ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antihyperlipidemic and Antiatherosclerotic Activity of Rimonabant in Wistar albino Rats.

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ABSTRACT

The present study was carried out to investigate antihyperlipidemic and antiatherosclerotic effect of rimonabant against hyperlipidemia induced by high fat diet in wistar albino rats. Wistar albino rats were divided in 6 groups of 6 rats in each. They were treated as follows. Group I received only high fat diet containing 4% cholesterol, 10% coconut oil, 1% cholic acid for 30 days. Group II received a normal diet for 30 days. Group III received high fat diet supplemented with rimonabant 2.5mg/kg for 30 days. Group IV received a high fat diet supplemented with rimonabant 5mg/kg for 30 days. Group V received a high fat diet supplemented with rimonabant 10mg/kg for 30 days. Group VI received a high fat diet supplemented with simvastatin 4mg/kg for 30 days. The activity was assayed by the measuring serum cholesterol, triglyceride, low density lipoprotein, very low density lipoprotein, atherogenic index, body weight, food intake, cholesterol excretion and serum high density lipoprotein. A decrease serum cholesterol, triglyceride, low density lipoprotein, very low density lipoprotein, atherogenic index, body weight, food intake and an increase cholesterol excretion and serum high density lipoprotein content was observed in the group III, IV, V and VI rats as compared to the group I rats. Thus it could be concluded that rimonabant posse's potent antihyperlipidemic and antiatherosclerotic activity.

Keywords:- Rimonabant, Lipoprotein, Hyperlipidemic diet, Obesity.

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ISSN: 0975-8585

INTRODUCTION

Coronary artery disease (CAD) is one of the most important causes of death all over world. Hyperlipidemia is one of the risk factors for CAD. Data show that 25–30% risk of CAD is reduced by treating hyperlipidemia [1]. The remedies which are available today either less potent or having less satisfactory results. Literature survey shows rimonabant have potent antiobesity activity [2], helps in cessation of smoking [3] and possesses antilipogenesis property [4].

Considering all above properties of rimonabant we have designed the study for evaluation of antihyperlipidemic and antiatherosclerotic activity of rimonabant, which is not scientifically proved yet.

MATERIAL AND METHODS

Drug and Chemicals:

Cholesterol, cholic acids (sigma chemicals, USA), Rimonabant (Zydus health care, Ahmadabad, India), Cholesterol, triglyceride, HDL-cholesterol estimating kits (RFCL Pvt. Ltd, Gudgeon, India), Citric acid, Sodium citrate, Dextrose, Adenosine di phosphate (ADP), Heparin (S.D. Fine chemicals, India).

Animals:

Adults Wistar albino rats of both sexes, eight weeks old, weighing 150-200 g were used in present investigation. The animals were maintained in propylene cages in the departmental Animal House Facility with 12 hrs light and dark cycle. Temperature was maintained at 25±3^{0}C. Feeding schedule consisted of rat pellet diet and water ad libitum. Daily intake of food was quantitated precisely. Prior to initiation of experiments, the entire experimental protocol was submitted to the Institutional Animal Ethical Committee, reviewed and the approval obtained as per CPCSEA guidelines (Registration No.651/02/C/CPCSEA).

Antihyperlipidemic activity:

Hyperlipidemic diet model:

The rats were divided into the following 6 groups each consist of 6 animals. The hyperlipidemia was induced by feeding hyperlipidemic diet (4% cholesterol, 1% cholic acid, 10% coconut oil) for 30 days [5].

Group I: Received 0.5% CMC with hyperlipidemic diet for 30 days
Group II: Received only 0.5% CMC for 30 days
Group III: Treated orally with rimonabant 2.5mg/kg/day along with hyperlipidemic diet for a period of 30 days.

Group IV: Treated orally with rimonabant 5mg/kg/day along with hyperlipidemic diet for a period of 30 days.
Group V: Treated orally with rimonabant 10mg/kg/day along with hyperlipidemic diet for a period of 30 days.
Group VI: Treated orally with simvastatin 4mg/kg/day along with hyperlipidemic diet for a period of 30 days.

All animals had free access to diet and water. The daily diet consumed by animals was calculated by subtracting the leftover diet the next day from the previous day's added diet. The body weight of each animal was recorded every day.

Collection of blood samples and biochemical analysis from serum

At the end of the experiments on the 30th, blood samples was collected 4 h after the last dose of administration using light ether anasthesia. Blood samples were collected separately from retro orbital sinus puncture into sterilized dry centrifugation tubes. Samples were allowed to stand for 30 min at 37˚C. The clear serum was separated at 2500 rpm for 10 min using centrifuge. The biochemical investigation was carried out to assess total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, very low density lipoprotein [6] and atherogenic index [7].

Determination of Cholesterol in Feces

During the last 3 days of the experiment, the rats were transferred to metabolism cages. Feces were collected, separated from the adhering hair and diet residue, and stored. Then dried at 60^{0}C for 12 h, and pulverized with a mill. Resultant powdered fecal matter was extracted with chloroform: methanol (2:1).This extract was than analyzed for cholesterol content in similar manner of the serum [5].

Histopathology

After the decapitation of the animals, the aorta were removed and fixed in 10% neutral-buffered formaldehyde solution. Fixed tissues were embedded in paraffin, cut into sections and placed on microscope slides. Slides were stained with hematoxylin and eosin for the histomorphological examination which was performed under light microscopy [8].

In vitro anti atherosclerosis activity:

Platelet anti-aggregation activity:

Platelet rich plasma (PRP) was prepared by centrifugation (1000 rpm for 5 min) of blood collected from normal aspirin free blood donors. 1.5 ml of acid citrate dextrose was used as anticoagulant for every 8.5 ml of blood. PRP was taken into siliconized glass cuvettes. Platelet poor plasma (PPP) collected by centrifugation (3000 rpm for 5 min) was kept as reference. The

cuvettes were incubated at 37 °C for 5 min. The aggregation was initiated by adding 20 µl of ADP (10 µM) to 1ml of PRP. The aggregation was recorded for 5 min at 600 nm. The effect of different concentrations (50–250 µg) of PPE was studied by incubation with PRP at 37 °C for 5 min before the addition of ADP [9]. Commercial heparin (20µg/ml) was used as reference standard. The maximal aggregation was recorded. The aggregation is expressed as % inhibition (*X*) calculated by using the following equation [10]:

$$X\ (\%) = (A-B)/A \times 100$$

Where, A = maximal aggregation of the control,
B = maximal aggregation of drug-treated PRP.

Anti-inflammatory activity:

Test solution (1ml) containing different concentration (50 - 250 µg/ml) of drug was mixed with 1ml of egg albumin solution (1mM) and incubated at 27 ± 1° C for 15 min. denaturation was induced by keeping the reaction mixture at 70 ° C in a water bath for 10 min. after cooling the turbidity was measured spectrophotometrically at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and taken the average [11].

Statistical Analysis

Results are presented as mean ±S.E.M. The data were tested by one-way ANOVA, followed by Dunnett's multiple comparison post test to identify significant difference. All analyses were performed using Graph Pad Prism statistical software. A level of $p < 0.05$ was considered significant.

RESULTS

Administration of rimonabant at 2.5 mg/kg/p.o. to hyperlipidemia induced animals resulted in a decreased of total cholesterol (19%), triglyceride (17.13%), LDL-cholesterol (22.7%), VLDL-cholesterol (16.79%) With 5 mg/kg/p.o. of rimonabant treatment, a further reduction in total cholesterol (23.8%), triglyceride (35.34%), LDL-cholesterol (25.57%), VLDL-cholesterol (35.44%) and atherogenic index (41.55%). With 10 mg/kg/p.o. of rimonabant treatment, a further reduction in total cholesterol (42.2%), triglyceride (46.37%), LDL-cholesterol (56.75%), VLDL-cholesterol (45.82%), atherogenic index (73.43%) and fecal cholesterol excretion (10.9%) and an increased in HDL-cholesterol was dose dependant and significant (Table 1). There was also significant reduction in body weight of rats those received different dose of rimonabant along with hyperlipidemic diet, (36% at 2.5 mg/kg/p.o., 28.57 at 5 mg/kg/p.o., 44.18% at 10 mg/kg/p.o.) (Table-2). There was also significant reduction in food intake of the hyperlipidemia induced animals, (45.83% at 2.5 mg/kg/p.o., 54.16% at 5 mg/kg/p.o., 64.16% at 10 mg/kg/p.o.) (Table-3).

ISSN: 0975-8585

Table 1: Effect of rimonabant on various biochemical parameters in hyperlipidemic rats

Group / Parameter	HFD	Control	HFD+ rimonabant 2.5 mg/kg	HFD+ rimonabant 5 mg/kg	HFD+ rimonabant 10 mg/kg	HFD+ simvastatin 4 mg/kg
Cholesterol level	250±6.6	88.5±4.5	202.5±12**	190.5±7.9**	144±6.4**	111±5.5**
Triglyceride level	258.43±5.2	125.07±6.9	214.16±11**	166.81±6.9**	138.66±9.4**	128.09±5.8**
HDL level	26.31±4	42±2.8	29±3.3	30.46±2.1	42.61±3**	58±2.6**
LDL level	171±5.7	21.35±1.2	132.17±4.3*	127.27±3.2**	74±8.2**	27.56±5.6**
VLDL level	51.68±1	25±1.3	43±2.1*	33.36±1.3**	28±1.9**	25.28±1.7**
Atherogenic Index	9.41±0.84	1.13±0.12	8.4±0.5	5.5±0.4**	2.5±0.37**	0.95±0.016**
Fecal cholesterol excretion	22±1.2	19±1.6	23±1.3	24±1.5	24.4±0.9*	25±1.9*

Value represents, Mean ± S.E.M. (n=6) ANOVA: Dunnett's Multiple Comparative test *p< 0.05, **p<0.01, as compared with HFD group.

Table 2: Effect of Rimonabant on body weight

Group	Initial	5th day	10th day	15th day	20th day	25th day	30th day
HFD	165±2.9	175±14	180±16	180±15	200±14	210±11	230±18
Control	166±4.3	167±2.5	160±0.49	160±6.4	160±6.7	170±8.2	160±6.9
Rimonabant 2.5 mg/kg	171±1.3	163±1.5	150±6.4*	140±11*	130±13**	130±14**	110±11**
Rimonabant 5 mg/kg	168±1.1	161±2.2	150±4.1	150±4.5*	140±4.9**	140±4.3**	120±3.5**
Rimonabant 10 mg/kg	172±1.8	158±1.9	140±6.7**	120±6.5**	120±5.0**	120±6.2**	96±3.2**
Simvastatin 4 mg/kg	173±2.1	157±5.8	150±4.0*	140±3.7*	130±3.6**	130±4.0**	120±4.7**

The above data clearly shows that significant weight increased in HFD treated group compared with control and significant weight reduction in rimonabant treated group compared with HFD treated group.
Value represents, Mean ± S.E.M. (n=6) ANOVA: Dunnett's Multiple Comparative test *p< 0.05, **p<0.01, as compared with control.

Table 3: Effect of Rimonabant on food intake in high fat diet treated rats

Group	5th days	10th days	15th days	20th days	25th days	30th days
HFD	22±2.2	27±1.67	28±2.4	26±0.92	29±2.1	24±1.21
Control	20±2.2	25±23.3	26±1.8	24±0.86	27±2.4	22±0.97
Rimonabant 2.5 mg/kg	11±0.71**	10±0.91**	8.6±0.40**	11±1.0**	11±1.2**	13±0.97**
Rimonabant 5 mg/kg	9±1.91**	8±0.87**	6.6±0.91**	9.4±1.6**	8.8±1.7**	11±0.54**
Rimonabant 10 mg/kg	7±1.54**	6±0.71**	4.6±0.54**	7.4±1.5**	6.8±0.65**	8.6±0.84**
Simvastatin 4 mg/kg	21±1.2	22±0.87	21±1.4	23±2.8	21±2.6	22±2.14

The above data clearly shows that significant decreased in food intake in rimonabant treated group compared with HFD treated group.
Value represents, Mean ± S.E.M. (n=6) ANOVA: Dunnett's Multiple Comparative test *p< 0.05, **p<0.01, as compared with control.

ISSN: 0975-8585

Platelet anti-aggregation activity:

The rimonabant (50–150 µg/ml) interestingly inhibited platelet aggregation. Greater inhibition of aggregation was noticed with increased inhibition of platelets aggregation (Table 4).

Table 4: Effect of rimonabant on in-vitro Platelet anti-aggregation activity using ADP

Groups	Inhibition of platelet aggregation (%)
Control	-
Rimonabant	
50 µg/ml	12.04±0.26
100µg/ml	20.23±0.67
150µg/ml	22.84±6.0
200µg/ml	32.05±4.2
250µg/ml	33.82±2.8
Heparin (20 µg/ml)	72.12±1.3

Inhibition of protein denaturation:

In *in vitro* protein denaturation assay rimonabant inhibited protein denaturation in concentration dependent manner (Table 5).

Table 5: Effect of Rimonabant on *in vitro* protein denaturation assays

Drug Rimonabant(µg/ml)	Inhibition of protein denaturation (%)
50	34.67±0.79
100	39.54±0.84
150	46.34±0.89
200	67.83±0.45
250	82.76±0.39

Histopathological Examination

No histological alterations in rat aorta were established in any of the six experimental groups (Figure 1).

ISSN: 0975-8585

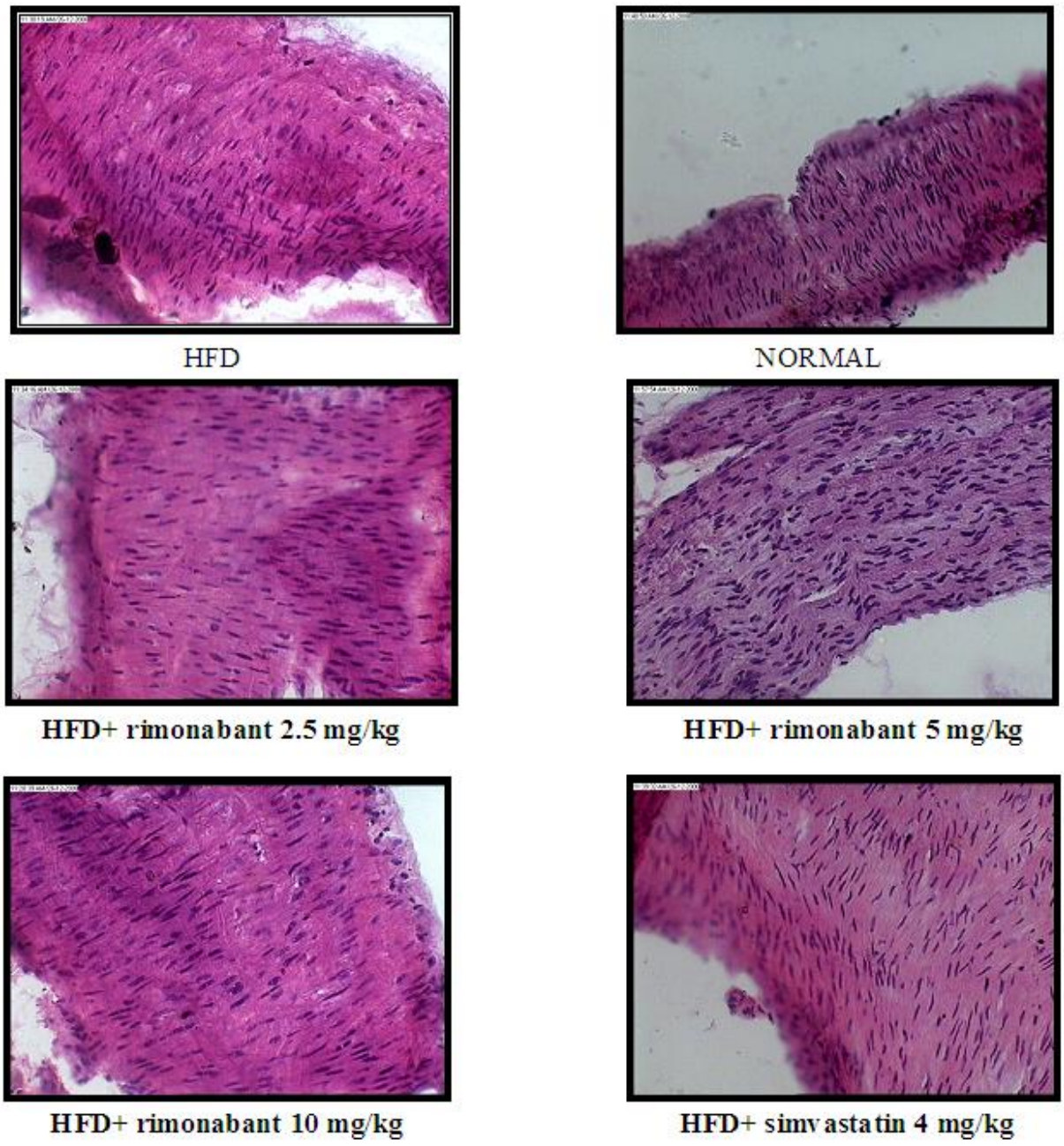

FIGURE 1: Histopathology of Aorta under light microscope

DISCUSSION

This study was done with the aim of evaluating antihyperlipidemic and anti atherosclerotic activity of rimonabant. We know that nutrition plays an important role in the etiology of hyperlipidemia and atherosclerosis. Several animal and human studies have confirmed the hyperlipidemic properties of saturated fatty acids and cholesterol which include

increasing total cholesterol and altering lipoprotein pattern and whose mechanisms remain under study.

Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess hypercholesterolemia related metabolic disturbances in different animal models. However, it is assumed that a high level of saturated fat in addition to cholesterol is required in the rat model. Increased cholesterol concentrations in plasma are, however, a cause of coronary atherosclerosis and increase risk of CAD [12]. High fat administration increases the biosynthesis of phospholipids possibly by a decrease in phospholipase activity or increased phospholipid turnover due to an onset of inflammatory process [13].

In this study, hyperlipidemia was induced in rats by adding cholesterol (4%), cholic acid (1%) and coconut oil (10%) to the diet for 30 days. Furthermore, body weights were significantly enhanced by the intake of a hyperlipidemic diet as compared to control rats, and it was accompanied by decrease body weights significantly those treated with rimonabant.

Determinations of the lipid profile in serum from rats fed a hyperlipidemic diet revealed higher levels of serum cholesterol and triglyceride, LDL-cholesterol, VLDL-cholesterol, Atherogenic index as compared to controls and marked decrease HDL-cholesterol in rats fed a hyperlipidemic diet as compared to control. But rimonabant treated groups with hyperlipidemic diet shows significant as well as dose dependant decreased serum cholesterol, triglyceride, LDL-cholesterol, VLDL-cholesterol and Atherogenic index as compared to hyperlipidemic diet and marked increased HDL-cholesterol as compared to hyperlipidemic diet [14].

Our results indicate that rimonabant is a potential therapeutic agent for hyperlipidemia because of its reduction of LDL-cholesterol levels. Experimental animals which consumed high dietary levels of cholesterol developed elevated LDL cholesterol levels and atherosclerosis (Yokozawa *et al*., 2003). This study also suggest that this effect is related to the change of lipid metabolism during the digestive process, in such a way as to prevent the accumulation of lipids inside the body, but allows them to be excreted through feces [15].

The antiplatelet therapy constitutes one of the best available tools for ameliorating the mechanisms related to atherogenesis and rimonabant interestingly inhibited platelet aggregation.

The risk of coronary heart disease (CHD) can also be lowered by treatment reducing the plasma cholesterol concentrations. Recent published studies have added to the evidence for a prethrombotic state in hyperlipidemia [16]. The consequence of plaque disruption in a coronary artery will depend partly upon the magnitude of the thrombotic response to this event. This is the rational for the antiplatelet and anticoagulant therapy in patients with CHD. Lipid lowering therapy may also be beneficial in this respect by reversing changes in the clotting pathway, fibrinolytic system and in blood platelets from hyperlipidemic patients [10]. Rimonabant is selective cannabinoid 1 (CB1) receptor antagonist. The newly discovered cannabinoid-1 (CB1)

receptor might be a potential therapeutic target for inducing weight loss as well as improving carbohydrate and lipid metabolism.

Atherosclerosis is an inflammatory disease. In inflammation protein denaturation occurs. Denaturation affects nearly all physico-chemical properties of protein molecules. To approach an understanding of the behavior of anti-inflammatory agents, several kinds of denaturing conditions should be imposed and several different parameters measured. In the present study we examined the influence of anti-inflammatory drugs on the denaturation of egg albumin induced by heat [17]. Rimonabant prevent the denaturation of protein in this study and shows the dose dependant prevention.

When activated by endocannabinoids, the CB1 receptor promotes lipogenesis in fat cells and inhibits the production of adiponectin, an adipose-derived cytokine that may have antidiabetic and antiatherosclerotic properties [4] and on the basis of that hypothesis we investigate its antihyperlipidemic and anti atherosclerotic effect. The first potent and selective CB1 receptor antagonist, Rimonabant was described in 1994 [18].

In conclusion, the rimonabant possesses antihyperlipidemic and antiatherosclerotic properties in rat. These properties show that Rimonabant is most useful in patients suffering from hyperlipidemia and atherosclerosis as well.

ACKNOWLEDGEMENT

The authors are grateful to Zydus health care, Ahmadabad, India for providing gift samples of Rimonabant.

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