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ORIGINAL ARTICLE

A Comparative Study for Investigation into Beneficial Effects of Ketoconazole and Ketoconazole + Cholestyramine Combination in Hyperlipidemia and The Complications Associated With It

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ABSTRACT
Cardiovascular diseases (CVDs) have been reckoned amongst the top reasons for early deaths in the country. One of the major risk factors for developing CVDs is hyperlipidemia, an elevated condition of lipid levels in the body. Hyperlipidemia has been known to speed up a process of hardening of the arteries called atherosclerosis that may prove fatal in the development of various CVDs. Many drugs are available in the market for treatment of hyperlipidemia. This study was done with an objective to have a better alternative for treating hyperlipidemia than the existing treatment. A combination of an anti-fungal drug ketoconazole was taken with cholestyramine, a bile acid sequesterant, under studies for treating hyperlipidemia and its complications in High Fat Diet induced hyperlipidemic rats. This was compared with a treatment of ketoconazole as a single drug. With both this treatment there was a significant lowering of serum lipid levels similar to that of the standard drug atorvastatin. Thus the results helped to conclude that ketoconazole can be helpful in improving lipid profile in hyperlipidemic conditions.
Keywords: *Hyperlipidemia, High Fat Diet, Ketoconazole, Atorvastatin, Cholestyramine*

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INTRODUCTION

Hyperlipidemia is a medical condition characterized by an elevation of any or all lipid profile or lipoproteins in the blood. It is the major cause of coronary artery disease, ischemic cerebrovascular disease, peripheral vascular disease, etc. Lipids are water-insoluble organic compounds, which are essential for many normal functions of living organisms: they are important components of cell membranes, they are used to store energy, and they play a significant role as enzyme co-factors, hormones, and intracellular messengers.[1] Of the many groups of lipids, three are most important from a clinical perspective: fatty acids, sterols (mainly cholesterol), and acyl-glycerols (mainly triglycerides).[1][2] Cholesterol is the main sterol in animal tissues. Dietary intake is the major source of cholesterol, but it can also be synthesized endogenously by the liver and other tissues. It plays a fundamental role in central metabolic pathways, such as bile acid metabolism and steroid hormone and vitamin D synthesis.[1][2] It is dangerous because the extra cholesterol circulating in the bloodstream forms the basis for plaque lining the arteries. Plaque slows the flow of blood through the arteries, which is especially dangerous when it occurs in the heart. Coronary artery disease can result in angina or a heart attack. During a heart attack, a section of the heart muscle receives no oxygen because blood circulation in the heart arteries is blocked by plaque. Plaque can also break off from an artery wall and circulate in the body, causing a stroke or peripheral arterial disease. Coronary artery disease (CAD) is the most common cause of congestive heart failure (CHF) in the developed world, accounting for 50% of cases. [3] In itself, high cholesterol does not cause symptoms. Many people do not discover that they have high cholesterol until after plaque has formed. Unless a person has regular checkups that include laboratory

testing, high cholesterol may silently cause plaque buildup in the arteries until symptoms of heart disease appear. Angina, heart attack, and stroke are all possible results of untreated high cholesterol.

This medical condition or problem divided into two subtypes which are: primary hyperlipidemia and secondary hyperlipidemia.

Primary or familial hyperlipidemia which is usually occurs as a result of genetic problems i.e., mutation within receptor protein. It is classified according to the Fredrickson classification, which is based on the pattern of lipoproteins on electrophoresis or ultracentrifugation. It was later adopted by the World Health Organization (WHO). It does not directly account for HDL, and it does not distinguish among the different genes that may be partially responsible for some of these conditions.[4]

According to "Frederickson" classification, there are five types of Hyperlipidemia:[4]

Type I - Raised cholesterol with high triglyceride levels

Type II - High cholesterol with normal triglyceride levels

Type III - Raised cholesterol and triglycerides

Type IV - Raised triglycerides, atheroma, and raised uric acid

Type V - Raised triglycerides

Acquired or secondary hyperlipidemia may mimic primary forms of hyperlipidemia and can have similar consequences. They may result in increased risk of premature atherosclerosis or, when associated with marked hypertriglyceridemia, may lead to pancreatitis and other complications of the chylomicronemia Syndrome, as a result of other underlining diseases like diabetes, renal failure, hypothyroidism and drug induced like corticosteroids, oral contraceptive, beta blockers etc.

Lipoproteins are spherical structures that consist of a hydrophobic core containing lipids (i.e. triglycerides and/or cholesterol esters), and an amphophilic (i.e. both hydrophobic and hydrophilic) outer layer of phospholipids, free cholesterol, and proteins that forms a protective envelope surrounding the lipid core.[1][2][5][6][7][8] It can be divided based on their hydrated density into the following major classes - chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).[9][10][11]

Drugs used in treatment of hyperlipidemia currently include statins (simvastatin, pravastatin), resins (cholecystyramine) and fibrates (gemfibrozil). Less commonly used drugs include nicotinic acid, probucol, clofibrate and colestipol. Fish oils have been advocated for the treatment of increased triglycerides (TG) but were found to raise low density lipoproteins (LDL). The statins have been used for almost a decade and have not produced untoward effects. Furthermore they are more efficacious than existing therapies and have a higher degree of patient acceptability. But along with this some side effects of statins like Myopathy including Myalgia, Myositis, Rhabdomyolysis, plasma creatinine kinase, abdominal cramps, constipation, diarrhea, heartburn, Hepatitis by elevating hepatic enzyme Alanine Amino Transferase (ALT) level in serum are found. This gave an influence in this research for getting a better alternative of it with similar efficacy and lesser side effects. [12][13][14]

Present study was focused on an anti-fungal drug ketoconazole to give anti-hyperlipidemic activity in preclinical studies done on High Fat Diet induced hyperlipidemic rats. Along with this cholestyramine was taken. Cholestyramine is an anionic exchange resin which is highly insoluble in nature. It's a polymer of divinylbenzene and polystyrene. It has a large molecular weight and large molecular structure. It does not get absorbed in the body throughout gastro-intestinal tract. Ketoconazole is an imidazole derivative antifungal agent developed for treatment of human mycotic infections and plays an essential role in antifungal chemotherapy. Ketoconazole was first discovered at Janssen **Pharmaceuticals. The IUPAC name of this molecule is 1-[4-[4-[[2-(2, 4-dichloropheny)-2-(imidazole-1-**ylmethyl)-1, 3-dioxolan-4-yl] methoxy] phenyl] piperazin-1-yl]ethan-1-one. Its chemical formula is C26H28Cl2N4O4 with a molecular weight of 531.43092 gm/mol. [15][16]

Ketoconazole is contains heterocyclic ring imidazole and interferes with fungal synthesis of ergosterol, a constituent of fungal cell-membrane as well as certain enzymes. It inhibits the enzyme cytochrome p-450 14 alpha demethylase (p45014DM). This enzyme participates in sterol biosynthesis pathway that forms ergosterol from lanosterol. Similarly similar pathway is followed in human cholesterol biosynthesis. From the same pathway of lanosterol dihydrosterols are synthesized and from dihydrosterols cholesterol is formed. So from this assumption was made as for the inhibition of 14- alpha demethylase in synthesis of ergosterol in fungus will also inhibit the cholesterol formation via same pathway thus lowering the level of cholesterol in serum.[17]

MATERIALS AND METHODS

Selection of animals

Male Wistar albino rats of weight 180- 200 gm were used for the present study. The animals were procured from animal house, Department of Pharmacology, School of Pharmacy R.K.University, Rajkot. Animals were house at a temperature of 24±2˚C and relative humidity of 30 – 70 %. A light and dark cycle was followed. All the experimental procedures and protocols used in the study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of School of Pharmacy, RK University and care of laboratory animals were taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).The animals were used after approval of IAEC protocol by Ministry of Social Justice and Empowerment, Government of India (Protocol No. RKCP / COL /RP/15/59).

Grouping of animals

Male Wistar rat with a weight ranging between 180-200gm were divided into five groups each consisting of 6 animals.

Group I: Normal control - Regular Normal low fat diet

Group II: Disease control - High Fat diet

Group III: Standard treatment - High Fat Diet+ Atorvastatin (dose 10mg/kg p.o)

Group IV: Treatment 1 - High Fat Diet + Ketoconazole (dose 300mg/kg p.o)

Group V: Treatment 2 – High Fat Diet + Ketoconazole (dose 200mg/kg p.o) + Cholestyramine (dose 200mg/kg p.o)

High fat diet is a hyper caloric diet and was prepared by mixing the below given constituents in fixed percentage. The mentioned quantity is for 1000 gm diet. The feed was prepared and given to animals with 25% fructose water. Diet was given for 21 days and the initial weight of the animals was noted. The weight gain was observed in rats by noting the weight on 7th, 11th, 21st and 27th day, therefore confirming the development of obesity in rats. On 21st day the High Fat Diet was stopped and the treatment protocol of the drug was given for 7 days. Study was continued for 28 days.

Table 1: High Fat Diet (HFD) composition [18]

Ingredient(g/kg)	HFD
Casein	100
Sucrose	260
Maize starch	300
Cellulose	30
Vegetable Ghee	15
Soyabean oil	200
Cheese	40
Vit. mix	10
Butter	40
DL-Methionine	2.5
Sodium chloride	2.5

A body weight for each group of rat was recorded on day 7, 14, 21 and 28 day during the period of experiment. The difference between mean body weights in each group was calculated to determine the change in the body weight between the first day and 28th day.[19] The daily food intake for each group of rats was measured at an interval of 7 days and expressed as mean daily food intake for each group of 6 rats. The blood samples were collected on 28th day, using Retro-Orbital Plexus method and the serum was separated by centrifugation at 5000 rpm for 30 minutes at stabilization.[14] The parameters like body weight, total Cholesterol, triglyceride, LDL-Cholesterol, VLDL-Cholesterol, HDL-Cholesterol, CK MB, SGOT, SGPT, serum Creatinine and Atherogenic Index were evaluated using the kits of Span Diagnosis.[20][21][22]

Statistical Analysis

To check the significance of the data obtained of the parameters evaluated following statistical tests were performed-

ANOVA - to see the variability within all the groups

TUCKEY'S TEST – to get the honest significance difference between all the groups

INSTAT SOFTWARE – to derive all the statistical terms like Standard Error of Mean (SEM), P-value, Standard Deviation, ANOVA, Degree of Freedom, etc.

RESULT AND DISCUSSION

Beneficial effect of ketoconazole on body weight in High Fat Diet induced hyperlipidemic rats

High Fat Diet when introduced to rats showed a significant difference from normal control group to that of disease control group. There was a significant increase in body weight from 271.66 ± 5.27 gm to 498.33 ± 4.77 gm at the end of 21 days. This increase in body weight proves that there was an induction of hyperlipidemia in rats after consuming High Fat Diet. After the treatment of 7 days with drugs there was a lowering in the body weight in standard group, treatment-1 and treatment-2. This decrease in the body weight indirectly gives an indication that there will be lowering of serum lipid level in the rats. (Table 2)

Table 2: Effect of Ketoconazole on body weight on Hyperlipidemic rats

Physical Parameter	NC			DC		STD				T-1			T-2		
	0	21	28	0	21	28	0	21	28	0	21	28	0	21	28
Body weight (gm)	271.66 ± 5.27	272.5 ± 2.81	272.5±2.14	260.0±2.28	499.16±3.74	489.16±15.07	260.0±3.87	498.33±4.77	482.5±2.14	260.33±2.38	498.33±4.77	477.5±1.11	260± 2.8	500±3.4	480±3.0

Beneficial effect of ketoconazole on serum total cholesterol

After consuming High Fat Diet there was a significant increase in serum total cholesterol level in the rats. The serum cholesterol in disease control group was found to be 248.08 ± 5.33 mg/dl compared to normal control group 118.08 ± 2.83 mg/dl. After 7 days of treatment, the outcome was with standard atorvastatin (172.15 ± 5.20 mg/dl) and ketoconazole (175.2 ± 5.20 mg/dl). There was no significant difference between T-1 group (175.02 ± 5.20 mg/dl) and T-2 group (174.358 ± 4.919 mg/dl) after the treatment. But we can say that there was a significant difference in the decrease in total cholesterol level in both the groups compared to disease control. So it helps in improving the lipid profile. (Table 3)

Beneficial effect of ketoconazole on serum HDL-Cholesterol level

High Fat Diet significantly reduced the serum cholesterol level in the disease control group (45.06 mg/dl) compared to normal control group (86.10 mg/dl). After the treatment of 7 days with Atorvastatin the serum HDL-Cholesterol was found to be increased (66.04 ± 3.50). Similarly with 7 days of treatment with Ketoconazole there was a significant increase in the HDL-Cholesterol level (60.02 ± 4.73). But there was no significant difference between T-1 group and T-2 group (61.15 ± 3.36). By this observation we can say that ketoconazole gives beneficial effect in increasing serum HDL-Cholesterol level and thus helps in improving lipid profile. (Table 3)

Beneficial effect of Ketoconazole on serum Triglyceride

High fat diet disease control rats exhibited high significantly increased serum triglyceride (229.71 ± 4.44 mg/dl) as compared to normal control group rats (90 ± 1.82 mg/dl). 7 days treatment with standard Atorvastatin (100.98 ± 4.44 mg/dl) and Ketoconazole (119.08 ± 5.49 mg/dl) showed a significant difference in the serum Triglyceride level compared to disease control group showed a significant difference in the serum Triglyceride level compared to disease control group. There was a non significant difference between T-1 group (119.08 ± 5.49) and T-2 group (116.04 ± 4.54). This clearly indicates that Ketoconazole at 300mg/kg gives beneficial effect on serum triglyceride and helps improving lipid profile. (Table 3)

Beneficial effect of ketoconazole on serum LDL cholesterol

High Fat Diet significantly increased the serum LDL cholesterol level in the disease control group (177.62 ± 5.28 mg/dl) compared to normal control group (63.59 ± 2.147 mg/dl). After the treatment of 7 days with Atorvastatin the serum LDL-Cholesterol was found to be decreased (113.34 ± 5.42 mg/dl). Similarly with 7 days of treatment with Ketoconazole there was a significant decrease in the LDL-Cholesterol level (115.35 ± 4.75 mg/dl) in T-1 group. Along with this it was found that there was a non significant difference between T-1 group (115.35 ± 4.75 mg/dl) and T-2 group (115.75 ± 7.82 mg/dl) after the treatment. By this observation we can say that ketoconazole gives beneficial effect in decreasing serum LDL-Cholesterol level and thus helps in improving lipid profile. (Table 3)

Beneficial effect of ketoconazole on serum VLDL cholesterol

High Fat Diet significantly increased the serum VLDL cholesterol level in the disease control group (45.48 ± 2.69 mg/dl) compared to normal control group (18.26 ± 1.133 mg/dl). After the treatment of 7 days with Atorvastatin the serum VLDL-Cholesterol was found to be decreased (20.45 ± 1.75 mg/dl). Similarly with 7 days of treatment with Ketoconazole there was a significant decrease in the VLDL-Cholesterol level (23.66 ± 1.85 mg/dl) in T-1 group. Along with this it was found that there was a non significant difference between T-1 group (23.66 ± 1.85 mg/dl) and T-2 group (23.33 ± 1.94 mg/dl) after the treatment. By this observation we can say that ketoconazole gives beneficial effect in decreasing serum VLDL-Cholesterol level and thus helps in improving lipid profile and manages hyperlipidemia. (Table 3)

Beneficial effect of ketoconazole on serum creatinine

High Fat Diet significantly increased the serum creatinine level in the disease control group (3.2 ± 0.36 mg/dl) compared to normal control group (63.59 ± 2.147 mg/dl). After the treatment of 7 days with Atorvastatin the serum creatinine was found to be decreased (1.01 ± 0.015 mg/dl). Similarly with 7 days of treatment with Ketoconazole there was a significant decrease in the creatinine level (1.061 ± 0.03 mg/dl) in T-1 group. Along with this it was found that there was a non significant difference between T-1 group (1.061 ± 0.03 mg/dl) and T-2 group (1.045 ± 0.019 mg/dl) after the treatment. By this observation we can say that ketoconazole gives beneficial effect in decreasing serum creatinine and thus can be said a lesser damage on kidney is found. (Table 3)

Beneficial effect of ketoconazole on serum SGOT

High Fat Diet significantly increased the serum SGOT level in the disease control group (64.92 ± 3.19 U/L) compared to normal control group (19.135 ± 0.99 U/L). After the treatment of 7 days with Atorvastatin the serum SGOT was found to be decreased (25.92 ± 3.51 U/L). Similarly with 7 days of treatment with Ketoconazole there was a significant decrease in the SGOT level (27.87 ± 3.01 U/L) in T-1 group. Along with this it was found that there was a non significant difference between T-1 group (27.87 ± 3.01 U/L) and T-2 group (27.53 ± 2.38 U/L) after the treatment. By this observation we can say that ketoconazole gives beneficial effect in decreasing serum SGOT and thus can be said a lesser damage to liver and heart. (Table 3)

Beneficial effect of ketoconazole on serum SGPT

High Fat Diet significantly increased the serum SGPT level in the disease control group (48.90 ± 2.75 IU/L) compared to normal control group (14.03 ± 0.86 IU/L). After the treatment of 7 days with Atorvastatin the serum SGPT was found to be decreased (20.16 ± 1.85 IU/L). Similarly with 7 days of treatment with Ketoconazole there was a significant decrease in the SGPT level (21.81 ± 3.01 IU/L) in T-1 group. Along with this it was found that there was a non significant difference between T-1 group (21.81 ± 3.01 IU/L) and T-2 group (21.70 ± 1.79 IU/L) after the treatment. By this observation we can say that ketoconazole gives beneficial effect in decreasing serum SGPT and thus can be said a lesser damage to liver. (Table 3)

Beneficial effect of ketoconazole on serum CK MB

High Fat Diet significantly increased the serum CK MB level in the disease control group (283.24 ± 5.07 U/L) compared to normal control group (89.22 ± 9.51 U/L). After the treatment of 7 days with Atorvastatin the serum CK MB was found to be decreased (103.88 ± 6.16 U/L). Similarly with 7 days of treatment with Ketoconazole there was a significant decrease in the CK MB level (104.82 ± 5.09 U/L) in T-1 group. Along with this it was found that there was a non significant difference between T-1 group (104.82 ± 5.09 U/L) and T-2 group (104.16 ± 6.78 U/L) after the treatment. By this observation we can say that ketoconazole gives beneficial effect in decreasing serum CK MB and thus can be said a lesser damage to heart. (Table 3)

Beneficial effect of ketoconazole on Atherogenic Index

High Fat Diet significantly increased the serum atherogenic index in the disease control group (4.507 ± 1.105) compared to normal control group (1.63 ± 0.108). After the treatment of 7 days with Atorvastatin the atherogenic index was found to be decreased (1.63 ± 0.14). Similarly with 7 days of treatment with Ketoconazole there was a significant decrease in the atherogenic index (1.90 ± 0.04) in T-1 group. Along with this it was found that there was a non significant difference between T-1 group (1.90 ± 0.04) and T-2 group (1.77 ± 0.05) after the treatment. By this observation we can say that ketoconazole gives beneficial effect in decreasing the atherogenic index and thus can be said as cardio protective action. (Table 3)

Table 3: Effect of Ketoconazole on various parameters

Biochemical Parameter	NC	DC	STD	T-1	T-2
Serum Total Cholesterol (mg/dl)	118.08 ± 2.83	248.51±5.33	172.15±5.20	175.02±5.20	174.358±4.919
Serum HDL-Cholesterol (mg/dl)	86.10±1.99	45.06±2.60	66.04±3.50	60.02±4.73	61.15±3.36
Serum Triglyceride (mg/dl)	90.09±1.82	229.71±4.44	100.98±4.98	119.08±5.49	116.04±4.54
Serum LDL-Cholesterol (mg/dl)	63.59±2.147	177.62±5.28	113.34±5.42	115.35±4.75	115.75±7.825
Serum VLDL Cholesterol (mg/dl)	18.26±1.133	45.48±2.69	20.45±1.75	23.66±1.85	23.33±1.94
Serum Creatinine (mg/dl)	0.988±0.017	3.2±0.36	1.01±0.015	1.061±0.03	1.045±0.019
Serum SGOT(U/L)	19.135±0.99	64.92±3.19	25.92±3.51	27.87±3.01	27.53±2.38
Serum SGPT(IU/L)	14.03±0.86	48.90±2.75	20.16±1.85	21.81±1.94	21.70±1.79
Serum CK MB (U/L)	89.22±9.51	283.24±5.07	103.88±6.16	104.82±5.09	104.166±6.78
Atherogenic Index	0.372±0.108	4.507±1.105	1.63±0.14	1.90±0.04	1.77±0.05

CONCLUSIONS

- We have confirmed anti-hyperlipidemic activity of ketoconazole (300 mg/kg, p.o.) in high fat diet induced hyperlipidemic rats.
- The probable mechanism for anti-hyperlipidemic activity of ketoconazole seems to be decreasing 29.55% of total cholesterol, 10% of LDL, 48.16% of triglyceride, 47.97% of VLDL and increases 24.92% of HDL.
- The probable mechanism for anti-hyperlipidemic activity of ketoconazole seems to be improving the complications associated with hyperlipidemia by decreasing 66.8% of creatinine, 57.07 % of SGOT, 55.39 % of SGPT, 62.99 % of CK MB and 57.84 % of Atherogenic Index.
- The assumed mechanism for anti-hyperlipidemic activity of ketoconazole was found to be inhibiting 14-alpha demethylase enzyme, which is one of the enzyme responsible for biosynthesis of cholesterol from dihydrosterols as well as responsible for inhibiting formation of ergosterol from lanosterol in fungi. Thus dihydrosterols are not formed and ultimately reduces biosynthesis of cholesterol.
- Ketoconazole can also be helpful in decreasing body weight.
- Ketoconazole when used in combination with cholestyramine which is one of the bile acid sequesterant agent no significant difference was found in anti-hyperlipidemic activity.

FUTURE ASPECTS

- Toxicity studies for ketoconazole can be done to check the extent of damage to body organs on prolong use.
- Clinical trials can be done to check the anti-hyperlipidemic activity of ketoconazole and to note the improvement in the lipid profile when exposed to humans.

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