ORIGINAL ARTICLE

Pharmacokinetic and Pharmacodynamic Interaction between Metformin and Fenofibrate in Animal Models

Chakali Ayyanna[1], T. Y. Sree Sudha[2], J. Anbu[3]

[1]Department of Pharmacology, CES College of Pharmacy, Kurnool, Andhra Pradesh, India, [2]Department of Pharmacology, AIIMS, Raipur, Chhattisgarh, India, [3]Department of Pharmacology, MS Ramaiah College of Pharmacy, Bengaluru, Karnataka, India

Abstract

Back ground: Diabetes mellitus and hyperlipidemia are the most common coexisting conditions across the world. Hence, to treat these types of patients both antidiabetic and antihyperlipidemic combination of drugs has to be advised. The most commonly used safe drugs metformin and fenofibrate combination has been selected for our study. Thus, this study was performed to investigate the pharmacokinetic and pharmacodynamic interactions between these two above mentioned drugs in animal models. **Materials and Methods:** Diabetes and hyperlipidemia in rats were induced by streptozocin and cholesterol rich diet regimen and thereafter treated with metformin and fenofibrate. The blood samples were collected to evaluate plasma drug concentrations using high-performance liquid chromatography. **Results:** Single dose of metformin and fenofibrate individually and concomitantly treated in diabetic hyperlipidemic rats. Bio-statistically no significant interactions found in pharmacokinetic parameters. In the pharmacodynamic interaction study, the combination therapy of metformin with fenofibrate shown a marked lipid profiles reduction when compared to fenofibrate alone treatment. **Conclusion:** It is proved in our pharmacodynamic study that concurrent administration of these two drugs has potential benefit in management of diabetes and hyperlipidemia. In addition, due to their in significant pharmacokinetic interaction, the combination therapy will be safe and highly advantageous to the patients.

Key words: Anti-diabetic, Fenofibrate, High-performance liquid chromatography, Hyperlipidemic, Metformin, Pharmacokinetics and Pharmacodynamics interactions, Streptozocin

INTRODUCTION

Diabetes mellitus is a metabolic disorder and oral hypoglycemics are the medications prescribed initially to every patient.[1] These drugs are[2-6] metabolized by cytochrome (CYP2C9) and CYP3A.[4] By inhibition or induction of similar enzymes, promotes drug interactions. Hyperlipidemia is an ailment portrayed by a rise of any or all lipid profile as well as lipoprotein in the blood.[7] The predominance of diabetes in India is alarmingly high, World Health Organization (WHO), assessed that in 2010 there were 50.8 million individuals with diabetes in India and by 2030 number is increased to 87 million.[8] Management of diabetes mellitus involves lifestyle modification and antihyperglycemic therapy.[9] Polypharmacy is a common practice all over the world to treat the chronic disorders such as diabetes mellitus and hyperlipidemia which occurs simultaneously. In such situations, multiple drug usage at a single time causes drug-drug interactions sometimes leading drug efficacy failure or serious adverse effects. These interactions most commonly seen with usage of drugs in common disorders such as diabetes, hyperlipidemia, and hypertension. Combination of drugs for both diabetes and hyperlipidemia sometimes may result in drug interactions and it may lead to life-threatening side effects of death because all these drugs are metabolized through the specific CYP enzyme pathway, particularly CYP8&9 or CYP3A4 or both. Combination of these groups of drugs may results in competition for the same enzyme.

Address for correspondence:
Mr. Chakali Ayyanna, Department of Pharmacology, CES College of Pharmacy, Kurnool - 518 218, Andhra Pradesh, India. Mobile: +91-9885020087.
E-mail: ayyannac5@gmail.com

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This competition may generally alter the pharmacokinetic or pharmacodynamic or both the characters of combinational drugs. The vast majority of the proof support the role of CYP isoenzymes will be a consideration of drug interactions. Among the few CYP enzymes families, the initial three, CYP1, CYP2, and CYP3 are engaged with human drug metabolism.[10,11] Hence, this study was undertaken, to identify both pharmacokinetic and pharmacodynamic drug interactions and the optimal combination of drugs (metformin and fenofibrate). The present study is planned to evaluate pharmacokinetic and pharmacodynamic parameters in hyperlipidemic- and streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Animals

Male Wister rats (weighing 200–220 g) were selected from animal house of Vels College of Pharmacy, Chennai. Animals were maintained under standard animal house conditions of 12/12 h day/night cycle at a temperature of 25±2°C and humidity 60±2%. Study was approved by the institutional animal ethical committee (XII/VELS/PCOL/27/2000/CPCSEA/IAEC/11.03.11).

Study design

Simultaneous estimation of metformin and fenofibrate by reversed phase high-performance liquid chromatography (RPHPLC) method[13]

The chromatographic analysis was performed on a Shimadzu LC10 HPLC system with ODS UV detector using phenomenex C18 column 250 mm × 4.60 mm at 258 nm accomplished with CLASS VP data station. The mobile phase consists of buffer and acetonitrile (50:50 v/v) with pH-6.2. The flow rate was fixed to 1 ml/min with sample volume 20 µl and the mobile phase was filtered through a 0.22 µ membrane and degassed using ultrasonicator, injected into HPLC system using Rheodyne injector. The column temperature was maintained at29±2°C. The HPLC analysis was carried out at room temperature 20°C. Different standard solutions of metformin 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 30, 40, and 50 µg/ml using water:acetonitrile (1:1 v/v) were used. The standard plasma solutions of different concentrations were used for constructing the calibration curve. Further 0.5 ml of metformin standard solution and 0.5 ml of precipitating agent (perchloric acid) were vortexed and centrifuged at 3000 rpm for 5 min and the supernatant separated and it was used for analysis. The same procedure was employed for fenofibrate also.

Pharmacokinetic analysis

Male Wistar rats (200–230 g) were divided into three groups with six animals in each group. For pharmacokinetic study, animals were treated as follows:[12]

Group 1: Metformin alone dispersed in 0.25% sodium carboxy methyl cellulose (NaCMC) once a day for 7 days.
Group II: Fenofibrate alone dispersed in 0.25% NaCMC once a day for 7 days.
Group III: Metformin and fenofibrate concomitantly once a day for 7 days.

The treatment given for 1 week and blood samples were collected at different time intervals for various pharmacokinetic analysis.

Collection of blood sample

The blood drawn through retro-orbital sinus and retained into the Eppendorf tube. Serum was obtained by centrifugation of blood samples. Centrifugation was performed using REMIULTRA, cooling centrifuge at 2500–3000 rpm for 5 min. Blood samples 0.5 ml on 1st and 8th day were collected at 0, 0.5, 1, 2, 4, 6, 8, and 24 h and equal ratio of saline added to replace the blood volume for each blood withdrawal.[13] All samples were stored at −4°C until pharmacokinetic and pharmacodynamics measurements were carried out.

Pharmacodynamic interaction study

Antidiabetic study

Adult albino rats of either sex weighing 150–200 g were chosen in the study. Animals divided into six groups, each group consists of six rats and was treated with water ad libitum. Animals were fasted for 18 h to avoid food and drug interactions and allowed access to water intake only till samples were collected. Diabetes induced to rats by streptozocin (STZ) 60 mg/kg through subcutaneous route.[14] Blood glucose levels above 260 mg/dl were considered for the study.

Collection of blood samples for estimation of cholesterol and triglycerides (TG)

Blood approx. 0.5 ml were collected in retro-orbital sinus of each of a group before and also at 4, 6, 8, 10, 12, 16, 18, 20, and 24 h after oral administration of the samples were collected into glass vials containing potassium oxalate and sodium fluoride as anti-coagulant. They were stored at −40°C in a refrigerator.

Antihyperlipidemic study

Male Wister rats (weighing 200–220 g) were selected and they were randomly divided into three groups each group contain six animals. Before introduction of hyperlipidemia in animals, the weight of the individual animal and plasma cholesterol levels were obtained. Then, standard cholesterol rich diet was administered up to 1 month. Standard cholesterol rich diet contains as follows.

Composition of cholesterol rich diet for induction of hyperlipidemia

40% ground nut powder
2% Cholesterol
58% normal rat feed, i.e., (34.8 g normal rat feed+24 g ground nut powder
0.5 ml of butter twice a day for 1 month

During the cholesterol rich diet feeding period, the animals were periodically checked for weight changes, quantity of food, and water consumption. Baseline readings were obtained for analyze the plasma samples of total cholesterol (TC), TG, high-density lipoprotein (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Plasma TG and TC, HDL-C were estimated by enzymatic method using spectrophotometer.

The elevated hyperlipidemic animals were divided into six groups and each group consists of six animals. A control non-hyperlipidemia animal group was studied for lipid profile before commencement of study to confirm and compare the induction of hyperlipidemia.

Drug treatment pattern[12]

The rats were grouped and treated as follows:

Group I: Diabetic rats served as control group (10 ml/kg i.p) normal saline solution.
Group II: Hyperlipidemia in rats as control group (10 ml/kg i.p) normal saline solution.
Group III: Metformin alone dispersed in 0.25% NaCMC once a day in diabetic rats.
Group IV: Fenofibrate concomitantly in hyperlipidemia in rats.
Group V: Metformin and fenofibrate concomitantly in diabetic rats.
Group VI: Metformin and fenofibrate concomitantly in hyperlipidemia rats.

Statistical analysis

All the values were exposed as mean ± SEM. The data were statistically analyzed by one-way ANNOVA followed by Dennett's test. The data of hematological parameters were analyzed using ANNOVA followed by Tukey multiple composition test. $P < 0.05$ were considered significant.

RESULTS

Pharmacokinetic parameters chromatography

The HPLC method for simultaneous estimation of metformin and fenofibrate was developed with better reproducibility, sensitivity, and accuracy. The calibration curves were constructed for both metformin and fenofibrate using the different graded drug concentration and ratio of chromatogram area of drug. The retention time of metformin and fenofibrate under the above-mentioned chromatographic conditions was 1.8 and 19.3 min, respectively.

The correlation co-efficient for metformin was 0.9995 with slope ×4473.8 and intercept of 211.5, whereas for fenofibrate, it was 0.999, slope ×4523.6, and intercept of 498.86 [Figures 1 and 2].

The % of drug recovery in the rat plasma samples collected at different concentrations was ranged as 78.4–94.18% and 87.9–99.2% for metformin and fenofibrate, respectively [Table 1].

The pharmacokinetic study of these drugs on simultaneous treatment showed a statistically significant raise in Cmax, AUC0-t, AUC0-∞, and T1/2 ($P < 0.05$). All the other parameters were decreased after 7 days of treatment.

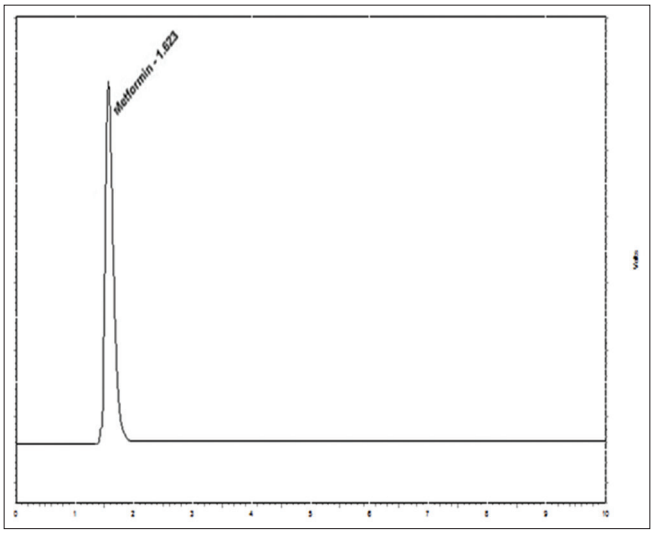

Figure 1: Standard calibration curve of metformin

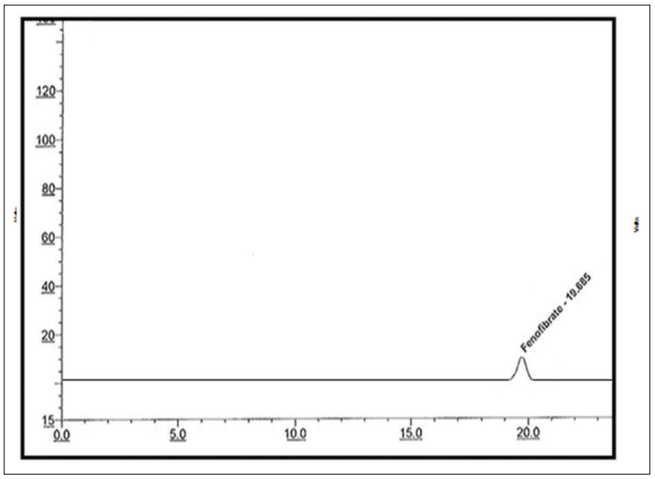

Figure 2: Standard calibration curve of fenofibrate

Pharmacodynamic interaction study

Anti-dabetic study

In the present study, after STZ treatment the blood glucose levels were elevated significantly and the metformin alone produced maximum reduction in blood glucose of 82.41% ($P < 0.01$) with doses of 30 mg/kg of body weight after 1 week of treatment with metformin 1 g/kg and also fenofibrate 120 mg/kg produced a significant ($P < 0.01$) reduction in blood glucose levels compared to control (80.22%).

Anti-hyperlipidemic study

Fenofibrate alone and combination of metformin-treated animals showed significant ($P < 0.05$) and favorable changes in the levels of TC, TG, HDL, and LDL after 0.5–1-h administration of day 1 and day 7. TG and LDL were achieved peak statistical significance on day 7th similarly HDL level increased from baseline from day 1 to day 7.

DISCUSSION

Simultaneous estimation of metformin and fenofibrate by RP-HPLC method

Under the chromatographic condition described the above retention times of metformin and fenofibrate were found to be 1.623 and 19.65 min, respectively [Figure 1 and 3]. The recoveries of sample from rat plasma were found to be 85.5–99% at the concentration range of 20–00 ugml, metformin and fenofibrate was 81.6–99.8 at the concentration of 5–50 ug ml, respectively [Table 2]. To determine linearity and the detection range of HPLC method, sample spiked with seven different concentrations. No discernible peaks were observed within the time frame in which metformin and fenofibrate. In the HPLC assay for the simultaneous quantification of metformin and fenofibrate, recovery, sensitivity, and linearity were satisfactory in the concentration range studied. The slope intercept and correlation coefficient for metformin was found to be 1812x + 5688.9 and R^2 = 0.999, whereas for fenofibrate was fond to be 4325.2x + 1621.6 and R^2 = 0.999 [Figures 4 and 5], respectively.

Table 1: Mean changes in concentration (µg/ml) of metformin (1 g/kg/p.o.) and fenofibrate (120 mg/kg/p.o.) alone and in combination at different time intervals in rats

Time (hour)	Metformin alone once daily		Fenofibrate alone once daily		Metformin with fenofibrate after concomitant treatment			
					Metformin		Fenofibrate	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0	0	0.13±0.01	0.00±0.00	0	0.00	0.16±0.00	0.00±0.00	1.00±0.01**
0.5	0.10±0.02	0.29±0.01	5.16±0.36	4.01±0.04	0.10±0.01	0.14±0.00	3.16±0.11	4.16±0.03
1	0.68±0.02	0.56±0.02	15.28±1.12	15.47±0.04**	0.29±0.01	0.16±0.01	12.00±0.06	12.00±0.03***
2	0.35±0.03	0.35±0.02	26.32±2.09	18.25±0.22**	0.41±0.02	0.43±0.02	21.18±0.13	16.10±0.17**
4	0.29±0.03	0.33±0.01	21.72±0.12	31.92±0.21**	0.34±0.00	0.30±0.01	0.11±0.12	6.29±0.06**
6	0.25±0.01	0.28±0.02	18.22±1.21	27.12±0.16**	0.16±0.01	0.20±0.02	8.33±0.24	8.14±0.11**
8	0.19±0.01	0.19±0.01	12.62±0.85	19.55±0.12**	0.09±0.00	o. 13±0.01	5.00±0.1	5.00±0.09**
24	0.13±0.01	0.18±0.01	5.18±0.46	5.45±0.04	0.00±0.00	0.00±0.00	0.00±0.00	4.00±0.05

Values are expressed as mean±S.E.M.; (n=6); (*P<0.05); [a]Comparison made between day 1 and 7 alone and in combination

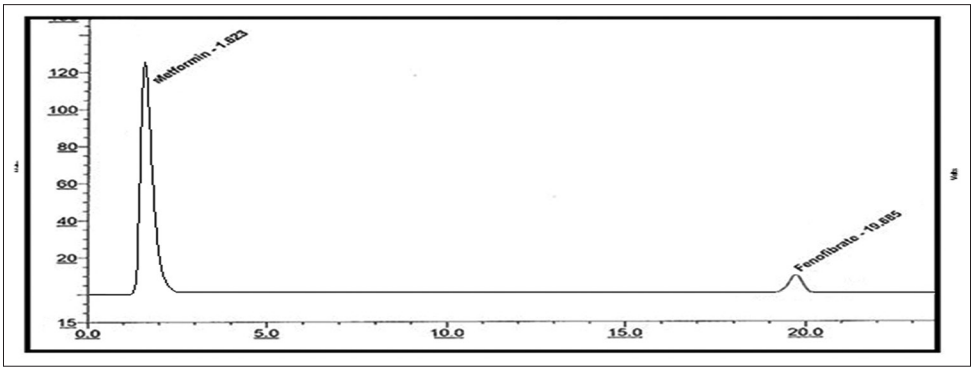

Figure 3: Chromatogram of combination of metformin and fenofibrate in pharmacokinetic study

Table 2: Pharmacokinetic changes of metformin (1 g/kg/p.o.) and fenofibrate (120 mg/kg/p.o.) alone and after concomitant administration in rats

PK Parameter	Metformin (1 g/kg/p.o.) alone once daily		Fenofibrate (120 mg/kg/p.o.) alone once daily		Metformin with fenofibrate after concomitant treatment			
					Metformin		Fenofibrate	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
C_{max} (ng/ml)	0.21±0.03	0.42±2.7	20.2±2.6	18.1±0.01	0.2±0.01	0.2±0.1	10.10±2.3	16.10±2.3**
T_{max} (h)	0.4±0.11	0.4±0.12	0.4±0.1	0.4±0.09	0.3±0.09	0.3±0.1	0.3±0.1	0.4±0.1
$AUC_{(0-t)}$ (ng.h/ml)	1.42±0.12	1.94±0.32**	78±3.0	80±3.1	1.24±0.10	1.20±0.27	36.2±2.09	59.2±4.0**
$AUC_{(0-F0A5)}$ (ng.h/ml)	200±0.15	120±0.20	80±2.6	70±2.7**	200±0.21	208±0.22	46.1±3.3	68.4±2.1**
$T_{½}$ (h)	2.95±6.02	1.96±0.11	1.25±0.2	1.10±0.02	2.42±3.24	3.00±0.12	1.0±0.1	1.2±0.02
Cl/f (L/h)	10.43±3.2	6.8±1.00**	1.3±0.3	1.6±0.02	14.12±2.2	8.0.±1.00	2.9±0.4	2.0±0.2**
Vd/f (L/kg)	18.25±1.12	10.16±1.12*	6.8±2.0.	6.5±2.1	16.00±1.48	15.20±2.08	6.0±1.0	6.0±2.0

Values are expressed as Mean±S.E.M.; (*n*=6); (**P*<0.05). [a]Comparison made between day 1 and 7 alone and combinational treatment

Table 3: Antidiabetic efficacy on administration of metformin alone and in combination with fenofibrate in diabetic rats

Treatment and dose	Glucose level (mg/dl)			
	1 day	3th day	5th day	7th day
Diabetic control	220 ± 9.00	252 ± 11.20	270 ± 15.50	282 ± 13.50
Metformin alone	124 ± 8.20 [a]	103 ± 10.21[a]	85 ± 13.45[a]	74 ± 10.50[a]
Metformin (1 g/kg) +Fenofibrate (120 mg/kg)	86.34 ± 8.50[a]	81.5 ± 14.25[a]	73 ± 12.60[a]	69 ± 17.55[a]

n = 6; Values are expressed as mean ± SEM. [a]*P* < 0.05; [b]*P* < 0.01 versus diabetic control. Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test

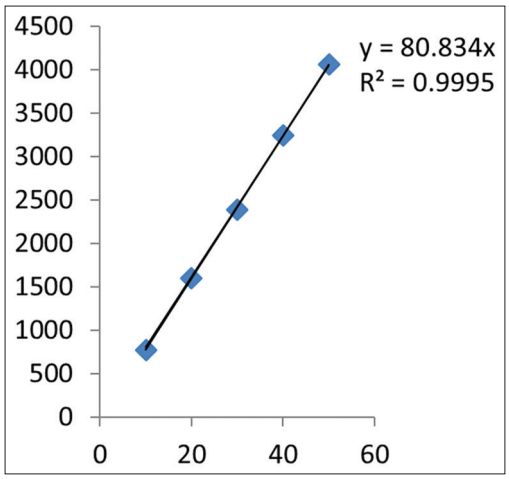

Figure 4: Chromatogram of metformin

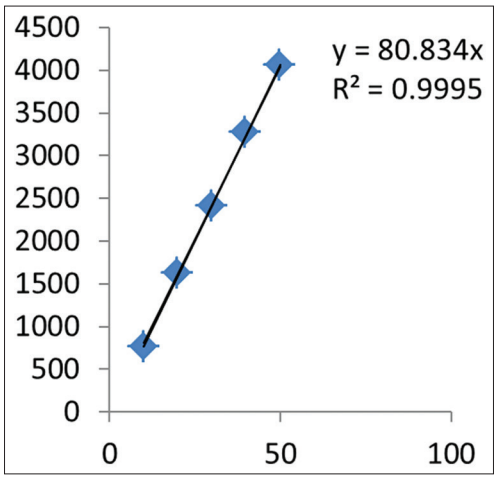

Figure 5: Chromatogram of fenofibrate

Pharmacokinetic interaction study

In the present study, the plasma concentration of metformin and fenofibrate alone and in combination were studied and the data were presents [Table 3]. It was observed that up on administration of metformin alone in rats for 1 week the plasma concentration was rapidly increased. However, there was no significant alteration noted in fenofibrate concentration. The combinational treatment of metformin peak plasma concentration was achieved after 2 h on day 1 and day 7 also. However, on 7th-day peak plasma concentration of metformin was significantly ($P < 0.01$) increased to 0.45±0.02 from 0.31±0.01 after 1 and 2 h of treatment with fenofibrate. Similarly, the fenofibrate concentration was also increased significantly ($P < 0.05$) when administered simultaneously with metformin

From the pharmacokinetic parameters studied, it was observed that C_{max} (0.21+0.03 to 0.30+0.1 ng/ml), AUC 0-t (1.42+0.12 to 2.09+0.32), and T ½ (2.95+6.02 to 19.6+0.11) of metformin were significantly ($P < 0.01$) increased on day 7 after alone treatment.

The fenofibrate alone after 1-week treatment showed no alterations in any of the pharmacokinetic parameters. There was slight decrease in the clearance (C1/f) rate of fenofibrate in combination compared with fenofibrate alone on day 1 and day 7, respectively. Similarly, there was a slight decrease in clearance (C1/f) of metformin in combination compared with metformin alone by 4.29% on day 1 and 4.6% on day 7, respectively. The half-life of fenofibrate was almost similar after alone and combination treatment on day 1 and day 7, all these changes were not statistically significant $P > 0.05$ in combinational drug treated group. The mean clearance (C1/f) was 10.43 ± 3.2 and 6.8 ± 1.00 L/h/kg, which was increased to 14.12 ± 2.2 and 8.0 ± 1.00 L/h/kg upon treatment of metformin with fenofibrate in rats on day 1 and day 7, respectively. Volume of distribution was increased 3–6 and 2–5-fold in metformin alone compared to metformin with fenofibrate group on day 1 and day 7, respectively [Table 4].

Table 4: Effect of metformin alone and in combination with fenofibrate on total cholesterol and triglyceride levels in streptozocin induced diabetic rats

Treatment and dose	Parameters (mg/dl)	
	Total cholesterol	Triglycerides
Normal 10 mg/kg of vehicle	93.0±4.40	145.0±4.82
Diabetic control	117.5±3.5[a]	123.5±6.36[a]
Metformin alone (1 g/kg)	83±4.10	150.2±12.80
Fenofibrate (120 mg/kg) +Metformin (1 g/kg)	71.80±5.30	120±7.25

n=6; Values are expressed as mean±S.E.M. [a]*P*<0.001; [b]*P*<0.01; [c]*P*<0.05 versus normal; [d]*P*<0.001 versus diabetic control

Pharmacodynamic interaction study

Antidiabetic study

Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the world. Diabetes mellitus is possibly the world's largest growing metabolic disease, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases. STZ a beta cytotoxin causes a massive destruction of beta cells of islets of Langerhans resulting in reduced synthesis and release of insulin. It is well established that metformin produce hypoglycemia by increasing the secretion of insulin from pancreas and this compound active in mild STZ-induced diabetes, whereas they are inactive in intense STZ diabetes. *In vitro* studies demonstrate that metformin is predominantly metabolized by CYP2C8, with a minor contribution by CYP2C9. Since there is no significant *in-vitro* inhibition of CYP1A2, 2A6, 2C19, 2D6, 2E1, 3Aor 3A, and 4A with metformin, there is a low probability of significant metabolism-based interactions with drugs metabolism-based interactions with drugs metabolized by these p450 enzymes. In the present study, after STZ treatment the blood glucose levels were elevated significantly and the metformin alone produced maximum reduction in blood glucose of 82.41% ($P < 0.01$) with doses of 30 mg/kg of body weight after 1 week of treatment [Table 5] with metformin 1 g/kg and also fenofibrate 120 mg/kg produced a significant ($P < 0.01$) reduction in blood glucose levels compared to control (80.22%). The percent reduction in blood glucose tended to be higher in the diabetic condition compared to the normal state. A significant range reduction ($P < 0.01$) in blood glucose after 4–8 h in metformin alone groups was calculated as 79.60–82.41% and in combination group 79.3–80.22% ($P < 0.001$) was observed when compared to diabetic control. Hence our results showed that metformin alone reduced blood glucose level in hyperglycemic animals. Similarly, the TC and TG levels were also normalized significantly in both metformin alone (95±4.13, 160.3±12.89) ($P < 0.01$, $P < 0.001$ when compared to normal and diabetic control, respectively)

Table 5a: Effect of metformin alone and combination with fenofibrate on cholesterol and triglycerides levels on day 1

Time (h)	Cholesterol level (mg/dl)		Significance (*P*<0.05)	Triglyceride (mg/dl)		Significance (*P*<0.05)
	Fenofibrate	Combination		Fenofibrate	Combination	
Normal	80.12±0.09	82.1±0.09	S	119.10±0.17	121.9±0.26	S
After induction	192.26±0.28	203.2±0.40	S	285.51±0.675	286.6±0.55	S
0.5	183.0±0.18	186.45±0.24	S	274.3±0.23	282.3±0.43	S
1	164.1±0.17	179.45±0.45	S	248.34±0.34	272.6±0.04	S
2	158.72±0.18	167.12±0.53	S	237.34±0.20	262.7±0.10	S
4	153.5±0.15	1600.±0.08	S	200.5±0.34	238.03±0.17	S
6	146.2±0.24	156.34±0.65	S	224.0±0.24	229.6±0.12	S
8	148.0±0.18	153.24±0.07	S	186.5±0.39	224.34±0.45	S
24	165.4±0.25	149.8±0.24	S	188.6±0.32	200.43±0.43	S

Table 5b: Effect of metformin alone and combination with fenofibrate on low-density lipoprotein and high-density lipoprotein on day 1

Time (h)	Low-density lipoprotein (mg/dl)		Significance ($P < 0.05$)	High-density lipoprotein (mg/dl)		Significance $P < 0.05$)
	Alone	Combination		Alone	Combination	
Normal	20.81 ± 0.15	22.26 ± 0.14	S	31.12 ± 0.158	33.5 ± 3042	NS
Induction	76.23 ± 0.36	81.5 ± 1.22	S	58.07 ± 0.25	54.71 ± 0.12	S
0.5	69.06 ± 0.34	74.34 ± 1.15	S	50.05 ± 0.23	57.75 ± 0.16	S
1	61.45 ± 0.37	70.16 ± 1.01	S	54.45 ± 0.56	64.15 ± 0.13	S
2	59.98 ± 0.21	66.76 ± 0.86	S	59.56 ± 2.01	66.86 ± 0.11	S
4	63.12 ± 0.23	64.78 ± 0.89	S	66.54 ± 1.76	58.98 ± 0.23	S
6	61.85 ± 0.34	63.34 ± 0.45	S	63.98 ± 1.05	56.08 ± 0.47	S
8	60.46 ± 0.24	64.56 ± 0.51	S	64.00 ± 1.19	68.56 ± 0.72	S
24	63.17 ± 0.2	64.46 ± 0.75	NS	68.94 ± 1.75	67.05 ± 0.92	NS

Table 5c: Effect of metformin alone and combination with fenofibrate on cholesterol and triglycerides levels on day 7

Time (h)	Cholesterol level (mg/dl)		Significance ($P<0.05$)	Triglyceride (mg/dl)		Significance ($P<0.05$)
	Alone	Combination		Alone	Combination	
Normal	119.23±0.21	132.34±0.01	S	141±0.22	128.5±0.42	S
0.5 h	167.00±1.31	142.41±1.15	S	174.64±0.35	166.5±0.34	S
1	130.23±1.23	122.86±1.10	S	153.43±0.42	122.5±0.42	S
2	132.34±1.25	118.3±1.11	S	148.24±0.74	120.65±0.20	S
4	140.46±1.30	116±1.20	S	150.12±0.37	118.87±0.86	S
6	43.43±1.45.	113.5±1.54	S	154.34±0.43	116.86±0.74	S
8	141.45±1.34	119.23±1.53	S	148.43±0.53	115.2±0.64	S
24	142.76±1.42	104.45±1.30	S	58.42±0.55	113.64±1.24	S

Table 5d: Effect of metformin alone and combination with fenofibrate on low-density lipoprotein and high-density lipoprotein on day 7

Time (h)	Low-density lipoprotein (mg/dl)		Significance ($P<0.05$)	High-density lipoprotein (mg/dl)		Significance ($P<0.05$)
	Alone	Combination		Alone	Combination	
Normal	29.83±0.42	35.66±0.21	S	52.12±1.42	63.01±0.12	S
0.5 h	34.11±0.32	34.75±0.45	S	56.41±1.52	64.10±0.10	S
1	45.86±0.44	34.65±0.34	S	55.24±1.32	64.55±0.11	S
2	43.54±0.20	33.45±0.54	S	4.21±0.94	65.34±0.10	S
4	42.56±0.23	31.23±0.78	S	4.43±0.96	65.12±0.12	S
6	4.16±0.45	30.13±0.45	S	4.65±0.96	66.20±0.13	S
8	41.54±0.23	29.56±0.42	S	57.43±1.06	66.45±0.34	S
24	45.26±0.45	29.65±0.24	S	7.43±1.09	66.82±1.45	S

and in combination with fenofibrate (71.83±5.34, 125±7.25) treated group, respectively, compared to control group but no existence of statistically significant difference among the treated group. STZ-treated animals receiving the metformin and fenofibrate showed rapid normalization of blood glucose levels in comparison to control and this could be due to possibility that some beta cells are still surviving.

Antihyperlipidemic activity

Fenofibrate alone and combination of metformin-treated animals showed significant ($P < 0.05$) and favorable changes in the levels of TC, TG, HDL, and LDL after 0.5–1-h administration of day 1 and day 7. After initiation of treatment with fenofibrate compared with combination with metformin

single dose/day, it was observed that the combinational treatment altered this parameter from base line in TG, HDL, and LDL achieved a statistical significance within 24 h (day 1). TG and LDL were achieved peak statistical significance on day 7th similarly HDL level increased from baseline from day 1 to day 7. There was statistically significant difference in TG, HDL, and LDL with treatment metformin in hyperlipidemic group compared with non-hyperlipidemic groups.

CONCLUSION

The present study based on the result obtained from kinetic study, it is evident that the single dose of metformin and fenofibrate individually and concomitantly treated in diabetic with hyperlipidemic rats did not show any bio-statistically significant interactions in its pharmacokinetic parameters. For the pharmacodynamic interaction study describes that combination therapy of metformin with fenofibrate and elicits a marked, lipid profiles reduction when compared to fenofibrate alone treatment. And also, in the development of HPLC method, it is concluded that the simultaneous determination of plasma concentration metformin and fenofibrate is possible.

Hence, it is proved that the concurrent administration of these two drugs has potential benefit in management of diabetes and hyperlipidemia. In addition, due to their in significant pharmacokinetic interaction, the combination therapy can be safe and highly advantageous in patients with diabetes and hyperlipidemia.

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