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The Role of Endocannabinoid System in the Obesity Induced Atherogenesis. What Are the Possible Mechanism/s Involved?

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Abstract

Objectives: The present work was designed to studythe role of endocannabinoid system in the obesity associated atherogenesis and trying to clarify its possible mechanism/s of action. **Methods:** Thirty adult male wistar albino rats were utilized in the present experiment. They were divided into three equal groups (10 rats each); Group 1: Lean control group, which were fed normal laboratory chow diet and gavaged once daily by dimethyl sulfoxide in a dose of 0.6ml/kg /day for 10 weeks. Group 2: Atherogenic diet group which were fed high fat diet and gavaged once daily by dimethyl sulfoxide as group 1. Group 3: Atherogenic diet treated group which were fed high fat diet and gavaged once daily by NIDA-41020 (a selective cannabinoid receptor 1 blocker) in a dose of 10mg/ kg /day for 10 weeks. Then body mass index (BMI), bleeding time, and total clotting time were assessed. After that, the animals were sacrificed and lipid profile, atherogenic index, bleeding time, platelet aggregation percentage, clot retractions, clotting time, prothrombin time (PT), activated partial thromboplastin time (aPTT), total & differential leukocytic counts and serum adiponectin levels were assessed in all groups. The aorta was obtained from each animal dissected and stained by haematoxylin/eosin and oil Red O staining for histological examination and detection of aortic thickness and foam cells deposition. **Results:** The laboratory investigations and histological examination revealed, significant increases in BMI, lipid profile, atherogenic index, platelet aggregation%, peripheral monocytic count, and aortic thickness in the high fat diet received group versus lean controls which were otherwise associated with significant decreases in total clotting time, PT, aPTT, serum HDL & adiponectin levels. These changes were significantly and profoundly inhibited by the administration of the cannabinoid receptor antagonist. **Conclusion:** The endocannabinoid system is involved in the atherogenic changes associated with obesity. These effects were attributed to interference with serum adiponectin level, dyslipidemia, hypercoagulability, increased platelet activation & peripheral as well as endothelial recruitment of monocytes. These effects were found to be via activation of cannabinoid 1 receptor.

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INTRODUCTION

Cannabinoids are the major active constituents of Cannabis sativa. They are oxygen-containing aromatic hydrocarbon compounds[1]. About seventy different naturally occurring Cannabinoids are now recognized [2]. Many tissues in the body can synthesize a group of these related unsaturated fatty acid derivatives named the endocannabinoids. They act as endogenous ligands for the Cannabinoid receptors and are involved in the regulation of different physiologic functions[3]. There are two specific Cannabinoid receptors, namely CB1 and CB2. CB1 receptors are expressed predominantly in the brain and peripheral tissues, including cardiac muscle, liver, gastrointestinal tract, vascular endothelium, and vascular smooth muscle cells [4,5]. While CB2 receptors are predominantly expressed on immune cells. Moreover, both receptors have been recently identified on endothelial cells, where their expression is regulated by the pro inflammatory cytokines[6]. Other tissues as adipocytes[7], platelets[8], and bronchial epithelium were found to express both CB1 and CB2 receptors as well [9].

Anandamide is considered the most active endocannabinoid receptor agonist[10]. The endocannabinoids are involved in diverse physiological functions, many of which are related to stress-recovery systems and to the maintenance of homeostatic balance[11]. Moreover, the endocannabinoid system is involved in neuro-protection [12], inhibition of nociception[13], and regulation of motor activities[14] as well as the modulations of the immune and inflammatory responses [15].

Atherosclerosis is a chronic inflammatory disease characterized by the build-up of lipids and cellular debris within arterial walls which is influenced by numerous biochemical factors[16]. During atherogenesis, monocytes adhere to sites of vascular endothelial injury and migrate into the vascular wall where they proliferate and differentiate into macrophages. In the intima, they ingest atherogenic lipoproteins, such as the oxidized low density lipoproteins (OxLDL), and their oxysterol constituents as 7-ketocholesterol and transform into foam cells. Activation of platelets is nowadays recognized as the essential step in promoting leukocyte adhesion and determining the progression of atherosclerotic lesion formation [17].

The links between white blood cells, platelets and atherogenesis are well established,[18] while the role played by cannabinoids in atherosclerosis still remains to be elucidated.

The secretion of endocannabinoids from the endothelium and their modulatory effects on immune functions and inflammation may point to their involvement and participation in the pathogenesis of atherosclerosis and thrombosis.

Several studies have detected a relation between Cannabinoids intake and ischemic heart disease[19,20]. Also, others have reported a procoagulatory effect for these compounds [21].However, Steffens et al [22] reported that cannabinoids can cause reduction in the development of atherosclerotic plaques in a murine knock out model of atherogenesis. Also, **Zolese et al**[23] showed that cannabinoids may protect the low density lipoproteins (LDL) from oxidation which is the most important step in the build-up of atherogenesis. It was believed that adipocytes play a critical role in the development of atherosclerosis. It was suggested adipokines could be the link between endocannabinoid system and atherosclerosis [24, 25].

The previous studies have demonstrated that, the activity of endocannabinoid system is up-regulated in obesity and is converted from a system which is intermittently and transiently activated, to a one chronically and persistently over activated[26]. This over activity not only promotes fat storage in the adipocytes, but can also be associated with insulin resistance (IR). The metabolic disturbances commonly occurring in patients with IR are atherogenic dyslipidemia, hypertension, glucose intolerance and pro-thrombotic state[27]. All of which are risk factors for atherosclerosis and other cardiovascular diseases.

Adiponectin is considered an important regulator in this field due to its distinct anti-atherogenic and anti-inflammatory properties in contrast with other adipokines [28]. It is suggested that, the disturbed secretion of adiponectin in obesity might, at least partly, account for the links between obesity, atherosclerosis and the endocannabinoid system.

It was found that, treatment with Rimonabant (a cannabinoid receptor blocker) eliminates the part of obesity controlled by the endocannabinoid system such as increased appetite, excessive hunger and food intake and also increases adiponectin level leading to increased fat metabolism. This could result in reducing cardiovascular risk factors through weight loss and improvement of other metabolic risk factors profile [29].

In face of these discrepancies and contradictory reports concerning the effects of endocannabinoids on blood coagulation and atherosclerosis, this study was designed to investigate the effects of "a cannabinoid receptor antagonist" (NIDA-41020) on platelet function, blood coagulability and lipid profile in an experimental model of obesity associated atherogenesis. Also, the changes in the adiponectin level and its relationship with platelet function were determined.

MATERIALS AND METHODS

Animals

A total number of thirty healthy, adult, male albino rats weighing 190-220 gms were used. The animals had a free access to water, were kept at room temperature and were maintained on a 12 h light/dark cycles. The rats were accommodated to animal house conditions for two weeks before the experiment were undertaken. They were kept in steel wire cages and divided into three equal groups (10 each); Group 1: Lean control group: They were fed normal laboratory chow diet consisting of 25.8% protein, 62.8% carbohydrates and 11.4% fat; about 12.6 KJ/g and were gavaged once daily by dimethyl sulfoxide (ADWIC Lab. Chemicals, Egypt) in a dose of 0.6ml/kg /day for 10 weeks[30,31]. Group 2: Atherogenic diet received group: They were fed high fat diet consisting of 16.45% protein, 25.6% carbohydrate and 58.0% fat in the form of cotton seed oil added to the laboratory chow diet; about 23.4KJ/g and were gavaged once daily with dimethyl sulfoxide as group 1 for 10 weeks [(30,31)]. Group 3: Atherogenic diet received group treated by NIDA-41020 (selective CB1 receptor antagonist): They were fed high fat diet and gavaged once daily by NIDA-41020 **(**Sigma Chemical, St. Louis, MO.) in a dose of 10mg/kg /day for 10 weeks[30, 31]. All investigations were conducted in accordance with the guiding principles for the care and use of research animals and were approved by the Institutional Research Board.

Methods:

At the start and the end of the experimental period, (2 hours after the last dose),[32] the rats were weighted in all groups by the digital balance, and their lengths were taken from nose to anus. Body mass index (BMI) was calculated using the following equation: BMI (gm / cm^2) = body weight (gm)/ length ^{2}(cm^2) [(33)].

-The bleeding time and the total clotting time: were performed according to Garcia-Manzano et al [34].

II- Blood Sampling:

General anesthesia was performed using sodium thiopental 50 mg/kg body weight intra peritoneally[35], then rats were sacrificed by decapitation and blood sample was collected and were used for determination of:

-Clot retraction: Which is expressed as the amount of serum extruded from a clot of 1 ml of blood after 45 min. of sampling incubated at 37ºC [36].

-Platelet aggregation: Two ml were collected in a plastic centrifuge tube containing sodium citrate buffer solution

(0.11 mol/ L) at ratio of one part sodium citrate to nine parts blood [1:9] [37]. Plasma was separated by centrifugation of blood at 3000 rpm for 15 min. The supernatant plasma was immediately used. Determination of platelet aggregation was done according to Marcus et al [38], using DiaMed kit described. Platelets were stimulated to aggregate by ADP. These aggregation were determined by optical density in turbo optical instrument (540 dual channel aggregometer). Maximum aggregation is recorded as a percentage.

Differential leukocytic count: Leukocyte populations were quantitatively assessed using automatized blood cell counter **(39)**.

Prothrombin time (PT) and activated partial thromboplastin time (aPTT): Were performed according to the method described by Ansell [37], using Dade-Behring Kit.

Lipid profile and serum adiponectin levels: Two ml were collected in a plastic centrifuge tube and allowed to coagulate then serum was separated by centrifugation of sample at 3000 rpm for 15 min. The supernatant serum was used for determination of total cholesterol levels (TC), high density lipoprotein cholesterol (HDL) and Triglycerides (TG) according to the method described by Tietz[40] (Cat. No. BK 8148 CGPO – PAP). LDL was calculated as follows: LDL=TC-HDL-TG/5.

Atherogenic index: was calculated as follows [TC – (HDL-C)] / (HDL-C)**[41]**.

Adiponectin: Rat adiponectin ELISA, (R&D Systems, Inc.-Minneapolis, MN 55413-USA) was used for determination of adiponectin concentration.

Sampling of tissues

After collection of blood samples, the thoracic and abdominal aorta were dissected, cleaned from adventitia and immersed in a phosphate buffered formalin solution**[30]**. Paraffin section were prepared and stained by haematoxylin/ eosin for histological examination of aortic strips and determination of their thickness and Oil Red O staining for detection of foam cells deposition. Stains were performed as per the standard protocols **[42]**.The thickness of the aorta was recorded using a simple bench microscope (Olympus CH-2). Then the value of the thickness from each sample was finally statistically compared.

Statistical analysis

Statistical analysis in this study was preformed utilizing the SPSS released 10.0 program for Windows (SPSS Inc. Chicago, IL, USA). All data were expressed as mean ± Standard Deviation ($\overline{x}$ ± SD). Analysis of

variance (one way ANOVA of F test) was used for comparison of means of more than two groups

RESULTS

Measurements of the body mass indices: BMI was measured at the start of the experiment and was approximately similar in different animal groups. At the end of the experiment, the BMI measurements had significantly increased in the atherogenic diet received group versus lean controls. This increase in the BMI by atherogenic diet was significantly reduced by the administration of the Cannabinoid receptor blocker NIDA-41020 for 10 weeks (Tab. 1).

Lipid profile and atherogenic index:

In the present study, Lipid profile demonstrated significant increases in the levels of TC, TG and LDL associated with an otherwise significant decrease in HDL in the blood samples obtained from atherogenic diet received group when compared to their levels in the lean control group. However, there were significant decreases in TC, TG, and LDL associated with a significant increase in the HDL in the blood samples obtained from the cannabinoid receptor antagonist treated rats when compared to group 2. In addition, by calculating the atherogenic index in group 2, we detected significant increases in their values versus lean controls, and these increases were significantly reduced by administration of CB1 blocker in the 3rd group (Tab.1).

Haemostatic parameters

As regards haemostatic changes, it was demonstrated a significant decrease in the bleeding time associated with statistically significant increases in platelet aggregation percentage and clot retraction activity in blood samples obtained from the rats which received the atherogenic dietary regimen for 10 weeks when compared to lean controls. These haemostatic changes were significantly reversed by administration of CBI receptor antagonist (Tab.2).

Furthermore, there were statistically significant shortening in clotting time, PT, and aPTT in the blood samples obtained fromatherogenic diet untreated group versus their values in lean controls, which were significantly increased back toward to normal control values by administration of cannabinoid 1 receptor blocker in the 3rd group (tab.2).

Differential leucocytic count (monocytic count): The present study revealed a significant increase in the Monocytecounts in the blood obtained from atherogenic diet received group when compared to their counts in thelean controls. This increase in monocytic count was significantly reversed toward the normal control value by

administration of CBI receptor antagonist (Tab.2).

Adiponectin levels

The levels of the adepokine "adiponectin" showed significant decreases in the atherogenic diet received group versus lean controls. However, these levels were significantly increased in the Cannabenoid receptor blocker treated group versus 2nd group (Tab.3).

The adiponectin levels were positively correlated with HDL in either 2nd or 3rd groups (r= 0.7812 and 0.7413 respectively with P<0.05). But it was negatively correlated with platelet aggregation percentages, where r= - 0.8550, and - 0.9340 respectively with P<0.05 in the same groups. This correlation pointed to the interrelation between the adiponectin levels, dyslipidemia, platelet aggregation and endocannabinoid system on the atherosclerotic enhancement during obesity.

Histological examination:

The histological findings of the aorta taken from atherogenic diet received rats showed prominent thickening of the media, splitting⬅ within the media by lipid, and extensive extracellular foam cell deposit⬏ on their outermost layer. The extracellular foam cells changed in certain areas to necrotic fibrous cap** with variable amount of cell loss (*fig. c& d).*These changes are absent in the aorta obtained from the control rats *(fig. a& b).* Administration of Cannabenoid receptor antagonist in atherogenic diet received rats led to decrease in their aortic thickness, disappearance of the foam cell deposit with reduction in the aortic connective tissues *(fig. e& f)*

Table (1): Body mass index (BMI), Total cholesterol (TC), Triglycerides (TG), Low density lipoproteins (LDL), High density lipoproteins (HDL) and Atherogenic (Athero.) index in all studied groups

			1st group (n=10)	***2nd group (n=10)***	***3rd group (n=10)***
BMI	Mean ± SD		0.48 ± 0.06	0.76± 0.05	0.50 ± 0.04
	L S D	**vs*1st group***		0.079**	0.025-
		vs*2nd group*			0.054*
TC (mg/dL)			61.89±1.93	79.46±8.1	70.30±10.1
	L S D	**vs*1st group***		17.60***	8.4**
		vs*2nd group*			9.20**
TG (mg/dL)			51.21±2.54	62.40±8.60	55.20±3.0
	L S D	**vs*1st group***		9.10***	3.10*
		vs*2nd group*			8.20**
LDL (mg/dL)			11.82±2.21	43.37±8.7	19.14±2.42
	L S D	**vs*1st group***		31.55***	7.32*
		vs*2nd group*			24.23***
HDL (mg/dL)			40.61±2.71	31.92±3.2	39.2±2.61
	L S D	**vs*1st group***		8.68***	1.40-
		vs*2nd group*			7.28***
Athero. Index			0.54±0.8	1.78±0.93	0.82±0.8
	L S D	**vs*1st group***		1.238***	0.61*
		vs*2nd group*			0.93**

Values are means± slandered deviation ($\bar{x}$ ± SD).
Least significant difference (LSD) among values was analyzed by one way ANOVA, When the Interaction was significant (P<0.05). P<0.05(*),P<0.01(**),P<0.001(***),and P>0.05(-).
1st group = Control lean group, 2nd group = Atherogenic diet received group and 3rd group = Atherogenic diet received group treated by Cannabinoid receptor blocker

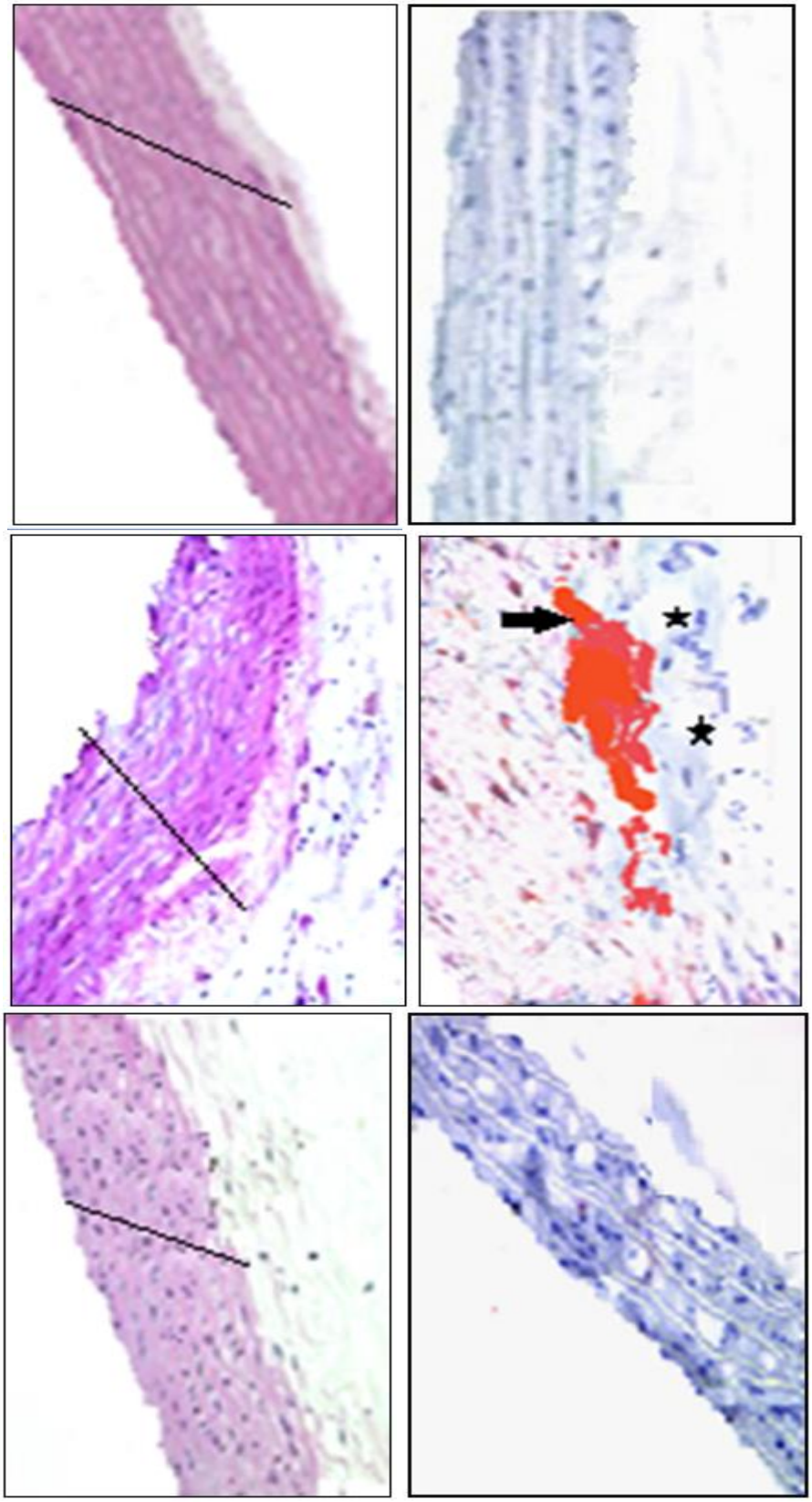

Fig. (1): Photographic illustration of Haematoxylin-Eosin and oil red O stained aortic samples (×400), taken from lean control (fig. a and b), atherogenic diet received group (fig. c and d) , atherogenic diet received group treated by Cannabinoid receptor antagonist (fig. e and f) groups

Table (2): Bleeding time(Bl.T),Platelet aggregation percentage (PA%),Clot retraction (CR), total clotting time (CT),prothrombin time(PT),activated partial thromboplastin time (aPTT),and monocytic count (MON.) in different studied groups

			1st group (n= 10)	*2nd group (n= 10)*	*3rd group (n= 10)*
Bl.T (Sec.)	Mean ± SD		10.77±1.51	6.98±0.75	9.03±1.16
	LSD	**vs *1st group***		3.79***	1.8*
		vs *2nd group*			2.05**
PA (%)	$\bar{x}$ ± SD		22.4±1.34	37±5.23	25.9±1.52
	L S D	**vs *1st group***		12.60**	1.50-
		vs *2nd group*			11.10**
CR (ml)	$\bar{x}$ ± SD		0.374±0.045	0.450±0.33	0.399±0.044
	LSD	**vs *1st group***		0.04*	**0.006-**
		vs *2nd group*			0.036*
CT (Sec.)	Mean ± SD		207±30	174.6±32	203.7±29.86
	L S D	**vs *1st group***		32.40*	3.30-
		vs *2nd group*			29. 10*
***PT* (Sec.)**	Mean± SD		13.49±0.77	9.85±2.10	11.69±2.18
	LSD	**vs *1st group***		3. 64***	1.8*
		vs *2nd group*			1.48*
***aPTT* (Sec.)**	Mean ± SD		19.49±2.98	9.34±1.5	14.5± 2.2
	L S D	**vs *1st group***		10.1***	4.9*
		vs *2nd group*			5.6**
Mon.coun ($\times 10^3/mm^3$)	Mean ± SD		0.58±0.08	0.85 ± 0.9	0.64±0.06
	L S D	**vs *1st group***		**0.23****	0.64-
		vs *2nd group*			0.168*

Values are means± slandered deviation ($\bar{x}$ ± SD).
Least significant difference (LSD) among values was analyzed by one way ANOVA, When the Interaction was significant (P<0.05). P<0.05(*),P<0.01(**),P<0.001(***),and P>0.05(-).
1st group = Control lean group, 2nd group = Atherogenic diet received group and 3rd group = Atherogenic diet received group treated by Cannabinoid receptor blocker

Table (3): Serum adiponectin level (Adipo.) , and Aortic (Aort.)Thickness in the different studied groups in different studied groups

			1st group (n= 10)	*2nd group (n= 10)*	*3rd group (n= 10)*
Aipo (ug/ml)	Mean ± SD		10.33±1.94	6.74±1.34	9.79±0.89
	LSD	**vs *1st group***		10.88**	7.83-
		vs *2nd group*			3.05**
Aort. Thickness (mm)	$\bar{x}$ ± SD		0.058±0.006	0.079±0.007	0.063±0.010
	L S D	**vs *1st group***		0.015**	0.007*
		vs *2nd group*			0.023**

Values are means± slandered deviation ($\bar{x}$ ± SD).
Least significant difference (LSD) among values was analyzed by one way ANOVA, When the Interaction was significant (P<0.05). P<0.05(*),P<0.01(**),P<0.001(***),and P>0.05(-).
1st group = Control lean group, 2nd group = Atherogenic diet received group and 3rd group = Atherogenic diet received group treated by Cannabinoid receptor blocker

DISCUSSION:

The present study revealed significant increases in measured BMI, blood cholesterols, triglycerides, low density lipoprotein and atherogenic index associated with significant decrease in high density lipoproteins in the rats which received high fat diet versus their values in the lean control group. These changes were significantly reversed by administration of the CB1 Blocker, which pointed to the participation of the endocannabinoids in the disturbed lipid metabolism and atherogenic dyslipidemia accompanied with obesity.

These results are in concordance with the previous studies carried on the experimental animals by Schafer et al[43] who found a significant increases in plasma

levels of triglycerides, total cholesterols and low-density lipoprotein cholesterols (LDL-C) in obese Zucker rats versus lean rats from the same species.

In addition, Cota et al [44] detected the presence of CB1 receptors in the primary adipocyte and reported that its activation could enhance lipogensis. Also, in other studies, it was demonstrated that lacking of CB1 receptor gene (CB1 _/_) and /or after the use of CB1 receptor blocking , there were exhibited reduction in the total fat mass, body weight, and enhanced lipolysis in mice through the induction of beta oxidation enzymes[45,46].

Moreover, in a clinical trial carried on obese patients with dyslipidemia. It was found that administration of a selective CB1 blocker (Rimonabant) affected the lipid profile by decreasing serum triglycerides, increasing high-density lipoprotein fraction, and improving the metabolic risk profile. These effects were independent to some extent of the weight loss achieved by Rimonabant[(47].

However, in contrast to our study, Steffens et al[48] found non-significant changes in serum lipid profile after administration of WIN 55212-2 (a synthetic cannabinoid receptor agonist with CB2 selectivity) in mice.

The differences between the present study and the pervious study could be explained by differences in the selectivity of the cannabinoid receptor activated , as selective CB1 receptor antagonist were used in our study. Moreover, the two studies were conducted on different animal species

On the other hand, the present study demonstrated a significant increase in the platelet activation represented by significant increases in platelet aggregation, clot retraction and reduced bleeding time, accompanied by increased peripheral monocytic count in the high fat diet received group, which were markedly inhibited by the administration of the cannabinoid receptor antagonist.

These results are in agreement with Catani et al [49] who detected the presence of the two known Cannabinoid receptor subtypes CB1and CB2 on the cell membrane of human platelets, suggesting that the platelets are target cells for the Cannabinoids action.

In addition, Tanikawa et al[50] demonstrated significant enhancement of ADP-induced platelet aggregation at lower concentrations in platelet-rich plasma from obese versus lean rats, which was attenuated by CB1 receptor antagonism.

Also, in humans Deusch et al [21] found that Delta-9-tetrahydrocannabinol (cannabinoid receptor agonist) in high concentrations activated the human platelets in vitro by increasing the expression of both glycoprotein IIb-IIIa and P- selectin on the platelets plasma membrane. The platelet

activation was mediated through transforming platelet membrane glycoprotein IIb-IIIa complexes into a conformational state which is considered a component for binding fibrinogen, and by integration of P-selectin into the cytoplasmic membrane of activated platelets as the internal α-granule[51], mediates heterotypic aggregate formation, and serves as a marker for platelet secretion and activation.

The activated platelets are the essential step in promoting leukocyte adhesion, the precursors of the foam cells of atherosclerotic lesion [52].

On the other aspect, the present results showed peripheral recruitment of the monocytes associated with histopathological deposition of the foam cells on the aortic endothelium and increased intimal thickness in the atherogenic diet untreated group which was inhibited after treatment by NIDA-41020.

These results are in agreement with Han et al (53) who detected the expression of CB1 and CB2 receptors in freshly isolated human monocytes and identified their up-regulation by CB1 receptor activation, as its activation differentiated monocytes into tissue macrophages.

Moreover, Schafer et al[54] demonstrated that the activated platelets released serum monocyte chemo-attractant protein1 (MCP-1) which triggered monocyte recruitment to the vascular wall. This protein was significantly increased in obese versus lean rats and decreased following treatment with Rimonabant**.** Also, Sugamura et al (55) identified CB1 expression in macrophages of advanced atheromas. As the atherosclerotic coronary artery sections from patients with unstable angina had significantly higher expression of CB1 receptors when compared to coronary artery sections from patients with stable angina.

in contrary to our results, the study of Steffens et al [56] who found that platelet aggregation, and macrophage chemotaxis were inhibited in vitro with a small dose of tetrahydrocannabinol (1mg/kg/day) and this effect was completely blocked by the specific CB2 receptor antagonist. In addition, Rajesh et al [57] demonstrated that CB2 antagonism prevents atherogenesis through decrease in the trans-endothelial migration of monocytes.

This controversy between the present study and the above mentioned studies could be explained by the differences in the experimental models of the study (in vivo versus in vitro), used dose of tetrahydrocannabinol, as its atherogenic protective effect has been proved to be dose dependent[56] and the selectivity of the cannabinoid receptor examined. In the present study we examine the activity of CB1 receptor by using selective CB1 blocker versus examination of CB2 receptors in the above mentioned study.

As regard the significant inhibitory effects of cannabinoid receptor blocker on the hyper-coagulable state induced by high fat diet in our obese model represented by significant reduction in the duration of clotting, PT, and PTT times versus lean controls. Stein and Goldman [58] reported that obesity has long been regarded as a risk factor for various coagulation abnormalities. As they documented that, plasminogen activator inhibitor-1, Von Willebrand factor, fibrinogen, factor VII and factor VIII were all present in higher levels in the obese population.

The studies conducted by Pal Pacher and Sabine Steffens[19] and Sauvanier et al[20] have established the presence of an association between cannabinoids intake and myocardial infarction as well as increased prevalence of juvenile onset thromboangitis-obliterans. In addition, Deusch et al [(21)] have reported a procoagulatory effect for these compounds.

So we can suggest that the possible atherogenic induced effect of the endocannabinoid system activation in obese model was through a triple pathway, firstly mediation of metabolic dyslipidemia, secondly through increased platelet activation & aggregation which participated in the local vascular atherosclerotic changes, thirdly by enhancement of hypercoagulability status.

Also, the present study detected a significant decrease in the serum adiponectin levels of obese rats versus their levels in lean controls, which were statistically increased by administration of the cannabinoid receptor antagonist. These adiponectin changes were negatively correlated with the increased platelet aggregation, and positively correlated with HDL in all studied group.

These results were supported by the data showing a decreased plasma level of adiponectin in patients with cardiovascular risk factors such as obesity and diabetes as well as patients with coronary heart diseases. Also, it was revealed that adiponectin could exhibit protective effects against development of arteriosclerosis through prevention of cholesterol transport in macrophages and decreased synthesis of the pro-inflammatory interleukins on the vascular endothelial surface thus reducing atherosclerosis. Moreover, other study demonstrated the improvement of the fibrinolytic activity by adiponectin and leptin during exercise [59, 60].

The in vitro study conducted by Gary-Bobo et al[61] reported that the cannabinoid receptor blocker; Rimonabant, stimulated mRNA expression and protein levels of adiponectin in cultured mouse 3T3 F442A preadipocytes. In addition, in vivo study carried by Maccarrone et al [62] found that the administration of Rimonabant at a

dose of 20 mg could increase the plasma adiponectin levels and decrease CRP in human beings.

However in contradiction with our results, Bobbert et al [63] found that the proteoly tic cleavage product of the adiponectin known as globular (gAPN), may induce opposite effects when compared to the endogenous full length form of adiponectin (fAPN). As the gAPN facilitated the atherogenic process in endothelial cells through inducing NF-kappa B transcription factor for pro-inflammatory genes**.**

This controversy between ours and their study may be explained by the types of adiponectin affected by the endocannabinoids. From our conducted results and other mentioned results, it is clear that endocannabinoids could be interfering with adiponectin expression which mediates dyslipidimia, hyper-coagulability and the endothelial inflammatory atherosclerotic changes occurred in obesity.

CONCLUSION

The present study concluded that the endocannabinoid system is involved in the atherogenic changes associated with obesity. These effects were attributed to interference with serum adiponectin level, dyslipidemia, hypercoagulability, increased platelet activation & peripheral as well as endothelial recruitment of the monocytes. These endocannabinoid induced effects were found to be via activation of cannabinoid 1 receptor .These findings could offer a new potential therapeutic option for this condition.

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