Journal of Education for Pure Science- University of Thi-Qar
Vol.9, No.2 (June, 2019)
Website: jceps.utq.edu.iq Email: jceps@eps.utq.edu.iq

DOI: http://doi.org/10.32792/utq.jceps.09.02.22

Study The Effects of Extracted Octacosanol and Atorvastatin on Leukocytes in Female Norwegian Rats.

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Received 11/04/2019, Accepted 09/05/2019, Published 02/06/2019

Abstract:

The present study was designed to compare some of hematological parameters in female norwegian rats (*Ratus norvigicus*) after administration of extracted octacosanol and atorvastatin. 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: Group.1 (n=8) (Control): only 0.٥ ml/ kg of Dimethyl Sulphoxide (DMSO). Group.2 (n=8) animals treated with (0.02 mg/ kg) of standard octacosanol. Group.3 (n=8) animals treated with (0.02mg/ kg) of octacocosanol extraction, Group. 4 (n=8) treated with only (0.02mg/ kg) of atorvastatine. The treatment continued for 30 days. All animals were then sacrificed and blood samples were collected in EDTA tubes for measuring of total and different count of WBC.

Keywords: *Rats, Octacosanol Extraction, Atorvastatin, Leukocytes, Neutophils.*

1. Introduction

Leukocytes are prerequisite for the development of atherosclerosis [1]. Preventing adherence of leukocytes to the endothelium results in improved endothelial function [2] and inhibition of plaque formation in arteries of animal model [3]. Many epidemiological studies have linked the leukocyte count to coronary artery disease (CAD) and its risk factor [4]. From all leukocytes, neutrophils are more associated with (CAD) [5]. Octacosanol is the major effective component of policasanol which forming about (62.9%). Policasanol is a mixture of higher aliphatic alcohols produces from isolation and purification of sugar cane wax (*Saccharum officinarum*) [6] . Octacosanol has antioxidative, lipid lowering, antithrombotic (antiplatelet) effects and concomitant coronary artery disease (CAD), prevent development of atherosclerosis and provides protection against free radical associated diseases, also decrease the thickness of adipose tissue by inhibition cholesterol biosynthesis [7] , sugarcane wax contains ≈ 60-70% octacosanol [8]. Besides these effects on lipid metabolism, policosanol also presents a wide range of pharmacological activities, such as acting as anti-inflammatory [9,10] , Identification of bioactive compounds from plants has become a highly active area of pharmaceutical research. Such compounds

have been applied in the treatment of different conditions, including anxiety, pain, and inflammation[11,12,13]. Thus, the evaluation of pharmacological effects on these conditions can be used as a strategy for discovering new drugs of plant origin. Statins are the inhibitors of hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase. They are mostly used to treat hyperlipidaemia [14]. 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 development of atherosclerosis [15] . They also have favorable effects on inflammation [16,17] . The present study was designed to compare the effects of octacosanol on leukocytes in female rat model with atorvastatin that currently present in market with certain side effects like rhabdomylysis, muscle weakness etc..

2. Materials and Methods

2.1. Experimental Animals

Thirty two healthy adult female Norwegian rats (*Rattus norvegicus*). 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.

2.2. Standard drug

Standard octacosanol was obtained from Purethentic Naturals/ USA.

2.3. 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) as in figure (1) and table (1).

2.4. Atorvastatin

Atorvastatin tablet 20mg were obtained from Actavis- UK

2.5. Experimental groups

Animals were divided into four equal groups: Group 1 (n=8) (Control): the first group animals were treated with only 0.5 ml/ kg of Dimethyl Sulphoxide (DMSO). Group 2 (n=8) the second group animals were treated with standard octacosanol at dose (0.02 mg/ kg) day. Group 3 (n=8) the third group animals were treated with extracted octacosanol at dose (0.02 mg/ kg) day. Group 4(n=8) the fourth group animals were treated with only atorvastatin at dose (0.02 mg/ kg) day.

2.6. 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

2.7. Hematological observation

The following parameters (Leukocytes, Neutrophils, Lymphocytes, and Monocytes) were all determined by a Celltac- α Hematology analyzer (Nihon Kohden Japan).

2.8. 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 $p \leq 0.05$ [18] .

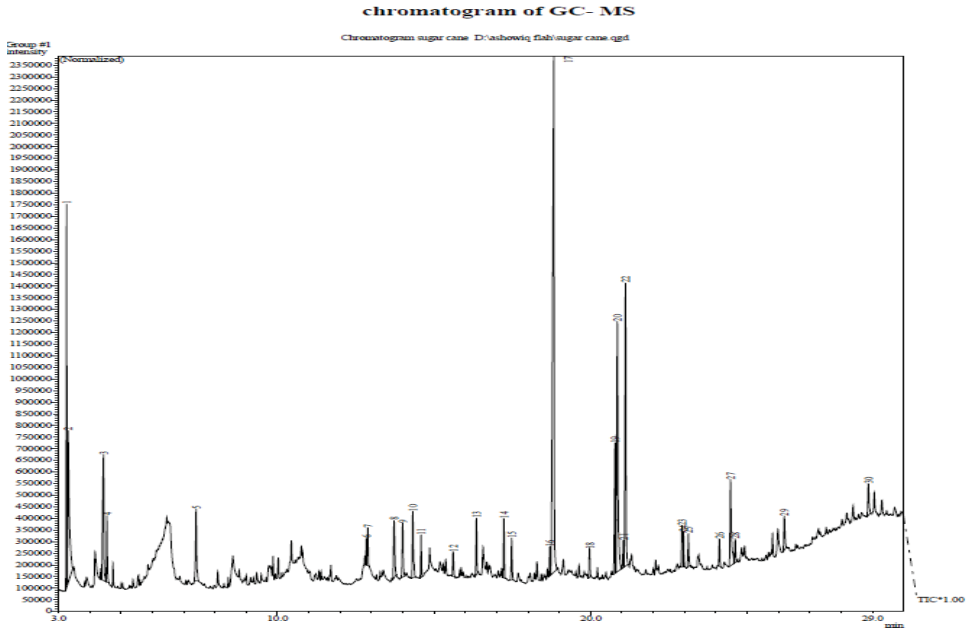

FIGURE 1: Gas Chromatography – Mass Spectrometry (GC/MS).

TABLE 1: Gas Chromatography – Mass Spectrometry (GC/MS).

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	3.260	2318582	7.61	2-Pentanone, 4-hydroxy-4-methyl-
2	3.307	2259484	7.42	Dimethylsulfoxonium formylmethylide
3	4.441	1527018	5.01	Dimethyl sulfone
4	4.549	489443	1.61	1,2-Cyclopentanedione
5	7.399	727224	2.39	Nonanal
6	12.839	185084	0.61	1-Dodecanol
7	12.882	261355	0.86	3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-
8	13.725	673166	2.21	.alpha.-D-Glucofuranose, 1,2-O-(1-methylethylidene)-
9	13.998	511002	1.68	Dodecanoic acid
10	14.321	676090	2.22	Diethyl Phthalate
11	14.584	318532	1.05	2,2-Dimethylpropanoic acid, heptadecyl ester
12	15.607	239738	0.79	8,16-Dimethyl-1,9-dioxacyclohexadeca-4,12-diene-2,10-dione
13	16.354	475458	1.56	Tetradecanoic acid
14	17.231	457081	1.50	2-Pentadecanone, 6,10,14-trimethyl-
15	17.479	374007	1.23	Phthalic acid, butyl undecyl ester
16	18.699	250193	0.82	Dibutyl phthalate
17	18.824	7380924	24.24	Pentadecanoic acid
18	19.967	254358	0.84	Heptadecanoic acid
19	20.783	1584327	5.20	9,12-Octadecadienoic acid (Z,Z)-
20	20.856	3123902	10.26	6-Octadecenoic acid, (Z)-
21	21.044	224439	0.74	9,12-Octadecadienoic acid, ethyl ester
22	21.116	2840050	9.33	Octadecanoic acid
23	22.924	409074	1.34	2(3H)-Benzofuranone, hexahydro-4,4,7a-trimethyl-
24	22.970	305745	1.00	4,8,12,16-Tetramethylheptadecan-4-olide
25	23.124	289843	0.95	Icosanoic anhydride
26	24.116	223199	0.73	Hexadecanoic acid, (2,2-dimethyl-1,3-dioxolan-4-yl)methyl ester
27	24.471	1103681	3.62	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
28	24.620	228458	0.75	1,2-Benzenedicarboxylic acid, diisooctyl ester
29	26.189	378137	1.24	Octadecanoic acid, 2,3-dihydroxypropyl ester
30	28.875	364018	1.20	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-
		30453612	100.00	

3. Results

WBC (or Leukocytes) count that illustrated in table (1) revealed that there were no significant differences in groups treated with extracted octacosanol and standard octacosanol as compared to control group, while we observed there were a significantly ($P \leq 0.05$) decreased in WBC count in groups treated with atorvastatin and standard octacosanol when compared with control group. For more details the differential diagnosis in the same table showed that the neutrophils count in groups treated with extracted octacosanol and standard octacosanol were not significantly differ from those in control group, whereas Neutrophils count in group treated with atorvastatin were significantly ($P \leq 0.05$) decreased than those in control group. Lymphocytes count in groups treated with standard and extracted octacosanol, were not significantly differ from those in control group, but Lymphocytes count in group treated with atorvastatin were significantly ($P \leq 0.05$) increased than those in control group. Monocytes count in groups treated with extracted octacosanol and atorvastatin were not significantly differ from those in control group, while Monocytes count in group treated with standard octacosanol were significantly ($P \leq 0.05$) increased than those in control group.

Table 1: Effects of Extracted Octacosanol and Atorvastatin on WBC count (Leukocytes, Neutrophils, Lymphocytes, and Monocytes) in all groups. Mean±SD, No. 6 animals for each group.

Groups / Par.	Control	Standard Octacosanol	Extracted Octacosanol	Atorvastatin	LSD
Leukocytes 10 9/L	8.99 ± 0.47 **a**	8.73 ± 0.65 **b**	8.94 ± 0.7 **a**	5.26 ± 0.40 **c**	0.14
Neutrophils 10 9/L	5.87 ± 0.73 **a**	4.13 ± 1.43 **a**	5.56 ± 0.41 **a**	1.3± 0.1 **b**	1.55
Lymphocytes 10 9/L	3.00 ± 1.44 **b**	3.07 ± 1.15 **b**	3.01 ± 0.20 **b**	3.6± 0.5 **a**	0.8
Monocytes 10 9/L	0.6 ± 0.1 **b**	0.93 ± 0.50 **a**	0.16 ± 0.05 **b**	0.4 ± 0.1 **b**	0.6

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

4. Discussion

The results of group treated with Extracted Octacosanol in the present study are in agreement with [19] who proved that WBC, Lymphocytes, Monocytes, and Granulocytes in group treated with policosanol were nearby normal ranges. While results of group treated with atorvastatin in this study are in agreement with [20] who noted the decrease in total white blood cell count and Neutrophils in dogs after atorvastatin treatment. In fact the systemic inflammation plays an important role in the etiopathogenesis of Congeted

Heart Failure CHF and CAD [21] . Although numerous inflammatory biomarkers are available, the WBC count on a standard complete blood count CBC is the most readily available nonspecific marker of systemic inflammation. In this study, significant decreases in total WBC (Leukocytes) and neutrophilcounts were seen in the Group 4 after atorvastatin administration may due to the case of rhabdomylysis and muscle weakness that concomitant with this type of drugs. Also [22] , proved that statins such as lovastatin were reported to cause hemolytic anemia . And any Drug-induced hematologic disorders like statins can affect any cell line, including white blood cells (WBCs), [23].

6. Conclusions

Extracted octacosanol kept some hematological parameters particularly Leukocytes in the normal ranges, and successfully use more than atorvastatin and without side effects. For this reason the extracted octacosanol successfully used in treatment CAD without serious side effects.

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