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A Study on the Immunological Changes in Adult Male Obese Rats and the Possible Immune Modulator Effect of Conjugated Linoleic Acid Supplementation

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Abstract

Background: Conjugated Linoleic Acid [CLA] is suggested as a novel drug to improve obesity.

Objectives: The aim of this study was to elucidate the possible role of CLA in ameliorating the pathophysiology in obese rats and modulate its immune function.

Methods: Forty adult male albino rats of local strain, weighing [120-160] grams each, were used. Rats were randomly divided into five equal experimental groups, control, CLA-supplemented, obese, CLA-prophylactic obese and CLA-treated obese groups obesity was induced by High Fat Diet [HFD] for 12 weeks. CLA was administered orally in a dose of 1500mg/kg body weight for 12 weeks. Initial and final Body Weight (BW) and Body Mass Index (BMI) and food intake were measured. Total Leucocytic Count (TLC) and its differential count and serum Tumor Necrosis Factor-alpha (TNF-α) were measured. Visceral fat and total white abdominal fat to each 100 [gm]/BW were measured. Caspase 3 expression was evaluated in the abdominal fat biopsies using immunohistochemistry.

Results: High fat diet resulted in deterioration and impairment of most measured parameters. CLA supplementation decreased final BW of obese rats, food intake, visceral fat, and total abdominal together with increased CD8 +, CD4+ T lymphocytes and eosinophilic %. Moreover, CLA supplementation decreased TNF-α in CLA-supplemented and CLA-prophylactic groups but not in CLA-treated group. High fat diet increased TLC and CD4 + T lymphocytes but decrease eosinophilic % and CD8+ T lymphocytes. In addition, CLA increased the positivity of caspase 3 in CLA-prophylactics and CLA-treated obese groups.

Conclusion: CLA supplementation, either as a prophylactic agent or a therapeutic one, to the high fat feed rats could ameliorate most of the detrimental effects of obesity, particularly those related to the immune system. This beneficial effect of CLA could be explained by its immune modulator, anti-inflammatory and pro apoptotic effects.

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Key Words: *High fat induced obesity – Conjugated linoleic acid – Immunity – Rats.*

Introduction

OVER the last few decades, the prevalence of obesity has been increased at an alarming rate both in developed and developing countries particularly among adolescents **[1]**. World Health Organization [WHO] in 2016, has reported that nearly 124 million children and adolescents and about 650 million adults are obese worldwide.

Obesity usually increases the risk of developing coronary heart disease, congestive heart failure, stroke, gall bladder disease, hepatic steatosis, sleep apnea, and cancer of endometrium, breast, prostate, and colon, therefore, WHO has defined obesity as one of the top ten global health problems **[2]**.

It was reported that obesity has been linked to impaired immunity and lower antibody response **[3]**. Also, obesity has been associated with a higher incidence of infections **[4,5]**.

Conjugated Linoleic Acid [CLA] is one of Polyunsaturated Fatty Acids [PUFA] that is found in dairy products and ruminant meats **[6]**. In the literature, it was found that CLA has powerful anti-adiposity functions in both humans and animals. This beneficial effect of CLA was a motive in this study to elaborate the possible immune modulator effect of this agent in obesity-induced immune dysfunction in an experimental model of high fat diet-induced obese rats.

Material and Methods

Experimental animals: Forty adult male albino rats of local strain, weighing [120-160] grams each

were used in this study. The rats were housed in a standard laboratory conditions with a natural 12/12 h light-dark cycle. The rats were kept in wire mesh cages [8 rats per a cage with dimensions 80 X 40 X 30cm] at a controlled temperature of 25 ±2ºC with free access to food and water ad libitum. The animals were housed in the Animal House at Faculty of Medicine, Menoufia University, from Nov. 2016-April 2017 throughout the whole study period. All experimental procedures were conducted in adherence to the international guide for caring and use of laboratory animals of the National Research Council [NRC] 2002, with the approval of the Ethics Committee of the Menoufia University.

Animal groups:

The rats were divided equally into five experimental groups, 8 rats in each of them.

1- Control group [C]: The rats were fed a normal standard commercial rat chow diet and were subjected to 0.5ml olive oil by intragastric oral gavage as a vehicle, once daily for 3 months.

2- CLA-supplemented group [CLAS]: The rats were supplemented with the normal standard commercial rat chow diet and were ingested with 1.5% CLA [equivalent to approximately 1500mg/kg BW per day] intragastric by oral gavage, once daily for 3 months **[7,8]**.

3- Obese group [O]: Diet induced obesity was experimentally obtained by feeding the rats with High Fat Diet [HFD] for 3 months **[9]**.

4- CLA -prophylactic obese group [CLAP]: The rats were supplemented with HFD concomitant with 1.5% CLA by oral gavage, once daily for 3 months.

5- CLA-treated obese group [CLAT]: In which rats were rendered obese by their supplementation with HFD for 3 months, afterwards they were supplemented with 1.5% CLA by oral gavage once daily for 3 months.

Drug and chemicals:

Tonalin® [CLA]: was obtained in the form of capsules, each one contain 2ml [1600mg CLA] [NBTY Manufacturing LLC., Bayport, NY11705, USA]. CLA was dissolved in olive oil **[10]**.

Preparation of HFD:

HFD was prepared according to American Institute of Nutrition [AIN]. The rats were fed semi-purified diet formula based on the AIN-93M **[30]**. HFD contained 20g of fat/100g of diet [19g of butter oil and 1g of soybean oil to provide essential fatty acids]. Compared to the standard commercial rat chow that provides 12.62kJ per gm of diet, this HFD provided 19.34kJ per gm diet, of which fat provides 7.74kJ [40% fat per weight of diet] **[10]**.

Experimental procedures:

1- *Anthropometric measures:*

- Initial and final Body Weight [BW] by using a digital balance.
- Initial and final Body Mass Index [BMI] were calculated {BMI=BW weight [gm]/length ^{2} [cm^2]. The cut off value of obesity is advocated when the BMI is more than 0.68gm/cm^2 **[11]**.
- Abdominal circumference **[11]**.

2- *Biochemical analysis:*

- At the end of the experiment, two ml blood samples were withdrawn from all rats through the retro-orbital route using heparinized capillary tubes. One ml of the obtained whole blood was collected in a tube containing EDTA for assessing:

A- Total leucocytic count.

B- Eosinophilic %.

C- CD4%, CD8% and CD4/CD8 ratio by flow cytometry [FITC/PE/Z0017483, El-Kser El-Ainy hospital].

- One ml of the blood was left to clot at room temperature in a water bath for 15 minutes, then it was centrifuged at 4000 rotation per minute [r.p.m.] for 5 minutes. The serum was collected and frozen at –20 until used for estimation of:

D- TNF-α, using a commercial ELISA kit, Catalog No. ERT2010-1 provided by Assaypro LLC CO USA.

3- *Immunohistochemical study of caspase-3:*

At the end of experiments, the rats were anesthetized by intraperitoneal injection of sodium phenobarbitol [40mg/Kg/BW]. Then laparotomy was done and total fat mass, the visceral pad of fat along the intestine was dissected and weighted and then processed for further immunohistochemical study caspase-3 [as a marker of apoptosis]. The primary antibody used was mouse monocolonal antibodies directed against caspase 3 (7ml) (lab vision, USA ready to use {cat.#MS-1123-R7}. From each case, multiple 4- µm-thickness sections were cut. The slides were subjected to subsequent steps of deparaffinization and rehydration. Antigen retrieval performed by boiling in citrate buffer saline [pH 6] for 20 minutes, followed by cooling at room temperature. Endogenous peroxidase activity was blocked by incubation with 6% H_2O_2

in methanol. The primary antibodies incubated overnight at room temperature. Immunoreactivity for caspase 3 was visualized using Envision system [Dako, Copenhagen, Denmark] with DAB chromogen as substrate and Mayer's hematoxylin as counterstain. Negative control was prepared by substituting the primary antibody with cross-matched isotopes. Positive tissue control for caspase 3 was Jurkat cells. Tonsil. Cytoplasmic staining in any number of cells was required to assign positive caspase 3 expression.

4- Weighting of visceral fat pads and total abdominal fat to each 100gm BW **[12,13]**.

5- Measurement of food intake **[14]**.

Statistics analysis:

The SPSS Version 23, [Armnok, NY: IBM Corp] was used for analysis of data. The results were expressed as mean ± Standard Deviation [SD]. The significance of differences between groups was determined by one-way analysis of variance [ANOVA] and post-hoc Tukey test was done. Chi-square test was used to study association between qualitative variables. p-values <0.05 were considered statistically significant.

Results

Anthropometric and fat mass measurement:

The present study showed that, final BW and final BMI of the obese group significantly higher compared to their matching control group parameters [p<0.05]. In initial body weight and initial BMI[before start of treatment] of CLAT group showed a significant increase compared to their matching control and obese groups parameters [$p<^{0.05}$].

A non-significant variation was seen in both initial and final BW and BMI between control group and both CLAS and CLAP group.

Whereas, CLA supplementation resulted in a significant decrease in final BW, final BMI CLAS, CLAP and CLAT group compared with obese group [p<0.05] (Table 1).

CLA administration resulted in a significant decrease [p<0.05] in visceral fat mass, total abdominal fat, Abdominal Circumference [AC] and % of body weight change in CLAS, CLAP and CLAT groups compared with obese group which showed a significant increase [p<0.05] in visceral fat mass, total abdominal fat to each 100 [gm] BW and % of body weight change when compared with control group Fig. (1).

Food intake measurement and biochemical analysis:

CLA supplementation resulted in a significant decrease [p<0.05] in food intake in CLAS, CLAP and CLAT groups compared with obese group. Also, CLAP and CLAT groups showed a significant decrease [p<0.05] in food intake compared with control group. A non-significant variation was seen in food intake between CLAS and obese groups compared with control group.

TNF-α significantly increased in obese and CLAT groups when compared with their value in control group. However, TNF-α significantly decreased in CLAS and CLAP groups compared with obese group. A non-significant variation was seen in TNF-α of CLAS and CLAP groups compared with control group.

CLA administration significantly decrease CD4/CD8 ratio in CLAS, CLAP and CLAT groups when compared with obese group which showed a significant increase in CD4/CD8 ratio compared with control group, (Table 2).

A non-significant variation was seen in TLC between control group and CLAS, CLAP and CLAT groups. In obese group, the TLC and $CD4^+$ T lymphocytes was significantly higher than their matching control group parameters [p<0.05]. CLAS, CLAP and CLAT groups showed a significant increase in $CD8^+$ and CD^+4T lymphocytes compared with corresponding results in both obese and control groups.

CLA administration resulted in a significant increase [p<0.05] in eosinophilic % in CLAS and CLAP groups compared with obese group which showed a significant decrease [p<0.05] in eosinophilic % when compared with control group. Figs. (2,3).

Caspase expression in different studied groups:

One rat out of eight in control group (12.5%), four rats out of eight in CLAS group (50%), one rat out of eight in obese group (12.5%), seven rats out of eight in both CLAP and CLAT groups (87.5%) showed cytoplasmic expression of caspase 3 in adipose tissue.

CLA supplementation resulted in a significant increase [p<0.05] in caspase 3 positivity level in CLAP and CLAT groups when compared with both control and obese groups (Table 3), Fig. (4).

Table (1): Inintial body weight (BW), final (BW), initial body mass index (BMI) and final (BMI) in control (C), CLA-supplemented (CLAS), obese (O), CLA-prophylactic obese (CLAP) and CLA-treated obese (CLAT) groups.

Parameter	C	CLAS	O	CLAP	CLAT
Initial BW (gm)	159.87± 12.82	158.62± 15.15 p<0.001 #	117.37±8.15 p<0.001 *	148.37±8.29 p<0.001 #	367.50±26.21 p<0.001 * p<0.001 #
Final BW (gm)	317.75±21.19	312.25±33.85 p 0.01 #	376.75±27.58 p 0.007 *	294.75±36.72 p 0.004#	232.50±22.08 p<0.001 * p<0.001 #
Initial BMI	0.36±0.02	0.35±0.02 p<0.001 #	0.28±0.01 p<0.001 *	0.34±0.02 p<0.001 #	0.73±0.04 p<0.001 * p<0.001 #
Final BMI	0.59±0.03	0.61 ±0.05 p 0.001 #	0.74±0.03 p 0.001 *	0.56±0.05 p<0.001 #	0.45±0.03 p<0.001 * p<0.001 #

Results are expressed as mean ± SD (n=8) significance was considered when p-value was <0.05. The marks * and # indicate that the values are significantly different when compared with corresponding values of C and O groups respectively.

Table (2): Food intake, tumor necrosis factor alpha (TNF- α), CD4/CD8 ratio in control (C), CLA-supplemented (CLAS), obese (O), CLA-prophylactic obese (CLAP) and CLA-treated obese (CLAT) groups.

Parameter	C	CLAS	O	CLAP	CLAT
Food intake (gm/d)	22.08±3.43	18.04±4.44 p 0.001 #	26.34±4.07	16.82±4.19 p 0.02 * p 0.001 #	17.42± 1.01 p 0.006 * p 0.001 #
TNF-α (ng/ml)	0.01 ±0.009	0.03±0.01 p<0.001 #	0.42±0.12 p<0.001 *	0.03±0.01 p<0.001 #	0.29±0.09 p 0.003 *
CD4/CD8 ratio	1.65±0.28	1.27±0.36 p<0.001 #	3.12±0.59 p<0.001 *	1.20±0.13 p<0.001 #	1.40±0.27 p<0.001 #

Results are expressed as mean ± SD (n=8) significance was considered when p-value was <0.05. The marks * and # indicate that the values are significantly different when compared with corresponding values of C and O groups respectively.

Table (3): Caspase 3 expression of positivity with inter group comparison in control (C), CLA-supplemented (CLAS), obese (O), CLA-prophylactic obese (CLAP) and CLA-treated obese (CLAT) groups.

Groups	C	CLAS	O	CLAP	CLAT
Caspase 3	1/8	4/8	1/8	7/8	7/8
Positivity	(12.5%)	(50%)	(12.5%)	(87.5%)	(87.8%)
p_1		0.28 {NS}	0.55 {NS}	0.01 {S}	0.01 {S}
p_2			0.28 {NS}	0.28 {NS}	0.28 {NS}
p_3				0.01 {S}	0.01 {S}
p_4					0.44 {NS}

Numbers of rats in each group was 8. $p1, p2, p3$ and $p4$ comparison with the C, CLAS, O, CLAP and CLAT groups, respectively, (S) Significant and (NS) Insignificant.

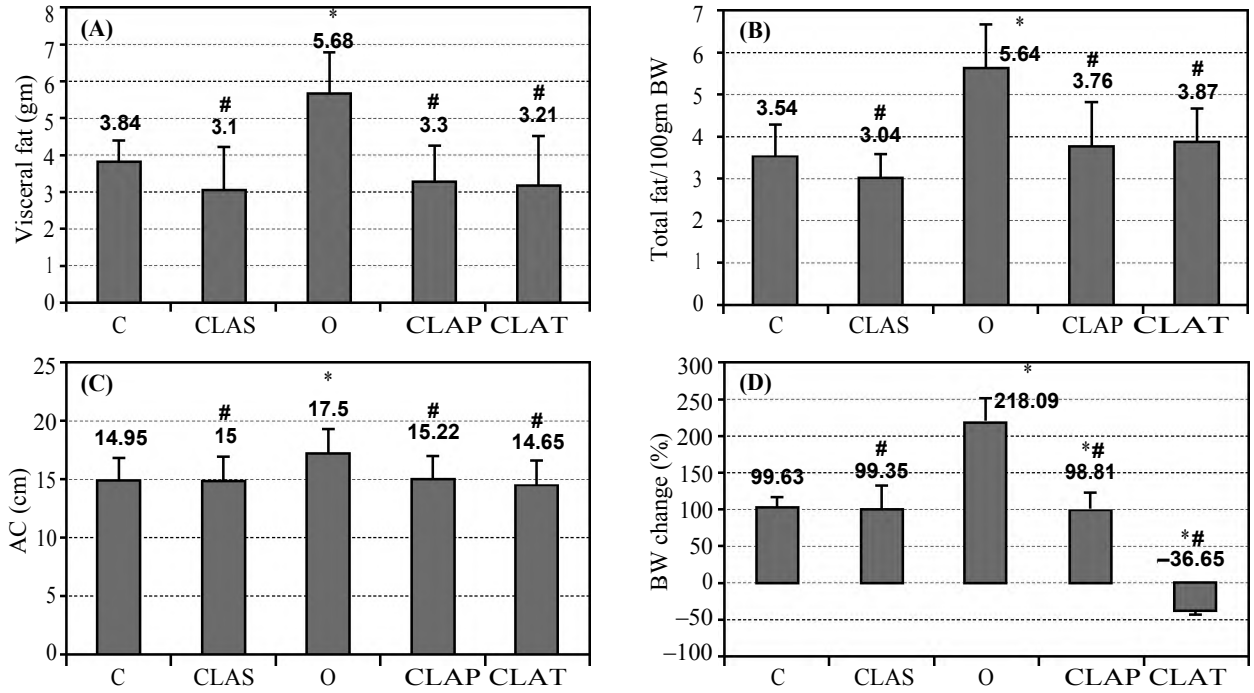

Fig. (1): Visceral fat (panel A) and total abdominal fat/100 (gm) body weight (panel B). Abdominal circumference (AC) (panel C) and body weight change % (panel D) in control (C), CLA-supplemented (CLAS), obese (O), CLA-prophylactic obese (CLAP) and CLA-treated obese (CLAT) groups. Results are expressed as mean ± SD (n=8). Error bars represent standard deviation. Significance was considered when p-value was <0.05. The marks * and # on top of columns indicate that values are significantly different, when compared with the corresponding values of control and obese groups respectively.

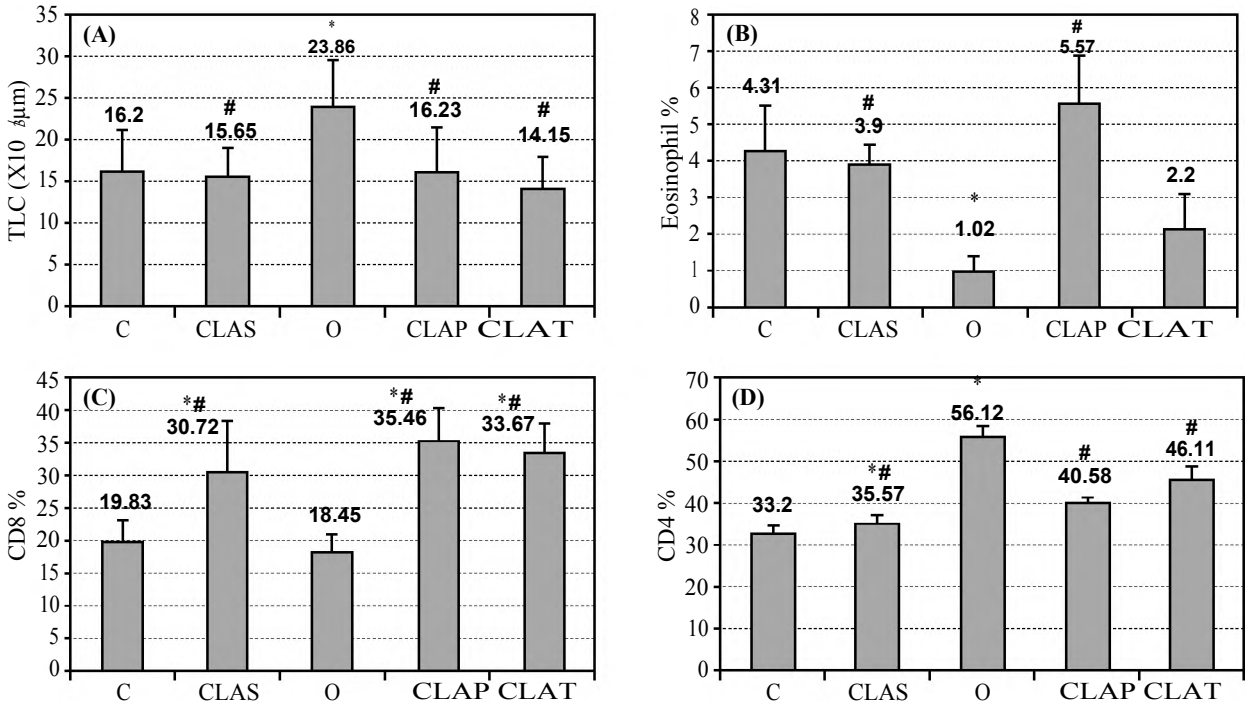

Fig. (2): Total leucocytic count (panel A), eosinophilic % (panel B), CD $^{+}$T lymphocytes (panel C) and CD4^{+}T lymphocytes (panel D) in control (C), CLA-supplemented (CLAS), obese (O), CLA-prophylactic obese (CLAP) and CLA-treated obese (CLAT) groups. Results are expressed as mean ± SD (n=8). Error bars represent standard deviation. Significance was considered when p-values was <0.05. The marks * and # on top of columns indicate that values are significantly different, when compared with the corresponfing values of control and obese groups respectively.

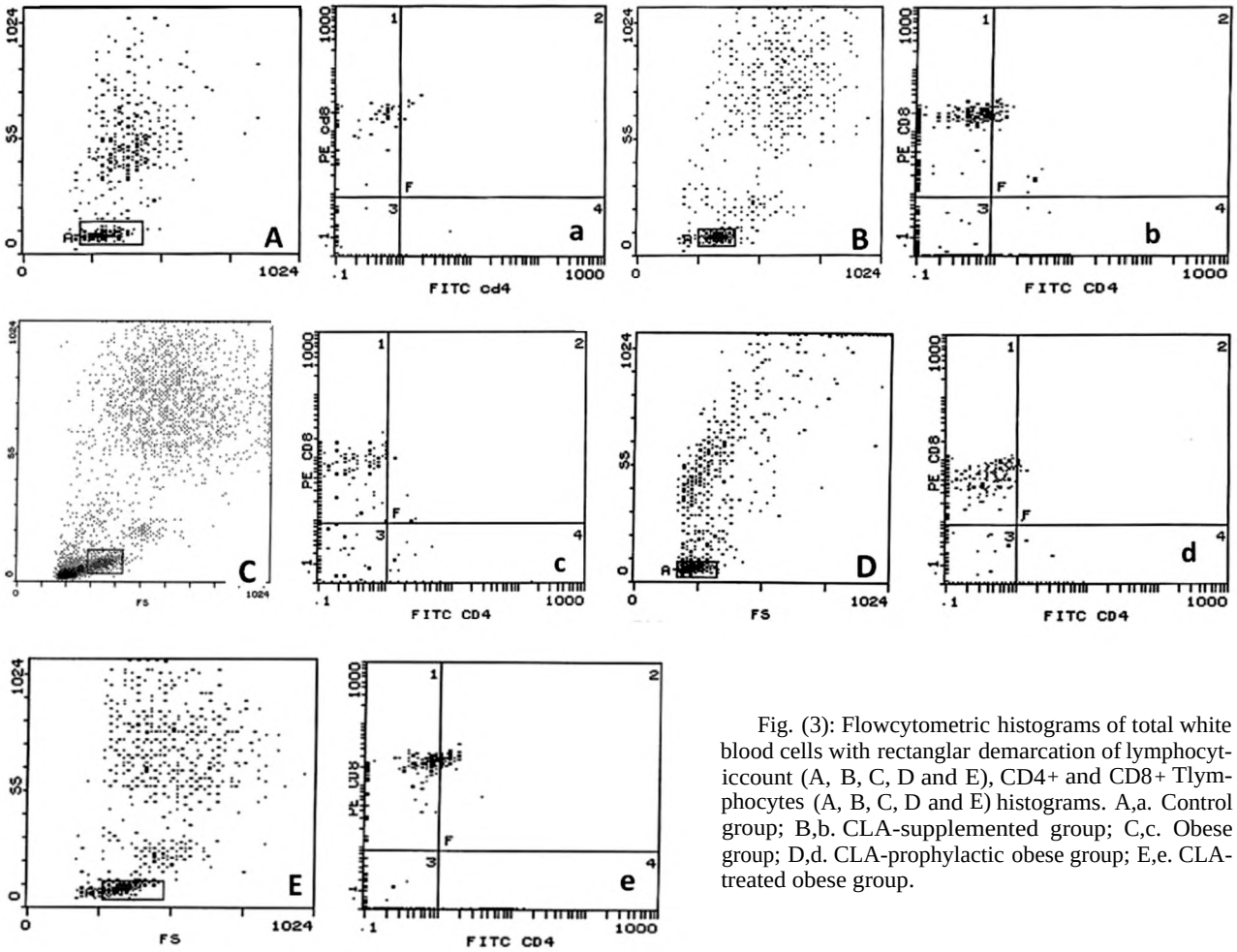

Fig. (3): Flowcytometric histograms of total white blood cells with rectanglar demarcation of lymphocyt-iccount (A, B, C, D and E), CD4+ and CD8+ Tlymphocytes (A, B, C, D and E) histograms. A,a. Control group; B,b. CLA-supplemented group; C,c. Obese group; D,d. CLA-prophylactic obese group; E,e. CLA-treated obese group.

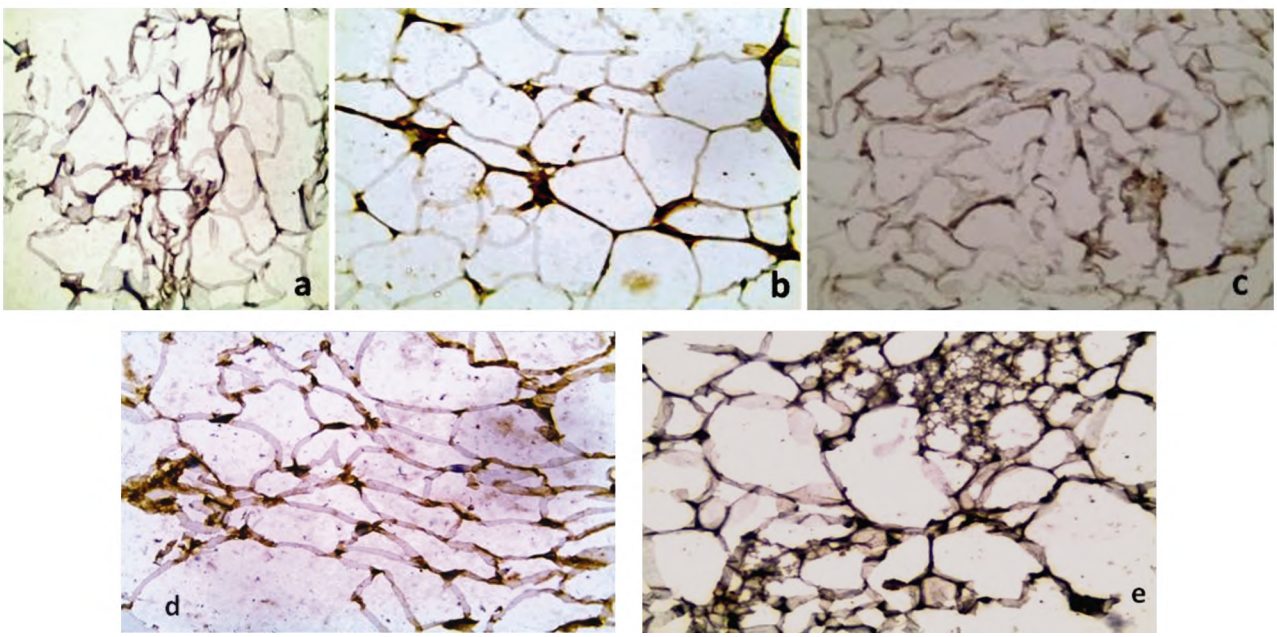

Fig. (4): Caspase 3 positivity in white adipose tissue of control, CLA-supplemented, obese, CLA-prophylactic and CLA-treated obese groups (A, B, C, D and E) respectively. CLA intervention significantly increase caspase 3 +ve (pre) adipocyte (D and E) (Immunohistochemival staining X400).

Discussion

The current study was designed to evaluate the possible modulating effect of Conjugated Linoleic Acid [CLA] on the change of food intake, Body Mass Index [BMI], percentage of body weight change, body weight, visceral fat, total abdominal fat/100gm body weight, Tumor Necrosis Factor alpha [TNF-0τ], total leochocytic count, eosinophils %, CD4+, CD8+ T lymphocytes, CD4/CD8 ratio and caspase 3 positivity, on High Fat Diet [HFD] induced experimental obesity in adult male albino rats.

In this work, there was a significant decrease in food intake of CLAS, CLAP, CLAT groups compared with obese group which showed the highest mean of daily amount of food intake. Also, CLAP and CLAT groups has a significant decrease in food intake compared with control group.

These results agreed with SO et al., **[15]** who reported that food intake was reduced in mice fed diet supplemented with CLA.

This significant decrease in food intake may be explained by that CLA exerts an inhibitory effect on hypothalamic appetite-regulating genes, reduced the expression of orexigenic neuropeptides like NPY and agouti-related protein which robustly increase food intake **[16]**.

Some contradictory results showed that CLA supplementation decrease body weight but not affect food intake **[17]**.

The major finding in our work is that supplementation of the HFD-induced obese rats with CLA resulted in a significant decrease in final BW, final BMI, % of BW change, Abdominal Circumference (AC), visceral fat and total abdominal fat to each 100gm BW in the CLAS, CLAP and CLAT groups compared with obese group which showed a significant increase in these parameters compared with control group.

The results obtained showed that CLA supplementation for 12 weeks ameliorates most of detrimental effect of high fat diet induced obesity in rats. This finding was concomitant with the results of Wendel et al., **[18]** who found that supplementation with CLA decrease BW and white adipose tissue mass.

In agreement with the previously-mentioned results, Dalia and Suzan **[19]** had reported that HFD for 8 weeks significantly increase BMI and AC in rats.

The significant decrease in final BW and final BMI following supplementation with CLA may be due to the effect of CLA in decreasing food intake as previously explained **[16]**. Also, this effect may be due to concomitant decrease visceral fat mass **[18]**.

Against these results, Guallier et al., **[20]** had reported that CLA decreased body fat mass but increased the lean body mass.

Visceral fat mass is more closely correlated with obesity-associated pathology than overall adiposity **[21]**. A significant reduction in visceral fat mass, total abdominal fat and AC in CLAS, CLAP and CLAT groups compared with obese group might explore the possible the role of CLA in reduction of body weight via reduction of abdominal fat mass.

This improvement in visceral fat mass and total abdominal could be explained by increase apoptosis in adipose tissue following CLA supplementation **[22]**.

Another mechanisms by which CLA decreases body fat mass is that CLA was reported to inhibit adipogenesis and pre adipocyte differentiation in animals **[23]** and in humans **[24]**.

Surprisingly, our study demonstrated the effect of CLA on caspase 3 positivity, there was a significant increase in positivity of caspase 3 in CLAP and CLAT groups compared with control and obese groups. These results were consistent with many authors who reported that CLA increased the level of apoptosis in pre adipocytes **[22,25,26]**.

TNF-0τ is a powerful pro-apoptotic factor secreted by fat in mammals **[27]**.

Renli et al., **[28]** reported that, CLA-fed pigs showed significantly increased TNF-0τ mRNA expression in their back fat tissue. However, the circulating concentration of TNF-0τ protein was significantly decreased.

In line with this finding, the current study showed that TNF-0τ in CLAS and CLAP groups significantly decreased when compared with obese group.

TNF-0τ in obese group showed a significant increased level when compared with control group, this increase may be due to chronic inflammatory state which occurs in obesity, as TNF-0τ is an inflammatory factor **[28]**. In addition, TNF-0τ increased in CLA-treated obese group, but mechanism of this increase is obscure.

As regard Total Leucocytic Count (TLC), our study showed a significant increase of this parameter in obese group compared with control group, also showed a significant decrease in TLC in CLAS, CLAP and CLAT groups compared with obese group.

These results were concomitant with David et al., **[29]** who reported that, obese subjects had higher TLC compared with non obese subjects.

The increased in TLC in obese group may be explained by impaired immune defenses and increased susceptibility to infections in obese subjects **[30]**.

Agreeing with Kelley and Erickson, **[31]** who reported that ,CLA supplementation did not alter the number of TLC in normal mammals, the present study showing non-significant change of TLC between CLAS group compared with control group.

In the present work, it was observed that there was a significant increase in CD8+ T lymphocytes in CLAS, CLAP and CLAT groups compared with control and obese groups. These results were consistent with previous reports that declared a CLA-induced linear increase in percentages of CD8+ lymphocytes **[32]**.

This finding may be explained by CLA-induced decline of PGE2 by interfering with the synthesis of arachidonic acid from linoleic acid. PGE2 was reported to have a suppressive effect on CD8+ lymphocyte proliferation **[33]**.

These results indicated that dietary CLA enhances cellular immunity by modulating phenotype and effector functions of CD8+ T lymphocytes which are involved in both adaptive and innate immunity **[34]**.

CD4/CD8 ratio was significantly decreased in CLAS, CLAP and CLAT groups compared with control and obese groups despite the significant increase in CD4+ T lymphocytes in all these groups. The significant increase in CD4+ T lymphocytes in obese group could be explained by the significant increase in CD4/CD8 ratio compared with control group.

These results were concomitant with some authors who reported that CD4+ Th1 cells increased with obesity **[32]** and CLA enhanced Tcell proliferation **[35]**.

Interestingly, the current study found that there was a significant decrease in esinophilic count in obese group compared with control group. Also, there was a significant increase in esinophilic count in CLAS and CLAP groups compared with obese group. These results were consistent with Wu et al., **[36]** who reported that, the number of eosinophils is reduced in adipose tissue of diet induced obesity in mice.

Our study demonstrated the increased pro-inflammatory state in obese group as noticed by increase in CD4+, TNF-ct and decrease in esinophils which act as anti inflammatory immune cell in adipose tissue. These were supported by previous findings that eosinophils are responsible for 90% of IL-4 expression and accelerate M2 macrophage polarization by secreting Th2 type cytokines such as IL-4 and IL-13 **[36,37]**.

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دراسة على التغيرات المناعية المصاحبة للسمنة فى ذكور الجرذان البالغين والدور المحتمل لتحسين المناعة بالإمداد بحمض اللينوليك المقترن

مقدمة: قد يؤثر النظام الغذائى العالى الدهن على بعض خلايا الجهاز المناعى وبعض القياسات المتعلقة بمؤشر كتلة الجسم.

مواد وطرق البحث: تم إجراء هذه الدراسة على أربعون من ذكور الفئران البيضاء من السلالة المحلية وتتراوح أوزانهم بين ١٥٠–٢٠٠ جرام لكل فأر، وتم تقسيمهم إلى خمس مجموعات متساوية (٨ فئران لكل مجموعة): المجموعة الضابطة والمجموعة الضابطة التى تناولت حمض اللينوليك المقترن لمدة ٣ شهور والمجموعة التى تعانى من السمنة الغذائية والمجموعة التى تعانى من السمنة الغذائية والمعالجة بحمض اللينوليك المقترن من بداية التجربة والمجموعة التى تعانى من السمنة الغذائية والمعالجة بحمض اللينوليك المقترن بعد ٣ شهور من تناول الغذاء عالى الدهن ولمدة ٣ شهور. وفى نهاية التجربة–بعد ١٢ أسبوع–تم قياس أوزان الفئران وطولهم من الأنف إلى الشرج وتم حساب نسبة التغير فى وزن الجسم وتم تصويم الفئران طوال الليل وسحب عينات دم من خلف حجاج العين لقياس عدد كرات الدم البيضاء ومكوناتها ونسبة سى دى ٨ وس دى الخاص بالخلايا الليمفاوية وأيضا تم فصل مصل الدم لقياس عامل نخر الورم ألفا وتم حساب متوسط كمية الطعام اليومية لكل فأر وأخيرا تم تخدير الفئران من جميع المجموعات وقياس محيط الجسم ثم إستئصال الشحوم البطنية وقياس وزنها وعمل تحليل نشاط الكاسبيس ٣ فى الخلايا الشحمية بواسطة مقايس تجارية.

النتائج: أظهرت نتائج هذه الدراسة أن إتباع نظام غذائى عالى الدهون لمدة ١٢ أسبوعا فى ذكور الفئران البيضاء تسبب فى زيادة ذات دلالة إحصائية فى جميع الدلائل. تسبب تعاطى حمض اللينوليك المقترن فى تحسن ذو دلالة إحصائية فى مؤشر كتلة الجسم ومحيط الجسم وعامل نخر الورم ألفا وتغيير ملحوظ فى عدد كرات الدم البيضاء ومكوناتها وزيادة ملحوظة فى نشاط الكاسبيس ٣.

الإستنتاجات: إعطاء حمض اللينوليك المقترن وقائيا أو علاجيا لذكور الفئران المسمنة غذائيا (المغذاة بوجبات عالية الدهن) يؤدى إلى تحسن ملحوظ فى الآثار الضارة الناجمة عن الوجبات عالية الدهن خاصة المرتبطة بالجهاز المناعى. هذا التأثر النافع لحمض اللينوليك المقترن من الممكن أن يرجع لتأثيره المغير المناعى، كمضاد للإلتهاب وكذلك كمساعد لموت الخلايا المبرمج فى الأنسجة الدهنية.