IJP (2025), Vol. 12, Issue 6 **(Review Article)**

Received on 30 May 2025; received in revised form, 24 June 2025; accepted, 25 June 2025; published 30 June 2025

A BRIEF REVIEW ON SCREENING MODELS FOR HYPERLIPIDEMIA

Shivanjali S. Lakhapate [*1], Omkar A. Devade [2], Laxmikant M. Purane [2] and Vivekkumar K. Redasani [1]

Department of Pharmacology [1], Department of Pharmacology [2], YSPM's Yashoda Technical Campus Satara - 415011, Maharashtra, India.

Keywords:

Hyperlipidemia, Chemically induced model, Gene manipulated animal model, *In-vitro* screening model

Correspondence to Author:
Shivanjali S. Lakhapate

Research Scholar,
Department of Pharmacology,
YSPM's Yashoda Technical Campus
Satara - 415011, Maharashtra, India.

E-mail: shivanjalilmpharm@gmail.com

ABSTRACT: Hyperlipidemia is a significant contributor to the global burden of cardiovascular diseases, such as coronary heart disease and stroke. Characterized by elevated plasma lipids, including cholesterol, triglycerides, and lipoproteins, hyperlipidemia is a prevalent health issue with increasing incidence in both developed and developing countries. According to the World Health Organization (WHO), raised cholesterol levels are linked to 4.5% of global deaths and account for 29.7 million disability-adjusted life years (DALYs) annually. The Fredrickson classification system, endorsed by WHO, categorizes hyperlipidemia into six types based on lipoprotein abnormalities, providing a foundation for understanding its diverse etiologies and clinical implications. Experimental induction of hyperlipidemia is pivotal for studying its pathophysiology and testing potential therapeutic interventions. Various screening methods have been developed, including the use of pharmacological agents, dietary modifications, and genetic models. This review discusses the epidemiology, classification, and methodologies used to induce hyperlipidemia in experimental settings, highlighting their relevance in advancing our understanding and management of this critical public health challenge.

INTRODUCTION: One of the biggest risk factors for the occurrence and severity of coronary heart diseases (CHDs) has been identified as hyperlipidemia [1]. The leading causes of death include hyperlipidemia, atherosclerosis, stroke, and coronary heart disease [2]. Elevated serum levels of total cholesterol (TC), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) and decreasing levels of high density lipoprotein (HDL) are the hallmarks of hyperlipidemia. Among these, ischemic heart disease is intimately associated with hypercholesterolemia and hypertriglyceridemia [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.12(6).478-82

Article can be accessed online on: www.ijpjournal.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.IJP.12(6).478-82

Epidermiology: There are already over three million persons in the US and Europe who have been diagnosed with hyperlipidemia, and the number is rapidly increasing. Usually a chronic, progressive condition, hyperlipidemia necessitates dietary and lifestyle modifications as well as the possible need for additional lipid-lowering drugs. Patients with premature coronary artery disease (CAD), which is defined as CAD that develops in males before the age of 55 to 60 and in females before the age of 65, have the highest degree of hyperlipidemia.

The incidence of hyperlipidemia is approximately 75–85% under the previously mentioned conditions, compared to approximately 40–48% in the control group of similar age, but without early coronary artery disease [4]. According to estimates, more than half of American adults Over 50% of American individuals are estimated to have raised

LDL levels, and it is hypothesized that less than 35% of those patients effectively control their higher LDL levels, indicating a disease that is not receiving enough treatment."Prevalence of dyslipidemia was significantly greater among whites than blacks (women, 64.7% vs. 49.5%; and men, 78.4% vs. 56.7%; P<.001 for both) and amongst men than women (P≤.02 in every ethnic group) [5].

It seems sense that, in contrast to rates in Europe and the US, the prevalence of hyperlipidemia and the ensuing coronary artery disease would be lower in nations with lower overall rates of obesity and saturated fat consumption Underweight or obese children under the age of two may develop secondary (non-genetic) pediatrichyperlipidemia [5].

Screening Models for Hyperlipidemia: Screening models for hyperlipidemia are essential for understanding pathophysiological mechanisms and evaluating potential therapeutic interventions. There are various screening models to induce hyperlipidemia it include the following

***In-vivo* Screening Models for Hyperlipidemia:**

Chemically Induced Models for Hyperlipidemia:

D-Fructose Induced Hyperlipidemia: The human liver possesses the capability to rapidly absorb and metabolize fructose. Historically, individuals consumed approximately 16 to 20 grams of fructose daily, primarily derived from fresh fruits. However, the westernization of diets has led to a significant rise in the intake of added fructose, with current daily consumption levels ranging from 85 to 100 grams. When the liver is subjected to elevated fructose levels, it initiates rapid lipogenesis and triglyceride accumulation, which can result in diminished insulin sensitivity and the development of hepatic insulin resistance and glucose intolerance. The metabolism of fructose has garnered substantial scientific attention due to these adverse effects. Interestingly, even small amounts of fructose can yield positive outcomes, such as reducing the glycemic response to glucose intake and enhancing glucose tolerance, without causing any changes in insulin responses, non-esterified fatty acids, or total lipid levels [6].

Mechanism of Action: By boosting hepatic denovo lipogenesis, encouraging triglyceride synthesis, and decreasing fatty acid oxidation, D fructose causes hyperlipidemia. It contributes to the development of hyperlipidemia by raising plasma triglycerides, secreting VLDL, and lowering HDL cholesterol levels [7, 8].

Mechanism of Induction: According to research on animals, consuming beverages with 25% fructose for 21 days significantly increased hyperlipidemia Therefore, 25% fructose was fed to female Wistar rats for 21 days in drinking water to cause hyperlipidemia [9].

Polaxomer 407 Induced Hyperlipidemia: P-407 is a block copolymer made up of hydrophilic polyoxyethylene units on either side of a core hydrophobic component. It can interact with lipid metabolic pathways because of its amphiphilic nature. The delivery of P-407 results in a marked increase in plasma triglyceride levels, which is significantly greater than the rise in total plasma cholesterol. Plasma triglyceride levels are almost five times greater than plasma cholesterol levels following a single P-407 injection [10].

Mechanism of Induction: Rats were given 300 mg/kg of poloxamer-407 reagent dissolved in normal saline (0.9%), which caused hyperlipidemia. 48 hours before to the final sample treatment, the P-407 solution was injected. In order to facilitate the P-407's dissolution using the cold approach, the agent was combined with saline to create the injectable solution, which was then chilled for the whole night [11, 12, 13].

Triton X-100-Induced Hyperlipidemia: Triton X-100 has been commonly used to induce the acute hyperlipidemia by blocking the removal of triglyceride and cholesterol in various animal models particularly the rat models, for the screening of synthetic or natural antihyperlipidemic drugs [14, 15]. Rats given 100 mg/kg of Triton X-100 had higher serum lipid profile values. It is commonly known that HDL protects against cardiovascular conditions, especially coronary artery disease [16]. Likewise, elevated serum LDL levels are associated with a higher chance of developing atherosclerosis After 24 hours of IP injection of Triton X-100, the levels of HDL in all groups decreased, while TC, triglyceride, LDL, and VLDL levels significantly increased [17].

Mechanism of Induction: After 18 hours of starvation, rats received a single intraperitoneal (IP) injection of 100 mg/kg Triton X-100 prepared in normal saline to cause hyperlipidemia. Rats with serum TC levels greater than 130 mg/dL were deemed hyperlipidemic after 24 hours, when serum triglyceride and cholesterol levels were measured. Following a 24-hour Triton X-100 injection (when hyperlipidemia was produced), treatment was initiated orally for 21 days [18].

Diet Induced Model for Hyperlipidemia: A diet-induced model of hyperlipidemia is a common method to simulate human-like hyperlipidemic conditions in animals for research purposes. This model typically involves feeding animals a specific diet high in fats, cholesterol, and sometimes sugar to induce metabolic alterations resembling human hyperlipidemia. There are varuoustyape of diet used it include the following

Atherogenic Diet for Rat: Animals were fed two types of rodent diets, an atherogenic rodent diet (ARD) for induction of the model of hyperlipidemia and a commercially available standard rodent diet (SRD) (VERSELE-LAGA, Nature Rat) used as a control. An atherogenic rodent diet was prepared from 75.0 % of commercially available Teklad TD. 02,028 (Western purified atherogenic diet with added cholesterol and cholic source) to which 15.0 % of lard and 10.0 % of coconut oil were added.

The exact ingredients of ARD and SRD used for the induction of the model of hyperlipidemia include Casein 146.25 Crude protein 17.5 DL-methionine 2.25 Crude oils and fats 8.5 Sucrose 244.095 Crude fibers 8.0 Corn starch 112.5 Crude ash 5.0 Anhydrous milkfat 157.5 Additives (per kilogram) Cholesterol 9.375 Vitamin A (I.U) 11,750 Cholic acid 3.75 Vitamin D3 (I. U) 1450 Cellulose 37.5 Vitamin E (mg) 90.0 Mineral Mix, AIN-76 (170915) 26.25 Fe (mg) 112.0 Calcium carbonate 3.0 Mn (mg) 86.0 Vitamin Mix, Teklad (40060) [19].

High fat Diet Induced Models: Sprague-Dawley male rats with an average body weight of 160-180 g were made hyperlipidemic by giving high-fat diet (HFD contained Cholesterol (2%), Cholic acid (1%), Dalda (20%), and Coconut oil (6%) as major constituents. Hyperlipidemia was confirmed by measuring the levels of serum lipids and lipoproteins in the rats [20].

PTU Induced Hyperlipidemia: It doesn't take long to complete this process. Hyperlipidemia (PTU) is brought on by propylthiouracil. PTU is a drug for hyperthyroidism. The result is hypothyroidism. which is defined by increased triglycerides, LDL, VDL, and total cholesterol.

Procedure: Six hours prior to the measurement of total cholesterol, VLDL, and LDL, a significant quantity of cholesterol is given to each group. There must be 32 rats, split up into five groups. PTU is given to all groups for seven days at a dose of 10 mg/kg of rat body weight, with the exception of the control group. PTU is given at a dose of 0.01 percent for seven days. Lastly, measurements are made of the total cholesterol levels in the liver extract, feces, and serum [21].

Gene Manipulated Animal Model for Hyperlipidemia:

Knockout (KO) Laboratory Animals: have been developed recently to investigate hyperlipidemia (HL). ApoE-KO mice, when maintained on a standard diet, exhibited mild HL, with total cholesterol (TC) levels at or below 200 mg/dL. However, when subjected to a cholesterol-rich diet, these mice demonstrated a greater susceptibility to HL compared to their normal counterparts, with significant elevations in plasma triglycerides (TG) and cholesterol, showing a fivefold increase in TG and a sixfold increase in cholesterol relative to normal animals. The apoE gene can be deleted using one of two genome editing methods. ApoE serves as a ligand for both the LDL receptor and lipoprotein receptor-related proteins, playing a vital role in the liver's capacity to clear residual lipoproteins. A genetic deficiency in apoE leads to human type III hyperlipoproteinemia, and even under a standard chow diet, animals lacking the apoE gene exhibited HL [22, 23].

Transgenic Animals (TG) Model: Exhibit overexpression of human apolipoprotein E (apoE), C-III genes, and B-100 within hepatic tissue, leading to hyperlipidemic conditions in these TG models. Specifically, plasma total cholesterol (TC) and triglyceride (TG) levels in human apo B-100

transgenic rabbits were found to be three times higher than those in their normal counterparts. While the levels of high-density lipoprotein cholesterol (HDL-C) and TC remained unchanged, the plasma TG levels in apo C-III transgenic animals were threefold greater compared to normal animals. Lipoprotein analyses indicated that the elevated TG in apo C-III transgenic rabbits was distributed among very low-density lipoprotein (VLDL) and chylomicrons (CM). Additionally, there have been observations of transgenic animals expressing human apo E2, which is associated with type III hyperlipoproteinemia, as well as apo E3, the most common isoform in humans. The presence of elevated levels of human apo E3 (greater than 20 mg/dL) in these transgenic animals resulted in significant mixed hyperlipidemia, characterized by increased levels of VLDL and low-density lipoprotein (LDL) [24, 25].

***In-vitro* Screening Model for Hyperlipidemia:**

***In-vitro* Assay using Caco-2 Cell Lines:** The lipid profile produced by human intestinal epithelium-like cells derived from the colon cancer cell line Caco-2 is assessed through a specific methodology. Initially, Caco-2 cells are cultured in well plates for a duration of two days in a medium that includes 10% fetal bovine serum, along with penicillin and streptomycin. Following this, the cells undergo differentiation for four days upon exposure to sodium butyrate at concentrations ranging from 0 to 5 mM. After the incubation period, the Caco-2 cells transform into intestinal epithelium-like cells, exhibiting numerous microvilli on their apical surface when observed under an electron microscope. Subsequently, the culture medium is enriched with sodium oleate, prompting the differentiated Caco-2 cells to secrete a lipoprotein profile into the medium through a microporous membrane, resulting in the release of four distinct classes of fractions: chylomicrons, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) [26, 27, 28].

Nitric Oxide (NO) Radical Scavenging Assay: The assay for scavenging nitric oxide (NO) radicals was conducted following A solution of 0.6 mL containing 10 mM sodium nitroprusside was combined with extract at varying concentrations ranging from 4 to 64 µg/mL. This mixture was incubated at a temperature of 25°C for a duration of 150 minutes, after which it was mixed with 1.0 mL of a pre-prepared Griess reagent, which consists of 1% sulfanilamide, 0.1% naphthyl ethylenediamine dichloride, and 2% phosphoric acid. Ascorbic acid and trolox served as standards for comparison. The absorbance of the resulting solution was measured at a wavelength of 546 nm. The percentage inhibition of NO radicals was calculated using the formula:

$$\% \text{ inhibition of NO radical} = [A0 - A1] / A0 \times 100,$$

Where A0 represents the absorbance prior to the reaction and A1 denotes the absorbance following the reaction with the Griess reagent [29, 30].

CONCLUSION: Nil

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Neil HA, Mant D, Jones L, Morgan B. & Mann JI: Lipid screening: Is it enough to measure total cholesterol concentration?. BMJ 1990; 301: 584–587. https://doi.org/10.1136/bmj.301.6752.584
2. Smith GD, Song F and Sheldon TA: Cholesterol lowering and mortality: The importance of considering the initial level of risk. BMJ. 1993; 306: 1367–73. doi: 10.1136/bmj.306.6889.1367.
3. Jackson R and Beaglehole R: Evidence-based management of dyslipidaemia. Lancet 1995; 346: 1440–2. doi: 10.1016/s0140-6736(95)92466-3.
4. Fredrickson DS: An international classification of hyperlipidemias and hyperlipoproteinemias. Ann Intern Med 1971; 75(3): 471-2. [
5. Ballantyne CM, Grundy SM, Oberman A, Kreisberg RA, Havel RJ, Frost PH and Haffner SM: Hyperlipidemia: diagnostic and therapeutic perspectives. J Clin Endocrinol Metab 2000; 85(6): 2089-112.
6. Ostos MA, Recalde D, Baroukh N, Callejo A, Rouis M and Castro G: Fructose intake increases hyperlipidemia and modifies apolipoprotein expression in apolipoprotein AI- CIII-AIV transgenic mice. J Nutr 2002; 132(5): 918–23.
7. Aeberli I, Hochuli M and Berneis K: Response to comment on: Aeberli *et al*.. moderate amounts of fructose consumption impair insulin sensitivity in healthy young men: A randomized controlled trial. diabetes care 2013; 150–156. Diabetes Care 2013; 36(7): doi:10.2337/dc13-0299
8. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA and Graham JL: Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. Journal of Clinical Investigation 2009; 119(5): 1322–34. doi:10.1172/jci37385

9. P. Hasimun, A. Sulaeman, Y. Mulyani, W. Nur Islami and F. Apriany Timika Lubis: Antihyperlipidemic Activity and HMG CoA Reductase Inhibition of Ethanolic Extract of *Zingiber cassumunar* Roxb in Fructose-Induced Hyperlipidemic Wistar Rats. Journal of Pharmaceutical Sciences and Research 2019; 11(5): 1897-1901.
10. Johnston TP & Palmer WK: Mechanism of Poloxamer 407-induced Hypertriglyceridemia in the Rat. Biochemical Pharmacology 1993; 46(6): 1037-1042. https://doi.org/10.1016/0006-2952(93)90668-M
11. Zanwar AA, Hegde MV, Rojatkar SR, Sonawane KB, Rajamohanan PR and Bodhankar SL: Isolation, characterization, and antihyperlipidemic activity of secoisolariciresinol diglucoside in poloxamer-407-induced experimental hyperlipidemia. Pharm Biol 2014; 52(9): 1094–1103. doi:10.3109/13880209.2013.877492
12. Jung HJ, Nam JH, Park HJ, Lee KT, Park KK and Kim WB: The MeOH extract of *Pleurospermumkamts chaticum* and its active component buddlejasaponin (IV) inhibits intrinsic and extrinsic hyperlipidemia and hypercholesterolemia in the rat, J. Ethnopharmacol 112 2007; 255–261, https:// doi.org/10.1016/j.jep.2007.03.012.
13. Johnston TP and Palmers WK: Mechanism of poloxamer 407-induced hypertriglyceridemia in the rat, Biochem. Pharmacol 1993; 46: 1037–1042
14. Kellner A, Correll JW & Ladd AT: Sustained hyperlipemia induced in rabbits by means of intravenously injected surface-active agents. Journal of Experimental Medicine 1951; 93(4): 373–384. https://doi.org/10.1084/jem.93.4.373
15. Fiser RH, Denniston JC, Rindsig RB & Beisel WR: Triglyceride secretion rates: Use of Triton WR 1339 in the rhesus monkey. Journal of Nutrition 1973; 104(2), 223–229. https://doi.org/10.1093/jn/104.2.223
16. Wilson PW, Abbott RD & Castelli WP: High density lipoprotein cholesterol and mortality: The Framingham Heart Study. Arteriosclerosis, Thrombosis, and Vascular Biology 1988; 8(6): 737–741. https://doi.org/10.1161/01.ATV.8.6.737
17. Warnholtz A, Mollnau H, Oelze M, Wendt M & Münzel T: Antioxidants and endothelial dysfunction in hyperlipidemia. Current Hypertension Reports 2001; 3(1): 53–60. https://doi.org/10.1007/s11906-001-0081-z
18. Keshetty V, Srinivas Pabba RG, Kandukuri JM and Allenki V: Antihyperlipidemic activity of methanolic extract of Garlic (*Allium sativum* L.) in Triton X 100 induced hyperlipidemic rats. J Pharm Res 2009; 2: 777–80
19. Pehlivanović Kelle B, Kulo Ćesić A, Ćustović S, Ćosović E, Lagumdžija D, Jordamović N & Kusturica J: Improvement of a diet-induced model of hyperlipidemia in Wistar rats: Assessment of biochemical parameters, the thickness of the abdominal aorta and liver histology. Journal of King Saud University - Science, 2024; 36: 103068.https://doi.org/10.1016/j.jksus.2023.103068:contentReference[oaicite:0]{index=0}.
20. Keshetty V, Srinivas Pabba RG, Kandukuri JM and Allenki V: Antihyperlipidemic activity of methanolic extract of Garlic (*Allium sativum* L.) in Triton X 100 induced hyperlipidemic rats. J Pharm Res 2009; 2: 777–80
21. Kakadiya Jagdish: Causes symptoms, Pathophysiology and Diagnosis of Atherosclerosis- A review. Pharmacology Online 2009; 420-442
22. Niimi M, Yang D, Kitajima S, Ning B, Wang C, Li S and Fan J: ApoE knockout rabbits: a novel model for the study of human hyperlipidemia. Atherosclerosis 2016; 245: 187-193.
23. Mahley RW: Apolipoprotein E: cholesterol transport protein withexpanding role in cell biology. J Sci 1988; 240: 622-630.
24. Ding Y, Wang Y, Zhu H, Fan J, Yu L, Liu G and Liu E: Hypertriglyceridemia and delayed clearance of fat load in transgenic rabbits expressing human apolipoprotein CIII. Transgenic Res 2011; 20: 867-875.
25. Huang Y, Schwendner SW, Rall SC, Sanan DA and Mahley RW: Apolipoprotein E2 transgenic rabbits: modulation of the type III hyperlipoproteinemic phenotype by estrogen and occurrence of spontaneous atherosclerosis. J. Biol. Chem 1997; 272: 22685-22694.
26. Ferruzza S, Rossi C, Scarino ML and Sambuy Y: A protocol for differentiation of human intestinal caco-2 cells in asymmetric serum-containing medium. Toxicol *In-vitro* 2012; 26: 1252-5.
27. Hussein O, Ismail A and FIdris O: Evaluation of Serum Lipid Profile Level and Gamma-Glutamyltransferase Activity as a Biomarker for Coronary Artery Disease in Sudanese Patients 2017; 7(10): 016-22
28. Nigam PK: Serum lipid profile: Fasting or non-fasting? Indian J Clin Biochem 2011; 26: 96-7.
29. Kumari S, Elancheran R, Kotoky J & Devi R: Rapid screening and identification of phenolic antioxidants in *Hydrocotyle sibthorpioides* Lam. by UHPLC–ESI-MS/MS. Food Chemistry 2016; 203: 521–529. https://doi.org/10.1016/j.foodchem.2016.02.10
30. Nugroho A: *Hydrocotyle sibthorpioides* Lam. (Pennywort) as a Source of Bioactive Compounds. Frontiers in Pharmacology 2017; 8: 438. https://doi.org/10.3389/fphar.2017.00438

How to cite this article:
Lakhapate SS, Devade OA, Purane LM and Redasani VK: A brief review on screening models for hyperlipidemia. Int J Pharmacognosy 2025; 12(6): 478-82. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.12(6).478-82.