Evaluation of Anti-Rheumatoid Arthritis potential Activity of Persimmon juice in female rats induced by Complete Freund's Adjuvant

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Abstract:

This study aimed to evaluate the potential activity of persimmon fruit juice (PFJ) against rheumatoid arthritis (RA). The study conducted on 35 female albino (Sprague-Dawley) rats weighing (170±10g)were randomly divided into five groups(7each) fed on basal diet the first group was the control negative and four other groups were injured with 1ml/kg of Freund's complete adjuvant into the left hind knee joint for7days to induce RA. They were re-divided as follows: group2 served as the control positive group and the three other groups were treated with different doses of PFJ (low)1ml, (high)2ml/kg bw and drugs Diclofenac sodium(DS)10mg/kg bw, respectively. The experiment lasted for 28 days. The chemical results displayed that the persimmon fruit had a high content of fiber, total phenolic, ascorbic acid content and antioxidant activity. While, the biological results revealed that the all treated groups by(low, high doses of PFJ and DS) showed a significant decrease in paw volume as well as improved biochemical and hematological analyses such as white blood cell count, erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, malondialdehyde, interleukin(IL)-6 and tumors necrosis factor(TNF)-α levels. While showed a significant increase in body weight, hemoglobin level, red blood cell count, and antioxidant enzymes in joint tissue. Histological examination of joints tissue confirmed the results of biochemical analyzes of blood. It can be recommended that the necessity consumption of persimmon fruit juice in the diets of peoples exposed to rheumatoid arthritis, because of their anti-rheumatoid arthritis and antioxidants properties, and that play important role of health status.

Keywords: Diospyros kaki; Diclofenac Sodium; Rheumatoid arthritis; Joints tissue and Experimental animals.

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Introduction

Rheumatoid arthritis (RA) is a clinical example of an autoimmune disorder induced by several factors. The global prevalence of RA is documented to be between 0.5 and 1% **(Otón and Carmona, 2019)**. It is most common in individuals within 25 and 55 old age. The incidence of RA in women more than men by three times **(Meroni *et al.*, 2018**). This disease initially injured the smaller joints, manifesting as erosive and destructive polyarthritis **(Ben *et al.*, 2022**). In animal models of arthritis, pro-inflammatory cytokines, such as (IL-1β, IL-6, and TNF-α) are conducted in all stages of the disease's pathogenesis, involving the development of autoimmunity, the preservation of a persistently inflammatory synovitis, and the deterioration of joint tissue next to the affected joint. Therefore, the immune-regulatory and tissue-destructive mechanisms that underlie the development and clinical manifestation of rheumatoid arthritis are combined by cytokines (**Andreev *et al.*, 2022**).

Complete Freund's adjuvant (CFA) has been linked to several inflammatory responses. Where it causes injection of CFA to the footpad of rats causes inflammation of the skin that confirmed by redness and swilling appear quickly 2 hours and peaks between 6 or 8 hours **(Nasuti *et al.*, 2019)**. Erythrocyte sedimentation rate (ESR), blood neutrophil, and leukocyte counts start to increase on the fourth day following CFA injection, and this first inflammation lasts for three to four days. A few weeks after the CFA injection, there is a subsequent development of edema and hyperalgesia in the ankle as a result of considerable synovial lining expansion and neutrophil infiltration **(Luo *et al.*, 2020)**.

Diclofenac sodium (DS) is a well-known non-steroidal anti-inflammatory drug (NSAID) with analgesic, antipyretic, and anti-inflammatory properties **(Orabi *et al.*, 2020)**. Long-term DS use can have serious adverse effects on heart and kidney **(Duarte *et al.*, 2019)**. Because of the limitations of these novel therapies and medicines, it is critical to develop natural products for the treatment of RA.

Persimmon fruit (*Diospyros kaki*) (PF) is an edible fruit that belongs to *Ebenacea* family with a sweet/ slightly tangy taste and fibrous to the soft texture. It is rich in vitamins, fibre, carbs, minerals, phenolic compounds and proteins **(Matheus *et al.*, 2020)**. Its high levels of polyphenols, carotenoids, ascorbic acid, and tannins suggest a range of health advantages. One trait that distinguishes the persimmon from other fruits is its high proanthocyanidins concentration, known as persimmon tannin (PT) **(Wang *et al.*, 2023)**. Both the fruit and the leaves of the PF have been reported to have significant biological significance, including antioxidant and anti-inflammatory properties, protection against rheumatoid arthritis, cholesterol reduction, and resistance to free radicals **(Ferrara, 2021)**. Therefore, this study was carried out to evaluate the potential activity of persimmon fruit juice compared with Diclofenac sodium drug against rheumatoid arthritis in female rats.

Materials and methods

Materials

Fruits: Ripe mature seedless persimmon (*Diospyros kaki*) were brought from local markets in Port Said, Egypt at the end of September 2023. The fruit had red-orange color, spherical shape with an average weight 150-170 gm. per fruit.

Chemicals: Basal diet, casein, cellulose, vitamins and minerals, were purchased from the General Company for Commerce and Chemicals, (Cairo, Egypt). Diclofenac sodium (Voltaren® SR 100 mg) and CFA were acquired from Merck Company, (Cairo, Egypt).

Kits: for biochemical estimations were provided from Gama Trade Company, (Cairo, Egypt).

Rats: 35 adult female (Sprague-Dawley) albino rats (weighing 170 ±10g) were bought from the National Research Center's animal house in Giza, Egypt.

Methods

Preparation of Persimmon juice

Fresh, spotless, and seedless date plum persimmons were chosen, and the leaves were manually removed and washed with drinkable water. There is no need to remove the fruit skin to acquire the pulp for the juice preparation; the ripe fruits (100 g) were combined in a food blender with 1000 ml distilled water for 10 minutes and filtered through a sieve to yield fruit juices at a concentration of 10%. To prevent discoloration, a solution of citric acid (0.1%) was added to the strained pulp before filling sterilized glass bottles. The juice was kept refrigerated at 4-8°C until it was used.

Chemical investigations

Moisture, protein, fat, ash, and crude fibre were measured in accordance with **A.O.A.C (2010)**. By using the differential, total carbohydrates were computed.

Total phenolic content (TPC) and Total flavonoids content (TFC): The Folin-Ciocalteu by method of **Figueira *et al*., (2014)** was used to determinate phenolic compounds. Gallic acid equivalents dry weight extract (mg GAE/g DW) was used to express the results. **Cam and Hisil, (2010)** method were used to ascertain the total flavonoid contents. The results were given in mg of catechin equivalents (mg/CE) per 100 g of persimmon.

Ascorbic acid content

The ascorbic acid concentration of the sample was ascertained using a spectrometric method with certain adjustments as described by **Figueira *et al*., (2014)**. Its content was stated in mg of ascorbic acid per 100 g persimmon.

Determination of Antioxidant Activity

The antioxidant activity was measured spectrophotometrically using the DPPH free radical scavenging capacity **(Haddouchi *et al*., 2014)**.

Determination of Phenolic and Flavonoid by HPLC

HPLC was used to determine the phenolic and flavonoid components in fresh persimmons using the methods developed by **Goupy *et al.*, (1999) and Mattila *et al.*, (2000)** respectively.

Induction of rheumatoid arthritis

RA was generated in rats by injecting 1ml/kg of CFA freshly prepared with saline intraperitoneally into the left hind paw, as described by **Khaled *et al.*, (2022)**.

Biological Experiment

Ethical approval: the study was started after approved from the research Ethics Committee of Nursing Faculty of Port Said University, code number: NUR (3-12-2023) (32).

After a week of acclimation in the animal house, the rats were randomly allocated into five groups. Group (I): (n =7) the rats fed a basal diet according to **(Reeves *et al.*, 1993)**, which served as a negative control group. Four other groups: (n=28) were fed basal diet and injected with 1 mL/kg of CFA into the left hind knee joint for induction of RA **(Khaled *et al.*, 2022)**. RA was developed at 7^{th} day after a CFA injection. By the end of the induction period (7 days), all of the rats had acquired RA symptoms such as edema, redness, stiffness, and trouble moving joints. From days 8 after CFA-induced RA rats divided into 4 equal groups (7 rats each) as following: group (2) served as a positive control, and the three other groups were given oral doses of PFJ (low); 1ml/kg bw, (high); 2ml/kg bw at 10% concentration and Diclofenac sodium at 10 mg/kg bw in 0.5% dimethyl sulfoxide as standard drug, respectively **(Alazragi and Baeissa, 2023)** for 21 days.

For 21 days (from the 8^{th} to the 28^{th} day), all animals were given the prescribed doses of juice orally or stander drug.

After the experiment was completed, blood samples from the orbital plexus were collected followed by centrifugation at 3000 rpm to extract the sera, which were then stored in at -80°C until they were used for analyses.

Biological Evaluation

The amount of food intake (FI) was recorded daily, while rat's weight was measured once a week to identify the gain in body weight. In according to **Chapman *et al.*, (1959)**, body weight growth (BWG %) and feed efficiency ratio (FER) are calculated using the following equation:

$$\mathbf{BWG\%} = \frac{\mathbf{Final\ body\ weight - Initial\ body\ weight}}{\mathbf{Initial\ body\ weight}} \times 100$$

$$\mathbf{FER} = \frac{\mathbf{weight\ Gain\ (g)}}{\mathbf{Feed\ intake\ (g)}}$$

Measurements of the volume of the Paw:

The volume of the hind paw was measured using a digital plethysmometer before CFA administration on day 1 and then every 7 days for the next 28 days **(Alzarea *et al.*, 2022)**.

Biochemical analysis

Complete blood count (CBC): Haemoglobin (Hb) and Hematocrite (Hct) were measured depending on the method of **Billett, (1990) and Bain *et al.*, (2016)**. Red blood cells (RBC) and white blood cells (WBC) were estimated according to the method **Fischbach, (2015)**. Erythrocyte sedimentation rate (ESR) was determined according to **Westergren, (1957)**. C-reactive protein (CRP) was evaluated using the method of **Voila *et al.*, (1981)** and Rheumatoid Factor (RF) was obtained using the method of **Johnson and Faulk, (1976)**. For assessing lipid peroxidation, the plasma level of Malondialdehyde (MDA) was determined according to **Draper and Hadley, (1990)**. Superoxide dismutase (SOD) activity was assessed according to **Spitz and Oberley, (1989)**. Catalase (CAT) was measured according to previous method of **Aebi, (1984)**. Glutathione peroxidase (GP_X) were measured method by **Moin, (1986)**. Pro-inflammatory cytokine (IL-6 and TNF-α) were evaluated using a commercially available kits in accordance the instructions of the manufacturer (Ray Biotech, Inc., Norcross, GA) **(Tran *et al.*, 2023)**.

Histopathology of left hind limb joint

Histopathological analysis was conducted as described by **Patel *et al*., (2021)** procedure. The joints of the rats' left hind limb were detached and immersed in formaldehyde (10%) for 24 hours. Dehydrated formalin-fixed tissue was embedded in paraffin wax, sectioned at 5 mm, and stained with hematoxylin and eosin (H&E). A light microscope was used to examine histopathological changes.

Statistical analysis

The data were expressed as mean ±SE, and the difference between means was examined using One-way ANOVA followed by a post hoc (Tukey's) test. The difference in values was considered statistically significant at ($P \leq 0.05$). **(Armitage and Berry, 1987)**.

Results and Discussion

The chemical composition of 100 g of edible part persimmon was shown in **Table (1)**. The moisture, carbohydrates, crude fiber, and ash were significantly ($p \leq 0.05$) high in fresh persimmon represent as (64.68%, 18.19 %, 3.6% and 2.35% respectively).The results showed persimmon fruit is full of nutritious substances which were in line with **El Makhzangy *et al*., (2023) and Ahmad *et al*., (2022)**. The quantities of these contents were also close to the findings of other researchers.

Table (1): Chemical composition of persimmon (100g) of the edible part

Nutrients	Crude Protein	Crude Fat	Ash	Carb.	Moisture	Crude Fiber
Fresh Persimmon	0.76	0.42	2.35	18.19	74.68	3.6

Each value represents the mean of three replicates.

The present work revealed that the fresh persimmon fruit had a significant quantity of the total phenols content was 298.03 mg/GAG, while the total flavonoid content was 225 mg/CE. Though, the ascorbic acid content was 17.95 mg/AA, while, antioxidant activity was 80.50 DPPH % as shown in **Table (2)**. These findings correspond with those of **El Makhzangy *et al*., (2023)** who discovered that persimmon fruit is a great

source of antioxidant. **Hu *et al*., (2022)** observed that the microwave hydrothermal extraction (MHE) processed persimmon juice had the most total phenolics, total flavonoids, and antioxidant activity. Similar, **Silva *et al*., (2021)** observed that the juice made via freeze concentration contained a high concentration of phenolic components and antioxidant activity. Furthermore, **Mochida *et al*., (2022) and Direito *et al*., (2020)** showed that ascorbic acid concentrations in persimmon extract ranged from 0.68 to 1.129 mg/100g in *Diospyros kaki* is a good source of ascorbic, which is also in accordance with the current results.

Table (2): Total phenolic compounds and ascorbic acid content of persimmon fruit

Sample / Parameters	Fresh Persimmon
Total phenols (mg/GAG)	**298.03**
Total flavonoids (mg/CE)	**225**
Ascorbic acid content (mg /AA)	**17.95**
Antioxidant activity (DPPH %)	**80.50**

Each value represents the mean of three replicates.

GAE: gallic acid equivalent; CE: Catechin equivalents; AA, ascorbic acid.

Tabulated data in **Table (3)** presented the types and concentrations of phenolic and flavonoid compounds in fresh persimmon. From this table, it was eleven phenolic compounds; Pyrogallol it was found in higher concentration (1835ppm) followed by catechol (83.35ppm) and benzoic acid (50.50ppm) in decreasing orders. Tyro sol recorded as the lowest value (3.34ppm) of the all detected phenolic compounds. These findings are consistent with those obtained by **El Makhzangy *et al*., (2023)**. Also, **Direito *et al*., (2019)** found to be gallic acid was the most plentiful phenolic compound in an aqueous persimmon fruit extract. Regarding, the flavonoid compounds that presented in abundance were, Hesperidin, Quercetrin, Luteolin, Narengin and Rutin in decreasing order. Rosmarinic acid was the lowest value of flavonoids (47.30 µ/100g). Our results are compatible with

El Makhzangy *et al.*, (2023). All these components are useful to human health because of their potential to prevent or control a variety of ailments.

Table (3): Types of phenolic and flavonoid compounds in fresh persimmon

Phenolic acids	(ppm)	Flavonoid compounds	(µ/ 100g)
Gallic acid	5.93	Luteolin	559.40
Pyrogallol	1835	Narengin	534.70
4-Amino benzoic	4.78	Rutin	104.40
Tyrosol	3.34	Rosmarinic	47.30
Chlorogenic acid	43.20		
Catechol	83.35	Hesperidin	2699
Caffein	49.29		
Caffeic acid	43.73	Hispertin	57.64
Vanillic	28.82		
Ferulic acid	27.45	Quercetrin	816.60
Benzoic	50.50		

Each value represents the mean of three replicates.

Table (4) showed the effects of PFJ (low, high) and Diclofenac sodium (DS) on paw volume (PV) in rheumatoid arthritis female rats. PV increased in all rats except the control negative group when measured on day 7. CFA administration caused alterations in rats such as paw oedema, paw swelling, weight loss, biochemical and histopathological abnormalities, indicating the development of inflammation. **Manen *et al.*, (2022)** reported that CFA-induced arthritic rat is the most extensively used animal model for RA because it closely resembles the characteristics of human RA. These findings are matched with **Alzarea *et al.*, (2022) and Noh, *et al.*, (2021)** whom demonstrated that CFA caused paw edema, as evidenced by 2-3-fold higher paw volume. On the other hand, administered oral doses of PFJ (low, high), and standard drug (DS), attenuated the PV in all tested periods. The maximum decrease PV was observed on day 28^{th} compared to the RA control. These results indicated that high doses of PFJ had a higher on PV

impact than low-dose, and the outcomes were prominent compared to drug DS. This could be due to persimmon's significant antioxidant properties and its anti-inflammatory effects. These findings are consistent with those obtained by **Direito *et al*., (2020) and Direito *et al*., (2019)** who discovered a reduction in edema volume induced by arthritis after consuming persimmon extract. In addition, **Manen *et al*., (2022) and Triastuti *et al*., (2021),** demonstrated that DS dramatically reduced paw oedema in comparison to the RA groups.

Table (4): Effect Persimmon fruit juice (PFJ) on paw volume in control (-ve) and rheumatoid arthritis female rat groups

Groups	Paw volume (ml)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control (-ve)	0.15±0.04^{b}	0.15 ± 0.01^{c}	0.16 ± 0.02^{d}	0.13 ± 0.01^{c}	0.14 ± 0.02^{e}
Control (+ve) CFA	0.13±0.04^{c}	1.04 ± 0.27^{a}	0.78 ± 0.04^{b}	0.86 ± 0.03^{a}	0.83 ± 0.03^{a}
CFA+PFJ (low)	0.16±0.04^{b}	1.08 ± 0.04^{a}	0.85 ± 0.04^{a}	0.40 ± 0.02^{b}	0.39 ± 0.03^{b}
CFA+PFJ (high)	0.17±0.04^{ab}	0.98 ± 0.04^{b}	0.89 ± 0.04^{a}	0.39 ± 0.02^{b}	0.21 ± 0.03^{d}
CFA + Diclofenac	0.18±0.05^{a}	1.04 ± 0.08^{a}	0.88 ± 0.04^{a}	0.41 ± 0.03^{b}	0.29 ± 0.03^{c}

Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at (P≤0.05).

The data in **Table (5)** demonstrate the changes in body weight, food intake (FI), and feed efficiency ratio (FER) of RA rats. The rats that were injection of CFA showed substantial body weight loss (FBW) and (BWG) compared to negative control with significant difference although FI in both CFA groups and normal rats was near. During the study period, water and food consumption were normal, indicating normal protein, lipid, and carbohydrate metabolism within the body **(Zhang *et al*., 2022)**. One of the evaluation factors in RA progression is body weight change **(Manen *et al*., 2022)**. These findings are consistent with **Alzarea *et al*., (2022) and Noh *et al*., (2021)** observed that CFA injection reduced body weight in rats when compared to the negative control. Furthermore, RA is linked to a decrease of lean body mass (**Nasuti *et al*., 2019)**.Contrarily, treatment with PFJ at low or high dosages and DS considerably ($p<0.05$) improved body weight and

alleviated RA, with better results observed at high doses of PFJ, due to attribute to PFJ's high phenolic and flavonoid components. These findings agree with **Mochida *et al.*, (2022)** found that the after being given dried persimmon increased the BW and food intake of diabetes rats. Similarly, **Ali *et al.*, (2020) and El-Tour and El-Wahsh (2019)** found that significant increase in BWG, FI, and EFR were seen in rats given persimmon fruits. Furthermore, it is worth noting that CFA+ Diclofenac group had the highest FI and FER value when compared to the negative control. Using DS as an anti-inflammatory medicine normalizes absorption, restoring body weight **(Alazragi, and Baeissa, 2023)**. Similar, **Manan *et al.*, (2022)** demonstrate that treatment with the standard drug (DS) restored body weight and improved nutrition absorption in comparison to CFA control rats, while alleviating the suffering caused by RA.

Table (5): Effect of Persimmon fruit juice (PFJ) on BWG%, FI and FER in control (-ve) and rheumatoid arthritis female rat groups

Parameters / Groups	IBW (g)	FBW (g)	BWG %	FI (g/day)	FER
Control (-ve)	**175.07±1.50^{a}**	**194.53±1.25^{a}**	**11.12±1.17^{a}**	**15.00**	**0.046±0.03^{a}**
Control (+ve) CFA	**169.53±1.60^{a}**	**167.22±1.09^{c}**	**-1.36±1.32^{e}**	**12.50**	**-0.007±0.05^{e}**
CFA+PFJ (low)	**170.13±1.10^{a}**	**178.97±1.27^{b}**	**5.20±1.15^{d}**	**13.00**	**0.024±0.04^{d}**
CFA+PFJ (high)	**172.70±1.20^{a}**	**183.96±1.06^{ab}**	**6.52±1.12^{c}**	**13.60**	**0.030±0.05^{c}**
CFA +Diclofenac	**174.32±0.78^{a}**	**190.87±2.19^{a}**	**9.49±1.81^{b}**	**14.50**	**0.041±0.01^{b}**

Initial body weight (IBW), Final body weight (FBW), Body weight gain (BWG%) ,food intake (FI) and feed efficiency ratio (FER). Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at ($P \leq 0.05$).

The data in the **Table (6)** demonstrated the impact of persimmon fruit juice (PFJ) on the hematological parameters of RA female rats. CFA-induced rheumatoid arthritis causes anemia, as evidenced by significantly ($p \leq 0.05$) lower Hb, HCT, and RBC counts, while increasing WBC count and ESR in comparison with the negative control group, due to hyperactive immune system **(Narendhirakannan and Limmy, 2012)**. Anemia, a

significant clinical characteristic of RA, may be related to reduce iron storage in the reticuloendothelial system after RA **(Naz *et al*., 2020)**. In addition, ESR and inflammatory markers are also indicators of rheumatoid activity. An increase in all of these measures verifies the onset of RA **(Manan *et al*., 2022)**. These findings are agreed with **Alzarea *et al*., (2022) and Akhtar *et al*., (2022)** whom showed that administration of CFA decreased hemoglobin levels and RBC counts while increased of the WBC count and ESR in comparison with the negative control group.

Contrarily, treatment with PFJ at low or high doses, and standard drug DS significantly ($p \leq 0.05$) had reversed the hematological changes induced by CFA in rats and improved Hb, HCT levels, and RBCs, led to inhibit the onset of anemia. The highest doses of PFJ and DS had the greatest inhibition in abnormal alterations in hematological and biochemical indicators (Table 6). As a result of its anti-inflammatory properties, PFJ may be a safer choice in this regard. These findings are consistent with **Kashif *et al*., (2017) and Yaqub *et al*., (2016)** reported that persimmon has been found to be useful in reducing inflammation and increasing blood circulation throughout the body, because of its phenolic compounds (particularly tannins), carotenoids, and vitamin C. The current findings are consistent with **Manan *et al*., (2022) and Akhtar *et al*., (2022)** indicated that DS administration at 10 mg/kg oral dose considerably changed hematological parameters, and led to inhibit the onset of anemia.

Table (6): Effect of Persimmon fruit juice (PFJ) on some hematological parameters in control (-ve) and rheumatoid arthritis female rat groups

Parameters / Groups	Hb (g/dL)	HCT (%)	RBCs (10^6/mm^3)	WBCs (10^3/mm^3)	ESR (mm/hr)
Control (-ve)	**16.05±0.50^a**	**40.86±0.15^a**	**9.04±0.07^a**	**5.01±0.03^e**	**4.46±0.42^d**
Control (+ve) CFA	**9.67±0.53^d**	**25.07±0.32^d**	**4.22±0.06^d**	**9.86±0.03^a**	**9.77±0.40^a**
CFA+PFJ (low)	**12.74±0.46^c**	**30.54±0.25^c**	**6.35±0.09^c**	**7.10±0.06^b**	**6.51±0.25^b**
CFA+PFJ (high)	**14.46±0.24^b**	**34.76±0.27^b**	**7.67±0.04^b**	**6.54±0.05^c**	**5.04±0.27^c**
CFA +Diclofenac	**14.83±0.22^b**	**36.47±0.20^b**	**7.98±0.08^b**	**6.03±0.04^c**	**4.59±0.60^d**

Hemoglobin (Hb), Hematocrit (HCT), red blood cell (RBCs), white blood cells (WBCs) and Erythrocyte Sedimentation Rate levels (ESR). Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at ($P \leq 0.05$).

Table (7) showed the effects of PFJ (low, high) and DS on CRP and RF in rheumatoid arthritis female rats. Increase of CRP and RF values above normally indicate active inflammation and RA development in CFA groups. The high levels of CRP and RF are associated with elevated levels of IL-6 and TNF-α which contribute to the acceleration of synovitis and tissue injury, which were line with the findings of **Hussain *et al.*, (2021)** observed that a significant elevate CRP and RF level in CFA-induced arthritic groups. CRP is an indicator of inflammation and tissue damage throughout the body. It is a reactive protein that is generated by the liver stimulated IL-6 and TNF-α in response to infection, damage, and inflammation **(Kalaiselvan and Rasool, 2016)**. In addition, the measurement of RF in serum is one of the most well- established biomarkers for assessing the severity of RA **(Talwar *et al.*, 2011)**.

Contrarily, treatment with PFJ at low or high doses, and DS, significantly reduced CRP and RF levels ($p \leq 0.05$) as compared to the CFA group. These findings revealed that high dosages of PFJ highly significance in the improvement CRP and RF levels than low doses. **Akhtar *et al.*, (2022) and Manan *et al.*, (2022)** showed that cytokines such as IL-6 and TNF-α, regulate growth of CRP and RF concentrations. These findings were agreed with **Direito *et al.*, (2020)** and **Zou *et al.*, (2014)** observed that persimmon administration reduce of CRP and reduce the inflammation associated with RA. Furthermore, **Manan *et al.*, (2022)** showed that standard drug (DS) reduced the level of CRP and RF in groups treated by formaldehyde injection. Similarly, **Hussain *et al.*, (2021) and Daram *et al.*, (2021)** disclosed that DS reduced CRP and RF levels in arthritic rats.

Table (7): Effect of Persimmon fruit juice (PFJ) on rheumatoid arthritis markers (CRP and RF) levels in control (-ve) and rheumatoid arthritis female rat groups

Parameters / Groups	CRP (mg/L)	RF (IU/mL)
Control (-ve)	12.75±1.30^{c}	10.18±0.40^{d}
Control (+ve) CFA	21.34±2.72^{a}	22.9±1.43^{a}
CFA+PFJ (low)	16.32±0.34^{b}	15.02±0.23^{b}
CFA+PFJ (high)	10.54±0.72^{d}	13.06±0.23^{c}
CFA +Diclofenac	7.54±0.50^{e}	9.72±0.60^{d}

C-Reactive protein (CRP) and Rheumatoid factor (RF). Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at ($P \leq 0.05$).

Table (8) illustrates that CFA-induced rheumatoid arthritis significantly increased MDA level ($p \leq 0.05$). Whereas, the antioxidant markers (SOD, CAT and GPx) in joint tissue were significantly reduced ($p \leq 0.05$) CFA-treated rats in comparison to negative group, indicating that CFA caused oxidative stress damage. These findings were agreed with **Alzarea *et al.*, (2022)** that suggests, an oxidative stress is an underlying key mechanism related with persistent inflammation in RA, which were line with the findings of **Hussain *et al.*, (2021)**. Similar, **Manan *et al.*, (2020) and Manan *et al.*, (2022)** confirmed this findings that antioxidants such as GPx, CAT, and SOD were significantly reduced in CFA-induced arthritis in rats compared to negative control.

Contrarily, treatment with PFJ at low or high doses, and standard drug (DS) distinctly attenuated the oxidative stress to rats of RA treated groups by decreasing (MDA) and increasing (SOD, CAT and Gpx) levels, significantly. These results indicate that persimmon has beneficial effects on oxidative stress damage, due to its antioxidant properties **(Ferrara, 2021)**. These findings are consistent with **Shin *et al.*, (2021)** that suggested consumption of ethanolic extract of *Diospyros kaki* (EED) decreased the production of MDA levels. Similarly, **Tian *et al.*, (2012) and Zou *et al.*,**

(2012) observed that an oral dose of persimmon at 100 or 200 mg/kg alleviated oxidative stress in mice by increasing (SOD, CAT and GPx) and decreasing (MDA). Furthermore, **Alzarea *et al.*, (2022) and Manan *et al.*, (2022)** showed that diclofenac sodium (10mg/kg) increased the concentrations of SOD and CAT While, MDA concentrations were significantly reduced in CFA -injected groups.

Table (8): Effect of Persimmon fruit juice (PFJ) on oxidative stress and antioxidant enzymes in control (-ve) and rheumatoid arthritis female rat groups

Parameters / Groups	MDA (nmol/mg)	SOD (U/ml)	CAT (nmol/ ml)	GP_X (U/ml)
Control (-ve)	8.30 ± 0.75^{c}	13.31 ± 1.06^{a}	12.45 ± 2.11^{a}	15.05 ± 0.35^{a}
Control (+ve) CFA	13.34 ± 1.4^{a}	6.04 ± 0.52^{e}	6.12 ± 0.05^{d}	6.86 ± 0.21^{c}
CFA+PFJ (low)	10.96 ± 1.03^{b}	9.13 ± 0.19^{d}	8.47 ± 0.02^{c}	10.53 ± 0.23^{b}
CFA+PFJ (high)	10.50 ± 1.30^{b}	10.24 ± 0.73^{c}	10.36 ± 0.07^{b}	11.04 ± 0.27^{b}
CFA +Diclofenac	8.70 ± 1.07^{c}	11.60 ± 0.87^{b}	11.85 ± 0.02^{a}	14.52 ± 0.29^{a}

Malondialdehyde (MDA), Superoxide dismutase (SOD); Catalase (CAT) and Glutathione peroxidase (GPx). Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at ($P\leq0.05$).

Table (9) showed that CFA injection were significantly ($p \leq 0.05$) increased proinflammatory cytokines (IL-6, and TNF-α) in joint tissue compared to negative control. An increase in all these indicators verifies the induction of RA **(Alzarea *et al.*, 2022)**. The results agree with **Grötsch *et al.*, (2019)** stated that CFA injection elicits T cells that activate monocytes and macrophages, releasing proinflammatory cytokines like IL-6 and TNF, which accelerate bone erosion, and cell death in arthritic rats.

On the other hand, treatment with PFJ at (low or high doses), and DS significantly ($p\leq0.05$) reduced this elevated concentration of proinflammatory cytokines in comparison to the CFA group. The most effective high dose of PFJ (2ml/kg BW daily at a concentration of 10%). Which reduced levels of (IL-6, and TNF-α) and the results were significant

when compared to the standard drug (DS). The current agree with **Park *et al.*, (2020)** observed that persimmon fruit has a high anti-inflammatory impact by blocking pro-inflammatory signaling pathways in cells (IL-1β, IL-6, TNF-α).

Similarly, **Direito *et al.*, (2019)** showed that administering persimmon extract (15 mg/kg /day) reduces the level of chronic inflammation and tissue damage associated with collagen-induced arthritis (CIA) in rats. This effect that is most likely due to the extract's antioxidant properties. **Seo *et al.*, (2015) and Kim *et al.*, (2013)** observed that Persimmon consumption of 200 mL (diluted 5 times with water) reduced pro-inflammatory cytokine production. Persimmon fruit juice found to be antioxidant as well as an inhibitor of neutrophil function, limiting the generation of harmful ROS that enhanced inflammatory, may be related to the presence of several phytochemicals such as polyphenols, tannin, carotenoids, and ascorbic acid **(El Makhzangy *et al.*, 2023 and Direito *et al.*, 2020)**. Furthermore, **Ruckmani *et al.*, (2017)** reported that Diclofenac decrease the elevated IL-6 and TNF-α levels.

Table (9): Effect of Persimmon fruit juice (PFJ) on the pro-inflammatory cytokines indicators in control (-ve) and rheumatoid arthritis female rat groups

Parameters / Groups	IL-6	TNF-α
	pg/ml	
Control (-ve)	28.83± 0.76^{d}	98.22±0.54^{e}
Control (+ve) CFA	70.36± 0.84^{a}	415.54± 0.47^{a}
CFA+PFJ (low)	52.87± 0.63^{b}	221.08± 0.38^{b}
CFA+PFJ (high)	43.23± 0.35^{c}	156.30± 0.43^{c}
CFA +Diclofenac	41.92± 0.25^{c}	132.52± 0.50^{d}

Interleukin-6 (IL-6) and Tumor necrotic factor-α (TNF-α). Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at (P≤0.05).

Histopathology examination

The examination of joints with light microscope from the control negative group 1st (Photo A) revealed normal joint histology free of degenerative or inflammatory alterations; it appeared to consist of two cartilage covered bone heads with synovial membrane lining the joint capsule internally and histopathological normal trabecular bone of the epiphysis. On the other hand, the joints of the control positive group 2nd (Photo B), revealed numerous alterations; the articular cartilage was thinner, and the synovia and joint capsule were substantially enlarged due to enlargement by inflammatory edema and exudate. The size and number of trabecular bones in the head were reduced. Concerning the group (3) (Photo C and C1) treatment with low dose of PFJ at 1 mL/kg bw orally once daily at a concentration of 10%, as show in (Photo C) had modest thinning in the articular cartilage covering the articular surface. The synovia and joint capsule appeared normal, however the epiphysis trabecular were thinner than in the negative control group. While (Photo C1) showed a normal joint, all of its components were normal with no obvious histopathological abnormalities. Regarding the group (4) both articular surfaces and synovia appeared normal by treatment with a high dose of PFJ at 2 mL/kg bw orally once daily at a concentration of 10%, as shown in (Photo D). The trabecular of the epiphysis was also normal. The group (5) that received Diclofenac sodium orally at 10 mg/kg bw (Photo E) recovered the best, as the joint was devoid of degenerative or inflammatory lesions and seemed histopathological normal.

According to **Alzarea *et al*., (2022) and Triastuti *et al*., (2021)** demonstrated that CFA injection caused edema, bone marrow inflammatory cell buildup, and cartilage degeneration in the hind paw. Contrarily, the treatment PFJ at (low or high doses) reversed the inflammatory response caused by the CFA. Histopathological findings confirm Persimmon fruit's anti-inflammatory and antioxidant effects against CFA-induced inflammation and arthritis. Our findings agree with **Direito *et al*., (2019)** demonstrate that the administration of persimmon extract attenuates the degree of chronic inflammation, tissue damage, and improves the histology

of CIA in rats, most likely due to the extract's powerful antioxidant properties.

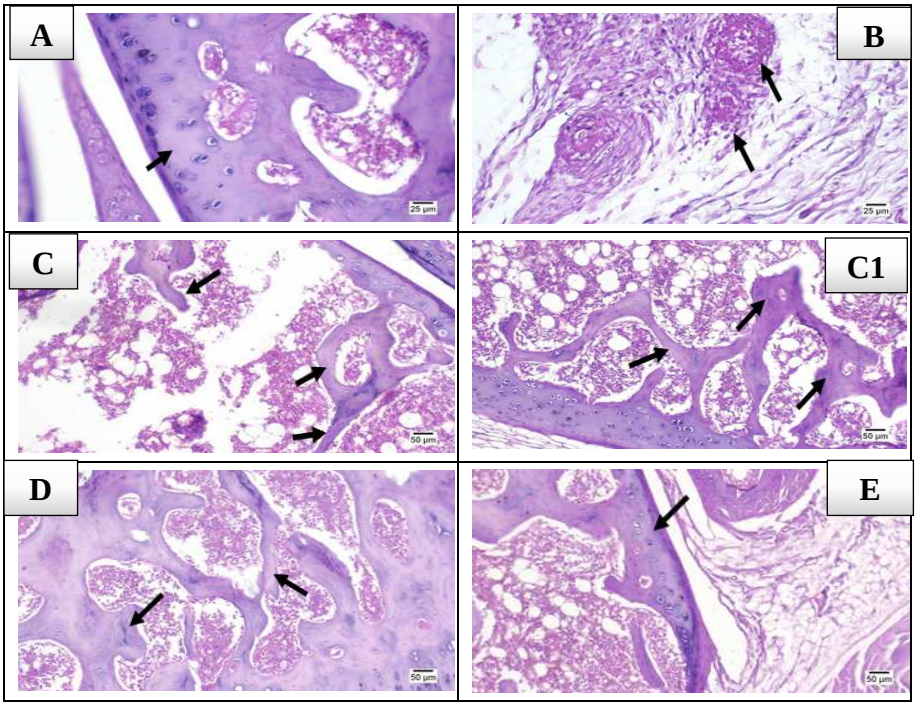

Photo (1): Photomicrograph of joint (H&E). A= Groups (1) (ve-), higher magnification showing normal cartilage (black arrow). B= Group (2) CFA; C and C1 = Group (3) CFA+PFJ (low); D = Group (4) CFA+PFJ (high); and E= Group (5) CFA +Diclofenac. magnification power (× 400).

Conclusion

In conclusion, the adding of Persimmon fruit juice in diets can reduce the rheumatoid arthritis properties in female rats manifested by increase antioxidant and reducing paw volume, weight loss, anemia, oxidative stress, normalizing pro-inflammatory cytokines (IL-6 and TNF-α levels), and stopping aberrant biochemical and histopathological changes. Thus persimmon juice has functional nutritional capacity for treatment of rheumatoid arthritis as the Diclofenac sodium drug.

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تقييم الفاعلية المحتملة لعصير الكاكى كمضاد لالتهاب المفاصل الروماتويدى المستحث في اناث الفئران بواسطة فروويند الكامل

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الملخص العربي:

كان الهدف من هذه الدراسة هو تقييم النشاط المحتمل لعصير فاكهة الكاكى ضد التهاب المفاصل الروماتويدى. اجريت الدراسة علي ٣٥ من اناث الفئران الالبينو سلالة (اسبراجو داولى) يتراوح اوزنهم (١٧٠±١٠جم) تم تقسيمهم بشكل عشوائى إلى خمس مجموعات (٧ فئران لكل منها) ، تم تغذيتهم على النظام الغذائى الأساسى ، اعتبرت المجموعة الأولى كمجموعة ضابطة سالبة ، وتم حقن الاربع مجموعات الاخرى بمساعد فروويند الكامل في مفصل الركبة الخلفي الأيسر١مل/كجم من وزن الجسم لمدة ٧ ايام لاحداث التهاب المفاصل الروماتويدي. تم إعادة تقسيمهم على النحو التالي: واعتبرت المجموعة الثانية كمجموعة ضابطة موجبة والمجموعات الثلاثة الأخرى تم معاملتهم بجرعات مختلفة من عصير الكاكى منخفضة (١ مل / كجم من وزن الجسم) ، مرتفعة (٢ مل/كجم من وزن الجسم) ، وديكلوفيناك الصوديوم بتركيز ١٠ملجم/كجم من وزن الجسم ، على التوالي واستمرت التجربة لمدة ٢٨ يومًا. أظهرت النتائج الكيميائية أن فاكهة الكاكى تحتوي على نسبة عالية من الألياف والفينول الكلي وحمض الأسكوربيك والنشاط المضاد للأكسدة ، بينما النتائج البيولوجية اشارت الى أن جميع المجموعات التي تلقت جرعات منخفضة اوعالية من عصير الكاكى اوديكلوفيناك الصوديوم اظهرت انخفاض ملحوظ فى حجم المخلب بالاضافة إلى تحسن فى القياسات البيوكيميائية والدموية مثل عدد خلايا الدم البيضاء ، معدل ترسيب كرات الدم الحمراء ، البروتين التفاعلى ، عامل الروماتويد ، المالوندهيد، ومستويات السيتوكينات المؤيدة للالتهابات (IL-6, and TNF-α) . بينما اظهرت زيادة ملحوظة فى وزن الجسم ومستوى الهيموجلبين وعدد خلايا الدم الحمراء والانزيمات المضادة للاكسدة فى انسجة المفصل. اكد الفحص النسيجى لأنسجة المفاصل نتائج التحاليل البيوكيميائية للدم. ويمكن التوصية بضرورة استهلاك عصير الكاكى فى النظام الغذائى للأشخاص المصابين بالتهاب المفاصل الروماتويدى بسبب خصائصه المضادة لالتهاب المفاصل الروماتويدى والمضادة للأكسدة والتى تلعب دورا هاما فى الحالة الصحية.

الكلمات المفتاحية :

فاكهة الكاكى، ديكلوفيناك الصوديوم ،التهاب المفاصل الروماتويدى، أنسجة المفاصل، فئران التجارب .

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