Wound healing activity of Cassia occidentalis
in albino Wistar rats

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Abstract
The wound healing property of methanolic crude extract of Cassia occidentalis L. leaves and a pure compound Chrysophanol isolated from it was evaluated in excision, incision and dead space wound models. The parameters studied included rate of wound contraction and the period of epithelialization in excision wound model. Tensile strength in incision wound model and granulation tissue dry weight in dead space model were assessed along with histopathological examinations. Chrysophanol was found to possess significant wound healing property than methanol crude extract. This was evident by decrease in the period of epithelialization, increase in the rate of wound contraction, skin breaking strength, granulation tissue dry weight content and breaking strength of granulation tissue. Histopathological study of the granulation tissue showed increased collagenation when compared to control group of animals. C.occidentalis can be a good source of wound healing compound.

Keywords: C. occidentalis, Chrysophanol, wound healing.

INTRODUCTION

The processes of wound healing involve a variety of biological responses, such as an acute inflammation, cellular proliferation and a contraction of the collagen lattice formed (Bodeker and Hughes, 1998; Suressh Reddy et al., 2002). Wounds caused by environmental insults such as mechanical and chemical injuries, may extend from the epidermis deep into the muscles depending on the severity of damage. Wound thus caused can be healed by a spontaneous process in the organism through a cascade of events, which starts by switching on various chemical signals in the body; this facilitates the restoration of anatomical continuity and function. While partial thickness wound heals by mere epithelialization, the healing of full thickness wound which extends through the entire dermis involves more complex well-regulated biological events resulting in the formation of hypertrophic scars (Clark, 1993; Sumitra et al., 2005) The healing process begins with the clotting of blood and is completed with re-modelling of the cellular layers of the skin. However, the wound healing process may be prevented by the presence of ROS (Reactive oxygen species) or microbial infection, since the type of cells to be first recruited to the site of injury is the neutrophil which is produced in response to cutaneous injury (Gupta et al., 2002) and which has a role in antimicrobial defense and may cause cellular damage by peroxidation of membrane lipids (Russo et al., 2002; Choi et al., 2009). Appropriate method for healing of wounds is essential for the restoration of damaged tissue anatomical continuity and disturbed functional status of the skin (Meenakshi et al., 2006). Research on wound healing agents is one of the developing areas in modern biomedical sciences and many traditional practitioners across the world particularly in countries like India and China have valuable information of many plants for treating wounds and burns (Kumar et al., 2007).

Nayak et al. (2006, 2009) have studied the wound healing potential of many medicinal plants from Trinidad. Many scientists around the world have recently reported on wound healing activity of some medicinal plants viz., Portulaca oleracea L. (growing in Jordan) in Mus musculus JVI-1 (Rashed et al., 2003), antioxidant and wound healing activity of Tephrosia purpurea (Marwah, et al., 2006) and n-hexane fraction
of methanol extract of *Centaurea sadleriana* (Csúpor et al., 2009).

*Cassia occidentalis* L. is extensively used in the indigenous and folklore medicine systems to treat hepatotoxicity. In Unani medicine it is used as an antidote of poisons, blood purifier, expectorant, anti-inflammatory agent and a remedy for the treatment of liver diseases (Kabiruddin, 1951). It is also an important ingredient of several polyherbal formulations marketed for liver diseases. Its roots, flowers, seeds and leaves have been employed in herbal medicine around the world (Kirtikar and Basu, 1933; Chopra et al., 1956; Nadkarni, 1976) for a variety of purposes such as laxative, expectorant, anti-malarial (Tona et al., 2001), hepatoprotective (Jafri et al., 1999), relaxant (Ajagbonna, 2001), anti-inflammatory (Sadique et al., 1987), analgesic, vermifuge and febrifuge. This study was aimed at investigating the wound healing effect of methanol extract of *C. occidentalis* and its isolated compound Chrysophanol in Albino Wistar rats.

**MATERIALS AND METHODS**

**Extraction, separation and purification of the compound**

Fresh leaves of *C. occidentalis* L. were collected from the premises of Loyola college Chennai, and it was authenticated by Dr. Ayyanar, taxonomist, Entomology research institute Chennai; a voucher specimen (ALC DB-27) was preserved. The leaves were shade dried, coarsely powdered and was used for extraction. The dried leaf material (3kg) was extracted with methanol (w/v 1:3) three times at room temperature for a week. The methanol crude extract was combined and concentrated to yield a residue (140 g) which was subjected to successive solvent partitioning to give chloroform (26g), methanol (114 g) soluble fractions. The methanol fraction showed wound healing activity. Thus, the methanol extract (100 g) was chromatographed on a silica gel column (100-200 mesh) using a gradient solvent system of chloroform: methanol (100: 0; 95:5; 90:10, 85: 15…………0: 100) to give 25 fractions. The fractions were combined based on the TLC profile. Finally 7 fractions were obtained. The fractions were screened for wound healing activity. Fourth fraction showed good activity and its yield was 10.6 g. It was further purified by column chromatography using chloroform: methanol solvent system. Fraction 2 showed single (yellow colour) spot on TLC over silica gel with CHCL3; methanol (9:1) as the developing solvent system. The yield was 4.2 g. The spot turned pink color on exposure to ammonia vapor or spraying with 5% alcoholic sodium hydroxide. It indicated the presence of anthraquinones. The pure compound was subjected to IR 1H and 13C NMR and MASS spectrum analyses. Mass spectra were taken on a JEOL-GC mate spectrometer. IR spectra were taken on a Perkin Elmer FT-IR (Spectrum One) spectrophotometer. In Nuclear Magnetic Resonance Spectroscopy Tetra Methyl Saline (TMS) was used as standard, which showed chemical shift value at zero on the δ scale. 1H and 13C NMR spectra were recorded with a JEOL 500 MHz FT NMR spectrometer (¹H), 500 MHz (¹³C) at Indian Institute of Technology, Chennai, India.

**Experimental design**

Healthy male Albino rats weighing 110-150g were procured from National Institute of Nutrition, Hyderabad, India, and the animal experiments were performed in accordance with legislation on welfare (CPCSEA). The animals were fed with a standard laboratory diet (Godrej Agro Food Industries, Bangalore, India) and were provided with clean drinking water ad libitum. The animals were aclimatized to laboratory conditions for a week prior to the initiation of the experiment. Twelve hours before the start of the experiment, rats were deprived of food, but given free access to water.

**Acute toxicity studies**

The acute toxicity of the extract was conducted by the method of Lorke (1983). Albino Wistar rats were divided into five groups, each divided in to six rats each, with each group receiving graded dose of 200, 400, 800, 1200 and 1600 mg/kg b.w of methanol crude extract and 250, 500, 750 and 1000 mg/kg b.w Chrysophanol orally. The animals were allowed access to food and water, and behavioral changes were observed over a period of 72 hours for sign of acute toxicity. The number of mortality caused by the compound within this period of time was observed in order to fix the lethal dose (LD₅₀).

**Excision wound study**

Excisions of wounds were made as described by Morton and Malone (1972). Animals were anaesthetized with anaesthetic ether and placed in operation table in its natural position. A circular wound of about 5cm was made on depilated ethanol-sterilized dorsal thoracic region of rats. Male Albino Wistor rats weighing 110-150g were divided into four groups of 6 rats each, Group I animals were considered as the control; Group II animals served as reference standard and were treated with Soframycin ointment; Group III animals were treated with 500 mg/kg body weight of methanol crude extract; and IV Group animals were treated with 100 mg/kg body weight of Chrysophanol. Methanol crude extract, Chrysophanol and Soframycin were topically applied once a day, till the epithelialization was complete. The wound contraction was studied by tracing the raw wound area
Wound healing activity of *Cassia occidentalis* subsequently on day 1, 4, 8, 12, 16, 18 and 21 on graph paper. Scar residue, area and time for complete epithelialisation were also measured. The percentage of wound closure and period of epithelialisation were recorded.

**Incision wound study**

The method of Ehrlich and Hunt (1968) was adopted for incision wound study. The animals were anesthetized under light ether and the back of the animal was shaved and washed with spirit. 6 cm long paravertebral parallel incision was made through the entire thickness of the skin on right side of vertebral column with the help of a sharp blade. The wounds were closed with interrupted sutures 1 cm apart. Male Albino Wistar rats weighing 110-150g were divided into four groups, each group containing six animals. Group I animals were considered as the control; Group II animals served as reference standard and treated with Soframycin ointment; Group III animals were treated with 500 mg/kg body weight of methanol crude extract; Group IV animals were treated with 100 mg/kg body weight of Chrysophanol. Methanol crude extract, Chrysophanol and Soframycin were topically applied once a day. The wounds were closed with interrupted sutures, which were removed on day 8 of the wounding. Wound breaking strength was measured on day 10 by adopting continuous constant water flow technique as described by Lee (1968); the breaking strength was expressed as the minimum weight of water necessary to bring about gaping of the area. Three such readings were recorded for a given incision wound and an average of six readings in each animal was used to calculate mean of breaking strength.

**Dead space model**

In Dead space model, male Albino wistar rats were divided into three groups, each group containing six animals. Group I served as control, which received vehicle (carboxymethyl cellulose) for 10 days. The animals of group II and III received oral suspensions of methanol crude extract 200 mg/kg body weight and Chrysophanol 30 mg/kg body weight for 10 days respectively. Under light ether anesthesia the wounds were inflicted by implanting sterile cylindrical grass piths measuring 2.5 cm in length and 0.3 cm in diameter in the groins (Turner 1965). The wounds were sutured and rats returned to individual cages after recovery from anesthesia. Excision of the granuloma from the surrounding tissue was performed on the 10th post wounding day. Granuloma surrounding one grass pith was excised and dried at 60°C overnight to record the dry weight of granulation tissue which was expressed as mg/100 g body weight as suggested by Dipasquale and Meli (1965). The granulation tissues were trimmed so as to obtain rectangular strip of 1.5 cm x 8 mm to measure its breaking strength, while the other was preserved in 10% formalin for histological examination. (Drury et al., 1980).

The animals were sacrificed after the experiment, and the healed regenerated tissues were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 hrs and processed for paraffin embedding. Sections of 5μ thickness were taken using a microtome. The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin (Galigher et al., 1971) and subjected to histopathological examination.

**Statistical analysis**

All the data were expressed as Mean±S.D. Tukey-Kramer multiple comparison test and one way analyses of variance (ANOVA) were performed. A value of P<0.05 was considered significant.

**RESULTS**

**Identification of compounds based on spectral evidence**

The purified compound was subjected to IR, 1H and 13C NMR and MASS spectral analysis. The compound was obtained as an yellow powder. The EI-MS spectrum showed a molecular ion peak at m/z (rel. int.): 253 [M+1]+ : 234,223, 194, 177, 166, 149, 136, 113, 102. IR spectrum showed chelated hydroxyl (3435 cm−1), chelated carbonyl (1627 cm−1), unchelated carbonyl (1676 cm−1), and aromatic system (3055,1606, 1568,1475 and 1453, 903,868, 839,815 and 753 cm−1): The 1H-NMR spectrum showed δH-2 = 7.05 (1H, brs), H-4 = 7.59 (1H, brs), H-5 = 7.77 (1H, brs), H-6, J= 8.5 Hz). The 13C NMR: δC Found: 162.65 (1), 124.32 (2), 149.32 (3), 121.31 (4), 133.56 (4a), 119.89 (5), 136.91 (6), 124.51 (7), 162.36 (8), 115.80 (8a), 192.42 (9), 113.66 (9a), 181.85 (10), 133.19 (10a), 22.24 (CH3). These spectral data suggested that the compound was an anthraquinone derivative. The structure of the compound was determined to be 1,8- dihydroxy-3-methyl anthraquinone (chrysophanol) (Fig. 1). The NMR spectral and physical data of compound were in good agreement with those reported in a previous paper (Kim et al., 1998).

![Figure 1: Chrysophanol isolated from *C. occidentalis*.](image-url)
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The results of the oral acute toxicity of Chrysophanol and methanol crude extract indicated that, there was no mortality or any toxic reaction until the end of the study. Therefore the LD_{50} value of the methanol crude extract was fixed as 1600 mg/kg b.w in methanol crude extract and 1000 mg/kg b.w in Chrysophanol.

![Figure 2: Macroscopic observations of excision wounds on day-4](image)

A- Group I - Normal control, B- Group II – Soframycin, C- Group III – Methanol crude extract of *C. occidentalis*, D- Group IV – Isolated compound chrysophanol

![Figure 3: Macroscopic observations of excision wounds on day-16.](image)

A- Group I - Normal control, B- Group II - Soframycin, C- Group III – Methanol crude extract of *C. occidentalis*, D- Group IV – Isolated compound chrysophanol

Rats treated with the pure compound Chrysophanol showed faster wound contraction such as 29.22% on day 4, 49.30% on day 8, 72.56%, on day 12 and 95.62% on day 16, with a short period of epithelialization (Table 1 [Supplementary data]). The results were similar (p<0.01) to the healing potential of soframycin treated group. Methanol crude extract treated groups also showed good wound contraction (p>0.05) i.e. 33 % of wound contraction on day 4 (Fig. 2), 42.74% of wound contraction on day 8, 65.20% of wound contraction on day 14 and 82.50% of wound contraction on day 16 (Fig. 3). Histopathological section of wounds exposed to Chrysophanol showed increased regenerated tissue, epithelialization, fibroblasts and new blood vessel formation. The rats treated with methanol extract promoted epithelialization, fibrosis with underlying inflammatory cells predominantly lymphocytes, fibroblasts and new blood vessels. The rats treated with soframycin showed normal architecture. The control rats showed ulceration, necrotic debris, neutrophils, lymphocytes and fibroblasts (Fig. 4).

![Figure 4: Microscopic observations of excision wounds.](image)

A- Group I - Normal control, B- Group II – Soframycin, C- Group III – Methanol crude extract of *C. occidentalis*, D- Group IV – Isolated compound chrysophanol

![Figure 5: Microscopic observation of incision wounds.](image)

A- Group I - Normal control, B- Group II – Soframycin, C- Group III – Methanol crude extract of *C. occidentalis*, D- Group IV – Isolated compound chrysophanol

The incision wound study was also carried out to measure the tensile strength of the regenerated tissue. Chrysophanol treated group showed significant breaking strength (417.40±0.57) which was more or less similar to soframycin treated group (421.23±0.12)
Wound healing activity of *Cassia occidentalis* (Table 2 [Supplementary data]). Methanol crude extract treated rats also showed better breaking strength (381.04±0.83) when compared to normal group. Histopathological section of incision wounds exposed to Chrysophanol revealed granulation tissue with plenty of fibroblasts and thick bundles of collagen tissue, which was comparable to that of reference drug soframycin. The rats treated with methanol extract showed granulation tissue macrophages and fibroblasts. Soframycin treated group of rats showed normal histopathological architecture. The control group of rats showed a mild re-epithelialization with chronic inflammatory cells (Fig. 5).

**DISCUSSION**

Many studies indicate that plant products are potential agents for wound healing and largely preferred because of the absence of unwanted side effects and their effectiveness (Jagetia *et al.*, 2003). In this report, we show for the first time that the topical application of methanol extract of *C. occidentalis* and a pure compound Chrysophanol, an anthraquinone derivative promoted wound healing activity in excision, incision and dead space models in rats.

Topical application of Chrysophanol improved wound contraction and closure, and the effects were distinctly visible starting from 4th post-wounding day. Methanol extract also improved the wound contraction and significantly shortened the period of epithelization. In incision wound model Chrysophanol and methanol extract treated group showed significantly increased breaking strength, which was more or less similar to the result of soframycin treated group and it was less in control animals. These results were in agreement with that of a previous study of Oomen *et al.* (2000) who reported that the external application of *Hydnocarpus* spp. paste significantly shortened the epithelialization.

The overall performances of topical application of plant extracts like *Centella asiatica*, *Vernonia arborea* (Manjunatha *et al.*, 2003) and *Moringa oleifera* (Hukkeri *et al.*, 2006) were almost close to the effectiveness of *C. occidentalis*.

In dead space model significant increase in dry weight of granulation tissue and tissue breaking strength was recorded in the animals treated with oral administration of Chrysophanol compared to methanol crude extract. Thus it appears that significant immune stimulation is involved in the wound healing processes. Dead space wound model was used to study the effect of the extracts on granulation and the healing process. Such wound models have been employed for qualitative and qualitative studies of wound healing studies. Breaking strength of granuloma indicated increased collagen maturation (Taranalli *et al.*, 2004). This result was in agreement with that of a previous study by Manjunatha *et al.* (2005) who reported that treatment with methanolic extract of *Vernonia arborea* significantly increased the breaking strength of granulation tissue in dead space wound model. It is also in agreement with Hukkeri *et al.* (2006) who reported that the rats treated with ethanolic extract of *Moringa oleifera* granuloma showed significant breaking strengths of granuloma tissue, compared to untreated control group.

**CONCLUSION**

Our study clearly showed that the methanol crude extract and the pure compound isolated from *C. occidentalis* promoted the wound healing activity significantly in all the three models (excision, incision and dead space). High rate of wound contraction, decrease in period of epithelialization, increased dry weight of granulation tissue, high breaking strength were observed in Chrysophanol treated group compared to methanol crude extract treated group.
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*Cassia occidentalis* had wound healing property. This could be a good source of wound healing compound.

References


