Evaluation of the wound healing activity of an ethanolic extract of Ceylon cinnamon in mice

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ABSTRACT: The present study was conducted to verify the effect of Ceylon cinnamon 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 1.5% of cinnamon and Group B a placebo containing 3%. Group C, as the control group, didn't receive any treatment and finally Group D received a blank placebo as the reference standard group. Wound healing was monitored on Days 3, 6, 9 and 14 and histological evaluation was carried out on the samples. The results show that cinnamon extract served to accelerate the wound healing process and specifically increased epithelialization in treatment groups compared to the other groups. Thus, this study demonstrates that Ceylon cinnamon may be effective in stimulating the enclosure of wounds.

Keywords: wound healing; Ceylon cinnamon; ethanolic extract; mice

Wounds are inescapable events of life, which arise due to physical or chemical injury or microbial infections. The healing of wounds often deviates from a normal course and under-healing, over-healing or failure of wounds to heal 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 healthcare, based on a combination of time honoured traditional usage and ongoing scientific research. Medicinal plants are coming into prominence because of the overuse of conventional medicines such as antibiotics which has resulted in the development of resistance in many infectious organisms. Thus, herbal preparations can be more effective than conventional medicines and their non-toxic nature means that they can be administered over long periods (Vinothapooshan and Sundar 2010).

Cinnamon has a long history of use as a spice and flavouring agent. Two types of cinnamon are commonly consumed, common cinnamon also called 'true cinnamon' or Ceylon cinnamon (*Cinnamon verum*, *C. zeylanicum*) and its related spice, cassia cinnamon (*C. 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 discussed as potentially harmful. While of medical value as it is a precursor to several anticoagulants, coumarin is moderately toxic to the liver and kidneys, with an LD50 of 275 mg/kg (Lungarini et al. 2008). With regard to safety, with the exception of one case report published by Westra et al. (1998), data obtained from clinical trials suggests that cinnamon, when consumed in doses used for food preparation, may be considered as safe (USFDA 2006).

Cinnamon exhibits diverse biological functions including anti-inflammatory (Lee et al. 2005), anti-oxidant (Lee et al. 2003; Singh et al. 2007), anti-microbial (Matan et al., 2006; Singh et al. 2007), and anti-diabetic effects (Khan et al. 2003; Qin 2003). Recently, the anti-tumour activity of cinnamon has been shown both *in vitro* (Schoene et al. 2005; Singh et al. 2009) and *in vivo* (Kwon 2009). In addition, cinnamon is rich in essential oils and tannins which inhibit microbial growth (Mau et al. 2001; Amara 2008). The most favoured chemical constituents of cinnamon are volatile oils (cinnamaldehyde, eugenol, cinnamic acid, and

weitherhin), mucilage, diterpenes and proanthocyanidins (Jayaprakasha et al. 2002).

In particular, 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 effective against a large number of other bacteria (Larhsini et al. 2001; Friedman et al. 2002; Burt and Reinders 2003; Cressy et al. 2003) and viruses (Kim et al. 2001). The aim of this study was to evaluate the wound healing activity of cinnamon and to determine the effects of the anti-inflammatory, anti-oxidant and anti-microbial characteristics of cinnamon's constituents on acceleration of the wound healing process.

MATERIAL AND METHODS

Preparation of the plant extract

An ethanolic (20%) extract of Ceylon cinnamon was prepared from a whole plant and the extract was filter purified. Treatment extracts were then gathered and prepared to 1.5 and 3% solutions in a blank placebo (Eucerin and Vaseline).

Experimental animals

Twenty-four male Wister rats (200–220 g) of approximately two months of age were used as experimental animals and were divided into four groups of six 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 course of the experiment the rats were administered a standard pellet diet (Pastor Institute, Iran) and water *ad libitum*.

Surgical procedures

After induction of anaesthesia with 2% xylazine and 10% ketamine (*i.m.* 60 mg/kg) rats were fixed in a ventral posture on a surgery table. The dorsal area from the scapula to the ilium were then scrubbed and prepared for surgery. Two circular, full thickness surgical wounds with diameters of 7 mm, 1 cm away from both sides of the backbone, and 5 cm away from each other were made with a 7 mm biopsy punch. Using this excisional wounding method, the epidermal, dermal, hypodermal and panniculus carnosus layers were removed completely (Luisa and DiPietro 2003).

Treatments

After the making of surgical wounds, all rats were randomly coloured with a non-toxic colour and divided into three groups. To Group A, an ointment comprising 1.5% cinnamon and to Group B an ointment of 3% cinnamon were administered. Group C as the control group did not received any treatment and finally Group D as the reference standard group received the blank placebo. All rats were monitored daily for 14 days and any wound fluid or evidence of infection or other abnormalities were noted. The study was approved by the ethics committee for animal experiments.

Histopathological study

Samples of healing tissue were taken on Days 3, 6, 9 and 14 from all four groups of animals and were processed for histological study. The samples were fixed in formalin and installed on slides, stained with Hematoxylin and Eosin and then analysed under a light microscope. The recorded parameters were scars, inflammatory cells, angiogenesis, congestion, fibroblast collagen density, fibrin and fibroblastic aggregation.

Statistical analysis

All values are reported as mean ± S.E.M. The statistical differences among groups were assessed using the Duncan multiple range test and analysis of variance (ANOVA). A value of $P < 0.05$ was considered significant. Statistical analysis was performed using the SAS for Windows software.

RESULTS

A: 1.5%, B: 3% C and D: control and placebo.

In this study the wound healing process in the control and placebo groups proceeded almost identically.

Angiogenesis, which is important factor in the first days of healing, was significantly higher in the treated group than in Group C on Day 3. Strikingly, epithelization in Group B was elevated significantly

Table 1. Effect of ethanolic extract of ceylon cinnamon on excisional wound model in mice

No.	Groups	Wound area (mm)			
		Day 3	Day 6	Day 9	Day 14
1	control	6.94 ± 0.02	6.81^{a} ± 0.02	6.21^{a} ± 0.03	5.2^{a} ± 0.07
2	placebo	6.93 ± 0.01	6.73^{b} ± 0.05	6.07^{b} ± 0.02	4.89^{b} ± 0.09
3	1.5%	6.92 ± 0.04	6.69^{b} ± 0.03	4.95^{c} ± 0.07	3.52^{c} ± 0.05
4	3%	6.91 ± 0.04	6.21^{c} ± 0.03	4.47^{d} ± 0.08	2.2^{d} ± 0.04
		ns	*	**	**

All expressed as mean and standard error mean (S.E.M). Mean in columns with different letters were significantly different (ns = not significant, $^{*}P < 0.05$ $^{**}P < 0.01$)

at Day 3, while in the other groups the epithelization rate was negative. The remaining parameters were almost identical at Day 3.

On the 6th day, epithelization in both A and B was higher than in Group C and wound healing was proceeding towards the chronic phase. However, in Group C healing was still in the acute phase. On the 9th day, there was no difference in parameters between the A and B Groups, in which wound healing was significantly better compared to Group C. Finally, the progression of wound healing on Day 14 in the B Group was better than in the A Group owing particularly to the lower levels of congestion and oedema in Group B. Overall, wound healing proceeded most effectively in Group B, in large part due to the excellent epithelization from the beginning of the wound healing process.

Wound enclosure

Overall, we show here that the topical application of Ceylon cinnamon extract at different concentrations (1.5 and 3%) elicited a significant ($P < 0.05$) reduction in the wound area (Table 1, Figure 1). On Day 3 there were no differences among the groups but three days later at Day 6 there were significant differences among all groups, although the placebo (6.73 ± 0.05) and 1.5% treatment (6.69 ± 0.03) groups showed no differences. On the 9th day of the study a significant difference was seen between the treatments groups (4.95 ± 0.07 and 4.47 ± .08), and the placebo (6.07 ± 0.12), and control (6.21 ± 0.03) groups. Finally, on the last day of the experiment the effectiveness of the treatment extract was completely obvious and, in particular the group administered the 3% treatment extract showed a huge contraction in wound size (2.2 ± 0.04) compared to the other groups. However, the 1.5% treatment group also had an effective impact on wound enclosure (3.52 ± 0.05).

DISCUSSION

The present study was carried out to evaluate the effects of cinnamon extract on the healing of exper-

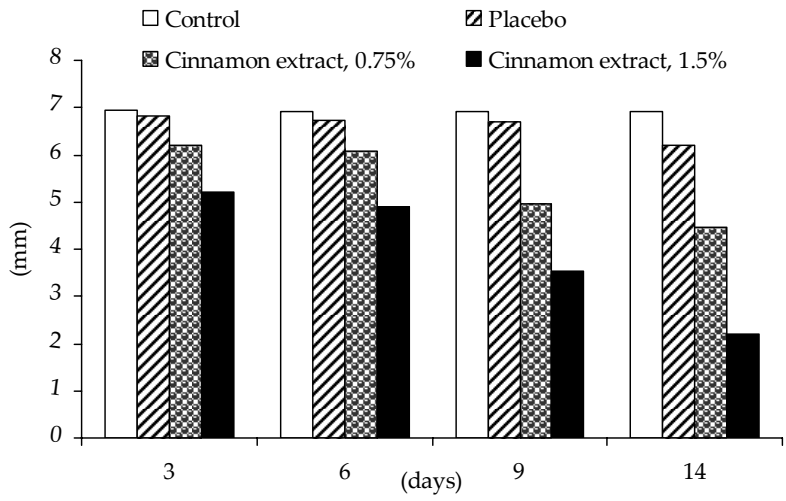

Figure 1. Shows the wound area (mm) of different groups over a period of 14 days

imentally 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, intervention at any one of these phases using drugs could eventually either promote or inhibit one or all phases of healing (Vinothapooshan and Sundar 2010).

Herbal drugs have come to be increasingly used worldwide because of their effectiveness and safety. Cinnamon is a medicinally useful plant with many therapeutic properties Stefan et al. (2009) reported an anti-oxidant activity of cinnamon essential oil while another study indicates that ceylon cinnamon essential oil inhibits hepatic 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase activity in rats, and suppresses lipid peroxidation via the enhancement of hepatic antioxidant enzyme activity (Lee et al. 2003). These characteristic antioxidant properties of cinnamon may serve to promote healing at the wound site. It is well known that diverse mechanisms may be involved in the genesis of inflammatory reactions. Eugenol showed similar anti-inflammatory effects to a COX antagonist (indomethacin; Huss et al. 2002; Kim et al. 2003), which helps to accelerate wound healing. It has been shown that cinnamon extract, be it water or ethanol-based, as well as its marked antioxidant properties, may also exert antimicrobial effects (Anderson et al. 2004; Blomhoff 2004; Kanuri et al. 2009), which may constitute a further basis for cinnamon's wound healing activity.

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 moulds and dermatophytes as well as the eggs and adult females of human head louse (Ooi et al. 2006). These constitute yet further properties of cinnamon which may serve to accelerate wound enclosure.

Thus, all these reported characteristics of cinnamon extracts promote wound healing and also more effective and faster wound contraction. In fact, anti-inflammation, antioxidant and antimicrobial are the main properties of remedies which accelerate wound healing. In conclusion, in the present study we demonstrate that cinnamon is effective in wound healing and that it improves conditions at the wound site to promote better enclosure and healing. We think that the basis of this wound healing ability likely lies in cinnamon's anti-inflammatory, antioxidant and antimicrobial functions which are mostly due to its essential oil, specially eugenol and cinnamoldeyde.

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