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Restoring Effect of Squalene Supplements on Rats Treated with Simvastatin

Thesis

Submitted to the Council of the College of Veterinary Medicine, University Of Kerbala in Partial Fulfillment of the Requirements for the Master Degree of Science in Veterinary Medicine / Physiology

by

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

﴿ فَتَعَٰلَى ٱللَّهُ ٱلۡمَلِكُ ٱلۡحَقُّۗ وَلَا تَعۡجَلۡ بِٱلۡقُرۡءَانِ مِن قَبۡلِ أَن يُقۡضَىٰٓ إِلَيۡكَ وَحۡيُهُۥۖ وَقُل رَّبِّ زِدۡنِى عِلۡمًا ﴾

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I hereby declare that this thesis is my origin work except for equations and citations which have been fully acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University of Kerbala or other institutions.

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/ / 2023

Dedication

To the one who fed me, watered me, and showered me with the abundance of grace, my creator and my beloved

To the one whose birth was a mercy to the worlds, and the ships of salvation, the Messenger of God, Muhammad, and the God of Purity, is my support

To the heart that overwhelmed me with love and tenderness, my father, may God prolong his life, and my late mother

To my support and my strength in life, the companionship of my life, and my path towards the future

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To the apple of my eye and my open flowers, my children Fatima, Hassan and Yaqin

To my arms, my loved ones, my dear brothers and sisters

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To the one who removed the fog of ignorance from me with the light of his knowledge and illuminated my path with the light of his knowledge from my teachers

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Summary

The study was undertaken to investigate how squalene suplement reversed the negative side effects of statins. The experiment was carried at the animal house of Veterinary Medicine / University of Kerbala during the period from (October 2022 to March 2023). Thirty adult male rats aged (2-3 months) weighting 260-300 gm were divided into two group, (experimental one) six rats dosage with normal saline only used as a negative control group and other (24 rats) administrated with cholesterol 1% for 28 days. Then the second group randomly divided to four groups (experimental two). The experiment lasted about during two months, the rats were administration as the following groups: First group: six rats used as a positive control group dosage with normal saline only after giving cholesterol 1% since 28 days, second group: six rats administrated with cholesterol 1% for 28 days then treated with simvastatin 40 mg/kg/BW dosage orally every day for 28 days, third group: six rats administrated with cholesterol 1% for 28 days then treated with squalene 2% orally every day for 28 days, forth group: six rats will have administrated with cholesterol 1% for 28 days then treated with simvastatin 40 mg/kg/BW dosage orally with squalene 2% every day for 28 days.

The cholesterol of the treated animals increased significantly at ($p<0.0001$) from that of the group that received just cholesterol without any additional treatment (positive control). As indicated, the outcomes significantly increased of creatine kinase (CK), hydrogen peroxide concentrations in simvastatin group while superoxide dismutase (SOD) concentrations significantly decreased in simvastatin group in male rats after four weeks of receiving cholesterol. Furthermore, the concentrations of creatine kinase (CK), hydrogen peroxide were significantly decreased in squalene

group and their combination of simvastatin with squalene, while SOD concentration increased significantly.

Histological changes in muscles demonstrate that cholesterol, and simvistatin treated rats showing muscle fibers atrophy, and significant pathological alterations represented in comparing to control group, however after squalene administration with simvistatin treated rats were able minimized or improved the pathological alterations condition of muscle fibers but not fully recovered.

It can be concluded that squalene interferes with testing animals' cholesterol levels. Squalene and simvastatin were found to be more effective together than simvastatin alone, as evidenced by the results showing that dietary supplementation with squalene at 2% level considerably reduced blood level cholesterol in experimental rats simultaneously with lowering creatine kinase. These findings imply that squalene does not interact with simvastatin via triggering feedforward cholesterol synthesis, but rather regulates the process offering a fresh opportunity to reduce myotoxicity.

Table of Contents

List of Figures

List of Tables

List of abbreviations

Abbreviations	**Meaning**
AMPK	activated protein kinase
ATP	Adenosine Triphosphate
CHD	coronary heart disease
CK	creatine kinase
CoA	acetyl-coenzyme A
CRD	Chronic Rnal Disorder
CVD	cardiovascular disease
CYP	cytochrome P3A450
DMD	Duchenne muscular dystrophy
FDPS	farnesyl diphosphate synthase
FPP	farnesyl pyrophosphate
FTase	Farnesyltransferase
GATM	glycine amidinotransferase
GGPP	geranylgeranyl pyrophosphate
GGTase	geranylgeranyl transferases

GPX4	Glutathione peroxidase 4
H&E	Hematoxylin and Eosin stain
HDL	High density lipoprotein
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
IPP	isopentenyl pyrophosphate
LDL	LOW density lipoprotein
mg/dl	Milligrams per deciliter
mg/kg	Milligrams kilogram
mol H2O2Eq/L	micromolar hydrogen peroxide equivalent per liter
µg/g	Microgram gram
MVA	Metabolic pathways of mevalonate
NADPH	Nicotinamide Adenine Dinuc
PVD	peripheral vascular disease
Q10	Ubiquinone
ROS	reactive oxygen species
SAMS	Statin Associated Muscle Symptoms

SOD	Superoxide dismutase
SQ	Squalene
STAT3	Signal Transducer and Activator of Transcription 3
TOS	Total oxidant status
VLDL	Very low density lipoprotein
VOO	virgin olive oil

Chapter One: Introduction

1. Introduction

Hypercholesterolemia is the most common cause of increased atherogenic risk, and both genetic disorders and a diet high in saturated fat and cholesterol contribute to our population's and many other developed countries' elevated levels, which are well-known risk factors for cardiovascular diseases (Reddy *et al.*, 2006; Vijayan *et al.*, 2018; Belete *et al.*, 2023). Cholesterol is synthesized from acetyl-coenzyme A (CoA) via more than 30 enzymatic reactions, making this pathway time- and energy-consuming (Abdullah *et al.*, 2018).

Statins are fungal-derived molecules that became powerful cholesterol-lowering medications by inhibiting the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase enzyme, a key step in the sterol biosynthetic pathway (Sirtori, 2014). Statin, a hypercholesterolemia treatment, is used by approximately 200 million people worldwide (Peyrel *et al.*, 2023). Some adverse muscle issues include muscle spasms, myalgia, a lack of strength, immune-mediated necrotizing myopathy, and, less commonly, rhabdomyolysis associated with utilizing statins. (Rosenson *et al.*, 2014; Mendes *et al.*, 2014; Stroes *et al.*, 2015), simvastatin being the most commonly implicated (Iatan *et al.*, 2023). Neuromuscular side effects make up about two thirds of all statin-related adverse events. Additionally, they can cause peripheral neuropathy or reveal an existing neuromuscular junction dysfunction (Mollazadeh *et al.*, 2021; Attardo *et al.*, 2022; Jawad *et al.*, 2022).

The phenomenon of ‘statin intolerance’ and the associated cessation of therapy (or reduction in dose) is related with intension risk of myocardial infarction and coronary heart disease and a combined concequence of myocardial infarction, stroke, or death (Orringer *et al.*, 2022). As a result, it's mandatory that practitioners have clear guidelines for handling muscle complaints in people on statin therapy. Among the key regulators of cellular metabolism produced by the mevalonate

pathway (MVA) include lipoproteins, dolichol, ubiquinone, squalene, or cholesterol derived compounds which inhibited by statins (Jawad *et al.*, 2022). Squalene (SQ) is a metabolite involved in the biosynthesis of cholesterol that is oxidized by monooxygenase in the early stages of metabolism. Squalene has a lot of interesting activities, including antioxidant and antitumor effects (Katdare *et al.*, 1997; Owen *et al.*, 2004; Martín-Peláez *et al.*, 2013; Guasch-Ferré and Willett 2021; Sánchez-Quesada *et al.*, 2022), lowering the serum cholesterol level (Trichopoulou *et al.*, 2000; Aguilera *et al.*, 2005) as well as having cardio-protective properties (Ward *et al.*, 2019), Squalene is the primary triterpene hydrocarbon found in virgin olive oil (Lou-Bonafonte *et al.*, 2018). In comparison to other vegetable fats, virgin olive oil (VOO) contains the most SQ (Newmark, 1999; Gaforio *et al.*, 2019; Gutiérrez-Luna *et al.*, 2022). However, cholesterol accumulation severely inhibits its monooxygenase activity (Yoshioka *et al.*, 2020). In order to treat hypercholesterolemia, targeting this biochemical process with SQ offers a promising therapeutic approach that can be used in combination with statins treatment.

Aim of the Study

The present study conducted to observe effect of squalene supplements on rats treated with simvastatin to avoid side effect of statin via performing the following objectives:

1- Serum cholesterol concentration.

2- Serum creatine kinase (CK) estimation.

4- Determination of serum free oxygen radicals (oxidants).

5- Determination of serum superoxide dismutase (SOD) concentration.

6- Histopathological changes of muscles.

Chapter Two: Review of the Related Literature

2. Review of the Related Literature

2.1. Hypercholesterolemia

Hypercholesterolemia is the medical term for elevated levels of cholesterol (Johansen *et al.,* 2021). Hyperlipoproteinemia represents a widespread health problem in the global population: indeed, levels of lipoproteins (Lp) >50 mg/dL have been found in 10–30% of the world population, with an estimated 1.43 billion people affected in the world (Tsimikas *et al.,* 2018). Lipids are macromolecules that serve as energy storage units, participants in intracellular signaling cascades, and critical elements of cell membranes. Cholesterol, sphingolipids, and glycerophospholipids are the three main lipid types present in membranes (Guerra and Issinger, 2020). Forty–ninety percent of the total cholesterol is found in the plasma membrane (Litvinov *et al.*, 2018). A crucial part of the plasma membrane of mammalian cells is cholesterol. Numerous cellular processes, including as cell signaling, as well as cell survival, development, and proliferation, depend on it (Rosenhouse-Dantsker *et al.,* 2022).

High blood cholesterol levels are referred as hypercholesterolemia. It is a combination of hyperlipoproteinemia and hyperlipidemia. A sterol, or type of a lipid, is cholesterol. All animal cells produce it because it belongs to one of the three main classes of lipids that are used to build membranes in animal cells. It is also a precursor to bile acid, vitamin D, and all steroid hormones. The bloodstream carries cholesterol around with it. It is a chemical that is necessary for the human body (Vijayan *et al.,* 2018).

Cholesterol is transported in the blood plasma by protein particles called lipoproteins because it is insoluble in water. Very low density lipoprotein (VLDL), Intermediate density lipoprotein (IDL), Low density lipoprotein (LDL), and High density lipoprotein are the different types of lipoprotein based on their density. Although all lipoproteins include cholesterol, but increasing levels of non-HDL lipoprotein, especially LDL-cholesterol, are linked to a higher risk of atherosclerosis and coronary heart disease (Kamstrup, 2021; Korneva *et al.*, 2021; Kosmas *et al.*, 2023). Higher HDL cholesterol levels, on the other hand, are protective. An elevated lipoproteins level is a strong, causal, and independent risk factor for CVD through multiple pathogenetic mechanisms: proatherogenic, prothrombotic, and proinflammatory (Enas *et al.*, 2019).

Experimental evidence from in vitro studies and animal models shows lipoproteins promotion of atherosclerotic plaque formation through various mechanisms, such as smooth muscle cell proliferation, foam cells formation, and increased expression of IL-8, a key mediator of plaque formation, from inflammatory cells (Boffa *et al.*, 2004; Deb and Caplice, 2004), lipoproteins can interact with the major components of the extracellular matrix (fibrin, fibronectin, proteoglycans, tretranectin, and beta2-glycoprotein) (Kapetanopoulos *et al.*, 2002). The binding of lipoproteins to fibrin has been proposed as the mechanism that allows for the delivering of cholesterol to sites of injury, where it is involved in the repairing of the vascular wall. It has also been observed that lipoproteins are able to bind oxidized phospholipids in plasma by forming covalent bonds. Levels of lipoproteins and oxidized phospholipids in plasma predicted the risk of the incidence of CVD (Boffa *et al.*, 2019).

Increased levels of LDL and non-HDL cholesterol in the blood can result from poor diet (Herfel, 2022), inherited (genetic) diseases associated with obesity

(Gomes *et al.,* 2023), or other diseases like diabetes or an underactive thyroid. Reducing dietary intake is advised to lower overall (Biondi *et al.,* 2019).

Genetic and observational evidence support a causal role of lipoprotein in the development of CVD, peripheral arterial disease and heart failure (Arathimos *et al.,* 2019). Hyperlipoproteinemia represents a widespread health problem in the global population (Vinci *et al.,* 2023). The main factor increasing atherogenic risk is hypercholesterolemia, and the raised levels of both our population and many other affluent nations worldwide are a result of both inherited disorders and diets high in saturated fat and cholesterol. Drugs that lower cholesterol levels were developed as a result of the identification of hypercholesterolemia as some risk factors (Chang *et al.,* 2023).

Hypercholesterolemia is the primary cause of atherosclerosis and conditions linked to atherosclerosis like coronary heart disease, ischemic cerebrovascular disease, and peripheral vascular disease (Roeters van Lennep *et al.,* 2023). Atherosclerosis is the accumulation of fats, cholesterol, and other substances in and around the artery walls, which increases the risk of cardiovascular disease (Istvan, 2002 and Wu *et al.,* 2022). Although the correlation between cholesterol, food, and coronary heart disease (CHD) was identified almost 50 years ago (Ding *et al.,* 2022), substantial epidemiological research and preclinical trials were needed to demonstrate that decreasing cholesterol was safe and decreased CHD death (Yusuf *et al.,* 2001). The primary cause of death is cardiovascular disease (CVD), this could be brought on by a poor lifestyle, inherited traits (Reyes-Soffer *et al.,* 2022), or both (Bhotla *et al.,* 2023).

2.2. Statins

Statins or 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) inhibitors belong to the class of lipid-lowering agents (Ramkumar *et al.,* 2016). HMG-CoA reductase is a rate-limiting enzyme that catalyzes an early mevalonate pathway reaction. Inhibiting HMG-CoA reductase lowers cellular cholesterol synthesis while also inhibiting the formation of other compounds produced via the same pathway. Coenzyme Q10, heme-A, and isoprenoids are a few of the additional products (Golomb and Evans, 2008 and Pinal-Fernandez *et al.,* 2018). Statins are used widely for the treatment of hypercholesterolemia. They lower LDL levels more than other cholesterol-lowering medications because they competitively inhibit HMG-CoA reductase. Angiographic investigations have shown that these chemicals slow the advancement of atherosclerosis and may even promote its regression (Tarsitano *et al.,* 2022).

Many clinical investigations found that these effects resulted in considerable decreases in cardiovascular morbidity and mortality (Vaughan *et al.,* 2000). Statins affect a number of processes, including the accumulation of esterified cholesterol in macrophages, the elevated level of endothelial NO synthetase, the reduction of inflammation, the improved stability of atherosclerotic plaques, the reactivation of platelet activity, and the coagulation process (Cimmino *et al.,* 2023). The beneficial properties of HMG-CoA reductase inhibitors are typically attributed to their ability to decrease endogenous cholesterol production by competingly blocking the main enzyme involved (Hunninghake, 1992). Considering mevalonate, the product of the HMG CoA reductase process, is the precursor for not just cholesterol but also many

other non-steroidal isoprenoidic substances, inhibiting this essential enzyme may have pleiotropic effects (Bezaire, 2023).

They are classified into two types: those that involve lipids directly and those that involve intracellular signaling pathway. The first group includes: reduction of cholesterol production, increased absorption and breakdown of low density lipoproteins (LDL), inhibition of lipoprotein synthesis, inhibition of LDL oxidation, and inhibition of scavenger receptor expression. Patients with dyslipidemia and even those with coronary artery disease, diabetes mellitus, stroke, blood hypertension, and chronic kidney disease with or without dyslipidemia are routinely recommended statins. Statins provide benefits that go beyond just lowering cholesterol levels, or "pleiotropic effects," such as anti-inflammatory, antioxidant, anti-proliferative, anti-apoptotic, cell cycle regulating, and immunomodulatory actions (Mohammadkhani *et al.*, 2019). Statins are fungus-derived compounds that were developed into effective cholesterol-lowering drugs by blocking the hydroxymethylglutarylcoA (HMG-CoA) reductase enzyme, a critical step in the sterol biosynthesis process (Sirtori, 2014). Statins may be categorized into two groups: lipophilic and hydrophilic, both of which exhibit "pleiotropic effects" (Oesterle and Liao, 2019). Lovastatin, Simvastatin, Fluvastatin, Atorvastatin, and Pitavastatin are examples of lipophilic statins that passively diffuse across cell membranes and are not particularly selective for hepatic tissues (Climent *et al.*, 2021).

Rosuvastatin and pravastatin, two hydrophilic statins, need active carrier-mediated transport since they cannot penetrate cell membranes. For hepatic tissues, they are more selective (Oesterle and Liao, 2019). The production of isopentenylpyrophosphate, dimethylallylpyrophosphate, geranyl-pyrophosphate, and

farnesylpyrophosphate, among other isoprenoid intermediates, may also be inhibited by statins. Isoprenoids are key players in membrane attachment signaling molecules and post-translational modifications (Perez-Matos *et al.*, 2017). It has been observed that some patients have had neuromuscular symptoms after the use of statins, which has been cited as one of the major reasons for stopping the use of statins and may, in turn, increase the risk of cardiovascular events (Alonso *et al*., 2019). Five- ten percent of people on statins have negative effects on their skeletal muscles (Pohjola-Sintonen and Julkunen, 2014).

Additionally, the use of statins lowers the production of isoprenoids such geranylgeranyl pyrophosphate and farnesyl pyrophosphate, which in turn lowers Rho-associated protein kinase (Hussien *et al.*, 2021). Statins' pleiotropic actions, which include immunological regulation, endothelial cell protection, and cardioprotection, are caused by the inhibition of protein prenylation (Al-Kuraishy *et al.*, 2020 and Hussien *et al.*, 2021). Similar to this, protein prenylation inhibition is associated with the emergence of negative consequences such myalgia, neuropathy, and hyperglycemia (Al-Kuraishy *et al.*, 2019). Effects Drug Pharmacodynamics Food may increase a drug's bioavailability by altering how it is absorbed, metabolized, or excreted. Known medication interactions with nutrients that turnover may eliminate this might result in overgrowth, unsuccessful treatment, this causes it to be tall. This results in either treatment failure or greater availability, which raises the possibility of budget drug swaps (Bloland *et al.*, 2003).

It could result in poisoning. The most popular and effective medications for treating hypercholesterolemia and managing people at higher risk for cardiovascular disease are statins (Schachter, 2005). At the first stage of cholesterol production,

statins function as the competitive inhibitors of 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase, preventing the conversion of HMG-CoA to mevalonate (Stancu and Sima, 2001; Goldstein and Brown, 2015). In individuals with and without diabetes who have proteinuria, PLANET I and II renal effects of atorvastatin and rosuvastatin (Waters, 2000). Although statins have a well-known positive impact, it is important to be aware of their adverse effects, which may range from mild and asymptomatic to severe and life-threatening, particularly for the function of the liver and kidneys (Behling *et al.,* 2017).

2.2.1. Various Statins

Statins come in two varieties: synthetic including atorvastatin, fluvastatin, cerivastatin, and rosuvastatin and natural lovastatin, mevastatin, etc. (Kadhim *et al.,*2020 and Al-Rubiay *et al.,* 2021). As semisynthetic statins, simvastatin and pravastatin are examples of statins generated via fermentation (Al-Kuraishy *et al.,* 2018). Additionally, statins are divided into lipid-soluble (such as atorvastatin) and water-soluble (such as rosuvastatin) categories based on their lipophilicity (Al-Kuraishy and Al-Gareeb, 2019 and Al-Kuraishy *et al.*,2021).

2.2.2. Simvastatin

Although all statins work in the same way, they are classified into two types based on their origin: fungus-derived lovastatin, simvastatin, mevastatin, and pravastatin, and synthetic atorvastatin, cerivastatin, fluvastatin, pitavastatin, and rosuvastatin. Statins are hepatoselective, meaning that the liver absorbs the majority of the statins ingested from the gastrointestinal system. The remaining molecules bind to plasma proteins with great affinity, resulting in systemic exposure to free drug (Bond *et al.,* 2022).

Simvastatin and atorvastatin can decrease LDL in those with homozygous familial hypercholesterolemia who lack functioning LDL receptors (Newman *et al.*, 1997). Simvastatin, pravastatin, and lovastatin come from the fermentation of fungi (Subhan *et al.*, 2016).

According to (Knoblauch *et al.*, 2013) study, simvastatin was given to mdx mice at low to moderate human equivalent dosages, and the results showed both short- and long-term improvements in total left ventricular function. Simvastatin's key advantages are the avoidance of cardiac fibrosis, prolonged improvement in diastolic function, and enhanced heart rate variability as a result of increased parasympathetic activity (Fontana, 2018).

Statins are categorised according to their improved potency and efficacy in decreasing plasma LDL cholesterol concentrations, as well as their physicochemical features. The classification of statins based on these two characteristics (Hussain *et al.*, 2023). The first generation of statins hit the market in the late 1980s and early 1990s (Egom and Hafeez, 2016 and Azemawah *et al.*, 2019). This statin generation includes pravastatin, lovastatin, and fluvastatin, and it has the lowest potency (Egom and Hafeez, 2016).

The second generation of statins includes simvastatin and atorvastatin, which are the best-selling statins on the market. Second-generation statins were more effective than first-generation statins, with daily doses of only 10 mg atorvastatin and 20 mg simvastatin causing greater than a 30% reduction in LDL compared to first-generation statins' 20-40 mg daily doses (Azemawah *et al.*, 2019 and Climent *et al.*, 2021). The third generation is referred to as "super statins," because it has the maximum potency and efficacy. These third-generation statins are used in high-risk

patients who are more prone to develop statin intolerance than previous statins on the market (Egom and Hafeez, 2016), as showen in figure (2-1).

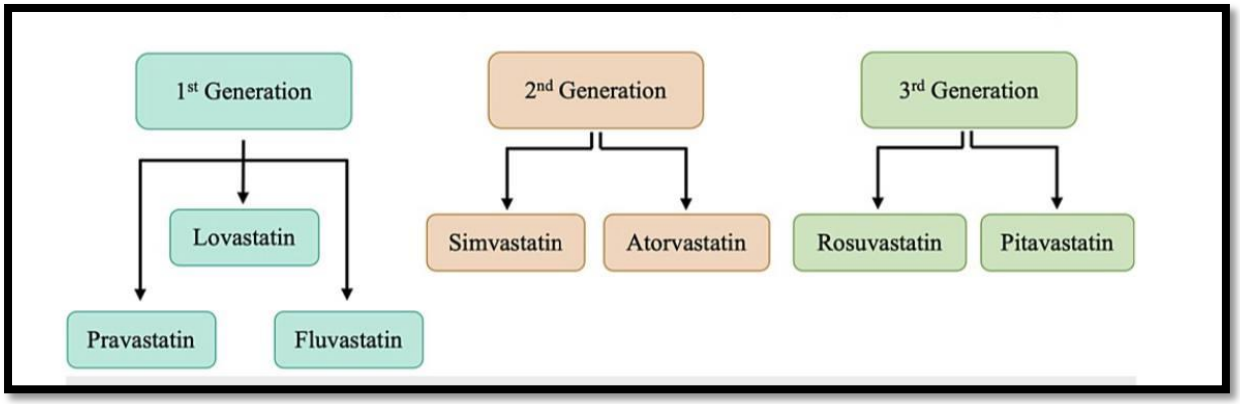


Figure (2-1): Classification of statins (Hussain *et al.,* 2023)

Statins can also be classed based on their physicochemical characteristics, such as hydrophilic or lipophilic, which refer to their capacity to dissolve in water or a lipid-containing solution. Lipophilicity and hydrophilicity can coexist. The absorption and excretion of a medication are eventually determined. In this regard, absorption is faster with Lipophilic medicines are easier to excrete in the kidney than hydrophilic statins (Climent *et al.,* 2021). The hydrophilic or Statins' lipophilic nature influences their entry into cells and transportation throughout the body. The majority of statins on the market today are lipophilic, with a few exceptions. Rosuvastatin and pravastatin are the only hydrophilic statins. Lipophilic statins can easily pass through and enter cell membranes, interacting with the acyl chains that surround them (Climent *et al.,* 2021 and Ramkumar *et al.,* 2016). Hydrophilic statins, on the other hand, remain bound to the polar surface of the cellular membrane. Protein transporters are normally required to enter the cell in order to inhibit HMG-CoA reductase (Climent *et al.,* 2021). Lipophilic statins are associated with more extrahepatic manifestations, such as a higher incidence of statin-related muscle complaints, since they can go deeper into the membranes (Climent *et al.,* 2021). Hydrophilic statins, on the other hand, are likely to have less muscle penetration and so pose a lesser risk. Still, an ongoing cohort research revealed that hydrophilic statins were not tolerated better (Althanoon *et al.*, 2020

and Climent *et al.,* 2021). Lipophilic statins have been theorized to have a higher risk of neurocognitive effects due to their enhanced capacity to pass the blood-brain barrier (BBB), (Climent *et al.,* 2021).

Simvastatin's clinical effects which reduced LDL by 35% compared to pravastatin's 28% reduction were substantially more pronounced (Stein *et al.,* 1998).

Chronic simvastatin administration in rabbits causes membrane hyperexcitability, which has previously been reported in muscle myopathies associated with reduced chloride conductance, impaired Cl^- ion channel function can affect membrane depolarization and change the resting membrane potential. (Sirvent *et al.,* 2008 and Tomaszewski *et al.,* 2011).

Statin-induced myopathy is more likely to occur at larger doses or with more powerful statins. A recent study looked at the incidence of statin-induced myopathy in patients and found that simvastatin had the greatest incidence while fluvastatin and rosuvastatin had the lowest (Egom and Hafeez, 2016).

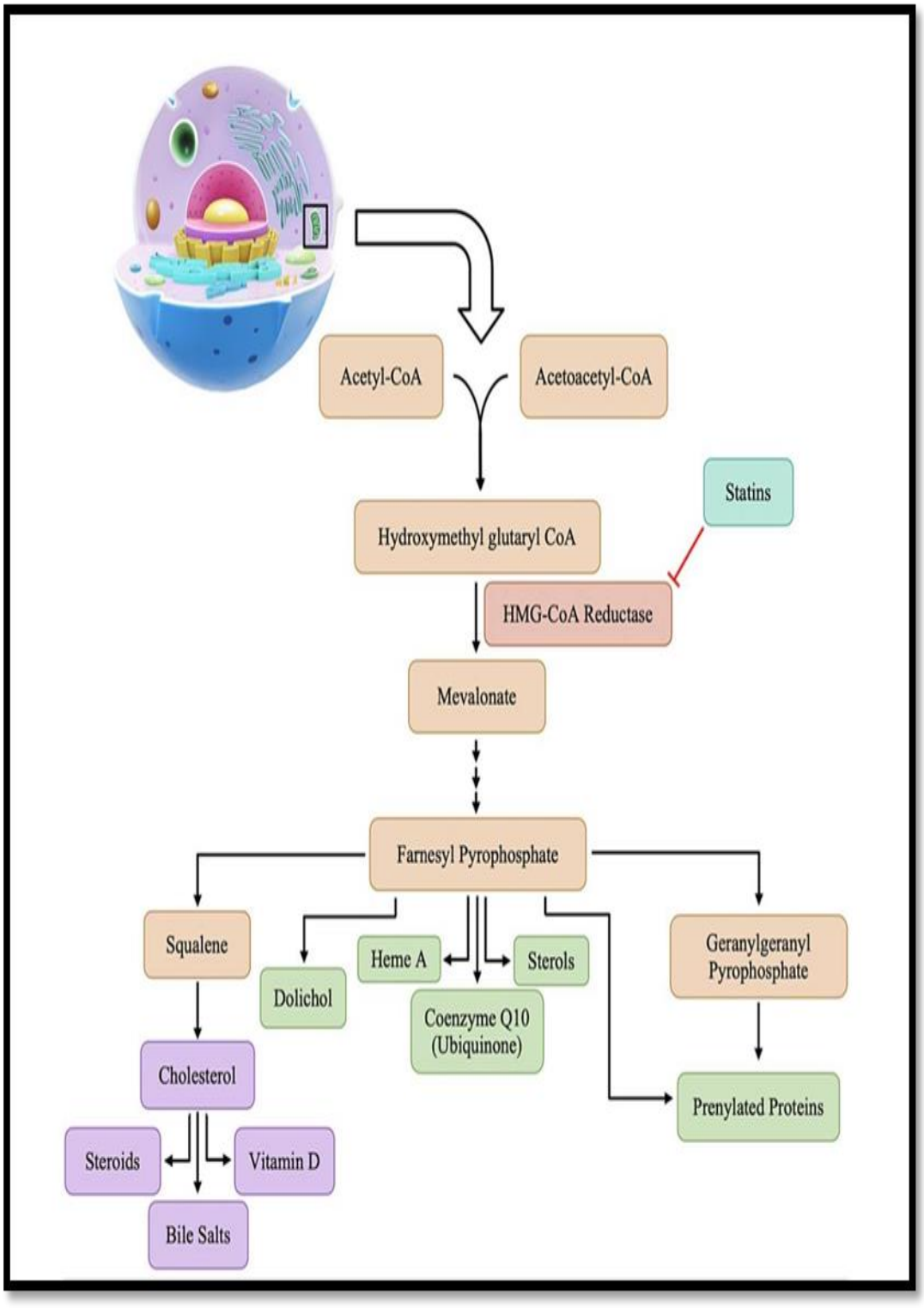
2.2.3. Mechanism Action of Statins

The enzyme HMG-CoA reductase, which catalyzes the rate-limiting step in cholesterol production, is competitively inhibited by statins. The mevalonate pathway is a critical metabolic pathway for supplying bioactive chemicals to cells, which is critical for cellular activities (Buhaescu and Izzedine, 2007). Cholesterol is synthesized in the cytoplasm and endoplasmic reticulum membranes of all human organs, particularly the liver, intestine, adrenal cortex, and reproductive tissue (Egom and Hafeez, 2016), as showen in figure (2-2).

Statins inhibit HMG-CoA reductase by a catalytic mechanism (Buhaescu and Izzedine, 2007). Statins will occupy the catalytic part of the enzyme HMG-CoA

reductase, which is ordinarily specifically bound to the substrate HMG-CoA, preventing the substrate from accessing the enzyme's active site (Istvan, 2002). As decreasing cholesterol levels is the primary therapeutic aim behind statin administration, decreased production of other downstream products is also observed (Pinal-Fernandez *et al.,* 2018). Statins activate the formation of microsomal HMG-CoA reductase and cell surface low-density lipoprotein (LDL) receptors, leading to an increased clearing of LDL from the bloodstream, expressed in lowered blood LDL levels ranging from 20-55% (Pinal-Fernandez *et al.,* 2018).

Although continuing cholesterol synthesis in human tissues, the liver creates around 70% of total body cholesterol. Blood cholesterol levels will start to decrease once the liver stops producing (Go *et al.,* 2013 and Egom and Hafeez, 2016). Following the formation of mevalonate, a number of events ensue that are catalyzed by many other enzymes, and the end products of these reactions include cholesterol, amongst many others. Cholesterol also aids in the creation of steroids, vitamin D, and bile salts, which are all required for regular body activities (Noh *et al.,* 2022).



(Figure 2-2): Role of statins inhibition cholesterol synthesis (Hussain *et al.*, 2023)

2.2.4. Adverse Effects of Statin

The adverse effect of statins varies based on both the dose and the type of statin used. Lipophilic statins have a greater ability to infiltrate membranes and have been related to statin-induced myopathies in addition to neurocognitive effects by considerably crossing the blood-brain barrier (Hussain *et al.,* 2023).

The risk of myopathy/rhabdomyolysis varies between statins due to varying pharmacokinetic profiles (Maji *et al.,* 2013). Muscle and liver damage are the most significant negative effects. All statins have a 1% incidence of transaminase elevations more than 3-fold, and this incidence is dosage dependent (Shepherd *et al.,* 1995; Sacks *et al.,* 1996; Downs *et al.,* 1998). Transaminase levels typically recover to baseline in two to three months after this occurs, therefore the medication should be stopped (Bradford *et al.,* 1991).

Myopathy, or muscle pain or weakening linked with creatine kinase (CK) levels more than 10 times the upper limit of normal, is the main side effect of statins (Sewright *et al.,* 2007). Fever and malaise are possible symptoms, and cases have been linked to increased serum statin medication levels. If myopathy is not identified and the medication is used, rhabdomyolysis and severe renal failure may occur (Pierce *et al.,* 1990). After confirmed instances of significant myopathy/rhabdomyolysis, the first statin, cerivastatin, was taken off the market in 2001 (McKenney *et al.,* 2006).

According to Jacobson, (2008), asymptomatic elevations of liver enzymes and musculoskeletal problems such myalgia, myopathy, and rhabdomyolysis were the

most significant side effects linked to statins, by using it, 5% of patients developed myalgia, 0.1% to 0.2% developed myopathy, and 0.01% developed rhabdomyolysis.

Statins have transformed the treatment of people who are at high risk of cardiovascular disease. The decision to utilize statins in patients with a history of cardiovascular disease is significantly more straightforward than the decision to give statins to high-risk individuals. Because of the pleiotropic nature of statin medications, the use of a statin in those with a history of cardiovascular disease may prevent the development of other disorders. However, statin therapy for high-risk individuals, such as those with other conditions such as immunosuppression, autoimmune diseases, and so on, must be much more carefully considered. In patients with such situations, statins' unfavorable profile may cause more harm than good (Figorilli *et al.,* 2023).

Because of the profile of adverse effects, healthcare practitioners must do a harm vs benefit analysis to determine whether statin medication should be delivered (Hussain *et al.,* 2023).

According to observational studies, myalgia, tiredness, and more severe muscle complaints with significant CK increases affect between 10 and 15 percent of statin users (Abd and Jacobson, 2011). Muscle disease and statins due to their recognized negative effects, statins are often avoided by people with muscle disease (Attardo *et al.,* 2022).

2.2.4.1. Myopathy

A generalized muscle disease known as myopathy or myositis discomfort associated with an increase in plasma creatine kinase (CK) concentration that is 10 times higher than the upper maximum of normal (Hamilton-Craig, 2001). Pain,

discomfort, weakness brought on by intense pain, and mobility restrictions are typically present. It is typically characterized by the presence of pain, soreness, weakness brought on by intense pain, and mobility restriction (Ruscica *et al.,* 2022). The development of myopathic symptoms in patients with normal CK levels has also been linked to statin therapy, proving that CK testing alone is insufficient to predict statin-associated myopathy (Mohaupt *et al.,* 2009).

In the absence of high CK levels, statin-taking patients' discomfort may potentially result from the structural breakdown of muscle fibers (Egom and Hafeez, 2016). Although myopathy is a class effect of statins, each statin has a different chance of causing it. The use of synthetic, powerful, and more lipophilic statins has generally been associated with a greater reporting of these muscle side effects. Although myopathy is a class effect of statins, each statin has a different chance of causing it. The use of synthetic, powerful, and more lipophilic statins has generally been associated with a greater reporting of these muscle side effects (Abd and Jacobson, 2011).

The manifestation of statin-induced myopathy varies from patient to patient, which could be attributable to a variety of variables. Advanced age, female gender, Asian ancestry, and diabetes are the most major risk factors for statin-induced myopathies. Excessive physical activity, muscle, liver, or chronic renal disease, medications that change statin plasma levels hypothyroidism that is uncontrolled, abdominal obesity, metabolic syndrome, and vitamin D insufficiency (Sewright *et al.*, 2007 and Bagley *et al.,* 2007). Each risk factor enhances the vulnerability to statin- induced bad effects by decreasing the individual's immunological response, making the individual more susceptible to unfavorable effects (Marx *et al.,* 2023).

2.2.4.2. Rhabdomyolysis

Rhabdomyolysis is one of the primary side effects of statins, particularly when used alongside fibrate lipid-lowering medications (Michaeli *et al.,* 2023). Myoglobinemia, myoglobinuria, and myoglobin-induced acute renal failure (oliguria, elevated plasma creatinine, potassium, and phosphorus) are symptoms of rhabdomyolysis. There is also a notable 50-fold rise in CK activity (Beltowski *et al.*, 2009). Patients receiving the statins atorvastatin, simvastatin, and lovastatin reported greater rates of rhabdomyolysis. In the more extreme cases, myopathy can take the form of rhabdomyolysis, a condition in which patients have CK levels > 10,000 IU/L (Golomb and Evans, 2008; Azemawah *et al.,* 2019). Rhabdomyolysis is a pathological syndrome in which muscle degeneration occurs and the circulatory system becomes infested with intracellular species such as myoglobin (Sewright *et al.,* 2007). This was brought on by a greater rate of statin metabolism by cytochrome P3A450 (CYP) isoenzymes in the liver (Banach *et al.,* 2015).

2.2.5. Monitoring for Muscle Toxicity

The measurement of CK at baseline might be helpful. All statins, small, clinically inconsequential elevations in transaminases and CK are frequently noticed (Illingworth and Tobert, 1994). Regular CK follow-up tests are typically not advised because severe myopathy typically develops quickly and is not preceded by long-term CK increases (Newman *et al.,* 2019). Patients should be advised to call their doctor if

they develop acute malaise, flu-like symptoms, or muscle discomfort or weakness. If this happens, statin therapy should be stopped and the CK level as soon as possible should be checked. After the CK level returns to normal, many specialists would suggest resuming treatment with a new statin, starting with a low dose and closely watching for symptoms and high CK (Bolster *et al.,* 2023).

2.3. Squalene

2.3.1. Squalene Synthesis

Squalene is a triterpene with the formula C30H50, an intermediate for the biosynthesis of phytosterol or cholesterol in plants/animals and humans, widespread in animal and vegetal kingdom. Scientists have discovered that, at the moment life appeared on Earth, microorganisms, and later in Precambrian the cells' membrane of higher organisms, contained, in a great proportion, squalene, a substance likely to be essential to their survival in that hostile environment free of oxygen (Terletskaya *et al.,* 2023).

In human body squalene is synthesized by the liver and is secreted in large quantities by the sebaceous glands. It is transported in the blood by the small and very small density lipoproteins. It is interesting to notice that squalene represents 12% of the lipids secreted by the sebaceous glands and it is not transformed in cholesterol (Li *et al.,* 2023).

The highest squalene concentrations in human is met in skin lipids, about 500 μg/g, and in the adipose tissue, 300 μg/g while in organs where the active

biosynthesis takes place, the concentration is much smaller, as in liver 75 μg/g or small intestine 42 μg/g (Kumar *et al.*, 2023).

Squalene was discovered in 1906 by the Japanese Researcher Dr. Mitsumaru Tsujimoto, an expert in oils and fats at Tokyo Industrial Testing Station. He separated the unsaponifiable fraction from the shark liver oil "kuroko-zame" and discovered the existence of a highly unsaturated hydrocarbon. Ten years later, Tsujimoto succeeded to obtain by fractional vacuum of the liver oil from two deep-sea shark species an unsaturated hydrocarbon, which he named "squalene" (Gohil *et al.*, 2019).

Squalene is a bioactive substance, which is mostly present in shark oils and olive oils. Due to its abundance, this substance is a perfect research subject for understanding its positive impacts on the human body (Klopčič *et al.*, 2023). In both plants and animals, it acts as an intermediary in the manufacture of phytosterol or cholesterol. Squalene content in humans is highest in newborns and starts to significantly decline between the ages of 30 and 40 (Popa *et al*., 2015). In human liver and skin are most of the squalene is made. Low density lipoproteins (LDL) are then responsible for carrying it through the blood, and finally secreted by the sebaceous glands in large quantities (Reddy and Couvreur, 2009).

2.3.2. Reduction of Oxidative Stress

Squalene synthase plays an important role in the cholesterol biosynthesis pathway as it is responsible for the flow of metabolites into either the sterol or the non-sterol branches of the pathway. The synthesis of squalene and its release in the endoplasmic reticulum protects the cell against lipid peroxidation, resulting in cell survival (Picón and Skouta, 2023). Squalene has emerged as a promising therapeutic target for

various diseases, including cancers, owing to its pivotal role in the mevalonate pathway and the antioxidant properties of squalene (Picón and Skouta, 2023).

In addition, variants of the squalene synthase gene appear to modulate plasma cholesterol levels in human populations and therefore may be linked to cardiovascular disease (Do *et al.*, 2009).

Squalene is not very susceptible to peroxidation and appears to function in the skin as a quencher of singlet oxygen, protecting human skin surface from lipid peroxidation due to exposure to UV and other sources of ionizing radiation. Supplementation of squalene to mice has resulted in marked increases in cellular and non-specific immune functions in a dose-dependent manner. Squalene may also act as a "sink" for highly lipophilic xenobiotics. Since it is a nonpolar substance, it has a higher affinity for un-ionized drugs. In animals, supplementation of the diet with squalene can reduce cholesterol and triglyceride levels. In humans, squalene might be a useful addition to potentiate the effects of some cholesterol-lowering drugs. The primary therapeutic use of squalene currently is as an adjunctive therapy in a variety of cancers (Kelly, 1999).

Conforti *et al.* (2005) reported an antioxidant effect of squalene in a model of lipid peroxidation of liposomes; the IC50 value for squalene was 0.023 mg/mL. An ethyl acetate extract of Amaranthus caudatus examined using the same method was 20-fold less active. The regeneration of a-tocopherol by squalene in photo-oxidation studies was suggested by Psomiadou and Tsimidou, (2002). This is in line with the hypothesis of Kohno *et al.* (1995).

Squalene showed slight antioxidant activity when assayed by the crocin bleaching method (Finotti *et al.*, 2000). In the same study, squalene demonstrated a

synergistic effect with a-tocopherol and bsitosterol. The authors suggested that squalene could act as a competitive compound in the crocin bleaching reaction, thereby reducing the rate of oxidation. Results of Dessi *et al.* (2002), showed that during temperature-dependent autoxidation and UVA-mediated oxidation, squalene acts mainly as a peroxyl radical scavenger (Seçmeler and Galanakis, 2019). Squalene has been reported to have beneficial health effects due to its antioxidant (Amarowicz, 2009), and anti-inflammatory properties (Martirosyan *et al.*, 2022 and Mirmiranpour *et al.*, 2022).

2.3.3. Therapeutic Uses of Squalene

The name came from the denomination of the sharks' family: Squalidae (Popa *et al.*, 2015). The primary triterpene hydrocarbon found in VOO is squalene (SQ). Compared to other vegetable fats, VOO is the source of SQ that is most abundant (Newmark, 1999). Squalene is known to have several intriguing properties, including antioxidant and anticancer actions (Owen *et al.*, 2004).

Producers of squalene Sharks are now recognized as one of the main sources of squalene (Randhir *et al.*, 2020 and Czaplicki *et al.*, 2012). Shark hunting has expanded quickly due to the high squalene content. As of now point being, with 90% use, the cosmetic business leads all other industries in squalene utilization. Sharks are in danger of becoming extinct because 2.7 million shark livers must be harvested each year to satisfy the squalene demands of the beauty business. Additionally, when chemical pollutants and marine pollution increase, so does the quality of shark squalene (Patel *et al.*, 2020 and Bagul, and Annapure, 2020). It is a difficulty for long-term manufacturing when squalene quality and quantity are so unpredictable.

Researchers have thought about finding alternate sustainable sources for the manufacture of squalene, nevertheless, owing to the unpleasant smell of squalene generated by sharks (Song *et al.,* 2010 and Yin *et al.,* 2018). Numerous plants, including rice bran, amaranth, wheat germ, and olive oil, have been shown to be capable of producing squalene by researchers (Spanova and Daum 2011; Czaplicki *et al.,* 2012; Rosales-Garcia *et al.,* 2017; Xie *et al.,* 2017). These plants are regarded as effective substitutes for sharks in the squalene synthesis process (Yarkent and Oncel, 2022).

Squalene is one of several bioactivities that has a strong inhibitory effect on mammary epithelial cells that exhibit abnormal hyperproliferation (Katdare *et al.,* 1997). In the first phases of metabolism, monooxygenase oxidizes SQ, a molecule involved in the production of cholesterol (Yoshioka *et al.,* 2020). However, the buildup of cholesterol significantly reduces its monooxygenase activity. The minor chemicals found in VOO have been connected to the bioprotective and anticancer properties of the substance (Costa and Paiva-Martins, 2022).

The bioactivity characteristics of isolated VOO compounds have been the subject of several research (Brenes *et al.,* 1999; Cicerale *et al.,* 2009; Warleta *et al.,* 2010), but VOO's health-promoting qualities may be ascribed to the interactions between its constituent nutrients, such as fatty acids and minor chemicals. Two of the minor components of VOO are HT and SQ, both of which have been isolated and shown to have a preventative effect on breast epithelial cells but no anticancer activity in highly invasive breast cancer cells (Warleta *et al.,* 2010 and Warleta *et al.,* 2011). They proposed the possibility that the well-known chemopreventive benefits of VOO

against breast cancer may not be attributable to a single component, but rather to the combined synergistic action of a number of minor chemicals (Vivanco *et al.*, 2023).

However, SQ, another significant component found in olives and VOOs, also has anticancer capabilities against a number of cancer types, including skin, breast, and colon cancer (Rao *et al.*, 1998 and Newmark, 1999).

Squalene furthermore has the benefit of being yet another small chemical that naturally occurs in VOO. It functions as an intermediary in the creation of cholesterol in animal cells as well. Therefore, the Mediterranean diet, which includes VOO as the major fat, might help people consume HT and SQ. Masumaru Tsujimoto found squalene in shark liver in 1916 (Rosales-Garcia *et al.*, 2017). Three terpene units make up this natural substance, which has the chemical formula C30H50 (6 unsaturated bonds) (Patel *et al.*, 2020 and Kim and Karadeniz, 2012). Because of its molecular makeup, it exhibits hydrophobic properties. Therefore, its density and molecular weight are 0.858 g/cm3 and 410.5 g/mol, respectively (Zhang *et al.*, 2017). It is created in the endoplasmic reticulum and either transported or stored in vesicles, vesicles connect to the cell membrane (Rosales-Garcia *et al.*, 2017 and Fagundes *et al.*, 2021).

Most animal research on CVD revealed that SQ had a favorable impact. Rats were used in several investigations (Khor and Chieng, 1997; Sabeena Farvin et al., 2004; Guillen *et al.*, 2008; Liu *et al.*, 2009; Preobrazhenskaya *et al.*, 2016; Micera *et al.*, 2020), mice in three studies (Khor and Chieng, 1997; Guillen *et al.*, 2008; Ravi Kumar *et al.*, 2016;), hamsters and rabbits in one study (Sabeena Farvin *et al.*, 2004; Liu *et al.*, 2009; Preobrazhenskaya *et al.*, 2016).

Producers of squalene Sharks are now recognized as one of the main sources of squalene (Czaplicki *et al.,* 2012 and Randhir *et al.,* 2020). Shark hunting has expanded quickly due to the high squalene content. As of now point being, with 90% use, the cosmetic business leads all other industries in squalene utilization. Additionally, when chemical pollutants and marine pollution increase, so does the quality of shark squalene (Yin *et al.,* 2018; Patel *et al.,* 2020; Bagul, and Annapure, 2020). Additionally, the composition of their liver oil varies depending on the weather (Song *et al.,* 2010). It is a difficulty for long-term manufacturing when squalene quality and quantity are so unpredictable. Researchers have thought about finding alternate sustainable sources for the manufacture of squalene, nevertheless, owing to the unpleasant smell of squalene generated by sharks (Weete *et al.,* 1997; Song *et al.,* 2010; Yin *et al.,* 2018). Numerous plants, including rice bran, amaranth, wheat germ, and olive oil, have been shown to be capable of producing squalene by researchers (Spanova and Daum, 2011; Czaplicki *et al.,* 2012; Rosales-Garcia *et al.,* 2017; Xie *et al.,* 2017). These plants are regarded as effective substitutes for sharks in the squalene synthesis process.

Squalene may be produced by microalgae, according to studies (Patil and Gogate, 2015; Dellero *et al.,* 2018; Randhir *et al.,* 2020). The use of these microbes as squalene suppliers has several benefits. For instance, their cultivation procedures may be effectively and simply handled.

2.3.4. Action of Squalene

Sources of squalene, it is important to look at natural items as alternatives for those who cannot take statins. It is important to look into the effects of squalene since

it is a necessary intermediary in the formation of phytosterol and cholesterol (SQ) that might be successful in reducing blood cholesterol and LDL-C. Evidence of SQ's protective benefits against CVD indicated that the mechanism was comparable to that of statins and was linked to the liver enzyme HMG-CoA reductase, which inhibits the conversion of acetyl CoA to cholesterol (Strandberg *et al.*, 1989). SQ has also been shown to have a broad range of biological properties, including the ability to fight aging, improve immunity, and enhance sexual function (Fang *et al.*, 2020).

The isoprenoid hydrocarbon's double bond structure made it possible for it to function both as a potent antioxidant and a natural antibacterial. Additionally, due to its biological makeup, SQ is very reactive when it comes to transitioning into its oxidized state, where it interacts with water's hydrogen ions and releases three unattached oxygen molecules to transform into its saturated form, squalane, C30H62. As a result of this interaction, oxygen enters the cells, intensifying cellular metabolism. metabolism, improving the performance of several organs including the liver and kidney (Kelly, 1999).

Chapter Three: Methodology

3. Methodology

3.1. Equipment and Instruments:

The tools and equipment that are applied in this research and their deliverers are shown in table (3-1).

Table (3-1): The equipment and instruments which were used in this study

NO.	Equipment & Instruments	Company	Country
1.	Analytical sensitive balance	Sartorius	Germany
2.	Balance for animals	Shimadu company	Japan
3.	Centrifuge	Hettich Roto fix11	Japan
4.	Digital camera	Toup Cam	China
18.	Electronic Balance	Metter company	Switzerland
5.	ELISA reader	Bio Kit	USA
6.	ELISA washer	Bio Kit	USA
7.	Freezer	Hitachi	Japan
8.	Incubator	BINDER	Germany
15.	Jell tube	AFMA-Dispo	Japan
16.	Latex gloves	Great glove	Malaysia
9.	Light microscope	Leica	China

10.	**Optical microscope with table PC**	**OPTICA**	**Italy**
11.	**Rotary macro tom**	**Leica**	**Germany**
12.	**Spectrophotometer**	**Labomed**	**UK**
14.	**Sterile syringes 5, 10 ml**	**PROTON**	**Malaysia**
13.	**Water bath**	**K.F.T.Lab. Equipment**	**Italy**

3.2. Chemicals:

Through table below (3-2) whole chemical agents and their deliverers that were applied.

Table (3-2): The chemicals were used in this study and their sources:

No.	Chemicals	company	Country
1.	**Chloroform**	**Noorbrok**	**England**
2.	**cholesterol**	**Avonchen**	**UK**
3.	**Creatine Kinase Activity Assay kit**	**SPINREACT**	**Spain**
4.	**Eosin-Hematoxilin Stain**	**Merck**	**Germany**
5.	**Ethanol**	**Merck**	**Germany**
6.	**Formalin 10 %**	**TEDIA Company**	**USA**

7.	**Normal saline**	**Labort**	**India**
8.	**Paraffin Wax**	**Merck**	**Germany**
9.	**Simvastatin**	**Pharma international**	**Jordan**
10.	**Squalene**	**Mothernest**	**England**
11.	**Xylole**	**Scharlau**	**Spain**

3.3. Animals

The experiment was carried at the animal house of Veterinary Medicine / University of Kerbala during the period from (October 2022 to March 2023). Thirty adult male rats (Rattua albicans) aged (2-3 months) weighting 260-300 gm were obtained from the animal house of Collage of Pharmacy, University of Kerbala., and the rats were reweighed every week throughout the experiment. Rats were grouped into five groups of six rats per cage. These animals were kept in suitable environmental condition with a temperature of around 25-28 C°, relative humidity 40 ٪- 60٪ conditions room with a 12:12 hour /light/dark cycle (Meyer *et al.,* 1982). The animals were housed in aplastic cage with diameters of 50×35×15cm. The feed given were pellets. The animals were kept for at last 10 days for acclimatization before experiment began. Each rat was weighed afterward, and cholesterol levels were evaluated.

3.4. Experimental design

Oral cholesterol of 1% was used to conduct research on male Albino rats and develop a model by increasing total blood cholesterol levels over four weeks. By sequence, simvastatin with/without squalene was administered for four weeks to check the effect of squalene on the activity of statin by decreasing side effects after the blood cholesterol level and creatine kinase had raised. As indicators for oxidative stress, the levels of cholesterol, creatine kinase, and hydrogen peroxide were assessed.

Thirty rats were divided two group, (experimental one) six rats dosage with normal saline only and other rats administrated with cholesterol 1% for 28 days. Then the second group randomly divided to four groups (experimental two).

The experiment lasted about during two months, the rats were administration as the following groups:

1- First group: Six rats used as a positive control group dosage with normal cholesterol 1% only at the first 28 days.
2- Second group: Six rats administrated with cholesterol 1% for 28 days then treated with simvastatin 40 mg/kg/BW dosage orally every day for 28 days.
3- Third group: Six rats administrated with cholesterol 1% for 28 days then treated with squalene 2% orally every day for 28 days.
4- Forth group: Six rats will have administrated with cholesterol 1% for 28 days then treated with simvastatin 40 mg/kg/BW dosage orally with squalene every day for 28 days.

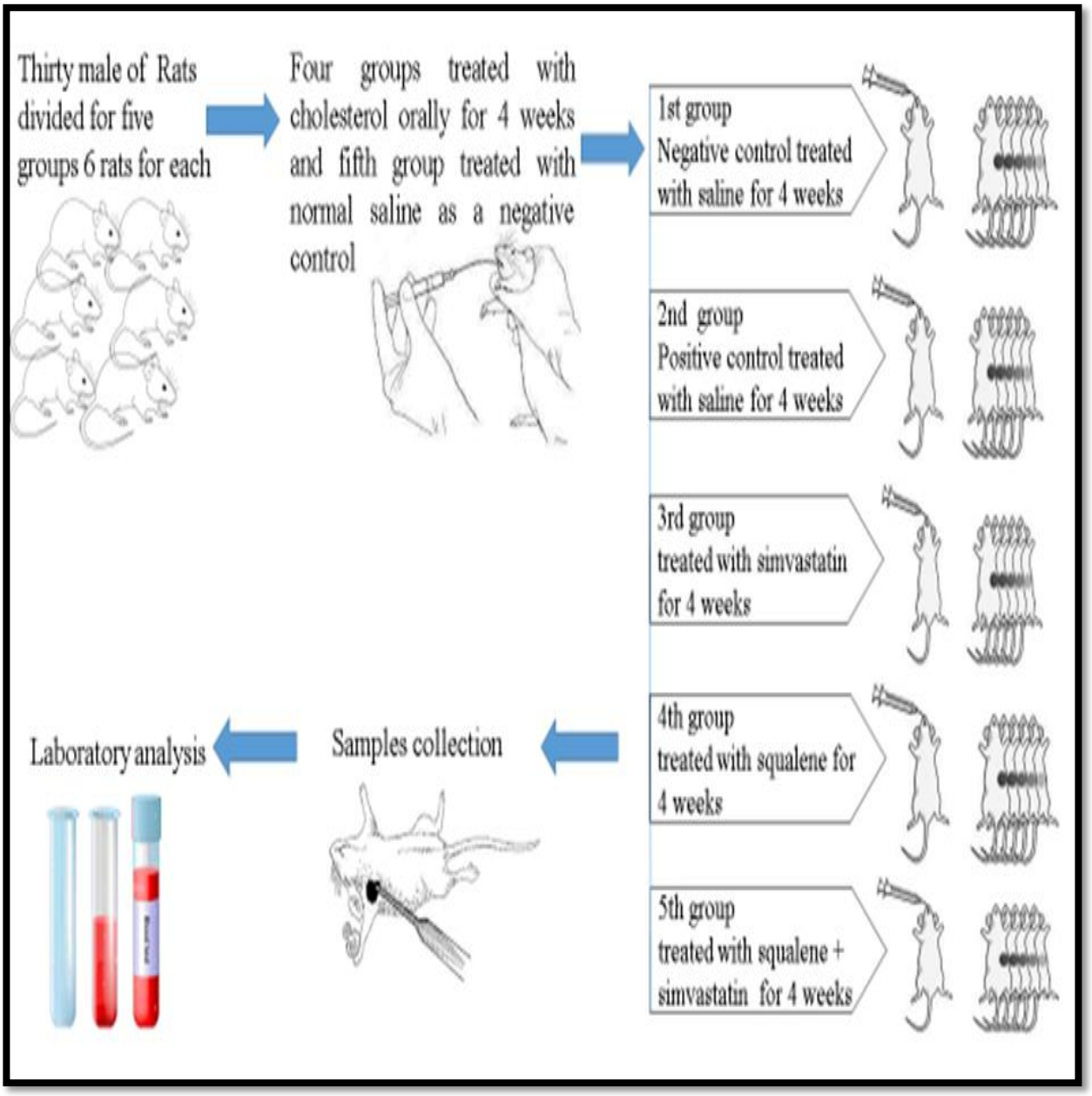


Figure (3-1): The experimental design graphically with timing details

3.5. Ethical approve

Each and every experimental technique was authorized by the College of Veterinary Medicine of kerbala and complied with the ethical approval number (UOK.VET.PH.2022.048).

3.6. Collection of blood

Rats used in study were sedated by putting them in a closed jar with cotton that had been saturated in chloroform before the following process, which involved carefully draining 3 ml of blood from the heart directly by using sterile syringes, went according to plan. After that, 3 ml of the blood are collected and put in a tube with anticoagulant. The first blood tube is then allowed to fully agglutinate for 30 minutes at room temperature in preparation for serum collection.

3.7. Biochemical traits

3.7.1. Calculating the Serum Cholesterol Concentration (in mg/dl): -

The amount of cholesterol in serum samples was determined using the reagents that were used and the serum samples that had already been collected. After mixing the serum sample with the reagents in the tube, the results were allowed to stand for five minutes at 37 Co. A sample was read using a spectrophotometer (Biotech, British) at a wavelength of 505 nm against the blank, and the absorbance of the standard was calculated.

Total Cholesterol concentration = (O.D sample)/ (O.D/ standard) × nn =200 mg/dl, as shown in appendix (I).

3.7.2. Serum Creatine Kinase Estimation: -

A simple and fast method for determining the levels of CK in various serum samples is provided by the Creatine Kinase Activity Assay kit (SPINREACT, Spain). A coupled enzyme reaction that produces NADPH, which is detected at 340 nm in proportion to the amount of Creatine Kinase (CK) activity present in the sample, is used in this test to quantify the activity of the enzyme. Creatine and ATP are produced during this process from phosphocreatine and ADP. Hexokinase utilizes the ATP produced to phosphorylate the glucose to make glucose-6-phosphate, which is then oxidized by NADP in the presence of glucose-6-phosphate dehydrogenase to produce NADPH and 6-phospho-D-gluconate. At pH 6.0, 1.0 mmole of phosphate can be transferred from phosphocreatine to ADP every minute by the enzyme known as one unit of CK, as shown in appendix (II).

3.7.3. Free Radicals of Oxygen (Oxidants) Estimation: -

A novel method created by Erel was used to calculate the Total Oxidant Status (TOS) of the sample. The ferrous ion-o-dianisidine complex is oxidized by oxidants in the sample to ferric ion. Glycerol molecules, which are abundant in the reaction medium, help to speed up the oxidation process. In an acidic media, the ferric ion and xylenol orange combine to form a colorful complex. The amount of oxidant molecules present overall in the sample is correlated with the color intensity, which is quantifiable spectrophotometrically. Hydrogen peroxide is used to calibrate the test, and the results are given in terms of micromolar hydrogen peroxide equivalent per liter (mol H2O2Eq/L), as shown in appendix (III).

3.7.4. Determination of Serum Superoxide Dismutase Concentration

The activity of the Superoxide dismutase (SOD) enzyme was defined according to Marklund and Marklund's method (1974), as shown in appendix (VI).

3.8. Histopathological Preparation

After the end of the experiment period muscles sample were taken after the animal sacrifice and they were immediately put into a 10% fixative formalin solution and left there for 48 hours before being processed. The samples were first dehydrated in graded alcohol at room temperature for 2 minutes each at 70%, 80%, 90%, and 100% concentrations before being submerged in xylene for 2 hours and then melted paraffin wax for 3 hours. The samples were then positioned and implanted in brand-new paraffin (paraffin blocks). To examine the sections under a microscope, the blocks were sectioned with a microtome at a thickness of 5 microne f. The sections were subjected of standard hematoxylin and eosin (H&E) staining procedures. Photomicro-graphs of each section were taken using a digital camera (canon, japan) using a light microscope to analyzethe sections under examination (Suvarna *et al.*, 2018), as shown in appendix (V).

3.9. Statistical Analysis

Results were analysed by using GraphPad Prism version 9.00 for windows. Results are expressed as mean +_ standard error of the mean (SEM). Comparison between means were med by using one way analysis of variance (ANOVA) test.

Chapter Four: Results and Discussion

4. Results and Discussion

4.1. Effect of simvastatin, squalene, and their combination on serum cholesterol concentration:

The current study shows that there is a significant increase (P<0.0001) in serum cholesterol levels (70.6±0.4) from that of the group that received just cholesterol without any additional treatment (positive control) as compared with control, simvastatin, squalene and simvastatin+squalene groups (54.8±1.71, 61±0.447, 85.2±0.916, 48.6±1.860) respectively as shown in table and figure (4-1).

High significant (P<0.0001) decrease in serum cholesterol in simvastatin, squalene and simvastatin+squalene groups as compared with cholesterol group, as shown in table and figure (4-1).

Table (4-1): Effect of simvastatin, squalene, and their combination on serum cholesterol concentration**.**

Groups / Parameters	Control	Cholesterol	simvastatin	Squalene	simvastatin+ Squalene
Cholesterol	54.8±1.71 D	70.6±0.4 B	61±0.447 C	85.2±0.916 A	48.6±1.860 E

-Values = Mean±SE

-Different letters represent significant differences between groups.

-Number of rats in each group = 6

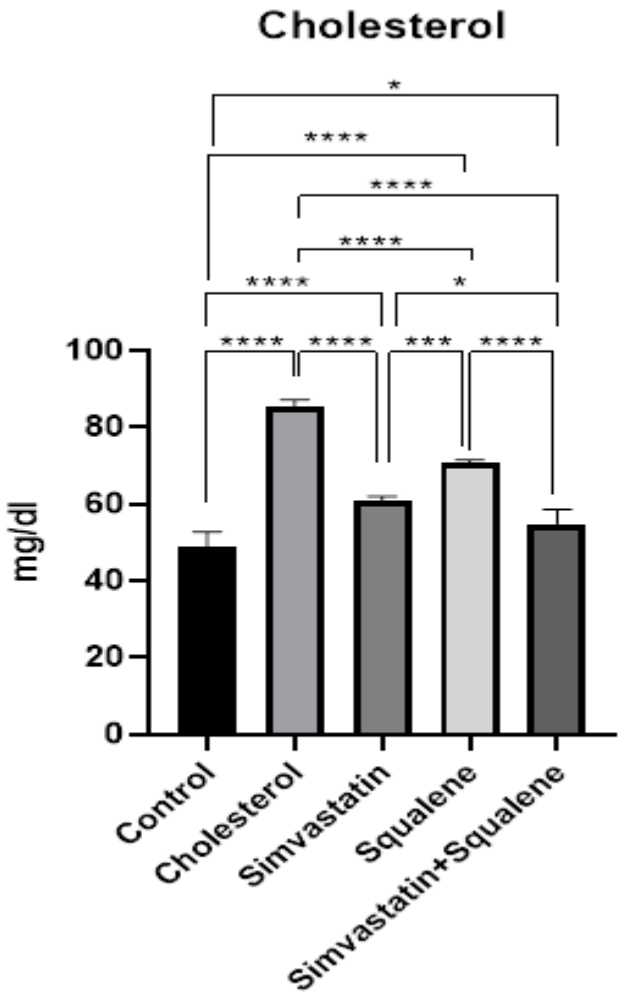


Figure (4-1): Serum cholesterol level in male rats following four weeks of receiving simvastatin with or without squalene. The level of serum cholesterol was significantly increased ($P<0.0001$) in the untreated group used as a control positive group compared to negative control. Treatment with simvastatin+squalene group was significantly different ($p<0.0001$) as compared to positive control. Data are expressed as mean ± SEM, n=6.

The outcomes show that squalene interferes with testing animals' cholesterol levels figure (4-1). The cholesterol of the treated animals differs significantly at ($P<0.0001$) from that of the group that received just cholesterol without any additional treatment (positive control).

A class of medications called statins is frequently prescribed to lower cholesterol. The main cause of statin discontinuation is the development of statin-associated muscle symptoms (Banach *et al.,* 2016 and Penson *et al.,* 2022), but despite their widespread use, discontinuation remain a significant gap (Ward *et al.,* 2019). Results of the present study showed that the primary outcomes were a reduction in levels of total cholesterol when treated with simvastatin and secondary outcomes were reductions the effect of statin-associated muscle myopathy when combination in simvastatin with squalene.

Recently, investigators have attempted to address the question of how statin may modulate myotoxicity phenotypes. A crucial metabolic pathway, the highly conserved mevalonate pathway produces sterol and nonsterol isoprenoids, which are essential for many cellular processes. The sterol isoprenoid cholesterol is an essential component for bile acids, lipoproteins, and steroid hormones, whereas nonsterol isoprenoids like dolichols and ubiquinone (coenzyme Q10) are essential for intracellular signaling, cell growth and differentiation, gene expression, protein glycosylation, and cytoskeletal assembly (Buhaescu *et al.,* 2007 and Muntean *et al.,* 2017). Farnesyl pyrophosphate and geranylgeranyl pyrophosphate are two other mevalonate pathway end products that contribute to cell growth, maintenance, and a reduction in apoptosis (Bitzur *et al.,* 2013 and Elam *et al.,* 2017).

Results of the study have shown agreement with Hansen *et al.* (2005) who, studied that rats given statins for four weeks, and it is possible that a longer treatment would result in muscle functional abnormalities. However, because statin myopathy can occur at any time during long-term statin treatment, it is important to monitor patients closely.

A compensatory upregulation in response to statin-induced inhibition was hypothesized to be reflected by disruption of genes related to downstream proteins of the mevalonate pathway and associated with cholesterol biosynthesis. Due to increased statin exposure and sensitivity, increased expression of these distal pathways may also indicate a complete blockade of the pathway (Elam *et al.,* 2017). The authors further suggest that persistent myalgia originates from cellular stress that affects the structural integrity and performance of skeletal muscle and its response to post inflammatory repair and regeneration (Elam *et al.,* 2017).

4.2. Effect of simvastatin, squalene, and their combination on serum creatine kinase (CK) cocentration:

The current study shows that there is simvastatin group very high significant increase ($P<0.0001$) in serum creatine kinase concentration (688.24±16.83) compared with other groups. On the other hand, cholesterol group (420.468±16.46) show high significant ($p<0.0001$) increase compared with squalene and combination simvastatin groups (467.04±14.74, 510.8±4.152) respectively. While lowest significant decrease ($P<0.0001$) in serum creatine kinase concentration in squalene, combination simvastatin and squalene groups (467.04±14.74, 510.8±4.152) compared with simvastatin group (688.24±16.83) as shown in table and figure (4-2).

While there is non-significant difference in serum creatine kinase cocentration between (control with squalene groups), (cholosterol with simvastatin and squalene) and (squalene with combination simvastatin and squalene) groups, as shown in table and figure (4-2).

Groups / Parameters	Control	Cholesterol	simvastatin	Squalene	simvastatin+ Squalene

Table (4-2): Effect of simvastatin, squalene, and their combination on serum creatine kinase (CK) levels.

CK	420.468±16.46 C	546.4±18.84 B	688.24±16.83 A	467.04±14.74 C	510.8±4.152 B

-Values = Mean±SE

-Different letters represent significant differences between groups.

-Number of rats in each group = 6

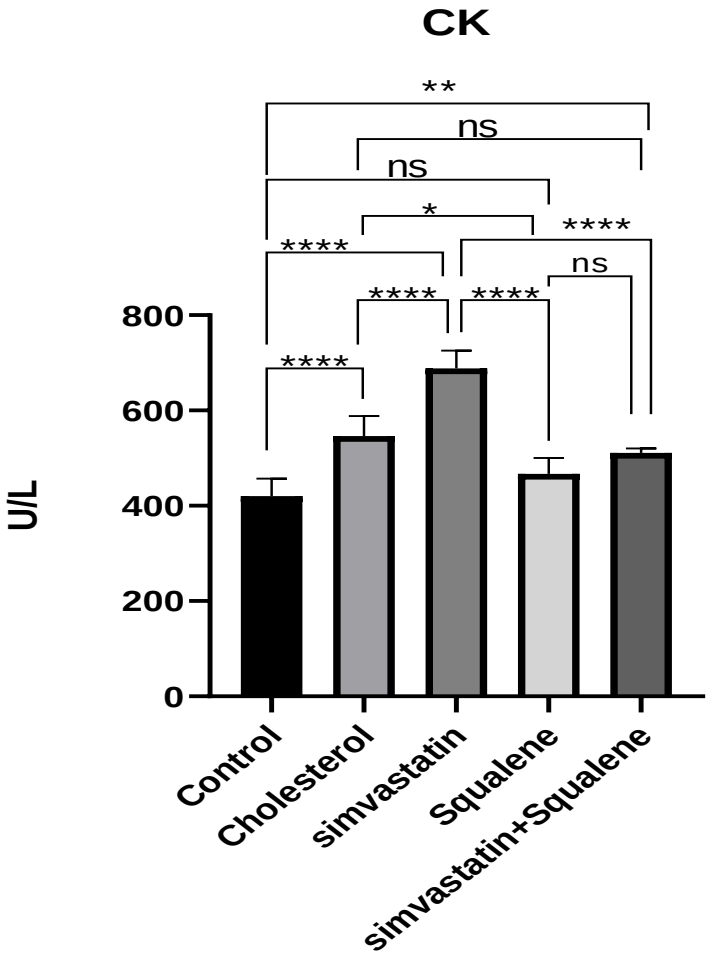


Figure (4-2): Serum creatine kinase level in male rats following four weeks of receiving simvastatin with or without squalene. The level of creatine kinase was significantly increased (P<0.0001) in the simvastatin. Data are expressed as mean ± SEM, n=6.

The study demonstrates that the effect of simvastatin on creatine kinase (CK) markers figure (4-2), which exhibit as muscle problem brought on statin exposure and manifested with elevated CK levels, after statin therapy and to understand the reduction effect of squalene on CK stress markers.

Skeletal muscle tissue is found throughout the body, and damage to it can have serious consequences (De Luca *et al.,* 1997). In registries and observational studies, the prevalence of statin-induced muscle symptoms ranges between 7% and 29% during first year of administration (Lotteau *et al.,* 2019).

Progressively worse statin-related myotoxicity phenotypes have been proposed. Beginning at asymptomatic CK elevation, they include tolerable and intolerable myalgia, myopathy, severe myopathy, rhabdomyolysis, and autoimmune-mediated necrotizing myositis (Alfirevic *et al.,* 2014), or at its worst, elevated creatine kinase, with some people reporting additional symptoms joint and stomach aches (Keen *et al.,* 2014 and Selva-O'Callaghan *et al.,* 2018). The link between statin-induced muscle symptoms and muscle pain has been studied by examining elevated CK are the symptoms of non-inflammatory myopathy (Bitzur *et al.,* 2013 and Collins *et al.,* 2016).

According to Osaki *et al.* (2015) study, postnatal myopathy with elevated CK levels, mitochondrial dysfunction, and necrosis can be seen in skeletal muscle-specific HMG-CoA reductase knockout mice.

The benefits of lowering LDL-c are highlighted by meta-analysis, which shows that every 1 mmol/L (38.7 mg/dL) drop in LDL-c is associated with a significant 22%

relative risk drop in major vascular and coronary events (Silverman *et al.*, 2016). This is supported by the Cholesterol Treatment Trialists Collaboration.

The enzyme glycine amidinotransferase, which is encoded by GATM, is necessary for the synthesis of creatinine, a key mechanism of energy storage in muscle is the phosphorylation of creatinine, the main downstream product of GATM (glycine amidinotransferase) activity, which is mediated by CK, a biomarker of statin myopathy.

An association between GATM and statin-induced myopathy, cellular cholesterol homeostasis, and energy metabolism has been suggested by genome-wide eQTL analysis of lymphoblastoid cell lines from simvastatin-treated participants (Luzum *et al.*, 2015).

Furthermore, statin-treated mice overexpressing lipoprotein lipase showed an increase in skeletal muscle mitochondria, cholesterol accumulation, and lipid droplets. Increased plasma creatinine phosphokinase, a marker of muscle damage, was also linked to this (Yokoyama *et al*., 2007). Statins have been demonstrated to be toxic to immature muscle cells more recently through a variety of mechanisms.

In C_2C_{12} myoblasts, the lactone forms of statins significantly reduced complex III activity, which decreased mitochondrial respiration and triggered apoptosis. Patients who presented with the term "Statin Associated Muscle Symptoms" (SAMS) were also found to have decreased complex II activity when examined in a clinical setting; this was most noticeable in patients who had rhabdomyolysis, the most serious type of muscle damage (Schirris *et al.*, 2015). Simvastatin and pravastatin's conversion from their inactive lactone to hydroxy acid forms can be impacted by changes in the

acid/base balance, such as those that occur in conditions of acidosis and alkalosis. This might be due to increased lipophilicity, which causes myotoxicity, acidic environments seem to keep statins in their lactone form, allowing for greater uptake by C_2C_{12} skeletal muscle cells (Taha *et al.,* 2016).

Simvastatin's enhanced association with nonpolar lipoprotein fractions and uptake through a lipoprotein lipase-mediated process contributed to the worsening of this process in the presence of hyperlipidemia (Taha *et al.,* 2017). Statin exposure can also impair muscle regeneration (Trapani *et al.,* 2012), and cause cell cycle arrest, as shown by in vitro and animal study (Singh *et al.*, 2013).

Reith *et al.* (2022), who was showed the statins' have negative effects on skeletal muscle (SAMS), refers to adverse effects in the skeletal muscle such as pain, weakness, and there were appeared during the first year of statin therapy, there was a 7% relative increase in muscle pain or weakness. Due to SAMS, it is frequently necessary to reduce the given dose, substitute statins, or even discontinue therapy entirely (Zhang *et al.,* 2013). It has been estimated that over 75% of SAMS appear within the first 12 weeks of treatment and that 90% occur within 6 months (Banach and Mikhailidis, 2018).

Remarkably, SAMS is more common in physically active people (Bruckert *et al.,* 2005; Noyes and Thompson, 2017). As a result, statin therapy must be tailored to each individual.

A study conducted by (Camerino *et al.*, 2021), exhibited not with standing the fact that patients with SAMS may have statin-induced myotoxicity, which manifests as muscle necrosis brought on by statin exposure and manifested with elevated creatine

kinase (CK) levels, after statin therapy, SAMS often presents with a variety of muscular complaints that may go away when the therapy is stopped.

Clinical manifestations of SAMS are quite varied and may range from rhabdomyolysis to a myopathy pattern, which is marked by muscular pain, cramps, and aches, weakness, and an elevated CK level (even 10 times higher than the upper normal limit) (Chahin and Sorenson, 2009). Myopathy often develops in individuals receiving high dosages of statins (Davis and Weller, 2021) particularly simvastatin 80 mg daily,

which results in greater plasma levels of active statin metabolites, particularly in the first year of therapy or after increasing the dosage. Our study also demonstrated that CK level were decreased, in combination of squalene with simvastatin in male rats.

4.3. Effect of simvastatin, squalene, and their combination on hydrogen peroxide (free radicals of oxygen):

The present study was exhibited a higher significant differences ($P<0.0001$) in hydrogen peroxide (free radicals of oxygen) between simvastatin (19.97±0.569) with other treaded groups. Furthermore, control (6.38±0.494) with cholesterol (8.05±0.365), and combined simvastatin with squalene (9.01±0.48) groups at the same table showed a significant decreased ($P<0.0001$). The hydrogen peroxide in squalene (14.1±0.59) and combined simvastatin + squalene groups recorded significant decreased as compared to simvastatin (9.01±0.48) groups as shown in table and figure (4-3).

On the other hand, there were no significant differences in control with simvastatin, and between squalene and simvastatin + squalene groups, as shown in table and figure (4-3).

Table (4-3): Effect of simvastatin, squalene, and their combination on hydrogen peroxide (free radicals of oxygen).

-Values = Mean±SE

-Different letters represent significant differences between groups.

-Number of rats in each group = 6

Groups / Parameters	Control	Cholesterol	simvastatin	Squalene	simvastatin+ Squalene
H2O2	6.38±0.494 C	8.05±0.365 C	19.97±0.569 A	14.1±0.59 B	9.01±0.48 C

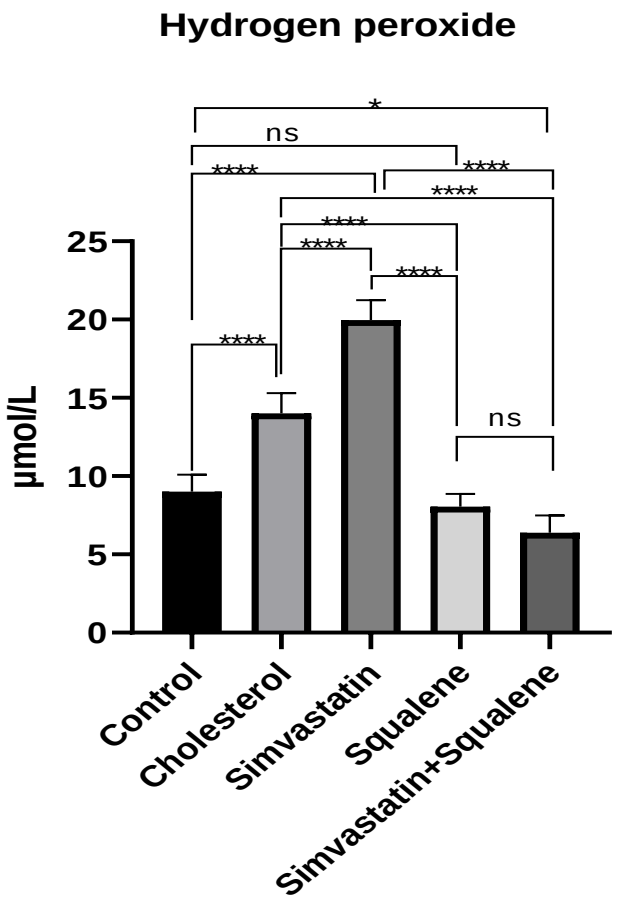


Figure (4-3): Serum hydrogen peroxide level in male rats following four weeks of receiving simvastatin with or without squalene. The level of serum hydrogen peroxide was significantly increased ($P<0.0001$) in the simvastatin group compared to positve control. Treatment with simvastatin+squalene group was significantly different ($P<0.0001$) as compared to positive control. Data are expressed as mean ± SEM, n=6.

The study showed that dietary squalene administration may decrease oxidative stress. Oxidative stress was used to describe the imbalance between the excessive production of cellular reactive oxygen species (ROS). Squalene suppressed hydrogen peroxide-induced as well as intracellular ROS content, as a result squalene reduced intracellular ROS levels.

Oxidative stress is the term used to describe the imbalance between the excessive production of cellular reactive oxygen species (ROS) and the reduced capacity of living organisms to counteract ROS through antioxidant system response. (Persson, *et al.,* 2014 and Chen *et al.,* 2021). For normal cellular functions and physiological processes, intracellular ROS production is required; however, when it exceeds the intrinsic antioxidant capacity. Oxidative stress results, seriously damaging cellular macromolecules (Nita and Grzybowski, 2016). The distinctive and stable triterpene structure of squalene, which enables it to efficiently scavenge dangerous free radicals, is closely related to its antioxidant properties (Kohno *et al.,* 1995). Squalene has been investigated in a number of cell lines for its potential role in quelling oxidative stress. Squalene protective qualities against oxidative damage have been proven in rodents (Farvin *et al.,* 2007; Motawi *et al.,* 2010; Gabás-Rivera *et al.,* 2014).

These beneficial effects are partially associated with a reduction in the oxidative and inflammatory stress in animals subjected to squalene therapy. Squalene suppressed hydrogen peroxide-induced protein carbonylation as well as intracellular ROS content caused by lipopolysaccharide incubation in mouse peritoneal macrophages and human promyelocytic leukemia cell lines (HL-60), respectively (Cárdeno *et al.,* 2015 and Yoshimura *et al.,* 2016).

Squalene reduced intracellular ROS levels, inhibited H_2O_2-induced oxidative injury and protected human mammary epithelial cells (MCF10A) against oxidative DNA damage (Warleta *et al.,* 2010).

Gabás-Rivera *et al.* (2020), rsearcher showed that dietary squalene administration may decrease oxidative stress in various mouse models.

Although new drugs (monoclonal antibodies, antisense oligonucleotides) are being developed, statins remain the first-line treatment option for hypercholesterolemia, making it critical to identify the causes of their toxicity.

Skeletal muscle cells are densely packed with mitochondria, which are metabolically active and susceptible to producing reactive oxygen species (ROS). Because of their high oxygen consumption, ROS produced as byproducts of mitochondrial oxidative phosphorylation are particularly damaging to the genome of skeletal muscle. Antioxidant enzymes found in myogenic cells include heme oxygenase-1, catalase, glutathione peroxidase, and superoxide dismutase. These enzymes have the ability to neutralize excessive ROS and play an important role in the regeneration process, influencing post-injury inflammatory responses and influencing differentiation by increasing satellite cell viability and proliferative capacity (Bouitbir *et al.,* 2012). By increasing proteolysis and/or inhibiting protein synthesis, oxidative stress can cause muscle atrophy. Additionally, lipophilic statins cause monocytes to produce ROS (Elam *et al*., 2017 and Isackson *et al*., 2018).

Bidooki *et al.* (2022), in particular, who found that squalene treatment significantly increased the mRNA level of Glutathione peroxidase 4 (Gpx4), a protein involved in the antioxidant defense that neutralizes oxidative stress.

Oxidative stress is the major etiopathological factor in myopathy, Hypercholesterolemia is characterized by elevated serum total cholesterol, low density lipoprotein, very low-density lipoprotein (LDL, VLDL) cholesterol and decreased high-density lipoprotein (HDL) levels. The consumption of synthetic drugs associated with a number of side effects. One of the proposed mechanisms of action

of such drugs is by enhancing cellular endogenous antioxidant enzymes (SOD, CAT, GSHPx), and nucleic acid biosynthesis (Rege *et al.*, 1999).

High level of circulating cholesterol and its accumulation in the heart tissue is usually accompanied by cardiovascular damage (Mediene-Benchekor *et al.,* 2001). Increased lipid peroxidation is thought to be a consequence of oxidative stress which occurs when the dynamic balance between prooxidant and antioxidant mechanism is impaired (Kumari and Menon, 1987). Reactive oxygen species (ROS) may attack any type of molecules, but their main target appears to be polyunsaturated fatty acids, which is the precursor of lipid peroxide formation (Gutteridge, 1982 and Rahman *et al.*, 2012).

Elevation of lipid peroxides in myopathic rats could be attributed to the accumulation of lipids in the muscles and the irreversible damage to the cell membranes. ROS are highly toxic byproducts of aerobic metabolism; react unfavorably with surrounding macromolecules resulting in severe cell and tissue damage. Squalene have been shown to inhibit lipid peroxidation formation in rat tissues and also inhibit the free radical production in the cells at various stages.

4.4. Effect of simvastatin, squalene and their combination on superoxide dismutase (SOD) estimation:

The current results were showed a significant increased ($P<0.0001$) of the serum superoxide dismutase (SOD) concentration in control, squalene, and combination of simvastatin and squalene groups (57.1±0.98, 36.56±1.29, 31.35±0.36) comparison with cholesterol (48.49± 237) and simvastatin (56.38±1.50) groups respectively as shown in table and figure (4-4).

Moreover, the superoxide dismutase (SOD) concentration lacked to significance among control vs. squalene groups and between cholesterol and simvastatin groups, as shown in table and figure (4-4).

Table (4-4): Effect of simvastatin, squalene and their combination on superoxide dismutase (SOD) estimation**.**

Groups / Parameters	Control	Cholesterol	simvastatin	Squalene	simvastatin+ Squalene

SOD	57.1±0.98 A	48.49± 237 B	56.38±1.50 A	36.56±1.29 C	31.35±0.36 C

-Values = Mean±SE

-Different letters represent significant differences between groups.

-Number of rats in each group = 6

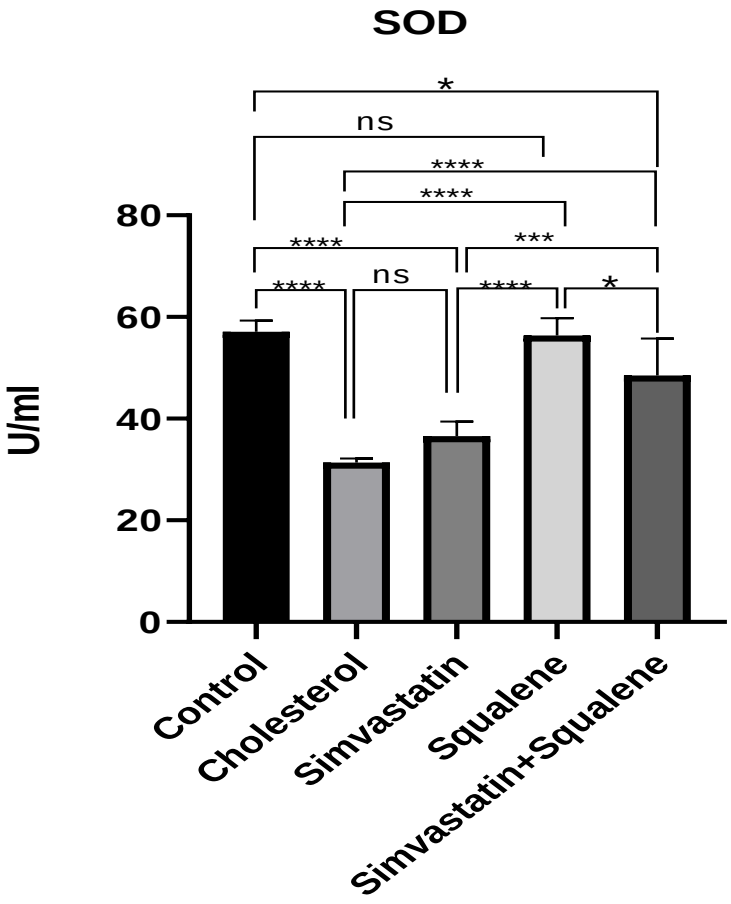


Figure (4-4): Serum superoxide dismutase (SOD) level in male rats following four weeks of receiving simvastatin with or without squalene. The level of serum superoxide dismutase (SOD) was significantly increased ($P<0.0001$) in the squalene and combination simvastatin+squalene groups compared to positve control. Data are expressed as mean ± SEM, n=6.

Antioxidants constitute the foremost defense system that limits the toxicity associated with free radicals. The equilibrium between antioxidants and free radicals is an important process for the effective removal of oxidative stress in intracellular organelles. However, in pathological conditions like myopathy, the generation of ROS can dramatically upset this balance with an increased demand on the antioxidant defense system. Free radical scavenging enzymes such as SOD, catalase, GPx, GRx and GST are the first line of cellular defense against oxidative injury. These enzymes are lowered due to enhanced lipid peroxidation.

Superoxide radicals generated at the site of damage in myopathy modulates SOD and catalase resulting in the lowered activities of these enzymes and accumulation of superoxide anion, which also damages the skeletal muscle cells. Rats pretreated with squalene showed increased activities of these enzymes which suggest that squalene may have the ability to prevent the deleterious effects induced by free radicals in statin-induced myopathy rats.

4.5. Histopathological Changes:

4.5.1. Muscles

The muscle of the control animals group showed normal histology figure (4-5) stained with (H&E), Photomicrograph of longitudinal section of skeletal muscle fibers of a control rat showing the normal morphology of parallel, cylindrical muscle fibers with visible transverse striations and multinucleated peripheral nuclei, and photomicrograph of cross section of skeletal muscle fibers of a control rat in figure (4-6) showing polygonal muscle fibers separated by endomysium. Bundles are separated by perimysium (collagen strands).

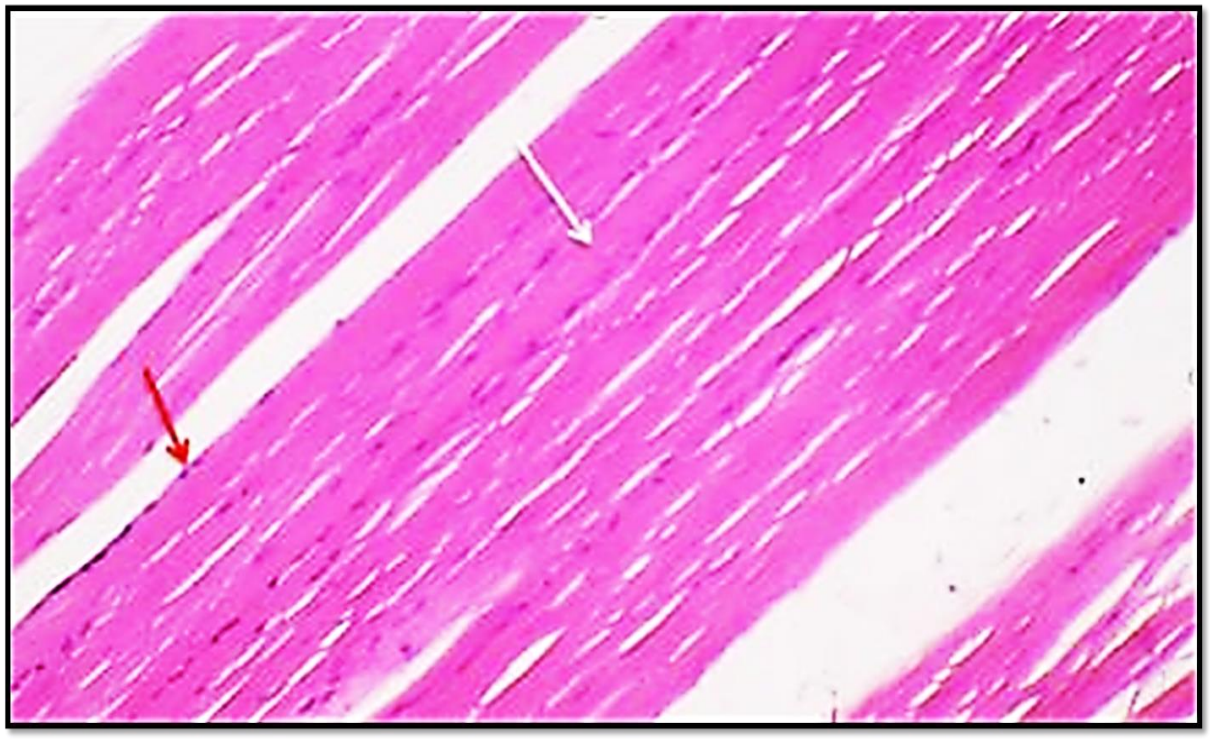


Figure (4-5): Photomicrograph of longitudinal section of skeletal muscle fibers of a control rat showing the normal morphology of parallel, cylindrical muscle fibers with visible transverse striations (white arrow) and multinucleated peripheral nuclei (red arrow) (H and E,10X).

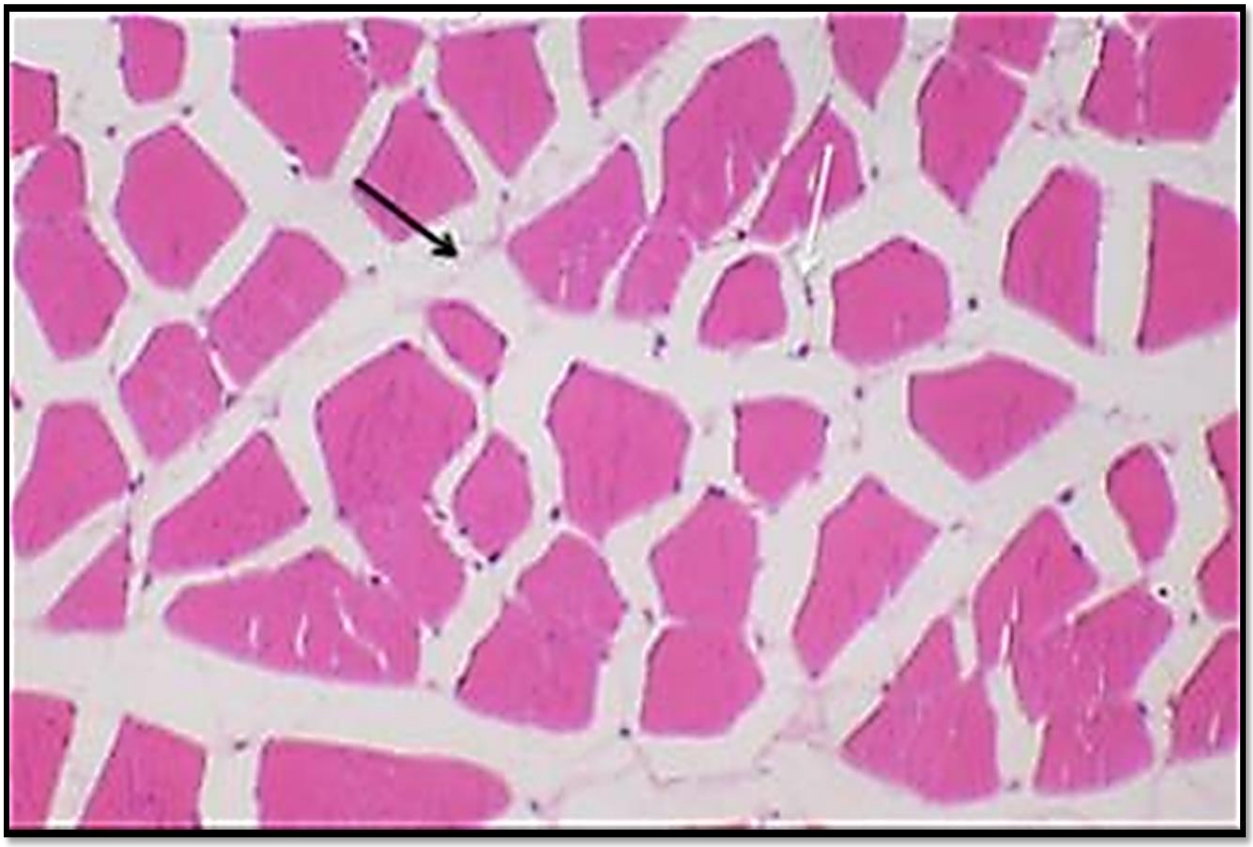


Figure (4-6): Photomicrograph of cross section of skeletal muscle fibers of a control rat showing polygonal muscle fibers separated by endomysium (white arrow). Bundles are separated by perimysium (collagen strands) (black arrow) (H and E,10X).

The histopathological examination from the second group of a longitudinal section of skeletal muscle fibers of cholesterol treated rat showed reverse parallel, branched, considerably thin muscle fibers with distinct striations, increased perimysium between bundles, (H and E, 10X), figure (4-7). Photomicrograph for cross section of skeletal muscle fibers of cholesterol treated rat showing muscle fibers atrophy, with significant extended endomysium, (H and E,10X), figure (4-8).

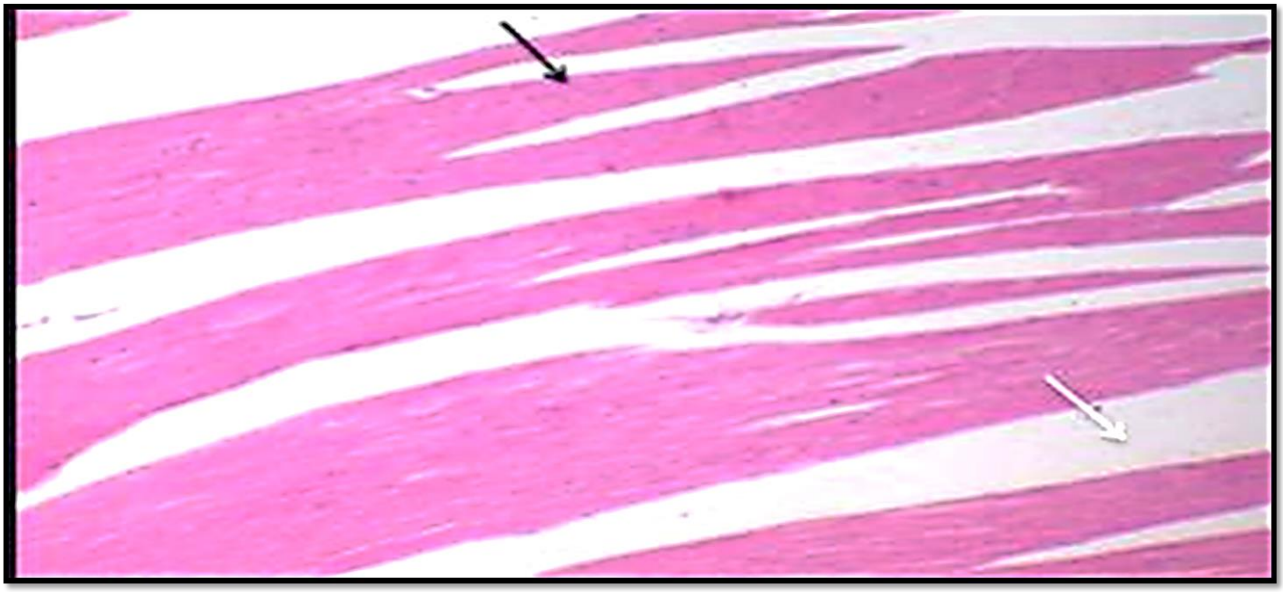


Figure (4-7): Photomicrograph for a longitudinal section of skeletal muscle fibers of cholesterol treated rat showing reverse parallel, branched, Considerably thin muscle fibers with distinct striations (black arrow), increased perimysium between bundles (white arrow). (H and E,10X).

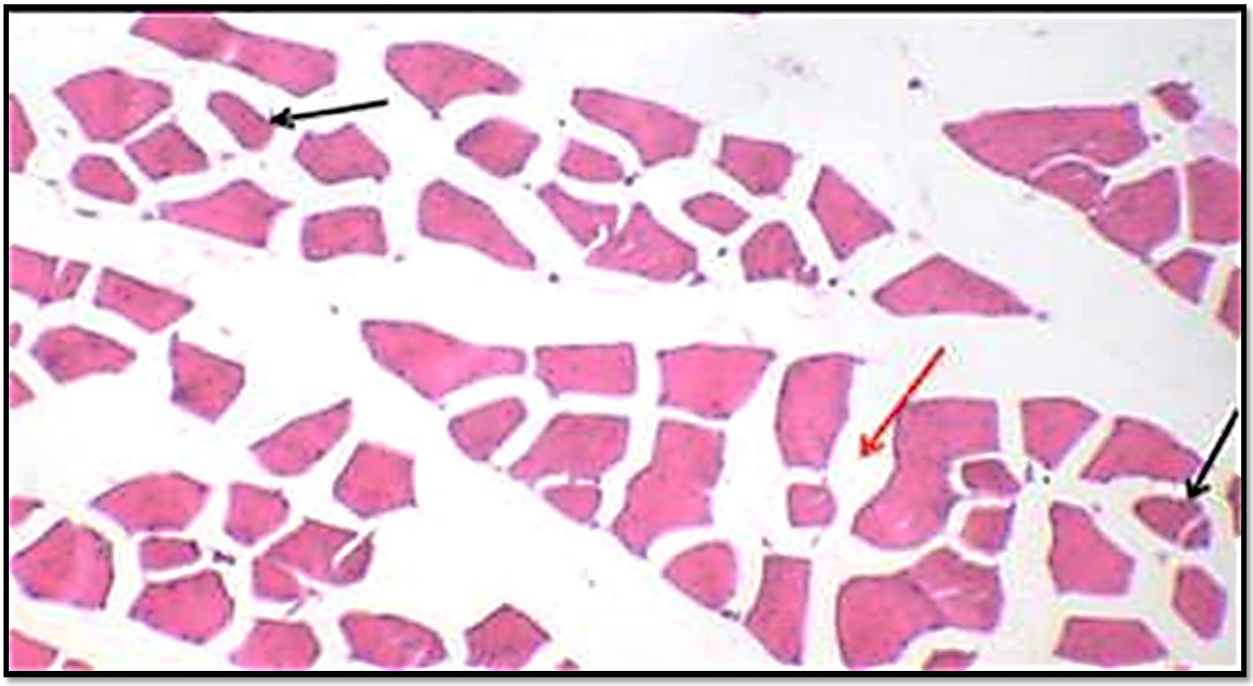


Figure (4-8): Photomicrograph for cross section of skeletal muscle fibers of cholesterol treated rat showing muscle fibers atrophy (black arrow), with extended endomysium (red arrow), (H and E,10X).

Statin treated group from the third group of longitudinal section of skeletal muscle fibers showed a significant pathological alterations represented by discontinuity and splitting in myofibrils, wavy appearance, marked atrophied myofibers with randomly arrangements of nuclei and mild infiltration of inflammatory cells, (H and E,10X), figure (4-9).

Photomicrograph of transverse section of skeletal muscle fibers of statin treated rat showing significant pathological alterations represented by sever dilation in perimysium with increased collagen strands, markedly atrophied myofibers decrease intracellular spaces, some myofibers appeared with no nuclei and mild infiltration of inflammatory cells, (H and E, 10X), figure (4-10), as compared to the normal histological structure of the muscle figures (1 and 2).

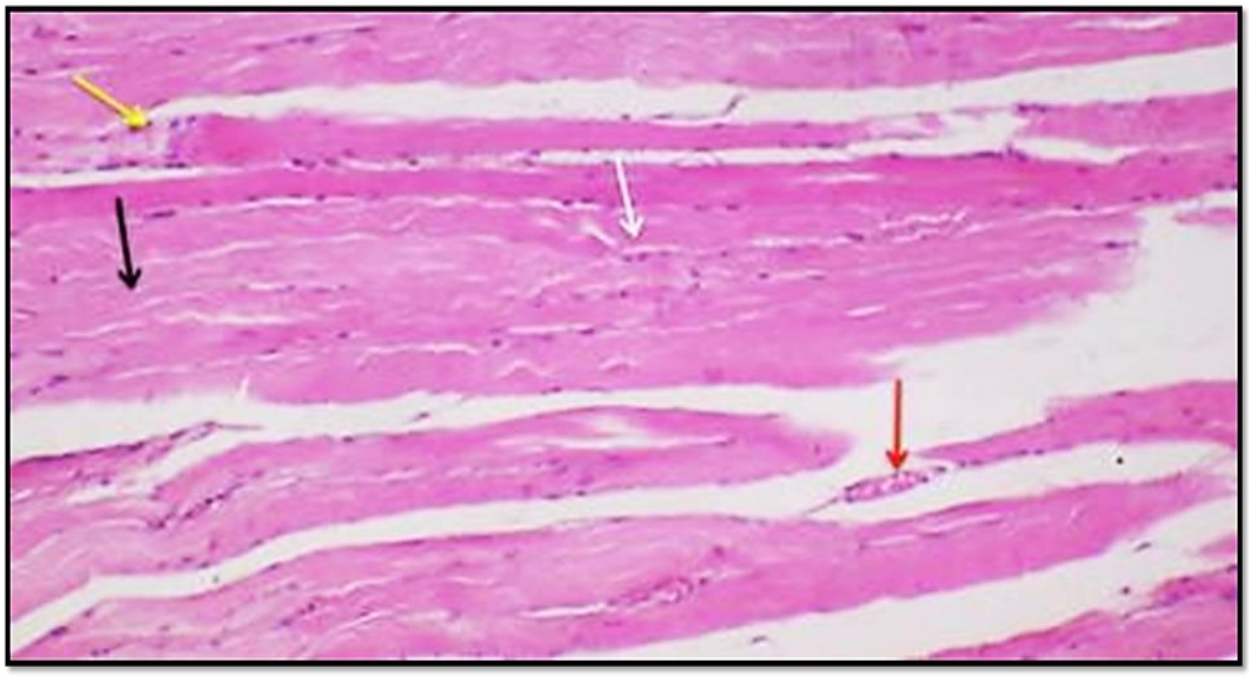


Figure (4-9): Photomicrograph of longitudinal section of skeletal muscle fibers of statin treated rat showing pathological alterations represented by discontinuity and splitting in myofibrils, wavy appearance (black arrow), marked atrophied myofibers (red arrow) with randomly arrangements of nuclei (white arrow) and mild infiltration of inflammatory cells (yellow arrow) (H and E,10X).

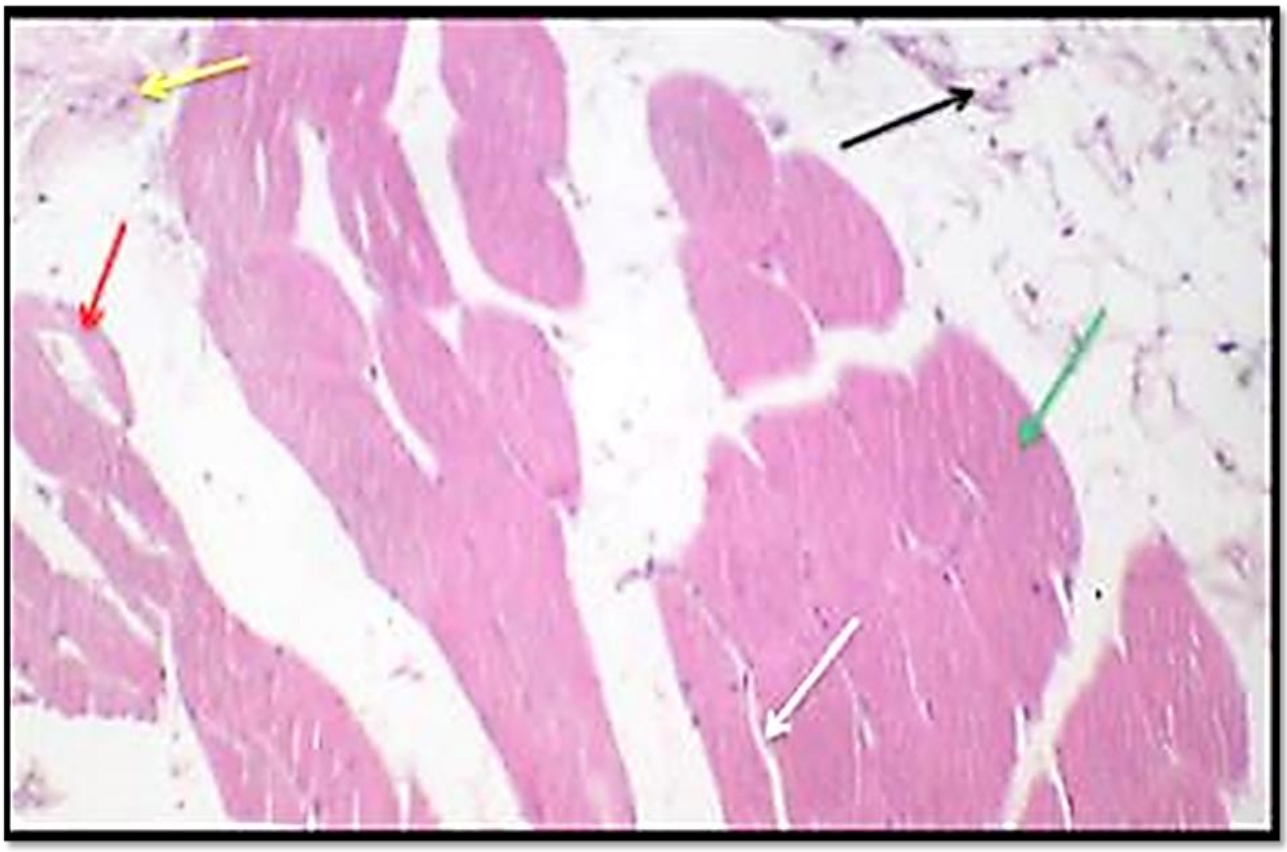


Figure (4-10): Photomicrograph of transverse section of skeletal muscle fibers of statin treated rat showing significant pathological alterations represented by sever dilation in perimysium with increased collagen strands (black arrow), marked atrophied myofibers (red arrow) decrease intracellular spaces (white arrow), some myofibers appeared with no nuclei (green arrow) and mild infiltration of inflammatory cells (yellow arrow) (H and E, 10X).

The photomicrograph for a longitudinal section of skeletal muscle fibers in figure (4-11) from a squalene treated rat which has parallel, cylindrical, considerably thin muscle fibers with significant striations, elongated and chain arranged nuclei, (H and E,10X). Photomicrograph for cross section of skeletal muscle fibers of squalene treated rat showing moderate increase in size, with significant extended nuclei and normal endomysium with collagen, figure (4-12).

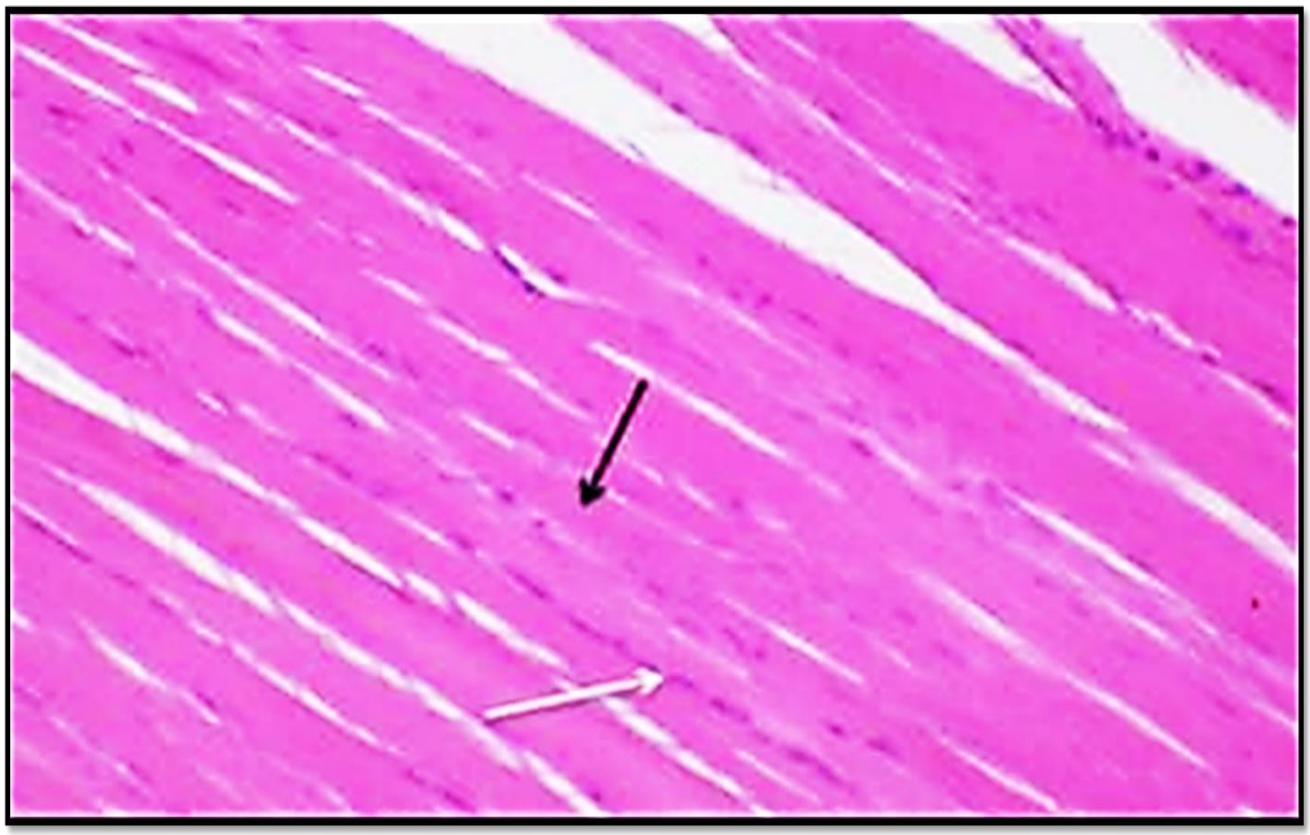


Figure (4-11): Photomicrograph for a longitudinal section of skeletal muscle fibers of squalene treated rat showing parallel, cylindrical, considerably thin muscle fibers with significant striations (black arrow), elongated and chain arranged nuclei (white arrow). (H and E,10X).

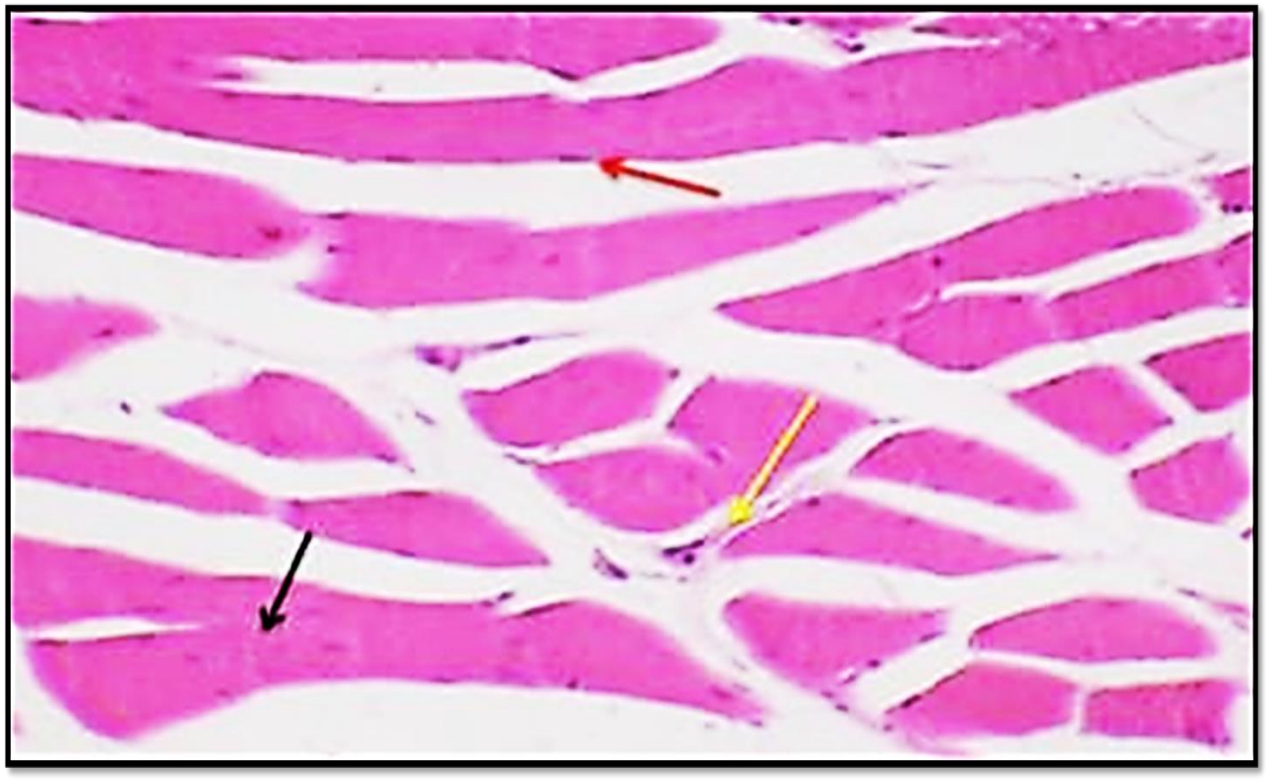


Figure (4-12): Photomicrograph for cross section of skeletal muscle fibers of squalene treated rat showing moderate increase in size (black arrow), with significant extended nuclei (red arrow) and normal endomysium with collagen (yellow arrow), (H and E,10X).

While photomicrograph for a longitudinal section of skeletal muscle fibers of simvistatin and squalene treated rat from fifth group showing reverse histological picture resembling control, parallel muscle fibers with distinct striations and nuclei forming a nuclear chain, (H and E,10X), figure (4-13). The figure (4-14) group photomicrograph for a transverse section of skeletal muscle fibers of statin and squalene treated rat shows a reverse histological picture improve to control, muscle fibers with distinct boundaries and acidophilic sarcoplasm, nuclei is peripherally located and significant collagen fibers in endomysium, (H and E, 10X).

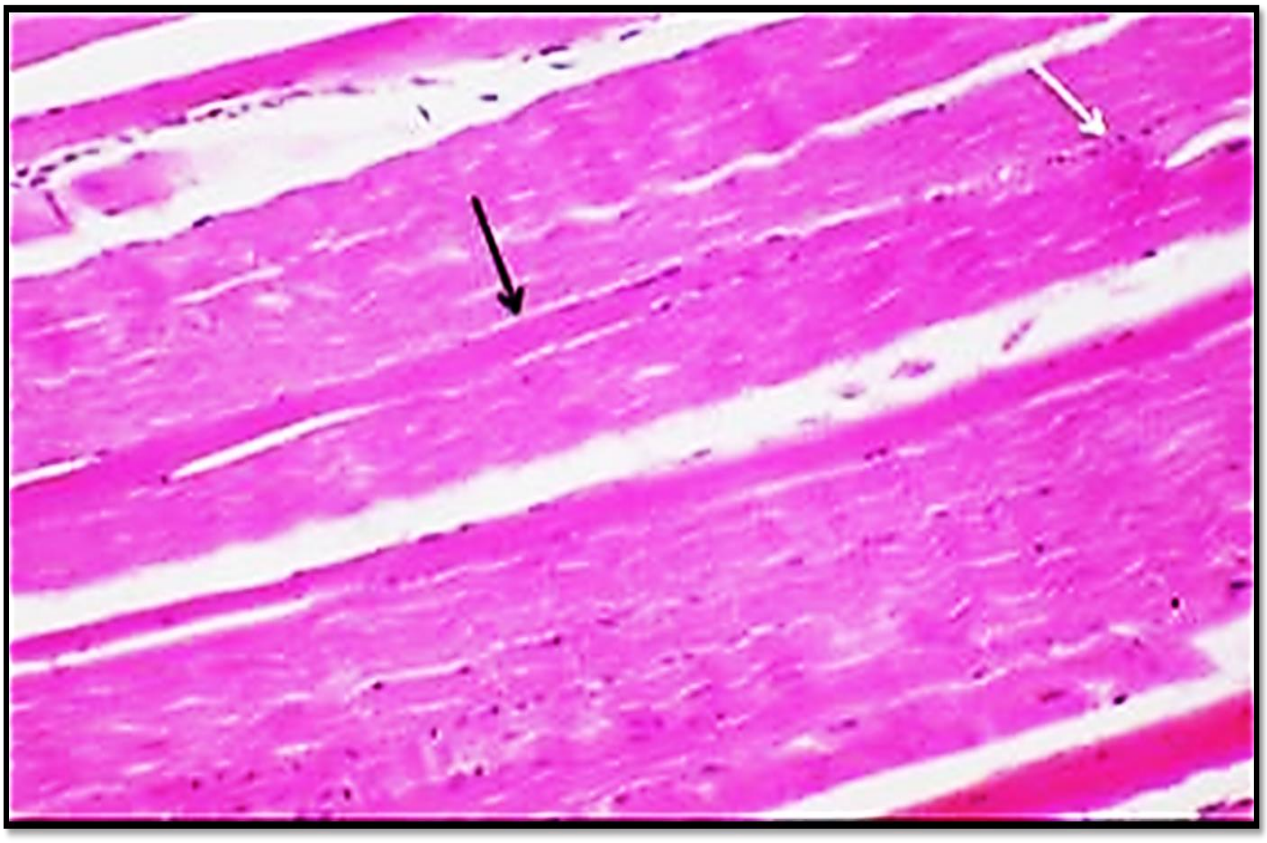


Figure (4-13): Photomicrograph for a longitudinal section of skeletal muscle fibers of statin and squalene treated rat showing reverse histological picture improve to control, parallel muscle fibers with distinct striations (black arrow) and nuclei forming a nuclear chain (white arrow), (H and E,10X).

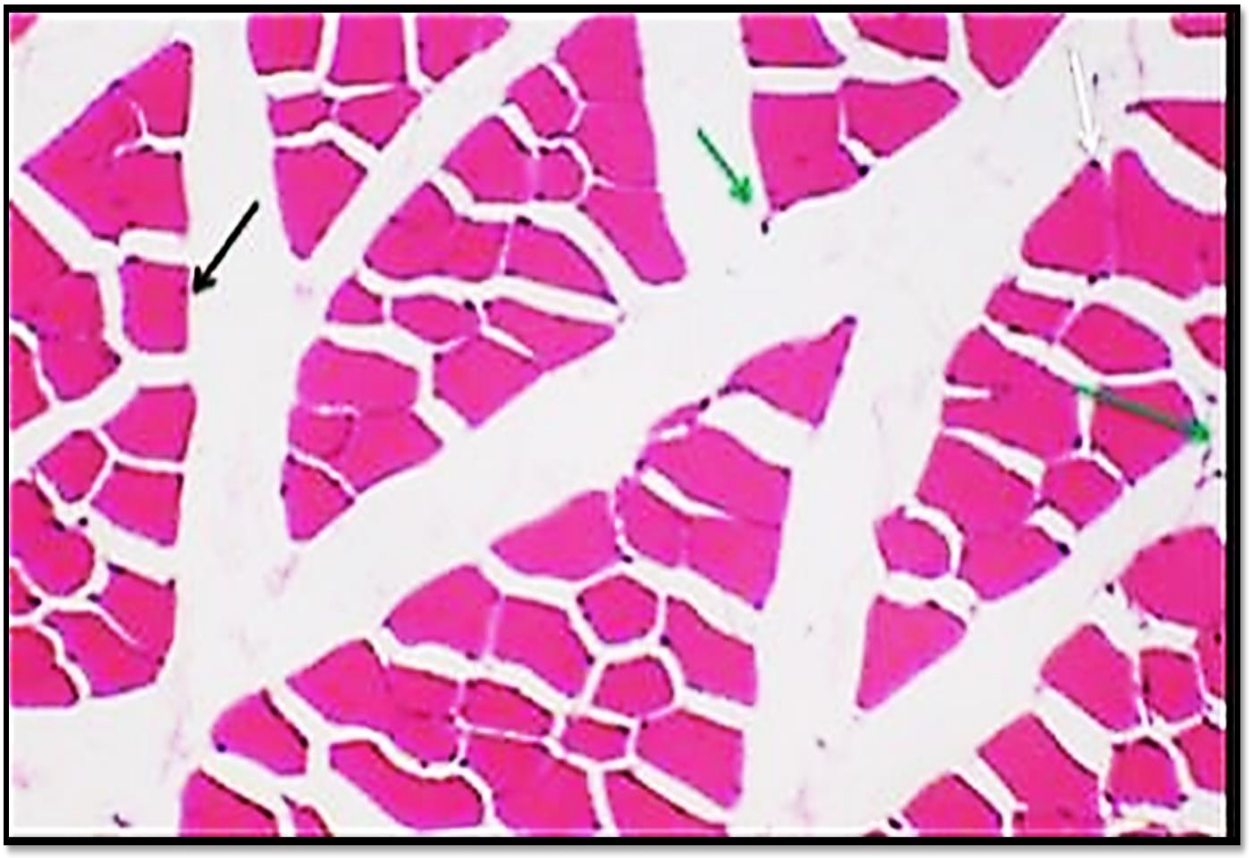


Figure (4-14): Photomicrograph for a transverse section of skeletal muscle fibers of statin and squalene treated rat showing reverse histological picture improve to control, muscle fibers with distinct boundaries and acidophilic sarcoplasm (black arrow), nuclei is peripherally located (white arrow) and significant collagen fibers in endomysium (green arrow), (H and E, 10X).

Cholesterol plays a critical role in the development and maintenance of all cell membranes, including those in skeletal muscles. A decrease in the amount of cholesterol in skeletal muscle cell membranes can cause them to become unstable and change the fluidity and excitability of ion channels. This can increase the sensitivity

of skeletal muscle to HMGCoA reductase inhibition (Auer *et al.,* 2016). As a result, myocyte damage and myopathy may result from altered sodium, potassium, and chloride channel function (Apostolopoulou *et al.*, 2015).

Statins and Muscle Disease People with muscle disease often avoid statins because of known side effects. The cause of statin-induced myopathy is unclear, although several mechanisms have been proposed, including increased oxidative stress (Sánchez-Quesada *et al.,* 2022), activation of the atrogynous-1 muscle wasting pathway (Reith *et al.,* 2022), and increased susceptibility to RyR1-induced Ca2+ leak in a malignant hyperthermia mouse model (Bouitbir *et al.,* 2012). Although statins may be myotoxic, one study suggests that statins are highly beneficial in skeletal muscle affected by underlying conditions such as ischemia, oxidative stress, and inflammation. Statins reduce oxidative stress, inflammation and fibrosis, all processes associated with functional muscle decline in muscular dystrophy, especially Duchenne muscular dystrophy (DMD). Simvastatin has been shown to improve muscle strength and fatigue resistance in DMD mice, in DMD, the leading cause of death is heart failure (Hanai *et al.,* 2007).

Statin intolerance is most frequently associated with a wide range of side effects in the skeletal muscle, the so-called "Statin-Associated Muscle Symptoms" (SAMS). SAMS are quite difficult to be diagnose and manage not only because there are no validated biomarkers or tests that can be used to confirm their presence, but also because muscle symptoms could originate from other comorbidities (Taylor *et al.,* 2018). However, a study carried out by (Camerino *et al.* 2021), showed that patients may have a statin-induced myotoxicity occurring as muscle necrosis due to statin exposure and manifesting with increased CK levels. The most important risk factors of SAMS are advanced age, female gender, Asian ethnicity, drugs altering statin

plasma levels, excessive physical activity, muscle, liver or chronic kidney diseases, uncontrolled hypothyroidism, abdominal obesity and metabolic syndrome, and vitamin D deficiency. The risk of SAMS is higher with lipophilic statins such as simvastatin, atorvastatin, and lovastatin, because of their ability to not selectively diffuse into extrahepatic tissues as skeletal muscles. Typically, SAMS manifests with different muscle symptoms occurring after statin treatment (with or without elevations of serum creatine kinase) that might resolve after its interruption. SAMS has a highly variable clinical presentation ranging from a myopathic pattern, characterized by muscle tenderness, cramping and muscle aches, weakness and increased CK level (even 10 times higher the upper normal limit), to rhabdomyolysis (Vrablik *et al.,* 2014). Myopathy usually appears in patients who receive high doses of statins, especially when taking simvastatin 80 mg daily, which lead to higher plasma levels of active statins metabolites, especially in the first year of treatment or after having increased the dosage. Muscle disorders are often reversible after statin withdrawal (Horodinschi *et al*., 2019). Skeletal muscle is made up of fast and slow twitch muscle fibers, which have different compositions and react in various ways to outside substances like statins.

Statin treatment causes massive necrosis in fast-twitch, glycotic type II-B muscle in animals, while sparing the slower-twitch, oxidative type I fibers (Muntean *et al.,* 2017). These changes were accompanied by ultrastructural changes to the muscle mitochondria, such as swollen mitochondria with disrupted cristae and increased vacuolation or degeneration, which led to vesicular bodies accumulating in the subsarcolemmal space (Draeger *et al.,* 2006). T-tubular system vacuolization in statin-treated patients has also been seen in human studies (Mohaupt *et al.,* 2009).

Chapter Five: Conclusions and Recommendations

5.1. Conclusions

1. The present study demonstrates that squalene offered significant protection against statin-induced myopathy through enhancement of antioxidant property without producing any cytotoxic effects.

2. Explain the causes of statin-induced myopathy, as a result of simvastatin effects, the reduction of cholesterol and increase in serum creatine kinase levels in muscle cell membranes may play an important role in the development of myopathy.

3. Squalene effectively reduced cholesterol, ROS, creatine kinase levels in serum male rats'.

4. Histologically the study showed muscles amelioration a defect when combination of squalene with simvastatin.

5.2. Recommendations

From the results of the present study, it can be recommended the following**:**

1. This study is encouraged to take CoQ10 and squalene while on simvastatin therapy.
2. Conducting the experiment on other laboratory animals such as rabbits, hamsters…etc.
3. Study the effect of squalene with the same dose of simvastatin and study it effect on the liver, kidneys, brain and other organs.
4. Studying the benefits effect of squalene in preserving DNA from the change that occurs as a result of giving a dose of simvastatin.

Chapter Six: References

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APPENDIXES

APPENDIXES

Appendix (I): Estimation of serum cholesterol concentration (mg/dl):

Principle:

Easter of cholesterol+H2O $Chol.\ estease$ Cholesterol + Fatty acids Cholesterol +O2 $Chol.\ oxidase$ Cholest-4-en-one+H2O2 H2O+4-Aminophenazone + phenol $peroxidase$ Quinonimine Reagent:

Reagent:

(1) Buffer solution: pipes PH 6.9 mmol/L, phenol 26 mmol/L

(2) vial of enzyme: cholesterol oxidase 300 U/L, peroxidase 1250 U/L, cholesterol esterase 300 U/L, 4-aminophenazone 0.4 mmol/L Reagent.

(3): cholesterol standard 200 mg/dl 1. Manual procedure: Cholesterol concentration in serum samples was measured according to the following:

a. Reagent and serum samples were brought to room temperature

b. Serum sample, blank and standard was treated as follows:

c. Tube contents were mixed and left to stand for 5 minutes at 37 C before reading

d. The absorbance of the standard was measured and a sample was read via spectrophotometer at wavelength 505 nm against the blank.

Tubes	Blank	Standard	Sample
Cholesterol standard (s)	-	10 ml	-
Sample	-	-	10 ml
Working Reagent	1ml	1ml	1ml

Calculation:

Result were calculated according to the following equation:

Total Cholesterol concentration = (O.D sample)/ (O.D/ standard) × nn =200 mg/dl.

Appendix (II): Estimation of Serum Creatine Kinase:

Product Description Creatine Kinase (CK), also known as phosphocreatine kinase, is an enzyme that catalyzes the transfer of one phosphate group from ATP to creatine generating phosphocreatine, an important energy reservoir in muscle and brain tissue. CK is a dimeric protein made up of B (brain) and M (muscle) subunits. Three isoenzymes, CK-MM, CK-MB, and CK-BB, have been observed. CK levels are elevated in various pathological conditions including myocardial infarction, rhabdomyolysis, muscular dystrophy, and renal failure. The Creatine Kinase Activity Assay kit provides a simple and direct procedure for measuring CK levels in a variety of samples such as blood, serum, and plasma. In this assay, Creatine Kinase activity is

determined by a coupled enzyme reaction resulting in the production of NADPH, measured at 340 nm, proportionate to the CK activity present in the sample. In this reaction, phosphocreatine and ADP are converted to creatine and ATP. The generated ATP is used by hexokinase to phosphorylate glucose resulting in glucose-6-phosphate, which is oxidized by NADP in the presence of glucose-6-phosphate dehydrogenase to produce NADPH and 6-phospho-D-gluconate. One unit of CK is the amount of enzyme that will transfer 1.0 mmole of phosphate from phosphocreatine to ADP per minute at pH 6.0. This kit has a linear range of 30–1,800 units/L CK activity.

Components
The kit is sufficient for 100 assays in 96 well plates.

Assay Buffer Catalog Number MAK116A	12 mL
Enzyme Mix Catalog Number MAK116B	120 µL
Substrate Solution Catalog Number MAK116C	1 mL
Calibrator Catalog Number MAK116D	150 µL

Reagents and Equipment Required

Ultraviolet Spectrophotometric multiwell plate reader · Clear 96 well flat-bottom plate suitable for use in UV absorbance assays (Catalog No. CLS3635 or equivalent). Precautions and Disclaimer This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices. Storage/Stability This kit is shipped on dry ice. Storage at –20 °C, protected from light, is recommended.

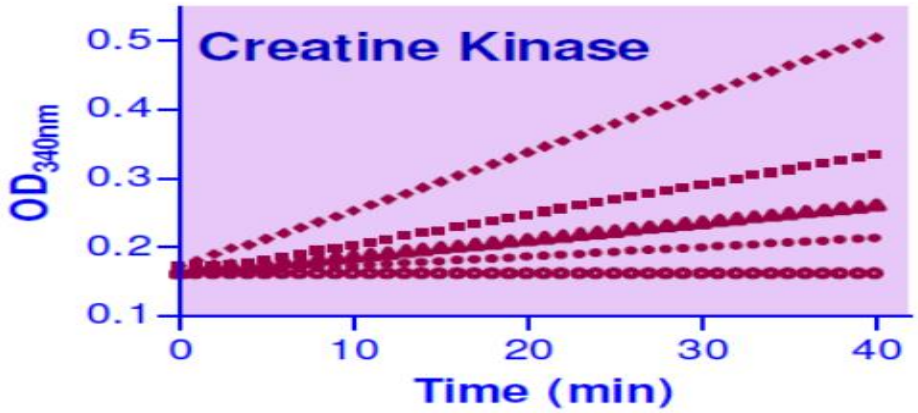
Procedure Sample Preparation Blood samples should be not being hemolyzed and assayed within 4 hours of collection if stored at room temperature and 12 hours if samples are stored at 2–8 °C. Alternatively, samples can be stored at –80 °C. Frozen samples can be thawed one time and may demonstrate some loss of activity. If turbidity is observed in the samples, centrifuge samples and used clear lysate for reactions. Tissue samples should be rinsed in phosphate-buffered saline, pH 7.4, to remove blood. Homogenize tissue (50 mg) in 200 mL of 50 mM postassium phosphate, pH 7.5, buffer. Centrifuge at 10,000 ´ g for 15 minutes at 2–8 °C. Use cleared supernatant for assay. Collect cells by centrifugation at 2,000 ´ g for 5 minutes at 2–8 °C. For adherent cells, do not harvest using proteolytic enzymes, instead use a cell scraper. Homogenize or sonicate cells in an appropriate volume of cold 50 mM potassium phosphate, pH 7.5, buffer. Centrifuge at 10,000 ´ g for 15 minutes at 2–8 °C. Remove supernatant for assay.

All samples can be stored at –80 °C for up to one month. Assay Reaction 1. This reaction can be carried out at either room temperature or 37 °C. Bring all components to room temperature or 37 °C before use. Prepare enough of the Reconstituted Reagent for each sample to be tested according to the scheme in Table 1. Each sample requires 100 mL of Reconstituted Reagent.

Reconstituted Reagent

Reagent	Volume
Assay Buffer	100 µL
Substrate Solution	10 µL
Enzyme Mix	1 µL

2. Transfer 110 mL of water into one well (Blank) and 100 mL of water plus 10 mL of the Calibrator into a separate well of a 96 well plate. 3. Transfer 10 mL of samples into separate wells. Add 100 mL of the Reconstituted Reagent to each sample well and tap plate to mix. 4. Incubate the samples at either room temperature or 37 °C. After 20 minutes, take the initial absorbance measurement at 340 nm (A340) initial. Note: CK is fully activated within 20 minutes by the glutathione present in the Substrate Solution. 5. Continue to incubate the plate at either room temperature or 37 °C for 20 additional minutes. Measure the (A340) final. Note; If the CK activity is expected to be higher than 300 units/L, measure the A340 at 5 minutes past the initial measurement. Calculations CK Activity (units/L) = (A340) final – (A340) initial 150 (A340) calibrator – (A340) blank where: 150 = equivalent activity (units/L) of the Calibrator when assay is read at 20 minutes and 40 minutes (20 additional minutes past initial reading). Note: If the CK activity is expected to be higher than 300 units/L, read A340 at 20 minutes and again at 25 minutes. To calculate the CK activity, replace [(A340) 40 min – (A340) 20 min] with [(A340) 25 min – (A340) 20 min] and replace the factor 150 with 600 in the above equation. Linear range: 30–1,800 units/L of CK activity One unit of CK is the amount of enzyme that will transfer 1.0 mmole of phosphate from phosphocreatine to ADP per minute at pH 6.0



Kinetics of CK reaction at 25 (solid circles), 50 (triangles), 100 (squares), and 200 (diamonds) units/L with Control (open circles).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For UV assays, use clear plates that are UV transparent or quartz plates.
Samples with erratic readings	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
	Samples measured at incorrect wavelength	Check the equipment and filter settings

Appendix (III): Free Oxygen Radicals (oxidants)

The TOS of sample was determined using a novel method, developed by Erel. Oxidants found in the sample oxidize the ferrous ion–o-dianisidine complex to ferric ion. By glycerol molecules the oxidation reaction is enhanced, which are richly found in the reaction medium. The ferric ion creates a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules found in the sample. The test is calibrated with hydrogen peroxide and the outcome are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H2O2Eq/L).

Principle of the assay

Reagent 1: Reagent 2 was prepared by dissolving 3.17 g of O-dianisidine dihydrochloride and 1.96 g of ferrous ammonium sulfate in 1000 mL of H2SO4 solution, 25 mM. The ending reagent was composed of 10 mM Odianisidine dihydrochloride and 5 mM ferrous ammonium sulfate. This reagent is stable for as a minimum 6 months at 4°C. Reagent 2: Reagent 1 was prepared by dissolving 8.18 g of NaCl and 114 mg of xylenol orange in 900 mL of H2SO4 solution, 25 mM. One hundred milliliters of glycerol were given to the solution. The ending reagent was composed of 140 mM NaCl, 150µM xylenol orange and 1.35 M glycerol. The pH value of the reagent was 1.75. This reagent is stable for as a minimum 6 months at 4°C.

Hydrogen peroxide: (100 µmol/L) was freshly diluted and standardized daily using a molar extinction coefficient of 43.6 M-1 cm-1 at 240 nm

Procedure

	Blank	Standard	Sample
Distilled water	50 µl	-----	------
Sample	------	-----	50 µl
Hydrogen peroxide	------	50 µl	------
R1	2 ml	2 ml	2 ml
Test tubes were mixed by vortex, and then add:			
R2	2 ml	2 ml	2 ml

Quietly mix the content of each tube after addition, allow standing at room temperature for 3 minute, read spectrophotometrically at 560 nm.

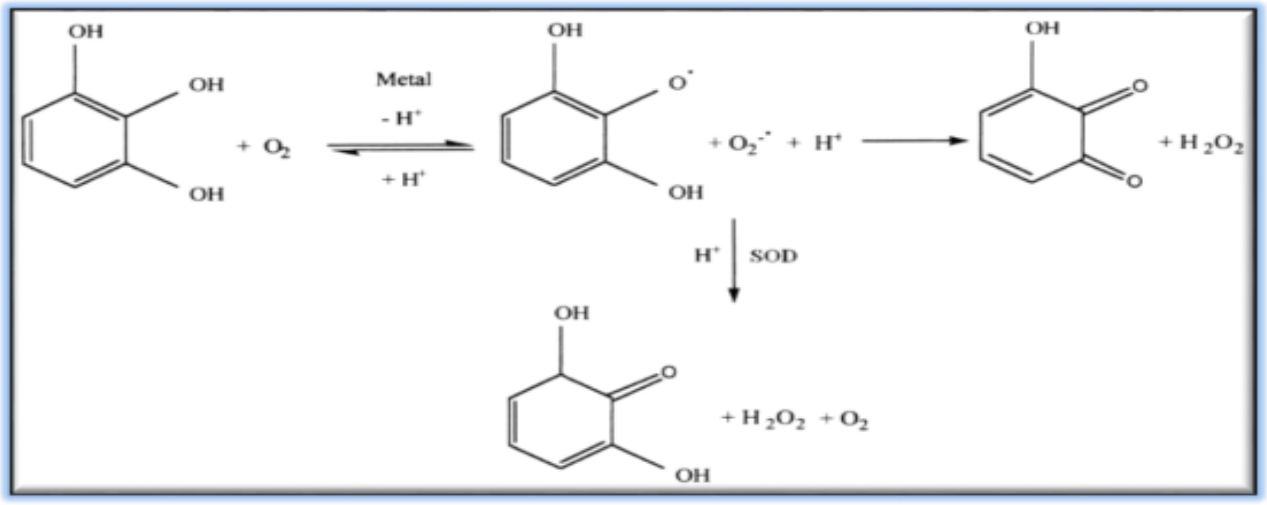
$$\text{Total oxidants status} = \frac{\text{A.test}}{\text{A.STD}} * Conc.of \quad STD$$

Appendix (IV): Superoxide Dismutase (SOD) Activity

Principle

(Cu-Zn) SOD activity was determined by use a simple and rapid method, based on the ability of the enzyme to inhibit the autoxidation of pyrogallol. The autoxidation of pyrogallol in the presence of EDTA in the pH 8.2 is 50%. The principle of this method is based on the competition between the pyrogallol autoxidation by $O_2^{\bullet -}$ and the dismutation of this radical by SOD [266].

Superoxide dismutase (SOD) provide an important role as cellular defense enzyme against free radical damage (Pillai and Pillai, 2002). SOD extracellular as antioxidant enzyme can catalyze the dismutation of the superoxide anion (the high reactive species) to O2and to H2O2 (the less reactive species). Then H2O2 can be destroyed by action of CAT or GPX reactions (Costa et al., 2009). O2- + O2- +2H- + SOD > H2O2 +O2 Three forms of SOD have been found: mitochondrial Mn-SOD, cytosolic Cu/Zn-SOD, and extracellular SOD (Lindberg et al., 2005; Ding & Dokholyan, 2008) Mn-SOD is the biological importance one. Cu/Zn-SOD is believed to play an important role as the first antioxidant defense line (Ding & Dokholyan, 2008).



Suggested Mechanism of Pyrogallol Autoxidation.

(Cu-Zn) SOD activities are expressed as units/ml. One unit of (CuZn) SOD activity being defined as amount of enzyme required to cause 50% inhibition of pyrogallol autoxidation [267]. 3.2.15.2 Reagents Preparation 1. Tris- EDTA buffer pH 8.2 A weight of 2.85 g of Tris and 1.11 g of EDTA-Na2 were dissolved in 1 liter of DW. 2. Pyrogallol Solution (0.2 mM) A weight of 0.252 g of pyrogallol was dissolved in a solution of 0.6 ml of concentrated hydrochloric acid diluted in 1 liter of Dw.

Procedure

Spectrophotometer was adjusted to read zero using Tris-EDTA buffer. Control and sample test tubes were prepared then pipetted into test tubes.

Reagents	Test (µl)	Control (µl)
Serum	50	-
Tris buffer	1000	1000
DW	-	50
Pyrogallol	1000	1000

Absorption was read at the wavelength of 420 nm against TrisEDTA buffer at zero time and after 1 minute of the addition of pyrogallol. 3.2.15.4 Calculation of SOD Activity ΔA control - ΔA test % Inhibition of pyrogallol autoxidation = X 100% ΔA control % inhibition of pyrogallol autoxidation (Cu-Zn) SOD Activity (U/ml) = 50%

Appendix (V): Histological Study

Histological Technique (E&H) Stain

The muscles of each animal were quickly removed and rapidly weighed then prepared for histological study according to Mescher method, (2010) with aid of the light microscope as the following steps:

*** Fixation** The specimen fixated in the formalin 10% for 24–48 hours. In front of the bone marrow sample placed in a Bouin‘s solution.

*** Washing and Dehydration** After fixation the specimens washed with water to remove the fixative in order to avoid the interaction between the fixative and staining materials used later. By dehydration the water had been completely extracted from

fragments by bathing them successively in a graded series of ethanol and water (70%, 80%, 90%, and 100% ethanol).

*** Clearing** Bathing the dehydrated fragments in solvent (Xylene) for 30–60 minutes, this step was repeated 3 times. As the tissues clearing, they generally became transparent.

*** Infiltration and Embedding** Once the tissue fragments were impregnated with the solvent, they were placed in melted paraffin in an oven, typically at 52 C. The heat causes the solvent to evaporate, and the space within the tissues becomes filled with paraffin

*** Sectioning** After holds from the oven, the specimen let at room temperature to be solid and removed from their containers in order to sectioning they were put in the rotary microtome and were sliced by the microtome, a steel blade into sections 5 micrometers thick. The sections were floated on water bath (50–55 oC), then transferred into glass slides coated with Mayers albumin as adhesive substance and left to dry.

*** Staining** The histological sections of the studied organs were stained with HematoxylinEosin stain. Staining Procedure

1. Deparaffinize sections, 2 changes of xylene, 10 minutes each.

2. Re-hydrate in 2 changes of absolute alcohol, 5 minutes each.

3. 95% alcohol for 2 minutes and 70% alcohol for 2 minutes. 4. Wash briefly in distilled water.

5. Stain in Harris hematoxylin solution for 8 minutes.

6. Wash in running tap water for 5 minutes.

7. Differentiate in 1% acid alcohol for 30 seconds. 8. Wash running tap water for 1 minute.

9. Bluing in 0.2% Ammonia water or saturated Lithium Carbonate solution for 30 seconds to 1 minute.

10. Wash in running tap water for 5 minutes.

11. Rinse in 95% alcohol, 10 dips.

12. Counterstain in Eosin-Phloxine solution for 30 seconds to 1 minute.

13. Dehydrate through 95% alcohol, 2 changes of absolute alcohol, 5 minutes each.

14. Clear in 2 changes of Xylene, 5 minutes each.

15. Mount with Xylene based mounting medium Trichrome stain (Modified masson's) procedure.

الخلاصـــــة

أجريت هذه الدراسة لمعرفة دور مكملات السكوالين على الجرذان المعاجة بالسمفستاتين وكيفية عكس العمل المحسن للسكوالين على الآثار الجانبية السلبية للستاتين. تم تنفيذ التجربة في بيت الحيوانات للطب البيطري / جامعة كربلاء خلال الفترة من (أكتوبر 2022 إلى مارس 2023). تم تقسيم ثلاثين جرذ من الذكور البالغين إلى مجموعتين الذين تتراوح أعمارهم بين (2-3 أشهر) ووزن 260-300غم، (التجربة الاولى) ستة من الجرذان جرعت مع محلول ملحي طبيعي فقط واستخدمت كمجموعة السيطرة السلبية لمدة 28 يومًا. اما بقية الجرذان 24 فجرعت بالكولسترول1% من وزن الجسم لمدة 28 يوماً. (التجربة الثانية) تمت بتقسيم الجرذان الأربع والعشرون الى أربع مجاميع ست جرذان لكل مجموعة فكانت المجموعة الأولى هي مجموعة الكولسترول والتي اعتبرت كمجموعة سيطرة إيجابية اما المجموعة الثانية جرعت بالسمفستاتين 40 ملغم/كيلوغرام من وزن الجسم والمجموعة الثالثة جرعت بالسكوالين 2% والمجموعة الرابعة جرعت بالسمفستاتين مع السكوالين.

زاد الكوليسترول في الحيوانات المعالجة بشكل كبير عند ($P < 0.0001$) من المجموعة التي تلقت الكوليسترول فقط دون أي علاج إضافي (التحكم الإيجابي)، كما ازادات التراكيز بشكل معنوي كبير لكل من الكرياتين كاينيز وبيروكسيد الهيدروجين في مجموعة سيمفاستاتين بينما انخفض تركيز السوبر اوكساید ديسموتيز بشكل كبير في مجموعة سيمفاستاتين في ذكور الجرذان بعد أربعة أسابيع من تلقي الكوليسترول. علاوة على ذلك، انخفضت تراكيز الكرياتين كاينز وبيروكسيد الهيدروجين بشكل ملحوظ في مجموعة السكوالين ومزيجها من سيمفاستاتين مع سكوالين، بينما زاد تركيز SOD بشكل كبير في تلك المجموعتين.

تُظهر التغيرات النسيجية في عضلات الجرذان المجموعتين المعالجة بالكوليسترول، والسيمفيستاتين ضمور ألياف العضلات، وتغيرات مرضية واضحة بالمقارنة مع مجموعة السيطرة، ولكن بعد إعطاء السكوالين للجرذان المعالجة بالسيمفيستاتين، لوحظ انها تقلل من حالة التغيرات المرضية أو تحسينها للالياف العضلية ولكن لم تستعاد بشكل تام.

وتشير النتائج إلى أن السكوالين يتداخل مع اختبار مستويات الكوليسترول في الحيوانات. حيث توصلت الدراسة الى ان السكوالين وسيمفاستاتين معاً أكثر فعالية من سيمفاستاتين وحده، كما يتضح من النتائج التي تبين أن المكملات الغذائية من السكوالين في مستوى 2 ٪ خفضت إلى حد كبير من الكوليسترول في مستوى الدم في الجرذان التجريبية في وقت واحد مع خفض الكرياتين كاينيز. هذه النتائج تشير إلى أن السكوالين لا يتفاعل مع سيمفاستاتين من خلال تفعيل تخليق الكوليسترول في التغذية، بل ينظم العملية التي توفر فرصة جديدة لتقليل السمية العضلية.

وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الطب البيطري
فرع الفسلجة والكيمياء الحياتية والأدوية

التأثير المحسن لمكملات السكوالين في الجرذان المعالجة بالسيمفاستاتين

رسالة مقدمة الى مجلس كلية الطب البيطري / جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في اختصاص علوم الطب البيطري/الفسلجة

بواسطة

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ميادة صاحب حسن

الأستاذ المساعد الدكتور

محمد جاسم جواد

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