EFFECT OF POLICOSANOL EXTRACT ADMINISTRATION ON LIPID PROFILE IN HYPERCHOLESTROLEMIC FEMALE RATS DURING LACTATION

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ABSTRACT

The present study designed to investigate the effects of the extracted policosanol on lipid profile of hypercholestrolemic lactating female rats (*Ratus norvigicus*). Forty eight female rat (10-12) weeks old,and weighting (200-250 gm) were used in the current study. After daily vaginal smear for 3 sequences estrus cycles, they mated with twenty four healthy adult fertile males weighting (280-350 gm), 24 of these females were induced to be hypercholestrolemic during the pregnancy,and others still healthy. Sugarcane were collected from Mesan province and policosanol was extracted from sugarcane wax. Immediately after birth the animals were divided into 6 groups: Group 1 (n=8) (Control) : Normal rats were given only 0.5 ml/ animal of Dimethyl Sulphoxide (DMSO) daily immediately after birth. Group 2 (n=8) hyperchol. (HC): Hpercholestrolemic rats were given only DMSO 0.5 ml/ animal daily immediately after birth. Group 3(n=8) (HC+SM): Hpercholestrolemic rats were given simvastatine 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 simvastatine at dose 20mg/kg BW/ day dissolvxsqqs ed in DMSO orally by gavages' immediately after birth. Group 5 (n= 8) (HC+ST. pol): Hpercholestrolemic rats were given standard policosanol at dose 20mg/kg BW/day orally by gavages' immediately after birth. Finally group 6(n=8)(HC+EX. pol): Hpercholestrolemic rats were given policosanol extraction at dose 20mg/kg BW/day orally by gavages immediately after birth. The treatment continued until weaning (30 days). All animals were then sacrificed

and blood samples were collected for measuring of lipid parameters. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) in all groups . Results revealed a significant improvement in TC level was recorded in (HC+SM) group and no difference was observed as compared with control group. While level of TC in (SM), (HC+ST.pol) and (HC+EX.pol) treated groups decreased significantly ($p \leq 0.05$) compared with control group, (HC) and (HC+SM) treated groups. No significant differences in HDL-C was observed between (HC+EX.pol) and control group. While the level of HDL-C still significantly lower in all other treated groups compared with control group. A significant reduction ($p \leq 0.05$) in LDL-C level was recorded in all treated groups compared with (HC) group. No significant differences were observed in LDL-C between (SM), (HC+ST. pol) and (HC+EX.pol) compared with control group. While the LDL-C still significantly higher ($p \leq 0.05$) in (HC+SM) compared with control group. Levels of triglyceride (TG) in (HC+SM),(HC+ST.pol) and (HC+EX.pol) treated groups recorded a significant elevation ($p \leq 0.05$) as compared with control group. While levels of TG in (SM) treated group were significantly decreased ($p \leq 0.05$) as compared with control group. Conclusion: local sugar cane can be used to extract the policosanol which has antihyperlipidemic effect on hypercholestrolemic lactating female rats.

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. In the plasma, it is carried in lipoproteins. These lipoproteins are divided into four important groups. 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 whereas (HDL-C) is not (1). Statins are the inhibitors of hydroxymethylglutaryl coenzyme A (HMG CoA) reductase. They are mostly used to treat hyperlipidaemia(2). Hypercholesterolemia during pregnancy affects fetuses in a high percentage, for this reason the percentage of affected offspring with cardiovascular diseases will be increased. Offspring from hypercholesterolemic animals usually affected with atherosclerosis more than those from normal animals. (3) . 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 (4). Many patients tolerate Statins in general, however they produce remarkable adverse effects particularly on skeletal muscle (5). Isolation and purification of sugar cane wax (*Saccharum officinarum*) produces a mixture of higher aliphatic alcohols called Policosanol. Policosanol shows cholesterol lowering and antiplatelet effects, and is being used as hypocholesterolemic drug. The present study aimed to compare between the effects of simvastatin, standared plicosanol and the extracted policosanol on lipid parameters of the hypercholesterolemic lactating female rats.

MATERIALS AND METHODS

Experimental Animals:

in the present study, the experiments were performed on 48 Norwegian white rats (*Rattus Norvegicus*) (10-12) weeks old, forty eight adult female rat weighting (200-250 gm) and twenty four healthy adult fertile males weighting (280-350 gm). 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. The animals were maintained under controlled optimum conditions light dark cycle (12/12) hours, at a temperature (25±4°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 (6) to establish their normal pattern of cyclical activity.

Preparation of Policosanol Extraction:

Sugar cane plant *(Saccharum officinarum*)were collected from Mesan province, Peels were manually scrapped and dried and stored in air tight container. Crude sugarcane wax extracted from supercritical CO2 technique was further purified by using polar aprotic solvent acetone. The resulting solid (wax) and supernatant were subjected to determine the purities and the content of policosanol by gas chromatography– mass spectrometry (GC/MS),**(**7).

Experimental Design:

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 and given by oral gavages until birth. The remaining 16 females were given only coconut oil in quantity equivalent to that is given to hypercholesterolemic animals and for the same period. Immediately after birth the animals were divided into the following 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): Hpercholestrolemic rats left without treatment, and were given only DMSO 0.5 ml/ animal daily immediately after birth.

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

Group 5 (n= 8) (HC+ST. pol): Hpercholestrolemic rats were given standard policosanol at dose 20mg/kg BW/day orally by gavages' immediately after birth,according to (8).**Group 6 (n=8)(HC+EX. Pol):** Hpercholestrolemic rats were given policosanol extraction at dose 20mg/kg BW/day orally by gavages' immediately after birth.The treatment continued until weaning (30 days old). All animals were then sacrificed, blood samples were collected and serum were separated to study the lipid profile.

Measurement of Serum Lipid Profile:

Total cholesterol **(**TC**)** was determined by using a special kit (BIOLABO cholesterol Kit , France). Colorimetric determination of the cholesterol was done as mentioned by (9).

High Density Lipoprotein Cholesterol (HDL-C) was determined by using special kit (BIOLABO / HDL - kit ,France).measured with total cholesterol reagent (10).

Triglyceride (TG) was determined by using a special kit (BIOLABO TG- KIT) according to method of (9).

LDL-Cholesterol concentration (LDL-C) can be calculated as the following equation (Ram, 1996)11: LDL= TC- (HDL+TG/5)

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 ± Standard deviation. All statistical tests were done by using statistical program SPSS(version 21.0) the level significant set on $p \geq 0.05$ (12).

RESULTS

The present results revealed a significant increase ($p \leq 0.05$) in TC, LDL-C and TG levels in hyperchol.(HC) group compared with the control group (Table 1). On the other hand, a significant decrease ($p \leq 0.05$) in HDL-C was recorded in (HC) group compared with control. A significant improvement in TC level was recorded in (HC+SM) group compared with control group. While level of TC in all other treated groups decreased significantly ($p \leq 0.05$) compared with control group, (HC) and (HC+SM) treated groups. No significant differences in HDL-C was observed between (HC+EX.pol) and control group. While the level of HDL-C still significantly lower in all other treated groups compared with control group. A significant reduction ($p \leq 0.05$) in LDL-C level was recorded in all treated groups compared with (HC) group. No significant differences were observed in LDL-C between (SM), (HC+ST. pol) and (HC+EX.pol) compared with control group. While the LDL-C still significantly higher ($p \leq 0.05$) in (HC+SM) compared with control group. Levels of triglyceride (TG) in (HC),(HC+SM),(HC+ST.pol) and (HC+EX.pol) treated groups recorded a significant elevation ($p \leq 0.05$) as compared with control group, but it remain significantly($p \leq 0.05$) lower in (HC+ST.pol) and (HC+EX.pol) treated groups compared with (HC) group. While levels of TG in (SM) treated group were significantly decreased ($p \leq 0.05$) as compared with control group.

Table (1): Animals lipid parameters (lipid profile): Total cholesterol, HDL, LDL and Triglyceride.

Par.s / Groups	TC mg/dI	HDL mg/dI	LDL mg/dI	TG mg/dI
Cont.	79.33 ± 4.54 **b**	51.83 ± 3.06 **a**	23.16 ± 2.85**cd**	27.00 ± 3.22 **d**
HC	118.16 ± 7.78 **a**	46.33 ± 7.00 **b**	44.5**0** ± 7.91 **a**	49.50 ± 4.27 **a**
HC+SM	84.33 ± 4.63 **b**	42.00 ± 4.47**bc**	37.66 ± 6.28 **b**	50.16 ± 7.78 **a**
SM	42.16 ± 5.70 **d**	22.66 ± 2.33 **d**	19.50 ± 2.94 **d**	20.00 ± 2.19 **e**
HC+ST. pol	68.50 ± 4.88 **c**	37.66 ± 3.07 **c**	27.83 ± 2.78 **c**	41.66 ± 4.32 **b**
HC+EX. pol	72.00 ± 7.29 **c**	56.00 ± 4.85 **a**	25.16 ± 3.06 **c**	35.83 ± 3.54 **c**
LSD	7.01	5.20	5.62	5.39

Results are expressed as mean± standard error of the mean (N=6)
Different letters refer to the significant differences at $P \leq 0.05$.

DISCUSSION

In the present study administration of cholesterol suspension produced elevation in serum level of total cholesterol, triglycerides, LDL-C and decrease in serum HDL-C level in (HC) group. This finding is in agreement with previous study (13), who demonstrated the increase of plasma cholesterol levels in rats by giving oral suspension of cholesterol .These effects may be attributed to increases the availability of acetyl- CoA, a precursor for cholesterol biosynthesis, this in turn increases the activity of HMG-CoA reductase - the rate determining enzyme in cholesterol biosynthesis- thus increasing the synthesis of cholesterol in the body . The cholesterol and fatty acids in the diet cause down regulation of LDL receptors either by changing hepatic LDL receptor activity or LDL-C production rate or both, this resulted in elevation of serum LDL-C level. Results of the present study are disagreement with previous study (14), who revealed that statins reduce blood levels of triglycerides and slightly increased levels of HDL-cholesterol. In the present study HDL –C levels in both (HC+SM) and (SM) treated groups were significantly decreased compared to control group, and triglyceride (TG) remained high and significantly increased in (HC+SM) treated group compared to control group. This increases the transfer of cholesteryl esters from HDL-C to triglyceride rich particles in exchange for triglycerides. This leads to increased serum concentration of triglycerides and decrease in serum concentration of HDL-C. Simvastatin in (HC+SM) treated group lowered TC nearby the levels of control group, and simvastatin in (SM) treated group lowered TC most than others. These findings are in agreement with previous study (2), who demonsrated that the statins like simvastatines are the inhibitors of hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase, and for this reason they are mostly used to treat hyperlipidaemia .

In the present study, the effect of policosanol on lipid profile in (HC+ ST.pol) and (HC+EX.pol) treated groups, these results are comparable to (15), who proved that policosanol has lipid-lowering and HDL-C elevating effects. These results are in agreement with (16), who explained that the effect of policosanol may inhibit hepatic cholesterol biosynthesis at a step before mevalonate generation, but unlikely to say by direct inhibition of HMG-COA reductase. In addition, these results are in agreement with,(17), who showed that administration of policosanol orally for one month inhibits hepatic cholesterol biosynthesis from tritiated water in normocholesterolemic rats, depending on the previous results that policosanol inhibited cholesterol biosynthetic pathway prior to mevalonate generation, suggesting an effect on HMG-CoA reductase, the key enzyme of cholesterol synthesis. However, the experiments performed to examine whether policosanol suppressed preformed reductase directly in microsomes showed that, when policosanol was added to the reductase assay at concentrations ranging from 5 to 50 mg/ml, the enzyme activity remained unchanged . Thus, there was no evidence of enzyme inhibition induced by policosanol (17). Also, policosanol addition does not interfere in the state of activation of the enzyme , since no major differences were observed in the fraction of expressed activity in control and policosanol treated hepatic microsomes. These results suggested that policosanol does not act on the reversible phosphorylation of the enzyme. This concluded, that the inhibitory effect of policosanol on hepatic cholesterol biosynthesis is not elicited by a direct action of policosanol on HMG-Co A reductase. Further research on the effect of policosanol on the cholesterol biosynthetic pathway is required to determine the primary site of action of

policosanol. Despite others confirming positive cholesterol-lowering effects of policosanol, still others having added elements to its mode of action. (18 ; 19). LDL-C oxidation is thought to be a necessary step in the development of atherosclerosis, studies on humans and rats show policosanol decreases LDL-C oxidation (20) . In a previous study, it has been mentioned that policosanol can inhibit cholesteryl ester transfer protein (CETP) activity, which is an atherogenic factor in serum, (15). However, many animal models such as mice, rats, and dogs lack serum cholesteryl ester (CE) transfer activity due to the absence of CETP, (21). This explains why many reports have not observed any changes in serum CE transfer activity due to PCO, even though HDL-C elevation via CETP inhibition is well known. Policosanol showed higher inhibitory ability against human cholesteryl ester transfer protein. This result suggests that policosanol-interfered with normal interactions between CE-donor (HDL-C), CETP, and CE acceptor (LDL-C). Regarding the detailed mechanism of CE transfer. (22) reported that CETP binds to HDL-C via hydrophobic interactions, because HDL-C surface lipid curvature generates a hydrophobic environment. Several reports on CETP inhibitors have suggested a putative mechanism for CETP inhibition on the basis of competitive interactions with CETP and HDL-C (٢٣ ; ٢٤). It is possible that aliphatic chains in policosanol interfere with binding between CETP and HDL-C(15). In Conclusion: local sugar cane can be used to extract the policosanol which has antihyperlipidemic effect comparing with standard policosanol and simvastatin which using for the same purpose.

تاثير مستخلص البوليكوسانول على صورة الدهون في إناث الجرذان مفرطة الكوليستيرول خلال فترة الرضاعة

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الخلاصة

صممت هذه الدراسة للتقصي عن تأثير مستخلص البوليكوسانول في بعض المعايير الدهنية لإناث الجرذان المختبرية من نوع(*Rattus norvegicus*) مفرطة الكوليسترول في فترة الرضاعة. استخدمت في هده الدراسة ثمانية وأربعون أنثى جرذ بأعمار تراوحت بين (١٠-١٢) أسبوع و بأوزان تراوحت ما بين (٢٠٠-٢٥٠) غرام. بعد عمل مسحات مهبلية يوميا لثلاث دورات شبق متتالية خضعت هذه الجرذان للتزاوج بذكور بالغة بعدد ٢٤ ذكر بأوزان تراوحت ما بين (٢٨٠-٣٥٠) غرام توزعت بمعدل ذكر واحد لكل ٢ من الإناث. بعد التأكد من حمل هذه الجرذان خضعت فقط ٢٤ من الإناث لاستحثاث فرط الكوليستيرول في الدم خلال فترة الحمل،والباقي تركن بدون استحثاث .استخلصت مادة البوليكوسانول التي استعملت في هذه الدراسة من قصب السكر بعد جمعه من محافظة ميسان وتجفيفه وطحنه ومعاينة مكوناته باستعمال تقنية كروماتوكرافيا الغاز GC/ MS. بعد الولادة مباشرة وفي فترة الرضاعة تحديدا قسمت الإناث إلى ٦ مجاميع هي: المجموعة الأولى عدد ٨ :حيوانات السيطرة (cont) حيوانات سليمة بدون استحثاث فرط الكوليستيرول أعطيت (٠.٥ مل / حيوان) من مادة(DMSO) يوميا وبدون معالجه، :المجموعة الثانية عدد ٨ : حيوانات مفرطة الكوليستيرول أعطيت فقط (٠.٥ مل / حيوان) من مادة

(DMSO) يوميا المجموعة الثالثة :(HC+SM) عدد ٨ حيوانات مفرطة الكوليستيرول أعطيت (٢٠ مل / حيوان) من مادة السمفاستاتين يوميا، :المجموعة الرابعة :(SM) عدد ٨ حيوانات غير مفرطة الكوليستيرول أعطيت (٢٠ مل / حيوان) من مادة السمفاستاتين يوميا، الخامسة :(HC+ST.pol) عدد ٨ حيوانات مفرطة الكوليسترول أعطيت (٢٠ مل / حيوان) من مادة البوليكوسانول القياسي يوميا، المجموعة السادسة :(HC+EX.pol) عدد ٨ حيوانات مفرطة الكوليسترول أعطيت (٢٠ مل / حيوان) من مستخلص البوليكوسانول يوميا، استمرت المعالجة يوميا لمدة ٣٠ يوم بعد الولادة. بعد ذلك تمت التضحية بالحيوانات وجمعت عينات الدم لغرض قياس المعايير الدهنية .النتائج: أظهرت النتائج تحسنا معنويا في مستويات الكوليسترول الكلي (TC) و البروتين ألدهني واطئ الكثافة (LDL-C) و البروتين ألدهني عالي الكثافة (HDL-C) والدهون الثلاثية (TG)في المجاميع المفرطة الكوليسترول المعاملة بالسمفاستاتين و البوليكوسانول القياسي والبوليكوسانول المستخلص من قصب السكر وبدرجات مختلفة مقارنة بمجموعتي الحيوانات مفرطة الكوليسترول والسيطرة.

الاستنتاجات :اعتمادا على النتائج أعلاه فانه يمكن استخدام مستخلص قصب السكر(البوليكوسانول) كمادة مثبطة للكولستيرول في إناث الجرذان المستحثة فرط الكوليستيرول أثناء فترة الرضاعة.

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