Applied Sciences Research Periodicals
February 2024, Vol. 2, No. 2, pp. 01-09

Evaluation of the Effect of Simvastatin, Atorvastatin, Rosuvastatin on Alcohol Induced Hypercholesterolemia in Female Wistar Rat

Nweke Luke Maduka[1], Ani Celestine Okafor[2], Anyaora Chisom Peace[1], Ezeaku Ifebuchechukwu Loveth[1], Emele Cindy Nneoma[1], Onah Emmanuel Sunday[3], Okolo Kenneth Obinna[4]

1. Department of Human Physiology, Faculty of Basic Medical Sciences. College of Medicine, University of Nigeria. Enugu Campus Nigeria
2. Department of Human Physiology, Faculty of Basic Medical Sciences. College of Medicine,
3. Department of Ophthalmology, Enugu State University Teaching Hospital, Parklane GRA Enugu, Nigeria
4. Pharmacology & Toxicology, Faculty of Pharmaceutical Sciences, Enugu State University of Science & Technology, Parklane GRA Enugu, Nigeria

Abstract:
This study evaluated the effect of simvastatin, atorvastatin and rosuvastatin in alcohol induced hypercholesterolemia (HCM) in female Wistar rat. Thirty (30) adults' female Wistar rat with body weight 180-210g were divided into 5 groups of 6 rats each. Hypercholesterolemia (HCM) was induced in rats through oral administration of alcohol for 2 weeks. Group A was received water and feed only and served as the normal control, Group B was induced with HCM without treatment, Group C, D and were induced with HCM + 3.5mg/kg of Simvastatin, HCM + 3.5mg/kg of Atorvastatin, HCM + 3.5mg/kg of Rosuvastatin respectively. The findings of this study revealed that, the use of simvastatin, rosuvastatin and atorvastatin for 14 days did not prevent weight gain in rats, all of the statin used increased high density lipoprotein cholesterol (HDL-C) levels, there was decrease in Triglyceride (TG) concentration by group E also a decrease in the low density lipoprotein cholesterol (LDL) levels in rosuvastatin .There was statistically significant difference ($P<0.05$) when compared with those of simvastatin group but there was no statistically ($P>0.05$) significant difference compared with atorvastatin group .It observed that Statins (Simvastatin, Atorvastatin and Rosuvastatin) could prevent alcohol-induced hypercholesterolemia and its related effects. Therefore, the study recommended among others that Government could also fund public health campaigns that could raise awareness about the risks of alcohol-induced hypercholesterolemia in both human and animal modelled study.

Keywords: Statin, Hypercholesterolemia, Lipid profile, Body weight

INTRODUCTION

Cardiovascular diseases (CVD) are one of the major health problems in the world and its increasing prevalence is threatening in the human health (Navaei et al, 2019). There are many prevention programs for CVD incidences, these disorders are the most common cause of mortality in several countries (Nadimi and Ahmadi, 2016). According to the findings of various studies, the cause of 40-45% of mortality is related to CVD. Based on the third report of World Health Organization (WHO), CVD (e.g., heart failure, stroke, and sudden cardiac deaths) is the cause of 12 million annual deaths worldwide (Jamshidi et al, 2017). CVD includes coronary diseases, brain artery diseases, and peripheral artery diseases (Nadimi and Ahmadi, 2016). In coronary diseases, vessels that provide blood to the heart is obstructed; a common CVD disorder. Coronary artery

obstruction is usually caused by arteriosclerosis (Jamshidi et al, 2017). The sediment of cholesterol, calcium, or other materials in the inner layer of the artery and connective tissue cause arteriosclerosis (Nadimi and Ahmadi, 2016).

The role of hypercholesterolemia as a major risk factor for coronary artery disease (CAD) has been proven (Nadimi and Ahmadi, 2016). Hypercholesterolemia also called high cholesterol is the presence of high levels of cholesterol in the blood (WHO, 2021). It is a form of hyperlipidemia (high levels of lipids in the blood), hyperlipoproteinemia (high level of lipoproteins in the blood), and dyslipdemia (any abnormalities of lipid and lipoprotein levels in the blood) (Ramsey, 2016). According to the WHO definition (2015), hypercholesterolemia would be included in all phenotype (Ramsey, 2014). It is a lipid disorder in which the low-density lipoprotein (LDL) or bad cholesterol is too high. Hypercholesterolemia is characterized by LDL cholesterol exceeding 159 mg/dl (Vander et al, 2018). Many developed countries have a high prevalence of hypercholesterolemia. Hypercholesterolemia is a complex disorder often due to multiple genetic defects and rarely due to a single genetic defect as in the case of familial hypercholesterolemia (Grundy and Stone, 2018). Because, hypercholesterolemia is associated with risk of developing atherosclerosis, much research has been devoted to understanding the genetic variants and environmental factors that contribute to elevated blood LDL cholesterol. There are several challenges to investigating gene-diet interactions in human.

METHODS

Experimental Animals

Thirty (30) adults female Wistar rats having an average weight of 180-210g were purchased from the Animal House in the Department of Anatomy, Enugu State University College of Medicine, Parklane and handled under standard temperature of within 28 ^{o}C and 50% relative humidity at 12-hour light/dark cycle. The animals were allowed to acclimatize with the new environment for two (2) weeks prior to experimental usage, during which they were fed with the standard livestock pellets produced by Guinea feed Nigeria Limited, Abuja, No E3 Zamfara Court Ficus Benjanin Street Gaguwa. Food and water were provided *ad libitum.*

Experimental Design

The Thirty adult female Wistar rats of 180-210g were used for the study (10-12 weeks old). Health assessment was conducted to ensure that the rats were free from any pre-existing health conditions. They were randomly assigned into five (5) different cages of six (6) rats each.

A. = Normal Control and received feed and water *ad libitum*
B. = HCM without treatment
C. = Hypercholesterolemia + 3.5 mg/kg of Simvastatin.
D. = Hypercholesterolemia+3.5mg/kg of Atorvastatin.
E. = Rosuvastatin Treatment Group: Hypercholesterolemia+3.5mg/kg of Rosuvastatin

Collection of Blood Samples

The collection of blood samples from the rat in each group was done by Cardiac Puncture method. The rats were dissected, followed by cardiac puncture after a mild anesthesia with chloroform. About 5-9mls of blood samples was collected in an Ethylenediaminetetraacetic acid (EDTA) tube from each group using a medical syringe. Serum was separated from the blood after clotting by centrifugation and then used for lipid analysis.

Lipid Profile Analysis

In the lipid profile analysis, tests were conducted using Zak method for serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides. Low-density lipoprotein cholesterol (LDL-C) was then calculated using a standard formula. Friedeward equation:

$$[\text{LDL-chol}] = [\text{Total chol}] - [\text{HDL-chol}] - [\text{TG}]/5$$

where [TG]/5 is an estimate of VLDL-cholesterol.

Test For Total Cholesterol (TC)

Material/reagents involved:

1. Serum
2. Ferric chloride reagent (250mg of pure cholesterol mixed with 100ml of 85% phosphoric acid)
3. Sulfuric acid

Procedure:

1. The serum was diluted at about 1:20 with distilled water
2. The standard cholesterol was diluted at about 1:20 with glacial acetic acid
3. Ethanol extract was reacted with ferric chloride - ethyl acetate and concentrated sulphuric acid (Zlatkis et al, 2004)
   Using three glass test tubes labelled – Test, Standard and blank respectively, the following procedure was conducted
4. The tubes were shaken for about 10 seconds in order to mix-up the contents and was dropped immediately drop the tube in a boiling water bath for about 90 seconds.
5. Cooled for about 5 minutes with running tap water and the absorbance was read at 560nm wavelength in the spectrophotometer. Finally, the formula was calculated thus:

Sample	Test	Standard	Blank
Ferric chloride reagent	5.0ml	5.0 ml	5.0 ml
Diluted serum	0.5ml	-	-
Diluted standard cholesterol	-	0.5ml	-
Distilled water	-	-	0.5ml

$$\frac{\text{Absorbance of test x concentration of the standard solution } (\frac{200\text{mg}}{\text{dl}})}{\text{Absorbance of the standard}}$$

Test For HDL-Cholesterol (HDL-C)

Materials/Reagents involved:

1. Serum
2. Cholesterol standard (100mg of pure cholesterol mixed with 100ml of glacial acetic acid)
3. Colour reagent (5.6g of 2,5 – dimethyl benzene sulphuric acid mixed with 200ml of glacial acetic acid and 300ml of acetic anhydride)
4. Phosphotungstic acid reagent (4.5g of phosphotungstic acid mixed with 50ml of water, add 16ml of IN NaOH and make up to 100ml with water)
5. Tris's buffer (1.21g of tris mixed with 90ml of water, then the pH was reduced to about 7.6 with IN HCL and diluted up to 100ml with water)

Procedure:

1. About 1ml of serum was pipetted in a test tube.
2. 0.1ml of phosphotungstic acid reagent was dropped and mixed very well
3. 0.05ml of magnesium chloride was added and mixed very well
4. Was Centrifuged at 2500rpm for 30 minutes
5. Carefully remove the clear supernatant with a Pasteur pipette
6. 2 drops of the colour reagent were added and allowed to be stable for 15 minutes
7. Absorbance was read at 560nM

Test for LDL-Cholesterol (LDL-C)

The LDL-Cholesterol was calculated from measured values of total cholesterol, triglycerides and HDL cholesterol according to the relationship: [LDL-chol] = [total chol] - [HDL-chol] - [TG]/5 where [TG]/5 is an estimate of VLDL-cholesterol and all values are expressed in mg/dL.

Test for Triglycerides

Material/Reagents involved*:*

1. Serum
2. Heptane
3. Isopropanol
4. Sodium methylate (50mg of sodium methylate diluted in 100ml of isopropanol)
5. Sulphuric acid (0.08N) – Prepared by mixing 2.2ml conc $H2SO4$ of about 36.N with 500ml of distilled water. The dilution should be up to 1 liter.
6. Sodium Periodate reagent (1.23g) of $Na1O_4$ was mixed with 100ml of 0.88N, about 5% v/v acetic acid. then stored in a brown bottle)
7. Acetylacetone reagent (0.75ml of acetyl acetone was dissolved with 2.5ml of isopropanol, then 2N of ammonium acetate (15.4%) was added to make the volume up to 100ml then stored in a brown at $4^{o}C$
8. Triglyceride standard (200mg of pure triolein was mixed with 100ml of isopropanol

Procedure:

1. Using three glass test tubes labeled-Test, standard and Blank respectively, the following procedures was conducted

Standard	Test	Standard	Blank
Serum	0.5ml	-	-
Triglyceride	-	0.5ml	-
Distilled water	-	0.5ml	0.5 ml
H_2SO_4 (0.08N)	1.0ml	1.0 ml	1.0 ml
Heptane	2.0ml	2.0 ml	2.0 ml

2. The tubes were shaken for 30 seconds to mix very well. It was made to stand for 10 minutes at room temperature for proper separation of two layers. Prepared respectively and the following procedure was done.

Sample	Test	Standard	Blank
Top solvent layer from respective tubes	0.2ml	0.2 ml	0.2 ml
Sodium methylate	3.0ml	3.0 ml	3.0 ml

3. The tubes were shaken using vortex
4. mixer to mix very well
5. Incubated at 60 degrees centigrade for 10 minutes and then cooled at room temperature
6. Colour development stage, which involves dissolving 0.1ml of periodate reagent to each tube and mixed very well. Then cooled at room temperature after 10 minutes of incubation at 60 degrees centigrade
7. The tubes were centrifuged and the upper phase liquid was transferred to another fresh tube
8. Using spectrophotometer, the absorbance of test and standard were read at N420nm against the blank
9. Finally, calculation was done by

$$\frac{Absorbance\ of\ test\ x\ 200}{Absorbance\ of\ standard} = Triglyceride\ (\frac{mg}{dl})$$

Statistical Analysis

Data generated was analysed with one-way analysis of variance (ANOVA) and presented as mean ±standard deviation using Statistical Package for Social Science version 20.0 (SPSS). P-value less than 0.05 ($p<0.05$) were considered statistically significant.

Ethical Approval

Ethical approval for the study was sought and obtained from Research and Bio-ethics Committee of the College of Medicine, University of Nigeria, Enugu Campus

RESULTS

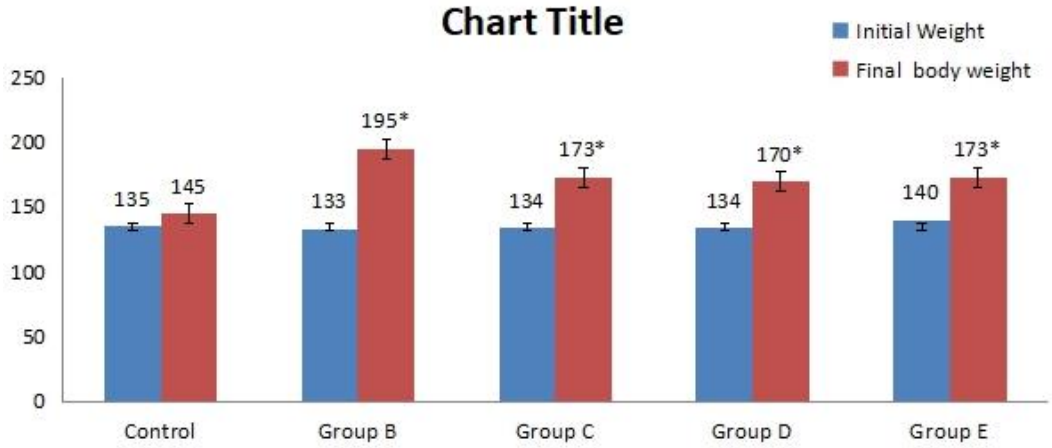

Fig 3.1 comparison of the initial body weight to the final animal body weight of the experimental groups

Table 3.1: Evaluation of the Effect of Statins on lipid profile parameters

GROUPS	HDL	LDL	TG	TC
A	45.50±4.50	100.00±4.00	118.00±13.00	125.00±5.00
B	38.00±3.00*	225.20±3.00*	199.00±1.00*	225.00±1.00*
C	55.00±1.00*	192.00±1.00*	183.50±0.50*	225. 20±0.20*
D	55.50±0.50*	189.50±0.00*	178.50±0.50*	226.50±0.50*
E	54.50±0.50*	180.50±0.50*	172.00±1.00*	228.95±0.05*

Data were analyzed and presented as mean±standard deviation. *P<0.05 showed a statistically significant

difference compared to the normal control group.

Result obtained from table 3.1 Revealed that; there was a significant increase in HDL in all the experimental groups B, C, D and E when compared to the control group. On the LDL, the result obtained showed a significant increase in LDL in all the experimental groups when compared to the control. TG showed a significant increase in all the experimental groups compared to the control. Whereas the result obtained by evaluating the TC showed a significant increase in all the experimental groups compared to the control. Therefore, there is a statistically significant difference in the mean Total Cholesterol, mean High density lipoprotein, mean Low density lipoprotein, mean Triglyceride in the normal control group and the experimental groups of female Wistar rat.

Table 3.2: Effect of Simvastatin, Atorvastatin and Rosuvastatin on Alcohol-Induced Hypercholesterolemia on lipid profile

GROUPS	HDL	LDL	TG	TC
A	38.00±3.00	225.20±0.20	199.00±1.00	225.00±1.00
B	47.50±4.50	100.00±4.00	118.00±13.00	125.00±5.00
C	55.00±1.0	192.00±1.00	183.50±0.50	225.20±0.20
D	55.50±0.50	189.50±0.50	178.50±0.50	226.50±0.50
E	54.50±0.50	180.50±0.50	172.00±1.00	228.950±0.05

Data were analyzed and presented as mean ± standard deviation. *P<0.05 showed a statistically significant difference compared to the normal control group.

Result obtained from table 4.2 showed a significant increase in HDL in all the experimental groups compared to the negative control (untreated group). While, on the LDL; result obtained showed a significant decrease in LDL in other experimental groups when compared to the negative control (untreated group). There was also a significant decrease in the level of TG in all the experimental groups when compared to the negative control (untreated group). TC showed a significant increase in all experimental groups when compared to the negative control group (untreated group)

Table 4.3: Percentage changes on the various parameters of lipid profile after administration of Rosuvastatin, Atorvastatin and Simvastatin

Lipid profile parameter (mgs%)	**Rosuvastatin group (%)**	**Atorvastatin group (%)**	**Simvastatin group (%)**
TG	20.13	13.14	9.97
HDL	18.31	7.11	4.56
LDL	44.25	35.56	25.17

HDL: High-density Lipoproteins, LDL: Low-density Lipoproteins, TG: Triglyceride

Table 4.3 revealed that there were no adverse events observed in any of the study groups. Rosuvastatin, Atorvastatin, and Simvastatin group did not deviate significantly from their baseline biochemical profile after 14 days of therapy.

DISCUSSION

This study sought to investigate the effect of Simvastatin, Atorvastatin and Rosuvastatin administration on the changes in body weight in alcohol-induced hypercholesterolemia in female Wistar rat. One of the widely known side effects of hypercholesterolemia is weight gain. Hypercholesterolemia-induced weight gain is caused by increased subcutaneous fat (Mulazimah,

2017). Changes in the body weight of rats can interfere with fat metabolisms whereas excessive fat consumption exacerbates the lipid disorder. As seen in fig. 1, there was an increase in the body weight of rats after the induction of hypercholesterolemia which occurred across the treatment groups. The induction of fat in rats increased body weight. In this study, the use of simvastatin, rosuvastatin, and atorvastatin for 14 days did not prevent weight gain in rats. Rats receiving simvastatin, rosuvastatin, and atorvastatin continue to experience a significant weight gain. This is in agreement with the study by (Dwi et al, 2022) who found out that the administration of simvastatin, rosuvastatin, and atorvastatin for 30 days increased the rats body weight and dyslipidemia, characterized by a significant decrease in low-density lipoproteins (LDL) and an increase in total cholesterol (TC) and triglycerides (TG) levels. The study revealed a notable observation, indicating that the administration of the three statins led to a significant increase in HDL levels in the experimental groups when compared to the control group. HDL is often considered the "good" cholesterol as it helps remove other forms of cholesterol from bloodstream. The significant change in HDL levels suggests that the statins did not negatively impact this beneficial cholesterol component in the rats. High levels of HDL cholesterol are associated with reduction in the risk of cardiovascular diseases and other related diseases. Raising HDL-C levels is another major factor known to reduce hypercholesterolemia risk (Brown et al, 2017). In the present study, all of the statins used increased the level of HDL-C compared to control group. Rosuvastatin had the least reduction in HDL-C and would thus be regarded as the most effective. However, the values for the statins were significant. In this instance, the findings in the current study are contrary to the study in the literature. For example, the STELLAR trial (Jones et al, 2003) found rosuvastatin (40mg) to be most effective on increasing HDL-C. As for the PULSAR (Clearfield et al, 2016) study, which investigated starting doses of rosuvastatin and atorvastatin, they found that the increase in HDL-C was significantly greatly statistically with rosuvastatin (10mg) than with atorvastatin (20mg). The study found a significant increase in LDL and triglyceride levels in all the experimental groups compared to the control group. Elevated LDL levels according to (Jonee, 2003) are associated with a decrease risk of cardiovascular disease, as LDL cholesterol is often referred as "bad" cholesterol. Additional, decreased triglyceride levels can lower the risk of atherosclerosis and heart-related conditions. The reduction in these lipid parameters suggests that the statins' administration have high efficacy in curbing the negative effects of alcohol-induced hypercholesterolemia in wistar rat. The lowering of triglycerides is another important goal in reducing hypercholesterolemia among CVD patients (Clearfield et al, 2016). In the current study, the greater reduction in TG was achieved by group E (Rosuvastatin group). Also, it is important to note that atorvastatin was the second highest reduction in TG. These findings are similar to the majority of studies in the literatures which have shown slightly higher reduction in TG in patients taking rosuvastatin in comparison to atorvastatin (Clearfield et al, 2016). It thus appears that, in relation to this factor (HG), that both rosuvastatin and atorvastatin are effective at reducing it.

Also, table 4.1 revealed that rosuvastatin was found to be the most effective statin at reducing LDL-C when compared with another statin. Indeed, it should be noted that rosuvastatin which is the latest statin to receive approved labeling by the food and drug administration, has been consistently found to be the most effective at reducing LDL-C level. In the most recent studies comparing its efficacy to another statins (Mckenney, 2015). Also, the results of the STELLAR trial year revealed that rosuvastatin was consistently, across all doses, the most effective at reducing LDL-C levels in comparison to all the other statins (Jones et al, 2003).

Table 4.2 indicated that the mean TG, and LDL-C levels were significantly reduced on therapy.

Simultaneously, the mean levels of HDL were highly significantly increased after treatment for with rosuvastatin, atorvastatin, and simvastatin, when compared to negative control group. Also, reduction of LDL levels in rosuvastatin group was statistically significant when compared with those of simvastatin group ($p< 0.05$) but was statistically non-significant when compared with atorvastatin group ($P> 0.05$). This is in line with the study carried by (Samir et al, 2019), who found out that rosuvastatin, atorvastatin and simvastatin were very effective in reducing the level of serum cholesterol, triglyceride and LDL after treatment. The results of their finding revealed that all the three statins used in the study increased the level of HDL significantly ($P< 0.001$). The findings of the current research study are in accordance with Pilot study with rosuvastatin conducted by (Glueck et al, 2006).

CONCLUSION

It was concluded that among the treatments, rosuvastatin was more effective in reducing LDL-C, TG, body weight and also more efficient in raising HDL-C of alcohol-induced hypercholesterolemia female Wistar rat. However, when comparing all the treatment groups, there was no significant difference found in the rats' body weight, TG, LDL-C and HDL-C levels after 14 days. It was also concluded that Statins (Simvastatin, Atorvastatin and Rosuvastatin) could prevent alcohol-induced hypercholesterolemia and its related effects.

The findings underscore the complexity of treating hypercholesterolemia in the presence of alcohol consumption and highlight the need for further research. Exploring alternative treatments, investigating different dosages, and considering combinatory therapies could offer promising avenues to address the challenges posed by alcohol-induced hypercholesterolemia.

ACKNOWLEDGEMENT

The Author would like to thank the Supervisor (Mr Nweke Maduka L) and Co-Supervisor (Dr Ani Celestine O) for their assistance, contributions, invaluable guidance which were instrumental in shaping this research

Conflict Of Interest

None to declare

REFERENCES

Clearfield MB, Amerena J, Bassand JP, Hernández García HR, Miller SS, Sosef FF, Palmer MK, Bryzinski BS (2006). Comparison of the efficacy and safety of rosuvastatin 10mg and atorvastatin 20mg in high-risk patient with hypercholesterolemia-prospective study to evaluate the use of low doses of the statin atorvastatin and rosuvastatin (PULSAR) *Trials*, 7(35):72-85.

Dwi A, Yulia Y, Mohammaf A, Gemini A, Latifah R, Muhammad a, & Aryadi M (2022). The effects of simvastatin, rosuvastatin, and Fenofibrate on the body weight and lip profiles of female rats treated with oral contraceptives and a high-fat diet. *Journal Kedokteran Hewan*, 16(3):88-93.

Glueck C, Aregawi D, Agloria M, Khalil Q, Winiarska MJ (2006). Rosuvastatin 5 and 10mg/d: A Pilot study of the effects in hypercholesterolemia adults unable to tolerate other statins and reach LDL cholesterol goals with non-statin lipid-lowering therapies. *Clinical Therapeutics* 28(6):933-942

Grundy SM & Stone NJ (2018). American heart association/ American college of cardiology multisociety guideline on the management of blood cholesterol. Primary prevention. *JAMA Cardiology* 4(5) 488-489

Jamshidi L, Seif L & Moradi M (2017). Prevalence and Association between Metabolic Syndrome and Ischemic Heart Diseases in Patients Admitted to the Hospitals of Hamedan University of Medical Science. *Journal of Islamic Azad university of Mashhad 6(*7):103-110.

Jones PH, Davidson MH, Stein EA, Bays HE, Mckenney JM, Miller E, Cain VA, Blasetto JW, & STELLAR Study Group (2003). Comparison of safety and efficacy of rosuvastatin versus atorvastatin, simvastatin and pravastatin across doses (STELLAR trial) *The American journal of cardiology.* 92(2): 152-160.

Mckenney J (2015). Efficacy and safety of rosuvastatin in treatment of dyslipidemia Ameic, Journal of Health System Pharmacy, 62(10):1033-1047.

Mulazimmah (2017). Perbedaan pengaruh penggunaan kontrasepsi pil kombinasi dan kontrasepsi IUD terhadap perubahan berat badan pada Akseptor di wilayah Puskesmas Sukorame. *(Jurnal Nusantara Medika).* 2(1): 23-32.

Nadimi AE & Ahmadi J (2016). Lipid abnormalities in urban population of Rafsanjan. *Journal of Diabetes metabolic Disorder 6(*3):149-154

Navael L, Mchrabi Y, Azizi F (2019). Epidemiology of hyperlipidemia, obesity & increased blood pressure in rural areas of Tehran Provence. Iran Journal of Endocrinal metabolism 7(2): 253-262.

Ramsey LB, Johnson SG, Caudle KE, Haidar CE, Voora D & Wilke R (2016). The clinical pharmacognetic implementation consortium guideline for SLCOIRI and simvastatin-induced myopathy: 2014 update. *Clinical Pharmacology and Therapeutics*, 96(4):423-428.

Samir M, Mirza S, Gade P, & Khandelwal P (2019) A comparative evaluation of safety and efficacy of rosuvastatin, simvastatin and atorvastatin in patient of eye 2 diabetes mellitus with dyslipidemia. *International Journal of Diabetes in Developing Countries*, 29(2):74-79.

World Health Organisation (2015). Cardiovascular disease. Retrieved www.who.int/cardiovascular-disease/resources/atlea/en/.

World Health Organisation (2021). World Health Organization model list of essential medication, 22nd list (2021). Geneva: World Health Organisation