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

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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± 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.

***Keywords*---**aqueous extract, Artemisia herba alba, procaine penicillin, healing, ear cartilage.

Introduction

Artemisia herba alba is belonging to family (Asteraseae), 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, anti-diabetic , 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 evaluate the role of this plant in treatment the wounds as compared with antibiotic procaine penicillin.

Materials and Methods

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.

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 [8]. At the same method Artemisia aqueous extract 10% and 15% were prepared.

Experimental design:

This study was carried out on 25 female wistar rats their ages ranged 32- 34 weeks, average body weights 260±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: -Control-group-(C) treated with normal saline.First treatment group-(T1) treated with5% of Artemisia aqueous extract, second treatment group-(T2) treated with 10% of Artemisia aqueous extract, third treatment group-(T3) treated with15% 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.

Statistical analysis

The results were analyzed statistically by using Mann – Whitney Test and the differences among means were regarded significant at P≤ 0.05[9].

Figure 1. The elastic cartilage hole of the left ear.

Results

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

Table- 1: The mean ± SE of the ear cartilage hole diameters (mm) after one, two and three weeks

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

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

Histopathological examination

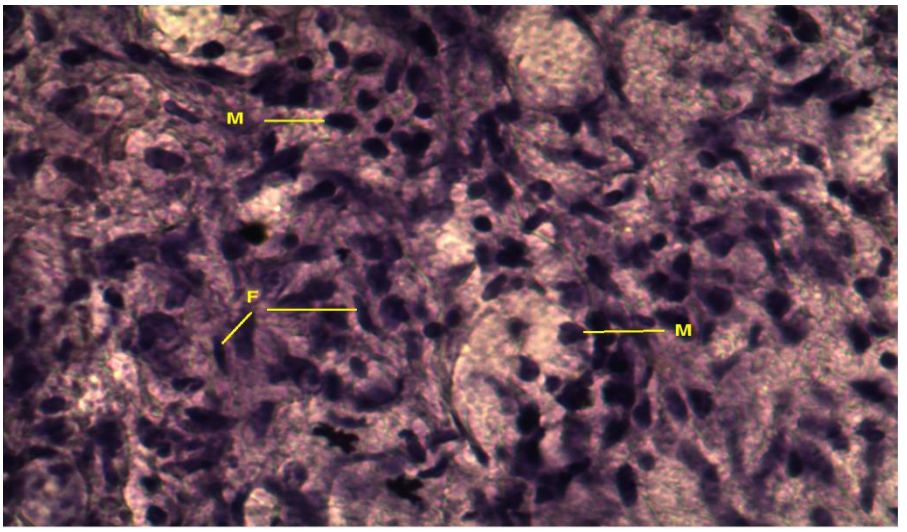

Figure 1. C- one week: Profuse fibrosis connective tissue (F) with mild infiltration of inflammatory cells mainly macrophages (M) in the site of incision X40.

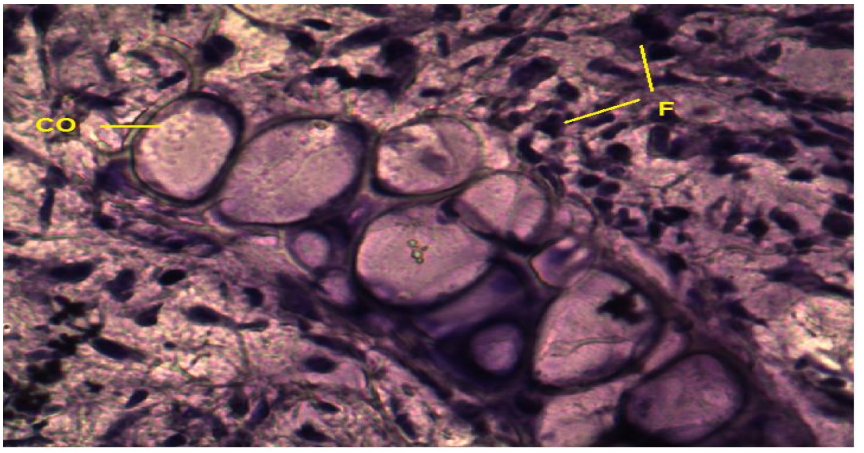

Figure 2. C- two weeks: Mild infiltration of inflammatory cells mainly macrophages (M) and there is marked vacuolation of chondrocyte (CO) in the edges of incision X 40.

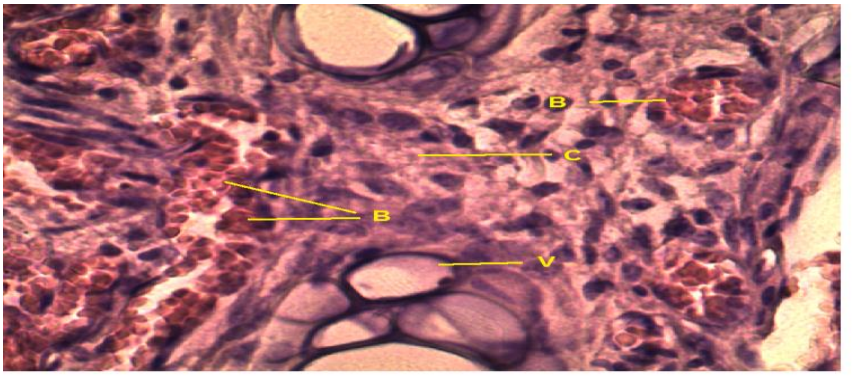

Figure 3. C- three weeks: Profuse vaculation of chondrocytes (V) with severe hemorrhage (B) and wide infiltration of inflammatory cells (C) X40

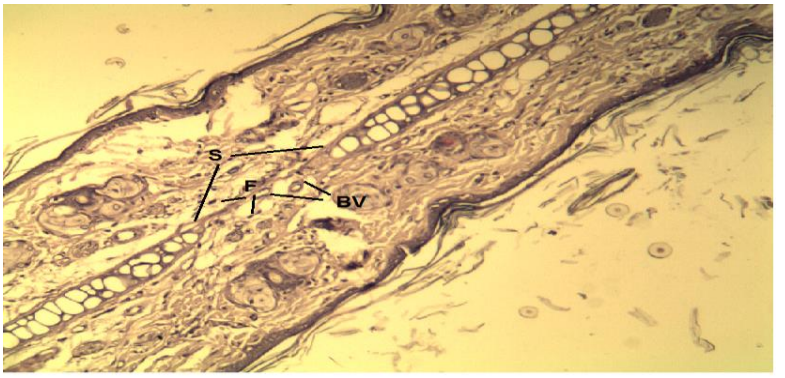

Figure 4. T1 – one week: Wide scar tissue (S) and presence of granulation tissue which characterized by fibrosis (F) and formation of new blood vessels (BV) complete absence of sweat gland and hair follicles X40

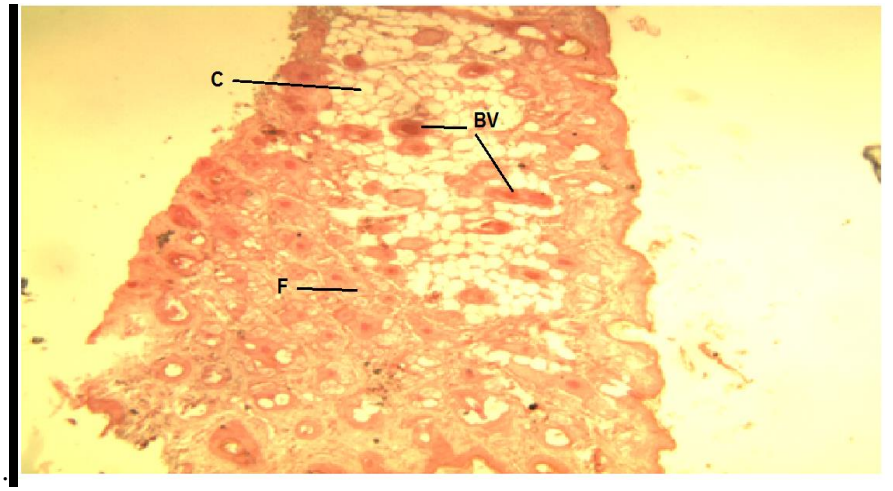

Figure 5. T1- two weeks Ruptured chondrocytes with thick walls (C), wide infiltration of inflammatory cells (F) and new blood vessels formation X40

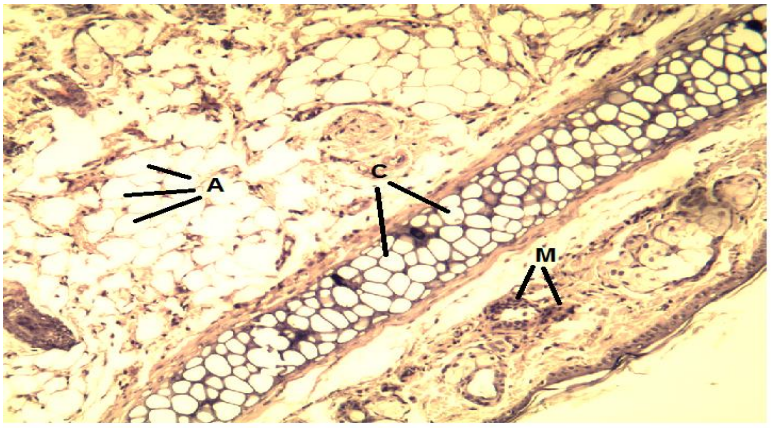

Figure 6. T1-three weeks: Complete healing and vaculation and hypertrophy of chondrocytes (C) profuse adipose tissue (A) and few infiltration of inflammatory cells (M) X40

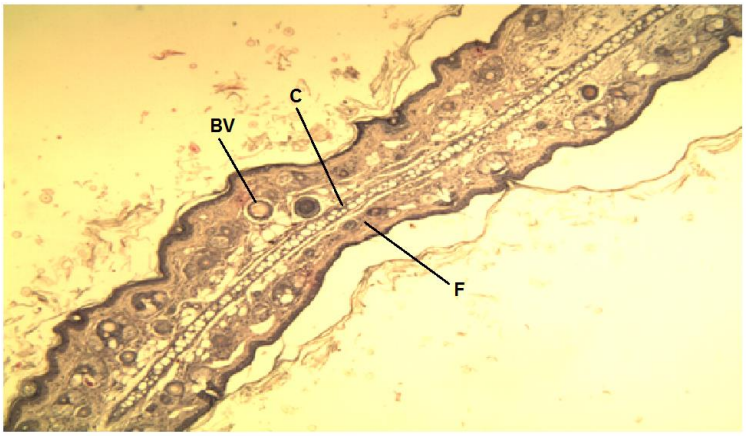

Figure 7. T2- one week: Vaculation of chondrocytes (C), wide distribution of inflammatory cells (F) and presence of blood vessels

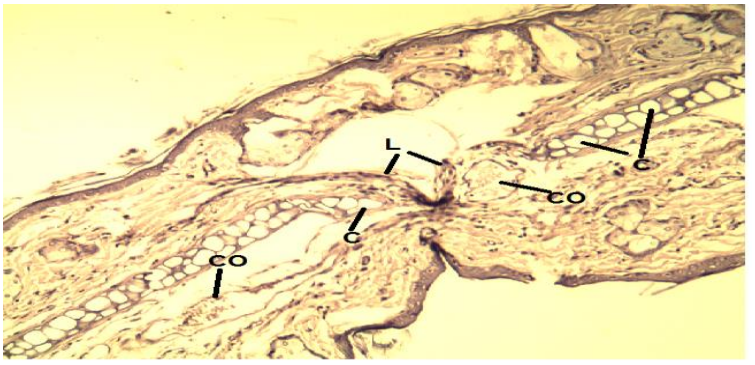

Figure 8. T2-two weeks: Presence of severe congestion (CO) and formation of collagen in the site of injury (L) with hypertrophy of chondrocytes (C) in both edges of injury X40.

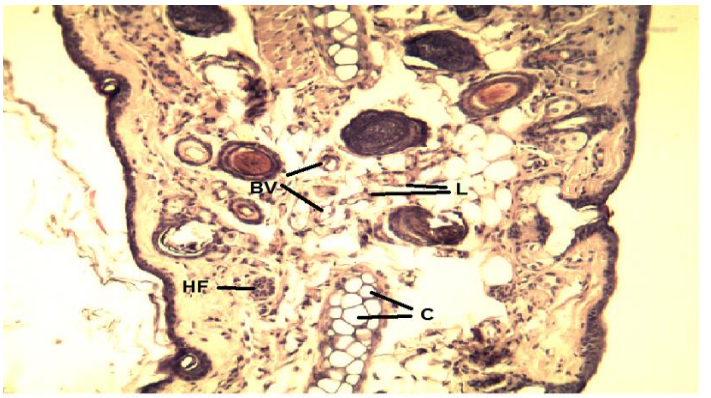

Figure 9. T2- three weeks: Wide scar tissue with profuse collagen (L) and formation of new blood vessels (BV) and hair follicles (HF) with hypertrophy of chondrocytes (C) X40

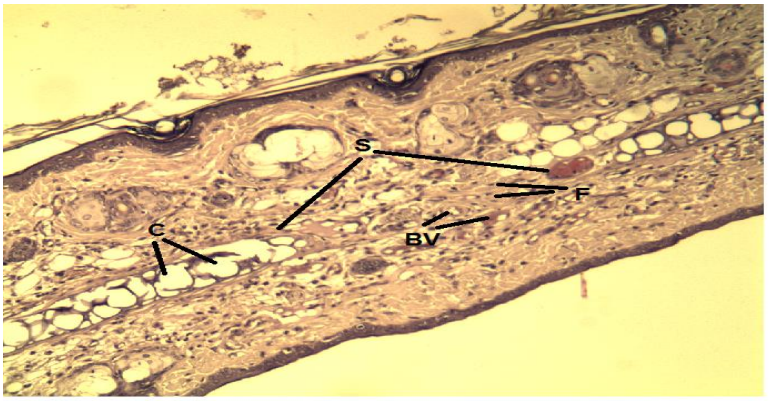

Figure 10. T3- one week: Wide scar tissue (S) with granulation tissue{ new blood vessels(BV) and fibrosis (F) }, ruptured chondrocytes (C) with thickened walls X40.

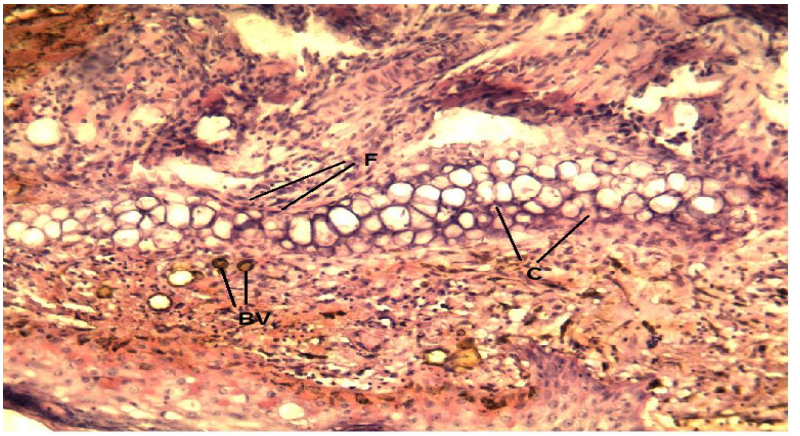

Figure 11. T3- two weeks: Wide infiltration of inflammatory cells (F), vaculation of chondrocytes (C) and formation of new blood vessels X40.

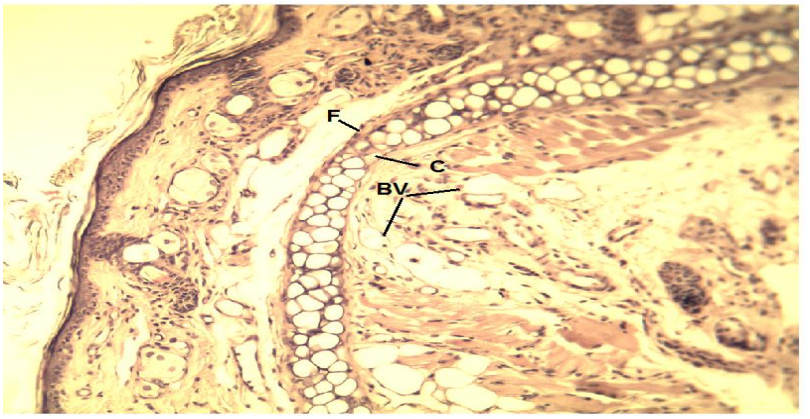

Figure 12. T3- three weeks: Complete healing and absence of scar tissue, both edges of cartilage fused together (C) and marked fibrosis (F), large blood vessels (BV) X40

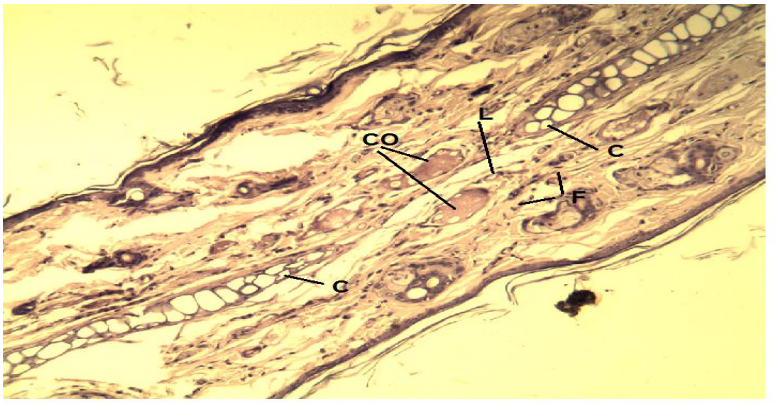

Figure 16. T4-one week: There is ruptured chondrocytes in both edges of cartilage (C) and severe congestion (CO) and presence of collagen (L) with mild fibrosis (F) X40.

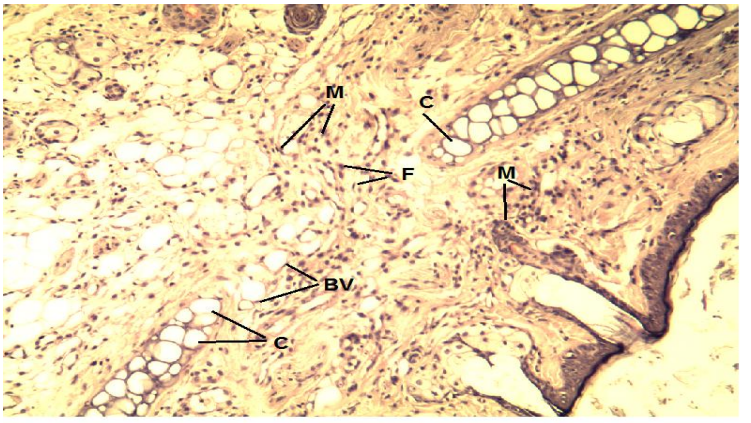

Figure 14. T4- two weeks: Profuse granulation tissue, new blood vessels (BV), proliferation of fibroblasts (F), infiltration of inflammatory cells (M), and mild hypertrophy of chondrocytes (C) X40.

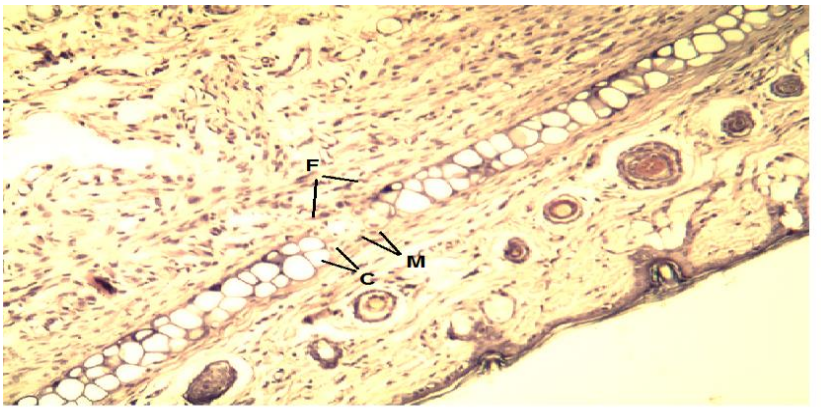

Figure 14. T4- three weeks: Narrow scar tissue and marked healing in the cartilage (C), the chondrocytes showed thickened wall and ruptured profuse fibrosis (F) and scattered inflammatory cells (M) X40.

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± 0.293 showed significantly at P≤ 0.05 the lowest mean± 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± 0.291, T1 (5% concentration) was 2.321± 0.170, and in T4 (procaine penicillin solution) was 2.392±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(10).

Conclusion

According to the results obtained from the present study. It could be concluded that the aqueous extract of Artemisia herba alba promote wound healing process due to their anti-oxidant and anti-microbial activities.

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