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Effect of *Rhus coriaria* extract on wound healing potential in *Sprague Dawley* rats

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Abstract

Background and objective: *Rhus coriaria*, a well-known spice grown in the Kurdistan region of Iraq, commonly known in the Middle East Region by (Sumac), is reported to enclose many medical benefits. This study assessed the effects of topical application of *Rhus coriaria* extract on the rate of wound closure, the Hydroxyproline (HXP) and Nitric Oxide (NO) levels were measured using the healed skin tissue homogenate the elemental composition of the plant was screened.

Methods: Adult male Sprague Dawley rats were topically treated with 0.2 mL of the vehicle (gum accacia), Intrasite gel (positive control), 100 and 200 mg/kg of *Rhus coriaria* extract.

Results: Wounds dressed with the extract and Intrasite gel healed significantly earlier than those with the vehicle. The high content of HXP and NO proves that the effect of *Rhus coriaria* extract on an excision wound model was significantly higher than that of the vehicle itself. The highest mineral contents were in Potassium, Calcium, Phosphorus, Magnesium and the extract was rich in energy content.

Conclusion: The current study concluded that *Rhus coriaria* showed high potential in wound healing activity.

Keywords: *Rhus coriaria;* Wound healing; Hydroxyproline; Nitric oxide**.**

Introduction

Rhus coriaria is a plant from family Anacardiaceae, commonly known as (Sumac) in the Mediterranean countries. It is a very popular spice, condiment and a major souring agent,[1] which found to possess different pharmacological activities such as antimicrobial[2] anti diabetic[3] hepato -protective[4] hypo-glycaemic[5] antioxidant,[6] DNA protective[7] and antibacterial activities.[8] Previous studies have reported that the extract of *Rhus* species may be a source of bioflavonoids that revealed the higher activity of this species.[9] Also, Sumac is a rich source of hydrolysabletannins,[6] Gallotannins.[10] Moreover, isomers of gallotannins and flavonoids were reported to be present in *Rhus coriaria*.[11] The physicochemical properties along with the mineral constituents of sumac were reported[12] also potassium, calcium, magnesium and phosphorus were found to be predominant elements in *Rhus coriaria* fruit. This study aimed to assess potential effects of *Rhus coriaria* on the enhancement of wound healing process in rats.

Methods

Plant Extraction:

Dried fruit part *Rhus coriaria* was used. For the preparation of ethanol extract, 100 grams of their fine powder was soaked in 1000 milliliters of ethanol for three days. After that, the mixture was filtered by filter paper (Whatman No.1) and extracted under compact pressure in a rotating evaporator.

Elemental composition:

500gm of the fine powder of *Rhus coriaria* was sent to the Consolidated Laboratory (M) Sdn. Bhd. Kuala Lumpur, Malaysia for the analysis of the minerals, fats and elemental compositions.

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***In vivo* wound healing:**

Rhus coriaria ethanol extract was dissolved by using the vehicle: gum acacia (a complex mixture of polysaccharides and glycoproteins) that was used after dissolving in normal saline as described by Zahra et al.[13] Two grams of gum acacia was dissolved in 100 ml of normal saline. From this, 10 ml of solution, which contains 200 mg of gum acacia, was used for dissolving one gram and two grams of *Rhus coriaria* ethanol extract. Intrasite gel: an amorphous gel, containing 2.3% of modified carboxymethyl cellulose (CMC) polymer with propylene glycol (20%) was used as the reference control. *Sprague dawley* adult male rats were randomly divided into four groups of 6 rats each. Each rat that weighed between 185–200 g were housed separately (one rat per cage). The animals were maintained on a standard pellet diet and tap water. The animals were anesthetized with a light dosage of ketamin and xylazine anesthesia. The skin was shaved by electrical shaver, disinfected with 70% alcohol. An area of uniform wound 2.00 cm in diameter was excised from the nape of the dorsal neck of all rats with the aid of round seal as described by Rawat et al.[14] Topical application of 0.2 ml of the vehicle (Gum acacia) was applied topically to the wounds of Group 1 rats twice a day. Group 2 rats were topically applied twice daily with 0.2 ml of 100 mg/kg of *Rhus coriaria* ethanol extract, and the Group 3 rats were dressed twice daily with 0.2 ml of 200 mg/kg *Rhus coriaria* ethanol extract. Group 4 rats were dressed twice daily with 0.2 ml of 200 mg/kg reference drug (Intrasite gel). The contraction of the wound area was measured. Wound areas were traced manually and calculated in square millimeters. The wound closure area of each animal was assessed by tracing the wound on days 0, 7 and 14 post-wounding surgery. The wound closure rate was expressed as the percentage of wound area compared with that on the post-operative day by using transparency paper and a permanent marker under general anesthesia (a mixture of Ketamine and Xylazil) as described by Abdulla et al.[15] The wound areas recorded were measured by using a graph paper. The percent wounds healing on these days were determined. The number of days required for falling of scar without any residual raw wound gave the period of epithelization. The wound area was measured immediately by placing a transparent tracing paper over the wound and tracing it out. The tracing paper was placed on 1 mm^2 graph sheet and traced out. The squares were counted and the area recorded.

Determination of Hydroxyproline and Nitric Oxide:

The wound tissue homogenate from each rat was prepared at 4ºC by using a teflon homogenizer (Polytron, Germany). After centrifugation at 4,500 rpm for 15 min at 4ºC, the supernatant was used for Nitric oxide (NO) and hyroxyproline (HXP) determinations using the NO and HXP assay kit (Cayman Chemical Co., USA).

Statistical analysis:

All values are expressed as mean ± S.E.M. and the statistical significance of differences among groups was assessed using Post Hoc test, one-way ANOVA. A value of P <0.05 was considered significant.

Results

Elemental composition

The chemical analysis of *Rhus coriaria* fruit part is shown in Table 1. The highest mineral contents were Potassium (544.3 mg/100g), Calcium (342.98 mg/100g), Phosphorus (109.88 mg/100g) and Magnesium (88.75 mg/100g), while it was rich in energy content (441 kcal/100g).

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In vivo wound healing test

The excision model was used, in which grossly wounds dressed with *Rhus coriaria* or with intrasite gel showed considerable signs of dermal healing and significantly healed faster compared to group received the vehicle (gum acacia). Table 2 shows the effects of *Rhus coriaria* ethanol extract on the percentage of wound healed on days post-surgery.

Table 1: Elemental composition of *Rhus coriaria* fruit part.

Test parameter	*Rhus coriaria*	Unit
Energy	441	kcal/100g
Total fat	18.1	g/100g
Carbohydrate	65.3	g/100g
Protein	4.2	g/100g
Cholesterol	<0.001	mg/100g
Dietary fibre	0.6	g/100g
Monosaturated fat	7.37	g/100g
Polysaturated fat	4.29	g/100g
Saturated fat	6.4	g/100g
Trans fat	<0.01	g/100g
Phosphorus (P)	109.88	mg/100g
Potassium (K)	544.3	mg/100g
Sodium (Na)	3.65	mg/100g
Zinc (Zn)	1.07	mg/100g
Calcium (Ca)	342.98	mg/100g
Copper (Cu)	0.55	mg/100g
Iron (Fe)	2.92	mg/100g
Magnesium (Mg)	88.75	mg/100g
Manganese (Mn)	1.04	mg/100g
Selenium (Se)	<0.002	mg/100g

Table 2: Effect of *Rhus coriaria* ethanol extract on percentage wound healing in the experimental rats.

Animal groups	Treatments	Percentage of wound closure on the day after surgery	
		Day 7	Day 14
group 1	0.2 ml/kg Gum accacia	19 [a]	22 [a]
group 2	100 mg/kg *Rhus coriaria*	59.67[b]	66.8[b]
group 3	200 mg/kg *Rhus coriaria*	61[b]	90[c]
group 4	0.2 ml/kg Intrasite gel	68.67[b]	86.4[b]

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The percentage of healing in the vehicle control group wounds was significantly lower than those of *Rhus coriaria* extract treated groups and intrasite gel wounds at P <0.05 (Figure 1). On day 14 post-surgery showed that wound dressed with *Rhus coriaria* extract showed comparatively less scar width at wound closure compared to the vehicle-treated group. Data expressed as Mean ± SEM, Mean values (n = 6) followed by different letters (a, b, and c) in a column are significantly different as compared to Gum acacia group (P ≤0.05).

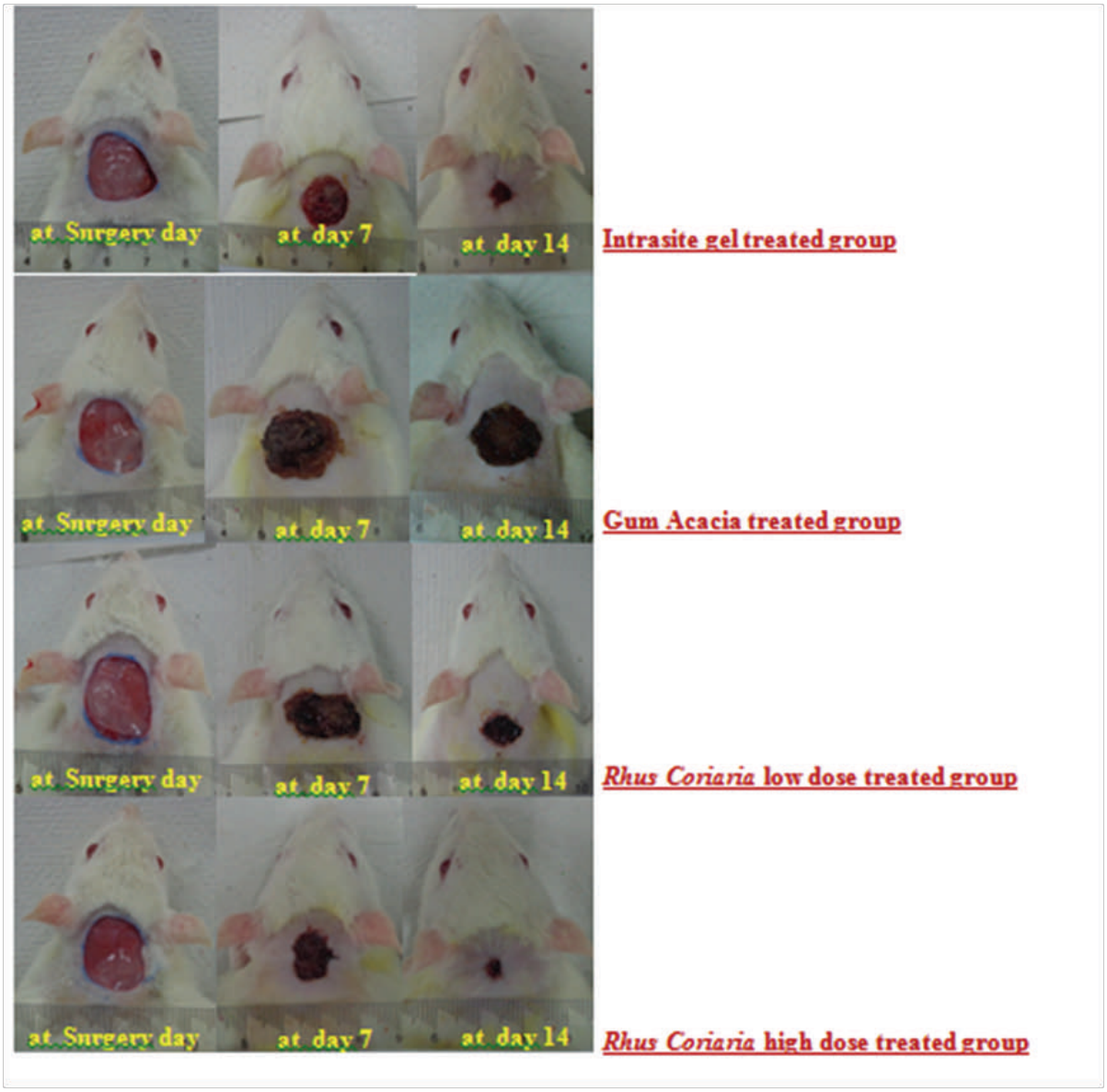

Figure 1: Gross appearance of wound healing on different days post-surgery. First row/ Rats treated with intrasite gel shows remarkably smaller wound closure area compared to vehicle. Second row/ Rats treated with 0.2 ml Gum acacia shows wide wound closure area. Third row/Rats treated with (100 mg/kg) of *Rhus coriaria* shows remarkably moderate wound closure area compared to vehicle. Fourth row/Rats treated with (200 mg/kg) of *Rhus coriaria* shows remarkably smaller wound closure area compared to vehicle.

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Treatment with *Rhus coriaria* ethanol extract significantly increased the hydroxyproline and nitric oxide levels as compared to the vehicle group as shown in Figure 2 and Figure 3.

Discussion

The wound healing achievement of *Rhus coriaria* may be due to the antioxidant content and the elevated free radical scavenging activity. The free radicals and reactive oxygen species made during tissue injury are theoretically concerned in late wound curing.[15-17] From the results, it is obvious that the treatment with the reference control (intrasite gel) significantly increased the collagen content represented as high level of hydroxylproline in comparison to the decreased levels of the vehicle control group (gum acaccia). Moreover, this study showed that *Rhus coriaria* was able to accelerate the wound healing process *in vivo* in doses of 100 mg/kg and 200 mg/kg based on the gross findings. Also, the high content of hydroxyproline and nitric oxide proves that the effect of *Rhus coriaria* extract on an excision wound model was significantly higher than that of the vehicle itself. These results were in agreement with the previous studies published elsewhere.[16,18] A previous study has shown that healing can be accelerated and enhanced by the use of specific wound dressing or care

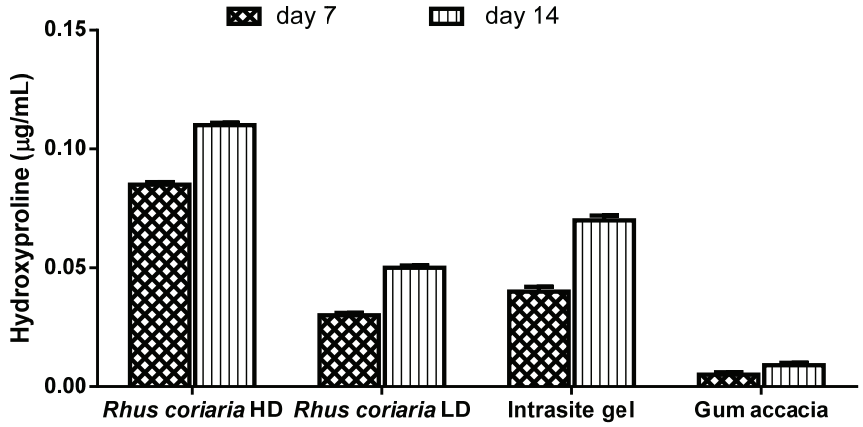

Figure 2: Hydroxyproline (HXP) levels in healed skin homogenates treated with 2% gum accacia, Intrasite gel, *Rhus coriaria* LD(100 mg/kg), *Rhus coriaria* HD (200 mg/kg*) P ≤0.05*.

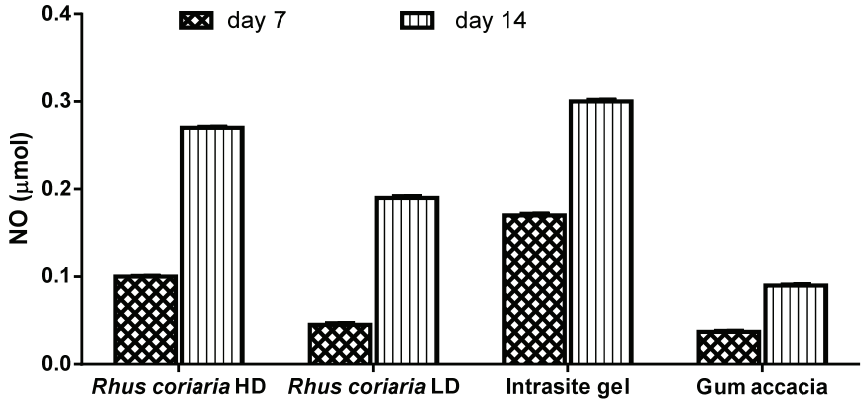

Figure 3: Nitric oxide (NO) levels in healed skin homogenates treated with 2% gum accacia, Intrasite gel, *Rhus coriaria* LD (100 mg/kg), *Rhus coriaria* HD (200 mg/kg) P ≤0.05.

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product and techniques and that it is not a passive process.[19] It has been observed that plant constituents can significantly accelerate the healing process and improve the quality of wound healing.[18] Numerous studies have shown that plant compounds could potentially be therapeutic agents to treat wounds.[17,18,20,21] The findings of this study are also in line with previous studies reported by various authors.[15,17]

Conclusion

Topical application of *Rhus coriaria* has an enhancing effect on the wound healing process through stimulating hydroxyproline production and scavenging NO. Therefore, *Rhus coriaria* is considered potentially useful in accelerating wounds, but additional research is needed to show the exact mechanisms of it.

Competing interests

The author declares that she has no competing interests.

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