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Introduction

According to the experiences in traditional medicine of the old civilization of Iraqis, Egyptians, Indians, and Chinese, they tried many herbal therapies.

A lot of people had fear knowledge about the medicinal plants, their processing and extraction to cure many diseases. These people got their knowledge from their forefathers and developed by many researchers. Different organic and inorganic chemicals present in a lot of herbs were used to defense against many pathogens. Aim of study was to compare the effects of Artemisia herba alba aqueous extract and Procaine penicillin on elastic cartilage defects healing.

The aim of the research was to study the use of modified Nanotherapy (Cellulose Platelet rich – Plasma) for wound healing in Iraqi Arabian Mares.

Wound healing

Wound healing is an intricate process in which the skin repairs itself after injury. In undamaged skin the epidermis (surface layer) and dermis (deeper layer) form a protective barrier against the external environment (1). When the barrier is broken, a regulated sequence of biochemical events is set into motion to repair the damage (2).

wound healing stages :

1-Hemostasis (blood clotting): Within the first few minutes of injury, platelets in the blood begin to stick to the injured site. This activates the platelets, They change into an amorphous shape, more suitable for clotting, and they release chemical signals to promote clotting. This results in the activation of fibrin, which forms a mesh and acts as "glue" to bind platelets to each other. This makes a clot that serves to plug the break in the blood vessel, slowing/preventing further bleeding (3,4).

2-Inflammation: During this phase, damaged and dead cells are cleared out, along with bacteria and other pathogens or debris. This happens through the process of phagocytosis, where white blood cells "eat" debris by engulfing it (5).

3-growth of new tissue: In this phase, angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction occur. In angiogenesis, vascular endothelial cells form new blood vessels. In fibroplasia and granulation tissue formation, fibroblasts grow and form a new, provisional extracellular matrix) by excreting collagen and fibronectin. Concurrently, re-epithelialization of the epidermis occurs, in which epithelial cells proliferate and atop the wound bed, providing cover for the new tissue. In wound contraction, myofibroblasts decrease the size of the wound by gripping the wound edges and contracting using a mechanism that resembles that in smooth muscle cells. When the cells' roles are close to complete, unneeded cells undergo apoptosis (6).

4-Maturation (remodeling): During maturation and remodeling, collagen is realigned along tension lines, and cells that are no longer needed are removed by programmed cell death, or apoptosis (7).

Platelets rich – Plasma (PRP):

Platelets rich – plasma is a concentrate of <u>platelet</u>-rich <u>plasma</u> <u>protein</u> derived from whole <u>blood</u>, <u>centrifuged</u> to remove <u>red blood cells</u>. PRP contains <u>cytokines</u> that can stimulate healing of <u>soft tissue</u> and joints and <u>growth factors</u>..

PRP has been used to encourage a brisk healing response across several specialties (8).

Materials and methods

The study was designed in the Department of surgery and obstetric / Veterinary Medicine College / University of Al- Qadisiyah from November 2017 to February 2018.

Extraction of cellulose from (frond midrib) of date palm :

Grinded of dry (frond midrib) to obtain its powder. The cellulose was extracted from the (frond midrib) powder by used conventional Soxhlet extractor with absolute ethanol 45°C for 6 hours. The extracted cellulose was dried by oven at 40 °C to evaporate the absolute ethanol and preserved at 4°C.



Fig.-1: Microfibril of date palm cellulose x 40.

Preparation of cellulose Platelets- rich plasma :

Whole blood 100 ml. were aspirated from the peripheral blood circulation (jugular vein) of each horse (autologous blood), twice centrifugation at 4000 rpm/ 10 minutes. The yellow layer (containing plasma, platelets and growth factors) was aspirated gently, added 10% calcium chloride then kept the solution in incubator at 37° c for 24 hours to activate platelets and release high

concentration of growth factors, cellulose mixed with PRP solution at ratio 2.5: 1 and preserved at -80 ° c .

Experimental design:

The study was conducted on six Iraqi Arabian mares lived in private stall. Their ages were ranged 9.5-12 years and their weights were ranged 275±5kg. These mares were divided randomly into three groups equally, treatment group-1 (TG1) treated with CPRP, treatment group-2(TG2) treated with PRP, and control group-3 (CG3), complete square cutaneous incisions 5 Cm² were done under routine surgical procedures in the back regions of all groups. CPRP was thawed at 37° C, daily for one week topical few drops were applied for incisions of TG1, at the same manner PRP for TG2 and normal saline 0.9% N for CG3.

4 – Measurement of wound contraction:

All incisions were measured daily for one week by drew them on transparent paper and measured the length of the side with caliper to calculate the rate of the wounds contraction.

Results

Wound contraction:

The wound contraction of TG1was the lowest value 3.8214 ± 0.3234 which was significantly variance than TG2 4.2500 ± 0.2034 and CG3 4.5000 ± 0.1577 as showed in table-1.

Table-1: show the length of the side of the square incisions of treatment groups for one week to measure their contractions.

Treatment	Length of the side of incisions
groups	
TG1	3.8214 ± 0.3234 A
TG2	$4.2500 \pm 0.2034 \text{ B}$
CG3	4.5000 ± 0.1577 B

*Different letters mean significant variance at $P \le 0.05$.

Macroscopic evaluation:

The macroscopic evaluation revealed clear differences between PRP and CPRP treatments, contraction and scar formation of the wounds .

Discussion

The world now is heading toward the biological remedies which considered as a revolutionary and effective cures for a number of diseases that the antibiotic and drugs, these days can't provide a treatment for it.

The aim of our research is to study the use of moderate Nanotherapy (Cellulose Platelets rich – plasma) for the wound healing in Arabian mares. Many researchers was try to mix nano-materials with PRP like purified fat graft(9) but our research according to our knowledge was the first study in the world.

Table-1 showed the gradual reducing of length of the side of the experimental square incisions of all groups but the superiority was for TG1 (3.8214 ± 0.3234) which treated with cellulose PRP, significantly variance than other groups. We believe that cellulose act as scaffold for fibroblast migration. This result was clear also with TG24.2500 \pm 0.2034 that treated with PRP only which may be the platelets content of PRP accelerate the regeneration of these cells and these platelets might be secreted growth factors which also played an essential role in accelerated the proliferation of tissue cells. In addition of this fact, the origin of the cellulose was the date palm plant that mean it's an cold material and there was no reaction of the immune system.

The contraction and the scar tissue formation were better in TG1 than TG2 as the macroscopic appearance. This result also might clear the effect the cellulose which extracted from plant source, cellulose nano-particles didn't showed any immune reactions like itching, redness, swollen....etc.

The histopathological evaluation of TG1 as showed in fig.-10 after three weeks, there was marked healing characterized by very narrow scar tissue, proliferation of epidermal layers in both edges of incisions, profuse collagen and fibrosis with mild infiltration of inflammatory cells and formation of new blood vessels, this result proved our explanations about the role of growth factors which released by platelets and the cellulose scaffold mechanism for cells growth.

Also this research shows the easy administration of a new therapy by local application as few drops only.

The results indicate clearly that the cellulose PRP was a new biological useful therapy for wound healing. Our research need more studies about the real effects of this new nano-materials which act in fact as a new scaffold for wound regeneration and the mechanism of the bioactive factors which represent in the PRP. Our research is very useful medically for veterinarians and medical companies.

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