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Research article

Effect of ethanolic extract of *Piper nigrum* Linn. fruits on pharmacodynamics of atorvastatin in rats

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ABSTRACT

Herb-drug interaction about oral antihyperlipidemic drugs is a challenging concept, since the consumption of food and other herbal drugs is not documented in patient's profile. With this aspect, the present study was designed to investigate the possible effect of Ethanolic extract of *Piper nigrum* Linn. fruits on Atorvastatin an oral antihyperlipidemic drug. The study was carried out to investigate the pharmacodynamics of atorvastatin (AT) alone, and in combination with Ethanolic extract of Piper nigrum Linn. fruits and Isolated Piperine in hyperlipidemic rats. The standard cholesterol diet was used to induce hyperlipidemia in Wister rats. The blood samples were collected (on 1st and 8th day) from AT alone and in combination with Extract (100mg/kg) treated and piperine (10mg/kg) treated groups and were analyzed for various lipid profiles. Atorvastatin caused a marked reduction in the lipid profiles in hyperlipidemic rats. The combination of AT and *Piper nigrum* Linn. fruits in hyperlipidemic rats produced a significant change in lipid profiles (pharmacodynamics). In this study, we investigated the effect of Ethanolic extract of *Piper nigrum* Linn. fruits on the efficacy of Atorvastatin (substrate for CYP3A4) in rats. These results suggest *Piper nigrum* Linn. fruits ingestion increases the efficacy of Atorvastatin by inhibiting intestinal CYP3A4 enzyme in albino wistar rats.

Key words: Atorvastatin, *Piper nigrum*, pharmacodynamics.

INTRODUCTION

Hyperlipidemia is an elevation of one or more of the plasma lipids, including cholesterol, cholesterol esters, triglycerides and phospholipids, in which statins plays an important role for the treatment[14].

Atorvastatin (AT) is a synthetic lipid-lowering agent. It is a selective competitive inhibitor of HMG-CoA reductase, which catalyses the conversion of HMG-CoA to mevalonate, an important rate-limiting step in cholesterol biosynthesis. It is used in the treatment of hyperlipidemia or cardiovascular complications like coronary heart disease.

Piperine, a major constituent of *Piper nigrum* Linn.fruit increases the oral bioavailability of cytochrome P450 (CYP) 3A4 substrates, and the mechanism of these interactions is mainly thought to be caused by the inhibition of CYP3A4 in the small intestine[1]. It has been reported that some herb drug interaction affects the oral bioavailability of drugs.

The standard cholesterol diet has successfully been used to induce hyperlipidemia in rats in previous studies, and it was chosen as the hyperlipidemic model due to its convenience, reproducibility and availability. The present study investigated the effect of Ethanolic extract of *Piper nigrum* Linn.fruit on pharmacodynamics of atorvastatin in hyperlipidemic rats.

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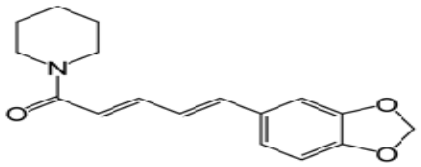
STRUCTURE OF PIPERINE



MATERIALS AND METHODS

Materials

Atorvastatin pure drug was a kind gift from Hychem Pvt Ltd, Hyderabad. Cholesterol kit (Enzymatic Method), HDL-C kit were procured from Qualigens Diagnostics, Mumbai. Triglycerides kit was obtained from E-Merck Limited, Mumbai, India. Piper nigrum Linn. fruits was purchased from the local market. Plant material was identified and authenticated by Botanist, Department of Botany.

Experimental Animals

Wistar albino adult rats weighing 200-220g were purchased from Sainath Agencies, 1-6-197/45/D, Bapujinagar, Musheerabad, (REG.No282/99/ CPCSEA), Hyderabad, India and housed in polypropylene cages in a room where the congenial temperature was 27°C ±1°C and 12 hrs light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet and water ad libitum. The protocol was approved by the Institutional Animal Ethical committee (IAEC) of Smt.Sarojini Ramulamma College of Pharmacy (R.No: 51/01-CPCSEA/2012/12).

Preparation of extract

20 g of fruit powdered was extracted with 250 ml Ethanol (95 %) in soxhlet apparatus[2]. The solution was filtered and kept under vacuum on a water bath at 60˚C. The extraction was continued untill the extraction completion. After completion of extraction the extract was filtered and kept under vacuum on a water bath at 60˚C concentrated under reduced pressure. That extract was stored in an airtight container in a refrigerator below 10°C. 20 ml of 10% alcoholic KOH was added with constant stirring to the concentrate. Then the extract was filtered and allowed the alcoholic solution to stand overnight were up on needles of piperine separated out. Separated piperine was collected and kept for drying.

Phytochemical screening

Phytochemical screening of the crude extract was carried out employing standard procedures [4], to reveal the presence of chemical constituents such as alkaloids, glycosides, flavonoids, tannins, terpenes, saponins, carotenoids, phyto sterols, fixed oils and others.

Acute toxicity

Initially, the Ethanolic extract of *piper nigrum* Linn. fruit was studied for acute oral toxicity as per revised OECD guidelines number 423. Ethanolic extract of *piper nigrum* Linn. fruit was devoid of any toxicity up to 2000 mg/kg in albino mice by oral route. Hence for further studies doses of 100 mg/kg po, of extract was used.

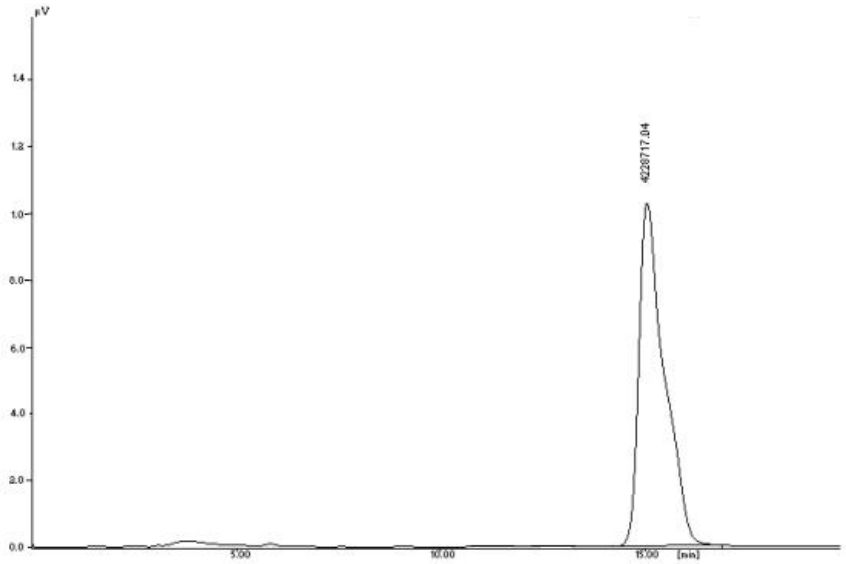
Chemical Constituents of the Extract and their Identification.

Piper nigrum Linn.fruit ethanol extract contain alkaloids, glycosides, flavones, saponins. The main constituents are Piperine 5-9%, di piperamide D and di piperamide E, piperidine5%. These constituents are identified by HPLC.

HPLC ANALYSIS

The HPLC analysis was performed using a shimadzu model – vp 135p2 equiped with a uv spectrophotometric detector set at 343 nm column : HiQ Sil C18W, flow rate: 1ml min $^{-1}$, injection volume 20 µL in methanol (HPLC grade).

Figure 1: HPLC chromatogram of ethanol extract of piper nigrum



PHARMACODYNAMIC INTERACTION STUDY IN HYPERLIPIDEMIC RATS.

Induction of hyperlipidemic in rats

Before induction of hyperlipidemia, the weight of the individual animal and plasma cholesterol levels were estimated. The standard cholesterol diet along with butter (0.5 ml twice a day) was administered for 30 days to induce hyperlipidemia. At the end of the one month the blood was withdrawn from tail vein to analyze (7) for lipid profiles (TC, TG, LDL-C and HDLC levels) to confirm the induction of hyperlipidemia.

The hyperlipidemic rats were divided into four groups of six rats in each group.

Group I: (Non-HL) Control group of Non-Hyperlipidemic rats received a dose of 1.5% CMC.

Group–II (Hyperlipidemic Control) - Hyperlipidemic rats were orally administered with 1.5% CMC.

Group-III (Hyperlipidemic Standard) Hyper-lipidemic rats were orally administered with Atorvastatin at dose of 6mg/kg.

Group– (Hyperlipidemic Test-1) – Hyperlipidemic rats were fed orally with extract at dose of 100mg/kg for Seven days followed by orally administered with Atorvastatin at a dose of 6mg/kg on 8th day.

Group–V (Hyperlipidemic Test 2) – Hyperlipidemic rats were fed orally with piperine at dose of 10mg/kg for seven days followed by orally administered with Atorvastatin at a dose of 6mg/kg on 8th day.

Collection of Blood samples

On 1st and 8th day, blood samples of 0.5 mL were withdrawn at different time intervals through retro-orbital sinus into heparinized eppendorff tubes at 0.5, 1, 2, 4, 6, 8 and 24 hrs and equal amount of saline was administered to replace blood volume at every blood withdrawal time. Plasma was obtained by immediate centrifugation of blood samples using cooling centrifuge at 3000 rpm for 5 minutes at room temperature. All samples were stored at 4°C until analysis[13].

Biochemical analysis

Plasma lipid levels include TC, TG and HDL-C were carried out using respective diagnostic commercial kits from Qualigens diagnostics, Mumbai, India and LDL-C in plasma was calculated as per Friedewald estimation(12-13), LDLC=TC-(TG/5+HDL)-C = mg/dl.

Statistical Analysis

The results were expressed as mean ± SD. Statistical comparisons for the pharmacodynamic study Non HL, HL, AT and Extract + AT, Piperine +AT groups were carried out using one-way ANOVA followed by Bonferroni's test. Differences below P<0.05 implied statistically significance[13].

RESULTS

Pharmacodynamic Study

The lipid profiles were estimated for all the groups on day1& day 8 at different time points. The average lipid profiles of atorvastatin alone and in combination with Extract and piperine were shown in table 2 & 3. Standard cholesterol diet effectively induced hyperlipidemia by increasing the plasma TC, TG and LDL-C levels and decreasing the plasma HDL-C levels. In groups of III, IV, V the lipid profiles were significantly changed than group II. Atorvastatin alone and in combination with Extract and piperine statistically reduced the hyperlipidemia.

Table 1: Pharmacodynamic parameters of Atorvastatin alone and in combination with Extract and Piperine on day 1 (n = 6)

PARAMETERS	TC	TG	LDL	HDL
I: Normal rats	83.16 ± 2.71	123.5 ± 2.16	21.5 ± 1.76	32.16 ± 3.06
II: HL Control	179.16 ± 3.97	264.5 ± 9.48	264.5 ± 9.48	56.5 ± 1.64
III:AT alone	163.16 ± 8.18	251.5 ± 10.09	61.33 ± 4.88	61.5 ± 2.42
IV: Extract + AT	153.83 ± 2.40	236.66± 10.76	51.83 ± 3.06	64.66 ± 1.75
V:Piperine +AT	145.73±1.30	225.55± 9.56	47 ± 2.06	68.55 ± 1.55

Table 2: Pharmacodynamic parameters of Atorvastatin alone and in combination with Extract and Piperine on day 8 (n = 6)

PARAMETERS	TC	TG	LDL	HDL
I: Normal rats	83.66 ± 2.91	125.8 ± 2.56	25.5 ± 1.96	33.18 ± 3.25
II: HL Control	175.16 ± 3.77	260.5 ± 9.35	72.16 ± 5.15	56.5 ± 1.64
III:AT alone	108.5 ± 1.37a	134.5 ± 1.04	30.66 ± 0.51	61.5 ± 0.83
IV: Extract + AT	106.83 ± 1.32a	119.83 ± 0.72	26.66 ± 2.42	64.66 ± 1.03
V:Piperine +AT	103.6±1.23	115.63± 0.52	24.54±1.76	68.55±1.55

Values are in mean ± SD (µg/ml);(n =6); p < 0.05 statistically significant

(TC=total cholesterol, TG=triglycerides, LDL =low density lipoprotein cholesterol and HDL= high density lipoprotein cholesterol)

Atorvastatin alone and in combination with Extract (100mg/kg) and piperine (10mg/kg) statistically reduced the hyperlipidemia. Atorvastatin alone and in combination with Extract and piperine significantly reduced the plasma cholesterol, triglycerides, low density lipoprotein-cholesterol levels in standard diet induced hyperlipidemic rats (P< 0.001), but the change in the lipid levels was more in Extract and piperine treated groups. There was a significant difference in change of lipid profiles in AT and Extract and piperine treated groups (P< 0.001) (table 1 & 2).

DISCUSSION

Atorvastatin lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-COA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic low density lipoprotein (LDL) receptors on the cell surface for enhanced uptake and catabolism of low density lipoprotein (LDL) and also decreases triglycerides (TG) levels but, increases high density lipoprotein - cholesterol .It was reported that the atorvastatin therapy produced a statistically significant changes in total cholesterol, LDL-C, TG, HDL-C within 24 hours .

Rats fed a standard cholesterol diet (coconut oil/cholesterol diet) develop hypercholesterolemia with increase in TG, LDL-C and HDL-C as shown in this and previous studies. In cholesterol fed rats, the increases in lipid levels are associated with diminished LDL receptor function and addition of oils containing saturated fatty acids causes a large down regulation of LDL receptor which led to higher cholesterol levels. Further, the amount of cholesterol returning to liver is increased and thus plasma HDL-C raises. The increase in plasma TG with this diet is due to the over production of VLDL . Treatment of standard cholesterol diet fed rats with AT and extract and piperine markedly decreased the plasma TC, TG and LDL-C levels relative to control animals.

The present results suggest that HMG-COA reductase inhibitors prevent the progression of hypercholesterolemia during treatment, though the plasma lipid levels remain much higher than in normal lipidemic rats. This may due to the decreased HMG-COA reductase activity and LDL receptor function in chronically fed cholesterol rats. The decrease in plasma lipid levels was more in Extract (100mg/kg) and Piperine (10mg/kg) treated group than atorvastain treated group. Enhanced antihyperlipidemic action of Extract and Pipeine treated Atorvastatin group over Atorvastatin alone treated group may be due to metabolic inhibition of Atorvastatin in intestine by the ethanolic extract of *Piper nigrum* Linn.fruit as Atorvastatin is metabolized by CYP3A4 and Extract inhibits CYP3A4 enzyme, thus leading to Herb-Drug interaction.

CONCLUSION

It was concluded that atorvastatin with Ethanolic extract of piper nigrum Linn. fruits alter the pharmacodynamics of Atorvastatin, significant change in lipid profile was observed in hyperlipidemic rats. The current study demonstrated this combination therapy produces marked reduction in total lipid profile levels which were compared to atorvastatin alone. The characteristic changes in pharmacodynamic profiles may be advantageous in the management of atherosclerosis.

Administration of Ethanolic extract of Piper nigrum Linn. fruits with Atorvastatin led to herb drug interaction and augmented the antihyperlipidemic activity of Atorvastatin significantly. Thus it is necessary to adjust the dose of Atorvastatin when it is administered with Piper nigrum Linn. fruits to minimize the adverse effects.

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