

## **Antihyperlipidemic Activity of Rimonabant on High Cholesterol Diet Induced Hyperlipidemia in Rats**

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### **Summary**

Hyperlipidemia is defined as increase in the lipid content in blood. The present study was conducted to evaluate the antihyperlipidemic activity of rimonabant on high cholesterol diet induced hyperlipidemia in rats. Hyperlipidemia was induced by giving high cholesterol diet (2% cholesterol, 1% sodium cholate and 2% coconut oil) for thirty days in standard rat chow diet. Rats on high cholesterol diet showed significant increase ( $p < 0.05$ ) in serum cholesterol, triglyceride LDL-C, VLDL-C, atherogenic index and decrease HDL-C levels. Pretreatment of rats with the rimonabant at doses of 2.5, 5 and 10 mg/kg showed significant decrease ( $p < 0.05$ ) in serum and tissue serum and tissue cholesterol, triglyceride, LDL-C, VLDL-C, atherogenic index and increase HDL-C levels. The activity of the rimonabant was comparable to the standard drug, simvastatin (4mg/kg, p.o.). Histological study showed that rimonabant caused decrease in aortic plaque as compared to high cholesterol diet fed rats. Thus the study demonstrates that rimonabant effective as an antihyperlipidemic agent.

**Key words:** Rimonabant, Hyperlipidemia, High cholesterol diet, Biochemical Parameters

### **Introduction**

Diseases of the cardiovascular system are the most common cause of death. Lifestyle changes have a significant impact on the health of the people. The modernization of societies appears to result in a dietary pattern that is high in saturated fats and refined sugars and is low in fibres content <sup>[1]</sup>. It is now established that hyperlipidemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications. Hyperlipidemia is a disorder characterized by the increase in blood lipoprotein or cholesterol levels.

Recent studies have shown that lipid associated disorders are not only attributed to the total serum cholesterol, but also to its distribution among different lipoproteins. The low density lipoproteins (LDL) are the major carriers of cholesterol towards tissues having atherogenic potential, while the high density lipoproteins (HDL) carry cholesterol from peripheral tissues to the liver. HDL thus gives protection against many cardiac problems and obesity. Although genetic factors recline behind these lipid disorders <sup>[2]</sup>.

Rimonabant appears to be a promising drug in an entirely new class called selective cannabinoid (CB1) receptor antagonists. The chemically described as N-peperidino-5(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide. Recent studies have demonstrated the beneficial effects of rimonabant in tackling obesity, smoking cessation and metabolic syndrome. The drug may be approved for treatment of obesity and smoking cessation <sup>[3]</sup>. Ongoing studies may provide information on its other clinical uses. In addition to weight loss, rimonabant is also reported to produce improvement in HbA<sub>1C</sub> levels and may be helpful in diabetes <sup>[4]</sup>.

The present study was hence designed to determine the antihyperlipidemic activity of rimonabant on high cholesterol diet induced hyperlipidemia in rats. In addition, we attempted to test and compare the possible action of rimonabant on high cholesterol diet induced hyperlipidemia in rats.

### **Materials and Method**

Three months old Wistar albino rats of either sex weighing 150- 250g were used for the study. The animals were procured from B.R. Nahata College of Pharmacy, Mandsaur. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2 °C and relative humidity of 30-70%. A 12/12 h light and dark cycle was followed. All animals were fed on standard balanced diet and provided with water ad libitum.

All the experimental procedures and protocols used in the study were reviewed and approved by the (IAEC) Institutional Animal Ethical Committee of Mandsaur Institute of Pharmacy, Mandsaur and were in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA). Registration No.MIP/IAEC/2010/005

**Toxicity Study** <sup>[5]</sup>: Doses of rimonabant were selected by using the references of various articles on rimonabant; further 7 days Anti-hyperlipidemic study was done using four different doses 5mg/kg, 10mg/kg and 20mg/kg of rimonabant. Among this dose 10mg/kg showed good anti-hyperlipidemic activity. So three different doses 2.5, 5, and 10 mg/kg of rimonabant were selected.

**High Cholesterol Diet (HCD) Induced Hyperlipidemia** <sup>[6]</sup>: Hyperlipidemia in rats was induced by administration of high cholesterol diet (2% cholesterol, 1% sodium cholate and 2% coconut oil) for thirty days in standard rat chow diet. Albino wistar rats of either sex (200-250g) were used. All the animals were divided into the six groups each group consists of 6 animals and they received the treatment as follows.

Group I: Normal diet for 30 days

Group II: High Cholesterol diet (4% Cholesterol +2% Cholic acid + 10% coconut oil) for 30 days

Group III: High Cholesterol diet + Standard drug (Simvastatin, 4mg/kg p.o.) for 30 days

Group IV: High Cholesterol diet + Rimonabant (2.5mg/kg p.o.) for 30 days

Group V: High Cholesterol diet + Rimonabant (5mg/kg p.o.) for 30 days

Group VI: High Cholesterol diet + Rimonabant (5mg/kg p.o.) for 30 days

**Biochemical estimation**: At the end of experimental period, rats were anaesthetized with ether. Blood samples were collected from orbital venous plexus in nonheparinized tubes, centrifuged at 3000 rpm for 20 minutes, and blood sera were then collected and stored at 4°C prior immediate determination of cholesterol, triglyceride, LDL-C, VLDL-C, HDL-C, atherogenic index. All of these parameters were measured using Automated Clinical Chemistry Analysis System, Dimension type RXL Max (Dade Behring Delaware, DE 19714, U.S.A.). Food intake and Weight gain in rats of each group were observed for 30 days. Samples of aorta were collected from the each group of animals for histopathology.

**Histopathological Examination**: For light microscopic examination, aorta from each groups were fixed with 10% buffered formalin, embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 4  $\mu$ m thickness and stained with haematoxylin and eosin.

**Statistical Analysis**: All the data expressed as mean  $\pm$  S.E.M and analyzed statistically using ANOVA followed by Dunnett test and compare with respective control group. A value was of  $P < 0.05$  was considered significant.

## Results

The food intake and weight was increased in high cholesterol fed diet rats as compared to normal control. Treatment with rimonabant showed significant decrease in food intake and weight gain as compared to high cholesterol fed diet rats (Table-1 and 2).

High cholesterol diet (HCD) fed rats produced significant increase ( $p < 0.05$ ) in serum cholesterol, triglyceride, VLDL-C, LDL-C, atherogenic index but significant decrease ( $p < 0.05$ ) in HDL-C level as compared to normal control rats (Table-2). Treatment with rimonabant showed significant reduction ( $p < 0.05$ ) in serum cholesterol, triglyceride, VLDL-C, LDL-C, atherogenic index but significant increase ( $p < 0.05$ ) in HDL-C as compared to high cholesterol diet fed rats (Table-3). Simvastatin significantly ( $P < 0.01$ ) reduced these levels near to normal. In the histopathological study high cholesterol diet fed rats exhibit atheromatous plaque as compared to normal control (Figures 1(a) and (b)). Treatment with simvastatin and rimonabant shows decrease in plaque size as compared to cholesterol control (Figures 1 (c), (d), (e) and (f)).

**Table 1:** Effects of rimonabant on food intake in High Cholesterol diet treated rats.

Groups	5 <sup>th</sup> days	10 <sup>th</sup> days	15 <sup>th</sup> days	20 <sup>th</sup> days	25 <sup>th</sup> days	30 <sup>th</sup> days
Normal	60±3.7	70±2.8	75±2.7	78±2.7	76±1.7	75±3.4
HCD	65±4.1	73±4.6	84±2.1	87±2.1	86±2.2	79±2.6
HCD+ Simvastatin(4mg/kg)	65±2.6	68±2.7	71±3.5*	74±3.2	69±3.9*	66±3.9*
HCD+ Rimonabant(2.5mg/kg)	56±2.8	53±2.8*	50±2.8*	47±2.8**	46±3.2**	43±3.5**
HCD+ Rimonabant(5mg/kg)	55±1.1	52±1.1**	48±2.1**	47±2.2**	43±2.0**	41±2.6**
HCD+ Rimonabant(10mg/kg)	50±1.1**	47±2.6**	45±2.3**	43±2.0**	39±1.65* *	37±2.4**

Value represents, mean ± S.E.M. (n=6)

ANOVA: Dunnett’s Multiple Comparative test \* $p < 0.05$ , \*\* $p < 0.01$  as Compared with HCD.

**Table 2:** Effects of rimonabant on body weight in high cholesterol diet treated rats.

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

Value represents, mean ± S.E.M. (n=6)

ANOVA: Dunnett's Multiple Comparative test \*p< 0.05, \*\*p<0.01 as Compared with HCD.

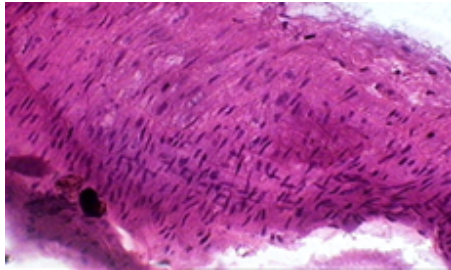
**Table 3:** Effects of rimonabant, simvastatin and HCD on different biochemical parameters.

Group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL Cholesterol (mg/dl)	VLDL Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	Atherogenic Index (mg/dl)
Control	88.5 ± 4.5	123.05 ± 6.4	21.09 ± 4.9	25.03 ± 1.3	42.15 ± 2.5	1.05 ± 0.12
HCD	250 ± 6.6	257.54 ± 5.2	170.68 ± 5.7*	51.55 ± 1.7	26.38 ± 4.0	9.41 ± 0.84
HCD+ Simvastatin(4mg/kg)	111 ± 5.5**	128.31 ± 3.7**	26.97 ± 6.5**	25.41 ± 0.7**	57.92 ± 2.6**	8.33 ± 0.51**
HCD+ Rimonabant(2.5mg/kg)	202.5 ± 9.1**	214.15 ± 11**	132.43 ± 12*	42.71 ± 2.1**	28.96 ± 3.3	5.54 ± 0.4
HCD+ Rimonabant(5mg/kg)	190 ± 6.4**	168 ± 6.4**	126.54 ± 11**	33.12 ± 1.3**	30.4 ± 2.1**	2.56 ± 0.37**
HCD+ Rimonabant(10mg/kg)	144 ± 5.2**	139.38 ± 9.4**	74.06 ± 8.2**	27.72 ± 1.9**	42.63 ± 3.0**	0.92 ± 0.34**

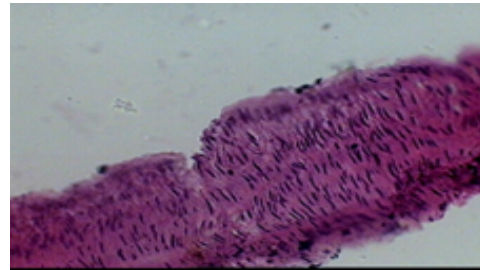
Value represents, mean ± S.E.M. (n=6)

ANOVA: Dunnett's Multiple Comparative test \*p< 0.05, \*\*p<0.01 as Compared with HCD.

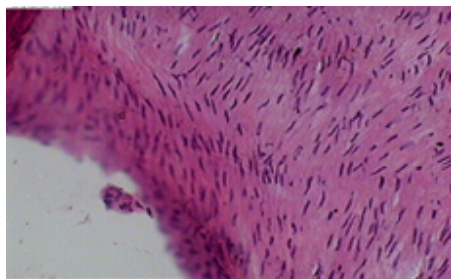
**Histology of Aorta:**



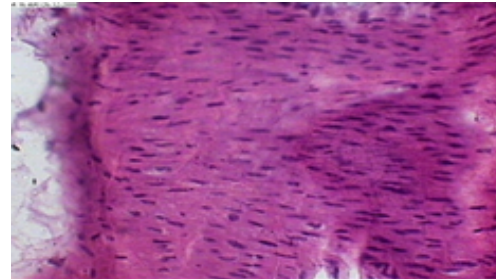
(a) High Cholesterol diet



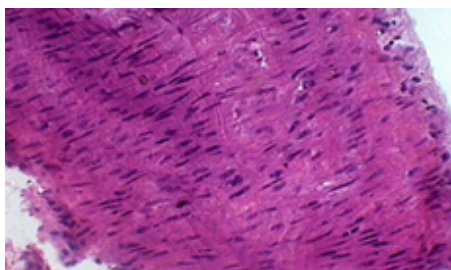
(b) Normal



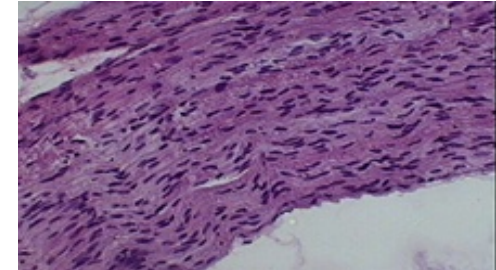
(c) HCD + Simvastatin (4mg/kg)



(d) HCD + Rimonabant (2.5mg/kg)



(e) HCD + Rimonabant (5mg/kg)



(f) HCD + Rimonabant (10mg/kg)

**Figure 1:** Aorta micrographs of high Cholesterol diet (a), normal (b), Simvastatin (c) and rimonabant (d, e, and f) treated rats. Original magnification X400.

**Discussion**

Hyperlipdemia has been documented as one of the causative factor for atherosclerosis, resulting in coronary heart diseases (CHD). Elevated cholesterol particularly LDL are the major reasons attributed to cardiovascular diseases. Accordingly to W.H.O by 2020, 60% of the cardiovascular causes will be of Indian origin<sup>[7]</sup>.

A high-fat diet causes cholesterol levels to increase in susceptible people, which leads to obesity. The weight gain in high cholesterol diet (HCD) group of rats was significantly higher than control rats reflecting the influence of high cholesterol diet [8]. Similarly, in present study there was significant weight gain in cholesterol control (HCD) as compared to normal control groups. Treatment with rimonabant significantly reduced the weight gain.

In the present study administration of high cholesterol diet to rats causes significant increases in tissue cholesterol, LDL-C, VLDL-C, triglyceride, atherogenic index and decrease HDL-C levels. Pretreatment of rats with the rimonabant at doses of 2.5, 5 and 10 mg/kg showed significant decrease ( $p < 0.05$ ) in serum and tissue serum and tissue cholesterol, LDL-C, VLDL-C, triglyceride, atherogenic index and increase HDL-C levels.

The decrease in serum triglyceride level is an important finding of this experiment. Recent studies show that triglycerides are independently related with coronary artery disease [9, 10]. Treatment with rimonabant shows significant decreased in triglyceride.

HDL is synthesized mainly in intestine and liver. HDL is considered to be a beneficial lipoprotein as it has an inhibitory effect in the pathogenesis of atherosclerosis. Low level of HDL is associated with high risk of coronary artery disease [11]. In the present study HDL-C level in both serum and tissue were significantly increased by rimonabant.

Atherogenic index indicates the deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronaries, aorta, liver and kidneys. The higher the atherogenic index, the higher is the risk of above organs for oxidative damage [12]. Treatment with rimonabant shows significant decreased in atherogenic index.

Histopathology of aorta of control groups administered with high cholesterol diet showed increases the plaque size in aorta, when compared to normal group (GI). Where as groups treated with rimonabant showed protective on aorta, these can be proofed by decreased the plaque size in aorta, more over with a slight change in morphology of aortic cells when compared to control group (G-II). Thus, histological architecture of rimonabant to prevent hyperlipidemia

### **Conclusion**

In conclusion, the present findings show that oral administration of rimonabant produces significant antihyperlipidemic effects in high cholesterol diet treated rats. Further investigations are required to explore exactly the mechanism action of rimonabant against high cholesterol diet physiological disturbances and histopathological changes. Finally, the present study identifies new areas of research for development of better therapeutic agents for heart, liver and other organs' dysfunctions and diseases.

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