Topical Anti-inflammatory and Wound Healing Activities of Herbal Gel of Ziziphus nummularia L. (F. Rhamnaceae) Leaf Extract

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Abstract: In this study, the anti-inflammatory and wound healing effects of Ziziphus nummularia (sedar) leaf alcoholic extract formulated gel on albino rats were determined. Carbapol and Dimethyl Sulfoxide (DMSO) were used to prepare gels containing 20 and 30% the ethanolic extract of Z. nummularia leaves. Topical application of 0.5 g gel formulation of the extract were used separately for anti-inflammatory and wound healing activities in wister albino rats by using Carrageenan induced paw edema and excision wound model, respectively. The extract gel formulations produced significant reduction of carrageenan induced paw edema and more rapid rate of wound reduction when evaluated with base control gel. The formulated gel 30% was relatively more active than marketed formulation (Betadine®) for wound repair. The anti-inflammatory and wound repairing study rationalize the traditional claim of Z. nummularia leaves extracts.

Key words: Ziziphus nummularia, gel formulations, anti-inflammatory, wound healing, diclofenac sodium, 10% w/w povidone-iodine

INTRODUCTION

Ziziphus nummularia family Rhamnaceae is one of five species belonging to the genus Ziziphus which are native of Saudi Arabia. It is commonly known in Arabic as seder. Moreover, it is routinely used in traditional medicine of many Asian countries. Fruits of Z. nummularia are used as laxative and astringent, while its leaves are used scavies, boils (Sher et al., 2010) and as expectorant (Ullah et al., 2010). The Charu (Rajasthan, India), rural people frequently used this plant for various disease conditions such as gastritis, rheumatism, diarrhea, fever, carbuncles, ulcers, abscess, boils and wounds as an alternative medicine (Parveen et al., 2007). It is used for lung inflammations and as anti-congestion at the traditional Arab herbal medicine in the eastern region of the Mediterranean (Azuizah et al., 2006). This plant is also used as antidiarrheal and anti-infective for skin by Local Community of Jodhpur District of Thar Desert (Goyal et al., 2011). Root bark powder mixed with cane sugar is taken with milk (3-5 g twice a day) to induce abortion (Shah et al., 2009). The plant is documented at western Kachchh, Gujarat (India) ethno medicinal plants for blood purification and vomiting (Patel et al., 2010). Antitumor activity is also documented for one naphthoquinone compound isolated from the plant as adjuvant for radiation therapy (Kumar et al., 2002). Leaves and seeds of this plant are used by ethno veterinarians at Al-Qassim region, Kingdom of Saudi Arabia for treatment of old wound of camels (Abbas et al., 2002). The paste of the plant leaves is used also by the ethno-veterinary medicine at the greater Cholistan desert (Pakistan) to cure the itch and chronic ulcerous wounds in animals (Khan, 2009). The plant is being used as antihelmentic in ethnoveterinary medicinal system of Pakistan (Bachaya et al., 2009, Nair and Chanda, 2006). Methanol extract of the aerial parts of this plant showed antioxidative, antibacterial and antifungal activities (Mahasneh, 2002, Chanda et al., 2011). This plant contains alkaloids (Shah et al., 1989), glycosides (Srivastava, 1984), flavonoids, tannins, steroids and saposomes (El-Shanawani, 1996), naphthoquinones (Kumar et al., 2002). The present study was planned to justify and validate the traditional anti-inflammatory and wound healing potential of Z. nummularia leaves extract in the form of gel in the experimental rats.

MATERIALS AND METHODS

Chemicals: Polyethylene glycol 200 and 4000, DMSO (Dimethyl sulfoxide), Glycerin, trigonolamine and ethanol were obtained from Merck and Co Inc (USA). Carragenaan, Carbapol-934 were supplied by Sigma (USA). Dicloflex® (diclofenac sodium gel) and Betadine® (10% w/w Povidone-Iodine).

Plant material: Ziziphus nummularia fresh leaves were collected in May, 2010 from Al-Kharj region of Saudi Arabia and were authenticated by Dr. Mohammad Atiqr Rahman, taxonomist of the
Preparation of extracts: The air dried leaves of *Z. nummularia* were powdered using grinder. The powder (500 g) was extracted with 2000 mL of 90% ethanol in a soxhlet apparatus at 70°C till exhaustion and then the solvent was filtered by using whatman-1 filter paper. The obtained extract was concentrated under reduced pressure at 40°C. The thick solution of extract was lyophilized using freeze dryer. The obtained dry powdered extract (40.5 g) was used for the experimental studies.

Animals: Wister albino rats (*n* = 200 g) were obtained from the college of Pharmacy experimental animal care centre, King Saud University, Riyadh. The animals were housed under constant temperature (22±2°C), humidity (55%) and 12 h light/dark condition. They were provided standard diet *ad libitum*. The conduct experiment and procedures exercised were approved by College of Pharmacy ethical committee, King Saud University, Riyadh, KSA.

Acute toxicity and determination of median lethal dose (LD<sub>50</sub>): LD<sub>50</sub> of the ethanol extract of *Z. nummularia* was determined according to the method illustrate by Tanko *et al.* (2008). Mice were divided into five groups (*n* = 6) and tested extract was administered orally in doses of 50 to 3000 mg kg<sup>–1</sup> body. Signs of acute toxicity and number of death per dose within 24 h were recorded and the LD<sub>50</sub> was calculated.

Preparation of topical formulations: Control base, 20 and 30% gel were separately prepared using *Z. nummularia* extract. Carbopol -1.6 g was mixed with an adequate amount of distilled water in three different beakers and kept in an oven at 100°C for 20 min to obtain a homogenous viscous mixture and then cooled to room temperature with continuous stirring (Srikarth *et al.*, 2008). Triethanolamine -10 mL was added drop-wise with continuous stirring using mechanical stirrer. A weighed amount of the extract (20 or 30 g) was added to the last two beakers separately whereas first beaker keep as such and mixed using glass rod. Other ingredients (DMSO; 10 mL, PEG-200; 1.5 g, PEG-400; 1.5 g) were added with constant stirring to prepare 100 g gel formulation.

**Anti-inflammatory activity:** The carrageenan-induced paw edema method was used for anti-inflammatory evaluation (Maswadeh *et al.*, 2006). Wister albino rats were divided into 4 groups (*n* = 5). The first (negative control) and second (positive control) groups were treated with the base gel and Diclofam<sup>®</sup> dosage form. Animals of the third and fourth groups were treated with the extract gel in concentrations of 20 and 30%, respectively. All treatments were applied to the planter surface of the left hind paw of rats by gentle rubbing of 0.5 g with the index finger. After one hour, a subplanter injection of 0.1 mL of 1% carrageenan in normal saline was injected into the treated paw of all rats. The volumes of the injected paws were measured in mL using a plethysmometer (Aptex, France) immediately before and 3 h following carrageenan injection. The percentage of anti-inflammatory activity was calculated using the following equation:

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\text{Percentage anti-inflammatory activity} = \frac{V_v - V_i}{V_i} \times 100
\]

where, *V* is the paw volume after 3 h carrageenan injection and *V<sub>i</sub>* is Initial paw volume.

**Wound healing activity:** The excision wound model was used to monitor wound contraction and wound closure time (Esimone *et al.*, 2005). Four groups of albino rats were used in the experiment, 5 animals each. At the beginning of the experiment, the dorsal skin of each rat was shaved with an electric clipper. After 24 h, all animals were anesthetized by diethyl ether and the shaved areas were sterilized with 70% alcoholic solution. A predetermined dorsal area (approximately 2.5 cm<sup>2</sup>) was excised using toothed forceps, scalpel and pointed scissors. A fresh surgical cutting edge was used for the perpendicular cut in each animal and during procedure the tension of skin was kept constant.

Wound of the first (negative control) and second (positive control) groups were treated with the base gel and Betadine<sup>®</sup>, respectively. Animals of the third and fourth groups were treated with the extract gel in concentration of 20 and 30%, respectively. Treatment with 0.5 g of the base gel, standard drug and the extract gels was performed by topical application on the wound surface once a day for 16 days. The wound areas were traced on 1 mm<sup>2</sup> graph paper immediately after the wound excision and every 4 days until healing was accomplished. The reduction in the wound size was calculated according to the following formula:
Wound contraction (%) = \( \frac{W_t - W}{} \times 100 \)

where, \( W_t \) is the wound area immediately after wound excision, \( W \) is the wound area on day \( t \).

**Statistical analysis:** Results are expressed as Mean±SEM. The data were compared between experimental groups by One-way ANOVA, student t-test using graphPad Prism 5 software. \( p \leq 0.01 \) was considered as significant.

**RESULTS**

**Pharmacological activities**

**Acute toxicity and determination of median lethal dose (LD\(_{50}\)):** All mice treated with the ethanol extracts of *Z. nummularia* in dose up to 3000 mg kg\(^{-1}\) survived during the 24 h of observation. The animals did not show visible signs of acute toxicity, therefore it suggested that the value of median lethal dose (LD\(_{50}\)) of the tested extract was much higher than 3000 mg kg\(^{-1}\).

**Anti-inflammatory activity:** In control rats, the mean reduction in paw volume at 3 h after subplantar injection of carrageenan was 2.28±0.06 mL (Table 1). Diclofenac, a potent anti-inflammatory drug and the extract gel 20 and 30% produced significant reduction of carrageenan-induced paw edema as compared to the control base gel group (0.44±0.06, 0.93±0.011, 0.68±0.09 mL, respectively). The inhibition was however less than that of the standard drug. 30% gel showed a markedly higher anti-inflammatory effect than 20% gel indicating that the efficacy is dose dependent.

**Wound healing activity:** The result of present study revealed that the topical application of the extract gel on the experimentally excised wound surface in concentration of 20 and 30% accelerate the wound healing process (Table 2). The percentage of wound contraction in the extract gel (20 and 30%) medicated groups were reduced by 63.04 and 64.56%, respectively on day 12 and 85.12 and 91.84% on day 16. The corresponding figures for the control animals were 44.88% (day 12) and 54.4% (day 16). The figures for the reference drug; Betadine\(^a\) were 59.74% (day 12) and 87.36% (day 16). The WC\(_{50}\) values showed that both formulations (20, 30% gel) produced a higher wound contraction rate (10.38, 9.53) than control base gel (14.53) and Betadine\(^a\) (10.82) (Table 3). The figure clearly signify that the wound healing effects of

![Fig. 1: Effect of topical application of *Z. nummularia* extract gel formulation (20%) gel on wound healing after 4th and 12th day](image)

20% (Fig. 1), 30% (Fig. 2) of *Z. nummularia* extract gel formulations and Betadine\(^a\) (Fig. 3) on 4th and 12th day.
Z. nummularia leaves were incorporated in carbopol gel and DMSO. Management of wound presents a major problem due to the high cost and side effects. Wound healing is a multifaceted sequence of events instigated by the stimulus tissues injury. A constructive stimulus may release some healing factors by wounding tissues. This sequence of physiologic measures occurs by a process of connective tissue repair. These events involve 4 phases: coagulation (prevents blood loss), inflammation, epithelial repair such as proliferation, mobilization, migration and differentiation and tissue remodeling and collagen deposition (Baie and Sheikh, 2000). Acute inflammatory phase produced in wounding condition followed by synthesis of extra cellular matrix which is afterward remodeled to form scar (Shetty et al., 2008). Gel formulations produced a healthier wound contraction in contrast with the ointment formulations (Srikanth et al., 2008). In addition the favorable effects on inflammation and wound healing by enhancing percutaneous by DMSO (Duimel-Peeters et al., 2003). Carrageenan induced inflammatory process is believed to be biphasic (Vinegar et al., 1987). The early phase seen at the first hour is recognized to the release of histamine and serotonin (Crunkhorn and Meacock, 1971). The next accelerating phase (swelling) is due to the release of bradykinin, prostaglandin and lysozyme. Our results revealed that administration of herbal gel and marketed formulation inhibited the rat paw edema and inhibition is probably due to chemical inflammation mediators. The results of the present investigation suggest that 20 and 30% extract gel formulations produced significant anti-inflammatory effect. The anti-inflammatory activity exerted by formulated gels suggests that they could have proceeded by disturbing prostaglandin, kinin, bradykinin and lysozyme synthesis. The presence of terpenes, glycosides and sterols in plants has been found to exert active anti-inflammatory effects (Chawla et al., 1987). More pronounced activity of the plants may be due to the presence of certain polar components such as flavonoids and glycosides (Kaith et al., 1996). Z. nummularia extract gel showed an inhibitory effect on carrageenan induced edema may be due to presence of phytochemical such as alkaloids, glycosides, flavonoids, tannins, sterols and saponins (El-Shanawawi, 1996; Shetty et al., 2008). The process of healing of wound could be influenced by some modification of above process (Rao et al., 2000). In our study the herbal gel of Z. nummularia leaves extract was found to have significant wound healing effect during the 16th days of study. WCG3 values showed that herbal formulations produced a higher wound closure rate than both negative and positive controls. The flavonoids and tannins of the plant are responsible for the free radical
scavenging activity, anti-inflammatory as well as improved regeneration and organization of the new tissue. The two classes are likely one of the important components in wound healing process (Leite et al., 2002; Karodi et al., 2009). Since, Z. nummularia grows abundantly especially in Asian countries, it could be a reasonably economical therapeutic agent for management of wound. This plant is used traditionally in gout, rheumatism, carbuncles, ulcers, abscess, boils and old wound diseases (Parveen et al., 2007; Goyal et al., 2011; Khan, 2009) as well as antioxidant and antibacterial activities of the aerial parts extract. Mahasneh (2002) and Chanda et al. (2011) supported the present finding of anti-inflammatory and wound healing activities. However, further tests are needed to explore the exact active principle(s) responsible for the anti-inflammatory and wound healing activities.

CONCLUSION

The alcoholic extract of Z. nummularia leaves formulated gels possessed anti-inflammatory property. In comparison with marketed products, it showed a good wound healing effects. The results of this study indicated that the local application of Z. nummularia formulated gels can be an effective medication for inflammation and wound healing.

ACKNOWLEDGMENTS

The authors are thankful to Mr. Malik Saud, Dr. Rais and to all members of the Pharmacology Department of College of Pharmacy, KSU for their help in using the animals and apparatus necessary for the pharmacological study.

REFERENCES


