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Anti-rheumatic effect of phloretin in combination with Plaquenil against rheumatic impact of Complete Freund's adjuvant (CFA) in adult male rats

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent inflammation of the joints, leading to progressive joint destruction. Phloretin (Ph) is a naturally occurring flavonoid with notable antioxidant and anti-inflammatory properties, suggesting potential efficacy in RA treatment. **Aim:** the aim of this study was to investigate the effect of phloretin (ph) either alone or in combination with Plaquenil (plq) against CFA-created arthritis in rats. **Methods:** 48 male rats were separated haphazardly into group (I): control; group (II): Plq; group (III): Ph; group (IV): CFA; group (V): Plq treated (Plq Tr); group (VI): Ph Tr; group (VII): Plq&Ph Tr; group (VIII): Ph protective (Ph Pr). At the end of the experiment, rats were sacrificed following blood collection, and their foot paw tissue was promptly extracted and preserved in 10% neutral formalin for histopathological examination. **Results:** There was a notable increase in RF, Anti-CCP, TNF-α, IL-6, MDA, GPT, GOT, Urea, and Creat levels, and a marked drop in TAC levels and X-ray showed marked narrowing of joint space and diffuse swelling of the surrounding soft tissue and histopathological examination showed changes in articular cartilage and destruction of the adjacent bone in the CFA group compared to the control. In contrast, administration of Ph alone or combined with Plq results in an improvement of all biochemical parameters in the treated and protective groups, and X-ray, besides histopathological examination. **Conclusion:** Ph either alone or in combination with Plq, exhibited significant anti-arthritic activity in a CFA-induced rheumatoid arthritis model.

Introduction:

Rheumatoid arthritis (RA) is a chronic, prevalent autoimmune inflammatory arthritis disorder that can result in persistent disability and joint damage in multiple joints [1]. Between 0.3% and 1% of people have RA, and it is more prevalent in developed nations and among women [2].Hydroxychloroquine (active ingredient of Plaquenil) is a 4-aminoquinoline derivative that is used as an antimalarial medication and, more commonly, as a disease-modifying anti-rheumatic drug (DMARD) to treat

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chronic inflammatory autoimmune disorders such as RA, sarcoidosis, Sjögren's syndrome, and systemic lupus erythematosus [3]. Plaquenil drug marks with safe, cheap, and efficient antimalarial medication; hydroxychloroquine also has antithrombotic, cardioprotective, antibacterial, and anti-neoplastic properties [4]. But long-term usage of the Plaquenil generates serious negative effects that could force these treatments to stop [5, 6]. The side effects of Plaquenil are retinopathy, cardiotoxicity, and neurotoxicity [7]. It has become a human commitment to continuously hunt for novel anti-arthritic medications that are efficacious and have a therapeutic profile that is tolerated after extended use. In this regard, the potential therapeutic benefit of phloretin is possibly because of its anti-inflammatory [8], antioxidant [9], antitumor [10], besides hepatoprotective properties [11].Phloretin (Ph) is a dihydrochalcone derivative classified under the flavonoid group, present in apples and strawberries, but is prevalent in apples [12]. By lowering the synthesis of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, and IL-17), phloretin successfully reduced joint inflammation and the degeneration of cartilage and bone while also lessening the severity of RA [13].The goal of the current investigation was to assess the anti-arthritic effect of phloretin and ph combined with Plq against the rheumatic impact of CFA in rats.

Materials and Methods:

Chemicals:

Phloretin with CAS number 60-82-2, molecular formula C15H14O5, molecular weight 274.29 g/mole, storage temperature 2-8°C, with purity 98%, was purchased from Acros Organics, Belgium.

Plaquenil (Hydroxychloroquine sulphate - 200 mg film-coated tablets) is a treatment for rheumatoid arthritis and was purchased from SANOFI Egypt.

Complete Freund's Adjuvant (CFA) (10 mL; Each ml of CFA comprises 0.85 mL of paraffin oil, 0.15 mL of mannide monooleate, and 1 mg of dried and heat-killed Mycobacterium tuberculosis) was bought from Sigma-Aldrich Chemical Co., USA.

Animals:

Forty-eight adult male Wistar albino rats (120 -140 g) were acquired from Alexandria University's Faculty of Agriculture. Throughout the investigation, the animals were housed for a week to acclimate to the conditions in the husbandry room, which was maintained at 24 ± 2°C with a light-dark cycle of 12:12 hours, fed standard laboratory chow with tap water ad libitum. The Institutional Animal Care and Use Committee at Fayoum University granted approval for the experimental methods and animal welfare, which followed Rules for the Caring and Utilization of Lab Animals precisely (Approval number: AEC2351). Efforts were made to reduce the number of animals used and to minimize the distress of animals.

Experimental design:

After an acclimation period of 1 week, forty-eight rats were separated haphazardly into 8 groups, six rats in each group. **Group I (control)**; rats received 1ml distilled water orally once a day for 3 successive weeks; **Group II (Plquenil group)(Plq gp)** rats received 1ml Plq (124 mg/kg BW/day) orally for 3 successive weeks [14]; **Group III (Phloretin group)(Ph gp)**rats received 1 ml Ph(25 mg/kg BW/day) orally for 3 consecutive weeks[12]; **Group IV(CFA gp)** Rats were given a sole subcutaneous dosage of CFA (0.1 ml) to cause arthritis in the left posterior foot paw's sub-plantar zone [15]; **Group V(Plaquenil Trated)(Plq Tr)** rats were injected with CFA, and 1 day thereafter, Plq was administered for 3 successive weeks; **Group VI (Ph Tr)** rats were injected with CFA, and 1 day thereafter, Ph was administered for 3 consecutive weeks; **Group VII (Plq&Ph Tr)** rats were

injected with CFA, and 1 day thereafter, Plq and Ph was administered for 3 successive weeks; **Group VIII (Phloretin Protective) (Ph Pr)** rats processed orally with Ph 2 consecutive weeks before and 3 successive weeks following CFA injection.

Sample collection

After the experimental period, all rats were allowed to fast for 12 hours. Euthanasia was performed using 5 g/kg ether (inhalation), and blood samples were collected in three tubes for each rat as follows: the first tube contained EDTA to ascertain white blood cell count, the second tube contained sodium citrate for the ESR assay, and in the third tube, serum was separated by centrifuging blood at 4000 rpm for 20 minutes after it was allowed to remain at 37ºC for 15 minutes. The serum was extracted and stored in plastic containers at -20ºC until it was required for additional biochemical analyses. Rats were sacrificed following blood collection, and their foot paw tissue was promptly extracted and preserved in 10% neutral formalin for histopathological examination.

Measurement of Body Weight (BW) changes

Every week from the beginning until the conclusion of the experiment, the animals' body weight was assessed using a digital weighing balance, and the starting and final BW were utilized to evaluate changes in BW [16].

Hematological Analysis

Rat blood samples were collected to assess the total white blood cells (WBCs) count, as blood cells were then suspended in WBC-diluting fluid and counted in a hemocytometer while being viewed under a light microscope [17]. Also, ESR assay was measured using the Westergren method [18].

Biochemical analysis

Specific rheumatoid biomarker assays

Serum rheumatoid factor (RF) and Serum Anti-Cyclic Citrullinated Peptide antibody (anti-CCP) assays were estimated by ELISA kits according to Kragstrup et al. [19] and Tampoia et al. [20], respectively.

Proinflammatory Cytokines

Serum interleukin-6 (IL-6) and Serum Tumor Necrosis Factor-alpha (TNF-α) assays as indicators of proteins from the acute inflammatory phase were assessed using sandwich ELISA kits obtained from BT LAB Co., according to Pirttila et al. [21] and Boehme et al. [22], respectively.

Oxidative stress biomarker assays

The quantitative estimation of lipid peroxidation was determined by measuring malondialdehyde (MDA) levels using the TBA method according to Esterbauer & Cheeseman [23]. Total Antioxidant Capacity (TAC) was determined using bio diagnostic kits in accordance with Koracevic et al. [24].

Liver function biomarker assays

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined using bio diagnostic kits according to the method explained by Reitman & Frankel [25].

Kidney function biomarker assays

Creatinine (Creat) and Urea were estimated according to Bartels et al. [26] and Fawcett & Scott [27], respectively.

X-ray and live images of the feet of rats

Rats were given anesthesia at the conclusion of the experiment, and X-ray images of their joints were taken at Fayoum University's faculty of dentistry. The images were assessed based on soft tissue swelling, joint space, and joint alterations [28]

Histopathological investigation

After dissecting the left hind limb, the skin and muscles were cut off, the digits were removed, and the ankles were preserved for two days in 10% buffered formalin. For a month, 10 milliliters of formaldehyde (37–40%) and 90 milliliters of distilled water were used to dissolve 5.5 grams of ethylene diamine

tetraacetic acid (EDTA) to decalcify. Until the samples were completely soft and decalcified, the EDTA was replaced every 48 hours. The ankle articulation was cut longitudinally in a sagittal plane along the center line. The samples were then dehydrated, cleaned, and paraffin-embedded. Following splitting at a 5 μm depth, H&E staining was carried out. [29].

Statistical analysis

The Statistical Package for the Social Sciences (SPSS software, version 16) and Microsoft Excel (version 10) were used on a personal computer to conduct the analysis. According to the method outlined by [30,31], the following analyses were carried out. All values are provided as mean ± standard deviation (SD) [32]. When p values are ≤ 0.05, the difference in means is significant.

Results

BW change:

There was a significant decline in the BWs in the CFA group compared with the control group. While the BWs increased significantly in all treated and protective groups compared to the CFA group (Table 1).

Hematological & Biochemical results

Our results displayed a significant rise in WBCs count in group IV compared with group I. But there was a significant reduction in WBCs count in V-IIIV groups compared to group IV (Table 2).

Our data presented a significant rise in ESR and serum levels of RF, anti-CCP, TNF-α, and IL-6 in group IV in comparison with group I. But compared with group IV there were significant decreases in all treated and protective groups (Table 2).

The existing data presented a significant decline in TAC levels and a significant rise in MDA in the CFA group compared with the control group. Compared to the CFA group (group IV), there was a significant increase in serum TAC levels and a significant decrease in MDA in V- IIIV groups (Table 3).

Our investigation displayed a significant rise in ALT and AST levels in the CFA group compared with group I, but the therapeutic and protective groups displayed a significant drop in ALT and AST in comparison with the CFA group (Table 3).

Our outcomes directed a significant rise in serum levels of Creat and Urea in the CFA group comparison with group I. Compared to the CFA group, all treated and protective groups showed significant decreases in serum levels of Creat and Urea (Table 3).

X-ray and live images of the feet of rats

The radiographic examination of ankle joints showed that the experimental control group (control group, plaquenil group, and Phloretin group) had all examined joints intact, as regards spaces and articulations. While the CFA group showed marked narrowing of joint space and diffuse swelling of the surrounding soft tissue, these changes weren't noticed in the experimental control groups. These changes were improved in Plq, Ph, and Plq&Ph treated groups and Ph Pr group, as there was no soft tissue swelling, and the joint space resembled normal (Figure 1).

Histological examination of ankle joints

(Figure 2)

Discussion

Rheumatoid arthritis is a long-term autoimmune disorder that mainly impacts the joints of the hands and feet and the membranes that surround them. Pannus development, immune cell infiltration, synovial hyperplasia, and the consequent destruction of bone and cartilage are its defining features [33]. Currently, the management of rheumatoid arthritis involves the prolonged use of pharmaceutical agents such as DMARDs, non-steroidal anti-inflammatory drugs (NSADs), and corticosteroids, which are designed to delay the structural deformities of RA or

manage symptoms [34]. Hydroxychloroquine, an example of DMARDs, is classified as an aminoquinoline medication; Initially, they were developed as medications to combat malaria, but can also be utilized for rheumatoid arthritis and other rheumatic diseases such as lupus [35]. In addition to their clarified side effects and expensive cost, approximately 30% of the patients did not respond to previous conventional treatments [36]. Because they are less expensive, less harmful, and have fewer adverse effects than synthetic medications, natural products are now preferred for treating arthritis [2]. The dihydrochalcone compound phloretin is only present in apples, pears, strawberries, and sweet tea [37]. It possesses several pharmacological properties, including anti-inflammatory, antioxidant that shields the skin from UV light damage, anticancer, and hepatoprotective [12].

The present study found significant weight loss after the injection of CFA because inflammation inhibits the intestines' ability to absorb nutrition [38]. Compared to the control group, the CFA group's total body weight decreased significantly. Our results strongly concur with Khawaja & El-Orfali's conclusions [16]. While the body weight increased significantly in the Plq Tr group in comparison with the CFA group, this result is consistent with Akhtar et al. [39]. Administration of Ph in Ph Tr and Ph Pr groups directed to a significant rise in the rats' body weight in comparison with the CFA group. This agrees with Wang et al. [40].

The investigation demonstrated that rats treated with CFA displayed an elevation in WBC count in comparison with the control group. this disagrees with Al-Iedani [41], but similar results were observed by Das et al. [38]

While the Plq Tr group showed a significant drop in WBC count in comparison with the CFA group, this agrees with Akhtar et al. [39]. In addition, Phloretin treatment caused a significant decline in WBC count in comparison with the CFA group, which is in line with Fathi et al. [42].

In our study, the ESR level in the CFA group increased significantly in comparison with the control group. Sharma et al. [43] supported this result. But treatment with Hydroxychloroquine in Plq Tr group directed to a significant retardation in ESR level in comparison with the CFA group, this concurs with Wang et al. [44]. Also, the administration of Ph in the Ph Tr and Ph Pr groups led to a significantly lower ESR than that in the CFA group. This agrees with Ramadan et al. [45].

In the present data, serum rheumatoid markers such as RF and anti-CCP increased significantly in the CFA group in comparison with the control group. The results reported here are close to those of El-Shiekh et al. [2]. Administration of Ph in Ph Tr group and Plq in Plq Tr group led to significant decreases in serum RF and anti-CCP, but combining the two treatments created a more significant drop in serum rheumatoid markers.

In consistent to Hallak et al. [46], there was a significant rise in TNF-α in the CFA group against the control group. In comparison with the CFA group, the Plq Tr group's TNF-α levels significantly decreased. This is supported by Akhtar et al. [39]. Additionally, Administration of Ph in the Ph Tr and Ph Pr groups resulted in significant decrease in TNF-α contrast to the CFA group, that supports Wang et al. [13]

IL-6 is essential to the pathophysiology of RA because it can promote oxidative stress, osteoclastogenesis, and pannus development [47]. Our results reported a significant increase in IL-6 in group IV against group I; these outcomes harmonized with Rafeeqi et al. [48]. while administration of ph displayed a

highly significant decline in IL-6 level in Ph Tr and Ph Pr groups in comparison with the CFA group. This is compatible with Wang et al. [13] and Hsu et al. [49]. Additionally, there was a significant decline in IL-6 levels in Plq Tr groups compared to the CFA group, which is in line with Silva et al. [50], Ulander et al. [51], and Wakiya et al. [52].

According to our findings, the CFA group's TAC significantly decreased in contrast with the control group. This matches El-Shiekh et al. [2] and Hajizadeh et al. [53]. But, administration of hydroxychloroquine in the Plq Tr group led to a significant rise in TAC in contrast with the control group. This is supported by Ahmed& Althanoon [54]. In addition, giving Ph to the rats in Ph Tr and Ph Pr groups caused a significant increase in serum TAC compared to the CFA group, which concurs with Khalifa et al. [55].

In line with Cellat et al. [56], our data displayed a significant rise in MDA in the CFA group against group I. However, the Plq Tr Group, which was given hydroxychloroquine, had a significant decline in MDA in comparison with the CFA group. Akhtar et al. [39] support our findings. Moreover, administration of Ph in Ph Tr and Ph Pr groups directed to a significant drope in MDA in comparison with the CFA group, Wang et al. [13] concur with this.

Our data demonstrated that the CFA group's ALT and AST levels were significantly higher than those of the control group. Costa et al. [57] corroborated this finding. But Plq Tr Group presented a significant reduction in ALT and AST levels in comparison with the CFA group, this result is supported by Akhtar et al. [39]. Additionally, Ph Tr & Ph Pr groups, which were given Ph, presented a significant reduction in ALT and AST levels in comparison to the CFA group. this result agrees with Zuo et al. [58], who found that Ph decreased ALT and AST levels in acute liver damage.

In the present data, serum urea and creat levels in the CFA group significantly rose in comparison with the control group; Cellat et al. [56] support this finding. While administration of hydroxychloroquine in the Plq Tr group led to a significant reduction in levels of serum urea and Creat levels in comparison with the CFA group. This is in agreement with An et al. [59], who found that hydroxychloroquine decreased serum creatinine levels and suppressed the inflammation of the renal interstitium. Additionally, treatment with Ph in the Ph Tr and Ph Pr groups causes a significant decline in urea and Creat levels compared to the CFA group. This is following Un et al. [60], who stated that by reducing serum urea levels, phloretin minimizes chronic renal dysfunction caused by hyperuricemia.

X-ray and live images of the feet of rats further verified Ph and Plq's anti-rheumatic effectiveness. Radiographs of the ankle joints of the CFA group were marked with joint space narrowing and soft tissue swelling, these changes were not found in the control group. This outcome supports Das et al. [38]. After treatment with Phloretin and Plaquenil, the changes were improved.

The biochemical alterations and X-ray examination were additional reinforced by histopathological explanations of the joints. The most degenerative changes in articular cartilage and destruction of the adjacent bone were found in the CFA group, this agree with Alves et al.[61]. Treatment with Ph and Plq improved the histological changes and made them more similar to normal. This is in line with Wang et al. [13].

Conclusion:

In conclusion, administration of phloretin alone or in combination with Plaquenil enhanced the biochemical and histological changes against CFA-induced RA in male adult rats. our study

showed that phloretin may have potent anti-arthritic properties that protect rats from rheumatic arthritis diseases.

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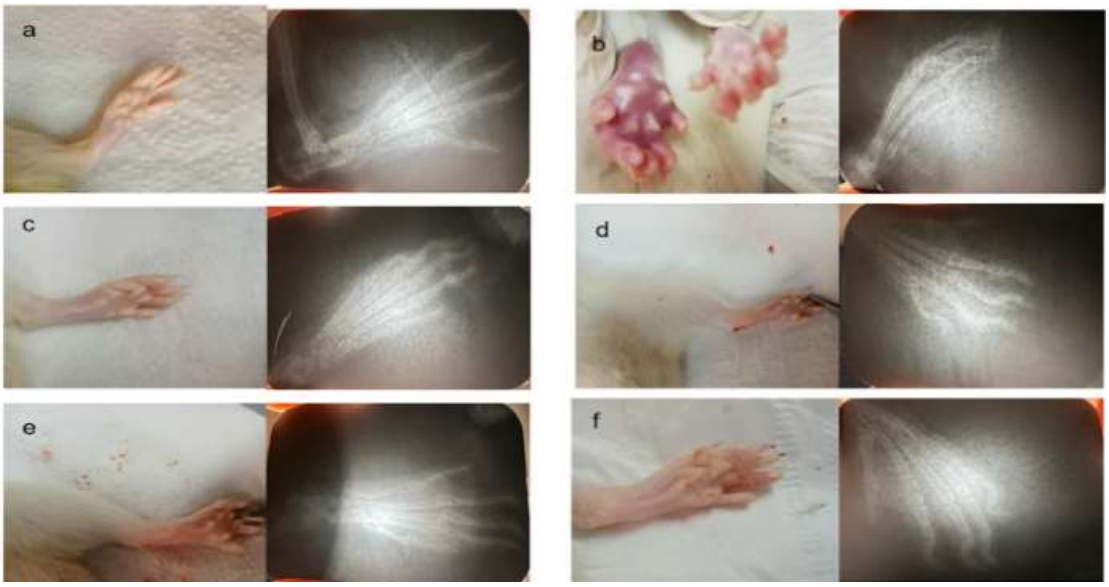

Figure (1): X-ray and live images of the feet of rats a experimental control group b CFA group c Plq treated group d Ph treated group e Plq&Ph treated group f Ph protective group.

Table (1): Mean ± SD of body weight (g) at the beginning and conclusion of the experiment in different groups

	Mean ± SD At the start	Mean ± SD At sacrifice	*P* value [a]	*P* value [b]	% of change
Group I (control)	132±2.1	172±5.3			30.3↑
Group II (Plq)	6.7±133	151±6.1	***0.001 >***		13.5↑
Group III (Ph)	3.7±132.5	174±3.6	0.463		31.3↑
Group IV(CFA)	133±5.7	122±4.7	***0.001>***		9.0↓
Group V(Plq Tr)	133±3.9	140±6.7	***0.001 >***	***0.001 >***	5.3↑
Group VI(Ph Tr)	132±2.9	156±6.5	***0.001***	***0.001 >***	18.2↑
GroupVII(Plq&PhTr)	5.2±133	151±4.5	***0.001 >***	***0.001 >***	13.5↑
Group VIII (Ph Pr)	132.5±3.3	173±4.2	0.724	***0.001 >***	30.1↑

P-value ≤ 0.05 indicates significance.
P-value > 0.05 indicates non-significance.
P^a indicates p-value against the control group.
P^b indicates p-value against the CFA group.

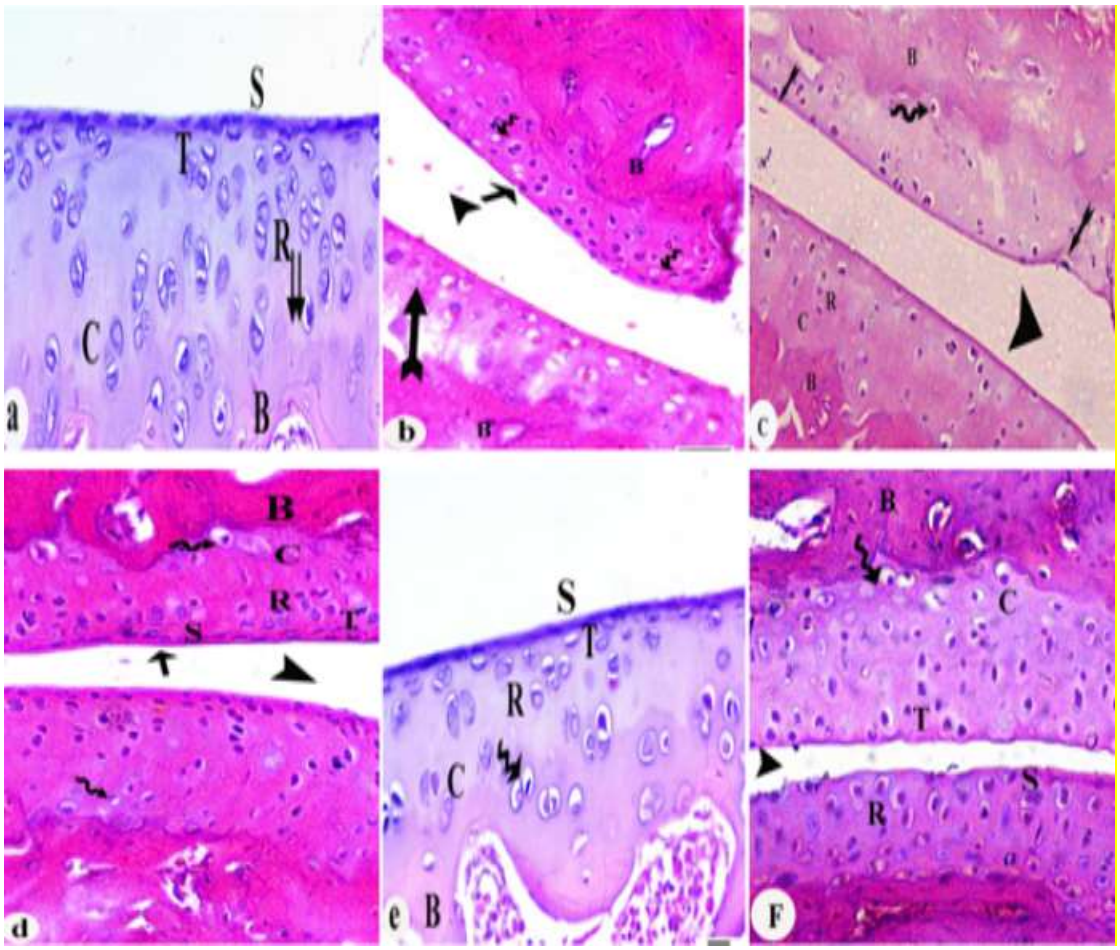

Figure (2): Photomicrograph of rat joints, **(a)** photomicrograph of the experimental control group (The control group, plaquenil group, and phloretin group) showed a typical articular surface histological structure. The articular cartilage was divided into 4 regions: 1st a superficial tangential region (S), 2nd transitional region (T), 3rd radial region (R), and 4th calcified region (C), including chondrocytes dispersed and superiorly enclosed by the line of tidemark (arrows). There was a calcified zone underneath the subchondral bone (B). (H&E x400), **(b)** Photomicrograph of the CFA group illustrated Pannus development (↑) which caused the articular cartilage to lose its smooth shape and developed a rough surface. Decreasing focal areas, absence(erosion) of calcified cartilage (⟶), degenerative changes in articular cartilage (▲), pyknotic nuclei in chondrocytes and vacant chondrocyte lacuna (↝), and destruction of the adjacent bone (B) were also noticed (H&E x200), **(c)** a photomicrograph of the Plq Tr group showed little improvements, but the articular cartilage had a little irregular surface and with smooth contour. Decreasing focal areas and calcified cartilage thickness (⟶), degenerative changes in articular cartilage (▲), pyknotic nuclei in chondrocytes and vacant chondrocyte lacuna (↝), and destruction of the adjacent bone (B) were also noticed (H&E x200), **(d)** photomicrograph of the ph Tr group showed a limited impact on joint structure following RA induction. The articular cartilage had a regular surface with a nearly smooth contour (↑). Decreasing chondrocyte lacuna and chondrocytes with pyknotic nuclei (↝) , and destruction of the adjacent bone (B) were also noticed. A basophilic matrix with chondrocytes inside its lacunae was seen in the articular cartilage, which seemed to be thicker (▲) (H&E x200), **(e)** Photomicrograph of the Plq&Ph Tr group showed a positive impact on the structure of joints following RA induction. The articular cartilage with a regular surface, a superficial tangential zone (S) with smooth surface, 2nd transitional zone (T), a 3rd radial zone (R); and 4th zone calcified zone (C) with decreasing chondrocytes lacuna and chondrocytes with pyknotic nuclei, and destruction of the adjacent bone (B) were also noticed. A basophilic matrix with chondrocytes inside its lacunae was seen in the joint cartilage, which seemed to be thicker (H&E x400), **(f)** photomicrograph of the ph Pr group showing little alterations in joint structure" The articular cartilage showed the presence of chondrocytes inside its lacunae in a basophilic matrix with nearly normal thickness of superficial tangential zone (S), 2nd transitional zone (T), a 3rd radial zone (R), and 4th zone calcified zone (C) with nearly normal chondrocytes with decreasing pyknotic nuclei and subchondral bone (B)contains inflammation (H&E x200).

Table (2): Mean ± S.D. of WBCs, ESR, RF, Anti-CCP, TNF-α, and IL-6 in the different groups

	WBCs (× 10^3 / mm^3)	ESR (mm/hr)	RF(IU/ml)	Anti-CCP (IU/ml)	TNF-α (ng/L)	IL-6 (ng/L)
Group I (control)	8.95±0.19	3.8±0.75	9.2±0.18	5.2±0.23	53.6±0.60	3.42±0.25
Group II (Plq)	8.10±0.26[a]	4.3±0.82	9.1±0.24	5.1 ±0.35	52.9±0.38	3.2±0.18
Group III (Ph)	9.15±0.48	3.7±0.52	9.0±0.29	4.9±0.49	53.0±0.35	3.35±0.19
GroupIV(CFA)	15.88±0.59[a]	9.2±1.17[a]	57.5±3.27[a]	25.6±1.27[a]	90.9±0.59[a]	8.4±0.34[a]
Group V(Plq Tr)	12.0±0.32[ab]	6.8±0.75[ab]	26.3±2.16[ab]	10.4±0.31[ab]	66.2±0.45[ab]	6.9±0.24[ab]
Group VI(Ph Tr)	13.0±0.24[ab]	6.3±1.03[ab]	30.8±1.47[ab]	11.4±0.35[ab]	70.4±0.91[ab]	6.5±0.30[ab]
Group VII(Plq&Ph Tr)	12.52±0.38[ab]	6.5±0.84[ab]	24.5±1.87[ab]	7.7±0.34[ab]	61.6±0.83[ab]	6.3±0.29[ab]
Group VIII (Ph Pr)	12.70±0.40[ab]	5.8±0.98[ab]	29±2.37[ab]	10.1±0.38[ab]	68.1±0.74[ab]	6.2±0.33[ab]

P-value ≤ 0.05 indicates significance
P-value > 0.05 indicates non-significance
[a] specifies a significant p-value versus the control group
[b] specifies a significant p-value versus the CFA group.

Table(3): Mean ± S.D. of TAC, MDA, ALT, AST, Urea, and Creat in the different groups

	TAC (mM/L)	MDA (nmol/ml)	ALT (U/L)	AST (U/L)	Creat (mg/dL)	Urea (mg/dL)
Group I (control)	0.47±0.026	10±0.31	30±2.37	107±2.61	0.43±0.023	25±0.47
Group II (Plq)	0.36±0.01[a]	26±0.93[a]	39.3±1.75[a]	122.5±2.43[a]	0.51±0.025[a]	29.7±0.62[a]
Group III (Ph)	0.54±0.02[a]	6.3±0.61[a]	29±2.19	104.7±2.94	0.42±0.029	24.4±0.79
GroupIV(CFA)	0.2±0.022[a]	53.8±0.20[a]	56.8±2.79[a]	150.3±2.16[a]	0.84±0.037[a]	44.9±0.76[a]
Group V(Plq Tr)	0.30±0.02[ab]	38.5±1.80[ab]	46±2.28[ab]	139.2±3.19[ab]	0.69±0.022[ab]	38.0±0.44[a b]
Group VI(Ph Tr)	0.37±0.01[ab]	22±1.13[a b]	37.2±3.06[ab]	124.3±3.14[ab]	0.59±0.033[ab]	34.5±0.43[a b]
GroupVII(Plq &Ph)	0.34±0.02[ab]	27±1.29[ab]	39.7±2.07[ab]	129.5±+1.8[ab]	0.63±0.019[ab]	36.0±0.34[ab]
Group VIII (Ph Pr)	0.39±0.02[ab]	18±1.00[ab]	36.2±1.47[ab]	118.8±2.86[b]	0.56±0.029[ab]	32.7±0.46[ab]

P-value ≤ 0.05 indicates significance
P-value > 0.05 indicates non-significance
[a] specifies a significant p-value versus the control group
[b] specifies a significant p-value versus the CFA group.