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EFFECT OF COMPETITIVE BINDING OF HYPOGLYCEMIC AGENTS TO HUMAN SERUM ALBUMIN ON DRUG PHARMACOLOGY

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ABSTRACT

For the highly protein-bound anti-diabetic drugs with a small volume of distribution, competitive binding to human serum albumin can significantly influence the pharmacological activity of drugs resulting in serious fluctuations in the blood glucose levels of diabetic patients. In this paper, competitive binding studies using fluorescence spectroscopic technique have been reported for a wide range of drug combinations involving oral hypoglycemic (anti-diabetic) agents. For the drug combinations used, such studies are not available in the literature. The results indicated that the combination of gliclazide and repaglinide with the studied competing drugs can increase the risk of hypoglycemia in diabetic patients and should be avoided. On the other hand, the corresponding combinations involving glimepiride and glipizide were found safe. The therapeutic efficacy of studied competing drugs, on the other hand decreased in the presence of antidiabetic drugs in most cases. Competitive binding mechanism based on the site-specificity and conformational changes in the human serum albumin molecule has been proposed.

Key Words: Competitive binding, hypoglycemic agents, fluorescence spectroscopy

INTRODUCTION

In combination therapy, the therapeutic response of a given drug depends to a large extent on the competition offered by the other drug in the combination for the binding sites on serum albumin. Such competition may alter the concentration of free drug available for pharmacological effect.[1-3] Simultaneous binding of more than one drug may also cause conformational changes in serum albumin (non-competitive interference). However, drug displacement interactions are clinically significant only for low clearance, highly protein bound drugs with small volume of distribution and a low therapeutic index.[4] Competitive binding studies involving some NSAIDs[5], antibiotics[6], anticoagulants[7], and antifungal drugs[8] have been reported.

Competitive binding of some antidiabetic drugs to human serum albumin has been reported by Judis[9] and others.[10-12] This class of drugs have special relevance for such studies due to the following reasons: i) the drugs used are highly bound to serum albumin with small volume of distribution, ii) combination therapy is very frequently employed in type II diabetes and iii) change in the concentration of free drug available for antihyperglycemic effect due to competitive binding, can result in serious fluctuations in the blood glucose levels of diabetic patients. However, detailed studies on various aspects of the competitive binding of a wide range of hypoglycemic agents to human serum albumin (HSA) are not available.

In the present work, competitive binding of four oral antidiabetic drugs in the presence of four categories of competing drugs as well as competitive binding of competing drugs in the presence of four antidiabetic drugs has been studied. Data has been expressed as association constants, percentage of drug bound and percentage of free drug in the absence and presence of competing drugs. Results have been interpreted in terms of the change in the percentage of free drug due to competitive binding and the competitive binding mechanism has been proposed.

MATERIALS AND METHODS

Competitive binding studies have been carried out for four oral antidiabetic drugs: gliclazide, glimepiride, glipizide and repaglinide in the presence of four categories of competing drugs; one non-steroidal anti-inflammatory drug (parecoxib sodium), two antibiotics (sparfloxacin and cefdinir), analgesic and antipyretic drug (paracetamol) and two antidiabetic drugs (metformin hydrochloride and repaglinide), using fluorescence spectroscopic technique. HSA was titrated with antidiabetic drugs in the presence of competing drugs. Reverse experiments, titration of HSA with the competing drugs in the presence of antidiabetic drugs, were also carried out. A total of forty six drug combinations were studied. HSA was also titrated individually with the antidiabetic drugs as well as the competing drugs. For the binding studies, protein concentration was kept fixed (10 μM) and the titrant drug concentration was varied from 5-70 μM in each case.

The following procedure, also reported in our previous publications[13-14], was used for data analysis. The fraction of protein binding sites occupied by drug, θ was taken as the ratio of the change in fluorescence intensity, ΔF at a given drug concentration and the maximum change corresponding to saturation of binding sites. The concentration of bound drug, $[D_b]$ is given by $n\theta[P_t]$, where n is the number of binding sites and $[P_t]$ is the total protein concentration. The number of moles of free drug, $[D_f]$ was obtained by subtracting bound drug concentration from the total drug added, $[D_t]$. Moles of drug bound per mole protein, $r = [D_b]/[P_t]$ and $[D_f]$ values were fitted to the Scatchard equation ($r/[D_f] = nK_a - rK_a$) and association constant (K_a) for the binding was determined from $r/[D_f]$ versus r plots (not shown). The reported data is an average of three measurements with standard error of mean less than 0.05×10^4 in all cases.

The percentage of free drug ($\alpha = [D_f]/[D_t] \times 100$) and percentage of drug bound ($\beta = [D_b]/[D_t] \times 100$) at different drug:protein ratios were calculated from the dissociation constant, K_d ($= 1/K_a$) using equations (1) and (2).

$$\alpha = \frac{K_d + [D_f]}{[P_t] + K_d + [D_f]} \times 100 \quad (1)$$

$$\beta = \frac{[P_t]}{[P_t] + K_d + [D_f]} \times 100 \quad (2)$$

RESULTS AND DISCUSSION

Binding of antidiabetic drugs in the presence of competing drugs

Association constants: Association constants for the binding of antidiabetic drugs in the absence and presence of competing drugs are given in Table 1. In most cases, the presence of the competing drug decreased the association constant of parent antidiabetic drug. However, increase/practically no change in the association constant was also observed in some cases.

Percentage of drug bound (β): The presence of competing drug decreased the percentage binding of antidiabetic drugs in most cases (Fig. 1 (a-d)). The effect of competing drug on the binding affinity of parent drug was more pronounced at low $[D_t]/[P_t]$ ratios which are physiologically more significant since the serum albumin concentration in blood is much higher than the drug concentrations used. Moreover, at low drug concentrations, higher affinity sites on protein are occupied. It is seen that in general, the effect of competing drug on the binding affinity of glipizide was least. A large decrease (up to 32%) in the percentage binding of antidiabetic drugs was observed at the lowest $[D_t]/[P_t]$ ratio in most other cases. Percentage of bound gliclazide, however, increased by about 15% in the presence of repaglinide.

Percentage of free drug (α): Competitive binding results in change in the concentration of free, pharmacologically active drug. Data in Table 2 shows significant increase in the percentage of free antidiabetic agent in most cases. For example, the percentage of free gliclazide increased from about 42 to 60-69% in the presence of sparfloxacin, parecoxib sodium, metformin hydrochloride and paracetamol; the percentage of free repaglinide increased from about 27 to 55-59% in the presence of cefdinir and parecoxib sodium while percentage of free gliclazide decreased from about 42 to 27% in the presence of repaglinide. In the case of glimepiride and glipizide, the change in the percentage of free drug was relatively less.

Change in the percentage of free drug (Δ α): To get a quantitative idea about change in the percentage of free drug due to competitive binding, Δα (= α′- α, where α′ and α are the percentage of free drug in the presence and absence of competing drug, respectively) was also calculated (Table 3). In general, the presence of competing drugs increased the concentration of free antidiabetic drugs. Also the effect of competing drugs on the concentration of

free gliclazide and repaglinide was much larger as compared to glimepiride and glipizide, in most cases. For the analysis of data in Table 3, less than 1% change was considered as no change, 1-5%, 5-10%, 10-20% and >20% change was considered as very small, small, large and very large change, respectively.

A very large increase (>20%) was observed in the percentage of free gliclazide in the presence of sparfloxacin, parecoxib sodium and metformin hydrochloride and free repaglinide in the presence of cefdinir, sparfloxacin and parecoxib sodium. A large increase (10-20%) was observed in the percentage of free gliclazide in the presence of paracetamol, free glimepiride in the presence of repaglinide and metformin hydrochloride and free repaglinide in the presence of glimepiride. The concentration of free gliclazide, however, decreased by 15% in the presence of repaglinide. In other cases, the change was relatively less. The results are summarized in Table 4.

Binding of competing drugs in the presence of antidiabetic drugs

Association constants: Association constants for the binding of four competing drugs (cefdinir, sparfloxacin, parecoxib sodium and paracetamol) in the absence and presence of the antidiabetic drugs are given in Table 5. The presence of antidiabetic drugs resulted in increase in association constants of competing drugs in most cases. Decrease in association constant was observed only in the binding of parecoxib sodium in the presence of gliclazide and glimepiride. Maximum increase in the association constant was observed for the binding of antibiotic drugs, cefdinir and sparfloxacin. Large increase was also observed in the binding of paracetamol in the presence of glimepiride.

Percentage of drug bound (β): The percentage of drug bound (β) versus ($[D_t]/[P_t]$) ratio plots (Fig. 2 (a-d)) showed increase in binding affinity in most cases. Decrease was observed only in the binding of parecoxib sodium in the presence of gliclazide and glimepiride.

Percentage of free drug (α): Significant decrease in the percentage of free drug was observed in many cases (Table 6). The percentage of free cefdinir decreased from about 80% to 61% in the presence of gliclazide and to about 54% in the presence of glipizide and repaglinide; percentage of free sparfloxacin decreased from 62% to about 34%-47% in the presence of various competing antidiabetic drugs, percentage of free paracetamol decreased from about 75 to 42% in the presence of glimepiride. In other cases, the effect of competing antidiabetic drug was not very significant.

Change in the percentage of free drug (Δα): The change in the percentage of free drug (Δα) (Table 7) was interpreted in the same way as described before. A very large decrease (>20%) was observed in the concentration of free cefdinir in the presence of glipizide and repaglinide, free sparfloxacin in the presence of glimepiride and glipizide and free paracetamol in the presence of glimepiride. A large decrease (10%-20%) was observed in the concentration of free cefdinir in the presence of gliclazide and free sparfloxacin in the presence of gliclazide and repaglinide. The concentration of free parecoxib sodium, however, increased by about 17% and 10%, respectively in the presence of gliclazide and glimepiride. In other cases, the change was not very significant. The results are summarized in Table 8.

Binding characteristics of the drugs used

To get an insight into the binding mechanism, it is necessary to understand the binding behavior of individual drugs.

Association constants: The association constant data (Tables 1 and 5) showed that the competitive binding results discussed above cannot be explained on the basis of the magnitude of association constants. For example, significant decrease in the binding affinity of antidiabetic drugs in the presence of competing drugs shows that the antidiabetic drugs are unable to displace competing drugs from their binding sites, in spite of their higher binding constants. Thus the general belief that drug with higher binding constant has the ability to displace the drug with lower binding constant is not valid here. It has also been reported by other workers[9, 15] that the degree of displacement is not directly related to the association constants of the drug and the displacing compound. Thus factors other than binding affinity are involved.

Site-specificity: Since HSA has two high affinity drug binding sites; site I and site II in subdomain II A and III A, respectively, site-specificity of the binding and competing drugs can also be an important factor in explaining the competitive binding mechanism involved. Site-specificity of the antidiabetic drugs used has been reported in our previous publications [13-14]. Site-specificity of the competing drugs was also determined by the fluorescence probe displacement method[16]. Results show that glimepiride, glipizide and sparfloxacin bind at both

site I and II whereas all other drugs are site II-specific.

Competitive binding mechanism:

Binding of antidiabetic drugs: Amongst the antidiabetic drugs used, the binding of gliclazide and repaglinide decreased significantly in the presence of competing drugs. Since both the antidiabetic drugs as well as the competing drugs are site II drugs, antidiabetic drugs could not displace the competing drugs in spite of their relatively higher association constants. Decreased binding results in significant increase in the percentage of free drug available for antihyperglycemic effect. Thus these combinations increase the risk of hypoglycemia in diabetic patients and should be avoided.

The binding of glimepiride and glipizide, on the other hand, is not much affected by the competing drugs. Since these two drugs bind at both site I and II, it appears that in the presence of site II-specific competing drugs, glimepiride and glipizide mainly occupy site I and therefore, the binding affinity of these drugs is not much affected. These results, therefore, indicate that combination of glimepiride and glipizide with the studied competing drugs is safe.

Binding of competing drugs: In general, the binding affinity of competing drugs increased in the presence of the antidiabetic drugs. The association constants of the competing drugs are lower than that of the antidiabetic drugs and therefore, lower affinity competing drugs are not expected to displace the higher affinity antidiabetic drugs from their binding sites. In the presence of competing drugs, antidiabetic drugs probably cause conformational changes in the HSA molecule thereby creating more binding sites or increasing the accessibility of existing sites. This explains the increase in binding of competing drugs in the presence of antidiabetic drugs. It is also possible that in the presence of antidiabetic drugs, the competing drugs bind at a different site and ternary drug-HSA complex is responsible for the conformational changes in the HSA molecule. Thus antidiabetic drugs decrease the therapeutic efficacy of competing drugs in most cases.

Conclusions

In general, the binding of the antidiabetic drugs decreased in the presence of various competing drugs resulting in a significant increase in the concentration of free, pharmacologically active drug for several drug combinations. Such combinations may produce serious fluctuations in the blood glucose levels of diabetic patients and should be avoided. On the other hand, combinations for which change in free antidiabetic drug concentration was small can be considered as safe. The binding of competing drugs, however, increased in the presence of the antidiabetic drugs and the resulting decrease in the concentration of free drug can reduce the therapeutic efficacy of competing drugs. Site-specificity and conformational changes in the HSA molecule could explain the competitive binding mechanism involved.

Table 1. Association constants for the binding of antidiabetic drugs with HSA in the absence and presence of various competing drugs.

Competing drug	Association Constant (K_a) x 10^4 M^{-1}			
	Gliclazide	**Glimepiride**	**Glipizide**	**Repaglinide**
Absence of competing drug	20.91	14.11	37.41	49.18
Cefdinir	16.89	14.75	28.50	11.36
Sparfloxacin	5.68	9.15	43.00	16.68
Parecoxib Sodium	7.17	9.16	31.96	10.26
Paracetamol	9.33	9.65	44.16	68.59
Repaglinide	31.25	7.22	39.62	-
Metformin hydrochloride	7.17	7.95	49.55	54.54
Gliclazide	-	-	-	50.49
Glimepiride	-	-	-	17.05
Glipizide	-	-	-	32.96

Table 2. Percentage of free antidiabetics (α) in the absence and presence of competing drugs.

Competing Drug	Percentage of free antidiabetic drug (α)			
	Gliclazide	**Glimepiride**	**Glipizide**	**Repaglinide**
None	41.9	52.0	31.1	26.8
Cefdinir	47.0	49.9	37.4	55.2
Sparfloxacin	68.9	59.9	30.8	48.2
Parecoxib Sodium	64.0	59.3	33.4	59.0
Paracetamol	59.9	59.0	29.0	23.5
Repaglinide	26.9	64.0	26.7	-
Metformin hydrochloride	64.1	62.3	24.8	21.6
Gliclazide	-	-	-	26.0
Glimepiride	-	-	-	45.4
Glipizide	-	-	-	32.0

Table 3. Change in the percentage of free antidiabetics (Δα) in the presence of competing drugs.

Competing Drug	Change in the percentage of free antidiabetic drug (Δα)*			
	Gliclazide	**Glimepiride**	**Glipizide**	**Repaglinide**
Cefdinir	+5.1	-2.1	+6.3	+28.4
Sparfloxacin	+27.0	+7.9	-0.3	+21.4
Parecoxib Sodium	+22.1	+7.3	+2.3	+32.2
Paracetamol	+18.0	+7.0	-2.1	-3.3
Repaglinide	-15.0	+12.0	-4.4	-
Metformin hydrochloride	+22.0	+10.3	-6.3	-5.2
Gliclazide	-	-	-	- 0.8
Glimepiride	-	-	-	+18.6
Glipizide	-	-	-	+5.2

* +/- sign refers to increase/decrease in the percentage of free drug.

Table 4. Summary of competitive binding results: change in the percentage of free antidiabetic drugs in the presence of various competing drugs.

Drug	Change in the percentage of free antidiabetics in the presence of competing drugs				
	Very large change (>20%)	**Large change (10-20%)**	**Small change (5-10%)**	**Very small change (1-5%)**	**No change (<1%)**
Gliclazide	Sparfloxacin, Parecoxib Sodium, Metformin hydrochloride	Paracetamol, Repaglinide	Cefdinir	-	-
Glimepiride	-	Repaglinide, Metformin hydrochloride	Sparfloxacin, Parecoxib Sodium, Paracetamol	Cefdinir	-
Glipizide	-	-	Cefdinir, Metformin hydrochloride	Parecoxib Sodium, Paracetamol, Repaglinide	Sparfloxacin
Repaglinide	Cefdinir, Sparfloxacin, Parecoxib Sodium	Glimepiride	Glipizide, Metformin hydrochloride	Paracetamol	Gliclazide

Table 5. Association constants for the binding of various competing drugs with HSA in the presence of the antidiabetic drugs.

Competing antidiabetic drug	Association Constant (K_a) x 10^4 M^{-1}			
	Cefdinir	**Sparfloxacin**	**Parecoxib Sodium**	**Paracetamol**
None	2.78	7.69	7.41	3.74
Gliclazide	8.49	16.69	3.07	3.74
Glimepiride	2.61	32.05	4.58	18.06
Glipizide	11.97	30.24	9.23	3.96
Repaglinide	11.62	18.87	8.66	4.34

Table 6. Percentage of free drugs (α) in the absence and presence of various antidiabetic drugs.

Competing Drug	Percentage of free drugs (α)			
	Cefdinir	**Sparfloxacin**	**Parecoxib Sodium**	**Paracetamol**
Absence of competing drug	79.9	62.0	62.4	75.2
Gliclazide	61.0	47.5	79.1	76.2
Glimepiride	80.9	34.8	72.2	41.6
Glipizide	53.6	34.2	59.0	74.9
Repaglinide	54.0	44.9	60.5	73.4

Table 7. Change in the percentage of free drugs (α) in the presence of various antidiabetic drugs.

Competing Drug	Change in percentage of free drugs (Δα)*			
	Cefdinir	**Sparfloxacin**	**Parecoxib Sodium**	**Paracetamol**
Gliclazide	-18.9	-14.5	+16.7	+1.0
Glimepiride	+1.0	-27.2	+9.8	-33.6
Glipizide	-26.3	-27.8	-3.4	-0.3
Repaglinide	-25.9	-17.1	-1.9	-1.8

* +/- sign refers to increase/decrease in the percentage of free drug.

Table 8. Summary of competitive binding results: change in the percentage of free drugs in the presence of various antidiabetic drugs.

Drug	Change in the percentage of free drugs in the presence of antidiabetic drugs				
	Very large change (>20%)	**Large change (10-20%)**	**Small change (5-10%)**	**Very small change (1-5%)**	**No change (<1%)**
Cefdinir	Glipizide, Repaglinide	Gliclazide	-	Glimepiride	-
Sparfloxacin	Glimepiride, Glipizide	Gliclazide, Repaglinide	-	-	-
Parecoxib Sodium	-	Gliclazide	Glimepiride	Glipizide, Repaglinide	-
Paracetamol	Glimepiride	-	-	Gliclazide, Repaglinide	Glipizide

Fig 1. (a). Percentage of gliclazide bound to HSA in the absence and presence of competing

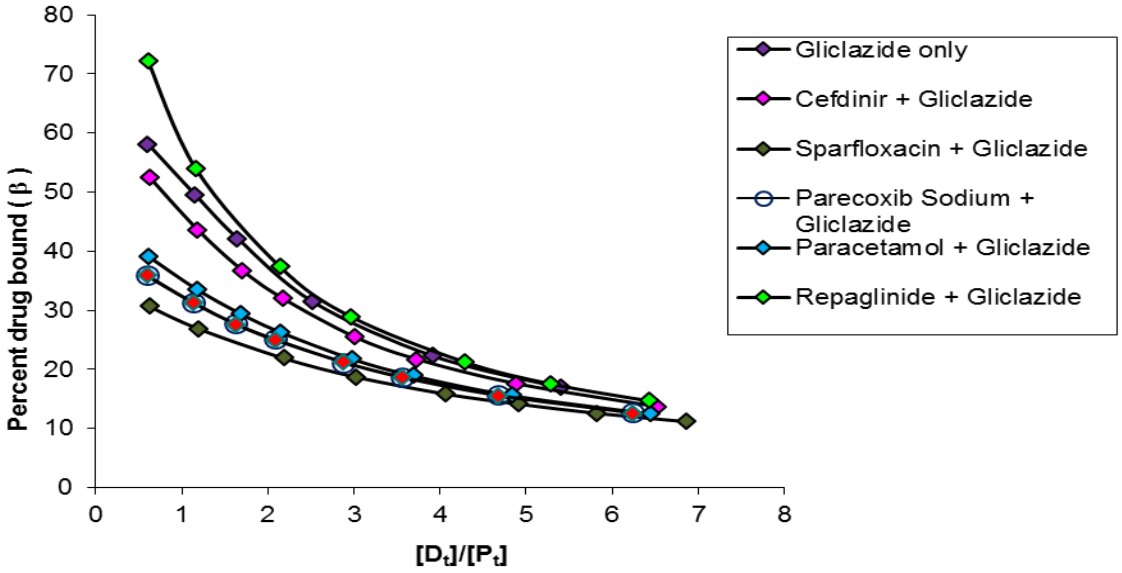


Fig. 1 (b) Percentage of glimepiride bound to HSA in the absence and presence of competing drugs.

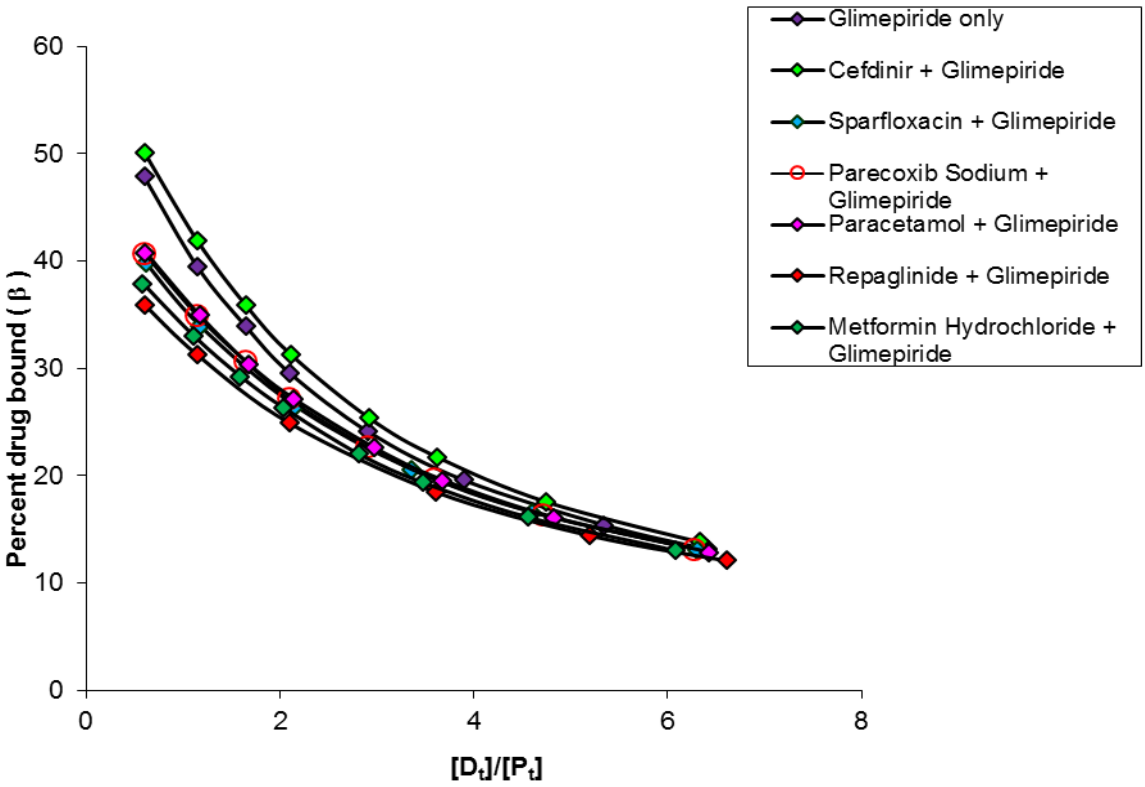


Fig. 1 (c). Percentage of glipizide bound to HSA in the absence and presence of competing drugs.

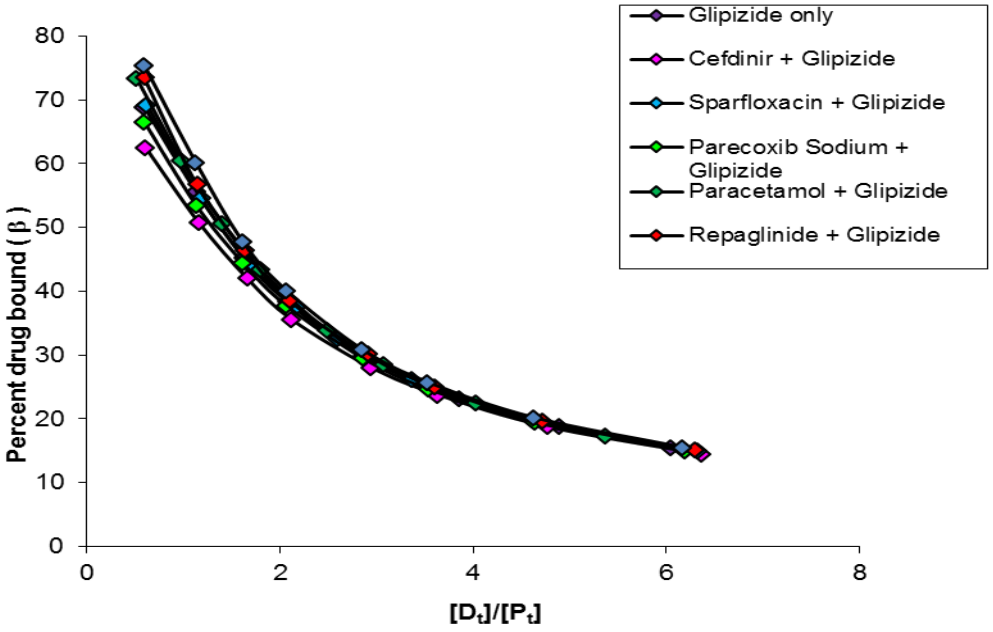


Fig. 1 (d). Percentage of repaglinide bound to HSA in the absence and presence of competing drugs.

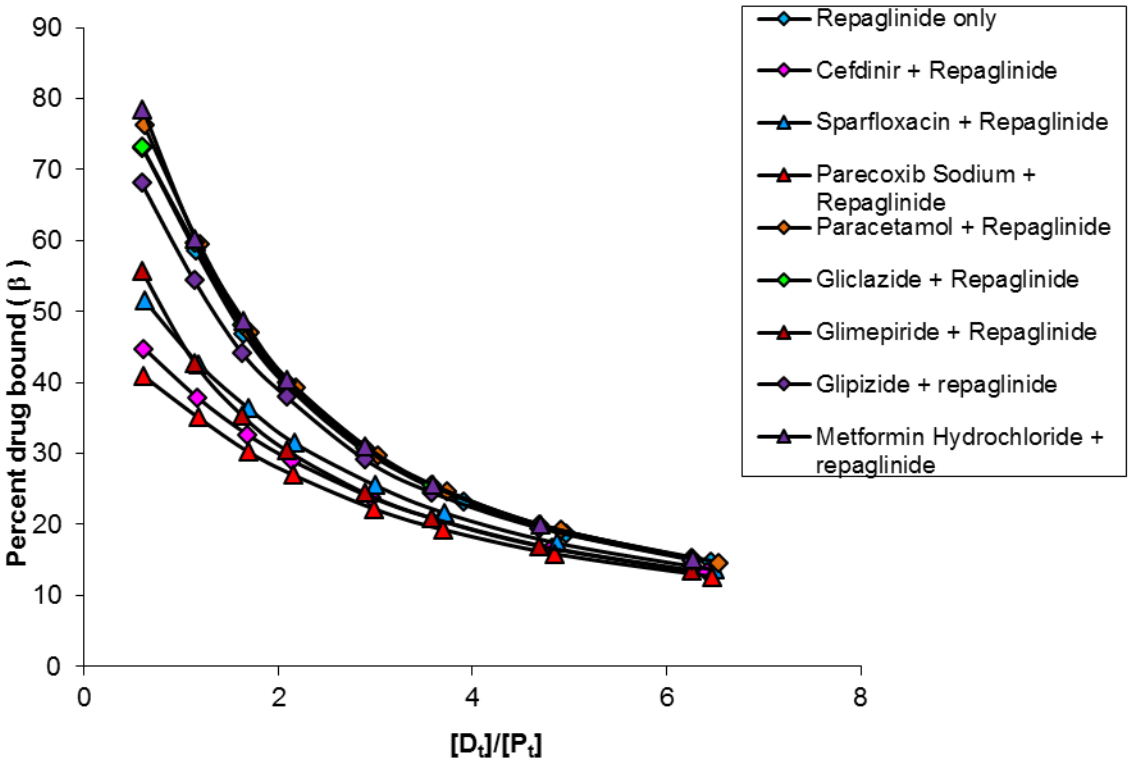


Fig. 2 (a). Percentage of cefdinir bound to HSA in the absence and presence of various antidiabetic drugs.

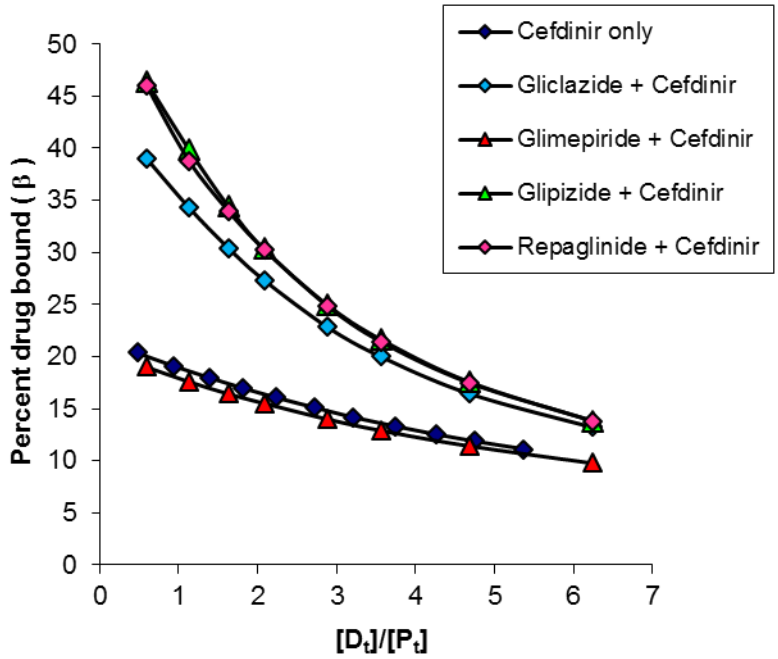


Fig. 2 (b). Percentage of sparfloxacin bound to HSA in the absence and presence of various antidiabetic drugs.

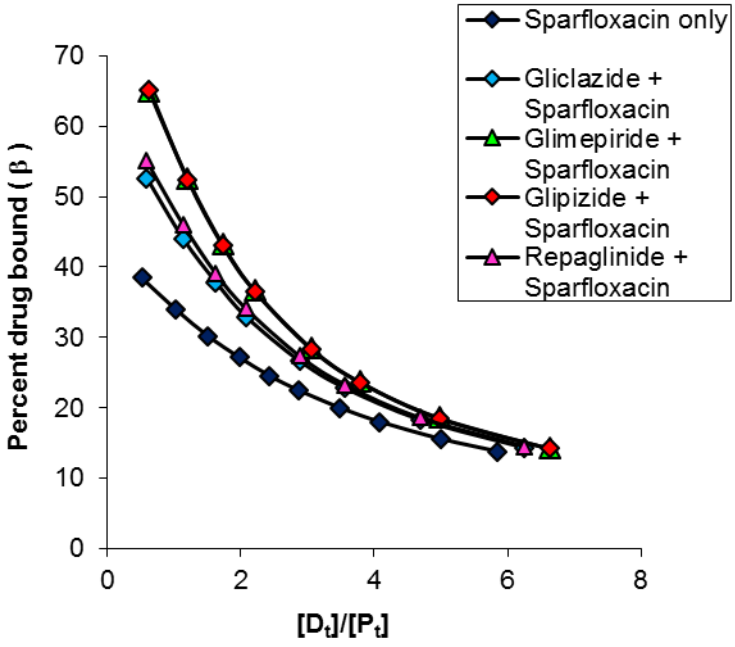


Fig. 2 (c). Percentage of parecoxib sodium bound to HSA in the absence and presence of various antidiabetic drugs.

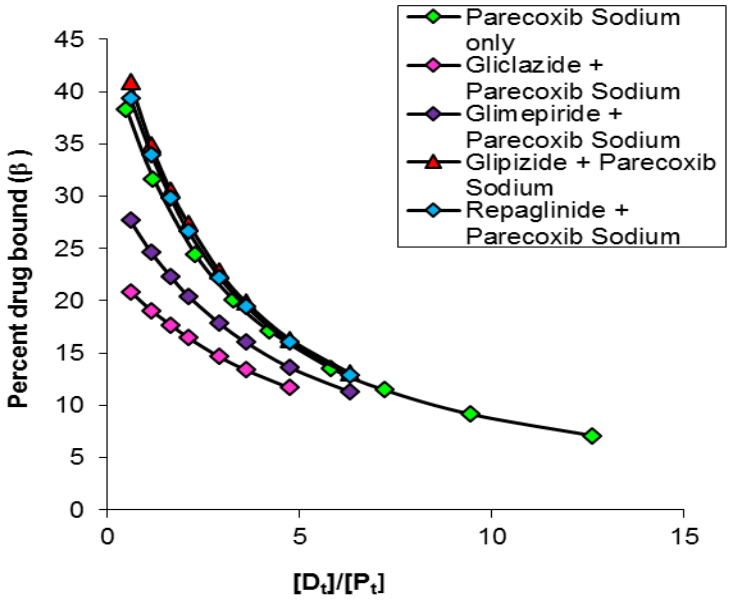
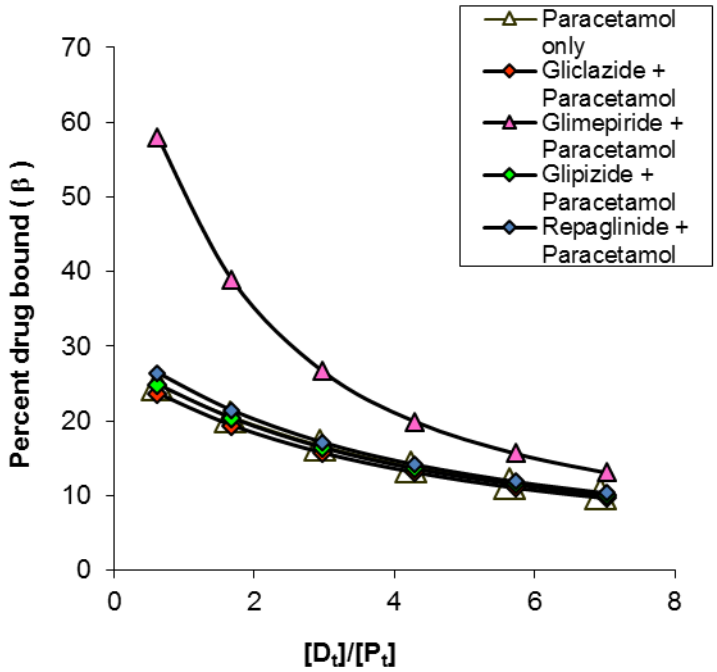


Fig. 2 (d). Percentage of paracetamol bound to HSA in the absence and presence of various antidiabetic drugs.



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