

Effect Of Policosanol Extract And Simvastatin On Some Liver Enzymes And Histopathology In Hypercholestrolemic Female Rats During Lactation

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Abstract

Hypercholesterolemia is usually known as the presence of high levels of cholesterol in the blood. Cholesterol is an amphipathic lipid and is naturally existed in the tissues and the plasma. In the plasma, it is carried in lipoproteins. These lipoproteins are High Density Lipoprotein-Cholesterol (HDL-C), Low Density Lipoproteins- Cholesterol (LDL-C), Very Low Density Lipoprotein- Cholesterol (VLDL), and chylomicrons. The last three groups are closely related with the hazard of coronary heart disease (CHD), whereas (HDL-C) is not. Policosanol is a mixture produces from extraction of sugar cane wax (*Saccharum officinarum*), this mixture shows cholesterol lowering and antiplatelet effects, and is being used as hypocholesterolemic drug.

Key Words: Rat-hypercholestrolemic, Policosanol- Simvastatine- AST, ALT, ALP & GGT, liver histopathology.

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الخلاصة

تعرف حالة فرط الكوليستيرول عادةً بأنها ارتفاع في نسبة كوليستيرول الدم , والكوليستيرول هو من الدهون الثنائية الجانب توجد بصورة طبيعية في الانسجة والبلازما. في البلازما يحمل الكوليستيرول بواسطة بروتينات دهنية, وهي بروتينات دهنية عالية الكثافة, بروتينات دهنية منخفضة الكثافة, بروتينات دهنية منخفضة الكثافة جداً و الشايلومايرون. ترتبط الانواع الثلاثة الاخيرة بأمراض القلب وتصلب الشرايين بينما النوع الاول ليس كذلك. يعرف البوليكوسانول بأنه مادة تنتج من استخلاص شمع قصب السكر من نوع (Saccharumofficinarum), هذه المادة لها خواص مخفضة للكوليستيرول ومضاد لتخثر الدم وقد استعملت كعلاج مخفض للكوليستيرول. صممت هذه الدراسة لإظهار تأثير مستخلص البوليكوسانول في بعض أنزيمات أكباداناث الجرذان المختبرية من نوع (Ratusnorvigicus) وكذلك التغيرات النسيجية الحاصلة في تلك الأكباد, في فترة الرضاعة. ثمانية واربعون انثى جردبأعمار تراوحت بين (١٠-١٢) اسبوعاً بأوزان تراوحت ما بين (٢٥٠-٢٠٠) غرام , قد استعملت في هذه الدراسة. بعد عمل مسحات مهبلية يومية لثلاث دورات شبقية متتالية خضعت هذه الجرذان للتزاوج بذكور بالغين وخصبين بعدد ٣٢ ذكر بأوزان تراوحت ما بين (٢٨٠-٣٥٠) غرام توزعت بمعدل ذكر واحد لكل ٢ من الاناث. بعد التأكد من حمل هذه الجرذان خضعت فقط ٢٤ من الاناث لإستحداث فرط الكوليستيرول في الدم خلال فترة الحمل والباقي تركن بدون استحداث .

Introduction

Hypercholesterolemia is usually known as the presence of high levels of cholesterol in the blood. Cholesterol is an amphipathic lipid and is naturally existed in the tissues and the plasma (Frederick, et al., 2010). In the plasma, it is carried in lipoproteins. These lipoproteins are divided into four important groups, they are High Density Lipoprotein-Cholesterol (HDL-C), Low Density Lipoproteins- Cholesterol (LDL-C), Very Low Density Lipoprotein- Cholesterol (VLDL), and chylomicrons. The last three groups are closely related with the hazard of coronary heart disease (CHD), whereas (HDL-C) is not (Frederick, et al., 2010). There are two different cases of increasing levels of lipoprotein groups in the blood they are hypercholesterolemia and hyperlipidemia. Policosanol is a mixture produces from extraction of sugar cane wax (*Saccharum officinarum*), this mixture shows cholesterol lowering and antiplatelet effects, and is being used as hypocholesterolemic drug. Statins are the inhibitors of hydroxymethylglutaryl coenzyme A (HMG CoA) reductase. They are mostly used to treat hyperlipidaemia (Winterfeld, et al. 2012). Statins that currently carried for clinical use include Atorvastatin, Fluvastatin, Lovastatin, Pravastatin and Simvastatin. These medications reduce the concentration of cholesterol intracellularly and cause increase the activity of (LDL-C) receptors that enhance the uptake and catabolism of LDL-C (Akiko, et al., 2003). Many patients tolerate Statins in general, however they produce remarkable adverse effects particularly on skeletal muscle (Camerino, et al., 2011). Liver enzymes are commonly referred to the liver function test and they usually reflect hepatic integrity or cholestasis rather than liver function (Edoardo et al., 2005). Constant elevations in hepatic transaminase AST occur by using Simvastatin, as liver enzyme aberrations (Merck and Dohme, 2010). Isolation and purification of sugar cane wax (*Saccharum officinarum*) produces a mixture of higher aliphatic alcohols called Policosanol. This mixture shows cholesterol lowering and antiplatelet effects and is being used as Policosanol and Simvastatin on some blood enzymes of the hypercholesterolemic lactating female rats.

Materials and Methods

The present study was carried out at the College of Veterinary Medicine, University of Basrah, The experiments in the present study were performed on forty eight adult females rat with (10-12) weeks old, weighting (200-250 gm) and twenty four healthy adult fertile males weighting (280-350 gm), which were used for this study. They were maintained in animal house 3 weeks for adaptation before the beginning of the experiments. Animals were housed in plastic cages with metal covers, containing bedding materials of fine wood which was kept dry and changed twice weekly.

The animals were maintained under controlled optimum conditions light dark cycle (12/12) hours, at a temperature ($25\pm 4^{\circ}\text{C}$). The diet was offered ad Libitum, and presented with tap water. Daily vaginal smear examination are made for the females for four consequences estrous cycles as described by Marcondes et al., (2002) to establish their normal pattern of cyclical activity. Sugar cane plant (*Saccharum officinarum*) were collected from Mesan province, peels were manually scrapped and dried under sunlight and stored in air tight container. Crude sugarcane wax extracted from supercritical CO_2 technique was further purified by using polar aprotic solvent acetone. The resulting solid (wax) and supernatant was subjected to determine the purities and the content of policosanols by gas chromatography– mass spectrometry (GC/MS). (Sirin, et al., 2016). At day one of pregnancy hypercholesterolemia was induced in 32 female by administration of cholesterol at dose 2.5 ml /kg (1%) daily dissolved in coconut oil given by oral gavages was continued until birth (Swati, et al., 2014). The remaining 16 females were given only coconut oil in quantity equal to that is given to hypercholesterolemic animals and for the same period. Immediately after birth the animals were divided into the following 6 groups:

Group 1 (n=8) (Control) : Normal rats were given only 0.5 ml/ animal of Dimethyl Sulphoxide (DMSO) daily without any treatment immediately after birth.

Group 2 (n=8) hyperchol. (HC): Hypercholesterolemic rats left without treatment, and were given only DMSO 0.5 ml/ animal daily immediately after birth.

Group 3 (n=8) (HC+SM): Hypercholesterolemic rats were given simvastatin at dose 20mg/kg BW/ day dissolved in DMSO orally by gavages' immediately after birth.

Group 4 (n=8) (SM) : Normal rats were given only simvastatin at dose 20mg/kg BW/ day dissolved in DMSO orally by gavages' immediately after birth

Group 5 (n= 8) (HC+ST. pol): Hypercholesterolemic rats were given standard policosanols at dose 20mg/kg BW/day orally by gavages' immediately after birth. (Noa, et al., 2003).

Group 6 (n=8) (HC+EX. pol): Hypercholesterolemic rats were given policanol extraction at dose 20mg/kg BW/day orally by gavages' immediately after birth. The treatment continued until weaning (30 days old), then all animals were sacrificed and blood samples were collected and serum were separated to study the liver enzymes as well as the livers were removed, and fixed in formalin 10% for histopathological examination..

Serum aspartate aminotransferase (AST) measurement (U/I). Aspartate aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine (Schumann & Klauke, 2003). By using AST kit from RANDOX/AST-PAP, UK.

Serum alanine aminotransferase (ALT) measurement (U/I).

Alanine aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl-hydrazine (Schumann & Klauke, 2003). By using ALT kit from RANDOX/AST-PAP, UK.

Serum alkaline phosphatase (ALP) measurement (U/I).

Serum Alkaline phosphatase is determined by using a special kit (BIOLABO SAS, ALP- Kit, France) according to (Tietz, 2006).

Serum Gamma glutamyltransferase (GGT) estimation (U/I).

Serum Gamma glutamyltransferase is determined by using a special kit from Erba Mannheim /GGT, United Kingdom. Kinetic colorimetric method according to Burtis, et al., (2012).

At the end of the experiment the animals were sacrificed and the livers were carefully removed and cleaned by normal saline and then fixed in 10 % buffered formalin for 24 hrs. Dehydrate the specimens by using graded series of ethanol and cleared in two changes of xylene and then embedded in paraffin wax. The thickness of section 5 μ m were cut by using a rotary microtome and then mounted on clean slides to histological examination after stained the sections by Haematoxylin and eosin (H&E) and evaluate the tissue structure under a light microscope (Mescher, 2010).

Statistical Analysis:

One-way ANOVA-test was used to determine the significant difference between subgroups. Differences between data were compared by least significant difference (LSD). All data were expressed as Mean $\pm$ Standard deviation. All statistical tests were done by using statistical program SPSS(21.0) the level significant set on $p \leq 0.05$ (Bryman and Cramer, 2012).

Results

The results as illustrated in table (1) revealed that levels of (AST) in (HC), (HC+SM), and (SM) treated groups were significantly ($P \leq 0.05$) increased as compared with control group. While no significant differences were observed in AST level in (SM), (HC+ST.pol) and (HC+EX.pol) treated groups compared with control group. ALT enzyme levels in (HC+SM) and (SM) treated groups were increased significantly ($P \leq 0.05$) compared with control group. No significant differences were recorded in ALT levels in (HC) and (HC+EX.pol) treated groups compared with control group. On the other hand the low significant ($P \leq 0.05$) ALT value was recorded in (HC+ST.pol) treated group as compared to control. Serum ALP levels decreased significantly ($P \leq 0.05$) in (HC) group compared

with control group. Moreover no significant differences were recorded in ALP levels in all other treated groups compared with control group. A significant increase ($P \leq 0.05$) in GGT levels was recorded in (HC) group compared with control group. However no significant differences in serum GGT levels were observed between (SM) and (HC) groups compared with control. Finally a significant reduction ($P \leq 0.05$) in serum GGT levels was recorded in (SM) and (HC+ ST. pol) groups compared with (HC) group, but still significantly higher ($P \leq 0.05$) than those of control group, GGT level in (HC+ EX. pol) treated group was not differ from those in control group.

Table (1):Effect of Policosanol treatment during lactation on liver enzymes: (AST, ALT, ALP, and GGT), in hypercholesterolemic female rats Mean $\pm$ SD, n= 6.

Par.s Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)
First group Cont.	55.00 $\pm$ 7.45 c	19.50 $\pm$ 1.87 c	222.00 $\pm$ 43.80a	0.53 $\pm$ 0.23 c
Second group HC	66.83 $\pm$ 10.15ab	18.66 $\pm$ 1.63 c	168.83 $\pm$ 40.63b	1.29 $\pm$ 0.19 a
Third group HC+SM	75.66 $\pm$ 6.86 a	28.83 $\pm$ 3.31 a	196.83 $\pm$ 26.51ab	0.62 $\pm$ 0.20 c
Fourth group SM	64.00 $\pm$ 11.82b	24.00 $\pm$ 4.14 b	220.00 $\pm$ 33.72a	0.96 $\pm$ 0.08 b
Fifth group HC+ST. pol	52.50 $\pm$ 9.22 c	10.16 $\pm$ 2.78 d	125.50 $\pm$ 13.33a	1.01 $\pm$ 0.12b
Sixth group HC+EX. pol	53.50 $\pm$ 8.57 c	19.00 $\pm$ 2.60 c	120.66 $\pm$ 18.73a	0.83 $\pm$ 0.19bc
LSD	10.79	3.45	37.09	0.21

Different letters refer to the significant differences at $P \leq 0.05$.

Histopathological examination of liver rats of control group showed normal hepatocytes radiated as hepatic plates from the normal central vein ; also the sinusoids empty into the central vein as shown in figure (1). While histopathological examination of liver of hypercholesterolemic (HC) group without treatment- showed absence of the normal radiation of hepatocytes as shown in fig. (2) in addition of

enlargement of nuclei of large number of hepatic cells with pyknosis and absence of nuclei of other number of hepatocytes. Also there is enlargement of the sinusoids this mean the hepatic tissue undergoing toward a degenerative changes ,the central vein is enlarged and congested. Liver of (HC+SM) treated group as in figure (3) showed sever congestion of the central vein , still there are vacuolated hepatocytes of less pyknosis nuclei with mild sinusoidal enlargement. While the section through liver of non-hypercholesterolemic (SM) treated group as shown in fig.(4) revealed better hepatocytes confirmation than hypercholesterolemic group with less vacuoles, better sinusoidal spaces, less pyknosis of hepatic nuclei, but still there is moderate central vein congestion. Section of liver of dams of (HC+ST. pol.) treated group as shown in fig. (5) showed moderate ameliorative changes represented by beginning of radiating hepatocytes architecture formation around the clear not congested central vein, there was less vacuolation; and normal sinusoidal spaces. Furthermore the histopathological examination of liver of (HC+EX.pol) treated group fig. (6) showed clear hepatocytes parenchymal ameliorative structure formation in spite of some cells had lost their nuclei and boundaries and fused with other cells. The central vein still congested , several hepatic cells nuclei are still pyknotic ,but sinusoidal spaces are normal.

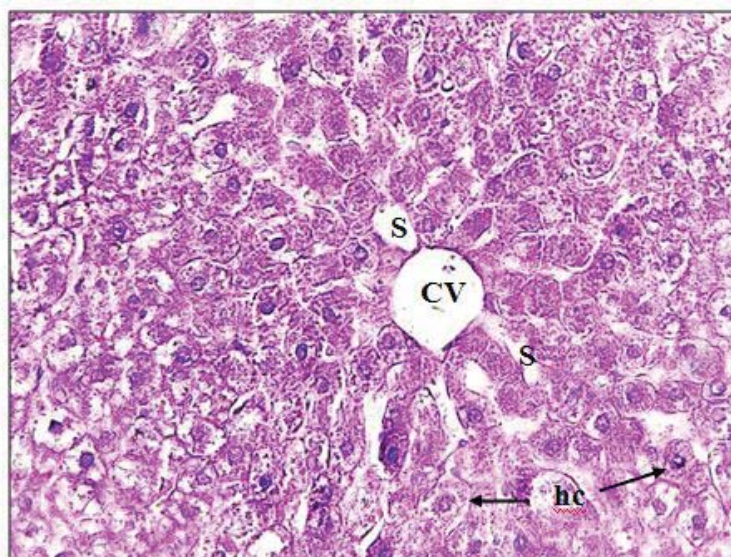


Fig. (1): Liver of control (Cont.) group showing normal hepatocytes (hc), radiated as hepatic plates from the normal central vein; also the sinusoids (S) emptied into the central vein (CV). (H& E stain 400X).

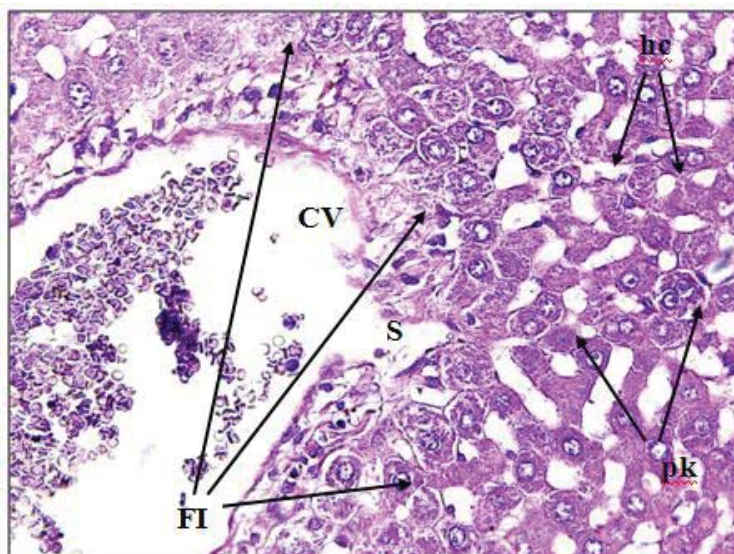


Fig. (2): Liver of (HC) group showing absence of the normal radiation of hepatocytes (hc), fatty infiltration (FI), enlargement of nuclei of large number of hepatic cells (pk) with pyknosis and absence of nuclei of other number of hepatocytes, enlargement of the sinusoids. The central vein is enlarged and congested. (H & E stain) 400 X.

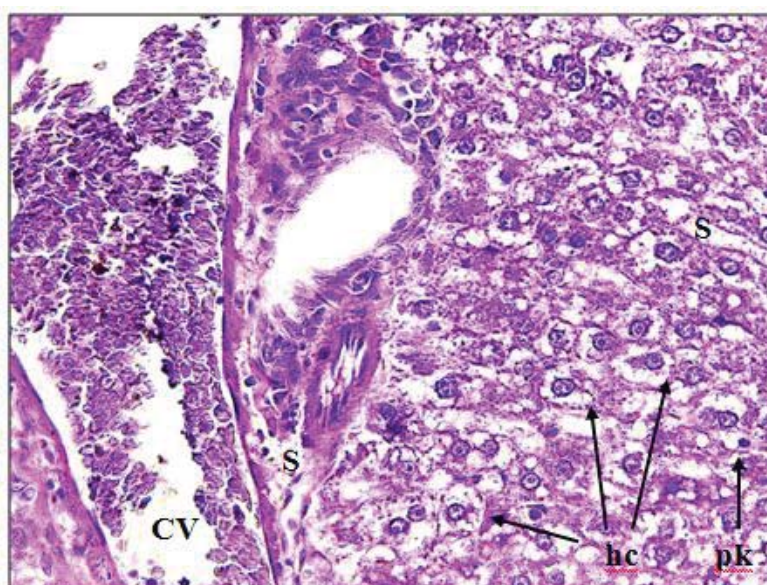


Fig. (3): Liver of (HC+SM) treated group showing sever congestion of the central vein (CV), still there are vacuolated hepatocytes of less pyknosis nuclei with mild sinusoidal enlargement (S). (H & E stain) 400 X.

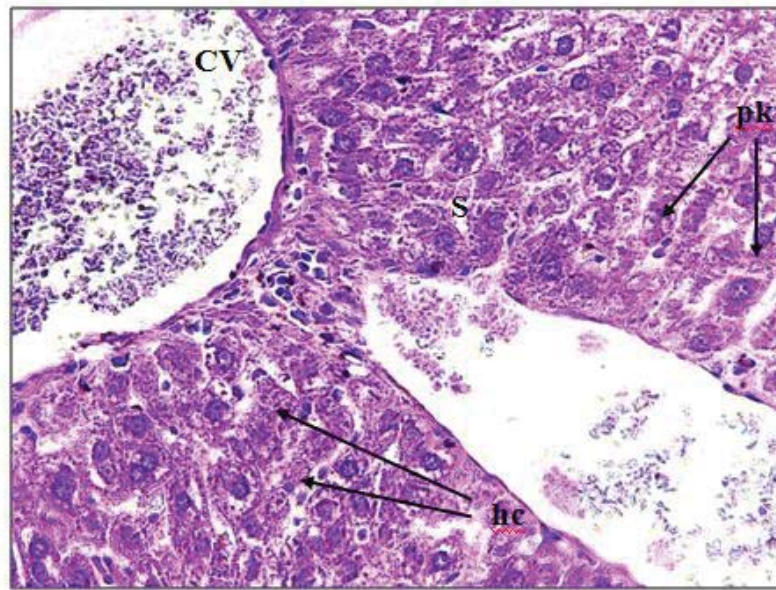


Fig.(4): Liver of (SM) treated group showing better hepatocytes (hc) confirmation than hypercholesterolemic group with less vacuoles; better sinusoidal spaces, less pyknosis of hepatic nuclei, moderate central vein congestion. (H & E stain) 400 X.

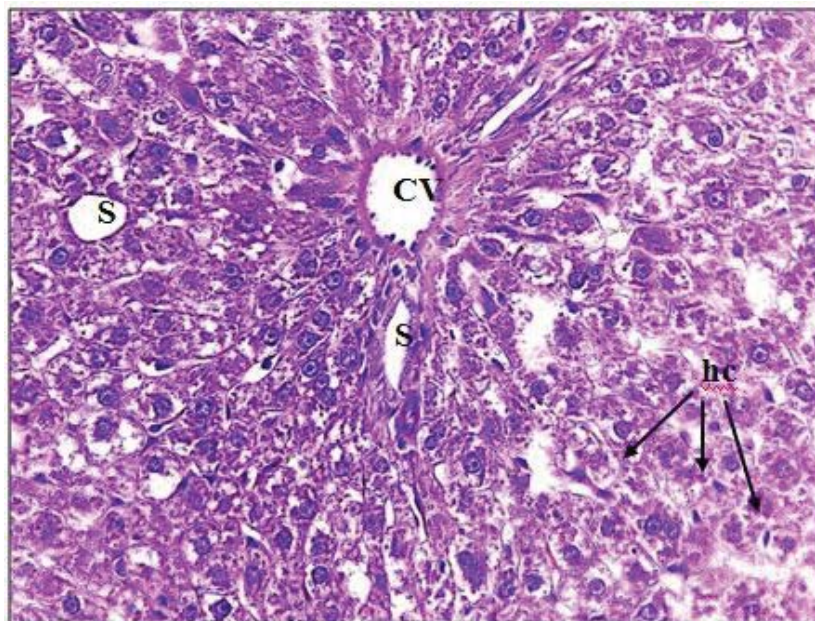


Fig. (5): Liver of (HC+ST.pol) treated group showing moderate ameliorative changes represented by beginning of radiating hepatocytes architecture formation, less vacuolation; and normal sinusoidal spaces. (H & E stain) 400 X .

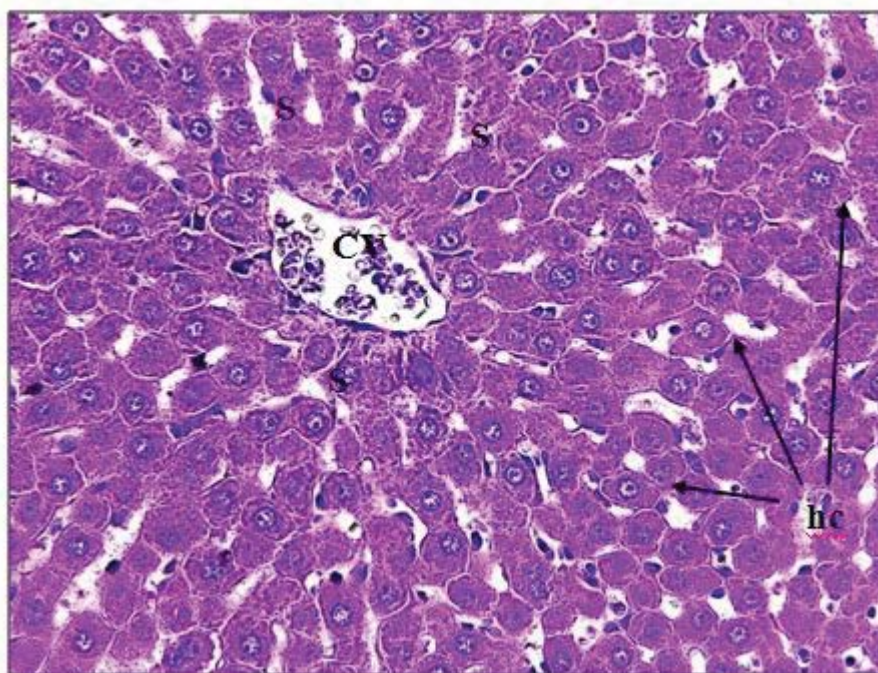


Fig. (6): Liver of (HC+EX.pol) treated group showing clear hepatocytes (hc) parenchymal ameliorative structure formation , some cells had lost their nuclei and boundaries and fused with other cells.. normal sinusoidal spaces (H & E stain) 400 X.

Discussion

Increasing of alanine aminotransferase ALT, aspartate aminotransferase AST and gamma glutamate GGT in (HC+SM) and (SM) treated groups in this study are in agreement with Thaparet al., (2013) who showed that statins have been associated with elevations in serum (ALT) and (AST) levels after statin administration to albino rats. They explained the effects of statin on liver and muscles, which are the sources of AST, and the higher sensitivity of ALT to hepatic inflammation. The potential mechanisms of statin induced myotoxicity include intracellular depletion of essential metabolites and destabilization of cell membranes, resulting in increased cytotoxicity. In addition, reduction in mevalonate metabolites by HMG-CoA reductase inhibitors would affect the activation of certain regulatory proteins responsible for the maintenance and mediation of apoptosis, Therefore, it was shown that statin administration to albino rats affected the liver, with elevation of transaminases (ALT being more specific to liver injury), and a complete normalization upon stopping the drug, suggesting no structural injury, a condition usually identified as “transaminitis”, which is asymptomatic, dose-related and reversible elevations in AST levels higher than those of ALT and not dropping to normal levels after discontinuing the drug, point to muscle involvement, leading to highlights the usually mild

nature of liver involvement, and the higher risk of statin myopathy, with the need to stop the drug instantly (Shehab and Gaballah , 2014)

Liver enzymes AST , ALT , ALP and GGT in (HC+ST.pol) and (H+CEX.pol) treated groups in this study either equal or significantly decreased compared with those in control group, these results are similar to the results of Janikula in 2002, who suggested that policosanol significantly lowered lipids without having any negative effects on liver enzymes this indicate decrease in liver damage. The enzyme activities shown indicated that gamma glutamyltransferase (GGT) levels were elevated in the serum of the in (HC.) group, this could be as a result of leakage of the enzymes into the serum as a result of damage to the integrity of the heart and liver. Elevated serum activity of these enzymes has been reported to be indicators of calculated risk of cardiovascular disease. (Otunola, et al.,2010).GGT has been reported to be a very strong risk factor, taking third place, for all forms of heart diseases and a possible indicator for early development of atherosclerosis. Ruttmann et al. (2005), reported a correlation between GGT and cardiovascular mortality indicating that the higher the elevation of GGT, the greater the risk of death. Microscopically the liver section of (HC) group in the present study revealed mild fatty infiltration and, other degenerative changes , these results are corresponding with the results of Swati et al.,(2014) and Olorunnisola et al.,(2012), who they found moderate to severe degree of fatty infiltration and mild degree diffuse granular degeneration in livers of rats fed with high-cholesterol diet. Lipid peroxidation is one of the indications of the oxidative stress in liver tissue. Hypercholesterolemia, enhances the free radical generation in various ways. Prime targets of oxygen free radicals (OFRs) attack are the polyunsaturated fatty acids in the membrane lipids causing lipid peroxidation which may lead to disorganization of cell structure and function, Dwivedi et al., (2014).The liver of (HC+SM) treated group in this study ,showed severe congestion of the central vein, and vacuolated hepatocytes. While liver section of (SM) treated group revealed better hepatocytes confirmation than hypercholesterolemic group with less vacuolation and better sinusoidal spaces, these results are in agreement with Wang, et al., in (2013) who showed that simvastatin causes slight sinusoidal fibrosis in rats. From another point of view these results are in contrast with conclusions of Sevgin and Alican in (2007) who demonstrated the beneficial effect of Simvastatin on damaged liver function in rats, this effect was explained to the action of Simvastatin by prevention of lipid peroxidation and then prevention liver fibrosis .Ameliorative change found in the liver tissues of (HC) groups treated with ST,Pol and EXT, Pol , are in consistent with Miriam, et al., in (2012), who revealed that the policosanol increased number of normal hepatocytes in rats livers after liver damage induction , also showed that policosanol has protective effects on liver against histological changes.

Also these results are in parallel with conclusions of Palayakotai in (2015) when proved the antioxidant effect of policosanol on rat liver microsomal lipid peroxidase. Furthermore these results are in comparable to results of Young Lee, et al.,(2016),who confirm that policosanol supplementation showed ameliorating fatty liver change in fish due to exerting antiapoptotic activity, resulting in increased cell replication and tissue regeneration.**Conclusion:** using of policosanol as antihyperlipidemic has less side effect on liver enzymes comparing with simvastatin using for the same purpose

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