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Enhanced antihyperlipidemic potential of gemfibrozil under co-administration with piperine



S. Mohanalakshmi^{a,*}, Shvetank Bhatt^a, C.K. Ashok Kumar^b

^a Amity Institute of Pharmacy, Amity University, Maharajpura (Opposite Airport), Gwalior, 474005, Madhya Pradesh, India

^b School of Pharmacy, Guru Nanak Institutions Technical Campus, Khanapur, Ibrahimpatam, Ranga Reddy Dist, Hyderabad, Telangana State, 501506, India

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ABSTRACT

Gemfibrozil is a well-known potent antihyperlipidemic drug with the capacity to lower triglyceride and cholesterol levels, which are responsible for most cardiovascular and cerebrovascular diseases. In addition, gemfibrozil has a potent activity at elevating the high density lipoprotein levels. However, this drug has a very short half-life of about 2 h and toxicity is observed in the liver as the dose increases. The drug piperine has the capacity to enhance the bioavailability of other drugs without altering their basic properties as well as improving their activity. In this study, we aimed to enhance the bioavailability of gemfibrozil as well as making it more potent and less toxic by applying piperine as a bio-enhancer. Thus, piperine was co-administered to rats with gemfibrozil and the antihyperlipidemic activity was tested when fed on a high fat diet. The results showed that co-administration of gemfibrozil with piperine decreased the elevated triglyceride and cholesterol levels to normal, and they performed significantly better than the individual drugs. Weight gain was controlled effectively by drug administration together with piperine compared with other groups. Hepatic function analyses demonstrated that the potentiation of gemfibrozil did not alter the hepatic function but instead it improved significantly by normalizing the elevated serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and alkaline phosphatase levels. The plasma drug concentration of gemfibrozil was studied over time, where the enhanced activity of the drug reached its C_{max} within 1 h of administration and the activated drug level was observed in the blood for 4 h.

1. Introduction

Hyperlipidemia is characterized by elevated serum and plasma lipid levels, and it is the highest risk factor for diseases that cause severe mortality and morbidity at present. Most heart diseases, vascular diseases, and cerebrovascular diseases are linked to elevated cholesterol and lipid levels in the blood [1]. A previous study predicted a massive rise of 25–30% in mortality throughout the world by 2020 but a decrease of 10% in the cholesterol level can reduce cardiovascular problems by 30%, and thus the death rate [2].

Gemfibrozil is an antihyperlipidemic drug used for the treatment of patients with Type IV and Type V hyperlipidemia. This drug is used for patients with risks for cardiovascular diseases, coronary vascular problems, and other lipid-related disorders. Gemfibrozil is most effective in patients who do not respond to a conventional weight loss diet, exercise, and regular antihyperlipidemic medicines. The drug effectively lowers

elevated levels of triglycerides (TG), very low density lipoprotein cholesterol, and total cholesterol (TC) in the serum [3,4].

The indicated dose of gemfibrozil is approximately 20 mg/kg and the tablets are marketed at 600 mg/tablet. The C_{max} estimated for gemfibrozil is $46 \pm 16 \mu\text{g/mL}$ and T_{max} is 2.2 ± 1.1 h. A slight elevation in T_{max} was noted in patients with liver disorders and the bioavailability was significantly lowered by almost 50%. A higher dose of the drug can be administered to achieve the required concentration in the blood with the greatest activity. Gemfibrozil is known to cause hepatic disorders, and thus it is necessary to maintain a low dose while improving the bioavailability of the drug. One of the options for achieving improved bioavailability and preventing hepatotoxicity is applying the drug concurrently with a bio-enhancer [5].

The importance of bio-enhancers of herbal origin is emphasized in Indian traditional medicine systems such as Ayurveda. They are well known to enhance the bioavailability of active drugs when used in low

* Corresponding author.

E-mail addresses: mohanaashok@gmail.com, smlakshmi@gwa.amity.edu (S. Mohanalakshmi), sbhatt@gwa.amity.edu, shvetankbhatt@gmail.com (S. Bhatt), ashokkumarck@yahoo.com (C.K. Ashok Kumar).

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doses, and thus the biological activity. Bio-enhancers can clinically lower the dose and dosage frequency, as well as often reducing the toxicity and adverse drug effects. A bio-enhancer may or may not possess any pharmacological activity but it enhances the bioavailability and efficacy of an active drug. Bio-enhancers typically enhance the oral absorption of the drug and its metabolism, or they may aid the conversion of inactive forms of drug molecules into active forms [6,7].

Many studies have investigated the effects of piperine on the absorption and metabolism of other drugs. Piperine is an effective anti-inflammatory, antioxidant, and hepatoprotective drug. In addition, it has been reported to enhance the bioavailability of curcumin and simvastatin as well as many other drug candidates while maintaining the activity of these drugs [6,8,9]. Thus, in the present study, we investigated the effectiveness of piperine at increasing the bioavailability of gemfibrozil without reducing the hepatic function.

2. Materials and methods

2.1. Chemicals and equipment

The drugs, chemicals, and biochemical assay kits used in the experiments were obtained from Sigma-Aldrich, India. The chemicals and solvents were high-performance liquid chromatography (HPLC) analytical grade. The water used in the analytical procedures was double distilled and filtered through a 0.25- μ m filter membrane.

2.2. Experimental animals

Sprague–Dawley rats were employed in this study. Male rats weighing 190–230 g were obtained from an animal supplier in Bengaluru, India. The rats were disease free and active according to the seller's documentation history. Rats were introduced into our laboratory setup and maintained at 23 °C with a light:dark cycle of 12:12 h. The animals were provided with standard pellet diet in their own polypropylene cages and water was allowed *ad libitum*. All of the experiments conducted on animals in this study received prior approval from the Institutional Animal Ethics Committee (IAEC), Sri Venkateswara College of Pharmacy, Chittoor and they followed the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) protocols.

2.3. Grouping and pretreatment

After an acclimatization period of 1 week, 10 rats were randomly assigned to groups with six rats in each group. Group 1 comprised normal control animals that received normal diet. Group 2 animals were fed with a high fat diet containing 10% fat and 2% cholesterol in addition to the normal pellet feed for about 30 days. The body weights were recorded before and after the experiments to ensure the induction of hypercholesterolemia.

2.4. Treatment and drug administration

The experiments were conducted for about 4 weeks and the groups received the following drug treatments: group 1 (NRML) served as the normal control; group 2 (HFF) served as the disease control and did not receive any drug treatment; group 3 (GEM) served as the drug control and received gemfibrozil alone at a dose of 20 mg/kg/day; group 4 (PIP) received piperine alone at a dose of 5 mg/kg/day; group 5 (GEM-PIP-5) received gemfibrozil and piperine at doses of 20 mg/kg/day and 5 mg/kg/day, respectively; group 6 (GEM-PIP-10) received gemfibrozil and piperine at doses of 20 mg/kg/day and 10 mg/kg/day, respectively; and group 7 (GEM-PIP-20) received gemfibrozil and piperine at doses of 20 mg/kg/day and 20 mg/kg/day, respectively [10].

The drugs were administered via oral gavage in corn oil. The NRML and HFF groups received corn oil during the course of the experiment. After 4 weeks, the body weights were recorded and the rats were then sacrificed under ether anesthesia by cervical dislocation.

2.5. Estimation of serum lipid parameters

Blood was withdrawn from the abdominal aorta and allowed to coagulate at room temperature. The serum was separated and collected after centrifugation at 3000 rpm for 10 min. The samples were stored in an appropriate manner at 4 °C and used to estimate lipid parameters comprising the total cholesterol (TC), TG, high density lipoprotein (HDL), and low density lipoprotein (LDL) contents.

2.6. Estimation of hepatic lipid and function parameters

Liver tissue (at least 0.5 g) was separated carefully from the rats and homogenized in phosphate-buffered saline (PBS, pH 7.2) to a concentration of 0.15 g tissue per 1 mL of PBS. The homogenate was subjected to centrifugation at 3000 rpm for 10 min and the supernatant was filtered and collected, before storing at 4 °C. The supernatant was used to estimate the hepatic lipid levels and in liver function assays to determine the serum glutamate serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) levels. The assays were performed using enzyme assay kits [11].

2.7. Estimation of gemfibrozil concentration in plasma

2.7.1. HPLC system configuration

The drug levels in the blood plasma were determined using reversed phase-HPLC. The HPLC system was manufactured by Shimadzu (Japan) and it was equipped with an LC-10 AT-VP system controller with an LC-20AT pump and SPD-10A ultraviolet (UV) detector. The system was coupled to a Phenomenex C18 analytical column (4.6 \times 250 mm) and the packed particle size was 5 μ m. A Rheodyne 7725-I auto-injector was used to inject 25 μ L of the plasma sample. Acetonitrile and 0.4% w/v phosphoric acid in distilled water were filtered through a 0.25- μ m filter membrane and used at a ratio of 53:47. A constant flow rate and temperature were maintained at 1.2 mL/min and 22 °C, respectively, throughout the analysis. UV detection was performed at 242 nm.

2.7.2. Deproteinization and preparation of plasma

On the last day of the experiment, the drugs were administered according to the protocol described above. The animals were anesthetized using ether and blood was withdrawn from the retro-orbital plexus at regular intervals of 1 h, 2 h, 4 h, and 8 h after drug administration. Next, 1 mL of the blood was transferred into a centrifuge tube and mixed with 50 μ L of freshly prepared 0.02% sodium EDTA solution. The samples were stored at –20 °C until use. The solution was mixed with an equal volume of acetonitrile and centrifuged at 3000 rpm for 10 min. The organic layer was collected and filtered using cellulose acetate filter papers, and injected into the HPLC system to estimate the gemfibrozil content [12,13].

2.8. Statistical comparisons

The results were analyzed using GraphPad Prism version 5.04 installed on Windows 10. The data were pooled and expressed as the mean \pm standard deviation, where n indicates the number of replicates in the experimental protocol. Statistically significant differences were estimated by one-way analysis of variance (ANOVA) using Dunnett's test and two-way ANOVA as applicable, and $P < 0.001$ and $P < 0.05$ were considered to indicate significant difference.

3. Results

3.1. Effects of co-administration of gemfibrozil and piperine on weight gain

3.1.1. Body weight variations

The results showed that a significant weight gain of 155 g occurred in the HFF group compared with the NRML group (41.25 ± 7.02 g), thereby indicating that hyperlipidemia was successfully induced over 30 days. In the GEM group, the weights of the rats were reduced to around 60.32 ± 6.09 g and they differed significantly compared with the NRML group, and similar results were obtained in the PIP group. The weights were normalized when gemfibrozil was used together with 5 mg/kg of piperine and they did not differ significantly compared to the NRML group. However, as the dose of piperine increased to 10 mg/kg and 20 mg/kg, the weight gain decreased dramatically and significant weight losses occurred of about -7.46 ± 1.32 g and -5.29 ± 0.75 g, respectively, compared with the initial weights, as shown in Table 1 and Fig. 1.

3.1.2. Hepatic weight variations

The weight of each rat liver was recorded and the weight of the liver was then calculated relative to 100 g of the initial body weight and the final body weight. The HFF group exhibited significant gains in the liver weight of 9.02 ± 0.92 g relative to the initial body weight and 4.47 g relative to the final weight. Thus, the liver tissue gained more weight and stored more fat and cholesterol in the HFF group. The relative liver weights were normal in the other groups and they did not differ significantly relative to the initial body weight and final body weight, as shown in Fig. 2.

3.2. Effects of co-administration of gemfibrozil and piperine on lipid parameters

3.2.1. Serum lipid profile

Treatment of rats with the high fat diet caused significant increases in the lipids estimated in the serum. The TG level in the serum increased to 4.51 ± 1.32 mmol/L in HFF rats and it was significantly higher compared with NRML rats. Treatment with piperine alone did not lower the elevated TG significantly and the value remained at 2.06 ± 0.98 mmol/L. No significant reduction in the TG level was found in the GEM group.

Table 1

Effects of gemfibrozil and piperine on weight gain in rats.

Group	Initial weight g	Final weight g	Weight gain g	Liver weight g	Relative to initial body g/100 g
NRML	225.27 ± 10.28	270.92 ± 12.91	41.25 ± 7.02	5.87 ± 1.21	2.60
HFF	201.76 ± 9.21	357.65 ± 15.05	155.46 ± 5.83*	9.02 ± 0.92	4.47 ^{a,b}
GEM	218.42 ± 11.63	282.74 ± 13.39	60.32 ± 6.09*	7.55 ± 0.83	3.45
PIP	211.75 ± 14.82	292.88 ± 16.29	80.04 ± 10.93*	7.18 ± 1.02	3.39
GEM-PIP-5	209.38 ± 17.21	253.45 ± 14.21	40.22 ± 3.33	6.54 ± 0.94	3.12
GEM-PIP-10	217.69 ± 12.78	210.82 ± 15.76	-7.46 ± 1.32 ^a	5.39 ± 1.11	2.47
GEM-PIP-20	212.01 ± 11.91	207.95 ± 13.42	-5.29 ± 0.75 ^a	5.27 ± 0.94	2.48

NRML: normal group; HFF: high fat diet induced group; GEM: gemfibrozil; PIP: piperine. The results are expressed as the rat weight ± standard deviation and n = 6. The results were analyzed using one-way ANOVA and statistically significant differences were determined with Dunnett's test, where * $P < 0.001$ indicates a significant difference compared with the NRML group. ^aNegative values indicate weight loss. ^b $P < 0.001$ indicates a significant difference compared with the liver weight relative to the initial body weight.

Effect of Gemfibrozil and Piperine on weight gain of rats

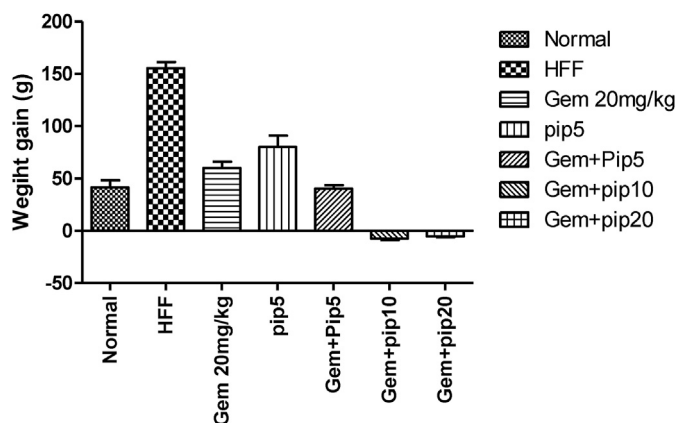


Fig. 1. Weight gain variations in rats treated with drugs.

Effect of Gemfibrozil and Piperine on Liver weight of rats

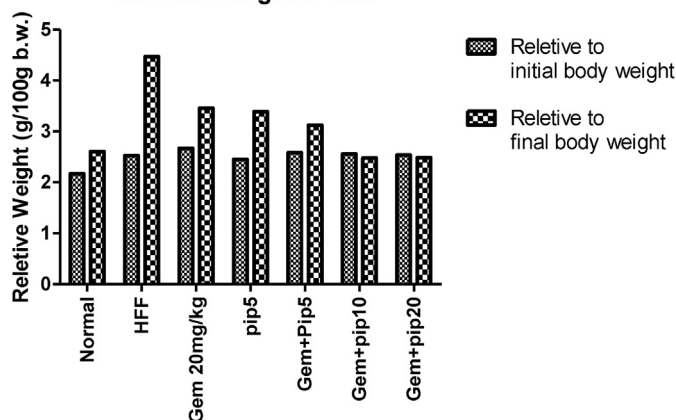


Fig. 2. Variations in liver weight relative to body weight in rats.

However, the co-administration of gemfibrozil with piperine at 5, 10, and 20 mg/kg significantly decreased the TG levels to 1.67 ± 0.87 , 1.29 ± 0.37 , and 1.22 ± 0.42 mmol/L, respectively. Similar changes were also detected in the TC levels. The TC level was 7.32 ± 1.97 mmol/L in the HFF group, whereas that in the NRML group was only 4.11 ± 1.04 mmol/L. Thus, the TC levels decreased significantly after the co-administration of gemfibrozil together with piperine at doses of 10 mg/kg and 20 mg/kg. Clear increases also occurred in the HDL levels, as indicated in Table 2, where the levels were elevated by treatment with gemfibrozil and by co-administration of piperine at all doses. Thus, treatment with piperine alone was not as effective as its co-administration with gemfibrozil. Therefore, the co-administration of piperine considerably increased the lipid lowering activity of gemfibrozil.

3.2.2. Hepatic lipid profile

The lipid parameters comprising TC and TG were estimated in the liver tissue homogenate and the results in Table 3 demonstrate the similarities of the serum lipid levels, except piperine had a greater activity compared with gemfibrozil alone. Gemfibrozil effectively lowered the blood lipid values but not the hepatic lipids. However, the co-administration of gemfibrozil with piperine obtained significantly higher activities compared with the individual drugs. The results were similar in both cases for TGs and TCs.

Table 2
Effects of gemfibrozil and piperine on serum lipid profile.

Group	TG (mmol/L)	TC (mmol/L)	LDL (mmol/L)	HDL (mmol/L)
NRML	1.95 ± 0.28	4.11 ± 1.04	1.15 ± 0.21	2.88 ± 0.76
HFF	4.51 ± 1.32**	7.32 ± 1.97**	5.47 ± 1.02**	1.45 ± 0.47*
GEM	2.06 ± 0.98	4.35 ± 1.21	3.04 ± 0.90*	2.73 ± 0.79
PIP	3.29 ± 1.03*	6.02 ± 1.72**	4.74 ± 1.01**	1.95 ± 0.64*
GEM-PIP-5	1.67 ± 0.87	3.86 ± 1.08	1.98 ± 0.88	2.89 ± 0.75
GEM-PIP-10	1.29 ± 0.37	3.22 ± 1.01	0.97 ± 0.16	3.45 ± 1.03
GEM-PIP-20	1.22 ± 0.42	3.18 ± 0.92	0.91 ± 0.13	3.51 ± 1.05

NRML: normal group; HFF: high fat diet induced group; GEM: gemfibrozil; PIP: piperine; TG: triglycerides; TC: total cholesterol; LDL: low density lipoproteins; HDL: high density lipoproteins. The results are expressed as the mean ± standard deviation and n = 6. The results were analyzed using one-way ANOVA and statistically significant differences were determined with Dunnett's test, where ***P* < 0.001, **P* < 0.05 indicates a significant difference compared with the NRML group.

Table 3
Effects of gemfibrozil and piperine on the hepatic lipid profile.

Group	TG (μmol/g)	TC (μmol/g)	SGOT (U/mol)	SGPT (U/mol)	ALP (U/mol)
NRML	18.02 ± 1.92	9.81 ± 1.21	135.56 ± 9.21	75.65 ± 5.49	34.36 ± 4.27
HFF	41.72 ± 5.28 ^a	35.47 ± 5.29 ^a	174.42 ± 12.02 ^a	110.37 ± 11.36 ^a	75.85 ± 8.65 ^a
GEM	22.87 ± 2.63*	18.76 ± 2.19*	165.56 ± 13.21	94.94 ± 8.04	63.48 ± 7.45
PIP	21.96 ± 3.20*	27.54 ± 4.65	151.43 ± 10.83	89.35 ± 8.46*	59.47 ± 5.38*
GEM-PIP-5	18.67 ± 2.02*	13.02 ± 1.28*	147.36 ± 9.04*	80.74 ± 9.75*	45.75 ± 4.35*
GEM-PIP-10	15.75 ± 1.25*	8.92 ± 1.25*	138.66 ± 10.52*	73.45 ± 8.39*	32.94 ± 4.33*
GEM-PIP-20	14.28 ± 1.22*	8.26 ± 1.34*	132.87 ± 9.36*	70.84 ± 7.57*	31.65 ± 3.27*

NRML: normal group; HFF: high fat diet induced group; GEM: gemfibrozil; PIP: piperine; TG: triglycerides; TC: total cholesterol; SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; ALP: alkaline phosphatase. The results are expressed as the mean ± standard deviation and n = 6. The results were analyzed using one-way ANOVA and statistically significant differences were determined with Dunnett's test, where **P* < 0.001 indicates a significant difference compared with HFF group and ^a*P* < 0.001 indicates a significant difference compared with NRML group.

3.3. Hepatic function test

Table 3 shows that significant increases occurred in the SGOT, SGPT, and ALP levels in the HFF rats, which were possibly due to the oxidative damage induced by the excessive lipids in hepatic tissues. These increases were reduced considerably by applying gemfibrozil and piperine together. These significant reductions in the enzyme levels indicate that the liver function was enhanced, but they remained above the normal range in the groups treated with drug or piperine alone. Thus, the concurrent administration of both drugs enhanced the liver activity. Interestingly, piperine had a dose-dependent enhancement effect on the hepatic activity, and even above a dose of 10 mg/kg.

3.4. Enhancement of plasma concentration of gemfibrozil

The plasma concentrations of gemfibrozil were estimated at different times after administration and the results are shown in Table 4. The peak concentration of gemfibrozil occurred at 2 h with 25.28 ± 2.17 μg/mL and the peak plasma concentration decreased by 50% after 4 h, thereby agreeing with the previously reported T_{1/2} and C_{max} values for gemfibrozil. After the co-administration of gemfibrozil with piperine at various

Table 4
Variations in plasma concentrations (μg/ml) of gemfibrozil over time.

Group	Time after drug administration			
	1 h	2 h	4 h	8 h
GEM	18.24 ± 1.86	25.28 ± 2.17	13.47 ± 2.10	2.03 ± 0.41
GEM-PIP-5	35.45 ± 4.27*	45.92 ± 5.43*	28.36 ± 2.18*	5.67 ± 0.89**
GEM-PIP-10	41.36 ± 5.45*	54.84 ± 6.38*	32.90 ± 3.21*	15.42 ± 1.21*
GEM-PIP-20	42.07 ± 5.39*	55.46 ± 6.32*	30.36 ± 3.07*	13.78 ± 1.12*

GEM: gemfibrozil; PIP: piperine. The results are expressed as the mean ± standard deviation and n = 6. The results were analyzed using one-way ANOVA and statistically significant differences were determined with Dunnett's test, where **P* < 0.001 indicates a significant difference compared with GEM group and ***P* < 0.05 indicates a significant difference compared with GEM group.

doses, the peak plasma concentration of 41.36 ± 5.45 μg/mL occurred within 1 h of administration, which was 100% higher than that when gemfibrozil was administered alone. The desired plasma concentration continued for a period of 4 h. There were no significant variations or increases in the concentration when the piperine dose exceeded 10 mg/kg. Thus, the co-administration of piperine and gemfibrozil significantly increased the drug concentration in terms of the plasma concentration and slowed its elimination, thereby agreeing with the enhanced activity described above. Fig. 3 illustrates the changes in the plasma concentration of gemfibrozil over time and after its co-administration with piperine.

4. Discussion

Piperine is a chemical moiety that is known to enhance the bioavailability and efficacy of drugs when administered concurrently. It is well established that piperine has synergistic effects on many antioxidant, antimicrobial, and anti-inflammatory drugs, as well as significantly influencing the metabolism of many drugs, especially those affected by first-pass metabolism. When administered orally, piperine inhibits hepatic aryl hydrocarbon hydroxylation, ethylmorphine-N-demethylation, 7-ethoxycoumarin-O-deethylation, and 3-hydroxy-benzo(a)pyrene glucuronidation [14]. Piperine inhibits glucuronidation to inhibit UDP-glucose dehydrogenase [15]. The inhibition of these metabolic processes by piperine increases the availability of the active forms of drugs in the bloodstream to enhance and extend their activity.

Piperine also enhances the bioavailability of the drugs by improving their solubility due to increased bile acid secretion. Piperine inhibits bile acid metabolism, thereby allowing bile acid to form micelles with the drug molecules, which enhances their solubility and absorption [15]. Piperine also increases the blood flow to the digestive tract to facilitate

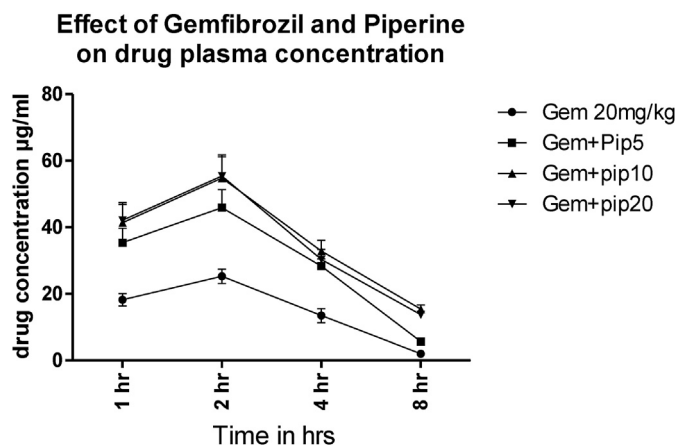


Fig. 3. Variations in plasma concentration of gemfibrozil over time.

increased drug absorption and bioavailability [15,16]. Piperine effectively inhibits the conjugation of glucuronic acid with drugs to reduce their renal clearance and enhance the duration of a drug's action [17].

Gemfibrozil is an antihyperlipidemic drug that acts by activating the alpha receptor activated by peroxisome proliferators, thereby altering the metabolism of lipids and leading to increases in the concentrations of HDLs and lipoproteins, as well as decreasing the removal of lipids (free fatty acid forms) from the liver. The decreased removal of fatty acids from the liver reduces the production of TG. Gemfibrozil increases the clearance of apoB to decrease the production of very low density lipoprotein cholesterol and the TG levels [18]. Gemfibrozil inhibits CYP2C8 to reduce the production and elevation of the lipid levels in the blood.

When used alone, piperine has an antihyperlipidemic activity as well as various other activities. Our results showed that piperine had a significant synergistic effect on the lipid lowering capacity of gemfibrozil. When used individually, piperine had a low activity compared with gemfibrozil, but the activity was significantly enhanced when applied at low doses of 5 mg/kg together with gemfibrozil. The activity also increased with the dose of piperine. However, interestingly, the synergistic effect of piperine was negligible when applied at doses over 10 mg/kg. There was no significant increase in the plasma concentration of gemfibrozil when applied with piperine at a dose higher than 10 mg/kg, and thus this can be considered the maximum effective dose for obtaining a synergistic effect with gemfibrozil.

Previous studies have shown that the C_{max} value for gemfibrozil is approximately 46 µg/mL but after its co-administration with piperine at 10 mg/kg and 20 mg/kg, we found that the C_{max} values were 54 µg/mL and 55 mg/mL, respectively, which are not significantly different, and thus a 10 mg/kg dose of piperine is probably the maximum dose that can obtain a synergistic effect with gemfibrozil. The T_{max} value reported previously for gemfibrozil is approximately 2.5 h [5]. In the present study, we found that the peak plasma concentration of gemfibrozil was reached within 2 h, thereby indicating that the pharmacokinetic parameters of the drug were clearly improved. Renal clearance of gemfibrozil occurs after approximately 1.5 h and up to 50% of the drug is eliminated in the form of conjugates [19]. It has also been estimated that 70% of the conjugates are excreted in urine. Another study suggested that glucuronidation of gemfibrozil is an important step that limits its activity and the conjugated form of the drug, gemfibrozil 1-O-β-glucuronide, is a metabolite-dependent inhibitor of CYP2C8 [20].

The enhanced activity of gemfibrozil when administered concurrently with piperine was due to the elevated plasma concentrations over 2 h after administration. Thus, we hypothesize that the inhibition of glucuronidation by piperine may prolong availability of gemfibrozil in its active and free form in the blood. The elevated plasma levels of the drug within 1 h can also be attributed to piperine enhancing the solubility of gemfibrozil and increasing its absorption.

Gemfibrozil was effective at lowering the TC and TG levels in the serum and liver [21]. The co-administration of piperine with gemfibrozil further enhanced its activity and significant reductions also occurred in the TG and TC levels, as well as increases in the HDL levels. No significant reductions occurred in the TG or TC levels when piperine was applied at a dose of 20 mg/kg, thereby indicating that the maximum effective dose is 10 mg/kg. Thus, increasing the dose of piperine can only enhance the activity of gemfibrozil up to this dose. Lipid metabolism typically occurs in the liver and the conversion of cholesterol to bile is an important pathway for the elimination of lipids. The decrease in the cholesterol levels in the liver suggests that increased lipid metabolism in the liver converted cholesterol into its metabolites and HDL, and this was supported by the elevated HDL levels in this tissue [22].

It has been suggested that increasing the dose of gemfibrozil can cause minor liver damage [23]. Thus, it is necessary to safely enhance the activity of gemfibrozil but without affecting other systems detrimentally. Our results suggest that the hepatic function improved according to the

reduced TG and TC levels in the liver tissue homogenate. Reductions in the levels of the hepatic enzymes comprising SGOT, SGPT, and ALP also occurred after the co-administration of gemfibrozil and piperine. The elevated levels of these enzymes might have been due to oxidative stress and the effects of drug administration on the liver, but their levels decreased significantly after the co-administration of piperine. In contrast to the highest synergistic antihyperlipidemic activity of piperine obtained at 10 mg/kg, the effect of piperine on the liver function in rats was dose dependent. Thus, the administration of 20 mg/kg of piperine together with gemfibrozil obtained the greatest hepatic function according to the SGOT, SGPT, and ALP levels.

Our HPLC results suggested that the concurrent administration of gemfibrozil with piperine enhanced the gemfibrozil levels in the blood to enhance the antihyperlipidemic activity. The T_{max} value for gemfibrozil was reached within 2 h and its activity was extended. The estimated lipid parameters showed that gemfibrozil decreased the levels of harmful lipids more effectively when co-administered with piperine compared with its individual administration. This effect can be attributed to the increased availability of the free drug in the blood for an extended period at a higher concentration. Various mechanisms have been proposed for the effect of piperine on enhancing the bioavailability of drugs. We found no indications of hepatic damage or changes in the liver function according to the liver enzyme levels. Piperine is a well-known hepatoprotective agent that might protect liver cells from the hepatic damage caused by free gemfibrozil.

5. Conclusion

The findings obtained in the present study indicate that gemfibrozil is a potent antihyperlipidemic drug and its concurrent administration with piperine can increase its activity by several times. We found that piperine acted as an effective bio-enhancer at doses up to 10 mg/kg according to *in vivo* activity assessments. Piperine increased the absorption and bioavailability of gemfibrozil but with no adverse effects on the liver. Further research is required to understand the molecular mechanisms responsible for this activity and to develop effective formulations by maintaining a suitable dose and dosing less frequently. The interactions between gemfibrozil and piperine also require further investigation to ensure that their co-administration is safe and effective.

Author contribution

S. Mohanalakshmi: Writing – original draft, Investigation, Writing – review & editing Dr. Shvetank Bhatt: Investigation Dr. C. K. Ashok Kumar: Methodology.

Declaration of interest

The authors declare that no financial or personal conflicts of interests influenced the research conducted in this study.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- [1] Asija, R., Singh, C., Hemlata, C., 2016. A comprehensive review on antihyperlipidemic activity of various medicinal plants. *Int. J. Curr. Pharm. Rev. Res.* 7 (6), 407–415. https://www.researchgate.net/publication/313661987_A_Comprehensive_Review_on_Antihyperlipidemic_Activity_of_Various_Medicinal_Plants.
- [2] Luijten, J., Van Greevenbroek, M., Schaper, N.C., Meex, S.J.R., Van der Steen, C., Meijer, L.J., de Boer, D., de Graaf, J., Stehouwer, C.D.A., Brouwers, M.C.G.J., 2018. Incidence of cardiovascular disease in familial combined hyperlipidemia: a 15-year follow-up study. *Atherosclerosis* 280, 1–6. <https://doi.org/10.1016/j.atherosclerosis.2018.11.013>.
- [3] FDA approved drug products: gemfibrozil oral tablets. https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/018422s058lbl.pdf.
- [4] Saku, K., Gartside, P.S., Hynd, B.A., Kashyap, M.L., 1985. Mechanism of action of gemfibrozil on lipoprotein metabolism. *J. Clin. Invest.* 75 (5), 1702–1712. <https://doi.org/10.1172/jci111879>.
- [5] Knauf, H., Kolle, E.U., Mutschler, E., 1990. Gemfibrozil absorption and elimination in kidney and liver disease. *Klin. Wochenschr.* 68 (13), 692–698. <https://doi.org/10.1007/BF01667018>.
- [6] Venkatesh, Sama, Nagula, Shilpika, Yenumala Padmavathi, Rajeswari Chiluka, Alavala, Ravi, Bolleddu, Rajesh, 2019. Effect of piperine on antihyperlipidemic activity and pharmacokinetic profile of simvastatin. *Inven. Rapid Pharm. Pract.* (1), 1–6, 2018. https://www.researchgate.net/publication/331285219_Effect_of_Piperine_on_Antihyperlipidemic_Activity_and_Pharmacokinetic_Profile_of_Simvastatin.
- [7] Singh, R., Devi, S., Patel, J.H., Patel, U.D., Bhavasar, S.K., thaker, A.M., 2009. Indian herbal enhancers: a review. *Phcog. Rev.* 3, 90–92. https://www.researchgate.net/publication/331162420_Indian_herbal_bioenhancers_a_review.
- [8] Tu, Y., Sun, D., Zeng, X., Yao, N., Huang, X., Huang, D., Chen, Y., 2014. Piperine potentiates the hypocholesterolemic effect of curcumin in rats fed on a high fat diet. *Exp. Ther. Med.* 8, 260–266. <https://doi.org/10.3892/etm.2014.1717>.
- [9] Khan, A., Mirza, Z.M., Kumar, A., 2006. Piperine. A phytochemical potentiator of ciprofloxacin against *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 50 (2), 810–812. <https://doi.org/10.1128/AAC.50.2.810-812.2006>.
- [10] Larsen, M.L., Illingworth, D.R., O'Malley, J.P., 1994. Comparative effects of gemfibrozil and clofibrate in type III hyperlipoproteinemia. *Atherosclerosis* 106 (2), 235–240. [https://doi.org/10.1016/0021-9150\(94\)90128-7](https://doi.org/10.1016/0021-9150(94)90128-7).
- [11] Lakshmi, V., Khanna, A.K., Sonkar, R., Mahdi, A.A., Agarwal, S.K., 2012. Antidyslipidemic and antioxidant property of piperine. *Nat. Prod. Ind. J.* 8 (7), 263–268. <https://www.tsijournals.com/articles/antidyslipidemic-and-antioxidant-property-of-piperine.pdf>.
- [12] Nakagawa, A., Shigeta, A., Iwabuchi, H., Horiguchi, M., Nakamura, K.I., Takahagi, H., 1991. Simultaneous determination of gemfibrozil and its metabolites in plasma and urine by a fully automated high performance liquid chromatographic system. *Biomed. Chromatogr.* 5 (2), 68–73. <https://doi.org/10.1002/bmc.1130050205>.
- [13] Kim, K., Jae, J.P., Hwang, H.R., Ban, E., Maeng, J.E., Kim, M.K., Piao, X.L., 2006. Simple and sensitive HPLC method for determination of gemfibrozil in human plasma with fluorescence detection. *J. Liq. Chromatogr. Relat. Technol.* 29 (3), 403–414. <https://doi.org/10.1080/10826070500452051>.
- [14] Atal, C.K., Dubey, R.K., Singh, J., 1985. Biochemical basis of enhanced drug bioavailability by piperine: evidence that piperine is a potent inhibitor of drug metabolism. *J. Pharmacol. Exp. Therapeut.* 232 (1), 258–262.
- [15] Mhaske, D.B., Sreedharan, S., Mahadik, K.R., 2018. Role of piperine as an effective bioenhancer in drug absorption. *Pharm. Anal. Acta* 9 (7), 591. <https://doi.org/10.4172/2153-2435.1000591>.
- [16] Reen, R.K., Singh, J., 1991. In vitro and in vivo inhibition of pulmonary cytochrome P450 activities by piperine, a major ingredient of piper species. *Indian J. Exp. Biol.* 29 (6), 568–573.
- [17] Johri, R.K., Thusu, N., Khajuria, A., Zutshi, U., 1992. Piperine-mediated changes in the permeability of rat intestinal epithelial cells. The status of gamma-glutamyl transpeptidase activity, uptake of amino acids and lipid peroxidation. *Biochem. Pharmacol.* 43 (7), 1401–1407. [https://doi.org/10.1016/0006-2952\(92\)90195-O](https://doi.org/10.1016/0006-2952(92)90195-O).
- [18] Mano, Y., Usui, T., Kamimura, H., 2007. The UDP-glucuronosyltransferase 2B7 isozyme is responsible for gemfibrozil glucuronidation in the human liver. *Drug Metab. Dispos.* 35 (11), 2040–2044. <https://doi.org/10.1124/dmd.107.017269>.
- [19] Knauf, H., Kölle, E.U., Mutschler, E., 1990. Gemfibrozil absorption and elimination in kidney and liver disease. *Klin. Wochenschr.* 68 (13), 692–698. <https://doi.org/10.1007/BF01667018>.
- [20] Ogilvie, B.W., Zhang, D., Li, W., Rodrigues, A.D., Gipson, A.E., Holsapple, J., Toren, P., Parkinson, A., 2006. Glucuronidation converts gemfibrozil to a potent, metabolism-dependent inhibitor of CYP2C8: implications for drug-drug interactions. *Drug Metab. Dispos.* 34 (1), 191–197. <https://doi.org/10.1124/dmd.105.007633>.
- [21] Shoman, M.E., Aboelez, M.O., Shaykhon, M.S.H.A., Ahmed, S.A., Elhady, A.R., Gamal O., 2020. New nicotinic acid-based 3,5-diphenylpyrazoles: design, synthesis and antihyperlipidemic activity with potential NPC1L1 inhibitory activity. *Mol. Divers.* 1–14. <https://doi.org/10.1007/s11030-020-10039-9>.
- [22] Zhang, Z., Wang, H., Jiao, R., Peng, C., Wong, Y.M., Yeung, V.S.Y., Huang, Y., Chen, Z.Y., 2009. Choosing hamsters but not rats as a model for studying plasma cholesterol-lowering activity of functional foods. *Mol. Nutr. Food Res.* 53, 921–930. <https://doi.org/10.1002/mnfr.200800517>.
- [23] Todd, P.A., Ward, A., 1988. Gemfibrozil. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in dyslipidaemia. *Drugs* 36 (3), 314–339. <https://doi.org/10.2165/00003495-198836030-00004>.