



IJCRR

Section: Healthcare

Sci. Journal Impact

Factor: 6.1 (2018)

ICV: 90.90 (2018)



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Effectiveness of Turmeric (*Curcuma longa* Linn) Extract Gel (EG) on Wound Healing in Female Rats (*Rattus Novergicus*)

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ABSTRACT

Objective: The purpose of this study was to identify differences in wound healing and wound healing time at each concentration of turmeric extract gel (*Curcuma longa* Linn) and base gel on days 3, 7 and 14, and identify the most effective turmeric extract gel for wound healing in cuts female rats (*Rattus Novergicus*).

Method: This study was an experimental laboratory using a post-test only with a control group. This study used 16 female rats, 3 treatment groups, and 1 control group (each group consisted of 4 mice). Female rats were injured in the back section by slashing along 1.5 cm with a depth of 0.2 cm. The treatment group was given turmeric extract gel with a concentration of 5%, 10%, 15%, and the control group was only given a gel base. This research was conducted for 14 days of topical turmeric extract gel by giving 2 times a day. The normality test uses the *Shapiro Wilk test*, then the *Kruskal Wallis test*, and continued with the *Post-Hoc Mann-Whitney*.

Results: The mean on the 3rd day of the EG group was 5% (0.95cm), the EG group was 10% (0.98 cm), the group was 15% (1.23 cm), and the control group (1.25 cm). On the 7th day EG 5% (0.58 cm), EG 10% (0.65 cm), EG 15% (0.73 cm) and the control group (1 cm). On day 14 showed wound healing in the treatment group, but the control group showed mean wound length on day 14 (0.15 cm). Test results on day 3 obtained $p = 0.007$, on day 7 obtained $p = 0.010$ and on day 14 obtained p -value = 0.017. P values on days 3, 7, and 14 showed significant differences with $p < 0.05$.

Conclusion: There are differences in wound healing and wound healing time at each concentration of turmeric extract gel. Turmeric extract gel concentration is the most effective healing wound cuts in female rats, namely the treatment group given EG 5%.

Key Words: Turmeric extract, Gel, Female rat, Wound

INTRODUCTION

Women delivery may experience problems when labour takes place including rupture of the birth canal or perineum. More than 85% of vaginal deliveries will experience perineal trauma and nearly 69% require.¹ The impact of perineal rupture on the mother such as infection of the bladder or on the birth canal. Bleeding can also occur due to the opening of blood vessels that do not close completely. Also, an increased risk of postpartum urinary retention, dyspareunia, and death can occur if treatment is slow.²⁻⁴

The World Health Organization (WHO) estimates that around three-quarters of the population of developing countries depend on traditional medicines (medicinal plants), for

their primary health care needs.³ In Indonesia, the use of plants around the house has happened since a long time ago, but only a little has been proven scientifically, so it needs to be proven scientifically so that the utilization of these plants is increasingly apparent. The main part of turmeric is the rhizome which contains two secondary metabolites of phenolic curcuminoids and essential oils. The main curcuminoids found in turmeric are curcumin, demetoxy curcumin, and bisdemetoxycurcumin.⁴ Research on the initial phytochemical content of the ethanol extract of *Curcuma longa* Linn⁷ and other studies showing the presence of curcumin, essential oils, tannins, alkaloids, saponins, flavonoids, terpenoids which have important pharmacological activities such as anti-mutagenic, anti-inflammatory, antibacterial, antipro-

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ISSN: 2231-2196 (Print)

ISSN: 0975-5241 (Online)

Received: 26.05.2020

Revised: 15.07.2020

Accepted: 25.08.2020

Published: 14.12.2020

tozoal, and properties antioxidant, and as an antiseptic, anticarcinogenic, anticoagulant, antifertility, antidiabetic, antifungal, antiviral, antifibrosis, antivenom, antiulcer, antiulcer, hypotensive, and hypocholesterolemia.⁵

Research using turmeric with topical administration in thirty rats for seven days there was no significant difference between the application of 5% curcumin and 0.9% Sodium Chloride in wound healing.⁶ Research that also discusses the preparation of turmeric in the form of a 10% turmeric extract ointment can accelerate the healing of female rats given for fourteen days.⁷ Another study that discussed *Curcuma longa* water extract which was applied topically provided an anti-inflammatory effect by suppressing endotoxin-induced uveitis in mice to reduce TNF- α activity.⁸

Based on this, it is necessary to do basic research on the effectiveness of turmeric gel extract (*Curcuma longa* Linn) in female rats (*Rattus Novergicus*) before it is applied to post-partum perineal wounds in humans.

MATERIALS AND METHODS

Research sites

The study was conducted in February 2020 to March 2020 and has received an ethical approval recommendation with the protocol number UH19121102. The study was conducted in four different places according to the stage of the study conducted. At the stage of turmeric processing and extraction carried out in the Phytochemical Laboratory of the Faculty of Pharmacy Hasanuddin University, and gel making at the Pharmacy Laboratory of the Faculty of Pharmacy Hasanuddin University, the animal testing stage at the Animal Laboratory of the Faculty of Medicine Hasanuddin University.

Materials

Plastic boxes (with husks) and wire for rats cages, surgical equipment (anatomical scissors for surgery, scalpels, anatomical tweezers, scissors), plastics, analytical scales, filter paper, glass funnel, mortar, 100 ml beaker glass, dropper, rotary evaporator, 75 gr plastic tube, cotton buds, gloves, tissue container, ruler, label, camera. The materials used in this study were, pet food (pallet), drinking water, Veet® cream, 70% ethanol, turmeric taken from Majene West Sulawesi Province, NaCMC, glycerin, propylene glycol, DMDM hydantoin, alcohol, aqua dest, ether, 70% alcohol, sterile gauze, sterile cotton.

Research Design

This research was a laboratory experimental method with experimental animals using a post-test with the control group.

Population and Sample

The population studied was female rats (*Rattus Novergicus*) Wistar strain. The number of samples used was sixteen rats

consisting of four each group taken randomly. The inclusion criteria were female mice (*Rattus Novergicus*), aged 2-2.5 months, bodyweight 150-300 grams, healthy body condition (active and not disabled) with exclusion criteria, namely sick mice, body weight decreased to less than 150 grams, and died during the study.

Procedure for the Making turmeric extract the gel

Simplicia: Making simplicia from turmeric was done by collecting turmeric. Turmeric rhizome as much as 9 kilograms, then cleaned from attached by using flowing water then the sample is cut into small pieces, then dried by aerating it until it contains water content below 10%.⁹

Simplicia Powder: Turmeric is ground until smooth and then sieved with a mesh size of 60 so that a smooth Simplicia sample of 500 gr is obtained, after which the sample is ready for extraction of the maceration method.

Yellow Turmeric Rhizome Extract (*Curcuma longa* Linn): Turmeric powder (*Curcuma longa* Linn) is extracted by the maceration method (ie using 70% ethanol solvent). In the maceration process, the turmeric is soaked with 70% ethanol at room temperature for 3 x 24 hours, every 1 x 24 hours stirring. After 3 x 24 hours soaked, then filtered using filter paper, while macerated again. The extract obtained is then evaporated using a rotary evaporator until it thickens, the resulting viscous extract is put into a porcelain cup then dried with the help of a water bath ¹⁰.

Making turmeric extract the gel: The extracted gel is made in 3 gel preparations, namely extracts with a concentration of 5; 10; and 15% (Table 1).

Table 1: Formulation of ingredients in the manufacture of turmeric extract gel

Material	Formula Composition (% b/v)			
	F I (Gel Base)	F II (EG 5%)	F III (EG 10%)	F IV (EG 15%)
Turmeric Extract	-	5	10	15
Alkohol	5	5	5	5
PG	5	5	5	5
Na. CMC	5	5	5	5
Gliserin	5	5	5	5
DMDM Hydan- toin	0.1	0.1	0.1	0.1
Aquadest	100	100	100	100

PG: Propylene Glycol; Na. CMC: Natrium Carboxy Methyl Cellulose; DMDM: dimethylimidazolidine.

The process of making a gel that is aquadest is heated to a temperature of 70 ° C, then put a gel-forming (Na. CMC) into the mortar and added the aquadest that has been heated then crushed using a mortar until it develops to form a gel,

add 5% glycerin and 5% propylene glycol, add DMDM Hydantoin 01% as a preservative, for turmeric extract a concentration of 5% is added to the gel that has been made then added to the alcohol everything is stirred until homogeneous, as well as for concentrations of 10% and 15%.

Animal Treatment

Sixteen (n=16) rats were selected and weighed before treatment. Mice are adapted first by giving (pallet) and given drinking libitum, put in a cage with the same conditions for 1 week. Experimental animals were divided into 4 groups, each group consisting of 4 mice. The part of the back that will be wound, cleaned its bristles or applied a hair loss thresher (Veet® cream) on the part that will be made a wound. Do hand washing then use clean gloves, then coat the moustache on the rat to be hurt. Furthermore, rats were eliminated using ether by inhalation. The rat was injured on its back using a sterile scalpel 1.5 cm long and 0.2 cm deep.¹¹

The control group, rats were only smeared with a gel base, and in three treatment groups, the wound was smeared with turmeric extract gel (*Curcuma longa* Linn) 2 times a day (7:00 am and 17:00) for 14 days. The application of the gel is done by using a sterile cotton bud to the wound.¹²

RESULTS

Effect of gel on wound healing

Data from this study were obtained from the results of the observation of wounds and determining the improvement of the wound healing process. One of them is done by measuring the length of the wound on days 3, 7, and 14 (Table 2). Table 2 shows the average wound length on day 0 of the treatment length of the wound length 1.5 cm, on the 3rd day there was a rapid reduction in wound length in the EG group of 5% (0.95 cm), then the EG group 10% (0.98 cm), and in the EG group 15% (1.23 cm), and in the control group (1.25 cm). On the 7th day the mean wound length was seen at EG 5% (0.58 cm), EG 10% (0.65 cm), then EG 15% (0.73 cm), and lowest in the control group (1 cm). At the end of treatment on day 14 showed wound healing in all treatment groups of turmeric extract gel, but different from the control group which showed mean wound length on day 14 (0.15 cm) (Figure 1).

Table 2: Average length of incision wounds by treatment group

Treatment group	Incision Length (cm)			
	Day 0 averages	Day 3 averages	Day 7 averages	Day 14 averages
Control	1.5	1.25	1	0.15
Extract Gel 5%	1.5	0.95	0.58	0
Extract Gel 10%	1.5	0.98	0.65	0
Extract Gel 15%	1.5	1.23	0.73	0

Description: Extract Gel: Turmeric Extract

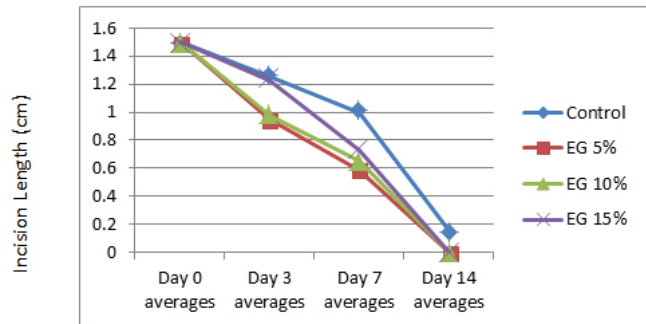


Figure 1: Graph of mean length of cut wounds of rats on days 3, 7 and 14.

Table 3 shows the results of the Kruskal Wallis test statistic on day 3, the value of P = 0.007 was obtained, on the 7th day P-value = 0.01 and on the 14th day P-value = 0.17 was obtained. Because the P-value < 0.05 there is a difference in wound length on the 3rd day, and the 7th, on the 14th day the value of P > 0.05, which means there is no difference in the length of the wound due to wound healing.

Table 3: Number of subjects per group, Median, minimum, and maximum of each group after giving turmeric extract gel, and P-value from Kruskal Wallis statistical results

Treatment group	N	Incision Length (cm)		
		3rd day	7th day	14th day
Control	4	1.25 (1.2-1.3)	1 (0.8-1.2)	0.15 (0-0.3)
EG 5%	4	0.95 (0.9-1)	0.6 (0.5-0.6)	0
EG 10%	4	0.95 (0.9-1.1)	0.65 (0.6-0.9)	0
EG 15%	4	1.2 (1.2-1.3)	0.75 (0.6-0.9)	0
P Value		0.007	0.010	0.017

EG: Turmeric Extract

Table 4: Mann-Whitney Post Hoc Results

Treatment group	Significance Test Results					
	3rd day	Sign	7th day	Sign	14th day	Sign
1 vs 2	0.018	S	0.017	S	0.047	S
1 vs 3	0.019	S	0.019	S	0.047	S
1 vs 4	0.495	NS	0.037	S	0.047	S
2 vs 3	0.752	NS	0.096	NS	1.000	NS
2 vs 4	0.017	S	0.044	S	1.000	NS
3 vs 4	0.017	S	0.222	NS	1.000	NS

S: significant, significantly different group (P < 0.05); NS: Non-Significant, groups that were not significantly different (P > 0.05); Group 1/control: gel base, 2: EG 5%, 3: EG 10%, 4: EG 15%

Table 4 Mann Whitney Post Hoc Test Day 3 showed significant differences between treatment groups where group 1/ control (gel base) was significantly different from group 2 (EG 5%), and group 3 (EG 10%), but not significantly dif-

ferent from the group 4 (EG 15%), group 2 with group 3 were not significantly different, while group 2 with group 4 appeared to be significantly different, and group 3 appeared significantly different from group 4. On day 7 showed group 1 was significantly different from group 2, 3, and 4, group 2 showed no significant difference from group 3, but group 2 was significantly different from group 4 and group 3 and 4 showed no significant difference. On day 14 it appears that group 1 is significantly different from all groups 2, 3, and 4, group 2 shows no difference with groups 3 and 4, group 3 with 4 show no significantly different results (Table 5).

Table 5: Wound Healing Time after turmeric extract gel

Treatment group	Time of wound healing
Control	> 14 days
EG 5%	≤ 14 days
EG 10%	≤ 14 days
EG 15%	≤ 14 days

EG: Turmeric Extract

Based on table 5 it can be seen that the wound healing time of all treatment groups containing turmeric extract when viewed at the end of the treatment ie on the 14th day experienced wound healing faster than the control group which only contained a gel base (Figure 2).

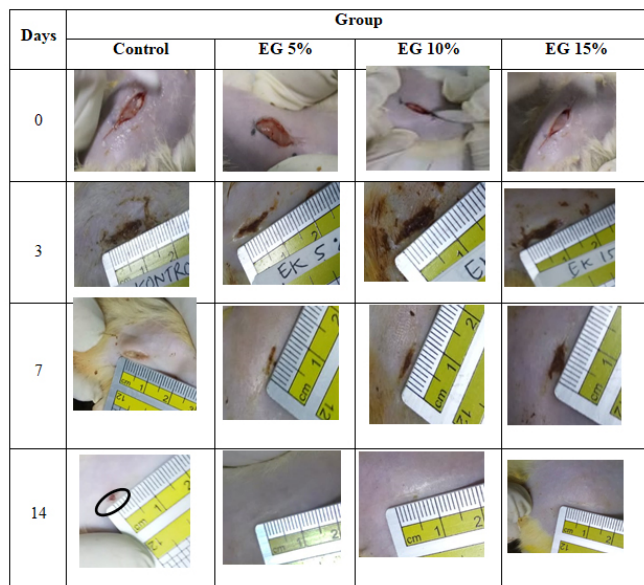


Figure 2: Wound healing activity.

DISCUSSION

The results of the measurement of wound length can be seen in Table 2 which shows that on the 3rd day there was a rapid shrinkage of the wound length in the EG group 5% (0.95

cm), then the EG group 10% (0.98 cm), the group 15% (1.23 cm) and the lowest is in the control group (1.25 cm). On the 7th day the mean wound length was seen at EG 5% (0.58 cm), EG 10% (0.65 cm), then EG 15% (0.73 cm), and lowest in the control group (1 cm). At the end of the treatment on the 14th day showed wound healing in all treatment groups containing turmeric (*Curcuma longa* Linn), but different from the control group which showed the average wound length on the 14th day (0.15 cm). This shows the shrinkage of the wound length is influenced by turmeric extract in the wound healing phase which consists of the inflammatory phase on the 3rd day, on the 4th day to the 14th day is the proliferation phase and the maturation phase lasts up to 1 year or more.¹²⁻¹⁴

The results of this study found that turmeric extract gel with the smallest concentration of 5% was effective against wound healing in mice. which showed similar results, where the concentration of turmeric rhizome ethanol extract gel which effectively healed the back skin of rabbits infected with *Staphylococcus aureus* ATCC 25923 was 5%. This is in line with research conducted by Oktasiana, 2018 turmeric rhizome ethanol extract gel which effectively healed the back skin of rabbits infected with *Staphylococcus aureus* ATCC 25923 was 5%.^{10,11}

Low concentrations are better than high concentrations because curcumin with high concentrations can increase the production of ROS (Reactive Oxygen Species) which produces oxidative stress so that it can inhibit the wound repair phase.¹⁴ ROS is also involved in wound healing, ROS acts as a signalling molecule and modulates cell growth, apoptosis, and differentiation. In low concentrations, ROS helps the signalling function. However, the presence of prolonged ROS at high concentrations produces oxidative stress, which can result in DNA damage. Oxidative stress is a factor that affects the wound healing process and generally inhibits tissue remodeling.¹⁵

The curcumin content of turmeric is believed to play an important role in the wound healing process. Curcumin can modulate physiological and molecular cells involved in inflammatory processes such as lymphocyte cells, monocytes, mast cells, neutrophils, and macrophages. Curcumin has antioxidant and anti-inflammatory properties to reduce the expression of inflammatory cytokines, shorten the inflammatory phase, and promote collagen synthesis, fibroblast migration, and differentiation^{16,17}. Accelerating the wound healing process in this case turmeric extract gel inhibits the expression of proinflammatory cytokines such as interleukin (IL) 1, 2, 6, 8, and 12, tumour necrosis factor-alpha (TNF-α), suppress NF-kappaB and COX-2 activation, suppress Reactive Oxygen Science (ROS), monocyte chemoattractant protein (MCP). In the proliferation phase, curcumin helps collagen synthesis, fibroblast differentiation. This fibroblast will accelerate the synthesis of new skin tissue to accelerate

wound closure.^{16,18} In the maturation or remodelling phase, curcumin increases type I collagen levels with the help of matrix metalloproteinase (MMP).¹⁹

Phenol compounds can denature bacterial cell proteins and damage bacterial cell membranes beyond repair. Saponins can increase pro-collagen synthesis, whereas tannins and flavonoids have antiseptic and antibacterial activity. Bioactive phytochemical compounds such as saponins, tannins, flavonoids can modulate one or more phases of the wound healing process. Besides, it is easily absorbed by superficial skin layers.²⁰⁻²²

CONCLUSION

In this study, it can be concluded that turmeric extract gel formula has effectiveness in healing cuts in female rats. The wound-healing effect when seen on the 14th day showed the same wound healing in all treatment groups with turmeric extract gel. The optimum wound healing effect of the wound when viewed from days 3, 7, and 14 is given by gel preparations with turmeric extract content of 5%.

ACKNOWLEDGMENT

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references to this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals, and books from which the literature for this article has been reviewed and discussed.

Conflict of Interest: Nil

Source of Funding: Nil

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