

STUDY AND DEVELOPMENT FOR THE PROPERTY OF GINGER IN WOUND
HEALING AND ULCEROGENIC ACTIVITY¹*Krunal Trivedi, ²Ankit Paliwal, ³Jayesh Dwivedi and ⁴Ayush Garg¹Research Scholar, Dept. of Pharmacology, Pacific College of Pharmacy, PAHER University, Udaipur-313024.²Associate Professor, Dept. of Pharmacology, Pacific College of Pharmacy, PAHER University, Udaipur-313024.³Professor, Dept. of Pharmaceutics, Pacific College of Pharmacy, PAHER University, Udaipur-313024.⁴Associate Professor, Dept. of Pharmacology, Pacific College of Pharmacy, PAHER University, Udaipur-313024.

Received on: 24/06/2022

Revised on: 14/07/2022

Accepted on: 04/08/2022

*Corresponding Author

Krunal Trivedi

Research Scholar, Dept. of
Pharmacology, Pacific
College of Pharmacy,
PAHER University, Udaipur-
313024.

ABSTRACT

Wounds have been defined as a disruption of cellular, anatomical, and functional continuity of a living tissue and healing is the interaction of a complex cascade. It may lead to the restoration of structural and functional integrity of strength of injured tissues. The present study shows the wound healing and ulcerogenic property of *Kaempferia rotunda* (Ginger). *Kaempferia rotunda* (Ginger) is a plant belonging to ginger family. China is native place and has a subcontinent to India. It is reportedly naturalized in Java, Malaysia and Costa Rica. *Kaempferia rotunda* (Ginger) plant has many medicinal uses in Ayurvedic and allopathic medicinal systems. This is also known as *bhumichampa*. In this study, evaluation of wound healing potential of the extract and rhizomes and tubers of *K. rotunda* (Ginger) and its effect influence wound to provide a less oedematous, less painful, adequate antioxidant effect for healing. Evaluate the wound healing potential of the ethanol extract and rhizomes and tubers of *K. rotunda* (Ginger) on surgical wound healing using a rat full thickness excision wound model. The active extract or essential oil with faster wound healing effect was further subjected for the active extract or essential oil with faster wound healing effect was subjected to successive solvent extractions with water immiscible organic solvents of increasing polarities and the wound healing potential of each fraction was then evaluated topically via excision wound healing model. Evaluate safety of the extracts, essential oil and the fractions after single and repeated dose (28-day) administration in animal model for safe wound healing and anti-ulcerogenic.

KEYWORDS: *Kaempferia rotunda* (Ginger), wound healing and anti-ulcerogenic, animal study.

1. INTRODUCTION

Human life revolves around the important concern of getting healthy and staying healthy. Over the ages, man has realized the immense potential of medicinal plants. This has led to the understanding of many of the underlying physical and chemical principles that account for the medicinal properties of plants.^[1,2] Plants have been an integral part of traditional medicine across the continents, since time immemorial.^[3,4] Wounds have been defined as a disruption of cellular, anatomical, and functional continuity of a living tissue. Healing is the interaction of a complex cascade which leading to the restoration of structural and functional integrity with regain of strength of injured tissues. Millions of people is been affected by acute and chronic wounds around the world.^[5,6] In the United States, Acute wounds are a common health problem around 12 million people around the world is affected and approximately 500,000 people is been hospitalized yearly. Chronic wounds are the major concerns of patient. Chronic wound affected large

number of people around the globe. This may reduce the quality of life. Around 6 to 8 millions peoples are suffer from chronic wound all over the globe.^[7,8]

Kaempferia rotunda (Ginger) is a plant belonging to ginger family. China is native place and has a subcontinent to India. It is reportedly naturalized in Java, Malaysia and Costa Rica.

Kaempferia rotunda (Ginger) plant has many medicinal uses in Ayurvedic and allopathic medicinal systems. This is also known as *bhumichampa*.^[9,10]

In India, this Plant is found in various region. These may seldom in the wild. The plant was grown in herbal nurseries. Sanskrit name *bhumichampa* implies the indigo-coloured flower shoots from within the soil.^[11,12]

2. MATERIAL AND METHOD

2.1 Preparation of crude rhizome extract of *K.rotunda* (Ginger)

Fresh rhizomes of *K. rotunda* were washed and chopped and powdered. The powder (1.0 kg) was successively extracted and stored at room temperature with 2.0 L of ethanol. At last, the filtrate collected and put them into a rotary evaporator and yield was found to be 9.85 % (w/w).

2.2 Preparation of crude tuber extract of *K.rotunda*(Ginger)

Fresh tubers of *K.rotunda*(Ginger) were collected, washed, cut into small pieces, dried and powdered. The powder (1.0 kg) was successively extracted and stored at room temperature with 2.0 L of ethanol. The extract was filtered. At last, the filtrate collected and put them into a rotary evaporator and the extract yield was found to be 4.85 % (w/w).

2.3 Phytochemical screening and quantitative analysis

The plant material of *K.rotunda*(Ginger) were dried in shade and powder. Eight grams of root powder was extracted in different solvents (aqueous, methanol, ethanol, chloroform and petroleum ether) in Erlenmeyer flasks. They were kept on orbital shaker for one day for complete extraction. Later, each solvent was filtered by using Whatman No.1 filter paper. From the resulting extract, 4-5 ml of each extract was used for phytochemical analysis.

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*(Ginger)

Alkaloids

The presence of alkaloids in root extracts (aqueous, methanol, ethanol, chloroform and petroleum ether) and the ethanolic extract of *K.rotunda*(Ginger) were tested by Mayer's and Dragendorff's reagents.

Mayer's reagent Test– Extracts of *K.rotunda* (Ginger) were treated with HCl solution. After that, 1 to 2-3 drops of Mayer's reagent was added and observed for the precipitation of yellow colour.

Dragendorff's reagent Test– Extracts of *K.rotunda* (Ginger) were dissolved in 10 ml of 1% HCl. Then, they were transferred to a water bath for few minutes and treated with 4-5 drops of Dragendorff's reagent, and observed for the orange reddish precipitation.

Saponins

foam test is used for the detection of saponins. Extract were mixed with water and the formation of froth is observed. It should be held for 15-20 min for positive result.

Terpenoids

The root extracts and the methanolic extract of *K.rotunda*(Ginger) was tested for the presence of terpenoids. Extracts of *K.rotunda*(Ginger) were dissolved in two or three granules of tin metal in 2 ml thionyl chloride solution and then, adding 1 ml of the extract into the test tube and observed for the formation of a pink colour.

Steroids

The presence of steroids in root extracts and the methanolic extract of *K.rotunda*(Ginger) was tested by Liebermann Burchard test and Salkowski test.

Liebermann Burchard Test– acetic anhydride is used for the mixing of all the extracts of *K.rotunda*(Ginger). At the side of the test tube concentrated sulphuric acid was added at the junction of two layers a brown ring is formed. Green coloration on upper layer and deep red colour in the lower layer indicate a positive test for steroids.

Salkowski Test– Extracts of *K.rotunda*(Ginger) were mixed with few drops of concentrated sulphuric acid. Acid was poured from the sides of the test tube and a brown ring is formed at the junction of two layers.

Flavonoids

The presence of flavonoids in root extracts and ethanolic extract of *K.rotunda*(Ginger) was tested by ferric chloride test and alkaline reagent test.

Ferric chloride Test– Extracts of *K.rotunda* (Ginger) were mixed with ferric chloride solution and observed for the formation of blackish red colour.

Alkaline reagent Test– Extracts of *K.rotunda*(Ginger) mixed with sodium hydroxide solution and observed to increase of yellow colour which would become colourless on addition of few drops of dilute hydrochloric acid.

Coumarines

Root extracts and the ethanolic extract of *K.rotunda* (Ginger) was tested for the presence of coumarines. Extracts of *K.rotunda*(Ginger) were mixed with few drops of 10% NH₄OH. Kept two spots on filter paper and examined under UV-light and then observed for intense fluorescence.

Tannins

The root extracts and the ethanolic extract of *K.rotunda*(Ginger) was done for the presence of tannins.

Gelatin Test– extracts of *K.rotunda*(Ginger) were mixed with gelatin solution and observed for the white precipitate for the presence of tannins.

2.4 Experimental animals

For this study, Wistar albino rats and Swiss albino mice were taken. According to NIH guidelines a standard housing condition was maintained.

2.4.1 Skin wounding and treatment with ointment formulations *via* topically

Male Wistar albino rats weighted and selected for the present study. The animals were inflicted with excision wounds. All the animals were anaesthetized. This was done for the creation of wounds with ketamine hydrochloride (30 mg/kg). The dorsal fur of the thoracic region of the animal, covering an area of about 1000 mm², was shaved and an impression was made on the ethanol-sterilized region. By using a surgical blade, circular wound area impressed.

2.4.2 Skin wounding and treatment with extracts *via* orally

On the dorsal thoracic region of male Wistar albino rats, a circular wound area was created. These animals then divided into five groups of twelve animals. Group I, 1ml of 1% Tween-80 were administered. The animals of group II, III and IV are administered the extract of doses 50, 100 and 200 mg/kg and Group V, the positive control group was administered with 1ml of Novamox dry syrup (25 mg/kg) once daily, from 0th day onwards till the wounds get completely healed.

2.4.3 Acute dermal toxicity of test samples

According to the guidelines of OECD, acute dermal toxicity test was conducted. Wistar albino rats (160-250 g) were selected. The fur was removed from trunk of all the animals. by using clipping or shaving of skin and only animals with intact skin. The animals divided into nine groups. Group I, the control animals (physiological saline). Group II, the vehicle control animals (ointment base) and the animals of Group III- Group IX has highest concentration of 20 % ointment. The gauze and tape were carefully removed. The change were observed on skin, eyes and mucous membranes, behavioural patterns, etc. during study Mortality was recorded.

2.4.4 Acute oral toxicity of test samples

According to the guidelines of OECD, acute oral toxicity studies were done. The test dose is 2000mg/kg was used. Around 18 hours of fasting animals were used for the experiment. In Group 1, 1ml of saline were administered. In group 2, the 1 % Tween-80 were administered or used as a vehicle. In group 3 to 9 the animals were orally treated with 2000mg/kg of single dose. After that all animals were allowed for food and water. The toxic symptoms are observed in first 4 hour of administration. After that animal were observed by 14 days. During the study mortality were observed. If any mortality were observed it will be expressed as LD₅₀.

2.4.5 Evaluation of repeated oral toxicity of dose after 28-day

This test was performed according to OECD guidelines. 23 group of both sex animals (male and female) were randomly selected and twenty-one treatment groups (n = 12 each; 6 males and 6 females). 1ml of saline were administered into normal control and vehicle control groups and the animals with test samples (KRR, KRTE, KRRP, KRRC, KRRB, KRRO and KRTO) with a dose of 250, 500 and 1000 mg/kg. for a period of 28 days animals were orally administered a single dose. This follow up is been observed for next 14 days. These animals then anaesthetized and blood sample collection were collected for haematological and biochemical analysis.

2.4.6 Ulcerogenic activity of *Krotunda* (Ginger) in Wistar albino rats

The anti-ulcerogenic activity of the test samples were evaluated *via* different ulcerative models induced by necrotising agents, non-steroidal anti-inflammatory drugs, water immersion stress and pylorus ligation in Wistar albino rats.

2.4.6.1 Gastric mucosal lesions

In gastric lesion model the gastroprotective activity of the test samples were assessed. Wistar albino rats were distributed into different groups with six animals each and they were fasted for 36 h with free access to water *ad libitum* prior to the experiment. The animals were grouped into normal control, vehicle (ulcer) control, different test samples and the positive controls treated groups. 1 ml of 1 % Tween-80 were orally administered. The animals of test sample treated groups were orally administered with the crude ethanol extract, different solvent fractions, essential oil, the chromatographic sub-fractions and the pure compound in separate experiments. The animals of positive control treated groups received orally ranitidine and omeprazole, 1 h prior to the experiment. After this pre-treatment, the animals were gavaged with 80 % ethanol to induce gastric ulcers.

2.4.6.2 Indomethacin-induced gastric ulcer

The indomethacin induced gastric ulceration was created as per the modified method. The animals were chosen into eight groups of rats. Group 1, the normal control and Group II, the Indomethacin control were orally administered with 1 % Tween - 80 as vehicle. The animals of group III and IV orally administered with KRR at a dose of 100 and 200 mg/kg. While the animals of Group V and VI were pre-treated with KRRC at a dose of 50 and 100 mg/kg respectively and the animals of Group VII and VIII were pre-treated with the positive control, ranitidine and omeprazole respectively. 30 min after drug administration, animals of group II - VIII were orally administered with 30 mg/kg indomethacin in a volume of 8 ml/kg of saline. 6 h after indomethacin administration, all the animals were euthanized by CO₂ inhalation and the stomachs were excised out.

2.4.6.3 Water immersion stress-induced gastric ulcer model

With *ad libitum*, animals were fasted for 36 h and grouped into eight groups with six animals each. Group 1, normal control and Group II, the ulcer control animals with 1% Tween – 80. Group III and IV animals were orally administered with KRR. While the animals of Group V and VI were pre-treated with KRRC and the Group VII and VIII animals were prior administered with the positive controls, ranitidine and omeprazole. After drug treatment animals from Group II- Group VIII allowed animals to swim in the glass cylinder. Then the animals were carefully removed from their experimental conditions and were euthanized by CO2 inhalation and the stomach tissue of each animal was excised.

2.5 Haematological parameters

Heparinised blood is used for the analysis of haematological parameters. Parameters are HGB, RBC, WBC) and PLT count were made by using an haematological analyser.

3. RESULT AND DISCUSSION

3.1 Phytochemical analysis

Table 1: Phytochemical screening of rhizomes of *K.rotunda*(Ginger).

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

3.2 Ointment formulation effect on topical application of *K.rotunda*(KRRD) containing ethanolextract concentration (1 %, 3 %, and 5 %) on excision wound model

The gross morphological changes observed in the vehicle treated control animals and the KRRD (1 %, 3 % and 5 %) and the positive control povidone-iodine treated groups were monitored from 0th day till the wounds get completely closed and was photographed and summarized. Topical treatment of wounds with KRRD at a concentration of 1 %, 3 % and 5 % demonstrated an increased wound closure rate in comparison with the vehicle treated control animals. In addition, the KRRD and povidone - iodine treated animals had less redness, swelling, and exudates. In between the 2nd and 3rd day post-surgery, a deep purple / black coloured scab tissue covering the entire surface of the open wound area appeared on the control and treated wounds. As the treatment of wounds with KRRD (1 %, 3% and 5%) continued, re-epithelialization progressed under this scab tissue in the wounded region and a reduction in the surface area was observed in all the animals in comparison with the vehicle treated control animals, while treatment of wounds with 5 % KRRD showed an increased wound closure rate and faster re-epithelialization in comparison with the control and other treated groups. This was followed by treatment of wounds with KRRD 3 % and 1 % treatment. In between 14th and 15th post wounding day, the wounds treated with 5 % KRRD. The newly formed tissue appeared to be thinner and paler than the surrounding tissue that was not excised. The treatment of wounds with the povidone-iodine treated group also showed a decrease in the mean wound area in comparison with the vehicle treated control animals during this period, while the wounds of the vehicle treated control animals were found to be still open and remained to be covered with the scab tissue on the surface of the wound bed during this period and it took more than 32 days for complete wound closure.

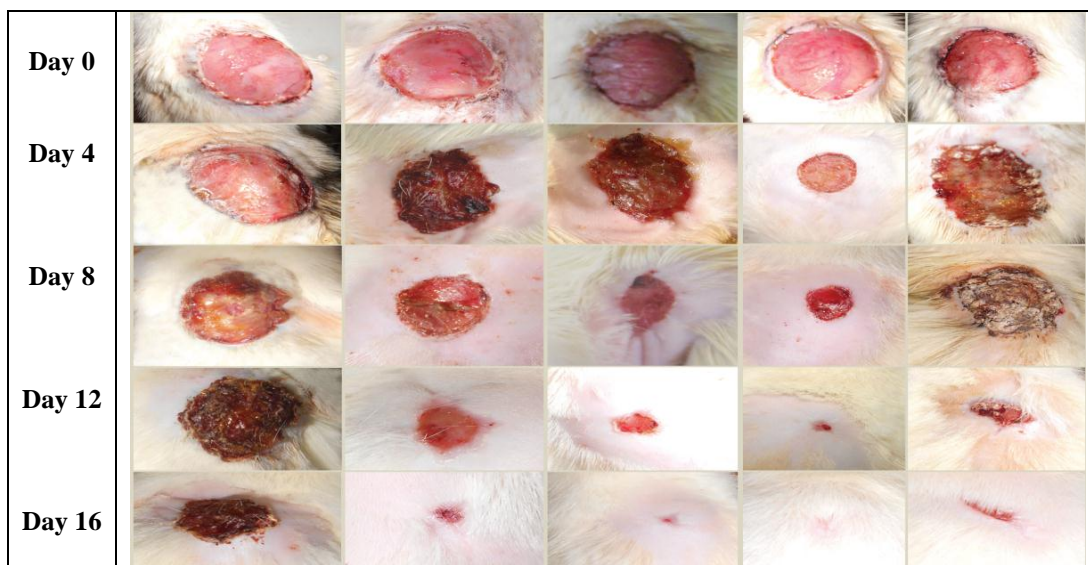


Figure 1: Photographic representation of wound closure treated control animals, KRRD 1%, 3% and 5% and povidone-iodine 5% treated rats on 0th, 2nd, 4th, 8th, 12th and 16th wounding days.

3.3 The gross morphology of the vehicle treated control, KRTD (1 %, 3 %, and 5 %) treated wound tissue

The gross morphological changes observed in the vehicle treated control animals and the KRTD (1, 3 and 5 %) and the positive control povidone-iodine treated groups were monitored from 0th day to till the wounds get completely closed and was photographed and summarized. The gross

examination of the wounded tissue showed that the topical treatment of animals with KRTD 1 %, 3 % and 5 % show a decreased dose dependent in comparison with the vehicle treated control groups and they were also associated with less redness, swelling, and exudates. Treatment of wounds with povidone-iodine show a significant decrease in mean wound area in comparison with the vehicle treated control animals.

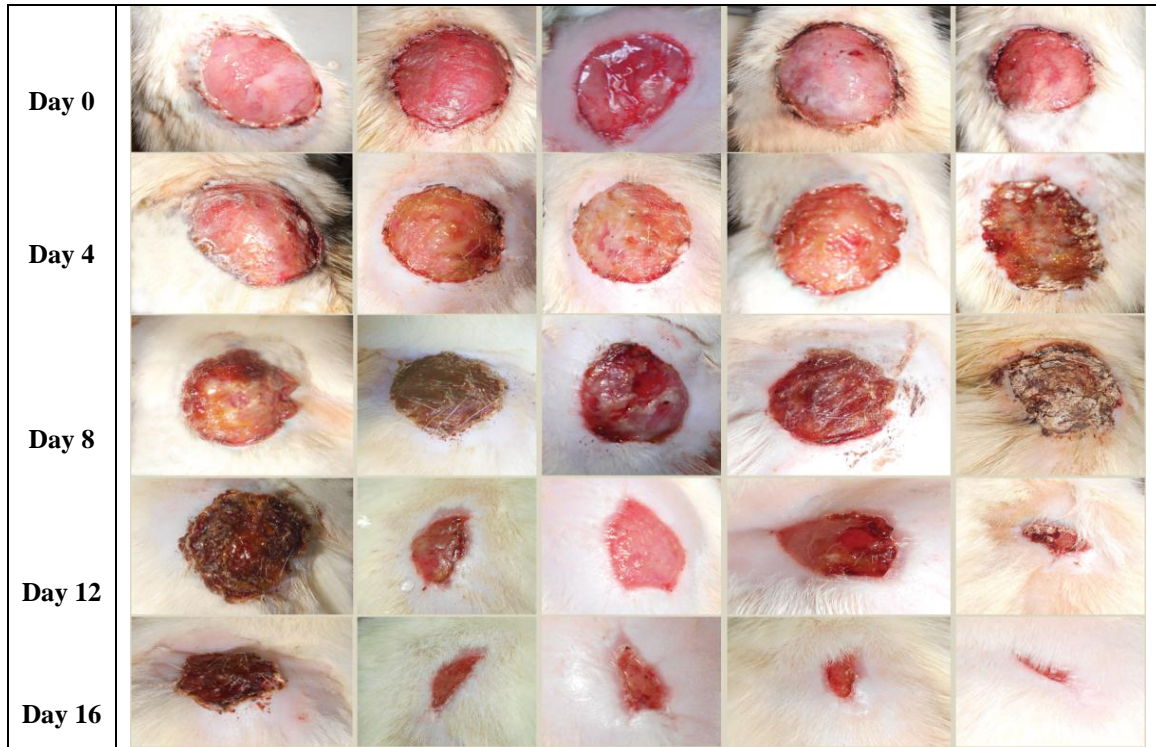


Figure 2: Photographic representation of wound closure treated control animals, KRTD 1%, 3% and 5% and povidone-iodine 5% treated rats on 0th, 2nd, 4th, 8th, 12th and 16th wounding days.

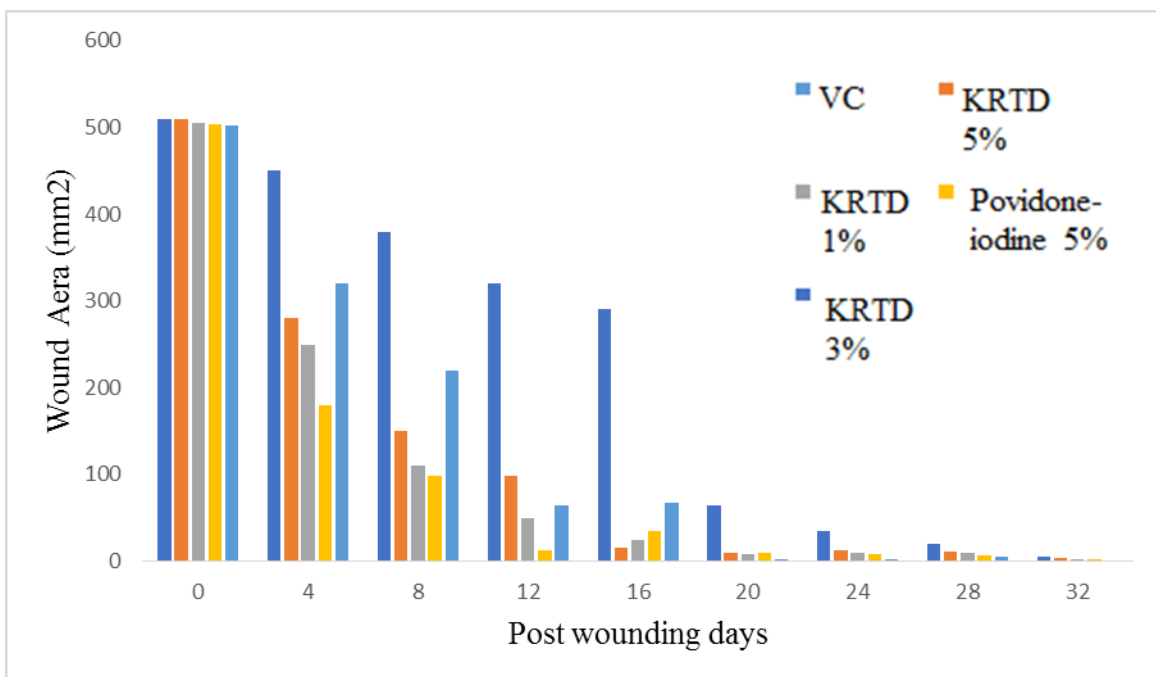


Figure 3: Ointment formulation effect of *Kaempferia rotunda* (Ginger) tuber (KRTD) on wound contraction.

3.4 Oral toxicity studies (repeated 28 day) of ethanol extract of the rhizome (KRR), tuber (KRT) of *K.rotunda*

No treatment-related toxicity signs and mortality observed in both sexes of rats orally for 28 days. There were no changes in eye, fur, skin, respiratory system and behavioural pattern.

Table 2: Haematological parameters of oral administration (28days) of ethanol extract of rhizome (KRR) and tuber (KRT) of *K.rotunda*(*Ginger*)in male rats.

Treatment	RBC ($10^6/\mu\text{l}$)	WBC ($10^3/\mu\text{l}$)	PLT ($10^3/\mu\text{l}$)	HGB (g/dl)
Control	8.68±0.65	14.85±1.56	840.18±65.48	15.89±1.85
Vehicle control	7.58±0.59	13.75±1.38	845.58±59.90	15.31±1.85
KRR (250mg/kg)	8.10±0.65	13.67±1.63	803.20±58.45	17.19±1.45
KRR (500mg/kg)	9.01±0.97	17.09±0.98	810.00±40.19	17.12±1.98
KRR (1000mg/kg)	6.89±0.86	15.18±1.98	899.02±55.98	18.78±1.94
KRT (250mg/kg)	7.12±0.58	15.98±0.89	890.98±33.98	15.26±1.09
KRT (500mg/kg)	8.09±1.08	15.98±1.03	835.00±56.04	17.89±1.04
KRT (1000mg/kg)	9.07±0.55	17.98±1.07	846.08±67.98	18.09±1.08

Table 3: Clinical haematological parameters of oral administration (28day) of ethanol extract of rhizome (KRR) and tuber (KRT) of *K.rotunda* in female rats.

Treatment	RBC ($10^6/\mu\text{l}$)	WBC ($10^3/\mu\text{l}$)	PLT ($10^3/\mu\text{l}$)	HGB (g/dl)
Control	7.90±1.09	15.98±0.98	820±78.09	15.98±0.98
Vehicle control	6.98±1.02	10.98±1.09	813±67.98	15.09±1.02
KRR (250mg/kg)	7.98±0.98	8.90±1.26	850±88.09	17.09±0.98
KRR (500mg/kg)	6.90±1.34	12.90±1.40	872±89.90	18.90±1.90
KRR (1000mg/kg)	7.80±0.89	15.90±0.89	890±88.90	16.89±1.88
KRT (250mg/kg)	7.89±0.90	13.89±1.89	769±89.89	15.90±1.89
KRT (500mg/kg)	6.66±0.89	15.89±1.70	888±69.80	17.89±1.66
KRT (1000mg/kg)	7.80±1.89	14.26±1.77	890±77.89	16.90±1.77

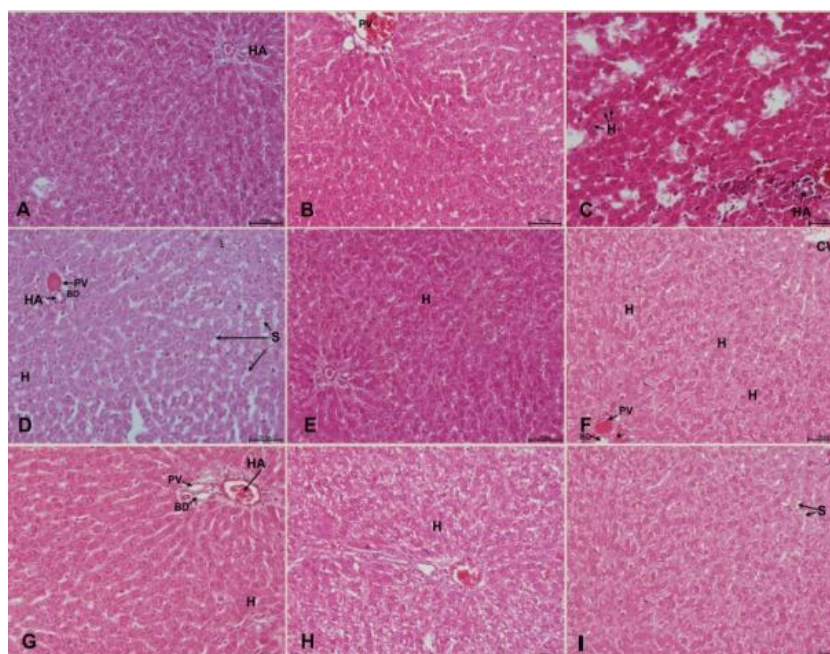


Figure 4: Photomicrographs of the rat liver sections stained with hematoxylin and eosin after a 28-day repeated oral administration of test drug. The strained sections of liver from KRR, KRT (1000mg/kg) treated animal.

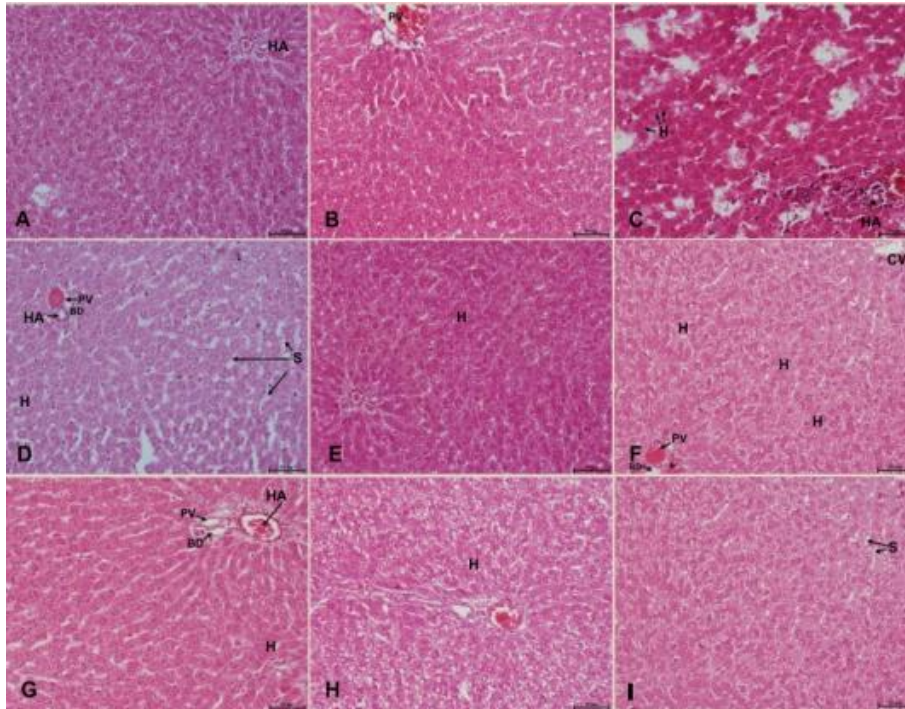


Figure 5: Photomicrographs of the rat spleen from normal control showing normal granular hemosiderin pigment with macrophages in the red pulp. The stained sections of spleen from vehicle treated control (1% Tween 80). The stained sections of spleen from KRR, KRT (1000mg/kg) treated animal.

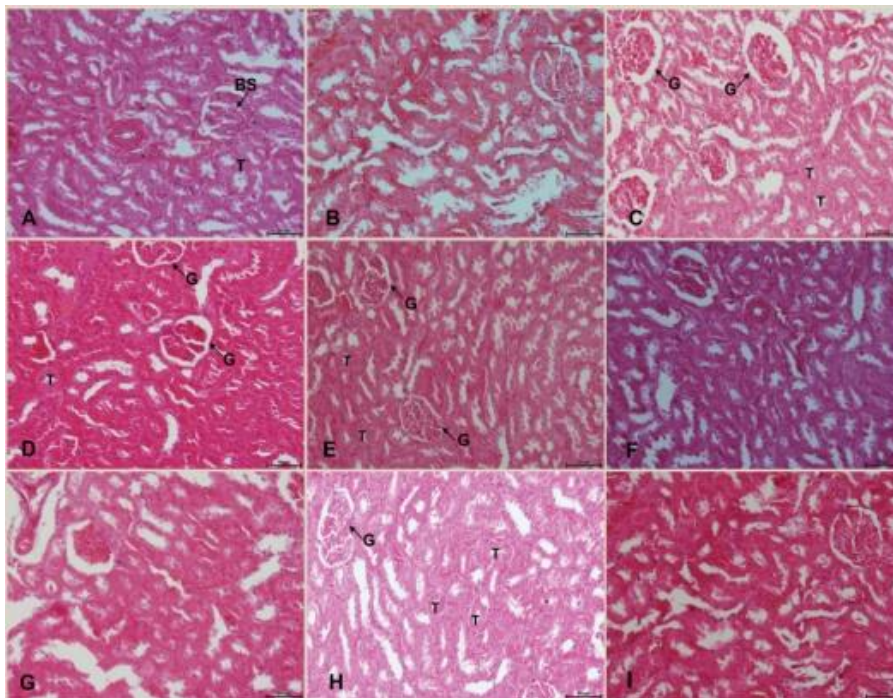


Figure 6: Photomicrographs of the rat kidney from normal control showing normal size of glomeruli with normal tubules. The stained sections of kidney from vehicle treated control (1% Tween 80) animals showing normal size of glomeruli with normal tubules. The stained sections of kidney from KRR, KRT (1000mg/kg) treated animal.

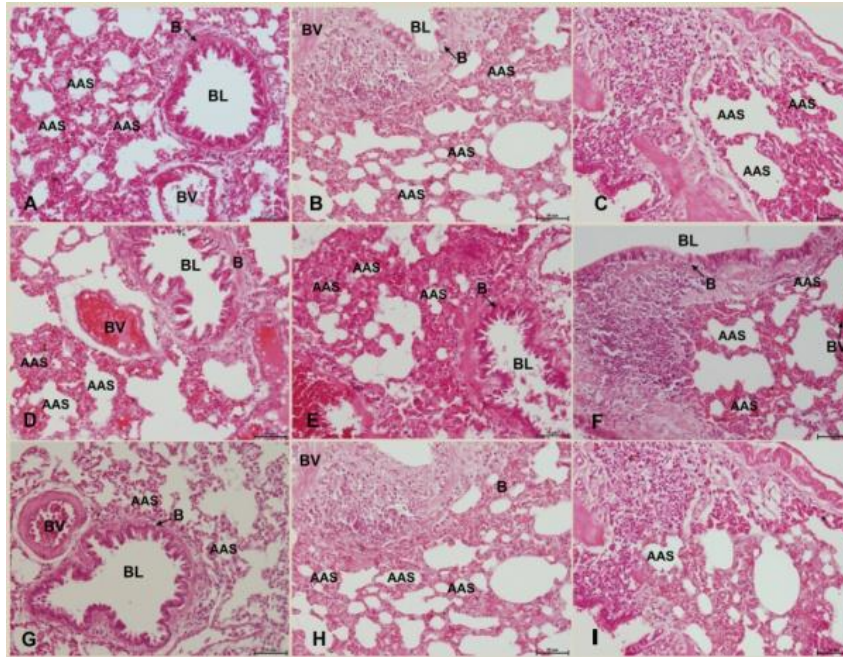


Figure 7: Photomicrographs of the rat lung from normal control showing normal lung architecture illustrating. The stained sections of lung from vehicle treated control (1% Tween 80) animals showing normal size of glomeruli with normal tubules. The strained sections of liver from KRR, KRT (1000mg/kg) treated animal.

3.5 The anti-ulcerogenic activity of the ethanol extract of rhizomes of *Kaempferia rotunda L.*(Ginger) on experimentally induced gastric ulcers in rats

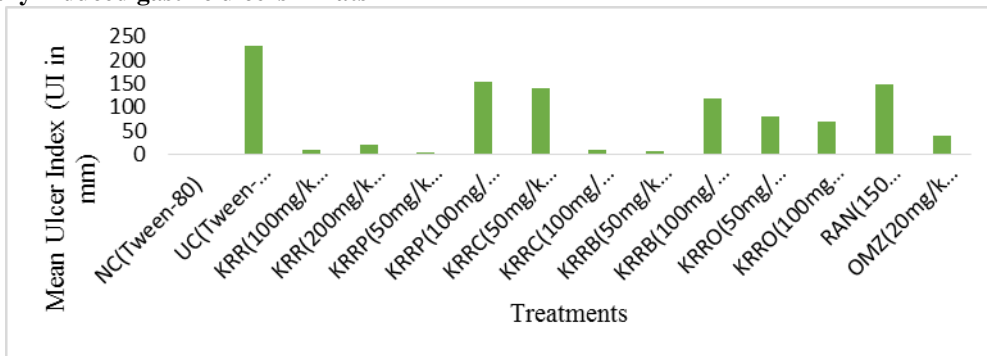


Figure 8: Effect of ethanol extract from rhizomes of *Kaempferia rotunda* (KRR, KRRP, KRRC, KRRB and KRRO) on ethanol (EtOH)-induced gastric ulcer in rats.

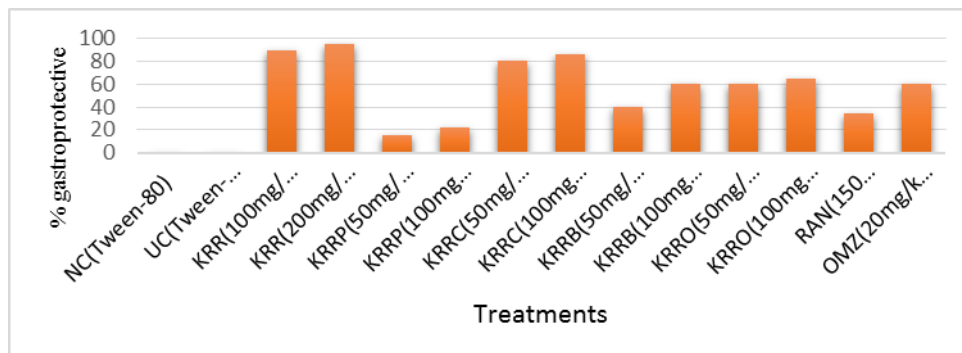


Figure 9: Effect of ethanol extract from rhizomes of *Kaempferia rotunda* (KRR, KRRP, KRRC, KRRB and KRRO) on degree of protection against ethanol (EtOH) – induced gastric ulcer in rats.

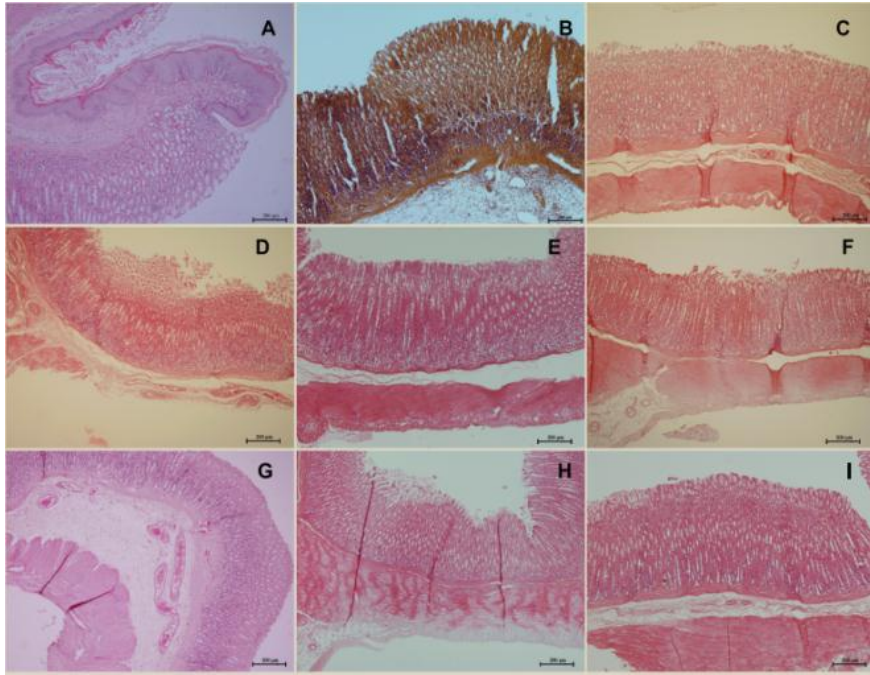


Figure 10: Histological evaluation of hematoxylin and eosin-stained sections of glandular stomach tissue from normal, ulcer control, KKR, KRRP, KRRC, KRRB, KRRO, RAN and OMZ pre-treated rats showing their effect on ethanol induced gastric lesions.

3.6 Effect of KRR and KRRC on NSAIDs induced gastric ulcer in Wistar rats

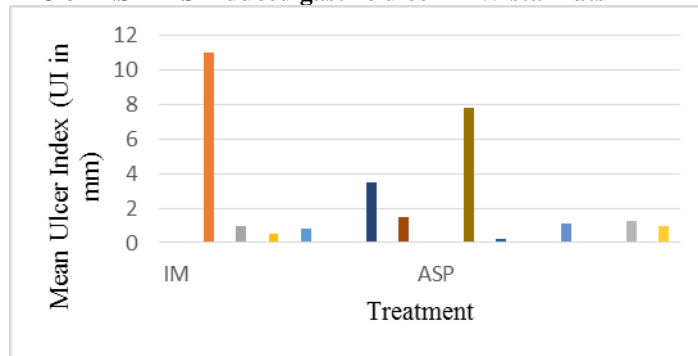


Figure 11: Effect of ethanol extract and chloroform fraction of the rhizomes of *Kaempferia rotunda* (KRR and KRRC respectively) on gastric lesions induced by NSAIDs (indomethacin (IM) and aspirin (ASP)).

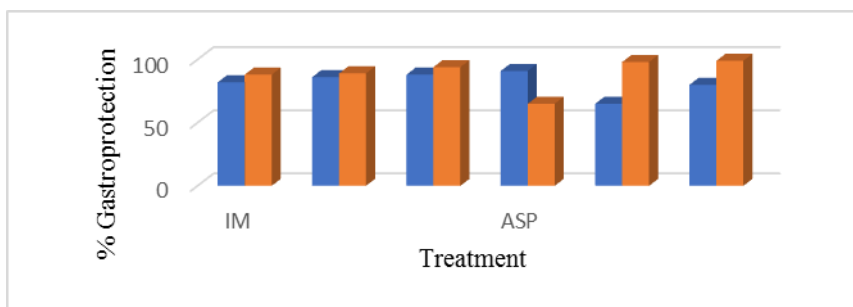


Figure 12: Effect of ethanol extract and chloroform fraction of the rhizomes of *Kaempferia rotunda* (KRR and KRRC respectively) on degree of protection against NSAIDs (indomethacin (IM) and aspirin (ASP)) induced gastric ulceration in rats.

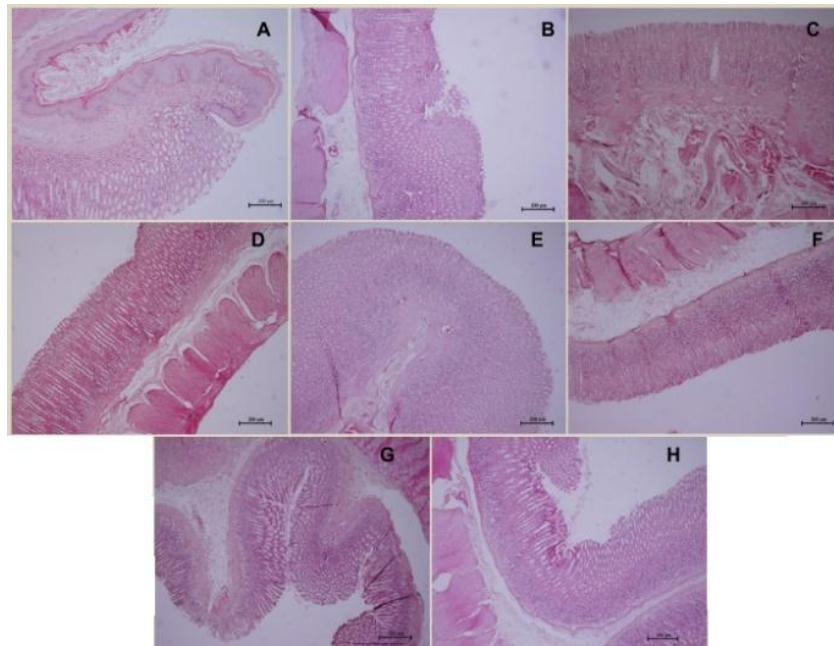


Figure 13: Histological evaluation of hematoxylin and eosin-stained sections of glandular stomach tissue from normal, ulcer control, KRR, KRRC, RAN and OMZ pre-treated rat.

3.7 Effect of KRR and KRRC on water immersion stress - induced gastric ulcer

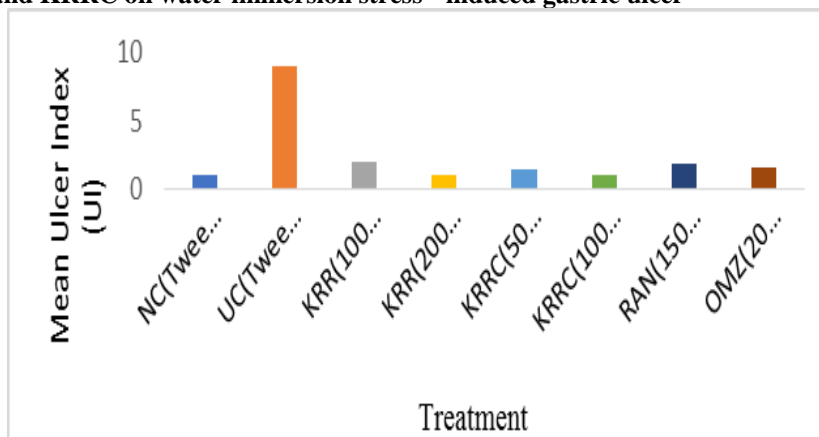


Figure 14: Effect of ethanol extract and chloroform fraction of the rhizomes of *Kaempferia rotunda* (KRR and KRRC respectively) in rats.

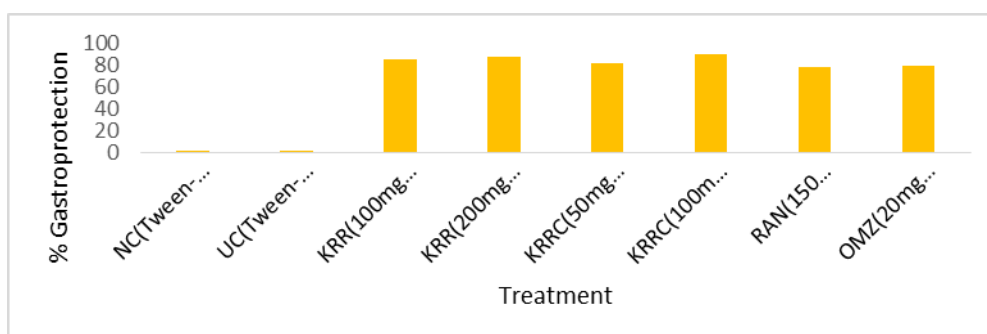


Figure 15: Effect of ethanol extract and chloroform fraction of the rhizomes of *Kaempferia rotunda* (KRR and KRRC respectively) in rats.

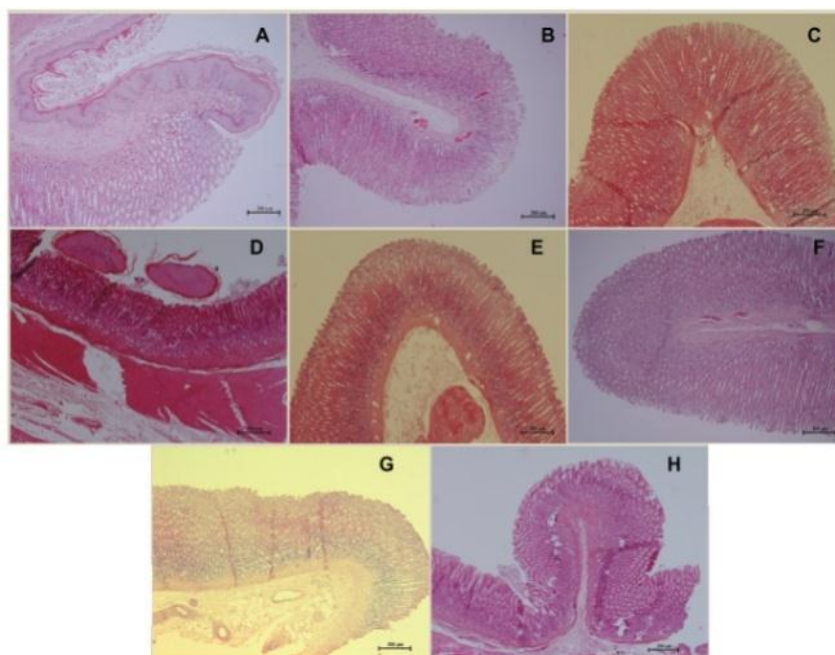


Figure 16: Histological evaluation of hematoxylin and eosin-stained sections of the glandular stomach tissue from normal, ulcer control, KRR, KRRC, RAN and OMZ pre-treated rats.

4. SUMMARY AND CONCLUSION

The present study was thus undertaken to evaluate the wound healing potential of the rhizomes and tubers of *K. rotunda* in Wistar albino rats. In the excision wound model, the wound healing potential of ethanol extract of the rhizome (KRR), tuber (KRT) were tested *in vivo*. The periodic assessment of wound area showed that the topical treatment of wounds with the ointment formulation containing ethanol extract of the rhizome and tuber (KRRD and KRTD respectively), the essential oil isolated from the rhizome and tuber (KRROD and KRTOD) and the oral administration of animals with ethanol extract of the rhizome and tuber (KRR and KRT) of *K. rotunda* significantly increased the wound contraction, collagen deposition and decreased the mean period of re-epithelialization dose dependently in comparison with their respective vehicle treated control animals. The wounds treated with KRRD showed more prominent healing with a faster wound contraction, re-epithelialization and excellent collagen deposition than the vehicle treated control, KRROD, KRR, KRTOD, KRTD, KRT and the positive controls povidone-iodine and novamox treated groups. The histological and histomorphometric evaluation carried out also supported the increased wound healing potential of KRRD.

Anti-ulcerogenic effect of ethanol extract of the rhizome (KRR), rhizome fractions (KRRP, KRRC and KRRB) and isolated from the rhizomes (KRRO) of *K. rotunda* was evaluated against ethanol-induced gastric ulcers in Wistar albino rats and the study demonstrated severe haemorrhagic lesions in the gastric mucosa of the ethanol administered ulcer control animals with decreased defensive factors such as gastric wall mucus and NP-SH content in association with ROS accumulation as

evidenced by increased lipid peroxidation with concomitant depletion of endogenous GSH level in the gastric mucosa. Treatment of animals with KRR, KRRO, KRRP, KRRC and KRRB showed a dose dependent significant increase in the mucosal defensive factors and offered a dose dependent significant protection to the adhered gastric mucosa. Treatment with KRR and KRRC was found to possess more prominent gastroprotective effect by inhibiting the loss of gastric mucosal integrity, endogenous GSH content and by decreasing the membrane lipid peroxidation in comparison with the ulcer control, the fractions (KRRP and KRRB) and the essential oil formulation (KRRO) of the rhizomes of *K. rotunda* and the positive controls (RAN and OMZ) pre-treated groups. The gastroprotective effect of the treated group was also supported by the histological evidences obtained from the study. However at 100 mg/kg, KRRC exhibited a greater degree of gastroprotection than all the treated groups indicating its increased cytoprotective effect against necrotising agent (ethanol)-induced gastric lesions.

5. REFERENCES

1. Huffman MA. Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. *Proc Nutr Soc*, 2003; 62(2): 371–81.
2. Girish Dwivedi, Shridhar Dwivedi. *History of Medicine: Sushruta – the Clinician – Teacher par Excellence*. National Informatics Centre, 2007; 224–225.
3. Ernst E. Herbal medicines: balancing benefits and risks. *Novartis Found. Symp*, 2007; 282: 154–67; 167–72, 212–8.

4. Schilling. Wound Healing. *Phys. Rev.*, 1968; 48(2): 374.
5. L.M.Wahl, S.M.Wahl: Inflammation, in I.K.Cohen, R.F.Diegelman, W.J. Lindblad (eds): *Wound Healing: Biochemical and Clinical Aspects*, Philadelphia, PA, W.B. Saunders, 1992; 40-62.
6. Kerstein MD. Overview of wound healing in a moist environment. *Am.J.Surg*, 1994; 167(1A): 2S-6S.
7. Linder R. Effect of dressing materials on wound healing. *Langenbecks Arch Chir Suppl Kongressbd*, 1998; 115: 694-7.
8. Bologna J.L. Jorizzo R.P. Rapini. *Dermatology*, 2008; 2(2): 2147–2158.
9. Khullar R, Saini S, Sethi N, Rana AC. Emulgel: A surrogate approach for topically used hydrophobic drugs. *Int J Pharm Biol Sci.*, 2011; 117-128.
10. Mohamed MI. Topical emulsion gel composition comprising diclofenac sodium. *AAPS J*, 2004; 6(3): 26.
11. Baibhav, Rana AC, Seema S, Vikas S. Emulgel: a comprehensive review on the recent advances in topical drug delivery. *International Research Journal of Pharmacy*, 2011; 2(11): 66-70.
12. Mohamad MI, Optimization of chlorphenesin emulgel formulation. *AAPS J*, 2004; 6(3): 1-5.