https://doi.org/10.31925/farmacia.2024.6.20 **ORIGINAL ARTICLE**

EFFECTS OF *CANNABIS SATIVA* L. SEED OIL ON PRO-INFLAMMATORY CYTOKINES AND LIVER AND KIDNEY FUNCTIONS IN OBESE FEMALE WISTAR RATS: A RANDOMIZED CONTROLLED TRIAL

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Abstract

Obesity is a global health concern linked to chronic diseases, including kidney and liver dysfunction. Functional foods with health benefits beyond basic nutrition are gaining attention as potential interventions. This study investigates the effects of hemp seed oil (HSO) on kidney and liver function and pro-inflammatory cytokines (IL-2, IL-6, TNF-α) in obese rats. Female Wistar rats (n = 84) were divided into four groups, fed a high-fat or control diet for 15 weeks, with subgroups receiving 10% HSO supplementation. At weeks 5, 10 and 15, 7 rats *per* group were euthanized, and blood samples were analysed. A high-fat diet increased inflammation and impaired kidney and liver function, while HSO supplementation improved levels of AST, ALT, uric acid, IL-2 and IL-6. These findings suggest HSO may counteract obesity-induced inflammation and organ dysfunction. Further research is needed to explore its therapeutic potential.

Rezumat

Obezitatea reprezintă o problemă globală de sănătate asociată cu boli cronice, inclusiv disfuncții renale și hepatice. Alimentele funcționale, cu beneficii pentru sănătate dincolo de nutriția de bază, atrag tot mai mult atenția ca intervenții potențiale. Acest studiu investighează efectele uleiului de semințe de cânepă asupra funcției renale și hepatice, precum și asupra citokinelor pro-inflamatorii (IL-2, IL-6, TNF-α) la șobolanii obezi. Experimentul s-a efectuat pe 84 de șobolani femele Wistar, care au fost împărțiți în patru grupuri. Au fost hrăniți fie cu o dietă bogată în grăsimi, fie cu dietă control timp de 15 săptămâni, sau au primit suplimentar ulei de cânepă. În săptămânile 5, 10 și 15, câte 7 șobolani din fiecare grup au fost sacrificați, iar probele de sânge au fost analizate. Dieta bogată în grăsimi a crescut inflamația și a afectat funcția renală și hepatică, în timp ce suplimentarea cu ulei de cânepă a îmbunătățit nivelurile de AST, ALT, acid uric, IL-2 și IL-6. Aceste rezultate sugerează că uleiul de cânepă poate contracara inflamația și disfuncția organelor, induse de obezitate. Sunt necesare cercetări suplimentare pentru a explora potențialul său terapeutic.

Keywords: hemp seed oil, IL-2, IL-6, obesity

Introduction

Obesity presents a growing critical global health issue that is linked to premature death and numerous non-communicable diseases. Obesity is associated with increased body inflammation [1]. Inflammation in obese individuals can contribute to the development of several diseases including type 2 diabetes, cardio-vascular disease, hypertension, non-alcoholic fatty liver disease (NAFLD) and chronic kidney failure [18].

In the body, inflammation is regulated by a complex network of signalling molecules, including key pro-inflammatory cytokines such as interleukins (ILs) and tumour necrosis factor-alpha (TNF-α) [12]. IL-2 functions as an immune-stimulating cytokine, facilitating the growth and activation of T immune cells, while IL-6 acts as a pro-inflammatory mediator, often elevated in response to injury and infection. Obesity and metabolic disorders frequently elevate IL-6 levels, fostering a pro-inflammatory status that can lead to chronic kidney and liver diseases such as NAFLD [15, 28]. Moreover, obesity increases TNF-α production, causing many obesity-related complications, including insulin resistance, cardiovascular disorders and liver dysfunction [20].

Functional foods have gained increasing attention as a potential strategy to manage obesity-related health complications, as they can reduce inflammation by providing specific nutrients that support the body's natural anti-inflammatory processes. Common anti-inflammatory nutrients include omega-3 fatty acids and polyphenols found in plants and their oil [10, 17].

Previous research has shown that dietary interventions can be an effective strategy for managing obesity-related health complications [1, 3, 7, 13, 26]. In this study, we aim to test the hypothesis that incorporating hemp seed oil into the diet can reduce inflammation and improve metabolic health in obese individuals. Specifically, we will examine the effects of hemp seed oil on levels of pro-inflammatory cytokines and markers of liver and kidney function in a randomized controlled trial of induced obese female rats.

Materials and Methods

Experimental design, animals, treatments and diets

Eighty-four 3-month-old females of Wistar female rats were divided randomly into four groups (n = 21 each). The first group was offered standard chow. The second group was given standard chow with 10% HSO added. The third group was offered a high-fat (HF) diet that contained standard chow 25%, sugar 15%, margarine 20% and powder milk 40%, and the fourth group was given an HF diet with 10% HSO added. Animals and standard chow were obtained from the animal house at the Jordan University of Science and Technology (JUST). All rats were housed individually in a room with controlled temperature (22 ± 2°C), humidity (55 ± 5%) and a 12-h light-darkness cycle throughout the study period (15 weeks). The rats underwent a one-week adaptation period and after were fed their respective diets for 15 weeks. The animal study protocol respected ARRIVE guidelines and was approved by the Institutional Ethics Committee of Jordan University of Science and Technology.

Blood and serum markers

After deep terminal aesthetic, rats were given a heart puncture to obtain blood samples, around 6 mL of blood was collected from each rat, which was then placed in gel test tubes and allowed to clot at room temperature for 20 minutes. The samples were then centrifuged for 10 minutes at 4°C and 4000 rpm. For use in upcoming tests, the serum was kept chilled in a freezer at -20°C. The Veterinary Health Centre at JUST conducted several studies using ELISA kits to see whether the HSO supplementation and food had any effect on health.

Data Analysis

Statistical analysis was performed using the MIXED procedure of SAS (version 8.1, 2000, SAS Inst. Inc., Cary, NC, USA). One-way analysis of variance was conducted for the normally distributed variables. The least-square difference (LSD) test was performed to determine group differences. A p-value of < 0.05 was considered statistically significant.

Results and Discussion

Liver function

There was no significant difference between all groups in total bilirubin levels at 5 and 15 weeks ($p > 0.05$; Figure 1).

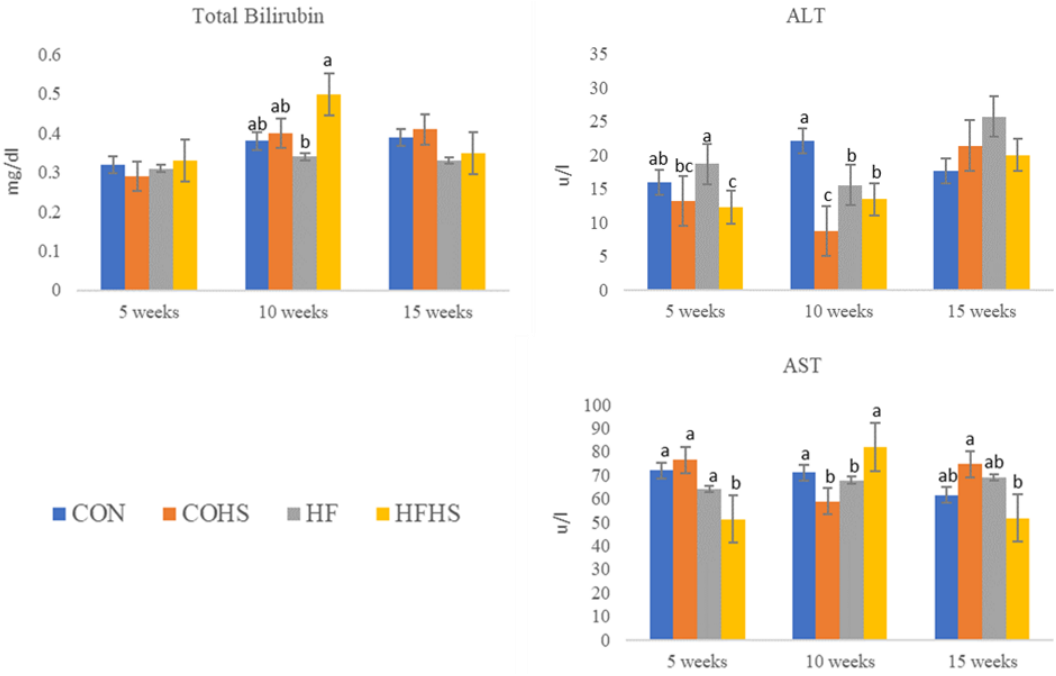

Figure 1.

Liver function biomarkers for Rat: Significant level at $p \leq 0.05$. All groups were compared with each other within the same week. CON: control diet group, COHS: control diet supplemented with 10% HSO group, HF: high-fat diet group, HFHS: high-fat diet supplemented with 10% HSO group, HSO: hemp seed oil. The same bar with different superscript letters is significantly different among groups within the same week ($p < 0.05$)

However, at week 10 serum bilirubin was lower ($p < 0.05$) for the HF group than the HFHS group. For the AST levels there was a significant difference between all groups and the HFHS group at week 5 ($p \leq 0.05$), at week 10, the CON and HFHS group had a significant difference toward the COHS and HF groups ($p \leq 0.05$). Nevertheless, the only significant difference observed at week 15 was between COHS and HFHS ($p \leq 0.05$). Serum ALT showed significant differences between CON and HFHS, COHS and HF and HF and HFHS groups at 5 ($p \leq 0.05$). At week 10, there was a significant difference between all groups except between the HF and HFHS groups. No statistically significant differences were observed between all groups at week 15 ($p > 0.05$).

Kidney function

There was a significant difference between the CON group and HF and HFHS groups in BUN levels at weeks 5, 10 and 15 ($p \leq 0.05$; Figure 2). However, no significant difference was found between the CON group and the COHS group in all slaughter times ($p > 0.05$). Creatinine levels showed a significant difference at week 5 only, and it was between the CON and COHS groups ($p \leq 0.05$). For uric acid levels, a significant difference was observed between the HF group and all other groups at week 5 ($p \leq 0.05$). At week 10, the only statically significant difference was between the HF group and COHS group ($p \leq 0.05$), no statically significant differences were observed at 15 weeks ($p > 0.05$).

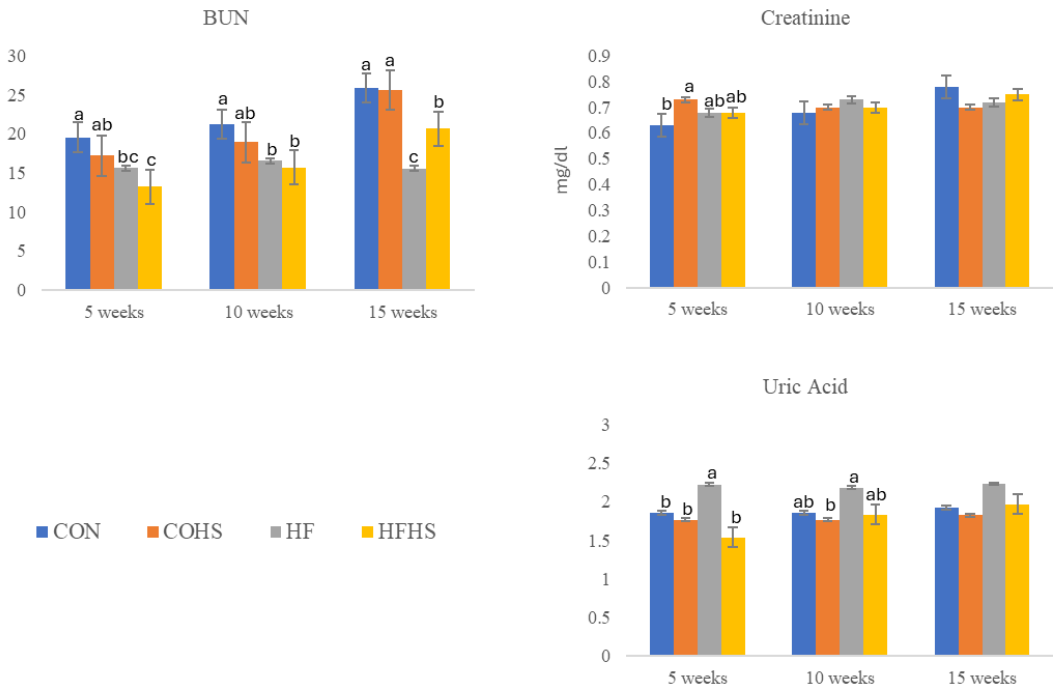

Figure 2.

Kidney function biomarkers for Rats: Significant level at $p \leq 0.05$. All groups were compared with each other within the same week. CON: control diet group, COHS: control diet supplemented with 10% HSO group, HF: high-fat diet group, HFHS: high-fat diet supplemented with 10% HSO group, HSO: hemp seed oil. The same bar with different superscript letters is significantly different among groups within the same week ($p < 0.05$)

Pro-inflammatory Cytokines

As shown in Figure 3, there were no statistically significant differences ($p > 0.05$) observed between any group in TNF-α. At week 15 there was a statically significant difference ($p \leq 0.05$) between the COHS group and HF group in IL-2 levels. Serum IL-6 content showed a significant difference ($p \leq 0.05$) between the HF group and all other groups at week 5 only, while at week 15, CON and COHS groups were significantly different ($p \leq 0.05$). C-reactive protein was tested for all groups at the three slaughter times, but a negative result was obtained always.

In our study, we confirmed the well-established connection between a high-fat diet and obesity. High-fat diets significantly contribute to obesity and are known to elevate serum liver enzymes, indicative of liver distress and compromised function [23]. Our results, as shown in Figure 1, align with these findings, revealing altered levels of ALT and AST enzymes in the HF group. Notably, supplementation with HSO led to a significant reduction in ALT and AST levels by week 15 compared to the HF group. This protective effect of HSO on the liver is supported by Hashemzadeh *et al.* along with other studies [4, 13, 26]. The potential hepatoprotective activity of HSO may be attributed to its cannabidiol content. Huang *et al.* demonstrated improved liver function in male mice treated with cannabidiol for non-alcoholic steatohepatitis induced

by a high-fat diet [15]. Also, it is noteworthy that Chen *et al.* observed that cannabidiol might reduce hepatocyte apoptosis by promoting autophagy flow [6], shedding light on the mechanisms through which cannabidiol may protect liver cells.

Interestingly, contrary to the reduced ALT and AST levels in the HFHS group, the COHS group exhibited a significant elevation in AST levels at week 15. This rise in AST levels in the COHS group may indicate distress in another organ, as ALT levels remained within the normal range, with no other signs of liver distress observed.

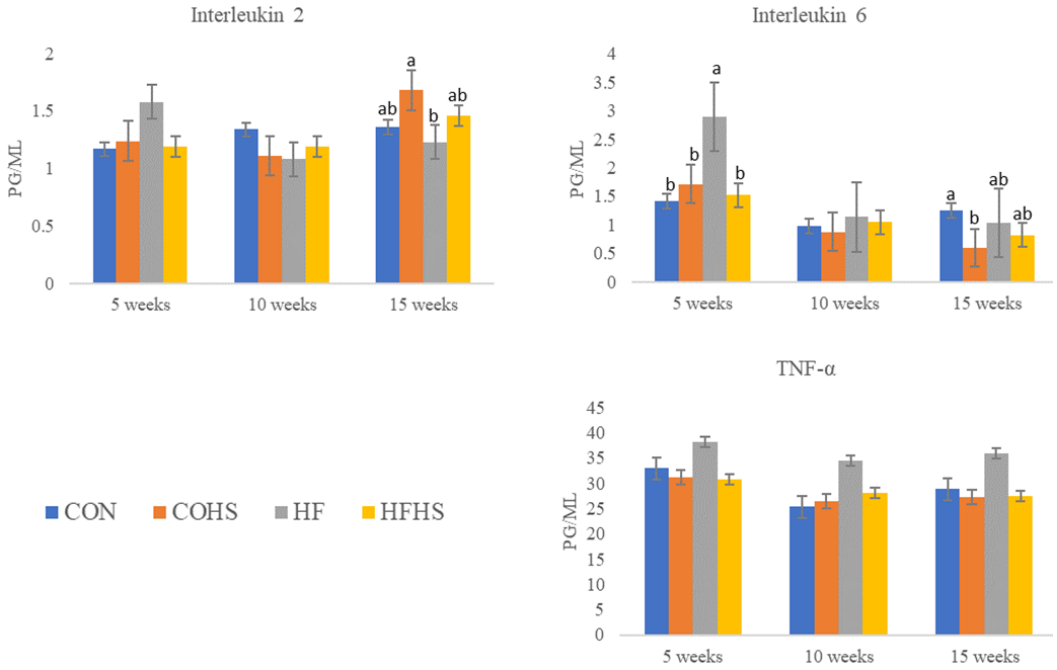

Figure 3.

Pro-inflammatory Cytokines for Rats: Significant level at $p \leq 0.05$. All groups were compared with each other within the same week. CON: control diet group, COHS: control diet supplemented with 10% HSO group, HF: high-fat diet group, HFHS: high-fat diet supplemented with 10% HSO group, HSO: hemp seed oil. The same bar with different superscript letters is significantly different among groups within the same week ($p < 0.05$).

Our findings at week 15 also revealed alterations in renal function biomarkers due to the high-fat diet, consistent with findings by Panchal *et al.* [23]. Specifically, we observed a slight elevation in uric acid, decreased BUN and no change in creatinine levels (Figure 2). These observations underline the impact of dietary composition, particularly the introduction of polyunsaturated fatty acids (PUFAs), on renal function. HSO, which boasts the highest PUFA content of 77.9% among 28 edible oils examined, may contribute to this effect [5]. PUFAs, especially omega-6, serve as precursors for prostaglandins, potent vasodilators that enhance blood flow dynamics [10, 11, 17]. Moreover, studies by Abou El-Soud *et al.* and Parker *et al.* demonstrated notable improvements in renal glomerular tissues in rats treated with caraway oil, which shares a similar omega-6 content with HSO (58.42 and 59.96 g/100g, caraway and hemp, respectively) [2, 24].

Alternatively, the observed results may be linked to the cannabidiol content of HSO. Recent research by Hokmabadi *et al.* revealed improvement in rats with induced renal impairment following the cannabidiol administration alongside the recommended dose of the antibiotic, suggesting activation of the FXR/Nrf2 pathway, which reduces inflammation and upregulates cannabinoid receptor 2 receptors [14]. Furthermore, Jiang *et al.* presented evidence suggesting a beneficial role for vitamin E in renal health [19].

Expanding our exploration of dietary influences on physiology, our study uncovered interesting insights regarding IL-2 levels. At week 15, the COHS group exhibited higher IL-2 levels compared to the HF group, consistent with findings by Vargas *et al.* and Meijer *et al.* Notably, our study suggests a potential long-term influence of HSO on IL-2 levels, warranting further investigation [22, 30]. The contrasting IL-2 responses between the HSO and HF groups could be attributed to the phenolic content in HSO, as elucidated by Ford *et al.* and Di Nunzio *et al.* as their results demonstrated a significant increase in IL-2 upon introducing phenolic compounds, which are known to downregulate cytokine expression from producing cells [8, 9]. Nevertheless, the precise cellular mechanisms underlying phenol-mediated effects remain unclear.

Our results (Figure 3) align with Ibrahim's 2018 study on 35 male rabbits regarding IL-6, showing elevated levels for the HF group and lower levels for the groups supplemented with HSO-containing phenols [16]. However, differences emerged regarding IL-2, TNF-α and C-reactive protein, where our observations differed from those of Ibrahim. These differences could be attributed to variations in dietary fat sources or other factors.

Obesity-induced by an HF diet can elevate TNF-α levels, as observed by Ibrahim et al. in 2018; previous studies have indicated that the administration of PUFAs can reverse this effect [16, 25]. While our results (Figure 3) agreed with this finding, the differences observed were not statistically significant, suggesting that a more extended period may be necessary to observe a more pronounced effect. The reduction in TNF-α levels may be linked to the PUFA content within HSO, as PUFAs have been shown to down-regulate TNF-α gene expression [25].

Furthermore, we hypothesize that the presence of phenolic compounds in HSO may contribute to a reduction in obesity-related inflammation through their anti-inflammatory properties. This hypothesis finds support in Taalab's 2019 study, which reported decreased serum levels of IL-6, IL-2 and TNF-α following the administration of cannabidiol to 440 epilepsy patients [29]. Taalab's study attributed this effect to cannabis' ability to attenuate pro-inflammatory status by modulating gene expressions. Our findings generally align with these results, except for IL-2 [29].

Our study's findings provide insights into the effects of HSO on inflammation, particularly in the context of obesity. Surprisingly, despite the known tendency of HF diets to elevate C-reactive protein levels in serum, our results did not reveal significant differences among the groups [7]. It is noteworthy that all groups, except for the CON group, share a common active substance: tocopherol (vitamin E). This prompts consideration of vitamin E's anti-inflammatory properties, as noted by Jiang *et al*., suggesting a potential link between vitamin E and reduced C-reactive protein secretion [19].

Furthermore, Abolghasemi's research in 2020 highlighted the relationship between obesity and increasing serum levels of TNF-α, AST, leptin, insulin and triglyceride, aligning with our present and previous findings [1, 3]. Building upon previous research, which established a connection between leptin and elevated cytokine levels [21, 27], we suggest that leptin presence may be a contributing factor to the cytokine levels observed in the present study.

Our study makes a significant contribution to the available data regarding the potential health benefits of HSO, particularly for individuals dealing with obesity-related inflammation. While few scientific studies have investigated the role of HSO in mitigating inflammation caused by obesity, our findings suggest that HSO may possess anti-inflammatory properties. However, further research is needed to elucidate the precise mechanisms behind these effects, including the examination of whether HSO reduces lipid peroxidation, enhances antioxidant enzymes, or inhibits proinflammatory cytokine production.

Conclusions

In conclusion, our study provides some evidence of the impact of dietary supplementation with hemp seed oil (HSO) on obesity-related inflammation and its associated health complications. Over the course of 15 weeks, our investigation, conducted on female rats induced into an obese state, yielded valuable findings. With a primary focus on exploring HSO's role as a functional food in reducing inflammatory responses and promoting health, our findings underline its potential as a dietary intervention.

A key observation from our study was the significant association between HSO supplementation and reduced AST and ALT levels, suggesting hepatoprotective properties that reverse some obesity damage. Additionally, HSO supplementation led to improvements in renal biomarkers, as evidenced by enhanced BUN and uric acid levels in the obesity-induced group. Notably, HSO also influenced IL-2 and IL-6 levels, indicating promising anti-inflammatory effects.

Our study contributes to the growing body of evidence supporting hemp seed oil (HSO) as a potential dietary intervention for individuals dealing with obesity-related inflammation. By elucidating the complex interactions between dietary components, inflammatory biomarkers and the therapeutic roles of HSO, we pave the way for more effective inflammation management strategies. However, a comprehensive understanding of the precise mechanisms underlying these effects remains important, highlighting the need for further exploration in this critical area. These findings hold promise for advancing our understanding of the intricate relationship between diet and inflammation in the context of obesity-related health complications, offering potential for personalized nutritional interventions aimed at reducing inflammation and improving overall health outcomes.

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Conflict of interest

The authors declare no conflict of interest.

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