Original Article

Comparative study of cannabinoid receptor 2 agonist and dexamethasone in experimentally induced rheumatoid arthritis

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ABSTRACT

To assess the impact of cannabinoid receptor 2-agonist (JWH-133) vs. dexamethasone in rats in a model of adjuvant-induced rheumatoid arthritis. The study utilized male albino rats weighing between 180 and 200 gm. The obtained data from the present study used dexamethasone orally daily for one 1week (1.5mg/kg), Cannabinoid receptor 2 agonists (JWH133) was administered as the other medication, at a dose of 4mg/kg I.P. injection daily for one week. Normal rats that received saline and DMSO showed insignificant changes in all measured parameters. CFA untreated group3 and group 4 showed significant increases in paw edema, rheumatoid factor and histopathological score, and reduction in cartilage thickness, and trabecular thickness. Treatment with dexamethasone orally or JWH133 I.P. decreased the elevated levels of these parameters and improved the decreased cartilage thickness and trabecular thickness. CFA-induced histopathological alterations were alleviated in rats given dexamethasone or a CB 2 agonist (JWH133), as well as in the combination group. CB 2 agonist (JWH133) was effective as dexamethasone in the treatment of RA. The combination therapy regimen of dexamethasone and JWH133 daily for one week produced more apparent improvement of arthritic paw edema, cartilage thickness, and histopathological score than the usage of each drug alone.

Keywords: Cannabinoid receptor 2 agonists, JWH133, Rheumatoid arthritis (RA), Complete Freund's adjuvant (CFA), Dexamethasone

Introduction

Rheumatoid arthritis (RA) is the most widespread chronic inflammatory joint disease in the industrialized world, with a prevalence of 0.5–1% [1-3]. Myopathy is a common condition in RA patients; it impairs the capacity to function and lowers the life quality for those who are afflicted [4]. It is an autonomous risk factor for several cardiac problems, with a 1.5-times higher CVD risk [5].

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CB2 is a cannabinoid receptor found in peripheral tissues that are predominantly on immune cells. CB2 is found in chondrocytes, osteocytes, fibroblasts, and FLSs, All of these cells have been linked to the development of RA [6]. The goal of this study was to see if cannabinoids receptor type 2 agonist (CB2) has any therapeutic potential in adult male albino rats with experimentally induced RA. To create a RA model, complete Freund's adjuvant (CFA) was employed.

Materials and Methods

Animals

In this study, 42 mature male Wistar albino rats weighing 180-200g were obtained from Cairo University's animal house. The animal's treatment regimen has been authorized by Cairo University's animal ethical committee. The procedure followed international norms for the care and handling of experimental animals. The Cairo University Institutional Animal Care and Use

Committee accepted the protocol for all experimental procedures according to ethical standards (31/October/2019-(CU III F 50 19 CU-IACUC).

Drugs and chemicals

Drugs used in this study included JWH-133 (Clinilab, Toccris Company. UK), Dexamethasone sodium phosphate 8 mg in 2 mL, ampoule (Dexamethasone®, Amriya Pharm. Egypt), and Thiopental sodium vial (50 mg/1mL) (Thiopental ®, Eipico. Egypt). Chemicals used in this study included Complete Freund's adjuvant (CFA), 10ml/10mg vial, (Sigma-Aldrich, USA), and Dimethylsulfoxide (DMSO 5%) (Biodiagnostic, Egypt) was used as a solvent for JWH133.

Experimental grouping and work design

This work was carried out on 42male albino rats weighing 180-200gm. The rats were split into seven groups, each with six rats. To develop rheumatoid arthritis, all groups from the group (3) to group (7) received a single S.C. injection of 0.1ml CFA into the plantar area of the right hind paw and were divided as follows: Group 1 (normal + oral DW): A single dosage of 0.1 mL physiological saline was injected subcutaneously into the plantar region of the right hind paw of each rat [7]. On the 15th day after hind paw injection, each rat has given 0.3 mL distilled water (DW) via oral gavage for seven days as a control [8]; Group 2 (normal + I.P., 5% DMSO): A single dosage of 0.1 mL physiological saline was injected SC. into the plantar surface of the right hind paw of each rat [7]. On the 15th day, after hind paw injection, each rat received 0.3 ml by I.P injection of DMSO 5% for 7days and serves as control; Group 3 (rheumatoid + oral DW): Each rat received a single SC injection of 0.1 ml CFA in the right hind paw's subplantar area [9]. On the 15th day, after hind paw injection, oral gavage of 0.3 mL distilled water was given to each rat for 7 days [8]; Group 4 (rheumatoid + I.P., 5% DMSO): A single SC injection of 0.1 ml CFA in the sub-plantar surface of the right hind paw was given to each rat [9]. On the 15th day, after hind paw injection, each rat received 0.3 ml by I.P injection of DMSO 5% for 7days**;** Group 5 (rheumatoid + dexamethasone treated): A single SC injection of 0.1 ml CFA into the subplanter area of the right hind paw was given to each rat [9]. On the 15th day, after hind paw injection, each rat received 0.3 ml of dexamethasone 1.5mg/kg orally for 7 days [10]; Group 6 (rheumatoid + CB2 agonist treated): Each rat received a single SC injection of 0.1 ml CFA in the right hind paw's sub-plantar area [9]. On the 15th day, after hind paw injection, For 7 days, each rat got 0.3 ml of JWH133 in a dosage of (4 mg/kg) via I.P injection [11]; Group 7 (rheumatoid +dexamethasone+CB2 agonist treated): Each rat received a single SC injection of 0.1ml CFA in the right hind paw's sub-plantar area [9]. On the 15th day, after hind paw injection, each rat received 0.3ml of dexamethasone 1.5mg/kg, orally in addition to a 7-day I.P injection of JWH133 at a dosage of 4 mg/kg [11].

A single SC injection of 0.1mL CFA was used to generate RA to test the effect of the cannabinoid receptor 2 agonist JWH-133 on adjuvant-induced rheumatoid arthritis (model). The following parameters and measurements were applied to the animals: detection of serum concentration of RF, measurement of paw edema, and histopathological studies of joints isolated from different studied groups was done. In all these groups the following parameters were investigated.

Measurement of paw volume

Using a plethysmometer, the right hind paw volumes of all animals were measured (immediately before and 14, 21 days after Freud's adjuvant injection, "days 0, 14, 21") [12]. The difference between the final and preceding paw volumes was used to calculate the change in paw volume at the tibiotarsal joint [13].

Blood samples collection

The rat tail vein was used to obtain venous blood samples. The samples were incubated at 37 degrees Celsius until the blood clotted, then centrifuged to separate the serum. At the end of the experiment (day 22nd), (after 24 hours from the last dose) rats were animal was euthanized by intraperitoneal injection of sodium thiopental (50mg/kg) [14].

Biochemical tests

Blood samples were taken from rat tails from all the animals in all groups. RF Kit (Omega diagnostics, inc., Scotland. United Kingdom) used for colorimetric determination of serum RF.

Separation of ankle and knee joints

When the experiment is finished, after receiving blood the animals were euthanized, the synovium, surrounding tissues, and bones of the knee joints were meticulously dissected, separated, and stored in well-sealed containers labeled with a 10% formalin solution (El-Gomhorya pharmaceutical company) [15].

Histopathological study

Histopathological study was carried out according to [16] method .ankle joints and knee joints were then kept in 10% nitric acid for 10 days for demineralization, as an essential step before tissue preparation. Sections were then transported again to 10 % formalin for 48 hours for fixation. Then tissues were embedded into paraffin blocks in a longitudinal orientation. 5–7 m thick slices were cut and stained with conventional Hematoxylin and Eosin (H&E) stains. After that, the sections were inspected and photographed for histological study.

Morphometric study

The morphometric study was done using Leica Qwin 500 software for measurement of cartilage thickness (by perpendicular line to detect height from the surface to point of junction with bone), and for measurement of trabecular bone thickness (across mid part of bone trabeculae in the epiphysis of bone, away from branching parts of the trabeculae, to avoid

error). The Histology department of the Faculty of Medicine conducted morphometric research and photographs. Data were collected and statistically analyzed.

Histopathological scoring system

In this study, an attempt was made to grade joint affection and/or preservation using the scoring methodology [17] and [18]. The histopathological scoring system included many parameters which were reduced cartilage thickness, cartilage erosions, reduced trabecular thickness, loss of matrix homogeneity, synovial hyperplasia, and inflammatory cells, and presence of debris and osteophytes in joint space.

In each group, each parameter was evaluated and compared to control values. For that parameter, control or values comparable to control were given a score of 0, and dispersion from the normal was given a score of 1, 2, or 3 depending on the degree of affection. The mean score for all parameters was then totaled up for each group, and the following grading was done based on the mean of the total scores in each group: Score (0-3) indicated normal; score (4-8) indicated mild RA; score (9-13) indicated moderate RA; score (14-18) indicated severe RA.

Examining serial non-overlapping fields over the whole specimen for all of the examined groups was done. Application of a semi-quantitative grading methodology to all four quadrants and several step sections throughout the joint. The severity of arthritis is indicated as a total of maximum values that can be aggregated for the entire joint: medial femoral condyle (MFC), medial tibial plateau (MTP), lateral femoral condyle (LFC), lateral tibial plateau (LTP), lateral femoral condyle (LFC), lateral tibial plateau (LTP) (LTP) [18].

Synovial hyperplasia according to [19]: Three aspects of chronic synovitis were assessed semi-quantitatively: lining cell layer expansion, synovial stroma cellular density, and leukocytic infiltration (from 0, absent to 3, strong).

Statistical analysis

The statistical software for the social sciences (SPSS) version 26 was used to code and input the data (IBM Corp., Armonk, NY, USA). To summarize the data, the mean and standard error of the mean were utilized. When comparing groups using analysis of variance (ANOVA) with multiple comparisons post hoc test, P-values less than 0.05 were considered statistically significant [20].

Results and Discussion

Effect of CB2 agonist 'JWH 133'on Paw edema

On day zero, there were no significant variations in the measurement of the mean right hind paw volume between the various study groups. The rats were examined on day 14 of CFA injection (Before Treatment) to detect the onset of paw edema which was detected after 8 days from CFA injection and progressed to reach the peak after 14 days of CFA injection on which the second measurements of right paw volume were taken **(Table 1)**.

Table 1. The mean volume (ml) of the right hind paw following subcutaneous injection of 0.1 ml saline or 0.1 ml CFA into the plantar surface of the right hind paw in the different studied groups. (On day 14 after induction of RA and before treatment), using the plethysmometer.

Groups		% Changes	Mean of paw volume ± SEM	Change in percentage compared to Group1	Change in percentage compared to Group 2	Change in percentage compared to Group 3	Change in percentage compared to Group 4	Change in percentage compared to Group 5	Change in percentage compared to Group 6	Change in percentage compared to Group 7
Normal groups	Group 1	Saline	0.192±0.010	-----	------	---------	--------	----------	---------	----------
	Group 2	Saline	0.190±0.008	1.04%	------	----------	----------	----------	----------	----------
Rheumatoid arthritis	Group 3	CFA	0.52±0.02[ab]	170.83%	----------	------	----------	-------	----------	----------
	Group 4	CFA	0.51±0.01[ab]	----------	168.42%	---------	-------	----------	----------	----------
	Group 5	CFA	0.53±0.03[ab]	---------	----------	1.92%	----------	--------	----------	----------
	Group 6	CFA	0.55±0.02[ab]	---------	----------	---------	7.8%	3.77%	---------	---------
	Group 7	CFA	0.57±0.02[ab]	----------	----------	9.61%	11.79%	7.54%	3.63%	------

- Values presented are the mean S.E.M. CFA; Complete Freund's adjuvant; (n = 6 rats/group).
a: Significant change compared to group 1 (P< 0.05)
b: Significant change compared to group 2 (P< 0.05)

Paw volume was assessed on day 21 in the various study groups, as indicated in **(Table 2)**. CFA-induced RA in untreated group 3 resulted in a substantial increase in the right hind paw mean volume when compared to the control group 1 value.

Furthermore, as compared to the same value in group 2, the untreated group 4 mean right hind paw volume increased significantly.

In CFA-triggered RA in group 5, dexamethasone therapy at a dosage of 1.5 mg/kg/day for 1 week resulted in a substantial reduction in the mean of right hind paw volume when compared to the equivalent value in the untreated group 3. In CFA-induced RA, JWH 133 therapy resulted in a significant reduction in the mean right hind paw volume in group 6 compared to the equivalent value in untreated group 4 (P0.05) **(Table 2, Figure 1a)**.

Table 2. The mean volume (ml) of the right hind paw in different studied groups on day 21, using the plethysmometer. For induction of RA, CFA was given SC in a single dose of 0.1ml into the plantar surface of the right hind paw.

	Groups	Mean of paw volume ± SEM	Change in percentage compared to Group1	Change in percentage compared to Group2	Change in percentage compared to Group3	Change in percentage compared to Group4	Change in percentage compared to Group5	Change in percentage compared to Group6	Change in percentage compared to Group7
Normal groups	Group 1 (control oral DW)	0.2±0.02	-----	----------	---------	-------	----------	----------	----------
	Group 2 vehicle (I.P DMSO)	0.19±0.01	5%	------	----------	----------	----------	----------	----------
	Group 3 (oral DW)	0.50±0.04[ab]	150%	--------	------	---------	----------	----------	----------
	Group 4 (I.P. DMSO)	0.52±0.02[ab]	----------	173.68%	----------	-------	----------	----------	---------
Rheumatoid groups	Group 5 (oral Dexa -1.5mg/kg)	0.40±0.02 [abcd]	-------	----------	20%	----------	--------	----------	----------
	Group 6 (I.P. JWH 133 -4mg/kg)	0.42±0.03 [abcd]	-------	----------	----------	-19.23%	5%	------	----------
	Group 7 (Dexa+ JWH 133)	0.29±0.01 [abcdef]	----------	----------	-42%	-44.23%	-27.5%	-30.95%	------

Values shown are the mean± S.E.M., Rats in groups (3 - 7) were subjected to CFA induced RA, CFA; Complete Freund's adjuvant, DW; Distilled water; DMSO; Dimethylsulfoxide, Dexa; Dexamethasone, JWH 133; Cannabinoid receptor 2 agonists. (n = 6/group).
a: Significant change compared to group 1 (P<0.05)
b: Significant change compared to group 2 (P<0.05)
c: Significant change compared to group 3(P<0.05)
d: Significant change compared to group 4(P<0.05)
e: Significant change compared to group 5 (P<0.05)
f: Significant change compared to group 6 (P<0.05)

Biochemical measurements

Effect of CB2 agonist 'JWH 133' on serum RF level

In CFA-induced RA in group 5, dexamethasone therapy resulted in a substantial decrease in mean serum RF level of 86.04 %compared to the equivalent value in untreated group 3 (P<0.05). JWH 133 therapy resulted in a significant decrease in the mean serum RF level of 83.94 % compared to the equivalent value in untreated group 4 (P<0.05) **(Table 3)**.

Table 3. Changes in the mean serum RF level (IU/ml) after oral treatment with Dexamethasone 'DEXA'(1.5 mg/kg/day), CB2 agonist 'JWH 133' I.P (4 mg/kg/day) & their combinations in CFA induced RA

Groups Serum RF (IU/ml)
G1(control oral DW) 4.33±0.56
G2(vehicle ,I.P DMSO) 4.35±0.49
G3(oral DW) 28.67±2.7[ab]
G4(I.P. DMSO) 28.03±2.29[ab]
G5 (oral Dexa) 4±0.37[cd]
G 6 (I.P .JWH 133) 4.5±0.63[cd]
G7(Dexa+ JWH 133) 3±0.37[cd]

Values shown are the mean± S.E.M., Rats in groups (3 - 7) were subjected to CFA induced RA, CFA; Complete Freund's adjuvant, DW; Distilled water; DMSO; Dimethylsulfoxide, Dexa; Dexamethasone, JWH 133; Cannabinoid receptor 2 agonists. (n = 6/group).
a: Significant change compared to group 1(P<0.05)
b: Significant change compared to group 2 (P<0.05)
c: Significant change compared to group3 (P<0.05)
d: Significant change compared to group 4 (P<0.05)
e: Significant change compared to group 5 (P<0.05)
f: Significant change compared to group 6 (P<0.05)

Histopathological assessment

Histopathological description

Control group 1 showed regular well-formed hyaline cartilage with smooth surface, homogenous matrix, as well as regular fibrocartilage. An evident tideline demarcating cartilage from bone was observed. Bone trabeculae were thick well-formed with interlacing bone marrow spaces. **(Figure 1, b1 and Figure 2a)**. Also, group 2 vehicle (DMSO) showed a regular

homogenous smooth surface of the articular hyaline cartilage. Fibrocartilage of menisci appears thick compact homogenous formed of parallel collagen bundles enclosing parallel rows of chondrocytes **(Figure 1, b2 and Figure 2b)**.

Untreated group 3 showed significant destruction of joint structure: articular (hyaline) cartilage surface was completely eroded; joint space was irregular and filled with debris and torn parts of fibrocartilage; destruction of bone trabeculae; and the cartilage thickness was markedly reduced. Cartilage cells were fewer and smaller in size compared to control group 1 **(Figure 1, b3 and Figure 2c)**. Moreover, group 4 was the same degree of joint destruction as seen in group 3. Cartilage showed full-thickness destruction. Bone trabeculae were separated and absent in wide regions of the field **(Figure 1, b4 and Figure 2d)**.

Significant improvement was seen in treated groups. Dexamethasone treatment group 5 showed smooth homogenous cartilage surface, clear joint space except for small regions which still showed the affection of cartilage **(Figure 1, b5 and Figure 2e)**. JWH 133 treatment in group 6 showed significant improvement as evidenced by homogenous cartilage surface structure, homogenous basophilic matrix, chondroblasts, and chondrocytes cell nests. There was a significant improvement of cartilage thickness and trabecular bone thickness compared to untreated group 4 while there was an insignificant change of both compared to treated group 5 **(Figure 1, b6 and Figure 2f)**.

Arthritis in group 7 showed maximum improvement with the restoration of the normal joint structure to near control appearance. Cartilage cells were numerous, similar to control, and had normal staining and activity **(Figure 1, b7 and Figure 2g)**.

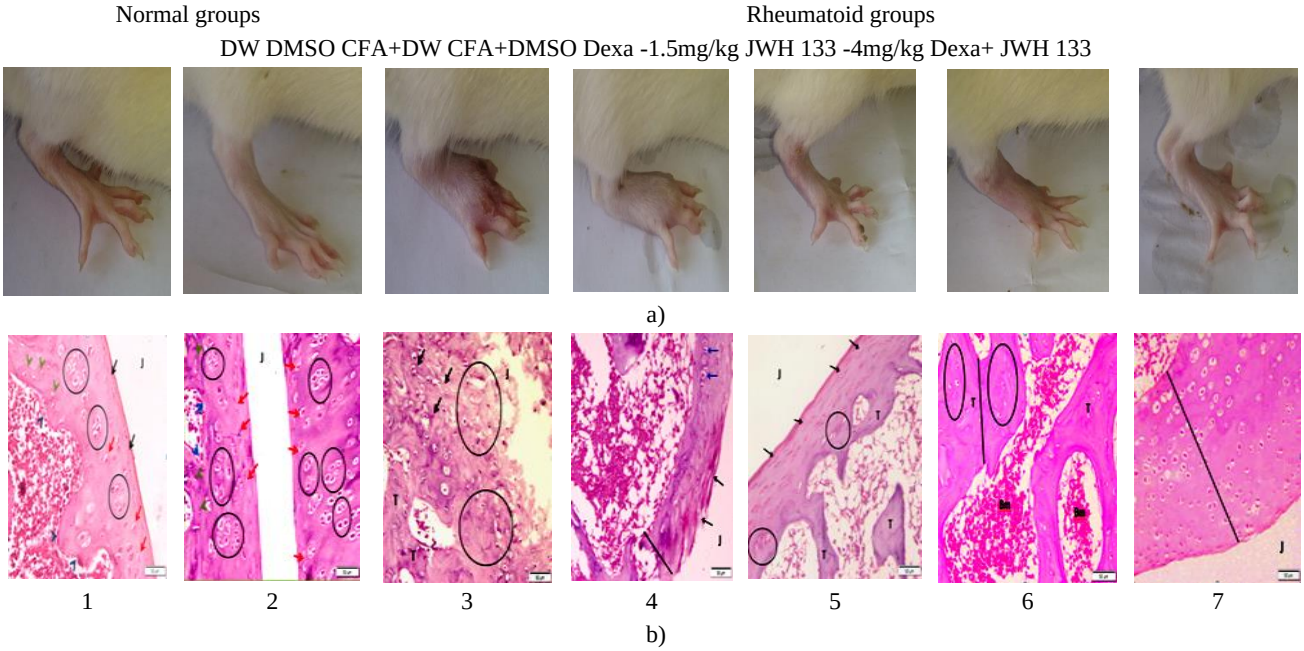
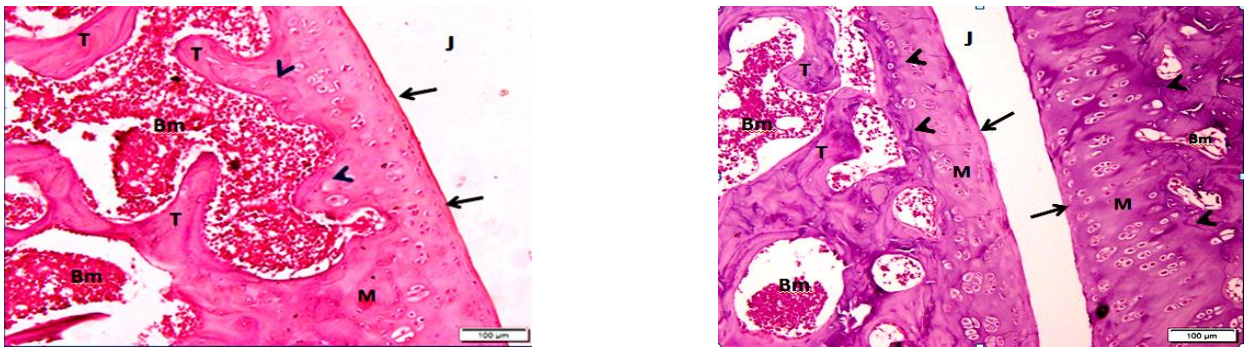
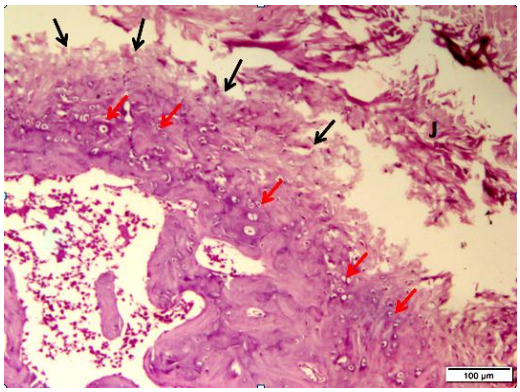


Figure 1. The effects of oral treatment with Dexamethasone "DEXA" (1.5 mg/kg/day), CB2 agonist "JWH 133'I.P (4 mg/kg/day), and their combinations in CFA-induced RA. CFA-induced RA was inflicted on rats in groups of three to seven. (a) Representative hind paw images (b) Representative haematoxylin and eosin (H&E) joint slices (scale bar 50m). CFA stands for complete Freund's adjuvant, DMSO; dimethylsulfoxide.





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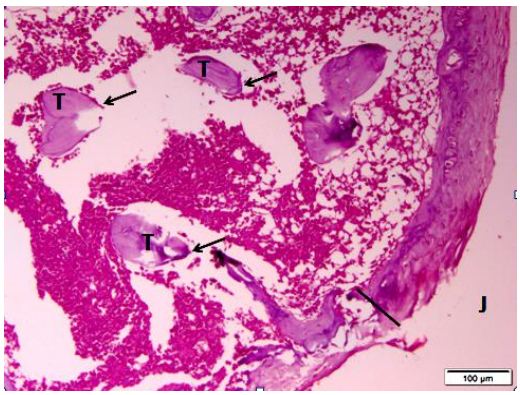

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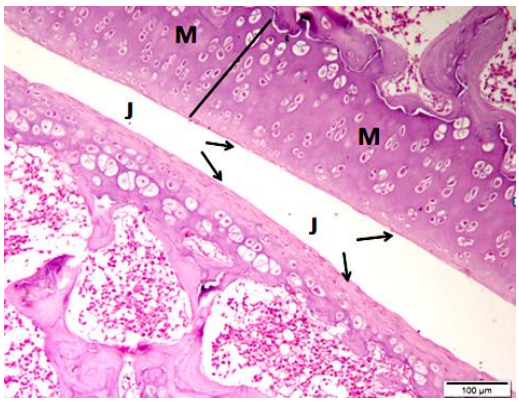

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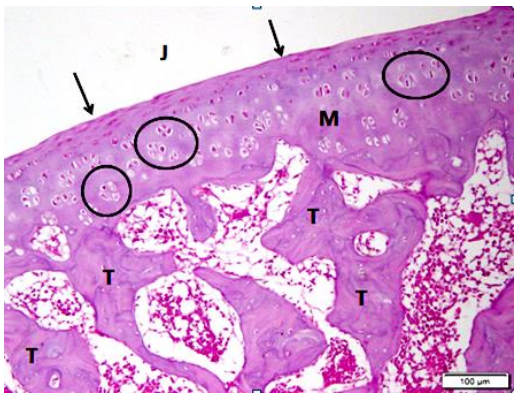

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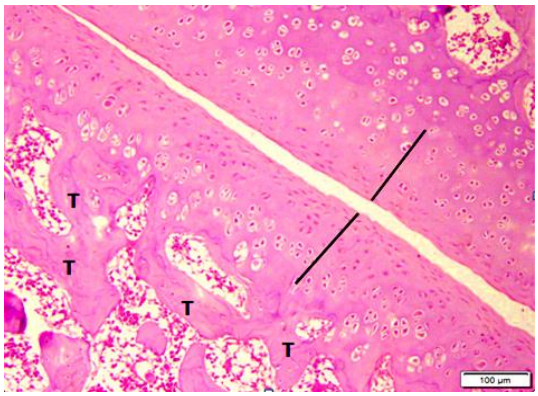

g)

Figure 2. Photomicrographs of segments of the rat knee joint stained with H&E in all groups (scale bar 100m). control group 1 (a) and Vehicle (DMSO) group 2(b) the joint space is regular well-formed cartilage with a smooth surface (black arrow), homogenous matrix (M), clear joint space (J), and evident line demarcating cartilage from bone (blue head arrow). Bone trabeculae (T) are thick well-formed with interlacing bone marrow spaces. CFA injected untreated group 3 (c) shows complete erosion of cartilage surface (black arrows) with an overall marked decrease of its thickness. Tissue debris fills joint space (J). Cartilage cells (red arrows) are fewer and smaller in size compared to control group 1. CFA and DMSO injected untreated group 4 (d) shows full-thickness cartilage destruction (black line). Bone trabeculae (T) are separated and absent in a wide region of the field (black arrow). group 5(e) shows significant improvement in cartilage thickness (black line), the surface is homogenous with a smooth surface (black arrows), clear joint space (J). Cartilage matrix is homogenous and basophilic (M). Effect of CB2 agonist (JWH 133) treatment in group 6(f) shows significant improvement as evidenced by increased cartilage thickness, regular cartilage surface structure (black arrow) .homogenous basophilic matrix (M), chondrocytes cell nests (black circle). Thick well-formed bone trabeculae (T) in meshwork structure are evident .joint space (J) is clear in most of the areas. combined CB2 agonist (JWH 133) treatment and Dexamethasone treatment for 7days on CFA induced RA in group 7(g) show maximum improvement with the restoration of the normal joint structure to near control appearance with the significant increase in cartilage thickness (black line) as well as bone trabecular thickness (T). T: bone trabeculae, M: matrix, J: joint space, black line: whole cartilage thickness, black circle: chondrocytes cell nests.CFA; complete Freund's adjuvant, DMSO; dimethylsulfoxide.

Histological scoring and grading

Group 7 treated with the combination of dexamethasone and JWH 133 had the best histological score assessment and was classified as a mild degree of RA that is equivalent to the control group. On the other hand, untreated groups 3 &4 had the worst histological score assessment, so classified as the severe grade of RA Moreover, dexamethasone-treated group 5 was classified as the moderate grade of RA while JWH 133 treated group 6, was classified as a mild grade of RA **(Table 4)**.

Table 4. Changes in the histopathological score after oral treatment with Dexamethasone 'DEXA' (1.5 mg/kg/day), CB2 agonist 'JWH 133'I.P (4mg/kg/day) & their combinations in CFA induced RA

Groups	Histological score
G1(control oral DW)	1.33±0.49
G2(vehicle ,I.P DMSO)	1.50±0.43
G3(oral DW)	14.58 ± 0.69^{ab}
G4(I.P. DMSO)	14.17 ± 0.60^{ab}
G5 (oral Dexa)	9.17 ± 0.70^{abcd}
G 6 (I.P .JWH 133)	8.00 ± 0.45^{abcd}
G7(Dexa+ JWH 133)	4.33 ± 0.33^{abcdef}

The values displayed are the mean S. E.M. of six animals in each group. Rats in groups (3 - 7) were subjected to CFA-induced rheumatoid arthritis. CFA; Complete Freund's adjuvant, DMSO; Dimethylsulfoxide, Dexa; Dexamethasone, JWH 133; Cannabinoid receptor 2 agonists.
a: Significant change compared to group 1($P<0.05$)
b: Significant change compared to group 2($P<0.05$)
c: Significant change compared to group 3($P<0.05$)
d: Significant change compared to group 4($P<0.05$)
e: Significant change compared to group 5($P<0.05$)
f: Significant change compared to group 6($P<0.05$)

Rheumatoid arthritis remains to be a serious public health concern worldwide and it is one of the most significant drains on healthcare medical resources. Up to this point therapy of RA, stays be unsatisfactory. The purpose of this research was to find out the new antiarthritic effect of stimulating cannabinoid receptor 2 in rat models induced by complete Freund adjuvant (CFA). CFA is frequently used to evaluate anti-arthritic therapeutic agents. This could be explained as the CFA model in rats mimics RA in humans [21].

In this study, Untreated CFA–induced RA in groups 3 and 4, showed a significant elevation in the mean paw volume measured by plethysmometer and a significant increase in mean RF. There were also significant histological alterations, such as cartilage and bone erosions, as well as decreased cartilage thickness and trabecular thickness. After the sub-plantar injection, the mycobacterial components in the CFA induce a strong immunological response in the rat paws via T-lymphocytes. They interact with dendritic cells, monocytes, and macrophages to trigger the synovial membrane to generate TNF-, IL-1c, and IL-6, the main pro-inflammatory cytokine [21].

However, as compared to untreated arthritic rats in group 3, dexamethasone-treated arthritic rats in group 5 exhibited a substantial decrease in paw volume. In agreement with the current study, dexamethasone decreases the synthesis of monocyte chemoattractant protein-1 (MCP-1) in cartilage cells, lowering the risk of macrophage infiltration in RA and might slowing disease progression, according to [22].

In the current work, arthritic rats in group 6, the paw edema was significantly reduced compared to the arthritic untreated group 4, JWH-133 is a specific CB2 agonist with 200 times greater binding for CB2 than CB1. The reduction in the mean volume of right hind paws may be explained by attenuation of inflammation and immune cell activation. Consistent with the current study, In CIA [23] found that CB2 agonist (4Q3C-10 mg/kg) significantly and dose-dependently reduced TNF-, IL-6, and IL-1 blood levels by 60% in mice treated with 4Q3C compared to mice in the control group.

In this study, the mean paw volume of the right hind paw of a CB2 agonist (JWH133) and dexamethasone-treated group 7 was significantly lower than that of untreated arthritic rats in groups 3 and 4. Also, there was a more significant reduction of the mean paw volume compared to arthritic treated in groups 5 &6. It could be due to the hypothesis of the complementary modes of action of both drugs that resulted in a greater antiarthritic synergistic effect more than anyone used alone. Additionally [24], justified this enhancement by revealing that CB2 inhibits the NLRP3 inflammasome stimulation and IL-1 production, enhancing apoptotic cell elimination and decreasing cellular reactivity in response to TNF or lipopolysaccharide (LPS)

The primary marker of RA clinical manifestation is RF. It includes toll-like receptor activation of B cells and numerous hereditary predispositions to arthritic disorders [25]. In this study, Untreated arthritic rats exposed to CFA in groups 3, 4, had substantially greater RF levels than non-arthritic control group-1 and vehicle group-2. Also, in agreement with this finding, [26]; [8] found significant increases in serum RF compared to the normal rats. Also, [14] evaluated the effect of Losartan in CFA- induced arthritis in rats, they found a rise in mean serum RF levels.

In the present study, compared to the untreated groups-3, the Dexamethasone-treated group-5 exhibited a significant reduction in the mean serum level of RF. Additionally, in agreement with the current study, [26] investigated dexamethasone at a dose of 2 mg/kg /day orally for three weeks in the AIA rat model and found a significant decrease in mean serum RF. Also, [27] showed that dexamethasone (0.05 mg/kg, orally), from day 0 to day 12 in the experimental model of RA results in a significant decrease in mean serum RF in CFA injected arthritic rats.

In the current study, the mean serum level of RF in arthritic rats in group-6 treated with CB2 agonist (JWH-133) was significantly lower. Up till now, no animal studies were conducted to demonstrate the effect of JWH133 (CB2 agonist) on RF. However, CB2 expression on B cells in RA synovial tissue was determined by [11]. They observed that administering JWH133 to CIA mice can impact B cells, resulting in a drop in anti-CII IgG1 antibody levels and an improvement in arthritis. As a consequence, they appear to be in line with the present study's findings.

In addition, compared to untreated arthritic rats in groups 3 and 4, there was a dramatic decrease in the mean serum level of RF in group-7, which received a combination of both CB2 agonists (JWH133) and dexamethasone. On the other hand, there was an insignificant reduction of the mean serum level of RF compared to treated arthritic rats in groups 5 &6.

As regard histopathological assessment in the current study, untreated arthritic rats subjected to CFA in groups 3, 4, showed significant elevation in histopathological score compared to non-arthritic control group-1 and vehicle group-2 in the form of synovial membrane hyperplasia (pannus formation),

inflammatory cell infiltration, articular surface damage, decreased cartilage thickness, degradation of the bone trabecular meshwork, decreased bone thickness, and presence of tissue debris in the joint space The histopathological score was significantly elevated, indicating a severe case of RA. Also, in agreement with these findings [28] and [29] they studied histopathological changes of rat joints after CFA injection and showed increased histopathological score and proliferating synovial membrane, which advocated the formation of RA.

Dexamethasone-treated arthritic mice in group-5 in this study revealed a significant reduction in histopathological score, significant increase in cartilage and trabecular bone thickness compared to untreated arthritic rats in group 3. In agreement with these findings, the overall thickness of the articular cartilage was increased by dexamethasone, especially the proliferative and mature layers, according to [30].

JWH 133 treated arthritic rats in group 6 in this study revealed a significant reduction in histopathological score. A significant increase in the mean trabecular bone thickness and cartilage thickness compared to untreated arthritic rats group 4. These findings were in agreement with, [31] and [23]. Improvement of the histopathological score may be explained by the that JWH133 was able to decrease CCL2 expression, a chemokine associated with recruiting of monocytes, macrophages, T-cells, and dendritic cells to inflammatory sites, according to [11].

Improvement of cartilage destruction and increased cartilage thickness in arthritic rats in group 6 in the current study may be explained by [32]. They showed that synthetic cannabinoid agonists, such as HU-210 and Win- 55,212–2, may suppress prostaglandin (PGE2) synthesis caused by IL-1 in bovine articular chondrocytes cultures and can prevent collagen and proteoglycan breakdown in cultures of bovine nasal cartilage.

In this study, treatment of both JWH133 and dexamethasone arthritic rats in group 7 showed a more significant decrease in the histopathological score, increase in cartilage thickness, and trabecular bone thickness compared to untreated arthritic rats in groups (group 3 and 4). In addition, as compared to groups that received each medication alone, there was a greater drop in the histopathological score and a greater rise in cartilage thickness (groups 5 and 6).as regard bone thickness there was an insignificant increase compared to groups 5 and 6.

Up till now, no animal studies were conducted to demonstrate the effect of combined jwh133 (CB2 agonist) and dexamethasone in RA treatment. Combined treatment is a popular therapeutic approach for RA that aims to improve treatment effects [33]. Furthermore, in the present work, combination treatment of both JWH133 (4mg/kg/day) and dexamethasone (1.5mg/kg/day) was administered to rats of group 7 for one week. This combination treatment induced a significant reduction in paw volume, a substantial decrease in the mean serum RF levels, and a significant improvement in histopathological scoring with the significant increase in both cartilage thickness and trabecular bone thickness. When compared to the comparable values in untreated arthritic rats in groups 3 and 4, the results in groups 3 and 4 were significantly higher.

As a result, these findings may imply that a combination therapy approach for RA is a reasonable and successful treatment modality. Therefore, it could be said that this interesting point needs further investigation and analysis in future researches to reach a definite, well-understood, and successful therapy for RA.

Conclusion

In conclusion, the current study looked at JWH133 as an immunomodulatory drug that suppressed synovial inflammation and cartilage loss in JWH133-treated rats by attenuating the inflammatory response at various levels. These findings contribute to a better understanding of the pathophysiology of immune-mediated inflammatory disorders, such as RA.

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Ethics statement: All procedures performed were in accordance with ethical standareds of the insititutional and national research committee and with 1964 Helsinki declaration.The procedure followed international norms for the care and handling of experimental animals. The Cairo University Institutional Animal Care and Use Committee accepted the protocol for all experimental procedures according to ethical standards (31/October/2019- (CU III F 50 19 CU-IACUC).

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