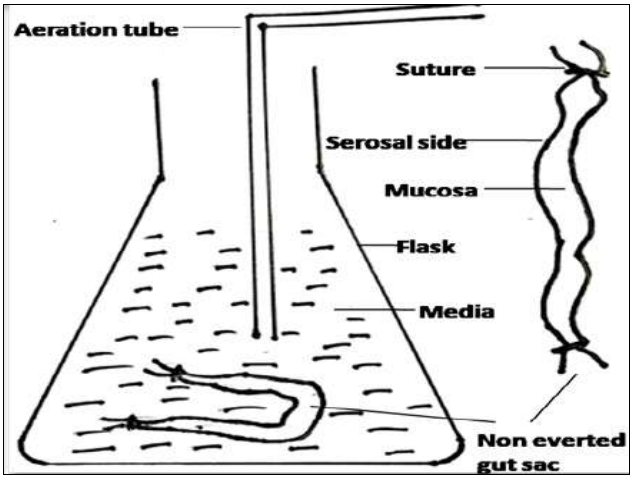

Fig 2: Schematic diagram of non-everted gut sac model

2.2.2 Preparation of tablet

Tablets were prepared by wet granulation method:

The drug and excipients were milled, and after proper mixing, a binder was added in a mixture to form a wet mass, which was then dried using a 6 to l2 mesh screen. Dry granules were then screened through 14 to 20 mesh screens before being mixed with lubricants and compressed into tablets. A single punch tablet compression machine was used to prepare batches. All the formulation batches were evaluated as per I.P. standards.

3. Results

3.1 Preliminary trial batches (tp1-tp6)

In TP1 to TP6 batches, ingredients like PVP K30, Drumstick, Linseed, Dates, Fenugreek and Basil were used as a binder. Lactose was used as a diluent. Magnesium stearate and Talc were used as a lubricant and glidant respectively.

Table 1: Preliminary trial batches

Ingredients (mg)	TP1	TP2	TP3	TP4	TP5	TP6
Metformin HCL	500	500	500	500	500	500
PVP K30	75	-	-	-	-	-
Drumstick	-	75	-	-	-	-
Linseed	-	-	75	-	-	-
Dates	-	-	-	75	-	-
Fenugreek	-	-	-	-	75	-
Basil	-	-	-	-	-	75
Lactose	160	160	160	160	160	160
Magnesium stearate	7.5	7.5	7.5	7.5	7.5	7.5
Talc	7.5	7.5	7.5	7.5	7.5	7.5
Total wt. (mg)	750mg	750mg	750mg	750mg	750mg	750mg

*All the weights are in mg

The preliminary trial batches (TP1-TP6) were prepared using different binders (PVP K30, drumstick, linseeds, dates, fenugreek, and basil). After evaluating all the parameters, it was observed that among the natural binders, the hardness, friability, and disintegration of batches TP2, TP5, and TP6 were determined to be below the acceptability standards according to I.P. 2007. However, the hardness of batches TP1, TP3, and TP4 was determined to be significant, and the friability of formulations was less than 1%. As a result, it met the acceptance criteria of I.P. 2007. The average weight variation of all formulations was determined to be close to their standard range, which was in accordance with IP standards. Because the disintegration

time of TP3 and TP4 was determined to be less and also the hardness was less as compared to that of TP1, so TP1 was selected for further study.

3.2 Formulation batches (F1-F4)

In F1-F4 batches piperine was used as P-gp inhibitor, pectin was used as diluent and it has also P-gp inhibitory role, PVP K30 was used as a binder, Magnesium stearate and talc was used as a lubricant and glidant respectively.

Table 2: Formulated batches

Ingredients (mg)	F1	F2	F3	F4
Metformin HCL	500	500	500	500
PVP K30	75	80	85	90
Piperine	100	150	200	250
Pectin	60	54	48	42
Magnesium stearate	7.5	8	8.5	9
Talc	7.5	8	8.5	9
Total wt. (mg)	750mg	800mg	850mg	900mg

*All the weights are in mg

The formulation batches (F1-F4) were prepared, and their evaluation was carried out. After evaluating all parameters, it was observed that the hardness, friability, and disintegration of all batches were significant; the friability of formulations was less than 1% and also the average weight variation and disintegration time of all formulation batches were determined to be within their acceptable range, in compliance with IP standards. An in-vitro drug release study was performed, and all (F1-F4) batches showed complete release in 30 minutes. As a result, these batches were selected for the ex-vivo study.

3.3 Permeation study of (F1-F4)

Table 3: Permeation study of Marketed formulation and F1-F4 batches

Time (mins)	Marketed Formulation	F1	F2	F3	F4
15	16.66	20.22	23.87	25.22	27.44
30	34.22	42.11	45.72	47.54	57.33
45	54.12	66.57	69.38	71.88	85.65
60	73.37	86.31	89.03	91.55	99.99
90	90.81	99.98	99.97	99.97	-
120	99.99	-	-	-	-
f2 value		63.65	60.87	56.51	35.16

3.3.1 Graphical representation of permeation study of marketed formulation and formulation batches (F1-F4)

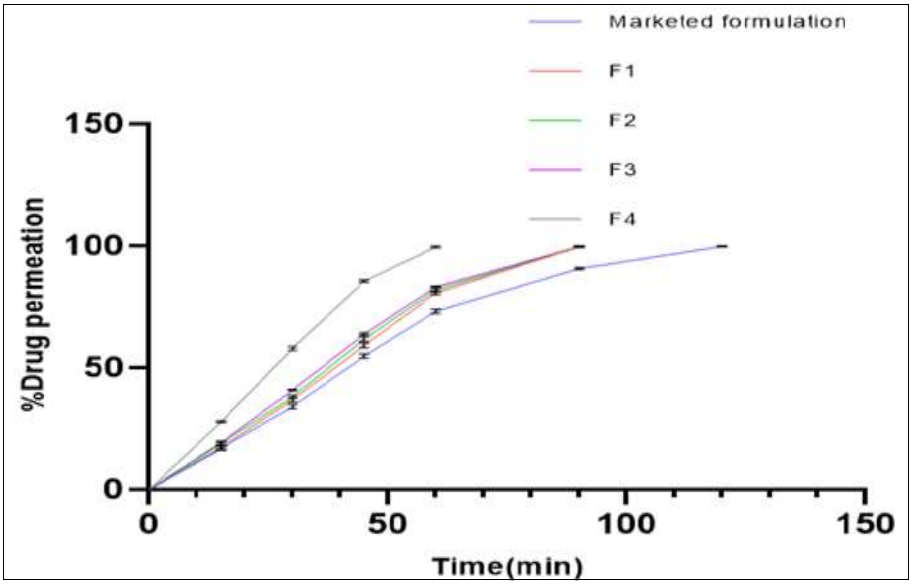

Fig 3: Comparision of drug permeation of marketed formulation with formulation batches

The data demonstrates that the drug transport from the intestine to the physiological solution in batches F1 (Piperine 100mg), F2 (Piperine 150mg) and F3 (Piperine 200mg) does not show a significant improvement, indicating that the amount of piperine in the formulation needs to be increased to achieve a significant difference in drug permeation. And, after increasing the quantity of piperine in the F4 (Piperine 250mg) batch, it was concluded that piperine demonstrated complete release with higher permeation in less time than marketed and F1-F3 batches with a significant difference in percentage drug permeation, which may be attributed to its competitive inhibition mechanism. As a result, the F4 batch was optimized for further study.

3.3.2 Kinetic modelling

Table 4: Release Pattern of optimized batch for Ex-vivo permeation study

Batches	Zero order R^2	First order R^2	Higuchi R^2	Korsmeyer Peppas R^2
F1	0.945	0.915	0.965	0.917
F2	0.962	0.900	0.968	0.918
F3	0.966	0.910	0.974	0.925
F4	0.977	0.922	0.978	0.918

After the selected (F1-F4) batches were fitted to zero order, first order, Higuchi model and Korsemeyer Peppas model to know the release mechanisms. It was observed that they follow nearly Higuchi controlled model which indicate transport of drug follows diffusion controlled path across the membrane and the it also follows nearly zero order release.

3.4 Optimized batch containing natural binders (F5-F8)

After Ex-vivo permeation study of marketed formulation and formulation batches, batch F4 (Piperine 250mg) was optimized for further study and piperine was used as a potent inhibitor at 250mg in the optimized formulation batches containing natural binders, Linseed and Dates was used as a natural binders, Pectin was used as diluent and it has also P-gp inhibitory role, Magnesium stearate and Talc was used as a lubricant and glidant respectively.

Table 5: Optimized batches

Ingredients (mg)	F5	F6	F7	F8
Metformin HCL	500	500	500	500
Linseed	100	-	50	75
Dates	-	100	50	75
Piperine	250	250	250	250
Pectin	130	130	130	80
Magnesium stearate	10	10	10	10
Talc	10	10	10	10
Total wt. (mg)	1000mg	1000mg	1000mg	1000mg

The optimized batches (F5-F8) were prepared, and their evaluation was carried out. After evaluating all parameters, it was observed that the hardness, friability, and disintegration time of batch F5 (Linseed 10%), F6 (Dates 10%) and F7 (Linseed 5% and Dates 5%) were determined to be below the acceptability standards according to I.P. 2007.So batch F8 (Linseed 15% and Dates 15%) was selected for stability study as the friability of formulation was less than 1% and also the average weight variation and disintegration time of batch was determined to be within their acceptable range, in compliance with IP standards. An in-vitro drug release study was also performed, and all (F5-F8) batches showed complete release in 30 minutes.

3.5 Accelerated stability study of optimized batch (F8)

Optimized formulation (F8) was subjected to accelerated stability study for 2 months and evaluated for hardness, friability, drug content, % CDR and compared with the previous results obtained for optimized formulation F8.

Table 6: Accelerated stability study of optimized formulation (F8)

Parameters	Initial	1 month	2 months
Hardness (kg/cm2)	7-8	7-8	7-8
Friability (%)	0.8	0.8	0.78
Percentage Cumulative Drug Release(%CDR)	99.99	99.98	99.87
Drug content (%)	99.98	99.87	99.76

It was observed that all the parameters were in range of I.P.2007 criteria.

4. Discussion

After increasing the quantity of piperine in the F4 (Piperine 250mg) batch, it was concluded that piperine demonstrated complete release with higher permeation in less time than marketed and F1-F3 batches with a significant difference in percentage drug permeation. As a result, the F4 batch was optimized for further study. The optimized batches (F5-F8) were prepared, and their evaluation was carried out. After evaluating all parameters, it was observed that batch F8 (Linseed 15% and Dates 15%) was selected for stability study as the friability of formulation was less than 1% and also the average weight variation and disintegration time of batch was determined to be within their acceptable range, in compliance with IP standards.

5. Conclusion

The purpose of this research work was to determine the efficacy of co-administered P-gp inhibitors, on Metformin absorption in an ex-vivo chicken non-everted gut sac model. In the current research work, piperine has shown significant enhancement in the drug permeation. Furthermore, the use of natural components decreases side effects.

6. Acknowledgement

For this Research work we don't have a scholarship or grant supporter.

7. Conflict of interest

There is no conflict of interest.

8. Funding

None to declare

9. Ethics approval

None to declare

10. References

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