A Comparative Study between Rosuvastatin and Nano-Rosuvastatin Effects on Liver in Mice that Consuming Diets Rich in Vegetable Oil

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Abstract

The study was designed to evaluate the histological changes occurring in mice liver subjected to a high-fat diet for 4 months, and to assess the effectiveness of Rosuvastatin drug in its two forms (free and nano) in alleviating these changes. Consumption of a high-fat diet (vegetable oil) resulted in mild to severe lymphocytes infiltration, sever lipid accumulation as macro and microvesicularsteatosis, necrosis, pyknotic nuclei. The histology of liver sectionsobtained from mice treated with rosuvastatin showed lower severity in comparing with positive control mice. Histological changes include infiltrated lymphocytes, pyknotic nuclei, apoptotic cells. Furthermore,Liver sections from the Rosuvastatin treatment group showed ameliorated steatosis when comparedto a high-fat diet treated group. Liver sectionsof thenanoRosuvastatintreated group showed much more improvement comparedtoRosuvastatin treated group.

Keywords: *High-fat diet (HFD), Mice, Rosuvastatin (ROS), Liver*

Introduction

A high-fat diet (HFD) is recognized as a critical factor that contributes to the epidemic of obesity[1]. Medically, obesity is described as a state of increase in body weight, more precisely of increased fat tissue mass. Several various variables influence 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[2]. Obesity can cause hyperinsulinemia, hyperglycemia, fat deposition, as well as resistance to insulin in the liver, all this, in turn, may affect normal liver function, ranging from raising the circulating liver enzyme levels and steatosis to local inflammation (steatohepatitis), cirrhosis, liver failure and even liver cancer[3,4]. Non-alcoholic fatty liver disease (NAFLD) seems to be the most prevalent cause of chronic liver disease, ranging from steatosis (lipid deposition in the liver particularly triglyceride) and non-alcoholic steatohepatitis (NASH) to cirrhosis (fibrosis) and hepatic failure[5].Rosuvastatin acts in the liver by the reversible competitive inhibition to the HMG-CoA reductase enzyme, the first and key rate-limiting enzyme of the cholesterol biosynthetic pathway (mevalonate pathway) by imitating 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; Rosuvastatin decreases plasma cholesterol levels by inhibiting cholesterol biosynthesis in the liver. As a result, the gene expression of the LDL receptor is increased[6]. Rosuvastatinis a poor water-soluble drug with oral bioavailability by only 20 per cent and this impacts its solubility rate and, in turn, its bioavailability[7]. Therefore, improving the dissolution of Rosuvastatin can lead to an improvement in its oral bioavailability[8].The principle of the nanotechnology field includes the 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[9].

Material and Method

Animals and groups: The currentexperiment was carried out on a total of 40 male albino mice, weighing about 25 to 30g each. The animals were purchased from the animal care centre of the Iraqi centre for cancer research, their ages ranged from 3.5 to 4 months. Animals were housed in controlled temperature conditions (25±3C°) and light-dark cycles for 12 hours. Mice were acclimatized for two weeks and had access to drinking water and a standard chow diet. During the experiment, animals were caged in large polypropylene cages. Mice were randomly divided into four major groups of ten (10) animals each. Group A (negative control) animal remained on stander chow (SC), group B (positive control) animals were fedahigh-fat diet (HFD) rich with vegetable fat, group C mice treated by rosuvastatin along with a high-fat diet (HFD- ROS), group D mice treated by nano-rosuvastatin along with high-fat diet(HFD-N.ROS). The composition of the typical diet used in this study is shown in table 1.

preparation of vegetable fat-rich diet: diet was prepared by adding 850g of powdered mice chow diet, to 150g vegetable fat to obtain homogeneous soft cake. The vegetable fat-rich diet preparation was modelled as a pellet of about 5g eachaccording to the method of [10,11]with some modifications

Rosuvastatin Solution Preparation: The rosuvastatin dose was taken 10mg as normal human adult dose, which was 0.02 mg/kg b.w. as mice dose according to the conversion chart[12] .Rosuvastatin of 10mg (AstraZeneca, UK) was crushed into a powder, then dissolved in distilled water to prepare rosuvastatin solution for oral administration.

Nanoparticle drug Solution Preparation: The hybrid nanoparticle solution was prepared by following the method described by kolekar[39]with some modification, at the end the hybrid drug was identified by using three methods, (FT-IR), (XRD) and (AFM).

Histological processing and staining: Ordinary histological processing was prepared for the liver to study the changes that may be found in animal groups as compared with the animals of the negative control group, processing and staining techniques were done[13].

Table 1: The percentages of standard chow and high-fat diet[10,11]

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

Results and Discussion

FT-IR spectrum of rosuvastatin (ROS): 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. As for the C-F band, it appeared at thefrequency 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[38].

FT-IR spectrum of rosuvastatinnanocomposite hybrid: 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**.** As for the vibrations of the C-F belt, it appeared at the frequency 1275cm^{-1}, thesecond 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[38] at 455 cm^{-1},as shown in figure 1.

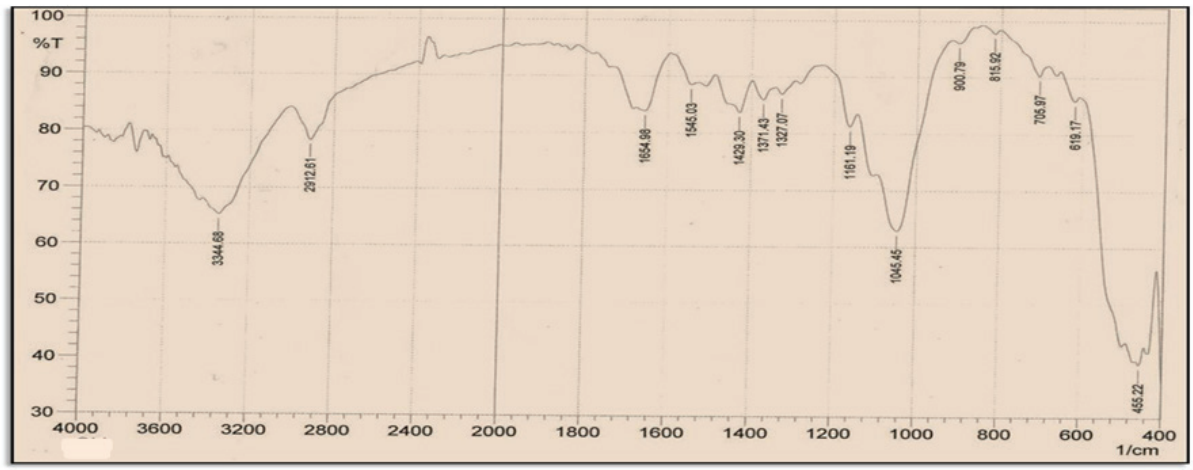

Figure 1: Infrared spectrum of ROS-ZnO

X-ray diffraction spectrum (XRD): The X-ray diffraction spectrum of both rosuvastatin nanoparticles ROS/ZnO and zinc oxide layer 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 (2) 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[37]. 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 rosuvastatincompound 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).

Examination with atomic force microscopy (AFM): As shown in Table (2), 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). The current result is closer to the result by[14],who found that the diameters of the prepared rosuvastatin hybrid nanocomposite were 67.21 nm. While a study by Li[15]mentioned that the average nanoparticle diameter of nanoparticle rosuvastatin was 98.4 nm.

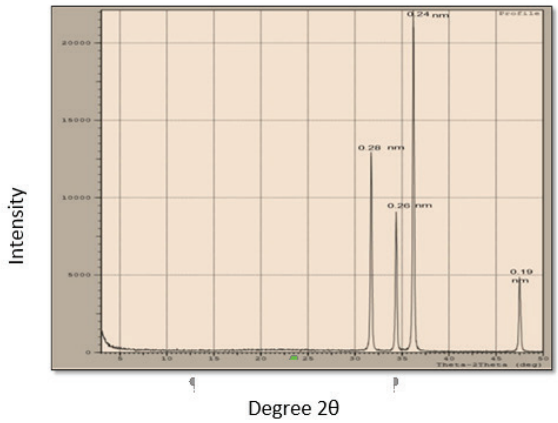

Figure 2: (XRD) of zinc oxide layers

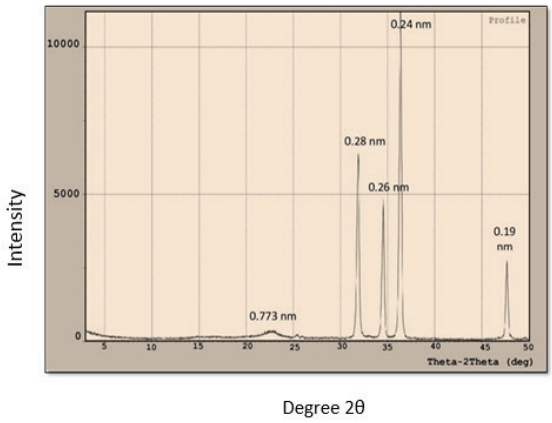

Figure 3: (XRD) of ROS-ZnO

Table (2): Diameters, sizes and particles of the (ROS-ZnO) hybrid nanocomposite after examining it with atomic force microscopy

Diameter r (nm)<	Volum e (%)	Cumulation (%)	Diamete r (nm)<	Volum e (%)	Cumulation %)	Diamete r (nm)<	Volum e (%)	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:73.50nm <= 50% Diameter:70.00nm					<= 10% Diameter: 0nm <=90% Diameter: 85.00nm			

The transforming of 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[16], make the drug more effective than the free form that showed improvement in solubility and oral bioavailability by overcoming the hepatic first-pass metabolism[17].

Histology of liver:Examination of livers sections obtained from mice consuming a high-fat diet (vegetable oil) showed, mild to severe lymphocytes infiltration; sever lipid

accumulation as macro and microvesicularsteatosis, necrosis, pyknotic nuclei as shown in (figure 4-B) While, the examination of the Eosin- hematoxylin stained section of the livers obtained from mice that received 10mg of rosuvastatinrevealed little infiltrate

lymphocytes, pyknotic nuclei, apoptotic cells.Also,the Rosuvastatin treatedgroup showed ameliorated steatosis, compare with the high-fat diet treated group as shown in figures (4-C1) and (4-C2). Figures (4-D1) and (4-D2) showed liver sections obtained from mice treated with nanoRosuvastatin showed much more improvement compare with the liver sections treated with Rosuvastatin

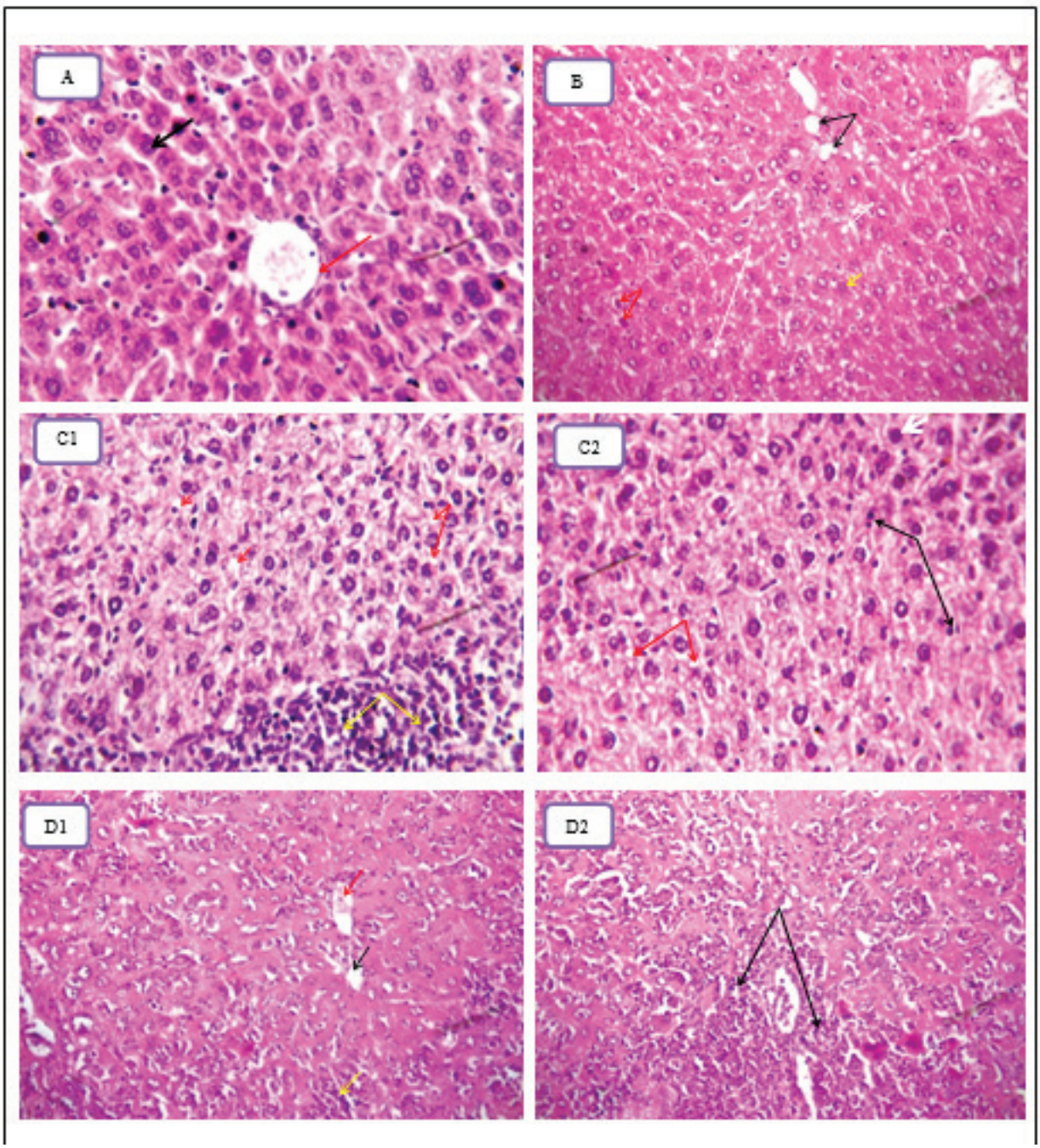

Figure 4:A:SC liver section showing, portal vein (red arrow), hepatocyte (black arrow),400x.B:HFD liver section showing, apoptotic cells (yellow arrow), macrovascularsteatosis (black arrow), microvascularsteatosis (white arrow), pyknotic nuclei (red arrow), 200x. C1:HFD-ROS liver section showing,high lymphocytes inflammatory cells (yellow arrow),microvascularsteatosis (red arrow), 400x. C2:HFD-ROS liver section showing, lipid deposition (red arrow), pyknotic nuclei (black arrow), apoptotic cell (white arrow), 400x.D1:HFD-N.ROSliver section showing, portal vein (red arrow), bile duct (black arrow), lymphocytes inflammatory cells (yellow arrow), 200x. D2:HFD-N.ROS liver section showing, lymphocytes inflammatory cells (black arrow), 200x. All Livers section stained by hematoxylin-eosin (H&E).

The fat accumulation in the liver is not completely understood, but it was suggested that when hepatic fatty acid availability surpasses the ability for elimination then they are stored as TG in the liver[18]. TG storage results in net fat retention which is a precondition for NAFLD progress[19]. There are many mechanisms by which fat can begin to accumulate in the liver, these include the increased delivery of fatty acids to the liver from adipose tissue lipolysis and elevated transmission of dietary fatty acids to the liver[20].

A study by Pan[21] explains that mice who consumed a high-fat diet for 12 weeks have developed fatty liver phenotypes, diabetes, accumulation of triglyceride in hepatocytes and an increase in total serum cholesterol. The livers of over 70 percent of these mice showed dispersed inflammatory zones of mononuclear cells with hepatocyte inflating, hepatocyte necrosis and increased lipid droplet. Another study by Hassan[22] , mentioned that feeding mice diet rich with fats for 6 weeks caused a various histological change in the 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 outcome of their experiments is in parallel with the result obtained by feeding mice with a high-fat diet for 4 months in the present study. The most acute variations noted in mice receiving the high-fat diet control group were huge numbers in the liver tissue of micro-vesicular steatosis and fibrosis. Results from the present study are in agreement with previous studies by VanSaun[23] and Hassan[22], which revealedthat feeding with a rich fat diet, 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[5]. One of the main mechanisms leading 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.The origin of elevated hepatic triglyceride level is 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 oxidation of the fatty acids[24] .Another principal mechanism demonstrated the hepatocellular injury[25]; 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 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 it is associated with NASH[26]. The oxidized LDL is in much competition with the surrounding tissues, which can cause inflammation leading to disease and damage to the body organ[27]. Another significant side through the pathogenesis of inflammation is apoptotic cell death, which has proven to play a significant role in NASH[28],it was found that OxLDL increases apoptosis by triggering apoptotic signalling cascades including the Fas signalling pathway[29]. Rosuvastatin has been reported to reduce the activity of hepatic lipase, which plays an important role in triglyceride level regulation in the blood by maintaining steady levels of HDL and LDL[30]. Another potential mechanism by which rosuvastatin raise HDL-C is the inhibition of cholesterol ester transfer protein (CETP), the increase in HDL level will promote and increase the clearance free cholesterol[31],The decrease in plasma LDL quantities was 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.Rosuvastatin 10mg/kg/day administration enhanced insulin sensitivity, diminishes liver steatosis and body weight, and enhanced circulating cholesterol and triglyceride levels in mice fed a high-fat diet[32]. Rosuvastatin decreased steatosis, presumably due to decreased FFAs input and increased FFAs output due to increased beta-oxidation[33]. Our main result is in agreement with Neto-Ferreira[34] and

Fraulob[32] , where they reported that administrated mice with 10mg/kg/day rosuvastatinalong with a high-fat diet for 5 weeks improve hepatic steatosis (microvesicular). This result is in agreement with Argo[35] and Hyogo[36] ,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 of statins in the improvement of serum aminotransferase levels.

Conclusions

The present study demonstratedthat consuming a high-fat diet induces hyperlipidemia stimulation with various histopathological changes in the liver tissues. Rosuvastatin treatment showed improvement in liver tissues; convert Rosuvastatin into nano form showed improve in solubility and oral bioavailability by overcoming the hepatic first-pass metabolism and which lead to makingthe drug more effective than the free form.

Conflict of Interest: None

Funding Source: Self

Ethical Clearance: the research approved by scientific and ethical committee at our department.

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