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Full Length Research Paper

Evaluation of the wound healing activity of *Cinnamomum zeylanicum* extract on experimentally induced wounds in rats

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The present study was undertaken to determine the effect of *Cinnamomum zeylanicum* on experimentally induced excision wounds in rats. Thirty-two rats were divided into four groups of eight rats each. Group A received a placebo containing 0.75% cinnamon, Group B received a placebo containing 1.5% cinnamon, Group C as a control group did not receive any treatment, and finally, Group D received blank placebo; Groups C and D were considered as one group (standard). The results were taken on days 3, 7, 14 and 21 and a histological evaluation was carried out on the samples. The result shows that cinnamon extract had a significant ($p<0.05$, $^{**}p<0.01$) effect on wound healing; it accelerated the healing process of the wounds. Significant enclosure rates were seen after 7 days of study. The present study proved that *C. zeylanicum* was effective in treating experimentally induced wounds and hasten healing, showing a dose-dependent treatment trend. It especially increased epithelialization in treatment groups compared to other groups.

Key words: *Cinnamomum zeylanicum*, induced excision, epithelialization, wound healing.

INTRODUCTION

Wounds are unavoidable events of life, which arise due to physical injury, chemical injury or microbial infections. The healing of wounds usually takes place in a direction away from its normal cause, as such, underhealing, over-healing or no healing of wounds is common. Research on drugs that increase wound healing is a developing area in modern biomedical sciences. Several drugs obtained from plant sources are known to increase the healing of different types of wounds (Biswas and Mukherjee, 2003). Herbal medicine has become an integral part of standard health care based on a combination of time-honored traditional usage and ongoing scientific research. Medicinal plants are becoming outstanding because many infectious organisms have developed resistance to conventional medicines like antibiotics such that they no longer respond to them. Herbal preparations can be more effective and much safer than conventional medicines. For the fact that they are non-toxic, they could be administered for a long period of time (Vinothapooshan and Sundar, 2010).

Cinnamomum zeylanicum (commonly referred to as cinnamon) has a long history of usage as spice and flavoring agents. Two types of cinnamon are used as spice: common cinnamon also called “true cinnamon” or “ceylon cinnamon” (*Cinnamomum verum* or *Cinnamomum zeylanicum*) and cassia cinnamon (*Cinnamomum aromaticum*) which is also known as “Chinese cinnamon” (Jellin, 2006a, b). Both common cinnamon and cassia cinnamon have been thought to be generally safe when ingested, however, in recent years, coumarin levels found in cassia cinnamon have been observed to be potentially harmful. Coumarin has medicinal values as it is a precursor to several anticoagulants; however, it is moderately toxic to the liver and kidneys, with an LD50 of 275 mg/kg (Lungarini

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et al., 2008). As regards safety, despite one case report published by Westra et al. (1998), data obtained from clinical trials suggest that cinnamon when consumed in doses used in food preparations could be considered safe (USFDA, 2006).

Cinnamon exhibits diverse biological functions including anti-inflammatory (Lee et al., 2005), antioxidant (Singh et al., 2007; Lee et al., 2003), anti-microbial (Singh et al., 2007; Matan et al., 2006) and anti-diabetic functions (Khan et al., 2003; Qin, 2003). Recently, the anti-tumor activity of cinnamon was shown both *in vitro* (Schoene et al., 2005; Singh et al., 2009) and *in vivo* (Kwon, 2009). Cinnamon is rich in essential oils and tannins which inhibit microbial growth (Mau et al., 2001; Amara et al., 2008). The most important chemical constituents of cinnamon are volatile oil (cinnamaldehyde, eugenol, cinnamic acid and weitherhin), mucilage, diterpenes and proanthocyanidins (Jayaprakasha et al., 2002).

Eugenol is widely used and well-known for its medicinal properties. It is active against oral bacteria associated with dental caries and periodontal disease (Cai and Wu, 1996), and a large number of other bacteria (Burt and Reinders, 2003; Larhsini et al., 2001; Cressy et al., 2003; Friedman et al., 2002) and virus (Kim et al., 2001). The aim of this study was to evaluate the wound healing activity of cinnamon and its constituents, which is based on its anti-inflammatory, antioxidant and anti-microbial characteristics that accelerate wound healing process.

MATERIALS AND METHODS

Plant material and preparation of the extract

The aqueous extracts of cinnamon and chamomile were prepared using 10 g of dried material/100 ml distilled water and boiled for 5 min at 100°C. Then, they were filtered and concentrated at 50°C under reduced pressure using a Rota vapor. The extracts were kept at -15°C until they were used in the experiment (Kassi et al., 2004).

Experimental animals

Thirty-two male Wister rats (180 to 200 g) of approximately three months old were used as experimental animals and were divided into four groups of eight rats. The animals were housed in standard environmental conditions of temperature (22 ± 3°C), humidity (60 ± 5%) and a 12 h light/dark cycle. During the time of the experiment, the rats were given standard pellet diet (Pastor Institute, Iran) and water *ad libium*.

Surgical procedures

After anesthesia induction with xylazine (2%) and ketamine (10%) (I.M: 60 mg/kg), the rats were kept in a ventral position on a surgery table. Then, the dorsal area from scapula to ilium was scrubbed and prepared for surgery. Two circular-shaped, full-thickness surgical wounds (7 mm in diameters on both sides of the backbone, 1 cm away from the backbone and 5 cm away from each other) were made with a biopsy punch of 7 mm. With this excisional wounding method, the epidermal, dermal, hypodermal and panniculus carnosus layers were removed completely (Luisa and DiPietro, 2003).

Treatment

After making the surgical wounds, all the rats were randomly colored with a non-toxic color and divided into three groups. All the extracts were applied daily on topical route. Ointments with 0.75% cinnamon extracts (CE) were applied to Group A, while those with 1.5% CE were administered to Group B. To Group C was administered a placebo with Eucerin Vaseline; Group D as the control group did not receive any treatment. All the rats were observed for 21 days. Daily observation was carried out and any wound fluid, evidence of infection or other abnormalities were noted.

Histopathological study

Samples of the healing tissues obtained on days 3, 7, 14 and 21 from all the groups of the rats were processed for a histological study. The tissue samples were placed in 10% buffer formalin for fixation. The histopathololological sections were prepared routinely after passing several stages, including dehydration by ascending concentrations of ethanol, clearing by xylene, impregnation and embedding by paraffin, and cutting of the sections by rotary microtome in 6 µm thickness. After placing tissue sections on glassy slides, they were stained with haematoxylin and eosin staining method. Histological changes in the sections were evaluated under light microscope with different magnifications of subjective lenses (4, 10 and 40). Recorded factors were congestion, inflammatory cells, kinds of inflammatory cells, angiogenesis, fibroplasia, scar, collagen density fibroblast, fibrin and fibroblastic aggregation.

Statistical analysis

All values were reported as mean ± S.D; the statistical differences among groups were assessed using Tukey's test and analysis of variance (ANOVA). A value of $p< 0.01$ was considered significant. Statistical analysis was performed using SAS 9.1 for Windows.

RESULTS

A: 0.75%, B: 1.5% and C: Control and placebo groups

The congestion and bleeding observed on the 3rd day was more in Group B than in Group A. There was no sign of epithelization among the groups. Angiogenesis was remarkably higher in both Groups A and B than in C group. On the 7th day, congestion, bleeding and edema factors were ignorable in all the groups. Wound healing trend improved more in Group B than in other groups and passed the acute phase. It was observed that epithelization was more prominent in Group B than in other groups, which shows the effectiveness of 1.5% CE on wound healing.

Epithelization stayed remarkably high in Group B on the 14th day, and the rate of angiogenesis which is one of the noticeable factors of wound healing trend was less in

Table 1. Effect of the treatments on wound healing in rats.

Group	Relative area of wound (mm^2)			
	3 day	7 day	14 day	21 day
A (0.75% cinnamon)	149.90	142.150[b]	94.856[b]	74.894[b]
B (1.5% cinnamon)	148.37	140.910[b]	76.848[c]	38.578[c]
C (Control)	151.02	149.138[a]	142.690[a]	138.585[a]
Significance	ns	**	**	**

*All expressed as mean and standard deviation (S.D). Means with different letters in a column were significantly different (ns: Not Significant *p<0.05, **p<0.01).

other groups than in the aforenamed group. Finally, on the 21st day, all factors except angiogenesis were approximately high in Group A compared to the other groups. In all, the best result was observed in the group that received 1.5% CE treatment, especially because of the effects which the CE had on epithelization.

Wound enclosure

The results of this study shown in Table 1 showed that three days after drug application, no difference was observed among groups, while on the 7th day, a significant difference was observed between the two treatment groups and the standard group, but no difference was observed between the treatment groups. The 14th day of the study demonstrated differences among all the groups as it was seen that wound healing was more effective in the treatment groups than the control group. The 21st day of the study showed the same results as did the 14th day. In both the 14th and 21st days, 1.5% concentration of cinnamon showed better healing effect compared to 0.75% concentration.

DISCUSSION

The present study was undertaken to evaluate whether CE promotes wound healing in experimentally induced wounds in rats. Collagenation, wound contraction and epithelization are crucial phases of wound healing. The phases of inflammation, macrophagia, fibroblasia and collagenation are intimately interlinked. Thus, an intervention on any one of these phases by drugs could eventually either promote or decrease one or all other phases of healing (Vinothapooshan and Sundar, 2010). We, therefore, evaluated the microscopic changes and wound enclosure during the different times of the study. The results indicate that wound healing showed a much better trend in groups which received treatments with CE than in groups which did not, showing a dose dependent effect of CE on wound healing. Moreover, epithelization which is a sign of wound healing was remarkably more in groups which received treatments with CE than in groups which did not.

Herbal drugs are currently used all over the world because of their safe and effective properties. Cinnamon is a useful plant which has lots of therapeutic functions (Štefan et al., 2009). The antioxidant activity of cinnamon's essential oil as shown in another study indicates that it inhibits the hepatic 3-hydroxy-3-methyl-glutaryl CoA (HMG-CoA) reductase activity in rats, and suppresses lipid peroxidation via the enhancement of hepatic antioxidant enzyme activities (Lee et al., 2003). This characteristic of cinnamon can promote wound healing based on the antioxidative activity of cinnamon on wound sites. It is well-known that different mechanisms may be involved in the genesis of inflammatory reactions. Eugenol shows similar anti-inflammatory effects on COX antagonist (indomethacin) (Huss et al., 2002; Kim et al., 2003) which helps to accelerate wound healing via the reduction of inflammation on wound sites and for better wound enclosure as is observed in our study where cinnamon extracts significantly (p<0.05, **p<0.01) promoted wound enclosure in groups treated with it. Although both treated groups experienced significant wound healing effects, wound contraction in our study showed a dose-dependent activity in which the highest dose (1.5%) had a pronounced effect on wound enclosure, particularly from the 14th day of the study. However, the best results were seen on the 21st day when ointment with 1.5% CE showed a more significant effect than that with 0.75% CE and the control group. It has been shown that CE, be it water or ethanol-based exhibits antioxidative properties and may also exert antimicrobial effects (Anderson et al., 2004; Blomhoff, 2004; Kanuri et al., 2009).

Indeed, cinnamaldehyde, a bioactive constituent found in cinnamon has been shown to possess considerable antibacterial activity against Gram positive and Gram negative bacteria in *in vitro* experiments (Lee and Ahn, 1998). Furthermore, cinnamaldehyde has also been shown to inhibit the growth of fungi, including yeast, filamentous molds and dermatophytes as well as the eggs and adult females of human head louse (Ooi et al., 2006) which is another potential effect of CE on wound remedy.

All these properties which are found in cinnamon are beneficial to wound healing in that they make for better and faster wound contraction. Characteristics such as

anti-inflammation, antioxidant and anti-microbial are the main factors of wound remedy which accelerate wound healing. In this study, we conclude that cinnamon is effective in wound healing as it promoted the conditions around the wound site for better enclosure and healing based on its anti-inflammatory, antioxidant and anti-microbial functions which are mostly because of its essential oil, especially eugenol and cinnamoldeyde.

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