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Submission date: 03-Aug-2020 12:03PM (UTC+0800)

Submission ID: 1365336479

File name: IIs_Post_Extraction_Tooth_In_Wistar_Rats_Rattus_Norvegicus.pdf (130.81K)

Word count: 2897

Character count: 15312

SNAKEHEAD FISH EXTRACT (*CHANNA STRIATA*) INCREASE THE NUMBER OF FIBROBLASTS CELLS POST EXTRACTION TOOTH IN WISTAR RATS (*RATTUS NORVEGICUS*)

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(Received 11 August 2019, Revised 17 October 2019, Accepted 25 October 2019)

ABSTRACT : Tooth extraction is an invasive action which causes dental tissue traumatic injury. The regeneration process is divided into four phases namely hemostasis, inflammatory, proliferating and remodeling. Fibroblast cells play important roles in the proliferating phase, with the ability to hasten wound healing using snakehead fish. This (*Channa striata*) contains albumin, Zn, Fe, Mg, Cu and unsaturated fatty acids as sources of nutrition which is beneficial to wound healing by increasing fibroblast cells. The objective of this research is to determine the effect of snakehead fish extract against the number of fibroblast in wound healing post extraction of Wistar rats. A total of 24 Wistar rats were divided into the control and treatment groups, with each further divided into three subgroups. On the 3rd, 5th and 7th day, they were histologically processed with HE staining. The statistical analysis showed significant differences between the control and treatment group. The extract of snakehead fish with a concentration of 100% increases the number of fibroblasts during tooth extraction in Wistar rats.

Key words : The snakehead fish extract, wound healing, fibroblasts cell.

INTRODUCTION

Tooth extraction is a procedure used to remove the teeth from the alveolar bone socket due to caries and periodontal diseases. Tooth loss is detrimental to patients because it reduces the efficiency of mastication, repositions the teeth, leads to temporal-mandibular joint and oral cavity problems. The results of a study conducted at Sam Ratulangi University Dental Hospital in 2012, showed 1389 cases of tooth extraction, with highest rate found in adults amounting to 837 cases (60.25%), 256 cases (18.43%) in children, 207 (14.90%) in the elderly and 89 cases (6.40%) in adolescents with a total number of 826 cases (59.48%) females and 563 cases (40.52%) male. The frequency distribution was based on pulp necrosis which had the highest extraction number of 787 cases (56.65%), followed by chronic periodontitis with a total of 180 cases (12.95%) (Rilly *et al*, 2012).

Tooth extraction is one minor surgical procedure performed by a dentist to remove the teeth from its socket. This most times leads to injury with various complications such as bleeding, swelling, dry socket and infections (Nur Permatasari *et al*, 2012).

The wound healing process is divided into several phases which are interconnected and mutually influenced prior to the formation of new tissues (Atik *et al*, 2012). Fibroblast cells are one of the cellular components and the main ingredient in the formation of collagen fibers. It disrupts the wound healing process.

There are several factors capable of accelerating the process, one of which is the presence of nutrients (Ryan *et al*, 2015). Snakehead fish is known and trusted by people as a food, which tends to accelerate this process with postoperative, childbirth and circumcision patients advised to consume it in large amount (Santoso *et al*, 2009).

Snakehead fish have been known to contain Albumin, Zinc (Zn), Copper (Cu), Iron (Fe), Magnesium (Mg) and unsaturated fatty acids, which plays an important role in the wound healing phase. Its albumin content is an animal antioxidant which tends to regulate osmotic pressure, intercellular transportation and anti-hrombosis. In addition, zinc work to improve the integrity of connective tissue and reduce cell membrane damage due to free radicals (ROS), while Fe binds oxygen in the blood and helps in the formation of collagen. Copper forms connective tissue,

bones and optimizes the work of antioxidants in the body. Unsaturated fatty acids in snakehead fish to regulate prostaglandin synthesis (Mustafa *et al*, 2012).

This study aims to determine the effect of snakehead fish (*Channa striata*) extract and the number of fibroblast cells in the wound healing process after extracting the teeth of a wistar strain rat (*Rattus norvegicus*).

MATERIALS AND METHODS

This is a laboratory experimental research with post-test control group design. The samples used were healthy male wistar strain rats (*Rattus norvegicus*) without clinical wounds, 2-3 months of age weighing from 200-300 grams. The total sample of 24 rats was divided into the treatment and control groups, with each further divided into 3 sub-groups, which were sacrificed on the 3rd, 5th and 7th day.

This study used 100% concentration of snakehead fish (*Channa striata*) extract gel with a dose of 0.1 ml for the treatment group and 0.5% CMC-Na gel for the control group.

The extract was prepared by weighing the snakehead which was weighed, cleaned and boiled at 70-80°C for 50 minutes. Furthermore, the boiled water was mixed with hexane solvent to separate the fat, with BHT 0.02% antioxidant added. It was dried with a freeze dryer for ± 24 hours and filtered to obtain a fine extract with 0.5% CMC-Na added to become a gel.

The control and treatment groups consists of 12 experimental animals, with the sub-group on the 3rd, 5th and 7th days, consisted of 4 male Wistar strain rats. In the control group, their mandibular incisors were extracted with 0.5% CMC Na gel, administered to close the wound for 3rd, 5th and 7th days. Also in the treatment group the extracted mandibular incisors and were given snakehead fish extract gel, to close the wound and was sacrificed on the 3rd, 5th and 7th days. Both groups were preceded with making tissue preparations and Hematoxyline Eosine (HE).

The tissue preparations of fibroblast cells were counted using binocular microscopy with 400 X magnification and ocular graticulae, which were placed inside the lens divided into 3 fields to avoid counting of repetitive male wistar rats. The number of fibroblast cells was read by counting the graticulae box in three fields from three pieces of tissue in each preparation, with the average values added. The data of this study were analyzed by distribution and statistical test of the independent t-test.

RESULTS

The results of the calculation of fibroblast cells were conducted by reading histology preparations on the mandibular left incisive tooth extraction socket of the wistar strain on the 3rd, 5th and 7th days. Fibroblast cell readings were performed using a 40 x magnification light microscope with the aim of determining the most extensive healing center on the preparation. It was increased by 400 x to clarify the object of observation which counted the number of fibroblast cells. Microscope images of fibroblast cells in tooth extraction sockets on the 3rd, 5th and 7th days in each treatment group is seen in Fig. 1.

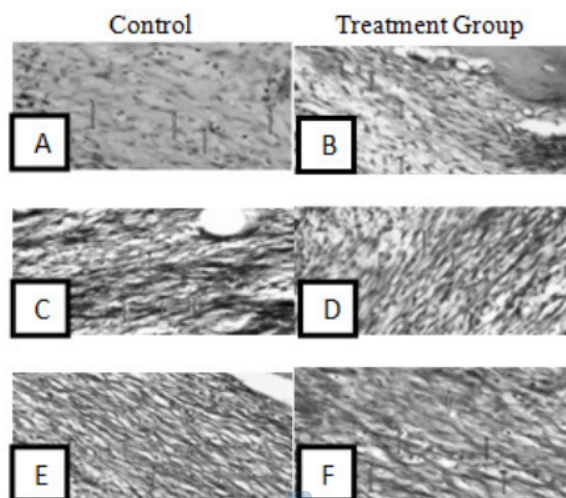


Fig. 1 : Post extraction socket, (A) control group on 3rd day, (B) treatment group on 3rd day, (C) control group on 5th day, (D) Treatment group on 5th day, (E) control group on 7th day, (F) Treatment group on 6th day Yellow arrow shows fibroblast cells on Hematoxyline Eosin staining, using Electron Microscope with 400 x magnification.

Table 1 : Descriptive statistics on each group on the 3rd, 5th and 7th days.

Descriptive Statistics			
Group	N	Mean	Std. Deviation
Control (C1)			
Treatment (T1)			
C1	5	22.74	2.30933
C2	5	45.08	2.52626
C3	5	75.02	3.59402
T1	5	36.32	1.67839
T2	5	59.06	1.23207
T3	5	100.32	7.72120

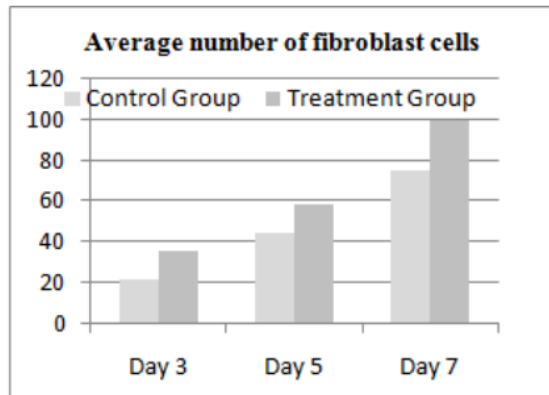


Fig. 2 : Average number of fibroblast cells on each group on the 3rd, 5th and 7th days.

The obtained research data showed differences in the treatment and control groups. On the 3rd day, the average number of fibroblast cells on the treatment group was 36.32 and 22.74 in the control. Similarly, on the 5th day, it was 59.06 in the treatment group and 45.08 in the control. It also happened on the 7th day with an average number of 100.32, for the treatment and 75.02 for the control group. This showed an increase in the number of fibroblast cells in the treatment group, which was given the extract of 100% snakehead fish gel when compared with the control group CMC-Na of 0.5% gel base. This is seen in Table 1 and Fig. 2.

Analysis of the data results were performed using the SPSS application. In the One Sample Kolmogorov Smirnov Test, the sample obtained C1 C2 C3 T1 T2 T3 using $p > 0.005$ significant, which shows that the variant in group was normally distributed. The homogeneity test using Levene's, obtained results which were above 0.05 ($p > 0.05$), indicating that the data were homogeneous. The results of tests using independent t-test obtained $P = 0.001 < 0.05$ and the average number of fibroblast cells in the control and treatment groups was significant on the 3rd, 5th and 7th days.

DISCUSSION

Teeth extraction is an invasion in the field of dentistry, which leaves wound with complex healing processes. This consists of 4 phases, namely hemostasis, inflammation, proliferation and remodeling. Some growth factors in the body, such as nutrition, greatly affect its healing time. This is attributed to the inadequate amount of protein and minerals in the blood, which plays a vital role in the formation of fibroblast cells.

Snakehead fish also contains protein, albumin and minerals such as Zinc (Zn), Copper (Cu), Iron (Fe), Mg and unsaturated fatty acids which have the ability to

accelerate the growth and formation of cells (Mustafa *et al.*, 2012).

Snakehead fish extract of 100% is the highest concentration which influences the mean of fibroblasts by 25% and 50% concentration. This shows that the higher the concentration, the higher its influence on the number of fibroblasts. The 100% snakehead fish extract has a higher impact because it contains more active substances capable of ameliorating the wound healing process (Putri *et al.*, 2015).

The extract in this study used a mixture of 0.5 % CMC-Na as base gel due to its non-toxic and high viscosity feature, thereby, making it difficult to dissolve easily in saliva (Rowe *et al.*, 2009). In this study, observations were made on the number of fibroblast cells and according to Majumdar (2005) research, it tends to increase on the 3rd day. This continues to increase prior to its peak on the 7th day. In this histo-pathological study, the numbers of fibroblast cells were observed on the 3rd, 5th and 7th days, with an increase in average in the control group.

Snakehead fish extract is used as the main therapeutic ingredient because they are easily available and affordable. It is one of the sources of animal antioxidants, known to contain albumin protein, which is 6.22% higher than other types of fishes. Albumin works by binding to Zn and acts as an antioxidant, while Zn maintains the immune system, by synthesizing proteins and maintaining the integrity of connective tissue. It also limits the membrane damage due to cleaning and the capturing process of Reactive Oxygen Species (ROS) during inflammation. ROS or free radicals are one of the causes of wound healing failure because its high number the lesion area causes inflammation which fails to heal (Mustafa *et al.*, 2012). Albumin also influences pro-inflammatory cytokines and the concentration of TNF- α , IL1, IL 6, CRP and MMP8 plasma in helping the wound healing process. This pro-inflammatory cytokine releases several growth factors, including PDGF, FGF, EGF, TGF-P, and TGF- α .

Snakehead fish is also rich in essential minerals for the body such as zinc (Zn), copper (Cu), iron (Fe) and unsaturated fatty acids (Santoso *et al.*, 2009; De Man *et al.*, 1997). Copper minerals contained in it, increases and produces fibroblast growth factor (FGF) which leads to an increase in fibroblast cells. Cu and Zn play a role in binding and optimizing the function of the enzyme superoxide dismutase (SOD) to reduce inflammation. Iron aids to deliver oxygen and in collagen synthesis during the wound healing process (Falanga *et al.*, 2003; Bernstein *et al.*, 1996; McCarley *et al.*, 1993; Williamson *et al.*, 2001).

The extract gel also contains unsaturated fatty acids, which play an important role in the regulation and synthesis of prostaglandin, a chemical mediator which arises during inflammatory activates macrophages. Therefore, the higher the level of prostaglandin, the faster the phagocytosis process and debris tissue cleansing, thereby accelerating the inflammation process (Sura *et al*, 2013).

The result of this study shows the average numbers of fibroblast on 3rd, 5th and 7th days, which are higher than the average number in the control group. Fibroblast cells in the treatment group were higher than the control which was administered with CMC Na gel without combining the snakehead fish extract. CMC Na gel only functions as a substance which provides stability without significant changes to tissue development (Khoswanto *et al*, 2010). It increases the number of fibroblast cells in the control group due to its ability to stabilize and not immune stimulant the wound healing process which continues physiologically.

Contents extract of snakehead fish increases the antioxidant activity of albumin which regulates the number of macrophages. Furthermore, minerals such as Cu, Fe, Mg and unsaturated fatty acids which play a role in reducing ROS and accelerating the inflammatory process are added to hasten the formation growth factors and healing process. The result of this study shows the number of fibroblast in the wound healing of socket post extraction process in the treatment group was higher than control group at the same day. However, further research needs to be carried out on the cytotoxic test using extract of snakehead fish and several concentrations to determine the safe support and proliferation of fibroblast cells.

CONCLUSION

The extract of snakehead fish tends to increase the number of fibroblasts in the wound caused by extracting the tooth of Wistar rat (*Rattus norvegicus*).

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