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Scientific Research  
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Biotechnology College  
Department of Agriculture Biotechnology



# Comparison the effect of aqueous extract of *Artemisia herba alba* and procaine penicillin on healing induced defect of the ear cartilage

A Research

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Qadisiyah in Partial Fulfillment of the Requirements for the Degree of Bachelor  
of Science in Agriculture Biotechnology Sciences

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَقَالَ  
رَبِّ زِدْنِي عِلْمًا

صدق الله العلي العظيم

سورة طه / الآية ١١٤

## **SUPERVISOR CERTIFICATE**

I certify that this research entitled (**Comparison the effect of aqueous extract of Artemisia herba alba and procaine penicillin on healing induced defect of the ear cartilage**), was prepared by **Mustafa Hamdan Mahmood** and **Ahmed Ibrahim Rashid** under my supervision at the College of Biotechnology /University of Al-Qadisiyah as required for the degree of Bachelor of Science in Agriculture Biotechnology Sciences.

**Prof. Karim Nasir Taher**

**// 2018**

## **CERTIFICATE OF INSTRUCTOR**

I certify that Mustafa Hamadan Mahmood and Ahmed Ibrahim Rashid has completed the fulfillment of his graduation research entitled **(Comparison the effect of aqueous extract of Artemisia herba alba and procaine penicillin on healing induced defect of the ear cartilage)** for the year 2017/2018 under our construction.

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Assist. Prof. Dr. Mohanad Mohamed Sahib*

*April 2018*

## **Certification of examining committee**

We, the members of the examining committee ,certify after reading this research entitled Comparison the effect of aqueous extract of Artemisia herba alba and procaine penicillin on healing induced defect of the ear cartilage examining the students in its content , we found it appropriate to award the degree of bachelor of science in agriculture biotechnology.

## **DEDICATION**

To The Women Whom The Prophet (Peace And Blessings Of Allah Be Upon Him And His Righteous Family) Recommended , The Woman That Without Allah Grace And Her Support I Wouldn't Be Standing Here Today ..... To My Mother

Mustafa & Ahmed

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## Abstract

This study was undertaken to investigate the effect of aqueous extract of *Artemisia herba alba* (Asteraceae) and procaine penicillin on healing induced defect of the ear cartilage. A total of (25)female rats which used in this study were distributed randomly into five treatment group(five animals per each). Under routine surgery, all rats had circular holes in the elastic cartilage of their ears. All treatment groups were treated with local application daily for one week. Control group(C) treated with normal saline only. First treatment group(T1) treated with 5% *Artemisia* aqueous extract, second treatment group(T2) treated with 10% *Artemisia* aqueous extract, third treatment group(T3) treated with 15% *Artemisia* aqueous extract, fourth treatment group(T4) treated with procaine penicillin. The results showed that there was no significant differences between all treatment group except between (T3) and other treatment groups( $1.857 \pm 0.293$ ). It could be concluded that concentration of aqueous extract at 15% (T3)affected significantly on reduce the diameter of ear defect after three weeks of treatment, the result of histo pathological test reveal that complete healing, absence of scar tissue, both edges of cartilage and fused together with marked fibrosis and formed large blood vessels in the cartilage healing site.

**Chapter**

**one**

**Introduction**

## Introduction

Artemisia herba alba is belonging to family (Asteraceae), this is one of the most common medicinal plants, It is used as a popular folk remedy due to their medicinal characters in addition to flavor[1]. The genus Artemisia involves several number of species (about 200-400) species. The plant is a greenish-silver perennial herb found throughout the northern half of the world. Its known as white worm wood and in Arabic known as (shih). This plant grow (20 to 40 cm) in height, the vegetative growth take place in autumn and the flowering starts from Sept. to Dec. It is known to have several bioactive components such as (Astermisinin) which is the most common component exist in the plant as well as coumarin, monoterpenes, flavonoids and phenolic compounds [2,3]. The plant is widely used for treatment of cold, coughing, intestinal disorder, bronchitis, diarrhea, hypertension, hepatic and rheumatic diseases, also as antioxidant, antidiabetic, antimicrobial agent and anthelmintic [4,5,6,7]. Because there were a few studies about the role of Artemisia herba alba in treatment wounds. The present study was carried out to shed light on the role of this plant in treatment the wounds as compared with antibiotic procaine penicillin.

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**Chapter**

**two**

**Literature**

**Review**

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## 2-1 Cartilage:

Cartilage is a connective tissue - matrix and cells. In cartilage we see a remarkable modification of structure and function. Sulphate compounds in the ground substance and a high proportion of water trapped in the ground substance allows cartilage to resist compression. The root word "chondro", meaning cartilage features in all terminology referring to cartilage [8].

The principle cell type in cartilage is the chondrocyte. Chondrocytes are surrounded completely by the matrix they produce. During preparation of sections the cells shrink, but the surrounding matrix remains firm. this creates the appearance of chondrocytes lying in cavities or "lacunae" in the matrix. Remember that in living cartilage there is no gap between the matrix and the cell [9].

## 2-2 types of cartilage

**2-2-1 Hyaline cartilage.** The word hyaline means "glassy". Fresh hyaline cartilage has a translucent appearance, hence the term.

**2-2-2 Elastic cartilage.** Due to a high proportion of elastic fibers and lamellae, this type of cartilage is yellowish in color in the fresh state.

**2-2-3 White fibrocartilage.** As the name suggests, a high proportion of collagen fibers character this cartilage. It may be described as a transitional stage between dense connective tissue and cartilage, and indeed, blends. Another special feature of cartilage is the absence of blood vessels in the main mass of the tissue [10]

("cartilage is avascular"). Hyaline and elastic cartilage is covered by a fibrous layer called perichondrium (= "around cartilage"). In hyaline and elastic cartilage the perichondrium carries the blood vessels of the cartilage. Exchange between blood and chondrocytes takes place through the matrix. The deeper layers of perichondrium also contains "chondrogenic" cells which become chondrocytes as they produce matrix. White fibrocartilage blends with the surrounding fibrous tissue, and therefore does not have perichondrium [11].

Histological features of cartilage are best understood with the example of hyaline cartilage [12].

### **2-3 Growth of cartilage :**

It has been mentioned that in the deep part of the perichondrium there are chondrogenic cells. These cells, when they become chondro blasts produce cartilage matrix around themselves. Cell growth and the new matrix (the latter more so) add to the cartilage mass.

Deeper down, chondrocytes can divide and grow. When a cell divides, for a while it appears as if there are two cells in one lacuna. The two daughter cells produce new matrix and gradually move away from each other. The two daughter cells may divide again before they move away, and we may see two, three or four cells in a single lacuna. Such cell groups are called cell-nests or isogenies groups [13]

## **2-4 Botanical aspect of Artemisia herba alba**

### **2-4-1 Morphology**

Artemisia herba alba is a greenish silver perennial dwarf shrub that grows (20-40)cm in height their stem rigid and erect , and leave, stems covered by wooly hair steppes of middle east and north Africa, the flowering is start from sept. to Dec. [14].

### **2-4-2 geographic distribution**

The genus Artemisia herba alba aromatic dwarf shrub grows in arid areas of the Mediterranean basin [15] .

### **2-4-3Taxonomy**

Artemisia herba alba Is classified as below

Kingdom: plantae

Class: magnoliopsida

Order : asterales

Family: Asteraceae

Genus :Artemisia L.

Species: Artemisia herba alba [16]

## **2-4-4 Bioactive components**

Artemisia herba alba

Contain many bioactive compounds such as :

- Artemisinin which is profound cytotoxicity against tumor cell [17].
- arglabin which is used for treating some types of cancer[18].
- Lactones :- The prominent natural products found in Artemisia species which are responsible for the importance of these plants in medicine and pharmacy. these compounds found in the aerial parts of plant.
- Flavonoid :- The flavonoid found in leaves and stem of Artemisia herba alba ranging from common flavone and flavonol glycosides to more unusual highly methylate flavonoids [19].
- Phenolic compounds: There are about eight polyphenol and related components

were isolated, such as chlorogenic acid isofraxidin, schaftoside , vicenin-2 etc. [20]

## **2-4-5 Biological effect of Artemisia herba alba can be summarized as bellow :**

- Antioxidant activity  
Artemisia herba alba contain large amount of Antioxidant compounds such as phenolic compounds which are used for prevention and treatment free radical-related disorders.
- Anti-fungal activity : This traits associated with two major volatile compounds isolated form the fresh leaves of the part such as carvone and piperitone [21].



- Antibacterial activity :-  
The principle component of the most active fraction was santolina alcohol [22]. These compounds were active against gram negative bacteria (E.coli, Salmonella typhi), so this plant could be considered as alternative for drug such as the Tetracycline's , chloramphenicol's that are currently used in the treatment of bacteria [23]
- Anti-diabetes activity  
Artemisia herba alba used in treat of diabetes mellitus [24]. [25]showed that administrate of Artemisia herba alba to male albino rats induced very significant decrease in glucose level ( $p < 0.05$ )

#### **2-4-6 Therapeutic effect of Procaine penicillin:**

Recently, surgeons have begun playing an active role in modulating the healing process with the pharm logical treatment of open wounds. Procaine has widespread usage in clinical practice and is also a component of mesotherapy cocktails due to its vasodilator and analgesic effects in anti-aging agents [26].

The infiltration of local anesthetics at high concentrations (2%) decreases the breaking strength of skin wounds in rats, but at a concentration of 0.5%, the differences are not significant [27].

Procaine did not cause any necrosis around the wound, did not retard wound healing, did not cause circulation deficiency, and did not reduce the breaking strength of the wound. Thus, this agent may be recommended for the healing of both open wounds and surgical wounds due to its effect on collagen accumulation. In addition, procaine can be used safely to reduce pain around the wound and to accelerate the healing of slow-to-heal wounds[28].



# **Chapter Three Materials and Methods**

## Materials and Methods

### 3- 1 . Collection of Artemisia herba alba plant:

Artemisia herba alba plant was collected from the gardens of the Biotechnology College / University of Al-Qadisiyah, washed well with distilled water three times, dryness in the laboratory at room temperature for three days, took the leaves only, grinded by electrical grinder and the powder preserved in cans court lid in the refrigerator until drawn.

### 3-2 . Preparation of Artemisia herba alba aqueous extracts:

To prepare Artemisia aqueous extract 5%, took 10 grams of Artemisia herba alba powder and mixed with 200 ml. distilled water used a blender and left for 24 hours at room temperature, filtrated it by using medical gauze to get rid of plankton, centrifuged at 3000 rpm / 10 min., filtration to get clear solution then the extract was put in oven 40m and save in the fridge until using [29]. At the same method Artemisia aqueous extract 10% and 15% were prepared.

### 3-3 . Experimental design:

The study was conducted on 25 female wistar rats Their ages ranged 32- 34 weeks, average body weights  $260\pm 10$  gm. in Biotechnology College from November 2017 to January 2018. and lived in special boxes under standard environment ventilation, light hours, food and drinking clean water ad libitum.

The experimental rats divided randomly and equally into five groups: 1-Control-group-(C) treated with normal saline.

First treatment group-(T1) treated with 5% of Artemisia aqueous extract, second treatment group-(T2) treated with 10% of Artemisia aqueous extract, third treatment group-(T3) treated with 15% of Artemisia aqueous extract, fourth treatment group-(T4) treated with procaine penicillin solution. at the same levels dose of artemisia aqueous extract

Under routine surgical approach 3mm in diameter holes were done in the elastic cartilages of the left ears of all rats. Control group was treated with three drops of normal saline ,while the first three treatment groups T1, T2, and T3 was treated topically and daily for one week with three drops of Artemisia aqueous extract with different concentrations ,yet T4 were treated at the same levels dose of artemisia aqueous extract with procaine penicillin(400000 IU/ vial). The diameters were measured daily for one week. Biopsies were taken from the edges of the holes at 1, 2, and 3 weeks for histo- pathological evaluation of the cartilages healing. Smears were done and stained with E&H stains and examined under light microscope X 40.

### **3- 4 . Statistical analysis:**

The results were analyzed statistically by using Mann – Whitney Test and the differences among means were regarded significant at  $P \leq 0.05$ [30].



Fig.-1: The elastic cartilage hole of the left ear.

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**Chapter**

**Four**

**Results**

## Results

The results presented in (Table.1).Showed that the mean value of diameter of ear cartilage holes for(T3) was  $1.857 \pm 0.293$ .it differs significantly ( $P<0.05$ ) after one week of treatment while T3, recorded the lowest value in diameter as compared to the other treatment groups.

Table- 1: The mean  $\pm$  SE of the ear cartilage hole diameters (mm) after three weeks of treatment.

Treatment groups	Mean $\pm$ SE
C	$2.535 \pm 0.148$ a
T1	$2.321 \pm 0.170$ a
T2	$2.107 \pm 0.291$ a
T3	$1.857 \pm 0.293$ b
T4	$2.392 \pm 0.162$ a

Values within column followed by different superscript letter are significantly different ( $p<0.05$ ) .

### Histopathological examination:

The results of histopathological examination of control groups in first week of experiment showed that Profuse fibrosis connective tissue (F) with mild infiltration of inflammatory cells mainly macrophages (M) in the site of incision X40.( Figure -1)

While the results of other treatment groups shown as bellow figures

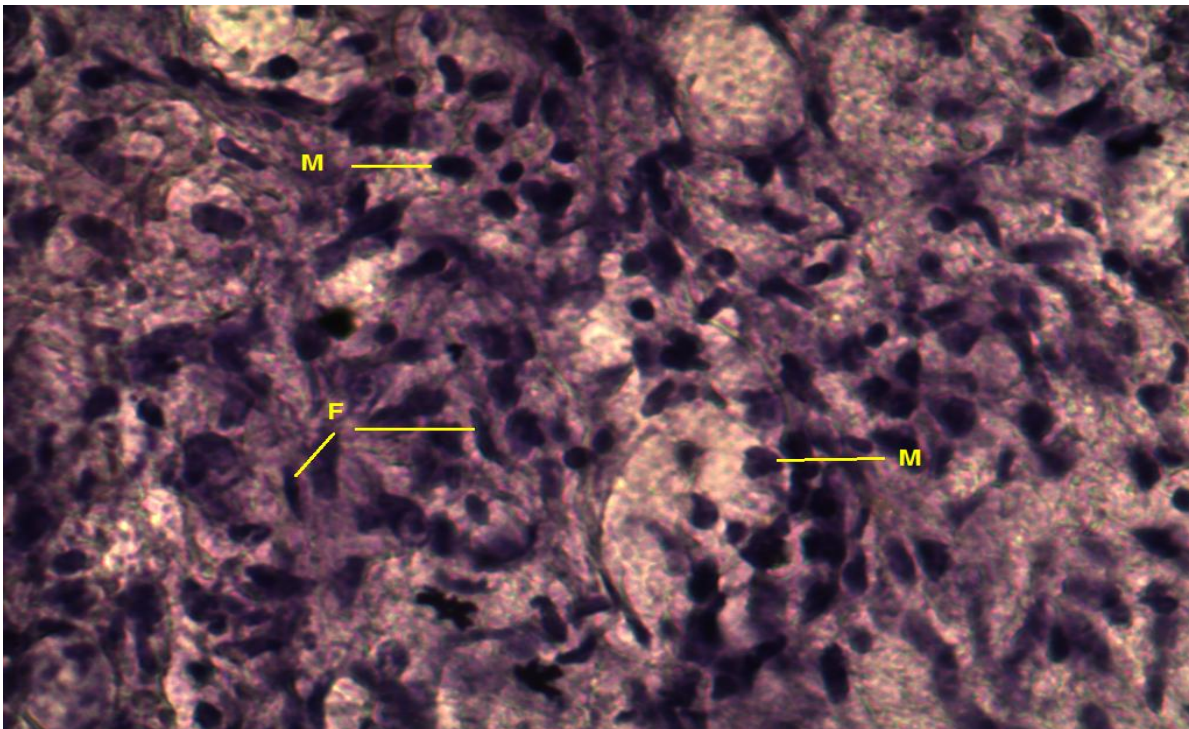


Fig.-1: C- one week: Profuse fibrosis connective tissue (F) with mild infiltration of inflammatory cells mainly macrophages (M) in the site of incision X40.



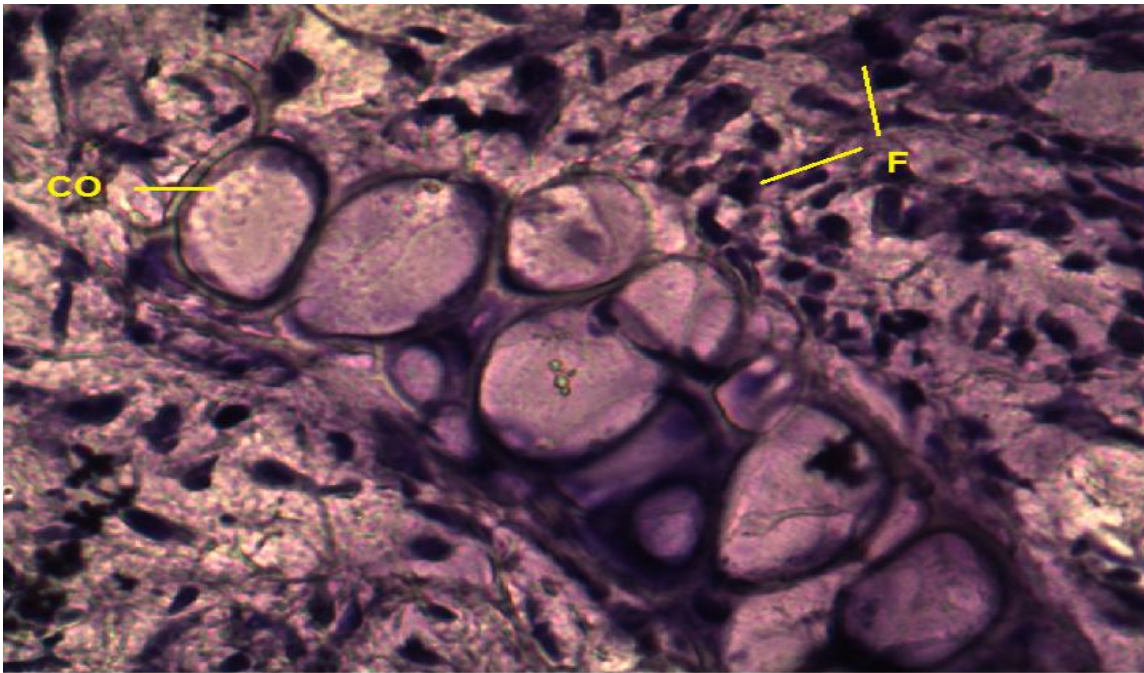


Fig.-2: C- two weeks: Mild infiltration of inflammatory cells mainly macrophages (M) and there is marked vacuolation of chondrocyte (CO) in the edges of incision X 40.

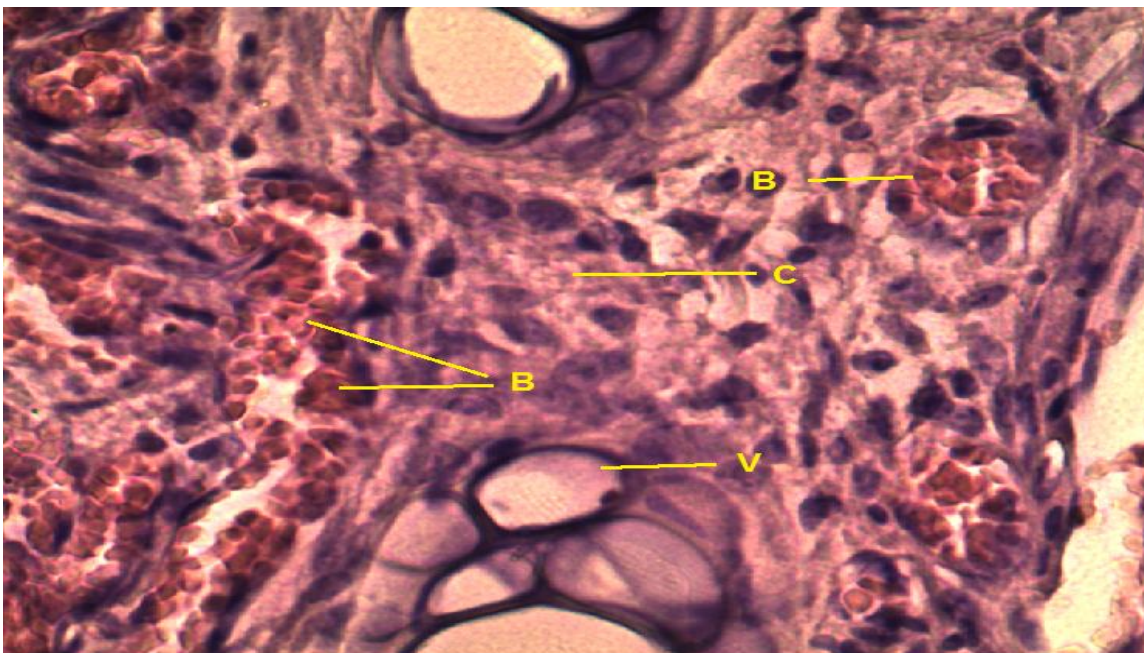


Fig.-3: C- three weeks: Profuse vacuolation of chondrocytes (V) with severe hemorrhage (B) and wide infiltration of inflammatory cells (C) X40.

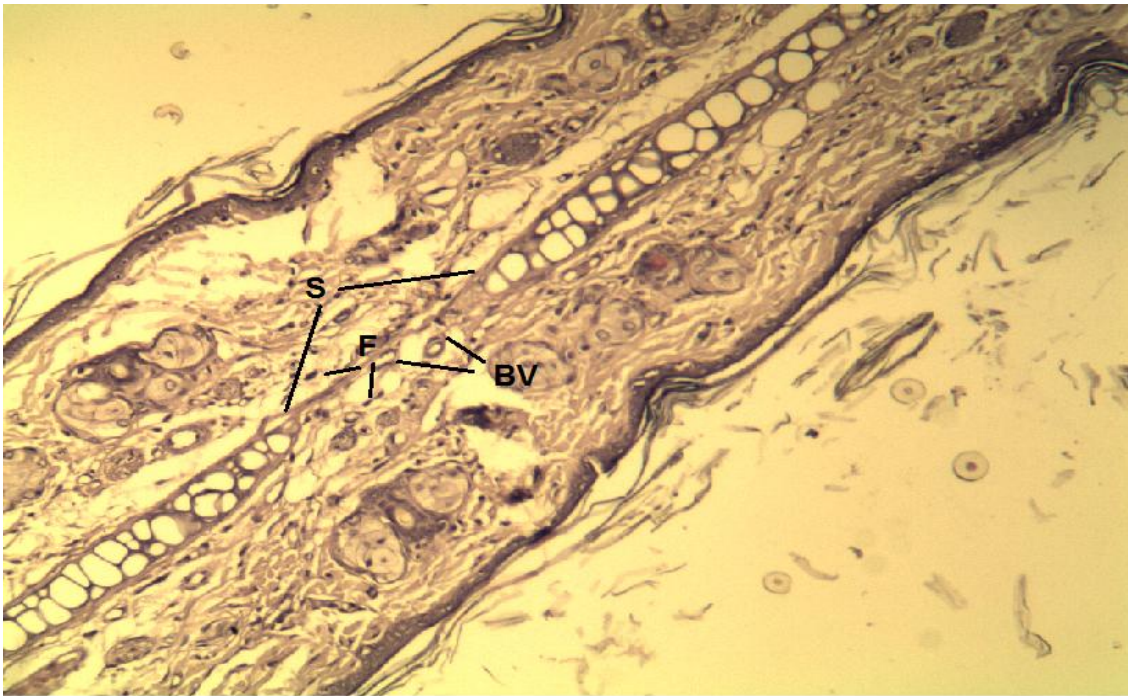


Fig.-4: T1 – one week: Wide scar tissue (S) and presence of granulation tissue which characterized by fibrosis (F) and formation of new blood vessels (BV) complete absence of sweat gland and hair follicles X40

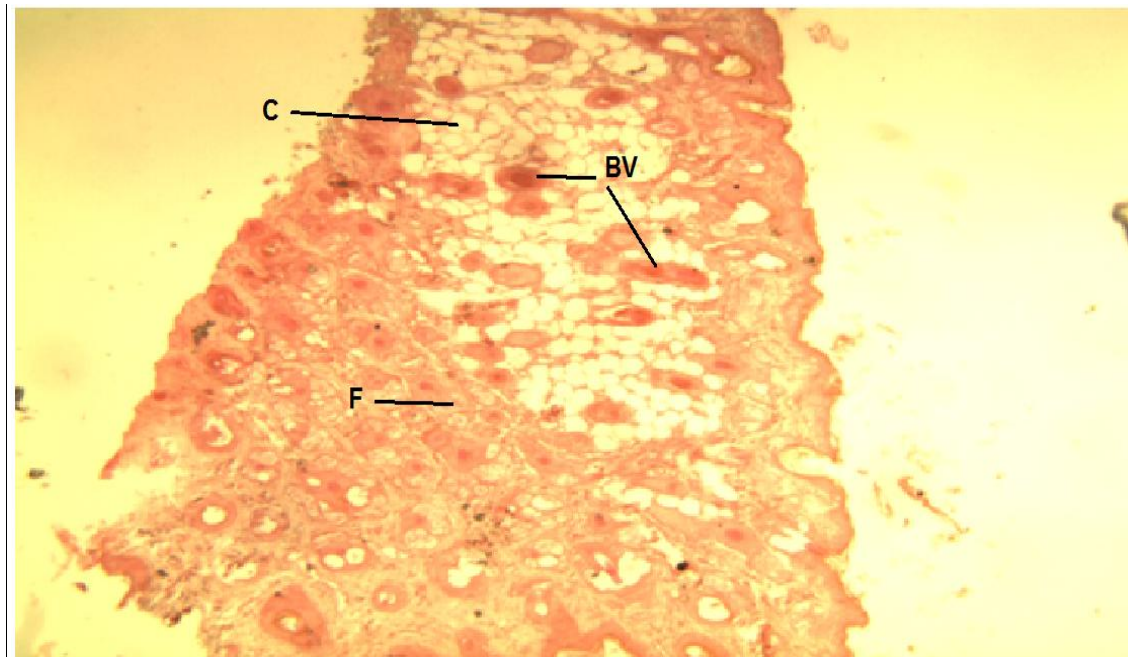


Fig.-5: T1- two weeks Ruptured chondrocytes with thick walls (C) , wide infiltration of inflammatory cells (F) and new blood vessels formation X40.:

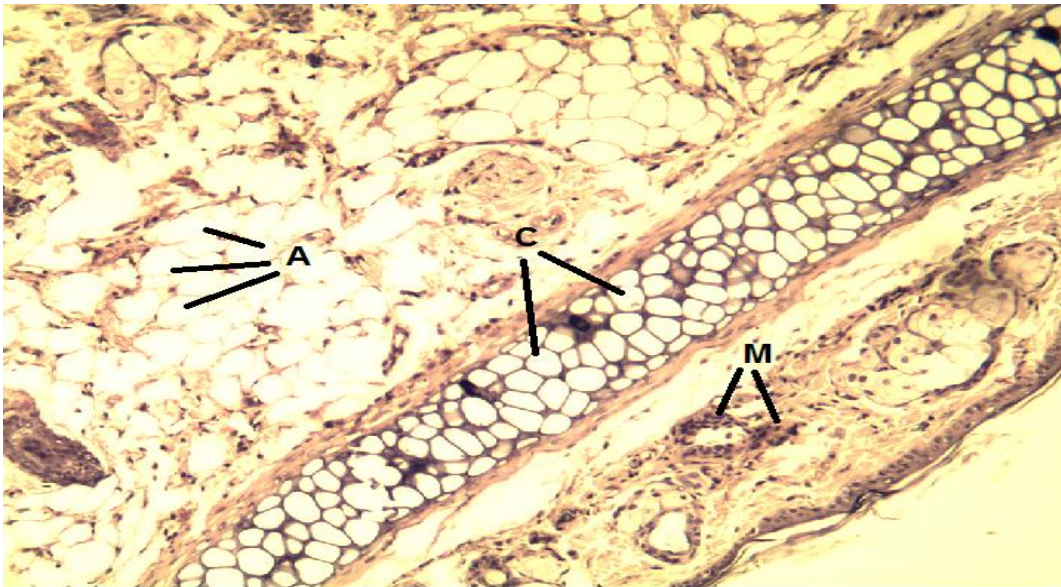


Fig.-6: T1-three weeks: Complete healing and vacuolation and hypertrophy of chondrocytes (C) profuse adipose tissue (A) and few infiltration of inflammatory cells (M) X40.

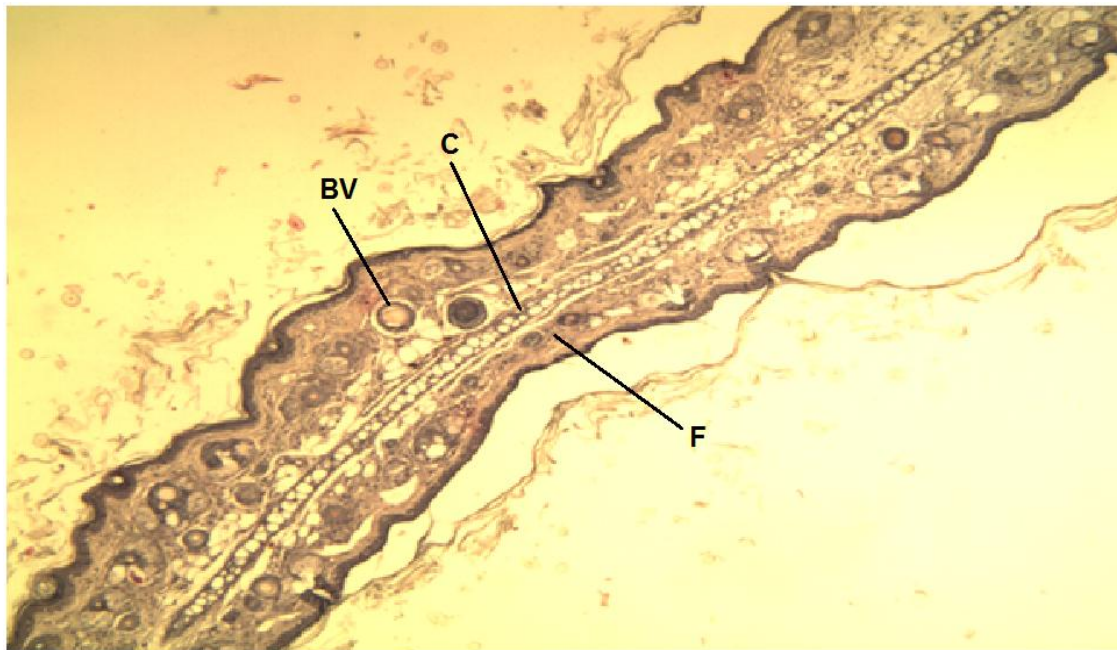


Fig.-7: T2- one week: Vacuolation of chondrocytes (C), wide distribution of inflammatory cells (F) and presence of blood vessels.

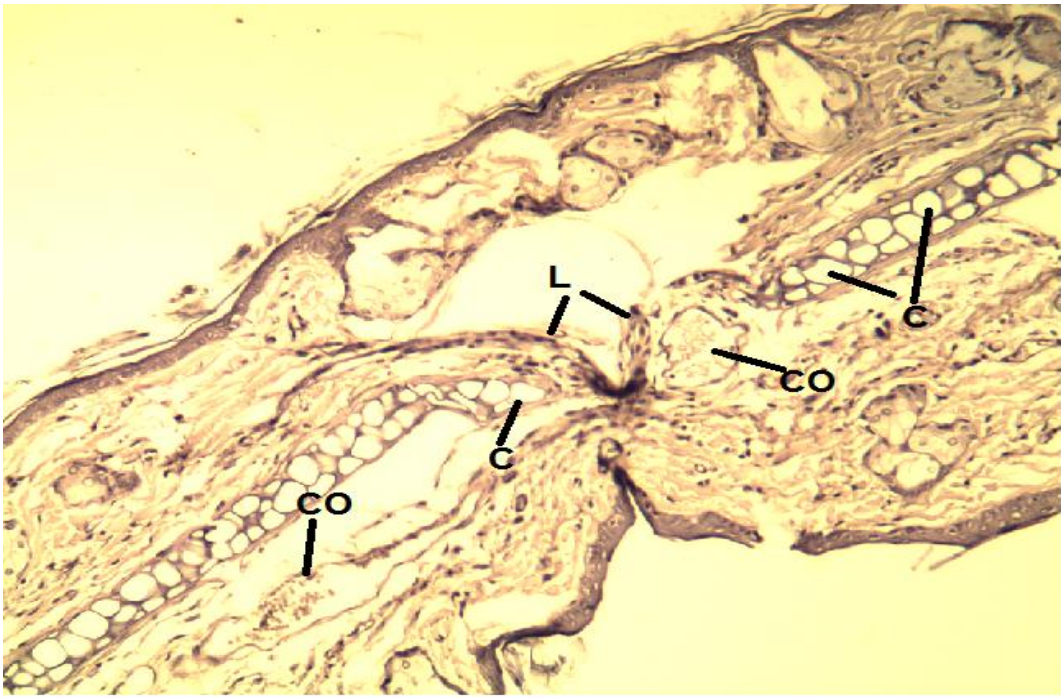


Fig.-8: T2-two weeks: Presence of severe congestion (CO) and formation of collagen in the site of injury (L) with hypertrophy of chondrocytes (C) in both edges of injury X40.



Fig.-9: T2- three weeks: Wide scar tissue with profuse collagen (L) and formation of new blood vessels (BV) and hair follicles (HF) with hypertrophy of chondrocytes (C) X40.

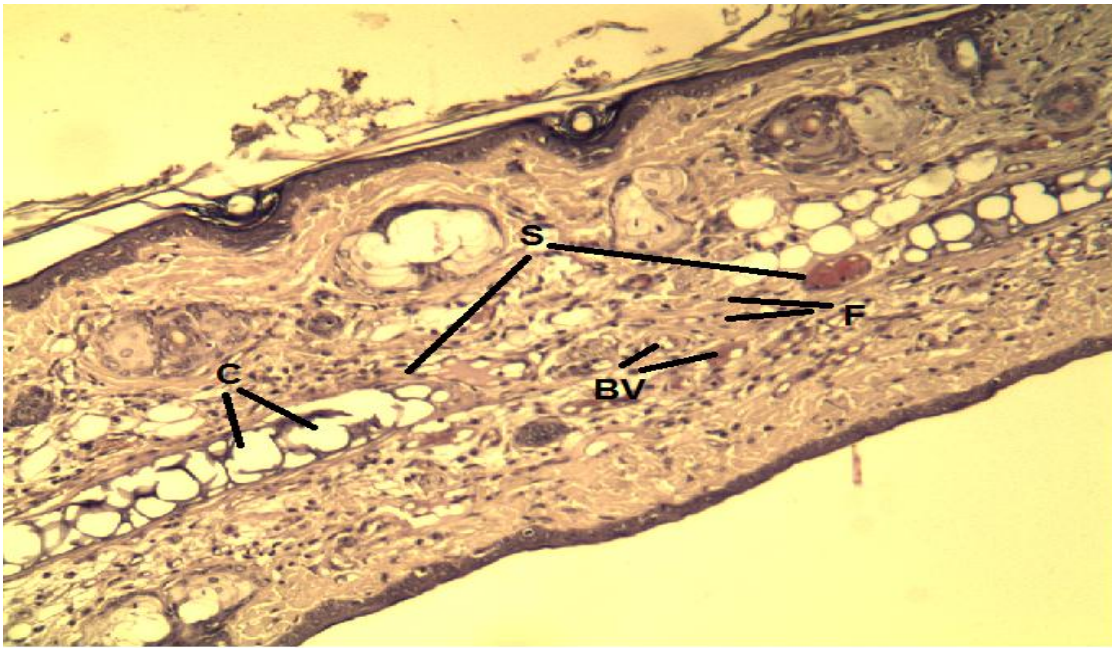


Fig.-10: T3- one week: Wide scar tissue (S) with granulation tissue{ new blood vessels(BV) and fibrosis (F) }, ruptured chondrocytes (C) with thickened walls X40.

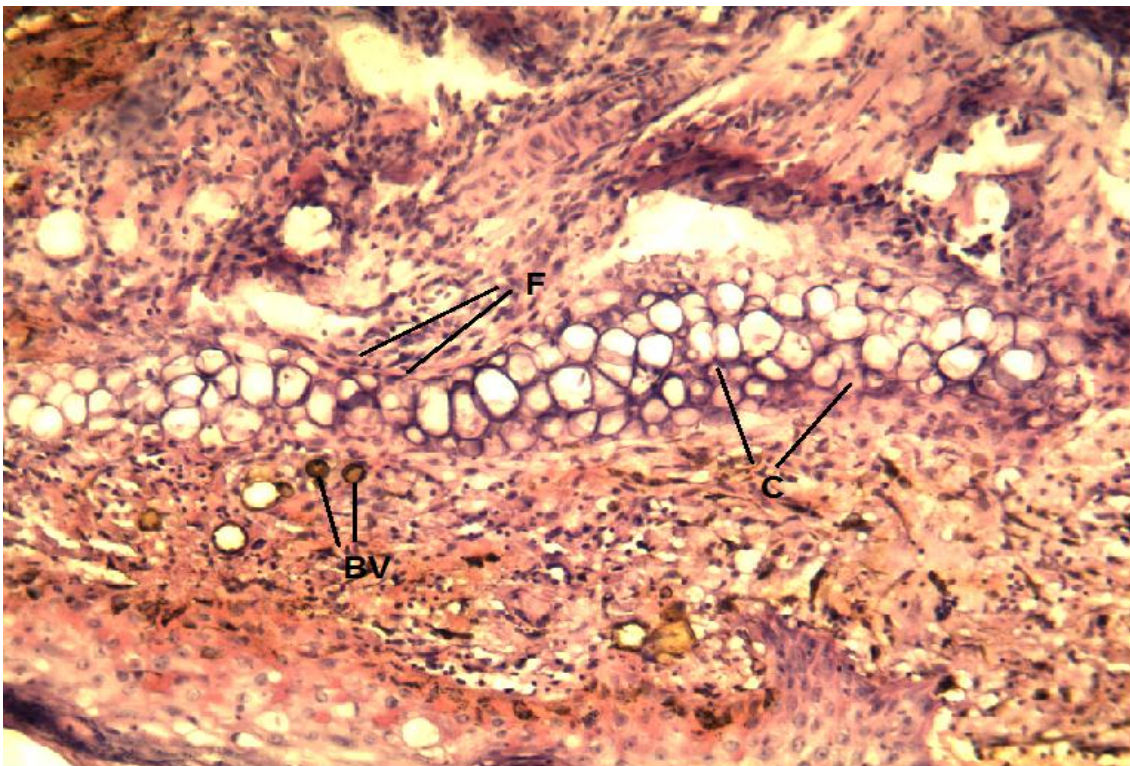


Fig.-11: T3- two weeks: Wide infiltration of inflammatory cells (F), vacuolation of chondrocytes (C) and formation of new blood vessels X40.



Fig.-12: T3- three weeks: Complete healing and absence of scar tissue, both edges of cartilage fused together (C) and marked fibrosis (F), large blood vessels (BV) X40.

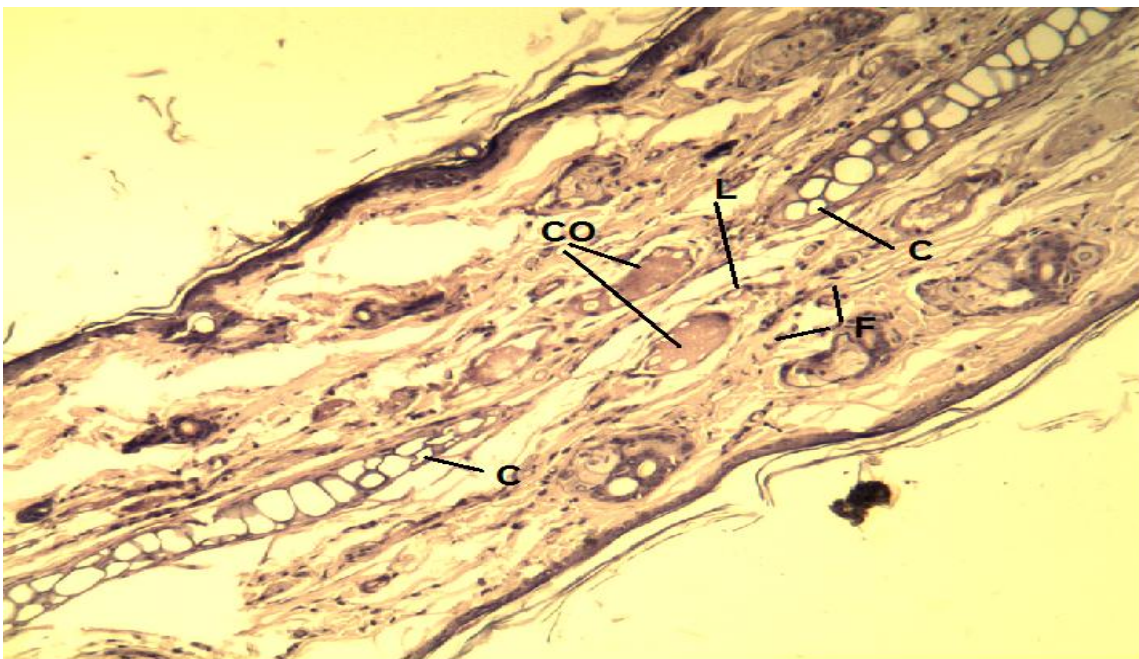


Fig.-13: T4-one week: There is ruptured chondrocytes in both edges of cartilage (C) and severe congestion (CO) and presence of collagen (L) with mild fibrosis (F) X40.

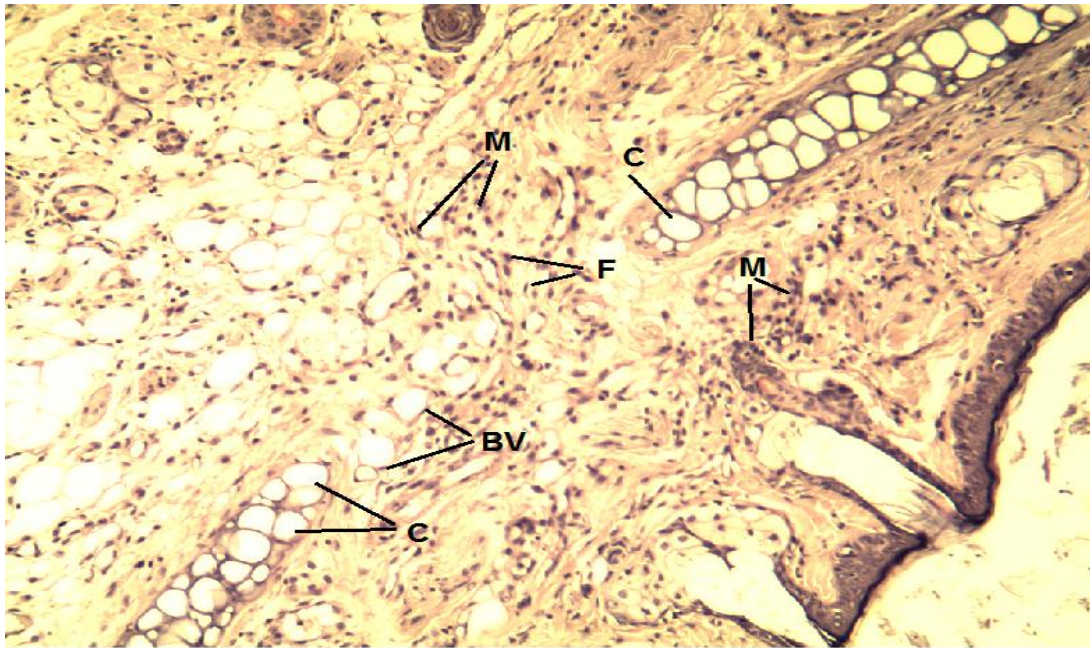


Fig.-14: T4- two weeks: Profuse granulation tissue, new blood vessels (BV), proliferation of fibroblasts (F), infiltration of inflammatory cells (M), and mild hypertrophy of chondrocytes (C) X40.

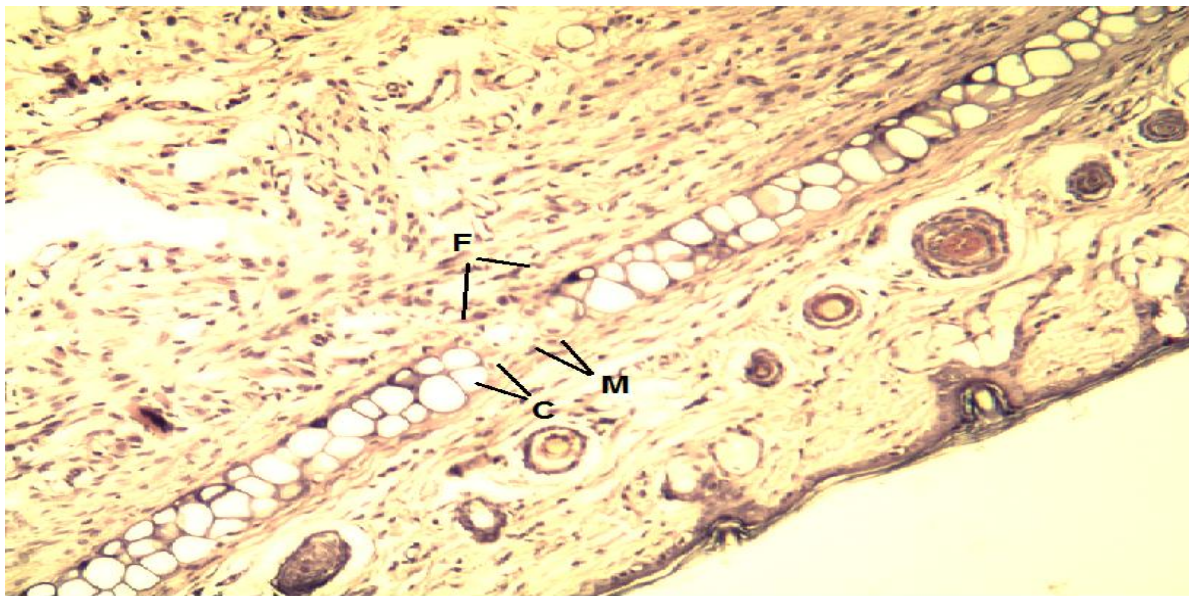


Fig.-15: T4- three weeks: Narrow scar tissue and marked healing in the cartilage (C), the chondrocytes showed thickened wall and ruptured profuse fibrosis (F) and scattered inflammatory cells (M) X40.

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# **Chapter five**

# **Discussion**

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## Discussion

Herbal medicine was very attractive therapy for several researchers in many countries. Our results showed very important new aspects of the aqueous extract of *Artemisia herba alba* for the first time when compare it with procaine penicillin on the elastic ear cartilage healing.

In the current work, to evaluate the healing of cartilage, contraction of wound measurement was an essential parameter, the way for evaluation of contraction was to measure the holes diameter. The diameter of cartilage hole of T3 which treated with aqueous extract of *Artemisia herba alba* at 15% concentration was  $1.857 \pm 0.293$  showed significantly at  $P \leq 0.05$  the lowest mean  $\pm$  SE value .

We believed that, this result was due to the concentration of 15% aqueous extract of *Artemisia herba alba* while in other groups T2 (10% concentration) was  $2.107 \pm 0.291$ , T1 (5% concentration) was  $2.321 \pm 0.170$ , and in T4 (procaine penicillin solution) was  $2.392 \pm 0.162$  which was nearly equal to T1 as showed in table-1.

The healing wound resulted from using *Artemisia herba alba* aqueous extract may be attributed to the presence of high content of crude flavonoids and phenolic compounds which act as antiseptic, anti-inflammatory, anti microbial action. These properties increase cell proliferation and reduced free radical production and stimulate wound contraction and epithelization period which helps in wound healing

Other parameter of cartilage healing evaluation was the histo pathological examination. Fig.-12 of T3 after three weeks showed complete healing, absence of scar tissue, both edges of cartilage were fused together with marked fibrosis and large blood vessels were formed in the cartilage healing site. While the fig.-15 of T4 after three weeks showed narrow scar tissue, marked healing in the cartilage, thick walls of chondrocytes, ruptured profuse fibrosis and scattered inflammatory cells.

A flavones from *Artemisia* induced the production of IL-10 and anti-inflammatory cytokine(31).

# **Chapter Six**

## **Conclusions And Recommendations**

**Conclusions:**

- 1 . The aqueous extract of Artemisia herba alba was a cheap useful herbal therapy.
- 2 . The aqueous extract of Artemisia herba alba had the superior effect on elastic ear cartilage healing than procaine penicillin effect.
- 3 . The aqueous extract of Artemisia herba alba as one of folk medicine drug had used topically and no need for any instruments to application.
- 4 . The aqueous extract of Artemisia herba alba was a safety therapy have no side effects in our study (itching, redness, pain....etc.)for the animals .
- 5- The aqueous extract of Artemisia herba alba promote wound healing process due to their anti-oxidant and anti-microbial activities.

**Recommendations**

- 1 . The aqueous extract of Artemisia herba alba effect need more studies of its therapeutic effects and the real mechanism of action.
- 2 . Use of aqueous extract of Artemisia herba alba in medicine regeneration of soft tissues.

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# **Chapter seven**

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أجريت الدراسة لمعرفة تأثير المستخلص المائي لنبات الشيح والبروكاءين بنسولين على التئام الجروح المستحدثة في غضروف الأذن.

شملت الدراسة (25) انثى جرذ حيث قسمت عشوائيا إلى خمسة مجاميع بواقع خمسة جرذان لكل مجموعة . تم عمل ثقب دائرية جراحيا في الغضروف المرن للأذن ولكل الجرذان. عولجت الجرذان موضعيا وبشكل يومي لمدة اسبوع حيث عولجت مجموعة السيطرة بمحلول ملحي متعادل, وعولجت المجموعة الأولى والثانية والثالثة بالمستخلص المائي لنبات الشيح وبتراكيز 5%,10%,15% على التوالي , أما المجموعة الرابعة فقد عولجت بمحلول البروكاءين بنسولين.

نستنتج من الدراسة بان تركيز المستخلص المائي 15% (المعاملة الثالثة) أثرت بشكل معنوي في تقليل قطر الجرح للأذن بعد ثلاثة اسابيع من المعالجة . كما ان نتائج الفحص النسيجي توضح الشفاء التام وغياب النسيج الندبي لكلا الحافتين وتكون اوعية دموية كبيرة في موقع الجرح.



جمهورية العراق  
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جامعة القادسية  
كلية التقانات الإحيائية  
قسم التقانات الإحيائية الزراعية



# مقارنة تأثير المستخلص المائي لنبات الشيح والبروكائين بنسلين على التئام العيوب المستحدثة لغضروف الأذن

بحث مقدم إلى  
مجلس كلية التقانات الإحيائية/ جامعة القادسية كجزء من متطلبات نيل  
درجة البكالوريوس في علوم التقانات الإحيائية الزراعية

من قبل  
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و

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بإشراف

الأستاذ

كريم ناصر طاهر

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