Review Article

Review Article on Animal Models in Preclinical Research in Hyperlipidemia

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Received: 18-10-2023; **Revised:** 22-12-2023; **Accepted:** 30-12-2023; **Published on:** 15-01-2024.

ABSTRACT

Several frequently used animal models of atherosclerosis are described in this mini review. Rats, Mice, rabbits, pigs, and non-human primates have been highlighted among them. Despite the fact that these animal models have helped us understand how atherosclerotic lesions form, we still don't have a suitable animal model for disease reversal. The present technique for screening method for antihyperlipidemic activity could be classified under in vitro and in vivo methods. In vivo methods include Triton, PTU-, Fructose & high fat diet induced hyperlipidemic models. *In vitro* methods include In vitro Assay using Caco-2 Cell Lines and Inhibition of HMG CoA Reductase. Hyperlipidaemia is a well-established risk factor for cardiovascular diseases and therefore, many animals model have been developed to mimic the human abnormal elevation of blood lipid levels. In parallel, extensive research for the alleviation of ischaemia/reperfusion injury has revealed that hyperlipidaemia is a major comorbidity that attenuates the cardioprotective effect of conditioning strategies (preconditioning, postconditioning and remote conditioning) and that of pharmacological interventions by interfering with cardioprotective signalling pathways.

Keywords: Preclinical research, animal models, hyperlipidaemia, cardiovascular diseases.

INTRODUCTION

Cardiovascular disease is the most common cause of mortality worldwide.[1] Hyperlipidaemia is the pathological state characterized by elevated levels of serum cholesterol and triglycerides (TGs) and it is considered as a major risk factor for cardiovascular disease. In fact, hyperlipidaemia is present in most patients with myocardial infarction. Chronic elevation of blood cholesterol results in the development of atherosclerosis but also exerts a negative impact on the myocardium by increasing oxidative stress, mitochondrial dysfunction and apoptosis. Moreover, hypercholesterolaemia induces microvascular dysfunction through nitro-oxidative stress and induction of inflammation, mechanisms which may also account for increased susceptibility of the myocardium to infarction.[2]

Various animal species have been used to study the effects of diet on cholesterol homeostasis and atherosclerosis including mice[3], hamsters, guinea pigs (Fernandez & Volek, 2006), rats, rabbits, minipigs and farm breed pigs. Mice and rats are resistant to spontaneous development of atherosclerosis but both diet and genetic manipulations render these rodents more susceptible to atherosclerosis development, while genetically altered strains respond to cholesterol feeding to a greater extent.[4] Some of these models, mainly mice and rats, have been employed for the investigation of hypelipidaemia's impact on myocardial ischaemia/reperfusion injury (I/R). However, a number of studies (including one in pigs) indicate that the effect of cardioprotective manoeuvres is blunted in these animals. In fact, high cholesterol diet (HCD)-induced hyperlipidaemia was the first comorbidity where the loss of cardioprotection was observed in rabbits and rats.[5]

1. Epidemiology

Every year due to coronary artery diseases 6,10,000 people passed away in United states of America, it is the major source of killing over 3,70,000 peoples per annum, around 7,35000 people suffers from cardiac arrest per year. Due to plaque rupture 75% of population suffers acute myocardial infarction; it is mainly observing man over 45 years, whereas in woman chances of myocardial infarction are increase after the age of 45 year. Due to the protecting action of female sex hormone the chances of atherosclerosis in woman is less than man, the protecting action of hormone is lost after menopause (above 50 years) Plaque rupture is said to cause 75 percent of acute myocardial infarctions, with the largest incidence of plaque rupture occurring in males over the age of 45, while the incidence increases in women after the age of 50.[6] In the recent study, in India about 60% population was suffering from coronary artery diseases or cardiovascular diseases. Around 10% population have admitted and some of fear about hospitalization. Around 22% patients suffering from these diseases are died Because of they were not provided therapy due to the lack of medical staff in Villages. They shouldn't give counselling and awareness about these diseases.[7]

Pathophysiology

The formation of atheromatous plaques is known as atherogenesis. It is characterised by arterial remodelling as well as the formation of plaques, which are fatty substances. Leukocytes such as monocytes and basophils, according to one theory, assault the endothelium of the arterial lumen in cardiac muscle for unknown reasons.[8] Atheromatous plaques occur in the tunica intima of the

artery wall, which is positioned between the endothelium and the tunica media, as a result of the inflammation. The bulk of these lesions are made up of excess fat, collagen, and elastin. When plaques form, just the wall thickens without constriction, stenosis of the artery entry, also known as the lumen; stenosis is a late occurrence that may never occur and is typically the consequence of recurring plaque rupture and healing responses, rather than the atherosclerosis process alone.[9]

Table 1: Blood cholesterol levels

Blood Cholesterol Levels iClinic

Type of Cholesterol	Category	Anyone ≤ 19 years mg/dL	Women ≥ 20 years mg/dL	Men ≥ 20 years mg/dL
Total Cholesterol	Normal value	<170	125 - 200	
	Border line	179 - 199	200 - 239	
	High	≥ 200	≥ 239	
Non-HDL	Normal value	<120	130	
	Border line	120 - 144	≥ 130	
	High	≥ 145		
LDL	Normal value	<100	100	
	Border line	110 - 129	Above optimal: 100 - 129	
	Borderline high	-	130 - 159	
	High		160 - 189	
	Very high		≥ 189	
HDL		>45	≥50	≥40

Inflammation is thought to play a role in all phases of atherosclerosis, according to recent research. A major component of atherosclerosis, in addition to inflammation, is the proliferation of vascular smooth muscle cells (VSMCs) (Fig. 1).

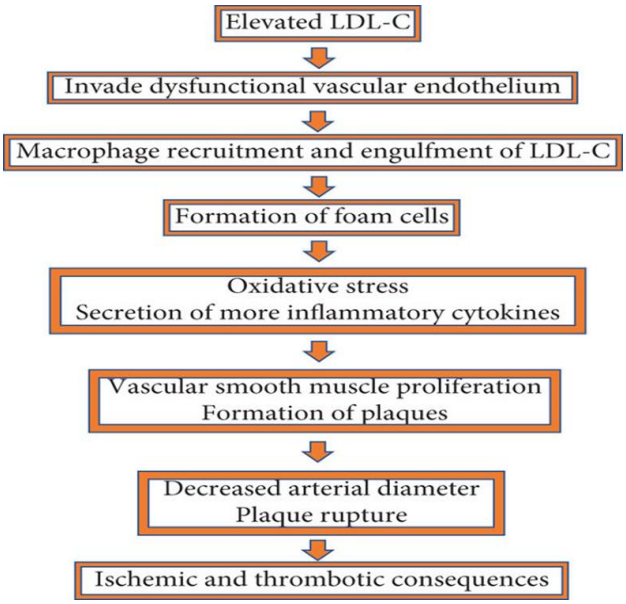

Figure 1: Pathology of hyperlipidaemia

In humans, a localised buildup of VSMCs inside the intima might be a prelude to the formation of a lesion. However, the precise role of VSMCs in atherosclerosis is still a matter of dispute. VSMCs may contribute to the formation of atheroma in early atherosclerosis by producing pro-inflammatory mediators such as monocyte chemoattractant protein 1 and vascular cell adhesion molecule, as well as synthesising matrix components necessary for lipoprotein retention. VSMCs, on the other hand, may play a role in preserving the plaque's integrity by forming a strong fibrous cap. Indeed, there is evidence of VSMC apoptosis, notably at the 'shoulder' area, in lipid-laden lesions with a thin and weak fibrous cap, which is linked with inflammation. Furthermore, the local inflammatory environment might increase collagenase expression while inhibiting proteolytic inhibitors.[10]

2. Induction animals' models of hyperlipidaemia :-

1. IN VIVO METHODS:-

Fructose Induced Hyperlipidaemia :-

- **Principle:-**

The human liver can quickly absorb and process fructose. For thousands of years, people ingested 16–20 grams of fructose each day, mostly from fresh fruits. Dietary westernization has led in considerable increases in added fructose, resulting in normal daily fructose consumptions of 85–100 grams. When the liver is exposed to significant amounts of fructose, it undergoes fast lip genesis and TG buildup, which leads to decreased insulin sensitivity and hepatic insulin resistance/glucose intolerance. Fructose metabolism has received considerable scientific focus due to these unfavorable consequences of fructose. Small catalytic amounts of fructose, it turns out, can have a beneficial impact, lowering the glycemic response to glucose loading and improving glucose tolerance. These effects are also found in the absence of any alterations in insulin responses, non-esterified fatty acid (NEFA), or total lipid levels.[11]

Triton Induced Hyperlipidaemia :-

By parenteral administration of Triton WR 1339 (isooctyl polyoxy ethylene phenol) in adult rats.

- **Principle:-**

In vivo screening technique of antihyperlipidemic activity is reported using Triton-induced hyperlipidemic rat model. Triton X-100 is a non-ionic surfactant which accelerates hepatic cholesterol synthesis and enhances intestinal lipid absorption by the emulsification process.

- **Procedure:-**

Albino Wistar rats (160–200 g) can be utilised to test hyperlipidemic activity in vivo. The animals are kept in polypropylene cages in a well-ventilated environment at a temperature of 25°C with a light/dark cycle of 12:12 hours. Throughout the trial time, standard pellet feed and filtered tap water should be supplied. The rats are split into three groups of twelve rats each at random. Except for the control group, triton is dissolved in normal saline to achieve a 5 percent concentration and administered at a dosage of 300 mg/kg to all rats.[12]

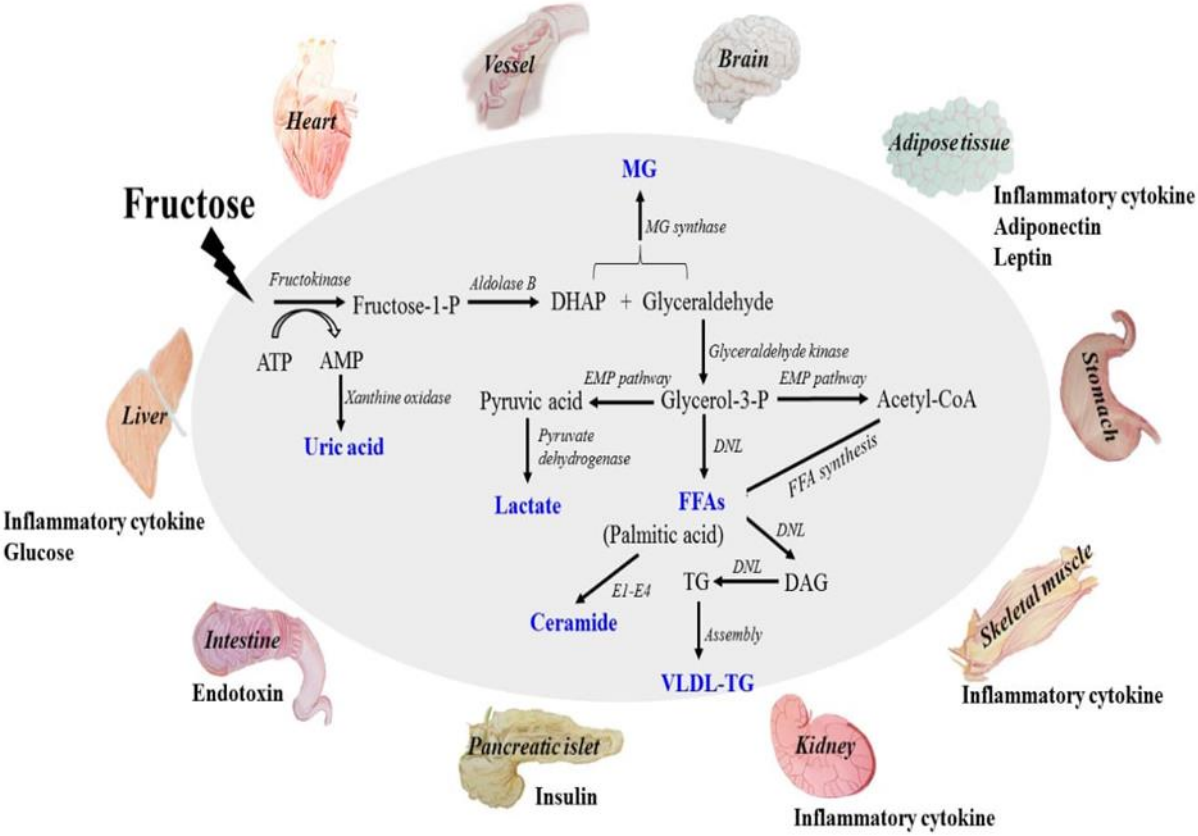

Figure 2: Lipid metabolism

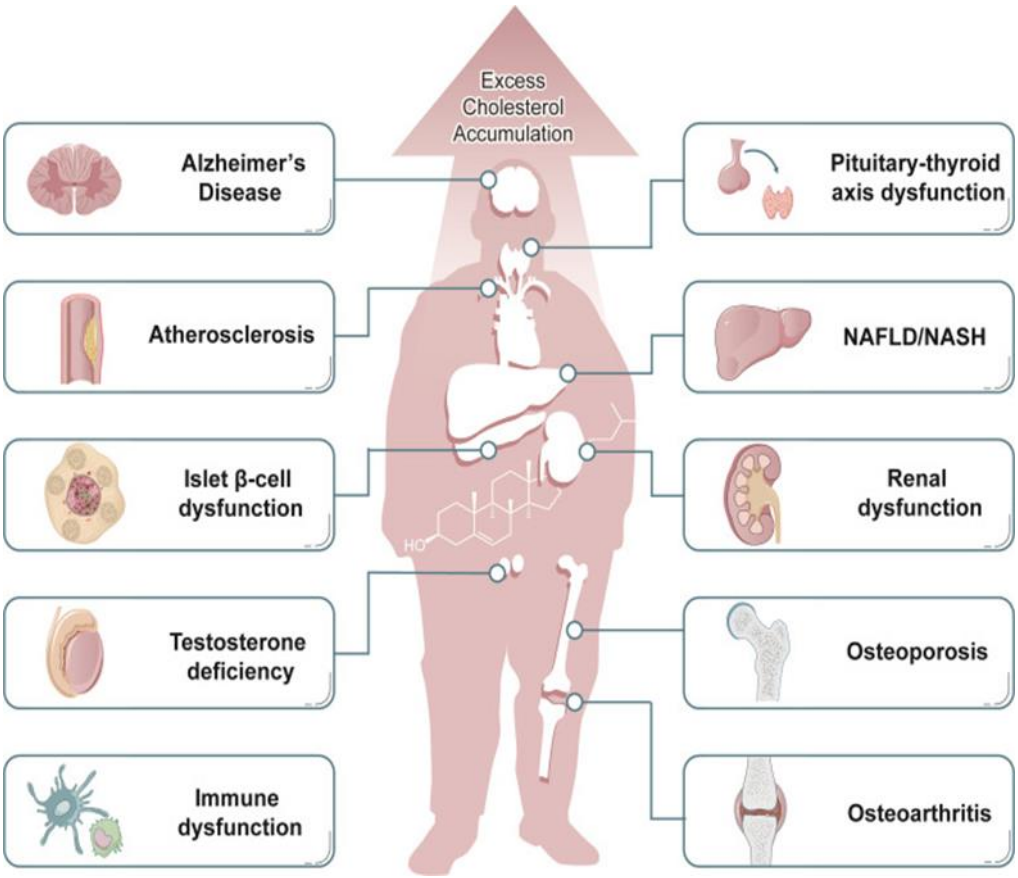

Figure 3: Excess cholesterol complications

Cholesterol Induced Hyperlipidaemia:-

- **Procedure:-**

Male Wistar rats (18 weeks old) were fed laboratory chow enhanced with 2% cholesterol or regular chow for 8 weeks in a room with 12-hour light–dark cycles and a constant temperature of 22±3°C.The HFD Consists of 95% commercial rat feed, 4% cholesterol & 1% Cholic acid. It is administered with vehicle for 21 days. After the completion of treatment, we have used to measure blood levels of LDL, VLDL, total cholesterol, and triglycerides.

High Fat Diet Induced Hyperlipidaemia:-

- **Principle:-**

The composition of HFD in 48 days is rat chow, Folic acid, cholesterol, dalda ghee. These are the parameters which have determined by blood collection method with different routes such as Retro orbital, tail vein collection. Lipid Profile- TC- Total Cholesterol, Triglycerides, High density lipid- Cholesterol, Low Density lipid- Cholesterol. Atherogenic Index of Plasma - It is detected by this formula; Log (triglyceride/ HDL- Cholesterol), myopathy marker- Creatine kinase marker or also known as creatine phosphokinase (CPK). CRP- C- reactive protein, TNF α, Interleukin – 6, Adiponactin mRNA , RB-4.

- **Procedure :-**

This model closely resembles hyperlipidemia in humans. This approach involves mixing a large amount of cholesterol with vegetable oil and administering it to all groups except the control group. Following the chronic treatment with high fat, the second group is given a conventional medicine, the third group is given a test sample, and the fourth group is given merely a regular diet as a control group.[13] Collect the blood sample by an appropriate technique under minor anaesthetic at the conclusion of the 30th day. Animals are sacrificed, and organs such as the heart, liver, aorta, pancreas, spleen, and kidney are isolated, weighed, and submitted to histopathological tests[14].

2. IN VIVO METHODS:

In vitro Assay using Caco-2 Cell Lines

The lipid profile released by human intestinal epitheliumlike cells from the colon cancer cell line Caco-2 is measured in this manner. Caco-2 cells are sown in well plates for two days in a mixture containing 10% foetal bovine serum, penicillin, and streptomycin. Caco-2 cells are differentiated for 4 days after being exposed to 0–5 mM sodium butyrate.[15] Caco 2 cells will be changed into intestinal epithelium-like cells after an incubation period, and plenty of microvilli may be seen on the apical side of the cell when seen under an electron microscope. The medium containing the cultured cells is then supplemented with sodium oleate, and the differentiated Caco-2 cells secrete a lipoprotein profile into the medium via a microporous membrane. Chylomicron, VLDL, LDL, and high-density lipoproteins are among the four class fractions released[16]

Inhibition of HMG CoA Reductase :-

HMG CoA reductase is a key regulatory enzyme in the production of cholesterol from acetyl CoA. 3-hydroxy, 3-methyl glutacyl CoA (HMG CoA) is converted to mevalonate by this enzyme. Cholesterol is not produced because this enzyme is inhibited. As a result, this in vitro model is used to assess a chemical moiety's hypolipidemic activity. This enzyme-inhibiting mechanism is how existing statin compounds show hypolipidemic action. Inhibition of HMG CoA reductase causes the liver to express LDL receptors, which decreases cholesterol levels in the blood.[17] This is a reaction that necessitates the presence of NADPH. Commercially available HMG CoA assay kits are available. HMG CoA reductase assay buffer, HMG CoA reductase, NADPH, and an inhibitor are included in this kit (atorvastatin).[18] The use of NADPH is assessed by a decrease in absorbance at 340 nm, which is measured by the assay kit. In addition, this sort of kit allows for the determination of pure enzyme activity.[19]

1. Diagnosis:-

Detection and diagnosis pattern of atherosclerosis. After the completion of physical examination by the family history or other depending on detection pattern then further test will be suggested by the doctor or RMP or pharmacists included:[20]

2. Blood Test:-

By this test check the blood cholesterol level & blood sugar it will be low, normal & higher the range. Cholesterol level is high it will be increasing the risk of atherosclerosis. C-reactive protein test is also done. To check the inflammation in the arteries[21]

3. Electrocardiogram:-

(ECG or EKG) Simple and painless test to record the signals in heart.

4. Echocardiogram:-

This test is done for uses sound waves to show how blood well moves in when the heart beat & through your arteries

5. Doppler Sound:-

Your doctor may use ultrasound Doppler equipment to take your blood pressure at different spots on your hand and leg. It will display the obstructions as well as the rate of blood flow through the arteries.[22]

6. Ankle- Brachial Index (ABI):-

Detect the comparison between the legs and arms blood pressure. Abnormal differences may be sign of peripheral vascular diseases, usually caused by atherosclerosis.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Timmis, A., Townsend, N., Gale, C., Grobbee, R., Maniadakis, N., Flather, M., … ESC Scientific Document Group. European Society of Cardiology: Cardiovascular disease statistics 2017. European Heart Journal, 2018;39:508–579. https://doi.org/10.1093/eurheartj/ ehx628

2. Andreadou, I., Iliodromitis, E. K., Lazou, A., Görbe, A., Giricz, Z., Schulz, R., & Ferdinandy, P. Effect of hypercholesterolaemia on myocardial function, ischaemia-reperfusion injury and cardioprotection by preconditioning, postconditioning and remote conditioning. British Journal of Pharmacology, 2017;174:1555–1569. https://doi.org/10.1111/ bph.13704

3. Temel, R., & Rudel, L. Diet effects on atherosclerosis in mice. Current Drug Targets, 2007;8:1150–1160. https://doi.org/10.2174/ 138945007782403847

4. Dillard, A., Matthan, N. R., & Lichtenstein, A. H. Use of hamster as a model to study diet-induced atherosclerosis. Nutrition & Metabolism (London), 2010;7: 1–12, 89. https://doi.org/10.1186/1743-7075-7-89

5. Fernandez, M. L., & Volek, J. S. Guinea pigs: A suitable animal model to study lipoprotein metabolism, atherosclerosis and inflammation. Nutrition & Metabolism (London), 2006;3:1–6, 17. https://doi.org/10. 1186/1743-7075-3-17

6. Badimon, J. J., Badimon, L., & Fuster, V. Regression of atherosclerotic lesions by high density lipoprotein plasma fraction in the cholesterol-fed rabbit. The Journal of Clinical Investigation, 1990;85:1234–1241. https://doi.org/10.1172/JCI114558

7. Shim, J., Al-Mashhadi, R. H., Sørensen, C. B., & Bentzon, J. F. Large animal models of atherosclerosis—New tools for persistent problems in cardiovascular medicine. The Journal of Pathology, 2016;238:257–266. https://doi.org/10.1002/path.4646

8. Shim, J., Al-Mashhadi, R. H., Sørensen, C. B., & Bentzon, J. F. Large animal models of atherosclerosis—New tools for persistent problems in cardiovascular medicine. The Journal of Pathology, 2016;238:257–266. https://doi.org/10.1002/path.4646

9. La Ville A, Turner PR, Pittilo RM, Martini S, Marenah CB, Rowles PM, et al. Hereditary hyperlipidemia in the rabbit due to overproduction of lipoproteins. I. Biochemical studies. Arteriosclerosis. 1978;7(2): 105–12.

10. Zhang Y, Koradia A, Kamato D, Popat A, Little PJ, Ta HT. Treatment of atherosclerotic plaque: perspectives on theranostics. J Pharm Pharmacol. 2019;71(7): 1029-43.

11. Ostos MA, Recalde D, Baroukh N, Callejo A, Rouis M, Castro G. Fructose intake increases hyperlipidemia and modifies apolipoprotein expression in apolipoprotein AI- CIII-AIV transgenic mice. J Nutr. 2002;132(5): 918–23

12. Jijith U.S, Jayakumari S. Screening methods for antihyperlipidemic activity: Drug invention Today. Drug intervention today. 2018;10(2): 257-259

13. Kakadiya Jagdish. Causes symptoms, Pathophysiology and Diagnosis of Atherosclerosis- A review. Pharmacology Online. 2009;420-442.

14. Lusis J. Aldons. Atherosclerosis NIH Public access. 2000;407(6807): 233-241.

15. Machhi J.P, Shah N.N. Study of antiatherosclerotic activity of polyherbal preparation using rat as an experimental animal model. International journal of pharmaceutical science & research. 2012;3(10):4010-4018.

16. Ferruzza S, Rossi C, Scarino ML, Sambuy Y. A protocol for differentiation of human intestinal caco-2 cells in asymmetric serum-containing medium. Toxicol In Vitro. 2012;26: 1252-5.

17. Hussein O, Ismail A, 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.

18. Nigam PK. Serum lipid profile: Fasting or non-fasting? Indian J Clin Biochem. 2011;26:96-7.

19. Gaddikeri K, Bhorgonde DD. Estimation of serum lipid profile patterns as a diagnostic marker in oral cancer and precancer. Asian Pac J Health Sci. 2016;3:59-62

20 Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of endothelial nitric oxide synthase by HMG coA reductase inhibitors. Circulation. 1998:97:1129-35.

21. Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. Science. 2001;292:1160 LP-1164.

22. J Hematol. Blood Disord Induce hyperlipidemia in rats using high fat diet investigating blood lipid and histopathology.Journal of hematological and blood disorder. 2018; 4(1): 1-5.