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# Efficacy of Curcumin in the healing of paracentesis in rats

Leman Birdane [a], Turhan San [b], Nuray Bayar Muluk [c,*], Dilek Burukoglu [d], Cemal Cingi [e]

[a] Yunus Emre State Hospital, ENT Department, Eskisehir, Turkey
[b] Istanbul Medeniyet University, Göztepe Training and Research Hospital, ENT Department, Istanbul, Turkey
[c] Kırıkkale University, Faculty of Medicine, ENT Department, Kırıkkale, Turkey
[d] Eskisehir Osmangazi University, Faculty of Medicine, Histology Department, Eskisehir, Turkey
[e] Eskisehir Osmangazi University, Faculty of Medicine, ENT Department, Eskisehir, Turkey

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ABSTRACT

*Objectives:* The present study was designed to investigate the possible beneficial effect of Curcumin (CMN) in healing of paracentesis in terms of wound thickness, sclerosis and closure by histological evaluation. To evaluate the efficacy of CMN, paracentesis was performed experimentally in the rats; and the results were presented histologically.

*Methods:* Sixteen, each 270–310 g weighted, healthy Sprague-Dawley female rats were included into the study. In both groups, paracentesis was performed into the eardrum bilaterally. In Group 1 (Paracentesis + Saline Group), saline drop was applied; and in Group 2 (Paracentesis + Curcumin group), Curcumin drop treatment was applied. Paracentesis area did not healed bilaterally in two rats (one in Group 1 and one in Group 2). Therefore, these two rats were excluded from the study. Histological examination performed in 14 rats and 28 temporal bones on the 15th day after the completion of drop treatment and closure of the paracentesis-area and wound healing were evaluated according to the histological examination criteria: Thickening of the tympanic membrane (ThicTM); and sclerosis.

*Results:* Both tympanic membrane thickening and sclerosis values of Paracentesis + Curcumin Group (Group 2) were significantly lower than those of the Paracentesis + Saline Group's (median: 2.0) ($p = 0.001$). Histological examination by light microscopy showed that in Paracentesis + Curcumin Group (Group 2), the structure of the tympanic membrane is near to the normal and decreased sclerosis was observed in connective tissue. Whereas in Paracentesis + Saline Group (Group 1), tympanic membrane thickening and connective tissue sclerosis were observed.

*Conclusions:* Curcumin improves wound healing process in paracentesis of TM. By using Curcumin drops, the closured paracentesis area was observed near to the normal eardrum; and thickness of the TM and sclerosis were less than the control, showing the improved healing at 15th day. The possible mechanisms may be anti-inflammatory effect, improving collagen deposition, and increasing fibroblast and vascular density in wounds thereby enhancing impaired wound healing.



## 1. Introduction

Curcumin (diferuloylmethane), a major active component of the dietary spice turmeric, has been used in Indian and Chinese medicine for treatment of various disease conditions. Curcumin is a polyphenol and possesses anti-inflammatory, antioxidant, antiproliferative, and wound healing properties [1,2]. The disease portfolio for this traditional medicine includes pain disorders, digestive diseases, menstrual difficulties, skin conditions, sprains, wounds, and liver disorders [3]. Curcumin has the potential to preserve food through its antioxidant property [4]. Laboratory studies have also confirmed that Curcumin possess antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and anti-cancer activities [5–7].

*In vivo*, wound healing is considerably more complex than *in vitro*, involving three (partially overlapping) phases: inflammation, tissue formation and remodeling [8]. Specific cell types migrate into the wound and then interact with other cells and the environment. During the inflammatory phase, neutrophils and macrophages migrate into the wound, removing foreign particles and bacteria and releasing cytokines which promote fibroblast migration and proliferation.

Another important aspect in wound healing is neovascularization which re-establishes perfusion to sustain the new tissues. It is

* Corresponding author at: Birlik Mahallesi, Zirvekent 2. Etap Sitesi, C-3 blok, No: 62/43, 06610 Çankaya/Ankara, Turkey. Tel.: +90 312 4964073/532 7182441; fax: +90 312 4964073.
*E-mail addresses:* nbayarmuluk@yahoo.com, nurayb@hotmail.com (N.B. Muluk).

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thus important to emphasize that fibroblast migration *in vivo* could depend on factors that cannot be included in an *in vitro* assay. Nevertheless, the strength of *in vitro* studies is in isolating effects of the tested NMs on direct biomechanical measures of the migration kinematics, since *e.g.* these substances could potentially interact with cytoskeletal activation proteins that control cell spreading, contraction or directionality, such as Rac, Rho and Cdc42 [6,9,10].

Curcumin has a beneficial effect on nuclear factor-kappa B (NF-κB). Curcumin inhibits NF-κB, subsequently reducing inflammation and thereby providing benefits for various skin diseases. Recent studies as reviewed here are unraveling previously unidentified pathways, unique molecular targets and novel mechanisms through which Curcumin may perform as a skin protectant [2,11].

The present study was designed to investigate the possible beneficial effect of CMN in the healing of paracentesis. To evaluate the efficacy of CMN, paracentesis was performed experimentally in the rats; and the results were presented histologically.

## 2. Materials and method

The study was conducted in University Faculty of Medicine of Eskişehir Osmangazi University. Adaptation and care of the animals and experimental study were performed at TICAM (Experimental Studies Center of Eskişehir Osmangazi University). During both adaptation and experiment periods, the animals were treated in compliance with the principles of the Declaration of Helsinki [12].

### 2.1. Animal subjects

Sixteen, each 270–310 g weighted, healthy Sprague-Dawley female rats were used in the present study but two were later excluded due to the fact that the paracentesis area were not healed at the time of the examination (day 15). The experimental protocol was reviewed and approved by Ethique Committee of Osmangazi University, Medical and Surgical Experimental Research Center (date: February 23, 2012 and number: 265). All the animal procedures were performed in accordance with the approved protocol.

Female rats were housed under the same conditions in temperature and humidity controlled room (20 ± 1 °C, 50 ± 10% relative humidity) and 14–16 h light/dark cycle conditions. Tap water and standard pelletized food are provided *ad libitum*.

### 2.2. Experimental design

Sixteen Sprague-Dawley female rats were randomizely divided into two groups:

1. Group 1 (Paracentesis + Saline group): The rat group, in which paracentesis was performed bilaterally; and after then saline drop treatment was applied ($n = 8$)
2. Group 2 (Paracentesis + Curcumin group): The rat group, in which paracentesis was performed bilaterally; and after then Curcumin drop treatment was applied ($n = 8$).

### 2.3. Method

1. *Operation*: During the experimental process, rats were sedated by 10 mg/kg xylazine and 30 mg/kg intraperitoneal injection of ketamine were used. In all rats, in both ears of each rats, paracentesis was performed (2 mm in length).
2. *Treatment modalities in Groups*:
   - Group 1: After paracentesis procedure, saline drop (0.9% NaCl, 3 drops) was given to these rats onto the gelfoam twice a day on the 1st day; and only saline drop was given twice a day on the 2nd and 3rd days.
   - Group 2: After paracentesis procedure, Curcumin drop (3 drops) was given to these rats onto the gelfoam twice a day on the 1st day; and only Curcumin drop was given twice a day on the 2nd and 3rd days (11 mg of powdered Curcumin was mixed with 1 ml DMSO (dimethylsulfoxide); and solution was prepared. Curcumin Extract (Sigma catalog number: C1386) was used).
3. Paracentesis area did not healed bilaterally in one rat of Group 1; and in one rat of Group 2. Therefore, these two rats were excluded from the study. At the end, 7 rats (14 temporal bones) in Group 1 and 7 rats (14 temporal bones) in Group 2 were taken to the next step of the study: Histological Examination Procedure described below.

### 2.4. Histological examination procedure

Fifteen days after the completion of saline or Curcumin drop treatments, all rats were sacrificed by giving 80 mg/kg pentothal as anesthetic agent (Fig. 1). Immediately after death, the temporal bones were removed, the otic bullae opened and placed in fixative (10% formalin). The temporal bones were decalcified in 5% formic acid [13]. They were deparaffinized and dehydrated by immersion into xylene twice for 10 min. Following dehydration in ascending series of ethanol (70, 80, 96, 100%), tissue samples were cleared in xylene and embedded in paraffin. Tissue sections of 5 μm were stained with hematoxylin–eosin (H–E). A minimum of 3–4 fields for each samples were examined and assigned for severity of changes by an observer blinded to the treatments of the animals [14]. Slides were examined by light microscopy with Entella Olympus BH-2 microscope; and photos were taken with Olympus DP-70 digital camera.

During histological examination, Thickening of the tympanic membrane (ThicTM); and Sclerosis (Sc) were evaluated by light microscopy. Severity of changes was assigned using scores of none (−), mild (+), Moderate (++) and Severe (+++) [14].

### 2.5. Statistical analysis

Statistical packet for SPSS (Version 16.0) was used for statistical evaluation. Mann–Whitney *U* was used to analyze the difference between Groups 1 and 2.

$p$-value $<0.05$ was considered as statistically significant.

## 3. Results

The histological examination results of the tympanic membrane (TM) in Groups 1 and 2 were demonstrated on Table 1. For

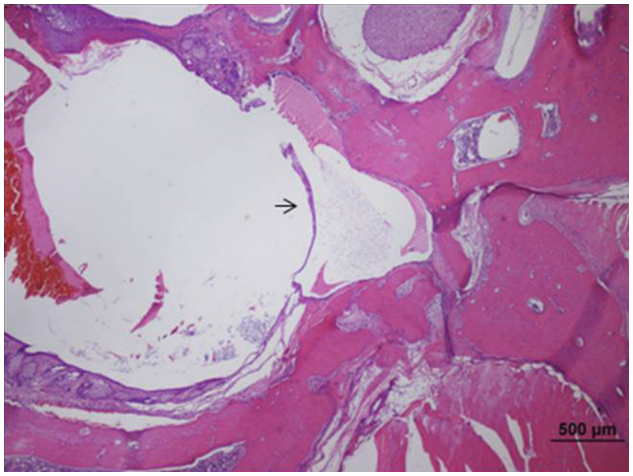


**Fig. 1.** In Paracentesis + Saline Group, tympanic membrane thickening (→) and connective tissue sclerosis are seen (H & E, scale bar: 500 μm).