Republic of Iraq

Ministry of Higher Education & Scientific Research

University of Kerbala - College of Science

Biology Department

Investigation of some Biochemical and histopathological changes in liver and kidney of diet induced obesity in male albino mice treated with rosuvastatin

A Dissertation

Submitted to the Council of the College of the Science, University of Kerbala, in Partial Fulfillment of the Requirement for the Degree of Master of biology

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2021 A.D 1442 A.H

Certification

I certify that this dissertation (Investigation of some Biochemical and histopathological changes in liver and kidney of diet induced obesity in male albino mice treated with rosuvastatin) was prepared under my supervision at the College of Science, University of kerbala, as a partial requirement for the degree of Master of Science in biology.

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بسم الله الرحمن الرحيم

صدق الله العلي العظيم

سورة طه الآيه (١١٤)

Dedication

To my parents

The reason of what I became today

Thanks for your great support and continuous care

To my sisters and my brother

I am really grateful to all of you

You have been my inspiration and my soul mates

Acknowledgement

I would like to express my sincere gratitude to my supervisor Asst. Prof. Dr. Jasem Hannon Hashim Al-Awadi for his guidance, and his scientific and moral support.

I would like to express special thanks of gratitude to Asst. Prof. Dr. Ali Abdul-Kadhim for his help and guidance during my project. I also would like to thank Prof.Dr. Zaid Hassan Abboud and Asst.Prof. Dr. Nasir Mirza Hamza Hassan, who helping me to complete my project, also all my thankful and gratitude to Asst. Prof. Dr. Kawkab Abdulla Hussein AL-saadi.

Finally, all thankful and gratitude to my family for their support and continuous care.

Summary:

The study was designed to investigate the biochemical and histological changes in mice that consuming diet rich with animal and vegetable fats that caused obesity, which treated with rosuvastatin in its two forms (free and nano) after induction obesity for one months (the total period of experiment for four months). For this purpose about 70 Albino mice were divided into seven equal experimental groups each of 10 animals. The first group fed on normal chow diet, it was called normal diet group, second and third groups supplied by high fat diet (HFD) which consists of 15% of animal and vegetable fats. Mice treated groups with 0.02mg/kg/day of rosuvastatin and nano rosuvastatin which proceed along with high fat diet for one month, include four groups (animal high fat diet and rosuvastatin, animal high fat diet and nano rosuvastatin, vegetable high fat diet and rosuvastatin, vegetable high fat diet and nano rosuvaststin). At the end of experiment period which continued for four months all mice were weighted and sacrificed and the following assays were performed which consist:: measurement of body weight (once monthly), biochemical assay to measurement the Total cholesterol(TC), Triglycerides(TG), High density lipoprotein(HDL), Low density lipoprotein(LDL) and Very low density lipoprotein(VLDL), As well as measuring liver enzymes levels Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), and histological study of the histopathological changes evaluation which correlated with obesity and rosuvastatin treatment, the histological study includes of the following organs: liver and kidney.

the result showed: There was significant increase in the final body weight ($p<0.05$) in the positive control groups (animal and vegetable high fats diet groups) as compared with negative control group, but mice

consuming diet rich with vegetable fat caused significant increase ($p<0.05$) in the body weight as compared with animal high fat diet groups. The results showed significant increase ($p<0.05$) in the level of TC, TG, LDL-C, VLDL-C, AST and ALT in positive control groups (animal and vegetable high fats diet groups) as compared with negative control group. TC, LDL-C and ALT enzyme levels were significantly ($P<0.05$) higher in vegetable high fat diet supplemented group comparatively with animal high fat diet group , while TG , VLDL-C and AST enzyme level were higher in animal high fat diet supplemented group comparatively with vegetable high fat diet group. Mice consuming high fat diet showed significant decrease ($p<0.05$) in HDL-C level in both positive control groups (animal and vegetable high fats diet groups) as compared with negative control group.

Treated with rosuvastatin showed no significant change in the body weight in positive control groups as compared with negative control group, while treated with nano rosuvastatin showed significant reduction ($p<0.05$) in the body weight of high fat diet groups compare with negative control group. Mice treated with rosuvastatin and nano rosuvastatin showed significant decrease ($p<0.05$) in the level of TC, TG, LDL-C, VLDL-C, ALT and AST in all treatment groups as compared with positive control groups (animal and vegetable high fat diet groups) and significant increase in HDL-C, but treated with nano Rosuvastatin showed significant decrease ($p<0.05$), in lipid profile level and liver enzyme level compare with rosuvastatin.

Changes in liver sections of mice consuming high fat diet (animal and vegetable fat) showed, mild to severe lymphocytes infiltration, sever lipid accumulation as macro and micro vesicular steatosis, necrosis, pyknotic nuclei. These changes were more sever in high animal fat liver section

comparatively with liver section of mice consuming diet rich with vegetable fat. Liver section treated with rosuvastatin showed change that was of less severity as compared with positive control mice these changes included: little infiltrate lymphocytes, pyknotic nuclei, and apoptotic cells, also rosuvastatin treatment showed ameliorated the steatosis, compare with high fat diet liver section. Liver section treated with nano rosuvastatin showed much more improvement compare with liver section treated with Rosuvastatin.

Kidney sections of positive control groups showed different histological changes, compared with negative control group these changes include: dilated in renal tubules, degeneration and necrosis in the cells lined renal tubules, abundant vascular congestion with thickening of the basement membrane, interstitial severe lymphocytes infiltration and widened interstitial space. Kidney section treated with rosuvastatin and nano rosuvaststin showed mild changes which include: congestion mild inflammatory lymphocytes infiltration, mild hyaline cast, necrosis, also the treated section showed normal architecture which described previously, normal glomeruli, normal capsular space and normal renal tubules.

List of Abbreviations:

4-AA	4 – Aminoantipyrine
4-AP	4 – Aminophenazone
ABCA1	ATP binding cassette transporter-A1
AF	Animal fat
AFM	Atomic Force Microscope
AHR	Aryl hydrocarbon receptor
ALT	Alanine aminotransferase
APO B	Apolipoprotein B
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BMI	Body mass index
CD36	cluster of differentiation 36
CDC	Centers for Diseas Control and Prevention's
CE	Cholesterol ester
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
CHE	Cholesterol esterase
CHOD	Cholesterol oxidase
CLA	Conjugated linoleic acid
CRP	C-reactive protein
CVD	Cardiovascular disease
CYP 450	Cytochrome P450
DCC	Deleted in Colorectal Cancer
ECM	Extracellular matrix
FFA	Free fatty acids
FT-IR	Fourier transform infrared spectroscopy
GK	Glycerolkinase
GPO	Glycerol-3-oxidasa
HDL	High-density lipoprotein
HFD	High-fat diet
HF-R10	High fat-Rosuvastatin 10mg
HF-R20	High fat-Rosuvastatin 20mg
HMG-CoA	Hydroxymethyl glutaryl coenzyme A reductase
HPG	Hypothalamic-pituitary-gonadal
IARC	International Agency for Cancer Research
IGFBP1	Insulin-like growth factor binding protein 1
IL	Interleukin

lbLDL-C	Large buoyant LDL particle
LCAT	Lecithin-cholesterol acyltransferase
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LPL	Lipoprotein lipase
MCP-1	Monocyte chemotactic protein-1
MDH	Malate dehydrogenase
NAFLD	Non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
OSA	Obstructive sleep apnea
OX-LDL	Oxidized low-density lipoprotein
PCSK9	Proprotein convertase subtilisin/kexin type 9
POD	Peroxidase
RCT	Reverse cholesterol transport
ROS	Reactive oxygen species
rTFA	ruminant trans fatty acid
sdLDL-c	Small dense LDL cholesterol
SR-BI	Class B scavenger receptor type I
SRE	Sterol regulatory element
SREBPs	Sterol regulatory element-binding proteins
TC	Total cholesterol
TG	Triglycerides
TGF 1	Transforming growth factor 1
TNF-α	Tumor necrosis factor-α
VEGF	Endothelial vascular growth factor
VF	Vegetable fat
VLDL	Very low density lipoproteins
WAT	White adipose tissue
WAT	White adipose tissue
WHO	World Health Organization
XRD	X-ray diffraction

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Introduction

Introduction:

Obesity is a chronic disease that has spread throughout the globe and threatens worldwide public health, clinically; it is characterized as a condition of increased body weight, more precisely of increased mass of fat tissue. It is related to an increased size and volume of fat cells and is usually accompanied, if not controlled, by serious health effects, several variables, including genetic, metabolic, environmental and psychological determines body weight and the storage of energy as triglycerides in adipose tissue, these factors eventually change the equilibrium between power consumption and expenditure (Romieu *et al.,* 2017; Engin, 2017).

High-fat diet (HFD) consumption and the type of fat considered a risk factor for the development of obesity (Rocha *et al.,* 2019). Overconsumption diet rich with fats, particularly hydrogenated vegetable oil that's rich with trans-fatty as a byproduct for hydrogenation process and animal fats, which naturally synthesis from cow and sheep milk that rich in saturated fat, these fats considered the major factor that contributes to excess weight gain and obesity growth (Buettner *et al.,* 2007). Energy consumption that exceeds metabolic requirements leads to lipogenesis and fat accumulation in white adipose tissue (WAT), the body's main fat storage spot. Overconsumption of dietary fat can lead to a relatively rapid increase in weight, as dietary fat is metabolized into free fatty acids, the primary substrate for triglycerides (TG), and then lipid synthesis (Botchlett and Wu, 2018).

In developed and developing nations, obesity is categorized as chronic and serious disease, affecting both adults and kids, according to the World Health Organization (WHO). Recent International Obesity Task Force study has shown that 1.7 billion individuals are subjected to health hazards linked to body weight, while the rise in the Body Mass Index

(BMI) is liable for more than 2.5 million deaths per year, which is anticipated to double by 2030 (Torjesen, 2007; Berghöfer *et al*., 2008). The most common measure of obesity used is obtained from the body mass index (BMI), BMI is achieved by splitting weight (in kilograms) by square height (in meters), the World Health Organization describes overweight and obesity as a BMI value higher than or equal to 30(kg/m2) (WHO, 2004).

Obesity is usually responsible for many chronic diseases, including cardiovascular issues, hypertension, diabetes and hyperlipidemia (Choudhury and Sanyal, 2004; Hristov *et al.,* 2019). In addition, obesity has been the most essential risk factor for complicated and chronic liver disorders (Sarwar *et al.*, 2018). These liver illnesses start as steatosis and can lead to steatohepatitis, cirrhosis, hepatocellular carcinoma and liver failure (Haynes *et al*., 2004; Mori *et al*., 2004; Saitta *et al.*, 2019). Insulin-resistant type 2 diabetes and hyperlipidemia have been recorded in most instances of the liver (Bugianesi *et al*., 2004).

In obesity condition kidneys showed demonstrate functional and morphological changes (Papafragkaki and Tolis, 2005). Some studies have shown the danger of chronic kidney disease (CKD) in patients with metabolic syndrome; obesity may also be correlated with breathing issues such as obstructive sleep apnea and the syndrome of obesity hypoventilation, obese people usually display decreased chest wall compliance, elevated breathing work, decreased total lung capacity and residual functional ability (Chen et al., 2004; Palaniappan et al., 2003). Obesity can also affect the functioning of the hepatobiliary system; obese people have a greater occurrence of gall bladder stones, especially cholesterol gallstones, In addition, Obesity may contribute to infertility, Furthermore, obesity is connected with issues of bones and joints such as

osteoarthritis and gout, It can also lead to skin issues, viz. Acanthosis nigricans (demonstrated by darkening and thickening of folds of skin on the neck, elbows and interphalangeal spaces), increased skin friability and greater risk of fungal infections (Cefalu *et al.,* 2015).

Research has shown that loss of weight and Physical activities are considered among the best treatment for the obese and over weighted (Tyson *et al.,* 2013), also using Hydroxymethyl glutaryl coenzyme A reductase (HMG-CoA) inhibitors (usually known as statins) which become one of the most widely specified lipid lowering drug, Generally, the effect of statins on the lipid profile is consistent between decreases in total cholesterol, LDL-C and triglycerides, and an increase in HDL-C, resulting in reduced fat deposition and reduced body weight gain.

Aims of the study

The present study was carried to evaluate the effect of animal and vegetable fats on some parameter of lab animals and the possibility of treated the lipidemia by using rosuvastatin drug by the following objectives:

1. Measurement of body weight changes.
2. Measurement lipid profile (TC, TG. HDL-C, LDL-C, VLDL-C) and liver enzymes (AST, ALT).
3. Investigate the histopathological changes in liver and kidney.
4. Study the efficiency of rosuvastatin in its two form (free and nano) to eliminate the favorable effect of high fat diet.

Chapter One
Literature review

Chapter One

1-Literature Review

1-1- Obesity definition and classification

A high fat diet (HFD) is recognized as a critical factor that contributing to the epidemic of obesity, medically, obesity is described as a state of increase in body weight, more precisely of increased fat tissue mass, It is correlated with increased fat cell size and number and is typically accompanied by serious health effects if not treated (Schrauwen and Westerterp, 2000; Bray *et al.*, 2004). Several various variables influences the body weight and energy storage as a triglyceride in adipose tissue, Eventually, these variables work by altering the balance between energy consumption and expenditure (Romieu *et al.*, 2017).

The World Health Organization (WHO) is using body mass index (BMI) to define adult obesity; it's described as body weights (kg) divided by measured square height (m2), Individuals with a BMI of 30 or higher are usually considered obese. A person with a BMI equal to or greater than 25 shall be regarded as overweight (WHO, 2012), as mentioned in Table (1-1)

Table (1-1): WHO adult BMI classification

Category	BMI(kg/m2)
Underweight	<18.5
Healthy weight	18.5–24.9
Overweight:	>=25.0
Preobese	25.0–29.9
Obese:	>=30.0
Obesity grade I	30.0–34.9
Obesity grade II	35.0–39.9
Obesity grade III	≥40.0

The BMI provides the most useful measure of obesity and overweight at the population level, since it is the same for both sexes and adults of all ages, but it's considered a rough index because it does not refer to the same amount of body fat in different individuals, The benefits of BMI are that it is easy to evaluate and the most commonly used measure in population-based research (Romero-Corral *et al*., 2008).

The child's weight is determined depending on the Centers for Disease Control and Prevention's (CDC) BMI-for age growth charts, rather than the adult BMI categories, this is because the body composition of children differs as they age and differs between boys and girls, as mentioned in Table (1-2) by (CDC, 2015).

Table (1-2): Weight status categories in children

Weight Status Category	Percentile Range
Underweight	Less than the 5th percentile
Normal or Healthy Weight	5th percentile to less than the 85th percentile
Overweight	85th to less than the 95th percentile
Obese	95th percentile or greater

1-2-Type of fats:

1-2-1-Hydrogenated vegetable oil:

Hydrogenated vegetable oil usually palm oil, which produced by chemical process called hydrogenation that performed by adds hydrogen atoms to the double bonds available in the vegetable oil, result in convert oil into solid in room temperature (Gómez-Cortés *et al.*, 2019). Hydrogenated vegetable oil rich with trans-fatty as byproduct for hydrogenation process which is about 60%, that medically proven the consumption of Trans-fat have adverse effect on health, including lipid profile level, insulin resistance, chronic inflammation, metabolic syndrome, diabetes, and cardiovascular disease (CVD) (Takeuchi and Sugano, 2017).

1-2-2-Animal fat:

Animal fats naturally synthesis from cow and sheep milk, it's rich with saturated fat (Jing *et al.*, 2019). Animal fats regard a good source of vitamins, particularly vitamin D (Schmid and Walther, 2013), and contain ruminant trans fatty acid (rTFA) that produced through biohydrogenation within ruminant animals of cows and sheep which possess health benefits, include: conjugated linoleic acid (CLA) which has antioxidant properties, such as anti-obesity, anti-carcinogenic, anti-atherogenic, anti-diabetic, anti-mutagenic, anti- hypertensive, immunomodulatory, apoptotic and osteosynthetic (Mehta *et al.*, 2015). Despite all benefits of animal fat but conceder healthy when consumption in moderate quantities, too much could too dangerous for health which are considered risk factors for CVD and could raise the cholesterol level (Sharma *et al.*, 2010).

1-3-Causes of overweight and obesity:

1-3-1-Genetic influence:

The role of genetics in obesity etiology has also been studied extensively. Recent research indicates that genetics contribute 40-70% to obesity as more than 50 genes are identified that are closely associated with obesity. More popularly, obese individuals have multiple genes that predispose them to obtain extra weight; one of these genes is the fat mass and obesity-associated gene (*FTO*) present in up to 43% of the people. Those with the fat mass and obesity-associated gene can face challenges in restricting their calorie consumption, the presence of this gene can increased hunger levels, excessive calorie consumption, reduced satiety, decreased feeding control and increased tendency to store excess fat (Sicat, 2018).

Study showed that the recognition and sequence of the *ob* gene, that encodes the peptide leptin, and finding that the mutation in this gene (*ob/ob*) causes the mice to suppress the hormone which appears to be the main cause of obesity in *ob/ob* mice (Acharya *et al.,* 2019). However, the rapid increase in the spread of obesity in the last years cannot have been justified by genetic modifications that theoretically could not have happened within such a short period of time, therefore some investigators point out that the difference in the rate of obesity in some population groups is linked to environmental factors, in particular diet and reduced physical activity (Sicat, 2018).

1-3-2-Environmental Origin:

Environmental effects on overweight and obesity are mainly related to food intake and physical activity behaviors (Sikorski *et al.,* 2011). The abundance of dulcet calorie-dense foods, In addition, aggressive and

developed marketing of food in mass media, supermarkets and restaurants, as well as huge portions of food served outside the home, promotes high calorie consumption. Many of our socio-cultural traditions encourage overeating and the favorable intake of high calorie foods (Qasim *et al.*, 2018). For many people, even if the calorie intake is not above the recommended level, the amount of calories spent on physical activity is insufficient to compensate for consumption. Individual attitudes and behaviors are critical to weight management in this obesity-promoting setting (Maddock, 2004).

1-4- Health Effects of obesity:

1-4-1- Hypertension:

Obesity increases the danger of death caused by hypertension, dyslipidemia, diabetes, coronary heart disease (CHD), stroke and cardiac failure (Jensen *et al.*, 2014; Alagiakrishnan *et al.*, 2016), Hypertension with obesity are both attributed to increase the risk of death from all these diseases including cardiovascular death (Berrington *et al.*, 2010; Whitlock *et al.*, 2009). The association among the excess adiposity and elevated blood pressure is well known, and obesity is rated about 65 to78% of main hypertension cases. The techniques through which obesity causes hypertension are complicated which include over activation of the sympathetic nervous system, stimulation of the renin-angiotensin-aldosterone system, alterations in adipose-derived cytokines, insulin resistance as well as structural and functional alterations in the renal system; weight reduction is the main objective to obesity-related hypertension therapy, but few patients achieve success only with nonpharmacological intervention (Omair and Travis, 2020).

1-4-2- Insulin resistance:

Diabetes and insulin resistance are closely related to the body mass index. The amount of nonesterified fatty acids, glycerol, cytokines proinflammatory markers, and many other substances involved in the progression of insulin resistance, is elevated in obese individuals (Al-Goblan *et al.,* 2014).Research to explain the mechanisms investigates the relationships between obesity and insulin resistance reinforces the theory that visceral, but not subcutaneous obesity leads to insulin resistance and increased risk of Type 2 diabetes. The processes through which insulin resistance develops in visceral obesity tend to be linked to excess fat accumulation in the liver, Excess lipid accumulation can lead to reduced insulin signaling by cell autonomous mechanisms, or by inflammation which increases in obesity (Hardy *et al.,* 2014). The processes that increase inflammation throughout obesity are not completely understood increased pro-inflammatory cytokine release in obesity lead to insulin resistance, Among these cytokines, tumor necrosis factor-α (TNF-α) consider the first cytokine known to be capable of stimulating insulin resistance in adipocytes in vitro (Castoldi *et al.,* 2016).

1-4-3- Cancer:

Over the previous years, compelling evidence shows that obesity and obesity-related diabetes are connected with a higher occurrence of some cancers (Park and Colditz, 2017; Wolin *et al.*, 2010).There is a lot of proof to support the connection between adult obesity and overweight, and many cancers. Surplus adiposity is also known to be the key risk factor for cancer, so that obesity along with physical inactivity are currently recognized as one of the most important risk factors for primary cancer besides the tobacco use (Park and Colditz, 2017; Wolin *et al.*,

2010). The International Agency for Cancer Research (IARC) working group reported that there is adequate proof that obesity lead to cause cancer of the esophagus (adenocarcinoma), stomach cardio, colon, rectum, liver, gallbladder, pancreas, breast (postmenopausal), corpus uterus (endometrium), ovary, kidney (renal cell), meningioma, thyroid and multiple myeloma (Lauby-Secretan *et al.*,2016 ; Park and Colditz, 2017).

The relationship between obesity and cancer can be clarified by differences in the metabolism of endogenous hormones (including insulin - like growth factors and sex steroids) that may impair the normal balance between cell proliferation, differentiation, and apoptosis, research suggests that chronic inflammation plays a key role in cancer risk, probably involving dietary content. (Irigaray *et al.*, 2007; IARC, 2002).

1-4-4-Liver disease:

Liver is the largest solid organ in adults, accounting for 2-3 per cent of body weight and 25-30 per cent of overall oxygen intake. Normal liver function is important for the maintenance of metabolic homeostasis, and there is a complex connection between the liver and adipose tissue to regulate the metabolism of carbohydrates, lipids and proteins. Obesity can cause hyperinsulinemia, hyperglycemia, fat deposition, as well as resistance to insulin in the live, all this, in turn, may affect the normal liver function, ranging from raise the circulating liver enzyme levels and steatosis to local inflammation (steatohepatitis), cirrhosis, liver failure and even liver cancer (Diehl and Day , 2017 ; Williams *et al.*,2013).

The term non-alcoholic fatty liver disease (NAFLD) is now used to describe this spectrum of hepatic abnormalities, it seems to be the most prevalent cause of chronic liver disease, ranging from steatosis (lipid

deposition in the liver particularly triglyceride) which can take one of two forms anatomically, based on the size of the lipid vesicles: microvesicular steatosis and macrovesicular steatosis (Catta-Preta *et al.*, 2011), and non-alcoholic steatohepatitis (NASH) to cirrhosis (fibrosis) and hepatic failure (Abel *et al.*, 2009; Brunt, 2004; Brunt and Tiniakos 2010), It is usually observed in visceral obesity, insulin resistance, dyslipidemia and high blood pressure patients (Kaser *et al.*, 2010). The NAFLD is generally distributed among the adult population, but there is also a growing incidence among the paediatric (estimated NAFLD prevalence of 3-10%) with reported NAFLD incidence levels of up to 80% in kids with obesity (Chalasani *et al.*, 2012; Giorgio *et al.*, 2013).

1-4-5-Kidney disease:

Obesity is a strong risk factor for the kidney disease development which contributes to the development of chronic kidney disease (CKD) Also referred to as chronic renal disease; it explains the progressive loss of kidney function. The kidneys flush out the waste and excess fluids from the blood, which are then excreted in your urine. If chronic kidney disease is at an advanced stage, harmful amounts of fluid, electrolytes, and waste will build up in the body. In individuals affected by obesity, a compensated process of hyperfiltration is used to meet the increased metabolic demands of increased body weight. Increased intraglomerular pressure can damage the structure of the kidneys and increase the risk of developing CKD over the long term (Kovesdy *et al.*, 2017; Nehus, 2018).

Obesity affects the development of CKD, among other causes, since it promotes to diabetic nephropathy, hypertensive nephrosclerosis and focal and segmental glomerulosclerosis. Overweight and obesity are correlated with hemodynamic, structural and histological renal modifications, in

addition to metabolic and biochemical modifications contributing to kidney disease, such as chronic inflammation, increased oxidative stress, and hyperinsulinemia. (Lakkis and Weir, 2018; Mascali *et al.,* 2016).

1-4-6- Gallstones:

The development gallstone chances in humans may significantly rise due to obesity, Cholesterol serves a main role in the development of gallstones, thought influence bile saturation which can cause the formation of gallstones (Zahra and Kaisrani 2019; Park et al., 2017). Gallstones are found to have higher incidence in women than in men (Aune *et al.,* 2015; Field *et al.,* 2001), The explanation for this gender gap is believed to be hormonal, for example during the pregnancy serum estrogen rises, which may promote biliary cholesterol saturation, leading to increased progesterone that may in turn inhibit gallbladder contraction (Novacek, 2006). Hypersecretion of cholesterol (linked to obesity) is a major pathogenic element; Studies have found that people who have obesity may have greater levels of cholesterol in their bile that may lead to cause gallstones (Aune *et al.,* 2015).

Gallstone disease also can be noticed among people who face rapid weight lose either by consume low caloric diets or surgeries, In such condition the liver secret additional cholesterol into the bile, also cholesterol which is activated from adipose tissue is released into the bile, which caused oversaturation of cholesterol and reduce gallbladder contraction, the rapid weight loss can also discourage the gallbladder from cleaning correctly (Erlinge, 2000). However, it has been suggested that the main cause of the development of gallstones is obesity instead of weight loss.

1-4-7- Obesity and respiratory system disorders:

Increased body weight and fat accumulation in the abdomen and chest wall may have a major impact on respiratory physiology, leading to reduce of lung function, mostly due to increased mechanical pressure on the thoracic cage and trunk (McClean *et al.*, 2008; Sebastian, 2013). Obesity is often associated with a host of various respiratory disorders, including obstructive sleep apnea, hypoventilation syndrome, asthma, and chronic obstructive pulmonary diseases (McClean *et al.*, 2008; Crummy *et al.*, 2008; Sin and Sutherland, 2008). Sleep apnoea is widespread respiratory disorder that characterized by recurrent episodes of cessation of respiratory airflow caused by occlusion in the upper airway during sleep, with aconsequent decreasein oxygen saturation (Romero-Corral *et al.*, 2010). Reducing weight has been shown to be connected with reduced the upper airway collapsibility in obstructive sleep apnoea (Sutherland *et al.*, 2011).

1-4-8- Dyslipidemia:

Abnormalities in lipid metabolism are very common in patients who are obese; approximately 60-70% of patients with obesity are dyslipidemic, Lipid abnormalities seen in obese patients include elevated triglyceride, very low density lipoproteins (VLDL), apolipoprotein B (Apo B) and low-density lipoprotein (LDL) that are commonly observed while High-density lipoprotein (HDL) cholesterol and Apo A-I levels are typically low (Bays *et al.*, 2013; Franssen *et al.*, 2011). The dyslipidemia is partly responsible for the increased risk of cardiovascular disease in obese patients; Low-density lipoprotein (LDL) plays a key role in the development and progression of atherosclerosis and cardiovascular disease. LDL consists of several subclasses of particles with different

sizes and densities, including large buoyant LDL-C (lbLDL-c), intermediate and Small dense LDL-C (sdLDL-c) (Hirayama and Miida, 2012; Ivanova *et al.,* 2017). These small dense LDL particles are regarded more pro-atherogenic than other LDL particles for a number of reasons (Berneis and Krauss, 2002).The (sd) LDL particles have lower affinity to the LDL receptor resulting in prolonged period of time in the circulation, In addition, these small particles more easily enter the arterial wall than large particles and then they bind to intra-arterial, which traps them in the arterial wall (Masuda *et al.,* 2017; Klop *et al.,* 2013).

1-5-Obesity treatment:

1-5-1-Change the lifestyle:

Weight loss is primarily dependent on reducing total caloric intake, not the dietary proportions of carbohydrate, fat, and protein (Sacks et al., 2009).The dietary therapy is based on the criteria of decreased energy intake and increased energy expenditure by assisting the patient in making healthier dietary and physical activity choices that will lead to a net negative energy balance, the original objective is to obtain a weight loss of 5% to 10% over the original 6 months of therapy (Wadden *et al.,* 2012). Physical activities are considered among the best treatment for the obese and over weighted patients, obesity is related to a sedentary lifestyle. Practice causes lipolysis, resulting in free fatty acid release from triglycerides stored in fat for use as an energy source by muscle, increasing energy consumption (Fujioka *et al.,* 2000).

1-5-2- Statins drugs:

Hydroxymethyl glutaryl coenzyme A reductase (HMG-CoA) inhibitors (usually known as statins) have become one of the most widely specified drug groups in the world since their introduction to the market. There are currently six statin medicines available on the market, Lovastatin, pravastatin and simvastatin are derived from fungal metabolites and have elimination half-lives of 1–3 h. fluvastatin, pitavastatin and rosuvastatin are fully synthetic compounds, with elimination half-lives ranging from 1h for fluvastatin to 20h for rosuvastatin (Saku *et al.*, 2011).

Statins are generally classified into hydrophilic and lipophilic groups based on tissue selectivity, Hydrophilic statins, like pravastatin and rosuvastatin, have minimal tissue intake except for the liver, and fewer side effects due to the lower dependence on the cytochrome p (450) enzyme, while the other stains drug like Atorvastatin, simvastatin, lovastatin and fluvastatin are Lipophilic statins and they are more susceptible to metabolism by the cytochrome P (450) system, Excepting pitavastatin, which is undergoing limited metabolism through this pathway (McKenney, 2003). Hepatoselectivity of statins is largely determined by their hydrophilic characteristics. Lipophilic statins tend to be more exposed to non-hepatic tissues, while hydrophilic statins are more specific to the liver. The reason for these differences in selectivity is that lipophilic statins can passively spread through cell membranes to many cell types, while hydrophilic statins use active transporters to hepatocytes, resulting in fewer unwanted side effects in other tissues (Brunton *et al.*, 2011).

The primary mechanism of action of Statins is the competitive reversible inhibition of HMG-CoA reductase, a rate-limiting stage in biosynthesis of cholesterol. Through the inhibiting of HMG-CoA reductase, statins eventually prevent the endogenous production of cholesterol. In addition, the resultant reduction in cholesterol concentration in the hepatocytes triggers an up-regulation of low-density lipoprotein (LDL)-receptor expression which stimulates the absorption of LDL and LDL-precursors from systemic circulation, consequently a significant proportion of LDL-C will clearance from the plasma (Young and Fong, 2012; Istvan and Deisenhofer, 2001). Secondary mechanisms of statin-induced lipoprotein reduction involve inhibition of hepatic synthesis of apolipoprotein B100, and decreased the synthesis and secretion of triglyceride-rich lipoproteins (Reyes-Soffer *et al.*, 2016; Laufs *et al.*, 2020).

Statins (or HMG-CoA reductase inhibitors) are a class of drugs that reduce cholesterol in individuals who have dyslipidemia (abnormal fats level in the blood) and thus are at risk for cardiovascular disease (Taylor *et al.*, 2013; Odden *et al.*, 2015). Statins have also shown additional pleiotropic effects further than their lipid-lowering effect; they enhance endothelial function, minimize inflammation and coronary artery thrombus, and lowering left ventricular mass, blood pressure, left ventricular fibrosis, cardiac-valve sclerosis, atrial fibrillation, and death rates in patients with diabetes and renal disease. Generally, the effect of statins on the lipid profile is consistent between decreases in total cholesterol, LDL-C and triglycerides, and an increase in HDL-C (Park *et al.*, 2010).

The statins drugs not only compete with the normal substrate in the enzyme active site, but they also alter the conformation of the enzyme

when it binds to its active site. This lead to prevent HMG-CoA reductase from acquires a functional structure. The modification in conformation at the active site makes these drugs very effective and specific (Corsini *et al.*, 1999; Kellick, 2017).

1-5-3- Rosuvastatin:

Rosuvastatin is synthetically prepared, The chemical name for it is bis[(E)-7-[4(4 fluorophenyl)-6-isopropyl-[methyl(methylsulfonyl)amino] pyrimidin5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt, as shown in figure (1-1). And the empirical formula is $(C_{22}H_{27}FN_3O_6S)_2Ca$. Rosuvastatin molecular weight is 1001.14 (Anders, 2002).

In the molecular structure of rosuvastatin, the presence of the polar hydroxyl and methane sulphonamide groups makes Rosuvastatin more hydrophilic (Fergus, 2003; Schachter, 2005). Rosuvastatin is a synthetic statin that represents a progress in the pharmacology and clinical properties of statins, which is one of the most powerful statins and is currently widely prescribed to prevent adverse cardiovascular events and to lower total cholesterol and LDL-cholesterol (Law *et al.*, 2003).

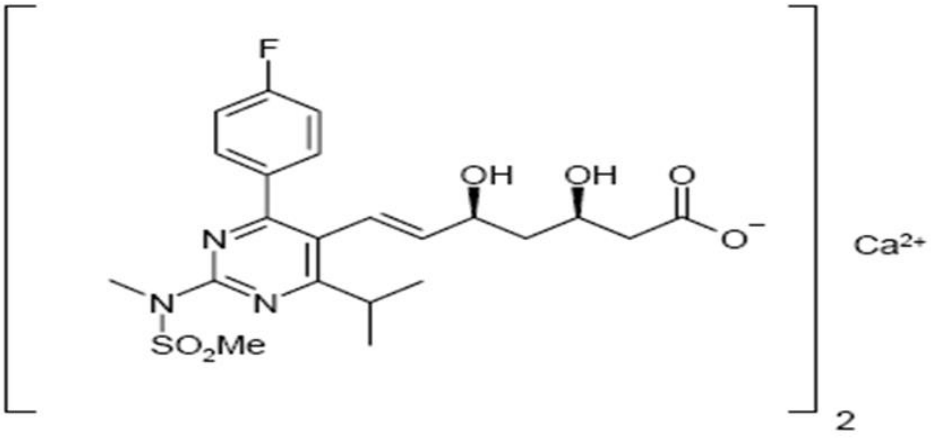

Figure (1-1): Chemical structure of rosuvastatin (FDA, 2010)

Rosuvastatin has the longest terminal half-life among statins which is about 20 houre and is only minimally metabolized by the cytochrome P450 (CYP 450) enzyme system (Goodman *et al.,* 2011). Comparable to other statins, rosuvastatin has a higher number of binding interactions with HMG-CoA reductase and a high affinity for the active site of enzyme, resulting in the most effective inhibition of cholesterol synthesis. Rosuvastatin is relatively hydrophilic and selectively absorbed and active in hepatic cells (Rosenson, 2003; McKenney, 2003). This results in decreased spread to non-hepatic cells, which reduces the possibilities for adverse events (McTaggart *et al.,* 2001).

1-5-4- Mechanism of action of rosuvastatin:

Rosuvastatin acts in the liver by the reversible competitive inhibiting 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), the rate-limiting enzyme in the biosynthesis of cholesterol through the mevalonate pathway (Armitage, 2007); Statins imitate the natural substrate molecule, HMG-CoA, and compete for binding to the active site of HMGCR enzyme. This competition slows down the rate of mevalonate production, the next molecule in the serial step to produce cholesterol, Therefore, in the presence of statins, the precursor HMG-CoA is not effectively processed forward to generate mevalonate, and ultimately blocking the pathway (Yoshihisa and Yuichi, 2006).

As compared to other statins rosuvastatin shows high activity in reduction the cholesterol and LDL-C level in the body by decreases plasma cholesterol levels by inhibiting cholesterol biosynthesis in the liver, as the level of free cholesterol in hepatocytes is reduced, the liver cells feel the lowered levels of liver cholesterol production with the use of statin and try to compensate by synthesizing more LDL receptors on the

cell surface to increase cholesterol uptake from serum, the result is cleavage of membrane-bound SREBPs (Sterol regulatory element-binding proteins) and translocation to the nucleolus, Protease induces this cleavage (Fergus, 2003).

The LDL receptor gene has a sterol-responsive element that is bounded by transcription factors, resulting in increased transcription and increased LDL receptor synthesis, there is a marked decrease in the degradation of LDL receptors and there are an increased number of LDL receptors on the hepatocyte surface, that plays an important role in the increased removal of LDL from the blood, resulting in lower blood LDL-C levels (Goodman *et al.*, 2011).

1-5-5-Histological effect of rosuvastatin:

Statins are a class of drugs used widely for the treatment of hyperlipidemia as well as for prevention of atherosclerosis and cardiovascular events with therapy duration of 30–120 days (Wainwright, 2005; Bjornsson *et al.,* 2012). Statins lower the circulating atherogenic lipoprotein level by inhibition the (HMG-CoA) reductase enzyme (McTaggart, 2003; Guthrie and Martin, 2007). Premarketing for statin drugs ,the biochemical clinical studies and initial toxicological studies in animals suggested that statins could cause hepatotoxicity, primarily elevations in serum aminotransferases levels induced with a need of liver enzymes monitori (Veillard and Mach, 2002; Famularo *et al.*, 2007).

As for rosuvastatin is considered a second-generation of cholesterol-lowering drug, with unique pharmacokinetic and pharmacodynamic properties (Nezasa *et al.,* 2002; Famularo *et al.,* 2007). Its chemical structure allows for additional HMG-CoA reductase enzyme-binding interactions that cause tighter binding, and in vitro studies have shown

substantial effective transport into hepatocytes, and this make rosuvastatin more selectively and efficiently than other statins which has generated considerable controversy regarding its safety particularly about its possible potential hepatotoxicity (Famularo *et al.,* 2007; Guthrie and Martin, 2007; Khan and Ibrahim, 2009). In spite of the fact that statins hepatotoxicity is well documented (Kaplowitz *et al.,* 2004), rosuvastatin stimulate hepatotoxicity has been placed into question (Bader, 2010). Pre-marketing studies have proposed that rosuvastatin may have minimum possibility to cause liver toxicity as compared with other statins (Davidson, 2007). Published data from the JUPITER study confirmed the efficacy of this statin in primary prevention for older patients with multiple risk factors and inflammation.

Rosuvastatin can cause deficiency in coenzyme Q10, which is mainly produced by means of in-vivo biosynthesis, a process that involves the enzyme HMG-CoA reductase. Coenzyme Q10 a natural antioxidant that is widely distributed across the human body; it is a lipid-soluble provitamin which is structurally similar to vitamin K. It is incorporated into the walls of mitochondria and functions in the transport of electrons and the production high-energy of adenosine triphosphate (ATP) compound (Siciliano *et al.,* 2007). Its concentration is elevated in tissues with high-energy needs, such as cardiac muscle, skeletal muscle, kidney and liver tissues.

Coenzyme Q10 depletion may lead to muscle energy starvation (a particular concern in the heart failure) (Neubauer, 2007), and caused skeletal muscle fiber damage which can cause kidney damage. This complication could be corrected through coadministration of rosuvastatin with coenzyme Q10 supplements (Mortensen *et al.*, 1997).

1-5-6- Nanocrystals rosuvastatin:

Nanotechnology will have an incredibly huge impact on our lives over the next century in very various fields such as medicine and pharmacy which includes conversion of materials to the nano dimension range from 0.1nm to 100nm by modifying their physical properties, which were used in pharmaceuticals to create a new innovative formulation concept for poorly soluble drugs (Alshora *et al.,* 2018), by using inorganic nanosize compounds that showed impressive antibacterial properties at very low concentrations related to their high surface-to-volume ratio and unusual chemical and physical characteristics, and are even more stable at high temperatures and pressures (Siddiqi *et al.,* 2018). Due to their high surface to volume ratio and high reactivity, metallic and metal-oxide nanostructures have shown amazing potential (Selim *et al.*, 2020). As one of the most common metal oxide nanoparticles, zinc oxide (ZnO) is commonly used in various fields because of its peculiar physical and chemical properties (Jiang *et al.,* 2018). Rosuvastatin is a poor water-soluble drug with oral bioavailability by only 20 per cent. It is categorized as a Class II drug by a biopharmaceutical classification system. The poor solubility of rosuvastatin impacts its solubility rate and, in turn, its bioavailability, improving rosuvastatin dissolution can thus lead to an improvement of its oral bioavailability. Thusly, several approaches to nanosizing were applied to enhance dissolution and bioavailability of rosuvastatin (Salah *et al.,* 2011).

Chapter Two
Materials and Methods

Chapter Two

2- Materials and Methods

2-1-Materials

2-1-1- Chemicals (Reagents and Stains): The general laboratory chemicals used in the study shown in table (2-1) with their suppliers:

Table (2-1): List of Chemicals

No.	Chemicals	Company (origin)
1	Rosuvastatin 10mg	AstraZeneca/United Kingdom
2	Zinc oxide (ZnO)	Fluka/Switzerland
3	Chloroform	BDH Chemical/England
4	formalin	Biosolve/USA
5	Xylen	Gainland Chem. Comp./England
6	Eosin Stain	BDH Chemical/England
7	Hematoxylin stain	BDH Chemical/England
8	Ethanol absolute	Gainland Chem. Comp./England
9	Paraffin wax paraplast wax.55-60c milting point	BDH Chemical/England
10	Animal fat	Local
11	Vegetable fat	Egypt
12	Triglyceride kit	spinreact comp. / Spain
13	HDL kit	spinreact comp. / Spain
14	Cholesterol kit	spinreact comp. / Spain
15	AST kit	spinreact comp. / Spain
16	ALT kit	spinreact comp. / Spain

2-1-2- Equipment and apparatus: The equipment's and apparatus used in this study are listed in table (2-2) with their suppliers:

Table (2-2): list of equipment's and apparatus

No.	Instrument	Company (origin)
1	Incubator	Binder/England
2	Sinsitive Balanc	Sartorius/Germany
3	Centrifuge	Hettich/Germany
4	Shaker Incubator	Labtech/Korea
5	Magnetic Stirrer with hot plate	Labtech/Korea
6	Glass slide	Mheco/China
7	Light Microscope	Motic/Germany
8	Disposable Latex Examination Gloves	Great Glove. Sde. Bhd/ Malaysia
9	Rotary microtose	Thermo/United Kingdom
10	Medical syringe	MFG.Co.Ltd /Saudi Arabia
11	Water bath	Tafesa/Germany
12	Spectrophotometer	Turdo/Korea
13	Gel clot Activator tubes	China
14	Fourier transform infrared spectrophotometer (FT-IR)	Perkin-Elmer 1725x/Japan
15	(X-Ray)diffraction	Shimadzu XRD-6000 powder diffractometer /Japan
16	Scan probe microscope	AFM model ,AA3000 Advanced Angstrom Inc/USA

2-1-3-Diet:

The Standard chow and High fat diet (HFD) used in this study are listed in table (2-3)

Table (2-3): The percentages of standard chow and high fat diet. (Abro *et al.*, 2008; Kumar *et al.*, 2009).

No.	Ingredients	Standard chow	HFD (Animal fat)	HFD(Vegetable fat)
		Percentage (g)	Percentage (g)	Percentage (g)
1	Casein	20	20	20
2	wheat starch	42	30	30
3	Maize starch	23	21.5	21.5
4	Cane sugar	10	10	10
5	Salt or mineral mixture	3.5	3	3
6	Vitamin Mixture cholesterol	1	1	1
7	Animal fat	-	15	-
8	Vegetable fat	-	-	15
9	Choline and Methionine	0.5	0.5	0.5
	Total (g)	100	100	100

2-2- Methods

2-2-1-Animals:

Seventy male albino mice were purchased from the animal care center of the Iraqi center for cancer research, their ages ranged from 3.5 to 4 months, While weight ranged from 25 to 30 g. Animals were kept in animal house of college of veterinary medicine /university of kerbala under controlled temperature conditions (25 ± 30C°) and light-dark cycles for 12 hours. The experiment preceded for 4 months, where mice acclimatized for two weeks and had access to drinking water and a standard chow diet; then divided into seven major groups, each of ten (10) animals, which caged in large polypropylene cages.

2-2-2- Experiment groups:

2-2-2-1- Diet groups:

1. Normal diet control group (negative control): In this group, the animal remained on normal diet (standard chow) for 4 months.
2. Animal fat diet control group (positive control): In this group animals were fed a high fat diet (HFD) rich with animal fat listed in table (2- 3) for 4 months.
3. Vegetable fat diet control group (positive control): In this group animals were fed a high fat diet (HFD) rich with vegetable fat listed in table (2-3) for 4 months.

2-2-2-2-Treatment groups:

1. Rosuvastatin and animal fat diet group: At the end of three months feeding on a diet rich with animal fat, each mouse in this group was administered orally 1ml of 0.02mg/kg/day of rosuvastatin for one month.
2. Nanoparticle rosuvastatin and animal fat diet group: At the end of three months feeding on a diet rich with animal fat, each mouse in this group was administered orally 1ml of 0.02mg/kg/day of nanoparticle rosuvastatin for one month.
3. Rosuvastatin and vegetable fat diet group: At the end of three months feeding on a diet rich with vegetable fat, each mouse in this group was administered orally 1ml of 0.02mg/kg/day of rosuvastatin for one month
4. Nanoparticle rosuvastatin and vegetable fat diet group: At the end of three months feeding on a diet rich with vegetable fat, each mouse in this group was administered orally 1ml of 0.02mg/kg/day of nanoparticle rosuvastatin for one month.

All animals in these groups at the end of 4 months were weighted and scarified after overnight fasting, then collecting blood sample to measure: Total Cholesterol (TC), Triglyceride (TG), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), also collect tissue samples to study the modification in liver and kidney tissues, as shown in figure (2-1).

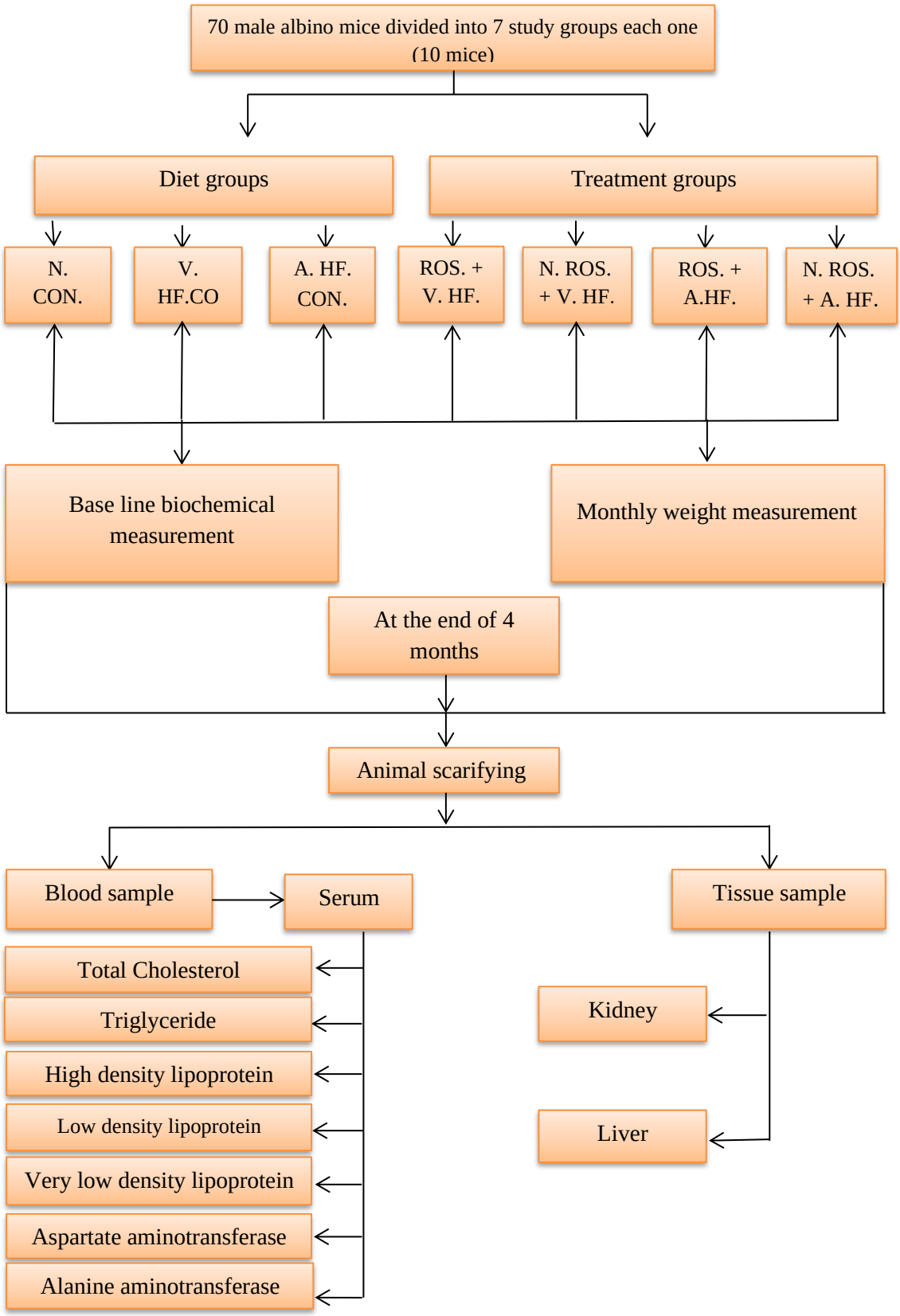

Figure (2-1) Study design

2-2-3- Preparations:

2-2-3-1-Preparation of animal fat- rich diet: diet was prepared by add 850g of powdered mice chow diet, to 150g of local animal fat, to obtain homogeneous soft cake. The animal fat- rich diet preparation was modeled as a pellet according to the method of (Abro *et al.*, 2008; Kumar *et al.,* 2009).

2-2-3-2- Preparation of vegetable fat- rich diet: diet was prepared by add 850g of powdered mice chow diet, to 150g vegetable fat to obtain homogeneous soft cake. The vegetable fat- rich diet preparation was modeled as a pellet according to the method of (Abro *et al.*, 2008; Kumar *et al.,* 2009).

2-2-3-3-Harris's Haematoxylin solution: The solution prepared by dissolving 1g of haematoxylin in 10 ml ethyl alcohol. 20g of potassium alum dissolved in 200 ml of DW and boiled. Haematoxylin then added and the solution boiled for ½ minutes. 0.5g of mercuric oxide added. The solution cooled rapidly and a few drops of acetic acid were added; it is optional but its inclusion gives more precise and selective staining of nuclei; (Bancroft *et al.,* 2013).

2-2-3-4- Eosin solution: Prepared by mixing 1gm. of eosin Y in 99ml ethanol. A little of acetic acid (0.5 ml to 1000 ml of the stain) then added to sharpen the staining (Bancroft *et al.,* 2013).

2-2-3-5- Rosuvastatin Solution Preparation: The rosuvastatin dose was taken 10mg as normal human adult dose, which was 0.02 mg/kg bw as mice dose according to the conversion chart (Ghosh, 1984). Rosuvastatin of 10mg (AstraZeneca, UK) was crushed in to powder, then dissolved in distill water to prepare rosuvastatin solution for oral administration.

2-2-3-6- Nanoparticle drug Solution Preparation: The hybrid nanoparticle solution was prepared by following the method described by (Kolekar *et al.*,2011) with some modification, by adding 50 ml of the prepared drug to zinc oxide solution (resulting from dissolving 1g of zinc oxide in 50ml ethanol), the mixture stir at room temperature and then washed with distilled water removed from the ions then precipitate was dried crushed in to powder, then dissolved in distill water to prepare solution hybrid for oral administration, as shown in figure (2-2)

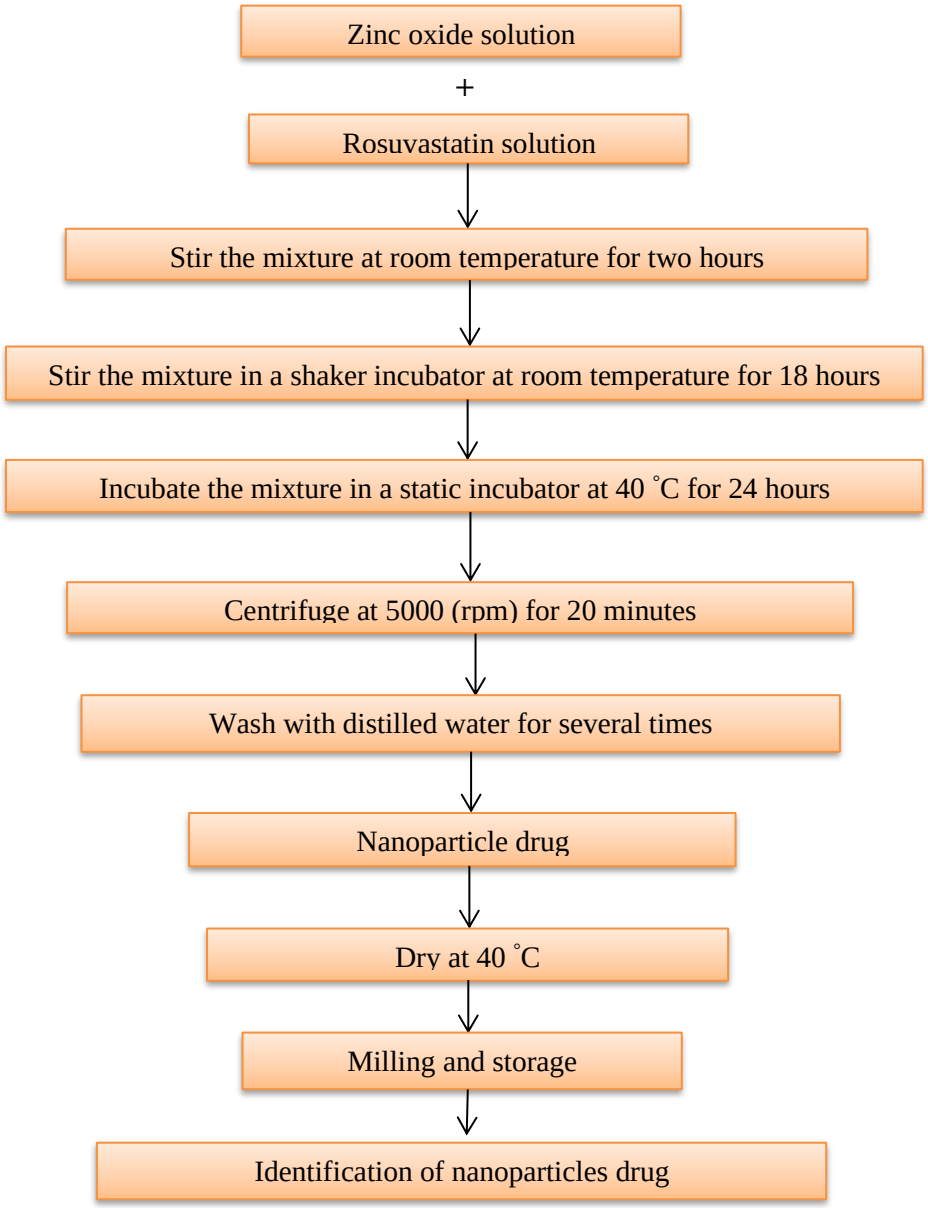

Figure (2-2) Preparation of the nanoparticle drug using the Sol-Gel ion exchange method

2-3- Identification of nanoparticles drug:

The hybrid drug was identified by using three methods, Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and Atomic Force Microscope (AFM).

2-3-1- Fourier transform infrared spectroscopy (FT-IR): The infrared spectrum of the nanoparticle and the free form of the drug were measured, as well as zinc oxide. the infrared spectrum was measured in a range of wave numbers (400-4000) cm^{-1} and all the visible beams were fixed with their wave numbers most of the major packages have also been identified.

2-3-2- X-ray diffraction (XRD): The identification was made by using X-Ray spectroscopy, which shows the difference in the thickness of the layer before and after the intercalation process for both the nanoparticle and zinc oxide by using Brack's Law $n\lambda = 2dSin\Theta$ to extract the value of the layer thickness (d) before and after the intercalation process. As the:

n: The rank of the crystalline level from which x-ray diffraction is present.

λ: X-ray wavelength used (copper tube).

Θ: X-ray diffraction angle.

d: The vertical crystal distance between two parallel levels.

2-3-3- Atomic Force Microscope (AFM): The atomic force microscope was used to examine the nanoparticle drug, and measure the diameters, sizes, and assemblies of the nanoparticles. The model was sent to College of Science / University of Baghdad for the purpose of examination.

2-4- Blood Sampling:

A bout 1ml of blood was collected by direct heart puncture after overnight fasting at the end of four months (second period of study), and after anesthetized of animal with chloroform. The blood was placed in gel test tube and left to stand for 30 minutes at room temperature to allowing clotting. The sera samples were prepared by centrifugation at 4000 rpm for 5 minutes to estimate the levels of TC, TG, HDL-C, LDL-C, AST and ALT) (biochemical assays).

2-4-1- Biochemical assays:

Measurement of serum total cholesterol and lipid profiles (TC, TG, HDL, LDL, VLDL ALT and AST) to determine baseline values and to check the induction of lipid, through the period of the study, the serum was prepared by centrifugation in microhematocrit centrifuge at 10000rpm for 10 minutes.

2-4-1-1- Measurement of serum cholesterol (TC):

1- Reagent composition

R 1 Buffer	PIPES pH 6,9	90 mmol/L
	Phenol	26 mmol/L
R 2 Enzymes	Cholesterol esterase (CHE)	300 U/L
	Cholesterol oxidase (CHOD)	300 U/L
	Peroxidase (POD)	1250 U/L
	4 – Aminophenazone (4-AP)	0,4 mmol/L
CHOLESTEROL CAL	Cholesterol aqueous primary standard 200 mg/dL. Contains Triton X-114 10-15%.	

2- Principle of the Method

The cholesterol present in the sample originates a coloured complex, according to the following reaction:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenona} + H_2O_2$$

$$2\,H_2O_2 + \text{Phenol} + \text{4-Aminophenazone} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

The intensity of the color formed is proportional to the cholesterol concentration in the sample (Naito, 1984).

3- Procedure

1. Assay conditions:

Wavelength: 505 nm (500-550)

Cuvette: 1 cm light path

Temperature: 37 °C /15-25 °C

2. Adjusted the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1,0	1,0	1,0
Standard (µL)	--	10	--
Sample (µL)	--	--	10

4. Mixing and incubate for 5min. at 37 °C or 10 min. at room temperature.

5. Reading the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 60 minutes.

5- Calculations

$$\frac{\text{(A) Sample} - \text{(A) Blank}}{\text{(A) Standard} - \text{(A) Blank}} \times 200\ (\text{Standard conc.}) = \text{mg/dL Cholesterol in the sample}$$

Conversion factor: mg/dL x 0.0258= mmol/L.

2-4-1-2-Estimation of Serum Triglycerides (TG):

1- Reagents composition:

R 1 Buffer	GOOD pH 7.5	50 mmol/L
	p-Chlorophenol	2 mmol/L
R 2 Enzymes	Lipoprotein lipase (LPL)	150000 U/L
	Glycerolkinase (GK)	500 U/L
	Glycerol-3-oxidasa (GPO)	2500 U/L
	Peroxidase (POD)	440 U/L
	4 – Aminophenazone (4-AP)	0.1 mmol/L
	ATP	0.1 mmol/L
Triglycerides Cal	Triglycerides aqueous primary standard 200 mg/dL	

2-Principle of the method

$$\text{Triglycerides} + H2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{Free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\textbf{Glycerol kinase}} \text{G3P} + \text{ADP}$$

$$\text{G3P} + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4-AP} + \text{P-chlorophenol} \xrightarrow{\text{POD}} \text{Quinone} + H_2O$$

The intensity of the color formed is proportional to the triglycerides concentration in the sample (Kaplan *et al.*, 1984).

3- Procedure

1. Assay conditions:

Wavelength: 505nm (490-550)

Cuvette: 1 cm light path

Temperature: 37 °C / 15-25 °C

2. Adjusted the instrument to zero with distilled water.

3. Pipette in to a cuvette:

	Blank	Standard	Sample
R (ml)	1.0	1.0	1.0
Standard (µL)	--	10	--
Sample (µL)	--	--	10

4. Mixing and incubate for 5min. at 37 °C or 10 min. at room temperature.

5. Reading the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

4- Calculation

$$\frac{\text{A sample}}{\text{A standard}} \times 200 \text{ (Standard conc.)} = \text{mg/dL cholesterol in the sample}$$

Conversion factor: mg/dL x 0.0258= mmol/L.

2-4-1-3- Estimation of serum high density lipoprotein-cholesterol (HDL-C):

1- Reagents composition:

R1	N,N-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid pH 6,6	100 mM
	N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS)	0,7 mM
	Cholesterol Esterase	≥ 800 U/L
	Cholesterol oxidase	≥ 500 U/L
	Catalase	≥ 300 U/L
	Ascorbic oxidase	≥ 3000 U/L
R2	N,N-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid pH 7,0	1,1 mmol/L
	4 – Aminoantipyrine (4-AA)	100 mM
	Peroxidase	≥ 3500 U/L

2- Principle of the Method:

Directly determination of serum HDL-C (high-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation of the sample (Shih *et al.,* 2000), the assay takes place in two steps.

1. Elimination of lipoprotein no-HDL

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHOD}} \text{Cholestenone + } H_2O_2$$

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

2. Measurement of HDL-C

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHOD}} \text{Cholestenone + } H_2O_2$$

$$2\ H_2O_2 + \text{HDAOS + 4-AA} \xrightarrow{\text{POD}} \text{Quinone Pigment + } 4H_2O$$

The intensity of the color formed is proportional to the HDL-C concentration in the sample.

3- Procedure:

1. Assay conditions:

Wavelength: 600 -700 nm

Cuvette: 1 cm light path

Temperature: 37 ˚C

2. Adjusted the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	Standard	Sample
R1 (µL)	300	300	300
standard (µL)	--	3	--
Sample (µL)	--	--	3

4. Mixing and incubate for 5 min at 37 °C.

5. Reading the absorbance (A1) of the samples and standard.

6. Add:

R2	Blank	Standard	Sample
	100	100	100

7. Mixing and incubate for 5 min. at 37 °C.

8. Reading the absorbance (A2) of the samples and standard, against the Blank.

9. Calculate the increase of the absorbance ΔA= A2 – A1.

4- Calculations

The HDL cholesterol concentration in the sample is calculated by using the following general formula:

$$\frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times \text{Standard conc.} = \text{mg/dL of HDL-C in the sample}$$

Conversion factor: mg/dL x 0.0259= mmol/L.

2-4-1-4- Measurement of serum low density lipoprotein-Cholesterol (LDL-C) and very low density lipoprotein (VLDL):

The LDL-C, VLDL concentrations were calculated from the Friedewald equation:

LDL -C = Total cholesterol (TC) – (HDL-C+ VLDL-C)

LDL = TC – (HDL + VLDL)

VLDL-C= Triglycerides /5, VLDL =TG/5

2-4-1-5- Measurement of serum aspartate aminotransferase (AST):

1- Reagents composition

R 1 Buffer	TRIS pH 7.8	80 mmol/L
	Lactate dehydrogenase (LDH)	800 U/L
	Malate dehydrogenase (MDH)	600 U/L
	L-Aspartate	200 mmol/L
R 2 Substrate	NADH	0,18 mmol/L
	α-Ketoglutarate	12 mmol/L

2- Principle of the Method

Reaction scheme is as follows:

$$\text{L-Aspartate} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{Glutamate} + \text{Oxalacetate}$$

$$\text{Oxalacetate} + \text{NADH} + \text{H+} \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^{+}$$

The rate of decrease in concentration of NADH, measure photometrically, is proportional to the catalytic concentration of AST present in the sample (Murray, 1984).

3- Procedure

Detailed Kenza 240TX procedure is available on reques

Wavelength: 340 nm

Temperature: 37 ˚C

Let stand reagents and specimen at room temperature

	Automated analyzer	Manual procedure
Reagent 1	200 µL	800 µL
Reagent 2	50 µL	200 µL
Mix. Wait for 15 sec then add		
Specimen		
Mix. After 60 sec, measure variation of absorbance per minute (Abs/min) during 180 sec		

4- Calculations

With Seric Muticalibrator:

$$\text{AST Activity} = \frac{(\Delta \text{ Abs/min) Specimen}}{(\Delta \text{ Abs/min) Calibrator}} \text{ x Calibrator Activity}$$

With Theoretical Factor:

Activity (U/L) = ΔAbs/min x Factor

$$\text{Factor} = \frac{\text{VR x 1000}}{\text{6.3 x VE x P}}$$

With:

VR = Total reactional volume (mL)

VE = Specimen volume (mL)

6.3 = Molar extinction coefficient for NADH at 340nm

P = Path length (cm).

2-4-1-6- Measurement of serum alanine aminotransferase (ALT):

1- Reagents composition:

R 1 Buffer	TRIS pH 7,8	100 mmol/L
	L-Alanine	500 mmol/L
R 2 Substrate	NADH	0,18 mmol/L
	Lactate dehydrogenase (LDH)	1200 U/L
	α-Ketoglutarate	15 mmol/L

2- Principle of the Method

$$\text{Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{Glutamate} + \text{Piruvate}$$

$$\text{Piruvate} + \text{NADH} + \text{H+} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^{+}$$

The rate of decrease in concentration of NADH measured photometrically is proportional to the catalytic concentration of ALT present in the sample (Murray, 1984).

3- Procedure

1. Assay conditions:

Wavelength: 340 nm

Cuvette: 1 cm light path

Constant temperature: 25 ˚C / 30 ˚C / 37 ˚C

2. Adjusted the instrument to zero with distilled water or air.

3. Pipette into a cuvette:

WR (mL)	1,0
Sample (µL)	100

4. Mixing and incubate for 1 minute.

5. Reading initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1 minute intervals thereafter for 3 minutes.

6. Calculate the difference between absorbance and the average absorbance differences per minute (Δ A/min).

4- Calculations

Δ A/min x 1750 = U/L of ALT

2-5- Histological processing and staining:

Ordinary histological processing was prepared for liver and kidney in order to study the changes that may be found in animal groups as compared with the animals of negative control group (Bancroft *et al.*, 2013); processing and staining technique was as follow:

A- Processing steps:

1-Tissues piece was fixed in 10% formalin.

2- Dehydration in different concentrations of ethanol (ascending series of 70, 80, 90, 100, 100%) (2 hours in each concentration).

3- Clearing with xylene (2 repeats, 2 hours in each repeat).

4- Infiltration with paraffin wax (at 60 ˚C) (two repeats, 2 hours in each repeat).

5- Embedding in paraffin wax and left at laboratory temperature.

6- Sectioning by the microtome (5mm thick sections).

B- Haematoxylin – Eosin (H&E) staining technique:

1- Deparaffinization of sections in xylene (two repeats 5 minutes each).

2- Hydrated by exposing to gradual concentrations of ethanol (100, 90, 80 and 70%) and then to D.W.

3- Stained in Harris's haematoxylin for 3 minutes.

4- Rinse in tap water for 2 minutes for bluing.

5- Differentiated in 1% acid alcohol for 5-10 seconds.

6- Stained in 1% eosin for 5 minutes.

7- Washed in running tap water for 2 minutes.

8- Dehydrated in gradual concentrations of ethanol (70, 80, 90 and 100%).

9- Cleared in xylene, and then mounted in D.P.X.

2-6-Statistical analysis:

The results were expressed in mean ± S.D. The statistical analysis of the obtained data was performed by using the Analysis of Variance (ANOVA) test at least significant differences (L.S.D) test, all analysis were done by using ready-made statistical SPSS.

Chapter Three
Results and Discussion

Chapter Three

3- Results and Discussion:

3-1- Diagnosis of prepared nanocomposites spectroscopic methods:

3-1-1-FT-IR spectrum:

3-1-1-1- Infrared spectrum of the rosuvastatin (ROS):

Figure (3-1) shows that the compound rosuvastatin in its free state exhibits many distinguished beams at certain frequencies. The wide and intense absorption beam at the frequency of 3346cm^{-1} is due to the vibration (alcoholic O-H stretch) bonds that intertwined with the (aromatic C-H stretch) bonds of the benzene ring, The weak absorption beam at frequency 2914cm^{-1} its belong to (aliphatic C-H stretch) bonds. The strong absorption beam at frequency 1660cm^{-1} is due to the asymmetric stretch vibration of the negative carboxylate group ion. As for the structural stretch vibration of the C=N bonds and the C=C bonds of the aromatic rings of pyrimidine and benzene, they appeared at the frequencies 1550cm^{-1} and 1435cm^{-1}, respectively.

The absorption beam at frequency 1381cm^{-1} is due to the asymmetric stretch vibration of the SO2 group in the sulfonamide unit, while the symmetric stretch vibration of the sulfone group was shown at frequency 1078cm^{-1}, as for the C-F band, it appeared at the frequency 1271cm^{-1}. The powerful absorption beam at frequency 1039cm^{-1} is due to the stretch vibration of the alcoholic C-O, as for the curvature of the aromatic C-H bonds outside the plane of the benzene ring, it appeared at frequencies 893cm^{-1}, 765cm^{-1}, 619cm^{-1}, The weak absorption beams at frequencies

593cm^{-1} and 449cm^{-1} are due to the structural stretch vibration of the aliphatic C-C single bonds (Bhokare and Marathe, 2020).

3-1-1-2- Infrared spectrum of zinc oxide:

The zinc oxide layers showed the unclear beam at the frequency (400 - 500) cm^{-1} and its distinguishing beam is the powerful beam shown at 443cm^{-1} which was due to the vibration of the metal bond Zn-O, Which was shifted towards the higher frequency when the nanocomposite was inserted, and it appeared at the frequency 455cm^{-1} (Voicu *et al.*, 2013). As shown in figure (3-2)

3-1-1-3- Infrared spectrum of rosuvastatin nanocomposite hybrid:

In the infrared spectrum of the nanocomposite, there were several shifts in absorption beams, most of them towards the lower frequencies, in addition to a change in the intensity of some beams,indicating the successful insertion of rosuvastatin between the zinc oxide layers as showed in figure (3-3). The wide stretching of the alcoholic O-H bonds interfering with the stretching of the aromatic C-H bonds appeared at frequency 3344cm-1 and its intensity decreased, and the C-H aliphatic bond is shifted to the frequency 2912cm^{-1}, the asymmetric stretch beam of the negative carboxylate ion also shifted towards the lower frequency at 1654cm^{-1} , Also, the structural frequency of the C=N bonds and the C=C bonds of the aromatic rings of pyrimidine and benzene decreased at the frequencies 1545cm^{-1} and 1429cm^{-1}, and their intensity decreased.

The spectrum also showed two absorption beams due to the asymmetric stretch of amide sulfone group the first shifted towards the lower frequency at 1371cm-1, while the new ones appeared at the frequency 1327cm^{-1}. As for the vibrations of the C-F belt, it appeared at the frequency 1275$cm^{-1,}$ a second beam of symmetric stretch vibration of the sulfone group appeared at frequency 1161cm^{-1}, which was shifted towards the higher frequency at 1105cm^{-1}, while the beam vibration of the alcoholic C-O stretch was shifted towards the higher frequency at 1045cm^{-1}. The extra-plane curvature of the C-H aromatic bonds appeared at different frequencies than in the free compound at 900, 815, 619,705cm^{-1}. The Zn-O band vibration beam was also shifted to a higher frequency at 455 cm^{-1} (Bhokare and Marathe, 2020).

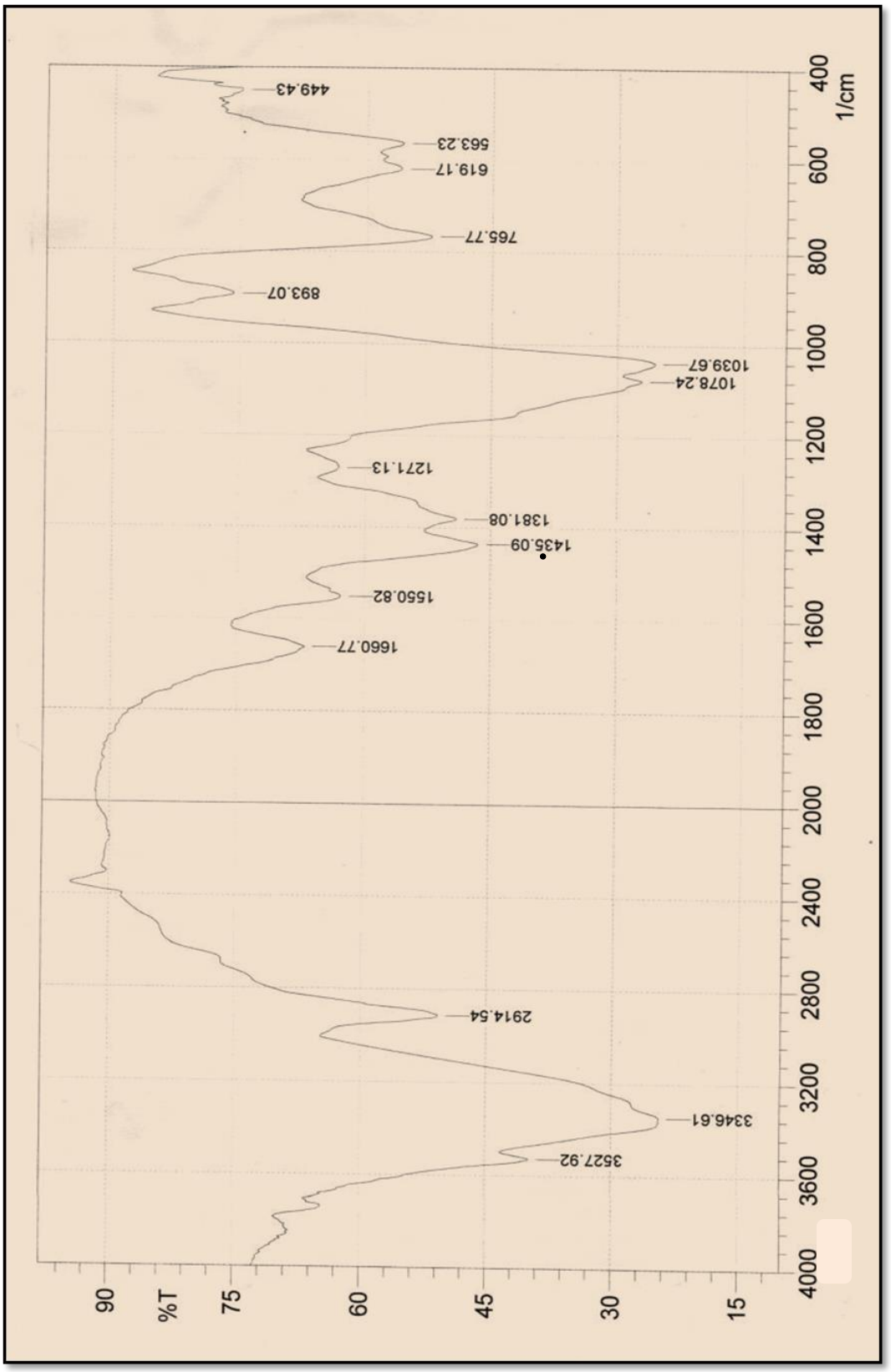

Figure (3-1): Infrared spectrum of rosuvastatin

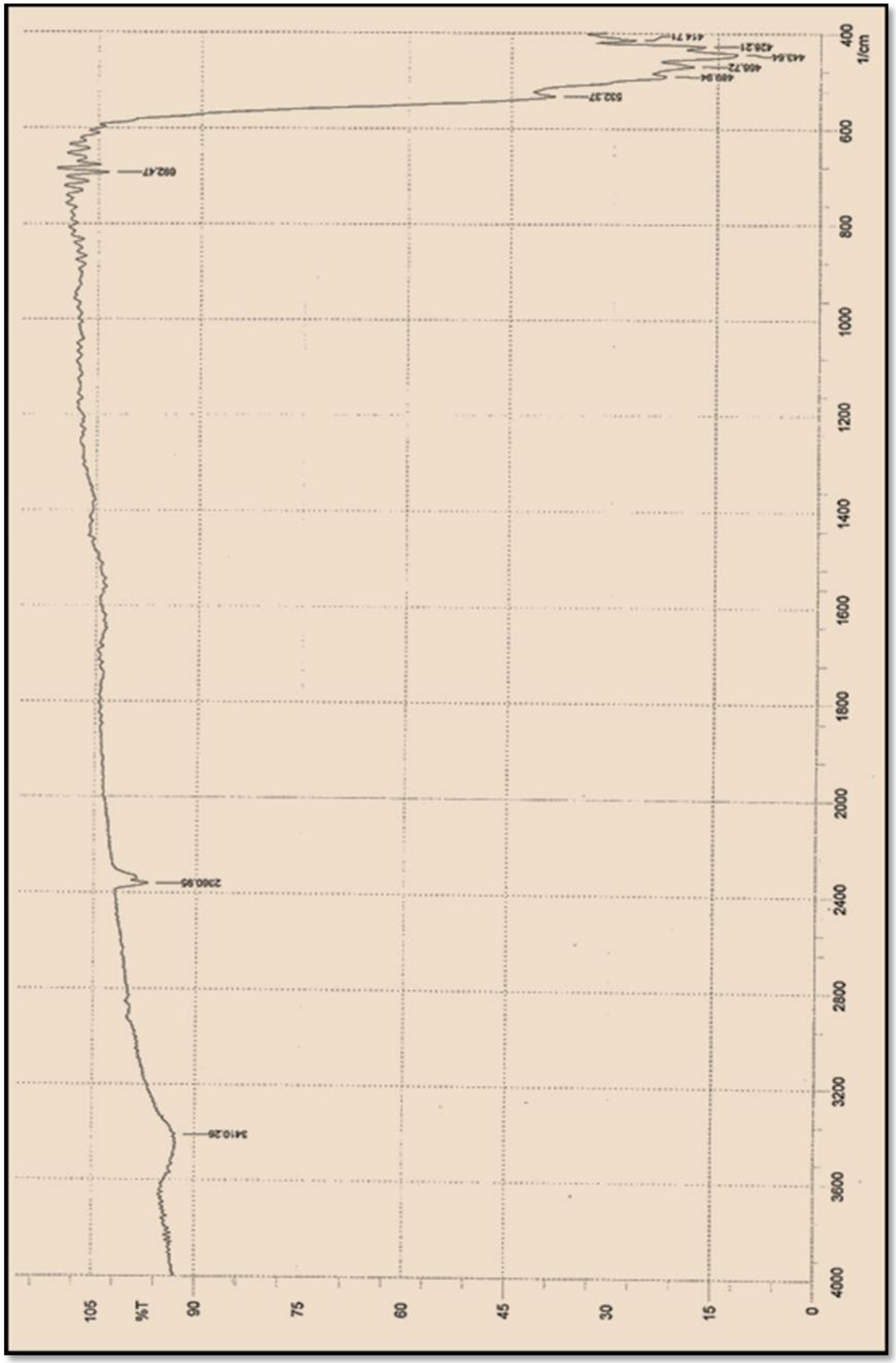

Figure (3-2): Infrared spectrum of zinc oxide

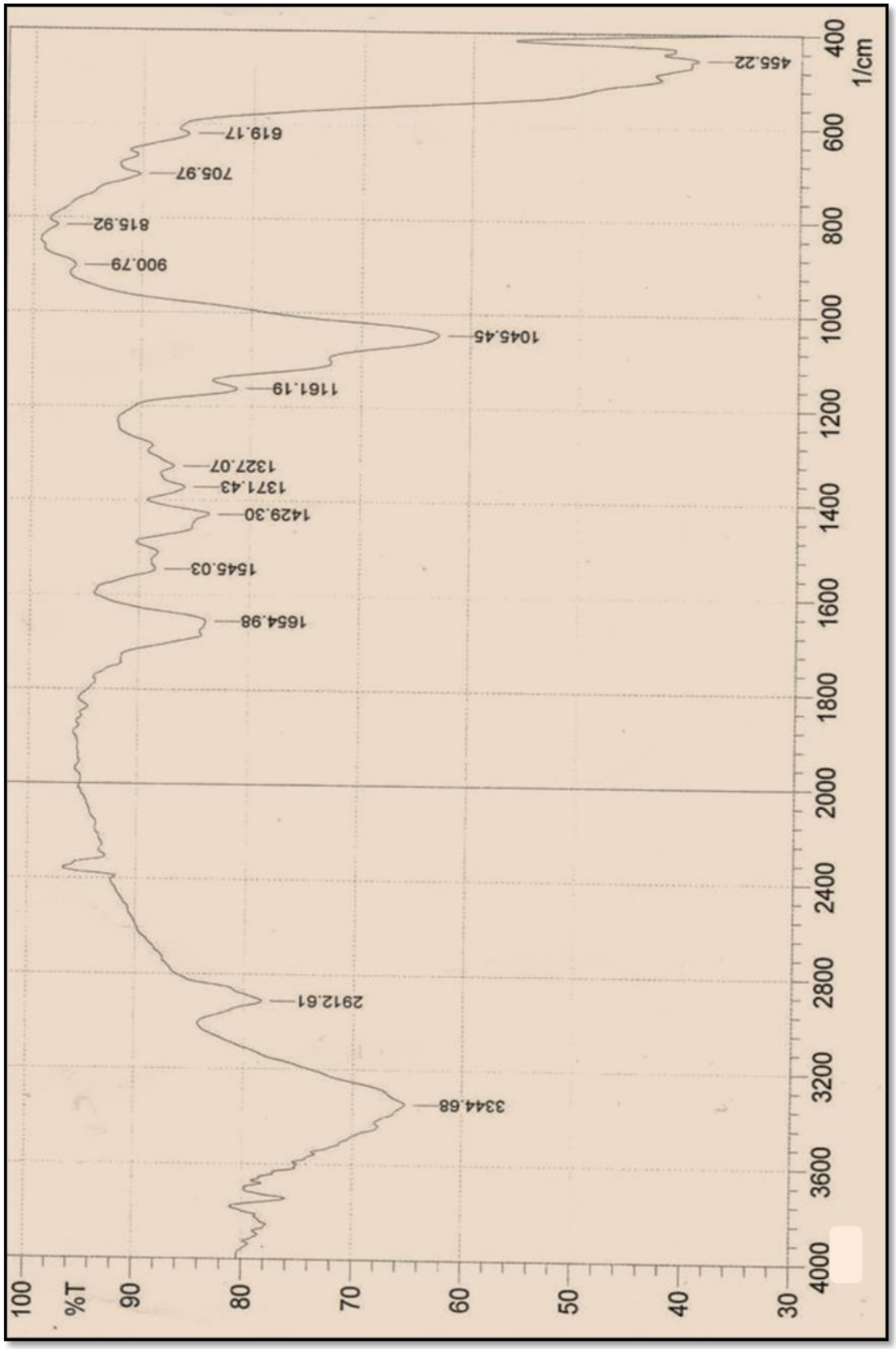

Figure (3-3): Infrared spectrum of ROS-ZnO

3-1-2- X-ray diffraction spectrum (XRD):

The X-ray diffraction spectrum of both rosuvastatin nanoparticles ROS/ZnO and zinc oxide layers ZnO were studied to find the difference in the thickness of the layer before and after the Rosuvastatin drug insertion between the zinc oxide layers using Brack's law.

Figure (3-4) shows the X-ray diffraction spectrum of zinc oxide and shows the diffraction of the levels (100) at the angle of 31.29° and has a crystalline distance (d) equal to 0.281nm and the plane (002) at an angle of 34.82° with a crystalline distance of 0.259 nm as for the plane (101) it appeared at the angle 36.29° and has a crystal distance of 0.247 nm (Voicu *et al.*, 2013).

When carrying out the ion exchange process between rosuvastatin and zinc oxide, a diffraction level was observed at the angle 23.3θ, indicating that the rosuvastatin compound was successfully inserted between the zinc oxide layers and thus formed a new nanocomposite. The value of the crystal distance (d) was 0.733 nm. As shown in figure (3-5).

3-1-3- Examination with atomic force microscopy (AFM):

The outer surface of the nanocomposite was studied using atomic force microscopy. Figure (3-6a) shows a 2Dimentinal image of the hybrid nanocomposite (ROS-ZnO) showing oval molecular assemblies. As for Figure (3-6b), it shows a 3Dimentinal image of a section from the surface of the hybrid nanocomposite (ROS-ZnO), in which observed the height of molecular clusters, which are about 28.88 nm, indicating the manufacture of a hybrid nanocomposite of rosuvastatin and zinc oxide.

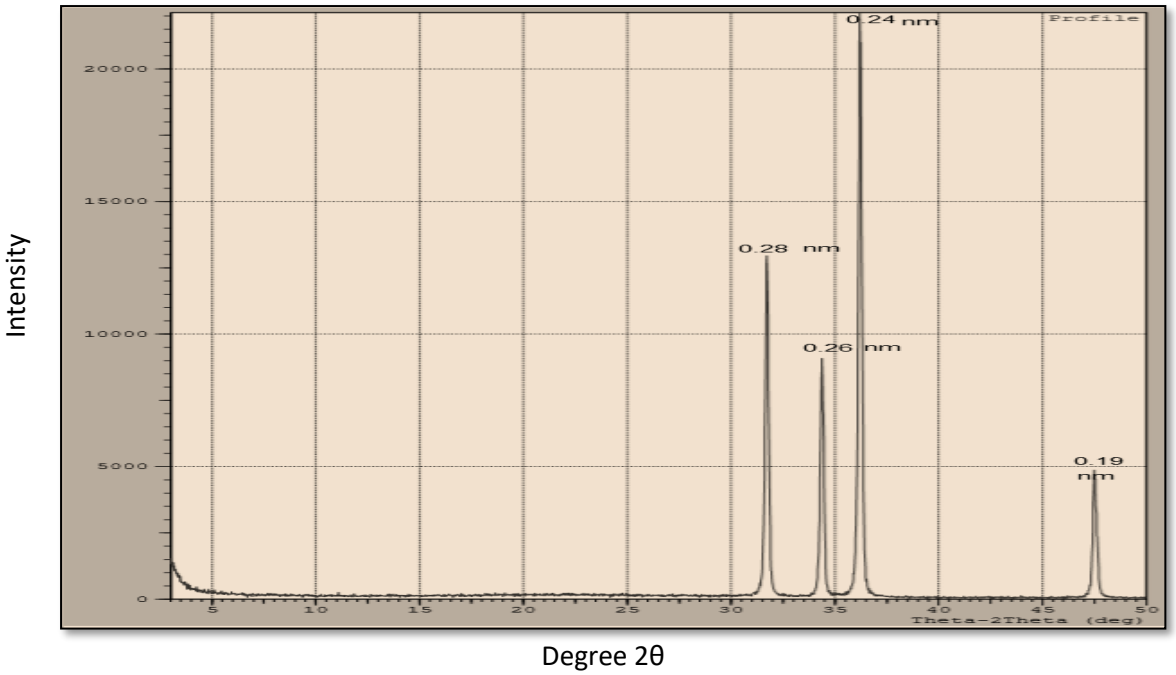

Figure (3-4): X-ray diffraction (XRD) spectroscopy of zinc oxide layers

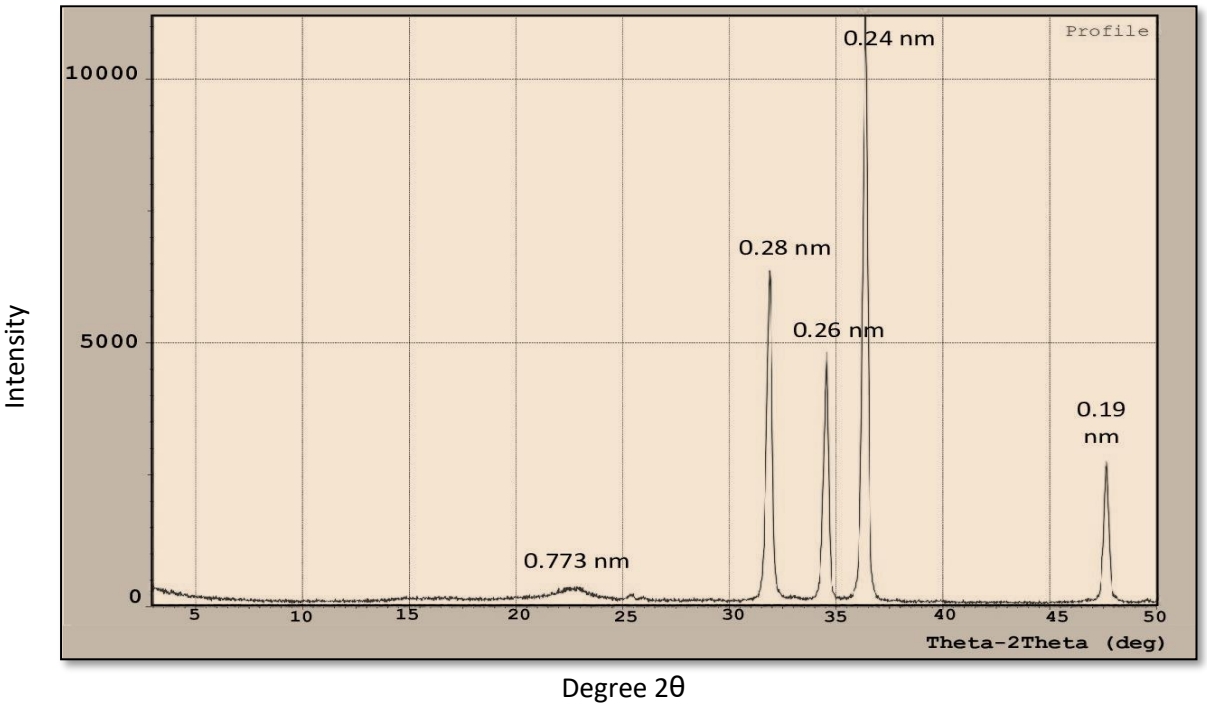

Figure (3-5): X-ray diffraction (XRD) spectrum of rosuvastatin hybrid nanoparticles (ROS-ZnO).

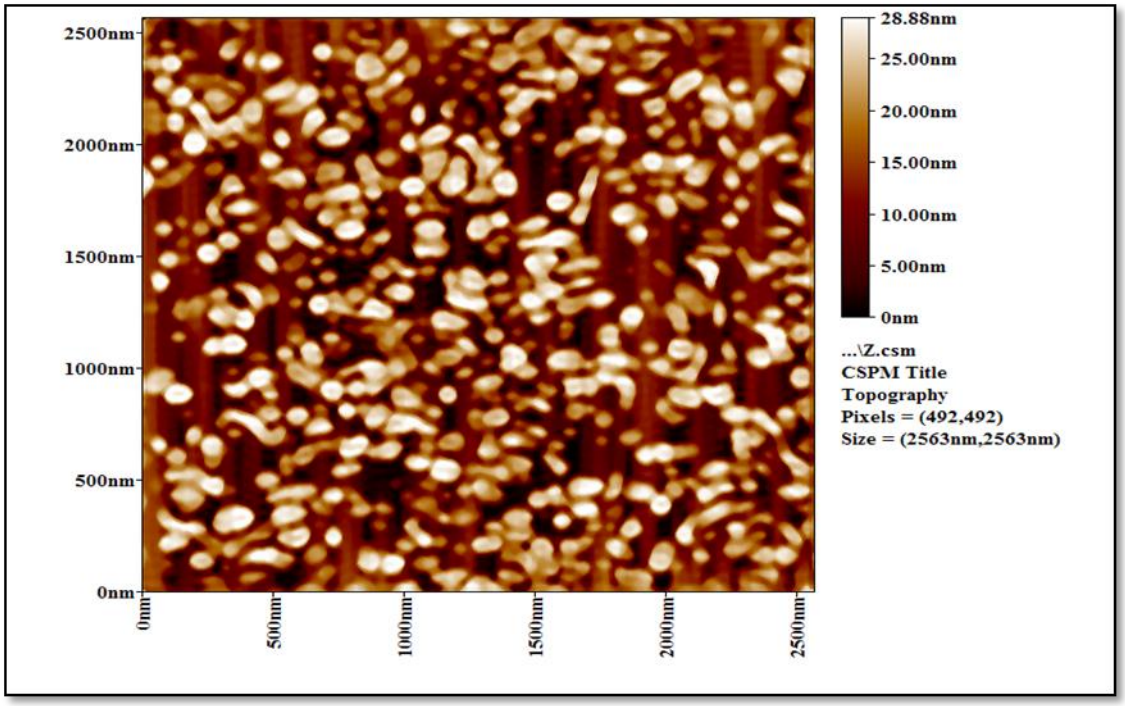

Figure (3-6a): 2Dimensional image of a hybrid nanocomposite (ROS-ZnO)

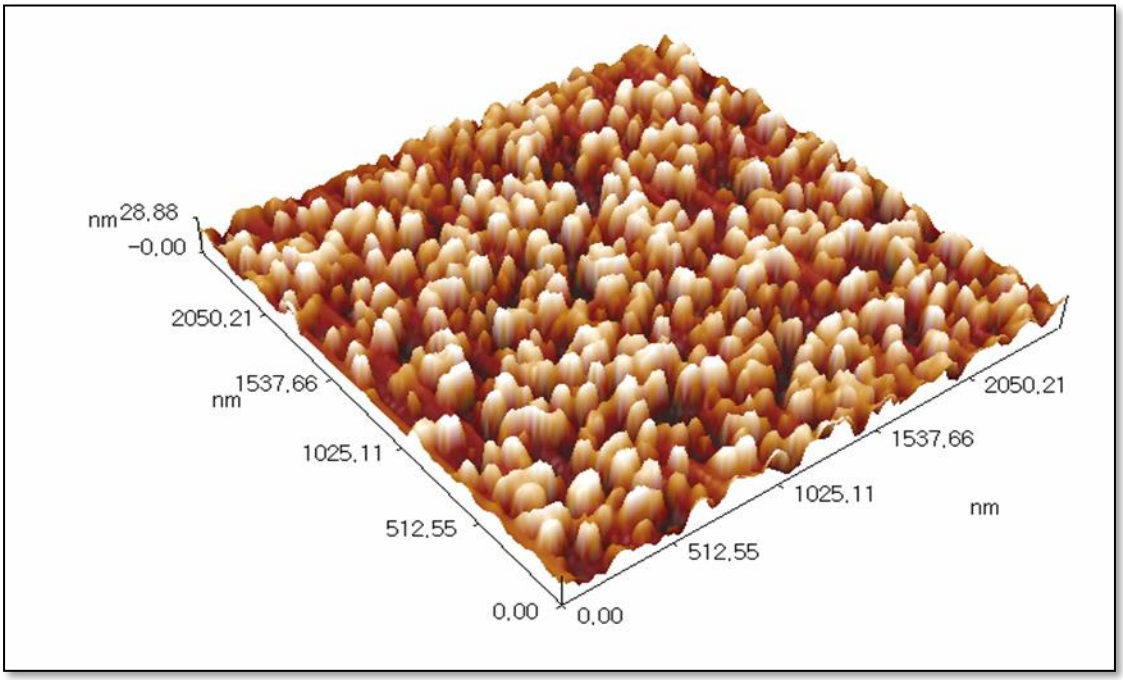

Figure (3-6b): 3Dimensional image of a hybrid nanocomposit (ROS-ZnO)

As shown in Table (3-1), the average particle sizes of the nanocomposite (ROS-ZnO) are around 73.5 nm. The preparation process of this compound led to obtaining minerals with diameters between (60-100 nm).

Table (3-1): Diameters, sizes and particles of the (ROS-ZnO) hybrid nanocomposite after examining it with atomic force microscopy

Diameter (nm)<	Volume (%)	Cumulation(%)	Diameter (nm)<	Volume (%)	Cumulation(%)	Diameter (nm)<	Volume (%)	Cumulation(%)
60.00	10.95	10.95	75.00	19.79	56.18	90.00	9.19	96.11
65.00	10.25	21.20	80.00	16.25	72.44	95.00	3.89	100.00
70.00	15.19	36.40	85.00	14.49	86.93			

Avg. Diameter: 73.50 nm	<= 10% Diameter: 0 nm
<= 50% Diameter: 70.00 nm	<=90% Diameter: 85.00 nm

The result obtained by our study is closer to Dudhipala and Veerabrahma, (2017) which the diameters of the rosuvastatin hybrid nanocomposite prepared in this study were 67.21 nm. While the result disagree with Li *et al.*, (2018) whom mentioned that the average nanoparticle diameter of nanoparticle rosuvastatin was 98.4 nm.

Transforming rosuvastatin to nanocomposite by using zinc oxide which characterized with less than 100 nanometers in diameters, and have a large surface area relative to their size and high catalytic activity (Kumar *et al.*, 2013), make the drug more effective than the free form that showed improve in solubility and oral bioavailability by overcoming the hepatic first-pass metabolism (Gabr *et al.*, 2018).

3-2-Body weight measurement:

As shown in table (3-2), results of this study showed changes in the body weight experimental animals and the changes represented by a gain in body weight between the final and initial body weight. There was no significant different in the initial body weight of negative and positive control groups, As well in the initial body weight of treatment groups. There was a significant increase in the final body weight ($p<0.05$) in the positive control groups (2 and 3) as compare with negative control group, but mice consuming diet rich with vegetable fat caused significant increase ($p<0.05$) in the body as compare with animal high fat diet groups.

There were no significant changes ($p>0.05$) in final body weight in both groups (4 and 6) which treated with rosuvastatin as compare with positive control groups (2 and 3), but there was significant changes ($p<0.05$) in final body weight in both (5 and 7) groups treated with nano rosuvastatin as compare with positive control groups (2 and 3), The result showed that nano resovastatin therapy was more effective than rosuvastatin therapy.

Table (3-2): Changes of means body weight in study groups

Groups		Initial body weight (gm.) (mean±S.D)	Final body weight (gm.) (mean±S.D)	Body weight gain (gm.) (mean±S.D)
Diet groups	1 N. Con.	25.42±2.09	27.42±1.50	1.99±0.60
	2- A.HF.CON.	25.46±2.84	40.31±1.92*	14.58±1.80
	3- V.HF.CON.	24.98±1.13	45.03±1.19*	21.05±1.12
Treatment groups	At the end of three months			
	4- A. HF- ROS.	33.25±1.22	38.90±2.17	5.65±0.96
	5- A. HF- N. ROS.	32.83±2.40	36.94±1.81*	4.11±0.69
	6- V. HF- ROS.	37.63±1.74	43.87±1.32	6.24±0.42
	7- V. HF- N. ROS.	37.28±2.00	41.30±1.03*	4.02±1.57

Data expressed as mean±S.D (N=10),* Significant differences ($p<0.05$). Aberrations: N. Con. Normal diet control, A.HF.CON Animal fat diet control, V.HF.CON Vegetable fat diet control, A. HF- ROS Rosuvastatin and animal fat diet, A. HF- N. ROS Nanoparticle rosuvastatin and animal fat diet, V. HF- ROS Rosuvastatin and vegetable fat diet, V. HF- N. ROS Nanoparticle rosuvastatin and vegetable fat.

Overnutrition is the biggest factor for excess weight gain and obesity development, which found there is positive relationship between the level of fat in the diet and body weight or fat gain (Romieu *et al.*, 2017). Previous studies have shown that HFD produces significant weight gain and total body fat, which is progressing to obesity (Yang *et al.*, 2007; Aslam *et al.*, 2010; Kim *et al.*, 2012; Navarrete *et al.*, 2015).

The weight gain in the high fat diet (HFD) group of mice was significantly higher than the control mice, demonstrating the impact of high fat diet (Navarrete *et al;* 2018). Similarly, in this study there was significant weight gain ($p<0.05$) in high fat diet control groups which fed on (a diet rich with animal and vegetable fats) for four months as compared to a normal control group as shown in table (3-2).

Supplemented diet with 15% fat feed to positive control mice resulted in a significant increase in final and gain body weight compared to negative control mice. Weight gain and adiposity are often attributed to eating a diet rich in high fats in animals (Hariri and Thibault, 2010; Ghibaudi *et al.*, 2002) and humans (Chooi *et al.*, 2019; Grundy, 2000). Similarly, we found that 15% of fats fed to animals for four months caused significant increases in body weight and adiposity, which caused increased intra-abdominal fat pad mass.

Energy consumption that exceeds metabolic requirements leads to lipogenesis and fat storage in white adipose tissue (WAT), the primary body fat storage site which result increase of subcutaneous, visceral and abdominal adiposity, Overconsumption of dietary fat can lead to relatively rapid weight gain, because dietary fat is metabolized into free fatty acids, the primary substrate for triglycerides (TG), and then lipid synthesis (Botchlett and Wu, 2018). By general, excessive dietary fat consumption leads to high blood fat circulation and thereby increases epididymal and perirenal adipose tissue and fat deposition in the liver, contributing to lipid metabolism and obesity disorders (Chen *et al.*, 2018). Many studies have shown a significant correlation between oxidative stress and obesity, which is definitely a consequence of excessive accumulation of fat, high levels of reactive oxygen species, compromised antioxidant defenses, and elevated levels of inflammatory adipokines are commonly seen in obese subjects (Manna and Jain, 2015).

There is a disagreement about the impact of high fat diet on mice weight. Simo´n *et al.,* (2000) reported that 15% of fats showed no significant weight gain or there was a linear weight increase between the control and obesity animals while, Rahman *et al.,* (2012) reported that 20mg of fats feed for 8 weeks showed a significant increase in the body

weight compared with control, Also The result obtained coincide with the finding of Karaji-Bani *et al.,* (2006) who studied the effect of 12% of fats for 4 weeks, These result harmonize with our study, which found that The body weight of high fat diet groups that feed 15% of high fat diet for 4 months was significantly higher compared to control group.

Obesity is related to increased energy intake and dietary fat preferences (Drewnowski, 1997). Additionally, the effects of the type of fat consumed on health have been discussed for years and vegetable oil on animal fats has been recommended (Covas, 2008). Animal models are frequently used to investigate the role of high-fat diets in human obesity (Buettner *et al.,* 2007). Many rodent studies use either animal fat, or hydrogenated vegetable oil (vegetable fat) In this research, have been studying the effect of high fat diet rich with animal fat and hydrogenated vegetable oil (palm oil) on weight gain and the development of obesity (Fujiwara *et al.*, 2014).

The animal and vegetable fats included as study groups since the high fat diets are almost always based either on animal or hydrogenated vegetable oils (Hu, 2011). Trans fatty acids are produced either by industrial hydrogenation with hydrogen gas and a metal catalyst or by biohydrogenation within ruminant animals of cows and sheep through partial hydrogenation and/or isomerization of cis unsaturated fatty acids from the feed through hydrogen generated during oxidation of substrates, along with bacterial enzymes as catalyst (Popkin, 2001; Mozaffarian *et al.,* 2004). Hydrogenated vegetable oil rich with trans fatty acid as byproduct of hydrogenation process, which contain trans isomers of oleic acid and a lot amount of elaidic acid in addition to small amount of vaccenic acid, while the animal fat is completely consists of fat (high in saturated fat) , and naturally contain small amount of trans fatty acid

particullary vaccenic acid and contain rich amount of conjugated linoleic acid (CLA) which is derived from the grass-fed cows and sheeps (Daley *et al.*, 2010), and is naturally rich source of vitamin D (Ljunghall *et al.*, 1987; Ortega *et al.*, 2009).

There are different opinions about the influence of vegetable and animal fats in weight gain and the development of obesity. Sylvester *et al.*, (1986) Mention that there was no significant differences in the mean body weight throughout the experiment among the different dietary groups. While Ponnampalam *et al.*, (2011) reported that animal feed diet rich with vegetable fats consumed more calories, accumulated more excess body fat than those fed with animal fat, this result harmonized with our study.

It is possible that in our study VF was a greater contributor to the increase in energy intake and adiposity more than animal fat. Since VF riche with trans-fatty acid, higher consumption of TFAs caused elevate the total cholesterol and LDL-C which lead to excess fat accumulation and increase the body weight (Micha and Mozaffarian, 2008). While in animal fat, which is contain large amount of CLA that indicate its can reduce body fat in a variety of ways, In mouse studies, it was found to decrease food intake, stimulate fat breakdown and inhibit fat synthesis (Bhattacharya *et al.*, 2006; Whigham *et al.*, 2007), Also animal fat naturally riched with lipid soluble vitamin D which that many studies reported the association between vitamin D and its ability to reduce the metabolic consequences associated with obesity (Ljunghall *et al.*, 1987; Ortega *et al.*, 2009). Study by Motard-Bélanger *et al.*, (2008) mentioned moderat consuming to animal fats can be extremely healthy. but like any fats, excess consuming to animal fat have adverse effect on health can

caused heart disease and weight gain., the study agreed with our result, that mice consume animal fats for four month has gained weight.

High fat diet mice groups treated with rosuvastatin in its two forms (free and nano) for one month along with high fat diet, the obtained results showed no significant effect on high fat diet mice groups treated with 10mg/kg/day rosuvastatin. Our results agreed with Neto-Ferreira *et al.*, 2013 were mentioned that mice administration rosuvastatin 10 mg/ kg/day dose along with high fat diet for five weeks there was insignificant decrease in the body weight as compared with 40 mg/kg/day dose, the study also mentioned that despite the fact that rosuvastatin therapy reduces the adiposity and the adipocyte size in the HF-R10 and HF-R20 groups, but mice treated with 20 mg/Kg/day and 40 mg/Kg/day dose of rosuvastatin had a preferable body weight control compared with groups treated with 10 mg/Kg/day of the drug.

Rosuvastatin is lipid lowering drug exhibits some unique pharmacologic properties, which has Significant LDL-C lowering capacity by blocking an enzyme that is needed by the body to make cholesterol (White, 2002), Rosuvastatin converting into nanosize range lead to improved solubility and oral bioavailability (Alshora *et al.*, 2018), which results in reduced lipid accumulation in body organs and lowered body weight. Another possible explanation for decreasing body weight gain from treatment with nano rosuvastatin was mentioned by Fraulob *et al.*, (2012) who noted out that administration of rosuvastatin (10 mg / kg / day) improved circulating cholesterol and triglyceride levels in mice fed an HF diet through Increase the expression and production of hepatic LDL receptors, resulting in increased blood removal of LDL-C and increased cholesterol degradation and catabolism from the body, and ultimately reduced fat deposition resulting in reduced body weight gain.

3-3- Serum lipid profiles in study groups (TC, TG, HDL-C, LDL-C and VLDL-C)

Lipid profile levels of all experimental groups were showed in table (3-3), There was significant difference ($p<0.05$) in serum TC,TG,HDL-C,LDL-C and VLDL-C In all experimental groups as compared with negative control group.

The results showed there was significant increase ($p<0.05$) in the level of TC, TG, LDL-C and VLDL-C in positive control groups (2, 3) as compared with negative control group. TC and LDL-C levels were significantly ($P<0.05$) higher in vegetable fat supplemented group (3) comparatively with animal fat group (2), while TG and VLDL-C level were higher in animal fat supplemented group (2) comparatively with vegetable fat group (3). Mice consuming high fat diet caused significant decrease ($p<0.05$) in HDL-C level in both positive control groups (2, 3) as compared with negative control group, but there was no significant different between the effect of animal and vegetable fats on HDL-C.

Treatment with rosuvastatin and nano rosuvastatin caused significant decrease ($p<0.05$) in the level of (TC, TG, LDL-C and VLDL-C) in groups (4, 5, 6, 7) comparatively with their respective positive control groups (2, 3), but nano rosuvastatin therapy caused significant decrease ($p<0.05$) in lipid profile level in treatment groups (5, 7) compare with groups (4, 5) that treated with rosuvastatin. Mice administration with 0.02mg/kg/day of free and nano rosuvastatin caused significant increase ($p<0.05$) in the level of (HDL-C) in groups (4, 5, 6, 7) comparatively with their respective positive control groups (2, 3), but nano rosuvastatin therapy caused significant increase ($p<0.05$) in lipid profile level in treatment groups (5, 7) compare with rosuvastatin in groups (4, 5).

The results obtained showed significant differences ($p< 0.05$) between the effect of rosuvastatin and nano rosuvastatin on the serum lipid value, but the medicine in its two forms was more effective in mice consuming diet rich with animal than vegetable fats.

Table (3-3): Effect of various treatments on serum lipid profiles

Groups	T.C. mg/dl (mean ± S.D)	T.G. mg/dl (mean ± S.D)	HDL-C mg/dl (mean ± S.D)	LDL-C mg/dl (mean ± S.D)	VLDL-C mg/dl (mean ± S.D)
1- N. Con.	89.67±2.52	105.67±10.69	47.33±2.52	21.20±3.47	21.13±2.14
2- A.HF.CON.	212.33±10.79	180.33±3.51*	29.17±1.04	147.10±10.98	36.07±0.70*
3- V.HF.CON.	230.33±5.03*	153.83±4.54	31.50±1.50	168.07±5.72*	30.77±0.91
4-A. HF-ROS.	172.00±3.61	126.00±4.00	39.50±1.50	107.30±4.29	25.20±0.80
5-A. HF- N. ROS	128.50±2.18*	106.33±5.69*	43.50±1.50*	63.73±2.47*	21.27±1.14*
6-V. HF-ROS.	152.00±4.58	120.67±4.51	36.83±1.76	91.03±5.45	24.13±0.90
7-V. HF- N. ROS.	141.67±4.51*	99.33±3.06*	41.50±0.50*	80.30±4.41*	19.87±0.61*

Data expressed as mean±S.D (N=10),* Significant differences ($p<0.05$). Aberrations: N. Con. Normal diet control, A.HF.CON Animal fat diet control, V.HF.CON Vegetable fat diet control, A. HF- ROS Rosuvastatin and animal fat diet, A. HF- N. ROS Nanoparticle rosuvastatin and animal fat diet, V. HF- ROS Rosuvastatin and vegetable fat diet, V. HF- N. ROS Nanoparticle rosuvastatin and vegetable fat, TC Total Cholesterol, TG Triglyceride, HDL-C High density lipoprotein-cholesterol, LDL-C low density lipoprotein-Cholesterol, VLDL-C Very low density lipoprotein.

The dysplidimia caused by feeding HFD (15% of fats) for four months resulted in several alterations in TC, TG, LDL-C, VLDL-C and HDL-C serum, which resemble to type IIa dysplidimia in humans (Ramasamy, 2016), as shown in the current study eating a high fat diet caused obesity, which has been shown to be associated with cardiovascular disease (CVD) such as arteriosclerosis, stroke and myocardial infarction. These conditions may affect the metabolism of lipoprotein and caused temporary increases in TC, TG and LDL-C levels and lower HDL-C level (Zhao *et al.,* 2005). The effect of HFD-induced hyperlipidemia may be due to the activity of the cholesterol biosynthesis rate-limiting enzyme, HMG-CoA reductase, which leads to stimulate the rate of cholesterogenesis (Rashid *et al.,* 2015).

In (2013) Cariou *et al.,* and in (2014) Jia *et al.,* were reported the relationship between PCSK9 (Proprotein Convertase Subtilisin Kexin type 9) and dysplidimia caused by high fat diet, Where they mentioned that the PCSK9 proximal promoter gene contains a functional sterol regulatory element (SRE) that react to changes in intracellular cholesterol levels. So eating HFD caused increased the concentration of PCSK9 result in degradation of hepatic LDL receptor and elevated the level of LDL-C.

Another study connects between the role of sympathetic nervous system and development of dyslipidemia as Masuo and Lambert, (2011) reported that A large part of the sympathetic nervous system mediated energy expenditure through thermogenesis mechanism by the lipolysis of triacylglycerol stored in adipose tissue through the coupling of β2-adrenoceptors found on the membrane of adipocyte in response to specific neural and hormonal stimuli. These stimuli are represented by catecholamines, which are considered to be the most potent lipolysis

regulators. The role becomes particularly important during exercise and use of fat as a fuel, but eating high-fat food with inactivity leads to decreased adrenal medullary activity and the development of dyslipidemia (increased circulating TG, LDL-C and decreased HDL-C).

In the present study, saturated fat results from eating a high fat diet, which considers best correlates with elevated the circulating serum LDL-C levels by suppressing hepatic receptor-dependent LDL uptake (Sacks *et al.,* 2017). Within excess amount LDL-C in blood cause oxidation, which occur when LDL cholesterol particles in the body react with free radicals; unstable molecules that are byproduct as a result of normal metabolism (Dipiro *et al.,* 2017), that lead to formation of oxidized-LDL-C, which is thought to foster the development of atherosclerosis and raises the risk of a heart attack or stroke (Suciu *et al.,* 2018).

Another possible reason in decreasing in Serum HDL-C and increase the serum level of LDL-C and TG in animals fed with High fat diet in this research may be due to deficiency in the activity of lecithin-cholesterol acyltransferase (LCAT) enzyme that mediated the formation of HDL particles, the maturation of HDL, involved in the esterification of cholesterol into cholesterol ester (CE) in the core of the HDL particle (Welsh *et al.,* 2019). Increase the activity of cholesteryl ester transfer protein (CETP) protein which synthesized by some body tissues, but mainly in adipose tissue, eating a high fat diet result in store the excess energy in the form of triglyceride in adipose tissue cells, It was reported that diets with high amount of fat, leads to increased activity, synthesis and secretion of CETP (Salerno *et al.*,2009) , in the serum CETP transfer cholesterol ester from HDL to VLDL and LDL, resulting in a decrease in HDL-C levels and increase LDL-C and TG (Mabuchi *et al.,* 2014).

Raising the level triglyceride in our study supported by the research of Lee *et al.*, (2020) who reported that mice consume animal fat increased blood level of triglyceride , The result has also been reinforced by Colandre *et al.,* (2003) and Ibrahim *et al.,* (2005) who have shown that trans fatty acids elevate triglyceride levels. Our results agreed with the report by Rahman *et al.,* (2018), Who found that consuming a diet rich in high fat resulted in a substantial increase in plasma fat levels, including LDL, while the HDL values insignificant among mice groups , which contradicts our findings, the study also showed that animal fats increase triglyceride levels compared with vegetable fats. These results are consistent with the Karanth and Jeevaratnam (2009) studies, which found that high fat diet intake significantly, increased the plasma lipid profile including LDL and decreased HDL. Other findings also indicated that HDL decreased when eating diet rich with animal and vegetable fats (Ibrahim *et al.,* 2005).

The result obtained in this study possibly due to the fact that hydrogenated vegetable oils considered rich in oleic acid and elaidic acid, two of the main trans isomers and alpha-linolenic acid generated throughout industrial hydrogenation of edible oils. All these industrial trans fatty acids have negative impacts on plasma lipoproteins, that causes an increase in levels of total cholesterol and LDL-C and decrease HDL-C, also contain smaller amounts of vaccenic acid as compared with animal fat (Mozaffarian *et al.,* 2006; Vermunt *et al.,* 2001). On the other hand animal fat vaccenic acid which is naturally produced Trans fatty acid, scientific proof Evidence from studies on animal model with dyslipidemia mentioned the beneficial effect of vaccenic acid in lipid lowering (Wang *et al.,* 2010). In addition animal fat conceder the main natural source rich with conjugated linoleic acid (CLA), study performed

on animal reported that CLA Reduces the risk of atherosclerosis by decrease LDL-C and increase HDL-C (Le ae *et al.,* 1994), also study by Lalithadevi *et al.,* (2018) reported that CLA has antioxidant properties, They function as free radical scavengers thus providing an protective mechanism toward membrane attacks by harmful oxygen free radicals and prevent the formation of oxidized LDL-C.

In the present research, both vegetable fat dietary and animal fat elevated levels of triglycerides, indicating that both forms of fat promote insulin resistance. There is evidence that peripheral insulin resistance is related to hypertriglyceridemia (Piatti *et al.,* 1995; Widén *et al.,* 1992).

Rosuvastatin administration to mice fed HFD resulted in a decrease in serum TC and LDL-C rates, whereas serum HDL-C rates were elevated, which is consistent with Adams *et al.,* 2014 and Ephraim *et al.,* (2016). Those results may be explained by the inhibitory action of rosuvastatin on the enzyme HMG-CoA reductase that catalyzes the conversion of HMG-CoA to mevalonate, a rate-limiting phase in the production of endogenous cholesterol that leads to a reduction in cholesterol intracellular stores, So this eventually leads to the up-regulation of LDL receptors located on the cell membrane, thereby improving the removing of LDL-C from the plasma (Stalker *et al.,* 2001). This impact of rosuvastatin on LDL-R can be regarded by hepatocytes as a feedback response to decreased hepatic cholesterol.

Rosuvastatin therapy could return HDL-C levels to their normal range by activation the reverse cholesterol transport (RCT) method, which transmit the excess amount cholesterol from extra-hepatic tissue which include (gastrointestinal tract, adipose tissue and macrophages) that have an exceptional role in the metabolism of cholesterol to the liver and also

improves the cholesterol absorption from the intestine, which could be regarded as the primary mechanisms of compensating for the deficiency of cholesterol in the liver during rosuvastatin therapy (Rigotti *et al.*, 2003; Moreno *et al.*, 2009).

The RCT mechanism stimulates the transfer of cholesterol from the peripheral cells to HDL, then HDL-C is transferred throughout the blood to the liver, and the cholesterol in HDL is converted to cholesterol ester (CE) by the enzyme lecithin-cholesterol acyltransferase (LCAT) and transported as CE in the center of the HDL particle to the liver, CE is finally excreted from the liver into bile or feces (Dietschy and Turley, 2002). Most researches have shown that rosuvastatin stimulate various components of the RCT process, which include ATP binding cassette transporter-A1 (ABCA1) and Class B scavenger receptor type I (SR-BI) (Moreno *et al.*, 2009).

A comparative study on dyslipidemia by using rosuvastatin mentioned by Deedwania *et al.*, (2005) they revealed that LDL-C decreasing were better with rosuvastatin 10 mg than atorvastatin 20 mg. Rosuvastatin 10, 20 and 40 mg were more efficient as compared with some higher doses of pravastatin and simvastatin. Also Rader *et al.*, (2003) reported that, using rosuvastatin increased HDL-C level compared with atorvastatin, simvastatin and pravastatin, while Triglyceride level decrease using rosuvastatin much more than other statins atorvastatin, simvastatin and pravastatin. Rosuvastatin have also been reported to reduce activity of hepatic lipase, whic plays an important role in triglyceride level regulation in the blood by maintaining steady levels of HDL and LDL (Barter *et al.*, 2010; Fox, 2015).

Another study by Clearfield *et al.,* (2006) mentioned that 10mg of rosuvastatin for 6 weeks of treatment reduced the LDL-C level more significantly than 10 mg of atorvastatin. Neto-Ferreira *et al.,* 2013 reported that 10 mg of rosuvastatin combaind with high fat diet for five weeks of treatment showed highest efficacy in increased the HDL-C and reduction of LDL cholesterol, improved the circulating levels of cholesterol and triglycerides in mice fed a HF diet.

In particular, nanoparticles are one of the most commonly studied carriers for enhancing the therapy of potent drugs. These can be used to increase the stability of trapped drugs in the presence of biological fluids, and to enhance uptake the by target cell (Peer *et al.,* 2007; Davis *et al.,* 2008). Because of rosuvastatin low solubility, low permeability and poor oral bioavailability. So Sathali *et al.,* (2013) and Gabr *et al.,* (2018) established that rosuvastatin convert to nanometer size range particles can enhanced the bioavailability of poor water-soluble by overcoming the hepatic first-pass metabolism and this makes the nano drug more effective than rosuvastatin.

3-4- Serum liver enzyme in study groups (ALT and AST):

Liver enzymes levels of all experimental groups were showed in table (3-4). The results showed there was significant increase ($p<0.05$) in the level of AST and ALT in positive control groups (2, 3) as compared with negative control group. ALT enzyme levels were significantly ($P<0.05$) higher in vegetable fat supplemented group (3) comparatively with animal fat group (2), while AST enzyme level were higher in animal fat supplemented group (2) comparatively with vegetable fat group (3). Mice administration with 0.02mg/kg/day of free and nano rosuvastatin caused significant decrease ($p<0.05$) in the level of (AST and ALT) in groups (4, 5, 6, 7) comparatively with their respective positive control groups (2, 3), but nano rosuvastatin therapy caused significant decrease ($p<0.05$) in lipid profile level in treatment groups (5, 7) compare with groups (4, 5) that treated with rosuvastatin.

Table (3-4): Serum liver enzyme in study groups (ALT and AST)

GROUPS	**AST U/L (mean ± S.D)**	**ALT U/L (mean ± S.D)**
1. N. CON.	142.33±10.02	36.00±4.00
2. A.HF.CON	393.33±16.07*	62.67±3.06
3. V.HF.CON.	372.33±4.16	81.00±5.57*
4. A.HF-ROS.	343.33±6.03	55.00±3.61
5. A.HF-N.ROS.	224.67±7.51*	50.67±3.06*
6. V.HF-ROS.	335.00±5.57	47.00±3.00
7. V.HF-N.ROS	303.33±15.04*	42.33±3.06*

Data expressed as mean±S.D (N=10),* Significant differences ($p<0.05$). Aberrations: N. Con. Normal diet control, A.HF.CON Animal fat diet control, V.HF.CON Vegetable fat diet control, A. HF- ROS Rosuvastatin and animal fat diet, A. HF- N. ROS Nanoparticle rosuvastatin and animal fat diet, V. HF- ROS Rosuvastatin and vegetable fat diet, V. HF- N. ROS Nanoparticle rosuvastatin and vegetable fat, AST Aspartate aminotransferase, ALT Alanine aminotransferase.

Abnormalities in liver function determine through the changes in the level of liver marker enzyme alanine transaminase (ALT) and aspartate aminotransferase (AST) in the bloodstream, elevated to the level in these enzymes in the blood represent an indication for liver damage (Byrne, 2012; Dyson *et al.,* 2014). ALT is the most accurate liver function indicator, but AST less accurate indicator since it is found in other tissues (Hanley *et al.,* 2004; Lee *et al.,* 2014). The present study accompanied with Kim *et al.,* (2017), were reported that feeding mice diet with 45% of fats for 15 weeks stimulate liver damage and increased serum level of alanine transaminase (ALT) and aspartate transaminase (AST) in high fat diet groups as compared with control group.

Studies by Saki *et al.,* (2011) documented that high-fat diet elevated the levels of serum hepatic enzyme, alanine aminotransferase (ALT) and aspartate aminotransferase (AST); this raise was as result to increase in the free radicals formation that activates lipid peroxidation, This result harmonized with our study, which shows that the high fat diet elevated the serum hepatic enzymes (ALT) and (AST) in both vegetarian and animal high fat diet groups.

Numerous researches demonstrate that NAFLD progression is related to the quantity of the visceral fat, total cholesterol, triglycerides, serum insulin and insulin resistance which caused elevated in liver enzyme level. Rosuvastatin (10 mg/ kg/ day) and nano rosuvastatin administration enhanced insulin sensitivity, diminishes liver steatosis and body weight, and enhanced circulating cholesterol and triglyceride levels in mice fed an high fat diet, all these factors caused decrease in ALT and AST levels (Fraulob *et al.,* 2012). Rosuvastatin decreased steatosis, presumably due to decreased FFAs input and increased FFAs output due to increased betaoxidation (Svegliati-Baroni *et al.,* 2006).

3-5-Histopathological study:

3-5-1- Histology of liver:

Liver section examination with microscopic revealed different changes between the study groups as compared to the negative control group. Liver sections of negative control group demonstrate the central vein enclosed by hepatocyte cords extending radially from the central portal vein. The following forms have been described among the nonparanchymal cells: bile duct, endothelial and kupffer cells are preferentially located in the region per portal. Standard hepatocytes with centrally rounded nuclei and homogeneous cytoplasm, flat endothelial cells around the central vein and sinusoids were identified in negative control groups as showed in figures (3-7).

Changes in liver sections of mice consuming high fat diet (animal and vegetable fat) which stained with hematoxylin-eosin stain showed, mild to severe lymphocytes infiltration, sever lipid accumulation as macro and micro vesicular steatosis, necrosis, pyknotic nuclei. These changes were more sever in high animal fat liver section comparatively with liver section of mice consuming diet rich with vegetable fat, figure (3-8).

Liver section treated with 10mg of rosuvastatin showed change that include little infiltrate lymphocytes, pyknotic nuclei, apoptotic cells.Also Rosuvastatin treatment showed ameliorated the steatosis, compare with high fat diet liver section, figure (3-9). Liver section treated with nano Rosuvastatin showed normal structure of liver as showed in figure (3-10)

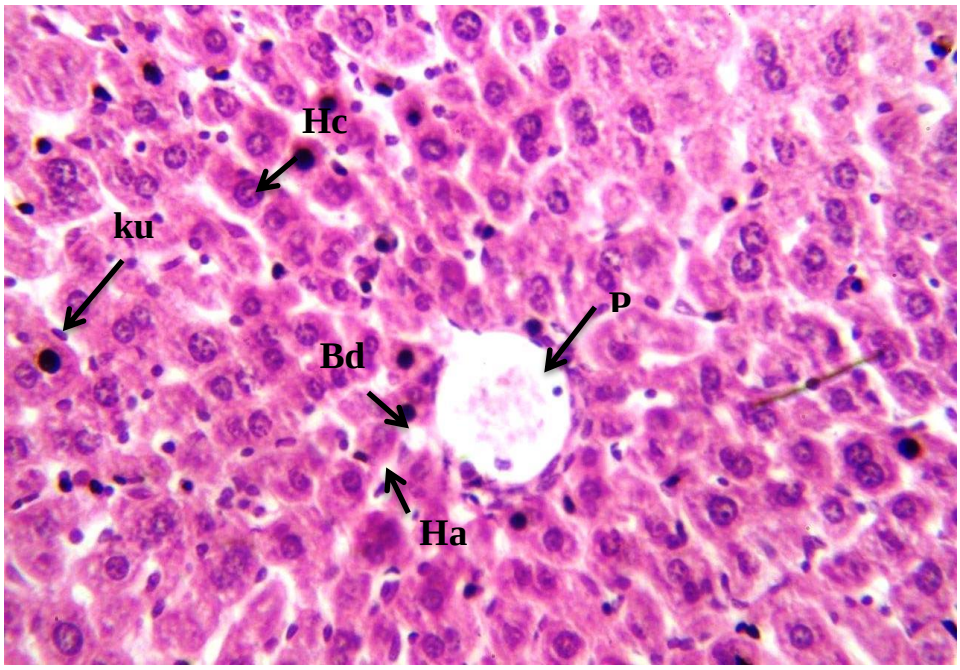

Figure (3-7): Liver section showed normal histological structure. Hepatocyte (Hc arrow), portal vein (Pv arrow), kupffer cell (Ku arrow), bile duct (Bd arrow), hepatic artery (Ha arrow), stained by heamatoxylin-eosin, at magnification 400x.

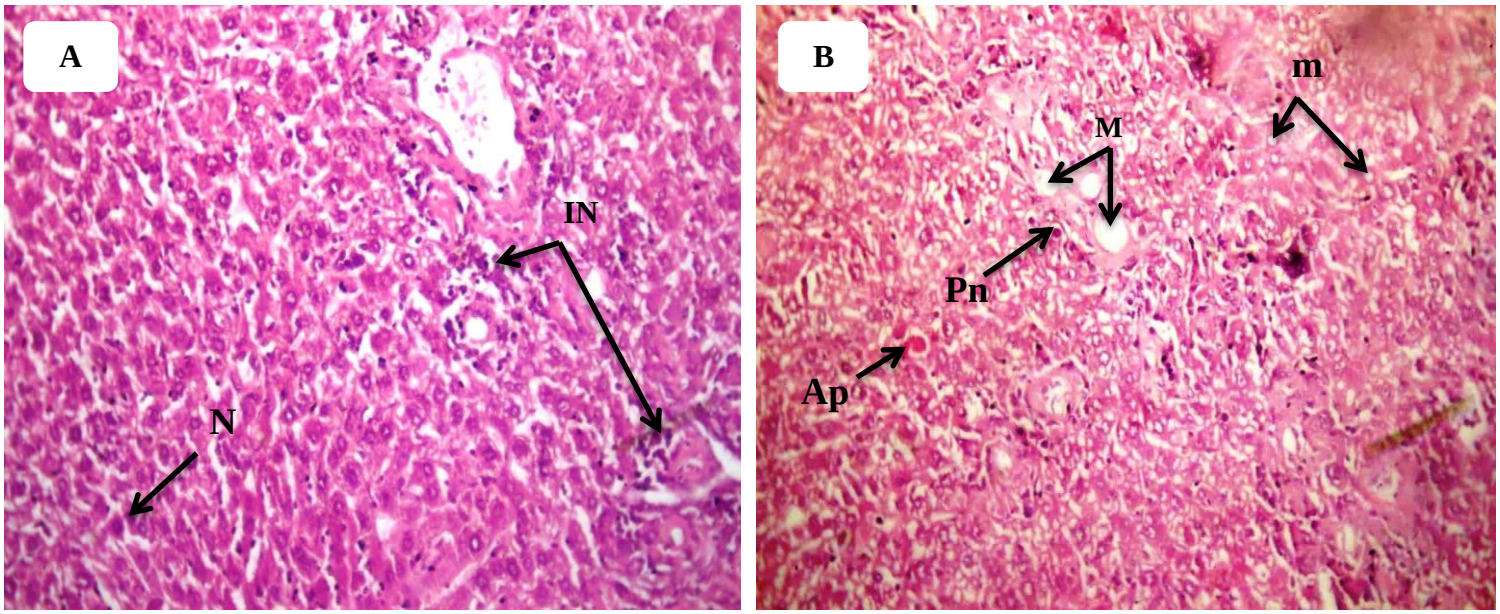

Figure (3-8): Photomicrograph of heamatoxylin-eosin stained liver section from animal high fat diet fed mice showed. A) Infiltration lymphocytes (IN rrow), necrosis (Ne arrow) at magnification 200x. B) Macro vascular steatosis (M arrow), micro vascular steatosis (m arrow), apototic cell (Ap arrow), pyknotic nuclei (Pn arrow), at magnification 200x.

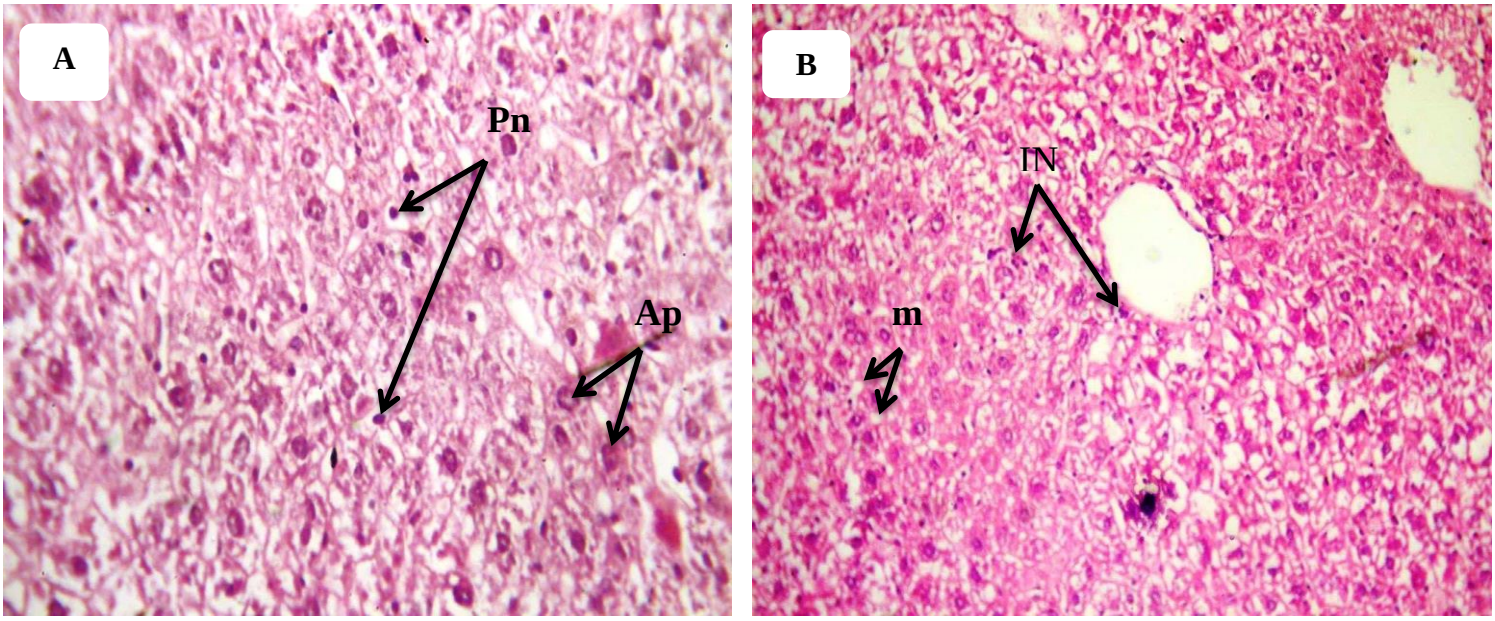

Figure (3-9): Effect of rosuvastatin supplementation on liver section in AHF diet fed mice showed: A) pyknotic nuclei (Pn arrow), apototic cell (Ap arrow). B) Infiltration lymphocytes (IN rrow), micro vascular steatosis (m arrow), stained by heamatoxylin-eosin, at magnification 200x.

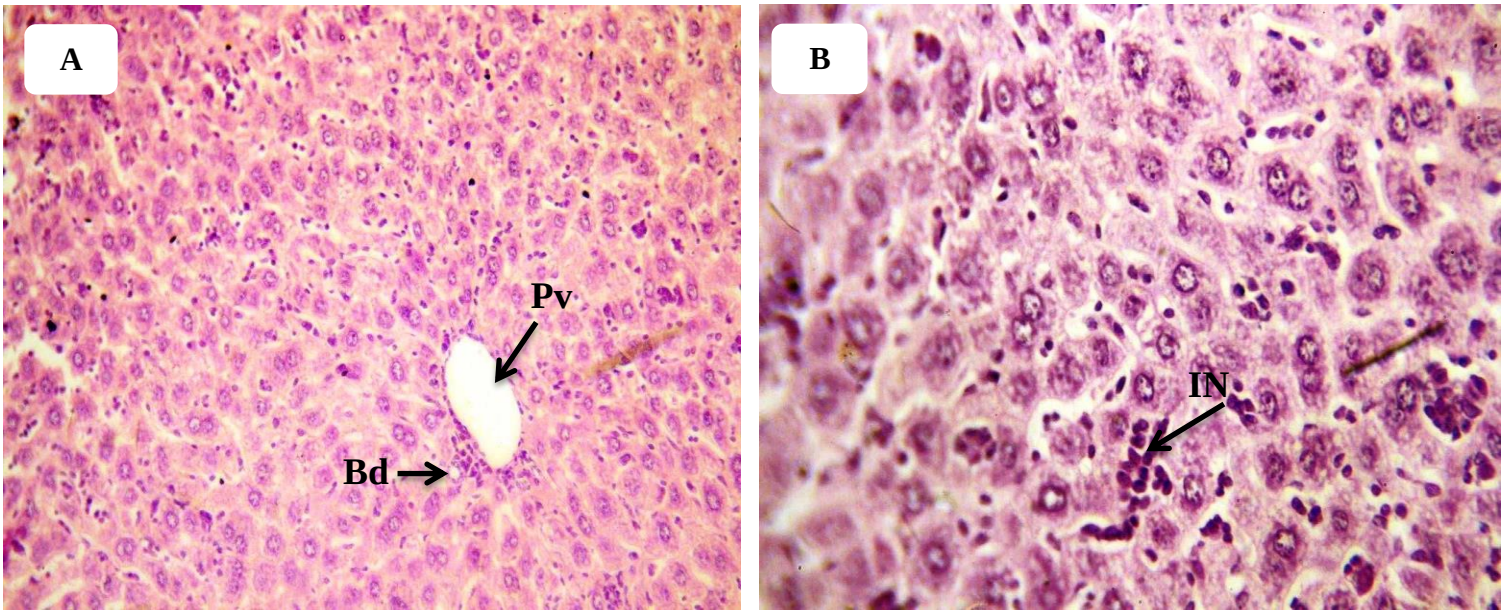

Figure (3-10): Effect of nano rosuvastatin supplementation on AHF diet fed mice showed improvement in liver section: A) portal vein (Pv arrow), bile duct (Bd arrow) at magnification 200x. B) Infiltration lymphocytes (IN arrow), stained by heamatoxylin-eosin, at magnification 400x.

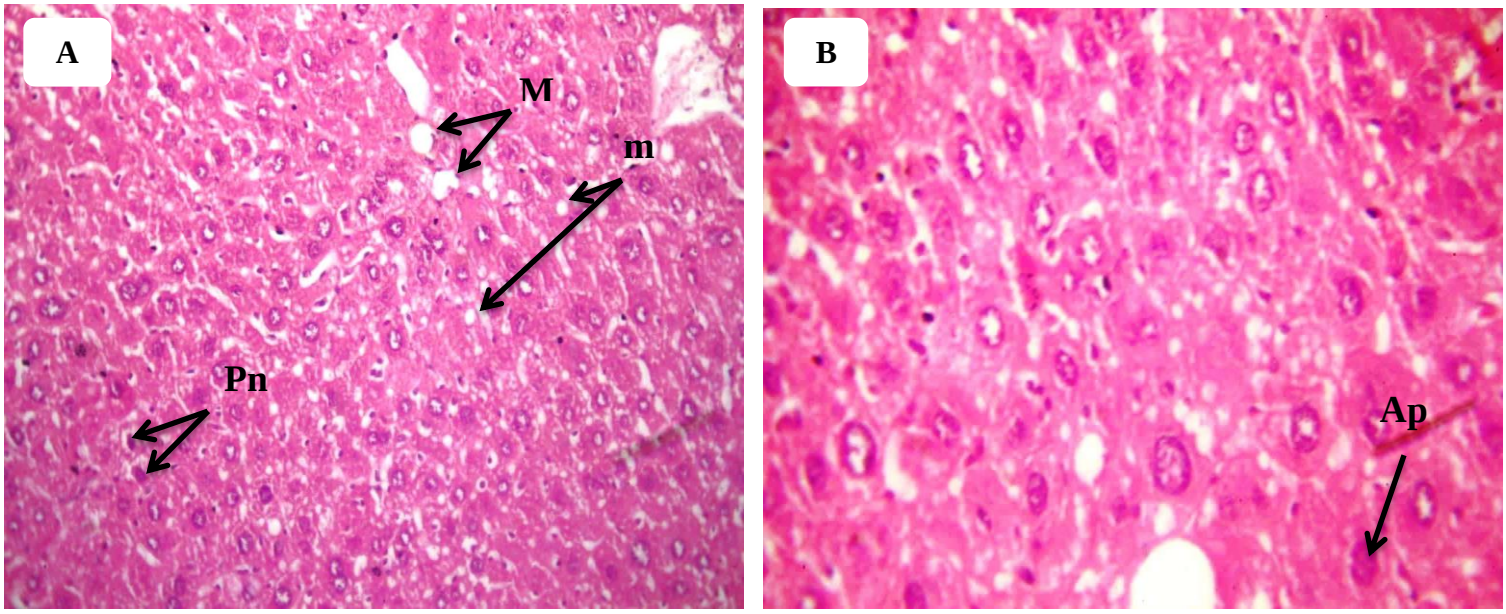

Figure (3-11): Photomicrograph of heamatoxylin-eosin stained liver section from vegetable high fat diet fed mice showed. A) Macro vascular steatosis (M arrow), micro vascular steatosis (m arrow), pyknotic nuclei (Pn arrow) at magnification 200x. B) Apoptotic cell (Ap arrow), at magnification 400X.

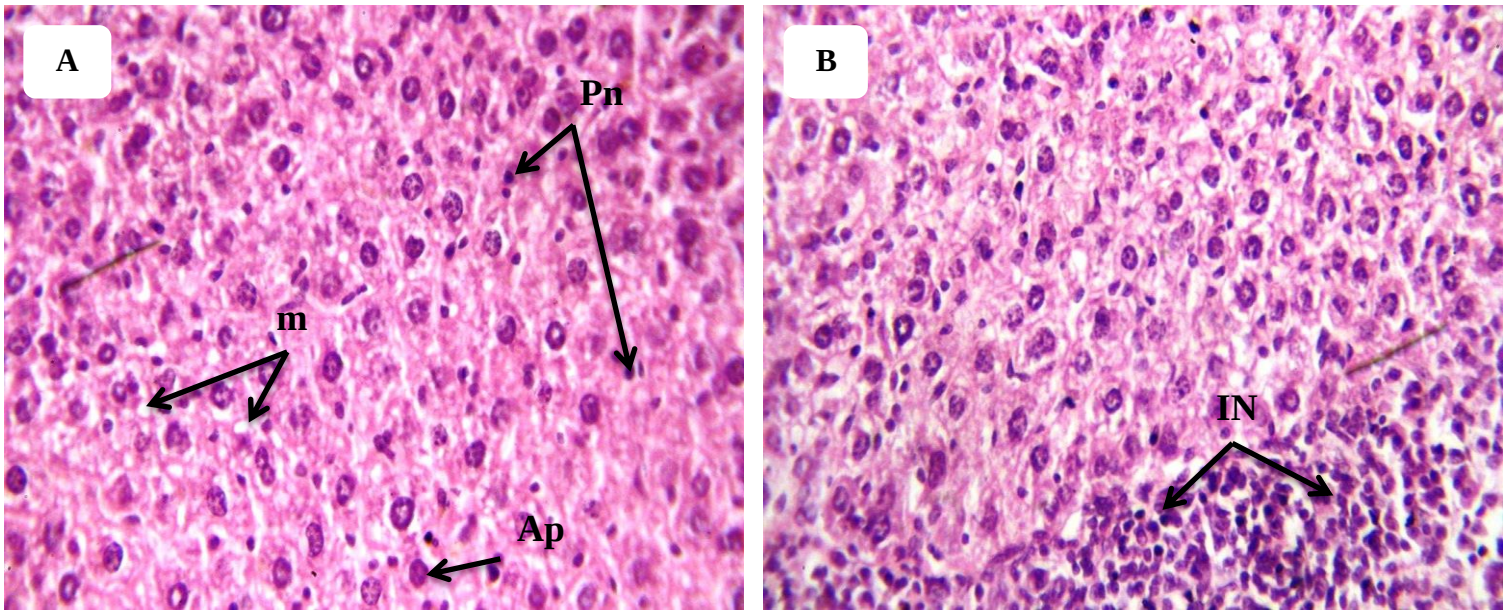

Figure (3-12): Effect of rosuvastatin supplementation on liver section in VHF diet fed mice showed: A) pyknotic nuclei (Pn arrow), apototic cell (Ap arrow), micro vascular steatosis (m arrow) at magnification 200x. B) Infiltration lymphocytes (IN rrow), stained by heamatoxylin-eosin, at magnification 200x.

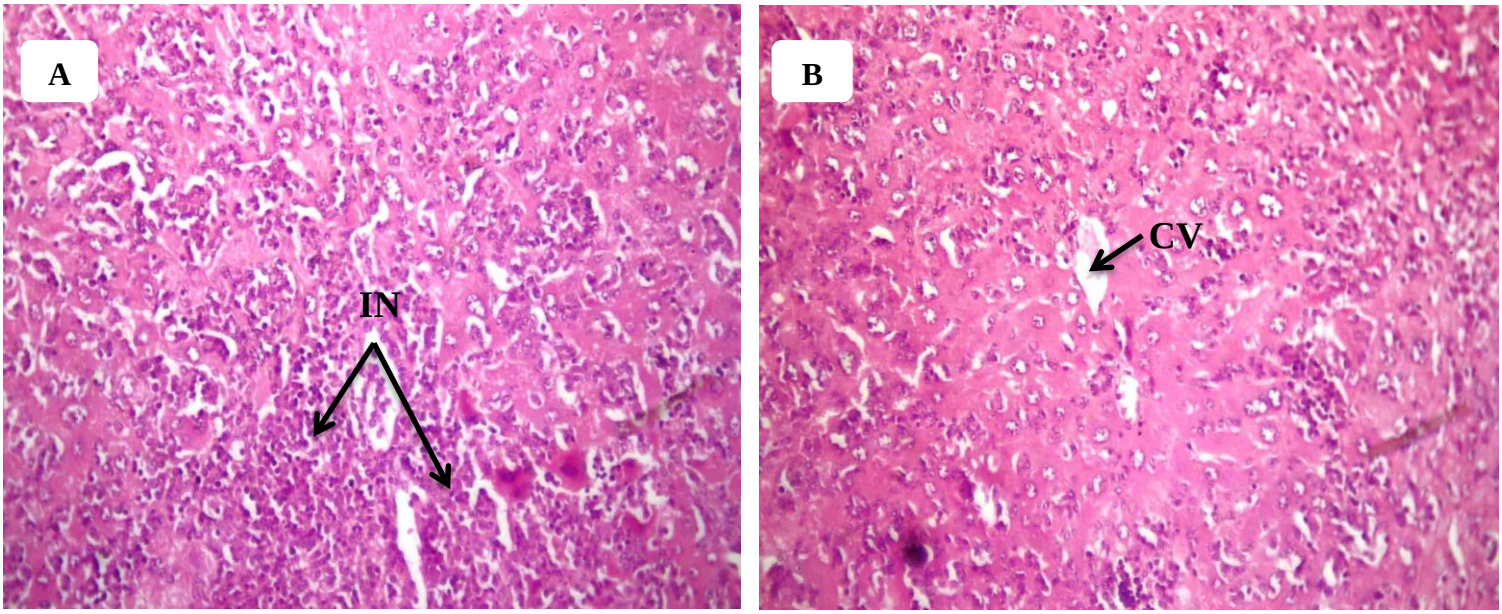

Figure (3-13): Effect of nano rosuvastatin supplementation on VHF diet fed mice showed improvement in liver section: A) Central vein (CV arrow) at magnification 400x. B) Infiltration lymphocytes (IN arrow), stained by heamatoxylin-eosin, at magnification 400x.

In regular conditions, the liver processes significant quantities of fatty acid (FA) on a daily basis but only stores minimal quantities in the form of TG, with stable TG content of fewer than 5 %. This is because FA acquisition levels by plasma absorption and de novo production within the liver are balanced through FA oxidation and plasma secretion levels as a very low-density lipoprotein enriched with TG (VLDL-TG), The comparatively small amounts of TG stored in the liver are situated in cytoplasmic lipid droplets (Browning *et al.*, 2004; Alves-Bezerra and Cohen, 2017).

Obesity caused by consuming high fat diet lead to fat accumulation in the liver is regarded as non-alcoholic fatty liver disease (NAFLD) and further develops from simple steatosis to nonalcoholic steatohepatitis (NASH) Which liver steatosis with inflammation, advanced fibrosis and cirrhosis (Ragab *et al.,* 2015). The fat accumulation in the liver is not

completely understood, but it was suggested that when hepatic fatty acid availability surpass the ability for elimination then they are stored as TG in the liver (Babin and Gibbons, 2009). TG storage results in net fat retention which is a precondition for NAFLD progress (Angulo, 2002). There are many mechanisms by which fat can begin to accumulate in the liver, including: increased delivery of fatty acids to the liver from adipose tissue lipolysis and elevated transmission of dietary fatty acids to the liver (Dowman *et al.*, 2010; Lavoie and Gauthier, 2006).

Study by Hassan *et al.,* (2018), mentioned that feeding mice diet rich with fats for 6 weeks caused various histological change in liver include micro and macro-vesicular steatosis , also revealed several ultrastructural changes in the form of several variable sized and formed lipid droplets within the hepatocyte cytoplasm. The study harmonized with our result that obtained by feeding mice high fat diet for 4 months.

Extra lipid accumulation in the liver cells is not only a mediator of metabolic syndrome and a lipid overload predictor, but also followed by a number of histological changes. Liver histology results showed various degrees of histological modification due to a large number of histological changes in various study groups which analogues with biochemical results obtained in the present research which including a decrease in HDL-C and elevated in TC, TG and LDL-C.

The most acute variations noted in mice high fat diet control groups, were huge numbers in the liver tissue of micro-vesicular steatosis and fibrosis, the result obtained harmonized with VanSaun *et al.,* (2009) and Hassan *et al.,* (2018) result, they mentioned that feeding rodent diet rich with fats, caused numerous ultrastructural changes in the shape of several lipid droplets of varying size and shape within the hepatocyte cytoplasm.

Hepatocyte vacuolation has been characterized as microvesicular and macrovesicular steatosis (Brunt and Tiniakos, 2010). Macrovesicular steatosis is demonstrated as imperfections in lipid delivery, metabolism, synthesis, and export. Nevertheless, microvesicular steatosis is connected with impaired beta-oxidation of fatty acids including mitochondrial cytopathies, which is the hallmark of liver diseases. In addition, cytoplasmic vacuolation was linked to lipid peroxidation due to oxidative stress that damages cell membranes as well as cell organel membranes, resulting in increased permeability and disruption of ion concentrations in cytoplasm and cell organelles (Ayala *et al.*, 2014).

One of the main mechanisms that lead to develop NAFLD caused by consuming a high fat diet is the rising of triglycerides in plasma and also in the liver, which noticed in the present result in high fat diet groups as compared with control groups. origin of elevated hepatic triglyceride level are due to consuming excess dietary rich with fats, caused elevated the synthesis of triglyceride in the liver from FFA created through de novo lipogenesis, promote FFA flow into the liver from lipolysis of adipose tissue, and subsequent transformation into triglycerides, decrease fatty acids lipid export from the liver through very low-density lipoprotein particles and minimize the fatty acids oxidation (Cheung and Sanyal, 2008). In addition elevated LDL-C and decrease HDL-C concider anathor characteristics connected with NAFLD (Koruk *et al.*, 2003).

Another principal mechanism demonstrated the hepatocellular injury (Wierzbicki and Oben, 2012); the direct mechanism includes direct cytotoxicity of the fatty acids on the hepatocytes resulting from excessive accumulation of intracellular fatty acids, whereas the indirect mechanism involves cytotoxic effects of lipid peroxidation of fatty acids. In addition,

oxidative stress is believed to be the main mechanism of hepatocellular injury, as many experimental studies have proven (Zhang *et al.*, 2018). Oxidative stress sources at NASH involve cytochrome P450, peroxisomal oxidation of fatty acid, mitochondrial dysfunction and inflammatory cytokines (Rolo *et al.*, 2012).

Cellular injury caused by high fat diet resulting from the activation of cytochrome P-450 in the liver, creating highly reactive radical free trichloromethyl. This in effect induces lipid peroxidation of the membrane in the presence of oxygen produced by metabolic leakage from the mitochondria leading to a loss of cell membrane integrity and damage to hepatic cells (Saki *et al.*, 2011).

In most extreme cases, the liver sections showed various degrees of hepatocyte degeneration, apoptotic cells and pyknotic nuclei; these changes possibly due to an increase in the potentially harmful form of cholesterol called oxidized low-density lipoprotein (LDL) formed in the body when the normal LDL cholesterol is destroyed by chemical interactions with free radicals and its associated with NASH (Gao *et al.*, 2017). The oxidized LDL is much competition with the surrounding tissues, which can cause inflammation leading to disease and damage to the body organ (Dipiro *et al.*, 2020), The evidence indicates that oxLDL plays an significant role in obesity-related inflammatory disorders like the atherosclerosis and cardiovascular disease (CVD) (Li and Mehta , 2005; Nishi *et al.*, 2002).

Oxidized low-density lipoprotein (oxLDL) has several harmful effects such as the conversion of macrophages into foam cells , resulting in excessive quantities of lipids building up in macrophages, contributing to a phenomenon called foam cell formation (Itabe *et al.*, 2011). OxLDL is

also inducing a dose-dependent increase in oxidative stress in cultivated cells, including the production of reactive oxygen species and lipid peroxidation products, which is believed to be a key factor in NASH development (Chen *et al.,* 2007; Gambino *et al.,* 2011).

Another significant side through the pathogenesis of inflammatation is apoptotic cell death, which has proven to play a significant role in NASH (Feldstein *et al.*, 2003; Wang *et al.,* 2008), it was found that OxLDL increases apoptosis by triggering apoptotic signaling cascades including the Fas signaling pathway (Takarada *et al.,* 2003). In addition, bioactive oxidized lipids have been observed in apoptotic cells, thus, given that oxLDL promotes apoptosis, oxLDL is not only an inflammatory cause, but also stimulates subsequent damage to the cells (Chang *et al* ., 2004).

Triglyceride is the strongest parameters that connected with fat accumulation in the liver (Chen *et al.,* 2019), triglyceride represents the basic of animal fat (Liu *et al.,* 2018), which lead to elevated triglyceride and much more sever effect in liver of mice consuming diet rich with animal fat compare with vegetable fat. mice with dysplidemia showed well improvement in liver histology after one months of rosuvastatin 10mg treatment, there were , modest changes or normal liver sections were noticed, these results could attributed to effects of rosuvastatin which decrease lipid profile level of (TG,TC,VLDL and LDL) specially oxidized-LDL, in additional to lipid peroxidation.

The potential mechanism by which rosuvastatin enhanced lipid deposition in liver by raise HDL-C through the inhibition of cholesterol ester transfer protein (CETP), the increased in HDL level will promote and increase the clearance of free cholesterol (Barter *et al.,* 2018), and the decrease in plasma LDL quantities that attributed to increased hepatic

LDL receptor expression, which resulted in greater hepatic removal of native and oxidized LDL-C from circulation and finally reduced lipid peroxidation both of these activities would result in reduced lipid deposition in hepatocytes (Chung *et al.,* 2010).

Our main result agree with Neto-Ferreira *et al.,* (2013) and Fraulob *et al.,* (2012), where reported that administrated mice with 10mg/kg/day along with high fat diet for 5 weeks caused decrease in TG, AST and ALT level and improve hepatic steatosis (microvesicular). The result has also agreed with Argo *et al.,* (2008) and Hyogo *et al.,* (2008), who demonstrated that statin therapy in patients with NAFLD and nonalcoholic steatohepatitis could decrease liver enzyme levels as well as hepatic steatosis, by the capability statins in improvement of serum aminotransferase levels.

3-5-2- Histology of kidney:

Microscopic examination of kidney sections stained by heamatoxylin - eosin stain showed different histological changes among the groups of the study compared with negative control group in high fat diet groups which include: dilated in renal tubules, degeneration and necrosis in the cells lined renal tubules, abundant vascular congestion with thickening of the basement membrane, interstitial severe lymphocytes infiltration and widened interstitial space.

Kidney section treated with rosuvastatin showed mild changes which include: congestion, mild inflammatory lymphocytes infiltration, mild hyaline cast, necrosis, while kidney section treated with nano rosuvaststin showed normal architecture which described previously, normal glomeruli, normal capsular space and normal renal tubules.

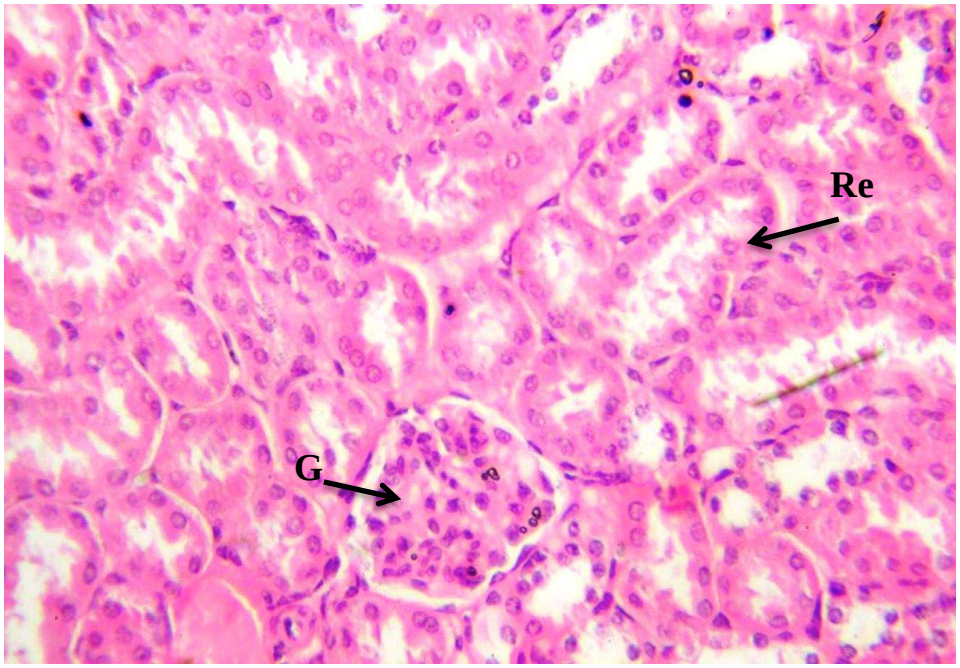

Figure (3-14): kidney section stained by heamatoxylin-eosin (H&E) of negative control group, showing normal architecture of renal tubules (Re arrow) and glomerulus (G arrow), at magnification 400x.

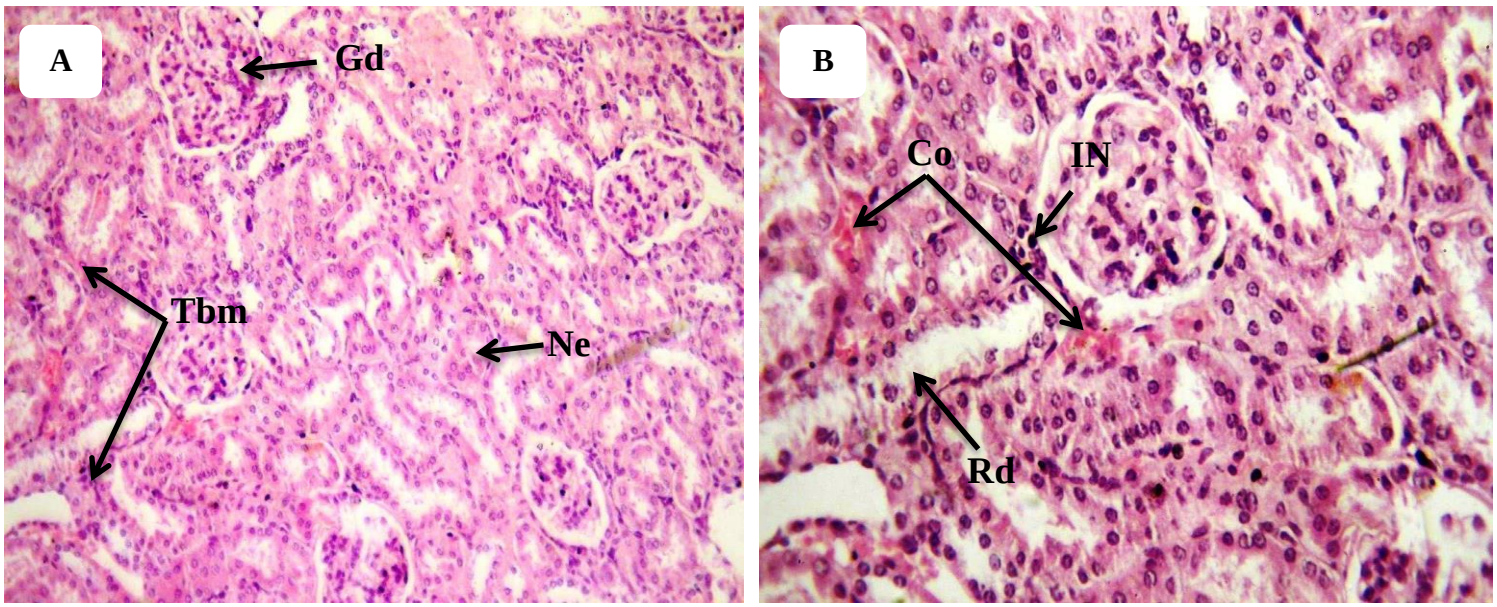

Figure (3-15): Photomicrograph of heamatoxylin-eosin stained kidney section from animal high fat diet fed mice showed: A) Thickened basement membrane (Tbm arrow), necrosis (Ne arrow), glomerulus degeneration (Gd arrow), at magnification 200x. B) Renal tubules dilatation (Re arrow), congestion (Co arrow), infiltration lymphocytes (IN arrow), at magnificatio 400x.

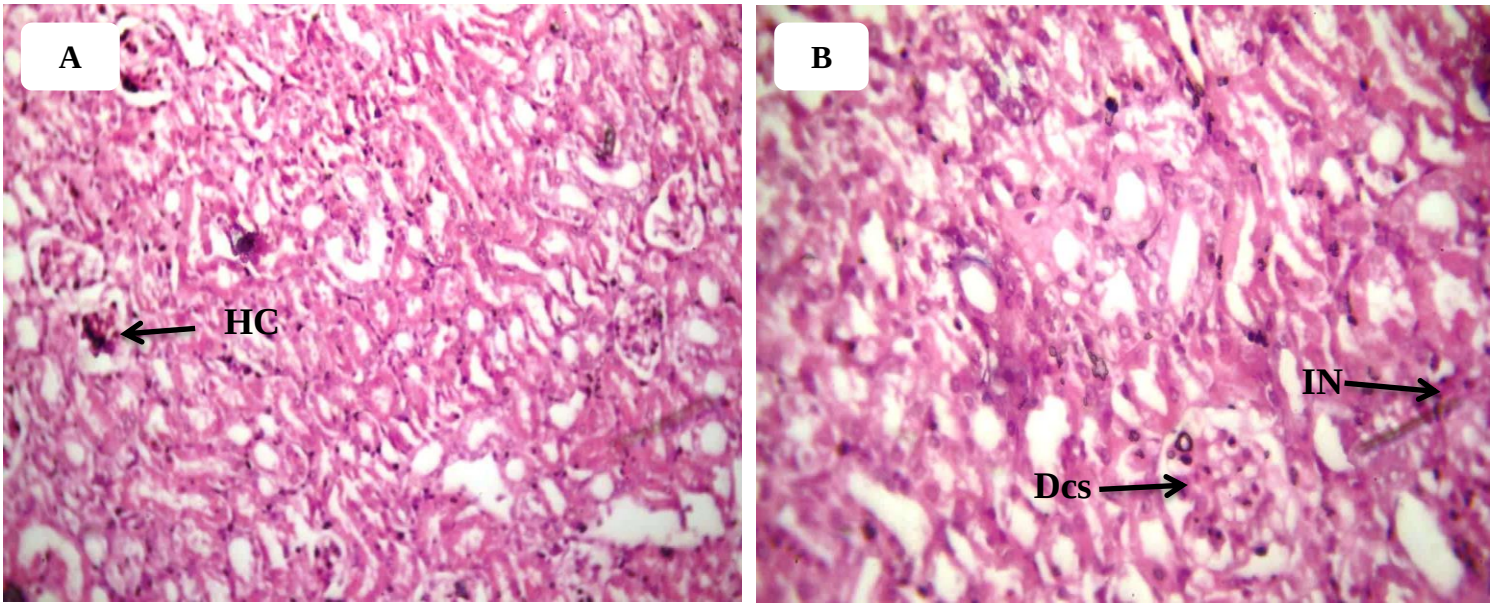

Figure (3-16): Effect of rosuvastatin supplementation on kidney section in AHF diet fed mice showed: A) hyaline cast (HC arrow), at magnification 200x. B) Decrease the capsular space of the glomerular capsule (Dcs arrow), infiltration lymphocytes (IN arrow), stained by heamatoxylin-eosin, at magnification 400x.

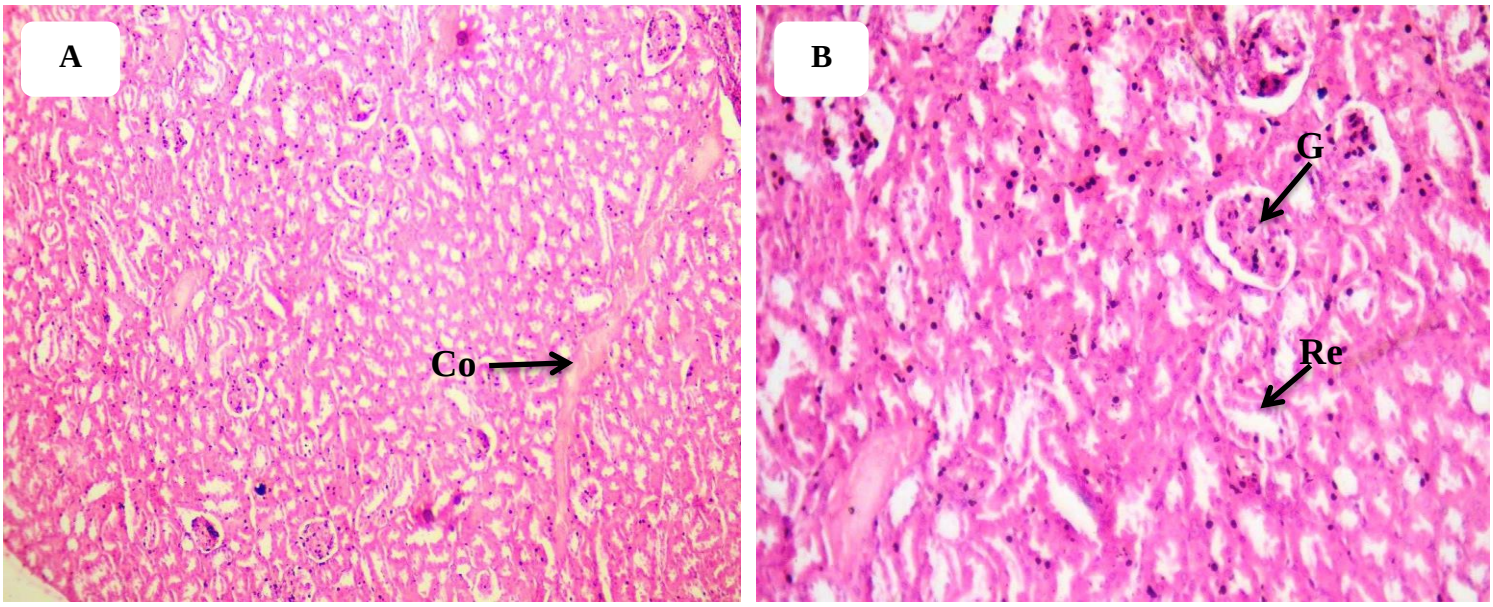

Figure (3-17): Effect of nano rosuvastatin supplementation on kidney section in AHF diet fed mice showed: A) Congestion (Co arrow), at magnification 100x. B) Normal architecture of renal tubules (Re arrow) and glomerulus (G arrow), stained by heamatoxylin-eosin, at magnification 200x.

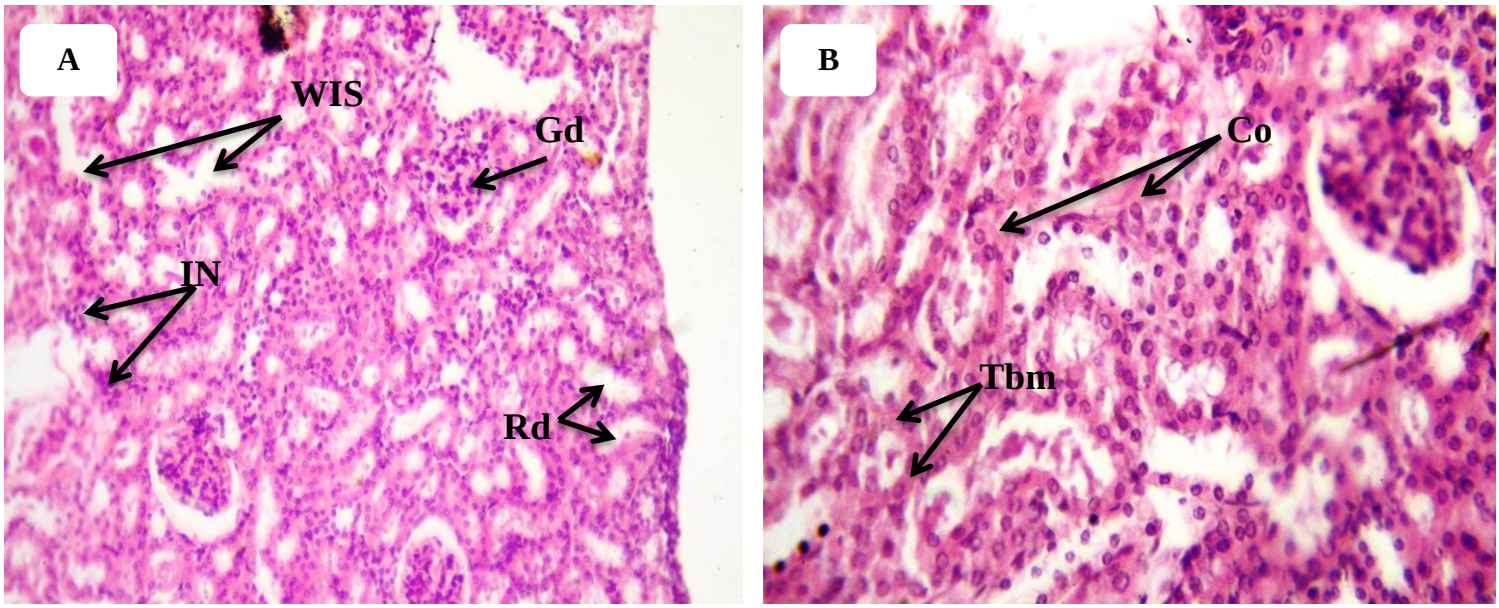

Figure (3-18): Photomicrograph of heamatoxylin-eosin stained kidney section from vegetable high fat diet fed mice showed: A) Renal tubules dilatation (Re arrow), infiltration lymphocytes (IN arrow) widened interstitial space (WIS arrow), glomerulus degeneration (Gd arrow), at magnification 200x. B) Congestion (Co arrow), thickened basement membrane (Tbm arrow), at magnification 400x.

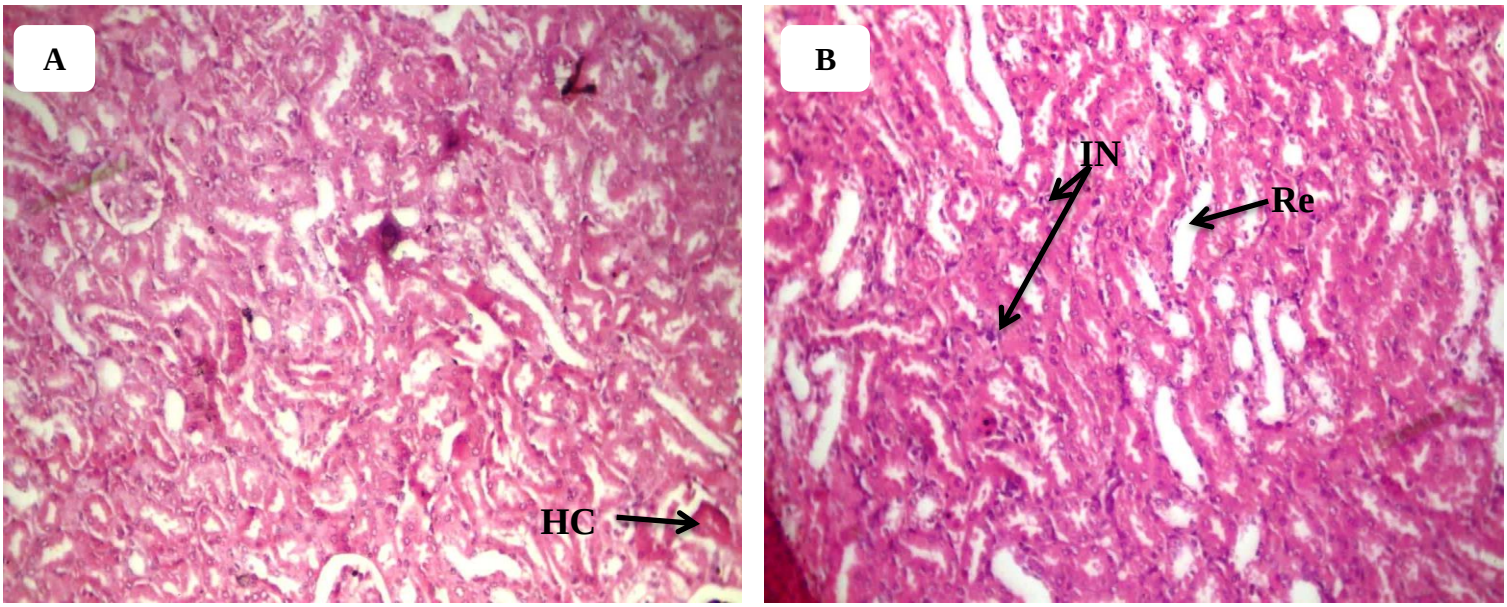

Figure (3-19): Effect of rosuvastatin supplementation on kidney section in VHF diet fed mice showed: A) hyaline cast (HC arrow), at magnification 200x. B) Infiltration lymphocytes (IN arrow), normal renal tubules (Re arrow), stained by heamatoxylin-eosin, at magnification 200x.

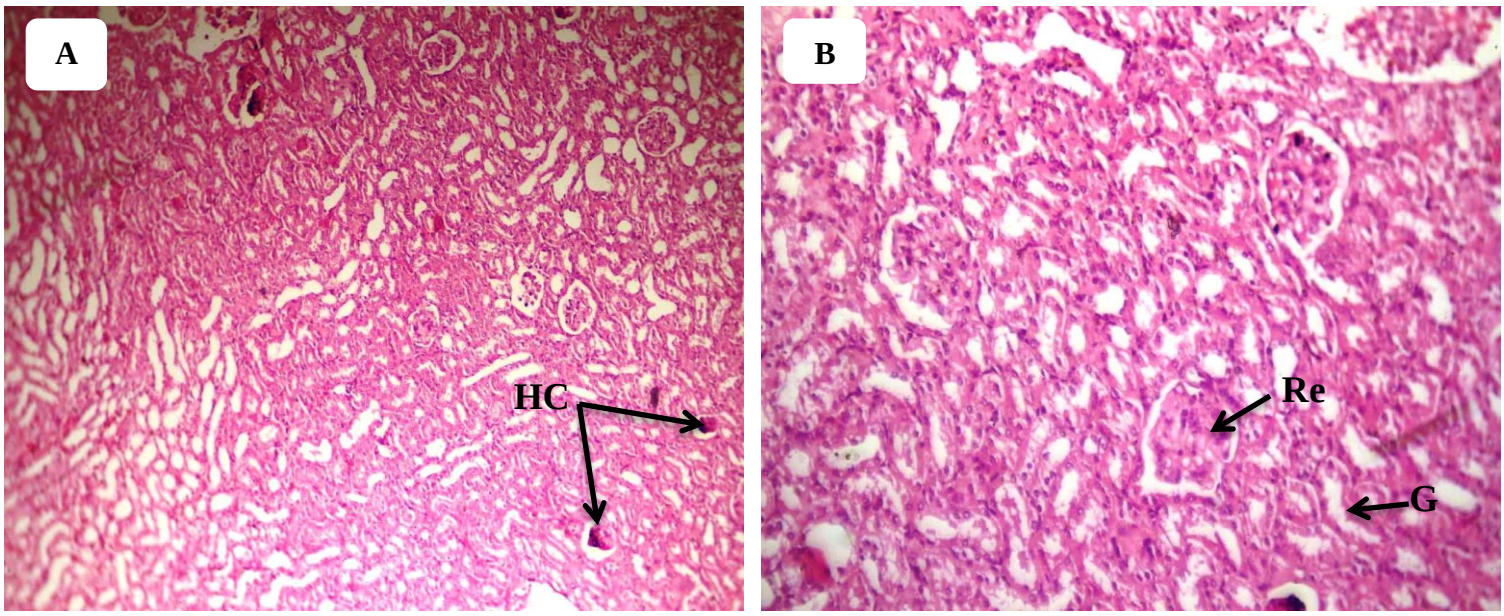

Figure (3-20): Effect of nano rosuvastatin supplementation on kidney section in VHF diet fed mice showed: A) hyaline cast (HC arrow), at magnification 100x. B) Normal architecture of renal tubules (Re arrow) and glomerulus (G arrow), stained by heamatoxylin-eosin, at magnification 200x.

The most common risk factors for chronic kidney disease (CKD) are obesity and hyperlipidemia, indicating that fat deposition in the renal parenchyma is detrimental to renal function **(**Gai *et al.*, 2019). Diet stimulate obesity is well documented as a significant risk factor for renal impairment. Severe consumption of high fat diet can cause abdominal obesity and can significantly modify the renal cortical structure of mice (Aguila & Mandarim-De-Lacerda, 2003; Armitage *et al.* 2005); degeneration of cells lined the renal tubules, as well as glomeruli, which could be contributing to lipid deposition that can directly affect the glomerular basement membrane, this slowly progressing cycle can be accelerated in case of metabolic disturbances that caused by obesity, such as inflammation or oxidative stress (Henegar *et al.*, 2001).

Dyslipidemia may also induce mesangial cell activation and proliferation, a technique similar to the smooth proliferation of muscle cells in the progression of atherosclerotic plaque (Dzau *et al.,* 2002), which is one of the essential pathological features of obesity-associated nephropathy, Extravagant, proliferation of mesangial cell can lead to glomerulosclerosis and a loss of renal function (Sun *et al.*, 2019).

Strong evidence has shown that the metabolic syndrome associated with obesity is a significant risk factor for glomerulopathy (Fang *et al.*, 2017; Yang *et al.,* 2017). Lipid metabolism disorders are generally connected with dyslipidemia and atherogenic lipoprotein glomerular accumulation, of which oxidatively alter low-density lipoprotein (ox-LDL) is a convincing contributor to glomerular mesangial proliferation, inflammation and extracellular matrix (ECM) growth, which eventually contributes to glomerulosclerosis and nephron loss (Kamanna *et al.*, 2008; Shen *et al.*, 2017).

Most research focused on the hypothesis of lipid nephrotoxicity based on the work of Moorhead *et al.,* (1982). The support this hypothesis claim that inflammation, reactive oxygen species (ROS) development and endogenous electrical stress can develop as a result of hyperlipidemia. There is evidence that accumulation of renal lipids can cause structural and functional changes in mesangial cells, products and proximal tubular cells that all participate in the function of the neuron (de Vries *et al.*, 2014). The lipid-induced toxicity also leads to the glomerulosclerosis evolution (Yang *et al.,* 2017), glomerulosclerosis induced by lipid can also result from combined stimulation of SREBP-1, transforming growth factor 1 (TGF 1), endothelial vascular growth factor (VEGF), and inflammatory pathways (Sun *et al.*, 2002; de Vries *et al.*, 2014).

CD36 is a multifunctional transmembrane glycoprotein mediating the absorption of oxidized LDL (Armesilla and Vega, 1994). CD36 considered the key mechanism of fatty acid uptake in the kidneys and its expression level significantly higher in kidney damage linked with deposition of lipids in renal; therefore CD36 appears to play a central role in the production and progression of Kidney damage (Kennedy *et al.*, 2013; Herman-Edelstein *et al.*, 2014).

HFD with chronic inflammation experienced significant renal degeneration and glomerular damage, suggesting that the obesity aggravation could itself worsen existing kidney damage, likely due to overexpression of CD36, TNF, IL-6 and monocyte chemotactic protein-1 (MCP-1) throughout inflammation; This results in thickening of the glomerular basement membrane, the extracellular matrix, and glomerulosclerosis (yang *et al.*, 2017). CD36 levels in macrophages were shown to associate with the intracellular ox-LDL levels (Febbraio *et al.*, 2000). Thus, lipid accumulation may correlate to the activation and inflammation of the mononuclear phagocyte system, which macrophage phagocytes can oxidize lipids and transform into foam cells, which in turn recruit more macrophages that aggravate lesions and lipid deposition (De Winther and Hofker, 2000). These adverse effects may be the cause of the appearance of congestion in the kidneys of hyperlipidemic mice.

High-fat diet induced substantial apoptosis in the renal tubular cells and triggered renal tubular cell injury, which could result from increased development of pro-apoptotic molecules, activation of pro-apoptotic signaling pathways and inhibition of anti-apoptotic signaling (Li *et al.*, 2015). The rise in ROS can stimulate apoptosis of renal tubular cells and kidney injury aggravates (Brezniceanu *et al.*, 2010), As well as significantly increased in the expression of Caspase-3 is an important

molecule that participates in apoptotic signal transduction which considered a key downstream enzymes in cellular apoptosis, that regulate and effect in the molecule apoptosis (Chen *et al.*, 2018).

Statins, drugs possess lipid-lowering impact that influences both total cholesterol and triglyceride (TG) levels and pleiotropic effects, including their ability to reduce inflammation and fibrosis, Statins therapy enhanced lipid deposition in proximal tubules, enhanced glomerular hypertrophy, raised nephrin expression and reduced desmin expression relative in obese rodent (Gotoh *et al.,* 2013). Because of the statins main mechanism of inhibiting HMG-CoA reductase activity, they have pleiotropic effects that include inhibition of cell proliferation and inflammation.also has been shown to enhance endothelial dysfunction and raise renal blood flow (Brosnahan *et al.,* 2017). Dyslipidemic mice treated with rosuvastatin showed improvement in the kidney tissue, presumably due to various mechanisms of oxidized-LDL reduction (Nishikido *et al.*, 2016). Chronic kidney disease is more likely to be associated with elevated levels of triglyceride and low density lipoprotein levels, which are risk factors for CVD (Parikh *et al.*, 2006), On the basis of scientific evidence; dyslipidemia is connected with tubulointerstitial and glomerular injuries, which can contribute to glomerulosclerosis.

Statins inhibit the activity of HMG-CoA reductase and thus play a beneficial role in the treatment of dyslipidemia (Satirapoj *et al.*, 2012). Rosuvastatin administration also Improvement of proinflammatory mediators, cell proliferation and decreased serum cholesterol levels all that lead to reduced lipid accumulation in the kidney (Agarwal, 2007; Gotoh *et al.,* 2013). Statins create a number of anti-inflammatory and vascular benefits that are independent of the reduction of cholesterol. Stem cells, for example, control various ischemic and degenerative

diseases and recent research has shown that statins can play a role in the modulation of stem cell functions (Romagnani *et al.*, 2007), similarly, vascular injury is characterized by impaired endothelial progenitor cell function and statin therapy can improve the regenerative capacity of the progenitor cells (Walter *et al.*, 2004).

A- Conclusions:

Study results indicate that consuming of animal and vegetable fats

1. Increase the body weight, changes the lipid profile level as a substantial rise in serum TC, TG, LDL-C, HDL-C and VLDL-C.
2. Elevate liver enzyme level AST and ALT.
3. The result showed that consuming high fat diet induces hyperlipidemia stimulation with various histopathological changes in the liver and kidney tissues.
4. Rosuvastatin treatment showed insignificant decrease in the body weight, significant decrease in lipid profile and liver enzyme level, and improvement in liver and kidney tissues.
5. Treatment with nano Rosuvastatin showed significant decrease in the body weight, lipid profile and liver enzyme level, also showed improvement in liver tissues much more effective than rosuvastatin. Treated with nano Rosuvastatin was much better than Rosuvastatin in its free form.

B-Recommendations:

1. Despite the beneficial compounds that found in the animal fat compared with vegetable fat, we recommend moderate using of animal fat, since it's basically fat so too much consume have an adverse effect on health.
2. Suggest further investigations involving a greater number of rodent and more organs like (heart, artery, spleen, skeletal muscle, adrenal glands and its hormones), also more criteria could provide reliable information on the use of animal and vegetable fats as supplements.
3. Using nano rosuvastatin in cholesterol treatment, which showed improvement in the body weight, lipid profile, liver and kidney tissues compare with rosuvastatin, and help to decrease the duration of treatment and probably rosuvastatin side effect.
4. Further investigations of some inflammatory parameters which related to dyslipidemia and rosuvastatin like (IL-1ß, IL-6, TNF-α).

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الخلاصة:

صممت هذه الدراسة لاكتشاف التغيرات الكيموحيوية والنسجية في الفئران المستهلكة لنظام غذائي غني بالدهون الحيوانية والنباتية والمسبب للسمنه, والتي عولجت بعقار الروزوفاستاتين بشكليه (الحر والنانوي) بعد استحداث السمنه لمدة شهر واحد. استخدم في هذه الدراسة 70 فأرا ابيض وزعت على سبعة مجاميع رئيسية كلا منها مكون من 10 افراد. المجموعة الأولى قدم لها غذاء اعتيادي سميت بمجموعة النظام الغذائي الاعتيادي، المجموعتان الثانية والثالثة قدم لهما غذاء احتوى نسبة عالية من المحتوى الدهني والتي بلغت 15٪ من الدهون الحيوانية والنباتية على التوالي لمدة أربعة أشهر. مجاميع الفئران المعالجة بعقار الروزوفاستاتين والنانو روزوفاستاتين 0.02 ملغم/كغم/يوم مع نظام غذائي عالي الدهون لمدة شهر واحد تضمنت اربعة مجاميع وهي:(غذاء غني بالدهون الحيوانية والروزيوفاستاتين ، غذاء غني بالدهون الحيوانية ونانو روسوفاستاتين ، غذاء غني بالدهون النباتية وروزيوفاستاتين ,غذاء غني بالدهون النباتية ونانو روزوفاستين). في نهاية التجربة والتي استمرت لمدة اربعة أشهر تم التضحية بالحيوانات واجريت اختبارات الدراسة والتي شملت حساب اوزان الجسم مره واحدة شهريا, الاختبارت الكيموحيوية والتي تضمنت حساب الكوليستيرول الكلي (TC) ,الكليسيريدات الثلاثية (TG) ,الدهون البروتينية مرتفعة الكثافة (HDL-C) ,الدهون البروتينة واطئة الكثافة (LDL-C) وواطئة الكثافة جدا(VLDL-C) , كذلك قياس مستويات انزيمات الكبد إنزيم ناقلة أمين الألانين (ALT) وإنزيم ناقلة الأسبارتات (AST)، اما الدراسة النسيجية فاشتملت على الأعضاء التالية (الكبد والكلى) لتقييم التغيرات النسيجية المرضية التي ترتبط بالسمنه وعلاج الروزوفاستاتين.

أظهرت الدراسة النتائج التالية: حدوث زيادة معنوية في وزن الجسم النهائي ($P < 0.05$) في مجموعة التحكم الموجبة (مجموعتي الدهون الحيوانية والنباتية) مقارنة بمجموعة السيطرة السلبية ، لكن الفئران التي استهلكت نظام غذائي غني بالدهون النباتية أظهرت زيادة معنوية ($P < 0.05$) في وزن الجسم مقارنتاً مع الفئران التي استهلكت نظام غذائي غني بالدهون الحيوانية. كما أظهرت النتائج زيادة معنوية ($p < 0.05$) في مستويات TC و TG و LDL-C و VLDL-C و AST و ALT وانخفاض في مستوى HDL-C في مجاميع السيطرة الإيجابية (مجموعتي الدهون الحيوانية والنباتية) مقارنة بمجموعة السيطرة السلبية, حيث ظهرت مستويات إنزيمات TC و LDL-C وانزيم ALT أعلى بشكل ملحوظ ($P < 0.05$) في المجموعة المستهلكة للدهن النباتي مقارنة بالمجموعة المستهلكة للدهن الحيواني في حين ظهرت مستويات TG, VLDL-C و انزيم AST أعلى في المجموعة المستهلكة للدهن الحيواني مقارنة بالمجموعة المستهلكة

للدهن النباتي ، كما أظهرت النتائج انخفاض معنوي ($P < 0.05$) في مستوى HDL-C في مجموعتي التحكم الموجبة المعتمدة على نظام غذائي غني بالدهون الحيوانية والنباتي مقارنتاً بمجموعة السيطرة السلبية.

أظهرت نتائج العلاج بعقار الروزوفاستاتين عدم وجود تغير معنوي في وزن الجسم في مجاميع السيطرة الموجبة مقارنتاً بمجموعة السيطرة السلبية ، في حين أظهر العلاج بالنانو روسوفاستاتين انخفاضًا في وزن الجسم في مجاميع النظام الغذائي عالي الدهون مقارنتاً بمجموعة التحكم السلبية. كما أظهرت النتائج انخفاضًا معنويًا ($p < 0.05$) في مستويات TC و TG و LDL-C و VLDL-C و AST و ALT للفئران التي عولجت بعقار الروزوفاستاتين والنانو روزوفاستاتين في جميع مجاميع العلاج مقارنتاً بمجاميع السيطرة الإيجابية (مجموعتي المستهلكة لنظام غذائي غني بالدهون الحيوانية والنباتية) وزيادة معنوية ($p < 0.05$) في مستوى HDL-C, لكن العلاج بالنانو روزوفاستاتين أظهر انخفاضًا معنويا ($p < 0.05$) أكبر بكثير في مستويات الدهون وانزيمات الكبد مقارنتاً مع روزوفاستاتين.

أظهرت المقاطع النسيجية لكبد الفئران التي استهلكت نظام غذائي عالي الدهون (الدهون الحيوانية والنباتية) مجموعة من التغيرات المتمثلة في، ارتشاحًا خفيفًا إلى شديدًا في الخلايا الليمفاوية ، وتراكم شديد للدهون مثل تنكس دهني حويصلي كبير وصغير ، وتنخر و موت الخلايا. حيث كانت هذه التغييرات أكثر حدة في مقاطع الكبد للفئران التي استهلكت غذاء يحوي على نسبة عالية من الدهون الحيوانية مقارنةً بمقاطع الكبد للفئران التي استهلكت نظامًا غذائيًا غنيًا بالدهون النباتية. فيما وجدت تغيرات مرضية نسجية الا انها بدرجة اقل حده في مقاطع الكبد للفئران المعالجة بالروسوفاستاتين ، وشملت هذه التغييرات: ارتشاح بسيط للخلايا الليمفاوية ، تنكز وموت للخلايا، كما أظهر علاج روسوفاستاتين تحسن في التنكس الدهني ، مقارنة مع مقاطع الكبد لمجاميع السيطرة الموجبة,كما اظهرت مقاطع الكبد المعالجة بالنانو روزوفاستاتين تحسنًا أكبر مقارنةً بمقاطع الكبد المعالج بالروزوفاستاتين. أظهرت مقاطع الكلى لمجاميع السيطره الإيجابية تغيرات نسيجية مختلفة مقارنة بمجموعة السيطره السلبية ، وتشمل هذه التغييرات: توسع النبيبات الكلوية ,تنكس وتنخر في الأنابيب الكلوية المبطنة بالخلايا , احتقان الأوعية الدموية الغزير مع سماكة الغشاء القاعدي , ارتشاح شديد للخلايا اللمفية تمدد في الأنابيب الكلوية واتساع المساحة الخلالية. أظهرت مقاطع الكلى المعالجة بالروزوفاستاتين والنانو روزوفاستاتين تغيرات طفيفة تشمل: احتقان دموي ، ارتشاح خفيف للخلايا الليمفاوية ، تنخر ووجود البروتين في البعض منها ، في حين أظهرت مقاطع الكلى المعالجة بالنانو روزوفاستاتين التركيب الطبيعي للكلى مثل ، الكبيبات الطبيعية ، مساحة المحفظة الطبيعية و الأنابيب الكلوية الطبيعية.

جمهورية العراق
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تحديد بعض التغييرات الكيموحيوية والمرضية النسيجية للكبد و الكلية المستحثه السمنه بالتغذيه في ذكور الفئران البيضاء المعالجه بالروزوفاستاتين

رسالة مقدمة الى

مجلس كلية العلوم/جامعة كربلاء

وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل

زهراء سعد حسين الخفاجي

بكلوريوس علوم الحياة/ جامعة كربلاء/2011

اشراف

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