Treatment Effect of Willow Leaves on Rheumatoid Arthritis in Chronic Inflammatory Disorders of Rats

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مجلة البحوث في مجالات التربية النوعية

معرف البحث الرقمي DOI: 10.21608/jedu.2021.57432.1196

المجلد السابع العدد 34 . مايو 2021

الترقيم الدولي

P-ISSN: 1687-3424 E- ISSN: 2735-3346

موقع المجلة عبر بنك المعرفة المصري https://jedu.journals.ekb.eg/

موقع المجلة http://jrfse.minia.edu.eg/Hom

العنوان: كلية التربية النوعية . جامعة المنيا . جمهورية مصر العربية

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ABSTRACT

This Study aimed to determinate some chemical composition of Willow leaves and investigates the treatment effect of Willow leaves powder and extract on injured rheumatoid arthritis in rats injected by 100µl of Complete Freund's Adjuvant (CFA) into left hind knee joint36 adult male Sprague Dawely rats weighing150-160g classified into control negative(five rats) and rheumatoid arthritis injured rats groups which were control positive group and four treated groups with 10&20% Willow leaves powder and 200 & 400 ppm /kg bw rat /daywillow leave aqueous extract (each five rats).The treatment period was designed for 4weeks .The chemical composition of Willow leaves showed higher value of protein and total dietary fiber in dry weight .Total Phenolic fractionations of Willow illustrated that the salycilic, gentisic, gallic and pyrogallol acids were the major compounds butthe major compoundsoftotal flavonoids compounds were reported thatrutin, hesperidin, naringin, apigenin, and luteolin.

The biological results in positive control groupprevealed that, there was a significant increase in the Malondialdehyde (MDA), creatinine, urea, aspartate amino transferase (ALT&AST) and TNF- alphathere was a significant decrease in the antioxidant enzymes Glutathione Reduced (GSH) and Superoxide activity (SOD) and IL-10compared with control negative control group inclusion of willow leaves powder and Willow leaves extract into the diets of rheumatoid arthritis treated rats improved the levels of all these biomarkers of liver ,kidney ,.antioxidant enzymes, TNF-alpha and IL-10 F. Histopathological examination of the rat knee confirmed this amelioration of health showing that consumption

of Willow leaves powder and Willow leaves extract can lower pathological changes in injured rheumatoid arthritis rats.

From the clear results, it could be demonstrated that administration of Willow leaves powder and their extract alleviates the harmful effect of rheumatoid arthritis CFA-induced rats.

Keywords: Willow leaves**,** rheumatoid arthritis, Kidney and liver functions,

INTRODUCTION

Chronic joint inflammatory disorders increased inflammation and oxidative stress of osteoporosis and rheumatoid arthritis cause histological changes. The medications used are effective pain relievers but are associated with side effects that affect life. Therefore, medicinal plants are used as traditional herbs, as they have an analgesic effect, and many of them have proven effective, so they can often be compared with traditional medicines. (**Dragos *et al.*, 2017**)

Rheumatoid arthritis (RA) is defined as the most common disease of inflammatory arthritis, as it affects the joints, and over time it causes great suffering to patients **(Khurana and Berney, 2005).**

Rheumatoid arthritis (RA) is mainly affecting peripheral joints. Medicines are acting as a sedative for rheumatoid arthritis for pain and it has dangerous side effects. Flavonoids have antioxidant, anti-inflammatory, and immune-modulating properties. In addition, it has powerful anti-inflammatory effects. Several dietary flavonoids have been found to act as analgesics in human rheumatoid arthritis and relieve symptoms of this inflammation **(Hughes *et al.*, 2017).**

Willows (*Salix safsaf* L.) is one of the most widely known and widespread species of the genus Salix. It is rich in a variety of biologically active substances (primarily phenolic compounds) and, since ancient times, has been used for medicinal purposes.

Also, it is known about the chemical composition of white willow and the medical usage of its organs and compounds, including medications developed from raw materials in different parts of the world **(Vasfilova, 2020).**

Various species from the genus Salix, or willow, were already in common use for pain relief in antiquity (**Vlachojannis *et al.*, 2009),** The inflammation-suppressing effect of willow extract relies, at least partially, on its ability to antagonize the activated monocytes, by blocking the activity of pro-inflammatory cytokines (TNFα), enzymes (COX-2), and mediators (NF-κB) (**Bonaterra *et al.*, 2010).**

Willow leaves decreased the inflammatory infiltrate and exudate and blocked the cytokine surge with potency at least equivalent to that of acetylsalicylic acid (ASA), which was better than ASA in reducing leukotrienes levels and in inhibiting COX-2, and as good as ASA in decreasing prostaglandins levels. Willow leaves influenced favorably oxidative stress increasing GSH and decreasing malondialdehyde levels more efficiently than ASA or celecoxib (a selective COX-2 inhibitor). Despite being more potent than ASA, on a molar basis, the salicin amount in willow leaves are much less than the salicylate content of ASA, suggesting that active principles other than salicin might play a role in the anti-inflammatory and antioxidative action of willow leaves, the polyphenols being among the candidates, at least regarding the protection against free radicals (**Khayyal *et al.*, 2011).** The ability to mitigate pro-inflammatory cytokines and oxidative stress was corroborated by still another study on the collagen-induced arthritis animal model (**Sharma *et al.*, 2011).**

This study aimed to investigate the effect of willow leaves powder or extract on ameliorating rheumatoid arthritis in rats injured with CFA

Material and Methods

Materials:

Leaves of willow (*Salix Safsaf* L) were collected from the trees which spread behind the branches canals of the Nile River at Egypt.

Kits for determination of all different parameters were purchased from Sigma-Aldrich Corp., MO, for use in analysis.

Complete Freund's Adjuvant (CFA) was purchased from Sigma, St. Louis, Mo.

Male albino rats (36 rats) with weight ranging from 150-160g were purchased from National Organization for Drug and Control Research, Giza, Egypt.

Thirty six healthy Sprague–Dawley albino rats were purchased from National Organization for Drug and Control Research, Giza, Egypt and they were kept under observation for one week before experiment and fed on the basal diet and water ad libitum for adaptation.

A basal rat diet was prepared according to **Pell *et al*. (1992)** and consisted of casein(20%), corn oil (8%), corn starch (31%) . sucrose (32%), mgcellulose (4%), salt mixture (4%) and vitamin mixture (1%)

Methods

Preparation of willow leaves

Willow leaves were properly washed under running water to remove adhering foreign particles, mud, dust, etc., dried at low temperature (50°-60° C), and mill under hygienic conditions to powder in an electric grinder. The powder so obtained was sieved twice to remove the coarse particles and stored in airtight containers until further analysis.

Determination of chemical composition of willow leaves

Chemical composition as protein, fat, crude fibers and ash content were determined in the gum Arabic according to the methods of **AOAC (2005)** and also, total carbohydrates were

determined by differences. Total dietary fibers, soluble and insoluble dietary fibers were determined in raw materials according to **Prosky *et al.* (1992).**

Estimation of total phenolic acids, total flavonoids compounds

The total phenolic content in the willow leaves extract was measured using the method of **Qawasmeh *et al.* (2012)** with Folin-Ciocalteu reagent. Gallic acid was used as standard (1 mg/ml) and the results were expressed as gallic acid equivalents (GAE mg/g of dry weight).

The total flavonoids content was determined by the method of **Eghdami and Sadeghi (2010).** The absorbance was measured against a blank solution at 510 nm and the total flavonoids content was expressed in terms of milligrams of quercetin equivalent per gram dry weight (mg QE /g of dry weight).

Quantitative determination of phenolic and flavonoids by HPLC:

Phenolic compounds were determined by HPLC according to the method of **Goupy *et al.* (1999).** HPLC Hew let Packard (series 1050) equipped with auto sampling injection, solvent degasser, ultraviolet (UV) detector set at 280 nm, and quaternary HP pump (series 1050). Hewlett Packard using a column Altman C18, 5mm (150mm x 4.6mm Alltech) the column temperature was maintained at 35°C. G Phenolic acid standard from sigma Co. was dissolved in a mobile phase and injected into HPLC

Biological experimental

Experimental rats were fed on a basal diet for 7 days and randomly divided into six groups six rats for each. The 1st main group was fed on a basal diet for another 4 weeks and considered as control negative rats.

The rats were divided into 6 groups (6 for each group). The negative control group was fed the basal diet only. The rest of the rats were injected with 100µl of Complete Freund's Adjuvant (CFA) into the left hind knee joint to induce rheumatoid arthritis

in rats'. After 7 days, secondary arthritis was induced by injecting 50 µL of CFA under the left hind knee joint according to **Narendhirakannan *et al.*, (2007)** then divided into 5 groups. A positive control group was also fed the basal diet only, while the other4 groups were fed basal diets containing 10&20%willow leaves powder substituted by fiber and 200&400ppm /kg bw rat /day willow leave aqueous extract orally by stomach tube for four week storage period

At the end of experimental, the blood samples were taken with drawn from the orbital plexus and centrifuged at 3000 rpm to obtain the sera after that, the sera were kept in a deep - freezer at -20°C until their analysis.

Serum the lipid peroxidation was determined calorimetrically as malondialdehyde (MDA) by **Yoshioka *et al.* (1979).** Moreover, the activity of the antioxidant enzymes, plasmasuperoxide dismutase (SOD) was measured in the serum according to the method of **Sairam *et al.* (2003)**, non-enzyme Glutathione (GSH) were measured in the serum by **Habig *et al*. (1974).**

Serum kidney function as creatinine and urea were estimated according to the method described by **Schirmeister (1964) Patton and Crouch (1977).**

Serum liver function as Alanine (ALT) and Aspartate (AST) transaminoferase determined according to the method described by **Reitman and Frankel (1957).**

Serum Selected cytokines IL-10 (Cat. No. BMS629) and TNF-α (Cat. No. BMS622) were determined by ELISA (Bio-tek Instruments, Inc.) using enzyme-linked immunosorbent method according to **Kandir and Keskin (2016) & Millena *et al.* (2004).**

Histopathology evaluation

The animals were sacrificed (after four weeks following CFA injection), and the knee joint was excised and fixed in 10%buffered formalin for 7 days. The knee joint was then

decalcified with nitric acid 10% for 27 hours. Tissues were sectioned and embedded in paraffin. Slides were prepared and stained with hematoxylin and eosin according to **Laste *et al.* (2016)**.

Statistical analysis

The obtained data were exposed to the analysis of variance. Duncan's multiple range tests at ($P \leq 0.05$) level was used to compare between means. The analysis was carried out using the ANOVA procedure of Statistical Analysis System **(SAS, 2004).**

RESULTS AND DISCUSSION

The chemical composition of willow leaves

Chemical constituents, as well as total dietary fiber, insoluble and insoluble dietary fiber, in addition, total phenolic acid and total flavonoid compounds were determined in willow leaves and the results are reported in Table (1). From the results, it could be noticed that the protein, crude fiber, total lipids, ash content were 11.35, 20.68, 2.89 and 14.51% on dry weight basis. Moreover total dietary fiber, insoluble and insoluble dietary fibers were 53.28, 38.45 and 14.83% on dry weight basis.

The resultant from the same table noticed that the total phenolic acid and total flavonoids compounds were 12.35±0.83 mggallic acid /g and 8.67±0.14mg Quercetin/g, respectively. **Jo Smith *et al*. (2014)** reported that leaves of willow tree contained 12.7 g/100g crude protein on a dry matter (DM), while the neutral detergent fiber, acid detergent fiber and lignin concentration were 57.3, 41.0 and 18.4 g/100g dry matter, respectively.

Table (1): Percent of Chemical composition and total fiber fraction in leaves powder and phenolic and flavonoids content in its extract

Chemical composition	Willow leaves	Chemical composition	Willow leaves
Crude protein %	11.35±0.86	Total dietary fiber%	53.28±4.81
Crude fiber%	20.68±2.14	Insoluble dietary fiber%	38.45±1.35
Total lipids%	2.89±0.06	soluble dietary fiber%	14.83±0.94
Ash content%	14.51±0.97	Total phenolic acids mg GAE /g of dry weight%	12.35±0.83
Total carbohydrates %	50.67±6.27	Total flavonoid compounds mg QE /g dry weight%	8.67±0.14

Values are mean and SD (n = 3).

Polyphenolic fractions of Willow leave extract:

Total phenolic and flavonoid compounds in willow leave extract were fractionated using HPLC apparatus and the results are tabulated in Table (2). Total phenolic acids had contained from salycilic, gentisic, gallic and pyrogallol acids were the major compounds (12.31, 11.35, 9.25 and 8.52 mg/100g, respectively). The medium compounds from willow leave extract cinnamic, ferulic and chlorogenic acids were 6.15, 5.73, and 5.41 mg/100g, respectively. The minor compounds were *p*-coumaricand coumarin acids, respectively.

Total flavonoids compounds were fractionated using HPLC and the results reported that rutin, hesperidin, naringin, apigenin, and luteolin were the major compounds (10.49, 9.12, 7.35, 6.77, and 5.13mg/100g, respectively). Meanwhile, kaempferol, catechin, epicatechin, and quercetin were 4.35, 3.58, 3.16, and 2.7313mg/100g, respectively.

Meena *et al.* (2006) observed that willow leaves had contained rich amounts from tannins, vitamins crude protein, crude fibers; minerals content.

Willow species synthesize low molecular phenolic glycosides, such as salicin (35 g/kg DM) and/or condensed tannin (CT, 38 g/kg DM) **(Pitta *et al.*, 2007).** Salicin and salicortin are

the primary salicylates found in white willow (*salixsafsaf*). They are metabolized by intestinal flora to saligenin **Julkunen-Tiitto and Meier (1992),** absorbed into the blood stream, and metabolized by the liver to salicylic acid; excretion is primarily through renal **(Bissett, 1994).**

Catechins, proanthocyanidins, and anthocyanins are present in the extract of willow leaves. Catechins are represented by (–)-epicatechin, (+)-catechin, and gallocatechin**(Esatbeyoglu *et al.*, 2010).**

Kalaivaniand Mathew (2010) indicated that the ethanol extract rich in polyphenolic compounds had potent antioxidant activity may be caused by the ability to be done antioxidant technique of the ethanol extract can be due to its hydrogen or electron-donating and direct free radical scavenging characteristics. Also, maybe due to the existence of the combined ring structures and hydroxyl groups, many polyphenolic compounds have the possibility to the role as antioxidants by scavenging free radicals participatory in oxidative processes meanwhile hydrogenation with oxidizing species **(Afsar *et al.*, 2018)**.

Table (2):Phenolic and flavonoids compounds of Willow leaves

phenolic acids	Quantification (mg/100 g dw)	Flavonoid compounds	Quantification (mg/100 g dw)
Gallic	9.25^{b}±0.81	Luteolin	5.13^{c} ±0.07
Pyrogallol	8.59^{b} ±0.75	Narengin	7.35^{b} ±0.14
gentisic	11.35^{a}±0.98	Rutin	10.49^{a}±0.94
chlorogenic	5.41^{c} ±0.07	Hisperidin	9.12^{a}±0.83
p-Coumaric	3.28^{d} ±0.05	Quercetin	2.76^{e}±0.05
Ferulic	5.73^{c}±0.06	Kampferol	4.35^{c} ±0.18
Cinnamic	6.15^{c}±0.08	Apegnin	6.77^{b} ±0.28
Coumarin	2.89^{d} ±0.01	Catechin	3.58^{d}±0.18
Salycilic	12.31^{a}±1.28	epicatechin	3.16^{d} ±0.14

Mean values in each raw having different superscript (a, b, c, d) are significant different at 0.05 levels.

Effect of willow leaves and its extract on oxidative stress in rats' chronic inflammatory disorders

The lipid peroxidation as Malondialdehyde (MDA), and the activity of the antioxidant enzymes Glutathione Reduced (GSH) and Superoxide activity (SOD) were determined in the different rats' groups' chronic inflammatory disorders and the results are reported in Table (3).

The results indicated that the MDA increased in control rats' positive rats' chronic rheumatoid arthritis by 3.01 m. mol/mg protein than control negative rats was 0.30 m. mol/mg protein. In addition, the rats fed on willow leaves at 10.0 and 20g from willow leaves powder substituted from the basal diet, the results found that decreased to 1.92 and 1.52 m. mol/mg protein, in groups 3 and 4, respectively. Meanwhile, the best results for treatment rats' chronic rheumatoid arthritis in groups were 5 and 6 fed on basal diet and taken orally at level 200 and 400ppm/kg body weight/ rat/dayfrom aqueous extract from willow leaves found that the rats' groups' results were decreased to 1.60 and 0.82 m. mol/mg protein, respectively.

MDA is a product of lipid peroxidation and the most representative indicator of the body's operating system, while SOD is a function to remove reactive oxygen species (ROS). These two factors indirectly affect the extent of the vitality of the body **(Bajpai *et al.*, 2017).** When tissue damage due to include hypoxia, therefore, large amounts of reactive oxygen species are produced, occurs to oxidative stress (OS**)** **(Kuksal *et al.*, 2017).** The main source of ROS in the respiratory chain complex is in the mitochondria by electron transfer to produce ATP (**Kadlec *et al.*, 2016**).

The results from glutathione (GSH) and superoxide dismutase (SOD) in the same table showed that the control rats positive were decreased to 2.04 and 5.58 m. mol/mg protein, compared with control rats negative which increased to 10.2 and

16.22 m. mol/mg protein, respectively. When the rats' chronic rheumatoid arthritis fed and taken orally willow leaves gave the best results for GSH and SOD especially the groups 4 and 6 which increased in GSH to 7.14 and 8.94 m. mol/mg protein, as well as, in SOD more increased to 11.90 and 14.08 m. mol/mg protein, respectively, this may be due to the willow leaves had contained rich amounts from dietary fiber, protein, and natural antioxidant.

GSH is the first-line resisting attack against lipid peroxidation and serves as anucleophilic co-substrate to glutathione transferases in the detoxification of xenobiotics (**Barber and Harris, 1994).**The uncontrolled production of ROS leads to a lower in SOD and CAT activities as well as GSH level as a consequence of their supersaturation and consumption through oxidative stress as well as loss through cellular lysis, which resulted from membrane lipid peroxidation (**Hassan *et al.*, 2001 and Islamov *et al.*, 2002).**

Table (3): MDA, GSH and, SOD, in experimental rat groups.

Groups	MDA (m. mol/mg/ protein	GSH (m. mol/mg/ protein	SOD (m.mol/mg/ protein)
Control negative	0.30^{e}±0.01	10.25^{a}±0.81	16.22^{a}±0.97
Control positive	3.01^{a}±0.10	2.04^{e}±0.04	5.58^{c}±0.43
Group 3	1.92^{b}±0.04	5.70^{d}±0.32	10.31^{b}±0.93
Group 4	1.52^{c}±0.03	7.14^{c} ±0.45	11.90^{ab}±0.97
Group 5	1.60^{c}±0.02	7.05^{c}±0.71	11.64^{ab} ±1.12
Group 6	0.82^{d}±0.01	8.94^{b}±0.83	14.08^{ab} ±1.08

Mean values in each raw having different superscript (a, b, c, d) are significant different at 0.05 levels.

Effect of willow leaves and its extract on kidney functionsin rats' chronic inflammatory disorders

The results from Table (4) showed that the effect of different diets on urea and creatinine on rat groups. The results found that the highest in urea, creatinine in control positive were 52.10 and 2.40 mg/dl, respectively, the increase in these parameters means the disorder in kidney functionsin rats' chronic inflammatory disorders**.**These results confirmed by **Cameron and**

Greger (1998) who found that the serum urea is an important test for knowing the conditions of the kidneys; therefore the increase in the urea level may indicate impairment of renal functions.

Moreover, the results from the rat group fed on basal diet plus fortified with 10.0 and 20.0% willow leaves and rat groups fed on basal diet and the aqueous extract from willow leaves was taken orally 200 and 400 ppm/kg body weight/ rat/day were decreased and gives the best results in rats group 4 and 6 which decrease in creatinine to 1.10 and 0.90 mg/dl and more decreased in urea to 41.50 and 39.01mg/dl, respectively. These decreases in the rat group due to the willow leaves had contained high amounts of natural antioxidant and dietary fiber lead to improve kidney functions.

Table (4): Kidney functionsin rats' chronic inflammatory disorders

Groups	Creatinine (mg/dl)	Urea (mg/dl)
Control negative	0.20^{d}±0.01	37.9^{c} ±2.45
Control positive	2.40^{a}±0.02	52.10^{a}±3.36
Group 3	2.20^{a} ±0.01	44.70^{b}±2.49
Group 4	1.10^{b}±0.01	41.50^{b} ±2.16
Group 5	1.01^{b} ±0.01	42.25^{b} ±2.38
Group 6	0.90^{c}±0.03	39.01^{c}±1.61

Mean values in each raw having different superscript (a, b, c, d) are significantdifferent at 0.05 levels.

Effect of willow leaves and its extract on liver functionsin rats' chronic inflammatory disorders

Liver functions as alanine (ALT) and aspartate (AST) transaminoferase, were determined in rat groups fed on different diets and the results are reported in Table (5). The results illustrated that the highest activity in the enzyme ALT and AST in control positive ware56.25 and 68.0 9 mg/dl, compared with the control negative was the lowest to 26.41 and36.01mg/dl, respectively. Whereas the groups 4 fed on basal diet plus fortified with 20.0% willow leaves and rats group 6 was taken orally from

willow leaves extract on 400ppm /kg body weight/day which decreased and nearly or equal to control negative rats group to 30.11 and 37.05 mg/dl, respectively, these results showed that the willow leaves had contained rich amounts of natural antioxidant which status improve liver enzymes activity. These results confirmed with **Narendhirakannan *et al.*,(2007**) who indicated that elevate in serum liver functions enzyme activities in control positive rats of arthritic rats are in approval with the described significant lowering in lysosomal the state of being of CFA treated rats (**Narendhirakannan *et al.*, 2007).**Moreover, an increased level of serum ALP activity in arthritic rats can be caused to elevate in bone erosion and periarticular osteopenia, since the enzyme is allowed through circulation in the course of bone resorption and formation (**Niino-Nanke *et al.*, 1998).**

The significant decrease/increase in the cellular toxicity markers (AST and ALP) and anti-oxidant defense systems (SOD, MID, and GSH), respectively induction emphasises the role of willow leaves in preventing organ damage through scavenging the free radicals (**Deng *et al.*, 2006).**

Table (5): Liver functions in rats' chronic inflammatory disorders

Groups	ALT (mg/dl)	AST (mg/dl)
Control negative	26.41^{d} ±1.91	36.01^{d} ±2.58
Control positive	56.25^{a}±2.68	68.09^{a}±4.26
Group 3	48.41^{b}±2.14	55.25^{b} ±3.12
Group 4	39.87^{c}±2.68	45.50^{c} ±2.58
Group 5	39.88^{c} ±2.94	44.80^{c} ±2.19
Group 6	30.11^{d}±2.11	37.05^{d} ±1.69

Mean values in each raw having different superscript (a, b, c, d) are significant different at 0.05 levels.

Effect of willow leaves and its extract on cytokines IL-10 and TNF-αin rat chronic inflammatory disorders

TNF- α is a major pro-inflammatory cytokine released from migrated macrophages during inflammation **(Rozza *et al.*, 2014).**

Results in Table (6) illustrated that the TNF-α in control positive rats chronic inflammatory disorders was the highest (424.25 pg/mg) may be due to inflammatory in knees compared with the control negative healthy rats group was 88.69 pg/mg.TNF-α is a pleiotropic cytokine and plays a critical role in both acute and chronic inflammation. It facilitates inflammatory cell infiltration by promoting adhesion of neutrophils and lymphocytes to endothelial cells (**Szekanecz *et al.*, 2000).**

Meanwhile, the rats fed on willow leaves at 10.0 and 20g from willow leaves powder substituted from the basal diet, the results found that decreased to 305.15 and 197.01 pg/mg, in groups 3 and 4, respectively. Meanwhile, the best results for treatment rats' chronic rheumatoid arthritis in groups were 5 and 6 fed on basal diet and taken orally at level 200 and 400ppm/ kg body weight/ rat/day from an aqueous extract from willow leaves found that the rats' groups' results were decreased to 200.14 and 109.26 pg/mg, respectively. These results confirmed with **Shanahan *et al.*, (2003)** who found thatwhen TNF-α is specifically lowering, theseverity of inflammation is reduced.

From the same Table, it could be found the IL-10 as an anti-inflammatory cytokine was decrease in control positive rats with chronic inflammatory disorders by 70.65 pg/mg, whilst, the control negative was the highest by 186.05 pg/mg. These results indicated that when IL-10 which lowering due to the rats' chronic inflammatory disorders, as well as the increasing IL-10 in the rats' group, proved the rats healthy. Furthermore, the best results to IL-10 was in the rats' group fed on basal diet and taken orally 400ppm/kg body weight/day was given 153.71 pg/mg this may be when fed and/or taken orally willow leaves when the IL-10 increased and also, inflammatory disorders were decreased in different rats groups. IL-10 (an anti-inflammatory cytokine) has been thought to be an upstream regulator that controls the progression of rheumatoid arthritis (RA) negatively. Also, IL-10

inhibits the cytokine production and release by activated macrophages (**Hisadome *et al*., 2000 and Yoshihara *et al*., 2000).**

Table (6): Serum cytokines IL-10 and TNF-αin rats' chronic inflammatory disorders

Groups	TNF- alpha (pg/mg)	IL-10 (pg/mg)
Control negative	88.69^{e}±6.29	186.05^{a}±5.26
Control positive	424.25^{a}±11.28	70.65^{e}±4.13
Group 3	305.15^{b}±9.26	100.00^{d}±9.28
Group 4	197.01^{c}±5.48	111.02^{c} ±10.22
Group 5	200.14^{c} ±10.26	115.01^{c}±11.35
Group 6	109.26^{d}±7.35	153.71^{b}±12.28

Mean values in each raw having different superscript (a, b, c, d) are significant different at 0.05 levels.

Histopathology evaluation

Microscopic examination of joints from control negative group 1st (**Photo1**) revealed normal histology of the joint and free from inflammatory or degenerative changes; it appeared consisted of two cartilage covered bone heads with synovial membrane lining the joint capsule internally and histological normal trabecular bone of the epiphysis. On contrary, the joints from control positive group 2nd (**Photo2**) showed various alterations; the articular cartilage was thinned, and the synovium and the joint capsule were greatly thickened due to expansion by inflammatory edema and exudate. The trabecular bone forming the head was decreased in size and number. Regarding the group (3) fed on basal diet substituted 10g willow leave from aqueous willow extract (**Photo3**) it exhibited mild thinning in the articular cartilage covering the articular surface. The synovium and the joint capsule appeared apparently normal, while the trabeculae of the epiphysis were thinner compared to the negative control group. Group (4) fed on basal diet substituted 20g willow leave (**Photo4**) showed normal joint; all components of the joint were normal without any detectable histological alterations. Concerning group (5) taken

orally 200 ppm/kg body weight /day (**Photo5**) both articular surfaces and the synovium were apparently normal. The trabecular of the epiphysis were normal as well. Group (6) taken orally 400 ppm/kg body weight /day (**Photo 6**) exhibited the best recovery, as the joint was free from degenerative or inflammatory lesions and appeared histologically normal.

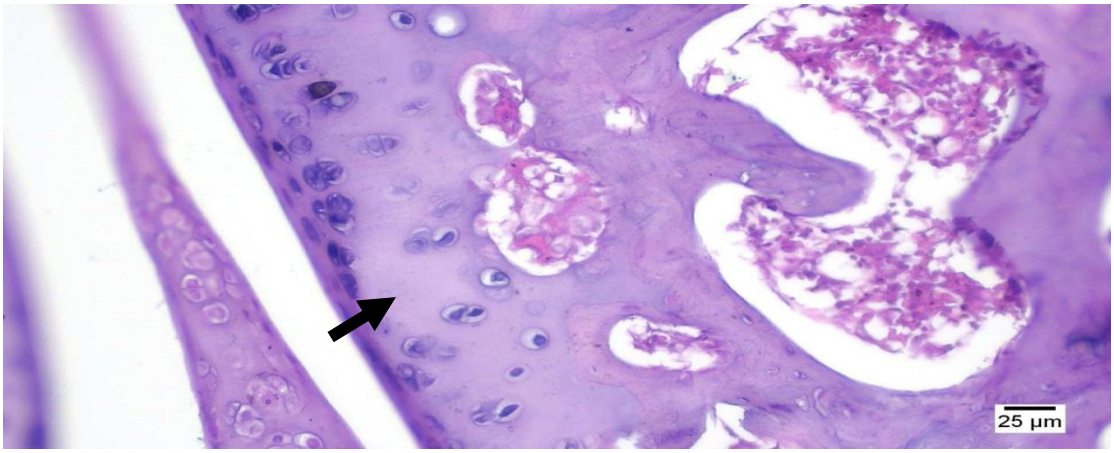

Photo (1): Photomicrograph of joint, negative control group, higher magnification showing normal cartilage (black arrow) (H&E).

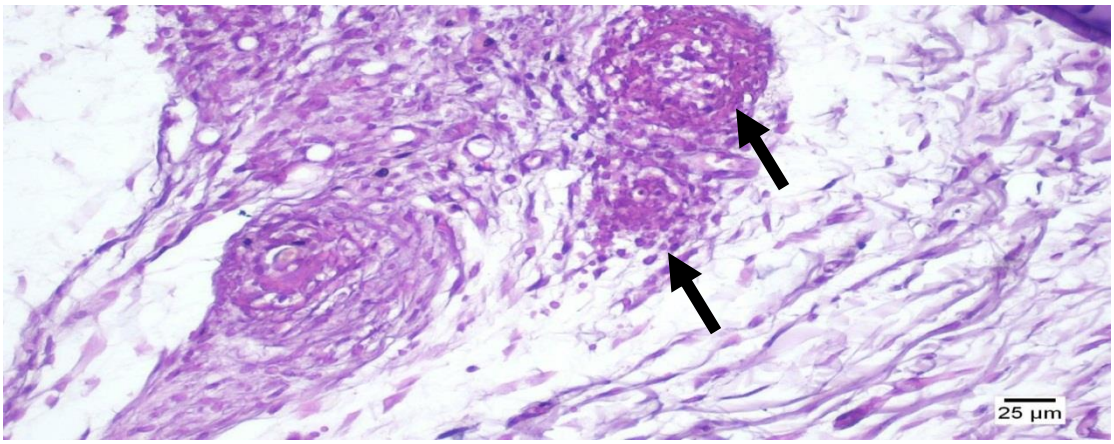

Photo (2): Photomicrograph of joint, positive control group, higher magnification showing perivascular inflammatory cells infiltration (arrows) and edema in the synovial membrane (H&E).

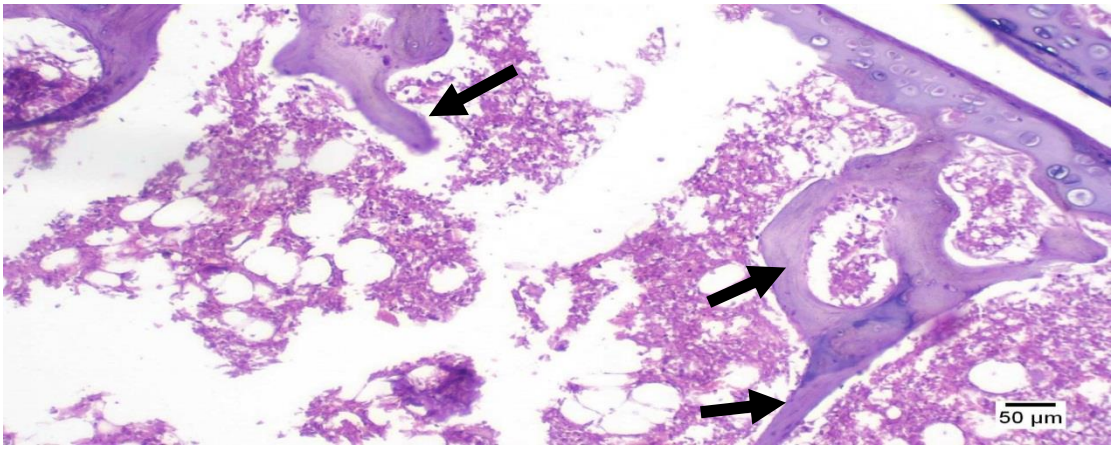

Photo (3): Photomicrograph of joint, group fed on basal diet substituted 10g willow leaves showing mild thinning in the trabecular bone (arrows) of the epiphyses (H&E).

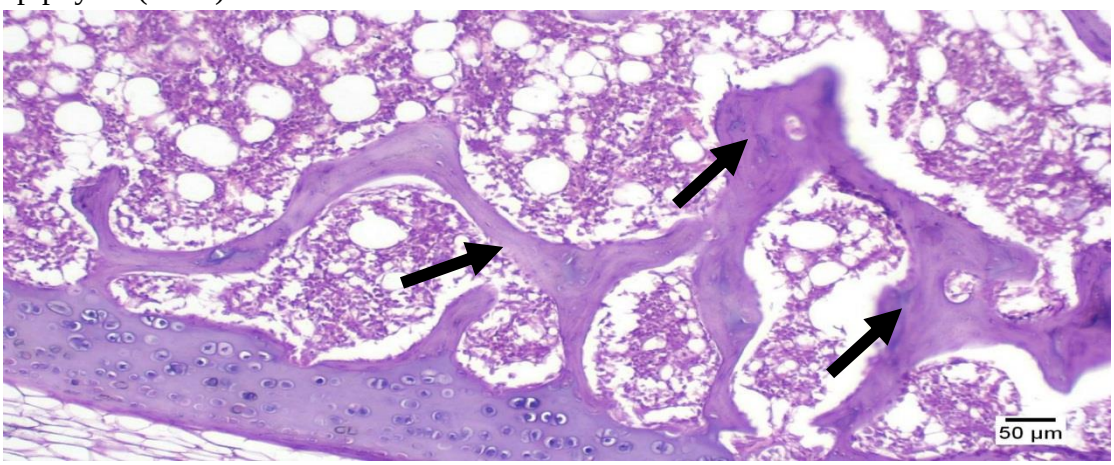

Photo (4): Photomicrograph of joint, group fed on basal diet substituted 20g willow leaves showing normal trabecular bone (arrows) of the epiphysis (H&E).

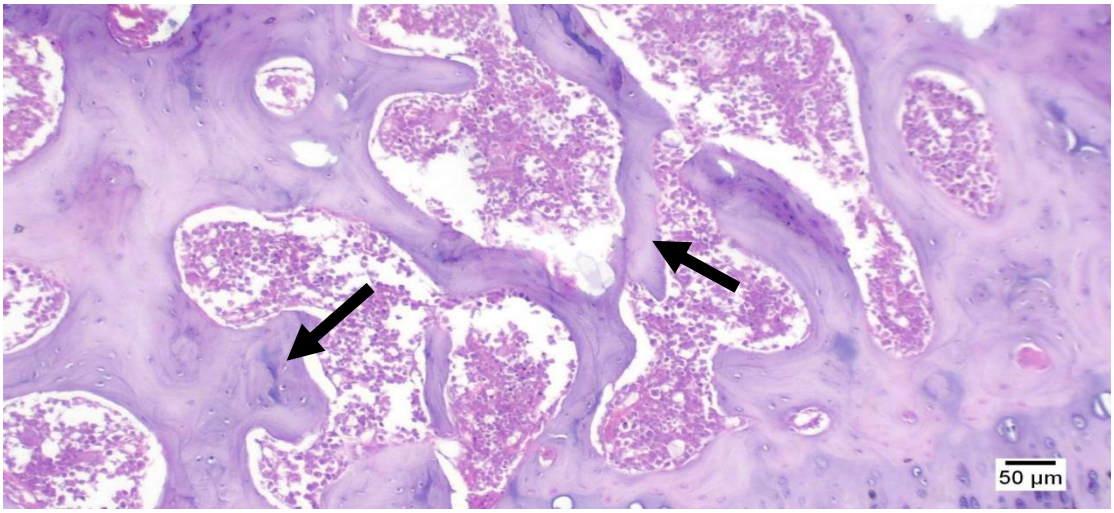

Photo (5): Photomicrograph of joint, group taken orally 200ppm/ kg body weight / day,, showing normal bone of the epiphysis (H&E).

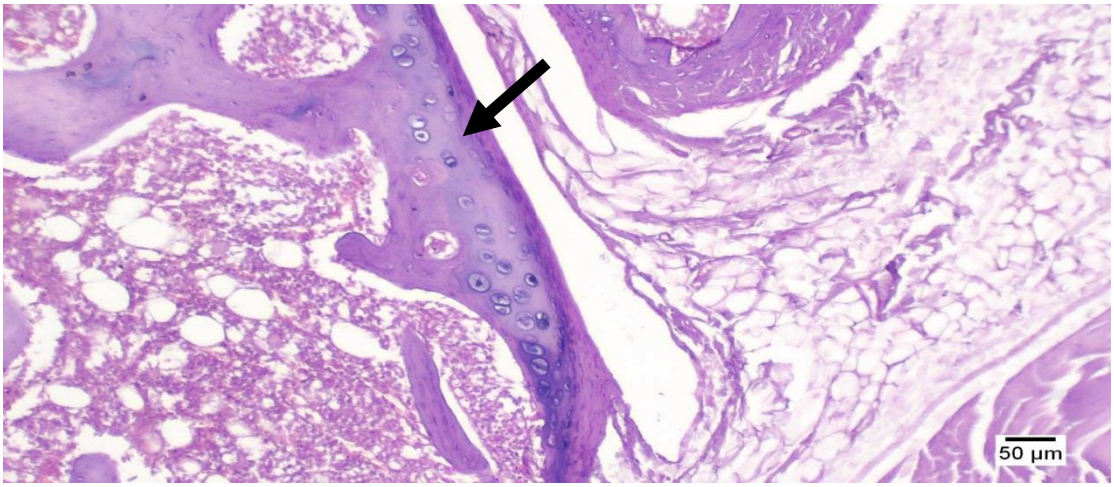

Photo (6): Photomicrograph of joint, group taken orally 400ppm/ kg body weight / day, higher magnification, showing normal synovium and joint capsule (H&E).

CONCLUSION

It could be concluded that the willow leaves had contained the highest amounts of protein and total dietary fiber, and also, phenolic acids content and flavonoid compounds which scavenging the free radical in the blood. Therefore, when fed the rats on 20.0 g willow powder and 200ppm willow leaves extract to lead the best results for on oxidative stress, liver and kidney

functions and also, cytokines (TNF-α and IL-10) in rheumatoid arthritis in rats' chronic inflammatory disorders.

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التأثير العلاجي لأوراق الصفصاف على التهاب المفاصل الروماتويدي في الاضطرابات الالتهابية المزمنة للفئران

تهدفت هذه الدراسة إلى تقدير بعض التركيبات الكيميائي لأوراق الصفصاف وتقييم التأثيرالعلاجي لمسحوق أوراق الصفصاف ومستخلصها المائي على التهاب المفاصل الروماتويدي في الفئران حيث تم اصابة الفئران بحقنها بمقدار 100 ميكرولتر من (CFA)في مفصل الركبة الخلفية اليسرى واجريت الدراسة علي 36 من ذكور الفئران البالغة من فصيلة سبراج داولي. المتراوح اوزنهم بين 150–160 جم وتم تقسيمهم إلى مجموعة الكنترول السالبة (ستة فئران) ومجموعة فئران مصابة بالتهاب المفاصل الروماتويدي وتم اعادة تقسيمهم الي مجموعة الكنترول الموجبة وأربع مجموعات تم علاجها بنسبة 10 و 20٪ مسحوق أوراق الصفصاف و 200 و 400 جزء في المليون / كجم من وزن الجسم / يوم من مستخلص أوراق الصفصاف المائي (كل مجموعة بها ستة فئران). وتم تحديد فترة العلاج لمدة 4 أسابيع. حيث أظهرت نتائج التركيب الكيميائي لأوراق الصفصاف قيمة أعلى للبروتين والألياف الغذائية الكلية في الوزن الجاف. كما أوضحت نتائج تحليل الفينولية الكلية للصفصاف أن أحماض الساليسيليك والجنتيسيك والجاليك والبيروجالول كانت المركبات الرئيسية بينما كانت نتائج تحليل مركبات الفلافونويد الكلية. روتين ، هيسبيريدين ، نارينجين ، أبيجينين ، ولوتولينو .أظهرت النتائج البيولوجية في مجموعة الكنترول الموجبة أنه هناك زيادة معنوية في المانولدهيد والكرياتينين واليوريا وأسبارتات امينو ترانس فيراز (ALT & AST) و TNF– alpha، وكان هناك انخفاض كبير في إنزيمات مضادات الأكسدة جلوتاثيون والسوبر اكسيد ديسميوتيزو IL–10 بالمقارنة بمجموعة الكنترول السلبية.

استهلاك المسحوق والمستخلص المائي لأوراق الصفصاف في الوجبات الغذائية للفئران المصابة بالتهاب المفاصل الروماتويدي أدى إلى تحسين مستويات كل هذه المؤشرات الحيوية للكبد والكلى وأنزيمات مضادات الأكسدة و TNF – alpha و IL–10 F. وأكد ذلك الفحص النسيجي لمفصل ركبة الفئران

توصي الدراسة بضرورة استهلاك مسحوق ومستخلص أوراق الصفصاف حيث يقلل التغيرات المرضية في الفئران المصابة بالتهاب المفاصل الروماتويدي

الكلمات المفتاحية .أوراق الصفصاف ، التهاب المفاصل الروماتويدي ، الاضطرابات الالتهابية المزمنة ، وظائف الكلى والكبد فى الفئران .