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COMPARATIVE STUDY OF WOUND HEALING ACTIVITY OF VARIOUS SPICE AND HERBS IN RATS

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Abstract: Wound is actually the disruption of cellular and anatomical discontinuity of tissue.In the present work five spices Sygyzium aromaticum(cloves), Cinnamomum zeylanicum(dalchini), Cinnamomum tamala (tejpatta),Myristica fragrans(nutmeg) a herb Ocimum sanctum (tulsi) were studied to see the effect of their common constituent Eugenol on the wound healing activity. Excision and incision wound models and dead space in Sprague Dawley rats were used to study complete epithelisation time, wound contraction , histopathological studies and tensile strength of wounds. The animals were divided into separate groups with 6 animals in each group. The time taken for wound contraction and complete epithelisation by various spices was significantly ($p<0.05$ to $p<0.001$) less as compared to control. Mean tensile strength of the 5 mentioned spices were significant greatly ($p<0.05$ – $p<0.01$) after 16 days when compared with control animals. The methanolic extract of all the above spices showed better granulation tissues, early and complete epithelisation and better tensile strength compared to control.

Keywords: Wound healing , topical ,methanolic ,epethelisation

Introduction:-

Wounds are the common medical problems faced by mankind since the life existed on the earth.[1] so there is a need to have some pharmacological agents which could promote and accelerate the process of wound healing.[2] Wound healing actually occurs as a fundamental response to tissue injury.[3]

Spices ,taken in the present work are studied to see their effect specifically for the excision and incision wounds on the rats.Spices are the natural plant or mixtures, in whole or ground form which are used to impart flavour and aroma to the food items.[4] Earlier they were thought to contribute mostly in seasoning , flavouring and enhancing the taste of foods,beverages and also they provide a

means to gustatory perfection and at the same time they contain such bioactive components that help to prevent various ailments as infections ,hypertension etc.[5]Sygyzium aromaticum (clove) a commonly available plant of class Myrataceae is found to have analgesic,anti microbial, anti oxidant , anti inflammatory property.Cinnamomum zeylanicum(dalchini),a spice easily available in Indian surrounding is found to have various activities like anti microbial, anti oxidant, anti inflammatory, anti diabetic property.Cinnamomum tamala commonly known as tejpatta also has shown various biological activities like anti diarrhoel,anti vomiting , anti oxidant , anti fungal .Ocimum sanctum or tulsi as it is commonly known is actually a miracle plant whose each and every part is useful medicinally .It acts as an anti stress agent,it is effective on the nervous system,on the reproductive system.Along with that it shows anti oxidant, anti ulcer activities. Earlier some work regarding wound healing activity has been done on dalchini and tulsi,but in them over all effect of the spice has been studied ,and here it is shown that the wound healing effect is due to its one of the components Eugenol .

The principle constituents of all the above mentioned spices and herbs are volatile oil, alkaloids and tannins. The constituents of the other components in them which can be used in Ayurveda for treatment of diverse aliments like skin disease digestion disorders antidote or sanke bite and scorpion sting.

A methanolic extract of all the above spices were found to have anti inflammatory analysis and anti microbial , anti diabetic prop.

The main aim for the present work is a study conducted in which the methanolic extracts of all the 5 spices orally and as an ointment showed that it is majorly due to the eugenol content in it. Since majority of wound healing activities are topical the present study is planned to know the effect of eugenol specially for wound healing activity and a comparative study is made of all the taken is done spices.

<es extraction with methanol for about 12-15 hrs at 70^{0}C. then the extract was filtered , distilled , concentration and gave a residue of 4.2 g 5.8 gm of each spice extract.

Preliminary phytochemical analysis : The presence and absence of different phytochemical constituted viz phenols , flavonoids alkaloids tannins sterols and triterpenes were recorded.

Preparation of juice

To evaluate wound healing activity in excision wound model, eugenol and methanolic extract of clove, tejpatta, dalchini, nutmeg and tulsi were formulated in ointment (3 % w/v) using simple ointment BP as base. 0.5 g of each of ointment was applied once daily to the animals. To determine wound healing activity by incision wound model and dead space wound model the animals were administered with eugenol (50 mg/kg, oral) or methanolic extract of clove (300 mg/kg, oral) or methanolic extract of tejpatta (600 mg/kg, oral) or methanolic extract of dalchini (1000 mg/kg, oral) or methanolic extract of nutmeg (600 mg/kg, oral) or methanolic extract of tulsi (450 mg/kg, oral).

IN VIVO WOUND HEALING ACTIVITY

Excision wound model

Rats were anaesthetized by giving ketamine hydrochloride (120 mg/kg, ip) and hairs were removed from the dorsal thoracic region of the rats. Separate group (n = 6) of rats were treated with ointment containing eugenol and methanolic extract of clove, tejpatta, dalchini, nutmeg and tulsi (3 % w/v) (50 mg/rat per day) or ointment base or povidone iodine ointment (50 mg/rat per day) every day topically for a period of 16 days. Wound contraction was monitored by tracing wounds on graph paper on the day of wounding and on day 4, 8, 12, 16 until total healing. Wound contraction was calculated as percentage reduction in wound area as an indicator of the rate of wound contraction. The period of epitheliliazation was calculated as the number of days required for falling off of the dead tissue without any residual wound (Morton and Malone, 1972).

Incision Wound Model

Two paravertebral incisions (6 cm long) were made through the full thickness of the skin on the either side of the vertebral column (Ehrlich and Hunt, 1969). Wounds

were closed with interrupted sutures, 1 cm apart. Separate group of rats were daily administered with saline (10 ml/kg, oral) or eugenol (50 mg/kg, oral) or methanolic extract of clove (300 mg/kg, oral) or methanolic extract of tejpatta (600 mg/kg, oral) or methanolic extract of dalchini (1000 mg/kg, oral) or methanolic extract of nutmeg (600 mg/kg, oral) or methanolic extract of tulsi (450 mg/kg, oral) or applied topically with povidone iodine ointment (50 mg/rat per day). The sutures were removed on day 7 and wound breaking strength was measured on 10th day of wounding by continuous water flow technique as described by Lee (1968). Briefly, a line was drawn 3 mm away from the either side of the wound and two forceps were applied on to the line. One of the forceps was fixed, while the other was connected to a freely suspended lightweight polypropylene graduated container through a string run over to a pulley. Water was flown into the container from the reservoir. Gradual increase in weight transmitted to the wound site by pulling apart the wound edges. Water flow was arrested as soon as the wound opened and the weight of water in container was noted to determine the mean wound breaking strength (n=6).

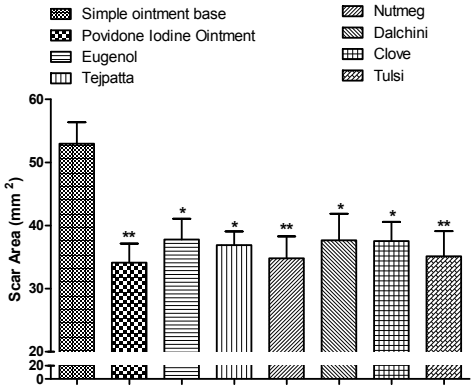
Dead Space Wound

Separate group of animals were implanted with two polypropylene tubes (0.5×2.5 cm^2 each) on the dorsal surface of either side of the lumbar region to create the wound (Patil and Kulkarni, 1984). Animals received saline (10 ml/kg, oral) or eugenol (50 mg/kg, oral) or methanolic extract of clove (300 mg/kg, oral) or methanolic extract of tejpatta (600 mg/kg, oral) or methanolic extract of dalchini (1000 mg/kg, oral) or methanolic extract of nutmeg (600 mg/kg, oral) or methanolic extract of tulsi (450 mg/kg, oral) or applied topically with povidone iodine ointment (50 mg/rat per day) from day 0 to 9th post-wounding day. On 10th post-wounding day the tissues from each implanted tube was dissected out along with the tube and granuloma breaking strength and the hydroxyproline content was estimated (Woessner, 1961).

Statistical Analysis- The results were analyzed using one way analysis of variance (ANOVA) with post hoc Dunnett test or Neumann-Keul test. $P < 0.05$ was considered to be statistically significant.

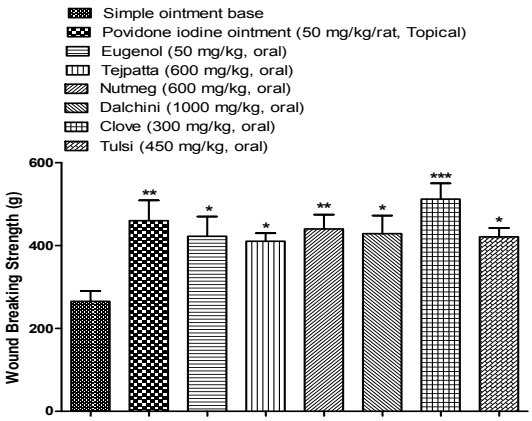
RESULTS AND DISCUSSION

Excision wound method



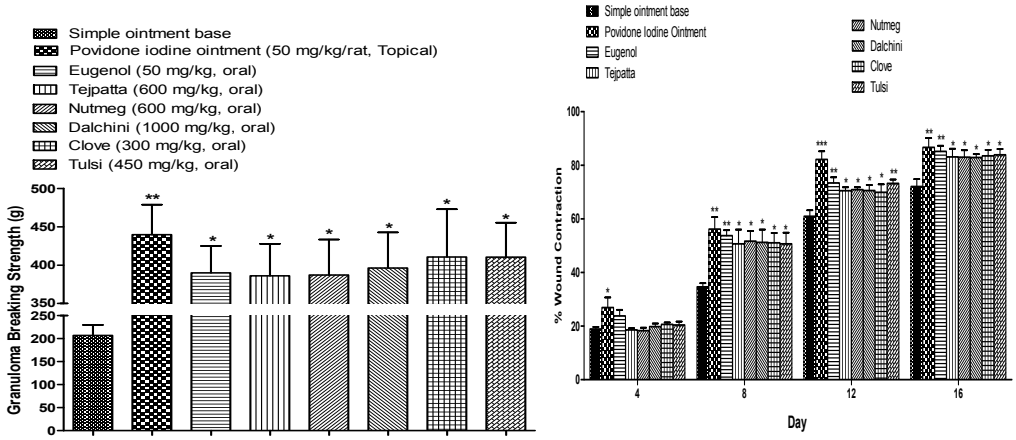
Topical application of the ointment prepared from eugenol and methanolic extract of clove, tejpatta, dalchini, nutmeg and tulsi significantly increased the percentage of wound contraction (Figure 1). In fact, topical application of eugenol and methanolic extract of clove, tejpatta, dalchini, nutmeg and tulsi ointment accelerated the progression of wound healing as compared with simple ointment treated animals [4 days- $F(7, 47) = 2.88$, $P < 0.05$; 8 days- $F(7, 47) = 2.73$, $P < 0.05$; 12 days- $F(7, 47) = 7.40$, $P < 0.001$; 16 days- $F(7, 47) = 3.00$, $P < 0.05$]. Moreover, eugenol and methanolic extract of clove, tejpatta, dalchini, nutmeg and tulsi ointment treated rats exhibited significantly reduced scar area on complete epithelization when compared with control animals [$F(7, 47) = 3.23$, $P < 0.01$].

Incision wound method



As depicted in Figure, the breaking strength of the incision wounds was significantly increased in eugenol (50 mg/kg, oral) ($P < 0.01$) or methanolic extract of clove (300 mg/kg, oral) ($P < 0.001$) or methanolic extract of tejpatta (600 mg/kg, oral) ($P < 0.05$) or methanolic extract of dalchini (1000 mg/kg, oral) ($P < 0.05$) or methanolic extract of nutmeg (600 mg/kg, oral) ($P < 0.01$) or methanolic extract of tulsi (450 mg/kg, oral) ($P < 0.05$) treated groups as compared to saline treated animals [$F (7, 47) = 3.70$, $P < 0.01$]. These results were comparable to the wound strength determined in povidone iodine treated.

Dead space wound method



As shown in Figure, significant increase in granuloma breaking strength in eugenol (50 mg/kg, oral) ($P < 0.01$) or methanolic extract of clove (300 mg/kg, oral) ($P < 0.05$) or methanolic extract of tejpatta (600 mg/kg, oral) ($P < 0.05$) or methanolic extract of dalchini (1000 mg/kg, oral) ($P < 0.05$) or methanolic extract of nutmeg (600 mg/kg, oral) ($P < 0.05$) or methanolic extract of tulsi (450 mg/kg, oral) ($P < 0.05$) when compared against saline treated animals [$F (7, 47) = 2.69$, $P < 0.05$]. In addition, there was significant increase in hydroxyproline content in eugenol (50 mg/kg, oral) ($P < 0.01$) or methanolic extract of clove (300 mg/kg, oral) ($P < 0.05$) or methanolic extract of tejpatta (600 mg/kg, oral) ($P < 0.05$) or methanolic extract of dalchini (1000 mg/kg, oral) ($P < 0.05$) or methanolic extract of nutmeg (600 mg/kg, oral) ($P < 0.05$) or methanolic extract of tulsi (450 mg/kg, oral) ($P < 0.05$) treated rats [$F (7, 47) =$

2.82, P < 0.05]. Topical application of povidone iodine ointment also showed significant increase in the granuloma breaking strength (P < 0.01) as well as hydroxyproline content (P < 0.01) as compared to control animals.

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