

# The effect of *Curcuma longa* extract on fibroblast count and collagen density in intestinal anastomosis: an experimental study on New Zealand rabbits



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## ABSTRACT

**Background:** Anastomotic leakage was the most feared complication after intestinal surgery because it could bring morbidity and mortality. *Curcuma longa* (turmeric) has been known for its effect on wound healing by increasing fibroblast count and collagen density. Nevertheless, its effect on intestine anastomosis has not yet been proven. This study was an experimental study on New Zealand rabbits to analyze the effect of *Curcuma longa* extract on fibroblast count and collagen density in intestinal anastomosis.

**Methods:** This study was a randomized control trial experimental study on a New Zealand white rabbit. The rabbits were divided into 2 groups randomly, the control group and the treatment group, which were given *Curcuma longa* extract for 4 days after laparotomy intestinal resection and anastomosis. Fibroblast count and collagen density of the anastomotic area were evaluated on the 5<sup>th</sup> day after the surgery.

**Results:** 36 New Zealand white rabbits underwent laparotomy intestinal resection and anastomosis, then randomly divided into 2 groups. On the 5<sup>th</sup> day, the subjects were evaluated. The analysis results showed that the control group had a lower mean count of fibroblasts (9.89) than the treatment group (27.11), with the Mann-Whitney analysis showing significant differences ( $p$ -value 0.000). The lowest score for collagen density in the control group was +2, with the highest being +3. While on the treatment group, the lowest score was +3, and the highest was +4. The mean value was lower in the control group (10.61) rather than in the treatment group (26.39), with the Mann-Whitney analysis showing significant differences ( $p$ -value 0.000).

**Conclusion:** *Curcuma longa* extract had an effect on intestinal anastomosis by increasing fibroblast count and collagen density.

**Keywords:** *Curcuma longa*, collagen, fibroblast, intestinal anastomosis.

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## INTRODUCTION

Anastomotic leakage was one of the most feared complications after intestinal surgery because it could bring morbidity and mortality.<sup>1-3</sup> There was a lot of research to evaluate factors that had a correlation with anastomotic healing for a better understanding of the cause of the leakage to prevent it.<sup>1</sup>

Factors that influence anastomotic healing can be technical or factors that correlate to patients' conditions, including nutritional status and medication that had been given. *Curcuma longa* has been known for centuries in South East Asia, India, and China as traditional medicine. A lot of experimental studies have been done

to prove the healing effect of *Curcuma longa* in various types of wounds, such as in skin, burnt, and nasal mucosal.<sup>2-6</sup>

The aim of this research was to study the mechanism of the healing effect of *Curcuma longa* in intestinal anastomosis by experimenting on New Zealand white rabbits.

## METHODS

This is a randomized trial experimental study using New Zealand rabbits by dividing the samples into two groups randomly after laparotomy intestinal resection and anastomosis had been done. The small intestine was resected and sutured with a simple interrupted

absorbable multifilament thread.

The treatment group was given *Curcuma longa* extract orally at 40 mg/kg body weight once daily for 4 days, while the treatment group was given nothing. After 5 days, all the samples underwent laparotomy again to pick the specimens of the anastomosed small intestine. We examine each specimen for fibroblast count and collagen density.

The specimens for counting the fibroblast were stained with *hematoxylin-eosin* under a light microscope with 400x magnification, and the fibroblasts were counted for each field of view. The stained procedure for the collagen density study used *Masson's Trichrome* with 100x magnification, and the collagen density

was measured based on the score invented by Novriansyah. Data from all specimens were then analyzed using SPSS 23.<sup>7</sup>

## RESULTS

The characteristics of the samples are shown in Table 1, and the statistic test for homogeneity is shown in Table 2, in which the result for Levene statistic > 0.05 means that all the samples were homogeneous. The Kolmogorov-Smirnov and Shapiro-Wilk normality test for fibroblast count and collagen density were shown in Table 3, with p-value < 0.05.

The fibroblast count and statistical analysis for each group are shown in Table 4. The lowest count in the control group was 2, while the lowest in the treatment group was 6. The highest count was 14, and it was in the treatment group. The Mann-Whitney analysis showed significant differences between the two groups (p-value 0.000).

The collagen density score was decided based on the Novriansyah score. The lowest score in the control group was +2 (Figure 1), while the lowest in the treatment group was +3. The highest score (+4) was found only in the treatment group. The Mann-Whitney analysis showed significant differences between the two groups (p-value 0.000). The details are shown in Table 5.<sup>7</sup>

## DISCUSSION

*Curcuma longa* (turmeric), chemically known as 1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione. It contained approximately 60-70% curcumin, 20-27% demethoxycurcumin, and 10-15% bisdemethoxycurcumin.<sup>8,9</sup> Turmeric extracts were taken from their rhizome, then chopped into small pieces, fried in the shade, then ground into powder form. The powder form was then extracted in methanol and followed the stages of the process to get its derivatives.<sup>8,9</sup>

Curcumin works in speeding up epithelial regeneration, controlling the inflammation process, and increasing fibroblast and vascular density. In the inflammation process, curcumin works in controlling pro-inflammation factors such as *Tumor Necrosis Factor Alpha* (TNF- $\alpha$ ). Curcumin also inhibits *Nuclear Factor  $\kappa$ B*

**Table 1. Samples characteristics distribution.**

Characteristic	Count (n)	Mean	Median	Minimum	Maximum
Age (month)	36	8.1	8.5	6	9
Weight (gram)	36	2235	2220	2025	2500

**Table 2. Homogeneity test.**

	p-value
Age (months)	0.131
Weight (gram)	0.648
Fibroblast	0.253
Collagen	0.015

**Table 3. Normality test.**

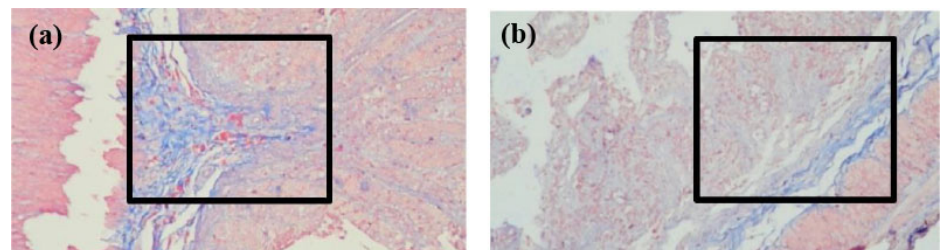
Group	Kolmogorov-Smirnov	Shapiro-Wilk
Fibroblast (per field of view)	0.000	0.001
Collagen (density score)	0.004	0.047
Control	0.000	0.000
Treatment	0.000	0.000

**Table 4. Fibroblast count.**

Fibroblast count (Per field of view)	Control group (n=18)	Treatment Group (n=18)	p-value
Lowest	2	6	
Highest	8	14	
Mean	9.89	27.11	0.000
Total Mean	178	488	

**Table 5. Collagen Density Score.**

Collagen Score	Control Group (n=18)	Curcuma Group (n=18)	p-value
Lowest	++	+++	
Highest	+++	++++	
Mean	10.61	26.39	0.000
Total Mean	191	475	



**Figure 1. Collagen Density Comparison. (a) Collagen in the control group and (b) Collagen in the treatment group (400x magnifying, Masson's Trichrome stain).**

(NF- $\kappa$ B) by suppressing kinase activity to control the inflammation process at the wound site.<sup>3,10,11</sup>

The previous study also showed that curcumin mediates fibroblast migration into the wound site to inducted granulation process. In the proliferation phase, curcumin works by increasing hydroxyproline and collagen synthesis. The higher the hydroxyproline concentrate,

the faster the cellular proliferation rate and collagen synthesis.<sup>12,13</sup>

This research was done to study the effect of *Curcuma longa* on intestinal anastomosis, particularly in the small intestine. The *Curcuma longa* extract was given to the treatment group orally after laparotomy intestinal resection and anastomosis with a dosage of 40 mg/kg body weight once daily for 4 days,

while the treatment group was given no *Curcuma longa* extract. We study the effect on fibroblast count and collagen density. A previous study had shown the effect of *Curcuma longa* on various types of wounds, such as in skin, burnt, and nasal.<sup>4-6</sup>

The specimens were stained in *hematoxylin-eosin* to look for fibroblast, then counted per field of view. The fibroblast count was higher in the treatment group rather than in the control group. The Mann-Whitney analysis showed a significant difference between the two groups which means that *Curcuma longa* extract had an effect on increasing fibroblast count.

The specimens were also stained in *Masson's Trichrome* with a 100x magnification light microscope to determine the collagen density score. This score is described by Novriansyah based on the density percentage of collagen fiber. 0 means no collagen was found. 1 means 10-25% of collagen density. 2 means 25-50% density. 3 means 50-75% collagen density. 4 means 75-100% collagen density.<sup>7</sup>

Higher collagen density is found in the treatment group, with the lowest density found in the control group. The Mann-Whitney analysis showed a significant difference between the group which means that *Curcuma longa* extract had the effect of increasing collagen density.

## CONCLUSION

Curcuma extract had an effect on intestinal anastomosis by increasing fibroblast count and collagen density.

## ETHICAL CONSIDERATIONS

This study was approved by the Animal Care and Use Committee (ACUC)

of Veterinary Faculty Universitas Airlangga with Ethical Clearance number 2.KEH.063.05.2022.

## CONFLICT OF INTEREST

No conflict of interest.

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## AUTHOR CONTRIBUTION

All authors have contributed equally from the conceptual framework, data acquisition, and data analysis until the study results are reported through publication.

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