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A Study Comparative of Some Haematological Parameters after Treatment by Extracted Octacosanol and Atorvastatin in Female Rats.

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Abstract

Hematological and serum parameters are a prerequisite to understand the normal functioning of the system and to confirm the nontoxic nature of the administered crude drug. Hematological parameters such as mean platelet volume (MPV) and RBCs count are associated with the increased risk for cardio vascular diseases CVD. Octacosanol is the major effective component of policosanol forming about (62.9%), it has antioxidative, lipid lowering, antithrombotic (antiplatelet) effects and provides protection against free radical associated diseases. Statins are the inhibitors of hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase. They are mostly used to treat hyperlipidaemia. Statins such as atorvastatin and some of medical plants have shown their beneficial effect on the cardiovascular disease (CVD) by virtue of their lipid lowering, antioxidant and cardioprotective effects. The present study was designed to compare some of hematological parameters after treatment of extracted octacosanol and atorvastatin in female Norwegian rats. Thirty two females rat with (10-12) weeks old-and weighting (200-250 gm) were used in the current study. Policosanol was extracted from sugarcane that obtained from Mesan province. Octacosanol is determined in this extraction by gas chromatography – mass spectrometry (GC/MS). Animals were divided into 4 equal groups: G.1 (n=8) (Control) : only 0.5 ml/ animal of Dimethyl

Sulphoxide (DMSO). G.2 (n=8) animals treated with (0.02 mg/ animal) of standard octacosanol . G.3 (n=8) animals treated with (0.02mg/ animal) of octacocosanol extraction, G. 4 (n=8) treated with only (0.02mg/ animal) of atorvastatine. The treatment continued for 30 days. All animals were then sacrificed and blood samples were collected in EDTA tubes for measuring of hematological parameters. **Results**: all hematological parameters (RBCs, Hb, MCV, MCH and MCHC) in group treated with extracted octacosanol were nearby the control group except platelet count and MPV level were lower than those of control group, while platelet count and MPV were higher in extracted group than those in the Atorvastatin group (all $P \leq 0.05$). In conclusion: The antiplatelet activity of the extracted octacosanol is more than atorvastatin and without side effects. So the extracted octacosanol successfully used in prevention of platelet aggregation and thrombosis in patients with CVD.

Keywords*:* Female Rats, Octacosanol Extraction, Atorvastatin, Hematological parameters, Platelet, mean platelet volume MPV.

دراسة مقارنه لبعض المعايير الدموية في إناث الجرذان البيضاء بعد معاملتها بمستخلص الاوكتاكوسانول والاتورفاستاتين.

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الخلاصة

تعتبر المعايير الدموية والمصلية من المتطلبات الاساسية لفهم وظائف الجسم الطبيعية وللتأكد من الطبيعة الغير سمية للمواد الدوائية. ترتبط المعايير الدموية عادة مثل اعداد كريات الدم الحمراء والصفائح الدموية ومعدل احجام تلك الصفائح بمخاطر بعض امراض القلب وتصلب الشرايين وحدوث الجلطات. يعتبر الاوكتاكوسانول المادة الاكثر فعالية في مستخلص البوليكوسانول (المستخلص من نبات قصب السكر) ويشكل نسبة, (62.9%) منه, ويمتاز الاوكتاكوسانول بأن له فوائد كمادة مضادة للأكسدة , خافضة للكوليسترول الضار ومانعة لتخثر

الدم ويتميز بأسلوب دفاعي عن الجسم ضد تكون الجذور الحرة. الستاتينات مثل الاتورفاستاتين والسمفاستاتين واللوفاستاتين وغيرها من الادوية التي تثبط عمل انزيم هيدروكسي ميثايل كلوتمايل كو ا المختزل (HMG-COA reductase) المهم في تكوين الكوليستيرول وهذه الادوية تستعمل عادة كمادة علاجية مخفضة للكولستيرول في الجسم وتعالج امراض القلب وتصلب الشرايين وتقلل من نسبة تخثر الدم و حدوث الجلطات .صممت الدراسة الحالية لمقارنه بعض المعايير الدموية في إناث الجرذان البيضاء بعد معالجتها بمستخلص الاوكتاكوسانول والاتورفاستاتين. استعملت في هذه الدراسة اثنان وثلاثون أنثى جرذ بأعمار تراوحت بين (10-12) أسبوع و بأوزان تراوحت ما بين (200-250) غرام. .استخلصت مادة البوليكوسانول التي استعملت في هذه الدراسة من قصب السكر بعد جمعه من محافظة ميسان وتجفيفه وطحنه ومعاينة الاوكتاكوسانول في هذا المستخلص باستعمال تقنية كروماتوكرافيا الغاز GC/ MS. قسمت الإناث إلى 4 مجاميع هي: المجموعة الأولى عدد 8 :حيوانات السيطرة (control) حيوانات سليمة أعطيت (DMSO) بجرعة 0.25 مل / حيوان. المجموعة الثانية عدد 8 اعطيت الاوكتاكوسانول القياسي بجرعة 0.02 ملغم/ حيوان , المجموعة الثالثة: عدد 8, اعطيت مستخلص الاوكتاكوسانول بجرعة 0.02 ملغم/ حيوان :المجموعة الرابعة:عدد 8 حيوانات اعطيت الاتورفاستاين بجرعة 0.02 ملغم/ حيوان , استمرت المعالجة يوميا لمدة 30 يوم. بعد ذلك تمت التضحية بالحيوانات وجمعت عينات الدم في انابيب تحتوي مادة EDTA لإجراء الفحوصات المختبرية لبعض المعايير الدموية .**النتائج**: كانت جميع المعايير الدموية(RBCs, Hb, (MCV, MCH , MCHC الخاصة بالمجموعة المعاملة بمستخلص الاوكتاكوسانول مقاربة لمجموعة السيطرة ماعدا اعداد الصفيحات الدموية ومعدل حجم تلك الصفيحات اقل مما في مجموعة السيطرة . بينما كانت اعداد الصفيحات Plt الدموية ومعدل حجم تلك الصفيحات MPV في مجموعة الاتورفاستاتين اعلى مما في مجموعة المستخلص التحليل الاحصائي لجميع المعايير كان ضمن مستوى معنوية ($P\leq0.05$). **الاستنتاجات** : مستخلص الاوكتاكوسانول اكثر كفاءة من الاتورفاستاتين في تقليل حدوث الجلطات الدموية لدى مرضى القلب وتصلب الشرايين.

الكلمات المفتاحية: اناث الجرذان. مستخلص الاوكتاكوسانول, الاتورفاستاتين, المعايير الدموية, الصفائح الدموية, متوسط حجم الصفائح MPV.

Introduction

Assessment of hematological and serum parameters is a prerequisite to understand the normal functioning of the system and to confirm the nontoxic nature of the administered crude drug. A number of medical plants have shown their beneficial effect on the cardiovascular disease

(CVD) by virtue of their lipid lowering, antioxidant and cardioprotective effects [1]. Hematological parameters such as mean platelet volume (MPV) [2], red cell distribution width [3], are associated with the increased risk for CVD. However, whether other parameters of the complete blood count (such as erythrocyte and its related parameters, including hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are also associated with the risk for CVD and whether the combination of these parameters with blood lipids can improve the ability to predict the risk For CVD are still unclear [4]. Mean platelet volume (MPV) which is a simple measure of platelet activation, has recently become an interesting topic in cardiovascular research. When platelets become activated (MPV) increases and change from quiescent discs to swollen spheres[5,6]. Octacosanol is one of policasanol components , policasanol is a mixture of higher aliphatic alcohols produces from isolation and purification of sugar cane wax (*Saccharum officinarum*) [7] . Octacosanol is the major effective component of policosanol forming about (62.9%)[8] , it has antioxidative, lipid lowering, antithrombotic (antiplatelet) effects and provides protection against free radical associated diseases sugarcane wax contains (60-70%) octacosanol [9] . Octacosanol may protect against cerebral ischemia by reducing theTxA2/PgI2 ratio [10] .Statins are the inhibitors of hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase. They are mostly used to treat hyperlipidaemia [11]. Statins that currently carried for clinical use include Atorvastatin, Fluvastatin, Lovastatin, Pravastatin, and simvastatin. Lowering of low-density lipoprotein (LDL) plasma levels has been shown to reduce primary and secondary cardiovascular events including myocardial infarction (MI), stroke, and all-cause mortality [12]. They also have favorable effects on platelet activation, endothelial function, inflammation, and coagulation cascade [13,14]. The present study was designed to compare the effects of octacosanol on some hematological parameters in female rat model with atorvastatin that currently present in market with certain side effects like rhabdomylysis, muscle weakness and affect the liver enzymes.

Materials And Methods

Experimental Animals:

Thirty two healthy adult female rats. Weights and age of animals were (200-250 gm) and (10-12) weeks respectively. 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±4°C). The diet was offered *ad Libitum*, and presented with tap water.

Standard drug :

Standard octacosanol obtained from USA.

Plant material collection and preparation of the extraction

Sugar cane plant *(Saccharum officinarum*) were collected from Mesan province , peels were manually scrapped and dried at 60°C for 24 hours and stored in air tight container, after that grinding sugar cane then extracted the policosanol, and determined its contents – particularly octacosanol- by using gas chromatography – mass spectrometry (GC/MS).

Experimental groups:

Animals were divided into 4 groups: all animals were treated orally as the following groups: The first group 1 (n=8) (Control): animals were given treatment orally only 0.25 ml/ animal of Dimethyl Sulphoxide (DMSO). The second group 2 (n=8) given standard octacosanol at dose (0.02 mg/ animal) day. The third group 3 (n=8) were given extracted octacosanol at dose (0.02 mg/ animal) day. The fourth group 4(n=8) were given only atorvastatin at dose (0.02 mg/ animal) day.

Criteria of observation :

At the end of the experiment that continued 30 days, animals of each group were euthanized and blood samples were collected in clean dry Eppendorf tubes containing EDTA as anticoagulant to be used for hemogram studies

Hematological parameters :

The following parameters RBCs, Hb, MCV, MCH, MCHC, Platelets count and MPV were all determined by a Celltac- α Hematology analyzer (Nihon Kohden Japan).

Statistical Analysis:

One-way ANOVA-test was used to determine the significant difference between groups. 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 $p \leq 0.05$ [15] .

RESULTS

RBC (Erythrocytes) count that illustrated in table (1) revealed that there was no significant difference in group treated with extracted octacosanol as compared to control group, while RBC count in groups treated with standard octacosanol and atorvastatin were significantly ($P \leq 0.05$) decreased than those in control group. Also in the same table results showed that the hemoglobin ratio (Hb) in group of extracted octacosanol was significantly ($P \leq 0.05$) increased than other groups even higher than the control group. While hemoglobin ratio in groups of standard octacosanol and atorvastatin was significantly ($P \leq 0.05$) decreased than the control groups . MCV in all treated groups were significantly ($P \leq 0.05$) higher than those in control. MCH in extracted and standard octacosanol were not significantly differ from those in control, but MCH in atorvastatin treated group was significantly ($P \leq 0.05$) lower than those of control group. MCHC levels in all treated groups showed a significant ($P \leq 0.05$) decrease than those of control group . It observed that platelets count in group treated with extracted octacosanol was significantly ($P \leq 0.05$) decrease than those of control group, but platelets count in other treated groups were not significantly differ from those in control group. MPV levels in all treated groups were significantly ($P \leq 0.05$) decrease than those of control group, and the group that treated with extracted octacosanol was the most significant ($P \leq 0.05$) decrease . All results are illustrated in table (1).

Table (1): Effects of extracted octacosanol and atorvastatin on hematological parameters for all groups.

Groups / Par.	Control First group	Standard Octacosanol second group	Extracted Octacosanol third group	Atorvastatin fourth group	LSD
RBC count 10 12/L	6.89 ± 0.28 **a**	5.71 ± 0.44 **c**	6.71 ± 0.35 **ab**	6.37 ± 0.32 **b**	**0.36**
Hb (g/L)	13.42± 0.50 **b**	10.67± 0.24 **d**	14.22± 0.40 **a**	12.25± 0.66 **c**	**0.48**
MCV(fl)	48.80 ± 4.98 **b**	55.72± 1.98 **a**	57.05± 4.04 **a**	59.16± 2.95 **a**	**3.98**
MCH (pg)	18.76 ± 0.56 **a**	17.9 ± 0.72 **ab**	18.92 ± 1.13 **a**	17.27 ± 2.30 **b**	**1.39**
MCHC(g/L)	38.98 ± 4.96 **a**	32.23 ± 0.91 **bc**	34.66 ± 4.47 **b**	29.11 ± 3.60 **c**	**3.89**
Plt. (× 10 9/L)	457 ± 105.18 **a**	437.5 ± 52.81 **ab**	361.62 ±60.21 **b**	435.12±112.81 **ab**	**87.47**
MPV (fL)	10.28± 0.57 **a**	7.01± 0.25 **bc**	6.6± 0.88 **c**	7.62± 0.70 **b**	**0.63**

*Different letters refer to a significant differences at **(P ≤ 0.05)**.

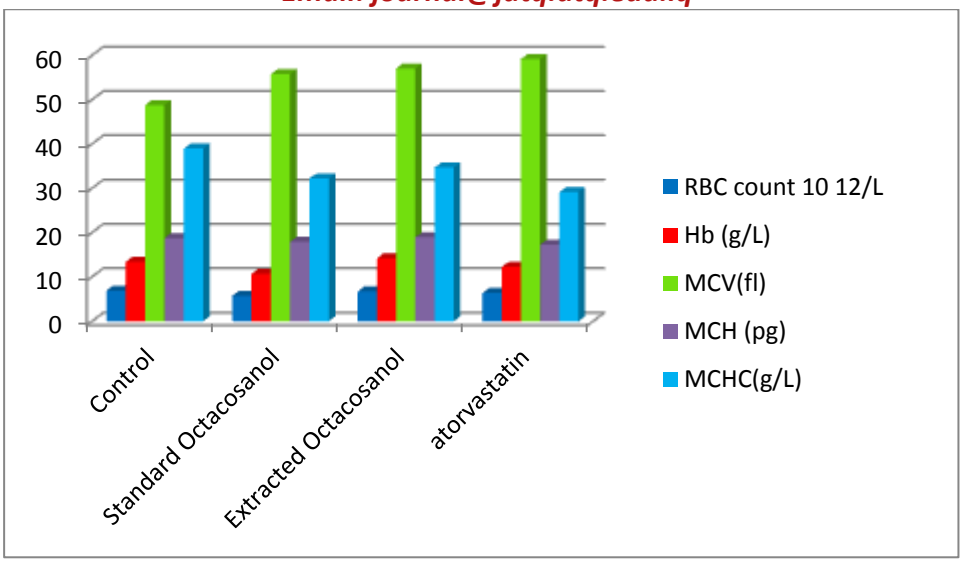

Figure 1: Results of RBC, Hb, MCV, MCH and MCHC, at the significant differences of $P \leq 0.05$ ($n = 8$).

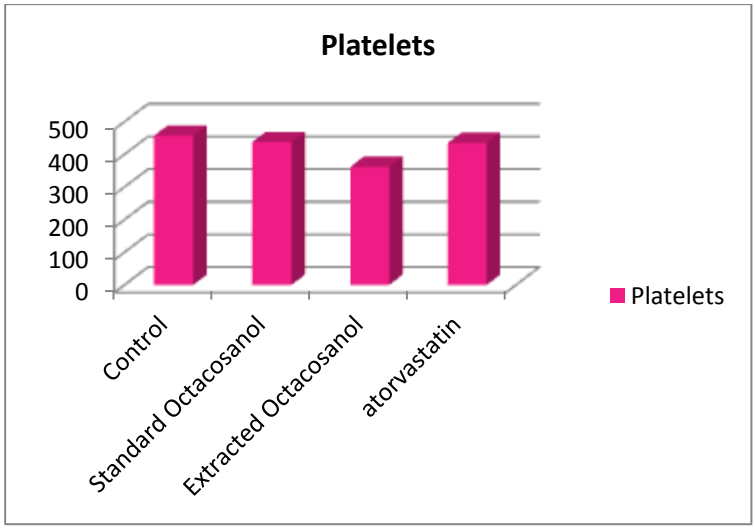

Figure 2: Results of Plt: platelets, at the significant differences $P \leq 0.05$ ($n = 8$).

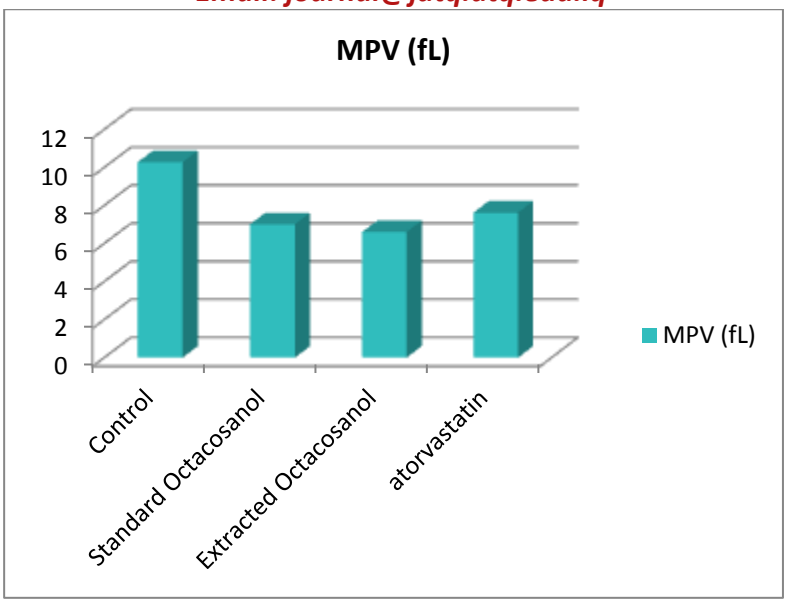

Figure 3: Results of MPV: mean platelet volume , at the significant differences
$P \leq 0.05$ ($n = 8$).

Discussion

Hematological parameters should be regularly monitored for patients with a risk for atherosclerosis and CVD. In the present study, the effects of octacosanol and atorvastatin on blood cell count were investigated, which may be helpful to guide the clinical therapy of these patients. Results of extracted octacosanol treated group in this search are in agreement with results of Wong, *et al*.,(2016) who proved all the haematological parameters of group treated with extracted octacosanol were found to be within the normal range except platelet count and MPV level [16]. Results of RBC count in groups of atorvastatin were in agreement with a study showed significantly decreasing RBC count and concomitant lowering hemoglobin, this may due to deformation of red blood cell (RBC) that may be related to ATP release from cells [17]. Membrane cholesterol has been shown to alter the properties of cell membrane such as fluidity and bending stiffness [18]. Hyperlipidemia is a risk factor of systemic atherosclerosis [19]. The platelet aggregation and cholesterol-rich lipoproteins have been found in the atherosclerotic plaques, which suggest

that such lipoproteins and activated platelets are involved in the pathogenesis of atherosclerosis [20]. Results of platelet in this search are in agreement with Wong, *et al.*, (2016) who proved that treatment with policosanol which extracted from rice bran inhibited platelet aggregation as comparable with the effect of aspirin, also the treatment with rice bran policosanol extract causes attenuation of serum thromboxane A2 and reduced *ex vivo* ADP-induced platelet aggregation without giving adverse effects. [16].

Antithrombotic effect of policosanols has been demonstrated in several animal species including rabbits and other rodents. Specifically, policosanols have shown to exhibit effects on platelet activation, endothelium turnover and foam cell formation[10] ,this may due to policosanol were shown to inhibit collagen induced decreases in platelet counts. In addition, policosanols administered were able to inhibit the formation of thromboxane A2 (TxA2, a potent platelet aggretory agent in both human and nonhuman species)[9].

The platelets transform from static dish into globular swelling shape after activation, resulting in MPV increase. The adhesion and aggregation of platelets increase significantly after activation. However, the platelets are basically at the rest state in the blood circulation of health subjects. In the prothrombotic state, platelets at rest state may be activated by various factors, which significantly increase the incidence of coronary heart complications and thrombosis. Therefore, reducing platelet activity is very important for the clinical treatment of coronary heart disease and the prevention of cardiovascular events [4].In this study MPV were significantly decreased after atorvastatins treatment. It is well known that MPV is an indicator of platelet activation and has been widely studied in CVD [21].

Extracted octacosanol kept some hematological parameters in the normal ranges, and successfully reduced platelet aggregation without giving adverse effects on the animal models as compared to atorvastatin, so the antiplatelet activity of the extracted octacosanol is more than atorvastatin and without side effects. For this reason the extracted

octacosanol successfully used in prevention of platelet aggregation and thrombosis in patients with CVD.

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