Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 4, 2013

Research Article

EVALUATION OF ANTIHYPERLIPIDEMIC AND ANTIATHEROSCLEROTIC POTENTIAL OF RIMONABANT IN EXPERIMENTAL ANIMALS

[1]KIRAN NARKHEDE*, [2]ASHISH MAHAJAN, [2] SAVITA D. PATIL

[1]Department of Pharmacology, G. N. College of Pharmacy, Sawarde, Dist-Ratnagiri, [2]Department of Pharmacology, R. C. Patel Institute of Pharmaceutical Education & Research, Near Karwand Naka, Shirpur, Dist-Dhule, Maharashtra, India 425405.
Email: kpnarkhede@gmail.com

Received: 06 Oct 2013, Revised and Accepted: 29 Oct 2013

ABSTRACT

Objectives: The objective of present investigation was to evaluate antihyperlipidemic and antiatherosclerotic potential of Rimonabant in experimental animals.

Materials and Methods: Hyperlipidemia was induced by High Cholesterol Diet (HCD). Wistar albino rats were used for the study and were divided in 6 groups of 6 rats in each. They were treated with high fat diet containing 4% cholesterol, 1 mL coconut oil, 1% cholic acid for 30 days. The test (Rimonabant) and standard drugs were administered orally for the period of 30 days. The test drug administered at the dose levels of 2.5, 5 and 10 mg/kg and Simvastatin was used as standard drug at the dose level of 4mg/kg b.wt./day. The diet consumed by animals and body weight was recorded and evaluated on daily basis. The Aorta was isolated from all the animals and subjected to histopathological evaluation. Biochemical investigations were carried out to assess serum level of total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, very low density lipoprotein and atherogenic index. Determination of Cholesterol in Feces was also evaluated. In vitro anti atherosclerotic activity was evaluated by Platelet anti-aggregation method and protein denaturation evaluation.

Result: High fat diet showed significant increase in body weight, lipid profile in serum. The Rimonabant and Simvastatin treated groups showed significant increase in HDL levels and decreased total cholesterol, triglyceride, LDL, VLDL, HDL ratio and AI (atherogenic index) significantly.

Conclusion: It could be concluded that Rimonabant posses significant antihyperlipidemic and antiatherosclerotic potential.

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

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 increasing morbidity and mortality from coronary heart disease is the biggest challenge to nutrionists and medical scientists all over the world.[2]

Hyperlipidemia, hypertension, obesity, raised coagulation factor and homocysteine are modified risk factors for atherosclerosis. Dyslipidemia is most common risk factor causes IHD in elderly population. The underlying mechanism of IHD involves the deposition of serum lipids in coronary arteries, and its resulting in decreased blood flow to cardiac muscles.[3] Literature survey has shown that Rimonabant have potent antiobesity activity[4], helps in cessation of smoking[5] and possesses anti lipogenesis property[6].

Considering all above properties of Rimonabant we have designed the model for evaluation of antihyperlipidemic and antiatherosclerotic activity, 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

Healthy Adult Wistar albino rats of both sexes, eight weeks old, weighing 150-200g were used in present investigation. The animals were maintained in groups in large specious propylene cages in the departmental Animal House Facility with 12 hrs light and dark cycles. 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 high fat diet (4% cholesterol, 1% cholic acid, 1mL coconut oil) for 30 days[7].

Group I: rats received 0.5% c.m.c with high fat diet for 30 days

Group II: rats received only 0.5% c.m.c. For 30 days

Group III: rats were treated orally with Rimonabant 2.5mg/kg b.wt./day along with high fat diet for a period of 30 days.

Group IV: rats were treated orally with Rimonabant 5mg/kg b.wt./day along with high fat diet for a period of 30 days.

Group V: rats were treated orally with Rimonabant 10mg/kg b.wt./day along with high fat diet for a period of 30 days.

Group VI: rats were treated orally with simvastatin 4mg/kg b.wt./day along with high fat 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 30thday, blood samples were 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 Remi centrifuge. The biochemical investigation was carried out to assess total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, very low density lipoprotein[8] and atherogenic index[9].

Determination of Cholesterol in Feces

During the last 3 days of the experiment, the rats were transferred to metabolic cages. Feces were collected, separated from the adhering hair and diet residue and stored[10] then dried at 60 ˚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[7].

Histopathological study

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.

***In vitro* anti atherosclerotic activity**

Platelet anti-aggregation activity:[11,12]

Platelet rich plasma (PRP) was prepared by centrifugation (1000 rpm for 5 min) of blood collected from normal aspirin free blood bank 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 ℃ 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 ℃ for 5 min before the addition of ADP. 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:

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

Where A= maximal aggregation of the control,

And B = maximal aggregation of drug-treated PRP.

Anti-inflammatory activity[13]

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 ℃ for 15 min. denaturation was induced by keeping the reaction mixture at 70 ℃ 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.

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

Body weight analysis and Serum lipid profile

HFD showed increased serum lipid profile, increased body weight and increased atherogenic index. Administration of Rimonabant at 2.5 mg/kg, p.o. dose to hyperlipidemia induced Wistar albino rats resulted in a decreased of total cholesterol (19% [p<0.01]), triglyceride (17.13% [p<0.01]), LDL-cholesterol (22.7% [p<0.05]), VLDL-cholesterol (16.79% [p<0.05]) (Table 1). With 5 mg/kg/ p.o. dose of Rimonabant a further reduction in total cholesterol (23.8% [p<0.01]), triglyceride (35.34% [p<0.01]), LDL-cholesterol (25.57% [p<0.01]), VLDL-cholesterol (35.44% [p<0.01]) and atherogenic index (41.55% [p<0.01]) was observed as shown in (Table 1). With 10 mg/kg/ p.o. dose of Rimonabant a further reduction in total cholesterol (42.2% [p<0.01]), triglyceride (46.37% [p<0.01]), LDL-cholesterol (56.75% [p<0.01]), VLDL-cholesterol (45.82% [p<0.01]), atherogenic index (73.43% [p<0.01]) and an increased in HDL-cholesterol (63% [p<0.01]) and increased fecal cholesterol excretion (10.9% [p<0.05]) was observed in dose dependant manner and significant as shown in (Table 1). There was also significant reduction in body weight of rats those received different doses of Rimonabant along with High Fat Diet, (36% [p<0.01] at 2.5 mg/kg/p.o. dose, 28.57% [p<0.01] at 5 mg/kg/p.o. dose 44.18%[p<0.01] and at 10 mg/kg/p.o. dose) (Table 2). There was also significant reduction in food intake of the hyperlipidemia induced animals, who received simultaneously administration of Rimonabant at a dose of (2.5 mg/kg/p.o., 5 mg/kg/p.o., 10 mg/kg/p.o), 45.83% [p<0.01], 54.16%[p<0.01], 64.16%[p<0.01] resp.) (Table 3) Significant increase in HDL level was found in 2.5, 5 and 10 mg/kg Rimonabant treated group. Significant reduction of calculated VLDL, AI found in both standard and Rimonabant treatment groups

Platelet anti-aggregation activity

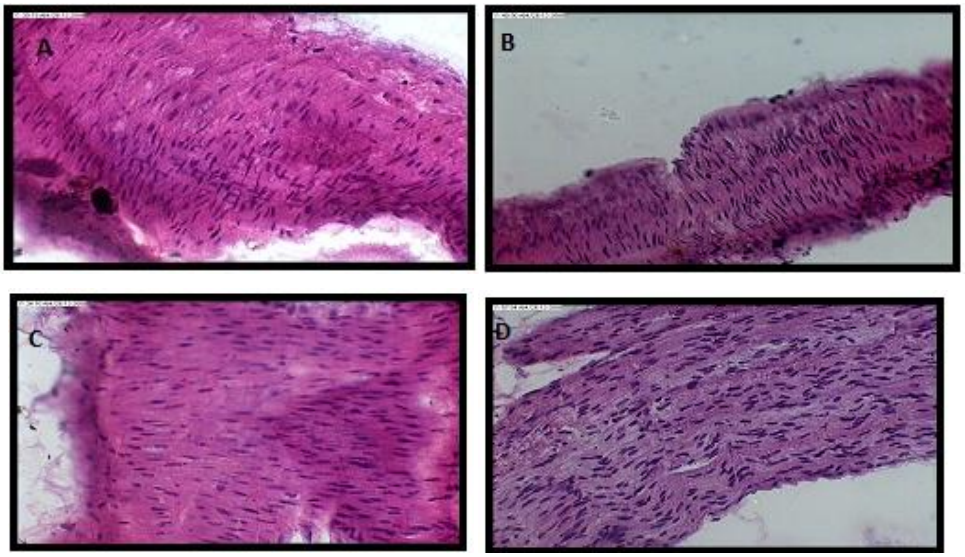
The Rimonabant (50–150 µg/ml) interestingly inhibited platelet aggregation. Greater inhibition of aggregation was noticed with increased inhibition of platelets aggregation shown in Table 4.

Inhibition of protein denaturation

The Rimonabant also inhibited protein denaturation shown in Table 5.

Histopathological Examination

No histological alterations in rat aorta were established in any of the six experimental groups



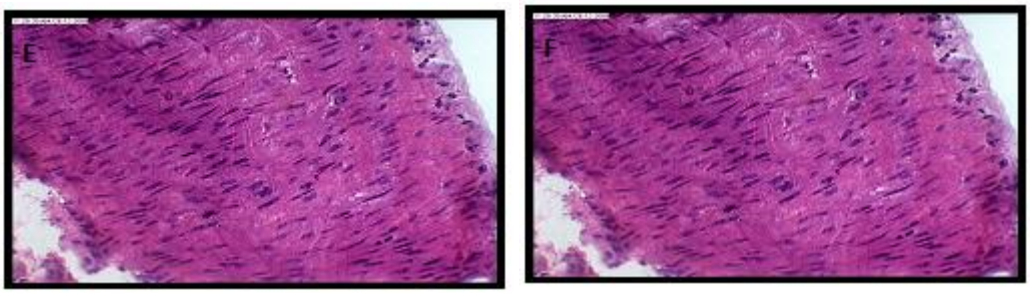


Fig. A: High fat diet control (HE 40 X): No increase in thickness of aortic valve leaflet in rats fed with high fat diet compared with normal group

Fig. B: Normal (HE 40 X): cross section of normal rat aorta shows normal thickness. Normal architecture of aorta

Fig. C: High fat diet + Rimonabant 2.5 mg/kg (HE 40 X): cross section of rat aorta of Rimonabant 2.5 mg/kg treated group shows no degeneration in the inner layer of aortic tissue

Fig. D: High fat diet + Rimonabant 5 mg/kg (HE 40 X): cross section of rat aorta of Rimonabant 5 mg/kg treated group shows no degeneration in the inner layer of aortic tissue

Fig. E: High fat diet + Rimonabant 10 mg/kg (HE 40 X): cross section of rat aorta of Rimonabant 10 mg/kg treated group with no change in cellular architecture

Fig. F: High fat diet + Simvastatin 4 mg/kg (HE 40 X): Showing normal cellular architecture

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

Groups	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-Cholesterol Level	26.31±4	42±2.8	29±3.3	30.46±2.1	42.61±3**	58±2.6**
LDL-Cholesterol Level	171±5.7	21.35±1.2	132.17±4.3*	127.27±3.2**	74±8.2**	27.56±5.6**
VLDL-Cholesterol 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 control.

Rimonabant treated groups with high fat diet shows significant as well as dose dependant decrease in serum cholesterol, triglyceride, LDL-C, VLDL-C and Atherogenic index and increased HDL- cholesterol.

Table 2: Effect of Rimonabant on body weight

Groups	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**

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

(Significant weight increase in HFD treated group compared with control and significant weight reduction in Rimonabant treated group compared with HFD treated group)

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

Groups	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

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

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

Table 5: Effect of Rimonabant on *in vitro* 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

DISCUSSION

Assessment of hypercholesterolemia-related metabolic disturbances in different animal models is possible due to cholesterol feeding which leads to elevated serum or tissue cholesterol levels. However, it is assumed that a high level of saturated fat in addition to cholesterol is required to induce hyperlipidemia in the rat model. Increased cholesterol concentration in serum is a cause of coronary atherosclerosis which ultimately increases the risk of CAD.[14] High fat administration increases the biosynthesis of phospholipids possibly either by decrease in phospholipase activity or increased phospholipid turnover due to onset of inflammatory process.[15]In the present study, hyperlipidemia was induced in rats by adding cholesterol (4%), cholic acid (1%) and coconut oil (1 ml) to the diet for 30 days.

Estimation of the serum lipid profile in rats receiving high fat diet revealed rise in the levels of serum cholesterol and triglyceride, LDL-C, VLDL-C, Atherogenic index ($p<0.01$) as compared to control, and decrease in HDL-C ($p<0.05$). Whereas Rimonabant treated groups with high fat diet shows significant as well as dose dependant decrease in serum cholesterol, triglyceride, LDL-C, VLDL-C and Atherogenic index and increased HDL- cholesterol. With increase in atherogenic index risk of coronary artery disease increases[16]

Our results indicate that Rimonabant may be effective as a therapeutic agent for hyperlipidemia due to reduction in LDL-cholesterol levels. Experimental animals which consumed high dietary levels of cholesterol developed elevated LDL-C cholesterol levels and atherosclerosis[14].

Elevated body weight due to intake of a high fat diet was found to be significantly decreased in the rats receiving Rimonabant along with high fat diet due to anorexic effect of Rimonabant which decreases food intake[17].

As Cannabinoid (CB1) receptor acts neuronally by reducing GI motility[18] and Rimonabant is cannabinoid receptor 1 antagonist[17] may due to this reason Rimonabant increases GI motility and increases faecal cholesterol excretion as well. Furthermore, there was also an increase in the cholesterol content of the fecal matter indicating that the Rimonabant promoted the excretion of cholesterol ($p<0.05$).

No histopathological changes in the aorta were established in present study. May be the 30 days feeding of rats with the 4% cholesterol, 1% cholic acid and 1mL coconut oil was not enough to induce morphological alterations.

Recent published studies have added to the evidence for a prethrombotic state in hyperlipidemia. 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 coronary artery disease. 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 [12]. Rimonabant inhibits platelet activation and reduces pro-inflammatory chemokines and leukocytes in Zucker rats [19].

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

Atherosclerosis is an inflammatory disease[20]. In inflammation, protein denaturation occurs[13] 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 are measured. In the present study we examined the influence of anti-inflammatory drugs on the denaturation of egg albumin induced by heat[21]. Rimonabant prevent the denaturation of protein in the present study and shows the dose dependant effect.

CONCLUSION

In conclusion, the findings in this study suggest that the Rimonabant possesses antihyperlipidemic and antiatherosclerotic Potential against high fat diet induced hyperlipidemia in experimental animals. These properties indicate its usefulness in obese patients with hyperlipidemia and or atherosclerosis.

ACKNOWLEDGEMENT

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

REFERENCES

1. Tajuddin, M. Nasiruddin, N. Ahmad. Effect of an Unani formulation on lipid profile in rat. Indian J Pharmacol 2006; 38:1:56-7.
2. Venu Pamidiboina, Rema Razdan, M.G.Hariprasad. Evaluation of the antihyperlipidemic, cardioprotective activity of a polyherbal formulation. Int J Pharmacy Pharm Sci. 2010; 2: Suppl 1:86-87.
3. Parasuraman S, Kumar EP, Anil Kumar, Emerson SF. Antihyperlipidemic effect of triglize, a polyherbal formulation. Int J Pharmacy Pharm Sci. 2010; 2:3: 118-119.
4. Zoë D,Thornton-Jones , Steven P, Vickers, Peter G, Clifton. The cannabinoid CB1 receptor antagonist SR141716A reduces appetitive and consummatory responses for food. Psychopharmacology 2005; 179:452-460.
5. John R. Hughes. Ethical concerns about non-active conditions in smoking cessation trials and methods to decrease such concerns. Drug and Alcohol Dependence 2009; 100: 187–193.
6. Mayer B. Davidson, Thomas A. Buchanan, Terrence F. Fagan, Mark Palangio. Metabolic Effects of Rimonabant in Overweight Patients with Dyslipidemia. Clinical insights in Diabetes 2005; 8:12: 1-4.
7. Hassarajani S, Chattopadhayay S, Souza T.D, Mengi S.A. Efficacy study of the bioactive fraction(F-3) of Acrous calamus in hyperlipidemia. Indian J Pharmacol 2007; 39:4:196-200.
8. William T. Friedewald, Robert I. Levy, Donald S. Fredrickson. Estimation of concentration of low density lipoprotein cholesterol in plasma, without the use of preparative ultracentrifuge. Clinical Chemistry 1972; 18:6: 499-502.
9. S. Muruganandan , K. Srinivasan , S. Gupta , P.K. Gupta , J. Lala. Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats. Journal of Ethnopharmacology 2005; 97:497-501.
10. D Subba Rao, n. Chandra Sekhara, M. N. Satyanarayana. Effect of Curcumin on Serum and Liver Cholesterol Levels in the Rat. Journal of Nutrition 1970; 100:1307-1316.
11. Chidambaram T. Kumarappan, T. Nageswara Rao, Subhash C. Mandal. Polyphenolic extract of Ichnocarpus frutescens modifies hyperlipidemia status in diabetic rats. Journal of Cell and Molecular Biology 2007; 6:2:175-187.
12. Mary N. K, B. H. Babu, J Padikkala. Antiatherogenic effect of Caps HT2, a herbal Ayurvedic medicine formulation. Phytomedicine 2003; 10: 474–482.

13. CT. Kumarappan, Rabish Chandra, Subhash C. Mandal. Anti-inflammatory activity of Ichnocarpus frutescens. Pharmacologyonline 2006; 3:201-216.
14. T. Yokozawa, A. Ishida, E. J. Cho, T. Nakagawa. The effects of Coptidis Rhizoma extract on a hypercholesterolemic animal model. Phytomedicine 2003; 10:17-22.
15. K. Vijaimohan , Mallika Jainu , K.E. Sabitha , S. Subramaniyam, C. Anandhan , C.S. Shyamala Devi. Beneficial effects of alpha linolenic acid rich flaxseed oil on growth performance and hepatic cholesterol metabolism in high fat diet fed rats. Life Sciences 2006; 79: 448–454.
16. Dobiasova, M, J. Frohlich. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FERHDL). Clinical Biochemistry 2001; 34: 583–588.
17. Wright S. M, C. dikkers, L. J. Aronne. Rimonabant: New Data and Emerging Experience. Current Atherosclerosis Reports 2008; 10: 71–78.
18. Sanger G.J. Endocannabinoids and the gastrointestinal tract: what are the key questions?British Journal of Pharmacology 2007; 152: 663–670.
19. Schafer A, Pfrang J. Neumuller J, Fiedler S, Ertl G, Bauersachs J. The cannabinoid receptor-1 antagonist rimonabant inhibits platelet activation and reduces pro-inflammatory chemokines and leukocytes in Zucker rats. Br J Pharmacol 2008; 154:5:1047-1054.
20. Ross R. Atherosclerosis an Inflammatory Disease. The New England Journal of Medicine 1999; 340:2: 115-126.
21. Grant N.H, Alburn H.E, Kryzanauskas C. Stabilization of serum albumin by anti-inflammatory drugs.Biochemical Pharmacology 1970; 19: 715-722.