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Wound healing activity of methanolic extract of the leaves of *Crataeva magna* and *Euphorbia nerifolia* in rats

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ABSTRACT

Crataeva magna and *Euphorbia nerifolia* have been shown to possess hepatoprotective activity and antioxidant property. The present works with these plants were undertaken with the premise that the drug promoting hepatoprotective activity and radical scavenging property could have effect on wound healing also. The wound healing property of the methanolic extract of the leaves of *Crataeva magna* (CNM) and *Euphorbia nerifolia* (ENM) were chosen to investigate in excision and incision wound models. The methanolic extracts of the two plants at the dose of 500 mg/kg/day by topically applying method. Healing was assessed by the rate of wound contraction, time until complete epithelialization, incision breaking strength, estimation of hydroxyproline and histopathological parameters. Complete wound contraction was shown by both the plants in the study period. In excision and incision wound models, all the test drugs showed significant ($P<0.001$) wound healing activities compared to the control. Moreover the CNM was found to possess significant wound healing activities than the ENM and had been observed to have equipotent wound healing activity as of the standard drug Framycetin.

INTRODUCTION

The elevation of free radical levels seen during the muscle/tissue damage is due to enhanced production of free radicals and decreased scavenging potential of the cells (Hebbar *et al*., 2013). Free radicals are reactive molecules involved in many physiological processes and wounds are physical injuries that results in variety of cellular and molecular sequelae (Savanth and Shah, 1998). Wound may extend from the epidermis deep into the muscle depending on the severity of damage. The basic principles of wound healing is a complex and intricate process initiated in respond to an injury that include restores the function and integrity of damage tissues, debriding nonviable tissue, maximizing tissue perfusion and oxygenation and proper nutrition (Whaley and Burt, 1996). Plant drugs are an indispensible part of traditional medicine. *Crataeva magna* (*C. magna*) belonging to family Capparidaceae is a multipurpose tree that is also used to increase appetite and for the treatment of various diseases e.g. rheumatism and nephrotoxicity, arthritis, urinary disorders (Shirwaikar *et al*., 2004). Leaves are deciduous 3 foliolate; petioles 3.8-7.6 cm long; leaflets 5-15 ovate, lanceolate, acute or acuminate, attenuate at the base, entire, glabrous on both surfaces, pale beneath and reticulately veined and has been reported that its methanolic extract had significant free radical scavenging property which may attribute to its phenolics, flavonoid and proanthocyanidine contents (Pattanaik *et al*., 2012).

The literature revealed that wide variety of medicinally important compounds including friedelin, diosgenin, sitosterol, butulic acid, dodecanoic anhydride, methyl pentacosanoate, kaemferol-3-O-α-D-glucoside and quercitin-3-O-α-D-glucoside have been reported from C. magna (Mantena *et al*., 2008).

The plant *Euphorbia nerifolia* (*E. nerifolia*) belonging to family Euphorbiaceae is extensively used in indigenous and folklore medicine to treat fever, vomiting, gastric irritation, inflammations and others. It is used for the treatment of various skin disorders and rheumatism. The literature revealed that *E. nerifolia* contains 24-methylenecycloartenol, euphorbol hexacosonate, Glut-5-en 3-ol, Glut-5(10)-en-1-one, Glut-5-en-3 beta-yet-acetate, taraxerol, friede-lan-3 alpha-ol, and -3 beta-ol and amyrin, dodecanoic anhydride, methyl pentacosanoate, kaemferol-3-O-α-D-glucoside and quercitin-3-O-α-D-glucoside (Nadkarni 1954 and Ilyas *et al*. 1998). There are no scientific studies in support of this traditional claim for its wound healing activity.

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Hence, an attempt was made to investigate wound healing potential of the leaves of the methanol extract of the plant *C. magna* (CNM) and *E. nerifolia* (ENM).

MATERIALS AND METHOD

Plant material

The fresh leaves of *E. nerifolia* and *C. magna* were collected during month of December 2012 from Balesore district, Odisha, India. The plant material was taxonomically identified and authenticated by botanist. A voucher specimen has been preserved in the herbarium of the Department of Pharmacognosy, School of Pharmaceutical Sciences, and Siksha O Anusandhan University, India. The leaves were picked and washed with water to remove dust particles and shade dried (25 °C ± 2 for 14 days).

Preparation of plant extract

The dried leaves of *C. magna and E. nerifolia* were ground to a coarse powder in a mechanical blender and each of 500 g put into in to a soxhelt apparatus and was extracted sequentially with petroleum ether, chloroform and methanol (Pattanaik *et al*., 2013). The yield of methanolic extract of each plant was found to be 12 % for *C. magna* and 10.5% for *E. nerifolia*.

Preliminary Phytochemical Test

The preliminary phytochemical test of the leaf extracts for the presence of alkaloids, flavonoids, terpenoids, glycosides, saponins and tannins was performed by the standard methods (Plummer 1984 and Pollock and Stevense *et al*. 1965).

Experimental animals-Adult albino Wistar rats (150 - 200 gm) of either sex (OUAT, Bhubaneswar) used in the experiment were allowed to acclimatize to the laboratory conditions for 7 days in acrylic cages prior to commencement of the experiment with 12hr day and night schedule at a temperature of 26±4 c. The animals were maintained with standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee and animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA 07/09/IAEC/SOAU).

Wound Models

Excision wound model

For excision wound study, the male albino rats were divided into four groups, each comprising six animals (Nayak *et al*., 2007 and Patel *et al*., 2009). They were starved for 12 hrs prior to wounding. Under light ether anesthesia, wounding was performed aseptically. A circular wound of about 5 cm diameter was made on depilated dorsal thoracic region of each animal, washed with normal saline and observed during the study. Wounds were traced on $1mm^2$ graph paper on the day of wounding and subsequently on 4, 8,12,16,18 and 21 days until healing processes were complete. Scar residue, area and time for complete epithelisation were recorded. Changes in wound area were calculated, giving an indication of the rate of wound contraction.

Treatment Group (Gr):

Group 1: control group only treated with vehicle (2% gum acacia) topically.

Group 2: standard group was treated with Framycetin (1%) ointment (Soframycin skin ointment, Aventis).

Group 3: Treated with 500 mg/kg of *E. nerifolia* methanolic extracts.

Group 4: Treated with 500 mg/kg of *C. magna* methanolic extracts.

No medication other than the extracts was given to the test groups.

Incision Wound Study

Animals were anaesthetized with light ether and the back of the animal was shaved and washed with spirit one mid dorsal incision (2 cm) was made through the full thickness of the skin on the middle of the vertebral column with the help of a sharp sterile blade. Wounds are closed using 4-0 number silk thread using a (No. 11) bend needle in interrupted sutures, 1cm apart. The sutures were removed on 7^{th} day. Wound breaking strength (WBS) was measured on 10^{th} post wounding day. The breaking strength was measured with a manually operated instrument in terms of weight (Lee 1968). The animals were treated with drugs as in excision wound model except that the treatment was given up to 9th day.

Estimation of hydroxyproline content

Under light ether anesthesia the wounds were inflicted by implanting sterilized grass piths of 2.5 cm length and 0.3 cm diameter in the region of axilla and groin to induce granuloma formation. The wounds were sutured and mopped with a saline swab.

The animals were treated with drugs except the control group for 9 days from the day of wounding. The granuloma tissues formed on implanted piths were dissected out on the 10th post wounding day. One of the pith was used to determine the tensile strength by the manually operated instrument in terms of weight, while the other pith containing the granuloma tissue was used for estimation of hydroxyproline content (Neuman and Logan 1950).

Histopathology Study

The histopathology study was carried on the section of granuloma tissue to observe the stages of keratinization, fibrosis, collagenation, epithelization and neovascularisation.

Statistical Analysis

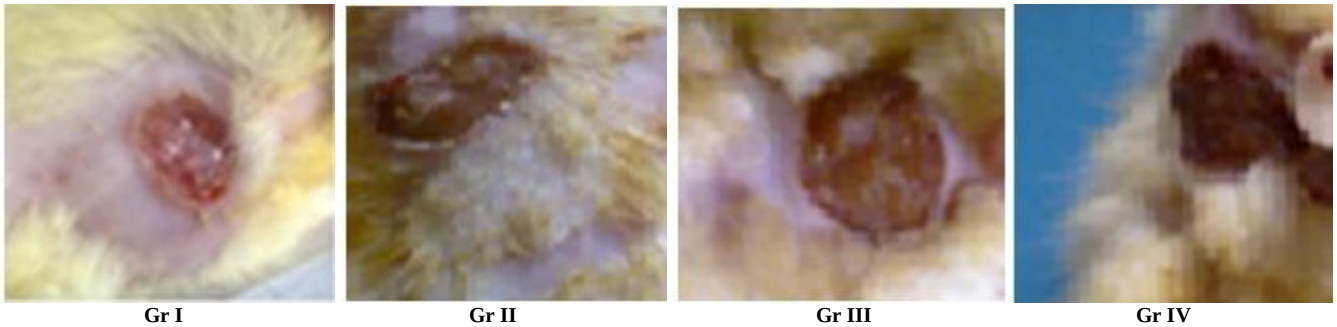
All the experiments were carried out in triplicates and the results are reported as mean ± standard error. The data were analyzed by one–way analysis of variance (ANOVA) and Tukey post test. P-values < 0.05 were considered significant.

Table. 1: Wound healing effect of methanolic extracts of *E. nerifolia (ENM) and C. magna (CNM)* on excision wound model.

Treatment Group (Gr)	Epithelization Period (days)	Wound closure (% of original area Treatment) in mm^2 on day						
		1	4	8	12	16	18	21
Control Gr-I	17.4±0.81	15.22±0.07	32.14±0.28	40.73±0.41	59.38±1.23	79.11±1.86	85.66±2.78	98.46±2.89
Framycetin 1%w/w Gr-II	12.5±0.43	19.28± 0.06	39.32±0.19	86.44±1.01	99.82±2.79	100.00±1.86	-	-
CNM 500 mg/kg Gr-III	12.9± 0.51	18.39±0.09	37.81±0.28	80.35±1.72	98.91±2.68	100.00±2.55	-	-
ENM 500 mg/kg Gr-IV	13.2±0.48	17.91 ±0.09	36.78±0.26	78.53±1.69	91.34±2.32	94.33±2.05	100.00±1.93	-

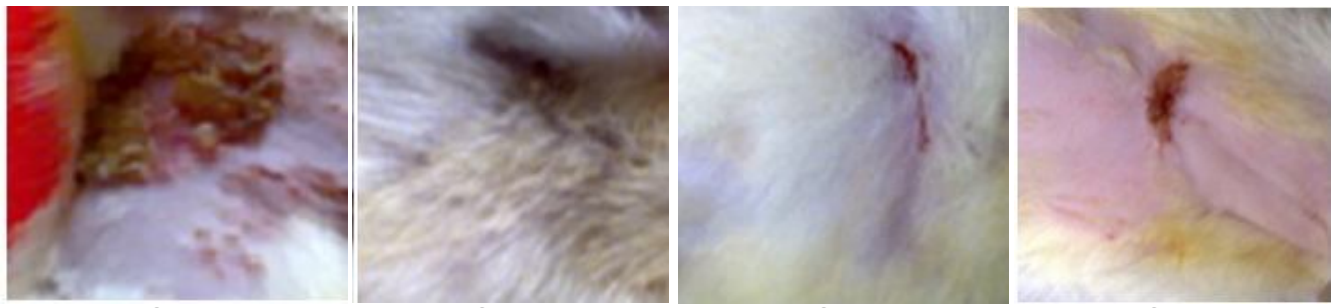
Table. 2: Wound healing effect of methanolic extracts of *E. nerifolia (ENM) and C. magna (CNM)* in incision and dead space wound models.

Treatment Group (Gr)	Incision breaking strength(g)	Hydroxyproline (µg/100g)
Control Gr-I	389.87±3.86	1401.22±0.98
Framycetin 1%w/w Gr-II	712.23±2.84	2439.61±0.87
CNM 500 mg/kg Gr-III	709.19±3.54	2310.16±1.19
ENM 500 mg/kg Gr-IV	700.31±3.67	2198.78±1.20



Gr I **Gr II** **Gr III** **Gr IV**

Fig. 1: Macroscopic Observation of Excision Wounds macroscopic observation of excision wounds.



Gr I **Gr II** **Gr III** **Gr IV**

Fig. 2: macroscopic observation of excision wounds on day-16 Gr I – normal control, Gr II-framycetin 1%w/w, Gr III- CNM500mg/kg. Gr IV-ENM500 mg/kg.

RESULTS AND DISCUSSION

Preliminary Phytochemical Test

The preliminary phytochemical analysis of the methanolic extracts of *Crataeva magna and Euphorbia nerifolia* showed the presence of the major phytoconstituents like tannins, saponins, flavonoids, alkaloids, cardiac glycosides, terpenoids and reducing sugars. Moreover there are plenty of research studies proved the potent wound healing activities was due to the presence of flavonoids and terpenoids which serve as a defensive agent against any pathogen (Hostettmann and Marston, 1995).

Wound healing activity

Excision wound model

Traditional medicines are always a better choice for the wound healing purpose because of their widespread availability, non-toxicity, absence of unwanted side effects and their effectiveness as crude preparations. Approximately one-third of all traditional medicines in use are for the treatment of wounds and skin disorders, compared to only 1–3% of modern drugs. Reports about medicinal plants affecting various phases of the wound healing process, such as coagulation, inflammation, fibroplasia, collagenation, epithelization and wound contraction are abundant in the scientific literature. The result of the excision wound healing model revealed that all the two groups of animals received the extracts of the two plants showed increased wound contraction continuously from 2^{nd} day to 21^{st} day or the day till they healed. The mean percentage closure of wound area was calculated on the 1, 4, 8, 12, 16, 18 and 21 post wounding days. All readings are found to be statistically significant and comparable with control (Table 1). The animals treated with 500 mg of methanolic extract of CNM shown the healing of wound completed within 16 days (Fig. 1 and 2) compared to 500 mg of methanolic extract of ENM took 18 days for complete wound. The epithelization period was found to be less 500 mg of methanolic extract of CNM (12.6 days) comparatively which was similar to Framycetin treated group (12.5 days). Hence the 500 mg of methanolic extract of CNM has similar potency of action like standard drug Framycetin in wound

healing. Wound healing is the process of epithelial renewal which involves the proliferation and migration of epithelial cells towards the center of the wound (Cotran *et al*., 1994; Mohan, 2005).

Incision Wound model

The result of the incision wound healing model revealed that the breaking strength was found to be higher in CNM treated group 709.19 g and it was equipotent to standard drug Framycetin group 712.23 g (Table 2). In incision wound model all the test drugs shown to have significant wound healing activity.

Hydroxyproline content

The CNM showed (Table-2) increased hydroxylproline content compared to the ENM and was significant wound healing property compared to Control.

Histopathological examination confirmed the mechanism of wound healing by increased deposition of collagen, fibroblast on the granulation tissue and revascularization. The CNM treated group showed similar stage of keratinization which is comparable to the effect of the standard drug Framycetin.

CONCLUSION

The present study explored the wound healing properties of leaves of the methanolic extracts of *C. magna* and *E. nerifolia* in experimental models. Here, both the plants shown to have significant wound healing activities in excision and incision models. The histopathological examination provided additional evidence for the experimental wound healing studies. It may be attributed to its anti-oxidant activity and presence of secondary metabolites which could be a good source of wound healing compound.

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