JCHR (2025) 15(1), 95-101

Journal of Chemical Health Risks

sanad.iau.ir/journal/jchr

ORIGINAL ARTICLE

Anti-Inflammatory Effects of Δ9 Tetrahydrocannabinol in Adipose Tissue of an Obese Rat Model

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(Received: 21 December 2022 Accepted: 7 November 2023)

KEYWORDS
Inflammation;
Obesity,
THC;
Rat

ABSTRACT: This study sought to investigate how Δ9 Tetrahydrocannabinol (THC) affects inflammation within the adipose tissue of an obese rat model. Female Sprague-Dawley rats, aged 4 weeks (total: 15 rats), were divided into four groups: Control, which received a standard diet; Obese, which were fed a high-fat diet (HFD); and THC, which received both THC treatment and an HFD. After a 16-week period, we measured their body weight and C-reactive protein (CRP) levels. Adipose tissue samples were collected to analyze the expression levels of Mcp, Tnf-α, and Il-1β mRNA. The obese rats showed a significant increase in body weight compared to the other groups, while the THC-treated group exhibited significantly lower weights than the obese rats. CRP levels were notably higher in the obese group than in the other groups, but CRP levels in the THC group were significantly lower than in the obese group. Expression levels of *Mcp, Tnf-α*, and *Il-1β* mRNA were markedly higher in the obese group compared to the control. In contrast, the THC group displayed significantly lower mRNA expression levels than the obese group. Treatment with a low dose of THC resulted in a reduction of inflammation markers in the adipose tissue of obese rats.

INTRODUCTION

In recent years, nutritional factors have gained significant attention in reproductive research, particularly due to the strong connection between obesity and polycystic ovary syndrome (PCOS). Obesity is closely linked to menstrual irregularities, hyperandrogenism, and hirsutism [1], making it a crucial consideration in understanding PCOS. The role of diet, especially in the occurrence and severity of PCOS, is evident [2]. Obesity has an impact on hormonal patterns and the levels of cytokines, leading to the emergence of metabolic syndrome, marked by issues such as glucose intolerance, insulin resistance, and inflammatory responses [3]. The rise in obesity can be attributed to the consumption of high-fat and high-sugar foods, resulting in excess calorie intake and the accumulation of adipose tissue within the body. With obesity becoming increasingly prevalent, it has emerged as a global issue affecting numerous countries and populations. The consequences of obesity extend to various health problems and severe conditions, including insulin resistance and metabolic syndrome, both intricately associated with inflammation.

Persistent low-level inflammation is identified by sustained increases in C-reactive protein (CRP) levels. This persistent condition represents a systemic and prolonged state marked by a slight elevation in inflammatory markers. Consequently, chronic low-grade inflammation leads to systemic and long-term pathological implications, often involving mild

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DOI: 10.22034/jchr.2023.1975620.1659

increments in inflammatory markers [4]. This state of chronic inflammation contributes to oxidative stress, thereby causing functional imbalances and even apoptosis [5]. Furthermore, chronic low-grade inflammation is believed to underlie the connection between hyperandrogenism, insulin resistance, and their subsequent effects [6]. This complex scenario is a consequence of the heightened production of inflammatory mediators, including cytokines, chemokines, and certain adipokines [5].

Cannabinoids are compounds derived from Cannabis sativa that have exhibited potent anti-inflammatory properties by regulating both cell-mediated and humoral immune responses [7, 8]. Phytocannabinoids, such as Δ9-tetrahydrocannabinol (THC) and Cannabidiol (CBD), contribute to these anti-inflammatory effects [7, 8]. Previous research has validated the efficacy and safety of THC and CBD in addressing inflammatory disorders [7, 9]. However, the impact of THC on the inflammatory profile of adipose tissue remains unexplored. Consequently, this study delves into the effects of low-dose THC using a rat model of obesity, with a specific focus on assessing its impact on inflammation-related properties.

MATERIALS AND METHODS

Chemicals

The chemicals used were obtained from Sigma-Aldrich in Steinheim, Germany, unless specified otherwise in the text.

In this experimental study, female Sprague-Dawley rats, aged 6 weeks and weighing 180-200 grams (n=15), were acquired from the Pasteur Institute of Iran. All the rats were given time to adapt to the animal facility, where they were kept under controlled conditions, including a 12-hour light and 12-hour dark cycle, a temperature range of 22-24°C, and a humidity level of 45±2%.

Experimental design

The rats were assigned randomly to one of four groups, each containing five rats: 1) Control: Animals were fed a standard laboratory diet with the following composition: 3.14 kcal g^{-1}, energy supply ratio: protein 21.5%, carbohydrate 65%, fat 4%. 2) Obese: Animals were fed a high-fat diet (HFD) comprising 5.3 kcal g^{-1}, energy supply ratio: protein 20%, carbohydrate 36%, fat 40%, cholesterol 1.25%, along with 23 g L^{-1} d-fructose and 18.9 g L^{-1} d-glucose, for 16 weeks.3) THC group: Animals received HFD for 16 weeks and were administered THC (10 mg kg^{-1} i.p) daily during the final four weeks. The rats' weights were measured weekly to track changes. Upon concluding the study, all the rats were euthanized in a humane manner by decapitation while under deep anesthesia, achieved with ketamine and xylazine (100 mg kg-1 and 10 mg kg-1 respectively). Subsequently, samples of adipose tissue were gathered.

C-reactive Protein (CRP) Assay

We used serological analysis to measure the levels of serum CRP. To do this, we employed an ELISA kit (specifically, Millipore's MILLIPLEX® MAP Rat/Mouse CRP Single Plex, USA).

Gene Expression Analysis

We extracted ovarian RNA using Trizol reagent from Qiagen (USA), following the manufacturer's instructions. Afterward, we treated the RNA with DNase I from Cinnagen (Iran). We utilized 500 ng of the extracted RNA, which exhibited an optical density ratio (260/280) exceeding 1.8, for cDNA synthesis. This process involved a cDNA Synthesis Kit from Fermentas (MD, USA), following the manufacturer's protocol.

For the evaluation of mRNA expression levels in the Tnf, Il1B, and Mcp genes, we employed an ABI StepOne real-time PCR machine from Applied Biosystems (ABI, USA) in conjunction with the Quanti Fast SYBR Green PCR Kit from Qiagen (Germantown, USA). Gene-specific primer sets were designed using AlleleID software version 7.5 by DBA Oligo, Inc. (USA), as specified in Table 1. All real-time polymerase chain reaction (PCR) procedures strictly adhered to the guidelines of the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE). Ef1 was designated as the reference gene. Ultimately, we calculated the relative expression fold using the 2-ΔΔCT method and included a no-template control (NTC) tube for each gene in all experiments.

Table 1. List of used primers

Primers	Accession number	Sequence	Tm (°C)	Product size
Tnf-F	NM_012675	5′ TCAGCCTCTTCTCATTCC′ 3	59.8	93
Tnf-R		5′ ACTTCTCCTCCTTGTTGG′ 3	60	
Mcp-F	NM_19190	5′ TGGTGGTCTGTGGTGCTAAG′ 3	66	89
Mcp-R		5′ AACTGGAGGCTTGGTAGAATGAG′3	66.2	
Il1b-F	NM_031512	5′ CACTATTCCTAATGCCTTCC′ 3	59.4	105
Il1b-R		5′ TCTGAGAGACCTGACTTG′3	59	
Ef1-F	NM_010106	5′ AGTCGCCTTGGACGTTCTT′ 3	67.56	124
Ef1-R		5′CCGATTACGACGATGTTGATGTG′3	65.60	

Statistical analysis

We performed data analysis using SPSS version 16 software on a Windows platform (SPSS Inc., Chicago, IL, USA). We employed one-way analysis of variance (ANOVA) along with Tukey's HSD post hoc test. The results are presented as mean values with accompanying standard deviations (mean ± SD). We applied a significance threshold of $P<0.001$.

RESULTS

Weight Changes

Initially, there were no notable differences in body weights among the different groups. However, starting from the fourth week of treatment, a significant increase in the rats' weight became evident in the obese group compared to the control group ($p<0.001$, Table 2). In contrast, no significant differences in the rats' weights were observed between the THC and control groups. It's worth highlighting that the obese group displayed the most substantial weight gain when compared to the other groups ($p<0.001$, Table 2).

CRP Assay

CRP levels exhibited a substantial increase in the Obese group compared to the other groups ($p<0.001$, Table 3). Furthermore, it's worth noting that the CRP level in the HFD group was significantly lower than that of the obese group. Conversely, there were no significant differences in CRP levels observed between the THC and control groups ($p<0.001$, Table 3).

Table 2. Body weight (gr) of the experimental groups.

Groups Weeks	Control	Obese	THC
6	199 ± 8	203 ± 10	204 ± 10
7	223 ± 7	228 ± 11	232 ± 13
8	237 ± 7	241 ± 10	247 ± 12
9	262 ± 5	265 ± 10	268 ± 5
10	292 ± 5	321 ± 6	328 ± 7
11	316 ± 5	346 ± 5	352 ± 8
12	338 ± 5	386 ± 5	385 ± 11
13	339 ± 5	395 ± 13	397 ± 19
14	364 ± 5^{a}	426 ± 7^{b}	428 ± 11
15	393 ± 5^{a}	454 ± 6^{b}	449 ± 24
16	411 ± 5^{a}	486 ± 7^{b}	473 ± 10
17	436 ± 6^{a}	513 ± 17^{b}	483 ± 5^{c}
18	476 ± 12^{a}	565 ± 12^{b}	521 ± 6^{c}
19	496 ± 13^{a}	588 ± 15^{b}	552 ± 13^{c}
20	516 ± 12^{a}	610 ± 11^{b}	570 ± 16^{c}
21	533 ± 12^{a}	638 ± 16^{b}	604 ± 13^{c}

The body weights of each group (n = 5/group) recorded weekly with a gram scale. Different letters indicate significant difference among groups ($p<0.001$). Values are mean ± SD.

Table 3. CPR level of experimental groups.

Groups	CRP (pg ml^{-1})
Control	14.02 ± 1.28^{a}
Obese	17.22 ± 0.85^{b}
THC	13.12 ± 1.07^{a}

Different letters indicate significant difference ($p<0.001$). Values are expressed as Mean ± SD.

Gene Expression Analysis

The outcomes of the real-time PCR demonstrated significant variations in Tnf-α relative mRNA expression levels among the experimental groups ($p<0.001$, Figure 1). Notably, no significant difference was observed between the Control and THC groups in this context. Relative expression levels of Il-1β exhibited a substantial increase in the obese group compared to the other groups ($p<0.001$, Figure 1). However, no significant difference was observed between the control and THC groups. Furthermore, the expression levels of Mcp-1 mRNA in the obese group showed a significant increase compared to the control and THC groups ($p<0.001$, Figure 1), while no significant difference was noted between the control and THC groups ($p<0.001$, Figure 1).

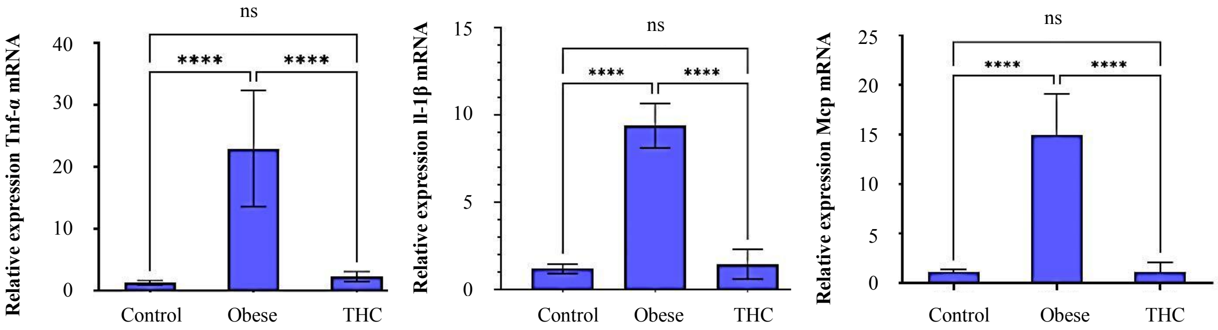

Figure 1. The mRNA expression levels of *TNF-α, Mcp1,* and *Il-1b* were measured and presented as relative fold changes (Mean ± SD) based on data from six independent experiments. Significant differences are denoted by asterisks ($p<0.001$).

DISCUSSION

The findings of the present study align with previous research regarding the features of the rat model employed, ensuring consistency [3, 10]. However, the novelty of this study lies in the introduction of a new dietary regimen for the rat model and the exploration of THC's impact on the inflammatory profile of adipose tissues. Successfully establishing an obese rat model using a novel HFD represents a significant achievement in this study. High-fat diets have been shown to induce various metabolic alterations in animals [11]. It is well-established that excessive weight is a key indicator of obesity [12], and in line with this, our results indicate that the HFD led to statistically significant weight gain, which is corroborated by prior research [10].

Obesity is widely acknowledged as a risk factor for metabolic syndrome [3, 11], which our study supports by demonstrating elevated inflammatory markers. We found a significant increase in CRP concentrations in the Obese group compared to the Control group, consistent with previous studies showing a correlation between CRP concentrations and both obesity severity and insulin sensitivity [4]. Adipose tissue-derived cytokine expression is believed to play a crucial role in low-grade chronic inflammation [13], further supported by our study's findings of increased gene expression of inflammatory markers in the obese group. This suggests that visceral adipose tissue may contribute to low-grade chronic inflammation [13], which may in turn explain the link between CRP and insulin sensitivity reported by Festa et al. [4]. Diet-induced oxidative stress and the subsequent inflammatory response via increased ROS production have been demonstrated [14].

The results of our study show that treatment with THC led to reduced weight gain. Despite the weight loss being relative and weight gain still present compared to the control group, our findings are consistent with previous reports that THC administration can mitigate the rate of weight gain in rats fed HFD, potentially through fat mass gain inhibition [15, 16]. This contrasts with the appetite-stimulating effects typically associated with THC, possibly explained by the low dosage employed in our study. Unlike synthetic cannabinoids, natural cannabis has been linked to improved insulin sensitivity, weight loss in HFD-fed obese rats, and reduced blood sugar levels [9, 17, 18]. It's worth noting that long-term THC administration has not been associated with weight gain [15-17]. Despite its appetite-stimulating potential, THC appears to be associated with a decreased risk of obesity and, consequently, insulin resistance [9, 16, 18]. Recent studies have suggested a significant correlation between cannabis consumption and lower insulin resistance [19, 20], with THC's protective mechanism on sugar levels and insulin resistance attributed in part to its antioxidative properties, which enhance insulin sensitivity by reducing ROS [21].

Our study's findings indicate the presence of inflammation, supported by significant increases in both circulating CRP levels and the mRNA expression of Tnf-α, Il1-β, and Mcp-1. THC is recognized as an anti-inflammatory agent with the ability to suppress cytokine production, inhibit Th-1 cells, and activate Th-2 cells. Furthermore, it has been demonstrated that THC can suppress pro-inflammatory cytokines such as IL-1α, IL-1β, IL-6, and TNF-α, which is in line with our results [22].

Our observations revealed reduced relative mRNA expression of Il1β, Tnfα, and Il-1β in adipose tissue following THC treatment. These findings are consistent with previous research, highlighting THC's capacity to decrease levels of pro-inflammatory cytokines such as TNF-α, interferon-c cytokine, and GM-CSF (Granulocyte-macrophage colony-stimulating factor), as well as downregulate the expression of Il-1a, Il-1b, and Il-6 [22]. Additionally, THC has demonstrated potential in mitigating intestinal inflammation in mouse colitis models [23]. Consequently, we can posit that THC's ability to alleviate inflammation in a rat model of PCOS may be attributed to its influence on the expression of pro-inflammatory cytokines.

CONCLUSIONS

In summary, this study establishes a new HFD as an effective tool for exploring metabolic features in an obese rat model and evaluating novel treatments. Moreover, the study provides evidence of THC's anti-inflammatory properties in an obese rat model. Administration of low-dose THC led to reductions in inflammatory cytokines in the obese rat model. These results hold potential for further investigation into the molecular mechanisms underlying the protective effects of low-dose THC against obesity-related metabolic dysfunction and its associated metabolic and endocrine complications.

Author Contributions

Conducted experiments: S.F.M. Conceived study, designed experiments, and authored the paper: S.Z. Analyzed data: M.N. Contributed reagents/materials/analysis tools: H.H.M.

ACKNOWLEDGEMENTS

This research did not receive any specific grants from funding agencies.

ETHICAL CONSIDERATION

All procedures were approved by The Laboratory Animal Ethics Committee of Damghan University (IR.BSDU.REC.1399.14).

Conflict of Interest

The authors declare no conflicts of interest that could influence the objectivity of the reported research.

Funding

This research did not receive any specific grants from

public, commercial, or nonprofit sectors.

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