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Anti-inflammatory and Wound Healing Activities of Herbal Gel Containing an Antioxidant *Tamarix aphylla* Leaf Extract

¹Hasan Soliman Yusufoglu and ^{1,2}Saleh Ibrahim Alqasoumi

¹Department of Pharmacognosy, College of Pharmacy, Salman Bin Abdulaziz University, Al Kharj 11942, Kingdom of Saudi Arabia

²Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia

Abstract: *Tamarix aphylla*, a traditionally used plant in the Middle Eastern countries, was screened for its potential antioxidant, anti-inflammatory and wound healing effect. Antioxidant activity of *T. aphylla* ethanolic leaf extract was measured by 1, 1, diphenyl, 2 picryl, hydrazyl and hydrogen peroxide free radicals scavenging method. Herbal gel formulations containing 15 and 25% *T. aphylla* extract in gel base, namely Carbapol-934, were prepared. Further herbal gel formulations were evaluated for its anti-inflammatory activity by carrageen an induced paw edema model and wound healing activity by excision wound model in Wister rats. Percentage, reduction in paw edema and wound contraction was measured in these models. The extract showed maximum scavenging activity i.e., 80.81 ± 0.29 and 67.76 ± 0.09 at $400 \mu\text{g mL}^{-1}$ by 1, 1, diphenyl, 2 picryl, hydrazyl and hydrogen peroxide free radicals scavenging method, respectively. In carrageen an induced paw edema model and excision wound model, the formulation showed optimum percentage inhibition of 53.07 and 89.6% with 25% gel formulation which was comparable to the standard Diclomax[®] and Betadine[®], respectively. The Present study concludes that *T. aphylla* leaves extract, possesses antioxidant, anti-inflammatory and wound healing activities.

Key words: *Tamarix aphylla*, antioxidant, herbal gel formulation, anti-inflammatory, wound healing

INTRODUCTION

Tamarix aphylla or related plant species (F. Tamaricaceae) (Quranic name = Athil), mention in the Quran, Ahadith and Islamic literature for the folk medicinal use as jaundice, bad evils, rheumatism, wound and abscesses (Marwat *et al.*, 2009). The several plants used in Qassim area of Saudi Arabia for the treatment of camel skin diseases, the *T. aphylla* is one of them. When butter and Vaseline, base formulation of this plant fine powdered applied over lesions once or twice daily for 3-7 days it cure mycotic or allergic dermatitis (Abbas *et al.*, 2002). *T. aphylla* is used in the Coastal Mediterranean region of Egypt, for astringent and eczema capitis (Heneidy and Bidak, 2004) and in the Eastern Mediterranean region for eye inflammation and fever (Azaizeh *et al.*, 2006). The methanolic extract of leaf and bark showed good antimicrobial and antioxidant activities (Vadlapudi *et al.*, 2009). The active constituents of *T. aphylla* are alkaloids, flavonoids, tannins and other polyphenolic compounds (Abbas *et al.*, 2002; Mahmoud and Sahar, 1994; Merfort *et al.*, 1992). The first glycosylated isoferulic acid, isoferulic acid 3-O- β -glucopyranoside, together with the

new phenolics, tamarixetin 3,3'-di-sodium sulphate and dehydrodigallic acid dimethyl ester have been characterized from a flower extract of *T. aphylla* (Nawwar *et al.*, 2009). Methanolic extract of *T. aphylla* is a rich source of flavonoids that appear to have a protective effect for human health (Shafaghat, 2010). Dichloromethane extract of *T. aphylla* showed significant antifungal, antibacterial, topical anti-tumor and nematocidal activities and provided a new source of further exploration in this respect (Mughal *et al.*, 2011; Abdel-Rahman and Saleh, 2006). The powdered leaves of *T. aphylla* are used to treat toothache and smoke from burnt leaves is passed over wounds to heal them (Kamal *et al.*, 2009). Healing of a wound is a complex and protracted process of restoring cellular structures and tissue layers. The wound healing process can be divided in three distinct phases the acute inflammatory phase, the proliferative phase and the remodeling phase (Shetty *et al.*, 2008). The religious Islamic literature and traditional data indicates the wound healing and anti-inflammatory properties, which is used by remote area of Saudi Arabia (Abbas *et al.*, 2002). Documented information of folkloric use in Saudi Arabia is lacking, thus the present study was aimed to

investigate claimed wound healing and anti-inflammatory used and supported their mechanism through antioxidant properties.

MATERIALS AND METHODS

Plant material: The fresh leaves of *T. aphylla* were collected in March, 2010 from Al-Kharj region of Saudi Arabia. The plant was authenticated by Dr. Mohammad Atiqur Rahman, taxonomist of the Medicinal and Poisonous Plant Research Centre (MAPPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen of this plant has been deposited at the herbarium of the Department of Pharmacognosy, College of Pharmacy, AL-Kharj University KSA.

Extraction: The air dried powdered leaves of *T. aphylla* (300 g) were macerated with 2000 mL of 90% ethanol for 3 days at room temperature. The obtained extract was filtered and concentrated under reduced pressure at 40°C. The thick solution of extract was lyophilized to produce 27.5 g, used for the various experimental studies.

Chemicals and drugs: Hydrogen peroxide, glycerin, triethanolamine and ethanol were obtained from Merck and Co. Inc (USA). Carrageenan, DPPH (1, 1, diphenyl, 2 picryl, hydrazyl), carbapol-934 gel were supplied by Sigma (USA). Diclomax® (1% Diclofenac sodium) and Betadine® (10% Povidone-Iodine) were purchased from a local pharmacy in King Khalid Hospital, Riyadh KSA.

Antioxidant activity: The antioxidant activity of the ethanolic extract of *Tamarix aphylla* was evaluated by using two *in vitro* assays, DPPH radical scavenging assay and of hydrogen peroxide scavenging assay.

Quantitative evaluation of the DPPH free radical scavenging activity: The ability of extract to scavenge free radical was examined *in vitro* towards DPPH stable radical using spectrophotometric method (Ruch *et al.*, 1989).

Quantitative evaluation of the hydrogen peroxide scavenging activity: The ability of the extract to scavenge hydrogen peroxide (H₂O₂) was determined using spectrophotometric method (Subhan *et al.*, 2008).

Gel formulations: 100 grams of Control base, 15 and 25% gel were separately prepared using *T. aphylla* extract. Carbapol, 1.5 g was mixed with an adequate amount of distilled water in three different mortars and allowed to soak for 24 h. Triethanolamine-10 mL was added drop-wise with continuous stirring using mechanical stirrer. A weighed amount of the extract (15 or 25 g) was added to

the last two mortars separately whereas first mortar keep as such and mixed using pestle.

Animals: Wistar rats (180-200 g) of either sex were obtained from the experimental animal care centre, college of Pharmacy, 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 with Purina chow diet and drinking water *ad libitum*. The experiments and procedures used were approved by the Ethical Committee of the College of Pharmacy, King Saud University, Riyadh, KSA.

Toxicity study of plant extract: Acute toxicity of the plant extract was determined in rats (Asif and Kumar, 2009). Doses of 50, 100, 300, 1000 and 2000 mg kg⁻¹ body weight were administered orally, to separate groups of rats (n = 5) after overnight fasting.

Anti-inflammatory activity: The Anti-inflammatory activity was evaluated in Wister albino rats using a carrageenan-induced paw edema test (Lira *et al.*, 2008). The rats were divided into 4 groups (n = 5). Rats of the 1st (negative control) and 2nd (positive control) groups were treated with the base gel and Diclomax® respectively. Animals of the 3rd and 4th groups were treated with the extract gel in concentrations of 15 and 25%, respectively. All treatments were applied to the planter surface of the left hind paw of rats by gentle rubbing of 500 mg with the index finger. After one hour, acute inflammation was induced by the subplanter injection of 0.1 mL of 1% carrageenan in normal saline 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:

$$\text{anti-inflammatory activity (\%)} = (V - V_1) / V_1 \times 100$$

where, V is the paw volume after 3 h carrageenan injection and V₁ is Initial paw volume

Wound healing activity: The excision wound model was used to monitor wound contraction and wound closure time (Bhat *et al.*, 2007). Four groups (n = 5) of albino rats were used in the experiment. 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 cm²) was excised (Fig. 1) using toothed

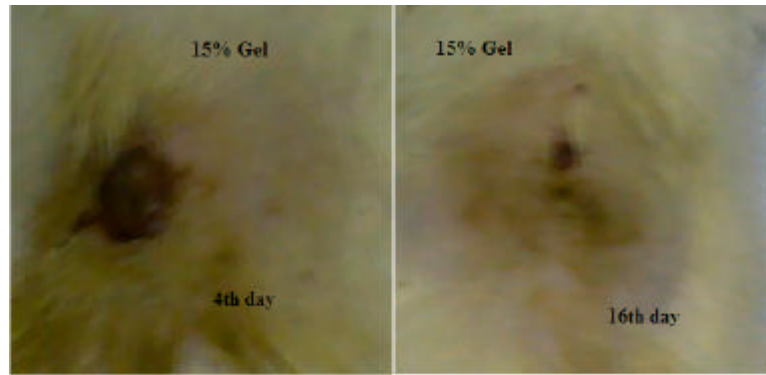


Fig. 1: Effect of topical application of *T. aphylla* extract gel formulation (15% gel) on wound healing after 4th and 16th day

forceps, scalpel and pointed scissors. A fresh surgical blade was used for the perpendicular cut in each animal and tension of skin was kept constant during the procedure. Wound of the 1st (negative control) and 2nd (positive control) groups were treated with the base gel and Betadine®, respectively. Animals of the 3rd and 4th groups were treated with the extract gel in concentration of 15 and 25%, respectively. Treatment with 500 mg of the base gel, standard drug and the extract gels was performed by application topical on the wound surface once a day for 16 days. The wound areas were traced on 1-mm² 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:

$$\text{Wound contraction (\%)} = (\text{Wi} - \text{Wt}) / \text{Wi} \times 100$$

Where: Wi = the wound area immediately after wound excision, Wt = the wound area on day t.

Statistical analysis: The results were expressed as Mean±SEM. The data were subjected to one-way ANOVA student t-test using graphPad Prism 5 software. p<0.001 was considered as significant.

RESULTS

Antioxidant activity: In the DPPH radical scavenging assay, the tested extract reduced DPPH free radical in a concentration dependent manner. The lowest percentage scavenging property of extract was 11.18±0.21%, found at 10 µg mL⁻¹ and highest percentage scavenging property was 80.81±0.29% found at 400 µg mL⁻¹ (Table 1). The H₂O₂ scavenging activity of extract was also concentration dependent. The lowest percentage scavenging property (11.78±0.05%) was found at

Table 1: Antioxidant activity of *T. aphylla* alcoholic leaf extract by 1, 1, diphenyl, 2 picryl, hydrazyl scavenging method

Concentration (µg mL ⁻¹)	Leaf extract (% inhibition)
10	11.18±0.21
20	23.47±0.37
50	48.06±0.13
100	62.98±0.05
200	76.36±0.05
400	80.81±0.29

Values are the average of triplicate experiment and represented as Mean±SEM

Table 2: Antioxidant activity of *T. aphylla* alcoholic leaf extract by hydrogen peroxide scavenging method

Concentration (µg mL ⁻¹)	Leaf extract (% inhibition)
50	11.78±0.05
100	22.47±0.35
150	36.61±0.67
200	48.37±0.02
400	67.76±0.09

Values are the average of triplicate experiment and represented as Mean±SEM

Table 3: Anti-inflammatory activity of *T. aphylla* alcoholic leaf extract formulated herbal gels by carrageenan induced hind paw oedema in rats

Drug	Volume of rat paw oedema (mL)	(%) inhibition
Control base	2.28±0.06	--
Diclomax®	0.44±0.06***	80.70
15% Gel	1.81±0.06**	20.61
25% Gel	1.07±0.01***	53.07

Values are Mean±SEM. n = 5 in each group, **p<0.01, ***p<0.001 when compared to control base

50 µg mL⁻¹ and highest percentage scavenging property (67.76±0.09%) was showed at 400 µg mL⁻¹ (Table 2).

Toxicity study: The obtained results indicated that different doses of *T. aphylla* extract (up to 2000 mg kg⁻¹) did not produced any symptoms of acute toxicity.

Anti-inflammatory activity: In control base the carrageenan-induced rat paw edema mean reduction at 3 h was 2.28±0.06 mL (Table 3). 1% Diclofenac sodium (Diclomax®) and extract gel 15 and 25% produce significant reduction of carrageenan-induced paw edema

as compare to control base gel groups (0.44±0.06, 1.81±0.06 and 1.07±0.01 mL). The inhibition was however less than that of the standard drug. The highest anti-inflammatory effect was recorded with the extract gel 25% with 53.07% paw swelling reduction.

Wound healing activity: The control base, 15 and 25% gel and Standard Betadine® were studied for percentage wound size reduction using rat excision wound model. The result of present study revealed that the topical application of the extract gel on the experimentally excised wound surface in concentration of 15 and 25% accelerate the wound healing process (Table 4). The percentage of wound contraction in the extract gel (15 and 25%) medicated groups were reduced by 9.6 and 11.2%, respectively on day 4 and 83.2 and 89.6% on day 16. The corresponding figures for the control animals were 4.9% (day 4) and 54.5% (day 16). The figures for the reference

drug; Betadine® were 6.19% (day 4) and 85.2% (day 16). The wound half closure time (WC₅₀) values showed that both formulations (15 and 25% gel) produced a higher wound contraction rate (11.06 and 10.53) than control base gel (13.92) (Table 5). The figure clearly signify that the wound healing effects of Betadine®, 15 and 25% extract-gel on 4 and 16th day (Fig. 2, 3).

Table 4: Time required for wound healing by *T. aphylla* alcoholic leaf extract formulated gel and standard Betadine® (10% Povidone-Iodine)

Treatment	Formulations (% wound contraction)			
	4 day	8 day	12 days	16 days
Control base	4.9±0.19	13.8±0.11	45.0±0.11	54.5±0.19
Betadine®	6.19±0.19**	21.8±0.11***	63.0±0.11***	85.2±0.16***
15% gel	9.6±0.11***	20.4±0.11***	58.0±0.25***	83.2±0.16***
25% gel	11.2±0.16***	34.0±0.15***	64.8±0.10***	89.6±0.11***

Each value is the Mean±SEM, n = 5. Values are the average of triplicate experiment. **p<0.01, ***p<0.001 when compared to control base

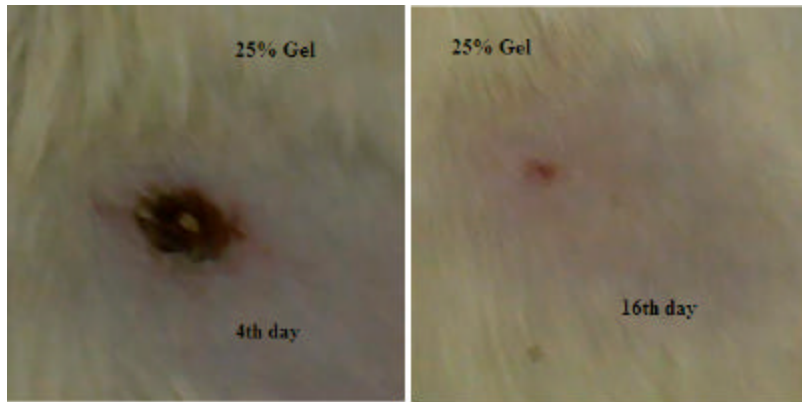


Fig. 2: Effect of topical application of *T. aphylla* extract gel formulation (25% gel) on wound healing after 4th and 16th day

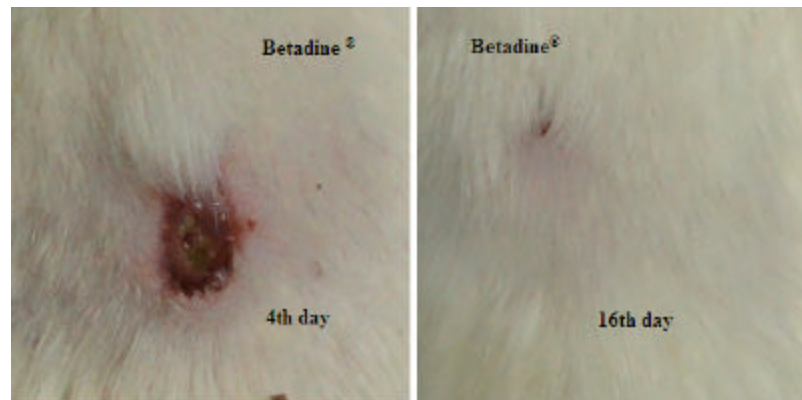


Fig. 3: Effect of topical application of Betadine® (10% Povidone-Iodine) on wound healing after 4th and 16th day

Table 5: Effect of *T. aphylla* alcoholic leaf extract gel and standard Betadine® (10% Povidone-Iodine) formulations on WC₅₀ (The wound half closure time)

Treatment	WC ₅₀ (Days)
Control base	13.92
Betadine®	10.82
15% gel	11.06
25% gel	10.53

Each value is the Mean±SEM, n = 5. Values are the average of triplicate experiment

DISCUSSION

Antioxidant activity: DPPH is a free radical, stable at room temperature, which produces a violet solution in ethanol that fades in the presence of antioxidant molecules. Recent studies demonstrated that the interaction of a potential antioxidant with DPPH free radical depends on its structural conformation. The number of DPPH molecules that are reduced seems to be correlated with the number of available hydroxyl groups (Cosio *et al.*, 2006). The use of DPPH provides an easy and rapid way to evaluate antioxidants, it abstracts the phenolic hydrogen of the electron-donating molecule and this could be the general mechanism of the scavenging action of anti-peroxidative (Ratty *et al.*, 1988). Based on DPPH free radical reduction results obtained from study, the antioxidant properties are dose dependent (Table 1). *T. aphylla* plant has active constituents like flavonoids, tannins, triterpene and other polyphenolic compounds (Mahmoud and Sahar, 1994; Merfort *et al.*, 1992). Our results strongly suggested that these phytochemicals are responsible for antioxidant activity. The ability of hydrogen peroxide to initiate lipid peroxidation is dependent on its ability to generate OH radical through the Fenton reaction (Kellogg and Fridovrich, 1975). The ability of plant extract to scavenge OH radical and superoxide anion those largely responsible for the damage of macromolecules hence, protected the macromolecules from oxidative damage (Ogunlana *et al.*, 2008). The result clearly indicates that the *T. aphylla* extract protect OH free radical in dose dependent mode (Table 2).

Toxicity study and gel formulation: Toxicity study revealed that doses up to 2000 mg kg⁻¹ of *T. aphylla* extract is safe for animals use, so 15 and 25% of extract was selected for gel formulations (Bhat *et al.*, 2007).

Anti-inflammatory activity: At the injured site carrageenan-induced oedema involves the synthesis or release of pain and fever mediators, including prostaglandins, especially the E series, histamines, bradykinins, leucotrienes and serotonin. Inhibitions of these mediators improve the inflammation and other symptoms. The edema which develops in rat paw started

between 0 to 2 h and reaches to maximum approximately after 3 h post injection of Carrageenan and then it begins to decline (Vinegar *et al.*, 1987). The probable mechanism of action of carrageenan induced edema is bi-phasic, the first phase is attributed to the release of histamine, serotonin, 5-HT and kinins in the first hour; while the second accelerating phase of swelling is related to the release of prostaglandin, bradykinins and lysozymes-like substances in 2-3 h (Di Rosa *et al.*, 1971; Brooks and Day, 1991). The present study clearly demonstrate that the 15, 25% and marketed (Dielomax®) gel formulations possesses a potent anti-inflammatory activity against carrageenan-induced rat paw edema (Table 3). Anti-inflammation is the first step in the wound healing, so these formulations were further tested against excise wound model.

Wound healing activity: Wound contraction is the process of mobilizing healthy skin surrounding the wound to cover the denude area. This centripetal movement of wound margin is believed to be due to the activity of myofibroblast (Gabbiani *et al.*, 1972). In recent years, oxidative stress has been implicated in a variety of degenerative process and diseases; these include acute and chronic inflammatory condition such as wound (Maier and Chan, 2002). In present study, using excision wound model, animal treated with the 15 gel and 25% gel showed significant decrease in wound area (Table 4) and order of WC₅₀ was 25% gel > Betadine® > 15% gel > control base gel (Table 5), the enhanced capacity of wound healing with the plant could be explained on the basis of anti-oxidant and anti-inflammatory effects of *T. aphylla*. Previous studies indicated that the major phytoconstituents present in *T. aphylla* are tannins, flavonoids, polyphenolics, isoferulic acid glycosides and triterpenes (Abbas *et al.*, 2002; Mahmoud and Sahar, 1994; Merfort *et al.*, 1992). The antioxidant and anti-inflammatory activities of flavonoids were believed to be one of the important mechanisms in wound healing (Marwah *et al.*, 2007) and tannin improved regeneration and organization of the new tissue and hasten the wound healing process (Leite *et al.*, 2002). The benefits of this plant for human health has been already mentioned in Islamic Religious books, i.e., toothache and wound healing. The recent study explored the antifungal, nematocidal and antibacterial and topical anti-tumor activities and provided a new source of further examination (Shafaghat, 2010; Mughal *et al.*, 2011; Kamal *et al.*, 2009). The results of wound contraction studies indicate that all the formulations enhance wound healing in open wounds due to antioxidant and anti-inflammatory properties.

CONCLUSION

T. aphylla alcoholic extract possessed antioxidant properties. When formulated gels were compared with marketed products for inflammation and excision wound conditions, showed a good anti-inflammatory and healing properties. Hence, these results suggest that the application of *T. aphylla* formulated gels can be an effective medication for inflammation, wound healing and provides a rationale use of the plant in inflammatory and injury conditions.

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