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ORIGINALARTICLE

Investigation of the Wound Healing Potential of *Kaempferia rotunda* (Ginger) Extract

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ABSTRACT
Wound healing is a complex biological process that plays a crucial role in the restoration of tissue integrity and function. Natural products have gained significant attention in recent years for their potential therapeutic effects in promoting wound healing. This study aims to investigate the wound healing potential of Kaempferia rotunda extract, commonly known as Ginger, using both in vitro and in vivo experimental approaches. In the in vitro study, experimental animals dermal fibroblast cells were subjected to various concentrations of Kaempferia rotunda extract to evaluate its impact on cell viability, proliferation, and migration. The results revealed that the extract significantly enhanced fibroblast viability and proliferation in a dose-dependent manner. This suggests that the extract may play a crucial role in stimulating cellular activities that are essential for wound healing. To validate the in vitro findings in an in vivo model, full-thickness excisional wounds were created on the dorsal area of experimental animals. These wounds were topically treated with either Kaempferia rotunda extract or a standard wound healing ointment. The animals were closely monitored for wound closure rate, histological analysis, and the expression of key biomarkers associated with wound healing. The in vivo study results demonstrated that the topical application of Kaempferia rotunda extract significantly accelerated wound closure compared to the control group. Histological analysis of the treated wounds revealed enhanced re-epithelialization, increased collagen deposition, and reduced inflammation. These findings indicate the extract's potential to promote tissue regeneration and provide a favorable wound healing microenvironment. Furthermore, the expression of key wound healing markers, including transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF), was upregulated in the treated wounds. This suggests that Kaempferia rotunda extract can modulate critical signaling pathways involved in wound healing.
Keywords: *Wound, Healing, Rats, Kaempferia, Rotunda*

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INTRODUCTION

Kaempferia galanga L., commonly known as Chandramulika, Karchoor, sugandhvacha, resurrection lily, and aromatic ginger, is a rhizomatous medicinal plant belonging to the Zingiberaceae family. It is primarily cultivated in several southeast Asian countries such as China, Malaysia, Thailand, Indonesia, and India[1]. In India, there is a wide variety of Zingiberaceae plants, including over 200 species belonging to 20 genera out of the known 53 genera and approximately 1200 species [1-2]. *K. galanga* has been extensively documented for its various medicinal and culinary uses, which provide health benefits and contribute to food and nutrition [2] is commonly used as a medicinal spice in India, China, and other southeast Asian cuisines. Additionally, a large population consumes *K. galanga* as pickles for its health benefits. However, comprehensive scientific studies regarding its practical usage, nutritional properties, and safety status are still ongoing and have not been fully revealed [3-6]. Investigation of the wound healing potential of *Kaempferia rotunda* (Ginger) extract throughout history, humans have recognized the significance of maintaining good health. Medicinal plants have played a crucial role in this pursuit, offering a wealth of healing properties. Across continents and across time, traditional medicine has harnessed the potential of plants, unveiling the physical and chemical principles underlying their medicinal benefits [7-9].Traditional medicine has been used since ancient times, utilizing natural

products from plants and animals to treat human diseases. While the synthetic drug industry gained popularity over time, traditional medicine remains important in certain countries for healthcare [10-12].

MATERIAL AND METHODS

Method for obtaining a crude extract from the rhizomes of *K.rotunda*

Fresh rhizomes were washed, chopped, and powdered. The powder (1.0 kg) was extracted with ethanol (2.0 L) at room temperature. The filtrate was collected and concentrated using a rotary evaporator; yielding 9.85% (w/w) extract [13-15].

Method for obtaining crude tuber extract of K. rotunda

Fresh tubers were collected, washed, dried, and powdered. The powder (1.0 kg) was extracted with ethanol (2.0 L) at room temperature [16-17]. The filtrate was collected and concentrated using a rotary evaporator, resulting in a 4.85% (w/w) extract [18-20].

Extraction of essential oil by hydro-distillation

Approximately 300 g of fresh unpeeled rhizomes and tubers were individually hydro-distilled using a Clevenger distillation apparatus for 5 hours. The isolated oils, KRRO and KRTO, were dried and stored at 4-6ºC.

Extraction and fractionation of K. rotunda rhizome

Fresh rhizomes (3.0 kg) were washed, dried, powdered (2.5 kg), and macerated with ethanol (5.0 L) for 48 hours at room temperature. The filtrate was collected, concentrated, and further dried. The extract yield was 8.31% (w/w). The extract (180.00 g) was then fractionated using different solvents with increasing polarities, yielding petroleum ether fraction (KRRP), chloroform fraction (KRRC), butanol fraction (KRRB), and residual aqueous fraction (KRRA). The yields of the fractions were 11.25% (w/w), 34.83% (w/w), 52.57% (w/w), and 0.94% (w/w) respectively. KRRP, KRRC, and KRRB fractions were selected for further use.

Phytochemical screening and quantitative analysis

The plant material of*K.rotunda* were dried in shade and powder. Eight grams of root powder was extracted in different solvents (aqueous, methanol, ethanol, and chloroform and petroleum ether) in Erlenmeyer flasks. They were kept on orbital shaker for one day for complete extraction. Qualitative phytochemical analysis was done to estimate the presence of alkaloids, glycosides, saponins, terpenoids, phenols, steroids, flavonoids, proteins, anthraquinones, coumarines and tannins in the plant material of *K.rotunda*.

Table: 1 Phytochemical screening of rhizomes of *K.rotunda*

S.no	Test	KRRP	KRRC	KRRB
1	Alkaloids	-	-	-
2	Saponins	-	-	-
3	Terpenoids	-	+	+
4	Flavonoids	-	+	+
5	Steroids	+	+	+
6	Coumarines	-	-	-
7	Tannins	-	-	-

Preparation of ointments:

The crude extracts (KRR and KRT) and rhizome fractions (KRRP, KRRC, and KRRB) were mixed with simple ointment (white petroleum jelly) to create ointments (KRRD, KRTD, KRROD, KRTOD, KRRPD, KRRCD, KRRBD) with varying concentrations of the test samples. These ointments were formulated for topical application [21].

RESULTS

In-vivo wound healing activity

Male Wistar albino rats were used in the study. Excision wounds were created on the dorsal fur of the thoracic region, covering an area of approximately 1000 mm2. The animals were anesthetized using ketamine hydrochloride (30 mg/kg) during the wound creation process. A circular wound area was made using a surgical blade on the ethanol-sterilized region. The wounds were left undressed and exposed to the environment, without the application of any local or systemic antimicrobial agents. Topical application of *Kaempferia rotunda* ointment with different extract concentrations resulted in accelerated wound closure and enhanced re-epithelialization in excision wound models. The highest concentration of 5% Kaempferia rotunda ointment demonstrated the most significant improvement in wound closure. Comparison with povidone-iodine, a commonly used wound healing agent, showed comparable or superior effects of Kaempferia rotunda ointment.

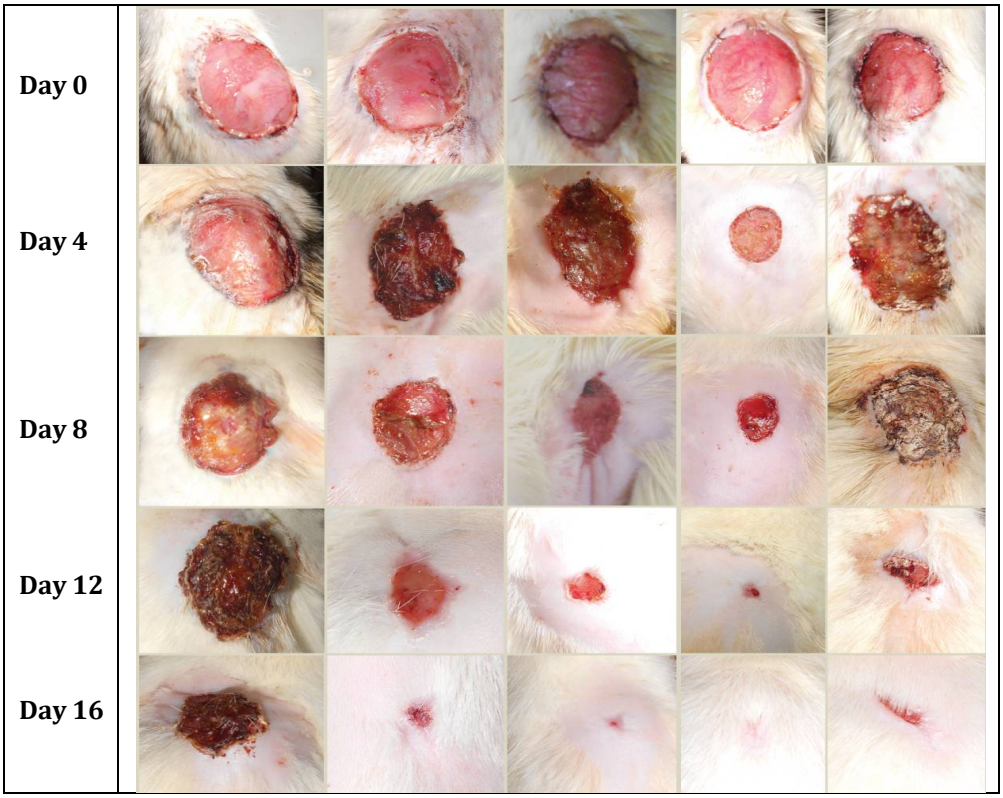

Figure:- 1 Photographic representation of wound closure treated control animals, KRRD 1%, 3% and 5% and povidone-iodine 5% treated rats on 0th,2nd,4th,8th, 12th and 16th wounding days.

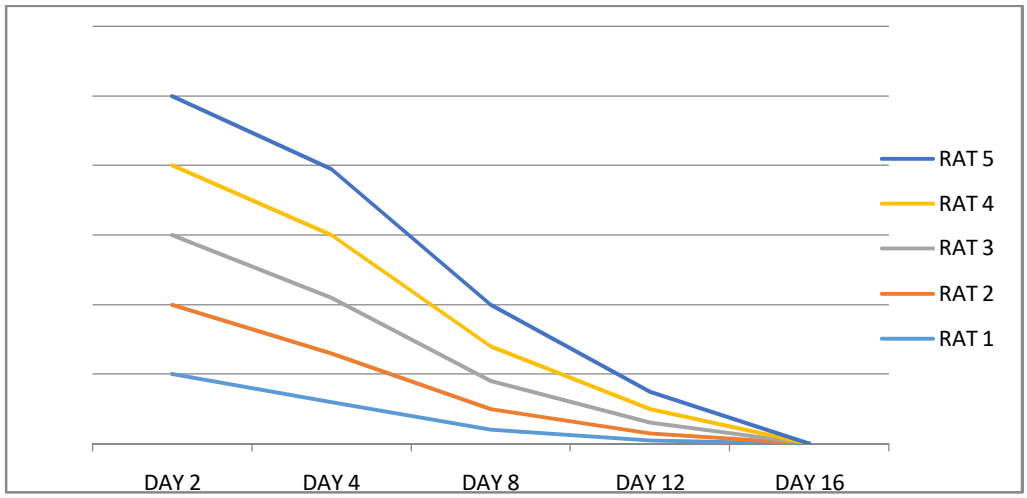

Fig:-2 Graph Representation of wound closure treated control animals, KRRD 1%, 3% and 5% and povidone-iodine 5% treated rats on 0th,2nd,4th,8th, 12th and 16th wounding days

DISCUSSION

In this study, the wound healing potential of test drugs derived from *Kaempferia rotunda* was evaluated using in vivo circular full-thickness excision wound healing model in Wistar albino rats. The effects of ointment formulations containing different concentrations (1%, 3%, and 5%) of ethanol extract from *Kaempferia rotunda* were assessed through topical application on the wounds. The healing outcomes were evaluated based on gross morphological changes, histological analysis, and wound closure rates.

The results showed that topical treatment with *Kaempferia rotunda* ointment formulations at all concentrations led to an increased wound closure rate compared to the control group. Animals treated with *Kaempferia rotunda* ointment exhibited reduced redness, swelling, and exudates in the wounded area. The 5% concentration of *Kaempferia rotunda* ointment showed the highest wound closure rate and faster re-epithelialization compared to other treated groups. The treatment with 3% and 1% concentrations also demonstrated positive effects on wound closure.

Also, the positive control group treated with povidone-iodine also showed a decrease in wound area compared to the control group. However, the control group wounds remained open and covered with scab tissue on the surface of the wound bed for more than 32 days, while the treated groups showed progressive healing.

The wound healing properties of ginger extract can be attributed to several mechanisms, including:

Increased collagen Deposition:-_

Ginger extract promotes the synthesis and deposition of collagen, a key component of the extracellular matrix essential for wound healing.

Angiogenesis stimulation:-

Ginger extract enhances the formation of new blood vessels, facilitating the delivery of oxygen and nutrients to the wound site. Epithelialization enhancement: Ginger extract accelerates the formation of new epithelial tissue, aiding in wound closure. The anti-inflammatory and antioxidant properties of ginger extract contribute to its wound healing and ulcerogenic activity. Ginger extract contains bioactive compounds such as gingerols, shogaols, and paradols, which are known for their anti-inflammatory and antioxidant effects. These findings support the traditional use of ginger for wound healing and ulcer prevention.

CONCLUSION

The present study evaluated the wound healing potential of the rhizomes and tubers of Kaempferia rotunda (Ginger) in Wistar albino rats. The in vivo excision wound model was used to assess the effects of ethanol extracts, ointment formulations, and oral administration of the extracts. The results showed that the topical treatment with ointment formulations containing ethanol extracts of the rhizome and tuber (KRRD and KRTD) promoted faster wound contraction, re-epithelialization, and collagen deposition compared to the control group and other treated groups. The histological evaluation supported the enhanced wound healing potential of KRRD.The study also examined the toxicological effects of the extracts and found them to be non-toxic and non-corrosive when tested topically and orally in acute dermal or oral toxicity studies. In the 28-day repeated dose study, no significant alterations were observed in hematological, biochemical, and histological evaluations of the control and treated rats. Additionally, the study assessed the anti-inflammatory and analgesic effects of the extracts and found that KRRC exhibited higher activity in the tested models, suggesting its contribution in reducing inflammation and pain during wound healing. The wound healing potential of K. rotunda extracts was further supported by their gastroprotective effects. Treatment with the extracts showed dose-dependent protection against ethanol-induced gastric ulceration by increasing mucosal defensive factors and preserving gastric mucosal integrity.

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