Use of Iraqi Castor (*Ricinus Communis*) Leaf Extract as Anti-Inflammatory in Treatment of Skin Wounds in Rabbits

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Abstract

The present study was investigate to study the effects of castor leaf extract on histological healing rates of skin wounds in rabbits. sixteen adult clinically healthy rabbits from both sexes weighing from (1.25- 2 kg) were used. All animals were created wound ($4cm^2$) full-thickness in abdominal region .The treated group (B) application of the extract on the wound to all animals while group A (control group) leave without treatment. On day 3, 7, 14 and 21 post operations collect skin tissue biopsy samples for histopathological examination each time two rabbits. The result revealed, Clinically, the rate of wound healing was same in two groups, no differences when section taken from the edge of the wounds, histopathological result was show: In 3rd day of skin wound healing group (A) (control group) show demarcation line consisted of polymorphonuclear (PMN), there was thickened at cut edges of epidermis with inflammatory cells, the inflammatory responses of group (B) (treated group) were observed in low range than the control groups. In 7th day of skin wound healing group

(A) show few small vessels were present and tensile strength of the tissue by organized collagen fibers, group (B) show good tensile strength, increased in small vessels and better wound healing. In 14th day of skin wound healing group (A) show vascular density was noticeable, group (B) show increased vascular density than in group (A).

Keywords: *Castor, Skin, Rabbits, wound healing. Anti-inflammatory*

Introduction

Ricinus communis, also called castor oil, is one of the most important shrub belonging to the family (Euphorbiaceae) and spread in tropical and subtropical regions[(1)].The castor are almost small tree 6 meters in length and they are spread globally in South Africa, India, Brazil and Russia[(2)]. In Iraq, it spreads in central and northern Iraq, as well as in the western regions[(3)]. All parts of the plant are used medically, the stem of the castor plant use as anticancer and antidiabetic[(4)], in Indian medicine, the roots of the castor plant and its five-lobed leaves were used in the treatment of various diseases such as skin ulcers, liver disorder, hypoglycemic and laxative [(5)]. The wound healing is a complex, interactive integrative process that commences right after injury invading cellular and chemotaxis activity[(6)].The wound is a physical injury of body that described by distraction normal continuity structures of body. Wound may cause damage in superficial structures skin and reach to structures underlying the skin[(7)]. The tolerance of injury was a varies with tissue type. Therapeutic considerations are based on the type of skin wound and often determine the amount of tissue damage[(8)]. The anti-inflammatory pharmacological activity of castor (Ricinus communis) observed due to the presence of phytochemicals like flavonoids, alkaloids and tannins that present in the plant extract and various biological activates[(9)]. The plant extract has antibacterial activity against Escherichia coli, Salmonella newport, Serratia marcescens, Streptococcus progens and Shigella flexneri[(10)],Bacillus subtilis and Staphylococcus aureus[(11)], Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, and Pseudomonas aeruginosa[(12)].

The aim of this study was to estimate the benefits of using Iraqi castor (Ricinus Communis) leaf extract in

skin healing in rabbits.

Materials and Method

This study was carried out on sixteen adult clinically healthy rabbits from both sexes weighing from (1.25-2 kg) were divided equally into two group A (control group) and group B (treatment group) .The animals were housed in the animal farm of the College of Veterinary Medicine, University of Karbala, maintained in individual cages under normal environment including climate, management and feeding all animals were subjected to the creation of a full wound (4 cm^2) in the abdomen after preparation for sterile surgery [13].

In this study used fresh leaves of Ricinus communis Linn. (Fam. Euphorbiaceae) with entire petiole. The leaves were collected from soada region in Karbala province, and washed after collection to remove the debris then dried this leaves and the dried leaves were powdered to get a coarse powder then moistened the dried powder material of Ricinus communis (200 gm) with adequate amount of methanol and subjected to soxhlet extraction 3 cycles of solvent and concentrated to get about 16 gm [14].

General anesthesia was induced by diazepam (diazepam 10). The ampoule contains 2 ml (10 mg/ml), Aleppo, pharmaceutical industries, Aleppo - Syria) as surgery at 1 mg/kg body weight. After 10 min syringe with zylazine (2% zylazine® contains 50 ml (20 mg/1 ml), Ceva Saute animal, Spain) at a dose rate of 10 mg/kg body weight. Ketamine® (10%) contains 10 ml Vet. Injection, Kepro Pharmaceuticals, Netherlands) at a dose rate of 50 mg/kg body weight. All these drugs are injectable [15].

On each animal, one full thickness (4 cm^2) was created in the abdominal area using a mold made from X-ray film for all animals and the wound tailor was sewn by 3-0 silk by intermittent horizontal stitching. after that application of the extract on the wound to all animals in group B (treatment group) while group A (control group) leave without treatment.

On day 3, 7, 14, and 21 collect skin tissue biopsy samples for histological examination of groups A and B group for each time two rabbits. The samples were fixed in 10% neutral formalin then cut into 5 μm thick sections and stained with hematoxylin and eosin (H & E), and investigated for microscopy [16].

Results and Discussion

All animals were noted to eat and behave normally in the first three days after surgery. Clinically, the rate of wound healing was same in all groups, no differences when section taken from the edge of the wounds.

The animals remained healthy, without clinical of infection after the surgery period. The macroscopic observation of all groups during the wounding confirmed the aseptic conditions. Wound healing is a complex biological method that takes place in all tissues and all organs of the body. Various cell types, including keratinocytes, neutrophils, macrophages, lymphocytes, fibroblasts and endothelial cells, are involved in this process [17].

In 3rd day of skin wound healing group (A) (control group) the inflammatory reaction was dominant. On the skin surface, necrosis of skin tissue was saturated as a consequence of mechanical damage . This stage was observed also under the tissue necrosis. The demarcation line consisted of polymorphonuclear (PMN), the epidermis was thickened at its cut edges with inflammatory cells, while in treated group show inflammatory responses at lower range than control groups (Figure. 1).

The inflammatory response is started very soon after the trauma on wound event, its the wound healing first phase. Wound and surrounding tissues become inflamed during the first phase and cells mostly neutrophils and monocytes are mobilized to infiltrate the clot and start the complex procedures of synthesis granulation tissue [18].

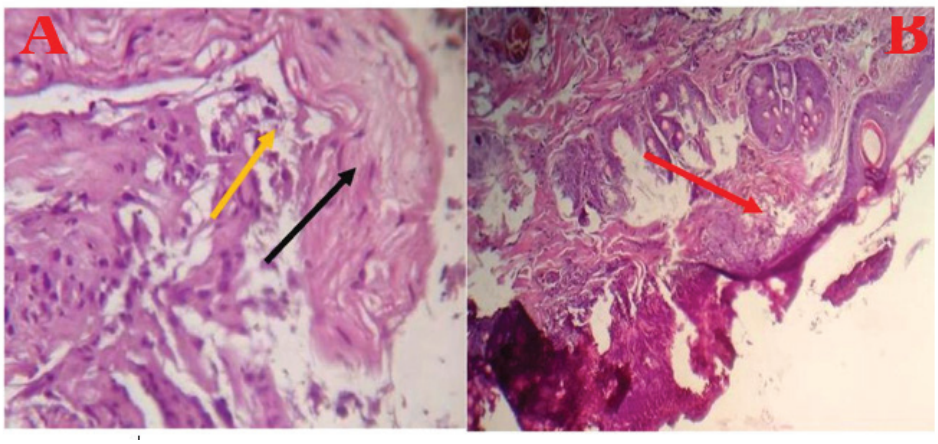
In 7th day of skin wound healing were increased new organized collagen bundles and relatively advanced epitheliumat the wound because treated with castor leaf extract (group B) when compared with non-treated wounds (group A). In treated tissues with castor leaf extract appear several small vessels (angiogenesis), whereas in control tissues was present only a few vessels. The tensile strength of the tissue originate from organized collagen fibers, the collagen was observed consistent arrangement with tissues in the treated group

that give good tensile strength and improved wound healing (Figure 2).

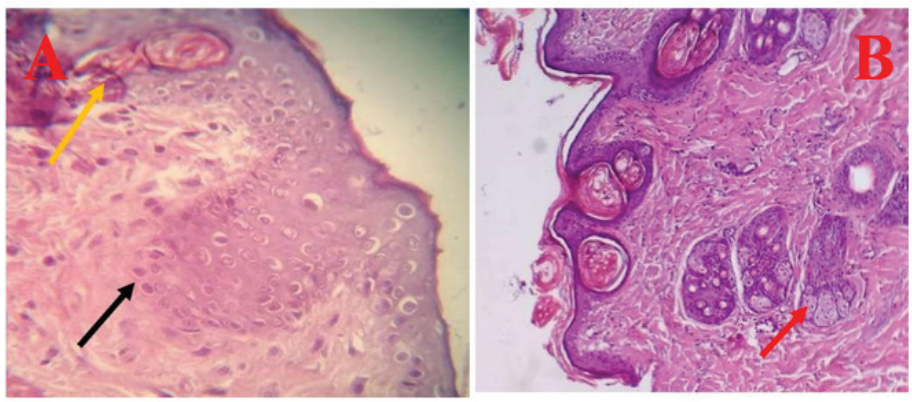
The cell surface binding edges of wound have to myofibril contraction, also present of fibroblasts other cells. A fibrin or fibrinogen was showed to interact specifically with platelets [19]. In 14th day of skin wound healing increased in the mean vascular density of the castor leaf extract treated groups(B) when compared with control groups(A). Consequently, it can be established that treated group by castor leaf extract improved angiogenesis at the wound beds when compared with non-treated groups (Figure. 3).

Small amount of voltage produced as long as the collagen bundle was subjected to stress was arrangement and absorption of collagen [20] .In 21th day of skin wound healing through rich neovascularization all wounds contained abundant fibroblasts and collagen bundles, that noticed only in the wounds treated with castor leaf extract (Figure 4).

Moreover, platelet concentrates comprise many chemotactic growth factors and powerful mitogenic, which regulate tissue repair key processes complex, including cell proliferation, chemotaxis, migration, cellular differentiation, and extracellular matrix synthesis [21] . According to the result of this study the effect of castor leaf extract in wound healing shown positive result in acceleration of epithelial migration, the angiogenic response and fill of tissue. Castor leaf extract accelerate healing both normal tissue and impaired wounds when locally applied.

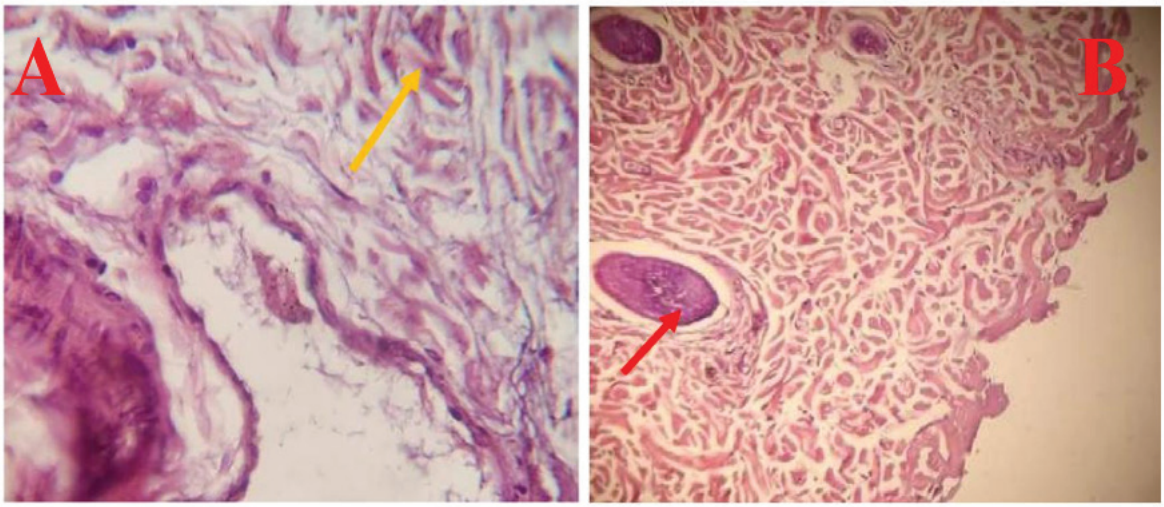


Fig(1) histological cross section of skin show: (A) 3rd day wound healing demarcation line consisted of polymorphonuclear (PMN) ( ), the epidermis was thickened at its cut edges with inflammatory cells ( ) , (B) 3rd day wound healing inflammatory responseswere observed in low range than the control groups ( ) (H &E stain) 40X.



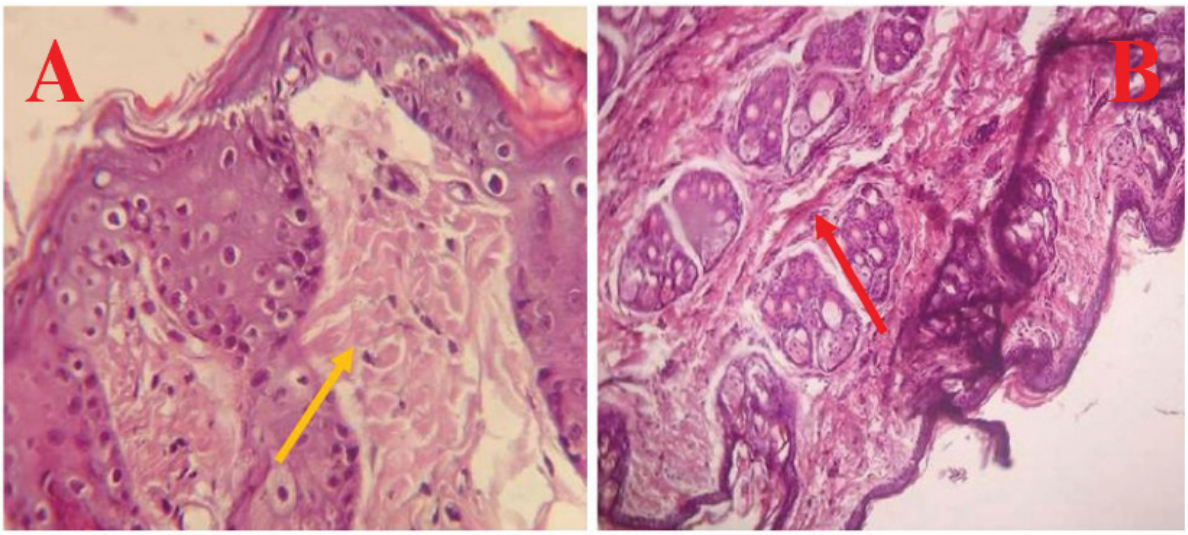
Fig(2) histological cross section of skin show: (A) 7th day wound healing which few small

vessels were present ( ), organized collagen fibers give tensile strength to the tissue( ) (B) 7th day wound healing increased in small vessels, good tensile strength hand improved wound healing ( ) . ((H &E stain) 40X.



Fig(3) histological cross section of skin show: (A) 14th day wound healing which vascular density was noticeable( ) (B) 14th day wound healing increased vascular density than in

(A) ( ). ((H &E stain) 40X.



Fig(4) histological cross section 21th day wound healing show:(A) increase of vascular density and collagen bundle ( ) . (B) through rich neovascularization wounds contained abundant fibroblasts and collagen bundles, ( ) (H &E stain) 40X.

Conclusion

The results of systematic review and hisological analysis shown that the time of wound healing in group was treated with castor leaf extract shorter than non-treated group.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Veterinary Medicine and all experiments were carried out in accordance with approved guidelines.

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